Journal of Chromatography Open 4 (2023) 100100

Contents lists available at ScienceDirect

Journal of Chromatography Open

journal homepage: www.elsevier.com/locate/jcoa

Solid-phase extraction combined with dispersive liquid-liquid microextraction for the analysis of glucocorticoids in environmental waters using liquid chromatography-tandem mass spectrometry

Lorenzo Antonelli, Chiara Dal Bosco, Massimo Giuseppe De Cesaris, Nina Felli, Elena Lucci *, Alessandra Gentili

Department of Chemistry, Sapienza University, P.le Aldo Moro 5, Rome 00185, Italy

ARTICLE INFO

Keywords:
Glucocorticoids
Corticosteroids
Deep eutectic solvents
Dispersive liquid-liquid microextraction
Environmental water

ABSTRACT

This paper illustrates an enrichment procedure based on the combination of solid phase extraction (SPE) with dispersive liquid-liquid microextraction (DLLME) for the determination of ng/L concentration levels of glucocorticoids in river water samples. In SPE-DLLME, the target analytes were adsorbed by loading a large volume sample (200 mL) into an OASIS HLB cartridge (60 mg). After their desorption with a small volume of ethyl acetate (500 μL), this eluate was used as the dispersant in the following DLLME, performed on 5 mL of water, using a low transition temperature mixture (LTTM) as the extractant (100 μL). The LTTM was obtained by the heat-mixing of choline chloride and sesamol in a molar ratio 1:3 (ChCl:Ses, 1:3); when cooled, it was liquid at room temperature, denser than water and immiscible with it. The SPE-DLLME approach significantly limits the use of high volumes of organic solvents, avoids the evaporation step, and allows one to achieve an enrichment factor greater than 2500. All extracts were analysed by high-performance liquid chromatography-electrospray-tandem mass spectrometry. Eleven glucocorticoids, including the epimers dexamethasone/betamethasone, were separated on a polysaccharide-based column, based on cellulose tris(3-chloro-4-methylphenylcarbamate) chiral selector. Recoveries ranged from 68 to 100% (spike level of 5 and 20 ng/L), with a LOD of 0.21-1.39 ng/L and a LOQ of 0.69-4.17 ng/L. The intra-day precision and inter-day precision were lower than 15%. After optimization and complete validation, the method was applied to analyze surface water samples, taken from River Tiber, to evaluate the effective applicability of the procedure and to establish the contamination levels for these substances.

Introduction

The acronym CECs is used to define "contaminants of emerging concern", i.e. synthetic or naturally occurring substances that are not commonly monitored in the environment but that have the potential to cause adverse effects on human and ecosystem health. CECs include different classes of chemicals such as drugs, diagnostic agents, antimicrobial agents, disinfectants, fragrances, flame retardants, pesticides, solvents, surfactants, and water disinfection by-products [1–4]. The release of CECs into the environment occurs through multiple pathways: effluents from sewage treatment plants, illegal dumpings, runoff from agricultural lands, and landfill leachates [5]. Wastewater treatment plants can only partially remove these compounds that, therefore, can be found at ng/L-μg/L concentrations into the receiving water body [6].

Many CECs are classified as EDCs (Endocrine Disrupting Compounds) due to their capability of altering human and animal development, growth, and reproduction [7]. This category of contaminants has not been regulated yet, as data on toxicity, bioaccumulation, occurrence, transport, and transformation are poorly known [8]. Steroids that are considered EDCs include androgens, oestrogens, corticosteroids, and glucocorticoids. These last ones can be natural or synthetic and are widely used as anti-inflammatories drugs for farm animals and pets [9] as well as for humans. Structural modifications of cortisol, a natural glucocorticoid, have led to the synthesis of a large number of compounds with increased anti-inflammatory properties [10]. Both natural and synthetic glucocorticoids are excreted as free or conjugated forms [11] and have been found in aquatic environments with total concentrations ranging between tens of pg/L to hundreds of ng/L [12,13]. Owing to

* Corresponding author.
E-mail address: elena.lucci@uniroma1.it (E. Lucci).

https://doi.org/10.1016/j.jcoa.2023.100100
Received 26 May 2023; Received in revised form 29 July 2023; Accepted 2 August 2023
Available online 9 August 2023

their low-level occurrence and complexity of environmental waters, their determination requires highly sensitive and selective methods. For this reason, liquid chromatography-tandem mass spectrometry (LC-MS/MS) [14–16] is the most reliable technique, but it needs to be anticipated by a sample pretreatment step to enrich the analytes and remove interferences. Thus far, a number of extraction procedures have been proposed, based on solid phase extraction (SPE) [17], magnetic solid phase extraction (MSPE) [18–20], micro-solid phase extraction (μ-SPE) [21], solid phase microextraction (SPME) [22], liquid-liquid extraction (LLE) [23], and liquid-liquid microextraction (LLME) [24]. Among all, SPE is the technique that allows for very high enrichment factors (EFs) (up to 40,000) and high recovery yields. However, when the target glucocorticoids are concentrated, the unremoved matrix interferences are condensed as well, causing the ion suppression of the analyte signal both using the electrospray (ESI) source and the atmospheric pressure chemical ionization (APCI) source [25]. Dispersive liquid-liquid microextraction (DLLME) is a miniaturized sample pre-treatment technique, well-known for its favourable features such as rapidity, simplicity, low cost, and good EFs (up to 1000). Its combination with a preliminary SPE step has been proposed to obtain an ultra-preconcentration technique for the analysis of some environmental contaminants [26,27].

