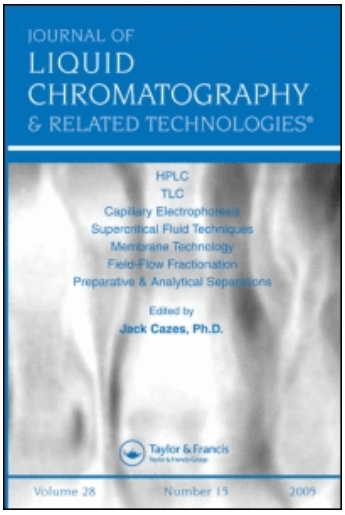

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Hajibrahim, Saleh K.(1981) 'Development of High Pressure Liquid Chromatography (HPLC) for Fractionation and Fingerprinting of Petroporphyrin Mixtures', Journal of Liquid Chromatography & Related Technologies, 4: 5, 749 — 764
To link to this Article: DOI: 10.1080/01483918108059971
URL: http://dx.doi.org/10.1080/01483918108059971

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(5), 749-764 (1981)

DEVELOPMENT OF HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC) FOR FRACTIONATION AND FINGERPRINTING OF PETROPORPHYRIN MIXTURES*

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ABSTRACT

A method is described which permits the analysis of fossil porphyrin mixtures by HPLC. This method involves the fractionation of the demetallated petroporphyrin mixtures on silica columns followed by rechromatography of the trapped fractions on ODS columns. This coupling of the two modes of HPLC provides a fast and effective method for the separation of isomeric porphyrins and for the petroporphyrin fingerprinting of geological (oil/shale) samples. Representative examples of analysis are discussed in terms of the potential applicability of the technique in the areas of structure elucidation and geochemical correlations.

INTRODUCTION

Porphyrin mixtures extracted from geological samples (petroporphyrins) typically contain series of nickel and vanadyl complexes of alkyl substituted petroporphyrins of two major skeletal types, etio (I, R=H or alkyl) and desoxophylloerythroetio (DPEP, II, R=H or alkyl) and a minor type, rhodo (tentatively assigned as III). Structures are shown in Figure 1. The alkyl substituents contain from ca. 6 to ca. 19

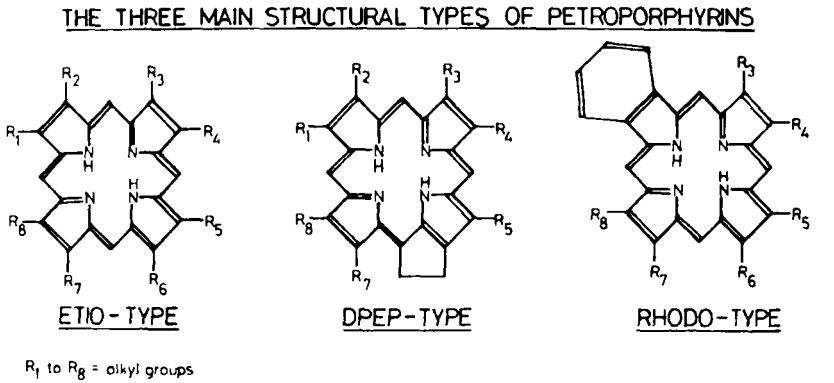

FIGURE 1. The three main structural types of petroporphyrins.

carbon atoms [1,2]. However, the exact nature of the substituents and the actual sites of substitution have not been fully determined [3]. In addition, structural isomers are known to occur in petroleum [2-9].

The chromatographic methods previously used to fractionate petroporphyrins (e.g. TLC, column chromatography) afford insufficient separation and are laborious and time-consuming, thus rendering them impractical for structure elucidation and geochemical correlation studies. Direct analysis of these pigments by gas-liquid chromatography is hindered by their involatility and/or thermal instability.

Recently, HPLC (silica columns) was introduced as a technique for the fractionation and fingerprinting of petroporphyrin mixtures [4]. However, this technique has two shortcomings: namely, incomplete separation of the components and the inability to resolve positional isomers. This work describes development of the said technique to overcome these shortcomings. It also discusses the potential applicability of the new method in structure elucidation of petroporphyrins and in

correlation of oils with oils and with source rocks based on their porphyrin distribution.

