JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Analysis of Trace Quaternary Ammonium Compounds (QACs) in Vegetables Using Ultrasonic-Assisted Extraction and Gas Chromatography−Mass Spectrometry

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Supporting Information

ABSTRACT: A reliable, sensitive, and cost-effective method was developed for determining three quaternary ammonium compounds (QACs) including dodecyltrimethylammonium chloride, cetyltrimethylammonium chloride, and didodecyldimethylammonium chloride in various vegetables using ultrasonic-assisted extraction and gas chromatography−mass spectrometry. The variety and acidity of extraction solvents, extraction times, and cleanup efficiency of sorbents were estimated to obtain an optimized procedure for extraction of the QACs in nine vegetable matrices. Excellent linearities (R^2 > 0.992) were obtained for the analytes in the nine matrices. The limits of detection and quantitation were 0.7−6.0 and 2.3−20.0 μg/kg (dry weight, dw) in various matrices, respectively. The recoveries in the nine matrices ranged from 70.5% to 108.0% with relative standard deviations below 18.0%. The developed method was applied to determine the QACs in 27 vegetable samples collected from Guangzhou in southern China, showing very high detection frequency with a concentration of 23−180 μg/kg (dw).

KEYWORDS: *quaternary ammonium compounds, vegetable, ultrasonic-assisted extraction, GC−MS, matrix effect*

INTRODUCTION

Quaternary ammonium compounds (QACs) are a major class of cationic surfactants containing molecules with at least one hydrophobic hydrocarbon chain linked to a positively charged nitrogen atom.[1] Because of hydrophobic cation-exchange and germicidal efficiency, QACs are extensively used as detergents, emulsifiers, fabric softeners, disinfectants, floating agents, etc.[2] The worldwide annual consumption of QACs was 700,000 tons in 2010,[3] which is expected to grow rapidly in the future. The large-scale application of QACs inevitably leads their release into various environmental compartments. Recently, QACs were ubiquitously detected in surface water, sediment, sewage effluent, municipal sludge, and soil.[4−9] Especially, the residual levels of QACs in sediment or sludge are up to several hundreds of mg/kg (dry weight, dw), much higher than those of traditional organic pollutants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs).[8,10]

Low biodegradation and potential persistence of QACs are suggested by their residues frequently detected in freshwater sediment.[8,10] Furthermore, QACs, as cationic surface active compounds, are generally more toxic than anionic and nonionic compounds.[11] They could cause unpredictably adverse ecological effects even at lower concentration (tens of μg/L to hundreds of μg/L).[12,13] According to a previous study, QACs were highly toxic to bacteria and ciliated protozoa with 50 percent effective concentrations (EC_{50}) varying from 0.11 to 70 μmol/L.[12] Two common QACs, benzalkonium chloride and dimethyldioctadecyl ammonium bromide, could cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations.[14] Meanwhile, QACs may pose a remarkable effect on the bioavailability and mobility of the other pollutants coexisting in environmental media.[8,15] For example, cetyltrimethylammonium bromide (CTAB) significantly inhibited the uptake and removal of nutrients by algae through declining algal cell activities.[16] Because of their potential persistence, high toxicity, and effect on the fate of coexisting contaminants, QACs have been extensively studied in aquatic environments including their occurrence, behavior, and toxicity assessment, etc.[16−22] Nevertheless, little relevant information on QACs in terrestrial environments, especially in soil-plant systems, was available until recently.[9,23−27]

QACs may be transferred into agricultural soil through land application of sewage sludge, use of quaternary ammonium herbicide, and irrigation of water containing QACs.[9,23,24] QACs were detected by the authors in both irrigation water (220 to 2000 μg/L) and agricultural soils (820 to 940 μg/kg, dw) neighboring a mining area, in Guangdong province, southern China.[5,9] Furthermore, QACs were also detected in agricultural products including fruits, cereals, etc.[25−27] However, research on the occurrence and risk assessment of QACs in vegetables is hardly available owing to the lack of routine quantitation strategies for determining trace QACs in vegetables. Although some methods for determining QACs in foodstuffs such as meat products, dairy products, fruits, and cereals have been developed, they focused mainly on benzylalkyldimethylammonium compounds (BACs) instead of alkyl QACs.[25−27]

Received: April 12, 2015
Revised: July 7, 2015
Accepted: July 12, 2015
Published: July 12, 2015

DOI: 10.1021/acs.jafc.5b01828
J. Agric. Food Chem. 2015, 63, 6689−6697

Additionally, matrix effects, which can significantly interfere with the accuracy of an analytical method,[28] were not studied in the previous literatures.

A liquid chromatography−mass spectrometry (LC-MS) technique has been developed to measure QACs in both environmental media and food products.[25−27,29,30] However, a gas chromatography−mass spectrometry (GC-MS) technique has only been developed to measure QACs in environmental media without food products.[5,9,31,32] GC-MS is available in ordinary laboratories much more commonly than LC-MS because of the cost. Furthermore, GC-MS performs higher chromatographic resolution than LC-MS because it possesses a long capillary column where analytes can be effectively separated, which is beneficial to improve the accuracy of an analytical method.[31,32] Therefore, we believe that the GC-MS technique is more appropriate, cost-effective, and practical to be developed for routine determination of QACs in vegetables.

