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Hydrocarbon speciation studies in ancient sediments by high temperature supercritical carbon dioxide extraction

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

**Hydrocarbon Speciation Studies in Ancient Sediments by High Temperature Supercritical
Carbon Dioxide Extraction**

**A thesis submitted in partial satisfaction of the
requirements for the degree of**

MASTER OF SCIENCE

IN

CHEMISTRY

by

Doraida Diaz

1996

To: _____ Dean: Arthur W. Herriott

College of Arts & Sciences

This thesis, written by Doraida Diaz, and entitled Hydrocarbon Speciation Studies in Ancient Sediments by High Temperature Supercritical Carbon Dioxide Extraction, having been approved in respect to style and intellectual content, is referred to you for judgement.

We have read this thesis and recommend that it be approved.

Rudolf Jaffe

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Date of Defense: July 29, 1996

The thesis of Doraida Diaz is approved.

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Florida International University, 1996

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To my parents, husband and son

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TABLE OF CONTENTS

	Page
ABSTRACT OF THE THESIS.....	ix
LIST OF FIGURES.....	xi
LIST OF TABLES.....	xvi
1. INTRODUCTION.....	1
1.1 Speciation of hydrophobic organic compounds in sediments.....	3
1.2 Supercritical Fluid Extraction (SFE).....	7
1.3 SFE of hydrocarbons from geological samples.....	11
1.4 Rock-Eval Pyrolysis.....	14
1.5 Pyrolysis-GC/MS.....	17
1.6 Hydrocarbon composition in shales. Biomarkers.....	19
1.7 Geosynthesis of alkylaromatics in sediments.....	27
2. EXPERIMENTAL	
2.1 Sample Preparation.....	29
2.2 Other materials.....	29
2.3 Instrumentation	
2.3.1 Supercritical Fluid Extractor.....	29
2.3.2 Rock-Eval Pyrolysis.....	31
2.3.3 Pyrolysis-GC/MS.....	32
2.4 Analysis	
2.4.1 GC/MS.....	33

2.5 Soxhlet Extraction.....	35
2.6 Supercritical Fluid Extraction	
2.6.1 Stepwise SFE.....	36
2.6.2 Temperature dependent SFE of individual samples.....	37
2.7 Heating experiments	
2.7.1 Thermal treatment of non-extracted sediments.....	37
2.7.2 Thermal treatment of pre-extracted sediments.....	38
2.8 Preliminary evaluation of alkylphenanthrene geosynthesis during HT-SFE.....	38
2.9 Rock-Eval Pyrolysis.....	39
2.10 Pyrolysis-GC/MS.....	39
2.11 Microscopy Study.....	40
 3. RESULTS AND DISCUSSION	
3.1 Identification of target analytes.....	41
3.2 Soxhlet extraction.....	41
3.3 SFE	
3.3.1 Stepwise SFE.....	50
3.3.1.1 Regular hydrocarbons.....	50
3.3.1.2 Biomarkers.....	55
3.3.2 Direct temperature effect on SFE extraction efficiency.....	62
3.3.1.1 Regular hydrocarbons.....	62
3.3.1.2 Biomarkers.....	65

3.3.3 Thermal treatment of non-extracted and pre-extracted sediment samples.....	69
3.3.4 Preliminary evaluation of methylphenanthrene geosynthesis during HT-SFE.....	73
3.3.5 Rock-Eval Pyrolysis of Posidonia Shale. Comparison with Kupferschiefer Shale.....	74
3.3.6 Pyrolysis-GC/MS.....	79
3.3.7 Microscopy Study.....	85
4. CONCLUSIONS.....	87
5. RECOMMENDATIONS.....	89
REFERENCES.....	90

ABSTRACT OF THE THESIS

HYDROCARBON SPECIATION STUDIES IN ANCIENT SEDIMENTS BY HIGH TEMPERATURE SUPERCRITICAL CARBON DIOXIDE EXTRACTION.

By

Doraida Diaz

Florida International University, 1996

Miami, Florida

Professor Rudolf Jaffé, Major Professor

Professor Kenneth G. Furton, Co-Major Professor

Results on the application of high-temperature supercritical carbon dioxide extraction (HT-SFE) to the study of hydrocarbon (HC) speciation in geological samples are presented. Ancient sediments were treated by stepwise extractions, and by using a fresh sample each time. SFE temperatures ranged between 50 and 350°C. Individual analytes showed to be speciated in different ways throughout the solid matrix because different fractions are extracted under different energy conditions. Aromatic HCs appear to have a stronger association with the matrix than the aliphatics due to their higher polarity and molecular planarity. No evidence of geosynthesis of alkylaromatics during HT-SFE was obtained. Tri- and mono-aromatic steranes were totally extracted at relatively

low temperatures while hopanes and steranes show a small fraction strongly interacting with the macromolecular organic structure possibly due to “trapping“ processes. The release of “trapped” HCs was confirmed by the “maturity inversion” effect observed for the molecular distribution of hopanes at the highest extraction temperatures. Branched compounds like pristane and phytane showed to have a weaker association with the organic matrix if compared with straight chain analytes. Fresh sample extracted by SFE at different temperatures confirmed the results obtained for the stepwise procedure. Rock-Eval Pyrolysis and Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) from SFE pre-extracted sediments showed that HCs extracted at the highest temperature levels are not dominated by pyrolysis products and may have been released from trapped conditions, through irreversible thermally induced structural changes of the matrix and/or rearrangements of the macromolecular organic matter possibly via sulfur bond cleavage. No significant changes of the matrix were apparent after HT-SFE as suggested by Rock-Eval Pyrolysis and Py-GC/MS experiments, as well as from Electron Scanning Microscopy (ESM) determinations.

List of Figures

	Page
1. Hypothetical Speciation of HOCs in Sediments and Soils.....	5
2. Proposed Structure of Sedimentary Organic Matter with Trapped HOCs.....	5
3. Phase Diagram for Carbon Dioxide.	8
4. Hildebrand Solubility, Density and Pressure of Supercritical CO ₂ at Different Temperatures.....	8
5. Rock-Eval Pyrolysis Technique.....	15
6. Composition of Disseminated Organic Matter in Sedimentary Rocks.....	21
7. Major Compounds in the Hydrocarbon Fraction of Oil Shales.....	21
8. Molecular Structures and Numbering Systems for Steranes and Hopanes.....	23
9. Origin of Hopanes in Sediments and Configurational Isomerization Processes in Hopanes During Diagenesis and Catagenesis.....	23
10. Typical Mass Chromatogram for m/z 191 of the Aliphatic Fraction of a Shale Extract.....	25
11. Aromatization of C-ring Monoaromatic Steranes.....	25
12. Schematic Diagram of High Temperature SFE System.....	30
13. GC/MS Pattern for n-alkanes (m/z=57) in Posidonia Shale SFE Extracts.....	42
14. GC/MS Pattern for Hopanes (m/z=191) in Posidonia Shale SFE Extracts.....	42

15. GC/MS Pattern for Naphthalene (m/z=128), C1-Naphthalenes (m/z=142), C2-Naphthalenes (m/z=156) and C3-Naphthalenes (m/z=170) in Posidonia Shale SFE Extracts.....	43
16. GC/MS Pattern for Phenanthrene (m/z=178), C1-Phenanthrenes (m/z=192), C2-Phenanthrenes (m/z=206) and C3-Phenanthrenes (m/z=220) in Posidonia Shale SFE Extracts.....	44
17. GC/MS Pattern for Dibenzothiophene (m/z=184), C1-Dibenzothiophenes (m/z=198), C2 - Dibenzothiophenes (m/z = 212) and C3- Dibenzothiophenes (m/z = 226) in Posidonia Shale Extracts.....	45
18. GC/MS Pattern for Steranes (m/z=217), Monoaromatic (m/z=231) and Triaromatic Steranes in Posidonia Shale SFE Extracts.....	46
19. N-alkanes Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	51
20. Naphthalene and Alkylnaphthalene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	51
21. Phenanthrene and Alkylphenanthrene/anthracene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	52
22. Dibenzothiophene and Alkyldibenzothiophene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	52
23. Anthracene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	54

24. GC/MS Chromatograms for Methylphenanthrene/anthracenes at Different Temperatures during Stepwise SFE of Posidonia Shale.....	54
25. Hopane Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	56
26. Sterane Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	56
27. (S/S+R) C-31 Hopane Ratio versus Temperature during Stepwise SFE of Posidonia Shale.....	56
28. GC/MS Chromatograms for C-31 α β Homohopanes (S and R) at Different Temperatures during Stepwise SFE of Posidonia Shale.....	58
29. Mono and Triaromatic Sterane Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.....	59
30. TA/(TA+MA) Ratio versus SFE Temperature during Stepwise SFE of Posidonia Shale.....	59
31. Prist/Phyt and C18/Phyt Ratios versus SFE Temperature during Stepwise SFE of Posidonia and Kupferschiefer Shales.....	61
32. GC/MS Chromatograms (m/z=57) Showing Relatives Abundances of Pristane, Phytane and C18 at Different Temperatures during Stepwise SFE of Posidonia Shale.....	63
33. Reproducibility Results for Stepwise SFE of Posidonia Shale.....	64
34. N-alkane SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Time.....	66

35. Naphthalene and Alkylnaphthalene SFE Extraction Recoveries Obtained Using a Fresh Sample at EachTemperature.....	66
36. Phenanthrene and Alkylphenanthrene SFE Extraction Recoveries Obtained Using a Fresh Sample at EachTemperature.....	67
37. Dibenzothiophene and Alkyldibenzothiophene SFE Extraction Recoveries Obtained Using a Fresh Sample at EachTemperature.....	67
38. Anthracene SFE Extraction Recoveries Obtained Using a Fresh Sample at EachTemperature.....	67
39. Hopane SFE Extraction Recoveries Obtained Using a Fresh Sample at EachTemperature.....	68
40. Sterane SFE Extraction Recoveries Obtained Using a Fresh Sample at EachTemperature.....	68
41. Mono and Triaromatic Sterane SFE Extraction Recoveries Obtained Using a Fresh Sample at EachTemperature.....	68
42. Reproducibility Results for SFE of Posidonia Shale Using a Fresh Sample at Each Temperature.....	70
43. Effect of Previous Thermal Treatment at 200 ^o C and 350 ^o C of Non - Extracted Posidonia Shale Samples during 0, 3, and 12 h on the 50 ^o C SFE Recovery.....	71
44. Effect of a Thermal Treatment on Some Lipid 50 ^o C SFE Recoveries Using a Pre-extracted Posidonia Shale Sample.....	72
45. Rock-Eval Pyrolysis Results for Posidonia and Kupferschiefer Shales.....	76

46. Posidonia and Kupferschiefer Shale Kerogen Type Determination from Rock-Eval Pyrolysis Data of Both the original and the SFE Extracted Sediment Samples.....	78
47. Py-GC/MS at 358 °C of Posidonia Shale for the Original and 350 °C SFE Extracted Sample.....	80
48. Py-GC/MS at 610 °C Following Py-GC/MS at 358 °C of Posidonia Shale for Original, 50 °C HT-SFE and 350 °C HT-SFE Samples.....	81
49. GC/MS Chromatograms for Phenanthrene/Anthracene (m/z = 178) and Methylphenanthrene/Anthracenes (m/z=192) obtained from 358 °C Py-GC/MS of Non-extracted and SFE Extracted Posidonia Shale Samples.....	83
50. GC/MS Chromatograms for Phenanthrene/Anthracene (m/z=178) and Methylphenanthrene/Anthracenes (m/z=192) Obtained from 610 °C Py-GC/MS of SFE Extracted Posidonia Shale Samples.....	84
51. Electron Scanning Microscope Photographs (1000 X) Obtained for the Original Posidonia Shale and for the Sediments Extracted at 150 °C and 350 °C in the SFE.....	86

List of Tables

	Page
I. Critical Properties of Common Supercritical Fluids.....	10
II. Peaks Identified for m/z=191 (Hopanes) Mass Chromatograms of Posidonia Shale SFE Extracts.....	47
III. Peaks Identified for m/z=217 (Steranes) Mass Chromatograms of Posidonia Shale SFE Extracts.....	47
IV. Peaks Identified for m/z=253 (Monoaromatic Steranes) Mass Chromatograms of Posidonia Shale SFE Extracts.....	48
V. Peaks Identified for m/z=231 (Triaromatic Steranes) Mass Chromatograms of Posidonia Shale SFE Extracts.....	48
VI. Analyte Recovery from Soxhlet Extraction of Posidonia Shale.....	49
VII. Reproducibility Results for Soxhlet Extraction of posidonia Shale.....	49
VIII. Increment Percentage Rates in Some Hcs Recoveries (related to 250 °C SFE) for 350 °C SFE and 50 °C SFE of Thermally Treated (350 °C, 1 h) Posidonia Shale Samples.....	72
IX. Rock-Eval Pyrolysis Data for Posidonia and Kupferschiefer Shale Extracted Sediments (Fresh Sample Each Time).....	75

1.INTRODUCTION

The extraction recovery of organic species from soils and sediments are critical in the identification and quantification of environmentally important compounds. Supercritical fluid extraction (SFE) has been generally accepted as an alternative to conventional solvent extraction methods, like Soxhlet extraction and sonication, for the recovery of a wide variety of compounds, including aliphatic and aromatic HCs from environmental matrices (Hawthorne, 1990; Furton, et al., 1994).

The SFE of analytes from solid matrices depends on several factors including the vapor pressure of the compounds, the solubility and the diffusion coefficient of the analytes in the supercritical fluid. However, for environmental solids, such as soils and sediments, the limiting factor is generally how analytes are associated with the sample matrix. The speciation of hydrophobic organic compounds (HOCs) within geological samples is based in their association with the organic matrix through adsorption, partitioning, "sieving", binding and "trapping" processes. Some of these solute-solid interactions result in the incorporation of the solute into the solvent non-extractable fraction. The unique capacity of supercritical fluids to penetrate into pores of solid matrices permits the differentiation between traditional solvent extractable or "free" HOCs and those "trapped" in, or strongly associated with, the organic matrix of the sample (Hawthorne, et al., 1993; Furton, et al., 1993). The application of high temperature in the procedure (HT-SFE) has proven to facilitate release of such compounds due to macromolecular structural rearrangements of the

matrix and increased desorption kinetics (Furton, et al., 1994).

This thesis deals with the application of HT-SFE, using carbon dioxide as a supercritical fluid, in order to study the speciation of some lipid components in Posidonia Shale by gradually applying increasing temperatures (stepwise SFE) and using the GC/MS for the analysis of the extracts. In addition, comparison with Soxhlet (using methylene chloride) recoveries was made.

To evaluate the SFE potential as an extraction technique it is important not only to chemically characterize the resultant extracts but to analyze the extracted samples to gain a better understanding on SFE effects on the organic matrix, specially in those cases where high temperatures were applied. Rock-Eval Pyrolysis and Pyrolysis-Gas Chromatography / Mass spectrometry (Py-GC/MS) are useful tools for this purpose because both methods have become widely used to obtain structural information on source rock organic matter as well as other reliable data of geological interest (Killops and Killops, 1993).

Rock-Eval data allows us to quantitatively evaluate the remaining “extractable” hydrocarbon fraction as well as the generation potential upon pyrolysis of previously extracted sediments, while Py-GC/MS provides additional information for the description of a kerogen sample in terms of the quantitative distribution of various molecular types in the kerogen macromolecule (Larter and Horsfield, 1993).

Therefore, a comparison of Rock-Eval Pyrolysis and Py-GC/MS data obtained for ancient sediments extracted using different temperatures (fresh sample each time SFE) was carried out to evaluate HT-SFE effects on a specific matrix at a given temperature level. A microscopy study of the extracted solid structures was also performed to observe possible physical or structural changes in the SFE extracted matrices. Additionally, some experiments were conducted to know whether or not the structural macromolecular changes induced in the matrix during HT-SFE had a reversible nature. A preliminary evaluation of the possibility of alkylaromatic hydrocarbon geosynthesis during this procedure was included in this study.

1.1 Speciation of hydrophobic organic compounds in sediments.

The speciation of lipids in recent and ancient sediments has been studied by a great number of investigators and, in general terms, has been classified into two categories as “free” and “bound” lipids. The former can be isolated via traditional solvent extractions, whilst the latter require more severe physical/chemical treatments for their release.

The interaction of HOCs with the organic fraction of sediments and soils has been widely accepted (Jaffé, 1991) and, although it has been suggested that this interaction is controlled by a partitioning process (Chiou, 1979,1985; Kyle, 1981 ; Karickhoff, 1979) it is also likely that some speciation changes take place after the partitioning step. Diagenetic processes may play an important role in controlling this association.

Partitioning processes, where the molecules can associate with the “exposed “ lipophilic surfaces , with humic micelles or “membrane-like” structures, may be the initial pathway of incorporation of these compounds. However, during early stages of diagenesis the organic matter undergoes a variety of chemical and physical changes primarily induced by microbial activity, characterized by biodegradation, polycondensation and insolubilization of natural compounds such as biopolymers, aminoacids, carbohydrates, lignins and lipids, which are then transformed into geopolymers such as humic and fulvic acids, humin and protokerogen (Tissot and Welte, 1986). During these microbially mediated processes, HOCs, which had previously partitioned into (and adsorbed on) the organic matter of the sediment, may become trapped in internal voids of the polymeric molecular sieve-type structures (Wershaw,1986) and/or within plugged or deep pores, becoming “non extractable” by conventional analytical methods therefore, turning “free” HOCs into “trapped” HOCs (Khan, 1982; Monthieux and Londain, 1987; Del Rio et al., 1989; Jaffé and Gardinali, 1990; Jaffé et al., 1992).

Figures 1 and 2 show the hypothetical speciation of HOCs in sediments and soils, and a proposed structure of sedimentary organic matter with “trapped” compounds (e.g. naphthalene), respectively.

Many compounds incorporated into the “bound” fraction can only be released by saponification (Cranwell et al., 1987; Albaijes et al., 1984), other chemical degradation processes (Trifilieff et al.,1991; Al-Lihaibi and Wolff, 1991) or by thermal methods (Khan,

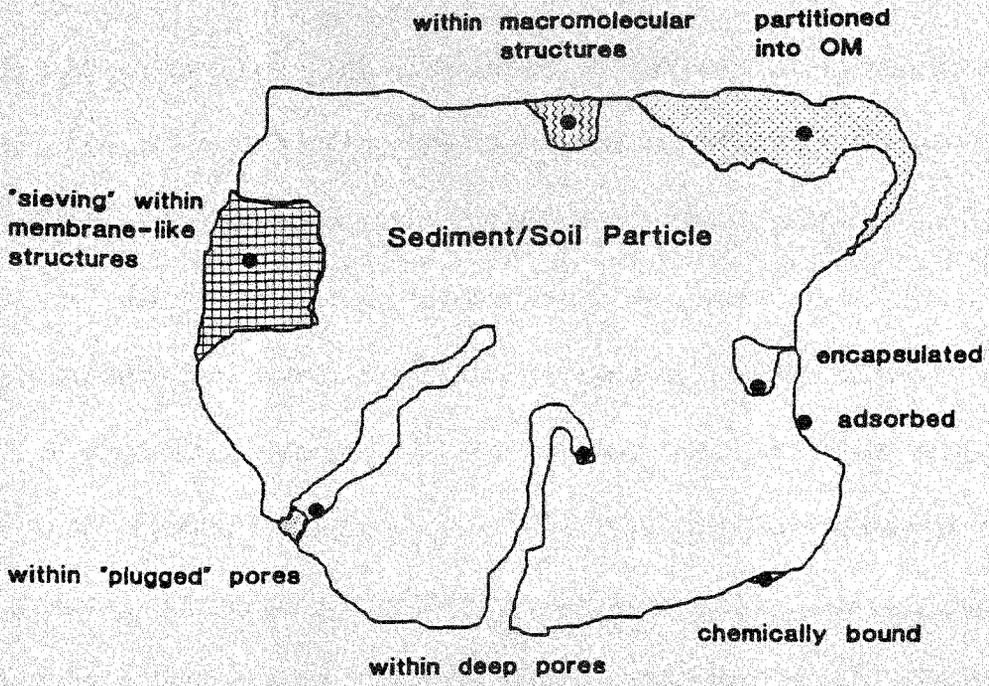


Fig.1. Hypothetical Speciation of HOCs in Sediments and Soils

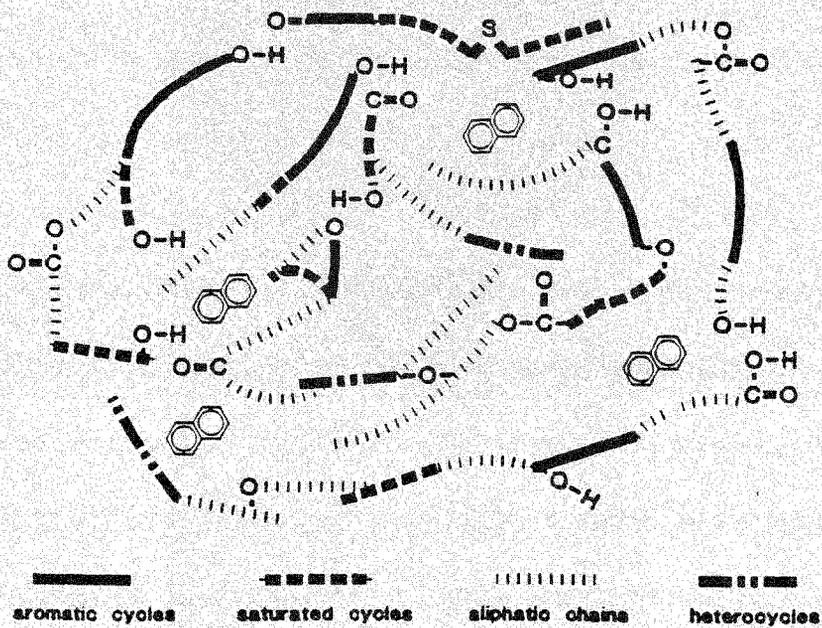


Fig.2. Proposed Structure of Sedimentary Organic Matter with Trapped HOCs (Naphthalene).

1982). Jaffé and coworkers (Jaffé and Gardinali, 1990) observed in ancient sediments the natural release of “trapped” molecules from the kerogen due to thermal rearrangement of the geopolymers, while bound compounds were released via thermal bond cleavage. Since the trapped material showed molecular distributions characteristic of organic matter of low thermal maturity, the authors suggested that the “trapping” of HOCs must occur at early stages of diagenesis. Apparently, the “trapped” and bound HOCs are exempt from biodegradation and thermal maturation due to the “protective effect” of the OM in which they are contained. Consequently, the molecular distribution of HOCs corresponding to the “free” vs. bound fractions can be different. Studies concerning the interaction of hydrophobic organics with humic substances have appeared in the literature (Khan and Schnitzer, 1992; Del Rio et al., 1989; Grimalt and Saiz-Jimenez, 1989) and the environmental significance of this interaction with respect to the fate and transport of organic pollutants has been stressed.

The SFE is a suitable experimental approach in the study of the environmental/geochemical processes involved in the “trapping” of hydrophobic organic molecules within the organic matrix of sediments due to the increased penetration of the solvent into the porous medium compared with that of the traditional extraction methods, especially when high temperatures are applied inducing structural rearrangements in the macromolecular organic matter that increase the availability of the solutes of interest to the extracting solvent. Preliminary studies made with shales (Furton et al., 1994 ; Jaffé et al., 1995) showed a promising potential for HT-SFE as an analytical tool in the investigation of the speciation of lipid

components in geological and environmental samples with a significant improvement in recoveries for certain compounds, precision and analysis time while using inexpensive, non-toxic CO₂.

1.2 Supercritical Fluid Extraction (SFE).

A substance that is above its critical temperature and pressure is defined as a supercritical fluid. The combined gas-like mass transfer and liquid-like solvating characteristics of supercritical fluids have led to their use as mobile phase for supercritical fluid chromatography (SFC) and as solvents in analytical scale SFE. Although the high solvating power of supercritical fluids was noticed more than a century ago (Hannay and Hogarth, 1879) it is not until recent decades that supercritical fluid extraction has been used as a practical separation technique (Klesper and Angew, 1979; Sie and Rijnders, 1967). SFE was eventually used as an alternative to distillation and solvent extraction in industry (Johnston and Penninger, 1989; Mc Hugh and Krukonis, 1986) but it was not applied in Chemistry on an analytical scale until recent years (Brigh and Mc Nally, 1992; Hawthorne, 1990).

Fig 3 shows the phase diagram for CO₂. T is called triple point indicating that three stages (solid, gas and liquid) of a single substance co-exist under the conditions of that particular pressure and temperature and C is termed critical point what means that above the pressure and temperature shown on C, there is a unique state-supercritical fluid.

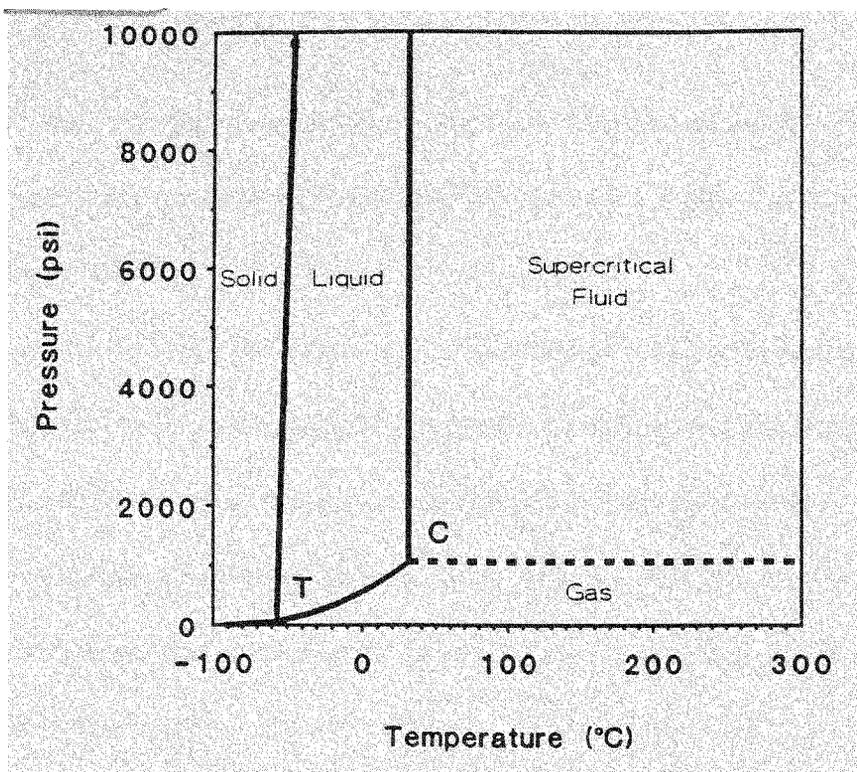


Fig. 3. Phase Diagram for Carbon Dioxide. T: Triple Point , C: Critical Point, C: Critical Point (Gong, 1995).

