NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Development of Chromatographic and Mass Spectrometric Techniques for the Analysis of Complex Mixtures Containing Aromatic Compounds

by

Hélène Perreault

Submitted in partial fulfillment of the

requirements for the Degree of

Doctor of Philosophy

at

Dalhousie University

Halifax, Nova Scotia

January 1992

ISBN 0-315-76711-1

DEDICATION

To my parents

CONTENTS

List of figures xi

List of tables xvii

Abstract xix

List of abbreviations xx

Acknowlegments xxii

1. **Introduction** 1

1.1 Polycyclic aromatic compounds (PACs) 1

1.2 Origins of PACs 2

1.3 Requirements for the analysis of PACs in complex samples 5

1.4 The Sydney Tar Pond 6

1.5 The Alberta oil sands 9

1.6 Main objectives of this work 12

2. **Experimental** 15

2.1 Sampling 15

2.1.1 Sampling of the Sydney tar pond sediment 15

2.1.2 Sampling of the Syncrude broken oil sand 16

2.1.3 Sampling of heavy gas oil (HGO) and pitch (PIT) samples 16

2.2 Extraction 17

2.2.1 Extraction of the organic matter from the tar pond and oil sand samples 17

2.2.2 HGO and PIT samples 18

2.3 Removal of elemental sulfur from the extracts 19

2.4 Fractionation 20
2.4.1 Fractionation of the tar pond extract (TPE) 20
2.4.1.1 Ramos and Prohaska method 20
2.4.1.2 Alumina fractionation scheme 22
2.4.1.3 Silica over alumina fractionation method 24
2.4.1.4 Attempts to separate PASHs using copper coated on silica 25
2.4.1.5 Second scheme for the separation of PASHs 27
2.4.1.6 Scheme for separation of PANHs 30
2.4.2 Fractionation of the Syncrude samples 34
2.4.2.1 Anion exchange chromatography 34
2.4.2.2 Alumina column chromatography 34
2.4.2.3 Silica over alumina column chromatography 37
2.5 Chromatography as the final determination method 38
2.5.1 Gas chromatography (GC) 38
2.5.2 High performance liquid chromatography (HPLC) 40
2.5.2.1 Reversed phase HPLC 41
2.5.2.2 Normal phase HPLC 43
2.5.3 Supercritical fluid chromatography (SFC) 46
2.5.3.1 Introduction to SFC 46
2.5.3.2 Capillary column SFC 47
2.5.3.3 Packed column SFC 48
2.6 Mass spectrometry (MS) 56
2.6.1 Interfacing 56

2.6.1.1 Interfacing GC to MS 56

2.6.1.2 Interfacing HPLC to MS 58

2.6.1.3 Interfacing SFC to MS 62

2.6.2 Ionization modes used in this work 67

2.6.2.1 Electron impact (EI) ionization 67

2.6.2.2 Chemical ionization (CI) 72

2.6.3 Selectivity enhancement 74

2.6.3.1 Study of the fragmentation of PANH molecular dications 75

2.6.3.2 Monitoring of the ionic signal of the half-integral mass dications of PANHs 76

2.6.3.3 GC/MS/MS 78

2.6.3.4 Constant neutral loss investigations 78

3. **Results and discussion: sampling** 80

3.1 Sampling of the Sydney Tar Pond sediment 80

3.2 Sampling of the Syncrude oil sand 80

4. **Results and discussion: extraction** 82

4.1 Sydney Tar Pond sediment and Syncrude oil sand sample 82

5. **Results and discussion: removal of the elemental sulfur** 84

6. **Results and discussion: fractionation techniques** 87

6.1 Fractionation of the TPE 87

6.1.1 Ramos and Prohaska method 87

6.1.2 Alumina fractionation method 89

6.1.3 Silica over alumina fractionation method 93

6.1.4 Copper-treated silica 99

6.1.5 Scheme for the separation of PASHs from other PACs 104

6.1.6 Scheme for separating PANHs from other PACs 110

6.2 Fractionation of the Syncrude samples 114

6.2.1 Anion exchange chromatography 114

6.2.1.1 Amberlyst-26 anion exchange resin 114

6.2.1.2 Anion exchange chromatography on cellulose 116

6.2.1.3 Silica coated with potassium hydroxide 116

6.2.2 Alumina fractionation scheme 123

6.2.3 Silica over alumina 127

6.2.4 Normal phase SFC fractionation 133

7. **Results and discussion: chromatography and standard MS interfacing** 140

7.1. Gas chromatography/mass spectrometry (GC/MS) 141

7.1.1 GC/MS of the TPE 142

7.1.1.1 Conventional GC/MS 142

7.1.1.2 High temperature GC/MS 152

7.1.2 GC/MS of the Syncrude samples 154

7.1.2.1 GC/MS of the HGO sample 154

7.1.2.2 GC/MS of the oil sand extract (OSE) sample 154

7.2 HPLC/MS 158

7.2.1 HPLC/MS of the TPE 160

7.2.1.1 Analysis of compounds in the range accessible to GC/MS 160

7.2.1.2 Analysis of the high molecular weight portion of the TPE 169

7.2.2 HPLC/MS of the Syncrude samples 179

7.2.2.1 HPLC/MS of the HGO sample 182

7.2.2.2 HPLC/MS of the OSE sample 197

7.2.2.3 HPLC/MS of the PIT sample 202

7.2.2.4 Overall comparison of the contents of the HGO, OSE and PIT samples as determined by reversed phase HPLC/MS 207

7.3 SFC/MS 209

7.3.1 SFC/MS of the TPE 209

7.3.1.1 Capillary column SFC/MS 209

7.3.1.2 Packed column SFC/MS 218

7.3.2 SFC/MS of the Syncrude samples 228

7.3.2.1 Reversed phase SFC/MS of the HGO sample 228

7.3.2.2 Normal phase SFC/MS of the HGO sample 231

8. **Results and discussion: selectivity enhancement** 239

8.1 Fragmentation of doubly charged ions of PANHs 239

8.1.1 Compounds containing a pyridine ring 240

8.1.2 Compounds containing a pyrrole ring 244

8.1.3 Compound discrimination 250

8.1.4 Selectivity enhancement 250

9. **Conclusions** 257

10. **Appendices** 262

10.1 I: GC/MS data obtained for the TPE sample 262

10.2 II: Reversed phase HPLC/MS data obtained for the TPE sample for compounds in the GC-amenable range 271

10.3 III: Reversed phase HPLC/MS results obtained for the TPE sample eluted in the acetonitrile-dichloromethane portion of the gradient 277

10.4 IV: Reversed phase HPLC/MS data obtained for the alkylated-PAH standard solution and for the Syncrude samples 289

10.5 V: Capillary column SFC/MS data obtained for the TPE 300

10.6 VI: a) Reversed phase and b) normal phase packed column SFC/MS data obtained for the HGO sample 303

10.7 VII: PAHs listed in Appendices I-IV as the reference structures of all the compounds tentatively identified in the mixtures 308

11. **Bibliography** 311

LIST OF FIGURES

1.1.1 Structures and acronyms of fluorene and anthracene derivatives 2

1.1.2 Structures of 3 different types of PAHs 4

1.4.1 Location of the Sydney Tar Pond 8

1.5.1 Location of Fort McMurray, Alberta 10

1.5.2 Schematic representation of the methods used by Syncrude Canada Inc. to process tar sands into crude oils 13

2.4.1 Method for the isolation of PASHs from other PACs 28

2.4.2 Method for the isolation of PANHs from other PACs 32

2.5.1 Mobile phase pressure and temperature programs in capillary column SFC/MS 49

2.6.1 Schematic representation of a GC/MS system 57

2.6.2 VG 20-250 quadrupole mass spectrometer 59

2.6.3 Finnigan 4500 GC/MS system 60

2.6.4 VG ZAB-EQ mass spectrometer 61

2.6.5 VG moving belt interface 63

2.6.6 VG belt sample depositor 66

2.6.7 Schematic representation of an electron impact ionization source 70

5.1.1 Effect of elemental sulfur removal on the aspect of GC/MS TICs obtained for the TPE sample 85

6.1.1 GC/MS TIC trace of the TPE PAC fraction obtained using the R&P method 91

6.1.2 GC/MS TIC trace of the TPE PAC fraction obtained using the alumina method 91

6.1.3 Mass spectra of the TPE fractions obtained using the silica over alumina method 96

6.1.4 RPLC-UV absorption chromatogram and RICs of the 15% benzene in pentane TPE fraction obtained using the silica over alumina method 97

6.1.5 GC/MS TIC chromatograms of TPE Fractions 2 and 9 obtained using the copper coated silica method 100

6.1.6 GC/MS RICs of PASHs found in TPE Fraction 1 of the copper-coated silica method 102

6.1.7 GC/MS TIC chromatogram of the TPE obtained before performing the PASH isolation method 106

6.1.8 NPLC/MS RICs of the TPE sulfone fraction obtained while performing the PASH isolation method 107

6.1.9 GC/MS TIC of the final TPE PASH fraction obtained using the PASH isolation method 109

6.1.10 GC/MS RICs of the final TPE PANH fraction of the isolation method for nitrogen containing aromatic compounds 113

6.2.1 Mass spectra of the fractions obtained using the Amberlyst-29 anion exchange resin method 115

6.2.2 Mass spectra of the fractions obtained using the KOH-silica method 118

6.2.3 RPLC-UV absorption chromatograms of the HGO chloroform fraction obtained using the KOH-silica method 119

6.2.4 RPLC-UV absorption chromatogram of the PIT chloroform fraction obtained using the KOH-silica method 119

6.2.5 Mass spectra of the fractions obtained using the KOH-silica method 121

6.2.6 Mass spectra of the HGO fractions obtained using the alumina fractionation method 124

6.2.7 NPLC-UV absorption chromatograms of the HGO fractions obtained using the alumina method 125

6.2.8 Mass spectra of the HGO fractions obtained using the silica over alumina method 128

6.2.9 RPLC-UV absorption chromatograms of the 3:1:1 methanol-ether-benzene fractions obtained using the silica over alumina method 130

6.2.10 Mass spectra of the 3rd, 8th and 15th HGO fractions obtained using the normal phase SFC method 134

6.2.11 RPLC-UV absorption chromatograms of some of the HGO normal phase SFC fractions 136

6.2.12 RPLC-UV absorption chromatograms of some of the OSE and PIT normal phase SFC fractions 138

7.1.1 GC/MS TIC and RIC chromatograms of PAHs obtained for the TPE sample 143

7.1.2 GC/MS RICs of PACs obtained for the TPE sample 147

7.1.3 Mass spectra of 3 sets of TPE PAH isomers, obtained by GC/EI-MS 149

7.1.4 Mass spectra of different TPE PACs, obtained by GC/EI-MS 151

7.1.5 High temperature GC/MS RICs obtained for the TPE extract 153

7.1.6 GC/MS TIC chromatogram of the HGO sample 155

7.1.7 GC/MS TIC and mass spectra of the OSE sample 157

7.2.1 RPLC/MS TIC trace of the TPE sample, mass spectrum of the belt background and structures corresponding to the belt peaks 161

7.2.2 RPLC/MS RICs of PACs contained in the TPE sample in the GC-amenable range 163

7.2.3 GC/MS and HPLC/MS RICs showing the complementary separation selectivity of the two methods 166

7.2.4 Mass spectra of PACs obtained by RPLC/MS 168

7.2.5 RPLC-UV absorption chromatogram and HPLC/MS RICs obtained for a mixture of high molecular weight PAH standards 171

7.2.6 RPLC-UV absorption chromatogram of the TPE sample 173

7.2.7 RPLC/MS RICs of the TPE sample obtained during the acetonitrile-dichloromethane portion of the gradient 174

7.2.8 RPLC/MS RICs of the TPE sample obtained during the dichloromethane portion of the gradient 175

7.2.9 Mass spectra of high M.W. PAHs obtained by RPLC/MS of the TPE sample 177

7.2.10 RPLC/MS RICs of a mixture of alkylated PAH standards 181

7.2.11 RPLC/MS RICs of some of the unsubstituted PAHs found in the HGO and in the TPE samples 183

7.2.12 RPLC/MS RICs of some of the alkylated PAHs found in the HGO sample 184

7.2.13 RPLC/MS RICs of the alkylated carbazoles found in the HGO sample 185

7.2.14 RPLC/MS RICs illustrating some of the differences between the HGO and TPE samples 186

7.2.15 RPLC/MS RICs of high M.W. HGO compounds obtained over the whole retention time range 189

7.2.16 Mass spectra obtained during the acetonitrile-dichloromethane portion of the RPLC/MS gradient for the HGO sample 191

7.2.17 RPLC/MS RICs (m/z 206) of some of the HGO normal phase SFC fractions 193

7.2.18 RPLC/MS RICs (m/z 242) of some of the HGO normal phase SFC fractions 195

7.2.19 Possible structures for polar compounds of M.W. 206 and 242 found in the HGO sample 196

7.2.20 RPLC/MS RICs of compounds found in the OSE sample 198

7.2.21 Possible structures of some of the compounds producing the RIC peaks in Figure 7.2.20 199

7.2.22 RPLC/MS RICs of high m/z values obtained over the whole retention time range for the OSE sample 200

7.2.23 Mass spectra obtained during the acetonitrile-dichloromethane portion of the RPLC/MS gradient for the OSE sample 201

7.2.24 RPLC/MS RICs of compounds found in the PIT sample 203

7.2.25 RPLC/MS RICs of high m/z values obtained over the whole retention time range for the PIT sample 204

7.2.26 Mass spectra obtained during the acetonitrile-dichloromethane portion of the RPLC/MS gradient for the PIT sample 206

7.2.27 Comparison of the RPLC/MS TICs obtained for the three Syncrude samples 208

7.3.1 Capillary column SFC/MS TIC and RICs obtained for PAHs contained in the TPE sample 211

7.3.2 Mass spectra obtained by capillary column SFC/MS for the TPE sample 217

7.3.3 Reversed phase SFC/MS RICs obtained for some of the PAHs found in the TPE sample 220

7.3.4 Reversed phase SFC/MS RICs obtained for some of the PAHs found in the TPE sample 221

7.3.5 Normal phase SFC/MS TIC and RICs obtained for some PAHs found in the TPE sample 224

7.3.6 Normal phase SFC/MS RICs obtained for some PAHs found in the TPE sample 225

7.3.7 Mass spectra of some high M.W. species found in the TPE sample, obtained by normal phase SFC/MS 227

7.3.8 Reversed phase SFC/MS of PACs found in the HGO sample 229

7.3.9 Reversed phase SFC/MS RICs of alkylated PAHs found in the HGO sample 230

7.3.10 Normal phase SFC/MS RICs of alkylated PAHs found in the HGO sample 232

7.3.11 Normal phase SFC/MS TIC and RICs of high M.W. compounds found in the HGO sample 234

7.3.12 Mass spectra of compounds contained in the HGO sample, obtained by normal phase SFC/MS 236

8.1.1 Fragmentation spectra of pyridine dications 241

8.1.2 Unimolecular fragmentation spectra of acridine and perdeuterated acridine dications 242

8.1.3 Energy diagram for the competing dissociation of the molecular dications of pyridine 245

8.1.4 Proposed structures involved in charge separation dissociation of the pyridine dications 246

8.1.5 Unimolecular fragmentation spectra of molecular dications of pyrrole-based aromatic compounds 247

8.1.6 Diradical structures proposed for dications of heterocyclic fused-hexagonal-ring aromatics and of pyrrole-based compounds 249

8.1.7 Low mass portion of CID fragmentation spectra of three groups of PANH isomers 251

8.1.8 Illustration of the selectivity enhancement achieved by monitoring the half integral m/z values of PANH dications 253

8.1.9 GC/MS/MS selected ion chromatograms obtained for a standard mixture of PANHs by monitoring the TIC of their CID fragmentations 254

LIST OF TABLES

2.5.1 Program used for RPLC/MS separations 42

2.5.2 Program used for NPLC/MS separations 45

2.5.3 Pressure program used for reversed phase SFC of a PAH standard mixture and the TPE sample (100% CO_2) 51

2.5.4 Pressure program used for reversed phase SFC of a PAH standard mixture and the TPE sample (10% methanol in CO_2) 52

2.5.5 Pressure program used for normal phase SFC of a PAH standard mixture (10% methanol in pentane) 53

2.5.6 Pressure and gradient program used for normal phase SFC of a PAH standard mixture (methanol in pentane) 54

2.5.7 Pressure and gradient program used for normal phase SFC of the TPE sample 55

2.5.8 Pressure and gradient program used for normal phase SFC of the HGO sample 55

2.5.9 Gradient and pressure program used for normal phase SFC fractionation of the HGO, OSE and PIT samples 56

2.6.1 Compounds studied for the fragmentation of their molecular dications 77

6.1.1 Weight of the TPE material recovered in different fractions using the R&P fractionation method 87

6.1.2 PAC/PAH ratios of the GC/MS peak areas obtained for the TPE PAC fraction (R&P method) 88

6.1.3 Weight of the TPE material recovered in different fractions using the alumina fractionation method 90

6.1.4 PAC/PAH ratios of the GC/MS peak areas obtained for the TPE PAC fraction (alumina method) 92

6.1.5 Material recovered from fractionation of the TPE using the silica over alumina method 94

6.1.6 Aromatic compounds found in TPE Fraction 3 (15% benzene in pentane, silica over alumina) and tentative identification by groups of isomers 98

6.1.7 PAC content of the 11 TPE fractions collected using the copper-coated silica method 103

6.1.8 PASHs found in the TPE by GC/MS 108

6.1.9 Comparison of the GC/MS peak intensities obtained for the basic fraction (Step 1) and for the PANH fraction (Step 7) of the PANH isolation scheme 111

6.2.1 Material recovered from the KOH-treated silica experiment for the HGO, OSE and PIT samples 122

6.2.2 Material recovered after elution of the HGO through alumina 126

6.2.3 Material recovered from the silica over alumina fractionation method for the HGO, OSE and PIT samples 132

7.2.1 High molecular weight PAH standards used in reversed phase HPLC-UV and -MS 170

7.2.2 Alkylated PAHs present in the standard solution for HPLC/MS analysis 180

7.2.3 Average retention time (scan number) of isomers contained in the alkylated-PAH standard solution 182

7.3.1 Mobile phases and modifiers used in SFC 215

ABSTRACT

Polycyclic aromatic compounds (PACs) are of great interest because they are widespread in the environment and comprise many potent mutagens and carcinogens. The main objective of this work was to characterize the PAC content of complex matrices. The materials studied in this work were a contaminated estuary sediment sample (Sydney Tar Pond, Nova Scotia), as well as samples obtained at three different stages of the processing of oil sands into synthetic crude oil (Syncrude Canada Ltd., Fort McMurray, Alberta). The characterization of the PAC content of each sample required development and application of specific sampling, extraction, fractionation and final analysis techniques. The application and development of open column chromatographic fractionation methods and chromatographic/mass spectrometric techniques was emphasized. The fractionation methods used varied depending on the origin of the samples. Gas chromatography, high performance liquid chromatography and supercritical fluid chromatography combined with mass spectrometry (GC/MS, HPLC/MS and SFC/MS) were used for final analysis of the PAC fractions. All three techniques were suitable for the analysis of the sediment sample; however, GC/MS failed in the characterization of oil sand-derived samples due to the low volatility of their components. The sediment material was found to contain mainly unsubstituted polycyclic aromatic hydrocarbons (PAHs), whereas the oil sand-derived samples contained alkylated-PAHs and alkylated carbazoles as the predominant species. Mass spectrometric selectivity enhancement for the detection of polycyclic aromatic hydrocarbons containing a nitrogen atom was also investigated using three different methods, each using the half-integral mass of the doubly charged molecular ions as the selectivity factor.

LIST OF ABBREVIATIONS

ACN: acetonitrile

APAH: amino-polycyclic aromatic hydrocarbon

CI: chemical ionzation

CID: collision-induced dissociation

CNL: constant neutral loss

CPAH: cyano-polycyclic aromatic hydrocarbon

Cx: Alkyl residue, where x= number of carbon atoms involved

DCM: dichloromethane

EI: electron impact ionization

Et-O-Et: diethylether

FID: flame ionization detector

FPD: flame photometric detector

GC: gas chromatography

HGO: heavy gas oil

HPLC: high performance liquid chromatography

HXPAH: hydroxylated polycyclic aromatic hydrocarbon

MeOH: methanol

MIKE: mass analyzed ion kinetic energy

MS: mass spectrometry

M.W.: molecular weight

NPAH: nitro-polycyclic aromatic hydrocarbon

NPLC: normal phase HPLC

OSE: oil sand extract

PAC: polycyclic aromatic compounds

PAF: polycyclic aromatic furan

PAH: polycyclic aromatic hydrocarbon

PAK: polycyclic aromatic ketone

PANH: polycyclic aromatic compound containing a nitrogen heteroatom

PAOH: polycyclic aromatic compound containing an oxygen heteroatom

PAQ: polycyclic aromatic quinone

PASH:polycyclic aromatic compound containing a sulfur heteroatom

PAX: polycyclic aromatic xanthene

PIT: pitch

RIC: reconstructed ion chromatogram

RPLC: reversed phase HPLC

SFC: supercritical fluid chromatography

SRS1J: Syncrude research sample 1J (PIT)

R&P: Ramos and Prohaska (8)

TIC: total ion chromatogram

TPE: tar pond extract

TS: tar sand

UV: ultra-violet

ACKNOWLEGMENTS

Sincere thanks are expressed to my research supervisors, Dr. P. Greig Sim of the Institute of Marine Biosciences (IMB) and Dr. Louis Ramaley of Dalhousie University, for their constant advice and support throughout the research and the writing of this thesis.

I would also like to express my appreciation to Dr. Robert K. Boyd of IMB for his precious guidance and encouragements during this project.

I wish to thank Dr. W. David Jamieson of Fenwick Laboratories (formerly of IMB) for first inviting me to pursue my Ph.D. studies at IMB (affiliated to Dalhousie), and Dr. Roger Foxall, the Director-General of IMB, for accepting me as a graduate student in the laboratories of the National Research Council.

I would like to sincerely thank Messrs. Glenn McCully, Donald Embree, Edward Dyer and William Hardstaff of IMB for their excellent technical and moral support during those three-and-a-half years.

I wish to acknowledge the help and assistance of all my other collegues of the Analytical Chemistry Section at IMB and to thank them for the stimulating research environment that they provided.

Great appreciation is expressed to Dr. Frank M. Benoit of Health and Welfare Canada, for his financial assistance during this project (Panel on Energy, Research and Development, Contract No. 57205).

Lastly, I would like to thank the Natural Sciences and Engineering Research Council (NSERC) for funding my two first years of research at IMB/Dalhousie University.

1. INTRODUCTION

1.1 Polycyclic Aromatic Compounds

Polycyclic aromatic compounds (PACs) are molecules with fused ring systems, most of which possess continuous circuits of π-electron density.

PACs are an important and widely occurring group of environmental pollutants. They are naturally present in petroleum, and are also produced by combustion and transformation of carbonaceous material; however most PAC production is due to anthropogenic activity. PACs are released in the environment by various processes such as combustion of wood in home fireplaces, coke oven emission, cigarette smoke and petroleum spills, contaminating both the atmosphere and water.

There is considerable interest in the analysis of these compounds in the environment because of their mutagenic and carcinogenic nature (1,2).

In terms of composition, PAC mixtures (naturally occurring or anthropogenic) range from simple to exceedingly complex, reflecting the thermal history of the sample and the nature of the precursors from which the PACs were formed.

PACs include a wide variety of compounds, among which polycyclic aromatic hydrocarbons (PAHs) are the most widely occurring in the environment. Substitution at various position on the aromatic rings by alkyl, nitro and other functional groups, or heteroatom substitution in the parent PAH with oxygen, nitrogen or sulfur provide a variety of different PACs. Figure 1.1.1 depicts structures for various classes of PACs. Each class is represented with a different acronym (3); for instance a polycyclic aromatic ketone is designated as PAK. In this figure fluorene is also named "166-PAH"; the compounds represented are fluorene and anthracene derivatives. The former, for instance, are designated by 166-PAx (x=K, NH, SH, etc). The isomers of benzo[a]pyrene, according to the same terminology (3), are 252-PAHs and their derivatives, 252-PAxs.

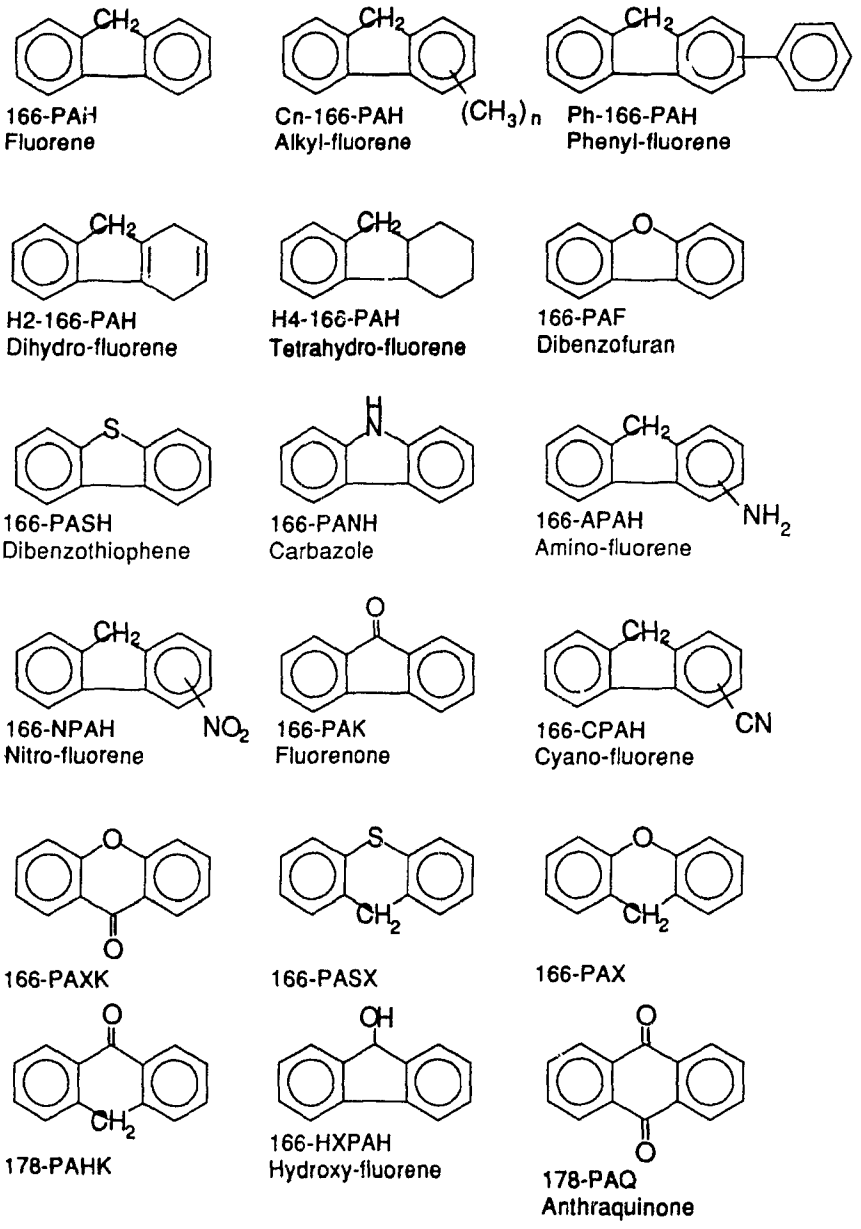


Figure 1.1.1: Structures and acronyms of fluorene and anthracene derivatives

Some rare minerals contain PAHs in almost pure form, whereas coal tar is estimated to contain some 30000 PACs, of which only a small number have been isolated and structurally identified (4).

Each given PAC from any class (with the exception of some smaller molecules) has more than one possible isomeric structure, and the number of possibilities increases with molecular weight. For example benzo[a]pyrene, a well known carcinogen, is one of 21 possible compounds containing five six-membered rings with a molecular weight of 252. In the case of alkylated PACs, isomeric possibilities increase in number with the length of the alkyl chain or the number of aliphatic carbon atoms placed around the aromatic system.

Rings are arranged in either catacondensed or pericondensed systems. Catacondensation involves ring connection in which two rings share a common bond, as in the structure of chrysene. Pericondensation may be thought of as condensation of new rings about the periphery of existing ones, to build new structures. It is well illustrated with pyrene, which has the same number of rings as chrysene, but where rings are attached to one another by more than one bond. The structures of chrysene and pyrene are shown in Figure 1.1.2. Another group of PACs features compounds whose rings are not fused, but rather attached together with a single covalent bond; the structure of biphenyl, also shown in Figure 1.1.2, provides a good example of this family of compounds.

1.2 Origins of PACs

PACs are formed during incomplete combustion and pyrolysis of almost any kind of organic material. They are continuously released in the environment from anthropogenic sources and also occur naturally. Anthropogenically produced PACs are found in processed petroleum, soot, coal and woodburning emissions, as well as automobile exhaust, waste incineration, cigarette smoke and even fried food. Naturally occurring PACs are present in coal, crude petroleum and soil.

Thermal formation of PACs by combustion yields a multitude of individual

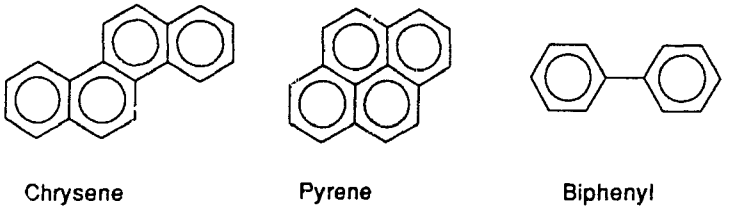


Figure 1.1.2: Structures of 3 different types of PAHs: chrysene (catacondensed), pyrene (pericondensed) and biphenyl (rings joined by a single bond).

compounds, with non-substituted PAHs as the predominant species. On the other hand PACs formed while treating fuels and lubricants by hydrocracking processes consist mainly of highly alkylated species.

Combustion causes PACs to be emitted as gases to the atmosphere, which later cool down and adsorb on particulate matter. Gaseous PACs also condense and form particles of almost pure substances. All these particles are dispersed by turbulence and transported by wind. They are then removed by settling, impaction and washout (5).

Marine sediments around industrial areas are often highly contaminated with PACs. Petroleum spills, atmospheric precipitation and fallout, waste dumping and sewage effluents are mainly responsible for their presence in lake and ocean sediments.

Many PACs are toxic and/or mutagenic in various biological systems. The toxicities of PACs are related to their specific structures and positions of ring substitution. For instance, the 1- and 9-methylphenanthrenes are mutagenic (6) while other isomers are moderately carcinogenic. Isomeric parent PAHs may also differ markedly in their activities: benzo[a]pyrene has significantly greater carcinogenicity than benzo[e]pyrene (6). So far,the carcinogenic activity of PACs has been reported to be related to the ease of oxidation of the aromatic rings (5). However, other theories are currently under investigation (7,8).

The occupational health hazards associated with high PAC exposure in industries such as coking asphalt have been well established (5). However, exposure of workers to the PAC byproducts of crude oil hydrocracking has not yet been assessed officially as dangerous and the subject is still under investigation.

1.3 Requirements for the analysis of PACs in complex samples

Analytical methods for PACs usually consist of procedures involving five steps:

1- Sampling of the sediment (or air particles or water)

2- Extraction of the organic matter from the sample matrix

3- Isolation of PACs from the organic extract

4- Separation of PACs into subgroups

5- Characterization and quantification of individual compounds contained in the subgroups ("final characterization")

A global PAC analytical method might thus involve a combination of techniques such as freeze drying for step 1, solvent extraction for step 2, fractionation by column chromatography for steps 3 and 4, and chromatography/mass spectrometry for step 5. Methods have already been developed in different laboratories (2,3,9-11) and a few are well established and commonly used (9-11).

With complex mixtures, the global analytical procedure should be as simple as possible, rapid and selective. It should provide a high overall resolution through the combination of preparative fractionation methods to isolate different classes of compounds, and high chromatographic efficiency and separation selectivity in the final characterization or analysis.

The main difficulty in PAC analysis is the extreme complexity of environmental matrices. Even after extensive fractionation, PAC fractions may still contain over a thousand compounds (12). These may overlap in the final chromatographic characterizations, which requires the establishment of very specific detection methods. This has led to the investigations of a variety of combinations of chromatographies and detection systems (13-16).

1.4 The Sydney Tar Ponds

The Sydney Tar Ponds are known as the largest waste chemical disposal site in Atlantic Canada (17). Coke was manufactured in Sydney, Nova Scotia, by destructive distillation of bituminous coal (lignite coal) via a process called the "by-product coke oven".

The ovens were arranged in series, separated by heating flues. Heat was supplied by external combustion and air was excluded from the coking process taking place in the ovens. Seventeen hours and high temperatures (ca. 1100°C) were

required to produce metallurgical coke. Most volatile compounds were driven off when the coke was heated; these compounds were collected and processed to reclaim chemicals and tars and to use the heating value of the coke oven gas. Certain processes produced high levels of emissions for short periods of time.

When coal was loaded into the ovens from the top, the top side oven doors were opened as the coal fell into the hot ovens. Higher than normal emissions of dust, smoke and gases occurred. When the coke was discharged, the oven doors at one end were opened and a large "ram" pushed the hot coke into open railway cars, and again higher than normal emissions occurred. The coke was then cooled, using water sprays. The water, upon hitting the hot coke, vaporized rapidly and was released to the atmosphere, carrying large quantities of fine dust with it.

Continuous emission sources at the coke plant were the combustion gases from the underfiring system for the coke ovens which burned coke oven gas, the boiler plant burning coke breeze or coal, and leakage from the ovens (5).

Ground water contamination of the ponds (later called Tar Ponds) is primarily due to deposition of dust produced by the fast quenching of the coke from the ovens, and also to various ancillary activities within the drainage basin of Coke Oven Brook. Solid by-products of the coking process were piled up at the city dump, the contents of which drained into Coke Oven Brook.

In June 1985, Acres International Ltd. completed a study which defined the extent of PAC contamination at certain sites around Sydney. The study also proposed a conceptual design for site remediation. Studied areas were the following, as shown on the map in Figure 1.4.1: South Arm, Muggah Creek, Coke Oven Brook, Wash Brook, Tar Plant, Boiler Site and their immediate surroundings. The proposed clean-up procedure involved excavation of contaminated sediments, incineration of sediments and elimination of all known ongoing inputs of PACs to these sites.

Coke Oven Brook flows through several areas which have been used as disposals for coke plants and tar plant wastes. The resulting input of PACs from Coke Oven Brook into Muggah Creek is in the order of 7.4 kilograms per year, based on conditions existing when the coke ovens are not operating. PAC inputs during

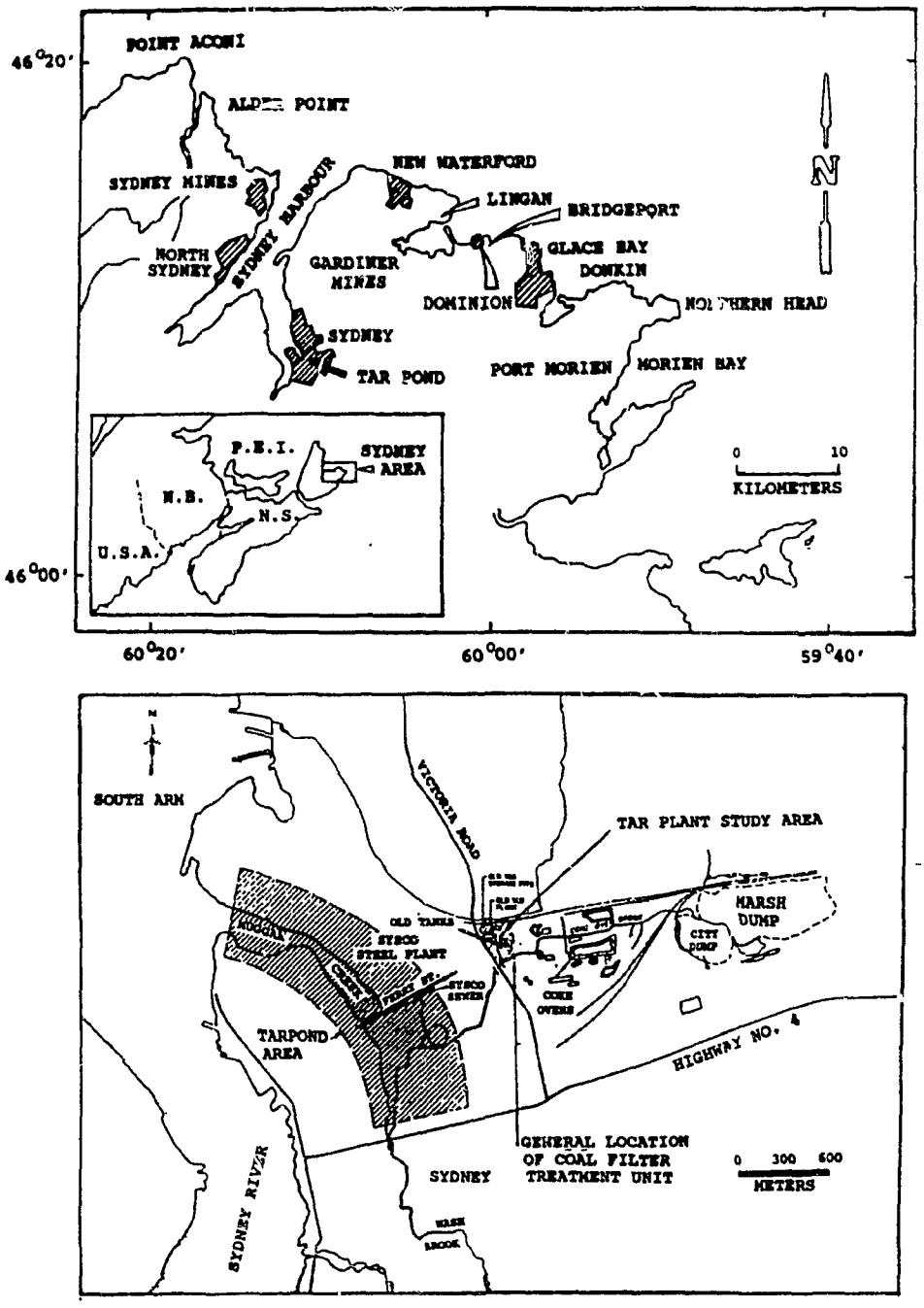


Figure 1.4.1: Location of the Sydney Tar Pond. Top: map of the Sydney area; bottom: closer view of Sydney.

periods of operation exceeded 3000 kilograms per year (4). Sediments containing more than 600 μg/g of PAC material on a dry weight basis are considered as contaminated (4).

1.5 The Alberta oil sands

The Alberta Oil Sands (Fort McMurray, Alberta, Figure 1.5.1) are refined by Syncrude Canada Limited. The company uses hydrocracking and other various processes to produce naphtha and light and heavy gas oils which are then transported through pipelines to other parts of Alberta and of Canada.

The oil sands are hidden beneath a layer of overburden up to 35 meters deep. The overburden is used to build dykes and dams, and to eventually fill the mine area. The oil sand vein averages 42 meters in thickness.

Electric power shovels load overburden (a mixture of sand, gravel, clay and silt into 150 tonne trucks. The overburden is stored for future reclamation of the mined land. Four draglines work around the clock to move 100 million tonnes of oil sand per year. The oil sand is piled in winnows for easy access by a bucketwheel reclaimer. Teaming up with the draglines to help move oil sand onto conveyor belts and into the extraction plant are four bucketwheel reclaimers, each of the length of a football field.

The feeder breakers, part of a mining auxiliary feed system, crush large chunks of oil sand too big for the conveyors to handle. This feed system can increase oil sand production by over 10 million tonnes a year.

The extraction plant uses steam, hot water, caustic and gravity to break bitumen away from the sand. Over 90% of the bitumen is recovered during the extraction.

The tailings oil recovery unit can recover up to three million barrels of bitumen per year historically lost to the tailing pond with the discharged water from extraction. A Naphtha recovery unit can capture $7x10^5$ barrels of naphtha escaping during the extraction process.

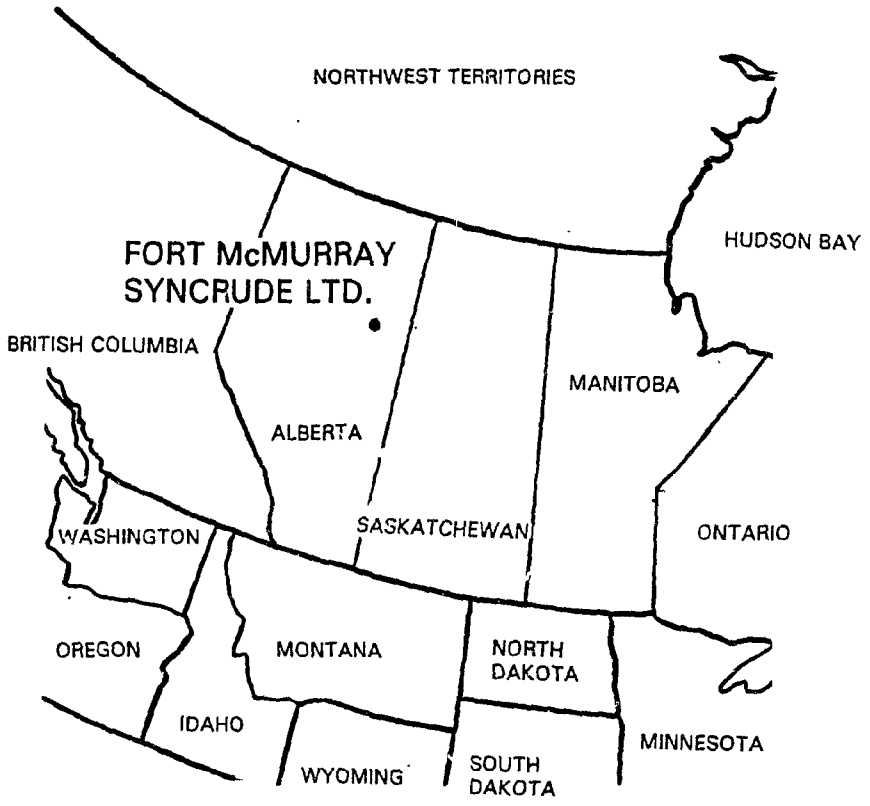


Figure 1.5.1: Location of Fort McMurray, Alberta, site of operation of Syncrude Canada Inc.

Most of the water required by the extraction process is recycled from the tailing pond, a 17 km^2 holding pond which prevents contaminated water from discharging into the watershed. Froth is diluted with naphtha before the remaining solids and water are removed. The diluent recovery process feeds bitumen to the cokers and hydrocracker and recycles diluent naphtha back into the froth bitumen for re-use.

In upgrading, cokers heated to 500°C break down the molasses-like bitumen in a process known as cracking. The resulting products, primarily naphtha and gas oil, become the main ingredients in the synthetic crude oil. Cracking produces fuel gases and butane, as well as coke, as by-products.

Coke, hot and flowing like fluid, is the agent which breaks the bitumen down - "cracks it"- into its component parts. As coke is also a by-product of the cracking process, some of it is burned in the coker, while the remainder is stored for future use.

The bitumen hydrocracker breaks down bitumen through hydrogen injection over a catalyst bed, a process different from the cokers. Of the resulting products, fuel gas, naphtha and gas oil are sent to the fractionator while the pitch is fed into the fluid cokers.

The fractionators separate the lighter oils, gases and distillate into three groups: fuel gas, which is diverted to the amine gas recovery unit, and naphtha and gas oil which are fed into the hydrotreaters. Impure naphtha and gas oil are treated with hydrogen in hydrotreaters to remove sulphur and nitrogen. The five hydrotreaters use hydrogen formed in hydrogen plants where a combination of steam and natural gas is reformed at 820°C.

The fuel gas, with a high hydrogen sulfide (H_2S) content, is sent to the Amine gas recovery units for H_2S removal. The cleansed "sweet" fuel gas is used in the utilities plant to produce steam and electricity for the Syncrude operation. H_2S is sent to the sulfur plants and sulfreen/tail gas units for conversion into safe, usable sulfur. Three sulfur plants and the sulfreen tail gas plant convert nearly 99% of the H_2S to liquid sulfur. The remaining 1% is emitted as sulphur dioxide (SO_2). Emissions are

managed by stringent government standards to minimize any effect on the environment. These environmental units allow Syncrude to significantly reduce SO_2 emissions even while increasing production.

The utilities plant supplies Syncrude with energy, $1.8x10^6$ kg/hr of steam, and sufficient electricity to power a city of 250000 people. Syncrude electricity is available to Fort McMurray in case of power outages. Treated gas oil and naphtha can either be blended directly into the pipeline, or stored in tanks to await shipment. Syncrude has storage capacity for over $7x10^6$ barrels of product (treated naphtha or gas oil). Synthetic gas oil (SGO) is created when naphtha and heavy gas oil are blended in the pipeline. The Alberta Energy Co. Ltd. pipeline carries the SGO to Edmonton, Alberta. Some of the SGO is processed in refineries around the city, while the remainder is shipped to refineries from Montreal to Vancouver (18).

Figure 1.5.2 is a schematic picture of the upgrading system described above. Steps 5 and 20 represent the origin of three samples studied in this present work. The broken oil sand (oily sediment), the heavy gas oil (HGO) (high boiling point distillation fraction of the gas oil) and the pitch (PIT, residue from distillation of the gas oil) were obtained from areas where workers are directly exposed to the products. Then samples are examined to investigate possible health hazards to which Syncrude workers might be exposed.

1.6 Main objectives of this work

The main objective of this project is to develop chromatographic and mass spectrometric methods for the characterization of aromatic compounds in complex mixtures or matrices. Several points are involved in this main objective:

- to select an extraction method suitable for a variety of matrices, with a high yield for both low and high molecular weight species,
- to develop open column chromatographic fractionation techniques specific to particular samples,
- to combine high resolution-high performance chromatography with mass

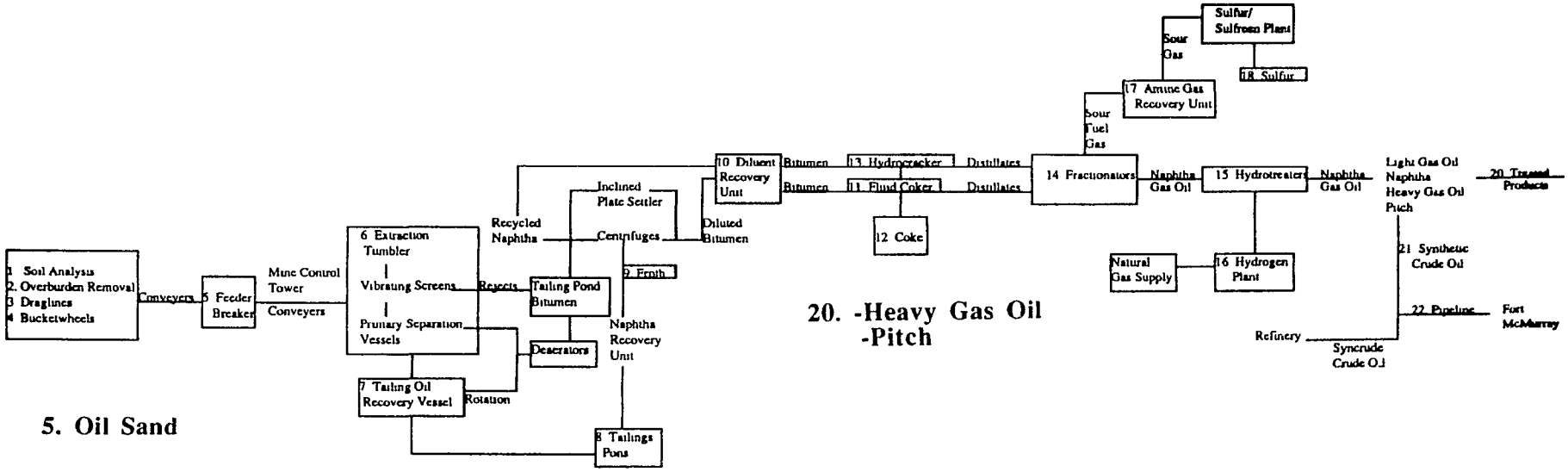


Figure 1.5.2: Schematic representation of the methods used by Syncrude Canada Inc. to process the broken tar sands into synthetic crude oils. The samples analyzed in the present work were obtained from Steps 5 and 20.

spectrometry for the analysis of final fractions,

- to use mass spectrometry as a universal or selective detection technique for the analysis of certain classes of aromatic compounds,
- to identify as many individual compounds as possible in fractions of interest, with the help of internal or external standards,
- to produce a qualitative pattern of the PAC content of the three sets of samples under investigation.

All points above are explored qualitatively, i.e. there is no emphasis on detection limits or concentrations of particular compounds in the samples. There is a primary need to obtain methods to characterize the overall contents of each sample; those methods are expected to be applied on a quantitative basis in future work.

2. EXPERIMENTAL

It is important to emphasize that sampling, extraction and fractionation are the basis for the analysis of compounds in complex matrices. The sample must be homogeneous, the extraction has to be efficient in removing the organic material from the matrix and fractionation should be successful in separating the extract into several defined fractions, some of which will contain the substances under investigation.

An inappropriate analytical clean-up procedure may give rise to many difficulties, from compound signals overlapping in the final determination to interference with the operation of the column.

2.1 Sampling

Four samples have been investigated in the course of this work: a Sydney tar pond sediment, an Alberta oil sand broken sediment, a heavy gas oil and a pitch sample. A sampling procedure is summarized here for each sample.

2.1.1 Sampling of the Sydney tar pond sediment

Bulk quantities of sediment were collected from Muggah Creek (see Figure 1.4.1, courtesy of Acres International Ltd., Halifax, N.S) and delivered in plastic buckets. A fraction the sediment material was freeze dried, then sieved to pass 60 mesh before being homogenized by tumbling it for one week in an electrically powered concrete mixer (about 0.4 m^3 capacity) which had been modified to minimize contamination of the contents by organic materials. Tests for homogeneity were carried out during the blending, by extracting small quantities of material by Soxhlet and performing gas chromatographic and high performance liquid chromatographic analyses of those extracts. Once the material was sufficiently homogeneous, it was subsampled and packed in precleaned all-steel cans of the air-

tight type normally used to contain household paint. Each can contained about 40 g of the sediment material. A series of randomly selected samples (15 g portions) were used in this work to test cleanup, fractionation and "final" characterization procedures.

2.1.2 Sampling of the broken Alberta Oil Sand

The broken oil sand sediment was obtained from Syncrude Canada Ltd., and was collected immediately after Step 5 as indicated in Figure 1.5.2. It was an oily sediment with a high content of water. According to the specifications of the company, bitumen and moisture content were 9.61% and 4.50%, respectively. Several 15 gram portions were spread in wide bottom dishes and allowed to dry under nitrogen for a week. The sediment was ground then for about 10 minutes each day during the week of drying (using mortar and pestle), to make sure that most of the material was exposed to nitrogen. Removal of the water left an oily solid, which was again ground as fine as possible. Grinding was rendered difficult due to easy agglomeration of solid particles with oil.

A detailed review on the sampling of sediments containing PACs has been published by B.P. Dunn (1).

2.1.3 Sampling of heavy gas oil and pitch samples

The heavy gas oil (HGO, a thick brown oil) was obtained directly from Step 20 in Figure 1.5.1. It was sampled from a line of treated products where workers can be in contact with the gas oil. The pitch sample (PIT, a black gummy solid) was obtained as a residue of distillation of Gas Oil, just before Steps 20 and 21. Both samples were used as received for fractionation, since they are homogeneous; furthermore, no extraction was necessary as samples were totally soluble in solvents such as chlorobenzene and dichloromethane.

2.2 Extraction

Methods for the extraction of PACs from sediments and particulate matter have been intensively studied. Blumer and Youngblood (19,20) described a Soxhlet extraction method with 1:1 benzene-methanol; also reported (21) was the extraction of organic matter from minerals containing PACs, achieved by stirring with hot benzene followed by brief ultrasonic agitation. Sonication has also been applied to soils (22). Chatot and co-workers (23) compared ultrasonic and Soxhlet methods for the extraction of PACs from atmospheric particles.

Teo and Watkinson reported on hexane extraction under sonication conditions for the characterization of pyrolysis tars from Canadian coals (24). Farrington and co-workers (25) used sonic assisted extraction with a sequence of solvents of increasing polarity, for the study of bitumens. Lauer et al. have used ultrasonic extraction as a step in characterizing coal tar distillation cuts (26).

A non-isothermal solvent extraction technique has been described for coal samples (27), where solvents were used in the liquid and supercritical fluid states. Supercritical fluid extraction of sediments has been well described (28), and commercial devices are currently available to perform this technique in a reproducible manner (29).

Brown et al. (30) reported a method which involves triple extraction of a wet sediment with 0.1 M HCl in 7:3 benzene-methanol using a homogenizer. Lane and Jenkins (31) gave a mechanistic description of microwave desorption of organic compounds from particulate matter.

2.2.1 Extraction of the organic matter from tar pond and oil sand sediments

The Soxhlet method is one of the most efficient techniques for the extraction of organic compounds adsorbed on the surface of particles in a sediment; it can be performed selectively by using different extraction solvents. Soxhlet extraction was selected as the method of choice to extract both tar pond and oil sand sediments, for

the following reasons:

- the setup is easy and bulk quantities of sediment may be extracted,
- strong solvents such as chlorobenzene may be used to extract high molecular weight (M.W.) compounds with high yields,
- the supercritical fluid extractor used in this laboratory allows the extraction of only 5g of material, and extraction yields for high molecular weight compounds were found to be lower than those obtained by Soxhlet extraction,
- no suitable ultrasonic or microwave extraction systems were available, and as Soxhlet extractors are currently used for PAH extractions in this laboratory, it was judged pointless to put together such systems.

Randomly selected 15 gram portions of tar pond and oil sand homogenized sediments were Soxhlet extracted with 150 mL of chlorobenzene for 48 hours. Chlorobenzene was selected for its ability to extract high molecular weight compounds with high yields (32). Tar pond and oil sand samples, once extracted, appeared as dark brown opaque solutions. The chlorobenzene was removed by vacuum, and samples were completely dissolved in 25 mL of dichloromethane.

2.2.2 Heavy gas oil and pitch samples

As these samples were homogeneous and completely soluble in chlorobenzene and dichloromethane, no extraction of the organic matter was required. Samples were used as received and proceeded through clean-up and fractionation steps as described below.

In the chemistry of bitumen, samples are routinely separated into two portions, asphaltenes and maltenes. The former by definition are heptane insoluble substances, whereas the latter are soluble in heptane and thus can be extracted by this solvent. A few publications report the separation of maltenes from asphaltenes by heptane extraction (33-36); however other workers have used hexane (37) and

pentane (38-40) for the same purpose. Separation of asphaltenes from maltenes was not used as an extraction procedures for the heavy gas oil and pitch samples studied in this work, for the following reasons:

- the need exists to characterize the overall contents of the samples rather than just the heptane soluble portion,
- the separation of asphaltenes and maltenes by solvent extraction is rather arbitrary ("operationally defined" fractions); there could be overlapping between several classes of compounds in these separations,
- instead of separating compounds into two arbitrary classes right from the start, it was judged better to attempt to split the sample into better defined fractions in later steps of the analysis scheme.

2.3 Removal of elemental sulfur from the extracts

Blumer (41) first reported removal of elemental sulfur by percolating sediment extracts over precipitated copper. The same technique was used by Giger and Schaffner (42) and by Visentini and Quilliam (43). The latter authors passed the extracts through a column containing 11 g of purified copper; the column was packed such that glass wool separated layers of copper powder. However, partial plugging of the column occurred and made the elution extremely slow. MacLeod and co-workers (44) performed desulfurization of a sediment extract by stirring with freshly cleaned granular copper and filtering. Sim et al. (45), according to a method described by Ramos and Prohaska (9), reported the elimination of elemental sulfur by passing sediment extracts through a short column of silica gel, topped with copper powder. The column, as well as retaining sulfur compounds, allowed retention of highly polar species on the silica adsorbent.

In this work, a copper-mercury amalgam was used to remove elemental sulfur from the sediment extracts to prevent interference in the gas chromatographic (GC), high performance liquid chromatographic (HPLC) and supercritical fluid chromatographic (SFC) analyses. The amalgam has proven to be faster and more

efficient than copper alone - and cleaner than mercury alone - to achieve this purpose (46). Sulfur removal was achieved by stirring the extract solutions with the amalgam, rather than percolation on an open column packed with the alloy. Stirring allows longer contact times than percolation for the amalgam and elemental sulfur to react and form solid CuS.

Tar pond and oil sand extract solutions (TPE and OSE, respectively) as well as dichloromethane solutions of HGO and PIT were treated for sulfur removal. The Cu-Hg amalgam was prepared as follows: copper powder (50 g) was cleaned by rinsing with 20% nitric acid followed by several rinses with water. Mercury (30 g) was added, followed by 10 mL of 30% aqueous HNO_3. Effervescence commenced upon the addition of the acid. Then deionized water (30 mL) was added. The mixture was stirred overnight, and was then allowed to settle before decanting the supernatant water. The amalgam was rinsed with acetone and hexane and dried with a stream of nitrogen. Immediately before use, the amalgam was reactivated by stirring with 30% nitric acid and rinsing in sequence with water, acetone and hexane.

A quantity of 1 g of amalgam was added to each extract solution (obtained from extracting 15 g of solid material), and the mixtures were stirred for one hour. Contents of the flasks were decanted and their volumes reduced to 5 ml on a rotary evaporator.

2.4 Fractionation

2.4.1 Fractionation of the tar pond extract (TPE)

2.4.1.1 Ramos and Prohaska method (9)

Silica gel is the form of silica most widely used in fractionation and its white color allows bands of eluting material to be seen clearly. The reactivity of silica depends upon the arrangement and number of hydroxyl and siloxane groups. The interaction of the adsorbate with adsorbent is thought to be through a π-electron

bonding interaction with the polar surface (47).

An early chromatographic procedure for the separation of the neutral fraction of environmental samples on activated silica gel (silicic acid), was devised by Rosen (48). Elution was with an alkane solvent to yield the aliphatic fraction, and benzene to yield the aromatics. Since then, silica column chromatography has been widely applied, namely to air pollutant PAHs adsorbed on particles (49), tobacco smoke condensate (50), river and lake sediment samples (42,44,51) and water and waste water samples (52,53). Gearing and co-workers have used silica as a thin layer chromatographic adsorbent as opposed to a column adsorbent (54).

The use of Sephadex LH-20 for the clean-up of environmental samples has been cited in many publications (3,55-62). Experiments using Sephadex LH-20 with different eluents showed that isocratic elutions are more successful than elutions where solvent polarity is increased, due to changes in the size of the Sephadex LH-20 bed with the nature of the solvent. Lee and co-workers (57-60) have shown that separation of PAHs using isopropanol on Sephadex LH-20 occurred, based on the number of aromatic rings in the compounds. Thus, naphthalene and alkyl naphthalenes (compounds with two rings) elute first, and increasing the number of rings yields longer retention times. Giger and Blumer (11) performed Sephadex LH-20 chromatography using 1:1 benzene-methanol to isolate PAHs from soil and sediment extracts. However, due to the well known carcinogenicity of benzene, Ramos and Prohaska (9) introduced a safer, contaminant free solvent system, through the use of a mixture of cyclohexane-methanol-dichloromethane (6:4:3). This Sephadex LH-20 method (9) is now a well established technique and is currently used in this laboratory (12,45,63)

In most analytical procedures involving Sephadex LH-20 columns, this type of size exclusion chromatography is used as a fractionation step following either alumina or silica column clean-up procedures, since these tend to yield overlapping aliphatic and neutral aromatic (PAH) fractions. Silica has been more widely used than alumina to fractionate extracts of pollutants.

The method reported by Ramos and Prohaska (9) was a two step procedure

that combined silica gel chromatography and Sephadex LH-20 chromatography using safe, contaminant free solvent systems to isolate polycyclic aromatic compounds from extracts of sediments and tissues from the marine environment. The method as reported has been modified slightly in our laboratories and is described below.

One TPE sample chosen randomly (0.2 g) was adsorbed onto 1 g of silica gel (100-120 mesh, Fisher Scientific Ltd.), and deposited on top of a 10 g silica gel column (20 mm i.d.). The extract was eluted with 3 mL of 20% dichloromethane in diethylether, followed with 20 mL of 40% dichloromethane in diethylether (Et-O-Et). Saturated hydrocarbons pass through the column with the 20% DCM/Et-O-Et eluent. The 40% DCM/Et-O-Et portion containing the PACs was brought over into dichloromethane and reduced to 1 mL in volume on a rotary evaporator.

The PAC fraction obtained from silica column chromatography was then rechromatographed on a pre-swollen Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemicals, Uppsala, Sweden) column (15 g, 25 mm i.d.) with 100 mL of a 6:4:3 mixture of cyclohexane, methanol and dichloromethane. The first 40 mL (aliphatic fraction) was discarded (the appearance of an azulene standard was used to establish the 40 mL volume (9,43)), and the last 60 mL (PAC fraction) was collected and taken over into dichloromethane, and reduced to a 10 mL volume using a rotary evaporator. The tar pond PAC fraction was then ready for final determination by GC combined with mass spectrometry (GC/MS), HPLC/MS or SFC/MS. A UV lamp was used to monitor fluorescence throughout the elution process. Fluorescence on the column or in the eluate was taken to indicate the presence of PACs.

2.4.1.2 Alumina fractionation scheme

As early as 1965, Sawicki and co-workers (64,65) used alumina column chromatography to isolate a fraction rich in PANHs from air particulate matter.

The separation of many PACs is more readily achieved using alumina than using silica adsorbents (66), although the use of alumina remains relatively less popular, because of two major disadvantages in alumina column chromatography:

sensitive compounds may decompose on the adsorbent, and reproducibility is difficult to achieve (2).

Various pretreatements, such as heating, grinding, and treatments with acids, alkalis and salts change the properties of natural alumina. Alumina is normally prepared by low temperature dehydration of aluminum hydroxide and is a mixture of γ-alumina and alumina monohydrate. The former is highly active when freshly prepared but rapidly loses its activity by adsorption of water when exposed to a damp atmosphere (67).

The natural lattice, made of hexagonal close-packed γ-alumina, can be altered by the pretreatments listed above. This alteration will result in aluminum cations being exposed at the surface. The O^- and Al^+ sites can adsorb protons or hydroxyl groups from acid or base treatment (67).

Most organic solutes, except possibly aliphatic hydrocarbons, are at least somewhat adsorbed by the polar alumina surface groups by π-electron bonding with aluminum cations exposed by alteration to the surface (67).

The method described here has been developed by Marr and Quilliam (3), although elution volumes presented here slightly modify the original procedure. It was used successfully by them to separate extracts of airborne particulate samples into fractions containing aliphatics, neutral aromatics, polar aromatics and very polar compounds (3). This fractionation method has been applied to the tar pond extract because of its selectivity in compound class separation.

A randomly selected TPE (0.17 g) was coated onto 1 g of neutral alumina (M. Woelm Eschwege, Germany). This was then packed onto a 10-g alumina column (neutral, activity I, dried overnight at 160°C).

Three fractions were obtained by passing solvents of increasing polarity through the column. The first fraction, which contained the aliphatic compounds, was collected by passing 30 mL of hexane through the column. The second overall fraction (PACs) was obtained by combining the fraction eluted with 80 mL of benzene with that eluted with 70 mL of chloroform (stabilized with 1% ethanol). The more polar compounds were collected by sequentially passing 50 mL of methanol and

50 mL of a methanol/water mixture (4:1) through the column. The PAC fraction (second) was concentrated by rotary vacuum evaporation and further taken up into 10 mL of dichloromethane. The PAC fraction was then ready for analysis.

2.4.1.3 Silica over alumina fractionation method

Hirsh et al. (68) obtained a very clean separation between saturates, monocyclic aromatics, dicyclic aromatics and polycyclic aromatic-polar compounds using this method. Although the technique does not effect the separation of polycyclic aromatics from polar compounds, it eliminates most aliphatic and highly alkylated monocyclic and dicyclic aromatic material from the sample matrix. This method has not been reported before for the analysis of polluted sediment samples and has for this reason been investigated here.

A dual packing column chromatography technique, this time using alumina over silica, has been described by Giger and Blumer (11) to characterize nearshore marine sediments.

The method described here has been applied successfully to the fractionation of high boiling petroleum distillates (68). As it has also been used in the present work to fractionate the Syncrude samples, a determination was made of the method's capabilities to fractionate a TPE sample.

A glass column (20 mm i.d.) was packed with 6 g of alumina (neutral, activity I, M. Woelm Eshwege, Germany) and covered with 4 g of silica gel (60-120 mesh, BDH Chemicals, Toronto). The 6:4 alumina/silica weight proportion was equivalent to a 1 to 1 volume ratio. An amount of 0.03 g of TPE was coated on 1 g of silica and placed onto the silica over alumina column. The TPE was eluted through the column with the following sequence of solvents:

- 35 mL pentane (aliphatics)
- 40 mL 5% benzene/pentane (aliphatics + monocyclic aromatics)
- 40 mL 15% benzene/pentane (aliphatics + dicyclic aromatics)
- 25 mL 6:2:2 methanol, diethylether, benzene (polycyclic aromatics)

- 250 mL methanol (very polar material and acids)

This elution pattern (gradient type) with increasing solvent strength should allow (68) aliphatics and monoaromatics to be eluted through the column very slowly, while polycyclic aromatic compounds are still retained. The 6:2:2 mixture then should elute PACs as a sharp band. Methanol removes the residual polars. In this work, the 15% benzene/pentane and 6:2:2 MeOH:Et-O-Et:Benzene fractions were kept for further analyses.

2.4.1.4 Attempts to separate PASHs using copper coated on silica

PAC fractions of TPE samples studied in this laboratory have been shown to contain many heterocyclic aromatic compounds, notably containing nitrogen and sulfur atoms. Attempts at using ligand exchange column chromatography to retain sulfur and nitrogen heterocyclic compounds and separating them from the rest of the sample matrix have been successful (69-78).

The method described here was developed in this laboratory, based on Nishioka and co-workers' earlier work (69). The method was an attempt to separate PASHs from PAHs and other PACs present in the mixtures. The authors of (69) used palladium chloride adsorbed on silica gel instead of copper sulfate as used in this work. However, solvent conditions used here were taken from (69). The same authors further used their method to characterize petroleum and coal-derived materials (70), followed by Andersson (71), who studied the retention properties of PASHs on the same adsorbent.

Thiophenic compounds have been isolated by retention on silver nitrate-coated silica columns by three different teams (72-74). Orr (75) investigated aqueous zinc chloride as a stationary phase for liquid-liquid chromatography of organic sulfides, and had earlier studied the separation of alkyl sulfides on stationary phases containing mercuric acetate (76). Ligand exchange thin-layer chromatography on silica gel loaded with mercury was performed by Kaimai and Matsunaga (77), to determine sulfur compounds in high boiling petroleum distillates.

Copper sulfate was chosen as the adsorbent modifier here because, added to the fact that this salt is readily available in any laboratory, copper (II) has coordination constants approaching those of palladium, zinc , mercury and silver.

The stationary phase consisted of silica gel (60-120 mesh, BDH Chemicals, Toronto) coated with copper sulfate ($CuSO_4.5H_2O$, BDH Chemicals, Toronto), and was prepared as follows: silica gel (100 g) was suspended in 500 mL of deionized water (Mixture 1). Copper sulfate pentahydrate was dissolved in 100 mL of deionized water; 5 mL of concentrated ammonium hydroxide was added, then another 5 mL was added dropwise until all copper precipitate was dissolved (Mixture 2). Mixtures 1 and 2 were mixed together and stirred gently in a fume hood for one week.

Supernatant water was decanted, and the blue copper-silica gel slurry was rinsed with water, acetone and hexane. The adsorbent was then dried under nitrogen in a wide bottom dish, and ground with mortar and pestle until homogeneous, for particle size and color. It was kept at 160°C in an oven for 24 hours prior to using.

A TPE sample (0.2 g) was adsorbed on 1 g of copper-coated silica and packed onto a 10 g column of the same adsorbent (glass column, 20 mm i.d.). The elution was performed in the isocratic mode, with a total volume of 110 mL of chloroform-hexane 1:1 mixture. Eleven fractions of 10 mL were collected. The rest of the material was eluted by passing 100 mL of 1:1 mixture of diethylamine/chloroform through the column in an attempt to break down any complexes formed between copper and the sulfur compounds and thereby mobilize any adsorbed compounds.

All eleven chloroform/hexane fractions were taken down to 1 mL by rotary vacuum evaporation, and to dryness under a gentle stream of nitrogen. They were then brought up to 10 mL in hexane.

The DEA/$CHCl_3$ 1:1 fraction was also taken to dryness on a high vacuum rotary evaporator, equipped with a condensation trap and brought up to 10 mL in hexane. All of the twelve samples were then ready for GC/MS analysis.

2.4.1.5 Second scheme for the separation of PASHs

A few research groups have developed methods for separating and/or characterizing PASHs in environmental samples (69-81). Among these methods, Nishioka et al. (80) used gas chromatography with flame photometric detection as a very selective way to screen sulfur compounds from other components in heavy oils. Wood and co-workers (81) performed chemical reduction and tandem mass spectrometry to analyze sulfur-containing fuel constituents. Other publications refer to PASH isolation by means of ligand exchange chromatography as described earlier (69-77).

Lee and co-workers (78,79) successfully separated PASHs from PAHs in coal tar and shale oil samples using the procedure summarized in Figure 2.4.1. This procedure has also been used in this work and will be described here. This method was selected because of the high yields of the oxidation and reduction reactions (78,79) and because the adsorbents and reagents required are readily available.

i) Preliminary

Part of the organic extract of 15 g of tar pond sediment (ca. 0.2 g of dry material) was adsorbed onto 0.5 g of activated silicic acid (100 mesh, Mallinckrodt, Paris). The adsorbed sample was then transferred to a 10 g silicic acid column. Hexane was passed through the column and the first 300 mL was collected. The hexane eluate was taken to dryness by vacuum rotary evaporation.

ii) Oxidation of PASHs into the corresponding sulfones

The dried hexane eluate from the silicic acid chromatography was brought up to 50 mL in benzene. Glacial acetic acid (50 mL) was added, and refluxing was started. Hydrogen peroxide (30%, 2 mL) was added over one hour, and refluxing was continued for 16 hours. The following reactions occur:

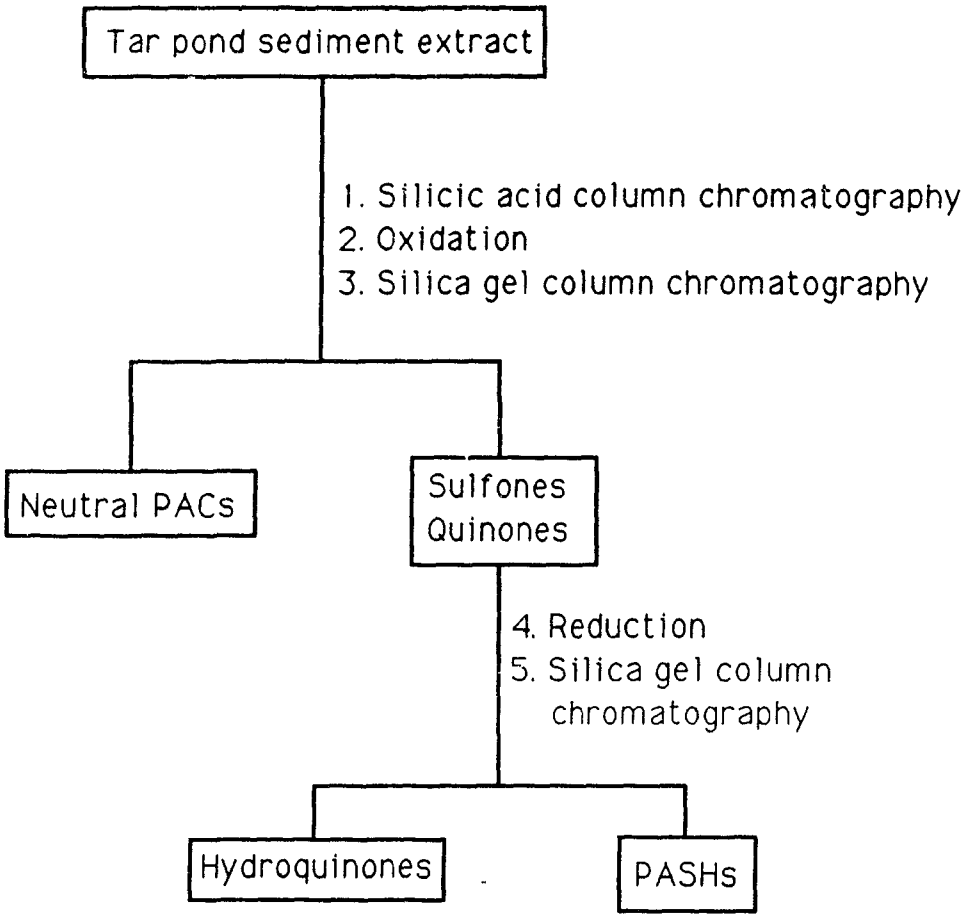
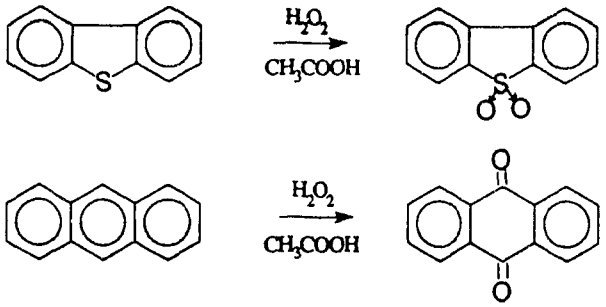


Figure 2.4.1: Method used for the isolation of PASHs from other PACs (78,79)



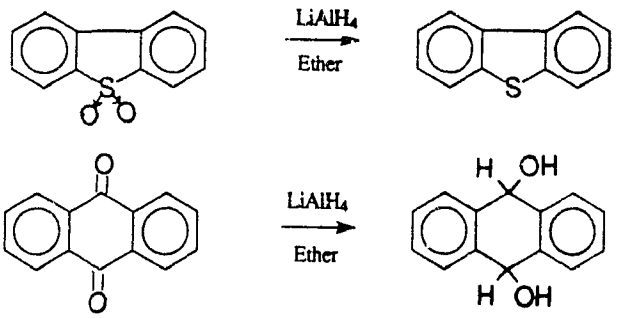
The mixture was cooled down and washed five times with 50 mL of deionized water in a separatory funnel to remove any excess of peroxide and acetic acid. The water washings were extracted twice with 50 mL of benzene. The benzene solutions were combined and evaporated down to 2 mL.

iii) Separation of the oxidized material from the unoxidized material

Material contained in the benzene solutions was adsorbed onto 1 g of silica gel (60-120 mesh, BDH chemicals, Toronto) and packed onto a 10 g silica gel column. The eluent used was benzene; the first 80 mL contained unoxidized compounds, most of which were PAHs. The oxidized compounds (sulfones and quinones) were collected by passing 200 mL of 1:1 benzene:methanol through the column. The benzene-methanol fraction was evaporated to dryness then brought up to 25 mL in anhydrous diethylether to form a suspension.

iv) Reduction of the sulfones to sulfides (PASHs)

The suspension from the step above was reduced by adding it dropwise to a refluxing suspension of 50 mL of ether containing finely divided $LiAlH_4$ powder. The addition was performed over one hour with constant stirring. The mixture was then refluxed for 2 hours. The reduction reactions were the following:



Water was added dropwise to decompose any $LiAlH_4$ in excess. The ether portion and inorganic precipitate plus water were divided in a separatory funnel. The precipitate was washed twice with 10 mL of ether and twice with 10 mL of dichloromethane. The ether and dichloromethane washings and solutions were combined and evaporated down to 2 mL.

v) Separation of the sulfides from hydroquinones formed by reduction of oxidized material

The fraction obtained from the previous step was adsorbed onto 1 g of silica gel, and eluted through a 10 g silica gel column with hexane only. The first 250 mL contained the PASHs. The hexane PASH solution was evaporated down to 10 mL and was ready for analysis.

Small aliquots were sampled before and after each step of this method to verify the efficiency of the process by chromatography/mass spectrometry analysis.

2.4.1.6 Scheme for separation of nitrogen compounds (PANHs)

PANHs, heterocyclic compounds containing one nitrogen atom, are ubiquitous in sediment samples such as the tar pond sediment. As those azaarenes have long been suspected of having carcinogenic properties, several methods of separating PANHs from miscellaneous matrices have been proposed. In 1968, Snyder et al. (82) developed a method to achieve the isolation of nitrogen and oxygen compounds in

petroleum. The first step of their complex fractionation technique involved alumina and cation exchange resin chromatographies, and a second step used alumina and silica column chromatographies to achieve the separation.

McKay, Webber and Latham (83) described the isolation of PANHs from high boiling petroleum distillates by a one step cation exchange column chromatography procedure. Snook and co-workers (84) improved their earlier work (82) by using silicic acid column chromatography and a gel chromatographic system to isolate indoles and carbazoles from cigarette smoke condensate. Gel chromatography was performed using Bio Beads SX-12, a neutral, porous styrene-divinylbenzene polymer as the stationary phase.

Burchill et al. (85) have described the isolation of the PANH fraction from coal liquefaction products by aqueous acid extraction. More recently, Yamauchi and Handa (86) isolated azaarenes from an extract of urban particulate matter using liquid-liquid partition and thin layer chromatography. Finally, Kamata and Motohashi (87) studied the separation of methyl substituted benz[c]acridine by cation exchange high performance liquid chromatography.

In order to isolate PANHs from analogous PAHs and other PACs, the method described below and summarized in Figure 2.4.2 was developed by Blumer and co-workers (88), but has been slightly modified to meet the needs of this work. This method has been selected because of its simplicity and of the quality of the results obtained by the authors (88). Each step involves simple equipment and can be performed routinely.

Prior to the procedure, the TPE sample (0.15 g) was chromatographed on a Sephadex LH-20 column, as described in Section 2.3.2.1 of the present work (9). The PAC eluate from Sephadex was chromatographed on acidic silica gel (10 g, 3% HCl). The adsorbent was prepared by adding concentrated HCl to silica gel (3% in volume) and allowing the mixture to stir on a rotary evaporator with very light vacuum, until all traces of water disappeared. The following solvents were passed through the column:

- 20 mL pentane (discarded: aliphatics)

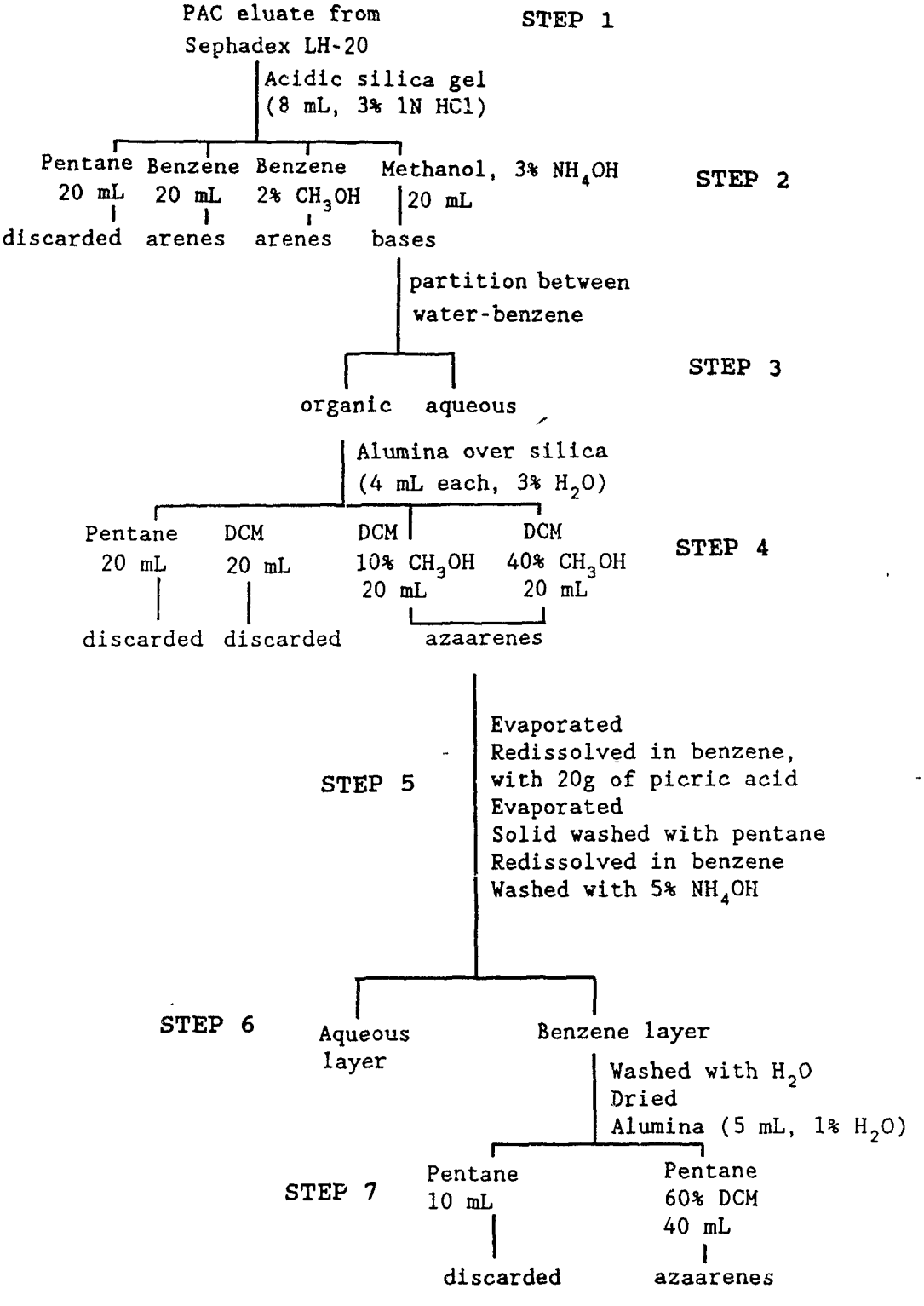


Figure 2.4.2: Method used for the isolation of PANHs from other PACs (88)

- 20 mL benzene (arenes)
- 20 mL benzene, 2% methanol (arenes)
- 35 mL methanol, 3% ammonium hydroxide (bases)

The last fraction was partitioned between water and benzene. The aqueous layer was re-extracted with benzene; benzene fractions were combined, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on alumina over silica gel (5 g each, 3% water). Deactivated silica and alumina used here were prepared by stirring the adsorbents with 3% of their volumes in water. The following solvents were used for the elution:

- 20 mL of pentane (Fraction A, aliphatics, discarded)
- 20 mL of dichloromethane (Fraction B, discarded)
- 20 mL of dichloromethane, 10% methanol (Fraction C, azaarenes)
- 20 of mL dichloromethane, 40% methanol (Fraction D, azaarenes)
- 25 mL of methanol (fraction E)*

Fractions C and D were combined, evaporated to dryness and re-dissolved in benzene containing 20 mg of picric acid. The solvent was evaporated under vacuum at room temperature, and the solid residue was washed three times with 2 mL of pentane, and the washings were discarded.

Dried solids were dissolved in benzene and washed three times in a separatory funnel with 5% ammonium hydroxide. The benzene layer was then washed with water, dried over sodium sulfate and evaporated.

The benzene fraction was then passed through a neutral alumina column (1% water, same preparation procedure as above); the sample was coated on 1 g of alumina on top of the column, and elution was performed as follows:

* This fifth step was added to the elution pattern since color was still left on the column. However, Fraction E was not combined with C and D.

- 10 mL of pentane (discarded)
- 40 mL of pentane, 60% dichloromethane[$]
- 40 mL of dichloromethane
- 40 mL 4:6 isopropanol:dichloromethane

The three last fractions were combined, solvents were evaporated, and samples were brought up to 10 mL in hexane.

2.4.2 Fractionation of the Syncrude samples

The methods described for fractionating the TPE in Sections 2.4.1.1 and 2.4.1.2 were not effective when directly applied to the Syncrude samples. Although they were tried, these methods led to poor chromatography in the final analysis as evidenced by too much peak overlap or bad chromatographic peak shapes. As terpenoic acids may be present in the Syncrude mixtures (89) and are expected to cause chromatographic interference, anion exchange chromatography was thought to be required prior to any fractionation method described in Section 2.4.1.

2.4.2.1 Anion exchange chromatography

As early as 1962, McCarthy and Duthie (90) presented a method for the separation of free fatty acids from other lipids. It consisted of running the original mixture through a column filled with KOH-treated silicic acid. Neutral lipids eluted with diethylether, while fatty acids were removed from the column with 2% formic acid in diethylether. Fifteen years later, Ramljak and co-workers (91) used potassium hydroxide treated silica gel for the rapid separation of acids from asphalts using a modified Soxhlet filled with the adsorbent. Acids were characterized by infrared spectroscopy, and results indicated the presence of a large proportion of free

$ From Reference 82, most azaarenes were present in this fraction, but not observed here.

carboxylic acids in the so called "acidic fraction" obtained from the asphalt. KOH-treated silica gel was also used by Amat et al. (92) and by Schmitter et al. (93) for acid removal; these authors also performed the extraction of bases from petroleum samples using silica modified with hydrochloric acid and polyphosphoric acid.

Desbene and co-workers (94) judged earlier procedures as being time consuming, and achieved preparative fractionation of petroleum heavy ends by ion exchange chromatography on resins; the selective extraction of bases and acids was performed using two columns packed respectively with Amberlite IRA 904 (anion exchange resin) and Amberlyst A15 (cation exchange resin).

Green et al. (95,96) later separated various petroleum distillates/residues into acidic, basic and neutral fractions, using nonaqueous ion exchange liquid chromatography, according to a technique described earlier (97).

Three anion exchange techniques have been used here, two using commercial column packings (resin and cellulose) and the other using KOH-treated silica. These techniques are described below.

i) Amberlyst anion exchange resin (98)

As Amberlyst-29 resin (98) was not available from the manufacturer, Amberlyst-26 anion exchange resin (100g, Rohm and Haas Company, Philadelphia, USA) was used instead, and when received had to be conditioned as follows:

- four washings with 10% HCl in methanol (v/v)
- rinsing with deionized water until washings are neutral on litmus paper
- stirring with 10% KOH/methanol for activation
- washing with deionized water until washings were neutral
- extracting by Soxhlet for 24 hours: -8 hours with methanol
  -8 hours with benzene
  -8 hours with hexane
- drying 24 hours at 40°C in a vacuum oven.

Chromatography: 1 g of oil sand extract (OSE), or 0.3 g of HGO or PIT samples were stirred in hexane for the extraction of asphaltenes (36). The mixtures were filtered; filtrates were evaporated, brought up to 20 mL in hexane and placed on 10 g Amberlyst-26 resin columns (2.5 cm i.d.). Hexane (300 mL) was passed through the columns to elute neutral compounds, and the remaining material was removed by passing 300 mL of isopropanol and 300 mL of 5% formic acid in methanol through the columns. The neutral fractions were chromatographed through Sephadex LH-20 for removal of aliphatics (See Section 2.4.1.1).

Blanks were run through resin columns as well, under the same conditions in order to see if any contamination occurred from the Amberlyst-26 resin.

ii) Anion exchange cellulose (Cellex-D, 0.62 meq/gram, Bio-Rad Laboratories, Richmond, CA)

The cellulose was conditioned by stirring with 10% KOH/methanol for 2 hours. It was then rinsed with deionized water until washings were neutral, then with isopropyl alcohol and hexane. The cellulose was allowed to dry for 48 hours in a heated (40°C) vacuum dessicator.

Chromatography: Four portions of 5 g of cellulose (3 samples, 1 blank) were packed in glass columns as hexane slurries. Samples consisted of 0.5 g of each material (OSE, HGO and PIT). Hexane (300 mL) was first passed through the columns, followed by isopropanol (300 mL) and 5% formic acid in methanol (300 mL). Hexane neutral fractions were run through Sephadex LH-20 columns (Section 2.4.1.1) to separate aromatics from aliphatics.

iii) Silica gel coated with potassium hydroxide (92,93)

Preparation and conditioning of the adsorbent: Potassium hydroxide (5 g) was dissolved in isopropyl alcohol (400 mL). Silica gel (50g, 60-120 mesh, BDH Chemicals) was added and the mixture was allowed to stir for 24 hours. Columns (10

g of adsorbent) were packed as slurries (the total of 50g was split approximatively into 5 columns). Each Syncrude sample (2 g of HGO and PIT, 0.83 g of OSE) and a blank were eluted with 70 mL of chloroform (neutral fraction) and 300 mL of 20% formic acid in chloroform (acidic fraction). Chloroform fractions were reduced in volume to 10 mL and small aliquots were analyzed before subjecting samples to further fractionation (See Section 2.4.2.3).

2.4.2.2 Alumina column chromatography

Aluminum oxide has been a less commonly used adsorbent than silica for the fractionation of petroleum heavy ends. Alumina column chromatography has generally been used as a minor step within a more elaborate fractionation scheme. For instance, Khorasheh and co-workers (39) have used alumina, according to a method described earlier (99), as a clean up step prior to silica over alumina fractionation. Allen et al. (100) separated non-distillable coal liquids into oils and 3 asphaltene subfractions on a fluorocarbon adsorbent, and one of the fractions was passed through a basic alumina column to separate nitrogen compounds from hydroxyaromatics.

Casalini et al. (101) used alumina adsorption liquid chromatography, according to a procedure proposed by Corbett (102), to accomplish fractionation of maltenes into saturates, aromatics and resins. In this method, heptane, toluene, toluene-methanol and trichloroethylene were used sequentially as eluents. Size exclusion chromatography was used as a further fractionation step.

The HGO sample was subjected to column chromatography on alumina, in the same way as described in Section 2.4.1.2, but with slight modifications. This method is expected to effect compound separation into aliphatics, neutral aromatics, polar aromatics and very polar compounds.

The sample (0.25 g) was coated on 1 g of neutral alumina (Activity I, Woelm, Germany) and placed onto a 10 g neutral alumina column. The modifications were made in the elution solvents, as follows:

- 50 mL of hexane (aliphatics), instead of 30 mL for TPE
- 70 mL of benzene (neutral aromatics), instead of 50 mL
- 100 mL of chlofoform (substituted PAHs), instead of 70 mL
- 50 mL of methanol-water 4:1

The benzene and chloroform fractions were not combined as in the TPE experiment; they were individually evaporated and brought over into 10 mL of dichloromethane.