Dodecyltrimethylammonium chloride (DTAC), cetyltrimethylammonium chloride (CTAC), and didodecyldimethylammonium chloride (DDAC) (Figure 1), the representatives of

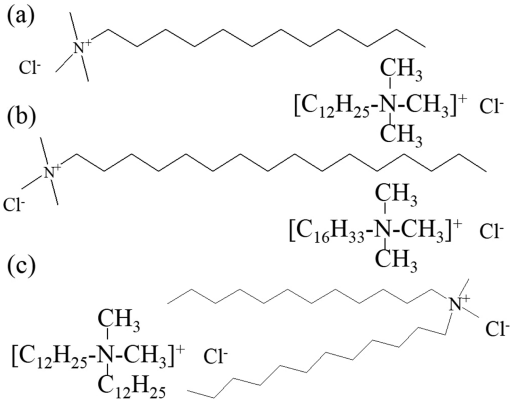

Figure 1. Structure of the three tested QACs: (a) dodecyltrimethylammonium chloride (DTAC), (b) cetyltrimethylammonium chloride (CTAC), and (c) didodecyldimethylammonium chloride (DDAC).

alkyl QACs with shorter alkyl chain lengths [C12−C16] as well as higher mobility and toxicity than other homologues [C18−C22],[11,13,33] have been detected in both agricultural soils and irrigation water as mentioned above.[5,9] Therefore, these QACs are most likely to be taken up by vegetables and consequently exhibit a health risk to humans through the food chain.[26,27,34]

The aim of the present study was to develop a reliable, sensitive, and cost-effective analytical method for routinely determining the three typical QACs (DTAC, CTAC, and DDAC) in various vegetables using ultrasonic-assisted extraction and GC-MS. This method is composed of sample pretreatment procedures optimized and reliable methodologies validated with linearity, matrix effect, precision, and accuracy. Acceptable results were obtained using this method to determine QACs in various real vegetables. To the best of our knowledge, this is the first study for the quantitation of QACs in various vegetables using GC-MS technique, and we believe it will effectively promote further research on QACs in soil−plant systems.

MATERIALS AND METHODS

Reagents. Standards DDAC (99%, purity) and CTAC (99%, purity) were purchased from Adamas (Emeryville, USA), and DTAC (99%, purity) was obtained from Aladdin (Seattle, USA). HPLC-grade solvents (including methanol, methyl trichloride, dichloromethane, and *n*-hexane) were purchased from Sigma-Aldrich (Steinheim, Germany). Neutral silica gel (200 mesh), neutral alumina oxide (200 mesh), and hydrochloric acid bought from Guangzhou Chemical Reagent Co., Ltd. (Guangzhou, China) were all of analytical grade.

Mixture stock solution (100 mg/L) of the three QACs (DTAC, DDAC, and CTAC) was prepared in methanol, which was kept in a refrigerator at 4 °C in the dark and used within one month. Standard working solutions of the three tested compounds at 20, 50, 100, 200, 500, 1000, and 2000 μg/L were prepared by diluting the stock solution with methanol when they would be used. Because matrix components in vegetables can influence detector responses, and the same vegetable species should have similar matrix effects,[28] nine vegetables including leafy vegetables (Chinese flowering cabbage, water spinach, and lettuce), root vegetables (white radish, potato, and carrot), and fruit vegetables (cucumber, pumpkin, and eggplant) were chosen to evaluate the matrix effects on the determination of QACs in vegetables. Correspondingly, matrice-matched standard solutions (20, 50, 100, 200, 500, 1000, and 2000 μg/L) were obtained by adding a blank vegetable sample extract to each diluted standard solution. All of these nine blank vegetables without the target QAC pollution were purchased from an organic vegetable farm where clean water and fertilizer without QAC residues were used.[28]

Sample Treatment. Approximately, 5 kg of each fresh vegetable sample was chopped and homogenized using a crusher (Jiu Yang Co., Ltd., China). An aliquot of about 1000 g of the sample was weighed and then lyophilized in a vacuum freeze drier (Jiangsu Hengfeng Equipment Manufacture Co., Ltd., China). Compared to the fresh vegetable sample, using a lyophilized vegetable sample was beneficial not only to minimize the sample volume and the reagent volume but also to shorten the extraction time.[28] The lyophilized samples were ground to a powder (0.45 mm) in a mill and stored in brown glass bottles. The spiked samples at three concentration levels (50, 200, and 1000 μg/kg) were obtained by adding 1 mL of mixture standard working solutions at 50, 200, and 1000 μg/L in methanol to 1.0 g of lyophilized blank vegetable sample powder, respectively. Afterward, the spiked samples were shaken continually to be homogenized and left for 12 h under a fume hood to evaporate the solvent at room temperature before analysis.

One gram aliquot of each lyophilized vegetable sample powder was weighed into 50 mL of Teflon centrifuge tube. Then, 10 mL of 0.1% acidified methanol (hydrochloric acid/methanol, v/v) was added into each tube. The tubes were shaken vigorously for 10 min with a constant temperature vibrator and then ultrasounicated for 10 min in a 100 W ultrasonic instrument (Kunshan Ultrasonic Instrument, Co., Ltd., China). The supernatant of each sample was collected after centrifugation at 8000 rpm for 5 min. The extraction was repeated twice again. All of the supernatants of each sample were combined and concentrated to almost 2 mL using a rotator vacuum evaporator (Shanghai Optical Instrument Factory, China) with 200 rpm at 40 °C. In order to remove interfering matrix components and other contaminants, neutral alumina oxide sorbent presoaked in methanol overnight was loaded into a glass column (30 cm height, 1 cm diameter) coupled with a flow control valve and filter cartridge until 8 cm height. The extracts were separately passed through the glass columns and eluted with 7 mL of methanol. The resulting elutes were collected and evaporated to near dryness, and then were redissolved with methanol, strained through 0.22 μm nylon filters, and transferred to sampler vials for GC-MS analysis.

