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A liquid chromatography–atmospheric pressure photoionization tandem mass spectrometric method for the determination of azaarenes in atmospheric particulate matter

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ABSTRACT

The development, optimization and validation of a liquid chromatography–atmospheric pressure photoionization tandem mass spectrometric (LC–APPI/MS/MS) method for the determination of 15 azaarenes (4-azafluorene, benzo[h] and -[f]quinoline, phenanthridine, acridine, 1-azafluoranthene, 4-azapyrene, benz[a]- and -[c]acridine, -10-azabenzo[a]pyrene, 7,9- and 7,10-dimethylbenz[c]acridine, dibenz[a,j]-, -[c,h] and [a,i]acridine) in airborne particulate matter is described. Each compound was detected and quantified operating in multiple reaction monitoring mode. Extraction of azaarenes was achieved using accelerated solvent extraction (ASE) with dichlormethane/methanol (50/50, v/v). After extraction, no additional clean-up procedure like solid phase or liquid/liquid extraction was necessary. Limits of quantification (S/N × 10) ranged from 0.2 pg/μl to 1.4 pg/μl, matrix dependent recoveries were between 57% and 94%, with relative standard deviations from 8% to 17%. Applicability of the method was demonstrated analyzing 10 samples of particulate matter ($PM_{2.5}$) collected in winter 2008. In all samples dimethylbenz[c]acridines as well as dibenzacridines were below the limit of quantification, concentration of the remaining analytes were in the range from 0.002 ng/m^3 to 0.356 ng/m^3.

1. Introduction

There is consistent epidemiological evidence that exposure to current levels of airborne fine particulate matter ($PM_{2.5}$, median aerodynamic diameter (Dp) ≤ 2.5 μm) is associated to increased mortality and morbidity among susceptible parts of the population like elderly persons with chronic respiratory or cardiovascular diseases, asthmatic subjects of all ages, and children [1–3]. Recent research on particle-induced health effects investigates the critical characteristics of particulate matter that are responsible for their biological effects. Besides physical properties like size and surface area the chemical composition of the particles probably determines the effects caused after their inhalation. This chemical composition of course varies greatly and depends on factors like emission sources, climate, season as well as duration and circumstances of atmospheric transport of the particles. Major components of urban PM are often organic compounds which are adsorbed on the surface cavities of a carbonaceous core [4,5]. One important class of these substances is represented by the well known and extensively investigated polycyclic aromatic hydrocarbons (PAHs). Azaarenes – a subclass of polycyclic aromatic nitrogen heterocycles (PANHs) – are derivatives of PAHs and their significance in air pollution has been recognized during the past century when elevated concentrations in urban atmospheres have been found [6–14]. They are generated and released mainly as the result of anthropogenic activities related to industrial discharge and incomplete combustion of organic material [15–17]. Azaarenes are classified as heterocycles composed of both an aromatic and a six-membered ring structure, in which a carbon is replaced by nitrogen [18]. The presence of this nitrogen atom introduces polarity into the molecule. Increased polarity leads to higher water solubility and bioavailability, which may result in more significant health effects, even at lower environmental concentrations compared to PAHs. A number of azaarenes, especially four- and five-ring compounds, show higher mutagenic, carcinogenic and toxic activity in comparison to their corresponding PAHs [19–25]. Due to their biological activity and their low environmental concentrations, reliable analytical methods for the determination of azaarenes in environmental samples are important. They can serve as helpful tools for the implementation of monitoring programs, the investigation of atmospheric levels, behaviour and fate of azaarenes in the atmosphere.

Methods for the determination of selected azaarenes in matrices like food, soil, sediment and aerosols are described in literature. Since relevant compounds can depend on the respective matrix,

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number and kind of substances analyzed varies. Common step for all methods is the extraction of the analytes, mainly performed by Soxhlet or ultrasonic extraction with organic solvents or solvent mixtures like dichlormethane, benzene, ethanol, hexane, toluene, ethyl acetate, methanol or acetonitrile [6–9,11–16,26–32]. Besides these methods other techniques like supercritical fluid extraction (SFE) and pressurized solvent extraction (PSE) are used seldom [26]. Depending on the sample, the organic extract normally contains a variety of substances – e.g., PAHs in higher concentrations – which can interfere with chromatographic separation and detection of the azaarenes. Hence the extract mostly must be purified or prefractionated prior to the succeeding steps. To accomplish this purpose different strategies are pursued. Due to the basic properties of azaarenes, liquid-liquid extraction (LLE) with acidic solutions is often applied [6–9,11,14,27,28,30]. Many authors use solid phase extraction (SPE), open column chromatography or high performance liquid chromatography (HPLC) on different materials such as alumina, silica gel, propylsulfonic acid, diatomaceous earth, and octadecylsilan [10,13,15,16,28–30,32–39]. In some cases different clean-up steps like SPE and HPLC are combined leading to pure extracts on the one hand, but to time consuming, error-prone and expensive analytical procedures on the other hand. Only very few authors described analytical methods without clean-up of organic extracts: Svabensky et al. developed a HPLC method which allows separation of PAHs and azaarenes in one chromatographic run, avoiding preceding fractionation steps but leading to run times about 120 min [39]. Delhomme et al. analyzed 10 azaarenes in atmospheric particulate matter using GC/MS in the selected ion monitoring mode (SIM) [12].

Final analysis of the pretreated extract is performed by gas chromatography (GC) mostly coupled with mass spectrometry (MS) [6–9,11–14,16,28–30,32–34], sometimes in combination with flame ionization detection (FID) [28,30], electron capture detection (ECD) [28,30] or nitrogen-phosphorus detection (NPD) [27,33]. GC/MS methods generally allow detection limits in the pg/μl range (ca. 2–4000 pg/μl [11,12]). Disadvantage of GC/MS systems can be the high sensitivity to the sample. Extracts containing solvents and compounds with higher boiling points are difficult to analyse.

