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Evaluation of direct sample injection as a fast, no-sample handling, approach for the LC-MS/MS monitoring of pharmaceuticals in different water matrices

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ABSTRACT

The determination of drug residues in water, particularly in environmental water, is a hot topic due to the usual presence of these emerging contaminants in the aquatic environment and their potential negative impact on water quality. The most widely approach applied at present for their determination is the use of a solid phase extraction (SPE) step followed by the LC-MS/MS measurement. This is due to the theoretical need to pre-concentrate the analytes and to produce "clean" sample extracts leading to less inferences in subsequent analysis. However, in LC-MS/MS based methods, the main interferences are not "visible" and are mostly due to matrix effects, which in fact are produced by unknown compounds that co-elute with the analytes and therefore not easily removed by SPE. As an alternative, an increasing trend is observed towards the use of direct injection (DI) of the samples, which is nowadays possible thank to the notable improvement in sensitivity of modern LC-MS/MS instrumentation.

In this work, both approaches, SPE and DI, have been evaluated for the determination of 16 pharmaceuticals in three different types of water: groundwater, surface water and effluent wastewater. The study has been performed by using pharmaceutical reference standards and their corresponding isotope-labelled internal standards (ILIS) for efficient matrix effects correction. Both methodologies have been compared in terms of matrix effects, sensitivity, and suitability for the analysis of real-world water samples. Our data show that DI is an efficient alternative to SPE, with satisfactory analyte recoveries for most pharmaceuticals, matrix effects even lower than in SPE, and sufficient sensitivity for most of applications. In addition, the absence of sample treatment minimizes potential errors associated with this step, and there is a notable saving in the analysis time and costs. The analysis of Quality Control (QC) samples included in different sample batch sequences has been used to support the feasibility of using DI in this type of analysis.

1. Introduction

The increasing interest of monitoring contaminants of emerging concern (CECs) can be justified by their constant presence in environmental samples. Pharmaceuticals are among the CECs of more worry because of the ecotoxicological risk that they have associated [1]. Moreover, in some cases, they can be bioaccumulated in living organisms, which represents a hazard for environment and human health [2–4]. Especially noteworthy is the case of antibiotics, since they are directly related with the proliferation of antibiotic resistant microorganisms [5–7].

The presence of pharmaceuticals in environmental water is a fact widely reported in the scientific literature [3,8–10], however until recently environmental regulations barely included the control of pharmaceuticals in aquatic samples. The European Union Watch List is used for EU-wide monitoring data of substances that can pose a hazard for aquatic environment and includes in the latest update [11] four antibiotics together with the antidepressant venlafaxine and its main metabolite. Nevertheless, the currently collected data on environmental relevance and toxicological effects seems to be not enough to develop water policies on pharmaceuticals yet [7].

Pharmaceutical contamination comes mainly from urban wastewater, which many times includes wastewater discharges from hospitals with high amounts of unchanged drugs and metabolites [1]. It is rather

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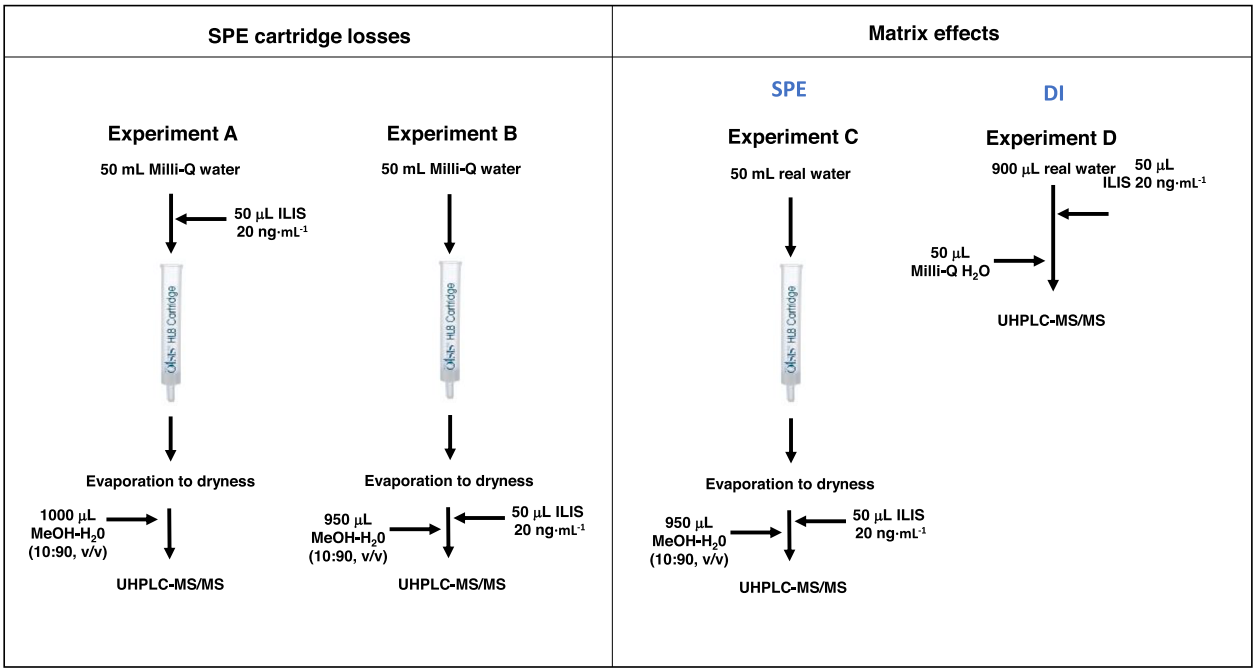

Fig. 1. Experimental design of the study for SPE cartridge losses (experiments A and B) and matrix effects in both SPE (experiment C) and DI (experiment D).

common to find high concentrations of pharmaceuticals in influent wastewater (IWW), reaching tens, even hundreds of ppbs, which, if not completely removed during the treatment processes, end up in the aquatic environment through wastewater treatment plant (WWTP) discharges [12]. The final concentrations of these CECs in the environment may vary a lot depending on the characteristics of the WWTP treatment, the flow of wastewater discharged, and the pharmaceutical consumption pattern of the population [13,14].

