Ionic liquid-based foam flotation followed by solid phase extraction to determine triazine herbicides in corn

Liyuan Zhang [a,b], Zhibing Wang [b], Na Li [b], Aimin Yu [b], Hanqi Zhang [b,*]

[a] College of Food, Heilongjiang Bayi Agricultural University, Xinfeng Lu 5, Daqing 163319, PR China
[b] College of Chemistry, Jilin University, Qianjin Street 2699, Changchun 130012, PR China

ABSTRACT

Ionic liquid-based foam flotation followed by solid phase extraction was developed for extraction of eight triazine herbicides, including cyanazine, metribuzin, desmetryn, secbumeton, terbumeton, terbuthylazine, dimethametryn and dipropetryn in corn samples. When the extraction was completed, the analytes were separated from the sample matrix by foam flotation and introduced into the solid phase extraction cartridge. The separation and concentration of target analytes were performed simultaneously. The analytes eluted from the solid phase extraction cartridge were determined by high performance liquid chromatography. The effects of experimental parameters of extraction and flotation were examined. Under the optimized experimental conditions, a good linearity was obtained in the range of 2.50–150 μg/kg. The detection limits of the triazine herbicides were in the range of 0.74–1.62 μg/kg. The present method was successfully applied to the analysis of corn samples. The recoveries ranged from 85.9% to 104.0% and the relative standard deviations were lower than 5.6%.

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1. Introduction

The triazine herbicides have been used widely throughout the world [1–3]. However, a large number of present researches have proven the toxicity of triazine herbicides [4–6]. On one hand, the triazine herbicides have been suspected to cause endocrine disruption, give rise to cancer and congenital defect and so on [7–10]. On the other hand, triazine herbicides and their metabolic degradation products could contaminate crop themselves and their surroundings environment, such as soil and water [11,12]. Therefore, the harm of triazine herbicides to humans and environment could not be ignored.

Many countries have formulated maximum residue limits (MRLs) of the triazine herbicides [13–15]. In the European Union (EU), MRLs for pesticides in corn, vegetable and other agriculture products are provided in several statutes (EU directive 2002/32/EC, Regulation (EC) No. 396/2005, Commission Directive 2008/149/EC and Commission Regulation (EU) No. 212/2013) and the MRLs of eight triazine herbicides were ranged from 0.05 to 0.1 mg/kg. The Environmental Protection Agency (EPA) requires that MRLs of triazine herbicides are 0.25 mg/kg for most agriculture products. The MRLs were used as an important technical barrier in import

and export of agricultural products. Therefore, effective control and detection of triazine herbicide residues are very important.

The extraction of triazine herbicides from a variety of matrices can be carried out by solid phase extraction (water, rice and corn, honey) [16–18], solid phase micro-extraction (water, soil) [19,20], magnetic solid phase extraction (water) [21], molecularly imprinted solid phase extraction (water, rice and onion) [12], liquid–liquid extraction (food) [22], microwave-assisted extraction (infant nutrient cereal-based foods) [23], matrix solid phase dispersion (milk) [24] and pressurized liquid extraction (potato, carrot, lettuce, zucchini, runner beans, oranges and wheat) [25]. Generally, high performance liquid chromatography (HPLC) [26] was applied to the determination of these polar and thermally stable herbicides. To our knowledge, the HPLC using reversed phase columns was widely used. In addition, gas chromatography [27], liquid chromatography–mass spectrometry [28], gas chromatography–mass spectrometry [29] and micellar electrokinetic capillary chromatography [30] were used to determine herbicide residues.

Ionic liquids (ILs) are semi-organic molten salts, which consist of organic cations and various anions. Because of their low vapor pressure, non-flammability, low melting point, good thermal stability and ability to dissolve various organic and inorganic substances, ILs have been widely used as extraction solvents in the recent years [31–33]. The IL-based aqueous two-phase system was widely applied for purification, extraction and biocatalysis [34]. The foam flotation (FF) based on IL was recently reported [35–37]. The FF coupled with solid phase extraction (SPE) was

* Corresponding author. Tel.: +86 431 85168399; fax: +86 431 85112355.
E-mail address: analchem@jlu.edu.cn (H. Zhang).

successful applied to the extraction, separation and enrichment of steroid hormones in water samples [35] and ginsenosides in extract of *Panax quinquefolius* L. [36]. The ionic liquid foaming-based solvent flotation was applied to the extraction of herbicides in yogurt [37]. In these researches, the foaming property of ILs was found and the factors that can influence foamability of the ILs were investigated. The FF based on ILs can be applied to the extraction, separation and concentration of analytes.

In this paper, IL-based FFSPE was applied for extraction of eight triazine herbicides, including cyanazine, metribuzin, desmetryn, secbumeton, terbumeton, terbuthylazine, dimethametryn and dipropetryn from corn samples. As far as we know, studies on extraction and detection of triazine herbicides mainly focused on the environmental samples and studies on the corn samples were rare. HPLC was applied to the determination eight triazine herbicides. ILs were used as the extraction solvents and foaming agents. The effect of experimental parameters were investigated and optimized.

2. Experimental

2.1. Chemicals and reagents

Cyanazine, metribuzin, desmetryn, secbumeton, terbumeton, terbuthylazine, dimethametryn and dipropetryn were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 1-Ethyl-3-methylimidazolium tetrafluoroborate ([C$_2$MIM][BF$_4$], > 98.0% purity), 1-butyl-3-methylimidazolium tetrafluoroborate ([C$_4$MIM][BF$_4$], > 99.0% purity), 1-hexyl-3-methylimidazolium tetrafluoroborate ([C$_6$MIM][BF$_4$], > 98.0% purity), 1-octyl-3-methylimidazolium tetrafluoroborate ([C$_8$MIM][BF$_4$], > 99.0% purity), 1-ethyl-3-methylimidazolium hexafluorophosphate ([C$_2$MIM][PF$_6$], > 98.0% purity), 1-butyl-3-methylimidazolium hexafluorophosphate ([C$_4$MIM][PF$_6$], > 98.0% purity), 1-hexyl-3-methylimidazolium hexafluorophosphate ([C$_6$MIM][PF$_6$], > 98.0% purity), 1-octyl-3-methylimidazolium hexafluorophosphate ([C$_8$MIM][PF$_6$], > 97.0% purity) and 1-hexyl-3-methylimidazolium chloride ([C$_6$MIM]Cl, 98.0%) were obtained from Chengjie Chemical Co. Ltd. (Shanghai, China). Methanol and acetonitrile of chromatographic grade were purchased from Dikma Technologies Inc. (USA). All other reagents of analytical grade were purchased from Beijing Chemical Factory (Beijing, China). Pure water was obtained with a Milli-Q water purification system (Millipore Co., USA).

