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Journal of Chromatography A, 1058 (2004) 81–88
JOURNAL OF CHROMATOGRAPHY A
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Determination of benzothiazoles from complex aqueous samples by liquid chromatography–mass spectrometry following solid-phase extraction

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Available online 15 September 2004

Abstract

A method was developed for the determination of benzothiazoles from municipal wastewater, which may originate from their use as corrosion inhibitors, vulcanization accelerators or fungicides. Extraction is performed by SPE using a polymeric sorbent, followed by LC–MS analysis with electrospray ionization. Due to the diversity of the analytes, two LC–MS runs are required: 2-aminobenzothiazole, benzothiazole and 2-methylthiobenzothiazole are detected in the positive ion mode, while benzothiazole-2-sulfonic acid (BTSA), 2-mercaptobenzothiazole and 2-hydroxybenzothiazole are determined in the negative ion mode. Limits of quantification range from 20 to 200 ng/L in treated wastewater. Reliable quantification with R.S.D.s below 25% is achieved by standard addition in a limited number of samples of each sample series. A significant reduction of matrix effects in positive electrospray ionization is achieved by reducing the flow directed into the ESI-interface. When applied to untreated municipal wastewater most analytes were found at concentrations in the high ng/L- to low μg/L-range with the most polar BTSA being the dominant compound of this class. Removal of benzothiazoles in biological wastewater treatment appears to be limited.

Keywords: Benzothiazoles; Water analysis; Matrix effects; Municipal wastewater

1. Introduction

Benzothiazoles are a class of high production volume chemicals that are employed in various industrial processes, mostly as derivatives with the 2-position of the thiazole ring being substituted. Vulcanization accelerators such as 2-morpholinothiobenzothiazole, *N*-cyclohexyl-2-benzothiazole sulfenamid and 2,2′-dithiobisbenzothiazole are derived from 2-mercaptobenzothiazole (MBT) [1–3]. 2-Thiocyanomethylthiobenzothiazole (TCMTB) is used as fungicide, e.g. in leather production [2] and paper industry and MBT itself may act as corrosion inhibitor. Accordingly a continuous release of benzothiazoles into the environment can be expected by industrial discharges from processes where benzothiazoles are involved. Further emissions may steem from the use of benzothiazole-containing consumer products like rubber materials. However, the importance of sanitary wastewater as a source of emission of benzothiazoles and the occurrence of these compounds in municipal wastewater is largely unknown.

Besides those benzothiazoles that are used in production processes, like TCMTB, MBT and its derivatives, the number of benzothiazole compounds of potential environmental relevance is much larger due to (bio-) transformation processes [4,5]. Indeed, those benzothiazoles first detected in aquatic samples were benzothiazole (BT) and 2-methylthiobenzothiazole (MTBT) [6–9], which are not used in industrial processes. However, most benzothiazole derivatives are polar and thermally labile, which has prevented their detection in the environment as long as gas chromatographic methods have been used.

High performance liquid chromatography (HPLC) provided a broader view, as also more polar benzothiazoles such as 2-hydroxybenzothiazole (OHBT), 2-mercaptobenzothiazole (MBT), 2-aminobenzothiazole (ABT) and benzothiazole-2-sulfonic acid (BTSA) [2,4,10,11] could be

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doi:10.1016/j.chroma.2004.08.081

detected, though sensitivity and selectivity of UV detection were limited.

Niessen et al. [1] investigated the coupling of HPLC with various MS techniques namely moving-belt, thermospray, particle-beam and did also tandem MS experiments with a thermospray interface and identified various nonpolar benzothiazolic by-products in industrially used formulations. With electrospray ionization (ESI) in the positive and negative ion mode a number of eight benzothiazoles became detectable by LC–MS with high sensitivity and selectivity [12]. This method allowed the direct analysis of industrial wastewaters but it was not sensitive enough for municipal wastewaters. Therefore, information on the occurrence and removal of benzothiazoles in municipal wastewater is still missing.

We here report on a method consisting of solid-phase extraction followed by LC–ESI-MS/MS that allows the detection of six benzothiazoles in municipal wastewaters and surface waters. This method enables to perform comprehensive studies on the occurrence and removal of benzothiazoles in municipal wastewater.