To study the occurrence of CECs in all type of water samples, the last advances of chromatographic techniques coupled to mass spectrometry (MS) have been crucial [15]. The most widespread approach currently applied for the determination of pharmaceuticals is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with a previous sample treatment based on solid phase extraction (SPE) [16–20]. Theoretically, the main objective of the SPE step is to pre-concentrate the analytes as well as obtaining cleaner extracts for the subsequent LC-MS/MS analysis. This is because pharmaceuticals are usually present at (sub) ppb levels and because SPE is expected to "clean up" the samples in order to better maintain chromatographic columns and mass analysers and to reduce matrix effects (ME) [21,22]. Despite these potential advantages, SPE step can also lead to analyte losses and to an increase of analytical errors associated with sample manipulation [21,23]. Although not usually reported, SPE not always decreases ME in LC-MS/MS analysis, because ME are due to different matrix components that co-elute with analytes [24,25], and such unknown components will be also pre-concentrated together with analytes along the SPE process. The direct injection (DI) of samples, even after previous dilution with ultrapure water in the case of more complex-polluted samples, such as untreated wastewaters, is an attractive alternative for analysis of CECs in water that is being increasingly used in the last years [9,12–14,26–30]. This approach is nowadays possible due to the notable improvement in sensitivity of the modern LC-MS/MS instrumentation. It allows to minimize sample treatment and much faster analysis, being a suitable approach for target quantitative analysis particularly in monitoring campaigns with a large number of samples to be analysed. Some works have used both methodologies to study the occurrence of contaminants in different water samples [31,32] and the performance of both methods [33], obtaining satisfactory results for direct injection.

In this work, SPE and DI have been evaluated and compared for the LC-MS/MS determination of 16 pharmaceuticals, selected as model compounds, in three types of water samples (groundwater, surface water and effluent wastewater). To this aim, different experiments using their corresponding isotopically labelled internal standard (ILIS) have been performed to evaluate matrix effects and potential losses associated with SPE procedure, calculating the accuracy of the procedure. Finally, real-world water samples have been analysed by both analytical methodologies in order to test their overall applicability and compare the concentration levels found for the pharmaceuticals selected. Special emphasis has been made to the robustness of the DI approach by analysing a notable number of Quality Control samples prepared in different water types along one year.

2. Materials and methods

2.1. Chemicals

A number of 16 pharmaceutical and their corresponding ILIS were selected as model compounds in this study (see Table S1 and Fig. S1). The criteria for this selection was to include at least one compound from different therapeutic families and the availability of its respective analyte-ILIS at our laboratory. In case of antibiotics, we selected several more compounds since they are of major concern in environmental studies. More details on reagents and chemicals are included in Supporting Information.

2.2. Instrumentation

A UPLC™ system (Acquity, Waters, Milford, MA, USA) interfaced to a triple quadrupole mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK) was used for analytical determination. The MS/MS conditions for analytes and ILIS are shown in Table S1.

The UPLC™ system (Acquity, Waters, Milford, MA, USA) was interfaced to a triple quadrupole mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK). LC separation was performed with a 100 × 2,1mm, 2,7 µm particle size CORTECS C_{18} analytical column (Waters). Column temperature was set to 40 °C. The mobile phases employed consisted of water (A) and MeOH (B) both with 0.1% HCOOH and 2 mM NH_4OAc, at a flow rate of 0,4 mL min^{-1}. The gradient program started

with 5% B, increased linearly to 95% of B for 9 min, increased at 9,1 min to 99% B and maintained at 99% B until minute 10. Finally, the gradient was held to initial conditions in order to re-equilibrate the column. 50 μL were selected as injection volume.

ESI was operated in positive ionization mode (ESI +) and capillary voltage and source temperature were set at 2 Kv and 150 °C, respectively. Drying and nebulizing gas was nitrogen (Praxair, Valencia, Spain). Desolvation gas flow was set to 1200 L h^{-1} and the cone gas to 250 L h^{-1}. Desolvation gas temperature was set at 650 °C. For operating in MS/MS mode, argon (99,995%; Praxair, Valencia, Spain) was used as collision gas at 0,13 mL min^{-1}). Three selected reaction monitoring (SRM) transitions were acquired for each compound (Table S1), and dwell time values ranging from 13 to 38 ms were applied in order to obtain 12 points per peak.

TargetLynx (MassLynx v. 4.1, Waters, Manchester, UK) software was used to process the quantitative data.

In order to confirm peak identity, the q_i/Q ratio, defined as the ratio between the signal of the confirmation transition (q_i) and the quantification transition (Q), was calculated as the mean value of the whole calibration curve injected in duplicate. This value was taken as reference. The ion ratio obtained in positive samples was compared with the reference value and the deviation calculated. A deviation ≤ 30% in at least one q_i/Q ratio (two ion ratios were available when three transitions were acquired) was taken as acceptance criteria to confirm the identity of a compound in sample.

