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Analytical Methods

Optimization of a phase separation based magnetic-stirring salt-induced liquid–liquid microextraction method for determination of fluoroquinolones in food

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ABSTRACT

Herein, we developed a novel integrated apparatus to perform phase separation based on magnetic-stirring, salt-induced, liquid–liquid microextraction for determination of five fluoroquinolones in animal-based foods by HPLC analysis. The novel integrated apparatus consisted of three simple HDPE (high density polyethylene) parts that were used to separate the solvent from the aqueous solution prior to retrieving the extractant. The extraction parameters were optimized using the response surface method based on central composite design: 791 μL of acetone solvent, 2.5 g of Na_2SO_4, pH 1.7, 3.0 min of stir time, and 5.5 min centrifugation. The limits of detection were 0.07–0.53 μg kg^{-1} and recoveries were 91.6–105.0% for the five fluoroquinolones from milk, eggs and honey. This method is easily constructed from inexpensive materials, extraction efficiency is high, and the approach is compatible with HPLC analysis. Thus, it has excellent prospects for sample pre-treatment and analysis of fluoroquinolones in animal-based foods.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are an emerging environmental concern, among which the fluoroquinolones (FQs) are the most important and growing class of potential environmental contaminants (Espinosa-Mansilla, Muñoz de la Peña, González Gómez, & Salinas López, 2006). FQs are widely used as antibacterial agents in human and veterinary medicines due to their broad spectrum activity against both Gram-positive and Gram-negative bacteria through inhibition of DNA gyrase (Gao et al., 2011). They have a common 4-oxo-1,4-dihydroquinoline skeleton, where the pharmacophore unit consists of a pyridine ring with carboxyl group, a piperazinyl group and a fluorine atom placed at positions 3, 6 and 7 (Gajda, Posyniak, Zmudzki, Gbylik, & Bladek, 2012). Fleroxacin (FLE), ofloxacin (OFL), norfloxacin (NOR) and ciprofloxacin (CIP) are third-generation FQs used in treating human and animal diseases, while enrofloxacin (ENR) is used only for treating animals diseases. These five FQs are used extensively in China clinical medicine, and thus they were chosen as the representative FQs analytes in this investigation (Wang, Zhou, & Zeng, 2005). With the overuse of these FQs in animal husbandry and aquaculture, they are widely detected in all kinds of environmental matrices, especially in animal-based foods such as milk (Xia, Yang, & Liu, 2012), eggs (Chu, Wang, & Chu, 2002) and honey (Gao, Zheng, Luo, Ding, & Feng, 2012).

To date, many methods have been developed for the determination of FQs, such as spectroscopy (Motwani, Chopra, Ahmad, & Khar, 2007), capillary electrophoresis (Lombardo-Agüí, García-Campaña, Gámiz-Gracia, & Blanco, 2010), spectrofluorometry (Du, Yang, & Wang, 2004; El-Kommos, Saleh, El-Gizawi, & Abou-Elwafa, 2003; Xia et al., 2012; Zhu, Gong, & Yu, 2008), potentiometric titration (Park, Jeong, Lee, Lee, & Baek, 2000) and high performance liquid chromatography (HPLC) (Ebrahimpour, Yamini, & Moradi, 2012; Vazquez, Vazquez, Galera, & Garcia, 2012) coupled with mass spectrometry (MS) (Garcés, Zerzanová, Kucera, Barrón, & Barbosa, 2006). Because of the interference from complex matrices in animal-based foods, these analytical methods often require extensive sample preparation. Accordingly, there is considerable interest in developing a cost-effective, efficient and reliable extraction method for the analysis of complex samples prior to FQ quantification.

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Several pre-treatment methods, including solid-phase extraction (SPE) (Hermo, Nemutlu, Kir, Barron, & Barbosa, 2008), liquid–liquid extraction (LLE) (Chu et al., 2002), stir bar sorption extraction (SBSE) (Huang, Yuan, & Lin, 2011), microwave-assisted extraction (MAE) (Hermo, Barron, & Barbosa, 2005), cloud point extraction (CPE) (Wu, Zhao, & Du, 2010) and supercritical fluid extraction (SFE) (Shim, Lee, Kim, Lee, & Kim, 2003) have been developed. Major limitations of these methods include time-consuming extraction procedures, low enrichment factor, tedious operation and creation of a large amount of hazardous organic solvent waste. In recent years, some novel liquid-phase microextraction (LPME) techniques, such as dispersive liquid–liquid microextraction (DLLME), ultrasound-assisted DLLME (Yan, Wang, Qin, Liu, & Du, 2011), ionic liquid-based homogeneous liquid–liquid microextraction (IL-HLLME) (Gao et al., 2011) and ion pair-based surfactant-assisted microextraction (IP-SAME) (Ebrahimpour et al., 2012) have been developed based on a ternary solvent system with the advantages of simplicity, speed, low cost, good recovery and high enrichment factors. However, a major drawback for the use of non-polar, water-immiscible, organic solvents in all type of LPME is their low dielectric constant, making extraction of polar or charges solutes relatively poor (Gupta, Archana, & Verma, 2009). More-polar solvents, such as acetonitrile and ethanol, which provide solubility for polar to non-polar compounds are frequently water-miscible and, thus, cannot be used in conventional LPME.

Salting-out is a process of electrolyte addition to an aqueous phase in order to increase the distribution ratio of a particular solute. The term also connotes reduction of mutual miscibility of two liquids by addition of electrolytes. Weak intermolecular forces, e.g., hydrogen bonds, between organic molecules or non-electrolytes and water are easily disrupted by the hydration of electrolytes. Salting-out assisted liquid–liquid microextraction (SALLME) is based on phase separation of water-miscible organic solvents from the aqueous solutions at high salt concentration (Tsai et al., 2009). It uses water-miscible organic solvents that, generally, have low toxicity and small amounts of salt that cause little pollution. Additionally, this method has the advantages of being simple and sensitive as well as using less solvents, and the product is compatible for subsequent analysis by HPLC (Cai et al., 2007; Myasein, Kim, Zhang, Wu, & Tawakol, 2009). In SALLME, a glass centrifuge tube is often used as the extraction device. However, collection and measurement of microliter volumes of organic phase are difficult because the thin layer of extract is difficult to retrieve from the wide diameter glass tube increasing extraction time. A few approaches have been reported for introducing extraction devices or vessels to classical DLLME that allows for the use of lower density organic solvent, using either a narrow-necked glass tube (Ye, Zhou, & Wang, 2007) or a glass vial (Cheng, Matsadiq, Liu, Zhou, & Chen, 2011). Hashemi, Beyranvand, Mansur, and Ghiasvand (2009) introduced a home-made narrow-necked glass tube for the effective collection of extractant, and inserted it into a centrifuge tube for centrifugation after extraction. Zhang, Shi, Yu, and Feng (2011), designed a special flask equipped with two narrow open necks with one having a capillary tip to facilitate the DLLME process. However, all of these glass-based devices are fragile and require special design, therefore their cost is relatively high and their commercial availability is limited (Wang, Cheng, Zhou, Wang, & Cheng, 2013).

