

A novel acrylamide modified primary-secondary amine analogue as impurities remover for determination of carbendazim and dimethyl phthalate in apples

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Abstract—A novel acrylamide modified Primary-Secondary Amine (PSA) analogue (acrylamide/3-aminopropyltriethoxysilane/silica) was prepared and used as impurities remover for pesticide residues (carbendazim and dimethyl phthalate) in apples by QuEChERS combined with pipette-tip micro solid-phase extraction method. The properties of acrylamide/3-aminopropyltriethoxysilane/silica were characterized by scanning electron microscopy and Fourier transform infrared spectroscopy. Under the optimized conditions, factors such as amount and type of adsorbents were investigated. The limit of detection and limit of quantity of the method were 0.012 µg/mL and 0.04 µg/mL for carbendazim, 0.023 µg/mL and 0.08 µg/mL for dimethyl phthalate, respectively. The recoveries of proposed method at three spiked levels analysis were 84.9-90.0% for carbendazim and 84.1-88.2% for dimethyl phthalate, respectively, with the relative standard deviation less than 2.9%. The acrylamide/3-aminopropyltriethoxysilane/silica as potential adsorbent showed strong ability for removing the matrix, and it achieved great ability to retain the targets. It will provide a new material choice for detecting pesticide residues in apple samples.

Keywords: Acrylamide, PSA, QuEChERS, Carbendazim, Dimethyl Phthalate

INTRODUCTION

Carbendazim (CAR) and dimethyl phthalate (DMP) [1] as pesticides have been extensively used in fruits, vegetables and other crops. The widespread presence of pesticide residues has potential risks to human health, animals and environment [2-8]. However, due to extremely low concentration and serious interference from matrices, qualitative and quantitative analysis of pesticide residues is considered to be a difficult task, so the pretreatment is critical before instrumental analysis [10,11]. QuEChERS (quick, easy, cheap, effective, rugged, and safe) method is a type of sample preparation method which has attracted wide attention [12]. Because its advantages such as safety, ease of operation, high recovery, high reproducibility and less cost, QuEChERS has already been proven to be useful in pesticide residue analysis [13-15].

Typically, QuEChERS method involves an initial extraction with acetonitrile (ACN) followed by an extraction/partitioning step after the addition of a salt mixture. An aliquot of the raw extract is then cleaned up by dispersive solid phase extraction (DSPE). After shaking and centrifugation, the final extract in ACN is directly amenable to determinative analysis by various analytical instruments, such as liquid chromatography (LC) and gas chromatography (GC) [16-19]. To achieve the goal of purification, adsorbents play a vital role in QuEChERS method. Commercial primary-secondary amine (PSA) is widely used in the traditional QuEChERS method for remov-

ing polar compounds [20]. PSA as a polar adsorbent has higher absorption capability and been widely used to remove various polar organic acids, polar pigments, some sugars and fatty acids [13,17]. However, commercial PSA is expensive and PSA has a relatively low recovery rate for some targets in complex samples. So this research considers synthesizing easily a low cost and PSA-like material. A novel acrylamide modified PSA analogue acrylamide/3-aminopropyltriethoxysilane/silica (AM/APTES/silica) was synthesized. This material has a functional group similar to PSA, a secondary amine group. At the same time, the primary amine group forms an amide group, which enhances the polarity of the material and increases the recovery of target. The pesticide residue analytical methods based on QuEChERS procedure have been studied widely. Although the original QuEChERS method could help to reduce amounts of co-extracted matrix; it is unsuitable for the cleanup of complex apple matrix. Testing complex samples can cause considerable damage to the instrument. Pipette-tip micro solid-phase extraction (MSPE) is a representative solid-phase extraction method because of its miniature device and use of reduced amount of reagents and time consumption [21,22], and it has been widely used for analysis.

