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Comparison of ASE with in-cell cleanup and the QuEChERS sample preparation methods for the analysis of pesticide residues in tea

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Abstract

The aim of the present work was to compare and choose the best method to extract incurred pesticide residues from green tea. Accelerated solvent extraction (ASE) with in-cell cleanup and the quick, easy, cheap, effective rugged and safe (QuEChERS) methods were tested on green tea samples with incurred beta-endosulfan pesticide. The extracts were analyzed by GC-MS/MS and the recovery and the precision of both methods were compared. The average recovery using ASE with the in-cell cleanup method was in the range of 89 to 92% which is better than that obtained using a QuEChERS method. Both the ASE with in-cell cleanup and the QuEChERS methods provided good precision with RSDs in the range of 12 to 15% and 17 to 18%, respectively. This finding indicates that the ASE method with the in-cell cleanup is more suitable for the accurate determination of pesticides incurred in tea.

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Introduction

The application of pesticides during tea planting is a common practice for pest and plant disease control. Under certain circumstances, however, residues of active ingredients may occur in the final product (processed tea), tea infusion and spent leaves (Kanrar *et al*., 2010; Pakade *et al*., 2013). In recent years, there have been several alerts concerning the presence of pesticide residues in tea exceeding the maximum residual limit (Cajka *et al*., 2012). Therefore, rapid and cost-effective methods for the determination of pesticide residues are required to protect consumers with regard to food safety. The key to a successful method of analysis for pesticides in tea is a technique that will thoroughly extract the pesticide residues from the complex matrices and determine how the interfering substances that co-extracted with the pesticides can be cleaned up (Kolberg *et al*., 2011). A high degree of precision and accuracy of the pesticide residue analysis was correlated with the sufficient removal of co-extractives that will cause matrix effects (Kruve *et al.*, 2008).

The QuEChERS (quick, easy, cheap, effective, rugged and safe) and ASE (accelerated solvent extractor) with in-cell cleanup methods have been introduced to reduce extraction time and solvent use substantially in pesticide analysis (Rajski *et al*., 2012; Feng *et al*., 2013). Apart from the method performance, both methods have resulted in new possibilities for sample treatment and such advantages as a reduction of the extraction time, minimal solvent usage and waste, ease of performance without requiring much training or technical skill and a lower usage of labware (Lambropoulou *et al.*, 2007). The ASE method with in-cell cleanup integrates the extraction and cleanup processes in a single step by adding cleanup sorbents along with the sample to the extraction cell. The addition of the cleanup sorbent, to the extraction cell together with the sample (dispersive solid phase extraction (d-SPE)) provides simultaneous extraction and cleanup (Labarta *et al*., 2012). In this approach, analytes are efficiently extracted from the sample at high pressure and temperature simultaneously and most of the coextracted compounds are retained in the sorbent. This method has demonstrated excellent performance for the extraction of multi-residue pesticides in tea with good recovery and precision and a low detection limit using a combination of the cleanup sorbents PSA and C_{18} (Haslina *et al*., 2015). The ASE with in-cell cleanup presented the advantages of higher extraction efficiency, a low cost of the extraction solvent and short analysis time. It has been used for the extraction of pesticide residues from complex sample matrices, such as fish and fish

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oil (Haglund *et al*., 2007), soil (Jia and Deng, 2008), pesticides in seaweeds (Rodríguez *et al.*, 2010), and mushroom compost (Labarta *et al.*, 2012).

The QuEChERS method was originally developed for extracting a wide range of pesticides from fruits and vegetables and also has been widely used for the analysis of pesticide in tea. The original method involves an initial extraction with acetonitrile followed by liquid-liquid partitioning with the addition of a mixture of anhydrous $MgSO_4$ and NaCl. Removal of residual water and polar residues is performed simultaneously using a dispersive solid phase extraction (d-SPE) cleanup in which the extract is mixed with primary secondary amines (PSA), C_{18} or other sorbents (Anastassiades *et al*., 2003). Rapid shaking is followed by solvent exchange, after which a second d-SPE step (with the same sorbents) is performed to reduce matrix interference during gas chromatography-mass spectrometry (GC-MS/MS) analysis (Steiniger *et al*., 2010).