Besides GC methods HPLC is often applied for separation and detection of azaarenes in different matrices. Older methods are based on ultraviolet or diode array detection, which suffer from low selectivity and high limits of detection (ca. 100 pg/μl) [10,26,34,35]. Selectivity and sensitivity can be significantly increased using fluorescence detection. With optimised programmed wavelength switching, detection limits in the range of approximately 0.05–20 pg/μl can be achieved [7,12,15,28,30,37–39]. Essential conditions for this sensitive and selective detection are a (pre)separation of substances with similar fluorescence characteristics on one side and a good peak resolution for exact wavelength switching on the other side. These demands exacerbate reliable HPLC-determination of azaarenes and led to strategies like extended separation times [39], two successive chromatographic runs with different gradient and wavelength programs [7,15], and a reduced number of target analytes [12,38].

Compared to the determination methods for azaarenes discussed above, HPLC coupled with tandem mass spectrometry (LC–MS/MS) offers fundamental advantages: Tandem mass spectrometry allows the generation of selective fragmentation patterns for every substance of interest, containing valuable structural information. Based on these fragmentation schemes, specific mass losses (so called transitions) can be used for identification and quantification leading to increased sensitivity and selectivity. Due to this selectivity clean-up and prefractionation procedures of the crude organic extract are often simplified or even not necessary. The filtered extract – in a compatible solvent – can be directly injected into the LC–MS/MS system. Furthermore, similar substances, which co-elute, but have different fragmentation patterns and thus different transitions, can be reliably identified and quantified without chromatographic separation.

Depending on the physico-chemical properties of the analytes, different interfaces and ionization techniques, namely electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are applied.

One method based on LC–MS/MS with electrospray ionization for the determination of 15 PANHs in soils is described [31]. However, ESI often is subject to ionization suppressions or enhancements due to matrix effects. Moreover it is primarily well suitable for the ionization of polar compounds. In recent studies APCI and especially APPI interfaces distinguish themselves by efficient ionization of non-polar or less polar compounds, a lower sensitivity to matrix effects, and a broad dynamic linear range of response [40–46].

To meet the requirements of performing fast and reliable analyses, e.g., during ambient monitoring campaigns, we decided to develop a LC–APPI/MS/MS method in combination with ASE-extraction for the determination of 17 relevant azaarenes (4-azafluorene, benzo[h] and -[f]quinoline, phenanthridine, acridine, 1-azafluoranthene, 4-azapyrene, benz[a]- and -[c]acridine, 10-azabenzo[a]pyrene, 7,9- and 7,10-dimethylbenz[c]acridine, dibenz[a,c]-, -[a,j]-, -[a,h]-, -[c,h] and [a,i]acridine) in ambient fine particulate matter. First experiments in our laboratory using an API 2000 coupled to an Agilent HP 1100 HPLC via an APCI source exhibited detection limits between 0.2 pg/μl and 10 pg/μl (S/N = 3) [47]. Because for real samples of particulate matter, this sensitivity is often not sufficient, we decided to change to an APPI interface. The increase in sensitivity was significant, with detection limit values between 0.05 and 0.92 pg/μl (S/N = 3). Due to internal, laboratorial reasons, the method was subsequently applied, optimised and validated on an API 3000. The results of these investigations are described in this paper. To our knowledge there are no literature reports on the use of LC–APPI/MS/MS-based methodology for the determination of azaarenes in any environmental matrix.

To demonstrate the applicability of the new method for the sensitive and reliable determination of azaarenes in particulate matter, samples of $PM_{2.5}$ were collected on 10 days in December 2008 with a High Volume sampler. Loaded quartz fibre filters were extracted using ASE and analyzed applying the described LC–APPI/MS/MS method.

2. Experimental

2.1. Chemicals

Formic acid p.a. 98–100% and methanol chromosolve was purchased from VWR International GmbH (Darmstadt, Germany), methanol optigrade for LC/MS from LGC Standards (Wesel, Germany), acetonitrile gradient grade for HPLC from Sigma–Aldrich (Munich, Germany), toluene Pestanal, dichlormethane Pestanal and acetone Pestanal from Riedel-de Haën (Honeywell Riedel-de Haën, Seelze, Germany). Water was generated by a Milli-Q Ultra Plus Water System, Millipore GmbH (Schwabach, Germany). Nitrogen used for the mass spectrometer and for evaporation during sample processing was taken from a central gas supply which is provided by a liquid nitrogen tank (Linde, Germany).

2.2. Standard substances

Acridine (Acr), benz [a]acridine (B[a]a), benz[c]acridine (B[c]a), dibenz [a,j]acridine (Db[a,j]a), dibenz [a,c]acridine (Db[a,c]a), dibenz [a,h]acridine (Db[a,h]a), dibenz[a,i]acridine (Db[a,i]a) and

10-azabenzo(a)pyrene (10-Abap) came from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Dibenz[c,h]acridine (Db[c,h]a), 4-azapyrene (4-Apy), 1-azafluoranthene (1-Afla) and 4-azafluorene (4-Aflu) were from the PAH research institute Dr. Schmidt (Greifenberg, Germany). Benzo[f]quinoline (B[f]q) and benzo[h]quinoline (B[h]q) were obtained from Ultra Scientific (North Kingstown, Rhode Island, USA). Phenanthridine (Phen), 7,9-Dimethylbenz [c]acridine (7,9-Dmeb[c]a) and 7,10-dimethylbenz[c]acridine (7,10-Dmeb[c]a) were purchased from Sigma–Aldrich, (Munich, Germany). Acridine-d9 (Acr-d9) and dibenz[a,j]acridine-d13 (Db[a,j]-d13) were obtained from LGC Standards (Wesel, Germany). The chemical structures and the molecular masses of the compounds studied are presented in Table 1.

B[c]a and B[a]a were purchased as 10 ng/μl solutions in iso-octane, 10-Abap, Db[a,h]a, Db[a,j]a, Db[a,i]a and Db[a,c]a as 10 ng/μl solutions in acetonitrile. Stock solutions of the other substances were prepared by weighing ca. 0.5 mg in 10 ml acetonitrile. From these stock solutions single standard solutions were obtained by dilution with acetonitrile. Calibration standards containing all compounds in concentrations ranging from 2 pg/μl to 109 pg/μl were prepared by mixing and diluting the single standard solutions in acetonitrile.