2.4.2.3 Silica over alumina column chromatography

The neutral fractions collected from KOH-coated silica gel chromatography (Section 2.4.2.1, iii) for the three samples (0.2 g of HGO and PIT, 0.55 g of OSE) were eluted through silica over alumina columns. The elution was achieved similarly to that described previously (Section 2.4.1.3, (68)), without any modification. Only the 6:2:2 MeOH:Et-O-Et:Benzene fractions were submitted to final analyses.

This method has been published by Hirsh et al. in 1972 (68), and has been widely employed in other laboratories since. Poirier and Das (103), Coulombe and Sawatzky (40), Wallace et al. (104) and Khorasheh et al. (39) have employed this technique to separate asphaltenes into saturates, monocyclic aromatics, dicyclic aromatics, polycyclic aromatics and polar compounds.

2.5 Chromatography as the final determination method

2.5.1 Gas chromatography

Capillary column gas chromatography is accepted as the most efficient method of separating many compounds of different classes. Although the resolution achieved with capillary GC is currently unparalleled among chromatographic methods, its use is limited to the separation of volatile and thermally stable compounds. In GC, the retention of solutes is not greatly affected by the nature of the mobile phase. Helium,

most commonly used as a carrier gas, is inert and will not interact with samples.

Selectivity and retention depend on the interactions between solutes and the stationary phase. Compound separation is governed by: the polarity of the stationary phase; its ability to form hydrogen bonds; its ability to form weak electron donor-acceptor complexes; and the volatility of the sample components. Retention is influenced by the chemical nature of the sample and stationary phase and the column temperature (105).

All gas chromatograms that will be presented in Sections 6, 7.1 and 8 of this work were obtained with two types of instruments. First, a Finnigan gas chromatograph, equipped with an on-column injector, was used for GC/MS analyses in conjunction with a Finnigan 4500 quadrupole mass spectrometer, which will be described later. Second, Hewlett-Packard 5890A gas chromatographs, also equipped with on-column injection systems, were used. Three different HP 5890A gas chromatographs were employed: the first equipped with a flame ionization detector (FID), the second interfaced to a VG Masslab 20-250 quadrupole mass spectrometer and the third connected to a VG-ZAB-EQ sector mass spectrometer.

For the analysis of neutral mixtures of PAHs or standards, a 30m x 0.25 mm i.d. DB-5 fused silica capillary column with a 0.25 μm thick stationary phase (J&W Scientific Ltd., Folsom, CA) was used. For PANH mixtures or more polar compounds, a DB-1701 (same dimensions and characteristics as the DB-5) was used. In all cases, helium was the carrier gas at a flow rate of 2-3 mL/min and the oven temperature was programmed as follows: from an initial temperature of 70°C, held for two minutes, the temperature was increased to 300°C at the rate of 4°/min., then kept at 300°C for 15 minutes. The injection volumes were 1 μL. For analyses using the FID, the detector was kept at 275°C.

For the analysis of higher molecular weight polycyclic aromatic compounds, high temperature gas chromatography was initially performed using the HP 5890A instrument equipped with a FID. A high temperature GC column as originally purchased was coated with aluminum ("SuperCap Series", High temperature Al-Clad fused silica capillary column, 10 m, "400" bonded methyl silicone, 0.25 mm i.d., 0.10

μm film thickness, Quadrex Corporation, New Haven, Connecticut). When GC conditions were optimized (see description of temperature program below), the aluminum-coated column was installed in the oven of the Finnigan gas chromatograph. Many difficulties occurred during this transfer, mainly due to the inflexibility of the aluminium coating, leaving the column with only 3 m of length to be used for the analysis. Helium was used at the same flow rate as above, and the temperature program was similar to that described previously except that the upper limit was increased to 375°C instead of 300°C. The final temperature was held for 15 minutes.

Another high temperature GC column was purchased (25m, methyl silicone, 0.25 i.d, 0.1 μm film thickness, J&W Scientific) and used on the Finnigan GC/MS system. However, even with temperatures programmed up to 350°C, this new column did not allow elution of PACs with molecular weights higher than allowed with a regular DB-5 capillary column.

In most laboratories where GC is applied to PAC analysis, the range of compounds studied is restricted to molecular weights lower than ca. 326, due to the low volatility of PACs beyond this range (3,49,106,107). High temperature gas chromatography has been successful in eluting and separating saturated C_nH_{2n+2} compounds with n up to 61 (M.W. 856) (108), but has not been applied successfully to the analysis of PACs heavier than 400 in molecular weights in other laboratories.

2.5.2 High performance liquid chromatography

All high performance liquid chromatography (HPLC) chromatograms presented in this work in Sections 6 and 7.2 were recorded using a Hewlett Packard 1090M system equipped with a ternary DR5 solvent delivery system.

For HPLC experiments with ultraviolet (UV) detection, a HP 1040M diode array detector (DAD) was used and data acquisition was controlled by a HP 79994A data system. Detection channels were set at 254, 280 and 300 nm, with bandwidths of 10 nm. The reference wavelength for automatic background subtraction was set

at 550 nm, with a bandwidth of 100 nm. Both reversed and normal phase HPLC were performed and corresponding results will be presented in this work.

For HPLC with on-line mass spectrometric (MS) detection, the HP 79994A data system was used to control the pumps of the liquid chromatograph, while data acquisition was performed by the MS data system. Interfacing the HPLC chromatograph with the mass spectrometer will be discussed in Section 2.6.1.2.

Certain experiments involved simultaneous UV and MS detection; this could be performed by splitting the column effluent in a 1:1 ratio between the DAD and the mass spectrometer. In such cases, data acquisition was performed simultaneously by both MS and HPLC data systems.

2.5.2.1 Reversed phase high performance liquid chromatography

Three different reversed phase HPLC (RPLC) columns were used, for similar analytical purposes, during the course of this project:

- A 250 X 4.6 mm i.d., 5 μm Vydac 201TP column (C_{18} octadecyl silane on Vydac TP silica base, Separations Group, Hesperia, CA)
- A 250 x 2 mm i.d., 5 μm Vydac 201TP column (same characteristics)
- A 250 x 4.6 mm i.d., 5 μm Supelco RP-C_{18} column (LC-PAH RP-C_{18} packing, Supelco Inc., Bellafonte, PA)

The gradient tabulated below (Table 2.5.1) was used with each of the three columns, for PAC separations.

All separations were performed at 25°C. The injection volume was 5 μL when using the 2 mm i.d. column, with a flow rate of 0.2 ml/min. When using both 4.6 mm i.d. columns, flow rates were set at 0.8 ml/min, to better match the requirements of the HPLC/MS interface, which will be described later. The injection volume was 20 μL when using the larger columns.

Analyses requiring elution of PACs up to molecular weight ca. 326 only were performed using the gradient tabulated above, but stopping at 55 minutes, i.e. not proceeding through the dichloromethane step. This first part of the complete gradient

program (involving water and acetonitrile only) was previously used in this laboratory for PAC analyses (12). The second part, incorporating dichloromethane as a strong solvent for high molecular weight aromatic compounds, was developed during this project based upon earlier work by Peaden and co-workers (109). Dichloromethane allowed the elution of PACs with molecular weights up to ca. 600 in the case of TPE, and up to 900 for the Syncrude samples OSE, HGO and PIT.

Table 2.5.1: Program used for reversed phase HPLC separations.

time (min)	% water	% acetonitrile	% dichloro-methane
0-45	40-0	60-100	0
45-55	0	100	0
55-105	0	100-0	0-100
105-120	0	0	100
120-121	0	0-100	100-0
121-122	0-40	100-60	0

The HPLC gradient initially developed by Peaden and co-workers (109) was used to analyze high molecular weight PACs in a carbon black sample. The solvent program consisted of operating isocratically for the first 15 minutes with a 1:1 mixture of water-acetonitrile, programming to 100% acetonitrile over the next 70 minutes, programming to 100% ethyl acetate over the next 130 minutes, and finally to 100% dichloromethane over the last 60 minutes. The duration of this gradient was 6 hours total. The same solvent progression was tried for the elution of the Tar Pond sample. Unfortunately, ethyl acetate does not provide good wetting of the surface of the belt used for HPLC/MS interfacing (vide infra). Since dichloromethane does wet the belt, the gradient was modified and optimized to yield the final program listed

above. This new gradient allowed elution of compounds in the same mass range as observed in (109), in one third of the time required in the original experiment.

A water-acetonitrile gradient (first part of the program) has been used in this laboratory (12) for the HPLC/MS of PACs, with high resolution mass spectrometry. Water-acetonitrile gradients in fact have been widely applied to PAC analyses by reversed phase HPLC of PACs on octadecylsilane columns (110-115).

Pure methanol and aqueous methanol mixtures, pure acetonitrile and aqueous mixtures, and pure ethyl acetate, tetrahydrofuran, chloroform and dichloromethane mobile phases have all been used with polymeric phases (see next paragraph) for shape selective separation of PACs. A series of gradients using these solvents allows shape selective separation from the alkyl-naphthalenes up through PAHs with 14 rings (116). Fetzer and Biggs (117,118) found that methanol had a higher separating capability than acetonitrile when used with a dichloromethane gradient.

There are two types of HPLC C_{18} reversed phases: monomeric phases and polymeric phases; the columns used in this work were of the latter type.

Monomeric phases are made by derivatizing silica with a monochloro-trialkylsilane, resulting in a single moiety on each derivatized silanol. Although forming a brushlike plane in organic media, the octadecyl moieties are very irregular in aqueous mobile phases.

Polymeric phases result from reaction of a di- or trichloro-octadecylsilane with the silanols. When one of these reacts with a silanol, further reactions can occur through the remaining chlorine atoms. Addition of water substitutes the residual chlorines by hydroxyl groups, which may then react with another alkylsilane. Repetition of the reaction yields a bonded alkyl substituted polysiloxane (116).

2.5.2.2 Normal phase high performance liquid chromatography

Normal phase HPLC has not been widely used for the analysis of PACs in sediments similar to the Tar Pond sample studied here, because GC and reversed phase HPLC provide more information very rapidly and are more specific. However,

normal phase HPLC has been the object of a few studies for the characterization of PACs in the environment. Separation of aliphatics from PACs, whose elution is achieved by the number of rings, has been studied with conventional silica HPLC columns (119). Chemically modified columns have also been used: diol-(120) and nitro-(121) bonded silica provided improved efficiency in normal phase separation of PAC standards.

Normal phase HPLC for the analysis of oils and petroleum samples has been extensively used over the last two decades. Samples of these types are often very complex, more so than samples from anthropogenic pollution. This complexity is due to the presence of numerous alkanes, alkylated aromatics, polar compounds, acids and bases. Gas chromatography generally fails to separate compounds of those complex mixtures and severe overlapping occurs of peaks in reversed phase HPLC.

Therefore, normal phase HPLC (NPLC) has been the technique of choice for the characterization of petroleum type matrices, and different types of packings have been used. One of the most spectacular separations by NPLC was achieved by Dark and McGough (122) in the characterization of asphalts. Amino bonded silica and plain silica columns were used in sequence and allowed the separation of asphaltenes into several subgroups: saturates, aromatics, polycyclic aromatics, monophenols, nitrogen heterocyclic aromatics, polyphenols and high molecular weight highly functionalized molecules.

Bollet and co-workers (123) used amino and cyano bonded phase columns to separate saturates, aromatics and polar compounds in heavy petroleum products. The aliphatics were passed through the amino column, and the retained aromatics and polar compounds were then separated by backflushing onto the cyano column.

The same type of compound class separation of Venezuelan vacuum residua was achieved by Carbognani and Izquierdo a few years later (33,36). Saturates, aromatics and resins were separated using silica and cyano-bonded silica normal phase columns.

Only one normal phase HPLC column was used during this work, a 300 x 4.6 mm i.d. μBondapak NH_2 Energy Analysis column (Waters, Milford, MA). This

column was used because it allowed a clean separation of neutral PAHs from more polar PACs such as PANHs. A flow rate of 1 mL/min was used when operating with the DAD, and 0.8 mL/min. was more suitable when performing HPLC/MS. A 20 μL sample loop was mounted on the system, and compounds were eluted according to the program in Table 2.5.2 (124).

Normal phase HPLC was tested as a means of further fractionation of samples to render analyses easier. However, larger scale normal phase HPLC was not

Table 2.5.2: Program used for normal phase HPLC separations. All gradients are linear.

time (min.)	% hexane	% dichloromethane
0-20	100	0
20-25	100-99.5	0-0.5
25-30	99.5-98	0.5-2
30-35	98-95	2-5
35-40	95-85	5-15
40-45	85-60	15-40
45-50	60-0	40-100
50-60	0	100
60-65	0-100	100-0

developed in the course of this project, since, as it will be shown later, reversed phase HPLC allows the separation of nitrogen containing and polar heterocyclic compounds from PAHs in a well defined manner. Thus this technique was not seen as a fractionation method, but rather as a complementary tool in studying complex

mixtures.

2.5.3 Supercritical fluid chromatography (SFC)

2.5.3.1 Introduction to SFC

SFC employs a mobile phase consisting of a highly compressed gas at or above its critical temperature and pressure. Above its critical point, a substance has density and solvating power approaching those of a liquid, but viscosity similar to that of a gas, and diffusivity intermediate between a gas and a liquid (125). The use of supercritical fluids as chromatographic mobile phases was first reported in 1962 by Giddings (126).

Carbon dioxide (CO_2) is commonly chosen as the mobile phase because it is non-toxic, easy to handle and it only requires moderate pressures and temperatures to reach its supercritical fluid state. The critical temperature of CO_2 is 31°C and its critical pressure is 7397 kPa. Pentane, another mobile phase used in SFC, has critical temperature and pressure of 196.6°C and 3374 kPa, respectively.

At the critical temperature of a substance, the vapor and liquid phases have identical densities. A gas cannot be liquefied above its critical temperature, irrespective of the pressure. Density, diffusivity and viscosity of a supercritical fluid result in good behavior for the transport of solutes through a column. The solubility increases with mobile phase density. Any given analyte has increased solubility in a supercritical fluid relative to solubility in a gas, and about the same solubility as in a liquid at the same temperature. Solute diffusion coefficients are greater in a supercritical fluid than they are in a liquid: higher analyte diffusivity leads to narrower chromatographic peaks, and also results in higher optimal linear velocities for supercritical fluids than liquids. The speed of analysis is thus higher in SFC than it is in HPLC.

Solutes migrate through a column as a function of mobile phase density, polarity and stationary phase composition. The strength of the mobile phase can be

modified by adding some polar agent in small proportions. So-called organic modifiers are added to supercritical fluids to reduce adsorption and to change selectivity (127). SFC can potentially bridge the gap between GC and HPLC, while retaining some positive attributes of both; it enables the elution of large, non volatile and thermally labile compounds.

SFC is developing along two lines: Capillary column and packed column SFC. The latter generally produces sharper chromatographic peaks than the former, thus providing better resolution.

Both capillary and packed column SFC modes have been studied for the purposes of this work and a description of experimental conditions will be given here.

2.5.3.2 Capillary column SFC

Peaden et al. (128) described a very robust instrumental set up that provided good separations of PAHs on a 100 μm i.d. capillary column containing a bonded poly(methylphenyl siloxane) stationary phase. The mobile phase was pentane, with pressure programming. On-column fluorimetric detection was used in order to minimize band broadening. The restriction system used allowed a constant flow of the eluent through the column, thus allowed reproducible retention times. Two examples of data obtained with this instrument were reported, one for a carbon black extract, the other for a coal tar sample. Both chromatograms showed reasonable separation of groups of isomers and of some individual compounds.

Heavy oils have been characterized by capillary SFC by Fuhr and co-workers (129). They used a 30m x 100 μm i.d. SPB-5 capillary column and carbon dioxide as the mobile phase with pressure programming. Detection was achieved with a flame ionization detector (FID). FIDs can tolerate flow rates in the order of 50 μL/min of liquid, expanding to a gas. Restriction was accomplished with a smaller i.d. linear fused silica capillary tubing attached to the end of the column. Individual peaks could be resolved for n-paraffins in waxes up to C_{90}. Chromatograms of mid-distillate (200-340°C) cuts of bitumen and heavy oil samples however showed no resolution of

individual components. Instead compounds were eluted in large unresolved envelopes.

The experiment reported here was conducted using a Brownlee Microgradient pump (Applied Biosytems Inc.) as a means of mobile phase pressure control. The supercritical fluid (CO_2) was fed to a Rheodyne 7520 valve, equipped with a 0.02 μL injection loop, and then through a 10m x 0.05 mm i.d. cross-linked DB-5 fused silica capillary column. Coated packings such as those used in GC cannot withstand SFC conditions unless they are cross-linked (130). Without the cross-linking process, stationary phases do not resist the high solvation power of supercritical fluids (131).

Decompression was achieved by pulling a restrictor at the end of the column, which was maintained inside a HP 5980 GC oven, and connected to either a HP FID or to the VG Masslab 20-250 mass spectrometer.

Carbon dioxide density was controlled by programming both pressure and temperature according to diagrams shown Figure 2.5.1. A simultaneous increase in pressure and drop in temperature featured in this program results in an asymptotic increase of carbon dioxide density (Figure 2.5.1 c) (132), thus favoring the elution of molecules of increasing size as the run proceeds.

2.5.3.3 Packed column SFC

Packed SFC columns are very similar to those used in HPLC. Their internal diameters vary from 320 μm (packed capillaries) to 4.6 mm. Stationary phases consist of bare and modified silica and alumina. Chemically bonded phases, which feature a monolayer of organic ligand on a silica surface are commonly used. C_8 and C_{18} reversed phases have been used, as well as more polar phases with cyano, amino or diol groups (133).

Three packed SFC columns were used for the purposes of this project:

- A 200 x 1 mm i.d. C_{18} column (5 μm, monofunctional, Applied Biosystems-Brownlee Labs, Santa Clara, CA)
- A 200 x 1 mm i.d. silica column (Spheri-5, 80 A pore, Applied Biosystems-Brownlee

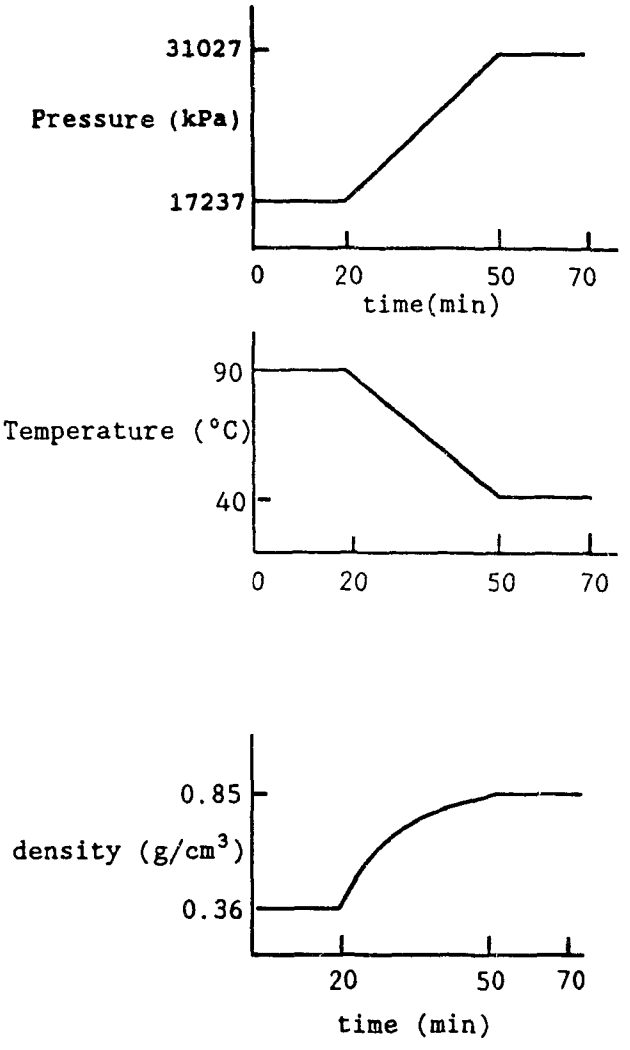


Figure 2.5.1: Mobile phase pressure (top) and temperature (center) programs used in capillary column SFC/MS to obtain asymptotic density programming (bottom)

Labs, Santa Clara, CA)

- A 250 x 4.6 mm i.d. silica column (Spheri-5, 80 A pore, Applied Biosystems-Brownlee Labs, Santa Clara, CA).

Different experimental procedures have been used with each column and will be described separately.

i) C_{18} column

Billie and Greibrokk (127) used gradient programming and combined gradient-pressure programming to analyze a mixture of 22 standard compounds (PAHs and nitrated PAHs) on a reversed phase C_{18} SFC packed column. Carbon dioxide was used as the mobile phase, with methanol as the organic modifier. Programming both pressure and % methanol in CO_2 yielded a clear separation of the 22 compounds present in the mixture.

For this work, an Isco Microgradient SFC pump (Isco Inc., Lincoln, Nebraska) was used to feed the mobile phase through the reversed phase column. The column was equipped with a Rheodyne 7520 SFC injector (2 μL loop) and was kept at 110°C in a column oven. Restriction at the end of the column was accomplished by pinching the end of a stainless steel capillary tube, to maintain flow rates between 75 and 300 μL/min. The SFC system was interfaced to the VG Masslab 20-250 mass spectrometer. Interfacing will be described later in Section 2.6.1.3.

Two successful separations were achieved for TPE and a standard mixture. The first separation was conducted using pure CO_2 as the mobile phase; it involved the type of programming tabulated in Table 2.5.3. Pressure programming was achieved using an Isco SFC data system, installed on a IBM compatible PC interfaced with the SFC system.

The second successful separation of TPE and standard mixture was accomplished using 10% methanol in carbon dioxide as an organic modifier. The program is given in Table 2.5.4.

Although both chromatographic runs (TPE and standard mixture) obtained

with methanol as a modifier were better than the corresponding ones obtained with carbon dioxide only, the use of methanol was not pursued since it seemed to strip some of the stationary phase off the column and thus plug the restrictor.

The HGO sample was then run through the C_{18} column with pure CO_2, and the first program described in Table 2.5.3.

As CO_2 and CO_2 modified with methanol did not allow elution of very large PACs (MW > 350), an attempt to use pentane as the mobile phase with the C_{18} column was tried and failed. The bonded stationary phase could not withstand the high solvating power of supercritical pentane.

Table 2.5.3: Pressure program used for the SFC separation of a PAH standard mixture and TPE. Mobile phase: 100% CO_2.

segment no.	initial pres. (kPa)	final pressure (kPa)	time (min.)
1	20685	20685	15
2	20685	27580	15
3	27580	34475	10
4	34475	48265	15
5	48265	48265	15

ii) 1 mm i.d. silica column

The idea of using unmodified silica in SFC came from the observation that supercritical pentane, required for eluting high molecular weight PACs, would not affect this stationary phase. It has been shown before (134) that normal phase packed column SFC could effect fractionation of diesel fuel samples into saturates, aromatics and polars. For this purpose, a 4.6 mm i.d. column packed with silica was

Table 2.5.4: Pressure program used for the separation of a standard PAH mixture and the TPE by packed column SFC. Mobile phase: 10% methanol in CO_2.

segment no.	initial pres. (kPa)	final pressure (kPa)	time (min.)
1	17238	17238	10
2	17238	20685	10
3	20685	41370	30
4	41370	41370	15

used. The mobile phase was carbon dioxide at constant pressure.

In the present work, the column was equipped with the same injection system as described above and a 2 m long, 50 μm i.d. fused silica capillary was connected to the end as a pressure restrictor. The system was kept at 230°C, above the critical temperature of pentane (196.6°C) and flow rates were on the order of 200 μL/min. Detection was achieved by mass spectrometry.

The technique was first developed with a standard PAH mixture containing compounds with a 166-278 molecular weight range (same standard mixture used with the C_{18} column). Using pure pentane as the mobile phase, at 3374 kPa, resulted in coelution of all standard compounds shortly after the solvent front. Thus methanol was employed as a modifier (a "bad" solvent, compared with pentane) in order to retain compounds on the column longer. Methanol (10%) in pentane was tried with several pressure programs to separate the standards, and the most suitable form of programming is presented in Table 2.5.5.

Pressure programming was still achieved using the SFC ISCO pump as described previously. As will be shown in Section 7.3.1.2, separations obtained under these conditions were not satisfactory when trying to analyze "real world" samples. Gradient and pressure programming had to be combined, and this combined form

Table 2.5.5: SFC pressure program used to separate compounds in a PAH mixture. Mobile phase: 10% methanol in pentane.

segment no.	initial pres. (kPa)	final pressure (kPa)	time (min.)
1	3348	3348	0.5
2	3348	6895	20
3	6895	6895	5

of programming was possible with a Brownlee Microgradient SFC syringe pump (Applied Biosystems Ltd.). Separation of compounds in the standard mixture was best achieved using the program listed in Table 2.5.6.

Separation of the TPE was possible using this program, although all compounds above mass 300 almost coeluted during the two last steps. After optimization with the TPE instead of the standard mixture, the most suitable combination of pressure/gradient programming was as listed in Table 2.5.7.

The HGO sample investigated in this part of the work was best separated using a different programming scheme; the program listed in Table 2.5.8 provided a reasonable separation of compounds contained in the HGO.

iii) **4.6 mm i.d. silica column**

Total ion chromatograms obtained using the 1 mm i.d. silica column strongly resembled those obtained when performing preparative normal phase HPLC. Development of a SFC preparative method was thus investigated using a 4.6 mm i.d. silica SFC column. The column was equipped with a Rheodyne 8125 injector (20 μL loop) and restriction was accomplished using a 1 m, 50 μm i.d. fused silica capillary.

Table 2.5.6: Pressure and gradient programming used for the SFC separation of a PAH standard mixture. Mobile phase: MeOH-pentane.

time (min.)	pressure (kPa)	% methanol in pentane	max. flow rate (μL/min.)
0-6	3448	10-4	150
6-9	3448-4137	4	150-200
9-12	4137-4826	4-2	200
12-15	4826-5516	2-1	200
15-20	5516-6895	1-0	200

Flow rates were in the order of 400-600 μL/min. The Brownlee pump was once again used to allow simultaneous pressure and gradient programming. After optimization with the HGO, the program given in Table 2.5.9 was used for fractionation of the HGO, OSE and PIT samples.

During the first 70 minutes, 14 fractions were collected at intervals of 5 minutes. Fractions 15a to 15d were collected at intervals of 10 minutes over the 40 following minutes. Fractions collected were then subjected to HPLC/UV and HPLC/MS analysis (off line).

Table 2.5.7: Pressure and gradient programming used for the SFC separation of the TPE. Mobile phase: MeOH-pentane.

time (min.)	pressure (kPa)	% methanol in pentane	max. flow rate (μL/min.)
0-60	3792	7.5	175
60-65	3792	7.5-5	175
65-70	3792	5-3	175
70-80	3792-4826	3-2	175-200
80-90	4826-5516	2	200
90-105	5516-6895	2-0	200-250

Table 2.5.8: Pressure and gradient programming used in SFC for the separation of the HGO sample. Mobile phase: MeOH-pentane.

time (min.)	pressure (kPa)	% methanol in pentane	max. flow rate (μL/min.)
0-24	3448	15	150
24-48	3448-6895	15	150-200
48-60	6895	15-5	200
60-65	6895	5-4	200
65-75	6895	4-0	200
75-80	6895-8274	0	200

Table 2.5.9: SFC gradient and pressure program used to separate the HGO, OSE and PIT samples. Mobile phase: MeOH-pentane.

time (min.)	pressure (kPa)	% methanol in pentane	max. flow rate (μL/min.)
0-70	3792	11	400
70-110	3792-6895	11-0	400-600
110-120	6895	0	600

2.6 Mass spectrometry

2.6.1 Interfacing

2.6.1.1 Interfacing gas chromatography to mass spectrometry

In each GC/MS experiment reported in this work, direct introduction of the GC capillary column into the ion source of the mass spectrometer was used to interface GC and MS (direct interfacing). The low helium flow rates (ca. 2-3 mL/min of gas) and the high pumping efficiency of the mass spectrometers together allow this type of interfacing. The column at the exit of the GC oven is fed through a heated, temperature controlled tube which is connected as an inlet to the ion source. This interface tube between the gas chromatograph and mass spectrometer is ca. 30 cm long, and is kept at 300°C. GC/MS analyses were performed on three different mass spectrometers: the VG Masslab 20-250 quadrupole, the Finnigan 4500 GC/MS system and the VG ZAB-EQ sector instrument. Figure 2.6.1 gives a schematic of the interfacing described above while Figures 2.6.2, 2.6.3 and 2.6.4 show diagrams of the

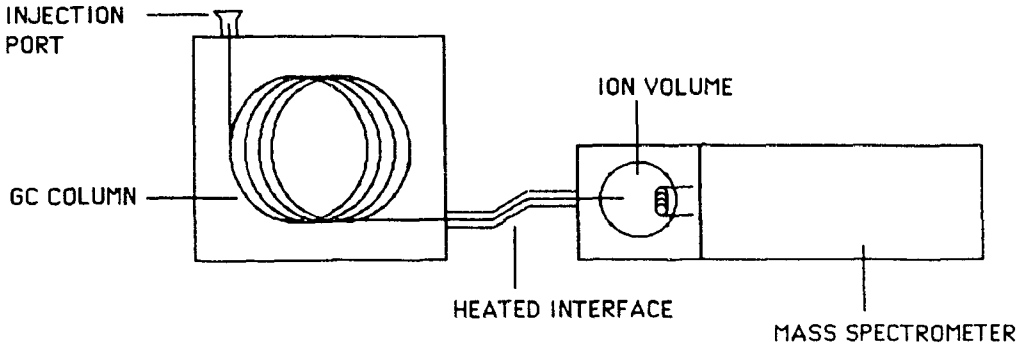


Figure 2.6.1: Schematic representation of a GC/MS system

mass spectrometers used.

In coupling a gas chromatograph to a mass spectrometer, it is required that the pressure be reduced from atmospheric to 10^{-5}-10^{-7} torr at the mass spectrometer inlet. Whenever sample size and column flow rate permit, the GC may be directly coupled to the mass spectrometer. Broadening of the chromatographic peaks due to reduced column pressure is minimal for a wall coated, open tubular column and the speed of analysis is increased. Direct coupling also minimizes losses and decomposition of thermally labile compounds (135). When, however, flow rates are too high for direct coupling, it is desirable to feed the column effluent to the mass spectrometer via a device that separates the carrier gas from the sample molecules. A jet separator is used for that purpose, using the fact that carrier gas molecules are lighter than sample molecules. Pressure restriction causes molecules to gain a supersonic velocity as they exit the column. As the solid angle of diffusion is inversely proportional to the mass, most of the helium is rejected while the heavier sample molecules are captured by the mass spectrometer interface (136).

2.6.1.2 Interfacing HPLC to mass spectrometry

In HPLC/MS, sample molecules must be extracted from a high pressure, mobile condensed phase and introduced into a mass spectrometer inlet at about 10^{-6} torr. Therefore, on-line coupling of the HPLC to the MS is limited by the effective high flow rates generated by HPLC. Depending on the solvent, a characteristic 1 mL/min flow rate from the HPLC columns expands to 100-1000 mL/min when vaporized (136).

The use of HPLC/MS interfaces based on mechanical transfer devices, such as a moving wire (137) or moving belt (135) has fallen into disfavor in recent years, due mainly to the popularity of the thermospray interface (138,139) and its discharge assisted variants (140). More recently, particle beam (or momentum separator) HPLC/MS interfaces have been introduced (141,142), permitting electron impact ionization spectra to be obtained for analytes of moderate polarity and thermal

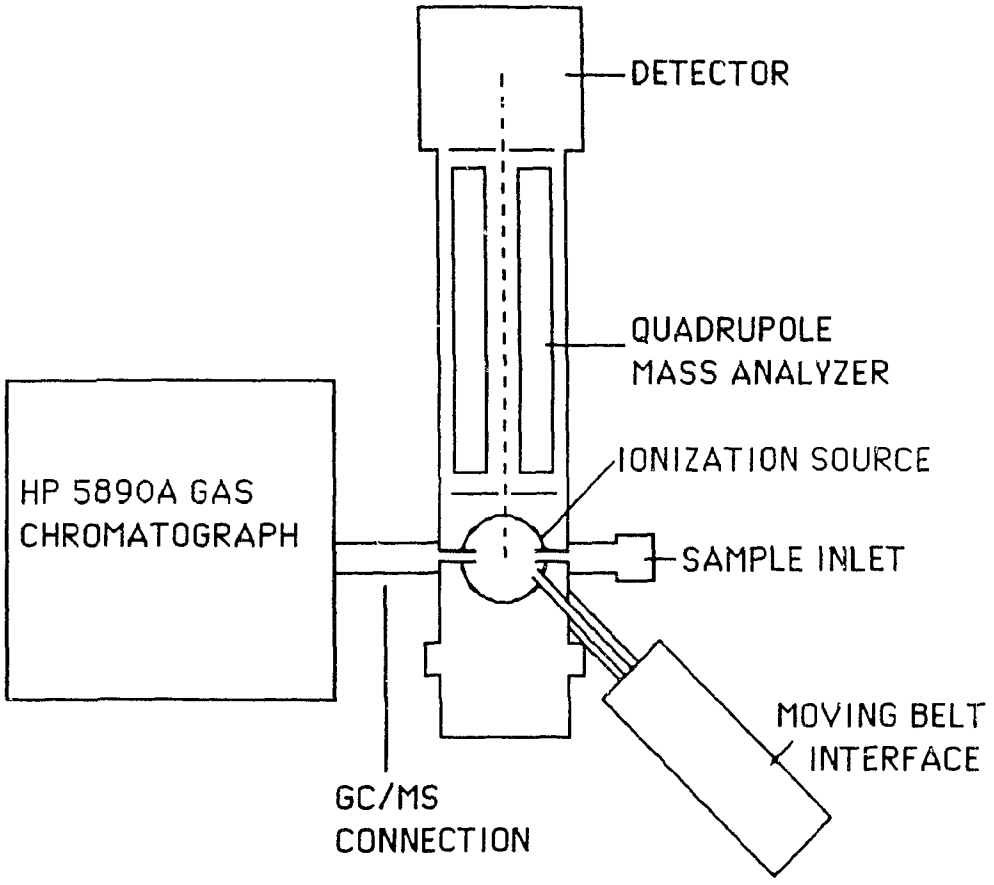


Figure 2.6.2: VG 20-250 quadrupole mass spectrometer

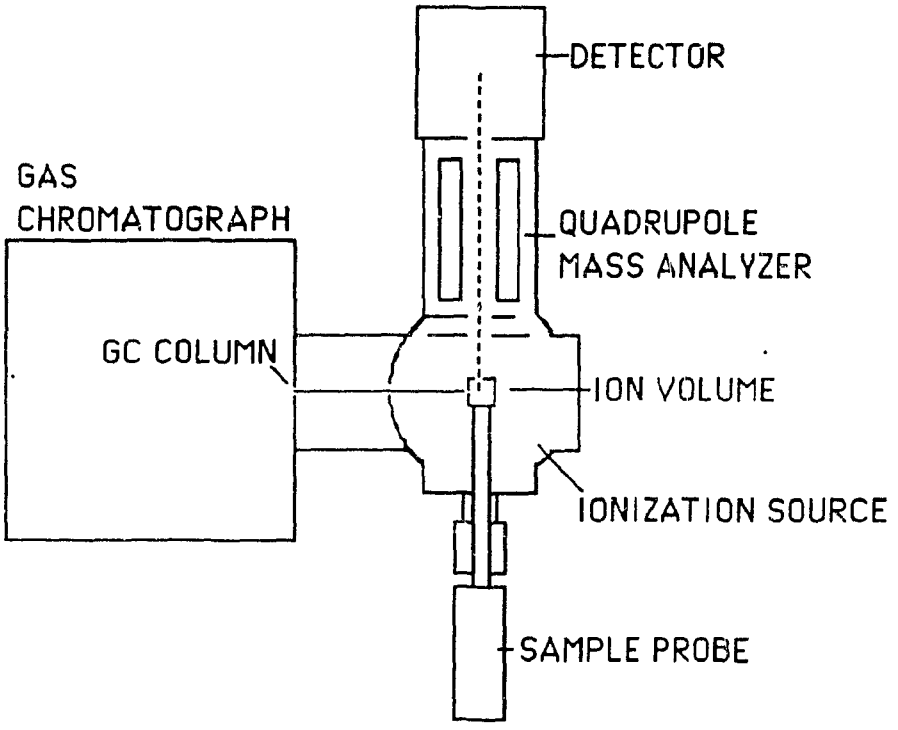


Figure 2.6.3: Finnigan 2500 GC/MS system

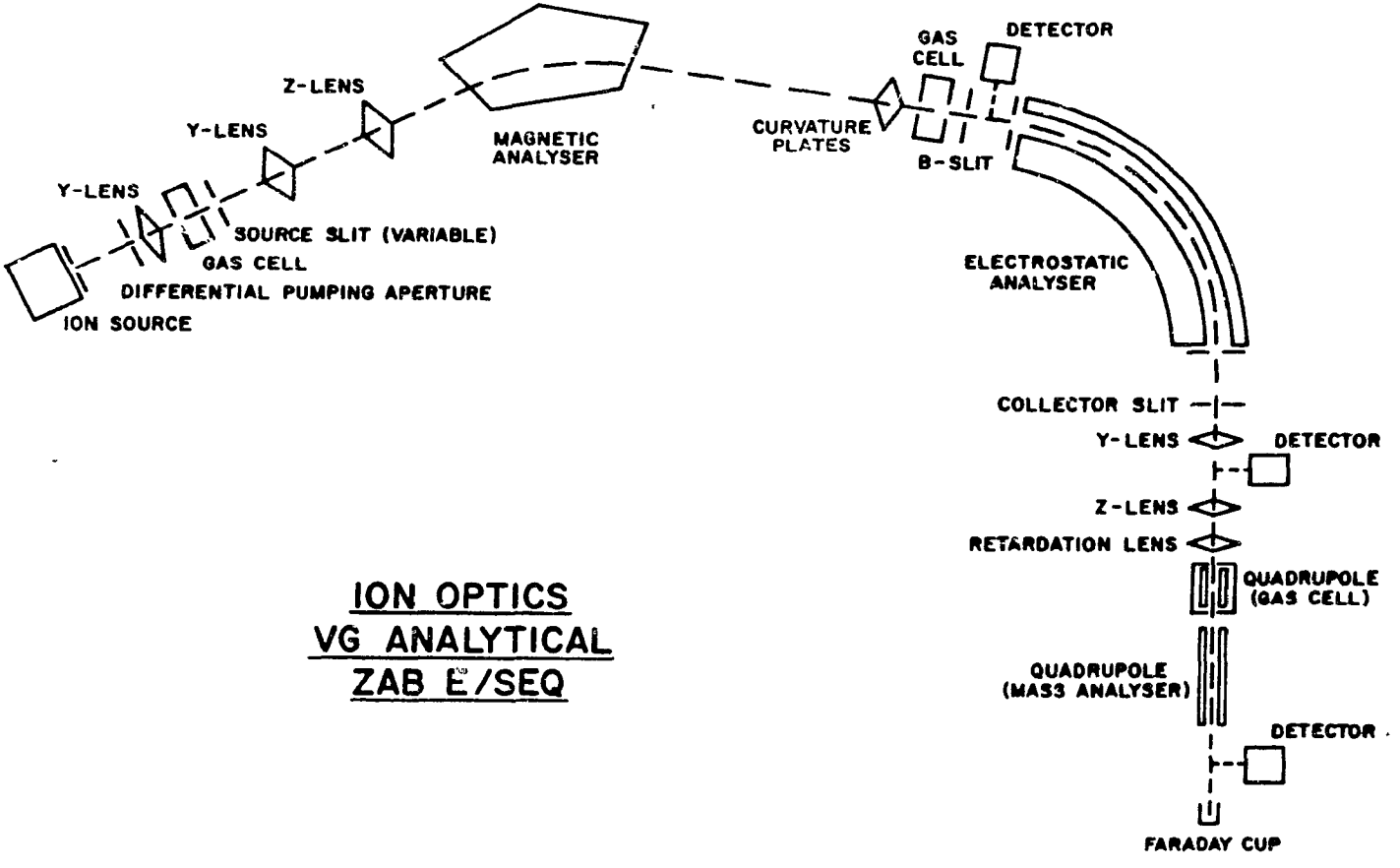


Figure 2.6.4: VG ZAB-EQ mass spectrometer. Not shown: gas chromatograph, sample inlets.

stability. Extremely polar and thermally labile analytes may be successfully analyzed by HPLC/MS using the related electrospray and ionspray (pneumatically assisted electrospray) interfaces (143,144), or by the use continuous flow fast atom bombardment (145,146). These newer techniques have largely supplanted the mechanical transport devices (135,137) and HPLC/MS interfaces based on direct liquid introduction (147,148). However, the moving belt has proven to be a reliable and efficient HPLC/MS interface for thermally stable analytes of moderate volatility, especially PACs. The experimental setup used for this work will be described here.

The column effluent with a flow rate of 0.8 mL/min. was transferred to a moving belt interface (VG Analytical, Manchester, UK), which delivers the sample directly into the ionization source block, using a spray depositor. The belt was operated at a speed of 1.6 cm/sec. Figure 2.6.5 is a schematic representation of a moving belt interface for HPLC/MS. The effluent is deposited directly on the belt via a nebulizing probe, and forms a thin (ca. 0.2 mm) film on the belt surface. It then passes through the preliminary solvent separation zone and into the interlocks, where most of the remaining solvent is removed. The eluate is flash evaporated directly into the ion source, by a heater mounted at the end of the interface device (nose heater). Because of the very rapid desorption, decomposition of thermally labile compounds is minimized. Any residual material is removed with a clean-up heater mounted on the belt device. The belt nose heater was operated at an indicator dial setting of 0.8 (80% of full power, the actual temperature cannot be measured). The belt clean-up heater was operated at 70% of full power.

The mass spectrometer used for HPLC/MS experiments in this work was the VG Masslab 20-250 instrument.

2.6.1.3 Interfacing SFC to mass spectrometry

Interfacing SFC/MS is not as easy to effect as interfacing GC/MS, due to the high equivalent gas flow rates generated in SFC. However mobile phase flow rates in SFC are lower than those in HPLC and hence interfacing is easier.

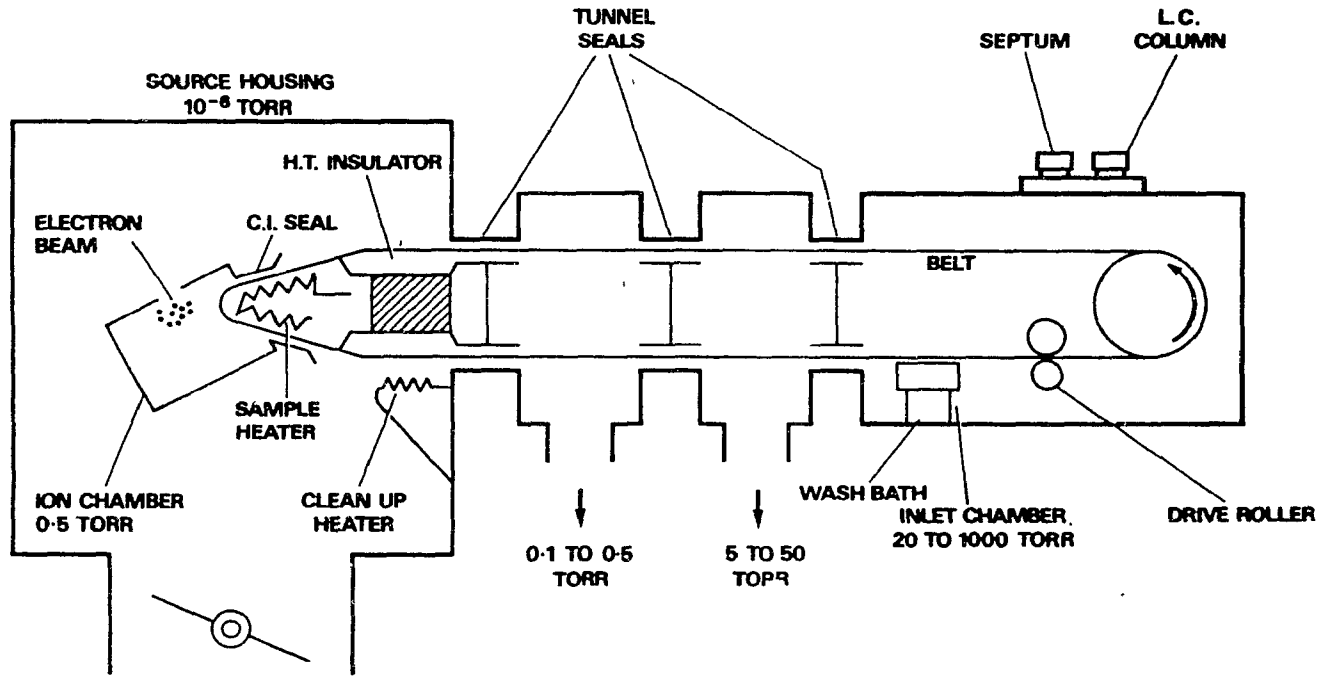


Figure 2.6.5: VG moving belt interface

For effective development of an SFC/MS interface, there are three prime requirements (149):

- The interface must be capable of handling the gas flow rates generated from the mobile phase
- The solute must be transported into the mass spectrometer ion source and ionization effected without thermal decomposition
- Maintenance of chromatographic integrity must be achieved using minimal dead volume couplings. Supercritical fluid chromatographic conditions must be maintained through the interface.

In capillary column SFC, typical flow rates from 50 μm i.d. columns lay between 0.5 and 5 μL/min as liquid; however, packed column SFC provides liquid flow rates of 100-600 μL/min. Capillary column SFC/MS and packed column SFC/MS must therefore be interfaced in two different ways, which will be described here.

i) Interfacing of capillary column SFC/MS

The liquid flow rates used with capillary SFC columns can be adequately handled by mass spectrometers configured for chemical ionization, due to their high pumping capacity.

Hawthorne and Miller (150) performed the SFC/MS of polycyclic aromatic compounds with a relatively simple capillary direct interface. The SFC column was inserted through the transfer line of a GC/MS system, with the restrictor tip of the column extended to within ca. 1 mm of the end of the interface probe. A commercial restrictor was used to control column flow at a gas flow rate of 1 to 3 mL/min.

Smith and co-workers (151,152) described a similar system, using two different modified direct fluid injection probes. In the probes, a zero dead volume connection was made to 100 um i.d. Pt-Ir tubing using a silver chloride melt as a sealant. The Pt-Ir tubing extended through the last 3 cm of the interface to the flow restrictor. One model of probe used micrometric adjustment of an orifice to control restriction;

for the second model, the Pt-Ir tube was pinched at the end to provide restriction. These two arrangements allowed no dead volumes and left only a short distance between the column end and the detector (mass spectrometer ionization source).

In the present work, the interface between column and ion source was direct, and corresponds to the same diagram as illustrated in Figure 2.6.1 for GC/MS. The main difference is the presence, in SFC/MS, of a restrictor, pulled manually at the end of the SFC column in the ionization block. The capillary column SFC system was interfaced to the VG Masslab 20-250 quadrupole mass spectrometer.

ii) Interfacing packed column SFC/MS

Direct interfacing of packed column SFC with a mass spectrometer capable of EI and CI is very difficult, due to the high mass flow rates generated in this technique. However, the moving belt has proven to be a very reliable interface for this mode of chromatography. Berry and co-workers (153) were the pioneers in combining packed column SFC with mass spectrometry using the moving belt interface. The thermospray deposition device originally designed for HPLC/MS was modified, and the technique was applied successfully to mixtures of xanthines and sulfonamides. Columns used had 4.6 mm internal diameter and were packed with an amino bonded phase. Mixtures of carbon dioxide with methanol or methoxy-ethanol were used as mobile phases. The same technique was used later in the same laboratory (154) for the analysis of indole alkaloids.

The moving belt system used in this work was described earlier in Section 2.6.1.2. It was operated under the same conditions (belt speed, power of the nose heater and of the belt cleaner). The need for a restrictor led to a modification of the original VG Analytical HPLC/MS spray depositor, as shown in Figure 2.6.6. The stainless steel capillary tube used for HPLC/MS was removed from the probe and replaced with a pinched stainless steel capillary (for CO_2 and CO_2/methanol experiments) or with a 1 or 2 m long fused silica capillary (50 μm i.d.), heated by a home made copper block heater (for pentane/methanol experiments). The amount

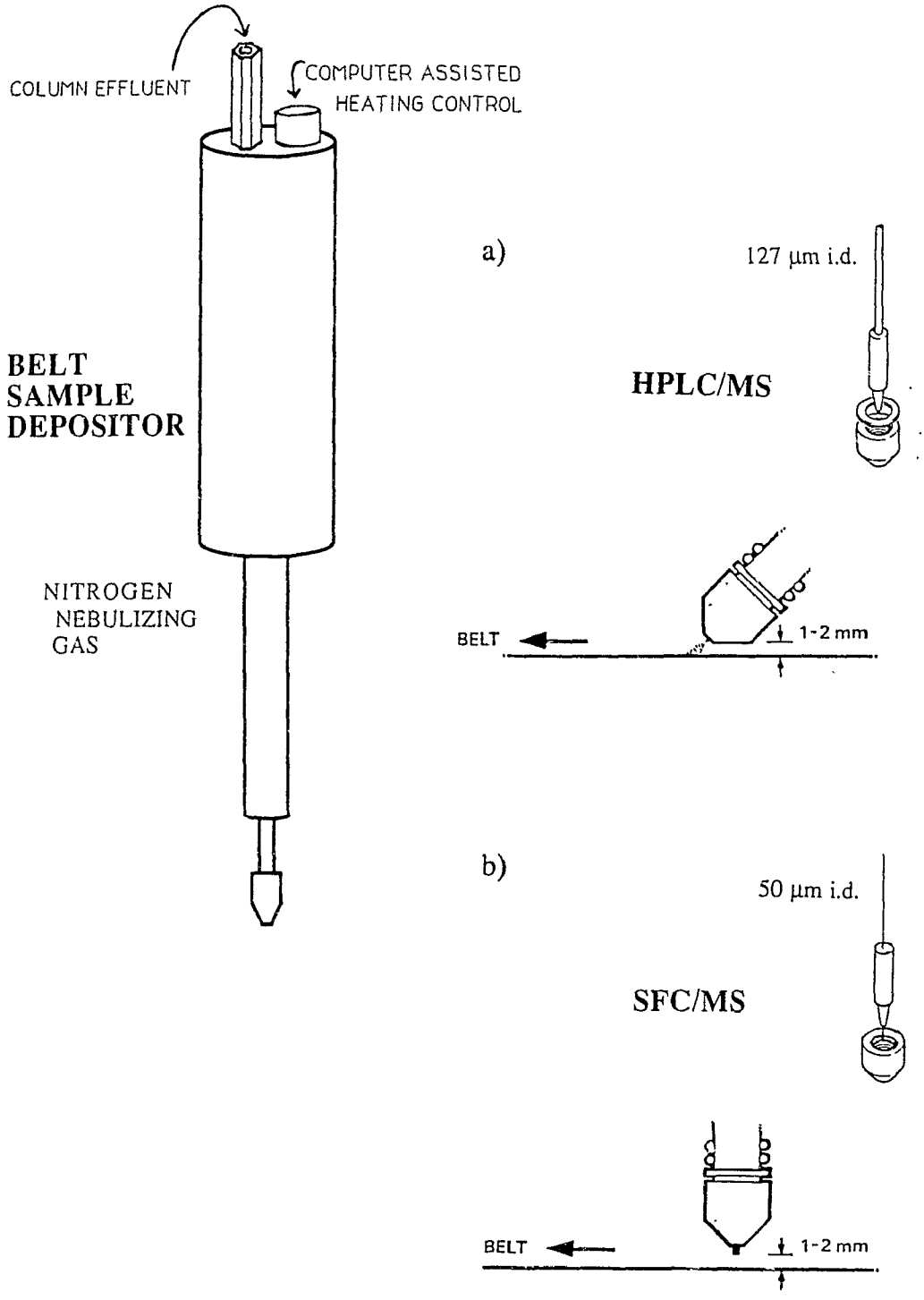


Figure 2.6.6: VG belt sample depositor. a) Original design for HPLC/MS. b) Modified design for SFC/MS.

of effluent deposited onto the belt was difficult to monitor, especially when using CO_2 as the mobile phase, since it was readily vaporized and thus invisible on the belt. When using pentane in SFC or other liquid solvents in HPLC, the interfacing was optimum when the liquid formed a thin, regular film on the belt, or when it formed fine droplets of equal sizes and evenly spaced onto the belt. Factors to optimize for good transfer of material were the pressure of nitrogen nebulizing gas, the temperature of the sample depositor tip and the flow rate.

In SFC, using 1 mm i.d. columns, flow rates were optimal at ca. 200 μL/min, and ca. 400 μL/min with the 4.6 mm i.d. column. Using no nitrogen nebulizing gas seemed to lead to better transfer of the effluent onto the belt; the effluent would otherwise be sprayed with a wide solid angle, too wide for the narrow surface of the belt. Temperature of the spray depositor block heater was increased as higher molecular weight compounds eluted from the column. Typical temperatures lay between 150 and 320°C. The sample spray depositor was mounted at 90° with respect to the belt for maximal transfer, as opposed to HPLC/MS where it is usually set with an angle of 45° relative to the belt. The mass spectrometer used was the VG Masslab 20-250.

Interfaces other than the moving belt have been used to couple packed SFC column to mass spectrometers. Sim and Elson (14,155) set up an interface by coupling a frit restrictor to the exit of the column with a zero dead volume union; the frit restrictor was positioned at the entrance of the ionization volume. More recently, packed SFC columns were interfaced to an atmospheric pressure ionization source by Anacleto et al. (156).

2.6.2 Ionization modes used in this work

2.6.2.1 Electron impact ionization

Production of positive ions by EI is a widely used technique. For thermally stable and moderately volatile analytes, it usually produces ions at the molecular

mass of the compounds. These molecular ions are designated as $M^{+\cdot}$ and result from the loss of one electron from the sample molecule M. Fragmentation of molecules also occurs following this energetic ionization process and often helps in elucidating molecular structure.

The reaction involved in the EI process results from the collision of a gaseous molecule M with a fast electron:

$$M + e^- \text{---}> M^{+\cdot} + 2e^- \qquad (5)$$

EI mass spectrometry is a very specific detection method for PACs, which are extremely stable under these ionization conditions.

Coupling chromatography (GC, HPLC, SFC) to EI mass spectrometry is one of the most reliable methods of identification of PACs in complex mixtures. The main advantage is that retention times and mass spectral data can be obtained in a single analysis.

The electron impact mass spectra of PACs are well characterized as being quite simple, mainly consisting of an intense molecular ion peak and small peaks due to ions which have lost one to four hydrogen atoms. Doubly charged molecular ions are quite common and are usually near 20% of the abundance of the molecular ion at 70 eV electron energy. For PAHs fragment ions due to the expulsion of acetylene (C_2H_2) are present, but in very low abundances.

Alkylated PAHs demonstrate the normal $(M-15)^+$, $(M-29)^+$, etc. fragmentation pattern for alkyl chains.

In most cases, differentiation of PAH isomers by their EI mass spectra cannot be done. Although slight differences are seen, they are not significant enough for positive identification within the experimental variations normally encountered when analyzing real samples. Even in the cases of isomers with very different structures (e.g. fluoranthene and pyrene), the mass spectra are most often indistinguishable.

The mass spectra of hydroaromatic compounds (fluorene, acenaphthene) show the ease of removal of protons from saturated carbons to give abundant $(M-1)^+$ ions.

The position of substitution of a heteroatom in the aromatic frame makes essentially no difference in the observed spectra. PACs with an attached functional group will normally yield mass spectra showing a fragment corresponding to the loss of this functional group; PAQs and PAKs produce spectra featuring losses of 28 (CO) (2).

Figure 2.6.7 shows a diagram of a typical EI ionization source, which includes:
- a tungsten filament, which becomes incandescent and emits electrons when a current is passed
- an electron trap, to attract electrons to the opposite side of the ionization chamber
- high voltage electrodes, to accelerate positive ions out of the source into the mass spectrometer
- collimating slits as well as focusing and beam centering electrodes to achieve proper deflection and transmission of the ion beam.

The ion repeller accelerates the ions, once formed, toward the exit slit. A magnetic field within the chamber is produced by a secondary magnet (not illustrated in Figure 2.6.7) and used to deflect and focus electrons between the filament and electron trap, increasing the probability of M colliding with an electron. This magnetic field is in the order of 100 Gauss (136).

The accelerating voltage, Va, can be varied from 1 to 10 kV for magnetic sector instruments. In the case of a quadrupole system, Va values usually lie between 10 and 50 volts.

The three mass spectrometers used in this work are operable in the EI mode. A few differences in operating these systems require a more detailed description of each system.

i) Electron impact ionization on the Finnigan 4500 system (GC/MS)

The Finnigan ionization source has EI and CI ion volumes. Those ion volumes are in fact chambers where ionization takes place. As the mass analyzer is a quadrupole, ions go through circular apertures rather then slits as shown in Figure 2.6.7 (sector instruments).

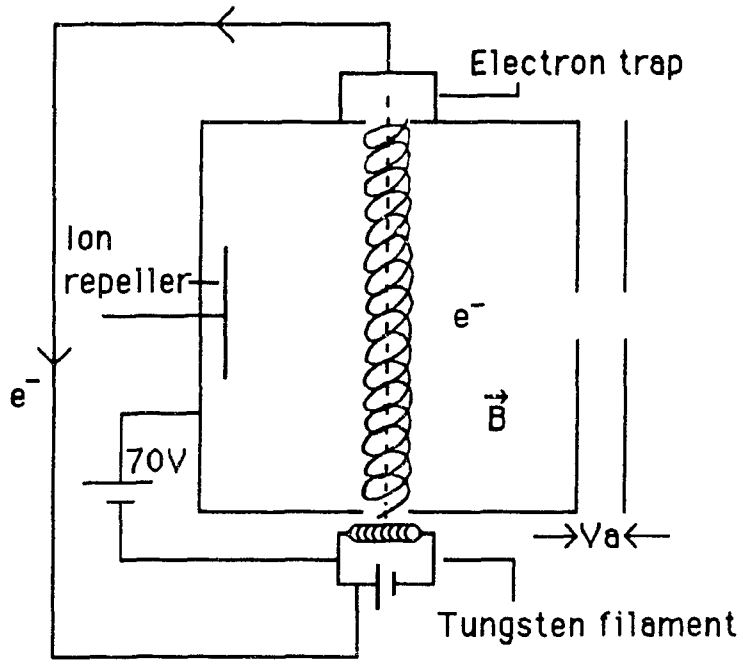


Figure 2.6.7: Schematic representation of an electron impact ionization source

The Finnigan EI exit aperture is fully open, avoiding pressure build-ups in the ion volume. In this work, the source was kept at 200°C and the quadrupole was scanned from 100 to 600 Da. at the rate of 1 scan/sec. Tuning and calibration were achieved using perfluorokerosene (PFK, high boiling point, PCR Chemicals, Gainesville, FLA). Sample introduction was performed via the GC/MS interface only.

The ionization source was operated with 70 eV nominal electron energy, with a 110 μA trap current. The detector was an off-axis continuous dynode electron multiplier equipped with a 3kV conversion dynode. Data were recorded with a SuperIncos data system.

ii) Electron impact ionization on the VG Masslab 20-250 mass spectrometer

The ionization source used for EI is equipped with an adjustable slit which can be either positioned for EI (large aperture) or CI (very small aperture). The source temperature was 275°C for most experiments unless specified otherwise, and the source was operated at 70 eV electron energy and 100 μA trap current.

Samples can be introduced into the ionization block in 5 different ways:
- direct probe insertion (solid samples). The sample is deposited in a glass capillary tube, which is introduced into the temperature programmable sample probe.
- reservoir inlet injection. Liquid compounds are injected in a reservoir inlet, which leaks its contents into the ion source via a heated capillary
- GC/MS, capillary column SFC/MS. Direct interfacing is used, as described earlier.
- HPLC/MS, packed column SFC/MS. Belt interface.
- spikes on the belt interface. Samples in solution can be spiked directly onto the belt with a syringe. Solvents are removed by differential pumping, before samples proceed into the ionization source.

The quadrupole was typically scanned from 100-900 Da. at the rate of 1 sec/scan (GC/MS and capillary column SFC/MS) and 3 sec/scan (other sample introduction methods). Calibration and tuning were achieved with PFK.

The VG Masslab 20-250 is equipped with a Galileo 4771 series electron multiplier, and data were recorded with a VG 11-250 data system.

iii) Electron impact ionization on the VG ZAB-EQ mass spectrometer

This tandem hybrid instrument of BEqQ configuration (magnet B, electric sector E, rf only quadrupole q and quadrupole mass filter Q) is similar to the ZAB-Q mass spectrometer described in detail previously (157).

EI ionization was effected with a nominal electron energy of 70 eV and a trap current of 100 μA. Samples were introduced either by a probe, by a liquid reservoir or by direct interfacing with GC.

When operating in the GC/MS mode, the source was kept at 275°C and the magnet was scanned at the rate of 1 sec/decade. For probe samples, the probe temperature was programmed to match the compound boiling points and the mass spectrometer was scanned at 10 sec/decade. Liquid samples could be directly injected via the reservoir, kept at 200°C. Conventional mass spectra of either solid or liquid samples were obtained at a magnet scan rate of 10 sec/decade. The source temperature was 250°C. In all cases, the accelerating voltage was 8 kV.

The ZAB BEqQ instrument is equipped with three detectors: an electron multiplier between B and E for single focusing magnet scans, a photomultiplier (scintillation detector) between E and q for experiments requiring double focusing, and an additional scintillation detector placed after Q for the detection of ions exiting the quadrupole. In scintillation detectors, ions are first converted to photons at a scintillator (charged plate covered with a very thin layer of aluminum to avoid charge overload), and the photons released are detected by a photomultiplier.

2.6.2.2 Chemical ionization (CI)

In some cases, EI produces unstable $M^{+\cdot}$ ions and only fragment ions are observed. It is more appropriate in those cases to ionize molecules by chemical

ionization, which is achieved by ion-molecule reactions (charge transfer or proton transfer occurring between a preionized reagent gas and the gaseous sample molecules). Such reactions take place at relatively high source pressures and involve the transfer of only a small amount of energy to the sample molecules. This leads to more stable quasi-molecular ions, and thus fragmentation occurs to a substantially lesser extent than it does with EI (158). Most commonly, ionization is achieved by proton exchange, from a preionized gas GH^+ to a sample molecule M:

$$GH^+ + M \text{ ---> } MH^+ + G \qquad (6)$$

This reaction will occur if the proton affinity of M is greater than that of G. Methane, propane, isobutane and ammonia are frequently used as reagent gases. Within the ionization chamber, those gases are first ionized by EI, to lead to primary ions. The latter transform into a reactive form (secondary ions) and then transfer protons to sample molecules.

The CI source is a modified version of the EI source, as the exit slit (or circular aperture) is narrowed down to allow pressure to build up inside the ionization chamber.

The electron beam energy is increased to ca. 200 eV, to allow electrons to penetrate the source in spite of the high local gas pressure. As sample M is introduced with a vapor pressure of ca. 10^{-6} torr, the amount of sample ionized by EI is negligible. The following scheme illustrates chemical ionization with ammonia as the reagent gas (159).

$$NH_3(g) + e^- \text{ ---> } NH_3^{+\cdot} + 2e^- \quad \text{(primary ion, EI)}$$
$$NH_3^{+\cdot} + NH_3 \text{ ---> } NH_4^+ + NH_2^\cdot \quad \text{(secondary ion, reagent)}$$
$$NH_4^+ + M \text{ ---> } MH^+ + NH_3 \quad \text{(protonated sample molecular ion)} \qquad (7)$$

Ammonia is one of the mildest reagents among commonly used gases, in that

it produces very little fragmentation of the analyte.

For the purposes of this work, ammonia-CI has been used as a means of differentiating molecular ions and fragments. When an EI mass spectrum contains many intense ions, there is a possibility that some of those are fragments from molecular ions, or unfragmented molecular ions. However, with ammonia-CI, which is a softer ionization technique than EI, most ions can be assigned as molecular ions. For instance, alkylated PAHs submitted to EI ionization tend to fragment easily, by losing methyl and methylene groups. If ionized by ammonia-CI, the same substances are more likely to stay intact upon ionization.

CI experiments were conducted on the VG Masslab 20-250 quadrupole; the source was kept at 200°C. The pressure of the ammonia gas plasma had to be optimized as to obtain the best $MH^+/M^{+\cdot}$ intensity ratio for a given analyte of interest. 2-Methylphenanthrene (M.W. 192) was used as the standard to optimize CI conditions.

As all initial experiments were performed in EI, only a few CI experiments (spikes on the moving belt) were conducted to verify the amount of fragmentation observed when analyzing the Alberta samples. No chromatographic/mass spectrometric data were acquired under CI conditions.

2.6.3 Selectivity enhancement

Since PACs usually occur in extremely complex mixtures in the environment, reliable analytical procedures must incorporate as much selectivity as possible in the fractionation, chromatography and detection strategies. This part of the work involved investigations of the behavior of dications formed from PACs by electron impact ionization. The objective of these investigations was to find some means of increasing the selectivity of mass spectrometric detection of PACs in complex environmental samples, based upon their propensity to form doubly charged ions.

It has been shown that useful improvements in signal/noise ratios in GC/MS analyses for PAHs were obtained by monitoring the first ^{13}C isotope peak of

molecular dications M^{2+}, i.e. at nominal "half-integral" mass values (16). Fragmentation reactions of PAH dications were studied (160) using the technique of tandem mass spectrometry (MS/MS), in the hope that some characteristic dissociations might provide the basis for a highly selective MS/MS procedure.

2.6.3.1 Study of the fragmentation of PANH molecular dications

This part of the present work is an extension of the PAH dication investigation (161) to dications of analogous PACs containing one nitrogen atom incorporated into the polycyclic framework (PANH) (158). PANHs are important constituents of tobacco smoke and have been linked to carcinogenic activity (160,162).

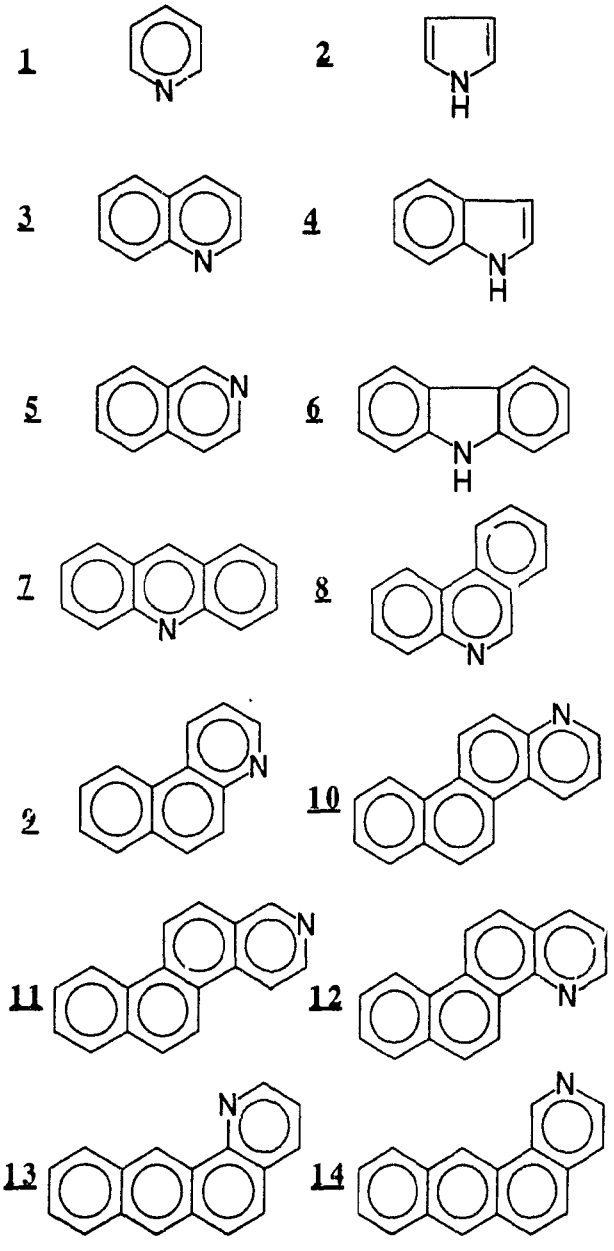
All experiments were conducted using a VG Analytical ZAB-EQ instrument, see Figure 2.6.4. The hybrid tandem mass spectrometer of Beqq configuration is equipped with a high field magnet and associated ion optics and with a VG-11 250J data system.

The electron impact ionization source was operated at 230°C, with a nominal electron energy of 70 eV and a trap current of 100 μA.

Solid compounds were introduced via a temperature controlled direct insertion probe, whereas liquids were injected via a molecular leak inlet.

Tandem mass spectrometry experiments were conducted using two complementary techniques to characterize the fragmentation reactions. In order to obtain unambiguous mass assignments, the ions to be monitored were accelerated out of the ionization source, mass selected by the double focusing combination of magnetic and electric sectors, and decelerated into the rf-only quadrupole reaction cell followed by the quadrupole mass filter. Both unimolecular and argon gas collision induced dissociations (CID) could be studied in the rf-only quadrupole collision gas cell q. The laboratory frame collision energies of these CID reactions lay in the range of 50-300 eV. These quadrupole experiments provided half mass unit mass resolution but no energy information.

Table 2.6.1: Compounds studied for the fragmentation of their molecular dications; **1** pyridine; **2** pyrrole; **3** quinoline; **4** indole; **5** isoquinoline; **6** carbazole; **7** acridine; **8** phenanthridine; **9** benzo[f]quinoline; **10** naphtho[2,1-g]quinoline; **11** naphth[2,1-f]isoquinoline; **12** naphtho[1,2-h]quinoline; **13** naphtho[2,3-h]quinoline; **14** naphth[2,3-h]isoquinoline.



In the complementary technique of mass analyzed ion kinetic energy spectroscopy (MIKES) (163), the ions accelerated out of the source are mass selected by the magnetic sector alone, and transmitted to the field free region between magnetic and electric sectors. The fragment ions formed there, by either unimolecular or collision induced reactions, are characterized by a translational energy spectrum obtained by scanning the electric sector field (163). A MIKES experiment thus provides energetic information on the fragmentations (particularly the kinetic energy release) and the widths of the peaks which contain this information can lead to difficulties of interpretation if peak overlap is severe. By using MIKES, doubly charged precursor ions of 8 keV translational energy (4 kV source potential) were induced to fragment under both unimolecular and CID (helium collision gas) conditions.

MIKE spectra provide only very poor mass resolution, particularly when charge separation (CS) reactions are involved. The information about kinetic energy release obtained from MIKE spectra is invaluable for establishing which fragment ions are the results of CS reactions since in such cases, there must be two fragment peaks with complementary fragment masses (sum of masses equals mass of the precursor) and with the same value deduced for the kinetic energy release. However, in some cases the z-discrimination effects (163) can be so severe that the low mass CS fragment is not observable, and in such cases the characteristically broad dished MIKES peak shapes are used to identify CS reactions. Quadrupole mass filter data are more accurate relative to mass assignment and are invaluable in such cases where a lack of resolution characterizes MIKE spectra.

The compounds studied in this Section are shown as 1-14 in Table 2.6.1. They all include only one nitrogen atom in the polycyclic aromatic framework.

2.6.3.2 Monitoring of the half integral mass PANH dications

A full scan GC/MS experiment was performed on the VG ZAB-EQ instrument. The sample under investigation was the TPE, and the GC capillary

column used was a DB-1701. The mass spectral resolution was relatively high (ca. 5000) to ensure good discrimination between half-integral and integral mass ion peaks. Post-run data analysis allowed the construction of RICs of m/z values corresponding to molecular cations and dications of the PANHs of interest.

2.6.3.3 GC/MS/MS experiments

The GC/MS conditions were as cited above in Section 2.6.3.2. The magnet was programmed to change from mass to mass as a function of time with the masses of interest corresponding to those of the molecular dications of PANH species. Collisions were induced in the rf-only cell, and the fragmentation spectra thus obtained were recorded at the rate of 2 sec/quadrupole scan and were used to characterize the species.

2.6.3.4 Constant neutral loss investigations

As a main conclusion of the results obtained from experiments described in 2.6.3.1, most molecular ions or dications of heterocyclic aromatic compounds containing nitrogen undergo unimolecular losses of HCN or HNC (27 mass units for the monocation, 13.5 for the dication). The loss of 13.5 is rather unique and represents the main or only low energy channel reaction observed for the dications.

The constant neutral loss (CNL) of 13.5 has thus been investigated as a potentially useful and selective analytical tool for screening PANHs in complex environmental mixture.

CNL experiments were conducted on the VG ZAB-EQ instrument, and losses of 13.5 occurring in the rf-only quadrupole collision cell were monitored. This was achieved by simultaneously scanning the magnet and the quadrupole mass filter, with the help of the VG 11-250J CNL software.

CNL scanning was performed at the rate of 10 sec/decade, first for detecting standard compounds (1-aza-benz[a]anthracene and acridine) by direct probe

introduction. The second experiment was achieved by running GC/MS (DB 1701 column) of a mixture of PAH and PANH standards: quinoline, acridine, 1-aza-benz[a]anthracene, naphthalene, phenanthrene, chrysene. In this case the scan rate was 1-2 sec/decade.

3. RESULTS AND DISCUSSION: SAMPLING

3.1 Sydney Tar Pond sediment

The procedure used to sample the tar pond sediment, once collected by Acres International Ltd. (1791 Barrington Street, Halifax, Nova Scotia), was performed at the Institute for Marine Biosciences, National Research Council of Canada (Halifax). This method was similar to that used previously as a standard procedure for the MACS (Marine Analytical Chemistry Standards) program (45) (See Section 2.1.1).

The powdered sediment obtained from the sampling process was very homogeneous and showed no trace of moisture or oily components. Several chlorobenzene extracts of different 15 g portions of the sediment showed a high degree of homogeneity from GC/MS analyses. The amount of organic matter extracted from the sediment was (10.0±0.8)%.

3.2 Broken Syncrude oil sand

The oil sand sample was not lyophilized since the procedure would not have eliminated the oils that rendered homogeneity difficult to obtain. As received from Syncrude Canada Ltd., the sample was marked to contain 9.61% bitumen and 4.50% water.

Drying several portions of 15 g under nitrogen (see Section 2.1.2) resulted in a loss of weight of (3.9±0.9)% from the original sample. Chlorobenzene Soxhlet extraction of these different portions of dried material yielded a percentage of combined bitumen and organic matter of (10±2)%. After extraction, the remaining material was removed from the filter and ground again to verify the absence of any residual oily components. The filters were found to contain only dried inorganic material.

The presence of oily components in the original sediment made sampling difficult due to the tendency of particles to agglomerate in oily chunks. This is

reflected by the higher standard deviation obtained for the organic content of the tar sand sample compared to that of the tar pond sediment.

4. RESULTS AND DISCUSSION: EXTRACTION

4.1 Sydney tar pond sediment and Syncrude oil sand samples

Chlorobenzene was used as the Soxhlet extraction solvent since it had previously been shown to be useful in the extraction of organic compounds of high molecular weights (32). In this work the interest was partly focused on high molecular weight compounds contained in the samples.

PACs are soluble in many organic solvents and several have been recommended for the Soxhlet extraction of solid environmental samples (23,164), e.g. acetone, benzene, cyclohexane, chloroform, methanol and other alcohols, acetic acid, benzene-methanol, petroleum ether, dichloromethane and tetrahydrofuran. The solvent can be chosen in order to obtain high extraction yields for either polar substances, high molecular weight compounds, or moderately polar PAHs, etc.

Extracting with a sequence of solvents of increasing strength is also common; for instance hexane, toluene, dichloromethane, chlorobenzene and 1,2,4-trichlorobenzene were used in sequence to Soxhlet extract carbon black and coal tar pitch samples (32).

In this laboratory, hexane was found to extract PACs with M.W. up to 350 only from tar pond sediments, and the organic matter extracted totaled only $(5.3 \pm 0.5)\%$ of the original sample. Dichloromethane extended the upper molecular weight range of the extracted PACs to ca. 450, which is comparable to supercritical fluid extraction (165).

As it will be shown in Section 7, chlorobenzene allowed compounds with molecular weights up to ca. 600 to be extracted from the tar pond sediment, and up to ca. 960 in the case of the oil sand sample. 960 Da. is not necessarily the upper limit in the sample, it was the upper limit detected by the mass spectrometer. Moreover, chlorobenzene brought the extraction yield up to 10% for both tar pond and syncrude samples.

For the few times that Soxhlet extraction has been reported for tar and oil

sands (90), it was performed using a sequence of solvents (32). The five classes of organic compounds found in oil sands (maltenes, asphaltenes, resins, saturates and aromatics) could be selectively separated by the extraction procedure. These types of samples are more often subjected to liquid-solid extraction by stirring. Pentane (38,40), hexane (36) or heptane (33-37) will dissolve the maltenes and leave asphaltenes as a residue.

The asphaltenes form the highest M.W. fraction of crude oils. When precipitated, they appear as shiny black amorphous solids (32). Nuclear magnetic resonance (NMR) and infrared (IR) studies have shown the presence of aromatic rings in the asphaltene fraction, as well as carboxylic groups and long alkyl chains. By definition, all saturates, aromatics, and resins that are not soluble in pentane, hexane or heptane belong to the category of asphaltenes.

For the purposes of this work, it was preferred to extract the chlorobenzene soluble portion as a first step and then worry about separation of compounds by classes. This work required that the heaviest possible compounds, as well as the most mutagenic and carcinogenic compounds would be separated from the solid, inorganic matter. Without knowing what fraction would contain these heavy or carcinogenic compounds, it was decided to perform a non selective extraction. According to what was obtained by extracting the tar pond sediment with hexane, PACs whose weights are above 350 were not removed from the sample, and thus are asphaltenes by definition. Those "asphaltenes" can, however, be extracted with chlorobenzene, and analyses revealed that they were PACs.

The situation is similar for the Syncrude samples: "asphaltenes" probably include PACs as well as saturates, which can be eliminated by fractionation. This result justified the extraction of the broken oil sand sample with chlorobenzene.

The aromatic portion of Alberta heavy oils has been shown to contain PAHs, PANHs and PASHs. The resin fraction is a complex mixture of heterocycles and carboxylic acids. Alkyl fluorenones and benzofluorenones as well as phenols have been identified in this class (32).

5. RESULTS AND DISCUSSION: REMOVAL OF THE ELEMENTAL SULFUR

Different methods have been studied earlier in order to remove elemental sulfur from PAC extracts. In this laboratory, the amalgam method described in Section 2.3 was reported to be more efficient than stirring the sample with copper powder or eluting the mixture through a column topped with a layer of copper (46).

Elemental sulfur constitutes a strong interference when performing high resolution chromatography/mass spectrometry of the samples, especially GC/MS. Sulfur (S_8) elutes in the same range as PACs of interest, producing a very broad and often saturated peak that overlaps with other peaks of the species under investigation. It is thus important to remove as much sulfur as possible, although the methods mentioned above (including the amalgam method) never achieve a 100% removal. It has been observed (166) that decomposition of thiaarenes (PASHs) is a possible cause of the continuous formation of elemental sulfur in the samples. Figure 5.1.1 compares two GC/MS total ion current (TIC) chromatograms obtained for a tar pond sediment extract, a) before and b) after sulfur removal with the copper-mercury amalgam. The top trace shows the high sulfur content in the sediment. The elimination of the sulfur interference, yielding the bottom trace, renders the identification of compounds much easier. The mass spectrum of S_8 is shown in c). Following the removal of sulfur, the extracts were passed through a Sephadex LH-20 column for the elimination of aliphatic compounds. The elimination was only partial, as shown by the overall background on both chromatograms. the ratio aliphatic/aromatic was higher in b) than in a).

Sulfur also interferes in capillary SFC/MS, but not in HPLC/MS (or HPLC/UV) and packed column SFC/MS. The S_8 molecule does not absorb in the UV, and its retention time in both reversed phase HPLC and SFC chromatographic modes does not correspond to that of any of the compounds under investigation.

Since the Syncrude samples studied in the present work were not amenable to GC and capillary column SFC analysis, sulfur never represented a real interference but was still removed prior to liquid chromatographic analyses as part

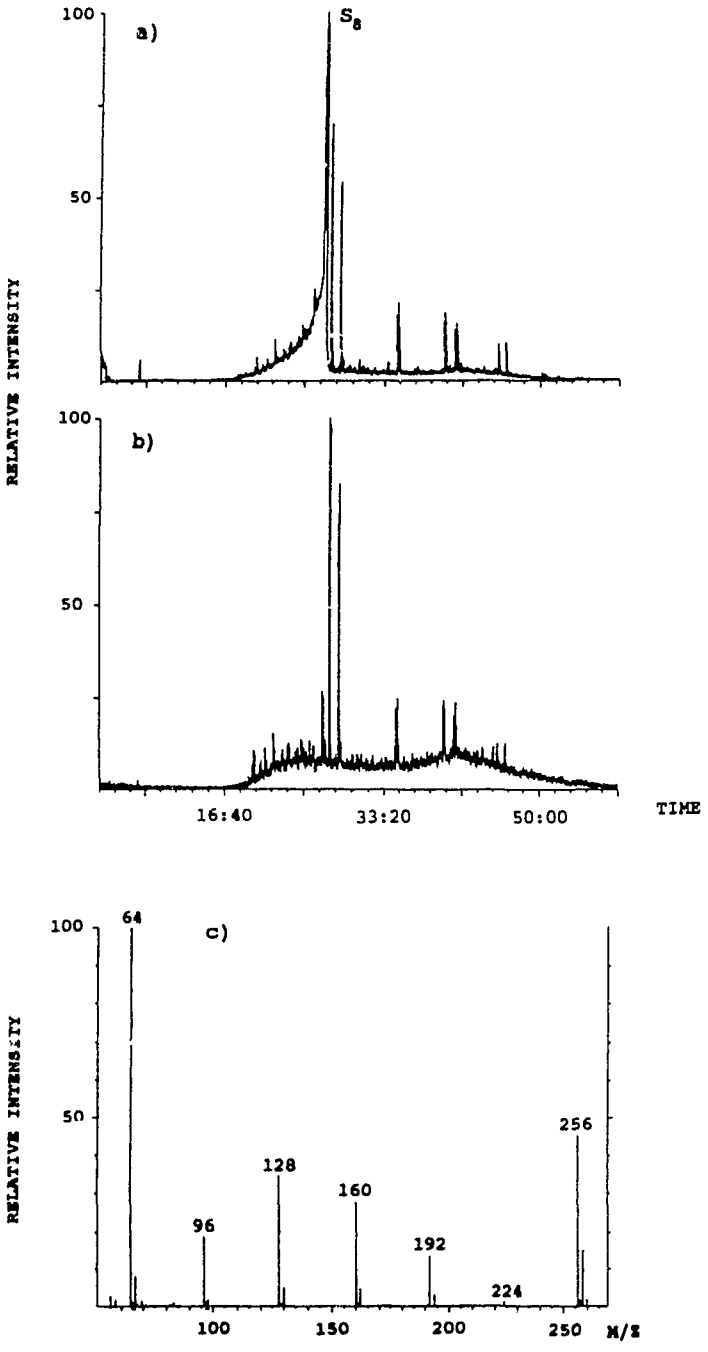


Figure 5.1.1: Effect of elemental sulfur removal on the aspect of GC/MS TICs obtained for the TPE sample; a) TIC without removal of S_8, b) after removal, c) mass spectrum of S_8 obtained from the top trace.

of the sample clean-up. According to the procedure presented in Figure 1.5.2 and as cited elsewhere (89), sulfur should be ubiquitously present in those samples.

6. RESULTS AND DISCUSSION: FRACTIONATION TECHNIQUES

6.1 Fractionation of the tar pond extract

6.1.1. Ramos and Prohaska method

A comparison was made between the two step fractionation method proposed by Ramos and Prohaska (9) and the alumina fractionation method which will be discussed in Section 6.1.2 (3). This comparison was based first on the extent of elimination of aliphatic substances from the samples, second on the amount of polar PACs (PAQs, PAKs, PANHs) eluted in the final PAC neutral fraction, and finally on the sample recovery levels.

Table 6.1.1 summarizes the relative weights of the fractions at both steps of the chromatography and Table 6.1.2 provides intensity ratios of polar PACs over parent PAHs obtained by GC/MS. Results of the latter table will be discussed together with those of Table 6.1.4 in Section 6.1.2.

Table 6.1.1: Weights of the material obtained in different fractions using the fractionation method of Ramos and Prohaska

column	fraction	weight (g)	% of total weight	% recovery
silica	aliphatics PACs	0.01555 0.17756	7.6 86.7	94.3
Sephadex LH-20	aliphatics PACs	0.01313 0.15962	7.4 89.9	97.3

weight of starting material: 0.20475 g

weight of material recovered: 0.18830 g

overall recovery: 91.9 %

Table 6.1.2: PAC/PAH ratios of the GC/MS peak areas obtained for the PAC fraction eluted using the method of Ramos and Prohaska

compound(s)	molecular weight	total area of GC/MS peaks	ratio PAC/PAH
fluorene (PAH)	166	1785	1
fluorenone (PAK)	180	1791	1.003
anthraquinone (PAQ)	208	7144	4.002
carbazole (PANH)	167	1616	0.916
benzofluorenes(PAHs)	216	13599	1
benzonaphtho furans (PAOHs)	218	19525	1.436
benzofluore-nones (PAKs)	230	19695	1.448
benzocarba-zoles (PANHs)	217	5113	0.376

Figure 6.1.1 shows a GC/MS TIC trace obtained for the PAC fraction obtained using the method of Ramos and Prohaska. The flat baseline indicates that most aliphatic compounds were removed. Aliphatics usually produce a very broad envelope ("hump") in the base line of GC (MS or FID) chromatograms.

The first step of this method, which consists of open column chromatography on silica gel, eliminated 7.6% of aliphatic material in weight from the original sample. The cut off point between aliphatics and aromatics was very difficult to determine due to the fast elution of the compounds with the strong solvent (20% dichloromethane in diethylether). A UV lamp was used to monitor the elution of the aromatics, which were collected as soon as fluorescence started to appear at the exit

of the column. A GC/MS analysis of the PAC fraction (collected with 40% dichloromethane in ether) revealed the presence of aliphatic material from observation of the characteristic envelope in the baseline of the TIC trace (not shown). The mass spectra obtained from this experiment also showed peaks characteristic of aliphatics. PACs were collected all together, with no discrimination for polar vs neutral material, and counted for 86.7% (in weight) of the original sample.

The second step of this fractionation method eliminated most of the residual aliphatic compounds from the PAC fraction. The aliphatics constituted 7.4% of the PAC fraction obtained in the first step. A GC/MS analysis of the PAC fraction (Figure 6.1.1) showed the presence of almost no aliphatics (flat baseline and pure PAC-like mass spectra). Open column chromatography on Sephadex LH-20 thus allowed completion of the aliphatic-aromatic separation procedure initiated using silica gel. The solvents used with the latter adsorbent are too strong to allow good efficiency in the chromatographic separation of the two groups of compounds.

However, the overall method yielded the isolation of a fraction which contains PACs of all varieties, from neutral to polar. Such a fraction is the type of sample required to obtain a qualitative profile of the overall PAC contents of the tar pond extract (TPE).

6.1.2 Alumina fractionation method

This fractionation method, described in Section 2.4.1.2 of this work, has been developed (3,43) as a one step way of separating aliphatics, neutral PACs (PAHs), substituted PAHs and polar compounds. The efficiency of separation and recovery levels of this method have been compared to the values obtained using the method described above in Section 6.1.1.

Table 6.1.3 summarizes the relative weights of the fractions eluted from the alumina column.

Table 6.1.3: Weights of the material obtained in different fractions using the alumina fractionation method

fraction	weight recovered (g)	% recovery
aliphatics	0.01040	6.1
PACs	0.14024	83.3
polars	0.01066	6.3

weight of starting material: 0.16983 g
weight of material recovered: 0.16130 g
% recovery: 95.7%

Hexane, as the first solvent passed through the column, eliminated only 6.1% of the total weight of the original sample in aliphatics, as compared to a total of 15% with the method of Ramos and Prohaska. Polar material constituted 6.3% of the sample. A GC/MS analysis of the combined PAH and substituted PAH fractions showed the presence of aliphatics (Figure 6.1.2). The polar fraction collected with methanol and methanol-water was not analyzed by GC/MS, due to the non ideal behavior of polar compounds in GC.

Table 6.1.4 gives the same intensity ratios as listed in Table 6.1.2 obtained from a GC/MS analysis of the PAC fraction.