2. Experimental

2.1. Chemicals

Benzothiazole (BT; 96%), 2-hydroxybenzothiazole (OHBT; 98%), glutathione (GSH; 98%) in reduced form and benzothiazole-6-carboxylic acid (BTCA; 96%) were purchased from Sigma–Aldrich Chemie (Steinheim, Germany); 2-mercaptobenzothiazole (MBT; p.a.) from Merck (Darmstadt, Germany); 2-aminobenzothiazole (ABT; 98%) from Fluka Chemie (Buchs, Switzerland) and 2-methylthiobenzothiazole (MTBT; pure) and 2-methylbenzothiazole (MeBT, pure) from Ferak (Berlin, Germany). Benzothiazole-2-sulfonic acid (BTSA) was kindly provided by Helen De Wever (Mol, Belgium). Methanol, acetone, formic acid and ammonium acetate were of HPLC grade. Ultrapure water was supplied by a water purification system ELGA maxima HPLC system (Elga, High Wycombe Bucks, UK).

2.2. Chromatographic separation

An HP 1100 (Agilent) system consisting of a membrane degasser, a binary high pressure gradient pump, an autosampler and a column heater and a diode-array detector was used for separation. Two separate chromatographic runs were performed, for the determination of BTSA, OHBT and MBT in the negative ion mode and for ABT, BT and MTBT in the positive ion mode [12]. The flow entering the MS was reduced to 1/15th by post-column splitting with a T-piece.

For the negative ionization mode separation was performed on a 3 mm × 125 mm Super-Sphere 100 C18e column with 4 μm particle size (Knauer, Berlin, Germany). Eluents were H_2O/MeOH 85:15 (A) and 10:90 (B), both containing 10 mM NH_4OAc. Gradient conditions were 0 min 15% B, 9 min 75% B, 9.5 min 15% B, 14 min 15% B with a flow rate of 0.5 mL/min and a column temperature of 40 °C. Sample volumes of 60 μL were injected.

The benzothiazoles detected in the positive ESI mode were separated on a Eurosphere C18 column (3 mm × 125 mm, 5 μm particle size) from Knauer, Berlin, Germany. Eluent A was H_2O/MeOH 80:20 and B was H_2O/MeOH 10:90, both containing formic acid at 0.1%. Gradient program started at 30% B and increased linearly to 65% B in 2 min and further to 80% B after additional 2.5 min at a flow rate of 0.5 mL/min. Then the gradient was kept stable for 4 min and afterwards decreased to the initial conditions of 30% B in 0.5 min. The system was ready for the next injection after 14 min.

2.3. Mass spectrometry

Analytes were detected by multiple reaction monitoring (MRM) using electrospray ionization mass spectrometry (ESI-MS) on a Quattro LC triple-stage quadrupole mass spectrometer (Micromass, Manchester, UK) based on a previously described method [12]. The source temperature was set to 120 °C and desolvation temperature was 220 °C. The gas flows for nebulizer and drying gas were 90 and 800 L/h, respectively. In the negative ESI mode the capillary voltage was 3.3 kV and in the positive ESI mode it was 3.6 kV. Argon gas pressure in the collision cell was 1.3e−3 mbar. Instrumental conditions as well as MRM transitions for the individual benzothiazoles are listed in Table 1.

2.4. Solid-phase extraction

Solid-phase extraction (SPE) was done with an Auto-Trace SPE Workstation (Zymark, Hopkinton, USA) using 200 mg Oasis HLB cartridges (Waters, Milford, USA) that contain a co-polymeric sorbent of divinylbenzene and *N*-vinylpyrrolidone. Cartridges were conditioned with 5.5 mL of methanol/acetone 6:4 and washed with 5 mL of pure water before extraction. Volumes of 100 mL of treated wastewater and 50 mL of untreated wastewater were extracted at a flow rate of 4 mL/min and cartridges washed with 0.5 mL water. Elution was performed with 7 mL methanol/acetone (6:4). A volume of 0.5 mL of pure water was finally added to the extracts as a keeper for the following solvent evaporation.

One hundred microliters of an internal standard solution (benzothiazole-6-carboxylic acid (BTCA) and methylbenzothiazole (MeBT) at 600 μg/L each) were added to the extracts. Solvent was evaporated to approximately 0.7 mL with a SpeedVac concentrator (Savant; Farmingdale, USA) at 45 °C. Half milliliter of pure water was added before injection into the HPLC.

