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Abstract

Heavy polycyclic aromatic hydrocarbons (HPAH) are known to cause undesirable effects in petroleum hydrocracking processes by deactivating the catalysts and accumulating themselves in the downstream of reactors. Polycyclic aromatic hydrocarbons with less than 7 rings (PAH) naturally contained in vacuum gas oils (VGO) act as precursors in the HPAH formation. However, getting a detailed quantitative characterisation of such polycyclic hydrocarbons has never been done until now because of the high chemical complexity of VGO. Thus an off-line comprehensive three-dimensional methodology was required to achieve a quantitative analysis: centrifugal partition chromatography (CPC) as the first dimension of separation, supercritical fluid chromatography (SFC) as the second dimension hyphenated to Fourier transform ion cyclotron resonance mass spectrometry as the third dimension. In this study, we demonstrated that the developed CPC method fractionated samples according to hydrocarbons alkylation degree, whereas our SFC method provided an elution order according to their double bond equivalent. Finally, high-resolution mass spectrometry (HRMS) brought crucial information on the identity of analytes and proved to be essential in the event of unresolved peaks from CPC and SFC chromatograms. To assess the ability of the three-dimensional method for quantification purposes, matrix effects were evaluated by spiking VGO samples with deuterated pyrene. A strong ion suppression phenomenon was highlighted when using only SFC/HRMS whereas no significant matrix effect was observed with CPC×SFC/HRMS approach. These experiments revealed the high potential of this innovative methodology to quantify both PAH and HPAH in VGO for the first time.

1. Introduction

Conversion of oil heavy distillates such as vacuum gas oils (VGO) in petroleum fuels is a topical energy issue, making enhancement of the existing process performances very challenging for refiners[1]. Among conversion processes, hydrotreating (HDT) and hydrocracking (HCK) processes are one of the main routes to transform a VGO to lighter valuable products with low sulphur and nitrogen contents. These high boiling point distillates (350-550°C) are ultra-complex mixtures which are composed of several thousands of diversified molecules including saturated and aromatic hydrocarbons as well as heteroatom-containing components (sulphur, nitrogen and oxygen compounds)[2–4]. Among these components, polycyclic aromatic hydrocarbons with 2-6 rings (PAH) present in VGO are known to be precursors for the formation of heavy polycyclic aromatic hydrocarbons (HPAH) during HCK process[5,6]. These latter are responsible for undesirable effects on catalyst activity and tend to accumulate over time in the downstream of the reactor. In order to predict the HPAH that are formed, process modelling requires quantitative input data on the characterisation of VGO feeds which have not been published to date.

One of the main historical analytical tool used for petroleum characterisation is gas chromatography (GC) and two-dimensional GC (GC×GC)[7–12]. However, these techniques are usually limited to polycyclic aromatic hydrocarbons with less than eight benzene rings (coronene) due to their low volatility[13–15]. At the opposite, liquid chromatography (LC) is a suitable technique for heavy products analyses. In the literature, some works have already proposed analytical methods to quantify HPAH in hydrocracked products using non-aqueous reversed-phase liquid chromatography (NARP) as was done by Panda *et al.*[16]. Yet, the authors explained that the developed method was restricted to the streams with low sulphur content and thus not applicable to most of VGO. Indeed, the molecular composition of a VGO introduced in the reactor significantly differs from the HCK products as a VGO contains more aromatic, more alkylated and more sulphur-containing HPAH than their products. Due to this complexity and the lack of LC resolution, one-dimensional liquid chromatography cannot be used alone. Although some LC×LC methods have been developed[17,18], no methodology has succeeded in a fully resolved chromatogram and allowed to determine the amount of polycyclic aromatic hydrocarbons in VGO. At the boundary of GC and LC, supercritical fluid chromatography (SFC) using carbon dioxide as the mobile phase is a promising tool for separation of non-volatile compounds in petroleum samples. At its beginning, this technique was mainly employed using capillary columns[19]. Nowadays, SFC is performed on packed columns using an organic modifier to increase the eluent strength[20,21]. Although no study has provided VGO characterisation using packed columns SFC, few publications have reported PAH separation for other applications[22–26]. Among these studies, some of them selected apolar stationary phases[23,25], while the others chose stationary phases with polar groups[22,24,26]. Zhang *et al.* compared the separation of 16 PAH from two to six rings on three kinds of stationary phase: BEH 2-EP, HSS C_{18} SB and CSH Fluoro-phenyl. Although some differences of selectivity were observed, elution order was according to the number of rings for the three columns. Finally, the authors selected BEH 2-EP column due to a better resolution[26]. After a screening of columns, two other teams selected the Torus 2-picolylamine column which corresponds to the new generation of the BEH 2-EP[22,24]. Concerning the mobile phase, a gradient elution using methanol or acetonitrile as co-solvent was preferred in all the studies. Zhang *et al.* noticed that among the co-solvents investigated (acetonitrile, isopropanol, methanol and hexane), only methanol succeed in a complete elution of the 16 PAH on a BEH 2-EP column.