In general, the [substituted PAH/PAH] abundance ratios are larger for the PAC fraction obtained using the Ramos and Prohaska (R&P) method than those obtained with the alumina fractionation scheme. The substituted compounds listed in Tables 6.1.2 and 6.1.4 are all more polar than their PAH homologs (fluorene, M.W. 166 and benzofluorenes, M.W. 216). With the R&P method, these moderately

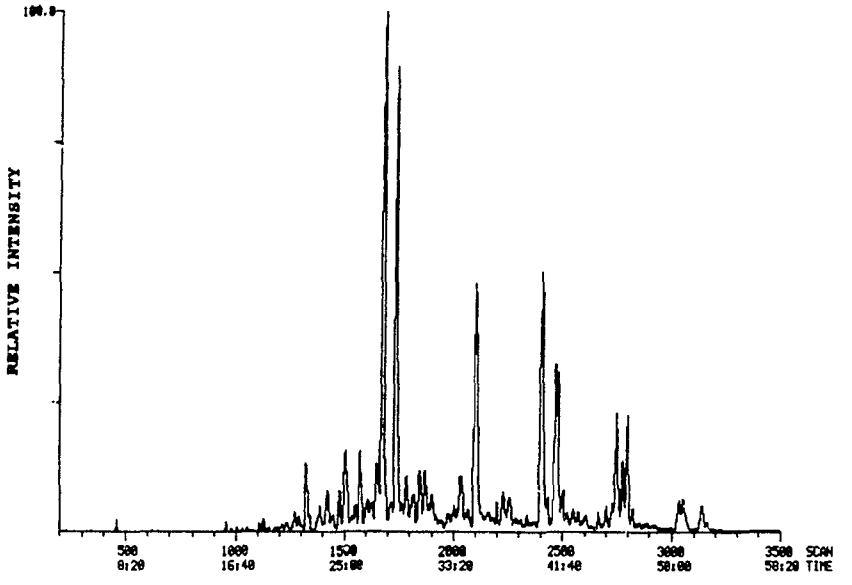


Figure 6.1.1: GC/MS TIC trace of the TPE PAC fraction obtained using the Ramos and Prohaska fractionation method (8)

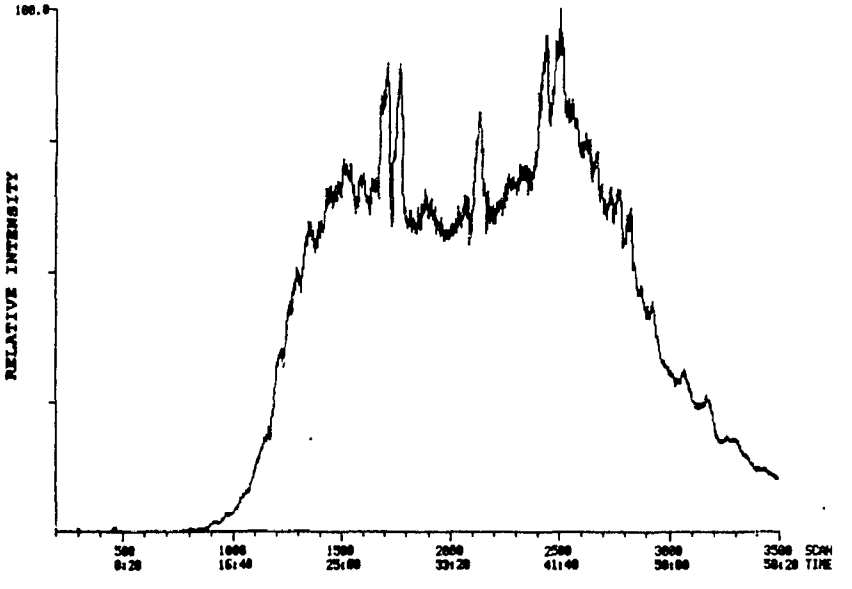


Figure 6.1.2: GC/MS trace of the TPE PAC fraction obtained using the alumina fractionation method (11)

Table 6.1.4: PAC/PAH ratios of the GC/MS peak areas obtained for the PAC fraction eluted using the alumina fractionation method

compound(s)	molecular weight	total area of GC/MS peaks	ratio PAC/PAH
fluorene (PAH)	166	1006	1
fluorenone (PAK)	180	326	0.324
anthraquinone (PAQ)	208	3371	3.350
carbazole (PANH)	167	796	0.791
benzofluorenes (PAHs)	216	7680	1
benzonaphtho furans (PAOHs)	218	6945	0.904
benzofluore-nones (PAKs)	230	3312	0.431
benzocarba-zoles (PANHs)	217	898	0.116

polar PACs are expected to elute with PAHs as a mixture, and with the alumina scheme they should elute in the substituted PAH fraction which was combined here with the PAH fraction to yield a single PAC fraction. Therefore, PAC fractions obtained from both methods should ideally have equivalent contents. However, smaller ratios obtained with the alumina method show that only a portion of the substituted PAHs were eluted with chloroform, while the other portion stayed on the column and was later eluted in the "very polar" fraction with methanol and methanol-water. This partition of compounds between the substituted PAH and the polar fractions has two major disadvantages:

- the substituted PAHs contained in the polar fraction cannot be analyzed by GC/MS because of the presence of other very polar compounds that are not suitable for GC analysis,
- the PAC fraction obtained by combining PAHs and substituted PAHs is incomplete and could not be used for quantitative analysis.

The R&P fractionation method is less time and solvent consuming than the alumina method, even if the former involves two steps of open column chromatography (silica and Sephadex). The volumes of solvent required in the latter are large and thus elution is performed slowly. Large solvent volumes also involve more working time to evaporate the solvents in order to get proper concentration for the final analyses.

The small volumes used in the R&P method make it more adaptable to routine analysis. This method was thus considered as better than alumina column chromatography for rapid preparation of samples containing a broad range of various PACs.

6.1.3 Silica over alumina fractionation method

Although Giger and Blumer (11) had reported an alumina over silica fractionation scheme as part of their complete clean-up scheme, the method discussed in this section and developed by Hirsh et al. (68) uses the same two adsorbents but in reverse packing order. This method has been developed to isolate PACs from crude oil extracts. According to the authors (68), the elution pattern, which features a slow increase in solvent strength, should allow fractionation of organic extracts into aliphatics, monocyclic aromatics, dicyclic aromatics, polycyclic aromatics and finally polar compounds.

Hirsh et al.'s method (68) was applied here to the TPE in order to test its efficiency to fractionate contaminated marine sediment samples as compared to oil sands and crude oils. Characterization of the fractions obtained was achieved by first spiking the moving belt with a few microliters of each fraction, and examining the

mass spectra obtained for each spike. Fractions were then run in reversed phase HPLC-UV (254 nm) and -MS (belt interface, EI).

Table 6.1.5 lists the recoveries obtained by the silica over alumina fractionation method for the TPE.

Table 6.1.5: Material recovered from the fractionation of the TPE with the silica over alumina method

fraction no.	eluent	weight (g)	% original weight
1	pentane	0.00112	3.2
2	5% benzene in pentane	0.00134	3.9
3	15% benzene in pentane	0.00897	26.1
4	3:1:1 MeOH-ether-benzene	0.01997	58.1
5	methanol	0.00125	3.6

weight of the original sample: 0.03437 g
weight the material recovered: 0.03265 g
overall recovery: 94.9%

The elution was monitored with a UV lamp throughout the process, and fluorescence started to appear in Fraction 4. Thus Fractions 1, 2 and 3 were considered to contain only aliphatics. Fraction 4, which should contain most of the aromatic material, was very fluorescent under the lamp and eluted as a very sharp, well-defined brownish band on the column.

The EI mass spectra obtained by spiking the belt with each of the fractions are shown in Figure 6.1.3. There seems to be only one compound present in the first fraction (pentane eluent); the molecular ion of this chlorinated compound undergoes a loss of 63, analogous to the characteristic loss of COCl from dioxins (167). This compound contains an odd number of nitrogen atoms according to its odd mass, and has not been further characterized for the purposes of the present work. Aliphatics were found in Fraction 2 (5% benzene in pentane), as well as the same compound found in Fraction 1. A mixture of both aliphatics and aromatics was found in Fraction 3 (15% benzene in pentane). Compounds such as phenanthrene and anthracene (M.W. 178) and pyrene and fluoranthene (M.W. 202) were unexpectedly eluted in this fraction, which are tri- and tetracyclic aromatic compounds.

Most of the polycyclic aromatic content was collected with the 3:1:1 solvent mixture in Fraction 4. The mass spectra shown in Figures 6.1.3d and e exhibit ions that correspond to well known PAHs, with molecular weights up to 550.

Figure 6.1.3e shows the mass spectrum obtained for Fraction 5, which was obtained with methanol. It contains a series of peaks that correspond to characteristic PAHs and PANHs as the predominant species, mixed with lower intensity peaks which are most probably produced by more polar compounds. PAHs should not be present in this fraction, and are probably residual compounds left behind from Fraction 4 as a result of column overloading. The PANH species found in Fraction 5 are fairly basic pyridine-based compounds and are found in Fraction 4 as well as a result of partition between the two fractions.

One advantage of using this fractionation method with the TPE is the apparent complete absence of aliphatics in Fraction 4, at the expense of losing some aromatics in Fraction 3. The major disadvantage is that the PANHs eluted in both Fractions 4 and 5, and that some PAHs were still eluted with methanol in Fraction 5.

Fractions 3 and 4 were then characterized by reversed phase HPLC using UV and mass spectrometric detection. Figure 6.1.4 shows the HPLC UV and some reconstructed ion chromatographic (RIC) traces obtained for Fraction 3 and of the

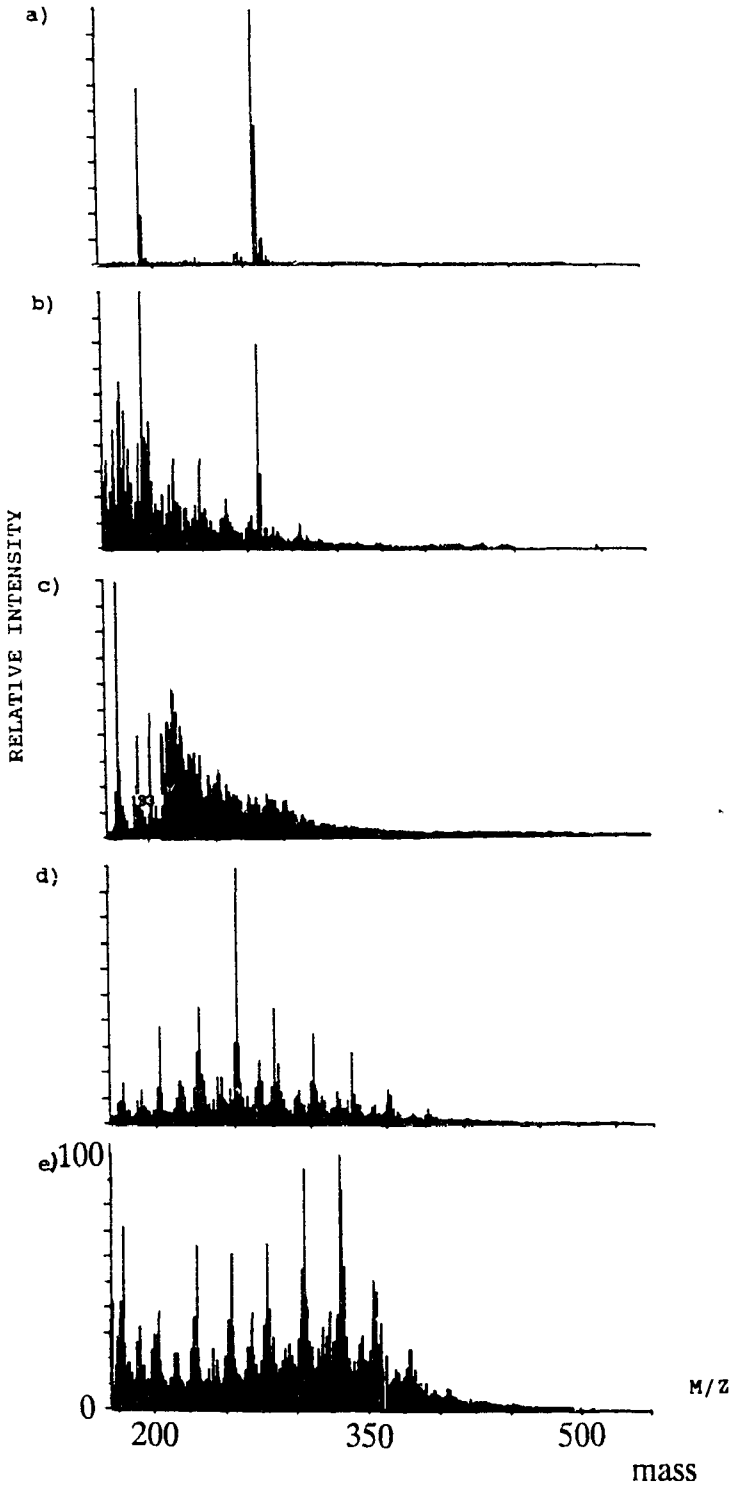


Figure 6.1.3: Mass spectra of the TPE fractions obtained using the silica over alumina fractionation method (11); a) pentane, b) 5% benzene in pentane, c) 15% benzene in pentane, d) 3:1:1 methanol-ether-benzene and e) methanol.

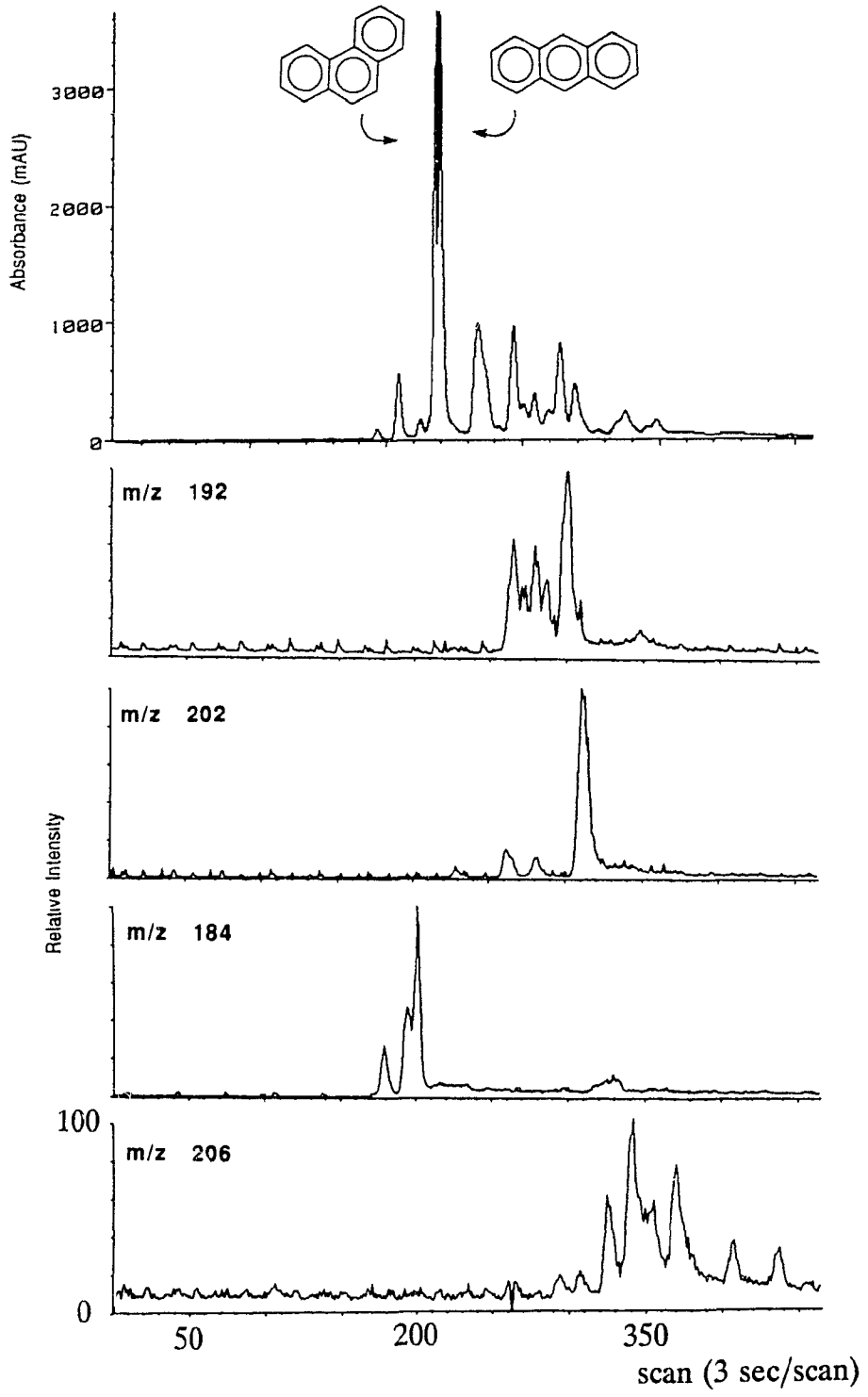


Figure 6.1.4: Reversed phase HPLC-UV absorption chromatogram (254 nm) and RICs of the 15% benzene in pentane TPE fraction obtained using the silica over alumina method

selected ion chromatograms of typical components of the mixture. Table 6.1.6 summarizes the tentative assignment of these compounds.

Table 6.1.6: Aromatic compounds found in Fraction 3 and tentative identification by groups of isomers

M.W.	compounds
178	phenanthrene-anthracene
180	methyl-fluorenes
181	methyl-carbazoles
184	dibenzothiophenes
190	4H-cyclopenta[def]phenanthrene
192	methyl-phenanthrenes/anthracenes
194	dimethyl or ethyl-fluorenes
198	methyl-dibenzothiophenes
202	fluoranthene-pyrene
203	azafluoranthenes
204	benzoacenaphthene
206	dimethyl or ethyl phenanthrenes/anthracenes
208	anthraquinone
212	dimethyl or ethyl-dibenzothiophenes

The tricyclic aromatic compounds listed in Table 6.1.6 represent four classes of PACs: PAHs, PAQs, PASHs and PANHs. The two latter classes include polar

compounds, it is thus unexpected that compounds such as anthraquinone and carbazole should be found in the diaromatic PAH fraction.

The HPLC-UV and -MS results obtained for Fraction 4 are not shown here, since they are qualitatively very similar to those obtained with the HPLC analysis for the R&P PAC fraction, which will be presented in Section 7.2.1.

The fractionation method described here is useful for the separation of aliphatics from aromatics in the TPE, except that the cut point is a whole fraction (Fraction 3) where both groups of compounds are found. In general, the same elution order was found (aliphatics-PAHs-polars), but there was a high extent of overlap between the PAH and polar fractions (3 and 4, respectively), and some polar compounds eluted as early as in Fraction 3.

This technique had been developed for the fractionation of crude oils and not estuary sediment samples. Since oils contain more aliphatic and alkylated aromatic material than samples such as the TPE, the unsatisfactory fractionation obtained here could be explained by the different matrix effects in the TPE yielding different retention behavior for the compounds on the silica over alumina column.

6.1.4 Copper-coated silica

This ligand exchange chromatographic experiment was an attempt to separate PASHs from other PACs in a TPE by coordinating them with copper on the adsorbent (69,70). As mentioned in Section 2.4.1.4, collection of eleven 10 mL fractions eluted with 1:1 chloroform-hexane was performed. A twelveth fraction was collected by passing a mixture of diethylamine and chloroform through the column. Each fraction was analyzed by GC/MS on the Finnigan 4500 GC/MS system. The original sample, a dark brown solution, was separated into two diffuse colored bands. The GC/MS analyses show that although some of the fractions were colorless, they still contained PACs and that the overall retention scheme was peculiar to the copper coated silica adsorbent.

Figure 6.1.5 shows two GC/MS TIC traces, obtained for Fractions 2 (top) and

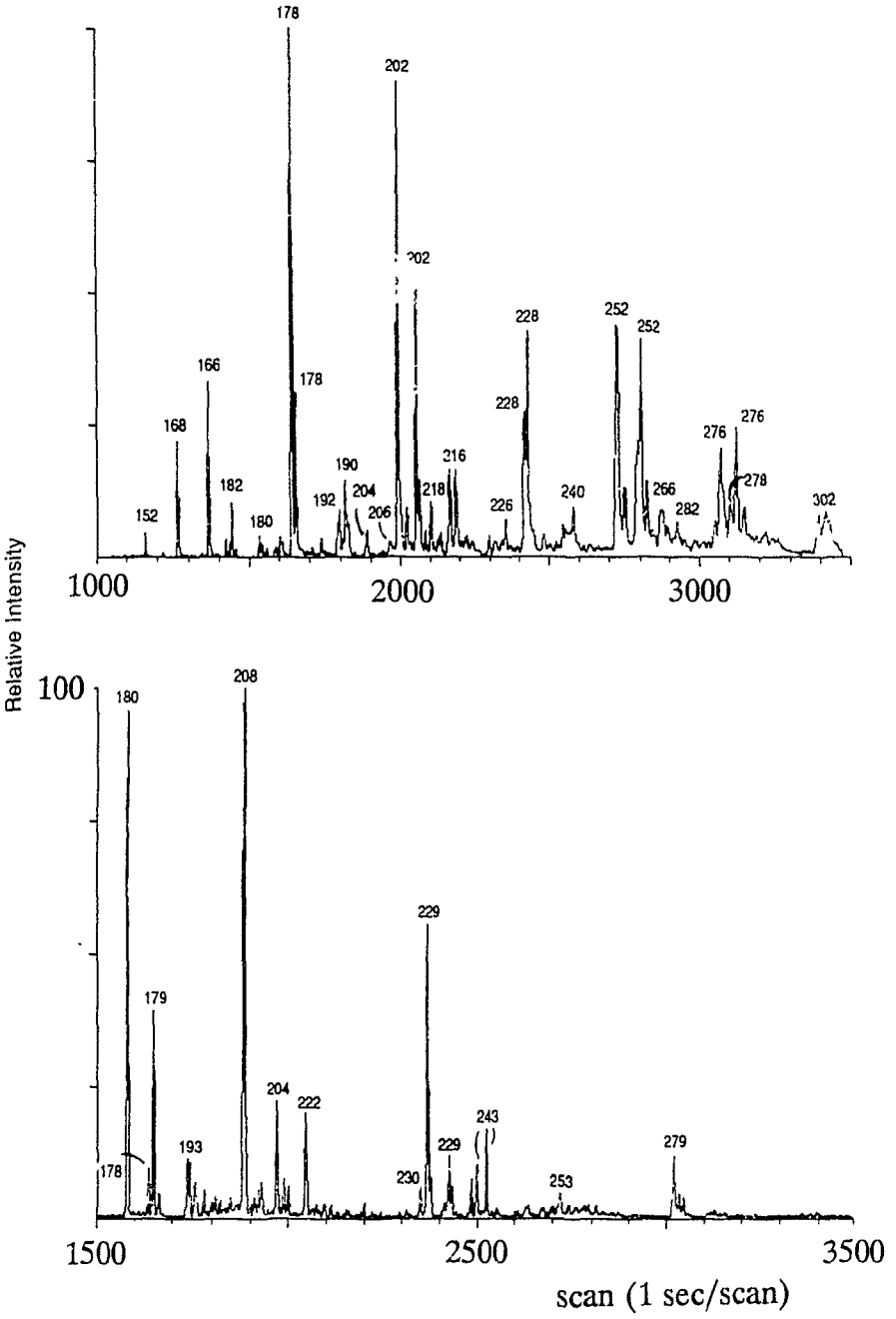


Figure 6.1.5: GC/MS TIC chromatograms of TPE Fractions 2 (top) and 9 (bottom) obtained using the copper-coated silica fractionation method

9 (bottom) respectively, with masses of compounds labelling the major peaks. Fractions 2 and 9 correspond to the darkest part of the elution of the two colored diffuse bands. The chromatograms of Figure 6.1.5 show that a very distinct separation between PAHs (Fraction 2) and PANH, PAKs and PAQs (Fraction 9) occurred. Between those two most concentrated fractions, other types of PACs were eluted and revealed by GC/MS. To simplify the presentation of the GC/MS results, Table 6.1.7 gives an overview of what was in each of the 11 fractions collected with chloroform-cyclohexane.

The concentrate of Fraction 12 (collected with diethylamine-chloroform) was also run on the GC/MS instrument. Before, a blank of concentrated diethylamine solvent was diluted in dichloromethane and run on the GC/MS. The resulting chromatogram showed intense peaks due to contaminants contained in diethylamine and thus Fraction 12 was highly contaminated as well. Theoretically, Fraction 12 should contain all material bound to copper on the stationary phase. But a background subtraction of the chromatogram obtained for the solvent from that of Fraction 12 revealed no peaks of interest.

In Figure 6.1.6, selected ion chromatograms corresponding to PASHs are shown. Most PASHs were eluted at the very beginning, in Fractions 1 and 2. The ligand exchange chromatographic process thus failed in separating PASHs from other PACs. This failure could be attributed to the lack of coordination sites on copper atoms due to the presence of bonded water molecules, or to excessive solvent strength. Snyder (168) reported that ligand exchange separations should occur only if the metal loaded adsorbent was stable physically and chemically and kept in strictly anhydrous conditions. Both components of the adsorbent prepared here (silica gel and copper sulfate) are easily hydrated, if not kept away from any source of moisture. In the procedure used to prepare copper-coated silica, rinsing with organic solvent as well as heating were used to remove the moisture, but removal might have been incomplete. On the other hand, the adsorbent might have absorbed moisture when handled while packing the column.

The idea of using copper sulfate instead of palladium chloride (69,70)

Table 6.1.7: PAC contents of the 11 fraction collected with cyclohexane-chloroform from the copper-coated silica column. Numbers in bold refer to the five most intense GC/MS peaks of each run.

fraction no.	M.W. of compounds producing major peaks in the TIC	possible 5 major compounds (and others)
1	154,166,168,180,196,**178**, 222,**190**,204,**202**,216,226, **228**,242,240,**252**,278,276, 302.	178-PAH, 190-PAH, 202-PAH, 228-PAH, 252-PAH, (PAHs).
2	152,168,**166**,182,181,**178**, 192,190,204,206,**202**,218, 216,226,**228**,242,240,**252**, 266,276,278,302.	166-PAH, 178-PAH, 202-PAH, 228-PAH, 252-PAH, (PAHs).
3	166,168,208,**202**,247,243, **242**,252,**253**,**280**,**278**,293, 303	202-PAH, 228-PAH-C1, 252-PANH, 266-PAH-C1, 278-PAH, (PAHs).
4	**153**,167,**228**,178,208,167, 223,209,222,**203**,217,**227**, 228,270,252,280,278,**303**.	152-PANH, HXPAH, 202-PANH, HXPAH, 302-PANH,(PANHS, HXPAHS).
5	**153**,**167**,181,193,194,**228**, 191,**203**,217,231,230,227, 228,244,254,253,269,267, 277,283,303.	152-PANH, 166-PANH, HXPAH, 202-PANH, 216-PAK, (PANHs, HXPAHs, PAKS).
6	**153**,167,194,**228**,191,**203**, 217,**230**,**227**,244,254,253, 267,303,326,342,353.	152-PANH, HXPAH, 202-PANH, 216-PAK, HXPAH, (PANHs, HXPAHs, PAKs).
7	153,167,179,180,194,**208**, 204,**203**,217,**229**,**230**,**228**, 227,244,243,241,257,254, 253,279,303.	166-PAQ, 202-PANH, 228-PANH, 216-PAK, HXPAH, (PANHs, PAKs, HXPAHs, PAQs).
8	**180**,**179**,193,**208**,204,222, **229**,**243**,302,326,253,316, 279,328.	166-PAK, 178-PANH, 166-PAQ, 228-PANH, 228-PANH-C1, (PAHs, PANHs, PAQs, PAKs).
9	**180**,178,**179**,193,**208**,**204**, 222,230,**229**,243,253,279, 303.	166-PAK, 178-PANH, 166-PAQ, 190-PAK, 228-PANH, (PAKs, PAQs, PANHs).
10	**180**,**179**,**208**,**204**,222,**229**, 243.	166-PAK, 178-PANH, 166-PAQ, 190-PAK, 228-PANH, (PAQ, PANH).
11	**180**,**179**,**208**,**204**,222,229, 243,**277**,279.	166-PAK, 178-PANH, 166-PAQ, 190-PAK, 276-PANH, (PAQ, PANHS).

HX-PAH: hydroxy-PAH C1: methyl, C2: dimethyl or ethyl, etc.

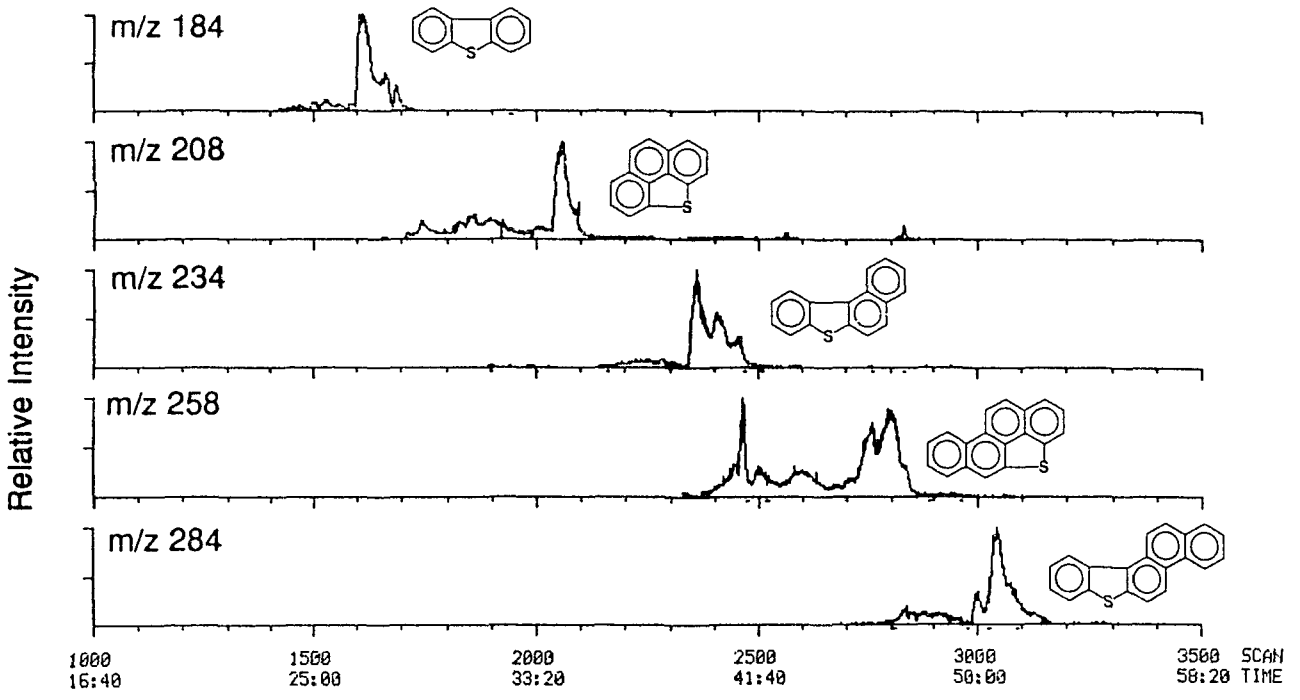


Figure 6.1.6: GC/MS RICs of PASHs found in TPE Fraction 1 of the copper-coated silica method

adsorbed at the surface of silica particles was taken from the work of Vogh and Dooley (72). These authors used a resin (Bio Rex 70) coated with $CuSO_4$ as the stationary phase, and n-pentane as the mobile phase to remove non-coordinated compounds. The strongly retained sulfides were then backflushed with a pentane-diethylether mixture. The solvent used here (1:1 cyclohexane-chloroform) was the same mobile phase used by Nishioka et al. on $PdCl_2$-coated silica (69,70), and is a much stronger solvent than pentane for PACs. The fact that in this experiment PASHs were eluted in the early fractions indicates that cyclohexane-chloroform was too strong to leave PASHs bonded on copper, and that the copper atoms were possibly already coordinated with water molecules.

However, this chromatographic method provided a good means of separating PACs into several classes, among which PAH+PASH, PANH, HXPAH, PAK, PAQ compounds were found.

6.1.5 Second scheme for the separation of PASHs from PACs

The detailed characterization of PASHs in environmental polluted samples is important because the human health effects of these compounds are a direct result of the specific structures of the individual components in the mixtures. Different isomers exhibit varying degrees of carcinogenic activity. For example, benzo[b]naphtho[1,2-d]thiophenes are not carcinogenic, benzo[b]naphtho[2,3-d] and [3,2-d] are moderately carcinogenic and benzo[b]phenanthro[3,4-d]thiophene is a very potent carcinogen (169).

Characterization of PASHs among other PACs in mixtures is possible using GC coupled to flame photometric detection (FPD) (123) or to mass spectrometry. FPD is extremely useful for screening sulfur containing compounds at a specific detection wavelength. However, non-linearity and quenching of the detector response becomes a serious problem when PASHs are present as trace components. PASHs appear as background peaks in a GC/MS TIC and coelute with major components of the sample. The separation of the sulfur heterocycle fraction from other PACs is

therefore the first step that should be accomplished if detailed structural information on the organosulfur compounds is desired. The isolation of this fraction is not an easy task because of the similarities in chemical properties between these compounds and other PACs. In conventional fractionation methods, PASHs and PANHs have always been isolated in the same fraction (123).

Figure 6.1.7 shows the GC/MS TIC trace (equivalent to a FID trace) obtained for the TPE sample. This trace contains information about all compounds eluted and detected by the mass spectrometer. PASHs corresponding to the labelled peaks have been tentatively identified using their retention indices (2,170) and are obviously only a few components of the mixture.

The method used to isolate PASHs from other matrix constituents was described in Section 2.4.1.5 and is briefly outlined here (78):

- Silicic acid column chromatography. All PACs were eluted through a silicic acid column, which retains lipids, pesticides and other very polar constituents of the environmental extract.
- Oxidation of PASHs into corresponding sulfones. This process also converts PAHs into quinones.
- Silica gel chromatography. Separation of the polar sulfones and quinones from unoxidized, moderately polar PACs.
- Reduction of sulfones and quinones to PASHs and hydroquinones, respectively.
- Silica gel chromatography. Separation of hydroquinones from PASHs.

The level of success obtained after each step of this method was evaluated using GC/MS or normal phase HPLC/MS. Figure 6.1.8 shows some normal phase HPLC/MS selected ion chromatograms obtained for the mixture after oxidation. Sulfones at m/z 166, 216 and 266 were monitored and correspond to the traces in the figure. Normal phase HPLC was chosen as the best analytical method at this stage, because sulfones are too polar and not volatile enough to be analyzed by GC. Moreover, they have a certain level of affinity for the normal phase column packing (sulfones are not usually retained on reversed phase HPLC columns). Normal phase HPLC does not provide very good selectivity and thus no resolution between the

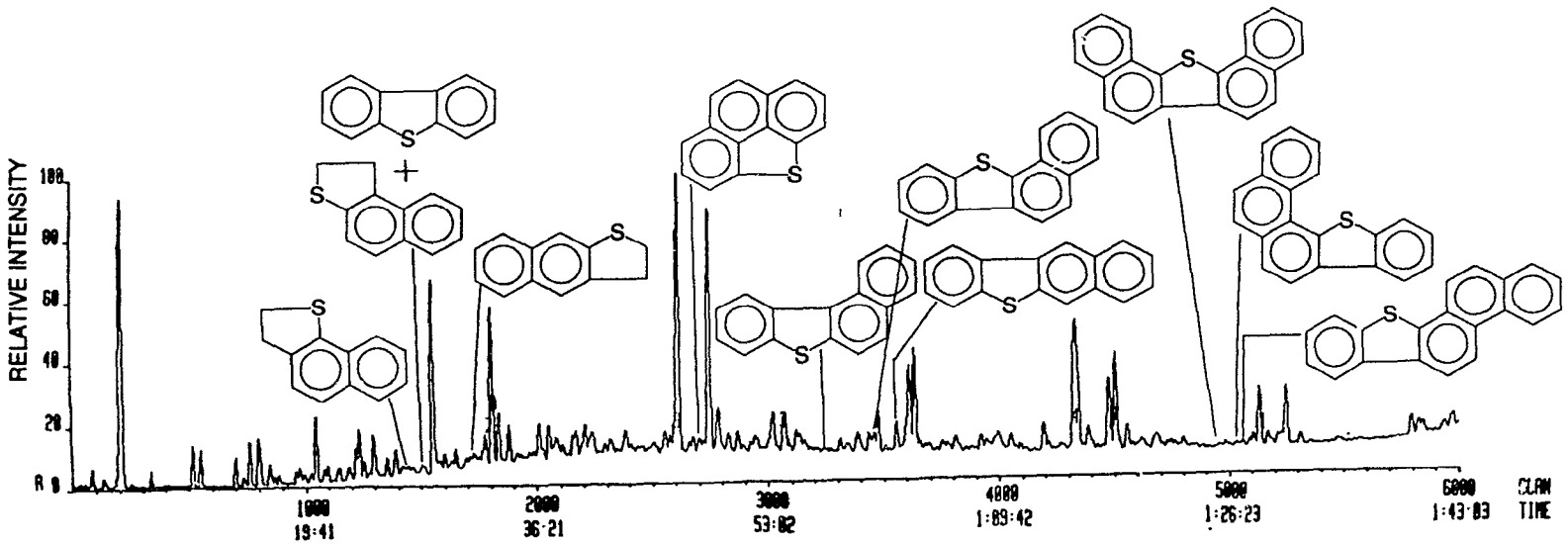


Figure 6.1.7: GC/MS TIC chromatogram of the TPE obtained before performing the PASH isolation method

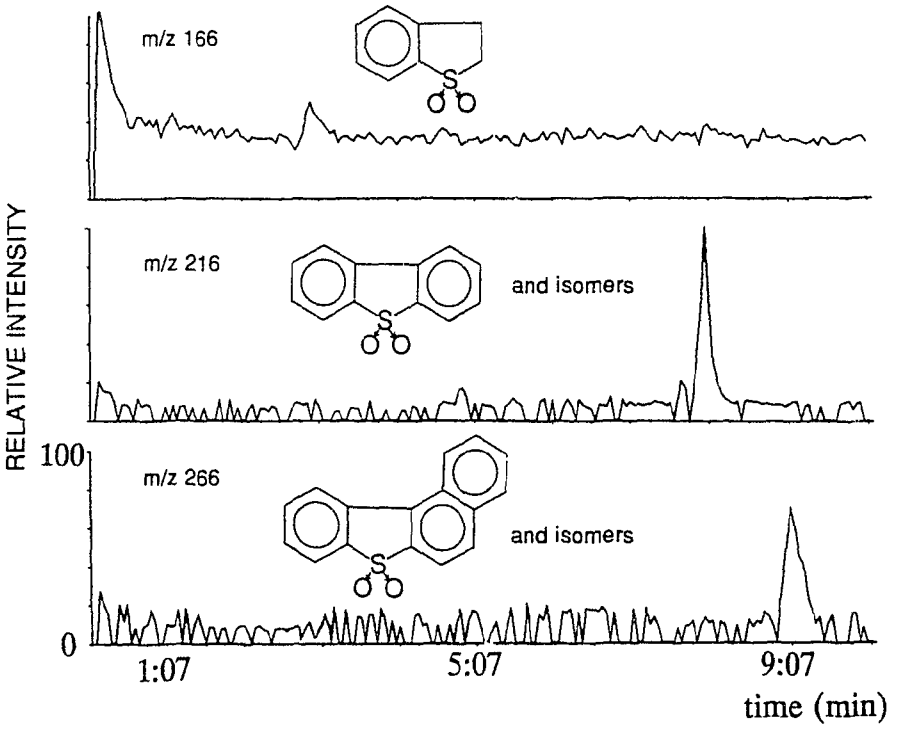


Figure 6.1.8: Normal phase HPLC/MS RICs of the TPE sulfone fraction obtained while performing the PASH isolation method

sulfone isomers. Each peak probably corresponds to a number of coeluting isomers.

Table 6.1.8: PASHs found in the TPE

peak no.	compound	peak no.	compound
1	naphthalene (IS)	20	9-me naphtho[2,1-b]thiophene
2	benzo[b]thiophene	21	tetrahydro dibenzothiophene
3	fluorene (IS)	22	1-me benzo[b]naphtho[1,2-d]thiophene
4	naphtho[1,2-b]thiophene	23	benzo[b]naphtho[2,1-d]thiophene
5	dibenzothiophene	23a	benzo[b]naph.ho[1,2-d]thiophene
6	naphtho[2,1-b]thiophene	24	phenanthro[4,3-b]thiophene
7	phenanthrene (IS)	25	chrysene (IS)
8	naphtho[1,2-b]thiophene anthracene	26	10-me benzo[b]naphtho[2,1-d]thiophene
9	5-me naphtho[2,1-b]thiophene naphtho[2,3-b]thiophene	27	3-me benzo[b]naphtho[2,1-d]thiophene
10	x-me naphtho[1,2-b]thiophene	28	8- or 10-me benzo[b]naphtho[1,2-d]thiophene
11	8-me naphtho[1,2-b]thiophene	29	3-me benzo[b]naphtho[1,2-d]thiophene
12	naphtho[2,3-c]thiophene	30	10-me benzo[b]naphtho[1,2-d]thiophene
13	4,6-dime dibenzothiophene	31	PASH, m/z 258
14	2-ethyl dibenzothiophene	32	pyreno[4,5-b]thiophene
15	2,8-dime dibenzothiophene	33	benzo[1,2]phenaleno[3,4-bc]thiophene
16	3,8-dime dibenzothiophene	34	pyreno[4,5-b]thiophene
17	fluoranthene (IS)	35	chryseno[4,5-bcd]thiophene
18	pyrene (IS)	36	3-me phenanthro[2,1-b]thiophene
19	hexahydro dibenzothiophene	37	dinaphtho[1,2-b:1',2'-d]thiophene

GC/MS was used after the final step and a global evaluation of the efficiency of PASH separation was possible by comparing the TIC of Figure 6.1.9 to that of the original mixture (Figure 6.1.7). In Figure 6.1.9, most major peaks correspond to internal standards, spiked in the sample to enable the calculation of the retention

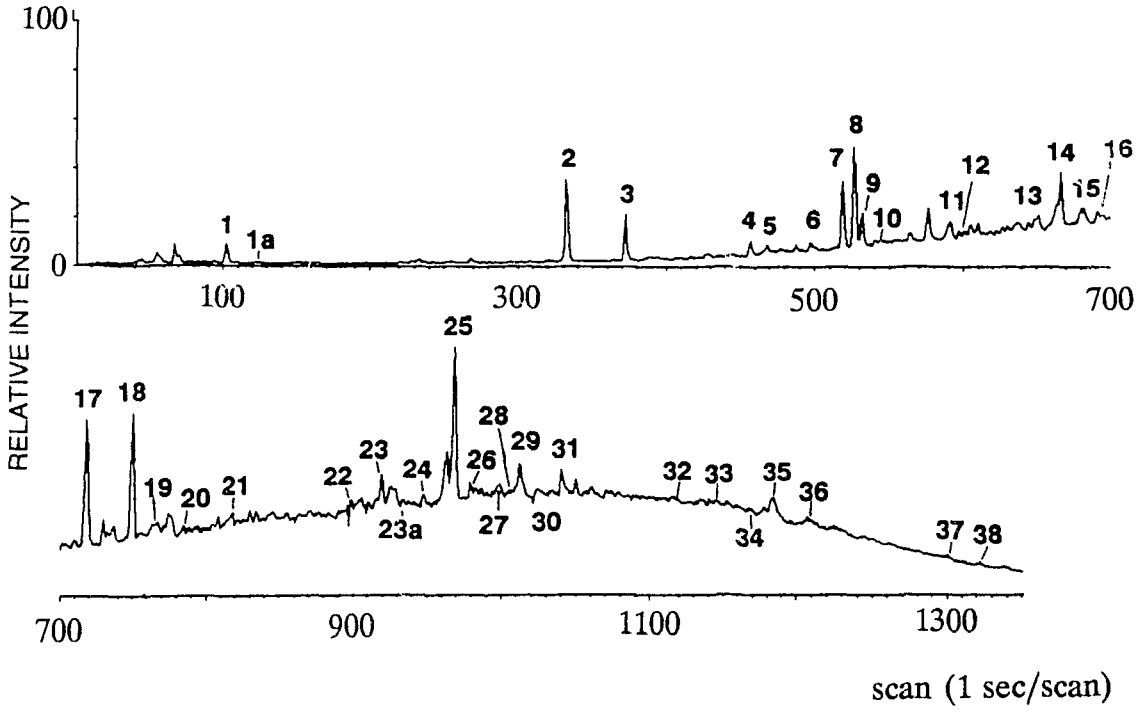


Figure 6.1.9: GC/MS TIC chromatogram of the final TPE PASH fraction obtained using the PASH isolation method. The numbers on the peaks refer to the compounds listed in Table 6.1.9.

indices of unknowns. All PASHs identified by retention index are listed in Table 6.1.8. Internal standards are labelled "IS" on the chromatogram of Figure 6.1.9a. Besides "IS" peaks, PASH peaks dominate, showing that PASHs were effectively isolated from the original TPE matrix.

This method involved several steps, each yielding a loss of material. PASH concentration in the final product mixture is thus extremely low. Other reasons for low overall reaction yield are:

- In order to oxidize only the sulfides, an equimolar ratio of oxidizing agent to the sulfur content is required and is difficult to determine in complex mixtures containing relatively low concentration of PASHs
- the reduction of sulfones back to PASHs requires a very strong reducing agent ($LiAlH_4$) which often reduces the unsaturated hydrocarbon portion of the compounds

The method did not yield a pure PASH mixture; it however allowed the simplification of the total ion chromatogram and rendered the identification of PASHs easier.

6.1.6 Scheme for separating PANHs

Some heterocyclic aromatic compounds containing nitrogen atoms (PANHs) have been found to cause health hazards because of their carcinogenicity (162); in fact many of these compounds are found in cigarette smoke condensate (2).

The method proposed and developed by Blumer and co-workers (88) to isolate pyridine-based and pyrrole-based PANHs was applied here with slight modification to the original procedures (See Section 2.4.1.6). The method was applied to a TPE extract with its aliphatic content already removed. This preliminary step was achieved by passing the Soxhlet extract through a Sephadex LH-20 column (9).

The first step of the method, which consisted of passing the aromatic eluate from the Sephadex through an acidic silica gel column, was done with no modification to the method described by Blumer et al. (88). GC/MS analysis of the

3% NH_4OH/methanol basic fraction confirmed the presence of PANHs as the dominant PACs, as revealed by the relative intensities of the GC/MS peaks listed in the third column of Table 6.1.9.

Table 6.1.9: Comparison of the GC/MS peak intensities obtained for the 3% NH_4OH in methanol fraction (acidic silica gel, Step 1) and for the PANH fraction, Step 7.

m/z	compounds (isomers of ...)	total int. of GC peaks (height) STEP 1	total int. of GC peaks (height) STEP 7
128	naphthalene	185	27
129	quinoline	34	257
178	phenanthrene	0	0
179	acridine	126	402
202	fluoranthene	93	75
203	thebenidine	122	469
216	benzofluorene	47	43
217	benzocarbazole	58	153
228	chrysene	84	49
229	benzacridine	261	623
252	benzo[a]pyrene	33	31
253	phenalenoquinoline	124	135
266	dibenzofluorene	0	0
267	dibenzocarbazole	29	76
276	benzo[ghi]perylene	0	0
277	phenanthroquinoline	38	92

After partition between water and benzene and drying of the organic layer over sodium sulfate, the solution was made up to 10 mL in benzene and split into 5 different portions of 2 mL which were run in turns through alumina over silica. Both fractions of MeOH/DCM (eluent) containing azaarenes were thus collected 5 times and later combined. This procedure was modified this way to avoid overloading that

would have occurred with using only one alumina over silica column.

The rest of the method (88) was performed as described in Figure 2.4.2. A GC/MS analysis of the combined MeOH/DCM fractions obtained in Step 4 was performed (results not shown), and showed an almost total elimination of neutral PAHs from the polar fraction containing the PANHs. No analysis was performed after the basic fraction was reacted with picric acid, since the presence of an excess of this acid was suspected and would have damaged the GC column. The presence of picric acid was confirmed during the final step of the method, which consisted of passing the polar fraction through alumina. After the elution, a yellow residue (picric acid) was retained at the top of the alumina column.

The fourth column of Table 6.1.9 presents the peak intensities (PANHs vs PAHs) obtained by GC/MS for the final PANH fraction after Step 7. Figure 6.1.10 shows some selected ion chromatograms obtained from the GC/MS run of the final PANH fraction.

This method allowed the isolation of PANHs with a low yield, since several steps are required which may possibly involve the loss of sample (especially while performing liquid-liquid partition). The data in Table 6.1.9 show that a good proportion of PAHs has been removed from the matrix through the overall process, leaving a clean mixture of PANHs for analysis.

Unlike PASHs, PANHs have chemical properties that are different from those of PAHs. They are more basic and thus their chromatographic behavior, whether using normal or reversed phase columns, is different. These differences offer a real advantage in the chromatographic/mass spectrometric determination of PANHs, viz. PANH peaks found in the reconstructed ion chromatograms (RICs) do not interfere with the first ^{13}C isotope peaks of the molecular ions of their parent PAHs. Separating PANHs from other PACs is not as critical as isolating PASHs for final characterization since PANHs can be easily determined as part of a mixture of PACs. The method described here was thus employed on an explorative basis, to see if it could be used routinely. The abundance of PANHs relative to PAHs was higher at the final step than at Step 1, but the overall concentration of PANHs was much

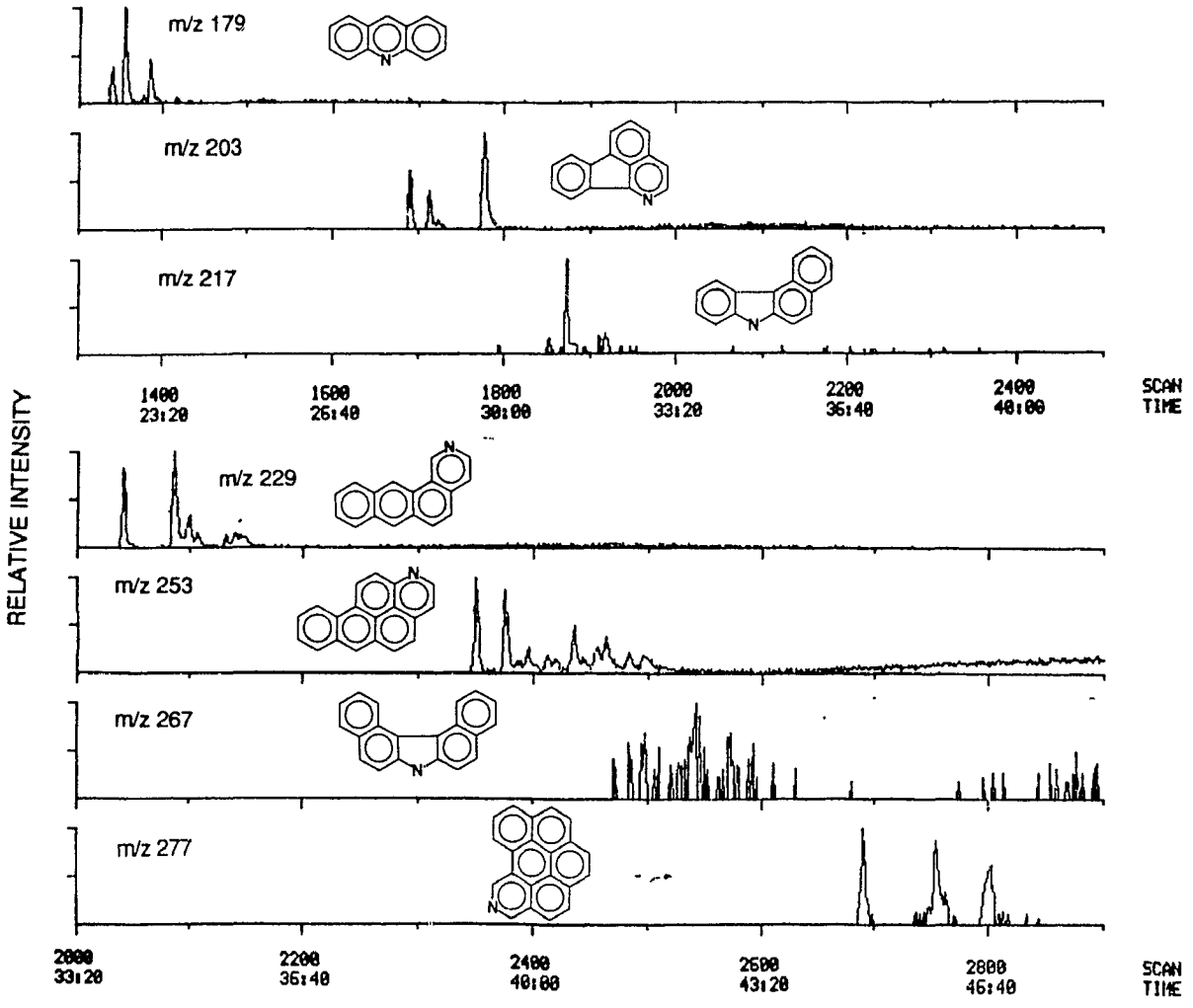


Figure 6.1.10:GM/MS RICs (PANHs) of the final TPE PANH fraction of the isolation method for nitrogen containing aromatic compounds

lower in the final fraction than it was in the original TPE due to the loss of sample throughout the procedure. The removal of PANHs from mixtures using this method was quite successful; the precipitation of these compounds with picrate ions as ammonium salts is very selective and efficient, although picric acid is known to be explosive and needs to be handled with extreme care.

In summary, this method was efficient in separating PANHs, but was not quantitatively useful, because of the high material losses associated with it.

6.2 Fractionation of the Syncrude samples

6.2.1 Anion exchange chromatography

6.2.1.1 Amberlyst-26 anion exchange resin (98)

Conditioning the Amberlyst resin as described in Section 2.4.2.1 did remove a large quantity of impurities. The originally dark-brown beads turned paler after each cleaning step and a yellowish residue was removed every time.

Prior to running the real sample through an Amberlyst column, the cleanliness of the stationary phase was verified. A blank was run under the same conditions as the HGO, and showed many impurities in belt-spike mass spectra (Figure 6.2.1a). The contaminants appeared to be long chain aliphatics (or carbo ylic acids). Figure 6.2.1b shows the mass spectra obtained for the HGO, after passing it through an Amberlyst-26 column. The blank and sample spectra of the first fraction are very similar, which can lead to confusion in the interpretation of the contents of the HGO fraction. The spectra of both other blanks reveal the high concentration of contaminants from the anion exchange resin. It is possible that some acidic compounds from the HGO was retained on the column, but their concentration is lower than that of the contaminants. The use of Amberlyst-26 was thus discontinued, but that of using ion exchange chromatography to remove acids was applied using other column packings (Sections 6.2.1.2 and 6.2.1.3).

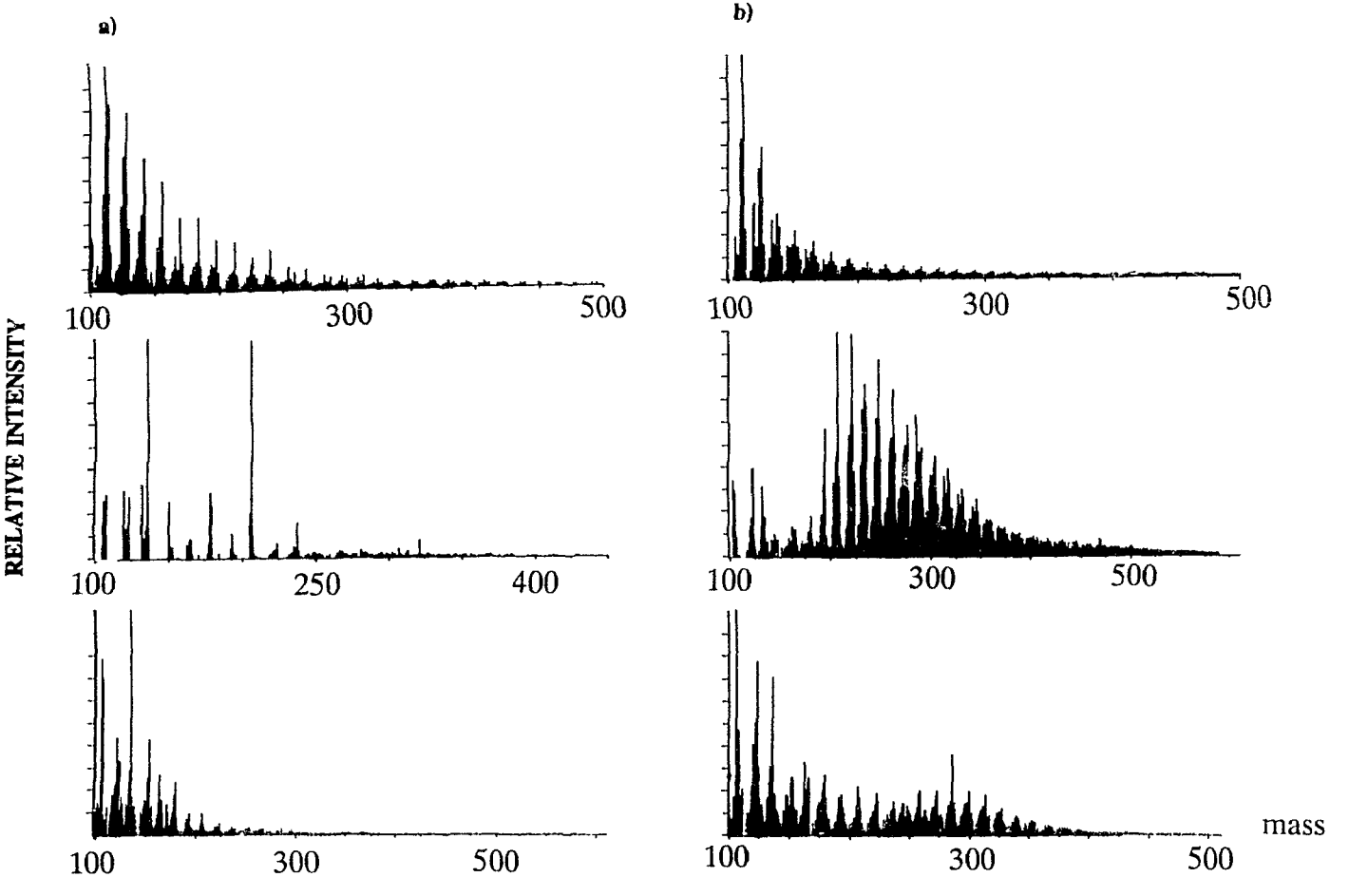


Figure 6.2.1: Mass spectra of the fractions obtained using the Amberlyst-29 anion exchange resin method; a) blanks, b) HGO. From top to bottom: fractions obtained with hexane, isopropanol and 5% formic acid in methanol.

The method originally developed by Jewell et al. (98) was used to remove the polar non-hydrocarbon compounds (acids, bases) from crude oils. The acidic and basic fractions were removed respectively with anion and cation exchange resins. The remaining hydrocarbon and neutral compounds were separated into saturate and aromatic fractions on silica gel. Only the anion exchange method for acid removal was used here, since bases include PANHs which are compounds of interest in this study. Amberlyst-29, used by the authors (98), and Amberlyst-26, used in this work, are both strongly basic macroreticular anion exchange resins. As the former is no longer commercially available, the advice from Aldrich Chemicals, supplier of Rohm and Haas products, was to use the latter resin, whose basicity is equivalent to that of Amberlyst-29 but whose porosity is smaller.

Nothing in Reference 98 was mentioned about the presence of contaminants in the resin, however it was implicit, with the extensive conditioning scheme used by the authors, that the resin was contaminated when received from the manufacturer. Repeating the conditioning cycle once again was seen as a possibility, but was judged to be too time consuming, since the time required for the conditioning was triple that required for the actual experiment.

6.2.1.2 Anion-exchange chromatography on cellulose

Similar contamination problems as observed using Amberlyst-29 occurred with cellulose. Blank samples contained compounds yielding mass spectra (belt spikes, not shown) similar to those obtained for the Amberlyst-blank samples (Figure 6.2.1a). The contaminants in the cellulose were too abundant to consider using this packing as a useful tool for retaining acidic compounds from the Syncrude matrices.

6.2.1.3 Silica coated with potassium hydroxide

The original KOH-treated silica method was developed by McCarthy and Duthie (90) for the rapid elimination of free fatty acids from lipids. These authors

had made unsuccessful attempts to perform this type of separation with ion exchange resins. They found that contamination from the resin ruined the samples and the methods were not reproducible due to the fast degeneration of the resins. They found in KOH-coated silica a reliable and clean stationary phase for acid-neutral separations. In the original method, neutrals were collected after one percolation through the modified silica column and the same procedure was used in this work. However, when Ramljak and co-workers (91) adapted the method to acid removal in asphalts, they used a chromatographic system that allowed recycling of the solvent for successive "extractions" of the neutrals from the column. Their chromatographic system resembled a Soxhlet extractor equipped with column packing instead of a thimble filter. A plot of percentage sample material recovered vs volume of solvent percolated through the columns for standard compounds naphthalene (neutral) and 1-naphthol (acidic) showed that more than one percolation with each solvent was necessary to extract a maximum of material.

In the experiment described here, no such equipment was available, and solvent recycling was simulated by using large volumes of chloroform to elute neutrals and of 20% formic acid in chloroform to elute acids.

The authors of Reference 92 analyzed the acidic fraction by infrared absorption spectrophotometry and came to the conclusion that this fraction contained a large proportion of free carboxylic acids. Thus, according to them, coating silica gel with KOH provided a good anion exchange stationary phase. The hydroxyl ions are very mobile and thus easily exchange with the anions of any carboxylic acids present in the Syncrude samples.

After finding no major impurities eluting in blanks (mass spectra, Figure 6.2.2a-b, two top spectra), this anion exchange method was then investigated for each of the three Syncrude samples. Figure 6.2.2a-b (bottom) shows belt spike mass spectra obtained for each fraction of the elution of the heavy gas oil (HGO) and Figure 6.2.3 shows the reversed phase HPLC/UV 254 nm trace obtained for the neutral fraction. The latter shows material eluting and separating over the first 40 minutes, then eluting as a continuum until the end of the run The mass spectrum of

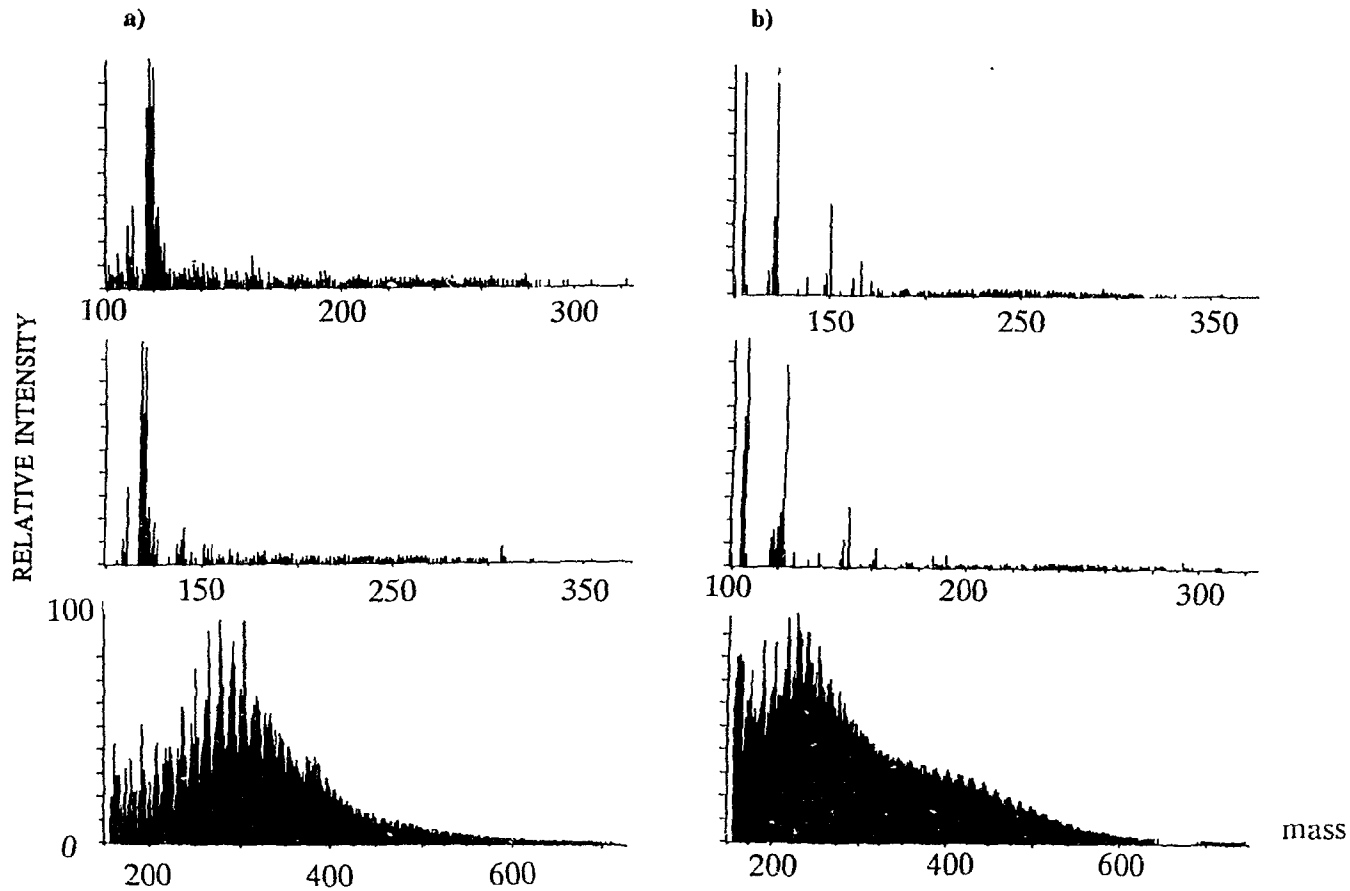


Figure 6.2.2: Mass spectra of the fractions obtained using the KOH-coated silica method. a) chloroform: solvent, blank and HGO (from top to bottom). b) 20% formic acid in chloroform: solvent, blank and HGO.

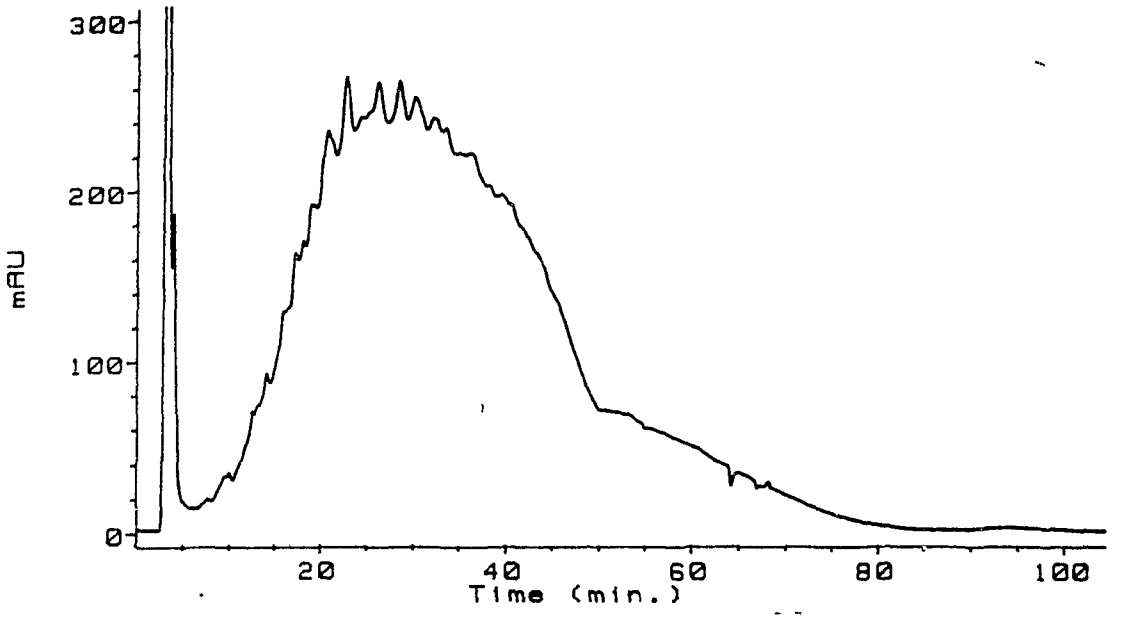


Figure 6.2.3: Reversed phase HPLC-UV absorption chromatogram (254 nm) of the HGO chloroform fraction obtained using the KOH-coated silica method

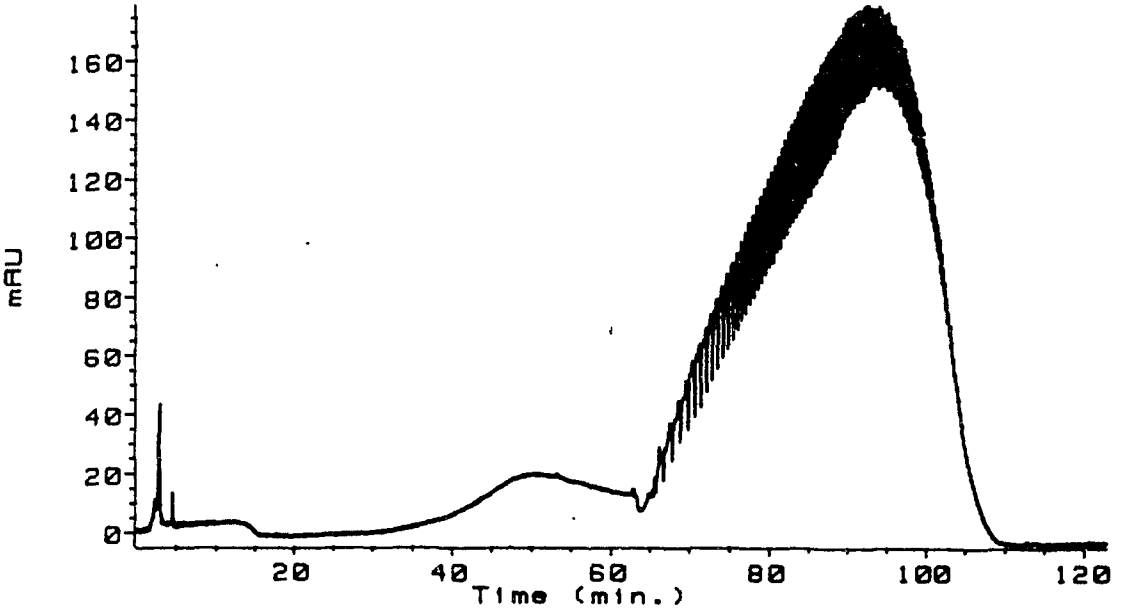


Figure 6.2.4: Reversed phase HPLC-UV absorption chromatogram (254 nm) of the PIT chloroform fraction obtained using the KOH-coated silica method

the 20% formic acid/chloroform fraction shows that, compared to its equivalent obtained for the blank sample, acidic or very polar substances were indeed contained in HGO and might have been interfering with other analytes of interest in the sample. For the purpose of this project, no further analysis was performed on the acidic fraction.

The oil sand extract (OSE) and pitch (PIT) samples were also passed through KOH-coated silica columns. Figure 6.2.5 shows the mass spectra associated with the fractions collected for those samples. Neutral and acidic fractions (eluted with chloroform and 20% formic acid/chloroform, respectively) contain compounds with a wide range of molecular weights (up to ca. 950 Da). Although the four spectra of Figure 6.2.5 have similar features, there was a gap on the columns between the elution of neutral and acidic fractions, however this gap was not as well defined as that observed for the HGO; this narrow gap could not guarantee the absence of overlap between the fractions.

The HGO is a clear, fluid sample, whereas the OSE and PIT are molasses-like dark solids. During elution through the columns, the latter two were very slow moving compared to the HGO, although the same amount of material was chromatographed. This delay in elution might have caused some degree of overlap between neutral and acidic fractions. Compounds in both latter fractions are within the same M.W. range and do have similar structures, as observed upon comparison of the mass spectra of Figure 6.2.5. Those compounds are most probably characterized by the presence of long aliphatic chains. If one was to effect the separation of asphaltenes and maltenes followed by the removal of acids, one would most certainly find acids and neutrals in both asphaltene and maltene groups.

The overall recoveries obtained from KOH-treated silica chromatography are listed in Table 6.2.1.

Good recoveries were obtained for the HGO and OSE; ratios between the amounts of neutral and acidic material are different because samples have different origins, which will be discussed later. In the case of the PIT sample (recovery of 80.7%), the whole of the acidic fraction could not be collected, due to plugging of

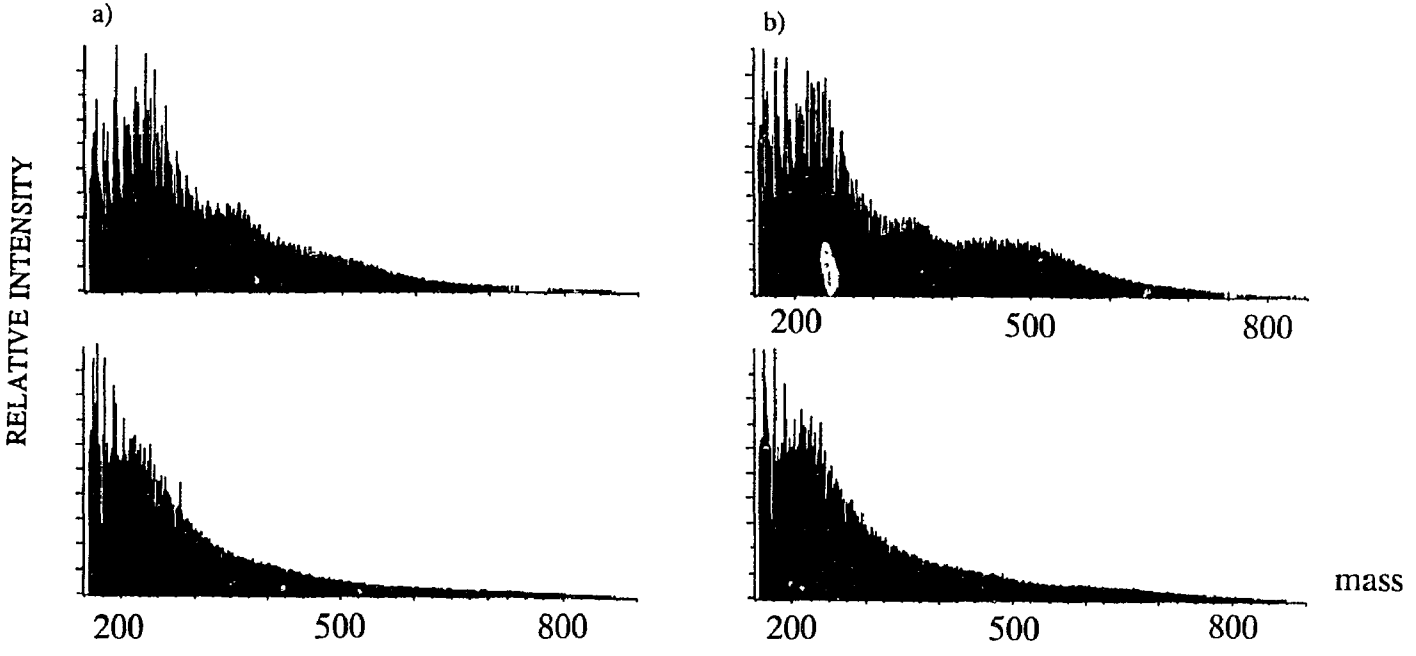


Figure 6.2.5: Mass spectra of the fractions obtained using the KOH-coated silica method; a) oil sand extract, b) pitch. Top: chloroform; bottom: formic acid in chloroform.

the column during the last stages of the elution. The sample however contained less neutral material than the HGO and OSE.

Table 6.2.1: Material recovered from the KOH-treated silica experiment

sample	initial weight (g)	weight of the $CHCl_3$ fraction(g)	weight of the $HCOOH/CHCl_3$ fraction(g)	overall % recovery
HGO	2.08409	1.98475 (95.2%)	0.01598 (0.8%)	96.0
OSE	0.83380	0.66450 (79.8%)	0.13115 (15.7%)	95.5
PIT	2.10490	1.54671 (73.5%)	0.15082 (7.2%)	80.7

Figure 6.2.4 shows the reversed phase HPLC/UV (254 nm) trace obtained for the neutral fraction of PIT sample. Compared to that of Figure 6.2.3, it contains less low molecular weight material (eluting in the first 40 minutes), whereas the high molecular weight end of the chromatogram indicates the presence of a relatively large amount of eluting material. The HPLC/UV (254 nm) trace of the neutral fraction of the OSE (not shown) is intermediate between those of the HGO and PIT samples, in that both portions of the chromatogram contain approximately equal amounts of material.

To summarize, the KOH-treated silica method allowed the separation of neutral material from acidic or polar compounds for the HGO, PIT and OSE samples. The mass spectra obtained by spiking material on the moving belt and HPLC/UV chromatograms have shown that all three Syncrude samples contain different proportions of light vs heavy compounds.

6.2.2 Alumina fractionation scheme

The alumina fractionation scheme described earlier (3,43) in Section 2.3.2 has been applied to the HGO. Figure 6.2.6 shows the mass spectra obtained for every fraction collected during the elution and figure 6.2.7 shows four normal phase HPLC/UV traces (254 nm), illustrating the chromatography of the aliphatic (containing some PAHs), neutral (PAHs), and polar (PANHs) fractions. The fourth chromatogram was obtained for a mixture of the PAH and PANH fractions. Table 6.2.2 gives recovery values.

The mass spectrum obtained for the aliphatic fraction (hexane) shows characteristic groups of peaks appearing every 14 m/z units from 100 to 775 Da. The weight of this fraction, according to Table 6.2.2, constituted more than 82% of the original sample, which is compatible with the expectation that an oil should contain aliphatics as the predominant species. This fraction may contain long chain alkylated aromatics as well, because the cut off point between fluorescent and non fluorescent material exiting the column was very diffuse due to similar chromatographic behavior between aliphatics and alkylated aromatics. Overlap was thus unavoidable; it is however impossible to determine whether or not aromatics are present in the hexane fraction from the observation of the mass spectrum. The normal phase HPLC/UV (254 nm) trace presented in Figure 6.2.7a was useful to confirm the presence of aromatic material.

The mass spectra of both benzene and chloroform fractions (PAHs and PAH derivatives, respectively) have the characteristic pattern showing the presence of aromatic compounds. The ions are separated into two groups, the singly charged (higher m/z) and the doubly charged (lower m/z) ions. In both spectra, ion peaks spaced by 14 m/z units are characteristic of the alkyl chains substituted on the aromatic nuclei. In both fractions, compounds with M.W. up to ca. 700 were detected by the mass spectrometer; both spectra are almost identical and no conclusion can be drawn about the composition of the corresponding fractions. Normal phase HPLC/UV however gives more information. The traces presented in Figure 6.2.7b-c

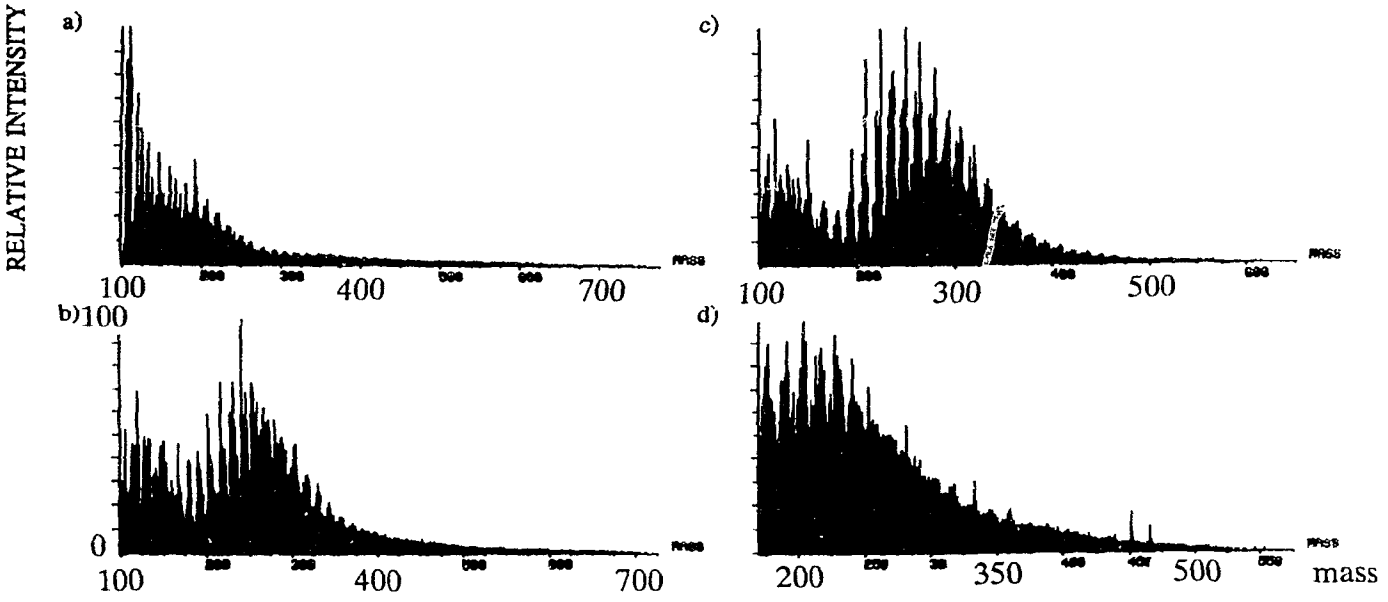


Figure 6.2.6: Mass spectra of the HGO fractions obtained using the alumina fractionation method; a) hexane, b) benzene, c) chloroform and d) methanol-water.

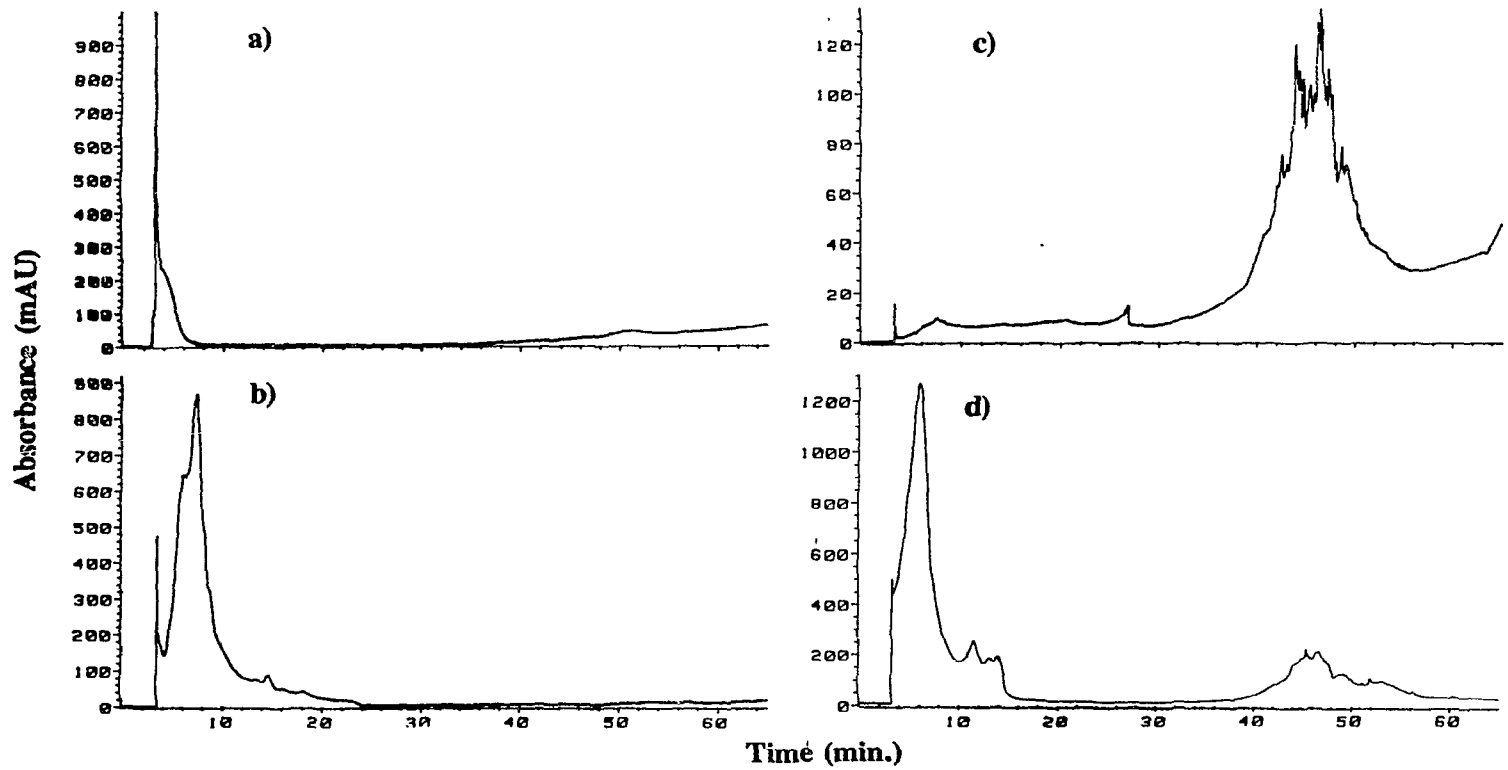


Figure 6.2.7: Normal phase HPLC-UV absorption chromatograms (254 nm) of the HGO fractions obtained using the alumina method; a) hexane fraction, b) benzene fraction, c) chloroform fraction and d) combination of benzene and chloroform fractions.

show that the separation was effected with good efficiency, since no overlapping was obtained between the benzene and chloroform fractions. Figure 6.2.7d shows the chromatographic trace obtained for a combination of both benzene and chloroform

Table 6.2.2: Material recovered after the elution of the HGO through an alumina column

fraction	weight of material recovered (g)	% recovery
hexane (aliphatics)	0.20254	82.3
benzene (PAHs)	0.02425	9.8
chloroform (PAH derivatives)	0.00665	2.7
methanol-water (polars)	0.00585	2.4

weight starting material: 0.24589 g
weight material recovered: 0.23929 g
recovery level: 97.2%

fractions, and it is observed that PAH derivatives are less concentrated than PAHs.

The mass spectrum of the polar fraction (methanol and methanol-water) is different from that of the other two. It shows the presence of aliphatic material with M.W. up to 550, and these compounds are most certainly substituted hydrocarbons because of their polarity. No further experiments were conducted to characterize this fraction.

This fractionation method yielded a good separation of the contents of the HGO into four subgroups, according to the mass spectra and NPLC/UV chromatograms presented in Figures 6.2.6 and 6.2.7. Table 6.2.2 shows that the content of PAHs and PAH derivatives was extremely low with respect to the aliphatic content. This can be partially attributed to the early elution of aromatics in the

hexane fraction, later verified by normal phase HPLC/MS. The separation between PAHs and derivatives seemed satisfactory. It will be shown later that the separation of these two fractions is not a prerequisite for final characterization using reversed phase HPLC/MS and thus their contents have not been further characterized at this stage.

6.2.3 Silica over alumina

This method was designed (68) for the separation of crude oils into subfractions containing aliphatics, monocyclic aromatics, dicyclic aromatics, polycyclic aromatics and polar compounds. Hirsch and co-workers performed the separation on a 2.5 cm x 2.4 m dual packed column and thus obtained a very clear cut separation between fractions. Unfortunately, a glass column with such proportions was not available in this laboratory and the experiment was performed using a 20 mm x 12 cm column. A consequence of using a shorter column is to decrease the efficiency of separation, viz. the cut points between fractions will not be as well defined as in Hirsch's experiment (Hirsch et al. found overlapping between monocyclic aromatics and dicyclic aromatics in spite of a 50 hours overall elution time). The procedure used here may yield less selective separations, but the experiment is performed in ca. 2 hours and thus can be used on a routine basis.

The samples separated using this method were the neutral fractions obtained from KOH-treated silica for the HGO, OSE and PIT.

The development of the method was performed with the HGO sample, whose fractions helped to determine what volumes of eluent to use. In Figure 6.2.8, mass spectra obtained by spiking the moving belt interface with an aliquot of each fraction of the HGO are presented. These spectra show that mainly aliphatic material is eluted in the three fractions collected with pentane and benzene. The molecular weights of these aliphatic compounds increase with the % benzene in pentane. One would expect to find monocyclic and dicyclic aromatic compounds in the second and third fractions, but as the HGO and PIT samples are high boiling point distillation

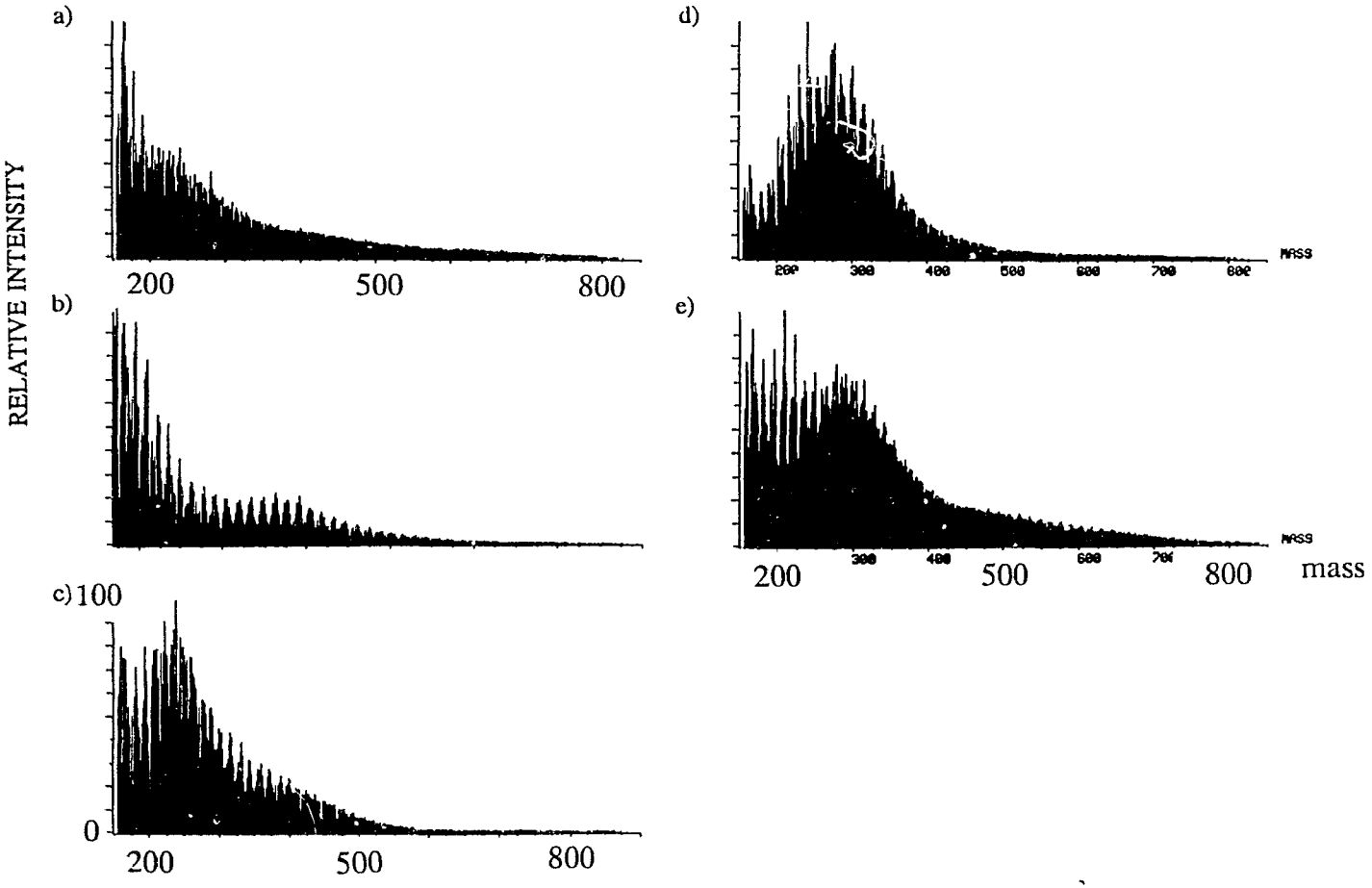


Figure 6.2.8: Mass spectra of the HGO fractions obtained using the silica over alumina method; a) pentane, b) 5% benzene in pentane, c) 15% benzene in pentane, d) 3:1:1 methanol-ether-benzene and e) methanol.

fractions, their content in benzenic and naphthalenic compounds should only include highly alkylated, relatively non volatile molecules. Unfortunately, such species produce mass spectra that are similar to those of aliphatics and thus the presence of highly alkylated mono and diaromatic compounds cannot be verified from inspection of the mass spectra (results not shown for PIT). The OSE sample should contain more volatile benzenic and naphthalenic compounds than the HGO and PIT, but the presence of aliphatics masks any trace of the latter in the spectra recorded for the second and third fractions (not shown).

