



Analysis of Cypermethrin and Permethrin in Celery Cabbage by Lanthanum-Modified Activated Alumina QuEChERS Method

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Received: 29 October 2013;

Accepted: 3 December 2013;

Published online: 30 September 2014;

AJC-16119

A new QuEChERS method based on lanthanum-modified activated alumina for the determination of cypermethrin and permethrin in celery cabbage was proposed. The lanthanum-modified activated alumina that prepared by glycerol complexing-assisted sol gel method using lanthanum as the modifier was employed as the adsorbent of QuEChERS for purification of cypermethrin and permethrin residues in celery cabbage coupled with gas chromatography detection and ethyl acetate as extraction solvent. Factors that affected QuEChERS efficiency were investigated including extraction solvent, extraction time, adsorbent kinds and adsorption time *etc.* The results showed that this lanthanum-modified activated alumina adsorbent could effectively purify the sample and eliminate the interferences originated from sample matrices. Under the optimized conditions, the fortified recoveries at three spiked levels were ranged from 91.6 to 110 % with RSD \leq 4.9 %. Good linearity of the two pesticides was ranged from 0.005 to 1.190 mg/g with $r \geq 0.9994$.

Keywords: QuEChERS; Permethrin, Cypermethrin, Activated alumina.

INTRODUCTION

Cypermethrin and permethrin are two kinds of pyrethroid pesticides used in agriculture to control insects. They are among the most toxic substances that contaminating food, environment, fruits and vegetables¹. Therefore, it is crucial to monitor cypermethrin and permethrin residues in fruits and vegetables.

At present, the determination of pesticide residues in complicated matrices was a formidable challenge mainly because of the trace level of analytes and a large amount of interferences, so the sample must initially be cleaned up before final analysis²⁻⁵. Generally, solvent extraction⁶, matrix solid-phase dispersion extraction⁷, gel-permeation chromatography⁸, emulsification-microextraction^{9,10}, solid-phase extraction^{11,12} and liquid-phase microextraction¹³ *etc.* were often employed for sample extraction and purification. However, from the practical point of view, these pretreatment procedures suffered from non-equilibrium extraction procedures or the small contact surface between the phases, which negatively affected the sample throughput and the enrichment factors.

QuEChERS (quick, easy, cheap, effective, rugged and safe) technique as a novel sample extraction and clean-up method was developed for analysis of multiple residues in agricultural products¹⁴. It was a streamlined and effective extraction and cleanup approach for analysis of various analytes in food

matrices^{15,16}. The QuEChERS procedures had the characters of few simple analytical steps, fast and easy to perform and less susceptible to errors¹⁷ and it could provide high recovery for a broad scope of pesticides¹⁸. In QuEChERS, extraction solvent and purification adsorbent were the main key factors that affected purification efficiency. The original extraction solvent of QuEChERS was acetonitrile with primary-secondary amine (PSA) as the adsorbent. For the higher toxicity of acetonitrile and the primary-secondary amine adsorbent only be good at removing of fatty acid, not suitable for sterol, pigment and vitamin elimination. Many modification/improvement work of the QuEChERS was proposed to ensure efficient extraction of the analytes, minimize the toxic of the extraction system or expand the spectrum of matrices covered. Of which, ethyl acetate, dry ice or acetate-acetonitrile *etc.* as extraction solvent and graphite carbon, C₁₈ or neutral alumina oxide *etc.* as adsorbent had been developed^{19,20}. C₁₈ and graphite carbon black was suitable for adsorption of sterol, pigment and vitamin. While neutral alumina oxide could eliminate part water solubility pigment and most of lipide pigment⁷. Though these measurement made a great improvement. The QuEChERS method still suffered from too complex samples analysis, new extraction system of QuEChERS was desired greatly.

The purpose of this work was to develop a new QuEChERS method for determination of cypermethrin and permethrin in celery cabbage sample coupled with GC-FID detection. In the

QuEChERS procedures, low toxicity solvent of ethyl acetate was employed as the extraction solvent with lanthanum-modified activated alumina as the adsorbent. The lanthanum-modified activated alumina adsorbent could effectively purify the sample and eliminate the sample matrices interferences. The proposed QuEChERS-GC method was suitable for extraction of cypermethrin and permethrin from complex vegetable samples.

