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Determination of polar 1H-benzotriazoles and benzothiazoles in water by solid-phase extraction and liquid chromatography LTQ FT Orbitrap mass spectrometry

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Abstract
A sensitive, reliable and robust method for the trace determination of six polar 1H-benzotriazoles and four benzothiazoles in drinking and surface water was developed. These compounds were extracted from water by solid-phase extraction and analyzed by Liquid Chromatography–Electrospray Mass Spectrometry using a linear ion trap–Orbitrap hybrid instrument at high resolution of 30,000 FWHM in the full-scan acquisition mode. At least one product ion was simultaneously detected in the linear ion trap at low mass resolution and was used for confirmation of compound identity.

The analytes and four internal standards were preconcentrated by solid-phase extraction at low pH. Positive electrospray ionization resulted in protonated molecular ions for all the 1H-benzotriazoles and benzothiazoles.

The mass accuracy was between −5 ppm at m/z 120 and −0.1 ppm at m/z 182 and did not change for more than 2 ppm over a sample sequence of 8 days of analysis time. The optimized method allowed quantifying six benzotriazoles and four benzothiazoles in samples of drinking and surface water down to method detection limits of 0.01 g/L. The recoveries ranged between 45 and 125% in ultrapure, drinking and surface water at a spiking level of 0.2 g/L; the repeatability was between 2 and 13%. All analytes showed a linear response between 0.01 and 2.0 μg/L.

In Dutch drinking water samples, the compounds 1H-benzotriazole, 4- and 5-methyl-1H-benzotriazole, 5,6-dimethyl-1H-benzotriazole, 5-chloro-1H-benzotriazole and benzothiazole were detected. The concentration levels ranged from 0.01 to 0.2 g/L. In surface waters, eight out of ten compounds tested were found to be present in concentration levels ranging between 0.1 and 1.0 μg/L. In addition, in effluents of two sewage treatment plants, eight out of ten compounds tested were present with maximum concentrations for 1H-benzotriazole of 8 μg/L and for methyl-1H-benzotriazole of 3 μg/L (summed concentration of two isomers).

This work demonstrates the excellent suitability of the LTQ FT Orbitrap mass spectrometer for this type of analysis.

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1. Introduction

The benzotriazoles and benzothiazoles are high production volume chemicals that find broad application in various industrial processes as well as in households. The group of 1H-benzotriazoles are complexing agents that are widely used as anticorrosives, e.g., in engine coolants, aircraft deicers, or anti-freezing liquids, and for silver protection in dish washing liquids. Benzothiazoles are employed in various industrial processes, including rubber manufacturing, and as corrosion inhibitors and fungicides. Accordingly, a release of benzothiazoles into the environment can be expected by industrial discharges from processes where benzothiazoles are involved. Further emissions may stem from the use of benzothiazole-containing consumer products like rubber materials (tires and shoes). The 1H-benzotriazoles and benzothiazoles are soluble in water, resistant...
to biodegradation, only partially removed in wastewater treat-
ment [1,2,3,4], and they may pass the water treatment processes
employed in the production of drinking water.

Most 1H-benzotriazole and benzothiazole derivatives are polar
and thermally labile, which has prevented their detection in
the environment as long as gas chromatographic methods have
been used. The compounds studied in the present work generally
have a log $K_{ow}$ below 2.1 (with the exception of methylthio-
benzothiazole with a log $K_{ow}$ of 3.1, see Table 1). Recently, LC–MS
and LC–MS–MS methods have been developed for their measure-
ments in environmental waters. A few reports about the occurrence
of 1H-benzotriazoles and benzothiazoles in aquatic compartments
have been published since 2003 [6,7]. Giger et al. developed a
LC–MS–MS method, using Oasis HLB SPE cartridges for extrac-
tion and concentration [5]. Kloepfer et al. [2,3] used a SPE-LC–MS
method and showed that benzothiazoles are present in samples
from both the influent and effluent of municipal wastewater treat-
ment plants (WWTP) in Berlin and are thus not completely removed
in the wastewater treatment. Weiss and Reemtsma [1] conclude
the same for 1H-benzotriazoles found in wastewater and surface
water in the Berlin region. The authors expect 1H-benzotriazole
and 4-methyl-1H-benzotriazole to be omnipresent contaminants
in the water cycle in Germany, due to their limited biodegradabil-
ity, as high concentrations of several micrograms per liter in treated
municipal wastewater were observed.