2.3. Samples

Six different water samples were selected for this study: 2 ground waters (GW1 and GW2), 2 surface waters (SW1 and SW2) and 2 effluent wastewaters (EWW1 and EWW2). All samples were collected in different places of Castellón province: GW1 was from an irrigation well (39°59′23.3″N, 0°13′44.0″W) and GW2 from a borehole near to a solid-waste treatment plant (39°59′44.9″N, 0°13′59.5″W); SW1 and SW2 were taken from Mijares river (39°59′55.3″N, 0°13′46.2″W) and from *Clot de la Mare de Déu* in Borriana (39°52′46.8″N, 0°03′31.3″W), respectively; EWW1 and EWW2 consisted of 24-h composite samples collected from the WWTP of Castellón. All samples were frozen and stored at −18 °C until their analysis.

2.4. Study design

Several experiments were carried out with the aim to compare the applicability of SPE and DI based methodologies (Fig. 1). All experiments were carried out by duplicate using the six samples selected in this study, all spiked at 0,1 ng $mL^{-1}$with analyte-ILIS instead of the analytes themselves to circumvent the problem associated with the usual presence of analytes in the "blank" samples used in this study.

2.4.1. SPE cartridge losses

SPE cartridge losses were evaluated using Milli-Q water spiked with ILIS before and after application of the SPE step:

- *Experiment A*: 50 mL of Milli-Q water was spiked with 50 μL of ILIS mixture of 20 ng mL^{-1} before being subjected to the SPE procedure.
- *Experiment B*: after passing 50 mL of milli-Q water through SPE cartridge and evaporated to dryness, the final residue was spiked with 50 μL of ILIS mixture of 20 ng mL^{-1} and then reconstituted with 950 μL MeOH:water (10:90, *v/v*).

Recoveries of each compound after SPE were calculated comparing the signals in Experiments A and B, according to Eq. (1).

$$\text{SPE Recovery}(\%) = \frac{\text{Signal of ILIS in Experiment A}}{\text{Signal of ILIS in Experiment B}} \text{ x } 100 \quad (1)$$

2.4.2. Matrix effect

Generally, matrix effect (ME) is evaluated by comparing the signal of samples spiked with the compound under study with the signal of the corresponding reference standard in solvent, both at the same concentration. However, as many real-world samples (particularly wastewater) contain the studied pharmaceuticals, the samples were spiked with analyte-ILIS instead of the native unlabeled compounds. The fact that isotopically-labelled compounds are not present in the "blank" samples and that their behavior is similar to the non-labelled analytes make this approach highly recommendable [7,30,34,35].

ME for SPE procedure was evaluated using *Experiment C*. After passing 50 mL of the water sample through SPE cartridge and evaporated to dryness, the final residue was spiked with 50 μL of ILIS mixture at 20 ng mL^{-1} and then it was reconstituted with 950 μL MeOH:water (10:90, *v/v*). In the DI procedure, ME was evaluated (*Experiment D*) spiking the water samples with 50 μL of ILIS mixture of 20 ng mL^{-1}, as described in section 2.5. In both cases, the response of ILIS in the samples was compared with the signal of ILIS prepared in solvent at the same level (final concentration in the extract/sample injected 0,1 ng mL^{-1}), according to Eq. (2).

$$\text{ME } (\%) = \frac{\text{Signal of ILIS in sample - Signal of ILIS in solvent}}{\text{Signal of ILIS in solvent}} \text{ x } 100 \quad (2)$$

ME lower than 25% was not considered significant, being much relevant when it was over 50% [30]. Moreover, positive values indicated signal enhancement, while negative ME values revealed that compounds suffered ion suppression.

2.5. Sample analysis

2.5.1. Direct injection (DI)

After performing the comparative study DI *vs* SPE, a set of samples was analyzed using DI without any pre-concentration step [12]. A volume of 2 mL of water sample was centrifuged at 12.000 rpm for 10 min. Then, 900 μL of the supernatant were spiked with 50 μL of 20 ng mL^{-1} ILIS solution and the final volume was adjusted to 1 mL, by adding 50 μL of Milli-Q water. Finally, 50 μL of the final solution were injected into the LC-MS/MS system.

2.5.2. Solid phase extraction (SPE)

The same samples analyzed by DI were also analyzed by SPE based on the procedure developed and validated by *Gracia-Lor et al.* [8]. Briefly, 50 mL of centrifuged water sample were spiked with 50 μL of 20 ng mL^{-1} ILIS solution and passed through an HLB cartridge (60 mg) by gravity, drying under vacuum for approximately 30 min. Then, the compounds under study were eluted with MeOH (2 × 2,5 mL). The eluate was evaporated to dryness at 40 °C under a gentle stream of nitrogen, and the residue was reconstituted to 1 mL MeOH:water 10:90 (pre-concentration factor × 50). Finally, 50 μL of the extract was injected into the LC-MS/MS system.

Both procedures were applied to the analysis of real-world samples of different origin and composition. In both cases, quantification was performed by means of calibration curves in solvent using relative responses to their corresponding ILIS. The limit of quantification (LOQ) was estimated from the lowest calibration level (LCL) (see Table S1), considering the pre-concentration factor in SPE (LCL/50). In DI, a positive was considered as detected, but not quantified, when its concentration level was below LCL. As regards SPE, a compound was considered as detected when its concentration level was below LCL/50, considering the pre-concentration factor of the procedure.). Furthermore, for a compound to be considered as detected, it was required that at least one q/Q ratio agreed with that of the reference standard (deviation ≤ 30%; see 2.2. Instrumentation).

