Analytical Methods

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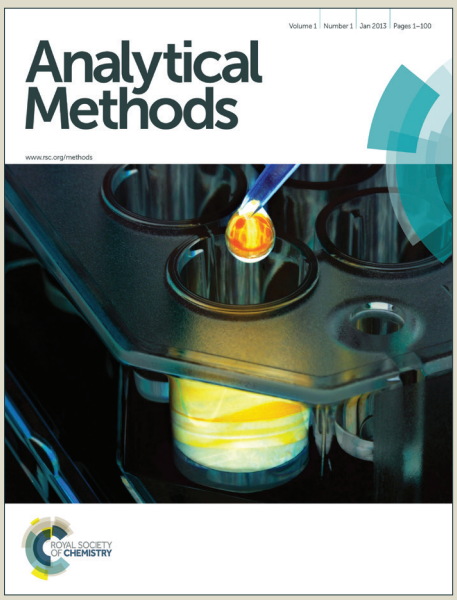

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Determination of five quaternary ammonium compounds in foodstuffs using high performance liquid chromatography-tandem mass spectrometry

Haiwei Cao[1], Mingqin Kang[1,2], Zhen Chen[1], Ling Li[2], Minghui Cui[1], Mingyan Chen[2], Hanqi Zhang[1] , Yinghua Wang[1*]

[1]*College of Chemistry, Jilin University, Changchun, P. R. China*

[2]*Jilin Entry-exit Inspection and Quarantine Bureau, Changchun, P. R. China*

* Corresponding author: College of Chemistry, Jilin University, 2699 Qianjin Street, Changchun 130012, PRChina.

E-mail address: wangyh.jlu.edu.cn (H.Wang).

Tel.: +8613159530365.

Abstract: The high performance liquid chromatography-trandem mass spectrometry was applied to the determination of the quaternary ammonium compoueds, including dodecyltrimethyl ammonium bromide, dodecyldimethylbenzylammonium chloride, tetradecyldimethylbenzylammonium chloride, hexadecyldimethylbenzylammonium chloride and didectyldimethylammonium chloride in pork, beerliver, apple, spinach, rice and white suger. The analytes were extracted using methanol-water mixture as extraction solvent, and further purified with Oasis WCX cartridge. The separation of quaternary ammonium compounds was performed on a CAPCELL PAK CR 1:4 column using acetonitrile–50 mmol /L ammonium format aqueous solution containing 0. 1 % formic acid as mobile phase in a gradient elution mode. The mass spectrometric detection was operated by using electrospray ion source in the multiple reaction monitoring mode. The results showed that the linear range for 5 quaternary ammonium compounds was 5.00～500. 00 μg/L, and the correlation coefficients were higher than 0. 995. The limit of quantification for the analytes was 10.00 μg/L. Six kind of real samples were analyzed and the recoveries of the analytes at three spiked levels were between 76.8 % and 102.5 % with the relative standard deviations of 1.09 %～8.33 %. The present method is simple, reliable and accurate and can be applied to the determination of quaternary ammonium compounds in foodstuffs.

Key words: high performance liquid chromatography–tandem mass spectrometry (HPLC-MS /MS) ; quaternary ammonium compounds; foodstuffs

1 Introduction

Quaternary ammonium compounds (QACs) contain a quaternary nitrogen . In the simplest case, alkyl chains of different lengths are attached to the nitrogen atom, although the quaternary nitrogen may also be part of a ring system (such as pyridine or piperidine) [1]. QACs belong to the group of cationic surfactants and are located at the phase boundary between the organic and water phase [2]. Quaternary ammonium surfactants are high-production-volume chemicals that constitute a large fraction of the cationic surfactant market[3,4]. The QACs are an economically important class of industrial chemicals. Because of their physical and chemical properties QACs are widely used as biocides, drugs and herbicides[5,6].Disinfectants based on QACs are widely used in hospital environments and the food industry due to their low toxicity to humans and animals[7,8]. The focus on safe food and production of refrigerated food has led to the increasing use of QACs in the food industry[8,9]. The QACs were also used as fabric softeners, hair conditioners, emulsifying agents and constituents of room deodorizers, and sanitizers[10–12,2]. However, in recent years quaternary ammonium disinfectant poisoning death events have been reported[13]. The toxic effects of quaternary ammonium germicides have been described in detail[14]. Therefore, it is necessary to study residues of QACs in food.

Benzalkonium chloride (BAC) is a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups have a chain length from C8 to C18 [15–17], This mixture is widely used as an active substance in anti-bacterial and antifungal products, such as preservatives and medical disinfectants[18,19]. The most common BACs are

C_{12}-BAC, C_{14}-BAC and C_{16}-BAC[20,21]. Other quaternary ammonium compounds, such as dodecyltrimethylammonium dromide and didectyldimethylammonium chloride are also commonly used as biocides and disinfectants.

BAC can be determined using liquid chromatograph (LC) with UV-detection in aerosol preparations [22]and treated wood[23]. The capillary electrophoresis(CE) also is an efficient separation method of ionic surfactants[24,1]. Recently, LC–MS was a widely applied for analyzing samples because MS has higher sensitivity and selectivity compared with LC detection[25–27,2]. Before determination of QAC, sample preparation was required. The solid phase extraction (SPE) was widely applied to perform the analyte concentration[28,29,5].

