Talanta 120 (2014) 158–166

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of nine benzotriazole UV stabilizers in environmental water samples by automated on-line solid phase extraction coupled with high-performance liquid chromatography–tandem mass spectrometry ☆

Runzeng Liu, Ting Ruan, Thanh Wang, Shanjun Song, Feng Guo, Guibin Jiang *

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Article history:
Received 23 August 2013
Received in revised form
16 October 2013
Accepted 19 October 2013
Available online 24 October 2013

Keywords:
On-line solid phase extraction
HPLC–MS/MS
Benzotriazole UV stabilizers
Water matrices

ABSTRACT

A method using automated on-line solid phase extraction coupled with a high-performance liquid chromatography–tandem mass spectrometry system was developed for the determination of emerging benzotriazole UV stabilizers (BZTs) in different environmental water matrices including river water, sewage influent and effluent. Water sample was injected directly and the analytes were preconcentrated on a Polar Advantage II on-line SPE cartridge. After cleanup step the target BZTs were eluted in back flush mode and then separated on a liquid chromatography column. Experimental parameters such as sample loading flow rate, SPE cartridge, pH value and methanol ratio in the sample were optimized in detail. The method detection limits ranged from 0.21 to 2.17 ng/L. Recoveries of the target BZTs at 50 ng/L spiking level ranged from 76% to 114% and the inter-day RSDs ranged from 1% to 15%. The optimized method was successfully applied to analyze twelve water samples collected from different wastewater treatment plants and rivers, and five BZTs (UV-P, UV-329, UV-350, UV-234 and UV-328) were detected with concentrations up to 37.1 ng/L. The proposed method is simple, sensitive and suitable for simultaneous analysis and monitoring of BZTs in water samples.

1. Introduction

UV stabilizers belong to a group of anthropogenic chemicals that have various applications in both commodities and industrial products to absorb ultraviolet radiation from sunlight [1–3]. In recent years, an increasing concern has been focused on this group of emerging pollutants due to their reported toxic effects [4–6]. Among the different UV stabilizers, benzotriazole UV stabilizers (BZTs), also known as Tinuvins, are one of the most important families. They are produced in large volumes and are used in materials such as building materials, automobile polymeric components, waxes, films, varnishes, shoes, cosmetic products and some sports equipment to prevent yellowing and degradation reactions by ultraviolet radiation [2,7,8]. Toxicity studies showed that direct contact with 2-(2-hydroxy-5-methylphenyl) benzotriazole (UV-P) might cause acute effects such as dermatitis and skin irritation problems [9]. Gender specific hematological and histopathological changes in liver, kidney, spleen and thyroid were observed after long-term repeat-dose toxicity study of 2-(3,5-di-tert-butyl-2-hydroxyphenyl) benzotriazole (UV-320) in rats [10]. Similar repeat-dose toxicity effects have also been found for 2-(3,5-di-tert-amyl-2-hydroxyphenyl) benzotriazole (UV-328) [11]. Moreover, researches have also demonstrated potential bio-accumulation of these compounds in aquatic organisms and birds [2]. Multi-residue surveys implied that these pollutants could be transported through aquatic systems and be bound in sedimentary deposits [12].

Effluents from wastewater treatment plants (WWTPs) are considered as one of the main sources of these substances to the environment [13,14]. Certain BZT compounds such as 2-(2-hydroxy-3-tert-butyl-5-methylphenyl)-5-chlorobenzotriazole (UV-326) have shown low removal efficiencies during WWTP processes and could therefore be discharged into the surrounding environment such as downstream of the sewage effluent receiving river [15]. The concentrations for BZTs in these aquatic environments could reach ng/L levels. Therefore, analytical methods for determining BZTs in complex water matrices require high sensitivity as well as high selectivity to avoid matrix interference. Currently, gas chromatography–mass

* Corresponding author. Tel.: +86 10 6284 9334; fax: +86 10 6284 9179.
E-mail address: gbjiang@rcees.ac.cn (G. Jiang).

0039-9140/$ - see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.10.041

spectrometry (GC–MS) is commonly used to determine BZTs and the pretreatment methods for environmental water samples mainly involve off-line solid-phase extraction (SPE) [16,17]. However, the existing GC–MS method is not very suitable for the determination of the BZTs which have low volatility and the off-line SPE methods require manual sample preparation, extraction and pre-concentration, which is time-consuming [17]. BZT homologs with high hydrophobicities suffer severe sorption losses from the complex pretreatment steps of off-line SPE methods. For example, highly hydrophobic BZTs such as UV-328 and 5-chloro-2-(3,5-di-tert-butyl-2-hydroxyphenyl) benzotriazole (UV-327) were reported to have low recoveries ($<50\%$) due to sorption losses in the complex off-line pretreatment procedures [17]. These problems can be somewhat solved by using on-line SPE coupled with a high-performance liquid chromatography–tandem mass spectrometer (HPLC–MS/MS).

On-line SPE method has the advantages of significant reduction of preparation time, high sample throughput and small sample volume requirement, which is suitable for high sensitivity and accuracy methods [18,19]. Several steps such as evaporation and re-dissolution are eliminated by direct coupling SPE to HPLC–MS/MS. The use of atmospheric pressure chemical ionization (APCI) has also been shown to be useful in HPLC–MS/MS for the analysis of less volatile BZT analogs, such as 2-[3,5-bis(1-methyl-1-phenylethyl)-2-hydroxyphenyl] benzotriazole (UV-234) and 2-(2-hydroxy-5-tert-octylphenyl) benzotriazole (UV-329) [20]. To our knowledge, very few works have been conducted to establish on-line SPE-HPLC–MS/MS methods for the analysis of the BZTs [21]. In this context, the main objectives of the present work were to develop, optimize and validate a fully automated on-line SPE-HPLC–MS/MS method and apply the developed method to the collected environmental water samples to assess the presence of the target BZTs.

2. Materials and methods

2.1. Materials

Chemical structure, abbreviations and other information are shown in Table 1 [22]. UV-P, UV-PS, UV-326, UV-327, UV-328, UV-329, and UV-350 were purchased from TCI (Tokyo, Japan). UV-320 was purchased from Dr. Ehrenstorfer (Augsburg, Germany), while UV-234 and surrogate standard (Allyl-bzt, 99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals have a purity of 97% or greater. Stock standard solutions (1 mg/mL) were individually prepared in methanol (MeOH) and intermediate solutions were prepared weekly from the stock standard solutions by appropriate MeOH dilution. HPLC-grade MeOH was supplied by J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water (18.3 $M\Omega \times cm$) was generated by a Milli-Q system (Millipore, Billerica, MA, USA).

