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Analytical Methods

A simple and rapid assay based on hot water extraction and liquid chromatography–tandem mass spectrometry for monitoring quinolone residues in bovine milk

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Abstract

A rapid, specific and sensitive procedure for determining residues of eight widespread used quinolone antimicrobials in bovine milk is presented. The method is based on the matrix solid-phase dispersion technique with hot water as extractant followed by LC/MS/MS. The entire sample treatment did not take more than 40 min. Hot water appeared to be an efficient extracting medium, since absolute recoveries of the analytes in milk were 77–90%. The method proved to be robust as matrix effects did not affect significantly the accuracy of the method, as evidenced by analyzing six different batches of milk. Using norfloxacin as surrogate analyte, the accuracy of the method at three different spike levels of the analytes in milk was 93–110% with RSDs not larger than 10%. On the basis of a S/N of 10, estimated LOQs of this method range from 0.3 to 1.5 ng/ml, well below the tolerance levels of quinolones in milk set by the European Union.

Keywords: Fluoroquinolones; Milk; Hot water extraction; Liquid chromatography–tandem mass spectrometry

1. Introduction

Antimicrobials are widely used in dairy cattle management for the treatment and prevention of diseases. The use of antimicrobials may result in drug residues being present in milk, especially if they are not used according to label directions. There are concerns that the widespread usage of antimicrobials may be responsible for the promotion of resistant strains of bacteria (Brady & Katz, 1988; Wegener, Aarestrup, Gerner-Smidt, & Bager, 1999). For this reason, both the EU commission (Commission Regulation (EC) No 508/1999 of 4th March 1999) and the USA Food and Drug Administration (Code of Federal Regulation, Title 21, 2006) have established maximum residue limits (MRLs) of antimicrobials in food.

Immunological or microbial inhibition screening tests are commonly used to determine if antibiotic residues are present in milk. Some drawbacks of screening tests are: they cannot identify which antimicrobials are present in milk, the presence of high somatic cell counts may result in false positives (Tyler et al., 1992; Van Eenennaam et al., 1993), and they may detect antibiotic residues at levels far below the officially mandated safe levels, resulting in the unnecessary destruction of the milk. Therefore, sensitive and specific chemical methods for the identification and quantitation of antibiotic residues in milk need to support screening tests. Public health agencies in many countries rely on detection by MS for unambiguous confirmation of contaminants in foodstuff.

In the past 15 years, the on-line combination of LC and MS has developed into a widely applied and routinely applicable detection and on-line identification approach for LC. The ease of operation and robustness of current LC/MS interfaces based on atmospheric-pressure ionisation enable the application of LC/MS in a large variety of analytical fields. In particular, the MS analysis of residues of antimicrobials in food has greatly benefited from

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these developments and has been the object of several reviews (Andreu, Blasco, & Picò, 2007; Di Corcia & Nazzari, 2002; Gentili, Perret, & Marchese, 2005; Hernández-Arteseros, Barbosa, Compañó, & Prat, 2002; Kotretsou, 2004; Niessen, 1998; Stolker & Brinkman, 2005).

Quinolones are a group of relatively new antimicrobials synthesized from 3-quinolone carboxylic acid. Quinolones show excellent activity against both Gram-positive and Gram-negative organisms, as well anaerobes. They act to inhibit DNA gyrase a key enzyme in DNA replication. Several quinolones were specifically developed for veterinary medicine, i.e., danofloxacin (DAN), enrofloxacin (ENR) and sarafloxacin (SAR). These drugs are used to treat respiratory and enteric bacterial infections in cattle and other food producing animals.

So far, three methods based on MS/MS detection aimed at monitoring residues of quinolones in bovine milk have been proposed. Volmer, Mansoori, and Locke (1997) conducted a quite interesting and exhaustive study concerning the potential of the LC/MS/MS technique for trace analysis of quinolones in several biological matrices, including milk. However, they made no effort in elaborating a simple and rapid sample preparation procedure by taking advantage of the high specificity offered by MS/MS detection and followed a previously reported laborious procedure developed for monitoring quinolones in milk by LC with UV detection (Hormazabal & Yndestadt, 1994). Later, Van Hoof et al. (2005) elaborated and validated a LC/MS/MS method for identifying and quantifying eight quinolones in muscle tissue, aquaculture products and milk. They made use of a conventional sample treatment protocol, that is milk protein precipitation, centrifugation, analyte extraction, another centrifugation step before clean-up of the extract with a C_{18} solid-phase extraction cartridge. Recently, a Spanish researcher group designed an original and innovative method based on capillary electrophoresis–tandem MS for determining eight quinolones in bovine raw milk (Lara, García-Campaña, Alés-Barrero, Bosque-Sendra, & García-Ayuso, 2006). The sample treatment elaborated by the authors is rather laborious as it consists of (i) milk defattening; (ii) analyte solid-phase extraction (SPE) by an Oasis MAX cartridge; (iii) eluate drying; (iv) a second SPE with an Oasis HLB cartridge, after residue reconstitution; (v) eluate drying; and (vi) filtration of the reconstituted residue before injection in the CE system.