In this study, a HPLC–MS/MS was developed and applied for determination of quaternary ammonium compounds in food samples. SPE was applied to the treatment of the samples. The determination of the analytes in food samples have not been reported. The present method has high sensitivity and is suitable for routine determination of these analytes in real samples.

2 Materials and methods

2.1 Reagents and chemicals

Quaternary ammonium compounds, including dodecyltrimethylammonium bromide (DTAB), dodecyldimethylbenzylammonium chloride (C_{12}-BAC), tetradecyldimethylbenzylammonium chloride (C_{14}-BAC), hexadecyldimethylbenzylammonium chloride (C_{16}-BAC) and didectyldimethylammonium chloride (DDAC) were obtained from Sigma–Aldrich

(Shanghai, China) and the purities of the compounds are greater than 98 %. The chemical structures of the compounds are presented in Fig.1. HPLC grade acetonitrile (ACN), methanol (MeOH) and formic acid (99 %) were purchased from Fisher Scientific (NJ , USA). Ammonium formate and ammonia solution (25 %) are of analytical grade and supplied by Merck(Shanghai,China). C18 (200 g/3mL) Sep-Pak cartridge was obtained from Supelco (Bellefonte, PA, USA). Oasis WCX (60 mg/3 mL), Oasis HLB (60 mg/3 mL) and Oasis MCX (60 mg/3 mL) SPE cartridges were purchased from Waters Corp. (Milford, MA, USA). Ultrapure water was obtained with a Milli-Q system (Millipore Co., MA, USA).

Stock solutions of individual compounds were prepared by dissolving each compound in methanol at a concentration of 1000mg/L . Mixed standard solution was prepared in methanol at 10 mg/L. Working solutions were obtained by dilution as required. All solutions were kept in glass vials and stored at 4 ℃.

2.2 apparatus

The Agilent 1100series HPLC system, equipped with a quaternary pump, auto sampler, degasser and column department (Agilent TechnologiesInc., USA) was used. Applied Biosystems API 4000 triple quadrupole mass spectrometer with electrospray ionization (ESI) interface and Analyst 1.5 software (AB SCIEX,USA) was used. Allegra^{TM}X-22R Benchtop Highspeed refrigerated centrifuge was purchased from Beckman coulter,Inc. (Calif.,CA, USA). KQ-250B Ultrasonic generator was purchased from Kunshan Instruments Inc. (Kunshan, Jiangsu, China). The Vortex-Genie 2 was purchased from Scientific Industries (NY, USA).

2.3 Samples

Samples (pork, beer liver, apple, spinach, rice and white sugar)were purchased from local supermarket (Changchun, China). The pork, beer liver, apple and spinach were chopped and homogenized with food processor. The rice samples were triturated and passed through the 2.0 mm sieve. The white suger was used directly. The spiked samples containing QACs were prepared by spiking the mixed working solutions into the samples and vortexing for 5 min. The resulting pork, beer liver, apple and spinach samples were stored at -18 ℃ and other samples were stored at 4 ℃ until analysis. Except for the experiments mentioned in Section 3.3.3, which were performed with all samples, all other results were obtained with rice sample.

2.4 Extraction and cleanup

2.00 g of sample was placed into a 50 mL polyterafluoroethylene centrifuge tube. 10.00 mL of methanol/water (90:10, v/v) was added into the tube. The sample was vortexed for 30 s, then sonicated for 15 min, and finally centrifuged at 10000rpm for 5min at -4 ℃. The supernatant was transferred to another 50 mL centrifuge tube. The residues were re-extracted with 10.00 mL of methanol/water (80:20, v/v). All supernatants were combined in the tube for further purification with Oasis WCX SPE cartridge.

The WCX cartridge was pre-conditioned with 3 mL of methanol, followed by 6 mL of water. Then, the resulting supernatant was loaded at a flow rate of 3 $mL \cdot min^{-1}$. The cartridge was washed with 3 mL of 5 % (v/v) ammonia solution and 3 mL of methanol. Next, the analytes were eluted with 6.00 mL of the mixture of formic

acid/methanol (2:98, v/v). The eluate was evaporated under a steam of nitrogen gas at 40℃. Finally, the residue was dissolved in 1.00 mL of ammonium formate aqueous solution (0.1 % formic acid)/ acetonitrile and filtered with a 0.22 μm nylon filter (Millipore, Carrigtwohill, Ireland) before analysis.

2.5 LC–MS/MS analysis

The chromatographic separation was performed on a CAPCELL PAK CR 1:4 (150 mm×2.0 mm, 5.0 μm) column in gradient elution mode. The mobile phase consisted of 0.1 % formic acid – 50 mmol·L^{-1} ammonium formate aqueous solution (A) and acetonitrile (B). The gradient program is as follows: 0～3.0 min: 45 % A; 3.0～10.0 min:45～10 % A; 10.0～13.0 min: 10 % A; 13.0～13.1 min: 10～45 % A; 13.1～20.0 min: 45 % A. The flow rate of mobile phase was set at 0.30 mL·min^{-1} and column temperature was kept at room tempreature. Injection volume was 10.0 μL.

Mass spectrometric analysis was carried out using an ESI source in positive ionization mode. The operation conditions are as follows: ion spray voltage (IS), 5500 V; source temperature at 550.0 ℃; curtain gas (CUR), 35.0 psi; ion source gas 1, gas 2 at 65.0 psi and 55.0 psi, respectively; collision-induced dissociation (CAD) gas, 8.0 psi. The Multiple reaction monitoring (MRM) mode was applied for quantitative analysis. The optimized parameter values for detecting quaternary ammonium compounds are shown in Table 1.

