*Journal of Chromatography*, 282 (1983) 501–507
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 102

# RAPID HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR HYDROCARBON CLASS SEPARATION IN ENVIRONMENTAL SAMPLES

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## SUMMARY

A high-performance liquid chromatographic method for the separation of saturated and aromatic hydrocarbon mixtures, found in environmental samples, has been developed. The separation is carried out on silica. A short analysis time is achieved by using a backflush technique. Fractions collected are further analysed by high-resolution gas chromatography. The usefulness of this technique is illustrated on petroleum-polluted environmental samples.

## INTRODUCTION

Concern about the fate and effects of petroleum hydrocarbons in the marine environment has stimulated increased research activity on all aspects of hydrocarbon pollution. An integral part of most studies is the analysis of water, sediments and organisms. The large number of analyses being performed in hydrocarbon monitoring programs around coastal refineries, petrochemical industries and off-shore production platforms necessitates rapid, accurate and reproducible analytical procedures.

Essential steps for the analysis of the complex mixtures of petroleum hydrocarbons in environmental samples are (i) the isolation of hydrocarbons from the sample matrix, (ii) the separation of hydrocarbons into individual compound classes and (iii) the identification and quantitation of individual components. A variety of analytical procedures have been employed for these tasks[1–5], most of them including a hydrocarbon class separation into aliphatic and aromatic fractions. These separations rely on classical liquid chromatography with gravity feed columns, packed with either deactivated silica gel or alumina overlying silica gel.

Aliphatics are often eluted with *n*-pentane and aromatics by increasing stepwise the polarity of the eluting solvent through the addition of benzene or dichloromethane. This separation technique has successfully been applied in a variety of petroleum pollution studies[6–9], but the procedure is time-consuming, requires large amounts of precleaned solvents and the columns have to be repacked for each sample.

The present study describes the application of high-performance liquid chromatography (HPLC) to the separation of aliphatics and aromatics in petroleum-

0021-9673/83/$03.00 

polluted environmental samples, and has been adapted to rapid sample throughput by the use of a backflush technique. HPLC[10–17] and medium-pressure liquid chromatography[18] have been applied earlier in the petroleum industry for hydrocarbon class separations and group type determinations in petroleum products. Special emphasis has been placed on possible effects of the matrix from the various environmental samples on the HPLC separation.

## EXPERIMENTAL

### *Preparation of chemicals and equipment*

Trace analysis of hydrocarbons in environmental samples requires control of background levels in chemicals and equipment. All chemicals are solvent-washed prior to use. Solvents (methanol, *n*-pentane, cyclohexane) are distilled over rectifying columns (50 cm). Distilled solvents are kept in precleaned all-glass containers. Prior to use, the hydrocarbon levels are determined by concentrating 100 ml of solvent to a volume of 50 μl, followed by gas chromatographic analysis.

All glass or steel equipment is rinsed with dichloromethane and heated at 600°C overnight. To avoid contamination from the air, all samples are prepared in a hydrocarbon-free atmosphere (laminar flow cabinet). Florisil (Supelco), used for sample clean-up, is washed with dichloromethane and distilled *n*-pentane, then activated for 12 h at 400°C. Prior to use, the Florisil is deactivated with 18% water.

### *Sample preparation*

Biological tissue (*ca.* 30 g wet weight) is homogenized in methanol in a Sorvall omnimixer (Sorvall, Newton, CT, U.S.A.) then 80 ml of 1 *M* methanolic sodium hydroxide are added and the material is saponified under reflux for 2 h. After cooling, internal standards are added. For determinations of *n*-paraffins, a series of six 1-chloroalkanes (1-Cl-$C_8$, 1-Cl-$C_{10}$, 1-Cl-$C_{12}$, 1-Cl-$C_{14}$, 1-Cl-$C_{16}$, 1-Cl-$C_{18}$; Fluka, Buchs, Switzerland) are used as internal standards. Correspondingly, for determinations of aromatics, perdeuterated biphenyl and anthracene (Stohler Isotope Chemical, Waltham, MA, U.S.A.) are added. The saponified material is filtered under suction. The filtered material is first washed with 50 ml hydrochloric acid reagent (2 *M* hydrochloric acid–methanol, 1:3), then with methanol and finally with *n*-pentane. The combined filtrates are extracted twice with 100 ml of *n*-pentane. The combined extracts are washed with 50 ml of water, dried over sodium sulphate and evaporated to *ca.* 5 ml under vacuum. Further evaporation of solvent to a volume of *ca.* 1 ml is accomplished under a light stream of nitrogen while keeping the sample at 30°C (aluminium warming block). The concentrated sample is transferred to a Pasteur pipette, filled with Florisil (7 × 0.5 cm I.D.), eluted with 10 ml of *n*-pentane or cyclohexane and the eluate is concentrated under a light stream of nitrogen to a volume of 200 μl. Sediment samples (*ca.* 50 g wet weight) are prepared by the same procedures as that described for biological tissue but without the homogenization step.