2.2. Sample collection

Twenty-four hour integrated samples ($n=8$) of sewage influent and effluent were taken from four different WWTPs in Shandong province, China. Samples were also taken from four rivers which receive sewage effluent from the WWTPs. Water samples were gathered in amber glass bottles and mixed with MeOH (sample/MeOH=7:3, v/v) to prevent targets absorbing on vessels. All samples were transported to laboratory immediately, centrifuged at 4000 rpm for 10 min to eliminate suspended solid matters, and stored at 4 °C in the dark until analysis.

2.3. Apparatus

The sample preparation and analysis system includes the UltiMate™ 3000 system (Dionex, USA) and the Quattro Premier XE triple-quadrupole mass spectrometer (Waters Inc., Milford, USA), which were controlled by the Chromeleon® Chromatography Management Software (v. 6.80, Dionex, USA) and MassLynx (v. 4.1, Waters Inc., Milford, USA), respectively. The UltiMate™ 3000 system consisted of a DGP 3600 M dual gradient pump, SRD 3600 solvent rack with integrated vacuum degasser, TCC-3200 thermostated column compartment with two two-position, six-port (2P-6P) valves and a WPS-3000TSL autosampler with high volume loop (2.5 mL) for injection.

2.4. On-line SPE

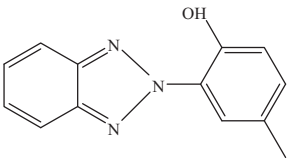
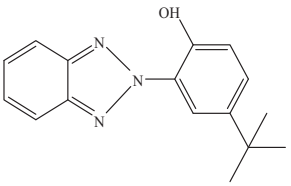
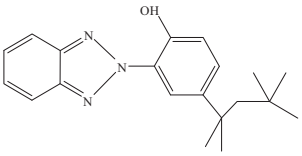
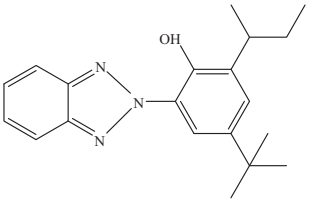
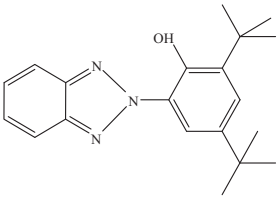
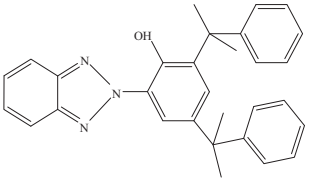
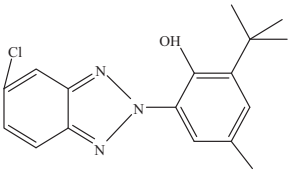
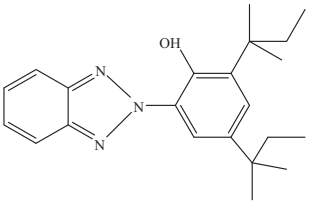
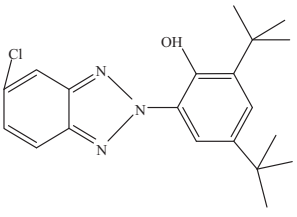
Fully automated on-line SPE system setup for on-line SPE consists of loading, injection and separation steps. Detailed on-line SPE conditions are presented in Table 2 and the valve position and instrumental configuration of the on-line SPE are shown in Fig. 1. In the first loading step, the cartridge Polar Advantage II was fitted into loading position of the Valco 6 port switching valve. After the cartridge was conditioned by 10 mL ultrapure water (25.1–30 min), 20 mL sample (contain 30% MeOH, pH=6) was loaded onto the cartridge using a 2.5 mL syringe and a 2.5 mL loop (WPS-3000TSL autosampler). Ultrapure water was used as loading solution. The left pump of the DGP 3600 M dual gradient pump was used to load the samples onto the cartridge at a flow rate of 2 mL/min. Analytes were retained on the SPE cartridge, sample matrices were flushed to waste, and the analytical LC column SymmetryShield was simultaneously equilibrated with the right pump. Further washing of the SPE cartridge was carried out with 1 mL of ultrapure water (0–0.5 min) in order to remove interferents such as inorganic salts [23]. In the second step (injection), the valve was switched to injection position at 0.6 min which coupled the SPE cartridge to the chromatographic column and transferred the analytes from the SPE cartridge to the analytical column. Then the targets absorbed on the SPE cartridge were eluted in back-flush mode by the mobile phase composition used in the chromatographic separation. The DGP 3600 M right pump was used to provide the gradient elution. In the third separation step, the analytes were separated in the analytical column and the valve was switched back to the loading position after 25.1 min to equilibrate the on-line SPE cartridge with ultrapure water prior to analyzing the next sample.

2.5. LC–MS/MS analysis

Two product ions for each BZT analyte were selected for quantification and confirmation. The HPLC–MS/MS experimental conditions were optimized based on our previous study with minor modification and were as follows [20]: A $150 \times 4.6\ mm^2$ SymmetryShield 5 μm C18 analytical column (Waters, Milford, MA) was chosen for separation. Column temperature was set at 40 °C. MeOH and ultrapure water were chosen as mobile phases. The flow gradient was initiated at a composition of 75:25 (MeOH/water, v/v) with a flow rate of 1 mL/min and held for 2.5 min. Then MeOH was linearly increased to 100% in 20 min and held for 2.5 min. Immediately after returning to the initial composition of 75:25, the column was allowed to re-equilibrate for 5 min giving a total run time of 30 min (Table 2). APCI was operated in positive mode with resolution tuned to 0.7 amu full-width half-maximum. The source and probe temperatures were optimized at 110 and 550 °C, respectively. The corona current was set at 3.0 μA, desolvation gas flow was 150 L/h, while argon pressure for ion collision was kept at 3.8×10^{-3} mbar. Detailed information on the optimized parameters and monitored ion transitions for each analyte is given in Table S1 (Supporting information).

