# Extraction and analysis of various benzothiazoles from industrial wastewater

## Oliver Fiehn, Thorsten Reemtsma *, Martin Jekel

*Department of Water Quality Control, Technical University of Berlin, Sekr. KF 4, Strasse des 17. Juni 135, D-10623 Berlin, FRG*

## Abstract

A method was developed for the analysis of benzothiazole, 2-mercaptobenzothiazole, 2-(methylthio)benzothiazole and 2-(thiocyanomethylthio)benzothiazole from industrial wastewater. It includes liquid–liquid extraction with ethyl acetate and toluene at pH 8.5, followed by liquid chromatographic (LC) analysis using a RP-18 column and an acetonitrile–water gradient with UV detection at variable wavelengths. LC analysis is compared with the potential of gas chromatography and its advantages are discussed. Solid-phase extraction appeared not to be suitable for all compounds. The method allows the determination of benzothiazole derivatives without further clean-up down to contents of about 5 $\mu$g l$^{-1}$, with recovery rates exceeding 90% for all compounds. Dissolved organic carbon contents of up to 900 mg l$^{-1}$ did neither interfere with extraction nor with chromatographic separation.

*Keywords:* Liquid chromatography; Extraction; Benzothiazoles; Wastewater; Waters

## 1. Introduction

Benzothiazole based substances have gained widespread application in industrial processes. 2-Mercaptobenzothiazole (MBT) and 2,2'-(dithiobis)benzothiazole (MBTS) are well-known vulcanization accelerators in the rubber industry [1]. MBT is also contained in metal finishing liquors [2]. 2-(Thiocyanomethylthio)benzothiazole (TCMTB) is widely used as fungicide for wood protection [3–5] and in the leather industry as substitute for chlorinated phenols, namely pentachlorophenol (PCP) [6–8]. Benzothiazole (BT)

is also mentioned as fungicide [9]. Correspondingly, benzothiazole and its derivatives are widely distributed and have been detected by screening analyses in industrial and municipal wastewater [10–12] as well as in various environmental compartments such as in groundwater and river water [9,13,14], tap water [14] as well as in landfill leachates [15], in atmospheric deposition [16], coastal sediments [1] and fish [17].

However, reports on the specific extraction and analysis of BT derivatives from aqueous media are rarely found. Warner et al. [10] employed liquid–liquid extraction (LLE) with dichloromethane for TCMTB and MBTS from spiked distilled water, but recovery from wastewater was poor. An on-line trace enrichment methodology for various pesticides including BT on PLRP-S

* Corresponding author.

polymer is reported by Liska et al. [9] for surface water monitoring. Analysis of BT derivatives by reversed-phase liquid chromatography (LC) [3,6,18] and of TCMTB and MBTS by normal-phase LC [10] is reported.

In order to determine these compounds in effluents of industrial processes involving BT derivatives as fungicides, and to study their behaviour in biological wastewater treatment, a method was required for the parallel extraction and analysis of several BT compounds from wastewater. We here report on the development of a suitable method for these purposes. LLE is compared with solid-phase extraction (SPE) and the potentials of LC and gas chromatography (GC) for the detection of BT derivatives are investigated. The use of the finally selected method is illustrated by its application on various wastewater samples. According to the obtained detection limit, the method appears not to be limited to industrial wastewater analyses.

## 2. Experimental

### 2.1. Chemicals and materials

TCMTB was received from the Dr. Eberle Co. (Tübingen). BT was obtained from Aldrich (Steinheim) and MBTS and MTBT from Ferak (Berlin). MBT and all solvents were purchased from Merck (Darmstadt).

Solid-phase extraction tubes of the following companies were investigated: 500 mg RP-18 (ww18) from Worldwide Monitoring (Horsham, USA); 400 mg Adsorbex RP-18 (mer18) from Merck; Supelclean LC-18 (Su18), Envi-18 (SuEn), LC-CN (SuCN), EnviCarb (SuCa), LC-8 (Su8) all as 500 mg cartridges from Supelco (Bellefonte, CA).