3 Results and discussion

3.1 Sample preparation

The some reagents, including water, methanol, ethanol, acetonitrile and acetone

were used as extraction solvents. The effect of type of extraction solvents on the recoveries of the analytes are shown in Fig.2. When water, ethanol and acetone are used as extraction solvents, recoveries of the target compounds are low. When methanol and acetonitrile were used, the recoveries of the analytes are high and close. Considering the toxicity of acetonitrile is higher than that of methanol, the methanol was used as the extraction soluent. The effect of methanol concentration was investigated and the experimental results are shown in Fig.3. When the methanol concentrations are lower 70 %, recoveries of all the analytes are low. High recoveries can be obtained for C_{12}-BAC, C_{14}-BAC, C_{16}-BAC and DTAB when 90% methanol is used as extraction solvent. However, the recovery of DDAC is low. The recoveries for C_{14}-BAC, C_{16}-BAC, DTAB and DDAC are high and the recovery of C_{12}-BAC is low when 80% methanol is used. In order to obtain high recoveries of all the analytes, the extraction was carried first using 90% methanol, and then using 80% methanol as extraction solvents.

The SPE cartridges including C18，Oasis HLB, Oasis WCX and Oasis MCX were used. When the standard solution was passed through the four types of cartridges the recoveries of analytes were examined and the experimental results are shown in Fig.4. The recoveries of QACs are very low when the C18 and Oasis MCX cartridges are used. The recoveries of QACs range form 78.7 % to 85.2 % when Oasis HLB was used. High recoveries (90.8 %~101.4 %) for all analytes were obtained when the WCX cartridge was used. Therefore, the WCX cartridge was selected for the following experiments.

The volume of elution solvent is also a crucial parameter that could have an effect on the recoveries of analytes. Under the same experimental conditions, 10.00 mL of formic acid-methanol (2:98, v/v) was used as the elution solvent. 1.00 mL of eluate was collected in a tube each time. The each eluate was analyzed and the analytical results are shown in Fig.5, the experimental results indicate that the all analytes can be completely eluted with 6.00 mL of elution solvent. However, because all elution solvent will be evaporated, so 10.00 mL of elution solvent should be used.

3.2 Optimization of HPLC–MS/MS determination

The MS/MS parameters were optimized by injecting a standard solution of 1 mg/L of each analyte directly into the MS system at a flow rate of 0.01 mL/min. The ESI positive and negative modes were evaluated. The results suggested that all QACs were ionized in positive ion mode, so positive ion mode was chosen. MS parameters for the target analytes were optimized in positive electrospray ionization full scan mode. The MS/MS conditions were adjusted in collision-induced dissociation (CID) mode under various collision energies. Each compound was detectable in the form of $[M\text{-}Cl]^+$ion except for DTAB, which forms a stable $[M\text{-}Br]^+$ ion. For these compounds, $[M\text{-}Cl]^+$ions and $[M\text{-}Br]^+$ion were selected as the precursor ions. Two MRM transitions were monitored and the most sensitive transition was chosen for quantitative analysis. The optimal parameters for each compound are shown in Table 1.

The HPLC conditions was studied with three analytical columns including Ultra C18 (150 mm×2.1 mm, 5 μm, RESCEK), Atlantis HILIC silica (100 mm×2.1 mm, 3

μm , Waters) and CAPCELL PAK CR 1:4 (150 mm×2.00 mm, 5 μm , SHISEIDO). Under the same LC gradient program and mobile phase composition, the resolution of the analytes obtained with the CAPCELL PAK CR 1:4 column is higher than that obtained with Ultra C18 and the Atlantis HILIC silica. The effect of the mobile phase on chromatographic sparation was studied. The effect of the concentration of formic acid and ammonium formate in moblile phase A was examined and the results indicated that when the concentrations of formic acid and ammonium format were 0.1% and 50 mmol·L^{-1}, respectively, the resolution was highest. So, 0.1 % formic acid–50 mmol·L^{-1} ammonium formate aqueous solution/ acetonitrile was selected as the mobile phase. Other parameters, such as column temperature, flow rate of mobile phase and injection volume of the sample were studied in order to obtain high resolution. The results showed that when the column tempreature was room temperature [18], the flow rate of the mobile phase was 0.3 mL/min and the volume of sample was 10.0 μL the resolution was highest.

3.3 Method validation

The linear range, limit of detection (LOD), limit of quantifaction (LOQ), the precision, and recovery of the present method were evaluated.

3.3.1 Linearity，LOD and LOQ

Under the optimal experimental conditions, the calibration curves were obtained for all the target compounds. From Table 2 it is seen that the linear range is from the 5.00 μg/L to 500.00 μg/L and the correlation coefficients are greater than 0.995. The LOD and LOQ for each analyte were deteminned as the lowest concentrations that

yield a signal-noise (S/N) ratio of 3.00 and 10.00, respectively. The LODs and LOQs are listed in Table 2.

3.3.2 Recovery and precision

The recoveries of the analytes were evaluated by analyzing the spiked samples at three spiked concentrations (10.00, 50.00 and 100.00 $\mu g \cdot kg^{-1}$). The results are summarized in Table 3. The average recoveries of DTAB, C_{12}-BAC, C_{14}-BAC, C_{16}-BAC and DDAC in spiked samples are from 76.8 % to 102.5 %. It can be noted that relative standard deviation values are lower than 8.33 % at three concentration levels. It was shown that the accuracy and the precision of the method developed are acceptable.