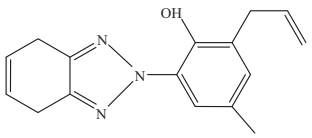
Please cite this article as: R. Liu, et al., Talanta (2013), http://dx.doi.org/10.1016/j.talanta.2013.10.041

Table 1
Analyte name, structure and other relevant data.

Acronym	Chemical name	Structure	CAS	Log K_{ow}[a]
UV-P	2-(2-hydroxy-5-methylphenyl) benzotriazole		2240-22-4	4.33
UV-PS	2-(5-tert-butyl-2-hydroxyphenyl) benzotriazole		3147-76-0	4.36
UV-329	2-(2-hydroxy-5-tert-octylphenyl) benzotriazole		3147-75-9	6.21
UV-350	2-(3-sec-butyl-5-tert-butyl-2-hydroxyphenyl) benzotriazole		36437-37-37-3	6.31
UV-320	2-(3,5-di-tert-butyl-2-hydroxypheny) benzotriazole		3846-71-7	6.27
UV-234	2-[3,5-bis(1-methyl-1-phenylethyl)-2-hydroxyphenyl] benzotriazole		70321-86-7	7.67
UV-326	2-(2-hydroxy-3-tert-butyl-5-methylphenyl)-5-chlorobenzotriazole		3896-11-5	5.55
UV-328	2-(3,5-di-tert-amyl-2-hydroxyphenyl) benzotriazole		21615-49-6	7.25
UV-327	5-Chloro-2-(3,5-di-tert-butyl-2-hydroxyphenyl) benzotriazole		3864-99-1	6.91

Please cite this article as: R. Liu, et al., Talanta (2013), http://dx.doi.org/10.1016/j.talanta.2013.10.041

Table 1 (*continued*)

Acronym	Chemical name	Structure	CAS	Log K_{ow}[a]
Allyl-bzt	2-(3-allyl-2-hydroxy-5-methylphenyl)-benzotriazole		2170-39-0	4.39

[a] Log K_{ow} values obtained from [20].

Table 2
On-line SPE and HPLC conditions.

Time (min)	Left pump			Right pump			Valve position
	MeOH (%)	H_2O (%)	Flow rating (mL/min)	MeOH (%)	H_2O (%)	Flow rating (mL/min)	
−11[a]	0	100	2.0	75	25	1.0	loading
0	0	100	2.0	75	25	1.0	Loading
0.5	0	100	2.0	75	25	1.0	Loading
0.6	0	100	0.1	75	25	1.0	Injection
2.5	0	100	0.1	75	25	1.0	Injection
22.5	0	100	0.1	100	0	1.0	Injection
25	0	100	0.1	100	0	1.0	Injection
25.1	0	100	2.0	75	25	1.0	Loading
30	0	100	2.0	75	25	1.0	Loading

[a] "−11" stands for the sample loading time before the start time (recorded as 0) of sample clean-up.

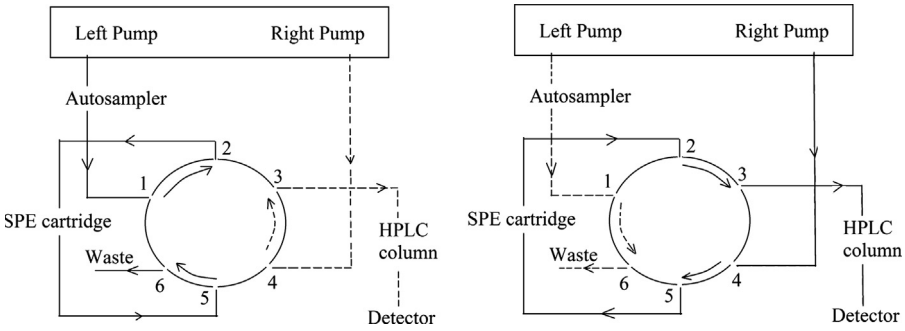


Fig. 1. Valco valve position and instrumental configuration of the on-line SPE. The left panel is loading position while the right is injection.

2.6. Method validation

Procedures were carried out to validate the proposed on-line SPE-HPLC–MS/MS analytical method. Blank samples of the WWTP influent, effluent and river water collected from Beijing were used during the method validation. To test the linearity range of the standard calibration curve, seven concentrations of standard mixtures (from method quantification limit to 500 ng/L) in different water matrices were injected in triplicate, and each point corresponded to the mean value obtained from three measurements. Since no target compounds were found in blank samples, the method detection limit (MDL) and method quantification limit (MQL) of each analyte in different water matrices were calculated based on the signal-to-noise (S/N) of 3 and 10 of 10 ng/L spiked corresponding water matrix samples, respectively. Recoveries of the target BZTs were studied by analyzing the blank influent, effluent and river samples spiked at 10 ng/L and 50 ng/L levels. The intra-day and inter-day relative standard deviations (RSDs) were assessed by replicate measurements of 50 ng/L of standard solution under optimum conditions within ($n=5$) and among days ($n=5$). When analyzing the collected water samples, one procedural blank (ultrapure water containing 30% MeOH) was added to every batch of six samples. In order to check for potential carry over effects, the mobile phase solution (MeOH/water=7:3, v/v) was routinely injected into the instrument after the high concentration of standard solution samples.

For the positive identification of the target BZTs, confirmation criteria published elsewhere was used [24,25]. In short, the analyte relative retention time should match that of the calibration standards at a tolerance level of 2%. A signal-to-noise ratio of 10:1 was set as a threshold for positive quantification. And the ratio of the quantification to the confirmation ions in real samples should be within 20% of that in the standards. For quantification of the analytes in the collected samples, the internal calibration curve method was used. Allyl-bzt was used as internal standard as reported in our previous study [20]. Five- to seven-point calibration curves were constructed from the on-line analysis of the different water matrices spiked with the standard mixture, using a least-squares linear regression analysis.

3. Results and discussion

3.1. On-line SPE optimization

Extraction efficiency of the on-line SPE procedure is mainly controlled by (1) the SPE cartridge, (2) sample loading flow rate, (3) pH value of the water sample, (4) MeOH ratio in the sample.

For the optimization procedures, 20 mL ultrapure water spiked with the target BZTs at the concentration of 50 ng/L was used as testing sample. In all optimization steps of this study, the extraction efficiencies were calculated by comparing the peak areas obtained in the on-line analysis of the water samples with the direct chromatographic analysis of equivalent amounts of the standard mixtures in MeOH.

