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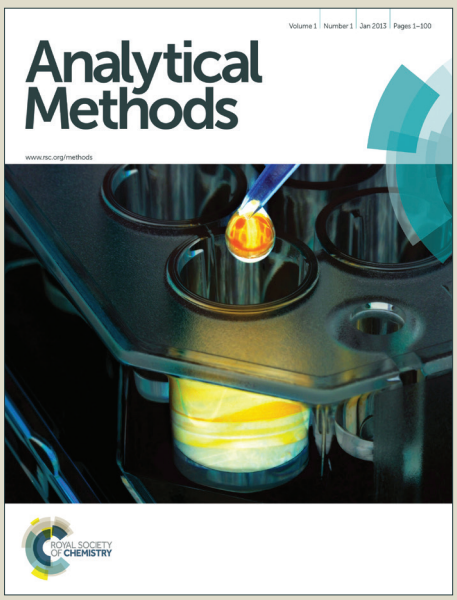

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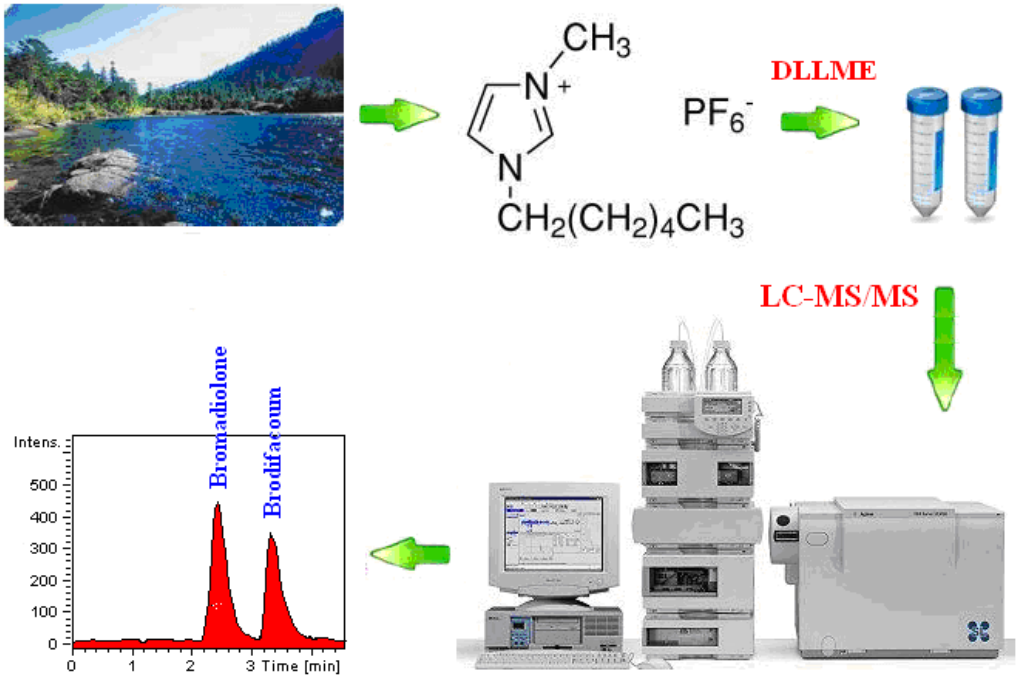

Analysis of trace bromadiolone and brodifacoum in environmental water samples by ionic liquid ultrasound-assisted dispersive liquid–liquid microextraction and LC–MS/MS

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Abstract: An ionic liquid-based ultrasonic-assisted dispersive liquid–liquid microextraction (IL-USA-DLLME) method was proposed for highly effective extraction of trace bromadiolone and brodifacoum in environmental water samples. The ionic liquid, 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6mim][PF_6] was quickly disrupted by ultrasonication and dispersed in water as fine droplets. At this stage, the analytes were extracted into the fine ionic liquid droplets. After centrifugation, the concentration of the enriched rodenticides in the sedimented phase was determined. Extraction conditions including extraction solvent, dispersive solvent volume, pH, ultrasonic time and centrifugation time were investigated thoroughly. Compared with the conventional solvent extraction, the proposed approach exhibited higher efficiency, which indicated the IL-USA-DLLME method was an efficient, rapid and simple sample preparation technique. Under optimized extraction conditions, the enrichment factors were up to 122~137 times, achieving the limits of quantitation, based on the signal-to-noise ratio (S/N) of 10, were 0.005 μg/L for both. The linearities for both analytes were in the range of 0.005~1.0 μg/L with a correlation coefficient>0.999, recoveries were between 88.8~98.3% with relative standard deviations (RSDs) between 1.1% and 9.3%. The proposed method shows high reproducibility, and it is suitable for analysis of trace bromadiolone and brodifacoum in environmental water samples.

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Key words: Ionic liquid ultrasound-assisted extraction; Dispersive liquid–liquid microextraction; Bromadiolone; Brodifacoum; LC-MS/MS; Environmental water.

