

# Liquid chromatography–atmospheric pressure laser ionization–mass spectrometry (LC-APLI-MS) analysis of polycyclic aromatic hydrocarbons with 6–8 rings in the environment

Jan B. Thiäner<sup>1</sup> · Christine Achten<sup>1</sup>

Received: 19 July 2016 / Revised: 18 November 2016 / Accepted: 28 November 2016 / Published online: 22 December 2016  
© Springer-Verlag Berlin Heidelberg 2016

**Abstract** A method has been developed for the sensitive and rapid analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples using liquid chromatography time-of-flight mass spectrometry as well as the selective atmospheric pressure laser ionization (APLI) process (LC-APLI-MS). Upon analyzing 34 PAHs, the limits of detection of this method were found to range from 0.008 to 1.824 pg (0.024 pg for benzo[*a*]pyrene). The method therefore provides 30-fold to 5,400-fold increased sensitivity compared with the established GC-MS technique. This LC-APLI-MS method was optimized for higher molecular weight PAHs (C<sub>24</sub>–C<sub>30</sub> PAHs with 6–8 rings), which are difficult to detect or cannot be detected by GC-MS. Using the LC-APLI-MS method, various 6- to 8-ring PAHs were detected in environmental samples for the first time. After developing the method, it was successfully validated in ruggedness tests. The concentrations determined by the LC-APLI-MS method were in good accord with the certified concentrations in three certified reference materials (contaminated soils and sediments). Upon applying the method to environmental samples, it was found that (1) the presence of dibenzo[*a,i*]pyrene and dibenzo[*a,h*]pyrene in urban soil samples could only be detected using LC-APLI-MS (i.e., not GC-MS) due to its high sensitivity, (2) a bituminous coal sample yielded 211 tentative peaks from aromatic compounds in the C<sub>24</sub>–C<sub>30</sub> range, and (3) eleven of those compounds occurred

in different environmental samples in similar patterns. Hence, 6- to 8-ring PAHs occur in solid environmental samples in which other 6-ring PAHs such as indeno[1,2,3-*cd*]pyrene or benzo[*ghi*]perylene may also be present. Some of these numerous higher molecular weight PAH compounds could have very high carcinogenic potential, which will need to be elucidated to ensure the reliability of PAH risk assessments.

**Keywords** Atmospheric pressure laser ionization · APLI · Polycyclic aromatic hydrocarbons · PAH · Method development · Environmental analysis · Matrix effect

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed in three different processes: (i) during anthropogenic or natural combustion processes such as the generation of domestic, agricultural, and industrial emissions and tobacco smoke, during waste combustion, as well as in wildland fires and volcanic eruptions (i.e., pyrogenic processes); (ii) during the diagenesis of coals or oils, caused by the effects of increased temperature and pressure on organic matter (i.e., petrogenic processes); and (iii) in plants and by microorganisms (i.e., biogenic processes) [1–4]. These sources may overlap, e.g., in low-rank coals (biogenic and petrogenic) or soils and sediments (petrogenic and pyrogenic). PAHs are ubiquitous and occur in soot, atmospheric particulate matter, crude oils, coals of different ranks, coal tars, soils (e.g., at manufactured gas plant sites, and at both urban and rural sites), sediments (e.g., from harbors, rivers, and lakes), sewage sludges, human and animal tissues, sea and glacier ice, and meteorites, among other substances [4–8].

PAHs are mutagenic at low concentrations [9]. Indeed, the dibenzopyrenes (five compounds, each with 6 rings) show

**Electronic supplementary material** The online version of this article (doi:10.1007/s00216-016-0121-9) contains supplementary material, which is available to authorized users.

✉ Christine Achten  
achten@uni-muenster.de

<sup>1</sup> Institute of Geology and Palaeontology—Applied Geology, University of Münster, Corrensstraße 24, 48149 Münster, Germany

very high carcinogenic potential [10]. Also, PAHs show a high diversity in terms of their structures (as indicated by the 211 compounds tentatively detected in the bituminous coal sample studied in the present work). It seems likely that some other higher molecular weight PAHs (HMW-PAHs, defined in this work as  $C_{24}$ – $C_{30}$  PAHs with 6–8 aromatic rings and  $m/z \geq 300$ ) may also be highly carcinogenic and occur in the environment. However, this is difficult to prove due to the lack of an appropriate analytical method to detect and quantify these very hydrophobic compounds.

PAHs are a group of up to a thousand different compounds, many of which are detected in the environment [11]. However, the only HMW-PAHs present in the 16 US EPA PAH list (i.e., a list of the 16 most important PAHs according to the US EPA) are indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene, and dibenzo[*a,h*]anthracene, and HMW-PAHs are only included in a limited number of reference materials. Thus, more research into their toxicity and occurrence in the environment is needed [12].

The established analytical method for PAHs is gas chromatography coupled with mass spectrometry (GC-MS). It is possible to achieve a limit of detection (LOD, signal to noise >3) of 10 pg (6 pg for benzo[*a*]pyrene) using GC-MS [13].

Due to the low volatility of HMW-PAHs, these compounds are difficult to transfer to the gas phase, so GC-MS is actually not particularly well suited to their analysis. Nevertheless, there are studies demonstrating the separation of HMW-PAHs using GC-MS with sophisticated preparation techniques such as sample fractionation and hyphenated liquid chromatography [14, 15]. Separation by liquid chromatography (LC) is possible and can be combined with MS, UV, or fluorescence detection, although the sensitivity of UV detection is too low to permit the detection of very small amounts of 6- to 8-ring PAHs. Atmospheric pressure ion sources are attracting increasing attention from researchers because they do not usually require sample cleaning steps. Different types of atmospheric pressure ion sources can be used to ionize PAHs: atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and atmospheric pressure laser ionization (APLI). These types of ion sources make use of different physicochemical effects, so there are differences between them in terms of the ions generated from PAHs. APCI is used to ionize small and semipolar molecules; an LOD of 275 pg for benzo[*a*]pyrene can be achieved using LC-APCI-MS [16, 17]. An improvement in PAH ionization efficiency was achieved with the development of the photo ion source [18]. This ion source was introduced by Robb et al. in 2000 [17], and uses a VUV lamp with  $h\nu = 10$  eV. The ionization potential (IP) is therefore below the IPs of the solvents usually used for liquid chromatography (IPs: water, 12.6 eV; methanol, 10.8 eV; acetonitrile, 12.2 eV) [19]. A dopant (toluene, chloro- or bromobenzene, or acetone) can also be supplied. The dopant is simultaneously ionized and

its charge is transferred to the analytes, leading to a two- to threefold increase in ion production [20] and an LOD of 2.6 pg for benzo[*a*]pyrene [21].