3.1.1. SPE cartridge

SPE adsorbent type is an important factor on the recoveries of the target analytes in the SPE process. When selecting proper SPE cartridge, the characteristics of the sorbent and the physical–chemical properties of the target analytes should be taken into account. For this purpose, four different trace enrichment cartridges were evaluated for their extraction efficiencies of the target BZTs: Oasis® HLB (particle size 25 μm, 4.6 mm × 20 mm, Waters), SolEx™ HRP (particle size 12 μm, 2.1 mm × 20 mm, resin amount 150 mg, Thermo Scientific), Acclaim® Polar Advantage II (PA II, particle size 3 μm, average pore diameter 120 Å, 3.3 mm × 33 mm, Thermo Scientific) and IonPac® NG I(particle size 10 μm, 4 mm × 35 mm, Thermo Scientific). The 50 ng/L spiked ultrapure water samples were loaded at a rate of 1 mL/min onto the different cartridges in triplicates. Results in Fig. 2(A) shows that the cartridge PA II provided the most satisfactory performance among the four candidates for all of the analytes, with recoveries ranging from 27% to 69% for UV-326 and UV-P, respectively. HLB cartridge showed inferior results with recoveries ranging from 21% to 62%, while HRP and NG I gave low recoveries for most of the analytes (13–47%). Many parameters of the SPE cartridge such as sorbent materials, sorbent mass and their particle sizes were demonstrated to affect the target recoveries [19,26]. PA II is a silica-based and polar-embedded cartridge designed for enhanced hydrolytic stability and allows enrichment of a wide variety of polar and non-polar analytes in large-volume water samples. The on-line HLB cartridge is packed with a balanced ratio of the monomers hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene, which is optimized for the extraction of a wide range of analytes. The PA II and the HLB which can absorb polar and non-polar analytes might be appropriated for the different BZT homologs. The HPR (packed with a divinylbenzene polymer with a hydrophilic bonded layer and has excellent retention property for polar and hydrophobic analytes) and the NG I (packed with neutral, macroporous and high-surface-area sorbent and is useful for traditional polymeric reversed-phase applications) might not be adequate for simultaneously trapping the nine target BZTs

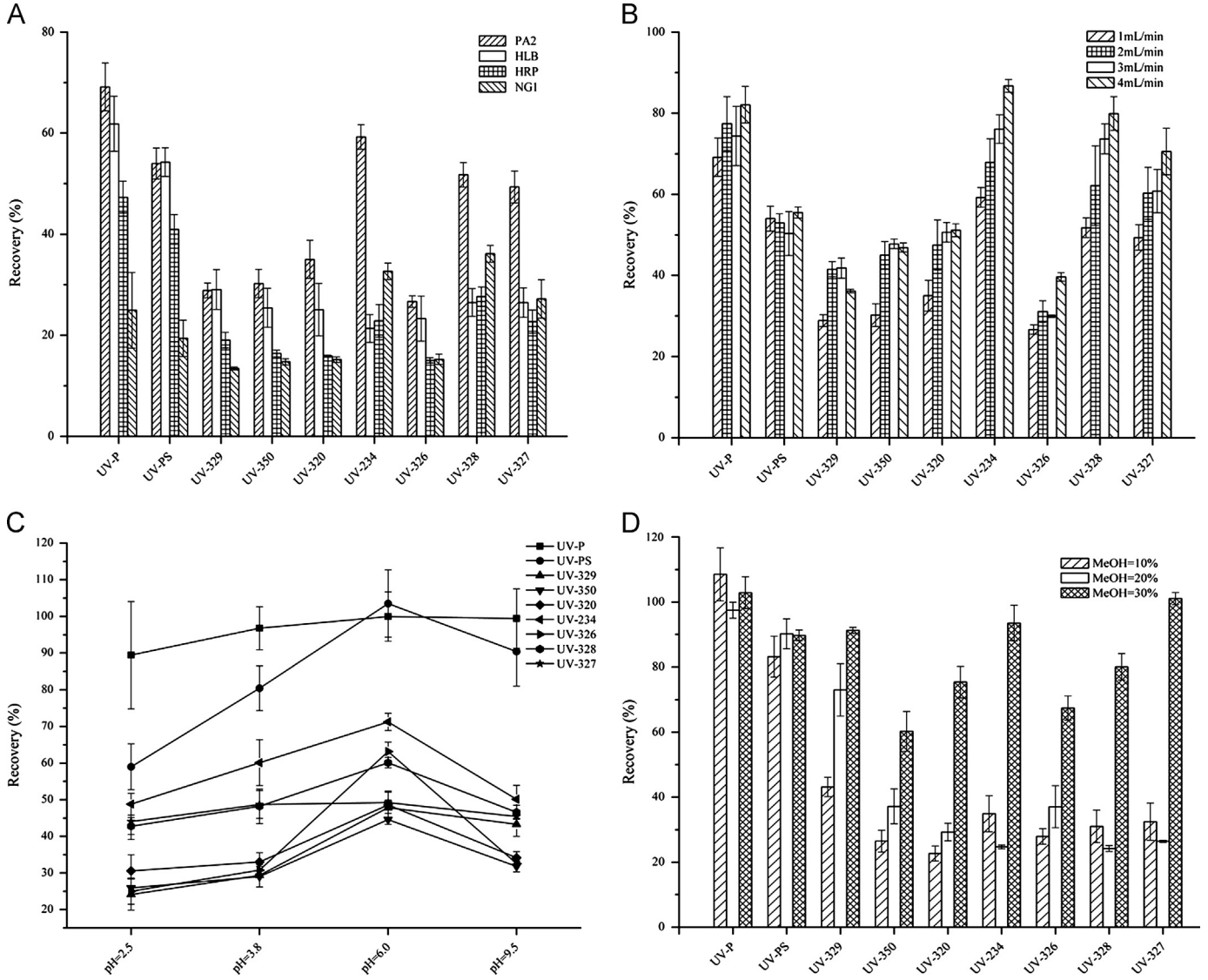


Fig. 2. Optimization of the affecting factors on on-line SPE including cartridge (A), loading flow rate (B), pH value (C) and MeOH ratio (D) ($n=3$).

