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Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography–tandem mass spectrometry

Arne Wick, Guido Fink, Thomas A. Ternes*

Federal Institute of Hydrology (BfG), Am Mainzer Tor 1, D-56068 Koblenz, Germany

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ABSTRACT

This paper describes the development of a multi-residue method for the determination of 36 emerging organic pollutants (26 biocides, 5 UV-filters and 5 benzothiazoles) in raw and treated wastewater, activated sludge and surface water using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The target analytes were enriched from water samples adjusted to pH 6 by solid-phase extraction (SPE) on Oasis HLB 200 mg cartridges and eluted with a mixture of methanol and acetone (60/40, v/v). Extraction of freeze-dried sludge samples was accomplished by pressurized liquid extraction (PLE) using a mixture of methanol and water (50/50, v/v) as extraction solvent followed by SPE. LC–tandem MS detection was compared using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in positive and negative ionization mode. ESI exhibited strong ion suppression for most target analytes, while APCI was generally less susceptible to ion suppression but partially leading to ion enhancement of up to a factor of 10. In general, matrix effects could be compensated using stable isotope-labeled surrogate standards, indicated by relative recoveries ranging from 70% to 130%. In wastewater, activated sludge and surface water up to 33 analytes were detected. Maximum concentrations up to 5.1 and 3.9 $\mu g\,L^{-1}$ were found in raw wastewater for the water-soluble UV-filters benzophenone-4 (BZP-4) and phenylbenzimidazole sulfonic acid (PBSA), respectively. For the first time, the anti-dandruff climbazole was detected in raw wastewater and in activated sludge with concentrations as high as 1.4 $\mu g\,L^{-1}$ and 1.2 $\mu g\,g\,TSS^{-1}$, respectively. Activated sludge is obviously a sink for four benzothiazoles and two isothiazolones, as concentrations were detected in activated sludge between 120 $ng\,g\,TSS^{-1}$ (2-n-octyl-4-isothiazolin-3-one, OIT) to 330 $ng\,g\,TSS^{-1}$ (benzothiazole-2-sulfonic acid, BTSA).

1. Introduction

In recent years, biocides and UV-filters have gained increasing interest as so called emerging contaminants since they are ingredients of various products used in every day life such as personal care products (PCPs), cleaning agents and paints and coatings [1,2]. Another crucial class are benzothiazoles which are mainly used as vulcanization accelerators and are present in all kinds of rubber made products [3]. As used mainly in rinse-off products, biocidal ingredients of PCPs and cleaning agents such as the preservative 1,2-benzisothiazolin-3-one (BIT) as well as water-soluble UV-filters such as benzophenone-4 (BZP-4) are discharged into municipal wastewater treatment plants (WWTPs) [4,5]. Biocides used as film-preservatives in paintings, coatings and roof sealings such as carbendazim and mecoprop reach WWTPs by leaching, washing of equipment and the disposal of unused products. In case of an incomplete removal all these contaminants are further discharged into the receiving waters [6,7].

Since biocides are biological active compounds applied to destroy or to inhibit the growth or action of organisms [8], even low environmental concentrations might have negative impacts on the aquatic environment. For example, triclosan has been shown to induce changes in the thyroid hormone-mediated process of metamorphosis of the North American bullfrog *Rana catesbeiana* and to cause a significant shift in the community structure of a natural river algae community at environmental relevant concentrations as low as 30 and 15 $ng\,L^{-1}$, respectively [9,10]. Carbendazim seriously effected the macroinvertebrate community in a freshwater microcosm in the low $\mu g\,L^{-1}$ range [11] and the antifouling irgarol was found to effect the community of macrophytes in mesocosms with EC_{50} values down to 0.2 $\mu g\,L^{-1}$ for the species *Myriophyllum verticillatum* [12]. UV-filters, such as benzophenone-1 (BZP-1) and

* Corresponding author. Tel.: +49 261 1306 5560; fax: +49 261 1306 5363.
E-mail address: Ternes@bafg.de (T.A. Ternes).

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benzophenone-2 (BZP-2), have been reported to show estrogenic activity [13,14].

Most of the currently available analytical methods for the determination of biocides and UV-filters in wastewater and activated sludge are based on GC-MS using a variety of derivatization techniques [6,15–21]. For isothiazolones, conazoles or water-soluble UV-filters analytical methods for identification and quantification in wastewater or activated sludge are hardly available. A GC–MS method for the measurement of isothiazolones in aqueous matrices was developed by Rafoth et al. [4]. The anti-dandruff ketoconazole was quantified together with other conazoles in surface water and wastewater using LC–MS/MS [22], but to our knowledge no analytical methods for the analysis of the anti-dandruff climbazole in any environmental matrices have been published so far.

UPLC–ESI/MS/MS was used recently to determine benzophenonic UV-filters in surface water, wastewater [23] and activated sludge [24]. The UV-filter PBSA was measured together with BZP-3 and BZP-4 in Spanish tap water, surface water and wastewater by Rodil et al. [5]. BZP-4 was found to occur in the $\mu g\,L^{-1}$ range in raw and treated wastewater and was also detected in 4 of 5 tap water samples at a mean concentration of $12\,ng\,L^{-1}$.

Nowadays, analytical methods based on LC–MS/MS offer a tool to identify and quantify compounds of medium to high polarity in all kinds of water bodies and solid matrices [25,26]. However, a serious drawback of LC–MS/MS methods is their susceptibility to matrix effects, e.g. the signal suppression or enhancement by matrix compounds entering the ion source at the same time. Matrix effects can strongly vary with the environmental matrix and result in poor analytical accuracy and reproducibility [22,27,28]. It has been reported that atmospheric pressure chemical ionization (APCI) is generally less sensitive to matrix effects than the more commonly used electrospray ionization (ESI) [29–33], but only few studies focused on matrix effects using APCI in direct comparison to ESI for different compound groups. Since especially for emerging contaminants stable isotope-labeled surrogate standards are often not available and only compensate but not reduce matrix effects, APCI was evaluated as an alternative ionization interface. Other measures which have been successfully used to reduce matrix effects such as changing the composition of the mobile phases, additional clean-ups and post-column switching [22,34–36] are often accompanied by compound losses if applied to multi-residue methods with a broad compound spectrum.

The objective of the current study was the development of a sensitive multi-residue method for the determination of biocides, UV-filters and benzothiazoles in surface waters, wastewater and activated sludge using LC–MS/MS. The challenge was to analyze the structurally diverse analytes from the same sample using a single extraction procedure. Matrix effects in the ESI and APCI interface were assessed for each analyte using post-extraction spikes. The suitability of the use of deuterated and ^{13}C-labeled surrogate standards for compensation of matrix effects was evaluated for both interfaces. The selected analytes are listed in Table 1.

2. Experimental

2.1. Chemicals

The following compounds were analyzed: dimethomorph, fenpropimorph, tridemorph, 2-n-octyl-4-isothiazolin-3-one (OIT), triclosan (purchased from Fluka, Buchs, Switzerland); imazalil, carbendazim, irgarol (purchased from Riedel-de Haen, Seelze, Germany); 1,2-benzisothiazolin-3-one (BIT), 3-iodo-2-propynyl-N-butylcarbamate (IPBC), triclocarban, benzothiazole (BT), benzothiazole-2-sulfonic acid (BTSA), 2-methylthiobenzothiazole (MTBT), 2-hydroxybenzothiazole (OHBT), 2-(4-morpholinyl) benzothiazole (morpholinyl-BT) (purchased from Sigma–Aldrich, Schnelldorf, Germany); thiabendazole, propiconazole, tebuconazole, climbazole, ketoconazole, diuron, isoproturon, mecoprop, terbutryn, terbuthylazine, N,N-dimethyl-N′-phenylsulfamide (DMSA, transformation product of dichlofluanide), N,N-dimethyl-N′-p-tolylsulfamide (DMST, transformation product of tolyfluanide), chlorophene (purchased from Dr. Ehrenstorfer, Augsburg, Germany); 2-methylthio-4-tert-butylamino-6-amino-s-triazine (M1, transformation product of irgarol) (purchased from Ciba Speciality Chemicals); 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) (purchased from Chemos, Regenstauf, Germany); phenylbenzimidazole sulfonic acid (PBSA), benzophenone-1 (BZP-1), benzophenone-2 (BZP-2), benzophenone-3 (BZP-3), benzophenone-4 (BZP-4) (kindly provided by Prof. Dr. J. Oehlmann, University Frankfurt).

The surrogate standards carbendazim-d_4, thiabendazole-d_6, propiconazole-d_5, tebuconazole-d_6, imazalil-d_5, terbutryn-d_5, terbuthylazine-d_5, diuron-d_6, isoproturon-d_6, mecoprop-d_3 were obtained from Dr. Ehrenstorfer (Augsburg, Germany), triclosan-$^{13}C_{12}$, triclocarban-$^{13}C_6$ from Cambridge Isotope Laboratories (Andover, MA, USA) and ketoconazole-d_8 from Campro Scientific (Berlin, Germany).

Methanol (picograde) and acetonitrile (HPLC gradient grade) were purchased from LGC Promochem (Wesel, Germany). Formic acid and sulfuric acid (both p.a.) were purchased from Merck (Darmstadt, Germany) and ammonium formate (purum grade) from Sigma–Aldrich (Schnelldorf, Germany). Pure water was obtained from a Milli-Q system (Integral 3/5/10/15, Millipore, Billericia, MA, USA).

Separate standard solutions of all analytes and surrogate standards were prepared in methanol at a concentration of 10 and $1\,\mu g\,mL^{-1}$, respectively and stored in the dark at 4 °C.

2.2. Sampling of wastewater, activated sludge, surface water and groundwater

The wastewater samples used for method validation derived from WWTP 1 serving approximately 320,000 population equivalents (PE). WWTP 1 consists of a mechanical treatment (screen, grit removal and primary clarifier), a trickling filter followed by an activated sludge treatment with nitrification and denitrification, phosphate removal and a final clarification. Grab samples were taken from the influent (after primary clarification) and from the final effluent on 11th March 2008. Samples from surface water were taken from the river Rhine in Koblenz (Germany) at river kilometre 590.3 on the same day as the wastewater samples. The sludge samples were taken from the activated sludge tank (nitrification zone) of WWTP 1 on 26th November 2008.

Additional wastewater samples were obtained from WWTP 2 serving approximately 307,000 PE with a treatment lane comparable to WWTP 1 but without a trickling filter prior to the activated sludge treatment. Grab samples were taken after grit removal prior to the primary clarifier and from the final effluent on 2nd July 2009. Furthermore, grab samples were taken on 1st September 2009 close to the mouth of two small tributaries of the river Main. The sampling point of stream 1 (Schwarzbach) and stream 2 (Wickerbach) was located about 3 and 0.1 km downstream the last discharge of a WWTP, respectively.

All samples were taken in solvent rinsed amber glass bottles and immediately cooled down to 4 °C until further sample preparation (within 1–2 days).

The groundwater used in this study was collected from a well in Koblenz-Arenberg (Germany). Measurements of groundwater blank samples subjected to the entire preparation and analysis procedure were included in every series of analysis and showed that the groundwater was pristine and free of all targeted analytes.

Table 1
Selected target analytes, abbreviations and properties. TP: transformation product.