Many studies dealing with petroleomic approach have applied Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/MS) which is one of the most powerful techniques to go deeper in the characterisation of heavy oils thanks to its ultrahigh-resolution[27–31]. Among the available ionisation sources, positive ion-mode atmospheric pressure photo-ionisation source ((+)APPI) seemed to be the most efficient one for HPAH ionisation[32–34]. Moreover, dopants are usually employed to increase the ionisation efficiency, such as toluene[35–37], chlorobenzene[38,39], anisole or a mix of several compounds[40,41]. However, mass spectrometry alone cannot resolve the complexity of petroleum samples mainly due to (1) ionisation discrimination and (2) presence of isomeric species. Sub-fractionation can be performed prior to mass spectrometry analysis, such as SARA fractionation method which separates samples into four major classes of compounds (Saturates, Aromatics, Resins and Asphaltenes) mainly based on solubility and affinity for absorption on solid granular packing column[42,43]. Basically, the first step consists in isolation by precipitation of the asphaltenes by adding an excess of alkane solvent (hexane or heptane). Then, the fraction that remains dissolved is injected on alumina and silica columns to adsorb resins and aromatics. The non-adsorbed remaining oil corresponds to saturated hydrocarbons. Then solvents such as toluene and dichloromethane are used to desorb the aromatics and resins fractions[44,45]. Although many sub-fractionation methods could be found in the literature such as gel permeation chromatography (GPC) fractionation[36], accurate quantification is still one of the main issues of mass spectrometry when using direct sample introduction. Another way to achieve an exhaustive characterisation and quantification is the hyphenation of separation technique(s) and mass spectrometry although MS scan speed can be a limiting factor.

In this work, in response to the chemical complexity of VGO and the requirement to quantify HPAH, two multidimensional separation approaches have been developed with hyphenation to (+)APPI/FT-ICR/MS. SFC, as well as centrifugal partition chromatography (CPC) separations, have been studied. The method development, in particular the choice of the two dimensions of separation, has been discussed as well as the mechanism of retention and the hyphenation conditions with high-resolution mass spectrometry (HRMS). Quantification of HPAH in VGO thanks to this method has been demonstrated for several industrial samples using deuterated pyrene to evaluate the matrix effect occurring in (+)APPI. The aim was to illustrate the actual input of an innovative three-dimensional (CPC, SFC and HRMS) methodology.

2. Experimental section

2.1. *Samples*

Three vacuum gas oils with different properties were selected to assess the three-dimensional methodology. Their characteristics are given in Table 1. One unconverted hydrocracked oil (UCO) coming from the hydrotreating and hydrocracking of VGO 1 was also investigated in this study. Thanks to the hydrotreating, which removes heteroatoms (oxygen, nitrogen, sulfur) in the presence of high pressure hydrogen, UCO sample is mainly composed of hydrocarbons.

Table 1: Physical and chemical properties of petroleum samples investigated in this work.

Sample	Sulfur content (mg/kg)	Nitrogen content (mg/kg)	Specific gravity at 15°C (g/cm^3)	Boiling points at 5 and 95% distilled (°C)
	ASTM D2622	ASTM D4629	ASTM D4052	ASTM D86
VGO 1	18921	1395	0.9284	394-581
VGO 2	25792	982	0.9244	352-544
VGO 3	24339	1250	0.9304	311-571
UCO	<10	<0.3	0.8628	390-568

2.2. *Chemicals*

All solvents were purchased from VWR (Fontenay-sous-Bois, France). Acetonitrile (ACN), heptane (HEPT), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF) and dichloromethane (DCM) were HPLC grade. Methanol (MeOH) used during SFC analyses was HPLC-MS grade. Anisole was ≥ 99% pure. Carbon dioxide SFC grade (99.97%) (B50 bottle under pressure) was purchased from Air Liquide (Paris, France).

HPAH standards and pyrene-d10 were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Anthracene, pyrene, chrysene, benz[a]antracene, perylene, benzo[ghi]perylene and coronene were prepared at 75 mg/L in DMSO as a model mix used for separation and detection method optimisations (Table S1).

2.3. *Centrifugal Partition Chromatography (CPC)*

CPC experiments were carried out with a SCPC100 associated to a Spot Prep II from Armen Instrument (Gilson Purification, USA). The system included a quaternary pump, an automatic sample injection with a 5 mL loop, a diode array detector (DAD) scanning from 200 to 400 nm and a fraction collector. A 131 mL column was used for this method development. Chromatographic data were managed using the Armen Glider CPC software. Experiments were conducted at room temperature.

The solvent system consisted of HEPT-DMSO-ACN 45/10/45% (v/v/v). Biphasic system solvent was prepared in a separatory funnel. Descendant elution mode allowed to use the heptane enriched phase as the stationary phase and the acetonitrile enriched phase as the mobile phase. The stationary phase ratio was measured at 69%. Samples were prepared as follows: 1 g of sample was diluted in 5 mL of 70% stationary phase / 30% mobile phase (w/w). After 10 min of equilibration with the mobile phase at 30 mL/min and 500 rpm, elution took place during the first 30 min of the run (2000 rpm, 10 mL/min of mobile phase), then the run ended with 10 min of extrusion (2000 rpm, 30 mL/min of stationary phase). One fraction was collected each minute (40 fractions in total per run). These fractions were evaporated to dryness and the dry extracts were dissolved in 300 µL of THF for further analyses with SFC.

2.4. *Supercritical Fluid Chromatography (SFC)*

SFC experiments were performed on an Acquity UPC² instrument (Waters, USA). The instrument control was performed by Empower 3 software (Waters). The mobile phase flow rate was 1.1 mL/min. The organic co-solvent was methanol ranging from 0 to 30% in 15 min. Prior to the gradient elution, the method started with an isocratic step of 5 min with 100% of CO_2. The injection volume was 1 µL. Three key parameters (type of stationary phase, back pressure and temperature) were

optimised (see part 3.1). The investigated columns were: Acquity UPC² BEH 2-EP, Acquity UPC² BEH, Acquity UPC² CSH Fluoro-Phenyl, Acquity UPC² HSS C_{18} SB and Acquity UPC² Torus 2-PIC columns (3 × 100 mm for all of them). The back pressure varied from 10.5 to 16.4 MPa and the temperature from 25 to 55°C. The final conditions were a back pressure of 10.5 MPa at 55°C with a Torus 2-PIC column (3 × 100 mm, particle size 1.7 µm, Waters). The UV detection wavelengths ranged from 210 to 400 nm. At the outlet of the diode array detector (DAD), a make-up solvent (anisole) was added at 100 µL/min. Finally, the flow was split, one part going to the mass spectrometer and the other one to the automated back pressure regulator (ABPR). A scheme of the commercial interface with tubing dimensions is presented in Figure S1.

