J Korean Soc Appl Biol Chem (2014) 57(6), 783–787
DOI 10.1007/s13765-014-4209-7

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Analysis of Abamectin Residues in Green Tea Using QuEChERS Method and Liquid Chromatography-tandem Mass Spectrometry

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Received: 8 July 2014 / Accepted: 18 September 2014 / Published Online: 31 December 2014

Abstract The present study estimated the residue levels of abamectin (B_{1a}) in green tea leaves and tea infusion. Samples were hydrated with water prior to extraction by using the quick, easy, cheap, effective, rugged, safe method and was analyzed with liquid chromatography-tandem mass spectrometry in positive ion mode. The matrix-matched calibration was linear over the concentration range of 0.01–2 mg/kg with determination coefficients (R^2) >0.995. Recovery rates at two spiking levels (0.1 and 0.5 mg/kg) ranged between 80.5–99.7% with a relative standard deviation <11%. The compound was stable at 20°C for 174 days with a recovery estimate of 109.9%. Although the maximum residue limit was not established by the Ministry of Food and Drug Safety, Republic of Korea, the limit of quantitation was very low at 0.01 mg/kg. The method was successfully applied to field incurred samples and detected residue of 0.02 mg/kg in green tea samples sprayed twice (7-3 days). Abamectin was not transferred to tea infusion.

Keywords abamectin · analysis · green tea · infusion · sample preparation · tandem mass spectrometry

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Introduction

Abamectin (ABM), a mixture of both avermectin B_{1a} (80%) and B_{1b} (20%), has been isolated following fermentation of a naturally occurring soil actinomycete *Streptomyces avermitilis* (MacBean, 2012). As an acaricide and insecticide, it is used to control the motile stages of mites, leaf miners, suckers, and Colorado beetles on ornamentals, citrus, pome fruits, nut crops, vegetables, and potatoes (MacBean, 2012). ABM stimulates gamma-aminobutyric acid, an inhibitory neurotransmitter, leading to paralysis, anorexia, and death of affected insects within a few days (MacBean, 2012). When exposed to light in water or as a thin film on biological surfaces such as leaves, it is degraded quickly by oxidative and photo-oxidative mechanisms (MacBean, 2012) with half-lives ranged of 4–21 h (Wislocki et al., 1989).

Tea represents a significant potential source of human exposure to chemical residues by virtue of the high application of pesticides to tea coupled with the average intake of 6 g of dried (made) tea per day per individual (Chen, 1985). One of the major disadvantages of pesticide use is that residues may remain on tea and may be transferred to the infusion/brew (Abd El-Aty et al., 2014; Cho et al., 2014) at concentrations higher than the maximum residue limits that might pose health hazards (Kumar et al., 2006). These effects may not be serious in adults, but could be aggravated in children, because they are likely a susceptible population to dietary pesticide exposure. Therefore, a simple, sensitive, and applicable approach for the detecting ABM in green tea and infusion is needed for a safety evaluation.

Detection of ABM either in a single or multiple residue levels in green tea is very limited. Yang et al. (2009) detected the residue of avermectins, including abamectin, emamectin, eprinomectin, ivermectin, doramectin, and moxidectin in tea using ultra-performance liquid chromatography-electrospray tandem mass spectrometry; however, no detailed information was available, because the full article was published in Chinese. Moreover, they

detected avermectins using liquid-liquid and solid-phase extraction, which are not attractive for an efficient routine analysis. Another study by Kottiappan et al. (2013) estimated the levels of ABM in black and dried green tea using high performance liquid chromatography (HPLC)-fluorescence detector. However, the method was tedious and time consuming due to derivatization and lack of method application.

Herein, we used QuEChERS as a quick, easy, cheap, effective, rugged, safe method for extracting ABM from green tea leaves followed by analysis using liquid chromatography-tandem mass spectrometry (LC/MS/MS). Transfer of the residue to a tea infusion was also evaluated.

Materials and Methods

Chemicals and reagents. Abamectin standard (purity: 97.6%) was supplied by Sigma-Aldrich (USA). HPLC-grade acetonitrile (MeCN) and dichloromethane were purchased from Burdick and Jackson (Korea). Ammonium acetate (purity: 99.9%) was obtained from Sigma-Aldrich. Sodium chloride (NaCl, purity: 99.5%), sodium sulfate (Na_2SO_4, purity: 99.0%), and anhydrous magnesium sulfate ($MgSO_4$, purity: 99.5%) were supplied by Junsei Chemical Co. Ltd. (Japan). Primary secondary amine (PSA), and C_{18} were obtained from Agilent Technologies (USA). All other chemicals and reagents were of analytical or HPLC grade.