Trimethylanilinium hydroxide (TMAH, 0.2 M in methanol) for methylation was purchased by Regis (Norton Grove, USA) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) for silylation by Fluka (Buchs, Switzerland). Glassfibrefilters were obtained from Schleicher and Schuell (Dassel, Germany) and 0.45-$\mu$m cellulose acetate filters from Sartorius (Göttingen).

Solvents were redistilled in an all-glass apparatus prior to use. Doubly distilled water was used for standard solutions.

### 2.2. Analytical apparatus and procedure

#### LC

LC analyses were carried out on a 30 cm x 4.6 mm i.d. Eurosphere 80 $C_{18}$ column, 5 $\mu$m (Knauer, Berlin) with an L-6200A gradient pump, an AS-2000 A autosampler and a T-6300 column thermostat (all from Merck-Hitachi, Darmstadt). 20 $\mu$l samples were injected. UV detection was performed with a SPD-10A UV–visible detector (Shimadzu, Kyoto) and data storage and processing with ChromStar V 3.11 software (Bruker, Bremen).

LC separation was performed with an acetonitrile–water gradient. Distilled water, containing 4 mmol $1^{-1}$ $NaH_2PO_4$ and adjusted to pH 4.5 with $H_3PO_4$ was employed as solvent A. Solvent B was an acetonitrile–solvent A mixture (90:10), acidified to pH 4.5 with $H_3PO_4$. Elution started with 70% B at a flow rate of 0.5 ml $min^{-1}$ and was linearly shifted to 90% B (flow rate, 0.5 ml $min^{-1}$) at 11 min. After 3 min of isocratic elution the gradient was shifted to 100% B and 1.0 ml $min^{-1}$ at 14.5 min. Separation was completed within 10 min of isocratic elution followed by 6 min of equilibration. Column temperature was held at 40°C.

UV detection was performed by time-programmed wavelength changes according to the absorption maxima of the individual compounds in the higher wavelength range: MBT was detected at 325 nm after 6 min, BT at a wavelength of 250 nm around 8 min, TCMTB and MTBT at 280 nm between 10 and 13 min, and MBTS at 280 nm after 19 min. These UV maxima are less affected by coeluting contaminants compared to the maxima in the low-UV range. No fluorescence activity of benzothiazole derivatives could be determined. The pH of the eluents has a significant influence on the retention times of the analytes. Moreover, quantification of MBT is strongly affected by the pH of the eluents. By raising the pH above 6.5, its absorbance maximum is shifted from 325 nm to 309 nm and the

absorbance at 325 nm is, hence, reduced to 10%. Therefore, LC eluents and sample solutions have to be buffered as given above.

*Identification*

Peak identification was based on the retention times of the corresponding standards and on the analysis of spiked samples. Comparison of the UV spectra gained by a diode array detector (DAD) supports peak assignment in critical cases. If no DAD is available, dual wavelength detection at the first and second maxima can be employed to ensure peak identity and purity. The absorbance ratio for MBT at 228. to 325 nm is 2.05, for BT it is 0.33 between 284 and 250 nm, for TCMTB it is 1.73 between 224 and 280 nm and for MTBT a ratio of 1.56 is recorded between the absorbance at 224 and 278 nm.

LC–mass spectrometry (LC–MS) was recently shown to be a powerful method for the identification of benzothiazoles [19], but it was not available to us. Collecting the LC peaks of interest and subsequent analysis by GC–MS offers an alternative route for some of the benzothiazole derivatives (see below).

*GC*

Gas chromatography was performed with a PE 8420 gas chromatograph (Perkin-Elmer, Überlingen) with a flame ionization detector (FID) on a 30 m × 0.25 mm i.d. SPB 5-column (Supelco) and Helium as carrier gas (112 kPa). Injector and detector temperatures were 250 and 300°C, respectively. Oven temperature was held at 100°C for 2 min, followed by a ramp rate of 6°C min$^{-1}$ to a final temperature of 280°C, which was kept constant for 10 min.

GC–MS was done on a HP 5989 A quadrupole mass spectrometer coupled with a HP 5890 II gas chromatograph (Hewlett-Packard, Böblingen, Germany) in the electron impact mode (EI) at 70 eV on a 30 m x 0.25 mm i.d. SPB5 column. Injector temperature was 250°C, GC–MS interface temperature was 280°C, and source temperature was kept at 200°C.