3.3.3 Analysis of real samples

In order to check the applicability of the present method, the method was applied to the determination of QACs in the six spiked samples including pork, beef liver, spinach, apple, rice and white sugar. The recoveries and precision of analytes in the six spiked samples are listed in Table 3. The total ion chromatogram of the extract of rice sample is shown in Fig.6. The typical MRM chromatograms of blank and spiked rice samples are shown in Fig.7.

3.3.4 Comparison of the present method with other methods

The performances of the present method were compared with those of other methods reported for determining QACs in foodstuffs. These methods include liquid–liquid extraction (LLE)[30]and QuEChERS [31]. The results are listed in Table 4. Compared with the reported methods,

when the present method was applied, the sample amount was smaller, the toxicity of the extraction solvent was lower, the operation was simpler. Considering the advantages, this study should be a satisfactory method.

4. Conclusions

In this study, a rapid and sensitive method was established for determination of QAC residues in foodstuffs. High clean up efficiency was obtained using WCX-SPE cartridge in the sample preparation. Under the selected experimental conditions, five kinds of quaternary ammonium compounds can be completely separated in 20 min. This method was validated with spiked samples and satisfactory recoveries were obtained. The present method is relatively simple and accurate, and can be applied for routine determination of these analytes in real samples.

References

[1] W. Buchberger, R. Schöftner. Electrophoresis. 24 (2003) 2111–2118.

[2] E. Martı´nez-Carballo, C. Gonza´lez-Barreiro, A. Sitka, N. Kreuzinger, S. Scharf, O. Gans. Environmental Pollution. 146 (2007) 543–547.

[3] X.L. Li, B.J. Brownawell. Environ. Sci. Technol. 44 (2010) 7561–7568.

[4] X.L. Li, B.J. Brownawell. Anal. Chem. 81 (2009) 7926–7935.

[5] O. N´u˜nez, E. Moyano, M.T. Galceran. J. Chromatogr. A 1058 (2004) 89–95.

[6] R. Castro, E. Moyano, M.T. Galceran. J. Chromatography A 914 (2001) 111–121.

[7] G. Vincent, M.C. Kopferschmitt-Kubler, P. Mirabel, G. Pauli, M. Millet.

Environ Monit Assess. 133 (2007) 25–30.

[8] G. Sundheim, S. Langsrud, E. Heir, A. L. Holck. International Biodeterioration. Biodegradation. 41 (1998) 235–239.

[9] L. Kröckel, W. Jira, D. Wild. Eur Food Res Technol. 216 (2003) 402–406.

[10] R. Ren, K.X. Li, C. Zhang, D.F. Liu, J. Sun. Bioresource Technology. 102 (2011) 3799–3804.

[11] N. Kreuzinger, M. Fuerhacker, S. Scharf, M. Uhlc, O. Gans , B. Grillitsch. Desalination 215 (2007) 209–222.

[12] U. Tezel, S.G. Pavlos tathis. Environ. Sci. Technol. 43(2009) 1342–1348.

[13] M. Hitosugi, K. Maruyama, A. Takatsu. Int J Legal Med. 111 (1998) 265–266.

[14] I. Ferrer, E.T. Furlong. Environ. Sci. Technol. 35 (2001) 2583-2588.

[15] Y.X.Yu, Y. Hieda, K. Kimura, T. Nishiyama, T. Adachi, Legal Medicine. 4 (2002) 232–238.

[16] K. Chanawanno, S. Chantrapromma, T. Anantapong, A. Kanjana-Opas, H.K. Fun. European Journal of Medicinal Chemistry. 45 (2010) 4199–4208.

[17] E. Martínez-Carballo, A. Sitka, C. González-Barreiro, N. Kreuzinger, M. Fürhacker, S. Scharf , O. Gans. Environmental Pollution. 145 (2007) 489–496.

[18] K. Kümmerera, A. Eitela, U. Brauna, P. Hubnera, F. Daschnera, G. Mascart, M. Milandri, F. Reinthaler, J. Verhoef. J. Chromatography A 774 (1997) 281–286.

[19] S.J. Prince, H. McLaury, L.V. Allen, P. McLaury. J.Pharmaceutical. Biomedical Analysis. 19 (1999) 877–882.

[20] M. J. Forda, L.W. Tetlera, J. Whiteb, D. Rimmer. J. Chromatography A 952 (2002) 165–172.

[21] J. Dudkiewicz-Wilczyńska, J. Tautt, I. Roman. J.Pharmaceutical. Biomedical Analysis 34 (2004) 909–920.

[22] T. Miyauchi, M. Mori, K. Ito. J. Chromatography A 1095 (2005) 74–80.

[23] O. Núñez, E. Moyano, M.T. Galceran. J. Chromatography A 946 (2002) 275–282.

[24] H. Sütterlin, R. Alexy, A. Coker 1, K. Kümmerer. Chemosphere 72 (2008) 479–484.

[25] A.V. Voorde, C. Lorgeoux, M. Gromaire, G. Chebbo. Environmental Pollution. 164 (2012) 150–157.

[26] E. Martıńez-Carballo, C. González-Barreiro, A. Sitka, N.Kreuzinger, S. Scharf, O. Gans. Environmental Pollution 146 (2007) 543–547.