Recently, a cheap, flexible and disposable polyethylene Pasteur pipette was introduced as an extraction devices for low-density solvent-based DLLME (Guo & Lee, 2011; Hu, Wu, & Feng, 2010). Cheng et al. (2011) developed an apparatus consisting of a dropper and a sample vial to perform extraction, separation and concentration of trace pesticides from solvents one step. The bulb end of the cut polyethylene dropper was inserted into the neck of the sample vial and the tip end of the polyethylene dropper was cut to an appropriate length (Wang et al., 2013). The plastic pipette afforded advantages of low cost, use of easily available materials and ease of operation. However, the major drawback of this apparatus is that the extracted organic phase was difficult to completely retrieve because the organic phase and aqueous solution were not separated prior to collection of the extractant. The repartition of extractant into the aqueous phase can occur over the relatively long retrieval time, which will result in a low extraction recovery.

To overcome the above-mentioned limitations of current methods, this study developed and optimized a novel integrated apparatus and methodology for extraction of FQs by means of a phase separation method based magnetic-stirring salt-induced liquid–liquid microextraction (PS-MSLM). The proposed PS-MSLM method was optimized for major operational factors (stirring time, pH, salt kind and volume, solvent kind and volume, and centrifugation time) using a response surface method (RSM) based on central composite design (CCD). The optimized method was compared with other commonly used LPME methods to evaluate its advantages and feasibility for determining trace levels of FQs in milk, honey and eggs. To the best of our knowledge, this integrated apparatus, designed to completely and rapidly separate the organic and aqueous phases prior to collection of the extractant, is the first reported use of this approach for determination of FQs in animal-based foods.

2. Experimental

2.1. Reagents and materials

Analytical standards for fleroxacin (FLE), ofloxacin (OFL), norfloxacin (NOR), ciprofloxacin (CIP) and enrofloxacin (ENR) were purchased from J&K Chemical Corporation (Shanghai, China) and used without further purification. HPLC-grade ethanol, methanol, ethyl acetate, acetonitrile and acetone were sourced from Merck Corporation (Shanghai, China). Salts (magnesium sulfate ($MgSO_4$), sodium sulfate (Na_2SO_4), ammonium sulfate ($(NH_4)_2SO_4$) and ammonium acetate (CH_3COONH_4)) with purities $\geqslant$99% were obtained from Aladdin Industrial Co. Ltd. (Shanghai, China).

Stock standard solutions (1000 μg mL^{-1}) for each FQ were prepared by dissolving each compound in methanol and stored at 4 °C. Stock solutions were diluted with methanol to prepare a secondary mix stock solution of 10 μg mL^{-1}. Mixtures of standard working solutions for extraction at different concentrations were prepared by dilution with Milli-Q ultrapure water (Millipore, Bedford, USA).

Milk, chicken egg and honey samples were produced by Jiangxin Milk Company, Ronghe Agricultural Product Company and Fujian XinZhiYuan Biological Company, China, respectively, and purchased from Baixin Supermarket, Wenzhou, China. These samples were mixed with a vortex mixer for 5.0 min and stored in amber bottles at 4 °C until analysis within 1 week.

2.2. Instrumentation

FQs were analyzed with an Agilent 1260 HPLC equipped with a fluorescence detector (FLD). A Zorbax Eclipse XDB-C_{18} column (150 mm × 4.6 mm, 5 μm particle size) was used and injections were performed manually using a 20.0-μL sample loop. The operating conditions were as follows: mobile phase, methanol–acetonitrile–water (15:5:80, v/v; water consisting of 3.4 mL orthophosphoric acid and 6.0 mL triethylamine per liter); flow rate, 0.8 mL min^{-1}; column temperature, 40 ± 1 °C; and excitation and emission wavelengths of 290 and 455 nm, respectively. Solutions were stirred with a model HJ-6A magnetic heater-stirrer with an 8 mm × 4 mm stirs bar (Jiangsu Jintan Medical Instrument Factory

(Jintan, China). Centrifugation used a model TDL-50C centrifuge from Anting Instrument Factory (Shanghai, China). Matrix density was measured by a JT-120S density meter from Jingtai Instrument Factory (Taizhou, China).

2.3. PS-MSLM procedure

A schematic of the integrated PS-MSLM procedure is shown in Fig. 1. This novel integrated device consists of three parts: (1) a high-density polyethylene (HDPE) centrifuge tube (8 cm × 1.6 cm external diameter, 1.4 cm internal diameter, Fig. 1A); (2) an inverted cut HDPE dropper (1 cm × 1.4 cm external diameter joined to a 3 cm length of capillary tube, Fig. 1K); and (3) a "V" HDPE capillary tube (10 cm × 0.5 cm internal diameter, Fig. 1K). The inverted cut disposable HDPE dropper was inserted into the centrifuge tube, and the "V" tube was easily attached/detached from the inverted HDPE dropper (Fig. 1H and I).

In operation, the sample solution was first added to the centrifuge tube followed by the solvent, which was water-miscible and lower density than water (Fig. 1A and C). After stirring and centrifugation, the sedimented proteins and other interfering compounds were discarded (Fig. 1B and C). Finally, an appropriate amount of salt was added to the remaining solution (Fig. 1D). After salting-out, and following stirring and centrifugation, the solvent floated on the top of the sample (Fig. 1E and F). The inverted HDPE dropper was then placed into the sample solution and the extractant was extruded through the tip of the dropper (Fig. 1G and H). When the extractant was fully transferred into the "V" tube, the "V" tube was detached and the extractant was collected with a micro-syringe (Fig. 1I). The extractant was then dried using a gentle nitrogen flow, dissolved with 50 μL of mobile phase and quantified by HPLC-FLD analysis (Fig. 1J).

For pre-treatment of food samples, 5 mL of milk, 1 mL of eggs (combined yolk and albumen) or 1 mL of honey were placed into triplicate 10 mL centrifuge tubes. Each sample was added using ultrapure water to obtain a final volume of 6 mL, and followed by acidification to pH 1.0 with sulfuric acid. The water-miscible organic solvent (500–1100 μL) was slowly introduced into the sample solution with a 1000-μL micropipette. After 2 min of magnetic stirring at 1400 rpm, the emulsion was centrifuged at 4000 rpm for 3 min resulting in sedimentation of protein impurities. The supernatant was transferred to another centrifuge tube and 2.0–4.5 g of salt was added followed by magnetic-stirring for 0–8 min at 1400 rpm and centrifugation at 4000 rpm for 0–8 min. Finally, the solvent was isolated as the top layer of the sample solution and recovered using the inverted dropper as described above.