Standard stock solutions for the triazine herbicides at the concentration level of 500 μg/mL were prepared in methanol and standard working solutions were prepared every week by diluting the standard stock solutions with methanol. Mixed standard working solutions at different concentrations were prepared by the same method as the standard working solutions. All the standard and working solutions were stored at 4 °C and protected from light.

2.2. Material and instrumentation

The 1200 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with multi-wavelength detector was used. Chromatographic separation of target analytes was performed on Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm i.d., 3.5 μm, Agilent, USA). The column temperature was kept at 30 °C and sample injection volume was 20 μL. The mobile phases A and B were water and acetonitrile, respectively. The gradient conditions are as follows: 0–5 min, 40–60% B; 5–10 min, 60–80% B; 10–15 min, 80–80% B; 15–25 min, 80–40% B. The flow rate of the mobile phase was 0.50 mL/min. The detection of the analytes was carried out at the
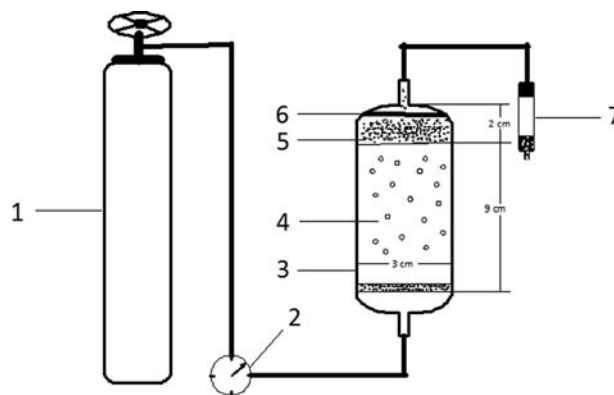

Fig. 1. The IL-based FFSPE system. (1) Nitrogen cylinder; (2) flowmeter, (3) flotation vessel; (4) sample solution; (5) foam; (6) glass cotton; (7) SPE cartridge.

wavelength of 228 nm. The reference wavelength and bandwidth were 360 nm and 4 nm, respectively.

The IL-based FFSPE system was assembled in our laboratory. A schematic diagram of the system is shown in Fig. 1. The glass flotation vessel is cylindrical in shape. The diameter and height are 3 cm and 11 cm, respectively. A 40 kHz, 100 W ultrasonic cleaner (KQ2200E Kunshan Ultrasonic Instrument Co. Ltd., Kunshan, China) was used in an extraction procedure. HR-2870 Philips mill (Philips, Zhuhai, China), CL-2 magnetic stirrer (Great wall, Zhengzhou, China) and RE-52AA vacuum rotatory evaporator (Yarong, Shanghai, China) were used.

Oasis HLB (3 mL, 100 mg) and MCX (3 mL, 100 mg) extraction cartridges were purchased from Waters (Milford, MA, USA). SPE cartridges, including SuperClean Al$_2$O$_3$ (3 mL, 100 mg), SuperClean C18 (3 mL, 100 mg), and SuperClean Si (3 mL, 100 mg), were purchased from Supelco (Bellefonte, PA, USA).

2.3. Sample preparation

Corn samples, including white waxy corn (Sample 1), yellow waxy corn (Sample 2), purple waxy corn (Sample 3) and color waxy corn (Sample 4) were purchased from local supermarkets in Daqing, Heilongjiang Province, in November 2012. All corn samples were pulverized using a HR-2870 Philips mill and passed through a 80-mesh sieve to obtain the powered samples. Except for the experiments mentioned in Section 3.2.3, which were performed with all four samples, all other experiments were performed with Sample 1. The fresh spiked samples containing triazine herbicides were prepared by spiking the stock standard solutions into milled corn mentioned and shaking for 3 min. To ensure the herbicides to be well distributed, a reasonable amount of acetone was added to moisten the milled corn and careful agitation was performed followed by an air-drying for 24 h at room temperature before extraction.

The aged spiked sample was prepared by the same method as mentioned above. Then the samples were kept in sealed bottle and stored for 1, 7, 14, 28, 56 and 70 days at 4 °C. All samples were refrigerated at 4 °C and stored at room temperature before use.

2.4. IL-based FFSPE

The IL-based FFSPE system is shown in Fig. 1. 10.0 g of corn sample, 30.0 μL of IL and 50.0 mL of water were placed into the polytetrafluoroethylene (PTFE) tube and blended with the CL-2 magnetic stirrer at 2400 rpm for 3.0 min to obtain a homogeneous mixture. The tube was immersed into the ultrasonic bath for 10.0 min at room temperature. After the extraction was completed, the mixture in the PTFE tube was transferred to a flotation vessel.

The PTFE tube was washed three times with 10 mL of water to collect the rests of extract and the washing water was also transferred to the flotation vessel. Meanwhile, the Oasis MCX SPE cartridge was conditioned by sequential washing with 4.00 mL of acetone and 2.00 mL of dichloromethane. Subsequently, the FF system and SPE system were connected as shown in Fig. 1. The carrier gas was passed through the mixture. The flow rate of the carrier gas was 500 mL/min. The foam flotation time was 10.0 min. The resulting foam was passed through the glass cotton, where the solid particles were not passed through, and introduced into the SPE cartridge. The foam is basically composed of the IL.