It was found that the claims of high recovery and reasonably good precision for most of the methods used to analyze pesticide residues in tea are usually based on the result of spiked or fortified samples (Feng *et al.*, 2013). Although this method was accepted by IUPAC for the recovery study, the actual recovered value of the analyte might be underestimated or overestimated when the recovery study was performed using the spiked or fortified samples (Wong *et al.*, 2008). This inaccuracy is observed because unlike the incurred sample, the pesticide in spiked samples only coats the surface of the tea and is not incorporated into the tea's structure (Betterncourt *et al*., 2003). The analyte in the spiked samples interacts differently from the one in incurred samples (Wong *et al*., 2008; Rajski *et al*., 2012). Studies on the analysis of pesticide residues in incurred tea samples are seldom reported. Therefore, the objective of this study is to compare the performance of both the QuEChERS and ASE methods with in-cell cleanup using incurred tea samples and to identify the most valuable method that provides the highest recoveries and good precision.

Materials and Methods

Chemicals and materials

HPLC-grade acetonitrile, acetone and hexane, glacial acetic acid (HOAc), anhydrous magnesium sulfate (MgSO4), and sodium acetate (NaAc) were obtained from MERCK (Darmstadt, Germany). The pure pesticide standard of beta-endosulfan was obtained from Sigma Aldrich (Steinheim, Germany). Triphenylphosphate (TPP) was obtained from Dr. Ehrenstorfer (Augsburg, Germany) and was used as an internal standard. The anhydrous $MgSO_4$ was heated in a muffle furnace for at least 5 h at 500ºC before use to remove phthalates and residual water. The primary secondary amine (PSA) and octadecyl (C_{18}) was obtained from Varian (Harbor City, USA). Cellulose filters (20 mm diameter) were purchased from Restek (Bellefonte, PA, USA) and Hydromatrix was obtained from Agilent Technologies (Santa Clara, CA, USA). The incurred tea sample (CCQM-K95) was obtained from the Hong Kong Government Laboratory (HKGL) which contained incurred beta-endosulfan was used as a quality control sample. Approximately 100 g of Sabah tea (organic tea) purchased from the market was used as a blank sample for preparation of matrix matched calibration standards.

Preparation of the standard solutions

The preparation of all standard solution was performed gravimetrically, whereby the determination of weights is used as a means of quantifying an analyte concentration in a mass/mass ratio. The weighing was made using a four decimal analytical balance. The individual pesticide stock standard solutions were prepared in acetonitrile by dissolving approximately 10 mg of the pure reference material of beta-endosulfan and triphenyl phosphate (TPP) into a pre-weighed 22 mL glass vial and dissolved in an appropriate mass of acetonitrile to give final mass fractions of 1000 and 130 µg g^{-1}, respectively. The solutions were stored in a refrigerator at a temperature of 4°C. The intermediate standard solutions were prepared by diluting the individual stock standard solution with acetonitrile to give a final concentration of 50 and 10 µg g^{-1}, respectively.

Matrix-matched calibration standard

For the calibration of the gas chromatography mass spectrometry (GC-MS/MS), matrix matched calibrations were employed to compensate for the matrix effects (Paya *et al*., 2007). Matrix-matched calibration standards were prepared fresh by fortifying the blank extract with the desired amount of the intermediate standard solution of pesticide, along with the addition of an internal standard (ISTD) to produce a final concentration of 0.2, 0.4, 0.7, 0.9 and 1.2 µg g^{-1} of beta-endosulfan and 1.5 µg g^{-1} for the ISTD.