No information is available until now about the distribution and
fate of the 1H-benzotriazoles and benzothiazoles compounds in
the Dutch water cycle. Therefore, the objective of this study was to
develop a method for the determination of six polar benzotriazoles
and four polar benzothiazoles in drinking and surface water on the
basis of solid-phase extraction and liquid chromatography–mass
spectrometry using a linear ion trap-Orbitrap hybrid instrument.
Our main focus was to explore the capabilities of the Orbi-
trap in the full-scan acquisition mode with high resolution to
evaluate if this instrument can achieve sensitivity, accuracy and
related performance characteristics required in quantitative anal-

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ysis of low level water contaminants. Nominal mass product ions were used for confirmation of the identity of the compounds.

This paper discusses the performance of the quantitative method for the determination of six 1H-benzotriazoles and four benzothiazoles in surface water and drinking water. The benzotriazoles selected include 1H-benzotriazole, 4-methyl-1H-benzotriazole, 5-methyl-1H-benzotriazole, 5,6-dimethylbenzotriazole and 5-chloro-1H-benzotriazole. Benzothiazole, 2-hydroxybenzothiazole, 2-aminobenzothiazole and 2-(methylthio)benzothiazole are the benzothiazoles that were investigated. Table 1 presents the structures of the compounds. The compound 2-mercaptopbenzothiazole was not studied due to its oxidation in aqueous solutions [3].

2. Experimental

2.1. Reagents and chemicals

1H-benzotriazole was purchased from Riedel-de-Haën (Sigma–Aldrich, Zwijndrecht, The Netherlands) and Acros (Boom, Meppel, The Netherlands) supplied 5-methylbenzotriazole, 5,6-dimethylbenzotriazole and benzothiazole. 5-chloro-1H-benzotriazole and 2-hydroxybenzothiazole were obtained from Aldrich (Sigma–Aldrich, Zwijndrecht, The Netherlands).

2-Aminobenzothiazole and 2-(methylthio)benzothiazole were supplied by Fluka (Sigma–Aldrich, Zwijndrecht, The Netherlands). Two compounds, 4-methylbenzothiazole and the internal standard (I.S.) 1H-benzotriazole-d₄, are not commercially available. Therefore they were synthesized by Mercachem (Nijmegen, the Netherlands). The reference standards were purchased in the highest purity available (96% or greater). The I.S. and a second one, the compound atrazine-d₃ (CDN, J.H. Ritmeester B.V., Utrecht, The Netherlands) were added to the sample extract to check the performance of the LC–MS analysis. 1H-benzotriazole-d₄, was used for correction of the calculated concentrations.

The compounds fenuron and chloroxuron (Dr. Ehrenstorfer, Boom, Meppel, The Netherlands) were used as internal standards to monitor the sample preparation process and were added to the water samples. Water used for LC–MS analysis was generated from an ultrapure water system from Millipore (Bedford, MA, USA) with a specific resistance of 18 MΩ cm. Ultra gradient acetonitrile/methanol (50:50; v/v) were prepared in a mixture of ultrapure water and acetonitrile (50:50; v/v). Stock solutions were stored at a temperature of −18 °C and working solutions at 4 °C. The ultrapure water was obtained by purifying demineralized water in a Milli-Q-system. All organic solvents were HPLC-grade. A 30% hydrochloric acid-solution was used and the quality of the formic acid was 98%. The sea sand had been glazed and ignited by the manufacturer.

