Talanta 99 (2012) 875–882

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Ultrasound-assisted ionic liquid/ionic liquid-dispersive liquid–liquid microextraction for the determination of sulfonamides in infant formula milk powder using high-performance liquid chromatography

Shiqian Gao [a], Xiao Yang [a], Wei Yu [b], Zhongling Liu [a], Hanqi Zhang [a,*]

[a] College of Chemistry, Jilin University, Qianjin Street 2699, Changchun 130012, PR China
[b] Department of Hand Surgery, China-Japan Union Hospital, Jilin University, Xiantai Street 126, Changchun 130033, PR China

ARTICLE INFO

Article history:
Received 7 May 2012
Received in revised form
8 July 2012
Accepted 18 July 2012
Available online 24 July 2012

Keywords:
Hydrophilic IL
Hydrophobic IL
Dispersive liquid–liquid microextraction
Infant formula milk powder

ABSTRACT

Ultrasound-assisted ionic liquid/ionic liquid-dispersive liquid–liquid microextraction (UA-IL/IL-DLLME) high-performance liquid chromatography was developed and applied to the extraction, separation and determination of sulfonamides in infant formula milk powder samples. The hydrophobic IL and hydrophilic IL were used as extraction solvent and dispersion solvent, respectively. The extraction procedure was induced by the formation of cloudy solution, which was composed of fine drops of $[C_6MIM][PF_6]$ dispersed entirely into sample solution with help of $[C_4MIM][BF_4]$. The purification of sample and concentration of target analytes were performed simultaneously. The introduction of ion-pairing agent (NH_4PF_6) was beneficial to the improvement of recoveries for IL phase and analytes. The experimental parameters of the UA-IL/IL-DLLME, including concentration of $[C_6MIM][PF_6]$ and $[C_4MIM][BF_4]$ in sample solution, ultrasound extraction time, pH value of sample solution and amount of ion-pairing agent (NH_4PF_6), were evaluated. The limits of detection for sulfamerazine, sulfamethizole, sulfachlorpyridazine, sulfamonomethoxine, sulfmethoxazole and sulfisoxazole were 2.94, 9.26, 16.7, 5.28, 3.35 and 6.66 μg kg^{-1}, respectively. When the present method was applied to the analysis of infant formula milk powder samples, the recoveries of the analytes ranged from 90.4% to 114.8% and relative standard deviations were lower than 7.5%. The proposed method was compared with the ionic liquid-homogeneous liquid–liquid microextraction, ionic liquid-ultrasound-assisted emulsification–microextraction and ionic liquid-temperature-controlled-DLLME. The results indicated that the proposed method is effective for the extraction of the sulfonamides in milk powder samples.

1. Introduction

Milk powder is an important type of food in daily life, the quality and security of which directly influences the health of customers. Over recent years, the continuous reports of milk powder-related disastrous events, such as the illegal addition of estrogen and the overuse of antibiotics, have raised extensive concern about the safety of milk powder. Sulfonamides (SAs) are a kind of synthetic antibiotics and commonly used in livestock husbandry for treating diseases and promoting growth [1]. Their overuse may lead to the contamination in different livestock products, which can give rise to an increase in the antibiotic resistance of pathogenic bacteria and result in food safety problems [2]. To protect public health and food safety, many governmental authorities have established the criteria of maximum residue limits (MRLs) for SAs in various foodstuffs, such as meat, milk and eggs [3–5]. European Union and US Food and Drug Administration (FDA) have provided that the total residues of SAs should not exceed 100 μg kg^{-1} in milk and dairy products/milk food, and stressed that infant formula sold to US consumers must be completely free of SAs [6–8]. Meanwhile, the amendment of the Canadian Food and Drug Regulations (1347-SAs) was published and emphasized that the combined residues of sulfonamides should not exceed 100 μg kg^{-1} in edible tissues and 10 μg kg^{-1} in milk [9].

Therefore, there is an urgent need to establish a rapid, effective and highly sensitive method for detecting target analytes at levels desired by regulatory authorities. Nevertheless, low concentration levels (below ng mL^{-1}) and matrix interference are two important problems for the SAs residue determination in dairy produces. Solid-phase microextraction (SPME) [10,11], stir bar sorptive extraction (SBSE) [12,13] and liquid phase microextraction (LPME) [14,15] were commonly used sample pretreatment techniques for the trace analysis of SAs in complex matrices.

* Corresponding author. Tel.: +86 431 85168399; fax: +86 431 85112355.
E-mail addresses: gaosq10@mails.jlu.edu.cn (S. Gao), analchem@jlu.edu.cn (H. Zhang).

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http://dx.doi.org/10.1016/j.talanta.2012.07.050

The SPME integrated the sample purification and target analytes preconcentration in one step, but generally, it is time-consuming and a large amount of solvent are required. LPME is a solvent-miniaturized procedure of LLE, which has a good preconcentration ability. However, the precision of this method was relatively poor due to the manually handling of small amount extraction solvent.

Dispersive liquid–liquid microextraction (DLLME) is a miniaturized LPME that uses microliter volumes of extraction solvent [16–19]. It was initially proposed by Rezaee et al. and has been introduced to the preparation of the complex matrix samples [16]. This method is based on a ternary component solvent system in which the water-immiscible organic solvent (extractant) and watermiscible organic solvent (disperser solvent) are injected into aqueous sample. The mixture is shaken and a cloudy solution is formed in the test tube. After centrifugation, the extract is taken with a micro syringe and analyzed. The advantages of DLLME are simplicity of operation, rapidness, low cost-effectiveness, high enrichment capabilities and environmental benignity. However, volatile organic solvents were still used as the disperser solvent in the DLLME. Ionic liquids (ILs) have gained significant attention owing to their unique properties, such as negligible vapor pressure, good thermal stability, tunable viscosity and miscibility with water and organic solvents, as well as good extractability for various organic compounds and metal ions [20,21]. In addition, their chemical and physical properties can be readily adjusted by suitable selection of cation and anion species [22]. Therefore, novel "green" organic solvents have got wide application in different areas of analytical chemistry, such as catalysis and synthesis [23–26], chromatography and extraction [27,28], electrochemistry and spectrometry [29–31]. IL based ultrasound-assisted emulsification–microextraction (IL-USAEME) have been developed for the extraction of triclosan and aromatic amines residues in water samples [32]. When ultrasonic energy was applied to solutions, the submicron droplet size was formed rapidly and the contact surface between both immiscible liquids was significantly enlarged. The formation of the homogenization emulsions accelerated the mass-transfer process between the involved phases and the extraction efficiency increase in short time.

