ELSEVIER

Journal of Chromatography A, 767 (1997) 163–169

JOURNAL OF CHROMATOGRAPHY A

Determination of seventeen polycyclic aromatic hydrocarbons in tobacco smoke condensate

G. Gmeiner*, G. Stehlik, H. Tausch

Austrian Research Centre Seibersdorf, A-2444 Seibersdorf, Austria

Received 10 September 1996; revised 25 November 1996; accepted 2 December 1996

Abstract

A method for the quantitative determination of polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke condensate has been developed and was applied to the analysis of the Kentucky Reference cigarette 1R4F. The procedure uses extraction of the filters with methanol, dilution with water, automated solid-phase extraction (SPE) on a C_{18} column for cleanup purposes and elution with cyclohexane prior to gas chromatography–mass spectrometry for quantification. Concentration values for 17 PAHs are given and compared to results of former publications.

Keywords: Tobacco; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a well known group of environmental carcinogens and are subject of intensive investigations. Their most important source is the incomplete combustion of organic materials [1–3]. Ingestion and inhalation are the main pathways into the human body.

The presence of environmental tobacco smoke (ETS) is a significant contributor to PAHs and derivatives in indoor air, where most of us spend more than 80% of our time [4]. Reduction of the amount of PAHs in ETS, mainstream and sidestream, is a driving force for the development of new sorts of cigarettes, in which the tobacco is heated, but does not actually burn [5].

ETS represents a very complex mixture of various classes of compounds, hundreds of PAHs occur in the mainstream and sidestream smoke [6]. The determination even of the most important components of this group of chemical substances presents a challenging task to the analytical chemist. This might be the main reason, why benzo[*a*]pyrene, the leading compound of this group and one of the most potential carcinogenic PAH, was determined exclusively or in combination with only two or three other PAHs. The sophisticated cleanup and determination methods reported by various authors indicate the complexity of the analytical problem: liquid–liquid extractions need large amounts of organic solvents, thus suffering from increased risk of sample contamination and substance losses due to the necessary evaporation procedure. Chromatographic preseparation by low pressure (LC) or high-performance liquid chromatography (HPLC) [1,6–11] is often necessary to resolve the desired fraction from the matrix.

During the last decade of environmental analysis solid-phase extraction has been established as a simple, fast and easy to automate option for selective

*Corresponding author.

PII S0021-9673(96)01092-8

extraction in environmental analysis. Some authors have used different types of cartridges for cleanup of ETS and mainly HPLC for final separation and quantification [1,12,13].

The objective of this investigation was to develop a cleanup and detection method suitable for the quantification of PAHs in mainstream and sidestream smoke. Furthermore, convenient automation of the cleanup procedure was made possible by using SPE. Gas chromatography–mass spectrometry (GC–MS) as the final analysis allowed for high resolution and sensitivity. The performance of the method is demonstrated by analysing the Kentucky reference cigarette 1R4F for the 17 PAHs recommended by the National Institute for Occupational Safety and Health (NIOSH).

2. Experimental

2.1. Chemicals

The native PAHs in certified quality were obtained from Dr. Ehrensdorfer (Augsburg, Germany). As ^{2}H-labelled internal standards, acenaphthene-d_{10}, naphthalene-d_{8}, chrysene-d_{12}, perylene-d_{12}, phenanthrene-d_{10} (Cambridge Isotope Laboratories, Andover, MA, USA) were used. Methanol and cyclohexane in p.a. quality (Merck, Darmstadt, Germany) were distilled before use. C_{18} ec-SPE columns (0.5 g, 3 ml) were received from ICT (Vienna, Austria). Water was obtained by purifying demineralised water using a Milli-Q system from Millipore (Belford, MA, USA). Nitrogen 4.6 and Helium 5.6 were purchased from Messer Griesheim (Gumpoldskirchen, Austria).

2.2. Equipment

For SPE-cleanup a Gilson ASPEC XL sample processor for solid-phase extraction (Villiers-le-Bel, France) was used. The GC–MS system consisted of a GC 8000 gas chromatograph and a MD 800 mass spectrometer from Fisons Instruments (Rodano, Italy). The smoking machine was a Borgwaldt (Hamburg, Germany). Ultrasonic extractions were performed on a Sonorex RK 510-bath from Bandelin Electronics (Berlin, Germany) with a frequency of 35 kHz at 700 W.

