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DETERMINATION OF NITROGEN-CONTAINING PAH's IN AEROSOLS BY LC/MS/MS

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ABSTRACT

A new method for the determination of 17 basic azaarenes and 5 amino-PAH's is presented. The analysis is performed using high performance liquid chromatography coupled with tandem mass spectrometry. As ionization technique the atmospheric pressure chemical ionization was employed. For the quantification of the analytes one precursor ion and two daughter ions per substance were selected. The procedure reaches LOQ from 2-50 pg/µl, corresponding to a concentration of 0.2-0.5 pg/m^3.

Key Words: Aerosol, Amino-PAH, APCI, Azaarene, LC/MS/MS

1. INTRODUCTION

Plenty of health hazard research questions have addressed the volatile and semi volatile nature of the ambient aerosol. However, the knowledge about more polar, less volatile substances still remains very limited. The N-PAH's belong to the chemical characteristics of the particulate air pollution responsible for health effects.
N-PAH's are commonly found in fossil fuels and their derivatives, although some may also form during combustion processes. They have been identified in tobacco smoke (Wynder and Hoffman, 1967), automobile exhaust (Grimmer, 1985), atmospheric particulates (Chen and Preston, 1998), crude oil (Grimmer et al., 1983), river and lake sediments (Wakeham, 1979), sewage (Bodzek et al., 1999) and charcoal-grilled meat (Janoszka et al., 2004).
Relative to their parent PAH's, the N-PAH's are more soluble in aqueous environments. Increased water solubility results in an increased potential for harm. For example, 1-aminopyrene demonstrates a 50-fold increase in mutagenic activity compared to the parent PAH, pyrene (Ho et al., 1981). Although N-PAH's are only present in trace amounts, care must be taken to avoid exposure due to their carcinogenic and mutagenic nature (Seixas et al., 1982, Matsuoka et al., 1982).
In order to identify and differentiate N-PAH's, many investigators have used the chromatographic techniques because of their high resolving power. These techniques have included gas (Schmitter et al., 1982, Kamata et al., 1992), thin layer (Kamata and Motohashi, 1987), supercritical fluid (Ashraf-Korassani and Taylor, 1988), and liquid chromatography (Colin et al., 1981, Schmitter et al., 1982, Borra et al., 1987, Motohashi et al., 1991, Carlsson and Östman, 1995, Wilhelm et al., 2000).

Both the low concentration level of these analytes and the complexity of the ambient aerosol matrix require the development of a selective and sensitive analytical method. Characterized by sensitivity and selectivity, the LC-MS/MS was until now not exploited for the analysis of N-PAH's.
In this study, in order to provide an analytical method which can be implemented in epidemiological studies, a high-throughput-LC/MS/MS-based method was developed for the determination of N-PAH's in aerosols.

2. EXPERIMENTAL

Materials investigated
The urban dust (SRM 1649a) is a certified reference material from the National Institute of Standards and Technology (Promochem, Wesel, Germany). This material was investigated for the presence of the analysed substances as a sample matrix. Two Chinese urban dust samples were also analyzed for the compounds.

Aerosol samples
The aerosols samples were collected on an intersection on a well-driven countryside highway near Munich. A high-volume sampler (HVS, Anderson) was used to collect filter samples of fine particulate matter ($PM_{2,5}$), operating for 24 hours at a rate of 800 L/min. Quartz-fibre filters, 203 mm x 254 mm (QF 20, Schleicher und Schuell) were used in the HVS. The filters were heated before use at 773 K for at least 12 hours in a muffle furnace (Heraeus, Hanau, Germany) to remove organic contaminants. After sampling the filters were wrapped in aluminium foil and placed in a desiccator at 4 °C until analysis.
2 aerosol samples, collected in January and February 2005, were tested for the presence of N-compounds, investigating the applicability of the LC/MS/MS for analysis of real samples.

