Analysis of Polycyclic Aromatic Hydrocarbons in Petroleum Vacuum Residues by Multiple Heart-Cutting LC Using the Agilent 1290 Infinity 2D-LC Solution

Application Note

Energy and Chemicals

Authors

Gerd Vanhoenacker, Mieke Steenbeke, Frank David, Pat Sandra, and Koen Sandra
Research Institute for Chromatography
Kennedypark 26
B-8500 Kortrijk
Belgium

Udo Huber
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

Polycyclic aromatic hydrocarbons (PAHs) were determined in a petroleum vacuum distillation residue using the Agilent Multiple Heart-Cutting (MHC) 2D-LC solution. The extract was analyzed in the first dimension by normal-phase LC. Well-defined fractions from this separation were stored in a set of sample loops, then transferred online to the second-dimension separation where the PAHs were separated from each other and from other sample constituents using reversed-phase LC on a dedicated PAH column. Detection was performed with diode-array detection (DAD) as the monitor detector after the first dimension, and with fluorescence detection (FLD) after the second dimension. An additional column switching valve enabled backflush of the first-dimension normal-phase column to remove highly polar components. The Agilent 1290 Infinity 2D-LC solution enabled automated, selective, and quantitative analysis of the PAHs in the complex petroleum vacuum distillation residue sample.

Agilent Technologies

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are monitored in petroleum products because of their higher toxicity and carcinogenic activity. The relative concentration of PAHs in a petrochemical product such as bitumen has an important impact on the total emission of hydrocarbons into the environment through hydrocarbon processing, combustion, construction (for example, roads), accidental leakage, and so on. Recent regulations have set limits on PAH content in various petroleum products, including vacuum distillates, vacuum residues, and bitumen[1].

Several methods are used for the determination of PAHs in high-boiling petroleum products. These include liquid-liquid fractionation followed by gravimetric determination[2], fractionation using column chromatography or SPE followed by GC-MS analysis[3], and offline size exclusion chromatography or normal-phase LC (NPLC), combined with reversed-phase LC (RPLC)[4,5]. These techniques often lack sensitivity or are highly labor-intensive and time-consuming. The determination of PAHs in high-boiling petroleum fractions is challenging due to the complexity of the matrix (including the presence of a polar fraction) and the low level of PAHs that need to be detected (<1 mg/kg). Online multidimensional chromatographic techniques can be of interest here. Comprehensive GC×GC has been used for group type separation of PAHs in petrochemicals, including high-boiling fractions[6,7]. With the GC×GC approach, PAHs fraction can be separated from the alkane or monoaromatic fraction, but analysis of trace levels of individual PAHs in a bulk of high-boiling alkanes is difficult.

Online LC-LC, combining NPLC with RPLC, could be an interesting approach. As demonstrated by offline approaches[4,5], NPLC is able to separate aromatic and polycyclic aromatic hydrocarbons from the bulk of the saturated alkanes and cycloalkanes. The fraction containing the PAHs can then be transferred to RPLC, while the retained polar fraction is backflushed. NPLC and RPLC offer excellent orthogonality. There are, however, two challenges in online hyphenation of NPLC and RPLC for PAH analysis. First, the mobile phases used in both modes are different and, in theory, not compatible. Second, the PAHs elute in a rather large window.

The Agilent Multiple Heart-Cutting (MHC) 2D-LC solution offers a smart valve setup that enables parking of multiple fractions from the first dimension and analyzing these sequentially as soon as the second dimension is ready for the next analysis. In this way, the wide elution window from the first-dimension separation can be split into multiple smaller fractions, thereby enhancing overall peak capacity and separation power. Moreover, this configuration also alleviates the problem of mobile phase incompatibility. The complete analytical process of fraction parking and transfer onto the second dimension is software controlled.

The power of the Agilent MHC 2D-LC solution is demonstrated by the analysis of PAHs in a petroleum vacuum residue. The method was validated (calibration, repeatability) for a selection of PAHs that also were quantified.

Experimental

Samples and sample preparation

The sample was a petroleum vacuum residue. The sample was dissolved at 200 mg/mL in *iso*-octane/cyclohexane 1/9 v/v prior to injection.

A PAH standard stock solution (PAH-Mix 25, YA20952500AB, Dr. Ehrenstorfer GmbH, Augsburg, Germany) containing 2,000 µg/mL PAHs in acetone/benzene was diluted in *iso*-octane/cyclohexane 1/9 v/v to the appropriate concentration.