EXPERIMENTAL

The demetallated porphyrin mixtures were isolated from their host environment as described previously [3,5]. The process is summarized in Figure 2.

The HPLC system is comprised of a Waters Assoc. ALC/GPC 244 Liquid Chromatography (Waters Assoc., Milford, Mass., U.S.A.) including second M6000A solvent delivery system, M660 programmer, M450 variable wavelength detector and U6K universal injector. Samples (2 µl; in $CHCl_3$) were introduced from a 10 µl syringe. Detection (uv/vis) was in all runs at 400 nm.

HPLC analyses were carried out on silica (Partisil-5, Whatman Ltd, Maidstone, Kent, U.K.) or ODS columns. The silica columns (stainless steel, 25 cm X 4.6 mm I.D.) were packed by the balanced density method [10]. The ODS columns, purchased prepacked, were Waters Assoc. µBondapak C18 or DuPont (Wilmington, Dela., U.S.A.) nonaqueous reverse phase (NARP).

The mobile phase, using gradient elution on the silica columns, was toluene and hexane (1:9 v/v) as as solvent A, and toluene and chloroform (1:1 v/v) as solvent B, programmed (40 min) from 20% to 100% B (curve 9) and a flow rate of 1.5 ml/min. Analyses under isocratic conditions were carried out using 30% B. The solvents used were HPLC grade (Ruthburn Chemicals, Scotland). The chloroform contained 0.5% EtOH (as preservative).

Analyses on the µBondapak C18 columns were carried out using acetonitrile:water (17:3 v/v; isocratic) as the mobile phase and a flow rate of 2 ml/min.

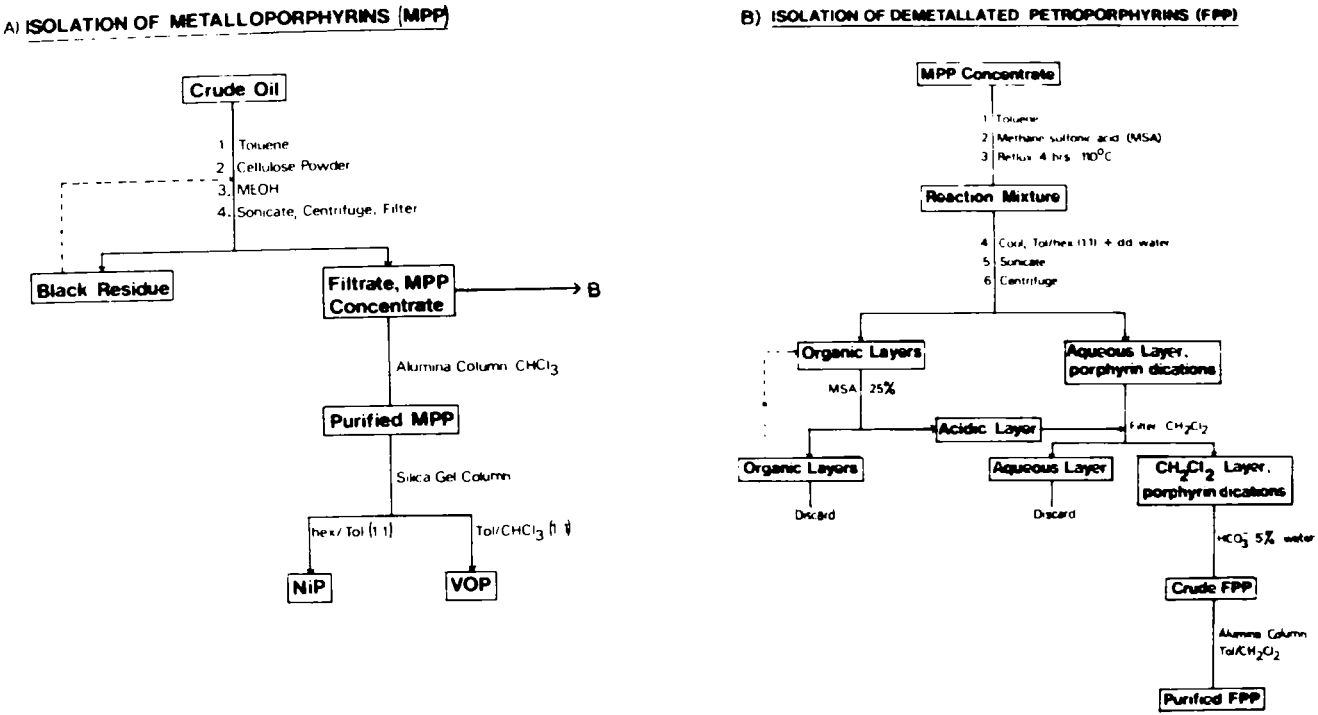

FIGURE 2. Isolation procedures for: A. metalloporphyrins from crude oils; B. demetallated petroporphyrins.