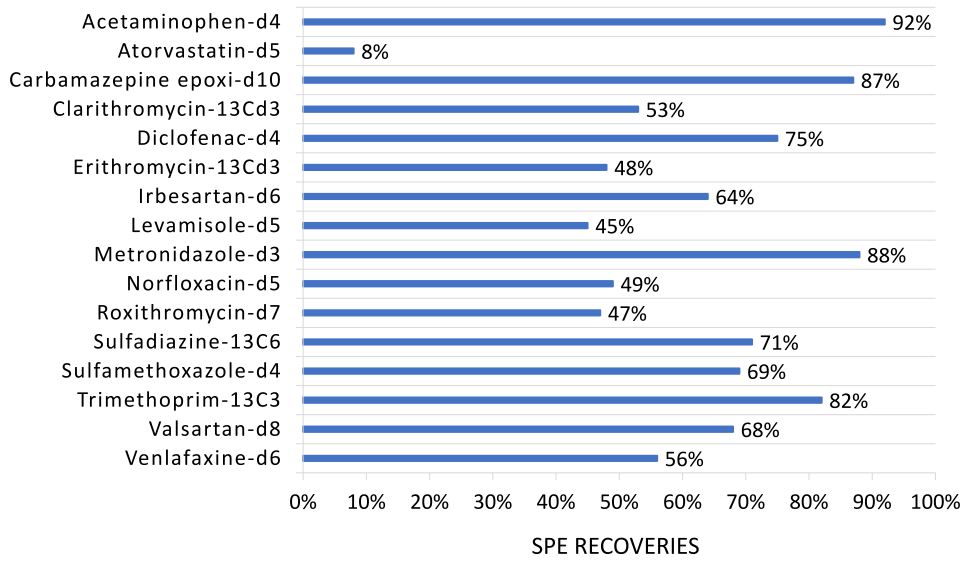

Fig. 2. Evaluation of SPE recoveries (according to Eq. (1) for analyte-ILIS in Milli-Q water spiked at 0.1 ng mL^{-1}.

2.6. Quality control

The reliability of DI methodology for the determination of pharmaceuticals in waters has been previously supported by analysis of quality control samples (QCs) performed in our own laboratory [9,12,36,37]. In this work, QCs consisted of selected samples fortified at two concentration levels (0,1 and 1 μg/L) that were analyzed together with the samples. QC preparation was as follows: an aliquot of 900 μL of centrifuged sample was taken, and then 50 μL of 20 ng mL^{-1} ILIS solution and 50 μL of the mixed working solution containing all analytes were added. QC recoveries ranging between 60 and 140% were considered satisfactory [38].

3. Results and discussion

3.1. Study of potential SPE losses

The use of SPE-based methodologies for the determination of organic compounds may lead to partial losses of analytes as a consequence of incomplete retention onto the cartridge, incomplete elution of analytes from the cartridge and/or partial analyte losses in the evaporation step usually performed to adjust the final volume and/or the solvent composition of the eluate. Moreover, the increase of experimental steps in sample treatment tends to also increase possible procedure errors and analyte losses. Although the errors associated with sample treatment can be corrected by using internal standards (IS) as surrogates, the ideal ISs, i.e. isotopically-labelled analogues, are not always available and/or are highly expensive. For this reason, before evaluating matrix effects, we tested possible losses of the analytes through the HLB cartridge (see section 2.4).

The SPE recoveries obtained for analyte-ILIS are shown in Fig. 2. Acetaminophen, carbamazepine, diclofenac, metronidazole and trimethoprim were almost completely recovered (≥75%), while the rest of compounds were just partially recovered (between 45 and 71%). Atorvastatin showed the lowest recovery (only 8%), a fact that agrees with previous data, probably due to its lipophilic behavior [34,39]. Nevertheless, when analyte losses along the SPE process are reproducible, the use of ILIS may allow an appropriate correction, but the challenge may become the sensitivity of the method. [39,40].

3.2. Study of matrix effects in the SPE/DI procedures

It is widely known that coeluting matrix components may compete in the ionization process of analytes, typically producing signal suppression in electrospray ionization (ESI). Some studies also explain the ion suppression due to the presence of less volatile compounds that can hinder the formation of droplets interfering in solvent desolvation [41]. The precipitation of interfering compounds with the analytes can affect the process of ionization as well [41–43]. In other cases, an enhancement of the signal may also occur. The exact mechanism of ion suppression or enhancement is not well known, since matrix effects can be caused by a wide range of factors as the type of matrix, the characteristics of the analyte or the sample treatment process [44].

One of the main objectives of the present work was to evaluate the ME for six different water samples spiked with ILIS at 0,1 ng mL^{-1} (see section 2.4), which were subjected to both SPE and DI procedures. Fig. 3 shows the average ME for every type of water (GW, SW and EWW). In general terms, most compounds were affected by signal enhancement when were analyzed by DI and by ion suppression when analyzed by SPE procedure, with a similar behaviour in the three types of water matrices. The highest signal enhancement in DI occurred for norfloxacin and venlafaxine for all the six water samples tested, while signal suppression occurred for nearly all compounds in the SPE procedure, especially in EWW. This data agrees with other publications that reported high signal enhancement for the analysis of norfloxacin by DI [33]. Our results are comparable to previous works that reported ion suppression for at least 75% of compounds in SPE-based methods [31,33–35]. In the case of DI, signal enhancement has been also reported for some compounds, but not as a general trend. In any case, it must be noticed that the use of reference (unlabeled) standards for evaluation of ME requires the "blank" subtraction when using spiked real-world samples (most of the "blank" samples contain the studied compounds, particularly wastewater samples), which clearly affects the recovery calculation [12,30–32,35,37,45,46]. This difficulty is overcome when ILIS are used for evaluation of matrix effects, as occurs in the present work.

In relation with the type of samples, in the case of GW matrix effects were more significant for SPE methodology, since 7 out of 16 compounds presented strong ME (>50%), while only 3 experimented strong ME using DI. The results for SW samples were fairly similar; however, in EWW analyzed by SPE, all compounds presented significant ion suppression while in DI remained in the range of low or moderate ME. The fact that ion suppression increases when the matrix becomes more complex has already been reported in other works based on SPE methods [34,37,39].