The analytes in the sample were diverted from the mixture to the SPE cartridge and then adsorbed onto the cartridge. The SPE cartridge was washed with 2.00 mL of dichloromethane at the flow rate of 2.00 mL/min for 1.00 min and the carrier gas N_2 was passed through the cartridge until all the dichloromethane was removed. The SPE cartridge was then eluted with 1.00 mL 5% ammonia methanol at the flow rate of 0.08 mL/min. The eluate was collected in a conical evaporating flask and evaporated to dryness at 35 °C in a vacuum rotary evaporator. Finally, the residue was dissolved in 0.20 mL methanol and then filtered through a 0.22 μm filter membrane. All samples were analyzed soon after preparation.

3. Results and discussion

3.1. Optimization of IL-based FFSPE

In the optimization of the experimental conditions, all the experiments were carried out in triplicate.

3.1.1. The effect of ionic liquid type

The selection of an appropriate extraction solvent is extremely important for the IL-based FFSPE. IL was used as a foaming agent and an extraction solvent. In our preliminary experiment, [C_2MIM][BF_4], [C_4MIM][BF_4], [C_6MIM][BF_4], [C_8MIM][BF_4], [C_2MIM][PF_6], [C_4MIM][PF_6], [C_6MIM][PF_6], [C_8MIM][PF_6] and [C_6MIM]Cl were used to examine the foaming ability. However, [C_2MIM][BF_4], [C_4MIM][BF_4], [C_2MIM][PF_6] and [C_4MIM][PF_6] have lower foaming abilities than the other ILs. The reason is mainly that foaming ability of ILs increases with the increase of alkyl chain length (n) at the 1-position of the cation. So the effect of [C_6MIM][BF_4], [C_8MIM][BF_4], [C_6MIM][PF_6], [C_8MIM][PF_6] and [C_6MIM]Cl on the recoveries was investigated. As can be seen in Fig. 2, the highest recoveries are obtained when [C_8MIM][BF_4] is used as the foaming agent and the extraction solvent. Therefore, [C_8MIM][BF_4] was selected in the work.

The effect of volume of [C_8MIM][BF_4] was also studied. As can be seen in Fig. 3, the recoveries increase with the increase of the volume from 10.0 to 30.0 μL and are almost unchanged when the volume ranges from 30.0 to 50.0 μL. So, 30.0 μL was selected.

3.1.2. The effect of extraction time

When the samples were intensely shaken only for a period of time without ultrasound irradiation, the recoveries of the target analytes were low. The ultrasound-assisted extraction was applied. Fig. 4 shows the change in the extraction recoveries of eight triazine herbicides versus extraction time. The results indicate that ultrasound-assisted extraction can result in high recoveries. In this work, ultrasound-assisted extraction time 10.0 min was necessary. Hence, 10.0 min was chosen as the optimal extraction time.

3.1.3. The effect of sample solution pH value

The triazine herbicides in the weak alkaline medium are stable. In the strong alkaline and acid conditions the foaming rate was too
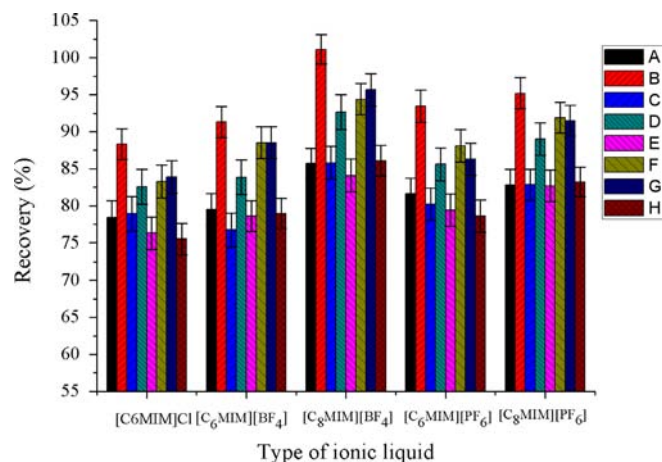

Fig. 2. Effect of ionic liquid type on the recoveries of the analytes. Volume of [C_8MIM][BF_4], 30 μL; extraction time, 10 min; pH value of sample solution, 9; flow rate of carrier gas, 500 mL/min; flotation time, 10 min; SPE cartridges, MCX (3 mL, 100 mg); washing solvent, dichloromethane; elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.

Fig. 3. Effect of volume of [C_8MIM][BF_4] on the recoveries of the analytes. Ionic liquid type, [C_8MIM][BF_4]; extraction time, 10 min; pH value of sample solution, 9; flow rate of carrier gas, 500 mL/min; flotation time, 10 min; SPE cartridges, MCX (3 mL, 100 mg); washing solvent, dichloromethane; elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.
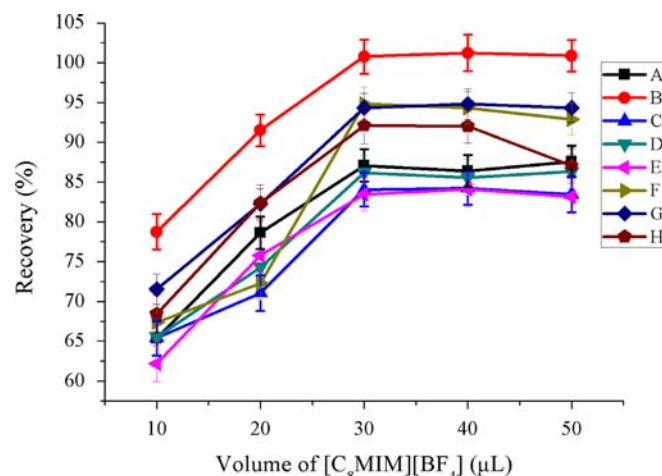
rapid, so that the retention time of foam in the sample solution was shortened and the loading capacity of IL foam was limited. Most of the analytes can be ionized at too high pH values, which can affect the recoveries and stability of the analytes. Therefore, in the present study, the effect of pH values ranging from 5.00 to 11.0 was investigated (Fig. 5). An increase of recoveries is observed when the pH increases from 5.00 to 9.00 and the recoveries slightly decrease when the pH increases from 9.00 to 11.00. The pH value of the sample solution was chosen as 9.00 in the experiment.

