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Analytical Methods

Magnetic effervescent tablets containing ionic liquids as a non-conventional extraction and dispersive agent for determination of pyrethroids in milk

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ABSTRACT

Conventional magnetic effervescent tablet has many drawbacks, such as not practicable for field processing, rapid moisture absorption, and poor tablet storage characteristics. Herein, we developed a novel magnetic effervescent tablet containing ionic liquid microextraction (MET-ILM) for determination of pyrethroids in dairy milk. It contains only Na₂CO₃ as an alkali source (no acidic source) in the Fe₃O₄ magnetic tablet; the CO₂-forming reaction is initiated in the acidic solution containing the analytes, and thus the prepared tablet can be stored for long time periods without deterioration. The combined action of extractant and sorbent vastly increase the extraction efficiency. The optimized procedure consisted of an effervescent tablet, 2:1 HCl:Na₂CO₃, and 60 µL [C₆MIM]PF₆ as extraction solvent. The LODs for five pyrethroids were 0.024–0.075 µg kg⁻¹ with recoveries of 78.3–101.8%. The RSDs were < 4.8% and < 6.3% for intra- and inter-day precisions. Overall, the method is very feasible for use in the field.

1. Introduction

Pyrethroids are synthetic pesticides which are widely applied as insecticides in stored grain, crops and indoor environments. The increasingly widespread use of pyrethroids in our daily lives has contributed to an increased hazard for pyrethroids residue transfer to food-producing animals, which may be acutely toxic and dangerous for human health (Chen et al., 2014; Stanislaw et al., 2013). Humans show somewhat unspecific symptoms after occupational exposure to pyrethroids. Male reproduction and development are primary concerns and children are especially regarded as being highly susceptible to risk from long-term exposure to pyrethroids (Saillenfait, Ndiaye & Sabaté, 2015).

As a result of widespread use, pyrethroid residues are known to migrate to foods, such as vegetable oil, eggs, fruits, vegetables, fish, and milk (Ciscato, Gebara & Spinosa, 2014; Daniela, et al., 2014; Meneghini et al., 2014; Pirsasheb, Fattahi & Shamsipur, 2013; Yu, Ang, Yang, Zheng & Zhang, 2017; Yu & Yang, 2017; Zhang, Wang, Lin, Fang & Wang, 2012). Milk and milk products are consumed regularly in our daily lives on account of their nutritional value and characteristic flavor. However, the presence of pyrethroid residues in milk gives rise to public health concerns because of pyrethroid accumulation from inhaled air,

veterinary treatment and contaminated cattle feed (Bushra, Samina & Shafiqur, 2014; Dallegrove, Pizzolato, Barreto, Eljarrat, and Barceló, 2016; Hamid, Wan, Mohd, and Hassan, 2016). Therefore, it is critical to monitor pyrethroid contents in milk due to its importance as a food source resulting in a growing demand to develop simple and efficient methods to analyze trace-level pyrethroids in milk samples.

Because direct determination of pyrethroids is not possible in complex milk samples, effective sample pretreatment and cleanup are required prior to instrumental analysis. Reported pretreatment methods include on-site dispersive liquid–liquid microextraction (DLLME) based on solidification of switchable solvents (Hu, Wang, Qian, Liu, et al., 2016), in-syringe low-density ionic liquid DLLME (Hu, Wang, Qian, Wang, et al., 2016), QuEChERS (quick, easy, cheap, effective, rugged and safe) (Luiz, Bolaños, González, Vidal & Frenich, 2011), membrane protected micro-solid-phase (Sajid, Basheer & Mansha, 2016), and directly suspended droplet microextraction (DSDME) (Liu & Min, 2012). These methods provide adequate efficiency, but have several limitations such as use of organic solvents, long processing time and simultaneous extraction of numerous interference compounds (Wang, Shu, Li, Yang & Qiu, 2016; Zainudin, Salleh, Mohamed, Yap & Muhamad, 2015).