EXPERIMENTAL

Cypermethrin and permethrin were obtained from Yangnong Chemical Co. Ltd. (Yangzhou, China). Ethyl acetate, aluminum sulfate, glycerol and lanthanum nitrate were obtained from Tianjin Jin Feng Chemical Co., Ltd. Dichloromethane, anhydrous MgSO_4 and acetonitrile were of analytical grades and purchased from Kermel Chemical Co. Ltd. (Tianjin, China). Neutral alumina oxide and basic alumina oxide (100-200 mesh) (obtained from National Pharmaceutical Group Chemical Reagent Co., Ltd.) were dried at 130°C for 12 h before use. All the other reagents used in the experiment were of the highest grade commercially available. Double deionized water was filtered with $0.45\ \mu\text{m}$ filter membrane before use.

Preparation of lanthanum-modified activated alumina:

Aluminum sulfate solution (200 mL, 1.28 g/mL) was adjusted to pH 7.5 by aqueous ammonium hydroxide (20 %) under vigorous stirring. After stirring for 2 h, the obtained $\text{Al}(\text{OH})_3$ was filtered and washed until none SO_4^{2-} existed, then it was adjusted to pH 4 by 0.1 % aqueous HNO_3 to obtain aluminium hydrogel. And then 306 g of glycerol and 2.7 g of lanthanum nitrate were added into the aluminium hydrogel and mixed sufficiently. The mixture was roasted at 540°C for 10 h to obtain lanthanum-modified activated alumina.

Instrumentation and conditions: Cypermethrin and permethrin were analyzed on a Shimadzu GC-2014 system equipped with a FID detector (Shimadzu, Japan) and DB-5 column (5 % phenyl-methylpolysiloxane $30\ \text{m} \times 0.53\ \text{mm} \times 1\ \mu\text{m}$). High-purity nitrogen (99.999 %) was used as the carrier gas with flow rate at 5 mL/min and split ratio of 2:1. A GH-300 high-purity hydrogen generator and GA-2000A air pump (Beijing ZXHL Technology Development Co. Ltd.) were used to supply hydrogen and air at the rate of 30 mL/min and 400 mL/min, respectively. The temperature-programmed mode was as follows: the initial oven temperature was set at 250°C for 2 min, then ascended to 280°C at the rate of $6^\circ\text{C}/\text{min}$ and held for 12 min. The injection port and detector temperatures were maintained at 285°C and 290°C , respectively.

Sample QuEChERS extraction: Different celery cabbage samples (80-100 g) were collected from the local markets and homogenized, then 3 g of the homogenized sample was mixed with the extraction solution (the mixture of 3 mL ethyl acetate and 0.5 g NaCl) and ultrasonicated for 2 min. Then the mixture was centrifuged at 4000 rpm for 5 min and the obtained supernatant (1 mL) was mixed with 0.2 g anhydrous MgSO_4 and 0.1 g lanthanum-modified activated alumina. It was ultrasonicated for 2 min, placidly placed for 5 min and centrifuged (4000 rpm) for 5 min. The obtained supernatant was filtered through a $0.45\ \mu\text{m}$ membrane and employed ($1\ \mu\text{L}$) for further analysis.

RESULTS AND DISCUSSION

Selection of extraction solvent: The selection of extraction solvent was critical to obtain an economic recovery of the target analytes from complex sample matrix. Generally, acetonitrile was the recommended solvent for QuEChERS and with the help of salt would be separated more easily from water. Ethyl acetate had the character of partial miscibility with water but not suitable for large lipids and waxes samples, for it would co-extracts with them leading lower recoveries and bad purification efficiency. Considered the characters of celery cabbage matrix, different extraction solvents including toluene, ethyl acetate, acetonitrile, *n*-hexane, acetonitrile-acetic acid (99:1, v/v), acetonitrile-toluene (1:1, v/v) and acetonitrile-ethyl acetate (1:1, v/v) were investigated to obtain the optimum extraction efficiency. Fig. 1 showed that acetonitrile and ethyl acetate had better ability for extraction cypermethrin and permethrin from celery cabbage. Considered the higher toxicity of acetonitrile, ethyl acetate was selected as the extraction solvent. Moreover, the effect of its volume on the extraction ratio of cypermethrin and permethrin was also investigated. Fig. 2 indicated that 3 mL