Please cite this article as: R. Liu, et al., Talanta (2013), http://dx.doi.org/10.1016/j.talanta.2013.10.041

which have different hydrophobicity. Besides, the material particle size should also affect the recoveries of the targets. Smaller particle size has larger surface area and could provide better interaction between the sorbent surface and the analytes in the water sample, which could eventually lead to better recoveries [26]. In this study, the high extraction efficiencies of PA II might be the result from a combination of suitable sorbent material characteristics and smaller particle size.

3.1.2. Sample loading flow rate

Another parameter that may affect the performance of the SPE procedure is the flow rate used for sample loading. Furthermore, the sample loading flow rate could affect the total analysis time of a sample. A higher sample loading flow rate can decrease the sample loading time which contributes to a more rapid sample detection. In this method, the sample was directly injected by an autosampler into a large-volume loop (2.5 mL), which was carried over by the mobile phase thereafter. Flow rates of 1, 2, 3 and 4 mL/min onto the PA II cartridge were tested in this study to help to understand how the loading flow rate affects the target recoveries during the on-line SPE process. Results in Fig. 2(B) shows that the recoveries for most BZTs increased along with the increase of loading flow rate. This is somewhat surprising because usually higher efficiencies are obtained at lower loading flow rate, which is attributed to the more efficient time for contact between the analytes and the sorbent surface [26]. The results might be because a high sample loading flow rate can decrease the residence time of the water sample in the glass vial before the SPE enrichment, and thus can decrease additional analyte loss by sorption onto the vessel, especially for highly hydrophobic BZTs [17]. These assumptions can be partly demonstrated by the fact that the recoveries of highly hydrophobic BZTs showed obvious increase (recoveries increased from 59%, 52%, 49% to 83%, 78%, 67% for UV-234, UV-328 and UV-327, respectively) when the sample loading flow rate increased from 1 mL/min to 4 mL/min, while the recoveries of low hydrophobic BZTs such as UV-P and UV-PS did not change much when the sample loading flow rate increased. Besides, the adsorption between BZTs and PA II might be strong such that there is efficient contact time between the analyte and the adsorbent at low flow rate, which makes the BZTs hard to be eluted from the SPE. However, high loading flow rate (3 and 4 mL/min) can occasionally lead to excessive pressure limit of the UltiMate™ 3000 system and interrupt the instrument analysis. Also, the high column pressure brought by high loading flow rate can damage the PA II cartridge and shorten the cartridge lifetime. In consideration of the recoveries, sample determination time and column pressure, 2 mL/min was eventually chosen as the sample loading flow rate. While the adsorption loss cannot be completely avoided by increasing the sample loading flow rate, this can be compensated by adding proper amounts of MeOH into the sample, which will be discussed in section 3.1.4.

3.1.3. pH value of the water samples

The pH value is also an important factor on the performance of the SPE procedure as it affects the compound charge state and their adsorption on the SPE cartridge [27]. In this study, the effect of the pH value was evaluated by determining the spiked ultrapure water samples at a pH range of 2.5–9.5, considering the PA I pH tolerance (1.5–10). Log pKa values of BZTs are between 7 and 10 [28], and the results shown in Fig. 2(C) suggest that the pH can affect the extraction efficiencies in the on-line SPE procedure. The recoveries of the target BZTs increased when the solution pH value increased from 2.5 to 6, whereas their recoveries decreased when the pH value further increased from 6 to 9.5. All of the BZTs showed highest recoveries when the pH=6, with recoveries varying from 45% (UV-350) to 110% (UV-PS). This might be because the charged state, which would enhance the compound solubility in water, made the targets hard to be adsorbed on the sorbent and thus lost in the SPE wash process [21].

3.1.4. MeOH ratio in water samples

Highly hydrophobic BZTs such as UV-328, UV-327 were reported with low recoveries ($<50\%$) by the off-line SPE process, mainly due to the sorption losses on glass vessels and polytetrafluoroethene connection during the complex pretreatment steps [17]. In order to avoid adsorption losses, increasing percentages of MeOH (10%, 20% and 30%) were added to the spiked water samples in the present on-line SPE process. Results in Fig. 2(D) shows that, for BZTs with relatively small log K_{ow} (4.33 and 4.36 for UV-P and UV-PS, respectively), the recoveries remained at high level percentages (83–109%) when the MeOH ratio changed from 10% to 30%. This might because BZTs with lower hydrophobicity are more water soluble and suffer less sorption losses. For the BZTs other than UV-P and UV-PS, whose log K_{ow} are relatively high (5.55–7.67), their recoveries were low (24–73%) when the MeOH ratio was 10% and 20%. However, when the MeOH ratio increased to 30% their recoveries dramatically increased to 60–101%. The great improvement of the recoveries for the targets with high hydrophobicity might suggest less adsorption losses on the vessels when the MeOH ratio increased.

Fig. 3 shows the different parameter effects on target recoveries for several representative compounds. This kind of plot shows the parameter effects with a line drawn between the low and the high recoveries of the corresponding parameters. The vertical length of the line is proportional to the recovery improvement of each factor in the extraction process. Results in Fig. 3 further shows the importance of MeOH ratio in increasing the recoveries of highly hydrophobic BZTs.

3.1.5. Breakthrough volume

Breakthrough volume is a vital factor for SPE cartridges, especially when large-volume and high concentration samples are loaded. In this study, the breakthrough experiment was conducted by loading two different amounts of analytes (4 ng and 10 ng) onto the PA II cartridge and comparing their recoveries. The amount of 4 ng (20 mL × 200 ng/L) was four times higher than the used amount of 1 ng (20 mL × 50 ng/L) in the above optimization procedures. In order to check whether 4 ng of the BZT compounds could lead to breakthrough, 10 ng BZTs were loaded onto the PA II cartridge. Results shown in Figure S1 (Supporting information) indicate that the recovery decrease mainly occurred for UV-234, UV-328 and UV-327 when the spiking amount increased from 4 ng to 10 ng, with recoveries decreasing from 110% (UV-234), 104% (UV-328) and 103% (UV-327) to 72% (UV-234), 72% (UV-328) and 73% (UV-327). From the above data we can speculate that no significant breakthrough occurred at the 4 ng spiking level, because if breakthrough occurred, then the recoveries of UV-234, UV-328 and UV-327 on 10 ng spiking level should be no more than 44% (110% × 4/10), 42% (104% × 4/10) and 41% (103% × 4/10), respectively.

