Profiling of Polycyclic Aromatic Hydrocarbons in Crude Oil with the Agilent 1290 Infinity 2D-LC Solution

Application Note

Energy and Chemicals

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Abstract

The Agilent 1290 Infinity 2D-LC Solution was used to profile the polyaromatic hydrocarbon (PAH) fraction from mineral oil using comprehensive two-dimensional liquid chromatography (LCxLC). The complexity of this fraction, consisting of nonsubstituted PAHs, alkyl-substituted PAHs, and heterocyclic PAHs, largely exceeds the peak capacity of a one-dimensional LC separation.

A combination of a cyanopropyl column in the first dimension and a dedicated PAH column in the second dimension provided good orthogonality, resulting in higher peak capacity. Detection was performed by parallel diode-array and fluorescence detection. This Application Note shows the potential of 2D-LC for profiling the polyaromatic fraction of mineral oils.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well-known contaminants in the environment and in food samples. PAHs mostly originate from natural and anthropogenic combustion processes. To date, most analytical methods for the trace-level analysis of PAHs in environmental samples (soil, sediment, water, air) and in food samples (mostly fatty foods), focus on a selected number (typically 16) of nonsubstituted polycyclic aromatic hydrocarbons, such as fluoranthene, chrysene, benzo(a)pyrene, and benzofluoranthenes. Analytical methods are based on GC/MS (including single quadrupole and triple quadrupole MS) or on HPLC in combination with diode-array detection (DAD) or fluorescence detection (FLD).

Carcinogenity of certain PAHs has been unequivocally demonstrated and, similar to polychlorinated dioxins and furans (PCDDs/PCDFs) and polychlorinated biphenyls (PCBs), toxicity equivalent factors (TEF) are used to measure the total contamination of a sample by PAHs.

More recently, concerns have been raised regarding the toxicity of alkyl-substituted PAHs. Indeed, in petroleum products such as diesel, mineral oils, and crude oils, the contribution of substituted PAHs to the total (poly)aromatic fraction is much larger than the contribution of the nonalkylated PAHs that are typically analyzed. This fact is, for instance, recognized by EFSA in their Scientific Opinion on Mineral Oil Hydrocarbons in Food[1]. For the analysis of mineral oil in food and in packaging material, GC-FID methods are used after a preseparation of the saturated hydrocarbons (mineral oil saturated hydrocarbons, MOSH) fraction from the aromatic fraction (mineral oil aromatic hydrocarbons, MOAH) using solid phase extraction or normal phase HPLC.

While measuring the MOSH fraction is well documented, good characterization of the aromatic fraction is still lacking. This is contradictory with the fact that toxicity of the aromatic fraction is substantially higher than that of the saturated hydrocarbon fraction.

The aromatic and polycyclic aromatic fraction contains nonsubstituted two to six-ring PAHs, alkyl-substituted PAHs, heterocyclic PAHs (for example, dibenzothiophene), alkyl-substituted heterocyclic PAHs and, possibly, more polar derivatives such as hydroxy-PAHs, amino-PAHs, and nitro-PAHs. Due to this high complexity, high resolution separation techniques are needed. MOSH and MOAH fractions are commonly analyzed by GCxGC and GCxGC/MS, but comprehensive LCxLC can be considered as an excellent complementary technique, especially since the high molecular weight PAHs (six rings) can be easily analyzed, and selective detection by fluorescence is very sensitive.

The combination of two separation modes, such as a ring number separation with a hydrophobicity separation, can be particularly useful for profiling the aromatic fraction in oils. This Application Note, illustrates the LCxLC approach using the Agilent 1290 Infinity 2D-LC Solution.

Experimental

Samples and sample preparation

The standard solution containing 16 PAHs in acetone/benzene at a concentration of 2 mg/mL each (PAH Mix 25, Dr. Ehrenstorfer, Augsburg, Germany) was diluted to 10 µg/mL in acetone.

From a crude oil sample, the polyaromatic fraction was isolated using a liquid-liquid partitioning between hexane and nitromethane.

A sample of 100 mg of crude oil was dissolved in 5 mL hexane. After dissolution, 5 mL of nitromethane was added and a liquid-liquid extraction was performed. The upper hexane fraction contained the saturated hydrocarbons bulk fraction. The lower nitromethane layer, containing the more polar aromatic fraction, was collected for analysis. For a crude oil sample, the aromatic fraction is typically 5 to 30 % of the total sample[1].

