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ORIGINAL PAPER

Simple column chromatography separation procedure for polycyclic aromatic hydrocarbons: controlling factor(s)

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Abstract
The present study proposes a simple one-step column and less reagent-consuming experimental procedure for separating aromatic hydrocarbons, especially polycyclic aromatic hydrocarbon compounds (PAHs), in crude oils. Thus, the research aimed to determine the best and reliable column chromatography technique and identify the main controlling factor (s) a successful PAHs separation into sub-fractions. We found that the choice of the type of column is the requirement for a successful column chromatography separation. Using alumina and silica-alumina at a ratio of 1:1 for the separation of the aromatic fraction of crude oil from the Termit basin (Niger) into sub-fractions, our analysis revealed that, less time also less reagent-consuming, silica-alumina (1:1) column is chosen to be the best among the two columns (alumina and silica-alumina 1:1) for separating PAHs into various sub-fractions. Apart from the type of column, we found that the diameter of alumina pores is the main factor controlling a successful separation of the aromatic compounds into sub-fractions. This factor controls the time and the volume of reagent ratios necessary. Thus, using the following consecutive ratios of petroleum ether:dichloromethane at 93:7 (6 ml), 90:10 (30 ml), and 75:25 (20 ml), respectively mono-aromatic, di-aromatic, and tri-aromatic sub-fractions were successfully recovered, whereas further addition of 12 ml of pure dichloromethane effectively recovers compounds with more than 3 aromatic rings. Finally, stable carbon isotope data obtained in this current study confirmed that the procedure proposed here provides a reliable stable carbon isotope measurement of individual PAH with an average standard deviation of 0.5‰.

Keywords Termit basin · Crude oil · Aromatic fraction · Separation procedure · Sub-fraction · Isotope data

Introduction

The organic component of crude oils is mainly composed of four fractions, namely, asphaltene fraction; saturated fraction; aromatic fraction; and the nitrogen, sulphur, oxygen (NSO) fraction. Defined as compounds composed at least of two benzene rings, polycyclic aromatic compounds (PAHs); the interest of this study, together with mono-aromatic compounds are the main constituents of the aromatic fraction of the crude oil. Alkylated aromatic hydrocarbons such as alkylbenzenes (ABs), alkylnaphthalenes (ANs), alkylphenanthrenes (APs), and biphenyls (BPs) are common constituents of petroleum and sedimentary organic matter (OM) (Maslen et al. 2011). The distributions of alkylated aromatic hydrocarbons are highly variable amongst oil samples, and they are often used to evaluate source, thermal maturity secondary alteration processes such as biodegradation and the migration pathway (Van Aarssen et al. 1999; Trolio et al. 1999; Asif et al. 2011). Conventionally, the relative abundances of saturated hydrocarbon biomarkers are widely used in preliminary oil and source rock geochemical assessment to obtain information about thermal maturity, depositional environment, and origin of organic matter (Yang et al. 2015; Xiao et al. 2019; Lu et al. 2021).

To obtain a more accurate and reliable information about crude oils, several researchers have developed their interest

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in aromatic hydrocarbon fractions because of their relatively high stability (Asif et al. 2011; Zhang et al. 2016; Li et al. 2018). Information provided by the relative abundance of some aromatic parameters have made PAHs, especially di-aromatic (naphthalenes and biphenyls) and tri-aromatic (phenanthrenes, dibenzothiophenes, dibenzofurans, and fluorenes) very important compounds in organic geochemistry due to their wide application in several disciplines. In the past, several authors (Alexander et al. 1985; Strachan et al. 1988; Van Aarssen et al. 1999; Asif et al. 2011), based on the application of PAHs, believed that these compounds could be used in the evaluation of sedimentary depositional environments, thermal maturity, oil-oil/oil-source correlation, and biodegradation. Polycyclic aromatic hydrocarbon (PAH) is a chemical compound containing only carbon and hydrogen with multiple aromatic rings. The group is a major subset of the aromatic hydrocarbons. The simplest of such chemicals are naphthalene, having two aromatic rings, and the three-ringed compounds anthracene and phenanthrene. Nowadays, researchers are mainly focused on the information provided by the stable carbon isotope data from the individual aromatic compound (Maslen et al. 2011; Mazeas and Budzinski 2001; Le Metayer et al. 2014). The information preserved in the stable carbon isotopic compositions of PAH compounds suggest a new application of PAHs by using compound specific isotope analysis (CSIA). CSIA, developed in the 1990s, has been widely used to probe the origins and diagenetic pathways of biomarkers in sediments at the molecular level (Jiang et al. 2013). Recent researches demonstrated that the stable carbon isotopic compositions of individual PAH compound were useful in crude oils characterization (Maslen et al. 2011; Le Metayer et al. 2014; Zhang et al. 2014; Chen et al. 2016). Unlike the conventional silica gel-alumina column chromatography method, principally used for separating the four main petroleum fractions, separating PAHs alone and achieving a high precision measurement of their stable carbon isotope composition is primarily dependent on the quality of separation method used. It is necessary because of their relatively lower concentration in the entire aromatic fraction (Kim 2004; Pule et al. 2012).

In order to determine the stable carbon isotope values of individual aromatic compound, different separation and purification methods or techniques of aromatic fraction separation such as normal-phase liquid chromatography (NPLC) and reverse-phase liquid chromatography (RPLC) were effective on preventing co-elution, peak overlap, and unresolved complex mixture (UCM) interferences (Wise et al. 1977, 1993; Wilson et al. 2016, 2017, 2018; Hayes et al. 2018). Normal-phase liquid chromatography (NPLC) has been used as a low-resolution fractionation technique for sample clean-up (Wilson et al. 2018). Without isolation of the isomeric fraction by normal-phase liquid chromatography (NPLC), it would be difficult, if not impossible, to quantify accurately isomers using reverse-phase liquid chromatography fluorescence (Wise et al. 2015). It is also important to note that gas chromatography-mass spectrometer (GC–MS) detection has become the method of choice for the determination of PAHs, even for routine analyses (Wise et al. 2015). In 1995, Budzinski and co-workers applied the high-performance liquid chromatography (HPLC) fractionation method on crude oils and organic extracts of rocks that allowed the isolation of tri-aromatic fractions and further analysis by gas GC–MS. Furthermore, separated aromatic fractions from some oil samples using medium pressure liquid chromatography (MPLC) on silica column before the fractions further were separated into five sub-fractions using high performance liquid chromatography (HPLC) to detect any maturity-related changes in their $\delta^{13}C$ values (Radke et al. 1998). Alumina/silica gel column chromatography, gel permeation chromatography, and thin-layer chromatography were developed for purification and to measure the stable carbon isotope ratios of PAHs more accurately (Kim 2004). A simple and fast multi-residue method based on high-pressure liquid chromatography related to a fluorescence detector (HPLC-FLD) has been developed for simultaneous determination of PAHs (Pule et al. 2012). In addition, high-performance liquid chromatography (HPLC), medium pressure liquid chromatography (MPLC), and thin-layer chromatography (TLC) have also been used to identify polycyclic aromatic compounds before obtaining the isotope values of each of these compounds from their aromatic fraction (Budzinski et al. 1995; Radke et al. 1998; Mazeas and Budzinski 2001; Kim 2004; Pule et al. 2012). Unfortunately, TLC involves a rather complicated procedure, making it time-consuming and laborious. After cooling in the desiccator, about 100 µl of the extract is applied as a thin band onto the TLC plate using a micropipette. The original sample band is concentrated on a very narrow streak by extending dichloromethane to the upper edge of the original sample band. Indeed, after drying the applied solvent, the plate is developed with a 3:2 (v/v) cyclohexane–toluene mixture, and the required PAH band (RF ~ 0.81) is located using a short wavelength (254 nm) ultraviolet light by comparison with the standard materials. The silica gel containing the PAHs is scraped off, and the PAHs are extracted with dichloromethane by sonication for 30 min three times. The extract is then filtered and rinsed through a small column filled with glass wool, sand, and sodium sulphate to remove silica gel and moisture (Kim 2004). The filtrates are then concentrated to 100 µl ~ 1 ml of hexane depending on the PAH concentration for isotope ratio composition analysis. Therefore, in order to get accurate results, an easier approach introduced the use of an aminopropyl (NH_2) stationary phase for the separation and isolation of PAHs from complex mixtures (Wise et al. 1977). These authors showed that NH_2 provided a convenient, reproducible HPLC fractionation of PAHs according to

the number of condensed aromatic rings. Thus, the retention data reported will provide the basis for developing normal phase liquid chromatography (NPLC) fractionation strategies to characterize complex mixtures (Wilson et al. 2017). Contrary to the silica and alumina, which used as adsorbents for routine NPLC fractionation of PAHs were shown to be unreliable because of high absorptivity and the influence of moisture content (Wise et al. 1977; Wilson et al. 2018). However, these methods presented above failed to propose a separation procedure for aromatic fractions into sub-fractions according to the ring class.

Indeed, very few researches proposed a procedure that use silica and alumina as components of the chromatography column that allow eluting of PAH sub-fractions according to the number of aromatic rings (Jiang et al. 2013; Le Metayer et al. 2014; Chen et al. 2016). Among them, Jiang et al. (2013) proposed an alumina column procedure that allowed their research to elute successfully alkylnaphthalenes free from other compounds. Le Metayer et al. (2014) used a silica column to separate mono-aromatic, di-aromatic, and tri-aromatic compounds. Chen et al. (2016) used also an alumina column procedure to separate aromatic fractions into mono-aromatic, di-aromatic, tri-aromatic, and more than 3 ringed sub-fractions. While these different researches provided reliable data, the authors failed respectively to propose a one-step column chromatography (Jiang et al. 2013), to elute each sub-fraction each free from other (Le Metayer et al. 2014), and to use the same reagent ratios to complete their different separation (Chen et al. 2016). In fact, Jiang and co-authors proposed a two-step alumina column to recover successfully all alkylnaphthalenes. Prior to the separation, glass tubes (15 cm length, 6 mm i.d.) were used as standard columns to separate the total aromatic hydrocarbons into sub-fractions. The bottom of each column was plugged with a small amount of cotton wool and then packed with dry, pre-activated alumina (approximately 6 cm column length). The prepared standard column was directly wetted and rinsed with petroleum ether before sample loading to ensure uniformity. According to the authors, with a 5.5 ml eluent of petroleum ether:dichloromethane (99:1, v:v), the first sub-fraction was collected and defined as AF1. The GC–MS data showed that the AF1 sub-fraction mainly contained mono-aromatics. The second sub-fraction was then eluted with a 5.5 ml of petroleum ether (P.E.):dichloromethane (DCM) (97:3 v:v) and defined as AF2B, which was directly followed by a pure dichloromethane eluent to recover the remaining aromatics (AF3C). Unfortunately, GC–MS monitoring revealed that some of the alkylnaphthalenes were present in AF3C, suggesting an overlap between AF2B and AF3C. For complete recovery of the alkylnaphthalenes from AF3C, an alternative solvent system using a column of the same size and with the same stationary phase was used. Petroleum ether:dichloromethane (8 ml, 90:10, v:v) was used to successfully recover the alkylnaphthalenes from fraction AF3C free of the tri-aromatic components. Le Metayer et al. (2014) attested that approximately 50–100 mg of crude oil was fractionated by silica gel liquid chromatography [column length used was 20 cm (silica) × 0.9 cm (i.d.)]. Silica for liquid chromatography was activated (overnight at 120 °C) and washed with n-pentane prior to use. The first aromatic sub-fraction was eluted with a solvent mixture of n-pentane:dichloromethane (19:1 v:v, 15 ml, corresponding to the solvent front). A second sub-fraction was eluted with a further 40 ml of the same solvent mixture, n-pentane:dichloromethane (19:1 v:v). A third sub-fraction was eluted with a mixture of n-pentane:dichloromethane (7:3 v:v, 40 ml). The target components were well separated with naphthalene the only exception, which was present in both the first and the second aromatic sub-fractions for several samples. According to Chen et al. (2016), glass tubes (15 cm × 6 mm i.d.) were used and packed with dry and activated alumina (ca. 6 cm column length). The first aromatic sub-fraction (mono-aromatic hydrocarbons) was eluted with hexane:dichloromethane (DCM) (99:1, 10 ml). A second sub-fraction (di-aromatic hydrocarbons) was two-time eluted with hexane:DCM (19:1, 15 ml; 9:1, 10 ml). A third sub-fraction (tri- and tetra-aromatic hydrocarbons) was four times eluted with hexane:dichloromethane (DCM) (8:1, 5 ml; 17:3, 8 ml; 4:1, 10 ml; 7:3, 10 ml). In addition, through the literature, none studies discussed clearly the factors that affect and control the successful separation of PAH compounds according to their number of aromatic rings.