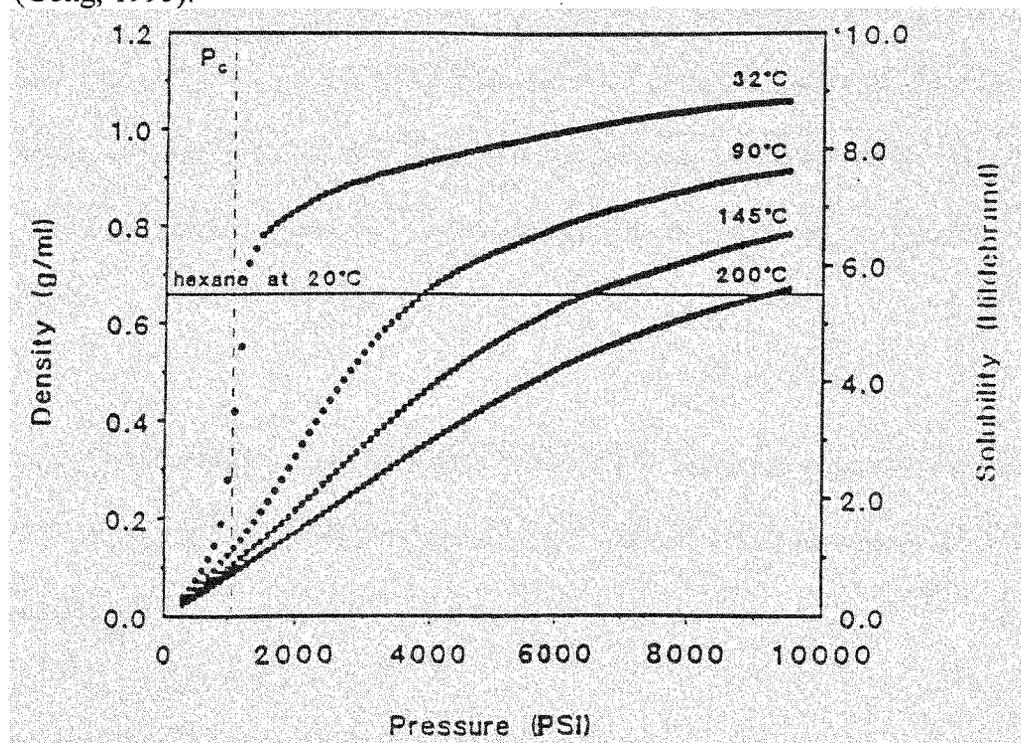


Fig.4. Hildebrand Solubility, Density and Pressure of Supercritical CO₂ at different temperatures. Comparison to hexane at 20 °C .

Reference from Lecture Notes of Advanced Chromatography (CHM 5156, Fall 1994, Department of Chemistry at FIU by Dr. Kenneth G. Furton).

A comparison of several characteristics of supercritical fluids with those of liquid solvents demonstrates the potential of SFE to approach the idealized goals for analytical extraction:

-The rate at which an extraction can be performed are ultimately determined by mass transfer limitations. Supercritical fluids have much better mass transfer characteristics than liquid solvents due to solute diffusivities an order of magnitude higher (10^{-4} vs. 10^{-5} cm^2/s) and viscosities an order of magnitude lower (10^{-4} vs. 10^{-3} $\text{N.s} / \text{m}^2$). While liquid solvent extractions take from several hours to days, a quantitative SFE are generally completed in 10-60 min.

-The solvent strength of a liquid is essentially constant regardless of extraction conditions but the solvent strength of a supercritical fluid depends on the pressure and temperature used in the procedure so it can be easily controlled. In Fig 4 the density, pressure and Hildebrand solubility of supercritical CO_2 at various temperatures is shown as well as a comparison to hexane at 20°C .

-Many supercritical fluids are gases at ambient conditions and the concentration steps needed for liquid solvent extracts are greatly simplified after SFE, saving time and avoiding the loss of more volatile analytes.

Other practical advantages of supercritical fluids is that most are inert, pure, nontoxic and inexpensive. On the other hand, as fluids such as CO_2 and N_2O have relatively low critical

temperatures (31°C and 36°C, respectively), SFE can be performed at low temperatures to extract thermally unstable compounds. Table 1 shows some physical properties of common supercritical fluids.

Table I- Critical Properties of Common Supercritical Fluids (Poole and Poole, 1991).

Fluid	(Name)	Tc (°C)	Pc (atm)	Dc (g/ml)
CO ₂	(Carbon Dioxide)	31.3	72.9	0.47
N ₂ O	(Nitrous Oxide)	36.5	72.5	0.45
SF ₆	(Sulfur Hexafluoride)	45.5	37.1	0.74
NH ₃	(Ammonia)	132.5	112.5	0.24
Xe	(Xenon)	16.6	58.4	1.10
n-C ₄	(Butane)	152.0	37.5	0.23
n-C ₅	(Pentane)	196.6	33.3	0.23
Cl ₂ F ₂	(Dichlorodifluoromethane)	111.8	40.7	0.56
H ₂ O	(Water)	374.4	226.8	0.34
Ch ₃ OH	(Methanol)	240.5	78.9	0.27

Recent concerns about the hazardous nature of many commonly used solvents, the cost of environmental dangers of waste solvent disposal and their emission into the atmosphere during sample concentration further support the development of alternative sample extraction methods such as SFE.

Supercritical CO₂ has been the choice for most SFE studies, primarily because of its attractive practical characteristics. In general terms, CO₂ is an excellent extraction medium for nonpolar species such as alkanes and terpanes, it is reasonably good for moderately polar species, including polycyclic aromatic HCs (PAHs), aldehydes, esters, etc., but is less useful for more polar compounds. Because of the practical difficulties in using polar fluids as ammonia for SFE, extractions of highly polar analytes have most often been done using CO₂

containing a few percent added organic modifier (Hawthorne, 1990; Taylor and Thomas, 1994).

1.3 SFE of HCs from geological samples. HT-SFE.

The development of novel analytical methods for the analysis of non-extractable organics from environmental samples will significantly improve pollution assessment, environmental fate and biodegradation studies. “Trapping” may be an important environmental pathway for HOCs, reducing their toxicity and bioavailability and may be a major sink for anthropogenic compounds in sedimentary and soil environments.

Any shallow rock that yield commercial amounts of oil upon pyrolysis can be classed as an oil shale. The organic matter in most shales appears to derive from marine or freshwater phytoplankton deposited in lakes, shallow seas, bogs or lagoons. For the generation of these shales or kerogenous rocks, the existence of anaerobic conditions for the preservation of the dead organisms in the original sedimentary environment is a requisite.

In the present study, ancient sediment (shale) samples containing only naturally occurring HOCs will be studied because by looking at specific compounds one may get a better insight into the geochemical process of incorporation of HOCs as trapped components in sediments (Trifilieff et al., 1992; Jaffé and Gardinali, 1990). The process itself should be similar for anthropogenic and biogenic compounds with similar physical-chemical characteristics (e.g.

octanol-water partition coefficient K_{ow} , and molecular planarity/shape).

The use of supercritical fluids for hydrocarbon extraction from source rocks or coals was originally studied from two different points of view. On one hand, extraction with supercritical fluids at a high temperature was used by Whitehead and Williams (1975), Kershaw (1977), Smith and Udseth (1983) with solvents such as toluene or pentane but, under these severe conditions, a partial breakdown of the organic matter may occur. On the other hand, extraction with supercritical fluids like CO_2 or light HCs was used by Galimov et al. (1985) and Barth et al. (1986) for the characterization of organic material from sedimentary rocks.

SFE has been applied to oil shales with industrial purposes to obtain soluble extracts (Kramer and Levy, 1988; Chong and McKay, 1987) using different solvent systems. Monin et al. (1988) and Kesavan et al. (1988) reported the successful use of carbon dioxide SFE in sedimentary rock extractions and Hopfgartner et al. (1990) concluded that SFE could advantageously replace solvent extraction for the determination of biomarkers in sediments. Multistage SFE of Kimmeridge Clay and Posidonia Shale formation samples, using plain or modified CO_2 , to obtain information of the part of the soluble organic matter that may preferentially enter into primary migration processes was reported by Greibroock et al. (1992).

It has been suggested that, to improve analyte recovery in SFE, it would be desirable to increase extraction temperature, to use polar supercritical fluids or to add polar modifiers to

non-polar fluids like CO₂ (Hawthorne et al., 1993). Lagenfeld et al. (1993) demonstrated that temperature was more important than pressure for achieving high extraction efficiencies when the interaction with pollutants molecules and sample matrices are strong and indicate that increasing SFE temperature may be a useful alternative to adding organic modifiers for achieving high extraction efficiencies from environmental samples. Similarly, Gong (1995) reported that temperature played a more important role than density of supercritical CO₂ and particle size in SFE of New Albany Shale samples. In fact, they found that HT-SFE can recover HOCs from shales previously exhaustively extracted by conventional methods (96 hr Soxhlet extraction).

Furton et al. (1994) showed the utility of SFE temperatures as high as 350⁰C for the rapid recovery of aliphatic and aromatic HCs from ancient sediments confirming the promising potential of this technique to determine geochemically important molecular parameters with a significant improvement in recoveries, precision and analysis time when compared with the traditional Soxhlet extraction. It was found in this study that high temperatures (250 to 350⁰C) followed by a lower temperature step (50⁰C) to increase supercritical fluid density are required to recover “trapped” HOCs from shales.

As was mentioned earlier, the focus of this study is the application of HT-SFE to the analysis of geological samples. Changes in the macromolecular structure of the organic matter, that can be induced via thermal processes may result in the release of previously non-extractable compounds which could led to a better understanding of the speciation of HOCs in ancient

sediments (like Posidonia Shale). At the same time, it is of great importance to analyze the effect of high temperature on the sample matrix, to evaluate the possibility of artificial maturation processes, due to pyrolysis of the kerogen, that could affect the HOC composition of the HT-SFE extracts. For this purpose, analytical pyrolysis methods like Rock-Eval Pyrolysis and Py-GC/MS will be applied to the extracted sediments.

1.4 Rock-Eval Pyrolysis .

Three factors: type of organic matter, amount of organic matter and thermal maturity, are important in the evaluation of potential petroleum source rocks. Rock-Eval Pyrolysis can provide useful information concerning those characterizations.

During this procedure (Fig. 5a), ground samples of sediment/rock are pyrolyzed in an inert atmosphere under varying temperature conditions (Espitalié et al., 1977). The sample is firstly heated to moderate temperatures (200 to 250⁰C) and the HCs already generated within the sample are volatilized. This group of compounds is quantified using a flame ionization detector and constitutes what is termed the S₁ measurement. The temperature is then progressively increased (up to approximately 550⁰C) until breakdown of the kerogen structure occurs (simulating catagenesis), and the resulting HCs are again determined by the flame ionization detector to provide the S₂ measurement. The flow of components emanating from the thermal breakdown of kerogen are usually split into two streams, so that oxygen containing components (specifically CO₂) could be determined by a thermal conductivity

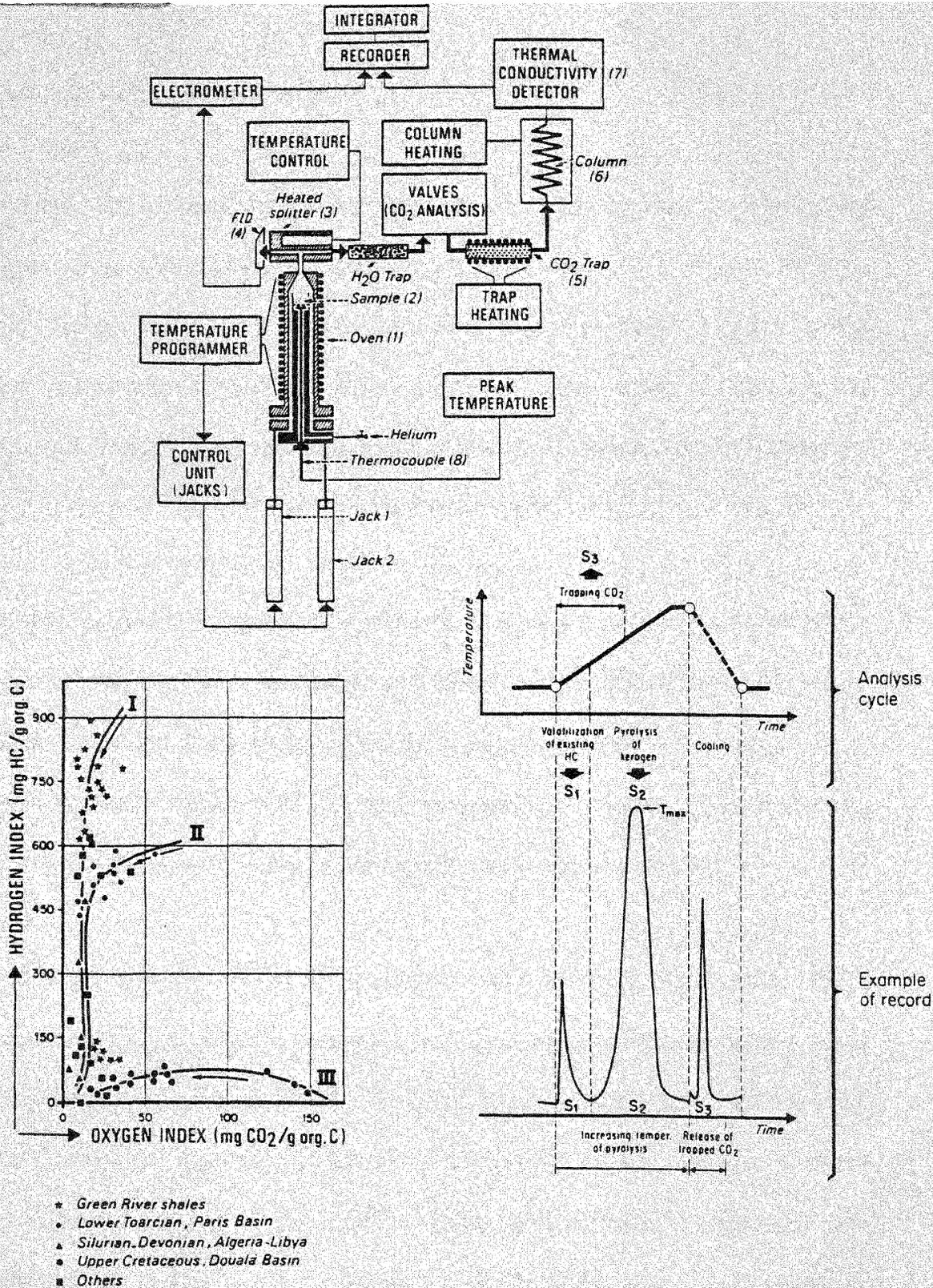


Fig. 5. Rock-Eval Pyrolysis Technique. a) Principle of the Rock-Eval Pyrolysis Device; b) Source Rock Type Classification Using HI and OI; c) Cycle of Analysis and Example of Record Obtained by the Method (Espitalié et al., 1977). Figures Taken from Tissot and Welte, 1986.

detector, providing the S_3 measurement.

For many years, Rock-Eval Pyrolysis has been used in oil exploration to predict the hydrocarbon generating potential of source rocks (Tissot and Welte, 1984; Sweeney et al., 1989; Burnham, 1987). The total amount of HCs that can be generated by a source rock is called “genetic potential” and is represented by $(S_1 + S_2)$ as a proportion of the amount of source rock. This information has been used to generate profiles of specific HOCs types with respect to the total petroleum potential in speciation studies (Jaffé and Gardinali, 1990).

Rock-Eval can yield very general information on the type of organic matter present by the use of the S_2 and S_3 values in combination with the total organic carbon (TOC) measurement (Radke et al., 1985). The hydrogen index (HI) is given by S_2 / TOC and the oxygen index (OI) by S_3 / TOC . A plot of HI vs. OI provides an analogue to the Van Krevelen diagram from which both organic matter type and maturity can be obtained (Fig. 5b).

As HCs are generated in increasing quantity with increasing temperature, the S_2 measurement decreases while S_1 increases. Hence (S_1/S_1+S_2) , known as the transformation ratio, increases with increasing maturity. Similarly, the temperature at which the maximum in the S_2 response is noted (T_{max}) increases with increasing maturity reflecting the higher thermal energy required to break the remaining bonds in kerogen associated with hydrocarbon generation during catagenesis. Fig. 5c shows the cycle of analysis and an example of records obtained by the Rock-Eval Pyrolysis method.

Etler et al. (1995) used Rock-Eval Pyrolysis to characterize samples extracted by a conventional chloroform extraction and by SFE, in a work designed to determine the experimental SFE conditions which would result in a bitumen of similar composition and concentration than that obtained by the traditional method.

Nowadays, improved Rock-Eval analyzers have been designed with expanded capability, enhanced flexibility and improved data input/output interface (Marquis et al., 1995). At the same time, these new models provide better estimations of OI, TOC content and kerogen types as well as the possibility of determining the mineral carbon in the source rock samples.

Recently, Rock-Eval Pyrolysis data from Kupferschiefer Shale SFE extracted samples has been obtained by our laboratory to study the Rock-Eval parameter behavior with increasing temperature levels in the extraction procedure. In the present report we will present a comparison of Kupferschiefer data with that obtained for Posidonia Shale under similar conditions.

1.5 Pyrolysis-GC/MS.

The chemical composition of kerogen is a complex function of its biochemical source-related mechanical composition (maceral composition), the diagenetic modification and its degree of thermal evolution. Bulk chemical analysis such as Rock-Eval Pyrolysis data has been useful in providing generalized chemical descriptions of kerogens, but only the use of

analytical pyrolysis as a kerogen structural analysis tool permits the description of kerogen in terms of the quantitative distribution of various molecular types in the macromolecular system.

Analytical Pyrolysis involves the on-line coupling of an inert atmosphere (He or N₂) pyrolyzer with a gas chromatograph (Py-GC) or with a mass spectrometer either directly (Py-MS) or via a GC column (Py-GC/MS) (Larter, 1984; Horsfield, 1984). In the evolution of this area of research, coupled use of Py-GC/MS and Py-MS techniques have proven to be more efficient (Maters et al., 1977; Larter, 1984) although to date most quantitative data are associated with Py-GC studies.

Most of the analytical pyrolysis have been performed using two main types of pyrolyzers: 1) Filament pyrolyzers, either resistively or inductively heated, with isothermal pyrolysis of submilligram samples in the temperature range 600-800°C for several seconds (Larter and Senftle, 1985) and 2) Furnace pyrolyzers which can pyrolyze larger samples either isothermally or, more typically, under programmed temperature conditions over the range 300 - 800°C, at heating rates usually similar to those of a Rock-Eval instrument (25°C/min) (Horsfield et al., 1989). Results from both systems appear to be qualitatively quite comparable. In this study we will use a Py-GC/MS with a filament type pyrolyzer.

Py-GC/MS permits the identification of a great variety of components in kerogen pyrolysates including light hydrocarbon gases (Giraud, 1970), inorganic gases (Larter, 1984; Eglinton

et al., 1988), alkylaromatic and aliphatic HCs, phenols, acids and oxygen-, nitrogen- and sulfur bearing heterocycles (Maters et al, 1977; Larter and Douglas, 1978; Sinninghe-Damste et al., 1989).

It is generally accepted that one of the major identified and readily quantifiable (GC/MS resolvable) kerogen pyrolysis product groups are saturated and unsaturated normal, branched and cyclic aliphatic HCs in the carbon number range of C_1 - C_{35} . Alkylated one to three -ring aromatic and naphthenoaromatic HCs with side chains from C_1 to C_{30} have been also well characterized as major pyrolysis products (Larter, 1984; Horsfield, 1984). Additionally, steroid and terpenoid HCs have been observed in high temperature pyrolysates of oil shales and coal kerogens by many authors (Gallegos, 1975, 1978; Eglinton, 1988).

The application of the Py-GC/MS technique to the original Posidonia Shale as well as to the SFE extracted samples will contribute both to a better characterization of this ancient sediment and to the analysis of possible HT-SFE effects on the kerogen that could be elucidated based on the pyrolysis products.

1.6 Hydrocarbon composition in shales. Biomarkers.

As was mentioned before, by the end of diagenesis the biogenic organic material in sediments either has been degraded and recycled by microorganisms or has been largely converted into insoluble polymeric material, such as kerogen, from which HCs are

produced (bitumen) with increasing burial and heating. Kerogen occurs in sedimentary rocks in the form of finely disseminated organic macerals being by far the most abundant form of organic carbon in the earth crust. The composition of disseminated organic matter in sedimentary rocks is shown in Fig. 6.

Bitumen is composed of three main fractions: asphaltenes, resins and HCs. The hydrocarbon fraction contains mainly aliphatic and aromatic HCs. Among the aliphatic HCs the predominating groups are the acyclic alkanes (normal and branched) and cycloalkanes. Acyclic isoprenoidal alkanes are also important constituents, particularly pristane and phytane. In the aromatic fraction, the major components are benzene, naphthalene, phenanthrene and their alkylated homologues. Sulfur-rich kerogens can give rise to significant quantities of thiophenic compounds like benzothiophenes, dibenzothiophenes, naphthobenzothiophenes and their alkyl derivatives. Some of these compounds are shown in Fig. 7.

Biological markers or biomarkers (Eglinton et al., 1964; Eglinton and Calvin, 1967) are the most useful molecular indicators at the stage of maturity corresponding to ancient sediments because their basic structures remain intact through processes associated with sedimentation and diagenesis. Biomarkers include acyclic isoprenoids like pristane and phytane, steroids, terpenoids and porphyrins and are found both, free in the bitumen and bound to the kerogen in petroleum source rocks. This makes them very useful in providing information about the organic matter present in source rocks, the environmental conditions during its deposition

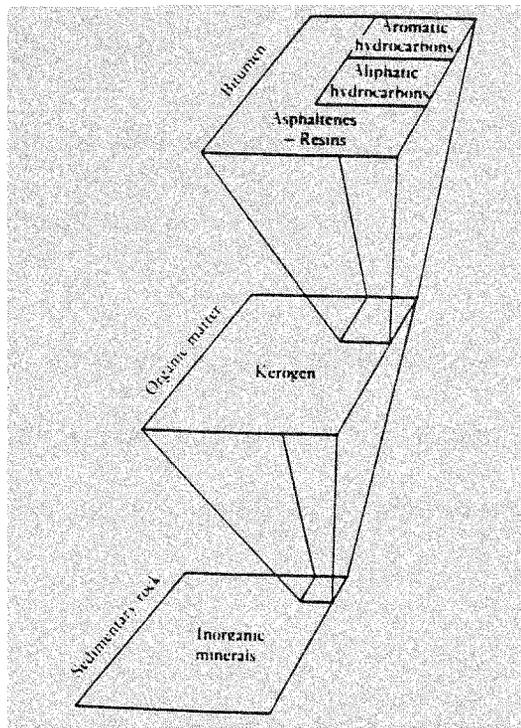


Fig. 6. Composition of Disseminated Organic Matter in Sedimentary Rocks (Tissot and Welte, 1984). Figure Taken from Killips and Killips, 1993.

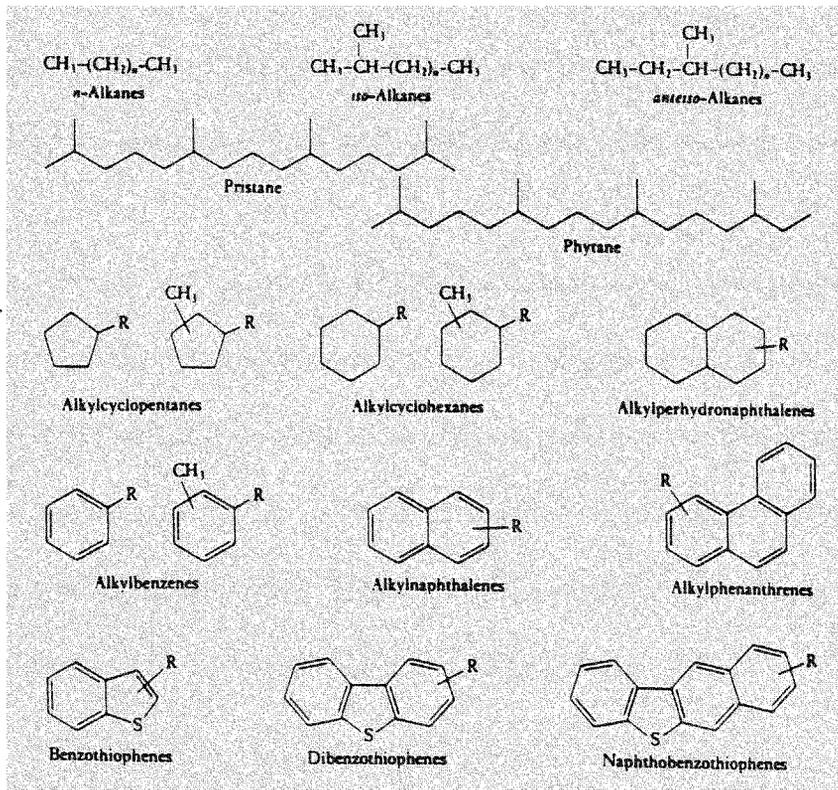


Fig. 7. Major Compounds in the Hydrocarbon Fraction of Oil Shales. Figure Taken from Killips and Killips, 1993.

and burial (diagenesis), the thermal maturity experienced by the rock (catagenesis), the degree of biodegradation, etc.

Known to be derived from the acyclic isoprenoid squalene, which is an ubiquitous component in organisms, steranes and hopanes (Fig. 8) (tetra and pentacyclitriterpenoids, respectively) can provide useful geochemical information in ancient sediments and sedimentary rocks .

Hopanes are abundant in sediments and petroleum because their precursor (bacteriohopanotetrol) is an important component of the bacterial cell membrane (Ourisson et al., 1984). Similarly, cholesterol, found in the membranes of all eukariotic cells (Rohmer,1987), is converted by dehydration and reduction to cholestane during diagenesis, and thus, like the hopanes, steranes are abundant in sediments, rocks and petroleum.

Pristane and phytane are commonly the most abundant acyclic isoprenoids in geological samples. Due to their large abundance and easy measurement by GC and GC/MS, their identification and quantitation is often utilized in organic geochemical studies. They have been used in the definition of thermal maturity indicators (Goosens et al., 1988; Van Grass et al, 1981), correlation parameters (Welte, 1966; Hagemann et al., 1975), and depositional environment indicators (Brooks et al.,1969; ten Haven et al., 1987).

Thermal maturity changes can be related to biomarker molecular distribution changes, such

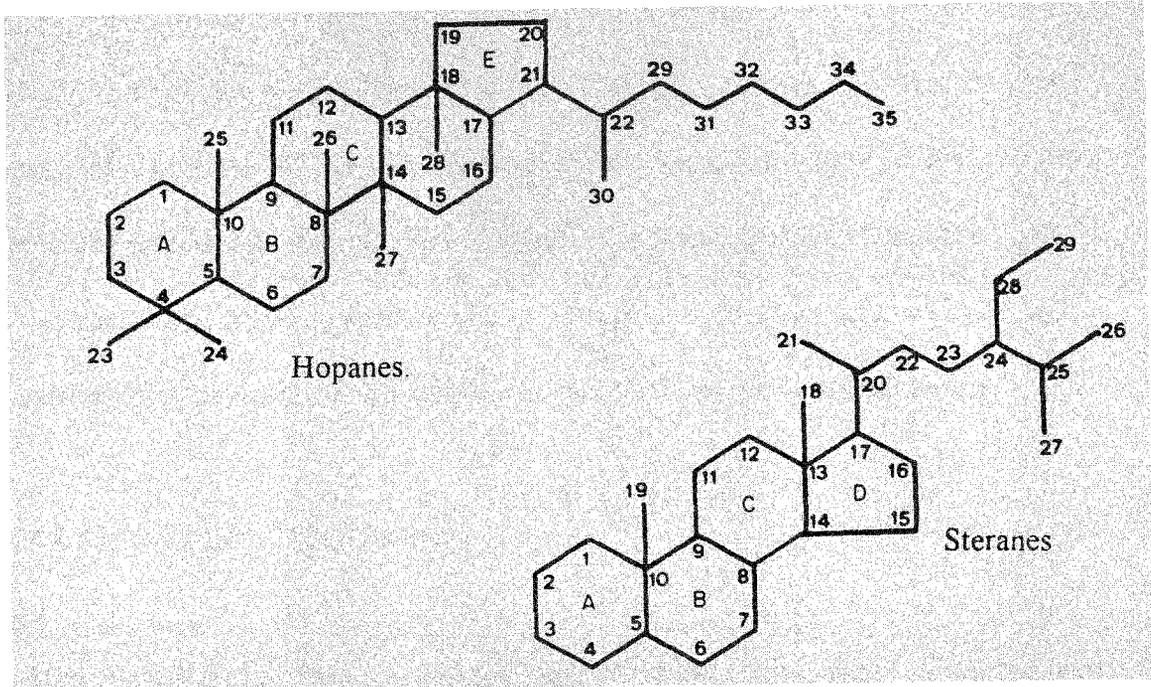


Fig.8. Molecular Structures and Numbering Systems for Steranes and Hopanes.

