Paper IV

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Normal phase high performance liquid chromatography for fractionation of organic acid mixtures extracted from crude oils

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Abstract

Crude oil contains such an extensive range of compounds that a complete analysis is impossible. Fractionation by chemical properties is often used to simplify analytical handling. This work presents a high performance liquid chromatography (HPLC) method using normal phase chromatography on a cyano-bonded phase column to separate acid extracts from crude oils into four fractions; non-polar compounds, saturated carboxylic acids, phenols and polyfunctional acids. The method has been developed both in analytical scale for characterisation of acid extracts, and in preparative scale to provide sufficient sample amounts for further analysis by complementary methods.

Keywords: HPLC; Cyano column; Normal phase; Crude oil; Petroleum acids; SPE

1. Introduction

Crude oil is formed by the slow thermal cracking of organic matter incorporated in sedimentary rocks [1], and therefore contains such an extensive range of compounds and molecular species that a complete chemical analysis is impossible to achieve. The components include a wide range of functionalised compounds in addition to the bulk hydrocarbons, and analysis of these fractions is even more challenging than for the hydrocarbons [2]. Gas chromatography (GC) is normally the chosen method when analysing crude oil. GC chromatograms often show a large hump, termed unresolved complex mixture (UCM), which makes the analysis very difficult. Sutton et al. [3] have estimated 250,000 unidentified compounds in the UCM of a biodegraded crude oil. Analysis of crude oils for specific compound types is thus a considerable challenge.

The characterisation of acidic compounds in petroleum is most often undertaken to explain a physical or technical property of the oils, like corrosion [4], emulsion stability [5] or wettability change of solid surfaces by adsorption [6,7]. The traditionally used measure of the acid content in petroleum samples is non-aqueous titration which gives a "Total Acid Number" (TAN) [8]. However, this value contains no information on the composition of the acids, and does not correlate well with, e.g. the degree of corrosion caused by oils of different acidities [9], so more detailed analyses are needed to correlate with the physical effects of the acids. At the other extreme of precision, recently developed methods that analyse very complex mixtures directly with no pre-treatment have been applied to petroleum acids. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) has been applied to heterocompounds in coal extracts [10] and naphthenic acids analysis [11], and gives a detailed overview of the distribution of acids in the sample, based on the molecular masses. These methods have a great capacity for determination of molecular compositions of such complex mixtures, but the challenge of relating the analytical data to the chemical properties of the components remains, and fractionation that separates the samples into fractions containing uniform chemical structures is still required for testing in the specific contexts. Thus, extensive work-up schemes are still needed for separating the sample into sub-fractions with similar chemical compositions.

Column chromatography (CC) and high performance liquid chromatography (HPLC) are useful techniques for this purpose. They are often used in normal phase mode to separate the hydrocarbon phase from the more polar fractions of the oil, e.g. in saturates–aromatics–resins–asphaltenes fractionation (SARA) [12]. In petroleum analysis, a silica stationary phase is

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often used for normal phase CC and HPLC separation, using a sequence of solvents with increasing polarity to elute the hydrocarbons and functionalised compounds as separate fractions [13,14]. However, the use of silica is limited by its very polar properties and tendency to irreversible adsorption of polar compounds, and recovery factors as low as 50% are considered acceptable [14,15]. The acid extracts analysed in this work contain compounds with high polarity, including the microbially produced biosurfactants that are of special interest due to their very strong surface active properties. A review of group separation of petroleum products by HPLC is published by Kamiński et al. [16]. However, the methods focus on fractionation of the hydrocarbons, and acidic compounds are addressed to a limited degree.

In previous investigations, ion pair HPLC has been used for separation of crude oil acids by acid strength [17]. This method uses a dynamic equilibrium on silica to control the retention of acidic compounds on the column, which makes it difficult to acquire stable retention time values and gives a long equilibrium time. Ion exchange chromatography has been used by Jones et al. [18], in the form of non-aqueous SPE ion exchange (SAX quaternary amine) for selective extraction of the carboxylic acids from crude oils. This procedure does not include sub-fractionation of the acids.

Reversed phase liquid chromatography using a silica column modified with C18 alkyl chains is often used to separate polar compounds. Lee et al. [19] have used non-aqueous reversed phase HPLC for separation of lipids. We have tested this method for analysis of the acids extracted from crude oil. Different solvents based on the gradient profile from Lee et al. were tested on a C18 column. The chromatogram showed that the sample components were separated to some degree, but no baseline separation of groups was obtained. In addition, non-polar compounds can probably be irreversible attached to the non-polar column material. Thus, the C18 column is not well suited for the samples we want to analyse.