Chemicals
1-aminonaphtalene (1ANaph), 2-aminofluorene (2AFl), 2-aminoanthracene (2AAnt), 6-aminochrysene (6AChr) and phenanthridine (Phe) were purchased from Sigma-Aldrich, Steinheim, Germany. 1-aminopyrene (1APyr) was from Fluka Chemie AG, Buchs, Switzerland. Acridine (Acr), benzo(a)acridine (BaA), benzo(c)acridine(BcA), dibenzo[a,j]acridine (DbajA), dibenzo[a,c]acridine (DbacA), dibenzo[a,h]acridine (DbahA), dibenzo[a,i]acridine (DbaiA) and 10-azabenzo(a)pyrene (10AbaP) came from Dr. Ehrenstorfer GmbH, Augsburg, Germany. 7,9-dimethylbenzo(c)acridine (7,9-DmbcA) and 7,10-dimethylbenzo(c)acridine (7,10-DmbcA) were from Sigma-Aldrich, Milwaukee, Wisconsin, USA.
Dibenzo[c,h]acridine (DbchA), 4-azapyrene (4APyr), 1-azafluoranthene (1AFlu) and 4-azafluorene (4AFl) came from the PAH research institute, Greifenberg, Germany. Benzo(f)quinoline (BfQ) and benzo(h)quinoline (BhQ) were from Ultra Scientific, North Kingstown, Rhode Island, USA.
Methanol was purchased from Merck KGA, Darmstadt, Germany, acetonitrile from Sigma-Aldrich, Steinheim, Germany, whereas the water came from a Milli-Q Ultra Plus Water System, Millipore GmbH, Schwalbach, Germany. BcA and BaA were purchased as 10 ng/µl solutions in iso-octane, 10AbaP, DbahA, DbajA, DbaiA and

DbacA as 10 ng/µl solutions in acetonitrile. Stock solutions were prepared with the other substances at a concentration of 100 ng/µl in acetonitrile. The base standards had a concentration of 10 ng/µl for amino-PAH's and 1 ng/µl for azaarenes, in acetonitrile, from which work standards were prepared by dilution.

Instrumentals

The Agilent Technologies HPLC 1100 Series tower (Palo Alto, California, USA) included a G1316A column oven, a G1329A autosampler with thermostat (G1330A), a G1311A quaternary pump and a G1322A degasser. The injected sample volume was 10 µl. Samples were chromatographed on a Gemini C18 (250 x 4.6 mm, 5µ) column from Phenomenex (Aschaffenburg, Germany) equipped with an equal-branded pre-column. Eluent A was 0.1 % formic acid in water and eluent B was methanol, with 800 µl/min flow. The gradient was increased from 70 % methanol to 90 % in 13 min, then to 100 % in 4 min and held for the next 8 min. Over the last 5 min the column was equilibrated prior to the next injection. The column oven had a temperature of 45 °C and the total analysis time was 30 min. The detection was carried out on an API 2000 Triple Quad mass spectrometer from Applied Biosystems (Toronto, Canada) equipped with an APCI ionization source. For system control and data acquisition the Analyst software version 1.4 from Applied Biosystems was used. Following source parameters were employed: TEM 475 °C, NC 2 µA, CUR 24 psi, CAD 5 psi, GS1 80 psi, GS2 50 psi. The focussing potential FP was optimized and fixed on 350 V.

Sample preparation

The urban dust sample was prepared by the procedure of Lintelmann et al. (Lintelmann et al., 2005). The Chinese urban dust samples were prepared as follows: 200 mg sample was weighed in a 75 ml centrifuge tube and 20 ml dichloromethane were added. The tube was closed and sonicated in an ultrasonic bath (Bandelin, Berlin, Germany) for two hours, shaking lightly every 15 minutes. The extracts were filtered on 597 1/2 folded paper filters (Schleicher and Schuell, Dassel, Germany), collected in 100 ml reduction flasks and reduced to a volume of ca. 1 ml on a VV 2000 Heidolph rotation evaporator (Kelheim, Germany) equipped with a Vacuubrand PC5 diaphragm pump (Wertheim, Germany). The residual solution was transferred in a 2 ml volumetric flask and completely evaporated under nitrogen stream in a Barkey (Leopoldshöhe, Germany) Vapotherm mobil S unit. 500 µl acetonitrile were added and sonicated shortly to support the dissolvation of the residue. The solution was filtered with a 0.2 µm Spartan 13/0.2 RC filter unit (Schleicher and Schuell) into an autosampler vial and subjected to LC/MS/MS analysis.

For each high-volume real sample one third of the filter was cut in small pieces, introduced in a 75 ml centrifuge bottle and sonicated with 40 ml methanol / dichloromethane 1:1 for 45 min. The samples were transferred in 100 ml reduction flasks and evaporated to a volume of 2 ml on the rotation evaporator. The residual solutions were transferred in volumetric flasks and evaporated to approx. 70 µl under a nitrogen stream. 30 µl acetonitrile were added, the samples were shortly sonicated and filtered in autosampler vials.

3. RESULTS AND DISCUSSION

Transitions and MS/MS parameters

The characteristic fragmentation pattern of each compound was used to build three transitions. The SIM (Single Ion Monitoring) as qualifier and the two most sensitive MRM (Multiple Reaction Monitoring) as quantifiers were selected for each compound. The precursor ions were found performing a Q1 total ion current scan for every substance from 50 up to 50 amu above their respective molecular weights.