3.1.4. The effect of carrier gas flow rate

In the process of foam flotation, target analytes are adsorbed on the surface of bubbles in the form of a monolayer and the formed foam amount and flotation efficiency are related to the flow rate of the carrier gas [38]. In this experiment, N_2 was used as a carrier gas and the effect of flow rate was investigated. The experimental results are shown in Fig. 6. When the flow rate is low, very less
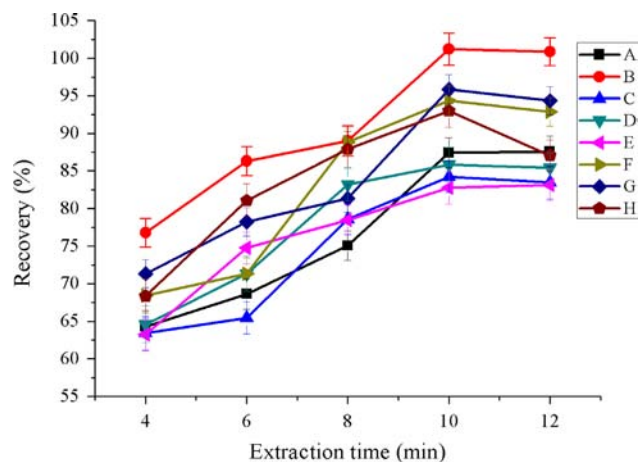
Fig. 4. Effect of extraction time on the recoveries of the analytes. Ionic liquid type, $[C_8MIM][BF_4]$; volume of $[C_8MIM][BF_4]$, 30 μL; pH value of sample solution, 9; flow rate of carrier gas, 500 mL/min; flotation time, 10 min; SPE cartridges, MCX (3 mL, 100 mg); washing solvent, dichloromethane; elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.
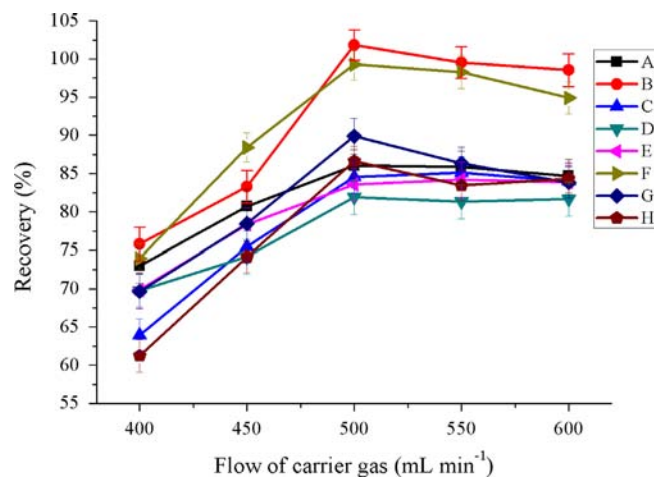
Fig. 6. Effect of flow rate of carrier gas on the recoveries of the analytes. Ionic liquid type, $[C_8MIM][BF_4]$; volume of $[C_8MIM][BF_4]$, 30 μL; extraction time, 10 min; pH value of sample solution, 9; flotation time, 10 min; SPE cartridges, MCX (3 mL, 100 mg); washing solvent, dichloromethane; elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.
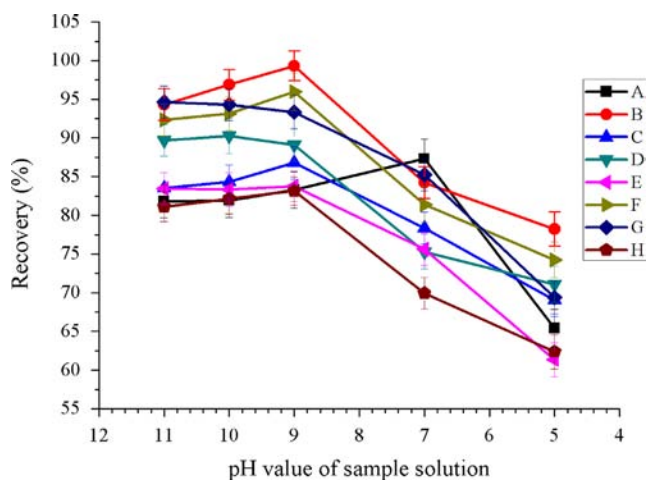
Fig. 5. Effect of pH value of sample solution on the recoveries of the analytes. Ionic liquid type, $[C_8MIM][BF_4]$; volume of $[C_8MIM][BF_4]$, 30 μL; extraction time, 10 min; flow rate of carrier gas, 500 mL/min; flotation time, 10 min; SPE cartridges, MCX (3 mL, 100 mg); washing solvent, dichloromethane; elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.

Fig. 7. Effect of flotation time on the recoveries of the analytes. Ionic liquid type, $[C_8MIM][BF_4]$; volume of $[C_8MIM][BF_4]$, 30 μL; extraction time, 10 min; pH value of sample solution, 9; flow rate of carrier gas, 500 mL/min; SPE cartridges, MCX (3 mL, 100 mg); washing solvent, dichloromethane; elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.
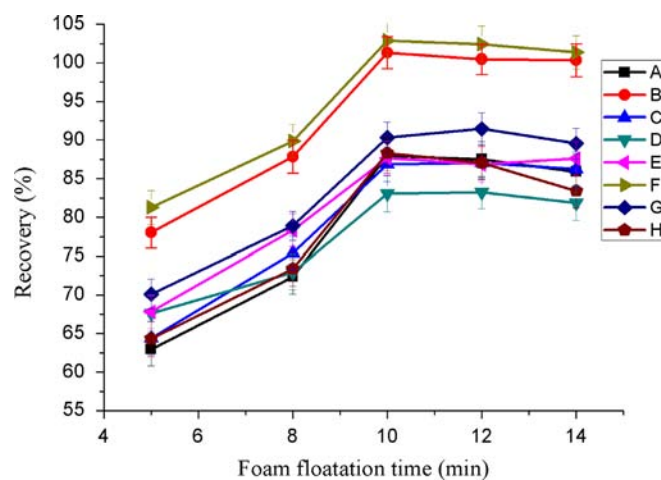
bubbles are formed, which will result in low recoveries. With the increase of the flow rate of the carrier gas, the amount of the foam increases and the recoveries are improved. However, when flow rate is too high, the vortex is generated in the solution and the bubbles of the solution surface can backed into the solution, which will result in low recoveries. In the experiments the flow rate of the carrier gas was selected as 500 mL/min.

