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Multiresidue analysis of fluoroquinolone antimicrobials in chicken meat by molecularly imprinted solid-phase extraction and high performance liquid chromatography

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ABSTRACT

This paper describes the synthesis of novel molecularly imprinted polymer (MIP) micro-beads for the selective extraction (MISPE) of six fluoroquinolone (FQ) antibiotics (enrofloxacin, ciprofloxacin, lomefloxacin, danofloxacin, sarafloxacin and norfloxacin) from chicken muscle samples and further analysis by high-performance liquid chromatography (HPLC) with fluorescence (FLD) or mass spectrometry (MS) detection. A combinatorial screening approach has been applied to select the optimal functional monomer and cross-linker formulation for polymer synthesis. The MIP prepared using enoxacin (ENOX) as the template – a mixture of methacrylic acid (MAA) and trifluoromethacrylic acid (TFMAA) as functional monomers and ethylene glycol dimethacrylate (EDMA) as the cross-linker – showed superior FQ recognition properties than the rest of the materials generated. MIP spherical particles were prepared using silica beads as sacrificial scaffolds. The polymers were packed in solid phase extraction (SPE) cartridges. The optimized MISPE–HPLC method allows the extraction of the antimicrobials from aqueous samples followed by a selective washing with acetonitrile/water (0.005% TFA, pH = 3.0), 20:80 (*v*/*v*) and elution with 5% trifluoroacetic acid in methanol. Optimum MISPE conditions led to recoveries of the target FQs in chicken muscle samples ranging between 68 and 102% and precisions in the 3–4% range (RSD, $n = 18$). The method has been validated according to European Union Decision 2002/657/EC, in terms of linearity, accuracy, precision, selectivity, decision limit (CCα) and detection capability (CCβ) by HPLC–FLD and HPLC–MS/MS. The limits of detection were improved using HPLC–MS/MS analysis and ranged between 0.2 and 2.7 μg kg^{-1} ($S/N = 3$) for all the FQs tested.

1. Introduction

Fluoroquinolones (FQs) are a family of synthetic broad-spectrum antimicrobial drugs with bactericidal action used both, in human and veterinary medicine. These antimicrobials are active against some Gram-negative and Gram-positive bacteria by inhibiting two bacterial enzymes, DNA gyrase (topoisomerase II) and topoisomerase IV, that play an essential role in bacterial DNA replication [1,2].

The evolution of food production systems from small farming units to large scale intensive production systems has been accompanied by an increasing administration of antimicrobials to food producing animals, to prevent and control the spread of infections in the farm. The use of these pharmaceuticals is restricted in many countries, especially those commonly applied in human medicine, which are forbidden for animal use. The European Union (EU) has set Maximum Residue Limits (MRLs), i.e., the maximum concentration of the residue that can be allowed in a food product obtained from a treated animal to avoid harmful effects in the consumers, for veterinary drugs residues in foodstuffs of animal origin, banning their use as growth promoters since January 2006 [3,4]. Nevertheless, the problem of antibiotic resistance is a global issue of growing concern that is related to both human and non-human antimicrobial usage, further complicated by the different rules and regulations, as well as livestock production methods, applied in different countries [5–8].

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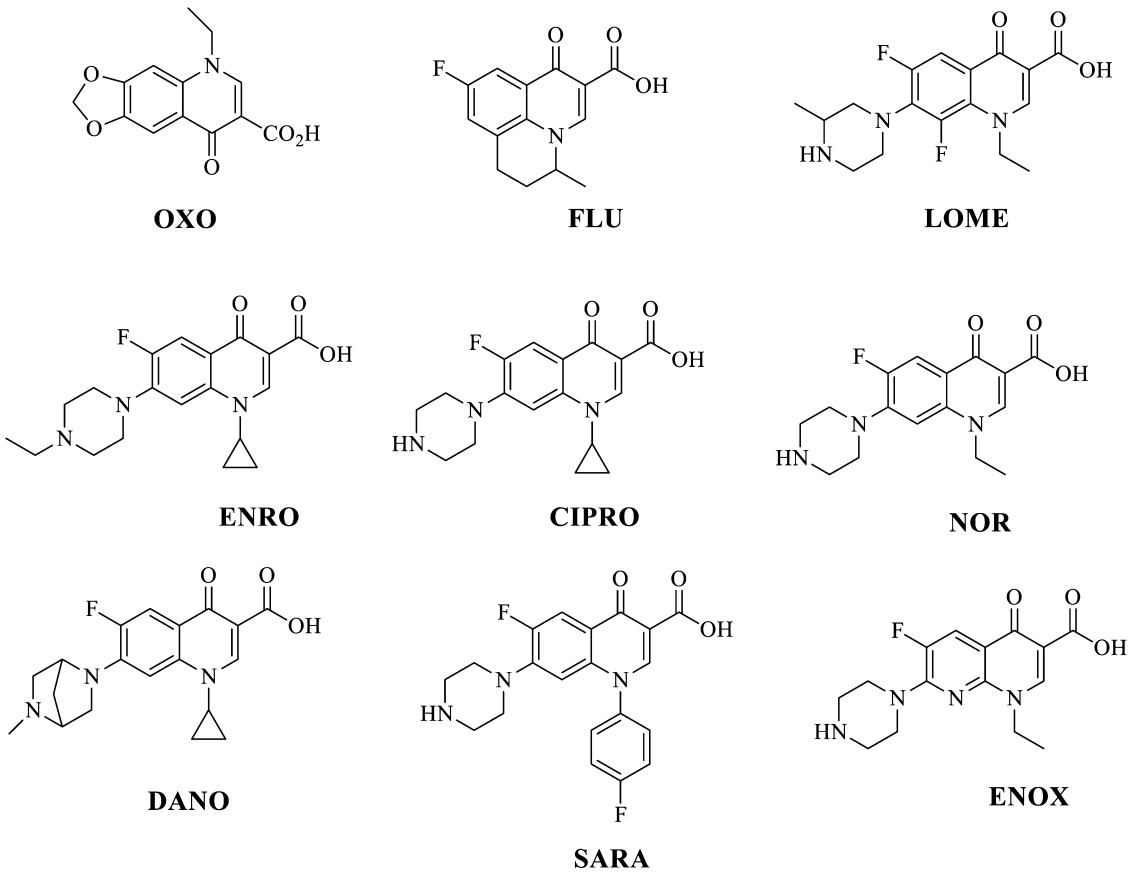