A built-in syringe-pump was used for infusion of standard solutions of substances with 10 ng/µl each into the APCI interface, with collision-activated dissociation CAD gas set at 0. No adducts were formed during ionization, therefore the molecular ion $[M+H]^+$ was chosen as precursor.

The ionization parameters of the parent ions, i.e., declustering potential DP, focussing potential, entrance potential EP, and collision cell entrance potential CEP were optimized automatically and used for the product ion scan by collision-activated dissociation gas on. For the two most abundant product ions the ionization parameters, i.e., collision cell energy CE and collision cell exit potential CXP were then manually optimized.

Figure 1 shows a typical product ion scan of Benzo(a)acridine. The ions at masses m/z 202.1 and 201.1 correspond to $[M+H - CH_3N]$ and $[M+H - C_2H_4]$, respectively. The MS/MS transitions of the 22 aromatic compounds studied are summarized in Table 1.

The APCI source parameters, i.e., nebulizer gas GS1, auxiliary gas GS2, temperature TEM, curtain gas CUR and collision gas CAD in Q3 were all optimized manually by flow injection experiments.

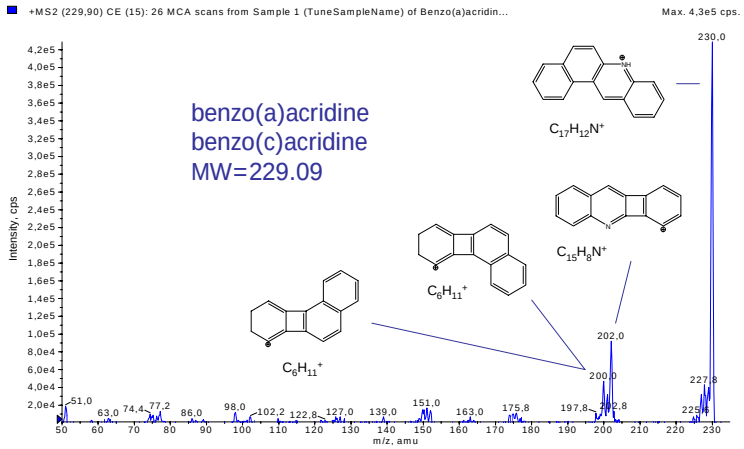

Figure 1. Typical product ion scan and fragmentation of the *m/z* 230.1

HPLC- method development

Based on the work of Bohn et al. (Bohn et al., 2004) experiments were conducted in order to reach a satisfying separation of the compounds. In principle, the baseline separation of all the compounds is not important, since the co-eluting compounds are resolved by the "two-dimensional" selectivity provided by the mass spectrometer.

Table 1. Selected transitions and parameters of the analysed compounds

Analyse	MW amu	Transition m/z	DP V	EP V	CEP V	CE V	CXP V
1ANaph	143.19	143.9 → 143.9	22	12	8	12	12
		143.9 → 126.9	25	11	8	33	11
		143.9 → 117.1	22	11	8	33	11
2AFlu	181.24	182.0 → 182.0	22	12	8	12	15
		182.0 → 165.1	20	10	8	33	14
		182.0 → 164.0	20	10	10	48	15
2AAnt	193.25	193.9 → 193.9	25	12	8	15	16
		193.9 → 177.0	28	9	7	34	15
		193.9 → 165.1	35	11	10	57	12
1APyr	217.27	218.1 → 218.1	25	10	10	14	21
		218.1 → 201.9	25	10	10	37	18
		218.1 → 188.9	22	10	11	68	15
6AChr	243.31	244.0 → 244.0	25	11	8	15	21
		244.0 → 227.1	25	9	7	38	19
		244.0 → 226.1	22	10	8	50	19
10Abap	253.31	254.0 → 254.0	20	10	8	15	21
		254.0 → 227.1	30	10	8	62	21
		254.0 → 226.1	60	10	8	65	21
4AFl	167.21	167.9 → 167.9	15	6	9	15	14
		167.9 → 138.9	20	6	9	40	10
		167.9 → 141.1	25	6	9	65	10
4APyr; 1AFlu	203.24	204.1 → 204.1	45; 20	12	7	15	17
		204.1 → 175.9	55	12	15	60	6
		204.1 → 151.1	55	12	15	60	6
BaA; BcA	229.28	230.1 → 230.1	25	10	10	20	21
		230.1 → 201.9	25; 20	10	15	55	18
		230.1 → 201.1	55; 75	10	15	65	18
DbaiA; DbajA	279.34	280.0 → 280.0	20	6	12	12; 10	12
		280.0 → 252.1	15; 20	8	8	65; 60	21
		280.0 → 251.1	47; 10	8	8	80	21
DbahA; DbacA	279.34	280.0 → 280.0	25	6	12	20	12
		280.0 → 252.1	15	8	8	65	21
		280.0 → 251.1	25	8	8	80	21
DbchA	279.34	280.0 → 280.0	25	8	8	12	21
		280.0 → 252.1	15	8	8	65	21
		280.0 → 251.1	25	8	8	75	21
7,9-DmbcA; 7,10-DmbcA	257.34	258.1 → 258.1	30	12	12	15; 12	12
		258.1 → 242.1	20	12	12	50; 55	15
		258.1 → 241.0	25; 40	12	12	55; 52	15
Acr; Phe	179.22	179.9 → 179.9	30; 25	12	10	15; 16	15
		179.9 → 151.9	20	11	6	50; 47	11
		179.9 → 126.9	25	11	6	55	11
BfQ; BhQ	179.22	179.9 → 179.9	25; 30	12	10	10; 15	15
		179.9 → 151.9	25; 20	11	6	53; 45	11
		179.9 → 126.9	15; 30	11	6	55	11