Instrumentation

An Agilent 1290 Infinity 2D-LC solution was used. The configuration is shown in Figure 1, and described in Table 1.

The mixer was removed in the first-dimension pump to reduce the delay volume. To have sufficient backpressure on the first-dimension separation, a calibration capillary (G1312-67500) was installed between the pump and the autosampler.

Software

Agilent OpenLAB CDS ChemStation Edition software, revision C.01.07 with Agilent 1290 Infinity 2D-LC software revision A.01.02.

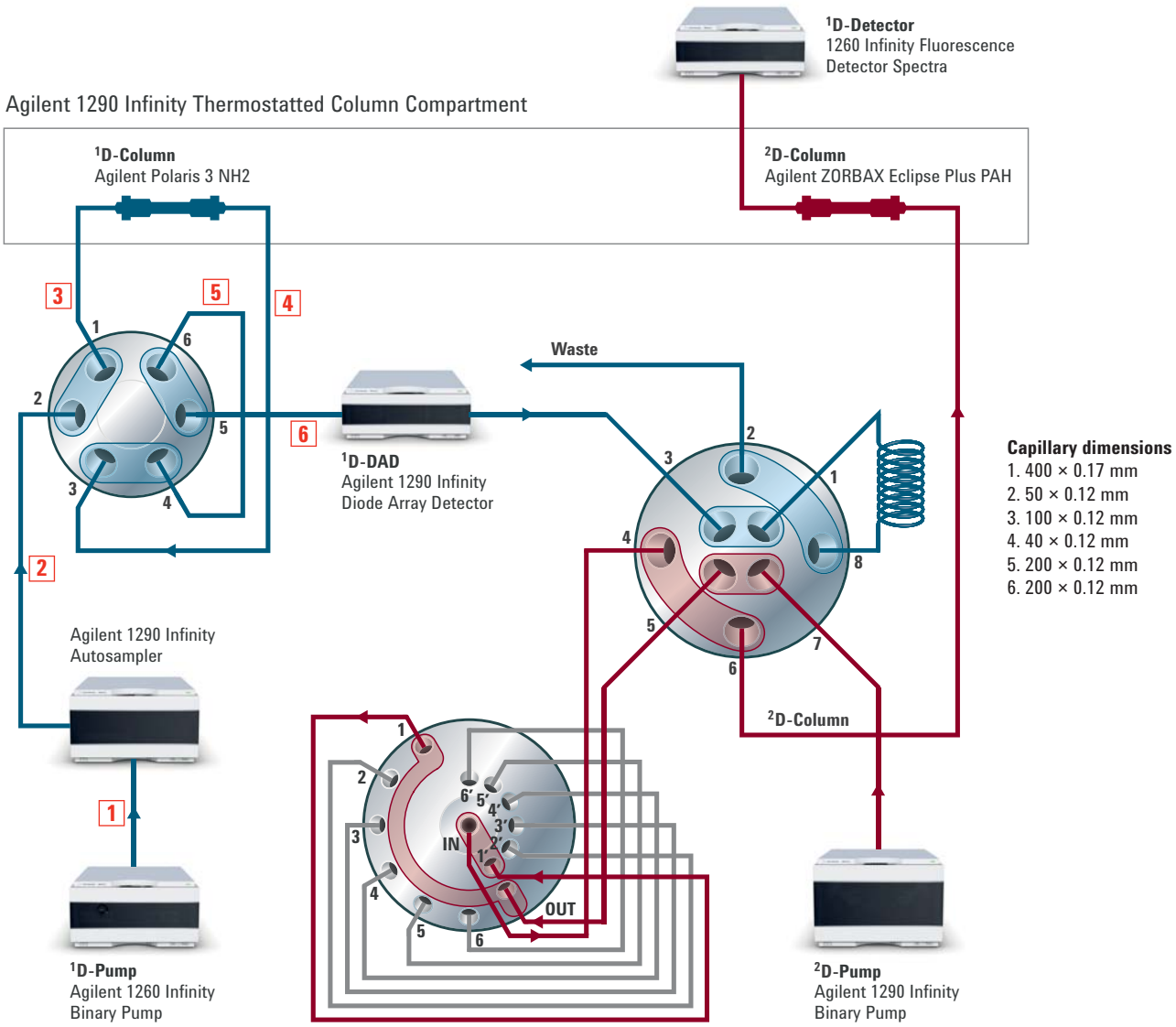

Figure 1. Configuration for multiple-heart cutting 2D-LC. Capillary dimensions:

Table 1. Instrumental configuration.

