Conclusion

Target factor analysis was successfully used to determine simultaneously the numbers, identities and concentrations of six aromatic compounds with overlapping UV spectra in mixtures. This work illustrates the potential usefulness of TFA in the direct analysis of the mixtures consisting of compounds similar to the aromatic compounds studied.

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Comparison of two different derivatization procedures for the determination of urinary chlorophenol excretions

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Abstract. A GC/MS method with enhanced sensitivity and specificity suitable for the determination of various chlorophenols in the urine of persons occupationally and environmentally exposed to diverse chlorinated aromatic hydrocarbons has been elaborated and compared with the method customarily used. After acid hydrolysis and steam distillation, followed by several clean-up steps, the chlorophenols have been derivatized with N-heptafluorobutyrylimidazole (HFBI), yielding ester derivatives. This procedure facilitates the detection of 3- and 4-monochlorophenol, 2,4- and 2,5-dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol as well as 2,3,4,6-tetrachlorophenol down to 0.1–1 µg/l, depending on the number of chlorine atoms. A comparison to the commonly used derivatization procedure with diazomethane revealed, except for 2,4,5-trichlorophenol, satisfactory conformity of both techniques. Due to an improvement of the detection limit by a factor of five for the monochlorophenols, for the first time the quantification of 3-monochlorophenol and the identification of 2-monochlorophenol as constituents of human urine have been possible as HFBI-derivatives. The excretion of mono-, di-, tri- and tetrachlorophenols in the urine of the general population could be confirmed.

Introduction

The determination of several mono-, di-, tri- and tetrachlorophenols in the urine of the general population was the unexpected result of two large scaled studies performed between 1989 [1] and 1992 [2]. The stress of the general population caused by these chlorophenols (CPs) is comparable with the exposure to pentachlorophenol, a substance extensively used as an indoor wood preservative in Germany until its ban in 1989. Although these findings were confirmed by subsequent works, the results should be verified by applying an analytical procedure with improved detection limits. Those can be achieved by a modified derivatization procedure followed by GC/MS analysis. An additional gain in sensitivity could be expected compared to the mass selective detector (GC/MSD) routinely used in our laboratory. Within the scope of the present study, the urinary excretion of 3- and 4-monochlorophenol (MCP), 2,4- and 2,5-dichlorophenol (DCP), 2,4,5- and 2,4,6-trichlorophenol (TCP) as well as 2,3,4,6-tetrachlorophenol (TECP) was recorded. Prior to analysis, the chlorophenols were either derivatized using diazomethane (DAM), according to the standard procedure of this laboratory, or with N-heptafluorobutyrylimidazole (HFBI).

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Material and methods

Collective

Urine samples from 16 male persons were collected postshift and stored frozen. The workers were occupied in different fields of technical attendance tasks in an incineration plant.

Reagents

N-heptafluorobutyrylimidazole was purchased from Pierce, Rockford, IL, USA. All other chemicals used were of the best available analytical grade and supplied from Merck, Darmstadt, FRG.

Analysis of HFBI derivatives

HFBI derivatives were measured using a GC/MS system from Hewlett Packard (Palo Alto, CA, USA) consisting of a HP 5890 Series II gas chromatograph connected to a HP 5989 A mass spectrometer.

GC conditions. Capillary column: HP Ultra 2, 25 m × 0.2 mm × 0.33 µm. Temperatures: injector: 280°C, column: 70°C, 3° C/min to 150°, 50°C/min to 280°C, isothermal for 5 min. Carrier gas: helium 5.0, inlet pressure 15 psi, splitless injection of 1 µl.

MS conditions. Temperatures: ion source: 200°C, quadrupole 100°C, interface: 300°C. Ion source pressure: 6×10^{-6} torr. Ionization: EI 70 eV, multiplier 2600 V. Mode: selected ion monitoring.

Analysis of methylether derivatives

Methylether derivatives were determined with a HP 5890 Series II gas chromatograph equipped with an automatic liquid

injection system HP 7673 A and a mass selective detector (MSD) HP 5970.