Name	Abbreviation	Application	CAS no	Formula	log K_{OW} [a]	pK_a [a]
Biocides						
Diuron		Herbicide	330-54-1	$C_9H_{10}Cl_2N_2O$	2.85	
Isoproturon		Herbicide	34123-59-6	$C_{12}H_{18}N_2O$	2.50	
Mecoprop		Herbicide	7085-19-0	$C_{10}H_{11}ClO_3$	0.1	3.74
Propiconazole		Fungicide	60207-90-1	$C_{15}H_{17}Cl_2N_3O_3$	3.72	
Tebuconazole		Fungicide	107534-96-3	$C_{16}H_{22}ClN_3O$	3.70	
Imazalil		Fungicide	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	3.82	6.5
Climbazole		Fungicide (anti-dandruff)	38083-17-9	$C_{15}H_{17}ClN_2O_2$	3.33[b]	7.5[b]
Ketoconazole		Fungicide (anti-dandruff)	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	4.30[b]	6.6[b]
Carbendazim		Fungicide	10605-21-7	$C_9H_9N_3O_2$	1.51	4.2
Thiabendazole		Fungicide	148-79-8	$C_{10}H_7N_3S$	2.47[b]	4.70[b]
Terbuthylazine		Herbicide	5915-41-3	$C_9H_{16}ClN_5$	3.04	2.0
Terbutryn		Herbicide	886-50-0	$C_{10}H_{19}N_5S_2$	3.65	4.3
Irgarol		Herbicide/algicide	28159-98-0	$C_{11}H_{19}N_5S$	3.72	4.1
2-Methylthio-4-tert-butylamino-6-amino-s-triazine	M1	TP of irgarol	30125-65-6	$C_8H_{15}N_5S$		
Dimethomorph		Fungicide	110488-70-5	$C_{21}H_{22}ClNO_4$	2.63	
Fenpropimorph		Fungicide	67564-91-4	$C_{20}H_{33}NO$	4.40	7.0
Tridemorph		Fungicide	24602-86-6	$C_{19}H_{39}NO$	6.99[b]	7.4[b]
1,2-Benzisothiazolin-3-one	BIT	Microbicide	2634-33-5	C_7H_5NOS	1.24[b]	
2-n-Octyl-4-isothiazolin-3-one	OIT	Microbicide	26530-20-1	$C_{11}H_{19}NOS$	2.45	
4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one	DCOIT	Microbicide	64359-81-5	$C_{11}H_{17}Cl_2NOS$	4.77[b]	
N,N-Dimethyl-N′-p-tolylsulfamide	DMST	TP of the fungicide tolyfluanide	66840-71-9	$C_9H_{14}N_2O_2S$		
N,N-Dimethyl-N′-phenylsulfamide	DMSA	TP of the fungicide dichlofluanide	4710-17-2	$C_8H_{12}N_2O_2S$		
3-Iodo-2-propynyl-N-butylcarbamate	IPBC	Fungicide	55406-53-6	$C_8H_{12}INO_2$	2.81[c]	
Triclosan		Microbicide	3380-34-5	$C_{12}H_7Cl_3O_2$	4.76[b]	8.0[b]
Triclocarban		Microbicide	101-20-2	$C_{13}H_9Cl_3N_2O$	5.10[b]	
Chlorophene		Microbicide	120-32-1	$C_{13}H_{11}ClO$	4.14[b]	9.6[b]
UV-filter						
Benzophenone-1	BZP-1	UV-filter	131-56-6	$C_{13}H_{10}O_3$	2.92[b]	8.0[b]
Benzophenone-2	BZP-2	UV-filter	131-55-5	$C_{13}H_{10}O_5$	2.08[b]	8.0[b]
Benzophenone-3	BZP-3	UV-filter	131-57-7	$C_{14}H_{12}O_3$	3.79[b]	8.0[b]
Benzophenone-4	BZP-4	UV-filter	4065-45-6	$C_{14}H_{12}O_6S$	0.39[b]	0.7[b]
PBSA	PBSA	UV-filter	27503-81-7	$C_{13}H_{10}N_2O_3S$	1.03[b]	4.9, 0.7[b]
Benzothiazoles						
Benzothiazole	BT	Vulcanization	95-16-9	C_7H_5NS	2.01[b]	1.2[b]
2-Methylthiobenzothiazole	MTBT	Vulcanization	615-22-5	$C_8H_7NS_2$	3.15[b]	2.5[b]
Benzothiazole-2-sulfonic acid	BTSA	Vulcanization	941-57-1	$C_7H_5NO_3S_2$	−0.39[b]	2.4, −1.0[b]
2-Hydroxybenzothiazole	OHBT	Vulcanization	934-34-9	C_7H_5NOS	2.12[b]	6.2[b]
2-(4-Morpholinyl) benzothiazole	Morpholinyl-BT	Vulcanization	4225-26-7	$C_{11}H_{12}N_2OS$	2.59[b]	4.5[b]

[a] C.D.S. Tomlin (ed.), The Pesticide Manual, British Crop Protection Council (BCPC), Farnham, UK, 10th ed., 1994.
[b] ALOGPS 2.1, Virtual Computational Chemistry Laboratory, 2007: http://www.vcclab.org/lab/alogps/.
[c] MRI. 1990. Analysis of Polyphase P-100 octanol/water partition coefficient (63-11). Final report. MRI Project No. 9555-F(01). Prepared for Troy Chemical Corporation Newark, NJ. MRI, Kansas City, MO.

2.3. Sample preparation and extraction

2.3.1. Aqueous samples

Solid-phase extraction (SPE) was used for compound extraction and enrichment from aqueous samples. Different types of adsorbents (Bakerbond C18, 500 mg, Mallinckrodt Baker, Phillipsburg, USA; Strata-X, 200 mg, 33 μm, Phenomenex, Aschaffenburg, Germany; Isolute ENV+, 200 mg, 90 μm, Biotage, Uppsala, Sweden; Oasis HLB, 200 mg, 30 μm, Waters, Milfort, USA; Oasis MCX, 60 mg, 30 μm, Waters, Milfort, USA; Strata-X-C, 200 mg, 33 μm, Phenomenex, Aschaffenburg, Germany), pH values of the samples and elution solvents were tested using 1000 mL of groundwater spiked with 100 ng L^{-1} of each analyte. Based on the these results, the following optimal SPE procedure has been established.

Water samples were filtered through glass fiber filters (GF 6, Whatman). For the solid-phase extraction 100 mL of raw wastewater, 200 mL of treated wastewater, and 1 L of surface water were adjusted to pH 6 with 3.5 M sulfuric acid and spiked with 200 ng of each surrogate standard. Oasis HLB cartridges (200 mg, 30 μm, Waters, Milfort, USA) were washed and conditioned with 1× 2 mL heptane, followed by 1× 2 mL acetone, 3× 2 mL methanol and 4× 2 mL groundwater (adjusted to pH 6 with 3.5 M sulfuric acid). The water samples were then passed through the pre-conditioned cartridges at a flow rate of approximately 5 mL min^{-1}. The solid-phase material was dried by a continuous nitrogen stream for approximately 1 h. Elution was accomplished with 4× 2 mL of a mixture of methanol and acetone (60/40, v/v). The extracts were evaporated to 500 μL under a gentle stream of nitrogen and filled up to a final volume of 1 mL with 0.1% formic acid.

2.3.2. Sludge samples

Extraction of sludge samples were conducted by pressurized liquid extraction (PLE). The solid part of the activated sludge was separated from the aqueous phase by centrifugation for 15 min at 4000 rpm. Subsequently, the sludge was freeze-dried and ground with a pestle. Approximately 200 mg of the dry sludge was weighed into 22 mL stainless steel extraction cells filled to one half with baked out sea sand before the internal standard mixture was added (1 μg g TSS^{-1}). After the solvent was completely evaporated, the cell was filled up with baked out sea sand (Riedel-de Haen, Seelze, Germany). The extraction was accomplished with a Dionex ASE 200 instrument (Sunnyvale, CA, USA). A variety of extraction solvents (water/methanol (50/50%, v/v), 100% methanol and 100% acetone) as well as extraction temperatures (80, 100, 120 °C) were tested to optimize the extraction efficiencies. The final PLE con-

ditions were as follows: prefill method; solvent, water/methanol (50/50%, v/v); equilibration, 5 min; static time, 10 min; flush volume, 120%; purge time, 60 s; static cycles, 4; temperature, 80 °C. The PLE extracts (~30 mL) were diluted with groundwater to a volume of 800 mL, adjusted to pH 6 with 3.5 M sulfuric acid and a SPE was performed as described above for the aqueous samples.

2.4. LC–MS/MS analysis

An Agilent 1200 Series (Agilent Technologies, Waldbronn, Germany) liquid chromatographic system consisting of a membrane degasser, binary high-pressure gradient pump, autosampler, and a column thermostat was used. Chromatographic separation was carried out on a Synergi Fusion-RP 80 Å column (150 mm × 3 mm, 4 μm) equipped with a SecurityGuard pre-column (4 mm × 3 mm) (Phenomenex, Aschaffenburg, Germany).

Two different LC–MS/MS methods (methods 1 and 2) were developed for the analysis of target analytes. For method 1, mobile phase A consisted of 10 mM ammonium formate buffer adjusted to pH 3.2 with formic acid, and acetonitrile with 0.1% formic acid served as mobile phase B. The applied gradient elution was as follows: start of the run with 0% B, kept isocratic for 1 min, increase to 30% B within 1 min, further increase to 80% B within 17 min, kept isocratic for 6 min, return to the initial conditions within 2 min which were hold for the last 5 min.

Using method 2, mobile phase A consisted of 0.1% formic acid, and acetonitrile served as mobile phase B. The applied gradient elution was as follows: start of the run with 0% B, kept isocratic for 1 min, increase to 40% B within 1 min, further increase to 80% B within 17 min, kept isocratic for 7 min, return to the initial conditions within 2 min which were hold for the last 5 min. For both methods, the flow rate was kept constant at 0.4 mL min^{-1} and the sample volume injected was 25 μL.

The HPLC was coupled to a tandem mass spectrometer (API 4000, Applied Biosystems, Foster City, CA, USA) operated in the positive (method 1) and in the negative ion mode (method 2) using multiple reaction monitoring (MRM). Electrospray ionization (ESI) as well as atmospheric pressure chemical ionization (APCI) were applied to compare sensitivity, recoveries and ion suppression of both ionization interfaces.

The ESI source conditions were adjusted as follows (values for negative ion mode (method 2) are given in parenthesis): collision gas, medium (medium); curtain gas, 15 psi (15 psi); ion source gas 1 and ion source gas 2, both 35 psi (40 psi); source temperature, 600 °C (550 °C); entrance potential, 10 V (−10 V); ion spray voltage 5.5 kV (−2.0 kV). The corresponding APCI source conditions were adjusted as follows (values for negative ion mode (method 2) are given in parenthesis): collision gas, medium (medium); curtain gas, 10 psi (15 psi); nebuliser current, 3 μA (−3 μA); ion source gas 1, 30 psi (30 psi), ion source gas 2, 35 psi (40 psi); source temperature, 450 °C (450 °C); entrance potential, 10 V (−10 V).

Two MRM transitions for each compound were monitored for quantification (transition 1) and confirmation (transition 2) of all target compounds. The compound specific parameters such as declustering potential, collision energy, and the cell exit potential were optimized individually for each compound in continuous flow mode via direct injection of standard solutions (200 ng mL^{-1}) solved in acetonitrile/water (90:10) at a flow rate of 10 μL min^{-1} and are listed together with the retention times, MRM transitions, transition intensity ratios and dwell times in Table 2. Only the data for ESI are shown, since for APCI the same transitions were selected and the compound specific parameters did not differ significantly. For most analytes dwell times were set to 25 ms. Higher dwell times of 100 ms were chosen for selected analytes for which relatively low sensitivities with ESI were observed.

2.5. Method validation

2.5.1. Recoveries

Determination of recoveries was assessed for the different matrices (groundwater, surface water, wastewater and activated sludge) at different concentration levels. Groundwater and surface water were spiked at a concentration level of 0.1 μg L^{-1}. WWTP effluent, influent and freeze-dried activated sludge were spiked at two concentration levels of 0.5 and 2 μg L^{-1}, 1 and 4 μg L^{-1} and 0.5 and 2 μg g TSS^{-1}, respectively. Due to the impossibility to obtain WWTP samples and surface water samples free of analytes, the background concentrations were determined in non-spiked samples ($n = 4$) and subtracted from the concentrations measured in the spiked samples. The relative recoveries describing the accuracy of the entire analytical procedure were calculated as the ratio of the spiked concentrations and the quantified concentrations. Deviations from the mean values are given as 95% confidence intervals ($n = 4$). The instrumental precision, determined as relative standard deviation (%RSD), was obtained from the repeated injection of a spiked groundwater extract during the same day (intra-day precision, $n = 5$) and on three different days (inter-day precision, $n = 3$).

2.5.2. Calibration curves and quantification limits

Calibration curves with 14 different calibration points ranging from 0.2 to 2000 ng L^{-1} were obtained by spiking 1000 mL of pristine groundwater. A constant amount of surrogate standards (200 ng) was added. Samples were then subjected to the SPE as described above. The linearity range was between 0.2 and 200 ng L^{-1}. A quadratic fitting ($y = ax^2 + bx + c$) with a weighing factor of $1/x$ was used from 200 to 2000 ng L^{-1}. The limit of quantification (LOQ) was defined as the second lowest calibration point in the regression as long as the calculated signal to noise ratio (S/N) of the compounds in the native sample extracts was >10 for the first transition (t1) used for quantification and >3 for the second transition (t2) used for confirmation. Taking into account the different sample volumes used for SPE and the sludge amount used for PLE, the LOQs for the influent, effluent and sludge samples were calculated by multiplying the LOQ achieved for the extraction of groundwater by a factor of 10, 5 and 5, respectively. Still the criteria of a S/N ratio >10 for quantification and >3 for confirmation had to be fulfilled. For confirmation, the S/N ratios of both transitions were determined using non-spiked extracts for analytes with background concentrations above the LOQs, whereas for analytes with background concentrations <LOQ spiked sample extracts were used (cp. Table A5, Supplementary data). For most analytes the S/N ratios were in accordance with the LOQs calculated from the enrichment factors. In a few cases the LOQs were individually adapted based on the determined S/N ratios.