Instrument	Part number
Agilent 1260 Infinity Binary Pump (first dimension)	G1312B
Agilent 1290 Infinity Binary Pump (second dimension)	G4220A
Agilent 1290 Infinity Autosampler	G4226A
Agilent 1290 Infinity Thermostatted Column Compartment with 2-position/6-port valve	G1316C
Agilent 1290 Infinity Valve Drive	G1170A
Agilent Multiple Heart-Cutting Single Upgrade Kit	G4242A
Six-column selector valve, 1200 bar (equipped with six loops of 40 µL)	5067-4142

Results and Discussion

For the determination of PAHs in a complex hydrocarbon matrix such as vacuum distillation residues or bitumen, a combination of NPLC and RPLC was used. Figure 2 shows the separation of a standard mixture of PAHs in NPLC mode. Table 2 gives the identity of the peaks. On the aminopropyl column, PAHs are separated according to the number of rings. Also, some separation is observed according to ring fusion within a group of PAHs with the same number of aromatic rings. Benzo(a)anthracene is, for instance, partly separated from chrysene.

This NPLC separation alone is, however, not suitable for the determination of PAHs in complex petroleum fractions. Figure 3 shows the overlay of the UV trace obtained for the analysis of a vacuum distillate residue and the PAH test mixture. In NPLC, the bulk of the hydrocarbons elutes unretained (fraction eluting before naphthalene, not visible in UV). For the sample, a large unresolved hump is detected, eluting between 4 and 18 minutes. This is the aromatic and polyaromatic hydrocarbon fraction. In addition, the sample also contains a polar fraction that is not eluted with 100 % heptane, but is backflushed. This backflush option is possible due to the installation of an extra valve in the system (see Figure 1). The use of column backflush is also preferred over the use of gradient elution in NPLC (using ether or isopropanol, for instance), since the latter typically requires long equilibration times at initial mobile phase conditions.

Method

Parameter	Value
First dimension	NPLC
Column	Agilent Polaris 3 NH2, 2.0 × 150 mm, 3 µm (p/n A2014150x020)
Solvent	Heptane
Flow rate	120 µL/min
Temperature	30 °C
Flow direction	0 to 18 minutes, normal direction (valve in TCC port 2 > 1, 3 > 4, 6 > 5) 18 to 55 minutes, backflush direction (valve in TCC port 2 > 3, 1 > 6, 4 > 5)
DAD detection	254/4 nm (Reference off) Peak width > 0.05 minutes (5 Hz)
Injection	2 µL (with needle wash, flush port, 3 seconds, isopropanol/methanol
Loop filling	
Valve and loop configuration	6+1 loops (cocurrent) 40 µL loops
Time segments	Timing varied according to targeted PAHs Four fractions of 0.33 minutes wide were taken
Second dimension	RPLC
Column	Agilent ZORBAX Eclipse Plus PAH, 4.6 × 100 mm, 3.5 µm (p/n 959961-918)
Solvent A	Water
Solvent B	Acetonitrile
Flow rate	0.3 mL/min
Idle flow rate	2 mL/min
Gradient	0–0.5 minutes, 20 to 40 %B 0.5–11 minutes, 40 to 100 %B 11–11.5 minutes, 100 %B 11.5–12.5 minutes, 20 %B
Temperature	30 °C
FLD detection	Multi-emission mode Peak width > 0.05 minutes (9.26 Hz) PMT Gain: 10 Detector wavelength varied according to targeted PAHs