In this paper, in order to reduce the consumption of the volatile organic solvent, two kinds of nonvolatile ILs, hydrophobic IL and hydrophilic IL, were used as extraction solvent and disperser solvent, respectively [33]. This approach is based on the emulsification of hydrophobic IL in an aqueous sample by ultrasound radiation and further separation of both liquid phases by centrifugation. Therefore, ultrasound-assisted ionic liquid/ionic liquid dispersive liquid–liquid microextraction (UA-IL/IL-DLLME) was developed for the extraction and enrichment of SAs in infant formula powder.

2. Experimental

2.1. Reagents and chemicals

Sulfamerazine (SMI), sulfamethizole (SMT), sulfachlorpyridazine (SCP), sulfamonomethoxine (SMM), sulfmethoxazole (SMX) and sulfisoxazole (SIA) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). The purity of all the compounds was higher than 98.0%. The chemical structures, p*K*a value and log *Pow* of the compounds are shown in Fig. 1. All standard substances were dissolved in acetonitrile to prepare 500 μg mL^{-1} of stock solutions. The working solutions were obtained by diluting the stock solutions with acetonitrile. Chromatographic grade acetonitrile was purchased from Fisher Scientific Company (UK) and pure water was obtained with a Milli-Q water purification system (Millipore Co., USA). Formic acid, orthophosphoric acid (99%), methanol, ethanol, acetone and acetonitrile are analytical-reagent grade and purchased from Beijing Chemical Factory (Beijing, China). 1-Ethyl-3-methylimidazolium tetrafluoroborate ($[C_2MIM][BF_4]$, > 98.0% purity), 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM][BF_4]$, > 99.0% purity), 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM][PF_6]$, > 98.0% purity), 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$, > 97.0% purity), 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$, > 99.0% purity) and ammonium

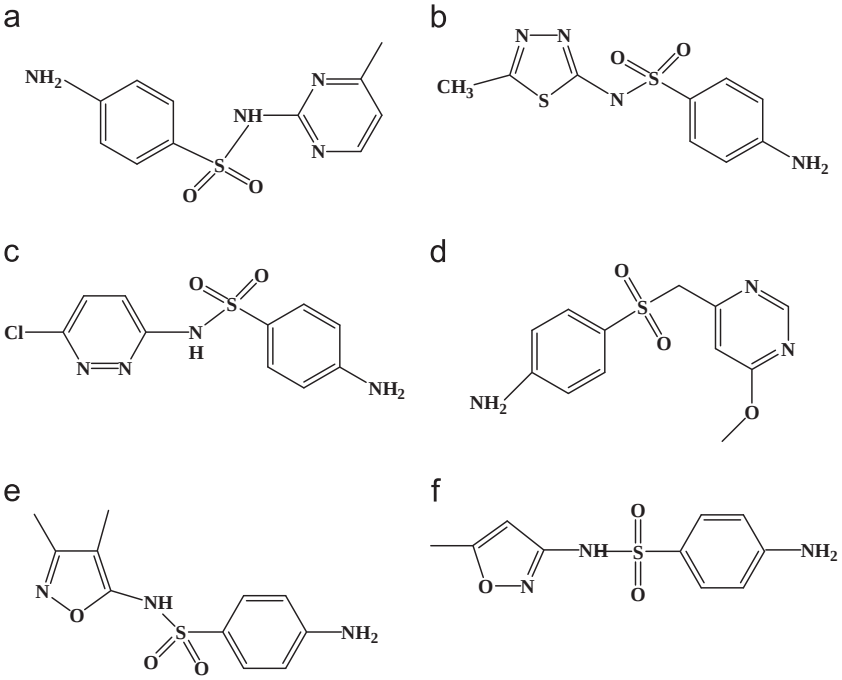

Fig. 1. Chemical structures of the analytes (a) SMI ($pK_{a1}=2.22\pm0.01$; $pK_{a2}=6.80\pm0.01$; log *Pow*=0.3); (b) SMT ($pK_{a1}=1.86\pm0.30$; $pK_{a2}=5.29\pm0.04$; log *Pow*=0.54); (c) SCP ($pK_{a1}=1.88\pm0.50$; $pK_{a2}=5.90\pm0.30$; log *Pow*=0.32); (d) SMM ($pK_{a1}=1.42$; $pK_{a2}=6.67$; log *Pow*=0.70); (e) SMX ($pK_{a1}=1.85\pm0.30$; $pK_{a2}=5.60\pm0.04$; log *Pow*=0.89); (f) SIA ($pK_{a1}=1.52$; $pK_{a2}=4.83$; log *Pow*=1.01).

hexafluorophosphate (NH_4PF_6, 98.0%) were obtained from Chengjie Chemical Co. Ltd. (Shanghai, China). The infant formula milk powders were purchased from local large-scale supermarket and stored at 4 °C.

2.2. Samples

In the study, five infant milk powder samples, including sweetened whole (sample 1), bovine colostrum (sample 2), premature infant formula (milk-based, sample 3), formula I (malt-based, sample 4) and formula II (oatmeal-based, sample 5) milk powders were analyzed. The fat in the above-mentioned samples was determined by the recommended method (GB 5413.3-2010 and 10765-2010) and the fat contents in samples 1–5 were 18.0, 1.30, 22.6, 16.0 and 8.3, respectively. Except for the experiments mentioned in Section 3.2.2, which were performed with all five samples, all other results were obtained with sample 1. The spiked samples containing SAs were prepared by spiking the working solutions into milk powder samples and stored at 4 °C for one week.

2.3. Instruments

The 1100 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with photodiode-array detector was used. Chromatographic separation of the SAs was performed on Zorbax Eclipse Plus-C_{18} column (150 mm × 4.6 mm, 3.5 μm, Agilent, USA) with a C_{18} guard column (7.5 mm × 2.1 mm I.D., 5 μm). The mobile phase consists of acetonitrile (0.1% formic acid) (A) and aqueous solution (0.1% formic acid pH = 3.0) (B). The gradient program is as follows: 0–10 min, 10%–30% A; 10–20 min, 30%–34% A; 20–21 min, 34%–35% A; 21–24 min, 35% A; 24–30 min, 35%–10% A. The flow rate of mobile phase was set at 0.5 mL min^{-1} and column temperature was kept at 40 °C. The injection volume was 10 μL. The monitoring wavelength was 270 nm. The reference wavelength and bandwidth were 360 and 4 nm, respectively. A 40 kHz, 100 W ultrasonic generator (KQ2200E Kunshan Ultrasonic Instrument Co. Ltd., Kunshan, China) was used to assist the microextraction. The phase separation was performed on high-speed freezing centrifuge (Allegra 64R, Beckman Coulter, Inc., USA).