In this study, AM/APTES/silica was synthesized and used as impurities remover in QuEChERS combined with MSPE (QuEChERS-MSPE) method for analyzing CAR and DMP in apple samples. The raw extractions were first cleaned up by QuEChERS, and then MSPE was used to further clean up. Morphology and structure information of AM/APTES/silica was characterized by Fourier transform infrared (FT-IR) and field emission scanning electron microscope (FE-SEM). The adsorption ability using QuEChERS-MSPE method was further optimized and developed. By coupling

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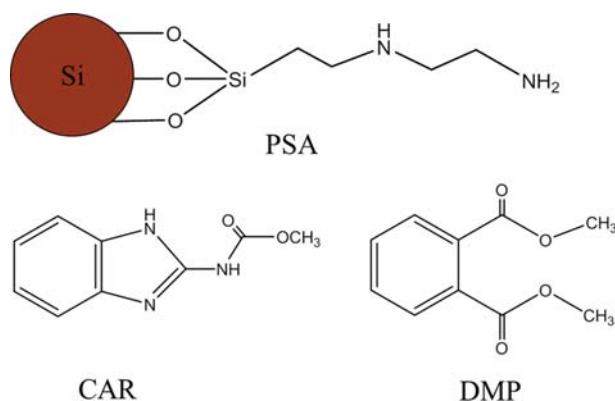


Fig. 1. Chemical structures of PSA, CAR and DMP.

with high performance liquid chromatography, a rapid and effective method for the analysis of pesticide residues in apple samples was established.

MATERIALS AND METHODS

1. Reagents and Materials

Carbendazim (CAR) and dimethyl phthalate (DMP) (Fig. 1) were bought from Energy Chemical. Co. Ltd. (Shanghai, China). Apples were purchased from the local market (Tianjin, China). Silica and 3-aminopropyltriethoxysilane (APTES) was from Dingkang. Co. Ltd. (Qingdao, China). Commercial PSA (Fig. 1) was purchased from Bona Bio-Technology. Co. Ltd. (Jinan, China). Acrylamide, methanol (MeOH), acetone and acetonitrile (ACN) were obtained from Guangfu Chemical Reagent. Co. Ltd. (Tianjin, China). Potassium bromide (KBr) was obtained from Concord Chemical Research Institute (Tianjin, China). Ultrapure water was from Hangzhou Wahaha Group Co. Ltd. (Hangzhou, China). All the other solvents used in the experiment were HPLC or analytical grade. All the samples were filtered before injection into the HPLC system.

2. Standard Solutions and Analytical Conditions

Standard stock solution of CAR and DMP (200 µg/mL) was prepared in methanol. Matrix standard solutions spiked CAR and DMP with 10 µg/mL and the desired concentration was prepared by dilution for further use. All solutions were stored in a glass vial under refrigeration at 4 °C.

The chromatography system consisted of LC-10ATVP pump and SPD-10AVP UV-VIS detector (Shimadzu, Suzhou, China), with the injector (10 µL sample loop). The analysis was performed on an Kromasil C₁₈ column (5 µm, 250×4.6 mm, RS tech Corporation, Germany) and Chromatography Data System N2000 (Surwit Technology, Hangzhou, China). Mobile phase was methanol : water (50 : 50, v/v) and flow rate was set at 0.7 mL/min. The wavelength of detector was set at 225 nm with injection volume 10 µL.

3. Synthesis of Polymers

3-1. Activation of Silica

Silica (10 g) was added into 6 mol/L hydrochloric acid (50 mL). After being stirred at reflux for 12 h, the silica was washed by deionized water until neutral and dried in a vacuum at 110 °C for 12 h.

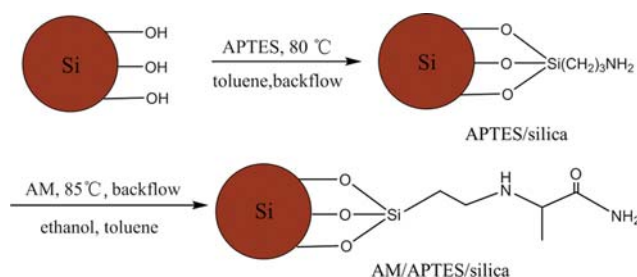


Fig. 2. Illustration of the preparation of APTES/silica and AM/APTES/silica.