A third dimension had to be established by means of chromatography, aiming at separating the isomers of the basic azaarenes, i.e., quinolines, benzoacridines, dibenzoacridines, dimethylbenzoacridines, 1-AFlu and 4APyr, showing identical transitions respectively (see Table 1). Bohn et al. used a BDS Hypersil C18 50 x 2.1 mm 3µ (Thermo Hypersil-Keystone) column, employing three separate methods for the determination of the basic azaarenes isomers with ammonium acetate 5 mM / methanol 50/50 as eluent. Further stationary phases like BDS Hypersil C18 50 x 2.1 mm 5µ, Luna Amino 50 x 2.1 mm 5µ, pH 1.5-10, Luna Cyano 50 x 2.1 mm 5µ, pH 1.5-7 and Gemini C18 Twin Technology 250 x 4.6 mm 5µ, pH 1-12 (Phenomenex), SunFire C18 150 x 3 mm 5µ, pH 2-8 and HILIC 150 x 4.6 mm 5µ, pH 1-6 (Waters), presented as appropriated for the separation of basic substances, were tested for the basic azaarenes. Combinations of methanol or acetonitrile with solutions and additives of pH values between 2 and 8 were also tested for the gradient elution. Figure 2 shows the possible co-elution of the azaarene isomers.

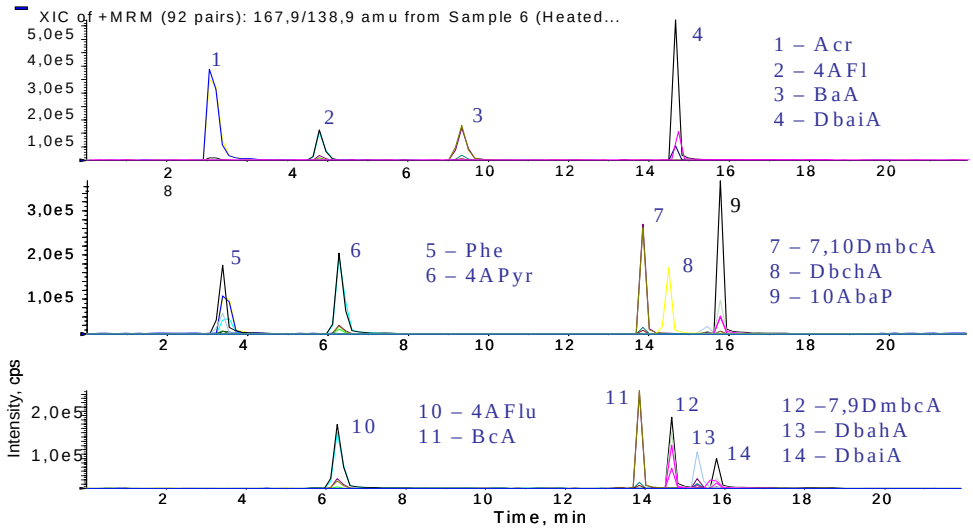

Figure 2. Elution of basic azaarenes using three separate methods on BDS 5µ column with ammonium acetate 5 mM/ methanol 50/50

