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SAMPLES



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**STUDY OF THE RETENTION OF BENZOTRIAZOLES, BENZOTHIAZOLES AND  
BENZENESULFONAMIDES IN MIXED-MODE SOLID-PHASE EXTRACTION IN  
ENVIRONMENTAL SAMPLES**

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**Highlights**

Benzotriazoles and benzothiazoles were selectively extracted in mixed-mode sorbents.

These neutral analytes showed ionic interactions with strong ion-exchangers.

Ionic interactions are promoted by induced charges in the analytes.

Two developed methods using cationic and anionic sorbents showed similar results.

Low matrix effect was obtained in environmental waters thanks to the washing step.

**Abstract**

In the present study, the capabilities of strong cation-exchange and strong anion-exchange sorbents for solid-phase extraction (SPE) have been evaluated for the selective retention of benzotriazoles (BTRs), benzothiazoles (BTs) and benzenesulfonamides (BSAs), which are a group of neutral analytes with interesting properties such as high polarity and the capability of delocalizing electron density. The retention of these analytes has been compared in both sorbents for the first time, using a SPE procedure specially designed to promote ionic retention of the analytes with the objective of including a washing step with an organic solvent to eliminate interferences retained by hydrophobic interactions.

As a result, ionic interactions between the analytes and both sorbents were observed, which allowed the successful introduction of a washing step using methanol in the SPE procedure even when most of the analytes were in their neutral state under SPE conditions. Consequently, a method was developed and further validated for each sorbent using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Apart from the development of an improved method, special attention was paid to the discussion of the interactions present between the sorbents and each group of analytes to explain how these analytes in their neutral state might develop ionic interactions with the sorbents. At the end, the use of these sorbents helped to simplify previous developed methods where hydrophobic/hydrophilic sorbents were used, obtaining enhanced results when evaluated in river water and effluent and influent wastewaters.

## 1. Introduction

Mixed-mode polymeric sorbents have been developed to be applied in solid-phase extraction (SPE) to address the limitations of hydrophobic/hydrophilic sorbents. These sorbents have a polymeric backbone capable of retaining compounds through reversed-phase interactions and ionic groups capable of retaining ions with the opposite charge so both types of interactions are combined in one sorbent. The ionic groups bonded to the polymer are commonly sulfonic or carboxylic acids groups, which promote strong cation-exchange (SCX) and weak cation-exchange interactions (WCX), respectively; or quaternary amine groups for strong anion-exchange interactions (SAX); or tertiary or secondary amine groups for weak anion-exchange interactions (WAX) [1, 2].

The presence of these ionic interactions allows basic or acidic compounds to be selectively extracted from complex matrices. These sorbents have been applied to extract several groups of compounds such as pharmaceuticals, drugs of abuse, herbicides, and biological compounds, among other analytes, from several biological, foodstuff and environmental matrices [1, 3-9]. Generally, the methods are developed to extract the ionic analytes selectively in the elution step, which can be eluted separately from interferences that are rinsed in previous washing steps. To do so, the selection of pH values and solvents is very important and it depends on the type of sorbent and the structure of the analyte.

Benzotriazoles (BTRs), benzothiazoles (BTs) and benzenesulfonamides (BSAs) include substances containing the skeleton of benzotriazole (BTR), benzothiazole (BT) and benzenesulfonamide (BSA), respectively (Table 1), and they are considered emerging contaminants with particular chemical structures [10]. Both BTRs and BTs are heterocyclic compounds comprising two fused aromatic rings, allowing the delocalization of the electron density throughout the entire molecule. Moreover, the presence of electronegative atoms in one of the rings introduces high polarity to the structure. Furthermore, BTR can exist as two tautomers and can give

resonance-stabilized anions and cations when treated with strong bases and electrophiles, such as all azoles and their bicycle derivatives [11]. In the case of BSAs, delocalization of the electrons only occurs on the benzene ring but they are very polar compounds thanks to the sulfonamide group. The properties of these three groups of compounds have become important in several industrial applications and, for this reason, they have become high production volume substances. Their applications include: flame and corrosion inhibitors, photosensitizers, intermediates in the production of pharmaceuticals and dyes, de-icing/anti-icing fluids, herbicides, fungicides and chemotherapeutic applications [12-15]. However, these compounds can reach environmental waters and, for this reason, they have been classified as emerging organic contaminants [10, 16, 17]. A number of toxicity studies have shown that BTR, 4-methyl-1-H-benzotriazole (4TTR) and 5-methyl-1-H-benzotriazole (5TTR) (these two usually measured as a total and referred to as tolyltriazole, TTR) are hazardous to plants. BTR is also mutagenic in some bacteria cell systems, potentially estrogenic in fish and a suspected human carcinogen, while TTR has been reported to be toxic to microorganisms [18].

The technique of choice for determining these compounds in environmental waters is liquid chromatography (LC) coupled to mass spectrometry in tandem (MS/MS) or high resolution mass spectrometry (HRMS) [10, 12-14, 19]. With regard to the sample treatment, most of the methods developed to determine BTRs, BTs and BSAs in these matrices use SPE with polymeric hydrophobic/hydrophilic sorbents (such as Oasis HLB or Strata-X) for the extraction of the analytes [10, 12-14, 20-23]. However, the retention that is achieved for the target analytes when using this type of sorbents is also gained by the interferences present in the matrix and, as a result, the methods proposed to determine these compounds exhibit a considerable matrix effect (ME) [10, 12, 13, 15, 19, 23]. For methods where ME was successfully reduced [20], the SPE procedure demanded an additional washing step that extended sample treatment and increased costs. For this reason, some attempts have been made to reduce this ME by using anion-exchange mixed-mode sorbents that claim to present more selectivity for anions [19, 20]. In one case [19], the

analytes were eluted with a mixture of organic solvents leaving the acidic interferences retained, a procedure that showed good results for river water and effluent wastewater but was less satisfactory for influent wastewater. In another study [20], the sorbent was dismissed because results of preliminary tests were not promising.

In the present study, the strong anion-exchange mixed-mode sorbent Oasis MAX is evaluated focusing its capabilities on selectively retaining a group of BTRs, BTs and BSAs, using conditions to promote ionic interactions between the sorbents and the analytes. Also, for the first time, the strong cation-exchange mixed-mode sorbent Oasis MCX is evaluated for these analytes and compared with the Oasis MAX, because ionic interactions with the analytes are feasible thanks to the structural properties of the analytes mentioned above. The aim is to reduce the ME compared with other methods but also to increase the knowledge and understanding for these types of sorbents as the selected analytes bare interesting properties. The two methods using Oasis MAX or Oasis MCX in SPE followed by LC-HRMS are developed and further validated.