2.5.3. Matrix effects

Ion suppression or enhancement was assessed using post-extraction spikes according to Matuszewski et al. [33]. Briefly, final sample extracts from groundwater, surface water and WWTP influent and effluent were divided into two aliquots of 200 μL. While one aliquot served as blank sample and was only supplemented with 50 μL of methanol, the other one was spiked with 50 μL of a 1 μg mL^{-1} compound standard solution resulting in a final spike concentration of 200 ng mL^{-1}. Similar to the calculation of the absolute recovery, the matrix effect (ME) was calculated as the percental ratio of the analyte peak area in the spiked sample ($PA_{post\text{-}spike}$) subtracted by the peak area in the non-spiked blank sample (PA_{blank}) to the peak area in a non-enriched external standard (PA_{EXT}):

$$ME = \left(\frac{PA_{post\text{-}spike} - PA_{blank}}{PA_{EXT}} \right) \times 100 \quad (1)$$

Table 2
Precursor, product ions, and retention times in LC–MS/MS detection (ESI, positive and negative ionization mode). (*) Analytes determined in negative ionization mode.

Recovery [%]	Retention time [min]	Transition 1[a] (t1) [m/z]	Transition 2[a] (t2) [m/z]	[t1]/[t2] (%RSD)	Dwell time [ms]	DP[b] (t1/t2) [V]	CE[b] (t1/t2) [eV]	CXP[b] (t1/t2) [V]
Biocides								
Diuron	12.1	235.0/72.1	233.0/72.1	0.8 (2)	25	50/50	30/30	12/11
Isoproturon	11.9	207.1/72.1	207.1/143.0	13.4 (12)	25	65/65	35/33	10/10
Mecoprop (*)	10.6	213.0/140.8	213.0/71.0	1.9 (3)	25	−50/−50	−18/−16	−11/−1
Propiconazole	16.1	342.1/159.1	344.1/161.1	1.6 (3)	25	76/76	45/37	14/12
Tebuconazole	15.1	308.1/70.0	310.1/70.0	2.3 (4)	25	66/81	49/45	6/4
Imazalil	11.3	297.1/159.0	297.1/201.0	1.8 (2)	25	76/76	31/25	12/12
Climbazole	12.1	293.1/69.0	295.1/199.0	1.7 (8)	25	50/60	37/23	10/10
Ketoconazole	12.3	533.1/491.1	531.1/244.1	1.2 (8)	25	125/110	46/47	10/10
Carbendazim	7.4	192.1/160.1	192.1/132.1	5.1 (14)	25	61/61	25/41	12/10
Thiabendazole	7.8	202.1/175.1	202.1/131.1	1.6 (4)	25	91/91	37/47	14/10
Terbuthylazine	13.3	230.1/174.1	230.1/104.1	7.7 (7)	25	61/61	25/45	14/6
Terbutryn	13.1	242.1/186.1	242.1/91.0	6.6 (7)	25	50/50	25/38	15/5
Irgarol	13.5	254.1/198.1	254.1/83.0	4.3 (6)	25	70/70	26/41	6/6
M1	10.3	214.1/68.0	214.1/110.1	1.9 (5)	25	50/50	53/37	3/10
Dimethomorph	13.5	388.1/301.1	388.1/165.1	4 (19)	25	105/105	28/43	16/15
Fenpropimorph	13.2	304.3/147.2	304.3/117.1	1.6 (6)	25	81/81	41/77	10/10
Tridemorph	16.3	298.4/130.2	298.4/98.2	1.5 (6)	25	86/86	35/41	10/8
BIT	7.9	152.0/105.0	152.0/108.8	0.8 (4)	100	71/71	33/31	7/8
OIT	15.0	214.1/102.0	214.1/84.0	37 (6)	25	70/70	21/56	6/10
DCOIT	18.9	282.0/170.0	284.0/172.0	1.4 (8)	25	60/60	22/22	12/12
DMST	11.2	215.1/106.1	215.1/79.1	2.6 (6)	25	43/43	20/39	6/6
DMSA (*)	8.7	199.0/90.9	199.0/154.9	1.5 (9)	100	−45/−45	−30/−21	−4/−8
IPBC	12.8	282.0/57.2	282.0/164.9	0.6 (3)	100	56/56	23/23	2/12
Triclosan (*)	15.2	287.0/35.0	289.0/35.0	1.5 (2)	100	−45/−45	−30/−32	−3/−3
Triclocarban (*)	15.1	313.0/159.9	315.0/161.9	1.7 (3)	25	−65/−65	−22/−18	−9/−15
Chlorophene (*)	13.2	217.0/35.0	217.0/180.9	1.1 (2)	100	−70/−70	−46/−26	−3/−10
UV-filter								
BZP-1 (*)	10.4	213.1/135.0	213.1/91.0	1.0 (2)	25	−70/−70	−28/−36	−11/−5
BZP-2 (*)	8.3	245.1/135.0	245.1/109.0	1.4 (3)	25	−50/−50	−22/−28	−9/−7
BZP-3	15.7	229.1/151.1	229.1/105.1	1.5 (2)	25	66/66	27/27	10/8
BZP-4 (*)	9.3	307.0/211.0	307.0/227.0	0.5 (4)	100	−90/−90	−46/−32	−15/−19
PBSA	7.0	275.0/194.0	275.0/166.0	2.8 (7)	25	100/100	43/67	14/12
Benzothiazoles								
Benzothiazole	10.0	136.0/109.0	136.0/64.8	2.3 (6)	100	61/61	33/49	8/6
MTBT	13.5	182.0/167.0	182.0/109.0	5.7 (4)	100	70/70	29/48	11/6
BTSA	7.2	216.0/134.0	216.0/90.1	2.9 (4)	25	66/66	33/53	10/8
OHBT (*)	8.1	150.0/42.0	150.0/121.8	3.1 (8)	100	−70/−70	−50/−26	−5/−1
Morpholinyl-BT	11.3	221.1/177.1	221.1/109.0	1.2 (2)	25	66/66	33/51	16/8
Surrogates								
Diuron-d_6	12.0	239.1/78.2	239.1/160.0	17.7 (4)	25	70/70	42/39	12/14
Diuron-d_6 (*)	10.2	236.9/185.9	239.1/188.1	2.0 (4)	25	−70/−70	−25/−25	−9/−9
Isoproturon-d_6	11.8	213.2/78.1	213.2/171.2	3.9 (7)	25	65/65	30/22	12/14
Mecoprop-d_3 (*)	10.6	216.1/71.0	216.1/143.9	26.6 (3)	25	−50/−50	−16/−20	−1/−11
Propiconazole-d_5	16.0	347.2/158.9	349.2/161.1	1.6 (2)	25	80/83	34/51	10/13
Tebuconazole-d_6	15.0	314.2/72.1	316.2/72.1	3.0 (3)	25	84/71	59/46	10/4
Imazalil-d_5	11.2	302.1/159.1	302.1/203.1	2.1 (3)	25	70/70	28/27	11/12
Ketoconazole-d_8	12.2	539.1/497.1	539.1/244.1	2.0 (5)	25	75/75	43/49	12/12
Carbendazim-d_4	7.3	196.2/164.2	196.2/136.2	5.7 (12)	25	70/70	26/42	12/11
Thiabendazole-d_6	7.7	208.2/136.2	208.2/181.2	1.3 (3)	25	90/90	47/37	8/16
Terbuthylazine-d_5	13.3	235.2/179.1	235.2/101.0	6.3 (5)	25	61/61	25/39	14/6
Terbutryn-d_5	13.1	247.1/191.1	247.1/91.1	4.0 (10)	25	50/50	24/41	12/5
Triclosan-$^{13}C_{12}$ (*)	15.1	298.9.0/35.1	301.0/37.1	3.5 (4)	100	−45/−60	−26/−31	−3/−1
Triclocarban-$^{13}C_6$ (*)	15.1	318.9/159.9	321.0/161.8	1.2 (3)	25	−60/−65	−20/−21	−9/−13

[a] Precursor ion/product ion.
[b] DP: declustering potential, CE: collision energy, CXP: collision exit potential.

ME values less than 100% indicate signal suppression, while values above 100% indicate signal enhancement due to the influence of matrix in the sample extracts in contrast to the non-enriched external standard prepared in 0.1% formic acid.

3. Results and discussion

3.1. Method development

3.1.1. Aqueous matrices

Highest absolute recoveries and reproducibility were achieved using the HLB material (cp. Table A1, Supplementary data) and was therefore chosen for further optimization of the SPE conditions: The effect of pH on the extraction efficiency was tested in a range of pH 5–8 (cp. Fig. 1(a)). Due to diversity of target analytes with a broad range of pK_a values, no optimal pH value for all analytes could be found. For most acidic analytes such as OHBT (pK_a 6.2) and benzophenone-1 (pK_a 8.0) the recoveries increased by up to 20% when increasing the pH from 5 to 8, whereas for some basic analytes, such as the morpholines fenpropimorph and tridemorph and for some neutral analytes such as the isothiazolones OIT and DCOIT, the recoveries decreased by a maximum of up to 30%. However, for most analytes no effect was observed or was statistically insignificant. Nevertheless, to ensure reproducible recoveries,

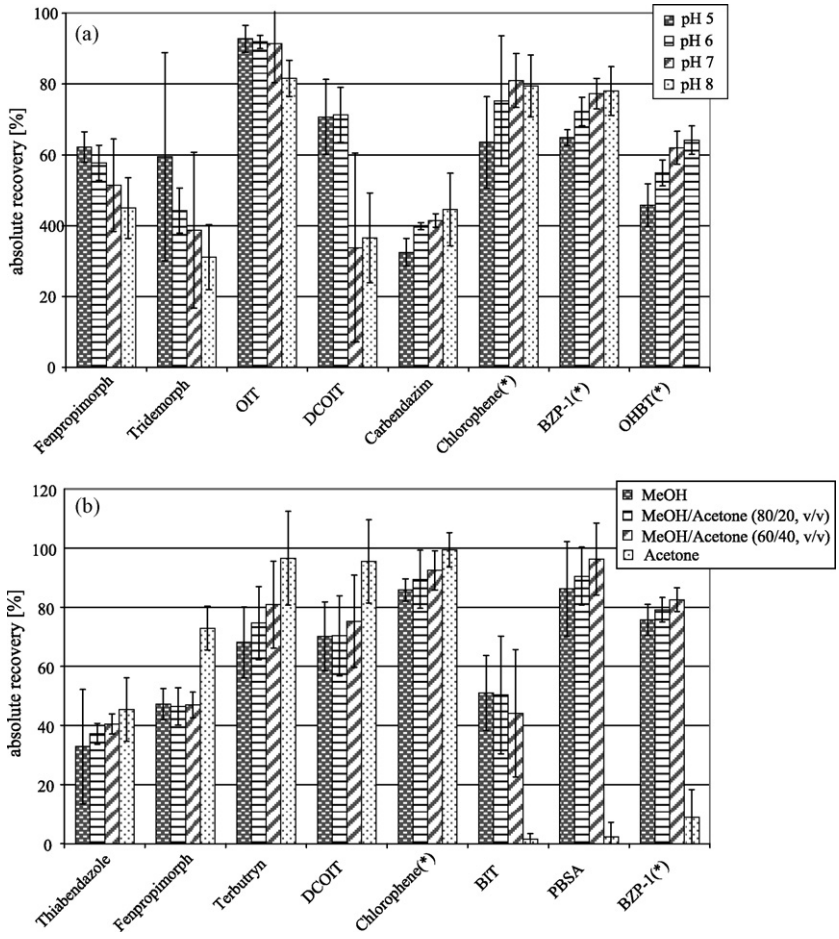

Fig. 1. Optimization of SPE conditions: influence of matrix pH (a) and the elution solvent (b) on the absolute recoveries of selected target analytes. The error bars indicate the 95% confidence intervals ($n = 4$). (*) Analytes determined in negative ionization mode.

the pH was adjusted to 6 as a compromise for the selected analytes.

Most analytes could be eluted efficiently with methanol (cp. Fig. 1(b)). Elution with acetone led to a slight increase of the elution efficiency for less polar compounds such as fenpropimorph and DCOIT. In contrast, the recovery of other analytes such as BIT and BZP-1 strongly decreased down to less than 10% when pure acetone was used. Thus, a mixture of methanol and acetone (60/40, v/v) was chosen, since it did not show the negative effect on the recovery of BIT and benzophenone-1 but at least slightly increased the recovery of compounds such as DCOIT and terbutryn.

3.1.2. Activated sludge

The PLE of the activated sludge was conducted using a mixture of methanol and water (50/50, v/v), 100% methanol and 100% acetone as extraction solvents.

With 100% acetone as extraction solvent certain analytes such as BIT and mecoprop could not be recovered at all. Highest recoveries were achieved with the mixture of methanol and water, which was therefore chosen for further analyses and method validation (cp. Fig. 2(a)). No significant increase of recoveries were obtained with an increase in PLE temperature from 80 to 120 °C (cp. Fig. 2(b)). Slightly higher recoveries were measured for example for carbendazim and BIT but could also be achieved by increasing the number of extraction cycles from 3 to 4. Since for some compounds such as diuron and propiconazole the recoveries even slightly decreased with higher temperatures, further extraction with PLE were conducted using 4 extraction cycles and an extraction temperature of 80 °C. Information regarding the results of the PLE tests for the examined target analytes not included in Fig. 2(a) and (b) are shown in Table A2 (Supplementary data).