2.5. *Hyphenation with Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR/MS)*

The chromatographic systems were hyphenated to a linear ion trap - Fourier-transform ion cyclotron resonance mass spectrometer (LTQ-FT-ICR, Thermo Scientific, Germany) equipped with a 7T magnet. Ionisation was carried out using APPI in positive mode. The mass range was set as *m/z* 98-1000. In order to have at least 10 points for each chromatographic peak, a resolving power of 12500 at *m/z* 400 and 2 µscans were used. External mass calibration was performed using Calmix from ThermoFisher Scientific. The mass accuracy was inferior to 2 ppm.

Toluene, acetone, anisole and a mix of toluene/anisole 50/50 (v/v) used as dopant were compared. Anisole showed the best HPAH ionisation efficiency. Its optimised flow rate for SFC/MS was 100 µL/min (flow rate before splitting). For desolvation and ionisation optimisation, a design of experiments consisting of a central composite design was conducted using the model mix of standards described in part 2.2. Five parameters were included: the APPI vaporiser temperature, flow rate of the sheath gas (also called nebulising gas), flow rate of auxiliary gas (drying gas), the ion transfer capillary temperature and voltage. Areas of HPAH extracted ion chromatograms (EIC) were used as responses. The optimised conditions were summarised in Table 2.

Table 2: Optimised MS parameters for SFC/MS analyses.

Parameters	SFC/MS	Parameter range
Sheath gas flow rate (A.U.)	10	10-60
Auxiliary gas flow rate (A.U.)	34	5-45
Sweep gas flow rate (A.U.)	0	/
APPI vaporiser temperature (°C)	348	200-450
Transfer capillary temperature (°C)	200	200-400
Transfer capillary voltage (V)	48	0-100
Tube lens voltage (V)	100	/

2.6. *MS data processing*

Data were processed using MZmine 2.40.1 software[46]. Raw data were directly imported into the software. After mass detection, ADAP chromatogram builder functionality[47] was applied. $[M]^{+\bullet}$ were the main formed ions. Finally, molecular formulae were assessed with the following conditions: $C_{1\text{-}100}H_{1\text{-}100}O_{0\text{-}1}S_{0\text{-}1}N_{0\text{-}1}$ and an accuracy of 2 ppm. Based on the formulae list obtained, several properties could be calculated such as the double bond equivalent (DBE) which represents the number of rings plus the number of double bonds. DBE values are calculated by the following equation for $C_cH_hO_oS_sN_n$ compounds:

(1) $DBE = \frac{c-2\times h+n+2}{2}$

3. Results and discussion

In this work, we intend to overcome the complexity of VGO samples and to propose a method able to achieve a quantification of PAH and HPAH contents in VGO feedstocks and hydrocracked products.

3.1. *Optimisation of SFC separation and hyphenation with HRMS*

The first step was to develop the separation of HPAH using SFC-UV. Apart from the well-known advantages of this analytical technique in comparison to LC (efficiency, analysis time, environmental impact), SFC is appropriate to petroleum samples which are rather apolar matrices containing aromatic hydrocarbons sometimes highly alkylated. Indeed, carbon dioxide which constitutes the SFC mobile phase can be assimilated to heptane in terms of eluent strength and thus can elute the most apolar compounds. Moreover, no sample preparation is needed at the opposite of LC[16].

Based on a previous study, methanol was selected as co-solvent[26]. Indeed, Zhang *et al.* showed that on BEH 2-EP column, among the co-solvent investigated (acetonitrile, methanol, isopropanol and hexane), only methanol was able to elute the 16 PAH, especially the benzo[ghi]perylene, the heaviest PAH studied. Some VGO samples could contain PAH having more rings than benzo[ghi]perylene does and thus could be strongly retained on the stationary phase. Methanol has also the advantage to have an ionisation energy of 10.84 eV and thus to be invisible in APPI using a krypton lamp.

For the optimisation of HPAH separation using SFC, five chromatographic columns (*i.e.* Acquity UPC² BEH 2-EP, Acquity UPC² BEH, Acquity UPC² CSH Fluoro-Phenyl, Acquity UPC² HSS C_{18} SB and Acquity UPC² Torus 2-PIC columns), five ABPR pressures (*i.e.* 10.5, 12.0, 13.0, 15.0 and 16.4 MPa) and three temperatures (*i.e.* 25, 40 and 55°C) were considered. All chromatographic columns had the same dimensions (3 x 100 mm) with a particle size of 1.7 µm, except for the HSS C_{18} SB (1.8 µm). In this way, the efficiency of the set of selected columns was considered equal and the chromatograms could be directly compared for the same analytical method.

In total, 75 conditions were applied to a PAH/HPAH model mixture. The best conditions were chosen according to two criteria. The first one concerned the retention time of pyrene which had to be eluted after the isocratic step with 100% CO_2 so that small aromatic compounds were not co-eluted with pyrene. The second criterion was the retention time of coronene, the heaviest HPAH in the model mixture (with 8 aromatic rings), which should be eluted with a maximum of 25% of MeOH considering possible that bigger compounds with a higher aromaticity could be present in industrial samples. Based on these constraints, only one condition remained possible: the Torus 2-PIC column working at 55°C with a back pressure of 10.5 MPa (Figure S2). The retention time behaviours of pyrene and coronene on Torus 2-PIC column according to the temperature and the pressure are represented in Figure 1. The temperature had more influence on the pyrene retention time at low pressure than at high pressure. In the same way, the pressure influenced the retention time more significantly at 55°C than at 25°C. Pyrene was eluted mainly with CO_2 at the beginning of the gradient (with 1% of MeOH), thus its retention can be explained thanks to the changes of density lines (in this study from 0.4 g/cm^3 to 0.95 g/cm^3)[48]. At the opposite, coronene was eluted with a mix of CO_2 and MeOH (with 21% of MeOH) which leads to a lower impact of the pressure and the temperature on its

retention time due to the lower mobile phase compressibility with a higher methanol content in the carbon dioxide.

