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Short communication

Simultaneous extraction of enrofloxacin and ciprofloxacin from chicken tissue by molecularly imprinted matrix solid-phase dispersion

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ABSTRACT

An ofloxacin molecularly imprinted polymer was synthesized and used as a dispersant of matrix solid-phase dispersion for the determination of enrofloxacin and ciprofloxacin in chicken tissue. The selected dispersant shows high affinity to enrofloxacin and ciprofloxacin in aqueous environment and could selectively enrich them from chicken tissue matrix. The extract was sufficiently clean for further chromatographic analysis without interferences from template leakage or chicken tissue matrix. Linearity ranged from 0.03 to 200 μg/g with the correlation coefficient $r^2 > 0.9993$. The recoveries of spiked chicken tissues were in the range of 82.7–96.6% for enrofloxacin and 88.7–102% for ciprofloxacin.

1. Introduction

Fluoroquinolone antibiotic residues of enrofloxacin (ENR) and ciprofloxacin (CIP) in muscle and tissue have proved to be potential risk for health [1]. Several methods had been proposed for determination of ENR and CIP in biological samples [2–5]. Generally, a preliminary extraction step by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) was employed to make the sample clean enough for further analysis. These processes are complicated, time-consuming, and need large amount of organic solvent. Moreover, the low selectivity caused by the endogenous components might contribute to interference with the analytes.

Matrix solid-phase dispersion (MSPD) is one of the most promising techniques to reduce matrix interferences [6]. It was found to be suitable for solid, semisolid and highly viscous biological samples pretreatment [7]. However, the common dispersants, such as C_{18}, C_8, silica, and florisil, lack selectivity for analytes: further improving the selectivity of MSPD was still a meaningful work [8].

Molecularly imprinted polymers (MIP) have specific molecular recognition properties for target molecules. But the presence of polar solvent, especially water could destroy the imprinted recognition, which obviously limited their further application in environmental and biological fields [9]. To improve the affinity of MIP in aqueous environment, porogenic solvents such as dichloromethane and methanol [10–12] were recommend to MIP preparation. A water-compatible MIP using trimethylolpropane trimethacrylate as crosslinker and methacrylic acid (MAA) as functional monomer was synthesized [13]. However, extensive washing was needed to remove the entire template from the polymer matrix. Further improve the selectivity and suppress the non-specific binding are still desired.

In this work, a new water-compatible MIP using 2-hydroxyethyl methacrylate (2-HEMA) as hydrophilic monomer and ofloxacin (OFL) as dummy template was prepared in water–methanol system. The obtained MIP was used as MSPD dispersant to extraction of ENR and CIP from chicken tissues.

2. Experimental

2.1. Reagents

ENR, CIP, and OFL were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Shandong, China). 2-HEMA was purchased from Aldrich (Steinheim, Germany). Ethylene glycol dimethacrylate (EDMA) was obtained from Shanghai Trading Co., Ltd. (Shanghai, China). All the other reagents used in the experiment were of the highest grade commercially available.

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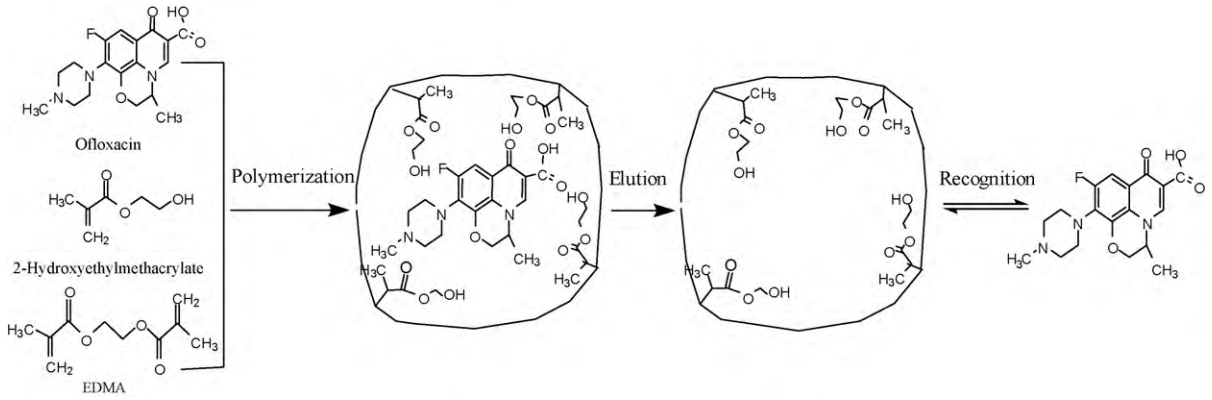

Fig. 1. Schematic illustration of the imprint formation and molecular recognition.