Instrument and Apparatus. Analysis of the three QACs was conducted on a Shimadzu-QP2010 Plus series gas chromatograph equipped with a triple quadrupole mass spectrometry (GC-MS). According to the optimized conditions of GC-MS, the three QACs were separated with a DB-5MS fused silica capillary column (30 m length × 0.25 mm diameter, 0.1 μm film thickness). Samples were injected in splitless mode with 1 μL volume. The carrier gas was high purity helium (99.9999%) at a flow rate of 1.0 mL/min. The temperatures of injector, detector, and ion source were set at 280 °C, 250 °C, and 250 °C separately. The column oven temperature program was set as follows: start at 100 °C for 2 min, increase to 200

DOI: 10.1021/acs.jafc.5b01828
J. Agric. Food Chem. 2015, 63, 6689−6697

Table 1. Comparison of Matrix-Matched Calibrations and Solvent Calibration at Seven Concentrations Ranging from 20 to 2000 μg/L

QAC	matrix	regression equation	R^2	linear range (μg/L)	slope ratio (matrix/methanol)	LOD (μg/kg)	LOQ (μg/kg)
DTAC	methanol	$Y^a = 1387477x^b + 30491$	0.999	20−2000	-	-	-
	Chinese cabbage	$Y = 1428112x - 61689$	0.998	20−2000	1.03	5.8	19.2
	water spinach	$Y = 1431568x - 60351$	0.997	20−2000	1.03	5.6	18.6
	lettuce	$Y = 1557865x - 8820$	0.995	20−2000	1.12	3.7	12.3
	pumpkin	$Y = 1629247x - 38187$	0.998	20−2000	1.17	5.8	19.4
	cucumber	$Y = 1763182x - 79974$	0.998	20−2000	1.27	3.3	11.0
	eggplant	$Y = 1227865x - 123104$	0.998	20−2000	0.88	3.8	12.7
	potato	$Y = 1432219x - 34051$	0.998	20−2000	1.03	5.5	18.4
	white radish	$Y = 1503215x - 82402$	0.999	20−2000	1.08	3.3	11.0
	carrot	$Y = 1772079x - 43264$	0.999	20−2000	1.28	4.5	15.1
CTAC	methanol	$Y = 1397583x + 25275$	0.999	20−2000	-	-	-
	Chinese cabbage	$Y = 1408233x + 12586$	0.999	20−2000	1.01	3.7	12.4
	water spinach	$Y = 1512305x + 8929$	0.997	20−2000	1.08	3.8	12.8
	lettuce	$Y = 1475279x + 82068$	0.992	20−2000	1.06	5.5	18.2
	pumpkin	$Y = 1484901x - 33443$	0.999	20−2000	1.06	3.3	11.0
	cucumber	$Y = 1725138x - 62511$	0.999	20−2000	1.23	1.3	4.4
	eggplant	$Y = 1396822x - 89874$	0.999	20−2000	1.00	1.3	4.2
	potato	$Y = 1719931x - 26713$	0.999	20−2000	1.23	2.4	8.0
	white radish	$Y = 1600135x - 68794$	0.999	20−2000	1.14	2.7	8.9
	carrot	$Y = 1955302x - 6349$	0.999	20−2000	1.40	6.0	20.0
DDAC	methanol	$Y = 620569x + 9729.4$	0.999	20−2000	-	-	-
	Chinese cabbage	$Y = 616255x - 18161$	0.999	20−2000	0.99	4.0	13.5
	water spinach	$Y = 598534x - 616$	0.995	20−2000	0.96	1.3	4.3
	lettuce	$Y = 671015x - 6623$	0.998	20−2000	1.08	1.2	3.9
	pumpkin	$Y = 616238x - 17129$	0.999	20−2000	0.99	1.6	5.4
	cucumber	$Y = 771454x - 30101$	0.999	20−2000	1.24	0.7	2.4
	eggplant	$Y = 592653x - 43187$	0.999	20−2000	0.96	0.8	2.7
	potato	$Y = 691922x - 5736$	0.996	20−2000	1.11	0.7	2.4
	white radish	$Y = 773358x - 45449$	0.999	20−2000	1.25	0.7	2.3
	carrot	$Y = 745671x - 11858$	0.999	20−2000	1.20	3.0	10.0

[a]Peak area produced by the standard QACs in a concentration. [b]Concentration of the standard QACs.

°C at a rate of 30 °C/min, and increase to 280 °C at a rate of 40 °C (held 4 min), in a total run time of 11.33 min. The mass spectrometer was operated using electron-impact (EI) mode (70 eV) in the selective ion monitoring (SIM) mode in order to improve detection limits of the three QACs. The target ions of DTAC, CTAC, and DDAC were 58, 58, and 212 m/z, respectively,[31] and their retention time in this analytical procedure was 5.31, 6.85, and 8.67 min, respectively.

Validation Study. Validation of the developed method was performed in terms of linearity, limit of detection (LOD), limit of quantitation (LOQ), specificity, accuracy, and precision using the nine vegetables (Chinese flowering cabbage, water spinach, lettuce, pumpkin, cucumber, eggplant, potato, white radish, and carrot). The linearity was studied for each of the tested three QACs by spiking their standards in methanol and the nine vegetable matrices, respectively, in triplicate at seven concentration levels (20, 50, 100, 200, 500, 1000, and 2000 μg/L). The calculated parameters of the linear regression equations (slope, determination coefficient (R^2), and intercept) are presented in Table 1. Matrix effects were determined by calculating the slope ratios of matrix-matched calibration curves to solvent one. The matrix dependent LOD and LOQ were determined using the blank and calibration standards of the tested nine vegetable matrixes. The LOD of the each QAC is the concentration that produces a signal-to-noise (S/N) ratio of three, whereas the LOQ is estimated based on the S/N ratio of 10. Specificity of the developed method was analyzed based on the absence of interfering peaks at the retention time of the three QACs in the nine blank sample matrices. Accuracy and precision of the developed method were tested using recovery assays performed on samples spiked at three concentrations (50, 200, 1000 μg/kg). Five replicates for each spiked sample were prepared on three different days. The precision of the developed method was expressed as the intraday (repeatability) and interday (reproducibility) relative standard deviation (RSD^a and RSD^b) for 5 and 15 replicates, respectively.