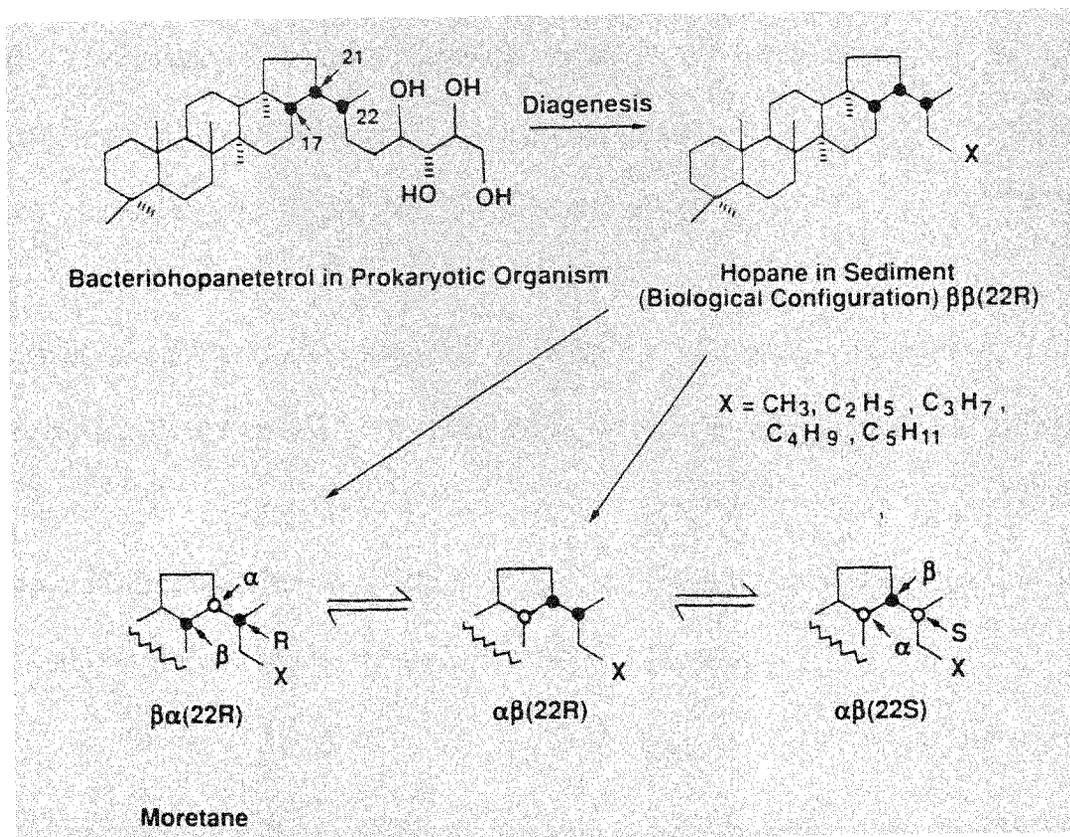


Fig. 9. Origin of Hopanes in Sediments and Configurational Isomerization Processes in Hopanes During Diagenesis and Catagenesis. Figure Taken from Peters and Moldowan, 1991.

as isomerizations at cyclic and acyclic centers in steranes, hopanes and other triterpanes, aromatisation of steroidal HCs and changes in relative abundances of acyclic isoprenoidal alkanes like pristane and phytane. We will refer here to three parameters that will be used in the characterization of Posidonia Shale SFE extracts in order to obtain information regarding the speciation of the related compounds in the matrix. These are described below:

A) % 22 S for $17\alpha(H)$, $21\beta(H)$ hopanes .

The bacteriohopanetetrol shows a $17\beta(H)$, $21\beta(H)22R$ ($\beta\beta R$) “biological” stereochemistry (Rohmer, 1987). Due to the fact that this stereochemical rearrangement is thermodynamically unstable, diagenesis of bacteriohopanetetrol result in the transformation of the $\beta\beta$ configuration to the $\beta\alpha$ and $\alpha\beta$ in the ring system and, similarly, the biological 22R converts to an equilibrium mixture of 22S and 22R $\alpha\beta$ -homologues (Fig. 9).

The homohopanes (hopanes with more than 30 carbon atoms) show an extended side chain with an asymmetric center at C-22 which results in two peaks for each homologue (22R and 22S) on the mass chromatogram of these compounds (Fig.10). Based on the above configurational isomerization, a ($22S/22S+22R$) ratio has been defined ranging from 0 to 60 % and can be calculated for any or all of the C31 to C35 hopanes.

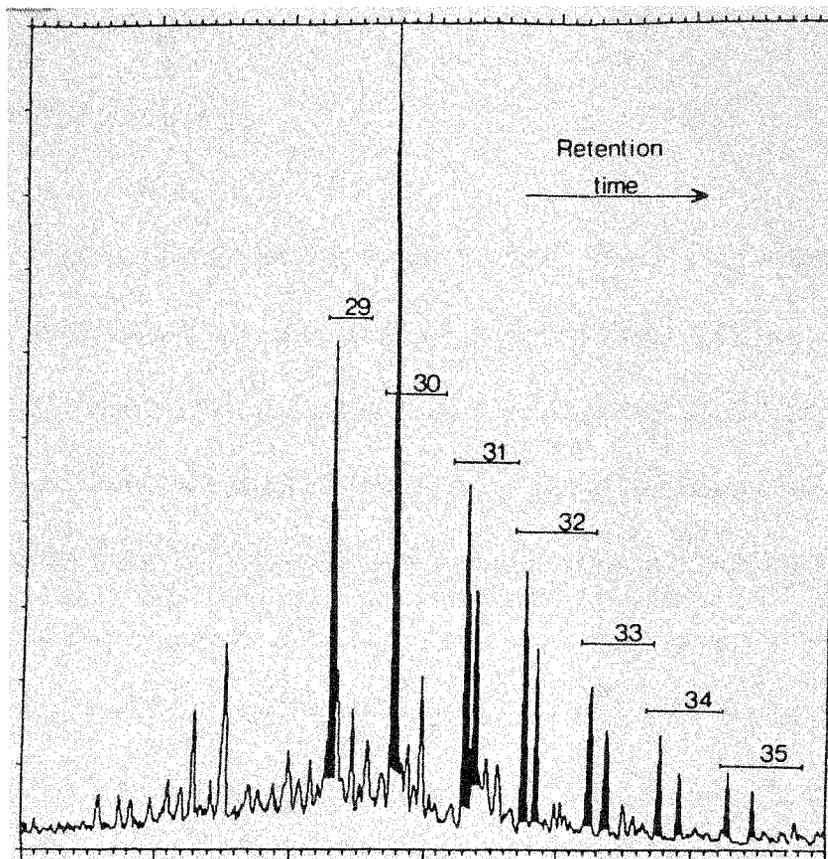


Fig. 10. Typical Mass Chromatogram for m/z 191 of the Aliphatic Fraction of a Shale Extract. 17α (H), 21β (H) - Hopanes are Shaded Black and Marked with their Carbon Number. Figure Taken from Peters and Moldowan, 1991.

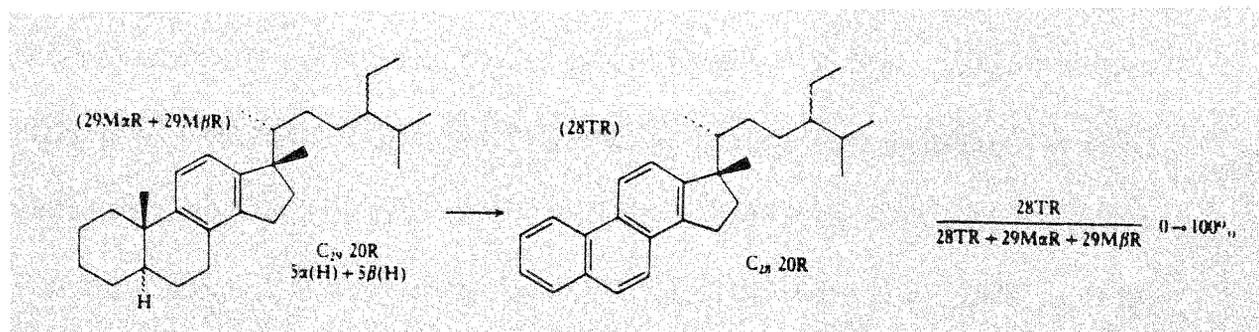


Fig. 11. Aromatization of C-ring Monoaromatic Steranes. Figure Taken from Killips and Killips, 1993.

B) % Tri/Monoaromatic steroids.

One of the major classes of diagenetic products of steroids is the C-ring monoaromatic steranes. The aromatization of C-ring monoaromatic steroidal HCs (MA) to ABC-ring triaromatic steroids (TA) occurs as temperature raises with increasing burial. It involves the loss of a methyl group at the A/B ring juncture (Fig. 11) yielding TA-steroids with one less carbon. Thus, the TA/(MA+TA) ratio which increases from 0 to 100 has been used as a thermal maturity parameter in source rock extracts and petroleum samples. It is possible to use the sum of all C27 to C29 C-ring MA-steroid peaks for MA and the sum of all C26 to C28 TA-steroid peaks in a chromatogram for TA to calculate this ratio, but the use of one specific transformation like the one shown in Fig. 11 is considered to be the more accurate (Killops and Killops, 1993).

C) Pristane/Phytane and C 18/Phytane ratios.

Pristane/Phytane (Prist/Phyt) and C 18 n-alkane/Phytane (C 18/Phyt) ratios have been defined as a depositional environment and as a thermally induced kerogen cracking indicators, respectively. It is generally accepted that the Prist/Phyt ratio does not change with maturity (Tang and Stauffer, 1995; Burnham et al., 1982; Didyk et al., 1978) until the kerogen begins to suffer thermal cracking during catagenesis. Then, additional pristane appears to be generated from kerogen causing a ratio increment. This increase is paralleled by an increase in n-alkanes relative to phytane and it is usually measured by determining the

C 18/Phyt ratio.

These ratios will be calculated for different SFE extracts to study the interaction of isoprenoidal compounds with the organic matrix compared to that of normal alkanes considering their different molecular shapes.

1.7 Geosynthesis of alkylaromatics in sediments.

The degradation of biogenic precursors in sediment maturation processes, largely by thermal bond breaking and dehydrogenation reactions, are generally considered responsible for the distribution of alkylaromatic HCs in sediment and petroleum (Smith et al., 1995). Alkylphenanthrenes have long been considered to be predominantly derived from terpenoids and steroids (Mair, 1964, Bendoraitis, 1974) while little is known about the origin of alkylanthracenes, which are commonly of much lower abundance than alkylphenanthrenes in sedimentary rocks. This observation corroborates their different origins.

Additionally it has been suggested that significant quantities of these compounds in sediments may be derived by methylation of parent aromatic HCs (Radke et al, 1982). For example, it has been reported that the abundance of methylphenanthrenes over phenanthrene increases during pyrolysis of Kimmeridge Clay and Brent coal samples heated to 350°C (Garrigues et al., 1990). Derbyshire and Whitehurst (1981) observed pyrene alkylation at 400°C due to transfer of carbon from the coal. Voigtmann et al. (1995) indicated the

likelihood of a geosynthetic, in addition to a precursor-directed, origin for the methylphenanthrenes in sediments. Smith et al (1995) demonstrated the synthesis of methylphenanthrenes, methylanthracenes and methylpyrenes by the reaction of their correspondent parent molecules with coals at 220-400°C. Therefore, it would be of interest to investigate the effect of speciation and temperature on the presence of these compounds in HT-SFE extracts. In other words, it would be important to elucidate whether the alkylaromatics extracted at high temperature SFE from Posidonia shale are present originally in the sediment, or if some fraction was produced through geosynthetic processes via methylation of the parent molecules. This would also allow for a better understanding of the origin of these compounds in the geosphere.

2.EXPERIMENTAL

2.1 Sample preparation.

Posidonia Shale, an ancient sediment from a quarry near Dotterhausen, Southwest Germany, was the sample used in this study. Before its use in the experiments, about 500 g of the sediment was pulverized and stored at 12^oC.

2.2 Other materials.

SFE/SFC grade carbon dioxide (99.9999 % purity) from Plumsteadville, PA, USA was used in all the SFE extractions. High purity solvents (Optima grade, Fisher Scientific, NJ, USA) were used in the present study and the glass wool was preextracted with methylene chloride before its use. All glassware was firstly washed in an ultrasonicator (Solid State / Ultrasonic Cleaning System Model FS-7652, Fischer Scientific, USA) with an special heavy duty liquid soap (Liqui-Nox, Alconox. Inc., NY, USA) dissolved in water during 1 hr at 90 ^oC, then it was rinsed out with deionized water and oven dried (120^oC). Finally, the glassware was covered with aluminum foil and was rinsed with metahylene chloride before use.

2.3 Instrumentation.

2.3.1 Supercritical Fluid Extractor.

Fig. 12 shows a schematic diagram of the SFE system used in the experiments. The sample

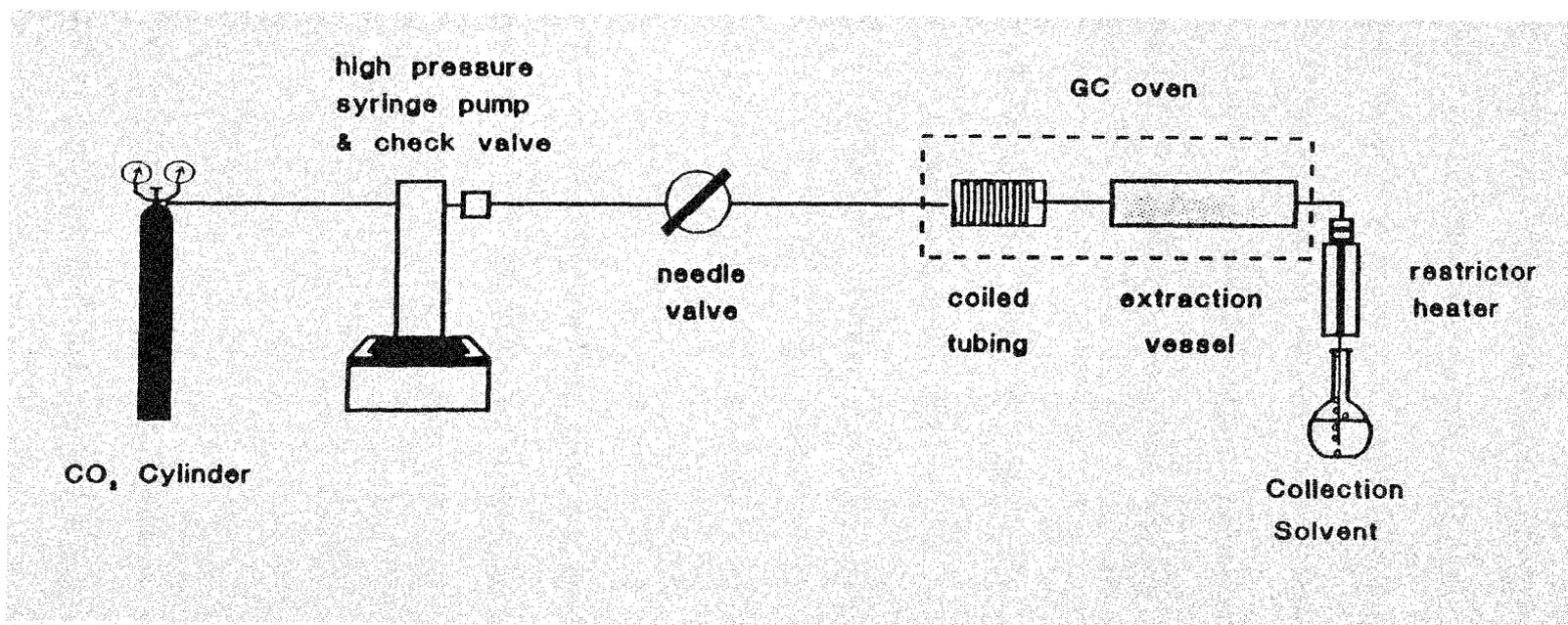


Fig.12. Schematic Diagram of High Temperature SFE System.

to be extracted was introduced in an empty stainless steel HPLC column (15 cm x 1 cm i.d. and 0.5 inch o.d.) with frits at the end (Keystone Scientific, Bellefonte, PA, USA). This sample holder was used as the extraction cell, and placed into a Varian model 3700 GC oven to be heated at the different temperatures under study. During the extractions, high pressure (5000 psi) CO₂ (SFE grade, Plumsteadville, PA, USA) delivered by a 260D syringe pump (ISCO, Lincoln, NE, USA), was passed through the extraction cell. The resultant extracts were collected in 20 ml vials with methylene chloride through a 10 cm (50 µm i.d., 375 µm o.d.) fused-silica linear restrictor (Polymicro Technologies, Phoenix, AZ, USA). The needle valve (Scientific Systems, Inc., State College, PA, USA) used to control a 1.0 ml/min CO₂ flow through the system was heated to 200°C with Silicone extruded heating tape controlled with a stepless temperature controller (Thermolyne, Dubuque, Iowa, USA).

2.3.2 Rock-Eval Pyrolysis.

The Rock-Eval Pyrolysis and TOC determinations were conducted by DGSI (The Woodlands, TX) using a LECO TOC and Rock-Eval II Pyrolysis unit as analytical procedures.

- Leco TOC:

TOC is determined by direct combustion. A 0.15 g sample, grounded so as to pass through a 60 mesh sieve to assure homogeneity, was carefully weighed, treated with concentrated

HCl to remove carbonates, and vacuum filtered on fiberglass filter paper. The residue and filter were placed in a ceramic crucible, dried and combusted with pure oxygen in a LECO EC-12 carbon analyzer at about 1000°C. The TOC was calculated as Wt %.

- Rock-Eval II Pyrolysis.

About 0.1 g of the same ground sample used for TOC determination was carefully weighted in a pyrolysis crucible and then heated to 300°C to determine the amount of free HCs that could be thermally desorbed (S_1) using an FID detector. In a similar way, the amount of HCs that are generated from kerogen during pyrolysis (S_2) was measured when the sample was heated in an inert environment from 300°C to 550°C at a heating rate of 25°C/min. At the same time as the S_2 analysis, carbon dioxide generated during pyrolysis is collected up to a temperature of 390°C, detected in a TCD and reported as S_3 (mg CO₂ / g sample). The temperature during pyrolysis peak (S_2) was reported as T_{max} . Hydrogen Index (HI) equals S_2/TOC and Oxygen Index (OI) equals S_3/TOC were plotted in a Van Krevelen type diagram (see Results and Discussion).

2.3.3 Pyrolysis-GC/MS.

The sample (2g) was placed in a steel press along with a Curie point wire (358°C) and pressed (15 atm) for 2 sec. The Curie point wire plus the sample were then inserted into a wide bore capillary tube which was then placed into a pyrolyzer unit that was activated (2

sec, interface temperature 250°C). The system is connected to a GC/MS. The GC was programmed from -40 to 310°C at a rate of 6°C/min, with a holding time of 5 min at 310°C. The GC was initially cooled down by a cryogenic cooling unit using liquid CO₂ . The MS mass scanning range was set between 50 and 600 mass units. After the thermally extracted HCs (358 °C) had been analyzed, the Curie point wire was removed and the residue was scrapped off and placed in the clean press. A small amount of the residue was applied to another Curie point wire (610°C) as above and the pyrolysable HCs were analyzed on the GC/MS. Before each analysis, the wires were cleaned in diluted HNO₃, distilled H₂O, ethyl acetate and methylene chloride using a sonicator in all the stages. (Note: These analyses were performed at the University of Liverpool, U.K.).

2.4 Analysis.

2.4.1 GC/MS.

The analysis of the Soxhlet and SFE extracts was performed by GC/MS. A HP5971A mass selective detector interfaced to an HP5890 Series II gas chromatograph (30 m x 0.250 mm of DB-5 MS fused silica, capillary column) was used. The GC/MS was firstly run in the Total Ion Current (TIC) mode to identify the compounds of interest in the extract, based on their characteristic mass spectral patterns. For the quantitative analysis, the GC/MS was run in the Selected Ion Monitoring (SIM) mode at an ionization energy of 70 ev and with a dwell time of 250 microseconds for each monitored ion. An internal standard (squalane) was

added to all SFE extracts. The response factor for the analytes by GC/MS was assumed to be 1. Therefore, the obtained data are semiquantitative in nature.

The GC temperature program began at 40°C during 1 min, followed by an increasing temperature rate of 6°C/min from 40°C to 300°C, with a final holding time of 20 min. The injector and detector temperatures were of 280 °C. The analytes monitored and the correspondent characteristic ions (m/z) are listed below:

-Normal alkanes, pristane and phytane, (57).

-Naphthalene, (128).

-C1-naphthalene, (142).

-C2-naphthalene, (156).

-C3-naphthalene, (170).

-Phenanthrene, anthracene, (178).

-C1-Phenanthrene, (192).

-C2-Phenanthrene, (206).

-C3-Phenanthrene, (220).

-Steranes, (217).

-Monoaromatic steroids (MA), (253).

-Triaromatic steroids (TA), (231).

-Dibenzothiophene, (184).

-C1-Dibenzothiophene, (198).

-C2-Dibenzothiophene, (212).

-C3-Dibenzothiophene, (226).

-Hopanes, (191).

-Deuterated phenanthrene, (188).

The (TA/MA+TA) ratio was calculated based on the sum of all C₂₆ to C₂₈ TA and the sum of all C₂₇ to C₂₉ C-ring MA steroid peaks in the chromatograms.

SFE extracts previously obtained for Kupferschiefer Shale were analyzed in the GC/MS for comparative purposes.

2.5 Soxhlet Extraction.

5 g sample of Posidonia Shale were weighed and transferred into a preextracted Cellulose thimble covering the sample with preextracted glasswool before placing it into the Soxhlet extractor. The samples was extracted with methylene chloride for 24 h and the resulting extract was concentrated first in a rotary evaporator followed by N₂ blowdown, to approximately 400 ul.

To eliminate the polar fraction of the extract (resins and asphaltenes), the sample was fractionated by column chromatography (Wang et al., 1994). A chromatographic column with a teflon stopcock (200 x 10.5 mm i.d.) was plugged with pre-extracted pyrex glass wool at the bottom, serially rinsed with methanol, hexane and dichloromethane, and allowed to dry. The column was then dry-packed with 3 g of activated silica gel and topped with about 0.5

cm of sand and about 0.5 cm of anhydrous sodium sulfate. The column was conditioned with 20 ml hexane and the eluent was discarded. Just prior to exposure of the sodium sulfate layer to air, Soxhlet extract was transferred onto the column using an additional 3 ml hexane to complete the transfer. All eluents up to this point were discarded. Hexane (12 ml) was used to elute aliphatic HCs (alkanes, triterpanes and steranes) while 15 ml of a toluene:hexane mixture (1:1) was used to elute target PAHs and their alkylated homologues. Both fractions were combined, concentrated and analyzed in the GC/MS after addition of a known amount of squalane as internal standard. The procedure was conducted in triplicate in order to establish the method reproducibility based on the standard deviation and coefficient of variation for some of the compounds.

2.6 Supercritical Fluid Extraction.

2.6.1 Stepwise SFE.

Stepwise SFE experiments were performed extracting a 3 g Posidonia Shale sample in the SFE using 5000 psi and a CO₂ flow of 1 ml/min, at seven successively increasing temperatures (50, 100, 150, 200, 250, 300 and 350°C) followed by a lower temperature step at 50°C for every temperature level and collecting the extract after each step. Every step consisted of a total extraction time of 1.5 h where 1 h corresponded to the SFE extraction at a given temperature and the remaining 0.5 h was divided between the initial heating time and a final 50°C period.

The seven fractions were collected and blown down separately using a $N_{2(g)}$ stream and the internal standard (squalane) was added before injecting them into the GC/MS for quantitative analysis of target analytes. The experiment was made in triplicate for the reproducibility study.

2.6.2 Temperature dependent SFE of individual samples.

Seven SFE experiments (5000 psi, 1.0 ml of CO_2/min) were conducted separately at different temperatures (50, 100, 150, 200, 250, 300 and 350°C) using 3g Posidonia Shale samples. Each extraction was performed during 1.5 h in a similar manner than the stepwise procedure steps (1 h at the temperature of interest and 0.5 h between the initial heating and the final 50°C step). In this case, the extracts were collected after each extraction experiment as well as the corresponding extracted sediments. The sample was changed for each experiment. The 50, 150, 250 and 350°C SFE extractions were performed in triplicate to determine the method reproducibility based on some of the studied compounds.

2.7 Heating experiments.

2.7.1 Thermal treatment of nonextracted sediments.

Two experiments were conducted with four thermally sealed borosilicate ampoules containing 3 g of Posidonia Shale each. For the first experiment, two of the ampoules were

placed into a GC oven (Varian model 3700) at 200°C and were analysed after 3 and 12 h of heating. The second experiment used the remaining two ampoules for a similar study but setting the GC oven temperature at 350°C. The thermally treated samples were then individually extracted in the SFE at 50°C during 1.5 h and the collected extracts were analysed in the GC/MS. A 50°C SFE extract obtained from the original (not thermally treated) shale was used as a control for comparative purposes.

2.7.2 Thermal treatment of preextracted sediments.

Three g of 250°C SFE-preextracted Posidonia Shale sample were kept in the sealed depressurized SFE extraction cell during 1 hr at 350°C in the GC oven; then a 50°C SFE extraction was performed (total extraction time = 1.5h) and the extract was analyzed in the GC/MS. Another 250°C SFE preextracted 3 g sample of Posidonia Shale was extracted at 50°C and used as a control.

2.8 Preliminary evaluation of alkylphenanthrene geosynthesis during HT-SFE.

In order to test possible geosynthesis of alkylaromatics during HT-SFE, 140 µl of a 1967.5 µg/ml deuterated phenanthrene solution (Supelco Inc., PA, USA) were diluted with methylene chloride in a 50 ml volumetric flask. This solution was poured on 6 g of the ground Posidonia Shale, so that a concentration of 40 µg of deuterated phenanthrene / g of shale was obtained. The sample was then swirled and rotoevaporated to dryness. Half of the

spiked sediment (3 g) was extracted in the SFE at 50°C while the other 3 g sample was extracted at 350°C. An internal standard (squalane) was added to the resultant extracts which were finally analysed in the GC/MS to compare the [deuterated phenanthrene]/[squalane] ratios for both extraction temperatures in order to assess if transalkylation to the deuterated standard did occur.

2.9 Rock-Eval Pyrolysis.

This technique was used to characterize both the original and the pre-extracted sediments. Rock-Eval Pyrolysis technique was applied to the extracted sediments obtained from the SFE at different temperatures, using a fresh sample each time. The TOC (Wt %), S1 (mg/g), S2 (mg/g), S3 (mg/g), Tmax (°C), HI and OI values were determined for each sample. The HI and OI values obtained for each extraction temperature were plotted in a Van Krevelen analogue diagram (see Results and Discussion).

2.10 Pyrolysis -GC/MS.

Sediment samples extracted by SFE using both a fresh sample each time (at 50°C, 250°C and 350°C) as well as the original nonextracted sediment, were analysed using the Py-GC/MS technique. Comparisons of the TICs and some specific ion chromatograms obtained at 358 and 610°C Py-GC/MS for each sample were performed (see 2.3.3).

2.11 Microscopy Study.

An original Posidonia Shale sample and sediments extracted at 150°C and 350°C (using a fresh sample each time SFE) were analyzed in an ISI Super III-A Scanning Electron Microscope (SEM). The samples were attached to aluminum stubs with silver paint and sputter coated with gold / palladium (60:40) in a Denton Vacuum Desk-1 sputter coater, and the photographic records were made using Polaroid 55 P/N film.