2.4. UA-IL/IL-DLLME

The milk powder sample was weighed accurately and added into 50 °C pure water. The ratio of solid to liquid was 1:8 (g/mL). The mixture of solid and liquid was shaken for 10 min to ensure that the solid sample was dissolved completely. 4 mL of above sample solution was placed into the 5 mL polytetrafluoroethylene (PTFE) tube. 20 μL of orthophosphoric acid and 70 μL of [C_6MIM][PF_6] were added into the tube and the mixture was intensely shaken for 5 min. 100 μL of [C_4MIM][BF_4] was added into the sample solution. The mixture was ultrasonically extracted for 10 min at 30 °C. Then 0.08 g of NH_4PF_6 was added into the solution and the resulting solution was ultrasonically shaken for 2 min. The resulting solution was centrifuged at 5 °C for 10 min at 15,000 rpm and the IL phase was deposited at the bottom of the tube (Fig. S1). Then the upper aqueous phase was removed completely. The IL phase was quantitatively transferred to 1.0 mL PTFE tube by using a 50 μL syringe. Then the IL was dilute with acetonitrile containing 0.1% formic acid to 200 μL. The resulting analytical solution was homogenized ultrasonically and filtered with 0.22 μm PTFE filter membrane before HPLC analysis.

3. Results and discussion

3.1. Optimization of UA-IL/IL-DLLME

In order to obtain high extraction efficiency, the influence of experimental parameters, such as types and amount of extraction solvent, the type and volume of disperser solvent, extraction time and temperature, sample solutions pH value and amount of ion-pairing agent, was investigated.

3.1.1. Selection of extraction solvents

Characteristics of ILs, such as solubility in water, the viscosity, extraction capacity and chromatographic behavior, play a key role in influencing the recovery and enrichment factor. It was necessary to consider the relationship of the extraction capacity and the length of alkyl chain of IL [34]. Therefore, three hydrophobic ILs, including [C_4MIM][PF_6], [C_6MIM][PF_6] and [C_8MIM][PF_6], were investigated. Their solubility in water was 18.8, 7.5 and 2.0 μg L^{-1}, respectively [35–37]. According to their solubility, 140 μL [C_4MIM][PF_6], 70 μL [C_6MIM][PF_6] and 40 μL [C_8MIM][PF_6] were selected as extraction solvents in the absence of dispersion solvent. The higher extraction recoveries were obtained when 70 μL of [C_6MIM] [PF_6] was used as an extraction solvent. The results indicated that the extraction recoveries obtained with the [C_6MIM][PF_6] and [C_8MIM][PF_6] were about equal. However, there were significant interference peaks in the chromatogram when [C_8MIM][PF_6] was used as the extraction solvent. On the other hand, the recovery obtained with [C_4MIM][PF_6] was lower than those obtained with [C_6MIM][PF_6] and [C_8MIM][PF_6]. The main reason is that the solubility of [C_4MIM][PF_6] in water is higher compared to [C_6MIM][PF_6]. Based on these results, [C_6MIM][PF_6] was selected as the extraction solvent.

The effect of volume of [C_6MIM][PF_6] on extraction recoveries was also studied when the volume of dispersion solvent ([C_4MIM][BF_4]) was 100 μL. As shown in Fig. 2, the recoveries show an increase with the increase of the volume from 40 to 70 μL. The recoveries are almost unchanged when the volume increases from 70 to 90 μL. Therefore, 70 μL of [C_6MIM][PF_6] was selected in the work.

3.1.2. Selection of dispersion solvents

The main criterion for the selection of the dispersion solvent is its miscibility with the extraction solvent and aqueous solution. In addition, the type of the dispersion solvent directly influences

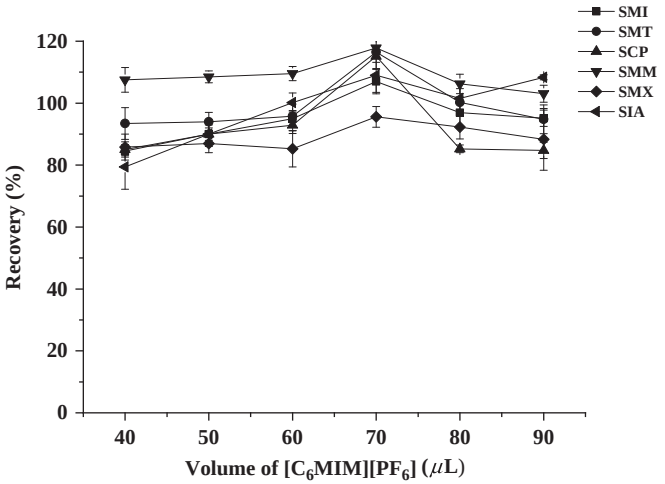

Fig. 2. Effect of [C_6MIM][PF_6] volume. Sample amount, 0.5 g; [C_4MIM][BF_4] volume, 100 μL; extraction time, 5 min.

the viscosity of the binary solvent. Thus, the reasonable selection of dispersion solvent can promote the production of droplet and improve the extraction efficiency of target analytes. The hydrophilic IL is miscible with the hydrophobic IL and water. When the hydrophilic IL was added into the aqueous solution containing hydrophobic IL, a distinct cloudy solution was formed in a short time. To study the effect of dispersion solvent, two hydrophilic ILs, including [C2MIM][BF4] and [C4MIM][BF4] and four conventional solvents, including ethanol, methanol, acetonitrile and acetone were considered. The optimal volumes of above-mentioned dispersion solvents were approximately 0.2, 0.1, 1.0, 0.7, 0.5 and 0.5 mL, respectively. The optimization of $[C_4MIM][BF_4]$ volume was shown in Fig. 3. The extraction recoveries were compared under the optimal volumes of dispersion solvents. As can be seen in Fig. 4, the difference of the recoveries obtained with 100 μL of $[C_4MIM][BF_4]$ and 500 μL of acetonitrile is not significant and the recoveries obtained with the other dispersion solvents are relatively low. On the other hand, the literature showed that the hydrophilic ILs with a short alkyl chain and $[BF_4]^-$ or Cl^- anion have relatively low toxicity [38,39]. Therefore, 100 μL was selected as the optimal volume of $[C_4MIM][BF_4]$.