3.1.5. The effect of flotation time

The flotation time can affect the recoveries of the analytes. As shown in Fig. 7, when the time is too short, the triazine herbicides in the sample mixture could not be taken out completely. The recoveries of all the analytes reached their maxima when the time was 10.0 min. Therefore, the flotation time was selected to be 10.0 min.

The formation of foams with ILs is related to the properties of the ILs and experimental conditions, such as the pH value of the system and the flow rate of the carrier gas. The long alkyl chain at the 1-position of the cation of IL, high and low pH value of the system and high flow rate of the carrier gas are beneficial to the formation of foams.

3.1.6. The effect of SPE cartridge type

The SPE cartridges have a significant effect on separation and enrichment of target analytes. The effect of type of the SPE cartridges, including Al_2O_3, MCX, C18, HLB and Florisil, was evaluated. The Al_2O_3 and Florisil cartridges belong to the medium polarity extraction cartridges. When the two cartridges were used, both some amounts of fat and the triazine herbicides could retain in these sorbents and other co-extracted compounds from the matrix were removed. Therefore, the two cartridges were not suitable for separation of triazine herbicides in the high fat content of corn samples.
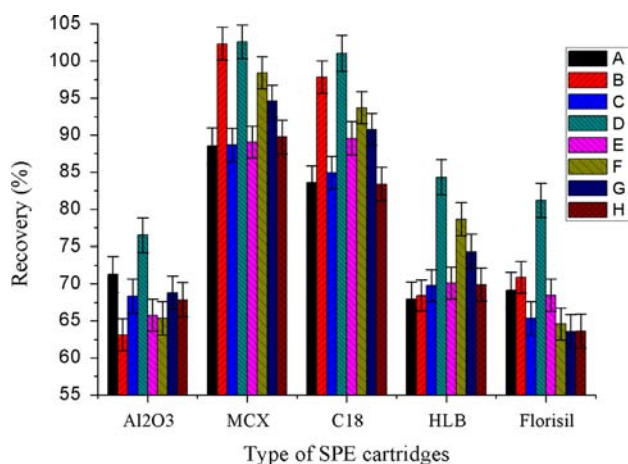
Fig. 8. Effect of SPE cartridges on the recoveries of the analytes. Ionic liquid type, [C$_8$MIM][BF$_4$]; volume of [C$_8$MIM][BF$_4$], 30 μL; extraction time, 10 min; pH value of sample solution, 9; flow rate of carrier gas, 500 mL/min; flotation time, 10 min; washing solvent, dichloromethane; elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.

Fig. 10. Effect of elution solvent on the recoveries of the analytes. Ionic liquid type, [C$_8$MIM][BF$_4$]; volume of [C$_8$MIM][BF$_4$], 30 μL; extraction time, 10 min; pH value of sample solution, 9; flow rate of carrier gas, 500 mL/min; flotation time, 10 min; SPE cartridges, MCX (3 mL, 100 mg); washing solvent, dichloromethane; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.
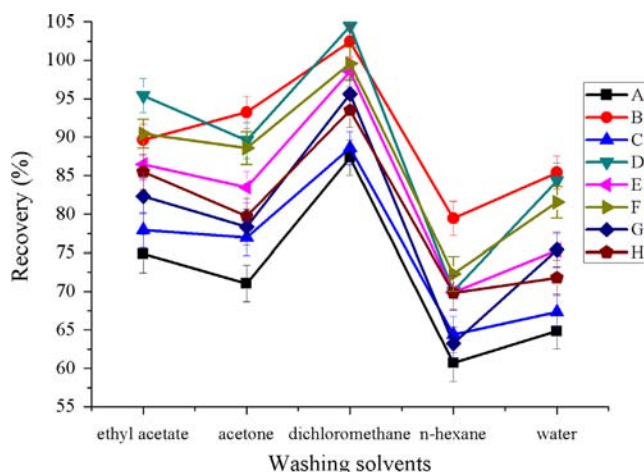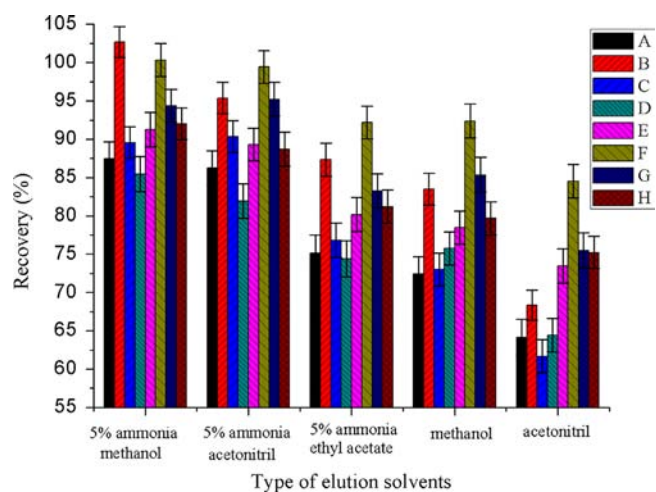
3.1.8. The effect of type and volume of elution solvent

In this experiment, the triazine herbicides were adsorbed on the MCX cartridge by hydrogen bonding and π–π interaction. The triazine herbicides are organic compounds and should to be eluted with organic solvents, such as methanol, acetonitrile, acetone and ethyl acetate. After a preliminary test, it was found that the triazine herbicides were well eluted from the MCX cartridge with the organic solvents containing ammonia. The reason may be that the analytes are easily soluble in the organic solvent and the interaction force between the analytes and the MCX cartridge decreases in the presence of ammonia. The effect of type of elution solvents, including methanol, 5% ammonia methanol, acetonitril, 5% ammonia acetonitril and 5% ammonia ethyl acetate, was investigated on the basis of previous investigation [39]. The detailed information can be found in Fig. 10. The recoveries are highest when 5% ammonia methanol is used as an elution solvent. Therefore, 5% ammonia methanol is most suitable.