Water samples (1–5) are extracted with *n*-pentane (3 × 40 ml). Internal standards (see biological tissue) are added to the combined extracts. The extracts are dried over sodium sulphate and evaporated to a volume of 200 μl.

*Apparatus*

The HPLC-system consisted of a M6000 A solvent delivery pump, U6K sample injection valve, M 720 system controller, M730 data module and a 401 refractive-index detector (all Waters Assoc., Milford, MA, U.S.A.). Flow reversal was carried out by using a M 7000 six-port valve (Rheodyne, Berkeley, CA, U.S.A.). The silica columns used were either 25 cm × 4,6 mm I.D. (Partisil 5 μm; Chrompack, Middelburg, The Netherlands) or 10 cm × 8 mm I.D. (Rad Pack 10 μm, Waters Assoc.). The mobile phase was either *n*-pentane or cyclohexane at a flow-rate of 1–2 ml/min.

Gas chromatographic (GC) analyses on HPLC fractions are carried out with a Carlo Erba gas chromatograph (Model 2100), equipped with a glass capillary column (20 m × 0.3 mm I.D., coated with OV-1) and a flame ionization detector.

## RESULTS AND DISCUSSION

*Chromatographic conditions*

Hydrocarbon class separation is an integral part of a complex analytical procedure (Fig. 1), necessary for the determination of low levles of hydrocarbon pollution in environmental samples. Of special interest in such investigations is the sepa-

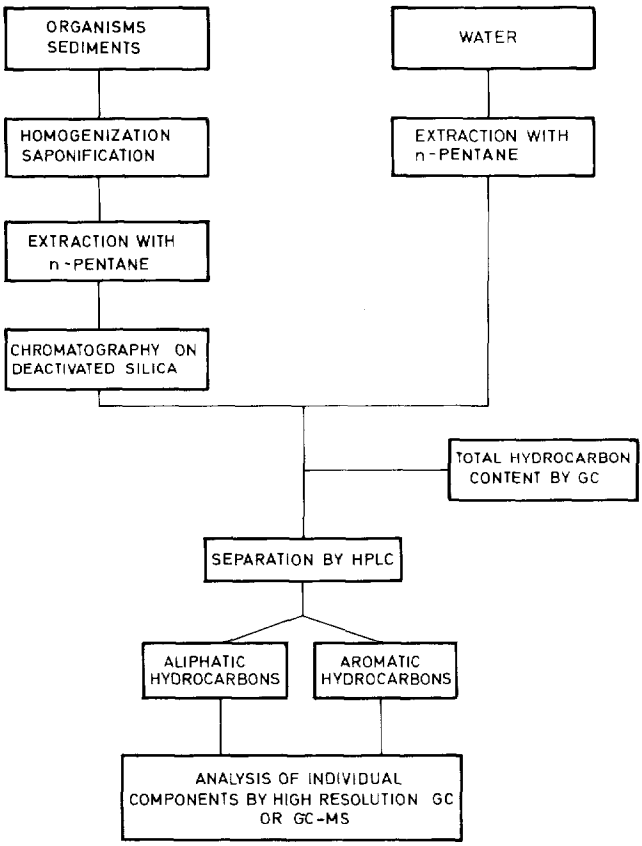


Fig. 1. Flow scheme of the total procedure for analysis of hydrocarbons in environmental samples.

ration and quantitation of selected aliphatic and aromatic compounds. Prior to the application of HPLC to environmental samples, the separation efficiency of silica columns was tested on model compounds and complex hydrocarbon mixtures (petroleum products). To obtain narrow fractions of aliphatics and aromatics, a HPLC system, allowing flow reversal, was assembled as shown in Fig. 2.