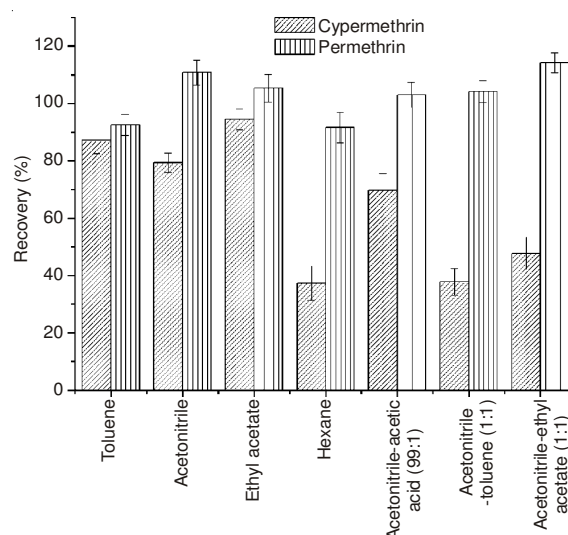


Fig. 1. Recovery obtained by different extraction solvent

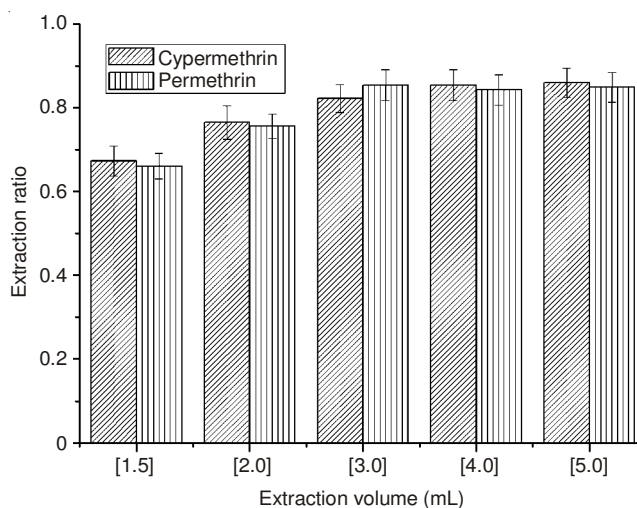


Fig. 2. Extraction ratio of different ethyl acetate volumes

ethyl acetate could provide the satisfactory recovery. If the volume of ethyl acetate was less than 3 mL, recoveries of the two analytes were increased with the increasing volume. While the volume of ethyl acetate was more than 3 mL, the extraction ratio was kept stable. Compromised the sensitivity and the amount of organic solvent, 3 mL ethyl acetate was used for further work and 0.5 g NaCl was added to induce phase separation.

In some instances, the pH of the extraction solvent must be controlled. For most of the pesticides, they are more stable at lower pH. In this work, pH of extraction solvent ranged from 1 to 11 was investigated and the results indicated that pH ranging from 1 to 8 had none obvious influence on the extraction efficiency of cypermethrin and permethrin. When the pH was more than 8, the extraction efficiency of cypermethrin and permethrin was decreased greatly with pH increased for ethyl acetate, cypermethrin and permethrin be decomposed. Due to the acidity of ethyl acetate being about 5, none pH adjustment was employed in extraction procedure.

Extraction time of extraction solvent: The interaction time between extraction solvent and sample was one of factors that affected extraction efficiency. Different extraction time was investigated and the results (Fig. 3) indicated that the mass transfer between extraction solvent (ethyl acetate) and the homogenized celery cabbage was very fast. It could reach equilibrium within 2 min. So extraction time of 2 min was employed for further work.