Instrumentation

An Agilent 1290 Infinity 2D-LC Solution with the following configuration was used for the experiments.

- Agilent 1290 Infinity Binary Pump, for first dimension (G4220A)
- Agilent 1290 Infinity Binary Pump, for second dimension (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector with standard flow cell (G4212A)
- Agilent 1260 Infinity Fluorescence Detector (G1321B)
- Agilent 1290 Infinity Valve Drive (G1170A)
- Agilent 1290 Infinity 2-position/4-port duo-valve for 2D-LC (G4236A)

Software

- Agilent OpenLAB CDS ChemStation Edition software, version C.01.07, with 1290 Infinity 2D-LC software, version A.01.02
- GC Image LCxLC Edition software for 2D-LC data analysis (GC Image, LLC., Lincoln, NE, USA)

Method

First dimension	
Column	Agilent ZORBAX SB-CN, 2.1 × 150 mm, 5 µm (p/n 883700-905)
Solvent A	Water
Solvent B	Methanol
Flow rate	100 µL/min
Gradient	40 %B at 0 minutes 100 %B at 80 minutes 100 %B at 85 minutes
Posttime	10 minutes at 40 %B
Column temperature	40 °C
Second dimension	
Column	Agilent ZORBAX RRHD Eclipse PAH, 3.0 × 50 mm, 1.8 µm (p/n 959757-318)
Solvent A	Water
Solvent B	Acetonitrile
Flow rate	2 mL/min
Idle flow rate	0.3 mL/min
Initial gradient	50 to 70 %B from 0 to 0.35 minutes 70 %B from 0.35 to 0.40 minutes 50 %B at 0.41 minutes
Gradient modulation	50 %B at 0 minutes to 100 %B at 70 minutes 70 %B at 0.35 minutes to 100 %B at 55 minutes
Column temperature	40 °C
Modulation	
Modulation on	7 to 85 minutes
Loops	Two 60-µL loops, cocurrent configuration
Modulation time	0.50 minutes
Injection[a]	
Volume	1 µL (injection program, mixed with 1-µL water plug)
Needle wash	5 seconds flush port (methanol/acetone)
Detection DAD[b]	
Wavelength	Signal 220/10 nm
Data rate	80 Hz
Detection FLD[b]	
Wavelength	Multi-emission mode Signal A: Ex 260 nm/Em 350 nm Signal B: Ex 260 nm/Em 430 nm Signal C: Ex 260 nm/Em 500 nm
Data rate	37.04 Hz
PMT Gain	7

[a] The samples were injected together with a water plug to avoid peak broadening/splitting due to the strong injection solvent.

[b] A zero-dead volume T-piece was installed at the outlet of the second dimension column to split the flow between the DAD and FLD. Red 0.12-mm PEEK tubing was used to connect to the detectors. The tubing length from the T-piece to the FLD was twice as long compared to the tubing going to the DAD, resulting in a DAD/FLD split ratio of about 2:1.

Results and Discussion

The LC analysis of PAHs is generally performed using dedicated PAH columns and DAD or FLD detection. Using this column chemistry with water/acetonitrile gradients results in complete separation of the 16 most important nonsubstituted PAHs. When this sample is analyzed on other reversed phase systems (other columns, other mobile phases), some coelution typically does occur, but the majority of analytes are separated. The analyses of PAHs in more complex samples (number of PAHs or complexity of the matrix) are significantly more challenging and require more chromatographic selectivity and separation power and, if possible, better detection selectivity. A comparison of the one-dimensional analysis of the standard mixture of 16 PAHs and the crude oil extract on the first dimension ZORBAX SB-CN column is shown in Figure 1 (chromatographic conditions are different from final LCxLC conditions). It is clear that the complexity of the real sample is far too high for one-dimensional separations. The complexity of the sample originates from the fact that the crude oil contains substituted PAHs next to the nonsubstituted PAHs, and that heterocyclic PAHs are also present. The substituted PAHs are mainly alkylated PAHs, and make up a large group of solutes taking into account variations in substitution degree, alkyl chain length, branching, and substitute position. All these result in extreme complexity and no chromatographic technique is available that would achieve full resolution of all possible individual compounds. For the characterization of the polyaromatic fraction, it is important to obtain information on the number of aromatic rings, relative substitution degree, and on the possible presence of more polar PAHs.