3-2. Synthesis of APTES/Silica

Activated silica (5 g) and APTES (5 mL) were added to a round bottom flask (100 mL), and anhydrous toluene (50 mL) was added as a solvent. The reaction was stirred at 80 °C and maintained for 7 h, and then allowed to temperature. The product (APTES/silica) was filtered and washed with anhydrous toluene, ethanol and acetone until no colloidal material was obtained. After these steps, APTES/silica was dried in a vacuum at 60 °C for 10 h.

3-3. Synthesis of AM/APTES/Silica

APTES/silica (1 g), acrylamide (0.5 g), triethylamine (two drops), absolute ethanol (30 mL) and toluene (30 mL) were added successively in a round bottom flask (100 mL), and the reaction was stirred at 85 °C for 15 h. The AM/APTES/silica was cooled and filtered, and washed successively by ethanol and deionized water and dried in a vacuum at 60 °C for 10 h. The preparation procedure of AM/APTES/silica is depicted in Fig. 2.

4. Characterizations of Polymers

The spectra of these materials were analyzed by VERTEX70 FT-IR (Bruker, Germany); the wave numbers of FT-IR measurement range were controlled from 400 to 4,000 cm⁻¹. The polymers were characterized by FT-IR with KBr pellet method. FT-IR disk process: 1 mg sample (silica, APTES/silica, AM/APTES/silica and commercial PSA, respectively) was ground together with 200 mg KBr for tablets, respectively. The percent of sample to KBr was 0.5%. The morphological microstructures of these materials were observed by FE-SEM (MERLIN Compact, ZEISS, Germany).

5. Sample Preparation

CAR and DMP were extracted from apple samples using a QuEChERS-MSPE method. The schematic procedure is shown in Fig. 3, which roughly summarizes the following steps: Apple samples were chopped with a domestic blender. For QuEChERS procedure, 10 g of the grinded sample was placed into a 50 mL centrifugation tube, 10 mL of ACN was added and vortexed for 1 min. 4 g of anhydrous MgSO₄ and 1 g of NaCl were added and the mixture was shaken vigorously for 2 min. After the centrifugation step (3,500 rpm, 20 min, 25 °C), the upper layer was collected. CAR and DMP were spiked into samples after the extraction. For the following clean-up step, a portion of 2 mL of the supernatant was transferred into a 10 mL centrifuge tube containing 300 mg of MgSO₄ and 60 mg of adsorbents (silica, APTES/silica, AM/APTES/silica and commercial PSA, respectively), and homogenized for 1 min. The mixture was centrifuged (3,500 rpm, 20 min, 25 °C) and 1 mL of the supernatant was taken into vials for the following MSPE