GC conditions. Capillary column: HP Ultra 2, 50 m × 0.22 mm × 0.33 µm. Temperatures: injector: 260°C, column: 50°C for 1 min, 10°C/min to 90°C, isothermal for 20 min, 2°C/min to 100°C, isothermal for 6 min, 10°C/min to 140°C, isothermal for 10 min, 10°C/min to 170°C, isothermal for 14 min, 25°C/min to 260°C, isothermal for 5 min. Carrier gas: helium 5.0, inlet pressure 21 psi, split 1:8, injection volume 5 µl.

MS conditions. Temperatures: ion source 150°C, quadrupole 100°C, interface: 280°C. Ionization: EI 70 eV, multiplier 2800 V. Mode: selected ion monitoring.

Clean-up procedure

16 different urine samples were worked up twice on different days. Except for the derivatization procedures, a detailed description of the sample preparation was given by Angerer et al. [2], taking into account all aspects of good laboratory practice. Briefly, 20 ml urine were spiked with 1 ml of an aqueous solution of 2,6-dibromophenol (100 µg/l) for internal standardization (IS). After acidification the sample was subjected to steam distillation. By that the analytes were separated from the urine matrix and combined with the application of a cation exchange column and a reversed phase column interferences due to physiological components could be minimized. The CPs were desorbed from the C-18 columns with 4 ml of a mixture of dichloromethane and heptane (1:1).

Table 1. Detection limits of several chlorophenols in human urine; derivatization was carried out either with diazomethane (DAM) or with N-heptafluorobutyrylimidazole (HFBI)

Compound	DAM [µg/l]	HFBI [µg/l]
3-MCP	n.d.[a]	0.1
4-MCP	0.5	0.1
2,5-DCP	0.2	0.1
2,4-DCP	0.2	0.1
2,4,6-TCP	0.3	0.3
2,4,5-TCP	0.3	0.3
2,3,4,6-TECP	0.5	1

[a] n.d.: not detectable

Methylation with diazomethane

The eluate was dried over anhydrous Na_2SO_4 and evaporated under a stream of nitrogen to a final volume of approximately 1 ml. 500 µl of a solution of diazomethane in toluene (prepared of 1.5 g N-nitroso-N-methylurea mixed with 5 ml aqueous 4 mol/l KOH and 30 ml toluene at a temperature below 5°C) were added. The final concentration of DAM (0.16 mol/l) turned out to be sufficient for a complete methylation. After 12 h the organic layer was separated from the Na_2SO_4 by centrifugation and the volume was reduced to 100 µl by evaporation under a gentle stream of nitrogen.

Acylation with N-heptafluorobutyrylimidazole

The eluate of the C-18 columns was dried over anhydrous Na_2SO_4 for 30 min. The organic layer was separated from the salt by centrifugation and 1 ml of toluene was added. The dichloromethane/heptane mixture was evaporated in a nitrogen stream and the toluene layer was filled up to a final volume of 3 ml. 100 µl of a toluene solution containing 20% HFBI (by volume) were added and the samples were placed in a water bath for 30 min at 60°C. After the derivatization, the samples were washed with 2 ml water. The organic layer was separated and evaporated to a final volume of 100 µl. Due to the sensitivity of the derivatives with respect to hydrolysis, the samples should be analysed within 24 h.

Calibration and quality assurance

Calibration was carried out for each analyte using spiked pool urine samples in the range from 0.5 to 15 µg/l. These samples were subjected to the same treatment as the urine samples. Spot checks were carried out of water blanks.

All calculations were based on the coefficient of the peak area of the chlorophenol divided by the peak area of the internal standard. For each analyte, the molecular ion was registrated.

Quality assurance for the methylethers was performed by the analysis of urine samples spiked with 10 µg/l 4-MCP, 2,6-DCP, 2,3,4-TCP and 2,3,5,6-TECP. The between-day imprecisions ranged from 9 to 11% for 4-MCP, 2,6-DCP and 2,3,5,6-TECP. For 2,3,4-TCP 5% between-day imprecision was determined.