APLI has been demonstrated to give excellent performance in the analysis of PAHs [22]. It provides enhanced sensitivity for two reasons: (1) the very high linear and two-photon cross-sections of the target analytes lead to saturation of the excitation and ionization transitions, even at moderate power densities, and (2) none of the LC solvents absorb the 248-nm laser radiation that penetrates the ion source. We previously reported that GC-APLI-TOFMS provided an LOD of 0.025 pg for benzo[*a*]pyrene [13]. Typical photon sources for laser ionization are Nd:YAG lasers ( $\lambda = 266$  nm, 4.6 eV) or excimer lasers with a repetition rate of up to 500 Hz (ArF:  $\lambda = 193$  nm, 6.3 eV; KrF:  $\lambda = 248$  nm, 5.0 eV; XeCl:  $\lambda = 302$  nm, 4.1 eV) [22]. These photon sources provide photons with energies that are much lower than the IPs of most molecules. As a consequence, the IP can only be reached through a two-step photon-energy accumulation process. In the case of the most favorable (1 + 1) resonance enhanced multiphoton ionization process (REMPI), the first photon ( $h\nu$ ) is absorbed by molecule M, leading to an excited electronic state  $M^*$  (Eq. 1). If the resonance conditions for the first absorption step are fulfilled and selective excitation occurs, a second photon can then be absorbed, producing a radical cation  $M^+$  (Eq. 2) [23]:



PAH selectivity is based on the large lifetimes of the resonant intermediate states that frequently occur in  $\pi$ -electron-rich resonance systems such as PAHs.

An APLI source for LC-MS was introduced by Constapel et al. in 2005 [20], and the first applications of LC-APLI in the field of crude oil characterization followed [24].

Almost nothing is known about the presence and toxicity of 6- to 8-ring PAHs in the environment, although the most toxic PAHs known are derivatives of 6-ring PAHs (dibenzopyrenes), and the probability of bay or fjord regions being present in the PAH increases with ring number. One obstacle is undoubtedly the lack of available reference standards for these numerous compounds. The aim of this study was therefore to begin to develop a method of determining 6- to 8-ring PAHs in environmental samples using LC-APLI-MS (currently still without reference standards except the compounds mentioned above). After developing the method, which included performing ruggedness tests and verifying the method via certified reference materials (CRM) for available reference standard compounds, matrix effects were investigated using a clean-up step. Finally, the method was applied to environmental samples.

## Experimental section

### Chemicals and standards

The solvents acetonitrile (ACN), *n*-hexane, dichloromethane (DCM), 2-propanol, methanol (MeOH), toluene (all Rotisolv GC or LC Ultra grade, purity  $\geq 99.8\%$ ; Carl Roth, Karlsruhe, Germany), acetonitrile (LC MS grade, purity  $\geq 99.9\%$ ; VWR, Radnor, PA, USA), and MilliQ water (Q-Pod with Millipak Polisher 40, 0.22- $\mu\text{m}$  membrane filter; Millipore, Darmstadt, Germany) were used.

A custom mixture (Chiron, Trondheim, Norway) of 34 PAHs in toluene contained (1) phenanthrene, (2) anthracene, (3) 9-methylphenanthrene, (4) 9-methylanthracene, (5) fluoranthene, (6) pyrene, (7) 9,10-dimethylanthracene, (8) 1-methylpyrene, (9) 7H-benzo[*c*]fluorene, (10) benzo[*ghi*]fluoranthene, (11) benzo[*c*]phenanthrene, (12) chrysene, (13) benzo[*a*]anthracene, (14) 5-methylchrysene, (15) 6-methylbenzo[*a*]anthracene, (16) 7,12-dimethylbenzo[*a*]anthracene, (17) perylene, (18) benzo[*b*]fluoranthene, (19) benzo[*j*]fluoranthene, (20) benzo[*e*]fluoranthene, (21) benzo[*k*]fluoranthene, (22) benzo[*a*]pyrene, (23) 6-methylbenzo[*a*]pyrene, (24) 3-methylcholanthrene, (25) dibenzo[*a,h*]anthracene, (26) indeno[1,2,3-*cd*]pyrene, (27) benzo[*ghi*]perylene, (28) anthanthrene, (30) dibenzo[*a,l*]pyrene, (31) dibenzo[*a,e*]fluoranthene, (32) dibenzo[*a,e*]pyrene, (33) dibenzo[*a,i*]pyrene, and (34) dibenzo[*a,h*]pyrene. Coronene (29) was purchased as solid material (Sigma-Aldrich, Schnellendorf, Germany), dissolved in ACN, and added to the custom PAH mixture. For quantification, a mixture of the 16 US-EPA PAHs (deuterated; Chiron) was used. Sodium sulfate (AnalaR, Normapur) was purchased from VWR.

### Certified reference materials and sample matrices

Three certified reference materials (CRM) and three different sample matrices were chosen for use in the analysis of known PAHs:

- (1) CRM of loamy, sandy soil from a former gasworks site in Berlin, Germany (BAM ERM-CC013a)
- (2) CRM of freshwater harbor sediment from the Netherlands (IRMM BCR-535)
- (3) CRM of marine sediment collected from Chesapeake Bay at the mouth of Baltimore Harbor, MD, USA (NIST 1941b)
- (4) Bituminous coal sample from Midland Valley Basin, near Edinburgh, UK [25]
- (5) Urban soil sample 1 (a mixture of coal ash, building rubble, limestone, marl, and wood) from the Ruhr region, Germany

- (6) Urban soil sample 2 (a mixture of waste rock, demolition waste, slag, and limestone) from the Ruhr region, Germany
- (7) Sediment sample from Lake Aasee in the center of Münster, Germany

A bituminous coal sample was chosen because PAHs are formed during coal diagenesis and bituminous coal is commonly characterized by 2–6 PAHs linked by methylene bridges. The number of aromatic units in the PAHs increases from three to four condensed rings in low-rank coals to about 30 fused rings in anthracite (high-rank coal) [4]. Hence, we would expect a bituminous coal to contain a broad range of  $\text{C}_{24}$ – $\text{C}_{30}$  PAHs. The bituminous coal sample used here was analyzed in a former study [25] and was found to include numerous PAH compounds.

### Extraction

In this study, all solid samples were extracted with pressurized solvent extraction [26] because this technique is fast, has a high extraction efficiency, requires less solvent than other techniques, and can be executed automatically.

The extractor (Speedextractor E-916; BÜCHI, Flawil, Switzerland) was flushed with DCM and toluene, as were the extraction cells and collection bottles. Additionally, the extraction cells were baked out at 300 °C and the metal frits and screws at 150 °C for at least 12 h before use. Forty-milliliter extraction cells were prepared using glass-fiber filters (glass microfiber filters, grade 698, retention 0.7  $\mu\text{m}$ , size 55 mm; VWR) and quartz sand (fat-free; BÜCHI). The cells were filled with 0.50 g of an urban soil sample, 30.00 g of the coal sample, or 1.00 g of a CRM sample (each sample was examined in triplicate). All samples were extracted with DCM using two cycles at 110 bar and 120 °C, and adjusted to a defined volume using a rotary evaporator. Toluene was used for GC and ACN for LC analysis.