As an intermediate polarity between the silica and the C18 column, a column material consisting of silica modified with cyanopropyl groups (cyano column) can be used. Such sorbents have been used in geochemical and petroleum analysis for group type fractionation [20] to avoid irreversible adsorption of asphaltenes and other polar oil components. This type of column is evaluated to be more suitable than silica for the analysis of the acids extracted from crude oil. Amino modified silica is also used for fractionation of polar petroleum constituents [21], but is not considered optimal for the acid fraction due to the added complexity of retention behaviour that can result from possible ion exchange behaviour on the amine groups.

The aim of this work is to develop an HPLC method to characterise the distribution of acidic organic compounds in crude oils, and to prepare fractions suitable for further analysis at molecular levels and also for testing of physical properties. This work presents an HPLC method using normal phase chromatography on a cyano-bonded phase column which provides a stable and fast separation of organic acids from crude oils into four well-defined fractions that correspond to the main types of acidic compounds; weak acids with no acidic protons, saturated carboxylic acids, phenols and polyfunctional acids. The method is developed both in analytical scale for characterisation of acid extracts, and in preparative scale to provide sufficient sample amounts for further analysis by complementary methods. The method is applied on a sample set of acid extracts from crude oils from the Norwegian continental shelf. These oils include both biodegraded and non-biodegraded oils.

The solvent programmes are modified from the solvent combinations conventionally used in petroleum group type separations [13,14,20], but they have been adjusted to give a slow, gradual increase in polarity to ensure good separation of the different acid types.

Two detectors are used: an evaporative light scattering detector (ELSD), which detects all compounds except low-boiling compounds that evaporate together with the solvent, e.g. certain phenolic compounds, and a UV detector that detects all molecules with suitable chromophores.

2. Experimental

2.1. Standards and samples

The standards used are of p.a. quality. This includes a commercial standard of naphthenic acids (technical purity, Aldrich) and a commercial biosurfactant, surfactin (purity approx. 98%, Sigma). Surfactin is a lipopeptide, and contains a seven-membered ring made up of four different amino acid units (leucine, glycine, valine and aspartic acid), linked with a hydroxy fatty acid. The biosurfactant rhamnolipid (0.25% in water) was provided by Professor I. Banat, University of Ulster [22]. Rhamnolipid is a glycolipid, and consists of the sugar structure rhamnose and hydroxy fatty acids. The solvents are all of HPLC or p.a. quality.

Acids are extracted from a sample set of eight crude oils, spanning from heavy biodegraded oils enriched in asphaltenes to light non-biodegraded oils. The oils originate from the Norwegian continental shelf and are supplied by Norsk Hydro ASA (seven oils) and Statoil ASA (one oil). The oils are marked with letters, B for biodegraded oils and S for sweet, non-biodegraded oils, followed by a number indicating production field and a letter denoting different wells or different batches within one field.

Two methods of acid extraction are used: an ion exchange method described by Mediaas et al. [23] and a liquid–liquid extraction described by Constantinides and Arich [24] and others [25,26]. These extraction procedures are also presented in a recent paper by Borgund et al. [27].

2.2. HPLC procedure

A P680 HPLC Pump (Dionex, California, USA) and a Rheodyne 7725 manual injector (Rheodyne, California, USA) with a 20 μl (analytical column) or 100 μl (semi-preparative column) loop are used for the analysis. Two types of detectors are used: a light scattering detector (ELSD, Sedex 55 Light Scattering Detector, France; operation temperature, 40 °C; nebulizing gas, nitrogen) and a UV detector (UVD340U Dionex, diode-array

Table 1
Gradient programme for HPLC analyses

Column	Time (min)	Hexane (%) (v/v)	DCM (%) (v/v)	MeOH (%) (v/v)
Analytical	0	97	3	–
	10	97	3	–
	20	70	30	–
	35	40	55	5
	40	–	100	–
	50	97	3	–
	65	97	3	–
Semi-preparative	0	97	3	–
	4	97	3	–
	8	90	10	–
	14	90	10	–
	18	70	30	–
	31	40	55	5
	35	–	100	–
	42	97	3	–
	55	97	3	–

detector, California, USA). Chromatograms from the UV detector at wavenumbers 230, 250, 280 and 300 nm are chosen for the characterisation of each sample. The chromatogram from a blank run is automatically subtracted from the sample chromatogram in order to remove the influence from the solvents. The laboratory data system used is Chromeleon (delivered by Dionex Softrun, California, USA).