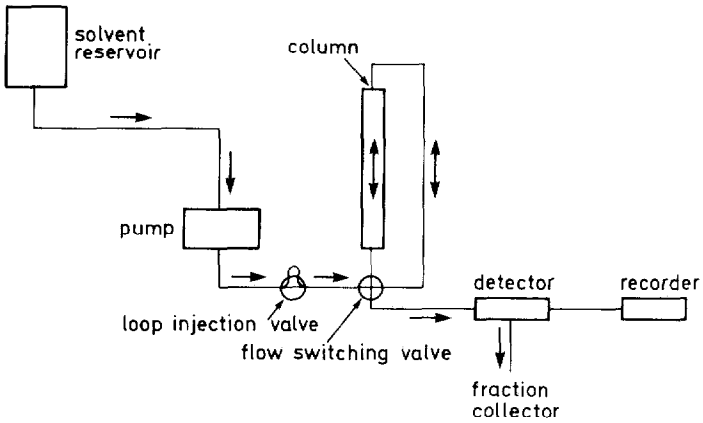


Fig. 2. HPLC system with flow-reversal equipment.

The model compounds chosen were 1-chlorooctadecane, as representative of the aliphatic fraction, and xylene, as representative of the aromatic fraction. The activity of the stationary phases was controlled by using anhydrous solvents that give baseline separations of the model compounds. The retention volumes were 3.90 ml for 1-chlorooctadecane and 4.75 ml for xylene on the Rad Pak column. The flow was reversed between the peaks of the two model compounds (retention volume 4.30 ml). Fig. 3 shows typical chromatograms of a fuel oil without (a) and with (b) flow reversal[19]. Complete baseline separation between aliphatic and more polar compounds is not achieved, probably due to relatively polar aliphatic constituents in the complex mixture of hydrocarbons in petroleum products. GC analyses of the HPLC fractions collected prior to and after flow reversal show complete separation of *n*-paraffins/branched alkanes from aromatic components. Since these compound groups are of major interest in environmental studies, the separation efficiency obtained under the HPLC conditions chosen is satisfactory.

*Application of HPLC to environmental samples*

Environmental samples, like water, sediments and biological tissue, often contain low levels of hydrocarbons, which necessitates isolation and enrichment of the total hydrocarbon fraction. The most common procedure is extraction of the different types of sample matrices by appropriate solvents. Extracts from sediments and biological tissue may often contain large amounts of biogenic components, compared to the amount of hydrocarbons. Typical examples of such natural compounds are various types of aldehydes, ketones, acids, carotenoids, lipids, etc. Direct separation of such extracts by HPLC causes overloading and rapid loss of the separating efficiency of the silica columns.

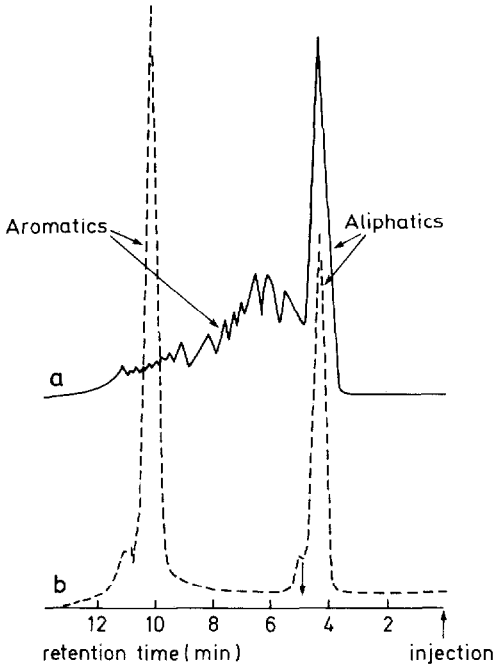


Fig. 3. Chromatograms of a fuel oil without (a) and with (b) flow reversal. Column: silica. Mobile phase: *n*-pentane. Detector: refractive index. The arrow indicates flow reversal.

To avoid problems with sample matrix effects and to protect the analytical columns, clean-up of the hydrocarbon extracts is necessary. This is achieved by filtration of concentrated sample extracts (< 1 ml) through deactivated Florisil. Deactivation of the Florisil with 18% water was found necessary to allow elution of polyaromatic hydrocarbons with *n*-pentane or cyclohexane, while the more polar biogenic components are retained by the column. The Florisil filtration procedure was standardized to elution with 10 ml of the solvent to allow complete elution of binaphthyl.