*UV absorbance and DOC*

UV absorption spectra of the benzothiazoles were measured on a Lambda-2 spectrophotome-

ter (Perkin-Elmer). An RF-551 LC fluorescence detector (Shimadzu) was employed for determining fluorescence activity. Dissolved organic carbon (DOC) of wastewater samples was determined with an Astro LiquiTOC 2001-MB analyzer (Foss-Heraeus, Hanau, Germany).

## 2.3. Extraction

Aqueous standard solution of 100 μg l$^{-1}$ MBT, 90 μg l$^{-1}$ MTBT, 117 μg l$^{-1}$ TCMTB and 175 μg l$^{-1}$ BT were prepared by adding appropriate amounts of a stock solution of the analytes in acetone to a dry flask. The acetone was allowed to evaporate and the dry residue redissolved in water by ultrasonification. 4 g l$^{-1}$ NaCl were added and the pH values adjusted (NaOH–H$_3$PO$_4$). 25 ml aliquots were employed for extraction.

MBTS was originally intended to be included in the analytical procedure. However, it was found to be poorly soluble in water and to decompose quickly. Mixtures of MBTS with distilled water at levels between 200 μg l$^{-1}$ and 4 mg l$^{-1}$ remained turbid after 30 min in an ultrasonic bath or stirring overnight. After filtration over a 0.45-μm cellulose acetate filter, the filtrates were extracted with dichloromethane at pH 6.5 and no MBTS was detectable. However, varying amounts of MBT and MTBT were detectable as well as several unidentified peaks. MBTS is, thus, suggested to be of minor importance in the context of industrial wastewater analysis. Astonishingly, Warner et al. [10] reported the recovery of MBTS up to 940 μg l$^{-1}$ from distilled water.

Wastewater samples from the different steps of a tannery wastewater treatment pilot plant [20] were stored frozen until analyzed, while effluents of a municipal wastewater treatment plant were instantly worked up. All samples were filtered over 0.45-μm cellulose acetate filters prior to extraction.

Due to possible photolytic reactions of TCMTB and MBT [18] all samples, standards and extracts were stored in the dark during work-up and, generally, exposed to daylight as shortly as possible (less than 60 min). Furthermore, hydrolysis of TCMTB to MBT was observed [18], with $t_{1/2}$ of

83 h at pH 9 and $t_{1/2}$ between 760 and 18800 h at pH 8. Therefore, the pH of aqueous samples must not be raised above 8.5.

### Solid-phase extraction

Standard solutions containing 4 g $l^{-1}$ NaCl and adjusted to pH 8.5 were extracted on the cartridges mentioned above at a flow rate of 2 drops $s^{-1}$. After a 2 ml wash with distilled water (4 g $l^{-1}$ NaCl, pH 8.5) ambient air was sucked through the columns for 1 min. The analytes were then sequentially eluted with one of the following solvent systems: (i) 2 ml methanol, 2 ml methanol–dichloromethane (20:80, v/v), 2 ml dichloromethane, 2 ml toluene or (ii) 2 ml acetonitrile, 2 ml acetone, 2 ml toluene. Work-up of solid phase extracts followed the procedure for LLE given below.

### Liquid–liquid extraction

LLE was performed at different pH values with three 5 ml portions of various solvents. The centrifuged organic phases were combined and reduced to approximately 0.5 ml with a rotary evaporator (50°C). The extracts were then evaporated to near dryness in 2 ml glass vials under a gentle stream of nitrogen at 50°C and redissolved in 1 ml of an acetonitrile–water mixture (60:40; v/v) buffered at pH 4.5 ($H_3PO_4$–$NaH_2PO_4$). Pure acetonitrile is also suitable as solvent. Owing to the volatility of BT, evaporation of the extracts to total dryness must be carefully avoided.