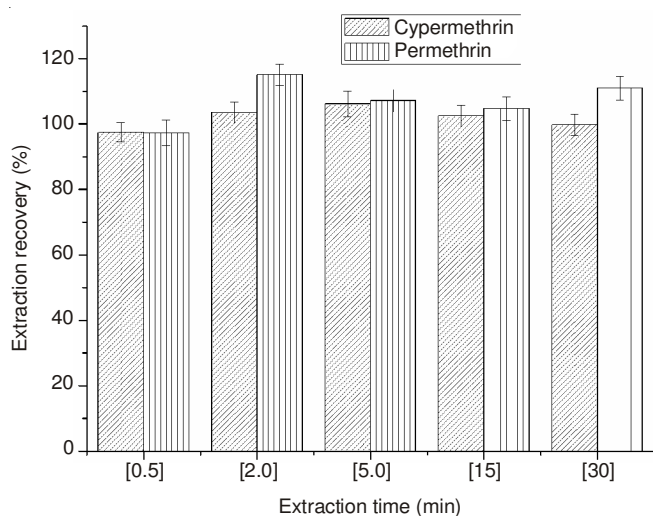


Fig. 3. Effect of extraction time on cypermethrin and permethrin recovery

Selection of adsorbent: The adsorbent was selected to retain matrix components, so a suitable adsorbent was a key factor for elimination of sample matrix impurities. An appropriate adsorbent and its amount should accord to the properties of matrix and the target compounds. To obtain the economic adsorbent, neutral alumina oxide, basic alumina oxide, diatomite, graphite carbon, lanthanum-modified activated alumina, activated alumina (it was prepared and treated in an identical manner as lanthanum-modified activated alumina, in absence of lanthanum nitrate), neutral alumina oxide-diatomite (1:1, g/g) and graphite carbon-diatomite (1:1, g/g) were taken into consideration except the original primary-secondary amine, which was considered expensive. Compared with the blank

(none adsorbent), diatomite adsorbent brought new interferences into sample, the purification and recovery of basic alumina oxide (62.6-71.5 %) was not satisfied. Graphite carbon (52.4-61.8 %) and activated alumina (59.4-60.7 %) had better clean-up efficiency, but both of them had strong adsorption to cypermethrin and permethrin, leading to a decreased recovery. Though lanthanum-modified activated alumina and neutral alumina oxide adsorbent had the least interference peaks, the lanthanum-modified activated alumina gave better recovery (91.6-110 %) than that of neutral alumina oxide (84.5-92.3 %). Compromised the clean-up efficiency and recovery, lanthanum-modified activated alumina was selected as the adsorbent of QuEChERS. Its amount (0.1-0.6 g) was investigated also and the results showed that the amount of lanthanum-modified activated alumina ranging from 0.1 to 0.4 g had not obviously influence on the two pesticides recoveries. However, when the amount was more than 0.4 g, the recovery of the two analytes would be gradually decreased. Compromised the recovery and the purification ability, 0.1 g lanthanum-modified activated alumina was used as the optimized amount. Additionally, 0.2 g anhydrous $MgSO_4$ was added into the sample simultaneously to remove much of the excess water, improve analytes partitioning and provide better clean up.

Adsorptive time of adsorbent: Adsorptive time (the time between the adsorbent and sample) must be suitable. Too short time, would make the impurities can't be completely adsorbed, while too long time, would lead to the impurities of adsorbent be dissolved out. After optimized, 5 min was employed as the compromised adsorbent time for sample impurities adsorption.

Method performance: The proposed QuEChERS-GC method was evaluated by linearity, precision, recovery, detection limits, inter-assay and intra-assay deviation. Under the optimum conditions, good linearities were ranged from 0.005 to 1.190 mg/g with linearity equation $y = 2.99 \times 10^6 X + 28799.51$ and $y = 4.13 \times 10^6 X + 37928.16$ (where Y was the peak area, X was the concentration of sample (mg/g)) for cypermethrin and permethrin, respectively ($r \geq 0.9994$). The detection limits based on $S/N = 3$ were 0.39 $\mu g/g$ and 0.43 $\mu g/g$ for cypermethrin and permethrin, respectively. Intra-assay and inter-assay precision were expressed by the relative standard deviation (RSD), five replicates of the control samples at three spiked levels in the same day and three different days were analyzed with results less than 5.6 and 7 %, respectively. Additionally, chromatogram (Fig. 4) of spiked sample indicated that QuEChERS-GC exhibited more clean eluents without interferences, which demonstrated the high selectivity of the proposed QuEChERS-GC method.

