



Application of a homemade silica-based mixed-mode ion-exchange sorbent for the determination of drugs in environmental water samples

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ARTICLE INFO

Keywords:

mixed-mode ion-exchange sorbent
homemade sorbent
drugs of abuse and pharmaceuticals
solid-phase extraction
environmental water samples

ABSTRACT

Two homemade silica-based mixed-mode ion-exchange sorbents with strong moieties were compared for the solid-phase extraction (SPE) of a group of pharmaceuticals, drugs of abuse and metabolites. The difference between sorbents was the presence of graphene microparticles embedded in the network and the best results were obtained with the sorbent that did not contain graphene.

The optimum SPE protocol of the non-graphene sorbent was: loading pH at 3, different loading volumes depending on the type of sample, 2 mL of MeOH as washing solvent.

The optimized method was validated for the analysis of water samples of environmental interest (river water, effluent wastewater and influent wastewater). Apparent recoveries ranged from 22 to 68%, matrix effect values were lower than ± 20 for most compounds, and method detection limits ranged from 1 to 28 ng/L and method quantification limits from 2 to 56 ng/L. Precision was obtained as percentual relative standard deviation (RSD%, $n=4$) and the repeatability and reproducibility between days were always lower than 18%.

The sorbent was used to determine the analytes in environmental water samples, where most compounds were found in the range from 2 to 180 ng/L in river samples, from 3 to 2547 ng/L in effluent wastewater samples and from 24 to 8086 ng/L in influent wastewater samples.

1. Introduction

Sample preparation is one of the most important steps in the analytical procedure. This step can improve both the sensitivity of the determination, since in most cases the analytes are preconcentrated, and the selectivity by removing matrix interferences. Moreover, analytical instruments (e.g., columns, mass spectrometer) are protected due to the removal of harmful species that can damage them [1,2].

For the analysis of liquid samples, the use of sorptive techniques has replaced classic liquid-liquid extraction (LLE) and one of the most representative of these techniques is solid phase extraction (SPE) [3] although modification on the format of SPE have led to other techniques [4–9]. In recent years, in compliance with the principles of Green Chemistry [10], sustainable supports such as fabric [11], wood [12] or paper [13] have been successfully explored as new sorptive materials.

The most used sorbents in SPE are polymer-based due to their good performance and the wide range of different characteristics. Being the most common ones Strata X (commercialized by Phenomenex) and Oasis HLB (commercialized by Waters) [14–16]. These sorbents are characterized for their capacity to retain a wide range of compounds due

to their hydrophilic-lipophilic balance. However, they can lead to low selectivity in the extraction.

The search for more selective extraction methods has provided new materials such as molecular imprinted polymers (MIPs) [17], mixed-mode ion-exchange sorbents [18] or aptamers [19]. The mixed-mode ion-exchange sorbents can perform both reversed phase and ion-exchange interactions. The compounds can engage in specific interactions (ion-exchange interactions) or non-specific interactions (hydrogen bonding, dipole-dipole, or Van der Waals forces). When a clean-up step is included, the non-specific interactions can be disrupted, rinsing the compounds retained with these interactions. Then, the compounds retained through ion-exchange interactions can be selectively eluted [18].

Mixed-mode ion-exchange sorbents can be acquired commercially. For example, Waters offers the polymer-based sorbents “Oasis MCX, WCX, MAX and WAX” which perform strong (MCX and MAX) and weak (WCX and WAX) ion-exchange interactions. There are also commercial mixed-mode ion-exchange silica-based sorbents such as Isolute HCX or Isolute HAX (Biotage). Apart from the commercial ones, in recent years, homemade mixed-mode ion-exchange sorbents have been developed [20]. Among these new sorbents, zwitterionic mixed-mode sor-

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benz has also been developed to extract simultaneously acidic and basic analytes, solving one of the main drawbacks of the commercial sorbents. These new sorbents developed are either polymer-based [21–23] or silica-based [24–27].

Mixed-mode ion-exchange sorbents are designed to increase the selectivity of the extraction of acidic/basic compounds. Pharmaceuticals and drugs of abuse are two groups in which most compounds are acidic or basic and there is increasing concern about their presence in environment [28,29]. These compounds reach aquatic systems and have an impact on human health and aquatic life [30,31]. They are released as parent compounds or metabolites through human waste or improper disposal [29,31] and in recent years their occurrence and effects have been evaluated [28,30–33].