Two types of BDS Hypersil Cyano columns and guard columns (Thermo Scientific, Massachusetts, USA) are used: an analytical column (250 mm × 4.6 mm, 5 μm) with a guard column (100 mm × 4 mm, 5 μm) and a semi-preparative column (250 mm × 10 mm, 5 μm) with a guard column (100 mm × 100 mm, 5 μm). The gradient programmes for the two columns are shown in Table 1. The flow rate is set to 0.5 ml/min for the analytical column and 2 ml/min for the semi-preparative column.

The samples are dissolved in dichloromethane (DCM):methanol (MeOH) 93:7 (v/v) to a concentration of approximately 10 mg/ml, giving 0.2 mg sample applied to the analytical column and 1 mg sample applied to the semi-preparative column.

Some of the acid extracts are run preparatively and fractions are collected for further analysis. The acid extract with a concentration of 10 mg/ml is run through the semi-preparative column five times, and five fractions are collected. The solvent in the fractions is evaporated under N_2-gas flow and the fractions are redissolved in a small volume (0.25–0.5 ml) of DCM:MeOH 93:7 (v/v).

2.3. SPE cyano columns

SPE cyano columns (Isolute SPE Cyano (end-capped), International Sorbent Technology, UK, 1000 mg sorbent mass, 3 ml reservoir volume) are tested to fractionate larger amounts of sample. In this procedure, the column is wetted with the first eluent, and approximately 30 mg of the sample is dissolved in 0.075 ml of DCM:MeOH 93:7 (v/v) before it is applicated onto the column. The solvents used are: hexane:DCM 90:10 (v/v), DCM:MeOH 93:7 (v/v), MeOH:DCM 70:30 (v/v) and MeOH:formic acid 95:5 (v/v). The volumes used are given in Table 2.

Table 2
Fractionation procedure for SPE fractionation

Fraction	Solvents	Volume of solvent (ml)	Compound types
1	Hexane:DCM 90:10 (v/v)	20	Non-polar and carboxylic acids
2	Hexane:DCM 90:10 (v/v)	30	Intermediate fraction
3	DCM:MeOH 93:7 (v/v)	20	Polyfunctional and phenols
4	MeOH:DCM 70:30 (v/v) + MeOH:formic acid 95:5 (v/v)	ca. 2 ca. 10	Highly polar compounds

2.4. FT-IR analysis

FT-IR analysis is performed on a Nicolet Protege 460 FTIR spectrometer (Thermo Scientific, Massachusetts, USA) with a Diamond Attenuated Total Reflection (ATR)-Dura sampler cell (from SensIR). The samples are dissolved in DCM:MeOH 93:7 (v/v). A small amount of sample (one droplet) is placed on the ATR diamond, and the solvent is evaporated before the spectra are recorded. The spectra are recorded from 600 to 4000 cm^{-1}, using 32 scans and a resolution of 4 cm^{-1}.

3. Results and discussion

3.1. Analytical cyano HPLC column

3.1.1. Standards

Standards having a wide range of polarities and different functionalities are run on the analytical HPLC column (250 mm × 4.6 mm, 5 μm) using both the ELS and UV detector. A list of the retention times for some standards is shown in Table 3. The standard octadecanoic acid is run six times, and 1,12-dodecanedioic acid is run 11 times. The standard deviation of the registered retention time for both standards is calculated to 0.2 min. The retention of standards is reproducible over a period of more than 6 months, within the standard deviation given.

Chromatograms from a mixture of three standards (octadecane, octadecanoic acid and 1,12-dodecanedioic acid) using the ELS detector, and four standards (phenol, octadecane, octadecanoic acid and 1,12-dodecanedioic acid) using the UV detector are shown in Fig. 1. The three standards octadecane, octadecanoic acid and 1,12-dodecanedioic acid are not visible using UV detection because they do not show UV absorbance.

The acid extracts are analysed using both the ELS and the UV detector, and the same HPLC-gradient as used for the standards is applied. Since the UV detector does not show all the compounds present in the samples, the ELS detector is used when comparing the acid profiles of different samples. However, the response for some phenolic compounds is weak on the

Table 3
Retention times for standards using the analytical HPLC column

Standards	ELSD detection		UV detection	
	Main peak RT (min)	Other peaks RT (min)	Main peak RT (min)	Other peaks RT (min)
Octadecane	6.5			
1,5-Dimethylnaphthalene	–		6.9	
Benzyl alcohol	7.0		7.3	
Phenanthrene	7.1		7.5	
2,6-Dimethylphenol	–		12.1	12.8
Benzoic acid	–		17.5	34.4
Dodecanoic acid	17.7			
Octadecanoic acid	17.8			
4-Methoxy benzyl alcohol			21.7	23.4
p-Cresol			27.7	27.1
Phenol			28.8	
2-Naphthol	32.0		32.0	
Naphthoic acid	32.8		33.8	30.4
1,12-Dodecanedioic acid	33.3			
o-Phthalic acid	35.3		37.5	35.2, 33.5
Surfactin	35.3			
Rhamnolipids	36.0	32.5, 33.0	33.7	33.5

ELS detector, probably due to evaporation of sample components together with the solvent. The UV detector is therefore useful for investigating the phenolic structures.