## 3. Results and discussion

### 3.1. Analysis

#### LC

Reversed-phase LC on $C_{18}$ phases has been previously employed for the analysis of TCMTB [3,6,21,22] and other BT derivatives [18]. The structures of the benzothiazole derivatives under investigation are given in Fig. 1. They are well separated within 21 min under the selected chromatographic conditions (Fig. 2). Owing to the poor solubility and stability of MTBS in water
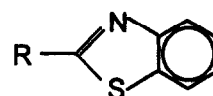


| | R |
|---|---|
| MBT | -SH |
| BT | -H |
| TCMTB | -S-CH₂-SCN |
| MTBT | -SCH₃ |
| MBTS | -S-MBT |

Fig. 1. Structure of the employed benzothiazole derivatives.

(see above), only MBT, BT, TCMTB and MTBT were included in further work.

UV calibration curves following LC separation are linear for MBT, TCMTB and MTBT from 4 to 1000 ng injected onto the column, whereas linearity for BT was observed above 30 ng. The detection limit ($S/N > 3$) and the more rigid method detection limits, derived from the relative standard deviation of the regression lines [23] are given in Table 1. While the first are in the range of 0.2 to 1 ng, the latter vary between 4 ng and 13 ng, with relative standard deviations of 3–5%. Assuming, that 25 ml aliquots of samples are extracted and redissolved in 500 $\mu$l of solvent
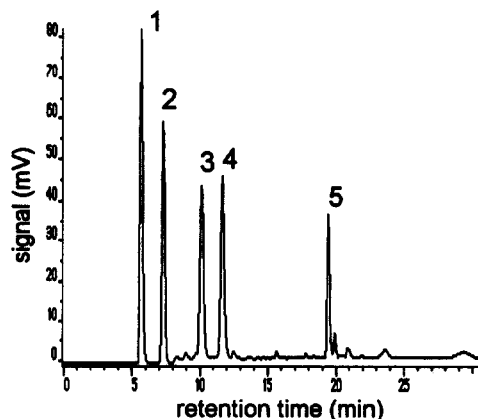


Fig. 2. Standard chromatogram of five benzothiazole components: 1 = MBT (44 ng), 2 = BT (180 ng), 3 = TCMTB (215 ng), 4 = MTBT (94 ng), 5 = MBTS (223 ng). Minor peaks are due to technical byproducts.

Table 1
Characteristics of UV detection of BT derivatives following LC separation (DL: detection limit; MDL: method detection limit; RF: response factor; R.S.D.: relative standard deviation, $r$: correlation coefficient of the linear regression)

|        | wavelength (nm) | DL (ng) | MDL (ng) | RF$\times10^{-4}$ (ng AU$^{-1}$) | R.S.D. (%) | $r$ |
|--------|------|-----|----|------|-----|--------|
| MBT    | 325  | 0.2 | 4  | 1.13 | 3.3 | 0.9960 |
| BT     | 250  | 1   | 13 | 4.84 | 4.7 | 0.9975 |
| TCMTB  | 280  | 0.5 | 5  | 6.09 | 3.5 | 0.9996 |
| MTBT   | 280  | 0.5 | 4  | 2.68 | 2.6 | 0.9987 |

prior to analysis, this results in a detection limit of 5 $\mu$g l$^{-1}$.

### GC

BT, MBT and MTBT are well detectable by GC-FID without derivatization. The detection limits ($S/N > 10$) under the above mentioned conditions are between 1 ng for MBT and 2 ng for BT and MTBT injected onto the column. However, peak tailing easily occurs, namely for MBT. These substances do not react with BSTFA. MBT is methylated with TMAH at the thiol S and at the ring N at a ratio of about 95:5. However, methylated MBT would interfere with the determination of originally occurring MTBT.

TCMTB was reported to be thermally labile and, therefore, not detectable by GC [3]. Correspondingly, only traces of the amount of TCMTB injected onto the column were detectable by GC–MS, provided that no derivatization reaction was performed prior to analysis. MBTS was not detectable by both, GC-FID and GC–MS.

Detection limits for BT, MBT and MTBT in GC-FID analysis appeared to be slightly lower than those of LC-UV. Nevertheless, LC analysis is favourable in wastewater analysis, since TCMTB and MBTS are not detectable by means of GC. Furthermore, wastewater extracts analyzed by GC-FID are usually too complex for reliable peak assignment. UV detection employed in LC (see above) appears to be more specific. Gas chromatography will be advantageous in the combination with MS detection for the structural elucidation of unknown BT derivatives and for environmental screening. Correspondingly, most reports on the appearance of BT derivatives were based on GC–MS screening analyses [9,11–16].