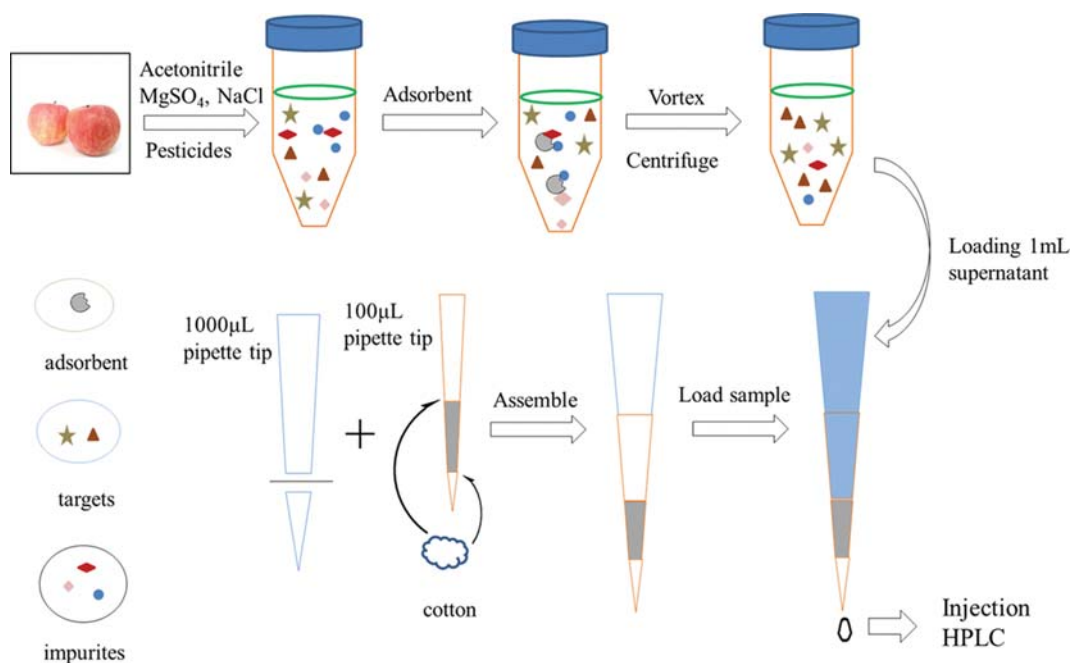


Fig. 3. Schematic procedure of QuEChERS-MSPE for the determination of CAR and DMP in apple.

procedure.

For MSPE procedure, 3 mg of different material was placed separately into the 100 μL pipette-tip using degrease cotton at both ends, avoiding different materials loss. 1 mL pipette-tip was then inserted into the smaller tip. Before extraction, the device was activated with 1 mL of methanol and 1 mL of water. Then 1 mL of the supernatant was loaded into the MSPE columns. The solution through the polymers was dried in a vacuum and the residues were dissolved in 1 mL ACN to be tested by HPLC.

RESULTS AND DISCUSSION

1. Preparation of Polymers

A novel acrylamide modified PSA analogue (AM/APTES/silica) was fabricated. The adsorbability of the AM/APTES/silica lies in its weak ion exchange properties, hydrophobic interaction and π electron accepting ability. Comparison with commercial PSA, AM/APTES/silica chose to enhance the polarity by grafting an amide group, and the modified amide group can effectively improve the specific recognition between targets and impurities. Since the non-shared electron pair of the amino nitrogen in the molecule forms a conjugated system with the π electron of the carbonyl group, then the electron cloud density on the nitrogen is reduced, the polarity of the N-H bond is increased. The difference of polarity between AM/APTES/silica and the target increased. According to the principle of "like dissolves like," the adsorption of AM/APTES/silica for the target will be weakened. There are organic acids in apple and the amide group in AM/APTES/silica can be effectively combined with organic acid. So it can further identify the targets by removing impurities in determination of pesticide residues (CAR and DMP) in apple.

2. Characterizations

The FT-IR spectra of silica, APTES/silica, AM/APTES/silica and

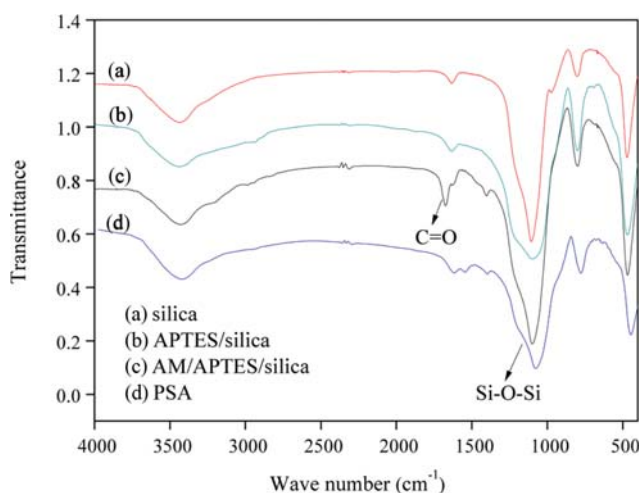


Fig. 4. FT-IR spectrum of silica, APTES/silica, AM/APTES/silica and PSA.