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In recent years, researchers have attempted to develop simple methods that use “green solvents” (e.g., ionic liquids and switchable solvents) to replace traditional volatile organic solvents to separate and concentrate pyrethroids (Chen et al., 2015). For example, the application of magnetic materials and effervescence reactions has recently gained much attention (Yang et al., 2016; Yu & Yang, 2017). A ternary solvent system is composed of target analytes, extraction agent and dispersive agent, and the magnetic sorbent is recovered using a simple magnet. The centrifugation or vortex step can be eliminated by using an external magnetic field. These methods can simplify experimental processing and vastly improve extraction efficiency. However, the effervescence step is often slow and not suitable for field use. In addition, the magnetic effervescent tablets have poor storage characteristics as the acid and alkaline salts in the magnetic effervescent tablets react during storage and absorb moisture.

In conventional microextraction methods, extraction and dispersive solvents are used to concentrate target analytes. Most of these methods employ organic solvents, which are potentially harmful to the environment and may lead to low analyte recovery as the disperser can dissolve the target analytes. Therefore, ionic liquids (ILs) were used in our new method as a “non-conventional extraction and dispersing agent”. ILs are regarded as green solvents due to their special properties, such as thermal stability, low vapor pressure, environmentally benign and good solubility for target compounds (Lu et al., 2011).

To address the limitations of traditional effervescence microextraction techniques, we developed a novel, environmentally-friendly and rapid method for determination of pyrethroids in milk samples. The method employs IL-mediated and effervescence-assisted microextraction for sample pretreatment. First, effervescent tablets were prepared with Fe_3O_4 magnetic nanoparticles, $[\text{C}_n\text{MIM}][\text{PF}_6]$ and sodium carbonate. Next, the magnetic effervescent tablet was placed in the acidified sample solution containing the analytes and the extraction agent was dispersed into the solution as a result of effervescence. Finally, the analytes sorbed to the magnetic nanoparticles were recovered using a magnet. Because this method does not rely on complex and environmentally sensitive devices or instrumentation, it can easily be performed in the field. The magnetic effervescent tablets are stable in storage because the acid and alkaline salts are not in contact with other in the effervescent tablets, and the Fe_3O_4 magnetic nanoparticles can be recovered and recycled. The newly developed method was optimized and applied to pyrethroid detection in dairy milks with different fat contents. The method is simple, rapid, environmentally benign and low cost making it an attractive technique for detection of trace pesticides in food and environmental samples.

2. Materials and methods

2.1. Chemicals and reagents

Standards for the five pyrethroid insecticides (bifenthrin, fenprothrin, permethrin (mixed isomers), deltamethrin (mixed isomers) and fenvalerate (mixed isomers)) and chromatographic-grade isooctane were purchased from Aladdin Reagent Co. (Shanghai, China). The chemical structures of the five pyrethroid insecticides are shown in Supplementary Fig. 1. Sodium chloride (NaCl), sodium carbonate (Na_2CO_3), acetone and hydrochloric acid (HCl , analytical grade) were supplied by Zhejiang Zhongxing Chemical Reagent Co. (Hangzhou, China). Magnetic Fe_3O_4 nanoparticles were purchased from Shanghai Mclean Biochemical Sci. & Technol. Co. (Shanghai, China). Three ionic liquids (purity > 99.0%) were gratis supplied by Shanghai Chengjie Chemical Co. (Shanghai, China): 1-butyl-3-methylimidazolium hexafluorophosphate ($[\text{C}_4\text{MIM}][\text{PF}_6]$), 1-hexyl-3-methylimidazolium hexafluorophosphate ($[\text{C}_6\text{MIM}][\text{PF}_6]$), and 1-octyl-3-methylimidazolium hexafluorophosphate ($[\text{C}_8\text{MIM}][\text{PF}_6]$). Ultrapure water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA).

Mixed standard solutions of pyrethroid insecticides were prepared

in isooctane at a concentration of $1000\text{ }\mu\text{g L}^{-1}$. A HCl solution (1.47 mol L^{-1}) was used to adjust sample pH. Six dairy milk samples were purchased from Wenzhou Baixin Supermarket (Wenzhou, China): full-fat milk (high calcium milk; Yili brand, Inner Mongolia, China), pure milk (6% fat content; Yili brand), half-skimmed milk (high calcium low fat milk; Yili brand), yogurt (2% fat content; Yili brand), skimmed milk (Shuangwaiwai brand; Wahaha, Hangzhou, China), and skimmed milk (zero fat content; Yili brand). Milk samples were stored in a $4\text{ }^\circ\text{C}$ refrigerator for 10 min, centrifuged at 3000 rpm for 5 min, filtered, and stored at $4\text{ }^\circ\text{C}$ before further analysis.

