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Determination of quinolones of veterinary use in bee products by ultra-high performance liquid chromatography–tandem mass spectrometry using a QuEChERS extraction procedure

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ABSTRACT

A reliable and rapid ultra high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) method has been developed for the determination of the eight quinolones of veterinary use regulated by European Union (marbofloxacin, ciprofloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin, flumequine and oxolinic acid). Chromatographic conditions were optimized in order to increase sample throughput and sensitivity. The antibiotics were detected by electrospray ionization in positive ion mode with multiple reaction monitoring (MRM) and MS/MS conditions were optimized in order to increase selectivity, selecting the corresponding product ions for quantification and identification. The separation was achieved in 3 min, using a Zorbax Eclipse Plus C18 column (50 mm × 2.1 mm, 1.8 μm), with a mobile phase of 0.02% aqueous formic acid solution and acetonitrile. A dispersive solid phase extraction methodology, often referred to as the "QuEChERS" (quick, easy, cheap, effective, rugged, and safe) method, was optimized for extraction of the quinolones from honey and also it was evaluated for other bee products such as royal jelly and propolis. The method was validated for each matrix in terms of linearity, trueness, precision, limits of detection (LODs) and quantification (LOQ). LODs ranged between 0.2 and 4.1 μg kg^{-1} with precision lower than 12% and satisfactory recoveries in most cases. The method was also applied for studying the occurrence of these antibiotics in several market samples.

1. Introduction

Quinolones (Qns) constitute one of the main groups of antibiotics used both in human and veterinary medicine for therapeutic purposes. The wide application range and the extensive use of Qns in veterinary medicine represent a potential hazard for human health; they can produce residues in foodstuffs [1], causing allergic reactions or antibiotic resistance in humans. Veterinary use of these compounds had been regulated by European Union (EU) and maximum residue limits (MRLs) have been established for eight Qns: marbofloxacin: MARBO; ciprofloxacin: CIPRO; danofloxacin: DANO; enrofloxacin: ENRO; sarafloxacin: SARA; difloxacin: DIFLO; flumequine: FLUME; and oxolinic acid: OXO; (see structures in Fig. 1) in different food matrixes of animal origin [2]. Antibiotic drugs are not authorized for the treatment of honey bees in the EU; thus, there are no MRLs established. However, it is certainly the case that antimicrobial drugs are authorized for the treatment of honey bees in many third countries [3]. Also, the incurrence of Qns in bee products could be produced by a wrong or illegal use of these antibiotics to treat bees. Despite the fact that their use are not allowed by EU, Qns, ENRO and CIPRO had been found in honey from third countries [4,5]. Therefore, sensitive methods for their determination in bee products are necessary.

Different methods have been published for the determination of several families of antibiotics in honey and royal jelly (e.g. sulphonamides [6,7], tetracyclines [8–10] or macrolides [11–13]), mainly using liquid chromatography tandem mass spectrometry (HPLC–MS/MS). In the case of Qns, some methods based on HPLC–MS have been developed for the analysis in honey of 16 Qns by using turbulent flow chromatography automated online extraction [14], 4 Qns by combining HPLC–MS with a stir rod sorptive extraction with monolithic polymer as coating [15] and, mainly of human use, 19 Qns using SPE [16] or 7 Qns in royal jelly by ultrasonic assisted extraction and HPLC with fluorescence detection [17]. There are no applications of the analysis of Qns in propolis. Also, multiclass/multiresidue LC–MS/MS methods have been proposed for the analysis of different veterinary drug residues in honey, such as nitrofurans [18] or 42 veterinary drugs, including tetracyclines, macrolides, aminoglycosides, β-lactams, amphenicols and sulphonamides [19]. Other multiclass methods included also some veterinary Qns, such as CIPRO, DANO, DIFLO, ENRO and SARA [20]. Recently, ultra high performance liquid

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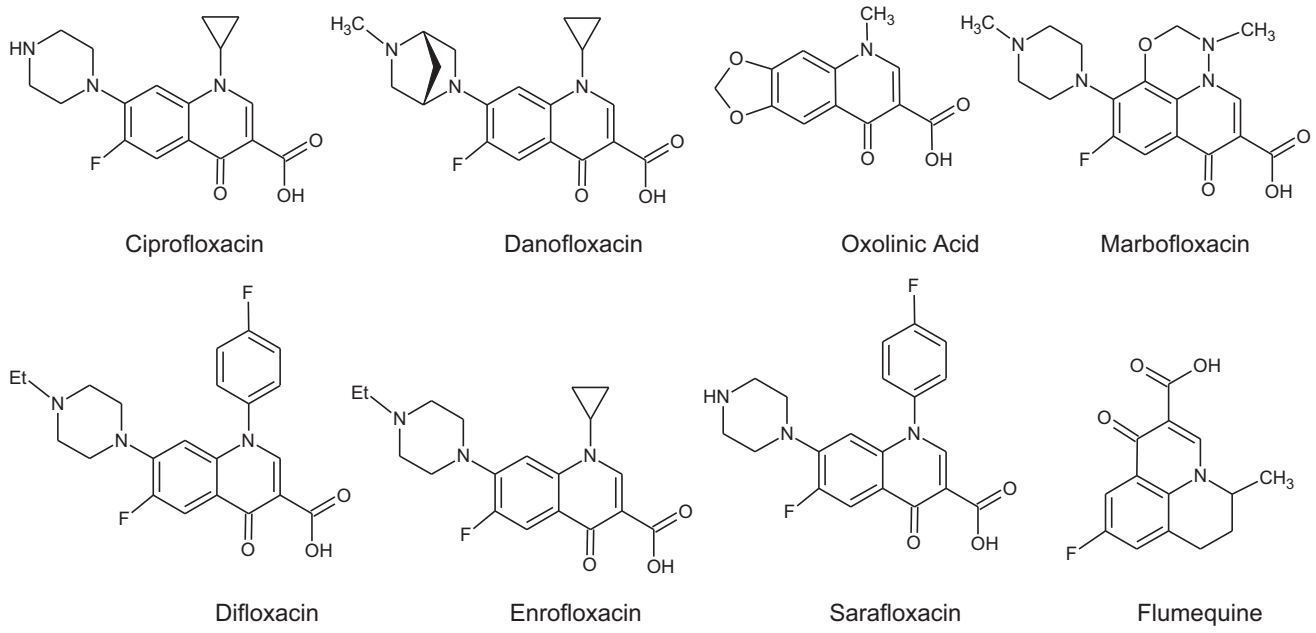