All of the compounds in the samples elute from the column within 47 min of the run, and the chromatogram is divided into four fractions: F_A, 0–10 min; F_B, 10–20 min; F_C, 20–32 min and F_D, 32–47 min.

The retention times of the standards illustrate the type of compounds that are expected to be present in the different fractions of the HPLC-run. The F_A-fraction contains the non-polar components. The weakest acids co-elute with the hydrocarbons and are included in what is termed the non-polar fraction. In the F_B-fraction we find saturated carboxylic acids, and the F_C-fraction contains phenols. The F_D-fraction contains polyfunctional compounds. Phthalic acid, rhamnolipids and surfactin are examples of compounds that elute in this part of the chromatogram. The benzoic acid peak is always accompanied by a smaller peak in fraction F_D. Different qualities and recrystallisation of the standard have been tested, but it still gives two peaks with similar UV spectra. The reason for the extra peak is not known, but some type of selective adsorption onto remaining Si–OH groups on the column or ion pair formation can be suspected.

On the polar cyano HPLC column the non-polar components elute first, while the more polar components are retained to some degree on the column and elute later. The results from the analysis of standards show that the phenolic compounds elute after the carboxylic acids. This indicates that aromatic structures are more strongly held back on the column relative to aliphatic structures, probably due to the aromatic compounds having stronger affinity for the cyano groups on the column material.

To confirm the elution of carboxylic acids from oils in the F_B-fraction, a representative standard for the mixture of compounds found in petroleum samples is also tested. This is a commercial standard of naphthenic acids, and it gives dominant peaks in the F_B-fraction and only small signals in the other fractions.

3.1.2. Analytical chromatography with ELS detector

A chromatogram of the acid extract from the biodegraded oil B4c using an ELS detector is shown in Fig. 2. The chromatogram contains three distinct peaks, and is divided into the four fractions (F_A, F_B, F_C and F_D) as described above. The major part of this sample elutes in the F_B-fraction, 10–20 min, corresponding to the standards of saturated carboxylic acids. The chromatogram also shows a component group eluting at 32.8 min, which indicates the presence of polyfunctional compounds. A small peak at 7 min indicates the presence of

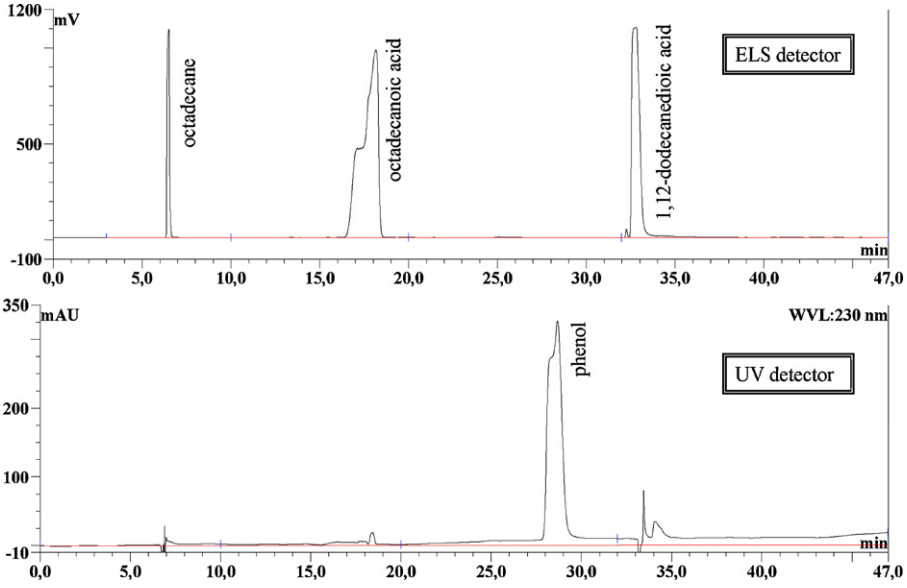

Fig. 1. Upper chromatogram, mixture of three standards (octadecane, octadecanoic acid and 1,12-dodecanedioic acid) using an analytical HPLC column and an ELS detector. Lower chromatogram, mixture of four standards (phenol, octadecane, octadecanoic acid and 1,12-dodecanedioic acid) using an analytical HPLC column and a UV detector.

