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Analysis of primary and secondary aliphatic amines in waste water and surface water by gas chromatography–mass spectrometry after derivatization with 2,4-dinitrofluorobenzene or benzenesulfonyl chloride

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Abstract

Two methods for the determination of aliphatic amines such as methylamine, dimethylamine, ethylamine, diethylamine, piperidine, pyrrolidine or morpholine in waste water and surface water at the sub-ppb level are presented. The methods are based on a derivatization of the amines within the aqueous medium with 2,4-dinitrofluorobenzene and benzenesulfonyl chloride, respectively. In both cases the derivatives are extracted with dichloromethane and measured by GC–MS. The performance data of the two methods are compared and the method which is based on the derivatization with benzenesulfonyl chloride is finally applied to industrial waste waters and surface waters in order to determine the rate of occurrence of aliphatic amines in the aquatic environment.

Keywords: Derivatization, GC; Water analysis; Dinitrofluorobenzene; Benzenesulfonyl chloride; Amines

1. Introduction

Low-molecular-mass aliphatic amines like methylamine, dimethylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, piperidine, pyrrolidine or morpholine are important intermediates in chemical and pharmaceutical industries. Some of them are produced in quantities of more than 100 000 tons per year in Western Europe. In addition to their industrial application, aliphatic amines may occur as biodegradation products of organic material like proteins and amino acids or other nitrogen-containing compounds. It is well known that secondary aliphatic amines can react with nitrite, forming carcinogenic nitrosamines. Up to now little information has been available on the occurrence of aliphatic amines in industrial waste waters and in surface waters.

Analysis of aliphatic amines in aqueous media is very difficult because of the high polarity of these compounds. Due to their high water solubility, enrichment of aliphatic amines by liquid–liquid or solid-phase extraction is not possible at the trace level. Furthermore, direct gas chromatographic analysis of aliphatic amines requires packed columns [1] or capillary columns coated with thick films of special materials [2,3]. Several GC and HPLC methods exist which contain a derivatization step prior to chromatographic analysis [4–21]. Neverthe-

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less, none of the methods described in literature were suitable for the trace level determination of aliphatic amines from water samples. Therefore we selected two promising methods, a derivatization procedure which was developed for the determination of low-molecular-mass aliphatic primary amines in urine by GC with flame photometric detection [17], and a derivatization procedure which is normally used for the analysis of amino acids [22] and improved their performance according to the requirements of water analysis at the trace level. Both methods consist of a derivatization of the aliphatic amines within the aqueous sample. The derivatives are transferred into an organic solvent and analysed by GC–MS. As will be shown, detection limits below 1 μg/l can be achieved with both methods.

2. Experimental

2.1. Chemicals

All amines under investigation such as methylamine, dimethylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, *n*-hexylamine, pyrrolidine, piperidine, morpholine and ethanolamine were of analytical grade and purchased by Fluka (Neu-Ulm, Germany). Methylamine and dimethylamine were used as hydrochlorides. 2,4-Dinitrofluorobenzene (>99%) and benzenesulfonyl chloride (>99%) were obtained from Aldrich (Steinheim, Germany). Dichloromethane of HPLC grade was purchased by Promochem (Wesel, Germany). All other chemicals such as hydrochloric acid (37%), sodium sulfate and sodium hydroxide pellets as well as sodium hydrogen carbonate were of analytical grade, and were obtained from Merck (Darmstadt, Germany) and Roth (Karlsruhe, Germany), respectively.

2.2. Equipment

GC analysis was carried out with a Hewlett-Packard Model 5890 gas chromatograph (Waldbronn, Germany) equipped with a split/splitless injector. Detection was done by a Hewlett-Packard MSD 5970 mass spectrometer using an ionization voltage of 70 eV. A fused-silica capillary column (35 m× 0.25 mm I.D., 0.25 μm film thickness) of DB5 type (J&W, Folson, CA, USA) was used. Helium with a purity of 99.9990% was used as carrier gas with an inlet pressure of $0.5 \cdot 10^5$ Pa corresponding to a flow-rate of 1 ml/min. Operating conditions were as follows:

For the GC separation of the 2,4-dinitrofluorobenzene derivatives, the temperature programme started at 140°C (held 3 min), set at 3°C/min from 140°C to 210°C, set at 10°C/min from 210°C to 290°C and held isothermally at 290°C for 5 min. The temperature programme used for the separation of the benzenesulfonamides started at 120°C (held 3 min), set at 5°C/min from 120°C to 220°C, set at 10°C/min from 220°C to 290°C and held isothermally at 290°C for 5 min. In both cases injector and detector temperature were 290°C.

