Talanta 105 (2013) 219–228

Contents lists available at SciVerse ScienceDirect

Talanta

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

A modified QuEChERS method for the determination of some herbicides in yogurt and milk by high performance liquid chromatography

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ARTICLE INFO

Article history:
Received 4 August 2012
Received in revised form
20 November 2012
Accepted 24 November 2012
Available online 1 December 2012

Keywords:
A modified quick, cheap, effective, rugged, and safe (QuEChERS) method
Milk
Yogurt
Triazine
Phenylurea

ABSTRACT

A modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method was applied to the extraction of triazines and phenylureas from milk and yogurt. The herbicides was extracted by the mixture of ethyl acetate and n-hexane and cleaned by primary secondary amine (10 mg/mL). The frozen-out centrifugation was applied to further remove fatty. The proposed method can achieve efficient extraction and cleanup. Some experimental parameters, such as extraction method, extraction solvent and adsorbent, pH of sample solution, extraction time and amount of primary secondary amine and sodium chloride were investigated and optimized. The precision and absolute recoveries of eight herbicides vary from 0.07 to 5.86% and from 78.9 to 99.9%, respectively. The detection limits for simeton, monuron, chlorotoluron, simetryne, atrazine, karmex, ametryne and propazine range from 0.15 to 0.35 ng/mL.

1. Introduction

Triazines and phenylureas are used worldwide as selective pre- and post emergence herbicides for the control of both grasses and broadleaf weeds in agriculture. The triazines also are applied for nonagricultural purposes such as soil sterilization and road maintenance [1]. Because the herbicides are chemically stable, they can remain active for several years in the environment, thus causing ecological damage. On the one hand, the herbicides may be transferred to ground and surface water, soil and atmosphere natural or factitious [2]. On the other hand, feed and water contamination may be indirect reasons for herbicides residues in animal products such as milk and yogurt. Herbicides residues in food could pose a risk to human health [3,4]. The European Union (EU) legislation harmonizes a maximum residue limits (MRLs) of the pesticides and fixes default value of MRLs at 0.01 mg/kg for human food (Commission Directive 2008/149/EC). Milk is traditional daily drink of human. In milk manufacture, the collected raw milk was transferred at low temperature and separated with a clarifier or separator to remove debris, sediment and some bacteria. A separator was used to separate the heavier milk fat to produce skim milk and yogurt. The milk is then fortified and sterilized commonly at the high temperature for a short period. To prevent fat separation or gel formation during storage, most milk is homogenized. At last, the milk can be on sale after packaging. And yogurt is an invaluable component in the diet of vulnerable population (infants, children, and the elderly) [5]. For yogurt manufacture, when the milk arrives, its composition is modified before using to make yogurt. This standardization process typically involves reducing the fat content by a separator and increasing the total solids by evaporating off some water or adding concentrated milk or milk powder. The other treatments are similar to milk production. And then yogurt is produced by bacterial fermentation of the treated milk. The bacteria metabolize certain compounds such as lactose in the milk producing the characteristic yogurt flavor [6]. Unfortunately, milk has been found to be contaminated with herbicides [7–9]. Recently, some high content of triazines which are far above MRLs have been detected in raw milk and infant formula by Angeles Garcia et al. [9]. So it is of essential importance to determine the herbicide residues both in milk and yogurt.

Sample preparation is critical in analysis of complex matrices analysis. To date, some sample preparation methods have been developed for the determination of pesticides in dairy products. Liquid–liquid extraction (LLE) [10] and solid-phase extraction(SPE) [11,12] are conventionally used methods. With the acceptable organic solvent consumption, matrix solid-phase dispersion (MSPD) [13] and quick, easy, cheap, effective, rugged, and safe (QuEChERS) method [8,14] rapidly developed in recent years. In addition, ionic liquid foaming-based solvent floatation (ILF-SF) [15] and diphasic dialysis extraction [9] have been reported. Additionally, some microextraction methods have also

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http://dx.doi.org/10.1016/j.talanta.2012.11.057

been developed, including headspace solid phase microextraction (HS-SPME) [16], carbon nanotube-reinforced hollow fiber solid-phase microextraction (CNTs-HF-SPME) [17] and microwave assisted ionic liquid microextraction (MAILME) [18]. In addition, Howard et al. have applied nano-solid phase dispersion to the extraction of analytes, in which the dispersion is maintained by Brownian motion and slow gravitational sedimentation [19–21]. Liquid chromatography (LC) is the preferred approach for monitoring these polar and thermally labile herbicides. Most of the known applications are based on high performance liquid chromatography (HPLC) coupled to conventional ultraviolet (UV) detection, diode array detection (DAD) and mass spectrometric (MS) detection. In addition, capillary electrophoresis (CE) [22] and gas chromatography (GC) [2,9] were applied.

The QuEChERS method has the advantages of safety, simplicity, affordability, effectiveness, efficiency and dependability of results. The QuEChERS method was developed by Anastassiades et al. in 2003 for the analysis of pesticides in fruits and vegetables [23]. This original method involves extraction with acetonitrile partitioned from the aqueous matrix using anhydrous $MgSO_4$ and NaCl and cleanup by dispersive-SPE(DSPE) with $MgSO_4$ and primary secondary amine (PSA). The approach is very flexible and has been modified several times since 2003. The QuEChERS method was modified with acid buffers for pH-sensitive pesticides. The acetate-buffering QuEChERS method became "AOAC Official Method 2007.01" [24] and the citrate-buffering version being named European Committee for Standardization (CEN) Standard Method EN 15662. Additionally, water was added to dry samples to obtain the necessary moisture [25]. However, the main disadvantage of the QuEChERS method is that it cannot achieve effective cleanup, especially for some complex matrices. Up to now, several modifications have been reported for the determination of pesticides and drugs in milk. Kinsella et al. used C18 adsorbent replacing PSA for determining anthelmintic drugs in milk [14]. Aguilera-Luiz et al. applied citrate and acetate buffers to extract pesticides in milk [8]. However, few reports focused on the modification of extraction solvent. As well known, the inefficient cleanup mostly is due to the polarity of acetonitrile. So the properly modification of extraction solvent can improve cleanup ability while at the same time keep the suitable extraction efficiency. Forsberg et al. [26] successfully applied a three-component variant solvent system of acetone, ethyl acetate and isooctane for the extraction of contaminants in fish issue. Aysal et al. [27] used ethyl acetate for the extraction of pesticides in vegetables and fruits. And ethyl acetate was also used by Banerjee et al. for determining pesticide residues in grapes [28]. But, so far, the modification of QuEChERS method in extraction solvent for determination of herbicides in milk or yogurt has not been reported.

In the work, to achieve an efficient cleanup and extraction, the mixture of ethyl acetate and n-hexane rather than acetonitrile was used for the extraction of herbicides in milk and yogurt. A small amount of NaCl replaced the salt mixture avoiding the salt agglomeration in the origin QuEChERS method. Moreover, the amount of PSA was reduced from 25 to 10 mg/mL. The frozen centrifugation was applied to remove the fatty matrix. The modified QuEChERS method can achieve a rapid, simple, cheap, efficient and clean extraction of herbicides in complex matrices.

