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Ion-paired extraction of cephalosporins in acetone prior to their analysis by capillary liquid chromatography in environmental water and meat samples

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ABSTRACT

Ion-pair extraction of cephalosporins from aqueous solution into acetone by the addition of ammonium sulfate to a 1:2 (v/v) acetone–water solvent was carried out followed by their determination using reversed-phase capillary liquid chromatography. The analytes included are cephoperazone, cefquinome, cephalexin, cephapirin, cephaloniun, cephamandole, cephazolin and cephadroxile. In order to form the ion-pair, hexadecyltrimethylammonium bromide (CTAB) was selected as cationic ion-pairing agent at a concentration of 0.9 mM using 10 mM phosphate buffer at pH 8 as the optimum condition for the aqueous solution. The applied methodology, named salting-out assisted liquid/liquid extraction (SALLE) involves the use of 1.25 g of ammonium sulfate as salting-out agent.

The separation of cephalosporins using a Luna C_{18} (150 mm × 0.3 mm, 5 μm, 100 Å) column was achieved under the following conditions: a gradient program combining solvent A (0.1% formic acid in water, pH 4) and solvent B (acetonitrile–methanol (50:50, v/v)), at a flow rate of 20 μl min^{-1}, column temperature 35 °C and injection volume 7 μl with UV detection at 250 nm. The limits of quantification for the studied compounds were between 4.3 and 22.7 μg/L for water samples and 4.1 and 73.3 μg/kg in the case of beef samples, lower than the maximum residue limits permitted by the EU for this kind of food. The developed methodology has demonstrated its suitability for the analysis of these widely applied antibiotics in environmental water and meat samples, including beef and pork muscle, with high sensitivity, precision and satisfactory recoveries.

1. Introduction

β-lactam antibiotics, including penicillins and cephalosporins, are one of the most widely applied groups of antimicrobial drugs in current veterinary and human practice. They are characterized by a beta-lactam ring in their structures, fused to a five-membered thiazolidine or a six-membered dihydrothiazine ring, respectively. The cephalosporin structure allows for more gram-negative activity than the penicillins. Improper use of these antibiotics may lead to residues in foods of animal origin and can cause human health risks [1]. β-lactam antibiotics possess an allergenic potential in pre-sensitised people and therefore it cannot completely be ruled out that high residue levels in food will evoke allergic reactions in highly sensitive individuals [2]. In this sense, maximum residue limits (MRLs) of antibiotic in foodstuffs of animal origin such as various animal tissues (e.g. muscle, liver, kidney, fat), milk or eggs have been recently revised by the European Regulation (EU) No. 37/2010 [3].

Also antibiotics are continuously being released into the environment mainly as a result of manufacturing processes and excreta [4,5,6] or from hospital and household discharges, getting into municipal wastewater treatment plants that cannot remove them [7,8,] and finally going into the environment via effluent or sludge [9,10]. The importance of the determination of antibiotic residues in environmental samples arises from the fact that they are suspected of being responsible of the appearance of bacterial strains that are resistant to them [11].

The low concentration levels and the complexity of the matrices of interest such as environmental water samples or foods of animal origin involve the development of sensitive and selective methods for β-lactam determination. High performance liquid chromatography (HPLC) has been widely used for the analysis of antibiotics in food and environmental samples, including β-lactams, mainly in combination with MS as has been stated in different reviews [12–15]. Also capillary electrophoresis (CE) has been used in combination mainly with DAD detection [16–18]. Some reviews about analytical methodologies for the detection of β-lactams have been recently

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published [19,20] and also specifically related to the analysis of cephalosporin antibiotics, using mainly reverse phase HPLC [21]. In food derived from animals they have been determined mainly in milk by HPLC-DAD; cephalexin using a C8 column [22], cephalexin and cefotaxime with a C18 column [23], four cephalosporins were analyzed by ion-paired gradient on a phenyl column [24] or eight of them were separated in 16 min, using a monolithic analytical column [25]. Using LC–MS it has been possible to develop multiresidue analytical methods for β-lactams including some cephalosporins in milk [26,27], muscle [27–29] or kidney [30,31].

In order to ensure the reliability and accuracy of the detection in complex matrices, suitable sample preparation methods are required before the instrumental analysis [32]. For the analysis of cephalosporins in HPLC, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most usual sample preparation modes [23,24,26,27] and recently pressurized liquid extraction (PLE) [29], matrix solid-phase dispersion (MSPD) [25] or dispersive solid phase extraction (d-SPE) procedures based on QuEChERS have been satisfactorily applied [28,30,31].