2.2. HPLC analysis

HPLC analysis was performed at 30 °C, using a Shimadzu HPLC system equipped with a LC-10A Multisolvent Delivery System, a DGU-12A on-line-degasser, a SCL-10Avp gradient controller and a SPD-M10Avp diode array detector (Shimadzu, Kyoto, Japan). A CLASS-VP workstation (Shimadzu, Kyoto, Japan) with an ODS C_{18} stationary phase (250 mm × 4.6 mm I.D., particle size 5 μm, Shimadzu, Japan). The wavelength of diode array detector was set at 277 nm. The mobile phase was acetonitrile–0.02 M tetrabutyl ammonium bromide aqueous solution (8:92, v/v; pH 2.60) with flow rate 1.0 ml/min.

2.3. Preparation of molecularly imprinted polymers

2.0 mmol OFL, 12 mmol 2-HEMA, 60 mmol EDMA, and 0.06 g α,α′-azobis (isobutyronitrile) were dissolved in appropriate solvent (methanol:water = 9:1, v/v). Polymerization was performed by thermal-initiated polymerization (53 °C for 48 h). The obtained polymers were grinded and sieved through a 32 μm sieve, and then suspended in acetone until the upper solution became clear. Finally, the particles were dried under vacuum and then put into a column and washed with methanol–acetic acid (4:1, v/v) to remove the templates. Non-imprinted polymer (NIP, in the absence of a template) was prepared and treated in an identical manner.

2.4. Procedure of matrix solid-phase dispersion

200 mg minced chicken tissue was placed into a porcelain mortar and gently blended with 200 mg of MIP particles until a homogeneous mixture was obtained. Using water as carried reagent, it was loaded into a cartridge, which was pre-packed with 45 mg of MIP particles. The cartridge was rinsed with 4.0 ml water and eluted with 3.0 ml acetonitrile–trifluoracetic acid (99:1, v/v). The eluent was evaporated at 30 °C to dryness under vacuum condition and the residue was re-dissolved in 0.5 ml of mobile phase for further HPLC analysis.

3. Results and discussion

3.1. Preparation of the water-compatible MIPs

In order to demonstrate the specific recognition for ENR and CIP in water condition, MIP using different proportions of methanol–water as porogenic solvent was evaluated. The results showed that the proportion of water in the polymerization mixtures had a critical effect on the pore properties and the surface area of the obtained polymer. Meanwhile, methanol–water (9:1, v/v) provided sufficient rigidity and desirable surface properties in the obtained polymers. Further increasing the water content would result in a flexible polymer with a small surface area. Conversely, lower water content would result in a high density polymer with small pore size. Schematic illustration of imprinting and molecular recognition processes is shown in Fig. 1.

3.2. Chromatographic evaluation of the MIP

The obtained MIP particles were packed into an HPLC column (200 mm × 4.6 mm I.D.) for chromatographic evaluation. No peaks of ENR, CIP, and OFL were observed within 60 min when methanol, water or acetonitrile were used as mobile phase. At the same time, the three analytes could be washed out from the blank column within 15 min, which indicated that the retention ability of the MIPs column to analytes was attributed to special imprinted recognition. When increasing the acidity in mobile phase, the affinity became weak. When the portion of trifluoracetic acid (TFA) in the mobile phase was up to 0.05%, all the analytes were be eluted within 5 min.

3.3. Different pretreatment of chicken samples

To demonstrate the selectivity of MIP-MSPD, C_{18} dispersant was also investigated. The recoveries of ENR and CIP on C_{18}-MSPD were 63.0–80.0% for ENR and 76.2–89.0% for CIP, while MIP-MSPD shows better recovery (82.7–96.6% for ENR, 88.7–102% for CIP) and cleaner extracts (Fig. 2), which demonstrated the higher selectivity and affinity of the obtained MIP to analytes.

3.4. The selectivity of MIP-MSPD

In order to avoid template leakage in the MIP, a structural analogue – OFL was imprinted as dummy template (to make a so-called "dummy MIP"). Fig. 2 indicated that any leakage of template during the MIP-MSPD could be distinguished from the analytes during the followed chromatographic separation. Furthermore, the recoveries of ENR and CIP (60–68%) on NIP-MSPD were lower than that on MIP-MSPD, which also indicated that MIP had higher affinity towards the target analytes.