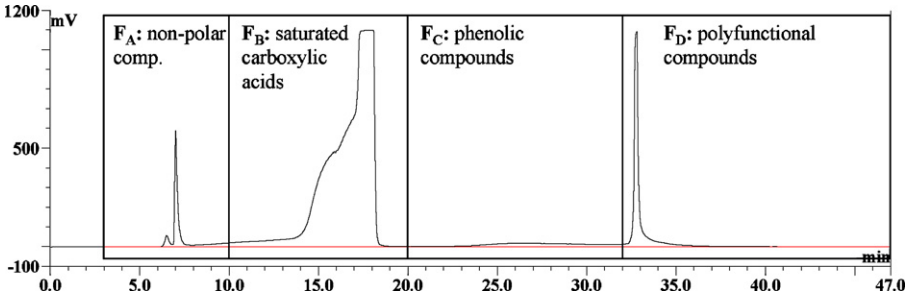

Fig. 2. A chromatogram of the acid extract from the oil B4c (ion exchange extraction), using an analytical column and an ELS detector.

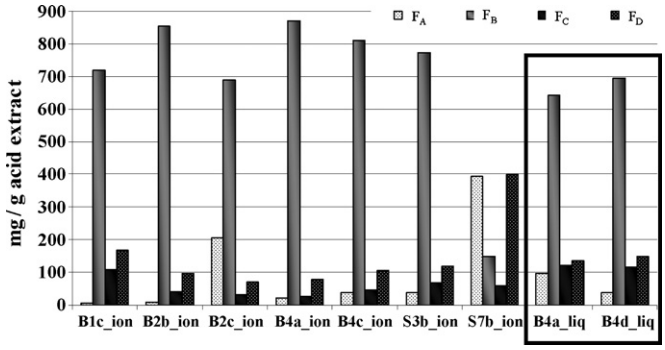

Fig. 3. The relative composition of acid extract fractions from different oils using an analytical HPLC column and an ELS detector. The oils marked with "ion" have been extracted by the ion exchange method, and the oils marked with "liq" have been extracted by the liquid–liquid extraction. Information about the extraction procedures can be found in Section 2.1.

non-polar compounds. The phenolic fraction, 20–32 min, has a broad band with low intensity and no distinct peaks in this chromatogram.

The acid distribution of all the samples is compared to each other by comparing the estimated amount of material found in each fraction calculated from the peak areas. The relative amounts are presented in a histogram shown in Fig. 3. Differences in acid profiles are clearly seen.

3.1.3. Analytical chromatography with UV detector

A chromatogram using UV detection of an acid extract of the biodegraded oil B4c is shown in Fig. 4. Like the chromatogram from the ELS detector of this extract (see Fig. 2), it contains three peaks. When using the UV detector the F_B-peak is not as prominent as the one detected by the use of ELS detector. This is reasonable as the alkanoic carboxylic acids give a weak UV response, as they are poor chromophores. In fraction F_C, there is still no strong peak for the phenols, but the area above the baseline is clearly larger than observed when using the ELS detector.

3.1.4. Linear range of detection

In the chromatogram resulting from ELS detection (Fig. 2), we can see that the second peak (F_B) is cut off at the top of the chromatogram, due to high concentration of the sample. One acid extract is run at different concentrations (1, 2, 3, 4 and 5 mg/ml) to find the range of linearity and the detection limit of the ELS detector for our samples. Response curves are made for the two largest peaks in the chromatogram, F_B and F_D, and linearity is observed up to a concentration of 4 mg/ml for both fractions. The two peaks give different slopes in the response curves, so the linearity is valid only within each fraction. A sample concentration of 5 mg/ml will overload the detector, as this point falls outside of the linear region. A standard (1,12-dodecanedioic acid) is also tested. For this standard a good six-point calibration curve over the range 0.05–1 mg/ml is obtained ($R^2 = 0.98$).

The sample concentration of 10 mg/ml that is used for the samples is thus outside the linear response range due to high concentrations of the largest peaks. However, this concentration of the sample was nonetheless used to ensure a sufficient response for the smaller peaks in the chromatogram.