2.2. GC analysis

The five pyrethroids were analyzed using an Agilent 7890 GC (Agilent Technologies, Wilmington, DE, USA) equipped with an electron capture detector (ECD), a splitless injector and an Agilent HP-5 fused-silica capillary column ($30\text{ m} \times 0.32\text{ mm i.d.}$ and $0.25\text{ }\mu\text{m}$ film thickness). The injection and detector temperatures were set at 260 and $300\text{ }^\circ\text{C}$, respectively. Oven temperature was initially held at $80\text{ }^\circ\text{C}$ for 3 min, increased to $200\text{ }^\circ\text{C}$ for 3 min at $15\text{ }^\circ\text{C min}^{-1}$, increased to $250\text{ }^\circ\text{C}$ for 2 min at $5\text{ }^\circ\text{C min}^{-1}$, increased to $280\text{ }^\circ\text{C}$ for 2 min at $8\text{ }^\circ\text{C min}^{-1}$, followed by a $10\text{ }^\circ\text{C min}^{-1}$ ramp to $300\text{ }^\circ\text{C}$, and held at $300\text{ }^\circ\text{C}$ for 2 min. Nitrogen (purity 99.999%) was used as carrier gas at a flow rate of 2 mL min^{-1} .

2.3. Preparation of magnetic effervescent tablets

For 10 tablets, a mixture of Na_2CO_3 (3.392 g), Fe_3O_4 nanoparticles (100 mg) and $[\text{C}_n\text{MIM}][\text{PF}_6]$ (60 μL) was ground into a fine and homogeneous powder. An aliquot (0.1866 g) of the above mixture was compressed into a magnetic effervescent tablet (8-mm diameter $\times$ 2-mm thickness) using a T5 Single Punch Press (Shanghai Pharmaceutical Equipment Co., Shanghai, China) and dried at $60\text{ }^\circ\text{C}$ for 1 h in a drying oven.

2.4. MET-ILM procedures

Fig. 1 shows the schematic diagram of the proposed MET-ILM procedure incorporating a non-conventional extraction and dispersive agent. An appropriate volume of standard solution (1.0 mg L^{-1}), 1.95 mL of 1.47 mol L^{-1} HCl and 2.0% (w/v) NaCl was homogeneously mixed in 8 mL pretreated milk samples in a centrifuge tube (Fig. 2a). Subsequently, a magnetic effervescent tablet was slowly placed in the centrifuge tube (Fig. 2b). This resulted in a large amount of bubble formation with the effervescence occurring from bottom to top in a $60\text{ }^\circ\text{C}$ water-bath (Fig. 2c). The effervescence procedure lasted for ca. 2 min and the extraction solvent, i.e., ionic liquid, was effectively dispersed by the release of CO_2 (Fig. 2d). Then, a magnet was placed at the bottom of the centrifuge tube, which attracted and isolated the ionic liquid-coated magnetic nanoparticles containing the sorbed analytes. The magnetic nanoparticles were gently settled until they were completely sedimented (Fig. 2e). The supernatant was discarded and $500\text{ }\mu\text{L}$ acetone was added to dissolve the analytes from the magnetic nanoparticles. The organic solvent was passed through a $0.22\text{ }\mu\text{m}$ filter, dried with a gentle nitrogen gas flow and dissolved in isooctane. Finally, $1.0\text{ }\mu\text{L}$ of the extraction solvent was injected into the GC-ECD for pyrethroid quantification.