On the selected first dimension SB-CN column, an interesting separation is obtained. The elution order for the 16 PAHs under the applied conditions differs significantly from the normal elution order in a classical RP-LC PAH analysis (as in Table 1). This opens perspectives for orthogonality in the 2D-LC setup.

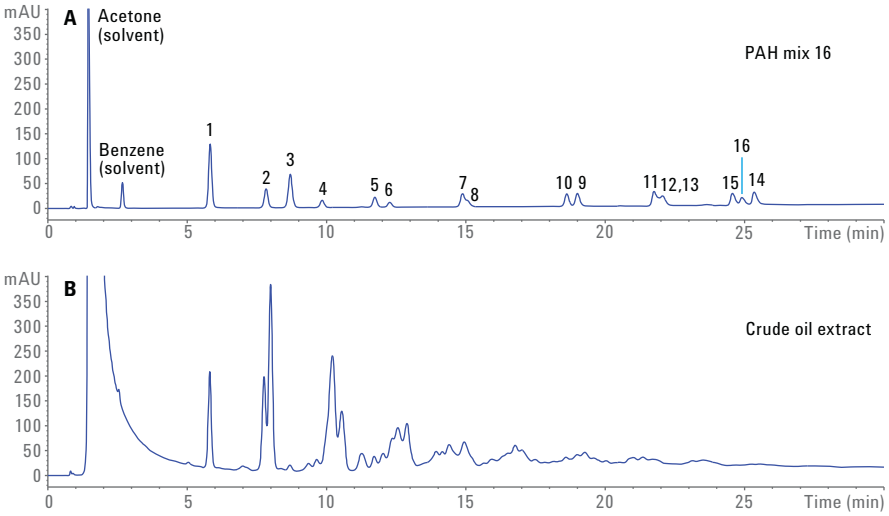

Figure 1. Comparison of a one-dimensional analysis of the PAH standard mix and sample extract. Column: Agilent ZORBAX SB-CN, 2.1 × 150 mm, 5 µm, Flow rate: 0.3 mL/min, Gradient: 40–100 % methanol in water from 0–40 minutes. Peak identities: see Table 1.

Table 1. Peak identities of the 16 PAHs (peak numbers are assigned according to the expected elution order on a PAH column).

Peak no.	Compound
1	Naphthalene
2	Acenaphthylene
3	Acenaphthene
4	Fluorene
5	Phenantrene
6	Anthracene
7	Fluoranthene
8	Pyrene
9	Benzo(a)anthracene
10	Chrysene
11	Benzo(b)fluoranthene
12	Benzo(k)fluoranthene
13	Benzo(a)pyrene
14	Dibenzo(ah)anthracene
15	Benzo(ghi)perylene
16	Indeno(1,2,3-cd)pyrene

Adapted chromatographic conditions and switching to UHPLC equipment could improve the resolution for the crude oil extract, but to drastically increase peak capacity, comprehensive 2D-LC will be more effective. A combination was made of the ZORBAX SB-CN column using a water/methanol mobile phase in the first dimension, with a classic PAH analysis setup in the second dimension (Eclipse PAH column with a water/acetonitrile mobile phase). On the dedicated PAH column, better separation was obtained within a group with equal ring number (for example, benzofluoranthene isomers).

The LCxLC contour plots obtained with DAD using the SB-CN/Eclipse PAH combination is shown in Figure 2B, and can be compared to Figure 2A, showing the 1D separation on the SB-CN column. Better separation was obtained for phenanthrene/anthracene, benzo(a)anthracene/chrysene, and benzofluoranthenes. Only benzo(ghi) perylene and indeno(1,2,3-cd)pyrene were still not completely separated. Next, the aromatic fraction of the mineral oil was analyzed using the same conditions. The LCxLC contour plot is shown in Figure 2C.

