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On-line solid-phase extraction coupled to hydrophilic interaction chromatography–mass spectrometry for the determination of polar drugs

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ABSTRACT

The present study describes the first fully automated method based on on-line solid-phase extraction (SPE) coupled to hydrophilic interaction chromatography–electrospray-mass spectrometry (HILIC–(ESI)MS) to determine a group of polar drugs that includes illicit drugs (such as cocaine, morphine, codeine and metabolites) and pharmaceuticals in environmental water samples. The SPE was performed using a highly retentive polymeric sorbent. The HILIC separation was optimised and the initial high organic content of the chromatographic mobile phase, was also suitable for the proper on-line elution of the analytes retained in the SPE column and for enhancing the ESI ionisation efficiency. This method allows the loading of samples of up to 250 ml of ultrapure water or 10 ml of environmental water samples spiked at low ng l^{-1} levels of the analytes. The method yields near 100% recoveries for all the analytes. The method was also validated with environmental water samples with linear ranges from 5 to 1000 ng l^{-1} and limits of detection $\leq$2 ng l^{-1} for most of the compounds.

1. Introduction

Different analytical methods have been developed to determine polar contaminants in environmental water samples. They usually combine an extraction technique with a separation technique followed by a powerful quantification technique such as mass spectrometry (MS) in order to determine the low levels of concentration usually found in environmental water samples [1–3].

Solid-phase extraction (SPE) is one of the preferred extraction techniques for isolating and enriching polar analytes in complex aqueous samples. One of the main advantages of SPE is the wide range of sorbents available, which covers a broad range of analyte properties including the polar analytes [4,5]. Moreover, the on-line coupling of the SPE to liquid-chromatography (LC) is well-established and has been applied to determining different types of analytes and samples [6,7]. The benefit of the on-line SPE–LC system is that it analyses all the eluate from the SPE extract, thus providing better preconcentration factors and sensitivity than the off-line systems.

Separation of the polar compounds is commonly achieved by reversed-phase liquid chromatography (RPLC), which starts with low levels of organic solvent. In on-line SPE–RPLC coupling, this might become a drawback because the low organic solvent content in the mobile phase might not have enough strength to elute the analytes trapped in the SPE precolumn (specially when high retentive sorbents are used), and the peak shape of the analyte could become broader during the chromatographic separation, which would lead to integration problems and eventually to increase the detection limit of the analytes [7]. One strategy to partially solve this is to use an LC instrument equipped with two pumps (one to pump the organic mobile phase and another to pump the aqueous mobile phase), so that only the organic solvent in the mobile phase passes through the SPE column to elute the analytes, before mixing with the aqueous mobile phase prior to entering to the column [8].

Hydrophilic interaction liquid chromatography (HILIC) has become popular in recent years for separating and determining polar analytes [9]. The HILIC term was first introduced by Alpert [10] in the 1990, which is based on a hydrophilic column eluted with a hydrophobic mobile phase (which contains a high percentage of organic solvent). This has the effect of increasing retention as the polarity of the solutes increases. Moreover, the highly organic mobile phases used in HILIC provide low column back pressure, increased ionisation efficiency for mass spectrometry (MS) detection [11–13] and offer a solution to the on-line SPE elution problems encountered with conventional RPLC separation systems that are caused by the low content of organic solvent in the mobile phase.

The present study focuses on this last advantage of high organic solvent mobile phases in HILIC technology and explores for the first time on-line SPE coupled to a HILIC column. To do this, we selected a group of polar illicit drugs and pharmaceuticals which are percolated through a highly retentive sorbent to be transferred on-line to the HILIC column. We then quantified the polar illicit drugs and

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Table 1
Compound retention time, optimised fragmentor voltage and ions selected for quantification and confirmation in SIM mode.