Data evaluation was done by a HP G1034C MS ChemStation software (Hewlett-Packard, Amsterdam, Netherlands).

2.3. Sample preparation

2.3.1. Derivatization with benzenesulfonyl chloride (BSC)

First, a clean-up with dichloromethane was carried out. Due to the application of some benzenesulfonamides as plasticizers using the derivatization method with BSC it is necessary to carry out a clean-up in order to ensure the absence of any benzenesulfonamide in the sample which would otherwise pretend the presence of the corresponding amine. Then 4 μl of an aqueous solution of 100 mg/l *n*-hexylamine as internal standard (I.S.) were added to 200 ml water sample in a 250 ml round-bottomed flask. The mixture was basified with 8 ml 10 *M* aqueous sodium hydroxide solution and 2 ml benzenesulfonyl chloride (BSC) were added. The flask was closed and set on an agitator for 30 min at room temperature. Then another 10 ml of 10 *M* sodium hydroxide solution were added and the mixture was agitated again for 30 min at 80°C in order to hydrolyze the excess of derivatization reagent. Subsequently the solution was cooled down with ice water and acidified under steady cooling with 18.5% aqueous solution of hydrochloric acid to pH 5.5. The mixture was extracted twice with 25 ml dichloromethane. Whereas the aqueous solution was

discarded, the organic phase was washed once with 15 ml 0.05 *M* aqueous sodium carbonate solution and dried with sodium sulfate. The solvent was evaporated to ca. 100 μl and 1 μl was injected into the gas chromatograph. The total time for a sample work-up was about two hours.

2.3.2. Derivatization with 2,4-dinitrofluorobenzene (2,4-DNFB)

After addition of 4 μl of an aqueous solution of 100 mg/l *n*-hexylamine as I.S., 200 ml water sample were basified in a 250 ml round-bottomed flask by addition of 2 ml 10 *M* aqueous sodium hydroxide solution. 1 ml 2,4-dinitrofluorobenzene was added, the flask was closed and set on an agitator for 60 min at room temperature and for 60 min in a hot water bath at 90°C in order to hydrolyze the excess of 2,4-DNFB. After the solution was cooled down to room temperature, it was acidified with 1 *M* hydrochloric acid to pH 6 and extracted twice with 25 ml dichloromethane. The extract was washed three times with 30 ml 0.1 *M* aqueous sodium hydrogen carbonate solution in order to separate the 2,4-dinitrofluorophenol which is a hydrolysis product of the derivatization reagent and can cause damage to the GC column. The aqueous solution was discarded and the dichloromethane extract was dried with sodium sulfate. After the solvent had been evaporated to ca. 100 μl, 1 μl was injected into the gas chromatograph. Employing this method, the total time for a sample work-up is about four hours.

2.4. Quantitative analysis

For quantitative analysis an internal standard was used. *n*-Hexylamine was chosen on the one hand because of its similar chemical nature to the compounds under investigation and on the other hand because of its low industrial application, so that one can ensure that natural waters and waste waters do not contain any *n*-hexylamine. Calibrations were performed by calculating the ratios between the peak areas of the respective derivatives of the aliphatic amines and the peak area of the *n*-hexylamine derivative. Additionally 1-chlorodecane can be used in order to check the performance of the GC–MS system.

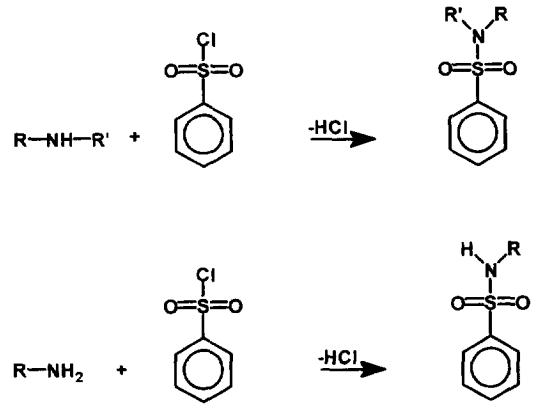

Fig. 1. Reaction of primary and secondary amines with benzenesulfonyl chloride.