3.5. Optimization of MIP-MSPD procedures

Several parameters (the ratio of sample to MIP sorbent (S/MIP), washing and elution solvent) affecting the efficiency of MIP-MSPD were investigated. To investigate the S/MIP, chicken tissue samples in a range of 0.05–0.60 g were applied to 0.20 g MIP particles. The result showed that the suitable S/MIP was 1:1 (g/g). When S/MIP was lower than 1:1 (g/g), stable recoveries were obtained. Other-

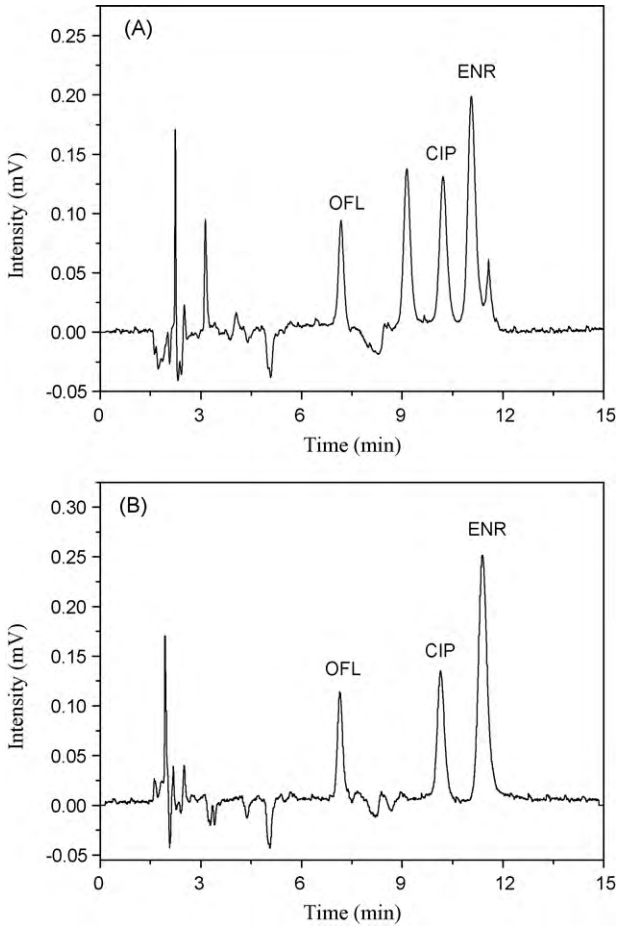

Fig. 2. Chromatogram of spiked chicken sample using different MSPD procedures. (A) MSPD with C_{18} dispersant; (B) MSPD with MIP dispersant; mobile phase: water–acetonitrile (92:8, v/v, with 0.02 M tetrabutyl ammonium bromide); flow rate: 1.0 ml/min; injection volume: 20 μl.

wise, higher S/MIP would result in too wet MSPD mixture to easily transfer it into the cartridge leading to lower recoveries.

The washing solvent should be compatible with the biological system and the subsequent chromatographic analysis, so water was selected as the washing solvent. When the water volume was lower than 4.0 ml, interferences from endogenic components were observed. However, if the water volume was higher than 4.0 ml, the recovery decreased. Therefore, 4.0 ml of water was employed.

A series of elution solutions, including water, methanol, and acetonitrile mixed with different proportions of TFA were investigated. The best recoveries of ENR and CIP were obtained using 3.0 ml of acetonitrile–TFA (99:1, v/v) as elution solution. The recoveries of ENR and CIP keep constant even further increasing the volume of elution solution from 3.0 to 7.0 ml. Additionally, the presence of TFA in elution solution could help ENR and CIP desorbed thoroughly. So 3.0 ml of acetonitrile–TFA (99:1, v/v) was used in the further work.

3.6. Validation of the methodology

Calibration curves were constructed by performing the linear regression analysis using the chromatographic peak areas versus the concentrations (0.03, 0.06, 0.1, 0.5, 2, 5, 10, 20, 50, 100 and 200 μg/g) of ENR and CIP. Good linearity was obtained throughout the concentration range, and the regression equations were $y = 1.35 \times 10^5 x + 1.68 \times 10^4$ for ENR and $y = 1.20 \times 10^5 x + 1.63 \times 10^4$ for CIP with the correlation coefficients (r^2) of 0.9993 and 0.9998,

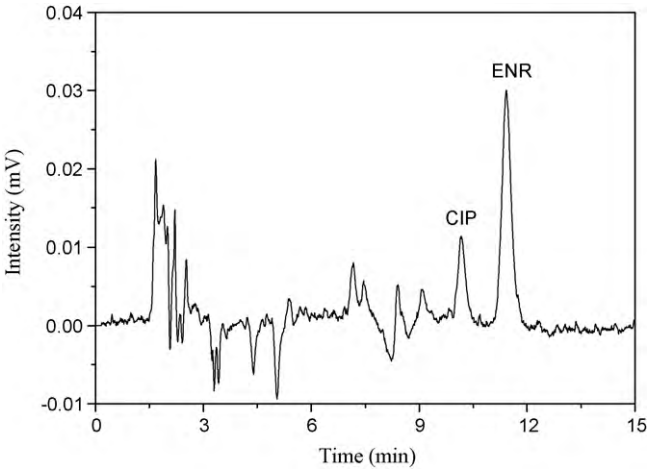

Fig. 3. Chromatogram of chicken samples after oral administration. Oral dose: 15 mg/kg; mobile phase: water–acetonitrile (92:8, v/v, with 0.02 M tetrabutyl ammonium bromide); flow rate: 1.0 ml/min; injection volume: 20 μl.