Results

Table 1 displays a comparison of the detection limits for the two different derivatization procedures, laying between 0.1 and

Table 2. Median values and ranges of urinary chlorophenol concentrations in 16 samples. Correlation was checked using Student's *t*-test

	Excretion of CPs in urine [µg/l]						
	3-MCP	4-MCP	2,4-DCP	2,5-DCP	2,4,5-TCP	2,4,6-TCP	2,3,4,6-TECP
HFBI							
Median	0.2	4.1	0.8	2.3	0.8	1.0	n.d.[a]
Range	0.1–0.4	1.8–10.2	0.4–14.6	0.5–59.6	0.6–3.3	0.5–11.5	
DAM							
Median	n.d.[a]	4.7	1.0	3.1	1.0	1.0	0.9
Range		2.0–11.1	0.3–13.4	0.7–63.4	0.5–3.2	0.3–8.7	0.6–3.8
Correlation coefficient	–	0.909	0.998	0.998	0.189	0.994	–

[a] n.d.: not detectable

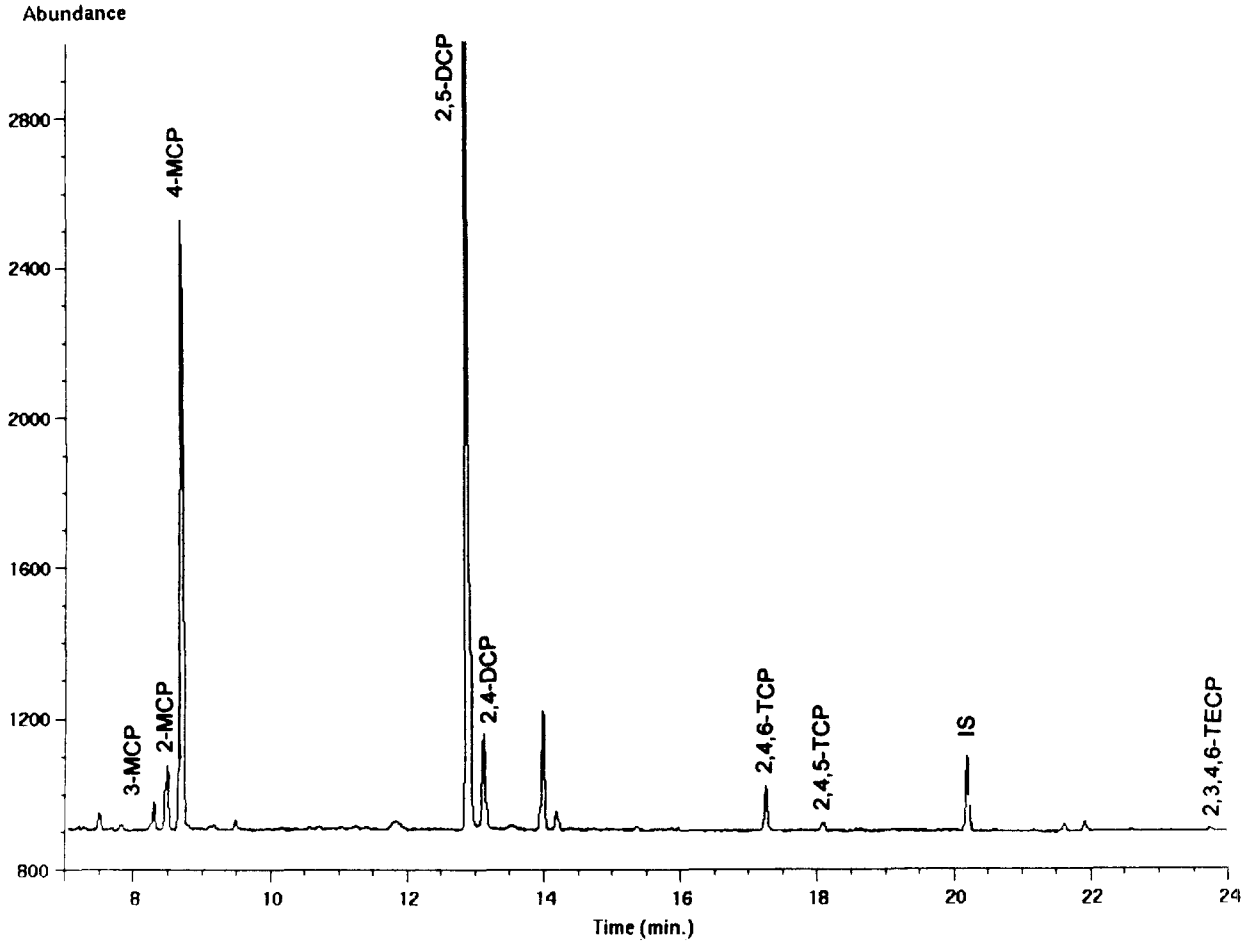

Fig. 1. Selected ion chromatogram of a urine sample derivatized with N-heptafluorobutyrylimidazole. Registrated ions were as follows: 324 for MCPs, 358 for DCPs, 392 for TCPs, 426 for TECP and 448 for 2,6-dibromophenol (IS). Concentrations of CPs in that sample: 0.3 µg/l (3-MCP), 4.5 µg/l (4-MCP), 20.2 µg/l (2,5-DCP), 2.3 µg/l (2,4-DCP), 2.2 µg/l (2,4,6-TCP), 0.8 µg/l (2,4,5-TCP), 0.9 µg/l (2,3,4,6-TECP)

1 µg/l. Methylethers were determined by GC/MSD, whereas the heptafluorobutyrylesters were analysed by GC/MS.

Table 2 summarizes median concentrations and ranges of the CPs excreted in the urine of 16 workers employed in an incineration plant. The evaluation of 3-MCP was, due to chromatographical interferences in the case of the methylethers, only possible for the HFBI derivatives. 2,3,4,6-TECP could only be detected in four samples derivatized with HFBI. For that reason, the correlation of both derivatization procedures was checked only for 4-MCP, 2,4- and 2,5-DCP, 2,4,5- and 2,4,6-TCP.

The correlation of both independent analyses provided, with one exception, satisfactory results. Except for 2,4,5-TCP, the error probability was found to be less than 0.001%.

Discussion