2.5. External sample calibration

For quantification standard addition before extraction was performed with four samples of each kind of matrix

Table 1
MS parameters for the detection of six benzothiazoles using electrospray ionization in the negative and positive ion mode and multiple reaction monitoring

Substance	*m/z* of molecular ion	CV (V)	MRM product ions	CE (eV)	Ion intensity ratio
Positive ionization $(M + H)^+$					
ABT	151	41	109	26	2.4
			65	35	
BT	136	46	109	26	2.2
			65	37	
MTBT	182	35	109	22	6.5
			65	34	
Negative ionization $(M - H)^-$					
BTSA	214	34	134	24	4.6
			58	34	
MBT	166	42	134	20	3.0
			58	32	
OHBT	150	36	42	31	2.4
			107	24	

(raw and treated wastewater) at three concentration levels. One hundred microliter volumes of the spike solutions were added, resulting in spiked concentrations of 20, 100, 300 ng/L for ABT, 80, 400, 1400 ng/L for BT and MBT and 100, 600, 1800 ng/L for MTBT, BTSA and OHBT. The response factor of each analyte was calculated as the average slope of the four linear regressions of the standard addition diagrams.

2.6. Method evaluation

2.6.1. Breakthrough experiments

Tap water and treated wastewater were spiked to a concentration of 200 μg/L (each benzothiazole) and 500 mL of these samples were extracted on 60 mg Oasis HLB cartridges under the conditions given above. During the extraction process the cartridge effluent was sampled every 50 mL and directly analyzed by LC–MS/MS.

2.6.2. Sequential elution test

Oasis HLB cartridges (200 mg) were loaded with six benzothiazoles (200 ng each) and eluted with different mixtures of methanol/acetone (pure methanol, 8:2 and 6:4) in steps of 1 mL. The 1 mL portions of the extracts were directly analyzed by LC–MS.

2.6.3. Limit of quantification (LOQ)

The LOQ ($S/N > 10$) has been determined by spiking samples prior to SPE and calculating the signal-to-noise ratio (S/N) in the quantification traces of the LC–MS/MS chromatogram.

2.6.4. Determination of recoveries

The determination of recovery rates was performed by adding all six benzothiazoles at three concentration levels either before or after SPE of pure water. Peak areas were compared to those of the directly injected standard solutions.

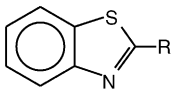
2.6.5. Post-column splitting

To study the effects of flow splitting on the sensitivity of the MS measurement raw and treated wastewater and pure water samples were spiked with the six benzothiazoles at three concentration levels. The spiked samples were directly analyzed by LC–ESI-MS with (1/15th) and without post-column splitting

3. Results and discussion

3.1. Solid-phase extraction

The target analytes (Fig. 1; Table 1) cover a wide range of polarity: from the moderately polar MTBT (log K_{ow} = 3.1 [4]) to BTSA and ABT that occur in ionic form at neutral pH. The strongly acidic BTSA was expected to be the most polar benzothiazole and breakthrough experiments proved that BTSA was indeed the first of the target analytes that could be detected in the effluent of the SPE cartridge. After an extracted sample volume of 50 mL BTSA was detected in the filtrate (Fig. 2a) from raw wastewater and tap water. For



R=	acronym	chemical name	CAS
positive ion mode			
-H	BT	Benzothiazole	95-16-8
$-NH_2$	ABT	2-Aminobenzothiazole	136-95-8
$-S-CH_3$	MTBT	2-Methylthiobenzothiazole	615-22-5
negative ion mode			
$-SO_3H$	BTSA	Benzothiazole-2-sulfonic acid	941-57-1
-SH	MBT	2-Mercaptobenzothiazole	149-30-4
-OH	OHBT	2-Hydroxybenzothiazole	934-34-9

Fig. 1. Structures and acronyms of investigated benzothiazoles.