Analytes	t_R (min)	Fragmentor voltage (V)	Quantification ion (m/z) $[M+H]^+$	Confirmation ion 1 (m/z)	Confirmation ion 2 (m/z)
Trimethoprim	6.2	150	291	275	261
6-Acetylmorphine	8.9	200	328	211	165
Cocaine	9.1	150	304	182	105
Benzoylecgonine	9.2	150	290	284	168
Codeine	10.2	150	300	243	215
Morphine	10.3	150	286	229	201
Atenolol	11.0	150	267	190	145
Dihydrocodeine	11.8	75	302	324	340

pharmaceuticals using an MS detector with electrospray ionisation (ESI). Very recently, an on-line SPE–HILIC–MS/MS method [14] was developed to determine folate catabolites in biofluids. In that case, however, the set-up, volume of sample loaded (100 μl) and aim of the coupling were pertinent to the type of analytes and samples analysed and, thus, different from the one presented in this study.

2. Experimental

2.1. Materials

The analytes selected for this study were two pharmaceuticals (trimethoprim and atenolol) and three illicit drugs and their metabolites: cocaine and benzoylecgonine (BE); morphine and 6-acetylmorphine; and codeine and dihydrocodeine. The pharmaceuticals were purchased from Sigma–Aldrich (St. Louis, USA). Standard solution of the illicit drugs and metabolites at a concentration of 1000 mg l^{-1} in methanol was obtained from Ceritilliant (Round Rock, TX, USA). Standard 1000 mg l^{-1} stock solutions were prepared for the two pharmaceuticals in methanol. All the stock solutions were stored at −20 °C in the dark. Working solutions of a mixture of all compounds were prepared in 1:1 MeOH:H_2O (v:v) and stored at 4 °C in the dark. The chemical structures, molecular weight, CAS number, log P and pK_a values of all the analytes are shown in Table S1 (electronic supplementary material).

Ultrapure reagent water purified by a Milli-Q™ gradient system (Millipore, Bedford, MA, USA) was used throughout. Acetonitrile and methanol (both HPLC grade) were purchased from SDS (Peypin, France). Analytical grade ammonium acetate, acetic acid, ammonium formate and formic acid, which were used to prepare the mobile phase, were from Aldrich. Mobile phases were filtered through a 0.22 μm nylon filter (Osmonics Inc., Minnetonka, MN, USA).

2.2. Instrumentation

The chromatographic system was an HP1100 series LC–MS selective detector (Agilent Technologies, Waldbronn, Germany) with an ESI interface. It was equipped with a degasser, a quaternary pump, a 20 μl loop injector and a column oven. The chromatographic column was a Fused-Core™ Ascentis Express HILIC (50 mm × 2.1 mm) with a particle size of 2.7 μm (Supelco, Bellefonte, PA, USA).

The on-line SPE precolumn was connected to the chromatographic system by means of a six-port switching valve (Rheodyne, Cotati, CA, USA). An HP1100 series isocratic pump (Agilent Technologies) was used to deliver the sample through a stainless-steel precolumn (20 mm × 2 mm I.D.) fitted with 2 μm stainless-steel frits, all purchased from Upchurch Scientific (Oak Harbor, WA, USA). This precolumn was packed manually using a packing funnel with 30 ± 1 mg of the sorbent.

2.3. Chromatographic conditions

The mobile phase was a mixture of solvent A: 15 mM CH_3COONH_4/CH_3COOH buffer at pH 4.5 and solvent B: acetonitrile. The gradient profile was 98% solvent B for the initial 2 min, then reduced to 80% solvent B in 10 min, and to 50% solvent B in 5 min, after which the mobile phase was returned to the initial conditions (98% solvent B) in 5 min (and held for 5 min to equilibrate the column for the following analysis). The flow-rate was 0.5 ml min^{-1} and the temperature of the column oven was set at 30 °C.

Flow injection analysis (FIA) was carried out to find the optimum conditions for each compound in the ESI-MS. The average conditions for all the compounds selected for the optimum performance of the ESI interface in the positive mode were: nebuliser pressure 40 psi, drying gas flow rate 13 l min^{-1}, drying gas temperature 350 °C, and capillary voltage 4000 V. Fragmentation voltages were defined individually and the values used are listed in Table 1. The ions selected for quantifying the analytes are also listed in Table 1. In SIM mode, the most abundant ion, which is usually $[M+H]^+$, was used for quantification and two other ions were used for confirmation.

