

HIGH THROUGHPUT QUANTIFICATION OF CEFUROXIME SODIUM IN CATTLE PLASMA BY LC/MS/MS

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Abstract

A simple, rapid and sensitive LC/MS/MS method for quantification of cefuroxime sodium in cattle plasma has been developed and validated. The analyte was extracted from plasma samples using protein precipitation with a mixture of perchloric acid 12% in acetonitrile. Separation was achieved using a Zorbax SB-C18 column using a mobile phase containing methanol: ammonium acetate 1mM in water (14:86 v/v). The detection was realized in MS-MRM mode using an Ion Trap mass spectrometer with electrospray negative ionization. The linearity domain was established between 63 to 6120 ng/ml. Accuracy and precision were less than -4.9% and 9.3% for intra-day assays and -6.9% and 9.3% for inter-day assays, respectively. The recovery ranged between 95 and 105%. The method is very simple and rapid and was used for analysis of cefuroxime sodium in cattle plasma for pharmacokinetics and drug residue analysis.

Rezumat

A fost dezvoltată și validată a metodă LC/MS/MS simplă și sensibilă de cuantificare a cefuroximei sodice din plasmă de bovină. Analitul a fost extras din probele de plasmă prin precipitare cu o soluție de acid percloric 12% în acetonitril. Separarea cromatografică a fost realizată pe o coloană Zorbax SB-C18 utilizând o fază mobilă alcătuită din metanol/soluție apoasă de acetat de amoniu 14/86 (v/v). Detecția a fost realizată prin spectrometrie de masă - MRM, utilizând o trapă ionică, ionizare electrospray negativă. Domeniul de liniaritate a fost stabilit între 63-6120 ng/ml plasmă. Pentru determinările efectuate în aceeași zi, acuratețea și precizia (CV%) au fost sub -4.9 și 9.3% în timp ce pentru determinările în zile diferite, sub -6.9 și 9.3%. Regăsirea analitului a fost între 95 și 105%. Metoda analitică dezvoltată este simplă și rapidă și a fost utilizată pentru determinarea cefuroximei în plasma de vacă în vederea analizei farmacocinetice și a determinării de urme de substanță.

Keywords: cefuroxime sodium, liquid chromatography, cattle plasma, mass spectrometry

Introduction

Cefuroxime sodium (Fig. 1) is a second generation cephalosporin antibiotic, used in both human and veterinary medicine with good results in the treatment and prophylaxis of bacterial infections [16].

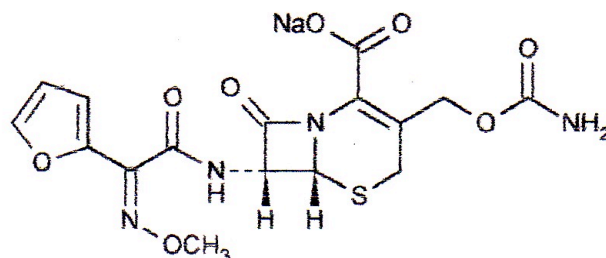


Figure 1
Chemical structure of cefuroxime sodium.

Cefuroxime sodium is widely used in the therapeutical practice due to its wide antibacterial spectrum (active on both G⁺ and G⁻ bacteria), its stability against beta-lactamases and its longer half-life than other cephalosporins ($t_{1/2}$ =80 minutes), which results in high active concentrations in plasma and tissues. Consequently, cefuroxime is frequently used in human medicine in the treatment of some collectivity type pneumonia and other severe infections [14]. Being active on *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, cefuroxime is used in veterinary medicine by intramuscular or intramammary administration for the treatment of mastitis in cows during lactating [2]. However, the active moieties of the drug which remain in the animal body or in the animal products (meat, organs, milk, eggs) are harmful for the human health if exceeds the normal limits and may cause allergies, disorders of the intestinal flora and can lead to the developing of resistant pathogen microorganisms [4,19].