Analyses on the NARP columns were carried out either isocraticly (chloroform:MeOH:water, 1:18:1 v/v; 2 ml/min) or using gradient elution with acetonitrile and water (17:3 v/v) as solvent A and acetonitrile, water and chloroform (18:1:1 v/v) as solvent B. Etio-type petroporphyrins were chromatographed using a linear program (100 min) from 15% B to 60% B, whereas the DPEP-type petroporphyrins were analyzed using a linear program (100 min from 50% B to 90% B. The flow rate in both cases was 2 ml/min and the temperature was ambient. Other operating conditions are indicated on the corresponding chromatograms.

The trapping of certain cuts or individual peaks from the silica column was carried out manually, and after the removal of the mobile phase, the fractions were rechromatographed on ODS columns. Peaks carbon numbers were assigned by coinjection with previously characterized petroporphyrins [11]. Peak area (%) was calculated by integration.

RESULTS & DISCUSSION

The HPLC fingerprints, on Partisil-5 silica column and gradient elution, of demetallated petro-porphyrin mixtures obtained from a wide variety of geological samples, showed the presence of seventeen distinct peaks [2]. The major differences in these fingerprints lie in the relative abundance of the peaks. The individual peaks for three of the samples were trapped for mass spectrometric (EI) analysis [11]. The data (e.g. Table 1) showed that the silica column enabled the separation of the etio-type (peaks 1 to 9 and 11, Table 1) from the DPEP-type (peaks 10 and 12 to 17, Table 1). It also separated structural-type

TABLE 1

Mass Spectral Characteristics of the Major Peaks in the Liquid Chromatogram of Demetallated Porphyrin Mixture (Ex. Boscan Oil)

Peak No.	t_R[a] (min)	Petroporphyrin Molecular Ions[b] Major component	Minor component(s) (%)[c]
0	2.5-7.5	$C_{32}E$	$C_{31}E(89)$; $C_{33}E(53)$; $C_{30}E(51)$; $C_{34}E(22)$
1	8.00	$C_{32}E$	$C_{30}E(65)$; $C_{29}E(55)$; $C_{33}E(40)$
2	9.00	$C_{29}E$	$C_{31}E(79)$; $C_{32}E(72)$
3	9.5	$C_{31}E$	--
4	11.00	$C_{30}E$	$C_{28}E(26)$
5	12.5	$C_{32}E$	--
6	14.25	$C_{29}E$	--
7	16.00	$C_{31}E$	--
8	18.00	$C_{28}E$	--
9	20.75	$C_{30}E$	--
10	23.75	$C_{30}D$	--
11	26.25	$C_{29}E$	--
12	29.00	$C_{31}D$	$C_{34}D(74)$
13	30.5	$C_{29}D$	$C_{33}D(71)$; $C_{30}D(53)$
14	33.00	$C_{32}D$	--
15	35.00	$C_{32}D$	$C_{30}D(60)$
16	37.00	$C_{31}D$	--
17	39.00	$C_{30}D$	--

a. Absolute retention time on Partisil-5 silica column using gradient elution. Reproducibility, ±1 min.

b. From the EI mass spectra of the trapped peaks expressed as carbon number; E = etio-type; D = DPEP-type.

c. Relative to the major ion = 100. The presence of the same porphyrins in the mass spectra of adjacent peaks is likely due to overlapping of the peaks rather than different isomers.

isomers. Thus, peaks 3 and 7 contained a C_{31}-etio; peaks 4 and 9 contained a C_{30}-etio; peaks 6 and 11 contained a C_{29}-etio; peaks 10 and 17 contained a C_{30}-DPEP; and peaks 12 and 16 contained a C_{31}-DPEP (Table 1). On the other hand, several of the peaks (e.g. 1, 2, 4, 12, 13, and 15, Table 1) correspond to more than one compound. Manipulation of the various chromatographic parameters failed to separate these compounds on Partisil-5 silica columns. In addition, all efforts to resolve positional isomers (e.g. Etio I and III) on the silica column were unsuccessful. These difficulties were overcome using ODS columns as described below.