2.4. Experimental design

The optimization experiments were randomized in order to minimize the effects of uncontrolled factors. As it was not possible to complete each experiment during a single work day, they were divided into two blocks and carried out in two sequential days to remove any variations caused by changes occurring over the course of the experiments (Sereshti, Izadmanesh, & Samadi, 2011). Four main factors, stirring time (A), pH (B), solvent volume (C) and centrifugation time (D), were chosen on the basis of the literature and preliminary experiments. For each variable, high and low set points were selected to construct an orthogonal design (Supplementary Table 1). Central composite design (CCD) was used to optimize values for each factor based on extraction recovery (ER). The CCD included 22 treatments in five levels ($-\alpha$, -1, 0, $+1$, $+\alpha$) for four factors, and consisting of two blocks (Supplementary Table 2). It contained an imbedded half-fraction factorial design ($N_f = 2^{f-1}$) with a set of center points (N_0) that was augmented with a group of "star points" ($N_\alpha = 2f$) that allow for estimation of curvature (Sereshti, Heravi, & Samadi, 2012), where "f" indicates the number of the experimental factors. As a result, the 22 treatments included 8 half-fraction factorial design points, 8 "star points" and 6 center points. The average extraction recovery (ER) was considered as the "experimental response" to evaluate the method performance (Sereshti et al., 2012), which was computed by Eq. (1):

$$\mathrm{ER} = \frac{C_{\mathrm{sed}} \times V_{\mathrm{sed}}}{C_0 \times V_{\mathrm{aq}}} \times 100 \quad (1)$$

where C_{sed} is concentration of the analyte in the sedimented phase; C_0 is the initial concentration of analyte in the sample solution; and V_{sed} and V_{aq} are the volumes of sedimented and sample solutions, respectively (Sereshti et al., 2012). A quadratic polynomial model Eq. (2) was used to predict the response of dependent variables for the ERs of FQs:

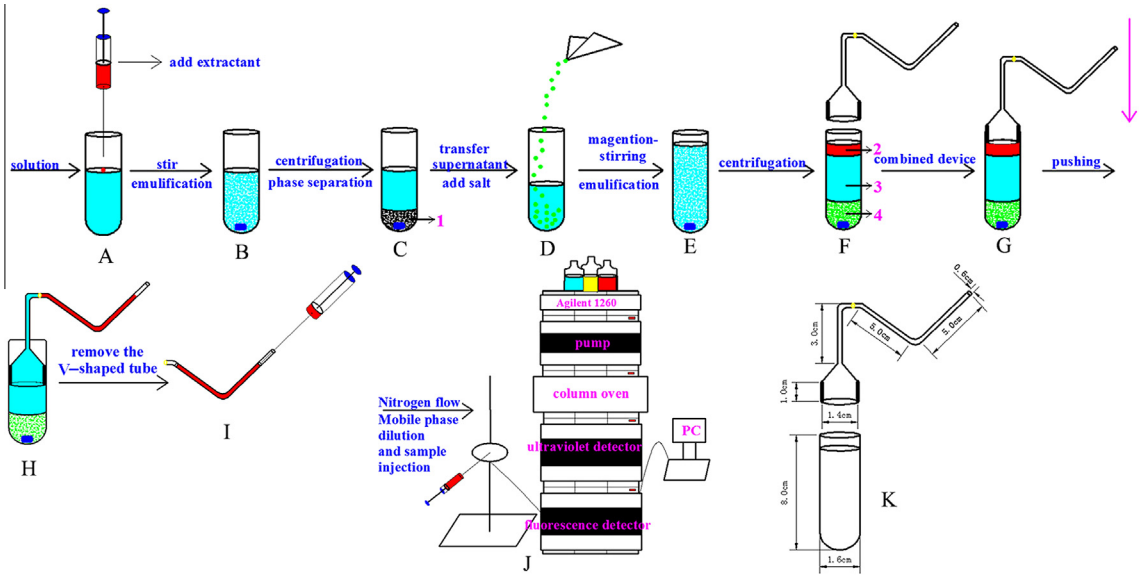

Fig. 1. The integrated apparatus and schematic procedure of PS-MSLM method. *Note:* each step in PS-MSLM procedures is described in the text.

$$Y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{ij=1(i\neq j)}^{6} b_{ij} x_i x_j + \sum_{i=1}^{4} b_{ii} x_i^2 \quad (2)$$

where Y is the dependent variable, x_i is the independent variable, b_0 is the intercept, b_i is the coefficient of linear effect, b_{ij} is the coefficient of interaction effect, and b_{ii} is the coefficient of the squared effect (Mohammadi et al., 2013). The software package Design-Expert 8.0.5 (Minneapolis, USA) was employed to analyze the data and experimental design. Analysis of variance (ANOVA) was used to evaluate the model and to obtain response surfaces for factor optimization.

3. Results and discussion

3.1. Selection of solvent and salt

In PS-MSLM, the selection of an appropriate solvent is based on basic requirements, such as lower density than water, miscibility with the aqueous phase, ease of phase separation in high salt concentrations, good chromatographic behavior, and high extraction efficiency for target analytes. According to these considerations, ethyl acetate, ethanol, methanol, acetonitrile and acetone were examined for their "salting-out" phenomena and extraction efficiencies for FQs (Supplementary Fig. 1). Using 5 ml sample and 0.8 ml solvent, we examined the salting-out effect of four salts ($MgSO_4$, Na_2SO_4, CH_3COONH_4 and $(NH_4)_2SO_4$) in the range 2–4.5 g. The methanol–water mixture did not show any phase separation even when the mixture was saturated with salts. Additionally, ethyl acetate and ethanol showed indistinct phase separation even after centrifugation. In contrast, water–acetonitrile and water–acetone mixtures gave a clear separation in the presence of all four salts under the conditions. Similarly, the volume of organic solvent-rich phase/water-rich phase after separation was 0.5/5.8 mL for water–acetonitrile and 0.5/5.6 mL for water–acetone. The highest ER was observed in water/acetone/Na_2SO_4 (94.7 ± 3.2%), followed by water/acetone/$(NH_4)_2SO_4$ (90.1 ± 2.7%) and CH_3COONH_4 (14.2 ± 1.5%) (Supplementary Fig. 1). As a result, acetone and Na_2SO_4 were chosen for subsequent experiments.