For the trace analysis of BT compounds by MS under single ion monitoring the fragment ions 108, (122) and 135 are suitable, whereas 108, 135 and 167 are characteristic for MBT derivatives.

### 3.2. Extraction

In recent years SPE is often favoured over LLE due to its ease of handling and low solvent consumption. SPE by on-line trace enrichment for the purpose of surface water pesticide residue analysis was reported [9]. The authors employed PLRP-S polymer, but of the four BT derivatives investigated here, only BT was included. The breakthrough volume was reported to exceed 30 ml. BT and MTBT have also been determined by SPE on $C_{18}$ phases within toxicity fractionation procedures applied to surface waters [24,25]. The completeness of extraction was, however, not investigated. The use of LLE, on the other hand, was investigated by Warner et al. [10] but limited to TCMTB and MBTS and recovery from wastewater was reported to be low.

We, therefore, investigated the use of SPE and LLE for the parallel extraction of BT and its

Table 2
Recovery (%) of benzothiazole derivatives (around 100 $\mu$g l$^{-1}$) from distilled water by liquid–liquid extraction with various solvents

| Solvent | MBT | | | BT | | | TCMTB | | | MTBT | | |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|
|         | pH 2 | pH 5 | pH8.5 | pH2 | pH5 | pH 8.5 | pH2 | pH5 | pH8.5 | pH2 | pH5 | pH 8.5 |
| Toluene | 2 | 3 | 95 | 19 | 98 | 98 | 96 | 96 | 83 | 99 | 85 | 61 |
| tert.-Butylmethyl ether | 18 | 8 | 13 | 6 | 20 | 10 | 95 | 78 | 85 | 58 | 108 | 63 |
| Dichloromethane | 40 | 68 | 72 | 15 | 61 | 70 | 103 | 105 | 89 | 48 | 54 | 103 |
| Ethyl acetate | 68 | 74 | 105 | 85 | 51 | 60 | 102 | 99 | 90 | 92 | 92 | 89 |

three derivatives MTB, TCMTB and MTBT from wastewater.

### Liquid–liquid extraction

LLE of the four compounds from distilled water was performed with four solvents at three pH values after the addition of 4 g $l^{-1}$ NaCl (Table 2). MBT is well extractable under weakly alkaline conditions with ethyl acetate or toluene; the latter should be employed for the extraction of BT. TCMTB and MTBT appear to be best extracted from acidic or neutral milieu by either solvent, but extraction at pH 8.5 with ethyl acetate still provided acceptable results (89 and 90%). Table 2 further indicates, that the relationship between the polarity of a solvent and the polarity of the analyte is complex. Toluene, although being one of the less polar solvents investigated, exhibits comparatively high recoveries namely with the most polar compounds. This might be due to the aromatic nature of toluene and the BT derivatives.

Corresponding to earlier reports [10], TCMTB is readily extracted by dichloromethane. However, it becomes evident that dichloromethane is a poor solvent for the extraction of the more polar compounds MBT and BT. This finding might explain some inconsistent results reported by Brownlee et al. [18].

Considering these results, seven replicate extractions were carried out at three different pH values subsequently employing 5 ml portions of ethyl acetate (twice) and toluene (once) after the addition of salt (4 g $l^{-1}$ NaCl).

While the recovery of BT, TCMTB and MTBT is between 94 and 97% at pH 6.5, MBT recovery
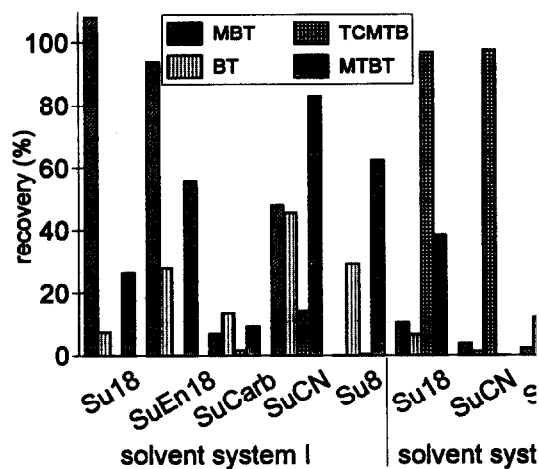


Fig. 3. Recovery of benzothiazole derivatives by solid-extraction. Solvent system I: MeOH–CH$_2$Cl$_2$–toluene vent system II: MeCN–acetone–toluene.

requires a pH of 8.5 (Table 3). Recoveries o first three components are still acceptable wit to 98% at this pH. Standard deviations for whole procedure are below 9%. Recoveries a 8.5 without NaCl are poor for all compor (Table 3).