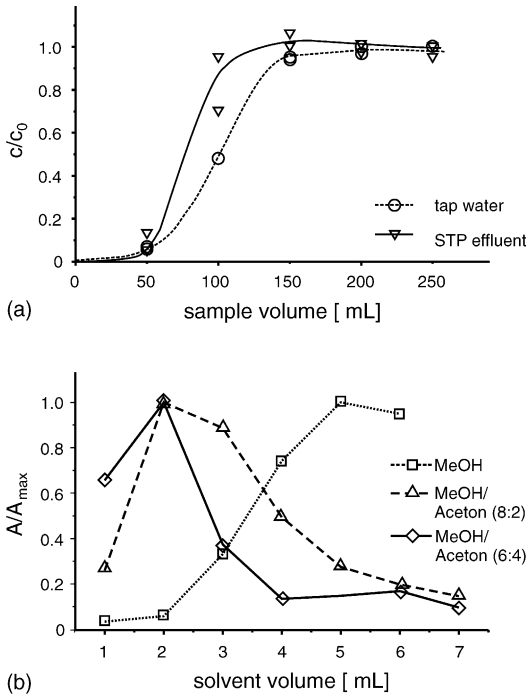


Fig. 2. (a) Breakthrough curve for BTSA (200 μg/L) in tap water and STP effluent using 60 mg Oasis HLB cartridges. (b) Elution of MTBT with different mixtures of methanol and acetone.

routine analysis the sorbent amount was increased to 200 mg to provide sufficient sorption capacity for 100 mL sample volumes.

Strongly binding polymeric sorbents like the Oasis HLB solid phase may require a higher elution strength for the desorption process. However, the eluent should not be too strong as to avoid unnecessary elution of more hydrophobic sample matrix. Indeed, pure methanol proved insufficient for the elution of the least polar MTBT (Fig. 2b). By gradually increasing the solvent strength to a 6/4 ratio of methanol/acetone, the desorption of MTBT is accomplished with 4 mL of eluent. This solvent mixture was used for routine analysis.

3.2. Preservation of MBT

The target analyte MBT contains a thiol functional group and is therefore prone to oxidation to the disulfide bis-(2-benzothiazolyl)-disulfide (MBTS) [4]. In preliminary experiments it was recognized that MBT concentrations decreased over a few days in aqueous solutions. Loss during SPE was even higher, probably due to the high surface area of the solid phase which is favourable for oxidation reactions. The putative oxidation product MBTS is difficult to detect and to quantify using ESI-MS. Moreover, it is quite hydrophobic and has never been detected from aqueous environmental samples before [12]. It was, therefore, not included in this analytical method. To protect MBT from oxidation during work up, we tested various reducing agents that are commonly applied in biochemical analysis for protection of thiol groups of proteins [13]. Of the seven reducing agents that were tested (1,4-dimercapto-2,3-butanediol, glutathione (GSH), 1,3-dimercaptopropane-2-ol, 2-mercaptoethanol, mercaptoacetic acid, mercaptoacetic acid sodium salt and cystin) glutathione (GSH) was most suitable, because the other reductants either exhibited high blank concentrations of MBT or showed poor protection properties.

A number of five samples of each raw and treated municipal wastewater samples, and two spiked pure water samples were analyzed for MBT with and without the addition of GSH (1 mL of a 0.1 M solution to 50 mL volumes of raw wastewater or to 100 mL of treated wastewater and pure water). The relative amount of MBT detected without adding GSH ranged from below 10% (raw and treated municipal wastewater) to 20% (spiked pure water) of what was detectable when GSH was added before any other step. With GSH being added before the extraction MBT concentrations of 68 and 120 ng/L were discernible in two of the samples of treated wastewater while MBT concentrations between 130 and 300 ng/L were found in four of the raw wastewater samples. Thus, the initial addition of GSH to a final concentration of 0.1–0.2 mM preserves MBT from being oxidatively removed from aqueous samples.

3.3. Analyte recovery

The final signal intensity of a given amount of a target analyte in SPE LC/MS procedures is dependent on the completeness of the extraction process and on the efficacy of the electrospray ionization [14,15]. For the optimization of an analytical method it is essential to distinguish between incomplete extraction and unfavourable ionization.