2.3. Sample preparation

2.3.1. Smoke condensate collection

Mainstream smoke was collected from 20 1R4F Kentucky Reference cigarettes following the procedures of DIN ISO 3402 for climatic conditions, DIN ISO 3308 for the smoking machine, DIN ISO 4387 for the collection of total particulate matter and DIN ISO 6488 for the determination of humidity. The glass fibre filters were placed in an Erlenmeyer flask and 100 ml of methanol was added. In this arrangement the samples were delivered to our laboratory for analysis.

2.3.2. Sample pretreatment

The procedure started by adding 1 ml internal standard solution in methanol (1 μg/ml) to the whole sample. Then, the mixture was shaken and treated for 15 min on an ultrasonic bath. 20 ml of the liquid phase was pipetted into a 100 ml volumetric flask, 15 ml of methanol were added. The sample was brought to 100 ml with Milli-Q water.

2.3.3. Solid-phase extraction

The C_{18} cartridges were conditioned with 8 ml methanol, followed by 8 ml water–methanol (65:35, v/v). 65 ml of the sample solution from Section 2.3.2 were loaded onto the SPE column with a flow of 15 ml/min. The column was washed with three times 10 ml water, followed by 10 ml of water–methanol (65:35, v/v). After drying with nitrogen (1.0 bar) for 5 min, elution was performed using 4 ml of cyclohexane. Preliminary experiments were carried out with toluene: due to its stronger elution power, about 1/3 of the volume was sufficient. Yet, the extracts were not clean enough for GC–MS analysis without further purification. All steps involving SPE were performed automatically with the ASPEC sample processor.

2.3.4. Preparation of standards

As the concentrations of the individual PAHs of the reference cigarette 1R4F vary over a range of two orders of magnitude, standard mixtures containing alike amounts for all substances were not

considered appropriate for an accurate calibration. Instead, the stock standard solution was composed to reflect approximately the relative concentrations of the individual components in the reference cigarette (which were predetermined roughly by analysing a small volume of the extract without further purification).

Table 1 shows the concentration of the stock standard solution, used for spiking purposes.

First of all a solution of 0.7 μg/l of deuterated PAHs in water–methanol (65:35, v/v) as internal standards was prepared. For working calibration standards used for the SPE-cleanup, a 250 ml aliquot of this internal standard solution was spiked with 250 μl stock standard. Additional standard solutions were prepared by dilution with internal standard solution (1/2, 1/10, 1/20, 1/100 and 1/200, v/v). All solutions underwent the extraction procedure, described above (Section 2.3.3). Recoveries were determined using external calibration with mixtures of labelled and unlabelled PAHs of the same stock solutions in cyclohexane.

Table 1
Ion masses (*m/z*) of the target PAHs and the deuterated internal standards

Compound	Ion masses (*m/z*)	Concentration (μg/ml)
Naphthalene-d_8	136	–
Naphthalene	128	109
Acenaphthylene	152	62
Acenaphthene-d_{10}	164	–
Acenaphthene	154	13
Fluorene	166	90
Phenanthrene-d_{10}	188	–
Phenanthrene	178	45
Anthracene	178	23
Fluoranthene	202	22
Pyrene	202	17
Chrysene-d_{12}	240	–
Benz[*a*]anthracene	228	9.0
Chrysene	228	9.1
Perylene-d_{12}	264	–
Benzo[*b*]fluoranthene	252	4.9
Benzo(*k*)fluoranthene	252	2.1
Benzo[*e*]pyrene	252	1.6
Benzo[*a*]pyrene	252	4.1
Indeno[1,2,3-*c,d*]pyrene	276	11
Benzo[*g,h,i*]perylene	276	4.4
Dibenzo[*a,h*]anthracene	278	8.2

The concentrations apply to the methanolic stock solution.

2.4. Gas chromatography and mass spectrometry

Separation was performed on a DB5MS fused-silica capillary column, 30 m×0.25 mm I.D.; 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA), protected by a guard column (DB1, 3 m×0.32 mm I.D., 0.5 μm film thickness, from the same manufacturer). Carrier gas was helium with an inlet pressure set to 1 bar. The temperature program was: 50°C held for 1 min, 25°C/min to 150°C, 5°C/min to 280°C, 2°C/min to 315°C held for 2 min at 315°C. The split/splitless injector was set to 250°C and 2 μl were injected with the split vent closed for 1 min.

The mass spectrometer was operated in the electron impact mode (EI) using 70 eV ionisation voltage. The ion source temperature was 250°C, and the GC–MS-interface set to 280°C. The analyses were performed by selected ion monitoring (SIM). The ion masses of the target compounds are summarised in Table 1.