Sample analysis: To illustrate a typical application of the developed QuEChERS-GC method, five kinds of celery cabbages that obtained from the local grocery stores were used for QuEChERS-GC analysis. None cypermethrin or permethrin was observed in any of the samples, which demonstrated that the misapplication of the two kinds of pesticide in celery cabbage at local area was not extensive. The recoveries of the two pesticides at three fortified levels indicated that the recoveries of cypermethrin and permethrin were ranged from 91.6 to 110 % with $RSD \leq 4.9$ % (Table-1). The chromatogram (Fig. 4) of the extract revealed that after the QuEChERS protocol, the samples were clean enough for direct GC analysis

TABLE-1
RESULTS OF CELERY CABBAGE SAMPLES (n = 5)

Analytes	Sample content (mg/g)	Spiked levels (mg/g)	Detected content (mg/g)	Recovery (%)	RSD (%)
Cypermethrin	Nd	0.010	0.011	110.0	2.8
		0.050	0.050	100.1	4.9
		0.149	0.162	108.4	2.4
Permethrin	Nd	0.010	0.009	91.6	3.9
		0.050	0.049	98.0	3.1
		0.149	0.163	109.6	2.5

Nd: Not detected

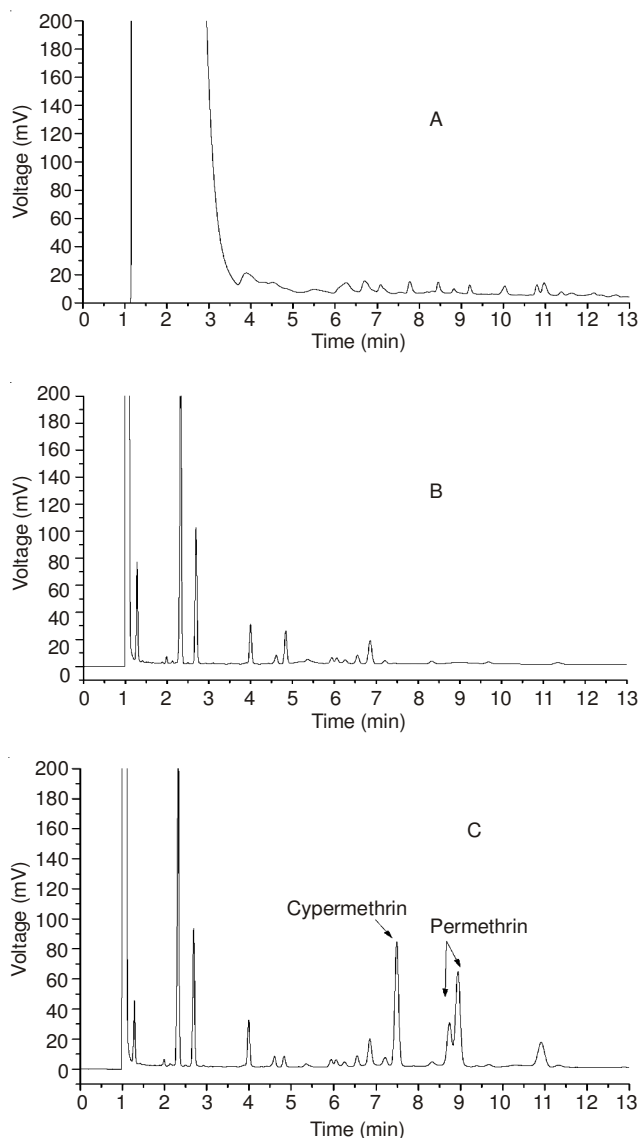


Fig. 4. Chromatogram of celery cabbage samples, (A: Unpurified sample B: Purified sample C: Fortified sample)

and no interferences originating from the human celery cabbage matrixes were observed. All this indicated that the method was reliable and could be used for the determination of trace cypermethrin or permethrin in celery cabbages.

Conclusion

A simple, low-cost and low toxicity QuEChERS-GC method for the determination of cypermethrin and permethrin pesticides in vegetables was developed. The method involved

extraction with ethyl acetate, liquid-liquid partition with addition of NaCl followed by dispersive solid phase cleanup with lanthanum-modified activated alumina adsorbent and anhydrous $MgSO_4$. After the QuEChERS protocol, the sample matrix interferences could be efficiently eliminated and the obtained sample was clean enough for direct GC analysis. The developed procedure gave satisfactory recovery (91.6-110 %) with RSD less than 4.9 % for the studied pesticides in celery cabbage. It was suitable for analysis of permethrin and cypermethrin residues in celery cabbages.

ACKNOWLEDGEMENTS

The project sponsored by National Natural Science Foundation of China (31301464), Natural Science Foundation of Hebei province (B2012201052, B2011104002).

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