commercial PSA are in Fig. 4. As the silica is relatively stable, the reaction of the silica coupling agent on the silica was carried out on only a few cell structures, so its FT-IR spectrum is similar to the FT-IR spectrum of the silica. Compared to the FT-IR spectrum of silica, it can be seen that for APTES/silica and AM/APTES/silica at near 2,950 cm^{-1} there is an absorption peak, indicating that the sample contains $-\text{CH}_3$ and $-\text{CH}_2-$. Besides, AM/APTES/silica displayed the characteristic peaks of $\nu(\text{C}=\text{O})$ at 1,690 cm^{-1} , while APTES/silica and silica exhibited none absorbed here. The strong band at near 1,095 cm^{-1} was characteristic for Si-O-Si anti-symmetric stretching vibration, which indicated that AM/APTES had been successfully loaded on the surface of SiO_2 . All of these indicated that AM/APTES/silica was well prepared.

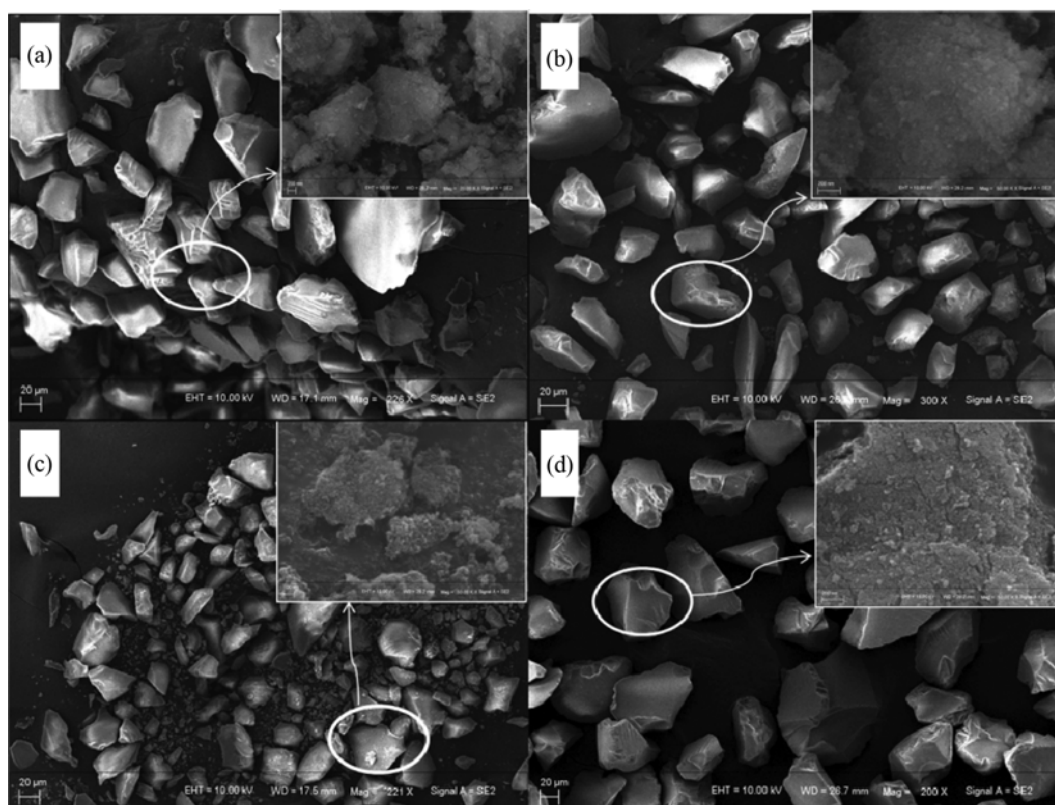


Fig. 5. FE-SEM of silica (a), APTES/silica (b), AM/APTES/silica (c) and PSA (d).