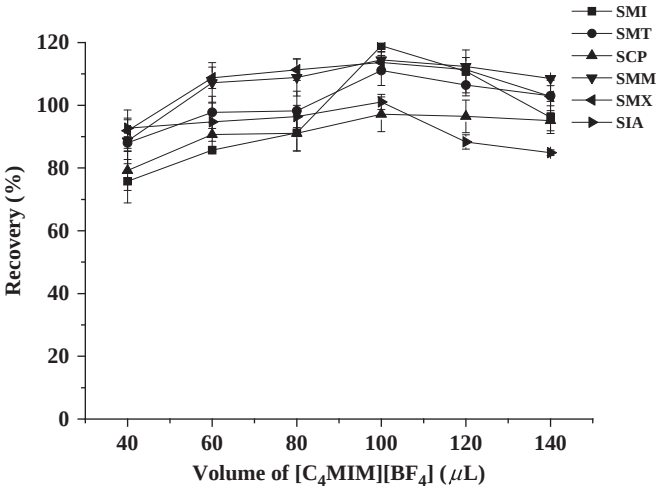

Fig. 3. Effect of $[C_4MIM][BF_4]$ volume. Volume of $[C_6MIM][PF_6]$, 70 μL; sample amount, 0.5 g; ultrasound extraction time, 5 min.

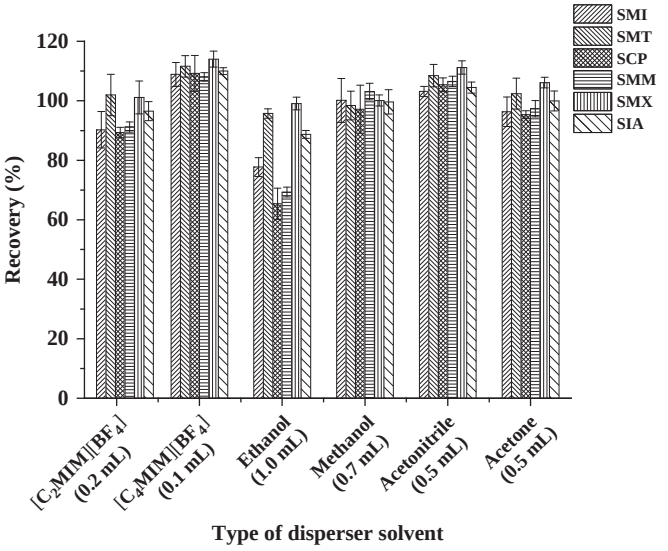

Fig. 4. Effect of dispersion solvent type. Volume of $[C_6MIM][PF_6]$, 70 μL; $[C_2MIM][BF_4]$, 0.2 mL; $[C_4MIM][BF_4]$, 0.1 mL; ethanol, 1.0 mL; methanol, 0.7 mL; acetonitrile, 0.5 mL; acetone, 0.5 mL; sample amount, 0.5 g; ultrasound extraction time, 5 min.

3.1.3. Selection of ultrasound extraction time

UA-IL/IL-DLLME is a type of equilibrium extraction, and the optimal extraction efficiency is obtained once the equilibrium is established. When the ultrasound irradiation was not applied and the sample was intensely shaken for 20 min, the extraction recoveries of the target analytes were 61.7%–80.0%. Therefore, the ultrasound irradiation was applied and the effect of ultrasonic extraction time on extraction efficiency was investigated in the range of 3–25 min (Fig. 5). The experimental results indicate that the extraction balance could be attained within 10 min and longer extraction time would not affect the extraction efficiency. It was probably because the content surface area between the $[C_6MIM][PF_6]$ and the aqueous solution was infinitely large. The extraction equilibrium can be achieved in short time and the phase-transfer of the target analytes was fast. Therefore, in further work, extraction time of 10 min was selected.

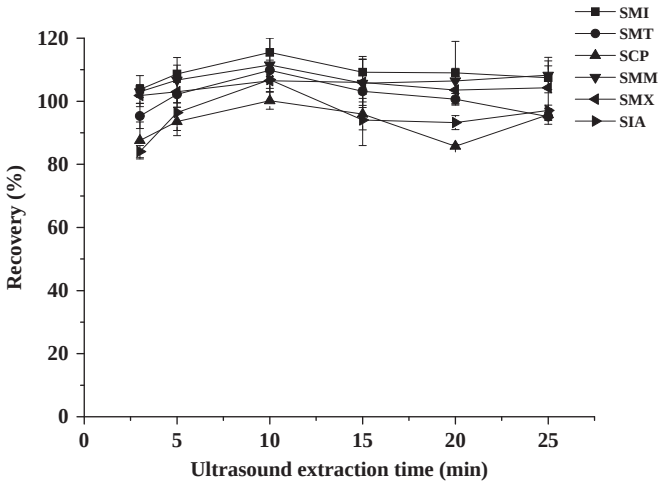

Fig. 5. Effect of ultrasound extraction time. Volume of $[C_6MIM][PF_6]$, 70 μL; $[C_4MIM][BF_4]$ volume, 100 μL; sample amount, 0.5 g.

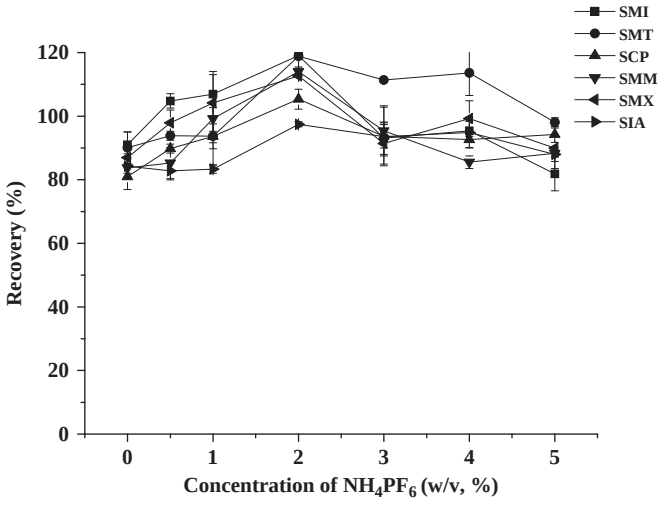

Fig. 6. Effect of NH_4PF_6 concentration. Volume of $[C_6MIM][PF_6]$, 70 μL; $[C_4MIM][BF_4]$ volume, 100 μL; sample amount, 0.5 g; ultrasound extraction time, 10 min.

3.1.4. Extraction temperature

Temperature has a significant effect on solubility and mass transfer. The effect of different temperatures on the extraction recovery was evaluated from 20 to 60 °C. The extraction recoveries increased with the increase of temperature from 20 to 30 °C, and then remaining constant up to 50 °C. The recoveries were decreased slightly when the temperature exceeds 50 °C. Therefore, the extraction temperature of 30 °C was chosen in this study.