The use of both HFBI and DAM for the derivatization of CPs results in some different reliability data. Compared to the methylation procedure, a decrease of the detection limits could be achieved especially for the mono- and dichlorophenols using HFBI. No improvements were possible for the tri- and tetrachlorophenols. This could be a consequence of the increasing number of chlorine substituents. Steric hindrance of the acylation reaction due to the bulky HFBI could result in a decreased derivatization yield. An increase in the specificity of the detection caused by the reduction of the analytical background noise was observable for all CPs derivatized with HFBI. This is demonstrated for one urine sample in Figs. 1 and 2.

The detection limits realized with HFBI are superior to those obtained by acylation with a mixture of acetic acid anhydride and pyridine, which only offer a detection limit of 10 µg/l for TCP and TECP [3]. On the other hand, a detection limit of approximately 0.005 µg/l for various DCPs and TCPs realized after a derivatization with diazoethane followed by chemical ionization and tandem mass spectrometry as reported by Holler et al. [4] could not be achieved after the derivatization with HFBI.

The advantages of the derivatization with HFBI like increased specificity and in some cases lowered detection limits, are confronted with the sensitiveness of the heptafluorobutyrylesters to traces of water or light. To gain reproducible results, it is necessary to analyse the samples within 24 h. Otherwise decomposition of the esters gives rise for inaccurate results. In contrast, methylether derivatives turned out to be stable for at least 8 weeks.

The application of both derivatization procedures on the 16 urine samples provided by one exception corresponding results, as revealed by the correlation coefficients in Table 2. In the case of 2,4,5-TCP, despite similar median values no correlation was found for that compound. One possible explanation therefore are falsified integration results due to interfering peaks in the case of methylether derivatives.