Field trial. To conduct the field trial, five experimental plots in an orchard field located in Bosung, Korea were used. Control samples were collected before pesticide application, which was carried out using a backpack motorized sprayer. An emulsion concentrate (AllStar®, 1.8% a.i., Kyung Nong, Seoul, Korea) was sprayed either once or twice to the green tea. Approximately 2 kg of green tea leaves were randomly collected from each plot in polyethylene bags, kept in ice, and then dried. The dried green tea leaves were blended, ground, and frozen at –21°C pending analysis.

Sample extraction of Dried green tea leaves. The original QuEChERS method (Anastassiades et al., 2003) was used with slight modifications to extract ABM from green tea leaves. Approximately 5 g green tea samples were weighed into a 50-mL Teflon centrifuge tube to which 20 mL MeCN and 10 mL water were added and vortexed for 5 min. Three grams of anhydrous $MgSO_4$ and 1 g of NaCl were added, vortexed again for 1 min, and centrifuged at 4,500 rpm for 5 min at 5°C. Eight milliliters of the supernatant was transferred to a 15-mL Teflon centrifuge tube containing C_{18} (400 mg) and PSA (400 mg). The tubes were vortexed for 0.5 min and centrifuged at 4,500 rpm for 5 min. Approximately 4 mL of the upper layer was transferred to a 20-mL vial and evaporated under nitrogen gas. The dried extract was redissolved in 1 mL of MeCN for LC/MS/MS analysis.

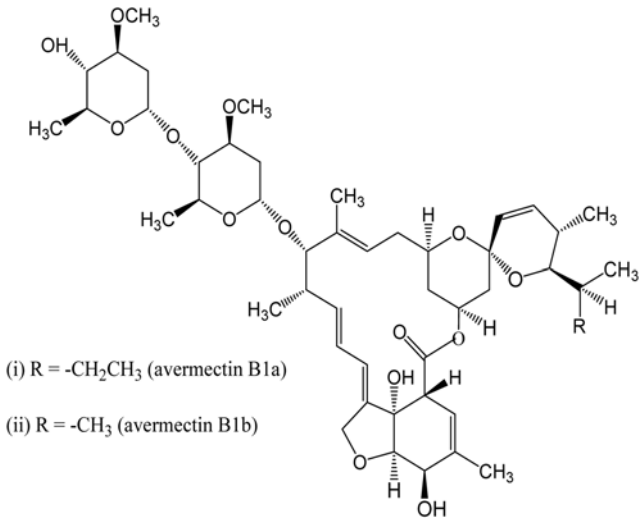
Green tea infusion. Five grams of dried green tea leaves were infused in 150 mL of boiling water (85±5°C) for 30 min. The infused samples were filtered via filter paper (Whatman, 110 mm diameter, no. 6) in a Buchner funnel. The filtrate was transferred to a 1000-mL separatory funnel. in to which a saturated sodium chloride solution (50 mL), water (100 mL), and dichloromethane (100 and 50 mL,) were added twice. The separatory funnel was vigorously shaken for 5 min and then left undisturbed until the organic layer separated from the aqueous layer. The organic layer was filtered with anhydrous sodium sulfate and then evaporated on a rotary vacuum evaporator (Buchi Rotavapor R-114) in a water bath (<40°C). The concentrated extract was dissolved in MeCN (5 mL) and then transferred to a 15-mL Teflon centrifuge tube containing C_{18} (100 mg) and PSA (200 mg). The tubes were vortexed for 0.5 min and centrifuged at 4,500 rpm for 5 min. The upper layer (1 mL) was aspirated for LC/MS/MS analysis. This methodology was developed in our laboratory.

LC/MS/MS analysis. The residual analysis of ABM in green tea and a tea infusion was conducted with an LC/MS/MS system, consisting of a Waters Alliance 2695 Separation Module and Waters TQ detector API tandem quadrupole mass spectrometer (USA). A Phenomenex-Gemini 3 μm C_{18} 100 Å column (50×2.0 mm; Torrance, USA) was used to separate the analyte from the samples. A binary solvent system, consisting of 0.1% ammonium acetate in water (A) and MeCN (B), was run in gradient mode. The mobile phase gradients (A and B) started at 95:5 for 0–1 min, ramped to 5:95 for 6 min, held at 5:95 for 6–9 min, ramped to 95:5 for 10 min, and maintained at 95:5 for 10–15 min. The flow rate was 0.25 mL/min, and column temperature was maintained at 35°C. An aliquot (5 μL) of the extracted sample solution was injected.