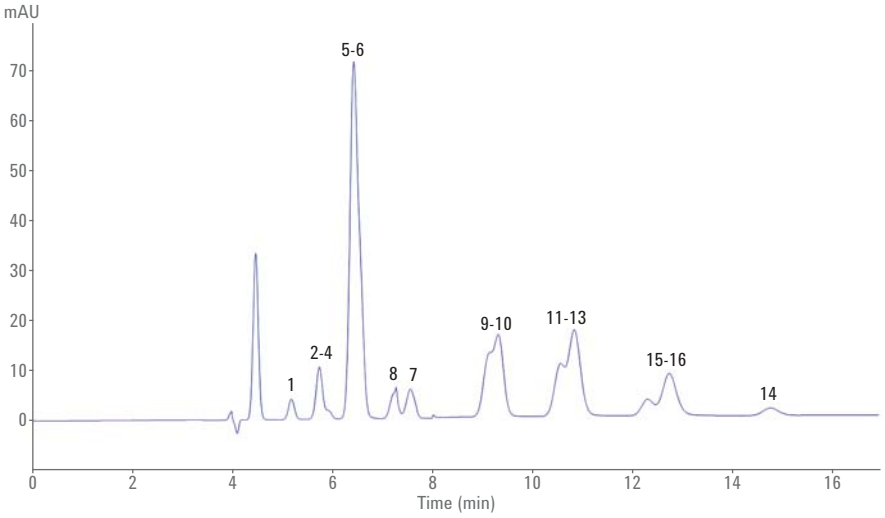

Figure 2. Analysis of a PAH standard mixture (1 ppm in iso-octane/cyclohexane 1/9 v/v, 2 µL injected) with the first dimension normal phase method on the Polaris NH2 column with heptane as mobile phase. Detection: DAD 254 nm.

Table 2. Target PAHs and used MHC and FLD settings (for second-dimension detector).

Normal-phase group	PAH	Compound	FLD excitation wavelength (nm)	FLD emission wavelength (nm)	MHC window (min)
	PAH1	Naphthalene	Not selected		
Group1	PAH2	Acenaphthylene	No FLD	No FLD	5.40–6.80
	PAH3	Acenaphthene	Not detected	Not detected	
	PAH4	Fluorene	255	318	
	PAH5	Phenanthrene	255	370	
	PAH6	Anthracene	255	405	
Group 2	PAH7	Fluoranthene	265	400	6.50–7.90
	PAH8	Pyrene	265	400	
Group 3	PAH9	Benzo(a)anthracene	277	395	8.70–10.10
	PAH10	Chrysene	277	380	
Group 4	PAH11	Benzo(b)fluoranthene	265	440	10.20–11.6
	PAH12	Benzo(k)fluoranthene	265	440	
	PAH13	Benzo(a)pyrene	265	415	
Group 5	PAH15	Benzo(g,h,i)perylene	295	420	11.70–13.10
	PAH16	Indeno(1,2,3-cd)pyrene	295	500	
Group 6	PAH14	Dibenzo(a,h)anthracene	265	400	13.80–15.20

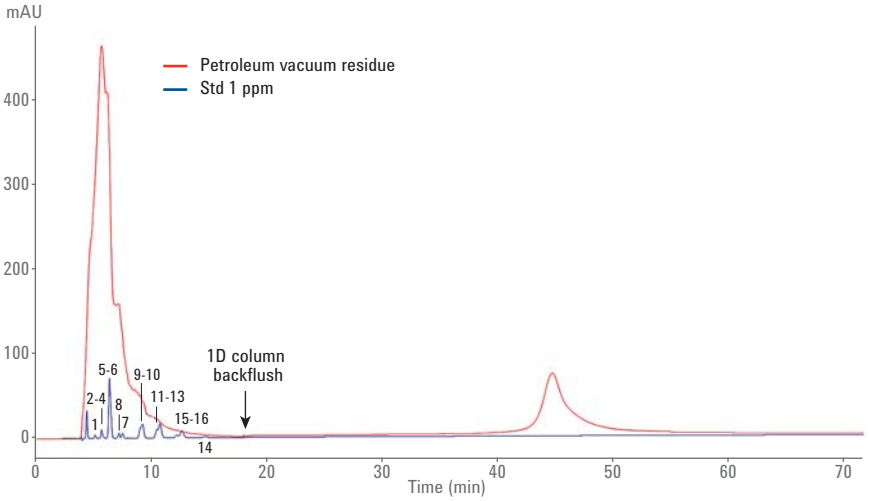

Figure 3. Analysis of the sample and a PAH standard mixture (1 ppm in iso-octane/cyclohexane 1/9 v/v, 2 µL injected) with the first dimension normal phase method on the Polaris NH2 column with heptane as the mobile phase. Detection: DAD 254 nm.