Brownlee et al. [18] observed enhanced hy ysis of TCMTB to MBT above pH 9. In sep: extractions of TCMTB-spiked wastewater a 8.5, however, MBT was not detectable, indic that TCMTB is stable at pH 8.5 during worl

### Solid-phase extraction

Solid phases of different polarity were ployed for solid-phase extraction (SPE), an samples adjusted to optimal conditions of (pH 8.5, 4 g $l^{-1}$ NaCl). The obtained result quite heterogenous (Fig. 3). MBT is well r ered on C$_{18}$ phases (Su18, SuEn) with methanolic solvent system (I), while TCM1 equally extractable by C$_8$ (Su8), Cyano (Si and C$_{18}$ phases (Su18) with the acetonitrile taining solvent system (II). MTBT was most ciently extracted (around 85%) on the cyano [ employing the methanolic solvent system, wh the recovery of BT was poor in all cases. deactivated carbon (SuCarb) is not advantag Extraction by an endcapped C$_{18}$ phase (w

Table 3
Recovery (%) and relative standard deviation (%) of benzothiazole derivatives (around 100 $\mu$g $l^{-1}$) from distilled water ($n = 7$)

| | NaCl | | | No salt |
|---|---|---|---|---|
| | pH 2 | pH 6.5 | pH 8.5 | pH 9 |
| BT | 89.4 (4.3) | 95.9 (7.2) | 88.1 (8.7) | 52 |
| TCMTB | 98.5 (1.2) | 96.9 (2.7) | 87.4 (5.5) | 70 |
| MTBT | 97.8 (8.1) | 94.4 (5.9) | 97.8 (3.7) | 83 |
| MBT | 51.9 (6.0) | 20.3 (3.8) | 104.3 (5.4) | 64 |

was comparable to the $C_{18}$ phase shown in Fig. 3. (Su18).

Correspondingly, MBT and TCMTB might be well extractable on $C_{18}$ reversed phases employing a methanol–acetonitrile solvent system. Under these conditions, however, the recoveries of MTBT and, moreover, BT will be insufficient.

The low recovery rates, however, appeared not to be due to incomplete adsorption of the analytes onto the solid phases. Filtrates of $C_{18}$, Cyano and $C_8$ SPE were reextracted by LLE at pH 8.5. A maximum of 6% of TCMTB and 8% of BT was recovered. On the other hand, additional elution of the cartridges with 2 ml portions of less polar solvents (cyclohexane, hexane) provided no additional amounts of benzothiazoles. The extremely poor recovery rates of BT, the most volatile BT derivative, might be due to the drying procedure applied before LC analysis. To some extent, this might also be true for MTBT, the second volatile compound. This problem would be avoided by on-line trace enrichment [9].

Considering these results SPE appears to be of limited value for the parallel extraction of MBT, BT, MTBT and TCMTB. Nevertheless, a method for the simultaneous quantitative extraction of MBT, BT and MTBT by solid phases would be of value for the monitoring of surface waters.

### 3.3. Application on wastewaters

#### LC analysis and extraction

LLE at pH 8.5 with ethyl acetate and toluene, followed by LC analysis of the BT derivatives was then applied on four different types of wastewater (industrial effluent, anaerobically treated, aerobically treated and effluent of a municipal wastewater treatment plant) spiked with the four BT derivatives between 90 and 175 $\mu$g l$^{-1}$.