3.1.5. Selection of pH values in sample solution

The pH value of solution can affect the ionization status and solubility of the analytes. Therefore, the effect of pH value of the sample solution in the range of 1.0–7.0 on the extraction recoveries was studied. The optimal extraction recoveries are obtained at pH 2.0. When the pH value of solution was exceeded to 2.0, the protein and fat are not deposited completely and the extraction recoveries are very low (27.8%–31.6%). At low pH, SAs would be protonated, which renders them easily soluble in the aqueous phase. On one hand, the log *Pow* (*Pow*, octanol–water partition coefficient) values of SAs are lower than 1.0, which means that the target analytes are easily soluble in water and not easily miscible with proteins and lipids [40–43]. At this condition, target analytes would easily distribute into the IL. Another interesting observation was that PO_4^{3-} can promote the phase separation between IL and aqueous solution [44]. Therefore, the pH value of 2.0 was optimal for the extraction.

3.1.6. Selection of ion-pairing agent

ILs are a class of non-molecular ionic solvents resulting from combinations of organic cations and various anions. Recently, a

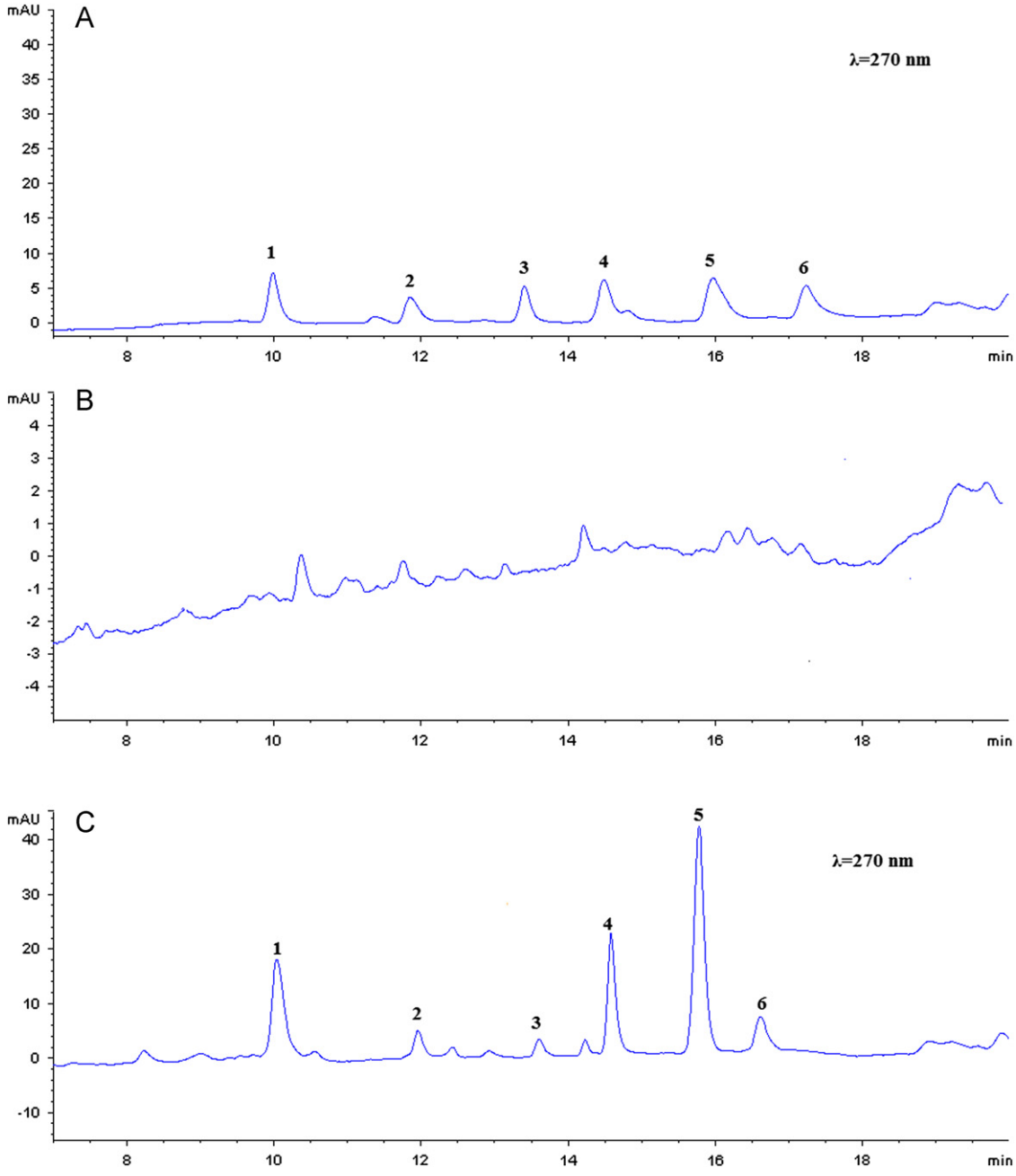

Fig. 7. Chromatograms of standard solution (A), milk powder sample (B) and spiked milk powder sample (C) 1, SMI; 2, SMT; 3, SCP; 4, SMM; 5, SMX; 6, SIA.Volume of $[C_6MIM][PF_6]$, 70 μL; $[C_4MIM][BF_4]$ volume, 100 μL; sample amount, 0.5 g; extraction time, 10 min; NH_4PF_6 concentration, 2.0%. The concentration of the analytes, 100 μg kg^{-1}.

novel microextraction technique ILs-based homogeneous liquid–liquid microextraction (IL-based HLLME) was developed for the extraction of antibiotics in the complex matrix samples [45]. In this method, a small amount of hexafluorophosphate (PF_6^-, as an ion-pairing agent) was added into the sample solution. A cloudy solution was formed as a result of formation of fine droplets of hydrophobic IL.

In this experiment, the partition equilibrium of target analytes in two-phase and the dispersion equilibrium of $[C_6MIM][PF_6]$ in aqueous solution were completed simultaneously. Considering there is residual cation ($[C_6MIM]^+$ and $[C_4MIM]^+$) in aqueous phase, the ion-pairing agent PF_6^- was introduced in order to deposit the residual cation. Thus the extraction recoveries of target analytes increase effectively.