Using only RPLC, it is not possible to accurately measure individual PAHs in the complex vacuum distillation residue. Figure 4 illustrates this, showing the FLD chromatogram obtained for the sample overlaid with the chromatogram of the PAH test mixture. Using RPLC on a dedicated PAH column, excellent separation of the PAH target compounds is obtained, but this separation is useless for the sample, as the fraction of aromatics and polycyclic aromatic hydrocarbons elute as a broad hump between 7 and 20 minutes. No individual target compounds can be measured. Obviously a multidimensional approach is needed here and, therefore, the online combination of NPLC and RPLC was tested. The first-dimension normal-phase analysis then mainly acts as a sample cleanup and fractionation for the second-dimension reversed-phase analysis.

From Figure 2, it can be seen that the target PAHs do not elute within a narrow band, and a partial separation between them is present in the first dimension. To completely transfer certain target analytes, long collection times (several minutes) are required. The consequence is that the volume of the fraction is increased significantly, and the injection of this fraction onto the second dimension leads to decreased chromatographic performance. Moreover, incompatibility between the two mobile phase systems, that is, heptane and water/acetonitrile in the first and second dimensions, respectively, will heavily aggravate this effect.

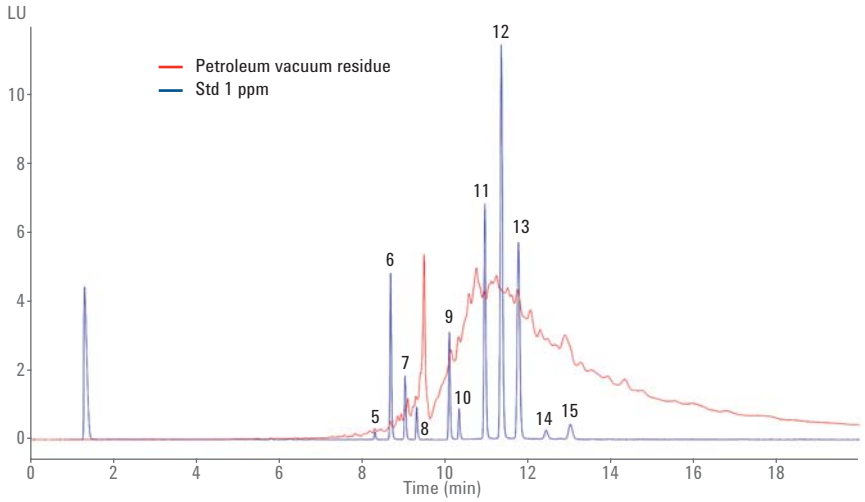

Figure 4. Analysis of the sample and a PAH standard mixture (1 ppm in acetone, 2 µL injected) with a typical PAH method using a water/acetonitrile gradient. This method is different from the second-dimension method in the MHC setup. Flow rate: 1.5 mL/min, gradient: 5 to 100 % acetonitrile in 10 minutes, detection: FLD, excitation/emission 260/440 nm.

The injection of large volumes of water-immiscible solvent onto a hydro-organic mobile phase system can lead to poor chromatographic efficiency and peak distortions. For large volume injections in LC, the sample solvent should be weaker than, and completely miscible with the initial mobile phase composition. In an ideal situation, there would be focusing of the solutes at the column inlet. This is typically observed when large portions of water, for example, are injected on a reversed-phase separation. For the online NPLC-RPLC combination, heptane is used as the first-dimension mobile phase, and thus, as the injection solvent onto the second dimension. This will affect the chromatography of this second dimension, especially if a large volume (wide heart-cut fraction) would be transferred. Therefore, measures must be taken to decrease or even overcome the negative effect. Several research groups have already reported on the applicability of the injection of large volumes of nonmiscible injection solvents[6,7]. This approach was applied here for the analysis of the petrochemical samples. Careful control of the separation conditions in combination with (relatively small) 40-µL fractions enables the efficient transfer of the fractions between the two dimensions.

This is illustrated by the analysis of fluoranthene and pyrene in the vacuum distillate residue sample, as shown in Figure 5. The region where the targets elute in the first dimension (7–8 minutes, see Figure 2) is parked in the MHC loops as four different fractions. Each of these fractions is then sequentially analyzed in the second dimension. After analysis of the first fraction, a blank gradient is performed to clean the flow path. The second-dimension chromatogram clearly shows the complexity of the sample, as each of the heart-cuts results in a densely populated second-dimension chromatogram. The target PAHs elute as sharp peaks in front of the clustered peaks, which are alkylated PAHs with the same number of aromatic rings (methyl-fluoranthenes, methyl-pyrenes, dimethyl-fluoranthenes, and so on). The same approach was used for the different PAH target solutes. These solutes could be detected, as illustrated in Figure 6, showing the overlaid FLD chromatograms for a selected heart-cut from an analysis of a sample and a PAH test mixture. The FLD settings were varied according to the specific PAH targeted.