In this present study, we aim to investigate the following: (1) how PAH compounds can be separated using the basic adsorbents, silica, and alumina, with an attempt to provide the most reliable and less time-consuming type of column chromatography by comparing and contrasting the performance of three types of column including alumina, silica-alumina (1:1), and silica. (2) Then, by progressively increasing the volume of reagents used on each column revealed reliable, determine the less reagent-consuming column chromatography. (3) Lastly, we hope to propose a separation procedure for PAH compounds into sub-fractions using common reagents (petroleum ether and dichloromethane) that will be followed by a GC–MS analysis to identify each PAH compound. The stable carbon isotope analysis of each compound will complete the procedure to confirm the reliability of the isotope measurements obtained. This research also identifies and discusses for the first time the main controlling factor of the repeatability of PAHs separation according to the number of rings using silica and alumina as component of the column chromatography.

Experimental

Samples

The crude oil sample used in this study was collected from the Sokor Formation reservoir in Termit basin located in the eastern part of the Niger Republic, southwards of the Republic of Chad (Xiao et al. 2019). The American Petroleum Institute (API) gravity of 29° with density values of 0.88 g/cm^3 characterizes the crude oil. In addition, the pristane/phytane ratio (Pr/Ph) value of the oil is > 1, while the gammacerane/hopane (Ga/C_{30}H) is < 0.20.

Reagents and materials

The reagents used in this study were mainly petroleum ether (P.E) at a boiling point of 30–60 ℃, dichloromethane (DCM), and methanol. The polarity of the reagent, which passed through the column, controls the relative rate at which compounds moved through the column. Generally, polar reagents react with polar molecules mixture on the adsorbent surface and effectively solvate the polar constituents. Purification of dichloromethane and petroleum ether was done by distillation.

Silica gel chromatography with a sieve particle size of about 0.149–0.177 mm (80–100 mesh) was activated for 8 h in an electric furnace at a temperature range of 140 ~ 150 ℃. After drying, it was allowed to cool and placed on a grinder bottle. Alumina chromatography (sieve particle size of 0.149–0.074 mm) (100–200 mesh), with a pore diameter of 17.4 nm, was also set up, and it was also activated for 4 h in a high-temperature electric furnace at a temperature of about 400 ~ 450 ℃, and it was removed and allowed to cool. It was subsequently kept in a grinder bottle. Silica gel (SiO_2) and alumina (Al_2O_3) are the two adsorbents commonly used by organic geochemists for column chromatography. Glass tubes (15-cm length, 7-mm inner diameter) were used as standard columns to separate the total aromatic hydrocarbons into sub-fractions in this study. The indoor temperature for the experiments was 10 ~ 30 ℃ under a relative humidity lower than 65%.

Separation of the total aromatic fraction from other crude oil fractions

The asphaltene fractions of the crude oils (80–100 mg) were the first to be removed before separating the other fractions. The de-asphaltened components of the crude oils were removed by precipitation using petroleum ether, and this was then followed by filtration of the asphaltene part using dichloromethane (approximately 6 ml is enough). The de-asphaltened crude oils were separated into saturated, aromatic, and NSO fractions using column chromatography (6 cm) with silica gel and alumina mixture (1:1). The saturate fractions were eluted with 40 ml of petroleum ether followed by aromatic fractions which were also eluted with petroleum ether and dichloromethane (1:2, 20 ml), and finally, the NSO fractions were eluted by using a mixture of dichloromethane and methanol (99:1, 20 ml).

Gas chromatography-mass spectrometry

Agilent 6890 gas chromatography and N-5975 IMSD were used for the identification of various molecular compounds. The column used was HP-5MS quartz capillary column (60 m × 0.25 mm × 0.25 µm film thickness), and the carrier gas was helium at a flow rate of 1 ml/min. The inlet temperature was set at 300 ℃, and the injection was done in a splitless mode. The heating of gas chromatographic oven temperature was initially programmed at a temperature of 80

Table 1 GC MS operating conditions for PAHs quantification

GC	Agilent 6890
Detector	N-5975 IMSD
Carrier gas	helium, 1 ml/min
Injector temperature	300 ℃
Injection volume	2 µl
Injection mode	splitless (1 mn 30)
Column	HP-5MS quartz capillary column (60 m × 0.25 mm × 0.25 µm film thickness)
Temperature program	Initial temperature is 80 ℃, maintained for 1 min
	Increase in temperature at 3 ℃/min until it reaches 310 ℃
Total run time	16 mn
Electron energy	70-eV
Acquisition mode	Selected ion monitoring (SIM)
Quality range of full scan	50–550 amu

℃, and this was maintained for 1 min; there was an increase in temperature at 3 ℃/min until it reaches 310 ℃. Electron ionization (EI) mode was adopted for mass spectrometry, and the electron energy was set at 70-eV. The acquisition mode was full scan although selective ion acquisition was also utilized to enhance feasibility, and the quality range of full scan is 50–550 amu (Table 1). To identify the spectrum of each PAH family, the mass charge of each component was used by matching their retention times with those of reference compounds as obtained from previous works (Budzinski et al. 1995; Asif et al. 2009; Jiang et al. 2013; Zhang et al. 2014).

Gas chromatography-isotopic ratio mass spectrometry (GC-IRMS)

The GC–MS results showed that the sub-fractions of di-aromatic, tri-aromatic, and compounds with more than 3 rings were free of unresolved complex mixture (UCM). Each compound was identified by comparison to previously published studies. After applying the latter procedure to the Termit oil samples using the silica-alumina (1:1) column, the stable carbon isotope analysis was carried out on the PAH compounds identified. An internal standard with known $\delta^{13}C$ value from the State Key Laboratory of Hydrocarbon Resources and Exploration, China University of Petroleum (Beijing), College of Geosciences, Beijing (102,249), was co-injected to determine their reproducibility and reliability of the isotope values.

The GC-IRMS system consisted of a Thermo Scientific Flash HT EA (elemental analyzer) interfaced with MAT 253 IRMS. Individual hydrocarbons were quantitatively converted to CO_2 and H_2O in the combustion reactor that was held at 980 °C. The CO_2 produced in the ion source was transferred to the mass spectrometer for the determination of the stable carbon ratios. The entire sub-fraction quantity (0.5 µl) was injected into the programmable temperature inlet with a septumless head working in split mode. The injection mode was held at a split ratio of 1 min 30 and an initial temperature of 50 °C. Through injection, the injector heated to 300 °C at a programmed rate of 700 °C/min was held at this temperature for the rest of the analysis. Helium was set at a flow rate of 1 ml/min. The sub-fraction components were separated on an HP-5MS quartz capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The temperature of the GC oven was initially held at 80 °C for 6 min, followed by a 15 °C/min ramp to 80 °C, then at a rate of 5 °C/min to 200 °C and was held there for 30 min.

The $^{13}C/^{12}C$ isotopic ratio was calculated by the integration of the masses 44, 45, and 46 ion current counts of the CO_2 peaks produced by the combustion (copper oxide reaction furnace at 850 °C) of hydrocarbons separated by gas chromatography (Table 2). A CO_2 reference gas (calibrated to Vienna Pee Dee belemnite) with a known $\delta^{13}C$ was pulsed into the mass spectrometer, and the isotopic composition of samples was reported in the δ notation relative to the reference gas. The accuracy of the data was routinely monitored with a set of standards of known isotopic composition before and after sample analysis. Briefly, the stable carbon isotope data were evaluated by analyzing a mixture of n- and isoprenoid alkanes with known $\delta^{13}C$ values. Each sample was analyzed at least three times, and the standard deviation (SD) of the replicates was calculated for each compound of interest to estimate the reproducibility.

Table 2 GCIRMS operating conditions for isotope measurements

GC	Thermo Scientific Flash HT EA
IRMS	MAT 253 IRMS
Combustion temperature	980 °C
Injection volume	0.5 µl
Injection mode	splitless
Carrier gas	Helium, 1 ml/min
Column	HP-5MS quartz capillary column (30 m × 0.25 mm × 0.25 µm film thickness)
Injector temperature	300 °C
Temperature program	80 °C for 6 min
	15 °C/min ramp to 80 °C
	5 °C/min to 200 °C
Total run time	30 mn

Results

Assessment of the types of column

The interaction between the compounds and the three types of column chromatography (alumina, silica-alumina 1:1, and only silica) used in this part of the present study was monitored. The size of the columns and the volume of reagents are expressed in centimeter (cm) and milliliter (ml), respectively. The column size was always approximately 6 cm for each, and the ratio of reagents is in percentage (%).

