ELSEVIER

Journal of Chromatography A, 923 (2001) 287–293

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Mass spectrometric detection in narrow-bore (0.10 mm I.D.) capillary chromatography
Fast, sensitive and selective analysis of polychlorinated biphenyls

A. Covaci*, P. Schepens

Department of Pharmaceutical Sciences, Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

Received 9 March 2001; received in revised form 9 May 2001; accepted 28 May 2001

Abstract

The combination of narrow-bore capillary gas chromatography with bench-top quadrupole mass spectrometric detection was evaluated for the determination of polychlorinated biphenyls (PCBs). The qualitative and quantitative performances of the system are illustrated by several analyses (PCB standards and human milk extracts). Capillary columns with different internal diameters (0.10, 0.18 and 0.22 mm, respectively) were compared for their ability to separate PCB congeners and the analysis time. Short run times (less than 7 min) were sufficient for complete separation of PCB congeners on a 0.10-mm internal diameter (I.D.) capillary column without any loss of resolution when compared with a 0.22 mm I.D. column. Good qualitative and quantitative data acquisition was possible with quadrupole mass spectrometer for run times of 8 min, but incomplete peak reading was observed when run times were reduced to 3–4 min. Selected ion monitoring and dwell times of 10 ms are necessary to obtain detection limits for individual PCB congeners as low as 0.4 pg μl^{-1} for standard solutions and 0.2 ng g^{-1} fat for milk extracts. By using cold splitless injection, relatively high volumes (1 μl) for narrow-bore capillaries could be injected without any peak distortion.

Keywords: Capillary columns; Injection methods; Polychlorinated biphenyls

1. Introduction

Since the introduction of capillary gas chromatography (GC), there has been a permanent request for faster and more sensitive analytical methods. From a theoretical point of view, the reduction of the internal diameter (I.D.) of the column is an attractive way towards shorter analysis times [1–3]. Furthermore, the use of narrow-bore capillary columns (I.D. less than 0.10 mm), near the conditions for minimum plate height allows a good chromatographic separation when structurally related compounds are analysed [3,4]. Despite a high number of samples to be analysed for organochlorine pollutants, only limited attention has been paid to the coupling of narrow-bore capillary columns with electron capture detectors or mass spectrometers. A 5 m CP Sil-8 column with 0.05 mm I.D. was used in combination with electron-capture detection (ECD) for the separation of polychlorinated biphenyls (PCBs) from different matrices and Aroclor mixtures [5]. However, relatively long run times (up to 15 min) were used for complete separation of PCB congeners from interfering materials. Separation of selected organochlorine

*Corresponding author. Tel.: +32-3-820-2702; fax: +32-3-820-2722.

E-mail address: covaci@uia.ua.ac.be (A. Covaci).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01001-9

pesticides was done in less than 4 min using a 10 m column with 0.10 mm I.D. and pulse-discharge ECD [6], but relatively high detection limits were obtained from water extracts. In another application [7], two 20 m DB-5 and DB-1701 capillary columns with 0.10 mm I.D. in combination with ECD were used for complete characterization of PCB congeners in sediment extracts. Fast GC using a 3 m column with 0.25 mm I.D. was used for PCB determination [8]. Besides an important loss of resolution, no improvement of detection limits was observed and restricted applicability to complex mixtures was observed. Although there is a need for improvement of selectivity for complex mixtures, the use of a selective detection method such as quadrupole mass spectrometry (MS) in combination with narrow-bore capillary columns was not fully investigated.

Here we show that narrow-bore (0.10 mm I.D.) capillary columns can be used in combination with quadrupole MS for the selective and sensitive determination of PCBs from human matrices. By using cold splitless injection, relatively large volumes (1 μl) for narrow-bore capillaries could be injected without any peak distortion. Thus, the technique can be used with success in trace analysis for environmental matrices.

2. Experimental

2.1. Chemicals and samples

PCB congeners (IUPAC Nos 28, 46, 52, 74, 99, 101, 105, 110, 118, 138, 143, 149, 153, 156, 170, 180, 187 and 194) were available in solution (10 ng μl^{-1} in isooctane) from Dr Ehrenstorfer Labs. (Augsburg, Germany). PCB 46 and PCB 143 were used as internal standards. Dilutions were made in isooctane in order to cover the entire range of PCBs expected in the samples. All solutions were stored at −20°C. All solvents (methanol, acetonitrile, hexane, dichloromethane, isooctane) were pesticide grade (Merck, Darmstadt, Germany). Human milk samples were collected at the University Hospital of Iassy, Romania. Sample preparation and clean-up were as previously described [9].