2.2. Solid-phase extraction

Preconcentration of the water samples was performed by comparing two different commercially available cartridges with adsorbent: Oasis HLB (5 mL glass cartridges, 200 mg) and Oasis HLB (6 mL polypropylene, 500 mg; Waters, Milford, MA, USA). A 5 mL glass or 6 mL polypropylene cartridge, filled with 4 gram of sea sand (Mallinckrodt Baker, Deventer, The Netherlands), was placed above the cartridge with adsorbent, in order to prevent the adsorbent from clogging. The SPE cartridges were conditioned successively with 10 mL of acetonitrile, 10 mL of methanol and 10 mL of ultrapure water (pH 2, except for the neutral pH experiments). The sea sand cartridges were conditioned in the same way, but the methanol was omitted, due to the absence of active sites. In order to examine the influence of the pH on the recoveries, spiked samples of drinking water and surface water were concentrated at a pH between 7.8 and 8.0, and at pH 2.

The internal standard compounds fenuron and chloroxuron were spiked to 1 L of the sample at a concentration level of 1 μg/L. The samples were acidified to pH 2 with 30% hydrochloric acid (except for the experiments at neutral pH) and the water samples were percolated through the cartridges with a flow of 10 mL/min. After sampling the water, the sea sand cartridges were removed and the SPE cartridges were washed twice with 5 mL of acidified water (pH 2). The cartridges were dried under vacuum for 90 min until dryness and eluted 3 times with 2.5 mL of organic solvent. Three different solvents were evaluated: acetonitrile, methanol and a mixture of acetonitrile/methanol (50:50; v/v). The eluates were concentrated to a final volume of 500 μL under a gentle flow of nitrogen. The temperature during the concentration step was evaluated (37, 45 and 56 °C). Lastly, the extracts were diluted with 500 μL internal standard solution (benzotriazole-d₄ and atrazine-d₃) in ultrapure water at a concentration level of 2 mg/L. The final concentration of the deuterated internal standards in the sample extract is 1 mg/L.

2.3. Chromatographic separation

The liquid chromatograph was equipped with a Surveyor autosampler model Plus and a Surveyor quaternary gradient HPLC-pump (Thermo Fisher Scientific, Breda, The Netherlands). The separation of the compounds was performed using a 250 mm × 4.6 mm i.d. Phenomenex Aqua C₁₈-column with 5 μm particles. The precolumn was used a 4.0 × 3.0 mm i.d. Phenomenex Security Guard column (both columns were supplied by Bester, Amsterdam, The Netherlands). These columns were conditioned in a column thermostat that was maintained at a temperature of 21 °C. 20 μL of the water/acetonitrile-extract was injected into the LC system and the analytes were separated using a linear gradient elution at a flow rate of 1.0 mL/min. The gradient started with 5% acetonitrile/95% water/0.05% formic acid, increased to 40% acetonitrile/60% water/0.05% formic acid in 15 min, then increased to 100% acetonitrile in 10 min, and was held constant for 10 min. Between the runs, the analytical column was re-equilibrated for 15 min.

2.4. Mass spectrometry

A linear ion trap-Fourier Transform (LTQ-FT) Orbitrap mass spectrometer (Thermo Electron, Bremen, Germany) was used. The ion trap part of this system was equipped with an Ion Max Elec-
trospray ionization (ESI) probe that was applied in the positive-ion mode for these compounds.

From the LC column effluent, 200 μL/min was introduced into the mass spectrometer, whereas the remaining 800 μL/min was directed to waste. The full-scan accurate mass spectra from 100 to 1000 Da, which were obtained at a resolution of 30,000 FWHM, were processed using the Xcalibur version 2.0 software. The total cycle time depends upon the resolution; at a resolution of 30,000 FWHM the total cycle time is about 0.55 s.

Before measuring the samples, accurate mass calibration was performed using flow injection of a 1,3,6-polytyrosine-solution (m/z 181, 507 and 997) in methanol/water (50/50; v/v) with 0.1% formic acid at a flow rate of 10 μL/min.

The conditions in the Electrospray positive-ion mode were: source voltage 3.6 kV, heated capillary temperature 275 °C, capillary voltage 30 V and tube lens 70 V.

In the LTQ component of the instrument, the FT part was set to 26 °C and helium was used as daping gas. All measurements were done using the automatic gain control (AGC) of the LTQ to adjust the number of ions entering the trap. Products ions were generated in the LTQ trap at a normalized collision energy setting of 35% and using an isolation width of 2 Da.

