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Validation of a liquid chromatography–high-resolution mass spectrometry method for the analysis of ceftiofur in poultry muscle, kidneys and plasma: A unique accuracy profile for each and every matrix

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ABSTRACT

A robust, selective and specific liquid chromatography–high resolution mass spectrometry (LC–HRMS) method was developed for the quantification of total residues of ceftiofur, an antibiotic belonging to the 3rd generation cephalosporins in plasma, muscle and kidney of poultry. Ceftiofur and conjugates in samples were firstly hydrolyzed with dithioerythritol into desfuroylceftiofur, which was then stabilized by derivatization with iodoacetamide into desfuroylceftiofur acetamide. Sample were then submitted to a solid phase extraction followed the accurate mass analysis of desfuroylceftiofur acetamide by LC–HRMS in full scan mode using a linear trap quadrupole (LTQ)-Orbitrap mass spectrometer with a resolving power 60,000 full width at half maximum (FWHM). The method was fully validated over a dosing range between 100 and 2000 $\mu g\,kg^{-1}$ (or $\mu g\,L^{-1}$) using the total error approach. Accuracy profiles a graphical decision-making tool were built by computing results of validation procedure with acceptance limits set at ±60%, and β-expectation tolerance intervals, *i.e.* the interval assuming to contain a β % of future measurements (β = 90% in this study). Total measurement error including trueness, repeatability and intermediate precision were evaluated. Relative bias of trueness was never exceeding the threshold of 6% in all matrices at all level of concentration. The mean relative standard deviation for repeatability was lower than 16% at all levels of concentration for all matrices; the mean relative standard deviation for intermediate precision was lower than 25% at all levels of concentration for all matrices. This validation approach proved that the method is reliable for the quantification in each and every matrix (*i.e.* plasma, kidneys and muscle of chicken) thanks to only one single regression model (*i.e.* linear) obtained from external calibration standards (without matrix) with deuterated labelled internal standard. The developed method was applied during a depletion study of ceftiofur in chicken tissues and plasma.

1. Introduction

Ceftiofur, a third generation cephalosporin β-lactam, is an efficient broad spectrum antibiotic legally used to treat bacterial respiratory infections in swine or lactating cows. Except for pigs and cattle the use of ceftiofur is not allowed in the European Union for other species although treatments have been reported to be used in poultry husbanding to prevent one-day old chicks from early mortality [1]. Ceftiofur can be administered by subcutaneous injection in day-old chicks or directly *in ovo*. This off-label use of ceftiofur may possibly contribute to an increase of bacterial resistance to extended-spectrum cephalosporins not only in poultry but also in humans [2–5].

Numerous studies about pharmacokinetics of ceftiofur in various animals were reported in literature (*e.g.* swine, iguanas, alpacas, domestic goats, camels, horses, cattle, mares, guineafowl, etc.) [6–15], however much less is known about pharmacokinetics of ceftiofur in poultry and avian species [16–18]. After parenteral administration ceftiofur is quickly metabolized to a reactive compound, the desfuroylceftiofur (DFC). DFC may directly bind to different proteins, and to amino acids such as cysteine or glutathione residues to form desfuroylceftiofur cysteine disulfide

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(DCCD), and desfuroylceftiofur glutathione, respectively, or may also form dimers of DFC [12,19–21].

Analytical methods targeting the control of the DFC generally start with a cleavage of all DFC-related compounds bearing a β-lactam ring through their disulfide or thioester bound activated by dithioerythritol (DTE) in alkaline conditions. The released DFC residues are submitted to a subsequent derivatization with iodoacetamide which convert DFC to a stable derivative, the desfuroylceftiofur acetamide (DCA). Such analytical procedure applying these chemical transformations further require successive cleaning steps of fluids or tissues extracts by solid phase extraction (SPE). This approach was developed for the analysis of cattle and swine tissues and of plasma using HPLC-UV firstly by Jaglan et al. [25] and modified by Beconi-Barker et al. [19]. Other alternative markers of ceftiofur like metabolites of CFT (*e.g.* DCCD), or marker residues produced after alkaline hydrolysis as described in Berendsen et al. [26–28] are detected by high performance liquid chromatography coupled to ultraviolet detector (HPLC-UV) [29] methods or by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) multi-residue methods [30–32].

Current trends in chemical residue analysis open the field toward high resolution mass spectrometry (HRMS) technologies. Full scan MS acquisition coupled to powerful analyzers (*e.g.* time of flight, Fourier transform orbital trap) becomes a promising tool for the detection and the confirmation of veterinary drugs residues at low $\mu g\,kg^{-1}$ level in complex matrices from animal origin [30,33–37]. This approach is also expected to match for the quantitative determination of various veterinary drug residues in residue depletion studies. Up to now, the residue depletion studies of the ceftiofur in avian species refer either to a microbiological method of analysis or to a HPLC-UV method [16,17].

The first aim of the present study was to develop a method for the quantification of ceftiofur residues in chicken tissues (*i.e.* muscle and kidneys) and in plasma. The other objective was to contribute to advanced knowledge from the existing depletion study. The analytical performances of the developed liquid chromatography–high resolution mass spectrometry (LC–HRMS) method have been evaluated using the total error approach. Accuracy profiles plotted by means of a graphical tool after a statistical process are built up in order to decide about the robustness of an analytical method as regard to the purpose it is dedicated to.