2.5. Statistical analysis

Experimental data were reported as mean $\pm$ SD (standard deviation, $n = 3$). Post-hoc Tukey test was used for multiple mean comparisons among different treatments (Fig. 3a). Dunnett test was applied for two mean comparisons among different treatments (Fig. 3d and f). All statistical analyses were conducted with SPSS 18.0 (SPSS, Chicago, USA) using a $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$

Fig. 1. Schematic diagram of MET-ILM procedures *Note:* (1) A, magnetic effervescent tablet formation; (2) B, dispersive process of magnetic effervescent tablets in aqueous solution; (3) C, elution and analytical processing of milk samples.

Fig. 2. Effervescence-forming photographs for each step of the MET-ILM method *Note:* a–e steps in MET-ILM procedures are described in detail in the text.

significance level, unless otherwise stated. Control was marked in figures.

3. Results and discussion

In this study, effervescence was chosen to disperse the extractant ionic liquid and magnetic nanoparticles to replace traditional dispersive organic solvents (often acetone, acetonitrile or methanol). Effervescence is effective and saves time compared to use of physical dispersive methods such as vortex or shaking (Abera, Francisco, Ana, Negussie, and Monsalud, 2015). In the MET-ILM process, HCl was first added to the aqueous solution as the acid source, and ILs were added in the magnetic effervescent tablets as extraction solvent. To achieve maximum extraction efficiency, a series of parameters were optimized (e.g., effervescent tablet composition, type and volume of ILs, elution solvent, salt addition, temperature and water-bath time). Because pyrethroids often have isomers, the average isomer peak cluster area for each pyrethroid species was quantified to assess extraction efficiency (Dallegrave, et al., 2016).

3.1. Composition of effervescent tablets

When HCl is added to the aqueous solution as the acid source, the effervescent tablets provide the alkali source for the extraction process. Sodium carbonate (Na_2CO_3) and sodium bicarbonate (NaHCO_3) are often selected as the alkali source (Guillermo, Rafael, Soledad & Miguel, 2011). However, NaHCO_3 absorbs moisture from the atmosphere, which decreases extraction efficiency for the analytes. Additionally, NaHCO_3 is not thermally stable as compared with Na_2CO_3 . Thus,

Fig. 3. Optimization of experimental variables in the MET-ILM method Note: (1) a, HCl:Na₂CO₃ ratio; (2) b, IL volume; (3) c, salt percentage; (4) d, elution solvent type; (5) e, water-bath time; (6) f, water-bath temperature.

Na₂CO₃ was selected as the CO₂ (alkali) source for the effervescent tablets.

Three tablet formulations were prepared to assess their extraction efficiency: (1) Tablet #1, Na₂CO₃ + ([C_nMIM]PF₆); (2) Tablet #2, Na₂CO₃ + Fe₃O₄; (3) Tablet #3, Na₂CO₃ + [C_nMIM]PF₆ + Fe₃O₄. Average peak areas indicating recoveries for the five pyrethroids were prominently higher for Tablet #3 (Supplementary Fig. 2A) compared with those of Tablets #1 and #2 (Supplementary Fig. 2B and C). This indicates that the mixture of IL and Fe₃O₄ had a higher extraction efficiency than their independent components alone. Because IL and Fe₃O₄ perform roles in adsorbing and extracting analytes, respectively, their interactive effects increased extraction recovery compared to their

independent use. As a consequence, the mixture of IL + Fe₃O₄ was selected as the optimum tablet formulation.

The ratio of HCl to Na₂CO₃ is also an important variable in optimizing MET-ILM procedures. For the 8-mm-diameter $\times$ 2-mm-thickness tablets (0.1866 g), a series of Na₂CO₃ amounts was investigated to assess reaction completeness in 1.47 mol L⁻¹ HCl solution. Based on reaction stoichiometry, a series of HCl:Na₂CO₃ molar ratios were analyzed: 1.5:1.0, 1.8:1.0, 2.0:1.0, 2.3:1.0 and 2.5:1.0. The five pyrethroids were effectively extracted with increasing HCl:Na₂CO₃ molar ratios up to 2.0:1.0 and then significantly decreased with a further increase in the molar ratio (Fig. 3a). Based on these results, a 2.0:1.0 M ratio of HCl to Na₂CO₃ was selected as the optimum ratio in the effervescent tablet.