2. Experimental

2.1. Reagents and chemicals

Simeton, monuron, chlorotoluron, simetryne, atrazine, karmex, ametryne and propazine were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The structures of these herbicides are shown in Fig. 1. Standard stock solutions for the herbicides at the concentration level of 100 μg/mL were prepared in acetonitrile. All of the stock standard solutions are stored in a refrigerator at 4 °C. Working standard solutions (10 μg/mL) were prepared every week by dilution of stock standard solutions in acetonitrile. Mixed working standard solutions were prepared in the same way as the working standard solutions. Chromatographic grade acetonitrile was from Fisher Scientific Company (Pittsburgh, PA, USA). Sorbents for DSPE included florisil, primary secondary amine (PSA) and C18-silica purchased from Beijing Agela Technologies Inc. (Beijing, China). Neutral silica gel (Si) was purchased from Qingdao Ocean Chemical Factory (Qingdao, China). Alumina (Al_2O_3) and basic alummia(B-Al_2O_3) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pure water was obtained with

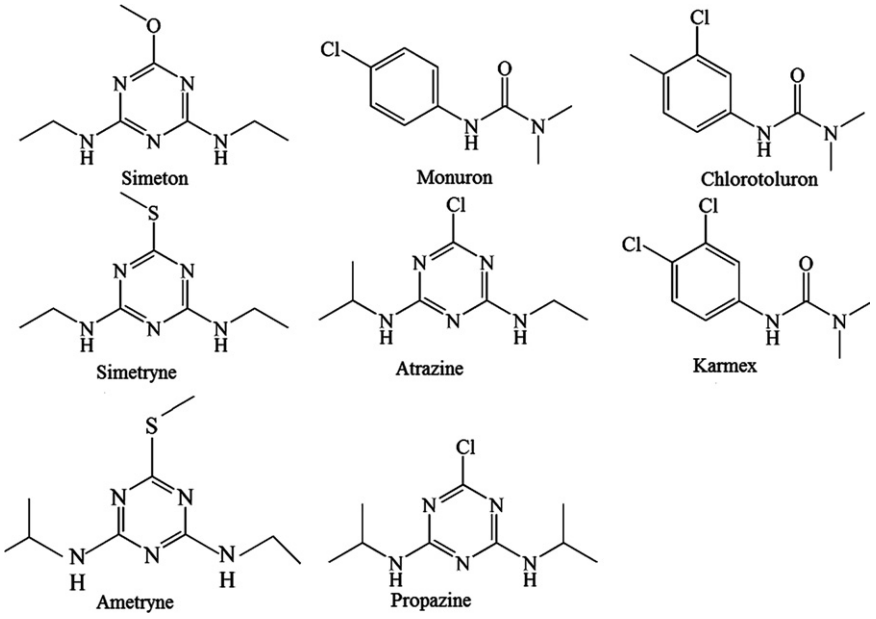

Fig. 1. Structures of triazines and phenylureas.

a Milli-Q water purification system (Millipore Co., USA). All other reagents were of analytical-reagent grade and from Beijing Chemical Factory (Beijing, China).

2.2. Sample collection and preparation

The milk and yogurt samples were purchased from local large-scale supermarket and stored at 4 °C. In the study, three kinds of yogurt samples, including plain (sample 1), low-fatty (sample 2) and no-sucrose (sample 3) yogurt, and three kinds of milk samples, including pure (sample 4), skim (sample 5) and pasteurization (sample 6) milk were used. The contents of main components in samples 1–6 provided by the manufactures are as follows: The concentrations of protein in samples 1–6 are 2.5, 2.3, 2.8, 3.0, 3.0 and 3.0 g/100 g; the concentrations of lipid in samples 1–6 are 2.8, 0.8, 2.9, 3.5, 0 and 3.3 g/100 g and the concentrations of carbohydrate in samples 1–6 are 6.2, 6.5, 3.5, 5.0, 5.1 and 4.5 g/100 g, respectively. Except for the experiments mentioned in Sections 3.2.3 and 3.2.4, all other results were obtained with sample 3. The experiments were performed with all six samples in Section 3.2.3 and with four samples except sample 3 and 6 in Section 3.2.4.

The fresh spiked samples containing triazines and phenylureas were prepared by spiking the mixed working standard solutions into yogurt and milk samples and shaking for 10 min.

The aged spiked samples(samples 1, 2, 4 and 5) was prepared by the method mentioned above except that the spiked sample was kept in sealed bottle and stored for 7, 14, 21 and 28 days at 4 °C, respectively.

2.3. Instrumentation

Chromatographic separation and determination of the herbicides were carried out on the 1100 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with the photodiode-array detector (DAD) and quaternary gradient pump. An eclipse XDB-C18 column (3.5 μm, 4.6 mm × 150 mm, Agilent, USA) was used.

The Allegra™ 64 R centrifuge was purchased from Beckman Coulter. SH-36 mixer (Zhenghui, Shanghai, China) were used in the extraction step. The RE-52AA vacuum rotatory evaporator (Yarong, Shanghai, China) was employed. The KQ-100DE ultrasonic cleaner (Kunshan, Jiangsu, China) and a modified household microwave oven (NN-MX25WF, Shanghai, China) were used.

2.4. Extraction procedure

Five millilitre of sample was added into 25 mL centrifuge tube and the pH value of sample was adjusted to 7 with 1 mol L^{-1} NaOH and 1 mol L^{-1} HCl and vigorously shaken for 10 s. Subsequently, 8 mL of extraction solvent (ethyl acetate: n-hexane = 1:1) and 0.3 g of NaCl were added into the tube and mixed for 2 min by the extraction method (vibration method). The sample was centrifuged at 15000 rpm for 5 min at 0 °C and the supernatant was decanted into another centrifuge tube containing 80 mg PSA. The mixture was mixed in mixer for 1 min and centrifuged at 4000 rpm for 8 min. Then, the supernatant was transferred into a glass flask, evaporated to dryness and redissolved with 250 μL of dissolved solvent (methanol). The resulting solution was centrifuged at 15000 rpm for 10 min at −10 °C. Finally, the supernatant was filtered with a 0.22 μm PTFE filter before injection.

2.5. HPLC conditions

Separation of the analytes was performed using gradient elution at a flow rate of 0.5 mL/min at 35 °C of column temperature. Mobile phases A and B are acetonitrile and water, respectively. The gradient conditions are as follows: 0–3 min, 25–30% A; 3–5 min, 30–40% A; 5–10 min, 40–48% A; 10–20 min, 48–50% A; 20–22 min, 50% A. The monitoring wavelength was set at 228 nm for simeton, simetryne, atrazine, ametryne and propazine and 245 nm for monuron, chlorotoluron and karmex.

2.6. The reference method

For comparison, a reference method was applied. The sample preparation was the same as mentioned in Section 2.2. The extraction procedure was mostly the same as described by Kinsella et al. [14]. Ten millilitre of sample were added into 30 mL centrifuge tubes. Then the sample was extracted with 12 mL of ACN in the presence of the mixture of 4 g of $MgSO_4$ and 1 g of NaCl. Sample was immediately shaken for 1 min and centrifuged at 2842 rpm for 12 min. The supernatant was transferred into a centrifuge tube containing 1.5 g of $MgSO_4$ and 0.5 g of C18. The sample was vortexed for 1 min and centrifuged at 2842 rpm for 10 min. 6 mL of the supernatant was transferred into a glass flask, evaporated to dryness and redissolved with 250 μL of methanol. The methanol was used as dissolved solvent while dimethyl sulfoxide(DMSO) was used in Kinsella's method.