1. Introduction

Bromadiolone and brodifacoum are the second-generation anticoagulant rodenticides for widely controlling mice, rats, and other rodents that pose a threat to public health, critical habitats, native plants and animals, crops, food and water supplies [1]. They are especially hazardous, highly toxic and interfered with blood clotting. Both rodenticides also greatly present human and environmental safety concerns, e.g. environmental water and soil. Therefore, it is necessary to develop an accurate, simple, fast and robust method to determine and monitor their levels in different environmental water matrices to protect against any possible health risks.

Sample preparation is one of the most crucial steps before instrumental analysis to obtain accurate and sensitive results in the analytical process. It is also the bottleneck especially for the trace analysis in the complex matrices. In general, sample preparation consists of extraction, cleanup and preconcentration of target compounds from a sample matrix. Today, most popular sample pretreatment methods, e.g. liquid–liquid extraction (LLE) and solid-phase extraction (SPE), have been used. Nevertheless, these traditional methods are laborious, time-consuming and require large volumes of samples and toxic organic solvents. Recently, much attention has been paid to the fast development of simplification and miniaturization, thus, several novel micro-extraction environmentally benign techniques are being developed to reduce the analytical run time, to increase the sample throughput and to improve the sensitivity of the analytical methods [2]. In 2006, an emerging technique named

dispersive liquid–liquid microextraction (DLLME) was developed, which is a novel sample-preparation technique offering high enrichment factors from low volumes of water samples [3]. DLLME has found wide acceptance because of several advantages, including simplicity of operation, rapidity, low cost and ease of method development, which made it available to virtually all analytical laboratories [4]. Today, some literatures with DLLME had reported for the successful determination of dye Brilliant Blue FCF (E133) in different kinds of food and cosmetic samples [5], linear alkylbenzene sulfonates (LASs) in water samples [6], ultraviolet filters in water samples [7], uranium(VI) in water samples [8], di(2-ethylhexyl) phthalate (DEHP) and its metabolite mono(2-ethylhexyl) phthalate (MEHP) in human urine samples [9], and balofloxacin in rat serum [10]. DLLME avoids many shortcomings of conventional methods and has been successfully applied for the pre-concentration of organic compounds in environmental and biological fluid samples [5-13].

With the development of science and technology, one kind of green and effective solvent media alternative of organic solvent named ionic liquids (ILs) has been introduced into the separation science, owning to their unique chemical and physical properties such as negligible vapour pressure, good thermal stability, wide liquidus range, good dissolving and extracting ability [14,15]. In recent years, ILs have been used as extraction solvents in liquid–liquid extraction (LLE) [16], liquid-phase microextraction (LPME) [17,18], solid-phase microextraction (SPME) [19,20] and dispersive liquid–liquid microextraction (DLLME) [5-13,21,22]. So far, many methods have been reported for the determination of bromadiolone and brodifacoum residues in diverse matrix [23-31], however, generally, these are time-consuming, expensive and unfriendly environmental. Recently, DLLME has attracted

increasing attention for its superior advantages of high enrichment factor, perfect recovery, low cost, rapid and easy operation [3, 11-13]. To our best knowledge, the simultaneous extraction of bromadiolone and brodifacoum from environmental water samples with DLLME using ionic liquid as extraction solvent has not been reported. The present paper aimed to develop a simple, fast, inexpensive, sensitive, and effective ionic liquid-based ultrasonic-assisted dispersive liquid–liquid microextraction (IL-USA-DLLME) method for simultaneous analysis of bromadiolone and brodifacoum in environmental water samples. In this paper, the organic solvent consumption of this technique is based on the current trends in green chemistry, a hydrophobic ionic liquid, 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6mim][PF_6], was chosen as the extraction solvent. The factors that affect the microextraction efficiency were investigated in detail, and the optimal conditions were established.

2. Experimental

2.1 Solvents and Materials

Bromadiolone and brodifacoum (purity>99.0%) were purchased from Dr. Ehrenstorfer, German. Both rodenticides individual stock solutions were prepared in methanol and stored in −20 °C freezer. The working mixed solutions of 20.0 mg/L were made by diluting with methanol. The ILs, 1-butyl-3- methylimidazolium hexafluorophosphate [C_4mim][PF_6] (purity>99.0%), 1-Hexyl-3-methylimidazolium hexafluorophosphate [C_6mim][PF_6] (purity>99.0%), 1-octyl-3-methylimidazolium hexafluorophosphate [C_8mim][PF_6] (purity>99.0%), were obtained from Shanghai Chengjie Chemical Co. Ltd.).

High-performance liquid chromatography (HPLC) grade acetonitrile, methanol, acetic acid, and ammonium acetate were purchased from Merck (Darmstadt, German). Deionized water (18.2 MΩ·cm) was obtained from the Milli-Q water purification system (Millipore, Molsheim, France). Analytical grade sodium chloride and formic acid were obtained from Sinopharm Shanghai Chemical and Reagent Ltd., Shanghai, China.

In this experiment, environment water samples including five river water samples (pH 5.83-7.45), five wastewater samples (pH 5.45-7.49) and five well water samples (pH 6.76-7.05) were analyzed. The river water samples were grabbed along five local rivers within three kilometers of each other. The wastewater samples were collected from the discharge outfall in five local municipal wastewater treatment plants. The well water samples were grabbed from five wells within one kilometer of each other in a local village. All water samples were collected in amber glass containers in 12th-15th March 2014, and were filtered immediately through 0.22 μm micropore membranes, then stored in the dark at 4 °C. The pH values of the water samples were adjusted to 6.0 prior to analyses.