To determine recovery rates, the procedure was applied to three extraction cells containing quartz sand spiked with the PAH mixture. All concentrations were corrected for these recovery rates. Procedures were performed using gastight syringes (Hamilton, Bonaduz, Switzerland) and 1.5-ml brown glass vials (Macherey-Nagel, Düren, Germany).

### Clean-up

An adapted SARA fractionation [25] was performed as clean-up procedure. First, 250  $\mu\text{l}$  of the sample extract were dripped onto silica gel, with the solvent evaporating at room temperature. For fractionation, a 5-ml glass pipette filled with glass wool and a 17-cm silica gel column were used. After adding 1 cm of sodium sulfate on top of the silica gel, the column was soaked with *n*-hexane. The dry silica gel loaded with the

sample was added and covered with quartz sand. The sample was then fractionated into three fractions using 5 ml of *n*-hexane, DCM, and MeOH.

### Analytical setup

The analytical setup consisted of a liquid chromatograph, an APLI source, and a time-of-flight mass spectrometer. The liquid chromatograph (Prominence<sub>XR</sub> UFLC; Shimadzu, Duisburg, Germany) was equipped with a Nucleodur C18 column (length: 100 mm, ID: 2 mm, particle size: 1.8 μm; Macherey-Nagel). The chromatographic separation was performed at 25 °C with a flow rate of 300 μl/min in gradient mode using MeOH (eluent A) and ACN (eluent B). Initially, 0% eluent B was changed to 100% within the first 4.5 min. Isocratic conditions with 100% eluent B were then maintained for 25 min, followed by 5 min of equilibration time. A volume of 15 μl was injected.

The APLI source (iGenTrax, Haan, Germany) was equipped with a pneumatically assisted sprayer (Bruker Daltonik, Bremen, Germany) and coupled to an excimer laser (ATL Lasertechnik, Wermelskirchen, Germany), which was operated at a repetition rate of 100 Hz and a pulse energy of 5 mJ at  $\lambda = 248$  nm (KrF\* emission, 5 eV). The laser beam was unfocused and directed vertically under the entrance of the APCI sprayer interface. The sprayer interface was heated to 450 °C and the nebulizer gas pressure was set to 3.6 bar.

PAHs were detected with a time-of-flight mass spectrometer (Maxis 3G UHR QTOF MS; Bruker Daltonik) in full-scan mode for positive ion polarity with the following settings: end plate offset -600 V, capillary 1,300 V, dry gas flow 6.0 l/min, dry gas temperature 260 °C, prepulse storage 30 μs, transfer time 5 μs, ion cooler 60 V<sub>pp</sub>, sampling rate 0.5 Hz. The mass spectrometer obtained nitrogen (99.5% purity) from a nitrogen generator (NGM 22; CMC Instruments, Eschborn, Germany). All MS settings as well as settings for the sprayer interface were adjusted to high mass resolution and high peak intensity for the relevant mass range of 128–424 Da.

GC-MS was performed using a GCMS-QP-2010Plus (Shimadzu, Duisburg, Germany) equipped with an Optima 5 MS Accent column (length: 60 m, ID: 0.25 mm, film thickness: 0.25 μm; Macherey-Nagel). Helium (99.999% purity; Westfalen AG, Münster, Germany) served as carrier gas, and a temperature program starting at 80 °C and rising to 320 °C at a rate of 4 °C/min was applied. This final temperature was held for 40 min. The injector temperature was 320 °C and splitless high-pressure injection (350 kPa for 1 min at a linear velocity of 31.9 cm/s) was used. A Combi PAL autosampler (Shimadzu) equipped with a 10-μl syringe (Hamilton) injected 1 μl for each acquisition.

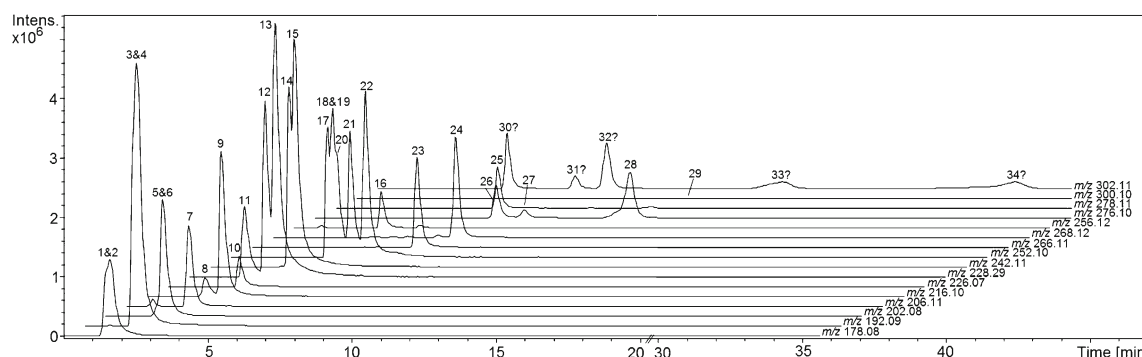
## Results and discussion

### Analysis of the PAH standard mixture and calibration

Initially, the mixture of 34 PAHs, including some commercially available 6-ring PAHs, was used for LC-APLI-MS method development. Method development focused on the detection of HMW-PAHs. The resulting extracted ion chromatograms are shown in Fig. 1. HMW-PAHs were detected and separated with high chromatographic resolution (in contrast to lower molecular weight PAHs, which were not of interest here).

Phenanthrene (1) and anthracene (2); 9-methylphenanthrene (3) and 9-methylanthracene (4); as well as benzo[*b*]fluoranthene (18), benzo[*j*]fluoranthene (19), and benzo[*e*]fluoranthene (20) were evaluated as summed concentrations.

Using the PAH mixture, calibration curves corrected with the internal standard were obtained for the range 0.1–100 pg/μl for LC-APLI-MS. The calibration range was covered by 13 calibration steps, where each calibration step was measured three times. This meant that each calibration fit almost always had  $r^2 > 0.99$  (the fit for benzo[*c*]fluorene had  $r^2 > 0.98$ ).



**Fig. 1** Chromatogram of the 34 PAH standard mixture analyzed with the LC-APLI-MS method after development at a concentration of 50 ng/ml

### Limits of detection

The limits of detection (LODs) ranged from 0.008 pg for 9-methylphenanthrene and 9-methylanthracene to 1.842 pg for coronene (Table 1). The LOD for benzo[*a*]pyrene was 0.024 pg. Using LC-APLI-MS, all compounds were detected at higher sensitivities than achieved with GC-MS. The increases in sensitivity ranged from 30-fold to 5,400-fold, with an average of about 1,000-fold. In a previous study, the coupling of GC to APLI yielded comparable sensitivities to our LC-APLI-MS method [13], indicating that the main reason for

the increase in sensitivity compared to GC-MS is the ionization process used rather than the chromatographic system. Nevertheless, the application of eluents with greater elution strength may produce sharper peaks with higher amplitudes which may lower the LODs in LC-APLI-MS.