In the present study, two homemade silica-based sorbents were compared for the SPE of a group of pharmaceuticals, drugs of abuse and metabolites followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The protocol of the best-performing sorbent was optimized, validated and compared with Oasis MCX. Finally, the method developed was applied to determine the analytes in environmental water samples.

2. Experimental

2.1. Reagents and standards

Solid standards of nicotine (NIC), cotinine (COT), morphine (MOR), ranitidine (RAN), codeine (COD), 4-acetamidoantipyrine (4-APY), caffeine (CAF), ketamine (KET), benzoylecgonine (BE), tramadol (TRA), cocaine (COC), venlafaxine (VEN), oxazepam (OXA), methadone (MET) and diazepam (DIA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The structures and pK_a of these compounds are presented in Table S1. Standard solutions of 1000 mg/L were prepared in methanol (MeOH) and stored in the dark at -20°C. Working solutions were prepared weekly in water/MeOH (80/20).

HPLC grade MeOH, MS grade water and MS grade acetonitrile (ACN) were acquired from Carlo Erba (Val de Reuil, France). Formic acid, hydrochloric acid, acetic acid and ammonium hydroxide were purchased from Sigma-Aldrich. Ultrapure water was obtained through a Millipore water purification system (Burlington, MA, USA).

2.2. Synthesis of the homemade mixed-mode ion-exchange sorbents

The synthesis and characterization of the homemade mixed-mode ion-exchange sorbents were deeply described in previous studies [26,27]. Briefly, SiO₂-SCX/SAX and SiO₂-G-SCX/SAX sorbents were synthesized using a sol-gel approach using tetramethyl orthosilicate, methyl trimethoxysilane, octadecyl trimethoxysilane, N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride, and 3-mercaptopropyl trimethoxysilane as building blocks. In the case of SiO₂-G-SCX/SAX, graphene microparticles were added to the reaction mixture. A scheme of the structures of the sorbents is presented in Figure S1. Both sorbents are based on silica modified with sulfonic groups and quaternary amines to perform strong ion-exchange interactions and with C₁₈ chains to perform reversed-phase interactions.

2.3. SPE procedure

For SPE with homemade sorbents, 200 mg of each sorbent was placed in an empty 6 mL SPE cartridge (Symta, Madrid, Spain) with two 10 µm polyethylene frits (Symta) above and below the sorbent. The sorbent was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 3.

The SPE protocol optimized for river samples was a loading volume of 100 mL adjusted to pH 3 with HCl, a clean-up step with 2 mL of MeOH and an elution step with 5 mL of 5% NH₄OH in MeOH. The eluate was evaporated to dryness using a miVac Duo centrifuge evapora-

tor (Genevac, Ipswich, UK) and reconstituted with 1 mL of water/ACN, (95/5, v/v). The reconstituted extracts were filtered with 0.45 µm PTFE (polytetrafluoroethylene) syringe filters (Scharlab, Barcelona, Spain). The same protocol was applied for wastewater samples, except for the loading volume, which was 50 mL for effluent wastewater and 25 mL for influent wastewater.

For Oasis MCX cartridge containing 500 mg of sorbent was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 3. Then 250 mL of river water (or 50 mL of influent wastewater) adjusted to pH 3 with HCl was loaded into the cartridge. The clean-up step was 5 mL of MeOH and the elution step 5 mL of 5% NH₄OH in MeOH. The eluate was treated with the same protocol described above.

2.4. Chromatographic conditions

A liquid chromatograph Agilent 1260 Infinity II coupled to a triple quadrupole mass spectrometer detector Agilent 6460 was used. The chromatographic column was Luna Omega 5 µm Polar C18 (150 mm x 3.0 mm, 5 µm particle size) with a precolumn (3 mm x 3 mm), both acquired from Phenomenex (Torrance, CA, USA). The mobile phase was water with 0.1% of HCOOH as solvent A and ACN with 0.1% of HCOOH as solvent B. The column was thermostated at 30°C, the flow rate was 0.4 mL/min and the injection volume was 5 µL.