2.2 Apparatus

An Agilent 1100 series LC-MSD Trap SL ion trap mass spectrometer (Agilent Technologies, Germany) was used, which equipped with a binary high-pressure pump, an automatic sample injector, an electrospray ionization (ESI) source. The LC-MSD Trap Software 4.2 (Bruker Daltonics, Bremen, Germany) was used to control the LC-ESI-MS/MS system and process the mass spectrometric data. A 40 kHz and 180 W SB-3200D ultrasonic water bath with temperature control (Ningbo Xinzi biological technology Co., Ningbo, China) was applied to emulsify the IL.

2.3 Extraction procedure

Accurately 5.0 mL of environmental water samples were transferred into a 10 mL glass centrifuge tube, then 54 μL of [C_6mim][PF_6] IL pre-mixed with 150 μL of methanol was added. The centrifuge tube was immersed into a SB-3200D ultrasonic bath for 5 min. During the ultrasonication, the IL was dispersed into the aqueous solution, and homogenous solution was achieved. There after the centrifuge tube was cooled with ice water, a cloudy solution was formed in the centrifuge tube and kept for 10 min. In this step, the suspicious existed rodenticides were extracted into the fine IL droplets. Then, the cloudy solution was centrifuged at 8434 × g at 0 °C for 10 min to disrupt the emulsions and separate the IL from the aqueous phase, the IL were sedimented at the bottom of the conical test tube. The upper aqueous phase was removed with a syringe, the residue was dissolved in 50 μL of methanol and 10 μL of solution was injected into the LC-MS/MS system for analysis.

2.4 Preparation of Standard Solutions

The standard stock solutions (1.0 g/L) of bromadiolone and brodifacoum were prepared in methanol. Appropriate serial dilutions of the bromadiolone and brodifacoum stock solution were made in methanol/2.0 mmol/L ammonium acetate solution (80:20, v/v) for spiking blank matrix. All solutions were stored at 4 °C in tightly closed bottles until use.

2.5 Method performance

The ionic liquid enrichment factor (*EF*) is calculated according to the following equation: $EF = C_{sed}/C_0$, where C_{sed} is the rodenticide concentration in the final ionic liquid phase, C_0 is the rodenticide concentration in the initial sample, respectively.

Meanwhile, the extraction recoveries (R_e) were calculated by

$$R_e(\%) = C_{sed} \times V_{sed} / (C_0 \times V_{aq}) \times 100 = EF \times V_{sed} / V_{aq}$$

where V_{sed} and V_{aq} are the final volume of sedimented phase and the volume of the aqueous sample, respectively. The C_{sed} was obtained from the calibration curve (0.005–1.0 μg/L) of the standard rodenticide solutions.

In this work, three ion liquids, such as $[C_4mim][PF_6]$, $[C_6mim][PF_6]$ and $[C_8mim][PF_6]$, were used as the extraction solvents, and different volumes (39, 44, 49, 54, 59, 64, 69, and 74 μL) with a constant volume of 150 μL of dispersive solution were investigated to evaluate their recoveries in 5.0 mL of water samples. The effect of pH (1.0–9.0), ultrasonic extraction time (1–10 min) and the centrifugation time (5–30 min) on the extraction recoveries was optimized.

Calibration curves at seven concentration levels (0.005, 0.010, 0.020, 0.10, 0.20, 0.50 and 1.00 μg/L) were prepared by spiking bromadiolone and brodifacoum standards into environmental water blanks. The standards were prepared according to the sample preparation procedure given above. Quality control (QC) samples at concentration levels of 0.010, 0.10 and 0.80 μg/L were prepared by spiking appropriate volume of bromadiolone and brodifacoum standard dilutions into well and river water blanks.

The extraction recoveries were obtained by performing six replicates with the QC samples under the optimized conditions. The limits of detection (LODs) and the limits of quantitation (LOQs) were determined at concentrations where the signal/noise ratios were equal to 3 and 10, respectively. The intra-day, and inter-day relative standard deviations (RSDs) were determined by the QC samples.

2.6 Liquid Chromatography and Mass Spectrometry Analysis

A reversed-phase Zorbax XDB-C18 column (150 mm×2.1 mm×5.0 μm, Agilent, USA) was used for separation. The analysis was conducted in isocratic elution by using the mobile phase of methanol/2.0 mmol/L ammonium acetate solution (80:20, v/v) at a constant temperature of 35 °C with a flow rate of 0.40 mL/min. The injection volume was 10 μL. Detection was carried out on an Agilent 1100 series LC/MSD Trap SL mass spectrometer in the negative mode with a full scan mass spectra over the *m/z* range 100~600 using a cycle time of 1 s, a capillary voltage of 3.5 kV, a capillary exit voltage of -165 V, a dry temperature of 325 °C, a high purity nitrogen (99.999%) dry gas of 9.0 L/min, a nitrogen nebulizer pressure of 60.0 psi and a dwell time of 200 ms. The ESI interface and mass spectrometer parameters were optimized by direct infusion of standard solution (1.0 mg/L) at 0.5 mL/h to obtain maximum sensitivity. In the MS/MS experiments, the deprotonated precursor molecular ions $[M–H]^-$ of bromadiolone and brodifacoum at *m/z* 527 and 523 were isolated and fragmented by helium gas collision in the ion trap. Throughout all the measurements bromadiolone and brodifacoum were detected by MRM with transitions of *m/z* 527→465 for bromadiolone, and *m/z* 523→477 for brodifacoum, respectively. Table 1 outlines the MRM parameters for bromadiolone and brodifacoum. The MRM peak areas were integrated for quantification.