### Method ruggedness, continuity test, and blanks

The ruggedness of the method was tested by slightly varying the values of critical method parameters. If these variations did not result in any notable deviations (i.e., if the error was <5%),

**Table 1** Limits of detection (LOD, signal to noise >3) for the 34 PAH mixture when analyzed by LC-APLI-MS and GC-MS. The standard mix was chosen due to the lack of commercially available 6- to 8-ring PAH standards. The calibration fit was based on 13 calibration levels, each measured in triplicate

No(s).	Compound(s)	LOD GC-MS (pg) [13]	LOD LC-APLI-MS (pg)	Fold increase in sensitivity compared to GC-MS (i.e., LOD GC-MS / LOD LC-APLI-MS)	Calibration fit $R^2$
1, 2	Phenanthrene + anthracene	5 + 9	0.030	464	0.9998
3, 4	9-Methylphenanthrene + 9-methylanthracene	1 + 5	0.008	800	0.9939
5, 6	Fluoranthene + pyrene	8 + 3	0.016	704	0.9915
7	9,10-Dimethylanthracene	9	0.023	385	0.9989
8	1-Methylpyrene	29	0.082	365	0.9961
9	7 <i>H</i> -Benzo[ <i>c</i> ]fluorene	32	0.012	2,673	0.9806
10	Benzo[ <i>ghi</i> ]fluoranthene	35	0.029	1,222	0.9983
11	Benzo[ <i>c</i> ]phenanthrene	31	0.026	1214	0.9951
12	Chrysene	10	0.014	728	0.9993
13	Benzo[ <i>a</i> ]anthracene	7	0.009	808	0.9982
14	5-Methylchrysene	33	0.023	1,438	0.9986
15	6-Methylbenzo[ <i>a</i> ]anthracene	35	0.018	1,951	0.9989
16	7,12-Dimethylbenzo[ <i>a</i> ]anthracene	14	0.023	608	0.9993
17	Perylene	14	0.024	580	0.9954
18, 19, 20	Benzo[ <i>b+j+e</i> ]fluoranthene	3 + 14 + 14	0.038	821	0.9994
21	Benzo[ <i>k</i> ]fluoranthene	5	0.025	197	0.9986
22	Benzo[ <i>a</i> ]pyrene	6	0.024	253	0.9994
23	6-Methylbenzo[ <i>a</i> ]pyrene	55	0.010	5,435	0.9995
24	3-Methylcholanthrene	40	0.010	3,982	0.9982
25	Dibenzo[ <i>a,h</i> ]anthracene	10	0.028	352	0.9995
26	Indeno[1,2,3- <i>cd</i> ]pyrene	8	0.254	31	0.9988
	6- to 7-ring PAHs				
27	Benzo[ <i>ghi</i> ]perylene	14	0.044	321	0.9978
28	Anthanthrene	50	0.029	1,707	0.9992
29	Coronene	-	1.824	-	0.9998
30	Dibenzo[ <i>a,l</i> ]pyrene	49	0.116	424	0.9990
31	Dibenzo[ <i>a,e</i> ]fluoranthene	40	0.052	772	0.9992
32	Dibenzo[ <i>a,e</i> ]pyrene	38	0.060	634	0.9998
33	Dibenzo[ <i>a,i</i> ]pyrene	68	0.177	384	0.9997
34	Dibenzo[ <i>a,h</i> ]pyrene	88	0.403	218	0.9997



the method was considered to be rugged. The injection volume of 15  $\mu\text{L}$  was varied by about  $\pm 5 \mu\text{L}$  and the temperature of the column oven (25  $^{\circ}\text{C}$ ) was changed by  $\pm 2 \text{ }^{\circ}\text{C}$ . A deviation in the elution strength was simulated by adding 5 vol.% MilliQ water to the eluent (Fig. 2a).

The long-term stability of the equipment and the response factors was monitored using a continuity test. The continuity test solution (50  $\text{pg}/\mu\text{l}$ ) was analyzed (followed by a blank) after five sample measurements or three calibration measurements, respectively (Fig. 2b). All measurements produced consistent results, indicating that the method can be considered rugged. An extraction blank using solely quartz sand and a clean-up blank using pure toluene without the PAH standard mix on silica gel did not show any peaks.

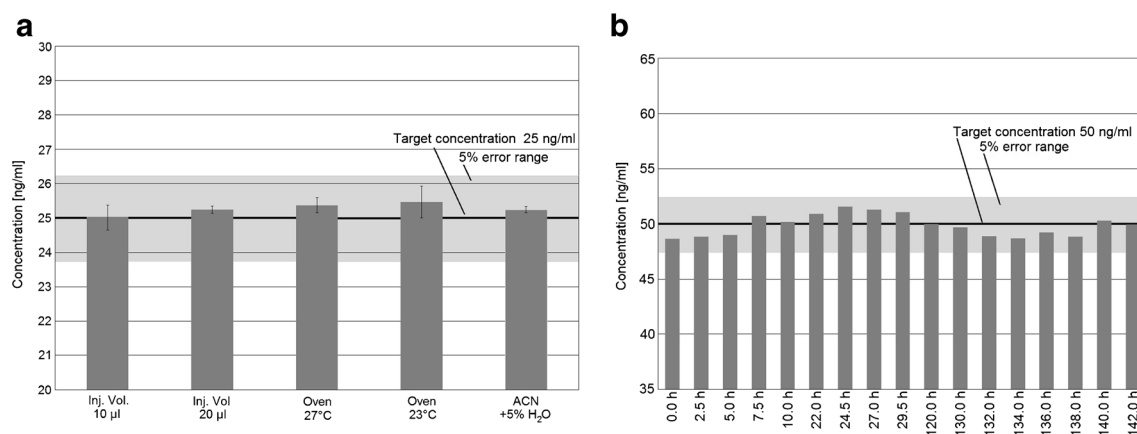
### Validation of the LC-APLI-MS method using certified reference materials

Method validation was performed using three CRMs, a soil sample, and two sediment samples. The results show that for the PAH-contaminated soil (CC013a), the measured concentrations for unfractionated samples were 81–128% of the certified concentrations, while the measured concentrations for fractionated samples were 76–114% of the certified concentrations (Fig. 3 and Tables S1–S3 in the “Electronic supplementary material,” ESM). Fractionation improved the agreement between the measured and certified concentrations for indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene from 124 to 104% and from 110 to 103%, respectively. All measured concentrations in the fractionated samples, except in the case of chrysene, agreed with the certified concentrations within the range of error. This is because the fractionation acted as a clean-up step in which matrix and unwanted compounds were removed, allowing the PAHs to be quantified more accurately [27]. The measured and certified concentrations in the

sediments (BCR535 and NIST 1941b) also agreed to an acceptable extent. In the harbor sediment (BCR535), a large deviation between the measured and certified concentrations was seen for benzo[*a*]anthracene. Benzo[*k*]fluoranthene in the CRMs was always underestimated by our measurements compared to the certified values. For the sediment samples, a fractionation-based clean-up procedure was not required because this sediment was a less complex matrix than the soil matrix. As the method was optimized for HMW-PAHs, the concentrations of phenanthrene and anthracene could only be measured as the sum of both; this was also the case for fluoranthene and pyrene. These summed concentrations could lead to a high divergence from certified values, so the validation of this method was limited to >4-ring PAHs. The results of its application to NIST 1941b shows that the LC-APLI-MS method provides accurate HMW-PAH concentrations (Fig. 3).