3. RESULTS AND DISCUSSION.

3.1 Identification of target analytes.

Figures 13 to 18 show characteristic GC/MS patterns for several compounds monitored in the different experiments. Tables II to V present the peak identification for hopanes, steranes, mono and triaromatic steranes. Compound identifications are based on mass spectral interpretations, comparison with literature spectra and chromatographic retention characteristics.

3.2 Soxhlet extraction.

The analyte concentrations obtained for the 24 h Soxhlet extraction of Posidonia Shale are shown in Table VI. These results will be compared later with results for stepwise SFE studies. The reproducibility study results are shown in Table VII for selected compounds. The coefficient of variation ranged in acceptable values (approximately 6.5 - 13 %). However, some analytes like naphthalene showed higher levels (approximately 17 %). The reproducibility of the Soxhlet, however, was found to be in the commonly observed range for this type of samples.

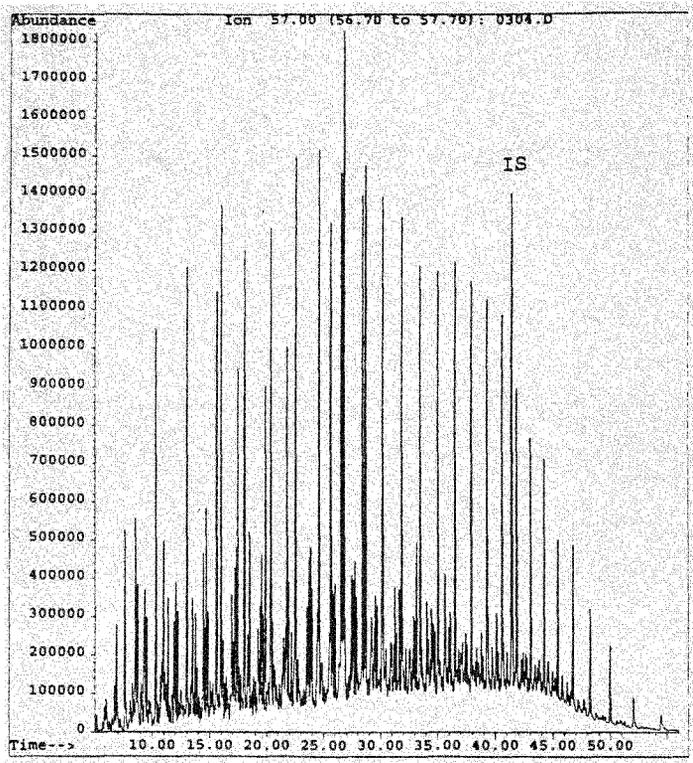


Fig. 13. GC/MS Pattern for n-alkanes ($m/z=57$) in Posidonia Shale SFE Extracts.

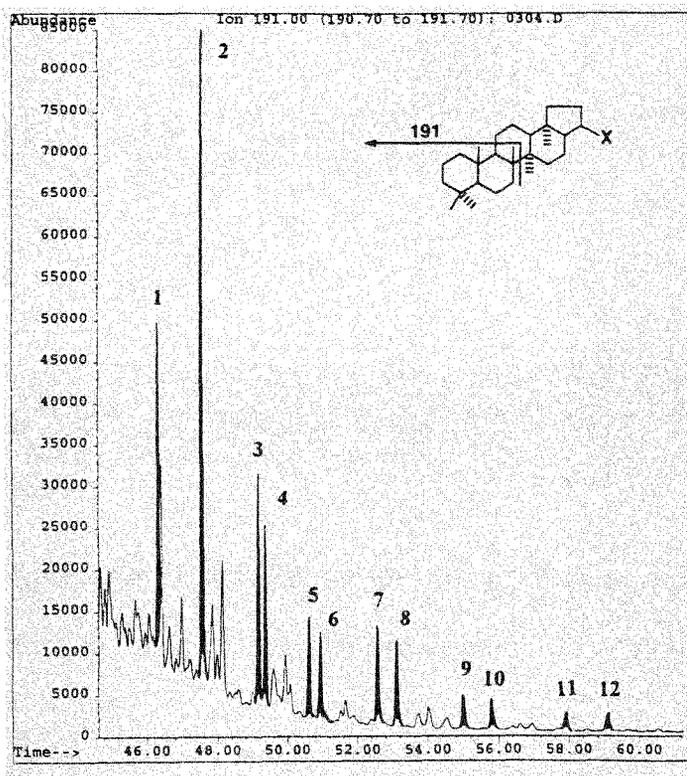


Fig.14. GC/MS Pattern for Hopanes ($m/z=191$) in Posidonia Shale SFE Extracts.

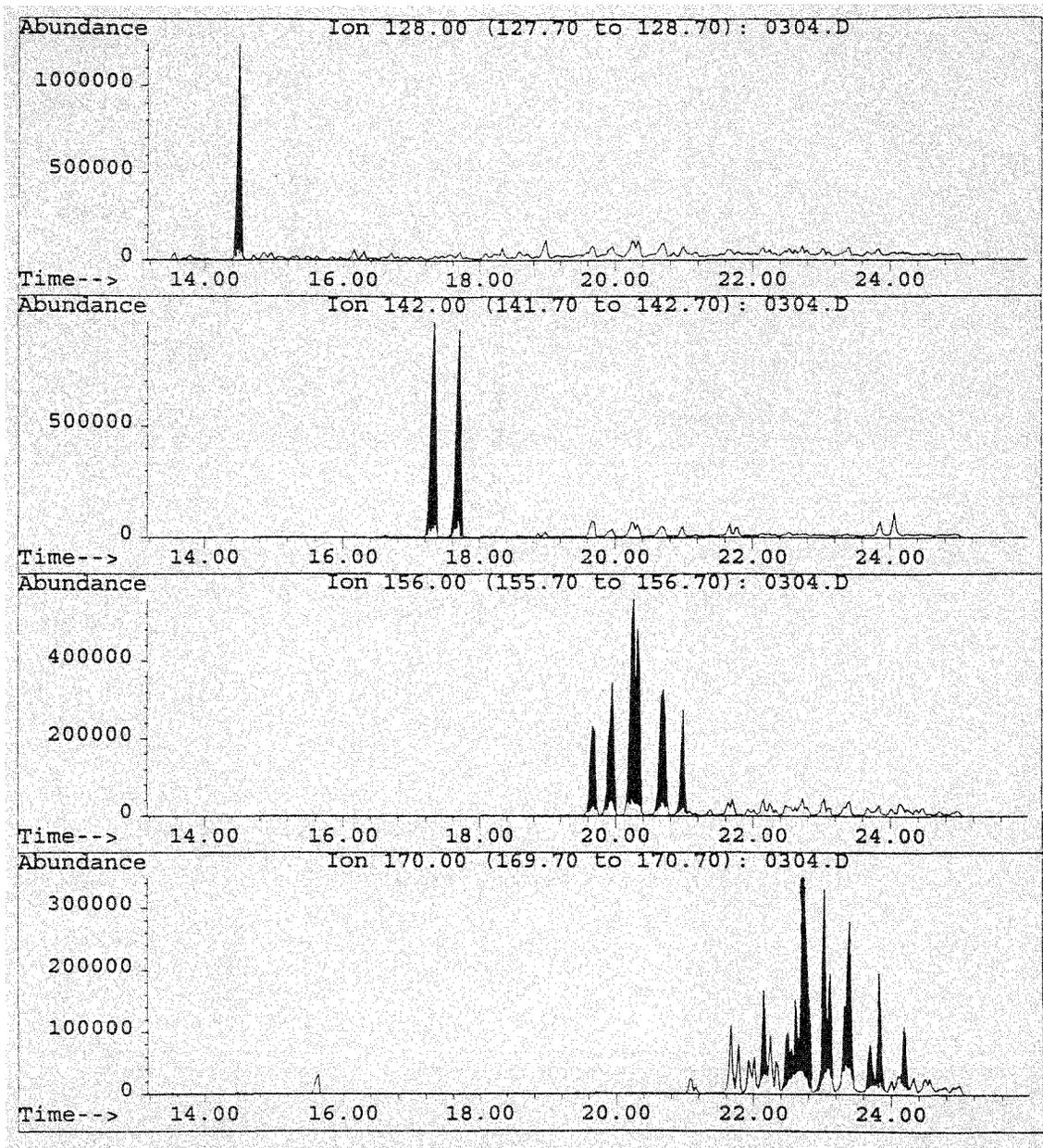


Fig.15. GC/MS Pattern for Naphthalene ($m/z=128$), C1-Naphthalenes ($m/z=142$), C2-Naphthalenes ($m/z=156$) and C3-Naphthalenes ($m/z=170$) in Posidonia Shale SFE Extracts.

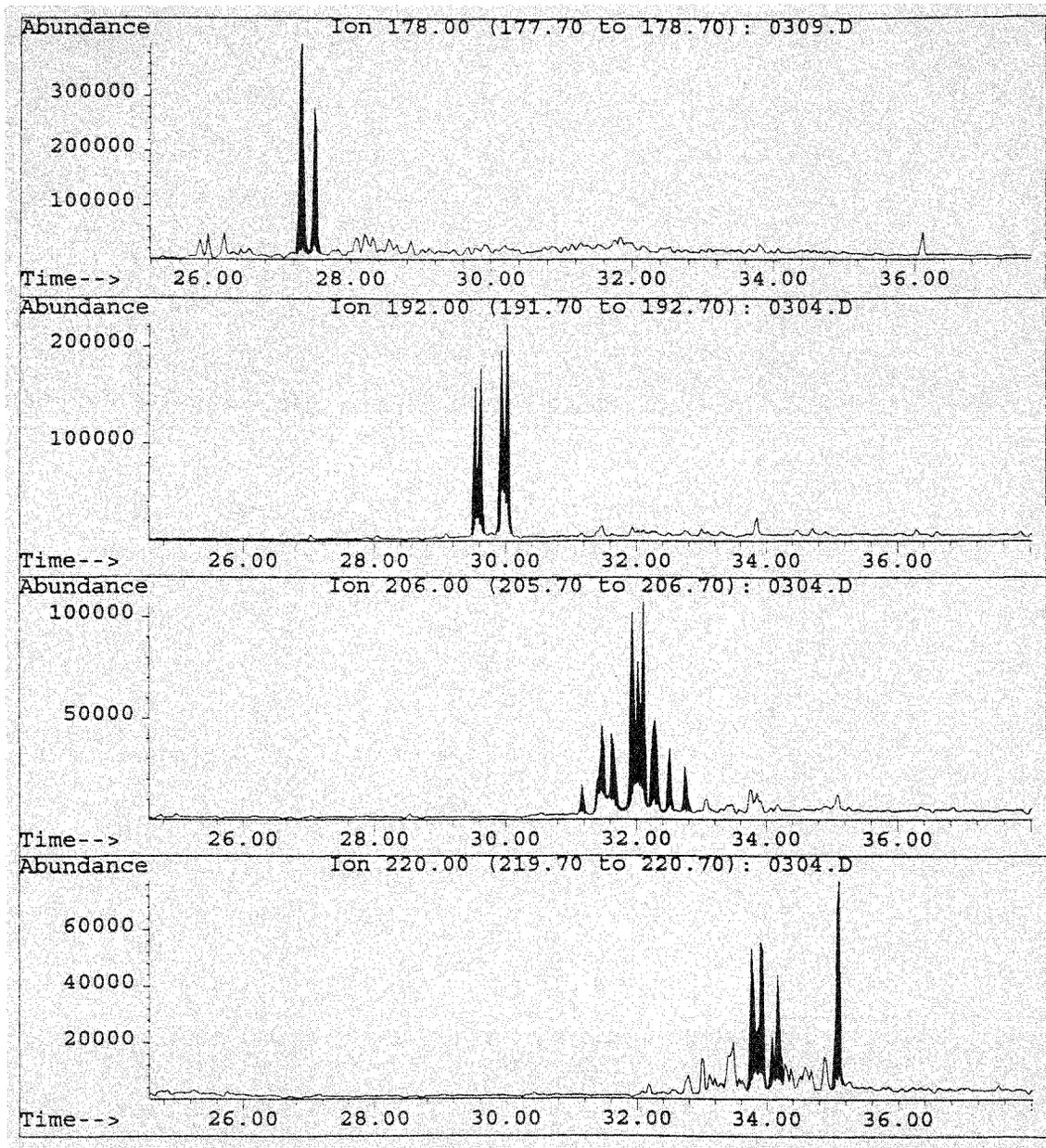


Fig.16. GC/MS Pattern for Phenanthrene and Anthracene ($m/z=178$), C1-Phenanthrene ($m/z=192$), C2-Phenanthrene ($m/z=206$) and C3-Phenanthrene ($m/z=220$) in Posidonia Shale SFE Extracts.

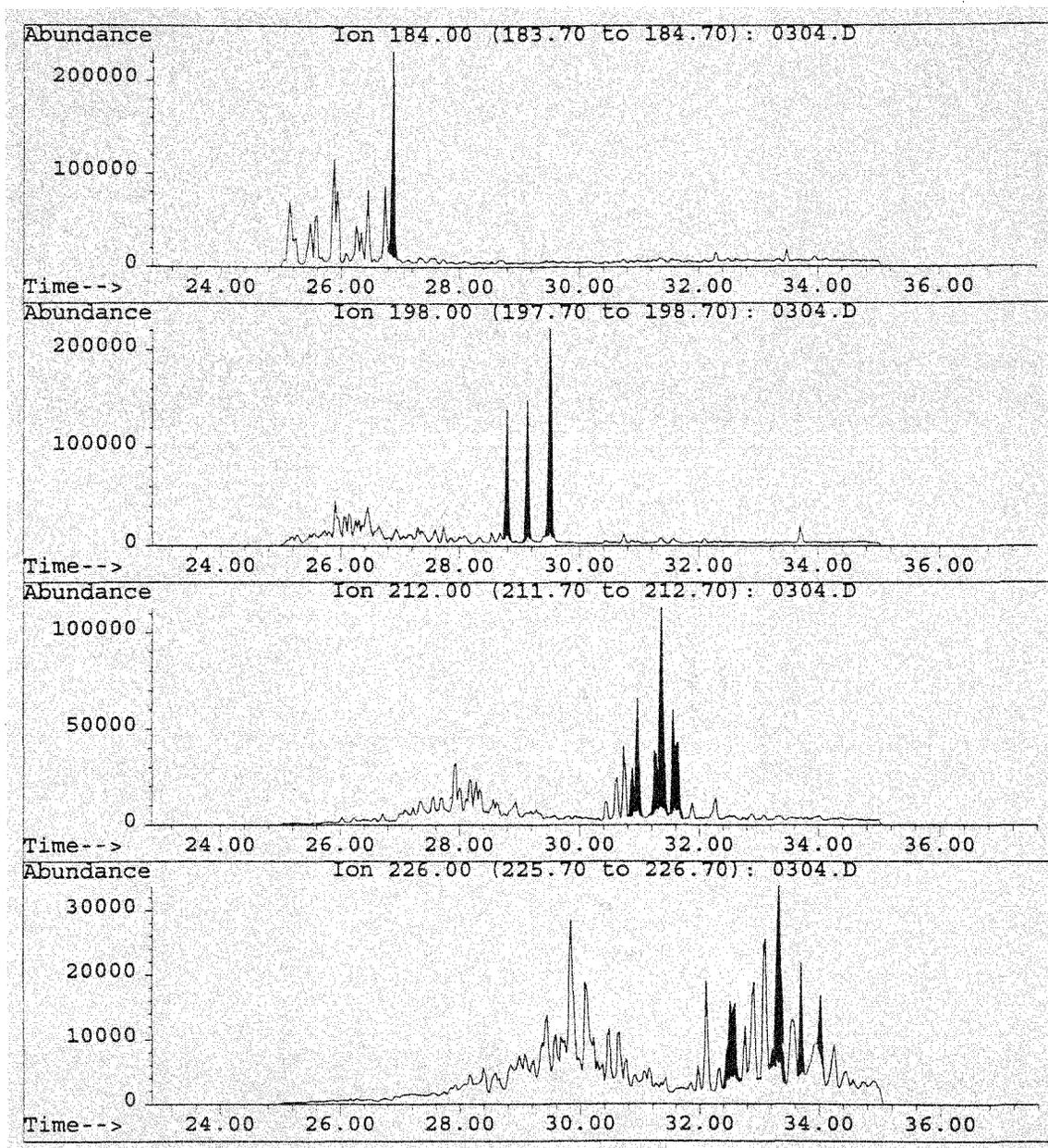


Fig.17. GC/MS Pattern for Dibenzothiophene ($m/z=184$), C1-Dibenzothiophene ($m/z=198$), C2-Dibenzothiophene ($m/z=212$) and C3-Dibenzothiophene ($m/z=226$) in Posidonia Shale SFE Extracts.

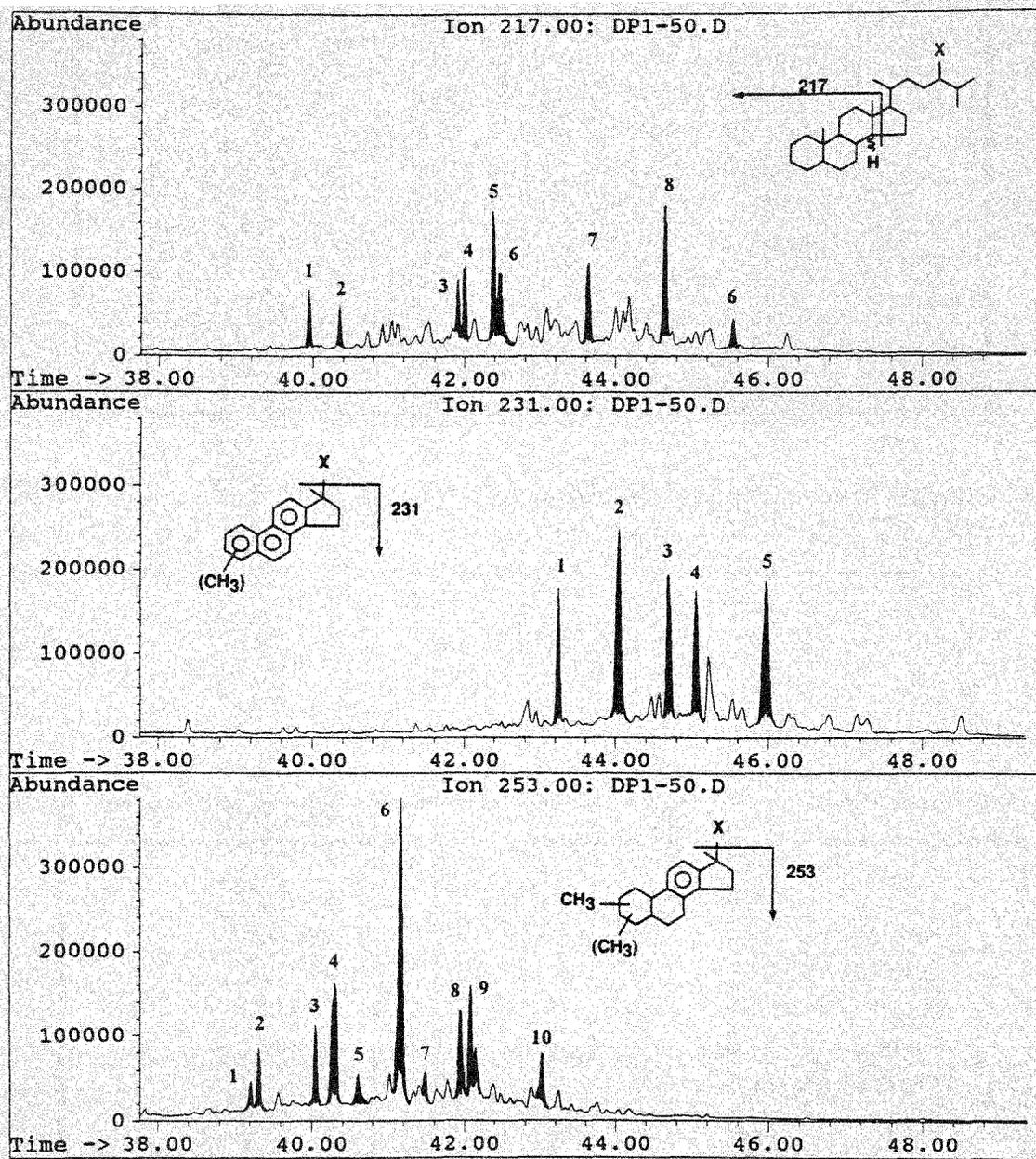


Fig. 18. GC/MS Pattern for Steranes ($m/z=217$), Monoaromatic ($m/z=231$) and Triaromatic ($m/z=253$) Steranes in Posidonia Shale SFE Extracts.

Peak	Formula	Name
1	C ₂₉ H ₅₀	17 α (h), 21 β (H)-30-Norhopane
2	C ₃₀ H ₅₂	17 α (h), 21 β (H)- Hopane
3	C ₃₁ H ₅₄	17 α (h), 21 β (H)-29-homohopane 22S
4	C ₃₁ H ₅₄	17 α (h), 21 β (H)-29-homohopane 22R
5	C ₃₂ H ₅₆	17 α (h), 21 β (H)-29-bishomohopane 22S
6	C ₃₂ H ₅₆	17 α (h), 21 β (H)-29-bishomohopane 22R
7	C ₃₃ H ₅₈	17 α (h), 21 β (H)-29-trishomohopane 22S
8	C ₃₃ H ₅₈	17 α (h), 21 β (H)-29-trishomohopane 22R
9	C ₃₄ H ₆₀	17 α (h), 21 β (H)-29-tetrakishomohopane 22S
10	C ₃₄ H ₆₀	17 α (h), 21 β (H)-29-tetrakishomohopane 22R
11	C ₃₅ H ₆₂	17 α (h), 21 β (H)-29-pentakishomohopane 22S
12	C ₃₅ H ₆₂	17 α (h), 21 β (H)-29-pentakishomohopane 22R

Table II- Peaks Identified for m/z=191 (Hopanes) Mass Chromatograms of Posidonia Shale SFE Extracts.

Peak	Formula	Name
1	C ₂₇ H ₄₈	C27 20S-13 β (H), 17 α (H)-diasterane
2	C ₂₇ H ₄₈	C27 20R-13 β (H), 17 α (H)-diasterane
3	C ₂₇ H ₄₈	C27 20S-5 α (H), 14 α (H), 17 α (H)-cholestane
4	C ₂₇ H ₄₈	C27 20R-5 α (H), 14 β (H), 17 β (H)-cholestane
5	C ₂₇ H ₄₈	C27 20S-5 α (H), 14 β (H), 17 β (H)-cholestane
6	C ₂₈ H ₅₀	C28 20S-5 α (H), 14 β (H), 17 β (H)-ergostane
7	C ₂₉ H ₅₂	C29 20S-5 α (H), 14 α (H), 17 α (H)-stigmastane
8	C ₂₉ H ₅₂	C29 20R-5 α (H), 14 β (H), 17 β (H)-stigmastane
	C ₂₉ H ₅₂	C29 20S-5 α (H), 14 β (H), 17 β (H)-ergostane
9	C ₂₉ H ₅₂	C29 20R-5 α (H), 14 α (H), 17 α (H)-stigmastane

Table III- Peaks Identified for m/z=217 (Steranes) Mass Chromatograms of Posidonia Shale SFE Extracts.

Peak	Formula	Name
1	C ₂₇ H ₄₂	5 β -Cholestane 20S
2	C ₂₇ H ₄₂	Diacholestane 20S
3	C ₂₇ H ₄₂	5 β -Cholestane 20R
	C ₂₇ H ₄₂	Diacholestane 20R
4	C ₂₇ H ₄₂	5 α -Cholestane 20S
5	C ₂₈ H ₄₄	5 β -Ergostane 20S
	C ₂₈ H ₄₄	Diaergostane 20S
6	C ₂₇ H ₄₂	5 α -Cholestane 20R
	C ₂₈ H ₄₄	5 α -Ergostane 20S
	C ₂₈ H ₄₄	5 α -Ergostane 20R
	C ₂₈ H ₄₄	Diaergostane 20R
	C ₂₉ H ₄₆	5 β -Stigmastane 20S
	C ₂₉ H ₄₆	Diastigmastane 20S
7	C ₂₉ H ₄₆	5 α -Stigmastane 20S
8	C ₂₈ H ₄₄	5 α -Ergostane 20S
9	C ₂₉ H ₄₆	5 β -Stigmastane 20R
	C ₂₉ H ₄₆	Diastigmastane 20R
10	C ₂₉ H ₄₆	5 α -Stigmastane 20R

Table IV- Peaks Identified for m/z=253 (Monoaromatic Steranes) Mass Chromatograms of Posidonia Shale SFE Extracts.

Peak	Formula	Name
1	C ₂₆ H ₃₂	Cholestane 20S
2	C ₂₆ H ₃₂	Cholestane 20R
	C ₂₇ H ₃₄	Ergostane 20S
3	C ₂₈ H ₃₆	Stigmastane 20S
4	C ₂₈ H ₃₆	Ergostane 20R
5	C ₂₈ H ₃₆	Stigmastane 20R

Table V- Peaks Identified for m/z=231 (Triaromatic Steranes) Mass Chromatograms of Posidonia Shale SFE Extracts.

Analyte	Conc. (ug/g)
C11-C19 n-alkanes	160.43
C20-C35 n-alkanes	66.26
Naphthalene	4.85
C1-Naphthalenes	16.60
C2-Naphthalenes	22.31
C3-Naphthalenes	17.36
Phenanthrene	4.32
Anthracene	0.00
C1-Phenanthrenes	8.64
C2-Phenanthrenes	8.28
C3-Phenanthrenes	4.23
Dibenzothiophene	2.23
C1-Dibenzothiophenes	5.61
C2-Dibenzothiophenes	4.77
C3-Dibenzothiophenes	2.35
Steranes	15.66
Monoaromatic Sterane	28.86
Triaromatic Steranes	40.88
Hopanes	12.72

Table VI- Analyte Recovery Obtained from Soxhlet Extraction of Posidonia Shale.

Analyte	Concentration (ug/g)			Mean (ug/g)	SD (ug/g)	CV (%)
	1	2	3			
C11-C19 n-alkanes	92.1	115.43	93.36	100.3	13.12	13.08
C20-C35 n-alkanes	42.16	52.36	45.45	46.66	5.21	11.16
Naphthalene	7.14	9.26	6.82	7.74	1.33	17.19
C1-Naphthalenes	27.73	24.15	22.86	24.91	2.52	10.13
C2-Naphthalenes	30.87	34.23	29.07	31.39	2.62	8.35
Phenanthrene	6.24	7.18	6.2	6.54	0.55	8.43
C1-Phenanthrenes	10.33	11.52	9.62	10.49	0.96	9.18
C2-Phenanthrenes	9.48	10.53	9.03	9.68	0.77	7.96
Hopanes	5.66	5.86	5.16	5.56	0.36	6.47

Table VII- Reproducibility Results for Soxhlet Extraction of Posidonia Shale

3.3 SFE.

3.3.1 Stepwise SFE.

3.3.1.1 Regular HCs.

The SFE temperature effect on the extraction efficiency of n-alkanes and aromatic HCs from Posidonia Shale samples is shown in Figures 19 to 23. Quantitative results for the stepwise SFE show that most of these compounds present higher recoveries at both end members of the temperature range (50 and 350°C) with lower recoveries at the intermediate temperatures. The same trend was observed in similar studies performed with Kupferschiefer Shale samples (Chen, 1996). Consistent increments in recoveries were reported for stepwise SFE of New Albany Shale (Furton, 1994) although it should be noted that in that case only three temperatures were studied.

The results suggest a non-linear temperature dependence of the extraction efficiency due to the fact that individual compounds present in this sample are speciated in different ways throughout the solid substrate. Therefore, different fractions of the same analyte were extracted under different energy (temperature) conditions. Obviously, those analytes (or analyte fractions) that are preferentially released at higher temperatures are likely to have a stronger interaction with the solid substrate or are released due to thermally induced structural changes in the matrix. This analyte-matrix association may be caused by "trapping" in porous media, microfractures and within the organic matter, or by chemical bonding to the matrix (possibly sulfur bonds).