The extraction of cephalosporins from aqueous media into non-polar organic solvent is a challenging task because of their high polarity and the instability of the β-lactam ring with respect to acid and alkali. LLE can be modified to extract polar organic compounds like cephalosporins using ion-paired extraction with a water-miscible organic solvent [33]. A phase separation occurs from a mixture of solvents containing water and water-miscible organic solvent like acetonitrile or acetone upon addition of an electrolyte to the mixture of solvents, i.e., salting-out, due to the decrease of the solubility of the organic solvent in the aqueous solution. The separated organic solvent contains water and salts, resulting in large donor and acceptor abilities compared to those of the corresponding pure organic solvent [34]. Thus, the solvent can easily extract ion-paired compounds of cephalosporins. The so called salting-out assisted liquid/liquid extraction (SALLE) is a cost-effective, time-efficient and easy-to-use sample preparation method [35].

The goal of this study is to develop a new method for the determination of cephalosporins for veterinary and human use in environmental and food matrices, like water and meat samples, based on the use of hexadecyltrimethylammonium bromide (CTAB) as ion-pairing agent to achieve a simple and effective treatment procedure. The sample treatment is based on the extraction of cephalosporin-CTAB compounds using a binary acetone–water mixture as extractant from water-salt solutions. A capillary HPLC method has been developed, showing several advantages compared to analytical HPLC, such as better resolution, lower detection limits and lower solvent consumption, being more environmentally friendly than conventional HPLC. The combination of a simple sample treatment such as ion-pair formation and SALLE with this miniaturized technique can provide a useful method for the monitoring of these antibiotics in routine analysis.

2. Experimental

2.1. Chemicals and standard solutions

All the reagents used were of analytical grade and the solvents of HPLC grade. The water used to prepare the solutions was purified with a Milli-Q system (Millipore, Bedford, MA, USA). Organic solvents such as acetonitrile (MeCN), methanol (MeOH) and acetone (ACO) were purchased from Merck (Darmstadt, Germany).

The formic acid (FA) used in the mobile phase was supplied by Sigma Aldrich (St. Louis, MO, USA). The pH was adjusted to 4.0 with sodium hydroxide (10 M), obtained from Merck.

Analytical standards of cephoperazone (CPR, CAS: 62893-20-3; purity: 94%) and cefquinome (CQ, CAS: 118443-89-3; purity: 95%), were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Cephalexin (CL, CAS: 15686-71-2; purity: 99.8%), cephapirin (CP, CAS: 24356-60-3; purity: 98%), cephaloniun (CLN, CAS: 5575-21-3; purity: 99.4%), cephamandole (CM, CAS: 30034-03-8; purity: 99.9%), cephazolin (CZ, CAS: 27164-46-1; purity100%) and cephadroxile (CD, CAS: 66592-87-8; purity: 100%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Stock standard solutions containing 1000 mg L^{-1} of each compound were prepared by dissolving accurately weighed amounts in ultrapure water and acetonitrile-water (50:50, v/v) in the case of CLN, and stored in darkness at 4 °C. Working standard solutions were made daily by diluting them with deionised water. The chemical structures, common names, abbreviations and pKa values of the selected compounds are listed in Fig. 1.

Hexadecyltrimethylammonium bromide (CTAB) and tetrabutylammonium bromide (TBABr) were purchased from Sigma Aldrich. The stock solution of CTAB (0.9 mM) and TBABr (6 mM) were prepared in water. Ammonium sulfate, $(NH_4)_2SO_4$ and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Panreac (Barcelona, Spain).

2.2. Instrumentation and software

The HPLC system consisted of an Agilent HP-1200 series capillary high performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump (20 μl min^{-1} maximum flow-rate), on-line degasser, autosampler (8 μl loop), column thermostat, and a diode array detector (DAD). ChemStation software (A.10.20 [1757] version) was used for data acquisition and processing.

The pH measurements were carried out in a pH-meter model Crison pH 2000 (Barcelona, Spain). A centrifuge Model Universal 320R (Hettich, Tuttlingen, Germany), a vortex-2 Genie (Scientific Industries, Bohemia, USA), an evaporator with nitrogen System EVA-EC from VLM GmbH (Bielefeld, Germany) and a blender (Taurus, Barcelona, Spain) were used for sample treatment.