3. Results and discussion

Primary and secondary aliphatic amines can be easily converted into their N-alkylbenzenesulfonamides and into their dinitrofluorobenzene derivatives using the procedures previously described. The reactions of primary and secondary amines with the respective derivatization reagent are summarized in Figs. 1 and 2. Tertiary amines do react neither with 2,4-DNFB nor with BSC. Consequently they cannot be determined using these methods. Primary and secondary aminoalcohols as well as diamines form derivatives with the respective derivatization agent, but only one amino group

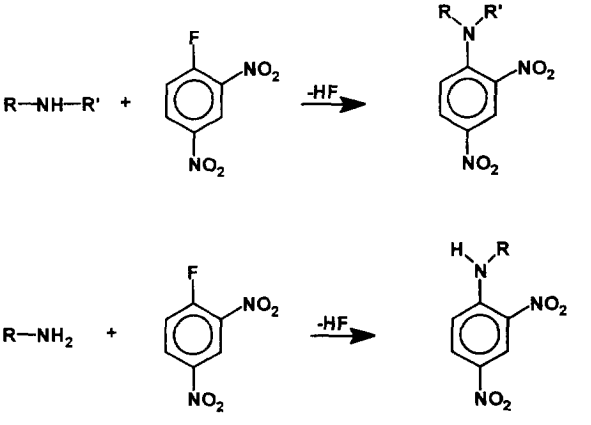

Fig. 2. Reaction of primary and secondary amines with 2,4-dinitrofluorobenzene.

reacts. The derivatives of aminoalcohols and diamines with BSC are too polar to be extracted from the water by dichloromethane. The 2,4-DNFB derivatives of ethanolamine and diethanolamine can be extracted, but recovery is poor and the GC separation of the respective compounds is incomplete. Therefore the detection limits are not satisfactory for trace-level analysis. For diethanolamine the detection limit is higher than 1 mg/l, for ethanolamine it is about 5 μg/l. For analysis of waste water the latter is acceptable but not for analyzing surface water. Diamines can neither be analyzed by derivatization with 2,4-DNFB nor with BSC.

Both 2,4-dinitrofluorobenzene derivatives and benzenesulfonamides have been found to be very stable under common laboratory conditions and no thermal decomposition was observed during GC analysis. As shown in Fig. 3 Fig. 4 each derivative eluted as a single peak and caused a definite response in the MS detector. For some peaks severe tailing was observed. The masses of the characteristic fragments using MS detection with electron ionization (EI) are summarized in Table 1 Table 2.

For both methods calibration was done for methylamine, dimethylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, pyrrolidine, piperidine, morpholine and ethanolamine using tap water which was spiked with different amounts of the compounds in question. In order to test the linearity of the calibration curve, amounts of the aliphatic amines ranging from 100 ng/l to 50 μg/l were derivatized and the amine derivatives were injected into the GC–MS system. For each compound a linear relationship could be observed from which detection limits as well as determination limits and regression coefficients were calculated. The respective data are presented in Table 3 for the BSC method and in Table 4 for the 2,4-DNFB method. The regression coefficients underline the good linearity which could be observed in the whole concentration range under investigation for each compound, especially for the derivatization with BSC. The numerical value for the detection limit and the determination limit of *n*-butylamine derivatized with BSC in Table 3 is obviously higher than that of the others. The reason is probably that *n*-butylbenzenesulfonamide is used as plasticizer and therefore it may cause a comparatively high blank value during sample work-up.