3. Results and discussion

3.1. Cleanup optimisation

3.1.1. Comparison of toluene and cyclohexane as eluent

In the preliminary elution experiments with standard mixtures toluene was used, which is known as an efficient eluent for PAHs adsorbed on C_{18}. In fact, only 1.3 ml of toluene are sufficient to elute more than 95% of the total amount from a 0.5 g C_{18} column. But the first test with a real tobacco sample revealed, that numerous interfering matrix compounds coeluted: Fig. 1 illustrates, as an example, the "forest" of peaks appearing at mass 188 (including the target compound phenanthrene-d_{10}, albeit not positively discernible). As toluene was the solvent, a different temperature program had to be used, which evidently caused shifts in the retention times. The analytical conditions are described in the legend of Fig. 1.

To avoid the involvement of additional purification steps, toluene was replaced by the more PAH-selective but less strong solvent cyclohexane as eluent. As expected, a larger volume was necessary for a complete recovery of the target compounds. To

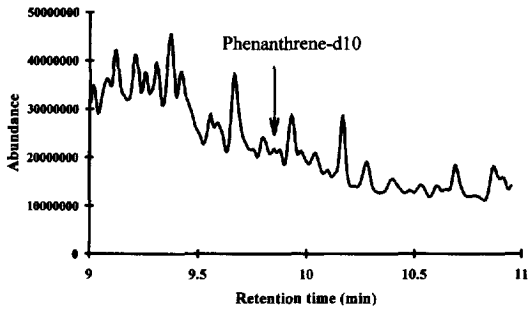

Fig. 1. Selected ion chromatogram (m/z=188). Sample: 1R4F cigarette smoke condensate. Eluent: 1.3 ml toluene. Temperature program: 83°C for 1 min, 15°C/min to 170°C held for 1 min, 5°C/min to 290°C, 10°C/min to 315°C held for 5 min.

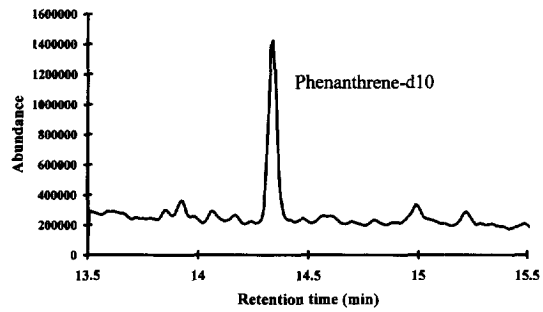

Fig. 3. Selected ion chromatogram (m/z=188). Sample: tobacco smoke condensate. Eluent: 4 ml cyclohexane. For analytical conditions see Section 2.4.

investigate the conditions in more detail, a PAH standard mixture in water–methanol was processed as described in Section 2.3.3 and 2 ml fractions were collected. The results presented in Fig. 2 show, that 4 ml of cyclohexane recovers more than 99% of the doped amount of PAHs.

By applying this optimised elution procedure to a real tobacco smoke condensate, a visually cleaner extract is obtained: Indeed, the region shown in the previous figure now exhibits much less contamination (Fig. 3). In particular, the well shaped peak of phenanthrene-d_{10} is now appropriate for quantitative evaluation.

3.1.2. Chromatograms

In Figs. 4–8 the mass traces for the native PAHs acquiring during analysis of a sample extract compared to the corresponding standard chromatograms, starting with naphthalene (2 rings, Fig. 4) and ending

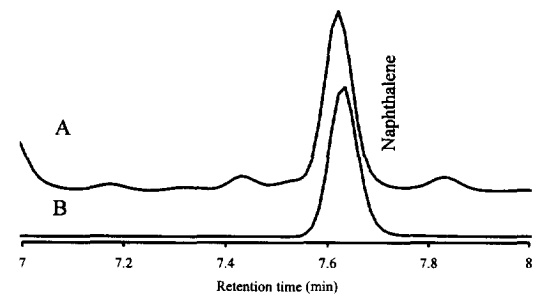

Fig. 4. Selected ion chromatogram, m/z=128. (A) Tobacco smoke condensate and (B) standard, both after cleanup as outlined in Section 2.3.

with the 6-ring PAHs in Fig. 8. These chromatograms demonstrate the effectiveness of the optimised workup method: virtually no contaminations interfere with the proper quantification of the target compounds. Most of the peaks are baseline-resolved,

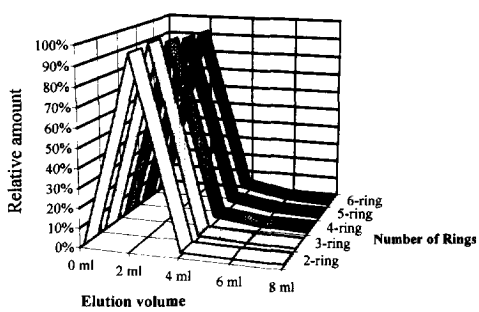

Fig. 2. Elution profile of PAHs, SPE on a C_{18}-column with cyclohexane as eluent.