Extraction by QuEChERS method

The QuEChERS method was designed for wet samples (more than 75% water). For dry products such as dry tea, water has to be added and given

sufficient time (more than one hour) to swell the matrix such that the sample pores are more accessible to the extraction solvent and therefore can permit the partitioning process between the aqueous and organic phases when the salts are added (Lehotay *et al*., 2011). Approximately 100 g blank tea sample and 3 g of CCQM-K95 sample were mixed with 600 mL and 18 mL of deionized water, respectively. Both samples were hand shaken and left for at least one hour at ambient temperature to give homogeneous slurry (paste). A sample of 15 g of each previously homogenized slurry was weighed in a pre-weighed 50 mL centrifuge tube. Fifteen milliliters of acetonitrile, containing 1% (v/v) of acetic acid, were then added to the sample, and the mixture was hand shaken for 1 minute. One milliliter of acidified acetonitrile is required for the extraction of 1 g of sample (Anastassiades *et al*., 2003). The acidified acetonitrile was prepared on a volume basis by adding 1 mL of glacial acetic acid and 99 mL of acetonitrile. Afterward, 50 µL of the 10 µg g^{-1} internal standard (ISTD) was added to the CCQM-K95 sample. The centrifuge tube was then weighed again to obtain the actual weight of the ISTD added. The cap was removed and a pre-weighed sample of 6 g powdered $MgSO_4$ and 1.5 g NaAc were poured slowly into the centrifuge tube. The tube was tightened securely and immediately hand shaken for 5 min until all of the powder was mixed with the liquid and the agglomerates were sufficiently broken up. It is important to tighten the cap well to avoid leakage. The addition of salt and $MgSO_4$ allowed a liquid-liquid partitioning process to occur between the aqueous phase and sample. The amount of $MgSO_4$ and salt required to be added was 0.4 g $MgSO_4$ and 0.1 g NaAc for each 1 g of sample (Anastassiades *et al*., 2003). The tube was then centrifuged for 5 min at 3000 rpm. A 10 mL aliquot of the extract (upper layer), was transferred into an empty centrifuge tube for the cleanup process.

The removal of residual water and cleanup were performed simultaneously by using dispersive solid-phase extraction (d-SPE) in which $MgSO_4$, primary secondary amines (PSA) and C_{18} sorbents were mixed with the extract (Lehotay *et al*., 2011). The PSA was employed to remove polar matrix components such as organic acids, certain polar pigments, fatty acids and sugar from the sample whereas the C_{18} helped to remove the pigment content in the matrix (Anastassiades *et al*., 2003). A pre-weighed mixture of 900 mg $MgSO_4$, 300 mg PSA and 150 mg C_{18} were added to the centrifuge tube containing a 10 mL aliquot of the extract. The tube was capped and hand shaken vigorously for 1 minute and subsequently centrifuged at 3000 rpm for 5 min. Next, 5 mL of the aliquot was transferred into an empty centrifuge for the final cleanup where a pre-weighed mixture of 150 mg $MgSO_4$, 50 mg PSA and 50 mg C_{18} was added. The tube was capped, hand shaken vigorously for 1 min and centrifuged at 3000 rpm for 5 min. An aliquot of the final extract was transferred into a clean vial for solvent exchange (Steiniger *et al*., 2010).

The larger evaporation expansion volume and low volatility of acetonitrile in the solvent for the final extract is not ideal for splitless injection in gas chromatography (Lehotay *et al*., 2005). Therefore, solvent exchange and concentration of the extract into a mixture of hexane and acetone (9:1) are employed. The aliquot of the extract was evaporated to dryness under a gentle stream of nitrogen at 40°C. A 0.9 mL mixture of hexane/acetone (9:1) was added and then 1 mL of the extract was transferred into a gas chromatography vial. The pesticide intermediate standard solution was added to the extract at this point for the preparation of matrix-matched calibration standard before analysis with GC-MS/MS (Steiniger *et al*., 2010).