Statistical Analysis. QAC concentrations from GC-MS analysis were calculated using postrun software of a Shimadzu-QP2010 plus series gas chromatograph. The data were exported to SPSS 21.0 (International Business Machines Co, USA) to determine the means and relative standard deviations (RSDs). Microsoft Excel 2010 was used to draw tables and figures.

RESULTS AND DISCUSSION

GC-MS Condition Optimization. The GC-MS conditions were optimized using the single and the mixture standard solutions of the three typical QACs (DTAC, CTAC, and DDAC) at 1000 μg/L. First, the mass spectral fragmentation pattern of each QAC was evaluated by analyzing the standard working solutions using EI mode at full-scan mode with the mass range of 50−300 m/z and at a rate of 3 scan/s. The three QACs were effectively converted into the corresponding tertiary amines by demethylation in the injection port at 280 °C as previously reported.[31,32] Then the tertiary amines were separated on a capillary column, depending on their alkyl chain lengths.[31] The target ions of DTAC, CTAC, and DDAC under the EI mode were 58, 58, and 212 m/z, respectively (Figure 2). Both the target ions at m/z = 58 ($[CH_2{=}N(CH_3)_2]^+$) and m/z = 212 ($[CH_2{=}N(CH_3)(C_{12}H_{25})]^+$) were derived from the α-cleavage of the carbon−carbon bond with respect to nitrogen.[31] In order to obtain an optimized separation of the tested QACs, three capillary columns (30 m × 0.25 mm × 0.25 μm) with

DOI: 10.1021/acs.jafc.5b01828
J. Agric. Food Chem. 2015, 63, 6689−6697

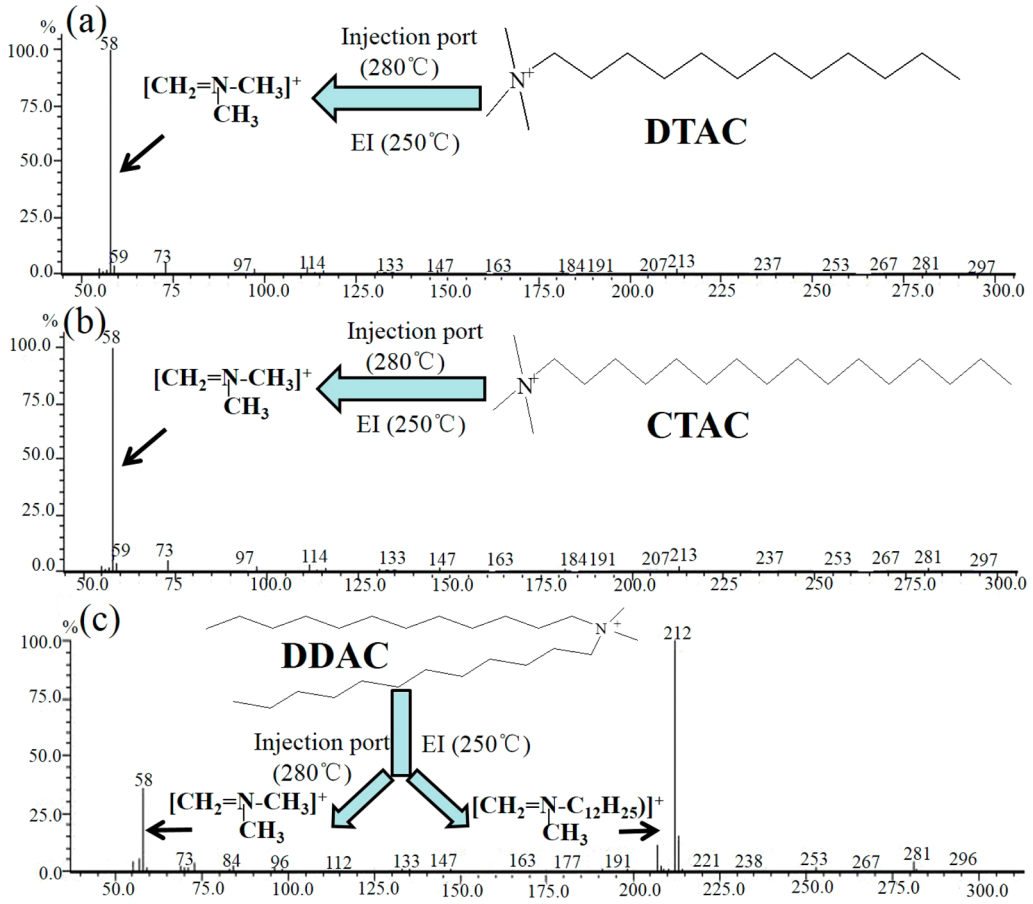

Figure 2. Product ion spectra of the three tested QACs: (a) DTAC, (b), CTAC, and (c) DDAC.

different stationary phases (i.e., DB-5MS consisted of 5% phenyl and 95% dimethylpolysiloxane, HP-1MS consisted of 100% dimethylpolysiloxane, and Rtx-35 consisted of 35% phenyl and 65% dimethylpolysiloxane) were compared. The best separation was obtained at the DB-5MS column with the appropriate proportion of phenyl and dimethylpolysiloxane. So the DB-5MS column was selected for the subsequent analysis. Furthermore, various temperature programs were investigated in order to achieve the best separation in the minimal time. By monitoring the target ions of the three QACs under the optimized GC-MS conditions, high response, remarkable selectivity, and excellent peak shape were obtained in a suitable analytical time (11.33 min), which are presented in Supporting Information, Figure S1.