## 2. Experimental

### 2.1. Reagents and standards

Solid standards of 1-H-benzotriazole (BTR) and four of its derivatives: 4-methyl-1-H-benzotriazole (4TTR), 5-methyl-1-H-benzotriazole (5TTR), 5,6-dimethyl-1-H-benzotriazole (XTR) and 5-chloro-1-H-benzotriazole (ClBTR); together with benzothiazole (BT) and its derivatives: 2-aminobenzothiazole (NH<sub>2</sub>BT), 2-hydroxybenzothiazole (OHBT) and 2-(methylthio)benzothiazole (MeSBT); and benzenesulfonamide (BSA) and the four derivatives: *ortho*-toluenesulfonamide (*o*-TSA), *para*-toluenesulfonamide (*p*-TSA), N-methyl-*para*-toluenesulfonamide (Me-*p*-TSA) and N-ethyl-*para*-toluenesulfonamide (Et-*p*-TSA) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Stock solutions of 1 mg/mL were prepared by dissolving each solid standard in methanol (MeOH) and were stored at -20°C. All working solutions of a mixture of all compounds were prepared weekly in ultrapure water and were stored at 4°C in the dark. The structure of the analytes, pK<sub>a</sub> values, log P values and masses of the molecular ions and the selected fragments are shown in Table 1.

Ultrapure water was obtained using a water purification system (Veolia, Sant Cugat del Vallès, Spain) and ultra-gradient HPLC grade MeOH and acetonitrile (ACN) were purchased from J.T. Baker (Deventer, The Netherlands). Acetic acid (CH<sub>3</sub>COOH) from SDS (Peypin, France), formic acid (HCOOH) from Sigma-Aldrich, hydrochloric acid (HCl) from Scharlab (Barcelona, Spain) and ammonium hydroxide (NH<sub>4</sub>OH) from Panreac (Barcelona, Spain) were used to prepare the mobile phase and the solutions for the SPE.

### 2.2. Sampling

Influent and effluent wastewater samples were collected from a Tarragona sewage treatment plant which operates with a primary and a secondary treatment. River



water samples were collected from the River Ebre in Catalonia. Water samples were collected in pre-cleaned bottles and were then stored at -20°C. Prior to analysis, the samples were filtered through a 0.45 µm glass-fibre filter (Fisherbrand, Loughborough, UK). The pH of the samples was adjusted to 3 or 7 with HCOOH before the SPE procedure.

### **2.3. Solid-phase extraction procedure**

Oasis MCX and Oasis MAX cartridges (500 mg) supplied by Waters (Milford, MA, USA) were used. The optimal conditions were: loading volume was 250 mL for river water and effluent wastewaters, and 100 mL for influent wastewaters.

Oasis MCX cartridges were conditioned with 10 mL of MeOH, followed by 10 mL of ultrapure water adjusted to pH 3 with HCOOH. Samples were also adjusted to pH 3 before being loaded into the cartridge. Two consecutive washing steps were used: 1) 5 mL of 5% HCOOH in aqueous solution and 2) 2 mL of pure MeOH. Finally, the analytes were eluted with 5 mL of 5% NH<sub>4</sub>OH in MeOH.

Oasis MAX cartridges were conditioned with 10 mL of MeOH, followed by 10 mL of ultrapure water adjusted to pH 7. Samples were loaded at pH 7 and the washing steps were as follows: 1) 5 mL of 5% NH<sub>4</sub>OH in aqueous solution and 2) 2 mL of pure MeOH. For the elution, 5 mL of 5% HCOOH in MeOH were used.

When analysing water samples, the extracts (in 5% HCOOH or 5% NH<sub>4</sub>OH in MeOH solutions) obtained after the elution were evaporated up to ~250 µL and taken to a final volume of 1 mL with ultrapure water.

### **2.4. Liquid-chromatography-high resolution mass spectrometry (LC-HRMS)**

Chromatographic analysis was performed with an Accela 1250 UHPLC system from Thermo Scientific (Bremen, Germany) that includes an automatic injector (Accela

Autosampler) and a quaternary pump capable of reaching up to 1,250 bar. The LC system was coupled with an Exactive Orbitrap<sup>TM</sup> mass spectrometer (also from Thermo Scientific) with a heated electrospray ionization (HESI) source and a HCD collision cell for the fragmentation of the analytes for their confirmation. The chromatographic column used was the Ascentis Express C<sub>18</sub> (100 mm x 2.1 mm i.d., 2.7 µm particle size, Fused-Core®) supplied by Sigma-Aldrich. The mobile phase was a mixture of solvent A (0.1% CH<sub>3</sub>COOH in H<sub>2</sub>O/ACN 98:2, v/v) and solvent B (MeOH). The gradient profile started with 0.5% of B and was held for 5.25 min, before being increased to 18% B in 3.5 min and held again for 1.25 min with a total time of 10 minutes. Subsequently, it was increased to 35% B in 6 min and then increased again to 100% in 4 min and held for 4 min before returning to the initial conditions in 1 min. The flow rate was 800 µL/min, the temperature of the column oven was set at 50°C and the injection volume was 20 µL.

In the HRMS instrument, the signal of the molecular ion, [M+H]<sup>+</sup> or [M-H]<sup>-</sup>, of each analyte was monitored to optimize the interface conditions in order to obtain the highest response for all of the analytes. This optimization was performed in full scan at high resolution (50,000 FWHM) in a mass range of 100 to 1,000 m/z. The optimal parameters for positive ionization were: skimmer voltage, 20 V; capillary voltage, 37.5 V and tube lens voltage, 90 V. In the case of negative ionization, they were: skimmer voltage, -25 V; capillary voltage, -40 V and tube lens voltage, -90 V. Other parameters were the same for both ionization modes: spray voltage, 4 kV; sheath gas, 55 AU (adimensional units); auxiliary gas, 20 AU; heater temperature, 400°C; capillary temperature, 350°C; and probe position adjustment: side to side, 0, vertical D and micrometer, 1.25.

For data acquisition, four time windows were used: two in negative mode (0-2.3 and 4.8-7.6 min) and two in positive mode (2.3-4.8 and 7.6-20 min) alternating two consecutive scan events each window. In all of the windows, the first scan event was a full scan at 50,000 FWHM with 250 ms of injection time while the second scan event was a fragmentation scan at 10,000 FWHM with 50 ms of injection time,

using an optimum collision voltage of 25 eV in the HCD cell in all windows. For quantification, the molecular ions were measured (with a mass extraction window of 5 ppm) and, for confirmation, the selected fragments and the ion ratios were taken into account.

### 3. Results and discussion

#### 3.1. LC-HRMS conditions

LC-HRMS conditions were adapted from a previous study [20] in which the analytes were determined in sewage sludge. The separation of some of the compounds is very difficult, so the most important parameters that were optimized in this previous study should be emphasized: the chromatographic column was also a Fused-Core® type; the *orto* and *para* isomers of the toluenesulfonamide (*o*-TSA and *p*-TSA) were determined as a mixture named TSA because chromatographic separation was not possible under optimal LC conditions. The separation of 4TTR and 5TTR was also very complex so, in order to obtain the best separation in this case, a percentage of 2% of ACN was added to the aqueous mobile phase and the oven temperature was increased to 50°C. In addition, the initial segment of the gradient profile was kept at low concentrations of organic solvent. The optimal parameters are detailed in Section 2.4 and the fragments selected for confirmation are described in Table 1. The instrumental limits of detection (ILODs) were the concentrations at which the signal to noise ratio (S/N) was 3 for the response of the fragment that showed the highest signal under collision conditions. The obtained values ranged between 1 and 10 µg/L. Instrumental limits of quantification (ILOQs) were the concentration corresponding to the first point of the calibration curve and they ranged from 1 to 25 µg/L (Table S1). Satisfactory linearity was observed in the range between the ILOQs and 1000 µg/L.