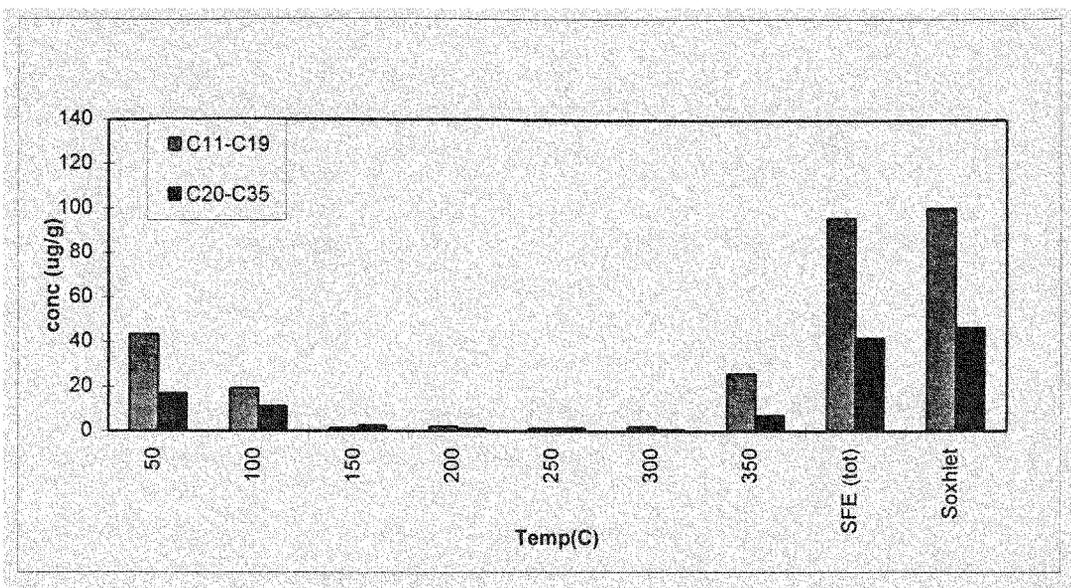


Fig.19. N-Alkanes Extraction Recoveries Obtained during Stewise SFE of Posidonia Shale.

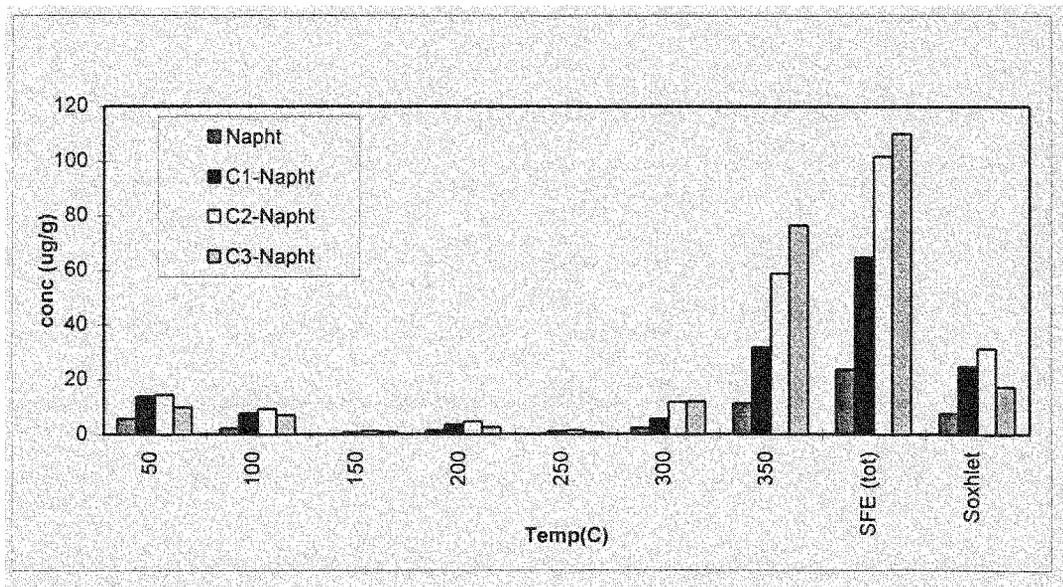


Fig. 20. Naphthalene and Alkyl naphthalene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.

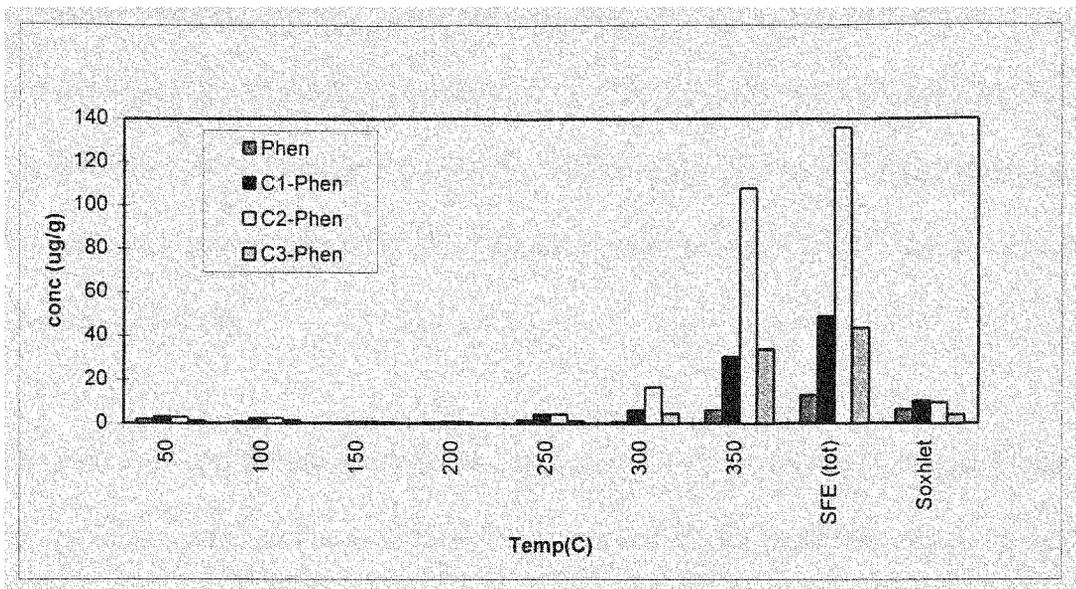


Fig. 21. Phenanthrene and Alkylphenanthrene/anthracene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.

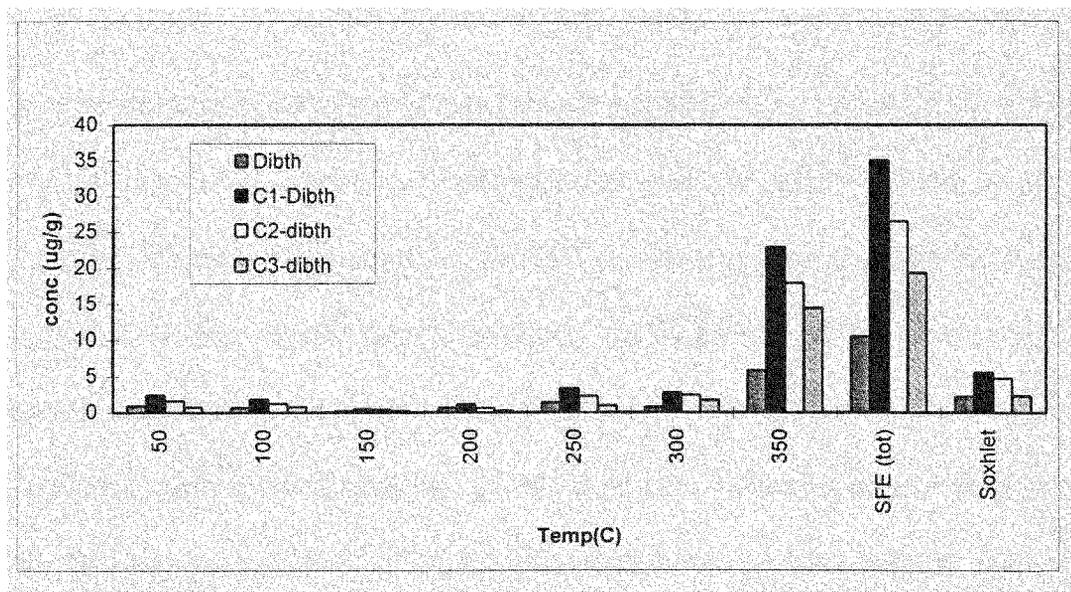


Fig. 22. Dibenzothiophene and Alkyldibenzothiophene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.

The extraction efficiency increment at 350°C (related to the correspondent 50°C recovery) was more pronounced for the PAHs with a 772, 3700 and 1933 % for C3-naphthalenes, C2-phenanthrenes and C3-dibenzothiophenes, respectively, which show the highest increments for each homologue serie at 350°C. This increment was only of 60% for short chain n-alkanes (C11-C19).

The corresponding Soxhlet recoveries (versus total HT-SFE) are shown in each Figure for comparative purposes. For n-alkanes similar extraction efficiencies are observed through both methods, while a dramatic increase in aromatic hydrocarbon recovery was obtained using the SFE relative to the traditional Soxhlet extraction method. Similar results have been reported by Furton et al (1994) for New Albany Shale.

The observed differences in the relative extraction efficiencies of aromatic HCs compared to that of aliphatics is probably due to the higher polarity (aromaticity), molecular size and geometry (planarity) of the aromatic compounds, all allowing for stronger interactions with the macromolecular organic matter. Obviously, the superiority of SFE if compared with Soxhlet, is evident for those analytes that are more closely associated with the matrix. Considering other practical advantages as saving of time and the use of an inert, pure, non-toxic and inexpensive fluid like CO₂, the SFE would be the method of choice even for the aliphatic compounds.

Anthracene was observed in extracts obtained in successive extractions only above 250

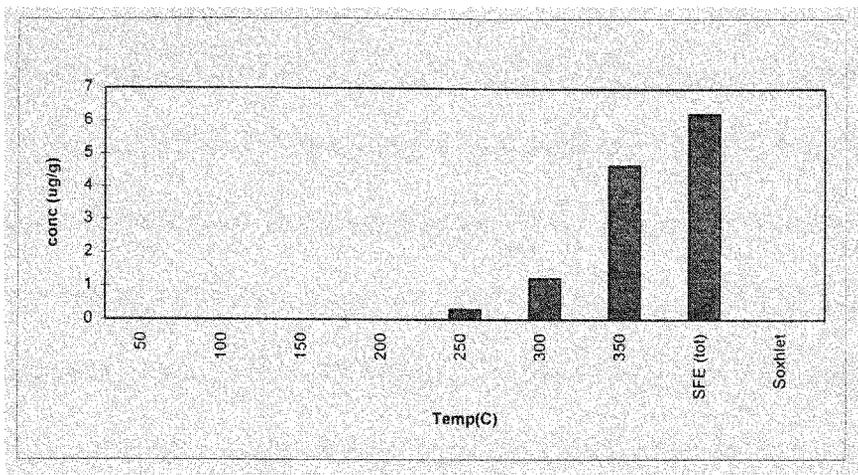


Fig. 23. Anthracene Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.

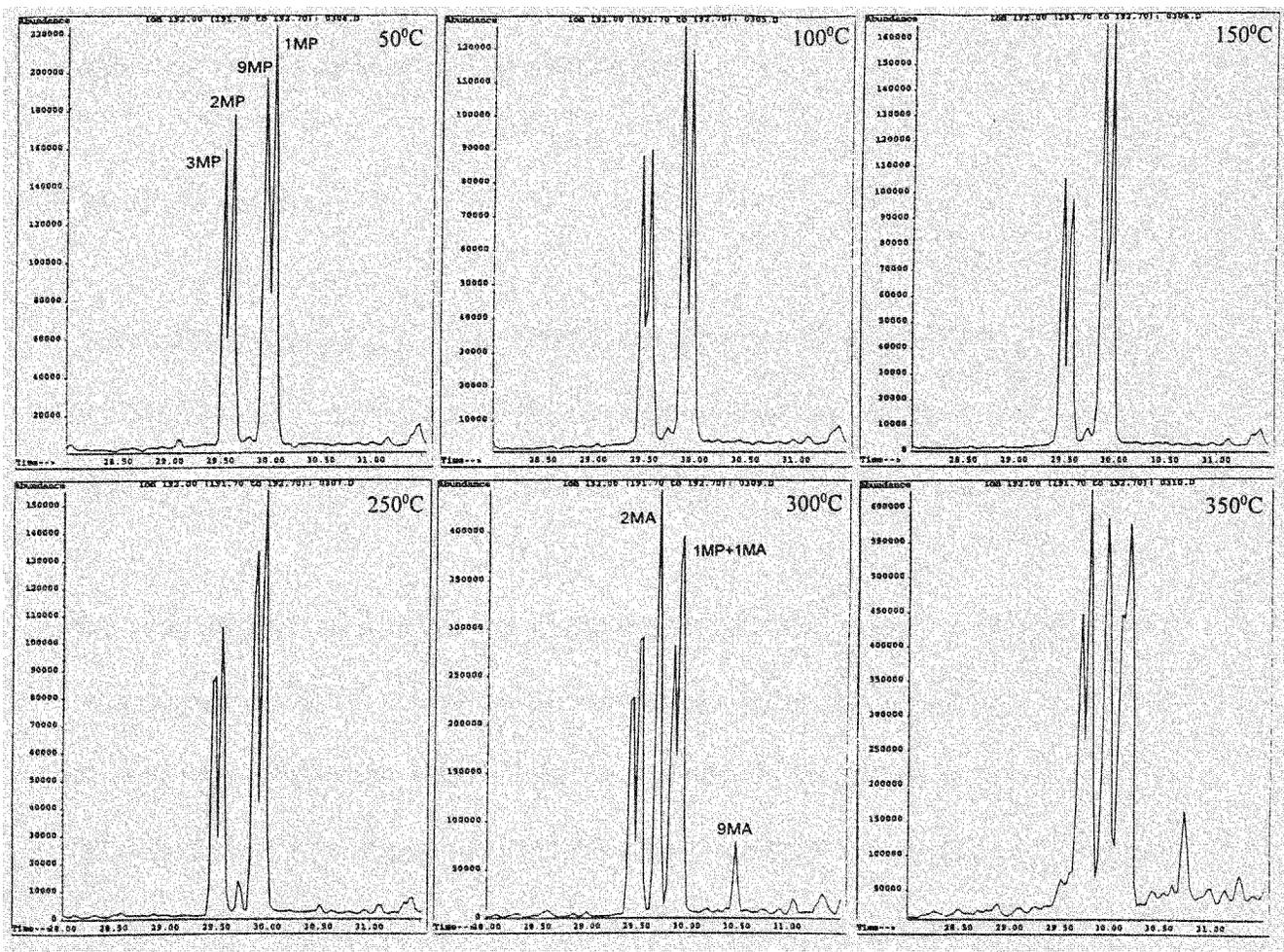


Fig. 24. GC/MS Chromatograms for Methylphenanthrene/anthracenes at Different Temperatures during Stepwise SFE of Posidonia Shale.

degrees (Fig. 23) and this behavior is consistent with those reported for Kupferschiefer and New Albany shales. In addition, compounds tentatively identified as methylanthracenes (Fig. 24) were observed at the same temperatures. These compounds apparently present a very strong association with the solid matrix, and seem to have a different origin than the corresponding phenanthrenes. The different behavior between phenanthrene and anthracene is likely to be associated to the difference in the geochemical characteristics of their precursor molecules. It has been suggested (Radke et al., 1982) that the anthracene molecule is less mobile than phenanthrene in the pore space of coals what could explain the fact that anthracene could only be recovered using HT-SFE.

3.3.1.2. Biomarkers

The total hopane and total sterane HT-SFE distribution patterns are very similar (Figures 25 and 26), showing a maximum at 100°C and only a slight increase at 350 °C . Total stepwise cumulative SFE recoveries were higher than the corresponding Soxhlet extraction values.

For both, hopanes and steranes, a small fraction appears to be strongly associated with the organic matter which is evident in the recovery increment at 350°C. This effect is also obvious from a molecular point of view, as suggested by the change of the trend of the (S/S+R) C-31 $\alpha\beta$ homohopane ratio with the extraction temperature shown in Fig. 27. Here, a decrease in thermal maturity is observed at the highest extraction temperature levels. This is an unexpected result, since all hopanes in the sample (Posidonia Shale) should have

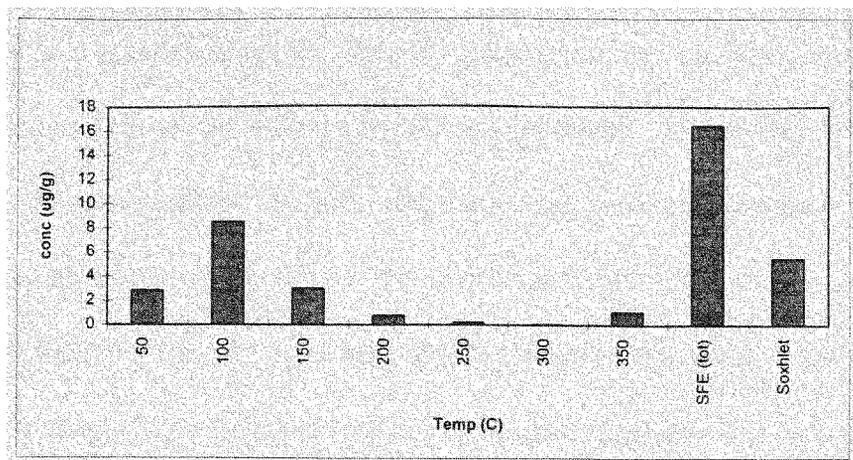


Fig. 25. Hopane Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.

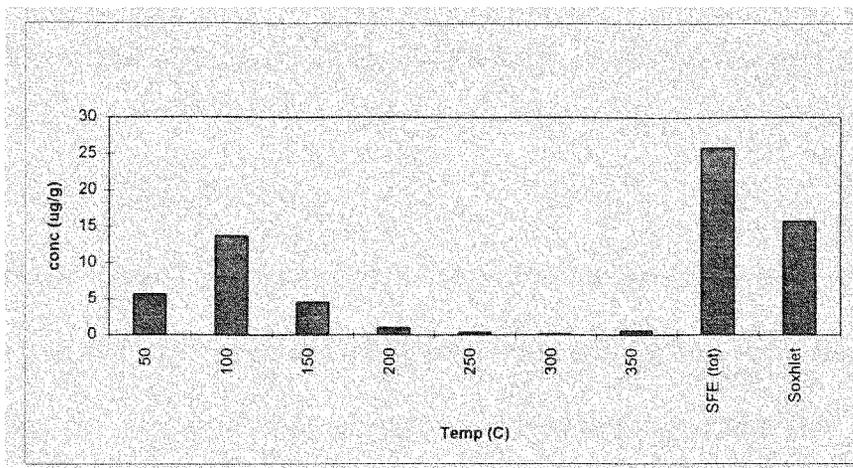


Fig. 26. Sterane Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.

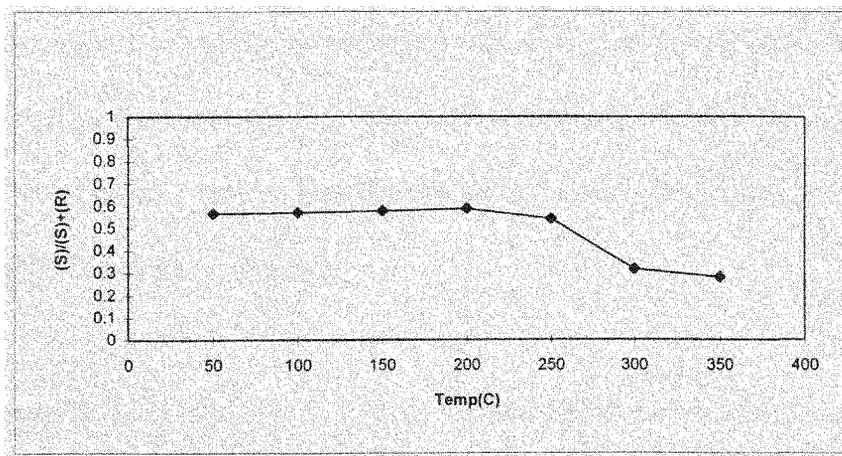


Fig. 27. (S)/(S+R) C-31 Hopane Ratio versus SFE Temperature during Stepwise SFE of Posidonia Shale.

undergone the same thermal history. However, such a maturity inversion has been reported for hopanoic acids in ancient sediments (Jaffé and Gardinali, 1990) and has been suggested to be due to steric protection (from diagenetic isomerization processes) of "trapped" biomarkers. Molecules of low thermal maturity stages (R isomers) may become "trapped" within or bound to the matrix at an early stage of diagenesis, and can only be released by high temperature induced structural rearrangements in the kerogen. The chromatograms for the hopanes (Figure 28) obtained at the different temperatures of stepwise SFE, clearly show the "maturity inversion" effect discussed above for the C31-homohopane (i.e. an increment in the relative ratio of 22 R vs. 22 S). Similar results were reported previously by Chen (1996).

Figures 29 and 30 show the mono and triaromatic steroids extracted at different temperature levels, as well as the relationship of the (TA/TA+MA) ratio with the extraction temperature. In this case the extraction efficiency shows a maximum at 100⁰C but no detectable concentrations of these compounds were observed at temperatures higher than 200⁰C. No evidence of strong matrix interactions of tri and monoaromatic steroidal HCs were observed because these analytes appear to be totally extracted at relatively low temperatures if compared with aliphatic and other aromatic hydrocarbon behavior (PAH). A weak analyte-matrix interaction could also explain the similar extraction efficiencies obtained for both Soxhlet and SFE extraction methods.

The increment in the (TA/TA+MA) ratio at 150 and 200⁰C may be due to the fact that the

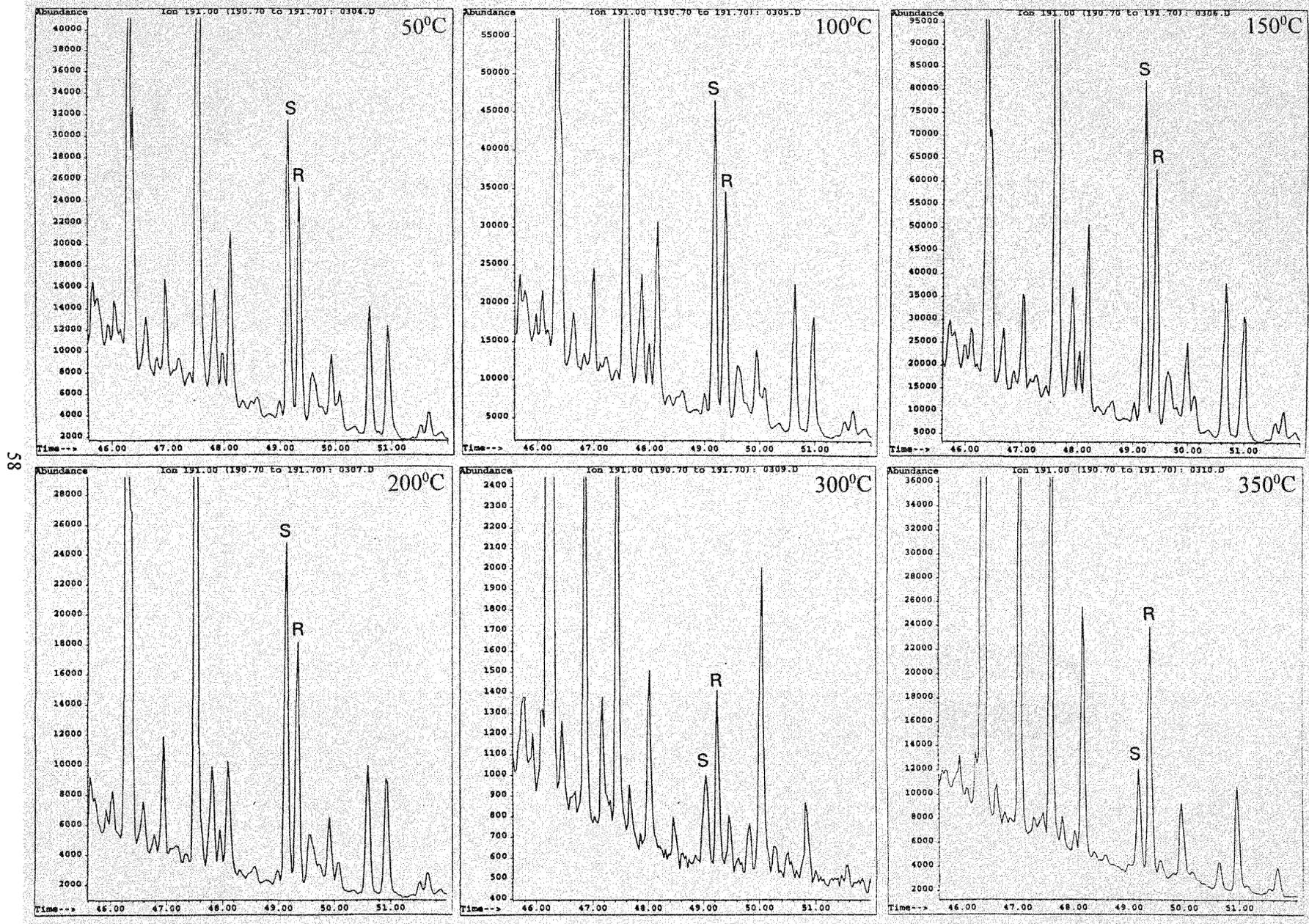


Fig. 28. GC/MS Chromatograms for C31 α β Homohopanes (S and R) at Different Temperatures during Stepwise SFE of Posidonia Shale.

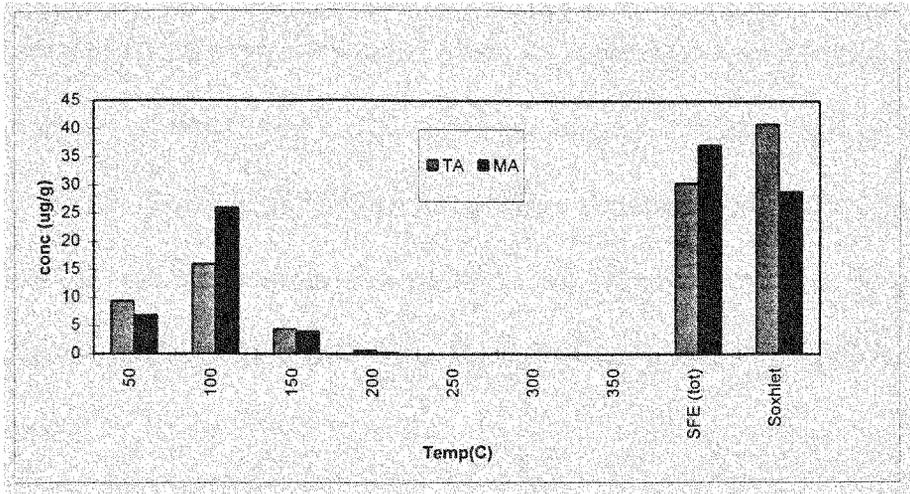


Fig. 29. Mono and Triaromatic Sterane Extraction Recoveries Obtained during Stepwise SFE of Posidonia Shale.