In addition, the effect of salt concentration on extraction efficiency was investigated by adding different concentrations of Na_2SO_4 (2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 g) to the 5.0 mL water/0.5–1.1 mL acetone system. Preliminary experimental results showed that when 1.5 g of Na_2SO_4 was added, no obvious phase separation occurred suggesting an insufficient amount to induce the salting-out process. As shown in Supplementary Fig. 2, a significant increase in ER from 82.4% to 95.3% occurred with increasing Na_2SO_4 concentrations from 2.0 to 2.5 g. However, with further additions of Na_2SO_4 from 2.5 to 4.5 g, the ER remained nearly constant (~94.2%), implying the occurrence of salt saturation. When salts were added in a non-saturated state, some researchers found that salt additions to the aqueous sample had differential effects on microextraction: it may enhance, not influence, or limit extraction (Wu, Tragas, Lord, & Pawliszyn, 2002). In these studies, the NaCl dissolved in the aqueous solution may have changed the physical properties of the Nernst diffusion film and reduced the rate of diffusion of the target analyte into the microdrop (Psillakis & Kalogerakis, 2001), thus affecting the extraction efficiency except for salting-out effect (Wang et al., 2012). In our study, the addition of 2.5 g of Na_2SO_4 was selected as the optimum salt amount for subsequent experiments.

3.2. Optimization of the PS-MSLM procedures using CCD

The experimental design matrix, which is composed of the number and order of the experiments, levels of factors in each experiment and the extraction recovery, is summarized in Supplementary Table 2. ANOVA was used to evaluate the significance of the model equation and related terms (Supplementary Table 3). The model was highly significant and the "probe > F" value for the "lack of fit component" was 0.2736, which means the other factors in this experiment had a small amount of interference and the model represents the data well. The significant model, with a "probe > F" value less than 0.0001, indicated that the equation is a good fit for representing the relationship between ER and the four main factors. Based on the significant effects for "probe > F" values <0.0500, it was concluded that A, B, C, D, AB, AC, AD, BC, A^2, B^2, C^2 and D^2 all showed significant effects. A second-order polynomial provided the strongest statistical fit and was considered as the best response surface model to fit the experimental data (Sereshti et al., 2012). As can be seen in the Eq. (3), there were four main effects (A, B, C and D), four two-factor interaction effects (AB, AC, AD and BC), and four curvature effects (A^2, B^2, C^2 and D^2):

$$Y = b_0 + b_1A + b_2B + b_3C + b_4D + b_5AB + b_6AC + b_7AD + b_8BC + b_9BD + b_{10}CD + b_{11}A^2 + b_{12}B^2 + b_{13}C^2 + b_{14}D^2 \quad (3)$$

with $b_0 = -58.22$; $b_1 = 2.31$; $b_2 = -2.01$; $b_3 = 0.32$; $b_4 = 7.27$; $b_5 = 0.10$; $b_6 = -1.54 \times 10^{-3}$; $b_7 = -0.24$; $b_8 = -1.23 \times 10^{-3}$; $b_9 = -0.064$; $b_{10} = 1.25 \times 10^{-4}$; $b_{11} = -0.43$; $b_{12} = -0.17$; $b_{13} = -1.78$ and $b_{14} = -0.54$Here Y is the extraction recovery, b_0 is the intercept and b_1 to b_{14} are parameter coefficients. The relationship between the related effect and the response is indicated by "+" or "−" for each coefficient. A "+" means the coefficient and the extraction recovery has a positive relationship, while a "−" means a negative relationship. The absolute value of the coefficients indicates the strength of the relationship between the coefficient and the extraction recovery (Y).

The goodness of fit for the polynomial model was expressed by the coefficient of determination (R^2, adjusted-R^2). The R^2 (0.9836) is a measure of the amount of variance around the average explained by the model. The adjusted-R^2 (0.9992) is the R^2 adjusted for the number of terms in the model, and it decreases as the number of terms in the model increases if those additional terms do not add value to the model (Sereshti et al., 2012). The high R^2 values indicated that we can use the model to analyze and optimize the effects of extraction conditions on ER. As can be seen from Supplementary Fig. 3a, most of the data points were scattered near the regression line, suggesting a good correlation between predicted and actual responses and a good fit for the quadratic model. In addition, the residual plots were scattered randomly (Supplementary Fig. 3b) indicating that the variance of the experimental measurements was constant for all values of Y.

In order to obtain more details of the experimental factors on the extraction recovery, 3D response surfaces and contour lines were plotted. These plots represent the relationship between the response and levels of two factors simultaneously, while holding the other factors fixed at their central levels (Sereshti et al., 2011). The 3D response surfaces and contour lines shown in Fig. 2 represent the relationship between recovery and the four experimental factors (stirring time, pH, solvent volume and centrifugation time). For example, Fig. 2a describes the 3D response surface and contour line for the effect of stirring time and pH on ER under fixed conditions of 800-μL extractant volume and 5-min centrifugation time. The ERs of FQs increased with increasing stir time from 0 to 3 min and pH from 1.0 to 1.7. However, with a further increase in stir time from 3 to 10 min and pH from 1.7 to 7.0, the ERs of FQs declined. Fig. 2b depicts the 3D response surface and contour line for the effect of stir time and solvent volume on ER when the pH and centrifugation time were set at 4.0 and 5 min, respectively. The maximum ER was observed at 3 min stir time

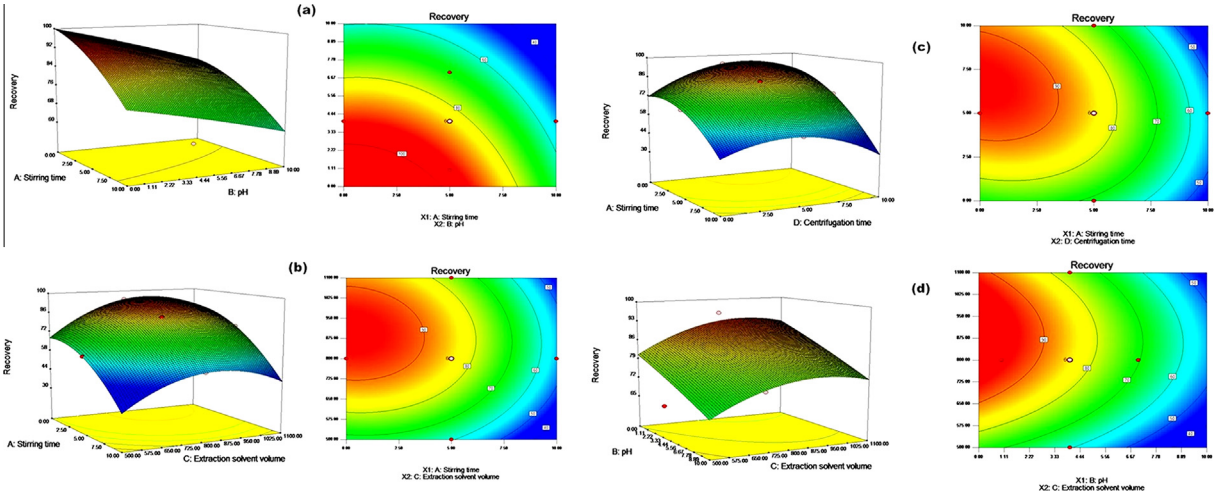

Fig. 2. (a) 3D response surface and contour plots for the stirring time and pH at constant concentration of solvent volume of 800 μL and centrifugation time of 5.0 min on the average extraction recovery, (b) 3D response surface and contour plots for the stirring time and solvent volume at constant concentration of pH of 4.0 and centrifugation time of 5.0 min on the average extraction recovery, (c) 3D response surface and contour plots for the stirring time and centrifugation time at constant concentration of pH of 4.0 and solvent volume of 800 μL on the average extraction recovery, (d) 3D response surface and contour plots for the pH and solvent volume at constant concentration of stirring time of 5.0 min and centrifugation time of 5.0 min on the average extraction recovery.