The gradient profile started with 5% of B for 3 min, and then the % of B was increased to 75% in 27 min, and to 100% in 4 min. Then, it was held at 100% for 3 min before being returned to the initial conditions in 2 min, where it was held for 4 min to stabilize the column.

The optimized conditions for the electrospray ionization source were N₂ flow rate of 12 mL/min, gas temperature of 340 °C, nebulizer pressure of 40 psi and capillary voltage of 2500 V in positive mode. The fragmentor potential for all compounds was 100 V and the collision energy for each product ion of each compound is presented in Table S2. The acquisition was performed in dynamic multiple reaction monitoring (dMRM) mode, using two transitions for each compound except for tramadol.

The instrumental linear range was obtained after analyzing the solutions with concentrations from 0.01 to 250 µg/L in triplicate. In all cases, two calibration curves were used, one for low concentrations and the other for high concentrations. In both cases, the determination coefficient (r^2) was always higher than 0.99. The instrumental limits of detection ranged from 0.01 µg/L (NIC, MET or TRA) to 0.25 µg/L (CAF or VAL), and the instrumental limits of quantification ranged from 0.05 to 0.5 µg/L.

2.5. Validation parameters

The method was validated in terms of apparent recovery at two levels of concentration, matrix effect, method limits of detection and quantification, and precision (repeatability and reproducibility between days).

The %R_{SPE} was obtained as the ratio between the experimental concentration after the SPE and the theoretical concentration of the standard solutions prepared in ultrapure water. When environmental samples were used, the apparent recoveries (%R_{app}) were calculated as the ratio between the experimental concentration after SPE and the theoretical concentration in the samples.

The matrix effect (%ME) was calculated with the following formula %ME = (C_{exp}/C_{the} x 100) - 100, where “C_{exp}” is the concentration obtained by spiking a non-spiked sample after SPE and “C_{the}” is the theoretical concentration. The signal is enhanced when %ME is positive, whereas when it is negative, it is suppressed.

The method limits of detection (MDL) and quantification (MQL) were estimated by applying the apparent recovery and the preconcentration factor to the instrumental limits due to the occurrence of some compounds in the samples.

Repeatability was obtained as %RSD (relative standard deviation) (n=4) by analyzing samples spiked at the same concentration on the

same day. The reproducibility between days was obtained as %RSD ($n=4$) by analyzing samples spiked at the same concentration on different days.

3. Results and discussion

3.1. Optimization of the SPE procedure

In previous studies, SiO₂-SCX/SAX was evaluated for the selective determination of six basic pharmaceuticals in environmental water samples [26], while SiO₂-G-SCX/SAX was applied for the determination of a group of benzothiazoles, benzotriazoles and benzenesulfonamides in environmental water samples [27] and for the selective determination of 2-aminobenzothiazole in environmental water, fish, and indoor dust samples [34]. The difference between SiO₂-SCX/SAX and SiO₂-G-SCX/SAX is the presence of graphene microparticles in the silica network. The graphene enhances the π - π interactions as was proved for benzothiazoles, benzotriazoles and benzenesulfonamides [27], with compounds with high π -density (benzothiazoles and benzotriazoles) and low π -density (benzenesulfonamides). Since all the selected analytes (except RAN) have at least one benzene ring, the effect of the graphene in the retention of compounds with low π -density is interesting to be evaluated.

Considering the structures and pK_a of the compounds (Table S1), the use of different loading pHs was evaluated. A total of 25 mL of ultrapure water standard solutions with a pH adjusted to 3, 5, 7 and 9 was percolated in both sorbents. A clean-up step of 1 mL of MeOH was included to rinse the compounds retained through non-specific interactions and increase the selectivity. The elution was performed with, consecutively, 5 mL of 5% NH₄OH in MeOH and 5 mL of 5% HAc in MeOH.