The first sub-fraction was eluted with 6 ml of petroleum ether and dichloromethane (98:2), and from this one, only mono-aromatic compounds were identified (Figs. 1a, 1b, and 1c). Then, petroleum ether:dichloromethane (93:7, 6 ml) followed. The GC–MS analysis showed that when petroleum ether:dichloromethane (93:7, 6 ml) was used, only mono-aromatic compounds were also present. The same observation was made for all the three columns (i.e., alumina, silica-alumina (1:1), and silica) (Figs. 1d, 1e, and 1f). It is directly followed by 8 ml of petroleum ether:dichloromethane (90:10). From the application of 8 ml of petroleum

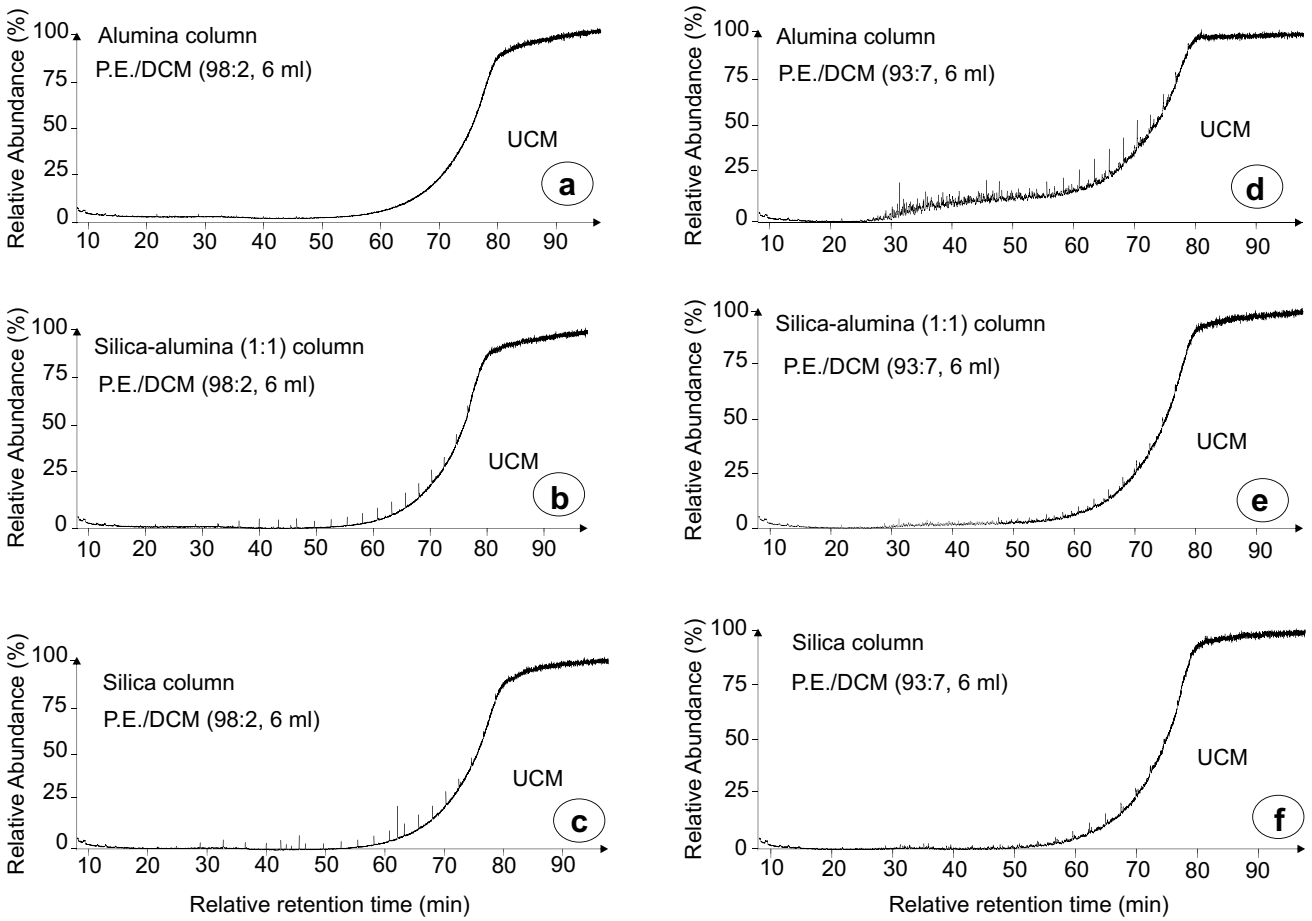

Fig.1 Chromatograms of alumina, silica-alumina (1:1), silica columns using 6 ml of petroleum ether:dichloromethane at ratio 98:2 and 6 ml at ratio 93:7

ether:dichloromethane (90:10), it was observed that the distribution of aromatic compounds in the alumina column is different from that of silica-alumina (1:1) and silica columns (Figs. 2a, 2b, and 2c). The chromatogram of the alumina column shows several alkylnaphthalenes (dimethylnaphthalenes and trimethylnaphthalenes) and biphenyls (methyl biphenyls and dimethyl biphenyls), while silica-alumina (1:1) and silica columns showed almost the same chromatogram composed only alkylnaphthalenes. Nonetheless, two biphenyl compounds were identified from silica-alumina (1:1) chromatogram (C2-biphenyl and C3-biphenyl) (Fig. 2a). It is obvious from those three different chromatograms that the presence of silica affects biphenyl compounds, which should have been eluted (Figs. 2a, 2b, and 2c).

Further application of 6 ml of petroleum ether:dichloromethane (83:17) revealed that both alumina and silica-alumina (1:1) columns display the similar distribution of compounds composed mainly of alkylnaphthalenes and alkylbiphenyls contrary to silica column, from which di-aromatic compounds were mixed with tri-aromatic compounds (Figs. 2d, 2e, and 2f). The GC–MS chromatograms obtained when 8 ml of petroleum ether:dichloromethane (80:20) applied to alumina and silica-alumina (1:1) columns also showed the similar distribution of compounds for both columns, although di-aromatic compounds dominated most of the peaks (Figs. 3a and 3b). Nevertheless, from the chromatogram of alumina column, two C_2-dibenzofurans were identified, whereas in that of silica-alumina (1:1) column, three alkyldibenzofurans, five alkylphenanthrenes, and three alkylfluorenes were identified (Figs. 3a and 3b). On the other hand, the chromatogram of the silica column showed a different distribution of compounds mainly composed of tri-aromatic compounds mixed to a lesser extent with the remaining di-aromatic compounds (Fig. 3c). The last fraction was also eluted with 6 ml of pure dichloromethane, and the resulting chromatograms revealed that both the alumina and silica-alumina (1:1) columns have similar compound distribution that is mainly composed of tri-aromatic compounds mixed with alkylchrysenes (Figs. 3d and 3e). The GC–MS analysis from only silica column showed a contrasting chromatogram dominated by alkylpyrenes, chrysene, alkylchrysenes, and benzo(e)pyrene (Fig. 3f). Furthermore,

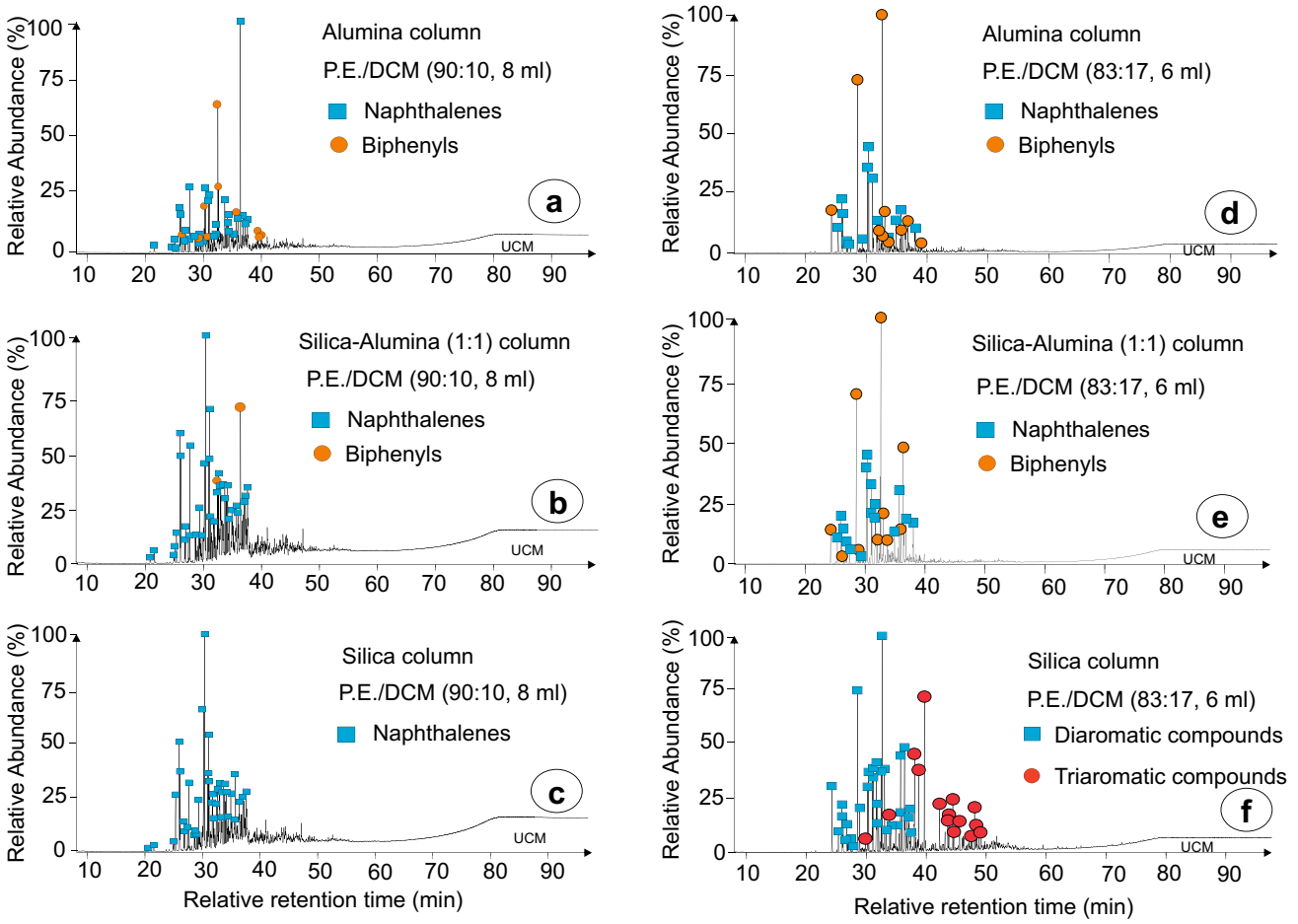

Fig.2 Chromatograms of alumina, silica-alumina (1:1), silica columns using 8 ml of petroleum ether:dichloromethane at ratio 90:10 and 6 ml at ratio 83:17

GC–MS analysis of this columns' test as applied to alumina, silica-alumina, and silica chromatograms showed that both alumina and silica-alumina (1:1) allowed the same gradual and organized distribution of PAH sub-fraction according to the number of aromatic rings (Figs. 2 and 3). From the silica column, it was difficult to separate di-aromatic from tri-aromatic compounds. In addition, the silica column was more time-consuming (35 min) compared to the alumina and silica-alumina (1:1) which lasted for 20 min and 25 min respectively to complete the procedure.

Ratio of reagents and related volume

The purpose of this section was to determine whether the same ratio and volume of reagents could be used for both columns (alumina and silica-alumina 1:1) and to determine whether the volume of reagents affects the proper separation of PAHs into sub-fractions according to the number of aromatic rings. Thus, the first sub-fraction eluted with petroleum ether:dichloromethane (93:7, 6 ml) was composed only of mono-aromatic compounds, while the second sub-fraction, which eluted with 16 ml of petroleum ether:dichloromethane (90:10) as indicated by GC–MS, revealed only the presence of di-aromatic compounds (alkylnaphthalenes and alkylbiphenyls) (Figs. 4a, 4b, 5a, and 5b). Further, 16 ml of petroleum ether:dichloromethane (80:20) directly used showed a mixing of the remaining di-aromatic compounds with tri-aromatic compounds. However, the concentration of di-aromatic compounds was dominant compared to the tri-aromatic compounds from the alumina column contrary to the silica-alumina (1:1) chromatogram that was dominated by tri-aromatic compounds (Figs. 4c and 5c). In the fourth sub-fraction, there was also a mixing of the remaining tri-aromatic compounds with those with more than 3 aromatic rings when recovered with 16 ml of petroleum ether:dichloromethane (50:50) (Figs. 4d and 5d). The recovery of this sub-fraction was then followed by using 12 ml of pure dichloromethane eluent that recovered the remaining compounds with more than 3 aromatic rings (Figs. 4e and 5e).