In this work, we present the development of a SPE-DLLME procedure to concentrate and clean up eleven common glucocorticoids, potentially occurring in trace and ultra trace in environmental waters. The linkage between the two techniques is the SPE solvent elution (ethyl acetate) that becomes the dispersant solvent in the following DLLME step, where a low transition temperature mixture (LTTM) is used as the extraction solvent and can be injected directly into the LC-MS/MS system. The use of both ethyl acetate, a recommended solvent according to CHEM21 guidelines [28], and the LTTM, composed of choline chloride andsesamol in a molar ratio 1:3 (ChCl:Ses, 1:3) [29,30], also provides the approach a green connotation, reinforced by the elimination of the evaporation steps at the end of both SPE and DLLME and by the limited volumes of solvents used. The chromatographic separation of glucocorticoids has been another challenge to overcome since the two epimers, dexamethasone (DEXA) and betamethasone (BETA), not only are indistinguishable via MS detection but are also difficult to separate at the baseline using common achiral columns. To this end, we developed a separation method on the chiral column Lux cellulose-2 under reversed-phase (RP) conditions, obtaining excellent results both in terms of enantioselectivity (for the pair of isomers) and chemoselectively (for the separation of the other glucocorticoids each other). Despite the important cost of chiral stationary phases, the perfect separation here achieved allowed a significantly improved quantitative analysis. The whole method was validated and applied to the analysis of samples taken from River Tiber (Isola Tiberina and Farfa Oasis), proving to be able to detect the low glucocorticoid concentrations.

Materials and methods

Reagents and chemicals

The standards employed for this work are: betamethasone (BETA), cortisone (CORT), cortisone acetate (CORT Ac), dexamethasone (DEXA), dexamethasone acetate (DEXA Ac), hydrocortisone (HCORT), hydrocortisone acetate (HCORT Ac), prednisolone (PREDLO), prednisone (PRED), flumethasone (FLU), triamcinolone (TRI) (Table S1 in the supplementary Material). They exhibit a purity of more than 98% v/v and were used without further purification. Choline chloride, with a purity greater than 98% v/v, and sesamol, with a purity greater than 99% v/v, and all organic solvents used (acetonitrile, methanol, acetone, and THF) were purchased from Sigma Aldrich S.r.l. (Milan, Italy). The water used for chromatographic analysis was purified and deionized using a Milli-Q Plus system (Millipore, Bedford, MA, USA).

Preparation of the working solutions

Standard solutions (stock solutions) were prepared by weighing the analyte standards in powder and diluting them with methanol at the concentration of 1 μg/μL. Working composite solutions were prepared by dilution with methanol from the stock solutions at concentrations useful for the purposes of the various stages of the experimental work. All solutions were stable for 6 months; the stock solution of TRI was analysed weekly to check a potential degradation [31]. All the solutions were stored at 4 °C.

Preparation of choline chloride-sesamol mixture

The preparation and characterization of the LTTM composed of ChCl and Ses in a 1:3 molar ratio has previously been reported by our research group [29,32]. Briefly, ChCl ($T_{melting}$ = 305 °C) is a highly hygroscopic salt that, before being used, was dried in a muffle furnace at 80 °C for 24 h. To prepare ChCl:Ses (1:3 molar ratio), about 1.648 g of ChCl and 4.837 g of Ses were weighed. The mixture was placed on a heating plate at about 50 °C until an amber viscous liquid was formed. This operation was carried out to accelerate the formation process that also occurs at room temperature but for a longer time. The mixture was then allowed to cool and stored at room temperature.

Sample collection

Four grab 2-L samples of water were collected from River Tiber, two of them near Isola Tiberina and the other two in the Farfa Oasis (a natural reserve near Rome). When not analysed within 24 h, the samples were kept at 4 °C in the dark until extraction.