As can be seen in Table 1, the retention of compounds decreased as the pH increased, and the retention of the analytes in both sorbents was acceptable when the loading pH was 3, with results being higher than 50% in most cases. It should be pointed out that some compounds showed a significant decrease in recoveries between pH 3 and pH 5. For instance, COT had a recovery of 55% at pH 3 and 11% at pH 5 when SiO₂-G-SCX/SAX was used. And RAN had a recovery of 83% at pH 3 and 38% at pH 5 when SiO₂-SCX/SAX sorbent was used. In general, pH 3 showed the highest recoveries, so it was selected for the subsequent experiments. However, three compounds presented low SPE recoveries at all pHs: OXA, 4-APY and CAF. The clean-up portion (MeOH) was analyzed and it was observed that these three compounds were retained in the sorbent but eluted with MeOH, meaning that they were retained mainly through reversed-phase interactions instead of through ion-exchange ones. Regarding the structures and pK_a of OXA, 4-APY and

CAF, these three analytes behaved as neutral compounds in the working pHs. Moreover, as CAF can present an aromatic character [35]. The π electronic density present in this compound is higher than in the other compounds, allowing it to interact better with SiO₂-G-SCX/SAX than with SiO₂-SCX/SAX, due to the π - π interactions. For this reason, the retention of CAF in SiO₂-G-SCX/SAX was better than in SiO₂-SCX/SAX, but unfortunately, π - π interactions are disrupted with MeOH. Since OXA, 4-APY and CAF did not presented ion-exchange interactions, the subsequent experiments were performed without these compounds.

In terms of performance, SiO₂-SCX/SAX showed better SPE recoveries for the compounds. For instance, NIC and MOR showed recoveries of 80 and 95% for SiO₂-SCX/SAX respectively, and 40% and 58% for SiO₂-G-SCX/SAX. The addition of graphene allows the sorbent to perform π - π interactions. However, the presence of graphene in the sorbent inherently reduces the number of ion-exchange sites. Therefore, SiO₂-SCX/SAX was selected as better exploited the ion-exchange interactions with most of the studied compounds, compared to SiO₂-G-SCX/SAX.

For the elution step, the acidic elution (5 mL of 5% AcOH in MeOH) was discarded since the analytes were retained through SCX interactions and they were rinsed with the basic elution (5 mL of 5% NH₄OH in MeOH).

This protocol was consistent with that used in studies which determined basic compounds. The use of commercial mixed-mode cation-exchange sorbents has been widely reported [29,33,36–40], Oasis MCX being the most commonly used. The studies on these sorbents used basic elution and an acidic pH for the loading solution to protonate, allowing them to interact with the sulfonic groups in the sorbent.

The clean-up step is important when working with mixed-mode ion-exchange sorbents because it increases the selectivity of the extraction. Higher clean-up volumes were evaluated (2 mL and 5 mL of MeOH). Figure 1 shows the results obtained for each clean-up protocol. The increase in the volume of MeOH reduced the SPE recoveries for most of the compounds. When the volume increased from 1 mL to 2 mL, this reduction was slight: for instance, %R_{SPE} (TRA) decreased from 96% to 79% and %R_{SPE} (MOR) from 95% to 82%. When 5 mL of MeOH was used in the clean-up step, most of the compounds showed a slight decrease in the SPE recoveries (e.g., TRA, VEN or VEN). But compounds such as NIC, KET and RAN showed a considerable decrease. Thus, 2 mL of MeOH was selected for the clean-up step. The clean-up step reported in several papers was based on water [33,37–40], leading to a less selective extraction.

The loading volume was evaluated with ultrapure water and was increased from 25 to 100 mL. The recoveries did not decrease more than 6% when 100 mL of ultrapure water was percolated and were higher than 60% for all compounds except MET and COT. Therefore, 100 mL was selected as the loading volume.

After optimization, the loading pH was 3, the clean-up was 2 mL of MeOH and the elution step was 5 mL of 5% NH₄OH in MeOH. The loading volume with ultrapure water was 100 mL. However, when the complexity of the samples increases, the loading volume should be reduced taking into account the breakthrough volume. In this sense, the loading of different volumes of river water, effluent wastewater and influent wastewater samples was tested. The volumes selected were 25 mL for influent wastewater samples, 50 mL for effluent wastewater samples and 100 mL for river samples.

3.2. Method validation

The method was validated for river water, effluent wastewater and influent wastewater samples according to the parameters described in Section 2.5.

The apparent recoveries were obtained at two levels of concentration for the three types of water and results are shown in Table 2. The apparent recoveries (discarding BE and DIA) ranged from 25 to 68% in river samples, from 28 to 65% in effluent wastewater samples and from 23 to 57% in influent wastewater samples. In the case of BE and DIA,

Table 1
SPE recoveries of the compounds when 25 mL of ultrapure water were percolated in each sorbent at different pH.