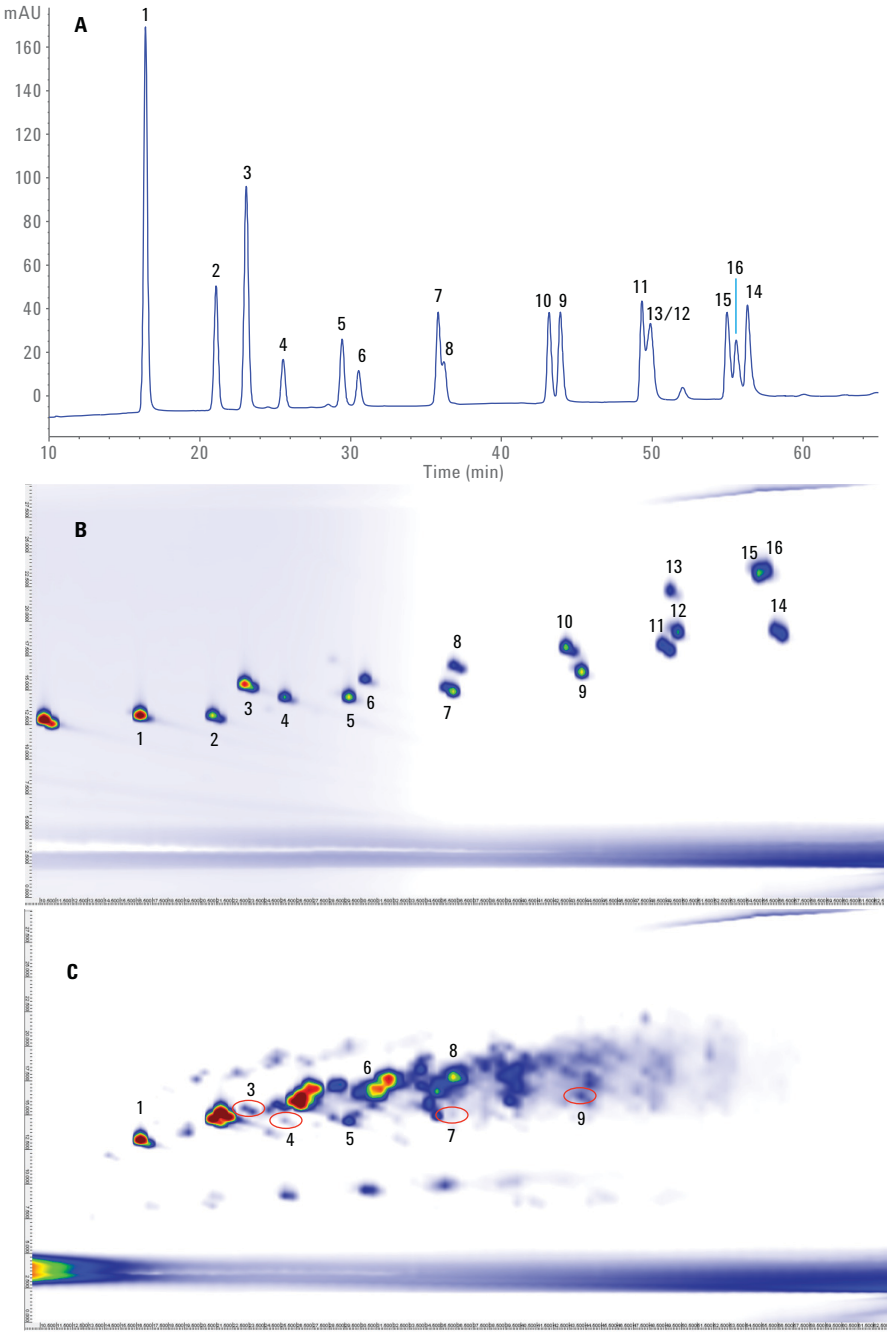

Figure 2. Comparison of a 1D-LC run of the standard mix (A), LCxLC run of the standard mix (B), and LCxLC run of the sample extract (C). Signal: DAD, 220 nm.

Figure 3, Figure 4, and Figure 5 show the LCxLC results from FLD using different emission wavelengths. Some additional series of compounds (probably substituted PAHs) are clearly detected. As an example, there is a series of compounds that elutes prior to the nonsubstituted PAHs on the second dimension column. This is clearly visible with FLD. There is also a considerable number of compounds that are more or less scattered around the PAHs present in the standard mix. Identification of the additional compounds in the mineral oil extract will require further investigation with, for example, hyphenation to MS using atmospheric pressure photoionization (APPI), but from their relative elution pattern it can be predicted that these are alkyl-substituted PAHs. These results clearly illustrate the high complexity of the PAH fraction of crude oil.

Although many compounds remain unidentified, the results clearly demonstrate the potential of the Agilent 1290 Infinity 2D-LC Solution for this type of analysis.