The analytes DCOIT and IPBC were recovered from activated sludge with efficiencies of <5%. These analytes could not be quantitatively extracted from activated sludge with the tested methods and were therefore excluded from further analyses.

3.2. Method validation

3.2.1. Aqueous matrices

In general, selected biocides, benzothiazoles and UV-filters can be analyzed with an acceptable accuracy in groundwater, surface water and raw and treated wastewater with ESI as well as APCI (cp. Table 3). The relative recoveries were in an acceptable range of 70–130% and the 95% confidence intervals were less than 25% for most target analytes.

ESI: Absolute recoveries were mainly below 70% in surface water and raw and treated wastewater and revealed significant ion suppression by natural matrix components in negative and positive ionization mode. For instance, absolute recoveries of carbendazim in Rhine water, treated wastewater and raw wastewater were as low as 18%, 8% and 10%, respectively. The strong ion suppression in surface water can be explained the larger extraction volume of 1 L instead of 100 mL for raw wastewater. Nevertheless, using appropriate surrogate standards to compensate for the signal reduction by ion suppression, the relative recoveries of the target analytes were mainly between 69% (DCOIT in treated wastewater) and 130% (BZP-3 in surface water) for all selected matrices.

Table 3
Recoveries of biocides, UV-filters and benzothiazoles measured with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in different aqueous matrices with 95% confidence intervals ($n = 4$). Groundwater and Rhine water was spiked with 0.1 μg L^{-1}, whereas raw and treated wastewater was spiked with 1 and 0.5 μg L^{-1}, respectively. (*) Analytes determined in negative ionization mode. ND: not determined.

	ESI								APCI							
	Groundwater		Rhine water		WWTP effluent		WWTP influent		Groundwater		Rhine water		WWTP effluent		WWTP influent	
Recovery [%]	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery
Biocides																
Diuron[a/a]	91 ± 4	105 ± 2	43 ± 3	104 ± 5	26 ± 1	100 ± 3	28 ± 1	99 ± 4	100 ± 9	95 ± 19	113 ± 9	106 ± 20	112 ± 5	89 ± 4	111 ± 7	96 ± 9
Isoproturon[b/b]	94 ± 2	105 ± 4	45 ± 4	101 ± 2	27 ± 1	96 ± 2	29 ± 1	96 ± 2	107 ± 4	103 ± 6	122 ± 5	98 ± 5	124 ± 5	97 ± 11	118 ± 9	95 ± 5
Mecoprop (*)[k/k]	94 ± 1	106 ± 4	51 ± 3	106 ± 6	32 ± 1	110 ± 17	51 ± 2	106 ± 7	103 ± 2	100 ± 8	122 ± 4	101 ± 15	116 ± 6	93 ± 10	110 ± 10	93 ± 10
Propiconazole[c/c]	105 ± 4	98 ± 1	96 ± 9	96 ± 3	61 ± 2	91 ± 5	49 ± 2	93 ± 7	103 ± 7	101 ± 6	120 ± 7	100 ± 10	121 ± 4	95 ± 4	120 ± 4	96 ± 4
Tebuconazole[d/i]	107 ± 6	104 ± 4	98 ± 9	103 ± 3	63 ± 2	97 ± 3	53 ± 2	91 ± 4	103 ± 7	96 ± 13	116 ± 4	95 ± 8	115 ± 7	100 ± 5	110 ± 10	100 ± 9
Imazalil[e/e]	81 ± 2	101 ± 3	40 ± 3	101 ± 2	22 ± 1	92 ± 4	22 ± 1	89 ± 3	96 ± 4	103 ± 3	132 ± 7	102 ± 3	139 ± 11	94 ± 7	104 ± 6	90 ± 7
Climbazole[j/ND]	86 ± 6	91 ± 5	69 ± 10	93 ± 16	61 ± 6	95 ± 4	57 ± 19	95 ± 16	ND	ND	ND	ND	ND	ND	ND	ND
Ketoconazole[f/ND]	71 ± 10	103 ± 11	57 ± 7	115 ± 24	55 ± 4	97 ± 8	48 ± 8	93 ± 10	ND	ND	ND	ND	ND	ND	ND	ND
Carbendazim[g/ND]	64 ± 2	107 ± 8	18 ± 1	116 ± 4	8 ± 1	117 ± 10	10 ± 1	122 ± 5	ND	ND	ND	ND	ND	ND	ND	ND
Thiabendazole[h/h]	66 ± 6	100 ± 2	21 ± 1	101 ± 4	10 ± 1	95 ± 7	16 ± 1	90 ± 7	97 ± 10	107 ± 10	95 ± 6	92 ± 9	86 ± 7	88 ± 8	95 ± 9	95 ± 13
Terbuthylazine[i/i]	99 ± 4	108 ± 2	64 ± 4	94 ± 2	39 ± 1	86 ± 1	34 ± 1	89 ± 3	102 ± 7	103 ± 10	117 ± 10	105 ± 8	109 ± 10	103 ± 8	99 ± 3	98 ± 5
Terbutryn[e/b]	95 ± 5	91 ± 6	51 ± 7	100 ± 17	29 ± 5	91 ± 17	23 ± 2	71 ± 7	100 ± 5	96 ± 5	140 ± 5	113 ± 7	142 ± 5	111 ± 14	120 ± 8	96 ± 10
Irgarol[e/b]	99 ± 3	98 ± 6	54 ± 4	108 ± 7	31 ± 2	101 ± 10	26 ± 1	82 ± 4	100 ± 5	92 ± 3	130 ± 4	101 ± 6	139 ± 6	104 ± 8	128 ± 16	98 ± 16
M1[e/b]	73 ± 1	101 ± 6	30 ± 3	84 ± 3	19 ± 1	85 ± 3	24 ± 1	108 ± 1	105 ± 6	101 ± 3	134 ± 3	107 ± 5	143 ± 7	111 ± 12	128 ± 6	102 ± 8
Dimethomorph[i/b]	108 ± 9	92 ± 5	96 ± 11	109 ± 12	57 ± 4	98 ± 7	62 ± 3	124 ± 6	107 ± 4	92 ± 4	147 ± 5	108 ± 5	149 ± 3	106 ± 10	132 ± 5	96 ± 3
Fenpropimorph[b/n]	78 ± 3	106 ± 3	44 ± 4	118 ± 4	23 ± 3	98 ± 9	23 ± 2	94 ± 10	93 ± 6	96 ± 6	110 ± 14	114 ± 15	90 ± 6	93 ± 6	82 ± 8	85 ± 9
Tridemorph[e/d]	35 ± 10	104 ± 28	21 ± 9	125 ± 39	11 ± 2	104 ± 21	9 ± 2	86 ± 16	39 ± 10	100 ± 20	43 ± 15	119 ± 70	38 ± 9	97 ± 40	61 ± 12	128 ± 27
BIT[e/n]	67 ± 8	99 ± 10	30 ± 3	90 ± 8	15 ± 1	73 ± 7	23 ± 1	110 ± 6	89 ± 17	103 ± 19	92 ± 13	107 ± 14	79 ± 15	92 ± 17	80 ± 14	93 ± 16
OIT[b/n]	93 ± 3	104 ± 3	66 ± 5	129 ± 5	35 ± 1	103 ± 3	26 ± 1	79 ± 1	105 ± 4	102 ± 4	109 ± 11	105 ± 11	95 ± 6	91 ± 5	103 ± 5	99 ± 5
DCOIT[e/n]	68 ± 21	83 ± 23	84 ± 11	198 ± 32	17 ± 3	69 ± 13	20 ± 4	78 ± 19	332 ± 174	70 ± 42	478 ± 24	107 ± 6	149 ± 25	28 ± 4	280 ± 96	58 ± 22
DMST[a/n]	89 ± 6	101 ± 6	39 ± 3	94 ± 5	24 ± 1	90 ± 4	27 ± 3	92 ± 9	88 ± 14	112 ± 11	77 ± 22	95 ± 18	73 ± 14	85 ± 7	81 ± 10	93 ± 15
DMSA (*)[k/n]	62 ± 1	100 ± 3	25 ± 1	76 ± 2	19 ± 2	93 ± 20	28 ± 1	83 ± 5	91 ± 16	97 ± 18	93 ± 16	99 ± 16	85 ± 7	90 ± 8	85 ± 14	90 ± 15
IPBC[j/ND]	93 ± 1	95 ± 3	63 ± 4	97 ± 4	40 ± 2	93 ± 4	40 ± 1	101 ± 1	ND	ND	ND	ND	ND	ND	ND	ND
Triclosan[l/l]	75 ± 31	102 ± 3	75 ± 8	102 ± 2	37 ± 2	98 ± 7	47 ± 10	95 ± 3	210 ± 127	107 ± 6	304 ± 45	106 ± 7	213 ± 38	99 ± 16	263 ± 31	93 ± 7
Triclocarban[m/m]	60 ± 33	98 ± 6	47 ± 10	105 ± 5	23 ± 2	100 ± 5	29 ± 3	102 ± 6	113 ± 20	113 ± 20	183 ± 54	111 ± 5	177 ± 44	107 ± 7	248 ± 38	110 ± 5
Chlorophene[a/n]	84 ± 20	104 ± 24	33 ± 2	120 ± 14	13 ± 2	90 ± 16	15 ± 4	108 ± 35	107 ± 24	110 ± 25	125 ± 15	128 ± 15	112 ± 14	112 ± 14	98 ± 14	99 ± 14
UV-filters																
BZP-1 (*)[a/a]	69 ± 5	114 ± 7	21 ± 1	98 ± 11	11 ± 1	93 ± 8	19 ± 1	180 ± 14	105 ± 4	99 ± 15	119 ± 10	102 ± 19	107 ± 4	83 ± 7	106 ± 9	93 ± 5
BZP-2 (*)[m/k]	23 ± 4	142 ± 71	6 ± 1	53 ± 7	6 ± 1	93 ± 15	9 ± 1	111 ± 6	163 ± 15	101 ± 16	257 ± 21	135 ± 25	230 ± 18	117 ± 12	198 ± 23	107 ± 18
BZP-3[j/n]	105 ± 20	95 ± 21	93 ± 25	130 ± 31	46 ± 6	102 ± 17	42 ± 5	100 ± 12	263 ± 134	84 ± 43	257 ± 62	82 ± 20	196 ± 48	62 ± 17	183 ± 86	59 ± 28
BZP-4 (*)[n/ND]	92 ± 3	107 ± 3	69 ± 6	81 ± 7	89 ± 9	105 ± 11	89 ± 3	105 ± 3	ND	ND	ND	ND	ND	ND	ND	ND
PBSA[a/–]	106 ± 3	101 ± 3	57 ± 1	100 ± 10	26 ± 11	66 ± 32	34 ± 6	96 ± 19	180 ± 60	ND	>1000	ND	>1000	ND	>1000	ND
Benzothiazoles																
Benzothiazole[d/n]	98 ± 8	95 ± 12	89 ± 6	97 ± 6	68 ± 7	109 ± 13	67 ± 12	124 ± 21	165 ± 34	102 ± 21	155 ± 41	96 ± 27	115 ± 75	72 ± 47	109 ± 38	70 ± 25
MTBT[n/n]	88 ± 11	91 ± 12	93 ± 8	98 ± 8	80 ± 10	86 ± 11	83 ± 9	87 ± 10	281 ± 31	104 ± 12	295 ± 59	110 ± 22	254 ± 109	96 ± 41	253 ± 56	94 ± 21
BTSA[a/ND]	108 ± 4	101 ± 3	35 ± 9	62 ± 9	24 ± 22	42 ± 57	46 ± 2	98 ± 12	ND	ND	ND	ND	ND	ND	ND	ND
OHBT (*)[k/n]	34 ± 2	99 ± 6	13 ± 1	70 ± 5	13 ± 3	102 ± 42	16 ± 1	84 ± 12	96 ± 15	115 ± 17	88 ± 5	105 ± 6	81 ± 33	93 ± 38	71 ± 33	83 ± 37
Morpholinyl-BT[e/n]	73 ± 2	94 ± 4	32 ± 1	84 ± 5	21 ± 1	89 ± 6	22 ± 1	94 ± 5	105 ± 3	106 ± 3	103 ± 5	104 ± 5	98 ± 7	99 ± 7	95 ± 3	97 ± 3

Indices (a–n) indicate the surrogate standards used for calculation of the analyte concentration by internal standard calibration for the measurement with ESI (first index) and APCI (second index). [a]Diuron-d_6, [b]isoproturon-d_6, [c]propiconazole-d_5, [d]tebuconazole-d_6, [e]imazalil-d_5, [f]ketoconazole-d_8, [g]carbendazim-d_4, [h]thiabendazole-d_6, [i]terbuthylazine-d_5, [j]terbutryn-d_5, [k]mecoprop-d_3, [l]triclosan-$^{13}C_{12}$, [m]triclocarban-$^{13}C_6$, [n]no surrogate.