Compound	SiO ₂ -G-SCX/SAX				SiO ₂ -SCX/SAX			
	pH 3	pH 5	pH 7	pH 9	pH 3	pH 5	pH 7	pH 9
NIC	40	44	30	31	80	65	67	62
COT	55	11	14	7	65	15	3	5
MOR	58	53	64	47	95	78	75	42
RAN	30	6	20	0	83	38	21	6
COD	63	44	57	44	94	87	76	45
4-APY	10	7	12	4	8	2	14	3
CAF	15	16	21	13	10	12	12	6
KET	30	38	27	16	77	72	52	24
BE	52	15	17	9	81	30	24	10
TRA	43	13	40	32	96	81	67	63
COC	54	8	35	21	88	55	48	33
VEN	72	65	58	47	92	84	78	62
OXA	17	12	18	10	18	10	15	9
MET	8	0	3	2	55	45	35	21
DIA	45	18	24	14	81	16	21	15

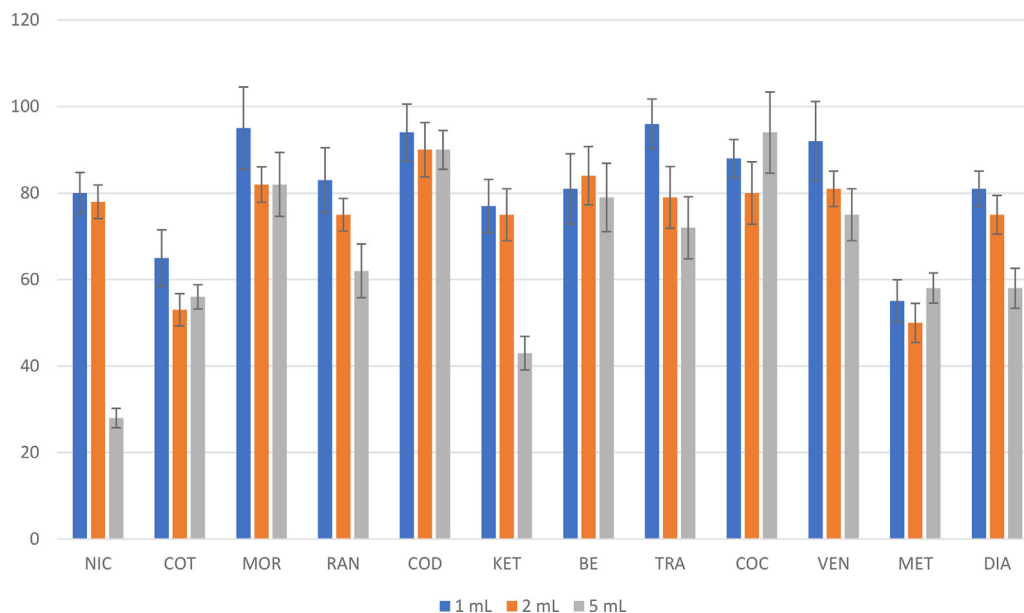


Figure 1. SPE recoveries of the compounds when 25 mL of ultrapure water was percolated evaluating different clean-up volumes.

Table 2

Apparent recoveries of the compounds at two levels of concentration and matrix effect in different matrices.

Compound	River			Effluent wastewater			Influent wastewater		
	%R _{app}		%ME	%R _{app}		%ME	%R _{app}		%ME
	0.1 µg/L	0.5 µg/L	0.5 µg/L	0.2 µg/L	1 µg/L	1 µg/L	0.4 µg/L	2 µg/L	2 µg/L
NIC	34	40	4	29	34	-1	-*	36	-17
COT	46	44	-5	42	39	9	32	30	1
MOR	31	25	-10	32	28	-3	24	23	-5
RAN	30	34	-10	41	49	-20	48	41	-17
COD	53	64	-10	53	60	-10	51	57	-17
KET	40	43	-10	34	37	-7	31	36	-8
BE	20	14	-1	14	17	-4	22	18	-8
TRA	58	65	-8	52	44	-10	-*	38	-14
COC	41	45	-7	48	43	-8	37	34	-13
VEN	52	57	-11	65	64	-18	46	42	-12
MET	59	68	1	63	58	-5	57	53	-15
DIA	25	22	-5	32	29	-9	22	26	-28

%RSD (n = 3) < 14%.