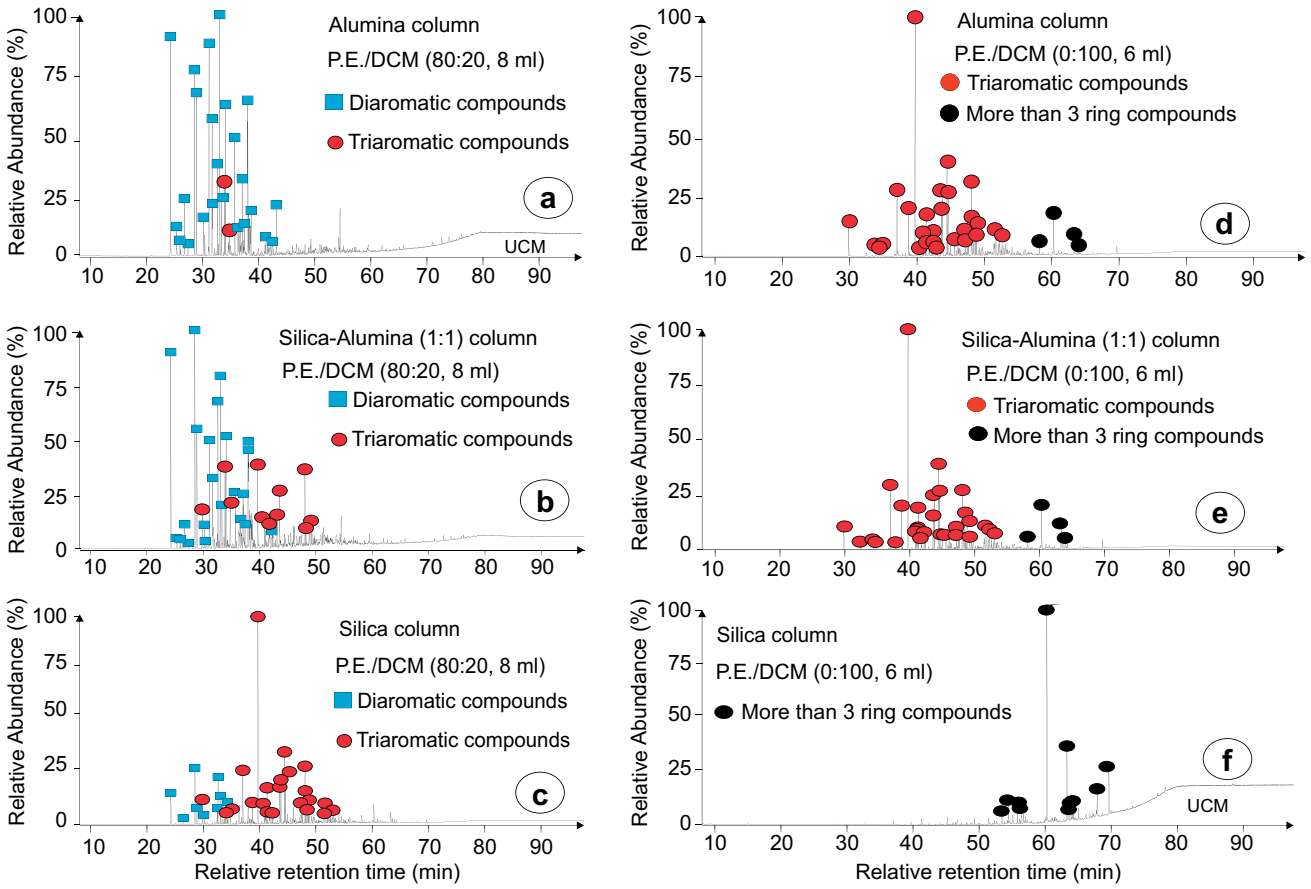

Fig.3 Chromatograms of alumina, silica-alumina (1:1), silica columns using 8 ml of petroleum ether:dichloromethane at ratio 80:20 and 6 ml at ratio 0:100

This test showed that petroleum ether:dichloromethane ratios of (90:10) and (80:20) could be used to recover di-aromatic compounds and petroleum ether:dichloromethane ratios of (80:20) and (50:50) could also be used to recover tri-aromatic compounds (Figs. 4 and 5). Thus, for a complete recovery, a second test was run by increasing the volume of each ratio, mainly petroleum ether:dichloromethane (90:10, 30 ml) and petroleum ether:dichloromethane (80:20, 30 ml) on both columns (alumina and silica-alumina 1:1) to recover successfully di-aromatic and tri-aromatic compounds (Figs. 6 and 7).

GC–MS analysis revealed that after recovering the first sub-fraction composed of mono-aromatic compounds using 6 ml of petroleum ether:dichloromethane (93:7) (Figs. 6a and 7a), the following petroleum ether:dichloromethane (90:10, 30 ml) does not recover all di-aromatic compounds using alumina column; however, it successfully recovers all the di-aromatics when silica-alumina (1:1) column is used (Figs. 6b and 7b).

The 30 ml of petroleum ether:dichloromethane (80:20) chromatograms revealed only the recovery of tri-aromatic compounds with alumina and silica-alumina (1:1) columns (Figs. 6c and 7c). Unfortunately, the 12 ml of pure dichloromethane finally used to recover the sub-fraction of compounds with more than 3 rings revealed the presence of remaining tri-aromatic compounds (Figs. 6d and 7d).

Elution of PAH sub-fractions

In this section, the procedures for separation of PAHs were based on the use of different reagent ratios and their related volume. After several laboratory tests, it was observed that a clear separation of PAHs was achieved when those are applied to alumina and silica-alumina (1:1) columns. The procedures were developed using a one-step column chromatography. Firstly, the total aromatic fractions from the Termit crude oil sample (10–20 mg) were introduced onto the top of alumina and silica-alumina (1:1) columns (Fig. 8) with a small volume petroleum ether (2 ml). Then, 6 ml of petroleum ether:dichloromethane (93:7) was used to elute the first sub-fraction of the aromatic compound. Results as shown in the chromatograms indicate that only mono-aromatic compounds were present and are free from other aromatic compounds (Figs. 9a and 10a). The second sub-fraction was then eluted with 40 ml and 30 ml of petroleum ether:dichloromethane (90:10) on alumina and

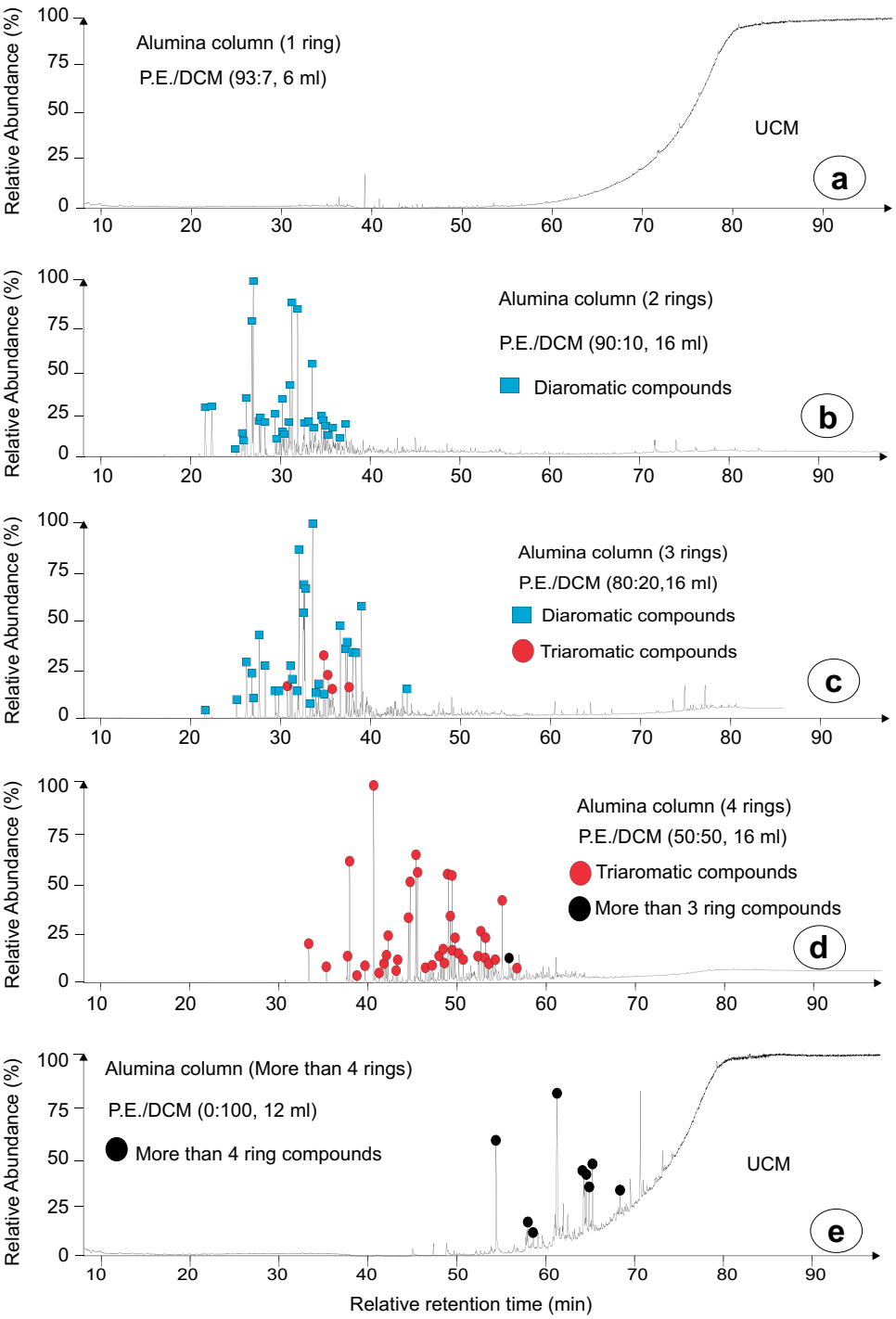

Fig.4 First assessment of the volume of reagents on the alumina column

silica-alumina (1:1) columns respectively (Figs. 9b and 10b). The GC–MS analysis showed that the ratio of reagents and their related volumes recover successfully all di-aromatics mainly naphthalene and biphenyl compounds and they are free from tri-aromatics and those with more than 3 rings (Table 3, Figs. 9b and 10b). The recovery of the following tri-aromatic sub-fraction was obtained using 25 ml of petroleum ether:dichloromethane mixture (75:25) on alumina column and 20 ml on silica-alumina (1:1) column (Table 4, Figs, 9c and 10c). For completing the separation, compounds with more than 3 rings were recovered using 12 ml of dichloromethane (Table 5, Figs. 9c and 10c).

It is important to mention that one of the reasons for this procedure involved in the separation of aromatic compounds into the number of ring is reducing the effect of the unresolved complex mixture (UCM). This procedure proposed in this current study reduces considerably the presence of UCM, and it frees almost completely each PAH sub-fraction from this factor (UCM).