The MS/MS system was run with positive electrospray ionization in multiple-reaction monitoring mode. Two MS/MS transitions were used to quantify and confirm the analyte. One transition with a higher intensity was selected for quantitation, and the other transition with a lower intensity was chosen for confirmation. The MS source conditions were as follows: capillary voltage, 4.0 kV; source temperature, 150°C; desolvation temperature, 350°C; desolvation gas (N_2) flow, 600 L/h; and cone gas (N_2) flow, 50 L/h. The optimized mass parameters for ABM are shown in Fig. 1. The Masslynx software ver. 4.1 program was operated for data analysis.

Method validation. A standard stock solution of ABM was prepared with MeCN at 100 mg/L. The working solutions for calibration curves and recovery tests were serially diluted with MeCN and stored at –40°C. To minimize the matrix effect (ME), matrix-matched standard solutions were used for analysis of ABM in green tea and the tea infusion. Linearity was estimated through at six points ranging from 0.01–2.0 mg/kg. Two different levels of ABM; 0.1 and 0.5 mg/kg; were fortified in uncontaminated green tea samples for recovery tests conducted in triplicate. Recovery rate was calculated by comparing the true value with the experimental value derived from matrix-matched calibration. To estimate the precision, relative standard deviation (RSD) (= standard deviation/mean × 100%) was calculated.

The slope of the matrix-matched calibration curve was compared



M.W.	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone (V)	CE (V)	Rt (min)
873	891	305 145	40	44 38	10.1

M.W., molecular weight.
CE, collision energy.
Rt, retention time.

Fig. 1 Chemical structure and optimized mass parameters for abamectin.

with that of the calibration curve created in pure solvent to estimate the ME in green tea and tea infusion. The ME was calculated using the following equation:

$$ME\% = \frac{\text{Slope of matrix-matched calibration} - \text{Slope of solvent calibration}}{\text{Slope of solvent calibration}} \times 100$$

Storage stability. To demonstrate the effects of storage on analyte stability, 0.5 mg/kg ABM concentration was spiked into blank samples and stored at 20°C for 174 days (n =3). The samples were extracted and analyzed as mentioned above.

Results and Discussion

Pesticide residue levels in green tea are generally higher than those in other commodities, because leaves have a wide surface area per mass unit. During tea manufacturing (drying process) leaves are condensed, resulting in increased pesticide concentration (Rajski et al., 2013). To achieve maximum extraction yield and satisfactory results, the green tea samples were hydrated with water (Cho et al., 2014). Rajski et al. (2013) reported that acetonitrile could extract relatively small amounts of non-polar substances, including fatty acids and waxes. The combined effect of both C_{18} and PSA sufficiently removed polar and moderately polar co-extractives.

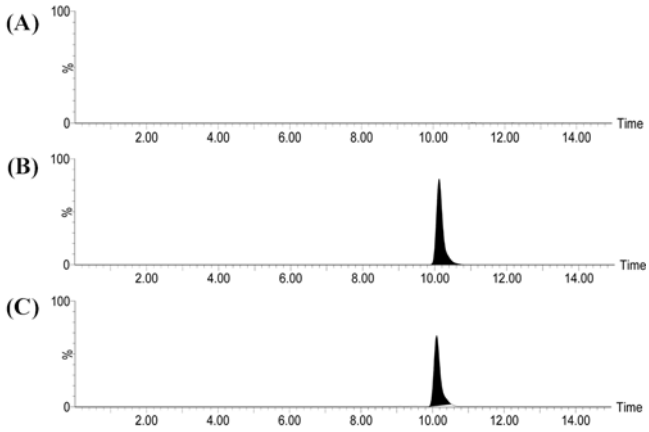


Fig. 2 Extracted ion chromatogram of (A) blank green tea; (B) matrix-matched standard of 1.0 mg/kg in a green tea extract, and (C) standard in solvent at 0.5 mg/kg.

MS/MS is generally more sensitive than MS alone for estimating the levels of an analyte in complicated matrices, because it is associated with higher and more stable signal-to-noise ratios as a result of specific fragmentations of isolated precursor ions and elimination of background noise (Yang et al., 2014). The first key point for detecting any analyte in MS is ionization. The physico-chemical properties of the analyte, adequate ionization source parameters, and mobile phase composition influence ionization performance during development of the LC/MS/MS method. In the present study, the use of ACN provided better chromatographic separation of ABM (Yang et al., 2014). Because ABM is sensitive to acid and base and was detected in an ammonium adduct form, an ammonium acetate buffer salt was added to the mobile phase. A gradient elution program was selected, because it provides baseline resolution of the analyte with a high detection signal and improved peak shape (Park et al., 2013). The selected program facilitated efficient separation of the analyte from matrix co-extractants, reduced the noise level and the risk for carry-over effects as well as column deterioration (Park et al., 2013).