2.2. GC–MS

A Hewlett-Packard (Palo Alto, CA, USA) 6890 GC system was connected via a direct interface to a HP 5973 quadrupole mass spectrometer. The interface temperature was set at 300°C. Helium was used as carrier gas. Samples were injected into an empty baffled liner (1.5 mm I.D.) of a Gerstel (CIS 4) programmable temperature vaporiser (PTV). Characteristics of columns used in this study together with injection parameters are presented in Table 1. The mass spectrometer was operated at 70 eV in the selected ion monitoring (SIM) mode. Dwell times were set to 10 ms for the 0.10 mm column and to 20–40 ms for the 0.18 and 0.22 mm columns, respectively. Two ions from the molecular ion cluster of each congener (M^+ and $[M+2]^+$) were monitored for each level of chlorination. Target ions were grouped following a similar procedure used for GC–MS analysis of PCB in 0.25 mm I.D. columns [10]. Retention times relative to the nearest internal standard, ion chromatograms and ratios between the monitored ions were used as identification criteria. A deviation of ion ratios of less than ±20% from the theoretical value was considered acceptable for identification. Hewlett-Packard Method Translation Software (1997) was used for calculation of optimal GC parameters for the narrow-bore capillary column.

3. Results and discussion

3.1. Injection in narrow-bore GC

3.1.1. Hot splitless

Only low injection volumes (typically 0.2–0.4 μl) can be injected by hot splitless in narrow bore columns without peak distortion [11]. Fast injection is needed to reduce discrimination for low volatile compounds. Because of low flow-rate (typically 0.3–0.4 ml min^{-1}), long splitless times are required, while liners with small internal diameter should be used (0.8–1.2 mm I.D.) to reduce the injector band broadening. Sufficiently strong focusing mechanisms (e.g. cold trapping and solvent effect) should occur [12]. Hot splitless can be used without any problems for columns of more than 0.15 mm I.D.

Table 1
Column characteristics and injection parameters

Parameter	Column		
	AT–5	AT–5	HT-8
Column description	5% phenyl–polydimethyl siloxane column	5% phenyl–polydimethyl siloxane column	1,7-dicarba-closo-dodecarborane–8% phenylmethyl siloxane column
Manufacturer	Alltech (Lokeren, Belgium)	Alltech (Lokeren, Belgium)	SGE (Zulte, Belgium)
Dimensions	10 m×0.1 mm, 0.1 μm	20 m×0.18 mm, 0.25 μm	50 m×0.22 mm, 0.25 μm
Carrier flow	0.4 ml min^{-1}	0.8 ml min^{-1}	0.7 ml min^{-1}
Oven program	90°C (1 min) at 50°C min^{-1} to 200°C (0.5 min), at 25°C min^{-1} to 250°C (0.2 min), at 75°C min^{-1} to 280°C (2 min)	90°C (1 min) at 35°C min^{-1} to 200°C (1 min), at 10°C min^{-1} to 250°C (0.5 min), at 50°C min^{-1} to 280°C (3 min)	90°C (1 min) at 15°C min^{-1} to 170°C (2 min), at 35°C min^{-1} to 290°C (14 min)
Injection type	Cold splitless	Hot pulsed splitless	Hot pulsed splitless
Injector program	100°C (0.1 min) at 700°C min^{-1} to 270°C (stay 7 min)	Pressure pulse 30 p.s.i., pulse time 1 min, 270°C	Pressure pulse 30 p.s.i., pulse time 1.20 min, 270°C
Injection volume	1 μl	1 μl	1 μl
Splitless time	1.00 min	1.00 min	1.25 min
Theoretical plates/m	8600	5300	3900
Theoretical plates (total)	86 000	106 000	195 000