3. Results and discussion

3.1. Optimization of the modified QuEChERS conditions

In this study, a modified QuEChERS method combined with HPLC-DAD was applied to the determination of herbicides in yogurt and milk samples. Some parameters that affect the extraction performance and efficiency were investigated and, then, the optimal conditions were selected.

3.1.1. Effect of extraction methods

The extraction methods including ultrasonic, microwave and vibration methods were investigated. The results are shown in Fig. 2. The vibration method gives higher peak area than the other methods and the ultrasonic method gives the lowest peak area. Due to the low polarity, extraction solvent can disperse better in vibration method compared to the other methods and thus extraction efficiency is better. Therefore, the vibration method was selected as extraction method for further studies.

3.1.2. Effect of extraction solvent and adsorbent

The extraction solvent and adsorbent can significantly affect the extraction and cleanup efficiency. To obtain the optimized parameters, four organic solvents, including acetonitrile (ACN), ethyl acetate/n-hexane(v/v, 1/1), ethyl acetate (EA) and dichloromethane (CH_2Cl_2), and six adsorbents, including PSA, C18, Al_2O_3, B-Al_2O_3, Florisil and Si, were investigated. A representative three-dimensional bar graphics is shown in Fig. 3. The extraction efficiency of herbicides obtained with ACN and ethyl acetate/n-hexane(v/v, 1/1) is close. The peak area is lowest obtained with CH_2Cl_2. The much cleaner chromatogram and lower baseline noise are obtained with ethyl acetate/n-hexane compared with those obtained with ACN. When the amount of PSA in ACN is 25 mg/mL, the cleanup result is also unsatisfactory and the peak area decreases. Compared to other adsorbents, PSA can achieve better cleanup efficiency. The optimum results can be achieved using the ethyl acetate/n-hexane (v/v, 1/1) as extraction solvent and PSA as adsorbent. Therefore, ethyl acetate/n-hexane (v/v, 1/1) and PSA were used in the following experiments.

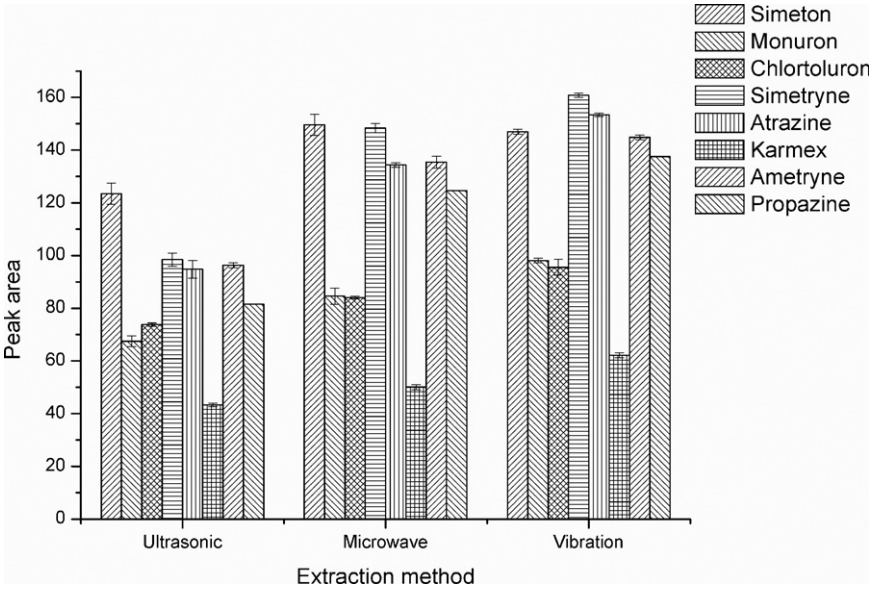

Fig. 2. Effect of extraction methods. Extraction solvent, 8 mL of ethyl acetate/ hexane(v/v, 1/1); pH of sample solution, 7; extraction time, 2 min; adsorbent, 80 mg of PSA; amount of NaCl, 0.3 g and spiked concentration, 25 ng/mL.

3.1.3. *Effect of pH*

The influence of pH value of sample solution over the range of 1–11 on the peak area was investigated. The results are shown in Fig. 4. The pH value of sample solution has a significant effect on extraction of triazine herbicides. The peak areas of triazines increase with the increase of pH from 1 to 7 and then decrease when the pH is higher than 7. Because the triazines are weak bases and can be hydrolyzed at excessively low pH value. And the pH value showed a minor effect on the extraction of phenylurea herbicides owing to their stable neutral structure. Thus, the pH value of 7 was chosen for the following experiments.

3.1.4. *Effect of extraction time*

The effect of extraction time was evaluated by performing assays in the 0.5 and 10 min range. The results shown in Fig. 5 indicate that the peak areas of analytes were highest when the extraction time was 2 min. And the peak areas changed slightly when the extraction time further prolonged. So the extraction time of 2 min was selected.

3.1.5. *Effect of volume of extraction solvent*

The effect of volume of extraction solvent was examined. The results are shown in Fig. 6. An increase in volume of extraction solvent ranging from 2 to 8 mL leads to an increase in the peak areas of analytes. And the peak areas are unchanged when the volume is larger than 8 mL. Therefore, the optimum volume of extraction solvent was 8 mL

3.1.6. *Effect of amount of PSA*

The effect of amount of PSA was investigated ranging from 0 to 250 mg. The results are shown in Fig. 7. The peak areas of analytes increase with increase of the amount of PSA when the amount is smaller than 80 mg, and slightly decrease when the amount is larger than 80 mg. The cleanup ability increases with the addition of PSA. And the cleanup ability changes slightly when the usage of PSA is more than 80 mg. But when large amount of PSA was used, it adsorbs certain herbicides resulting in low extraction efficiency. Therefore, 80 mg of PSA is suitable.

3.1.7. *Effect of amount of NaCl*

The effect of ionic strength was studied by adding NaCl ranging from 0 to 1.5 g. The results are shown in Fig. 8. The peak areas of analytes increase slightly with the increase of NaCl from 0 to 0.3 g and decrease with the increase of NaCl when the amount of NaCl is larger than 0.3 g. The addition of salt into sample solution can decrease the solubility of analytes and be benefit to phase separation. Hence, the peak areas of analytes increase with the addition of NaCl. However, when the amount of NaCl is excessively large, the viscosity of sample solution increases, which will weaken the mass transfer of the analyte to extraction solvent. Therefore, 0.3 g of NaCl was selected.

3.1.8. *Effect of kind and volume of dissolved solvent*

The kind and volume of dissolved solvent was also evaluated. There was no obviously difference in the peak areas of herbicides obtained with methanol and acetonitrile. But the chromatographic peak shapes obtained with methanol was better than that obtained with acetonitrile, especially the early chromatographic peaks. So methanol was chosen. Subsequently, the volume of methanol was optimized. An optimized volume of methanol was 250 μL.