SPE-DLLME procedure

Glucocorticoids were extracted from 200 mL of river water. Each sample was preliminarily filtered on a 1.5-μm pore size Whatman GF/C glass fiber pad (Maidstone, U.K.) to prevent particulate matter from clogging the SPE cartridge. The SPE cartridges (Oasis HLB 3 cc Vac Cartridge, 60 mg Sorbent per Cartridge, 30 μm, from Waters, Milan, Italy) were fitted into a 12-port Visiprep SPE vacuum manifold (Supelco, Milan, Italy) to allow liquids, used in the several SPE steps, to be forced through them under reduced pressure from a peristaltic pump. Initially, each cartridge was washed with 2 mL of methanol, and conditioned with 5 mL of milli-Q water. After loading the sample, the SPE cartridge was dried under vacuum for 2 min to remove the residual water; then the analytes were eluted with 500 μL of ethyl acetate. Five mL of milli-Q water containing NaCl (50 mg/mL) was placed in a 15-mL centrifuge tube to which 100 μL of ChCl:Ses (the extractant) and 500 μL of ethyl acetate from the SPE step (the dispersant) were immediately added. The mixture was then placed on a vortex for 1 min at 2500 rpm, at the end of which a cloudy solution was formed. After centrifugation at 8000 rpm for 5 min at 20 °C, the supernatant was recovered and the obtained extract (70 μL) was directly injected into the HPLC-MS/MS system (2 μL).

Liquid-chromatography-tandem mass spectrometry

Liquid chromatography was conducted using an HPLC Series 200- LC Pump system (Perkin Elmer, Norwalk, CT) equipped with a Series 200 Autosampler and Vacuum Degasser. The analytes were separated by using the column Lux cellulose-2 (4.6 mm x 250 mm, 3 μm; Phenomenex, Torrance, California). The chromatographic elution was performed by using water (50%) and acetonitrile (50%), both containing 10 mM formic acid at the flow rate of 0.4 mL/min.

The HPLC/autosampler system was coupled to a tandem mass spectrometer (ABI-Sciex 4000 $Q_{trap,}$ Toronto, Canada), equipped with a Turbo V source provided with an electrospray probe (ESI), operating in

positive ion mode. The ultra-pure nitrogen from a generator (Parker-Balston model 75A74, Haverhill, MA, USA) connected to a compressor (Jun-Air 4000-40 M, Bromsgrove, UK) was used as curtain gas (Flow = 1.5 L/min) and CAD gas (fragmentation gas at a pressure of 4×10^{-5} Torr), while the air generated by the same compressor was used as drying tube gas (Flow = 8 L/min) and nebulizer gas (Flow = 2 L/min). The temperature was set at 450 °C. A voltage of +5500 V was applied to the source capillary. LC-MS/MS chromatograms were acquired in the Multiple-Reaction-Monitoring mode (MRM), selecting two ion-precursor/ion-product transitions for each analyte. Table 1 lists the LC-MS parameters used for the identification of the analytes in the real matrices: retention time, two MRM transitions, and the corresponding ion ratio. The software used to acquire and process the LC-MS data was Analyst 1.6 (Ab Sciex S.r.l, Milan, Italy).

Method validation

The SPE-DLLME-HPLC-MS/MS method was validated through the evaluation of quantitative and qualitative parameters. For each of the eleven glucocorticoids, recovery, precision intra-day and inter-day, limit of detection (LOD), limit of quantitation (LOQ), sensitivity, carry over, and identification power.

The analytes were quantified using the external calibration method. For each calibration curve (matrix-matched calibration curve), five 200-mL aliquots of water were spiked pre-extraction with increasing concentrations of the analytes (1, 10, 20, 40, 60, 100 ng/L); an unspiked aliquot was used as a "zero sample". The curves were constructed by linear regression, reporting the area of the peak chromatographic VS the spike level. The carryover effect was evaluated by injecting methanol after the calibrator at the highest concentration value on the calibration curve.

For each analyte, LODs were calculated as the fortification level of a blank sample, spiked pre-extraction, capable of providing a signal three times more intense than the noise. The limit of quantification (LOQ) was estimated in a similar way but considering a S/N = 10. For the calculation of both the LODs and LOQs five replicates were carried out.

The recovery rates, intra-day precision and inter-day precision of the eleven glucocorticoids from river waters were evaluated by extracting five blank samples spiked pre-extraction with the analytes at 5 and 20 ng/L. For each analyte, the recovery yield was calculated applying the area method, based on the comparison with the analytical response of an extract from a blank spiked post-extraction with the same nominal concentration of the analytes and used as a reference sample:

$$R\% = \frac{A_{pre-extraction}}{A_{post-extraction}}$$

The intra-day and inter-day precision were assessed as the relative standard deviations associated to the calculated recoveries within one analytical session and over two analytical sessions, respectively. The EF was calculated according to the following equation:

$$EF = \frac{C_{analyte\ in\ the\ final\ extract}}{C_{analyte\ in\ the\ urine\ sample}}$$

Table 1
LC-MS parameters for the identification of the target analytes in the real samples. Retention time and ion ratio were calculated as mean of six replicates.