### 3.2. Mixed-mode solid-phase extraction

BTRs, BTs and BSAs are compounds with relatively high polar character due to the presence of the triazole, thiazole and sulfonamide groups, and also the first two groups have an important delocalized electron density [11]. With this in mind, two commercially available strong ion-exchange mixed-mode sorbents, Oasis MAX and Oasis MCX, were selected to evaluate their capability of establishing ionic interactions with the analytes induced by the strong character of the quaternary amine or the sulfonic group of the sorbents. Weak ion-exchange sorbents were not selected because their functional groups are not permanently charged (as is the case with the strong ion-exchange sorbents) and pH conditions not only affect the analytes but also the sorbent, adding more parameters to be controlled. Furthermore, preliminary tests showed no selective ionic interactions with any of the analytes.

Because of the chemical differences between the strong anionic and cationic exchange sorbents, the conditions for the SPE procedure also differ from each other [24]. When using anionic sorbents, a neutral pH value must be used to load the sample because the analytes must be in their anionic form to establish ionic interactions with the quaternary amine of the sorbent [7]. Likewise, when using cationic sorbents, the analytes must be loaded in their cationic form (usually adjusted to pH 3) to allow interaction with the sulfonic acid groups [25, 26]. The formation of strong ionic interactions allows the possibility of adding different washing steps to the SPE procedure in order to clean the extracts from interferences. An aqueous wash at the selected pH can be used to eliminate the most water-soluble compounds that are poorly retained in the sorbent by hydrophobic interactions and further activates the ionic interactions between the ionic compounds and the sorbent. Subsequently, the second washing step with an organic solvent is added to rinse all of the neutral compounds that are only retained by hydrophobic interactions. Finally, an elution step is needed to turn the analytes back to their neutral state using organic solutions at suitable pH conditions.

The performance of both sorbents and the differences in the ionic retention of the selected analytes will be discussed in the following sections.

### 3.2.1. Cation-exchange sorbent

Based on the most commonly used conditions, the following starting procedure was selected to optimize the SPE conditions in this sorbent: loading 100 mL of ultrapure water adjusted to pH 3 spiked at 25 µg/L with the mixture of analytes; two washing steps and elution as described in Section 2.3. It should be highlighted that preliminary studies without the washing step showed good recoveries (between 87% and 115%) for all of the analytes.

The starting conditions used for the Oasis MCX sorbent succeeded in retaining and consequently eluting the compounds which was surprising because all of the analytes were in their neutral state, with the exception of NH<sub>2</sub>BT which was positively charged (Table 1). In order to evaluate its effect on retention, the pH of the sample was adjusted to 2 (using HCl) before being loaded into the cartridge, rather than 3 as initially established. As a result, no significant differences were observed between both pH values. These observations agree with the fact that all of the analytes remain unchanged at both pH 2 and 3, according to their  $pK_a$  values. In order to avoid using relatively extreme conditions, pH 3 was selected as optimal.

Table 2 shows the recoveries obtained in the washing and elution fractions when using the starting SPE conditions described above. BSAs eluted in the second wash fraction corresponding to the pure MeOH wash. Clearly, these compounds interact with the sorbent through hydrophobic interactions which is expected as they are in their neutral state at pH 3. In contrast, BTRs and BTs were completely retained during the washing step and rinsed in the elution fraction even when most of them were also in their neutral state. These compounds seem to be retained to

the sorbent through ionic interactions that are more significant than hydrophobic interactions. It was interesting to observe these differences between groups of analytes with regard to their interactions with the sorbents, because one of the aims of the present study was to explore the capabilities of these sorbents for these compounds with interesting properties. It can be observed that the analytes with the capacity of delocalizing electron density (BTRs and BTs, Figure 1) were able to stabilize partial electron charges to interact with the ionic group of the sorbent, while BSAs behaved as neutral compounds, probably because they have no ability to delocalize electron density.

In a subsequent test, a higher volume of MeOH (5 mL) was employed for the second wash. Table 2 also details the distribution of the analytes between both the washing fraction (when using 2 or 5 mL of MeOH) and the eluting fraction. When increasing the volume of MeOH, CIBTR and OHBT were rinsed in the washing fraction instead of the elution fraction, probably because weaker ionic interactions were involved. The electronegativity of the groups attached to the BTRs and BTs seems to have an important effect on the ionic interactions that are formed. In this case, this effect might be detrimental, because retention was decreased, as shown by CIBTR, with the presence of the chloride group, and by OHBT, with oxygen being more electronegative than nitrogen and sulfur. Thus, 2 mL of MeOH was selected as second washing step.

As the analytes showed satisfactory recoveries (60% to 115%), a higher loading volume (250 mL) was tested using 2 mL of MeOH in the second washing step. When increasing the loading volume, all analytes showed similar recoveries, however, MeSBT was more evenly distributed between the washing and eluting fraction, so recovery in the elution fraction decreased (65%). For this reason, higher volumes were not further tested and 250 mL was established as the highest loading volume to be tested in environmental waters. Higher volumes of eluting solvent were not tested further because it was observed that the volume initially selected (5

mL) was enough to elute the analytes completing the mass balance of the overall SPE procedure.

### 3.2.2 Anion-exchange sorbent

In the case of this sorbent, the following starting procedure was selected: loading 100 mL of ultrapure water adjusted to pH 7 spiked at 25 µg/L with the mixture of analytes; two washing steps and elution as described in Section 2.3. Preliminary studies without the washing step showed good recoveries (between 85% and 106%) for all of the analytes.

For this sorbent, the starting conditions were also successful in retaining and consequently eluting the analytes, even when all of them were in their neutral state at the selected pH. As before, the pH of the sample was changed to evaluate its effect on retention. The sample was adjusted to pH 11.5 (with NaOH) instead of 7 as established in the starting conditions, because most of the BTRs, BTs and BSAs (except for BT, NH<sub>2</sub>BT and MeSBT) can donate a proton at pH values between 8 and 11, depending on the analyte (see pK<sub>a</sub> values in Table 1). Theoretically, retention should be higher at pH 11.5 for those compounds that donate a proton to become negatively charged. However, no differences were observed between the two pH values, so apparently the interactions occurring during the extraction are independent from the donation of the proton. This further confirms that retention is probably controlled by inducing charges in the analytes that are stabilized by resonance rather than actual ion-exchange. Thus, it was decided to adjust the samples to pH 7 in order to avoid using extreme pH conditions.