The current legislation establishes maximum limits for antibiotics residues [19]. According to this, the detection of antimicrobial residues in animal products is compulsory, taking into consideration their elimination period from the human body. The official methods of analysis include microbiological methods (test screening) [19] and also chromatographic analytic methods [18].

High performance liquid chromatography (HPLC) or liquid chromatography/mass spectrometry (LC/MS) methods are currently widely used in qualitative and quantitative analysis of drugs in biological fluids

[5,8,12,13]. There are already some published methods in the literature for quantification of cephalosporins from mixtures, biological matrix, antibiotic residues from biological liquids or stability studies, but they usually have long run-times and laborious sample preparation methods like liquid-liquid extraction or solid phase extraction [1,3,6,7,9-11]. The aim of this work was to elaborate and validate a simple, rapid, selective and reproducible method that allows the estimation of cefuroxime sodium in cattle plasma for using in pharmacokinetics and drug residue determinations.

Materials and methods

Reagents

Cefuroxime sodium as standard reference substance was purchased from Sandoz GmbH. HPLC grade acetonitrile and methanol and analytical grade ammonium acetate and perchloric acid 70% were purchased from Merck (Darmstadt, Germany). HPLC grade water was deionized with Milli-Q and then filtered using Milli-Q Academic, Millipore water purification system (Milford, MA, USA).

Standard solutions

A stock solution of cefuroxime with concentration of 12.75 mg/ml was prepared by dissolving appropriate quantity of cefuroxime sodium in methanol. All the concentrations were expressed as cefuroxime acid. A working solution was obtained by diluting a specific volume of stock solution with plasma. This was used to spike different volumes of plasma blank, finally providing eight plasma standards with the concentrations ranged between 63 and 6120 ng/mL. Accuracy and precision of the method was verified using cattle plasma standards with concentrations of 63, 191, 382, and 765 ng/mL cefuroxime acid.

Chromatographic and mass spectrometry systems and conditions

The HPLC system was an 1100 series model (Agilent Technologies) consisted of a binary pump, an in-line degasser, an autosampler, a column thermostat, and an Ion Trap SL mass spectrometer detector (Brucker Daltonics GmbH, Germany). Chromatographic separation was performed at 45°C on a Zorbax SB-C18 100 mm x 3 mm i.d., 3.5 µm column (Agilent Technologies), protected by an in-line filter. The detection of cefuroxime was in MS/MS using an electrospray negative ionization (ESI negative). The mass spectrometer operated using nitrogen as drying gas (12 L/min, 350°C) and as nebulising gas (60 psi). The capillary was set at 4000V. The ion transition was monitored as follows: m/z 423 $\rightarrow$ (m/z 207+318+362).

Mobile phase

The mobile phase consisted of a mixture of water containing 1mM

ammonium acetate and methanol 86:14 (v/v), each component being degassed before elution for 10 min in an ultrasonic bath. The pump delivered the mobile phase at 1 ml/min. In order to maintain the ion source clean, the column effluent was diverted to waste in the first minute of analysis.

Sample preparation

Standard and test plasma samples were prepared as follows, in order to be chromatographically analyzed. In an Eppendorf tube, to 0.2 ml plasma, 0.1 ml perchloric acid solution (12%, in acetonitrile) was added. The tube was vortex-mixed for 10 s and then centrifuged for 3 min at 12000 rpm. A volume of 0.2 ml of final solution was transferred in an autosampler vial and 10 μ l were injected into the HPLC system.