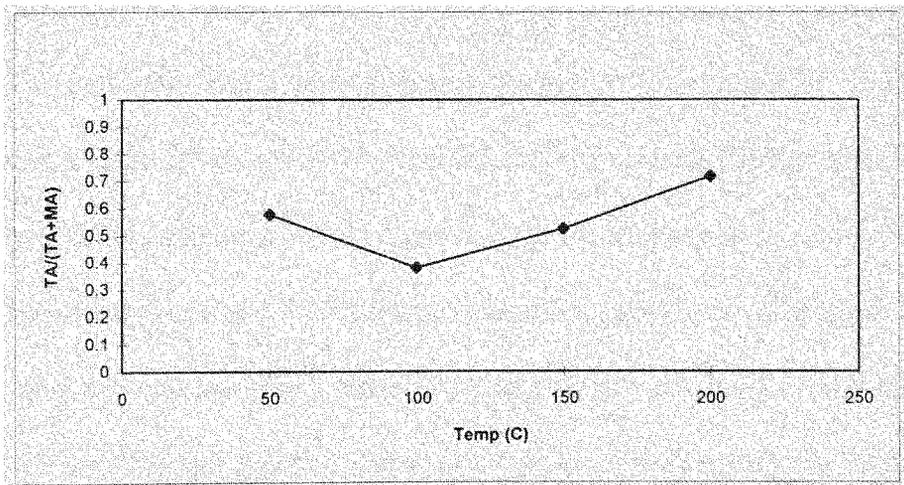


Fig. 30. TA/(TA+MA) Ratio versus SFE Temperature during Stepwise SFE of Posidonia Shale.

higher aromaticity of the triaromatic steroids increases their possibilities to get trapped into the porous macromolecular structure or to have a stronger surface interaction with the matrix when compared with the monoaromatics, which are expected to have a weaker association. The absence of a “maturity inversion” effect for aromatic steranes might be due to their aromatic nature. These compounds present a higher planarity compared to hopanes which may allow the “trapped” molecules to suffer the aromatization process in the pores, while the configurational isomerization in the side chain of the “trapped” hopanes is expected to be inhibited.

The behavior of the Prist/Phyt and C 18/Phyt ratios with increasing SFE extraction temperatures is shown in Figure 31 for Posidonia and Kupferschiefer shales. For both sediments, the Prist/Phyt ratio remains practically constant in the experimental temperature range, possibly with a slight increase at 350°C for Kupferschiefer. This increment is not likely to be significant, and is probably due to the experimental variability. Therefore, there is no evidence of a difference in the interaction of the macromolecular organic structures with any of these two isoprenoidal compounds. It is interesting to note that the Prist/Phyt ratio values are higher than 1 for Posidonia shale which is evidence of an initial oxygenated depositional environment where phytol was preferentially oxidized to form phytanic acid forming pristane through decarboxylation reactions. On the other hand, the Prist/Phyt ratios for Kupferschiefer shale ranged below 1 (except for 350°C) which could be interpreted as the existence of more reducing environment at early depositional stages where phytol was prone to be quickly hydrogenated into dihydrophytol which through dehydration and

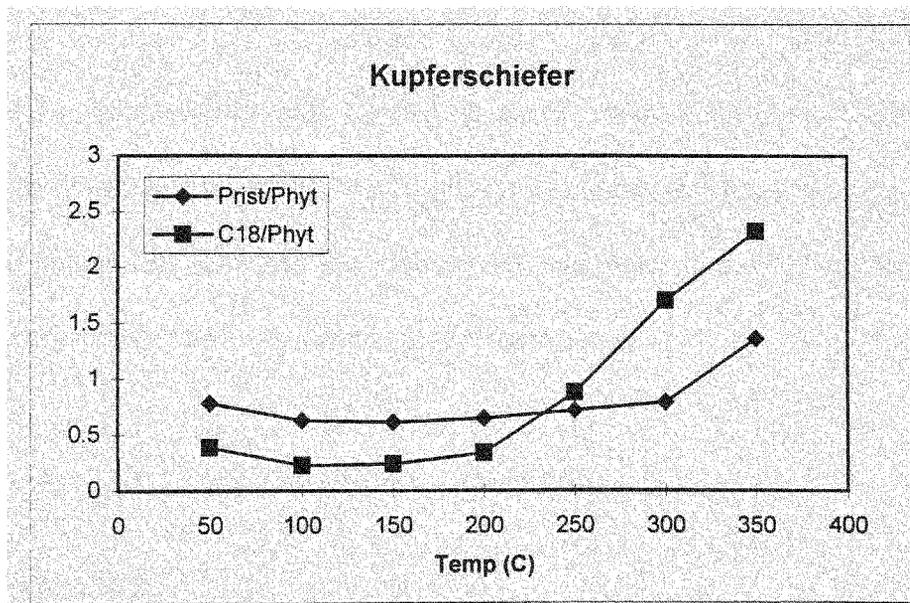
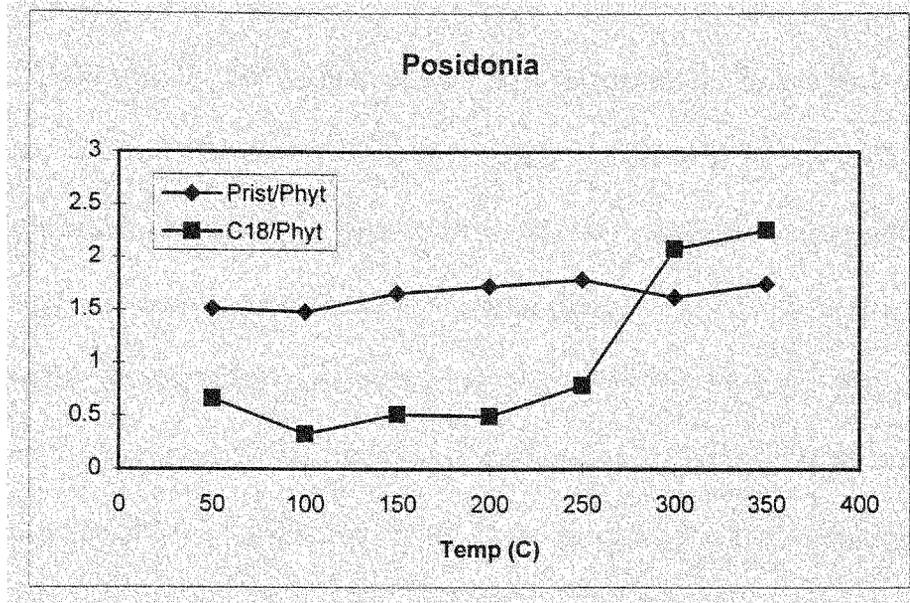


Fig. 31. Prist/Phyt and C18/Phyt Ratios versus SFE Temperature during Stepwise SFE of Posidonia and Kupferschiefer Shales.

hydrogenation reactions was converted to phytane.

In contrast, while the C 18/Phyt ratio doesn't show accentuated changes with extraction temperatures below 250°C for any of the two shales, a dramatic increment is observed at temperatures higher than 200°C for both samples. This is possibly due to the existence of a much stronger association with the matrix for normal alkanes such as C 18 than for branched compounds like phytane and pristane that are not going to be easily "trapped" into the porous system due to their more "bulky" molecular conformation. The relative abundances of phytane, pristane and C 18 alkane are shown in the chromatograms corresponding to the different temperatures used in the stepwise procedure (Figure 32). Generation of C 18 alkane due to thermal cracking is unlikely at these "low" temperatures, and in fact, no n-alkenes were observed.

Results for the reproducibility study for the stepwise SFE method are plotted in Figure 33. The standard deviation and variation coefficient values are acceptable, considering the great number of factors that influence the experimental variability in the SFE system. These variations do not affect the observed trends discussed above.

3.3.2 Direct temperature effect on SFE extraction efficiency.

3.3.2.1. Regular HCs.

The results obtained for n-alkanes and aromatics from HT-SFE using a new, non-extracted,

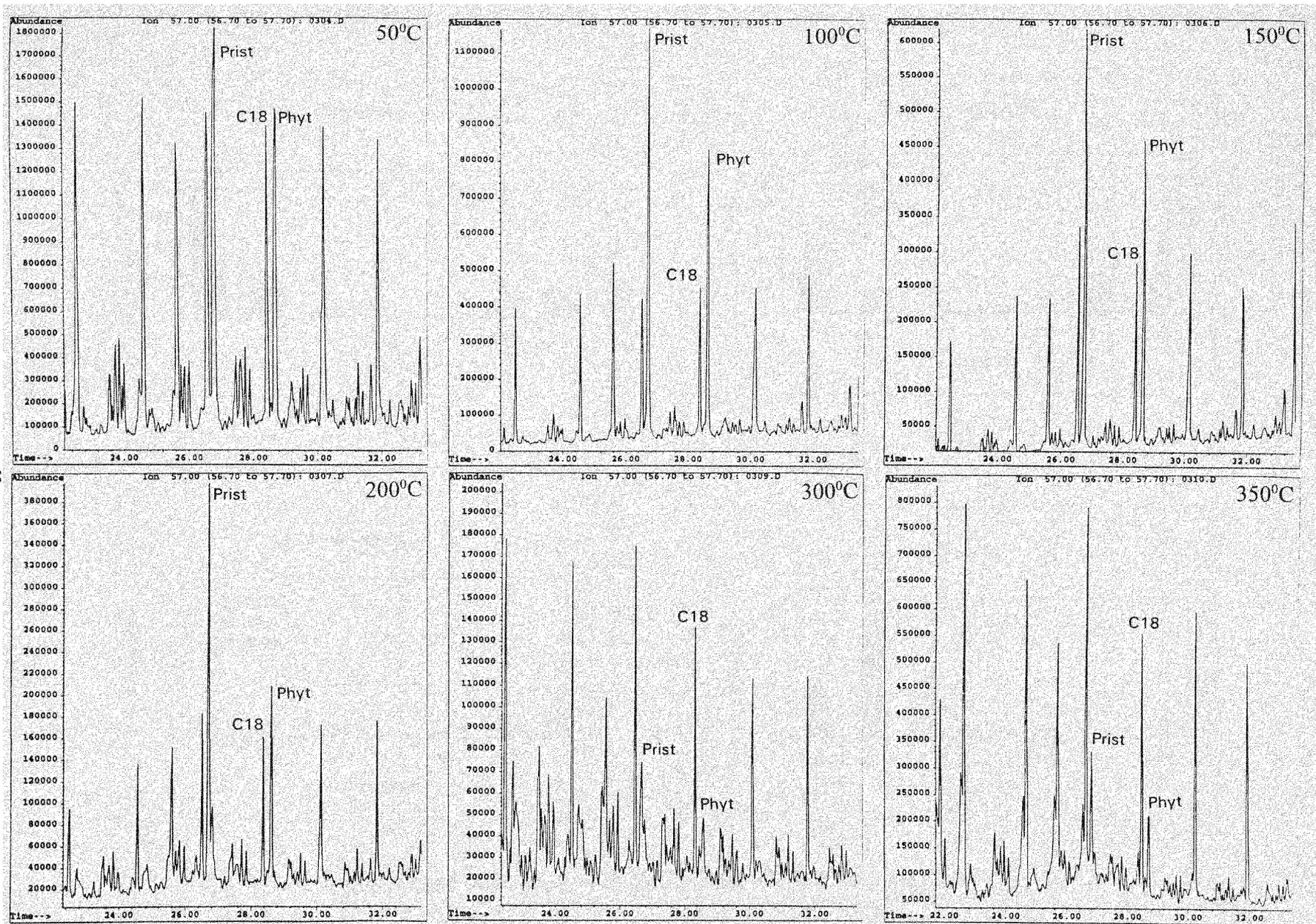


Fig. 32. GC/MS Chromatograms (ion 57) Showing Relative Abundances of Pristane, Phytane and C18 at Different Temperatures during Stepwise SFE of Posidonia Shale.

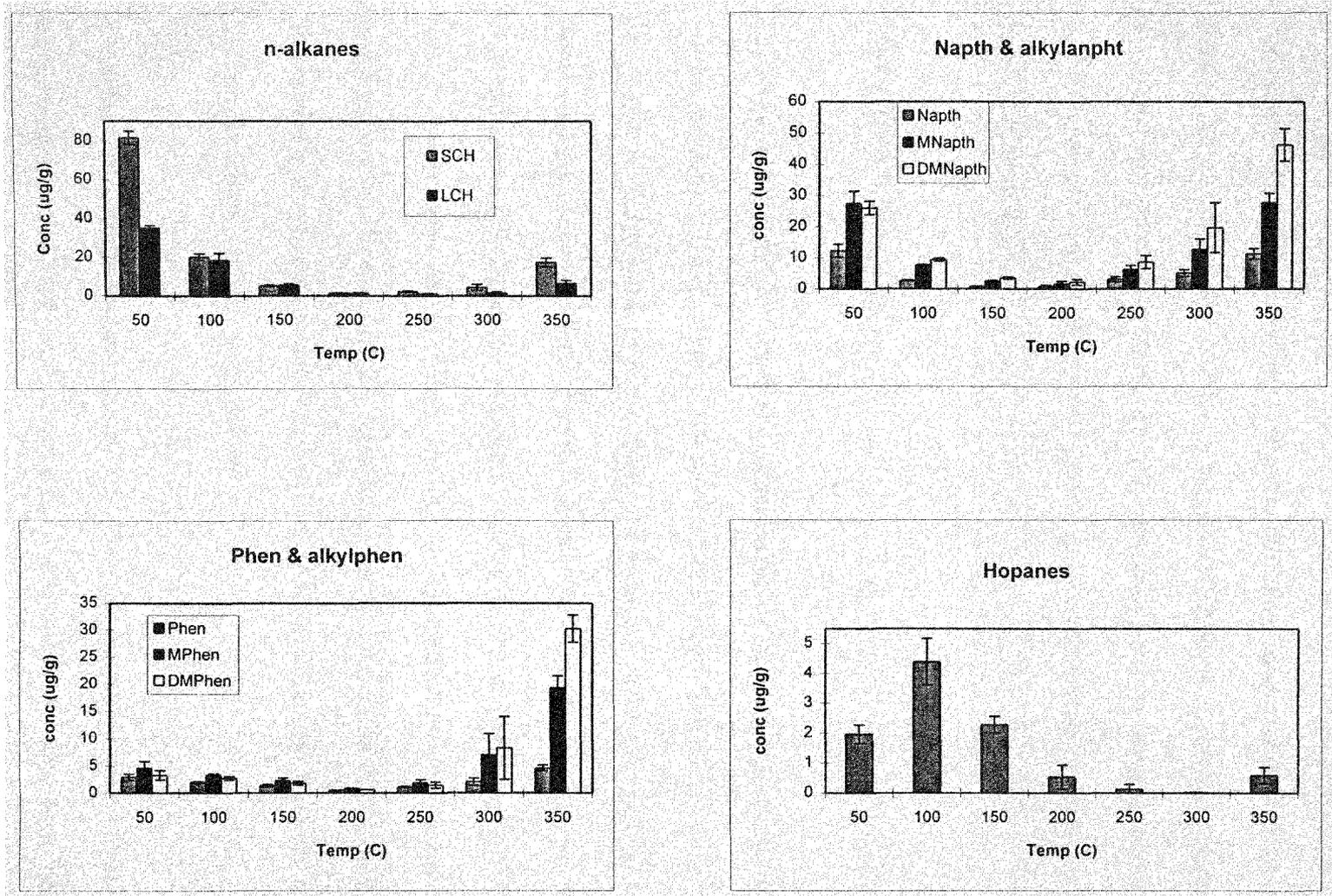


Fig. 33. Reproducibility Results for Stepwise SFE of Posidonia Shale.

sample each time (Figures 34 to 38) show that the PAHs have more accentuated extraction efficiency increments with increasing temperature than n-alkanes. This corroborates the observations already discussed for the stepwise SFE experiment, that the PAHs have a larger fraction that is strongly associated with the matrix. Similarly, anthracene (Figure 38) and methylanthracenes were detected only for extractions made at temperatures higher than 250°C. In general terms, temperature of extraction seems significantly more important for PAHs than for aliphatic HCs.

3.3.2.2. Biomarkers

For hopanes and steranes there is no apparent effect of the extraction temperature on the analyte recovery (Figures 39 and 40). It should be noted that , during these experiments, the samples were individually extracted during 1.5 h, collecting the total extracts. This could have overshadowed some effects that are evident in the stepwise procedure where the time is cumulative and the sample remained exposed to consecutively higher temperatures during a much longer period of time, during which the extracts were collected after each temperature level. However, for the stepwise experiments it was found, that the bulk of the biomarkers was extracted at relatively low temperatures. Therefore, as in the case of the alkanes, the above result is not surprising

Similarly, the pattern for extracted mono and triaromatic steroids (Figure 41) at increasing temperatures shows a 100°C maximum and remains practically constant from a temperature

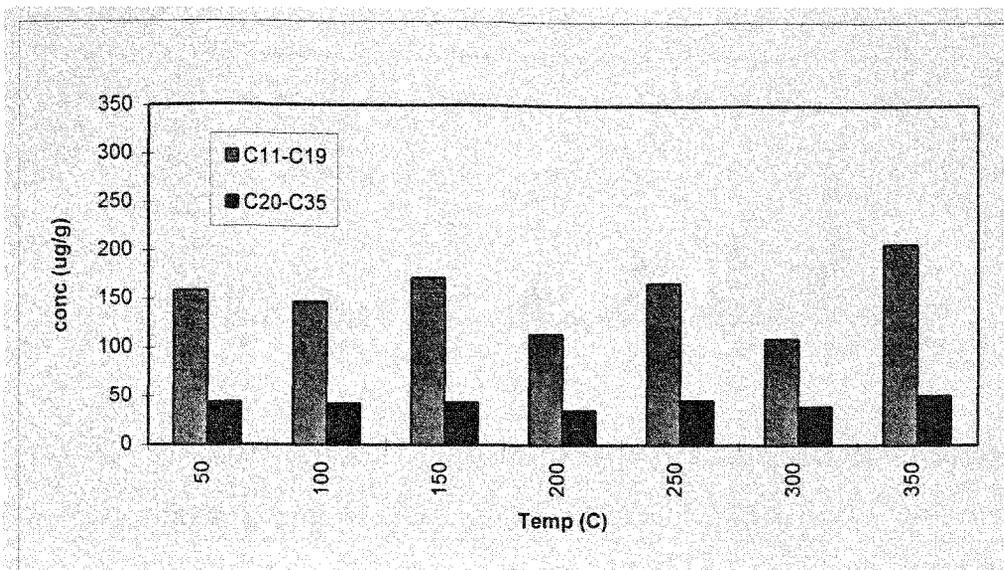


Fig. 34. N-Alkane SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

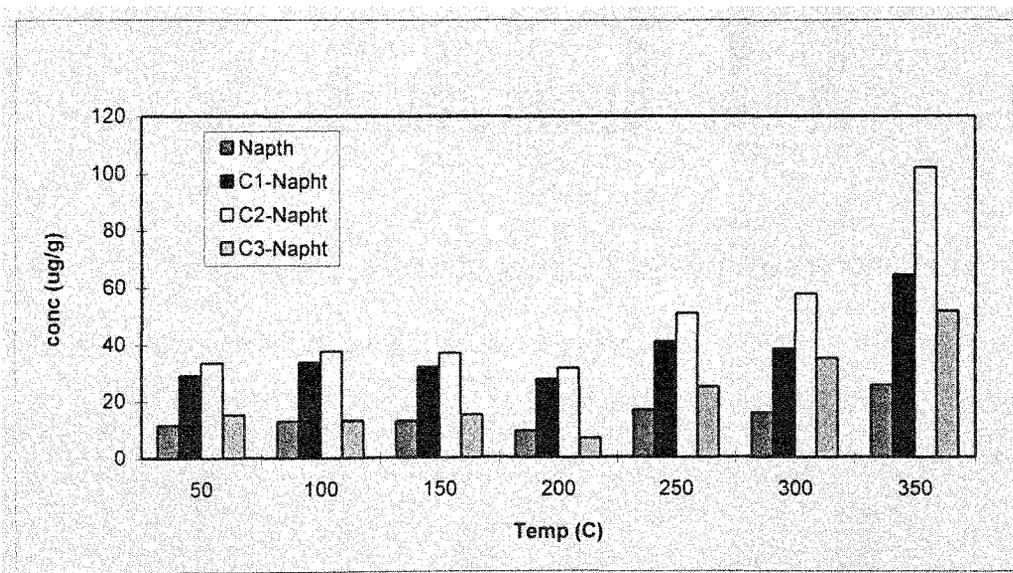


Fig. 35. Naphthalene and Alkyl naphthalene SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

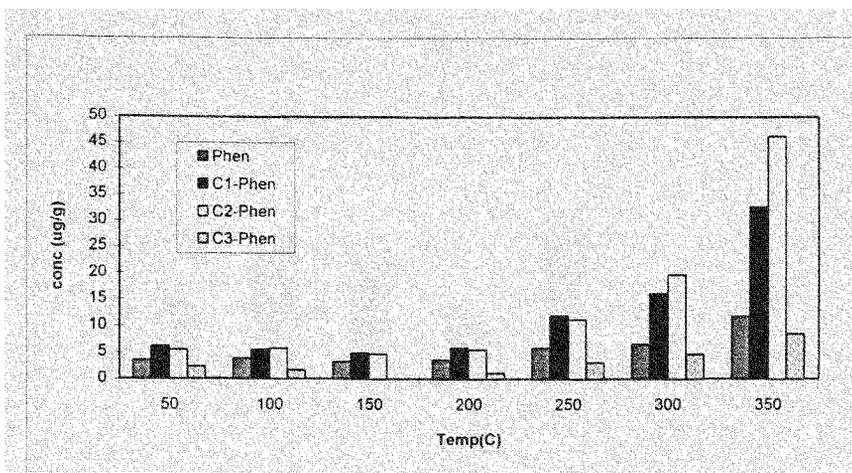


Fig. 36. Phenanthrene and Alkylphenanthrene SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

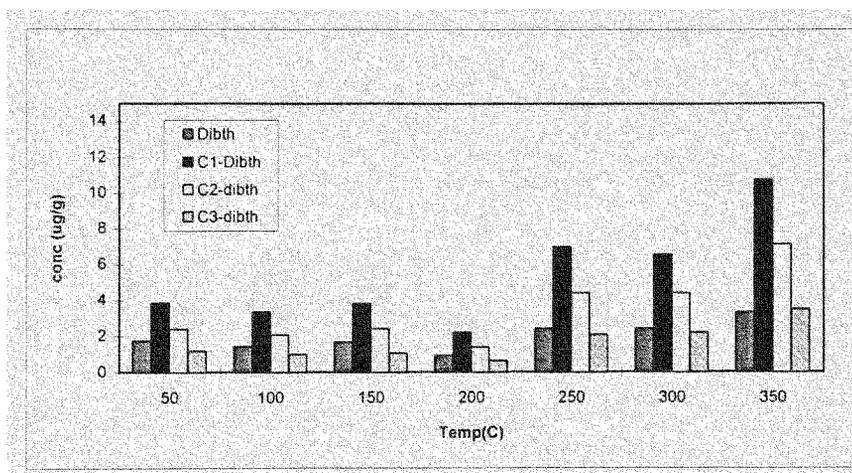


Fig. 37. Dibenzothiophene and Alkyldibenzothiophene SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

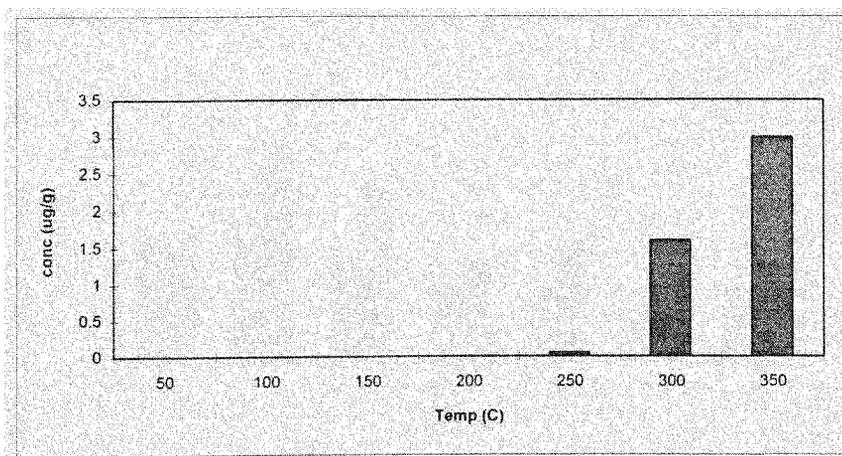


Fig. 38. Anthracene SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

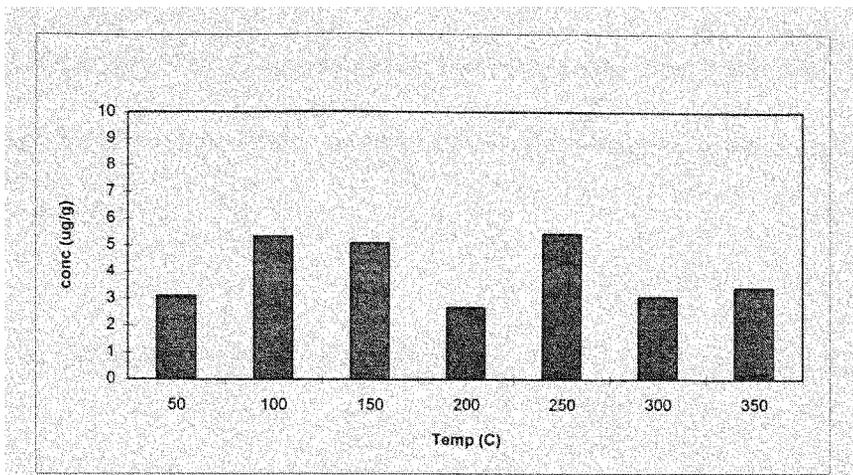


Fig. 39. Hopane SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

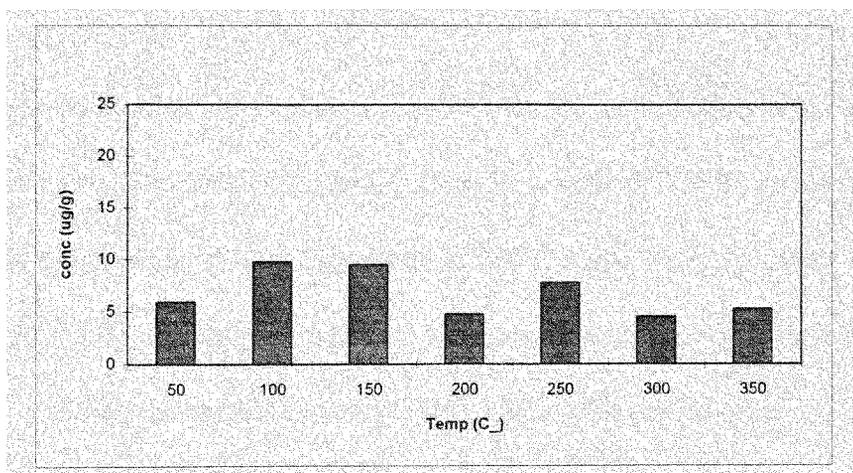


Fig. 40. Sterane SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

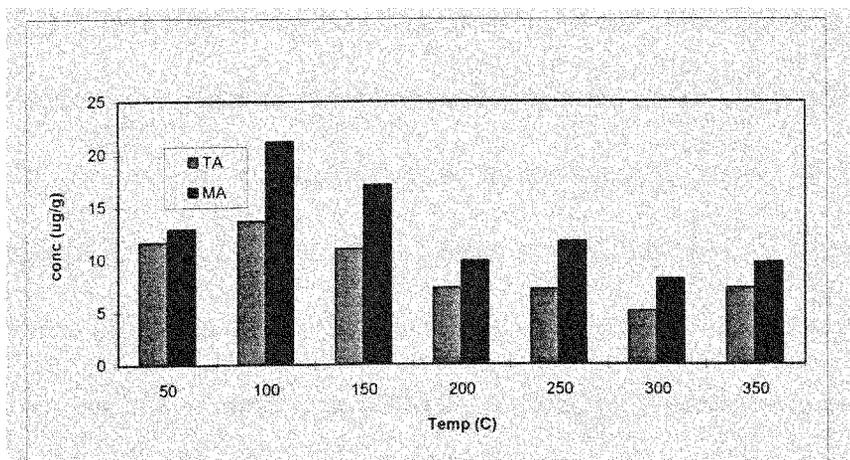


Fig. 41. Mono and Triaromatic Sterane SFE Extraction Recoveries Obtained Using a Fresh Sample at Each Temperature.

level of 200°C and on which is perfectly consistent with the results reported and discussed for the stepwise procedure where no detectable concentrations of these compounds were found after 200°C.