2.3. Samples of particulate matter

Samples of particulate matter ($PM_{2.5}$) were collected on the campus of the Helmholtz Zentrum Muenchen, Munich, Germany during 10 days from 11th to 23rd of December 2008. Filter samples were obtained using a high-volume sampler HVS (Anderson, USA) which was operated for 24 h at a flow rate of ca. 800 l/min. For particle collection, 203 mm × 254 mm quartz fibre filters (Whatman International Ltd., Maidstone, England) were placed in the HVS after heating them for at least 12 h in a circulating air oven from Nabertherm (Lilienthal, Germany) at 500 °C. After sampling the filters were wrapped in aluminium foil and stored in a dessicator at 4 °C until analysis.

2.4. Sample preparation

All parts (scalpel, forceps, support, etc.), used for the sample preparation were rinsed with acetone before use. Filters were cut into four pieces using a scalpel, which off two parts were separately extracted and analyzed by LC–APPI/MS/MS. The residual parts were stored for analysis of additional organic pollutants. Homogenous load of the filters in the HVS was investigated and ensured during previous studies [48].

Accelerated solvent extraction (ASE) was carried out using a Dionex ASE 200 (Idstein, Germany). Filters were folded twice and placed into the extraction cells (11 ml) which were closed at each end with a cellulose fibre filter (Dionex, Idstein, Germany). A PTFE-filter (0.2 μm, 25 mm, Sartorius, Goettingen, Germany) was placed on the outlet frit of the cell, to protect the capillary system of the ASE against plugging by small particles. Extraction of samples occurred during a 5 min heating phase, followed by three static cycles of 5 min at 100 bar and 100 °C, respectively. Flush volume was 150%, purge time 300 s. Dichloromethane/methanol 50:50 (v/v) was used as extraction solvent. The extract was reduced to ca. 0.3 ml in a Büchi Syncore platform, (Büchi Labortechnik AG, Flawil, Switzerland). The residual volume was placed in a 1-ml volumetric flask and carefully evaporated to dryness in a Barkey vapotherm mobil S (Barkey GmbH, Leopoldshöhe, Germany) under a gentle stream of nitrogen. The residue was dissolved in 1 ml acetonitrile, filtered through a 0.2 μm Spartan 13/0.2 filter unit (Whatman, Dassel, Germany) into an autosampler vial, and subjected to LC–APPI/MS/MS analysis.

2.5. Instrumentation

LC–MS/MS analyses were carried out with an API 3000™ tandem quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany) which was equipped with a standard atmospheric pressure photoionization (APPI) source and a HP 1100 HPLC system (G1316A column oven, G1329A autosampler with thermostat (G1330A), G1311A quaternary pump and a G1322A degasser, Agilent Technologies, Böblingen, Germany). Instrument control, data acquisition and analysis were performed with Analyst software version 1.4.2. Dopant eluent was supplied by an ABI 140D solvent delivery system (PerkinElmer, Rodgau-Jügesheim, Germany).

2.6. Chromatographic conditions

The optimised, final chromatographic conditions were as follows:

Samples of 10 μl were processed on a Gemini C18 (250 mm × 2.0 mm, 5 μm) column from Phenomenex (Aschaffenburg, Germany) equipped with an equal-branded pre-column. Eluent A was 0.1% formic acid in water (pH 2.3) and eluent B methanol. A methanol gradient from 5% methanol to 60% in 0.2 min, to 70% in 3.4 min, to 90% in 9 min, and to 100% in 10 min was performed at 45 °C and 400 μl/min. Over the last 7 min the column was equilibrated for the next injection. Total analysis time was 30 min.

2.7. MS/MS conditions

The API 3000™ triple stage quadrupole mass spectrometer was coupled to the HPLC system with an atmospheric pressure photoionization (APPI) interface. The APPI source was operated in positive mode with a krypton filled UV lamp emitting photons at 10.0 eV. All source parameters were optimised manually by HPLC injection experiments resulting in following settings: The source temperature was 350 °C, source voltage was 1200 V. For the gases nitrogen was used in all cases. Nebulizer gas was set to 5.17 bar, curtain gas and auxiliary gas were adjusted to 8 (arbitrary units used in the instrument). For the multiple reaction monitoring mode collision activated defragmentation (CAD) gas was set to 7 (arbitrary unit). Lamp gas was adjusted at 1 l/min. Toluene was used as dopant at 10% flow rate (40 μl/min) from the HPLC flow (400 μl/min). Scan time for each transition was fixed at 50 ms, sufficient for obtaining the 10 points necessary per peak. Operation parameters and monitoring transitions for the analytes are listed in Table 2. The LC–APPI/MS/MS instrument was operated in multiple reaction monitoring (MRM) mode for quantification.

3. Results and discussion

3.1. Method optimisation

3.1.1. Mass spectrometry

The characteristic fragmentation pattern of each compound was consulted for establishing the transitions used for the multiple reaction monitoring method. For every compound, the two most intensive mass losses (transitions) were chosen. The first transition served as quantifier, the second mass loss was used as qualifier. The precursor ions were found performing a quadrupole 1 (Q1) total ion current scan for every substance from 50 amu up to 50 amu above their respective molecular weights, in order to verify the possible formation of cluster products. A syringe pump Harvard pump 11 (Harvard apparatus, Holliston, USA) was used for direct infusion of standard azaarene solutions at a flow rate of 200 μl/min and concentrations of 100–1000 ng/ml each into the APPI interface with

Table 1
Analyte abbreviations, structures and molecular masses.

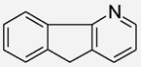
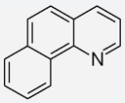
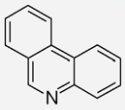
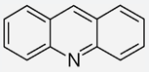
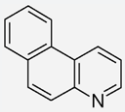
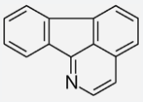
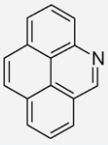
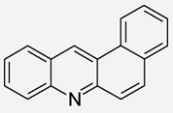
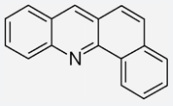
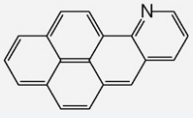
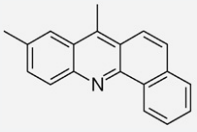
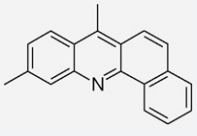
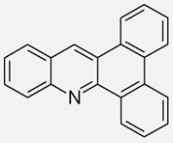
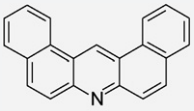
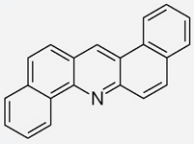
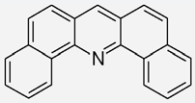
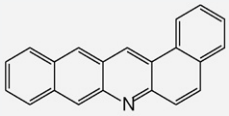
Compound	Abbreviation	Structure	Molecular mass
4-Azafluorene	4-Aflu		167
Benzo[h]quinoline	B[h]q		179
Phenanthridine	Phen		179
Acridine	Acr		179
Benzo[f]quinoline	B[f]q		179
1-Azafluoranthene	1-Afla		203
4-Azapyrene	4-Apy		203
Benz[a]acridine	B[a]a		229
Benz[c]acridine	B[c]a		229
10-Azabenzo[a]pyrene	10-Abap		253
7,9-Dimethylbenz[c]acridine	7,9-Dmeb[c]a		257
7,10-Dimethylbenz[c]acridine	7,10-Dmeb[c]a		257
Dibenz[a,c]acridine	Db[a,c]a		279