### Comparison of LC-APLI-MS with the established GC-MS method

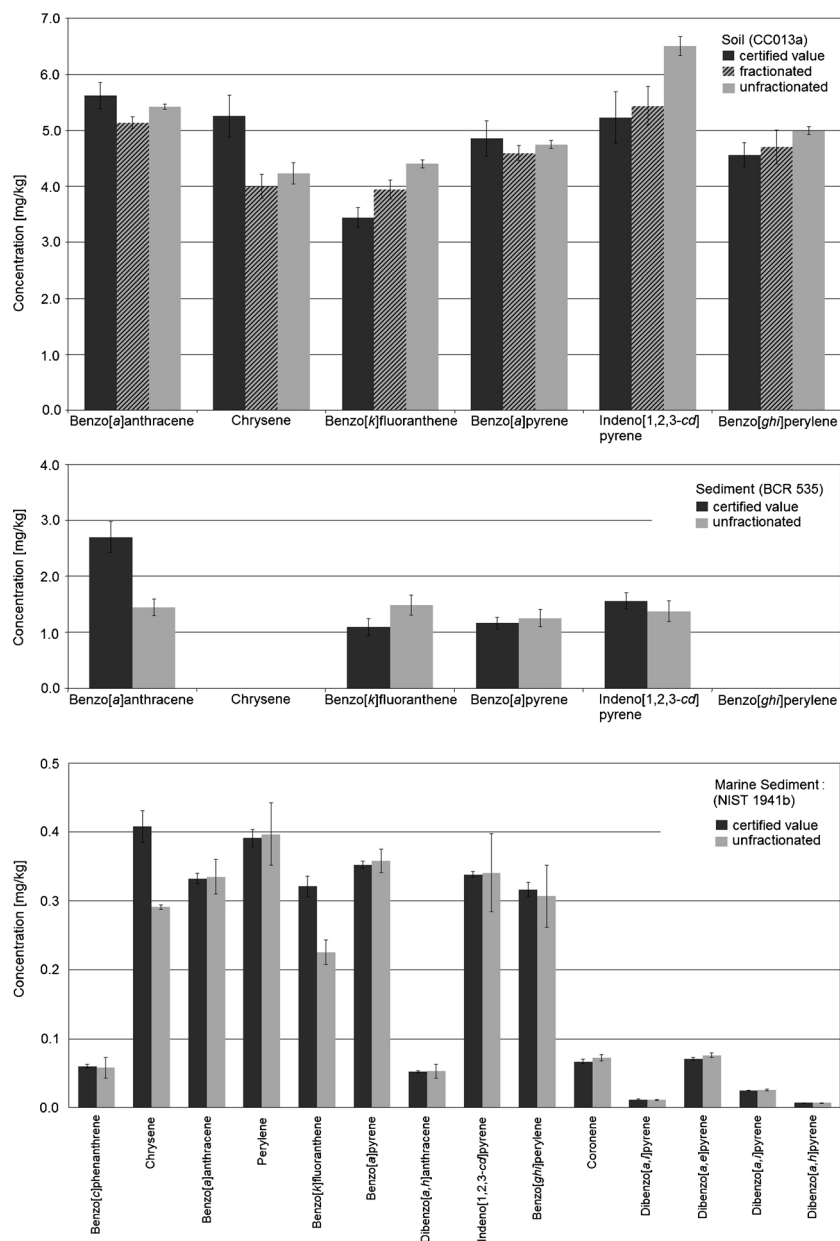
The urban soil samples 1 and 2 showed total concentrations of all 34 PAHs of 80 and 46  $\text{mg}/\text{kg}$ , respectively, and thus represented strongly and weakly contaminated soil samples from an urban district. Figure 4 shows that, again, the concentrations yielded by LC-APLI-MS and GC-MS were in good agreement for urban soil 1 (measured concentrations were 62–162% of the corresponding certified values). Both positive and negative deviations from the certified concentrations were seen when using both methods, but there was no general tendency for a particular method to under- or overvalue the concentrations. To gauge the accord between the results from the newly developed LC-APLI-MS and those from the established GC-MS technique, paired *t*-test calculations were performed for the urban soil data. Using such *t*-test calculations, it was possible to check whether the two methods gave



**Fig. 2** **a** Method ruggedness test: results for benzo[*a*]pyrene following variations in the injection volume (15  $\mu\text{L}$ ) and of the oven temperature (25  $^{\circ}\text{C}$ ) as well as the elution strength ( $n = 3$ , error bars indicate standard

errors) are shown as an example. **b** Continuity test: results for benzo[*a*]pyrene over 142 h are shown as an example

**Fig. 3** Validation using three certified reference materials ( $n = 3$ ): polycyclic hydrocarbons in soil (CC013a), freshwater harbor sediment (BCR 535), and organics in marine sediment (NIST 1941b). The errors in all the certified concentrations were given in the corresponding certificates for the CRMs, whereas the errors in the measured concentrations were calculated as the standard error of three independent measurements ( $n = 3$ )



significantly different results. The critical value for the 16 comparable substances in Fig. 4 (16 degrees of freedom) is  $t_{16} = 2.12$  ( $P = 0.05$ ). For urban soil 1,  $|t| = 1.03$ ; for urban soil 2,  $|t| = 1.11$ . For both samples, the calculated value of  $|t|$  was below the critical value of  $t_{16} = 2.12$  ( $P = 0.05$ ), indicating that the methods do not differ significantly at the 5% level ( $P = 0.05$ ).

All concentrations and the agreement between those given by LC-APLI-MS and GC-MS are shown in Tables S4 and S5 in the ESM.