The reproducibility study for 50, 150, 250 and 350°C SFE extractions are shown in Figure 42 .

3.3.3 Thermal treatment of nonextracted and preextracted sediment samples.

Figure 43 shows the effect of a previous thermal treatment of the nonextracted sample at two temperature levels (200 and 350°C) during 3 and 12 h on the 50°C SFE recovery. To expose the Posidonia shale samples to a temperature of 200°C doesn't seem to cause any effect on the final 50°C SFE recoveries for n-alkanes, hopanes, naphthalene, phenanthrene and alkyl aromatics in the experimental time range used, while a thermal treatment at 350°C causes consistent increments in extraction efficiencies with the time of exposure. This suggests, that irreversible thermally induced rearrangements in the matrix took place during the 3 hour heating at 350°C. Considering that this effect could be probably observed in shorter periods of time, we decided to perform an experiment under different conditions (See Experimental, section 2.7) with a pre-extracted sediment to minimize the effect of high analyte concentrations in the SFE extracts. The influence of a holding step of 1 h at 350°C on a sediment previously extracted at 250°C is shown in Figure 44. The extraction recovery increments obtained for n-alkanes, naphthalene, phenanthrene, dibenzothiophene and

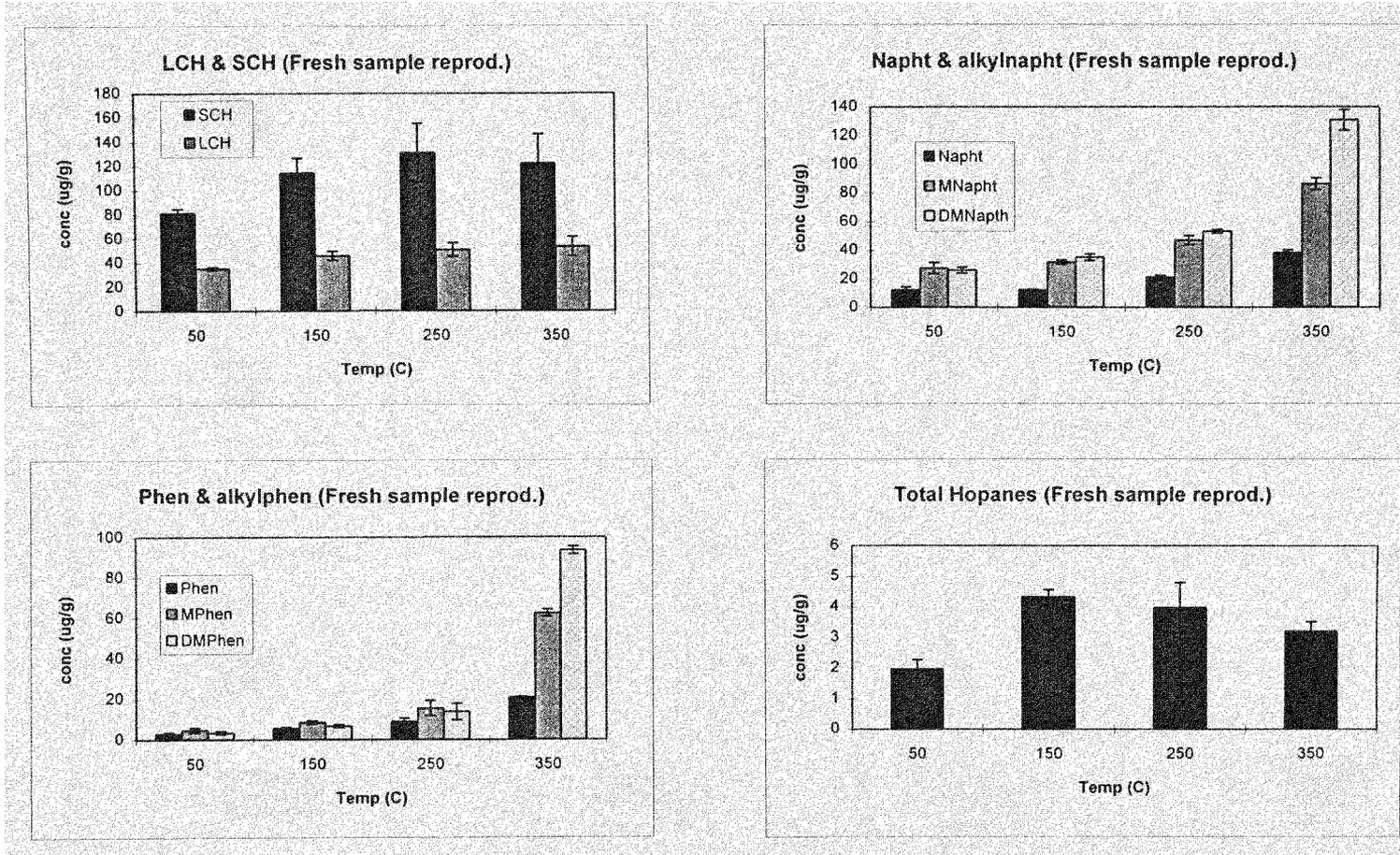


Fig. 42. Reproducibility Results for SFE of Posidonia Shale Using a Fresh Sample Each Time

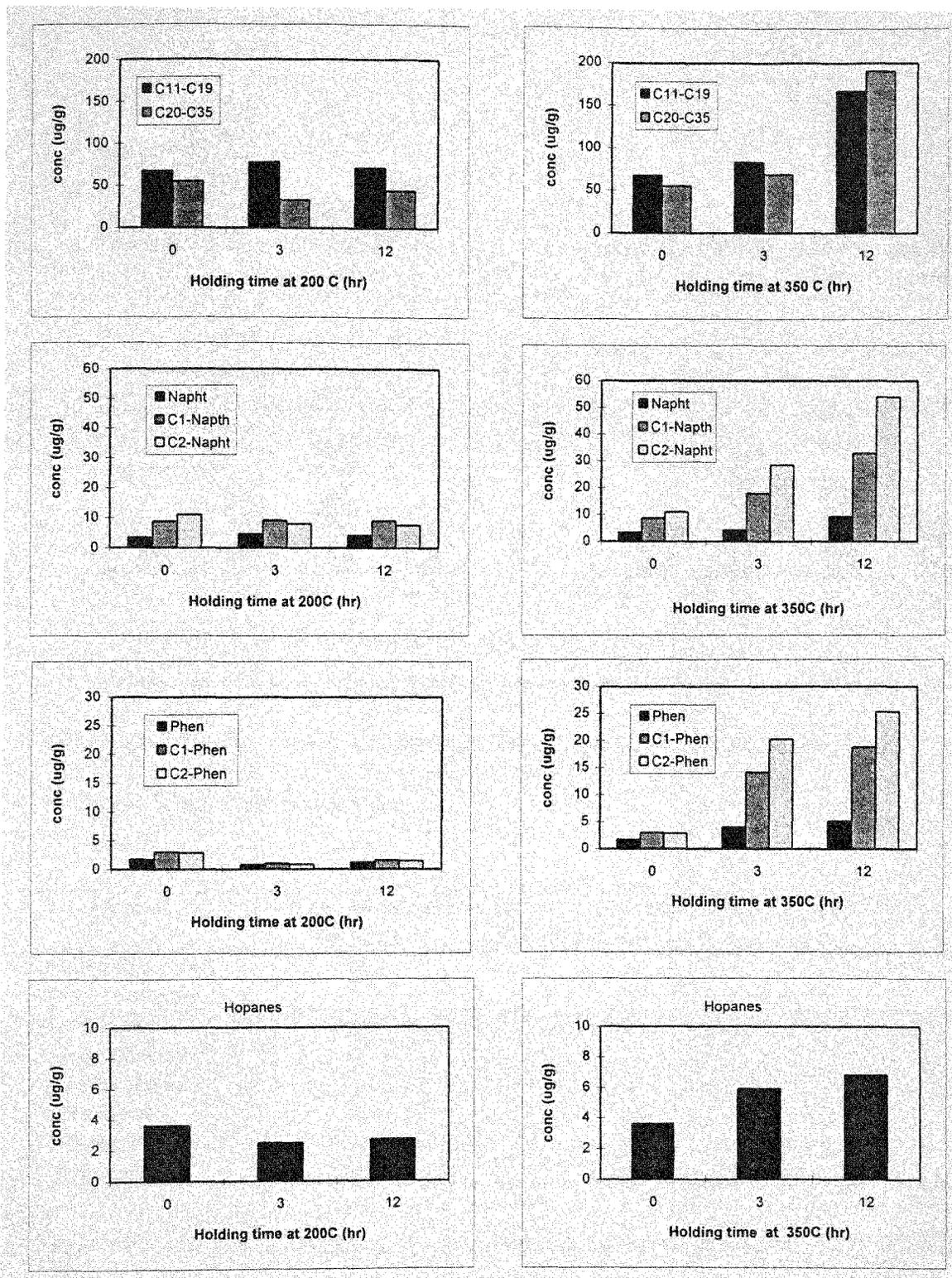
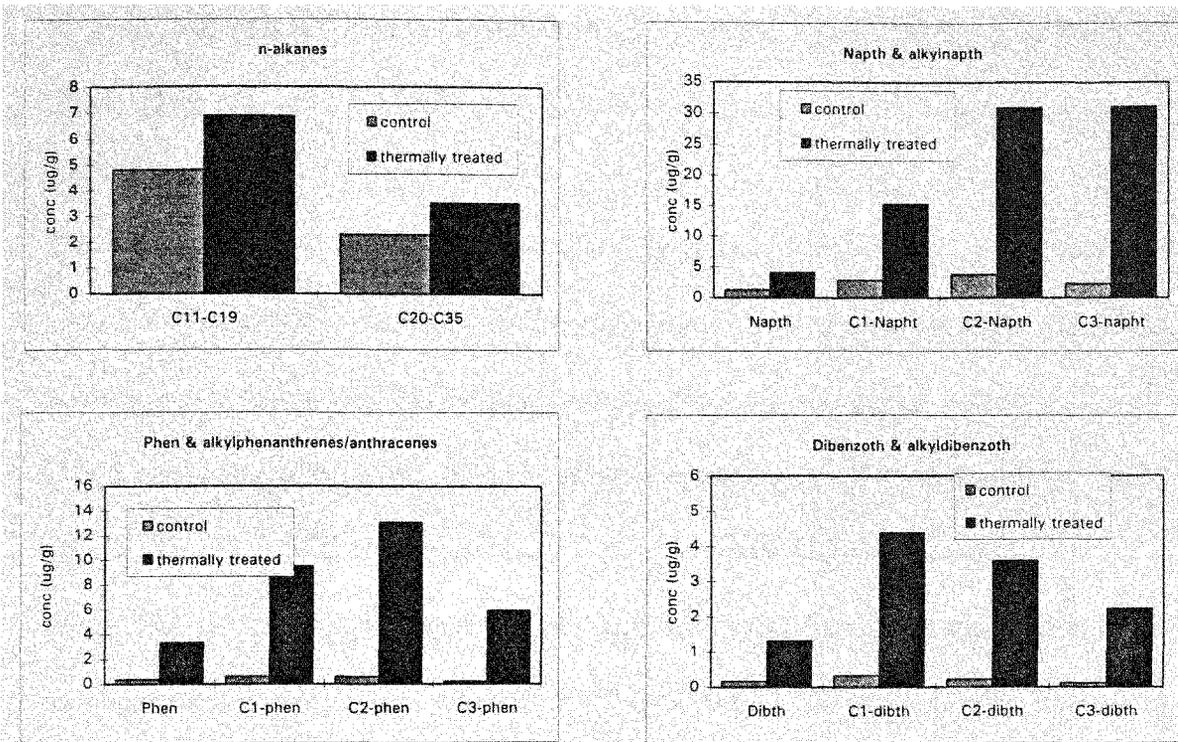


Fig. 43. Effect of Previous Thermal Treatment at 200 °C (left side) and 350 °C (right side) of Non-Extracted Posidonia Shale Samples during 0, 3, and 12 h on the 50 °C SFE Recovery



Control: 50°C SFE extraction recoveries of the 250°C SFE pre-extracted sediment.

Thermally treated: 50°C SFE extraction recoveries of the 250°C SFE pre-extracted sediment after thermal treatment at 350°C during 1 hour.

Fig. 44. Effect of a Thermal Treatment on Some Lipid 50°C SFE Recoveries Using a Pre-extracted Posidonia Shale Sample.

Compound	Increment percentage rate (%) related to 250 °C SFE recoveries	
	350 °C SFE	50 °C SFE of thermally treated sediment
C11-C19 n-alkanes	24	4
C20-C35 n-alkanes	11	8
Naphthalene	52	24
C1-naphthalenes	57	37
C2-naphthalenes	101	61
Phenanthrene	102	58
C1-phenanthrenes	173	79
C2-phenanthrenes	321	119

Table VIII- Increment Percentage Rates in Some HCs Recoveries (related to 250 °C SFE) for 350 °C SFE and 50 °C SFE of Thermally Treated (350 °C, 1h) Posidonia Shale Samples.

alkylated homologues suggest the existence of irreversible thermal effects on the solid matrix after only 1 hour at 350°C.

These results confirm the significant temperature contribution to the HT-SFE recoveries obtained from ancient sediments. At the same time, it was observed that the increment percentage rate related to 250°C SFE hydrocarbon recoveries using fresh sample each time, is higher if considering 350°C SFE extraction efficiencies than for the thermally treated (350°C, 1h) 50°C SFE recoveries (Table VIII) indicating that the combined effect of a supercritical fluid passing through the solid during the application of high temperature yields the high extraction recoveries reported in HT-SFE studies. However, heating alone does induce a noticeable effect on the post-thermal treated SFE recoveries at 50°C.

3.3.4 Preliminary evaluation of methylphenanthrene geosynthesis during HT-SFE .

As discussed earlier, large increments in SFE extraction efficiencies for PAHs with increasing temperatures were observed. Thus, it is of great interest to explore the possibility of methylphenanthrene geosynthetic processes during HT-SFE by methylation of the parent phenanthrene molecule, considering the presence of high concentrations of aliphatic HCs as methyl group sources during thermal treatment of kerogen.

Samples were spiked with deuterated phenanthrene, in order to observe possible transmethylation of this compound during HT-SFE at elevated temperatures. The ratio

[Deuterated phenanthrene] / [squalane] was 1.3715 for the 50°C SFE and 1.3831 for 350°C SFE suggesting that no phenanthrene methylation occurred in the Posidonia shale sample under these experimental conditions. However, it should be considered that spiked samples cannot give absolutely reliable results due to the fact that such analytes are not exposed to the same active sites as the equivalent native compounds. The experiment however, shows that such transmethylation probably does not occur to a significant degree. The transmethylation reactions observed by Smith and co-workers (Smith et al., 1995), transmethyations of up to about 14 % of the parent hydrocarbon, but occurred when the samples were subjected to much more extended heating times (up to 66 hours). Therefore, it is unlikely that under much shorter exposure times (1 hr) this process would have a significant effect on the formation of alkylaromatics. In addition, the parent compounds in that study (Smith et al., 1995) were spiked in high amounts into the sample, unlike the present study where they were added in amounts similar to the native compounds.

3.3.5 Rock-Eval Pyrolysis of Posidonia Shale. Comparison with Kupferschiefer Shale.

The Rock-Eval Pyrolysis data obtained for non-extracted and SFE extracted Posidonia and Kupferschiefer samples is shown in Table IX. Figure 45 shows the S_1 and S_2 behavior related to the SFE extraction temperature for both shales. A dramatic decrease in S_1 is observed from the original non-extracted sample to the 50°C SFE extracted sediment and no further changes occurred up to 250°C and 300°C (for Posidonia and Kupferschiefer, respectively). At the high temperature end, only a slight decrease is noted particularly for

Posidonia

SFE TEMP (C)	TOC (Wt%)	S1 (mg/g)	S2 (mg/g)	S3 (mg/g)	Tmax (C)	HI	OI
0	10.27	4.47	83.54	0.52	428	813	5
50	9.97	0.97	76.28	0.8	427	765	8
100	10.18	0.71	77.19	0.63	424	758	6
150	9.3	0.71	76.34	0.64	422	821	7
200	9.19	0.73	77.85	0.6	424	847	7
250	9.24	0.26	74.14	0.61	426	802	7
300	9.19	0.19	74.63	0.5	426	812	5
350	9.81	0.16	74.17	0.51	425	756	5

Kupferschiefer

SFE TEMP (C)	TOC (Wt%)	S1 (mg/g)	S2 (mg/g)	S3 (mg/g)	Tmax (C)	HI	OI
0	3.09	0.37	15.29	1.16	417	495	38
50	3.21	0.17	14.88	1.15	418	464	36
100	2.86	0.16	14.86	1.21	419	520	42
150	3.14	0.18	14.8	1.31	415	471	42
200	3.06	0.18	16.02	1.37	418	524	45
250	2.92	0.18	14.92	1.29	415	511	44
300	3.19	0.11	13.47	1.24	418	422	39
350	3.22	0.15	11.01	0.73	423	342	23

Table IX- Rock-Eval Pyrolysis Data for Posidonia and Kupferschiefer Shale
Extracted Sediments (Fresh sample each time SFE).

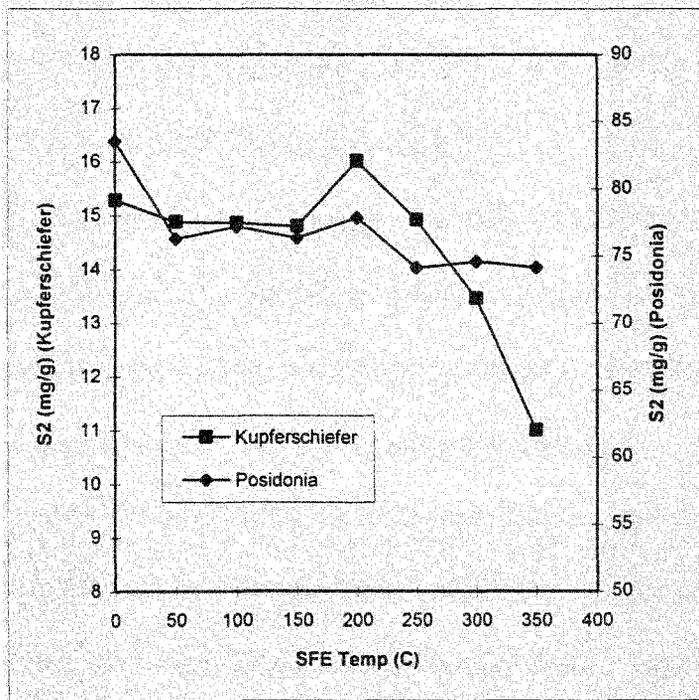
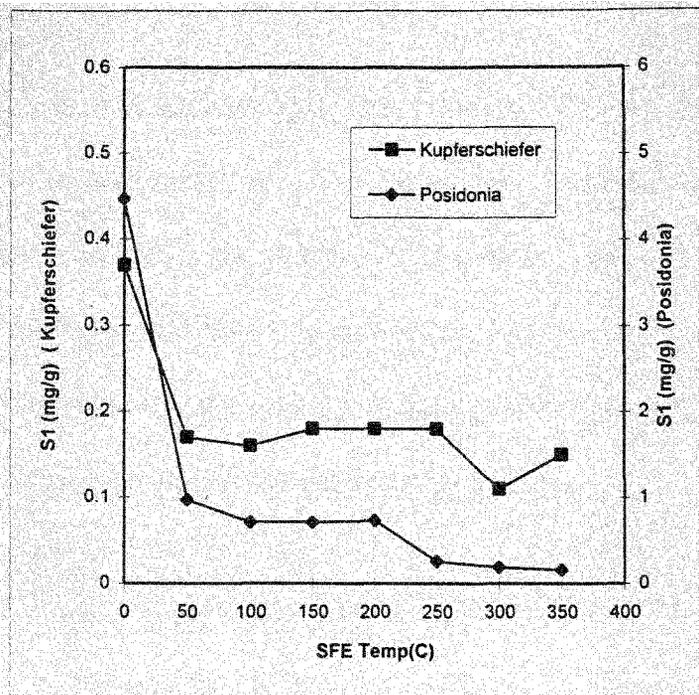


Fig. 45. Rock-Eval Pyrolysis Results for Posidonia and Kupferschiefer Shales.

Posidonia Shale. This may indicate that some “trapped” HCs are extracted at these temperatures, which are commonly part of the S₁ measurement. Similarly, the values of S₂ remained relatively constant for all extraction temperatures, except for the two highest temperatures for the Kupferschiefer Shale. Again, some very strongly matrix associated HCs could have been removed by the HT-SFE. In this case, compounds which normally form part of the S₂ measurement, but are not pyrolysis derived.

These data confirm the stepwise SFE results suggesting that most of the extractable HCs can be recovered under mild SFE conditions (relatively low temperature) and indicates that the HCs extracted at high temperatures are not dominated by pyrolysis products. It is important to notice that no alkenes produced by pyrolysis were identified neither for Posidonia nor for Kupferschiefer SFE extracts. The observed reduction for S₁ and S₂ values at higher temperatures might be caused by the extraction of significant amounts of "trapped" HCs during the HT-SFE procedure. Such release (particularly in the case of S₂ for Kupferschiefer shale) may be caused by the thermally induced structural changes and/or rearrangements of the macromolecular organic matter possibly via sulfur bond cleavage.

To further investigate the thermal effect during HT-SFE on the sample matrix, the HI and OI values obtained from the original Posidonia and Kupferschiefer Shales were plotted in a Van Krevelen analogue diagram presented in Figure 46. The HI of 813 and the OI of 5 for Posidonia shows that the organic matter of this shale is composed of type I kerogen, known to be derived from lacustrine depositional environments, while the HI of 495 and the OI of

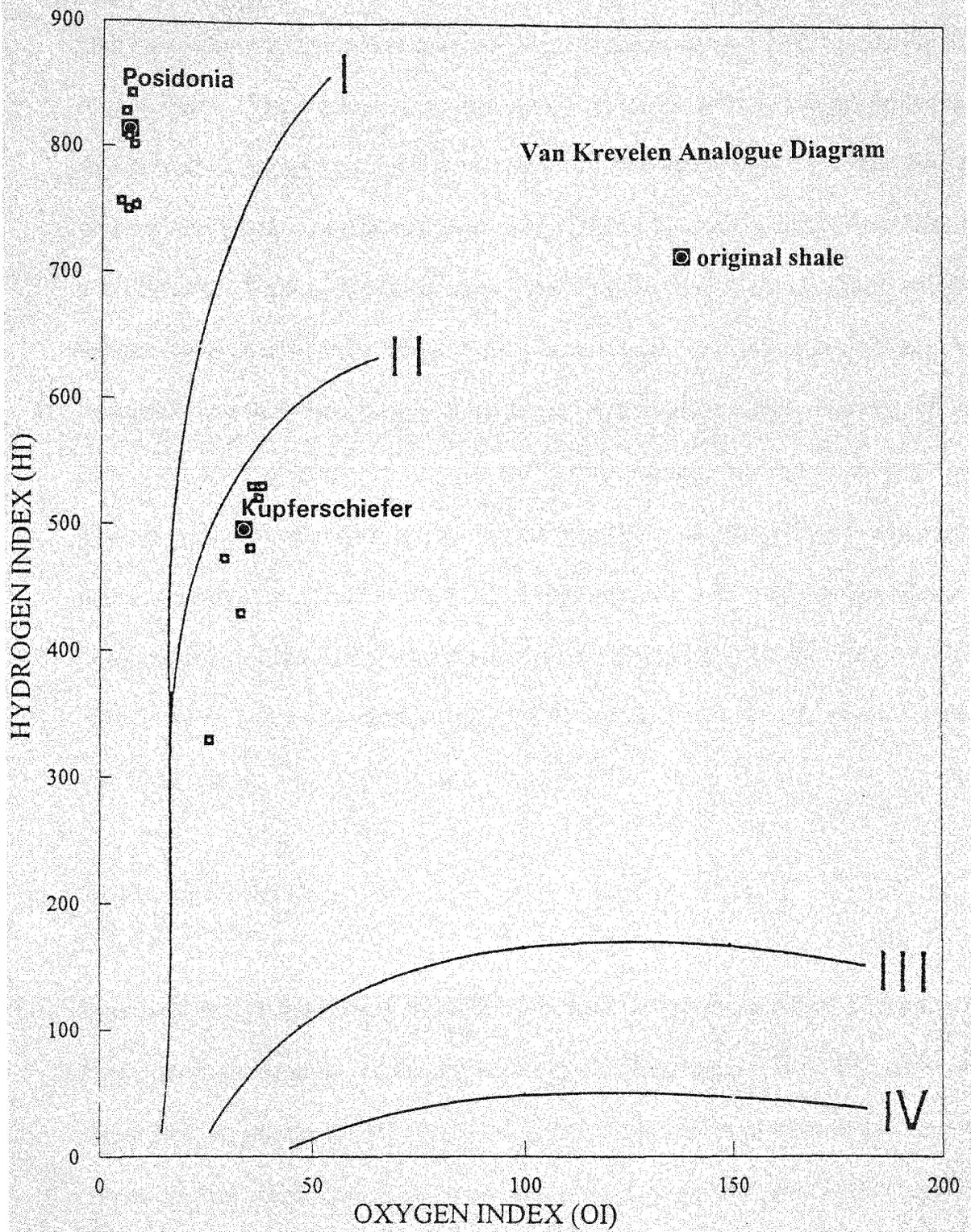


Fig. 46. Posidonia and Kupferschiefer Shale Kerogen Type Determination from Rock-Eval Pyrolysis Data of Both the Original and the SFE Extracted Sediment Samples.

38 obtained for Kupferschiefer Shale coincides with an immature type II kerogen (marine origin). The SFE procedure does not appear to affect the Rock-Eval Pyrolysis potential in determining the kerogen type of a given source rock because no significant changes were observed for HI and OI in sediments extracted at different SFE temperatures (Table VIII, see also Figure 46). While no difference was observed for the pre-extracted Posidonia samples for the relative position on the Van Krevelen diagram, some more noticeable difference was observed for the two highest temperatures for the Kupferschiefer sample. Both the 300 and 350 °C extracted samples showed “apparent” thermal maturity increments, although only slight ones. This could be caused by the fact that this shale contains significantly less organic matter compared to Posidonia (% TOC of approximately 3 vs. 10), and the release of “trapped” HCs could have more severe impact on its matrix bulk characteristics. Such an effect however seems only minor during HT-SFE and will be further addressed below (See Py-GC/MS data).

3.3.6 Pyrolysis-GC/MS.

Figures 47 and 48 show some of the TICs obtained for non-extracted and SFE extracted Posidonia Shale samples for the sequential Py-GC/MS procedure at 358°C and 610 °C respectively. In general, the 358°C step distributions show higher hydrocarbon abundances for the original shale which is obviously due to the previous extraction of these analytes during the SFE. This effect is particularly evident in the lower abundance of low molecular weight compounds, and n-alkanes which have been preferentially recovered during the SFE.