Identification of the compounds was performed with the accurate mass of the protonated molecule within a mass window of 7 ppm together with one product ion (nominal mass). The retention times of the compounds were compared to those of the compounds in the calibration standard solution of the final analysis.

Quantification of the compounds was obtained by using a 7-points external calibration curve ranging between 0.01 and 1.0 mg/L, corresponding to 0.01–1.0 μg/L water. The solutions for the calibration curve were prepared in acetonitrile/water (50/50; v/v). Peak areas of the external calibration curve were corrected for the internal standard benzotriazole-d4. The recoveries of the internal standards fenuron and chloroxuron were used to check the performance of the extraction procedure, whereas atrazine-d5 was measured to check the performance of the LC–MS analysis. Table 1 shows the chemical structure, chemical name, elemental composition, CAS number, predicted log KOW value [14] and the accurate masses of the protonated compounds.

### 2.5. Method evaluation

The performance of the method was evaluated in terms of linearity, recovery, limit of detection, limit of quantification and repeatability. In addition, matrix effects were studied and quantified. Furthermore, confirmation of the target compounds was assessed based on concept of the use of identification point criteria [10].

The optimized method was used for the analyses of different drinking waters, surface waters and WWTP efﬂuents originating from different locations in The Netherlands.

### 3. Results and discussion

#### 3.1. Extraction optimization

In order to examine the influence of the pH on the recovery, spiked samples of drinking water and surface waters were concentrated at neutral pH (between 7.8 and 8.0) and pH 2. Besides the two pH values, two spiking levels (0.1 and 1.0 μg/L) were examined. The samples were processed, as described earlier in Section 2, using 6 mL glass cartridges packed with 200 mg Oasis HLB and acetoni terol as elution solvent. The recovery of all benzotriazoles at neutral pH was less than 50%, whereas the recovery at pH 2 was in the range 50–110%. For the benzothiazoles studied, the recovery of only 2-amino benzothiazole decreases from >50% to <30% when lowering the pH to 2, due to the basic properties of this compound. The recovery of the other benzothiazoles was not significantly affected by the pH (difference less than 5%).

The average recovery of all the compounds (both benzotriazoles and benzothiazoles) extracted at neutral pH is 42%, whereas the average recovery at pH 2 is 65%. In further experiments, water samples were acidified to pH 2.

In the second set of experiments, the effects of the amount of adsorbent and the solvent composition for the elution of the SPE-cartridges were investigated. Therefore, two different cartridge volumes of the Oasis HLB type and three different compositions of elution solvents were evaluated. Drinking water, spiked with 0.1 μg/L of the target compounds, was processed as described in Section 2. Besides the use of cartridges that were filled with 200 and 500 mg Oasis HLB, the elution solvents acetoni terol, a mixture of acetoni terol/methanol (50:50; v/v) and methanol were assessed. All experiments were performed in duplicate and the average recoveries are shown in Table 2. The results shows that target compound recoveries are higher than 68% when using 500 mg adsorbent and the acetoni terol/methanol elution solvent. Every other combination causes a recovery below 34% for one or more compounds. Therefore, 500 mg adsorbent and a mixture of acetoni terol/methanol (50:50; v/v) were used in the further evaluation of the performance of the method.

To study the possible loss of compounds during the evaporation step of the sample extracts, three different evaporation temperatures were investigated (37, 45 and 56 °C). To that end, 7.5 mL of a...
standard solution of all the compounds in acetonitrile at a concentration level of 0.1 mg/L was evaporated to a final volume of about 0.5 mL. The recovery of all compounds was in the range of 95–100% related to a standard solution of 0.1 mg/L in acetonitrile. In conclusions the temperature of the evaporation step did not influence the recovery of the analytes. For practical reasons, a temperature of 45°C for the evaporation step was selected.

3.2. Method performance

A chromatographic separation of the isomers 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole was achieved with the chromatographic conditions used (see Section 2). The individual detection of these two isomers is important due to their differences in toxicity and degradation behavior [13].