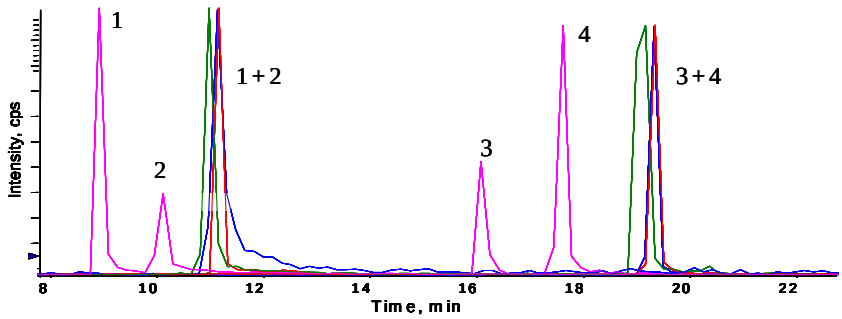

Figure 3. Separation of azaarene isomers

Only on the Gemini column the separation of these isomers succeeded. Figure 3 presents the separation of 1-azafluoranthene (1) and 4-azapyrene (2), respectively 7,9- (3) and 7,10-dimethylbenz(c)acridine (4). Beginning with 60 % acetonitrile and

using pH=8 (ammonium acetate + ammonia), pH=6.5 (10 mM ammonium acetate) or pH=3 (ammonium formiate + ammonia) the two couples of compounds could not be separated. The separation occurred finally with 0.1 % formic acid (pH=2.3), showing that both the pH value and the presence of ammonia ions influence the chromatographic separation of these substances.

The most problematic isomers are the dibenzoacridines, DbahA and DbacA, for which a partial separation could be reached using methanol instead of acetonitrile. Methanol is a protic solvent and interacts with the residual silanol groups of the stationary phase, resolving in this way the two compounds (Figure 4). A further factor, which can be employed in such delicate separation problems, is the oven temperature. In this study, setting the oven temperature on 45 °C amended their chromatographic separation further on. Their quasi co-elution is determined primarily by the steric hindrance of the nitrogen site, and the baseline separation can only be achieved by analysis times longer than 40 minutes, which however would not accomplish the goals of this work. The calculated resolution of the two peaks is 1.0, also less than 1.5 (the value normally used as the benchmark for baseline resolution), but sufficient for the correct integration of the peaks.

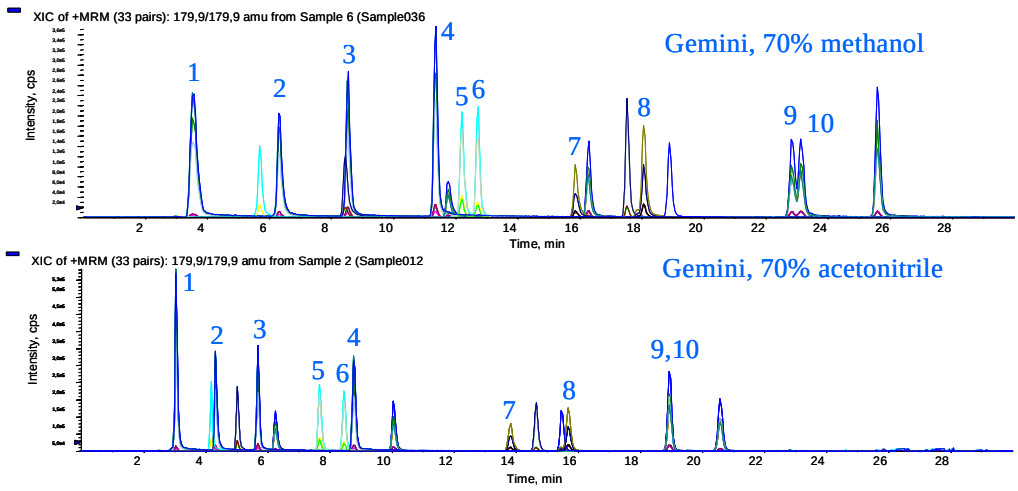

Figure 4. Comparison of organic eluents for the separation of the basic azaarenes
1 – acridine, 2 – benzo(f)quinoline, 3 – phenanthridine, 4 – benzo(h)quinoline,
5 – benzo(a)acridine, 6 – benzo(c)acridine
7 – 7,9-dimethylbenzo(c)acridine, 8 – 7,10-dimethylbenzo(c)acridine
9 – dibenzo(a,h)acridine, 10 – dibenzo(a,c)acridine

Figure 5 presents the optimized separation of the analysed compounds, with a 10 µl sample volume injected and a concentration of 250 pg/µl.
Enhancing the response is also possible by increasing the volume of sample injected. The injection of a double sample volume, 20 µl, do not affect the peak shape and produce also a signal increase of 12 to 80 %, therefore can be successfully applied for further analysis.