2.7 Storage stability studies

The stability of bromadiolone and brodifacoum in wastewater samples (collected from the discharge outfall in local wastewater treatment plants) was investigated. The samples for the storage stability studies were fortified with two levels at 0.05 μg/L and 0.50 μg/L, and held in sealed 25 mL ampoules at either 4 °C or 25 °C in the dark. Aliquots of sample type

were extracted and analyzed, as described above, immediately following preparation (day 0) and on days 3, 7, and 10.

3. Results and Discussion

3.1. Optimization of extraction conditions

3.1.1 Selection of the extraction solvent

The selection of an appropriate extraction solvent was a very critical process for IL-USA-DLLME. In general, the extraction solvent must have low solubility in water, a higher density than that of water and a high affinity with the target analytes [32]. Based on these considerations, the effect of extraction solvent including $[C_4mim][PF_6]$, $[C_6mim][PF_6]$ and $[C_8mim][PF_6]$ on the extraction recovery for both target analytes was examined for IL-USA-DLLME when using methanol as dispersive solvent. As shown in Fig. 2, a higher peak area signal was observed for both analytes when $[C_6mim][PF_6]$ was used as extraction solvent. This observation could be attributed to the fact that the relatively high hydrophobicity (the solubility of $[C_nmim]$ $[PF_6]$ (n=4, 6, or 8) in water was 18.8 g/L, 7.5 g/L, and 2.0 g/L, respectively [34]) and low viscosity (the viscosity of $[C_nmim]$ $[PF_6]$ (n=4, 6, or 8) in water was 450, 585, and 685 cP, respectively [34]). Hence, $[C_6mim][PF_6]$ was selected as the extraction solvent for subsequent experiments. It has been reported that ultrasonic radiation could accelerate various steps of the analytical process in liquid samples [33], in ultrasound-assisted liquid-phase microextraction, ultrasonic agitation makes the extractant completely disperse in aqueous phase and form vesicles to achieve efficient extraction. In this work, ultrasonic agitation was used as the dispersing technique in the water phase.

3.1.2 Effect of extraction solvent volume

The curves of the analyte extraction recovery versus the different volume of $[C_6mim][PF_6]$ are shown in Fig. 3(a). It can be seen that the extraction recoveries for both bromadiolone and brodifacoum initially increased to reach a peak value at 54 μL and then gradually decreased as the amount of $[C_6mim][PF_6]$ was increased. This is probably because large extraction solvent droplets rapidly deposited at the bottom of the tube caused low extraction efficiencies. In contrast, the *EFs* showed a continuous decreasing trend from 122-fold to 38-fold for bromadiolone and 137-fold to 43-fold for brodifacoum, respectively. Consequently, 54 μL was used as the optimum volume of extraction solvent because the highest recoveries were obtained and the *EFs* were acceptable.

3.1.3 Selection of dispersive solvent

The extraction solvent will disperse as very fine droplets when rapidly injected with a dispersive solvent into the aqueous sample, and will decrease the interfacial tension between the two phases to accelerate the the target analytes dispersing in water samples [35]. To form a fine dispersive phase for the extraction, a suitable dispersive solvent is required. Therefore, several dispersive solvents were studied, such as methanol, acetonitrile, and acetone. With 54 μL of $[C_6mim][PF_6]$ as an extraction solvent, a normal cloudy phase system was formed with all three dispersive solvents. Methanol is the most miscible with bromadiolone, brodifacoum and water. The results of extraction recoveries indicated that the highest extraction recovery was obtained by using methanol as dispersive solvent. Therefore, methanol was chosen as the dispersive solvent in the present studies.

3.1.4 Effect of dispersive solvent volume

The volume of the dispersive solvent is one of the key parameters in DLLME procedures.

This parameter directly affects the formation of the cloudy phase solution and the degree of dispersion of the extraction solvent in the aqueous phase, thus affecting the extraction recoveries. To study this effect, the volume of methanol was changed between 50 and 250 μL, as shown in Fig. 3(b). The experimental phenomena show that tiny droplet formation and cloudy state are stable when using all the selected volumes of dispersive solvent, and an increase in the volume of the dispersive solvent (150-250 μL) resulted in a decrease in the quantity of the sedimented phase. A possible explanation may be that, at high volumes, the solubility of analytes in water increases, which causes the extraction solvent to be hard to separate from the disperser and the aqueous solution. Moreover, the extraction efficiency decreased by increasing the methanol from 150 to 250 μL. Therefore, 150 μL of methanol was chosen as the optimum volume of dispersive solvent. The small quantity of organic solvent used during USA-DLLME is one of the most remarkable advantages of this technique.