3.1.2. Cold splitless

Peak distortion can be observed in hot splitless when volumes of more than 0.4 μl are injected into a 0.10 mm capillary column [11]. However, up to 2 μl can be injected in cold splitless, before distortion is observed. There is no solute discrimination and the limiting factor is the volume of liquid which can be handled in the liner. If sample volume is too high, the liquid may be shot through an empty liner. The use of a baffled liner, with higher contact surface, allows the handling of volumes of a few microliters. No fast injection is mandatory. The injector should be kept at low temperature (to be optimized for each solvent used) for a longer time. In this way, part of the solvent is already transferred before the analytes enter the column, thus allowing an efficient solvent effect [12]. However, at too low an initial oven temperature, excessive solvent recondensation occurs. Initial inlet and column temperatures did not influence greatly the area of the peak, but seriously affected their peak shape, especially for volatile analytes (serious distortion). Using isooctane as solvent, best conditions were 90 and 100°C for the oven and injector initial temperatures, respectively. No solvent focusing effect occurred at oven temperatures of 120°C or higher.

For low splitless times (0.5–0.75 min), analytes are incompletely transferred to the column, resulting in low areas. For splitless times higher than 1 min, similar areas were obtained for high volatile compounds (hexachlorobenzene and PCB 28), while the increase in area was more evident for low volatile compounds (e.g. PCB 180 and 194) (Fig. 1). However, splitless times longer than 1 min lead to a >25% increase in the peak width, which affected some separations. A splitless time of 1 min was used in further experiments.

A standard mixture of PCBs in isooctane was injected in cold splitless into a 10 m AT-5 capillary column with 0.10 mm I.D. (Fig. 2A). The resulting

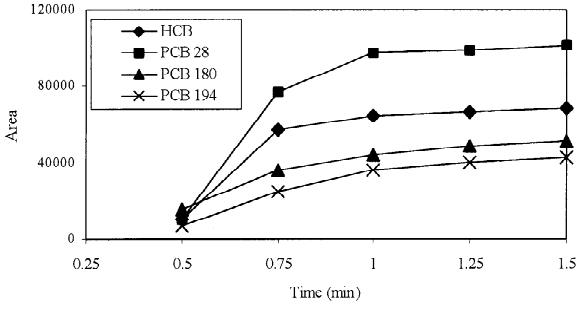

Fig. 1. Influence of splitless time (for cold splitless injection) on the area of hexachlorobenzene (HCB) and PCB 28, 180 and 194. Oven and injector conditions as presented in Table 1, column AT-5, 10 m×0.10 mm I.D.