Selectivity. The presence of potential interference in the LC/MS/MS chromatograms was monitored by running a control blank sample of green tea extract. The absence of chromatographic components at the same retention time of the analyte suggested no intrinsic interference (Fig. 2).

Linearity and ME. Because significant suppression effects (green tea, 82.13%; tea infusion, 63.2%) were detected for ABM in green tea and tea infusion; matrix-matched calibrations were used for quantitation. Curves over the concentration range of 0.01–2 mg/kg were linear with R^2 >0.995 (Table 1).

Recovery and storage stability. Recovery rates at two spiking levels (0.1 and 0.5 mg/kg) were comparatively low in tea infusion (80.5–96.3%) compared to that from the green tea extract (96.4–99.7%); however, both fell within the acceptable range (70–120%) specified by SANCO, 2009. Repeatability expressed as RSD was <11%. ABM was stable under storage at –20°C for 174

Table 1 Calibration curve, determination coefficient (R^2), recovery, limit of detection (LOD), limit of quantitation (LOQ), storage stability, and matrix effect of abamectin in green tea extract and a tea infusion (n =3)

Sample	Calibration curve	R^2	Recovery % (mean ± RSD)		LOD (mg/kg)	LOQ (mg/kg)	Storage stability % (mean ± RSD)	Matrix effect (%)
			0.1 (mg/kg)	0.5 (mg/kg)				
Green tea	Y=2,278.352−57.147	1.0	96.4±10.0	99.7±9.2	0.003	0.01	109.9±0.8	-82.13
Infusion	Y=4,688.264+821.156	0.995	80.5±10.3	96.3±7.0			NT	-63.22

NT: not tested.

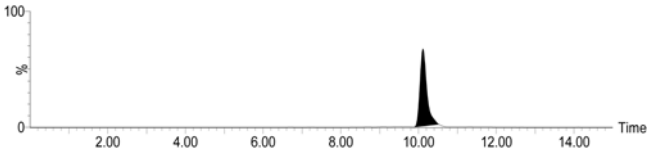


Fig. 3 Extracted ion chromatogram for abamectin under storage conditions.

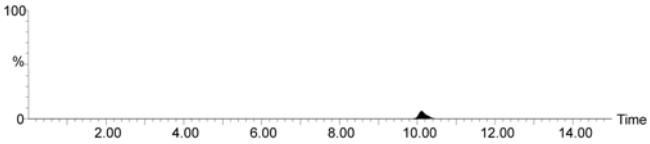


Fig. 4 Extracted ion chromatogram of field incurred sample.

days, as demonstrated by a satisfactory recovery (109.9%) (Fig. 3) **Limit of detection and limit of quantitation (LOQ).** The method was sensitive with low limits of detection (0.003 mg/kg) and quantitation (0.01 mg/kg) (Table 1). Although, the maximum residue limit (MRL) has not yet been established by the Ministry of Food and Drug Safety, Republic of Korea, we believe that this level is sufficient for estimation of a residue with an expected MRL between 0.02–0.05 mg/kg.

Method application. The method developed was applied to field incurred green tea samples; only samples treated twice (7-3) contained residues higher than the LOQ (Fig. 4). None of the infused tea contained residues higher than the LOQ (Table 2). This was expected as ABM was detected in very small amount or even nil. Additionally, ABM has slightly lower water solubility and moderate K_{ow} (4.4) (MacBean, 2012) that could interfere with the movement of the residue during brewing. The extraction rate of pesticide residues is dependent on their water solubility (Nagayama, 1996; Sharma et al., 2008; Gupta and Shanker, 2009), and the leaching ratio varies with the type of pesticide but not with the residue concentration (Nagayama, 1996). In line with our findings; Cho et al. (2014) found that cyhalothrin, flufenoxuron, and bifenthrin are not transferred to the infusion (almost nil).

In conclusion, the method developed using QuEChERS and LC/MS/MS had satisfactory performance criteria and was appropriate for analysis of ABM residues in green tea and its infusion. The mere presence of pesticide residues in tea does not necessarily mean that the tea has become toxic and would pose a health hazard. Monitoring tea brew residue is needed to set realistic MRL for public health safety and trade (Abd El-Aty et al., 2014).

Table 2 Residue levels of abamectin in incurred-green tea and a tea infusion

Spray time	Days after treatment	Residue level (Mean ± SD, mg/kg)	
		Green tea	Tea infusion
1	7	<0.01	<0.01
1	3	<0.01	<0.01
2	14-7	<0.01	<0.01
2	7-3	0.02±0.01	<0.01

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