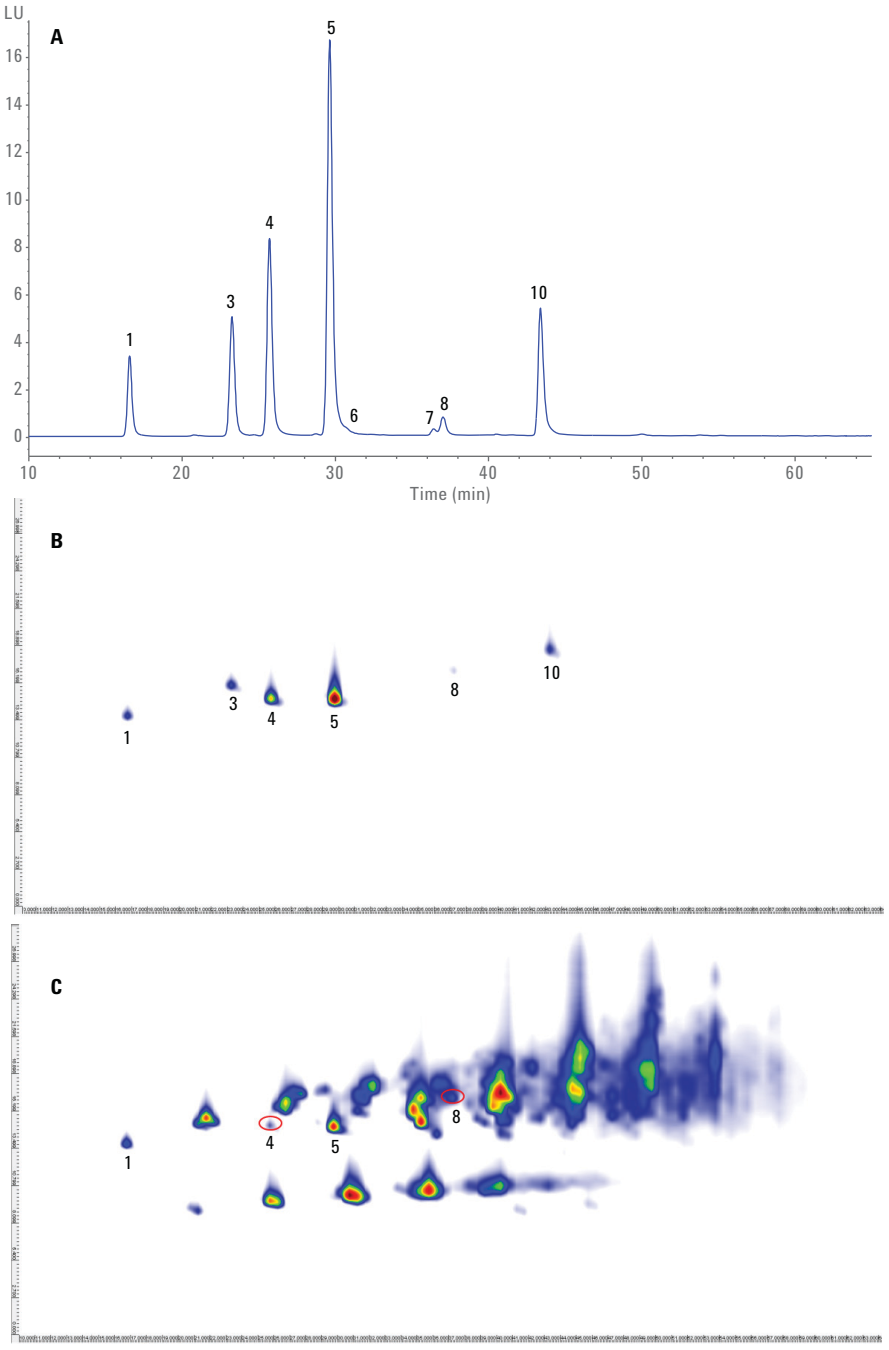

Figure 3. Comparison of a 1D-LC run of the standard mix (A), LCxLC run of the standard mix (B), and LCxLC run of the sample extract (C). Signal: FLD, Ex 260 nm/Em 350 nm.