Number of peak identification	Standard	Retention time (min)	MRM transition[a] (*m/z*)	Ion ratio[b] (%)
1	Triamcinolone	10.79 ± 0.04	395/357 395/375	40
2	Flumethasone	14.74 ± 0.03	411/121 411/253	53
3	Prednisolone	17.45 ± 0.04	361/147 361/343	40
4	Dexamethasone	18.99 ± 0.05	393/355 393/373	60
5	Hydrocortisone	19.63 ± 0.03	363/327 363/121	69
6	Prednisone	19.63 ± 0.03	359/323 359/341	40
7	Betamethasone	21.15 ± 0.04	393/355 393/373	51
8	Cortisone	21.90 ± 0.03	361/121 361/163	45
9	Dexamethasone-acetate	31.04 ± 0.03	435/309 435/415	37
10	Hydrocortisone-acetate	31.04 ± 0.03	405/309 405/327	92
11	Cortisone-acetate	38.51 ± 0.03	403/343 403/163	67

[a] The first line reports the most intense MRM transition (quantifier) and the second line the least intense one (qualifier).

[b] The ion ratio (relative abundance) between the two MRM transitions is calculated as qualifier intensity /quantifier intensity.

Results and discussions

Optimization of the chromatographic separation

The chromatographic separation of glucocorticoids is especially challenging in relation to the pair epimers BETA and DEXA. These are diasteroisomers which have opposite configuration at only one stereogenic center, differing in the orientation of the methyl group on the C-16 position (Table S1). With the aim of separating the eleven glucocorticoids selected in this study, several chromatographic columns (XTerra MS C18, 4.6 × 250 mm, 5 µm; Cosmosil Cholester, 4.6 × 250 mm, 5 µm; Lux cellulose-2 (4.6 × 250 mm, 3 µm), and mobile phases (water/methanol; water/acetonitrile with and without formic acid) were tested by injecting a working standard solution at a concentration of 2.5 ng/µL (5 µL injected). The choice was mainly based on the chromatographic resolution of the two epimers.

Using XTerra MS C18, a greater selectivity was achieved with acetonitrile as the organic modifier. Both acetonitrile (solvent A) and water (solvent B) were acidified with 10 mmol/L HCOOH to suppress the dissociation of weakly acid groups (17α- and 21-hydroxyl groups) and to support ES ionization of keto groups [31]. The best separation was obtained working in gradient elution, being 1 mL/min the flow rate: t_0-t_{10}: 35% A; t_{10}-t_{11}: 35-60% A; t_{11}-t_{12}: 60-100% A; t_{12}-t_{15}: 100% A. Although most of the analytes were well-separated working in gradient elution, the separation of BETA and DEXA was only partial (R = 0.5). Lowering the temperature to which the column was kept from 25 to 15 °C, the resolution could be improved (R = 0.7) but not in a significant way.

Cosmosil Cholester is a RP-HPLC column with cholesteryl bonded silica microparticles, which provides equivalent hydrophobicity like that of traditional C18 columns with approx. 20% of carbon content. Such a stationary phase offers strong stereoselectivity for hydrophobic compounds, resulting very effective in the separation of geometrical isomers, positional isomers, or for molecules whose separation needs great planarity selectivity. Nevertheless, when the Cosmosil Cholester was applied for the separation of the eleven glucocorticoids using the same elution gradient optimized for XTerra MS C18, the epimers were substantially overlapped. Further adjustments of the elution gradient could not improve the chromatographic resolution.

Based on the results obtained by other researchers for the separation of DEXA and BETA by using the column Lux i-cellulose-5, 4.6 × 250 mm, 5 µm (the chiral selector being cellulose tris(3,5-dichlorophenylcarbamate) [33], we also tested a polysaccharide column but with a different chiral selector, i.e. cellulose-2 [cellulose tris (3-chloro-4-methylphenylcarbamate) and cellulose-3 [cellulose tris

(4-methylbenzoate)], and based on smaller particles (3 μm instead of 5 μm). Polysaccharide stationary phases are well-known to provide excellent performance in terms of both enantioselectivity and chemoselectivity [34]. To this end, an isocratic RP method was applied, using water and acetonitrile, both of them containing 10 mM formic acid, at a flow rate of 0.4 mL/min; the column temperature was maintained at 25 °C. After testing different composition percentages, the best separation was obtained using a 50:50 ratio since the greater the water% the higher the retention time of the analytes. Under these conditions, DEXA and BETA were well separated (R=2.5) as well as most of the analytes. The partial co-elutions obtained for DEXA/HCORT (R=0.7) and BETA/CORT (R=0.7) were not a problem since they were MS-solved. Fig. 1 shows the LC/MRM chromatogram of a working standard solution (0.1 ng/μL) under the optimized conditions.

Optimization of the extraction method

In this work, the SPE and DLLME combination was devised to make more sustainable the SPE procedure: *i*) by using recommended/green solvent systems (ethyl acetate and the LTTM ChCl:Ses); *ii*) by employing small volumes of solvents (in the order of hundreds of microliters); and *iii*) by avoiding evaporation steps. This combination allows one not only to obtain high EFs but also the treatment of complex matrices. To reach high extraction recoveries and EFs, the SPE and DLLME conditions were carefully optimized by applying an OVAT (One Variable At a Time) approach. For the experiments, 200-mL aliquots of blank river water (samples taken from River Tiber in the Farfa Oasis) were spiked with the working solution of the eleven glucocorticoids to obtain a concentration of 0.1 μg/L.