Sample Extraction Optimization. To obtain an optimized sample extraction procedure, recovery tests for each single-variable (variety and acidity of extraction solvents, extraction times, and cleanup solvent) using QAC-spiked lettuce samples at 1000 μg/kg were carried out. Sequentially, the obtained procedure was evaluated in the other eight vegetable samples. First, four commonly used solvents (methanol, dichloromethane, methyl trichloride, and *n*-hexane) were separately investigated in recovery tests to get a satisfied extraction solvent for the three QACs. As the results exhibit in Figure 3 a, both recoveries (60%−77%) and RSD (<10%) of the three QACs were satisfied when methanol was used as the extraction solvent, while low recoveries of QACs were observed when using methyl trichloride and *n*-hexane as extraction solvent. QACs are cationic compounds tending to be extracted by polar solvents according to the similarity−intermiscibility theory.[9] Therefore, the highest recovery was obtained when using methanol with the strongest polarity among the four tested solvents in the present study.

As reported in the literature, the acidity of the extraction solvent significantly influences the extraction efficiency of QACs in the sample matrix (water and sediment).[29,30] So, recovery experiments using methanol as the extraction solvent with acidity ranging from 0.05% to 0.5% (hydrochloric acid/methanol, v/v) were conducted. Results showed that recoveries of the three QACs increased with increasing acidity of methanol under lower acidity (<0.1%, v/v) (Figure 3b). However, lower recoveries were observed with further increase in acidity from 0.2% (v/v) to 0.5% (v/v). Appropriate increase in acidity of methanol promoted the transfer of QACs from the sample matrix to the methanol phase because this approach not only improved methanol's polarity but also increased the concentration of hydrogen ion (H^+) which competed with the cationic compounds (i.e., QACs) in activated sorption sites of the sample matrix.[9,32] However, excessive increase in the acidity of methanol decreased recoveries of the three QACs because additional water entered the methanol solvent with the addition of hydrochloric acid, which heavily interfered with separation and extraction of the three QACs from the sample matrix.[9] So, methanol acidified with 0.1% of hydrochloric acid (v/v) was selected as the extraction solvent for subsequent experiments.

The influences of extraction times (once to three times) on recoveries of the three QACs were also investigated. As shown in Figure 3c, recoveries of all of the three QACs increased with increasing extraction times, and three times of extraction gained recoveries up to 96%−100%.