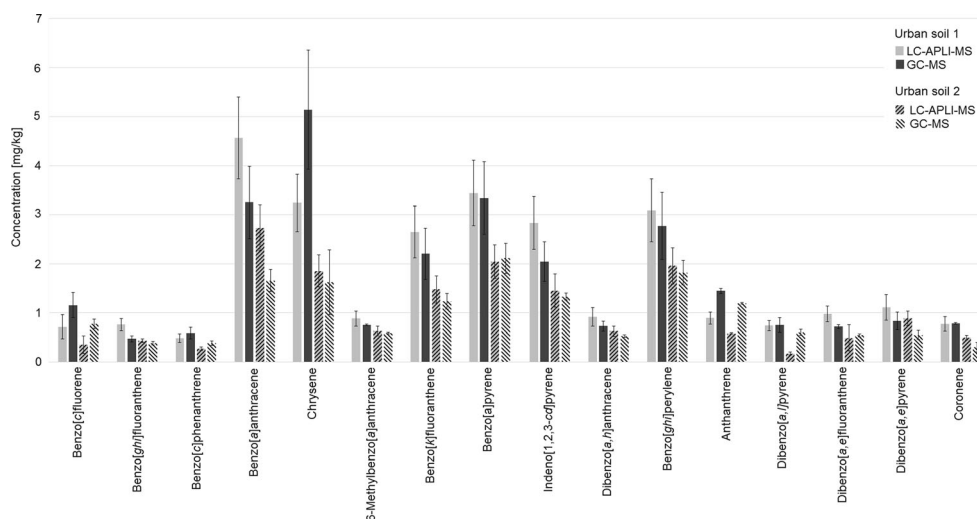
Performing a method comparison for the numerous alkylated PAH derivatives appears to be difficult task because alkylated PAHs are separated into several single peaks with GC, whereas these alkylated PAHs coelute when using LC

and are therefore measured as one peak. As a consequence, 1-methylpyrene and 7,12-dimethylbenzo[a]anthracene can interfere with isomers, meaning that the concentrations of these PAHs found using the two techniques cannot be compared.

Perylene was overvalued in the LC data, which we assume is attributable to the coelution of an unknown isomer with perylene. This overvaluation occurred with both samples, corroborating this hypothesis. In contrast, it is clear that the overvaluation of dibenzo[a,i]pyrene in urban soil sample 2 by GC can be attributed to an coeluting isomer, because a peak shoulder is observed.

Note that the dibenzo[a,i]pyrene and dibenzo[a,h]pyrene in urban soil sample 1 were only detected using LC-APLI-MS (not GC-MS), due to its high sensitivity. 5-Methylchrysene, 3-

**Fig. 4** Concentrations of PAHs in urban soil samples 1 and 2, as determined by LC-APLI-MS and GC-MS. The PAH concentrations determined with LC-APLI-MS basically match with those given by GC-MS, with deviations of 62–162% seen (details are given in Tables S4 and S5 of the ESM). Some of these deviations relate to the different chromatographic methods used in the methods and the influence of coelution



methylcholanthrene, and 6-methylbenzo[*a*]pyrene were not detected with LC-APLI-MS or GC-MS. Subsequent work will focus on the further optimization of the LC method, especially in relation to the separation of alkylated PAHs, which are commonly present in soil samples from industrial areas.

#### Application of LC-APLI-MS to a coal sample and comparison of PAH patterns

A total of 2,318 benzoic PAHs with 6–8 aromatic rings have been enumerated [28], and there are thousands of alkylated 6- to 8-ring PAHs or 6- to 8-ring polycyclic aromatic compounds that include heteroatoms such as nitrogen, sulfur, or oxygen in their aromatic structures or functional groups. The concentrations of PAHs in environmental samples usually decrease with increasing number of aromatic rings, making PAHs with large numbers of rings difficult to detect [5]. LC-APLI-MS is a suitable method for determining these small amounts of C<sub>24</sub>–C<sub>30</sub> PAHs at the chemical formula level via their mass-to-charge ratios. As an example, in the bituminous coal sample used in this study, 211 tentative 6- to 8-ring PAH compounds were detected (Table 2 and Figs. S1 and S2 in the ESM). The peaks were assigned by

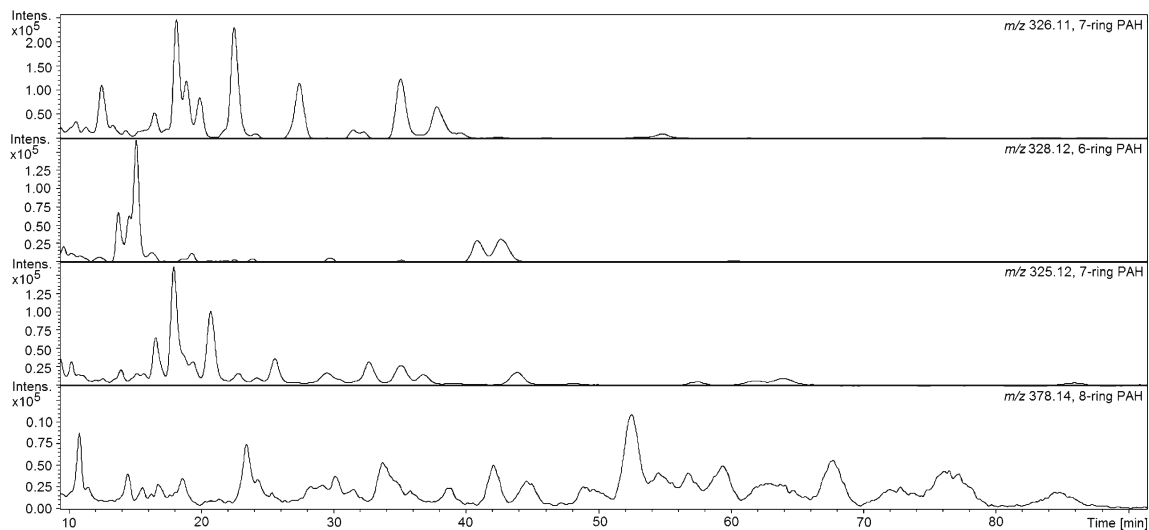
software and by hand based on peak shape and signal-to-noise ratio ( $S/N > 3$ ). In line with expectations, it was observed that compounds with eight rings elute later than compounds with seven or six rings (Fig. 5). Subsequent studies will aim at the identification of these compounds, which could be achieved using UV–VIS absorbance or fluorometric detection prior to splitting the flow to MS detection, because different isomers behave differently in both cases.

The environmental matrices (soils, sediments, and a coal sample) showed high numbers of peaks for  $m/z$  326 (Figs. 5 and 6). The PAH patterns and the relative peak intensities were quite similar in the bituminous coal, the soil (CRM CC013a), and the sediment (Aasee sediment and CRM BCR 535) samples. This is shown in Fig. 6 (taking into account the retention time shift of 2.3 min). Utilizing the most dominant peaks (e, i, j, k, m, and p, as well as q and r in two samples) that form a recurrent pattern, it is possible to correlate the peaks in the samples. The occurrence of similar PAH patterns in different environmental samples suggests that selected compounds probably occur consistently, which is also seen for several lower molecular weight PAHs.