Fig. 4 displays the liquid chromatogram of an extract from a spiked wastewater sample. All components are clearly detected and no interference with other coextracted contaminants was observed. The detection of the first peak (MBT) at 325 nm is advantageous within this respect, since potential polar contaminants are not detected at this wavelength. Furthermore, extraction under weakly alkaline conditions minimizes
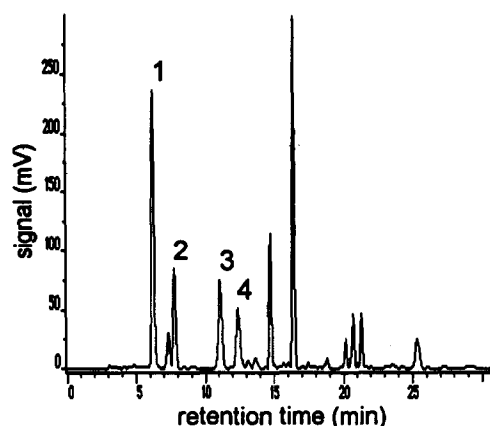


Fig. 4. Liquid chromatogram of an extract of spiked wastewater (for peak numbers and amounts see Fig. 2).

the coextraction of anionic compounds, which form a substantial portion of dissolved organic compounds in biologically treated wastewaters.

The recoveries of BT derivatives from the four types of wastewaters varied between 93% for MBT, 87% for BT, 95% for MTBT and 92% for TCMTB with relative standard deviations of 5 to 9%. These results correspond with those obtained for distilled water. The recovery of BT derivatives was, thus, neither affected by inorganic constituents of the wastewater, nor by its DOC content, which varied between 18 mg l$^{-1}$ in the effluent of the municipal wastewater treatment plant and 900 mg l$^{-1}$ in the untreated tannery wastewater.

#### Reproducibility

Real, not spiked, samples of tannery wastewater and the effluents of the tannery wastewater treatment pilot plant were analysed in duplicate within two months to examine the reproducibility of the whole analytical procedure (Table 4). Although originally applied within the tanning process, no TCMTB was detectable. This corresponds with the reported hydrolysis of TCMTB under alkaline conditions [18], such as in tannery effluent. Instead of TCMTB, the BT derivatives MBT, BT and MTBT were detected. Contents varied between 650–690 $\mu$g l$^{-1}$ of MBT in the untreated and anaerobically treated wastewater

Table 4
Content of BT derivatives in tannery wastewater samples ($\mu$g $l^{-1}$) and difference between duplicates (%) (TCMTB: below detection limit)

|  | Untreated | | Anaerobic | | Aerobic | |
|---|---|---|---|---|---|---|
|  | Content ($\mu$g $l^{-1}$) | Diff. (%) | Content ($\mu$g $l^{-1}$) | Diff. (%) | Content ($\mu$g $l^{-1}$) | Diff. (%) |
| MBT | 655 | 4.9 | 687 | 2.0 | 36 | 13.9 |
| BT | 10.5 | 4.8 | 99 | 6.1 | 5.5 | 9.1 |
| MTBT | 39 | 7.7 | 115.5 | 8.2 | 24.5 | 6.1 |

and 5.5 $\mu$g $l^{-1}$ of BT after the aerobic treatment. The deviation from the mean between duplicates is in the range of 2 to 14%, with an average of 7%. The method, thus, proves to be reliable over a concentration range of two orders of magnitude, even in extremely complex matrices such as untreated tannery wastewater.

## 4. Conclusions

A new method is developed for the analysis of benzothiazole, 2-mercaptobenzothiazole, 2-(methylthio)benzothiazole and 2-(thiocyanomethylthio)benzothiazole from industrial wastewater. 2.2'-(Dithiobis)benzothiazole, originally included in these investigations, was shown to be of comparatively limited relevance in aquatic environment, due to its low solubility and fast decomposition in water.

Highest recovery for all BT derivatives in LLE was obtained after the addition of 4 g $l^{-1}$ NaCl at pH 8.5 with ethyl acetate, followed by toluene. Recovery from different wastewaters was shown to exceed 90%. It was not affected by DOC contents of up to 900 mg $l^{-1}$ in a tannery effluent. LLE with dichloromethane, formerly employed in other reports, proved to be insufficient for the more polar substances MBT and BT. Furthermore, SPE appeared not to be suitable for the parallel extraction of all components.