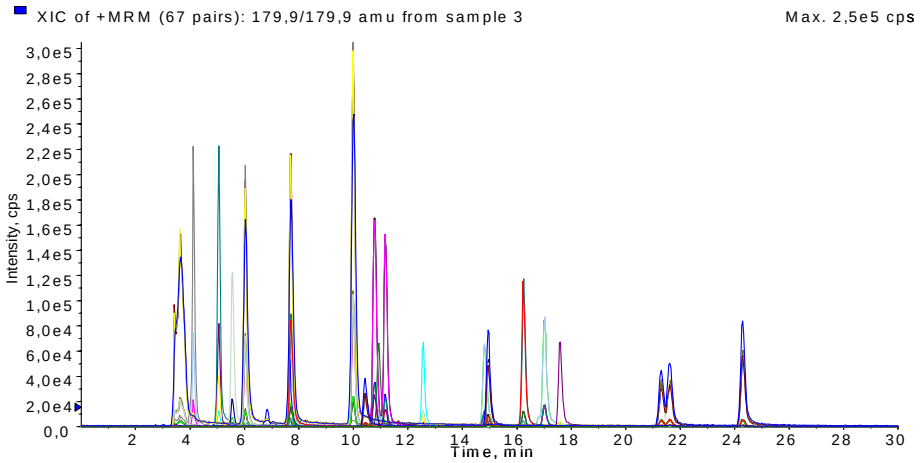

Figure 5. Optimized chromatogram of a standard mixture with 250 pg/µl

Real samples

The analysis of the SRM 1649a (urban dust) showed a considerable content of quinolines (Figure 6). The two Chinese urban dust samples were not revealing the analysed compounds, but the extraction method employed for this purpose is not optimized. The two high-volume filter samples contained 2 amino-PAH's and 10 basic azaarenes. The results are presented in Table 2. The limits of quantification were calculated using the signal/noise value delivered by the Analyst software.

Table 2. Analysis data of filter samples

	Retention time min	January pg/m^3	February pg/m^3	LOQ pg/µl
Acr	3.55	124	431	26
2AFlu	4.02	0	0	6
1ANaph	4.96	1	173	2
4AFl	5.46	1	5	5
BfQ	5.93	6	13	5
2AAnt	7.54	22	33	51
Phe	7.61	6	8	5
BaA	7.61	3	5	7
BhQ	9.92	7	12	5
DbaiA	10.3	0	0	51
4APyr	10.7	5	8	7
1APyr	10.9	0	0	4
1AFlu	11.1	6	10	10
6AChr	12.5	0	0	5
7,10-DmbcA	14.7	0	0	9
DbajA	14.9	0	0	14
BcA	16.2	4	7	11
7,9-DmbcA	17.0	1	1	7
10AbaP	17.6	0	0	8
DbahA	21.0	0	0	17
DbacA	21.8	0	0	17
DbchA	24.0	0	0	27

The stability of the retention time was checked by spiking one of the Chinese samples and comparing the observed values with the standard ones. All the retention times determined in the spiked sample were comprised in a 30 seconds retention time window, fixed during the creation of the quantification method.

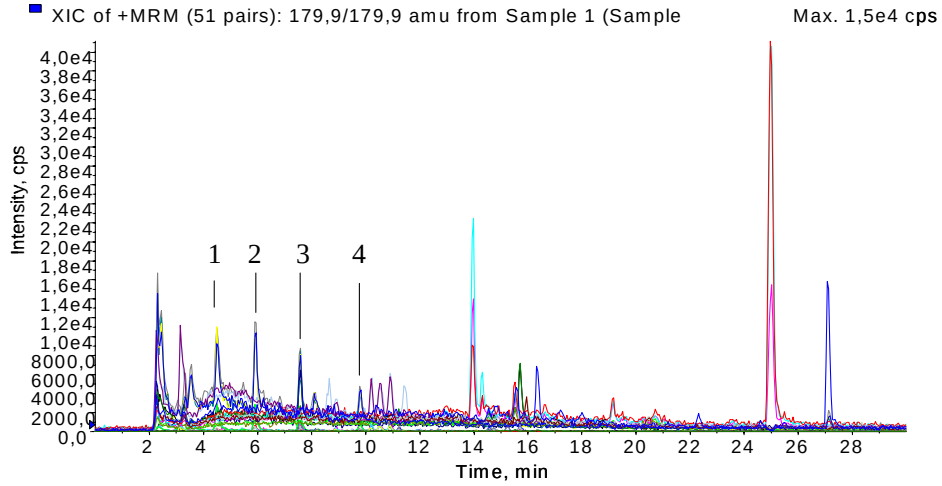

Figure 6. Chromatogramm of SRM 1649a, 1- acridine 18 ppb, 2- BfQ 16 ppb, 3-phenantridine 11 ppb and 4- BhQ 5 ppb.