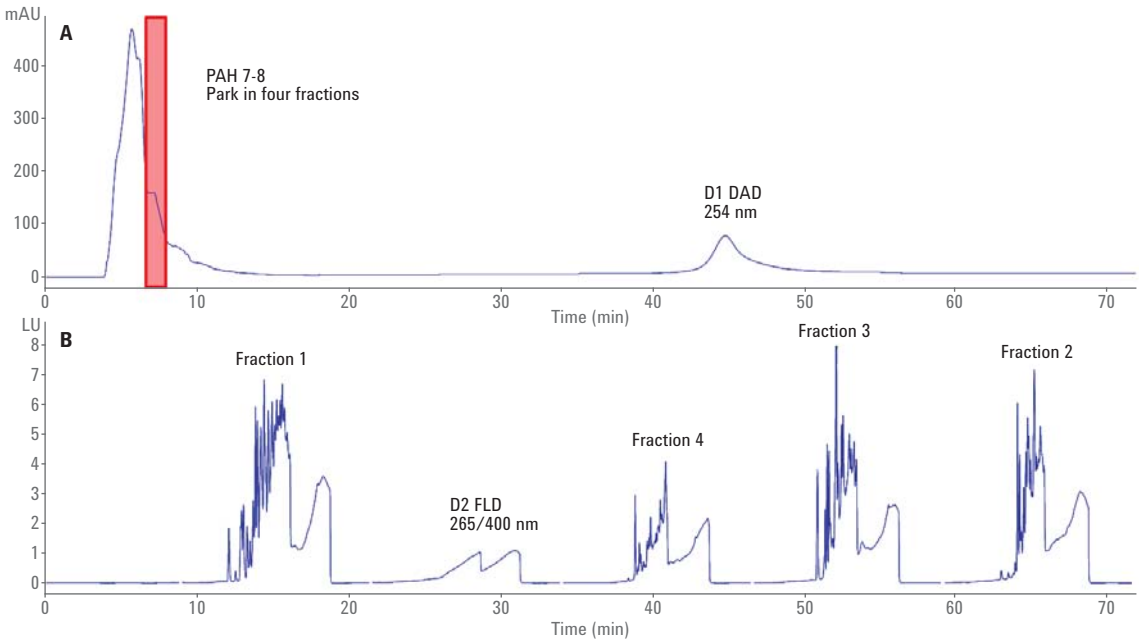

Figure 5. MHC analysis of fluoranthene and pyrene in the oil vacuum distillate sample. Top trace: first dimension separation, detection: DAD 254 nm. Bottom trace: second dimension analyses of the heart-cuts, detection: FLD excitation/emission 265/400 nm.