Extraction by accelerated solvent extraction (ASE) with in-cell cleanup method

Extractions were performed with a Dionex ASE 300 (Dionex, Sunnyvale, CA, USA) equipped with a 13 mL cell. The cell loading was performed in the following sequence. First, the cellulose filter was placed at the bottom of the cell. Next, the pre-weighed adsorbents (0.3 g of PSA and 0.15 g of C_{18}) were added and topped by the cellulose filter. The sample was spiked with 50 µL of TPP at a concentration of 130 µg g^{-1}, placed in the cell and then topped with a cellulose filter. Finally, the cell was filled to the top with Hydromatrix to fill the vacant volume and another cellulose filter was placed on the top. The cell was tightly closed and inserted into the cell tray for the extraction (Haslina *et al*., 2015).

The extraction was performed using the following ASE parameters as described previously. The extraction temperature, 120°C; extraction pressure, 1500 psi; heating time, 5 minute; static time, 10 min; purge time, 60 s; extraction solvent, acetone-hexane (2:1, v/v); flush volume, 60% and static cycles, 2. The extracts were collected in the collection vessel, concentrated to 1 mL with a gentle stream of nitrogen at 40°C, and transferred into a vial for the GC-MS/MS analysis (Feng *et al*., 2013).

GC-MS/MS analysis

The GC-MS/MS system consisted of a ThermoFinnigan Gas Chromatography, an AS

Table. 1. The measured value of beta-endosulfan in CCQM-K95 sample using QuEChERS and ASE with in-cell cleanup method

Precision (Days)	Intra-day		Inter-day	
Extraction method	QuEChERS*	ASE with in cell cleanup*	QuEChERS*	ASE with in cell cleanup*
Concentration ($\mu g\ g^{-1}$)	465	687	382	754
	528	735	454	504
	492	652	396	499
	574	629	603	477
	397	748	454	680
	420	632	365	747
	574	721	588	669
	392	478	471	747
	473	728	390	730
	397	701	529	694
Mean ± SD	471±71	671±80	463±85	650±112
Recovery, %	65	92	63	89
RSD %	15	12	18	17
P- value (precision)	0.449			
P- value (method)	0.001		0.001	

*Shows significant difference among both methods

200 autosampler and a Polaris Q ion trap mass spectrometer (San Jose, CA). The data acquisition and processing were performed using X-calibur software. The pesticides were separated on a DB-5MS (30 m x 0.25 mm i.d., 0.25 µm film) capillary column from Agilent. The splitless mode was used for the injection. The oven temperature was held at 80°C for 2 min, and then heated to 280°C at a heating rate of 15°C/min and maintained at that temperature for 8 min. Helium was used as the carrier gas with a constant flow rate of 1.5 mL/min. The injection port temperature and transfer line temperature were maintained at 260 and 280°C, respectively. The ion source temperature was set at 250°C and the injection volume was 1 µL. The mass spectrometer was operated using electron impact (EI) ionization with 70 eV electron impacts (Steiniger *et al*., 2010). The MS/MS detection method was first performed by the injection of beta-endosulfan and TPP in full scan mode at a concentration of 1.2 $\mu g\ g^{-1}$ to obtain their retention times and select their parent ion for quantification.

Results and Discussion

The assigned value of incurred sample CCQM-K95 as reported by the Government Laboratory Hong Kong is 730 ± 12 $\mu g\ g^{-1}$. This value was determined by three Metrology Institute in the APMP.QM-P15 inter-laboratory comparison under Asia Pacific Metrology Programme (APMP). The recovery and precision of the method in this study were measured based on the average recovery of triplicate analysis of the CCQM-K95 sample measured on the same day (intra-day) and for a period of four days (inter-day). The average recovery and the precision achieved for both methods for intra-day and inter-day analysis are listed in Table 1.

One-way ANOVA were used to analyze for a significant difference between measured values of CCQM-K95 obtained from both method and between inter-day and intra-day, while two-way ANOVA was used to evaluate any significant effect on methods and times. A probability value of 0.05 (95% level of confidence) or 0.01 (99% level of confidence) were considered to denote a statistically significant difference while the greater F-value indicates the greater significant effect of a respective factor. The results shown in Table 1 indicated that there was a significant difference ($P < 0.05$) observed for the accuracy between both methods for both intra-day and inter-day.