Therefore, a series of standard addition experiments was performed in which the addition of known amounts of target analytes at three concentration levels was performed before and after the extraction. The response factors of the linear calibrations obtained by these standard addition experiments were compared to that obtained for the pure standard solutions (R1). A lower (or higher) response by standard addition into the extract (R2) as compared to R1 can be ascribed to matrix effects in electrospray ionization. If lower response factors are obtained for standard addition before extraction (R3) as compared to R2 this would indicate incomplete recovery in the extraction process.

Corresponding to the response factor R2 normalized to R1 (Fig. 3a) only weak signal suppression occurs for BT, MBT and OHBT all of which exhibit relative response factors between 0.9 and 1.05. Stronger effects are discernible for BTSA, ABT and MTBT with relative response factors between 0.6 and 0.7 already in pure water. Since these extracts did not contain any sample matrix, signal suppression may be ascribed to either organic released by acetone from the extraction cartridge or to the GSH added to stabilize MBT.

Fig. 3b displays the SPE recovery by R3 response factors normalized to R2. For ABT, BTSA and OHBT the recovery ranged from 85 to 105%. The high recovery of the two most polar analytes (BTSA and ABT) proves that the capacity of

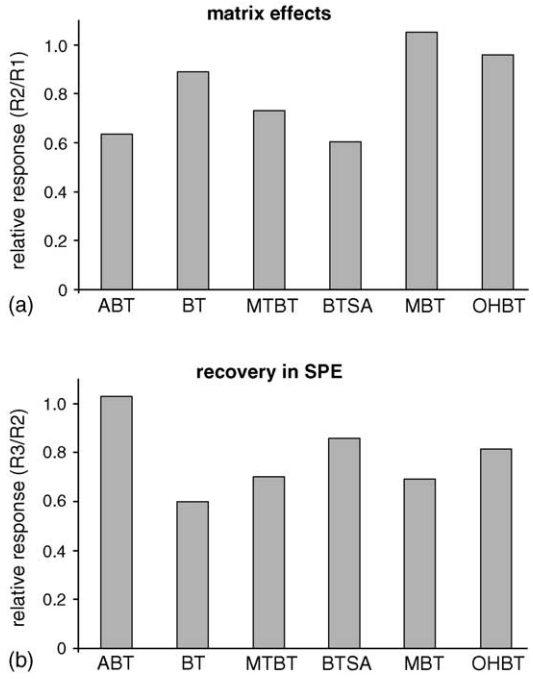


Fig. 3. (a) Matrix effects in the electrospray ionization, measured by the response factor (R2) for standard addition into a water extract (relative to the pure standard solution (R1)); (b) recovery by SPE, measured by the response factor (R3) for standard addition before the extraction (relative to standard addition after extraction (R2)).

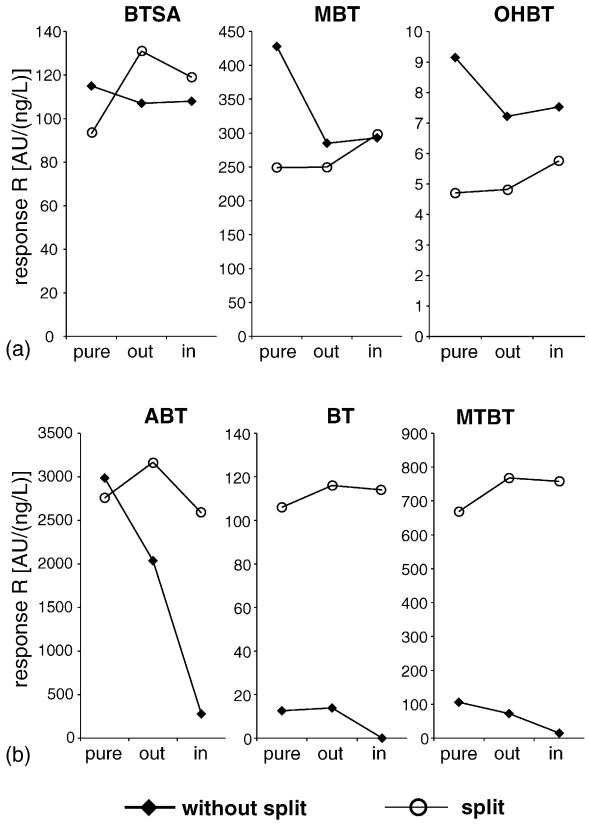


Fig. 4. Response factors of six benzothiazoles in water (pure), treated wastewater (out) and raw wastewater (in) by ESI-MS detection with and without post-column splitting. (a) BTSA, MBT and OHBT are analyzed in the negative ion mode; (b) ABT, BT and MTBT in the positive ion mode.