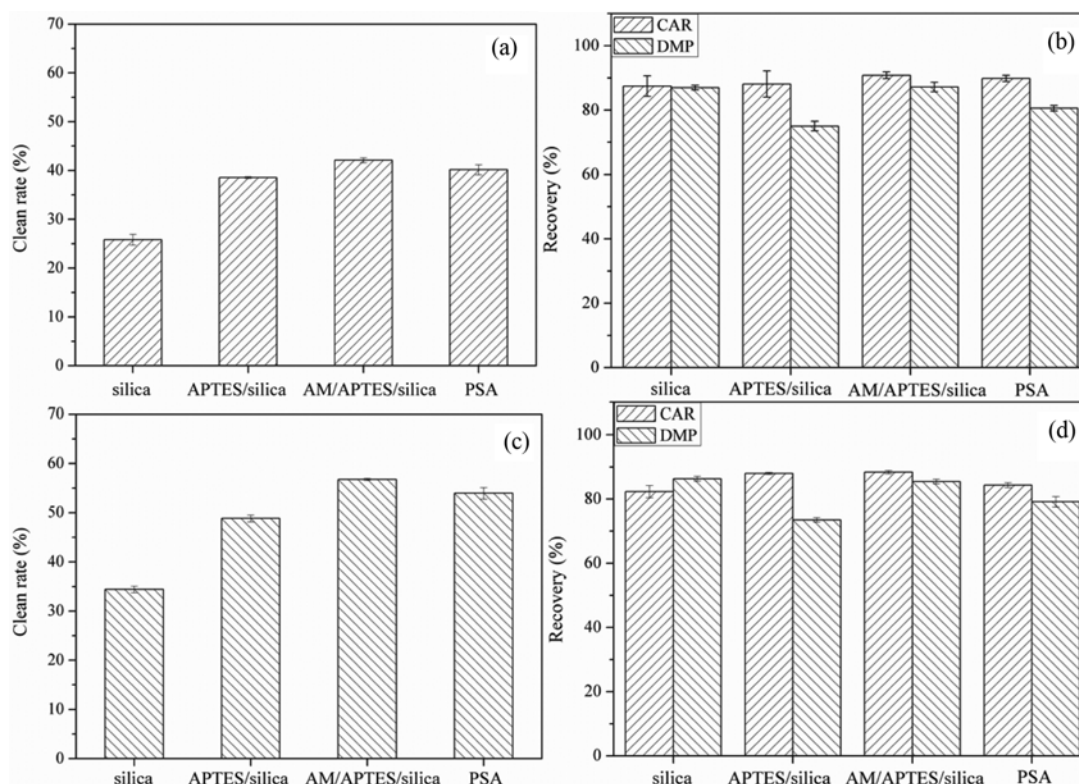


Fig. 6. Clean rate (a) and recovery (b) of CAR and DMP by four adsorbents in QuEChERS method; clean rate (c) and recovery (d) of CAR and DMP by four adsorbents in QuEChERS-MSPE method.

The micro-morphological analysis of silica, APTES/silica, AM/APTES/silica and commercial PSA was performed by FE-SEM (Fig. 5). From Fig. 5(a), silica had an irregular shape and its surface was smooth. From Fig. 5(b), APTES/silica maintained similar appearance and particle size of silica. The APTES/silica did not destroy the structure of the silica itself, and the reaction was carried out only on the surface of the silica. In Fig. 5(c), the rougher the surface of AM/APTES/silica, the looser skeleton decreases the mass-transfer resistance from mobile phase to stationary phase. From Fig. 5(d), commercial PSA has an irregular shape, and its surface is rough. FE-SEM was successfully used to observe the morphologies of block polymers, which are important parameters to evaluate polymerization stability and reproducibility.

3. Evaluation of QuEChERS Procedure

3-1. Effect of Type of Adsorbents

To evaluate the usefulness of AM/APTES/silica adsorbent, its performance was compared with silica, APTES/silica and commercial PSA. This experiment examined the effect of the adsorbent from two aspects. On one hand, clean-up efficiency is important in the method. Under the same condition, the purification results of these four adsorbents are exhibited in Fig. 6(a)-(b). All the four adsorbents have adsorption capacity for impurities; silica has the weakest and the other three adsorbents have better adsorption capacity for impurities. AM/APTES/silica has the best clean ability of the four adsorbents.