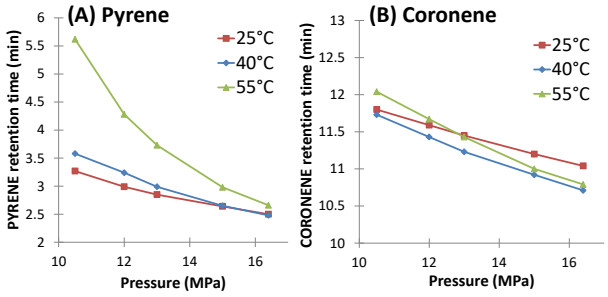

Figure 1: Retention times of pyrene (A) and coronene (B) on Torus-2PIC column according to the column temperature and the back pressure.

Finally, repeatability of the analysis under optimised conditions (55°C, 10.5 MPa) was evaluated on HPAH model mixture retention times. Indeed, in the optimised conditions a small change in temperature or pressure could lead to a modification of the mobile phase density and thus a modification of the retention time. Intra-day variability was assessed on 5 injections done on the same day. Inter-day variability was measured using 5 injections on different days. The relative standard deviations (RSD) of retention time of pyrene were 2.9% and 0.3% for intra-day and inter-day variabilities respectively. The RSD of coronene retention time were 0.2% and <0.0% for intra-day and inter-day variabilities respectively.

The SFC-UV method was applied to industrial samples: one VGO and one UCO both diluted six times by weight in toluene (Figure 2).

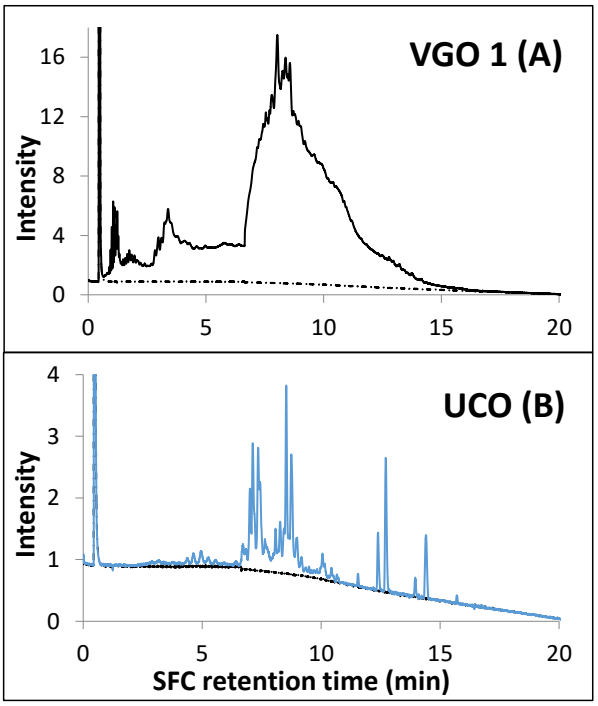

Figure 2: SFC max plot chromatograms (250-400 nm) of (A) VGO 1 and (B) UCO. Dot lines represent a blank with toluene injection.

As it can be seen in Figure 2, our SFC-UV method succeeded in the separation of aromatic hydrocarbons for the UCO sample whereas the resolution was not sufficient to obtain a resolved chromatogram for the VGO sample. Thus, SFC-UV could not be employed alone to quantify PAH and HPAH in VGO. To go further, hyphenation of SFC with HRMS was investigated to add a dimension of separation according to the mass to charge ratio of analytes. It should be noted that mass resolution was decreased to 12500 in order to have a sufficient scan speed and thus enough points to well describe the chromatographic peaks. Usually in petroleomics, the use of high-resolution mass spectrometry is a key feature to be able to resolve very small mass differences between isobars. Among the compounds that are usually found in petroleum products ($C_xH_yN_nO_oS_s$), the smallest mass difference is between C_3 and SH_4 (0.0034 Da). To distinguish this small difference, a resolution of 89000 is required for a *m/z* of 300. With direct introduction, this resolution can only come from mass spectrometry. However, with hyphenated techniques, in this work SFC/MS, the resolution of SFC allowed to work with a lower MS resolution. Indeed, two isobars can be differentiated if they elute at different retention times. For example, $C_{20}H_{28}S$ (*m/z* measured 300.1907) eluted at 2.7 min while $C_{23}H_{24}$ (*m/z* measured 300.1874) eluted at 10.1 min. Moreover, the mass accuracy of 2 ppm allowed the correct identification with only one possible chemical formula with the criteria presented part 2.6. Indeed, in the previous example, the mass difference represents 10 ppm.

In this work, SFC was hyphenated to HRMS with (+)APPI source. Since HPAH have an ionisation energy (IE) smaller than 10 eV (7.43 eV for pyrene as an example[40]), their direct ionisation is performed thanks to photons provided by the krypton lamp; however, a dopant is usually employed to increase the ionisation efficiency. Among the studied dopants (*i.e.* acetone, toluene, anisole and a mix of anisole/toluene 50/50 (v/v)), anisole at a flow rate of 100 µL/min provided the best sensitivity, which was in good accordance with previous works [40,41]. For example, the intensities of pyrene and coronene were respectively 50 and 25 times higher with anisole than when using toluene as a dopant (Figure S3). Desolvation and ionisation parameters were optimised with a design of experiments approach (see part 2.4).