*Natural occurrence higher than spiked concentration.

the good SPE recoveries (84 and 81%) obtained with ultrapure water decreased significantly when the matrix was more complex, and the apparent recoveries ranged from 14 to 26%. The apparent recovery of NIC and TRA when the influent wastewater samples were spiked at 0.4 µg/L could not be obtained because these compounds were present at levels higher than the spiked values.

As can be observed in Table 2, the %ME ranged from -11 to +4 in river samples, from -20 to +9 in effluent wastewater samples, and from -17 to +1 in influent river samples, except for DIA. The sorbent proved to be selective since the matrix effect for all compounds was lower than ±20% except for DIA in influent wastewater (-28%). Yuan *et al.* [36] obtained values between -33 and +14% for KET, MOR, 4-APY, COD, COC and BE (among others) in influent wastewater samples when they used Oasis MCX.

The MDLs and MQLs are presented in Table S3. For river samples, the MDLs were lower than 7 ng/L and the MQLs lower than 15 ng/L. For effluent wastewater samples, the MDLs were lower than 17 ng/L and the MQLs lower than 34 ng/L. And for influent wastewater samples, the MDLs were lower than 28 ng/L and the MQLs lower than 56 ng/L. In all cases, the compound with the highest limit was NIC, whereas RAN, COC and MET presented limits lower than 10 ng/L in the three matrices.

In the case of precision, repeatability (% RSD, n = 4) was lower than 13% and reproducibility between days (% RSD, n = 4) was lower than 18% for all compounds in all the matrices.

Different cartridges were used during the study without any differences among them, which confirms that inter-cartridge reproducibility. The cartridges were reused during the study and more than 15 environmental samples could be extracted in the same cartridge.

3.3. Comparison with Oasis MCX

Since SiO₂-SCX/SAX behaved like a mixed-mode strong cation-exchange sorbent (SCX), it was interesting to compare it with Oasis MCX, a commercial mixed-mode SCX sorbent. The sorbent was compared for river and influent wastewater samples (being the least and the most complex samples) applying the procedures described in Section 2.3. The procedure for Oasis MCX was adapted from a previous study performed in the group [41] where seven drugs of abuse and their metabolites were determined in river, effluent wastewater and influent wastewater samples. In that study the loading volume was 250 mL for river samples and 100 mL for influent wastewater samples, 5 mL of MeOH as washing step and 15 mL of 5% NH₄OH in MeOH as elution

Table 3

Apparent recoveries and matrix effect of Oasis MCX for river water (0.5 µg/L) and influent wastewater (2 µg/L) samples.

	River (250 mL)		Influent wastewater (50 mL)	
	%R _{app}	%ME	%R _{app}	%ME
NIC	52	-23	13	-20
COT	87	-12	51	-46
MOR	91	-6	87	-32
RAN	79	+13	88	-3
COD	100	-5	87	-35
BE	81	+16	82	-34
KET	45	+30	68	-12
TRA	84	+14	78	-25
COC	79	-34	72	-33
VEN	22	+10	27	-26
MET	76	+16	54	-18
DIA	72	+8	62	-24

%RSD (n = 3) < 12%.

step. In the present study, the loading volume with influent wastewater samples was reduced to 50 mL and the elution volume was reduced to 5 mL.

Table 3 shows the apparent recoveries and matrix effect obtained for both types of samples being most apparent recoveries above 40%. The recoveries in both matrices are largely similar, except for NIC and COT. As expected, the matrix effect is higher in influent wastewater samples because of their complexity, except for NIC, RAN, KET and COC.