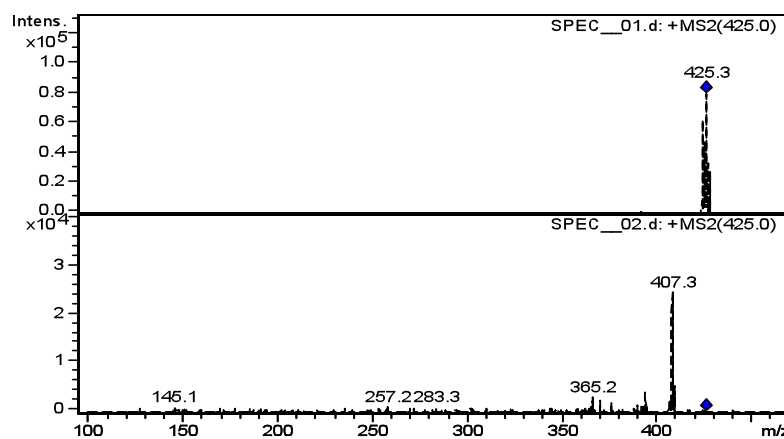
Validation

The method validation [20,21] involved verifying specificity by using six different plasma blanks. The linearity of the peak area against standard concentration was verified between 63-6120 ng/ml cefuroxime by the least squares analysis. The applied calibration model was a quadratic one: $y = ax^2 + bx + c$, weight $1/y^2$, where y is peak area and x , concentration. Distribution of the residuals (% difference of the back-calculated concentration from the nominal concentration) was investigated. The calibration model was accepted, if the residuals were within $\pm 20\%$ at the lower limit of quantification and within $\pm 15\%$ at all other calibration levels and at least 2/3 of the standards met this criterion.

The limit of quantification was established as the lowest calibration standard with an accuracy and precision less than 20%. The intra- and inter-day precision (expressed as coefficient of variation %, CV%) and accuracy (relative difference % between found and theoretical concentration, bias%) of the assay procedure were determined by the analysis in the same day of three samples at each of three levels of concentration in the considered concentration range and one sample of each in three different days, respectively. The recoveries at each of previous levels of concentration were measured by comparing the response of the treated plasma standards with the response of standards in water with the same concentration in cefuroxime as the final extract from plasma standards.

Results and discussion

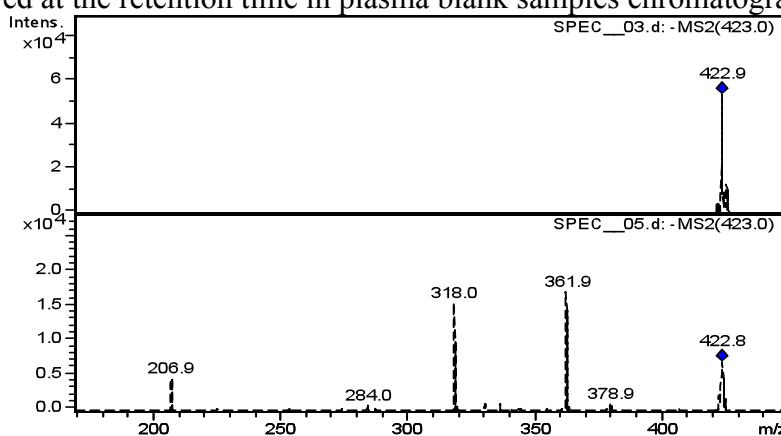
Two ionization modes were tested for detection of cefuroxime, electrospray positive and negative ionization, respectively. The obtained mass spectra of cefuroxime are presented in Fig. 2 and 3, respectively.