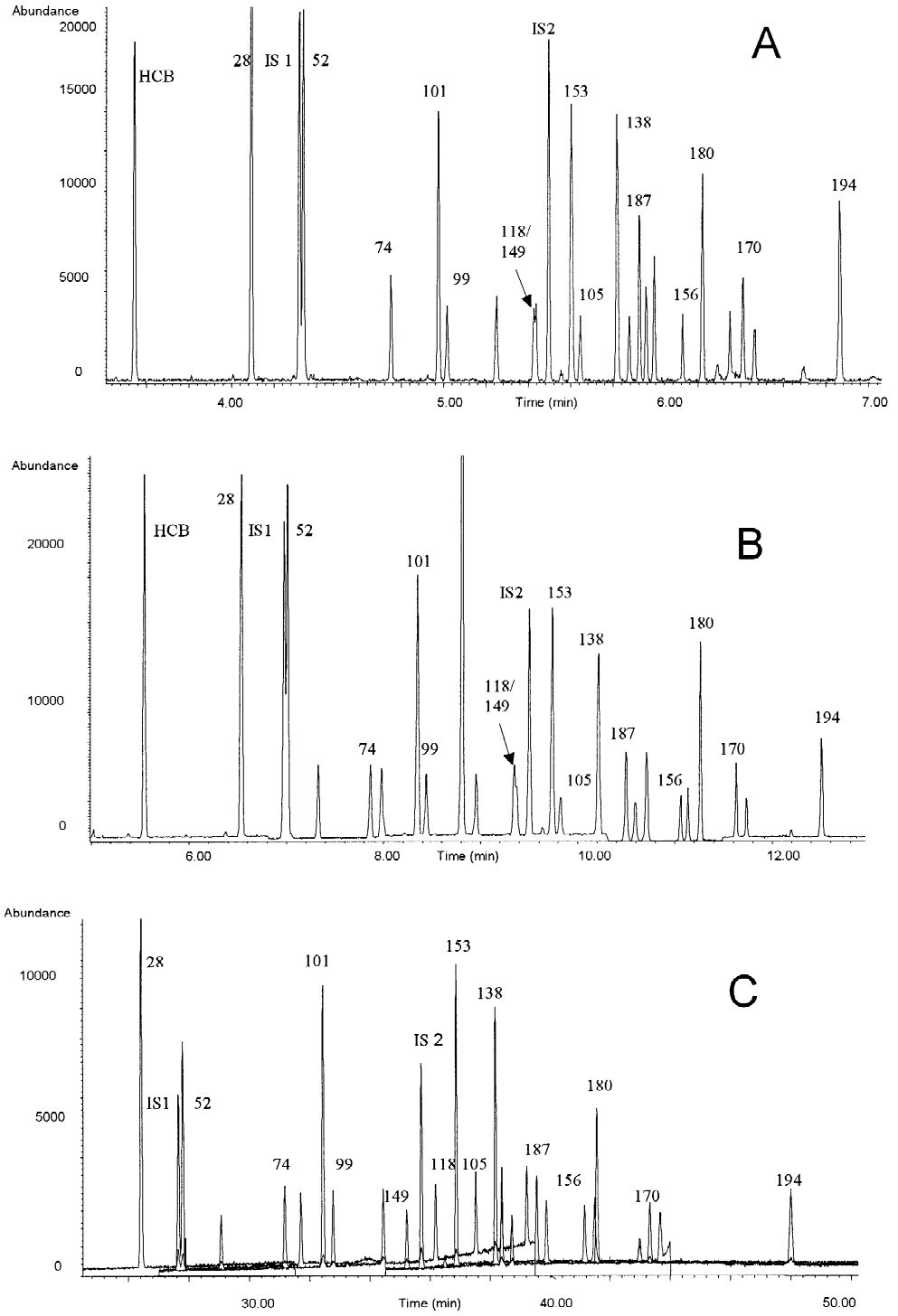

Fig. 2. Separation of a PCB standard mixture (IUPAC numbering) on three different capillary columns: 10 m×0.10 mm I.D. (chromatogram A), 20 m×0.18 mm I.D. (chromatogram B) and 50 m×0.22 mm I.D. (chromatogram C).

chromatogram could be compared with similar chromatograms obtained by injecting the same PCB standard solution into a 20 m AT-5 column with 0.18 mm I.D. and 50 m HT-8 column with 0.22 mm I.D. (Fig. 2B and C, respectively). No loss of resolution was observed for PCB congeners (see pairs PCB 101/PCB 99 and PCB 153/PCB 105), while run times were reduced from 50 to 7 min (a reduction of more than 85% of the analysis time).

3.1.3. Large volume injection

On programmable temperature vaporizers, large sample volume introduction is possible. For semi-volatile compounds (e.g. PCBs), optimisation of the method is not as critical as for volatile analytes. To minimise losses of volatile analytes, low solvent amounts should still be present in the injector, before the closure of the vent line [12]. A procedure involving the injection of 8×5 μl extract was already demonstrated for the determination of various pesticides from water using similar equipment to that described in the Experimental section (see above) [13]. Thus, it is now possible to increase sample capacity by an order of magnitude, which will allow lower limits of detection. The procedure is currently being tested for the analysis of PCBs in human body fluids [14].

3.2. Quadrupole MS detection for narrow-bore GC

The use of narrow-bore columns (less than 0.10 mm I.D.) requires a fast scanning detector, which should also be sensitive and selective. The use of specific mass spectrometers (magnetic sector [15] and time of flight [16]) has already been demonstrated for short run times (<3 min). Due to lower scanning rates, quadrupole MS can only be used for reasonable run times (>5 min). Selected ion monitoring and low dwell times (10 ms) are a requirement for a higher number of scans across the chromatographic peak, thus a better peak shape.