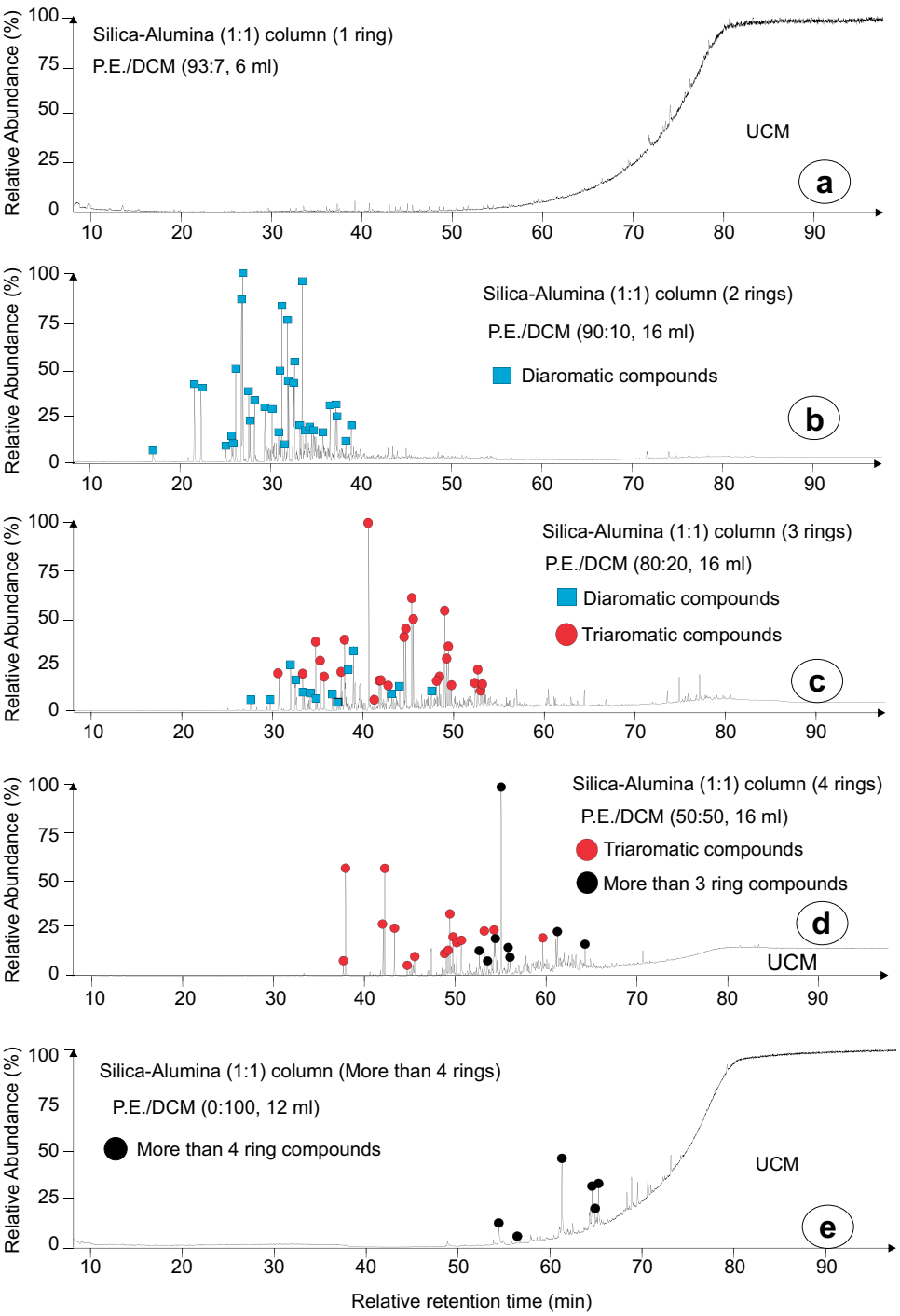

Fig.5 First assessment of the volume of reagents on the silica-alumina (1:1) column

The $\delta^{13}C$ values of individual aromatic compound

The $\delta^{13}C$ values of the individual PAH compound in the crude oil sample from the Termit basin ranged from − 32.2‰ to − 23.6‰ for alkylnaphthalenes and from − 29.7‰ to − 25.9‰ for alkylphenanthrenes. The $\delta^{13}C$ values of fluorene, phenanthrene, and 4-methyldibenzofuran were − 26.3‰, − 28.6‰, and − 25.3‰ respectively (Table 6). The $\delta^{13}C$ values of compounds such methylnaphthalenes and those with more than 3 rings were not obtained due to their low concentration in the sample. For the stable carbon isotope data, samples analyzed typically had an average standard deviation of 0.5‰.

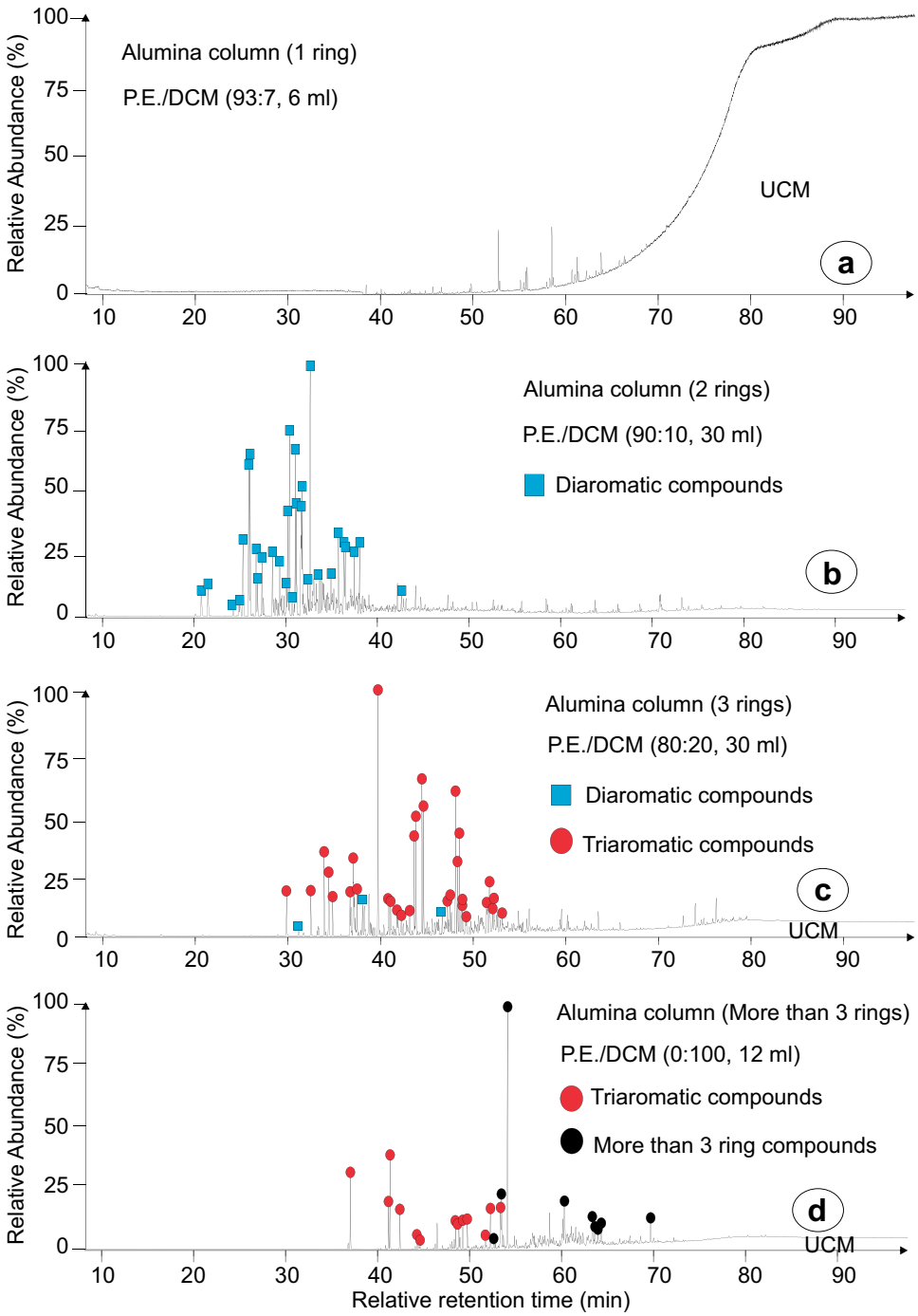

Fig.6 Second assessment of the volume of reagents on the alumina column

Discussion

Reliability of column for separation of PAHs into sub-fractions

Several type of columns have been used for the separation of crude oils or source rock extracts into fractions (Asif et al. 2009, 2011; Wang et al. 2014; Lai et al. 2018; Xiao et al. 2019). However, it seemed difficult to achieve a complete mono-/di-/tri-aromatic separation since the literature revealed a few authors (Jiang et al. 2013; Le Metayer et al. 2014; Chen et al. 2016) who attempted due to overlap in the elution bands for different aromatic ring types. It is obvious that the relationship between the type of column and the reagents used play an important role in PAH separation. Indeed, the non-polar substances were retained poorly on the polar column and therefore elute early, while more polar substances were retained for a longer time and, as a result, elute in the order of increasing polarity (Sinioja 2016). Although silica and alumina are known as polar sorbents, Fig. 2 a, b,