The first SPE experiments were performed to optimize the only SPE step in terms of sample volume, sample pH, sample ionic strength, type, and volume of eluent.

With the aim of defining the proper sample volume to treat, different sample volumes (50, 100, 150, 200, and 250 mL) were processed on Oasis HLB cartridges (60 mg), fitted into a 12-port Visiprep SPE vacuum manifold. Good recoveries were obtained for all the analytes (80-100%) in all cases, without incurring in breakthrough phenomena. For the elution was used methanol (3 mL); then, the eluate was concentrated in a water bath at 40 °C under nitrogen flow till to a final of 100 μL, and 2 μL was injected into the HPLC-MS/MS system. A sample volume of 200 mL was selected as the best compromise in terms of both high EF and reasonable time of processing.

Even if glucocorticoids exhibit weakly acid groups, such as 17α- and 21-hydroxyl groups, the acidification of the sample volume does not provide a recovery increase. For this reason, the sample pH was not adjusted.

In order to combine SPE with DLLME, the elution solvent of SPE was chosen to satisfy the requirements necessary to act also as the dispersant during the DLLME step. Besides methanol, the other tested solvents were: acetone, ethyl acetate, and acetonitrile. By eluting with 1 mL of each solvents and using ChCl:Ses (see Section 2.3 Preparation of choline chloride-sesamol mixture), recoveries were: 60-100% (ethyl acetate), 60-87% (acetone) and 60-86% (acetonitrile and methanol), showing that ethyl acetate is more efficient than acetone and acetonitrile. Moreover, according to the classification based on guidelines CHEM21 [28], ethyl acetate is greener (classified as recommended solvent) than acetone (classified as recommended but with fewer scores), methanol and acetonitrile (these last ones classified as problematic solvents). Other trials were carried out to study the ionic strength effect by using different concentrations of NaCl, ranging from 0 to 10% (w/v). The results showed that the salt addition in the explored range produced a positive effect on the phase separation, making the extractant withdrawal easier and maintaining high the recovery yields; NaCl at 5% was the condition providing the best results. Finally, the last evaluation was about the necessary volume of the dispersant: halving the elution volume of ethyl acetate (500 μL), mean recoveries (three replicates) were unvaried. It was concluded that a volume of 500 μL was sufficient to desorb the trapped analytes from the SPE column and to disperse the extractant adequately during the DLLME.

Results of the method validation

Tables 2 and 3 list all the calculated figures of merit for the main validation parameters.

Table 2 reports, for each analyte, the equation of the calibration curve, and the determination coefficient R^2. The slope of each curve provides the sensitivity for a specific analyte; among all, BETA, DEXA, and PREDLO are the glucocorticoids detected with the better sensitivity, while HCORT Ac was the one with the lowest sensitivity. The values of R^2 are all greater than 0.99. No carryover effect was observed injecting methanol after the calibrator at the highest concentration value on the calibration curve.

Recoveries were greater than 68% at the lowest spike level and greater than 70% at the highest spike level. Intra-day precision and inter-day precision were always lower than 15% regardless of the spike level to which were calculated.

The SPE-DLLME procedure was able to reach EFs varying from 1971 to 2857, which is the maximum achievable under the established extraction conditions (see Table 3).

Comparison with other extraction methods

Table 4 lists the main figures of merit of some recent methods having some analytes in common with this work. As far as LOQ is concerned, the method based on double SPE is the one providing the lowest values [12] together with the SPE method by Shen et al. [13]. Depending on the analyte, our method shows similar figures as other methods [12,13,19] not only in terms of LOQs but also in terms of precision and recovery, with the exception of the method of Herrero et al. [14] that exhibits very good precision. Our method displays slightly lower yields for a few of the analytes but at a very low spike level (5 ng/L) which was not applied for such calculation in the other methods.

Concerning the extraction time, our procedure allows one to reach high EFs, is more rapid, does not involve an evaporation step, and uses very small volumes of organic solvents which are classified “recommended” by guidelines CHEM21; thus, it is safer for the operator and with a minimal environmental impact due to the use of the neoteric solvent. Also, the method by Huang et al. [19] is quick, does not require the evaporation step, and consumes small volumes of methanol, whose total amount, however, is difficult to define precisely because it was not specified by the authors.

Application to real samples

The validated method was finally applied to analyze real samples from Tiber river. The samples from the Farfa Oasis did not show the occurrence of glucocorticoids at detectable levels. On flowing through Rome, the Tiber River receives effluents from three sewage treatment plants. Traces of FLU (see Fig. 2) were found in the water samples collected after Isola Tiberina, where is also located an important hospital equipped with its own sewage treatment plant. Such an occurrence could also be explained considering that the samples were gathered from a zone (the center of Rome) with a great anthropogenic impact.