3.1.9. *Effect of volume ratio of ethyl acetate to hexane*

The solvent composition can affect the polarity of solvent and thus the extraction efficiency. The volume ratio of ethyl acetate to hexane ranging from 8:2 to 2:8 was investigated. The results are shown in Fig. 9. As can be seen the peak areas increase with the descrease of the ratio of ethyl acetate to hexane from 8:2 to 5:5 and then decreases when the ratio is lower than 1. So the mixture of ethyl acetate/n-hexane (v/v, 1/1) was selected.

3.1.10. *Effect of dryning process*

The effect of dryning process on recoveries of the herbicides was investigated. Blank sample was extracted and cleaned by the method mentioned in Section 2.4. The obtained supernatant was spiked. Then the spiked solution was dried and treated by

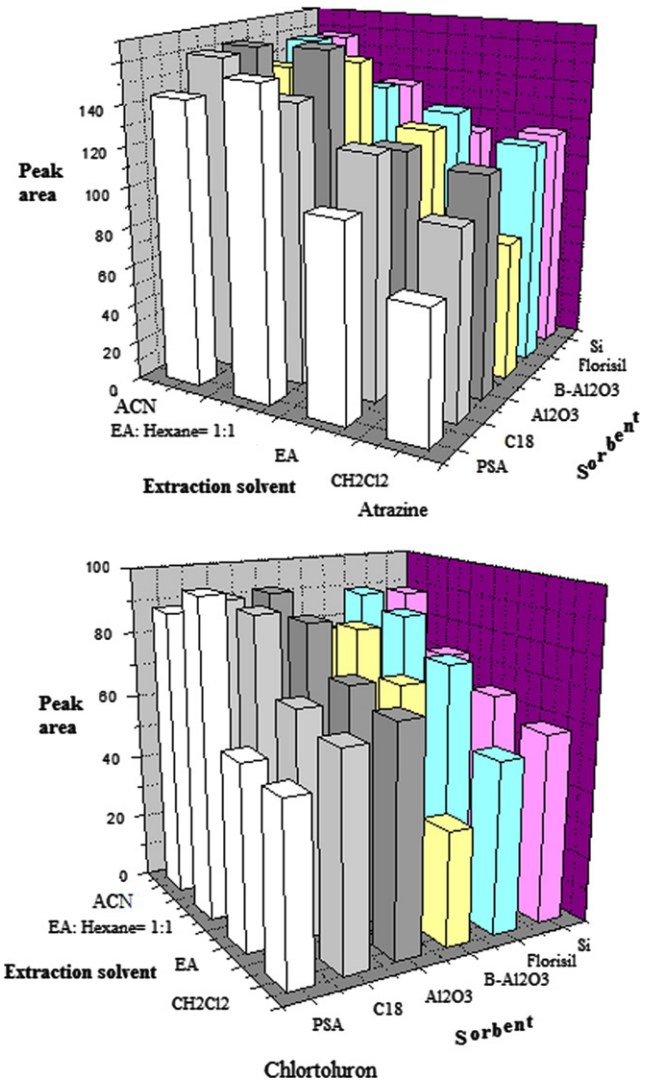

Fig. 3. Effect of extraction solvent and sorbent. Extraction method, vibration; volume of extraction solvent, 8 mL; pH of sample solution, 7; extraction time, 2 min; amount of adsorbent, 80 mg but 150 mg/mL anhydrous MgSO4 especially for ACN; amount of salt, 2 g anhydrous MgSO4 and 0.5 g NaCl for ACN and 0.3 g of NaCl for other solvents and spiked concentration, 25 ng/mL.

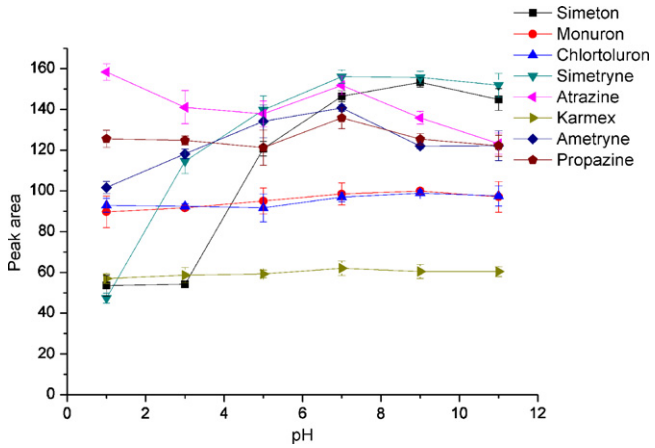

Fig. 4. Effect of pH value of sample solution. Extraction method, vibration; extraction solvent, 8 mL of ethyl acetate/hexane(v/v, 1/1); extraction time, 2 min; adsorbent, 80 mg of PSA; amount of NaCl, 0.3 g and spiked concentration, 25 ng/mL.

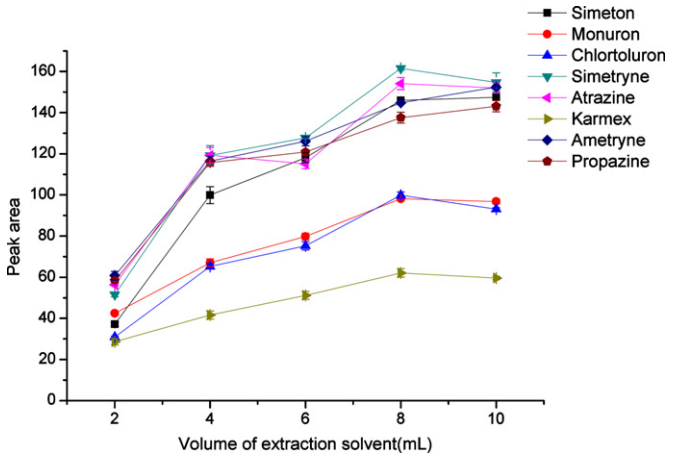

Fig. 6. Effect of volume of extraction solvent. Extraction method, vibration; extraction solvent, ethyl acetate/hexane(v/v,1/1); pH of sample solution, 7; extraction time, 2 min; adsorbent, 80 mg of PSA; amount of NaCl, 0.3 g and spiked concentration, 25 ng/mL.

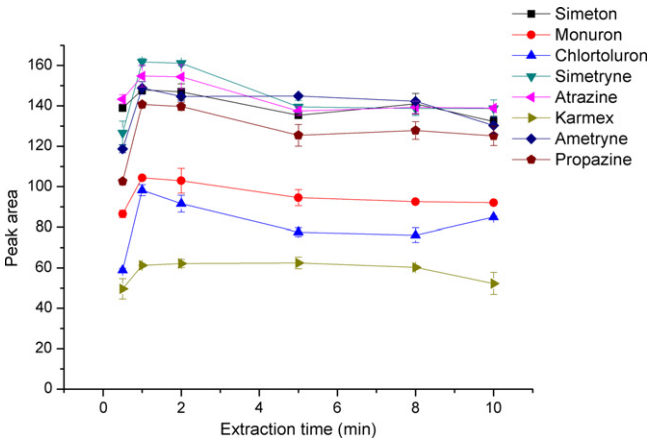

Fig. 5. Effect of extraction time. Extraction method, vibration; extraction solvent, 8 mL of ethyl acetate/hexane(v/v, 1/1); pH of sample solution, 7; adsorbent, 80 mg of PSA; amount of NaCl, 0.3 g and spiked concentration, 25 ng/mL.