Figure 3 presents the LC records for peak 12 (Table 1) on Partisil-5 silica, μBondapak C18 and NARP columns. The drastic improvement in the separation of the components on the ODS columns over the silica column is self-evident. The comparison of Figure 3a with 3b shows that the NARP column affords better separation of the porphyrins in question than the μBondapak C18 column, although the analysis time on the NARP column is approximately twice as long. Attempts to obtain better resolution on the μBondapak C18 column by using gradient elution were unsuccessful. The major advantage of the NARP column, however, was its ability to resolve positional-type isomeric alkyl porphyrins as seen in Figure 4a, which shows the separation of etio I and III standard porphyrins. These isomers have the same structure except the methyl and ethyl substituents in position 7 and 8 (1, Figure 1) are reversed. Similarly, the two C_{30} etio isomers in which the two ethyl substituents (the other substituents are methyl groups) are located on adjacent pyrrole rings (i.e. horizontal) or on opposite ones (i.e. diagonal) were resolved on the NARP

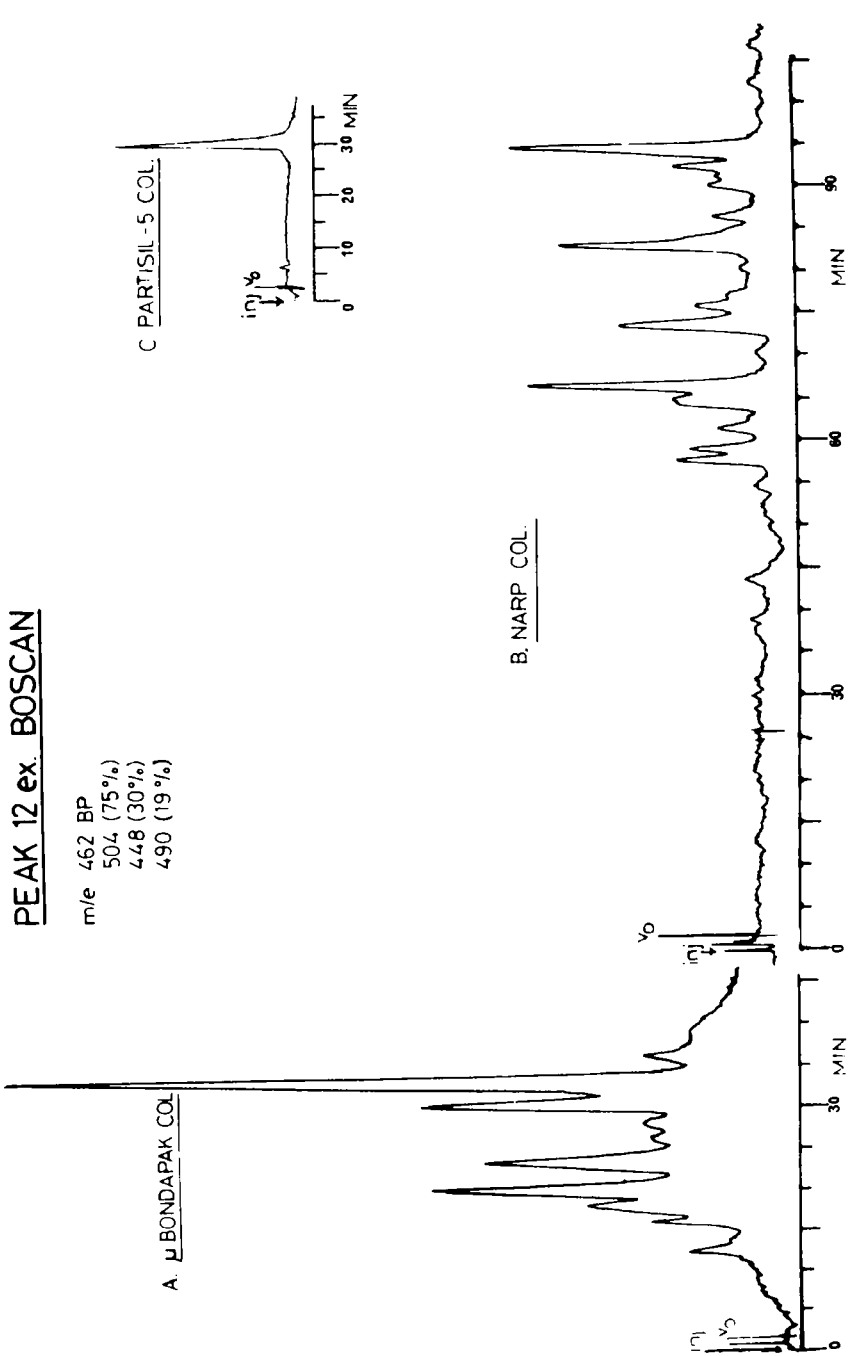

FIGURE 3. LC traces for peak 12 ex. Boscan oil (Table 1) on: A. μBondapak C18; B. NARP (gradient elution); C. Partisil-5 silica (gradient elution) columns.

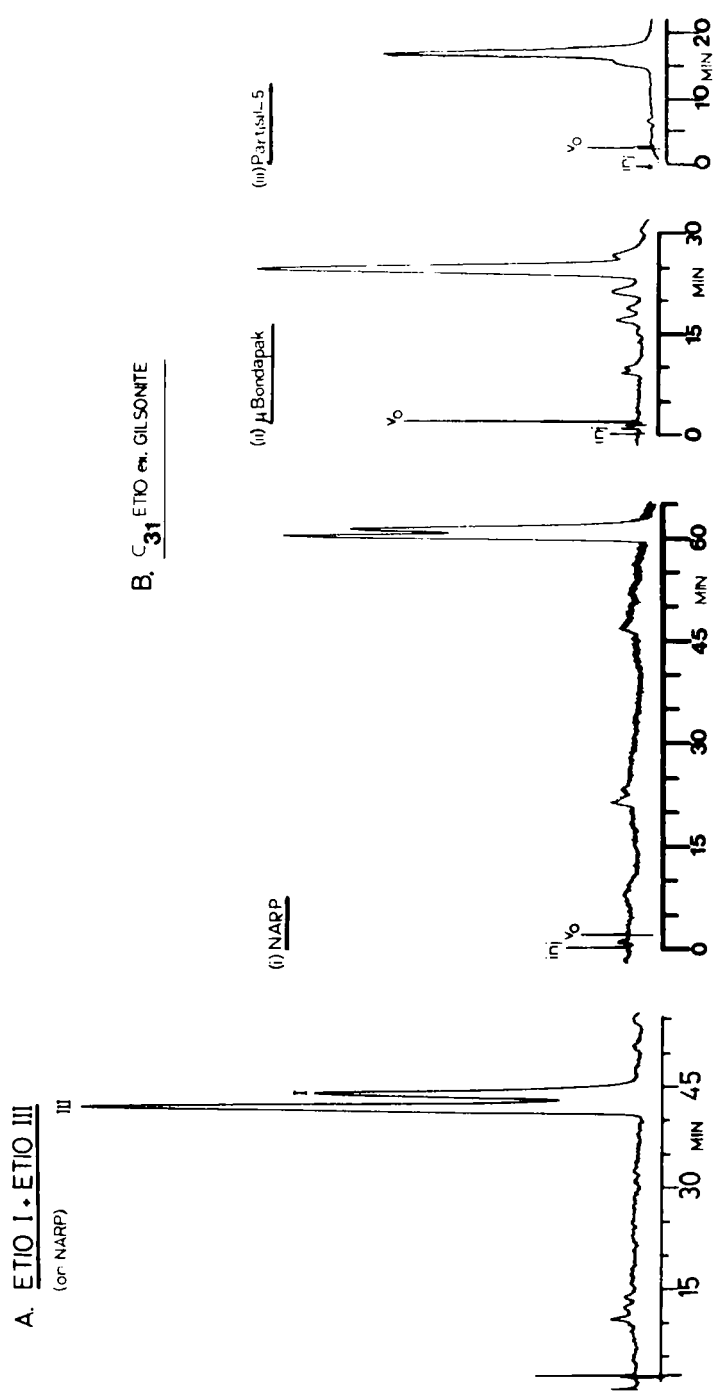

FIGURE 4. A. Separation of etio I and III standard porphyrins on NARP column (isocratic conditions). B. LC traces of C_{31} etio porphyrin (ex. Gilsonite) on: (i) NARP (gradient) elution); (ii) μBondapak C18; (iii) Partisil-5 silica (gradient elution) columns.

