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Novel dummy molecularly imprinted polymers for matrix solid-phase dispersion extraction of eight fluoroquinolones from fish samples

Xiaoli Sun[a,b], Jincheng Wang[a], Yun Li[a], Jiajia Yang[a,b], Jing Jin[a], Syed Mazhar Shah[a], Jiping Chen[a,*]

[a] Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

A series of novel dummy molecularly imprinted polymers (DMIPs) were prepared as highly class-selective sorbents for fluoroquinolones. A non-poisonous dummy template, daidzein, was used for the first time to create specific molecular recognition sites for fluoroquinolones in the synthesized polymers. The influence of porogen polarity on dummy molecular imprinting effect was studied. The DMIP prepared using dimethylsulfoxide–acetonitrile (1:1.8, v/v) as porogen achieved the highest imprinting factors (IF) for fluoroquinolones over a range of IF 13.4–84.0. This DMIP was then used for selective extraction of eight fluoroquinolones (fleroxacin, ofloxacin, norfloxacin, pefloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and gatifloxacin) from fish samples based on dummy molecularly imprinted matrix solid-phase dispersion (DMI-MSPD). The extracted fluoroquinolones were subsequently analyzed by high-performance liquid chromatography (HPLC) equipped with a fluorescence detector (FLD). The developed method had acceptable recoveries (64.4–102.7%) and precision (RSDs: 1.7–8.5%, $n = 5$) for determination of fluoroquinolones in fish samples fortified at levels of 10 and 100 ng g^{-1}. The limits of detection (LODs) for identification of eight fluoroquinolones ranged between 0.06 and 0.22 ng g^{-1}. The results demonstrated great potential of the optimized method for sample preparation in routine analysis of trace fluoroquinolones in fish samples.

1. Introduction

Fluoroquinolones (FQs) are one of the most important classes of synthetic antibiotics, which show excellent activity against pathogenic Gram-negative and Gram-positive bacteria, as well as anaerobes [1]. The extensive use of fluoroquinolones in food-producing animals has raised concerns about the potential risks of their residues in foodstuffs of animal origin [2] or environmental matrix [3–5]. These residues can be directly toxic or can elevate the pathogen resistance levels and possible allergic hypersensitivity reactions in humans [6,7]. Maximum residue limits (MRLs) for several FQs have been established in many countries for animal foods [8]. Therefore, monitoring of FQ residues in livestock, poultry, fish and other animal products for human consumption is greatly significant.

Currently, the most used methods for fluoroquinolone analysis are based on high-performance liquid chromatography (HPLC), mainly coupled with fluorescence, ultraviolet (UV) or mass spectrometric (MS) detection [9–11]. Different sample preparation methods have been developed for the analysis of fluoroquinolones in biological samples, such as liquid extraction (LE), protein precipitation (PP), liquid–liquid extraction (LLE), solid-phase extraction (SPE), supercritical fluid extraction (SFE) and so on [8,12]. The main limitations of these methods include the relatively low recoveries, the tedious and time-consuming extraction and cleanup processes, the use of a large amount of toxic organic solvents and the low specificity toward fluoroquinolones. Hence alternative sample preparation methods which can tackle these issues are highly desirable for the extraction and enrichment of fluoroquinolones.

Matrix solid-phase dispersion (MSPD), first introduced in 1989 by Barker et al. [13], is one of the most promising techniques for the simultaneous disruption, extraction and cleanup of solid, semisolid or viscous samples [14]. MSPD involves blending a viscous, solid or semi-solid sample with an appropriate sorbent (silica, florisil, alumina, C18, C8, etc.) followed by packing, washing and elution. In MSPD, the procedures of homogenization, disruption, extraction and cleanup are combined into one simple process, thus

* Corresponding author. Tel.: +86 411 84379562; fax: +86 411 84379562.
E-mail address: chenjp@dicp.ac.cn (J. Chen).

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greatly reducing analysis time and solvent use and increasing sample throughput. In recent years, MSPD has been widely used in the pre-treatment of food, plant, animal tissues, human biological samples, environmental samples and cosmetics [15]. However, due to the lack of special selectivity, MSPD using traditional sorbents was confronted with the difficulty of selectively extracting target analytes from complex samples.

Molecular imprinting is a rapidly developing technique for preparation of polymers with excellent molecular recognition properties, which has been found effective in a variety of extraction techniques such as molecular imprinted solid-phase extraction (MI-SPE), molecular imprinted solid-phase microextraction (MI-SPME), molecular imprinted stir-bar sorptive extraction (MI-SBSE), molecular imprinted matrix solid-phase dispersion (MI-MSPD) [16] and so on. Fluoroquinolone-imprinted polymers have been applied for their enrichment and purification from complex samples such as milk [17], eggs [18], urine [19], fish [20], serum [21], water [22,23] and soil [24]. Good recoveries as well as high selectivity have been obtained due to the good molecular recognition properties of molecularly imprinted polymers (MIPs). However, all the FQs-MIPs reported previously were prepared using one (pefloxacin [25], gatifloxacin [26], ciprofloxacin, ofloxacin [27] and enrofloxacin) or two (levofloxacin–ciprofloxacin [28]) of fluoroquinolones as templates, where possible leakage of template molecules still happened even after exhaustive washing steps. Template leakage could have a serious impact on the accuracy of analytical method [29] or made it unsuitable for simultaneous analysis of the whole class of fluoroquinolones. This problem has become one of the major areas of concern in sample pretreatment methods employing MIPs [30]. The use of a dummy molecule presents an easy solution to circumvent this problem since any leakage will be different from the analyte [31].