3.2. Method validation

Results from validation procedures of the analytical method are shown in Table 3 and 4. As listed in Table 3, the least-squares linear regression analysis showed good linearity within the range from MQL to 500 ng/L with correlation coefficients (r^2) higher than 0.99 for all of the BZTs studied in the three water matrices. Sensitivity is an important parameter for analytical methods,

Please cite this article as: R. Liu, et al., Talanta (2013), http://dx.doi.org/10.1016/j.talanta.2013.10.041

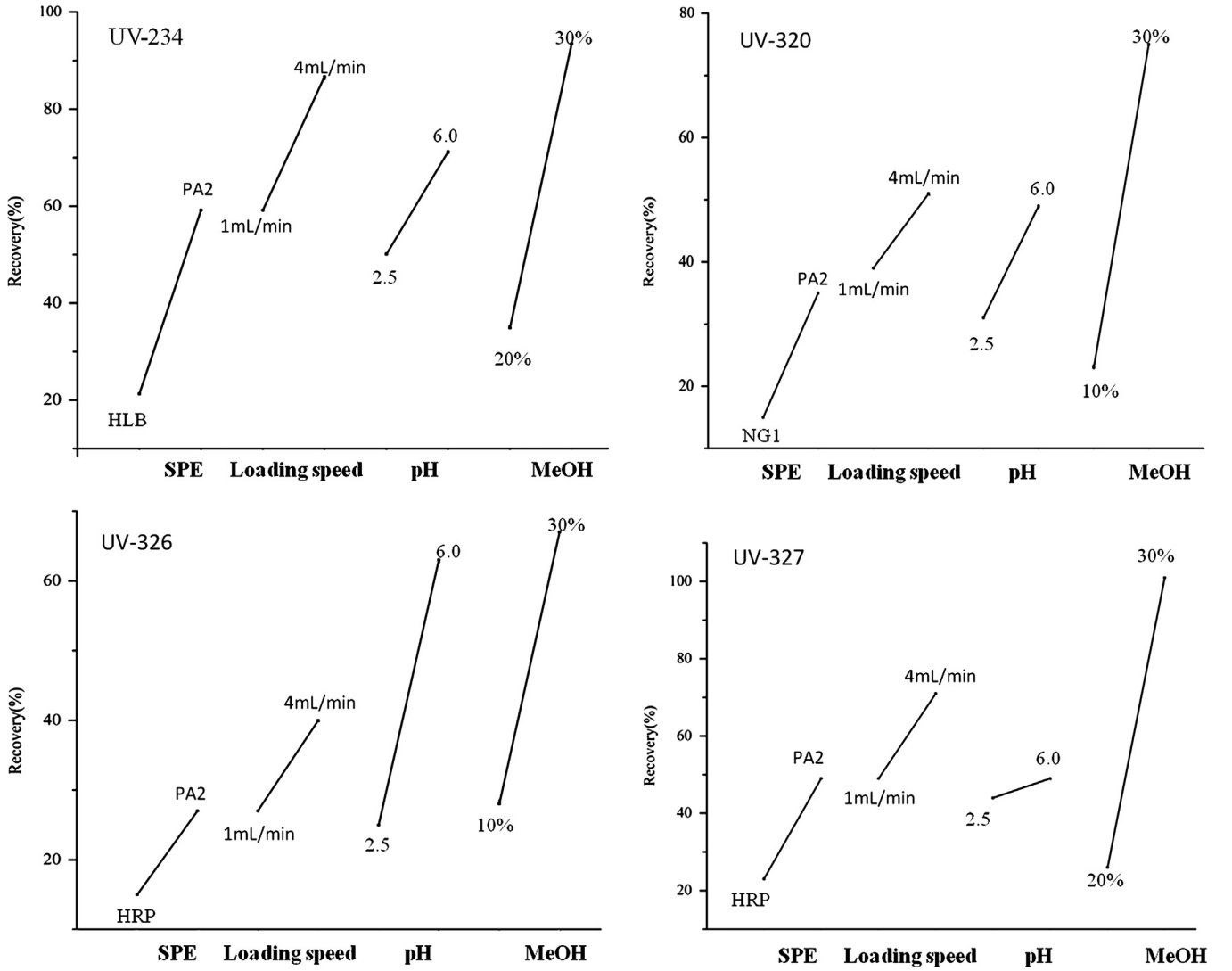


Fig. 3. Parameter effects on recoveries of some representative compounds including UV-234 (log K_{ow}=7.67), UV-320 (log K_{ow}=6.27), UV-326 (log K_{ow}=5.55) and UV-327 (log K_{ow}=6.91).

Table 3
The linearity, MDLs, MQLs and RSDs (50 ng/L level) of the nine target BZTs.

Compound	RSD (%, n=5)		r^2			MDL (ng/L)			MQL (ng/L)		
	Intra-day	Inter-day	Influent	Effluent	River	Influent	Effluent	River	Influent	Effluent	River
UV-P	5	6	0.995	0.994	0.999	0.95	1.68	0.84	3.15	5.59	2.81
UV-PS	2	15	0.998	0.996	0.999	1.68	2.17	1.53	5.60	7.15	5.11
UV-329	1	1	0.998	0.995	0.998	2.08	1.25	1.38	6.92	4.16	4.61
UV-350	10	6	0.999	0.993	0.997	0.56	0.61	0.66	1.88	2.04	2.21
UV-320	6	7	0.995	0.997	0.998	0.66	0.74	0.83	2.20	2.48	2.78
UV-234	6	4	0.990	0.991	0.990	0.39	0.28	0.21	1.31	0.92	0.68
UV-326	6	6	0.993	0.994	0.998	1.68	1.63	1.23	5.60	5.42	4.10
UV-328	5	8	0.997	0.995	0.990	1.30	0.33	1.32	4.33	1.09	4.40
UV-327	2	13	0.990	0.993	0.991	1.31	1.44	1.24	4.35	4.78	4.14

especially when analyzing low concentration samples. Results in Table 3 shows high sensitivity with MQLs in the range of 1.31–6.92 ng/L for sewage influent, 0.92–7.15 ng/L for sewage effluent and 0.68–5.11 ng/L for river water. MDLs were < 2.17 ng/L in the three different water matrixes, which were lower than those by the off-line SPE method (up to 3.0 ng/L) as reported previously [15]. Therefore, these results proved that a high sensitivity could also be obtained despite using a small sample volume (20 mL) in the on-line SPE strategy, as the target BZT analytes in the whole sample were transferred into the chromatography system. The intra-day and inter-day RSDs ranged from 1% to 15%, suggesting good reproducibility of the fully automatic on-line SPE protocol. As shown in Table 4, the recoveries of the target BZTs at 10 ng/L spiking level varied from 84% to 113%, 77% to 111% and 78% to 106% for sewage influent, effluent and river water, while their recoveries obtained at 50 ng/L spiking level were from 80% to 103%, 76% to 114% and 84% to 110% for sewage influent, effluent and river water, respectively. The comparable results from the different spiking