3.2.1. Confirmation

For confirmation of target compounds, LC relative retention time criteria (retention time window <2.5%) and mass spectrometric identification criteria need to be fulfilled. The latter being based on the concept of identification points [10]. For accurate mass screening using ToF or Orbitrap MS instruments, no criteria are described and recently some proposals were made by Nielen et al. [11]. For high resolution screening (resolution ≥20,000 and a mass spectrometric error (ME) of 0.2 mg/L for each compound. The results were corrected for the blank values. Table 5 shows the results of these experiments.

No significant matrix effect was observed in drinking and surface water, except for the compounds 2-aminobenzothiazole (signal enhancement about 50%) and 2-hydroxybenzothiazole (signal suppression about 25%).

3.3.1. Overall recovery and precision

The recovery and precision of the overall method were evaluated by analyzing extracts of ultrapure drinking water and surface water by LC-Orbitrap MS, spiked before extraction with 0.025 and 0.2 and 1.0 μg/L, respectively, of the analytes. For the 0.025 and 0.2 μg/L level, seven replicates were analyzed. For the 1.0 μg/L spiking level, only two replicates were analyzed, therefore the precision at that level is less accurate.

### Table 3

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<td>2-(Methylthio)benzotriazole (m/z 182)</td>
<td>C7H6NS</td>
<td>168.98579</td>
<td>C6H6NS</td>
<td>0.0</td>
<td>SCH3</td>
</tr>
</tbody>
</table>
was used for the quantification of the compounds. In order
3.4. Linearity
0.2
ing water and 2–13% in surface water at a concentration level of
standard deviations of 3–7% in ultrapure water, 4–8% in drink-
be explained by signal enhancement (ME = 1 10% in surface water at
pure water. In surface water, the recoveries ranged from 57% for
water, the recoveries were in general slightly higher than in ultra-
losses of analytes
do also occur during sample extraction and treatment.
The reproducibility of the total procedure was good with relative
standard deviations of 3–7% in ultrapure water, 4–8% in drink-
water and 2–13% in surface water at a concentration level of
0.2 µg/L.

3.4. Linearity
A 7-point standard calibration curve in water/acetonitrile
(50/50) was used for the quantification of the compounds. In order
to evaluate the linear range, the R² of the concentration–response
curve was calculated for the analytes in ultrapure water and in
extracts of ultrapure, drinking water and surface water, spiked before extraction. In total, 10 analytes in 4 matrices were analyzed.
The concentrations ranged between 0.01 and 1.0 µg/L (sample). The
R² ranges invariably between 0.952 and 1.000. The lowest value for
the R² was observed for the compound 5-chloro-1H-benzotriazole
(averaged value over the 4 matrices is 0.994). In the majority of
cases (31 of 40), the R² was higher then 0.996. This means that the
optimized LC–MS method can be used for the quantitative analyses
of the analytes in the range 0.01–1.0 µg/L in both drinking water and
surface water.

3.5. Mass accuracy
While for identification of unknowns the mass accuracy is of
largest importance [9], this is not a strict prerequisite for target anal-
ysis such as in this study, as the mass error could be accounted for
from the injection of calibration solutions. Still, the mass accuracy
should be stable over a whole analytical sequence to ensure accu-
rate peak detection and integration. To study the mass accuracy,
the drift in mass error of the target compounds with low molecu-
lar mass over a time period of 8 days was evaluated. The accurate
mass was derived from the apex of the chromatographic peak. The
accurate mass at the start of the experiments and after 3 and 8
days are presented in Table 8. The concentration of the compounds
is 0.1 mg/L. The mass spectrometer was calibrated externally (see
Section 2) before the start of the experiments. No recalibration was
performed during the experiments.

In general, the mass error for all the compounds ranged from
−0.1 to −4.8 ppm for all studied ions and was at lowest the
least m/z range. The mass accuracy of the compound with the lowest
molecular mass (1H-benzotriazole, m/z 120) was −4.8 ppm. After

Table 5
Matrix effect (ME; %) according to Matuszewski et al.[9] for target compounds and internal standards in different types of water. Concentration tested 1.0 µg/L; n=2. ME < 100%: signal suppression, ME > 100%: signal enhancement.