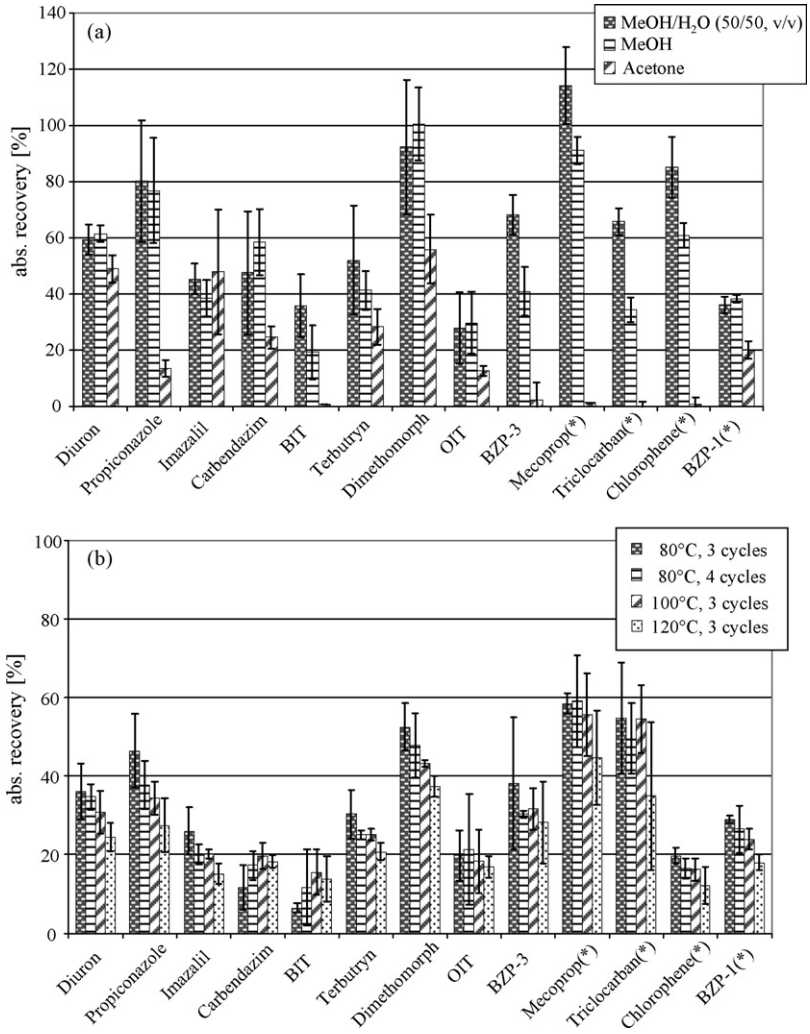

Fig. 2. Optimization of the PLE: influence of different extraction solvents (a) and extraction temperatures (b) on the absolute recoveries of selected target analytes. The error bars represent the 95% confidence intervals ($n = 4$). (*) Analytes determined in negative ionization mode.

Lower relative recoveries and high confidence intervals of $66 \pm 32\%$ and $42 \pm 57\%$ determined in treated wastewater for PBSA and BTSA, respectively, can be attributed to the high original background concentration in comparison to the spiked analyte concentration. Using a higher spike concentration of $4\ \mu g\ L^{-1}$ yielded acceptable relative recoveries of $80 \pm 13\%$ (PBSA) and $78 \pm 12\%$ (BTSA) (cp. Table A3, Supplementary data).

However, for DCOIT, BZP-1 and BZP-2 the standard addition method had to be used for quantification in some matrices, since the matrix effects could not be sufficiently compensated in every tested matrix by stable isotope-labeled surrogate standards. Whereas the use of imazalil-d_5 for DCOIT and diuron-d_6 for BZP-1 led to significantly elevated relative recoveries of $198 \pm 32\%$ in surface water and $180 \pm 14\%$ in raw wastewater, respectively, the relative recovery of BZP-2 in surface water was too low ($53 \pm 7\%$) using triclocarban-$^{13}C_6$ for compensation.

APCI: Using APCI, three analytes (carbendazim, IPBC and BTSA) could not be quantified due to insufficient ionization efficiency. In all selected matrices the absolute recoveries were higher than 70% for most analytes measured with APCI (cp. Table 3). Thus, in most cases the matrix did not suppress the ionization using APCI in contrast to ESI. However, for certain analytes measured in the positive ionization mode such as for BZP-3 and MTBT, significantly elevated absolute recoveries above 100% were determined. The UV-filter PBSA could not be quantified, since the absolute recoveries were even higher than 1000% in wastewater and Rhine water. Similarly, absolute recoveries up to 300% were obtained with negative ionization for triclosan, triclocarban and BZP-2. These elevated recoveries for some of the analytes indicated that the sample matrix can lead to a significant ion enhancement using the APCI interface. However, in most cases the use of appropriate surrogate standards and the internal standard calibration led to acceptable relative recoveries in the range of 70% (DCOIT in surface water) to 135% (BZP-2 in surface water).

Only for DCOIT and BZP-3 no appropriate surrogate standards could be assigned to compensate for the ion enhancement in raw and treated wastewater when using APCI, and thus standard addition had to be applied. Since for DCOIT the absolute recoveries were even higher in groundwater, the internal calibration without use of surrogate standards led to low relative recoveries of $58 \pm 22\%$ and $28 \pm 4\%$ for DCOIT in raw and treated wastewater, respectively. BZP-3 was detected with relative recoveries of $62 \pm 17\%$ and $59 \pm 28\%$ in raw and treated wastewater, respectively.

For both ionization interfaces, the method validation already indicated that the matrix significantly affected the absolute recoveries of most of the selected analytes. Since the matrix content and composition is variable, it is crucial to determine the individual relative recoveries in complex matrices at least for all those analytes for which labeled surrogate standards are not available. In those cases where no surrogate can sufficiently compensate

Table 4
Recoveries of biocides, UV-filters and benzothiazoles measured with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in activated sludge with 95% confidence intervals ($n = 4$). The activated sludge was spiked with 0.5 μg g TSS^{-1} and extracted using PLE with MeOH/H2O (50/50, v/v). (*) Analytes determined in negative ionization mode. ND: not determined.

	ESI		APCI	
Recovery [%]	Absolute recovery	Relative recovery	Absolute recovery	Relative recovery
Biocides				
Diuron[a/a]	28 ± 2	88 ± 9	115 ± 21	99 ± 13
Isoproturon[b/b]	34 ± 3	111 ± 11	134 ± 23	114 ± 12
Mecoprop (*)[k/k]	114 ± 14	112 ± 13	118 ± 26	113 ± 4
Propiconazole[c/c]	84 ± 4	105 ± 12	150 ± 17	127 ± 11
Tebuconazole[d/d]	84 ± 6	115 ± 14	143 ± 9	119 ± 26
Imazalil[e/e]	27 ± 3	106 ± 14	127 ± 39	95 ± 21
Climbazole[e/c]	36 ± 8	136 ± 28	259 ± 176	138 ± 25
Ketoconazole[e/b]	28 ± 6	119 ± 11	101 ± 54	97 ± 48
Carbendazim[g/ND]	18 ± 2	114 ± 23	ND	ND
Thiabendazole[h/h]	9 ± 1	118 ± 19	90 ± 8	88 ± 25
Terbuthylazine[i/i]	48 ± 3	107 ± 12	150 ± 37	114 ± 25
Terbutryn[j/j]	29 ± 7	104 ± 31	142 ± 62	100 ± 43
Irgarol[j/j]	25 ± 1	101 ± 8	150 ± 14	116 ± 20
M1[j/j]	17 ± 3	84 ± 16	169 ± 20	115 ± 9
Dimethomorph[n/n]	99 ± 5	90 ± 5	195 ± 21	119 ± 13
Fenpropimorph[e/i]	23 ± 2	101 ± 8	114 ± 12	86 ± 6
Tridemorph[e/d]	13 ± 1	87 ± 5	64 ± 16	98 ± 22
BIT[g/b]	17 ± 3	96 ± 28	62 ± 24	92 ± 20
OIT[h/b]	16 ± 3	110 ± 33	41 ± 15	43 ± 14
DCOIT[e/n]	<2	ND	<2	ND
DMST[d/n]	49 ± 4	84 ± 10	100 ± 11	103 ± 12
DMSA (*)[n/a]	83 ± 9	91 ± 10	117 ± 37	107 ± 28
IPBC[j/ND]	<2	ND	ND	ND
Triclosan (*)[l/l]	18 ± 35	101 ± 57	36 ± 111	116 ± 11
Triclocarban (*)[m/m]	66 ± 5	108 ± 11	53 ± 10	107 ± 11
Chlorophene (*)[l/l]	85 ± 11	96 ± 18	75 ± 36	112 ± 28
UV-filters				
BZP-1 (*)[m/k]	36 ± 3	74 ± 9	109 ± 27	105 ± 7
BZP-2 (*)[m/n]	18 ± 1	99 ± 11	236 ± 70	128 ± 36
BZP-3[i/i]	49 ± 3	104 ± 14	95 ± 18	86 ± 10
BZP-4 (*)[l/ND]	93 ± 15	114 ± 28	ND	ND
PBSA[i/ND]	67 ± 8	118 ± 19	ND	ND
Benzothiazoles				
Benzothiazole[e/b]	68 ± 16	89 ± 29	60 ± 50	67 ± 49
MTBT[e/b]	80 ± 11	90 ± 5	84 ± 22	90 ± 20
BTSA[h/ND]	17 ± 7	99 ± 25	ND	ND
OHBT (*)[l/k]	53 ± 6	105 ± 24	94 ± 23	104 ± 15
Morpholinyl-BT[i/b]	46 ± 6	92 ± 16	113 ± 16	102 ± 12

Indices (a–n) indicate the surrogate standards used for calculation of the analyte concentration by internal standard calibration for the measurement with ESI (first index) and APCI (second index). [a]Diuron-d_6, [b]isoproturon-d_6, [c]propiconazole-d_5, [d]tebuconazole-d_6, [e]imazalil-d_5, [f]ketoconazole-d_8, [g]carbendazim-d_4, [h]thiabendazole-d_6, [i]terbuthylazine-d_5, [j]terbutryn-d_5, [k]mecoprop-d_3, [l]triclosan-$^{13}C_{12}$, [m]triclocarban-$^{13}C_6$, [n]no surrogate.

for the matrix effects, the standard addition method has to be used.

3.2.2. Activated sludge

ESI: Except for DCOIT and IPBC the selected biocides, benzothiazoles and UV-filters could be analyzed with an acceptable accuracy in activated sludge taken from the nitrification tank of a conventional WWTP using ESI in the positive and negative ionization mode (cp. Table 4). Consistent with the results from the aqueous matrices, relatively low absolute recoveries as low as 9% for thiabendazole were determined in activated sludge. Using appropriate surrogate standards to compensate for the underestimation presumably caused mainly by ion suppression, for most analytes relative recoveries between 74% (BZP-1) and 119% (ketoconazole) could be achieved. Only for climbazole a slightly elevated recovery of 136 ± 28% was determined. The precision given by the 95% confidence intervals were mainly less than 25%. The high confidence interval of ± 57% determined for triclosan can be explained by the high background concentration of approximately 2.7 μg g TSS^{-1}. For the higher spiking level of 2 μg g TSS^{-1} an acceptable relative recovery of 102 ± 21% was obtained (cp. Table A4, Supplementary data).

APCI: The use of APCI revealed an ion enhancement for many analytes leading to absolute recoveries significantly higher than 100% (cp. Table 4). Nevertheless, for most compounds the relative recoveries were within the range of 86% (BZP-3 and fenpropimorph) to 128% (BZP-2) due to the use of surrogate standards. A slightly elevated value of 138 ± 25% was only determined for BZP-2, whereas the relative recoveries were too low for BT (67 ± 49%) and OIT (43 ± 14%). Thus, the used surrogate standard isoproturon-d_6 could not sufficiently compensate for the low absolute recoveries of BZP-2 and BT. The relatively high 95%-confidence intervals revealed a lower precision of the APCI measurement in comparison to ESI.