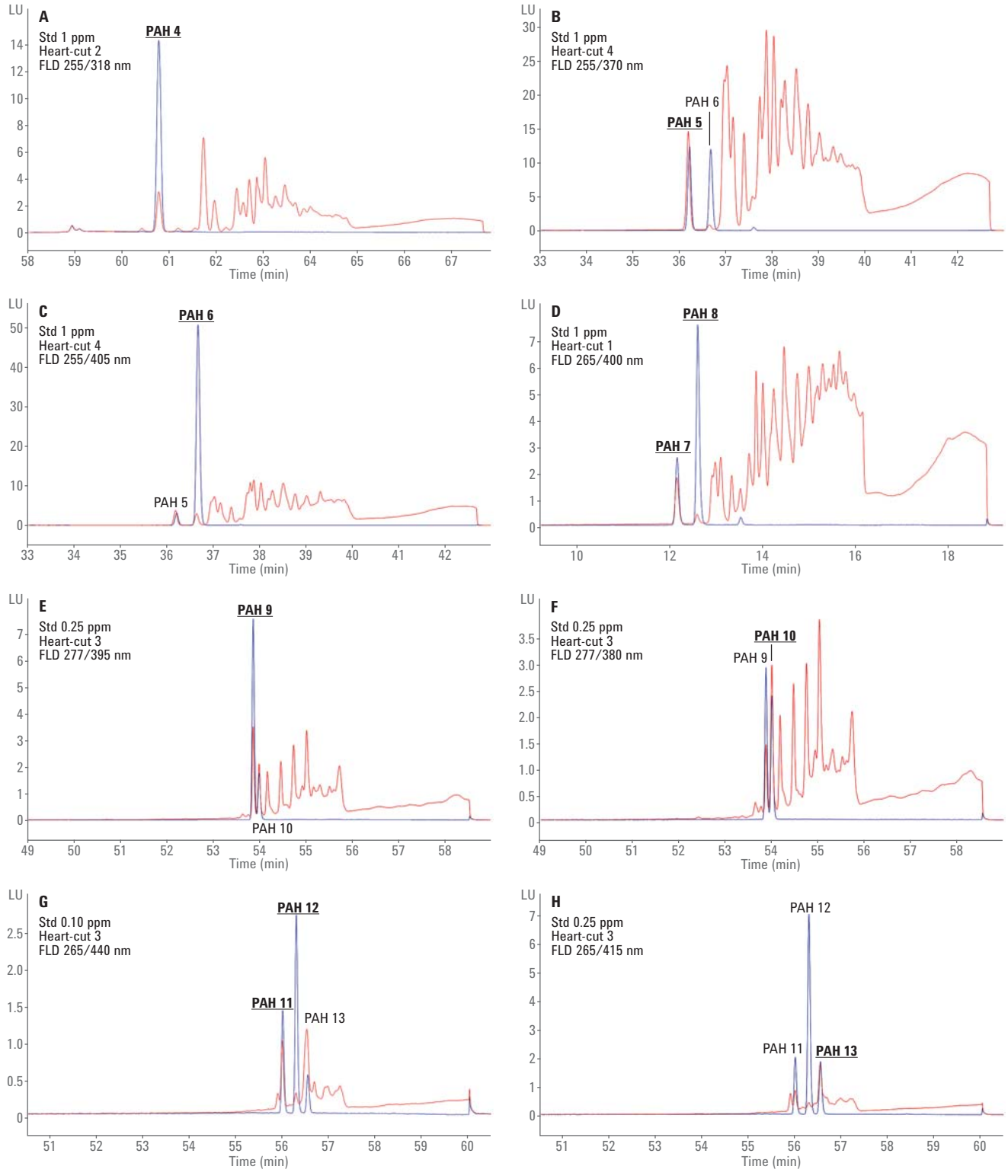

Figure 6. Examples of the second dimension analyses for the selected PAHs (see Table 2). Blue trace = standard solution, Red trace = sample. The FLD wavelengths were optimized for each individual PAH. The relevant PAH is in bold and underlined. PAH 14 was not detected. The heart-cuts can originate from different analyses depending on the PAH of interest, see Table 2. Figure is continued on next page.

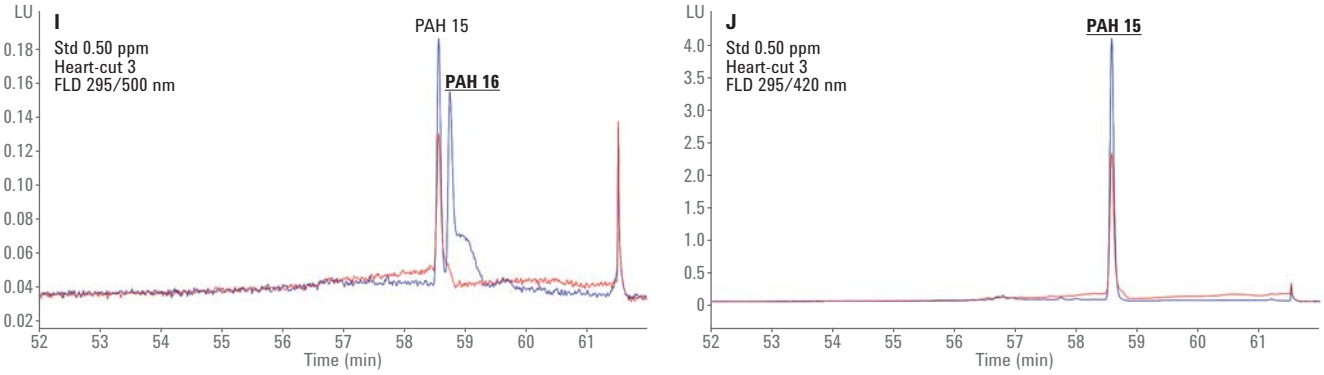

Figure 6. Continued from previous page.