Polycyclic aromatic and highly functionalized high molecular weight compounds are probably found in the 3:1:1 methanol-ether-benzene fractions, according to the spectra of the fourth fractions (HGO only shown). The mass spectra associated with the methanol fractions surprisingly resemble those obtained for the acidic fractions collected from the KOH-silica columns (Figure 6.2.2 for HGO, Figure 6.2.5 for OSE and PIT). This observation emphasizes that compounds eluted with methanol at this stage are the overlapping acidic and polar materials eluted in the neutral fraction during the anion exchange chromatographic process for acid removal. The mass spectra of the very polar fractions seem to show the presence of polar aromatic material.

Figure 6.2.9 compares the reversed phase HPLC/UV 254 nm traces obtained for the methanol-ether-benzene fractions of the three samples. These chromatograms show the elution of aromatic material (neutral and moderately polar). The three chromatograms are clearly divided into two parts: the water-acetonitrile part (first 45 minutes), which will be called the low M.W. part. The second part corresponds to the acetonitrile-dichloromethane gradient, and will be called the high M.W. part. The top trace of Figure 6.2.9 shows that the HGO contains reasonable amounts of low M.W. material which chromatographs with some peak separation. Compared to the chromatogram of Figure 6.2.3, it is observed that the removal of aliphatics using the present procedure yielded a better separation of the peaks. In RPLC, aliphatic material elutes as a continuum, due to the high solubility of the aliphatics in the C_{18} chains of the reversed phase stationary phase. The second part of the top trace

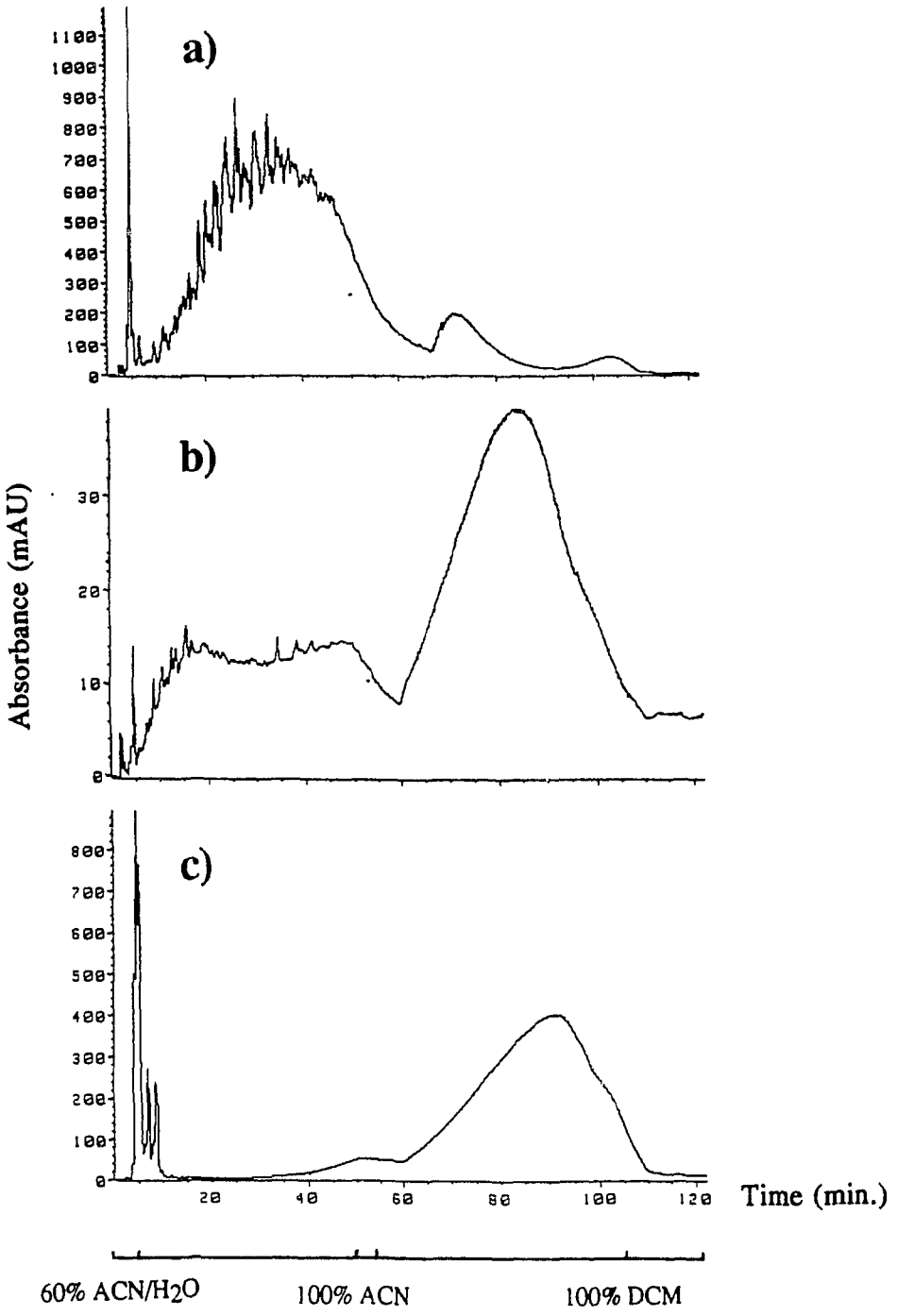


Figure 6.2.9: Reversed-phase HPLC-UV absorption chromatograms (254 nm) of the 3:1:1 methanol-ether-benzene fractions obtained using the silica over alumina method; a) HGO, b) OSE and c) PIT.

corresponds to the elution of high M.W., highly functionalized aromatic molecules. These compounds, although in low concentration in the HGO, behave more like aliphatics than aromatics on a C_{18} column in that they elute continuously as soon as dichloromethane is introduced in the gradient.

In the center trace of Figure 6.2.9, the chromatogram obtained for the OSE shows a different picture. There is a much lower concentration of low M.W. aromatics, followed by heavy material eluted with dichloromethane. This high M.W. material is also poorly chromatographed in that it is all eluted as a broad envelope with no well defined peaks. The ratio of the [Heavy material/Light material] overall intensities is greater than one (ca. 2-3), according to the absorbance at 254 nm. Chromatograms plotted at different wavelengths, 280 and 310 nm (not shown), are similar to that at 254 nm shown here.

In the bottom trace of the same figure, there is almost a complete absence of low M.W. material, but a high concentration of heavy, highly functionalized compounds. The Heavy/Light ratio reaches ca. 50, while it was around 0.2 for HGO. These observations can be related to the origin of the samples. The PIT and HGO are both distillation fractions from the same process (Section 1.5). The PIT is the heaviest distillation fraction and the HGO, the second heaviest. There is overlapping between these distillation fractions, i.e. heavy compounds found in the HGO should have remained in the PIT, and light compounds found in the HGO should have been distilled in the light as oil fraction. The OSE has not been treated by any process other than breaking and grinding, thus contains a mixture of low and high molecular weight compounds (wide range of volatilities).

According to the retention times of "light" and "heavy" portions of each chromatogram, the bulk of light material should be of the same nature for the three samples since it all elutes between 10 and 50 minutes. And as the bulk of heavy compounds elutes between 60 and 110 minutes for each sample, it is expected to find compounds of the same nature in this second part of the chromatograms as well. A mass spectrometric investigation will provide more detail on these assumptions (Section 7.2.2).

Table 6.2.3 summarizes the overall recoveries obtained using the silica over alumina method.

The percentage values indicated in this table are the ratios of the weight recovered in each fraction to the weight of the original sample. The aromatic fraction contains most of the material in the case of the PIT, whereas the contents of the OSE and HGO are dominated by aliphatic compounds. The silica over alumina

Table 6.2.3: Material recovered from the silica over alumina fractionation method for the HGO, OSE and PIT samples

sample	weight starting material,g	weight pentane fraction,g	weight 5%benzene in pentane fraction,g	weight 15% benzene in pentane fraction,g
HGO	1.01056	0.02756 (2.7%)	0.27649 (27.4%)	0.26842 (26.6%)
OSE	0.55264	0.33481 (60.6%)	0.01348 (2.4%)	0.00587 (1.1%)
PIT	1.00823	0.10758 (10.7%)	0.00791 (0.8%)	0.02592 (2.6%)

sample	weight 3:1:1 MeOH-ether-benzene fraction,g	weight methanol fraction,g	% recovery (overall)
HGO	0.39480 (39.1%)	0.00595 (0.6%)	96.3
OSE	0.05138 (9.3%)	0.06824 (12.3%)	85.7
PIT	0.71471 (70.8%)	0.00791 (0.8%)	85.6

method has eliminated non-aromatic material from the samples to a certain extent, to provide a cleaner fractionation of aromatics (3:1:1) to be further analyzed by chromatographic/mass spectrometric experiments (Section 7.2.2).

At this stage, it is worthwhile comparing the results obtained in the last section (alumina column chromatography) to those presented here for the fractionation of the HGO sample. With alumina, the ratio aliphatic/aromatic obtained was 6.6:1 while it was 1.4:1 with silica over alumina. This great difference is most probably due to difficulty in separating compounds whose properties are between those of purely aromatic and those of strictly aliphatic compounds. Neither of the two fractionation methods can be claimed as better than the other at this stage and thus there is a high level of uncertainty with respect to the real aliphatic and aromatic content of the HGO sample. It can be concluded however that aliphatic material is more abundant than aromatics in the HGO. Qualitatively, the silica over alumina method offers better visualization of the elution of fractions than does the alumina method. For instance, the 3:1:1 aromatic fraction is eluted as a very sharp band which is easy to observe from the color of the compounds and from the fluorescence on the column. The fractions eluted using the alumina method appear as more diffuse bands.

6.2.4 Supercritical fluid chromatographic fractionation

The HPLC/UV chromatograms presented in Figure 6.2.9 show that the samples are still very complex and contain an appreciable number of compounds, even after fractionation. Therefore, normal phase SFC fractionation, as detailed in Section 2.5.3.3, was performed on the 3:1:1 fraction of the HGO, OSE and PIT samples in order to obtain simpler samples for the final characterization. Belt spike mass spectra (recorded in both EI and ammonia CI modes) were obtained for each of the 15 fractions of the three samples, and showed no major differences whether they were acquired in EI or CI. Some of the spectra obtained for the HGO are shown in Figure 6.2.10. From this figure, the general trend is an increase in the

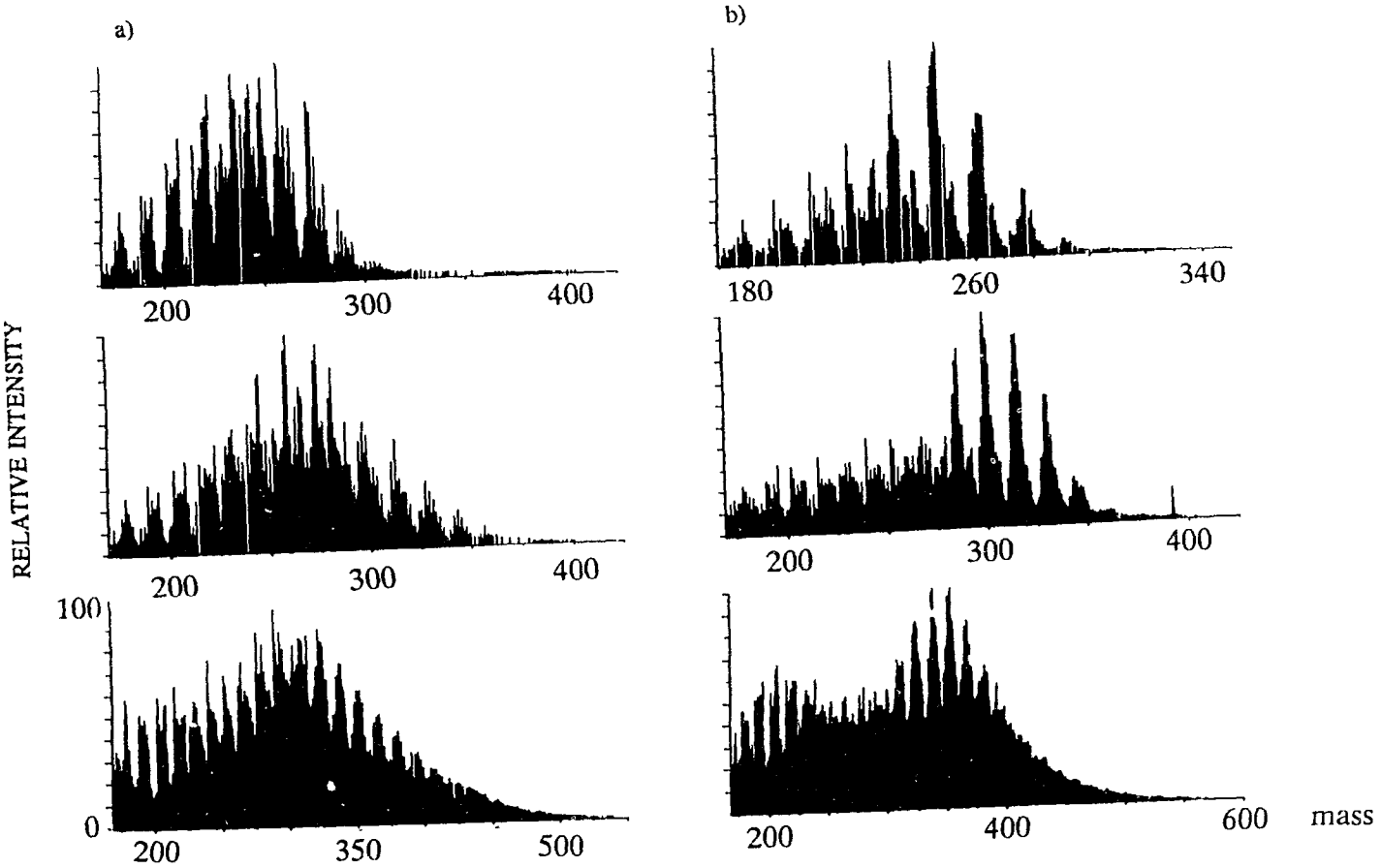


Figure 6.2.10:Mass spectra of the 3rd, 8th and 15th HGO fractions (from top to bottom) obtained using the normal phase SFC method; a) EI and b) ammonia-CI.

molecular weight of compounds collected as a function of time (also true for the OSE and PIT). For the 14 first fractions, collected with 11% methanol in pentane at 3792 Kpa, the increment in molecular weight from one fraction to the next corresponds to the addition of 14 mass units (one methylene group), as the alkylated chains connected to the aromatic nuclei increase by one carbon atom in length. Fraction 15, whose elution was performed at 6895 Kpa and with 100% pentane, contained high molecular weight material that was left adsorbed on the column when using 11% methanol in pentane. The EI and CI mass spectra of this fraction show that the heaviest detectable compound has a mass of only ca. 550. This is due to a lack of sensitivity with respect to the small amount of sample collected (for each sample, a total of 3 x 20 μL, divided in 15 fractions). It will be shown later (Section 7.3.2.2) that the heaviest detectable compound in the aromatic fraction of the HGO has a molecular weight of ca. 960.

A reversed phase HPLC/UV trace (254 nm) has been obtained for each SFC fraction of the HGO, and for a few fractions of the OSE and PIT. Some of these traces are presented in Figure 6.2.11. In spite of the low concentrations of compounds in each of the fractions, it is clearly observed that the envelope of eluting material is moved toward higher HPLC retention times as the SFC fraction number increases. Compounds in Fraction 2 are eluted in the 8-20 minutes time period of the HPLC run. The chromatogram of Fraction 3 shows an increment in the average retention time to ca. 14-27 minutes. In proceeding through to fraction 7, the same trend applies. Compounds of higher molecular weights, eluting with dichloromethane in HPLC, start to appear in Fraction 9, although this feature is not shown in the figure. The [high M.W./low M.W.] intensity ratio was ca. 0.1 for this fraction. The HPLC chromatogram of Fraction 11 shows a more significant proportion of high molecular weight compounds, in a high [M.W./low M.W.] ratio of ca 0.5. However, those heavy compounds do not separate, but instead elute in a broad envelope. Moving up to Fraction 13, the ratio increases to ca. 4 and remains the same for Fraction 14. Although high M.W. substances start to elute in Fraction 9, low M.W. compounds (chromatographed with acetonitrile/water in HPLC) still display their

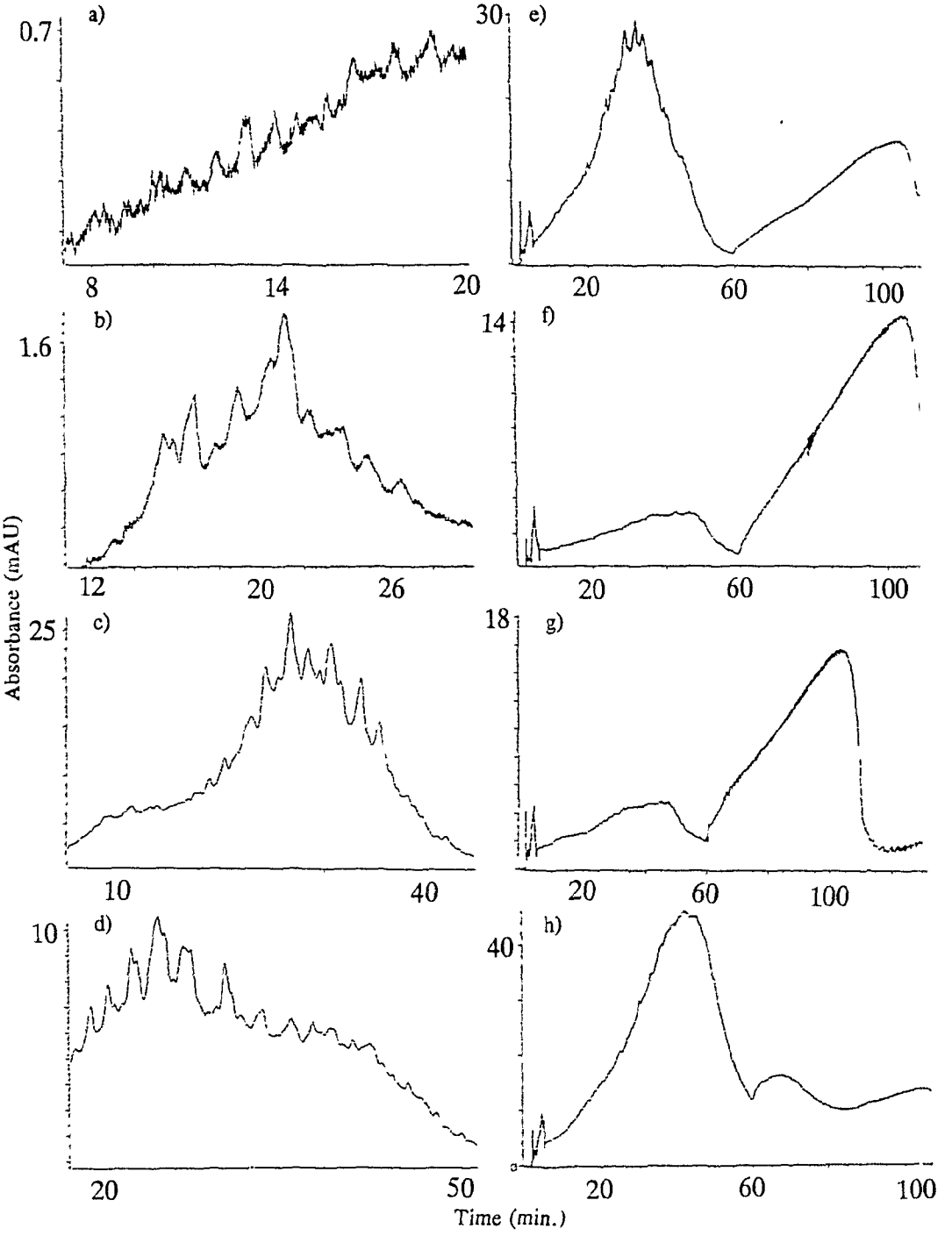


Figure 6.2.11:Reversed phase HPLC-UV absorption chromatograms (254 nm) of some of the HGO normal phase SFC fractions; a) Fraction 2, b) 3, c) 7, d) 9, e) 11, f) 13, g) 14 and h) 15.

progressive elution profiles toward higher HPLC retention times, without defined cut points. Surprisingly, Fraction 15 has a lower [high M.W./low M.W.] ratio than Fractions 13 and 14; low M.W. material was thus left adsorbed on the column throughout the SFC run and was eluted with 100% pentane toward the end of the experiment.

The heavy end of the HPLC chromatogram of Fraction 15 shows two envelopes, which were not observed for earlier fractions. The first envelope contains material that separates, while the second envelope is a continuum. The observation of low M.W. material and of two high M.W. envelopes for Fraction 15 indicates that some of the material contained in the HGO is strongly adsorbed on the silica column, until a strong solvent (pure pentane) is used to remove it. This undesirable retention of compounds was the result of a compromise between eluting all compounds together with pure pentane and not eluting any high M.W. compounds with carbon dioxide. Methanol, used here as the "bad" solvent to help retain compounds on the column, has too much affinity for the analytes and for the column at the same time.

This SFC fractionation technique is useful for separating light compounds from heavy compounds, thus avoiding the latter being passed through HPLC columns every time that a sample is injected. The method is not useful with respect to the analysis of the heavy end of the HGO sample, as these substances are still eluted in broad envelopes. The results of HPLC/MS studies of the SFC fractions will be presented in Section 7.2.2.

Some HPLC/UV chromatograms of the SFC fractions obtained from the OSE and PIT samples are presented in Figure 6.2.12. The traces associated with the OSE emphasize the fact that this sample contains very little low M.W. material, while the high M.W. compounds are more concentrated, as shown in the chromatogram of Fraction 10. The HPLC/UV traces of Fractions 2, 3, 4 and 5 exhibit low intensity envelopes in the water-acetonitrile part of the HPLC elution. Figure 6.2.12-f shows that the fifth SFC Fraction of PIT (to compare with that of HGO) only contains traces of low M.W. compounds. However, the chromatograms of Fractions 9-14 (not

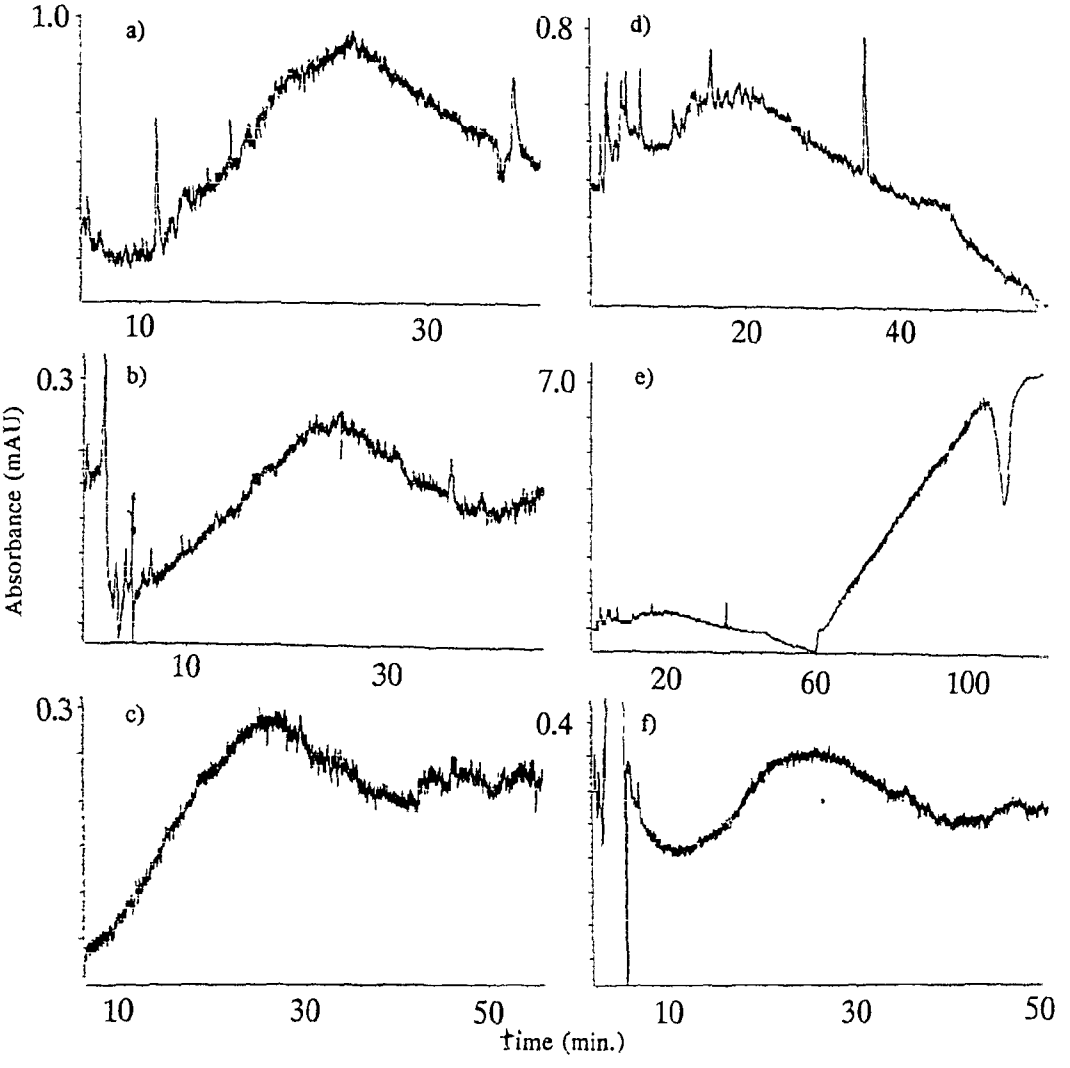


Figure 6.2.12:Reversed phase HPLC-UV absorption chromatograms (254 nm) of some of the OSE and PIT normal phase SFC fractions. a) OSE 2, b) OSE 3, c) OSE 5, d) OSE 10, e) OSE 14 and f) PIT 15.

shown) show a continuous elution of high M.W. material, with increasing intensity as the SFC fraction number goes up. Interestingly, the shape of the envelopes of high M.W. material does not change from fraction to fraction, i.e. the same compounds seem to be eluted in proportionally increasing concentrations.

This SFC/MS fractionation method was no real help in the analysis of the high M.W. constituents of the three Syncrude samples: it allowed dilution of those heavy compounds by separating them into several "identical" fractions of lower concentration in respect to that of the original samples. The technique was more useful in isolating low M.W. compounds (alkylated PACs from 150 to 400 Da.) from those of the heavy ends (highly functionalized compounds) in order to provide cleaner fractions for HPLC analyses.

The application of this technique has shown that pentane, as a SFC mobile phase, is appropriate for elution of the high M.W. substances contained in the Syncrude samples without damaging the column. HPLC/MS (Section 7.2.2) will provide more answers than HPLC/UV with respect to the nature of compounds contained in the very complex Syncrude samples.

7. RESULTS AND DISCUSSION: CHROMATOGRAPHY AND STANDARD MS INTERFACING

The combination of specificity and universality allowed by mass spectrometry compared to other detection techniques is very helpful in the analysis of complex mixtures. Mass spectrometry interfaced with chromatographic systems can provide simultaneous universal and specific detection. The latter is provided by the RICs and the former is obtained from the TIC chromatogram. Mass spectra, available from any scan acquired during a chromatographic run, are very helpful in giving information on the nature of the eluting compounds.

The technique that is the most commonly used for the analysis of PACs in environmental samples is GC/MS. The very high efficiency of capillary columns coupled with the molecular weight information available from the mass spectrometer makes compound identification and quantification possible in many cases. However, there are problems associated with the use of GC/MS techniques for the analysis of PACs.

First, stationary phases most commonly employed for the analyses do not adequately separate some compounds of interest from isomeric interferents. For instance, many laboratories use DB-5 (phenyl methyl siloxane) or equivalent stationary phases in their columns. On these columns, some isomers (chrysene and triphenylene, M.W. 228; benzo[b]-, [j]- and [k]-fluoranthenes, M.W. 252) are poorly resolved, if resolved at all.

A second problem is the somewhat limited mass range of PACs amenable to GC separation. A DB-5 column or its equivalent is limited to ca. 350°C under temperature programming conditions; this limits the upper mass of the PACs analyzable to ca. 326 Da.

The combination of HPLC and mass spectrometry (HPLC/MS) is very powerful in the characterization of PACs in environmental matrices. The principal advantage provided by HPLC/MS is the great range of mobile phase and stationary phase parameters which can be varied to obtain the desired selectivity. There is a

greater variety of stationary phases available commercially as compared to bonded phase capillary GC. Bonded reversed phases are generally more selective for the separation of isomers than GC columns. Although the efficiency of HPLC columns is lower than that of GC columns, the increased selectivity of the former, when coupled with a mass spectrometer, can provide information that may not be available from GC/MS experiments. Moreover, HPLC separations are conducted at or near room temperature so that low volatility and thermal fragility are no longer problems.

The combination of supercritical fluid chromatography and mass spectrometry has recently undergone a surge in popularity, and this development is still continuing (156,171). However, in most of the work published in SFC/MS for PAC analysis, the mass range of the analytes was restricted to that accessible by GC/MS (150-152). SFC/MS should ideally combine the high efficiency provided by the GC columns with the ability to analyze thermally labile, polar and non volatile samples provided by HPLC.

7.1 Gas chromatography/mass spectrometry

When developing a method for the fractionation of PACs, GC/MS is a fast and handy method to use for the characterization of the fractions and the determination of the efficiency of the method. Many laboratories (including this one) keep gas chromatographs interfaced permanently to mass spectrometers. DB-5 capillary columns (phenyl-methyl-siloxane) are frequently used to analyze moderately polar compounds such as PACs. GC/MS with a DB-5 column can provide information on many PAC fractions, except those fractions whose contents are very polar or non volatile. To analyze PANH fractions for instance, a DB-1701 (phenyl-isopropyl-methyl siloxane) capillary column is more appropriate than a DB-5.

GC/MS is also an invaluable tool in the detailed characterization of the PACs contained in the final fractions.

For the purposes of this work, the development of every fractionation method applied to the TPE has been performed using GC/MS in order to characterize each

fraction. GC/MS analyses could not be conducted in the case of the Syncrude samples because of the high boiling points of the compounds present in those mixtures. Spikes on the moving belt (EI) and HPLC/MS were used instead of GC/MS to characterize the fractions. The disadvantage of using HPLC/MS or SFC/MS for quick characterization of fractions is that setting up the instrumental systems can take a long time, while the fractionation steps must be performed quickly to minimize sample loss or decomposition.

The role of GC/MS in the characterization of fractions has been explained and discussed earlier in Section 6.1.

The results presented and discussed below were obtained from GC/MS "final" characterization of PAC fractions.

7.1.1 GC/MS of the TPE

7.1.1.1 Conventional GC/MS

The "final" fraction studied extensively here was obtained from the Ramos and Prohaska method described in Section 2.4.1.1.

Figure 7.1.1-a shows the total ion current (TIC) trace obtained by GC/MS for this sample. At first observation, this chromatogram seems fairly complex and many peaks of low intensity are lost in the background. This TIC trace is similar to what would be obtained using a flame ionization detector and shows the mass spectrometer acting as a universal detector. For a more specific analysis, reconstructed ion chromatograms (RICs) at nominal m/z values are easily obtained from the data system. Figure 7.1.1 (b-e) shows RICs corresponding to PAH species commonly found in polluted sediment samples. Some of these compounds (e.g. fluoranthene and pyrene at m/z 202) produce intense peaks that are also observable in the TIC trace, whereas other less abundant compounds produce TIC background level peaks which are better revealed by the RICs. Featured among the latter compounds are dibenzo[def,mno]chrysene (m/z 276, RIC not shown, scan no. 5310)

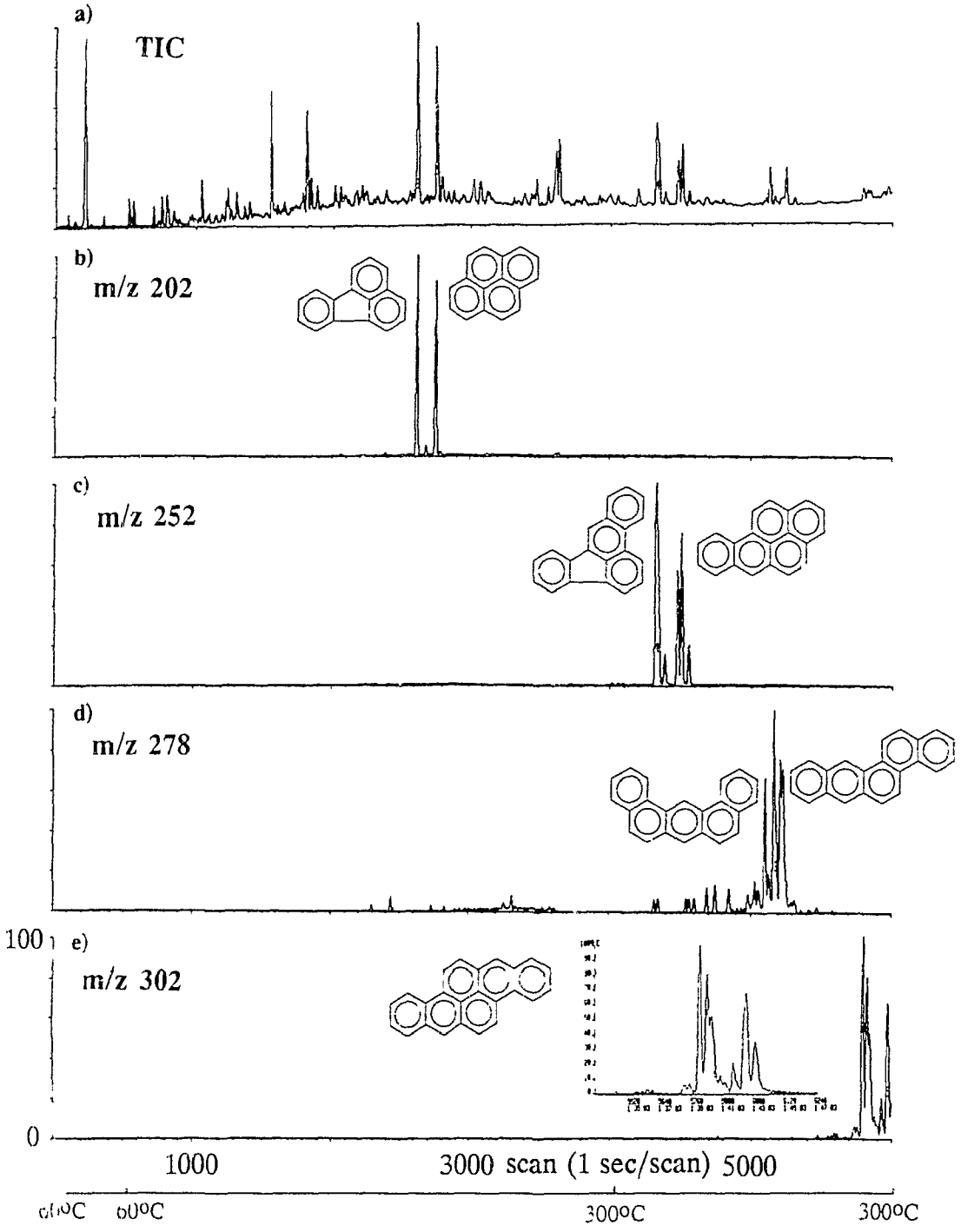


Figure 7.1.1: GC/MS a) TIC chromatogram and b) RICs (PAHs) obtained for the TPE sample (DB-5 column)

and dibenz[a,j]anthracene (m/z 278, scan no. 5106). Tentative identifications of the PACs detected in this GC/MS run have been made, according to the method described below (3).

- All reconstructed ion chromatograms were plotted from m/z 115 to 350 (nominal mass). Significant peaks were detected, corresponding scan numbers were determined and peak heights were measured. These data were entered as three columns (m/z, scan no., peak height) in a Lotus 1-2-3 spreadsheet and were then sorted by scan no. The resulting spreadsheet is found in Appendix I.

- Standard perdeuterated compounds (fluorene-d_6, m/z 176, scan 2598; benz[a]anthracene-d_{12}, m/z 240, scan 3613 and benzo[a]pyrene-d_{12}, m/z 264, scan 4334) were located in the RICs corresponding to their m/z value. Perdeuterated compounds were used as internal standards; their non-deuterated homologs are important constituents in the sample, and are easily located in the chromatogram due to their intense peaks. Perdeuterated compounds elute slightly ahead of their homologs on a DB-5 capillary column (166). To find fluorene, one looks for the fluorene-d_{10} peak in the m/z 176 RIC (not shown) and finds a major peak at scan 1219. Non deuterated fluorene elutes shortly after, and its peak is found at scan 1554 in the m/z 166 RIC.

- Once the non deuterated homologs have been identified (e.g. fluorene, m/z 166, scan 1554; fluoranthene, m/z 202, scan 2610; benz[a]anthracene, m/z 228, scan 3613; benzo[a]pyrene, m/z 252, scan 4507), compounds with preassigned retention indices are located: naphthalene, m/z 128, scan 334, I=200; phenanthrene, m/z 178, scan 1807, I=300; chrysene, m/z 228, scan 3636, I=400; picene, m/z 278, scan 5231, I=500. As chrysene and picene have several isomers, locating them on the respective RICs was difficult; it was necessary to compare the order of elution of isomers with those obtained from chromatograms of similar samples (10,42,49,172) and from data sets obtained using the same method (3). The retention indices of RIC significant peaks were calculated by simply fitting a linear function between two peaks with preassigned indices. Thus "I = a*scan# + b" functions were fit between the peaks of naphthalene and phenanthrene, phenanthrene and chrysene, and

chrysene and picene. These linear functions were used to calculate indices for all the peaks included in the corresponding ranges.

- By comparing the calculated indices with those published by Lee and co-workers (106,168), some of the PACs present in the TPE could be tentatively identified. Most calculated retention indices (RIs) could not be matched with any literature value, probably because they had been calculated linearly over a wide range of data. The spline curve function used by Marr and Quilliam (3) is more satisfactory than linear interpolation for calculation of RIs of unknowns. Comparison with FID chromatograms obtained for other sediment samples (4,11,42,45,49) was necessary and extremely useful in making the tentative identification of compounds. It is observed from Appendix I that there is a systematic deviation between the assigned and calculated RIs. From RI=200 to 260 (literature values), non-alkylated and unsubtituted PAHs show a systematic error in their RIs, ranging between 6 and 10 units. From RI=260 to 300, the differences decrease and the use of calculated RIs is more reliable. In the 300-350 RI range, non systematic deviations are observed. This can be due to an increasing number of possible isomers, and to the impossibility of finding any listing of all of those isomers in the literature. For I=400-500, deviations of up to 6 units are observed, probably due to the linear interpolation method of calculating RIs. PASHs, PANHs, PAOHs and PAQs were only tentatively assigned as their calculated RIs were quite far from the literature values.

All the results from this GC/MS investigation are summarized in Appendix I, a Lotus 1-2-3 spreadsheet with data sorted by scan number. They will be discussed later in this section.

In general, boiling point is the most important factor for determining the retention in GC. However, interactions of the analytes with the stationary phase are still not well understood. It is a well known fact that retention times increase with the number of aromatic rings, therefore larger PAHs whose structures include more rings will be retained longer. Further insights into structural relationships can be obtained upon the comparison of two major groups of isomers: Parent PAHs with different ring fusion, and alkylated PAHs with different alkyl chain lengths and positions.

In the former case (e.g. benzo[e]pyrene and benzo[a]pyrene), elution will generally be controlled by boiling point. Although there might be minor changes in the column selectivity, the order of elution is generally preserved with stationary phases of different polarities. Even if the differences among parent PAH molecules are minor, somewhat longer retention times tend to be associated to less "angular" molecules with the same number of rings and molecular weight (2). Thus phenanthrene, an angular molecule, is eluted slightly ahead of anthracene, its linear homolog. As a similar example in the series of isomers of M.W. 252, benzo[j]fluoranthene elutes first, followed by benzo[k]fluoranthene, benzo[e]pyrene, benzo[e]pyrene and perylene (2).

In the case of alkylated PAHs, a change in position of an alkyl group can produce distinguishable chromatographic behavior in capillary column GC (2). For instance, ethylated PAHs will be eluted after their dimethylated isomers. Among dimethyl-PAH isomers, coelution is often observed and column selectivity, which depends upon the nature of the stationary phase, might somewhat influence the elution order.

From Appendix I, most non-alkylated PAH structures within an isomeric group have been identified with the exception of PAHs of M.W. 302. The RIC at m/z 302 shows at least 10 isomers, eluting between scans 5700 and 6100. There are 66 isomers of mass 302 listed in the literature (2); it is thus difficult to determine, with nothing in the literature for comparison, which isomer each peak corresponds to.

Figure 7.1.2 shows RICs related to PACs other than unsubsituted PAHs. Isomers of these compounds were difficult to differentiate and identify. The m/z 208 RIC covers at least four different classes of PACs: phenanthrothiophenes, anthraquinones, trimethyl-fluorenes and dimethyl-fluorenones (173). These compounds could be readily identifiable by high resolution mass spectrometry using their respective accurate masses to plot RICs (m/z 208.035, 208.052, 208.125 and 208.089, respectively). Such high resolution experiments must be performed using a sector mass spectrometer and would involve a major decrease in sensitivity to achieve

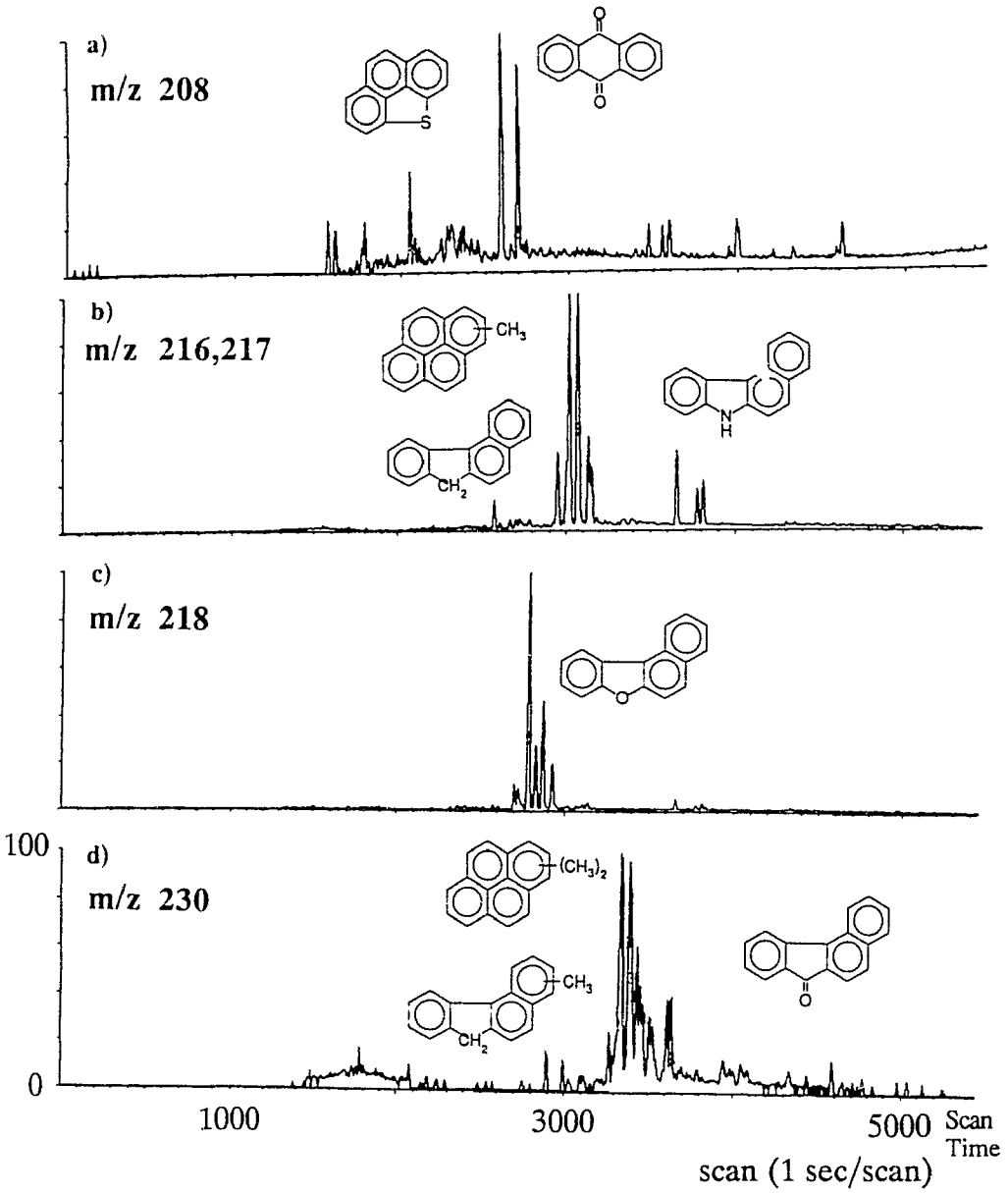


Figure 7.1.2: GC/MS RICs (PACs) obtained for the TPE sample

the desired resolution (ca. 12000). Unless quantitative analyses are required for specific target compounds interfering with others at the same nominal mass, experiments using chromatography combined with high resolution mass spectrometry are not performed.

The trace at m/z 217 corresponds to the elution of 3 benzocarbazoles, [c], [a] and [b] in elution order. Five intense peaks due to $(M+1)^{+\cdot}$ first isotope peaks of the molecular ions of methyl-pyrenes and methyl-fluoranthenes appear in this RIC. The RICs of m/z 216 and 217 were added to form a single trace for simpler presentation. M.W. 230 compounds also include different types: benzofluorenones (M.W. 230.073), dimethyl-pyrenes and dimethyl-fluoranthenes (M.W. 230.109).

Trimethyl naphthalenes (M.W. 170) produce a number of peaks of known retention times but these could not be assigned to specific isomers. The more general nomenclature "128-PAH-C3" refers to alkylated naphthalenes with 3 carbon atoms in the alkyl function(s). Ethyl-methyl-naphthalenes, trimethyl-naphthalenes and propyl-naphthalenes are all included in this category.

A large number of compounds could not be identified; their peaks are labelled with an asterisk in Appendix I. Such compounds are either substances whose mass/retention index combination was not found in the literature, or coeluting compounds producing very complex mass spectra. The chromatographic peaks related to these unknowns had low intensities in general and tended to be buried under more intense peaks.

Figure 7.1.3 shows the EI mass spectra of three sets of two PAH isomers, showing that they cannot be differentiated using this detection technique. This trend applies to almost all PAHs: the EI mass spectra of isomers are almost identical.

When polycyclic aromatic substances are ionized by electron impact, the resulting $M^{+\cdot}$ ions are very stable due the delocalized π electrons. EI spectra of unsubstituted PAHs exhibit a molecular ion $M^{+\cdot}$, a doubly charged molecular ion M^{2+} (appearing at M/2), and only very few fragments. These features are rather useless from the qualitative standpoint of determining structures. A good alternative to EI for isomer discrimination is the use of negative ion chemical ionization with

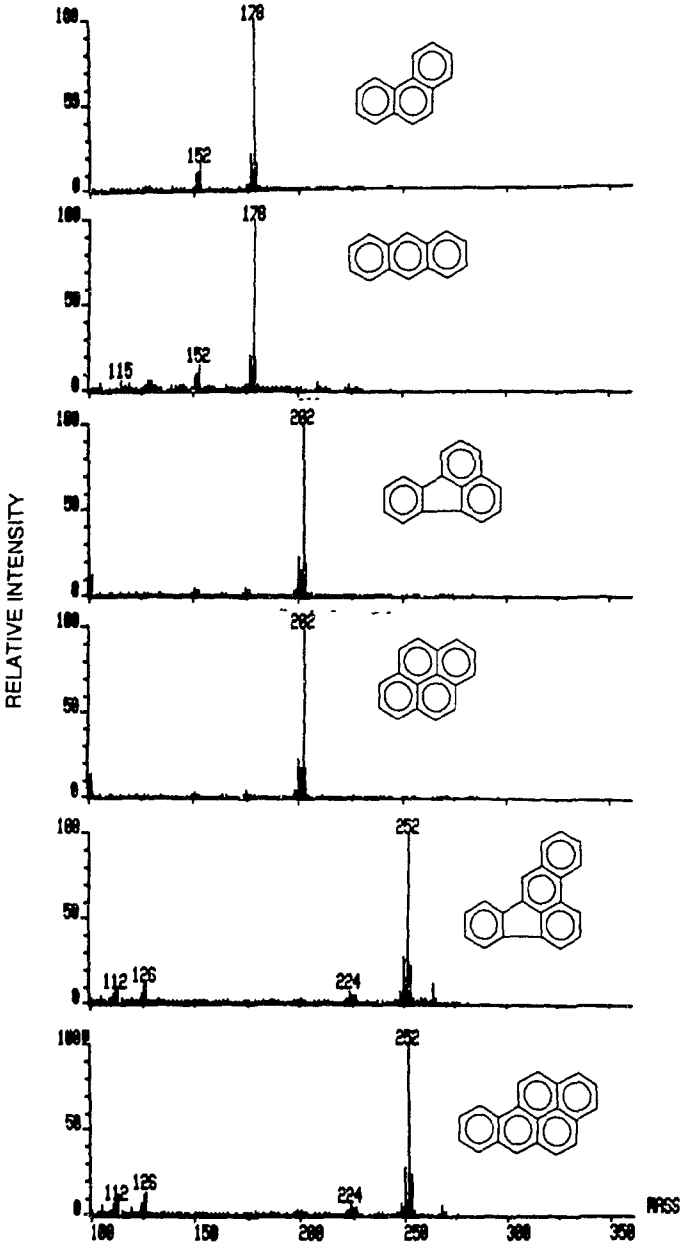


Figure 7.1.3: Mass spectra of 3 sets of TPE PAH isomers, obtained by GC/EI-MS

carbon dioxide, which allows the differentiation of isomers to a certain extent (13,155). Another way of addressing the task of isomer discrimination with mass spectrometry is to perform tandem mass spectrometry (MS/MS) experiments in order to study the fragmentation of molecular ions or molecular dications (16,158,161,174). This alternative will be discussed in Section 8.1.3.

The EI mass spectra of some PAH derivatives are given in Figure 7.1.4. When the compounds are alkylated, their $M^{+\cdot}$ ions are accompanied by abundant $(M-15)^{+\cdot}$ fragments resulting from the loss of a methyl group (e.g. Figure 7.1.4-f, 11-methyl-benzo[a]fluorene). The spectra of PAHs containing a methylene group such as benzo[c]fluorene exhibit a $(M-H)^{+\cdot}$ ion, often as intense as the $M^{+\cdot}$ ion. The molecular ions of PAKs and PAQs lose a CO moiety (136) under EI conditions and show abundant $(M-28)^{+}$ ions. Some specific fragmentation patterns are also observable for PAOHs, HXPAHs, NPAHs, APAHs, etc. EI mass spectrometry yields easy distinction between derivatized and plain PAHs. However, isomers of derivatized PAHs are still not differentiable using this technique.

The GC experimental conditions required to separate PACs are well established (3,10,42,45,49); accordingly, the separations performed here were of very good chromatographic quality. The column efficiency and resolution obtained were good with respect to the peak shapes and the number of separated isomers eluting in relatively short time periods.

The low resolution mass spectrometric RICs are invaluable for the detection of compounds with specific nominal masses, but do not allow any discrimination between compounds with the same nominal mass but different empirical formulae. This problem may be overcome with the use of high resolution mass spectrometry. It is important to note that the instrument used for this GC/MS acquisition, a quadrupole mass analyzer, only allowed low resolution experiments.

This qualitative experiment allowed the tentative identification of a variety of compounds and gave a good idea of the overall content of the TPE. Processing of the data obtained from this experiment was inspired by the work of Marr and Quilliam (3), who had verified the presence of ca. 500 compounds in a Hamilton

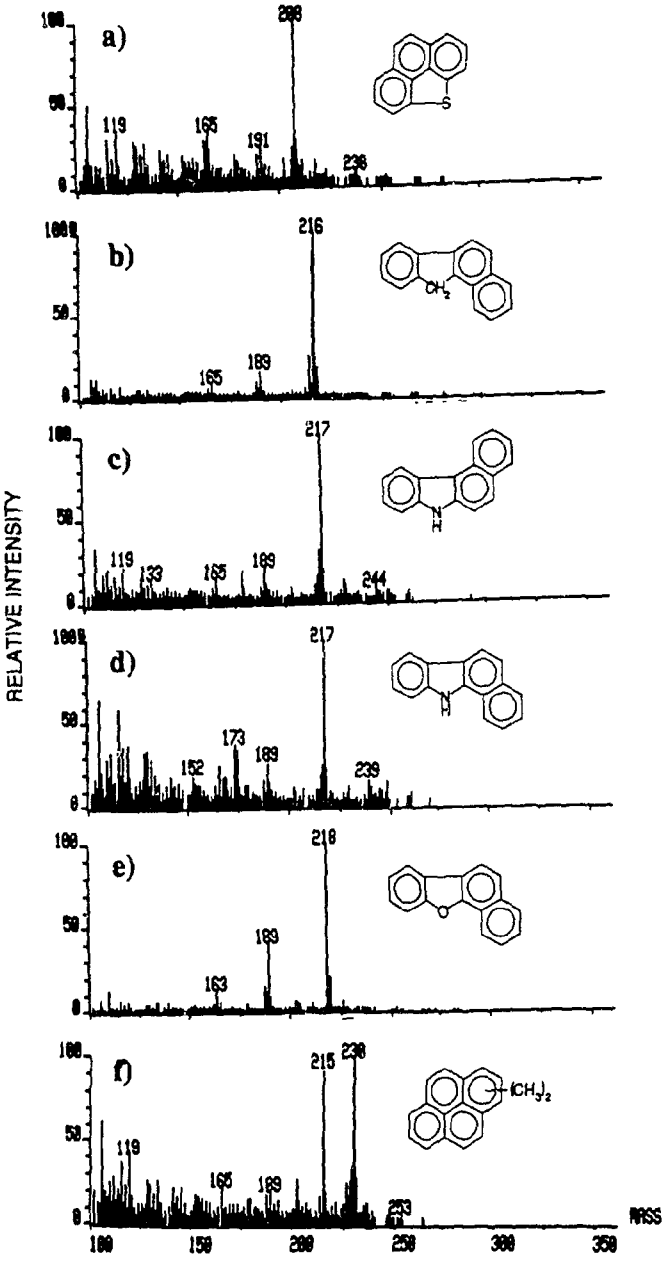


Figure 7.1.4: Mass spectra of different TPE PACs, obtained by GC/EI-MS

airborne particulate sample. Among these compounds, ca. 100 were identified.

7.1.1.2 High temperature GC/MS

The materials used in columns intended for use in high temperature GC must be temperature stable. Polyimide-clad fused silica columns are most commonly used. They are inert and easy to handle due to their flexibility. The drawback is the risk of oxidation of the polyimide at temperatures above 370°C. To overcome this temperature limitation, Lipsky and Duffy (175) introduced aluminum-clad fused silica columns. These were claimed to withstand temperatures far above 400°C. However in this laboratory, such a column became brittle after having been used once at 400°C. Bemgard and co-workers (176) have made the same observation. Metal columns, borosilicate glass columns, stainless steel armored fused silica columns and metal columns coated with a few μm of fused silica also feature among the most recent developments in high temperature GC. Very recently, Bemgard et al. (177) have compared ten commercially available and two laboratory made high temperature GC columns and could, in most cases, elute PACs with M.W. up to 400.

Figure 7.1.5 shows the gas chromatographic data obtained using a 2m long high temperature GC/MS column (see Section 2.5.1 for details). The M.W. range of the compounds eluted runs from 154 (acenaphthene) to 402 (isomers of tetrabenzo[a,c,fg,op]tetracene). Although the elution of compounds heavier than those amenable to regular GC was achieved, no resolution at all was observed for any group of isomers, due to the shortness of the column. This high temperature GC column, coated with aluminum for better protection against the heat, became extremely brittle after heating. Due to the high cost, no other similar columns were ordered.

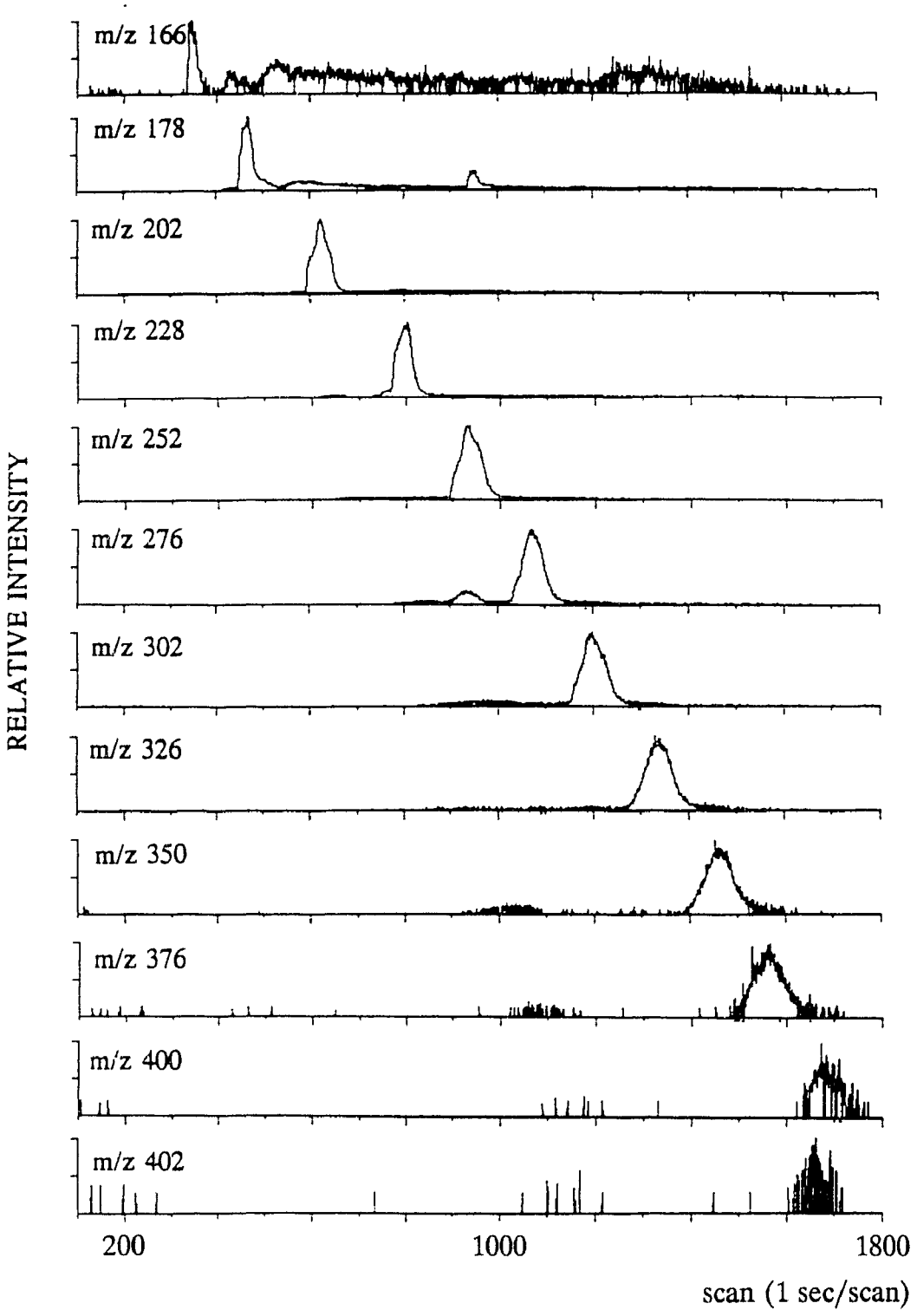


Figure 7.1.5: High temperature GC/MS RICs obtained for the TPE extract (Al-Clad column)

7.1.2 GC/MS of the Syncrude samples

7.1.2.1 GC/MS of the HGO sample

The HGO sample is a high boiling point (> 343°C) fraction obtained from the distillation of hydrocracked products (Section 1.5). However, it is possible to observe overlapping between the distillation fractions (lighter compounds in heavy ends and vice-versa) and thus GC/MS can be used to detect any volatile compounds present.

An aliquot of the HGO PAC fraction, obtained from silica over alumina column chromatography, was injected on a DB-5 column, mounted inside the GC oven of the Finnigan 4500 GC/MS system. As the column could not be heated beyond 350°C, most material was expected to stay on the column and therefore it was decided to not sacrifice a brand new GC column and to use an old one instead.

The results obtained are shown in Figure 7.1.6. In the TIC trace, no chromatographic separation is observable. Compounds whose boiling point is below 350°C were eluted as a broad envelope and their mass spectra (not shown) indicated the coelution of several alkylated PACs with masses ranging from 200 to 800, as detectable by the mass spectrometer. The other portion of the sample (B.P. > 350°C) was never eluted. The column was reconditioned by cutting off the injection end and by successive injections of dichloromethane under conditions of temperature programming. The reconditioning was successful.

No further attempt to use GC/MS was made for the HGO and PIT samples, the PIT corresponding to a higher temperature distillate cut than HGO.

7.1.2.2 GC/MS of the OSE sample

Group-type fractionation of petroleums and alternative fuels has been studied by Lancas and co-workers (178). They tentatively identified 20 compounds contained in an isolated dicyclic aromatic fraction of Brazilian sugarcane bagasse liquefaction products. These compounds were naphthalenes, C1 and C2-naphthalenes, biphenyl,

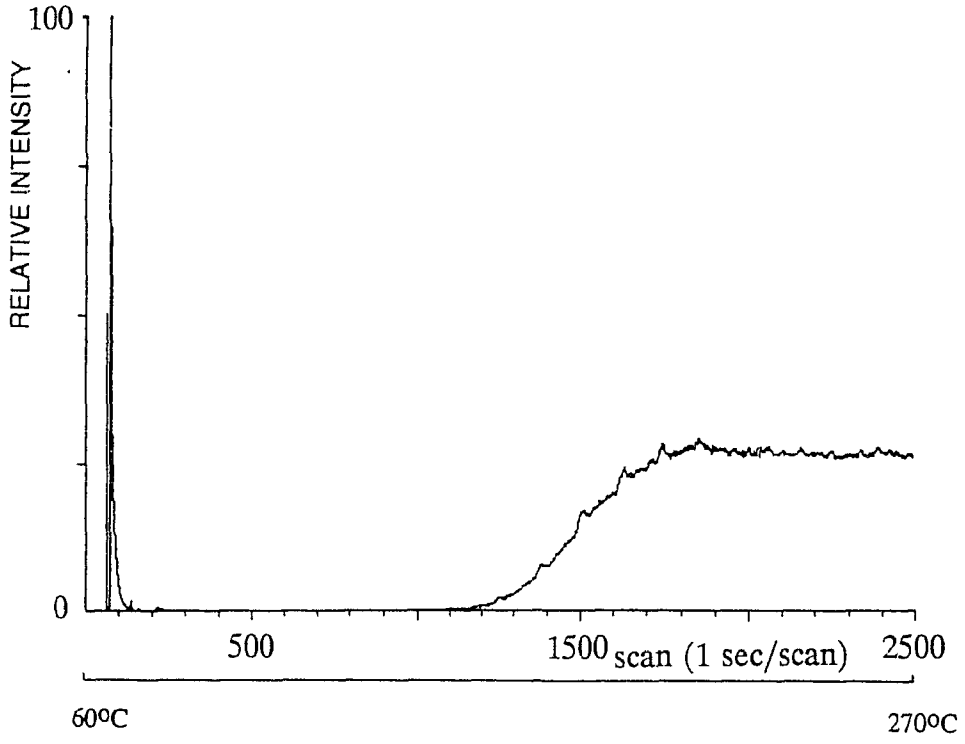


Figure 7.1.6: GC/MS total ion chromatogram of the HGO sample (column: DB-5)

acenaphthene, C1-acenaphthene and dibenzofuran. Lauer and co-workers (26) improved the characterization of coal tar distillation cuts (200-500°C) using acidic silica gel column chromatography and GC/MS as the final determination method. C0-C3-naphthalenes, fluorenes, phenanthrenes, pyrenes, benzofluorenes as well as C0-C2-biphenyls, acenaphthenes and dibenzofurans were among the species identified in these samples (26). These findings (26,178,179) are quite important in relation to this work, because they are useful in the characterization of the contents of the HGO, OSE and PIT. Comparing the GC/MS results obtained for the OSE to those of references 27 and 176 shows the similarity in the nature of compounds present in the OSE and in the two other oil-type samples (26,178). Lauer et al. (179) extended their study to the characterization of neutral and basic nitrogen containing aromatic compounds in an isolated fraction of the same sample. Alkyl quinolines, azaacenaphthenes, indenopyridines, azafluoranthenes and carbazoles were among the species characterized.

The OSE has not been subjected to any distillation process and therefore should contain compounds with lower molecular weights and boiling points than the HGO sample and these compounds might be characterizable by GC/MS. Here again, an old DB-5 column was used in order not to overload a brand new one with non-volatile material.

Figure 7.1.7-a shows the first part of the resulting GC/MS TIC trace. The second part of the chromatogram is not displayed, as it formed a large envelope, identical to that obtained for HGO. The main peaks in Figure 7.1.7-a were mass-assigned using their corresponding mass spectra, some of which are shown in b. A short list of compounds detected is given below.

- C2-benzenes (M.W. 106)
- C3-benzenes (M.W. 120)
- Indole (M.W. 117)
- C4-benzenes (M.W. 134)
- C1-indan (M.W. 132)
- Naphthalene (M.W. 128)

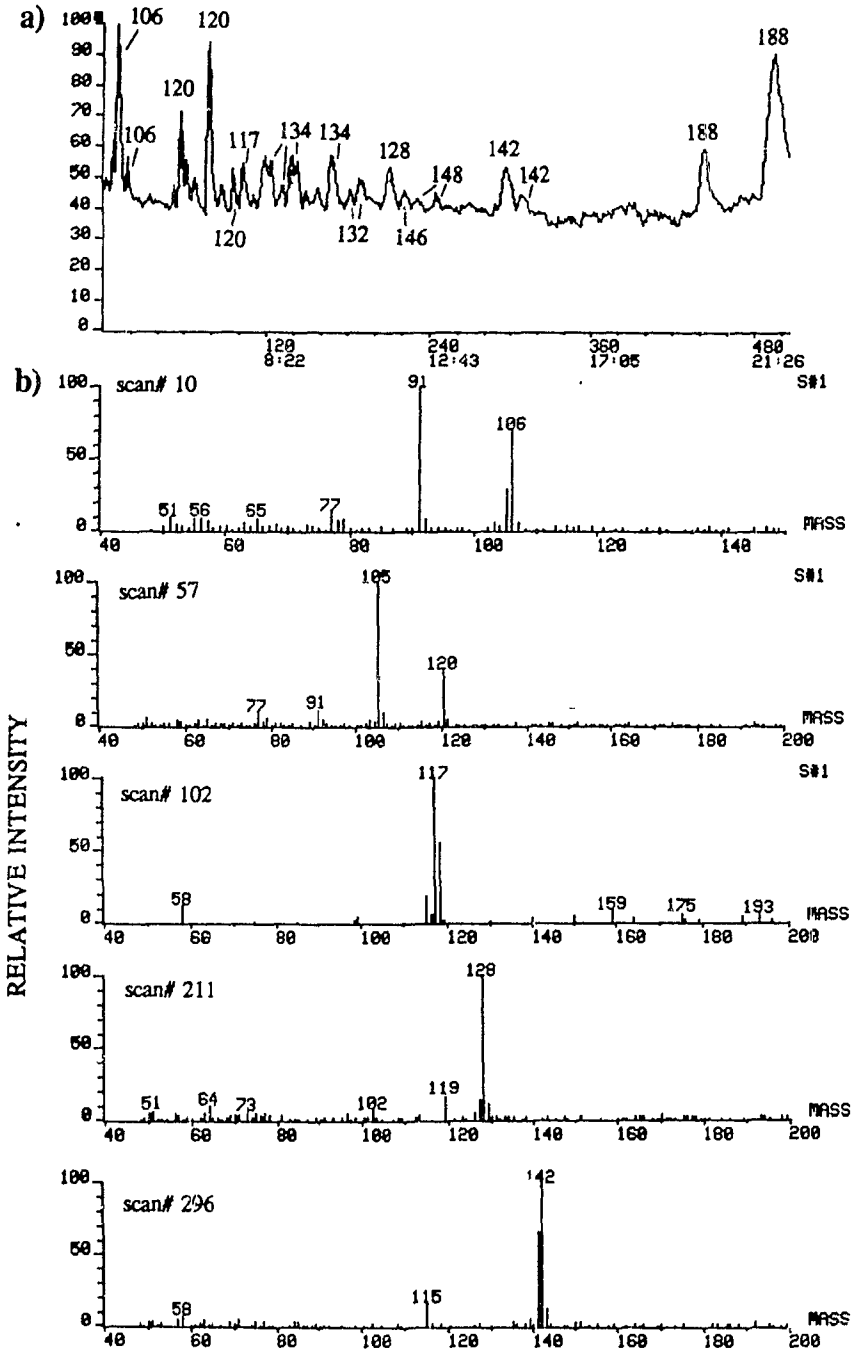


Figure 7.1.7: GC/MS a) TIC and b) mass spectra of the OSE sample. The numbers on the peaks correspond to the masses of the eluting compound(s).

- C2-indan (M.W. 146)
- C5-benzenes (M.W. 148)
- C1-naphthalenes (M.W. 142).

Serious efforts to identify each compound in this chromatogram were not pursued, since no internal standards were present in the mixture to allow the calculation of retention indices. Moreover, the compounds of interest for the purposes of this project are contained in the high boiling point portion of the samples. An examination of the very low boiling point portion of the OSE gives a good appreciation of the nature of the sample in general, i.e., of what to expect from the analysis of the heavier ends.

7.2 HPLC/MS

HPLC/MS is one of the most powerful techniques for the characterization of polycyclic aromatic compounds in complex matrices. Normal phase HPLC separates PACs into more groups (number of rings and polarity), whereas reversed phase HPLC allows greater selectivity in the separation.

The moving belt has proven to be a reliable and efficient HPLC/MS interface for the analysis of PACs (12). By use of a post column split whereby one half of the eluent was passed through a photodiode array spectrometer (DAD) and the other half delivered to a mass spectrometer via the moving belt interface, it was possible to obtain simultaneous EI mass spectra and UV-visible spectroscopic data (12). However, in that reported work, the heaviest compounds to be characterized were the group of PAHs of M.W. 302. Further work on the same sample (180) has extended the mass range to include PACs of M.W. up to ca. 350.

Other workers have used a combination of the moving belt interface with HPLC to characterize compounds with M.W. up to ca. 380 in heavy petroleum streams (145).

In all cases (12,145,180,181) it was emphasized that the ability to monitor the separation on-line, via the moving belt interface, offered significant advantages over

off-line MS analysis of collected fractions. These advantages include the ability to quantify many different species in one run using reconstructed ion chromatograms. The same approach also avoids the problem of how to decide when to collect fractions. A major advantage of the belt is the facility with which both conventional EI (low or high resolution) and CI mass spectra can be obtained.

Most applications of the moving belt interface to the HPLC analysis of PACs have been limited to compounds of molecular weights not much higher than the range accessible by normal GC/MS (300 Da. or so, although modern column developments are raising the upper limit). The sole motivation for pursuing HPLC/MS analysis of compounds in this mass range is the differing chromatographic selectivities that may thus be obtained. The separation of individual members of sets of isomers into clearly resolved chromatographic peaks, as can be done by reversed phase HPLC, makes quantification of target compounds more reliable.

Development of HPLC methods based on UV and/or fluorescence detection has involved separation of PACs of much higher molecular mass. Peaden et al. (128) reported on the separation of PACs of M.W. up to ca. 450 in a Carbon Black extract. They used a gradient elution scheme, beginning with 1:1 acetonitrile-water, proceeding through 100% acetonitrile, to ethyl-acetate and finally to 100% dichloromethane. The separation was monitored by fluorescence and by off-line MS analysis of the collected fractions.

Other workers (182-183) have applied both reversed phase and normal phase HPLC to the separation of compounds of M.W. up to ca. 600 in coal tar extracts, again monitoring the separation by UV absorption or fluorescence and by off-line EI mass spectrometry of collected fractions.

In a further series of papers, Fetzer and Biggs (117,118,184) have developed separation schemes for large PACs of M.W. up to 450. They have made extensive use of mass spectrometry for off-line confirmation of molecular mass by field desorption MS of compounds present in selected fractions.

The common feature of all these studies is that the on-line monitoring was done by various optical spectroscopic techniques, with all mass spectrometry being

done off-line.

The results reported here are all obtained from the HPLC on-line mass spectrometric monitoring of PACs in Sydney tar pond extracts and Syncrude samples.

7.2.1 HPLC/MS of the TPE

The TPE PAC fraction obtained from the Ramos and Prohaska method (9) was analyzed by HPLC/MS, first using a conventional elution gradient for PACs amenable to GC/MS analysis (12).

A second HPLC/MS analysis of the same sample was performed, in order to examine the high M.W. compounds present in the mixture; compounds up to mass 402 had been observed using high temperature gas chromatography (Section 7.1.1.2). The results obtained from these two series of investigations will be presented in different sections.

7.2.1.1 Analysis of compounds in the range accessible to GC/MS

If the TIC trace obtained by GC/MS (Figure 7.1.1-a) resembles that of a FID, the TIC trace obtained by HPLC/MS using the moving belt interface (Figure 7.2.1a) certainly does not match a clean 254 nm UV absorption trace (12). Instead it appears as a noisy chromatogram with intensity maxima repeated every 115 seconds, the time required for a full cycle of the belt.

Continuous mass spectral noise is produced by the ionization of the polyimide coating of the belt, which produces phthalate-related ions at m/z 113,149,167 and 279, as indicated in Figure 7.2.1b. These ions should have constant intensities throughout the chromatographic run, as the belt composition is expected to be homogeneous. However, the RIC at m/z 279 (not shown) has the same cyclic intensity pattern as the TIC and thus it is thought that some parts of the belt are more heavily contaminated with volatile material than others. The cyclic noise is also due to the belt nose heater, whose heating cycle has about the same duration as that

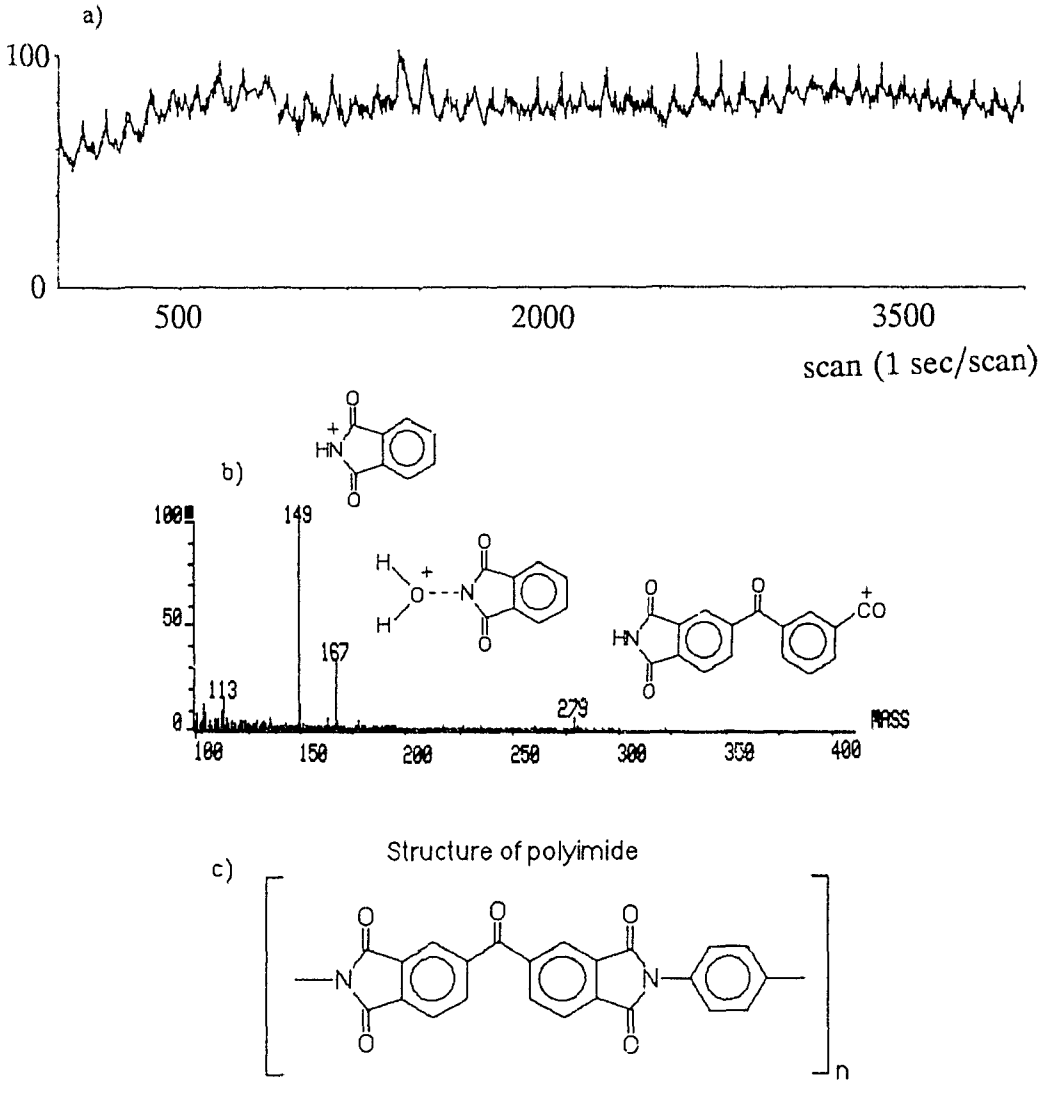


Figure 7.2.1: a) Reversed phase HPLC/MS TIC trace (TPE); b) mass spectrum of the belt background with structures corresponding to the peaks at m/z 149, 167 and 279; c) structure of polyimide.

of one belt cycle. However, the relative intensities of the ions in the belt background mass spectra remain constant throughout a chromatographic run. Therefore the belt background spectra can easily be subtracted from any spectrum of interest in order to obtain clear data. Fortunately, the belt background noise does not interfere with the RIC plots, as shown in Figure 7.2.2.

The elution orders observed in reversed phase HPLC are not the same as those observed in GC. General trends for separations on C_{18} reversed phase HPLC columns have been described (2). Solutes are retained by the hydrophobic (or less polar in general) stationary phase in order of decreasing polarity. Besides hydrophobic phase solubility effects, some residual adsorption and modifying effects of the mobile phase are likely to occur, for instance the production of a "binary" phase through the entrapment of some solvent molecules within the surface structure. There is no particular difference in the basic retention order of parent PAHs on reversed phases, as compared with adsorbents or conventional stationary phases (2). The hydrophobic interaction is, however, very important in the retention of alkylated PAHs. Compounds with side chains are more soluble in hydrophobic stationary phases and thus retained longer in reversed phase systems. Alkyl substitution thus causes an appreciable increase in retention, due also to the decreasing solubility in the polar mobile phase (e.g. acetonitrile-water mixtures). Reversed phase HPLC is therefore preferable for separating compounds with different side chain number and/or length.

It has been observed that for compounds with the same number of rings, the elution proceeds as follows, in order of retention time: PAH derivatives (polar), PAHs and alkylated PAHs. For instance, indeno[1,2,3-ij]isoquinoline (1-azafluoranthene) elutes ahead of fluoranthene which is followed by C1-fluoranthenes. The elution order among unsubstituted PAHs goes by increasing number of rings, however the reason for differing retention times within a set of isomers with the same number of rings is still not well understood. Wise and co-workers (185) have proposed a dependence of the retention in C_{18} reversed phase HPLC on the shape

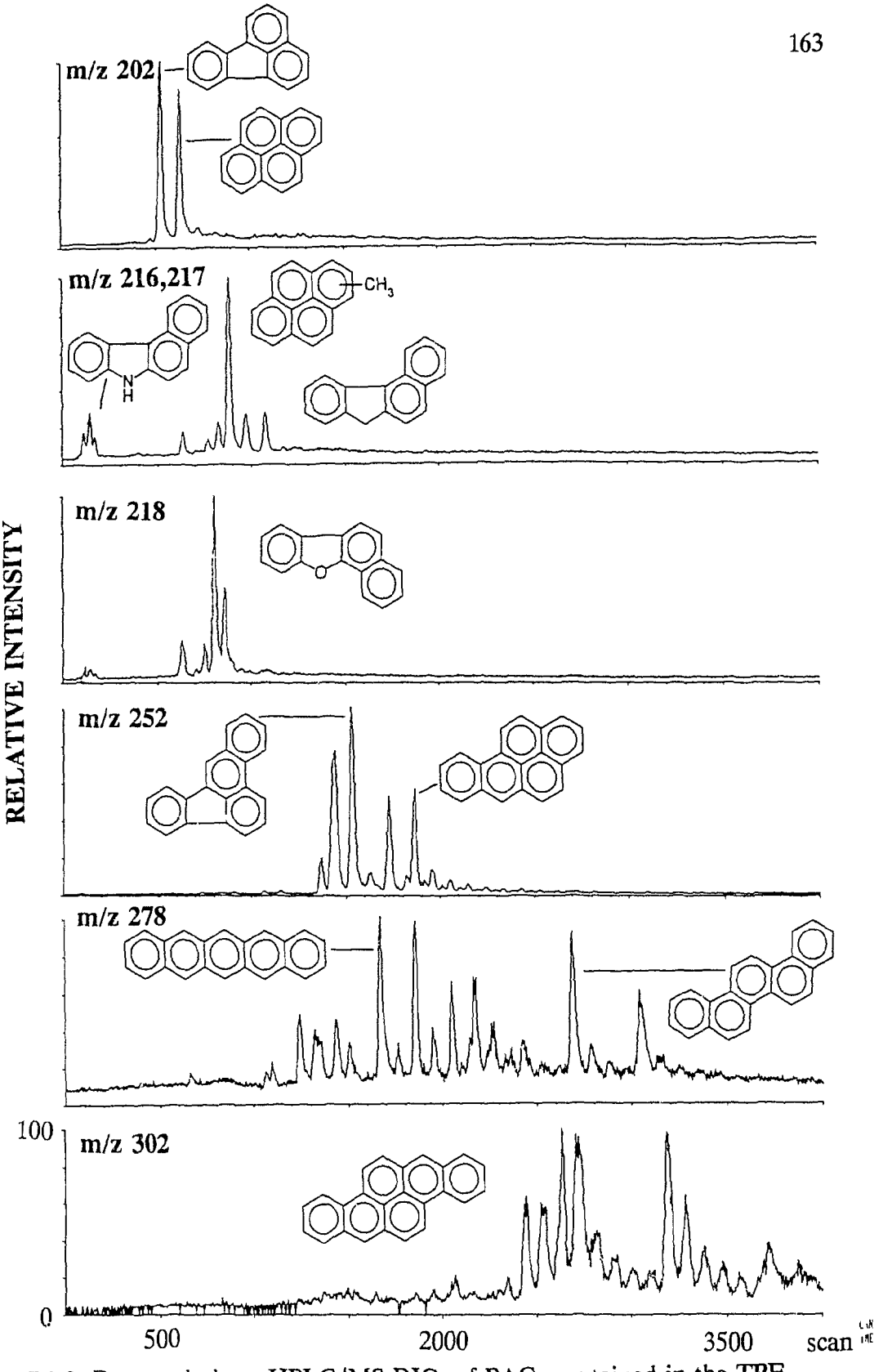


Figure 7.2.2: Reversed phase HPLC/MS RICs of PACs contained in the TPE sample in the GC-amenable range

ratios in the parallelograms that best frame the PAH planar structures. It has also been shown (2) that the order of elution of PAH isomers (on the same stationary phase) can be modified by changing the composition of the mobile phase, the elution gradient and the column temperature.

The HPLC/MS peaks observed in Figure 7.2.2 are wider than GC/MS peaks (Figures 7.1.1 and 7.1.2), as diffusivity of solutes in liquid phases is lower than in gas phases. However, reversed phase HPLC has the distinct advantage of greater selectivy in terms of isomer discrimination. To illustrate this point, the separation of isomers of M.W. 302 by HPLC as compared to GC offers a good example. In GC/MS, all the isomers are eluted over a period of 6 minutes in ten well defined peaks of ca. 40 seconds width. In HPLC/MS, the m/z 302 chromatogram is obviously more spread and sixteen peaks of ca. 73 seconds width elute over a period of 28 minutes. HPLC allows the resolution of 6 more isomers than GC. The same type of discussion also applies to the separation of isomers of M.W. 252 and 278.

Although isomer discrimination is improved using reversed phase HPLC compared to GC, the identification of individual isomers was more difficult in HPLC for the following reasons:

- UV spectra obtained from the DAD were those of mixtures of coeluting compounds and thus could not be compared to those of standards
- EI mass spectra do not help in discriminating PAC isomers
- reversed phase HPLC retention index systems are not as well established as they are in GC. May and co-workers (186) have developed an aqueous PAH standard reference material and their group is presently working at developing a complete RI system for PAHs.

A few reports have been published on isomer discrimination using HPLC. Quilliam and Sim (12) used HPLC/MS simultaneously with UV to confirm compound identities in PAC environmental samples; Wise et al. (187) compared the selectivities of different C_{18} reversed phase HPLC columns for PAH separation, and particular attention was given to isomers of mass 228 (triphenylene, benz[a]anthracene, chrysene) and 242 (methyl-chrysenes). The authors confirmed the

identity of compounds by comparing their retention times with those of standards obtained from previous runs: no UV or mass spectra were used for identification purposes.

The reversed phase HPLC/MS results obtained here are summarized in Appendix II, a Lotus 1-2-3 spreadsheet that contains retention time and mass spectral information. Each ion labelled with an asterisk is most probably a molecular ion. Most of the labelled ions could not be identified; some of them were however assigned names such as "128 PAH-C1" (methyl naphthalene), etc. The tentative identification of compounds was achieved by comparing RICs with those found in the literature (12) and with HPLC/UV chromatograms (12,109,113-115,187).

Compounds with M.W. 216, 217 and 218 are of particular interest because each of the three nominal masses corresponds to a different class of PACs. Compounds with M.W. 216 are benzofluorenes, methyl-pyrenes and methyl-fluoranthenes (PAHs); compounds with M.W. 217 are benzocarbazoles (PANHs) and finally M.W. 218 relates to dibenzofuran and its isomers (PAOHs). The selectivity of separation among these three groups of isomers is quite different from that observed in GC. Figure 7.2.3 illustrates the differences in selectivity obtained for those compounds between GC/MS and HPLC/MS. In GC/MS, M.W. 218 PA-furans (PAFs) and PA-xanthenes (PAXs) are eluted first, immediately followed by M.W. 216 PAHs. M.W. 217 PANHs come shortly after, clearly separated from both other groups. In HPLC/MS, M.W. 216 PAHs are eluted second, well after the 217's and slightly ahead of M.W. 218 PAFs and PAXs. Benzocarbazoles of M.W. 217 are eluted very early in the run, clearly showing the influence of the hydrophobicity and selectivity of the reversed stationary phase.

Appendix II is a compilation of data as obtained by scan number, i.e. as a function of time. Since each scan number corresponds to a different mass spectrum, it is possible to track down the main molecular ion(s) and fragment(s) and it is thus easy to recognize unsubstituted PAHs and their derivatives. As an example, the mass spectrum at scan 630 has only one major ion, m/z 202. There is hence a strong possibility that this compound is an unsubstituted PAH (no fragment). However, in

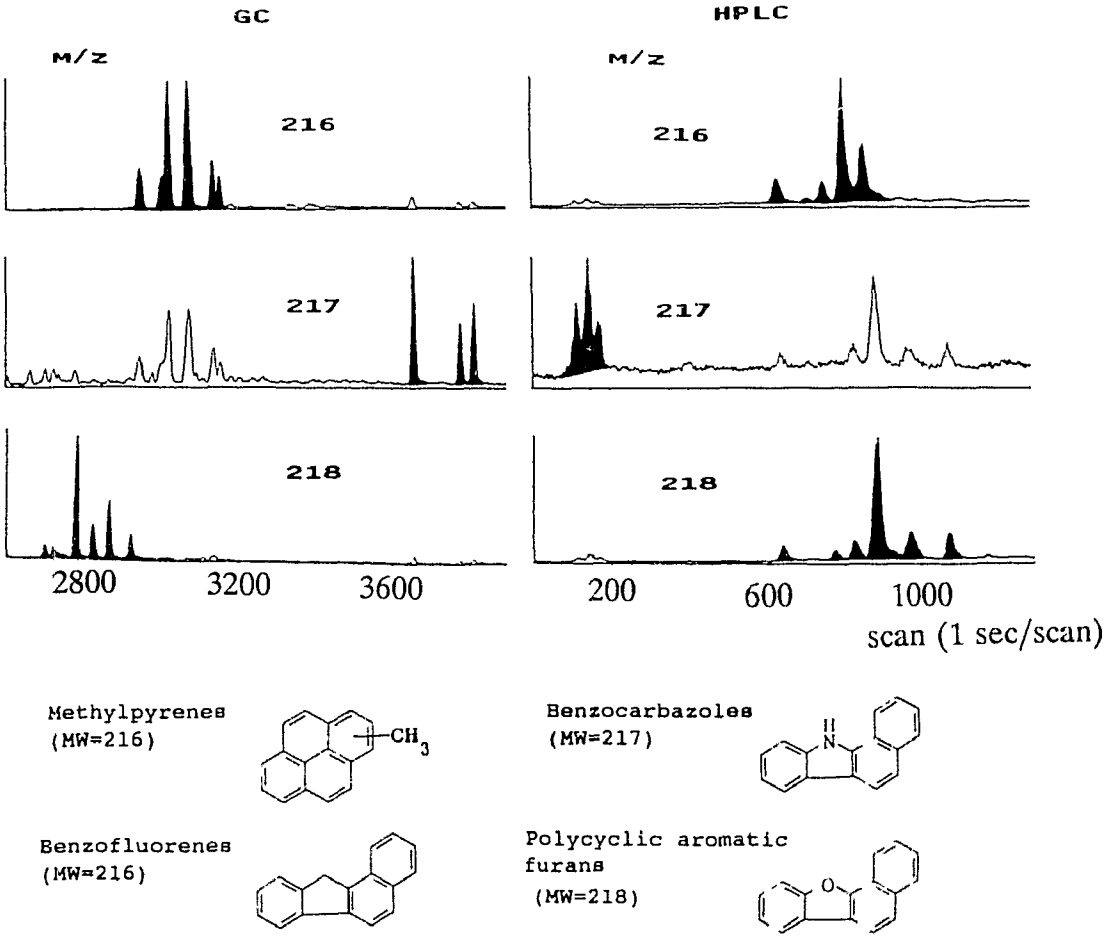


Figure 7.2.3: GC/MS and HPLC/MS RICs (m/z 216, 217 and 218) showing the complementary separation selectivity of the two methods for different classes of PACs contained in the TPE.
Shaded peaks are those due to molecular ion species.
Some example structures are also given.

scan 758, the presence of two ions, 206 and 191, differing by 15, indicates an alkylated compound, and the same comment applies to scan 904 (m/z 210 and 195). Scans 1657 and 1850 (m/z 265, 266), 194 (m/z 165, 166) and 1074 (m/z 215, 216) all show the characteristic combination of equal intensity $M^{+\cdot}$ and $(M-H)^{+\cdot}$ ions of compounds containing a methylene group.