3.3. Matrix effects (ME)

3.3.1. Aqueous matrices

In order to reduce matrix effects, different enrichment volumes of 1, 0.2 and 0.1 L were chosen for groundwater and surface water, treated wastewater and raw wastewater, respectively. Nevertheless, for most of the selected compounds ion suppression was still significantly higher in raw and treated wastewater in comparison to water from the river Rhine. In this study, the influence of matrix

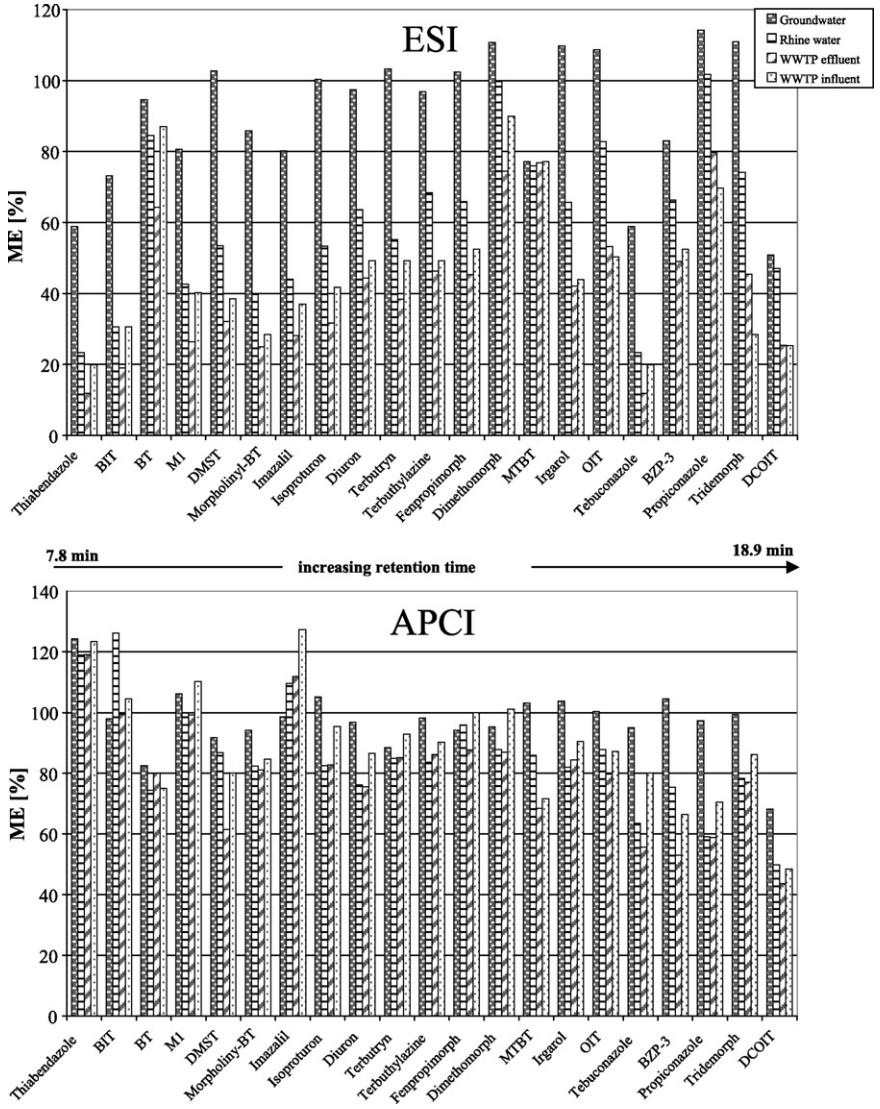

Fig. 3. Matrix effects (ME) determined for biocides, UV-filters and benzothiazoles spiked into extracts of groundwater, Rhine water and raw and treated wastewater at a concentration of 200 ng mL^{-1} using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in positive ionization mode. The analytes are ordered from left to right according to their increasing chromatographic retention time. Results for the analytes PBSA, BTSA, carbendazim and IPBC are not shown since they could not be analyzed using APCI.

effects on the absolute recovery was evaluated independently from any other factor by spiking the analytes into final extracts of groundwater, Rhine water and raw and treated wastewater. The ME determined in the different aqueous matrices are shown for all analytes which could be analyzed with ESI and APCI in Fig. 3 (positive ionization mode) and Fig. 4 (negative ionization mode). It can be seen that for most analytes the ME values determined with ESI for groundwater were not significantly different from 100%. However, for a few compounds such as thiabendazole, DCOIT and BZP-2 relatively low ME values of less than 60% indicate that even groundwater with a very low DOC of ~0.6 mg L^{-1} cannot a priori be regarded as being free of any interference from matrix components. As expected in regard to lower absolute recoveries in aqueous matrices with higher matrix loads, ME values were significantly decreased in Rhine water and even more in raw and treated wastewater. ME values of less than 40% were determined for 10 out of 21 analytes measured in the positive ionization mode and for all analytes measured in the negative ionization mode in at least one of the wastewater samples. Strongest ion suppression with ME values of 10 to 15% were observed for thiabendazole, tebuconazole and BZP-2. Consistent with these results, strong matrix effects of down to 15% were also observed by Marín et al. [37] in diluted leachates for carbendazim, thiabendazole, imazalil, diuron, isoproturon, terbuthylazine and terbutryn using UPLC–ESI/MS/MS.

In Fig. 3 the ME values of the target analytes are shown in order of their retention time. In general, for analytes with a retention time below 12 min ME values were lower than for compounds eluting afterwards. Thus, a correlation between ME and retention time can be assumed. Consistent with these results, Dijkman et al. [34] showed that the salinity caused strong ion suppression of early eluting acidic pesticides measured with ESI, while the DOC hardly effected their absolute recoveries. However, since the correlation of ME and retention time cannot explain the relatively high ME values for benzothiazole and the low values for DCOIT, the influence of matrix components cannot be exclusively related to the retention time of the target analytes.

Using APCI, all ME values were above 40% and for 15 from 21 analytes values between 74% (benzothiazole) and 127% (imazalil) were determined (cp. Fig. 3). This confirmed that APCI is quite less sensitive to matrix effects than ESI for the target compounds measured in the positive ionization mode. These results consist with other studies reporting APCI being the favourable ionization source

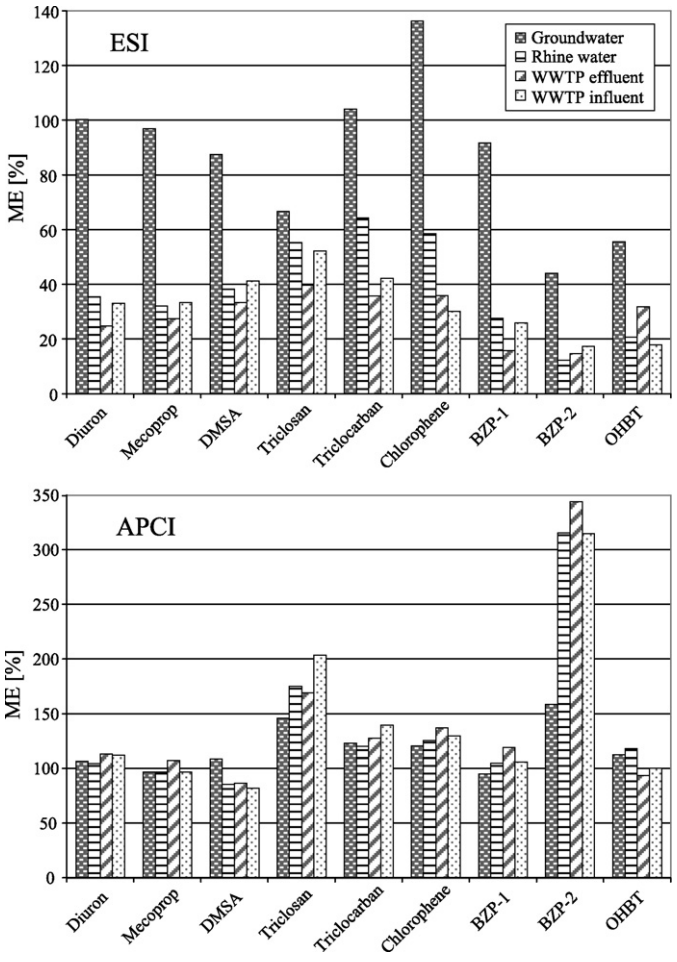

Fig. 4. Matrix effects (ME) determined for biocides, UV-filters and benzothiazoles spiked into extracts of groundwater, Rhine water and raw and treated wastewater at a concentration of 200 ng mL^{-1} using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the negative ionization mode.

regarding the reduction of ion suppression [31–33]. However, for triclosan and BZP-2 measured in the negative ionization mode (cp. Fig. 4), highly elevated ME values of 204% and 344% were found, respectively. These results illustrate that significant ion enhancement can occur with APCI for certain analytes, even in groundwater. Zhao and Metcalfe [38] reported an ion enhancement measuring neutral pharmaceuticals in wastewater using APCI. This differential behaviour of the ionization interfaces under identical conditions was also reported by Liang et al. [39], who examined the influence of stable isotope-labeled surrogate standards on the response of the target analytes and vice versa.

3.3.2. Activated sludge

In Fig. 5, ME values for activated sludge given as the absolute recoveries in post-extraction spikes are compared with the absolute recoveries determined in samples spiked prior to PLE (pre-extraction spikes) to assess both matrix effects and the extraction efficiency of the PLE. Consistent with the results for wastewater, ME values down to 20% (thiabendazole) showed that the measurement of sludge extracts with ESI was also strongly influenced by ion suppression. However, in extracts of activated sludge ME values below 40% were only determined for a lower number of 6 analytes indicating a slightly lower ion suppression compared to the tested wastewater matrices. In contrast, when using APCI as ionization interface ion enhancement was even more pronounced for activated sludge than for wastewater. ME values above 150% were observed for 17 analytes with a maximum ME value of 200% (climbazole). Higher 95% confidence intervals of the post- and pre-extraction spikes indicated a lower precision of the APCI measurement for the sludge extracts.

The comparison of the ME values (absolute recovery in post-extraction spikes) with the absolute recoveries in the pre-extraction spikes revealed that for most analytes absolute recoveries can be attributed mainly to matrix effects. However, for thiabendazole, fenpropimorph, tridemorph, BIT and OIT significantly higher absolute recoveries determined in the post-extraction spikes in comparison to the pre-extraction spikes with both ionization interfaces indicated losses during sample preparation, presumably by an incomplete sludge extraction.

3.4. ESI versus APCI

The ratio of the signal intensity ($R_I = I_{APCI}/I_{ESI}$) measured in an external standard was compared with the ME ratio ($R_{ME} = ME_{APCI}/ME_{ESI}$) measured in an influent sample (cp. Table 5) to decide which ionization source should be preferred for the target analytes.

If the product of these ratios ($R_I \times R_{ME}$) is significantly higher than 1, APCI leads to higher signal intensities in the samples and consequently to lower LOQs as long as the background noise is not increasing. Table 5 shows that BT, MTBT and OHBT were measured with approximately 20 times higher signal intensities in the tested influent samples using APCI due to a higher sensitivity and with respect to OHBT also due to avoided matrix effects. For the degradation product DMSA 3 times lower ion suppression and 13 times higher sensitivity led to a 26-fold signal increase. For all

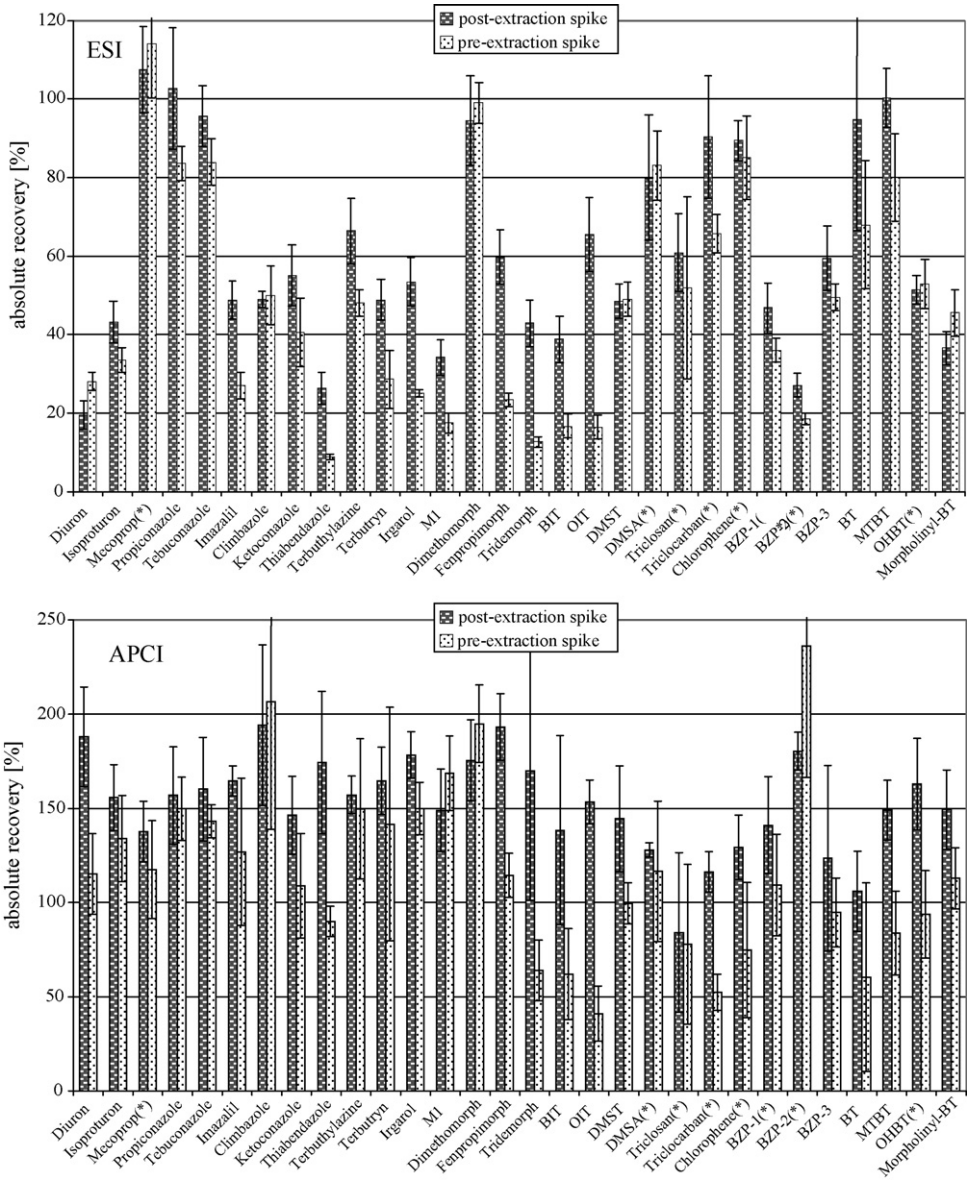

Fig. 5. Absolute recoveries determined for biocides, UV-filters and benzothiazoles spiked into freeze-dried secondary sludge before PLE (pre-extraction spikes) and prior to LC–MS/MS analysis (post-extraction spikes) using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the positive and negative ionization mode. The absolute recoveries determined in the pre-extraction spikes refer to activated sludge spiked with 2 $\mu g\,g\,TSS^{-1}$ for triclosan, climbazole and ketoconazole (due to their high background concentrations) and to activated sludge spiked with 0.5 $\mu g\,g\,TSS^{-1}$ for all other target compounds. The absolute recoveries determined in the post-extraction spikes refer to sample extracts spiked at a concentration of 200 $ng\,mL^{-1}$ for all analytes. The error bars represent the 95% confidence intervals ($n = 4$). (*) Analytes determined in negative ionization mode.