Matrix effects

Considerable problems of the LC/MS/MS analysis are the so-called matrix effects. Their origins are complex, and the main problem sources are the organic and inorganic molecules present in the sample (Antignac et al., 2005).

The matrix effects underlie to the geometry of the ionization source and the kind of ionization (Souverain et al., 2004). Further influencing factors are for example the

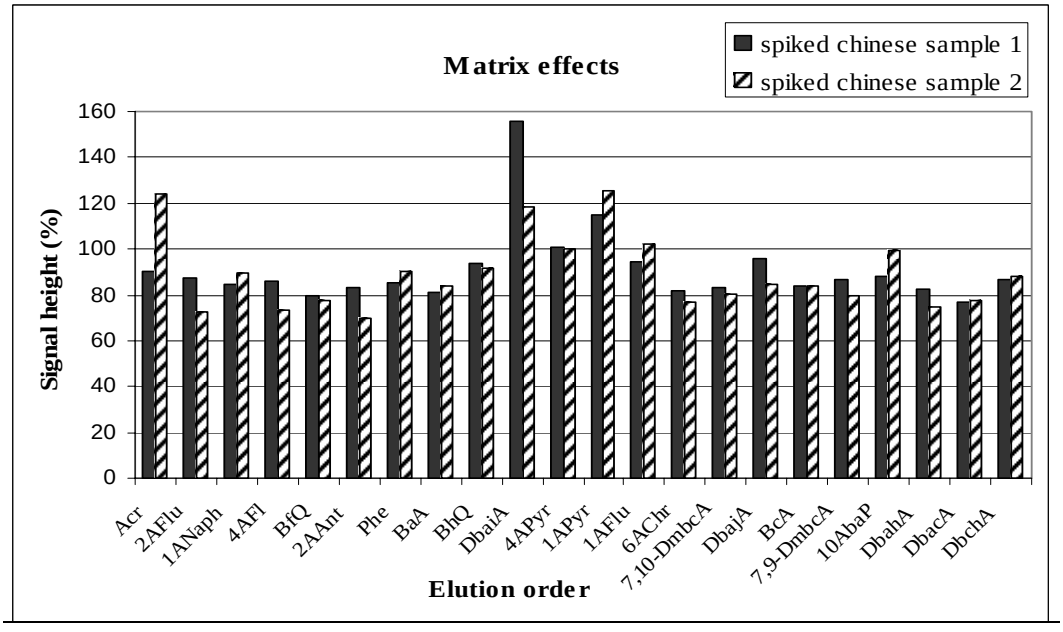

Figure 7. Ionization suppression and enhancement due to matrix compounds

physical and chemical properties of the analytes. As outputs, detection capability, repeatability, linearity and quantification are affected.
To verify the presence of matrix effects, the two Chinese sample extracts were spiked each with 250 pg/µl standard mixture, in triplicate, and analysed. Figure 7 shows the calculated recovery reported to the standard sample. Usually, the matrix effects lead to ionization suppression; however, in this study the enhancement of the ionization was also detected, but only for some compounds.

4. CONCLUSION

A new, unique method is presented for the determination of 5 amino-PAH's and 17 basic azaarenes, with good separation of isomers and limits of quantification between 2 and 50 pg/µl. The short analysis time allows the use of this method to complement the monitoring of epidemiological studies. Here, due to high sample throughput, only individually adjusted methods can be employed.
Further experiments are necessary for the development of a sample preparation procedure which allows the separation of co-extracted matrix compounds. The matrix effects have to be also studied and eliminated or at least compensated. For this purpose, the use of matrix-matched standards will be the most convenient choice.