It can be concluded that ME was in general more important (> ± 25%) in the SPE procedure than in DI, a fact that may be surprising given

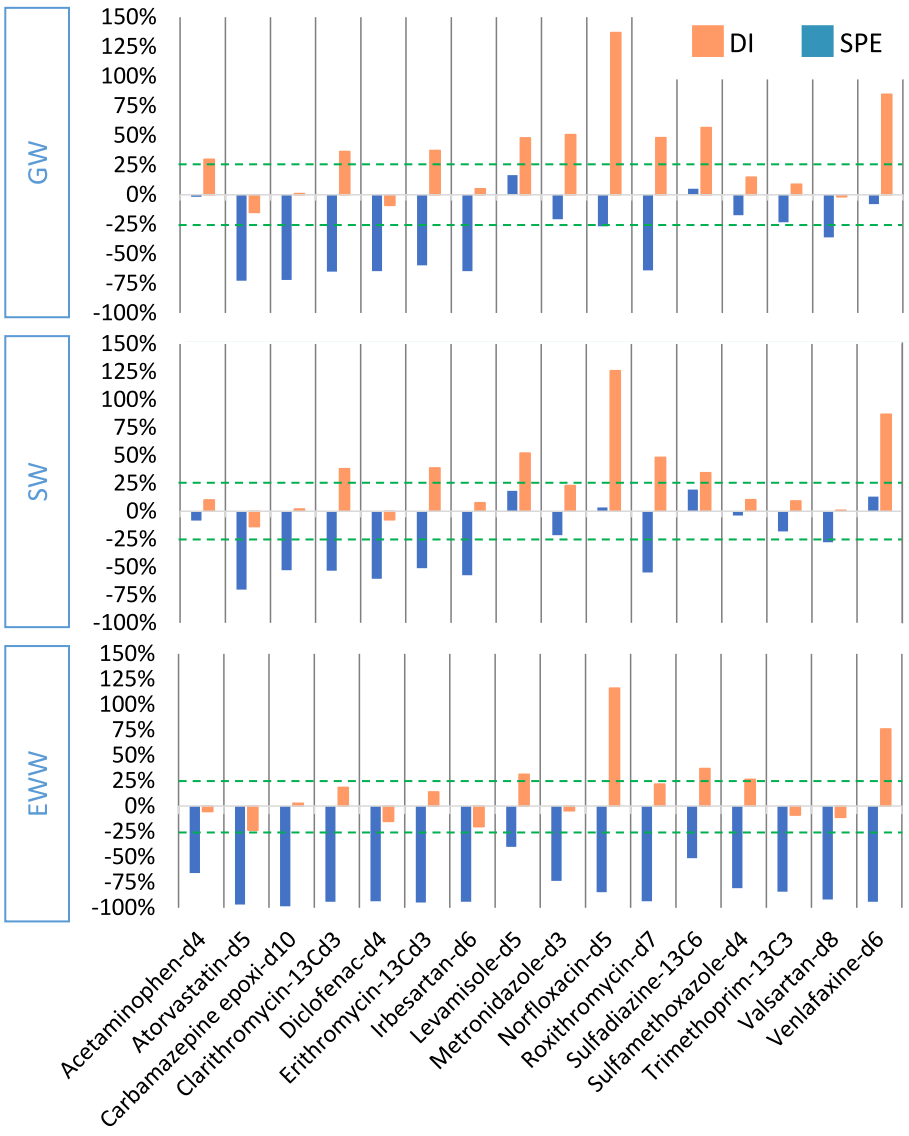

Fig. 3. Matrix effects for each sample type and compound after DI and SPE procedures. Each value corresponds to the average of two samples of each type (GW, SW and EWW).

Table 1
Concentration (ng/L) of pharmaceuticals analyzed in different water samples by SPE and DI methodologies.

	GW1		GW2		SW1		SW2		EWW1		EWW2	
Compounds	SPE	DI	SPE	DI	SPE	DI	SPE	DI	SPE	DI	SPE	DI
Acetaminophen	71	40	7,4	–	0,94	–	1,1	–	–	–	35	d
Atorvastatin	–	–	–	–	–	–	–	–	16	5,9	6,9	d
Carbamazepine	–	–	5,2	5,1	0,23	–	d	–	94	66	119	53
Clarithromycin	–	–	–	–	–	–	–	–	69	45	48	17
Diclofenac	–	–	–	–	2,4	–	1,6	–	356	299	722	460
Erythromycin	–	–	–	–	–	–	–	–	69	50	48	22
Irbesartan	–	–	–	–	0,15	–	1,4	–	528	601	493	422
Levamisole	–	–	–	–	–	–	–	–	35	33	34	23
Metronidazole	d	–	d	–	–	–	–	–	77	78	32	23
Norfloxacin	d	–	–	–	d	–	–	–	52	100	87	87
Roxithromycin	d	–	0,87	–	–	–	–	–	d	–	–	–
Sulfadiazine	–	–	–	–	–	–	–	–	d	–	1,4	–
Sulfamethoxazole	d	–	0,30	–	d	–	–	–	25	26	44	36
Trimethoprim	–	–	0,33	–	–	–	0,18	–	124	134	34	25
Valsartan	–	–	d	–	0,57	–	4,1	–	2304	3489	2417	2303
Venlafaxine	d	–	0,48	–	d	–	0,80	–	371	443	368	313

d. detected at concentration level below LOQ.
-. not detected.