column (LC not shown). These are the first reported chromatographic separations of alkyl porphyrin position isomers. Figure 4b contrasts the LC traces for the polar C_{31} etio porphyrin (e.g. peak 7, Table 1) isolated from Gilsonite on the three different columns used in this study. This petroporphyrin gave a doublet only on the NARP column (Figure 4bi).

Attempts to fingerprint the total demetallated petroporphyrin mixtures on either of the ODS columns ran into two major difficulties, namely the separation of the DPEP- from the etio-type porphyrins and of the structural isomers was lost. The second difficulty is illustrated by Figure 5 which depicts the LC records on μBondapak for two C_{29} etio isomers (e.g. peaks 6 and 11, Table 1). The large difference between their retention times on the silica column was almost lost when co-chromatographed on the μBondapak C18 column (Figure 5c). Likewise, it was not possible to achieve good separation of structural isomers on the NARP column, under the conditions used.

The above results clearly indicated the need to fractionate the petroporphyrin mixtures on silica prior to their analysis on the ODS columns. The examples discussed in Figures 3 and 4 demonstrate the efficiency of this technique for the separation of isomeric porphyrins, a prerequisite to their structure elucidation. The technique could also be used to fingerprint petroporphyrin distributions for oil-oil and oil-source rock correlations. The total demetallated porphyrin mixture from an oil (or shale) sample is first fractionated on Partisil-5 silica column, trapping three fractions (Figure 6). The criteria used in selecting these fractions were:

(i) The cutting points fall between reasonably separated peaks to minimize their overlapping. Tests