[27] X.T. Peng, Z.G. Shi, Y.Q. Feng. J. Chromatography A 1218 (2011) 3588–3594.

[28] J.L. Martínez Vidal, A. Belmonte Vega, F.J. Sánchez López, A. Garrido Frenich. J. Chromatography A 1050 (2004) 179–184.

[29] P. Bassarab, D. Williams, J.R. Dean, E. Ludkin, J.J. Perry. J. Chromatography A 1218 (2011) 673–677.

[30] Determination of Quaternary Ammonium Compounds (QACs) in Food

Products.http://www.iib.org.tr/files/downloads/PageFiles/%7B6089aa41-ac9f-4d71-8fe9-8621a6851e4d%7D/Files/4_Notice%20Labor.pdf.

[31] J. Hepperle, E. Schüle, D. Kolberg, E. Scherbaum. News Analytik. Publikationsdatum: 12.03.2014.

Figure caption

Fig. 1 Structures of quaternary ammonium compounds .

Fig. 2 Effect of the type of extraction solvent from rice samples at concentration of 50.00 μg·kg^{-1}.

Fig.3 Effect of the different methanol aqueous solution from rice samples at concentration of 50.00 μg·kg^{-1}.

Fig.4 Recoveries of analytes obtained with different SPE cartridges from rice samples at concentration of 50.00 μg·kg^{-1}.

Fig.5 Recoveries of analytes obtained with different volume of elution solvent from rice samples at concentration of 50.00 μg·kg^{-1}

Fig.6 Total ion chromatogram of the extract of rice sample at the level of 50.00 μg·kg^{-1} .

Fig.7 Typical MRM chromatograms of blank rice (A) and spiked rice sample at the level of 50.00 μg·kg^{-1} (B).

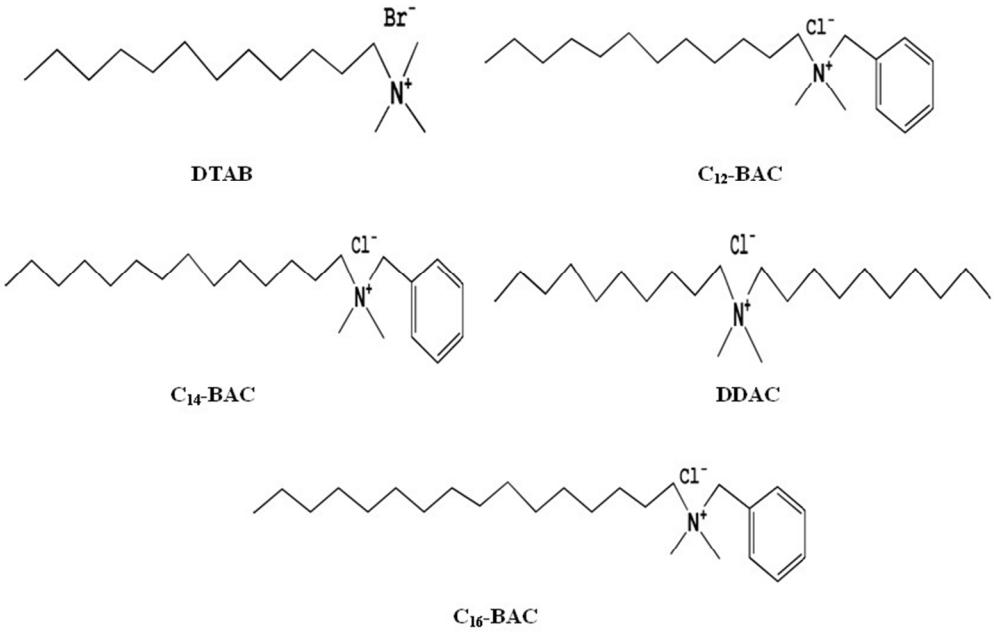

Fig.1 Structures of quaternary ammonium compounds .
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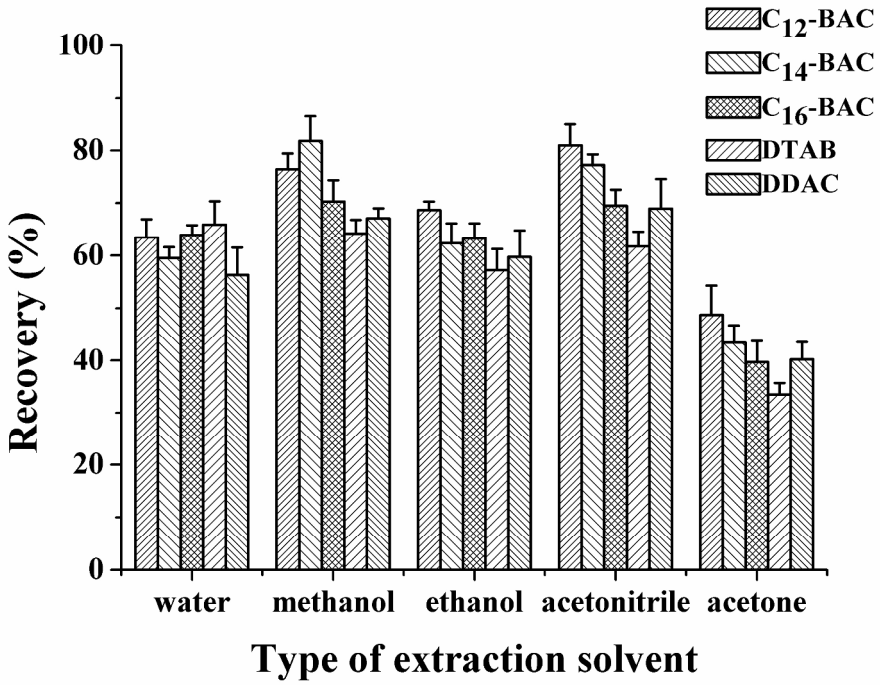