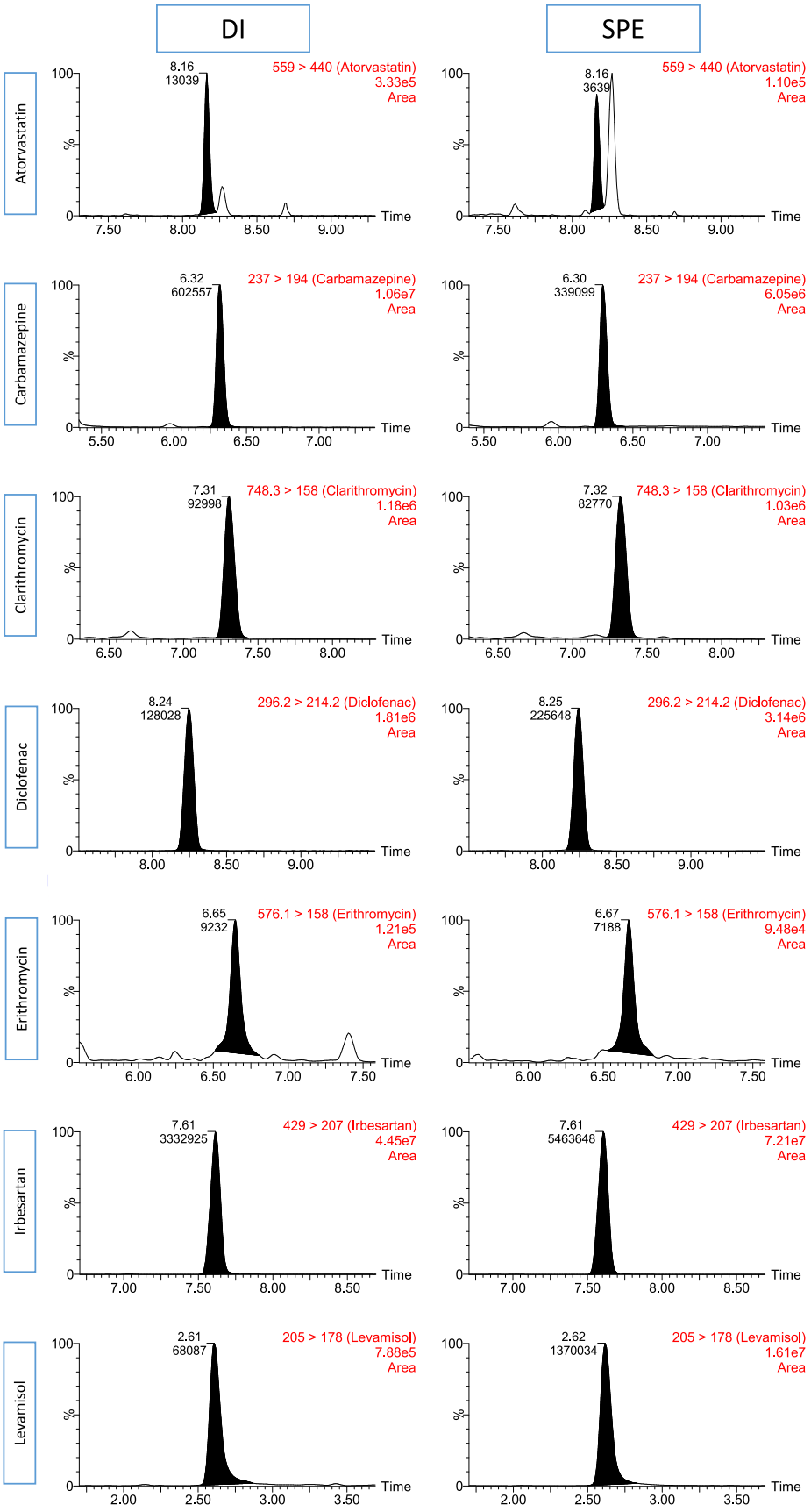

Fig. 4. LC-MS/MS chromatograms for compounds quantified in sample EWW1 by DI and SPE-based procedures.