Table 1(*Continued*)

Compound	Abbreviation	Structure	Molecular mass
Dibenz[a,j]acridine	Db[a,j]a		279
Dibenz[a,h]acridine	Db[a,h]a		279
Dibenz[c,h]acridine	Db[c,h]a		279
Dibenz[a,i]acridine	Db[a,i]a		279

CAD gas set at 0 (arbitrary units). For all substances investigated a scan of standard solutions displayed dominant pseudo-molecular ions $[M+H]^+$. The ionization parameters of these precursor ions, i.e., declustering potential (DP), focussing potential (FP), and entrance potential (EP) were optimised automatically. Afterwards a product ion scan was performed to find out the fragmentation pattern of the analytes. In the collision cell (quadrupole 2, Q2) collision activated dissociation of the pseudo-molecular ions was carried out using nitrogen as collision gas (7, arbitrary unit) and 35 eV as collision energy. Transitions used for quantification and identification were based on the following fragmentation characteristics: 7,9- and 7,10-Dimethylbenz[c]acridine presumably lost CH_4 $[M+H-16]^+$, the other azaarenes investigated all lost probably CH_2N $[M+H-28]^+$ in the first, most intensive, fragmentation step. These first transitions were used as quantifier (cf. Table 2).

Less intensive mass losses – second transitions, used as qualifier – were probably due to elimination of C_4H_5 $[M+H-53]^+$, C_2H_5N $[M+H-43]^+$, CH_4 $[M+H-16]^+$ and a fragment of m/z 102 $[M+H-102]^+$, which cannot be clearly ascribed. Representative product ion scans are shown in Figure S-1, supplementary material.

Instrument parameters, declustering potential (DP), focussing potential (FP), entrance potential (EP), collision cell energy (CE) and the collision cell exit potential (CXP), were manually optimised for the selected transitions by repeated HPLC injections at 400 μl/min.

Different solvents with ionization potential less than 10 eV can be used as dopant for the photoionization, whereas acetone and toluene are the preferred ones [43]. Both were tested in this study by comparing the resulting signal/noise (S/N) values after HPLC injection of the standard mixture. Delivering a considerably lower S/N, toluene was chosen for the further work. For optimization of the toluene flow, which is recommended to be between 10% and 15% of the HPLC flow, the S/N values for different toluene flows between 5% and 20% of the HPLC flow were compared. The optimal toluene flow was found to be 10% of the HPLC flow, corresponding to 40 μl/min.

3.1.2. Chromatography

Due to the presence of isomers, the selectivity of the MRM mode of the mass spectrometer was not sufficient. A further dimension had to be established by means of chromatography, aiming at separating exactly these isomeric compounds.

Because of the mass-based mode of analysis, there was no need for a clear chromatographic separation of compounds with different transitions. Thus, shortening the analysis time was possible.

For the isomers of the quinolines, benzoacridines, dibenzoacridines, 7,9- and 7,10-Dmeb[c]a, 1-Afla and 4-Apyr, a chromatographic separation is necessary, because they have respectively identical transitions (see Table 2). Separation of the isomers was developed by assistance of the Drylab Software (DryLab®, Molnar-Institut, Berlin, Germany). Most isomeric analytes could be baseline separated on a Gemini C18 column with a methanol gradient, except the isomers of dibenzoacridine, Db[a,h]a and Db[a,c]a. For these analytes only a partial separation could be reached applying these conditions (Fig. 1). Besides column packing material, solvent and flow, another factor which can be employed in separation problems is the temperature. Setting the oven temperature at 45 °C amended the chromatographic separation of the two compounds on. Their co-elution is determined primarily by the steric hindrance of the nitrogen site, and a baseline separation can only be achieved by analysis times longer than 40 minutes, which however would not accomplish the goals of this work. As a result the reliable quantification of these two substances is not possible with the method described.

Acridine as well as the internal standard acridine-d9 always showed a slight peak splitting in standards and real samples. This observation was made for different columns and chromatographic conditions. It may be caused in the ionization source. Nevertheless the peaks are highly reproducible (cf. validation results in Table 4) and can be therefore accepted.

Fig. 1 shows MRM chromatograms obtained analyzing a standard solution applying the optimised LC–APPI/MS/MS conditions.

3.1.3. Sample extraction

For practical reasons, one of our objectives was to extract three compound classes – PAHs, nitro-PAHs and azaarenes – within one extraction procedure. A routine ASE method, using hexane/acetone as solvent for the extraction of PAHs and nitro-PAHs was applied, but the recoveries for the azaarenes were not satisfactory (30–70%). Extraction of azaarenes from aerosol samples is mainly achieved applying dichloromethane or dichloromethane containing mixtures as extraction solvent [8–16,24]. Therefore the ASE method (described in detail in [49]), was modified by changing the extrac-

Table 2
Multiple reaction transitions, retention times and optimized compound dependent operation parameters for LC–APPI–MS/MS determination of azaarenes.