Figure 7.2.4 shows some EI mass spectra obtained for PACs from the same HPLC/MS data set. Once the background noise has been subtracted, they are clean and identical to those obtained by GC/MS. The interference-free mass spectrum of an isomer of M.W. 302 is compared to its analog without background subtraction; while in the former m/z 302 is the most intense ion, this ion is barely observable in the latter.

The HPLC conditions used for this characterization were well established; acetonitrile-water was found to be the most suitable mobile phase for reversed phase HPLC of low molecular weight PACs and has been used in many laboratories (2,12,113,185). The flow rate used to obtain the data presented here was 0.6 mL/min, adjusted to provide the best transfer of material onto the moving belt, and a source pressure suitable for EI conditions. However, the optimal flow rate for a 4.6 mm i.d. column is 1 mL/min, which could not be handled well by the pumping system of the mass spectrometer.

In summary, reversed phase HPLC/MS of PACs in the GC amenable range has been shown to be complementary to GC/MS, giving rise to the following observations.

- Certain compounds from different classes of PACs have different retention behaviors in HPLC and GC. For instance, compounds of two different classes may coelute in GC, and be reasonably well separated in HPLC, or vice-versa.
- The identification of compounds by GC/MS can be easily achieved due to the availability of many retention index tables. RPLC/MS compensates for its lack of universality (availability of Ris) by very good selectivity for isomer discrimination on a C_{18} column.
- Even if isomer discrimination is improved by using HPLC/MS instead of GC/MS,

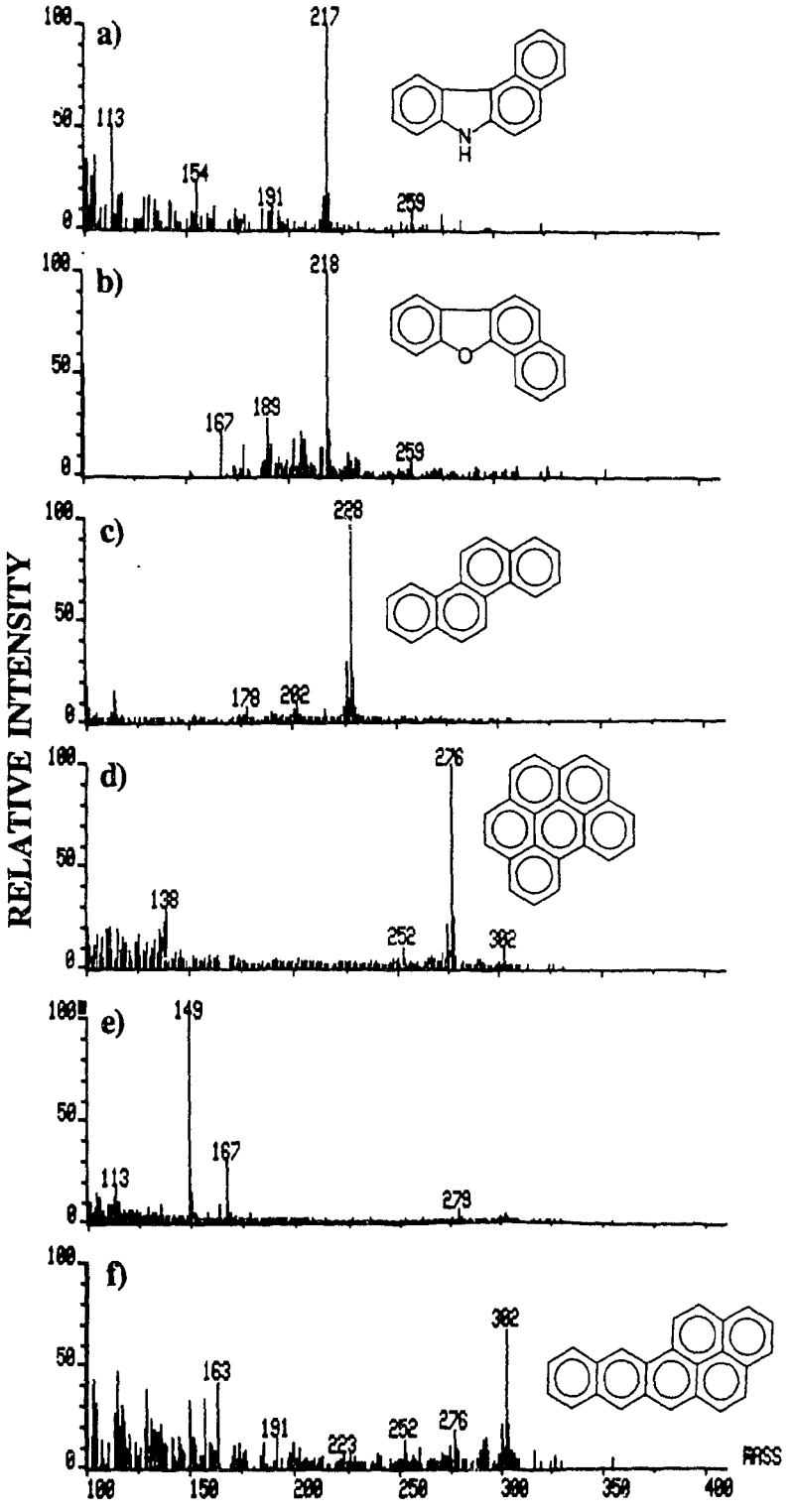


Figure 7.2.4: Mass spectra of PACs obtained by reversed phase HPLC/MS. The spectra in e) and f) respectively correspond to the background non-subtracted and subtracted spectra of a PAH species of M.W. 302.

extensive coelution still occurs and can only be partially overcome in the analysis by plotting the RICs.

7.2.1.2 RPLC/MS analysis of the high M.W. portion of the TPE

As a preliminary to the HPLC/MS analysis of the high M.W. portion of the TPE PAC fraction obtained from the Ramos & Prohaska method (9), a series of experiments were performed with standard materials available in this laboratory. The structures of the standards studied are shown in Table 7.2.1. The results are presented in Figure 7.2.5, where the simultaneously acquired (via a 1:1 post column split) UV absorption and RIC chromatograms of a synthetic mixture of standards are shown. The numbers that label the chromatographic peaks correspond to those of Table 7.2.1. This work showed that, at least for compounds of M.W. up to 424, no significant belt memory effects were observed for sample deposited onto the belt via the spray deposition device. This observation is somewhat surprising, in view of the low volatility of many PAHs of high molecular mass. Interestingly, belt memory effects were observed in cases where the same compounds were spotted directly onto the belt using a syringe. Under the HPLC gradient conditions employed, all of the compounds shown in the RICs of Figure 7.2.5 were eluted in acetonitrile-dichloromethane mixtures or in 100% dichloromethane (DCM). Therefore, DCM solutions were used for spotting onto the belt, in order to provide a fair comparison with HPLC/MS conditions. It appears that the mode of deposition has a profound effect on the extent to which compounds can adhere to the polyimide belt. Memory effects are also observed if the temperature of the ionization source is kept too low. In some applications, it may be necessary to increase the source temperature during the later stages of the chromatographic run. Keeping a high temperature from the start of a run may cause poor transfer of some PACs of lower molecular weight and higher volatility into the ionization volume.

Peak widths in the RICs were identical to those found in the UV absorption chromatogram, a further indication of good transfer by the belt interface.

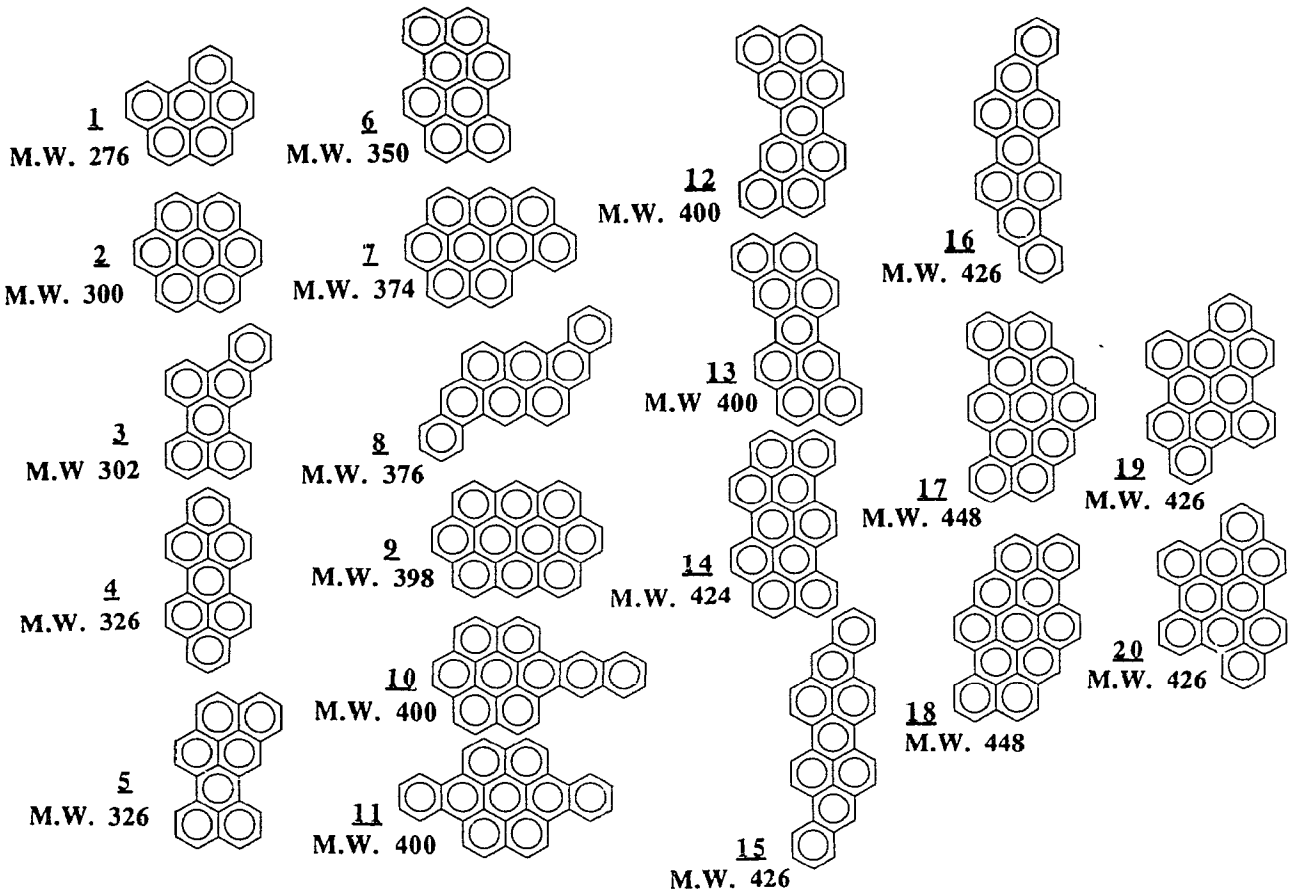


Table 7.2.1: High molecular weight PAH standards used in reversed phase HPLC-UV and -MS

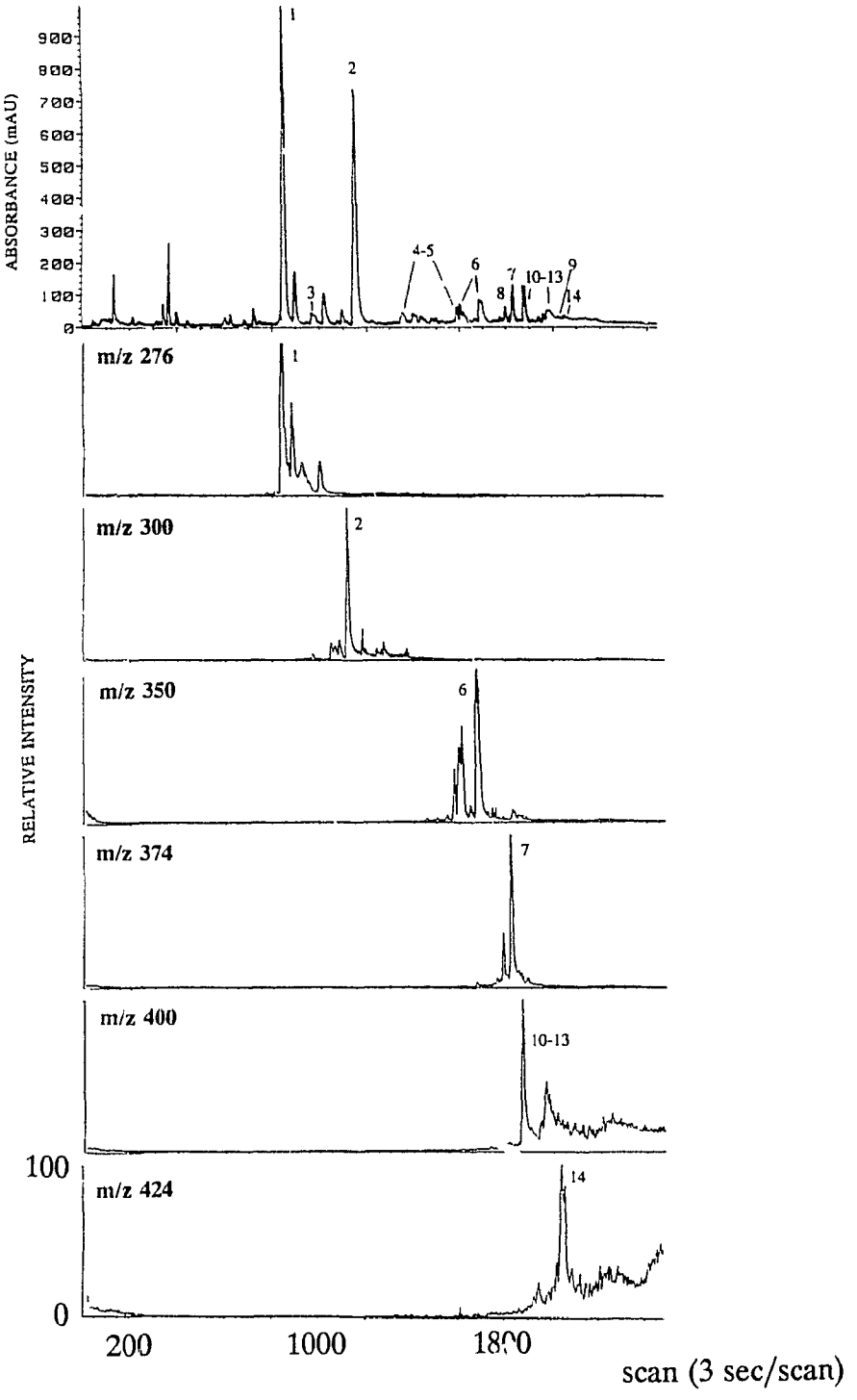


Figure 7.2.5 Reversed phase HPLC-UV absorption chromatogram (254 nm, top trace) and HPLC/MS RICs obtained for a mixture of high molecular weight PAH standards. Numbers next to the peaks refer to Table 7.2.1.

It is also apparent that many of the standards currently available contain significant amounts of isomeric impurities, which can severely hamper attempts to quantify individual compounds. Compounds of mass 426 were included in the standard mixture, but were never observed in the chromatogram (either HPLC/MS or HPLC/UV) and thus they might have decomposed to smaller compounds, giving rise to extra peaks in both UV and TIC traces.

Typical mass spectra of some of the standard compounds (not shown) are similar to those of smaller PAHs, in that they are dominated by molecular ions.

Similar series of chromatograms, obtained from an analysis of the TPE and covering the mass range 300-580 Da, are shown in Figure 7.2.7 and 7.2.8, while the 254 nm UV absorption trace is presented in Figure 7.2.6. These results show that the moving belt interface is an effective way of providing on-line monitoring of HPLC separations of these compounds with masses up to at least 580 Da. It is also worth noting that using chlorobenzene as the extraction solvent leads to a high recovery of high M.W. PACs. This observation had been made elsewhere in the context of petroleum chemistry (32).

As a qualitative indication of the apparently good chromatographic behavior, the column efficiency may be calculated from the results of Figures 7.2.5 and 7.2.8. For benzo[ghi]perylene (m/z 276, elution time 42.2 minutes, k' = 13.1) in Figure 7.2.5, the effective number of theoretical plates was calculated from the UV chromatogram peak width as 9888. For the unknown of m/z 580 (elution time 86.5 min., k' = 27.8, Figure 7.2.8), the number of plates was calculated from the mass chromatogram peak width to be 9604. In other words, no significant evidence of belt memory effects nor of degradation of column performance during the chromatographic run was observed over the full range of PAC analytes.

It also appears that the column performance, in terms of isomer separation, is better for the cata condensed than for the peri condensed systems. Thus, PAHs of M.W. 328 Da (cata) elute over a longer retention time range than those of m/z 326 Da (peri), although the numbers of isomeric possibilities are comparable. The same situation is observed for the related pairs of isomeric sets of m/z 352 and 350. The

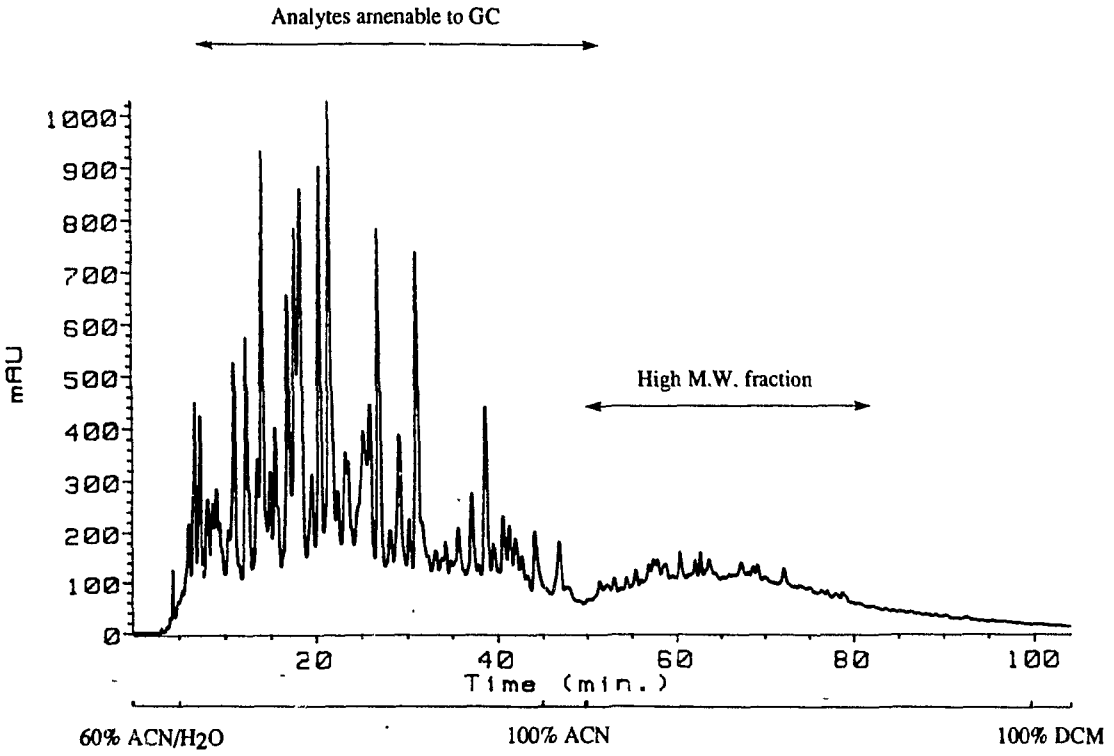


Figure 7.2.6: Reversed phase HPLC-UV absorption chromatogram (254 nm) of the TPE sample

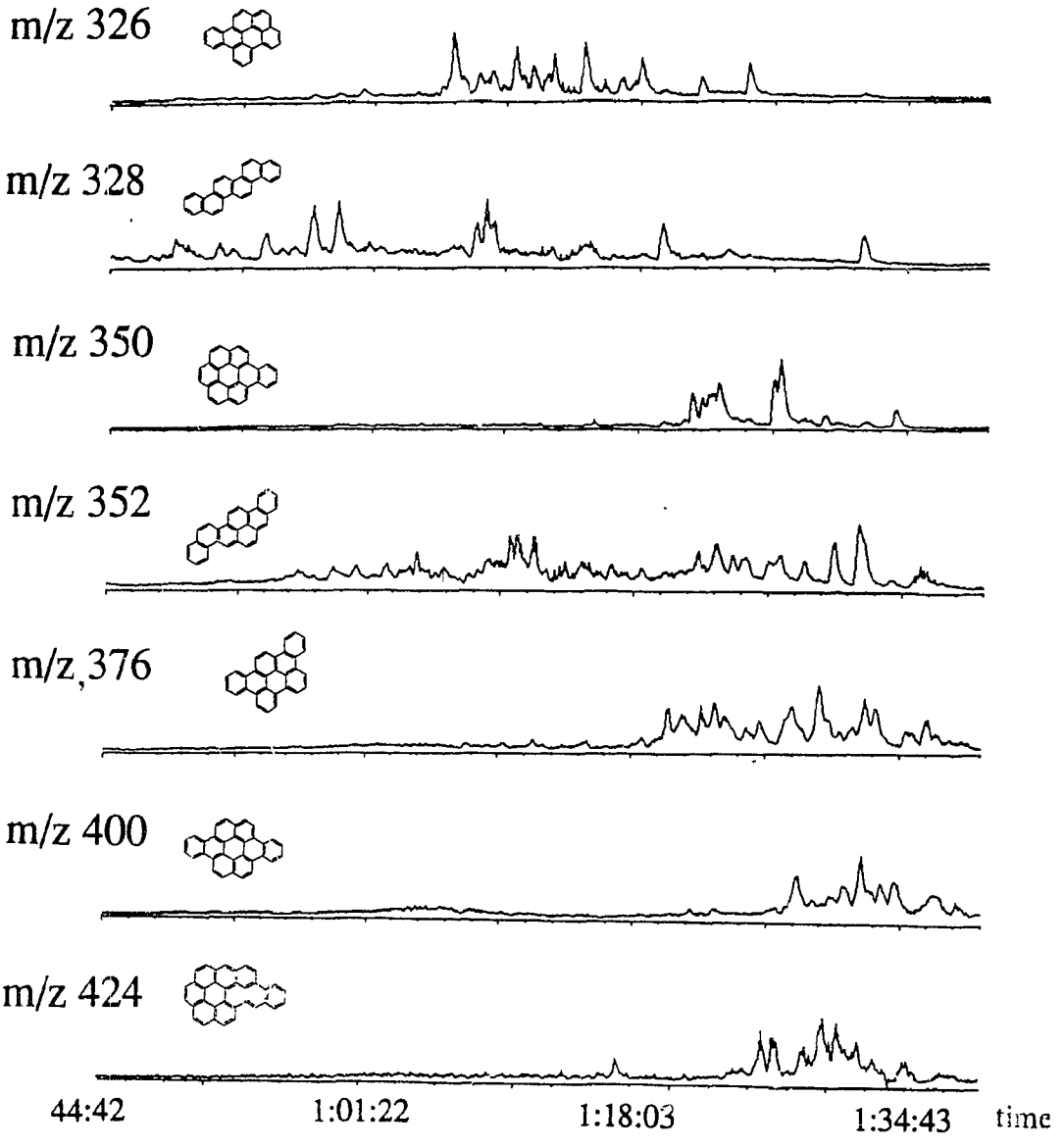


Figure 7.2.7: Reversed phase HPLC/MS RICs of the TPE sample obtained during the acetonitrile-dichloromethane portion of the gradient

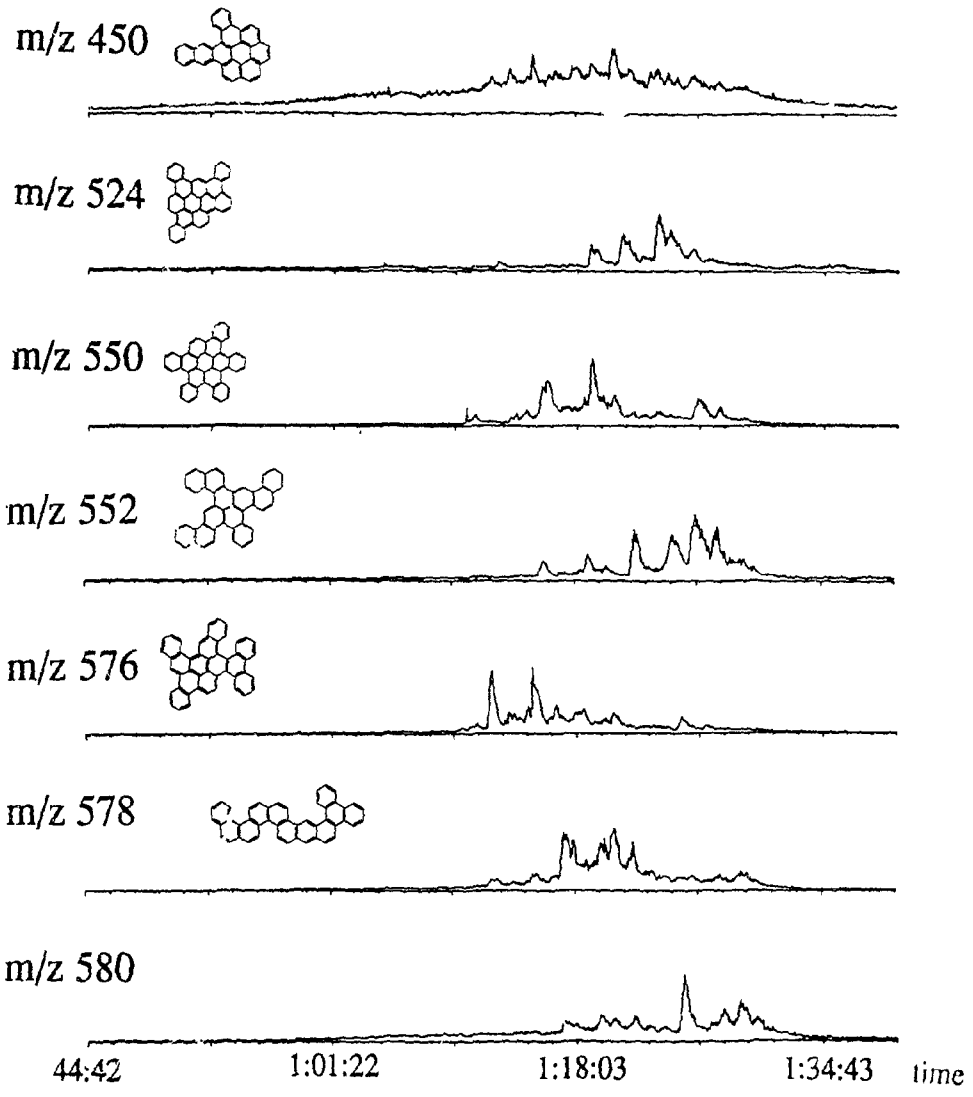


Figure 7.2.8: Reversed phase HPLC/MS RICs of the TPE sample obtained during the dichloromethane portion of the gradient

picture is less clear for heavier compounds, probably as a consequence of the rapidly increasing numbers of isomeric possibilities, but the general trend does persist.

Also, in general, compounds with molecular masses in the ranges 300-400 and 500-600 Da are apparently better separated than are their homologs in the 400-500 Da range, in that chromatographic peaks are sharper and better resolved. A comparison of mass chromatograms of m/z 350, 450 and 550 in Figures 7.2.7-8 illustrate this point. A reason for this apparent feature may be that the 400-500 Da compounds seem generally less concentrated in the sample than are the 300-400 and 500-600 Da groups. The lower signal to noise levels affect the observed peak shapes (ratio of peak heights to widths), making the separation appear, upon casual inspection of the chromatograms, poorer than it actually is. Some idiosyncrasy of the coking process, the type of coal used or some selective condensing process in the Tar Pond may explain the lower concentration of the 400-500 group compared to the others.

The apparently improved separation of cata condensed systems over that of peri condensed PAHs is almost certainly a manifestation of the well known molecular shape selectivity (separation as a function of the "length to breadth" ratio or relative planarity of the compound) of polymeric reversed phase columns (185). It is interesting that this known effect can be observed on molecules as large as those monitored in the present section of this work.

Work has been proceeding on identification of the actual compounds present but has been hampered, as noted elsewhere (109), by the lack of suitable standards. The ability of the belt interface to permit acquisition of good quality EI mass spectra of even larger compounds in the Tar Pond extract is evident from spectra shown in Figure 7.2.9. Each of these was obtained at the crest of one of the peaks in the mass chromatograms in Figures 7.2.7-8, and are shown as acquired, without background subtraction.

Because of the complexity of the sample, these mass spectra frequently show contributions from molecular ions of several different compounds. This is a consequence of the relatively low column efficiency (as compared to that obtainable

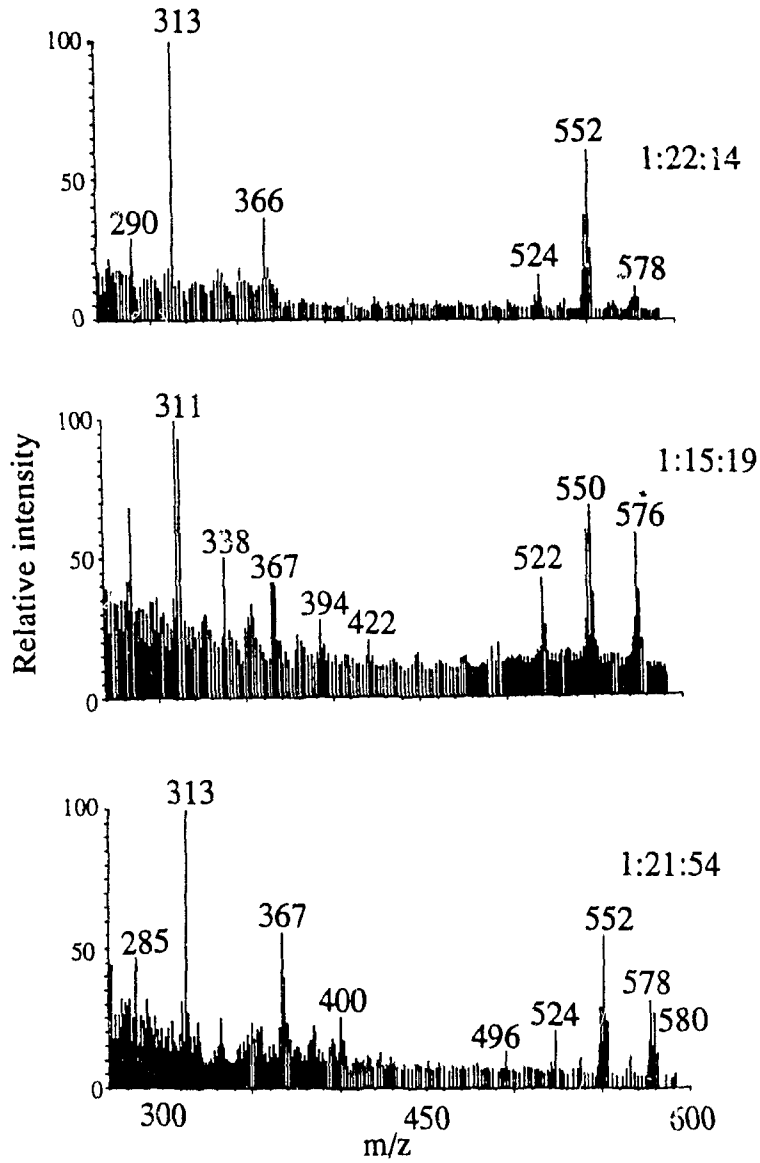


Figure 7.2.9: Mass spectra of high M.W. PAHs obtained by reversed phase HPLC/MS of the TPE sample. Numbers on the right indicate the retention times.

in GC) of the HPLC separation. However, in many cases such as those of compound(s) of m/z 350 eluting at 76.92 minutes and of compound(s) of m/z 550 eluting at 76.02 minutes, a single ion dominates the spectrum during that particular scan. And although the reversed phase HPLC employed here often yields coelution of many compounds, individual members of sets of isomers are generally widely dispersed in time. This is a prerequisite, together with the availability of authentic pure standards, for the identification of individual compounds.

The results obtained from this chromatographic run are summarized in appendix III; none of the compounds, with the exception of coronene at m/z 300 (the only possible PAH structure at this mass), has been identified. Mass spectral data as a function of time however show that compounds are continuously eluted throughout the run in discrete peaks rather than envelopes. Several high mass compounds, whose chromatograms were not shown in Figures 7.2.7-8, provided high intensity chromatographic peaks and mass spectra. The m/z values in the left column of the spreadsheet are bracketed within delimited scan ranges, which should ideally correspond to single scans. A RIC peak that is 67 seconds wide at half height, as is typical in this HPLC/MS experiment, will cover at least 22 scans (3 sec/scan). During these 22 scans, other compounds are likely to coelute and no valley will be observed between the peaks. This explains why, in Appendix III, placing these multi-scan segments into contiguous groups was difficult to achieve and very often compound peaks would be spread between such groups. This trend is apparent from scan 3790 up to scan 3810, where the brackets have been set with a high uncertainty level to delimit "single scans". Other scan ranges offer the same difficulty in assigning to every compound a single scan throughout the whole chromatographic run. This difficulty shows that the method of compiling chromatographic/mass spectrometric data in a spreadsheet becomes less convenient to use with large peak widths than it is with narrow peaks. However when characterizing mixtures as complex as the TPE, the spreadsheet is still an invaluable tool to study the overall contents of the sample.

To summarize, combining the moving belt mass spectrometric interface with reversed phase HPLC can afford a useful method for the on-line monitoring of

separations of large PACs in complex environmental samples. Since the mass spectra of PACs are dominated by molecular ions, the technique offers a good combination of retention and mass spectral data which can be invaluable in characterizing such samples, although unambiguous compound identification remains elusive due to the lack of authentic standards. With the additional information possible from the simultaneous acquisition of UV-visible spectra from a DAD, a powerful profiling technique can be realized (12). With a suitably chosen limited series of standard compounds, it may be possible to extend the retention index classification of PACs, well established for GC (106), to the HPLC determination of these compounds.

7.2.2 HPLC/MS of the Syncrude samples

It has been observed, from the HPLC/UV results presented in Section 6.2, that PACs are less abundant in the Syncrude samples than they are in the TPE. The HGO sample contains a reasonable amount of material at relatively low molecular weight which produces acceptable chromatography in reversed phase HPLC. This sample however contains many very high molecular weight compounds, whose elution is characterized by a large envelope in the HPLC chromatogram. The OSE contains less low molecular weight material ($150 < M.W. < 300$) than the HGO, and more heavy material that is difficult to resolve. The third sample, a pitch residue from the distillation of hydrocracked products (PIT), has been shown to contain high molecular weight material only, which is expected since the sample should boil at temperatures above 500°C.

It was necessary to take a closer look at the contents of these three samples using HPLC/MS, since no significant results could be obtained by GC/MS. The GC/MS analysis of the volatile compounds contained in the OSE (Section 7.1.2) showed the presence of alkylated PAHs. Similar compounds are thus expected to be found by HPLC/MS. The HGO, OSE and PIT samples chosen for HPLC/MS analysis are the PAC fractions obtained from the silica over alumina chromatography method (Section 2.4.1.3).

Table 7.2.2: Alkylated PAHs present in the standard solution for HPLC/MS analysis

compound	molecular weight
3-methylfluorene	180
1-methylanthracene	192
9-methylanthracene	192
2-methylphenanthrene	192
9-ethylfluorene	194
9,10-dimethylphenanthrene	206
3,6-dimethylphenanthrene	206
2-ethylanthracene	206
9,10-dimethylanthracene	206
1-methylpyrene	216
2-t-butylanthracene	234
9,10-dimethylbenz[a]anthracene	256

As a preliminary experiment, a solution of standard alkylated PAHs was prepared in acetonitrile, for the purpose of comparing the HPLC/MS retention times of the standards to those of the unknowns. The standard compounds used are listed in Table 7.2.2.

These compounds all have relatively low molecular weights and thus this solution will help in identifying sets of isomers in the first part (water-acetonitrile) of the HPLC/MS run. Figure 7.2.10 shows the HPLC/MS RICs obtained for the m/z values of the compounds listed in Table 7.2.2. For more convenience, the average retention times for each set of isomers, instead of individual retention times, are listed in Table 7.2.3. These values, as well as the RICs of Figure 7.2.10, will be referred to later to help in identification of some of the sets of isomers in the

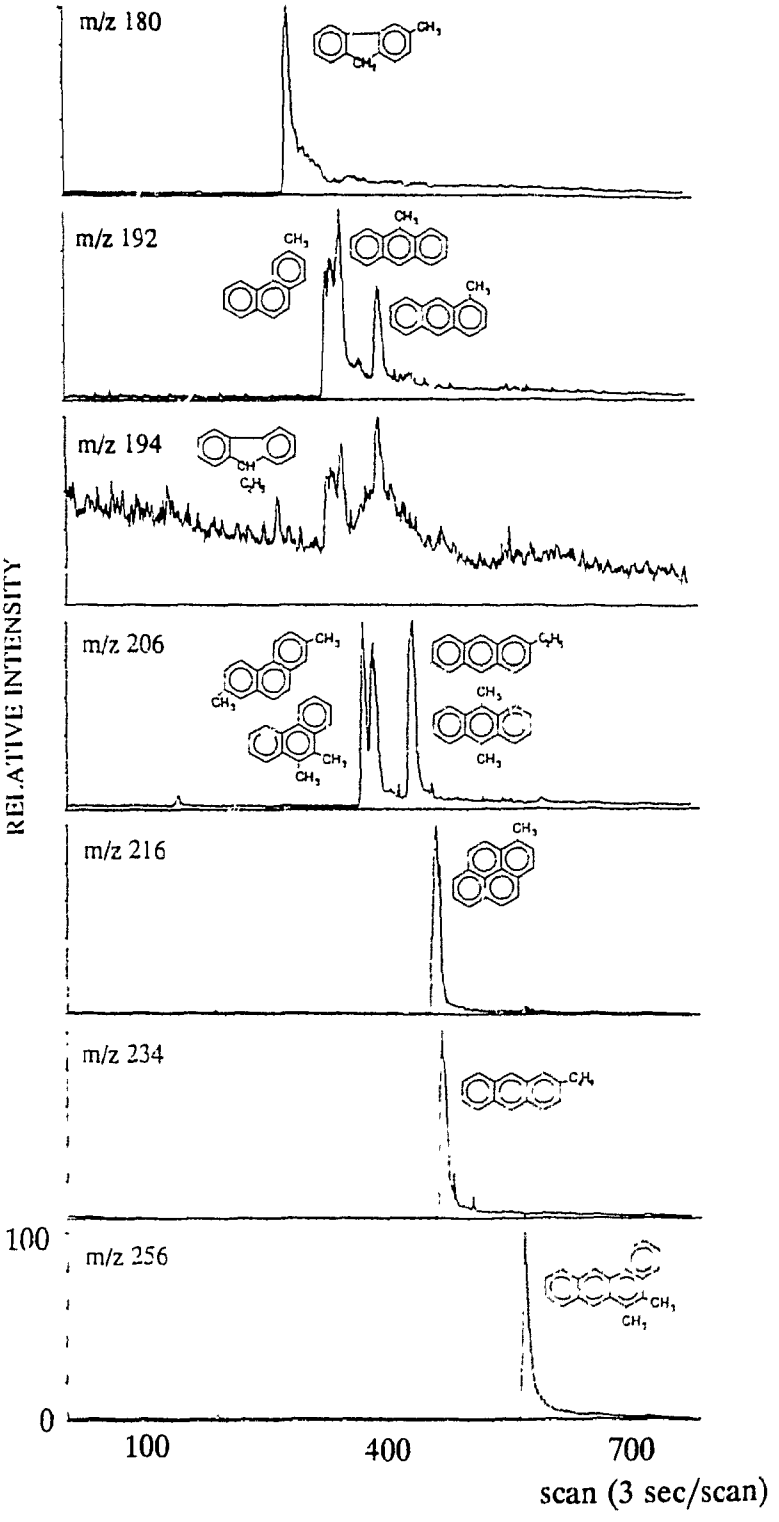


Figure 7.2.10:Reversed phase HPLC/MS RICs of a mixture of alkylated-PAH standards

Table 7.2.3: Average retention scan no. of isomers contained in the standard solution

M.W. of isomer set	retention scan number
180	282
192	360
194	265
206	410
216	460
234	468
256	571

unknowns. Retention in Table 7.2.3 is given as scan number and can be multiplied by 3 sec/scan to obtain the retention times in seconds.

7.2.2.1 HPLC/MS of the HGO sample

i) PAC fraction obtained from silica over alumina chromatography

In order to present the results clearly, the HPLC/MS data on this fraction have been divided into two parts: water-acetonitrile gradient (low M.W. compounds) and acetonitrile-dichloromethane gradient (high M.W. compounds).

Figures 7.2.11-14 shows some RICs obtained for the low M.W. part of the chromatographic run. Some of the traces (Figure 7.2.11) are readily compared to RICs obtained for the Tar Pond sample, for a better characterization of the unknowns. The complete HPLC/MS results obtained for the HGO as compared with the TPE and the standard alkylated PAH mixture are given in Appendix IV. In that Lotus 1-2-3 spreadsheet, only retention times and qualitative mass spectral

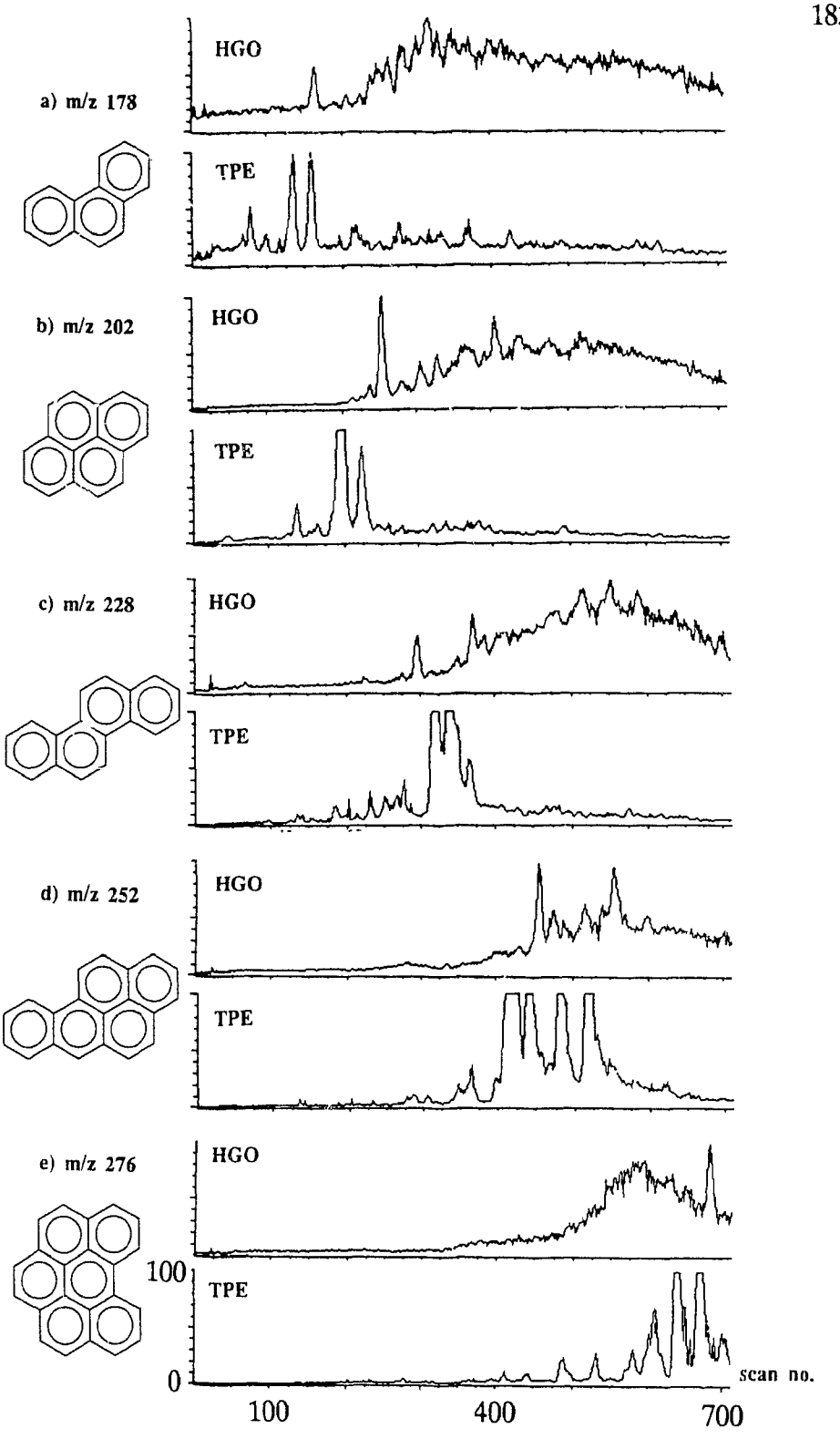


Figure 7.2.11:Reversed phase HPLC/MS RICs of some of the unsubstituted PAHs found in the HGO and in the TPE samples (low M.W. portion)

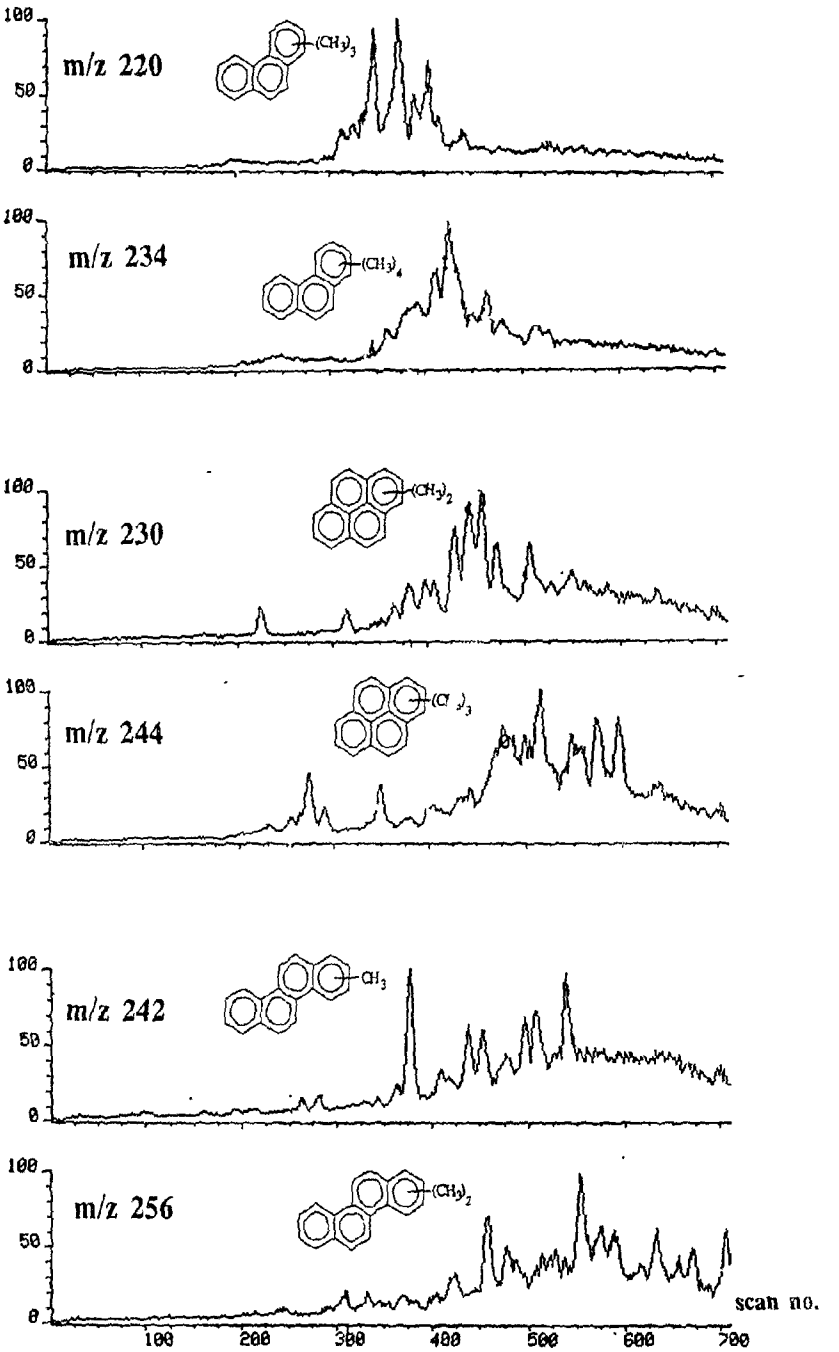


Figure 7.2.12:Reversed phase HPLC/MS RICs of some of the alkylated PAHs found in the HGO sample (low M.W. portion)

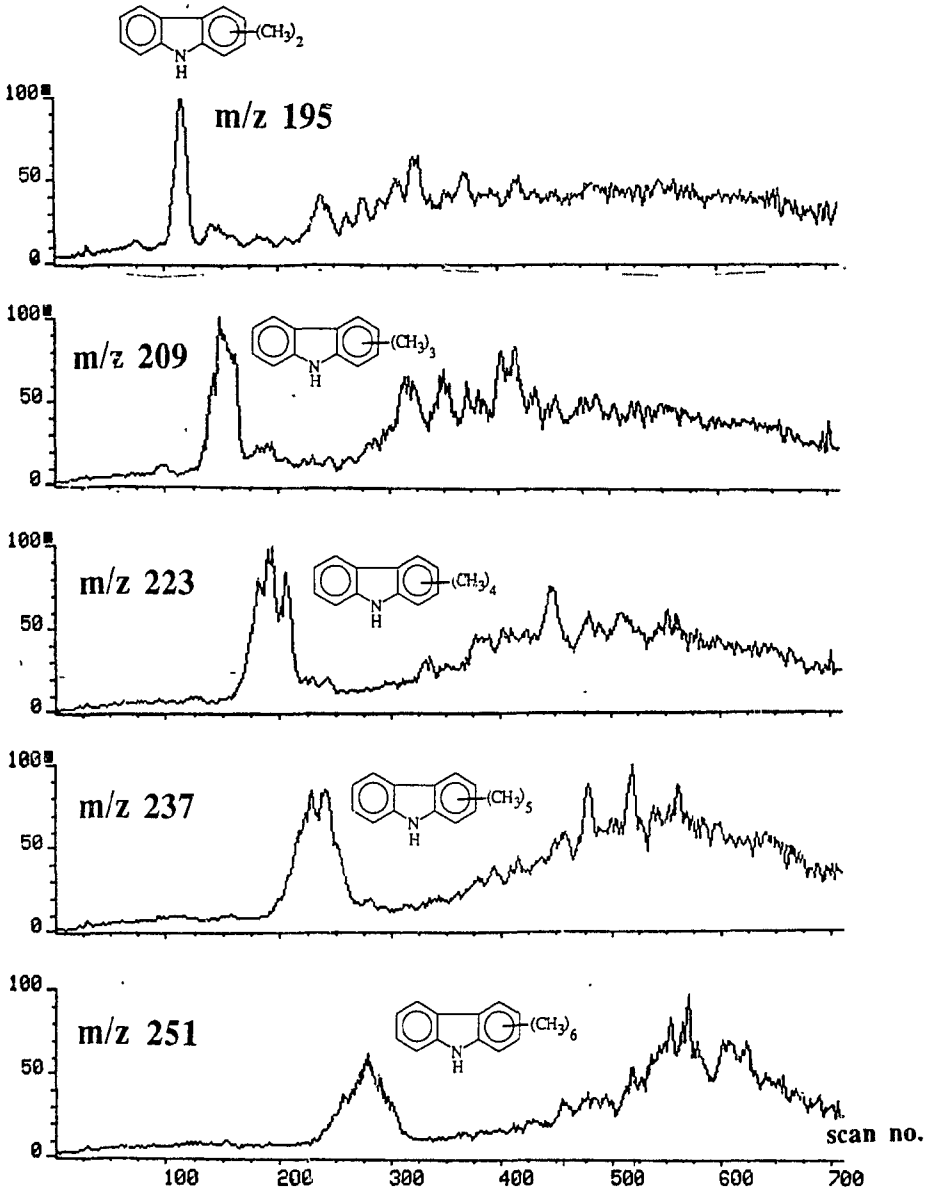


Figure 7.2.13:Reversed phase HPLC/MS RICs of the alkylated carbazoles found in the HGO sample

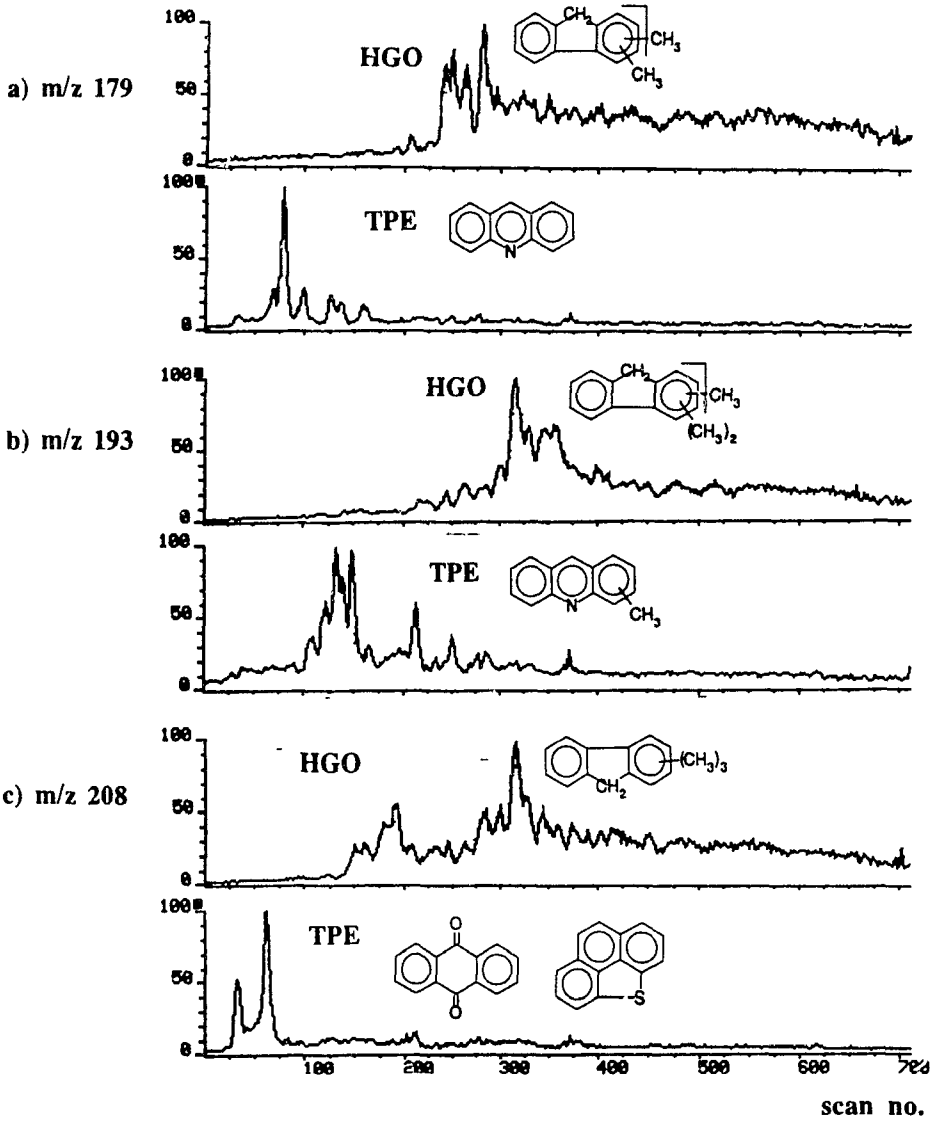


Figure 7.2.14:Reversed phase HPLC/MS RICs illustrating some of the differences between the HGO and TPE samples. Each pair of RICs at the same m/z value shows that different species with the same mass are found in the samples.

information are compiled. The peak intensities were not reported since the compilation was made by groups of isomers instead of individual compounds.

In Figure 7.2.11, the RICs are related to unsubstituted PAHs found in both HGO and TPE samples; their retention times are modified but are most certainly modified due to matrix effects. These compounds have not been assigned individual structures. Instead, a general structure was given for each set of isomers. It is interesting to note the low intensity of PAH related peaks compared to the intensities obtained for the TPE. For instance phenanthrene and anthracene are identified with certainty in the TPE from their two well separated and intense peaks appearing in the RIC at m/z 178. In the case of the HGO, the m/z 178 trace exhibits a single low intensity peak, whose nature is difficult to determine due to the matrix effects affecting the retention times. Other unresolved peaks that appear after PAH 178 during the run are due to fragmentation of heavier species. The same discussion also applies to other unsubstituted PAHs at m/z 202, 228, 252, 276, 278 and 302 (not all shown here).

Figure 7.2.12 shows a series of RICs related to alkylated PAHs, which are more abundant than unsubstituted PAHs in the HGO sample. Compounds with up to 4 carbon atoms in alkyl chains are detected and the RICs, from the standpoint of signal to noise ratio, show better quality than those obtained for unsubstituted PAHs.

These observations are in agreement with the GC/MS results obtained for the OSE (Section 7.1.2), where the predominant compounds were low molecular weight alkylated aromatics.

Figure 7.2.13 shows some RICs of alkylated carbazoles with alkyl chains containing between 2 and 6 carbons. The intensities of the alkyl-carbazole peaks lay between those of PAHs and those of alkylated PAHs. Carbazoles are more polar than PAHs and thus are eluted earlier in a RPLC run. This shows that separation of PAHs from PANHs is not absolutely necessary prior to a RPLC/MS experiment, because the peaks of PANHs do not interfere in time with those of the corresponding parent PAHs. As the length (or the number of carbon atoms) of the alkyl chain(s) increases, there are more possible isomers, which explains the

deterioration of the peak shapes. Isomers are not well resolved and thus are coeluted in broad peaks.

In Figure 7.2.14, differences between the TPE and HGO samples are emphasized by the comparison of mass chromatograms that differ drastically from one sample to the other. A very noticeable difference is observed in the traces of m/z 193. The peaks observed in the TPE trace are associated with isomers of methylacridine, as depicted in the figure. The m/z 193 peaks obtained for the HGO result from the loss of a methyl from C3-fluorenes at m/z 208. Another good comparison between the TPE and HGO samples can be made with the RICs at m/z 208. The TPE trace exhibits peaks produced by 9,10-anthraquinone and its isomers and by a naphthothiophene, while the HGO RIC mainly represents the elution of trimethylfluorenes. A general comparison between the HPLC/MS results obtained for the HGO and the TPE allows the following observations:

- PAQs and PAKs are more obviously present in the TPE than in the HGO
- unsubstituted PAHs are abundant in the TPE, and barely detectable in the HGO
- alkylated compounds are predominant in HGO and the situation is reversed for the TPE
- the TPE contains many pyridine-based PANHs (e.g. acridine) whereas the HGO contains many alkylated pyrrole-based PANHs (e.g. C2-C6-carbazoles)
- alkylated compounds in the HGO tend to elute in broad peaks due to coelution of many isomers
- the presence of many alkylated compounds in the HGO induced some fragmentation and thus noisier RIC baselines are observed than in the case of TPE

Figure 7.2.15 shows a few RICs obtained over the whole HPLC/MS run, for high M.W. compounds. Compounds with masses between 300 and 600 commence elution with 100% acetonitrile, and continue to be eluted with the introduction of dichloromethane. Compounds whose masses lay between 600 and 700 are eluted in the ACN-DCM part of the gradient, only. These compounds are chromatographed but do not separate sufficiently and accordingly broad envelopes instead of peaks characterize their elution. These broad envelopes result from the elution of many

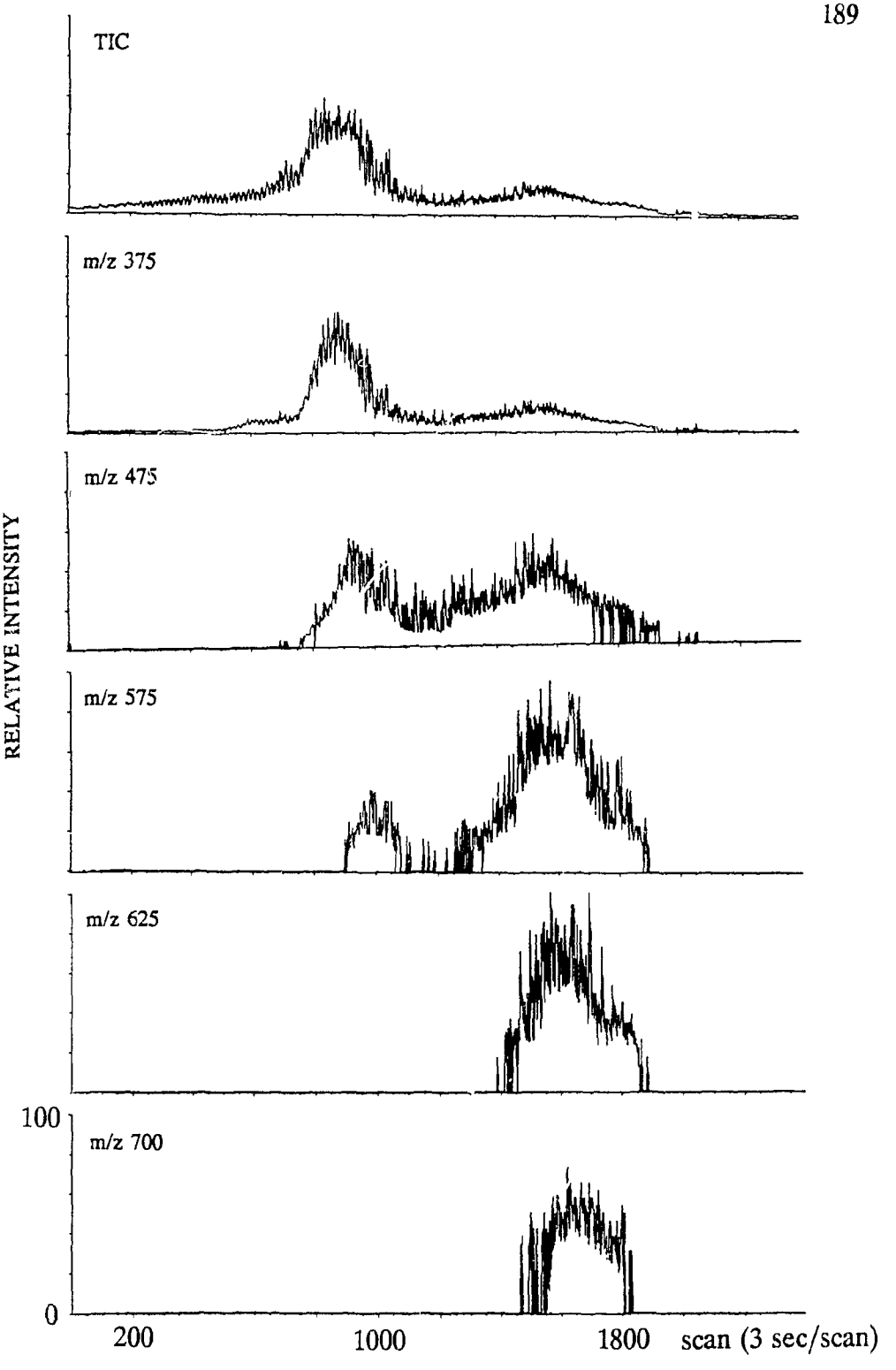


Figure 7.2.15:Reversed phase HPLC/MS RICs of high M.W. HGO compounds obtained over the whole retention time range (low + high M.W. portions) showing that compounds above mass 350 chromatograph without separation

isomers whose chromatographic behavior is too similar for them to be eluted in discrete peaks.

Two attempts have been made to "clean-up" the high M.W. unresolved part of the HPLC separation. The first attempt was to slow down the rate at which the solvent gradient was varied from 100% acetonitrile to 100% dichloromethane. This change was effected over 100 min. instead of 50 min. as in Table 2.5.1. This resulted in broader RIC envelopes, but still with no compound resolution and with longer times of analysis. The second attempt was to use a solvent weaker than dichloromethane to effect the second part of the gradient, as inspired by the work of Peaden and co-workers (109). Ethyl acetate and tetrahydrofuran were used as replacements for dichloromethane. Neither solvent however provided uniform wetting of the moving belt, which is required to maintain chromatographic integrity and preserve peak shapes. It was thus decided that no further work would be done to improve the elution pattern of high M.W. compounds, at least for the purposes of this thesis. The water-ACN-DCM method developed and presented here was very successful in chromatographing the high M.W. substances in TPE as seen in Section 7.2.1.2, and less successful here. This situation emphasized the great difference in the nature of the TPE and Syncrude samples.

The mass spectra in Figure 7.2.16 were taken at different retention times during the elution of the "large envelope" of DCM soluble compounds. These spectra exhibit a peak at every nominal mass, with no ion peak being particularly predominant. The only facts that would lead one to assign these compounds as PACs are:

- the fractionation process used for the HGO indicates that the polarity range of these high M.W. compounds is similar to that of PACs,
- their elution time range corresponds to that of the high M.W. PAHs of the TPE,
- the low M.W. compounds (MW < 310) of the same sample are PACs or alkylated PACs.

The results shown in this section are qualitative and speculative but show, in order of decreasing concentration, the presence of alkylated PAHs, PANHs

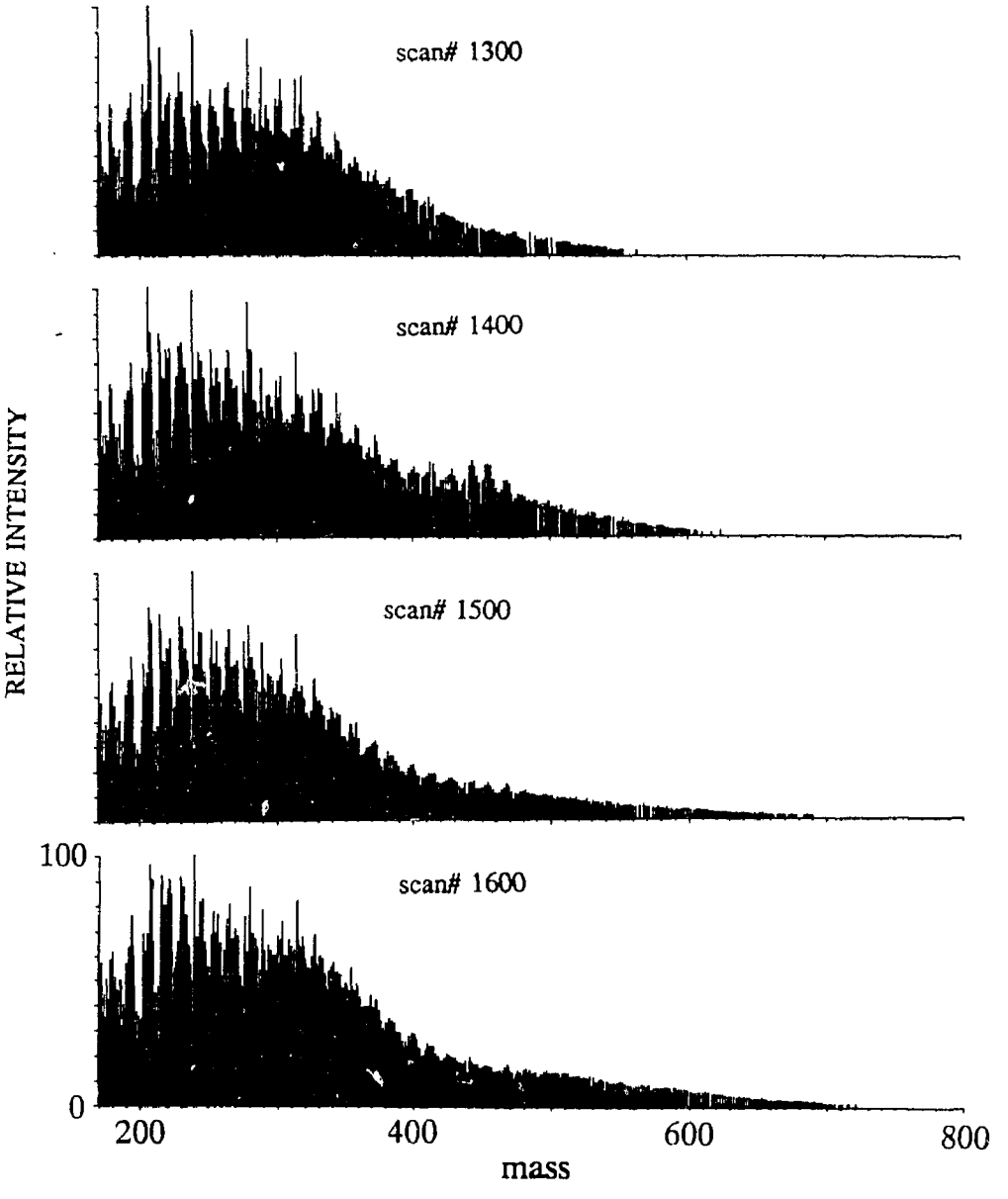


Figure 7.2.16:Mass spectra obtained during the acetonitrile-dichloromethane portion of the reversed phase HPLC/MS gradient for the HGO sample. These spectra feature a peak at every nominal m/z value, showing that the high M.W. compounds contained in the HGO sample do not separate.

(carbazoles) and unsubstituted PAHs in the HGO sample. Although none of the low molecular weight compounds was positively identified, this profiling study of the low M.W. portion of the HGO was successful and allowed a partial characterization of the sample. Chromatographic peaks were observed up to mass 310 in the RICs, and beyond this mass all the RICs resembled broad envelopes. Compounds in the 310-950 mass range all have similar chromatographic behavior, most probably due to long alkyl chains that shift their behavior toward the aliphatic and away from the aromatic. Their HPLC separation was unsuccessful, but profiling studies were still possible.

ii) SFC fractions of the silica over alumina PAC fraction

As described in Section 2.3.2, fifteen normal phase SFC fractions were collected from the PAC fraction of HGO. The HPLC/UV results of some of the fraction were shown in that section; the corresponding HPLC/MS results are presented here. As the SFC fractions had quite low concentration levels, the TIC traces of each individual fractions did not show any chromatographic peaks because the belt background was more intense than the actual analyte signals. The TICs are therefore not presented here.

Figure 7.2.17 shows the RICs of m/z 206 (C2-phenanthrenes and anthracenes) obtained by HPLC/MS of Fractions 3, 5, 9 and 15. The first three traces show that these alkylated PAHs reach their highest concentration in Fraction 5, and that the retention times are quite reproducible. Fraction 15, however, contains a compound which differs considerably, in terms of retention time, from C2-phenanthrenes and anthracenes. The former compound is more polar than the latter as it is eluted earlier, but could not be identified either from HPLC/MS runs of PAC standards or TPE. Possible structures of polar compounds of M.W. 206 were obtained from a PAC atlas (188) and in fact there are only four possibilities, shown in Figure 7.2.19a.

High resolution mass spectrometry would help identify the proper structure; the accurate molecular mass of the first species is 206.0366, whereas the others have

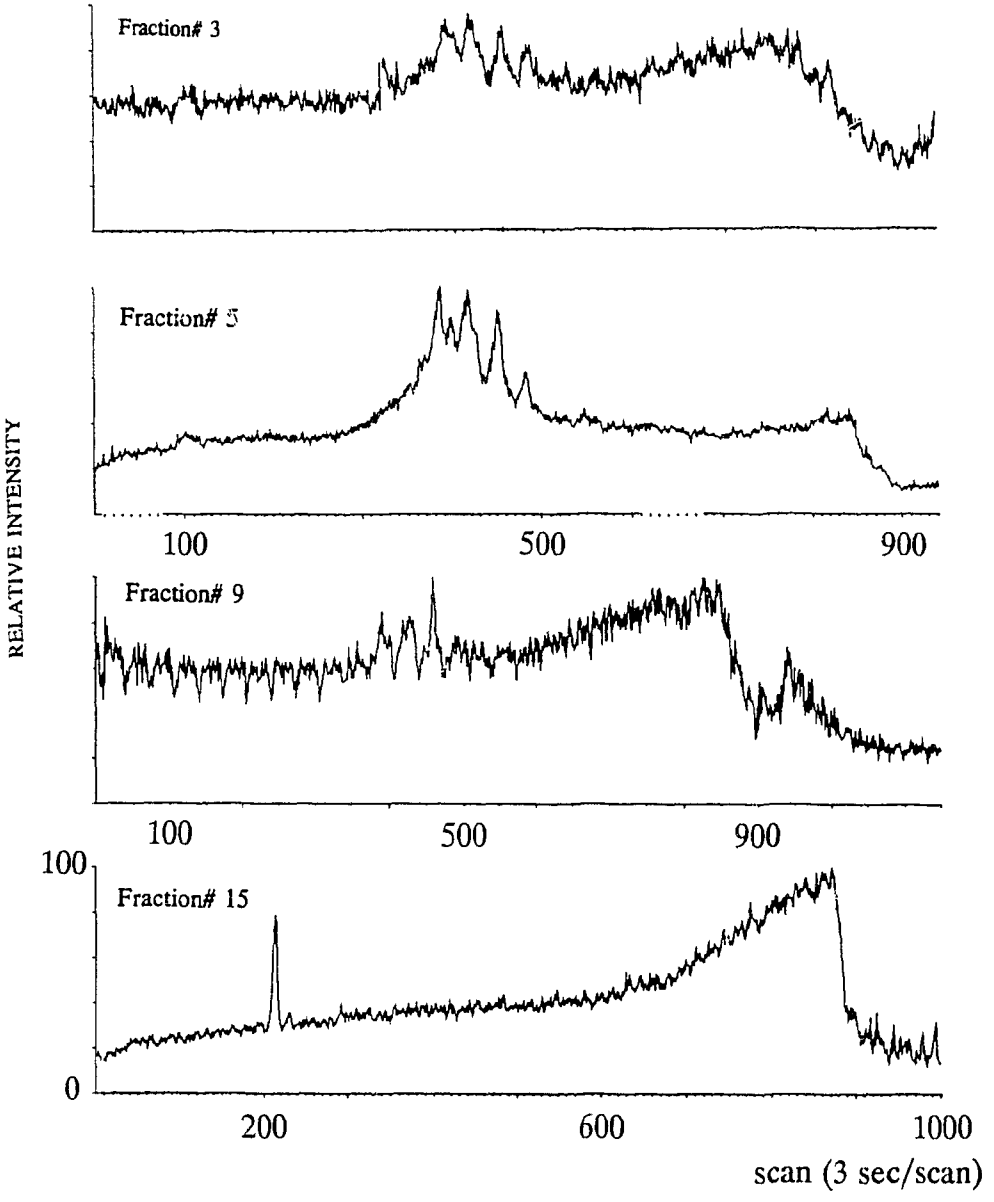


Figure 7.2.17:Reversed phase HPLC/MS RICs (m/z 206) of some of the HGO normal phase SFC fractions

a M.W. of 206.0729. Unfortunately, these HPLC/MS experiments were performed in low resolution mode, the only mode possible on the VG Masslab 20-250 quadrupole instrument. Again the minimal resolution of ca. 5700 required by the mass spectrometer to differentiate the two accurate masses involves an important decrease in sensitivity, which can seriously hamper the global analysis of the sample.

The RICs of m/z 242 (C1-chrysenes, C1-benzanthracenes) are displayed in Figure 7.2.18. These methylated compounds of M.W. 242 are first observed in Fraction 9, and were at their highest concentration in Fraction 15. The quality of the chromatography obtained for those species is good in terms of isomer discrimination. In Fraction 5, other species with M.W. 242 had a retention time of ca. 9 minutes in HPLC/MS. Once again, a few possible polar structures for these polar substances were obtained from the PAC atlas (188). Figure 7.2.19-b shows 3 proposed structures. These structures all have the same empirical formula and thus exact molecular mass of 242.0729 and, for the reasons discussed above, have not been confirmed by high resolution mass spectrometry.

Other examples are summarized in Appendix IV. According to those tabulated results, normal phase SFC fractionation did not separate the original sample into clear cut fractions of distinct compositions; instead it allowed the collection of fractions containing species of increasing molecular weights as a function of the collection time, with a high extent of overlapping between fractions. Both examples illustrated by the RICs of Figures 7.2.17-18 show the overlapping. They also show that more than one class of PACs have the same nominal mass but elute at different times, with polar species first. SFC fractionation was necessary for the observation of those polar species, which otherwise were not observed in the HPLC/MS runs of the original sample.

The selectivity of the column used here (silica, 4.6 mm i.d.) was not as good as that of the column originally used to develop the elution scheme (silica, 1mm i.d.). However, the main advantage of this method is that it enables the elimination of very high molecular weight compounds (Fractions 13-14) from the sample. These heavy fractions thus are not injected with the rest of the sample every time that an analysis

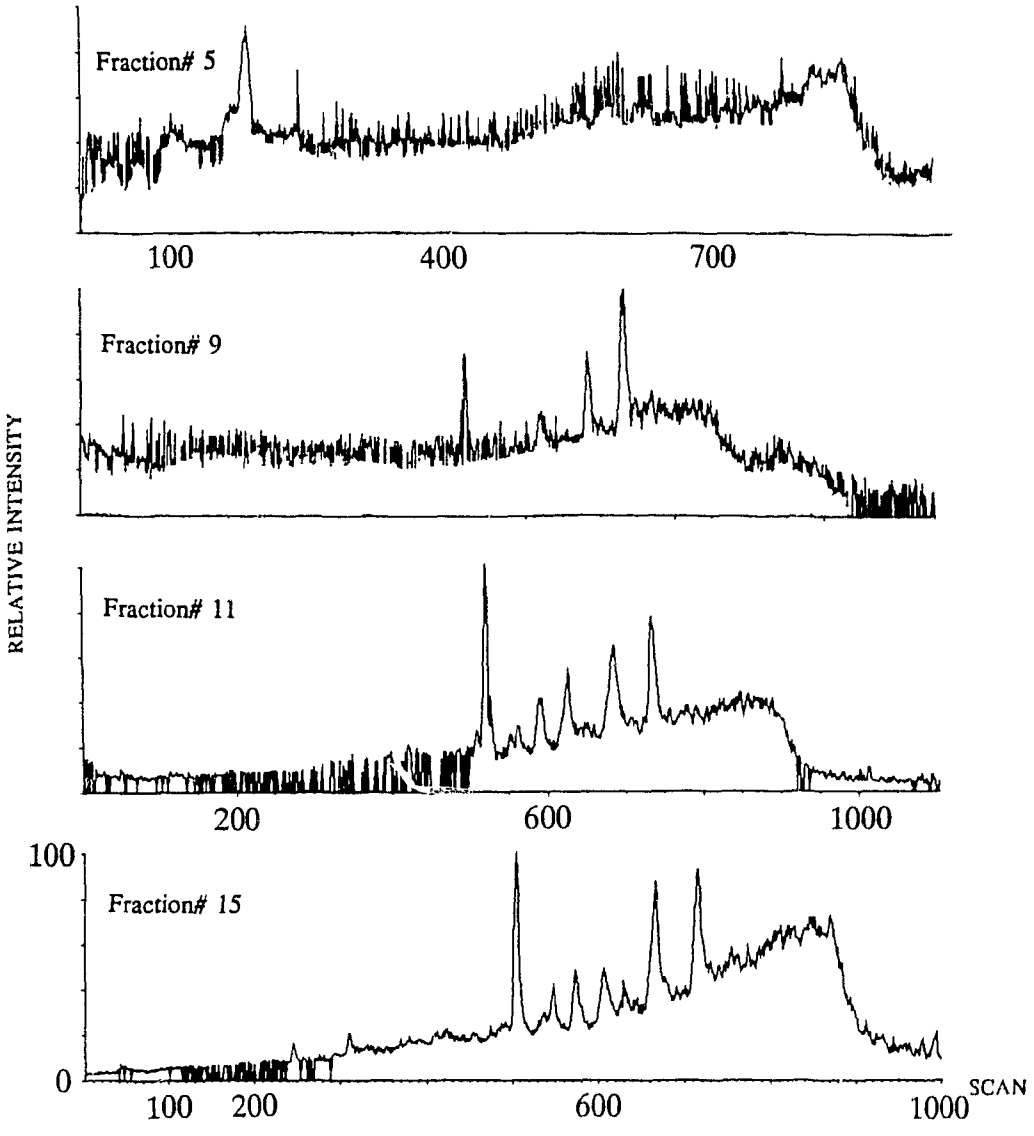


Figure 7.2.18:Reversed phase HPLC/MS RICs (m/z 242) of some of the HGO normal phase SFC fractions

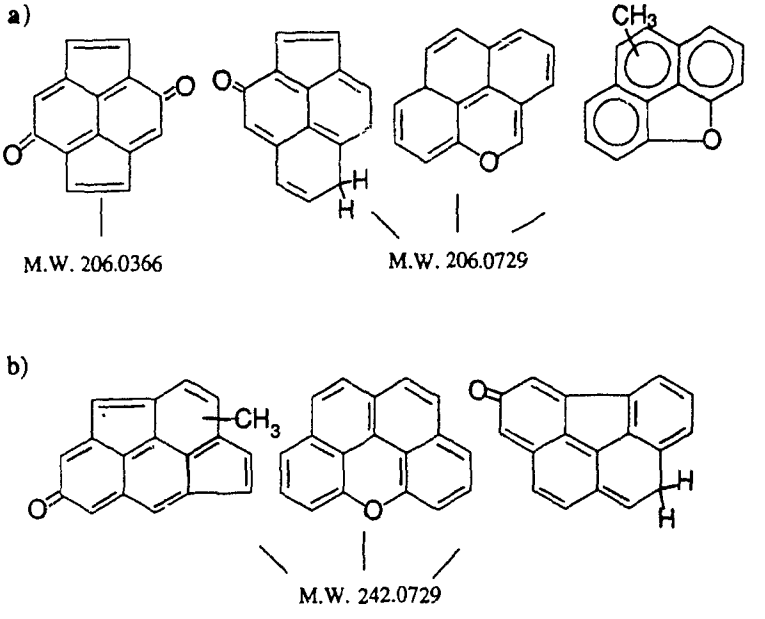


Figure 7.2.19:Possible structures for polar compounds of molecular weight a) 206 and b) 242 found in the HGO sample

of the low M.W. PACs contained in the HGO is required. Running those heavy compounds through a column with every single injection may damage the column since drastic conditions and longer analysis times are needed to elute them.

7.2.2.2 HPLC/MS of the OSE sample

Only the PAC fraction obtained from the silica over alumina method was analyzed by HPLC/MS. The reason for not analyzing every normal phase SFC fraction by HPLC/MS may be found from inspection of the HPLC/UV chromatograms of these fractions (Figure 6.2.12): the concentration of aromatic species eluting during the first part of the HPLC gradient is too low to make HPLC/MS useful.

Figure 7.2.20 presents some RICs obtained for the OSE PAC fraction. The first trace is that of m/z 206; the peak in this trace is due, according to its retention time, to the same polar compound found in the HGO sample, and no peaks due to C2-phenanthrenes/anthracenes appear. The retention times of the peaks in the m/z 230 RIC (C2-fluoranthenes/pyrenes) of the OSE are in good agreement with those of HGO (see Appendix IV). Compounds of M.W. 244 (C3-fluoranthenes/pyrenes) also offer similar chromatography for the HGO and OSE; C2-chrysenes and C2-benzanthracenes (m/z 266 Da.) are well represented in OSE and elute with good isomer discrimination (not shown). The RICs of some nitrogen containing species (m/z 235, 283 and 295) show that good chromatography was obtained; some proposed structures for these compounds are shown in Figure 7.2.21.

These results are summarized, together with those obtained for the HGO and the HGO SFC fractions, in Appendix IV.