3.2. Selection of IL type and volume

In MET-ILM procedures, an ideal IL extractant should have good chromatographic behavior, low solubility in aqueous solution, and high extraction capability for pyrethroids. Based on these criteria, three ILs, [C₄MIM]PF₆, [C₆MIM]PF₆ and [C₈MIM]PF₆, having contrasting hydrophobicity were selected for evaluation. Different alkyl chain length leads to different viscosities (450 cP for [C₄MIM]PF₆, 585 cP for [C₆MIM]PF₆ and 710 cP for [C₈MIM]PF₆), and water solubilities ([C₄MIM]PF₆ (18.8 µg L⁻¹) > [C₆MIM]PF₆ (7.5 µg L⁻¹) > [C₈MIM]PF₆ (2.0 µg L⁻¹)). Higher IL solubility and viscosity is expected to lead to weaker extraction efficiency. [C₄MIM]PF₆ showed the poorest extraction efficiency (< 40%) among three ILs, possibly due to its relatively high solubility in water. While [C₈MIM]PF₆ had the highest viscosity, it did not easily transfer the analytes resulting in poor extraction efficiency (50–80%). [C₆MIM]PF₆ provided the highest extraction recovery (78–102%). Its higher recovery may be explained by its mid-range viscosity and hydrophobicity characteristics that result in its low solubility and ease of separation from the aqueous phase (Rankke, Othman, Fan, & Müller, 2009). Based on these results, [C₆MIM]PF₆ was selected as the fortified IL in the effervescent tablets.

To optimize the [C₆MIM]PF₆ amount, a series of [C₆MIM]PF₆ volumes (30–70 µL) was added to the effervescent tablets to evaluate extraction efficiency. Higher extraction efficiency was obtained with increasing volumes from 30 to 60 µL (Fig. 3b); however, a sharp decline in extraction efficiency (20–30%) occurred as the volume increased from 60 to 70 µL. Considering the effervescent tablet formation process, it appears that when [C₆MIM]PF₆ volume exceeded 60 µL that IL volume was lost from the tablet during the tablet-pressing procedure resulting in the lower extraction efficiency. Therefore, 60 µL was chosen as the optimum IL volume.

3.3. Influence of salt addition

Due to salting-out effects, salt additions often improve extraction efficiency (Hu, Wang, Qian, Liu, et al., 2016). However in some instances, salt additions to the aqueous sample at non-saturated levels may have no influence, or even limit extraction efficiency. In these cases, NaCl dissolved in the aqueous solution may change the physical properties of the Nernst diffusion film and reduce rates of target analyte diffusion into the microdrop (Wang et al., 2016; Gao et al., 2015). In this investigation, NaCl (1–5%, w/v) was added to examine its effect on extraction efficiency. Extraction efficiency for the target analytes was remarkably improved with an increase of salt concentration from 1 to 2% (Fig. 3c). This may result from the small amount of salt playing an active role on demulsification, which may improve mass-transfer of the analytes. With increasing salt concentrations (3–5%) the extraction efficiency decreased or remained relatively unchanged. Reduced extraction efficiencies may result from the higher salt concentrations increasing viscosity and ionic strength, which subsequently decrease mass-transfer of target analytes. Based on these findings, a 2% NaCl concentrations was employed for the aqueous phase.

3.4. Selection of elution solvent

In MET-ILM procedures, the elution solvent is an important parameter because it separates ILs and analytes from the magnetic nanoparticles. The optimal solvent provides high solubility and fast migration of pyrethroids from magnetic nanoparticles. Five solvents (acetone, isooctane, acetonitrile, ethanol and methanol) were investigated for their desorption properties. Acetone gave the highest extraction recoveries for the five analytes, likely due to higher solubility of [C₆MIM]PF₆ in acetone (Fig. 3d). Thus acetone was deemed the best solvent for subsequent experiments.