Calculating the minimum detection limits (MDL) as three times baseline noise, the values for the

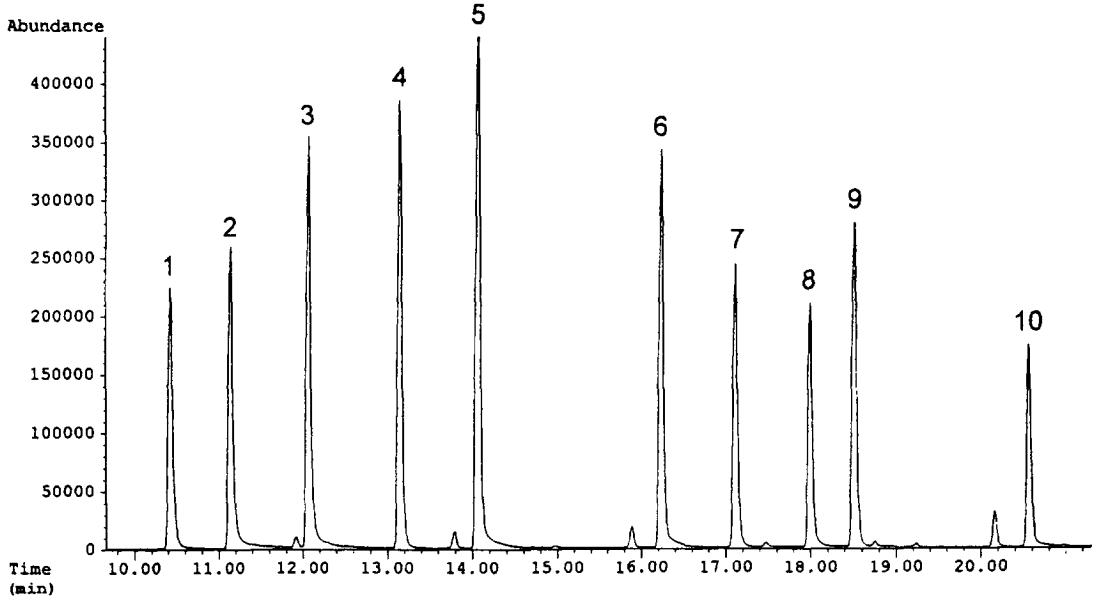

Fig. 3. Gas chromatogram of aliphatic primary and secondary amines after derivatization with benzenesulfonyl chloride. Peaks: 1=dimethylamine, 2=methylamine, 3=ethylamine, 4=diethylamine, 5=*n*-propylamine, 6=*n*-butylamine, 7=pyrrolidine, 8=morpholine, 9=piperidine, 10=*n*-hexylamine (I.S.). Concentrations are 25 μg/l each.