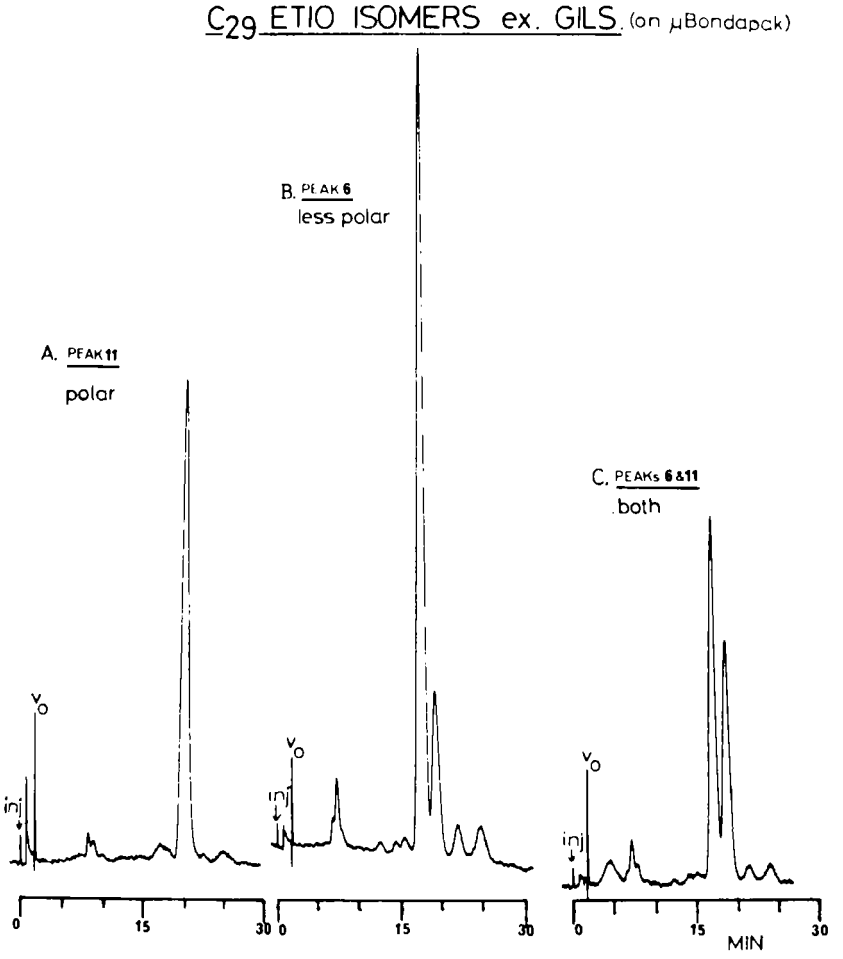

FIGURE 5. LC traces of C_{29} etio isomers (ex. Gilsonite) on the μBondapak C18 columns. A. polar isomer (e.g. peak 11, Table 1). B. less polar isomer (e.g. peak 6, Table 1). C. a mixture of both isomers.

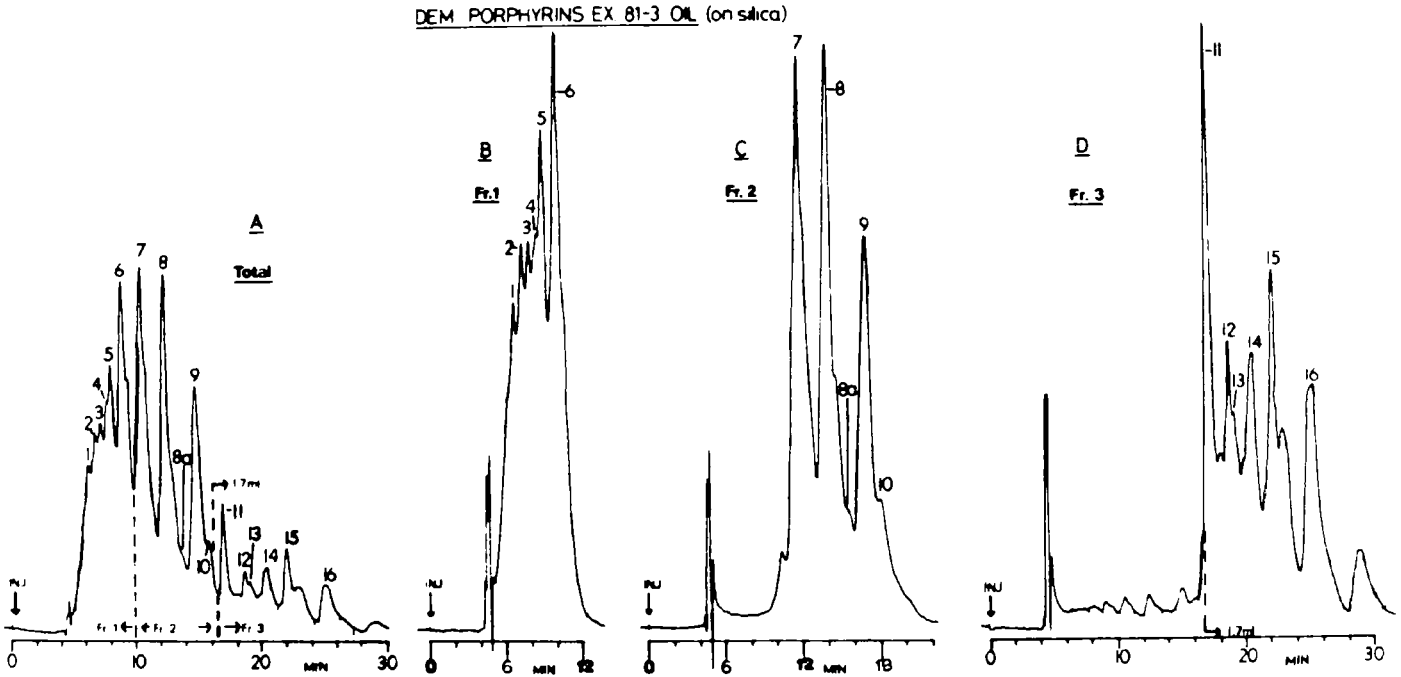

FIGURE 6. A. Fractionation of demetallated porphyrin mixture (ex. 81-3 oil) on Partisil-5 silica column under isocratic conditions. Flow rate: 1 ml/min increased to 1.7 ml/min after 16 min. B, C, D: LC traces of the three fractions (Fr. 1 to 3, respectively) trapped from the silica column as indicated on Figure 6A. Chromatographic conditions as for Figure 6A, except the flow rate was increased to 1.7 ml/min after 16 min for part D only.

were made to assure that the amount of overlapping was insignificant.