It has already been shown [17] that quadrupole mass spectrometers can be operated for qualitative fast GC analysis in full scan acquisition mode and that excellent quantitative data can be obtained in selected ion monitoring. Thus, for conditions described in Table 1, the peak width at the base is maximum 1.2 s for the 10 m column. Assuming that a peak is sampled 8–10 times to obtain a good peak shape, a minimum of 120 ms will be necessary for one chromatographic point. When using dwell times as low as 10 ms, a maximum of 12 ions can be monitored in one acquisition window. For PCB determination, this is not a problem due to specific ions for each level of chlorination. In these conditions, detection limits for individual PCB congeners as low as 0.4 pg μl^{-1} for standard solution and 0.2 ng g^{-1} fat for milk extracts could be achieved. The observed gain in sensitivity of capillary GC–MS by decreasing the column diameter might be explained by a reduction of the noise level by faster scanning (less background ions detected). Moreover, the gas flow through narrow-bore columns is much less than from conventional ones (proportional to the square diameter of the column for constant plate numbers) and so is the column bleeding mass flow. The use of low flow-rates is compatible with the pumping capacity of most systems. The quadrupole mass spectrometer is easy to use and maintain and can be easily changed for routine operation if needed.

3.3. Quantitative PCB analysis

Human milk extracts were injected on the 0.10 and 0.18 mm columns with quadrupole MS in selected ion monitoring mode (Fig. 3). No loss of resolution for critical PCB pairs was observed. Similar quantitative data (Table 2) were obtained on both columns after injection of a certified reference material extract (CRM 450 — PCBs in powdered milk). Very small quantities can be detected on narrow-bore columns, because sharper peaks result in a higher signal and, thus, in a better S/N ratio when a fixed amount of sample is introduced [1].

4. Conclusions

Narrow-bore columns (less than 0.10 mm I.D.) emerged from the need for faster and more efficient separations. Extremely narrow peaks (peak width of less than 1 s) are obtained and detectability is favored. Relatively large volumes (1 μl) could be injected in cold splitless. Narrow-bore capillaries can be used in combination with quadrupole mass spec-