3.1.5 Effect of ultrasonic extraction time

Ultrasound treatment is a key factor in UAS-DLLME, which directly influences the levels of dispersion. Therefore, the ultrasound irradiation was applied and the effect of ultrasonic extraction time on extraction efficiency was investigated in the range of 1–10 min. The experimental results indicate that the extraction balance could be attained within 5 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 finite. The extraction equilibrium can be achieved in short time and the phase-transfer of the target analytes was fast. Therefore, extraction time of 5 min was selected in further work.

3.1.6 Effect of centrifugation time

During USA-DLLME processing, ultrasonic agitation causes the extractant to disperse completely throughout the aqueous phase and to form vast organic vesicles to obtain efficient extraction. Centrifugation was used to break down the cloud solution and to deposit the sediment phase in the centrifuge tubes. The centrifugation time on the extraction efficiency in the range of 5–30 min was tested at 8434 × g at 0 °C. The extraction efficiency for the analytes was increased gradually within the centrifugation time from 5 min to 10 min, and afterward was held during 10 min to 30 min. Therefore, 10 min was adopted as the centrifugation time for treatment of the samples in this study to obtain a separated biphasic system with the highest possible recovery and efficiency.

3.1.7 Effect of pH

In the IL-USA-DLLME method, the pH of the sample solution is a very important factor which affects the formation of a complex with sufficient hydrophobicity and the subsequent extraction. 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 1.0–9.0 on the extraction recoveries was studied. As can be seen in Fig. 3(c), the extraction recoveries were increased as the pH increasing from 1.0 to 4.0, substantially unchanged from pH 4.0 to 7.0, and then declined with the pH higher than 7.0. The results showed that the best extraction recoveries were obtained at pH 6.0 for both bromadiolone and brodifacoum. This could be explained that at the pH of 4.0 to 7.0, the analytes probably existed in their ion forms (p*K*a 4.04 for bromadiolone and 4.50 for brodifacoum, respectively), which could be beneficial for their distribution into the ion liquid phase. Therefore, pH 6.0 was optimal for extraction.

3.1.8 Influence of ionic strength

In general, addition of sodium chloride to an aqueous solution increases its ionic strength, which reduces the solubility of the analytes in the sample solution and improves recoveries. Therefore, experiments were performed in which different amounts of sodium chloride (0–8%, w/v) were added, while all other experimental conditions were kept constant, and the effect on the recoveries was observed. The experimental results illustrated in Fig. 3(d). It showed that as the level of sodium chloride added was increased from 0 to 2%, the recoveries for both bromadiolone and brodifacoum increased accordingly. However, continuing to increase the salt concentration resulted in the decrease of extraction efficiency. Based on these results, 2% (w/v) sodium chloride was chosen as the optimal salt concentration in the IL-USA-DLLME procedure.

3.2 Validation of the proposed method

Under the optimized condition, bromadiolone and brodifacoum in water samples were extracted with an enrichment factor of 122-fold and 137-fold, respectively. The linearities for both bromadiolone and brodifacoum were in the range of 0.005-1.0 μg/L with correlation coefficients (R^2) more than 0.999. The linear regression equations were Y=3963.5X−1.52 for bromadiolone, and Y= 3217.3X−1.14 for brodifacoum (Y=Peak area; X=Concentration, μg/L). The limits of detection (LODs) at a signal-to-noise ratio of 3 were 0.0015 μg/L, and the limits of quantitation (LOQs) at a signal-to-noise ratio of 10 were 0.005 μg/L for both bromadiolone and brodifacoum.

The recoveries and RSDs were listed in Table 2. It shows that the recoveries are in the range of 88.8–95.0% for bromadiolone, and 91.0–98.3% brodifacoum. The intra-day RSDs

were in the range of 1.1–6.6%, and the inter-day RSDs were in the range of 3.9–9.3%. These excellent results indicate that the present approach is a simple and sensitive procedure to determine bromadiolone and brodifacoum at trace level in water samples.

3.3 Comparison with other methods

The performance of the presented method is compared with that of other methods reported for the determination of bromadiolone and brodifacoum, as shown in Table 3. In comparison with the other reported methods, the proposed method shows comparatively low limits of quantitation (0.005 μg/L for both bromadiolone and brodifacoum) and low volumes of the use of volatile organic solvents by relying on an ionic liquid as the green extraction solvent. These characteristics are of key interest for laboratories doing routine trace analyses.

3.4 Storage stability studies

The stability of bromadiolone and brodifacoum was evaluated by the percentage of the amount measured in the wastewater to the initial concentration. The obtained results were listed in Table 4. It can be seen that both bromadiolone and brodifacoum are stable within 3 days in the wastewater under the tested conditions, and statistically distinguishable after 7 or 10 days. The higher variability at 7 or 10 days may be an experimental artifact, but may reflect variable changes in wastewater sample. The rodenticide degradation in the wastewater may be attributed to microbial or other action, however, the real degraded reason is required to be further studied.