DOI: 10.1021/acs.jafc.5b01828
J. Agric. Food Chem. 2015, 63, 6689−6697

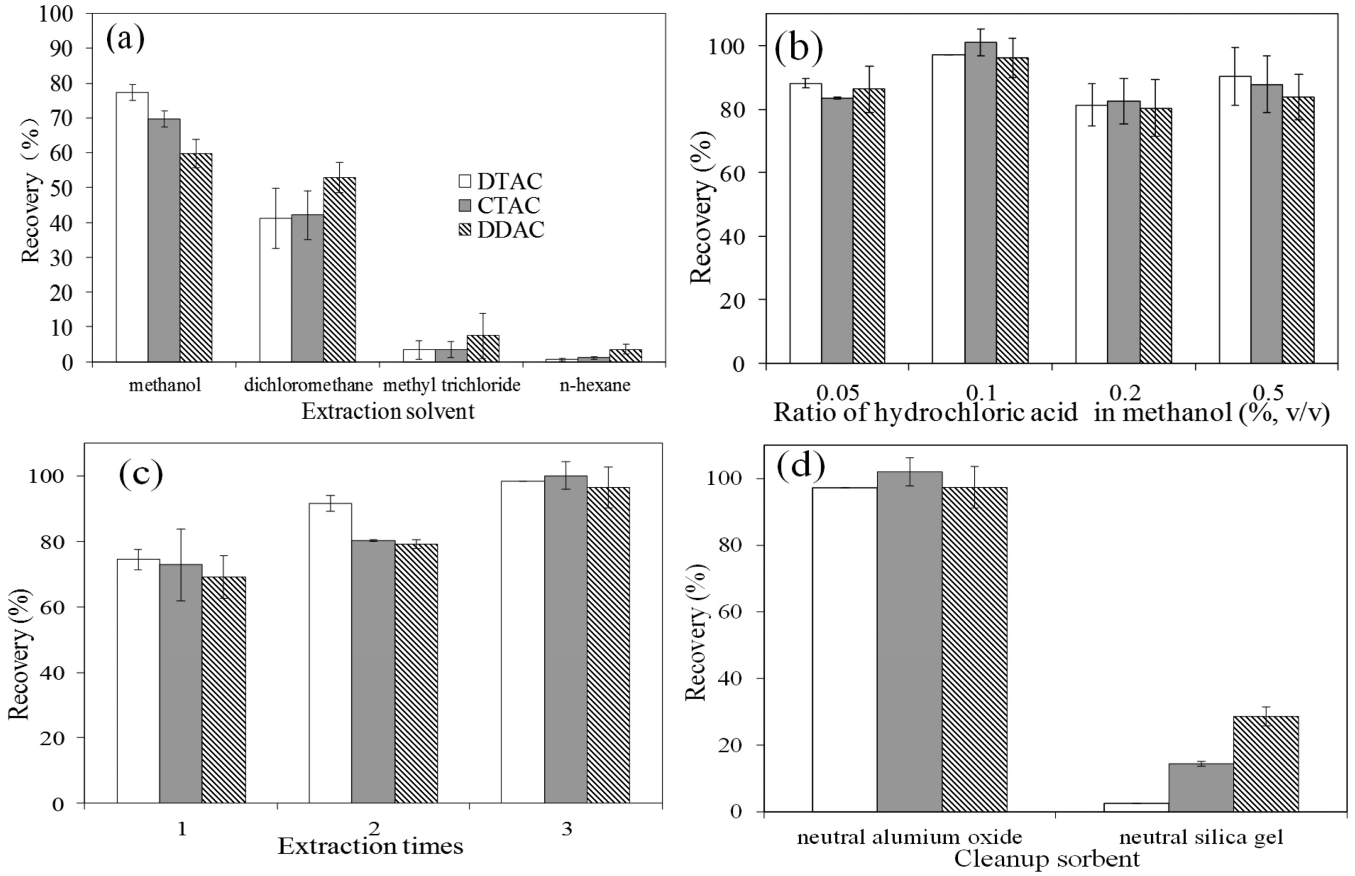

Figure 3. Effects of extractant (a), extractant acidity (b), extraction time (c), and cleanup sorbent (d) on the recovery of QACs in lettuce sample spiked with 1000 μg/kg.

In the cleanup procedure, two commonly used sorbents, neutral silica gel and neutral aluminum oxide filled to 8 cm height in a glass column (30 cm height, 1 cm diameter), were separately tested to obtain a satisfactory cleanup effect for the vegetable matrix. Unfortunately, as shown in Figure 3d, neutral silica gel used as the cleanup sorbent presented low recoveries for the three QACs (<30%). However, when neutral aluminum oxide was used, satisfactory recoveries (97%−102%) of the three QACs were observed. As we know, the negative polar silanol group (Si−OH), which is the main functional group of neutral silica gel, could be strongly bonded to positive polar chemicals especially cationic compounds.[9,35] Furthermore, the positive polarity of the three QACs decreased in the order of DTAC > CTAC > DDAC because increasing length and branch structure of the carbon chain remarkably decreases the polarity of QACs.[11] Thereby, the QACs with positive charges were more difficult to elute from neutral silica gel, and recoveries decreased with increasing polarity of the three QACs when using neutral silica gel as cleanup sorbent. Conversely, neutral aluminum oxide is suitable to separate positive polar chemicals from the sample matrix because the active-sites of aluminum oxide with the positive functional aluminum−oxygen group (Al−O) can hardly retain positive polar chemicals but can remove matrix compounds like lipid and chlorophyll via sorption.[9] Therefore, neutral aluminum oxide was selected as the cleanup sorbent in the present study.

Once the pretreatment procedure of the three QACs in lettuce had been optimized, it was used to determine the analytes in other eight vegetable samples including Chinese flowering cabbage, water spinach, pumpkin, cucumber, eggplant, potato, white radish, and carrot. Satisfactory recoveries between 72% and 108% were obtained in Chinese flowering cabbage, cucumber, potato, white radish, and carrot. However, lower recoveries (52%−70%) in water spinach, pumpkin, and eggplant were observed, but they were still acceptable as to the complex vegetable matrix.[28] Especially, when the eluant volume of the cleanup procedure was adjusted from 6 to 7 mL, satisfactory recoveries were obtained for the three QACs in all of the vegetables.

Method Validation. *Linearity, LOD, and LOQ.* The peak areas of the QACs target ions obtained from MS mode were applied to achieve the linearity, LOD, and LOQ. The linearity was estimated by preparing different calibration curves (methanol, and various vegetable matrixes including Chinese flowering cabbage, water spinach, lettuce, pumpkin, cucumber, eggplant, potato, white radish, and carrot) at seven concentrations ranging from 20 to 2000 μg/L for each compound. The regression equations and coefficients of determinations (R^2) of the standard solution curves and the nine matrix-matched curves are presented in Table 1. Good linearities (R^2 > 0.992) of the three QACs were obtained in all cases. The LODs and LOQs of the three compounds ranged from 0.7 to 6.0 μg/kg (dw) and 2.3 to 20.0 μg/kg (dw), respectively. As for specificity, no responses were observed in the procedure blank and the vegetable blank, indicating a high specificity of MS determination (Supporting Information, Figure S2).

Matrix Effect. The presence of matrix effects in the analysis of trace organic pollutants in complex samples by GC-MS is

DOI: 10.1021/acs.jafc.5b01828
J. Agric. Food Chem. 2015, 63, 6689−6697

Table 2. Recoveries (n = 15), RSD[a](%), and RSD[b](%) for the Three Tested QACs from Various Matrices at Three Spiked Levels