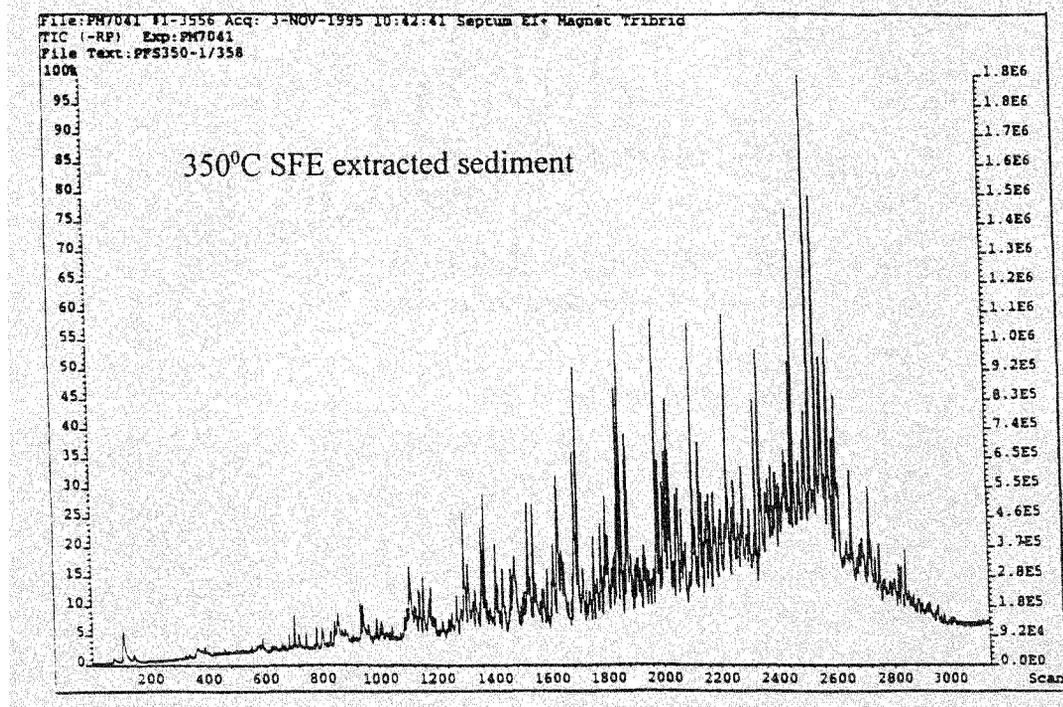
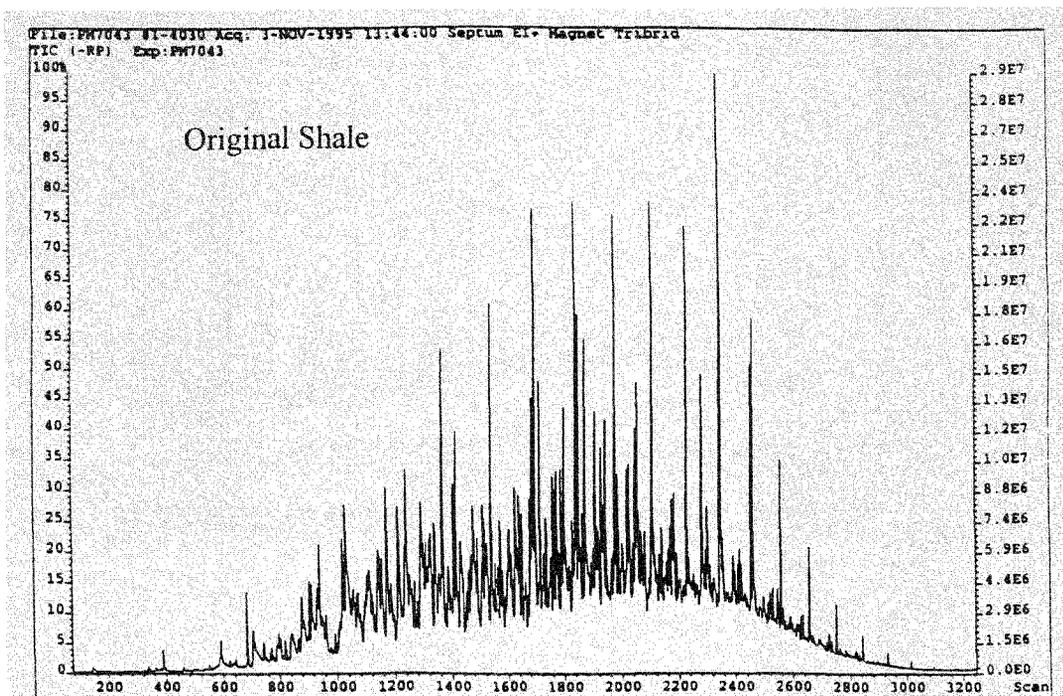


Fig. 47. Py-GC/MS at 358°C of Posidonia Shale for the Original Sample (top) and 350°C SFE extracted sample (bottom).

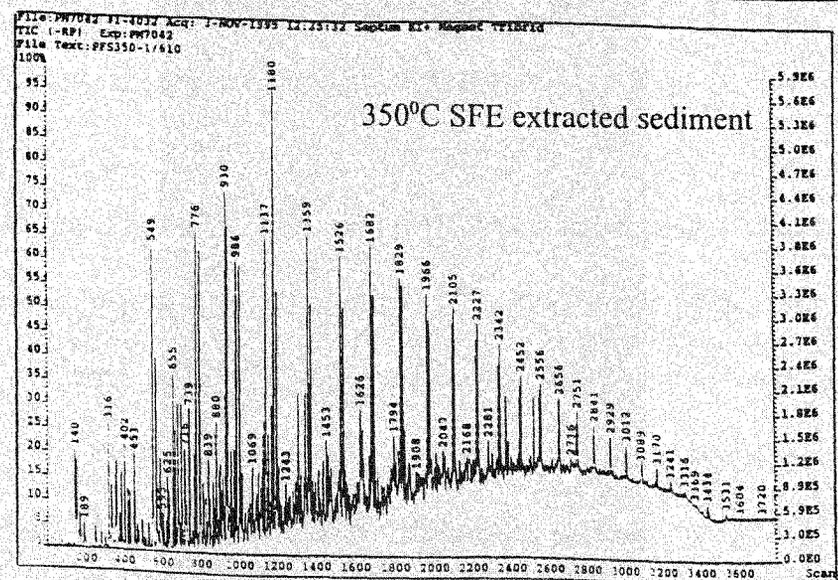
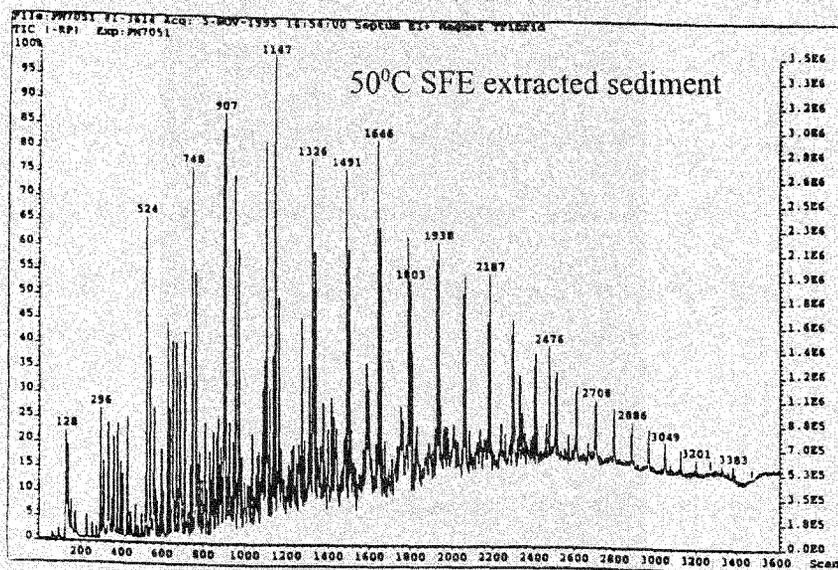
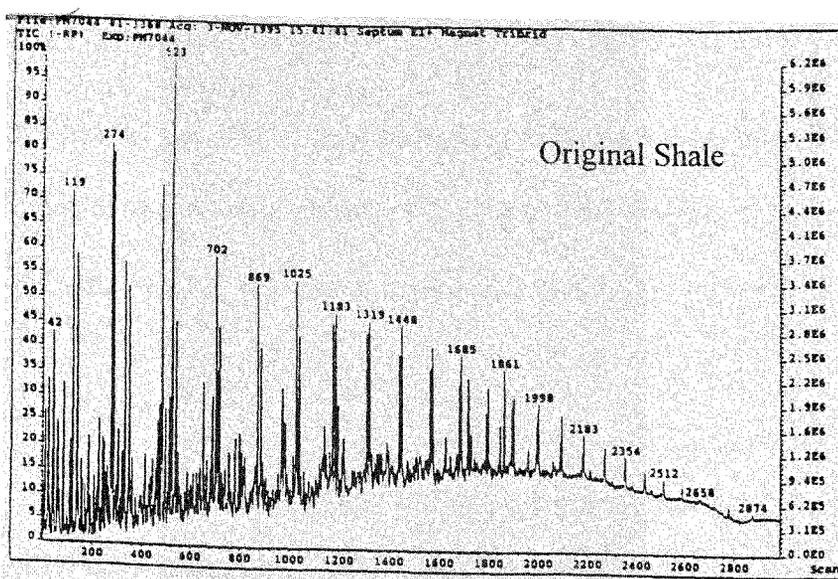


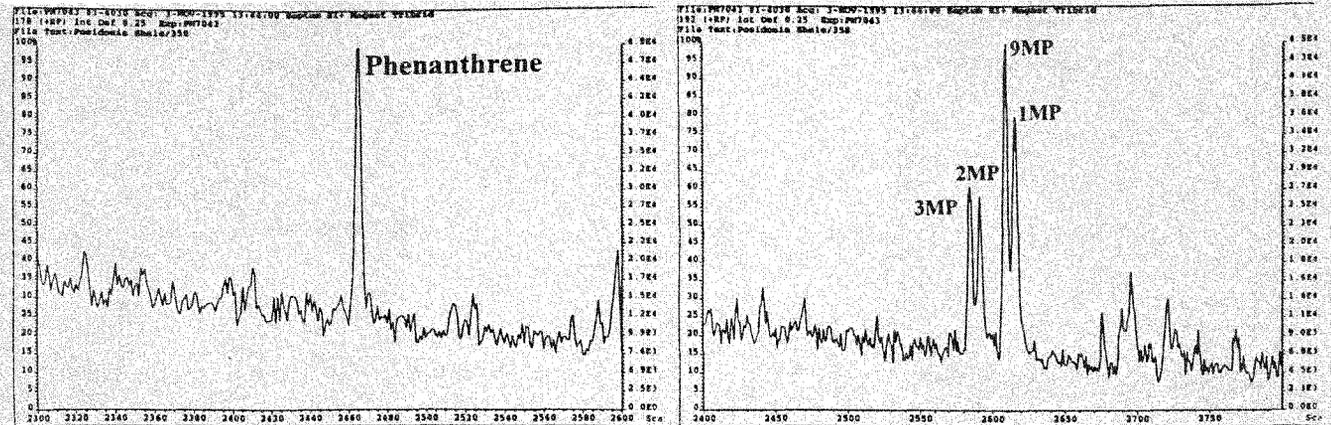
Fig. 48. Py-GC/MS at 610°C Following Py-GC/MS at 358°C of Posidonia Shale for original (top), 50°C HT-SFE (middle) and 350°C HT-SFE (bottom) samples.

The Py-GC/MS trace of the 350 °C pre-extracted sample consists mainly of cyclic and aromatic HCs, in agreement with the stepwise HT-SFE data. No evidence of pyrolysis of the matrix was observed at this temperature, since no n-alkenes were apparent in the chromatograms.

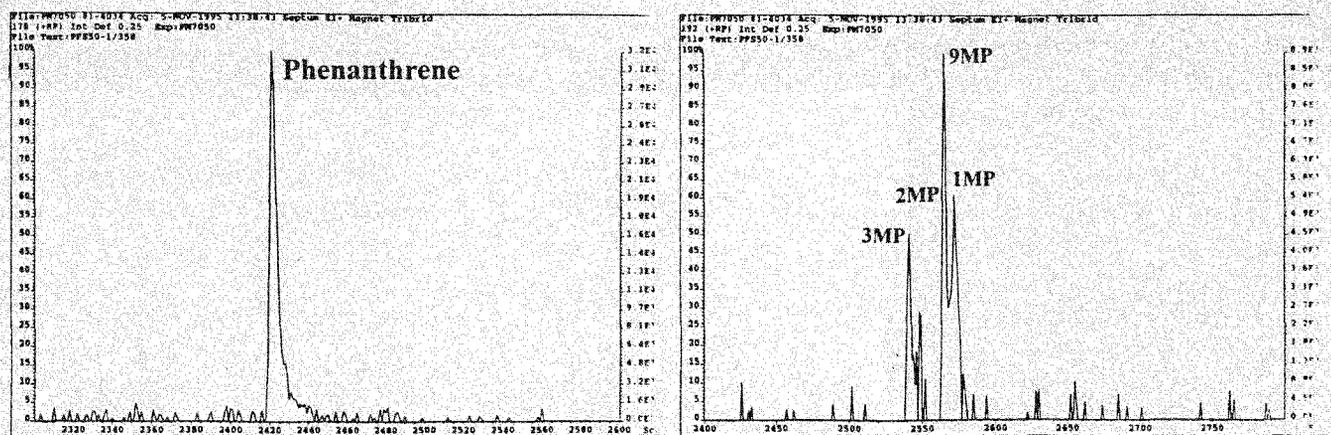
No noticeable differences between the 610°C TICs were observed for the original shale and the 50°C and 350°C SFE extracted samples, except a slight lower abundance for the low molecular weight HCs in the SFE extracted shales (Figure 48). The double peak pattern (indicating alkene presence) is characteristic of pyrolyzed matrices. The great similarity between the 610°C Py-GC/MS data for both original shale and 350 °C HT-SFE is an indication that HT-SFE does not cause significant changes in the composition of the matrix of the sample.

Some ion chromatograms for phenanthrene, anthracene ($m/z = 178$) and methylphenanthrene / anthracenes ($m/z = 192$) are shown in Figures 49 and 50 for 358°C and 610°C Py-GC/MS steps correspondent to the original shale and to SFE extracted sediments.

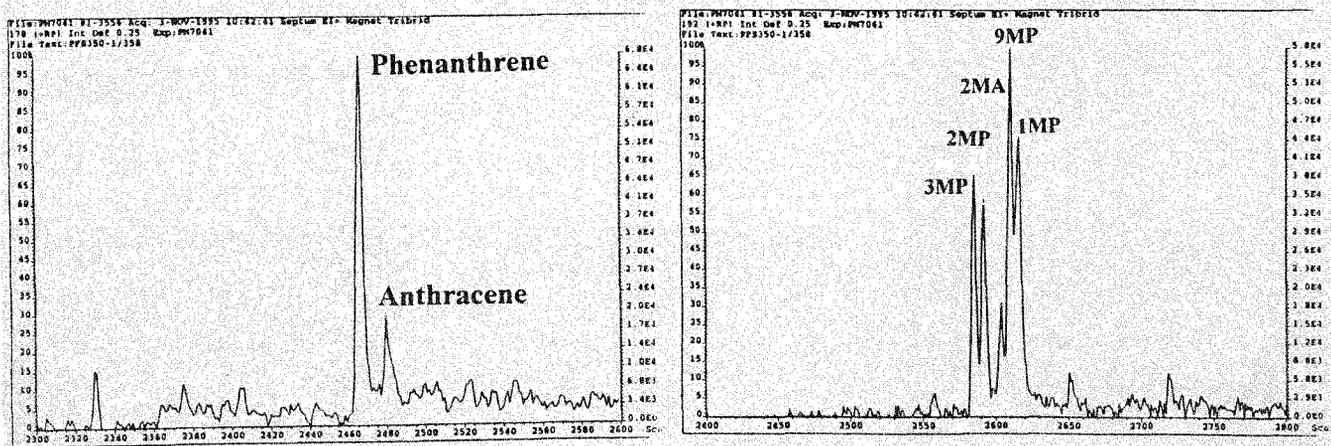
For the original and the 50°C SFE extracted shale sample only phenanthrene and methylphenanthrene peaks were detected at 358°C Py-GC/MS, while anthracene and methylanthracenes were additionally observed for the 350°C SFE extracted shale as a consequence of the temperature induced rearrangements in the matrix during the SFE extraction. This apparently irreversible structural change in the matrix allowed for additional



Original Shale

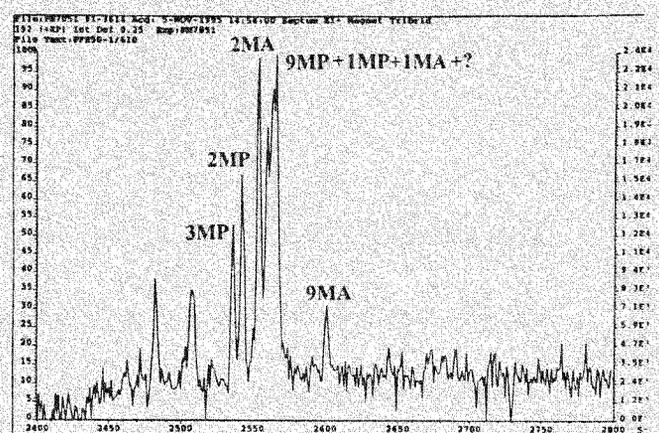
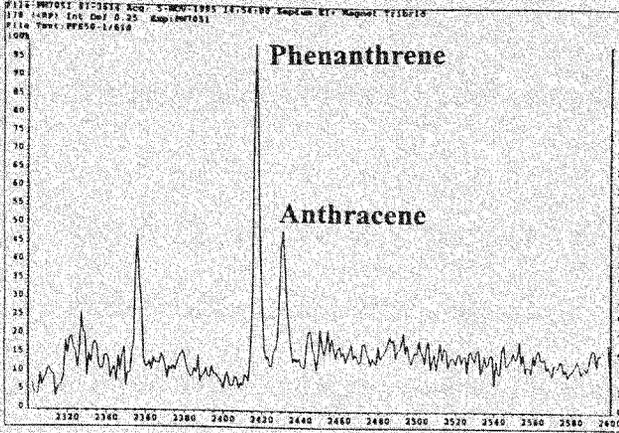


50°C SFE extracted sediment

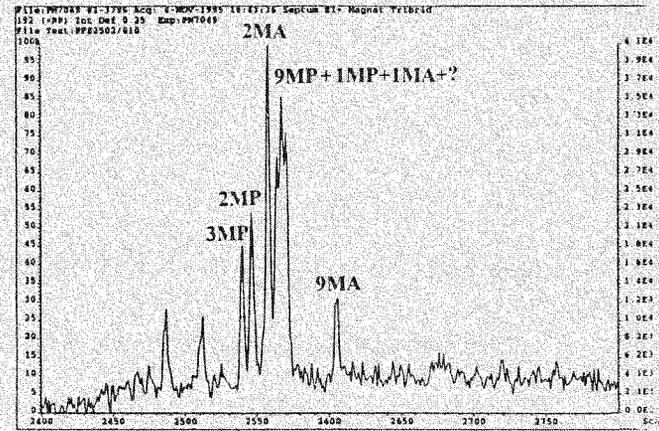
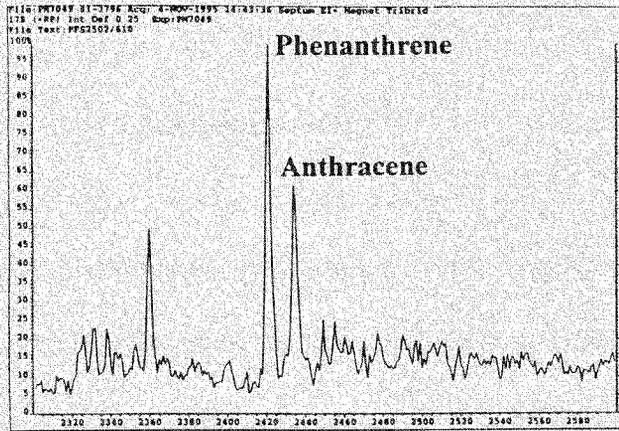


350°C SFE extracted sediment

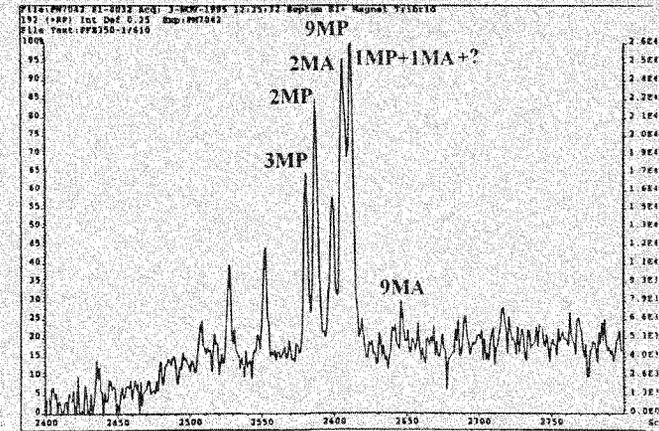
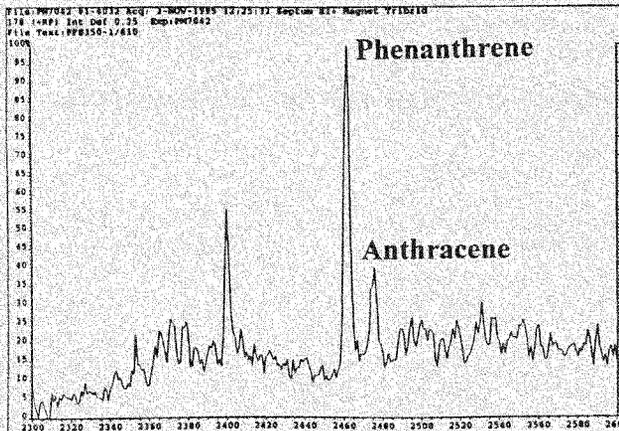
Fig. 49. GC/MS Chromatograms for Phenanthrene/Anthracene ($m/z=178$) and Methylphenanthrenes/Anthracenes ($m/z=192$) obtained from 358°C Py-GC/MS of non-extracted and SFE extracted Posidonia Shale Samples.



50°C SFE extracted sediment



250°C SFE extracted sediment



350°C SFE extracted sediment

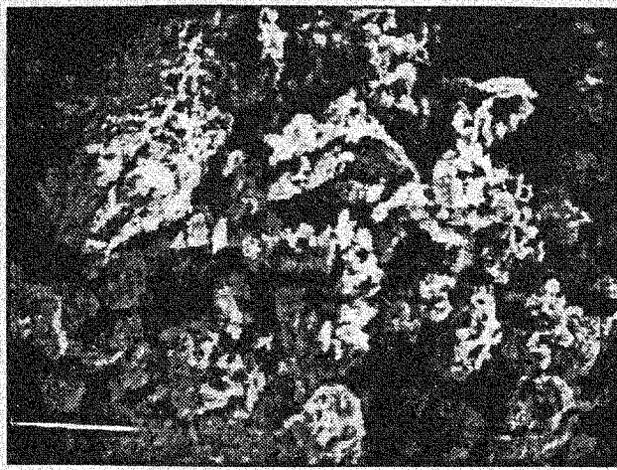
Fig. 50. GC/MS Chromatograms for Phenanthrene/Anthracene ($m/z=178$) and Methylphenanthrenes/Anthracenes ($m/z=192$) obtained from 610°C following 358°C Py-GC/MS of SFE extracted Posidonia Shale Samples.

phenanthrene/anthracene to be thermally desorbed from the sample.

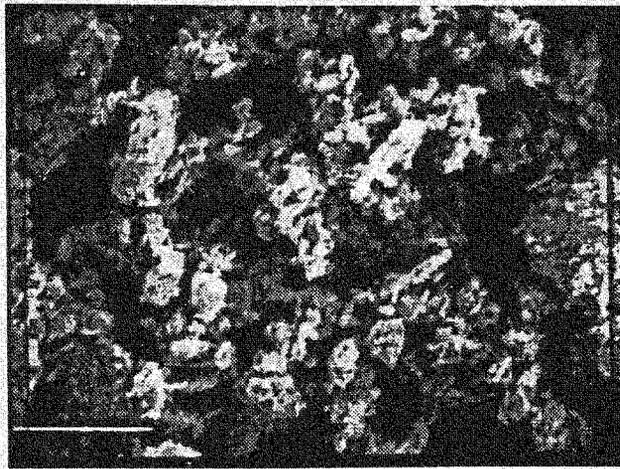
The 610°C Py-GC/MS experiment yielded both phenanthrene and anthracene and methylphenanthrenes/methylanthracenes for all samples including the original sample. Clearly, this temperature is elevated enough to induce structural changes of the matrix and release (and possibly produce via thermal synthesis) these compounds. Interestingly, no evidence of HT-SFE induced compositional changes in the matrix were apparent.

3.3.7 Microscopy Study.

In order to assess possible structural (physical) effects due to HT-SFE, Electron Scanning Microscope photographs (1000 X) were obtained for the original Posidonia Shale and for the SFE extracted sediments at 150 °C and 350 °C Figure 51. No differences were observed at this magnification level neither between the original shale and the SFE extracted ones nor between sediments extracted at different temperatures. It is likely that higher magnification is required to adequately assess such changes.



Original Posidonia Shale



150 °C SFE extracted sediment



350 °C SFE extracted sediment

Fig 51. Electron Scanning Microscope Photographs (1000 X) Obtained for the Original Posidonia Shale and For the sediments extracted at 150 °C and 350 °C in the SFE.

4. CONCLUSIONS

(A)- Individual analytes are speciated in different ways throughout the organic matrix of ancient sediments. Those fractions that are preferentially extracted under higher energy conditions (higher temperatures) have a stronger interaction with the organic system due to “trapping” processes in the porous media (or microfractures) or by chemical bonding to the matrix (possibly via sulfur bonds).

(B)- The higher SFE extraction efficiencies obtained at the highest temperature levels for aromatic HCs compared to that of aliphatics is probably due to the higher polarity (aromaticity), molecular size and geometry (planarity) of the aromatic compounds, all allowing for closer associations with the macromolecular organic matter. This could also explain the higher recoveries obtained for PAHs during the SFE compared to those observed for the Soxhlet extraction method. Branched compounds, like acyclic isoprenoidal alkanes, are less likely to get “trapped” into the matrix than straight chain analytes.

(C)- Anthracene and compounds identified as methylanthracenes are detectable in SFE extracts only for extraction temperatures of 250⁰C and above indicating a very strong association with the organic macromolecular structure. They are released only after thermal rearrangement of the matrix.

(D)- The release of “trapped” HCs during HT-SFE was confirmed by the inversion of

molecular thermal maturity parameters like the (S/S+R) ratio for C 31 -homohopane for the highest temperatures. The relatively more “immature” signal at 350°C HT-SFE clearly demonstrates that biomarkers such as hopanes can be sterically hindered from isomeric transformations during diagenesis/catagenesis while in the “trapped” form.

(E)- Thermally induced matrix rearrangements in ancient sediments during HT-SFE appear to have an irreversible nature. However, HT-SFE does not significantly affect the chemical composition of the matrix.

(F)- Rock-Eval Pyrolysis and Pyrolysis-GC/MS data suggest that most of the extractable HCs can be recovered at relatively low temperatures and indicate that the HCs extracted at high temperatures are not dominated by pyrolysis products but probably by “trapped” compounds that were released during the HT-SFE procedure . In addition, the data strongly support the fact that no major chemical changes in the matrix occurs during HT-SFE.

(G)- No evidence for alkylaromatic geosynthesis from the parent molecules during HT-SFE was obtained. The high abundance of these compounds in the HT-SFE are due to their speciation in the sediments.

(H)- In general terms, HT-SFE is suggested as an excellent tool for speciation studies of HCs in ancient sediments. It provides reliable and reproducible data useful in organic geochemical studies.

5. RECOMMENDATIONS

Based on the analysis of the results obtained during this study we would recommend:

. To study the speciation of organic compounds in ancient sediments by the addition of modifiers during the SFE procedure.

. To correlate the environmental fate of naturally occurring compounds in ancient sediments to that of the same analytes with an anthropogenic origin by carrying out speciation studies in polluted sediments using HT-SFE.

. To evaluate the possibility of alkylaromatic geosynthesis during the HT-SFE of sediments previously spiked with labeled parent molecules and kept during long time periods under different temperature conditions.

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