Table 1
Intra-day and inter-day precision of five FQs ($n = 6$) by the proposed method.

Analytes	Intra-day precision (RSD%, $n = 6$)			Inter-day precision (RSD%, $n = 6$)		
	Low	Medium	High	Low	Medium	High
FLE	2.1	1.8	1.1	5.1	3.5	2.1
OFL	5.2	1.6	1.0	5.0	4.9	2.7
NOR	4.9	4.1	1.2	5.5	3.7	3.2
CIP	5.3	2.2	1.3	6.0	4.3	4.0
ENR	3.1	2.8	0.7	4.3	3.5	2.4

Note: (1) "high" indicates 48.5 μg kg^{-1} for milk, 45.5 μg kg^{-1} for eggs and 35.2 μg kg^{-1} for honey; (2) "medium" indicates 19.4 μg kg^{-1} for milk, 18.2 μg kg^{-1} for eggs and 14.1 μg kg^{-1} for honey; and (3) "low" indicates 9.7 μg kg^{-1} for milk, 9.1 μg kg^{-1} for eggs and 7.0 μg kg^{-1} for honey, respectively.

and 791 μL of solvent. With further increases in stir time (3–10 min) and pH (1.7–7.0), the ERs decreased sharply.

Fig. 2c demonstrates the 3D response surface and contour line for the effect of stir time and centrifugation time on the ERs when the pH and extractant volume were set at 4.0 and 800 μL, respectively. When the stir time increased from 0 to 3 min and the centrifugation time increased from 0 to 5.5 min, the ERs gradually increased. The maximum ER was observed at approximately 3 min stir time and 5.5 min centrifugation time. Under the fixed conditions of 4.0 min stir time and 5.0 min centrifugation time, the effects of pH and solvent volume on the ERs were evaluated (Fig. 2d). With increasing pH from 1.0 to 1.7 and extractant volume from 500 to 791 μL, the ER reached a maximum point, and then quickly declined with the further increases of pH (1.7–7.0) and solvent volume (791–1100 μL). After rigorous analysis of the interaction factors in Fig. 2, the optimal set points for the four parameters were determined to be 3 min stir time, pH = 1.7, 791 μL solvent volume and 5.5 min centrifugation time.

3.3. Method evaluation

Under the optimized experimental conditions determined in this study, the performance of PS-MSLM was evaluated for linear range, limits of detection (LOD), precision and ER (Table 2). The coefficients of determination (R^2) for linearity of standard curves for the five FQs were in the range of 0.9989–0.9998. The limits of detection (LODs at S/N = 3) for milk, egg and honey samples were in the range 0.09–0.16 μg kg^{-1} for FLE; 0.35–0.47 μg kg^{-1} for OFL; 0.34–0.53 μg kg^{-1} for NOR; 0.11–0.21 μg kg^{-1} for CIP and 0.07–0.10 μg kg^{-1} for ENR. The linear dynamic range (LDR) was 0.50–500 μg kg^{-1} for FLE and CIP, 1.50–500 μg kg^{-1} for OFL and NOR and 0.25–250 μg kg^{-1} for ENR. The precision study was carried out in six parallel experiments by determining the intra- and inter-day RSDs (relative standard deviations) at three fortification levels of FQs. The RSDs varied between 0.65% and 5.28% for intra-day analysis, and ranged from 2.05% to 5.99% for inter-day analysis (Table 1).

3.4. Analysis of animal-based foods

The PS-MSLM method was applied for the determination of five FQs in milk, egg and honey samples. Fig. 3 illustrates a typical chromatogram for milk, egg and honey samples at fortification levels of 9.7, 9.1 and 7.0 μg kg^{-1}, respectively, for the five FQs using the optimized PS-MSLM method. The relative recovery (RR) was used to appraise the analytical performance of the optimized method following Eq. (4):

$$RR = \frac{C_{found} - C_{real}}{C_{added}} \quad (4)$$

where C_{found}, C_{real}, and C_{added} are the concentrations of analyte in the final solution after addition of a known amount of a standard into the animal-based food sample, the concentration of analyte in the food sample, and the concentration of a known amount of the standard which was spiked into the food sample, respectively. The results showed that the concentrations of FLE, OFL, NOR and

Table 2
The analytical performance of the PS-MSLM pretreatment method.

Sample	Analyte	Regression equations	Correlation coefficient (R^2)	Linear range (μg kg^{-1})	LOD (μg kg^{-1})
Milk	FLE	$y = 0.2946x - 0.0588$	0.9997	0.50–500	0.133
	OFL	$y = 0.0386x - 0.0310$	0.9997	2.00–500	0.466
	NOR	$y = 0.0937x - 0.0047$	0.9998	2.00–500	0.521
	CIP	$y = 0.4223x - 0.0956$	0.9996	1.00–500	0.198
	ENR	$y = 0.8438x - 0.5344$	0.9989	0.50–250	0.092
Eggs	FLE	$y = 0.2947x - 0.0538$	0.9997	1.00–500	0.158
	OFL	$y = 0.0384x - 0.0406$	0.9993	2.00–500	0.449
	NOR	$y = 0.0395x + 0.0293$	0.9997	2.00–500	0.526
	CIP	$y = 0.4242x - 0.3726$	0.9994	1.00–500	0.209
	ENR	$y = 0.8465x + 0.0444$	0.9995	0.50–250	0.104
Honey	FLE	$y = 0.2811x + 0.1471$	0.9993	0.50–500	0.092
	OFL	$y = 0.0372x - 0.0081$	0.9992	1.50–500	0.349
	NOR	$y = 0.1009x - 0.0108$	0.9998	1.50–500	0.344
	CIP	$y = 0.3959x + 0.2001$	0.9997	0.50–500	0.113
	ENR	$y = 0.8531x + 0.2383$	0.9991	0.25–250	0.067

Note: (1) LOD was calculated according to S/N = 3; (2) "μg L^{-1}" was converted to "μg kg^{-1}" on the basis of the following matrix densities: 1.03 g mL^{-1} for milk; 1.11 g mL^{-1} for egg; and 1.42 g mL^{-1} for honey, respectively.