Some RICs were also obtained for the entire run; the RICs presented in Figure 7.2.22 are some of those of m/z 375 to 575, and the TIC trace is shown as well. Most of these RICs follow the pattern of the TIC trace, i.e., a first group is eluted in the first 50 minutes and a second group in the ACN-DCM part of the gradient. Above m/z 400, only compounds eluable in DCM are observed. There are,

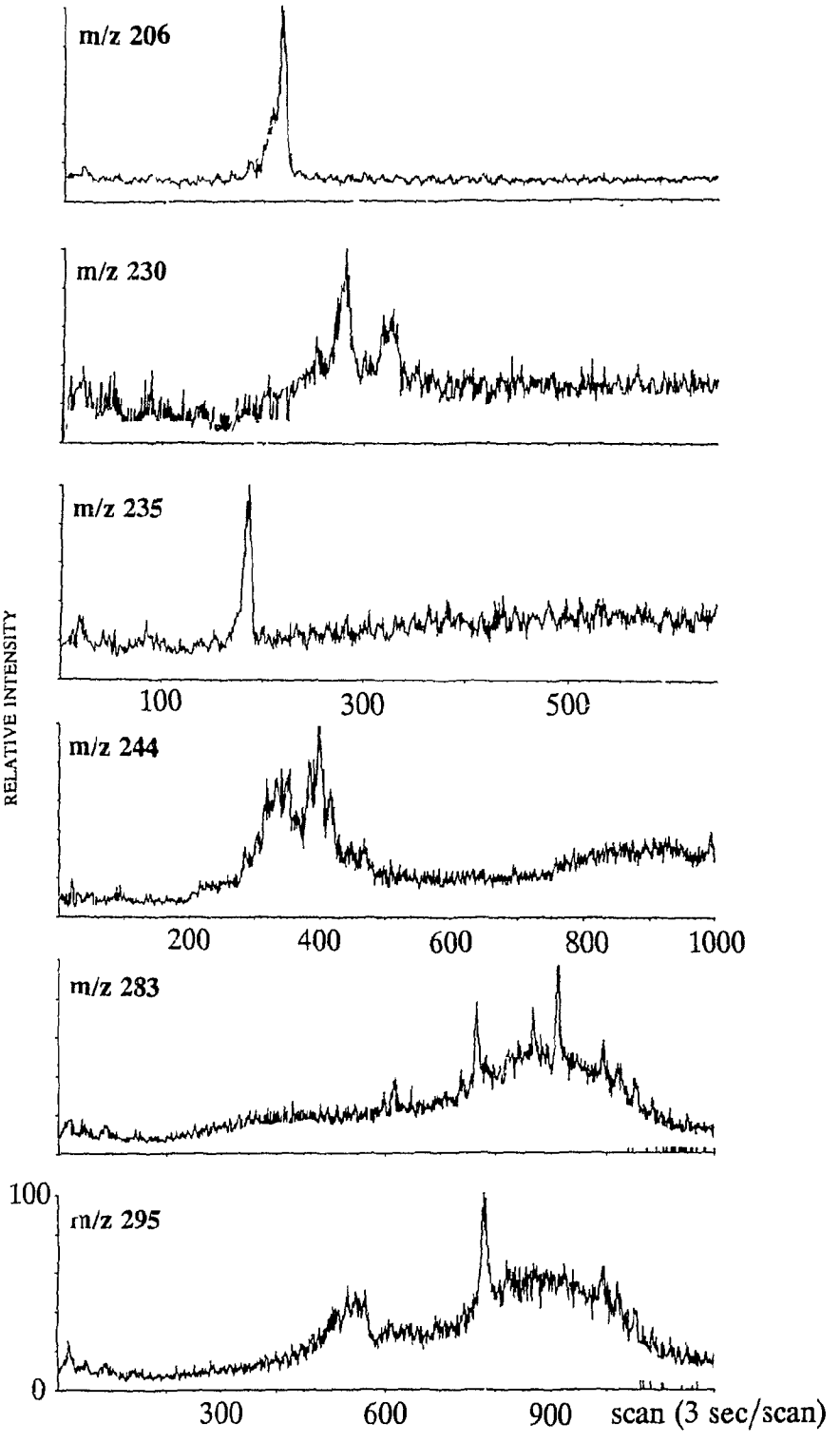
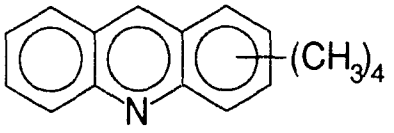
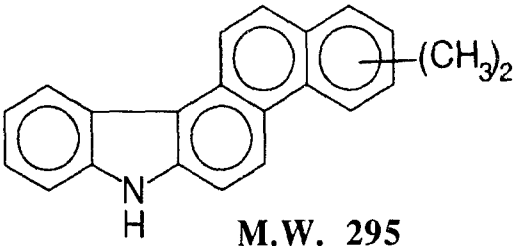


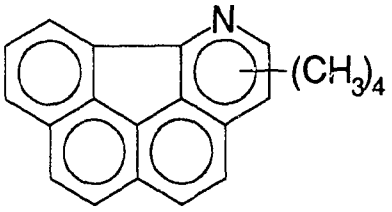
Figure 7.2.20:Reversed phase HPLC/MS RICs of compounds found in the OSE sample (low M.W. portion)



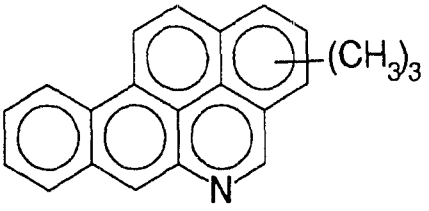
M.W. 235



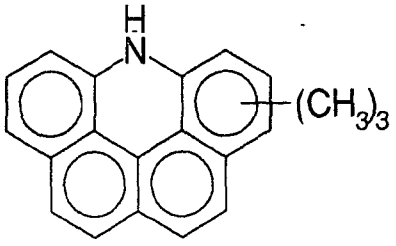
M.W. 295



M.W. 283



M.W. 295



M.W. 283

Figure 7.2.21:Possible structures of some of the compounds producing the RIC peaks in Figure 7.2.20

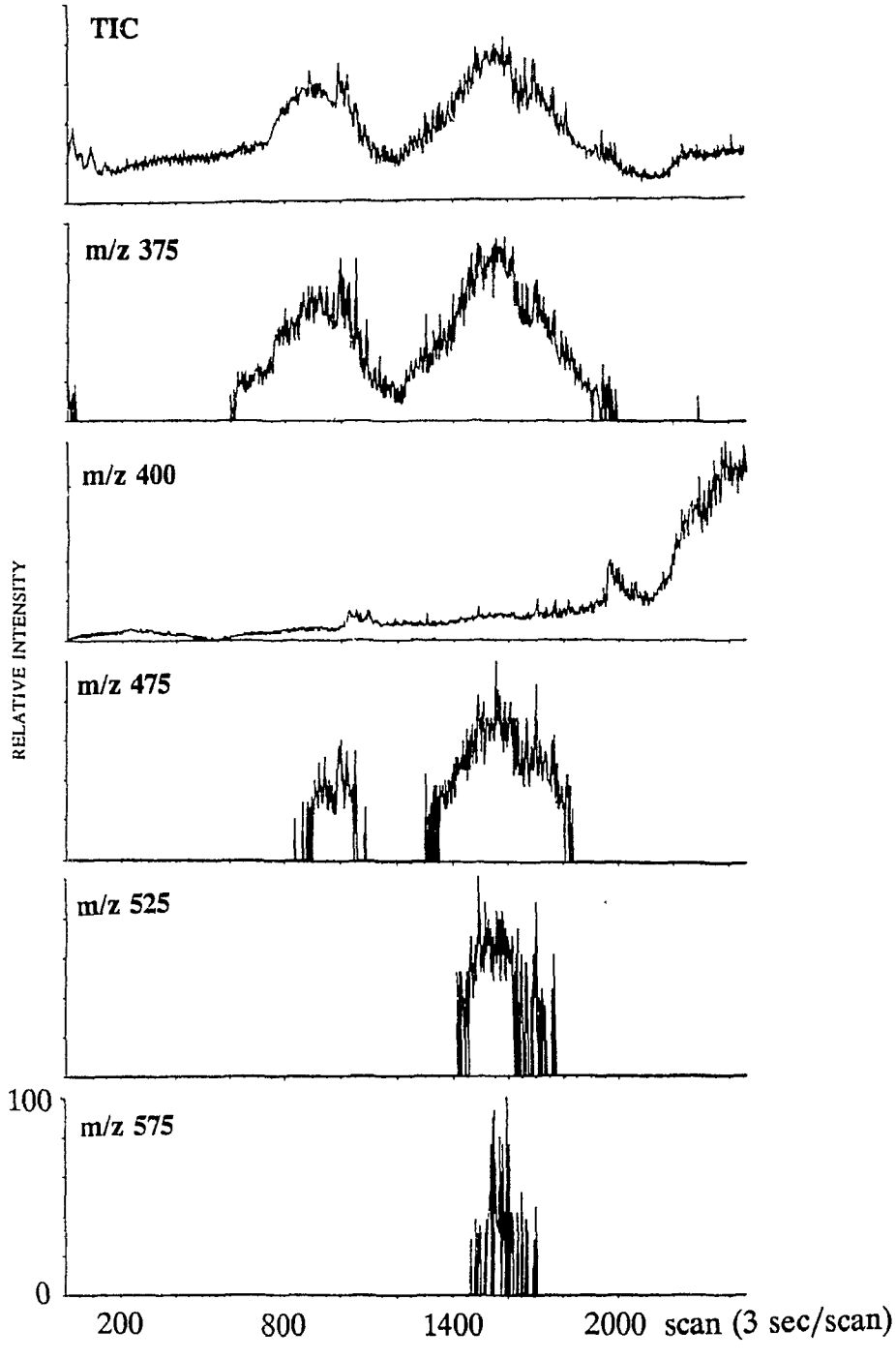


Figure 7.2.22:Reversed phase HPLC/MS RICs of high m/z values obtained over the whole retention time range (low + high M.W. portions) showing that compounds above mass 350 chromatograph without separation (OSE).

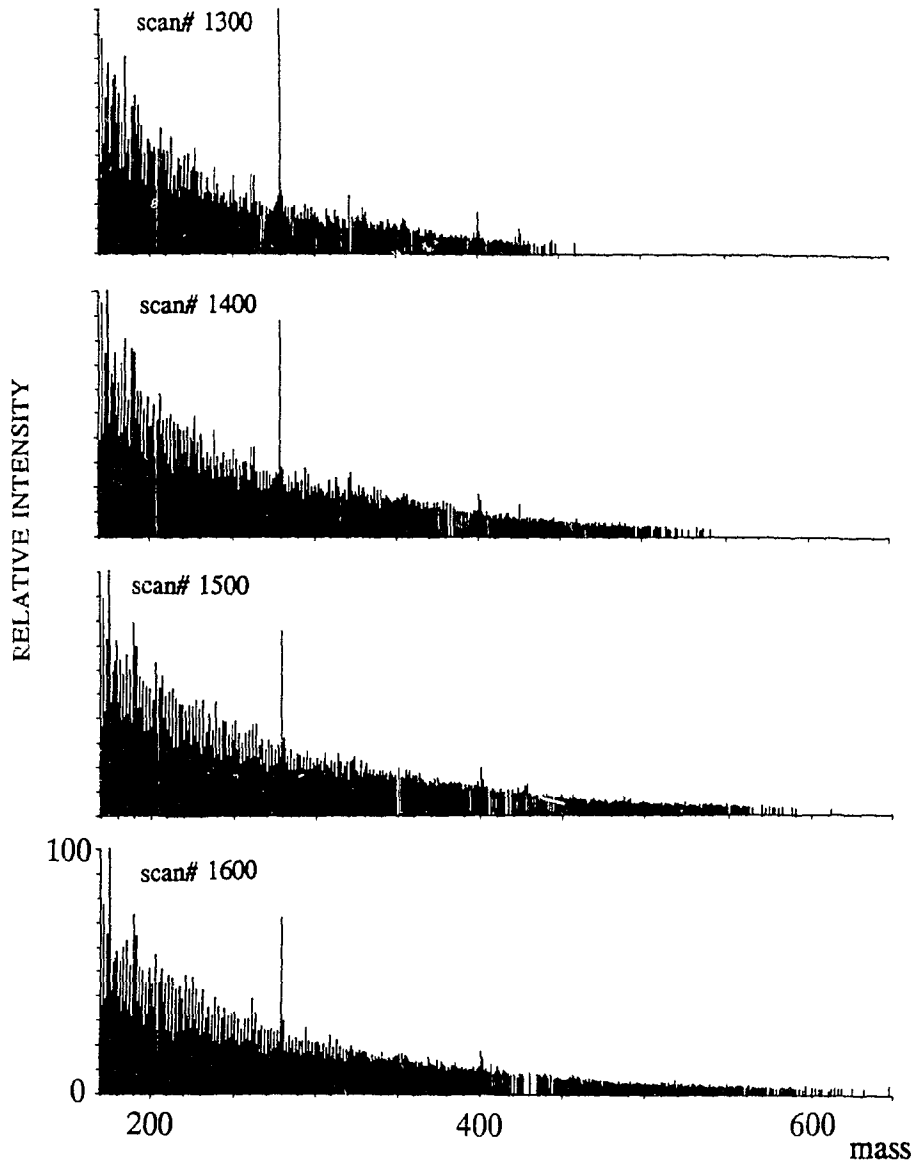


Figure 7.2.23:Mass spectra obtained during the acetonitrile-dichloromethane portion of the reversed phase HPLC/MS gradient for the OSE sample. These spectra feature a peak at every nominal m/z value, showing that high M.W. compounds contained in the OSE sample do not separate.

however, no well resolved chromatographic peaks in the second part of the elution, as observed with HGO previously. The mass spectra shown in Figure 7.2.23 are taken from the ACN-DCM portion of the HPLC chromatogram, and as observed for HGO, there are peaks at every nominal mass, due almost certainly to highly alkylated PAH molecular ions and fragments.

7.2.2.3 HPLC/MS of the Pitch sample

As in the case of the OSE, no HPLC/MS data were recorded for the normal phase SFC fractions of the PIT. In the HPLC/MS RIC data obtained for the silica over alumina PAC fraction of this sample, a few significant peaks were observed in the water-ACN part, some of which are presented in Figure 7.2.24. The peaks in the m/z 195 trace are those of C2-carbazoles and the RIC of m/z 202 shows the elution of fluoranthene or pyrene. The peak appearing in the RIC of m/z 206 does not belong to C2-phenanthrenes/anthracenes, but to that same polar species observed in the HGO (SFC fraction 15) and OSE. Compounds of M.W. 218 (possibly C2-4H-Cyclopenta[def]phenanthrenes or dibenzofurans) elute with moderate isomer discrimination, while the M.W. 243 compounds are eluted in two intense groups of peaks, the first group most certainly related to C1-azachrysenes and C1-azabenzanthracenes. Those compounds were not observed in the HGO or the OSE. The second group of peaks is thought to arise either from 214 PANH-C2 or 228 APAH species. The RIC of m/z 279 (not shown) is that of the highest m/z value to show any form of chromatographic separation.

These results from the low M.W. elution part of the PIT HPLC/MS run are tabulated with those of HGO, HGO (SFC fractions), and OSE in Appendix IV. The identification of some of the compounds has been attempted.

Figure 7.2.25 shows, together with the TIC chromatogram, a few RICs covering the entire elution time range. Up to m/z 500, these traces generally follow the TIC trace, and from m/z 600 up to 750, compounds are eluted only during the second (DCM) part of the gradient. The highest m/z value that produced a

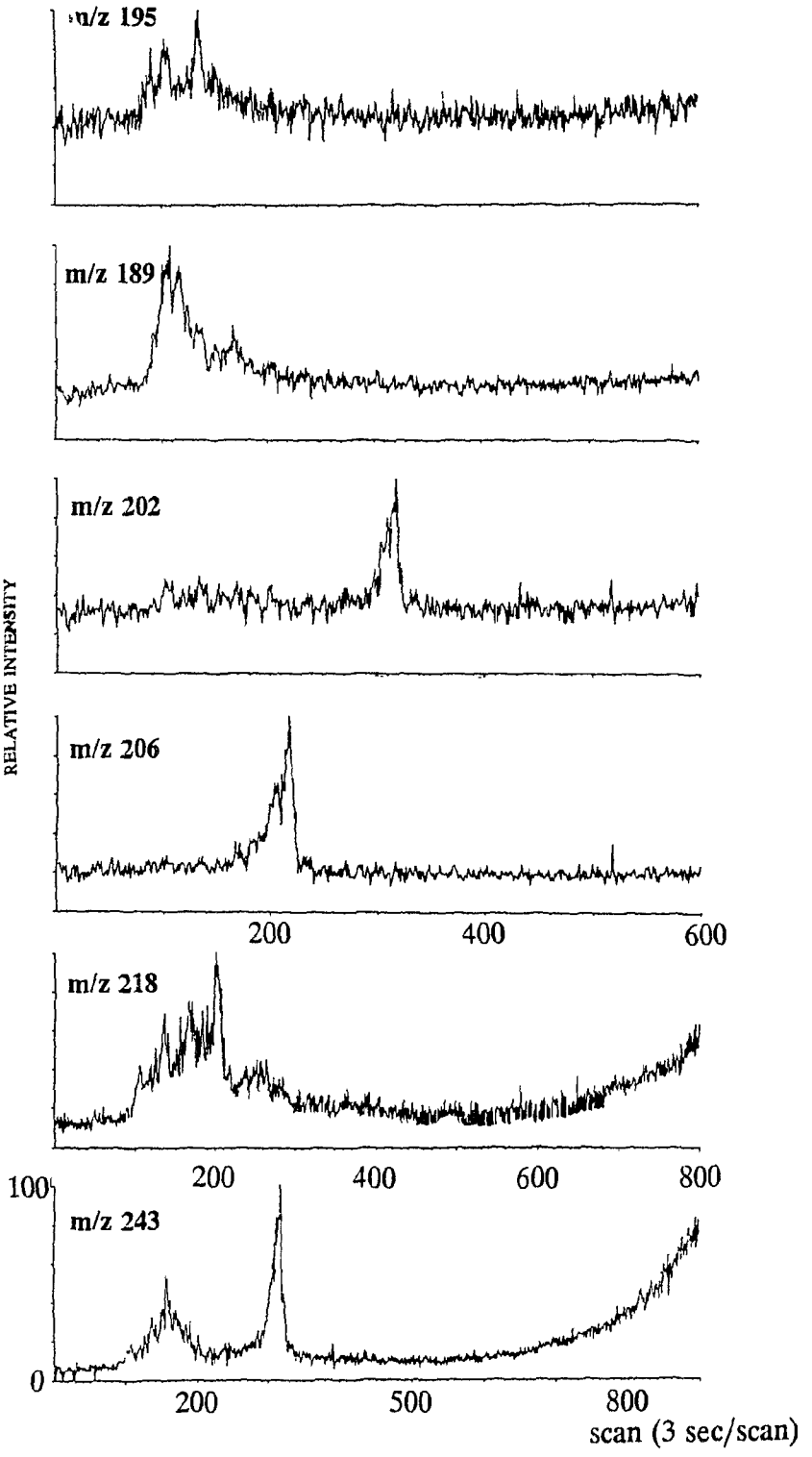


Figure 7.2.24:Reversed phase HPLC/MS RICs of compounds found in the PIT sample (low M.W. portion)

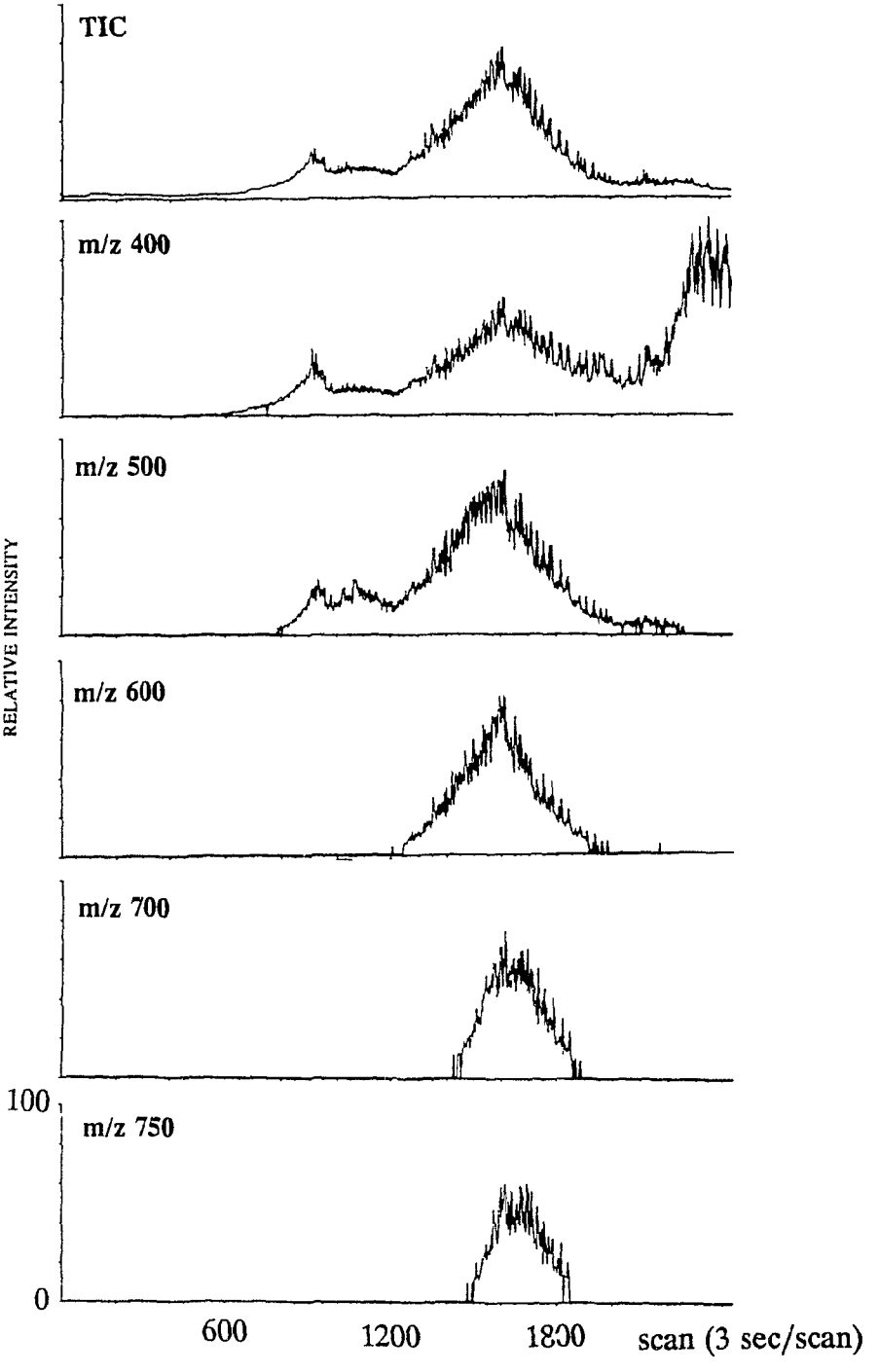


Figure 7.2.25:Reversed phase HPLC/MS RICs of high m/z values obtained over the whole retention time range (low + high M.W. portions) showing that compounds above mass 350 chromatograph without separation (PIT).

reasonable RIC was ca. 850 Da. The sharp peaks observed on each of the RICs are not real chromatographic peaks, but rather spikes due to the evaporation of the residua of droplets of dichloromethane solution off the belt in the ion volume of the mass spectrometer. All chromatograms of Figure 7.2.25 resemble the TIC trace, with the exception of that of m/z 400. The same observation is also valid for the OSE. Compounds of M.W. 400, according to the HPLC/MS of PAH standards (Section 7.2.1.2), elute in the very late stages of the gradient program. The last isomer of M.W. 400 of the standard solution is eluted at 1:40 (Figure 7.2.5), while it takes 2 hours to be eluted for the OSE and PIT. The standards and unknowns of M.W. 400 most probably belong to different classes of compounds, unless matrix effects have drastically modified their retention times. Their aromaticity was confirmed by observation of the m/z 200 trace (doubly charged molecular ion of species of M.W. 400) which matched the m/z 400 trace perfectly. The incomplete elution of compounds of M.W. 400, even after 2 hours of chromatography, suggests that a solvent stronger than DCM should be used after the latter. Benzene, toluene or chlorobenzene are possible candidates, but their properties have not been investigated in the course of this work.

Figure 7.2.26 shows four mass spectra obtained from the heavy end of the chromatogram. They are still characterized by a continuum of peaks over the whole mass range scanned.

Again, some optimization would possibly improve the separation of the compounds in this heavy end. It would involve extremely long analysis times by expanding the gradient in time or modifying the solvent system. As for the type of columns used, C_{18} reversed phase HPLC columns have been shown to be the most selective for PAC analysis, after pyrenylethyl (PYE) silica gel columns (189,190). The latter have been shown to yield very good isomer discrimination among sets of alkylated PACs. However, PYE columns are sensitive to the nature of the sample and of the mobile phase, in terms of degradation; thus one would normally not risk injection of a complex mixture such as PIT on these columns because of the drastic mobile phase conditions required for their elution.

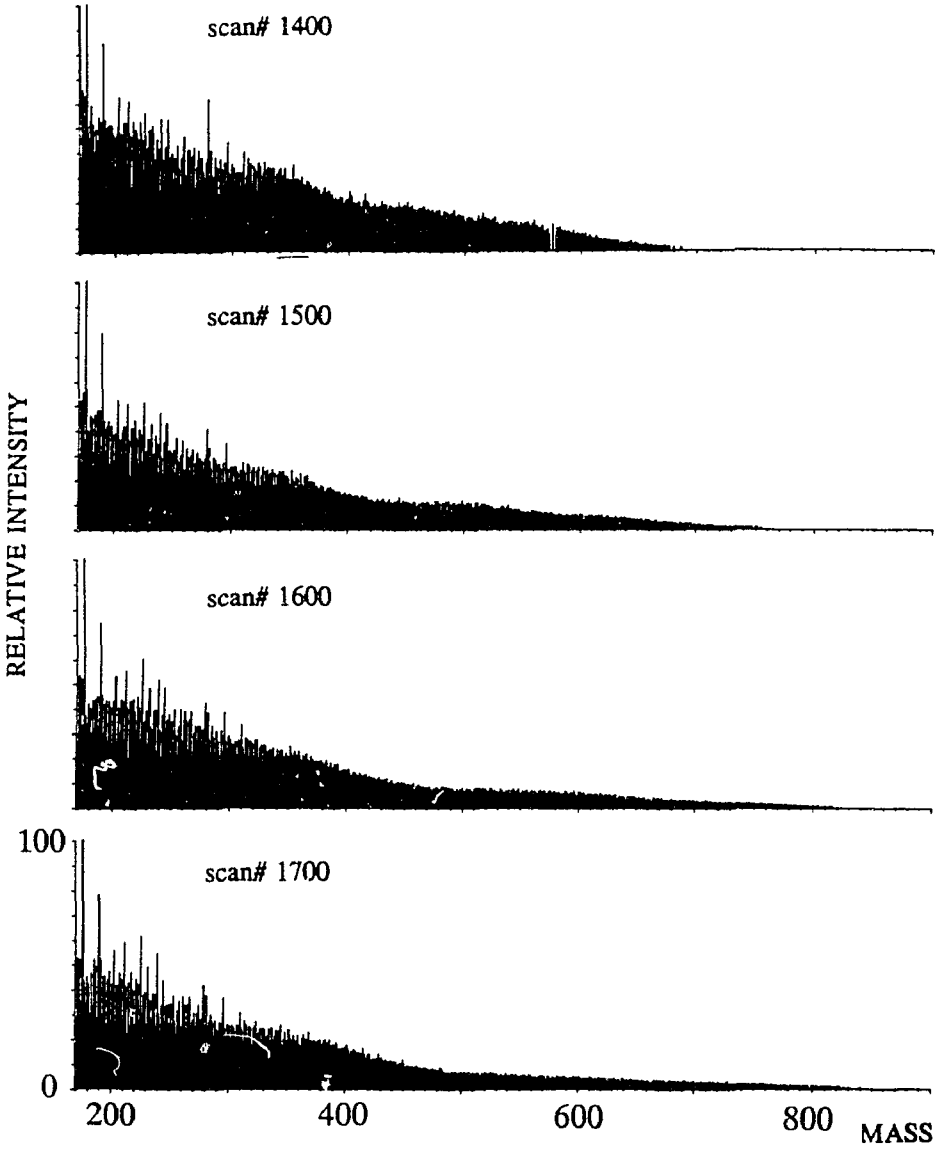


Figure 7.2.26:Mass spectra obtained during the acetonitrile-dichloromethane portion of the reversed phase HPLC/MS gradient for the PIT sample. These spectra feature a peak at every nominal m/z value, showing that high M.W. compounds contained in the PIT sample do not separate.

7.2.2.4 Overall comparison of the contents of the HGO, OSE and PIT samples as determined by reversed phase HPLC/MS

A comparison of the contents of each sample can be obtained by a detailed observation of Appendix IV, where elution time ranges are tabulated for every significant peak obtained from the RICs of each sample. Without inspection of the spreadsheet, the difference between the three samples is easily visualized by comparing the HPLC/UV chromatograms (Figure 6.2.9) as well as the HPLC/MS TICs (Figure 7.2.27). The ratio [high M.W./low M.W] obviously increases in the order HGO-OSE-PIT; this feature is observed in both UV and TIC trace comparisons. The characteristic envelopes of heavy material are centered around the same retention times from one sample to the next.

The data in Appendix IV have been sorted in respect with m/z values first; the second sorting factor is the origin of the samples. OIL1 and OIL2 are the PAC fractions obtained from two fractionation experiments both performed using silica over alumina column chromatography. OIL#n samples are the normal phase SFC fractions obtained from the OIL2 sample, where n=no. of the fraction. TS (Tar Sand) refers to the OSE. SRS1J (Syncrude Research Sample #1-J) corresponds to the PIT. The third column of the spreadsheet gives the retention time ranges expressed in scan numbers (3 sec/scan). The fourth column lists the names of the compounds whose tentative identification was possible (176).

There is a predominance of alkylated compounds; the species whose retention times increase regularly with mass are almost certainly unsubstituted and alkylated-PAHs. Odd mass ions correspond to the $(M-15)^+$ fragments of alkylated species when the peaks in the RICs of both $M^{+\cdot}$ and $(M-15)^+$ coincide in time. When no $M^{+\cdot}$ trace can be matched to that of the odd m/z value, the odd mass ions are almost certainly due to nitrogen containing species. In such cases, several compound identities are proposed in the fourth column of Appendix IV, although no particular structures can be absolutely assigned. The compounds with even masses and whose retention times are shorter than those of PAHs are more polar than the latter. In most cases, there

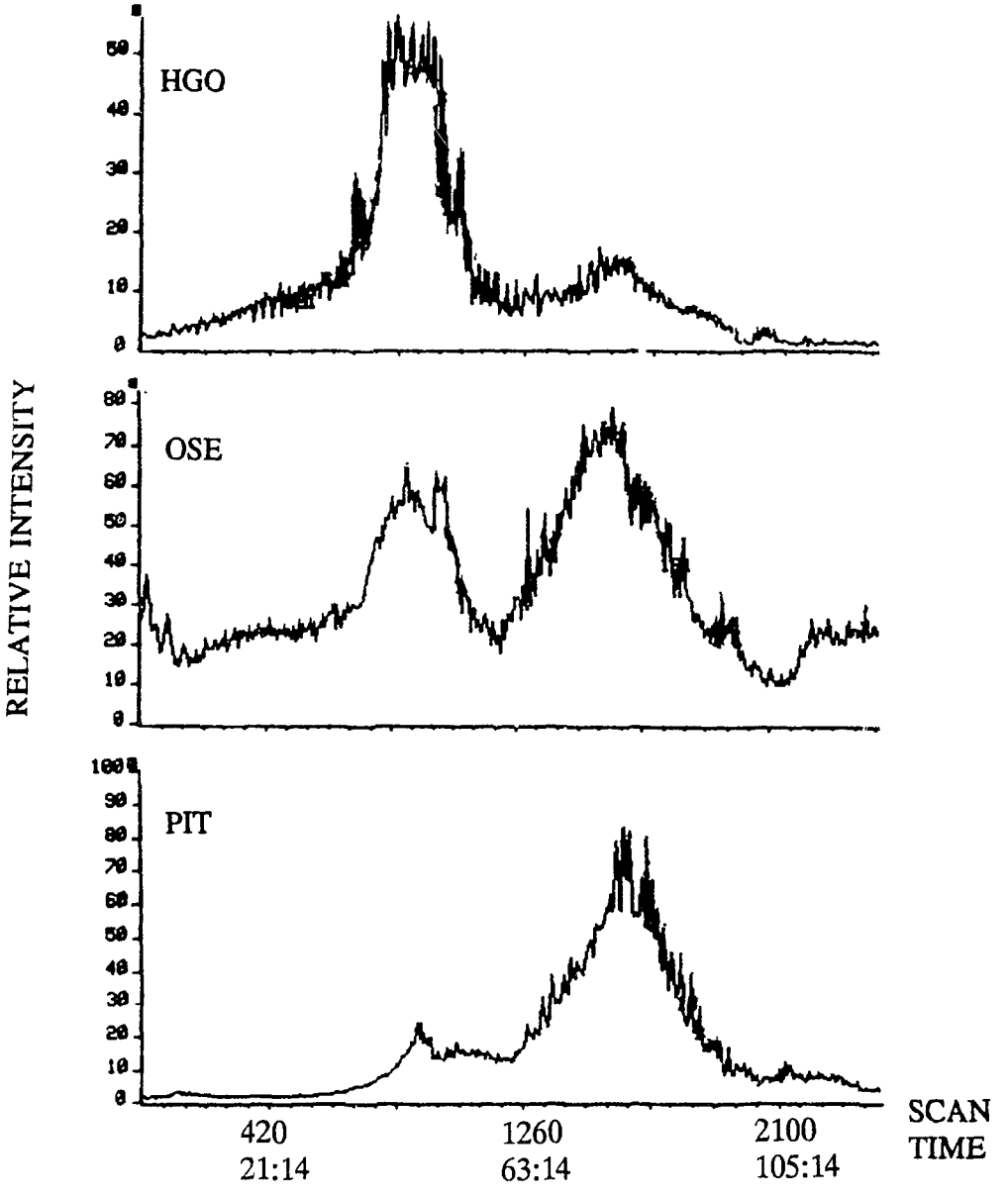


Figure 7.2.27:Comparison of the reversed phase HPLC/MS TICs obtained for the three Syncrude samples (HGO, OSE and PIT) emphasizing the different proportions of low M.W. vs. high M.W. material in the samples

are several possible PAK, PAQ, PAHK etc., structures available from the PAC atlas (188) to describe those polar species. However, their definitive identification was not possible here.

The nature of the compounds found in the lighter portion of each sample is in good agreement with the results obtained by Lancas et al. (178), McLean and Hsu (181) and Lauer et al. (26,179) for petroleum distillation cuts. All these authors found that alkylated PAHs were the main constituents of these samples.

The results obtained from the second part of each HPLC/MS run (High M.W. part) have not been tabulated since most of the RICs showed the same pattern; the most information obtained about the heavy ends was presented in the mass spectra of Figures 7.2.16, 7.2.23 and 7.2.26 for the HGO, OSE and PIT samples, respectively.

7.3 Supercritical fluid chromatography/mass spectrometry

Supercritical fluid chromatography, employed with capillary or packed columns, should ideally combine many of the advantages of GC and HPLC. SFC should allow the elution of volatile, non-volatile and thermally labile compounds with resolution and selectivity somewhere between that obtained using GC and HPLC. With proper interfacing between the SFC system and the mass spectrometer, EI mass spectrometry should provide detection at sensitivities comparable to those obtained using GC/EI-MS and HPLC/EI-MS.

7.3.1 SFC/MS of the TPE

7.3.1.1 Capillary column SFC/MS

Direct interfacing of the capillary column supercritical fluid chromatographic system with the VG Masslab 20-250 mass spectrometer was quite simple in theory, but difficult and time consuming in practice. An appropriate flow restrictor had to be pulled at the end of the column and inserted into the ion volume by passing it

through the GC/MS interface device without breaking the brittle end. The restrictor was pulled directly from the end of the column to avoid any dead volume or leak from connectors. Commercial restrictors were available and could be connected to the end of the column with a zero-volume union, but the flow rates thus produced were too high for direct interfacing to the mass spectrometer. Those flow rates are more appropriate for FID detection. The very narrow restrictors pulled manually for capillary column SFC/MS tended to plug quite easily, either due to column bleeding or deposition of organic sample material from several SFC/MS runs. As the pressure of carbon dioxide was increased, the gas solidified at the end of the restrictor, causing particles of dry ice to be sputtered into the ionization source. Those particles caused significant background noise, because they were sputtered at irregular intervals, during which any flow was stopped. Each sputtering event allowed sample material out of the column, producing spikes in the SFC/MS RICs.

The TIC and RICs obtained by capillary column SFC/MS of the TPE are shown in Figure 7.3.1. The TIC trace shows a slight increase in the baseline intensity with time. This is partially due to column bleeding increasing with CO_2 pressure and to sputtering of $[CO_2]_n$ clusters, whose masses build up with pressure. Those clusters appear at the m/z values of 132, 176, 220, 264, 308, 352, $(44)_n$,... in the mass spectra recorded at high CO_2 pressures (24000-31000 Kpa).

RICs of unsubstituted PAHs contain well defined peaks up to m/z 252. Above this mass, serious sensitivity problems were encountered. Compounds of M.W. 276 and 278 were detected, but with very poor signal to noise ratios and PAHs of M.W. 302 could not be detected at all. The poor sensitivity and chromatography in general are most certainly due to the interfacing method, since the same SFC system was first tested with a flame ionization detector and provided better chromatography than when using mass spectrometric detection.

The main differences between GC/MS and SFC/MS direct interfacing are the nature of the mobile phase and the need for a flow restrictor in SFC/MS. The gas flow rates generated by GC or SFC (using a restrictor) in the ion volume should be about equivalent, thus there seems to be no real reason why there would be such a

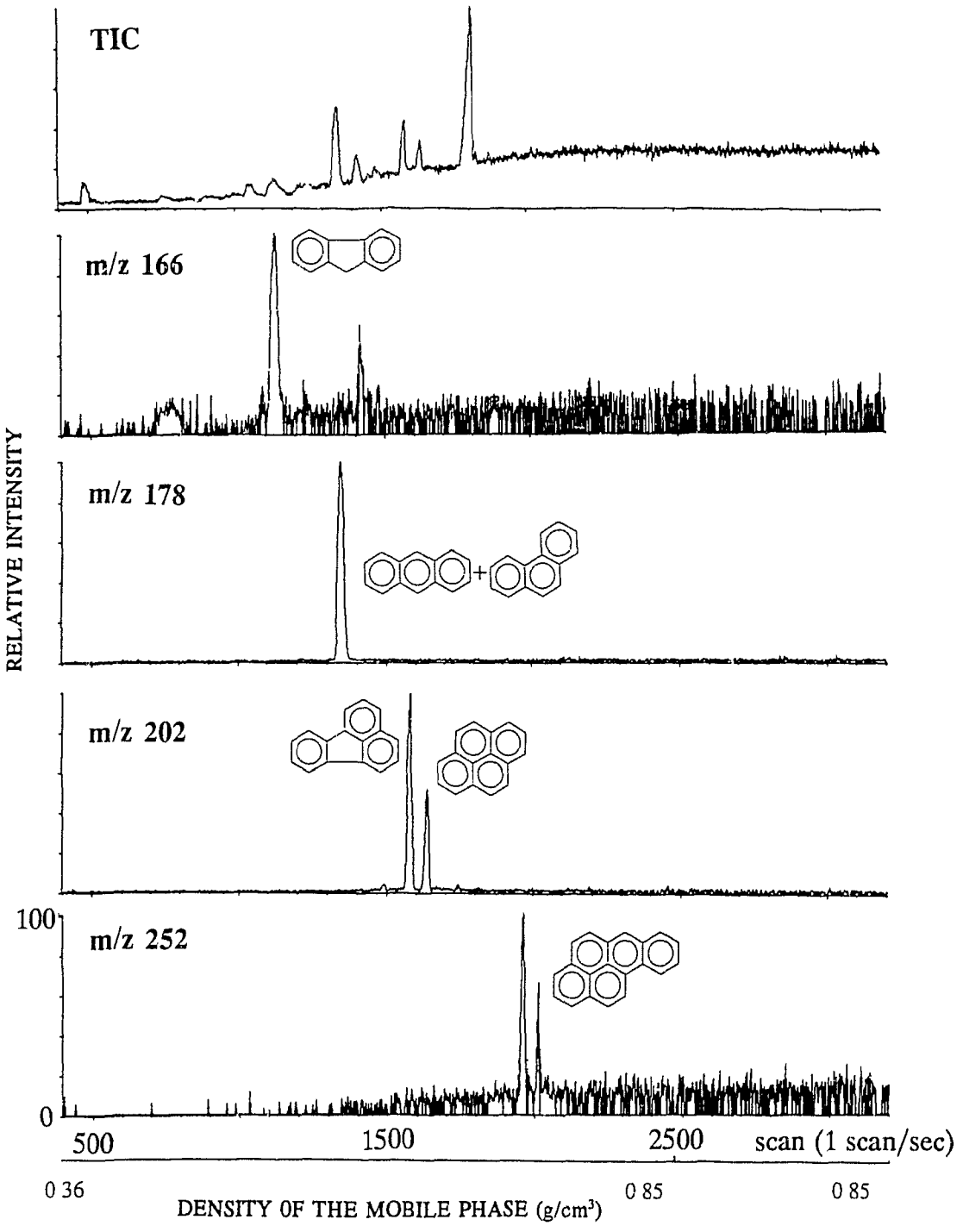


Figure 7.3.1: Capillary column SFC/MS TIC and RICs obtained for PAHs contained in the TPE sample

loss of material in capillary column SFC/MS. Practical reasons however come to mind:

- in GC/MS, no restrictor is necessary, the column effluent exits the unmodified, open tubular end of the column and thus is targeted directly at the center of the ion volume, minimizing the loss of analytes;
- in SFC/MS, the restrictor introduced into the ionization volume was pulled manually, and may have sprayed the column effluent at an uncertain angle and thus the analytes may have been targeted in a slightly different direction from that of the optimal ionization area. This angle was probably accentuated as the CO_2 pressure increased, since deposition of analytes and accumulated dry ice would partially block the effluent from being sprayed into the ion volume. This has been observed by running the SFC pressure program with the restrictor outside of the ion source, and monitoring the sample deposition on a sheet of dark paper;
- the flow rates at the end of GC columns are relatively stable and decrease slightly throughout the runs. However in SFC, the mobile phase flow rate increases with pressure during the course of a run, as the pressure is programmed. At high pressures, sputtering of CO_2 clusters causes irregular (intermittent) flow rates;
- the rotary and diffusion pumps of mass spectrometers can withstand helium better than carbon dioxide, which is more corrosive.

The SFC/MS interfacing method used in this work obviously requires some modifications in order to provide stable flow rates and spraying of the column effluent in the proper direction. A direct fluid injection (DFI) probe designed by Smith and co-workers (152) features a drilled orifice used as a pressure restrictor. A 0.5-2.0 μm hole is drilled in 13 μm thick stainless steel, and a small tin gasket is used to make a tight seal between the probe tip and the pressure restrictor, resulting in a dead volume estimated to be of the order of 0.01 μL. A second DFI probe design (152) is similar but terminates in a 0.2-0.5 mm length capillary restrictor (Pt-Ir tubing). The restrictor is formed by crimping the end of the tubing to obtain the desired flow rate. This restriction also provides an effective zero volume interface. Both DFI probes allow the column effluent to focus in the optimal ionization zone

of the source, and overcome the problem of the formation of solid CO_2 beads since the probe is heated.

However, building probes like those described above constituted a whole project on its own because major modifications to the ionization source and to the source housing were required. Therefore, no further improvements of the capillary column SFC/MS interface were attempted for the purposes of this thesis.

The low intensity RICs (e.g. m/z 216, not shown) show several very narrow, randomly scattered peaks that are not due to real compounds. These spikes are produced by the irregular sputtering of the mobile phase. Games et al. (149) proposed a way to overcome this problem, via coupling a frit restrictor at the end of the column; such restrictors are now commercially available and provide accurate and reproducible flow rates. Their flow rates in the pressure ranges used for the experiments described here were however too high to be handled by the pumping system of the mass spectrometer.

The very small volume of sample injected was another factor that restricted sensitivity. The volume injected was the maximum possible loading of the column without observing peak broadening due to column overloading.

Even when the SFC/MS peak widths lie between those obtained by GC/MS and HPLC/MS, the resolution and selectivity achieved here are not sufficient to separate the isomeric species phenanthrene and anthracene (m/z 178). Some of the compounds of M.W. 252 are also unresolved, since only 2 well defined peaks were obtained as compared to 5 by GC/MS and 9 by HPLC/MS.

Some SFC/MS parameters were adjusted in order to optimize the general quality of the chromatography: flow rate (pressure controlled) and composition of the mobile phase, temperature of the column and quantity of analyte injected were all optimized.

The flow rate of the mobile phase was restricted by the pressure requirements of the mass spectrometer pumping system. The Brownlee syringe pump used for this work did not give a precise reading of the flow rate; instead it allowed one to restrict the flow to a maximal value, which in this case was 100 μL/min. Varying this

maximal value for the purposes of optimization from 50 to 500 μL/min caused no major changes in the chromatography, which indicates that the actual flow rate was under 50 μL/min. Known flow rates of 50 μL/min obtained using the ISCO pump were compared qualitatively with those exiting the capillary column. This comparison led to the conclusion that the flow rates employed here are of the order of 10-20 μL/min at the beginning of the pressure program and increase to ca. 50 μL/min toward the end. The maximum flow rate on the Brownlee pump was thus left constant at 100 μL/min, in case the actual value would exceed 50 μL/min at high CO_2 pressure.

The optimal mobile phase composition was found to be 100% CO_2, after a few attempts to use methanol as an organic modifier. For a still unknown reason, the use of methanol tended to plug the restrictor, narrowing its internal diameter down until nothing could pass through. Column bleeding due to stripping of the stationary phase is presently the best explanation, but no evidence was obtained to support this hypothesis. Ideally, the cross-linked DB-5 stationary phase should survive drastic conditions such as methanol in carbon dioxide at 35000 kPa. Organic modifiers are added to CO_2 to reduce adsorption of the analytes on the stationary phase and to change the column selectivity. Billie and Greibrokk (127) have improved the SFC of PAHs and nitro-PAHs by adding 2% methanol to CO_2. They found that NPAHs were not soluble in supercritical carbon dioxide and that their elution was possible only if a polar agent was added to the mobile phase. Several possible mobile phases and modifiers have been used by other workers (125) and are listed in Table 7.3.1.

Although there seem to exist many possible combinations of mobile phase-modifiers, these are restricted by the respective physical properties of the compounds, because their critical pressures and temperatures must be compatible and both should be miscible. Moreover, if the SFC pump is equipped with only one solvent inlet, the mobile phase and modifier must be pre-mixed either in a gas cylinder or a solvent reservoir. Mixtures in gas cylinders are available commercially, although they are expensive. Room temperature liquid-liquid mixtures are easy to prepare in the laboratory but generally require high temperatures to attain their supercritical

Table 7.3.1: Mobile phases and modifiers used in SFC

mobile phases	modifiers
pentane, ammonia, nitrous oxide, CO_2, butane, SF_6, xenon, CCl_2F_2, CHF_3.	acetonitrile, 1,4-dioxane, diethylether, diisopropylether, pentane, hexane, butane, ethane, ethylene, methanol, isopropyl alcohol, N_2O, THF.

state, and SFC columns cannot be subjected to excessive temperatures in order to avoid deterioration of the stationary phases. Carbon dioxide is easily available, easy to handle, non toxic and is the most widely used mobile phase in SFC. It has also been shown to be a good eluent for neutral and moderately polar PACs (127) in the GC-amenable range.

As the mobile phase density is the "driving force" which influences elution of compounds in SFC, column temperature programming had to be achieved in conjunction with mobile phase pressure (density) programming, because the density of supercritical fluid CO_2 decreases with temperature (125). As the temperature increases, CO_2 becomes more like a gas (lower solvating properties) and the elution of volatile, low M.W. compounds is favored. Heavy, non-volatile compounds are eluted at lower temperatures, when supercritical fluid CO_2 more closely resembles a liquid. In fact the temperature dependence of retention in SFC is very complex and has been described by Yonker and co-workers (177). Low temperatures and high pressures (high density) towards the end of the chromatographic experiment will allow elution of large molecules, under conditions that resemble HPLC more than GC. At the beginning of a run, high temperatures coupled with low pressures (low density) will allow elution of small, volatile, CO_2 soluble molecules. GC conditions are therefore almost reproduced.

Throughout a chromatographic run, the density is usually increased to allow

the elution of compounds with a wide range of volatilities, solubilities and molecular weights. Pressure vs. density isotherms are not linear functions, as shown by Fields and Lee for pentane (132). Since in this work pressure, but not density, was indicated by the SFC pump, the density values were calculated from pressure-density isotherms available for CO_2 from the literature (191). In another publication, Fields and Lee (192) compared linear density programming, linear pressure programming and asymptotic density programming for the separation of compounds in mixtures of homologs, and found the latter mode of programming afforded separations with regular time intervals between homologous compounds. It was thus decided that the density program of CO_2 described in Section 2.5.3 would be used for this experiment.

The effect of solute concentration on retention in SFC has been investigated by Yonker et al. (193). They reported that at high CO_2 pressures, low M.W. nitro-PAHs eluted later if their concentrations were above a certain threshold value, which varied with the pressure. For the purposes of this work, the sample concentration and volume were adjusted so as not to overload the SFC column (qualitatively, from inspection of the peak shapes). While optimizing this parameter, no changes in retention times were observed as a function of concentration for PAHs; peak tailing could, however, indicate overloading of the column.

The results obtained from the RICs in Figure 7.3.1 and all other RICs from m/z 128 to 276 are tabulated in Appendix V. As no retention index table and no comparable SFC/MS results were available from the literature, the compounds were assigned hypothetical identities, based on the nature of the species already found by GC/MS and HPLC/MS. Since only a few RICs contained significant peaks, Appendix V is relatively shorter than the spreadsheets obtained for the GC/MS and HPLC/MS analyses of the same sample.

Figure 7.3.2 shows some of the SFC/EI-MS mass spectra, whose background noise could not be subtracted due to the continual variation in the intensity and nature of the noise. The quality of these spectra is thus poor compared to that obtained by GC/MS and HPLC/MS.

Of all techniques used in this work, capillary column SFC/MS is the one that

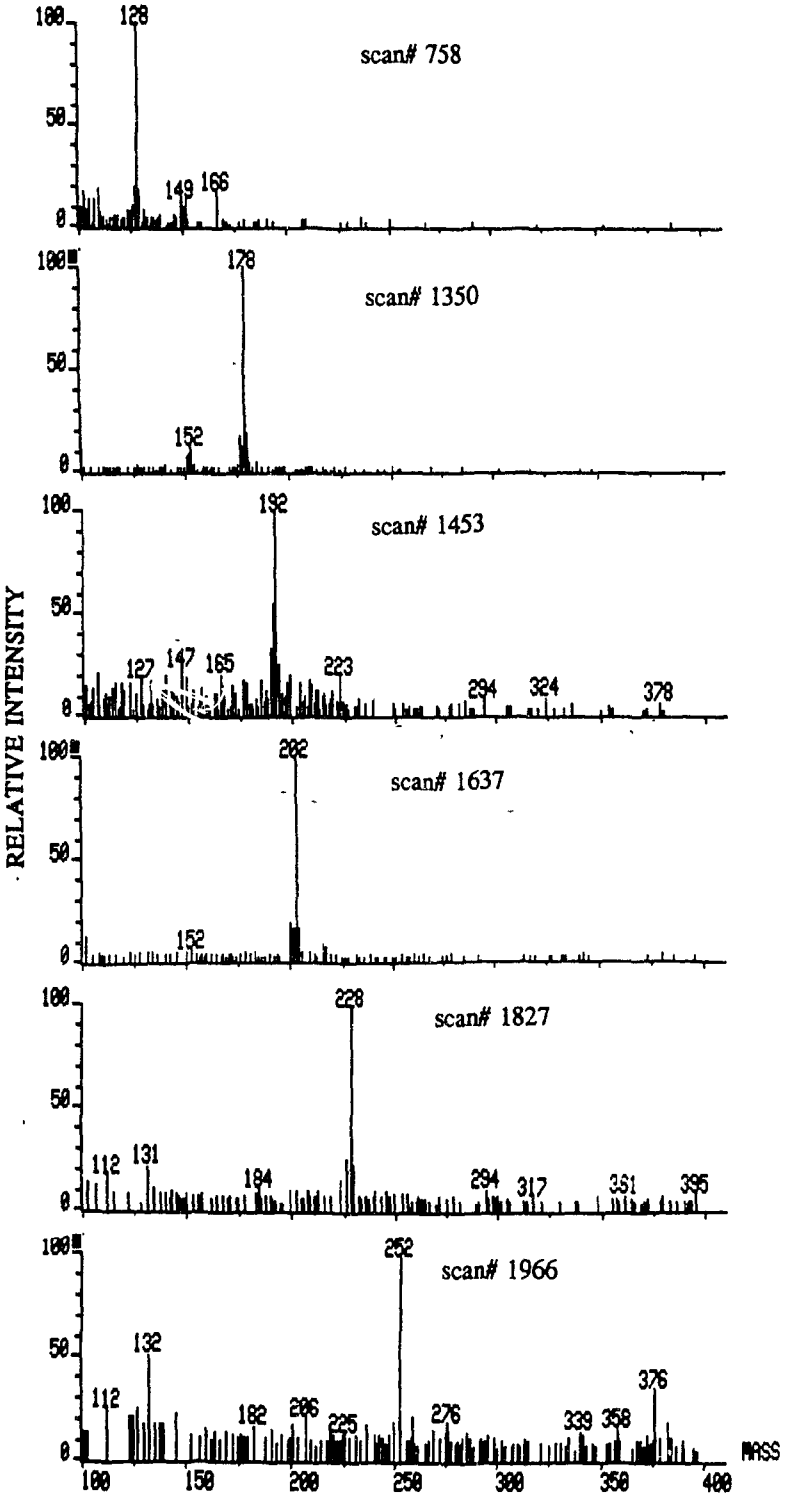


Figure 7.3.2: Mass spectra obtained by capillary column SFC/MS for the TPE sample

requires the most optimization and that yields the least reproducibility in results due to the limited life time of the restrictors. The results obtained here show that fewer compounds could be identified by SFC/MS than HPLC/MS or GC/MS. The SFC/MS system behaved like a GC/MS system with very low column efficiency and low yield of sample transfer to the ionization volume. Therefore, capillary column SFC/MS has not been used for the analysis of the Syncrude samples.

7.3.1.2 Packed column SFC/MS

i) Packed C_{18} reversed phase column

One of the advantages of packed column over capillary column SFC/MS is that it offers a greater column loading capacity. However, multidirectional paths undertaken by sample molecules within the stationary phase yield wider peaks and less column efficiency in general.

The column used here had an internal diameter of 1 mm and thus column capacity was limited; a 1 μL injection loop was used. The choice of the mobile phase, the optimization of the moving belt interface parameters and of the pressure program were all performed using a solution of PAH standards. Two sets of conditions were found suitable for PAC analysis: in the first, 100% CO_2 was used and in the second, 10% methanol in CO_2, commercially pressurized in a gas cylinder, was employed as the mobile phase. In both sets of conditions, pressure programming was used (see Section 2.5.3).

Although 10% methanol in CO_2 yielded better separation of PAHs in the TPE than pure CO_2, both sets of conditions were retained, because the presence of methanol was suspected of stripping the C_{18} stationary phase off the column. In fact, the continuous use of methanol in CO_2 slowly contributed to plugging the restrictor, which had to be replaced at the expense of non-reproducible retention times. The results presented here are obtained from a data set acquired using 10% methanol in CO_2; the results obtained with 100% CO_2 will not be shown here. The latter are

quite similar to the presented results, with slightly longer retention times and wider chromatographic peaks.

Figures 7.3.3-4 show a few RICs related to PAHs and alkylated PAHs. The highest m/z value that yields a chromatogram with defined peaks is 378 Da. It was hoped that this technique would provide for the analysis of high M.W. PACs as observed by HPLC/MS (Section 7.2). However, it only covered the mass range of compounds analyzable by high temperature GC/MS (Section 7.1.1.2). The column efficiency was poor in terms of isomer discrimination. Although M.W. 202 fluoranthene and pyrene are almost baseline separated, the isomers of chrysene at m/z 228 all elute in two unresolved peaks, while the RIC obtained by HPLC/MS shows 4 peaks for the same ion trace. The same situation is observed with the isomers of PAHs of M.W. 252, 278, and with most sets of isomers. The RICs at m/z 276 and 302 exhibit two main peaks, very well separated for m/z 276 and less defined in the case of m/z 302. If one now refers to the same m/z traces obtained by reversed phase HPLC/MS (Section 7.2), one observes two distinct groups of compounds, each, however, eluting with distinct peaks. As the elution order should remain the same from reversed phase HPLC to reversed phase SFC, one expects the first and second SFC/MS peaks of m/z 276 to cover at least 2 and 3 isomers, respectively. Applying the same rationale to m/z 302, both SFC/MS peaks should contain at least respectively 6 and 4 isomers. These comparisons show a great difference in column selectivity between HPLC and SFC. The lesser selectivity obtained using SFC with respect to HPLC may be due to the different nature and state of the solvent employed from one technique to the other.

Data have not been tabulated here, due to the lack of chromatographic efficiency and selectivity. Most chromatographic peaks in the RICs correspond to many coeluting isomers. This packed column SFC/MS technique is not, as it should be, complementary to GC/MS and RPLC/MS but it is useful to confirm the presence of certain groups of compounds already identified by the two latter techniques. The SFC/MS experiments reported here can be conducted more rapidly than HPLC/MS runs, and require less attention from the experimentalist. Reversed

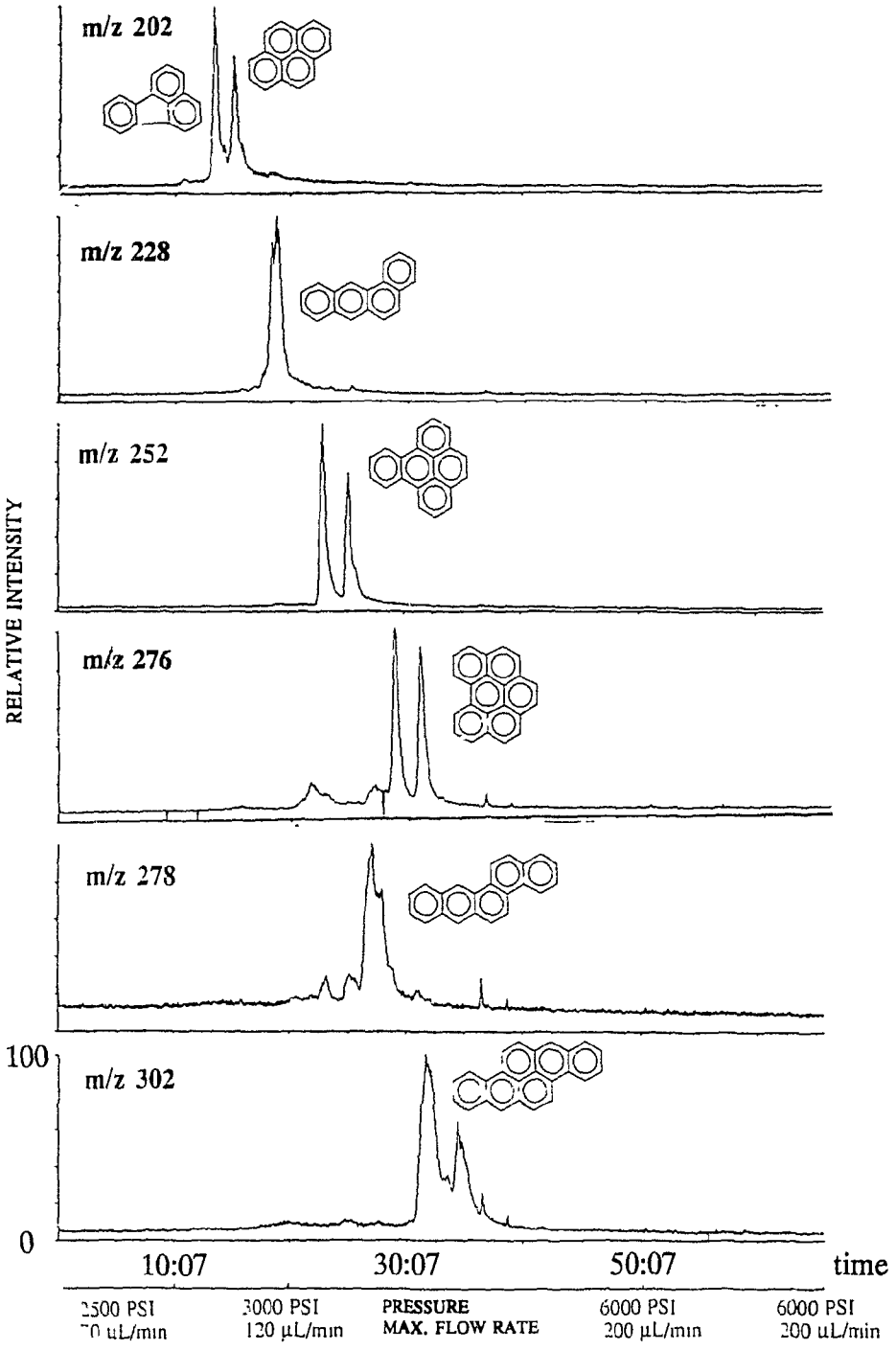


Figure 7.3.3: Reversed phase SFC/MS RICs obtained for some of the PAHs found in the TPE sample

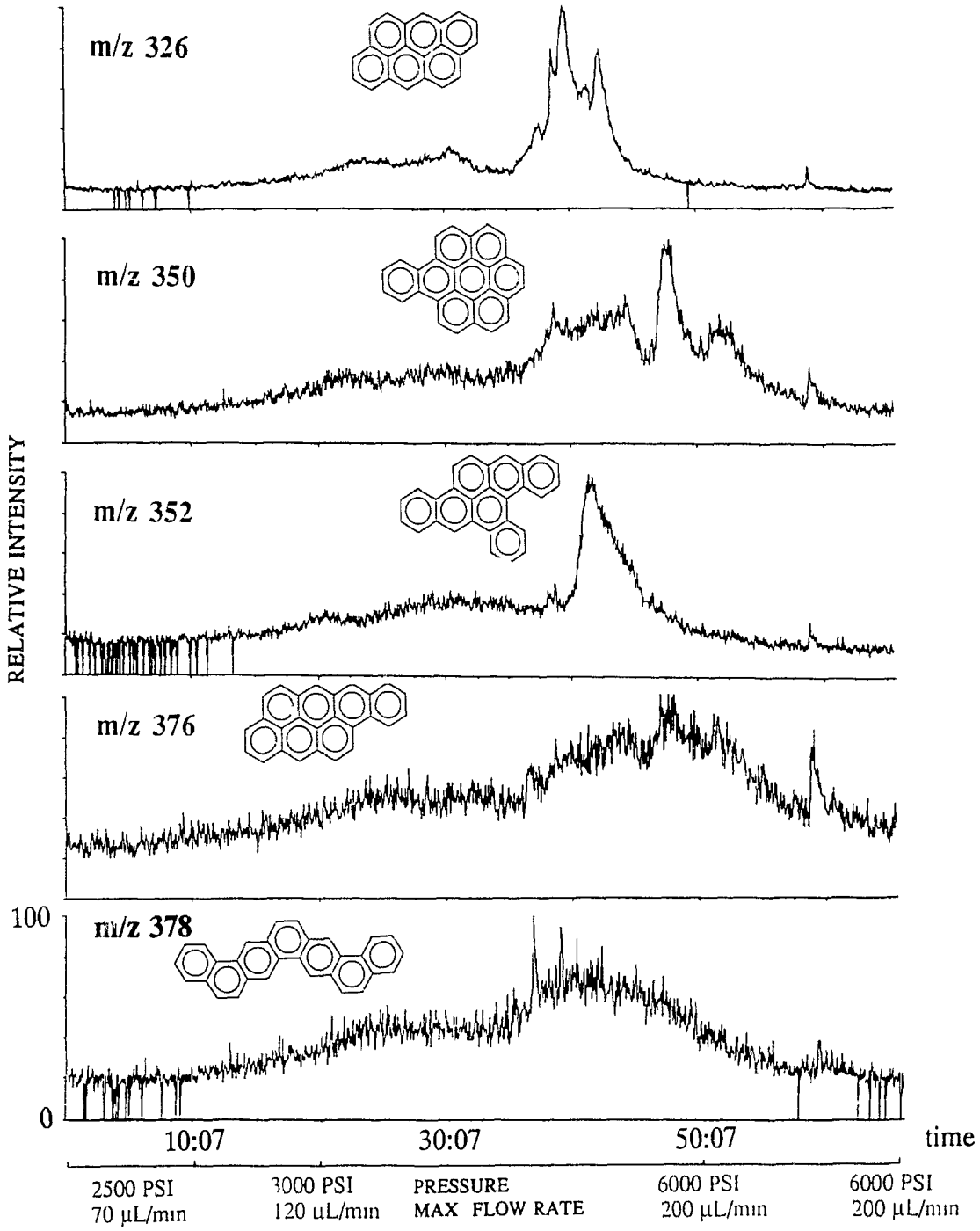


Figure 7.3.4: Reversed phase SFC/MS RICs obtained for some of the PAHs found in the TPE sample

phase packed column SFC/MS can therefore be used on a routine basis as a profiling technique. Interfacing packed column SFC to the mass spectrometer actually requires less effort than interfacing HPLC/MS. As CO_2 exits the restrictor as a supercritical fluid, the solutes are deposited onto the belt and the mobile phase is readily transformed into a gas and thus no liquid is left on the belt. The temperature of the sample depositor, critical in HPLC/MS to obtain a good spraying on the belt, has to be set to ca. 50°C at the beginning of the experiment, to avoid CO_2 freezing at the restrictor. This temperature can be kept constant for the rest of the run. Moreover, the optimization of the method showed that no nitrogen nebulizing gas is required because the latter scatters the effluent away from the center of the belt.

Packed column reversed phase SFC/MS is thus a robust technique and can be used as a rapid characterization technique for mixtures of PACs of moderate M.W. Carbon dioxide, even modified with methanol, has obviously not enough solvent strength to promote the elution of very high M.W. PACs. The addition of methanol however decreases the time of analysis and yields better peak shapes, but unfortunately contributes to plugging of the restrictors after a few chromatographic runs.

ii) Packed 1 mm i.d silica column

Since CO_2, either pure or modified with methanol does not have enough solvent strength to allow the elution of high M.W. PACs, the use of supercritical pentane as a mobile phase on the reversed phase SFC C_{18} column was attempted. Unfortunately, pentane stripped off some of the stationary phase. It was then decided that the use of a silica column with pentane would be more appropriate.

The eternal problem of pressure restriction in interfacing SFC to MS was rendered very minor using pentane, whose critical pressure is only 3448 kPa. There is thus a low pressure drop across the column, making restriction less difficult to effect than with CO_2. A 50 μm i.d. fused silica capillary was attached to the end of the column and kept the flow rate down within a 50-500 μL/min range, depending

on the length of the capillary. As supercritical pentane exited the capillary restrictor, it was deposited onto the belt as a liquid which provided perfect and uniform wetting of the polyimide surface. The liquid pentane was quickly evaporated due to its high volatility and left a uniform layer of eluate moving toward the ionization source. Again, no nitrogen nebulizing gas was required. The temperature of the sample depositor was increased manually as the run progressed; this was not a critical parameter for sample transfer, because pentane did not freeze at the end of the restrictor as CO_2 did.

Optimization experiments were conducted using the mixture of PAH standards. The use of 100% pentane originally yielded very rapid elution of all standards (including M.W. 278 picene) in a overall time period of 5 minutes. Since pentane had too much solvent strength, methanol was added as a "bad solvent" in order to slow down the elution of the compounds. After optimization, it was found that 7.5% methanol in pentane at a pressure of 3729 kPa, a temperature of 230°C and a flow rate of 175 μL/min provided optimal conditions for the separation of the compounds in the standard mixture. However, as the standards only went up to 278 in molecular weight, a "high M.W." part of the program had to be optimized using the real tar pond sample. Two other programming steps were added to the original segment: at 7.5% MeOH/pentane, the pressure was linearly increased from 3792 to 7584 kPa and from there, the %MeOH was linearly decreased to zero, to allow the elution of the high M.W. and methanol insoluble compounds. The column temperature was kept constant at 230°C throughout the run.

Figures 7.3.5-6 show the TIC trace and some RICs of typical PAHs. The TIC trace has a very interesting profile, in that it shows the separation of compounds by groups ("slices"). Similar features are commonly observed when performing normal phase HPLC of PACs (113). In Figure 7.3.5, the first group of compounds (eluted from 0 to 15 minutes) include, according to the RICs, species with M.W.<278. The second group corresponds to compounds with 300<M.W.<326, and the third group is characterized by isomers of PAHs of M.W. 350 and 352. Compounds of the fourth group, eluted with 100% pentane, have molecular weights of 376 and above (Figure

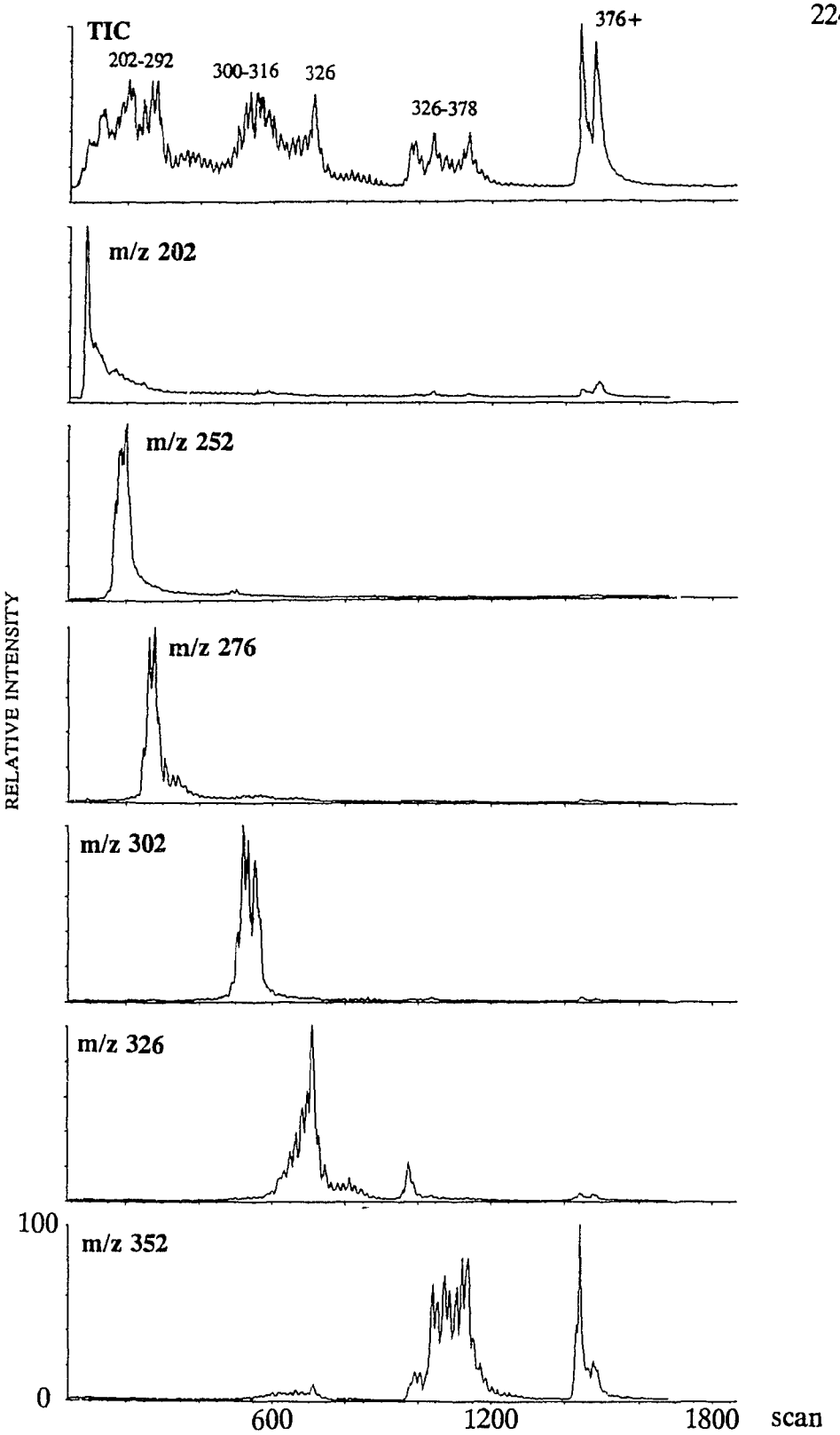


Figure 7.3.5: Normal phase SFC/MS TIC and RICs obtained for some PAHs found in the TPE sample. The numbers next to the TIC peaks indicate the mass or mass range of the compounds eluted.

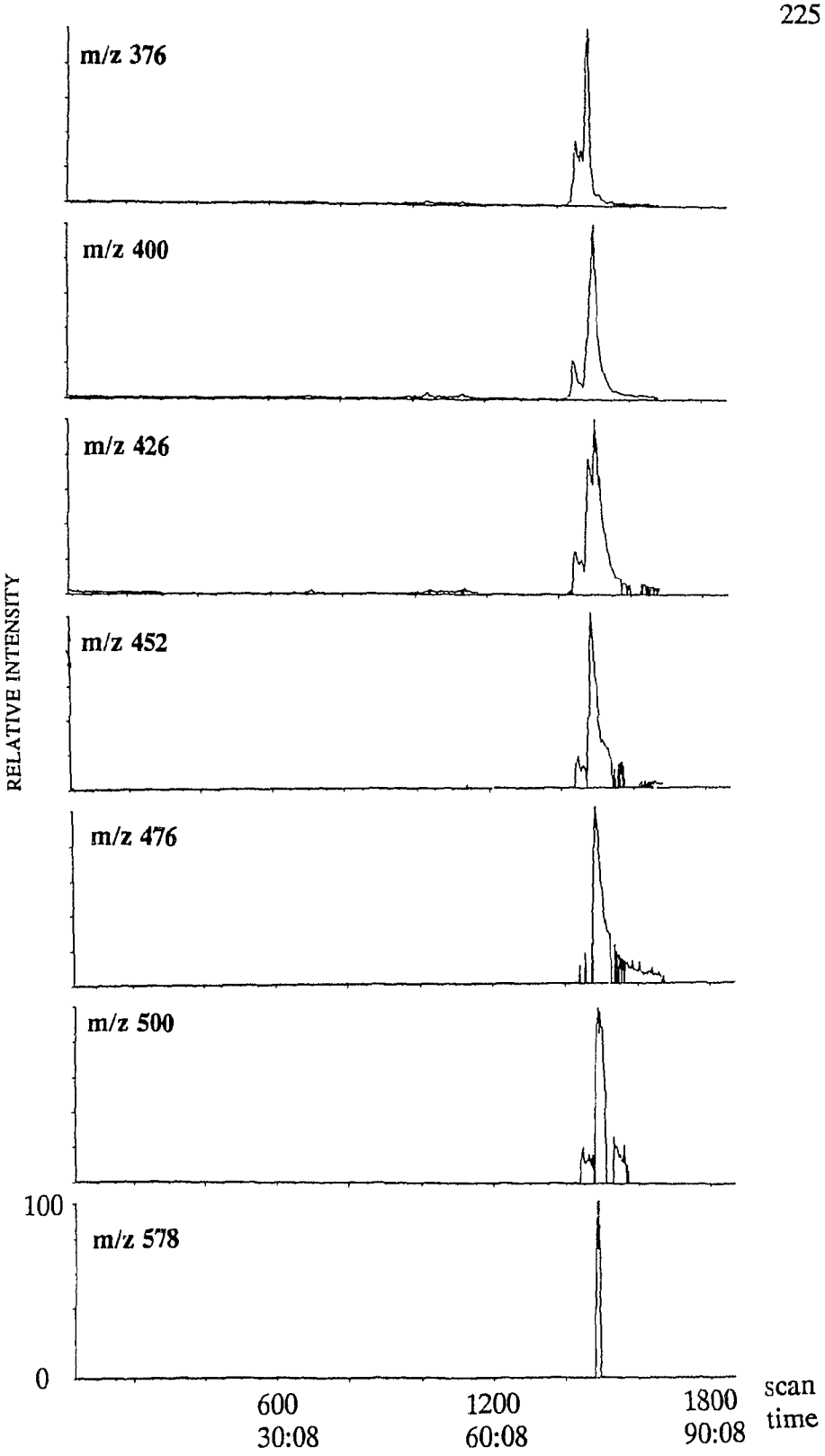


Figure 7.3.6: Normal phase SFC/MS TIC and RICs obtained for some PAHs found in the TPE sample.

7.3.6).

One can observe a certain extent of partition of some compounds between two groups. The best example to illustrate this is shown by the RIC at m/z 352. The isomers at this mass are effectively partitioned between the third and fourth groups, because some of these compounds were left on the column even after increasing the pressure to 7584 kPa; they were eluted in the next group with the decrease of the %MeOH in pentane. The RIC at m/z 350 (not shown) has the same aspect and shows that most compounds of M.W. 350 were eluted in the fourth group. The species of M.W. 350 are probably less methanol soluble than those of M.W. 352.

If one refers to Section 7.2.1.2 (RPLC/MS results) one finds that compounds of M.W. 350 are eluted with a higher DCM:ACN ratio than required for the elution of compounds of M.W. 352. Therefore the species at 350 have less affinity for polar solvents (acetonitrile, methanol) and more affinity for non-polar solvents (dichloromethane and supercritical pentane) than the species at 352. The heaviest compounds detected using this method are the series of isomers of M.W. 578, whose structures are catacondensed. The mass spectra of Figure 7.3.7 were obtained during the last part of the run. They show the presence of high M.W. PAHs, with a peak at almost every m/z value due to the high extent of coelution.

This type of chromatographic separation by groups, well illustrated by the TIC trace of Figure 7.3.5, is interesting because it renders fractionation and sample collection possible by SFC. Although performed on a micro-scale level here, this experiment would easily be transformed into a larger scale fractionation method, using a larger column and a larger injection loop. Such a method has been tried in this laboratory for the HGO, OSE and PIT samples and will be discussed later. For the TPE, it was decided not to attempt any normal phase SFC or HPLC fractionation, since the RPLC/MS characterization of the whole PAC fraction yielded satisfactory results. Fractionation of the TPE would have been rather time consuming, first because of the need to perform several successive fractionations in order to collect enough sample for further analysis, and second because a lengthy analysis is required for each fraction collected. The duration of a reversed phase

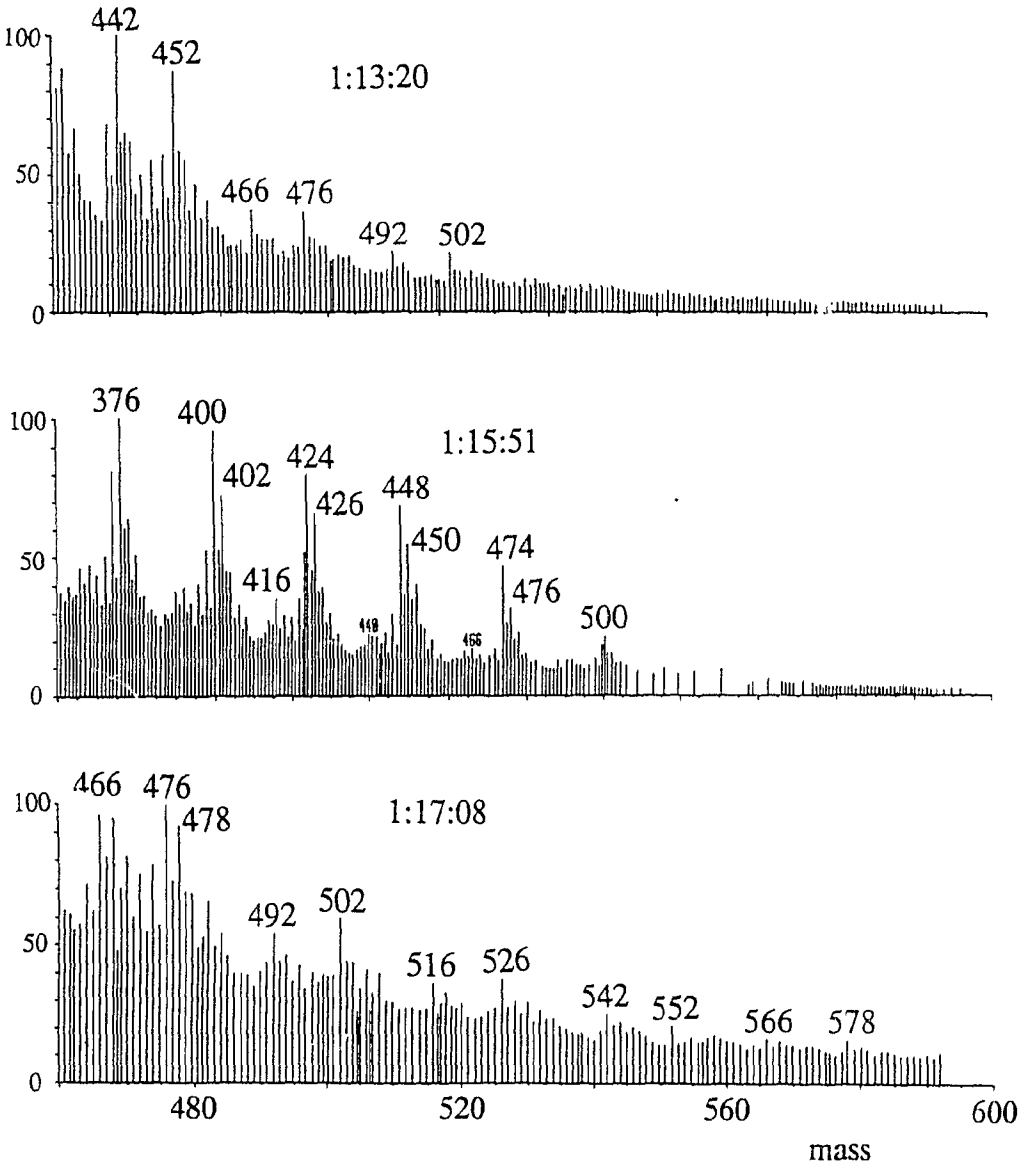


Figure 7.3.7: Mass spectra of some high M.W. species found in the TPE sample, obtained by normal phase packed column SFC/MS

HPLC/MS run is enough to allow the elution of all PACs without major interferences in the RICs. RPLC/MS allows the recognition of the PACs of different classes and also yields good isomeric discrimination.

7.3.2 SFC/MS of the Syncrude samples

The HPLC/MS data obtained for the HGO, OSE and PIT show that these samples are much more complex than the TPE, especially in the heavy ends. The complexity of the samples and thus the difficulty in obtaining good chromatographic characterization justify the need for using a complementary analytical technique (SFC/MS). Reversed phase SFC/MS is not expected to clarify the problem of separating the complex heavy ends; however normal phase SFC/MS, operated under proper gradient and pressure programming conditions, is expected to allow the elution of high M.W. compounds, as observed for the TPE in Section 7.3.1.2.

7.3.2.1 Reversed phase SFC/MS of the HGO

As the restrictor tended to plug due to bleeding of the C_{18} column when methanol-CO_2 mixtures were used, pure CO_2 was used as the mobile phase for the separation of the HGO. Figures 7.3.8-9 present a few RICs obtained in this mode. The highest m/z value that provides a reasonable chromatogram is 282 Da. (not shown). Above this m/z value, all the RICs form broad envelopes without any well-defined peaks. A direct comparison between the reversed phase HPLC/MS and SFC/MS of the HGO clearly demonstrates the lower level of efficiency and selectivity achieved using the latter. The peaks observed in Figures 7.3.8 and 7.3.9 have tailing ends because no methanol was in the mobile phase to help in desorbing compounds from the stationary phase.

The RIC at m/z 178 (not shown) does not have any well-defined peak, whereas the corresponding RIC obtained by RPLC/MS shows the presence of either phenanthrene or anthracene. The HPLC/MS trace at m/z 220 shows that ca. 8

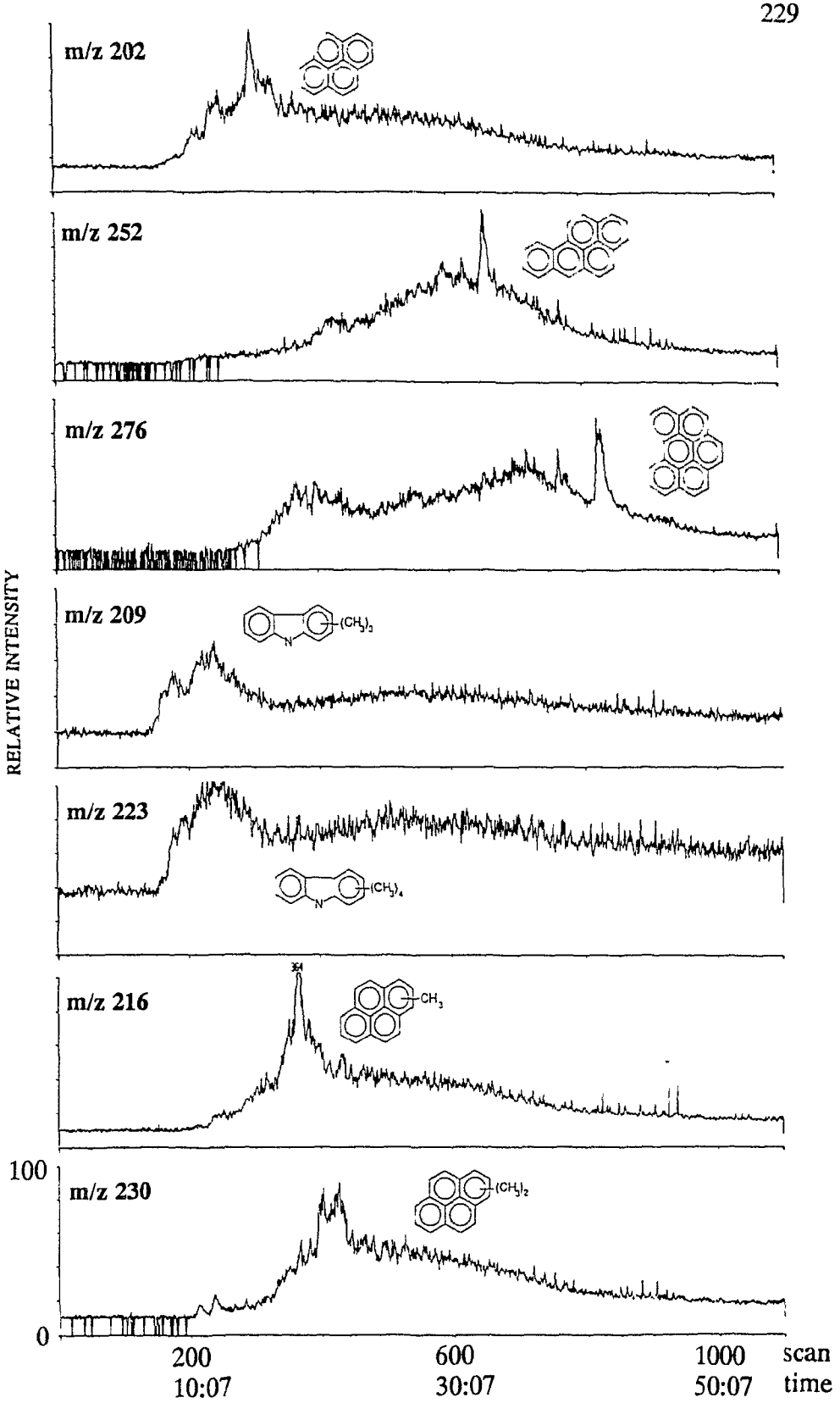


Figure 7.3.8: Reversed phase SFC/MS RICs of PACs found in the HGO sample

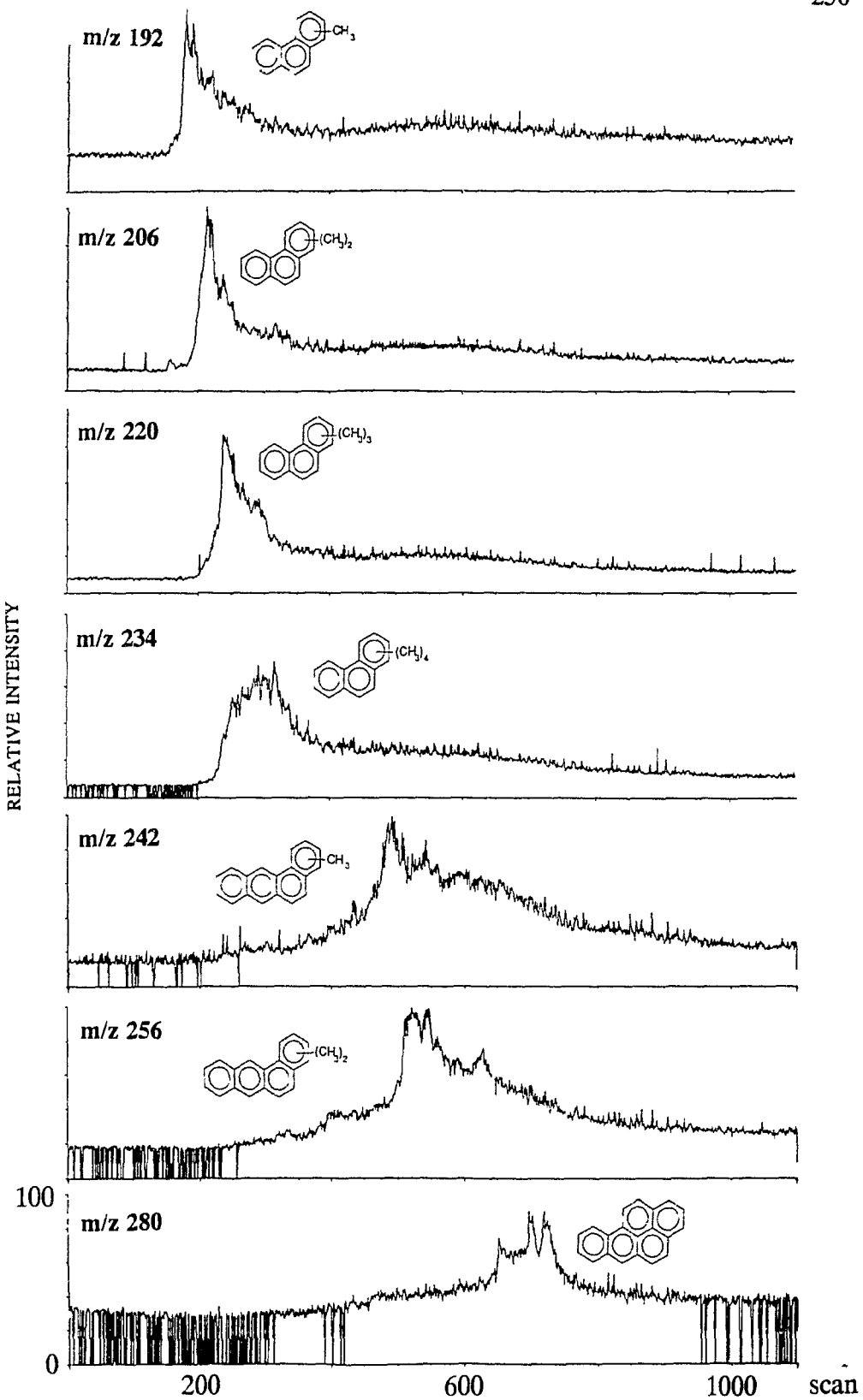


Figure 7.3.9: Reversed phase SFC/MS RICs of alkylated PAHs found in the HGO sample

compounds were separated, which were probably C3-phenanthrenes and anthracenes. However, the m/z 220 chromatogram recorded using SFC/MS exhibits 3 very broad peaks for the whole group of isomers. The same type of comparison applies to some of the other HPLC and SFC/MS chromatographic traces shown in Figures 7.2.11-14 and 7.3.8-9.

The results obtained for the reversed phase SFC/MS of the TPE show that the presence of methanol in the mobile phase has the effect of sharpening peaks and making retention times shorter for higher M.W. substances. It allowed the elution of the latter compounds with M.W. up to 378. Reasonable chromatographic quality was thus obtained using 10% methanol/CO_2 as the mobile phase, however the restrictors tended to plug after a few runs. The absence of methanol allowed the restrictors to be more functional, at the expense of lower quality chromatography, viz. peak tailing and long retention times. In an attempt to compensate for the absence of methanol, higher mobile phase pressure conditions were used throughout the SFC/MS run of the HGO. The pressure was programmed from 20685 to 51713 kPa, with the latter value corresponding to the maximum pressure under which the SFC injection valve could be operated. However, these high pressure conditions still did not allow proper desorption of material from the stationary phase, yielding poor resolution and poor peak shapes.

Since the OSE and PIT samples have lower concentrations of low M.W. PACs, and since the reversed phase SFC/MS of the HGO did not allow elution of compounds of M.W. > 282, no attempts have been made to analyze the OSE and PIT samples using this technique.