Conclusion

The purpose of this work was to develop a green version of the DLLME sample preparation method for the determination of substances such as corticosteroids from aqueous samples. In this regard, an LTTM based on choline chloride and sesamol was prepared (ChCl:Ses, 1:3). Eutectic solvents and their related low transition temperature mixtures represent one of the main areas of modern chemical research since they

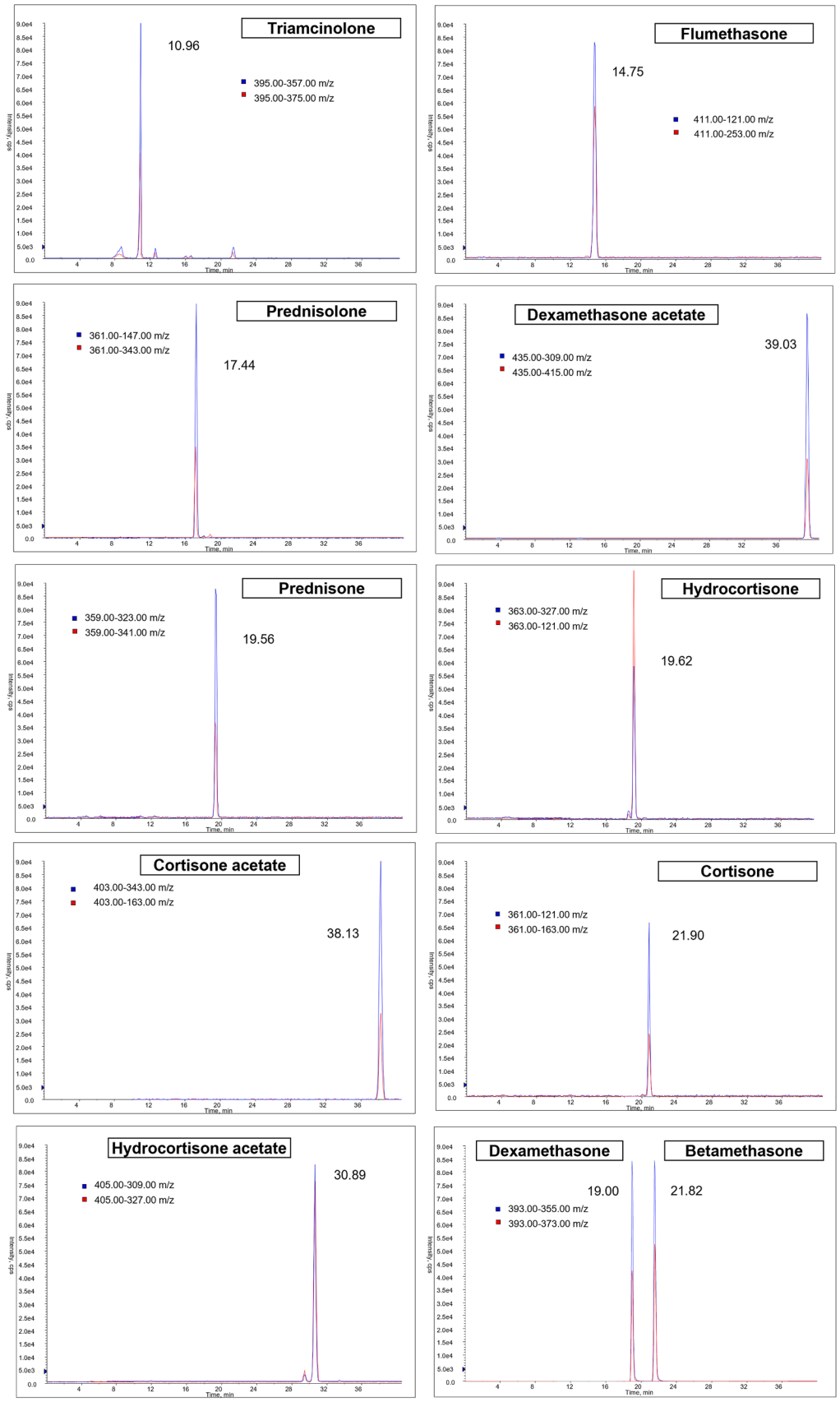

Fig. 1. Selected reaction monitoring chromatograms of a composite working standard solution (200 pg injected).

Table 2
Linear regression parameters.