2.4. Solid-phase extraction procedure

The in-house synthesised hypercrosslinked HXLPP sorbent (see the synthetic procedure detailed in [15] for HXLPP2), which had previously shown excellent results in the on-line SPE extraction of polar analytes [8], was laboratory packed (30 ± 1 mg) into a 20 mm × 2 mm I.D. stainless-steel precolumn used for on-line trace enrichment in the SPE process.

The final protocol was as follows: the SPE precolumn was conditioned with 5 ml of MeOH and 5 ml of ultrapure water adjusted at pH 4.5 with acetic acid; the water sample was adjusted to pH 4.5 (with acetic acid) and volumes ranging from 10 to 250 ml were passed through the conditioned precolumn. The flow-rate was 3 ml min^{-1} in all these steps. For the environmental water samples, the sorbent in the precolumn was washed with 5 ml (for river water) or 10 ml (for waste water) of ultrapure water before elution of the analytes. The retained analytes were desorbed from the SPE sorbent using the HILIC mobile phase (which is continuously passing through the SPE precolumn while the LC analysis is performed) in the gradient profile of the chromatographic system, and in the back-flush direction to reduce band-broadening. The unsplit eluate was transferred on-line to the analytical HILIC column.

Environmental water samples (river water and effluent waste water from a treatment plant) were filtered through 0.45 μm nylon membranes (Osmonics Inc.) before the SPE step to eliminate the particulate matter, after which they were adjusted to pH 4.5 with acetic acid.

3. Results and discussion

To study the different parameters of the automated on-line SPE–HILIC–MS system, we selected a group of polar compounds

whose polar character had presented problems in the SPE extraction, in the RPLC separation or in the on-line SPE–RPLC coupling. The analytes selected were three illicit drugs and their metabolites (cocaine and benzoylecgonine; morphine and 6-acetylmorphine; codeine and dihydrocodeine), and two pharmaceuticals: trimethoprim and atenolol.

3.1. HILIC–MS conditions

HILIC separation can combine electrostatic and partition mechanisms, which contribute to varying degrees depending on the particular conditions employed. HILIC separations can be influenced by the type of column, the nature of the buffer and the pH of the mobile phase [16]. In the present study, the column used was a Fused-Core™ Ascentis Express HILIC, which has a bare silica stationary phase. Moreover, its Fused-Core™ particle size enables it to work under ultra performance LC conditions using a conventional LC instrument. The influence of the aqueous mobile phase that included the variables pH and ionic strength was evaluated for the HILIC optimisation. When a volatile salt is required, then preferred buffers for HILIC are typically acetic acid, formic acid and their ammonium salts because they are both volatile and soluble in high percentages of organic solvent [17]. With this in mind, we tested the following different aqueous mobile phases: ultrapure water adjusted at pHs 3 and 4.5 with acetic acid, and the buffers $HCOONH_4$/HCOOH at pH 3 and CH_3COONH_4/CH_3COOH at pH 4.5, both at different salt concentrations (2, 5, 10 and 15 mM). All of these aqueous mobile phases were combined with acetonitrile as the organic mobile phase. From the results (data not shown), all the buffered aqueous mobile phases performed better (since they were able to separate the analytes, and each analyte appeared as one single peak) than the ultrapure water that had been merely adjusted at the fixed pH, which clearly indicates how the ionic strength of the mobile phase contributes to the ionic exchange separation mechanisms that participate in the HILIC separation. Moreover, the higher the salt concentration the better the peak shape performance [18], the aqueous mobile phase being buffered at 15 mM salt concentration when the slimmest peaks and lower retention time in all the studied compounds were obtained. Acetate buffer yielded better HILIC separation than formate buffer, because with the formate buffer peaks of some analytes overlapped or had very low retention times, which made it more difficult to quantify. Another casuistry is with cocaine, which under conditions different than the chosen ones appeared in the form of two peaks. Therefore, 15 mM CH_3COONH_4/CH_3COOH buffer at pH 4.5 was selected as the optimum aqueous mobile phase for separating the selected analytes.