**Figure 2**

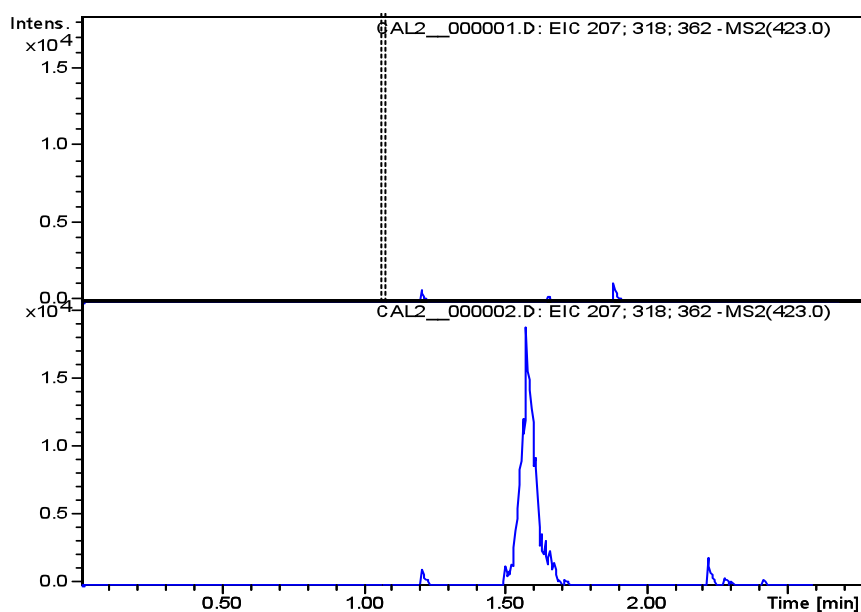
Ion mass spectra of cefuroxime in electrospray positive ionisation (upper spectra- full scan, lower spectra- fragmentation).

The MS detection of cefuroxime in negative mode is about 5 times more sensitive than in positive mode as the analyte has an easily ionizable carboxylic moiety by proton loss, so the negative detection mode was chosen.

The detection of cefuroxime was carried out by adding multiple fragments from the MS spectrum in order to improve the overall signal (the sum of ions with m/z 207, 318 and 362) (Fig. 3). The retention time of cefuroxime was 1.6 min (Fig. 4) and no significant interference was observed at the retention time in plasma blank samples chromatograms.

**Figure 3**

Ion mass spectra of cefuroxime in electrospray negative ionisation (upper spectra- full scan, lower spectra- fragmentation).

**Figure 4**

Chromatograms of a blank (upper) and a LLOQ plasma standard with 63 ng/ml cefuroxime.

The calibration curves showed linear response over the range of concentrations used in the assay procedure. The inter- and intra-day precision, accuracy and recovery results are showed in Table I and Table II. The lower limit of quantification was established at 63 ng/ml cefuroxime. Precision and accuracy at quantification limit were 8.1% and -1.6% for intra-day determinations and 8.9% and -2.0% for inter-day determinations, being in agreement with validation guidelines [20,21]. The recovery was consistent and ranged between 95 and 105% (Tables I and II).

Table I
Intra-day precision, accuracy and recovery (n = 5) for cefuroxime

c_{nominal} ng/ml	Mean c_{found} ng/ml ($\pm$ S.D.)		Precision (CV %)	Accuracy %	Recovery % ($\pm$ S.D.)	
63.75	62.75	5.09	8.1	-1.6	95.9	8.2
191.25	181.90	16.84	9.3	-4.9	103.1	9.7
382.50	369.55	17.30	4.7	-3.4	100.9	4.8
765.00	774.87	31.89	4.1	1.3	105.2	4.4

Table II

Inter-day precision, accuracy and recovery (n = 5) for cefuroxime

C_{nominal} ng/ml	Mean c_{found} ng/ml (± S.D.)		CV %	Bias %	Recovery % (± S.D.)	
63.75	62.47	5.59	8.9	-2.0	99.8	8.6
191.25	181.36	5.92	3.3	-5.2	104.6	4.9
382.50	356.05	23.38	6.6	-6.9	96.5	4.2
765.00	717.97	67.09	9.3	-6.1	95.4	5.3

Conclusions

The proposed method proved to be rapid, accurate and precise for the quantitative determination of cefuroxime in cattle plasma, having the advantage of selectivity due to MS detection, high throughput due to both simple plasma preparation and short analysis time. Without using an internal standard and applying a simple sample preparation by protein precipitation, a specific and efficient analysis of plasma samples could be performed. The method was used for pharmacokinetic analysis of cefuroxime and for drug residue analysis in cattle.

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Manuscript received: November 7th 2009