Fig. 2 Effect of the type of extraction solvent from rice samples at concentration of 50.00 μg·kg−1.
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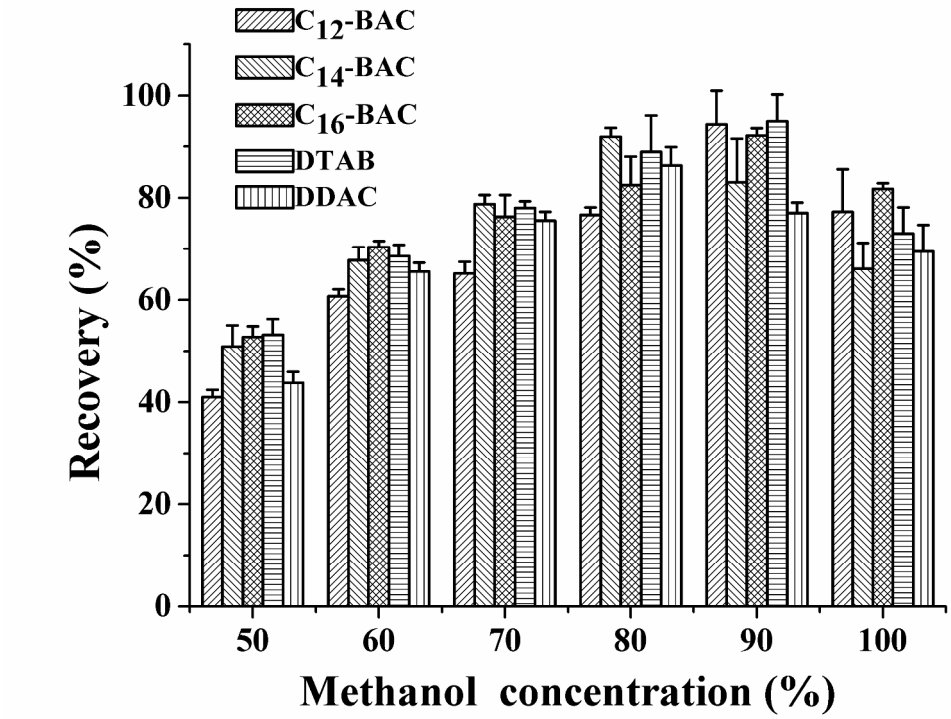

Fig.3 Effect of the different methanol aqueous solution from rice samples at concentration of 50.00 μg·kg−1.
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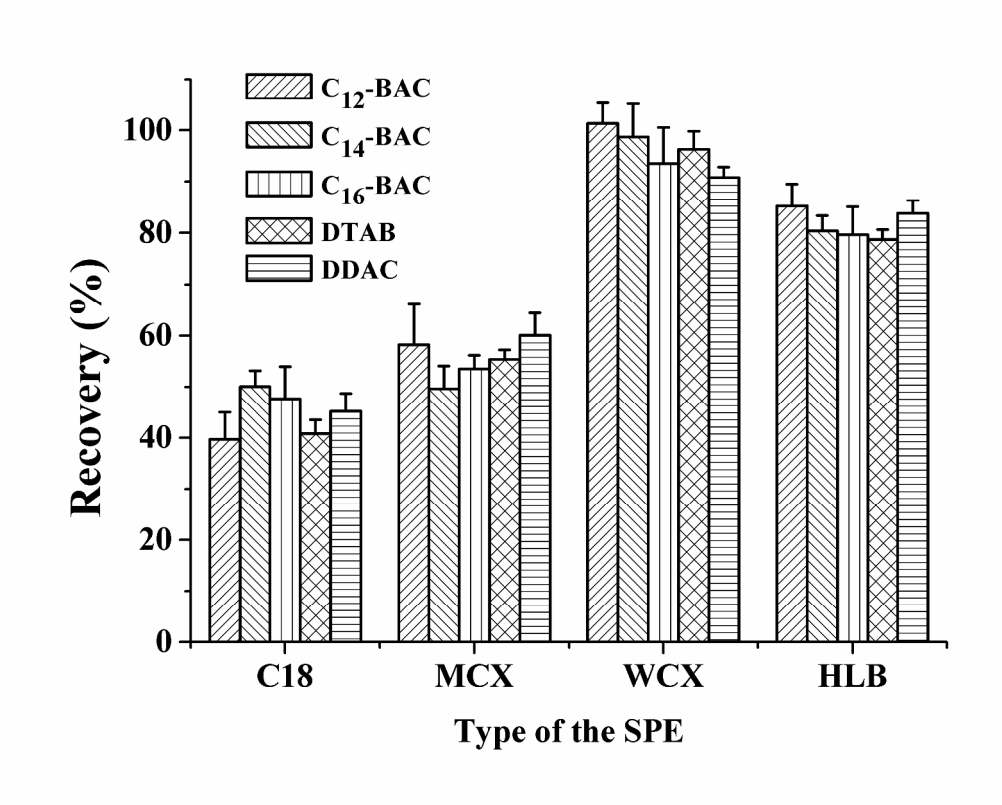