Once the mobile phase had been fixed, different separation gradients were tested. All of these started with a high percentage of ACN ranging from 98% to 80% because the higher the organic mobile phase content in the HILIC separation, the higher the retention of the polar compounds [11]. However, it should also be taken into account that a low percentage of water is needed for a sufficient hydration of the stationary phase particles [19]. Finally, the optimum separation of the analytes in the HILIC column was carried out using the gradient profile described in Section 2.

Under these optimum conditions, the temperature (25, 30, 35 and 45 °C) and flow rate (0.25, 0.5 and 0.75 ml min^{-1}) were also optimised. Regarding to temperature, the only difference of note being that as the temperature increased (i.e. at 35 and 45 °C), the retention time of some of the compounds decreased, as expected, which led to overlapping between them. In the end, 30 °C was selected because it provides the best separation of the analytes under controllable temperature. As for the flow rate, 0.5 ml min^{-1} was selected, mainly because it is suitable for the column inner diameter (i.e. 2.1 mm), morphology (fused-core) and size (2.7 μm) of the particles, and also because it provides the best separation profile in the shortest time. Table 1 lists the retention time of the studied analytes under the optimum separation conditions.

Specific MS parameters, such as nebuliser pressure (40 psi), drying gas temperature (350 °C), drying gas flow (13 l min^{-1}), capillary voltage (4000 V) that provided the best response and spectrum as a compromise among all the studied analytes, were selected. The fragmentor voltage (150 V for all the analytes, with the exception of acetylmorphine, 200 V, and dihydrocodeine, 75 V) was optimised for each compound separately. Table 1 lists both the fragmentor voltage and the ions selected for compound quantification.

All the compounds showed good linearity ($r^2 \geq 0.997$) when they were directly injected at low μg l^{-1} levels in ultrapure water. The linear range was 1–1000 μg l^{-1} for morphine, atenolol and dihydrocodeine; 5–500 μg l^{-1} for trimethoprim; and 1–500 μg l^{-1} for the remaining compounds. The limits of detection (LODs) calculated at a signal-to-noise ratio (S/N) ≥ 3, were 0.2 μg l^{-1} for all the compounds, expect for trimethoprim (1 μg l^{-1}). It should be highlighted that low instrumental LC–MS concentration levels were detected because the HILIC separation provided a better response than the response provided by conventional RPLC using a C_{18} column, as has already been reported [20,21]. For instance, the HILIC conditions enhanced the response signals by up to 8 times in the case of cocaine and 2 times in the case of benzoylecgonine [20].

3.2. SPE optimisation

As stated above, HILIC separation presents a series of advantages in the determination of polar analytes (i.e. ability to separate the polar analytes with enhanced sensitivity) [13,20]. Moreover, the initial gradient separation in the mobile phase with a high organic solvent content is an added advantage in on-line SPE coupling because the chromatographic mobile phase also acts as an elution solvent for the SPE.

3.2.1. Loading sample conditions

The initial experiments performed to optimise the SPE conditions were done by percolating 10 ml of ultrapure water spiked with the analyte mixture at 0.2 μg l^{-1} through the SPE precolumn.