2.3. HPLC conditions

The chromatographic separation was performed on a Luna C18 (2) (150 mm × 0.3 mm, 5 μm, 100 Å) from Phenomenex (supplied by Jasco Analítica, Spain). A gradient program was used, combining eluent A (Milli-Q water with 0.1% FA, pH: 4) and eluent B (MeCN–MeOH (50:50, v/v)) as follows: 5% B (0 min) to 25% B (10 min), 30% B (15 min), 45% B (20 min) at a flow rate of 20 μl min^{-1}, keeping the column temperature at 35 °C. The injection volume was 7 μl and all the compounds were eluted within 22 min. The UV detector was set at 250 nm. Quantification was performed using corrected peak area as analytical signal.

2.4. Sample preparation procedures

2.4.1. Water samples

Spring water collected from Sierra Arana (Granada, Spain) and river water from a local river (Paules, Granada, Spain) were analyzed. The water samples were collected directly in PVC bottles; they were filtered through nylon membranes with 0.2 μm pore size and were stored at 4 °C in the dark.

A volume of 200 mL of the natural water samples was spiked with different concentration levels of standard solutions of the analytes (CL, CP, CLN, CQ, CZ, CPR and CM) and an adequate amount of K_2HPO_4 was dissolved in order to obtain a 10 mM phosphate buffer at pH 8. An aliquot of 2.0 mL was taken and 200 μL of CTAB (0.9 mM) were added and stirred in vortex during 1 min. in order to form the ion-pair. Afterward, 1 mL of acetone and 1.25 g of ammonium sulfate were added. The mixture solution

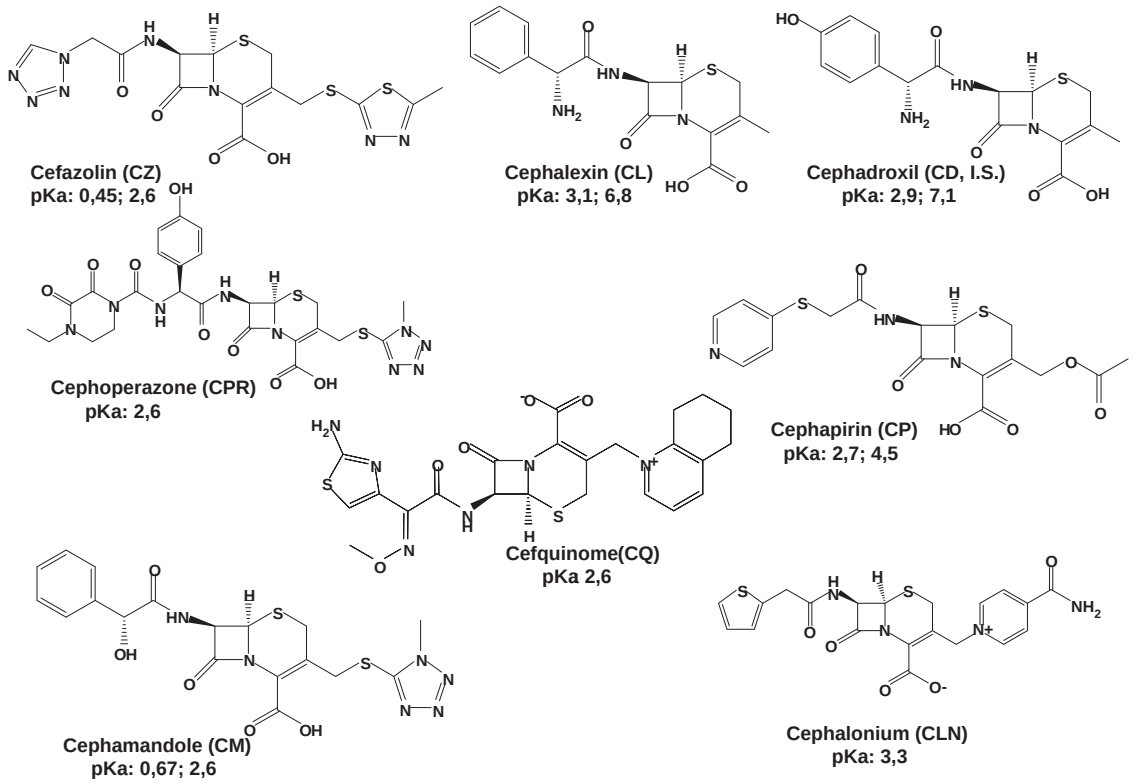

Fig. 1. Chemical structures, common names, abbreviations and pKa values of the studied cephalosporin antibiotics.

was well mixed in vortex during 1 min. and centrifuge at 4500 rpm during 5 min to obtain the separation of the organic phase. The upper phase was evaporated at 30 °C under a gentle nitrogen current to eliminate the organic solvent and reconstituted in 200 μL of water with CD (1 mg/L) as internal standard. These solutions were filtered through 0.2 μm nylon membrane and injected into the capillary HPLC system for analysis.