The results obtained for Oasis MCX can be compared with the ones obtained for SiO₂-SCX/SAX (Table 2) even though the amount of sorbent and the loading volume were not the same. For Oasis MCX and SiO₂-SCX/SAX, the amount of sorbent was 500 mg and 200 mg, respectively. Likewise, for Oasis MCX the loading volume was 250 mL and 50 mL for river water and influent wastewater, respectively, and for SiO₂-SCX/SAX it was 100 and 25 mL. As far as the results of river samples are concerned, the recoveries with Oasis MCX were higher than the ones with SiO₂-SCX/SAX. These differences were more pronounced with compounds like MOR or BE. Moreover, in terms of the matrix effect, SiO₂-SCX/SAX showed better results than Oasis MCX, with the %ME being lower in almost all compounds. The greatest differences were NIC, BE and COC. Similar trends were obtained for influent wastewater samples.

It should be highlighted that SiO₂-SCX/SAX is silica-based and Oasis MCX is polymer-based whose core might interact with the compounds through non-specific interactions, while the silica-based sorbents tend to perform fewer non-specific interactions, showing lower matrix effect. In this line, Oasis MCX presented higher recoveries while SiO₂-SCX/SAX presented a lower matrix effect, so SiO₂-SCX/SAX proved to be more selective than Oasis MCX.

There are other studies where Oasis MCX was used for the determination of similar compounds. For instance, in the study previously mentioned [41], the apparent recoveries for MOR, COC, COD and MET ranged from 82 to 154% for river samples and from 55 to 128% in influent wastewater samples. However, the matrix effect observed was significantly higher, ranging from +8 to +41% in river samples and from -20 to -57% in influent samples, even though the influent samples were diluted 1:5 to reduce the matrix effect.

There are more studies where these analytes were determined using Oasis MCX. For example, Wang *et al.* [40] obtained recoveries ranging from 85 to 111% for COC, MOR and COD in river samples. Krizman *et al.* [38] determine MOR, COD, TRA, MET and 4-APY (among others), and obtained recoveries ranging from 73 to 109% in river water, from 73 to 116% in effluent wastewater and from 79 to 97 in influent wastewater samples. It should be pointed out that both studies used deuterated compounds as internal standards, then the relative recoveries are reported.

Table 4

Range of concentrations (ng/L) obtained after the analysis of river water, effluent wastewater and influent wastewater samples (n=4).

	River (100 mL)	Effluent wastewater (50 mL)	Influent wastewater (25 mL)
NIC	54 – 180	160 – 310	956 – 8086
COT	10 – 13	20 – 36	160 – 1453
MOR	<MQL - 15	12 - 51	323 - 383
RAN	<MDL	8 - 10	25 - 103
COD	9 – 13	20 -72	66 - 153
KET	<MDL	28 – 58	24 - 127
TRA	40 - 80	1311 - 2547	1058 - 2166
COC	2 - 14	3 - 25	408 - 1222
VEN	15 - 30	234 - 697	373 - 1245
MET	2 - 14	99 - 210	84 - 189
DIA	<MDL - 7	38 – 72	54 -428

3.4. Analysis of environmental samples

Four samples from river water (Ebro River, Spain), effluent wastewater (Tarragona, Spain) and influent wastewater (Tarragona, Spain) were analyzed with the optimized method. The analytes were quantified by applying the external calibration curves described above and taking into account the %R_{app} and the dilution factor. It was decided not to use the matrix-matched calibration curves because of the natural occurrence of the compounds in the samples and the low matrix effects obtained (Table 2).

Table 4 shows the concentrations obtained. In the river samples, all compounds were detected except for RAN and KET in all samples and DIA in two samples. NIC was present at the highest concentrations, from 54 to 180 ng/L. The concentration of TRA was also high, ranging from 40 to 80 ng/L. Wang *et al.* [40] quantified COC, MOR and COD in river samples from China. MOR was not detected, COC was found between 0.05 and 13 ng/L, similar to the concentration observed in the present study (2 – 14 ng/L), while COD was found in lower concentrations (0.8 – 1.8 ng/L versus 9 – 13 ng/L). Briudes *et al.* [42] quantified TRA, MET, COD, COC and VEN in samples from the Seine River (France). VEN (3 – 27 ng/L), COC (MQL – 8 ng/L), COD (3 – 14 ng/L) and TRA (12 – 90 ng/L) were quantified at similar levels, while the concentration of MET was slightly lower (0.5 – 1.8 ng/L). RAN and VEN were quantified in similar samples from the Ebro River in a previous study [26], where RAN concentration ranged from 24 to 32 ng/L and VEN from 32 to 72 ng/L.