A short method validation was carried out on a selection of PAHs to demonstrate the usefulness of the 2D-LC method (see Table 3). PAHs 9 to 13 were selected as target analytes. The calculations were carried out after summing the areas from the four heart-cuts for each of the PAHs. Standard mixtures with different concentrations (0.05 to 1 ppm) were injected once to determine the linearity. The linearity was excellent, with R^2 values above 0.9999 for all compounds. The calibrations were then used to determine the concentration of these PAHs in the sample extract. A 1-ppm standard solution was injected five times using the method for PAHs 11 to 13, and repeatability of injection was calculated. RSD values were all below 2 %.

Table 3. Validation and quantitative results for the selected PAHs.

PAH	Calibration (R^2)	Area precision (RSD%)	Concentration extract (ppm)
PAH9 Benzo(a)anthracene	1.00000		0.093
PAH10 Chrysene	0.99998		0.272
PAH11 Benzo(b)fluoranthene	0.99999	0.83	0.085
PAH12 Benzo(k)fluoranthene	0.99998	0.90	0.012
PAH13 Benzo(a)pyrene	0.99997	1.37	0.077

All data is based on the area sum of four heart-cuts
Calibration: 0.05, 0.1, 0.25, 0.5, 1 ppm (one injection each)
Repeatability (only PAH 11 to 13): 1 ppm (five consecutive injections)
Concentration extract: Detected concentration in sample

Conclusions

The Agilent 1290 Infinity Multiple Heart-Cutting 2D-LC solution is a valuable tool to determine PAHs in complex petrochemical matrices. A combination of normal-phase in the first dimension and reversed-phase in the second dimension provided orthogonality and resolving power. The addition of a backflush valve enabled removal of retained polar solutes from the first-dimension column. The separation of the selected PAH fractions on the reversed-phase second-dimension column enabled the detection and quantification of selected PAHs. In addition, information on alkyl-PAHs was also obtained. The method was tested for linearity and injection precision, and showed excellent performance.

References

1. More information on PAHs and their distribution in the environment and in bitumen can be found at: https://www.umweltbundesamt.de/sites/default/files/medien/publikation/long/4395.pdf and at http://monographs.iarc.fr/ENG/Monographs/vol103/mono103-001.pdf

2. IP346, Determination of polycyclic aromatics in unused lubricating base oils and asphalthene free petroleum fractions - Dimethyl sulphoxide extraction refractive index method **1992**.

3. European Committee for Standardization, EN16143 Petroleum products – Determination of content of benzo(a)pyrene (BaP) and selected polycyclic aromatic hydrocarbons (PAH) in extender oils – Procedure using double LC cleaning and GC/MS analysis **2013**.

4. Gilgenast, E.; *et al.* Sample preparation procedure for the determination of polycyclic aromatic hydrocarbons in petroleum vacuum residue and bitumen, *Anal. Bioanal. Chem.* **2011**, *401*, 1059–1069.

5. Saravanabhavan, G.; *et al.* A multi-dimensional high performance liquid chromatographic method for fingerprinting polycyclic aromatic hydrocarbons and their alkyl-homologs in the heavy gas oil fraction of Alaskan North Slope crude, *J. Chromatogr. A* **2007**, *1156*, 124–133.

6. Von Mühlen, C., Zini, C.A., Caramão, E.B., Marriott, P.J., Applications of comprehensive two-dimensional gas chromatography to the characterization of petrochemical and related samples, *J. Chromatogr. A* **2006**, *1105*, 39–50.

7. Boursier, L.; *et al.* Complete elution of vacuum gas oil resins by comprehensive high-temperature two-dimensional gas chromatography, *J. Chromatogr. A* **2013**, *1280*, 98–103.

8. Medvedovici, A.; *et al.* Retention phenomena induced by large volume injection of solvents non-miscible with the mobile phase in reversed-phase liquid chromatography, *J. Liq. Chromatogr. Relat. Technol.* **2007**, *30*, 199–213.

9. Loeser, E.; Babiak, S.; Drumm, P. Water-immiscible solvents as diluents in reversed-phase liquid chromatography, *J. Chromatogr. A* **2009**, *1216*, 3409–3412.

www.agilent.com/chem

Published in the USA, February 1, 2016
5991-6549EN

Agilent Technologies