The effect of NH_4PF_6 was investigated in the range of 0%–5% and the results are shown in Fig. 6. The extraction recoveries increase with the increase of amount of NH_4PF_6 from 0% to 2%, and then decrease slightly when the amount of NH_4PF_6 is higher the 2.0%. PF_6^- is beneficial to the formation of $[C_6MIM][PF_6]$ and the amount of sedimentary IL phase increases. Meanwhile, the addition of salt can promote the phase separation successfully. However, the amount of IL phase decreased when the amount of NH_4PF_6 exceeded 2.0%. The reason may be that $[C_6MIM][PF_6]_2^-$ can be formed and dissolved in the aqueous phase. Therefore, the content of ion-pairing agent NH_4PF_6 selected was 2.0%.

3.2. Analytical performances

The working curves were constructed by plotting the peak areas measured versus the concentrations of analytes in the spiked samples. The milk powder sample 1 was used for preparing the working curves. As shown in Fig. 7(B), the SAs in sample 1 were undetectable and there were not interference peaks in the chromatogram of sample 1. Therefore, sample 1 could be used for the method validation. The slope and intercept of the linear regression equations, the residual standard deviations (*Sy/x*) and correlation coefficients are listed in Table 1. The limits of detection (LODs) and quantification (LOQs) indicated in Table 1 are determined as the lowest concentration yielding a signal-to-noise (*S/N*) ratio of 3 and 10, respectively. The concentrations of the target analytes in the extract are higher than the LOQs and lower than upper limits of determination for the present method. So the LOQs and linear regression equations are appropriate to the goal of the proposed method. However, the sum of the LOQ values for the six antibiotics is higher than the MRL of total antibiotics in milk (100 ng g^{-1}). When the antibiotics coexist in a sample the present method is limited.

Repeatability was evaluated by determining target analytes in spiked milk powder samples. Precision was evaluated by measuring relative standard deviations of intra- and inter-day tests. The intra-day precision was determined by analyzing the samples in five replicates in one day. The inter-day precision was achieved by analyzing the samples once a day in five consecutive days. The results are presented in Table 1 and the results indicate that the present method has good repeatability.

Long-term stability of the analytes in infant formula milk powder during sample storage was evaluated. The spiked samples were prepared according to the method mentioned in Section 2.2. All experiments were performed in five replicates. The results shown in Table 2 indicate that the SAs in milk powder samples are stable for the period of eight weeks when stored in 4 °C. The recovery can be calculated as follows:

$$\text{Recovery} = \frac{\text{Change in the amount of measured analyte}}{\text{Amount of analyte spiked into the sample}} \times 100\%$$

The recoveries and RSD values range from 92.8% to 116.1% and 0.7% to 7.5%, respectively. It can be concluded that the SAs in the milk powder samples were stable for at least two months.

Table 1
Regression equations, LODs and LOQs.

Compound	Regression equation ($n=5$)	Correlation coefficient	Linear range (μg kg^{-1})	*Sy/x*	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Intra day[a] precision (RSD, %, $n=5$)	Inter day[a] precision(RSD, %, $n=5$)
SMI	$A=(-2.06\pm1.05^b)+(0.46\pm0.005^c)c$	0.9996	15.0–396.0	1.61	2.94	9.8	2.2	2.0
SMT	$A=(0.71\pm5.84^b)+(0.08\pm0.02^c)c$	0.9987	32.9–432.0	6.80	9.26	30.9	3.5	5.6
SCP	$A=(-0.87\pm0.45^b)+(0.10\pm0.002^c)c$	0.9992	55.6–556.0	0.55	16.7	55.7	2.2	3.4
SMM	$A=(-1.76\pm1.65^b)+(0.37\pm0.007^c)c$	0.9990	19.6–466.0	2.54	5.28	17.6	2.2	4.9
SMX	$A=(0.35\pm0.59^b)+(0.90\pm0.003^c)c$	0.9999	12.1–442.8	0.92	3.35	11.2	2.0	1.0
SIA	$A=(-0.70\pm1.41^b)+(0.20\pm0.006^c)c$	0.9993	26.3–448.8	2.17	6.66	22.2	1.0	4.1

[a] Assays at 100 μg kg^{-1}.
[b] Standard deviation of intercept.
[c] Standard deviation of slope.

Table 2
The recoveries of the analytes in spiked sample 1.

Added (SMI, SMT, SCP, SMM, SMX, SIA, μg kg^{-1})	Stored time (weeks)	SMI		SMT		SCP		SMM		SMX		SIA	
		Recovery (%)	RSD (%, $n=5$)	Recovery (%)	RSD (%, $n=5$)	Recovery (%)	RSD (%, $n=5$)	Recovery (%)	RSD (%, $n=5$)	Recovery (%)	RSD (%, $n=5$)	Recovery (%)	RSD (%, $n=5$)
100, 100, 100, 100, 100, 100	1	94.1	2.3	101.5	4.2	95.8	2.5	100.4	2.4	99.3	5.0	109.8	6.1
	2	96.2	1.8	100.2	2.2	97.6	5.3	102.1	6.4	98.0	2.1	106.4	2.9
	4	104.8	3.5	116.1	6.4	95.5	3.5	101.3	6.8	98.4	3.5	107.0	2.8
	6	100.2	5.7	96.3	1.6	100.0	3.6	106.9	2.5	100.0	3.8	103.3	3.8
	8	95.3	5.1	97.8	5.0	96.7	2.9	101.2	2.0	95.8	2.2	101.1	3.6
150, 150, 150, 150, 150, 150	1	106.8	0.7	98.5	1.7	101.1	7.3	107.4	4.3	99.7	4.2	97.7	4.2
	2	108.6	5.2	97.7	2.5	105.4	7.1	92.8	1.8	96.4	6.6	102.3	7.5
	4	106.1	3.0	100.1	4.8	102.6	1.7	100.8	4.0	98.0	7.1	92.9	5.0
	6	95.6	3.8	104.0	4.1	99.0	3.2	99.7	3.1	103.0	1.9	97.9	3.9
	8	99.7	7.2	98.0	2.5	98.2	5.4	100.1	3.6	101.1	2.8	100.2	2.2

Table 3
Analytical results of milk powder samples ($n=5$).