the SPE cartridges was not exceeded and no breakthrough occurred. Recovery of BT, MBT and MTBT remained limited with 60–70%. For MBT a certain extent of oxidation cannot be excluded, although GSH was added to preserve the thiol moiety. For MTBT and BT some loss by evaporation may have occurred, as these two are the least polar and most volatile benzothiazoles in this series. Anyhow, a series of standard addition experiments was performed for calibration in routine analysis. This approach accounts for both, signal suppression in electrospray ionization and incomplete recovery from SPE.

3.4. Post-column splitting

As Fig. 3a has illustrated matrix effects in benzothiazole detection with electrospray MS can be significant. Attempts were made to reduce these effects, which decrease the instrumental sensitivity and complicate the quantification. It has been mentioned previously that ion suppression in electrospray ionization decreased when the flow rate was reduces and a smaller amount of sample entered the electrospray interface in a given time period [16]. This can be easily achieved by post-column splitting, with only a limited portion of the column effluent being directed into the electrospray interface. Splitting should not results in generally decreasing signal intensities when ESI is used, as signal intensity was reported to be concentration dependent rather than mass dependent [17,18]. Post-column splitting would also reduce the accumulation of non-volatile residues in the interface, thus improving instrumental stability during long series of analyses.

The effects of a post-column splitting on the response factors of all six benzothiazoles are displayed in Fig. 4. For the negative ion mode, response factors do not differ significantly with and without splitting (Fig. 4a), but a slight decrease in the analyte signals may be visible after splitting. In this ionization mode, however, the matrix effects were generally low with weak differences in the response factors between pure water, treated wastewater (out) and raw wastewater (in). The less selective positive ionization mode shows a completely different pattern (Fig. 4b). Here the post-column splitting is clearly beneficial, with much higher response factors obtained for MTBT and BT. For ABT splitting significantly reduces matrix effects in the raw and treated wastewater. From these results it appeared advantageous to perform the LC–MS analyses with post-column splitting to reduce suppressive effects of the different sample matrices, namely in the positive ion mode.

4. Quantification

The whole method, consisting of GSH addition, SPE and LC–MS with post-column splitting was evaluated by a series

of standard addition experiments. Standard addition was performed into eight samples (four raw wastewaters and four treated wastewaters) with three levels of analyte addition for all six analytes. In routine analysis such standard addition experiments are performed with some representative samples of each series. From these standard additions the response factors of the analytes can be calculated and applied to all samples of a series.

This approach has been called external sample calibration [15]. It is much more reliable than external calibration as it considers matrix effects [15,19] but it is less laborious than standard addition into each sample. However, the matrix of different samples of each series should not differ too much to provide reliable results by external sample calibration [15].

From these experiments eight calibrations are obtained for each analyte, four in raw wastewater and four in treated wastewater. Fig. 5 summarizes these results by displaying the average response factors and their variability for the six analytes in each of the two matrices.

For most analytes, the response factors differ strongly between the two sample matrices and they are not consistently lower in raw wastewater with its high organic load as compared to treated wastewater. Rather MBT and OHBT are detected with higher sensitivity in raw wastewater (+50 and +40% as compared to treated wastewater) what demonstrates the possibility of signal enhancement due to matrix effects. For two of the analytes detected in the positve ion mode, BT and MTBT, the response factors are comparable for both sample matrices, whereas only the two most early eluting analytes, BTSA in the negative ion mode and ABT in the positive ion mode, show the expected stronger suppression in the highly loaded untreated wastewater (−20 and −50%). It appears that matrix effects are not easily predictable and could result in suppressing or enhancing the ionization efficacy.