Conclusion

This Application Note shows the potential of the Agilent 1290 Infinity 2D-LC Solution for profiling PAHs in the aromatic fraction of mineral oils using parallel diode-array and fluorescence detection. The method is useful for profiling PAHs in crude oils, bitumen, and other mineral oils. The combination of the presented 2D-LC method with MS using APPI ionization should facilitate further structure elucidation of the detected PAH compounds.

Reference

1. EFSA Journal 2012; 10(6):2704, European Food Safety Authority http://www.efsa.europa.eu/fr/search/doc/2704.pdf

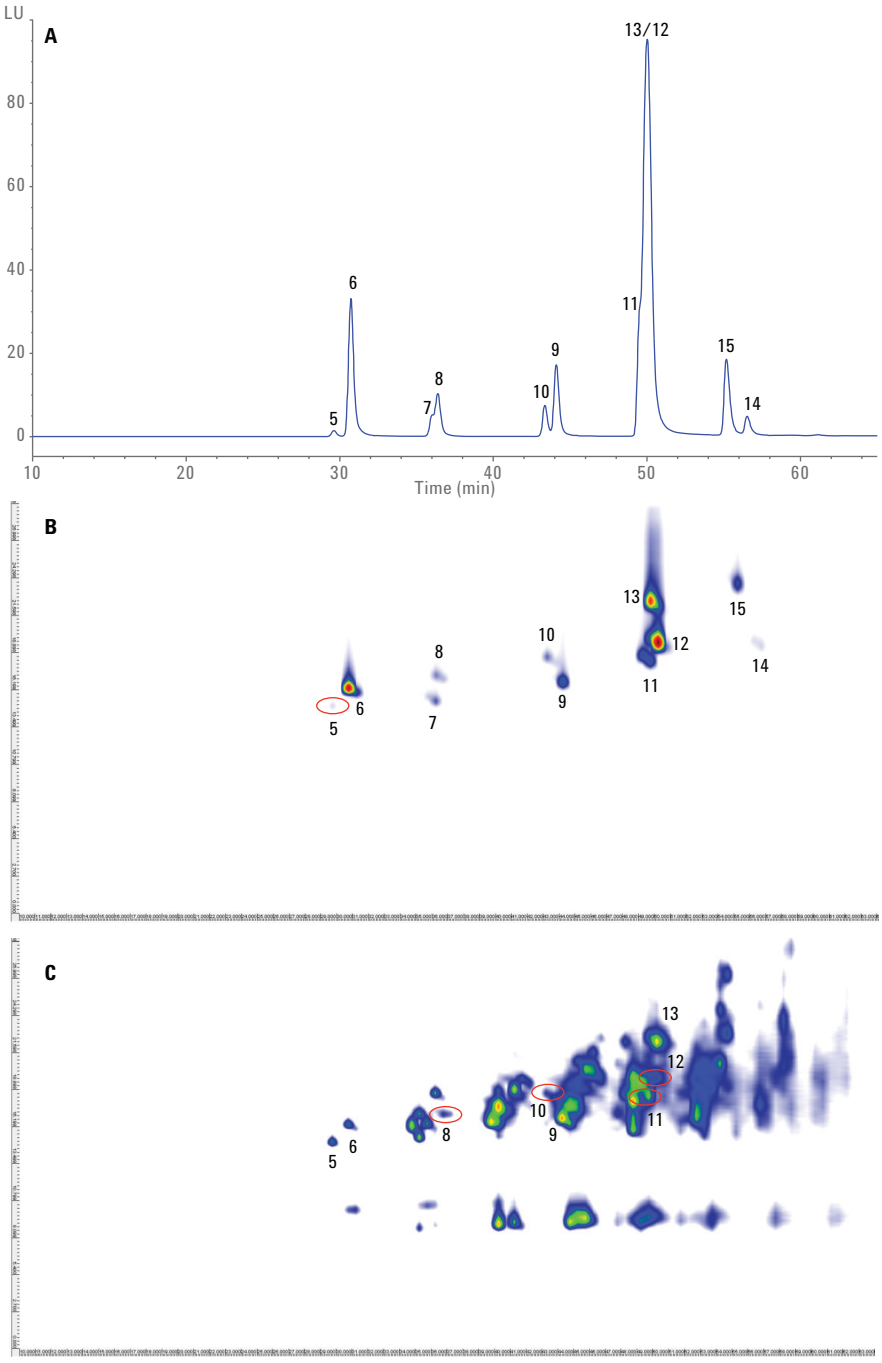

Figure 4. Comparison of a 1D-LC run of the standard mix (A), LCxLC run of the standard mix (B), and LCxLC run of the sample extract (C). Signal: FLD, Ex 260 nm/Em 430 nm.