**Table 2** Tentatively identified compounds in the bituminous coal sample studied in this work, ordered by number of rings and  $m/z$

Number of aromatic rings	Possible number of PAHs [28]	Mass-to-charge ratio	Chemical formula	Number of tentatively identified peaks
6	82	$m/z$ 302	C <sub>24</sub> H <sub>14</sub>	17
6	82	$m/z$ 328	C <sub>26</sub> H <sub>14</sub>	41
7	368	$m/z$ 300	C <sub>24</sub> H <sub>12</sub>	5
7	368	$m/z$ 326	C <sub>26</sub> H <sub>14</sub>	33
7	368	$m/z$ 352	C <sub>28</sub> H <sub>16</sub>	34
7	368	$m/z$ 378	C <sub>30</sub> H <sub>18</sub>	37
8	1868	$m/z$ 350	C <sub>28</sub> H <sub>14</sub>	15
8	1868	$m/z$ 376	C <sub>30</sub> H <sub>16</sub>	29



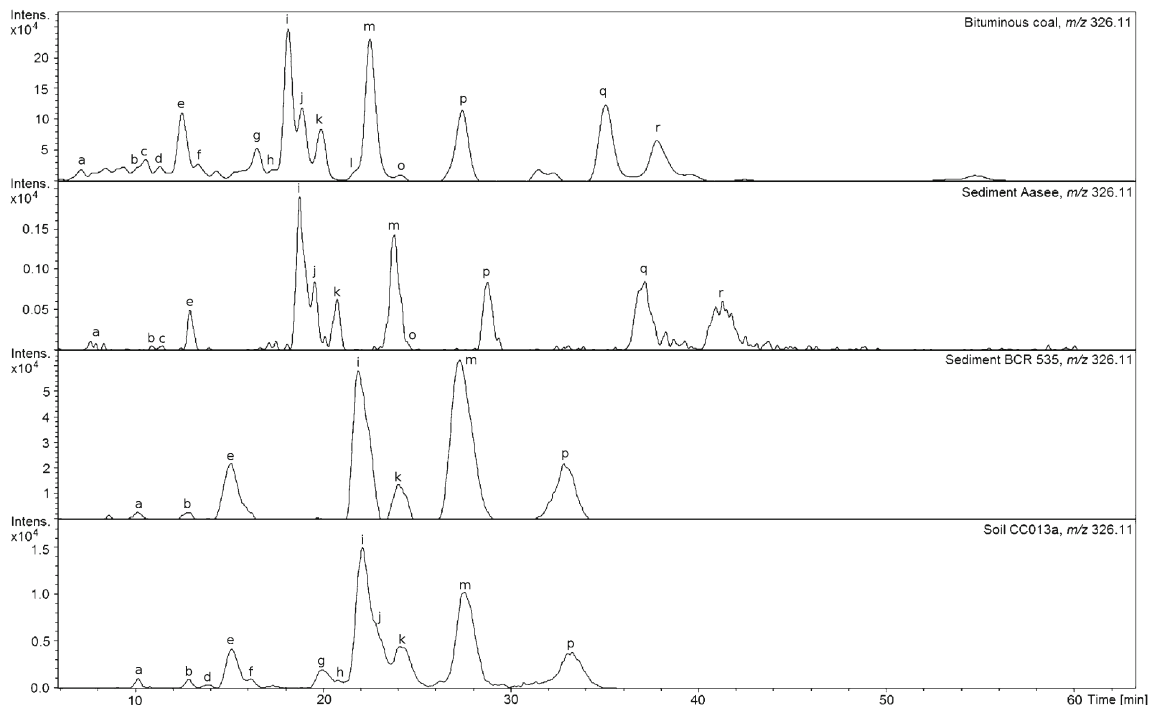


**Fig. 5** Chromatogram showing a selection of peaks from tentative compounds with six, seven, and eight aromatic rings in the bituminous coal sample from Scotland. The full list of tentative peaks is given in Table 2.

## Conclusions

In this study, an LC-APLI-MS method for the detection of higher molecular weight PAHs ( $C_{24}$ – $C_{30}$  PAHs with 6–8 aromatic rings,  $\geq m/z$  300) in environmental samples was developed. The method was found to be rugged and to provide plausible concentrations for known 6-ring and other PAHs when it was applied to certified reference materials. LC-APLI-MS is roughly 1,000 times more sensitive than the established GC-MS technique. Upon applying APLI for the

first time, 211 tentative 6- to 8-ring PAHs were detected in a bituminous coal sample. Similar PAH patterns were also seen in the high molecular weight range, suggesting that these compounds could commonly occur in environmental samples. It is currently unclear if other highly toxic PAH compounds besides the dibenzopyrenes (which are about 10 times more toxic than benzo[*a*]pyrene) also commonly occur in environmental samples; if they do, there may be implications for risk calculations. Further study is warranted in order to identify the compounds detected here and their potential toxicities,



**Fig. 6** Chromatograms for  $m/z$  326. Eleven tentative compounds occur in different environmental samples with similar PAH patterns. The shift in retention time was caused by the replacement of the capillary tubing

because this LC-APLI-MS technique cannot differentiate between isomers.