2.5. Meat samples

Beef and pork samples were purchased from a local market in Granada (Spain). A representative portion of sample (250 g of meat muscle) was chopped and homogenized before fortification by adding the appropriate volume of the working standard solutions of the cephalosporins of veterinary use (CL, CP, CLN, CQ, CZ and CPR). After fortification, the samples were kept in dark during 15 min.

Portions of 2.0 g of crushed meat samples were extracted with acetonitrile, by adding 4 mL and mixed in vortex during 1 min. After that, the samples were centrifuged at 9000 rpm for 5 min., the supernatants were transferred to vials and were brought to dryness under a gentle nitrogen current at 30 °C. The dry residues were redissolved in 2.0 mL of phosphate buffer at pH 8 (10 mM) and subjected to the treatment described above for water samples. In this case the final dry residues were dissolved in 250 μL of water containing CM (1 mg/L), a cephalosporin for human use, as I.S. in this case. These solutions were filtered through 0.20 μm nylon membrane and injected into the capillary HPLC system for analysis.

3. Results and discussion

3.1. Optimization of the chromatographic separation

Considering a previous work concerning the separation of cephalosporins by analytical HPLC [25] we checked acetonitrile, methanol and mixtures of both of them as the organic phase and formic and acetic acid in different ratios, both in the aqueous and in the organic fraction of the mobile phase. For the best separation, a mixture of acetonitrile/methanol (50:50, v/v) was selected as organic fraction (eluent B) and water with 0.1% of formic acid was fixed as aqueous fraction (eluent A)

The pH of the aqueous fraction of the mobile phase was tested at 3.0, 3.5, 4.0, 4.5 and 5.0 and finally the selected pH value in eluent A was 4.0 adjusted with sodium hydroxide (10 M), because satisfactory resolutions between peaks were obtained. Gradient mode was selected in order to get a better resolution in a shorter analysis time. The flow rate was also optimized testing values from 11 to 20 μL/min, selecting 20 μL/min as the optimum value because the retention times were lower and sensitivity was not affected. Temperature was varied from 15 to 40 °C selecting a final value of 35 °C. Injection volume was increased from 1 to 7 μL and 7 μL was selected as optimum value in order to increase sensitivity without any loss of resolution. The separation of the compounds was carried out within 22 min.

3.2. Optimization of the extraction procedure

3.2.1. Water samples

Cephalosporins are quite polar compounds, with low pKa values (see Fig. 1) due to the presence of carboxylic groups. Under neutral conditions they are in anionic form and highly soluble in aqueous solution, resulting in poor extraction efficiency. Based on the method proposed by Kukusamude et al. [33] for the extraction of three penicillins, we accomplished the extraction and preconcentration of the selected cephalosporins from aqueous media using an ion-pairing agent and a water-miscible organic solvent.

For the optimization of the sample treatment in water a volume of 200 mL of the natural water samples was spiked with different concentration levels of standard solutions of the analytes and an adequate amount of K_2HPO_4 was dissolved in order to obtain a