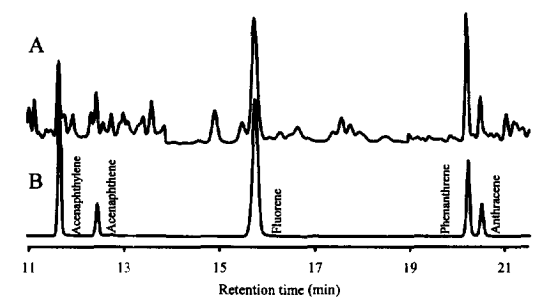

Fig. 5. Selected ion chromatogram, m/z=152 (acenaphthylene), 154 (acenaphthene), 166 (fluorene) and 178 (phenanthrene and anthracene). (A) Tobacco smoke condensate and (B) standard, both after cleanup as outlined in Section 2.3.

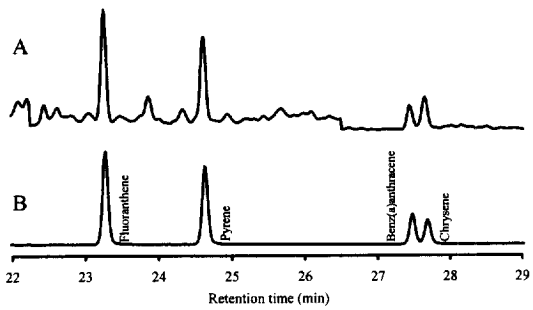

Fig. 6. Selected ion chromatogram, $m/z=202$ (fluoranthene and pyrene) and 228 (benz[*a*]anthracene and chrysene). (A) Tobacco smoke condensate and (B) standard, both after cleanup as outlined in Section 2.3.

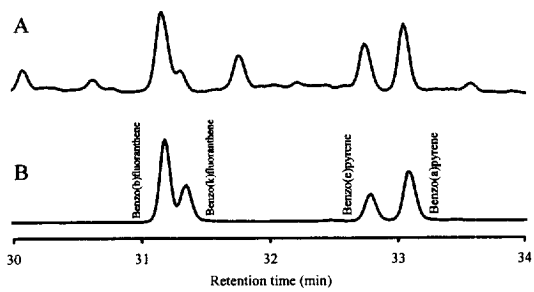

Fig. 7. Selected ion chromatogram, $m/z=252$. (A) Tobacco smoke condensate and (B) standard, both after cleanup as outlined in Section 2.3.

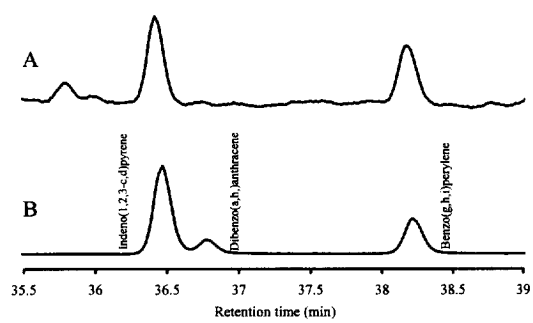

Fig. 8. Selected ion chromatogram, $m/z=276$ (indeno[1,2,3-*c,d*]pyrene and benzo[*g,h,i*]perylene) and 278 (dibenzo[*a,h*]anthrazene). (A) Tobacco smoke condensate and (B) standard, both after cleanup as outlined in Section 2.3.

including the labelled internal standards (not shown in the figures).

Concentration values for the analysed PAHs are given in Table 3.

3.2. Validation results

To describe quantitatively the analytical performance of the entire procedure, certain quality criteria were determined [14]. In, Table 2, the lower working range limit (LWR), upper working range limit (UWR), both representing the lowest resp. highest concentration of the calibration standards, the correlation coefficient (CC), the relative standard deviation for the entire procedure (S.D.P) and the recovery rate (R) are given.