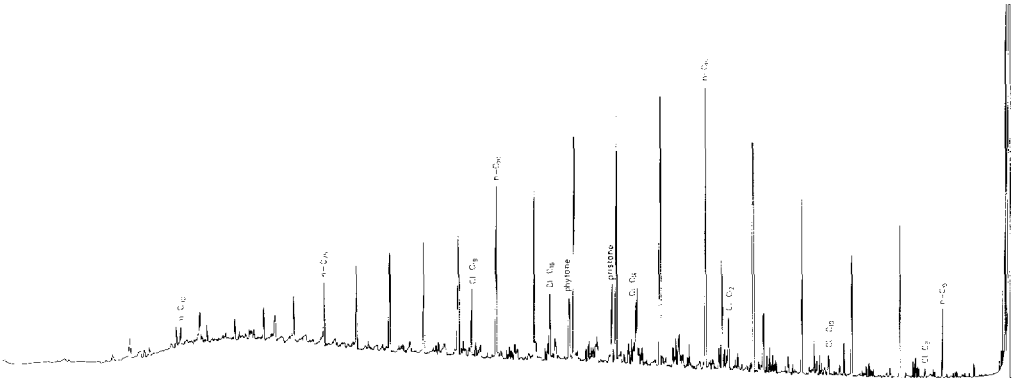


Fig. 4. Gas chromatogram of the aliphatic HPLC fraction, isolated from petroleum-polluted limpets (1-chloroalkanes added as internal standards).

The eluents obtained by the clean-up procedure were evaporated to a volume of 200 $\mu$l under a light stream of nitrogen. The total precleaned and concentrated extracts were injected into the HPLC column. Hydrocarbon class separations were successfully performed with the flow-reversal technique. Figs. 4 an 5 show typical gas chromatograms of the aliphatic and aromatic HPLC-fractions. The hydrocarbons were isolated from limpets, naturally exposed to weathered Ekofisk Crude oil.

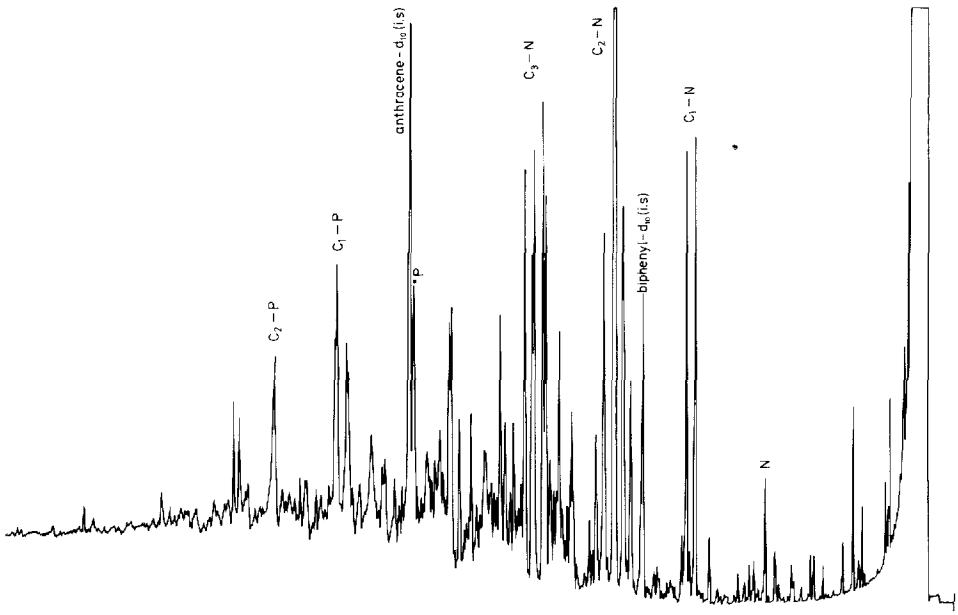


Fig. 5. Gas chromatogram of the aromatic HPLC fraction, isolated from petroleum-polluted limpets. (Internal standards are perdeuterated biphenyl and anthracene. N = Naphthalene; $C_1$-N, $C_2$-N, $C_3$-N = mono, di- and trialkylated naphthalenes; P = phenanthrene; $C_1$-P, $C_2$-P = mono-, dialkylated phenanthrenes, all identified by GC–MS).

Since fractions obtained by HPLC were subjected to further analyses, including GC–mass spectrometric (MS) analysis, care had to be taken to preclude contamination. Procedural blanks and standard mixtures analyzed routinely did not indicate any contamination. Cross-contamination of environmental samples analyzed in series was not a problem with the present method, as proved by frequent checks of the HPLC fractions by GC.

## CONCLUSIONS

The rapid HPLC technique described is suitable for group separations of aliphatic and aromatic hydrocarbons, isolated from environmental samples. Prior to HPLC, sample extracts need to be cleaned by chromatography to remove polar biogenic constituents. The technique is rapid, not only because separation times are short, but also because samples can be processed in series without delays between analyses. The procedure should be adaptable to automation.

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