When using the starting procedure for the Oasis MAX sorbent, all of the analytes were recovered in the eluting fraction, except for Me-p-TSA and Et-p-TSA, which were distributed between both the washing and eluting fractions (Table 2). When the volume of the pure MeOH washing step was increased to 5 mL, all of the BSAs were collected in this washing fraction, together with NH<sub>2</sub>BT and BT. Thus, similar

to the Oasis MCX sorbent behaviour, as BSA has no delocalized charges, they are not able to be retained in any of the mixed-mode sorbents through ionic-exchange mechanisms. Considering the lack of ionic interactions between both sorbents and the BSAs, it was decided to eliminate them from the target list and develop a more selective method for the compounds for which the sorbents display ionic interactions, which is the case of the BTRs and BTs. The latter have the property of delocalizing the electron densities throughout both aromatic rings that might induce dipoles that ionically interact with the amine groups of the sorbent. Compared to Oasis MCX, the Oasis MAX sorbent showed less retention for NH<sub>2</sub>BT and BT (eluting during washing when employing 5 mL of MeOH), while it presented higher retention for CIBTR and OHBT (Table 2). In the Oasis MAX, the electronegativity of the groups attached to the analytes seems not to have a negative effect on the ionic interactions responsible for retention, as CIBTR and OHBT were retained when washing with 5 mL of MeOH. BT showed less retention than its derivatives substituted with electronegative functional groups, suggesting that the effect might actually be favourable. The behaviour of NH<sub>2</sub>BT might be explained separately from the rest, because this compound is protonated at pH 3 (pK<sub>a</sub> 3.94 in Table 1) but it is neutral at pH 7, explaining the increased retention observed in Oasis MCX, in contrast to the decreased retention observed in Oasis MAX. These results are in agreement with a previous study [20] where Oasis MAX was compared with the hydrophilic/hydrophobic sorbent Oasis HLB. Even when pH and sorbent weight differ from the present study, the loss of BSAs, NH<sub>2</sub>BT and BT was also observed in the washing fraction of 6 mL of MeOH.

As BTRs and BTs were not lost using 2 mL of MeOH in the second wash, this volume was used for further optimization, but it was compared again using environmental samples, as will be discussed in the following sections. A higher sample volume (250 mL) was tested and similar results were obtained to those with 100 mL recoveries, except for MeSBT, which was partially rinsed in the washing fraction. For this reason, no higher volumes were tested further.



As can be seen, the ionic interactions promoting the retention of BTRs and BTs on Oasis MCX and Oasis MAX sorbents are not based on ion-exchange *per se* [27], as these molecules do not develop a clear ionic charge under SPE conditions. This affirmation is evidenced by the fact that different pH values of the loading sample showed no significant differences. However, the analytes are capable of developing partial charge densities that might easily interact with the ionic groups of the sorbents, allowing the retention of the analytes even when a MeOH washing step was used. This capability of delocalizing electron density throughout the whole molecule is due to the conjugation of both aromatic rings (Figure 1). Some observations suggest that the strong character of the ionic groups present in the sorbents plays an important role in the promotion of the ionic interactions between the analyte and the sorbent. These groups might induce ion-dipole interactions that are feasible thanks to the capabilities of the analytes to stabilize partial charges. As a result, these sorbents showed the capacity of retaining the selected compounds through ionic interactions in spite of being neutral under SPE conditions. When using 2 mL of MeOH as a second washing step, both sorbents showed similar results being slightly better for the Oasis MAX. However, their behaviour with environmental samples might lead to a clearer comparison and for this reason they will be further compared in the following section.

### 3.2.3. Evaluation in environmental samples

In this section, the previously described SPE procedure was tested on river water and wastewaters in order to evaluate the %ME obtained using HESI in LC-HRMS, with these more selective sorbents. Both sorbents were initially tested in influent wastewater because differences in ME would be more evident in such a complex matrix. The procedure followed was the same as previously described for ultrapure water, but after the elution, the extracts in 5% HCOOH or 5% NH<sub>4</sub>OH in MeOH solutions were evaporated up to ~250  $\mu$ L and taken to a final volume of 1 mL with ultrapure water. Evaporation to dryness was not possible as BT and MeSBT were

lost because of their volatility, but it was observed that no losses were found when evaporating up to ~250  $\mu$ L.

As expected, the extraction was affected by the sample matrix and therefore the volume of sample loaded into the cartridges was reduced from 250 mL to 100 mL. Table 3 shows the results obtained for both sorbents when percolating 100 mL of influent wastewater spiked at 5000 ng/L with the mixture of BTRs and BTs and adjusted to pH 3 (Oasis MCX) and to pH 7 (Oasis MAX).

The recovery named as  $\%R_{SPE}$  expresses the yield of the extraction procedure only and it was calculated as the ratio between the concentrations obtained for samples spiked before and after the SPE procedure, respectively, both calculated from a calibration curve prepared with pure standards.

The ME was calculated as  $\%ME = [(C_{exp}/C_{theo}) \cdot 100] - 100$ , where the  $C_{exp}$  was the concentration of analytes when the sample was spiked after the SPE procedure (just before injection into the LC-HRMS system) calculated from the interpolation in a calibration curve prepared in pure standards; and the  $C_{theo}$  was the theoretical concentration of analytes in the final volume (1 mL). The value obtained refers to the percentage of the response that is suppressed or enhanced. The recovery, referred to as  $\%R_{apparent}$ , was calculated by interpolating the response of the analytes when the sample was spiked before the SPE in a calibration curve prepared in pure standards. This recovery indicates the losses of the overall analytical procedure including the extraction itself and the influence of the matrix in the response. In every case, response of the analytes present in the blanks was subtracted from the response obtained for the spiked samples.

The  $\%R_{SPE}$  recoveries reported in Table 3 were similar in both sorbents except for certain differences (also observed in ultrapure water), such as the case of  $NH_2BT$ , which showed less retention (40%) in the Oasis MAX cartridge than in the Oasis MCX sorbent (86%).

The %ME values obtained for both sorbents were also similar. The %ME obtained for the Oasis MCX sorbent ranged from -32% to 24%, while values obtained for the Oasis MAX sorbent were between -29% and 0%. It can be seen that signal suppression predominates over enhancement, as only BT presented positive values of %ME. In order to observe the effect of the MeOH washing volume on the %ME, the second wash step of the SPE procedure was performed with 5 mL of pure MeOH instead of 2 mL. For the Oasis MCX sorbent, %ME values ranged between -28% and 10%, indicating that no significant decrease in %ME is observed when increasing the volume of MeOH wash. Furthermore,  $\text{NH}_2\text{BT}$  and BT were collected in the wash fraction. Likewise, in the case of the Oasis MAX sorbent, the differences in the %ME were not significant when increasing the volume of MeOH, so the change does not compensate the fact that more analytes were also rinsed in the wash fraction. It can be observed that the first millilitres of MeOH are the most important in eluting hydrophobic interferences and cleaning the matrix. Thus, for both sorbents, it was decided to use 2 mL in the second wash with MeOH. The recoveries obtained for influent wastewaters corresponding to the whole method ( $\%R_{\text{apparent}}$ ) are also detailed in Table 3. As can be seen,  $\%R_{\text{apparent}}$  values are similar to or slightly lower than the  $\%R_{\text{SPE}}$  values, as expected since %ME was low.