**Acknowledgments** The authors would like to thank Prof. Dr. Thorsten Benter and his working group (University of Wuppertal, Institute of Physical and Theoretical Chemistry), who loaned us an excimer laser when the laser belonging to our working group underwent maintenance. The authors also thank Dr. Michael Denneborg (ahu AG Wasser Boden Geomatik, Aachen) for the urban soil samples, and Prof. Dr. Jan Schwarzbauer (RWTH Aachen University, Laboratory for Organic-Geochemical Analysis of the Institute of Geology and Geochemistry of Petroleum and Coal) for the bituminous coal sample.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Ravindra K, Sokhi R, Van Grieken R. Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. *Atmos Environ*. 2008;42:2895–921. doi:10.1016/j.atmosenv.2007.12.010.
- Sakai R, Siegmann HC, Sato H, Voorhees AS. Particulate matter and particle-attached polycyclic aromatic hydrocarbons in the indoor and outdoor air of Tokyo measured with personal monitors. *Environ Res*. 2002;89:66–71. doi:10.1006/enrs.2002.4355.
- Godoi AFL, Ravindra K, Godoi RHM, Andrade SJ, Santiago-Silva M, Van Vaeck L, et al. Fast chromatographic determination of polycyclic aromatic hydrocarbons in aerosol samples from sugar cane burning. *J Chromatogr A*. 2004;1027:49–53. doi:10.1016/j.chroma.2003.10.048.
- Achten C, Hofmann T. Native polycyclic aromatic hydrocarbons (PAH) in coals—a hardly recognized source of environmental contamination. *Sci Total Environ*. 2009;407:2461–73. doi:10.1016/j.scitotenv.2008.12.008.
- Lima ALC, Farrington JW, Reddy CM. Combustion-derived polycyclic aromatic hydrocarbons in the environment—a review. *Environ Forensic*. 2005;6:109–31. doi:10.1080/15275920590952739.
- Yang Y, Ligouis B, Pies C, Achten C, Hofmann T. Identification of carbonaceous geosorbents for PAHs by organic petrography in river floodplain soils. *Chemosphere*. 2008;71:2158–67. doi:10.1016/j.chemosphere.2008.01.010.
- Baumard P, Budzinski H, Garrigues P, Dizer H, Hansen P. Polycyclic aromatic hydrocarbons in recent sediments and mussels (*Mytilus edulis*) from the Western Baltic Sea: occurrence, bioavailability and seasonal variations. *Mar Environ Res*. 1999;47:17–47. doi:10.1016/S0141-1136(98)00105-6.
- Becker L, Glavin DP, Bada JL. Polycyclic aromatic hydrocarbons (PAHs) in Antarctic Martian meteorites, carbonaceous chondrites, and polar ice. *Geochim Cosmochim Acta*. 1997;61:475–81. doi:10.1016/S0016-7037(96)00400-0.
- Bojes HK, Pope PG. Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas. *Regul Toxicol Pharmacol*. 2007;47:288–95. doi:10.1016/j.yrtph.2006.11.007.
- Sadiktsis I, Bergvall C, Johansson C, Westerholm R. Automobile tires—a potential source of highly carcinogenic dibenzopyrenes to the environment. *Environ Sci Technol*. 2012;46:3326–34. doi:10.1021/es204257d.
- Garrigues P, Achten C, Andersson J. Polycyclic aromatic compounds. 35th ed. Philadelphia: Taylor & Francis; 2015.
- Andersson J, Achten C. Time to say goodbye to the 16 EPA PAHs? Toward an up-to-date use of PACs for environmental purposes. *Polycycl Aromat Compd*. 2015;35:330–54. doi:10.1080/10406638.2014.991042.
- Stader C, Beer FT, Achten C. Environmental PAH analysis by gas chromatography–atmospheric pressure laser ionization–time-of-flight–mass spectrometry (GC-APLI-MS). *Anal Bioanal Chem*. 2013;405:7041–52. doi:10.1007/s00216-013-7183-8.
- Bergvall C, Westerholm R. Determination of 252–302 Da polycyclic aromatic hydrocarbons in standard reference materials 1649a urban dust and 1650b and 2975 diesel particulate matter by accelerated solvent extraction–HPLC–GC–MS. *Anal Bioanal Chem*. 2008;391:2235–48. doi:10.1007/s00216-008-2182-x.
- Ona-Ruales JO, Sharma AK, Wise SA. Identification and quantification of seven fused aromatic rings C<sub>26</sub>H<sub>14</sub> peri-condensed polycyclic aromatic hydrocarbons in a complex mixture of polycyclic aromatic hydrocarbons from coal tar. *Anal Bioanal Chem*. 2015;407:9165–76. doi:10.1007/s00216-015-9084-5.
- Thurman EM, Ferrer I, Barceló D. Choosing between atmospheric pressure chemical ionization and electrospray ionization interfaces for the HPLC/MS analysis of pesticides. *Anal Chem*. 2001;73:5441–9.
- Anacleto J, Ramaley L, Benoit F, Boyd R, Quilliam M. Comparison of liquid chromatography mass spectrometry interfaces for the analysis of polycyclic aromatic compounds. *Anal Chem*. 1995;67:4145–54.
- Pérez S, Barceló D. Determination of polycyclic aromatic hydrocarbons in sewage reference sludge by liquid chromatography–atmospheric-pressure chemical-ionization mass spectrometry. *Chromatographia*. 2001;53:475–80. doi:10.1007/BF02491606.
- Robb D, Covey T, Bruins A. Atmospheric pressure photoionization: an ionization method for liquid chromatography–mass spectrometry. *Anal Chem*. 2000;72:3653–9.
- Robb DB, Blades MW. State-of-the-art in atmospheric pressure photoionization for LC/MS. *Anal Chim Acta*. 2008;627:34–49. doi:10.1016/j.aca.2008.05.077.
- Cai S-S, Syage JA, Hanold KA, Balogh MP. Ultra performance liquid chromatography–atmospheric pressure photoionization–tandem mass spectrometry for high-sensitivity and high-throughput analysis of U.S. Environmental Protection Agency 16 priority pollutants polynuclear aromatic hydrocarbons. *Anal Chem*. 2009;81:2123–8. doi:10.1021/ac802275e.
- Constapel M, Schellenträger M, Schmitz OJ, Gäb S, Brockmann KJ, Giese R, et al. Atmospheric-pressure laser ionization: a novel ionization method for liquid chromatography/mass spectrometry. *Rapid Commun Mass Spectrom*. 2005;19:326–36. doi:10.1002/rcm.1789.
- Boesl U. Laser mass spectrometry for environmental and industrial chemical trace analysis. *J Mass Spectrom*. 2000;35:289–304.
- Lababidi S, Panda SK, Andersson JT, Schrader W. Direct coupling of normal-phase high-performance liquid chromatography to atmospheric pressure laser ionization Fourier transform ion cyclotron resonance mass spectrometry for the characterization of crude oil. *Anal Chem*. 2013;85:9478–85. doi:10.1021/ac400670s.
- Meyer W, Seiler T-B, Reininghaus M, Schwarzbauer J, Püttmann W, Hollert H, et al. Limited waterborne acute toxicity of native polycyclic aromatic compounds from coals of different types

compared to their total hazard potential. *Environ Sci Technol.* 2013;47:11766–75. doi:10.1021/es401609n.

26. Richter BE, Jones BA, Ezzell JL, Porter NL. Accelerated solvent extraction: a technique for sample preparation. *Anal Chem.* 1996;68:1033–1039.
27. Cho YJ, Na J-G, Nho N-S, Kim SH, Kim S. Application of saturates, aromatics, resins, and asphaltenes crude oil fractionation for detailed chemical characterization of heavy crude oils by Fourier transform ion cyclotron resonance mass spectrometry equipped with atmospheric pressure photoionization. *Energy Fuels.* 2012;26:2558–65. doi:10.1021/ef201312m.
28. Herndon W. On enumeration and classification of condensed polycyclic benzenoid aromatic hydrocarbons. *J Am Chem Soc.* 1990;112:4546–7.



**Jan Thiäner** is a PhD student from the Department of Applied Geology at the Institute of Geology and Paleontology, University of Münster (Münster, Germany). His main scientific interest is the investigation of aromatic compounds in complex environmental matrices. He specializes in application development using mass spectrometry coupled to various ionization techniques, including multimode ionization techniques.



**Christine Achten** is Head of the Department and Laboratory of Applied Geology at the Institute of Geology and Paleontology, University of Münster (Münster, Germany). She has been working for several years on the analysis, source apportionment, and fate of polycyclic aromatic compounds in the environment, with a special focus on bioavailability. More recently, she has studied applications of laser ionization in environmental analysis and other polar trace pollutants in the aqueous water cycle at the river catchment scale.