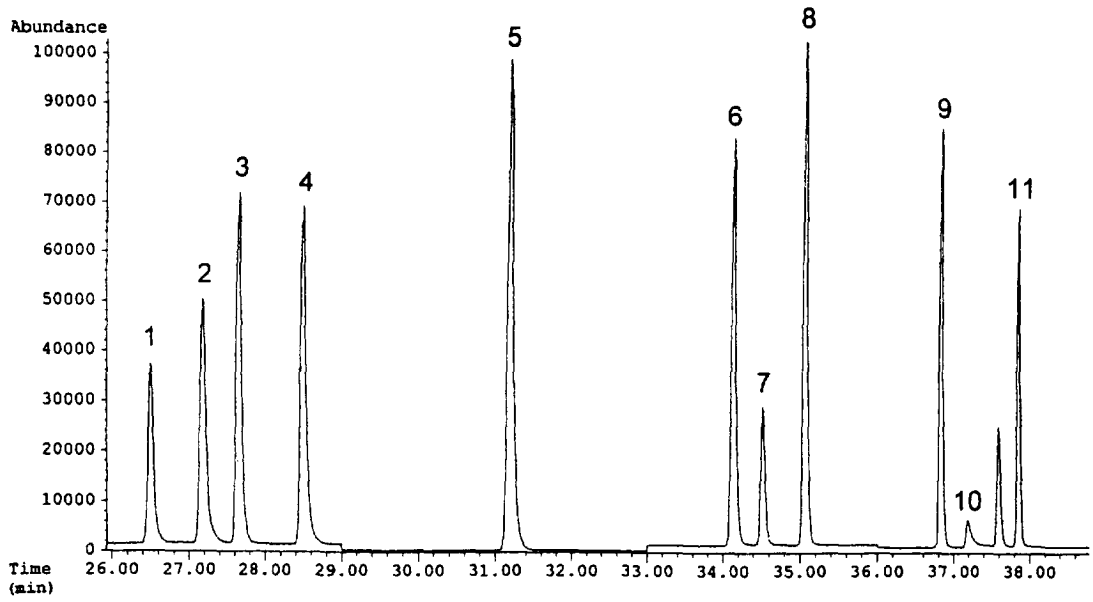

Fig. 4. Gas chromatogram of aliphatic primary and secondary amines after derivatization with 2,4-dinitrofluorobenzene. Peaks: 1= dimethylamine, 2=methylamine, 3=diethylamine, 4=ethylamin, 5=*n*-propylamine, 6=*n*-butylamine, 7=morpholine, 8=piperidine, 9= pyrrolidine, 10=ethanolamine, 11=*n*-hexylamine (I.S.). Concentrations are 25 μg/l each.

derivatization method with BSC were below 100 ng/l, as shown in Table 3. Using the derivatization method with 2,4-DNFB for the primary and secondary aliphatic amines the numerical value would be about 500 ng/l (see Table 4). Because of the poor recovery for the extraction procedure of the derivative, the value obtained for ethanolamine was ca. 2 μg/l and thus significantly higher. In all cases the determination and detection limits might possibly decrease by using more than 200 ml water sample for the derivatization. Due to the practicability, larger volumes were not used. The determination of recoveries for both derivatization procedures was not possible as neither the 2,4-DNFB derivatives nor the BSC derivatives of the aliphatic amines are commercially available. A large-scale synthesis and isolation of the derivatives was not attempted.

Table 1
Main mass fragments of the 2,4-dinitrofluorobenzene derivatives of some primary and secondary aliphatic amines (underlined: mass fragments used for selected ion monitoring (SIM), bold: mass fragments chosen for quantification)

Compound	Fragment masses *m/z*
Methylamine	78, 93, 104, 105, **197**
Dimethylamine	136, 148, 166, 194, **211**
Ethylamine	78, 118, 150, **196**, 211
Diethylamine	164, 196, 222, **224**, 239
n-Propylamine	104, 150, 166, **196**, 225
n-Butylamine	104, 150, 179, **196**, 239
n-Hexylamine (I.S.)	78, 150, 180, **196**, 267
Pyrrolidine	189, 190, **220**, 237, 254
Piperidine	189, 204, 216, **234**, 251
Morpholine	177, 190, 219, **236**, 253
Ethanolamine	78, 104, 150, **196**, 227

Table 2
Main mass fragments of the N-alkylbenzenesulfonamides of some aliphatic primary and secondary amines (underlined: mass fragments used for selected ion monitoring (SIM), bold: mass fragments chosen for quantification)

Compound	Fragment masses *m/z*
Methylamine	77, 106, 107, 141, **171**
Dimethylamine	77, 78, 120, 141, **185**
Ethylamine	77, 78, 141, **170**, 185
Diethylamine	77, 78, 141, **198**, 213
n-Propylamine	77, 78, 141, **170**, 199
n-Butylamine	77, 141, 158, **170**, 213
n-Hexylamine (I.S.)	77, 100, 141, 158, **170**, 241
Pyrrolidine	70, 77, 141, 146, 210, **211**
Piperidine	77, 84, 141, 184, 224, **225**
Morpholine	77, 86, 141, 184, 196, **227**

Table 3
Minimum detection limits calculated as three times baseline noise, statistical detection limits, determination limits and regression coefficients (R^2) of the derivatization procedure with benzenesulfonyl chloride

Compound	Minimum detection limit (μg/l)	Statistical detection limit (μg/l)	Determination limit (μg/l)	R^2
Methylamine	0.10	0.30	0.44	0.9981
Dimethylamine	0.10	0.22	0.32	0.9969
Ethylamine	0.10	0.24	0.36	0.9996
Diethylamine	0.10	0.57	0.85	0.9894
n-Propylamine	0.10	0.47	0.71	0.9931
n-Butylamine	0.10	1.2	1.7	0.9919
Pyrrolidine	0.10	0.49	0.73	0.9989
Piperidine	0.10	0.40	0.59	0.9968
Morpholine	0.10	0.50	0.74	0.9961