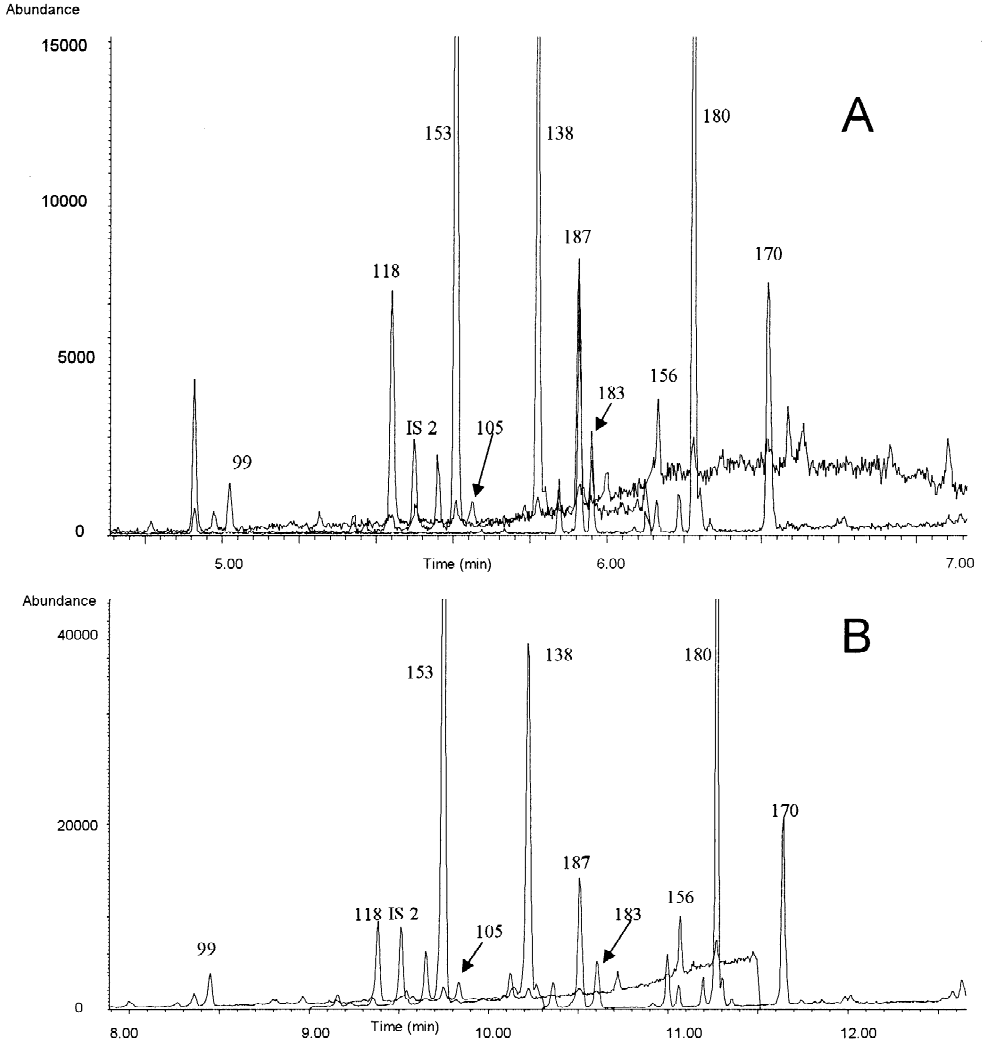

Fig. 3. GC–MS chromatograms of human milk extract on 10 m×0.10 mm I.D. (chromatogram A) and 20 m×0.18 mm I.D. (chromatogram B). IUPAC numbering for PCB congeners.

Table 2
PCB concentrations (ng g^{-1}) in CRM 450 (PCBs in powder milk) obtained on two AT-5 capillary columns with 0.10 and 0.18 mm I.D.

Compound	Certified values	AT-5, 20 m×0.18 mm	AT-5, 10 m×0.10 mm
PCB 118	3.3±0.4	3.2±0.3	3.1±0.3
PCB 153	19.0±0.7	18.3±1.6	18.6±0.6
PCB 156	1.62±0.20	1.51±0.02	1.56±0.08
PCB 180	11.0±0.7	11.2±0.9	11.4±1.0

trometer for the rapid determination of PCBs from human matrices.

References

[1] C.A. Cramers, P.A. Leclercq, CRC Crit. Rev. Anal. Chem. 20 (1988) 117.
[2] L.M. Blumberg, J. High Resolut. Chromatogr. 20 (1997) 679.
[3] L.M. Blumberg, J. High Resolut. Chromatogr. 20 (1997) 597.

[4] C.A. Cramers, H.G. Janssen, M.M. van Deursen, P.A. Leclercq, J. Chromatogr. A 856 (1999) 315.
[5] P.G. Van Ysacker, H.G. Janssen, H.M.J. Snijders, C.A. Cramers, J. High Resolut. Chromatogr. 18 (1995) 397.
[6] L.S. de Jager, A.R.J. Andrews, Analyst 125 (2000) 1943.
[7] E.J. Reiner, K.A. MacPherson, R. Brunato, T. Chen, M.A. Bogard, A.R. Boden, G. Ladwig, Organohalogen Comp. 45 (2000) 17.
[8] J.S. Alvarado, J. Silzer, F. Lemley, M.D. Erickson, Anal. Commun. 34 (1997) 381.
[9] A. Covaci, C. Hura, P. Schepens, Chromatographia, in press.
[10] M.D. Erickson, Analytical Chemistry of PCBs, CRC Press, Boca Raton, FL, 1997.
[11] P.G. Van Ysacker, H.M.J. Snijders, H.G. Janssen, C.A. Cramers, J. High Resolut. Chromatogr. 21 (1998) 491.
[12] K. Grob, Split and Splitless Injection in Capillary GC, Hüthig, Heidelberg, 1993.
[13] M. Hada, M. Takino, T. Yamagami, S. Daishima, K. Yamaguchi, J. Chromatogr. A 874 (2000) 81.
[14] A. Covaci, unpublished results.
[15] P.A. Leclercq, H.M.J. Snijders, C.A. Cramers, K.H. Maurer, U. Rapp, J. High Resolut. Chromatogr. 12 (1989) 652.
[16] B. van Bavel, J. Hughes, S. Davies, H. Wingfors, G. Lindström, Organohalogen Comp. 40 (1999) 293.
[17] C.C. Grimm, S.W. Lloyd, L. Munchausen, Am. Lab., June (1996) 18S–18W.