Fig. 1. Molecular structures of the FQ antibiotics tested: norfloxacin (NOR), ciprofloxacin (CIPRO), danofloxacin (DANO), enrofloxacin (ENRO), sarafloxacin (SARA), enoxacin (ENOX), lomefloxacin (LOME), oxolinic acid (OXO) and flumequine (FLU).

FQs are commonly applied in intensive farms for poultry production, where they are administered as oral solutions for therapeutic use only and under prescription of a veterinary surgeon. Enrofloxacin (ENRO) is the most widely applied FQ for such application but, flumequine (FLU), danofloxacin (DANO), difloxacin (DIFLO), oxolinic acid (OXO) and sarafloxacin (SARA) have also been licensed in the EU as veterinary medicinal products for poultry treatment [9] (Fig. 1).

The U.S. Food and Drug Administration (FDA) has banned the use of ENRO for the treatment of bacterial infections in poultry since 2005, as there was scientific evidence of the emergence of Campylobacter resistant species in chickens and turkeys treated with the antimicrobial [8]. These findings have also prompted the European Commission to initiate a referral procedure for all veterinary medicinal products containing quinolones and FQs, for all animal producing species, to promote their prudent use across the EU [10].

Therefore, whether banned or allowed with a MRL, it is important to have efficient methods available for monitoring FQ residues in poultry samples to prevent the presence of undesired hazards for human health and to comply with legislation. Current methods are mostly based in liquid chromatography (LC) with diode array (DAD), fluorescence (FLD), or mass spectrometry (MS) detection [11,12] after a clean-up step usually based on solid-phase extraction (SPE) [13,14].

Molecularly imprinted polymers (MIPs) are human-made materials that are able to selectively recognize a particular chemical in the presence of closely related interfering species, as they contain specific recognition sites with a shape and geometry of functional groups complementary to those present in the template molecule [15]. The use of MIPs as SPE sorbents, called MISPE, for the selective extraction of FQ antimicrobials from food or environmental samples has grown significantly in the last few years [16-34] (Table 1S, Supplementary material). However, so far, only a few methods have been reported describing the application of MISPE for the determination of FQs in foodstuff and, in particular, in chicken muscle samples. Some limitations encountered with the MIPs described in the literature for such applications include, the selection of FQs that can be found in foodstuffs of animal origin as template molecules, for example, ENRO or CIPRO, that can result in false positives; the use of polymers prepared by bulk polymerization, which leads to particles with irregular shapes and broad size distribution with limited performance as SPE sorbents or; the lack of method validation (see Table 1S, Supplementary material).

In the present paper we describe the synthesis of novel MIP beads imprinted with enoxacin (ENOX), using methacrylic acid (MAA) and 2-(trifluoromethyl)acrylic acid (TFMAA) as functional monomers and ethylene glycol dimethacrylate (EDMA) as crosslinker. Microspherical polymer particles have been synthesized using porous silica beads (40–75 μm) as sacrificial scaffolds for polymerization [35,36]. The MIP has been applied to the analysis of ENRO, DANO, CIPRO, NOR, LOME and SARA in chicken muscle samples using sample clean-up by MISPE followed by HPLC with fluorescence (FLC) or mass spectrometry (MS/MS) detection. The MISPE–LC–FLD or MS/MS methods have been validated according to the Commission Decision 2002/657/EC [37] for the analysis of FQs in chicken muscle samples.