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Development of a solid-phase extraction liquid chromatography tandem mass spectrometry method for benzotriazoles and benzothiazoles in wastewater and recycled water

Clara H. Loi*, Francesco Busetti, Kathryn L. Linge, Cynthia A. Joll

Curtin Water Quality Research Centre, Department of Chemistry, Curtin University, GPO Box U1987, Perth, Western Australia 6845, Australia

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ABSTRACT

Two methods employing solid-phase extraction and liquid chromatography tandem mass spectrometry were developed for the analysis of benzotriazoles (BTs) and benzothiazoles (BThs), compounds which are commonly found in a large variety of commercial and household products. The first method was able to detect 7 BTs and 7 BThs, the largest suite of BTs and BThs analysed in a single method to-date, but could not distinguish between the isomers, 4-methylbenzotriazole (4-MeBT) and 5-methylbenzotriazole (5-MeBT). Therefore, a second method was developed to achieve the chromatographic separation of 4-MeBT and 5-MeBT. The methods were validated for ultrapure water and secondary wastewater, and method limits of detection (MLD) for BTs and BThs (for the primary method) ranged from 0.1 to 58 ng L^{-1} for ultrapure water, and 2 to 322 ng L^{-1} for secondary wastewater. For the secondary method, MLDs for 4- and 5-MeBT ranged from 8 to 12 ng L^{-1} for ultrapure water, and 388 to 406 ng L^{-1} for secondary wastewater. Analysis of secondary wastewater and reverse osmosis (RO) treated water from an advanced water recycling plant in Australia is presented, and represents the first reported data from the analysis of BTs and BThs in recycled water. Some of these compounds were found to persist through wastewater treatment and incompletely removed by RO treatment. Benzotriazole (BT), 4-MeBT, 5-MeBT and 2-(methylthio)benzothiazole were detected in secondary wastewater, however the latter compound was not quantifiable. Concentrations of BT and tolyltriazoles (TTs, i.e. sum of 4- and 5-MeBT, detected with the primary method) in secondary wastewater were 3.3 (±0.02) and 2.8 (±0.04) μg L^{-1}, respectively. These same compounds were also detected in the post-RO water samples at concentrations of 974 (±28) ng L^{-1} for BT and 416 (±34) ng L^{-1} for TTs. 2-Hydroxybenzothiazole was also detected in the post-RO water samples, however it was not quantifiable. Removal efficiencies for RO treatment were calculated to be between 70% and 85% for BT and TTs.

1. Introduction

Benzotriazoles (BTs) and benzothiazoles (BThs) are widely used in industrial and household products. They are classified as high volume production chemicals, with an estimated worldwide production of BTs in excess of 9000 tonnes/year [1,2]. Benzotriazoles are generally used as anticorrosive additives, and can be found in dishwashing detergents, metal-cutting fluids, antifreeze products, such as aircraft de-icers, cooling and hydraulic fluids, and brake fluids [1,3,4]. Other uses of BTs include: complexing agents for chemical intermediates for pharmaceuticals, dyes and fungicides, as ultraviolet stabilisers, and for photographic applications for antifogging purposes [2,5]. Benzothiazoles are also used as corrosion inhibitors, as well as in the processing of other industrial products, such as herbicides and fungicides, though they are predominantly used as vulcanisation accelerators in rubber production [6,7].

The wide use of BTs and BThs has resulted in their detection in a range of water types, including surface water [1,8], drinking water [9,10], and primary and secondary wastewaters [5,6]. These occurrences demonstrate not only their high solubility in the aqueous phase, but suggest some resistance to conventional wastewater treatment [1,5]. The logarithmic octanol water distribution coefficients ($\log K_{ow}$) for BTs range from 0.69 to 2.06 for 1-hydroxybenzotriazole and 5,6-dimethylbenzotriazole, respectively [4,9], while for the BThs, the $\log K_{ow}$ of 2-(methylthio)benzothiazole and 2-hydroxybenzothiazole is 1.63 and 2.35, respectively [11]. These low $\log K_{ow}$ values are indicative of their high water solubility.