Among the parameters that had to be optimised were the sample conditions. This was necessary so that the analytes could be retained in the SPE material and separated in the HILIC column afterwards. The basic character of the analytes studied (see Table S1) meant that we first had to acidify the sample, so that all the analytes studied were in their ionic form. When the SPE sample was adjusted to pH 3, the subsequent separation of the analytes in the HILIC column were not the same as achieved under HILIC separation alone; however, at pH 4.5, the retention times of all the analytes were as expected and their SPE recoveries were high. The problems at pH 3 might be attributed to the non-compatible conditions of the analytes once in the HILIC column. To further investigate the best sample conditions for SPE, we tested a sample buffered at similar conditions to those used in the aqueous mobile phase (i.e. 15 mM CH_3COONH_4/CH_3COOH buffer at pH 4.5). However, under these conditions, a huge peak at the beginning of the chromatogram appeared. This peak might be attributed to the ions present in the SPE sample, which were enriched during the SPE loading. This indicates that the ionic strength of the sample solution is not suitable for the following HILIC separation. It must be concluded from these results that the sample adjusted at pH 4.5 is the most suitable for determining polar drugs by on-line SPE–HILIC.

The results of these experiments also indicate that the unsplit HILIC mobile phase is suitable for eluting the retained analytes in the SPE material. This is because the LC instrument only has one quaternary pump which pumps the unsplit mobile phase into the SPE precolumn. Another feature is that the peak width after SPE is

Table 2
% Recovery values of the analytes studied when 100 and 250 ml of ultrapure water spiked with the analyte mixture at 1 and 0.4 ng l^{-1}, respectively, were on-line SPE–HILIC–MS analysed. For the experimental conditions, see text.

Analytes	% Recovery	
	100 ml	250 ml
Trimethoprim	109	108
6-Acetylmorphine	102	100
Cocaine	105	106
Benzoylecgonine	102	104
Codeine	80	82
Morphine	92	92
Atenolol	101	95
Dihydrocodeine	97	96

% relative standard deviations (RSD) ($n = 3$) were lower than 5%.

comparable to that obtained by direct injection alone, which also helps the analytes to be properly quantified. This feature is also shared by elution conditions with a high organic solvent content.

3.2.2. Volume of the loading sample

Once the parameters that affect the SPE and the HILIC separation had been optimised, the next step was to test the effect of increasing the sample volume in order to determine the highest sample volume possible and therefore, decrease the limits of quantification. Table 2 lists the recovery values when 100 and 250 ml (the highest volumes) were spiked with the analyte mixture at 1 ng l^{-1} and 0.4 ng l^{-1}, respectively, and then percolated through the SPE sorbent on-line connected to the HILIC column. The results show that the recoveries were near to 100% for all the analytes and for all the volumes tested, except for codeine, whose recovery decreased to about 80%. These recovery values are similar to those found when 200 ml of ultrapure water spiked with a group of illicit drugs at 50 ng l^{-1} were extracted via off-line SPE using Oasis HLB (200 mg) as sorbent. In that case, morphine, 6-acetylmorphine and codeine provided values near to 100% [22]. Therefore, the recovery results obtained in the present study are significantly better if one takes into account that we were able to on-line percolate through about 30 mg of sorbent as high volume as 250 ml of sample with higher recoveries. In another study [23] a 5 ml sample of ultrapure water spiked at 50 ng l^{-1} with similar illicit drugs was percolated on-line through PLRP-S precolumn (10 mm × 2 mm) followed by RPLC. This study also provided recovery results of almost 100% in the case of cocaine and benzoylecgonine, but gave decreased recovery results for morphine (69%) and 6-acetylmorphine (55%). These results also showed that the HXLPP resin performed better than other sorbents. Another feature is that on-line SPE–HILIC–MS can be used to determine these polar drugs at low ng l^{-1} levels. These levels are comparable to those achieved when these analytes are determined using off-line SPE–HILIC [21] or RPLC [22] with the powerful tandem MS detection. Thus, the limits of quantification (LOQs) for all the illicit drugs studied, which included morphine [22], 6-acetylmorphine [21,22], cocaine [21] and benzoylecgonine [21] were at the low ng l^{-1}.