Subsequently, the method was evaluated in river water and effluent wastewaters using both sorbents. The results obtained are detailed in Table 4. The %ME values obtained for 250 mL of river water and effluent wastewater samples were lower than those obtained for influent wastewater samples, even when a higher volume of sample was employed. For the Oasis MCX sorbent, %ME values ranged from -4% to 20% and -11% to 11% for river water and effluent wastewater samples, respectively. In the case of the Oasis MAX sorbent, %ME values ranged between -19% and 16% and between -25% and 6% for river and effluent wastewater samples, respectively. It can be seen that the %ME obtained was very low for this type of matrices when performing the washing steps proposed in the SPE procedure. It was also noticeable that, in contrary to influent samples, river samples showed enhancement in most of the cases. In general, a %ME below 20% can be

considered not significant, hence, the values obtained in this study in environmental waters is quite acceptable for these types of matrices.

The %ME found for these compounds in complex environmental waters was lower than or similar to several studies. Herrero *et al.* [20] placed a Florisil cartridge (500 mg) in series after the Oasis HLB cartridge to obtain clearer extracts. They reported comparable values (from -29% to 4%) to those obtained here, but as they needed the additional Florisil cartridge while the present procedure uses only the mixed-mode sorbent, the present study shows the advantage of a simplified SPE procedure. It must be pointed out that in the same study [20], the performance of the Oasis MAX in effluent wastewater was compared with the Oasis HLB, but results were unsatisfactory because the procedure used was not optimized. In the study by Carpinteiro *et al.* [19], the Oasis MAX sorbent was used, but focusing on retaining ionic interferences of the matrix rather than the actual analytes. In their method, the analytes were collected with a mixture of MeOH and acetone, while acidic interferences remained retained in the sorbent through ionic interactions. Using this procedure, they obtained clearer extracts, reporting no ME for river samples and a small decrease in the response for effluent wastewater samples. Nevertheless, influent wastewater samples and industrial waters showed high ME (up to around -50%) and standard addition or matrix-matched calibration was suggested.

For river water and effluent wastewater samples, the %R<sub>apparent</sub> were from 77% to 105% and between 54% and 92%, respectively, when the Oasis MCX sorbent was used. In the case of the Oasis MAX sorbent, %R<sub>apparent</sub> values for river and effluent wastewater samples were from 65% to 93% and between 44% and 87%. As the matrix has a low effect on the response for these samples, the %R<sub>apparent</sub> were almost the same recoveries obtained for the %R<sub>SPE</sub>.

Up to this point, both sorbents exhibit the capacity to interact with the analytes, allowing washing with pure MeOH to achieve low %ME, even when the compounds

are not ionic *per se*. Hence, both sorbents were further compared during the method validation in order to evaluate whether there was any difference with regard to their validation parameters.

### 3.3. Method validation

The two methods based on the Oasis MCX and Oasis MAX sorbents were validated in the environmental waters using the SPE procedure detailed in Section 2.3 for each case. As some of the analytes (such as 4TTR and 5TTR) were present in the blanks at relatively high concentrations, the use of matrix-matched calibration curves for quantification was not possible. Considering that %R<sub>apparent</sub> values were satisfactory (Table 4) and that the repeatability of the method with both sorbents was great, quantification in the environmental waters was proposed using an external calibration method, taking into account the %R<sub>apparent</sub>.

As an example, the %R<sub>apparent</sub> values obtained for effluent samples during the validation (evaluated for two levels of concentration, 100 ng/L and 1,000 ng/L) will be discussed in more detail. For the Oasis MCX sorbent, the %R<sub>apparent</sub> obtained when the sample was spiked at 100 ng/L were from 69% to 97%, except for CIBTR (51%), while values obtained at 1,000 ng/L were from 52% to 89%. In the case of the Oasis MAX sorbent, the %R<sub>apparent</sub> at low level ranged between 78% and 111%, except for CIBTR (51%) and, at the high level, were from 67% to 91%, except for BT (47%). It can be seen that results were very similar to those obtained when spiking 2,000 ng/L during optimization (Table 4). Most of the %R<sub>apparent</sub> obtained were satisfactory, except for the few compounds that displayed less retention on the sorbents. The %R<sub>apparent</sub> obtained for river samples and influent samples were also in agreement with the values obtained during optimization, therefore, they were considered reliable for quantification purposes. This proved to be satisfactory when evaluating the %R<sub>relative</sub> values (spiking a fresh volume of effluent wastewater sample and analysing it as unknown to compare the concentration obtained with the

expected value) for two different levels of concentration, 100 ng/L and 1,000 ng/L, obtaining values from 85% to 110%.

For both sorbents, the repeatability and detection and quantification limits of the method were evaluated. Repeatability was also evaluated for two levels of concentration (100 ng/L and 1,000 ng/L) and was expressed as the relative standard deviation (%RSD,  $n=5$ ). Only the higher level was considered for those analytes that were present in the blanks. It was observed that repeatability was excellent, as it was lower than 19% in every case. As example for effluent samples and for the lower concentration, values ranged between 2% and 19% and, for the higher concentration, the values were between 3% and 13%. Similar results were observed for both sorbents and the different matrices with regard to repeatability.

The method limits (MDLs and MQLs) were estimated from the instrumental limits (ILODs and ILOQs, referred to in Section 3.1) and the  $\%R_{\text{apparent}}$  values. In more detail, ILODs and ILOQs were multiplied by 100% and divided by the  $\%R_{\text{apparent}}$  values. The result of this calculation was subsequently divided by the volume of the sample and the final result was expressed in ng/L. For both sorbents, results were very similar, observing that, for river samples, MDLs ranged from 2 to 60 ng/L and, for effluent and influent wastewaters, they were between 3 and 50 ng/L and between 10 and 225 ng/L, respectively. MQLs ranged from 5 to 155 ng/L for river samples and from 6 to 190 ng/L and from 15 to 565 ng/L for effluent and influent wastewater samples, respectively. In every case, it was found that the higher limits corresponded to BT, OHBT and MeSBT. These results are similar to those found in the literature, even when the detectors used were not the same. Using their LC-MS/MS method, Carpinteiro *et al.* [19] found LOQs that ranged from 2 to 286 ng/L in river water and wastewaters, also finding the higher limits for BT, OHBT and MeSBT. In the LC-MS/MS method developed by Loi *et al.* [14], LOQs between 6 and 1072 ng/L were reported for their selected group of BTRs and BTs in secondary wastewater, finding higher values for BT and OHBT. Jover *et al.* [22] found LOQs between 31 and 99 ng/L for river samples and between 62 and 198

ng/L for wastewater samples, when using TOF-MS detection. Thus, obtained values were in the same order of magnitude of several studies summarized in the literature [10]. During this section, differences between both sorbents were not substantial; therefore, their performance will be compared during the application of the method.