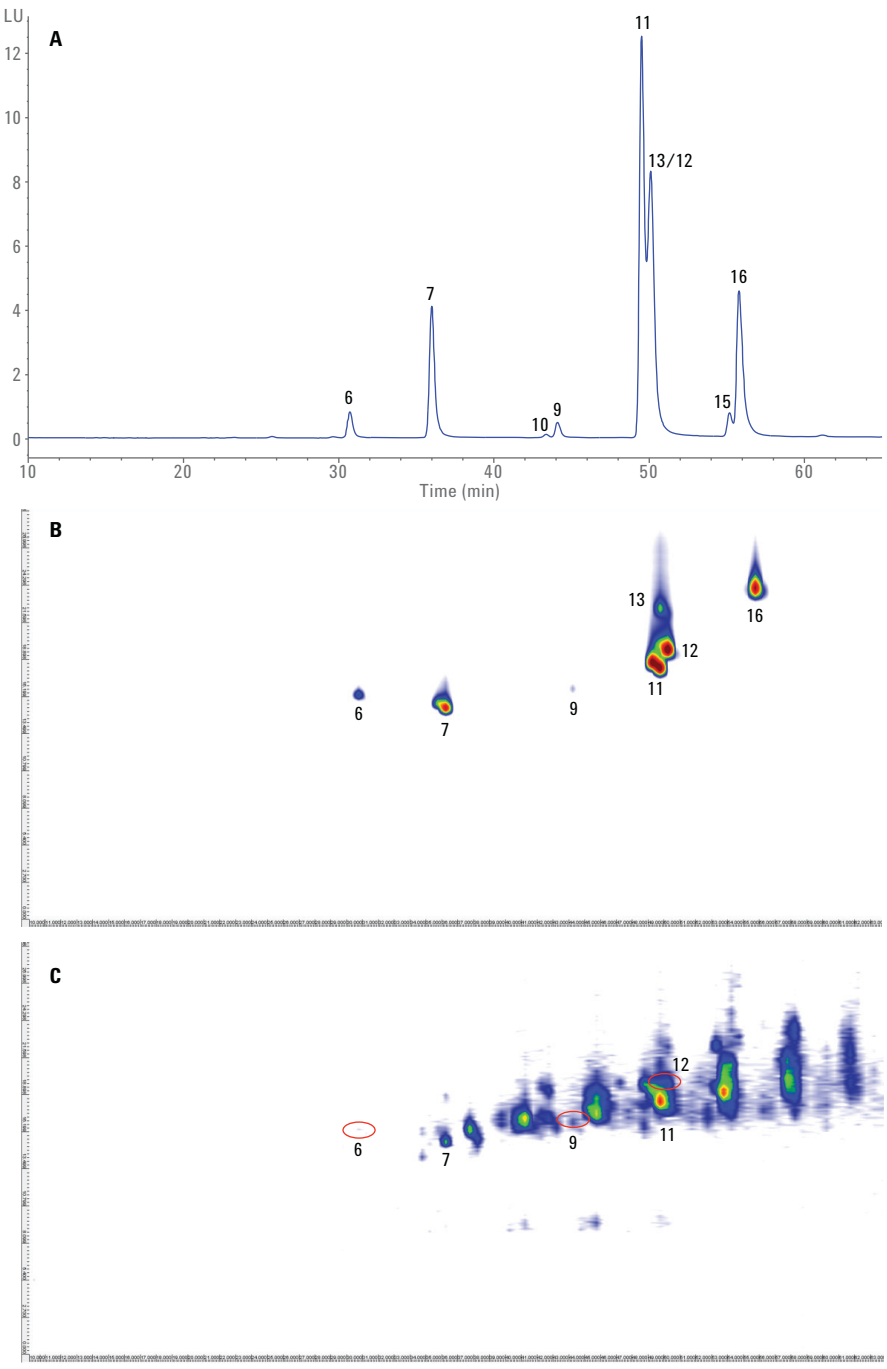

Figure 5. Comparison of a 1D-LC run of the standard mix (A), LCxLC run of the standard mix (B), and LCxLC run of the sample extract (C). Signal: FLD, Ex 260 nm/Em 500 nm.

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