3.3. Application to real samples

Our next aim was to use on-line SPE–HILIC–MS to analyse water samples from the Ebre river and from the effluent water of a waste water treatment plant (WWTP). To do so, we first percolated a 10 ml sample of river water that had been adjusted at pH 4.5 and spiked with the mixture of the analytes at 0.2 μg l^{-1}. Although a chromatogram was acquired under the SIM mode and at high levels of concentration, its peaks were difficult to integrate and the % of recovery for all the analytes ranged from 0 to 20%. As expected, a further increase in the sample volume to 50 ml made the problem worse: the recoveries were lower, which was attributable to the increase in organic and inorganic matter when a higher volume of sample was loaded, and an enhanced interference once in the HILIC column. These problems might be attributed to the type of HILIC column used, that is bare silica Fused-Core™ Ascentis Express HILIC column, which has a very low surface area. This low specific surface area resulted in it is becoming overloaded easily, reducing the amount of sample that could be processed. This problem could be overcome by using a totally porous HILIC column. This solution will be considered in further studies. In the present study, 10 ml of environmental water sample was selected as a compromise between the matrix effect when coupling to the HILIC column and sensitivity. In fact, in most studies where the SPE is on-line coupled to RPLC, the volume of real sample percolated is lower than 10 ml. Selecting such a low volume is justified because higher sample volumes may negatively affect the method's sensitivity by ionisation suppression effects [23,24].

We studied the effect of a clean-up step before the elution of the analytes from the precolumn to determine if it would remove the salts from the SPE precolumn and prevent them from entering the HILIC column. To do this, we tested a clean-up solution consisting of 5 ml of ultrapure water adjusted at pHs 4.5, 7 and 9.5. The solutions adjusted at both pHs 4.5 and 7 performed well as cleaning solutions; they provided chromatograms with less presence of inferences and the % of recovery was similar to that found with ultrapure water. Moreover, the results were best when the cleaning solution was adjusted to pH 7, because the solution was able to remove interferences such as humic and fulvic substances that could also interfere in the HILIC separation. Table 3 lists the recovery values provided when a 10 ml sample of Ebre river water spiked with the analyte

Table 3
% Recovery values of the analytes studied when 10 ml of each of different environmental water samples were spiked with the analyte mixture at different levels and on-line SPE–HILIC–MS analysed without and with a clean-up step involving aqueous solution at pH 7. For the experimental conditions, see text.

Analytes	% Recovery			
	Ebre river water			EWWTP water
	10 ml at 200 ng l^{-1}		10 ml at 10 ng l^{-1}	10 ml at 25 ng l^{-1}
	No washing	Washing with 5 ml H_2O, pH 7		Washing with 10 ml H_2O, pH 7
Trimethoprim	2	92	88	81
6-Acetylmorphine	0	78	77	72
Cocaine	0	102	107	86
Benzoylecgonine	8	99	97	103
Codeine	0	84	83	76
Morphine	2	96	92	97
Atenolol	18	98	101	83
Dihydrocodeine	7	95	98	74

% RSD ($n = 3$) were lower than 10% when the % recovery > 20%.

Table 4
Validation parameters of the on-line SPE–HILIC–MS method with Ebre river water samples.

Analyte	Linear range (ng l^{-1})	LODs[a] (ng l^{-1})
Trimethoprim	5–500	2
6-Acetylmorphine	10–500	5
Cocaine	5–500	2
Benzoylecgonine	5–1000	2
Codeine	2–500	1
Morphine	5–1000	2
Atenolol	10–1000	5
Dihydrocodeine	5–1000	2

[a] LODs determined from the S/N ≥ 3 method.

mixture at 0.2 μg l^{-1} was percolated without and with the addition of a previous washing step with 5 ml of water adjusted at pH 7. Once we had selected the pH, we tested it to see if adding 5% methanol to the solution would further improve its cleaning ability; however, when the methanol was added, there was some losses of the analytes and the recoveries decreased. Increasing the volume of the washing solution to 10 ml only provided positive results in the case of more complex samples, such as effluent water. Therefore, we selected as washing solution 5 ml of aqueous solution at pH 7 for river water and 10 ml of the same solution for effluent water.