### 3.4. Application to environmental samples

Three different samples of river water, effluent and influent wastewaters were analysed in both sorbents in order to compare the results obtained. The range of concentrations found is shown in Table 5. It can be seen that the results obtained did not show considerable differences between the sorbents. Almost all of the compounds were found at significant levels in the analysed samples. BTR, 4TTR and 5TTR presented the highest concentrations of all of the compounds, while CIBTR and MeSBT were found in concentrations below the LOQs.

In influent wastewater, BTR, 4TTR and 5TTR showed the highest concentrations of all of the analytes (up to 1980 ng/L for BTR, Table 5). Lower concentrations were found for BT (up to 767 ng/L) and OHBT (up to 450 ng/L) and low concentrations of XTR and NH<sub>2</sub>BT could be successfully quantified. The results found for this type of sample are in line with the concentrations found in other studies [10]. Herrero *et al.* [20] found concentrations of BTR, 4TTR and 5TTR ranging between 392 and 2212 ng/L in influent wastewaters collected from the same Tarragona sewage treatment plant. Concentrations of OHBT (199 ng/L) and NH<sub>2</sub>BT (10 ng/L) were also around the same order of magnitude, while XTR and BT levels found were lower than their LOQ. In influent wastewater samples collected also from a sewage treatment plant in Spain, Carpinteiro *et al.* [19] found average levels of BTR, TTR, OHBT and BT of 2,470, 2,100, 150 and 830 ng/L, respectively.

In the case of effluent wastewaters, it was found that concentrations of BTR, 4TTR and 5TTR were very similar to those obtained for influent wastewaters. In effluent wastewaters, concentrations were slightly higher, probably because samples were

collected randomly on different days. BT, NH<sub>2</sub>BT and OHBT showed concentrations lower than those obtained in influent wastewaters, suggesting partial removal or degradation, as expected after the treatment process. Some of these results are in agreement with the literature, such as the study by Asimakopoulos *et al.* [12], in which the removal efficiencies of BTRs and BTs were studied in a treatment plant in Greece. They found low removal rates for BTR and TTR (4TTR and 5TTR), ranging from 25% to 68%, and high removal rates for OHBT. However, they highlighted that there is a discrepancy in the literature concerning the removal efficiencies of these analytes, especially in the case of BTs, so further comparison is not reliable.

In the case of river waters, relatively high concentrations of BT (up to 286 ng/L) were found compared to other compounds. Herrero *et al.* [20] reported a concentration of 60 ng/L for the River Ebre. The range of concentrations found for this compound in rivers in Spain was between 30 and 200 ng/L [10]. Lower concentrations of the rest of the compounds were found, ranging from 24 to 101 ng/L. It can be seen that these analytes are present in rivers, due to anthropological sources, such as industrial spills or incomplete removal in treatment plants. The applicability of both methods was confirmed, as several compounds were found and quantified in the samples analysed.

#### 4. Conclusions

The mixed-mode strong cation-exchange and anion-exchange sorbents can successfully retain the group of BTRs and BTs studied, with enough strength to allow washing with pure MeOH in order to develop a more selective extraction method. This was possible because the analytes and the ionic groups of the



sorbent establish ionic interactions, which seem to be stabilized by the resonance along both aromatic rings of the compounds capable of delocalizing electron densities. The strong character of the groups attached to the sorbents might have induced partial charges in the analytes promoting ion-dipole interactions. In contrary, the selected BSAs were always rinsed in the washing fractions probably because only hydrophobic interactions are involved in their retention. For this reason, these analytes were not further subject and only BTRs and BTs were more deeply studied. These types of interpretations are important in the development and evaluation of the mixed-mode sorbents used in analytical applications as they help to understand their capabilities to retain not only ionic analytes but also compounds that may develop partial charges.

It has been demonstrated for the first time that mixed-mode cation-exchange and anion-exchange sorbents can retain BTRs and BTs through ionic interactions even when they are neutral under working conditions, confirming that these sorbents not only work with pure ionic analytes but also neutral ones that are highly polarisable.

No substantial differences were observed in their performance when both sorbents were compared in river water and effluent and influent wastewater samples. Thanks to the possibility of including a pure MeOH washing step, the %ME obtained was around 20% or lower for wastewater samples and was practically negligible for river samples. The developed method using either Oasis MCX or Oasis MAX followed by LC-HRMS provided better results compared to other studies found in the literature.

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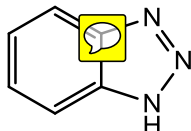
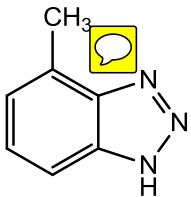
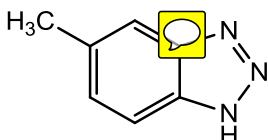
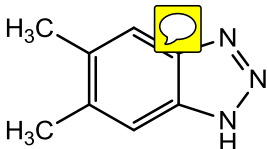
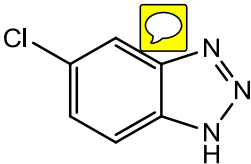
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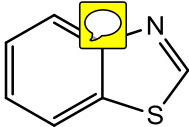
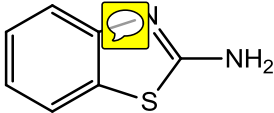
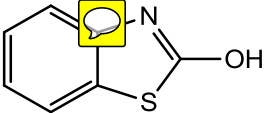
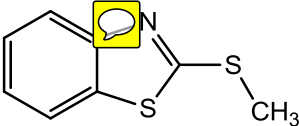
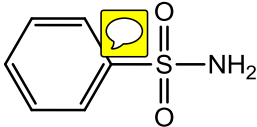
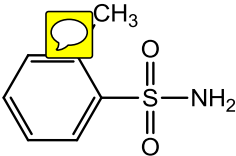
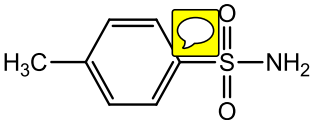
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**Table 1.** Chemical structure, pKa, log P, and exact masses of the studied analytes.