The effect of volume ranging from 400 to 1200 μL was tested when 5% ammonia methanol was used as the elution solvent. If the volume of 5% ammonia methanol is too small, the triazine herbicides cannot be eluted completely. For this reason, the column was eluted with 1.00 mL of 5% ammonia methanol.

3.2. Evaluation of the method

3.2.1. Linearity

The calibration graphs were drawn by plotting the peak areas (A) measured versus the concentrations (C) of the target analytes in the spiked samples. The linearities were investigated in the optimum IL-based FFSPE conditions, and the linear regression equations and correlation coefficients are listed in Table 1. As can be seen, good linearities were obtained with correlation coefficients of > 0.9991.

3.2.2. LODs and LOQs

The limits of detection (LODs) and quantification (LOQs) are indicated in Table 1. The LODs and LOQs are obtained by following equations:

$$LOD = 3\sigma/k, \quad LOQ = 10\sigma/k$$

where σ is the standard deviation of blank signal, which is obtained by analyzing the blank sample 11 times and k is the slope of the calibration graph.

Fig. 9. Effect of washing solvent on the recoveries of the analytes. Ionic liquid type, [C$_8$MIM][BF$_4$]; volume of [C$_8$MIM][BF$_4$], 30 μL; extraction time, 10 min; pH value of sample solution, 9; flow rate of carrier gas, 500 mL/min; flotation time, 10 min; SPE cartridges, MCX (3 mL, 100 mg); elution solvent, 5% ammonia methanol; volume of elution solvent, 1 mL; (A) cyanazine, (B) metribuzin, (C) desmetryn, (D) secbumeton, (E) terbumeton, (F) terbuthylazine, (H) dimethametryn, and (G) dipropetryn.

It was found that C18 and HLB cartridges were blocked easily in the FF process. The results shown in Fig. 8 indicate that the recoveries of triazine herbicides are highest when a MCX cartridge was used. The MCX is a polymer matrix adsorbent containing phenyl and sulfo groups. The analytes can be adsorbed on the MCX by hydrogen bonding and π–π interaction. The adsorption of triazine herbicides on MCX cartridge should be strong. The MCX cartridge was selected as a SPE cartridge in the experiment.

3.1.7. The effect of type and volume of washing solvent

In order to eliminate impurities and improve the recoveries, the washing step was necessary. Therefore, five popular solvents with different polarities, including dichloromethane, n-hexane, ethyl acetate, acetone and water were used. The experimental results indicate that the nonpolar and strongly polar solvents were not suitable. As shown in Fig. 9, the recoveries are highest when dichloromethane is used as a washing solvent. So, dichloromethane was selected as a washing solvent in further work.

Table 1
Analytical performances.

Triazine herbicides	Regression equations	Correlation coefficient	Linear range (µg/kg)	LODs (µg/kg)	LOQs (µg/kg)
Cyanazine	$A=1.2027c+0.3955$	0.9997	5.00–150.00	1.5	5.0
Metribuzin	$A=1.6764c+0.1761$	0.9996	5.00–150.00	1.4	4.8
Desmetryn	$A=2.5079c+0.838$	0.9996	2.50–150.00	0.7	2.5
Secbumeton	$A=1.4150c+1.9202$	0.9995	5.00–150.00	1.6	5.4
Terbumeton	$A=2.5151c+1.5058$	0.9999	2.50–150.00	0.7	2.5
Terbuthylazine	$A=2.9280c+0.7264$	0.9993	2.50–150.00	1.1	3.8
Dimethametryn	$A=2.6110c+0.0301$	0.9993	2.50–150.00	1.4	4.7
Dipropetryn	$A=2.4894c+0.0940$	0.9999	2.50–150.00	1.5	5.0

Table 2
Analytical results of corn samples.