(ii) The two major etio-type structural isomers fall in different fractions.

(iii) The etio-type compounds are separated from the DPEP-type.

Retention times and internal markers were used to ensure that the cutting points for different mixtures were always the same. The internal markers used were etio I and C_{30} etio standard compounds which coeluted with peaks 6 and 9, respectively (Figure 6). To ensure reproducibility of retention times (and to shorten the analysis time by eliminating the need to equilibrate the column after each run) fractionation was carried out under isocratic conditions. These conditions were developed so as not to compromise the degree of separation achieved using gradient elution (see Table 1). However, peaks in Figure 6 and Table 1, having the same number, do not necessarily correspond to the same porphyrin compound(s).

After removing the solvents, the silica fractions are then fingerprinted on the NARP column. A case study is presented in Figure 7, depicting the fingerprints of the three silica fractions on the NARP column. Visual inspection of Figures 6 and 7 clearly shows the separation obtained on the NARP column is far better than that obtained on the silica column, under the conditions used, with respect to fractions 1 and 2. However, retention time data showed that, had the total porphyrin mixture not been pre-fractionated on the silica column, several of the peaks from the different fractions would co-elute from the NARP column. This was confirmed by injecting the total mixture or co-injecting fractions 1 and 2 on the NARP column.

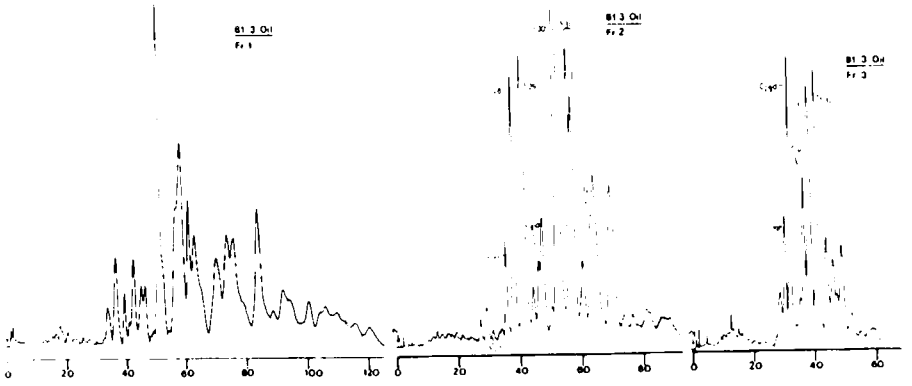

FIGURE 7. A. LC fingerprint on NARP (gradient elution) of the silica fractions ex. 81-3 oil in Figure 6.

CONCLUSIONS

The method reported here for HPLC analyses of fossil porphyrins provides for the separation of isomeric porphyrins as well as for the petroporphyrin fingerprinting of their host environments. Used in conjuction with existing analytical techniques, this method should prove useful in determining the structure of single petroporphyrins and in relating the crude oils and source rocks.

The results obtained in this study clearly demonstrate the need to employ more than one phase of LC when analyzing complex mixtures such as fossil porphyrins.

ACKNOWLEDGEMENTS

This work started at the University of Bristol and was supported in part by Chevron Oil Field Research Company (COFRC) California, U.S.A. The following kindly provided the geological samples used in this study: the American Gilsonite Company supplied the Gilsonite bituman; Dr. W.K. Seifert (COFRC) provided the sample of 81-3 oil; and Shell Netherland provided the Boscan oil sample.

The gifts of etio I and III and of the two C_{30} etio isomers were kindly supplied by Drs. J.G. Erdman (Phillips Petroleum) and A.W. Johnson (University of Sussex, England).

I am particularly grateful to Prof. G. Guiochon (Ecole Polytechnique, Paliseau, France) for the use of lab facilities to pack the Partisil-5 silica columns and for very useful discussions.

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* This work was presented in part at the 13th International Symposium on Chromatography, June 30-July 4, 1980, Cannes, France.