Considering the beta-endosulfan pesticide that was recovered from the CCQM-K95 sample, the lowest recoveries were obtained with the QuEChERS method with an average recovery of 64% (the average of recovery from intra-day and inter-day). On the contrary, the highest recoveries were obtained with the ASE method with in-cell cleanup with an average recovery of 91% (the average of recovery from intra-day and inter-day analysis). It was determined that the ASE method with in-cell cleanup yield the highest recovery compared to QuEChERS method with a 28% difference. This finding confirms the ability of the ASE method with in-cell cleanup to extract out most of the beta-endosulfan pesticide residues in tea. The low recovery obtained from the QuEChERS method could possibly be due to analyte loss during the extraction process, which was contributed by the loss of approximately 50% of the extract volume to the sorbent in the centrifuge tube and during the process of transferring the aliquot in the initial and final extraction step (Lehotay *et al*., 2011). This QuEChERS method was not fit for extraction

of an incurred sample and requires modification. Therefore, many modifications were made to the original QuEChERS method such as applying the SPE cartridge for further cleanup (Chen *et al.*, 2011), replacing $MgSO_4$ with calcium chloride in the cleanup step (Rajski *et al*., 2012), and an addition of new types of adsorbents (Chen *et al*., 2014). The method has been developed to further improve results for the determination of pesticides in tea.

The recovery obtained from the QuEChERS method in this study was found to be lower than that of the previous study. It was reported that the recovery achieved using this method ranged between 82 to 130% (Steiniger *et al*., 2010). However, this result is not comparable because the recovery study of the previous method relied upon the results of spiked samples. The pesticides in spiked samples coated the surface of tea and possibly were not incorporated into the tea structure therefore possibly explaining the high recovery results obtained (Steiniger *et al.*, 2010). It is difficult to determine the best extraction conditions only using spiked samples because in the real sample, the pesticides levels are increased in tea by spraying the tea plants while they are growing. Thus, the pesticide was incorporated and tightly bound into the tea cell structure (Guan *et al.*, 2013).

In the accelerated solvent extractor (ASE) system, the samples are extracted under static conditions, where the extraction solvent is held in the cell for controlled time periods to allow sufficient contact between the solvent and tea sample for efficient extraction. The combination of elevated temperatures and pressure enables the solvent to penetrate the pores of the matrix, reducing the effect of the matrix interaction and allowing the analyte to be removed from the matrix (Richter *et al*., 1996). Therefore, the combination of elevated temperatures and pressures in accelerated solvent extraction (ASE) system allows extraction to occur rapidly and completely thus giving a high recovery value (Feng *et al*., 2013). A study has been reported on the extraction of incurred pesticides of alpha-endosulfan, beta-endosulfan and bifenthrin in a tea sample using the ASE system followed by the gel permeation chromatography and solid-phase extraction for the cleanup method. In the study, complete extraction was achieved under the optimized ASE conditions with recovery values of 96 to 101% (Hu *et al*., 2008; Feng *et al*., 2013).

The precision of the QuEChERS and the ASE method with in-cell cleanup was assessed by the repeatability (intra-day analysis) and intermediate precision (inter-day analysis). The repeatability was assessed by the analysis of duplicate CCQM-K95 samples on the same day. The relative standard