Sample	Added (SMI, SMT, SCP, SMM, SMX, SIA, μg kg^{-1})	SMI		SMT		SCP		SMM		SMX		SIA	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Sample 1	15, 35, 60, 20, 15, 30	99.4	4.4	86.3	1.8	92.5	2.1	95.5	1.9	89.9	3.1	95.3	3.8
	100, 100, 100, 100, 100, 100	100.3	3.8	98.7	7.0	111.3	4.6	106.7	3.7	105.1	2.4	98.7	3.5
	150, 150, 150, 150, 150, 150	105.3	4.7	101.7	6.5	105.2	5.8	97.6	2.9	100.5	7.6	107.3	2.3
Sample 2	15, 35, 60, 20, 15, 30	104.3	4.9	95.9	4.3	94.2	5.0	97.9	5.9	91.7	3.8	99.2	5.8
	100, 100, 100, 100, 100, 100	98.5	7.2	95.8	3.0	106.3	4.3	98.2	1.8	103.1	2.6	95.4	1.6
	150, 150, 150, 150, 150, 150	114.8	1.9	106.2	2.0	104.5	4.7	103.9	3.5	100.7	6.2	96.5	4.2
Sample 3	15, 35, 60, 20, 15, 30	96.2	4.3	100.7	4.0	101.4	3.5	105.0	4.7	98.9	2.8	100.7	6.6
	100, 100, 100, 100, 100, 100	98.3	2.5	104.7	1.9	103.5	2.2	98.9	2.3	103.6	4.6	101.6	2.9
	150, 150, 150, 150, 150, 150	102.1	3.6	113.1	3.4	116.9	2.1	105.7	4.1	105.8	1.5	96.2	4.2
Sample 4	15, 35, 60, 20, 15, 30	97.0	2.0	98.3	3.1	100.9	2.2	109.0	1.7	100.2	2.6	105.7	4.7
	100, 100, 100, 100, 100, 100	97.9	0.7	103.9	3.7	101.7	2.0	105.9	4.4	105.5	3.8	101.3	1.4
	150, 150, 150, 150, 150, 150	103.0	5.0	105.9	3.8	103.1	4.6	104.6	5.8	97.5	3.3	93.1	1.6
Sample 5	15, 35, 60, 20, 15, 30	98.7	4.4	96.8	3.6	96.6	3.8	99.7	2.8	104.2	3.6	97.7	3.0
	100, 100, 100, 100, 100, 100	104.6	5.5	97.6	3.1	101.4	4.7	108.1	1.7	98.5	3.9	90.4	4.9
	150, 150, 150, 150, 150, 150	94.6	5.9	101.7	4.2	105.2	2.4	109.7	7.8	96.5	4.1	100.2	6.0

Table 4
Comparison of UA-IL/IL-DLLME with other methods.

Extraction method	Extraction mode	Amount of $[C_6MIM][PF_6]$ (μL)	Amount of dispersion (mL)	Temperature (°C)	pH	Extraction time (min)	Amount of salt (%, w/v)	References
IL-HLLME	Ultrasound	70	na.	25	2.0	10	10.0, NH_4PF_6	[45]
IL-UAEME	Ultrasound	70	na.	30	2.0	15	11.0, NaCl	[46]
IL-TC-DLLME	Microwave	60	na.	30	2.0	5	8.0, NaCl	[47]
UA-IL/IL-DLLME	Ultrasound	70	0.1, $[C_4MIM][BF_4]$	30	2.0	10	2.0, NH_4PF_6	This work

3.2.1. Matrix effect

The many highly molecular compounds present in the milk powder samples can influence the chromatographic signal of the target analytes. Therefore, a statistical comparison between the standard curve and working curve should be made. The Student's test was applied and the statistical analysis indicated that the difference between the slope of working curve and standard curve is significant ($P<0.05$). The result indicates that the matrix effect exists and the quantitative determinations should be carried out by the working curve procedure, especially standard addition procedure.

3.2.2. Analysis of samples

In order to evaluate the applicability of the present method, this method was applied to the determination of the residues of the SAs in some milk powder samples. The SAs in the spiked samples were determined and the results are listed in Table 3. As can be seen, the present method provides good recoveries (86.3%–116.9%) and acceptable precision ($\leq$7.8%). The chromatograms of the standard solution, milk powder sample and spiked milk powder sample are shown in Fig. 7. The results shown in Fig. 7 indicate that the reproducibility of the retention time should be satisfactory.

3.2.3. Comparison of UA-IL/IL-DLLME with other methods

The present method was compared with the IL-HLLME, IL-UAEME and IL-TC (temperature-controlled)–DLLME. The extraction parameters that affect extraction efficiency for IL-HLLME, IL-UAEME and IL-TC-DLLME were evaluated in this work. The optimal extraction parameters are shown in Table 4. The extraction recoveries of target analytes obtained by the reference methods are shown in Fig. 8. The results indicate that the present method achieve higher extraction efficiency than the other methods. Therefore, UA-IL/IL-DLLME-HPLC is proposed for the simultaneous extraction and determination of SAs in dairy products.

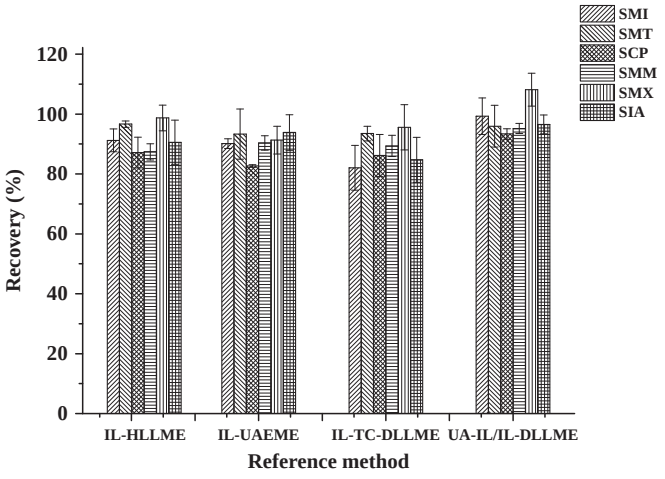

Fig. 8. Extraction recoveries of target analytes obtained by IL-HLLME, IL-UAEME, IL-TC-DLLME, and UA-IL/IL-DLLME.

4. Conclusions

The UA-IL/IL-DLLME was successfully applied to the extraction of the SAs from milk powder samples. The recovery of IL phase increases because of the introduction of the ion-pairing agent. So

it seems possible to extend this method to the extraction of SAs in other similar samples by varying the extraction conditions.

Acknowledgment

This work was supported by Program for New Century Excellent Talents in University (no. NECT-10-0443) and Science and Technology Developing Foundation of Jilin Province (no. 20100356).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.07.050.