Analyte	Regression equation[a] $m \pm s_m t_{(0.05;6)}$	$q \pm s_q t_{(0.05;6)}$	R^2
Triamcinolone	29.82 ± 0.28	22.82 ± 0.18	0.9947
Flumethasone	25.27 ± 0.52	19.83 ± 0.82	0.9953
Prednisolone	90.36 ± 0.71	54.30 ± 0.55	0.9938
Dexamethasone	96.08 ± 0.42	50.29 ± 0.86	0.9948
Hydrocortisone	46.09 ± 0.29	19.70 ± 0.98	0.9960
Prednisone	35.31 ± 0.51	26.31 ± 0.68	0.9937
Betamethasone	90.51 ± 0.48	45.02 ± 0.27	0.9932
Cortisone	61.70 ± 0.98	41.05 ± 0.94	0.9971
Dexamethasone-acetate	28.93 ± 0.35	19.24 ± 0.95	0.9956
Hydrocortisone-acetate	18.09 ± 0.63	14.06 ± 0.97	0.9952
Cortisone-acetate	60.92 ± 0.45	40.03 ± 0.22	0.9942

[a] Mean of six independent analyses.

show numerous properties in agreement with the twelve principles of green chemistry. In analytical chemistry, the advantages of using DESs, as an alternative to the classical chlorinated solvents used in the DLLME, lie in the negligible toxicity to the operator and the environment. In addition, the lower vapor pressure avoids a possible alteration of the concentration of analytes in the final extract. DESs can be directly injected into the column, thus also avoiding the possibility of analyte degradation during evaporation, which results necessary when common chlorinated solvents are used as a result of their limited compatibility with the mobile phase used in RP chromatography. The extraction method, coupled with the highly sensitive HPLC-MS/MS hyphenation, was suitably optimized and validated to extract eleven corticosteroids in water samples. The method was successfully applied to the assay of corticosteroids in river waters, and showed their presence in some samples, raising suspicion of actual risk from environmental contamination.

Table 3
LLOQ, enrichment factor, recovery, precision, and accuracy.

ANALYTES	LOD (ng/L)	LOQ (ng/L)	Enrichment factor[a]	Recovery[b]% Spike levels		Intra-day precision[b] (RSD%) Spike levels		Inter-day precision[b] (RSD%) Spike levels	
				5 ng/L	20 ng/L	5 ng/L	20 ng/L	5 ng/L	20 ng/L
Triamcinolone	0.44	1.45	2543	88	90	10	7	13	11
Flumethasone	0.54	1.89	2257	78	80	7	7	11	11
Prednisolone	0.58	1.79	1971	68	70	7	5	8	6
Dexamethasone	0.30	0.96	2857	100	100	12	10	14	13
Hydrocortisone	1.30	4.03	2000	68	72	8	8	15	10
Prednisone	1.39	4.17	2143	74	76	9	6	14	13
Betamethasone	0.30	0.94	2128	71	78	8	8	10	8
Cortisone	0.21	0.69	2172	72	80	8	7	7	7
Dexamethasone-acetate	0.72	2.23	2514	86	90	10	7	11	8
Hydrocortisone-acetate	0.82	2.46	2600	88	94	12	8	13	10
Cortisone-acetate	0.48	1.58	2585	89	92	9	8	9	9

[a] The enrichment factor has been reported as mean values of data obtained for the two spiking levels;.
[b] Recovery and precision were calculated by preparing five replicates at each spike level.

Table 4
Comparison of the main figures of merit of some recent extraction methods aimed at the extraction of glucocorticoids from river water.

Method (common analytes)	Common analytes	Enrichment factor	Recovery%	RSD%	LOQ (ng/L)	Types and volumes of organic solvents; Evaporation	Extraction time (min)	Reference
SPE/SPE-UHPLC-MS/MS	FLU, PREDLO, DEXA, HCORT, PRED, BETA, CORT	Up to 500	81.3–108.6 (5 and 20 ng/L spike levels)	3.4–5.9	0.019–0.029	Hexane: 5.4 mL MTBE: 10.5 mL Methanol: 11.5 mL Ethyl acetate: 0.6 mL Tot[a]: 28 mL Evaporation	> 1h	[12]
SPE-UPLC-MS/MS	FLU, PREDLO, DEXA, HCORT, PRED, BETA, CORT, DEXA-Ac, HCORT-Ac, CORT-Ac	Up to 5000	71–122 (10 and 50 ng/L spike levels)	5–17	0.03–0.13	Ethyl acetate: 9 mL Acetonitrile: 9 mL Methanol: 0.2 mL Tot[a]: 18.2 mL Evaporation	1h	[13]
SPE-UPLC-MS/MS	BETA, CORT, HCORT, DEXA, FLU, PRED, PREDLO	Up to 500	82–99 (10 ng/L spike level)	1–4	1.5	Methanol: 15 mL Acetonitrile: 0.2 mL Tot[a]: 15.2 mL Evaporation	1 h	[14]
Magnetic SPE-HPLC-MS/MS	FLU, DEXA, CORT, PRED	Up to 100	80.6–117 (5, 50, 500 ng/L spike levels)	1.2–7.2	0.19–0.46	Methanol: not specified to condition the sorbent CH_3OH/acetic acid (99.4/0.6, v/v): 0.5 mL+ not defined volume to rinse the sorbent Tot[a]: > 1 mL No evaporation	< 1 h	[19]
SPE-DLLME-HPLC-MS/MS	BETA, CORT, CORT Ac, DEXA, DEXA Ac, HCORT, HCORT Ac, PREDLO, PRED, FLU, TRI	Up to 2857	68–100 (5 and 20 ng/L, see Table 3)	5–14	0.69–4.17	Methanol: 2 mL Ethyl acetate: 0.5 mL Tot[a]: 2.5 mL ChCl:Ses 1:3: 100 μL No evaporation	< 1 h	This work

[a] Tot: it is referred to the total consumption of organic solvents.