3.5 Sample Analysis

To evaluate the applicability of the proposed method in real samples, fifteen environmental water samples (river water, wastewater and well water) were extracted using

the described IL-USA-DLLME methodology and analyzed by the LC–MS/MS. The obtained analytical results show that only a river water sample from a location along the Yao River was detectable for brodifacoum (0.56 μg/L), and the other samples were not detectable (below the limit of quantitation, 0.005 μg/L). The MRM chromatograms from both blank and Yao River samples are shown in Fig. 4. The further investigation indicated that the contaminated source polluted by brodifacoum was originated from a local rodenticide factory, which discharged illegally its untreated wastewater into the Yao River. The obtained results show that the proposed method is reliable and can be used for the determination of trace bromadiolone and brodifacoum in environmental water samples.

4. Conclusions

In this work, a simple and sensitive IL-USA-DLLME method combined with LC–MS/MS was developed for rapid determination of bromadiolone and brodifacoum in environmental water samples. An ultrasound-assisted microextraction process was used to shorten the extraction time and accelerate dispersion of the extraction solvent into the sample solution, which led to enhanced extraction efficiency. The experimental results indicated that the method could provide low LOQs, good linear ranges, and good enrichment factors within a very short time. Therefore, the method proved to be a useful tool for rapid determination of both bromadiolone and brodifacoum in environmental water samples.

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Captions to the Figures and Tables

Figure captions

Fig. 1 Chemical structures of bromadiolone (a) and brodifacoum (b).

Fig. 2 Effect of extraction solvent on the peak area of both bromadiolone and brodifacoum (n = 3). Extraction conditions: sample volume: 5 mL; spiked concentration, 1.0 μg/L; extraction solvent volume, 54 μL; dispersive solvent, 150 μL of methanol; sample pH, 6.0; ultrasonication time, 5 min; cooling time, 10 min; centrifugation time, 5 min at 8434 × g.

Fig. 3 Effect of volume of extraction solvent [C_6mim][PF_6] (a), volume of dispersive solvent (b), sample pH (c) and sodium chloride (d) on the recoveries of both bromadiolone and brodifacoum (n=3). Extraction conditions, (a): dispersive solvent, 150 μL of methanol; (b): extraction solvent, 54 μL of [C_6mim][PF_6]; (c): extraction solvent, 54 μL of [C_6mim][PF_6]; dispersive solvent, 150 μL of methanol; (d): extraction solvent, 54 μL of [C_6mim][PF_6], 150 μL of methanol; the other conditions as in Fig. 2.

Fig. 4 Typical chromatograms from both the blank (a, c) and the spiked (0.05 μg/L) environmental water samples (b, d).

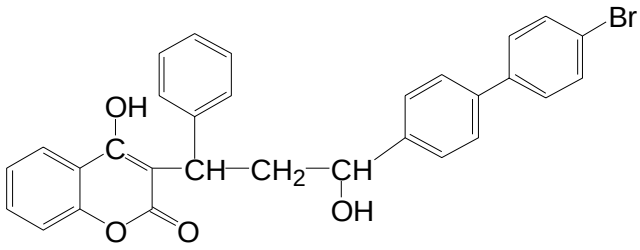
Table captions

Table 1 MRM parameters for bromadiolone and brodifacoum.

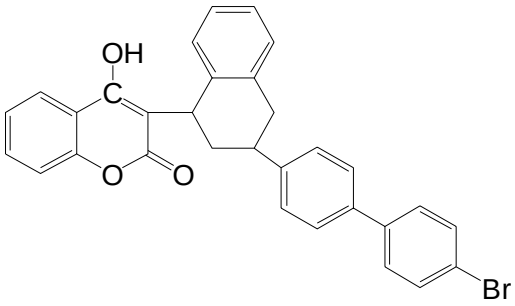
Table 2 Recoveries and RSDs of the proposed method.

Table 3 Comparison of the proposed method with other reported methods for extraction and determination of bromadiolone and brodifacoum.

Table 4 Stability assessment for bromadiolone and brodifacoum (n=3)

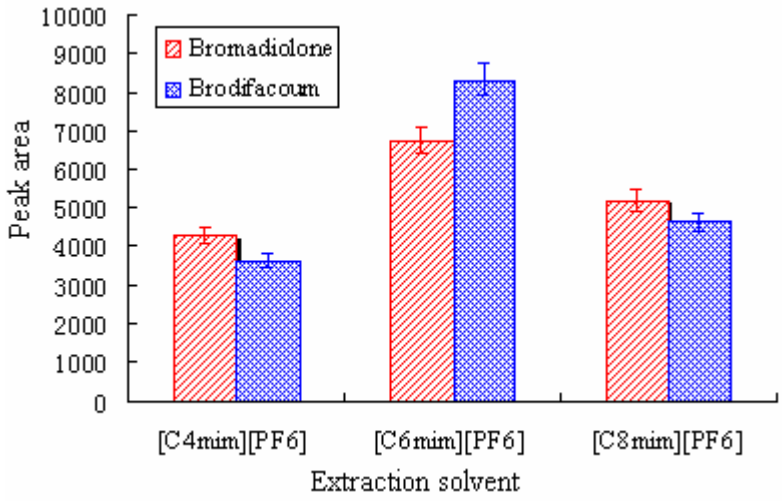


(a)



(b)

Fig. 1 Chemical structures of bromadiolone (a) and brodifacoum (b)