Under the optimum protocol for environmental water samples, the performance of the method was applied by spiking the samples with lower concentration levels of the analytes. Table 3 also lists the recovery values of the analytes when the 10 ml sample of river water and the effluent water sample were spiked with the analyte mixture at 10 ng l^{-1} and 25 ng l^{-1}, respectively. As can be seen, the data for the environmental water samples are excellent, with recovery values close to 90% for river water (except for 6-acetylmorphine, 77%) and close to 80% for effluent water (the lowest % recovery values again being obtained for 6-acetylmorphine at 70%). These high recovery results also indicated that the matrix has hardly any effect on this type of determination, and the ion suppression/enhancement was not significantly affecting the quantification of these analytes in environmental water samples. These recovery values are higher than others reported in the literature. For instance Hummel et al. [25] reported lower recovery values when a group of opioids and cocaine metabolites (including benzoylecgonine – 42%-, codeine – 64%-, dihydrocodeine – 70%- and morphine -43%) were determined in effluent water by extracting 200 ml of effluent water sample spiked with the analyte mixture at 1 μg l^{-1} and using an off-line Oasis HLB SPE cartridge followed by RPLC–MS/MS. In another study [23], the recoveries obtained were no higher than 60% when on-line SPE–RPLC–MS/MS was applied to 5 ml of sewage water spiked at a concentration of 50 ng l^{-1} with a group of illicit drugs (including cocaine, benzoylecgonine, morphine and 6-acetylmorphine). These low recoveries were due to a combination of low sorbent retention and matrix effect, problems that did not arise in the methodology presented in this work.

Fig. 1 shows representative SIM chromatograms from the analysis, obtained under optimum conditions, of Ebre river water spiked with 10 ng l^{-1} of the analyte mixture.

The on-line SPE–HILIC–MS method was validated using 10 ml of Ebre river water samples and following the whole procedure developed in the SIM mode. Table 4 reports the performance of the method. The calibration curve was linear in the concentration range with r^2 higher than 0.99. The limits of quantification (LOQs) for each compound were taken as the lowest concentration level of the calibration curve, which ranged from 2 to 10 ng l^{-1}. The LODs calculated as the S/N ≥ 3 ratio were 2 ng l^{-1} for all the compounds except for codeine (1 ng l^{-1}), 6-acetylmorphine and atenolol (5 ng l^{-1}). The sensitivity of the method is enhanced in an on-line system because