Table 4
Minimum detection limits calculated as three times baseline noise, statistical detection limits, determination limits and regression coefficients (R^2) of the derivatization procedure with 2,4-dinitrofluorobenzene

Compound	Minimum detection limit (μg/l)	Statistical detection limit (μg/l)	Determination limit (μg/l)	R^2
Methylamine	0.50	0.64	0.95	0.9692
Dimethylamine	0.50	0.48	0.72	0.9940
Ethylamine	0.50	0.65	0.97	0.9888
Diethylamine	0.50	0.91	1.3	0.9507
n-Propylamine	0.50	0.61	0.91	0.9671
n-Butylamine	0.50	0.74	1.1	0.9817
Pyrrolidine	0.50	1.2	1.7	0.9743
Piperidine	0.50	0.42	0.63	0.9938
Morpholine	0.50	1.1	1.7	0.9656
Ethanolamine	2.0	5.0	7.0	0.9895

Table 5
Relative standard deviations (R.S.D.) of both derivatization procedures, using 2,4-dinitrofluorobenzene or benzenesulfonyl chloride, respectively

Compound	Derivatization with 2,4-DNFB		Derivatization with BSC	
	R.S.D. (%)	R.S.D. (%) for GC–MS analysis	R.S.D. (%)	R.S.D. (%) for GC–MS analysis
Methylamine	21	12	8.9	4.9
Dimethylamine	19	18	8.5	6.5
Ethylamine	34	14	7.2	6.3
Diethylamine	35	16	8.6	4.4
n-Propylamine	14	14	3.7	5.5
n-Butylamine	22	12	6.4	5.8
Pyrrolidine	25	18	7.7	7.6
Piperidine	16	15	3.1	3.1
Morpholine	31	28	3.1	2.9
Ethanolamine	19	17	–	–

Table 6
Concentrations of aliphatic amines in waste water of an industrial plant after derivatization with 2,4-dinitrofluorobenzene and benzenesulfonyl chloride, respectively

Compound	Derivatization with 2,4-DNFB	Derivatization with BSC
	c (μg/l)	c (μg/l)
Methylamine	30	20
Dimethylamine	70	80
Ethylamine	20	10
Diethylamine	30	20
n-Propylamine	<5	3
n-Butylamine	<5	<1
Pyrrolidine	<5	<1
Piperidine	<5	<1
Morpholine	20	15
Ethanolamine	60	–

The reproducibility of both methods was examined as follows: Five different solutions of the aliphatic amines were derivatized with a concentration of 1 μg/l which is close above the calculated statistical determination limit of most of the inspected compounds. For ethanolamine a concentration of 5 μg/l was chosen. A 1 μl volume of the dichloromethane extracts of these samples was injected in series to