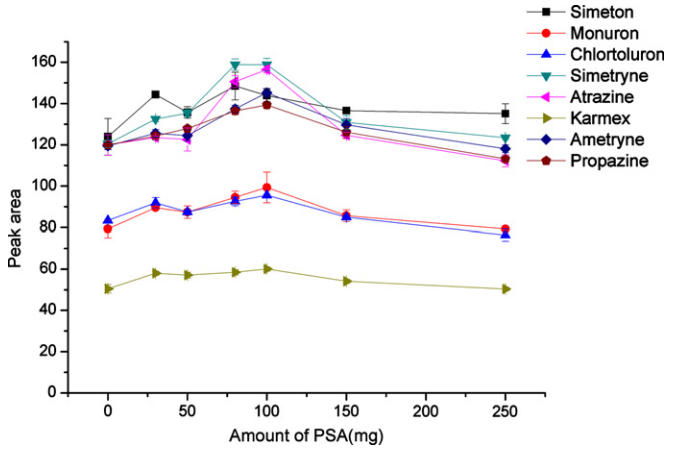

Fig. 7. Effect of amount of PSA. Extraction method, vibration; extraction solvent, 8 mL of ethyl acetate/hexane(v/v, 1/1); pH of sample solution, 7; extraction time, 2 min; amount of NaCl, 0.3 g and spiked concentration, 25 ng/mL.

the method mentioned above. The results shown in Fig. 10 indicate that there is no loss of the analytes due to the drying process.

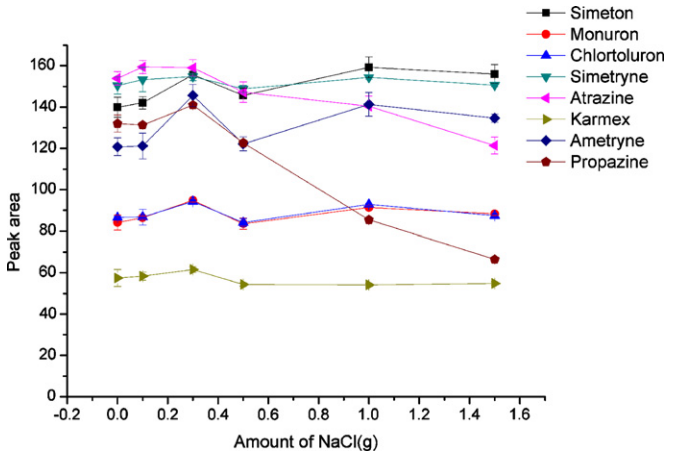

Fig. 8. Effect of amount of NaCl. Extraction method, vibration; extraction solvent, 8 mL of ethyl acetate/hexane(v/v, 1/1); pH of sample solution, 7; extraction time, 2 min; adsorbent, 80 mg of PSA and spiked concentration, 25 ng/mL.

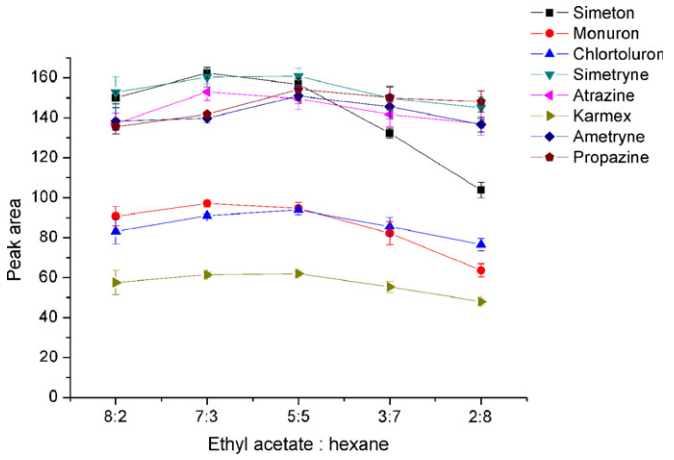

Fig. 9. Effect of the volume ratio of ethyl acetate to hexane. Extraction method, vibration; volume of extraction solvent, 8 mL; pH of sample solution, 7; extraction time, 2 min; adsorbent, 80 mg of PSA, amount of NaCl, 0.3 g and spiked concentration, 25 ng/mL.

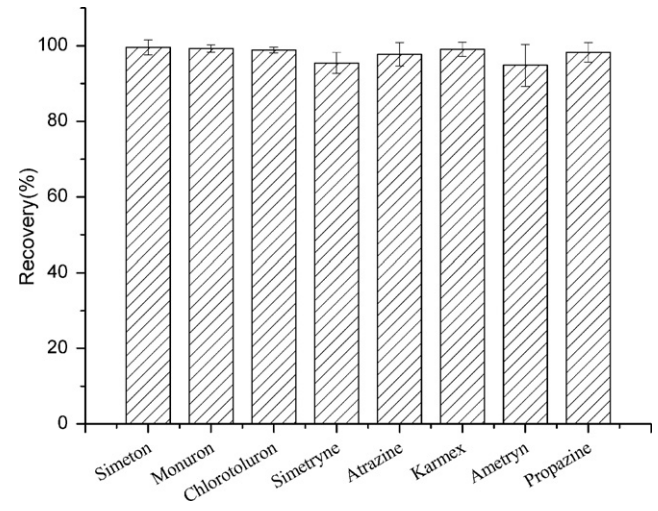

Fig. 10. Effect of dryning process. extraction solvent, 8 mL of ethyl acetate/hexane(v/v, 1/1); pH of sample solution, 7; extraction time, 2 min; adsorbent, 80 mg of PSA; amount of NaCl, 0.3 g and spiked concentration, 25 ng/mL.

3.2. Method validation

3.2.1. Limits of detection and quantification

The working solutions were prepared by diluting the mixed standard solutions with acetonitrile. The working solution for eight herbicides at seven concentration levels, 0.016, 0.021, 0.031, 0.063, 0.25, 1 and 2 μg/mL were used to prepare the standard curves. 20 μL of working solutions were introduced into HPLC and analyzed under the chromatographic conditions mentioned above. The standard curves were obtained by plotting the peak areas versus the concentrations of analytes in working solutions. The curves are used to calculate the recoveries. The linear regression equations and correlation coefficients are listed in Table 1. The correlation coefficients range from 0.99993 to 0.99998.

The working curves were constructed by plotting the peak areas measured versus the concentrations of analytes in the sample solution. The curves are listed in Table 2 and used to evaluate the limits of detection (LODs) and quantification (LOQs). LODs and LOQs of the herbicides were estimated by analyzing spiked samples at low concentrations. LODs and LOQs were calculated on the basis of a signal-to-noise (S/N) ratio of 3 and 10, respectively. The LODs for triazine herbicides and phenylurea

Table 1
Standard curves.

Analyte	Regression equation	Correlation coefficient	Liner range (μg/mL)
Simeton	$A=315.87c-3.11$	0.99993	0.016–2.0
Monuron	$A=226.84c+2.60$	0.99993	0.021–2.0
Chlortoluron	$A=209.78c-1.43$	0.99998	0.021–2.0
Simetryne	$A=356.32c-1.24$	0.99993	0.016–2.0
Atrazine	$A=337.91c-1.84$	0.99997	0.016–2.0
Karmex	$A=127.44c-1.00$	0.99997	0.031–2.0
Ametryne	$A=339.12c-1.02$	0.99996	0.016–2.0
Propazine	$A=364.69c-1.34$	0.99996	0.016–2.0

Table 2
Working curves.