Fig.4 Recoveries of analytes obtained with different SPE cartridges from rice samples at concentration of 50.00 μg·kg−1.
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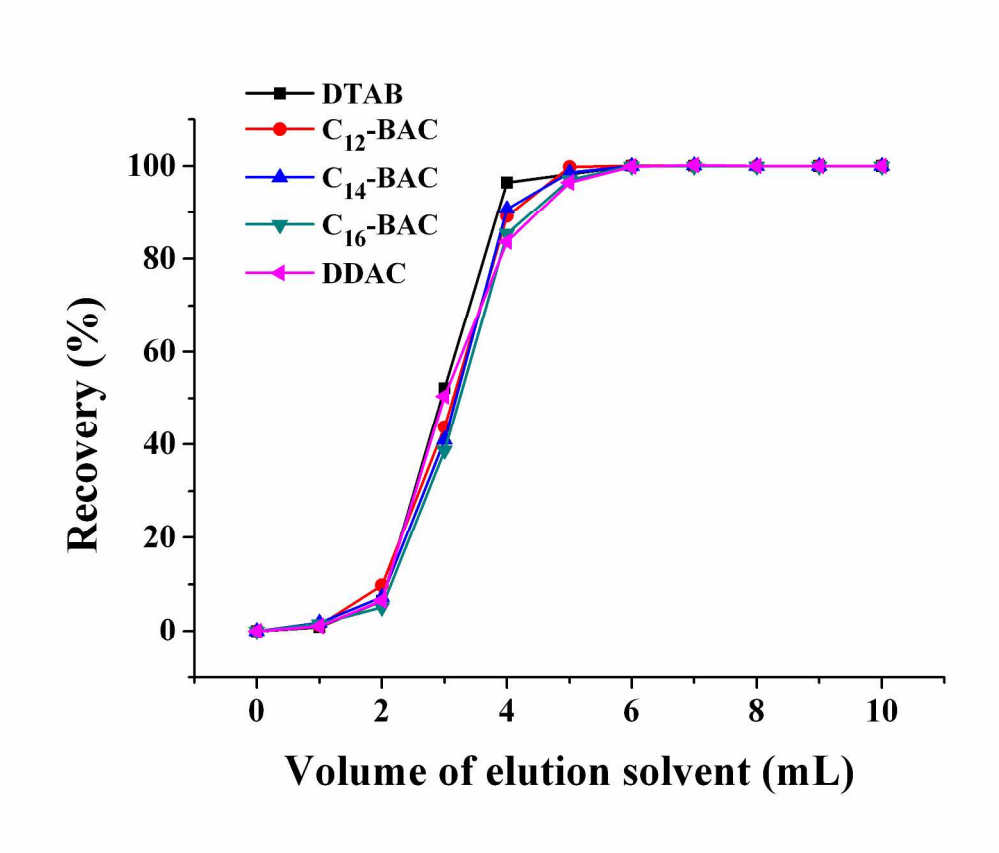

Fig.5 Recoveries of analytes obtained with different volume of elution solvent from rice samples at concentration of 50.00 μg·kg−1
270x230mm (300 x 300 DPI)

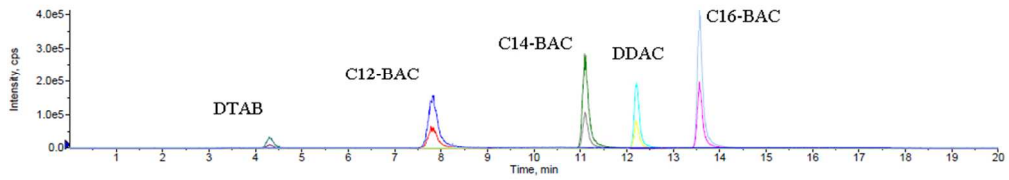

Fig.6 Total ion chromatogram of the extract of rice sample at the level of 50.00 μg·kg−1 .
266x71mm (96 x 96 DPI)