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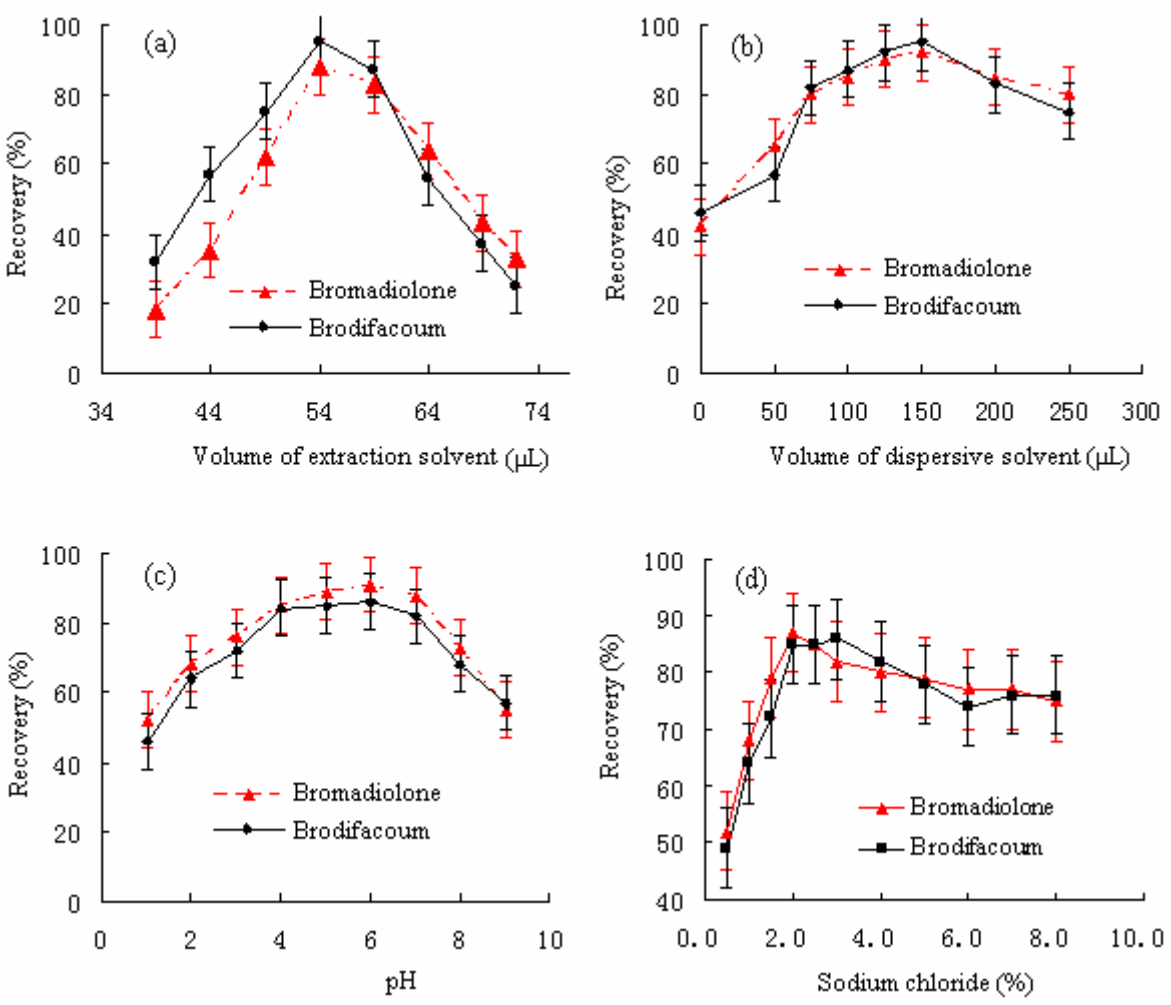

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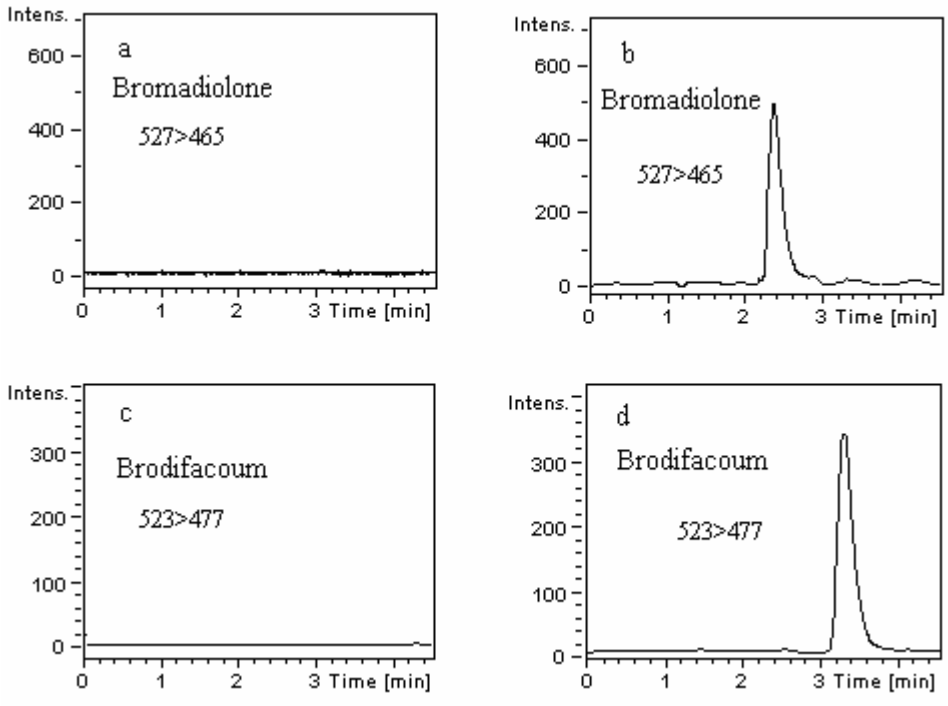