Analyte	Regression equation	Correlation coefficient	Liner range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Simeton	$A=5.74870c+1.3403$	0.9997	0.55–100	0.16	0.53
Monuron	$A=3.99577c-1.46393$	0.9998	0.78–100	0.21	0.72
Chlortoluron	$A=3.82826c+0.7875$	0.9983	0.78–100	0.18	0.60
Simetryne	$A=6.3310c+0.38209$	0.9998	0.55–100	0.15	0.51
Atrazine	$A=6.28751c-4.70448$	0.9998	0.78–100	0.15	0.49
Karmex	$A=2.51712c-1.31917$	0.9999	1.56–100	0.35	1.16
Ametryne	$A=5.69270c+1.19577$	0.9998	0.78–100	0.19	0.62
Propazine	$A=5.56774c+0.65174$	0.9998	0.78–100	0.19	0.63

Table 3
Intra- and Inter-day precision.

	Intra-day ($n=5$)		Inter-day ($n=5$)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Simeton	92.0	1.21	91.6	1.48
Monuron	86.2	2.33	87.0	2.39
Chlortoluron	91.5	0.87	90.4	2.53
Simetryn	90.4	1.45	87.0	1.25
Atrazine	85.8	1.38	88.3	1.91
Karmex	93.8	3.12	95.1	2.96
Ametryne	83.2	0.79	83.7	1.10
Propazine	82.7	1.38	84.6	1.37

Table 4
Analytical results for fresh spiked samples.

Sample	Added (ng/mL)	Simeton		Monuron		Chlortoluron		Simetry		Atrazine		Karmex		Ametryne		Propazine	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	10	98.1	2.89	84.0	1.97	98.0	5.29	86.3	2.23	85.5	2.67	94.3	5.19	81.7	1.01	90.7	1.62
	70	92.7	1.47	86.2	1.40	99.0	1.74	86.9	0.82	89.0	0.74	91.1	2.82	81.2	0.34	91.3	0.92
2	10	99.8	2.59	83.9	1.55	97.9	1.64	87.0	0.57	82.8	0.18	87.6	3.96	82.3	3.55	90.9	0.69
	70	89.4	1.03	83.6	0.97	99.4	1.79	85.4	0.48	83.7	1.44	97.5	2.80	83.0	0.70	90.8	0.52
3	10	98.7	0.84	85.3	0.24	99.0	2.00	96.8	1.11	87.7	0.43	98.8	5.85	81.4	1.56	94.1	1.86
	70	91.1	1.22	84.5	0.15	98.3	1.76	83.4	0.99	86.4	1.50	99.5	2.66	80.3	0.50	87.3	1.28
4	10	97.7	1.15	82.2	3.42	98.6	1.38	87.2	1.06	89.8	0.25	99.3	1.83	86.4	2.77	94.1	2.52
	70	93.6	1.76	86.1	0.54	99.3	0.56	88.1	0.47	91.2	0.52	99.2	1.28	81.5	0.66	90.6	1.05
5	10	98.4	1.81	81.4	1.92	98.3	2.34	85.6	0.75	88.3	2.15	96.6	6.55	87.0	1.38	99.1	0.35
	70	92.9	0.54	90.1	1.32	91.4	1.69	89.8	0.51	91.2	0.91	99.2	1.14	84.6	1.47	92.3	1.22
6	10	97.1	1.82	80.2	1.69	95.9	2.70	89.0	0.64	83.5	1.46	98.2	4.78	83.4	0.90	92.5	1.89
	70	92.0	1.03	88.2	1.54	99.3	1.09	88.7	1.40	92.3	0.17	96.2	3.19	86.0	1.04	87.7	0.93

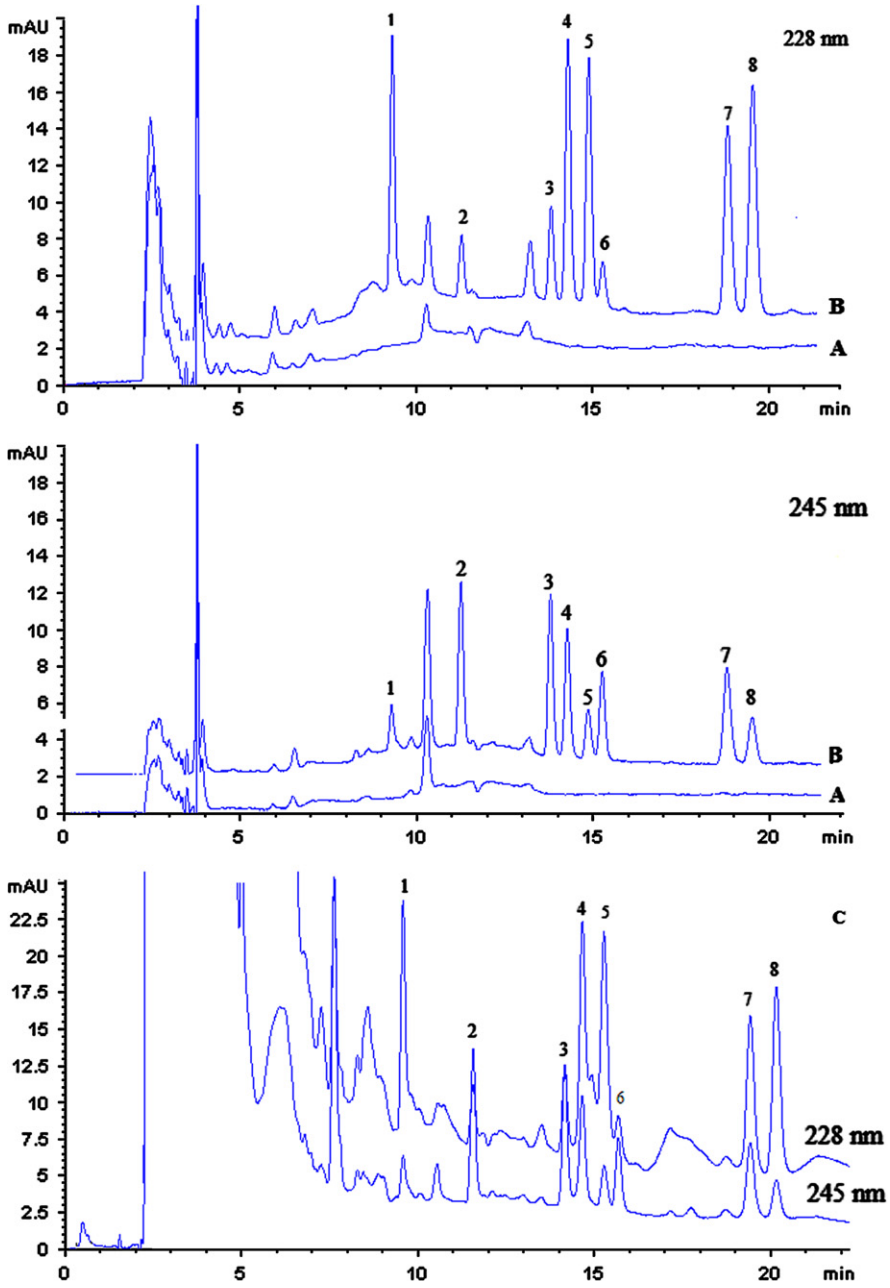

Fig. 11. Chromatograms of sample 3 (A) and spiked sample 3 (B) obtained by the present method and spiked sample 3 (C) obtained by the reference method 1, simeton; 2, monuron; 3, chlorotoluron; 4, simetryne; 5, atrazine; 6, karmex; 7, ametryne and 8, propazine.