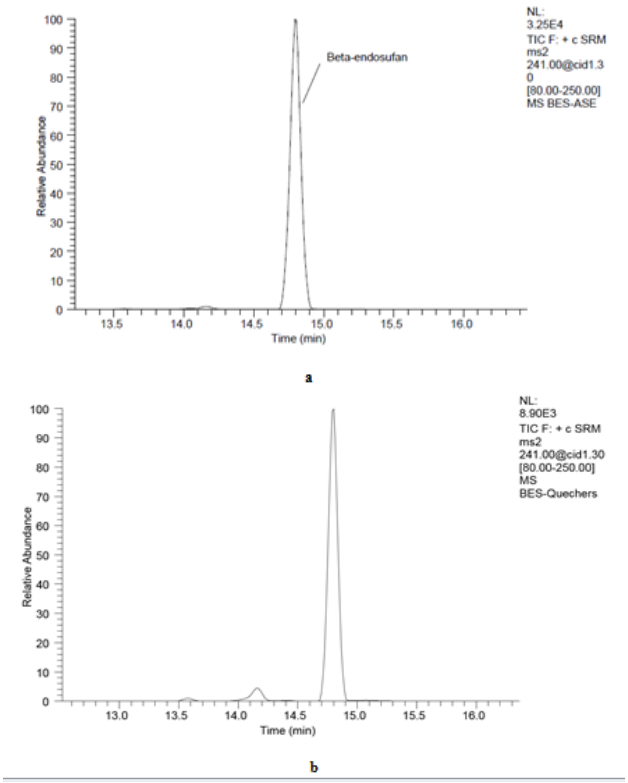

Figure 1. (a) Total ion chromatogram (TIC) for beta-endosulfan of CCQM-K95 sample obtained from ASE with in-cell cleanup method (b) Total ion chromatogram (TIC) for beta-endosulfan of CCQM-K95 sample obtained from QuEChERS cleanup method

deviation (RSD) values of 15 and 12% were achieved for QuEChERS and ASE with in-cell cleanup, respectively. The intermediate precision shows the variations from day-to-day analysis. The intermediate precision in this study was based on the mean repeatability values of duplicate CCQM-K95 samples for a period of four days. The RSD values of 18 and 17% were achieved for QuEChERS and ASE with in-cell cleanup, respectively. The precision of both methods was satisfactory (referring to relative standard deviation values (RSD) below 20%) and was also considered satisfactory according to the method validation guidelines (SANCO, 2006). This confirms the ability of gas chromatography mass spectrometry (GC/MS/MS) to provide good precision in this measurement for both methods. The repeatability and intermediate precision obtained from both methods was shown in the Table 1.

Analysis of variance (ANOVA) was used for both methods to determine if there was any difference in the precision of the method. The measured values of the CCQM-K95 sample obtained from both methods between the intra-day and inter-day measurement were compared. The results shown in Table 1 indicated that no significant difference ($P > 0.05$) was observed for the precision of both methods. It shows

that both methods gave a reproducible result between the intra-day and inter-day precision.

No interfering compounds were detected in both chromatogram as shown in Figure 1a and 1b. The results suggested that the combination of sorbents PSA and C_{18} was able to remove the co-extractives from the final sample extract. This finding is in agreement with the previous study and that makes primary secondary amines (PSA) and C_{18} sorbents as the most commonly used sorbents for cleanup the tea extracts (Kanrar *et al.*, 2010; Steiniger *et al.*, 2010; Cajka *et al.*, 2012). It was reported that PSA helps to remove acidic components and certain pigments and sugars whereas the C_{18} was shown to be effective in retaining the chlorophyll and minimizing pesticides losses (Paya *et al.*, 2007; Lehotay *et al*., 2011). The intensity of the beta-endosulfan peak obtained from the ASE method with in-cell cleanup (Figure 1a) was higher than the intensity of those obtained from the QuEChERS method (Figure 1b). This proves the higher recovery results of beta-endosulfan achieved from the ASE method with in-cell cleanup.

Conclusion

The accelerated solvent extraction (ASE) method with in-cell cleanup with gas chromatography tandem mass spectrometry determination for incurred pesticide residue in tea was demonstrated to be an efficient technique for the simultaneous extraction and cleanup of incurred pesticide residues in tea. This simple, rapid, effective and environmental friendly method could be applied to the routine analysis of pesticides in tea.

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