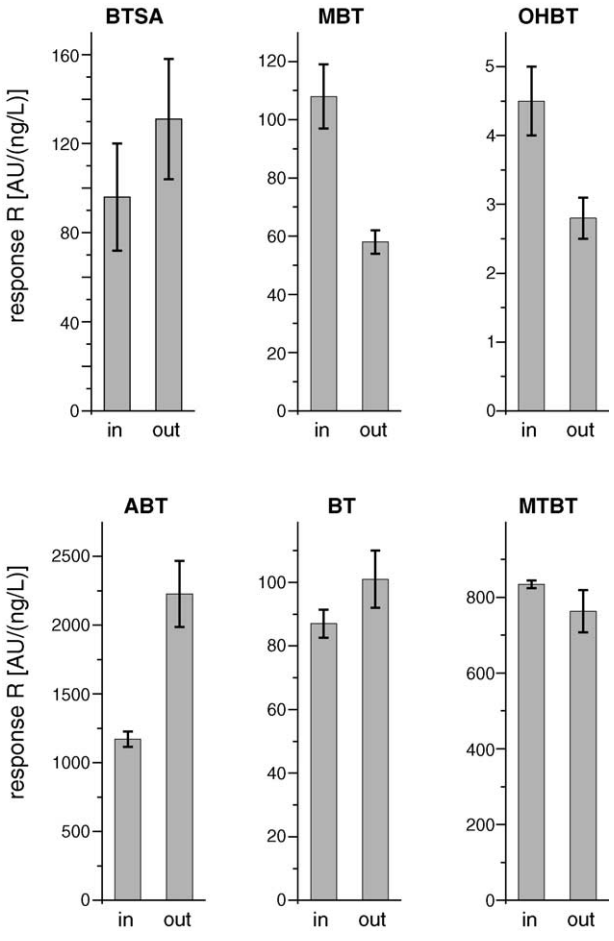


Fig. 5. Mean response factors (n = 4) of six benzothiazoles obtained by standard addition into samples of raw wastewater (in) and treated wastewater (out). Variability between samples displayed as standard deviation.

It should be noted that these standard addition experiments cover the whole analytical process including SPE. As raw and treated wastewaters were not analyzed together, differences between the response factors may also be due to day-to-day variation in instrumental sensitivity.

Another important aspect of Fig. 5 is the difference in the response factor between the four samples of each series, illustrated by the error bars. If the variability between the samples becomes too high, standard addition has to be performed with each sample of a series to obtain reliable quantitative data. The variability ranges from ±1% for MTBT to ±25% for BTSA. A maximum variability of 25% for the response factor in untreated wastewater is still acceptable. Provided that this is a true statistical error and no systematic error, correct average results will be obtained for a series of samples using the external sample calibration approach.

4.1. Detection limits

Instrumental detection limits (IDL) using post-column splitting and limits of quantification (LOQ) for the whole method including SPE were detected (Table 2). The IDL (S/N > 3) of the investigated benzothiazoles range from 15 pg of ABT injected onto the LC column to values between 35 and 50 pg for BT, MTBT, MBT and BTSA. The sensitivity for OHBT is relatively low with an IDL of 270 pg. The LOQs are generally higher in the raw wastewater than in the treated wastewater (Table 2). LOQs of 25–200 ng/L for benzothiazoles in treated municipal wastewater are fully satisfactory,

Table 2
Instrumental detection limits (IDL; S/N > 3) for the LC–MS analysis (MRM detection) from pure standard solutions and limits of quantification (LOQ; S/N > 10) from raw and treated wastewater (ww) for the whole analytical process including SPE

Substance	IDL pure solution (pg)	LOQ (ng/L)	
		Treated ww	Raw ww
ABT	15	25	65
BT	50	100	420
MTBT	35	40	75
BTSA	35	90	140
MBT	35	50	120
OHBT	270	200	620