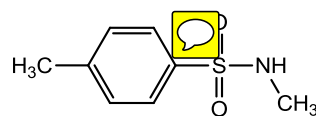
| Compound                            | Formula                                        | Structure                                                                            | pKa <sup>a, b</sup> | log P <sup>a</sup> | Molecular ion (m/z)             | Fragment Formula (exact mass, m/z)                                                                                                                                                                      |
|-------------------------------------|------------------------------------------------|--------------------------------------------------------------------------------------|---------------------|--------------------|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Benzotriazole (BTR)                 | C <sub>6</sub> H <sub>5</sub> N <sub>3</sub>   |    | 8.38                | 1.44               | [M+H] <sup>+</sup><br>120.05562 | C <sub>5</sub> H <sub>5</sub> <sup>+</sup><br>(65.03858)<br>C <sub>6</sub> H <sub>6</sub> N <sup>+</sup><br>(92.04948)                                                                                  |
| 4-methyl-1H-benzotriazole (4TTR)    | C <sub>7</sub> H <sub>7</sub> N <sub>3</sub>   |    | 8.74                | 1.82               | [M+H] <sup>+</sup><br>134.07127 | C <sub>6</sub> H <sub>7</sub> <sup>+</sup><br>(79.05423)<br>C <sub>6</sub> H <sub>5</sub> N <sub>2</sub> <sup>+</sup><br>(105.04472)<br>C <sub>6</sub> H <sub>5</sub> <sup>+</sup><br>(77.03858)        |
| 5-methyl-1H-benzotriazole (5TTR)    | C <sub>7</sub> H <sub>7</sub> N <sub>3</sub>   |    | 8.74                | 1.98               | [M+H] <sup>+</sup><br>134.07127 | C <sub>6</sub> H <sub>7</sub> <sup>+</sup><br>(79.05423)<br>C <sub>6</sub> H <sub>5</sub> N <sub>2</sub> <sup>+</sup><br>(105.04472)<br>C <sub>6</sub> H <sub>5</sub> <sup>+</sup><br>(77.03858)        |
| 5,6-dimethyl-1H-benzotriazole (XTR) | C <sub>8</sub> H <sub>9</sub> N <sub>3</sub>   |   | 8.92                | 2.28               | [M+H] <sup>+</sup><br>148.08692 | C <sub>7</sub> H <sub>7</sub> <sup>+</sup><br>(91.05423)<br>C <sub>7</sub> H <sub>9</sub> <sup>+</sup><br>(93.06988)<br>C <sub>6</sub> H <sub>5</sub> N <sub>2</sub> <sup>+</sup><br>(105.04472)        |
| 5-chloro-1H-benzotriazole (ClBTR)   | C <sub>6</sub> H <sub>4</sub> ClN <sub>3</sub> |  | 7.46                | 2.13               | [M+H] <sup>+</sup><br>154.01665 | C <sub>5</sub> H <sub>4</sub> Cl <sup>+</sup><br>(98.99960)<br>C <sub>6</sub> H <sub>4</sub> N <sup>+</sup><br>(90.03382)<br>C <sub>5</sub> H <sub>4</sub> <sup>37</sup> Cl <sup>+</sup><br>(156.01370) |

|                                                     |                                                 |                                                                                      |       |      |                                 |                                                                                                                                                                                           |
|-----------------------------------------------------|-------------------------------------------------|--------------------------------------------------------------------------------------|-------|------|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Benzothiazole (BT)                                  | C <sub>7</sub> H <sub>5</sub> NS                |    | 0.85  | 1.90 | [M+H] <sup>+</sup><br>136.02155 | C <sub>6</sub> H <sub>5</sub> S <sup>+</sup><br>(109.01065)<br>C <sub>5</sub> H <sub>5</sub> <sup>+</sup><br>(65.03858)                                                                   |
| 2-aminobenzothiazole (NH <sub>2</sub> BT)           | C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S  |    | 3.94  | 1.88 | [M+H] <sup>+</sup><br>151.03244 | C <sub>6</sub> H <sub>5</sub> S <sup>+</sup><br>(109.01065)<br>C <sub>5</sub> H <sub>5</sub> <sup>+</sup><br>(65.03858)<br>C <sub>6</sub> H <sub>6</sub> SN <sup>+</sup><br>(124.02155)   |
| 2-hydroxybenzothiazole (OHBT)                       | C <sub>7</sub> H <sub>5</sub> NOS               |    | 10.41 | 1.81 | [M+H] <sup>+</sup><br>152.01646 | C <sub>6</sub> H <sub>6</sub> SN <sup>+</sup><br>(124.02155)<br>C <sub>6</sub> H <sub>6</sub> N <sup>+</sup><br>(92.04948)<br>C <sub>6</sub> H <sub>5</sub> S <sup>+</sup><br>(109.01065) |
| 2-(methylthio)benzothiazole (MeSBT)                 | C <sub>8</sub> H <sub>7</sub> NS <sub>2</sub>   |    | 1.22  | 2.84 | [M+H] <sup>+</sup><br>182.00927 | C <sub>7</sub> H <sub>5</sub> S <sub>2</sub> N <sup>+</sup><br>(166.98579)<br>C <sub>6</sub> H <sub>5</sub> S <sup>+</sup><br>(109.01065)                                                 |
| Benzenesulfonamide (BSA)                            | C <sub>6</sub> H <sub>7</sub> NO <sub>2</sub> S |   | 10.08 | 0.49 | [M-H] <sup>-</sup><br>156.01247 | C <sub>6</sub> H <sub>6</sub> N <sup>-</sup><br>(92.05057)<br>SO <sub>2</sub> NH <sup>-</sup><br>(78.97335)                                                                               |
| o-toluenesulfonamide and p-toluenesulfonamide (TSA) | C <sub>7</sub> H <sub>9</sub> NSO <sub>2</sub>  |  | 10.17 | 1.13 | [M-H] <sup>-</sup><br>170.02812 | C <sub>7</sub> H <sub>8</sub> N <sup>-</sup><br>(106.06622)<br>SO <sub>2</sub> NH <sup>-</sup><br>(78.97335)                                                                              |
|                                                     |                                                 |  | 10.20 | 0.97 |                                 |                                                                                                                                                                                           |



n-methyl-p-toluenesulfonamide  
(Me-*p*-TSA)

C<sub>9</sub>H<sub>13</sub>NSO<sub>2</sub>



11.86

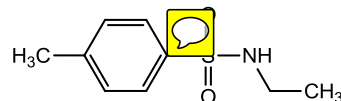
1.91

[M+H]<sup>+</sup>  
186.05832

C<sub>7</sub>H<sub>7</sub><sup>+</sup>  
(91.05423)

n-ethyl-p-toluenesulfonamide (Et-  
*p*-TSA)

C<sub>8</sub>H<sub>11</sub>NSO<sub>2</sub>



11.67

1.91

[M+H]<sup>+</sup>  
200.07398

C<sub>7</sub>H<sub>7</sub><sup>+</sup>  
(91.05423)

<sup>a</sup> Scifinder Database: Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2015 ACD/Labs)

<sup>b</sup> In all cases the pK<sub>a</sub> values indicated are referred to the loss of the proton from the neutral form with the exception of BT, NH2BT and MeSBT for which the pK<sub>a</sub> values are referred to the loss of the proton from the conjugated acid.