10 mM phosphate buffer at pH 8. An aliquot of 2.0 mL was taken and subjected to the sample treatment. Firstly, TBABr and CTAB were checked as cationic ion-pairing agents, finding that CTAB provides higher extraction efficiency in terms of recovery. To select the optimum amount of CTAB the concentration was kept constant at 0.9 mM, below the critical micelle concentration (CMC) which is 1 mM, and 100, 200, 400, 600 and 800 μL were added. It was found that 200 μL was enough to form the ion-pair. In order to select the most appropriate organic solvent for the extraction of the ion-pair formed we tested ethanol, acetone, 2-propanol, tetrahydrofuran and ethyl acetate finding that the best recoveries were obtained with acetone. The acetone volume was studied by adding 0.5, 1.0, 1.5, 2.0 and 2.5 mL. Finally 1.0 mL of acetone was found to be enough to extract the ion-pair from 2.0 mL of aqueous solution, as is shown in Fig. 2. As salting-out agent, ammonium sulfate was used because in preliminary experiments it was found that using this salt, the volume of organic phase obtained was higher than that obtained when using sodium chloride (a common salting-out agent). The amount of ammonium sulfate was tested by adding 0.5, 0.75, 1.0, 1.25 and 1.5 g of salt; 1.25 g of salt was found to be the optimum value because it provided the best extraction efficiency with a saturated solution of salt. The cephalosporin-CTAB ion-pairs can be more efficiently extracted into the acetone phase in comparison with the original polar forms of these compounds, increasing the recovery values. The optimized extraction procedure named ion-pair salting-out assisted liquid/liquid extraction (IP-SALLE) was used for the application of the method in water samples. Fig. 3A shows a chromatogram of a blank of water sample, using spring water as representative matrix. No cephalosporins or other interferents were found comigrating with the analytes. Fig. 3B provides the profile for the spring water sample spiked with 100 μg/L of each studied cephalosporin.

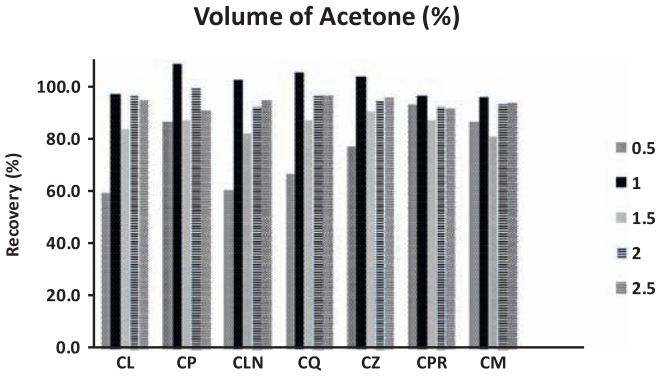

Fig. 2. Comparison of recoveries obtained for each cephalosporin using different volumes of acetone for the extraction of the ion-pair.

3.2.2. Meat samples

In the case of the meat, a portion of 2.0 g of beef muscle spiked with 200 μg/Kg of each cephalosporin was used to evaluate the extraction efficiency. In this case it was necessary to extract the cephalosporins from the matrix before the formation of the ion-pair. Acetone, acetonitrile and mixtures of them (2:8, 4:6, 5:5, 6:4, 8:2, v/v) were checked as extraction solvent; finally 100% acetonitrile provided the best extraction efficiency. The volume of acetonitrile was studied by adding 0.5, 1.0, 2.0, 4.0 and 6.0 mL, finding that 4.0 mL of acetonitrile was the optimum value. After that the organic extract goes to dryness under a gentle nitrogen current at 30 °C and the procedure follows as it is described above, redissolving the dry residues in 2.0 mL of phosphate buffer (10 mM) at pH 8. Fig. 4A shows a chromatogram of a blank of beef sample. No cephalosporins were found and some endogenous interferents were separated from the selected compounds. Fig. 4B provides the profile for a beef sample spiked with 200 μg/kg of each studied cephalosporin.

3.3. Validation of the IP-SALLE-capillary HPLC method

3.3.1. Calibration curves and performance characteristics

The method was characterized for different types of matrices of environmental water and meat. With this purpose, calibration curves in the presence of matrix were obtained using spring water and muscle beef samples spiked with the subsequent calibration levels before sample treatment. In all cases, a blank sample was analyzed in order to check if any of the analytes were already present at a concentration above the limit of detection or if some interferences could comigrate with the analytes.

Spring water samples were used for the establishment of matrix calibration curves for the analysis of environmental waters. The calibration levels for the analytes (CL, CP, CLN, CQ, CZ, CPR and CM) were 5, 25, 50, 100, 150 and 200 μg /L of each analyte in the sample. In all cases 1 mg/L of CD was added as I.S. For muscle beef samples, the matrix-matched calibration curves of the cephalosporins for veterinary use (CL, CP, CLN, CQ, CZ and CPR) were established from six levels corresponding to concentrations in sample of 25, 100, 200, 300, 400 and 500 μg/kg of each cephalosporin. In this case 1 mg/L of CM was added as I.S.