Compound	Precursor ion $[M+H]^+/m/z$	Product ion[a] $/m/z$	Retention time/min	DP[b]/V	FP[c]/V	CE[d]/eV	CXP[e]/V
4-Aflu	168	139	5.4	26	75	61	8
	168	115				53	8
B[h]q	180	152	8.4	54	130	49	8
	180	127				55	6
Phen	180	152	6.9	54	130	49	8
	180	127				55	6
Acr	180	152	4.6	54	130	49	8
	180	127				55	6
Acr-d9	189	159	4.6	54	130	51	8
	189	133				55	6
B[f]q	180	152	5.7	54	130	49	8
	180	157				55	6
1-Afla	204	176	9.2	51	130	54	10
	204	151				59	8
4-Apy	204	176	8.8	51	130	54	10
	204	151				59	8
B[a]a	230	202	6.9	71	190	55	11
	230	128				57	7
B[c]a	230	202	12.7	71	190	55	11
	230	128				57	7
10-Abap	254	226	13.8	66	170	61	12
	254	202				68	10
7,9-Dmeb[c]a	258	242	12.8	61	160	56	6
	258	215				71	12
7,10-Dmeb[c]a	258	242	11.1	61	160	56	6
	258	215				71	12
Db[a,c]a	280	252	16.6	70	190	61	6
	280	264				62	6
Db[a,j]a	280	252	11.5	70	190	61	6
	280	264				62	6
Db[a,h]a	280	252	16.3	70	190	61	6
	280	264				62	6
Db[c,h]a	280	252	18.0	70	190	61	6
	280	264				62	6
Db[a,i]a	280	252	8.6	70	190	61	6
	280	264				62	6
Db[a,j]a-d13	293	263	11.5	70	190	61	6
	293	259				62	6

For abbreviations of the azaarenes see Table 1.
[a] The most abundant fragment ion (transition) is listed first.
[b] Declustering potential.
[c] Focussing potential.
[d] Collision energy.
[e] Collision cell exit potential.

tion solvent from hexane/acetone to methanol/dichloromethane, resulting in acceptable recoveries for all analytes of interest, including the azaarenes.

3.2. Method validation

Calibration plots with standard solutions (concentrations between 2 pg/μl and 109 pg/μl, $n = 6$) were set up. The regression coefficients were always > 0.994 and the intercepts with the y-axis did not deviate significantly from the origin. Calibration plots are shown in Figure S-2, supplementary material.

To determine accuracy and precision, recoveries were investigated. For the matrix independent recovery blank quartz fibre filters were heated at 500 °C for at least 12 h in a circulating air oven, cut into ten pieces and spiked with standard solutions resulting in concentrations from 2 pg/filter part to 109 pg/filter part. Sample processing as described in Section 2.4 was performed and the aliquots of the acetonitrile solutions were analyzed by LC–APPI/MS/MS. Peak areas of the chromatograms of the spiked samples were compared with peak areas of the diluted spike standards. In a similar way matrix dependent recoveries were evaluated. Quartz fibre filters after a 24 h collecting period were cut into ten pieces. Two parts were extracted and analyzed without spiking, two parts respectively were spiked with one concentration. The fortified samples (concentrations: 2 pg/filter part to 109 pg/filter part) as well as the non-spiked parts of the filter were extracted and analyzed. Peak areas used for calculation were corrected for the values of the non-spiked filter parts.

As taken from Table 4, recoveries for the most analytes are better for matrix dependent recoveries. This effect was already observed during previous studies in our laboratory [48], and it is probably caused by two reasons: For the matrix independent

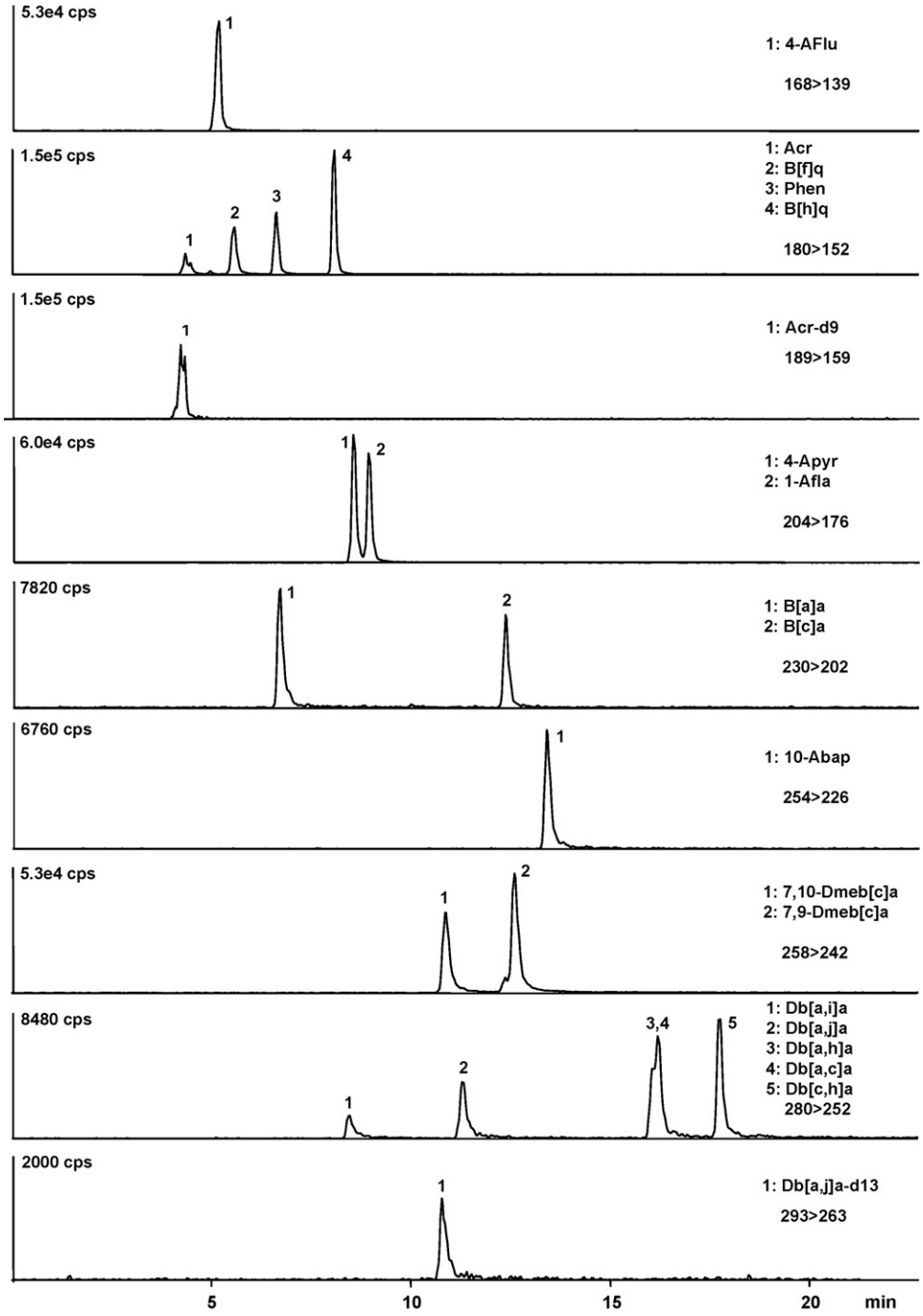


Fig. 1. MRM chromatograms of a standard solution of azaarenes. Concentrations are listed in Table 3.