		DTAC			CTAC			DDAC		
matrix	spiked level (μg/kg)	recovery	RSD[a]	RSD[b]	recovery	RSD[a]	RSD[b]	recovery	RSD[a]	RSD[b]
Chinese cabbage	50	76.6	5.2	5.3	78.0	7.9	9.7	81.1	5.8	5.7
	200	73.4	4.9	4.5	85.6	8.4	12.6	91.9	5.8	6.3
	1000	102.8	7.0	7.8	93.9	9.8	9.8	94.6	7.6	6.9
water spinach	50	73.6	6.3	5.6	71.8	7.9	7.4	72.0	7.0	7.8
	200	79.4	5.8	5.3	76.9	8.4	9.5	75.1	4.8	4.3
	1000	89.8	5.5	4.7	81.1	3.4	3.9	90.6	3.6	5.1
lettuce	50	82.3	7.6	8.3	79.7	8.7	8.5	73.6	9.0	8.5
	200	75.8	3.8	4.1	82.1	2.1	2.2	73.1	2.2	2.6
	1000	97.1	6.1	6.7	101.2	9.2	8.9	96.2	9.5	8.5
pumpkin	50	88.8	9.1	8.4	82.7	9.8	9.9	84.0	10.9	10.2
	200	106.2	7.6	7.1	107.9	9.9	10.4	100.5	8.1	7.8
	1000	98.1	6.0	5.5	82.4	4.8	4.4	92.6	8.2	7.5
cucumber	50	73.3	6.0	7.7	70.5	9.0	8.6	79.7	9.2	8.4
	200	74.3	3.2	6.4	72.5	6.9	6.3	85.3	5.5	4.8
	1000	72.3	5.8	5.1	82.7	6.6	6.6	98.1	8.3	7.8
eggplant	50	73.0	5.9	6.2	72.1	6.0	6.9	71.2	5.1	5.3
	200	81.1	4.3	4.5	71.3	2.9	5.2	77.8	2.4	2.6
	1000	72.3	4.6	4.8	72.9	4.3	5.1	70.5	3.7	3.8
potato	50	86.2	7.2	6.2	73.3	5.1	4.2	75.5	8.8	9.0
	200	90.2	4.1	3.8	77.1	3.2	3.4	87.0	5.9	6.9
	1000	108.0	4.5	4.2	84.1	4.5	4.1	86.7	7.3	6.6
white radish	50	80.8	4.9	4.8	81.3	8.7	8.5	79.1	8.1	7.5
	200	91.4	5.0	4.9	90.4	7.7	7.5	85.3	5.4	5.0
	1000	94.5	2.4	2.3	96.5	5.3	5.3	96.6	6.7	6.3
carrot	50	80.8	6.2	4.5	79.2	13.0	15.3	82.2	10.4	14.1
	200	77.3	3.5	2.2	85.2	18.0	15.7	81.2	11.3	11.3
	1000	100.4	3.9	3.7	89.1	7.9	7.3	99.7	7.5	7.0

[a]Intraday precision (n = 5). [b]Interday precision (n = 15).

well-known, which may exert a strong impact on the precision and accuracy of analyte quantitation.[36,37] As referred to in previous research, the matrix effects can suppress or enhance detector response compared with the standard response in pure solvent.[36−39] Usually, the extent of matrix effect depends on the analytes, type and amount of matrix, and sample preparation procedure, etc.[40] Therefore, in the present study, various matrix effects were evaluated by calculating the slope ratio of the matrix-matched standard calibration curves to the solvent one. An accepted ranking criterion was applied according to Zhu et al., i.e., at the slope ratio value under 0.9, the matrix effect suppressed detector response; at the slope ratio value between 0.9 and 1.1, the matrix effect was negligible; at the slope ratio value above 1.1, the matrix effect enhanced the detector response.[38] Dada in Table 1 indicated that matrix effects on all of the three QACs in two matrices (Chinese flowering cabbage and water spinach) was negligible since the slope ratios of the matrix/methanol ranged from 0.96 to 1.08. However, matrix effects slightly enhanced the detector response of DTAC in lettuce, pumpkin, cucumber, and carrot but slightly suppressed that in eggplant. Matrix effects on CTAC and DDAC were negligible in five matrices (Chinese flowering cabbage, water spinach, lettuce, pumpkin, and cucumber) but enhanced the detector response to various extents in the other four matrices (cucumber, carrot, potato, and white radish). Generally, matrix effects originate from insufficient removal of the interfering constituents such as fatty acids, pigments, and phospholipids in the sample matrix.[40,41] However, the mechanism underlying these matrix-effects are still not completely understood and need to be further investigated.[38] Therefore, in order to obtain more realistic results in various samples in the present study, matrix-matched standard calibrations were applied to eliminate the matrix effects.

Precision and Accuracy. The recovery and RSD of the three QACs were measured to evaluate the precision and accuracy of the proposed method by spiking with the QACs at three different concentrations (50, 200, and 1000 μg/kg) in the blank sample and then analyzing them in quintuplicate (Table 2). The precision of the method expressed as RSD was determined by both repeatability and reproducibility experiments. The repeatability RSD[a] (intraday precision, n = 5) was obtained by calculating the standard deviation of the recoveries for the spiked samples run during the same day. The reproducibility RSD[b] (interday precision n = 5, for three distinct days) was also measured by analyzing the spiked samples. Detailed recovery, RSD[a], and RSD[b] data of the three QACs analyzed in nine vegetable samples are presented in Table 2. The recoveries in various vegetable matrices at three spiked concentrations (50, 200, and 1000 μg/kg) were between 70.5% and 108.0% (n = 15), and the RSD[a] (n = 5) and RSD[b] (n = 15) for the proposed method ranged from 2.1% to 18.0%, and 2.2% to 15.7%, respectively, which meet DG SANCO/12459/2011 guidelines (recovery at the range of 70% to 120%, and RSD lower than 20%).[37] These results indicated that the proposed method achieved satisfactory precision and accuracy.