References

[1] S. Thiele-Bruhn, J. Plant Nutr. Soil Sci. 166 (2003) 145–167.
[2] M.J. García-Galán, M.S. Dáaz-Cruz, D. Barceló, Trends Anal. Chem. 28 (2009) 804–819.
[3] M.X. Zhou, Chin. J. Vet. Drug 37 (2003) 15.
[4] Proceedings of the National Conference on Interstate Milk Shipments NCIMS, M-I-05-06, Frankfort, KY, USA, 27 September 2005.
[5] Z.X. Cai, Y. Zhang, H.F. Pan, X.W. Tie, Y.P. Ren, Ionic liquid in Synthesis. J. Chromatogr. A 1200 (2008) 144–155.
[6] European Commission, Council Directive 178/2002, Off. J. Eur. Commun. L 031 (2002) 1.
[7] L. Alban, E.O. Nielsen, J. Dahl, Prev. Vet. Med. 83 (2008) 115–129.
[8] FDA, New Guidance for Industry on Antimicrobial Drugs for Food Animals. GFI#152. Released October 23, US Food and Drug Administration, 2003, p. 35.
[9] 〈http://www.gazette.gc.ca/rp-pr/p1/2011/2011-02-26/html/reg6-eng.html〉.
[10] G.Z. Fang, J.X. He, S. Wang, J. Chromatogr. A 1127 (2006) 12–17.
[11] J.G. Huang, J.J. Liu, C. Zhang, J.J. Wei, L. Mei, S. Yu, G. Li, L. Xu, J. Chromatogr. A 1219 (2012) 66–74.
[12] C. Huertas, J. Morillo, J. Usero, I. Gracia-Manarillo, Talanta 72 (2007) 1149–1156.
[13] C.H. Yu, B. Hu, Talanta 90 (2012) 77–84.
[14] C.Y. Lin, S.D. Huang, Anal. Chim. Acta. 612 (2008) 37–43.
[15] M.R. Payán, M.A.B. López, R. Fernandez-Torres, M.V. Navarro, M.C. Mochón, J. Chromatogr. B 879 (2011) 197–204.
[16] M. Rezaee, Y. Assadi, M.R.M. Hosseini, E. Aghaee, F. Ahmadia, S. Berijani, J. Chromatogr. A 1116 (2006) 1–9.
[17] M.A. Farajzadeh, M. Bahram, J.A. Jönsson, Anal. Chim. Acta. 591 (2007) 69–79.
[18] M. Rezaee, Y. Yamini, S. Shariati, A. Esrafili, M. Shamsipur, J. Chromatogr. A 1216 (2009) 1511–1514.
[19] S. Berijani, Y. Assadi, M. Anbia, M.R. Milani Hosseini, E. Aghaee, J. Chromatogr. A 1123 (2006) 1–9.
[20] C.F. Poole, J. Chromatogr. A 1037 (2004) 49.
[21] J.F. Liu, J.A. Jonsson, G.b. Jiang, Trends Anal. Chem. 24 (2005) 20–27.
[22] D.B. Zhao, Y.C. Liao, Z.D. Zhang, Clean 35 (2007) 42–48.
[23] P. Wasserscheid, T. Welton, Wiley-VCH Verlag, Weinheim, 2003.
[24] V.I. Parvulescu, C. Hardacre, Chem. Rev. 107 (2007) 2615–2665.
[25] A. Paczal, A. Kotschy, Monatsh. Chem. 138 (2007) 1115–1123.
[26] Z.C. Zhang, Adv. Catal. 49 (2006) 153–237.
[27] Aurora Martín-Calero, Verónica Pino, H.Juan Ayala, Venerando González, Ana M. Afonso, Talanta 79 (2009) 590–597.
[28] M. Bian, Z.J. Zhang, H. Yin, J. Pharmaceut Biomed. Anal. 58 (2012) 163–167.
[29] A. Chagnes, A. Diaw, B. Carre, P. Willmann, D. Lemordant, J. Power Sources 145 (2005) 82–88.
[30] A. Diaw, A. Chagnes, B. Carre, P. Willmann, D. Lemordant, J. Power Sources 146 (2005) 682–684.
[31] M. Taggougui, M. Diaw, B. Carre, P. Willmann, D. Lemordant, Electrochim. Acta 53 (2008) 5496–5502.
[32] N. Cabaleiro, F. Pena-Pereira, I. de la Calle, C. Bendicho, I. Lavilla, Microchem. J 99 (2011) 246–251.
[33] R.S. Zhao, X. Wang, F.W. Li, S.S. Wang, L.L. Zhang, C.G. Cheng, J. Sep. Sci. 34 (2011) 830–836.
[34] W.Y. Ma, Y.B. Lu, R.L. Hu, J.H. Chen, Z.Z. Zhang, Y.J. Pan, Talanta 80 (2010) 1292–1297.
[35] J.G. Huddleston, A.E. Visser, W.M. Reichert, H.D. Willauer, G.A. Broker, R.D. Rogers, Green Chem. 3 (2001) 156–164.
[36] S.K. Chun, S.V. Dzyuba, R.A. Bartsh, Anal. Chem. 73 (2001) 3737–3741.
[37] J.F. Liu, N. Li, G.B. Jiang, Y.G. Chi, Y.Q. Cai, Q.X. Zhou, J.T. Hu, Anal. Chem. 75 (2003) 5870–5876.
[38] S. Viboud, N. Papaiconomou, A. Cortesi, G. Chatel, M. Draye, D. Fontvieille, J. Hazard. Mater. 215–216 (2012) 40–48.
[39] J.S. Torrecilla, J. García, E. Rojo, F. Rodríguez, J. Hazard. Mater. 164 (2009) 182–194.
[40] J.Y. Pailler, A. Krein, L. Pfister, L. Hoffmann, C. Guignard, Sci. Total Environ. 407 (2009) 4736–4743.
[41] W.M. Meylan, P.H. Howard, J. Pharm. Sci. 84 (1995) 83–92.
[42] S. Mompelat, B. Le Bot, O. Thomas, Environ. Int. 35 (2009) 803–814.
[43] P.K. Jjemba, Pharma-Ecology, John Wiley & Sons, Inc., 2008, p. 47.
[44] S.H. Li, C.Y. He, H.W. Liu, K.A. Li, F. Liu, J. Chromatogr. B 826 (2005) 58–62.
[45] S.Q. Gao, H.Y. Jin, J.Y. You, Y. D., N. Zhang, Y. Wang, R.B. Ren, R. Zhang, H.Q. Zhang, J. Chromatogr. A 1218 (2011) 7254–7263.
[46] J.N. Sun, Y.P. Shi, J. Chen, J. Chromatogr. B 879 (2011) 3429–3433.
[47] S.Q. Gao, J.Y. You, X. Zheng, Y. Wang, R.B. Ren, R. Zhang, Y.P. Bai, H.Q. Zhang, Talanta 82 (2010) 137101377.