3.5. Water-bath time

Water-bath time was defined as the interval between placement of the magnetic effervescent tablet into the centrifuge tube and the initiation of magnetic nanoparticle removal from the sample using a magnet. Upon bubble formation, ILs require some time to become well dispersed into fine droplets in the sample; however, long water-bath times often result in loss of solution from the centrifuge tube due to excessive foaming. Consequently, water-bath times of 1–5 min were evaluated to assess extraction efficiencies. Peak extraction efficiencies for the target analytes improved with increasing water-bath times from 1 to 2 min, reached a maximum at 2 min, and then gradually decreased at times greater than 2 min (Fig. 3e). As a consequence, a 2 min water-bath time was employed in subsequent experiments.

3.6. Water-bath temperature

To assess the effect of water-bath temperature on extraction efficiencies, a broad temperature range (30–70 °C) was tested. The extraction efficiencies for pyrethroids increased rapidly with the increase of temperature from 30 to 60 °C, except for permethrin. These results suggest that higher temperatures assist in the dispersion of ILs by producing more CO₂ and faster reaction kinetics. However, increasing temperature from 60 to 70 °C led to significantly decreased extraction efficiencies for fenvalerate, deltamethrin, fenpropathrin and bifenthrin (Fig. 3f). This likely results from changing distribution coefficients of pyrethroids with the IL or water phases, which are closely related to temperature. Solubility increases appreciably at higher temperatures, which greatly increases the probability that pyrethroids will transfer from the IL phase to aqueous phase and consequently decrease the extraction efficiency. On the basis of these findings, 60 °C was selected as the optimum water-bath temperature.

3.7. Analytical performance metrics

Based on the optimization studies, the optimal parameters for the MET-ILM procedures were summarized as 2.0:1.0 of HCl:Na₂CO₃, 60 µL [C₆MIM]PF₆ as extraction solvent, acetone as elution solvent, 2 min water-bath duration at 60 °C and 2% NaCl. Under optimized conditions, a series of analytical indices were determined to validate the efficacy of the newly developed method: extraction recovery (ER), precision, linear range (LR), limits of detection (LODs) and limits of quantification (LOQs). The linear range was 0.08–400 µg kg⁻¹ for bifenthrin, 0.11–400 µg kg⁻¹ for fenpropathrin, 0.25–400 µg kg⁻¹ for permethrin, 0.09–400 µg kg⁻¹ for deltamethrin and 0.08–400 µg kg⁻¹ for fenvalerate with regression coefficients (R²) ranging from 0.9962 to 0.9998 (Table 1). The LODs (signal-to-noise (S/N) ratio of 3) were in the range 0.024–0.075 µg kg⁻¹, while the LOQs (S/N ratio of 10) were in the range 0.08–0.25 µg kg⁻¹. The precision of the method, expressed as relative standard deviation (RSDs), was assessed by a series of replicate experiments. The intra-day and inter-day precisions at three concentrations (low = 10 µg kg⁻¹, medium = 50 µg kg⁻¹ and high = 100 µg kg⁻¹) were evaluated using six replicate samples. The RSDs of the five pyrethroids were < 4.8% for intra-day repeatability precision, and no greater than 6.3% for inter-day reproducibility precision. Recoveries were examined at three spiked levels (low = 10 µg kg⁻¹, medium = 50 µg kg⁻¹ and high = 100 µg kg⁻¹) to analyze the accuracy of the method. The spiked extraction recoveries for the five pyrethroids ranged from 78.3 to 101.8% with standard deviations (SDs) of 0.2–6.9% under the optimized extraction conditions (Table 2).

3.8. Analysis of milk samples

The MET-ILM method combined with GC-ECD was applied to the analysis of five pyrethroids in milk samples with different fat contents

Table 1

Performance characteristics of the MET-ILM method combined with GC-ECD. Note: (1) R^2 indicates coefficient of determination; (2) LR is linear range; (3) LOD indicates limit of detection ($S/N = 3 \mu\text{g kg}^{-1}$); (4) LOQ denotes limit of quantification ($S/N = 10 \mu\text{g kg}^{-1}$); (5) RSD is abbreviation of relative standard deviation ($n = 6$, low = $10 \mu\text{g kg}^{-1}$, medium = $50 \mu\text{g kg}^{-1}$ and high = $100 \mu\text{g kg}^{-1}$).