To validate the ability of the SFC/MS method to quantify HPAH in UCO and VGO, samples were spiked with pyrene-d10 at several concentrations. To evaluate the matrix effect which could occur into the ionisation source, a calibration curve of pyrene-d10 prepared in THF was plotted as a reference. As shown in Figure 3, a linear calibration curve with MS detection was obtained between 10 and 350 mg/L (black squares). The spiked UCO data (green stars) were well located close to the calibration curve meaning that matrix effect was not observed or negligible. However the spiked VGO suffered from strong ionisation suppression. As an example, VGO 1 spiked at 200 mg/L of pyrene-d10 showed an area 10 times lower than the area obtained with the standard solution. This ionisation suppression effect was due to many co-eluted compounds in SFC entering the ionisation source at the same time and thus leading to ionisation competition (Figure S4). VGO are clearly more complex matrices than UCO products as they are composed of sulphur-containing compounds, nitrogen molecules as well as highly alkylated compounds. Thus, hyphenation of one dimension of separation, *i.e.* SFC, with HRMS is not a suitable solution to quantify PAH and HPAH in VGO. A supplementary dimension of separation was required to increase the global resolution of the method and to decrease the number of co-eluted compounds.

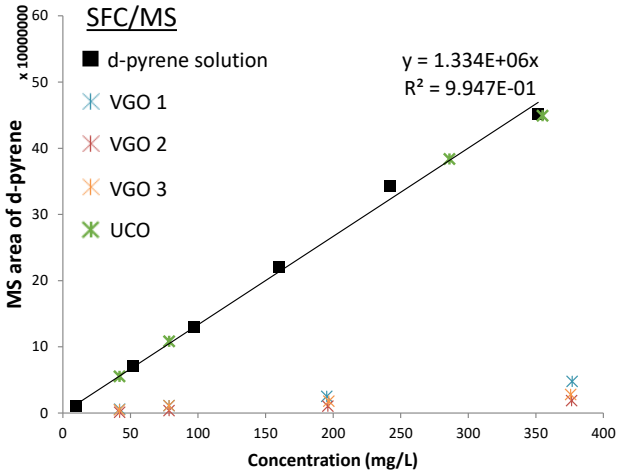

Figure 3: MS area of pyrene-d10 as a function of concentration for model solutions (black squares) and VGO and UCO samples spiked with pyrene-d10 according to SFC/MS analysis.

3.2. *Multidimensional approach with hyphenation to mass spectrometry*

3.2.1.Centrifugal partition chromatography as a first dimension

CPC is a liquid-liquid separation technique based on the partition coefficient (K) of analytes in a biphasic system. This method has two main advantages. The first one is that loss of sample in the CPC system is limited thanks to (1) the absence of irreversible adsorption on a solid stationary phase and (2) the extrusion mode which allows to recover the whole sample at the end of a run. The second advantage is the versatility of CPC with a large choice of biphasic solvents systems. Although this analytical technique has a lower efficiency than SFC or LC separation, a different and complementary separation mechanism to SFC could be expected.

Selection of the most suitable solvent system is a critical step: for a successful CPC separation, sample components have to be ideally distributed in equal parts between the mobile and stationary phases (0<K<3). In this study, the 'best solvents system' approach was used to optimise the solvents system[49]. DMSO is already known to be a good solvent for HPAH[16], so the biphasic solvents system was built based on this latter. Heptane was selected as the less polar solvent and acetonitrile as the more polar solvent. Partition of the sample was confirmed by injecting the upper and lower phases in SFC-UV (not shown). The HEPT-DMSO-ACN proportions were 45/10/45 (v/v/v). This system was stable with a complete separation of the two phases after shaking smaller than 30 s (demixing time also called t_d).

In order to increase the sample loading capacity in the CPC column, a ternary diagram was built with sample, stationary phase and mobile phase proportions as axes (Figure 4). To do so, three mixtures with different proportions of mobile and stationary phases were prepared: 70/30, 45/55 and 30/70 of stationary phase/mobile phase (w/w). Then, several amounts of sample were added: from 0.5 to 2 g representing 10, 20, 30 and 40% (w/w) of the global solvent system weight. Finally, demixing times were measured for each mixture and plotted on the ternary diagram in Figure 4. This diagram represents an area where only one phase can be obtained (t_d > 30 s) and another zone involving two phases (t_d < 30 s). When only one phase is observed, the solvents system in equilibrium inside the CPC column may be disrupted during the sample injection. This study showed that 1 g of VGO diluted

in a mixture 70% stationary phase/30% mobile phase (w/w) can be loaded without disruption of the solvent system.

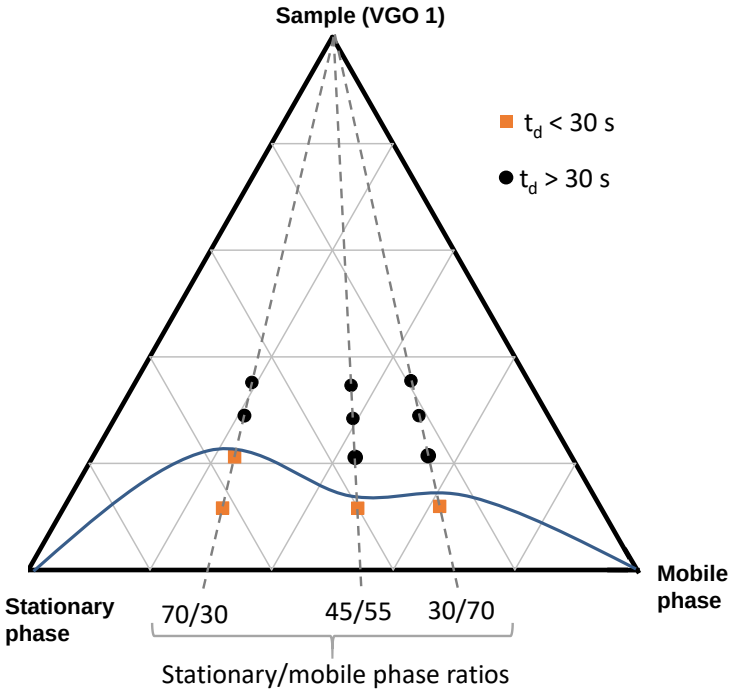

Figure 4: Ternary diagram (weight proportions) of VGO 1, stationary phase and mobile phase.