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Ultrapure water</th>
<th>Drinking water</th>
<th>Surface water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H-benzotriazole</td>
<td>103</td>
<td>118</td>
<td>95*</td>
</tr>
<tr>
<td>4-Methyl-1H-benzotriazole</td>
<td>102</td>
<td>126</td>
<td>110*</td>
</tr>
<tr>
<td>5-Methyl-1H-benzotriazole</td>
<td>95</td>
<td>109</td>
<td>108*</td>
</tr>
<tr>
<td>5,6-Dimethyl-1H-benzotriazole</td>
<td>108</td>
<td>118</td>
<td>114*</td>
</tr>
<tr>
<td>1-Hydroxybenzotriazole</td>
<td>87</td>
<td>103</td>
<td>106</td>
</tr>
<tr>
<td>5-Chloro-1H-benzotriazole</td>
<td>89</td>
<td>99</td>
<td>101*</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>100</td>
<td>114</td>
<td>106*</td>
</tr>
<tr>
<td>2-Aminobenzothiazole</td>
<td>132</td>
<td>153</td>
<td>143</td>
</tr>
<tr>
<td>2-Hydroxybenzothiazole</td>
<td>91</td>
<td>74</td>
<td>78*</td>
</tr>
<tr>
<td>2-(Methylthio)benzothiazole</td>
<td>100</td>
<td>98</td>
<td>101*</td>
</tr>
<tr>
<td>Fenuron</td>
<td>76</td>
<td>70</td>
<td>66</td>
</tr>
<tr>
<td>Chloroxuron</td>
<td>101</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>1H-benzotriazole-d₄</td>
<td>85</td>
<td>103</td>
<td>106</td>
</tr>
<tr>
<td>Atrazine-d₅</td>
<td>88</td>
<td>83</td>
<td>84</td>
</tr>
</tbody>
</table>

a Corrected for blank value.

After isolation with Oasis HLB, the extracts were measured in
duplicate and the recoveries were calculated and corrected for the
I.S. 1H-benzotriazole-d₄. Table 6 shows the average recoveries at three concentration levels together with the standard deviation (RSD). No correction for the matrix effect is performed.

The overall recovery numbers are equal to or below the matrix
effect numbers, indicating (1) that the matrix effect is an important
contribution to the overall recoveries and (2) that losses of analytes
do also occur during sample extraction and treatment.

At a concentration level of 0.2 µg/L, the recovery in ultrapure
water ranged from 45% for 2-(methylthio)-benzothiazole up to
97% for the compound 5,6-dimethyl-1H-benzotriazole. In drinking
water, the recoveries were in general slightly higher than in ultra-
pure water. In surface water, the recoveries ranged from 57% for
1-hydroxybenzotriazole up to 125% for 4-methyl-1H-benzotriazole.
The relatively high recovery of 4-methyl-benzothiazole (125%) can
be explained by signal enhancement (ME = 110% in surface water at
a spiking level of 1.0 µg/L) due to matrix effects.

The reproducibility of the total procedure was good with relative
standard deviations of 3–7% in ultrapure water, 4–8% in drinking
water and 2–13% in surface water at a concentration level of
0.2 µg/L.

3.4.1. LOD and LOQ
Quantification and detection limits were determined using a
signal-to-noise approach. The limit of quantification was estimated
for the concentration of compound that results in a signal-to-noise
ratio of 10:1. The limit of detection corresponds to the concentration
that results in a signal-to-noise ratio of 3:1.

Hereto, a standard dissolved in ultrapure water, and extracts
of ultrapure water, drinking water and surface water, spiked after
extraction with 0.025 mg/L of the analytes, were analyzed 7 times.
The signal-to-noise ratios were measured and the average values
are presented in Table 7. The data in this table show that the limit
of detection ranges between <1 and 3 ng/L for ultrapure water, from
<1 to 8 ng/L for drinking water and from <1 to 10 ng/L in surface
waters. The limit of quantification ranges between 1 and 17 ng/L for
ultrapure water, 1 and 9 ng/L for drinking water and between 1 and
33 ng/L for surface waters.