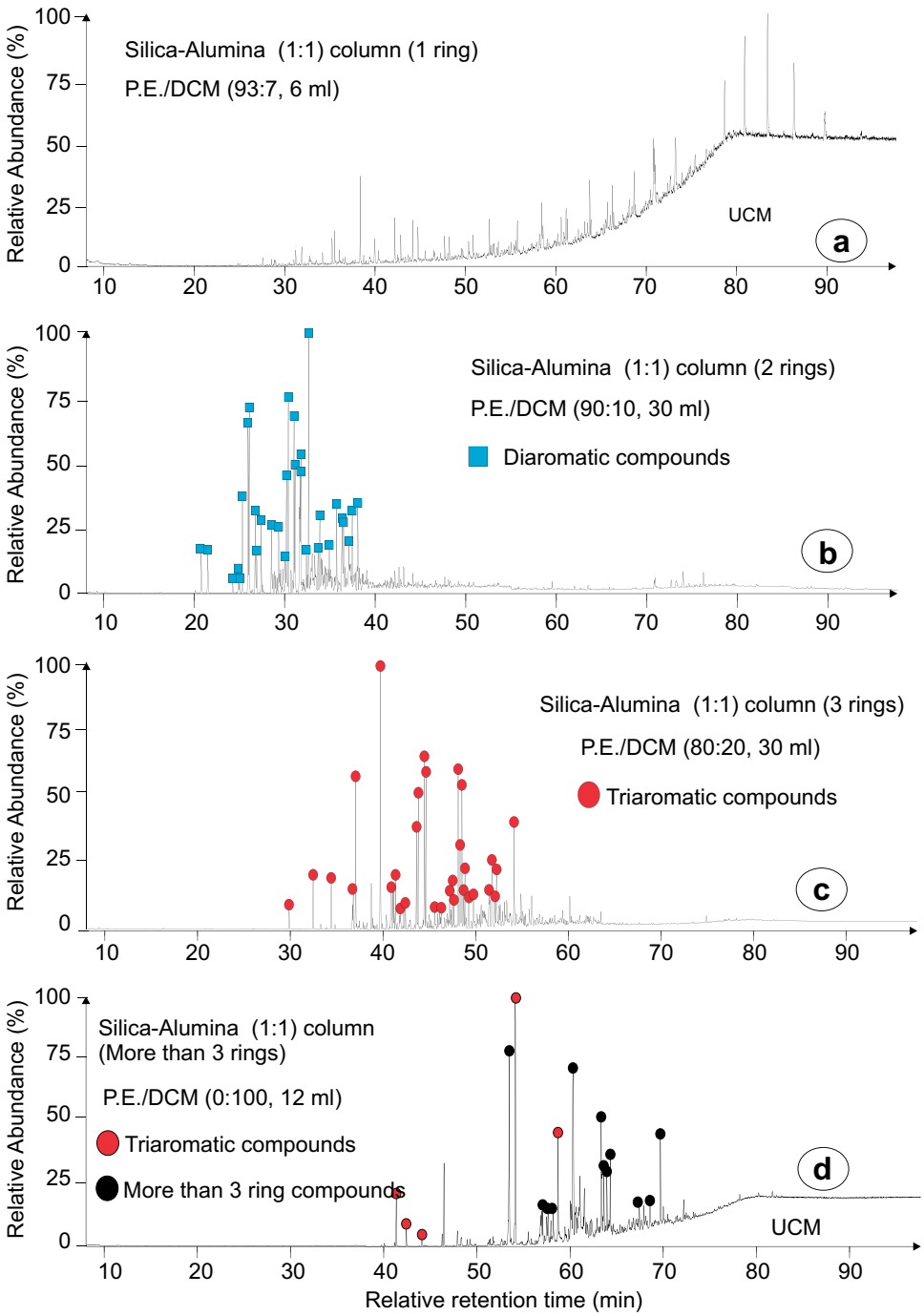

Fig.7 Second assessment of the volume of reagents on the silica-alumina (1:1) column

and c showed that silica eluted quickly naphthalene compounds than biphenyls that are non-polar compounds contrary to alumina gel that eluted both compounds. This observation suggests that alumina as a stationary phase is more reliable than silica gel for PAHs separation. Accordingly, gradient elution with unmodified silica columns should also be avoided especially if localizing solvents such as propanol are used because of their "strong" polar behavior (Sinioja 2016). In our study, the localizing solvent used was dichloromethane, and it is a polar reagent. Indeed, a localizing solvent is a reagent that competes for surface retention sites with the analyte, i.e., elution of analytes becomes independent on the interactions between mobile phase and analyte, but on strong retention of the mobile phase to the stationary phase. Thus, this can lead to chromatographic problems, e.g., the analyte will be poorly retained on the column; therefore, poor separation and low retention factor values would be obtained (Sinioja 2016). Figures 2 c and f and 3c revealed that whatever the ratio of reagents used on a silica column, the separation of di-aromatic and tri-aromatic compounds

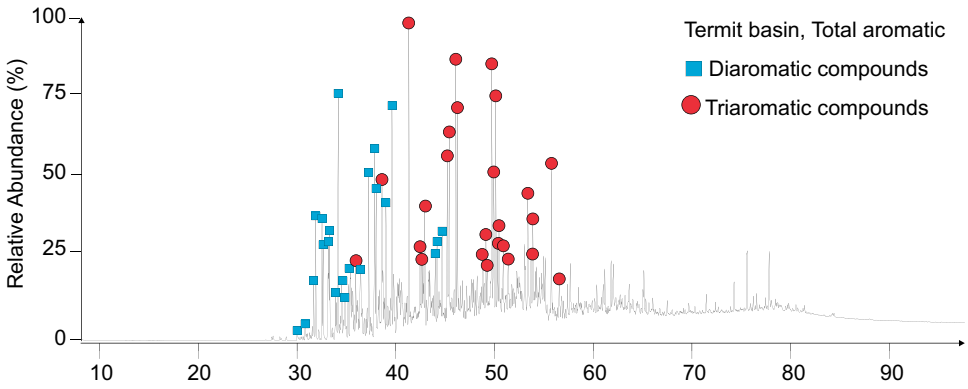

Fig. 8 Total aromatic fraction of crude oil from Termit Basin

remains difficult. In addition, using only silica as column takes longer compared to alumina and silica-alumina (1:1) columns. This is related mainly to its morphology. With respect to silica sample, the micrograph indicates an amorphous gel of non-porous material besides a slight porosity (Faramawy et al. 2016). Thus, the lack of pores could make silica gel less permeable to the reagents. Instead, the GC–MS chromatograms of alumina and silica-alumina (1:1) columns also revealed a gradual and organized elution of PAH compounds according to the number of aromatic rings. Jiang et al. (2013) pointed out that alkylnaphthalenes were successfully eluted using alumina column chromatography. The micrograph of alumina shows an amorphous phase containing different types of pores. For silica-alumina samples, it was indicated that porosity increases by increasing the volume of alumina (Faramawy et al. 2016). The increase in porosity makes the column more permeable; therefore, the more the alumina contains in the column, the less the time it consumes. Ravanbakhsh and co-authors showed that silica-alumina (5:5 g or 1:1) and (5:10 g or 1:2) tend to have higher degree of reliability with PAHs of higher molecular weight recovered from PCB compounds (Ravanbakhsh et al. 2007). Figure 2 (a, b, d, e) and Figure 3 (a, b, d, e) revealed that silica-alumina (1:1) shows the same distribution of PAH compounds as alumina. Both columns allow a gradual and progressive elution of individual compound according to the aromatic ring and their polarity. This observation suggests that silica-alumina (1:1) and alumina column are reliable for PAH separation. The test of the columns showed that a reliable column should not contain more silica than alumina gel. Thus, in addition to alumina and silica-alumina (1:1), silica-alumina (2:4) and silica-alumina (1:5) could be reliable types of column for PAHs separation into sub-fractions; however, they have not been tested in this study.

Relationship between the volume of reagents and column

The decrease in the surface area and pore volume induced by the removal of the hydroxyl group led to a decrease in adsorption, and the surface acquires more hydrophobic properties (Faramawy et al. 2016). The hydrophobic character due to the decrease in the surface area and pore volume does not allow the silica to retain any compound, and this makes elution of each sub-fraction with less solvent possible. It has been stated that the removal of hydroxyl group from the surface of silica leads to a decrease in the adsorption; thereby, the surface acquires more hydrophobic properties (Boudreau and Cooper 1989). In contrast, in the alumina column where the surface area and pore volume increase after thermal treatment, more compounds are retained, and they have to be removed using much reagent volume than what is used in the silica.

The ratio of petroleum ether:dichloromethane (97:3, 5.5 ml) was used by Jiang et al. (2013) for eluting di-aromatic compounds. As observed in this present study, by increasing the rate of dichloromethane that is a polar reagent rather than petroleum ether, which is non-polar, with different chromatograms, it was revealed that petroleum ether:dichloromethane (93:7, 6 ml) did not elute any PAH. Figure 2 a, b, and c revealed that petroleum ether:dichloromethane ratio of (90:10) is the minimum ratio required for elution of di-aromatic compounds. Indeed, Jiang and co-authors stated that petroleum ether:dichloromethane (8 ml, 90:10, v:v) was used to successfully recover the alkylnaphthalenes free of the tri-aromatic components. However, Figs. 9 and 10 b and c showed that alumina column was more reagent consuming (90:10, 40 ml) than silica-alumina column (90:10, 30 ml) to recover di-aromatic compounds successfully. This result attests that the same ratio of reagents can be used on alumina and silica-alumina (1:1) columns for the separation of PAH but not the same volume. Even if the successful recovery of di-aromatic compounds obtained by Jiang et al. is finally obtained in this study, both results are different by the volume of solvents applied; however, the differences in volumes used are understandable. Indeed, there are three types of alumina: alumina with micropores (diameter < 2 nm), alumina with mesopores (diameter between 2 and 50 nm), and alumina with macropores (diameter > 50 nm) (Morin 2014). It has been shown that, because of the high absorptivity of

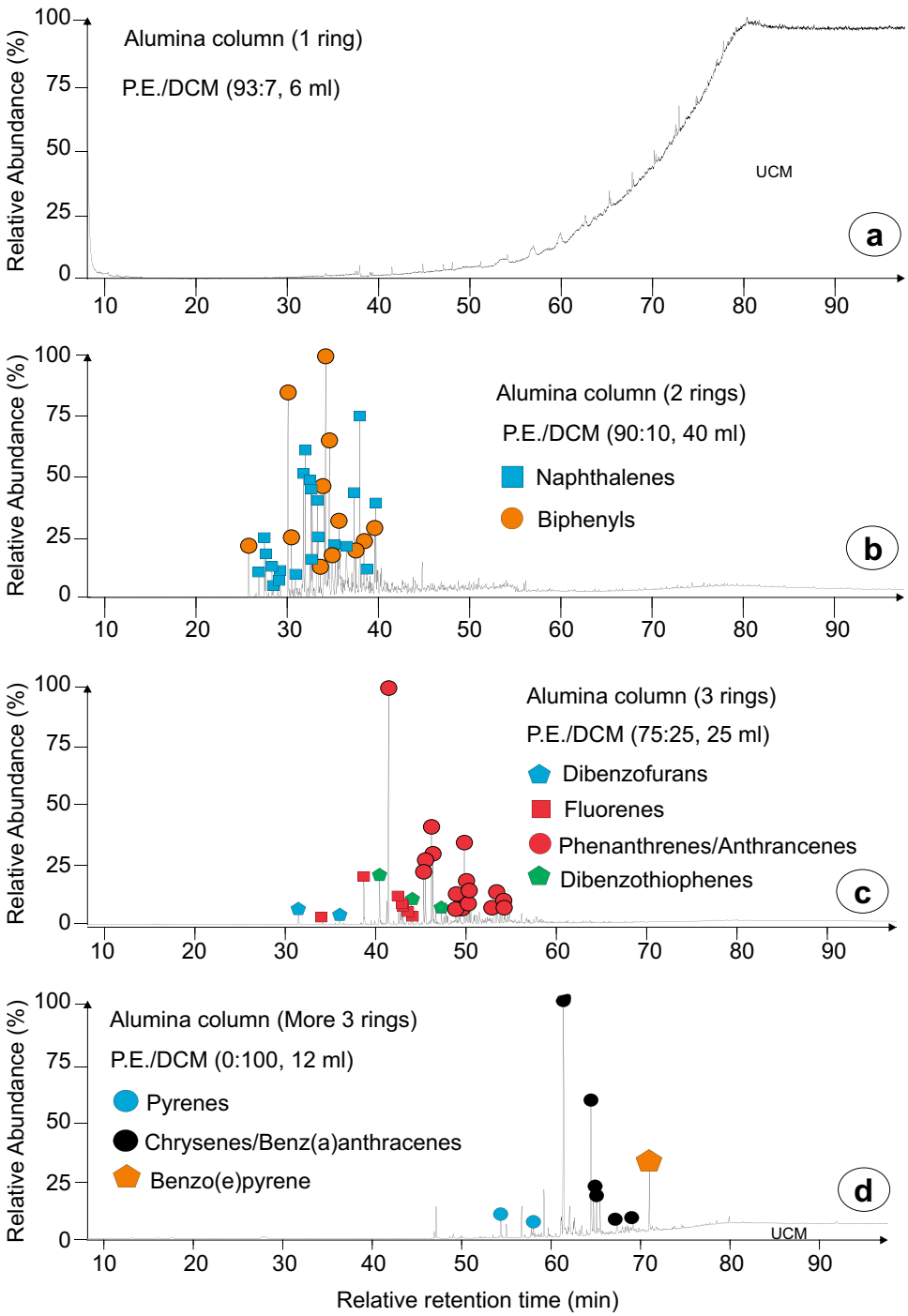

Fig.9 Separation of the aromatic fraction into sub-fractions using alumina column

silica and alumina, the use of these classical adsorbents for routine NPLC fractionation of PAHs is unreliable (Wise et al. 1977, 1993). However, the difference in volume of petroleum ether:dichloromethane (90:10) used by Jiang and co-authors and the volume used in the present study to elute successfully di-aromatic compounds shows that the pore diameter of alumina, which conditions that the volume of pores in the column chromatography is the factor affecting the reproducibility of the process. These observations show that the diameter of pores of alumina is the most important and the main factor defining the success of the separation procedure. Thus, the higher the diameter of the pores in the alumina, the higher the volume of solvents used for the separation. On the contrary, the smaller the diameter of the pores, the smaller the volume of solvents required. The pores' diameter of alumina used for this present study belongs to the mesoporous range (17.4 nm). The results obtained by Jiang and co-authors were certainly made by using a type of