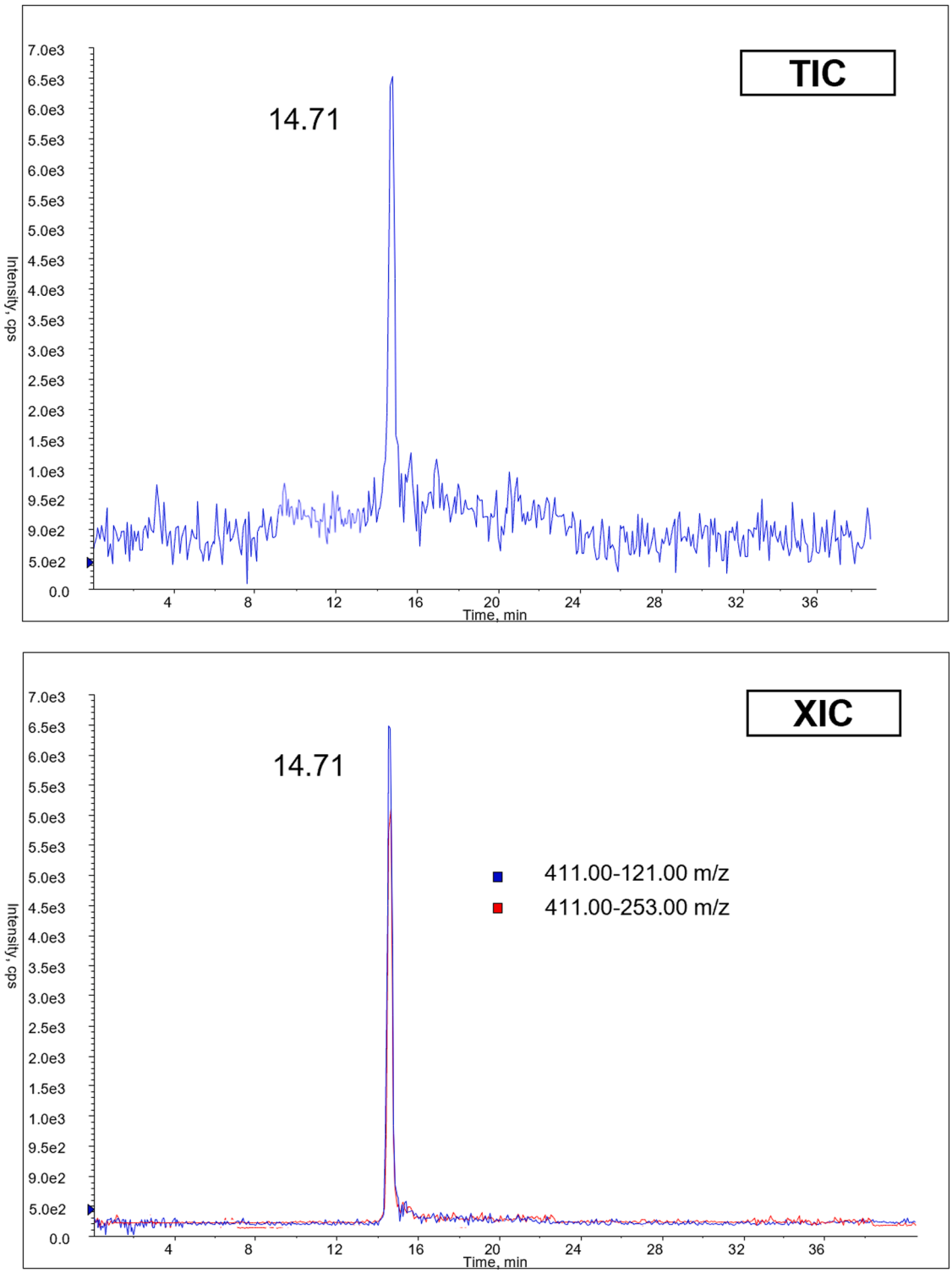

Fig. 2. HPLC-MRM chromatogram obtained from Tiber River in January 2023.

CRediT authorship contribution statement

Lorenzo Antonelli: Writing – original draft. **Chiara Dal Bosco:** Writing – original draft. **Massimo Giuseppe De Cesaris:** Writing – original draft. **Nina Felli:** Writing – original draft. **Elena Lucci:** Writing – review & editing. **Alessandra Gentili:** Supervision, 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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcoa.2023.100100.

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