In both matrix-matched calibration curves the relative corrected peak area (as the ratio of analyte peak area to I.S. peak area) as a function of the analyte concentration was considered. Each concentration level was prepared by duplicate and injected by triplicate. Satisfactory determination coefficients confirmed that

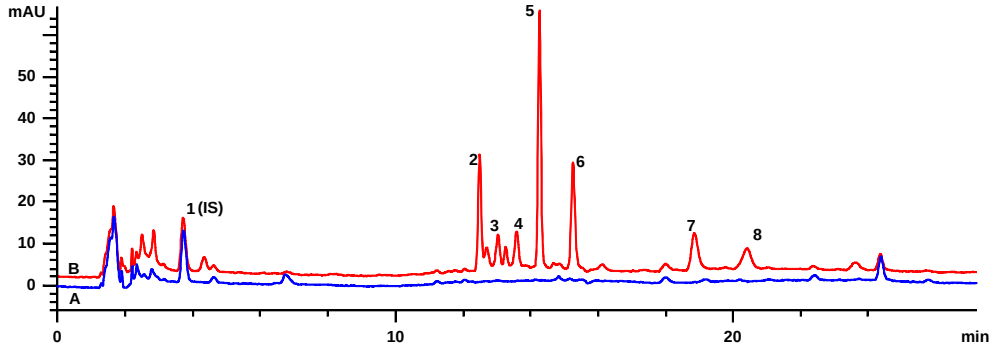

Fig. 3. (A) Chromatogram of a blank of water sample. (B) Chromatogram of water sample spiked with 100 μg/L of each studied cephalosporins. Peak identification: 1: CD as I. S; 2: CL; 3 CP; 4: CLN; 5: CQ; 6: CZ; 7: CPR; 8: CM.

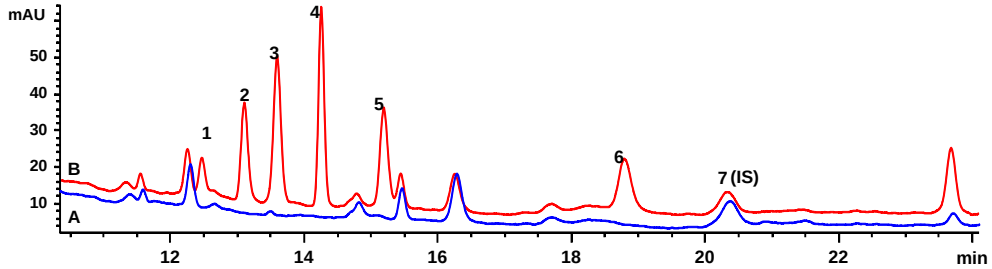

Fig. 4. (A) Chromatogram of a blank of beef sample. (B) Chromatogram for a beef sample spiked with 200 μg/kg of each studied cephalosporins. Peak identification: 1: CL; 2 CP; 3: CLN; 4: CQ; 5: CZ; 6: CPR; 7: CM as I.S.

Table 1
Statistical and performance characteristics for the studied cephalosporins in each kind of sample.

Compounds	Intercept	Slope	R^2	LOQ (μg/L)	Linear dynamic range (μg/L)	CCα (μg/L)	CCβ (μg/L)
Spring water							
CL	−0.0093	0.0119	0.996	10.6	10.6–200	10.7	21.3
CP	0.0407	0.0038	0.992	19.4	19.4–200	13.9	27.7
CLN	0.033	0.0044	0.991	22.7	22.7–200	17.5	35.0
CQ	0.0596	0.0209	0.997	4.3	4.3–200	8.4	16.9
CZ	0.0053	0.0115	0.995	11.6	11.6–200	11.7	23.5
CPR	−0.0637	0.0067	0.993	6.8	6.8–200	13.2	26.5
CM	0.034	0.0043	0.992	14.9	14.9–200	14.6	29.1
Beef muscle[a]							
CL	−2.28	0.3284	0.991	73.3	73.3–400	236.1[b]	672.2[b]
CP	25.7	0.981	0.993	28.2	28.2–400	80.6[b]	211.3[b]
CLN	−3.2	1.72	0.993	25.0	25.0–400	30.5	61.1
CQ	−7.67	1.68	0.997	15.5	15.5–400	71.8[b]	193.6[b]
CZ	73.3	1.19	0.991	4.1	4.1–500	41.1	82.1
CPR	3.14	1.04	0.994	33.8	33.8–400	29.0	58.1