7.3.2.2 Normal phase SFC/MS of the HGO

As a conclusion to the last section, reversed phase SFC/MS did not allow the elution of compounds with M.W. higher than 282. Therefore, pentane was used as the mobile phase to enable the elution of high M.W. compounds in the normal phase SFC of the HGO. Figure 7.3.10 shows some of the RICs obtained by normal phase

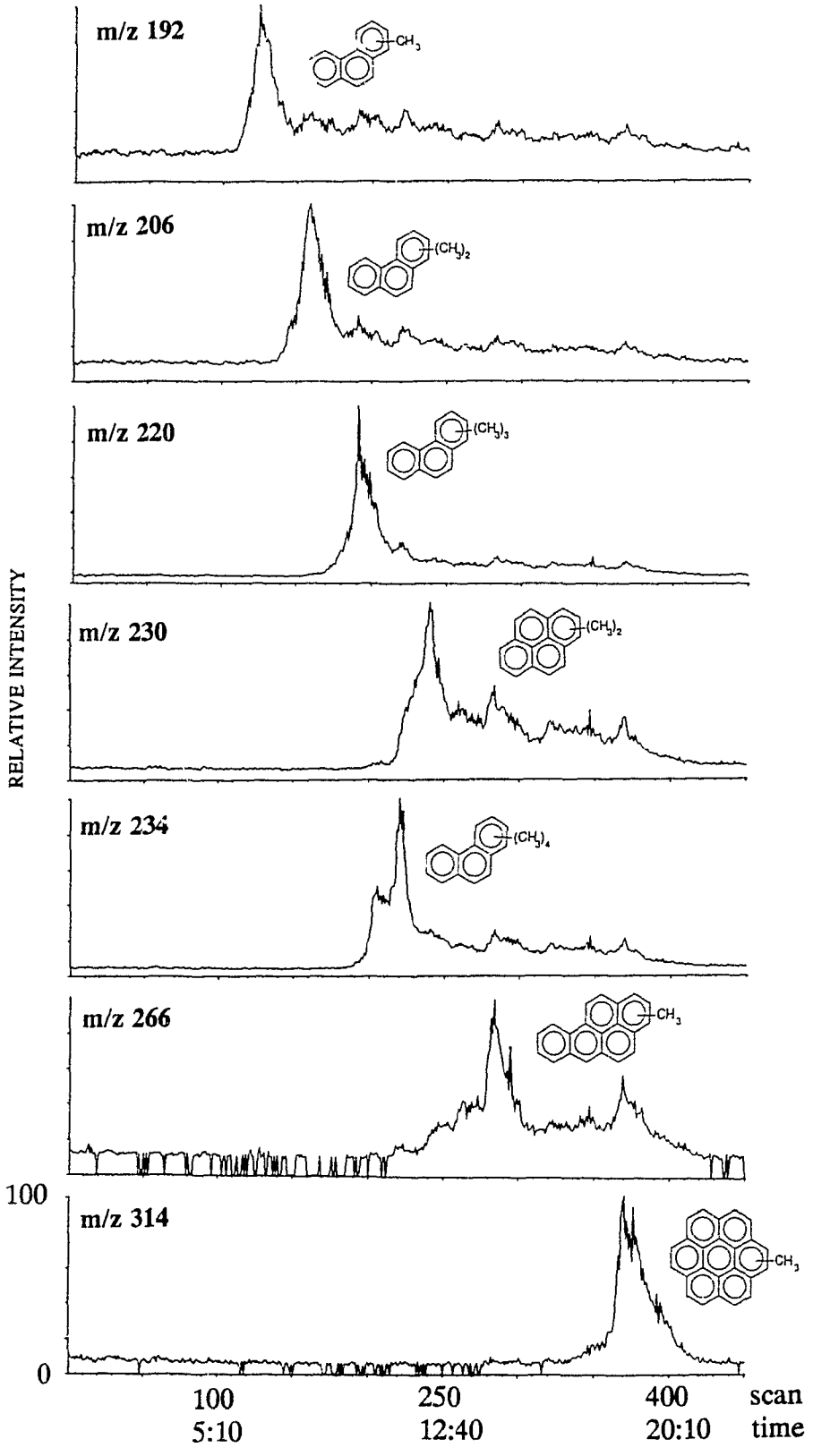


Figure 7.3.10:Normal phase SFC/MS RICs of alkylated PAHs found in the HGO sample

SFC/MS of the sample. The compounds, throughout the run, eluted in two distinct groups: low M.W. and high M.W. The chromatograms of Figure 7.3.10 are those of compounds of the first group. The conditions of pressure and gradient programming were modified from those used for the SFC/MS of the TPE, because the latter conditions caused low M.W. compounds of the HGO to be eluted too rapidly and with almost no separation. When the program was re-optimized for the HGO, however, it yielded reasonable chromatographic quality, about equivalent to that obtained with the reversed phase SFC/MS of the same sample. The main advantage of using normal phase over reversed phase SFC/MS is the increment in the range of M.W. of the compounds analyzable. In the low M.W. compound group, normal phase SFC/MS made possible the observation of peaks of species with M.W. up to 314, compared to 282 in reversed phase SFC.

The proposed structures of one possible isomer for each m/z value are shown with their respective RICs. A more complete tabulation of these results is presented in Appendix VI, which offers a comparison of the results obtained by reversed and normal phase SFC/MS obtained for the HGO sample.

Figure 7.3.11 shows the TIC and RICs of high M.W. species detected over the whole elution range. The compounds eluted in this group have molecular weights ranging from 314 to 600. The RIC at m/z 314 is the only trace to show a partition of isomers between the first and second groups of compounds. The TIC trace shows that the high M.W. compounds elute in three subgroups, each subgroup represented by a wide peak in that part of the chromatogram. The first subgroup principally includes compounds whose M.W. lay between 314 and 320 and compounds within the 320-370 M.W. range produce the second peak. In the mass range of 370-440, the compounds are partitioned between the second and third subgroups, and the compounds whose M.W. exceeds 440 were found in the third subgroup only. No data were tabulated for these compounds of M.W. 314-600. As the mass spectra of the latter exhibited ions at every nominal m/z value, a listing of all ions with their corresponding retention times and intensities would not have been appropriate for presentation in a spreadsheet. Attempts have been made to expand the second part

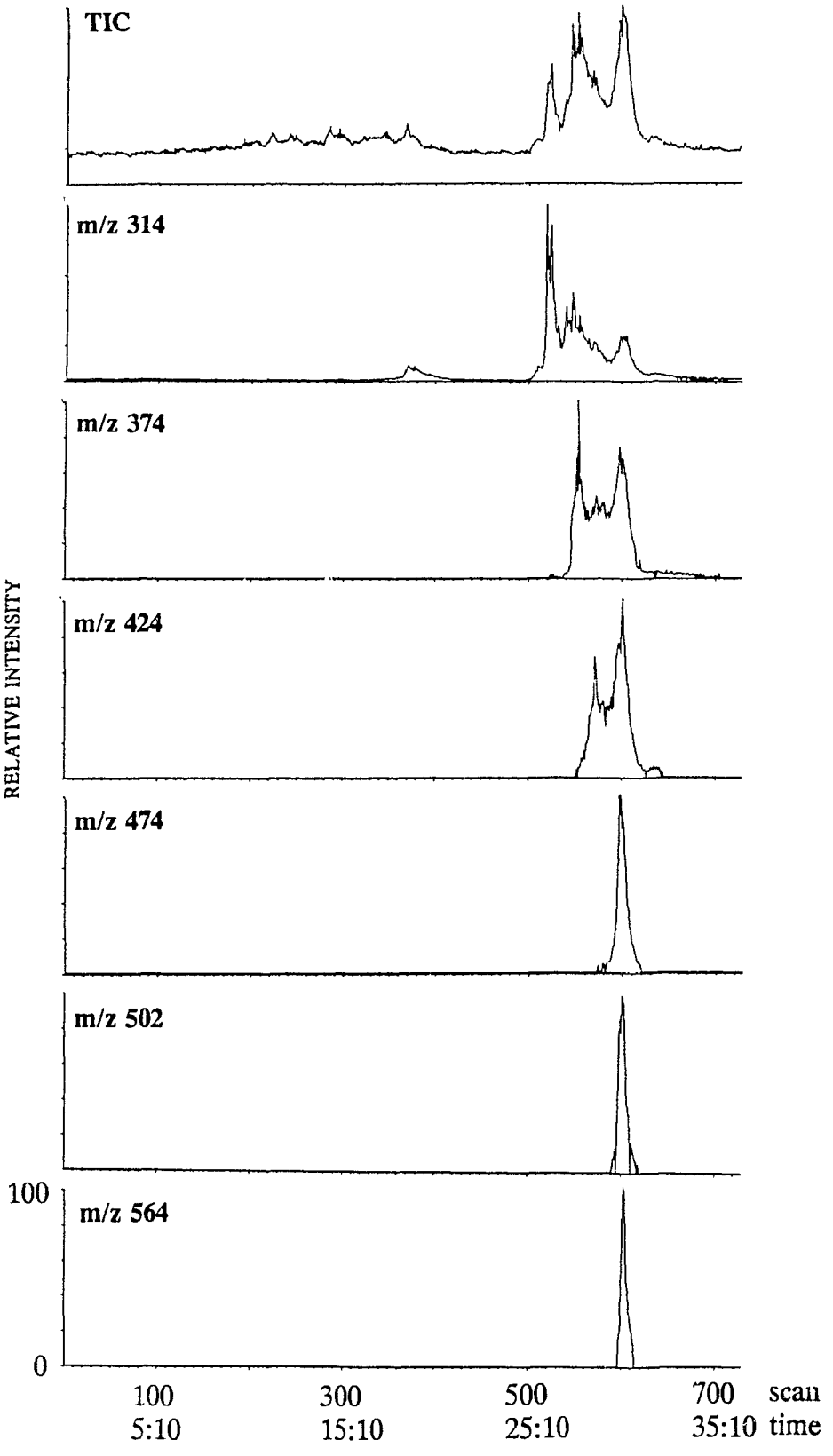


Figure 7.3.11:Normal phase SFC/MS RICs of high molecular weight compounds found in the HGO sample

of the chromatogram in time, using a slower drop of the % methanol from 15 to 0. These attempts caused the three peaks produced by high M.W. compounds to overlap and to form a broad envelope. Continuous elution of compounds in a longer analysis time was thus observed. The EI mass spectra acquired during these longer SFC/MS runs still exhibited peaks at every mass and thus were useless for compound identification (Figure 7.3.12).

The main achievement of normal phase SFC/MS was the separation of the sample into two main groups of compounds, low and high M.W. The first group was eluted over a long enough period of time to possibly be separated into several "slices" of compounds. The compounds in the low M.W. group yielded reasonable SFC chromatographic quality and corresponded to the first group of compounds eluted with acetonitrile-water in reversed phase HPLC experiments. The second (high M.W.) group is eluted as a continuum of compounds, whose resolution was not achieved even with attempts to improve the chromatographic conditions. Similarly, this second group corresponds to the high M.W. group eluted in RPLC.

The normal phase SFC method was adapted to larger scale fractionation of the HGO, OSE and PIT samples by coupling a 250 x 4.6 mm i.d. silica column to a 20 μL loop and to the Brownlee pump. The fractionation technique developed here was still at a small scale level and the fractionation of a given sample had to be carried out at least three times in order to collect reasonable amounts of material for further RPLC analyses. The results obtained using this method were presented earlier in Section 6.2.4.

The normal phase microscale experiment (1 mm i.d. silica column) was also used with the OSE and PIT samples (results not shown). The features of the resulting TIC chromatograms resembled those observed in the HPLC/UV traces of the same samples, with respect to the intensity ratios of [high M.W/low M.W], which increased in the order HGO-OSE-PIT. However, the TIC traces obtained by normal phase SFC/MS as compared with their RPLC/MS equivalents had consistently higher [high M.W./low M.W.] ratios. This distinction is due to the parameters governing the sample transfer onto the belt by interfacing HPLC/MS and SFC/MS and to a

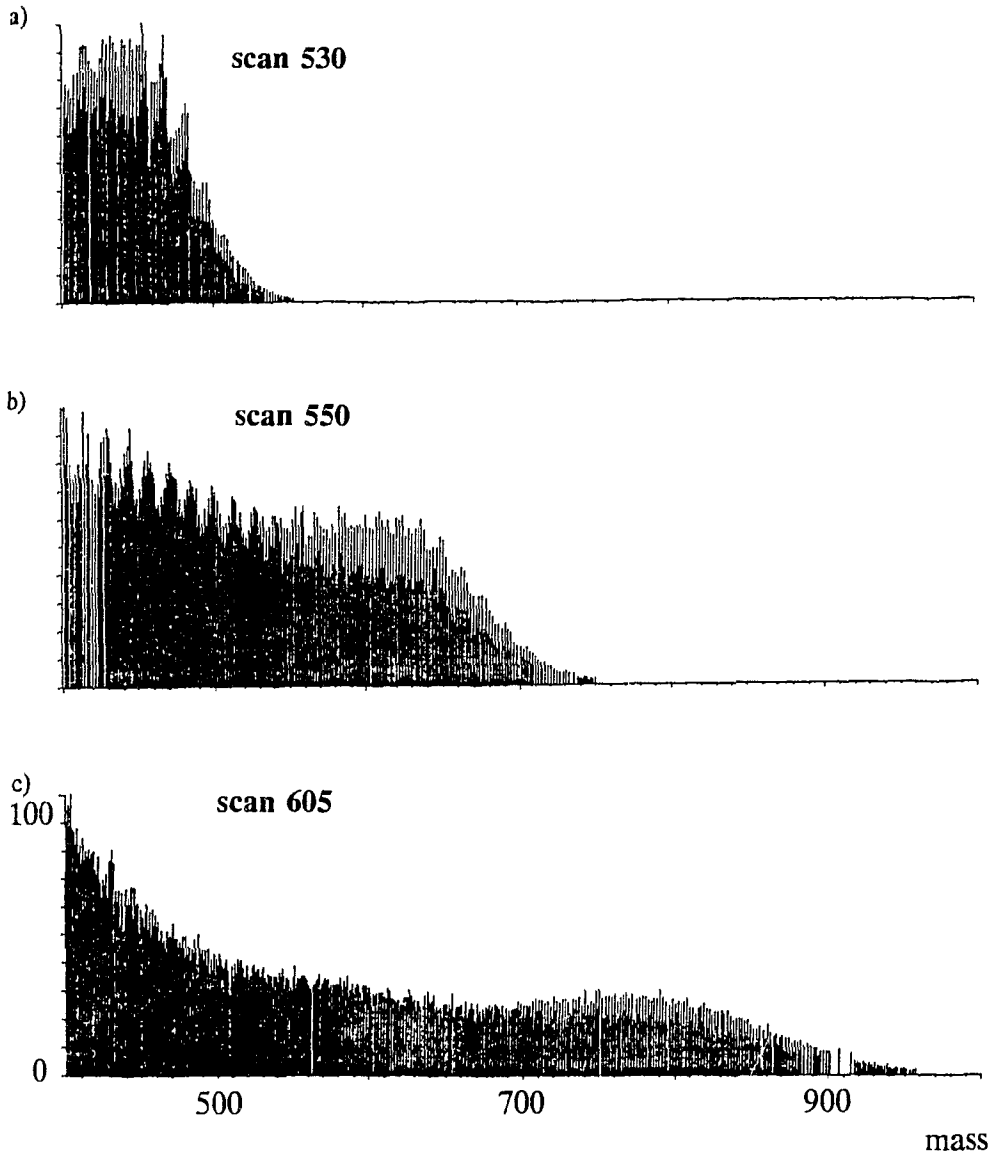


Figure 7.3.12:Mass spectra of compounds contained in the HGO sample, obtained by normal phase SFC/MS (high M.W. portion of the run)

different partition of the compounds between the so-called "low M.W." and "high M.W." portions of the samples with different modes of chromatography.

In general, compounds whose M.W. lay between 330 and 600 in the HGO, OSE and PIT were hardly separable by chromatography and their mass spectra as observed from chromatographic/mass spectrometric runs were not assignable to any particular compound due to the presence of too many molecular and fragment ions. These compounds are thought to be highly alkylated and highly functionalized PAHs, in which the mass of the side chains far exceeds that of the polycyclic aromatic moieties. One would thus expect their chromatographic behaviour to be somewhere between that of purely aliphatic compounds and that of aromatic substances. It has been observed in this laboratory that long chain aliphatic compounds elute as continua in RPLC, whereas pure aromatic compounds elute as sharp peaks. The high affinity of highly alkylated aromatics for the C_{18} reversed phases (hydrophobicity) is rationalized by the high solubility of the long alkyl chains in octadecyl moieties. The hydrophobicity of aromatic compounds increases with the degree of alkylation, which explains why the high M.W. portions of the HGO, OSE and PIT are eluted with dichloromethane (and not acetonitrile and water) in RPLC. The compounds are eluted when they are more soluble in the acetonitrile-dichloromethane mixture than they are in the octadecyl chains.

The behavior of these compounds in normal phase SFC seems to depend on their solubility in the mobile phase. If the nature of their interactions with the silica stationary phase were the main retention factors, those high M.W. species would be eluted first due to their hydrophobicity. In fact, when 100% pentane was first tested as a mobile phase, the whole contents of the samples were eluted in a period of 5 minutes, showing the lack of interaction with the stationary phase. When hydrophilic methanol was added to pentane, the highly hydrophobic species were not eluted until the % methanol was dropped to zero.

Normal phase SFC offers the advantage of possible elimination of the high M.W. end of the samples by fractionation. The clean, low M.W. samples thus obtained are easy to chromatograph using any technique without contaminating the

column every time with highly alkylated and hydrophobic species. The latter compounds require drastic conditions in either RPLC or normal phase SFC to be eluted from the columns. In reversed phase SFC using CO_2 as the mobile phase, they never make it through the column and thus contaminate the stationary phase. It is therefore advantageous to eliminate high M.W. fractions from oils and pitches prior the final analysis of the low M.W. portion.

8. RESULTS AND DISCUSSION: SELECTIVITY ENHANCEMENT

8.1 Fragmentation of doubly charged ions of PANHs

This work on PANH dications was initiated with the aim of achieving some degree of isomer discrimination for PANHs by mass spectrometry. It is the continuation of a similar investigation on PAH dications (161). In that previous work, unimolecular and collision induced dissociation (CID) reactions of molecular dications of PAHs were investigated. For larger PAHs (three rings or more), the unimolecular charge separations, characteristic of smaller aromatic dications, were unimportant relative to expulsion of the neutral species C_2H_2 (acetylene). These findings were rationalized by two principles:

- The necessity of avoiding anti-aromaticity implied in oxidizing a $(4n+2)\pi$-electron aromatic system to a 4n system (194).
- Expulsion of neutrals from larger PAH dications are possibly radical-site initiated whereas charge separation reactions for smaller PAH dications are likely to be charge site induced reactions (161,195).

The present work is an extension of the PAH dication study (161) to compounds in which a nitrogen atom is incorporated into one of the aromatic rings (PANHs).

Another objective of this part of the work was to enhance selectivity for the analysis of PANHs among other PACs by monitoring their half mass doubly charged molecular ion or specific constant neutral losses during a chromatographic/mass spectrometric experiment. This idea was inspired from earlier work achieved in this laboratory (45) where selectivity enhancement was observed in terms of useful improvements of S/N ratio in GC/MS analyses, when monitoring the half mass doubly charged ions of the first ^{13}C isotope peak of PAHs. Subsequently a similar result was obtained by monitoring non dissociative electron capture collisions of the dications (2E mass spectra, (196)).

The analogous PANHs investigated here are important constituents of tobacco

smoke and have been linked to carcinogenic activity (160,162). They are also found in polluted marine and estuary sediment samples (197).

Monitoring specific fragmentation reactions of PANH molecular dications, for instance neutral losses, is helped by understanding why fragmentation occurs and what type of fragmentation to expect. Therefore, fragmentation behavior of molecular ions of PANHs under unimolecular and CID conditions has been studied. The following points were investigated:

- Stabilization of PANH vs PAH dications via charge localization on the heteroatom.
- Fragmentation behavior of PANH dications based on their structure (pyridine-based vs pyrrole-base compounds). It might be expected that the extent of charge localization on the nitrogen atom should differ for these two PANH classes.
- Possibility of some isomer discrimination of PANHs using tandem mass spectrometry (MS/MS) of their dications as an analytical tool.
- Fragmentation behavior as a function of size.

8.1.1 Compounds containing a pyridine ring

For pyridine-based compounds, the formation of an aromatic sextet in the 6 membered ring upon double ionization does not require participation of the nitrogen lone electron pair. Naive considerations would suggest some degree of charge localization on the nitrogen atom, accommodated through the loss of lone pair electrons without disruption of the aromatic π system.

In Figure 8.1.1a, it is observed that pyridine dications undergo unimolecular charge separation, yielding two even electron species, at m/z 28 and 51, respectively. For larger PANHs of the pyridine-based series (two rings or more), charge separation is largely dominated by the expulsion of apparent mass 13.5; Figure 8.1.2a shows this feature for acridine. This reaction can only correspond to expulsion of a neutral fragment of mass 27 Da, leaving a doubly charged fragment ion. Repeating the experiment with acridine-d_9 (perdeuterated) confirmed the expulsion of neutral HCN (or HNC) rather than C_2H_3 because the neutral loss was found to be 28 instead of

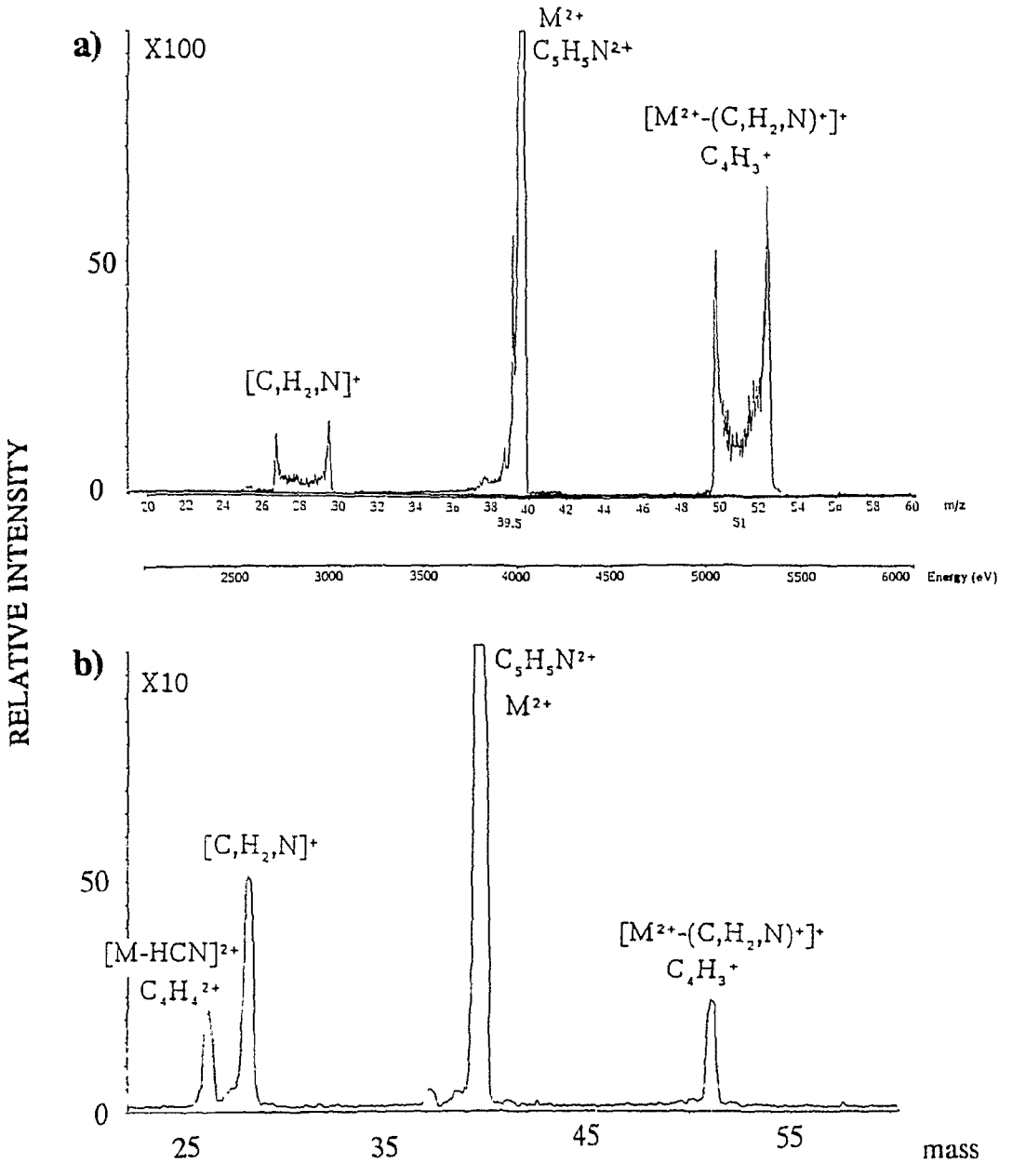


Figure 8.1.1: Fragmentation spectra of pyridine **1** dications (m/z 39.5); a) M.I.K.E. spectrum, unimolecular fragmentation conditions; b) fragmentation in the rf-only quadrupole collision cell, C.I.D. conditions.

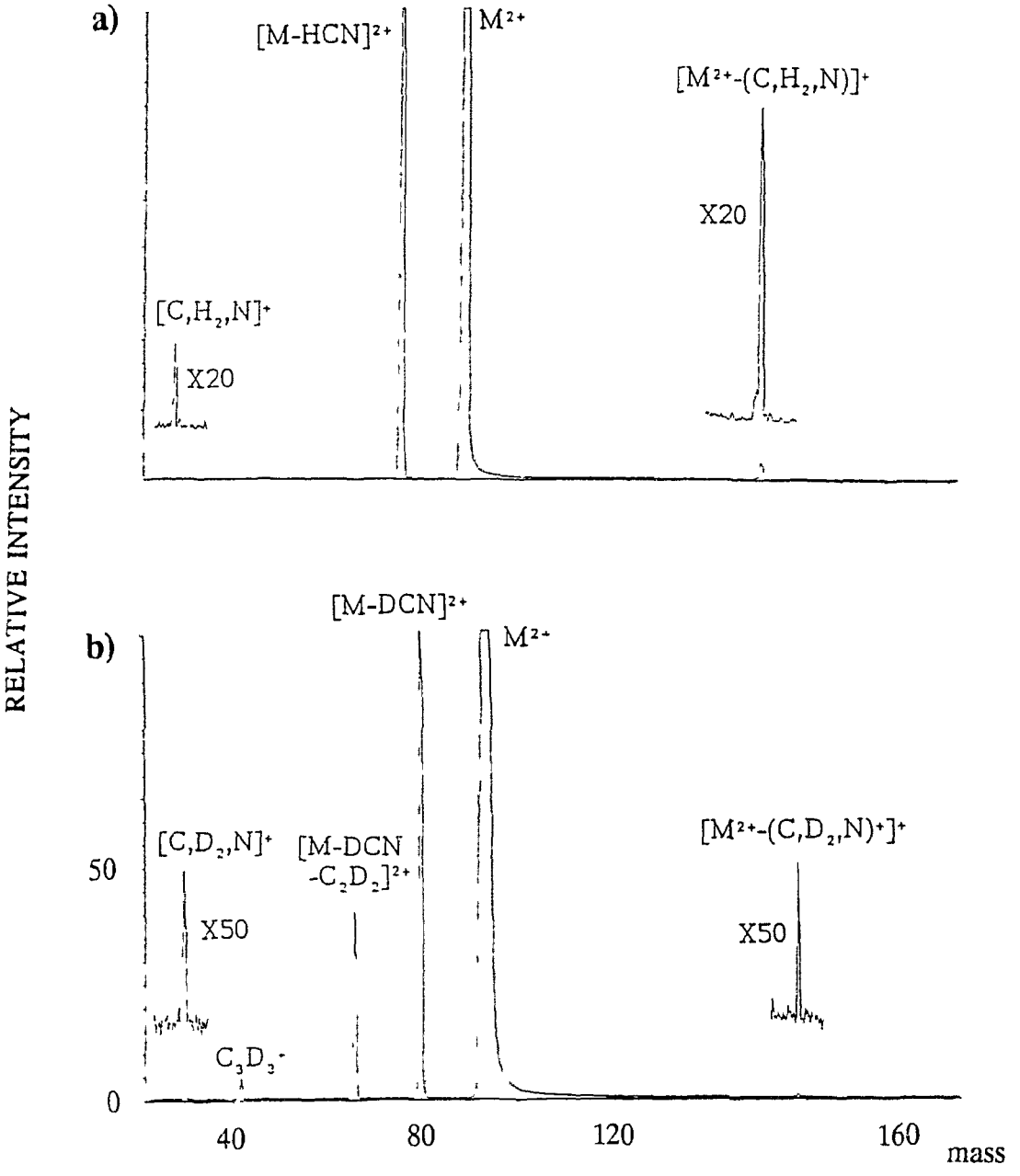


Figure 8.1.2: Unimolecular fragmentation spectra of acridine and perdeuterated acridine dications (rf-only quadrupole cell); a) acridine-H_9 (m/z 89.5), b) acridine-d_9 (m/z 94).

the 30 expected for the latter possibility (Figure 8.1.2b). The facile loss of this neutral species strongly argues against charge localization on the nitrogen atom in these M^{2+} ions. Subsequent losses from the $[M\text{-}HCN]^{2+}$ ions correspond to expulsion of neutral C_2H_2, and these $[M\text{-}HCN]^{2+}$ fragment ions appear to be indistinguishable from the $[M\text{-}C_2H_2]^{2+}$ ions formed for analogous PAHs (161). The loss of HCN (or HNC) from the pyridine dication can be observed under CID conditions (Figure 8.1.1b).

For PAHs (161) it has been shown that, with the exception of the benzene dications which preferentially fragmented via charge separation mechanisms, the PAH dications all expelled neutral acetylene molecules, an apparent loss of 13 m/z units. This highly unusual apparent neutral loss could form the basis of a strategy for selective detection of PAHs. In the previous work on PAHs (161), the competition between charge separation (CS) and neutral expulsion (NE) fragmentation mechanisms for dications was rationalized in terms of charge site initiated mechanisms dominating the behavior of the smaller systems (benzene and alkylated homologs), and with radical-site initiation taking over for the larger PAH systems (naphthalene and larger molecules), which can support a significant contribution from canonical structures with diradical character.

Dications of fused ring systems incorporating a pyridine ring (sp^2 nitrogen atom) behaved in a fashion similar to that of their PAH analogs, except that the neutral species expelled in NE fragmentation was invariably HCN (or HNC). Unimolecular CS reactions were dominant for the dication of pyridine (yielding a $[C,H_2,N]^+$ fragment ion), although the NE reactions could be induced by collisional activation (158) (not observed in the case of benzene). The NE reaction (loss of HCN) dominated the fragmentation channels of these M^{2+} ions for bicyclic and larger systems, though the CS reaction was observed at very low intensity even for the tetracyclic systems (158). Those observations are consistent with the diradical hypothesis provided that the tendency for the radical site on a sp^2 hybridized ring system (C or N), to initiate fragmentations by NE reactions, parallels the radical site's tendency to donate electrons. This hypothesis is entirely consistent with the general trend (nitrogen has much greater electron donating ability) well established

(198) for fragmentation of (singly charged) radical cations.

It is helpful to apply thermodynamic and structural considerations to explain the predominance of charge separation over neutral expulsion for pyridine dications, and vice versa for dications of larger PANHs.

Values of ΔH_f were obtained for products of CS and NE from pyridine dications (199-203) and the resulting energy diagram is given in Figure 8.1.3. It suggests that the charge separation reaction is strongly exothermic and that the loss of neutral HCN (or HNC) is endothermic by some 184 kJ/mol. Therefore this diagram can explain unimolecular charge separation, and also the fact that collisions are required for the expulsion of HCN from the pyridine dications. Thermodynamic values for quinoline, isoquinoline and other PANHs involving more than one ring were not available from the literature.

It is reasonable also, according to previous considerations (161) to advance the hypothesis that, for dications of pyridine based compounds, charge separation reactions are induced at the charge site, and involve electronic rearrangements only. Figure 8.1.4 illustrates two possible pathways of charge separation.

Concerning the preferential loss of HCN (or HNC) over the neutral species C_2H_2, radical site stabilization by localization on nitrogen should account for this observation. Reactions shown in Figure 8.1.4 account for the principle of avoiding antiaromaticity and of diradical character stabilization by localization of an unpaired electron on the nitrogen atom. This electron resides in a bonding orbital, and is thus expected to trigger expulsion of HCN (or HNC).

8.1.2 Compounds containing a pyrrole ring

Under the experimental conditions used in the present work, pyrrole did not yield observable molecular dications; accordingly, Figure 8.1.5 shows data for indole and carbazole only. The unimolecular dissociation of indole dications (Figure 8.1.5a) yields three CS reactions (ion pairs [90,27], [89,28] and [78,39]) and two NE reactions (ions at m/z 45.0 and 45.5). The nature of the latter was confirmed by MIKES

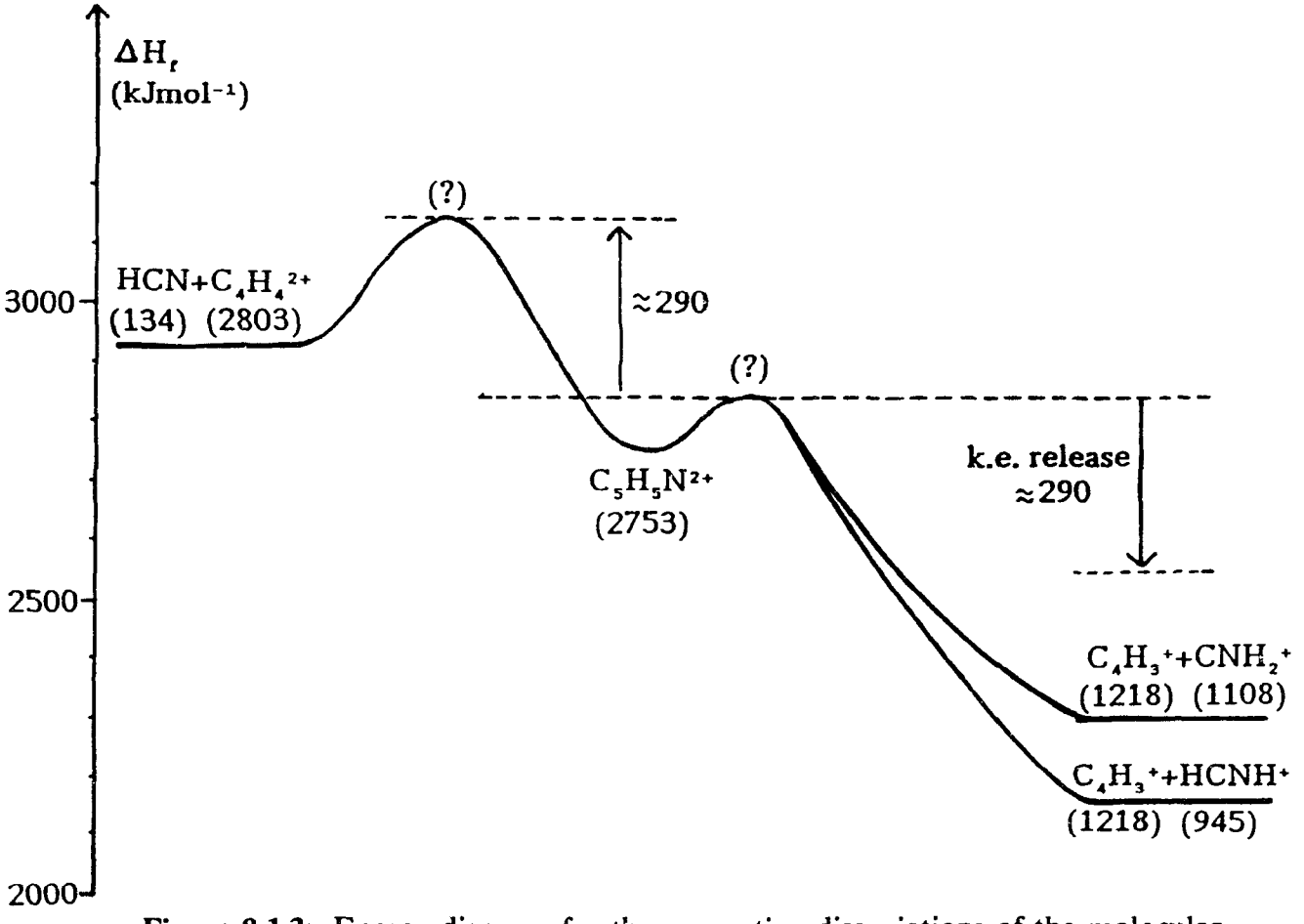


Figure 8.1.3: Energy diagram for the competing dissociations of the molecular dications of pyridine, $C_5H_5N^{2+}$. The value of the kinetic energy release was measured from Figure 8.1.1-a.

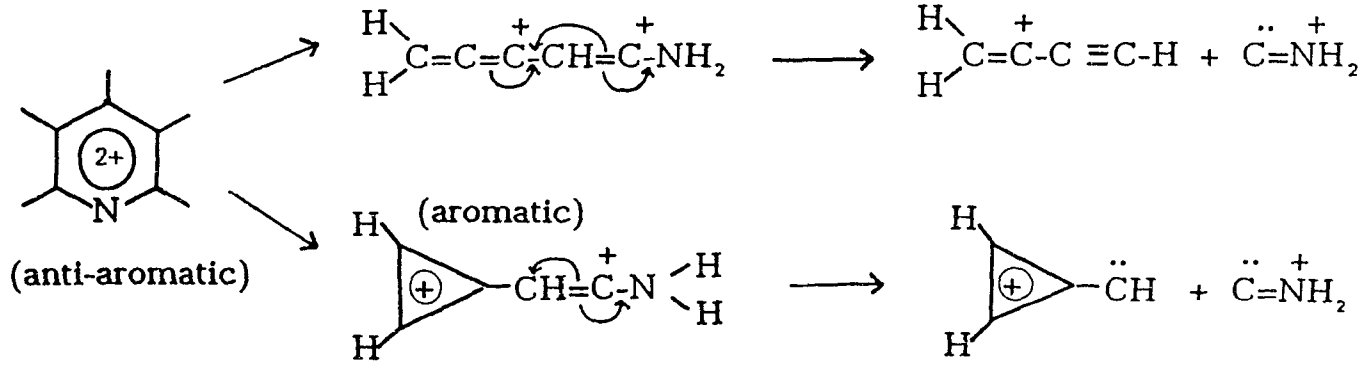


Figure 8.1.4: Proposed structures involved in charge separation dissociation of the pyridine dications

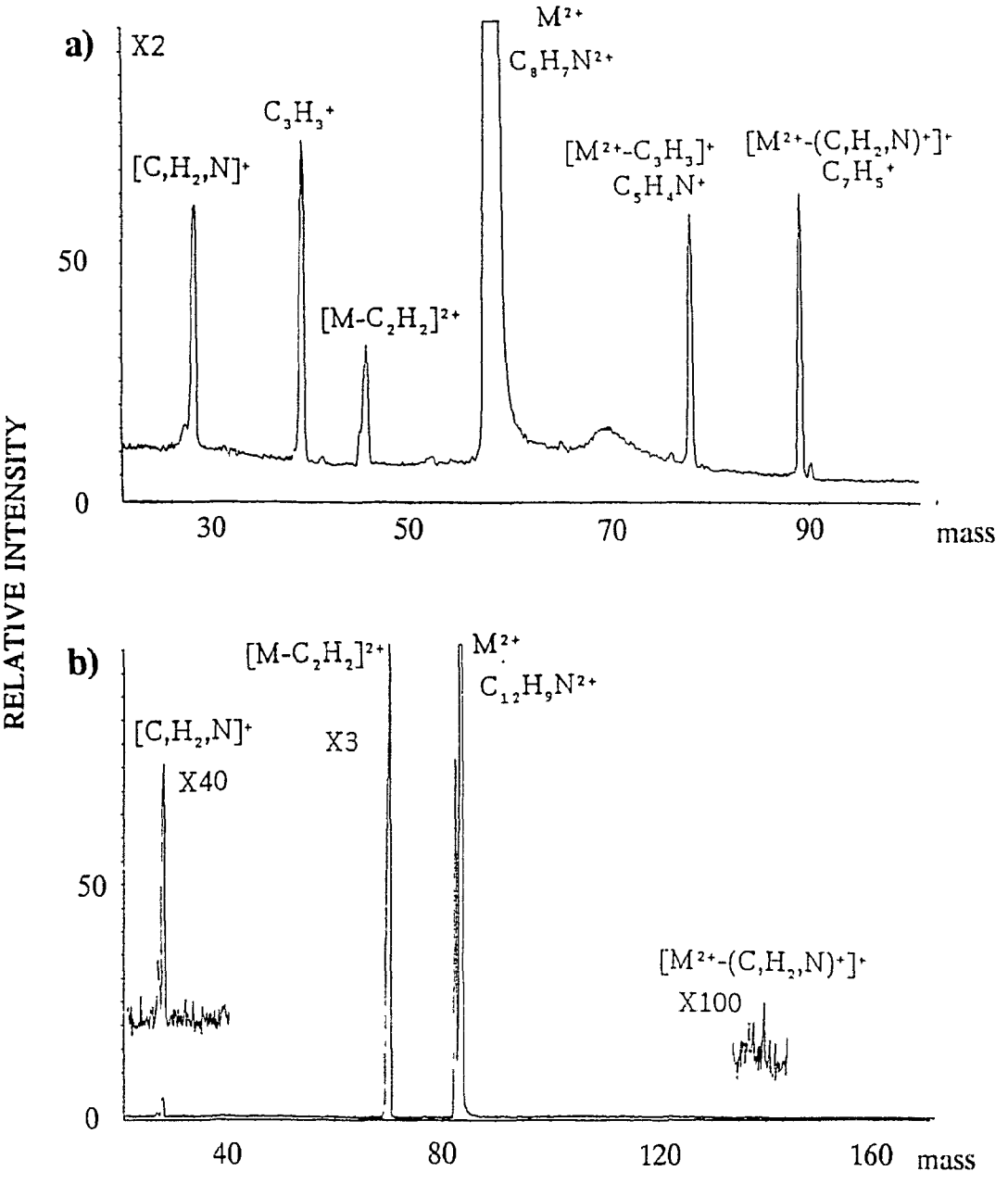


Figure 8.1.5: Unimolecular fragmentation of molecular dications of pyrrole-based aromatic compounds (dissociations in the rf-only collision cell); a) indole (m/z 83.5), b) carbazole (m/z 83.5).

experiments (166). The more intense of the CS reactions yielded fragments interpretable as the even electron species $[C,H_2,N]^+$ and $C_3H_3^+$ at m/z 28 and 39, respectively. The CS product at m/z 27 has been interpreted as $C_2H_3^+$.

From the preferential loss of C_2H_2 over HCN (or HNC), the behavior of indole dications upon unimolecular dissociation is somewhat similar to that of the aniline dications (194). In this respect, indole resembles an aromatic amine more than a fused ring heterocyclic aromatic compound. On comparison with the result for carbazole (Figure 8.1.5b), the same trend as noted for the six membered ring compounds is also apparent here, viz. the neutral expulsion dominates the charge separation reactions, though reactions of the latter type which yield ions at m/z 27 and 28 are still apparent. Loss of C_2H_2 dominates the spectrum and loss of HCN is not observed at all.

Rationalization proposals for the dissociation of dications of pyridine based compounds also account for the qualitative difference in behavior observed for dications of pyrrole-based compounds. One important feature is the decrease of the extent to which neutral expulsions dominate charge separations, relative to pyridine-based compounds with the same number of fused rings. In the case of indole, charge separation dominates, while it is extremely uncompetitive in the case of quinoline (not shown, spectrum similar to that of acridine, Figure 8.1.2a). Another important feature is the nature of the neutral expulsion reactions, which, for pyrrole-type compounds, involve loss of C_2H_2 in preference to HCN, however without excluding the latter.

These differences can be accommodated within the diradical hypothesis, according to Figure 8.1.6. In a pyrrole ring, a radical cation site on the nitrogen atom can be achieved by removing one of the lone pair electrons. Such an uncoupled electron in a lone pair orbital is probably less efficient in triggering fragmentation than one in a bonding orbital. Canonical forms as shown in Figure 8.1.6 would be expected to preferentially lose C_2H_2 from the radical site localized in the bonding orbital within the hydrocarbon region.

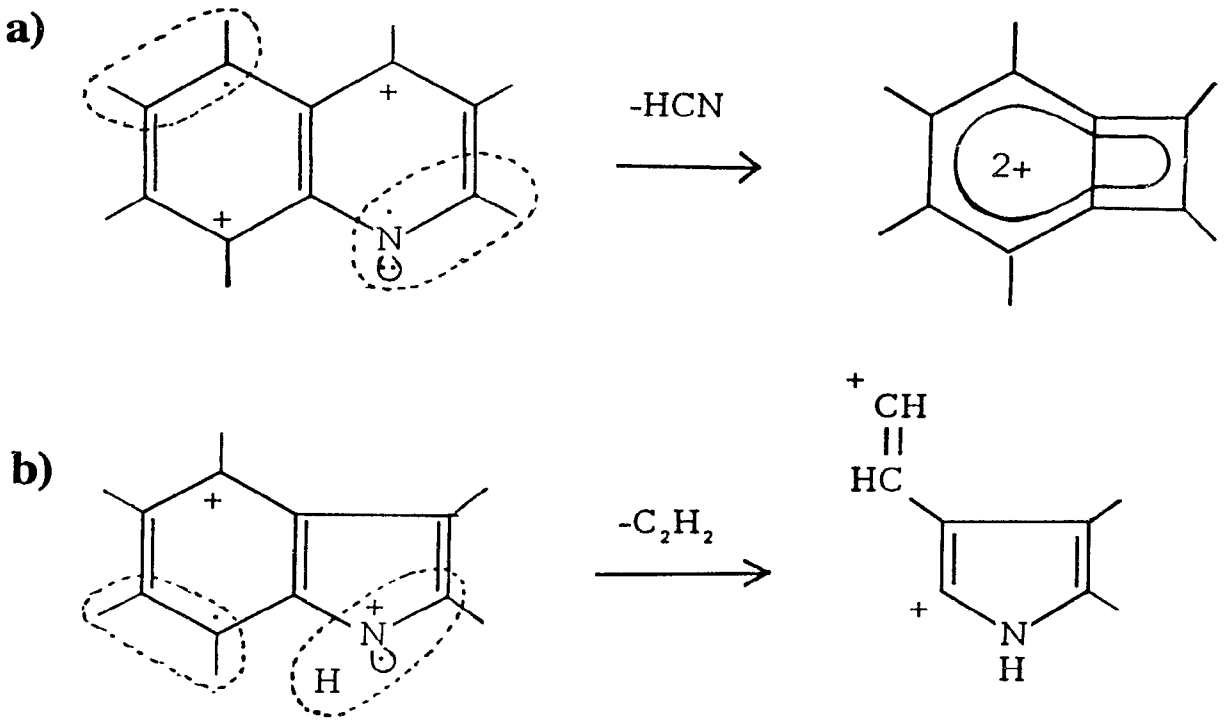


Figure 8.1.6: a) Diradical structures proposed for dications of larger heterocyclic fused-hexagonal-ring aromatics, with radical site stabilization on nitrogen, triggering expulsion of neutral HCN; b) diradical structure proposed for the dications of pyrrole-based compounds, with radical site stabilization in the hydrocarbon region, triggering expulsion of neutral C_2H_2.

8.1.3 Isomer discrimination

Earlier attempts in achieving some degree of isomer discrimination for PAHs by mass spectrometry were disappointing (161,204). In the context of the present work, differentiation of PANH isomers was observed to a limited degree. For instance in the CID spectra of M^{2+} of quinoline and isoquinoline, the relative intensities of ions in the low mass part of the spectra were different on a constant and reproducible basis (Figure 8.1.7a). In the case of the three-ring compounds, a linear isomer, acridine, was distinguishable from the two others, but the latter could not be distinguished from one another (Figure 8.1.7b). The four-ring compounds were also disappointing in this regard. 4-azachrysene was distinguishable with a particularly intense m/z 27 fragment ion, and some minor differences were observed between other isomers (Figure 8.1.7c). On the whole, the CID spectra of isomeric PANH dications did not provide much structural information.

8.1.4 Selectivity enhancement

Observations obtained from studying unimolecular and CID fragmentation of PANH dications revealed that highly selective monitoring of these compounds in real world samples can be achieved in three distinct fashions.

The first consists of extracting, following chromatographic/mass spectrometric full scan experiments, the RICs at the specific half integral m/z values related to the dications of PANHs, in order to reduce background and thus increase S/N ratios in the detection of these compounds. PACs with even masses are not likely to produce half integral mass ions, with the exception of the first ^{13}C isotope peaks of the molecular dications. However, the intensity of those first ^{13}C isotopes peaks of PAH dications is normally weak (in the order of [no. carbon atoms x 1.1%]). For instance, PAHs with four six-membered rings such as chrysene have an empirical formula of $C_{18}H_{12}$. The first ^{13}C isotope peak would have ca. 20% of the molecular ion intensity. Only if PAHs are very concentrated compared to PANHs could half integral mass

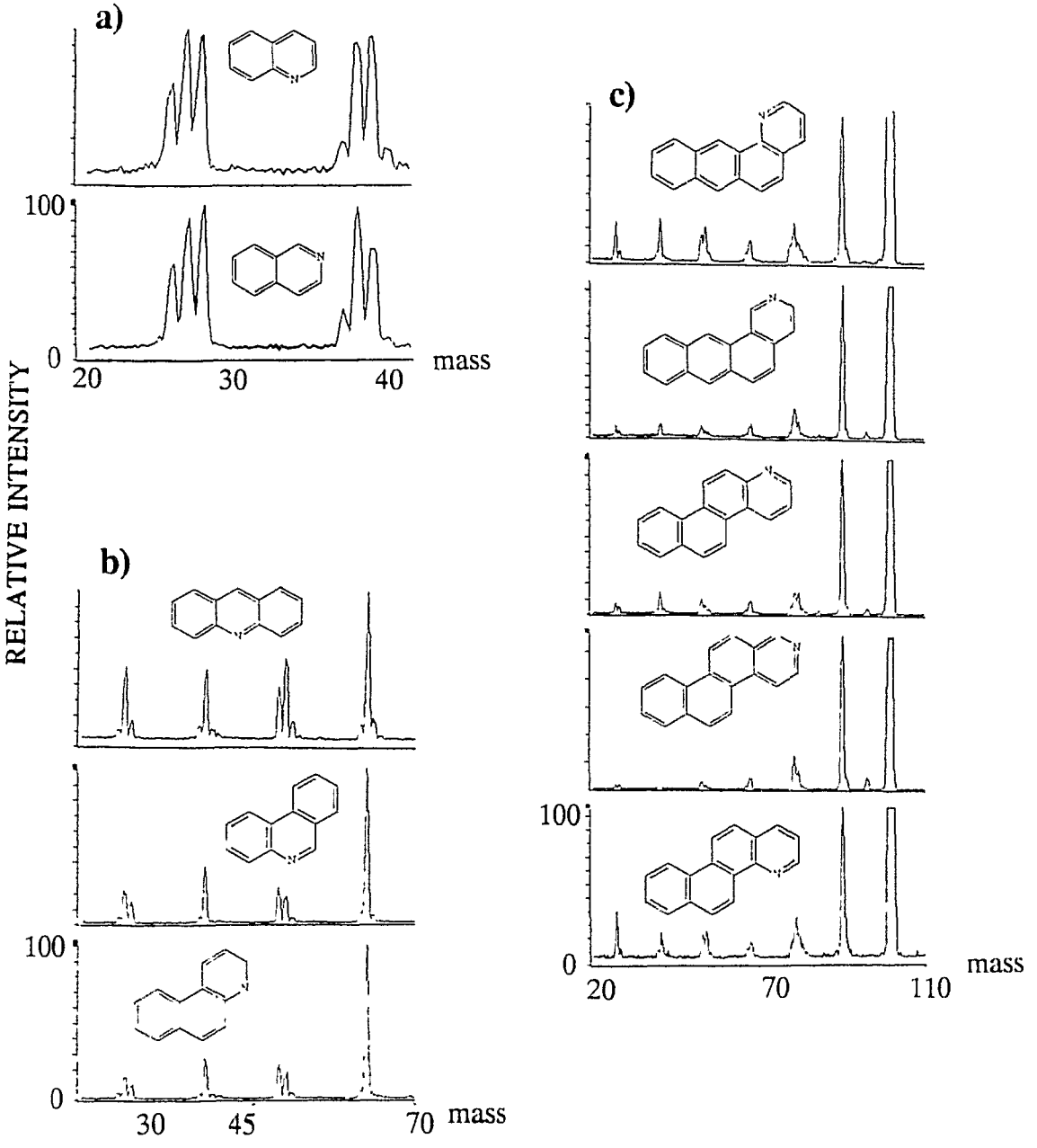


Figure 8.1.7: Low mass portion of CID fragmentation spectra of three groups of PANH isomers (dissociations in the rf-only quadrupole collision cell); a) two-ring compounds, b) three-ring compounds, c) four-ring compounds.

ionic signals interfere in the selective analysis of PANHs.

However, when either reversed phase HPLC or capillary column GC are used as means of introducing samples into the mass spectrometer, PANHs and homolog PAHs (e.g. acridine, M.W. 179 and its parent anthracene, M.W. 178), whose doubly charged ions at m/z 89.5 could interfere, elute at different times in both chromatographic modes. In GC/MS, with a DB-5 or DB-1701 capillary column, PANHs of M.W. 179 are eluted much later than phenanthrene and anthracene, and the opposite situation occurs using RPLC/MS. Moreover, it is less probable for a coeluting substance to produce fragment ions at 89.5 Da. than at 179 Da in this particular example.

Figure 8.1.8 shows how the monitoring of PANH dications at m/z 64.5 can help verifying the presence of quinoline-type (M.W. 129) species in an environmental polluted sample whose sulfur content has not been removed. The RIC at m/z 129 shows peaks due to naphthalene ($[M+1]^{+\cdot}$), quinoline and isoquinoline ($M^{+\cdot}$), as well as a peak due to the $[(M+1)^{+\cdot}]$ ion of the S_4 fragment species of S_8. As S_4 does not produce doubly charged ions detectable by the mass spectrometer, the m/z 64.5 trace is useful for the discrimination of PANHs quinoline and isoquinoline vs. sulfur. This approach was used earlier in this laboratory, with the ^{13}C isotope peaks of PAH dications (45).

A second method of achieving selectivity enhancement consists of performing GC/MS/MS experiments. Figure 8.1.9 shows the GC/MS trace (bottom) obtained for a mixture of PANH standards. Column bleeding, toward the end of the run, acts to decrease the S/N ratio in the detection of the four-ring compounds. Traces at the top of the figure result from monitoring the TIC obtained from MS/MS fragmentations of the species' respective molecular dications. These traces were obtained by setting the magnet to let ions of a specific m/z value pass and by scanning the quadrupole analyzer in order to collect all the unimolecular fragmentation products (and the unreacted molecular dications) of specific doubly charged species. The magnet was set to change mass between the elution of each group of isomers. A disadvantage of using this method is that the retention times

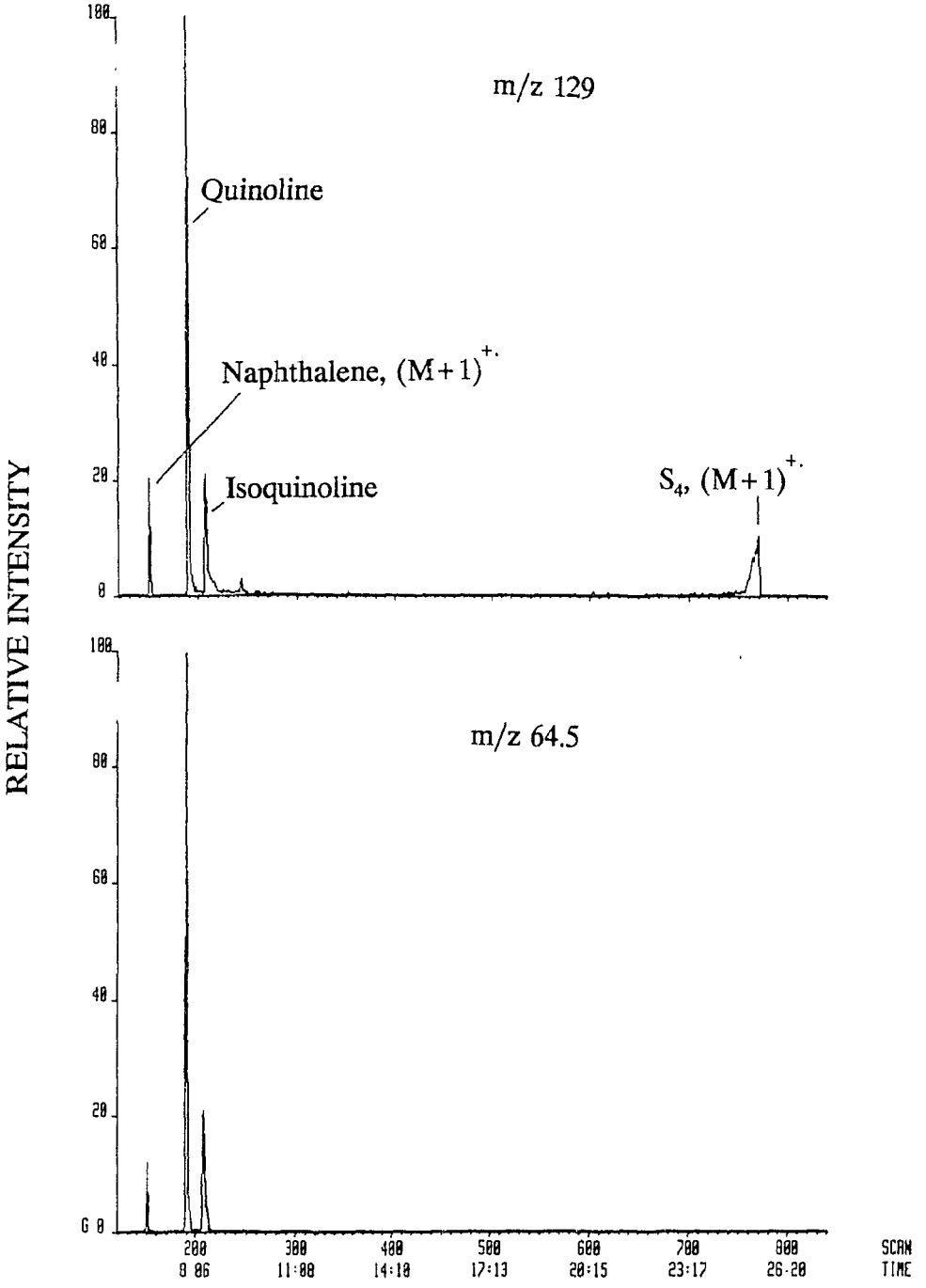


Figure 8.1.8: Example of the selectivity enhancement achieved by monitoring the half integral m/z value of PANH dications

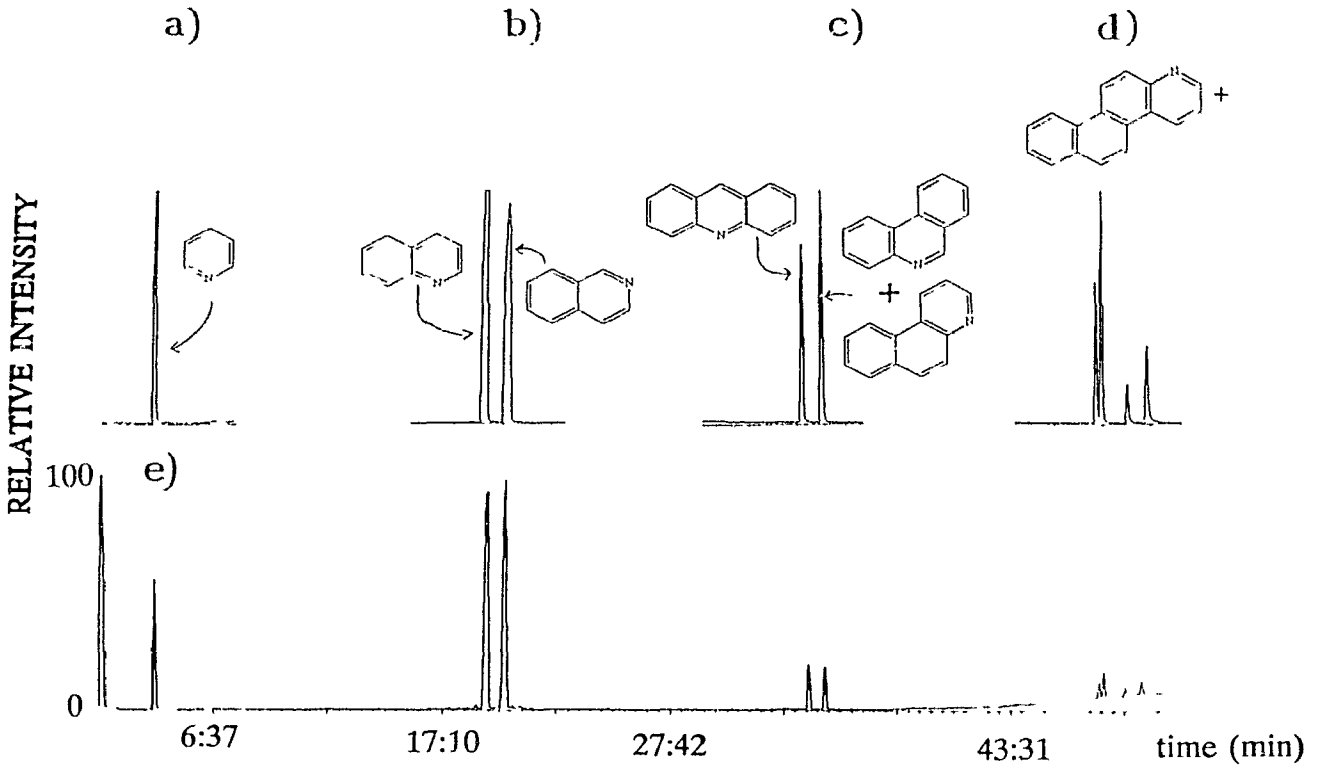


Figure 8.1.9: GC/MS/MS selected ion chromatograms obtained for a standard mixture of PANHs by monitoring the TIC of their CID fragmentations; a) pyridine **1** (m/z 39.5), b) quinoline **3** and isoquinoline **5** (m/z 64.5), c) acridine **7**, phenanthridine **8** and benzo[f]quinoline **9** (m/z 89.5), d) four-ring compounds **10**-**14** (m/z 114.5), e) conventional GC/MS TIC chromatogram. The MS/MS fragmentation spectra obtained at the apex of peaks in traces a to d are identical to those presented in Figure 8.1.1-b and 8.1.7.

must be known in order to program the magnet to jump from mass to mass at predefined times. The main advantage obtained from this technique is the possibility of comparing the MS/MS spectra of each eluted species and thus to achieve isomer discrimination to a certain extent.

A third way of achieving selectivity enhancement of PANHs vs other PACs is to monitor the constant neutral losses (CNL) of their molecular dications. Doubly charged molecular ions of PANHs that contain a pyridine ring (sp^2 nitrogen) undergo the very unusual apparent loss of 13.5 Da. (HCN or HNC) under either unimolecular or CID conditions. Dications of pyrrole-based compounds lose both neutral species of apparent masses of 13 (acetylene) and 13.5 (HCN). Monitoring the constant neutral loss of apparent mass 13.5 throughout a chromatographic/mass spectrometric experiment on a "real world" sample would yield peaks corresponding to the elution of only pyridine and pyrrole-based PANHs. On the other hand, monitoring the constant neutral loss of apparent mass 13 from the dications would allow the detection of PAHs (160) and pyrrole-type PANHs.

In a two step GC/MS experiment, the first step involving CNL of 13.5 and the second the loss of 13, the following observations would help in recognition of the nature of the species:

- if peaks are observed for CNL of 13.5 only, species are pyridine-based PANHs
- if peaks are observed for CNL of 13 only, species are PAHs
- the presence of peaks in both CNL 13.5 and 13 traces indicates that the compounds are pyrrole-based PANHs.

Attempts to perform CNL experiments were successful for the characterization of standard PANHs introduced in the mass spectrometer as probe samples, because the large amount of material ionized provided intense peaks for molecular and fragment dications associated with losses of neutral species HCN and/or C_2H_2 (scan rate 10 sec/decade). However, the CNL experiments partially failed when the samples were introduced from the gas chromatograph for the study of PANHs in a standard mixture. The rapid scan rate required when performing GC/MS (1-2 sec/decade), combined with the short duration of the individual GC peaks (15-30

seconds) made almost impossible the detection of PANHs using this technique. In "real world" samples such as the TPE, PANHs are in relatively low concentration with respect to PAHs, and thus the high intensity of PANH ions required to perform the CNL experiment is hardly attainable. Work is still proceeding on this technique, in the hope that it will be adaptable to the GC/MS of environmental samples.

9. CONCLUSIONS

The work performed in this project shows that the choice of extraction, clean up, fractionation and "final" determination methods must be made according to the nature and origin of the samples. A comparison of the methods used to obtain the "final fractions" from the tar pond sediment and from the Syncrude samples provides a good example to support that statement.

The techniques used to sample, extract, clean up and fractionate the tar pond sample were based on established methods previously developed for the analysis of PACs in sediments or airborne particles. The complexity of the tar pond sample did not require major new fractionation techniques prior to analysis. Modifications were however needed to obtain better overall fractionation. These modifications were made in the nature of the adsorbents used, in the sample/adsorbent weight ratios, and in the volumes and proportions of solvents used in the fractionation procedures. These modifications contributed improvements in the analyses.

It was found that a modified version of the fractionation scheme proposed by Ramos and Prohaska (9) was the method of choice to apply to the TPE, in terms of separation of aliphatics from aromatics. Most aliphatic material was eliminated from the extract by passing it through Sephadex-LH20, after it had already been passed through a silica column. A modification of the alumina chromatographic method developed by Marr and Quilliam (3) was more efficient in separating PAHs from polar PACs, but less so in separating aliphatics from aromatics. The contents of the PAC final fractions obtained in either case varied only in terms of the relative concentrations.

The oil sand samples were much more complex. The techniques used to sample, extract, clean up and fractionate the three Syncrude materials were also modified versions of methods published earlier, although significant modifications were involved. The best technique found for the fractionation of those very complex samples is a two step method, each step corresponding to a highly modified version of a published method. The method of choice consists first of passing the extracts

through a KOH-treated silica column to eliminate the acidic material, and then of eluting the acid-free fraction through a silica over alumina column. This method allowed good separation of aliphatics from aromatics, and the latter were eluted with no separation between neutral and moderately polar PACs. The alumina column chromatographic method did not allow removal of the acidic components from the mixture; it, however, allowed a separation between neutral PAHs and more polar PACs, such as PANHs. However the latter separation was not required since PAHs and PANHs were differentiable by the chromatography/mass spectrometry in the final analysis step.

Some of the analytical methods used for the "final" PAC fractions were not applicable to every sample as expected. The analyses performed by GC/MS yielded good resolution and good efficiency of separation for PACs of M.W.<326 contained in the TPE. GC/MS was also constantly used to monitor the PAC content during the development of fractionation methods for the TPE. The Syncrude "final" fractions, due to their high content of non volatile compounds, were not amenable to GC/MS analysis. A reasonable chromatogram was obtained for the volatile part of the OSE, whereas the HGO yielded a trace that resembled a broad envelope more than a chromatogram. GC/MS was therefore not employed for the analysis of the Syncrude samples.

The capillary column SFC/MS, tried here for the first time with the TPE, was successful in separating compounds amenable to GC/MS. It yielded poorer resolution and sensitivity than GC/MS, and its use was made difficult due to problems associated with direct interfacing. The technique was therefore not used as a routine method, neither was it applied to the analysis of the Syncrude samples.

Reversed phase packed column SFC/MS, also tried for the first time, was successful to a certain extent, viz. compounds with M.W. up to 378 could be detected in the TPE, and up to 282 in the HGO. Supercritical carbon dioxide, pure or modified with methanol, did not have enough solvating power to elute the high M.W. compounds present in the TPE and Syncrude samples. This technique, although as presently used does not provide good resolution and separation efficiency, is suitable

for rapid qualitative profiling of the sample contents (150-380 mass range).

Another innovation presented in this work, normal phase packed column SFC/MS on silica with pentane as the mobile phase, was more successful. It provided a suitable way of eluting the high M.W. compounds (up to mass 950) present in the samples. The separations obtained using this technique resembled normal phase HPLC chromatograms, in that compounds were eluted by groups. This technique, first performed at the microscale level, was then adapted to larger portions in order to fractionate the Syncrude samples and thus eliminate the heavy ends which otherwise interfered in every analysis.

Reversed phase HPLC/MS, with the gradients developed here, was certainly the best method for the final separation of samples. HPLC/MS using a moving belt interface provides excellent results for the characterization of these complex samples. This was used for the first time here for on-line analysis of high M.W. PACs in TPE and the analysis of oil sand samples. In the case of the TPE, lower resolution was obtained than in GC/MS, but this was compensated by the high selectivity of the C_{18} column, which yielded excellent isomer discrimination. The mass range of the compounds analyzable also increased drastically from GC/MS to HPLC/MS, pushing the higher mass limit from 326 to 580 Da. For the analysis of the Syncrude samples, RPLC/MS also led to good chromatography, and certainly helped to clarify the unsatisfactory results obtained in the attempts to run GC/MS. Acceptable resolution and good isomer discrimination were obtained for compounds with $128 < M.W. < 320$, eluted in the first part of the water-ACN-DCM gradient (first part: water-ACN only). In the second part however (ACN-DCM only), compounds in the 320-950 mass range were eluted as a continuum, without apparent good chromatographic separation. Attempts to improve the chromatography in this second part of the gradient met with little success.

The results obtained from all these chromatographic/mass spectrometric analyses allowed a qualitative profile to be constructed for each of the samples studied in this work.

The Sydney tar pond sediment extract (TPE) contains unsubstituted PAHs

as the predominant species (M.W. 117-580 Da observed in this work); alkylated analogs are second in importance, followed by PANHs, PASHs, PAQs and PAKs. Some of the low M.W. compounds were tentatively identified from the retention indices and elution patterns found in the literature, whereas high M.W. compounds could only be assigned possible structures, due to the lack of suitable standards available for PACs in the 300-600 mass range.

The PAC fraction of the lighter portion of the Syncrude heavy gas oil (HGO), a distillation fraction from hydrocracking products, was found to contain alkylated (from C1 to C6) PAHs as the predominant species, followed by alkylated dibenzothiophenes, alkylated carbazoles and unsubstituted PAHs. Alkylated species are present naturally but are also most certainly produced in the cracking processes (90). The heavier end of the HGO is characterized by high M.W. (320-950), highly functionalized molecules, whose chromatographic behavior was between that of aliphatic and aromatic compounds. Compounds in the lighter end of the HGO were tentatively identified, whereas those in the heavy end remained listed as unknowns due to the extreme complexity of the mixture.

The Pitch (PIT) sample, the distillation residue of the same hydrocracking products, only contains the heavy compounds. No light and volatile compounds were expected to be found in the PIT, since its contents boil at temperatures above 500°C.

The Oil Sand Extract (OSE) gave an interesting picture in terms of contents as compared with the two prior samples. The sample, not having been subjected to distillation, contained some volatile aromatic compounds, easily amenable to GC analysis (M.W. 106 to 178), which mainly consisted of alkylated benzenes and naphthalenes. In the 178 to 320 M.W. range, compounds generally similar to those found in the HGO were detected, however at very low concentrations. The low abundance of alkyl-PAHs can be explained by the fact that the OSE was not a hydrocracking product and thus only contained the naturally occurring substances. The compounds found in the heavy end were similar in nature and chromatographic behavior to those found in the HGO and PIT. It can be pointed out that samples of natural origin such as the OSE can be much more complex than those whose origin

is anthropogenic, a case well illustrated by the tar pond sediments.

There certainly are health hazards associated with the exposure to the compounds present in the samples studied in this work, especially in the Sydney tar pond sediment. The latter contains higher concentrations of unsubstituted PAHs than the Syncrude samples, and some of these compounds are potent carcinogens and mutagens. Phenanthrene, benz[a]anthracene, chrysene and benzo[a]pyrene, as well as the PANH species quinoline and acridine, figure among the carcinogens found in the Sydney sediment. As these compounds are at least somewhat soluble in water, there is a possibility that they could be removed from sediment matter and may contaminate nearby waters. Some alkylated PAHs found in Syncrude samples, especially in the HGO, could be carcinogenic as well; 7,12-dimethylbenz[a]anthracene is a possible example. The carbazoles, also found in cigarette smoke condensate, are also suspected of having tumorigenic activity. However, those compounds are found in very low abundance levels in the Syncrude samples. And as the latter samples are not very volatile, the Syncrude employees who supervise the distillation process of hydrocracked products may only be vulnerable to the lower boiling point distillation fractions, which have not been studied in this work. The heavy ends of the three Syncrude samples investigated here may contain carcinogenic substances, which could not be verified due to the impossibility of separating and identifying the species chromatographically.

The efforts in developing mass spectrometric techniques for the detection and isomer discrimination of PANHs based on the use of their molecular dications yielded positive results. MS/MS coupled with selected ion monitoring, when operated on-line with GC separation, allowed the detection of PANHs among other interfering compounds with improved S/N ratios. Isomer discrimination using MS/MS was successful to a certain extent in that fragmentation patterns varied between isomers. However, the variation was not enough to normally allow positive identification. Constant neutral loss experiments are also successful for PANH detection with probe samples, but could not be used with chromatographic sample introduction due to the need for high scan speeds. This techniques is still under development.

10. APPENDICES

10.1 Appendix I: GC/MS data obtained for the TPE sample and sorted by scan number (retention time)

m/z	M(*)	sc#	int.	RIlit.	RIexp.	tentative id.
131	*	33	363		179.58	116-PANH-C1
147	*	81	3697		182.84	
136	*	209	41911		191.52	
128	*	334	3136	200.00	200.00	naphthalene (128-PAH)
147	*	502	85		211.40	
142	*	516	4425	218.14	212.35	128-PAH-C1
147	*	532	116		213.43	
142	*	549	4016	221.04	214.58	128-PAH-C1
154	*	698	3915	233.96	224.69	biphenyl
141		732	849		227.00	
156	*	732	333	236.08	227.00	128-PAH-C2
141		740	269		227.54	
156	*	740	111	236.56	227.54	128-PAH-C2
128		759	724		228.83	
141		759	2649		228.83	
156	*	759	3584	237.58	228.83	128-PAH-C2
128		795	820		231.27	
141		795	3718		231.27	
156	*	795	3612	237.71	231.27	128-PAH-C2
128		803	531		231.82	
141		803	2100		231.82	
156	*	803	2389	240.25	231.82	128-PAH-C1
156	*	831		240.66	233.72	128-PAH-C1
167	*	831	112		233.72	
168	*	831	152		233.72	166-PAH-H2
128		843	386		234.53	
141		843	1765		234.53	
156	*	843	1500	240.72	234.53	128-PAH-C2
141		849	452		234.94	
156	*	849	555	243.55	234.94	128-PAH-C2
152	*	864	1378	244.63	235.95	acenaphthylene (152-PAH)
141		883	823		237.24	
156	*	883	528	243.57	237.24	128-PAH-C2
153		959	1040		242.40	
154	*	959	933	251.29	242.40	acenaphthene (152-PAH-H2, 154-PAH)
168	*	971	1372		243.21	154-PAH-C1
173		977	379		243.62	
188	*	977	153		243.62	
167	*	993	598		244.71	carbazole (166-PANH)
156	*	1002		244.98	245.32	128-PAH-C2
173		1012	194		245.99	
188	*	1012	90		245.99	
155		1022	155		246.67	
170	*	1022	391		246.67	128-PAH-C3

139		1045	600		248.23	
168	*	1045	9916	257.17	248.23	dibenzofuran (166-PAF)
155		1082	862		250.74	
170	*	1082	738		250.74	128-PAH-C3
155		1095	1152		251.62	
170	*	1095	968	263.31	251.62	128-PAH-C3
155		1148	1009		255.22	
170	*	1148	953	265.90	255.22	128-PAH-C3
155		1190	849		258.07	
170	*	1190	797		258.07	128-PAH-C3
174		1219	2700		260.04	
176	*	1219	2766		260.04	fluorene-d10 (166-PAH-D10)
165		1234	4885		261.05	
166	*	1234	4885	268.17	261.05	fluorene (166-PAH)
181		1253	620		262.34	
196	*	1253	350		262.34	154-PAH-C3
155		1255	906		262.48	
170	*	1255	782		262.48	128-PAH-C3
165		1296	1352		265.26	
166	*	1296	1352		265.26	166-PAH
181		1296	3351		265.26	
196	*	1296			265.26	154-PAH-C3
152	*	1306	583		265.94	
181	*	1306	1306		265.94	166-PANH-C1
181		1353	1392		269.13	
182	*	1353	1389		269.13	154-PAH-C2
152		1390	795		271.64	
181		1390	2371		271.64	
182	*	1390	2151		271.64	154-PAH-C2
181		1419	515		273.60	
182	*	1419	496		273.60	154-PAH-C2
184	*	1448	200		275.57	naphthothiophene (166-PASH)
167	*	1464	299		276.66	
184	*	1509	312		279.71	166-PASH
180	*	1554	4366	284.89	282.76	9,10-dihydroanthracene (178-PAH-H2)
195		1554	13959		282.76	
210	*	1554	4000		282.76	154-PAH-C4
180	*	1560	300	287.09	283.17	9,10-dihydrophenanthrene
165		1580	748		284.53	
180	*	1580	470	288.21	284.53	166-PAH-C1
165		1599	816		285.82	
180	*	1599	470	289.03	285.82	166-PAH-C1
207		1599	408		285.82	
222	*	1599	169		285.82	166-PAH-C4
193		1607	118		286.36	

208	*	1607	180		286.36	166-PAH-C3
165		1647	712		289.07	
180		1647	437		289.07	
195		1647	1503		289.07	
210	*	1647	500		289.07	154-PAH-C4
181		1717	544		293.82	
196	*	1717	180		293.82	154-PAH-C3
184	*	1726	1451		294.43	166-PASH
181		1735	3300		295.04	
196	*	1735	340		295.04	154-PAH-C3
195	*	1763	632		296.94	166-PANH-C2
181	*	1776	1836		297.82	166-PANH-C1
207		1776	350		297.82	
222	*	1776	180		297.82	166-PAH-C4
229		1776	350		297.82	
244	*	1776	175		297.82	
207		1782	500		298.23	
222	*	1782	200		298.23	166-PAH-C4
229		1782	220		298.23	
244	*	1782	200		298.23	
209		1800	1700		299.45	
224	*	1800	700		299.45	166PAX-C3,166-PAF-C4
178	*	1808	22252	300.00	300.00	phenanthrene (178-PAH)
178	*	1836	6846	301.69	301.52	anthracene (178-PAH)
179		1881	798		303.98	
194		1881	588		303.98	
209		1881	2110		303.98	
224	*	1881	1055		303.98	166-PAX-C3,166-PAF-C4
207		1916			305.90	
222	*	1916			305.90	166-PAH-C4
179	*	1930	372	304.04	306.66	acridine (178 PANH)
210	*	1930	100		306.66	202-PAH-H8
179	*	1969	425	307.94	308.79	phenanthridine (178-PANH)
210	*	1969	250		308.79	202-PAH-H8
207		1979	270		309.34	
222	*	1979	114		309.34	166-PAH-C4
167	*	1988	942		309.83	152-PANH-C1
179	*	2003	350	307.94	310.65	benzo[f]quinoline (178-PANH)
165		2011	1500		311.09	
180		2011	873		311.09	
195	*	2011	3400		311.09	166-PANH-C2
178	*	2052	1027		313.33	176-PAH-H2
207		2052	1872		313.33	
222	*	2052	646		313.33	166-PAH-C4
203	*	2064	515		313.99	1-azafluorene (202-PANH)
195		2072	350		314.43	

210	*	2072	350		314.43	166-PAQ-C1,166PAXK-C1,152-PAQ-C2,166-PAX-C2,166-PAF-C3
235		2072	150		314.43	
250	*	2072	129		314.43	190-PASX-C2,190-PASH-C3,178-PAQ-C3,178-PAHK-C4
207		2077	200		314.70	
222	*	2077	200		314.70	166-PAH-C4
229	*	2077	248		314.70	228-PANH
235		2077	100		314.70	
250	*	2077	100		314.70	same as scan# 2072
207		2082	829		314.98	
222	*	2082	397		314.98	166-PAH-C4
195	*	2091	425		315.47	166-PANH-C2
198	*	2091	400		315.47	166-PASX
220	*	2091	105		315.47	178-PAH-C3
195		2110			316.51	
210	*	2110	175		316.51	same as scan #2072
223		2110	180		316.51	
238	*	2110	80		316.51	166-PAQ-C3,166-PAXK-C3,152-PQA-C4,166-PAX-C4
192	*	2152	1522	319.46	318.80	PAH-178-C1
192	*	2166	2152	320.17	319.57	PAH-178-C1
192	*	2192	960	321.57	320.99	PAH-178-C1
209		2192	250		320.99	
224	*	2192	500		320.99	same as scan#1881
190	*	2205	2178	322.08	321.70	4H-cyclopenta[def]phenanthrene
192	*	2219	1125	323.06	322.47	178-PAH-C1
209		2219	200		322.47	
224	*	2219	100		322.47	same as scan#1881
192	*	2233	1225	323.17	323.24	178-PAH-C1
209		2233	250		323.24	
224	*	2233	125		323.24	same as scan# 1881
195	*	2258	644		324.60	166-PANH-C2
193		2275	297		325.53	
208	*	2275	170		325.53	166-PAK-C2,152-PAH-C4,166-C3
209		2297	270		326.74	
224	*	2297	130		326.74	same as scan#1881
193	*	2313	310		327.61	phenylindole
208	*	2313	450		327.61	same as scan#2275
209		2313	745		327.61	
224	*	2313	400		327.61	same as scan#1881
236	*	2313	150		327.61	190-PASX-C1,190-PASH-C2,178-PAQ-C2,166-PAK-C4,178-PAHK-C3
221		2318	549		327.88	
236	*	2318	250		327.88	same as scan2313
197		2324	200		328.21	
212	*	2324	570		328.21	166-PASH-C2
221		2375	400		331.00	

236	*	2375	150		331.00	same as scan#2313
204	*	2384	1506		331.49	methyl-4H-cyclopent[def]phenanthrene
212	*	2384	700		331.49	166-PASH-C2
234	*	2406	650		332.70	hexahydrochrysene (228-PAH-H6)
191		2429	200		333.96	
206	*	2429	141	337.05	333.96	178-PAH-C2
212	*	2458	350		335.54	166-PASH-C2
191		2475	261		336.47	
206	*	2475	220	337.50	336.47	178-PAH-C2
191		2480			336.75	
206	*	2480	211	337.83	336.75	178-PAH-C2
178		2490			337.29	
193	*	2490	178		337.29	178-PANH-C1
197		2490			337.29	
212	*	2490	170		337.29	166-PASH-C2
191		2505	200		338.11	
206	*	2505	423	339.23	338.11	178-PAH-C2
191		2538			339.92	
206	*	2538	239	346.26	339.92	178-PAH-C2
191		2551	501		340.63	
206	*	2551	778		340.63	178-PAH-C2
223		2551	400		340.63	
238	*	2551	150		340.63	166PAQ-C3,166PAXK-C3,152-PAQ-C4,166-PAX-C4
191		2571	301		341.72	
206	*	2571	324		341.72	178-PAH-C2
217		2571	410		341.72	
232	*	2571	200		341.72	190-PAK-C2,202-PAHK-C1,216-PAF-C1
206	*	2589	400		342.71	178-PAH-C2
208	*	2597	963		343.15	166-PAH-C3
212	*	2597	4643		343.15	fluoranthene-d10 (202-PAH-D10)
202	*	2600	4500		343.31	202-PAH
202	*	2610	38175	344.01	343.86	fluoranthene (202-PAH)
211		2656	430		346.37	
226	*	2656	503		346.37	166-PASH-C3
202	*	2668	832		347.03	202-PAH
202	*	2674	1879		347.36	202-PAH
208	*	2699	814		348.73	190-PASH,178-PAQ
218	*	2724	423		350.09	216-PAF
202	*	2744	34063	351.22	351.19	pyrene (202-PAH)
218	*	2783	4588		353.32	216-PAF
211		2793	200		353.87	
226	*	2793	307		353.87	166-PASH-C3
205		2812	685		354.91	
220	*	2812	112		354.91	178-PAH-C3
218	*	2827	1270		355.73	216-PAF

218	*	2869	2117		358.02	216-PAF
205		2887	400		359.01	
220	*	2887	412		359.01	178-PAH-C3
218	*	2925	917	361.38	361.09	benzo[kl]xanthene (216-PAX)
261		2941	50		361.96	
276	*	2941	207		361.96	216-PASH-C3
216	*	2948	1124	366.74	362.35	benzo[a]fluorene (216-PAH)
216	*	3002	919	369.38	365.30	benzo[b]fluorene (216-PAH)
216	*	3007	933		365.57	216-PAH
216	*	3014	1658		365.96	216-PAH
216	*	3021	3515		366.34	216-PAH
232	*	3040	417		367.38	214-PASH,202-PAQ,216-PAX
216	*	3070	3515	369.54	369.02	4-methylpyrene (202-PAH-C1)
232	*	3111	400		371.26	same as scan#3040
232	*	3119	800		371.70	same as scan#3040
216	*	3134	1297	370.15	372.52	2-methylpyrene (202-PAH-C1)
232	*	3134	800		372.52	same as scan#3040
216	*	3152	838	373.55	373.50	1-methylpyrene (202-PAH-C1)
277		3252	180		378.97	
292	*	3252	90		378.97	190-PASXK-C4
230	*	3272			380.07	216-PAH-C1
234	*	3272	166		380.07	178-PAH-C4
277		3308	420		382.04	
292	*	3308	220		382.04	190-PASXK-C4
230	*	3339	574		383.73	216-PAH-C1
215		3385	500		386.25	
230	*	3385	500		386.25	216-PAH-C1
215		3391	600		386.58	
230	*	3391	555		386.58	216-PAH-C1
215		3398	425		386.96	
230	*	3398	425		386.96	216-PAH-C1
230	*	3437	324		389.09	216-PAH-C1
234	*	3437	1546		389.09	216-PASH
226	*	3453	1377		389.97	226-PAH
226	*	3468	520		390.79	226-PAH
228	*	3468	574		390.79	228-PAH
226	*	3476	782		391.23	226-PAH
228	*	3476	1411	391.39	391.23	benzo[c]phenanthrene (228-PAH)
234	*	3502	356		392.65	216-PASH
230	*	3510	166		393.09	228-PAH-C2
230	*	3527			394.02	228-PAH-C2
195	*	3556	1974		395.60	166-PANH-C2
234	*	3556	570		395.60	216-PASH
246	*	3574	600		396.59	214-PASX,216-PAQ,216-PAXK

258	*	3574	500		396.59	240-PASH
226	*	3583	782		397.08	226-PAH
240	*	3598	2888		397.90	benz[a]anthracene-d12 (228-PAH-D12)
208	*	3600			398.01	178-PAQ
228	*	3602	1114		398.12	228-PAH
236	*	3602	900		398.12	190-PASXK
228	*	3613	9882	398.50	398.72	benz[a]anthracene (228-PAH)
228	*	3636	11471	400.00	400.00	chrysene+triphenylene (228-PAHs)
217	*	3654	1042	401.81	401.11	benzo[a]carbazole (216 PANH)
228	*	3681	352	408.30	402.81	naphthacene (228-PAH)
258	*	3698	353		403.88	240-PASH,228-PAQ
242	*	3754	889	412.72	407.40	11-methylbenz[a]anthracene (228-PAH-C1)
217	*	3775	535		408.72	benzo[c]carbazole (216-PANH)
242	*	3783	170	414.37	409.22	1-methylbenz[a]anthracene (228-PAH-C1)
217	*	3810	691	410.12	410.92	benzo[b]carbazole (216 PANH)
242	*	3853	196	416.32	413.62	1-methyltriphenylene (228-PAH-C1)
242	*	3879	167	416.50	415.26	9-methylbenz[a]anthracene (228-PAH-C1)
242	*	3897	366	416.63	416.39	3-methylbenz[a]anthracene (228-PAH-C1)
242	*	3920	1700	418.10	417.83	3-methylchrysene (228-PAH-C1)
242	*	3941	863	418.80	419.15	2-methylchrysene (228-PAH-C1)
239		3958	600		420.22	
240	*	3958	622		420.22	240-PAH
239		3974	400		421.23	
240	*	3974	755		421.23	240-PAH
242	*	3974	288	420.61	421.23	6-methylchrysene (228-PAH-C1)
239		3984	644		421.85	
240	*	3984	644		421.85	240-PAH
242	*	3984	235	420.83	421.85	4-methylchrysene (228-PAH-C1)
239		3995	868		422.55	
240	*	3995	822		422.55	240-PAH
242	*	4006	463		423.24	228-PAH-C1
254	*	4017	421		423.93	240-PAH-C1
254	*	4059	351		426.57	240-PAH-C1
254	*	4093	457		428.71	240-PAH-C1
195		4195	1073		435.12	
220	*	4195	415		435.12	176-PAQ-C1,190-PAF-C2,190-PAX-C1
252	*	4318	2616		442.85	252-PAH
252	*	4331	12677	441.74	443.66	benzo[b]fluoranthene (252-PAH)
264	*	4331	1267		443.66	benzo[a]pyrene D-12 (252-PAH-D12)
252	*	4338	10141		444.10	252-PAH
264	*	4338	1000		444.10	264-PAH
252	*	4343	9251		444.42	252-PAH
268	*	4363	275		445.07	238-PAQ,238-PAXK,252-PAHK,266-PAF

266	*	4611	685		461.26	252-PAH-C1
208	*	4622	148		461.95	178-PAQ
266	*	4622	371		461.95	252-PAH-C1
266	*	4664	269		464.59	252-PAH-C1
266	*	4683	870		465.79	252-PAH-C1
266	*	4747	230		469.81	252-PAH-C1
282	*	4762	153		470.75	264-PAH-C1
264	*	4798	624		473.01	264-PAH
276	*	5087	726		491.18	276-PAH
278	*	5106	900		492.37	bibenz[a,j]anthracene (278-PAH)
276	*	5133	6744	494.50	494.07	indeno[123-cd] pyrene(276-PAH)
278	*	5168	1354	495.45	496.27	dibenz[a,h]anthracene (278-PAH)
278	*	5184	529	495.01	497.27	dibenz[a,c]anthracene (278-PAH)
278	*	5214	1016	497.66	499.16	benzo[b]chrysene (278-PAH)
278	*	5227	973	500.00	500.00	picene (278-PAH)
276	*	5250	6744	501.32	501.42	benzo[ghi]perylene (276-PAH)
276	*	5310	1037	503.91	505.19	dibenzo[def,mno]chrysene (276-PAH)
306	*	5389	45		510.16	278-PAH-C2
290	*	5445	132		513.68	276-PAH-C1
306	*	5445	39		513.68	278-PAH-C2
302	*	5794	1623		535.61	302-PAH
302	*	5797	1957		535.80	302-PAH
302	*	5826	1800		537.62	302-PAH
302	*	5845	1000		538.82	302-PAH
306	*	5892	59		541.77	278-PAH-C2
302	*	5925	400		543.85	302-PAH
300	*	5940	940		544.79	coronene (300-PAH)
302	*	5977	1174		547.11	302-PAH
302	*	5979	1174		547.24	302-PAH
302	*	6009	300		549.13	302-PAH
252	*	4389	1950	442.56	447.31	benzo[k]fluoranthene (252-PAH)
252	*	4406	280		448.38	252-PAH
268	*	4406	566		448.38	same as scan# 4365
252	*	4471	1800		452.46	252-PAH
268	*	4471	971		452.46	same as scan# 4363
252	*	4479	7216	450.73	452.97	benzo[e]pyrene (252-PAH)
268	*	4479	722		452.97	same as scan# 4363
252	*	4507	10044	453.44	454.72	benzo[a]pyrene (252-PAH)
252	*	4516	521		455.29	252-PAH
268	*	4524	418		455.79	same as scan# 4363
252	*	4556	2535		457.80	252-PAH
230	*	4581			459.38	216-PAK,214-PAX
266	*	4603	334		460.76	252-PAH-C1