Please cite this article as: R. Liu, et al., Talanta (2013), http://dx.doi.org/10.1016/j.talanta.2013.10.041

levels further suggested that the optimized method was adequate for the analysis of BZTs at different concentrations in different water sample types. The absence of carry-over effect was also demonstrated by injecting 20 mL of the mobile phase solution (MeOH/water=7:3, v/v) at random time intervals. Besides, no target background contaminates or obvious recovery decrease was found when the cartridge was used for more than 100 times.

Table 4
The spike recoveries (mean ± SD, %, $n=3$) of BZT analytes in different water matrices.

Compound	Recovery at 10 ng/L spiking level			Recovery at 50 ng/L spiking level		
	Influent	Effluent	River water	Influent	Effluent	River water
UV-P	113 ± 11	111 ± 14	106 ± 8	103 ± 5	114 ± 5	110 ± 2
UV-PS	98 ± 2	99 ± 1	94 ± 1	99 ± 2	90 ± 2	92 ± 5
UV-329	86 ± 2	100 ± 3	87 ± 2	91 ± 3	92 ± 1	98 ± 8
UV-350	99 ± 17	97 ± 1	98 ± 2	83 ± 3	76 ± 6	87 ± 5
UV-320	97 ± 6	98 ± 1	95 ± 1	99 ± 8	97 ± 5	97 ± 3
UV-234	94 ± 6	90 ± 10	78 ± 1	98 ± 5	81 ± 6	84 ± 1
UV-326	84 ± 1	77 ± 3	82 ± 4	84 ± 2	87 ± 4	95 ± 6
UV-328	101 ± 3	89 ± 5	91 ± 3	80 ± 2	96 ± 4	84 ± 1
UV-327	102 ± 13	91 ± 7	83 ± 1	88 ± 3	95 ± 2	96 ± 1

Table 5
Concentrations (ng/L) of the detected BZTs in the collected samples ($n=3$).

Samples		UV-P	UV-329	UV-350	UV-234	UV-328
WWTP 1	Influent	37.1 ± 2.6	n.d.	1.9 ± 0.4	5.9 ± 0.5	2.6 ± 0.2
	Effluent	15.9 ± 1.4	n.d.	n.d.	2.1 ± 0.3	0.60[b]
WWTP 2	Influent	16.4 ± 1.3	n.d.	n.d.	2.6 ± 0.3	n.d.
	Effluent	7.1 ± 0.8	n.d.	n.d.	0.46[b]	n.d.
WWTP 3	Influent	9.9 ± 1.1	3.8	2.8 ± 0.6	6.3 ± 0.5	2.9 ± 0.3
	Effluent	7.2 ± 0.9	n.d.	2.2 ± 0.3	3.7 ± 0.4	0.60[b]
WWTP 4	Influent	10.9 ± 1.1	n.d.	n.d.	0.66 [b]	n.d.
	Effluent	7.4 ± 0.6	n.d.	n.d.	0.46 [b]	n.d.
River 1		n.d.[a]	n.d.	n.d.	n.d.	n.d.
River 2		n.d.	n.d.	n.d.	n.d.	n.d.
River 3		8.1 ± 0.7	n.d.	n.d.	n.d.	n.d.
River 4		n.d.	n.d.	n.d.	n.d.	n.d.

[a] n.d. Not detected.
[b] Concentration at 1/2 MQL.

3.3. Application

The optimized method was applied for the analysis of the nine target BZTs in the three different water matrices in triplicates. For the sewage influent and effluent samples, five BZTs including UV-P, UV-329, UV-350, UV-234 and UV-328 were detected at different frequencies and concentrations (Table 5). Representative chromatograms of the detected analytes are shown in Fig. 4. Among the five detected analytes, UV-P and UV-234 were found in all sewage water samples with concentrations varying from 7.1 to 37.1 ng/L and 0.46 to 6.3 ng/L, respectively. UV-328 was found in WWTP 1 and WWTP 3 influent and effluent with concentration from 0.6 to 2.9 ng/L. UV-350 was found in three of the eight sewage water samples with concentration from 1.9 to 2.8 ng/L, while UV-329 was detected in only one sample (WWTP 3 influent) at the concentration of 3.8 ng/L. For the four river samples, only UV-P was found in River 3 at concentration of 8.1 ng/L. The different BZT concentrations and profiles among the different WWTP samples may indicate different usage of the UV stabilizers among the sampling regions.

4. Conclusion

In this study, a new on-line SPE-HPLC–MS/MS method was developed to analyze nine BZTs in different water matrices including sewage influent, effluent and river water. An automated on-line pretreatment and analysis protocol was developed using the on-line SPE sample processor UltiMate™ 3000 system and the Quattro Premier XE triple-quadrupole mass spectrometer. The PA II cartridge showed higher target recoveries compared with other cartridges. pH=6 was the most appropriate pH value for water samples. Adding 30% MeOH to the water samples can dramatically increase the recoveries of hydrophobic BZTs due to less adsorption losses on the vessels when the MeOH added. The flow rate of 2 mL/min was selected to load the samples into the cartridge, taking the recoveries, sample analysis time and column pressure into consideration. The optimized SPE-HPLC–MS/MS method provided very low MDLs