Until now, there has been no report about the use of dummy template that does not belong to fluoroquinolones for the preparation of dummy molecularly imprinted polymers (DMIPs) for fluoroquinolones. This work presents the first attempt of using daidzein as a non-poisonous dummy template for the imprinting of fluoroquinolones. The synthesized DMIPs were used as selective MSPD sorbents for simultaneous determination of eight fluoroquinolones in fish samples. The selectivity, recovery and precision of the developed method were also evaluated.

2. Experimental

2.1. Chemicals and reagents

Daidzein was obtained from Zhongxin Pharmaceuticals (Tianjin, China). Enoxacin (ENO), norfloxacin (NOR), ciprofloxacin (CIP), pefloxacin (PEFX), ofloxacin (OFL), lomefloxacin (LOM), fleroxacin (FLX), enrofloxacin (ENR) and gatifloxacin (GAT) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The structures of these fluoroquinolones and daidzein are shown in Fig. 1. Diethylstilbestrol (DES), ethylene dimethacrylate (EDMA) and trifluoroacetic acid (TFA) were purchased from J&K Chemical Ltd. (Beijing, China). The initiator 2,2′-azobisisobutyronitrile (AIBN) was supplied by Aladdin Chemical (Shanghai, China). 4-Vinylpyridine (4-VP) was obtained from Acros (NJ, USA). HPLC-grade acetonitrile and methanol were purchased from Fisher (Fair Lawn, NJ, USA). Dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) were purchased from Tianjin Bodi Chemical Engineering Co., Ltd. (Tianjin, China). Dimethylformamide (DMF) was obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Purified water by Milli-Q Plus water purification system (Millipore, Bedford, with a 70 mm × 0.22 μm filter) was used throughout the experiments.

2.2. Preparation of imprinted and non-imprinted polymers

The dummy molecularly imprinted polymers were prepared using different porogens. The composition of the prepolymerization mixtures are presented in Table 1. The dummy template daidzein (0.2542 g, 1 mmol) was dissolved in 5.6 mL of porogen in a 10 mL thick-walled glass tube. The functional monomer 4-VP (0.42 mL, 4 mmol), cross-linking monomer (EGDMA) (3.8 mL, 20 mmol) and the initiator (AIBN) (0.04 g) were then successively added to the above solution. The solution was sonicated and saturated with dry nitrogen for 10 min before the glass tube was sealed. The tube was then placed in a water bath at 60 °C for 24 h. The obtained DMIPs were crushed, ground, sieved (particle size range: 38.5–63.0 μm), sedimented in acetone and dried under vacuum. The template was extracted by extensive washing with methanol–acetic acid (9:1, v/v). The non-imprinted

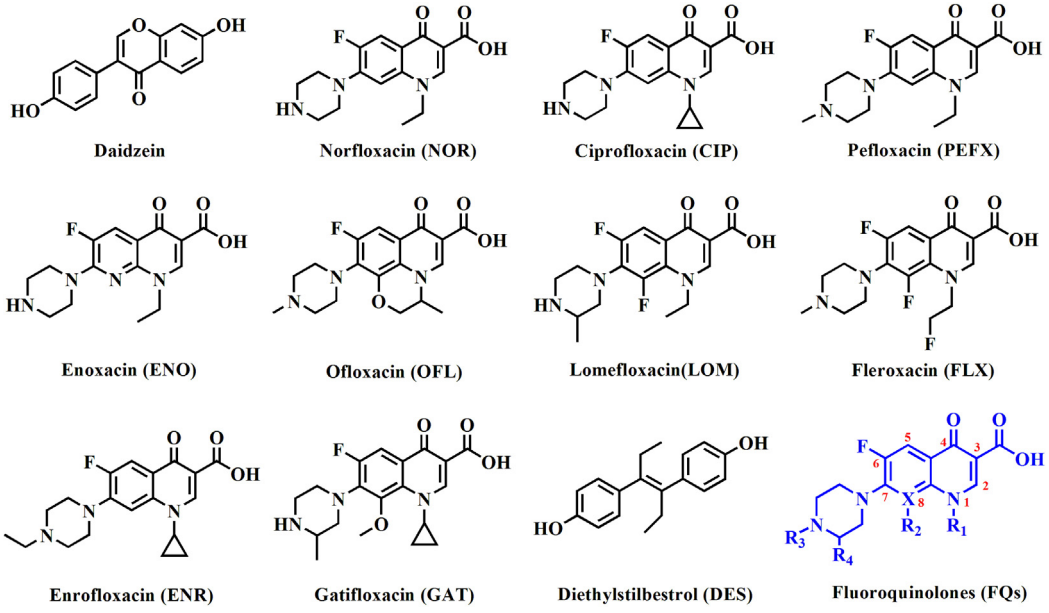

Fig. 1. Chemical structures of the dummy template, structure analogue and selected fluoroquinolones.