3.5. Mass accuracy

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Ultrapure water</th>
<th>Drinking water</th>
<th>Surface water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H-benzotriazole</td>
<td>38(11)</td>
<td>75(4)</td>
<td>81</td>
</tr>
<tr>
<td>4-Methyl-1H-benzotriazole</td>
<td>74(5)</td>
<td>76(4)</td>
<td>90</td>
</tr>
<tr>
<td>5-Methyl-1H-benzotriazole</td>
<td>54(7)</td>
<td>81(3)</td>
<td>89</td>
</tr>
<tr>
<td>5,6-Dimethyl-1H-benzotriazole</td>
<td>86(4)</td>
<td>97(2)</td>
<td>100</td>
</tr>
<tr>
<td>1-Hydroxybenzotriazole</td>
<td>99(3)</td>
<td>68(2)</td>
<td>67</td>
</tr>
<tr>
<td>5-Chloro-1H-benzotriazole</td>
<td>84(4)</td>
<td>84(3)</td>
<td>107</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>71(6)</td>
<td>62(3)</td>
<td>65</td>
</tr>
<tr>
<td>2-Aminobenzothiazole</td>
<td>61(4)</td>
<td>77(3)</td>
<td>61</td>
</tr>
<tr>
<td>2-Hydroxybenzothiazole</td>
<td>70(4)</td>
<td>76(2)</td>
<td>89</td>
</tr>
<tr>
<td>2-(Methylthio)benzothiazole</td>
<td>24(5)</td>
<td>45(7)</td>
<td>52</td>
</tr>
</tbody>
</table>

a n=2.
b Corrected for the blank value.

The drift in mass error of the target compounds with low molecu-
lar mass was derived from the apex of the chromatographic peak. The
accurate mass at the start of the experiments and after 3 and 8
days are presented in Table 8. The concentration of the compounds
is 0.1 mg/L. The mass spectrometer was calibrated externally (see
Section 2) before the start of the experiments. No recalibration was
performed during the experiments.

In general, the mass error for all the compounds ranged from
−0.1 to −4.8 ppm for all studied ions and was at lowest the
least m/z range. The mass accuracy of the compound with the lowest
molecular mass (1H-benzotriazole, m/z 120) was −4.8 ppm. After
8 days there was a drift in mass accuracy of ∼1.7 ppm. The mass accuracy of the compound with the highest molecular mass (2-(methylthio)benzothiazole (m/z 182)) was ∼0.1 ppm. Typically, the mass error did not change more than ∼2 ppm over the course of a sequence of more than 250 injections corresponding to a total analysis time of 8 days. Any effect of the signal intensity on mass accuracy was not noticed, indicating the large dynamic range of the Orbitrap MS with respect to mass accuracy. Similar results were obtained by Krauss and Hollender [12], who used the Orbitrap for the trace level determination of low molecular weight nitrosamines.

On the basis of these results, peak integration for quantitative analysis was done from ion chromatograms extracted for each ion at a range of ±7 ppm around the theoretical m/z value. If a better mass accuracy at the low m/z range would be needed, a lower calibration mass would be required than that of m/z 181 in the routine calibration solution.

### 3.6. Application to real water samples

Ten samples of drinking water were collected in 2007 from ten different locations. Also ten different surface water locations and effluents from two sewage treatment plants, originating from all over The Netherlands, were sampled and analyzed with the method developed. All samples were stored at 4 °C for a maximum period of 7 days in green glass bottles prior to analysis. In drinking water samples (originating from surface waters) from The Netherlands, the compounds 1H-benzotriazole, 4- and 5-methyl-1H-benzotriazole, 5,6-dimethyl-1H-benzotriazole, 5-chloro-1H-benzotriazole and benzothiazole were detected (see Fig. 1). The concentration levels ranged from 0.01 for the compounds 5-methyl-1H-benzotriazole, 5,6-dimethyl-1H-benzotriazole, 5-chloro-1H-benzotriazole and benzothiazole to 0.2 μg/L for 1H-benzotriazole and 4-methyl-1H-benzotriazole. In particular, the latter two compounds have the potential to reach drinking water prepared either directly from surface water or from surface water via bank filtration and should, therefore, be included in future monitoring studies. These results are in accordance with a study on occurrence of benzotriazoles and benzothiazoles in different samples from the Berlin region, reported by Weiss and Reemtsma [1], who also found the highest concentrations for 1H-benzotriazole and 4-methyl-1H-benzotriazole. The concentration of the isomer 5-methyl-1H-benzotriazole is significantly lower due to its lower microbial stability [1].