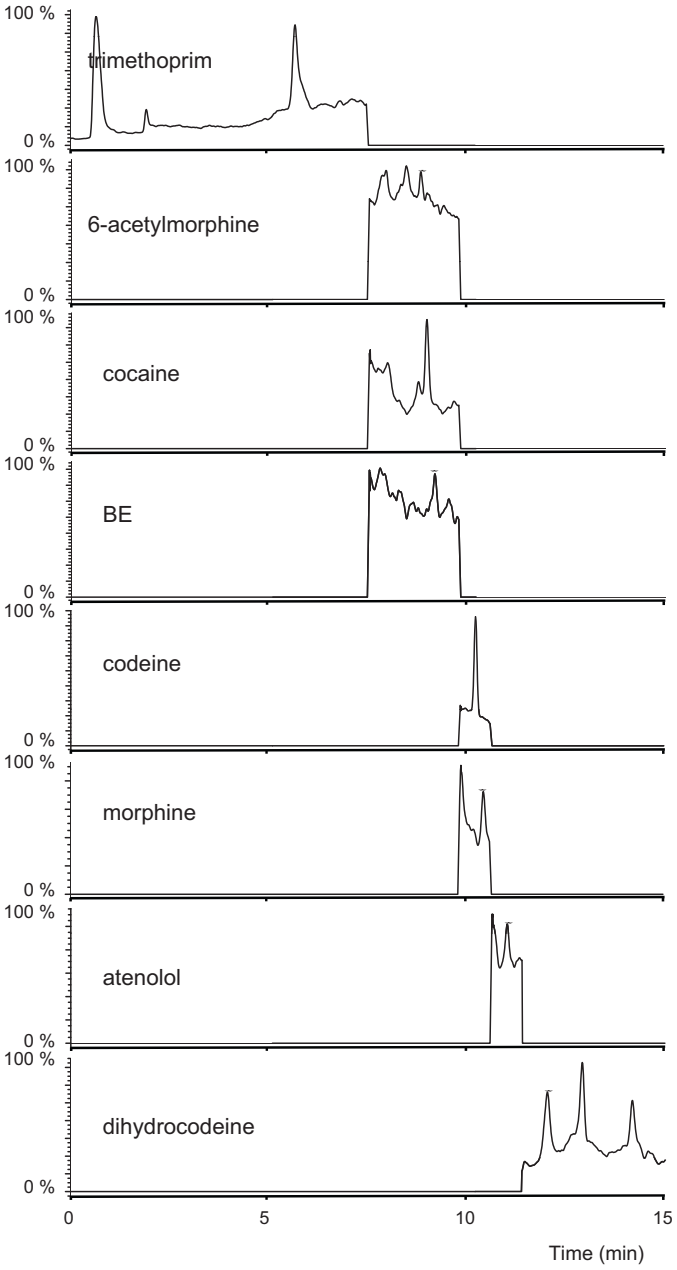

Fig. 1. SIM chromatograms obtained from on-line SPE–HILIC–MS applying a washing step that involves 5 ml of aqueous solution at pH 7 to a 10 ml sample of Ebre river water spiked with 10 ng l^{-1} of the analyte mixture ("*" denotes the peak of the analyte of interest).

the whole sample is directly transferred to the chromatographic system, rather than one aliquot of the final extract, as occurs in an off-line system. However, this sensitivity could be further enhanced if the system was connected to a tandem MS/MS detector. In any case, the LOQs and LODs reported with the present methodology are comparable to those found with off-line SPE–RPLC–MS/MS systems [21,22].

The repeatability and reproducibility between days were determined by spiking three replicates of river water sample at 10 ng l^{-1}, and the results obtained, expressed as a % of relative standard deviation (%RSD), were less than 9% and 12%, respectively.

To quantify the effluent water samples, we used the same calibration curve as for river water because the recoveries obtained with both sample matrices were similar and we could not discern any matrix effect in any of the samples. Moreover, the repeatabil-

ity and reproducibility between days were determined by spiking three replicates of EWWTP sample at 25 ng l^{-1} and were also shown to be similar, and the %RSD obtained was lower than 11% and 15%, respectively.

To demonstrate the applicability of the optimised method, different EWWTP samples from the tertiary treatment and river water samples were analysed, and different analytes were found (which were confirmed by a ratio of ion abundances lower than ±20%). River water samples contained cocaine (8.5 ng l^{-1}) and benzoylecgonine (10.9 ng l^{-1}). Atenolol could also be identified, but its concentration was below the LOQs. Trimethoprim (17.8 ng l^{-1}), codeine (41.4 ng l^{-1}) and benzoylecgonine (10.2 ng l^{-1}) were found in the EWWTP, and also cocaine at the level below to the LOQs. It should be pointed out that these results are in agreement with the results reported by Pedrouzo et al. [26], who analysed similar samples using off-line SPE and UPLC–MS/MS, which is more sensitive technique.

4. Conclusions

The present study describes for the first time the use of on-line SPE coupled to HILIC technology to determine polar drugs at low ng l^{-1} in environmental water samples. The coupling takes advantage of the high organic solvent content in the mobile phase, which is suitable for both SPE elution and retention in HILIC mode.

After we had optimised the variables that affect the whole automated system, the method was successfully used to analyse different water samples spiked with the analyte mixture at low ng l^{-1}.

With the present fully automated on-line SPE–HILIC–MS method the sensitivity levels reached are comparable to those reported for off-line SPE LC–MS/MS systems. These positive results are the basis for further research into enhancing the sensitivity of the coupling based on on-line SPE–HILIC–MS/MS system in order to quantify polar analytes at even lower levels.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.12.028.

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