The analysis of BTs and BThs in water by methods such as liquid chromatography tandem mass spectrometry (LC–MS/MS), liquid chromatography with UV detection, two-dimensional gas

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* Corresponding author. Tel.: +61 08 9266 9387; fax: +61 08 9266 3547.
E-mail addresses: c.loi@curtin.edu.au, loihclara@gmail.com (C.H. Loi).

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chromatography time-of-flight mass spectrometry and gas chromatography tandem mass spectrometry have been previously reported [1,5,11–17]. Some of these methods have been limited to only the analysis of BTs [5,12,15] or only BThs [18]. Often the isomers, 4- and 5-MeBT, are not chromatographically separated and instead reported as a sum of the isomers, tolyltriazoles [5,12], or only one of the isomers is reported [14,15]. Deuterated standards have not been used in most of the previously reported methods. Analytical methods that include both BTs and BThs, have been limited to only a total suite of 5–11 BTs and BThs, and deuterated standards were not employed in these methods, though for some studies standard addition was used to overcome matrix effects [1,11,14,16,17]. The development of a method for the detection of 6 BTs and 4 BThs in drinking and surface waters, using LC–MS with a linear ion trap-Orbitrap hybrid instrument, has been previously published [9]. In this method, one deuterated BT compound, [^{2}H4] BT, was used as an internal standard rather than a surrogate standard [9].

While Australia imports less than 100 tonnes/year of BTs, and between 300 and 400 tonnes/year of BThs (Personal communication, National Industrial Chemicals Notification and Assessment Scheme, NICNAS, March 17, 2010), these compounds are also present in many imported product mixtures. The occurrence of BTs and BThs has been frequently reported in European studies, however there is only one previously published study in Australian waters, where occurrence data for only the BTs in a municipal wastewater treatment plant was presented [15]. There is therefore a need for more occurrence data for BTs and BThs in Australian waters. In this study, we present a new LC–MS/MS method for the analysis of 7 BTs and 7 BThs, which is, to our knowledge, the largest suite of BTs and BThs to be analysed in a single method to-date. Furthermore, we present an additional method developed for the chromatographic separation of the isomers, 4- and 5-MeBT, which coelute in the primary method. We focus particularly on the method development, optimisation and validation of an improved solid-phase extraction (SPE) pre-concentration procedure. This new SPE–LC–MS/MS method has allowed analysis of the occurrence of BTs and BThs in secondary wastewater influent and post-reverse osmosis (post-RO) effluent from an Australian advanced water recycling plant, and is the first such study of BTs and BThs in RO-treated recycled water.

2. Experimental

2.1. Standards and chemicals

1*H*-Benzotriazole (BT), 5-methyl-1*H*-benzotriazole (5-MeBT), 5,6-dimethyl-1*H*-benzotriazole hydrate (5,6-DiMeBT), 5-chlorobenzotriazole (5-ClBT), benzothiazole (BTh), 2-(methylthio) benzothiazole (2-MTBTh), 2-hydroxybenzothiazole (2-OHBTh), 2-mercaptobenzothiazole (2-MBTh), 2-aminobenzothiazole (2-ABTh), 5-chloro-2-methylbenzothiazole (5-Cl-2-MeBTh) and 5-chloro-2-mercaptobenzothiazole (5-Cl-2-MBTh) were purchased from Sigma–Aldrich (New South Wales, Australia). 4-Methylbenzotriazole (4-MeBT), 1-hydroxybenzotriazole (1-OHBT) and 1-butylbenzothiazole (1-BBT) were purchased from Toronto Research Chemicals (North York, Canada), AK Scientific (California, USA) and Waterstone Technology LLC (Indiana, USA), respectively. The surrogate standards, [^{2}H4] 1*H*-benzotriazole (BT-d_4), [^{2}H3] 4-methylbenzotriazole (4-MeBT-d_3), [^{2}H6] 5-methylbenzotriazole (5-MeBT-d_6) and [^{2}H4] benzothiazole (BTh-d_4) were purchased from Toronto Research Chemicals (North York, Canada). The internal standards, [^{2}H5] atrazine (atrazine-d_5) and [^{2}H10] carbamazepine (carbamazepine-d_{10}) were purchased from Sigma–Aldrich (New South Wales, Australia) and CDN Isotopes (Quebec, Canada, distributed by SciVac, Hornsby, Australia), respectively. Methanol (MeOH) and acetonitrile (ACN) (ChromHR grade) were purchased from Mallinckrodt Baker (New Jersey, USA). Formic acid (purity 99%) and concentrated hydrochloric acid (32%, HCl) were both purchased from Ajax FineChem (New South Wales, Australia). Ultrapure water was purified using an ion exchange system (IBIS Technology, Perth, Australia), followed by an Elga (High Wycombe, UK) Purelab Ultra system with a 0.2 μm filter (Elga, High Wycombe, UK).