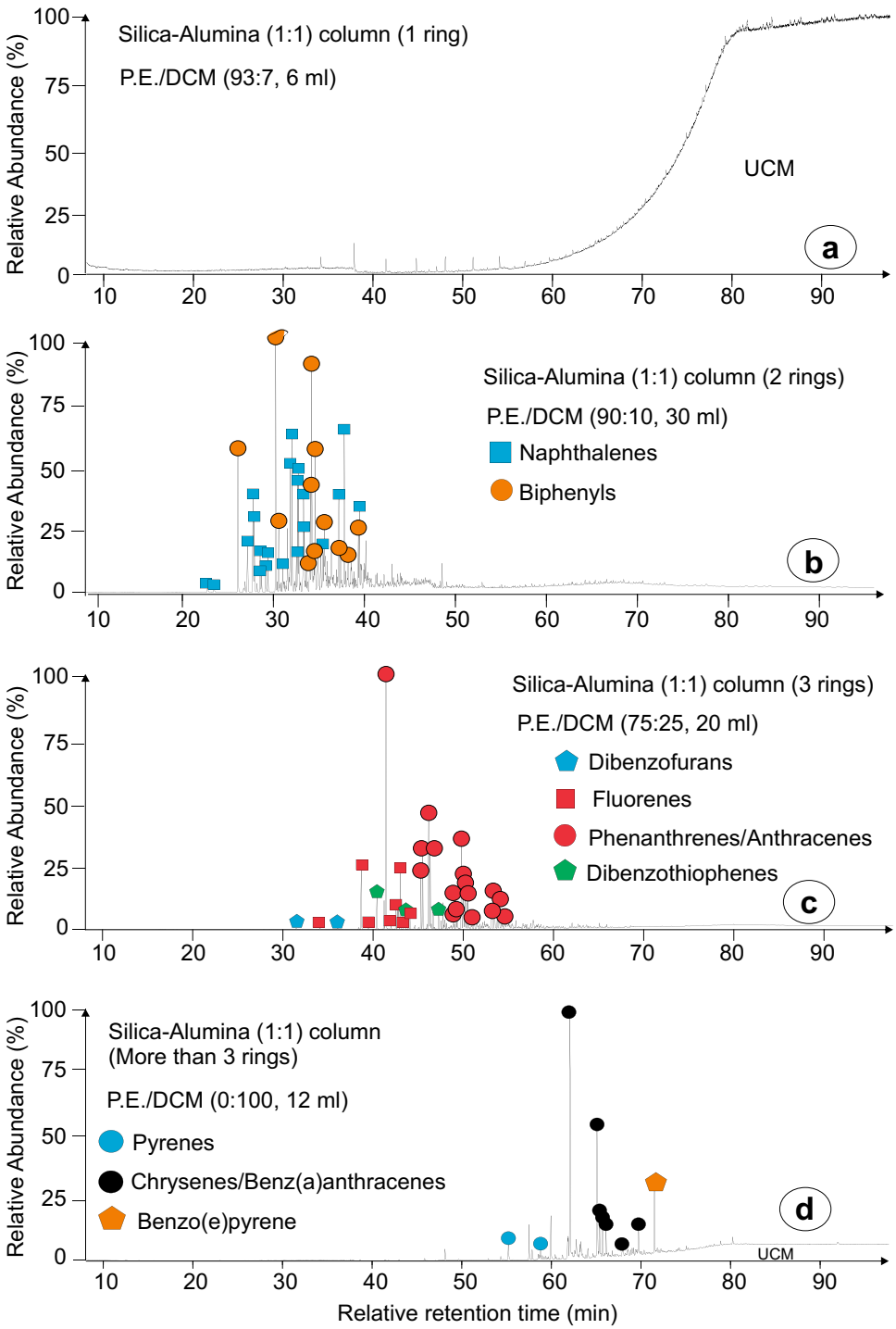

Fig.10 Separation of the aromatic fraction into sub-fractions using silica-alumina (1:1) column

alumina with a pore diameter smaller than 17.4 nm. After evaluating different column chromatography conditions for the separation of the analytes from each other, the combination of two sorbents silica-alumina (5:10 g) for separation of PAHs and silica-alumina (5:5 g) for separation of PCBs was chosen as the best of tested (Faramawy et al. 2016). In our case, despite the fact that silica-alumina (1:1) column is slightly time-consuming than alumina column, Figs. 9 and 10 show that it is the best of the two columns for the separation of PAH compounds in terms of volume of reagents necessary to complete the whole process.

Thus, for separating aromatic fraction into mono-, di-, tri-aromatic, and compounds with more than 3 ring sub-fractions, silica-alumina (1:1) column (approx. 6 cm) is wet with 5 ml of petroleum ether and then dried by using a pump to remove the remnant of petroleum ether. This was followed

Table 3 Identification of di-aromatic compounds in crude oil by GCMS

Number	Compound	Abbr	Number	Compound	Abbr	Number	Compound	Abbr
	Termit sub-fraction of 2 rings							
1	2,6-Dimethylnaphthalene	2,6- + 2,7-DMN	17	2,3,6-Trimethylnaphthalene	2,3,6-TMN	33	1,2,3,4-Tetramethylnaphthalene	1,2,3,4-TeMN
	2,7-Dimethylnaphthalene		18	1,2,7-Trimethylnaphthalene	1,2,7- + 1,6,7-TMN	34	C4-Naphthalene	C4-N
2	1.3-Dimethylnaphthalene	1,3- + 1,7-DMN		1,6,7-Trimethylnaphthalene		35	C4-Naphthalene	C4-N
	1,7-Dimethylnaphthalene		19	1,2,6-Trimethylnaphthalene	1,2,6-TMN	36	C4-Naphthalene	C4-N
3	1,6-Dimethylnaphthalene	1,6-DMN	20	C3-Naphthalene	C3-N	37	C4-Naphthalene	C4-N
4	1,4-Dimethylnaphthalene	1,4- + 2,3-DMN	21	1,2,5-Trimethylnaphthalene	1,2,5-TMN	38	C4-Naphthalene	C4-N
	2,3-Dimethylnaphthalene		22	3,4'-Dimethylbiphenyl	3,4′-DMBiph	39	3,4-Dimethylbiphenyl	3,4-DMBiph
5	1,5-Dimethylnaphthalene	1,5-DMN	23	C4-Naphthalene	C4-N	40	1,4,5,8-Tetramethylnaphthalene	1,4,5,8-TeMN
6	1,2-Dimethylnaphthalene	1,2-DMN	24	1,3,5,7-Tetramethylnaphthalene	1,3,5,7-TeMN	41	3,5,3′-Trimethylbiphenyl	3,5,3′-TMBiph
7	3-Methylbiphenyl	3-MBiph	25	1,3,6,7-Tetramethylnaphthalene	1,3,6,7-TeMN	42	3,5,4′-Trimethylbiphenyl	3,5,4′-TMBiph
8	4-Methylbiphenyl	4-MBiph	26	1,2,4,6-Tetramethylnaphthalene	1,2,4,6- + 1,2,4,7- + 1,4,6,7-TeMN	43	C3-biphenyl	C3-Biph
9	C3-Naphthalene	C3-N		1,2,4,7-Tetramethylnaphthalene		44	3,4,3′-Trimethylbiphenyl	3,4,3′-TMBiph
10	C3-Naphthalene	C3-N		1,4,6,7-Tetramethylnaphthalene		45	3,4,4′-Trimethylbiphenyl	3,4,4′-TMBiph
11	C3-Naphthalene	C3-N	27	1,2,5,7-Tetramethylnaphthalene	1,2,5,7-TeMN	46	2,2′-Diethylbiphenyl	2,2′-DEBiph
12	C3-Naphthalene	C3-N	28	2,3,6,7-Tetramethylnaphthalene	2,3,6,7-TeMN			
13	1,3,7-Trimethylnaphthalene	1,3,7-TMN	29	1,2,6,7-Tetramethylnaphthalene	1,2,6,7-TeMN			
14	1,3,6-Trimethylnaphthalene	1,3,6-TMN	30	1,2,3,7-Tetramethylnaphthalene	1,2,3,7-TeMN			
15	C3-Naphthalene	C3-N	31	1,2,3,6-Tetramethylnaphthalene	1,2,3,6-TeMN			
16	1,4,6-Trimethylnaphthalene	1,4,6- + 1,3,5-TMN	32	1,2,5,6-Tetramethylnaphthalene	1,2,5,6- + 1,2,3,5-TeMN			
	1,3,5-Trimethylnaphhalene			1,2,3,5-Tetramethylnaphthalene				