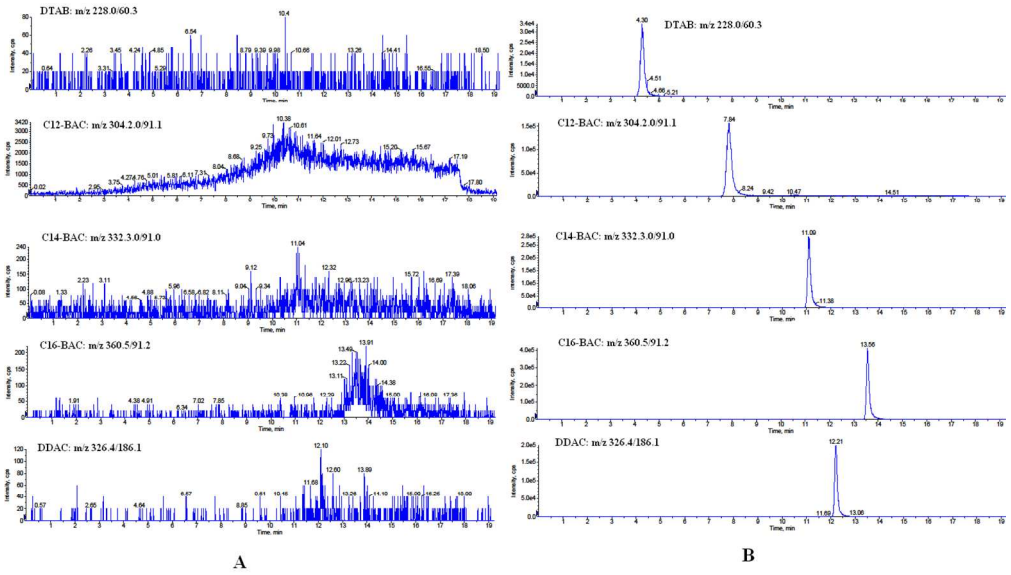

Fig.7 Typical MRM chromatograms of blank rice (A) and spiked rice sample at the level of 50.00 μg·kg−1 (B).
399x234mm (96 x 96 DPI)

Table 1 MS/MS parameters of the quaternary ammonium compounds.

Compounds	Precursor ion m/z	Production ions m/z	CE (eV)	DP (V)	Retention time(min)
DTAB	228.0	57.0	33	90	4.89
		60.3*	42	90	
C_{12}-BAC	304.0	91.1*	46	50	7.34
		212.3	29	50	
C_{14}-BAC	332.3	90.0*	50	95	11.25
		240.0	31	95	
C_{16}-BAC	360.5	91.2*	60	100	14.83
		268.3	32	100	
DDAC	326.0	57.0	40	60	18.46
		186.1*	53	60	

*** Quantitative ion.**

Table 2 Linear equation, correlation coefficient, linear range, LOD and LOQ

Analyte	Regression equation	Correlation coefficient	Linear range (μg/L)	LOD (μg/kg)	LOQ (μg/kg)
C_{12}-BAC	$A = 1.44e^5c + 2.05e^6$	0.9988	5.00-500.00	3.00	10.00
C_{14}-BAC	$A = 1.38e^5c + 2.54e^6$	0.9976	5.00-500.00	3.00	10.00
C_{16}-BAC	$A = 1.62e^5c + 4.81e^6$	0.9955	5.00-500.00	3.00	10.00
DTAB	$A = 3.05e^4c + 1.75e^5$	0.9997	5.00-500.00	3.00	10.00
DDAC	$A = 1.06e^5c + 1.48e^6$	0.9996	5.00-500.00	3.00	10.00

Table 3 Recoveries and precision for determining the analytes in spiked samples

Analyte	added (μg/kg)	C_{12}-BAC		C_{14}-BAC		C_{16}-BAC		DTAB		DDAC	
		Recovery/%	RSD%	Recovery/%	RSD/%	Recovery/%	RSD/%	Recovery/%	RSD/%	Recovery/%	RSD/%
Pork	10.00	91.1	6.59	89.8	7.87	89.6	4.15	90.0	7.44	84.7	8.33
	50.00	96.2	4.17	96.3	3.75	98.3	3.15	98.3	3.47	94.8	4.68
	100.00	99.7	3.03	98.2	2.55	99.7	3.09	102.1	2.35	99.1	2.49
Beer liver	10.00	99.5	4.76	88.4	4.86	81.4	4.81	87.8	7.95	76.8	4.59
	50.00	98.6	5.22	96.1	5.16	90.5	5.34	100.5	3.63	98.3	6.53
	100.00	92.0	5.90	90.7	8.33	87.2	4.72	89.3	6.86	90.7	3.77
Apple	10.00	95.3	1.89	84.5	2.72	83.7	2.38	80.4	3.72	85,6	3.88
	50.00	102.5	3.18	98.4	1.09	90.5	1.45	84.3	1.73	98.3	3.26
	100.00	99.3	2.60	100.1	1.39	101.5	2.19	85.6	3.89	94.4	2.75
Spinach	10.00	88.1	3.04	90.0	3.22	82.6	5.44	84.1	2.70	83.5	3.02
	50.00	92.8	3.28	90.3	2.20	92.6	2.28	88.7	3.97	84.8	2.72
	100.00	96.2	2.75	95.7	3.48	87.3	2.83	83.2	4.00	90.3	5.09
Rice	10.00	89.0	3.16	83.3	1.97	83.3	3.72	82.1	2.34	86.1	2.84
	50.00	98.4	1.84	92.4	1.60	92.5	2.16	94.3	1.95	92.4	1.42
	100.00	93.6	3.44	86.7	2.70	92.4	3.14	84.7	2.82	94.7	3.67
White suger	10.00	94.1	4.63	95.2	3.20	96.6	3.61	98.3	3.48	97.3	3.61
	50.00	98.7	4.42	100.3	2.53	98.1	2.04	99.6	2.52	98.1	2.04
	100.00	97.5	2.73	97.7	3.47	97.3	6.51	94.2	4.63	81.4	6.51

Table 4 Comparison of the present method with other methods

	LLE+SPE	LLE	QuEChERS
Sample amount (g)	2.00	5.00	10.00
Extraction solvent	10mL of methanol-water(9:1)→ 10mL of methanol-water(8:2)	5 mL of NaCl solution→ 100mLof Acetonitrile-water (7:3)- 0.1% Formic acid	10 mL of acetonitrile→ 5.5 g of mix of salts
Quantification	External standard	External standard	Internal and external standards
Reference	This work	[30]	[31]