Table 5
Analytical results for the aged spiked samples.

Sample	Added (ng/mL)	Stored time (day)	Simeton		Monuron		Chlortoluron		Simetry		Atrazine		Karmex		Ametryne		Propazine	
			Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	10	7	98.0	3.14	78.9	0.92	98.3	1.13	90.7	1.42	84.6	2.07	90.7	5.41	90.1	1.99	87.7	1.75
		14	98.9	1.52	79.5	1.18	98.8	1.84	88.9	1.69	84.8	2.06	90.9	2.47	87.6	1.11	91.2	0.15
		21	99.2	1.51	80.8	1.94	93.5	1.55	89.2	1.12	86.2	2.82	99.0	3.51	84.6	2.03	96.2	2.32
		28	98.1	3.31	81.9	1.78	92.5	1.08	91.7	2.10	88.4	2.75	99.0	3.51	88.5	1.02	91.0	0.69
	70	7	93.9	0.53	84.3	1.67	97.2	0.77	86.5	1.18	85.9	1.36	94.8	0.95	83.9	0.86	92.7	0.95
		14	96.0	0.47	84.0	0.22	99.2	1.62	87.4	1.15	84.2	1.37	95.3	1.54	85.1	0.86	91.3	0.38
		21	96.0	1.07	87.0	2.05	97.1	1.56	90.6	0.91	88.7	1.50	96.6	0.58	85.7	0.99	93.2	0.12
		28	92.9	0.49	85.8	2.17	98.4	1.37	87.7	0.82	87.1	0.55	97.4	1.97	83.2	1.52	90.4	0.53
2	10	7	99.7	0.83	79.7	0.65	99.0	0.75	90.7	0.94	88.0	0.95	93.7	1.96	85.4	1.14	93.8	2.01
		14	99.8	2.09	80.2	1.95	99.6	0.98	86.7	1.32	85.3	0.53	92.7	1.76	85.4	0.96	93.3	1.50
		21	98.1	0.17	79.4	4.20	99.3	3.78	89.3	1.36	87.1	2.18	90.7	4.05	85.6	1.22	94.2	1.41
		28	99.9	0.17	79.1	1.45	98.2	0.49	88.5	3.15	87.2	2.27	93.5	3.93	85.5	0.26	93.1	1.13
	70	7	90.0	0.62	85.1	0.28	99.2	1.66	87.7	0.23	88.4	0.52	95.0	1.54	84.1	0.53	91.3	0.32
		14	88.4	0.41	83.1	0.38	98.5	1.48	85.8	0.07	84.0	1.54	93.7	1.87	83.1	0.71	91.1	0.87
		21	91.1	0.90	87.0	1.33	99.9	0.72	89.2	1.14	87.3	0.36	95.8	1.32	83.7	1.58	91.6	1.09
		28	90.1	0.47	86.3	0.76	98.4	0.77	89.1	1.29	83.4	0.11	91.0	0.84	82.4	0.78	90.3	0.62
4	10	7	99.5	2.76	81.8	1.40	98.9	1.96	94.2	0.38	90.5	2.02	97.4	1.05	92.4	0.57	97.5	0.43
		14	99.1	3.16	84.8	0.74	99.0	1.09	91.3	0.86	89.4	1.62	99.0	2.68	86.9	1.46	95.1	0.44
		21	99.5	2.93	80.2	1.43	97.4	0.97	89.2	1.84	87.7	0.61	94.1	5.86	84.8	0.26	90.2	1.47
		28	99.0	1.77	79.4	1.05	98.5	2.51	88.0	1.38	88.2	0.86	96.4	2.12	84.2	0.98	87.2	2.16
	70	7	95.9	1.22	87.6	0.41	99.0	1.00	88.9	0.50	90.9	0.29	95.9	1.73	84.6	0.02	91.3	0.67
		14	97.8	0.69	88.5	0.69	96.5	0.95	88.3	0.32	90.0	0.84	98.2	0.23	84.4	0.81	92.4	0.42
		21	97.9	0.62	87.5	0.59	99.9	1.71	88.3	0.60	88.4	0.02	95.8	3.15	84.6	0.20	93.6	0.24
		28	96.2	0.75	86.4	0.36	97.6	1.65	88.2	0.83	86.6	0.29	94.2	1.29	81.6	1.11	89.6	0.27
5	10	7	99.5	2.26	83.5	1.62	97.2	3.62	94.8	1.81	92.7	0.41	96.8	2.11	90.8	0.08	99.2	0.49
		14	98.4	2.32	82.3	3.54	98.2	1.71	90.6	0.87	89.3	1.87	99.0	1.03	88.2	2.55	97.2	2.16
		21	99.7	2.84	80.9	0.52	98.8	2.42	90.9	0.55	88.9	1.11	99.5	2.03	88.3	1.36	92.1	2.50
		28	99.9	1.67	82.3	0.25	96.2	0.26	89.5	1.59	87.8	3.03	98.2	4.78	85.8	0.09	88.8	2.12
	70	7	99.0	1.06	86.4	1.37	97.7	1.56	89.7	0.91	93.0	0.57	98.7	2.14	86.2	0.84	91.6	0.58
		14	99.8	0.30	86.2	1.21	99.2	1.09	86.8	0.59	88.1	0.43	96.7	0.73	84.2	0.19	92.7	0.56
		21	99.3	0.18	88.1	0.60	98.4	0.70	89.2	0.86	90.4	0.88	97.6	1.16	84.4	0.56	92.4	0.20
		28	94.7	1.27	85.0	1.58	99.4	0.64	87.3	0.18	90.6	0.13	95.0	1.07	81.3	0.84	88.3	0.04

Table 6
Comparison of the proposed method with the reference method.

Method		This work	Reference
Extraction solvent		8 mL of ethyl acetate/n-hexane	12 mL of acetonitrile
Clean-up efficiency		Good	Insufficient
Adsorbent		0.08 g PSA	0.5 g C18+1.5 g $MgSO_4$
Partition salt		0.3 g NaCl	4 g $MgSO_4$+1 g NaCl
	simeton	0.16	0.94
	mounron	0.21	0.37
	chlorotoluron	0.18	0.31
LOD (ng/mL)			
	simetryne	0.15	0.26
	atrazine	0.15	0.28
	karmex	0.35	0.46
	ametryn	0.19	0.29
	propazine	0.19	0.25

herbicides range from 0.15 to 0.35 ng/mL. The LOQs of all analytes are much lower than MRL default value mentioned above. So the LOQs are appropriate to the goal of the proposed method.