the other analytes the sensitivities of the APCI measurement were similar or significantly lower compared to ESI and only for thiabendazole, BIT, morpholinyl-BT, mecoprop and BZP-2 the positive effect of lower ion suppression slightly overweighed the negative effect of a decreased sensitivity. However, for many analytes such as thiabendazole and BIT the background noise was significantly higher in the APCI chromatograms and thus higher analyte signals did not result in lower LOQs. This is particularly obvious for thiabendazole in Fig. 6. Looking at the peak heights of one transition of thiabendazole, the chromatograms of a standard solution show 5 times higher signals using ESI in comparison to APCI. The comparable peak heights in the chromatograms of the raw wastewater extracts show that the lower sensitivity was compensated by an approximately 5 times lower matrix effect using APCI. But the chromatograms of a non-spike sample extract show that the S/N ratio was approximately 4 times lower with APCI due to a higher background noise. Accordingly, the comparison of the S/N ratios calculated for all analytes and matrices using either the background concentrations in non-spiked sample extracts or different spiked amounts (Table A5, Supplementary data) revealed similar or lower S/N ratios using APCI except for benzothiazole and MTBT. For these analytes the LOQs were assessed from the S/N ratios to be 4 times lower for influent and sludge samples using APCI, whereas for all other analytes up to 10 times higher LOQs were determined (cp. Table 5).

Higher values of the product of ME ratio and sensitivity ratio were observed for many analytes when measuring activated sludge samples with APCI due to a strong ion enhancement. However, in comparison to the influent samples no further product values higher than 1 were determined except for diuron and BZP-3. Moreover, the stable isotope-labeled surrogates could not completely compensate for ion enhancement by the APCI interface and the precision was lower compared to the ESI measurement (cp. Table 4 and Fig. 5).

Table 5
Comparison of the ratio of matrix effects (R_{ME}) determined in an extract of WWTP influent and activated sludge using APCI (ME_{APCI}) and ESI (ME_{ESI}) with the ratio of the signal intensities (R_I) determined in an external standard using APCI (I_{APCI}) and ESI (I_{ESI}) together with respective LOQ values. (*) Analytes determined in negative ionization mode. ND: not determined.

		WWTP influent						Activated sludge					
	R_I	ME_{ESI} [%]	ME_{APCI} [%]	R_{ME}	$R_{ME} \times R_I$	LOQ_{ESI} [ng L^{-1}]	LOQ_{APCI} [ng L^{-1}]	ME_{ESI} [%]	ME_{APCI} [%]	R_{ME}	$R_{ME} \times R_I$	LOQ_{ESI} [ng L^{-1}]	LOQ_{APCI} [ng L^{-1}]
Biocides													
Diuron	0.31	42	86	2.1	0.64	5	20	19	188	9.9	3.1	2.5	25
Isoproturon	0.15	44	96	2.2	0.33	10	50	43	156	3.6	0.54	5	5
Mecoprop (*)	0.44	33	97	2.9	1.3	20	20	108	138	1.3	0.57	10	10
Propiconazole	0.47	70	71	1.0	0.48	10	20	103	157	1.5	0.71	5	10
Tebuconazole	0.78	83	80	1.0	0.75	5	20	96	160	1.7	1.3	5	5
Imazalil	0.10	37	127	3.4	0.33	20	50	49	165	3.4	0.34	5	50
Climbazole	0.10	ND	ND	ND	ND	10	20	49	194	4.0	0.4	5	10
Ketoconazole	0.12	ND	ND	ND	ND	50	50	55	146	2.7	0.32	25	25
Carbendazim	ND	16	ND	ND	ND	5	ND	32	ND	ND	ND	5	ND
Thiabendazole	0.32	20	123	6.2	2.0	5	10	26	174	6.7	2.1	2.5	5
Terbuthylazine	0.57	49	90	1.8	1.0	5	10	66	157	2.4	1.4	2.5	2.5
Terbutryn	0.48	49	93	1.9	0.90	5	10	49	165	3.4	1.6	2.5	5
Irgarol	0.08	44	91	2.1	0.17	5	20	54	178	3.3	0.26	2.5	10
M1	0.11	34	110	3.2	0.36	5	10	34	149	4.4	0.48	2.5	5
Dimethomorph	0.67	90	101	1.1	0.76	10	10	94	176	1.9	1.3	5	5
Fenpropimorph	0.12	53	100	1.9	0.23	5	10	60	193	3.2	0.38	2.5	5
Tridemorph	0.05	28	86	3.0	0.14	20	100	43	170	4.0	0.2	25	100
BIT	0.89	31	104	3.4	3.0	100	200	39	138	3.5	3.1	50	100
OIT	0.29	50	87	1.8	0.51	10	20	66	153	2.3	0.67	10	25
DCOIT	0.52	25	48	1.9	1.0	10	10	31	160	ND	ND	ND	ND
DMST	0.12	39	80	2.1	0.25	20	200	49	145	3.0	0.36	10	100
DMSA (*)	13	41	82	2.0	26	50	200	80	128	1.6	21	25	50
IPBC	ND	49	ND	ND	ND	50	ND	49	ND	ND	ND	ND	ND
Triclosan (*)	0.13	52	204	3.3	0.44	20	50	61	84	1.4	0.18	10	100
Triclocarban (*)	0.04	42	140	4.3	0.18	5	5	90	116	1.3	0.05	2.5	5
Chlorophene (*)	0.18	30	130	4.3	0.78	10	50	89	129	1.4	0.25	10	50
UV-filters													
BZP-1 (*)	0.19	26	106	4.1	0.77	5	50	47	141	3.0	0.57	2.5	5
BZP-2 (*)	0.08	17	315	18.1	1.5	5	5	27	180	6.7	0.54	2.5	5
BZP-3	1.4	52	66	1.3	1.8	50	50	59	124	2.1	2.9	25	50
BZP-4 (*)	ND	26	ND	ND	ND	10	ND	81	ND	ND	ND	5	ND
PBSA	ND	42	ND	ND	ND	10	ND	85	ND	ND	ND	5	ND
Benzothiazoles													
Benzothiazole	19	87	75	0.86	17	200	50	95	106	1.1	21	100	25
MTBT	18	77	72	0.93	17	50	10	100	149	1.5	27	25	5
BTSA	ND	44	ND	ND	ND	20	ND	65	ND	ND	ND	10	ND
OHBT (*)	3.2	18	100	5.6	18	200	200	51	163	2.8	9.0	100	100
Morpholinyl-BT	0.77	28	85	3.0	2.3	10	20	37	149	4.0	3.1	2.5	10

3.5. Method application

Since the comparison of the interfaces revealed a better performance of ESI with regard to lower LOQs and the susceptibility of APCI to ion enhancement influencing accuracy and precision, ESI was chosen as the preferred ionization source for quantifying the target analytes in the different matrices. A summary of the method validation data is given in Table A6 (Supplementary data).

3.5.1. Occurrence in wastewater and surface water

The most prominent biocides in the influents of both sampled WWTPs were the anti-dandruff climbazole and the bacteriostatics chlorophene and triclosan with maximum influent concentrations of 1350 ± 70, 664 ± 55 and 841 ± 31 ng L^{-1}, respectively (cp. Table 6). Climbazole was also the biocide found at the highest concentrations in both WWTP effluents and in stream 2 (Wickerbach) with maximum concentrations of 443 ± 11 ng L^{-1} (WWTP 2) and 530 ± 70 ng L^{-1} (Wickerbach), respectively. This emphasizes the importance of this biocide as a biological active micropollutant emitted by WWTPs, which was up to now not considered in other studies. The concentrations of the antifouling agent irgarol ranged from 6 to 22 ng L^{-1} in both sampled WWTP effluents and streams and were therefore significantly above the environmental quality value of 2 ng L^{-1} proposed by the German Working Group on water issues of the Federal States and the Federal Government (LAWA) [40].

All selected water-soluble UV-filters were detected in the influents of both WWTPs. Highest concentrations were determined for the sulfonic acids PBSA and BZP-4. In WWTP 2 sampled during summer time, BZP-4 and PBSA were detected in the influent at concentrations as high as 5130 ± 140 and 3890 ± 170 ng L^{-1}, respectively. Maximum effluent concentrations of 572 ± 15 ng L^{-1} (WWTP 1) and 1820 ± 240 ng L^{-1} (WWTP 2) for BZP-4 and PBSA, respectively, show the importance of WWTPs for the emission of these water-soluble UV-filters into the receiving water, even at least BZP-4 seems to be significantly removed by the treatment processes. Similar concentrations for BZP-4 were also reported by Kasprzyk-Hordern et al. [23] and Rodil et al. [5] in wastewater from WWTPs in Wales and Spain, respectively. Consistent with the high effluent concentrations, BZP-4 and PBSA were also the dominant analytes detected in all surface water samples. In the Wickerbach sampled close downstream (~100 m) of a discharging WWTP, stream concentrations were as high as 1980 ± 130 ng L^{-1} (BZP-4) and 3240 ± 140 ng L^{-1} (PBSA).

Consistent with the study by Kloepfer et al. [41], the selected benzothiazoles were detected in the high ng L^{-1} to the low μg L^{-1} range in influents and effluents with BTSA being the most prominent analyte.

Table 6
Concentrations of biocides, UV-filters and benzothiazoles determined in grab samples of activated sludge, wastewater (influent and effluent) and surface water. Sludge samples were taken from WWTP 1 on the 26th November 2008 ($n = 4$), wastewater samples from WWTP 1 on 11th February ($n = 4$) and from WWTP 2 on 2nd July 2009 ($n = 3$). Surface water samples were obtained from the river Rhine on 11th March 2008 ($n = 4$) and from two streams on 1st September 2009 ($n = 3$). Samples were measured with LC–MS/MS using ESI in the positive and negative ionization mode. The range indicates the 95% confidence interval. (*) Analytes measured in negative ionization mode. ND: not determined.