Chromatograms of CPC separation for the four samples and a model mixture at 75 mg/L are shown in Figure S5. CPC fractionations of UCO and VGO 1 samples were repeated twice to ensure the repeatability of the method. The seven HPAH investigated in the model mixture were non-alkylated compounds, also called 'chemical family heads', eluting between 7 and 14 min with a maximum intensity of UV signal observed at around 12 min. For VGO samples, analytes were eluted all along the run:

- from the dead volume at 6 min for compounds which stayed mainly in the mobile phase,
- during all the elution mode (from 6 to 30 min) for compounds which were partitioned between the mobile and stationary phases,
- until the extrusion of the stationary phase (from 30 to 40 min) for those having higher affinity for the heptane enriched phase than the acetonitrile enriched phase. These later might correspond to highly alkylated compounds.

The choice of the solvents system was finally validated by the fact that the occupation of the chromatographic space was complete.

Then each minute a fraction was collected and re-injected off-line with the SFC-UV/HRMS method.

3.2.2. Organisation of the 2D chromatograms

Once SFC-UV/HRMS analysis had been performed comprehensively on each CPC fraction, 2D contour plots were built either with UV signal (Figure S6) or with MS signal using a home-made software. For instance, bidimensional chromatograms involving base peak chromatograms (BPC) are presented in Figure 5. 2D map for UCO sample (Figure 5A) showed better resolved peaks than 2D map for VGO 1

(Figure 5B) as it was expected due to the difference of complexity between these two industrial samples.

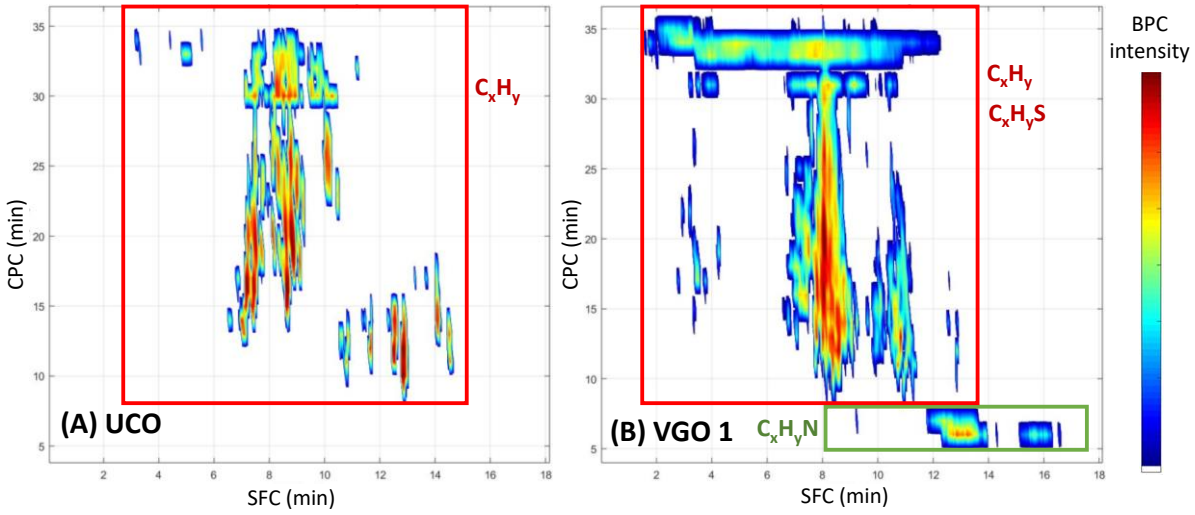

Figure 5: CPCxSFC/HRMS 2D contour plots obtained from the BPC of UCO (A) and VGO 1 (B) obtained from CPC×SFC/(APPI+)HRMS analysis

To go deeper in the understanding of the organisation of 2D plots, MS data were processed as described in section 2.7. For UCO sample, only C_xH_y compounds were found whereas, for VGO sample $C_xH_yS_1$, $C_xH_yN_1$ as well as few $C_xH_yO_1$ analytes were detected. Indeed, the UCO sample was hydrotreated, which allowed to remove the nitrogen and sulphur components. Based on the list of chemical formulae, the evolution of DBE according to the number of carbon atoms (nC) was plotted for C_xH_y family with colour scales for CPC and SFC retention times (Figure 6). The graphs DBE = f(nC) for the chemical families are presented in Figure S7. In this study, CPC was used as a first separation dimension and was governed by the number of carbon atoms in the alkyl chain (from left to right on Figure 6A). Indeed, for a given DBE, compounds with no alkylation were eluted first, and then followed by those with one CH_2, two CH_2, etc. This retention mechanism is driven by the partition coefficient of analytes that are distributed in CPC between the stationary and mobile phases. The more a compound is alkylated, the more its partition will be in favour of the stationary phase. The same trend was observed for the $C_xH_yS_1$ class of molecules. PAH and PAH-S-containing compounds eluted between 9 and 35 min whereas nitrogen compounds which were not partitioned in the stationary phase, were eluted at the beginning between 5 and 10 min.