In the effluent wastewater samples, all compounds were quantified (Table 4), being TRA, VEN and NIC the compounds present at the highest concentrations. The concentration of the other compounds was lower than 100 ng/L (except MET). Gómez-Canela *et al.* [43] determined RAN, TRA, VEN and DIA (among others) in effluent wastewater samples from Barcelona (Spain). TRA was also the most occurring compound (141 – 1200 ng/L), VEN and DIA were observed at similar concentrations (104 – 820 ng/L and 52 – 100 ng/L) and RAN was detected at higher levels (26 – 97 ng/L). Masemola *et al.* [33] determined MOR and COC (among others) in effluent wastewater samples from Florida (USA) at significantly higher concentrations of 65 – 142 ng/L and 265 – 328 ng/L, respectively. It should be highlighted that the differences between concentrations from different origins may be due to the influent water treatment carried out at wastewater treatment plants.

In influent wastewater samples, all compounds were quantified in all samples. Figure 2 shows a chromatogram of an influent wastewater sample. Some compounds were quantified in concentrations higher than 1 µg/L. This was the case of NIC and TRA in all samples and COT, COC and VEN in some. Krizman *et al.* [38] determined MOR, COD, TRA and MET (among others) in influent wastewater samples from Split, Croatia). The concentration of TRA was significantly lower (586 – 717 ng/L) while the concentration of COD was higher (237 – 625 ng/L), and the concentration of MOR and MET was similar (142 – 445 and 71 – 94

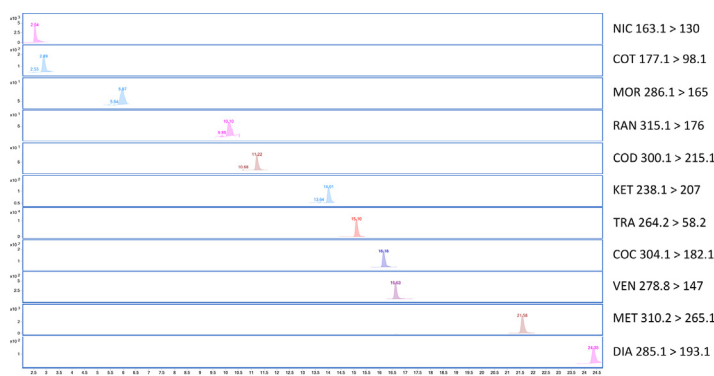


Figure 2. Chromatogram of an influent sample extracted using SiO₂-SCX/SAX.

ng/L). Gilart *et al.* [44] determined MOR, RAN, COD, MET and COC in samples from the same wastewater treatment plant (Tarragona, Spain). The concentration of RAN was significantly higher (1028 – 3293 ng/L), and the concentration of COC was slightly lower (163 – 500 ng/L). The concentrations of MOR, COD and MET were similar. It can be unexpected that in some cases, such as TRA or MET, the concentrations in effluent are similar or higher than the concentrations found in influent samples. It can be explained since the samples analyzed were collected in different periods of time, so the effluent and the influent were not related.

4. Conclusions

Two homemade silica-based materials were compared for the solid-phase extraction of a mixture of basic pharmaceuticals, drugs of abuse and metabolites. The materials were functionalized with the same ionic groups and the difference was the presence of graphene in the silica network in one of the sorbents.

The presence of graphene did not enhance the retention of the analytes, since it hindered the specific ion-exchange interaction with the analytes.

Good validation parameters were obtained for different types of sample (river water, effluent wastewater and influent wastewater). The homemade sorbent, SiO₂-SCX/SAX, showed higher selectivity (lower matrix effect) but lower capacity (lower recoveries) than Oasis MCX. The method developed for SiO₂-SCX/SAX was successfully applied to determine the analytes in river water, effluent wastewater and influent wastewater samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the Spanish Ministry of Economy, Industry and Competitiveness, the Spanish State Research Agency, and the European Regional Development Fund (ERDF) (PID2020-114587GB-I00) for their financial support. A. Moral would also like to thank Universitat Rovira i Virgili (URV) for his PhD grant (2020PMF-PIPF-33).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.sampre.2023.100074](https://doi.org/10.1016/j.sampre.2023.100074).

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