Some naturally contaminated samples from a ceftiofur residue depletion study in plasma, muscle and kidney samples were analyzed with the developed method in order to demonstrate the applicability of the method in routine.

2. Materials and methods

2.1. Chemicals, reagents and solutions

2.1.1. Analytical reference standards and standard solutions

Ceftiofur hydrochloride was obtained from Sigma–Aldrich (France) and isotopically labelled internal standard (ceftiofur-d3 hydrochloride) was purchased from Toronto Research Chemicals (North York, Ontario, Canada), both standards having a declared purity > 98%. Stock solutions of ceftiofur (CFT) and deuterated ceftiofur d_3 (CFT-d_3) were individually prepared at a concentration of $0.5\,mg\,mL^{-1}$ in HPLC grade methanol and ultrapure water 50/50 (V/V) and were stored at −18 °C for use within 6 months. Intermediate standard solutions of ceftiofur ($0.625–10\,\mu g\,mL^{-1}$). Working solutions of ceftiofur-d_3 ($1.25\,\mu g\,mL^{-1}$) were individually prepared from intermediate solutions ($10\,\mu g\,mL^{-1}$) which were prepared from stock solutions in a pH 7.0 ammonium acetate buffer solution (10 mM). Intermediate and work solutions of ceftiofur-d_3 were stored at +4 °C within 2 weeks.

2.1.2. Chemicals and reagents

Analytical reagent grade heptafluorobutyric acid (HFBA) was obtained from Fluka (St. Quentin Fallavier, France). HPLC grade acetonitrile (ACN) and methanol, and isooctane were purchased from Merck (Darmstadt, Germany). 99% 1,4-dithioerythritol (DTE) and iodoacetamide (IAA) with a declared purity ≥ 99% were supplied by Aldrich (St. Quentin Fallavier, France). Ammonium bicarbonate (Fluka, St. Quentin Fallavier, France), ammonium acetate (Fisher Scientific St. Quentin Fallavier, France), 30% ammonia solution (Merck, Darmstadt, Germany) and sodium hydroxide pellets (Merck, Darmstadt, Germany) were used for buffer solutions preparation. Purified water was prepared using a Milli-Q water system (Millipore, Molsheim, France).

Ammonium bicarbonate buffer pH 9 (10 mM) solution used for the DTE solution preparation and ammonium bicarbonate buffer pH 8.5 solution used during the solid phase extraction step were prepared by dissolving 0.79 g of ammonium bicarbonate in 1 L of purified water and were adjusted to the expected pH with 30% ammonia solution. Ammonium acetate buffer pH 7 (10 mM) was prepared by dissolving 0.76 g of ammonium acetate in 1 L of purified water and was adjusted to pH 7 with 30% ammonia solution.

A 0.4% (w/v) DTE solution was prepared by dissolving 1.0 g of DTE in 250 mL of ammonium bicarbonate buffer pH 9. A 14% (w/v) IAA solution was prepared by dissolving 14.0 g of IAA in 100 mL of the ammonium acetate buffer pH 7 solution. 0.4% DTE and IAA solution were kept during 2 weeks between +2 and +8 °C.

20.0 g of sodium hydroxide pellets was added to 100 mL of purified water to prepare a 5 M sodium hydroxide solution. This solution was then 500-fold diluted in purified water to obtain 0.01 M sodium hydroxide solution.

2.2. Sample preparation

Concentrations of CFT (Fig. 1) and its related residues were quantified in plasma, muscles, kidneys samples thanks to an analytical protocol simplified and adapted from Beconi-Barker et al. [19,38] and Becker et al. [38]. The principle of this method is based on the cleavage of CFT and all desfuroylceftiofur (DFC, Fig. 1) conjugates and DFC bound macromolecules into DFC by hydrolysis with DTE. DFC is then stabilized by derivatization with IAA to form the desfuroylceftiofur acetamide (DCA) which was extracted and purified by SPE and then analyzed by LC–HRMS (Fig. 1).

2.2.1. Hydrolysis and derivatization

Breast and thigh muscle, kidney and plasma samples were defrosted at the day of their analysis before undertaking their preparation.

Once defrosted, 0.5 g of minced muscle or 0.5 g of minced kidney sample was accurately weighted and 0.5 mL of plasma was transferred into 50 mL polypropylene centrifuge tubes. 80 μL of $1.25\,\mu g\,mL^{-1}$ internal standard solution of CFT-d_3 was added to each sample (muscle, kidney and plasma) tube. Exclusively for muscle samples, 2 mL of isooctane were added to each centrifuged tube in order to extract lipids from samples.

Hydrolysis of CFT and its related residues into desfuroylceftiofur (DFC) was performed by adding 7 mL of 0.4% DTE solution to each sample centrifuge tube followed by rotary-stirring the tubes for 1 min. Sample tubes were placed (incubated) in a water-bath regulated at 50 °C for 15 min. Following the hydrolysis, the sample tubes were centrifuged at 2400 × *g* for 10 min (5–10 °C) and the supernatant (kidney and plasma samples) was transferred into new clean tubes before derivatization. For muscle samples, the supernatant containing isooctane was eliminated before the transfer of the aqueous phase into new clean tubes. Derivatization of DFC residues into desfuroylceftiofur acetamide (DCA) was then