[a] MRLs established by the Commission Regulation (EU) N° 37/2010 in beef muscle sample: CL: 200 μg/Kg; CP: 50 μg/Kg; CQ: 50 μg/Kg; CLN, CZ, CPR: Not established.
[b] Obtained at the MRLs which are specified for beef muscle.

responses were linear over the studied range for the samples. All the statistical parameters and performance characteristics are shown in Table 1. Limits of quantification (LOQs) have been calculated using *S*/*N* of 10. As can be seen, for all the studied compounds, very low LOQs were obtained. It must be highlighted that LOQs for beef muscle samples were below the legislated MRLs [3], established at 50–200 μg/kg. The LOQs obtained in water for all the studied compounds by applying this procedure are at the low μg/L levels. No regulation exists for this kind of emerging pollutants in waters.

In order to complete the validation procedure, CCα and CCβ were calculated for water and beef muscle by the calibration curve procedure [36]. This procedure allowed the determination of both parameters for non-permitted and permitted veterinary drug substances. It can be observed in Table 1 that in spring water samples, CCα ranged from 8.4 to 17.5 μg/L and CCβ from 16.9 to 35.0 μg/L were obtained. In the case of beef muscle samples, for permitted substances with a MRL set at 200 μg/kg (cephalexin) CCα and CCβ were lower than 240 and 675 μg/kg, respectively, whereas for substances with MRL set at 50 μg/kg (cephapirin and cefquinome) CCα values were over 70 μg/kg and CCβ values were about 200 μg/kg.

3.3.2. Precision study

The precision of the whole method was evaluated in terms of repeatability and intermediate precision. Repeatability was assessed on the same day by means of repetitive application of the sample treatment and separation procedure to spring water samples (three experimental replicates) at concentration levels of 25, 100 and 150 μg/L, and 50 (75 μg/Kg for CL), 200, 400 μg/Kg for beef muscle samples, and each one was injected in triplicate (instrumental replicates). Intermediate precision was assessed for five consecutive days with one sample (spring water or beef muscle) for each concentration level and injected by triplicate. The results, expressed

Table 2
Precision study (RSD%) of the proposed method for different concentration levels.

	Intraday			Interday		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Spring water samples						
CL	5.0	6.8	2.1	7.8	6.4	5.9
CP	5.4	3.7	3.7	6.5	3.9	7.4
CLN	3.4	3.3	3.2	8.0	5.8	6.0
CQ	3.7	4.2	3.0	7.2	3.8	6.4
CZ	4.1	3.7	4.1	9.5	3.6	9.9
CPR	3.5	3.0	2.3	8.9	6.1	6.6
CM	2.6	3.4	2.1	5.2	7.1	6.6
Beef muscle samples						
CL	6.8	3.7	2.3	7.5	7.3	8.4
CP	2.4	5.1	4.4	8.4	8.9	6.4
CLN	1.3	7.4	2.7	7.1	6.9	9.2
CQ	0.8	5.8	3.3	9.2	7.9	9.6
CZ	2.1	5.0	2.0	5.8	6.5	5.5
CPR	6.6	8.3	2.7	7.4	8.4	8.7

For water samples: Level 1: 25 μg/L. Level 2: 100 μg/L. Level 3: 150 μg/L.
For beef samples: Level 1: 50 μg/Kg (75 μg/Kg for CL). Level 2: 200 μg/Kg. Level 3: 400 μg/Kg.
RSD, Relative standard deviation (n=9 for intraday study; n=15 for interday study).

as relative standard deviation (RSD) of relative peak areas, are given in Table 2. As it can be observed, acceptable precision was obtained in all cases.

3.3.3. Trueness of the method: recovery studies

In order to check the trueness and the applicability of the proposed methodology for the analysis of cephalosporins, recovery experiments were carried out in different analyte-free kinds of water samples and meat samples. River water samples spiked with each one of the analytes at three different concentration levels, (25, 100 and 150 μg/L) were analyzed. Three replicates were prepared at each concentration level, and each one was injected in triplicate. Previously the samples were extracted following the above-described treatment and a sample blank was also analyzed in order to confirm that the analyzed samples were free of the cephalosporins. The identification of the compounds was based on both their migration times and the absorption spectra. The obtained values of the recovery study in river water samples for each compound and the corresponding RSD (%) are shown in Table 3.