Corn	Herbicides	Spiked level (10 µg/kg)			Spiked level (50 µ/kg)			Spiked level (100 µg/kg)		
		Recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)	Recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)	Recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)
1	Cyanazine	87.3	3.4	5.6	86.3	3.2	4.6	88.4	3.0	3.2
	Metribuzin	101.1	2.7	4.3	104.0	2.0	3.2	103.4	1.9	2.3
	Desmetryn	92.1	3.2	5.4	91.2	2.7	3.2	91.3	2.4	3.1
	Secbumeton	92.7	3.5	5.4	94.0	2.8	3.5	94.8	2.6	3.2
	Terbumeton	91.4	2.9	3.7	93.3	2.4	3.5	92.5	2.2	2.8
	Terbuthylazine	101.4	2.1	3.5	102.2	2.1	3.4	101.2	1.8	2.3
	Dimethametryn	96.8	2.7	3.9	96.8	2.6	3.8	93.5	2.5	3.4
	Dipropetryn	94.2	3.1	4.9	95.3	2.5	3.8	93.8	2.8	3.6
2	Cyanazine	87.1	3.7	4.2	85.9	3.2	4.2	88.0	3.2	4.2
	Metribuzin	103.2	2.9	3.9	102.3	2.2	3.5	103.4	2.2	3.1
	Desmetryn	101.2	3.2	4.2	91.5	2.6	3.8	92.2	2.3	3.6
	Secbumeton	93.3	3.5	4.8	93.3	2.9	3.6	93.9	2.8	3.6
	Terbumeton	92.4	3.4	4.3	93.1	2.1	3.4	94.6	2.1	3.1
	Terbuthylazine	101.7	2.4	3.5	102.5	2.0	2.8	102.3	1.8	2.8
	Dimethametryn	96.7	2.8	3.3	95.6	2.4	3.5	95.9	2.2	3.3
	Dipropetryn	94.4	2.9	3.8	93.9	2.6	3.8	94.3	2.7	3.8
3	Cyanazine	86.6	3.5	4.1	87.3	3.1	4.1	87.9	3.1	4.3
	Metribuzin	102.1	2.5	3.5	103.3	2.1	3.6	103.1	1.9	2.8
	Desmetryn	91.5	3.3	4.6	91.3	2.3	3.5	92.4	2.4	3.5
	Secbumeton	91.9	3.5	4.4	92.8	2.4	3.5	91.9	2.7	3.6
	Terbumeton	89.8	3.1	4.6	90.4	2.3	3.3	89.5	2.6	3.5
	Terbuthylazine	101.4	2.9	3.8	101.2	2.5	3.6	101.0	2.3	3.4
	Dimethametryn	94.3	3.0	3.8	93.7	2.4	3.5	94.6	2.6	3.6
	Dipropetryn	91.6	3.4	4.2	90.8	2.7	3.7	92.3	2.8	3.7
4	Cyanazine	88.0	3.9	5.4	89.7	3.1	4.3	85.9	3.1	3.8
	Metribuzin	104.0	3.1	4.9	103.6	2.1	2.4	102.7	1.9	2.8
	Desmetryn	91.6	3.5	4.9	90.5	2.4	3.3	89.5	2.6	3.8
	Secbumeton	94.9	3.4	4.9	94.2	2.7	3.7	92.8	2.5	3.4
	Terbumeton	93.6	3.3	4.8	94.4	2.4	3.8	93.5	2.4	3.6
	Terbuthylazine	101.5	3.2	4.8	101.4	2.2	3.7	98.7	2.2	3.3
	Dimethametryn	96.4	3.5	5.0	97.3	2.5	3.7	96.6	2.2	3.4
	Dipropetryn	94.9	3.7	5.2	93.4	2.8	3.6	92.2	2.5	3.5

The LOQs of the eight triazine herbicides are lower than the MRLs of the herbicides, which range from 0.05 mg kg^{-1} to 0.1 mg kg^{-1}. The present method is appropriate to practical application.

3.2.3. Analysis of samples

To evaluate the accuracy and applicability of the present method, four samples were analyzed. The eight triazine herbicides in four corn samples were undetectable. The analytical results of the spiked samples are listed in Table 2. Table 2 shows the mean recoveries of the herbicides ($n=5$) for each spiked level. The recoveries of all triazine herbicides at three concentration levels are from 85.9% to 104.0%. The satisfactory results were obtained.

The intra-day relative standard deviation (RSD) was obtained by analyzing spiked sample five times in one day. The inter-day RSD was obtained by analyzing spiked sample once each day over five consecutive days. RSD values are lower than 5.6% for all the analytes at all concentration spiking levels (Table 2). The typical

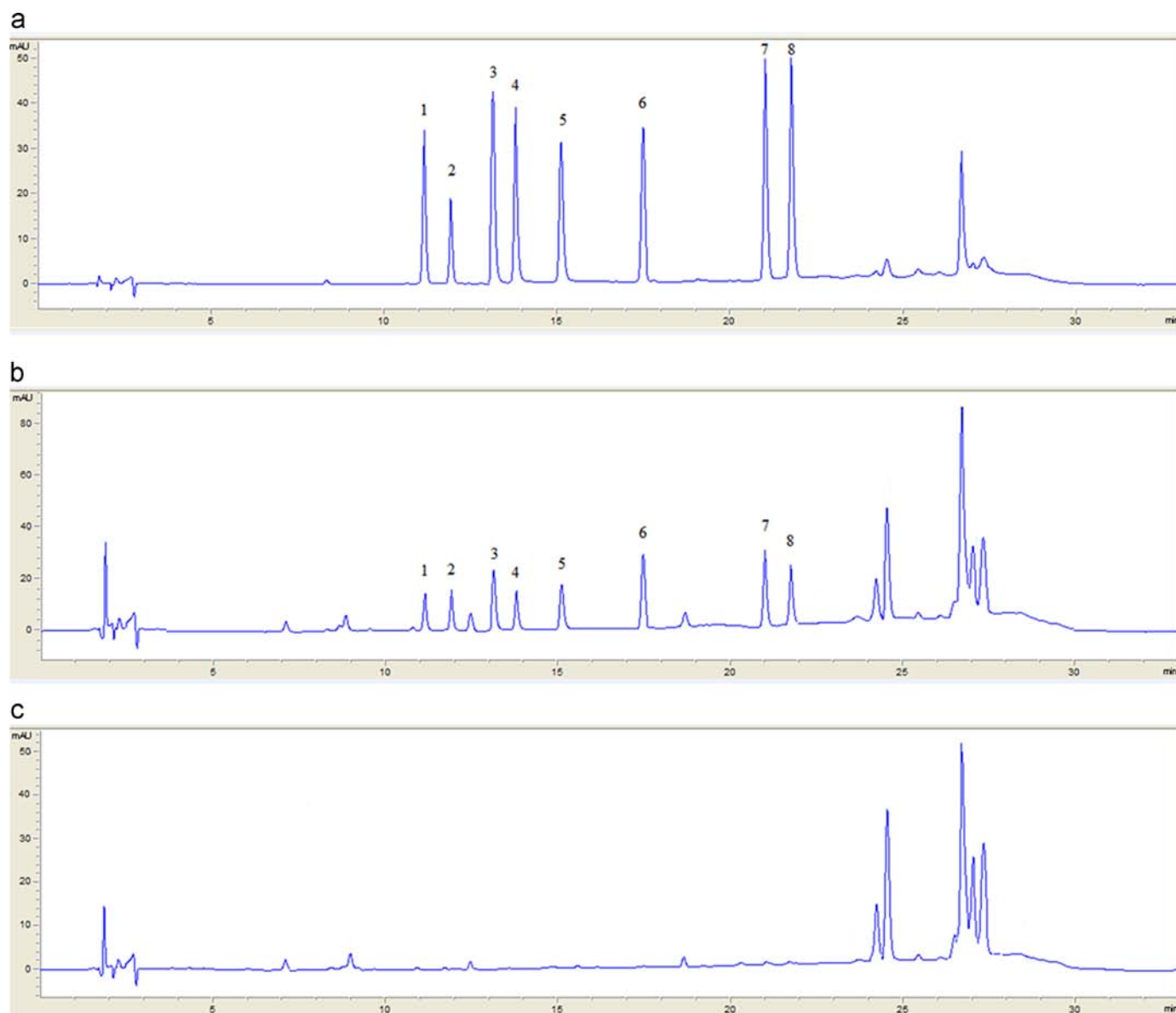chromatograms of standard solution, spiked sample 1 and sample 1 are shown in Fig. 11.