At the opposite of CPC separation mechanism, SFC retention times were mainly correlated to the DBE for C_xH_y family as well as for $C_xH_yS_1$ molecules (by other means depending on the number of rings of the hydrocarbons). This mechanism of retention is similar to those in normal phase chromatography[50]. For nitrogen compounds, they eluted between 10 and 18 min in SFC.

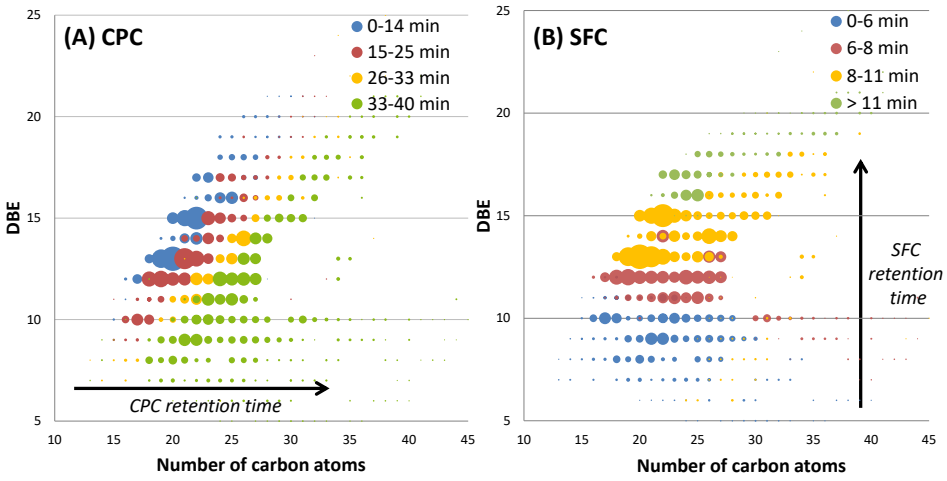

Figure 6: DBE as a function of the number of carbon atoms for C_xH_y compounds detected in VGO 1. Colour scales represent the retention times of (A) CPC, (B) SFC and size of the points is related to the intensity of the compounds on MS spectra.

The complementarity of CPC and SFC separation mechanisms allowed to obtain a well-organised 2D chromatogram. The off-line hyphenation of CPC and SFC enabled the fractionation of samples, firstly according to the alkylation degree of the hydrocarbons and sulphur compounds and secondly according to their rings number. Nitrogen components were well separated from the other analytes by eluting at the beginning of the CPC separation (Figure 5). Finally, for compounds still being co-eluted in both CPC and SFC dimensions, the use of HRMS was necessarily required to distinguish such molecules according to their *m/z* ratio.

To demonstrate the interest of such well organised 2D separation, 2D chromatograms of only PAH and HPAH compounds (*i.e.* C_xH_y family without sulphur or nitrogen-containing compounds) were plotted for UCO and VGO 1 samples in Figure 7. On these 2D plots, DBE ranges were highlighted thanks to colour boxes. The location of analytes in these boxes indicates the alkylation degree: at the bottom, components are non or slightly alkylated whereas, at the top, components are highly alkylated. By comparing the two samples, several interesting observations could be done. First, as it was mentioned in the introduction, HPAH are formed during the hydrocracking process. Indeed, on the 2D chromatogram of the UCO sample, components with DBE of 18 and 19 were present while they were not detected for the VGO sample. Then, the CPC retention time of analytes with a DBE of 13 was different for UCO and VGO. For the UCO, this family eluted between 15 and 35 min in CPC, which indicates alkylated compounds. At the opposite, for the VGO, this family eluted between 10 and 20 min. To bring additional information, UV spectra of compounds coming from the UCO and the VGO were compared between them and to standard molecules (Figure S8). It appeared that compounds with a DBE of 13 in the UCO sample showed a spectrum similar to that of pyrene. This observation was also true for the family with a DBE of 14. In opposition, compounds with a DBE of 13 in the VGO had a spectrum similar to that of chrysene. The hypothesis which could be done based on all this information was that compounds with DBE of 13 and 14 from the UCO belong to pyrene family but with additional naphthenic rings which might explain the difference of DBE. Conversely, in the VGO sample, these compounds may correspond to chrysene family with more or less alkylations.

These conclusions can only be done thanks to the combination of the three dimensions of separation developed in this study. For example, mass spectrometry alone could not have made the difference between the components of the family with a DBE of 13.