Pork muscle samples were spiked with a mixture of each one of the analytes at three different levels (75, 200 and 400 μg/Kg) in the case of CL, (50, 200 and 400 μ/Kg) for CPR and 25, 100, and 200 μg/Kg for the rest of the analytes. Each level was prepared by triplicate and it was injected three times. In order to check the presence of interferences, blank samples were subjected to the proposed method and no matrix peaks were found comigrating with the analytes. The obtained values of the recovery study for each compound and the corresponding RSD (%) are shown in Table 3 for pork muscle samples.

The proposed method provides satisfactory results in terms of both, trueness and precision for different kind of water and meat of different origins, so demonstrating method accuracy for the analysis of these compounds in these matrices.

Table 3
Recoveries of cephalosporin antibiotics from each kind of samples spiked at different concentration levels.

	Level 1		Level 2		Level 3	
	R (%)	RSD (%)	*R* (%)	RSD (%)	*R* (%)	RSD (%)
Spring water						
CL	76.5	6.3	97.8	5.5	99.9	2.4
CP	90.1	6.2	91.8	6.8	100.8	4.7
CLN	83.9	4.2	93.7	6.8	102.9	5.0
CQ	87.2	7.3	93.1	6.9	103.9	4.6
CZ	81.6	3.0	95.0	7.2	106.6	3.8
CPR	92.7	5.0	95.9	7.0	109.1	5.2
CM	97.7	5.4	96.8	3.9	105.4	4.9
River water						
CL	79.7	4.8	100.2	5.6	105.9	2.9
CP	80.6	6.9	105.5	2.4	104.2	2.8
CLN	73.5	4.5	104.1	3.3	97.0	5.3
CQ	88.2	6.6	104.3	2.7	104.0	3.2
CZ	87.8	4.8	104.3	3.4	107.5	3.4
CPR	84.2	5.8	106.8	2.9	105.7	2.7
CM	102.5	5.4	104.5	3.8	105.1	3.2
Beef muscle						
CL	81.6	6.8	67.9	3.7	70.1	2.3
CP	67.5	2.4	69.5	5.1	68.7	4.4
CLN	74.7	1.3	76.0	7.4	77.2	2.7
CQ	77.1	0.8	78.6	5.8	78.1	3.3
CZ	75.4	2.1	76.7	5.0	77.2	2.0
CPR	87.2	6.6	82.3	8.3	71.3	2.7
Pork muscle						
CL	70.8	9.4	60.9	3.1	62.7	8.3
CP	99.7	8.2	78.1	3.7	93.2	6.7
CLN	85.1	9.6	72.7	7.9	87.2	6.8
CQ	83.2	9.2	75.0	4.6	92.6	4.8
CZ	91.5	6.4	84.9	7.7	91.3	5.3
CPR	107.6	4.9	87.5	10.1	99.8	2.2

For water samples: Level 1: 25 μg/L. Level 2: 100 μg/L. Level 3: 150 μg/L.
For beef samples: Level 1: 50 μg/Kg (75 μg/Kg for CL). Level 2: 200 μg/Kg. Level 3: 400 μg/Kg.
For pork samples: Level 1: 75 μg/Kg for CL, 50 μg/Kg for CPR and 25 μg/Kg for CP, CLN, CQ and CZ. Level 2: 200 μg/Kg for CL and CPR and 100 μg/Kg for CP, CLN, CQ and CZ. Level 3: 400 μg/Kg for CL and CPR and 200 μg/Kg for CP, CLN, CQ and CZ.

4. Conclusions

A sensitive and simple procedure combining IP-SALLE and capillary HPLC with DAD detection has been developed and validated for the analysis of cephalosporins of human and veterinary use (cephalexin, cephapirin, cephalonium, cefquinome, cephazolin, cephoperazone, and cephamandol) in the case of water samples and only of veterinary use (cephalexin, cephapirin, cephalonium, cefquinome, cephazolin, and cephoperazone) in the case of meat samples. Prior to the HPLC analysis, the method applied an ion-pair formation (IP) step necessary for the efficient extraction and preconcentration of the cephalosporins. The preconcentration factor achieved in the sample treatment and the use of a miniaturized technique allow us to obtain very low quantification limits and good resolution, showing the proposed method as an efficient and easy alternative for the monitoring of these antibiotics. According to the results obtained in the precision and trueness studies, the method is accurate for the analysis of waters of different origins and animal products and could be satisfactorily applied as a routine procedure to quantify cephalosporins in laboratories of environmental contamination or food quality and safety control.

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