3.2.2. Precision and recovery

The intra- and inter-day precisions of the proposed method were obtained by performing the whole analytical procedure at the fortified concentration of 50 ng/mL. The intra-day precision was obtained by analyzing a sample five times in one day. The inter-day precision was obtained by analyzing a sample once a day over five consecutive days. The intra-day and inter-day precisions were expressed as the relative standard deviations (RSDs). Subsequently, the extraction mean recoveries were obtained. Table 3 shows the RSDs data and absolute recoveries. The results show acceptable RSD values, ranging from 0.79 to 3.12% and from 1.10 to 2.96% for intra- and inter-day, respectively. The absolute recoveries range from 82.7 to 93.8% and from 83.7 to 95.1% for intra- and inter-day, respectively.

3.2.3. Analysis of samples

In order to evaluate the applicability of the modified QuEChERS method, spiked yogurt (Sample 1–3) and milk samples (Sample 4–6) at two concentration levels (10 and 70 ng/mL) were analyzed. No target analytes were detectable in the six milk and yogurt samples. The results are listed in Table 4. As can be seen the proposed method provides good recoveries (80.2–99.8%) and acceptable precisions ($<5.85\%$). The chromatograms of blank sample 3 and spiked sample 3 are shown in Fig. 11.

3.2.4. Stability

The long term stability of the analytes in yogurt and milk was evaluated. The extraction of herbicides in fortified milk and yogurt samples was carried out at 7, 14, 21 and 28 days, respectively. The fortified samples were stored at 4 °C until analysis.

All experiments were performed in three replicates. The results are listed in Table 5. The absolute recoveries and RSD values range from 78.9 to 99.9% and 0.07 to 5.86%, respectively. It can be seen that the herbicides in the yogurt and milk samples were stable for at least 28 days.

3.2.5. Comparison of the present method with the reference method

For comparison, the modified QuEChERS method developed by Kinsella et al. was referred to as the reference method and the obtained chromatogram of spiked sample 3 obtained with the reference method is shown in Fig. 11(C). It can be seen that there are many interference peaks and high baseline noise in the chromatogram obtained by the reference method. Although the usage of adsorbent in reference method is 6 times than that in our work, the cleanup ability is insufficient in reference method. It can deduce that the adjustment of extraction solvent plays an important role in improving cleanup ability. The present method and reference method can be compared in terms of extraction solvent, clean-up efficiency, adsorbent, partition salt and LODs. The comparison of the two methods is listed in Table 6. It can indicate that compared with the reference method, the present method has some advantages in the salt and adsorbent consumption and clean-up efficiency. In additions, the LODs of the herbicides obtained by the present method are lower than those obtained by the reference method. These results indicate that this present method is a useful method for the extraction of herbicides in milk and yogurt samples and the modification of extraction solvent is meaningful and effective.

4. Conclusions

The proposed method based on QuEChERS method was proved to be effective for extraction of triazine and phenylurea herbicides from milk and yogurt samples. The absolute recoveries of herbicides are acceptable. The extraction method overcame the drawback of insufficient clean-up of original QuEChERS method and can be applied to the analysis of real samples. It could be considered that this method is very promising and can be applied to the extraction of pesticides from other complex matrices by varying the extraction parameters.

References

[1] D. Barcelo, Analyst 116 (1991) 681–689.
[2] J.J.B. Nevado, C.G. Cabanillas, M.J.V. Llerena, V.R. Robledo, Microchem. J. 87 (2007) 62–71.
[3] F.D. Campos-Pereira, C.A. Oliveira, A.A. Pigoso, E.C. Silva-Zacarin, R. Barbieri, E.F. Spatti, M.A. Marin-Morales, G.D. Severi-Aguiar, Ecotoxicol. Environ. Saf. 78 (2012) 170–177.
[4] C. Federico, S. Motta, C. Palmieri, M. Pappalardo, V. Librando, S. Saccone, Mutat. Res.—Genet. Toxicol. Environ. Mutagenesis 721 (2011) 89–94.
[5] C. Yague, A. Herrera, A. Arino, R. Lazaro, S. Bayarri, P. Conchello, J. AOAC Int. 85 (2002) 1181–1186.
[6] 〈http://www.madehow.com〉.
[7] I. Baranowska, H. Barchanska, A. Pyrsz, Chemophere 60 (2005) 1590–1599.

[8] M.M. Aguilera-Luiz, P. Plaza-Bolaños, R. Romero-González, J.L. Martínez Vidal, A.Garrido Frenich, Anal. Bioanal. Chem. 399 (2011) 2863–2875.
[9] M. Angeles Garcia, M. Santaeufemia, M.Julia Melgar, Food Chem. Toxicol. 50 (2012) 503–510.
[10] S.M. Goulart, M.E.L.R. de Queiroz, A.A. Neves, J.H. de Queiroz, Talanta 75 (2008) 1320–1323.
[11] L. Balduini, M. Matoga, E. Cavalli, E. Seilles, D. Riethmuller, M. Thomassin, Y.C. Guillaume, J. Chromatogr. B 794 (2003) 389–395.
[12] S. Seccia, S. Albrizio, P. Fidente, D. Montesano, J. Chromatogr. A 1218 (2011) 1253–1259.
[13] C. Yague, S. Bayarri, R. Lazaro, P. Conchello, A. Arino, A. Herrera, J. AOAC Int. 84 (2001) 1561–1568.
[14] B. Kinsella, P. Byrne, H. Cantwell, M. McCormack, A. Furey, M. Danaher, J. Chromatogr. B 879 (2011) 3707–3713.
[15] N. Li, R. Zhang, L. Nian, R.B. Ren, Y.Q. Wang, H.Q. Zhang, A.M. Yu, J. Chromatogr. A 1222 (2012) 22–28.
[16] M. Rodrigues, P.R.R. Mesquita, L.S. de Oliveira, F.S. de Oliveira, A. Menezes Filho, P.A. de, P. Pereira, J.B. de Andrade, Microchem. J. 98 (2011) 56–61.
[17] Y. Yang, J. Chen, Y.P. Shi, Talanta 97 (2012) 222–228.
[18] S.Q. Gao, J.Y. You, X. Zheng, Y. Wang, R.B. Ren, R. Zhang, Y.P. Bai, H.Q. Zhang, Talanta 82 (2010) 1371–1377.
[19] A.G. Howard, N.H. Khdary, Analyst 130 (2005) 1432–1438.
[20] N.H. Khdary, A.G. Howard, Analyst 136 (2011) 3004–3009.
[21] N.H. Khdary, A.E. Gassim, A.G. Howard, Anal. Methods 4 (2012) 2900–2907.
[22] R. Carabias-Martinez, E. Rodriguez-Gonzalo, E. Miranda-Cruz, J. Dominguez-Alvarez, J. Hernandez-Mendez, Electrophoresis 28 (2007) 3606–3616.
[23] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412–431.
[24] S.J. Lehotay, J. AOAC Int. 90 (2007) 485–520.
[25] T.D. Nguyen, K.J. Lee, M.H. Lee, G.H. Lee, Microchem. J. 95 (2010) 43–49.
[26] N.D. Forsberg, G.R. Wilson, K.A. Anderson, J. Agric. Food Chem. 59 (2011) 8016–8108.
[27] P. Aysal, A. Ambrus, S.J. Lehotay, A. Cannavan, J. Environ. Sci. Health B 42 (2007) 481–490.
[28] K. Banerjee, D.P. Oulkar, S. Dasgupta, S.B. Patil, S.H. Patil, R. Savant, P.G. Adsule, J. Chromatogr. A 1173 (2007) 98–109.