3.2.4. Stability

Long-term stability of the analytes in corn samples was evaluated. The spiked samples were prepared and treated according to the methods mentioned in Sections 2.3 and 2.4. All experiments were performed in five replicates.

The experimental results are listed in Table 3. The recoveries of the analytes range from 81.8% to 102.1% and the RSDs for determining the analytes range from 2.1% to 3.6%. The results shown in Table 3 indicate that the triazine herbicides in corn samples are stable for the period of 8 weeks when stored in 4 °C

3.3. Comparison of different extraction methods.

The present method was compared with the methods reported for the extraction of triazine herbicides from corn samples

Fig. 11. Chromatograms (a) standard solution (10 µg/mL for each analyte), (b) spiked sample 1 (50 µg/kg for each analyte), (c) blank sample 1. (1) Cyanazine, (2) metribuzin, (3) desmetryn, (4) secbumeton, (5) terbumeton, (6) terbuthylazine, (7) dimethametryn, and (8) dipropetryn.

Table 3
Stability of the herbicides.

Herbicides		1 week	2 weeks	4 weeks	8 weeks	10 weeks
Cyanazine	Recovery (%)	85.8	86.9	85.3	84.6	73.7
	RSD (%, $n=5$)	3.6	3.4	3.2	3.2	3.4
Metribuzin	Recovery (%)	102.1	102	102	100	94.4
	RSD (%, $n=5$)	2.2	2.8	2.4	2.1	2.3
Desmetryn	Recovery (%)	92.4	91.7	91.8	90.5	83.3
	RSD (%, $n=5$)	2.9	2.8	2.4	2.6	2.6
Secbumeton	Recovery (%)	93.0	93.2	91.6	81.8	74.7
	RSD (%, $n=5$)	3.2	3.3	2.9	3.1	3.4
Terbumeton	Recovery (%)	92.3	91.6	91.7	90.2	84.3
	RSD (%, $n=5$)	2.4	2.8	2.2	2.3	2.4
Terbuthylazine	Recovery (%)	101	100	99.5	97.3	91.3
	RSD (%, $n=5$)	2.3	2.5	2.3	2.6	2.4
Dimethametryn	Recovery (%)	97.2	96.9	95.5	94.4	84.6
	RSD (%, $n=5$)	2.7	2.5	2.5	2.2	2.3
Dipropetryn	Recovery (%)	93.7	92.7	91.4	90.0	79.9
	RSD (%, $n=5$)	2.8	2.4	2.3	2.6	3.0

Table 4
Comparisons of the present method with other methods.

Matrices	Extraction method[a]	Sample (g)	organic solvent (mL)	Spiked level (µg/kg)	Recovery (%)	LOD (µg/kg)	LOQ (µg/kg)	Reference
Cereals	DMAE	1.00 g	1.10	10–500	80.0–102.0	1.1–1.5	3.5–4.8	[13]
Rice and corn	SPE	5.00 g	5.00	10–10,000	75.3–104.3	1.0–32.0	1.0–96.0	[17]
Cereal-based food	PMAE	2.00 g	20.0	5–500	66.2–88.6	No report	0.9–2.0	[23]
Cereal and vegetable	SPE	7.00 g	65.0	100	87.0–114.0	10.0–15.0	No report	[25]
corn	IL-based FFSPE	10.0 g	1.20	10–100	85.9–104.0	0.7–1.6	2.5–5.4	This work

[a] DMAE, dynamic microwave-assisted extraction; PMAE, pressurized microwave-assisted extraction.

Table 5
Statistical analysis of recovery.

Methods	Analytes	n	Recovery	s	$\bar{s}$	t	
IL-based FFSPE	Desmetryn	4	94.0	3.3	3.6	2.6	$t_{(0.001,12)} = 4.318$
DMAE		10	88.5	3.7			
IL-based FFSPE	Terbumeton	4	91.3	3.2	8.9	0.43	$t_{(0.001,8)} = 5.041$
PLE		6	88.8	11			

(Table 4) [13,17,23,25]. Because desmetryn and terbumeton were simultaneously determined by the present method and another method listed in Table 4. The comparison of recoveries of the two analytes was made by statistical analysis. Student's t test was applied. The results of statistical analysis are shown in Table 5. The results indicate that there is no significant difference ($P < 0.001$) between the recoveries obtained by the present method and DMAE or PLE. However, it is obvious that compared with the other methods when the present method was applied the volume of the organic solvent is smaller and the sample amount is bigger. The separation and enrichment of target analytes were performed simultaneously.

4. Conclusions

The IL-based FFSPE was successfully applied to the extraction, separation and enrichment of eight triazine herbicides from corn samples. The factors which can affect the IL-based FFSPE were investigated. The advantage of the IL-based FFSPE is the low consumption of classical organic solvent. The present method could be applied directly to solid samples and large volume liquid samples. The separation and enrichment were achieved simultaneously. So it seems possible to extend this method to the extraction of triazine herbicides in other similar samples by varying the extraction conditions.

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