Single compound stock solutions of BTs and BThs were prepared in MeOH (1000 ng μL^{-1}), while working solutions (10 ng μL^{-1} and 100 ng μL^{-1}) were prepared in ultrapure water. A mixed working solution of all BTs and BThs (10 ng μL^{-1}) was prepared in ultrapure water. An additional mixed working solution of only 5 BThs (consisting of BTh, 2-MBTh, 2-OHBTh, 5-Cl-2-MBTh and 5-Cl-2-MeBTh; 100 ng μL^{-1}) was also prepared in ultrapure water, as these compounds had higher detection limits compared to other BTs and BThs tested. Individual surrogate and internal standard stock solutions were prepared in MeOH (1000 ng μL^{-1}). Mixed surrogate standard working solutions (consisting of BT-d_4, 4-MeBT-d_3, 5-MeBT-d_6 and BTh-d_4; 10 ng μL^{-1} for all; and a separate mixed surrogate solution with BT-d_4, 4-MeBT-d_3, 5-MeBT-d_6 at 10 ng μL^{-1} and BTh-d_4 at 100 ng μL^{-1}) were made in ultrapure water. A working solution of the internal standard, atrazine-d_5 (10 ng μL^{-1}) or carbamazepine-d_{10} (10 ng μL^{-1}) was made in ultrapure water. All solutions were stored at room temperature unless the manufacturer had specified otherwise.

2.2. Solid-phase extraction

Three different solid-phase extraction (SPE) cartridges were trialled for sample pre-concentration: Oasis HLB (500 mg/6 mL, Waters, Massachusetts, USA), Strata-X (500 mg/6 mL, Phenomenex, New South Wales, Australia) and Isolute ENV+ (500 mg/6 mL, International Sorbent Technology, Mid Glamorgan, UK). An automated Aspec Xli extractor (Gilson, Middleton, USA) was used for SPE cartridge conditioning and elution, as described in Table 1. Before sample loading, the mixed surrogate standard working solution (100–1000 ng L^{-1} for deuterated BTs and 1–10 μg L^{-1} for BTh-d_4, depending on sample type) was added to the sample and the sample pH was adjusted to 2 with concentrated hydrochloric acid. Samples (volume processed: 1 L for ultrapure water and post-RO water samples; 250 mL for secondary wastewater samples) were loaded onto the SPE cartridges using two 8-channel off-line peristaltic pumps (Gilson, Middleton, USA), at a flow rate of 5 mL min^{-1}. The SPE cartridges were then gently dried under vacuum on a manifold system (Supelco, Bellefonte, USA) for 30 min. Analytes were eluted into 12 mL glass test tubes, and the extract concentrated to near dryness (200–300 μL) under a gentle nitrogen gas flow on a dry block heater (Ratek 30D, Boronia, Australia) set at 45 °C. The internal standard solution, atrazine-d_5 (10 ng μL^{-1}; 10–25 μL), was added to the final extract, and the sample extract was diluted to a volume of 500 μL with MeOH/ultrapure water (30:70 v/v). The extract was then transferred into a 2 mL HPLC amber glass vial with a PTFE-lined screw cap, and stored at 4 °C until analysis.

Table 1
Solid-phase extraction (SPE) conditioning and elution procedure for BTs and BThs.

SPE procedure	
Conditioning Flow rate = 5 mL min^{-1}	10 mL ACN 10 mL MeOH 10 mL ultrapure water (pH 2 adjusted with HCl)
Elution Flow rate = 1 mL min^{-1}	3 × 3 mL ACN:MeOH (50:50 v/v), 1 min delay 2 mL ACN:MeOH (50:50 v/v), 9 mL air push (flow rate = 10 mL min^{-1})