Table 1
Recoveries of ENR and CIP in spiked chicken samples ($n = 5$).

Spiked level	2.0 μg/g		10 μg/g		50 μg/g	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Enrofloxacin	82.7	5.0	88.3	4.9	96.6	4.0
Ciprofloxacin	88.7	6.7	101	6.5	102	5.3

respectively. The limits of detection (LOD) based on S/N = 3 were 0.008 and 0.009 μg/g for ENR and CIP, respectively, which were below the maximum residue limits (0.1 μg/g of animal derived food) established by Ministry of Agriculture of China (No. 235). The intra-day precision were 4.0–5.6% for ENR, 5.5–6.7% for CIP ($n = 5$) and the inter-day reproducibility in three different days were 4.9–6.4% for ENR, 6.2–7.6% for CIP.

3.7. Application to real chicken samples

The applicability of the method was evaluated from chickens receiving ENR in an oral dose at a level of 15 mg/kg body mass. After two days, they were analyzed by the proposed method (Fig. 3). The levels of ENR and CIP were 0.54 μg/g and 0.072 μg/g, respectively and the recoveries from the spiked samples ranged from 82.7 to 102% (Table 1). Additionally, 20 chicken samples randomly collected from local markets were also analyzed with no residue of ENR or CIP being observed.

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References

[1] N. Gorla, H. Garcia Ovando, I. Larripa, Chromosomal aberrations in human lymphocytes exposed in vitro to enrofloxacin and ciprofloxacin, Toxicol. Lett. 104 (1999) 43–48.
[2] X. Zhou, D. Xing, D. Zhu, Y. Tang, L. Jia, Development and application of a capillary electrophoresis–electrochemiluminescent method for the analysis of enrofloxacin and its metabolite ciprofloxacin in milk, Talanta 75 (2008) 1300–1306.
[3] F.J. Lara, A.M. Garcia-Campana, F. Ales-Barrero, J.M. Bosque-Sendra, L.E. Garcia-Ayuso, Multiresidue method for the determination of quinolone antibiotics in

bovine rawmilk by capillary electrophoresis–tandem mass spectrometry, Anal. Chem. 78 (2006) 7665–7673.
[4] M.A. Bimazubute, E. Rozet, I. Dizier, P. Gustin, Ph. Hubert, J. Crommen, P. Chiap, Liquid chromatographic determination of enrofloxacin in nasal secretions and plasma of healthy pigs using restricted access material for on-line sample clean-up, J. Chromatogr. A 1189 (2008) 456–466.
[5] A. Espinosa-Mansilla, A. Muñoz de la Peña, D. González Gómez, F. Salinas López, Determination of fluoroquinolones in urine and serum by using high performance liquid chromatography and multiemission scan fluorimetric detection, Talanta 68 (2006) 1215–1221.
[6] Y. Yang, B. Shao, J. Zhang, Y. Wu, J. Ying, Analysis of eight free progestogens in eggs by matrix solid-phase dispersion extraction and very high pressure liquid chromatography with tandem mass spectrometry, J. Chromatogr. B 870 (2008) 241–246.
[7] Q.-H. Zou, Y. Liu, M.-X. Xie, J. Han, L. Zhang, A rapid method for determination and confirmation of the thyreostats in milk and urine by matrix solid-phase dispersion and gas chromatography–mass spectrometry, Anal. Chim. Acta 551 (2005) 184–191.
[8] S.A. Barker, Matrix solid phase dispersion (MSPD), J. Biochem. Biophys. Methods 70 (2007) 151–162.
[9] F. Qiao, H. Sun, H. Yan, K.H. Row, Molecularly imprinted polymers for solid phase extraction, Chromatographia 64 (2006) 625–634.
[10] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Novel enrofloxacin imprinted polymer applied to the solid-phase extraction of fluorinated quinolones from urine and tissue samples, Anal. Chim. Acta 562 (2006) 145–151.
[11] E. Turiel, A. Martin-Esteban, J.L. Tadeo, Molecular imprinting-based separation methods for selective analysis of fluoroquinolones in soils, J. Chromatogr. A 1172 (2007) 97–104.
[12] H. Yan, K.H. Row, G. Yang, Water-compatible molecularly imprinted polymers for selective extraction of ciprofloxacin from human urine, Talanta 75 (2008) 227–232.
[13] H. Yan, F. Qiao, K.H. Row, Molecularly imprinted-matrix solid-phase dispersion for selective extraction of five fluoroquinolones in eggs and tissue, Anal. Chem. 79 (2007) 8242–8248.