Satisfactory results were obtained for almost all compounds: The relative standard deviation over the entire procedure is below 10% for all target compounds, most of them attaining values below 5%. The correlation coefficient is better than 0.99 for all PAHs. With the exception of one compound, recoveries exceed 70%. The comparatively low rate of 57% obtained for dibenzo[*a,h*]anthracene is the main reason for the slightly higher standard deviation and the moderate correlation coefficient of this compound.

3.3. Results for the Kentucky Reference cigarette 1R4F

Results for the reference cigarette 1R4F are summarised in Table 3: six independent smoke condensate samples were analysed, each corresponding to 20 cigarettes. For any target PAH the relative standard deviation is less than 12%, except for dibenzo[*a,h*]anthracene, whose concentration is close to the lower limit of the working range. In this table, the issued standard deviations include the uncertainty of the condensate collection procedure.

Naphthalene is the dominating compound, followed by fluorene and phenanthrene. The high-molecular-mass PAHs (4–6 rings) exhibit rather small concentrations compared to those containing only 2 and 3 rings. The PAH-pattern corresponds fairly well to that found in indoor air affected by environmental tobacco smoke [4,5].

Table 2
Performance parameters for the entire procedure, representing 10 calibration points within the working range

Compound	LWR (ng/cig.)	UWR (ng/cig.)	CC	S.D. P (%)	R (%)
Naphthalene	7.8	1560	1.000	1	92
Acenaphthylene	4.4	879	0.992	8	106
Acenaphthene	1.0	186	1.000	1	91
Fluorene	6.5	1290	1.000	2	95
Phenanthrene	3.2	636	1.000	1	91
Anthracene	1.6	323	0.999	2	93
Fluoranthene	1.6	311	0.993	7	86
Pyrene	1.2	240	0.996	5	86
Benz[*a*]anthracene	0.7	129	0.998	4	79
Chrysene	0.7	130	0.999	3	80
Benzo[*b*]fluoranthene	0.4	69	0.998	4	91
Benzo[*k*]fluoranthene	0.2	30	0.994	7	84
Benzo[*e*]pyrene	0.1	24	0.999	3	94
Benzo[*a*]pyrene	0.3	59	0.998	4	92
Indeno[1,2,3-*c*,*d*]pyrene	0.8	154	0.998	4	72
Benzo[*g*,*h*,*i*]perylene	0.3	63	0.999	3	74
Dibenzo[*a*,*h*]anthracene	0.6	118	0.990	9	57

3.4. Scope and limitations of the method

The method presented in this work is characterised by high specificity and ample sensitivity. As it covers a wide concentration range, it is well suited to the analysis of tobacco smoke condensate collected from different brands of cigarettes. The procedure was tested by smoking 20 1R4F cigarettes onto one filter and using 20% of the entire methanolic extract for cleanup and analysis. However, the method can easily be adapted to samples containing either higher or lower PAH amounts: for example by working up 35% of a raw extract derived from 60 cigarettes, the detection limits were lowered by the factor of 5. This way much lighter cigarettes than the 1R4F could be investigated achieving essentially the same performance.

As any evaporation step is avoided, there is less risk of loosing volatile analytes such as naphthalene. Moreover, the background level decreases (the method blank invariably is a matter of concern due to the almost ubiquitous presence of PAHs in our environment).

By using only a small amount of organic solvent, toxicity and waste problems are minimised, too.

Both the obtained specificity and the sensitivity adhere to a great extent on the use of MS–SIR detection method: The relatively high costs of the necessary instrumentation (which moreover requires a highly skilled operator) obviously has to be considered as a certain disadvantage.

Table 3
223Concentration values and precision for the target PAHs in the Kentucky Reference cigarette 1R4F; each value represents 3 repeated determinations

Compound	Mean (ng/cig.)	S.D. (ng/cig.)	R.S.D. (%)
Naphthalene	236	19.3	8
Acenaphthylene	50.4	3.9	8
Acenaphthene	25.3	1.3	5
Fluorene	119	4.7	4
Phenanthrene	110	3.8	3
Anthracene	38.1	2.3	6
Fluoranthene	46.2	2.0	4
Pyrene	33.2	1.5	5
Benz[*a*]anthracene	13.2	0.5	4
Chrysene	21.8	0.9	4
Benzo[*b*]fluoranthene	8.6	0.2	3
Benzo[*k*]fluoranthene	1.5	0.1	5
Benzo[*e*]pyrene	4.0	0.1	3
Benzo[*a*]pyrene	7.9	0.2	3
Indeno[1,2,3-*c*,*d*]pyrene	3.5	0.4	11
Benzo[*g*,*h*,*i*]perylene	2.5	0.3	12
Dibenzo[7*a*,*h*]anthracene	0.6	0.1	21