5. AKNOWLEDGEMENTS

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REFERENCES

Antignac, J.-P., de Wasch, K., Monteau, F., De Brabander, H., Andre, F., Le Bizec, B., 2005. The ion suppression phenomenon in liquid-chromatography-mass spectrometry and its consequences in the filed of residue analysis. Anal. Chim. Acta 529, 129-136)
Ashraf-Khorassani, M. and Taylor, L.T., 1988. Chromatographic behaviour of polar compounds with liquid vs. supercritical fluid mobile phases. J. Chromatogr. Sci. 26, 331-336
Bodzek, D., Dobosz, C., Janoszka, B. and Konecki, J., 1999. Quantification of nitrogen-containing polycyclic aromatic compounds in sewage sludge by TLC-densitometry. J. Planar Chromatogr.—Mod. TLC 12, 265-268
Bohn, P., Wüst, A., Matuschek, G., 2004. Contribution of basic and neutral N-compounds to the adverse health effect of ambient aerosols. Poster at the International Workshop on Organic Speciation in Atmospheric Aerosol Research, April 5-7, Las Vegas, Nevada, USA
Borra, C., Wiesler, D. and Novotny, M., 1987. High-efficiency microcolumn liquid chromatography separation and spectral characterization of nitrogen-containing polycyclics from fossil fuels. Anal. Chem. 59, 339-343
Carlsson, H. and Östman, C., 1995. Retention mechanisms of polycylic aromatic nitrogen heterocyclics on bonded amino phases in normal-phase liquid chromatography. J. Chromatogr. A 715, 31-39

Chen, H. and Preston, M.R., 1998. Azaarenes in the Aerosol of an Urban Atmosphere. Environ. Sci. Technol. 32, 577-583
Colin, H., Schmitter, J.M. and Guiochon, G., 1981. Liquid chromatography of azaarenes. Anal. Chem. 53, 625-631
Grimmer, G., 1985. PAH- Their Contribution to the carcinogenicity of various Emissions. Toxicol. Environ. Chem. 10, 171-181
Grimmer, G., Jacob, J. and Naujack, K.W., 1983. Characterization of CH_2-homologous azaarenes in petroleum by capillary gas chromatography and mass spectrometry. Anal. Chem. 55, 2398-2404
Ho, C.H., Clark, B.R., Guerin, M.R., Barkenbus, B.D., Rao, T.K. and Epler, J.L., 1981. Analytical and biological analysis of test materials from the synthetic fuel technologies. Mutat. Res. 85, 335-345
Janoszka, B., Warzecha, L., Blaszczyk, U. and Bodzek, D., 2004. Organic compounds formed in thermally treated high-protein food, part II: azaarenes. Acta Chromatogr. 14, 129-141
Kamata, K. and Motohashi, N., 1987. Separation of methyl-substituted benz[*c*]acridines by reversed-phase high-performance thin-layer chromatography.
J. Chromatogr. 396, 437-440
Kamata, K., Motohashi, N., Meyer, R. and Yamamoto, Y., 1992. Determination of 7-methylbenz[*c*]acridines by capillary gas chromatography with electron-capture detection. J. Chromatogr. 596, 233-239
Lintelmann, J., Fischer, K., Karg, E., Schröppel, A., 2005. Determination of selected polycyclic aromatic hydrocarbons in aerosol samples by high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 381, 508-519
Matsuoka, A., Shudo, K., Saito, Y., Sofuni, T. and Ishidae, M., 1982. Clastogenic potential of heavy oil extracts and some aza-arenes in Chinese hamster cells in culture. Mutat. Res. 102, 275-283
Motohashi, N., Kamata, K. and Meyer, R., 1991. Chromatographic separation and determination of carcinogenic benz[c]acridines in creosote oils. Environ. Sci. Technol. 25, 342-346
Schmitter, J.M., Ignatiadis, I. and Guiochon, G., 1982. Capillary gas chromatography of azaarenes II. Application to petroleum nitrogen bases. J. Chromatogr. 248, 203-216
Seixas, G.M., Andon, B.M., Hollingshead, P.G.and Thilly, W.G., 1982. The aza-arenes as mutagens for Salmonella typhimurium. Mutat. Res. 102, 201-212
Souverain, S., Rudaz, S., Veuthey, J.-L., 2004. Matrix effect in LC-ESI-MS and LC-APCI-MS with off-line and on-line extraction procedures. J. Chromatogr. A 1058, 61-66
Wakeham, S.G., 1979. Azaarenes in recent lake sediments. Environ. Sci. Technol. 13, 1118-1123
Wilhelm, M., Matuschek, G. and Kettrup, K., 2000. Determination of basic nitrogen-containing polynuclear aromatic hydrocarbons formed during thermal degradation of polymers by high-performance liquid chromatography-fluorescence detection. J. Chromatogr. A 878, 171–181
Wynder, E.L. and Hoffman, D., 1967. Tobacco and Tobacco Smoke, Academic Press, New York