3.2. Semi-preparative HPLC cyano column

3.2.1. Standards

A list of the retention times for some standards run on the semi-preparative HPLC column is shown in Table 4. Some of the standards are run several times, and variations in the retention times from 0.04 to 0.5 min are found for the different standards.

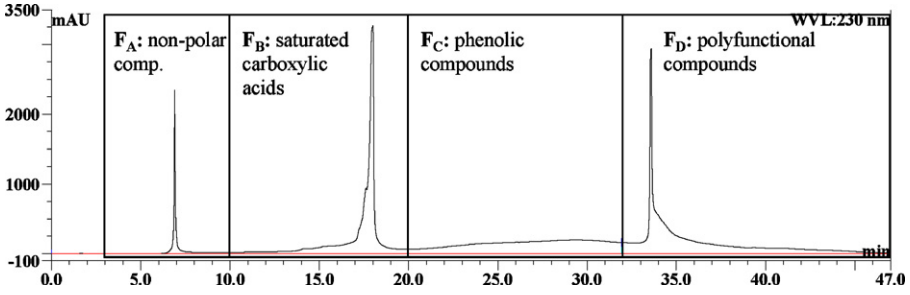

Fig. 4. A chromatogram of the acid extract of the oil B4c (ion exchange method) using an analytical HPLC column and a UV detection at wavelength 230 nm.

Table 4
Retention times for standards using the semi-preparative HPLC column

Standards	ELSD detection		UV detection	
	Main peak RT (min)	Other peaks RT (min)	Main peak RT (min)	Other peaks RT (min)
1,5-Dimethylnaphthalene	–		7.8	
Benzyl alcohol	7.9		7.9	
Phenanthrene	7.9			
2,6-Dimethylphenol	–		14.3	
Octadecanoic acid	17.7		–	
Dodecanoic acid	19.1		–	
Benzoic acid			19.7	31.1
4-Methoxy benzylalcohol	–		20.2	23.5
p-Cresol			27.1	
Phenol			28.4	
Naphthoic acid	30.3		31.0	
2-Naphthol	30.6		31.0	
1,12-Dodecanedioic acid	30.9			
Phthalic acid	32.9		33.5	
Rhamnolipids	33.3	30.3, 30.7	31.0	
Surfactin	33.3			
HPMC	36.6		–	

HPMC, hydroxy propyl methyl cellulose.

All of the compounds in the samples elute from the column within 40 min after injection. The HPLC system uses an additional 15 min for the solvent system to return to the starting conditions. The chromatogram is divided into five fractions: F_A, 3–12.5 min; F_{B1}, 12.5–19 min; F_{B2}, 19–22.5 min; F_C, 22.5–30 min and F_D, 30–40 min.

As for the analytical HPLC column, the retention times of the standards indicate the type of compounds that can be found in the different fractions of the HPLC-run. The F_A-fraction contains non-polar components. The F_B-fraction is divided into F_{B1} and F_{B2}, where carboxylic acids are found in both fractions, and F_{B2} contains more aromatic compounds. The F_C-fraction contains phenols. The F_D-fraction represents polyfunctional compounds, like phthalic acid, rhamnolipids and surfactin. The standard methoxy benzylalcohol elutes in the F_{B2} peak, while it elutes in the F_C-fraction using the analytical column.

3.2.2. Semi-preparative chromatography with ELS detector

A chromatogram of the acid extract of the biodegraded oil B2b, using an ELS detector is shown in Fig. 5. The chromatogram contains four peaks, and the chromatogram is divided into five fractions, as described in Section 3.2.1. There is no baseline separation between the second and third peak, but a division is set at 19 min to separate this peak into two fractions. The F_{B1} peak has the largest peak area in the chromatogram, and no clear peaks are found in the F_C-fraction.

The mobile phase flow through the column using the semi-preparative column is set to 2 ml/min. This overloads the vaporisation unit in the ELS detector. Thus, quantitative results are not obtained, and the detector is only used to confirm the fraction limits before preparative use.

3.2.3. Semi-preparative chromatography with UV detector

A chromatogram of the acid extract of the biodegraded oil B2b, using an UV detector, is shown in Fig. 6. This chromatogram contains three major peaks. The second peak (F_{B1}) is much smaller using the UV detector compared to the results from the ELS detector of the same extract in Fig. 5. This is due to the high proportion of carboxylic acids that cannot be detected using the UV detector.

The recovery from the semi-preparative HPLC column is above 70%, and sometimes exceeds 100% on a weight basis.