Fig. 1. Chemical structure of the different quinolones studied.

chromatography tandem mass spectrometry (UHPLC–MS/MS) methods for the determination of antibiotics in honey have been reported, including six macrolides [12] and a mixture of macrolides, tetracyclines, sulfonamides and Qns (MARBO, ENRO, DANO, DIFLO and SARA) [21]. However, as far as we know, no UHPLC–MS/MS methods specifically for the determination of the eight regulated Qns for veterinary use neither the analysis of these compounds in royal jelly and propolis have been proposed.

UHPLC technique shows several advantages compared to conventional HPLC, associated with the use of columns of less than 2.0 μm porous stationary phase able to withstand very high pressures, which allows an increased efficiency with a shortened analysis. UHPLC provides higher peak capacity, greater resolution, increased sensitivity and a higher speed of analysis and it is recommended especially to reduce analysis time and sample preparation [22,23], mainly in combination with MS/MS.

Concerning sample treatment, several methods have been proposed for the determination of Qns in different sample matrixes, being solid phase extraction (SPE) the most common methodology [24], reported also for the analysis of Qns in honey [16,21]. More recently, new methodologies showing higher selectivity and efficiency, being less time-consuming or environmentally friendly have been proposed for the determination of Qns in different matrixes, such as molecular imprinted polymers in milk [25–27] and kidney [27], dispersive liquid–liquid microextraction in water [28], turbulent flow chromatography automated online extraction in honey [14] or ultrasonic-assisted extraction combined with SPE for clean-up in royal jelly [17]. In the last few years, a fast and inexpensive extraction method named QuEChERS (quick, easy, cheap, effective, rugged and safe) has shown its usefulness in the analysis of residues in foods, presenting some advantages, such as its simplicity, minimum steps, and effectiveness for cleaning up complex samples. QuEChERS methodology involves two steps: the first one is an extraction step based on partitioning via salting-out extraction involving the equilibrium between an aqueous and an organic layer, and the second one is a dispersive SPE step that involves further clean-up using combinations of $MgSO_4$ and different sorbents, such as C18, primary–secondary amine (PSA) or graphitized carbon (GCB) to remove interfering substances [29,30]. This sample treatment has been extensively used for extraction of pesticides residues in vegetables, but it has been extended to other residues and matrixes [31]. QuEChERS has been used for the determination of veterinary residues (including Qns) in water [32], animal tissues [33,34], milk [25,35,36] and eggs [37]. However, as far as we know, it has not been used for the analysis of bee products.

The purpose of this work is the development of a simple, sensitive, selective and efficient UHPLC–MS/MS method for the simultaneous determination of the eight Qns of veterinary use regulated by EU (MARBO, CIPRO, DANO, ENRO, SARA, DIFLO, FLUME and OXO) using a simple and fast extraction procedure (QuEChERS methodology), optimized for honey and evaluated in other bee products such as royal jelly and propolis, that reduces sample handling and increase sample throughput.

2. Materials and methods

2.1. Chemicals and reagents

Solvents were LC–MS grade and Qns were analytical standard grade. Ultrapure water (Milli-Q Plus system, Millipore Bedford, MA, USA) was used to prepare buffer and standard solutions. Sodium hydroxide and sodium dihydrogen phosphate monohydrate were obtained from Panreac-Química (Madrid, Spain). Formic acid eluent additive for LC–MS, acetonitrile (ACN) and water were obtained from Sigma Aldrich (St. Louis, MO, USA). Formic acid (analysis grade) was supplied by Merck (Darmstadt, Germany). DANO, SARA and DIFLO were supplied by Riedel-de Haën (Seelze, Germany), FLUME by Sigma Aldrich and MARBO, CIPRO, ENRO and OXO by Fluka (Steinheim, Germany).

Individual stock standard solutions (100 mg L^{-1}) of each Qn were prepared by dissolving the appropriate amount of each analyte in ACN/0.02% formic acid aqueous solution (50/50, v/v) and were stored in the dark at 4 °C. Formic acid (analysis grade) was added to each standard to increase solubility of analytes in this solvent mixture. Under such conditions, they were stable for at least 1 month. Working solutions (containing all Qns) were prepared daily from dilution of the individual stock solutions with Milli-Q water.

A 30 mM phosphate buffer solution pH 7.1 was prepared by dissolving 2.07 g of dihydrogen phosphate monohydrate in 500 mL of water and the pH was adjusted with 4 M NaOH solution. A 0.02%