Pesticides	R^2	LR ($\mu\text{g kg}^{-1}$)	LOD ($\mu\text{g kg}^{-1}$)	LOQ ($\mu\text{g kg}^{-1}$)	Intra-day RSD (%)			Inter-day RSD (%)		
					Low	Medium	High	Low	Medium	High
Bifenthrin	0.9998	0.08–400	0.024	0.08	4.2	3.0	2.1	4.8	3.7	3.4
Fenprothrin	0.9976	0.11–400	0.033	0.11	3.2	3.1	2.8	5.1	3.0	2.5
Permethrin	0.9985	0.25–400	0.075	0.25	3.0	2.9	2.3	6.3	4.2	2.9
Deltamethrin	0.9962	0.09–400	0.027	0.09	2.7	1.7	1.6	4.4	2.7	1.9
Fenvalerate	0.9997	0.08–400	0.024	0.08	4.8	3.1	2.2	4.3	4.2	3.2

(two full-fat, two half-skimmed and two skimmed). The five pyrethroids were below their respective detection levels in the full-fat and half-skimmed milks (Supplementary Table 1). However, deltamethrin and fenvalerate were detected at 0.13 ± 0.003 and $0.11 \pm 0.004 \mu\text{g kg}^{-1}$, respectively, in skimmed milk sample #2. Although deltamethrin and fenvalerate were detected in the milk samples, their residual levels were far below the maximum residual limit (0.02 mg kg^{-1}) by GB 2763-2014. Fig. 4 shows the GC-ECD chromatograms for the five pyrethroids at fortification levels of 0 and $0.25 \mu\text{g kg}^{-1}$ in full-fat, half-skimmed and skimmed milks. Lipid content has a great effect on the extraction recoveries for analytes, and thus it should be removed as complete as possible prior to microextraction procedures. In previous reports, many methods have been utilized to clean up lipids such as liquid–liquid extraction (Taghvaei, Piravivanak, Rezaei, & Faraji, 2016), low-temperature freezing cleanup method (Chen et al., 2009; Liu et al., 2009) and solid phase extraction (Mojtaba, Najmeh & Mahnaz, 2016). In this investigation, the milk samples were firstly stored at 4°C , then centrifuged to remove proteins, and the subsequent MET-ILM procedures had the role in purifying lipid. Although no additional method was adopted to remove lipid prior to MET-ILM, the satisfied recoveries (78.3–101.8%) for pyrethroids proved the feasibility of this proposed method.

3.9. Comparison of new MET-ILM method with related methods

In the new MET-ILM method, the extraction process was performed using a centrifuge tube, a magnet and a lab-prepared reagent tablet. Tablets can be prepared in advance and maintained for a long time in a desiccator without any deterioration. Therefore, analytical operations are very simple and practical for use in the field. As compared with liquid–liquid extraction (LLE) and solid-phase extraction (SPE), the new method requires no organic solvents or centrifugation and vortex steps.

The new MET-ILM method achieved lower LODs for pyrethroids (0.024 – $0.075 \mu\text{g kg}^{-1}$), than LLE-Freezer-GC-ECD (0.25 – $0.75 \mu\text{g kg}^{-1}$) (Simone, Queiroz, Neves & Queiroz, 2008), LLE-Floril-GC-ECD (15 – $60 \mu\text{g kg}^{-1}$) (Zehring & Herrmann, 2001), and QuEChERS-GC-ECD (0.1 – $0.8 \mu\text{g kg}^{-1}$) (Gao, Zhang, Wang, Hua & Zhang, 2010), and comparable LODs with SPME-GC-ECD (0.003 – $0.56 \mu\text{g kg}^{-1}$) (Maria et al., 2008). Importantly, the new MET-ILM method attains better ERs (78.3–101.8%) than those of LLE-Floril-GC-ECD (30–92%), SPME-GC-ECD (69–139%), and SPE-DLLME-GC-MS (66–105%) (Mojtaba, et al., 2016), and comparable ERs with LLE-Freezer-GC-ECD (84.0–93.0%). The traditional methods such as LLE, SPE and SPME show limitations, e.g. tricky steps, large amounts of toxic organic solvent and disposable adsorbents. However, this proposed method is environmentally benign with reusable adsorbents and using “green solvents” (ionic liquids) to replace traditional volatile organic solvents to separate and concentrate pyrethroids, which can decrease the consumption of organic solvent to the least. The pretreatment time for the MET-ILM method is relatively short (ca. 30 min) and the convenient collection of magnetic adsorbents by a magnet can avoid using tedious operation processes such as centrifugation and filtration. Most importantly, the prepared tablet can be stored for long time periods without deterioration which can substantially reduce the pretreatment time and workload for sample preparation. Also, this method does not rely on complex and environmentally sensitive devices or instrumentation, and can easily be performed in the field. Overall, the new MET-ILM is simple, time-saving, accurate/sensitive/reproducible, no requirement of expensive instrument and environmentally benign (Supplementary Table 1). It therefore has great potential for application in food testing and environmental detection of trace pesticides under both laboratory and field conditions.