The derivatization with HFBI offers a reliable alternative to the methylation procedure. The discussed sensitiveness of the

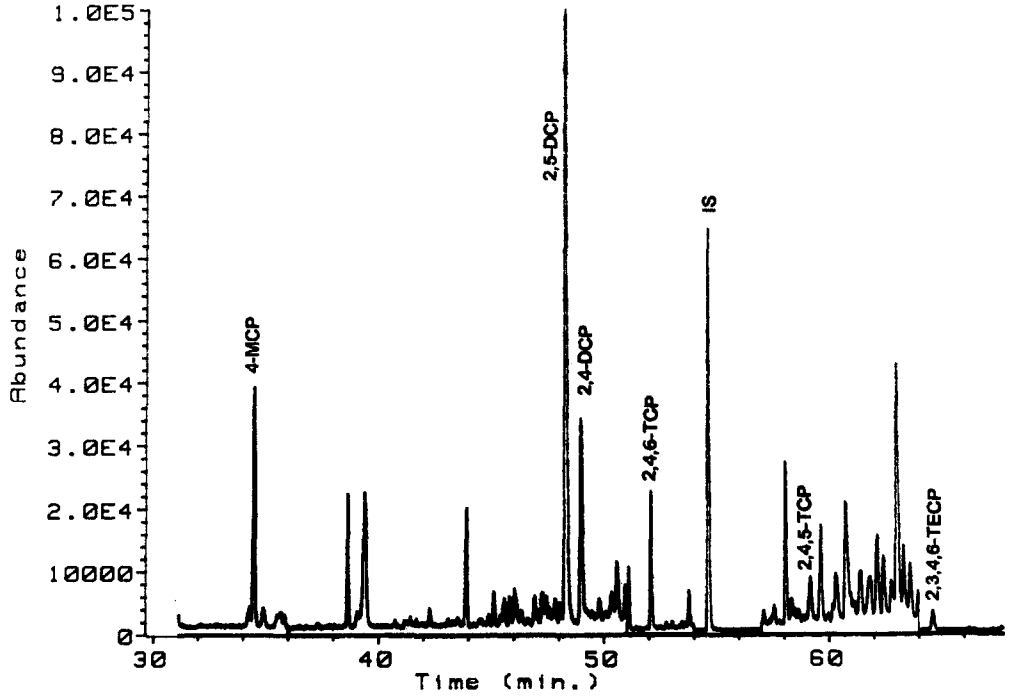

Fig. 2. Selected ion chromatogram of a urine sample derivatized with diazomethane. Registrated ions were as follows: 142 for MCPs, 176 for DCPs, 210 for TCPs, 244 for TECP and 268 for 2,6-dibromophenol (IS). Concentrations of CPs in that sample: 5.2 µg/l (4-MCP), 24.0 µg/l (2,5-DCP), 2.2 µg/l (2,4-DCP), 1.9 µg/l (2,4,6-TCP), 1.0 µg/l (2,4,5-TCP), 1.2 µg/l (2,3,4,6-TECP)

HFBI derivatives to hydrolysis and the comparability of the results obtained after the methylation with DAM makes the latter assay more suitable for the routine analyses of chlorophenols. A certain decrease in sensitivity and specificity has to be tolerated in this case.

Besides the known excretion of 4-MCP, 3-MCP could be determined in the urine samples by the application of the HFBI derivatization procedure. Moreover, 2-MCP could be identified in human urine. That isomer seems to occur to a slightly higher extend than 3-MCP. Further studies are necessary to estimate the exact contribution of 2-MCP to the overall amount of monochlorophenols in human urine.

The median values of the chlorophenols determined in the urine samples are within the range reported by Hill et al. [1] and Angerer et al. [2] to occur in the urine of the general population. This means, that the investigated workers showed no occupational stress caused by mono-, di-, tri- and tetrachlorophenols. This result is in line with the findings of an earlier study, where the internal exposure to various organic compounds in a waste incinerator was investigated [5]. Except for 2,4- and 2,5-DCP, no elevated levels of CPs were found in workers compared to control groups.

Vice versa these results confirm that the general population eliminates mono-, di-, tri- and tetrachlorophenols in amounts comparable to pentachlorophenol. The implications of these findings with respect to environmental health have still to be evaluated.

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