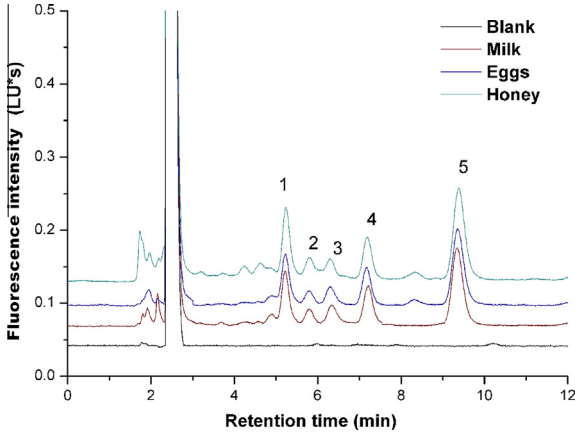

Fig. 3. Chromatogram of analytes obtained by the proposed PS-MSLM method under optimized conditions. *Note:* (1) FLE; (2) OFL; (3) NOR; (4) CIP; (5) ENR. Experimental conditions: (a) the milk, eggs and honey samples were fortified by FQs at 10 μg L^{-1}; (b) the blank samples indicated honey; (c) pH of 1.7, centrifugation time of 5.5 min, stirring time of 3.0 min, Na_2SO_4 of 2.5 g and solvent volume of 791 μL.

CIP were all below their respective detectable level in the milk, egg and honey samples. However, ENR was detected in the range of 2.93–4.58 μg kg^{-1} and 6.83–9.84 μg kg^{-1} in milk and egg samples, respectively (Table 3). For the three spiked levels, the RRs for the five FQs were in the range of 92.1–102.3% for milk, 92.2–105.0% for eggs and 91.6–104.0% for honey. These results collectively demonstrate that the optimal PS-MSLM method can be effectively used to analyze trace levels of FQs in animal-based foods with high precision and accuracy.

3.5. Comparison of PS-MSLM with other pretreatment methods

The PS-MSLM method developed and optimized in this study was compared with other methods from the literature, such as combined with liquid–liquid extraction (LLE) (Ho, Sin, Tang, Chung, & Siu, 2004), pressurized liquid extraction (PLE) (Herranz, Moreno-Bondi, & Marazuela, 2007; Luo, Ma, & Feng, 2010), dispersive solid-phase extraction (DSPE) (Pena-Pereira, Lavilla, & Bendicho, 2010), magnetic solid-phase extraction (MSPE) (Xu, Jiang, Lin, & Jia, 2012) and solid-phase extraction (SPE) (Rose, Bygrave, & Stubbings, 1998). Results were compared with reference to sample preparation time, LOD and ER (Supplementary

Table 3
Analytical performance for FQ quantification by the optimized method in milk, egg and honey samples (mean ± SD, $n = 6$).

FQs	Milk				Eggs				Honey			
	Blank	Added (μg kg^{-1})	Found (μg kg^{-1})	RR (%)	Blank	Added (μg kg^{-1})	Found (μg kg^{-1})	RR (%)	Blank	Added (μg kg^{-1})	Found (μg kg^{-1})	RR (%)
FLE	ND	9.7	9.30 ± 0.81	95.8	ND	9.1	8.34 ± 0.51	92.2	ND	7.0	6.67 ± 0.57	94.7
	ND	19.4	18.17 ± 0.37	93.6	ND	18.2	16.70 ± 0.55	92.7	ND	14.1	12.96 ± 0.47	91.9
	ND	48.5	49.64 ± 0.91	102.3	ND	45.5	44.26 ± 0.77	98.3	ND	35.2	34.76 ± 0.77	98.7
OFL	ND	9.7	9.36 ± 0.35	96.6	ND	9.1	9.08 ± 0.69	100.9	ND	7.0	6.45 ± 0.35	91.6
	ND	19.4	19.07 ± 0.16	98.2	ND	18.2	18.27 ± 0.27	101.3	ND	14.1	13.45 ± 0.23	95.5
	ND	48.5	48.47 ± 0.61	99.8	ND	45.5	45.51 ± 0.77	101.0	ND	35.2	34.69 ± 0.61	98.5
NOR	ND	9.7	9.19 ± 0.28	94.5	ND	9.1	9.47 ± 0.35	105.0	ND	7.0	7.27 ± 0.59	103.3
	ND	19.4	18.97 ± 0.25	97.7	ND	18.2	18.20 ± 0.25	100.1	ND	14.1	14.60 ± 0.38	103.6
	ND	48.5	48.76 ± 0.79	100.4	7.11 ± 0.67	45.5	51.93 ± 0.62	100.9	ND	35.2	36.73 ± 0.89	104.0
CIP	ND	9.7	8.94 ± 0.54	92.1	ND	9.1	9.43 ± 0.57	104.7	ND	7.0	6.85 ± 0.29	97.4
	ND	19.4	19.15 ± 0.32	98.6	ND	18.2	16.97 ± 0.31	94.2	ND	14.1	13.73 ± 0.15	97.5
	ND	48.5	47.62 ± 1.00	98.1	ND	45.5	44.13 ± 1.07	98.0	ND	35.2	34.27 ± 0.69	97.3
ENR	4.28 ± 0.62	9.7	10.01 ± 0.67	93.8	6.83 ± 0.83	9.1	10.12 ± 0.73	102.2	ND	7.0	7.22 ± 0.41	93.3
	2.93 ± 0.26	19.4	21.60 ± 0.41	96.1	7.41 ± 0.64	18.2	24.86 ± 0.59	96.8	ND	14.1	13.04 ± 0.24	92.6
	4.58 ± 0.98	48.5	53.58 ± 0.66	100.9	9.84 ± 0.73	45.5	53.05 ± 1.23	96.7	ND	35.2	34.48 ± 0.95	97.8

Note: (1) ND and RR represent non-detectable level and relative recovery, respectively; (2) "μg L^{-1}" was converted to "μg kg^{-1}" on the basis of the following matrix densities: 1.03 g mL^{-1} for milk; 1.11 g mL^{-1} for egg; and 1.42 g mL^{-1} for honey, respectively.

Table 4). The sample preparation time for PS-MSLM is much shorter (~8.5 min) than those of MSPE (2 days), DSPE (91.5 min), LLE (16 min) and PLE (21 min and 15 min), as shown in Supplementary Table 4. The LODs for PS-MSLM-HPLC-FLD were in the range of 0.07–0.53 μg kg^{-1}, which were comparable with those of MSPE, and lower than those of LLE, PLE, DSPE and SPE. The RRs of PS-MSLM (91.9–105.0%) were much higher than other referenced methods (ca. 80–90%), with the exception of LLE (28–129%), PLE (69–107%) and MSPE (84.0–106%). Additionally, the PS-MSLM method gave higher precision with RRs very close to 100%. The higher precision could be explained by low repartitioning of extractant into the aqueous solution during collection as a result of complete separation of extractant from the aqueous solution prior to collection.