recovery, a heated and thus activated quartz fibre filter was used. The activated surface can catalyze degradation of reactive analytes and/or decrease extraction efficiency due to increased interactions between surface and azaarene. The collected matrix and its constituents can have a stabilizing effect on reactive substances.

Matrix-dependent recoveries range from 57% to 94%, with relative standard deviations from 8% to 17%, meaning that during routine analysis regular recovery experiments have to be performed, or that for compounds with low recoveries also internal standards have to be found.

To investigate the precision of the whole method, one loaded filter was cut into ten pieces. Seven parts were spiked with one azaarene standard (one concentration level, analyte concentrations: 4 pg/filter part to 15 pg/filter part), extracted and analyzed. Relative standard deviations were good with values between 4% and 11% (Table 4).

For evaluation of the precision of the LC–APPI/MS/MS determination step, excluding sample preparation, one loaded filter was spiked (analyte concentrations: 4 pg/filter part to 15 pg/filter part), extracted and the extract was analyzed on five consecutive days for ten times every day. Imprecision is low with relative standard deviations from 2% to 9% for the within series imprecision and from 1% to 6% for the between-day imprecision.

It should be pointed out that – following the concept of Matuszewski et al. [50] – strictly speaking the results for matrix dependent recoveries reflect a combined effect of analyte loss dur-

Table 3
Concentrations of the azaarene standards in the MRM chromatograms depicted in Fig. 1.

Compound	Concentration/pg/μl
4-Aflu	13.6
Acr	11.2
B[f]q	8.8
Phen	8.8
B[h]q	8.8
Acr-d9	11.0
4-Apy	4.0
1-Afla	11.2
B[a]a	4.0
B[c]a	4.0
10-Abap	13.6
7,10-Dmeb[c]a	4.0
7,9-Dmeb[c]a	4.0
Db[a,i]a	10.4
Db[a,j]a	15.2
Db[a,h]a	4.0
Db[a,c]a	4.0
Db[c,h]	4.0
Db[a,j]a-d13	16.0

ing sample preparation and signal suppression or enhancement due to matrix effects (ME). ME can cause considerable problems of LC–MS/MS analysis methods. Their origins are complex, but the major sources are co-eluting organic and inorganic components which can affect the ionization process of the analytes. Matrix effects are subject to the geometry of the ionization source and the kind of ionization. Further confounding factors are the physical and chemical properties of the analytes. As outputs especially detection capability, and quantification are adversely affected [51,52]. To investigate the ME for the described method, sample extracts in acetonitrile – obtained after sample preparation of a loaded quartz fibre filter – were spiked with standard solutions leading to concentrations between 4 pg/μl and 16 pg/μl. To calculate the ion suppression or enhancement due to the matrix effect, peak areas of the chromatograms of the spiked samples were compared with peak areas of the diluted spike standards. Values calculated (Table 4) demonstrate that significant matrix effects can be observed only for the dibenzacridines. Fortunately, deuterated internal standards acridine-d9 and Dibenz[a,j]acridine-d13 show similar behaviour as their non-deuterated analogues concerning fragmentation, imprecision, recoveries and matrix effects as well.

Table 5
Concentrations of azaarenes in the MRM chromatograms shown in Fig. 2.

Compound	Concentration/pg/μl	Concentration/ng/m^3
4-Aflu	3.3	0.012
Acr	62.0	0.225
B[f]q	8.4	0.031
Phen	6.4	0.023
B[h]q	3.7	0.014
4-Apy	7.9	0.029
1-Afla	5.9	0.021
B[a]a	2.5	0.009
B[c]a	6.4	0.023
10-Abap	1.3	0.005

Therefore they are well suited for correction of real-sample effects during routine analysis.

Limits of quantification were automatically calculated by the Analyst software for every analysis on basis of the signal/noise ratio (S/N × 10). In routine analysis all peaks with a S/N above 10 were declared as positive results. Mostly quantification limits estimated analyzing real samples of particulate matter were in the range between 0.2 and 1.4 pg/μl.

Compared to recent analytical methods (cf. Section 1, Introduction), the LC–APPI/MS/MS method presented offers the following advantages for the determination of azaarenes in particulate matter:

ASE extraction, absence of additional clean-up or prefractionation steps, and low flow rates (0.4 ml/min) lead to short analysis times as well as to reduced amounts of organic waste lowering the total costs. Low limits of quantification, high selectivity, good reliability and last but not least the broad range of target substances relevant for ambient particulate matter make the method attractive for monitoring campaigns with high sample numbers on one hand, and for the investigation of behaviour and fate of selected azaarenes in the environment on the other hand.

3.3. Samples of particulate matter—method application

In winter 2008 ambient particulate matter ($PM_{2.5}$) was collected on the campus of the Helmholtz Zentrum Muenchen with an Anderson High Volume sampler. Quartz fibre filters were loaded on 10 working days (11th–23rd of December) during 24 h, respectively. Filter samples were treated as described in Section 2.4 and azaarene concentrations were determined. The results obtained in this short

Table 4
Recoveries, imprecision and matrix effect of the LC–APPI/MS/MS method for the determination of azaarenes in particulate matter.