	Sludge [ng g TSS^{-1}]		WWTP influent [ng L^{-1}]			WWTP effluent [ng L^{-1}]			Surface water [ng L^{-1}]			
	LOQ	WWTP 1	LOQ	WWTP 1	WWTP 2	LOQ	WWTP 1	WWTP 2	LOQ	Rhine	Stream 1	Stream 2
Biocides												
Diuron	2.5	24 ± 18	5	23 ± 5	68 ± 7	2.5	25 ± 4	182 ± 15	0.5	9.9 ± 0.8	32 ± 9	24 ± 4
Isoproturon	5	<LOQ	10	39 ± 3	6.6 ± 1.3	5	58 ± 5	50 ± 2	1	18 ± 1	7.9 ± 0.6	113 ± 2
Mecoprop (*)	10	<LOQ	20	252 ± 18	37 ± 6	10	203 ± 16	72 ± 14	2	10 ± 1	126 ± 21	14 ± 3
Propiconazole	5	12 ± 2	10	16 ± 4	<LOQ	2.5	14 ± 1	10 ± 2	0.5	5.1 ± 0.5	5.6 ± 1.4	6.0 ± 0.6
Tebuconazole	5	<LOQ	5	<LOQ	8.9 ± 2.8	2.5	3.6 ± 0.3	6.4 ± 1.6	0.5	2.4 ± 0.2	5.9 ± 1.2	11 ± 1
Imazalil	5	23 ± 7	20	<LOQ	<LOQ	5	<LOQ	6.0 ± 0.9	1	<LOQ	2.6 ± 0.3	6.6 ± 0.4
Climbazole	5	1160 ± 80	10	475 ± 44	1350 ± 70	5	312 ± 12	443 ± 11	1	ND	47 ± 4	530 ± 70
Ketoconazole	25	328 ± 45	50	<LOQ	90 ± 15	25	<LOQ	<LOQ	5	ND	<LOQ	<LOQ
Carbendazim	5	8.5 ± 0.8	5	41 ± 6	143 ± 26	2.5	48 ± 4	88 ± 14	0.5	18 ± 1	94 ± 22	84 ± 4
Thiabendazole	2.5	6.7 ± 4.0	5	<LOQ	13 ± 2	2.5	4.7 ± 0.6	13 ± 1	0.5	0.7 ± 0.1	18 ± 3	5.4 ± 2.0
Terbuthylazine	2.5	<LOQ	5	<LOQ	18 ± 2	2.5	<LOQ	33 ± 1	0.5	2.4 ± 0.1	13 ± 1	2.9 ± 0.3
Terbutryn	2.5	59 ± 55	5	26 ± 3	116 ± 10	2.5	28 ± 4	123 ± 7	0.5	5.6 ± 0.3	51 ± 4	169 ± 12
Irgarol	2.5	3.7 ± 1.0	5	21 ± 3	<LOQ	2.5	22 ± 2	6.3 ± 0.8	0.5	1.0 ± 0.1	11 ± 1	6.8 ± 0.8
M1	2.5	<LOQ	5	9.1 ± 3.2	<LOQ	2.5	9.2 ± 1.2	10 ± 1	0.5	0.7 ± 0.1	12 ± 2	3.1 ± 1.0
Dimethomorph	5	<LOQ	10	<LOQ	18 ± 2	5	<LOQ	8.9 ± 1.0	1	<LOQ	<LOQ	2.5 ± 0.7
Fenpropimorph	2.5	<LOQ	5	<LOQ	<LOQ	2.5	<LOQ	<LOQ	0.5	<LOQ	<LOQ	<LOQ
Tridemorph	25	<LOQ	20	<LOQ	<LOQ	10	<LOQ	<LOQ	2	<LOQ	<LOQ	<LOQ
BIT	50	179 ± 64	100	<LOQ	<LOQ	50	<LOQ	<LOQ	10	<LOQ	<LOQ	37 ± 17
OIT	10	120 ± 85	10	11 ± 1	<LOQ	5	<LOQ	<LOQ	1	<LOQ	<LOQ	<LOQ
DCOIT		ND	10	<LOQ	<LOQ	5	<LOQ	<LOQ	1	<LOQ	<LOQ	<LOQ
DMST	10	<LOQ	20	<LOQ	<LOQ	10	<LOQ	16 ± 2	2	<LOQ	4.7 ± 1.5	6.3 ± 1.9
DMSA (*)	25	<LOQ	50	<LOQ	<LOQ	25	<LOQ	48 ± 14	5	5.7 ± 0.9	22 ± 11	31 ± 3
IPBC		ND	50	<LOQ	<LOQ	25	<LOQ	<LOQ	5	<LOQ	<LOQ	<LOQ
Triclosan (*)	10	2730 ± 90	20	372 ± 10	841 ± 31	10	162 ± 25	12 ± 5	2	3.3 ± 0.6	18 ± 1	268 ± 7
Triclocarban (*)	2.5	116 ± 10	5	<LOQ	12 ± 1	2.5	<LOQ	<LOQ	0.5	<LOQ	3.5 ± 0.5	3.5 ± 0.6
Chlorophene (*)	10	322 ± 17	10	664 ± 55	216 ± 13	5	181 ± 14	<LOQ	2	<LOQ	3.4 ± 1.4	4.8 ± 1.8
UV-filters												
BZP-1 (*)	2.5	5.1 ± 1.5	5	43 ± 4	488 ± 19	2.5	12 ± 1	<LOQ	0.5	0.9 ± 0.3	2.2 ± 0.7	29 ± 2
BZP-2 (*)	2.5	11 ± 2	5	35 ± 6	93 ± 10	2.5	14 ± 3	<LOQ	0.5	<LOQ	1.8 ± 1.9	6.7 ± 2.4
BZP-3	25	132 ± 23	50	195 ± 31	518 ± 55	25	96 ± 12	<LOQ	5	<LOQ	<LOQ	47 ± 29
BZP-4 (*)	5	29 ± 7	10	2120 ± 220	5130 ± 140	5	572 ± 15	105 ± 11	1	51 ± 5	332 ± 11	1980 ± 130
PBSA	5	<LOQ	10	275 ± 27	3890 ± 170	5	316 ± 25	1820 ± 240	1	48 ± 3	1310 ± 200	3240 ± 140
Benzothiazoles												
Benzothiazole	100	265 ± 67	200	1120 ± 150	394 ± 75	100	313 ± 30	<LOQ	20	<LOQ	158 ± 6	560 ± 82
MTBT	25	157 ± 62	50	170 ± 24	379 ± 25	25	453 ± 27	261 ± 34	5	13 ± 1	119 ± 15	838 ± 25
BTSA	10	326 ± 147	20	1490 ± 220	1280 ± 90	10	2040 ± 90	393 ± 23	2	71 ± 8	1640 ± 240	2800 ± 490
OHBT (*)	100	307 ± 17	200	806 ± 26	619 ± 61	100	512 ± 68	<LOQ	20	<LOQ	199 ± 17	671 ± 55
Morpholinyl-BT	2.5	5.3 ± 1.2	10	20 ± 5	10 ± 2	2.5	19 ± 1	9.0 ± 1.5	0.5	0.8 ± 0.1	5.9 ± 2.4	5.9 ± 0.8

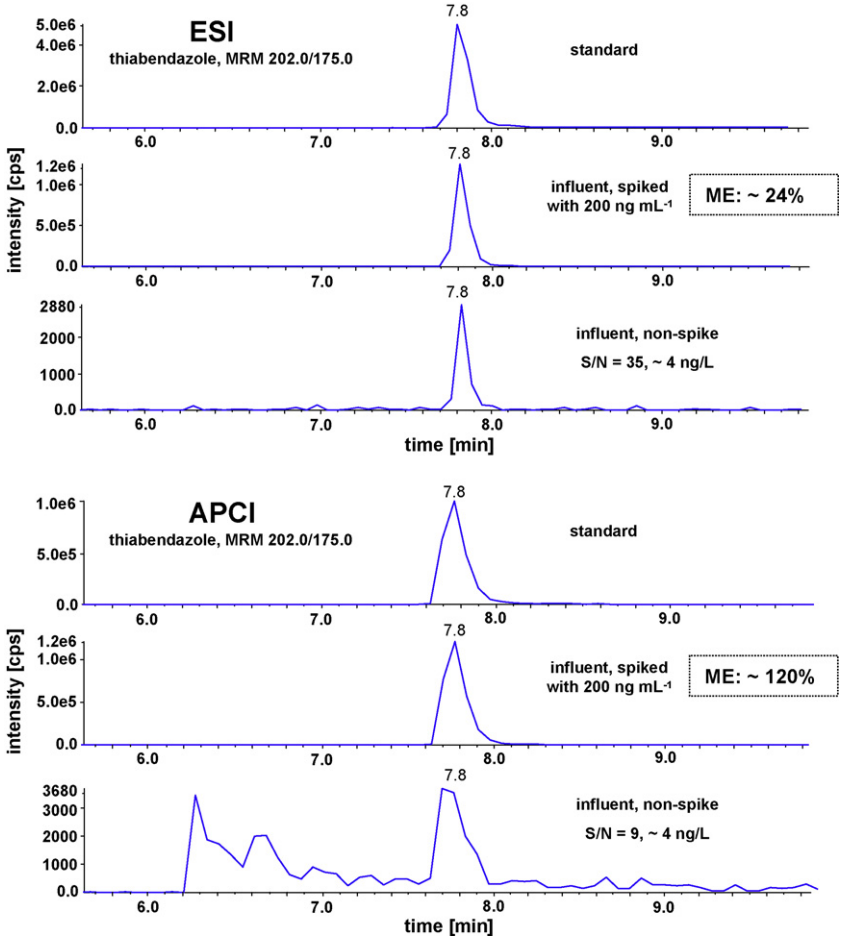

Fig. 6. ESI and APCI MRM chromatograms of thiabendazole in a standard solution (200 ng mL^{-1}) in comparison to non-spiked and spiked (200 ng mL^{-1}) extracts of WWTP influent. Matrix effects (ME) were calculated by dividing the peak height for thiabendazole of the spiked sample extracts by the peak height for thiabendazole of the standard solution according to Eq. (1).

3.5.2. Occurrence in activated sludge

The dominant target analytes found in activated sludge were the bacteriostatics triclosan (2730 ± 90 ng g TSS^{-1}) and chlorophene (322 ± 17 ng g TSS^{-1}) as well as the anti-dandruffs climbazole (1160 ± 80 ng g TSS^{-1}) and ketoconazole (328 ± 45 ng g TSS^{-1}) (cp. Table 6). The preservatives BIT and OIT, which were both below the LOQ in the wastewater samples, were found at concentrations of 179 ± 64 and 120 ± 85 ng g TSS^{-1}, respectively.

Concentrations of the selected polar UV-filters were quite low ranging from <LOQ (PBSA) to 132 ± 23 g TSS^{-1} (BZP-3), while the polar benzothiazoles with K_{OW} values <3 could be detected in activated sludge at considerable concentrations ranging from 157 ± 62 ng g TSS^{-1} (MTBT) to 326 ± 147 ng g TSS^{-1} (BTSA).

To our knowledge, this is the first time that the anti-dandruffs climbazole and ketoconazole, the isothiazolinones BIT and OIT as well as the benzothiazoles BT, MTBT, OHBT and BTSA were measured and detected in activated sludge.

4. Conclusions

A multi-residue method for the determination of 26 biocides, 5 water-soluble UV-filters and 5 benzothiazoles in activated sludge, raw and treated wastewater, and surface water has been developed using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the positive and negative ionization mode. Special emphasis has been made in this work to study the matrix effects in the different sample matrices using ESI and APCI. Ion suppression using ESI was identified to significantly reduce absolute recoveries of most target analytes, and thus making the use of appropriate labeled surrogate standards crucial to achieve acceptable relative recoveries in the range of 75–125%. Even APCI was shown to be less susceptible to ion suppression, the use of surrogate standards was needed for some of the target analytes to compensate for significant ion enhancement. The advantage of higher absolute recoveries when using APCI as ionization source was overweighed for most analytes by lower sensitivities and partly by higher background noise leading to higher LOQs. However, for benzothiazole and MTBT 4 times lower LOQs were determined in matrix containing samples using APCI. It can be concluded that the choice of ionization source depends on the target analytes and the matrices. In case ion suppression is significantly lower in comparison to ESI and no significant increase of the background noise occurs, APCI should be preferred to ESI if

(i) sensitivity is comparable or higher to ESI or less sensitivity does not equal higher responses due to less ion suppression and/or
(ii) no appropriate surrogates (e.g. stable isotope-labeled analytes) are available and ion enhancement does not occur or can be compensated by an internal calibration due to similar relative matrix effects.

For certain groups of analytes, APCI could be definitely more suitable than the still more commonly used ESI and should be evaluated in regards to matrix effects even a measurement of non-enriched external standards reveals less sensitivity. However, this study indicate that for multi-residue methods including a broad

spectrum of analyte groups applied to different complex matrices ESI is favourable. If stable isotope-labeled surrogate standards are not available for every analyte, the matrix effects have to be determined for every analyte/matrix combination to assure the appropriate compensation of the matrix effects.

A first application of the ESI method revealed that, besides the benzothiazoles, the analytes used in ingredients of PCPs such as the biocides climbazole and triclosan and the UV-filters BZP-4 and PBSA were the dominant analytes in the analyzed wastewater samples from urban WWTPs. These data indicate that, in addition to the more prominent analytes such as triclosan, the parabenes or the musk fragrances, high amounts of ingredients of PCPs are emitted by WWTPs which are still not included in monitoring programs such as the water-soluble UV-filters or the anti-dandruff climbazole. The proposed environmental quality value of 2 ng L^{-1} for irgarol proposed by LAWA was found to be exceeded by a factor of ten in a WWTP effluent indicating that WWTPs have to be considered as important point sources in regard to this quality norm.

The benzothiazoles BT, MTBT, OHBT and BTSA as well as the isothiazolinones BIT and OIT were detected for the first time in activated sludge in the mid ng g TSS^{-1} range. This shows that analytical methods for water and sludge phase are crucial to correctly assess the fate (sorption and biotransformation) in WWTPs even for these relatively polar analytes with log K_{OW} values <3.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.079.

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