Table 2

Analytical performance of the MET-ILM method in milk samples ($n = 3$)

Analytes	Spiked level ($\mu\text{g kg}^{-1}$)	Full-fat milk	Half-skimmed milk	Skimmed milk
		Recovery $\pm$ SD (%)	Recovery $\pm$ SD (%)	Recovery $\pm$ SD (%)
Bifenthrin	10	89.7 ± 4.8	88.1 ± 4.5	85.8 ± 1.6
	50	99.8 ± 4.1	97.3 ± 0.2	94.5 ± 0.3
	100	94.0 ± 5.4	101.8 ± 7.4	97.8 ± 0.5
Fenprothrin	10	84.2 ± 2.3	79.5 ± 0.5	79.2 ± 1.9
	50	78.9 ± 4.0	83.5 ± 0.4	87.3 ± 2.4
	100	83.6 ± 3.0	92.3 ± 1.1	78.8 ± 2.6
Permethrin	10	83.4 ± 2.7	94.7 ± 4.4	98.4 ± 6.9
	50	86.2 ± 3.1	92.5 ± 2.5	91.8 ± 2.5
	100	80.7 ± 3.2	89.7 ± 1.4	80.7 ± 2.6
Deltamethrin	10	96.4 ± 2.0	93.3 ± 5.0	93.1 ± 3.4
	50	78.3 ± 0.7	101.6 ± 1.1	99.1 ± 0.3
	100	86.7 ± 0.7	85.2 ± 4.4	85.7 ± 6.8
Fenvalerate	10	93.2 ± 2.0	88.5 ± 3.5	81.7 ± 5.4
	50	89.1 ± 1.4	97.1 ± 4.0	92.9 ± 4.4
	100	79.1 ± 1.7	82.1 ± 3.4	84.4 ± 1.1

Fig. 4. GC chromatograms of five pyrethroids in milk samples *Note:* (1) A, Full-fat milk at spiked level of 0.25 $\mu\text{g kg}^{-1}$; (2) B, Half-skimmed milk at spiked level of 0.25 $\mu\text{g kg}^{-1}$; (3) C, Skimmed milk at spiked level of 0.25 $\mu\text{g kg}^{-1}$; (4) 1, bifenthrin; 2, fenpropathrin; 3–4, permethrin; 5–6, fenvalerate; 7–8, deltamethrin.

4. Conclusions

We developed a novel and simple MET-ILM method for analysis of pyrethroids in milk samples based on magnetic effervescent tablet-assisted extraction. Because of the usage of ILs and only an alkali source in the magnetic tablets, the MET-ILM method gave high extraction efficiency for pyrethroids and the tablet was stable during long-term storage. Pyrethroids were recovered on the IL-coated magnetic nanoparticles allowing analyte separation with a magnet, thus avoiding the necessity for centrifugation or vortex steps. The new method exhibits several important advantages such as environmentally benign, short extraction time, easy operation practical for field use, and good recovery/sensitivity/accuracy. As a consequence, this newly developed MET-ILM method provide a promising method for separation and pre-concentration of pyrethroids in complex matrices and its simple operation makes it practical for field use.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.foodchem.2018.06.099>.

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