Table 4 Identification of tri-aromatic compounds by GCMS

Number	Compound	Abbr	Number	Compound	Abbr	Number	Compound	Abbr
Termit sub-fraction of 3 rings								
1	Fluorene	F	21	9-Methylphenanthrene	9-MP	32	1,8-Dimethylphenanthrene	9-MP
2	9-Methylfluorene	9-MF	22	1-Methylphenanthrene	1-MP	33	1,2-Dimethylphenanthrene	1-MP
3	4-Methyldibenzofuran	4-MDBF	23	2-Ethylphenanthrene	2-EP + 9-EP + 3,6-DMP	34	C2-phenanthrene	C2-P
4	3-Methylfluorene	3-MF		9-Ethylphenanthrene		35	4,5-Dimethylphenanthrene	4,5-DMP
5	2-Methylfluorene	2-MF		3,6-Dimethylphenanthrene		36	1,3,6-Trimethylphenanthrene	1,3,6- + 1,3,10- + 2,6,10-TMP
6	1-Methylfluorene	1-MF	24	1-Ethylphenanthrene	1-EP		1,3,10-Trimethylphenanthrene	
7	4-Methylfluorene	4-MF	25	2,6-Dimethylphenanthrene	2,6- + 2,7-DMP		2,6,10-Trimethylphenanthrene	
8	Dibenzothiophene	DBT		2,7-Dimethylphenanthrene		37	1,3,7-Trimethylphenanthrene	1,3,7- + 2,6,9- + 2,7,9-TMP
9	Phenanthrene	P	26	3,5-Dimethylphenanthrene	3,5-DMP		2,6,9-Trimethylphenanthrene	
10	C2-fluorene	C2-F	27	2,10-Dimethylphenanthrene	2,10- + 1,3- + 3,10- + 3,9-DMP		2,7,9-Trimethylphenanthrene	
11	9-Ethylfluorene	9-EF		1,3-Dimethylphenanthrene		38	1,3,9-Trimethylphenanthrene	1,3,9- + 2,3,6-TMP
12	C2-fluorene	C2-F		3,10-Dimethylphenanthrene			2,3,6-Trimethylphenanthrene	
13	2,3-Dimethylfluorene	2,3-DMF		3,9-Dimethylphenanthrene		39	1,6,9-Trimethylphenanthrene	1,6,9- + 1,7,9- + 2,3,7-TMP
14	C2-fluorene	C2-F	28	1,6-Dimethylphenanthrene	1,6- + 2,8- + 2,5-DMP		1,7,9-Trimethylphenanthrene	
15	C2-fluorene	C2-F		2,8-Dimethylphenanthrene			2,3,7-Trimethylphenanthrene	
16	C2-fluorene	C2-F		2,5-Dimethylphenanthrene		40	1,3,8-Trimethylphenanthrene	1,3,8-TMP
17	C2-fluorene	C2-F	29	1,7-Diimethylphenanthrene	1,7-DMP	41	2,3,10-Trimethylphenanthrene	2,3,10-TMP
18	4-Methyldibenzothiophene	4-MDBT	30	2,3-Dimethylphenanthrene	2,3-DMP	42	1,6,7-Trimethylphenanthrene	1,6,7-TMP

Table 4 (continued)

Number	Compound	Abbr	Number	Compound	Abbr	Number	Compound	Abbr
19	3-Methylphenanthrene	3-MP	31	4,9-Dimethylphenanthrene	4,9- + 4,10- + 1,9-DMP	43	1,2,6-Trimethylphenanthrene	1,2,6-TMP
20	2-Methylphenanthrene	2-MP		4,10-Dimethylphenanthrene		44	1,2,7-Trimethylphenanthrene	1,2,7- + 1,2,9-TMP
				1,9-Dimethylphenanthrene			1,2,9-Trimethylphenanthrene	

by putting aromatic fraction onto the top of the column using 2 ml of petroleum ether. Thus, the isolation of mono-aromatic sub-fraction was completed using 6 ml (2 ml dropped in the column 3 times) of petroleum ether:dichloromethane (93:7). The mono-aromatic elution was then followed by the separation of di-aromatics. However, before removing the container of each sub-fraction obtained during this process, it is important to use pump to dry the column to recover the remaining solvent mixture. Then, allow 2 drops of mixture of reagents mentioned below into the container to ensure that all compounds of the sub-fraction are removed. The di-aromatic sub-fraction was eluted with 30 ml (2 ml, 15 times) of petroleum ether:dichloromethane (90:10) on silica-alumina (1:1) column to elute all the di-aromatic compounds. The tri-aromatic sub-fraction using silica-alumina (1:1) column was successfully achieved using 20 ml of petroleum ether:dichloromethane (75:25). Compounds with more than 3-ring sub-fractions were completely recovered with 12 ml of pure dichloromethane (Fig. 10).

Table 5 Identification of compounds with more than 3 rings by GCMS

Number	Compound	Abbr
Termit sub-fraction of more than 3 rings		
1	1-Methylpyrene	1-Mpyr
2	4-Methylpyrene	4-Mpyr
3	C2-pyrene	C2-pyr
4	C2-pyrene	C2-pyr
5	C2-pyrene	C2-pyr
6	C2-pyrene	C2-pyr
7	C2-pyrene	C2-pyr
8	Chrysene	Chr
9	3-Methylchrysene	3-Mchr
10	2-Methylchrysene	2-Mchr
11	6-Methylchrysene	6-Mchr
12	1-Methylchrysene	1-Mchr
13	C2-chrysene	C2-chr
14	C2-chrysene	C2-chr
15	C2-chrysene	C2-chr
16	Benzo(e)pyrene	B(e)pyr

Potential geochemical significance of the isotope measurements and separation procedure

Several purification procedures of the aromatic fraction leading to isotope measurements of the individual PAH compounds have been completed in previous researches (Mazeas and Budzinski 2001; Yanik et al. 2003; Kim 2004; Sun et al. 2005; Jiang et al. 2013; Chen et al. 2016). The results revealed that good reproducibility, as uncertainties between three independent assays performed, were lower than 0.5‰ (Mazeas and Budzinski 2001). Thus, for carbon isotope data, samples analyzed in duplicate had an average standard deviation of 0.1 to 0.3‰ (Sun et al. 2005). For individual compound or combined peaks, method precision ranged between 0.08 and 0.43 ‰ (Kim 2004). In fact, the precision for replicate analyses was usually less than 1‰, although there were some exceptions (Yanik et al. 2003). From the present study, the precision of the replication for individual compound ranged between 0.1 and 0.9‰ with an average deviation of 0.5‰. The results obtained from the previous studies show that the isotope measurements provided by this present procedure are reliable and therefore could be used for obtaining further information.

Indeed, the study of the isotopic measurements of individual aromatic compound is mainly to assess the effects of the different source inputs, the thermal maturity, and the biodegradation (Mazeas et al. 2002; Maslen et al. 2011; Le Metayer et al. 2014; Chen et al. 2016; Cesar and Grice 2017). Thus, the degree of methylation is an important factor controlling the $\delta^{13}C$ values of AHs; however, for the alkylnaphthalenes, this ^{13}C depletion was also found to be related to thermal maturity (Le Metayer et al. 2014). Although, the $\delta^{13}C$ values of the substituted aromatic compounds display a positive trend with increasing maturity, the $\delta^{13}C$ values of the unsubstituted aromatic compounds (naphthalene and phenanthrene) shows no significant variation with increasing maturity (Chen et al.

Table 6 GCIRMS $\delta^{13}C$ values of individual PAH compound in crude oil from Termit basin

Compounds	Crude oil	
	$\delta^{13}C$ (‰)	Standard deviation
2-MN	-	-
1-MN	-	-
2,6 + 2,7-DMN	− 32.2	0.5
1,6-DMN	− 28.0	0.9
1,4 + 2,3 + 1,5-DMN	− 29.7	0.3
1,2 + 1,8-DMN	− 29.7	0.1
1,3,7-TMN	− 25.6	0.9
1,3,6-TMN	− 31.2	0.5
1,2,6-TMN	− 23.6	0.1
1,2,5-TMN	− 26.5	0.2
1,3,6,7-TeMN	− 28.4	0.2
Biph	-	-
3-MBiph	-	-
4-MBiph	-	-
F	− 26.3	0.2
1-MF	-	-
DBF	-	-
4-MDBF	− 25.3	0.6
P	− 28.6	0.9
3-MP	− 26.9	0.2
2-MP	− 28.6	0.8
9-MP	− 26.1	-
1-MP	− 29.7	0.3
2,10 + 1,3 + 3,10 + 3,9-DMP	− 27.4	0.8
1,,6 + 2,9 + 2,5-DMP	− 28.0	0.1
1,7-DMP	− 25.9	0.1
2,3-DMP	− 27.7	0.8

2016). However, the difference in the $\delta^{13}C$ values among alkylnaphthalene were showed mostly to result from the diverse source inputs with distinct stable carbon isotope compositions and/or the isotopic fractionations during the diagenetic evolution of their natural product precursors (Jiang et al. 2013). Previous studies revealed that crude oils from Yogou source rocks showed some characteristics of crude oils from marine origin when those of Sokor source rocks were revealed to be from terrigenous origin (Harouna and Philp 2012; Zhao and Li 2016). The discovered oils in the Termit basin are dominantly derived from YSQ2 and YSQ1 source rocks, with minor contribution of YSQ3 source rocks (Xiao et al. 2019). Indeed, all oils (including oils from Sokor Formation) mainly fall into the marine facies zone, deposited in a reducing environment with the mixed or main algal organic matter input (Xiao et al. 2019). The $\delta^{13}C$ values of 1,6-DMN and 1,2,5-TMN have been shown to be a useful tool for assessing the marine and terrigenous source contribution (Le Metayer et al. 2014). Our results correlate with the observation of Maslen and co-authors that attested that oils from marine input have the most negative $\delta^{13}C$ values for 1,6-DMN and 1,2,5-TMN (< -26‰); in contrast, oils with a terrigenous source exhibit high $\delta^{13}C$ values for 1,6-DMN and 1,2,5-TMN (> -26‰) (Maslen et al. 2011). Therefore, Table 6 showing the $\delta^{13}C$ values of each of these two compounds reveals a predominance of marine organic matter source input in this sample; however, the presence of 1,2,6-TMN (-23‰) suggests a contribution of terrestrial sourced organic matter, indicative that this crude oil could be from a mixed organic matter source. However, our research is an ongoing study and needs more samples in order to draw an overall conclusion.

For the purpose of this ongoing project, only a normal oil was used for the different tests. However, the conclusions provided might be useful and used as a base for future procedures adapted to other types of oils if necessary. Therefore, the future researches that will aim to establish a specific separation procedure for their crude oils' aromatic fractions should first use the less time and less reagent-consuming column, which according to the results provided by this present is certainly silica-alumina (1:1) column. Nonetheless, the choice of the diameter of the alumina pores is the most important in the phase of the separation of the aromatic fraction into sub-fractions. In the case, the chosen diameter was lower than the one used in this research, and the related volume should be less. On the other hand, if the diameter is higher, the volume should be more, but some primary tests had to be made to determine the right volume needed.

Summary and conclusion

The present study provides an experimental procedure for separating PAHs with the main factor(s) controlling their separation. Our results revealed that physical and chemical properties of the column affect the separation of PAHs into various sub-fractions according to the number of aromatic rings. Our laboratory test results showed that the type of column used is the factor that conditions the success of PAHs separation. Based on the time and the volume of reagents required, the separation achieved by using silica-alumina (1:1) column is chosen to be the best of the two columns used (alumina and silica-alumina 1:1). Thus, the successful separation with silica-alumina (1:1) was completed using successive petroleum ether:dichloromethane ratios of 93:7 (6 ml), 90:10 (30 ml), and 75:25 (20 ml), recovering mono-aromatic, di-aromatic, and tri-aromatic sub-fractions respectively, and further addition of 12 ml of pure dichloromethane recovered compounds with more

than 3 aromatic rings. Furthermore, as well as the type of column used, this present study shows that the diameter of the pores of alumina used for the separation is the most influencing factor that controls the successful separation of the aromatic compounds into sub-fractions. Through these results, this research proposes an experimental procedure for the separation of PAH compounds tending to have higher reliability for stable carbon isotope measurements. The GC-IRMS analysis on the separated fractions provided stable carbon isotope measurements of PAH compounds with an average standard deviation of 0.5‰.

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Declarations

Conflict of interest The authors declare that they no competing interests.

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