4. Conclusion

This study developed a new and simple integrated apparatus for extraction and quantification of five FQs in animal-based foods. The novel integrated apparatus consisted of three simple HDPE parts that were used to separate the solvent from the aqueous solution prior to retrieving the extractant. This technique reduces repartitioning of extractant into the aqueous phase during collection, decreases organic phase-collection time and improves extraction efficiency. As compared with other methodologies, the PS-MSLM method developed and optimized in this study has several advantages, such as high extraction efficiency, easily constructed with inexpensive HDPE materials, laboratory accessibility, short extraction time and compatible for subsequent HPLC analysis. It was successfully applied to determine five FQs with high RRs (91.9–105.0%) and low LODs (0.07–0.53 μg kg^{-1}) in milk, egg and honey samples. As a result, the PS-MSLM method developed and optimized in this study has excellent prospects for sample pretreatment and quantification of trace levels of FQs in animal-based foods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.11.132.

References

Cai, Y. Q., Cai, Y. E., Shi, Y. L., Liu, J. M., Mou, S. F., & Lu, Y. Q. (2007). A liquid–liquid extraction technique for phthalate esters with water-soluble organic solvents by adding inorganic salts. *Microchimica Acta, 157*, 73–79.

Cheng, J., Matsadiq, G., Liu, L., Zhou, Y. W., & Chen, G. (2011). Development of a novel ultrasound-assisted surfactant-enhanced emulsification microextraction method and its application to the analysis of eleven polycyclic aromatic hydrocarbons at trace levels in water. *Journal of Chromatography A, 1218*, 2476–2482.

Chu, P. S., Wang, R. C., & Chu, H. V. (2002). Liquid chromatographic determination of fluoroquinolones in egg albumen and egg yolk of laying hens using fluorometric detection. *Journal of Agricultural and Food Chemistry, 50*, 4452–4455.

Du, L. M., Yang, Y. Q., & Wang, Q. M. (2004). Spectrofluorometric determination of certain quinolone through charge transfer complex formation. *Analytica Chimica Acta, 516*, 237–243.

Ebrahimpour, B., Yamini, Y., & Moradi, M. (2012). Application of ionic surfactant as a carrier and emulsifier agent for the microextraction of fluoroquinolones. *Journal of Pharmaceutical and Biomedical Analysis, 66*, 264–270.

El-Kommos, M. E., Saleh, G. A., El-Gizawi, S. M., & Abou-Elwafa, M. A. (2003). Spectrofluorometric determination of certain quinolone antibacterials using metal chelation. *Talanta, 60*, 1033–1050.

Espinosa-Mansilla, A., Muñoz de la Peña, A., González Gómez, D., & Salinas López, F. (2006). Determination of fluoroquinolones in urine and serum by using high performance liquid chromatography and multiemission scan fluorimetricdetection. *Talanta, 68*, 1215–1221.

Gajda, A., Posyniak, A., Zmudzki, J., Gbylik, M., & Bladek, T. (2012). Determination of fluoroquinolones in eggs by liquid chromatography with fluorescence detection and confirmation by liquid chromatography–tandem mass spectrometry. *Food Chemistry, 135*, 430–439.

Gao, Q., Zheng, H. B., Luo, D., Ding, J., & Feng, Y. Q. (2012). Facile synthesis of magnetic one-dimensional polyaniline and its application in magnetic solid phase extraction for fluoroquinolones in honey samples. *Analytica Chimica Acta, 720*, 57–62.

Gao, S. Q., Jin, H. Y., You, J. Y., Ding, Y., Zhang, N., Wang, Y., et al. (2011). Ionic liquid-based homogeneous liquid–liquid microextraction for the determination of antibiotics in milk by high-performance liquid chromatography. *Journal of Chromatography A, 1218*, 7254–7263.

Garcés, A., Zerzanová, A., Kucera, R., Barrón, D., & Barbosa, J. (2006). Determination of a series of quinolones in pig plasma using solid-phase extraction and liquid chromatography coupled with mass spectrometric detection: Application to pharmacokinetic studies. *Journal of Chromatography A, 1137*, 22–29.

Gupta, M., Archana, J., & Verma, K. K. (2009). Salt-assisted liquid–liquid microextraction with water-miscible organic solvents for the determination of carbonyl compounds by high-performance liquid chromatography. *Talanta, 80*, 526–531.

Guo, L., & Lee, H. K. (2011). Low-density solvent-based solvent demulsification dispersive liquid–liquid microextraction for the fast determination of trace levels of sixteen priority polycyclic aromatic hydrocarbons in environmental water samples. *Journal of Chromatography A, 1218*, 5040–5046.

Hashemi, P., Beyranvand, S., Mansur, R. S., & Ghiasvand, A. R. (2009). Development of a simple device for dispersive liquid–liquid microextraction with lighter than water organic solvents: Isolation and enrichment of glycyrrhizic acid from licorice. *Analytica Chimica Acta, 655*, 60–65.

Hermo, M. P., Barron, D., & Barbosa, J. (2005). Determination of residues of quinolones in pig muscle: Comparative study of classical and microwave extraction techniques. *Analytica Chimica Acta, 539*, 77–82.

Hermo, M. P., Nemutlu, E., Kir, S., Barron, D., & Barbosa, J. (2008). Improved determination of quinolones in milk at their MRL levels using LC–UV, LC–FD, LC–MS and LC–MS/MS and validation in line with regulation 2002/657/EC. *Analytica Chimica Acta, 613*, 98–107.

Herranz, S., Moreno-Bondi, M. C., & Marazuela, M. D. (2007). Development of a new sample pretreatment procedure based on pressurized liquid extraction for the determination of fluoroquinolone residues in table eggs. *Journal of Chromatography A, 1140*, 63–70.

Ho, C., Sin, D. W. M., Tang, H. P. O., Chung, L. P. K., & Siu, S. M. P. (2004). Determination and on-line clean-up of (fluoro)quinolones in bovine milk using column-switching liquid chromatography fluorescence detection. *Journal of Chromatography A, 1061*, 123–131.

Huang, X. J., Yuan, D. X., & Lin, Q. M. (2011). Preparation of cation-exchange stir bar sorptive extraction based on monolithic material and its application to the analysis of soluble cations in milk by ion chromatography. *Journal of Chromatography A, 1217*, 2667–2673.

Hu, X. Z., Wu, J. H., & Feng, Y. Q. (2010). Molecular complex-based dispersive liquid–liquid microextraction: Analysis of polar compounds in aqueous solution. *Journal of Chromatography A, 1217*, 7010–7016.