On the other hand, as shown in the inset of Fig. 6(b), APTES/silica has the worst recovery for the two targets of the four adsorbents. Commercial PSA has a good recovery for CAR, but the recovery of DMP is not better than AM/APTES/silica. AM/APTES/silica and silica had good recoveries for two targets. The experimental results show that AM/APTES/silica had a good effect in clean-up efficiency while ensuring good recoveries of analytes. There is a reason that the amide group can enhance the polarity of AM/APTES/silica, reducing the loss of the targets (CAR and DMP: weak-polar) in the clean-up step. On the whole, AM/APTES/silica was considered to be the best adsorbent in this experiment.

3-2. Effect of Amount of Adsorbent

To assure sufficient clean-up efficiency and recovery of the tar-

geted analytes, the amount of AM/APTES/silica was investigated from 20 to 120 mg in the QuEChERS method. The apple sample was purified by various amounts of the adsorbent, and three independent experiments were repeated. As shown in Fig. 7, with 60 mg adsorbent, the recovery of DMP was higher than others and CAR also had a great recovery. When the adsorbent was more than 60 mg, the amount of removal of impurities did not significantly change. Hence considering the clean-up efficiency and recoveries of targets, 60 mg AM/APTES/silica was chosen for the QuEChERS clean-up step.

4. Evaluation of QuEChERS-MSPE Method

In the coupled QuEChERS-MSPE method, 60 mg adsorbent was added in the first clean-up step, and 3 mg adsorbent was added in the pipette-tip MSPE procedure. The result is shown in Fig. 6(c)-(d). AM/APTES/silica not only has the best clean-up efficiency of the four adsorbents, but also it keeps good recoveries of the targets. It proved that AM/APTES/silica is the best adsorbent in this experiment.

AM/APTES/silica as adsorbent was used for comparing the QuEChERS method and QuEChERS-MSPE method. As shown in Fig. 8, the clean rate was 56.78% in the combined method, with the clean rate 42.11% in the single method. From Fig. 6, the recovery of CAR was 85.44% and DMP was 88.37% in the combined method, and the recovery of CAR was 90.83% and DMP was 87.18% in the QuEChERS method. In all, the QuEChERS-MSPE method has the advantage of high clean-up ability of impurities with better recoveries of two targets.

5. Validation Method

The AM/APTES/silica as adsorbent to absorb various impurities in the apple was studied, and validation of the method under the optimized condition was achieved. The calibration curves are in the range of 0.1-100.0 $\mu\text{g/mL}$ for CAR and 0.1-100.0 $\mu\text{g/mL}$ for DMP. Their regression equations are $Y=7.11 \times 10^4 X - 3.91 \times 10^4$ ($R^2=0.9990$) and $Y=5.76 \times 10^4 X + 5.72 \times 10^4$ ($R^2=0.9977$), respectively (X is concentration; Y is peak area). Based on signal-to-noise ratio of 3 and 10, the limit of detection (LOD) and limit of quantity (LOQ) of the method are 0.012 $\mu\text{g/mL}$ and 0.04 $\mu\text{g/mL}$ for CAR,

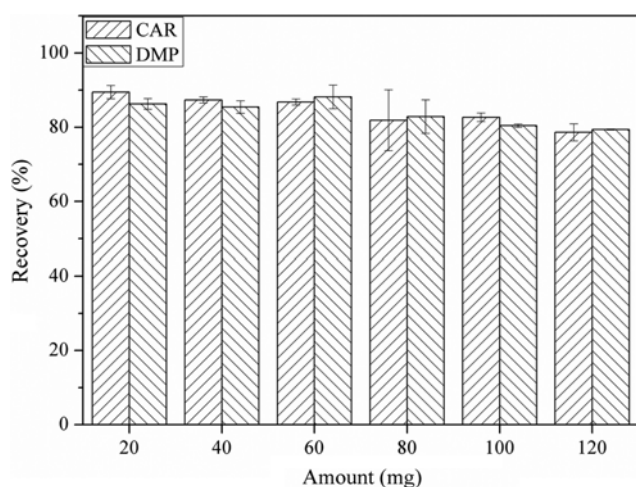


Fig. 7. Effect of different amounts of AM/APTES/silica on recoveries.