3.3. FT-IR analysis of fractions from preparative HPLC

Acid extracts are run preparatively and the fractions are collected for further analysis with FT-IR. The results from the FT-IR analysis confirm the separations observed in the standards: fraction F_A contains non-polar compounds and fraction F_{B1} and F_{B2} contain carboxylic acids. The precise functional composition of polyfunctional compounds is difficult to determine using FT-IR analysis, but acidic functionalities are clearly present, as seen by a strong absorption peak at 1705 cm^{-1} [16].

3.4. SPE cyano columns

SPE columns are used to fractionate a sample into four sub-fractions. Even after optimisation of the eluent composition, the SPE columns cannot reproduce the fractionation on the HPLC cyano columns. For a sequence of 11 SPE fractionations, an average recovery of 113 wt% was obtained. The SPE sub-fractions are analysed on an analytical cyano HPLC column (see Section 3.1) and the chromatograms are shown in Fig. 7.

The first SPE sub-fraction primarily contains non-polar compounds and carboxylic acids. The second sub-fraction is an intermediate fraction, with a small contribution from all fractions. The third sub-fraction primarily contains the phenols and

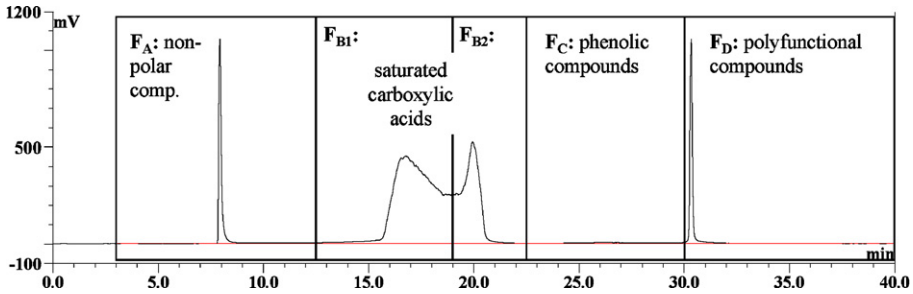

Fig. 5. A chromatogram of the acid extract of the biodegraded oil B2b, using a semi-preparative HPLC column and an ELS detector.

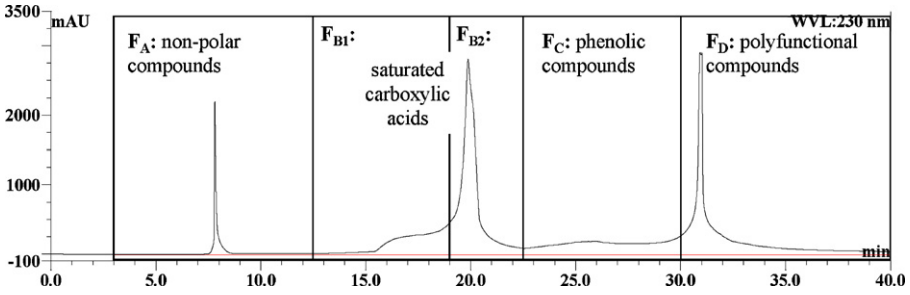

Fig. 6. A chromatogram of the acid extract of the biodegraded oil B2b, using a semi-preparative HPLC column and UV detection at wavelength 230 nm.

the polyfunctional compounds. The last highly polar eluent solvent is used to make sure that all the organic material is eluted from the column, and is collected as sub-fraction four. This solvent was added after visual inspection of the column showed that some coloured material still was adsorbed. The resulting fraction does not correspond to any of the HPLC fractions, and contains a small amount of polyfunctional components.

As illustrated in Fig. 7 the produced sub-fractions are not sufficiently uniform to be useful in precise analysis on Gel Permeation Chromatography (GPC), FT-IR and GC–MS to get