10.2 Appendix II: Reversed phase HPLC/MS data obtained for the TPE sample for compounds in the GC-amenable range, sorted by scan number (retention time)

m/z	M(*)	sc#	int.	tentative identification
154	*	100	171	acenaphtene (154-PAH)
217	*	111	375	benzocarbazole (216-PANH)
168	*	140	619	dibenzofuran (166-PAF)
217	*	143	650	benzocarbazole (216-PANH)
217	*	169	216	benzocarbazole (216-PANH)
184	*	187	54	dibenzothiophene 166-PASH)
165		194	351	
166	*	194	359	fluorene (166-PAH)
184	*	268	118	166-PASH
222	*	268	83	166-PAH-C4
178	*	289	3087	phenanthrene (178-PAH)
230	*	310	80	216-PAK
230	*	331	80	216-PAK
181	*	332	359	166-PANH-C1
231	*	337	216	216-PANH-C1
156	*	344	179	128-PAH-C2
204	*	362	83	202-PAH-H2
203	*	364	82	202-PANH
181	*	385	216	166-PANH-C1
231	*	385	81	216-PANH-C1
165		393	124	
180	*	394	137	166-PANH-C1
179	*	398	318	178-PANH
178	*	403	1516	anthracene (178-PAH)
217		407	72	
232	*	407	38	216-PAF-C1
189		415	435	
190	*	415	490	4h-cyclopenta[def]phenanthrene
241	*	438	124	240-PANH
257	*	439	72	228-PANH-C2
165		455	125	
180	*	455	116	
195		455	134	
210	*	455	65	154-PAH-C4
204	*	470	535	128-PAH-Ph
192	*	476	481	178-PAH-C1
202	*	476	205	202-PAH
212	*	487	118	166-PASH-C2
230	*	487	64	202-PAH-C2
195	*	495	559	166-PANH-C2
165		500	258	
180	*	500	218	166-PAH-C1
196		501	136	
211	*	501	62	140-APAH-C4

192	*	507	172	178-PAH-C1
155		508	150	
170	*	511	138	128-PAH-C3
202	*	531	9307	fluoranthene (202-PAH)
195	*	551	145	166-PANH-C2
208	*	572	309	178-PAQ
195	*	585	145	167-PANH-C2
195	*	595	145	167-PANH-C2
192	*	602	688	178-PAH-C1
218	*	627	618	216-PAF
202	*	630	7528	pyrene (202-PAH)
216	*	638	481	202-PAH-C1
202	*	658	934	202-PAH
202	*	668	1334	202-PAH
267	*	689	122	266-PANH
218	*	708	132	216-PAF
200	*	712	109	128-PAQ-C3
228	*	716	952	228-PAH (benzo[c]phenanthrene)
202	*	721	411	202-PAH
227	*	721	467	226-PANH
252	*	736	173	238-PAK
173		742	150	
174	*	742	135	116-PAQ-C2
218	*	749	503	PAF
191		758	194	
206	*	758	125	178-PAH-C2
192	*	771	430	178-PAH-C1
215		774	263	
216	*	774	321	216-PAH
226	*	790	564	226-PAH
228	*	793	2116	triphenylene (228-PAH)
202	*	796	137	202-PAH
218	*	803	2958	216-PAF
202	*	820	135	202-PAH
215		828	482	
216	*	828	535	216-PAH
234	*	837	236	216-PASH
218	*	858	1148	216-PAF
208	*	861	107	190-PASH
215		886	2892	
216	*	886	3582	216-PAH
208	*	888	125	190-PASH
212	*	896	296	166-PASH-C2
250	*	902	1534	178-PAQ-C3

195		903	112	
210	*	904	108	166-PAQ-C1
252	*	913	173	252-PAH
254	*	915	366	240-PAH-C1
226	*	947	1635	226-PAH
215		973	613	
216	*	973	802	216-PAH
232	*	989	189	190-PAH-C3
210	*	997	297	166-PAQ-C1
228	*	1029	7087	benz[a]anthracene (228-PAH)
232	*	1068	227	190-PAH-C3
215		1074	657	
216	*	1074	748	216-PAH
234	*	1079	464	216-PASH
211		1087	154	
226	*	1087	197	166-PASH-C3
195	*	1099	395	166-PANH-C2
232	*	1106	227	190-PAH-C3
195	*	1111	395	166-PANH-C2
240	*	1111	588	240-PAH
242	*	1111	388	228-PAH-C1
228	*	1138	5501	chrysene (228-PAH)
254	*	1155	314	240-PAH-C1
220	*	1170	184	178-PAH-C3
232	*	1177	393	190-PAH-C3
226	*	1207	226	226-PAH
242	*	1212	465	228-PAH-C1
228	*	1242	317	228-PAH
278	*	1245	276	278-PAH
242	*	1252	186	228-PAH-C1
231		1261	205	
232	*	1261	378	190-PAH-C3
242	*	1274	217	228-PAH-C1
234	*	1292	1252	216-PASH
226	*	1313	197	226-PAH
215		1321	109	
230	*	1321	201	216-PAH-C1
278	*	1324	204	278-PAH
242	*	1331	969	228-PAH-C1
242	*	1351	310	228-PAH-C1
253		1361	556	
254	*	1361	680	240-PAH-C1
252	*	1369	2250	benzo[k]fluoranthene (252-PAH)
239		1388	569	
240	*	1388	827	240-PAH
211		1426	96	
226	*	1426	282	166-PASH-C3
242	*	1426	388	228-PAH-C1

252	*	1439	8999	benzo[e]pyrene (252-PAH)
242	*	1458	651	228-PAH-C1
266	*	1520	510	252-PAH-C1
252	*	1527	11770	benzo[b]fluoranthene (252-PAH)
242	*	1548	295	228-PAH-C1
246	*	1552	194	214-PASH-C1
248	*	1577	133	216-PASH-C1
242	*	1629	465	228-PAH-C1
252	*	1631	1038	perylene (252-PAH)
268	*	1631	796	240-PAH-C2
242	*	1645	620	228-PAH-C1
265		1657	206	
266	*	1657	255	266-PAH
276	*	1674	175	276-PAH
278	*	1674	623	278-PAH
252	*	1726	6057	benzo[j]fluoranthene (252-PAH)
265		1741	91	
266	*	1741	136	266-PAH
258	*	1772	257	240-PASH
265		1778	115	
266	*	1778	340	266-PAH
263	*	1819	458	
242	*	1822	775	228-PAH-C1
252	*	1822	865	252-PAH
264	*	1822	637	264-PAH
265		1850	161	
266	*	1850	128	266-PAH
278	*	1859	603	278-PAH
252	*	1861	6403	benzo[a]pyrene (252-PAH)
266	*	1879	230	252-PAH-C1
252	*	1913	433	252-PAH
242	*	1924	155	228-PAH-C1
266	*	1930	128	252-PAH-C1
278	*	1948	204	278-PAH
266	*	1959	425	252-PAH-C1
252	*	1961	1384	252-PAH
268	*	1968	551	240-PAH-C2
252	*	2011	346	252-PAH
266	*	2024	213	252-PAH-C1
276	*	2039	184	276-PAH
252	*	2050	692	252-PAH
266	*	2050	170	252-PAH-C1
276	*	2058	200	276-PAH
266	*	2125	213	252-PAH-C1
252	*	2147	433	252-PAH
276	*	2167	394	276-PAH

278	*	2167	388	278-PAH
282	*	2198	200	264-PASH
252	*	2242	190	252-PAH
278	*	2248	102	278-PAH
276	*	2266	2624	benzo[ghi]perylene (276-PAH)
266	*	2328	170	252-PAH-C1
252	*	2334	173	252-PAH
268	*	2343	514	240-PAH-C2
276	*	2367	656	276-PAH
276	*	2429	2843	indeno[123-cd]pyrene (276-PAH)
302	*	2443	272	302-PAH
276	*	2524	743	276-PAH
302	*	2527	258	302-PAH
266	*	2556	213	252-PAH-C1
276	*	2619	394	276-PAH
290	*	2632	156	276-PAH-C1
302	*	2632	444	302-PAH
278	*	2688	562	278-PAH
276	*	2707	306	276-PAH
302	*	2707	358	302-PAH
278	*	2783	153	278-PAH
276	*	2809	131	276-PAH
302	*	2814	143	302-PAH
290	*	2828	115	276-PAH-C1
276	*	2933	350	276-PAH
278	*	3031	204	278-PAH
278	*	3048	327	278-PAH
302	*	3188	444	302-PAH
302	*	3281	251	302-PAH
302	*	3376	129	302-PAH
300	*	3403	247	coronene (300-PAH)
302	*	3721	143	302-PAH

10.3 Appendix III: Reversed phase HPLC/MS data obtained for the TPE sample for compounds eluted in the acetonitrile-dichloromethane portion of the gradient (sorted by scan number)

m/z	M(*)	scan#	abs.int.
278	*	1737	3160
278	*	1810	3160
278	*	1937	2633
278	*	2027	2633
276		2195	6553
278	*	2195	13165
276		2384	6553
278	*	2384	15799
276		2574	6553
278	*	2575	11849
300		2575	1962
301		2575	1815
302	*	2575	4306
303		2575	1200
276	*	2625	6553
276	*	2697	9660
278	*	2705	14482
304		2707	703
306	*	2707	2141
308	*	2746	2139
309		2746	648
328	*	2746	1963
329		2746	494
276	*	2787	65535
278	*	2795	3159
304	*	2829	2810
305		2829	279
316	*	2845	690
304	*	2880	2810
278	*	2897	2106
328	*	2914	1682
329		2914	494
276	*	2959	65535
300		2963	5886
301		2963	1815
302	*	2963	17222
303		2963	4801
304	*	3003	7026
305		3003	1676
308		3004	1222
318	*	3025	705
431		3038	720
432		3038	171
430	*	3039	1958
428	*	3046	234
300		3075	5886
301		3075	1815
302	*	3075	17222
303		3075	4801
308		3090	611
328	*	3091	2243
329		3095	740
330	*	3144	809
304	*	3165	3513
305		3165	1117
300		3178	7848
301		3178	2420
302	*	3178	23681
303		3178	6601
318	*	3230	1762
319		3230	380
344		3235	289
278	*	3246	26331
276	*	3252	6553
354	*	3256	498
300		3277	6867
301		3277	2118
302	*	3277	23681
303		3277	6601
328	*	3277	3926
329		3277	1728
308		3280	917
311	*	3280	1071
311	*	3286	6425
316	*	3298	1839
318	*	3298	1410
308	*	3318	1222
304	*	3360	5621
305		3360	1117
328	*	3368	4206
329		3368	1481
330		3368	1348
352	*	3370	986
304	*	3408	2810
305		3408	838
316	*	3419	2069
318	*	3419	2114
329	*	3423	1234

276	*	3435	26214
352	*	3459	986
353		3459	374
324		3466	1034
325		3466	406
326	*	3466	1619
430	*	3477	1566
431		3477	432
306	*	3486	1070
308	*	3500	917
318	*	3500	1057
278	*	3520	19748
276	*	3527	6553
308	*	3538	1528
307		3548	858
306	*	3549	2498
378	*	3549	680
304	*	3577	2810
352	*	3577	1479
301		3586	3328
302	*	3586	43056
303		3586	12002
318	*	3595	1410
311	*	3602	2142
312		3602	512
300	*	3608	10791
304	*	3620	7026
305		3620	1257
302	*	3632	17222
303		3632	4801
316	*	3636	2989
318	*	3636	2819
319		3636	951
387	*	3647	365
330	*	3664	1348
524	*	3668	774
359	*	3672	528
413	*	3673	774
511		3683	257
300	*	3688	19620
301		3688	6050
340	*	3690	703
352	*	3690	1972
353		3690	499
510	*	3690	362
314	*	3695	2677
315		3704	1723
316	*	3711	1379
508	*	3731	616
509		3731	188
540	*	3731	9362
541		3731	3471
542		3731	2901
543		3731	945
314	*	3735	3347
363	*	3739	405
544	*	3745	602
430	*	3750	3916
431		3750	1152
432		3750	1368
510	*	3767	634
336	*	3777	449
462	*	3780	64
308		3790	611
309		3790	756
310		3790	467
312		3790	512
313	*	3790	3950
344	*	3790	346
352	*	3790	493
508	*	3790	616
509		3790	94
543	*	3790	756
314	*	3794	6024
315		3794	1723
538		3797	1258
541		3797	2430
542	*	3797	3031
316	*	3798	1379
300	*	3800	5886
301		3800	2420
320		3800	806
321		3800	815
322		3800	1697
323		3800	1273
325		3800	1622
326	*	3800	16193
327		3800	3173
536	*	3802	376
324		3803	4135
302	*	3807	19375
303		3807	5401

540	*	3810	7490
367	*	3813	1311
312	*	3826	1280
569		3839	1746
570	*	3839	2798
571		3842	241
391	*	3850	310
365	*	3857	3116
413	*	3862	332
459		3863	86
311	*	3866	6425
312		3866	1024
339	*	3877	1057
340		3877	938
510	*	3887	453
515		3888	248
328	*	3891	2804
329		3891	1234
320		3893	644
321		3893	652
322	*	3893	1212
323		3893	909
324	*	3893	2068
325		3893	1082
326	*	3893	6477
327		3893	1851
430	*	3898	783
431		3898	288
432		3898	684
508	*	3901	246
313	*	3909	2963
316		3914	1379
315		3921	1378
328	*	3928	5608
329		3928	1728
538		3928	629
540	*	3928	5149
541		3928	1736
542		3928	1451
309	*	3932	1080
546	*	3932	6496
547		3932	524
545		3933	500
365	*	3936	1780
571		3937	407
572	*	3937	1434
573		3937	161
574		3937	78
311	*	3938	4283
312		3942	1024
324		3944	2068
325		3944	1082
326	*	3944	6477
327		3944	1851
520		3944	260
544	*	3962	241
544	*	3971	662
570	*	3971	1048
569		3973	319
339	*	3989	2536
394	*	3991	344
413	*	3991	332
533		3995	160
393	*	3996	1315
534	*	3997	222
340	*	4003	938
316	*	4016	920
494	*	4018	337
493		4021	115
337	*	4028	978
573		4029	777
574	*	4031	1613
324		4032	2585
326	*	4032	9716
327		4032	2645
391	*	4033	310
473		4033	61
322	*	4034	1333
323		4034	818
325		4034	1082
352	*	4035	2465
353		4035	1247
500	*	4036	202
585		4036	116
586	*	4036	270
311	*	4045	8566
312		4048	1792
459		4048	65
458	*	4049	79
547		4049	1957
548	*	4049	2505
365	*	4053	2893

421	*	4053	438
524	*	4053	95
544	*	4063	450
545		4063	298
352	*	4068	2465
353		4068	1247
354		4068	415
339	*	4076	1268
518	*	4082	363
519		4084	138
313	*	4089	1975
582	*	4095	120
421	*	4097	438
315	*	4098	1034
324		4100	1809
325		4100	1082
326	*	4100	6477
327		4100	1851
573		4101	163
574	*	4101	323
561		4103	146
562	*	4104	345
506	*	4112	114
522	*	4120	814
523		4120	246
516	*	4122	124
545		4122	421
546		4122	674
547		4122	347
548	*	4122	752
572	*	4122	604
575		4122	217
588	*	4122	120
502	*	4124	158
337		4131	761
340	*	4131	938
352	*	4131	2465
353		4131	1247
544	*	4134	709
520	*	4135	260
391	*	4141	310
365	*	4153	1780
339	*	4155	2114
393	*	4158	1315
394		4158	344
450	*	4158	149
576	*	4158	3770
577		4158	1310
520	*	4170	208
521		4170	103
322		4171	848
323		4171	818
324		4171	2326
325		4171	946
326	*	4171	8906
327		4171	2380
522	*	4175	3254
523		4175	1229
524		4175	509
572	*	4187	966
313	*	4196	5925
367	*	4201	2621
573		4204	380
574	*	4209	484
309		4231	864
310		4231	622
311	*	4231	5354
312		4231	1280
490	*	4232	237
494	*	4232	289
502	*	4232	356
545		4232	450
546		4232	992
548	*	4232	5872
549		4232	2505
422	*	4233	1641
423		4233	405
450	*	4233	297
451		4233	148
473		4235	81
476	*	4235	283
490	*	4242	77
393	*	4250	751
478	*	4252	157
365	*	4253	2003
466	*	4260	135
519		4271	182
520		4282	369
522	*	4282	651
574	*	4282	807

575		4282	311
322		4287	970
323		4287	818
324		4287	3101
325		4287	1352
326	*	4287	12954
327		4287	3438
534	*	4294	515
550	*	4295	1808
551		4295	763
562	*	4295	302
352	*	4300	1479
572	*	4302	163
544	*	4305	189
339	*	4307	1691
544	*	4316	120
506	*	4320	114
548	*	4324	2283
549		4324	752
502	*	4325	305
575		4325	544
576	*	4325	3770
577		4325	1638
531		4326	174
546	*	4326	397
450	*	4328	495
451		4328	197
478	*	4330	236
337		4334	1087
338		4334	763
340	*	4334	1406
391		4334	310
393		4334	939
422	*	4334	656
423		4334	243
463		4334	83
490	*	4334	190
566	*	4334	429
563		4337	258
550	*	4350	6327
551		4350	1907
496	*	4369	133
552	*	4371	579
587		4375	120
313	*	4377	6913
552		4379	2018
550	*	4381	7230
551		4381	2288
311	*	4387	4819
312		4387	768
520		4388	130
521		4388	276
522	*	4388	2603
523		4388	983
318	*	4405	1057
576	*	4415	1508
352	*	4418	986
574	*	4420	233
478	*	4423	236
341	*	4430	1501
324		4432	1034
325		4432	541
326	*	4432	5668
327		4432	1322
395	*	4435	959
396		4436	172
522	*	4440	976
523		4440	492
580	*	4443	235
578	*	4451	2174
313	*	4452	2963
564	*	4460	312
578	*	4460	993
452	*	4461	88
545		4465	181
546	*	4465	337
547		4465	198
572	*	4465	362
573		4465	163
496	*	4474	934
497		4474	59
495		4475	307
536	*	4480	483
393	*	4485	1127
522	*	4490	246
578	*	4495	1902
579		4495	710
339	*	4500	1902
450	*	4500	198
322		4502	727
323		4502	727
324		4502	2585
325		4502	811
326	*	4502	9716
327		4502	2645
521		4506	138
522	*	4506	1302
353	*	4513	1122
368		4513	1654
369	*	4513	2799

370		4513	608
564	*	4514	468
538	*	4515	154
532	*	4523	104
335	*	4524	739
336		4524	449
338		4524	610
391	*	4524	206
352	*	4525	740
562	*	4531	172
573		4531	326
574		4531	807
575		4531	544
576	*	4531	1320
516	*	4532	124
536	*	4548	515
524	*	4552	774
367	*	4554	2621
553		4554	675
438	*	4555	136
466	*	4555	80
492	*	4555	96
522	*	4555	2291
552	*	4555	2270
310		4562	622
313	*	4562	4938
476	*	4563	354
506	*	4563	585
548	*	4563	1503
365	*	4564	2226
421		4564	438
422	*	4564	1050
424		4564	222
452	*	4564	226
464	*	4564	138
547		4564	2610
550	*	4564	9038
551		4564	3814
492	*	4567	75
450	*	4568	198
451		4568	148
452		4568	196
311	*	4569	3748
478	*	4570	315
477		4572	61
489		4572	95
490	*	4572	157
502	*	4572	203
506		4572	116
519		4572	156
524	*	4572	677
525		4572	143
309	*	4573	1080
312		4573	896
464	*	4573	45
422	*	4574	486
522	*	4580	488
523		4580	307
524	*	4580	2036
328	*	4584	3365
329		4584	1234
341	*	4594	1072
580	*	4597	353
338	*	4600	763
395	*	4601	480
564	*	4610	312
510	*	4612	188
578	*	4617	1630
579		4617	710
376	*	4642	3031
377		4642	970
532	*	4642	70
564	*	4642	312
338	*	4643	763
393	*	4646	1127
576		4650	1131
577		4650	655
578	*	4651	2717
579		4651	993
550	*	4652	1526
450	*	4653	545
451		4653	197
506	*	4653	77
478	*	4654	315
546	*	4654	794
534	*	4656	201
496	*	4657	276
549		4657	501
550	*	4657	4519
478	*	4658	92
492	*	4660	209
580	*	4660	353

574	*	4661	777
496	*	4662	1334
538	*	4668	771
526	*	4684	191
566	*	4684	413
524	*	4686	2800
525		4686	967
356	*	4699	497
357		4699	260
346		4700	503
349		4700	1199
350	*	4700	8685
351		4700	2682
376	*	4700	2424
377		4700	970
480	*	4702	191
498	*	4706	59
496	*	4710	1067
497		4710	430
521		4710	172
522	*	4710	488
523		4710	369
494	*	4712	38
422	*	4719	438
478	*	4720	236
502	*	4722	102
520	*	4722	182
492	*	4725	157
506	*	4725	334
507		4725	77
324		4727	1034
325		4727	676
326	*	4727	4858
327		4727	1587
534		4736	80
578	*	4737	1902
579		4737	710
580		4737	353
352	*	4740	1972
508		4748	228
510	*	4748	283
552	*	4748	4035
553		4748	1350
564	*	4748	351
508	*	4751	123
552	*	4756	475
313	*	4762	2963
351		4798	3353
354		4800	664
352		4805	2465
353		4805	1247
496	*	4809	534
497		4809	246
368	*	4810	1654
376	*	4810	3031
377		4810	1212
510	*	4810	236
358	*	4811	278
538	*	4815	424
526	*	4828	334
366	*	4830	1049
422	*	4833	438
450	*	4833	297
478	*	4833	394
479		4833	92
365	*	4835	1113
395	*	4835	640
424	*	4838	222
339	*	4839	1691
438	*	4839	136
464	*	4839	193
465		4839	45
480	*	4839	319
481		4839	81
491		4839	104
494	*	4839	193
506	*	4839	418
507		4839	116
519		4839	182
520		4839	482
522		4839	1627
523		4839	615
524	*	4839	5090
525		4839	1934
548	*	4839	501
364	*	4841	420
376	*	4850	1818
377		4850	727
496	*	4850	400
497		4850	123
483		4853	34
568		4858	284
566	*	4859	620
516	*	4862	99

352	*	4865	2219
574	*	4873	484
575		4873	389
504	*	4875	127
478	*	4882	236
520		4883	345
522		4883	1139
523		4883	492
524	*	4883	3054
525		4883	967
526		4883	239
464	*	4885	165
356	*	4893	662
313	*	4900	3950
322		4902	848
324		4902	2068
326	*	4902	8097
327		4902	2380
357	*	4902	173
508	*	4902	286
509		4902	123
552	*	4902	3783
367	*	4903	3932
552	*	4904	1350
553		4904	475
325	*	4905	676
352	*	4916	2465
424	*	4919	630
538	*	4920	1006
588	*	4920	90
572	*	4921	241
538	*	4922	540
464	*	4925	83
376	*	4930	1212
564	*	4930	312
575		4930	350
576	*	4930	1131
577		4930	655
580	*	4940	1175
581		4940	405
395	*	4941	800
566	*	4946	517
582	*	4946	107
341	*	4952	1179
353		4953	997
368	*	4953	1157
526	*	4966	95
364	*	4972	755
402	*	4972	594
339	*	4973	1268
340		4973	820
510	*	4975	71
524	*	4975	2036
525		4975	484
538	*	4975	424
450	*	4978	198
534	*	4979	166
376	*	4980	1818
377		4980	970
534	*	4980	100
535		4980	93
313	*	4983	5431
452	*	4987	301
466	*	4987	147
492	*	4988	261
410	*	4990	458
424	*	4990	332
452	*	4990	168
464	*	4990	138
478	*	4990	394
479		4990	92
424	*	4991	1259
550	*	4994	4067
506	*	4995	668
507		4995	194
548	*	4995	1253
551		4995	1907
552	*	4995	5044
553		4995	1929
554		4995	594
361		4996	650
362	*	4996	1394
365		4996	1335
508	*	4997	286
520	*	4998	182
316	*	5000	690
352	*	5000	2219
402	*	5000	594
363	*	5001	540
354	*	5002	581
367	*	5002	3277
344		5006	462
345		5006	260
346		5006	804

347		5006	676
348		5006	2974
349		5006	1599
350	*	5006	10856
351		5006	3688
310	*	5008	467
400	*	5015	3154
401		5015	1204
402		5015	594
516	*	5021	99
305	*	5023	1117
333	*	5023	603
544	*	5027	421
545		5027	225
574	*	5029	155
320	*	5033	322
344		5033	519
345		5033	312
346		5033	1005
347		5033	743
348		5033	3398
349		5033	2132
350	*	5033	13027
351		5033	4694
352		5040	2219
544	*	5059	452
545		5059	225
319	*	5064	571
534	*	5075	80
478	*	5080	236
492	*	5080	209
549		5080	752
550	*	5080	3615
551		5080	1526
552	*	5080	4035
553		5080	1640
506	*	5084	418
507		5084	116
508		5084	257
367	*	5085	2621
464	*	5085	83
424	*	5088	630
313	*	5095	3950
376	*	5100	3637
377		5100	1212
424	*	5100	332
578	*	5100	470
579		5100	173
342	*	5120	666
398	*	5121	608
399		5121	214
566	*	5122	413
353		5133	623
352	*	5137	1972
356		5141	1159
426	*	5156	292
578	*	5165	588
534	*	5177	120
334	*	5179	382
580	*	5179	173
496	*	5180	267
340	*	5181	703
452	*	5190	168
350	*	5200	4342
351		5200	1341
377		5202	1697
376	*	5203	4243
378		5211	680
402	*	5225	890
366	*	5226	839
403		5233	336
412	*	5238	119
352	*	5246	2958
353		5246	997
380	*	5250	218
387		5282	274
388	*	5282	434
389		5282	229
380	*	5289	218
400	*	5289	2103
398		5298	365
401		5303	602
372	*	5309	1314
354	*	5310	332
370		5310	203
373		5310	621
374	*	5310	4959
375		5310	1638
426	*	5310	292
366	*	5326	629
328	*	5333	2804
329		5333	987
353		5338	1496
352	*	5342	4437

578	*	5345	411
389	*	5352	153
398		5352	608
402		5352	594
400	*	5357	4206
396		5358	257
399		5358	214
400	*	5358	1204
376	*	5372	3637
377		5372	1212
496	*	5383	267
497		5384	215
366		5390	839
368		5400	661
390		5401	200
370		5406	1014
373		5406	1656
374	*	5406	16529
375		5406	5461
371		5408	606
372		5410	2627
376	*	5418	3031
377		5418	727
400	*	5433	2103
401		5433	401
358	*	5450	464
359		5450	176
380	*	5450	218
351	*	5459	2012
346		5460	402
347		5460	270
349		5460	666
350	*	5460	4342
348		5461	1274
398		5479	486
399		5481	142
400	*	5481	2629
401		5481	602
391		5483	103
334	*	5492	318
364	*	5496	336
386	*	5503	131
314	*	5509	13387
315		5509	3446
379	*	5509	860
490	*	5510	116
397		5516	409
412	*	5516	357
376	*	5530	2424
377		5530	1454
378		5530	680
409		5530	509
410	*	5536	1505
411		5536	272
410	*	5552	1054
337	*	5553	869
338		5553	534
355	*	5553	800
371	*	5553	455
490	*	5561	116
351	*	5568	4694
352		5568	1479
365		5568	1335
366		5568	1049
369	*	5568	5598
370		5568	1622
383		5568	1298
384	*	5568	3277
385		5568	808
371	*	5577	1061
516	*	5586	99
319	*	5589	380
333	*	5589	528
335	*	5592	517
323	*	5595	636
325		5595	406
377		5604	727
376	*	5605	2727
400	*	5627	2103
401		5627	602
398		5637	365
364	*	5650	336
505		5651	185
506	*	5654	251
507		5654	77
363		5660	540
516	*	5675	50
305	*	5678	1397
422	*	5685	162
424	*	5689	665

425		5689	300
520		5690	413
207	*	5693	1372
518	*	5693	996
487	*	5699	101
488		5701	35
519		5704	415
400	*	5710	1314
401		5710	201
321	*	5715	326
335		5715	517
337	*	5715	1087
363	*	5715	945
364		5719	336
396	*	5719	343
424	*	5750	554
425		5750	250
379	*	5765	573
381		5765	554
382		5765	291
398	*	5765	486
399		5765	427
414	*	5765	383
428	*	5779	350

10.4 Appendix IV: Reversed phase HPLC/MS data obtained for the alkylated PAH standard solution and for the Syncrude samples, sorted by m/z

m/z	sample	scan no.	tentative identification
178	oil1	264	178-PAH
179	oil1	325-380	194-15
179	oil2	200,330-400	178-PANH,194-15
180	STD	282	3-methylfluorene (166-PAH-C1)
180	oil1	,215,241,291-378	152-PAH-C2,166-PAH-C1
180	oil2	140-200	152-PAH-C2
181	oil1	186,426	152-PANH-C2,FRAGMENT
181	oil2	160	152-PANH-C2
184	oil1	185	166-PASH
184	oil2	100,140	128-PAH-C4
184	oil#5	100-140,170	128-PAH-C4,166-PASH
189	oil2	320-530	204-15
189	oil#5	370-550	204-15
189	SRSIJ	100-140,170	190-1
190	OIL1	314-500	190-PAH,176-PAH-C1,116-PASH-C4
190	OIL2	320-530	190-PAH,176-PAH-C1,116-PASH-C4
190	OIL#5	380-550	190-PAH,176-PAH-C1,116-PASH-C4
190	SRS1J	100-140,170	116-PASXK-C2
191	OIL1	315-432	190+1,206-15
191	OIL2	310-470	190+1,206-15
191	OIL#5	390-480	190+1,206-15
192	STD	360	178-PAH-C1
192	OIL1	314-344	178-PAH-C1
192	OIL2	310,340,230	178-PAH-C1,190-PAF
192	OIL#5	300-340	178-PAH-C1
193	OIL1	415-473	192+1
193	OIL2	420-470	192+1
194	STD	265	9-ethylfluorene (166-PAH-C2)
194	OIL1	216-379	166-PAH-C2
194	OIL2	75-280,330-380	152-PAH-C3,166-PAH-C2,209-15
194	OIL#5	200	152-PAH-C3,166-PAH-C2
194	TS	180	152-PAH-C3
195	OIL1	214	166-PANH-C2
195	OIL2	190-200	166-PANH-C2
195	OIL#5	200	166-PANH-C2
195	OIL#9	200	166-PANH-C2
195	SRS1J	135	152-PANH-C3
197	OIL1	310-442	212-15,198-1,140-PANH-C4
197	OIL#5	150,190,430	212-15,140-PANH-C4
197	OIL2	430,465	212-15
198	OIL1	311,338	197+1,166-PASH-C1
198	OIL2	100,330,350	140-PAF-C4,140-PAX-C3,166-PASH-C1
198	OIL#5	190	140-PAF-C4
199	OIL#5	190	198+1
199	OIL2	350	200-1
200	OIL2	350	140-PASH-C3

200	OIL#5	360	140-PASH-C3
200	OIL#9	370	140-PASH-C3
200	SRS1J	230	140-PASX-C2,128-PAQ-C4
201	OIL2	247	202-1
201	OIL#9	360	202-1
201	SRS1J	135	176-CPAH,128-NPAH-C2
202	OIL1	353	202-PAH
202	OIL2	247	
202	OIL#5	350	202-PAH
202	OIL#7	360	202-PAH
202	OIL#9	360	202-PAH
202	OIL#11	360	202-PAH
202	SRS1J	300-320	202-PAH
203	OIL1	314-431	202+1,218-15
204	OIL2	560,280-400	190-PAH-C1,176-PAH-C2,190-PAK
204	OIL#7	530-600	190-PAH-C1,176-PAH-C2
204	OIL#11	525-600	190-PAH-C1,176-PAH-C2
204	TS	180	116-PASXK-C3
205	OIL2	380-530	206-1,220-15
205	OIL#5	400-575	206-1,220-15
205	OIL#7	460-560	206-1,220-15
205	OIL#11	475-560	206-1,220-15
205	TS	220	190-PANH-C1,176-PANH-C2
205	SR1J	220	190-PANH-C1,176-PANH-C2
206	STD	410	178-PAH-C2
206	OIL1	386-455	178-PAH-C2
206	OIL2	360-470	178-PAH-C2
206	OIL#3	380-500	178-PAH-C2
206	OIL#5	380-500	178-PAH-C2
206	OIL#9	400-475	178-PAH-C2
206	OIL#15A	210	190-PAX,190-PAF-C1,176-PAQ,176-PAHK-C1
206	TS	200-220	190-PAX,190-PAF-C1,176-PAQ,176-PAHK-C1
206	SRS1J	200-220	190-PAX,190-PAF-C1,176-PAQ,176-PAHK-C1
207	OIL2	300	178-PANH-C2
207	OIL#3	500	222-15
207	OIL#5	330-600	222-15
207	OIL#11	310,400	222-15
207	TS	200-220	206+1
207	SRS1J	200-220	206+1
208	OIL1	60-292,400-425	223-15,166-PAH-C3
208	OIL2	30-300,400-460	223-15,166-PAH-C3
208	OIL#3	400-500	166-PAH-C3
208	OIL#5	00-300,400-450	223-15,166-PAH-C3
208	OIL#11	250-375	223-15
208	OIL#14	250-350	223-15
208	TS	170,220-290	178-PAQ,223-15
208	SRS1J	165	178-PAQ,178-PAHK-C1,166-PAK-C2

209	OIL1	248	166-PANH-C3
209	OIL2	200-260	166-PANH-C3
209	OIL#3	130-200	224-15,166-PANH-C3
209	OIL#5	70-250	224-15,166-PANH-C3
209	OIL#7	100-250	224-15,166-PANH-C3
209	OIL#11	225-270	166-PAH-C3
209	OIL#14	240	166-PAH-C3
211	OIL1	405-516	226-11
211	OIL2	400-540,590	226-11
211	OIL#3	50-200,440-500	226-11
211	OIL#5	00-200,430-570	226-11
211	OIL#7	470-570	226-11
211	OIL#9	450-580	226-11
211	OIL#11	530-580	226-11
212	OIL1	403-450	166-PASH-C2
212	OIL2	405,425,450	166-PASH-C2
212	OIL#3	430-480	166-PASH-C2
212	OIL#5	430-560	166-PASH-C2
212	OIL#7	450	166-PASH-C2
212	OIL#9	450-560	166-PASH-C2
215	OIL2	400-480	216-1
215	OIL#5	400-500	216-1
215	OIL#7	450,700	216-1,230-15
215	OIL#9	470,500	216-1,230-15
215	OIL#11	475-750	216-1,230-15
215	OIL#14	475	216-1
216	STD	460	1-methylpyrene (202-PAH-C1)
216	OIL1	410-462	202-PAH-C1
216	OIL2	400-480	202-PAH-C1
216	OIL#5	450,500	202-PAH-C1
216	OIL#7	450,500	202-PAH-C1
216	OIL#9	465,500	202-PAH-C1
216	OIL#11	465,500	202-PAH-C1
216	OIL#14	465	202-PAH-C1
216	OIL#15A	630-720	216-PAH
217	OIL1	410-541	202-PANH-C1
217	OIL2	00-480,510-550	202-PANH-C1,216-PANH
217	OIL#5	380-550	202-PANH-C1,216-PANH
217	OIL#7	420-700	202-PANH-C1,216-PANH
217	OIL#9	500,550	202-PANH-C1,216-PANH
217	OIL#11	450-700	202-PANH-C1,216-PANH
217	OIL#14	500,700	202-PANH-C1,216-PANH
218	OIL1	350-505	190-PAH-C2,176-PAH-C3
218	OIL2	400,420-480	190-PAH-C2,176-PAH-C3
218	OIL#5	350-500	190-PAH-C2,176-PAH-C3
218	OIL#7	400-500	190-PAH-C2,176-PAH-C3
218	OIL#9	425,500	190-PAH-C2,176-PAH-C3

218	OIL#11	425-850	190-PAH-C2,176-PAH-C3
218	OIL#15A	620-850	190-PAH-C2,176-PAH-C3
218	SRS1J	00-200,230-280	216-PAF
219	OIL2	480-600	234-15
219	OIL#5	450-550	234-15
219	OIL#7	550-700	234-15
219	OIL#11	550-850	234-15
219	SRS1J	100-200	190-PANH-C2
220	OIL1	400-515	178-PAH-C3
220	OIL2	450-580	178-PAH-C3
220	OIL#5	450-600	178-PAH-C3
220	OIL#7	450-600	178-PAH-C3
220	OIL#9	460-600	178-PAH-C3
220	OIL#1	460-600	178-PAH-C3
221	OIL2	70,350-400,430-480	236-15,178-PANH-C3
221	OIL#5	350-550	236-15,178-PANH-C3
221	OIL#11	50-300,480,550	236-15,178-PANH-C3
221	SRS1J	160-200	178-PANH-C3
222	OIL2	270-350	166-PAH-C4
222	OIL#3	300-520	166-PAH-C4
222	OIL#5	330-540	166-PAH-C4
222	OIL#11	300-425	166-PAH-C4
222	TS	270-350	166-PAH-C4
222	SRS1J	150-170	178-PAQ-C1,166-PAK-C3,178-PAHK-C2
223	OIL1	294-310	166-PANH-C4
223	OIL2	250-300	166-PANH-C4
223	OIL#5	200	166-PANH-C4
223	OIL#11	260-340	166-PANH-C4
223	TS	200-320	166-PANH-C4
224	OIL2	50-300,490-630	166-PAX-C3,166-PAF-C4
224	OIL#3	170,500-540	166-PAX-C3,166-PAF-C4
224	OIL#5	170,500-540	166-PAX-C3,166-PAF-C4
224	OIL#7	700,750	?
224	OIL#11	725,787	?
224	OIL#14	730	?
225	OIL2	40-200,520-640	240-15
225	OIL#5	80-250,550-620	240-15
225	OIL#7	550-800	240-15
225	OIL#9	550,610	240-15
225	OIL#11	620	240-15
225	SR1J	320	240-15
226	OIL1	445-524	166-PASH-C3
226	OIL2	480-620	166-PASH-C3
226	OIL#3	80-200,500-540	140-PAQ-C4,166-PASH-C3
226	OIL#5	80-200,500-620	140-PAQ-C4,166-PASH-C3
226	OIL#7	500-600	166-PASH-C3
226	OIL#9	500-575	166-PASH-C3

226	OIL#11	410,525-750	226-PAH,166-PASH-C3
226	OIL#15A	410,510	226-PAH,166-PASH-C3
226	SRS1J	230	140-PAQ-C4
227	OIL#5	180,540	227-PANH,226+1
228	OIL1	396,470	228-PAH
228	OIL2	400,500	228-PAH
228	OIL#11	425,525	228-PAH
228	OIL#15A	00,500,630-670	228-PAH,FRAGMENTS
229	OIL#7	550-700	FRAGMENTS
229	OIL#11	575-750	FRAGMENTS
230	OIL1	480-60[illegible]	202-PAH-C2,216-PAH-C1
230	OIL2	550,62[illegible]	202-PAH-C2,216-PAH-C1
230	OIL#7	570-670	202-PAH-C2,216-PAH-C1
230	OIL#11	575-690	202-PAH-C2,216-PAH-C1
230	OIL#15A	50-330,570-640	202-PAH-C2,216-PAH-C1
230	TS	250,270,325	216-PAK,214-PAX
230	SRSIJ	210,310	216-PAK,214-PAX
231	OIL1	500-642	246-15
231	OIL2	240,270	202-PANH-C2
231	OIL#7	6[illegible]0-700	246-15
231	OIL#11	[illegible]0-675	202-PANH-C2,246-15
231	OIL#14	260,650-725	202-PANH-C2,246-15
231	TS	180,230-250	202-PANH-C2,246-15
232	OIL1	403-643	190-PAH-C3
232	OIL2	440,570	190-PAH-C3
232	OIL#5	425-575	190-PAH-C3
232	OIL#7	450-660	190-PAH-C3
232	OIL#11	450-700	190-PAH-C3
232	OIL#15A	510-630	190-PAH-C3
232	TS	180,250	216-PAX,216-PAF-C1
233	OIL1	537-650	248-15
233	OIL#7	540-680	248-15
233	OIL#11	525-750	248-15
233	TS	180,250	190-PANH-C3,176-PANH-C4
234	STD	468	2-T-butylanthracene (178-PAH-C4)
234	OIL1	460-563	178-PAH-C4
234	OIL2	510-600	178-PAH-C4
234	OIL#5	500-670	178-PAH-C4
234	OIL#7	500-690	178-PAH-C4
234	OIL#9	525-626	178-PAH-C4
234	OIL#11	550-725	178-PAH-C4
234	TS	180,300-380	190-PAF-C3,190-PAX-C2
235	OIL1	304	178-PANH-C4
235	OIL2	280-330	178-PANH-C4
235	OIL#11	300	178-PANH-C4
235	TS	180	234+1

236	OIL2	300-450	190-PASH-C2
236	OIL#5	380-550	190-PASH-C2
236	OIL#7	320-420	190-PASH-C2
236	OIL#11	300-400	190-PASH-C2
236	OIL#15A	300-380	190-PASH-C2
236	TS	180,280-400	190-PASX-C1,178-PAQ-C2,166-PAK-C4
237	OIL1	315-355	166-PANH-C5
237	OIL2	280-360	166-PANH-C5
237	OIL#7	250-350,660	166-PANH-C5
237	OIL#9	300-350,675	166-PANH-C5
237	OIL#14	300-375	166-PANH-C5
237	TS	240-380	166-PANH-C5
238	OIL2	570-630	166-PAX-C4
238	OIL#5	600-650	166-PAX-C4
238	OIL#7	600-650	166-PAX-C4
238	OIL#11	00-650,750-850	166-PAX-C4
238	OIL#15A	200,500,550	166-PAQ-C3,166-PAX-C4
238	TS	220	166-PAQ-C3
238	SRS1J	200,220	166-PAQ-C3
239	OIL#5	30-260,600-675	238-PANH,238+1
239	OIL#7	650-750	238+1
239	OIL#9	525,600-750	238+1
239	OIL#11	515,600-750	238+1
240	OIL1	500-575	226-PAH-C1.166-PASH-C4
240	OIL2	580-650	226-PAH-C1,166-PASH-C4
240	OIL#5	575-670	226-PAH-C1,166-PASH-C4
240	OIL#7	575-680	226-PAH-C1,166-PASH-C4
240	OIL#9	610-750	226-PAH-C1,166-PASH-C4
240	OIL#11	610-750	226-PAH-C1,166-PASH-C4
240	OIL#15A	500,600-700	226-PAH-C1,166-PASH-C4
241	OIL#5	240,620	240-PANH,256-15
241	OIL#9	525-775	256-15
241	OIL#11	525-750	256-15
241	SRS1J	310	240-PANH,226-PANH-C1
242	OIL1	477-639	228-PAH-C1
242	OIL2	500-600	228-PAH-C1
242	OIL#9	510-725	228-PAH-C1
242	OIL#11	525-750	228-PAH-C1
242	OIL#15A	510-730	228-PAH-C1
243	oil2	250	228-PANH-C1
243	OIL#7	650-760	258-15
243	OIL#11	510,700-850	258-15
243	SRS1J	130-200,310	228-PANH-C1
244	oil2	300-400	202-PAH-C3
244	OIL#7	-390,470,630-770	202-PAH-C3,216-PAH-C2

244	OIL11	,475,550-600,625-820	202-PAH-C3,216-PAH-C2
244	OIL#15A	00-400,620-780	202-PAH-C3,216-PAH-C2
244	TS	300-430	202-PAH-C3
245	OIL2	280-350	216-PANH-C2
245	OIL#11	350,600-800	216-PANH-C2,260-15
245	OIL#14	300-500	216-PANH-C2
245	TS	220-330	216-PANH-C2
246	OIL1	475-685	190-PAH-C4,216-PAF-C2,214-PASH-C1
246	OIL2	450-630	190-PAH-C4,216-PAF-C2,214-PASH-C1
246	OIL#7	500-740	190-PAH-C4,216-PAF-C2,214-PASH-C1
246	OIL#11	^500-810	190-PAH-C4,216-PAF-C2,214-PASH-C1
246	OIL#15A	330,530-750	190-PAH-C4,216-PAF-C2,214-PASH-C1
246	TS	270-350	190-PAH-C4,216-PAF-C2,214-PASH-C1
248	OIL1	525-680	216-PASH-C1,190-PAF-C4,190-PAX-C3
248	OIL2	340-500	216-PASH-C1,190-PAF-C4,190-PAX-C3
248	OIL#7	580-700	216-PASH-C1,190-PAF-C4,190-PAX-C3
248	OIL#11	00-450,600-750	216-PASH-C1,190-PAF-C4,190-PAX-C3
248	OIL#15A	80-480,600-750	216-PASH-C1,190-PAF-C4,190-PAX-C3
249	OIL1	363	178-APAH-C4
249	OIL2	300-400	178-APAH-C4
250	OIL2	590	250-PAH,190-PASH-C3
250	OIL#5	450-600	250-PAH,190-PASH-C3
250	OIL#11	40,625,740-800	250-PAH,190-PASH-C3
250	OIL#15A	610,640,740	250-PAH,190-PASH-C3
250	SRS1J	630	250-PAH,190-PASH-C3
251	OIL1	378	166-PANH-C6
251	OIL2	340-400	166-PANH-C6
251	OIL#11	-400,625,740-800	166-PANH-C6,266-15
252	OIL1	556-656	252-PAH
252	OIL2	590	252-PAH
252	OIL#7	660-700	252-PAH
252	OIL#11	25,650,725,750	252-PAH
252	OIL#15A	07,63,710,730	252-PAH
253	OIL#11	625,730	252+1
254	OIL2	560	226-PAH-C3,238-PAX,240-PAH-C1
254	OIL#15A	400,450,575	226-PAH-C3,238-PAX,240-PAH-C1
254	TS	350-420	226-PAH-C3,238-PAX,240-PAH-C1
255	OIL#9	610-825	270-15
255	OIL#11	500,625-800	270-15
256	STD	571	9,10-dimethylbenz[a]anthracene (228-PAH-C2)
256	OIL1	560-806	228-PAH-C2
256	OIL2	,700-730,800-860	228-PAH-C2
256	OIL#9	,650,725-760,840	228-PAH-C2
256	OIL#11	450,620-925	228-PAH-C2
256	OIL#15A	525-900	228-PAH-C2
258	OIL1	28,490,575,650	202-PAH-C4,216-PAH-C3
258	OIL2	360-500	202-PAH-C4,216-PAH-C3

258	OIL#7	430-800	202-PAH-C4,216-PAH-C3
258	OIL#11	40-460,600-850	202-PAH-C4,216-PAH-C3
258	OIL#15A	00-600,625-800	202-PAH-C4,216-PAH-C3
258	TS	350-580	202-PAH-C4,216-PAH-C3
258	SRS1J	310,710	228-PAQ,202-PAH-C4,216-PAH-C3
259	OIL2	340-430	202-PANH-C4
259	OIL#11	50-600,650-850	274-15
259	TS	340-400	202-PANH-C4
260	OIL2	750-850	214-PASH-C2
260	OIL#7	570-720	214-PASH-C2
260	OIL#9	600-850	214-PASH-C2
260	OIL#11	600-875	214-PASH-C2
260	OIL#15A	00-425,600,860	216-PAX-C2,214-PASH-C2
260	TS	280-420	216-PAX-C2,216-PAF-C3
262	OIL2	400-500	190-PAX-C4,216-PASH-C2
262	OIL#7	600-840	216-PASH-C2
262	OIL#11	600-875	216-PASH-C2
262	OIL#15A	00-600,650-875	216-PASH-C2
263	OIL2	70-480,700-720	238-CPAH,262+1
263	OIL#11	650,740,760	262+1
264	OIL1	643-693	190-PASH-C4,250-PAH-C1,264-PAH
264	OIL2	70-480,700-720	190-PASH-C4,250-PAH-C1,264-PAH
264	OIL#11	80-420,725-850	190-PASH-C4,250-PAH-C1,264-PAH
264	OIL#15A	400,725-870	190-PASH-C4,250-PAH-C1,264-PAH
265	OIL#11	740-850	?
265	TS	770,870,900,1000,1050	?
266	OIL1	650-701	252-PAH-C1
266	OIL2	700-750	252-PAH-C1
266	OIL#7	730-800	252-PAH-C1
266	OIL#11	730-875	252-PAH-C1
266	OIL#15A	710-860	252-PAH-C1
266	TS	770-1050	252-PAH-C1
267	OIL#11	725-875	266+1
267	OIL#14	740-825,925	266+1
268	OIL1	625-701	226-PAH-C3
268	OIL#11	740-900	226-PAH-C3
268	OIL#15A	500-850	226-PAH-C3
269	OIL#11	700-850	268+1
270	OIL2	800	228-PAH-C3
270	OIL#9	650-825	228-PAH-C3
270	OIL#11	650-900	228-PAH-C3
270	OIL#15A	640-880	228-PAH-C3
271	OIL2	310-400	228-PAH-C3
272	OIL#9	625-850	216-PAH-C4
272	OIL#11	500-875	216-PAH-C4
272	OIL#15A	500-850	216-PAH-C4
273	OIL2	390-510	216-PANH-C4,274-1

274	OIL2	390-500	216-PAX-C3
274	OIL#7	650-800	214-PASH-C3
274	OIL#11	700-850	214-PASH-C3
274	OIL#15A	50-625,700-875	216-PAX-C3,214-PASH-C3
275	OIL2	390-510	250-CPAH
276	OIL1	782	276-PAH
276	OIL2	835	276-PAH
276	OIL#14	880	276-PAH
276	OIL#15A	875	276-PAH
276	TS	50-1050 (825)	276-PAH
276	SRS1J	620	276-PAH
277	OIL2	400-540	276-PANH
278	OIL1	524	264-PAK
278	OIL#15A	50,700,825,850	264-PAK,278-PAH
278	TS	640	278-PAH
280	OIL1	756	252-PAH-C2
280	OIL2	805	252-PAH-C2
280	OIL#15A	740-900	252-PAH-C2
280	TS	500	264-PAX
282	OIL1	700-750	226-PAH-C4,240-PAH-C3
282	OIL#11	00-900,600-700	226-PAH-C4,240-PAH-C3
282	OIL#15A	25-700,775-875	226-PAH-C4,240-PAH-C3
284	OIL#11	750-900	228-PAH-C4
284	OIL#15A	725-875	228-PAH-C4
285	OIL2	350-400	228-PANH-C4
285	OIL#11	750-900	284+1
286	OIL#15A	540-875	240-PASH-C2
286	TS	600-1000	240-PASH-C2
287	OIL2	450-550	216-PANH-C5
288	OIL2	500-600	214-PASH-C4
288	OIL#15A	40-650,725-870	214-PASH-C4,288-PAH
290	OIL2	950	276-PAH-C1
290	OIL#14	800-850,990	276-PAH-C1
290	OIL#15A	0-875,990-1000	276-PAH-C1
291	OIL2	475-600	276-PANH-C1
292	OIL#14	600-835	278-PAH-C1
292	OIL#15A	00-675,725-860	278-PAH-C1
294	OIL2	825,900	266-PAH-C2
294	OIL#11	850-900	266-PAH-C2
294	OIL#15A	825-850	266-PAH-C2
294	TS	775-825	266-PAH-C2
295	OIL#14	800-1000	294+1
295	TS	500-575,775	266-PANH-C2,294+1
296	OIL#11	600-900	240-PAH-C4
296	OIL#15A	600-875	240-PAH-C4
296	TS	650-1050	240-PAH-C4
298	OIL#11	750-900	266-PASH-C1

299	OIL2	400-450	228-APAH-C4
301	OIL2	520-620	?
301	OIL#14	750-850,925	?
302	OIL1	579,661,797	302-PAH
302	OIL2	700	302-PAH
302	OIL#11	600-810 (720)	302-PAH
304	OIL2	650-750,1050	290-PAH-C1,276-PAH-C2
306	OIL2	525-650	278-PAH-C2
306	OIL#14	650-850	278 PAH-C2
308	OIL#14	910-1050	266-PAH-C3
308	TS	800-900	266-PAH-C3
310	OIL#15A	740-875	?
310	TS	800-1000	308+2
314	OIL#14	750-850,930	300-PAH-C1
314	OIL#15A	725-875	300-PAH-C1
314	TS	800-1000	300-PAH-C1
316	OIL#11	637-900	302-PAH-C1
320	OIL#14	750-825	278-PAH-C3
330	OIL#14	750-820	302-PAH-C2
330	TS	1000-1050	302-PAH-C2

10.5 Appendix V: Capillary column SFC/MS data obtained for the TPE sample, sorted by scan number

m/z	M(*)	sc#	int.	tentative identification
128	*	758	250	naphthalene (128-PAH)
153		906	100	
154	*	906	70	acenaphthene (154-PAH)
153		991	100	
168	*	991	40	154-PAH-C1
139	*	1043	151	
154	*	1046	130	biphenyl
153		1055	119	
168	*	1055	376	154-PAH-C1
180	*	1127	120	152-PAH-C2
165		1131	287	
166	*	1131	257	fluorene (166-PAH)
167		1146	56	
182	*	1146	189	154-PAH-C2
181	*	1148	245	166-PANH-C1
165		1218	150	
180	*	1218	120	166-PAH-C1
181		1240	71	
196	*	1240	114	154-PAH-C3
165		1247	140	
180	*	1247	270	166-PAH-C1
160	*	1300	100	
184	*	1343	200	166-PASH
178	*	1348	3092	phenanthrene+anthracene (178-PAHs)
153	*	1353	110	152-PANH
153	*	1401	110	152-PANH
208	*	1413	291	178-PAQ
180		1413	279	
192	*	1421	336	178-PAH-C1
192	*	1453	157	178-PAH-C1
189		1477	202	
190	*	1477	156	190-PAH
204	*	1488	198	190-PAH-C1
202	*	1580	2347	fluoranthene (202-PAH)
160	*	1600	1500	
192	*	1600	112	178-PAH-C1
218	*	1612	399	216-PAF
215		1630	109	
216	*	1630	82	216-PAH
202	*	1637	1224	pyrene (202-PAH)
215		1661	121	
216	*	1661	118	216-PAH
232	*	1671	108	216-PAF-C1

202	*	1739	680	acephenanthrylene (202-i AH)
230	*	1743	170	216-PAH-C1
234	*	1793	130	216-PASH
164	*	1799	110	
160	*	1800	2899	
192	*	1808	1546	178-PAH-C1
224	*	1809	400	166-PAF-C4
256	*	1809	1448	238-PASH
228	*	1811	400	228-PAH
228	*	1827	441	228-PAH
276	*	1913	65	276-PAH
252	*	1966	261	252-PAH
252	*	2016	157	252-PAH
276	*	2140	100	276-PAH
276	*	2173	100	276-PAH

10.6 Appendix VI: a) Reversed phase and b) normal phase packed column SFC/MS data obtained for the HGO sample, sorted by scan number (Retention time)

a) Reversed-phase-SFC data

m/z	M(*)	avg ret time (scan no)	tentative identification
195	*	150	166-PANH-C2
179		160	
194	*	160	166-PAH-C2
178	*	175	178-PAH
197	*	175	140-PANH-C4
193		185	
208	*	185	166-PAH-C3
209		185	
194	*	200	166-PAH-C2
195		200	
197		200	
212	*	200	166-PASH-C2
211		200	
218	*	210	216-PAF
197		220	
212	*	220	166-PASH-C2
211		220	
226	*	220	166-PASH-C3
227		220	
191		225	
206	*	225	178-PAH-C2
209	*	225	166-PANH-C3
208	*	240	166-PAH-C3
235	*	240	178-PANH-C4
211		245	
220	*	245	178-PAH-C3
226	*	245	166-PASH-C3
227		245	
202		250	
203	*	250	202-PANH
223	*	250	166-PANH-C4
228	*	250	228-PAH
232	*	250	216-PAX,216-PAF-C1
246	*	290	214-PASH-C1,216-PAX-C1,216-PAF-C2,190-PAH-C4
234	*	300	178-PAH-C4
202	*	305	202-PAH
232		320	
233		325	
248	*	325	216-PASH-C1,190-PAF-C4
217		330	

218	*	340	190-PAH-C2,176-PAH-C3
217		380	
232	*	380	190-PAH-C3,176-PAH-C4
231		400	
246	*	400	190-PAH-C4,176-PAH-C5
229		420	
230	*	420	202-PAH-C2,216-PAH-C1
242	*	500	228-PAH-C1
258	*	500	240-PASH,216-PAH-C3
268	*	525	226-PAH-C3,240-PAH-C2
282	*	550	264-PASH,226-PAH-C4
253		600	
268	*	600	same as scan 525
280	*	700	266-PAH-C1,252-PAH-C2
282		700	
278	*	775	278-PAH

b) Normal phase SFC data

m/z	M(*)	scan no.	tentative identification
180	*	90	166-PAH-C1
178	*	100	178-PAH
179		100	
194	*	100	166-PAH-C2
195	*	105	166-PANH-C2
197		105	
179	*	120	178-PANH
190		125	
191		125	
193		125	
206	*	125	178-PAH-C2
208	*	125	166-PAH-C3
197		140	
211		140	
212	*	140	166-PASH-C2
190		155	
191	*	155	
206	*	155	178-PAH-C2
226	*	170	166-PASH-C3
211		170	
190	*	190	190-PAH,176-PAH-C1

202	*	190	202-PAH
203		190	
211		190	
226	*	190	166-PASH-C3
218		190	
226	*	190	166-PASH-C3
220	*	190	178-PAH-C3
180		200	
195	*	200	166-PANH-C2
194		205	
208		205	
209	*	205	166-PANH-C3
234	*	205	178-PAH-C4
235		205	
195		220	
190		220	
191	*	220	190-PANH,176-PANH-C1
218	*	220	216-PAF
232		220	
233		220	
234	*	220	178-PAH-C4
235		220	
202		225	
203	*	225	202-PANH
217	*	225	216-PANH
223	*	225	166-PANH-C4
237	*	225	166-PANH-C5
248	*	232	216-PASH-C1,190-PAF-C4
229		240	
230	*	240	202-PAH-C2,216-PAH-C1
231		240	
232		240	
233		240	
264	*	240	264-PAH,250-PAH-C1,190-PASH-C4
268	*	240	226-PAH-C3,240-PAH-C2
246	*	245	190-PAH-C4,176-PAH-C5
248		245	
249		245	
278	*	250	278-PAH
229	*	265	228-PANH
246	*	265	SAME AS SCAN 245
278	*	265	278-PAH
227	*	280	226-PANH
278	*	280	228-PAH
282	*	280	226-PAH-C4

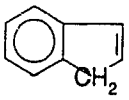
229	*	285	228-PANH
230		285	
231		285	
246	*	285	
249		285	
252	*	285	252-PAH
258	*	290	240-PASH,216-PAH-C3
276	*	290	276-PAH
280	*	295	266-PAH-C1,252-PAH-C2
304	*	350	290-PAH-C1
282	*	365	266-PAF-C1,266-PAX
304	*	365	
253		370	
268	*	370	266-PAF,238-PAF-C2,238-PAX-C1
314	*	370	300-PAH-C1

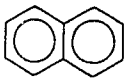
10.7 Appendix VII: PAHs listed in Appendices I-VI as the reference structures of all compounds tentatively identified in the mixtures

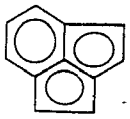
Nomenclature: PACs are named with respect to the structure of the most similar parent PAH. As an example, Table 10.1 lists the name of possible compounds which are identified as 166-PAx and 178-PAx (x=H, NH, SH, F, X, Q, ...) in this work. Cn (n=1,2,3,...) refers to the number of carbon atoms in the alkyl side chains.

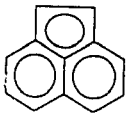
Table 10.1: Compound names related to the nomenclature used in this work

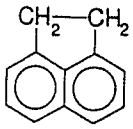
Nomenclature name	**Name of one possible compound**
166-PAH	fluorene
178-PAH	phenanthrene
178-PANH	acridine
166-PASH	dibenzothiophene
166-PAF	dibenzofuran
166-PAX	6H-dibenzopyran
178-PAQ	anthraquinone

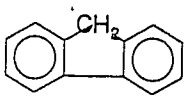
116-PAH 

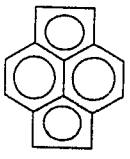
128-PAH 

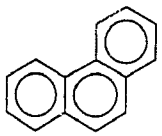
140-PAH 

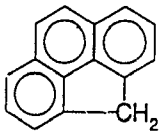
152-PAH 

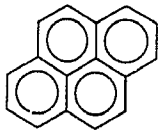
154-PAH 

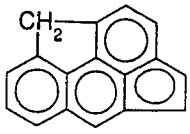
166-PAH 

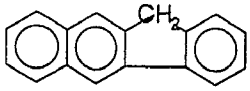
176 PAH 

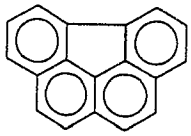
178-PAH 

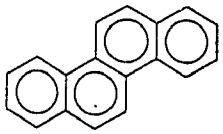
190-PAH 

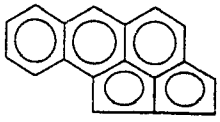
202-PAH 

214-PAH 

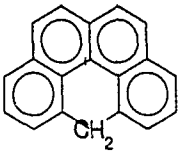
216-PAH 

226-PAH 

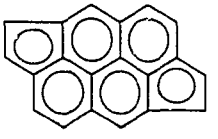
228-PAH 

238-PAH 

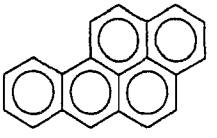
240-PAH



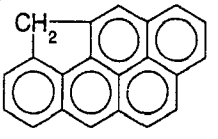
250-PAH



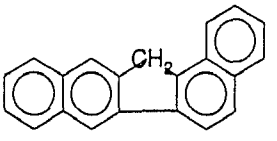
252-PAH



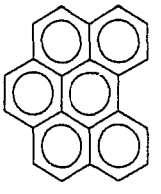
264-PAH



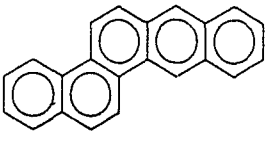
266-PAH



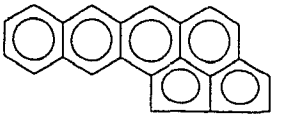
276-PAH



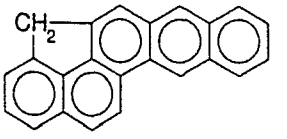
278-PAH



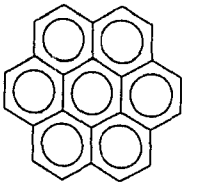
288-PAH



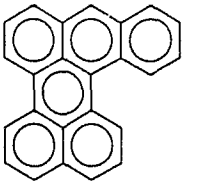
290-PAH



300-PAH



302-PAH



11. BIBLIOGRAPHY

1. B.P. Dunn, in "Handbook of Polycyclic Aromatic Hydrocarbons", A. Bjorseth Ed., Dekker, N.Y., 1983, 439.
2. M.L. Lee, M.V. Novotny and K.D. Bartle, "Analytical Chemistry of Polycyclic Aromatic Hydrocarbons", Academic Press, N.Y., 1981, Chap. 1-8.
3. J.C. Marr, Ph.D. Thesis, Chemistry Department, McMaster University, Hamilton, Ontario, 1989.
4. W. Schmidt, in "Polycyclic Aromatic Hydrocarbons and Astrophysics" A. Leger et al. eds., D. Reidel Publishing Company, 1987, 149.
5. L. Atwell, P. Hennings, J. Kozac, M. Morin and C. Oldreive, Environment Canada, Environmental Protection Service, Ambient air PAH study, Sydney, Nova-Scotia. Surveillance report EPS-5-AR-84-7, Atlantic region.
6. M. Nishioka, H.C. Chang and M.L. Lee, Environ. Sci. Technol., **20**, 1986, 1023.
7. J.M Flesher and S.R. Myers, Proc. 11th Intl. Symp. Polynucl. Arom. Hydrocarbons, Gaithersburg, MD, M. Cooke, K. Loening and J. Merritt, Eds., Battelle Press, Columbus, OH, 1987, 271.
8. R.L. Cerny and M.L. Gross, Proc. of the 39th ASMS conf. mass spectrom. allied topics, Nashville, Tennessee, May 19-24, 1991, 1265.
9. L.S. Ramos and P.G. Prohaska, J. Chromatogr., **211**, 1981, 294.
10. D.W. Later, M.L. Lee, K.D. Bartle, R.C. Kong and D.L. Vassilaros, Anal. Chem. **53**, 1981, 1612.
11. W. Giger and M. Blumer, Anal. Chem. **46**, 1974, 1663.
12. M.A. Quilliam and P.G. Sim, J. Chromatogr. Sci., **26**, 1988, 160.
13. P.G. Sim and C.M. Elson, Rapid Commun. Mass Spectrom. **2**, 1988, 137.
14. J.C. Fjelsted, R.C. Kong and M.L. Lee, J. Chromatogr. **279**, 1983, 449.
15. A.P. D'Silva and V.A. Fassel, Anal. Chem. **56**, 1984, 985A.
16. P.G. Sim, W.D. Jamieson and R.K. Boyd, Rapid Commun. Mass Spectrom. **1**, 1987, 28.
17. Sydney Tar Pond working group, Environment Canada, Sydney Tar Pond

remediation program, Draft terms for contract projects, 09/23/86.

18. Syncrude Canada Ltd., "Facts and Figures", Advertizing Brochure, 1989.
19. M. Blumer and W.W. Youngblood, Science, **188**, 1975, 53.
20. W.W. Youngblood and M. Blumer, Geochim. Cosmochim. Acta, **39**, 1975, 1303.
21. M. Blumer, Chem. Geol. **16**, 1975, 245.
22. J.W. Farrington and P.A. Meyer, Environ. Chem. **1**, 1975, 109.
23. G. Chatot, M. Castegnaro, J.L. Roche and R. Fontanges, Analytica Chemica Acta **53**, 1971, 259.
24. K.G. Teo and A.P. Watkinson, Fuel **66**, 1987, 1123.
25. J.W. Farrington, A.C. Davis, M.E. Tarafa, A.M. McCaffrey, J.K. Whelan and J.M. Hunt, Organic Geochemistry **13**, 1988, 303.
26. J.C. Lauer, D.H.V. Hernandez and D. Cagniant, Fuel **67**, 1988, 1273.
27. A. Wilhelm and K. Hedden, Fuel **65**, 1986, 1209.
28. S.B. Hawthorne, Anal. Chem. **62**, 1990, 633A.
29. Hewlett Packard, "The HP 7680A Supercritical Fluid Extractor", Advertizing Brochure, 1991.
30. F.S. Brown, M.J. Baedecker, A. Nissenbaum and I.R. Kaplan, Geochim. Cosmochim. Acta **36**, 1972, 1185.
31. D.A. Lane and S.W.D. Jenkins, Proc. 10th Intl. Symp. Polynucl. Arom. Hydrocarbons, M. Cooke and A.J. Dennis Eds., Battelle Press, Columbus, OH, 1988, 489.
32. J.C. Fetzer, Proc. 11th Intl. Symp. Polynuc. Arom. Hydrocarbons, Gaithersburg, MD, M. Cooke, K. Loening and J. Merritt, Eds., Battelle Press, Columbus, OH, 1987, 259.
33. L. Carbognani and A. Izquierdo, J. Chromatogr. **484**, 1989, 399.
34. P.R. Walter, A. Williams and K.D. Bartle, Fuel **68**, 1989, 520.
35. A. Izquierdo, L. Carbognani, V. Leon and A. Parisi, Fuel Sci. & Technol. Int. **7**, 1989, 561.
36. L. Carbognani and A. Izquierdo, Fuel Sci. Technol. Int. **8**, 1990, 1.

37. Z. Changming, L. Aiying, L. Yongsi and S. Zengmin, Am. Chem. Soc., Div. Pet. Chem. **34**, 1989, 240.

38. R.L. Melville, D.M. Trauth and K.K. Robinson, Am. Chem. Soc., Div. Pet. Chem. **34**, 1989, 635.

39. J.R. Woods, L.S. Kotlyar, D.S. Montgomery, B.D. Sparks and J.A. Ripmeester, Fuel Sci. Technol. Int. **8**, 1990, 149.

40. S. Coulombe and H. Sawatzky, Fuel **65**, 1986, 552.

41. M. Blumer, Anal. Chem. **29**, 1957, 1039.

42. W. Giger and C. Schaffner, Anal. Chem. **50**, 1978, 243.

43. J. Visentini, Honours Thesis, McMaster University, Chemistry Department, Hamilton, Ontario, May 1987.

44. W.D. McLeod Jr., D.W. Brown, R.G. Jenkins and L.S. Ramos, Fate Eff. Pet. Hydrocarbons Mar. Ecosyst. Org. Proc. Symp. 1976 (Pub. 1977), 385.

45. P.G. Sim, R.K. Boyd, R.M. Gershey, R. Guevremont, W.D. Jamieson, M.A. Quilliam and R.J. Gergely, Biomed. Environ. Mass Spectrom. **14**, 1987, 375.

46. F. Khorasheh, M.R. Gray and I.E.D. Lana, Fuel **66**, 1987, 505.

47. C.H. Giles and I.A. Easton, Adv. Chromatogr. **3**, 1966, 67.

48. A.A. Rosen and F.M. Middleton, Anal. Chem. **27**, 1955, 790.

49. S.A. Wise, B.A. Benner, S.N. Chesler, L.R. Hilpert, C.R. Vogt and W.E. May, Anal. Chem **58**, 1986, 3067.

50. R.L. Stedman, R.L. Muller, L. Lakpite and W.J. Chamberlain, Chem. Ind. (London), 1966, 394.

51. S.E. Hamilton, T.S. Bates and J.D. Cline, Environ. Sci. Technol. **18**, 1984, 72.

52. R.C. Lao, R.S. Thomas, M. Oja and L. Dubois, Anal. Chem. **45**, 1973, 908.

53. R. Riggin, P. Starp and S. Billets, Proc. 7th Intl. Symp. Polynucl. Arom. Hydrocarbons, M. Cooke and A.J. Dennis Eds., Battelle Press, Columbus, OH, 1983, 1021.

54. J.N. Gearing, P.J. Gearing, T.F. Lytle and J.S. Lytle, Anal. Chem. **50**, 1978, 1833.

55. H.J. Klimish, Frenesius Z. Anal. Chem. **264**, 1973, 275.

56. A.R. Jones, M.R. Guerin, and B.R. Clark, Anal. Chem. **49**, 1977, 1766.

57. M. Novotny, M.L. Lee and K.D. Bartle, J. Chromatogr. Sci. **12**, 1974, 606.

58. M.L. Lee, K.D. Bartle and M. Novotny, Anal. Chem. **47**, 1975, 540.

59. M.L. Lee, M. Novotny and K.D. Bartle, Anal. Chem. **48**, 1976, 405.

60. M.L. Lee, M. Novotny and K.D. Bartle, Anal. Chem. **48**, 1976, 1566.

61. G. Grimmer and H. Bohnke, Frenesius Z. Anal. Chem. **261**, 1972, 310.

62. D. Hoffman and E. Rathkamp, Anal. Chem. **44**, 1972, 899.

63. H. Perreault, L. Ramaley, P.G. Sim and F.M. Benoit, Rapid Commun. Mass Spectrom., **5**, 1991, 604.

64. E. Sawicki, J.E. Meeker and M.J. Morgan, Int. J. Air Water Pollut. **9**, 1965, 291.

65. E. Sawicki, S.P. McPherson, T.W. Stanley, J. Meeker and W.C. Elbert, Int. J. Air Water Pollut. **9**, 1965, 515.

66. B.S. Das and G.H. Thomas, Anal. Chem. **50**, 1978, 967.

67. T.D. Searl, F.J. Cassidy, W.H. King and R.A. Brown, Anal. Chem. **42**, 1970, 956.

68. D.E. Hirsh, R.L. Hopkins, H.J. Coleman, F.O. Cotton and C.J. Thompson, Anal. Chem. **44**, 1972, 915.

69. M. Nishioka, R.M. Campbell, M.L. Lee and R.N. Castle, Fuel **65**, 1986, 270.

70. M. Nishioka, M.L. Lee and R.N. Castle, Fuel **65**, 1986, 390.

71. J.T. Andersson, Anal. Chem. **59**, 1987, 2207.

72. J.W. Vogh and J.E. Dooley, Anal. Chem. **47**, 1975, 816.

73. R.R. Heath, J.H. Tumlinson, R.E. Doolittle and A.T. Proveaux, J. Chromatogr. Sci. **13**, 1975, 380.

74. W.F. Joyce and P.C. Uden, Anal. Chem. **55**, 1983, 540.

75. W.L. Orr, Anal. Chem. **39**, 1967, 1163.

76. W.L. Orr, Anal. Chem. **38**, 1966, 1558.

77. T. Kaimai and A. Matsunaga, Anal. Chem. **50**, 1978, 268.

78. M.L. Lee, C. Willey, R.N. Castle and C.M. White, Proc. 4th Intl. Symp. Polynucl. Arom. Hydrocarbons, A. Bjorseth and A.J. Dennis Eds., Battelle

Press, Columbus, OH, 1980, 59.

79. C. Willey, M. Iwao, R.N. Castle and M.L. Lee, Anal. Chem. **53**, 1981, 400.

80. M. Nishioka, J.S. Bradshaw, M.L. Lee, Y. Tominaga, M. Tedjamulia and R.N. Castle, Anal. Chem. **57**, 1985, 309.

81. K.V. Wood, R.G. Cooks, J.A. Laugal and R.A. Benkeser, Anal. Chem. **57**, 1985, 692.

82. L.R. Snyder, B.E. Buell, H.E. Howard, Anal. Chem. **40**, 1968, 1303.

83. J.F. McKay, J.H. Weber and D.R. Latham, Anal. Chem. **48**, 1976, 891.

84. M.E. Snook, R.F. Arrendale, H.C. Higman and O.T. Chortyk, Anal. Chem. **50**, 1978, 88.

85. P. Burchill, A.A. Herod and C.A. Mitchell, Chromatographia, **21**, 1986, 67.

86. T. Yamauchi and T. Handa, Envir. Sci. Technol. **21**, 1987, 1177.

87. K. Kamata and N. Motohashi, J. Chromatogr. **498**, 1990, 129.

88. M. Blumer, T. Dorsey and J. Sass, Science **195**, 1977, 283.

89. AOSTRA, Technical Handbook on Oil Sands, Bitumens and Heavy Oils, L.G. Hepler and C. Hsi Editors, Alberta Oil Sands Technology And Research Authority, 1989.

90. R.D. Carthy and A.H. Duthie, J. Lipid Research **3**, 1962, 117.

91. Z. Ramljak, A. Solc, P. Arpino, J.M. Schmitter and G. Guiochon, Anal. Chem. **49**, 1977, 1222.

92. M. Amat, P. Arpino, J. Orrit, A Latter and G. Guiochon, Analusis **8**, 1980, 179.

93. J. Schmitter, I. Ignatiadis, P. Arpino and G. Guiochon, Anal. Chem. **55**, 1983, 1685.

94. P.L. Desbene, D.C. Lambert, P. Richardin, J.J. Basselier, A.Y. Huc and R. Boulet, Anal. Chem. **56**, 1984, 313.

95. J.B. Green, J.W. Reynolds and S.K.T. Yu, Fuel Sci. Technol. Int. **7**, 1989, 1327.

96. C.D. Pearson and J.B. Green, Fuel **68**, 1989, 456.

97. J.B. Green, R.J. Hoff, P.W. Woodward and L.L. Stevens, Fuel **63**, 1984, 1290.

98. D.M. Jewell, J.H. Weber, J.W. Bunger, H. Plancher and D.R. Latham, Anal. Chem. **44**, 1972, 1391.

99. M.L. Selucky, Y. Chu, T.C.S. Ruo and O.P. Strausz, Fuel **56**, 1977, 369.

100. T.W. Allen, R.J. Hurtubise and H.F. Silvert, Fuel **66**, 1987, 1024.

101. A. Casalini, A. Mascherpa and C. Vecchi, Fuel Sci. Technol. Int. **8**, 1990, 427.

102. L.W. Corbett, Anal. Chem. **41**, 1969, 576.

103. M.A. Poirier and B.S. Das, Fuel **63**, 1984, 361.

104. D. Wallace, D. Henry, K. Pongar and D. Zimmerman, Fuel **66**, 1987, 44.

105. G.R. Peters, National Research Council of Canada, Atlantic Research Laboratory, Internal Report, Sept. 1986.

106. M.L. Lee, D.L. Vassilaros, C.M. White and M. Novotny, Anal. Chem. **51**, 1979, 768.

107. C.G. Blanco, J. Blanco, P. Bernard and M.D. Guillen, J. Chromatogr. **539**, 1991, 157.

108. Quadrex Corporation, Information Package Provided with High Temperature GC Columns, New Haven, Connecticut, 1990.

109. P.A. Peaden, M.L. Lee, Y. Hirata and M. Novotny, Anal. Chem. **52**, 1980, 2268.

110. J. Chmielowiec and H. Sawatzky, J. Chromatogr. Sci. **17**, 1979, 245.

111. K. Ogan, E. Katz and W. Slavin, Anal. Chem. **51**, 1979, 1315.

112. K. Ogan and E. Katz, J. Chromatogr. **188**, 1980, 115.

113. S.A. Wise, W.J. Bonnett and W.E. May, Proc. 4th Intl. Symp. Polynucl. Arom. Hydrocarbons, A. Bjorseth and A.J. Dennis Eds., Battelle Press, Columbus, OH, 1980, 791.

114. S.A. Wise, L.C. Sander, H.C.K. Chang, K.E. Markides and M.L. Lee, Chromatographia **25**, 1988, 473.

115. L.C. Sander and S.A. Wise, J. High Resol. Chromatogr./Chromatogr. Commun. **11**, 1988, 383.

116. J.C. Fetzer, in "Chemical Analysis of Polycyclic Aromatic Compounds", T. Vo-Dinh Ed., Chemical Analysis Series, **101**, Wiley Interscience, New York, 1988,

Chapter 3, 59.

117. J.C. Fetzer and W.R. Biggs, J. Chromatogr. **295**, 1984, 161.

118. J.C. Fetzer and W.R. Biggs, J. Chromatogr. **386**, 1987, 87.

119. A. Marcomini, B. Paroni, R. Donazzolo and A.A. Orio, Marine Chemistry **18**, 1986, 71.

120. A.M. Siouffi, M. Righozza and G. Guiochon, J. Chromatogr. **368**, 1986, 189.

121. E.P. Lankmayr and K. Muller, J. Chromatogr. **170**, 1979, 139.

122. W.A. Dark and R.R. McGough, J. Chromatogr. Sci. **16**, 1978, 610.

123. C. Bollet, J.C. Escalier, C. Souteyrand, M. Caude and R. Rosset, J. Chromatogr. **206**, 1981, 289.

124. J.F. Anacleto, personal communication.

125. K.D. Bartle, in "Supercritical Fluid Chromatography", R.M. Smith Ed., RSC Chromatography Monographs, 1988, 1.

126. J.C. Giddings, Sep. Sci. **1**, 1966, 73.

127. A.L. Billie and T. Greibrokk, J. Chromatogr. **349**, 1985, 317.

128. P.A. Peaden, J.C. Fjeldsted, M.L. Lee, S.R. Springston and M. Novotny, Anal. Chem. **54**, 1982, 1090.

129. J. Fuhr, L.R. Holloway and C. Reichert, Fuel Sci. Technol. Int. **7**, 1989, 643.

130. K. Grob and G. Grob, J. Chromatogr. **213**, 1981, 211.

131. D. Leyendecker, in "Supercritical Fluid Chromatography", R.M. Smith Ed., RSC Chromatography Monographs, 1988, 53.

132. J.C. Fjeldsted, W.P. Jackson, P.A. Peaden and M.L. Lee, J. Chromatogr. Sci. **21**, 1983, 222.

133. P.J. Shoenmakers, in "Supercritical Fluid Chromatography", R.M. Smith Ed., RSC Chromatography Monographs, 1988, 102.

134. C.M. White and R.K. Houck, Technical Bulletin no. 201, Suprex Corporation, Pittsburg, PA.

135. W.H. McFadden, H.L. Schwartz and S. Evans, J. Chromatogr. **122**, 1976, 389.

136. P.A. White and G.M. Wood, "Mass Spectrometric Applications in Science and Engineering", John Wiley and Sons, 1986, 772 p.

137. R.P.W. Scott, C.G. Scott, M. Munroe and J. Hess, J. Chromatogr. **99**, 1974, 395.

138. C.R. Blakely, J.J. Carmody and M.L. Vestal, J. Amer. Chem. Soc. **102**, 1980, 5931.

139. C.R. Blakely and M.L. Vestal, Anal. Chem. **55**, 1981, 750.

140. D. Zackett, G.J. Gallis and P.J. Savickas, Proc. 34th Annual ASMS Conf. Mass Spectrom. Allied Topics, Cincinnati, OH, 1986, 3.

141. R.C. Willoughby and R.F. Browner, Anal. Chem. **56**, 1984, 2623.

142. J.A. Apffel and R. Nordman, Proc. 36th Annual ASMS Conf. Mass Spectrom. Allied Topics, San Fransisco, CA, 1988, 1318.

143. M. Yamashita and J.B. Fenn, J. Phys. Chem. **88**, 1984, 4451.

144. A.P. Bruins, T.R. Covey and J.D. Henion, Anal. Chem. **59**, 1987, 2642.

145. Y. Ito, T. Takeuchi, D. Ishii and M. Goto, J. Chromatogr. **358**, 1976, 201.

146. R.M. Caprioli, T. Fan and J.S. Cottrell, Anal. Chem. **58**, 1986, 2949.

147. J.A. Apffel, U.A.T. Brinkmann and R.W. Frei, Anal. Chem. **55**, 1983, 2280.

148. E.D. Lee and J.D. Henion, J. Chrom. Sci. **23**, 1985, 253.

149. D.E. Games, A.J. Berry, I.C. Mylchreest, J.R. Perkins and S. Pleasance, in "Supercritical Fluid Chromatography", R.M. Smith Ed., RSC Chromatography Monographs, 1988, 159.

150. S.B. Hawthorne and D.J. Miller, J. Chromatogr. **468**, 1989, 115.

151. R.D. Smith, W.D. Felix, J.C. Fjeldsted and M.L. Lee, Anal. Chem. **54**, 1982, 1883.

152. R.D. Smith, J.C. Fjeldsted and M.L. Lee, J. Chromatogr. **249**, 1982, 231.

153. A.J. Berry, D.E. Games and J.R. Perkins, J. Chromatogr. **363**, 1986, 147.

154. J. Balsevich, L.R. Hogge, A.J. Berry, D.E. Games and I.C. Mylchreest, J. Nat. Prod. **51**, 1988, 1173.

155. C.M. Elson and P.G. Sim, Rapid Commun. Mass Spectrom. **4**, 1990, 37.

156. J.F. Anacleto, L. Ramaley, R.K. Boyd, S. Pleasance, M.A. Quilliam, P.G. Sim and F.M. Benoit, Rapid Commun. Mass Spectrom. **5**, 1991, 149.

157. A.G. Harrison, R.B. Mercer, E.J. Reiner, A.B. Young, R.K. Boyd, R.E. March

and C.J. Porter, Int. J. Mass Spectrom. Ion Processes **74**, 1986, 13.

158. H. Perreault, L. Ramaley, F.M. Benoit, P.G. Sim and R.K. Boyd, J. Phys. Chem. **95**, 1991, 4989.
159. M.J. Bertrand, Chemistry 6500, "Mass Spectrometry", Class Notes, University of Montreal, 1987.
160. M. Dong, I. Schmeltz, E. Lavoie and D. Hoffmann in "Hydrocarbons", Volume 3 of "Carcinogenesis, a Comprehensive Survey", P.W. Jones and R.I. Freudenthal Eds., Raven Press, New York, 1978, 97.
161. P.G. Sim, W.D. Jamieson and R.K. Boyd, Org. Mass Spectrom. **24**, 1989, 327.
162. T. Vo-Dinh, in "Chemical Analysis of Polycyclic Aromatic Hydrocarbons", T. Vo-Dinh Ed., Wiley-Interscience, New York, 1989, Chap. 1, 1.
163. R.G. Cooks, R.M. Caprioli, J.H. Beynon and G.R. Lester, "Metastable Ions", Elsevier, New York, 1973, 165-173.
164. T.W. Stanley, J.E. Meek and M.J. Morgan, Environ. Sci. Technol. **1**, 1967, 927.
165. S. Karunanithy, Personal Communication.
166. This Laboratory, Unpublished Results.
167. F. Benoit, Personal Communication.
168. L.R. Snyder, Anal. Chem. **41**, 1969, 314.
169. B.D. Tilak, Tetrahedron **9**, 1960, 76.
170. D.L. Vassilaros, R.C. Kong, D.W. Later and M.L. Lee, J. Chromatogr. **252**, 1982, 1.
171. T.L. Chester and J.D. Pinkston, Anal. Chem. **62**, 1990, 394R.
172. National Research Council of Canada, Atlantic Research Laboratory, Internal Report on Third Order Chromatography, 1988.
173. M.A. Quilliam, P.G. Sim, J.C. Marr, C.M. Elson and H.Perreault, Presented at the 5th Montreux Symposium on LC/MS, Friburg, FRG, 1988.
174. P.G. Sim, R.K. Boyd, W.D. Jamieson, Proc. 11th Intl. Symp. Polynucl. Arom. Hydrocarbons, Gaithersburg, MD, M. Cooke, K. Loening and J. Merritt Eds., Battelle Press, Columbus, OH, 1987, 847.
175. S.R. Lipsky and M.L. Duffy, J. High Resoln.

Chromatogr./Chromatogr. Commun. 9, 1986, 376.

176. A. Bemgard, B.O. Lundmark and A. Colmsjo, Proc. 13th Intl. Symp. Polyn. Arom. Hydrocarbons, Bordeaux, 1991, in press.

177. C.R. Yonker, B.W. Right, P.C. Petersen and R.D. Smith, J. Chem. Phys. **89**, 1985, 5526.

178. F.M. Lancas, E. Carrilho, G.H.N. Deane and M.C.F. Camilo, J. High Resoln. Chromatogr./Chromatogr. Commun. **12**, 1989, 368.

179. J.C. Lauer, D.H. Valles Hernandez and D. Cagniant, Fuel **67**, 1988, 1446.

180. M.A. Quilliam and S.A. Wise, Unpublished Results.

181. M.A. McLean and C.S. Hsu, Proc. 38th ASMS Conf. Mass Spectrom. Allied Topics, Tucson, AZ, 1990, 605.

182. G.P. Blumer and M. Zander, Z. Anal. Chem. **288**, 1977, 277.

183. M.L. Lee and R.A. Hites, Anal. Chem. **48**, 1976, 1890.

184. J.C. Fetzer and W.R. Biggs, J. Chromatogr. **322**, 1985, 275.

185. S.A. Wise, M.J. Bonnett, F.R. Guenther and W.E. May, J. Chromatog. Sci. **19**, 1981, 457.

186. W.E. May, J.M. Brown, S.N. Chester, F.R. Guenther, L.R. Hilpert, H.S. Hertz and S.A. Wise, NBS Special Publication no. 519, Proc. 5th Material Res. Symp., Gaithersburg, MD, 1978.

187. S.A. Wise, W.J. Bonnett and W.E. May in "Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects", A. Bjorseth and A.J. Dennis Eds., Battelle Press, Columbus, OH, 1980, 791.

188. M.A. Quilliam, "PAC Atlas", McMaster University, Hamilton, Ontario.

189. E.R. Barnhart, D.L. Ashley, V.V. Reddy and D.G. Patterson Jr., J. High Resoln. Chromatogr./Chromatogr. Commun. **9**, 1986, 528.

190. E.R. Barnhart, D.G. Patterson Jr, N. Tanaka and M. Araki, J. Chromatogr. **445**, 1988, 145.

191. CRC Handbook of Chemistry and Physics, R.C. Weast Ed., 64th edition, CRC Press, Boca Raton, FL, 1983-1984.

192. S.M. Fields and M.L. Lee, J. Chromatogr. **349**, 1985, 305.

193. C.R. Yonker, R.W. Gale and R.D. Smith, J. Chromatogr. **389**, 1987, 433.

194. J.M. Curtis, K. Vekey, A.G. Brenton and J.H. Beynon, Org. Mass Spectrom. **22**, 1987, 289.

195. F.W. McLafferty and M.M. Bursey, Chem. Commun. 1967, 533.

196. M.R. Robinson, D.E. Bostwick, L.E. Abbey and T.F. Moran, Rapid Commun. Mass Spectrom., **2**, 1988, 210.

197. M.G. Ondrus and T.R. Steinheimer, J. Chromatogr. Sci. **28**, 1990, 324.

199. H.M. Rosenstock, K. Draxl, B.W. Steiner and J.T. Herron, J. Phys. Chem. Ref. Data Suppl., **6**, 1977.

200. S.L. Lias, J.E. Bartmess, J.F. Liebman, J.L. Holmes, R.D. Levin and W.G. Mallard, J. Phys. Chem. Ref. Data Suppl. **17**, 1988.

201. M. Rabrenovic, C.J. Proctor, T. Ast, C.G. Herbert, A.G. Brenton and J.H. Beynon, J. Phys. Chem. **87**, 1983, 3305.

202. K. Krogh-Jesperson, P.v.R. Schleyer, J.A. Pople and D.J. Chemer, J. Am. Chem. Soc. **100**, 1978, 4301; **103**, 1981, 2589.

203. J. Chandrasekhar, P.v.R. Schleyer and K. Krogh-Jespersen, J. Comput. Chem. **2**, 1981, 356.

204. S.J. Pachuta, H.I. Kenttamas, T.M. Sack, R.L. Cerny, K.B. Tomer, M.L. Gross, R.R. Pachuta and R.G. Cooks, J. Am. Chem. Soc. **110**, 1988, 657.