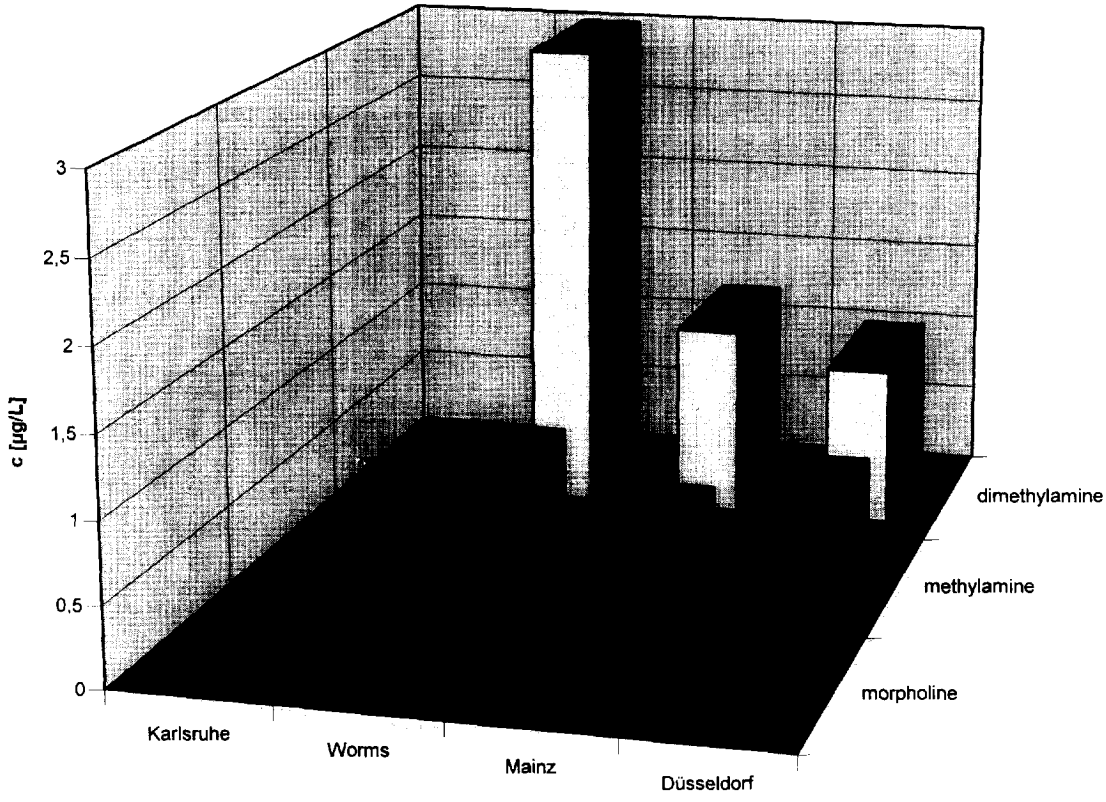

Fig. 5. Concentrations of aliphatic amines in river Rhine in September 1995.

give information about the relative standard deviation (R.S.D.) of the whole analytical method (derivatization procedure plus GC–MS analysis). Additionally one extract was injected five times in succession in order to have data about the R.S.D. of the GC–MS analysis. As shown in Table 5, the relative standard deviations of the derivatization method with BSC yielded values to less than 10% for each compound, whereas the reproducibilities of the method with 2,4-DNFB as derivatization reagent were calculated in a range between 10 and 30%.

Comparing the R.S.D. of the whole procedure to the R.S.D. of the pure GC–MS analysis one can recognize that for both methods the main origin of the limited reproducibility is the gas-chromatographic part. In contrast to this the derivatization process seems to be very reproducible.

Comparing the BSC derivatization method to the 2,4-DNFB method one recognizes that the former method is more appropriate for the determination of aliphatic amines from sewage, surface and drinking waters, since it presents the more sensitive method which additionally delivers more reproducible results. In addition, the handling of the derivatization method with BSC turned out to be simpler and faster, so that further investigations were essentially done using this procedure. However, in order to receive information about the occurrence of ethanolamine in waste water samples, the more suitable method is the derivatization with 2,4-DNFB.

Table 6 shows an example in which we compared the concentrations of aliphatic amines in waste water of an industrial plant determined by both methods. For this purpose the water sample was diluted 1:10