Compound	Matrix independent recovery/% (RSD[a]/%) $n = 10$	Matrix dependent recovery/% (RSD[a]/%) $n = 10$	Imprecision RSD[a]/% $n = 7$	Matrix effect/% $n = 2$	LOQ[c, d]/pg/μl (S/N × 10)
4-Aflu	41 (18)	63 (9)	4	93	0.41
B[h]q	43 (20)	63 (9)	4	106	0.26
Phen	64 (10)	78 (8)	5	100	0.43
Acr	58 (12)	80 (11)	7	100	1.42
Acr-d9	55 (11)	57 (13)	10	98	n.d.[b]
B[f]q	69 (9)	78 (8)	5	102	0.51
1-Afla	85 (8)	80 (8)	5	105	0.23
4-Apy	81 (7)	83 (8)	5	113	0.24
B[a]a	77 (4)	82 (9)	7	105	0.63
B[c]a	83 (6)	89 (9)	7	120	1.19
10-Abap	83 (5)	74 (17)	7	120	0.35
7,9-Dmeb[c]a	83 (6)	85 (9)	5	125	0.28
7,10-Dmeb[c]a	82 (6)	87 (10)	6	125	0.46
Db[a,j]a	76 (7)	94 (13)	11	134	0.57
Db[c,h]a	81 (6)	90 (11)	5	118	0.73
Db[a,i]a	67 (6)	79 (11)	11	150	1.40
Db[a,j]a-d13	78 (6)	93 (10)	8	135	n.d.[b]

[a] RSD: relative standard deviation.
[b] not determined.
[c] Limit of quantification.

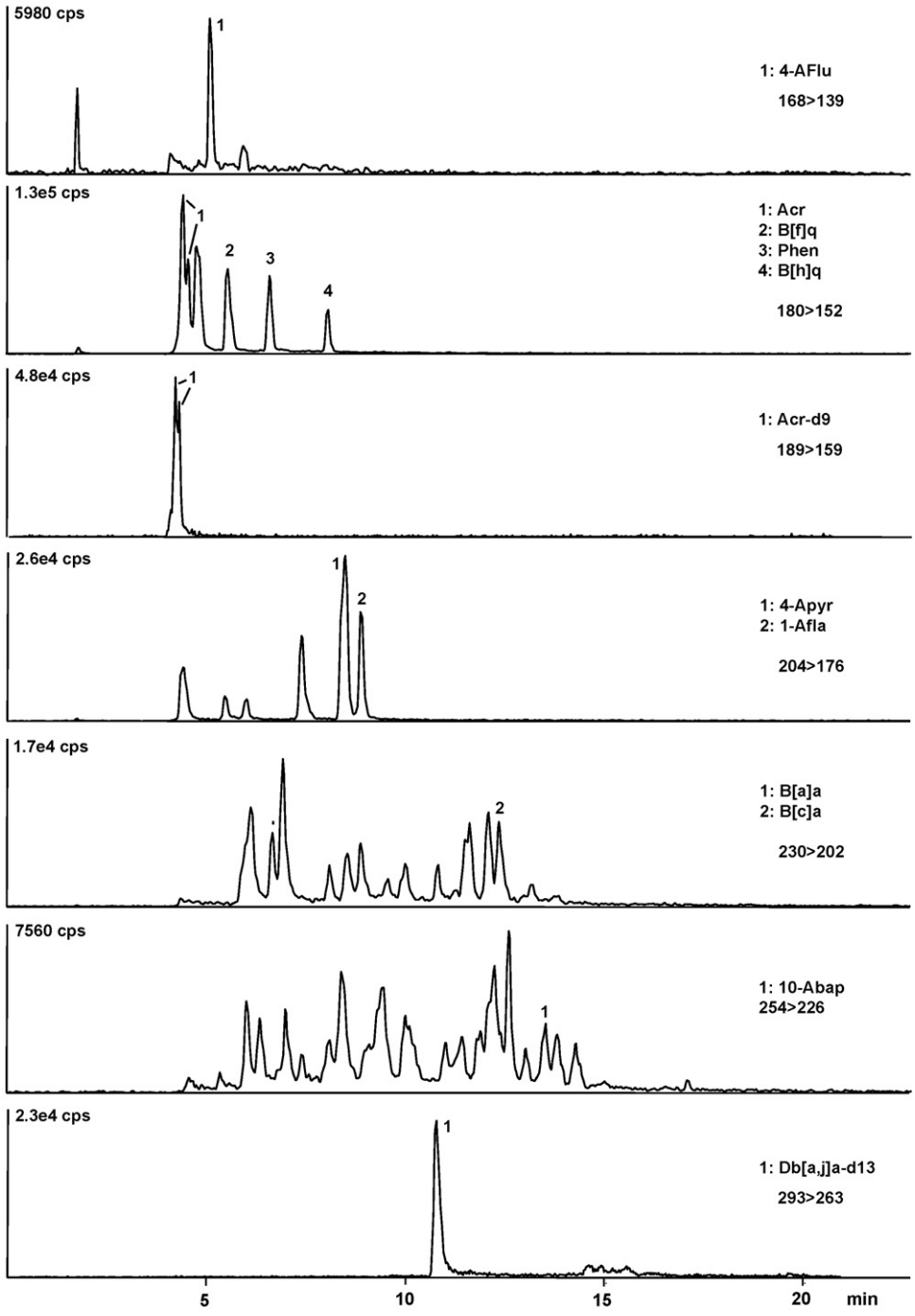


Fig. 2. MRM chromatograms of an extract of particulate matter ($PM_{2.5}$). Concentrations are listed in Table 5.

study were not corrected for recovery. Applying a routine, established HPLC–FD method for PAH-analysis in particulate matter [49], PAH content of the samples was also determined.

The area of the Helmholtz Zentrum Muenchen is a semiurban site located on the northern outskirts of Munich. The shortest distance to the city border is about 0.4 km. The sampling site is expected to be mainly influenced by traffic emissions from gasoline and diesel engines. But in addition to traffic sources emissions from industrial processes, domestic and communal heating can be transported to and collected by the High Volume sampler. It should be pointed out, that using the sampling technique described, only the particle bound part of the aerosol is grasped, azaarene content of the vapour phase is neglected in this study.