Fig. 4 Typical chromatograms from both the blank (a, c) and the spiked (0.05 μg/L) environmental water samples (b, d).

Table 1 MRM parameters for bromadiolone and brodifacoum

Item	Bromadiolone	Brodifacoum
Precursor ion (*m/z*)	527	523
Product ion for detection and quantification (*m/z*)	465	477
Additional ions for confirmation (*m/z*)	509, 491, 389	371, 373
Width (*m/z*)	2.0	2.0
Cutoff mass (*m/z*)	146	141
Collision induced dissociation (V)	1.50	1.50
Retention time (min)	2.51	3.37

Table 2 Recoveries and RSDs of the proposed method

Compound	Added /(μg/L)	Found [a]/(μg/L)		Recovery /%		RSD /%			
						Intra-day		Inter-day [b]	
		Well water	River water	Well water	River water	Well water	River water	Well water	River water
Bromadiolone	0.010	0.0093±0.0005	0.0095±0.0005	93.0	95.0	5.4	5.2	9.3	5.8
	0.10	0.092±0.005	0.092±0.005	92.0	92.0	5.4	5.4	7.2	7.9
	0.80	0.755±0.012	0.741±0.015	94.4	88.8	1.6	2.0	3.9	6.4
Brodifacoum	0.010	0.0091±0.0006	0.0091±0.0005	91.0	91.0	6.6	5.5	6.7	7.6
	0.10	0.095±0.004	0.091±0.005	95.0	91.0	4.2	5.5	7.9	8.9
	0.80	0.786±0.009	0.761±0.012	98.3	95.2	1.1	1.6	5.2	6.8

[a] n=6, expressed as mean ± S.D.

[b] n=3 replicates per day ×5 days within a week period, expressed as mean ± S.D.

Table 3 Comparison of proposed method with other reported methods for extraction and determination of bromadiolone and brodifacoum.

Compound	Sample	Extraction [a]	Detection	LOQ	Ref.
Bromadiolone, brodifacoum	Serum	LLE with phosphate buffer (0.5 mL, pH 5.5), 5% (v/v) ethanol in ethyl acetate (5 mL), and 1% (w/v) trichloroacetic acid (0.5 mL), cleanup with Florisil SPE column	Photodiode array (325 nm) and fluorescence detection	Photodiode array: Bromadiolone, 50 μg/L; Brodifacoum, 20 μg/L; Fluorescence detection: Bromadiolone, 5.0 μg/L; Brodifacoum, 1.0 μg/L	[24]
Bromadiolone, brodifacoum	Plasma	LLE with Toxi-tube B	LC-ESI/MS/MS	Bromadiolone, 25 μg/L; Brodifacoum, 20 μg/L	[25]
Bromadiolone, brodifacoum	Water	Direct injection	LC-ESI/MS/MS	Bromadiolone, 0.07 μg/L; Brodifacoum, 0.07 μg/L.	[26]
Bromadiolone, brodifacoum	Serum	LLE with 1-chlorobutane, buffer pH 4.2	LC-ESI/MS/MS	Bromadiolone, 5 μg/L; Brodifacoum, 5 μg/L	[27]
Bromadiolone, brodifacoum	Whole blood	LLE with ethyl acetate	LC-ESI/MS	Bromadiolone, 0.5 μg/L; Brodifacoum, 0.5 μg/L	[28]
Bromadiolone, brodifacoum	Whole blood	LLE with ethyl acetate	LC-ESI/MS/MS	Bromadiolone, 0.5 μg/L; Brodifacoum, 0.5 μg/L	[29]
Bromadiolone, brodifacoum	Environmental water	IL-USA-DLLME	LC-ESI/MS/MS	Bromadiolone, 0.005 μg/L; Brodifacoum, 0.005 μg/L	Proposed method

[a] LLE, liquid-liquid extraction.

Table 4 Stability assessment for bromadiolone and brodifacoum (n=3)[a]

Compound	Added (μg/L)	Day 0		Day 3		Day 7		Day 10	
		4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
Bromadiolone	0.05	99±3	97±5	97±7	89±3	82±4	76±6	42±3	31±2
	0.50	101±3	99±4	97±5	92±5	86±4	81±5	70±2	48±3
Brodifacoum	0.05	97±2	96±6	96±7	86±4	82±7	76±6	67±3	29±2
	0.50	95±4	96±5	96±7	89±5	86±6	82±5	76±2	48±3

[a] Expressed as the mean percentage of the amount measured in waste water to the initial concentration with a standard deviation ($\bar{x}$ ±s, n=3).