The determination methods of QACs in food stuffs using LC-MS/MS have been recently developed.[25−27] Compared with these methods (recoveries, 72%−112%; RSDs, 1.4%−

DOI: 10.1021/acs.jafc.5b01828
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Table 3. Concentrations of the Three QAC Variants in Real Vegetables

		DTAC		CTAC		DDAC		∑QACs	
vegetable[a]	water content (%)	detection rate (%)	range (dw[b]/fw[c]) (μg/kg)	detection rate (%)	average ± SD (dw/fw, μg/kg)	detection rate (%)	average ± SD (dw/fw, μg/kg)	detection rate (%)	average ± SD (dw/fw, μg/kg)
Chinese cabbage	94.8	100	25 ± 16/ 1.3 ± 0.8	100	67 ± 27/3.5 ± 1.4	100	15 ± 11/0.8 ± 0.5	100	107 ± 36/ 5.6 ± 1.9
water spinach	91.2	0	ND[d]	100	23 ± 16/2.0 ± 1.4	0	ND	100	23 ± 16/2.0 ± 1.4
lettuce	95.0	0	ND	100	29 ± 17/1.3 ± 0.8	100	5 ± 4/0.2 ± 0.2	100	34 ± 19/1.6 ± 0.9
pumpkin	88.5	0	ND	100	25 ± 12/2.9 ± 1.4	100	9 ± 6/1.0 ± 0.7	100	34 ± 16/3.9 ± 1.8
cucumber	83.2	100	18 ± 10/ 3.0 ± 1.8	100	23 ± 11/3.9 ± 1.8	100	4 ± 3/0.7 ± 0.4	100	45 ± 21/7.6 ± 3.4
eggplant	93.2	0	ND	100	28 ± 8/1.9 ± 0.5	100	6 ± 2/0.4 ± 0.1	100	34 ± 9/2.3 ± 0.6
potato	80.1	0	ND	100	41 ± 12/8.2 ± 2.3	100	15 ± 6/3.0 ± 1.1	100	56 ± 14/ 11.1 ± 2.8
white radish	92.8	100	30 ± 13/ 2.2 ± 0.9	100	122 ± 29/ 8.8 ± 2.1	100	28 ± 15/2.0 ± 1.0	100	180 ± 41/ 13.0 ± 3.0
carrot	90.1	0	ND	0	ND	0	ND	0	ND

[a] $n = 3$ for each vegetable. [b] Dry weight. [c] Fresh weight. [d] Not detected.

17%; LODs, 0.4−6 μg/kg), the present method for determining QACs in vegetables using GC-MS provided the comparable precision and accuracy as well as the LODs (recoveries, 70.5%−108%; RSDs, 2.1%−18.0%; LODs, 0.7−6 μg/kg). Furthermore, the sample amount used (1 g) and analytical time (11.33 min) in the present method were smaller than those of previous methods (sample amount, 2−10 g; analytical time, 14−16 min). In addition, the GC-MS instrument used in the present study is available more commonly in ordinary laboratories than LC-MS. Therefore, the developed method is more cost-effective and practical for routine determination of the three QACs in vegetables.

Method Application. To estimate its practicality, the developed method was applied to measure the real vegetable samples ($n = 27$; Supporting Information, Figure S3) including Chinese flowering cabbages, water spinaches, lettuces, pumpkins, cucumbers, eggplants, potatoes, white radishes, and carrots. These samples with water contents between 80.1% (potato) and 95.4% (lettuce) were collected from several local markets in Guangzhou, southern China, a metropolitan city with high daily consumption of vegetables. Three quality controls at 50, 200, and 1000 μg/kg were performed for each sample in the analysis procedure.

As shown in Table 3, the three QAC compounds were detected in all of the tested vegetable samples (except carrot) with a concentration of 23−180 μg/kg (dry weight, dw). The detection frequency and concentration range (dw) of the three QACs were 33.3% and ND-30 μg/kg for DTAC, 88.9% and ND-122 μg/kg for CTAC, and 77.8% and ND-28 μg/kg for DDAC, respectively. CTAC was one of the dominant varieties of industrial QAC products and was frequently detected in sludge and agricultural soil,[8,9] which likely explained the highest frequency and concentration in the vegetable samples among the three tested QACs. As for the tested vegetable samples, all of the three QACs were detected in cucumber and white radish at concentrations of 4 to 122 μg/kg (dw). Both CTAC and DDAC were detected in lettuce, pumpkin, eggplant, and potato with concentrations of 5 to 41 μg/kg (dw). Only CTAC was detected in water spinach at concentrations of 23 μg/kg (dw). However, the concentration of all of the three QACs in carrot was lower than LOQs.

In conclusion, a reliable, sensitive, and cost-effective method was developed for routine determination of trace QACs (DTAC, CTAC, and DDAC) in vegetables using ultrasonic-assisted extraction and GC-MS. This method performed an accurate determination of the three QACs in a short time (11.33 min) with excellent specificity. Using a simple procedure consuming only 1 g of lyophilized sample for ultrasonic-assisted extraction, neutral aluminum oxide cleanup, and GC-MS analysis, the method can be applied to simultaneously analyze the three common QACs in various vegetable samples at μg/kg level with recoveries ranging from 70.5% to 108.0% and RSDs below 18.0%. The proposed method was used to successfully determine QACs in real vegetable samples collected from several markets in a metropolitan city of southern China. QACs were detected in almost all of the samples with a concentration of 23−180 μg/kg (dw). More studies are needed to further estimate human health risk of exposure to QACs through edible terrestrial plants, particularly vegetables, in the future.

■ ASSOCIATED CONTENT

Supporting Information

Typical GC-MS chromatograms of the three tested QACs in methanol standard solution (1000 μg/L), in blank vegetable samples and spiked vegetable samples (1000 μg/L), and in real vegetable samples. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b01828.

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Funding

This work was supported by the National Natural Science Foundation of China (41071211, 41173101, 41301337, and 41273113), the project of Guangdong Natural Science Foundation (S2011010001083 and 2011020003196), the project on the Integration of Industry, Education and Research of Guangdong Province (2013B0906001), and the High-Level Talents Program of Guangdong Higher Education Institutions granted to C.H.M.

DOI: 10.1021/acs.jafc.5b01828
J. Agric. Food Chem. 2015, 63, 6689−6697

Notes

The authors declare no competing financial interest.

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