Table 4
Comparison of results for 1R4F

	Tomkins et al. (1985) [11]	Risner (1988) [8]	Risner (1991) [7]	Dumont et al. (1993) [13]	Evans et al. (1993) [6]	This work
Benzo[*a*]pyrene	6.6	6.4	9.2	8.5	5.3–8.2	7.9
Benz[*a*]anthracene			10.5			13.2
Benzo[*b*]fluoranthene					3.9–7.6	8.6
Benzo[*k*]fluoranthene					1.8–3.2	1.5

3.5. Comparison of the results with other publications

The results correspond well to the rare available information on PAH concentrations in the mainstream smoke of the Kentucky reference cigarette 1R4F.

Table 4 shows a comparison to the data accessible in the open literature.

4. Conclusion

A method has been developed for the determination of up to 17 PAHs in the mainstream smoke of cigarettes and was applied to the analysis the Kentucky reference cigarette 1R4F. For the entire procedure and all target compounds, the relative standard deviations were $<10\%$ and the correlation coefficients ≥ 0.99. Except for one compound, namely dibenzo[*a*,*h*]anthracene, recoveries exceeded 70%. Although the work presented only deals with mainstream smoke, the method is also suitable for sidestream smoke [15]. The more than ten times higher PAH-concentrations in sidestream smoke [6] require only an adequate dilution of the sample. The possibility to automate the solid-phase extraction procedure allows a cost effective monitoring of PAHs in cigarette smoke.

Acknowledgments

We gratefully thank Dr. Klus and Mr. Begutter from Austria Tabak for supplying tobacco smoke condensates and for helpful discussions on the field of tobacco analysis. Gratitude is also expressed to Prof. E. Schmid of the Analytical Institute of the University of Vienna for supporting this work.

References

[1] M.L. Lee, M.V. Novotny and K.D. Bartle, Analytical Chemistry of Polycyclic Aromatic Compounds, Academic Press, New York, 1991.
[2] M.N. Kayali and S. Rubio-Barroso, J. Liq. Chromatogr., 18 (1995) 1617.
[3] J.P. Buchet, M. Ferreira Jr., J.B. Burrion, T. Leroy, M. Kirsch-Volders, P. Van Hummelen, J. Jacques, L. Cupers, J.P. Delavignette and R. Lauwerys, Am. J. Ind. Med., 27 (1995) 523.
[4] J.C. Chuang, G.A. Mack, M.R. Kuhlman and N.K. Wilson, Atmos. Environ., 25B (1991) 369.
[5] C.H. Risner and J.M. Conner, J. Liq. Chromatogr., 14 (1991) 437.
[6] W.H. Evans, N.C. Thomas, M.C. Boardman and S.J. Nash, J. Sci. Total Environ., 136 (1993) 101.
[7] C.H. Risner, Beitr. Tabakforsch., 15 (1991) 11.
[8] C.H. Risner, J. Chromatogr. Sci., 26 (1988) 113.
[9] E.R. Schmid, G. Bachlechner, K. Varmuza and H. Klus, Fresenius J. Anal. Chem., 322 (1985) 213.
[10] R. Williams, C. Sparacino, B. Petersen, J. Bumgarner, R.H. Jungers and J. Lewtas, Int. J. Environ. Anal. Chem., 26 (1986) 27.
[11] B.A. Tomkins, R.A. Jenkins, W.H. Griest, R.R. Reagan and S.K. Holladay, J. Assoc. Off. Anal. Chem., 68 (1985) 935.
[12] L.A. Gundel, K.R.R. Mahanama and J.M. Daisey, Environ. Sci. Technol., 29 (1995) 1607.
[13] J. Dumont, F. Laroque-Lazure and C. Iorio, J. Chromatogr. Sci., 31 (1993) 371.
[14] DIN 38 402, Teil 51, Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, Allgemeine Angaben (Gruppe A), Kalibrierung von Analysenverfahren, Auswertung von Analysenergebnissen und lineare Kalibrierfunktionen für die Bestimmung von Verfahrenskenngrößen (A 51), Beuth Verlag Berlin, Mai 1986.
[15] G. Gmeiner, unpublished work.