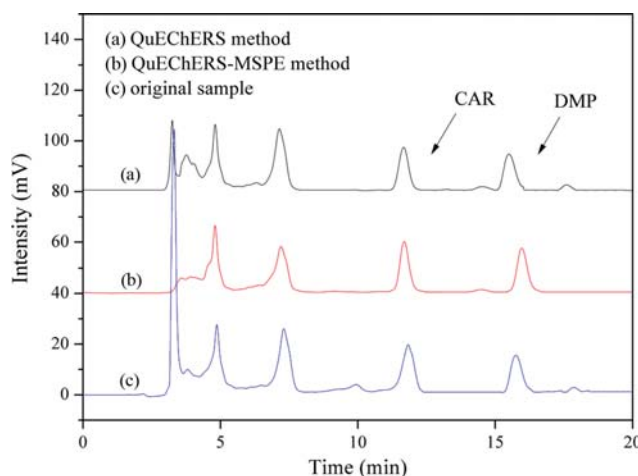


Fig. 8. The chromatograms of samples with QuEChERS method, QuEChERS-MSPE method and original sample.

Table 1. Recoveries of CAR and DMP in sample detected by QuEChERS-MSPE (n=3)

Analytes	Spiked	Intra-day		Inter-day	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
CAR	0.5	84.9	2.1	90.0	2.2
	5	86.0	1.6	86.8	0.7
	50	87.7	0.3	87.4	2.3
DMP	0.5	88.2	0.4	84.7	2.0
	5	87.1	2.9	84.1	2.3
	50	87.8	1.2	85.2	1.5

Table 2. Recoveries of analytes in 7 fruit samples (the concentrations of the spiked analytes were 10 µg/mL, n=3)

Sample	CAR		DMP	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Watermelon	89.4	2.0	98.3	1.5
Tomato	86.2	0.7	103.0	3.5
Pineapple	78.4	1.0	87.8	2.4
Strawberry	77.2	1.1	96.1	0.1
Grape	79.1	1.7	93.6	0.3
Orange	94.3	0.2	92.4	2.3
Pear	73.1	1.3	88.9	0.6

0.023 µg/mL and 0.08 µg/mL for DMP, respectively. The method recoveries were evaluated by performing three replicates of the spiked samples (0.50, 5.00, 50.00 µg/mL, respectively) in the intra-day and inter-day. As shown in Table 1, the method recoveries of CAR were in the range of 84.9–90.0% with associated intra-day and inter-day RSDs ranging from 0.3 to 2.3%. The method recoveries of DMP were in the range of 84.1–88.2% with associated intra-day and inter-day RSDs ranging from 0.4 to 2.9%. These results illustrated that the developed method provided a wide linearity range, high sensitivity and good repeatability for the target analytes.

6. Analysis of Samples

The proposed method was further applied to examine the AM/APTES/silica as adsorbent in seven fruits samples including watermelon, tomato, pineapple, strawberry, grape, orange and pear, which were executed by the proposed method (Table 2). The results showed that the recoveries ranged from 73.10 to 94.32% for CAR and the recoveries ranged from 88.89 to 103.01% for DMP, respectively. The reasons for the difference in the data are due to different components of the fruit with different pH values, and it can affect the solubility of the targets, and the adsorptivity of the adsorbents on the targets was also different.

CONCLUSION

An acrylamide modified PSA analogue (AM/APTES/silica) was synthesized by organosilane coupling agent method and was used as adsorbent for the QuEChERS-MSPE method. The adsorbability of the AM/APTES/silica lies in its weak ion exchange proper-

ties, hydrophobic interaction and π electron accepting ability. The SEM image revealed that the AM/APTES/silica possessed a rough structure. The obtained AM/APTES/silica had a good effect in clean-up efficiency and showed higher recoveries than silica, APTES/silica and commercial PSA. The established method exhibited excellent properties with good linearity, low LOD and LOQ. In general, AM/APTES/silica as adsorbent opens a new way for the determination of CAR and DMP in apple sample, and the proposed method can be extended to other types of fruits and analytes.

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