Lombardo-Agüí, M., García-Campaña, A. M., Gámiz-Gracia, L., & Blanco, C. C. (2010). Laser induced fluorescence coupled to capillary electrophoresis for the determination of fluoroquinolones in foods of animal origin using molecularly imprinted polymers. *Journal of Chromatography A, 1217*, 2237–2242.

Luo, Y. B., Ma, Q., & Feng, Y. Q. (2010). Stir rod sorptive extraction with monolithic polymer as coating and its application to the analysis of fluoroquinolones in honey sample. *Journal of Chromatography A, 1217*, 3583–3589.

Mohammadi, A., Tavakoli, R., Kamankesh, M., Rashedi, H., Attaran, A., & Delavar, M. (2013). Enzyme-assisted extraction and ionic liquid-based dispersive liquid–liquid microextraction followed by high-performance liquid chromatography for determination of patulin in apple juice and method optimization using central composite design. *Analytica Chimica Acta, 804*, 104–110.

Motwani, S. K., Chopra, S., Ahmad, F. J., & Khar, R. K. (2007). Validated spectrophotometric methods for the estimation of moxifloxacin in bulk and pharmaceutical formulations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 68*, 250–256.

Myasein, F., Kim, E., Zhang, J., Wu, H., & Tawakol, A. (2009). Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC–MS/MS. *Analytica Chimica Acta, 651*, 112–116.

Park, H. R., Jeong, G. Y., Lee, H. C., Lee, J. C., & Baek, G. M. (2000). Ionization and divalent cation complexation of quinolone antibiotics in aqueous solution. *Bulletin of the Korean Chemical Society, 21*, 849–854.

Pena-Pereira, F., Lavilla, I., & Bendicho, C. (2010). Liquid-phase microextraction techniques within the framework of green chemistry. *Trends in Analytical Chemistry, 29*, 617–628.

Psillakis, E., & Kalogerakis, N. J. (2001). Application of solvent microextraction to the analysis of nitroaromatic explosives in water samples. *Journal of Chromatography A, 907*, 211–219.

Rose, M. D., Bygrave, J., & Stubbings, G. W. F. (1998). Extension of multi-residue methodology to include the determination of quinolones in food. *Analyst, 123*, 2789–2796.

Sereshti, H., Heravi, Y. E., & Samadi, S. (2012). Optimized ultrasound-assisted emulsification microextraction for simultaneous trace multielement determination of heavy metals in real water samples by ICP–OES. *Talanta, 97*, 235–241.

Sereshti, H., Izadmanesh, Y., & Samadi, S. (2011). Optimized ultrasonic assisted extraction-dispersive liquid–liquid microextraction coupled with gas chromatography for determination of essential oil of *Oliveria decumbens* Vent. *Journal of Chromatography A, 1218*, 4593–4598.

Shim, J. H., Lee, M. H., Kim, M. R., Lee, C. J., & Kim, I. S. (2003). Simultaneous measurement of fluoroquinolones in eggs by a combination of supercritical fluid extraction and high pressure liquid chromatography. *Bioscience Biotechnology and Biochemistry, 67*, 1342–1348.

Tsai, W. H., Chuang, H. Y., Chen, H. H., Huang, J. J., Chen, H. C., Cheng, S. H., & Huang, T. C. (2009). Application of dispersive liquid–liquid microextraction and dispersive micro-solid-phase extraction for the determination of quinolones in swine muscle by high-performance liquid chromatography with diode-array detection. *Analytica Chimica Acta, 656*, 56–62.

Vazquez, M. M. P., Vazquez, P. P., Galera, M. M., & Garcia, G. M. D. (2012). Determination of eight fluoroquinolones in groundwater samples with ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction prior to high-performance liquid chromatography and fluorescence detection. *Analytica Chimica Acta, 748*, 20–27.

Wang, H. L., Yan, H., Wang, C. J., Chen, F., Ma, M. P., Wang, W. W., et al. (2012). Analysis of phenolic pollutants in human samples by high performance capillary electrophoresis based on pretreatment of ultrasound-assisted emulsification microextraction and solidification of floating organic droplet. *Journal of Chromatography A, 1253*, 16–21.

Wang, X. H., Cheng, J., Zhou, H. B., Wang, X. H., & Cheng, M. (2013). Development of a simple combining apparatus to perform a magnetic stirring-assisted dispersive liquid–liquid microextraction and its application for the analysis of carbamate and organophosphorus pesticides in tea drinks. *Analytica Chimica Acta, 787*, 71–77.

Wang, Y. S., Zhou, L. M., & Zeng, H. (2005). Relationships between mechanism of adverse reactions and structure for fluoroquinolones. *Journal of Adverse Drug Reaction, 6*, 405–407 (in Chinese).

Wu, H., Zhao, G. Y., & Du, L. M. (2010). Determination of ofloxacin and gatifloxacin by mixed micelle-mediated cloud point extraction–fluorimetry combined methodology. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 75*, 1624–1628.

Wu, J. C., Tragas, C., Lord, H., & Pawliszyn, J. (2002). Analysis of polar pesticides in water and wine samples by automated in-tube solid-phase microextraction coupled with high performance liquid chromatography–mass spectrometry. *Journal of Chromatography A, 976*, 357–367.

Xia, Q. H., Yang, Y. L., & Liu, M. S. (2012). Aluminium sensitized spectrofluorimetric determination of fluoroquinolones in milk samples coupled with salting-out assisted liquid–liquid ultrasonic extraction. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 96*, 358–364.

Xu, S., Jiang, C., Lin, Y. X., & Jia, L. (2012). Magnetic nanoparticles modified with polydimethylsiloxane and multi-walled carbon nanotubes for solid-phase extraction of fluoroquinolones. *Microchimica Acta, 179*, 257–264.

Yan, H. Y., Wang, H., Qin, X. Y., Liu, B. M., & Du, J. J. (2011). Ultrasound-assisted dispersive liquid–liquid microextraction for determination of fluoroquinolones in pharmaceutical wastewater. *Journal of Pharmaceutical and Biomedical Analysis, 54*, 53–57.

Ye, C. L., Zhou, Q. X., & Wang, X. M. (2007). Improved single-drop microextraction for high sensitive analysis. *Journal of Chromatography A, 1139*, 7–13.

Zhang, P. P., Shi, Z. G., Yu, Q. W., & Feng, Y. Q. (2011). A new device for magnetic stirring-assisted dispersive liquid–liquid microextraction of UV filters in environmental water samples. *Talanta, 83*, 1711–1715.

Zhu, X. S., Gong, A. Q., & Yu, S. H. (2008). Fluorescence probe enhanced spectrofluorimetric method for the determination of gatifloxacin in pharmaceutical formulations and biological fluids. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 69*, 478–482.