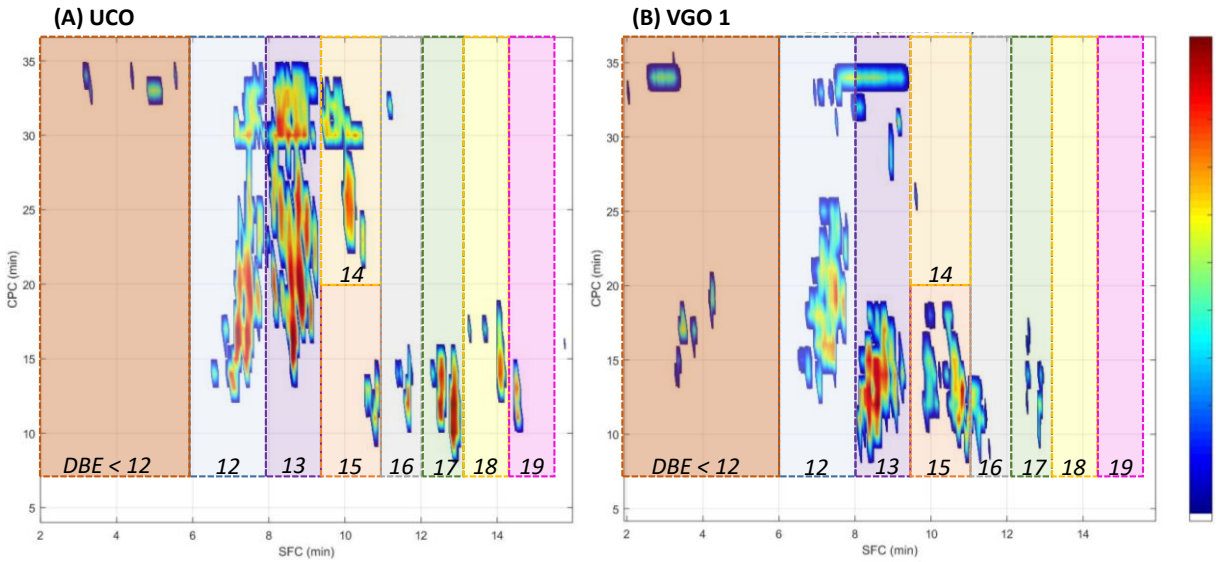

Figure 7: CPCxSFC/HRMS 2D contour plots of (A) UCO and (B) VGO 1 for C_xH_y compounds. Colour boxes represent the DBE which is indicated at the bottom of each area.

3.3. *Input of CPC×SFC/HRMS method for PAH and HPAH quantification*

As regards quantitative analysis, we focused on a specific CPC fraction (fraction #12 eluting between 11 and 12 min) to demonstrate how relevant the off-line CPCxSFC/HRMS method can be to determine PAH and HPAH contents in such petroleum matrices. Fraction #12 was selected because pyrene eluted in this fraction with the highest signal. Each fraction #12 resulting from the CPC separation of VGO1, VGO2 and VGO3 was individually spiked with pyrene-d10 at several concentrations as already done in the previous part dedicated to SFC/MS (see part 3.1). Figure 8 represents the MS peak areas of EIC chromatograms according to the pyrene-d10 concentration. At the opposite of spiked VGO analysed with SFC/MS, all spiked fractions obtained with the multidimensional approach did not show ionisation suppression and were aligned with the calibration curve prepared in THF. These results demonstrated that the use of two dimensions of separation is mandatory to limit the competition between analytes during the ionisation step and thus to limit the matrix effect in mass spectrometry detection. Thereby this unique hyphenation of the three dimensions allows access to the quantification of the HPAH in the VGO samples. Although it remains some works to fully validate the quantification of PAH and HPAH in VGO, at present, the developed methodology is one of the most promising solutions to progress in the VGO composition knowledge as well as in modelling hydrocarbon conversion processes.

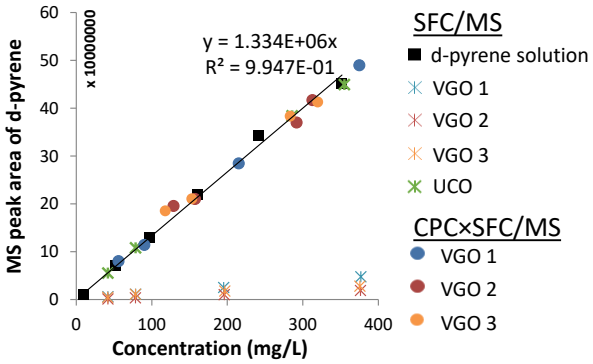

Figure 8: MS area of pyrene-d10 as a function of concentration for model solutions (black squares) and VGO and UCO samples spiked with pyrene-d10 according to SFC/MS analysis (stars) and according to CPCxSFC/MS (circles).Conclusion

In this work, we have demonstrated that our SFC/MS method was well adapted to quantitatively characterise PAH and HPAH in unconverted oils. However, for more complex liquids such as vacuum gas oils, a different and disruptive analytical strategy was needed. A multidimensional CPCxSFC/HRMS approach was developed for the separation of PAH and HPAH in VGO. CPC offered considerable advantages. After building a ternary diagram with VGO, CPC stationary and mobile phases as axes, 1 g of sample was injected without disrupting the solvent system. Also, limited loss of sample could occur thanks to the extrusion mode at the end of the run. This first CPC dimension was able to separate analytes according to their alkylation degree. In a complementary way, SFC in a second dimension gave a separation according to the DBE. Thus CPCxSFC separation provided well chemically organised 2D chromatograms, which is a step towards getting a better knowledge of the composition and reactivity of such complex petroleum matrices. Finally, spiked VGO samples with pyrene-d10 highlighted the ionisation suppression which occurred in SFC/HRMS and demonstrated that CPC×SFC/HRMS method prevented the matrix effect. Based on this novel CPC×SFC/HRMS method, quantification of PAH and HPAH is for the first time possible. Such a detailed and quantitative method has not yet been described in the literature and will be of a great interest for describing the VGO reactivity in hydrocracking processes for fuels production.

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Supporting Information

Model mix for separation and detection method optimizations. Scheme of the SFC-UV/MS interface. Retention times of pyrene and coronene for the different screening conditions in SFC. Extracted Ion Chromatograms of (A) pyrene (*m/z* 202.07-202.08) and (B) coronene (*m/z* 300.09-300.10) using toluene (red) or anisole (black) as dopant. Mass spectra of spiked UCO (A) and spiked VGO 1 (B) using SFC/MS method. CPC chromatograms at several UV wavelengths for HPAH model mixture, UCO and the three VGO. CPC×SFC-UV max plots (between 250 and 400 nm) of UCO (A) and VGO 1 (B). DBE = f(nC) for the different chemical families detected in VGO 1. UV spectra of compounds with a DBE of 13 detected in (A) VGO and (B) UCO, compared to (C) standard of pyrene and (D) standard of chrysene.

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