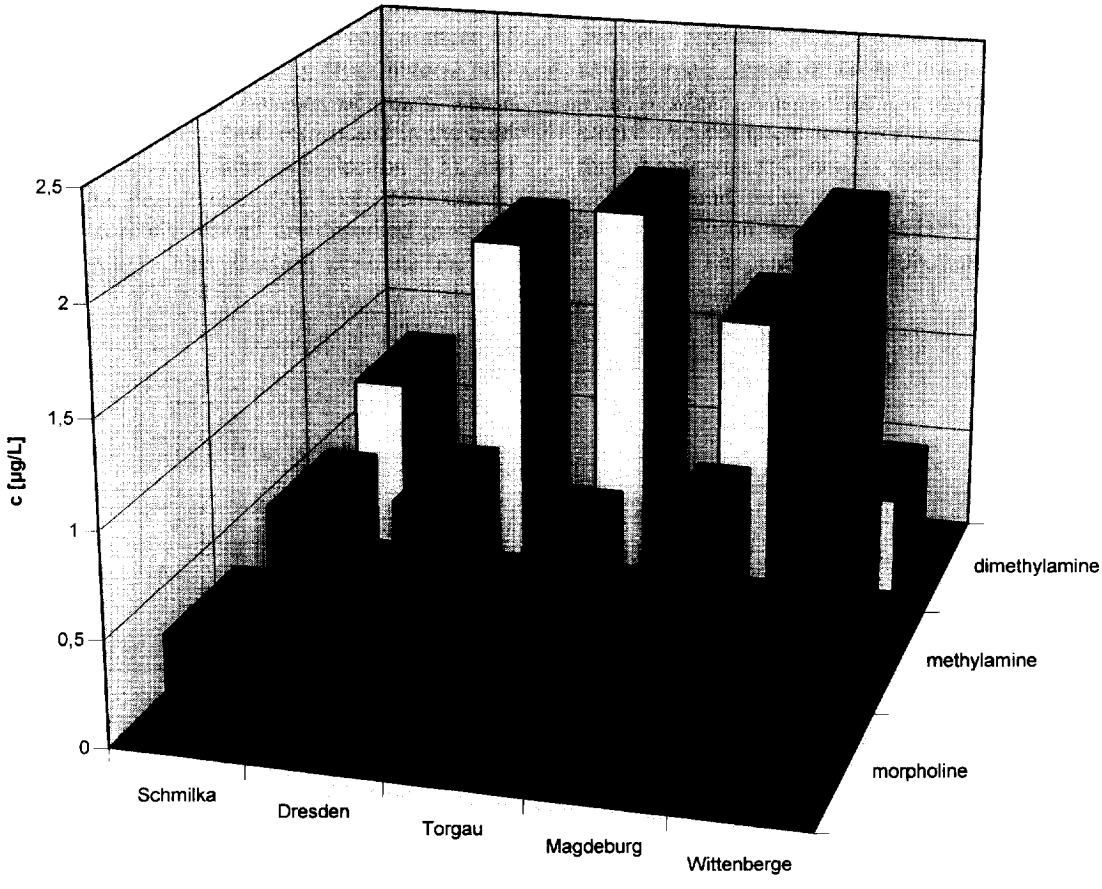

Fig. 6. Concentrations of aliphatic amines in river Elbe in September 1995.

prior to analysis. The results presented here which were confirmed by further measurements show a good correspondence within the limits of accuracy, which proves the efficiency and reliability of both analytical methods for the determination of primary and secondary aliphatic amines.

Both methods developed have been successfully applied to waste water of industrial plants. In several waste water samples most of the compounds of interest could be traced. The waste waters under investigation contained particularly methylamine, dimethylamine, morpholine and ethanolamine in concentrations up to 100 μg/l. Furthermore, ethylamine and diethylamine were found in concentrations up to 30 μg/l. Pyrrolidine and piperidine have been found in industrial waste waters at lower concentrations.

In order to get information about the occurrence of aliphatic primary and secondary amines in surface waters, several German rivers were examined using the derivatization method with BSC, although by this means ethanolamine can not be detected. Because of the comparatively low sensitivity of the derivatization method with 2,4-DNFB, ethanolamine could not be found in any natural water. Besides, this compound was found to be efficiently degraded by microorganisms [23] so that any anthropogenic pollution would be difficult to trace. Figs. 5 and 6 show some results of the most important aliphatic amines, methylamine, dimethylamine and morpholine, in the rivers Rhine and Elbe in September 1995. In the Rhine, dimethylamine was found up to 3 μg/l whereas methylamine and morpholine were found at a concentration level of about 1 μg/l. In the Elbe, the concentrations of both methylamine and dimethylamine were about 2 μg/l. These findings could be confirmed by further measurements. Occasionally ethylamine and diethylamine were found in both Rhine and Elbe in concentrations below 1 μg/l.

In conclusion, the measurements demonstrated that low-molecular-mass primary and secondary aliphatic amines can be precisely determined in waste water and surface water by GC–MS as their 2,4-DNFB derivatives or their benzenesulfonamides. The derivatization method with BSC was found to be more selective and sensitive, and waste water as well as surface and drinking water samples can be analyzed well. We believe that both methods provide a powerful tool in environmental monitoring of the compounds in question. First measurements showed that the aliphatic amines under investigation were found to be widespread in the aquatic environment.

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