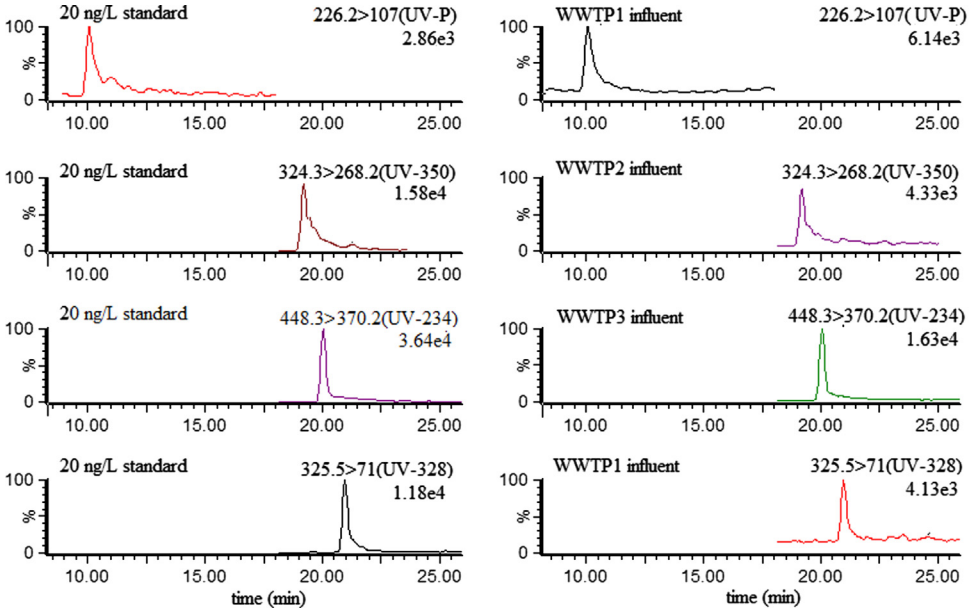


Fig. 4. HPLC–MS/MS MRM chromatograms of detected analytes. Left panels show 20 ng/mL standard while right ones are for collected samples.

Please cite this article as: R. Liu, et al., Talanta (2013), http://dx.doi.org/10.1016/j.talanta.2013.10.041

for these BZTs in types of water matrices, with MDL values ranged from 0.21 to 2.17 ng/L. Among the nine targets, five BZTs (UV-P, UV-329, UV-350, UV-234 and UV-328) were detected with concentrations up to 37.1 ng/L. This optimized method is practical and easy, and could be used for routine analysis of BZTs in river waters and influents and effluents from WWTPs.

Acknowledgments

This work was jointly supported by the National Natural Science Foundation (21207140, 20890110, 20621703) and the National Basic Research Program of China (2009CB421605).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.10.041.

References

[1] I. Carpinteiro, B. Abuin, I. Rodriguez, M. Ramil, R. Cela, J. Chromatogr. A 1217 (2010) 3729–3735.
[2] H. Nakata, S. Murata, J. Filatreau, Environ. Sci. Technol. 43 (2009) 6920–6926.
[3] L. Vidal, A. Chisvert, A. Canals, A. Salvador, Talanta 81 (2010) 549–555.
[4] D.L. Giokas, A. Salvador, A. Chisvert, Trends Anal. Chem 26 (2007) 360–374.
[5] S.D. Richardson, T.A. Ternes, Anal. Chem. 77 (2005) 3807–3838.
[6] M.S. Díaz-Cruz, D. Barceló, Trends Anal. Chem. 28 (2009) 708–717.
[7] Z. Zhang, N. Ren, Y.F. Li, T. Kunisue, D. Gao, K. Kannan, Environ. Sci. Technol. 45 (2011) 3909–3916.
[8] L.R. Gaspar, P.M. Campos, Talanta 82 (2010) 1490–1494.
[9] T. Yamano, M. Shimizu, T. Noda, J. Health Sci. 47 (2) (2001) 123–128.
[10] M. Hirata-Koizumi, N. Watari, D. Mukai, T. Imai, A. Hirose, E. Kamata, M. Ema, Drug Chem. Toxicol. 30 (2007) 327–341.
[11] U.S. Environmental Protection Agency. Available from: http://iaspub.ep a.gov/oppthpv/quick search.display?pChem=100705 (accessed 26.04.13).
[12] C.M. Reddy, J.G. Quinn, J.W. King, Environ. Sci. Technol. 34 (2000) 973–979.
[13] M. Vosough, N.R. Mojdehi, Talanta 85 (2011) 2175–2181.
[14] P. Gago-Ferrero, M.S. Diaz-Cruz, D. Barcelo, Chemosphere 84 (2011) 1158–1165.
[15] Y.S. Liu, G.G. Ying, A. Shareef, R.S. Kookana, Environ. Pollut. 165 (2012) 225–232.
[16] Y. Kameda, K. Kimura, M. Miyazaki, Environ. Pollut. 159 (2011) 1570–1576.
[17] I. Carpinteiro, B. Abuin, I. Rodriguez, R. Cela, M. Ramil, Anal. Bioanal. Chem. 397 (2010) 829–839.
[18] P.B. Fayad, M. Prévost, S. Sauvé, Talanta 115 (2013) 349–360.
[19] M.J. Garcia-Galan, M.S. Diaz-Cruz, D. Barcelo, Talanta 81 (2010) 355–366.
[20] T. Ruan, R. Liu, Q. Fu, T. Wang, Y. Wang, S. Song, P. Wang, M. Teng, G. Jiang, Environ. Sci. Technol. 46 (2012) 2071–2079.
[21] S. Montesdeoca-Esponda, Z. Sosa-Ferrera, J.J. Santana-Rodriguez, Anal. Bioanal. Chem. 403 (2012) 867–876.
[22] US EPA (2011). Estimation Programs Interface Suite™ v4.10. United States Environmental Protection Agency, Washington,DC, USA.
[23] F. Hernandez, J.V. Sancho, O. Pozo, A. Lara, E. Pitarch, J. Chromatogr. A 939 (2001) 1–11.
[24] R. Liu, T. Ruan, T. Wang, S. Song, M. Yu, Y. Gao, J. Shao, G. Jiang, Talanta 111 (2013) 170–177.
[25] Commission Decision 2002/657/EC of 12 August 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, OJ no. L 221, 2002.
[26] S. Rodriguez-Mozaz, M.J.L.d. Alda, D. Barcelo, Anal. Chem. 76 (2004) 6998–7006.
[27] N. Masque, R.M. Marce, F. Borrull, J. Chromatogr. A 793 (1998) 257–263.
[28] http://ibmlc2.chem.uga.edu/sparc/. (accessed 03.03.13).

Please cite this article as: R. Liu, et al., Talanta (2013), http://dx.doi.org/10.1016/j.talanta.2013.10.041