**Table 2.** % Recoveries or %R<sub>SPE</sub> obtained when 100 mL ultrapure water were percolated in Oasis MCX and Oasis MAX sorbents using either 2 mL or 5 mL of MeOH in the washing step.

|                                  | <i>Oasis MCX</i> |         |                  |         | <i>Oasis MAX</i> |         |                  |         |
|----------------------------------|------------------|---------|------------------|---------|------------------|---------|------------------|---------|
|                                  | <u>2 mL MeOH</u> |         | <u>5 mL MeOH</u> |         | <u>2 mL MeOH</u> |         | <u>5 mL MeOH</u> |         |
|                                  | wash             | elution | wash             | elution | wash             | elution | wash             | elution |
| <b>Benzotriazoles</b>            |                  |         |                  |         |                  |         |                  |         |
| BTR                              |                  | 95      |                  | 99      |                  | 103     |                  | 97      |
| 4TTR                             |                  | 93      |                  | 110     |                  | 101     |                  | 100     |
| 5TTR                             |                  | 97      |                  | 92      |                  | 111     |                  | 106     |
| CIBTR                            |                  | 99      | 100              |         |                  | 91      |                  | 101     |
| XTR                              |                  | 103     |                  | 107     |                  | 107     |                  | 108     |
| <b>Benzothiazoles</b>            |                  |         |                  |         |                  |         |                  |         |
| BT                               |                  | 73      |                  | 115     |                  | 89      |                  | 90      |
| NH <sub>2</sub> BT               |                  | 90      |                  | 85      |                  | 88      |                  | 111     |
| OHBT                             |                  | 60      | 110              |         |                  | 104     |                  | 104     |
| MeSBT                            |                  | 102     |                  | 114     |                  | 104     |                  | 73      |
| <b>Benzenesulfo-<br/>namides</b> |                  |         |                  |         |                  |         |                  |         |
| BSA                              | 86               |         | 84               |         |                  | 88      |                  | 73      |
| TSA                              | 80               |         | 87               |         |                  | 102     |                  | 80      |
| Me-p-TSA                         | 78               |         | 109              |         | 35               | 77      |                  | 104     |
| Et-p-TSA                         | 80               |         | 99               |         | 47               | 68      |                  | 105     |

%RSD (n=3) <13%

**Table 3.** %ME, %R<sub>SPE</sub> and %R<sub>apparent</sub> obtained when 100 mL of influent wastewater spiked at 5000 ng/L were percolated in both Oasis MCX and Oasis MAX sorbents.

|                       | Oasis MCX |                   |                        | Oasis MAX |                   |                        |
|-----------------------|-----------|-------------------|------------------------|-----------|-------------------|------------------------|
|                       | %ME       | %R <sub>SPE</sub> | %R <sub>apparent</sub> | %ME       | %R <sub>SPE</sub> | %R <sub>apparent</sub> |
| <b>Benzotriazoles</b> |           |                   |                        |           |                   |                        |
| BTR                   | -21       | 77                | 61                     | -22       | 77                | 59                     |
| 4TTR                  | -11       | 96                | 85                     | -19       | 82                | 66                     |
| 5TTR                  | 0         | 87                | 86                     | -7        | 84                | 77                     |
| CIBTR                 | -13       | 67                | 58                     | -21       | 64                | 50                     |
| XTR                   | 0         | 89                | 92                     | 0         | 82                | 80                     |
| <b>Benzothiazoles</b> |           |                   |                        |           |                   |                        |
| BT                    | 24        | 85                | 105                    | 0         | 52                | 52                     |
| NH <sub>2</sub> BT    | -25       | 86                | 64                     | -18       | 40                | 40                     |
| OHBT                  | -32       | 58                | 40                     | -29       | 90                | 63                     |
| MeSBT                 | -22       | 66                | 52                     | -26       | 86                | 64                     |

%RSD (n=3) <16%

**Table 4.** %ME and %R<sub>apparent</sub> obtained when 250 mL of river and effluent wastewater samples spiked at 2000 ng/L were percolated in both Oasis MCX and Oasis MAX sorbents.

|                       | Oasis MCX |                        |          |                        | Oasis MAX |                        |          |                        |
|-----------------------|-----------|------------------------|----------|------------------------|-----------|------------------------|----------|------------------------|
|                       | River     |                        | Effluent |                        | River     |                        | Effluent |                        |
|                       | %ME       | %R <sub>apparent</sub> | %ME      | %R <sub>apparent</sub> | %ME       | %R <sub>apparent</sub> | %ME      | %R <sub>apparent</sub> |
| <b>Benzotriazoles</b> |           |                        |          |                        |           |                        |          |                        |
| BTR                   | 14        | 96                     | -9       | 67                     | 5         | 75                     | -2       | 90                     |
| 4TTR                  | 12        | 103                    | -6       | 74                     | 4         | 87                     | -15      | 82                     |
| 5TTR                  | 13        | 103                    | -1       | 92                     | 11        | 89                     | -14      | 79                     |
| CIBTR                 | 14        | 87                     | -6       | 61                     | 5         | 77                     | -22      | 66                     |
| XTR                   | 20        | 105                    | 11       | 90                     | 16        | 92                     | 2        | 83                     |
| <b>Benzothiazoles</b> |           |                        |          |                        |           |                        |          |                        |
| BT                    | 12        | 92                     | 9        | 72                     | 8         | 65                     | 6        | 52                     |
| NH <sub>2</sub> BT    | 3         | 103                    | -7       | 86                     | -6        | 65                     | -10      | 44                     |
| OHBT                  | 19        | 77                     | -11      | 54                     | 2         | 72                     | -23      | 68                     |
| MeSBT                 | -4        | 83                     | -2       | 67                     | -19       | 93                     | -25      | 69                     |

%RSD (n=3) <20%

**Table 5.** Range of concentrations (ng/L) obtained when river, effluent and influent wastewater samples were analysed using the validated method, based on mixed-mode SPE followed by LC-HRMS.

|                       | River (ng/L) |            | Effluent (ng/L) |             | Influent (ng/L) |            |
|-----------------------|--------------|------------|-----------------|-------------|-----------------|------------|
|                       | Oasis MCX    | Oasis MAX  | Oasis MCX       | Oasis MAX   | Oasis MCX       | Oasis MAX  |
| <b>Benzotriazoles</b> |              |            |                 |             |                 |            |
| BTR                   | 24 - 27      | < LOQ - 30 | 544 - 2342      | 421 - 2067  | 954 - 1978      | 936 - 1980 |
| 4TTR                  | < LOQ - 15   | < LOQ - 18 | 994 - 1697      | 666 - 1308  | 743 - 1236      | 743 - 1210 |
| 5TTR                  | 28 - 43      | 30 - 47    | 971 - 1881      | 768 - 1610  | 687 - 1253      | 694 - 1043 |
| CIBTR                 | < LOQ        | < LOQ      | < LOQ           | < LOQ       | < LOQ           | < LOQ - 10 |
| XTR                   | < LOQ        | < LOQ      | < LOQ - 162     | < LOQ - 128 | < LOQ           | < LOQ - 58 |
| <b>Benzothiazoles</b> |              |            |                 |             |                 |            |
| BT                    | 176 - 286    | 218 - 221  | 243 - 272       | 265 - 358   | 286 - 431       | 474 - 767  |
| NH <sub>2</sub> BT    | 31 - 32      | 41 - 43    | 30 - 34         | 55 - 59     | 70 - 71         | 155 - 160  |
| OHBT                  | 86 - 94      | 91 - 101   | 123 - 182       | 92 - 136    | 410 - 450       | 347 - 408  |
| MeSBT                 | < LOQ        | < LOQ      | < LOQ           | < LOQ       | < LOQ           | < LOQ      |

**Figure captions**

**Figure 1.** Structure of the Oasis MCX and Oasis MAX sorbents and resonance structures of benzotriazole (BTR) and benzothiazole (BT).