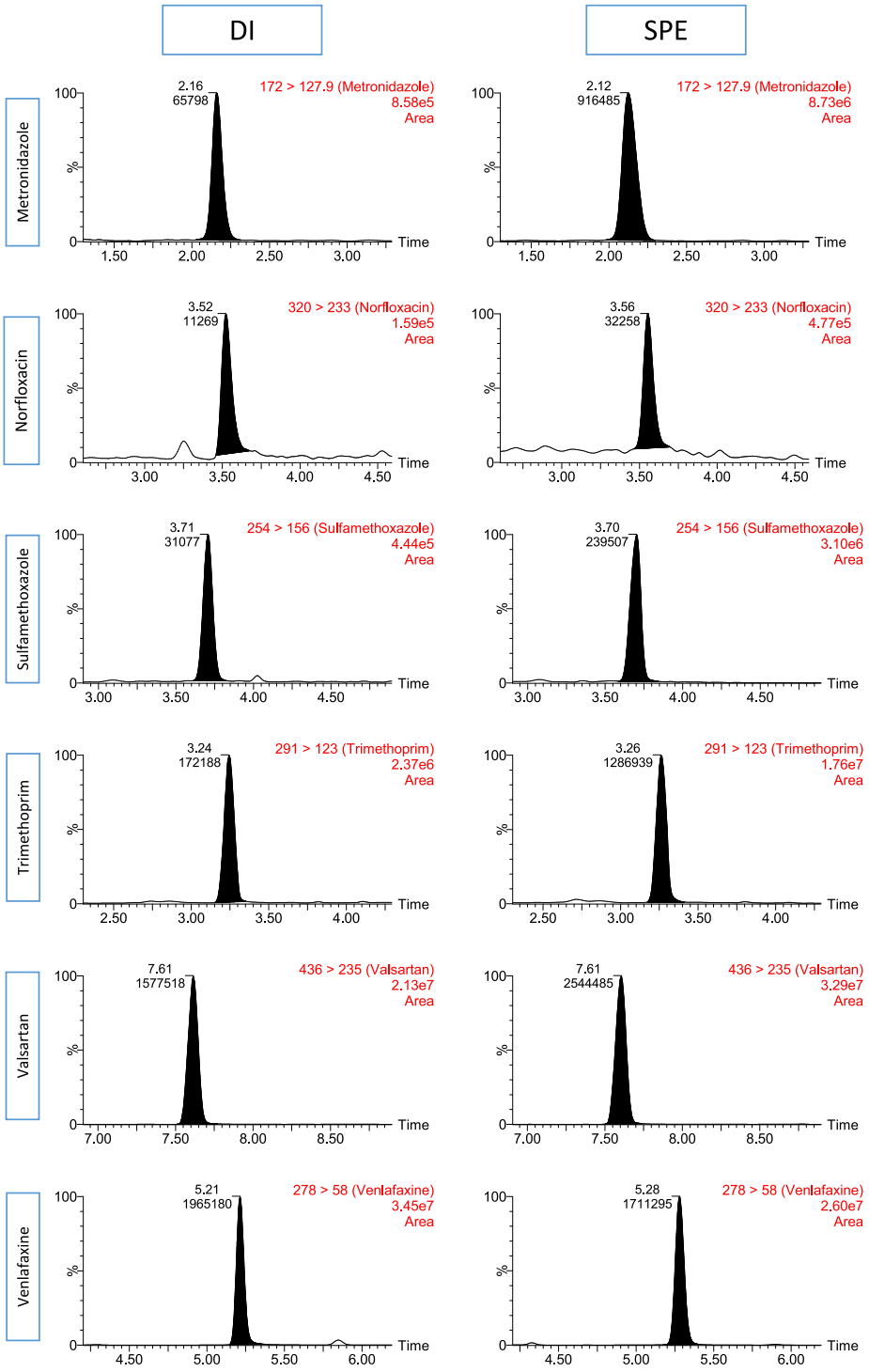

Fig. 4. (*continued*).

the general perception that SPE involves a cleanup of samples, thereby expecting to minimize matrix effects. Certainly, SPE is widely applied as clean-up step in many analytical procedures and also as pre-concentration technique. However, it must be considered that not only the analytes are preconcentrated but also the coeluting matrix interferents. In this way, although cleaner extracts are in general obtained, which helps to better maintenance of the instruments avoiding the injection of dirty extracts/samples, the use of SPE does not ensure a minimization of ME. On the contrary, SPE extracts might be subjected to ME higher than water samples directly injected in the instrument. In summary, DI seems an excellent alternative to SPE due to its speed, the absence of sample treatment (therefore, less analytical errors associated with this step), and even less matrix effects than in SPE. The less sensitivity reached due to the absence of a pre-concentration step may be partially compensated with the signal enhancement (versus signal suppression in SPE) and with the use of modern LC-MS/MS instruments every time more and more sensitive. In any case, the pharmaceutical concentrations commonly present in medium-highly polluted surface waters and urban wastewaters are above 10 ng/L, which can be perfectly determined by DI- LC-MS/MS QqQ as demonstrated in this work and in recent studies of our own research group [7,9,12,36,37].

Table 2
Mean recoveries (RSD % in brackets) of QCs analysed by DI-LC-MS/MS along one year in different types of water samples.

	EWW (n = 7)		SW (n = 13)		GW (n = 4)	
	0,1 μg/L	1 μg/L	0,1 μg/L	1 μg/L	0,1 μg/L	1 μg/L
Acetaminophen	91 (17)	99 (23)	89 (15)	89 (14)	89 (8)	87 (25)
Atorvastatin	91 (17)	92 (25)	105 (13)	105 (12)	91 (14)	95 (10)
Carbamazepine	93 (26)	78 (15)	100 (19)	68 (27)	90 (24)	*94 (38)*
Clarithromycin [(1)]	112 (24)	129 (35)	79 (48)	109 (32)	102 (14)	120 (35)
Diclofenac	107 (11)	106 (15)	110 (8)	109 (9)	99 (19)	99 (30)
Erythromycin	89 (33)	108 (16)	96 (35)	106 (18)	95 (15)	101 (23)
Irbesartan	115 (18)	103 (10)	108 (17)	112 (16)	98 (5)	97 (8)
Levamisole	106 (21)	*116 (31)*	101 (14)	110 (12)	94 (6)	91 (26)
Metronidazole [(2)]	135 (26)	124 (14)	128 (21)	136 (23)	120 (26)	137 (36)
Norfloxacin	127 (19)	101 (33)	*181 (79)*	140 (32)	84 (53)	88 (14)
Roxithromycin [(1)]	113 (36)	*149 (51)*	131 (98)	138 (185)	106 (30)	***175 (62)***
Sulfadiazine	140 (41)	129 (31)	118 (44)	117 (26)	88 (57)	105 (45)
Sulfamethoxazole	109 (10)	109 (9)	101 (9)	103 (13)	98 (23)	101 (24)
Trimethoprim	129 (46)	140 (46)	125 (17)	124 (18)	106 (44)	97 (28)
Valsartan	105 (16)	105 (19)	106 (21)	100 (19)	117 (17)	113 (2)
Venlafaxine	91 (18)	99 (12)	97 (13)	109 (12)	85 (7)	96 (7)

In **bold** and **italic**, recoveries out of the range 60–140%.
[(1)] Analyte-ILIS was only available in the last analysis performed. Data shown correspond to a lower number of QCs analysed: EWW (n = 4); SW (n = 4) and GW (n = 3).
[(2)] Data obtained without ILIS, as its reference standard was not available at the time of the analysis.