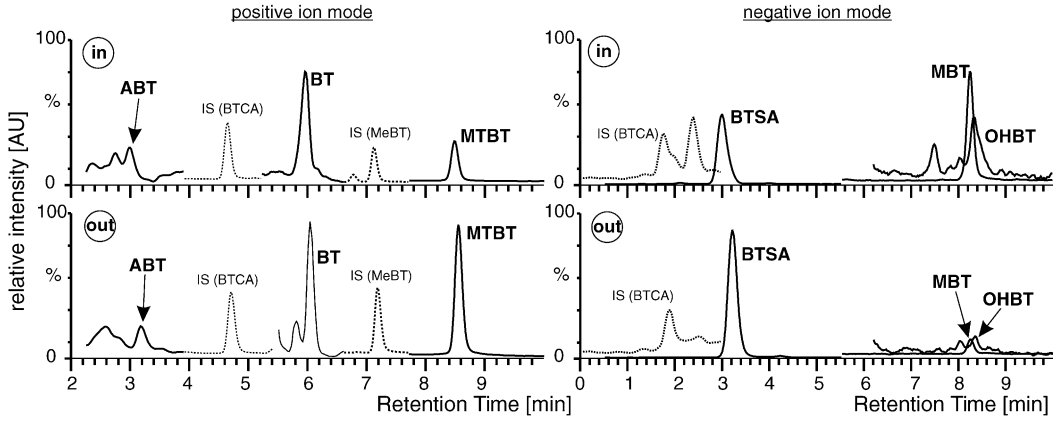


Fig. 6. Chromatograms obtained from raw wastewater (in) and treated wastewater (out); left side chromatograms from positive ESI mode, right side chromatograms from negative ESI mode.

but they may still be too high to allow detection of benzothiazoles from surface waters. However, one can expect that matrix effects are weaker in surface water than in wastewater, which would increase the analyte response and further decrease the detection limits.

In literature, there are only two methods reported for the detection of a number of benzothiazoles from water: as compared to a liquid–liquid extraction followed by LC–UV analysis [2] with LOQs ranging from 4 to 13 μg/L this LC–MS approach is not only much more selective but also significantly more sensitive. It is also one order of magnitude more sensitive than a direct injection LC–MS procedure [12].

4.2. Application to municipal wastewater

The method was finally applied to a series of 24 h composite samples from the influent and effluent of a municipal wastewater treatment plant. In all cases benzothiazoles were determined in the samples and the method proved to be robust, as clear signals were usually obtained. Chromatograms of an influent and effluent sample are exemplarily displayed in Fig. 6. Each MRM trace is scaled between the two chromatograms to illustrate the relative signal intensities between the two samples. In the raw wastewater (Fig. 6, top) all benzothiazoles except ABT showed clear signals, with concentrations ranging from 180 ng/L for MTBT to 1700 ng/L for BTSA. ABT seems neglegible in both influent and effluent samples.

In the effluent (Fig. 6, bottom) MBT and OHBT are reduced to below the LOQ. The concentration of BT is reduced by 50% in the effluent sample. However, its MRM signal is not reduced since less suppression occurred in the treated wastewater (bottom) as compared to the raw wastewater (top). A similar effect is visible for BTSA, where the effluent signal increased as compared to the influent, though the concentration remained unaltered in this case. For MTBT, a marked signal increase is visible that corresponds to a doubling of the concentration during wastewater treatment. This increase, likely, originates from the methylation of MBT by bacteria [20]. The most polar BTSA appears to be the dominant benzothiazole as previously found for an industrial wastewater treatment [21].

5. Conclusions

A method was developed for the LC–MS/MS determination of six benzothiazoles from municipal wastewater at concentrations levels above 20 ng/L (ABT) and 200 ng/L (OHBT). A critical aspect in method development were the very diverse physico-chemical properties of the analytes, from anionic to cationic and from very polar to moderately polar, including a thiol that was prone to oxidation.

Solid-phase extraction using a polymeric sorbent was used for analyte enrichment and two LC–MS runs using positive and negative electrospray ionization. A reducing agent was added to prevent oxidation of the thiol MBT.

Reliable quantification with R.S.D.s below 25% is achieved by standard addition in a limited number of samples of each sample series. It was shown that signal suppression due to matrix components in positive electrospray ionization can be drastically reduced by reducing the flow directed into the interface to approximately 30 μL/min.

All analytes except ABT were found in untreated municipal wastewater at concentrations in the high ng/L- to low μg/L-range with the most polar BTSA being the dominant compound of this class. The use of LC–MS is, thus, essential to investigate the behaviour of benzothiazoles in municipal wastewater treatment.

Acknowledgements

Financial support by the German Ministry for Research and Education (Fkz. 02WA0123) and by the European Union through the project P-THREE (EVK1-CT-2002-00116) is gratefully acknowledged. We are grateful to Jutta Jakobs for laboratory assistance.

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