Analysis of BT derivatives is best performed by LC with UV detection and time-programmed wavelength switching. Dual-wavelength detection supports peak assignment and monitors peak purity. The resolution and detection of BT deriva-

tives is not deteriorated by coextracted dissolved organic compounds, even from highly loaded wastewater. Although GC-FID is more sensitive than LC-UV, it is limited to MBT, BT and MTBT. Chromatograms are, however, more complex and peak assignment is less reliable unless coupled to mass spectrometry.

The final procedure, consisting of liquid–liquid extraction and analysis by LC with UV detection provides detection limits around 5 $\mu$g $l^{-1}$. Reproducibility was around ±7% in wastewater analyses yielding between 5 and 700 $\mu$g $l^{-1}$ of BT derivatives. Due to the obtained detection limits, this method is suggested not to be limited to the analysis of industrial wastewater.

## References

[1] R.B. Spies, B.D. Andresen and D.W. Rice Jr., Nature, 327 (1987) 697.
[2] J.K. Reichert, J. Lochtman, R.J. Müller and I. Könen, Jahrestagung der FG Wasserchemie in der GdCh, Badenweiler, 1993.
[3] C.R. Daniels and E.P. Swan, J. Chromatogr. Sci., 25 (1987) 43.
[4] J.C. Dacre, NTIS, AD-A144526 (1984).
[5] M.J. Kennedy, Analyst, 111 (1986) 701.
[6] W.M. Fowler, A.E. Russell, I.H. Kruger and S.C. Pinchuck, J. Soc. Leather Technol. Chem., 71 (1987) 100.
[7] E. Heidemann, Das Leder, 39 (1988) 9.
[8] H. Gattner, W. Lindner and H.-U. Neuber, Das Leder, 39 (1988) 66.
[9] I. Liska, E.R. Brouwer, A.G.L. Ostheimer, H. Lingeman, U.A.T. Brinkman, R.B. Geerdink and W.H. Mulder, Int. J. Environ. Anal. Chem., 47 (1992) 267.
[10] J.S. Warner, T.M. Engel and P.J. Mondron, NTIS, PB 85-189025 (1985).
[11] L. Braunstein, K. Hochmüller and K. Spengler, Vom Wasser, 73 (1989) 167.

[12] W. Elsäßer, E. Gilbert and S.H. Eberle, Acta Hydrochim. Hydrobiol., 29 (1992) 82.

[13] A.M. Dietrich, D.S. Millington and Y.-H. Seo, J. Chromatogr., 436 (1988) 229.

[14] J. Rivera, F. Ventura, J. Caixach, M. de Torres and A. Figueras, Int. J. Environ. Anal. Chem., 29 (1987) 15.

[15] J.F. Barker, J.E. Barbash and M. Labonte, J. Contam. Hydrol., 3 (1988) 1.

[16] D.I. Welch and C.D. Watts, Int. J. Environ. Anal. Chem., 38 (1990) 185.

[17] G. Runge and H. Steinhart, Agribiol. Res., 43 (1990) 155.

[18] B.G. Brownlee, J.H. Carey, G.A. MacInnis and I.T. Pellizzari, Environ. Toxicol. Chem., 11 (1992) 1153.

[19] W.M.A. Niessen, C.C. McCarney, P.E.G. Moult, U.R. Tjaden and J. van der Greef, J. Chromatogr., 647 (1993) 107.

[20] E. Genschow and W. Hegemann, GWF, Wasser-Abwasser, 134 (1993) 262.

[21] A. Bugby, C. Parbery, R.L. Sykes and C.D. Taylor, J. Soc. Leather Technol. Chem., 74 (1990) 134.

[22] C. Parbery and C.D. Taylor, Analyst, 114 (1989) 361.

[23] W. Funk, V. Damman and G. Donnevert, in Qualitätssicherung in der Analytischen Chemie, VCH, Weinheim, 1992.

[24] L.P. Burkhard, E.J. Durhan and M.T. Lukasewycz, Anal. Chem., 63 (1991) 277.

[25] J.R. Amato, D.I. Mount, E.J. Durhan and M.T. Lukasewycz, Environ. Toxicol. Chem., 11 (1992) 209.