3.3. Sample analysis

The six water samples used for the ME evaluation were analyzed by both SPE and DI methodologies for quantification of the pharmaceuticals under study, following the procedures previously reported, subjected to validation and quality control (see [8] for SPE; [12] for DI). Calibration was performed with standards in solvent. Quantification was made with the Q transition using relative responses analyte/ILIS in both calibration standards and samples, and the two additional q transitions were used for confirmation of the identity of the compound detected in samples (see section 2.2. and Table S1 for more information). The addition of ILIS mix allowed the correction of possible errors associated with sample treatment, especially in SPE (ILIS were added as surrogates before SPE treatment), as well as with ME. The results obtained are shown in Table 1.

As expected, the wide majority of positives were found in EWW samples, evidencing the incomplete removal efficiency of WWTPs [9,12,36,37]. The most abundant compounds were valsartan, irbesartan, and diclofenac, whose poor elimination in conventional WWTPs has been already reported [36,47]. As expected, positives found in SW and GW samples were less frequent and with low concentrations, as they were below 5 ng/L, except for acetaminophen in samples GW1 and GW2, and carbamazepine in GW2. These low concentrations hampered quantification by DI in most cases, as DI was only applicable for analyte concentrations above 5–10 ng/L. On the contrary, DI was much efficient for EWW analysis, where almost all compounds could be quantified by both SPE and DI.

As an example, Fig. 4 shows the LC-MS/MS chromatograms for compounds quantified in effluent wastewater (sample EWW1) using both methodologies. It can be seen that similar chromatograms were obtained, with good peak shape and enough signal for quantification, although peak areas were obviously higher after pre-concentration with SPE. However, such pre-concentration might also lead to an increase in the signal of the matrix interferents, as occurs in the case of atorvastatin.

With the aim to know the equivalence of the concentration levels obtained by SPE and by DI, a paired *T*-test (p-value < 0,05) was applied for all compounds and samples that could be quantified by both methods. The P-value was 0,66 indicating that the null hypothesis (H0) could be accepted with a 95% of confidence, and showing no significant differences between both methodologies. Thus, DI and SPE could be indiscriminately used when preconcentration is not crucial to achieve the levels present in samples. However, for "clean" samples with pharmaceuticals concentrations near or below 5–10 ng/L, the 50-fold preconcentration reached by SPE was required for their detection and quantification.

3.4. DI quality controls

To support data reported in this paper, we collected data from QCs analyzed along one year in a monitoring program based on DI-LC-MS/MS. A total of 22 QCs were analyzed corresponding to different water samples (7 EWW, 13 SW, 4 GW), all prepared at 0,1 μg L^{-1} and 1 μg/L. Table 2 shows the average recoveries and RSDs obtained for the different QCs. It can be seen that mean recoveries were satisfactory with values between 60 and 140 % for the three types of waters. The worst results were for roxithromycin with two values out of this range, and high coefficients of variation, in part due to the low number of data available. For this compound and for clarithromycin their analyte-ILIS were not available in the first analyses performed, and thus the number of QCs analysed with ILIS correction was lower, particularly in EWW samples (n = 4). The QC recovery for norfloxacin was also out of range for SW, which also presented elevated coefficients of variation. It must be noticed that average data do not correspond to replicates of the same sample (i.e. repeatability) but to the average of individual data for different samples analyzed along one year; therefore, higher variations must be expected in the reproducibility, expressed as RSD. In any case, norfloxacin was found one of the most problematic compounds in terms of method performance.

The results obtained in the compilation of QCs obtained along one year for different samples and using instruments under variable conditions of use, support the applicability of DI as a reliable analytical approach for the LC-MS/MS determination of pharmaceuticals in water samples, as long as it is not necessary to reach extremely low concentration levels (i.e. applicable for analyte concentrations above 5–10 ng/L).

4. Conclusions

In this work, SPE using Oasis HLB cartridges and direct injection have been applied to different water matrices to evaluate their suitability for the LC-MS/MS determination of 16 selected pharmaceuticals. Compounds under study included 8 antibiotics and 8 pharmaceuticals from different families, including antihypertensives, analgesics and anti-inflamatories, among other families. To this aim, matrix effects have

been evaluated, as well as potential analyte losses along the SPE process, obtaining the overall performance of both methodologies. Signal enhancement was consistently observed in the DI procedure, while ion suppression was observed in SPE. In general, matrix effects were more noticeable in the SPE procedure, and increased when it was applied to EWW due to their higher matrix complexity, a fact that was not observed in the DI analysis of the same samples. In addition, several pharmaceuticals were partially lost in the SPE process, with recoveries below 70% for 10 out of 16 compounds, emphasizing atorvastatin that showed average recovery of 8%. Both methodologies were applied to real-world water samples and their performance was compared, concluding that DI was useful for quantifying analytes at concentrations higher than 5–10 ng/L, although a pre-concentration with SPE was required (in this work, pre-concentration factor was × 50) for very low concentrations.

In order to support the applicability of DI, the QCs recoveries obtained along one year of analysis in a large number of samples have been reported, showing satisfactory recoveries. Data provided in this paper demonstrate that DI is a fast, low-cost and reliable alternative, with minimal sample manipulation, compared to conventional SPE preconcentration for the LC-MS/MS determination of pharmaceuticals in waters. The importance of using analyte-ILIS is also emphasized, as it allows an efficient matrix effects correction in this type of sample matrices.

CRediT authorship contribution statement

Claudia Simarro-Gimeno: Resources, Investigation, Formal analysis, Visualization, Writing – original draft. **Borja Garlito:** Investigation, Formal analysis. **Elena Pitarch:** Funding acquisition, Investigation, Writing – review & editing. **Félix Hernández:** Funding acquisition, Supervision, Visualization, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2023.108985.

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