In Fig. 2 MRM chromatograms of azaarenes extracted from particulate matter ($PM_{2.5}$) are shown. The analytes detected are separated and they can be identified due to chromatographic separation and specific transitions. It is obvious that with increasing molecular size the number of isobaric compounds – probably mainly isomers – is also increasing. This observation underlines several points: On the one hand identification and quantification of a higher number of (isomeric) azaarenes seems to be important. On the other hand the identification and use of marker or tracer compounds – perhaps like acridine, or one representative analyte for each ring size or azaarene compound class – will be more helpful, because the enormous number of possible isomers can probably not be separated and quantified with acceptable speed and relia-

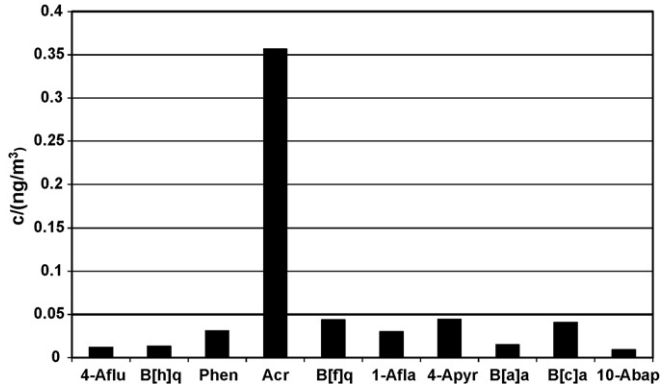


Fig. 3. Concentrations of azaarenes determined in ambient particulate matter ($PM_{2.5}$) collected on 15th December 2008.

bility. Here more studies which investigate correlations between relevant compounds and identify possible markers are needed.

Fig. 3 shows a typical concentration pattern of the azaarenes detected in the real samples. Dibenzacridines and dimethylbenz[c]acridines were below the limit of quantification in all samples. The profiles of the sampling days all look very similar. This impression is supported considering correlation coefficients between the single substances and acridine, which exhibits highest concentrations in all samples. Correlation coefficients (ρ) were between 0.75 and 0.92 indicating a good correlation between acridine and the analytes found in the samples. To support the assumption that acridine can really be useful as a tracer or marker substance for azaarenes, further investigations at different sites and seasons are necessary because meteorological conditions and sources of course significantly influence the pattern. But also the correlation coefficients (ρ) between the other azaarenes are mainly > 0.9, and never < 0.6. This can mean that the main sources as well as alterations during particle transport and sampling remained relatively constant over the sampling period. Similar observations were made by Chen et al. who compared 2-, 3-, and 4-ring azaarenes and also found significant correlations for the concentrations during the sampling period [9].

In Fig. 4 sums of PAH and azaarenes on the sampling days as well as mean values for daily temperatures and precipitation are depicted. Due to the short sampling period there is no significant correlation between ambient mean temperature and azaarene concentration. The same is true for the relation between azaarene concentration and precipitation, although highest values were found on days without rain or snow and vice versa. Taking into account the PAH-concentration, a clear relation for these 10 days is found, the respective analyte sums correlate with $\rho = 0.960$. This supports the assumption that PAHs and azaarenes have similar sources and behaviour in the environment. Absolute concentrations of azaarenes are mostly approximately one tenth of the PAH concentration, with values between 0.002 ng/m^3 and 0.356 ng/m^3 for single compounds, and between 0.12 ng/m^3 and 0.60 ng/m^3 for the sum of the analytes. Comparing these levels with results in the literature it can be realized that they are in a similar range, with a tendency to lower concentrations. Chen et al. described values in the range from 0.4 ng/m^3 to 3.7 ng/m^3 in Liverpool, Osborne et al. measured concentrations from 0.007 ng/m^3 to 3 ng/m^3, also in Liverpool [9,14]. Warzecha et al. published azaarene concentrations between 1.19 ng/m^3 and 2.29 ng/m^3 in particulate matter collected in the Upper Silesia region collected in the summer season [13]. In these samples acridine also always exhibited highest values. Delhomme et al. analyzed azaarenes in particles collected in Strasbourg during one year, covering all seasons. They found concentrations between 0.8 ng/m^3 and 3 ng/m^3. In accordance to the other studies, concentrations were highest in the winter period [12]. Higher azaarene concentrations during colder periods can have several reasons. The two most significant can be increased use of wood burning, domestic and communal heating on one hand; shifting of the gas/particle partition of azaarenes towards an increased part of particle bound analytes in colder seasons compared to warmer periods on the other hand [9,12].

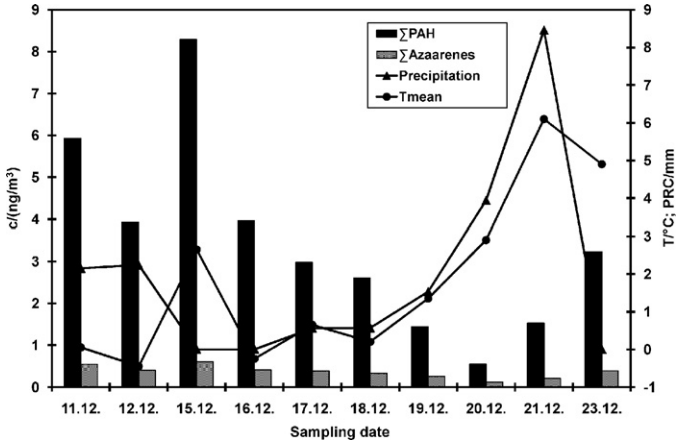


Fig. 4. Sums of azaarene- and PAH-concentrations determined in ambient particulate matter ($PM_{2.5}$) in December 2008. Mean values of temperature and precipitation on the sampling days are shown as lines to guide the eyes.

It must be taken into consideration again that only ten samples were collected to support practicability of the LC–APPI/MS/MS method described in this paper. To obtain data sets allowing more detailed and reliable interpretations concerning sources, behaviour in the environment, meteorological influences and identification of potential marker substances, more studies with higher sample numbers have to be performed.

4. Conclusion

A LC–APPI/MS/MS method for the determination of 15 azaarenes in particulate matter was developed, optimised and validated. The method is characterized by high sensitivity and selectivity due to the use of the multiple reaction monitoring mode for detection and quantification. Sample preparation is fast and efficient consisting only of ASE extraction and solvent exchange. Validation results underline good reliability and practicability of the described method allowing its routine use during monitoring programs. To demonstrate applicability of the LC–APPI/MS/MS method ten samples of $PM_{2.5}$ were collected during December 2008 and successfully analyzed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.029.

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