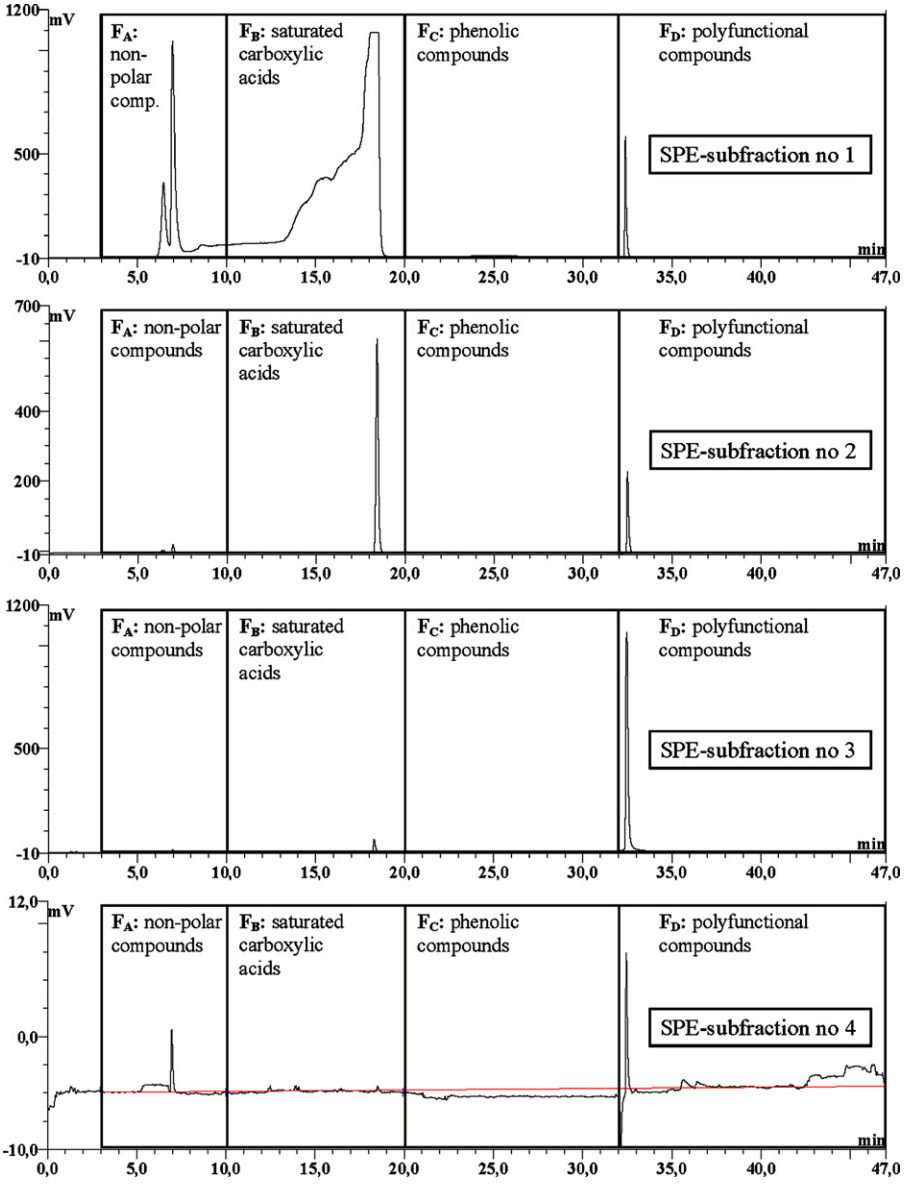

Fig. 7. HPLC chromatograms of the sub-fractions from the SPE fractionation of an acid extract from B4c. The sub-fractions were dissolved in 29 ml of DCM:MeOH 93:7 (v/v) prior to the HPLC analysis. Note the different scales on the *Y*-axis.

structural information. However, the SPE procedure can be used to rapidly separate large amounts of the acid extracts into rough fractions for physical testing.

4. Conclusions

A method for separating acid extracts from crude oils into four distinct fractions has been developed using normal phase chromatography on a cyano-bonded stationary phase with a gradient elution program using hexane, dichloromethane and methanol. The method fractionates the acid extract into non-polar compounds, saturated carboxylic acids, phenols and polyfunctional compounds. The fractions are still too complex for identification of individual components, but are suitable for comparison of the acid profiles of different crude oils. ELS and UV detection give slightly different profiles due to differences in response for the compounds groups. The separation is reproducible over time.

A very similar fractionation is obtained using the semi-preparative cyano column. The main difference is a slightly better resolution in the saturated carboxylic acid fraction, but baseline separation is not obtained. The amounts used are sufficient for collection of fractions for further analysis by FT-IR, GPC, GC–MS and LC–MS. The simplification of the acid composition in each fraction thus enables the more precise analysis of the molecular structure by these other techniques. The recovery of the sample components seems quite good (above 70% by weight).

Rough fractions for testing of physico-chemical properties can be produced using corresponding SPE columns. However, the fractionation is not comparable to the HPLC procedure, as the non-polar compounds and carboxylic acids co-elute, and a very strong eluent is required to elute the most polar compounds from the sorbent.

The methods described are simple and rapid, and complement the existing methods of fractionation of petroleum acids [14,17] because they provide reproducible fractions of acids with similar chemical compositions that are suitable for further testing and can provide a simplified sample for spectroscopic characterisation or more detailed chromatographic analysis, e.g. LC–MS.

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