### Table 7

Limits of detection (LOD) and limits of quantitation (LOQ) in standards and extracts.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Standard</th>
<th>Extract of spiked ultrapure water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOD a (ng/L)</td>
<td>LOQ b (ng/L)</td>
</tr>
<tr>
<td>1H-benzotriazole</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>4-Methyl-1H-benzotriazole</td>
<td>&lt;1</td>
<td>2</td>
</tr>
<tr>
<td>5-Methyl-1H-benzotriazole</td>
<td>&lt;1</td>
<td>2</td>
</tr>
<tr>
<td>5,6-Methyl-1H-benzotriazole</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>1-Hydroxybenzotriazole</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>5-Chloro-1H-benzotriazole</td>
<td>&lt;1</td>
<td>2</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>2-Aminobenzothiazole</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>2-Hydroxy-benzothiazole</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>2-(Methylthio)benzothiazole</td>
<td>&lt;1</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Drinking water</th>
<th>Surface water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOD a (ng/L)</td>
<td>LOQ b (ng/L)</td>
</tr>
<tr>
<td>1H-benzotriazole</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>4-Methyl-1H-benzotriazole</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>5-Methyl-1H-benzotriazole</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>5,6-Methyl-1H-benzotriazole</td>
<td>&lt;1</td>
<td>2</td>
</tr>
<tr>
<td>1-Hydroxybenzotriazole</td>
<td>&lt;1</td>
<td>3</td>
</tr>
<tr>
<td>5-Chloro-1H-benzotriazole</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>2-Aminobenzothiazole</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2-Hydroxy-benzothiazole</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>2-(Methylthio)benzothiazole</td>
<td>&lt;1</td>
<td>1</td>
</tr>
</tbody>
</table>

* s/n = 3.  
* s/n = 10.
Fig. 1. LC–MS extracted-ion chromatograms (positive ion mode, mass window 7 ppm) of five benzotriazoles in a drinking water sample with concentrations ranging from <0.01 to 0.1 μg/L.

Fig. 2. LC–MS extracted-ion chromatograms (positive ion mode, mass window 7 ppm) of five benzotriazoles and three benzothiazoles in a surface water sample with concentrations ranging from <0.01 to 0.5 μg/L.
In surface waters, eight out of ten compounds were detected (see Fig. 2), with concentrations ranging between 0.1 and 1.5 µg/L. The compounds 1-hydroxybenzotriazole and 2-aminobenzothiazole were not detected in surface water.

In the effluents of both sewage treatment plants, eight out of ten compounds tested were detected and the maximum concentrations were observed for 1H-benzotriazole (8 µg/L) and methyl-1H-benzotriazole (summed concentration of two isomers: 3 µg/L).

4. Conclusions

In Dutch drinking water samples, the compounds 1H-benzotriazole, 4- and 5-methyl-1H-benzotriazole, 5,6-dimethyl-1H-benzotriazole, 5-chloro-1H-benzotriazole and benzothiazole were detected. The concentration levels ranged from 0.01 to 0.2 µg/L. In surface waters, eight out of ten compounds tested were actually observed in concentration levels ranging between 0.1 and 1.0 µg/L. In addition, in the effluents of two sewage treatment plants, eight out of ten compounds tested were detected with maximum concentrations for 1H-benzotriazole of 8 µg/L and for methyl-1H-benzotriazole of 3 µg/L (summed concentration of two isomers). It can be concluded that 1H-benzotriazoles and benzothiazoles are omnipresent polar contaminants in the Dutch water cycle.

High resolution mass spectrometry coupled to LC is a very powerful combination for screening purposes. The application of accurate mass screening described in this article demonstrates that current day analytical instrumentation is well equipped to meet the challenges posed by newly emerging polar chemicals.

References