Recently, we have proposed two sensitive LC/MS confirmatory methods for determining residues of sulfonamide antibacterials (Bogialli, Curini, Di Corcia, Nazzari, & Polci, 2003) and aminoglycoside antimicrobials (Bogialli et al., 2005) in milk. These methods involve a simple and rapid sample treatment procedure that couples positive features of the matrix solid-phase dispersion (MSPD) technique, i.e. simplicity and intimate contact between the extractant and the matrix, to those offered by heated water as extractant. Besides to be a cheap and environmentally friendly solvent, water is able to selectively extract analytes by suitably controlling the extraction temperature (Hawthorne, Yang, & Miller, 1994). In essence, this method consists of: (i) dis-

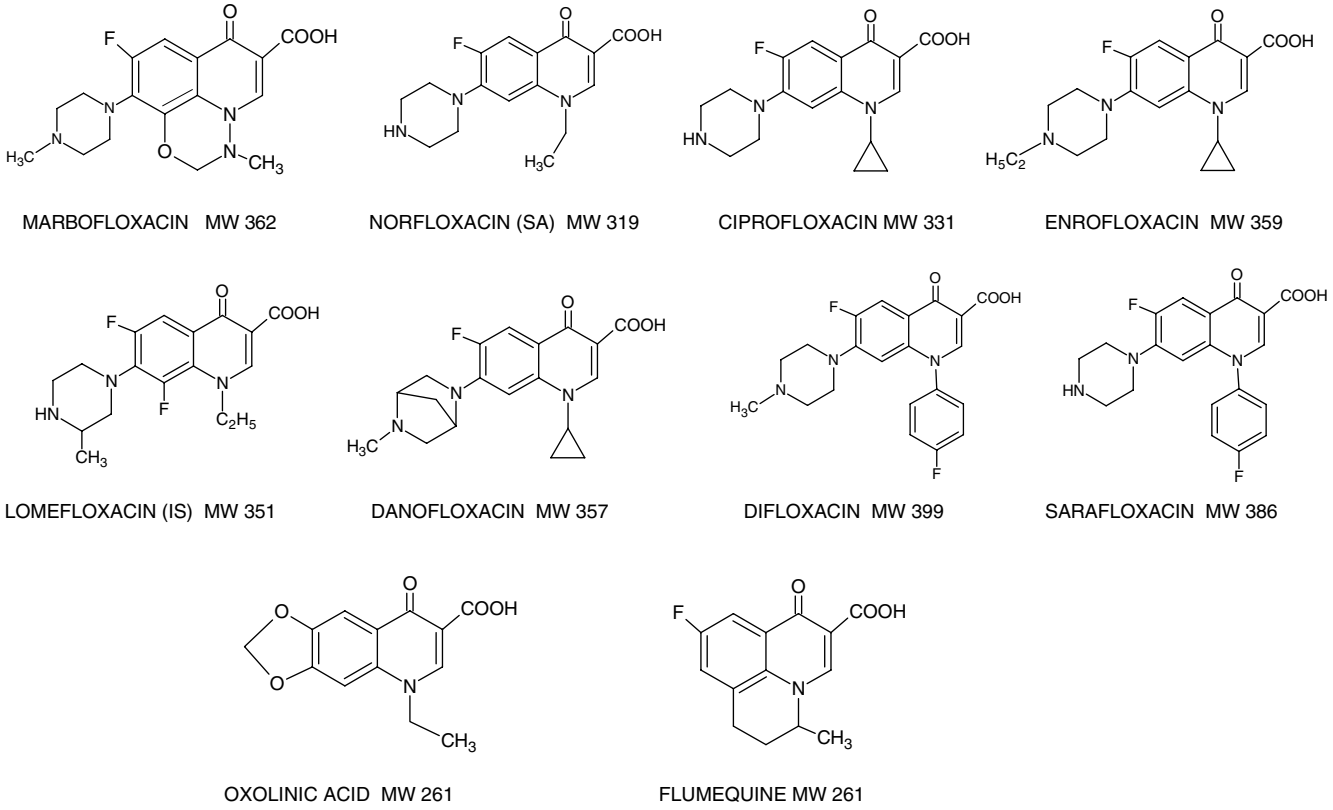

Fig. 1. Chemical structures of selected quinolone antimicrobials.