UNIVERSITI PUTRA MALAYSIA

ANALYSIS OF MULTICLASS PESTICIDE RESIDUES IN TEA USING ACCELERATED SOLVENT EXTRACTION WITH IN-CELL CLEANUP AND GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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By

HASLINA ABDUL KADIR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

August 2015

This thesis is especially dedicated to

My beloved husband and children who always there for me

Mohamad Shahabudin Bin Said
Nursyaza Irdina Bt Mohamad Shahabudin
Nur Khalisha Zahra Bt Mohamad Shahabudin

To my beloved families

Abdul Kadir Bin Yusof
Zainap Bt Hussin
Mohd Yusri Bin Abdul Kadir
Haslinda Bin Abdul Kadir
Muhammad Rizzuan Bin Abdul Kadir

Without whose support and trust this study could never have been completed

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: **Associate Professor Faridah Binti Abas, PhD**
Institute : **Bioscience**

The application of pesticides in tea plantations is a common practice to control pests and plant diseases. Chemical pesticides are excessively used by the farmers in order to meet the increasing demand for food for the fast growing population. Due to a lack of a proper, continuous and efficient monitoring, farmers are facing severe quality related problems when exporting agricultural products and various problems related to human health being reported. The existing situation urgently demands for the implementation of advanced analytical procedures for accurate monitoring of pesticides in agriculture products and which would be needed for the management of the existing pesticide problem. Therefore, the main objective of this study was to develop an efficient method for analysis of pesticide residues in tea. The current trend in pesticide residue analysis is the use of multiresidue methods that not only provide a simultaneous determination of multiple pesticides but also applicable for analysis of a large number of samples. Despite advances in the development of high performance analytical instrumentation for determination, sample preparation still an important part of obtaining accurate quantitative results.

QuEChERS and ASE with in-cell cleanup methods are amongst the modern sample preparation methods developed to overcome the drawback of traditional approach for pesticide analysis. There are many study reported for analysis of multiresidue pesticide analysis using QuEChERS method and the original version of this method also has been modified in order to establish efficient extraction and cleanup procedure for pesticide determination in tea. On the other hand, ASE with in-cell cleanup method has been successfully applied in pesticides analysis but it has not been applied for extraction of pesticides in tea. However, both methods had shown high performance for the analysis of multiresidue pesticides in tea, but their extraction performance based on recovery value was evaluate using spiked sample. Unlike incurred sample, the pesticide in spiked samples only coats the surface of matrices and may not be bound up in the tea cell structure. The first part of this study is to compare the extraction efficiencies of the QuEChERS and ASE with in-cell cleanup methods by using the reference material of incurred pesticide in tea. The extracts were analysed by gas chromatography-tandem mass spectrometry (GC-MS/MS). Following this, the selected method for analysis of multiresidue pesticides in tea was validated and subsequently apply to determine the occurrence of pesticides in tea samples. In addition, the uncertainty of pesticides measurement using ASE with in-cell cleanup method was also quantified and estimated based on top down approach.

ASE with in-cell cleanup method gave an average recovery of 91% with a 17% RSD whereas the QuEChERS method gave an average recovery of 64% with a 18% RSD. The recovery obtained from the QuEChERS method was outside the requirement set by the validation guidelines implying that the method is not reliable for extraction of pesticides in tea. Therefore, the ASE with in-cell cleanup method was selected and further validate for sample preparation of multiresidual pesticide analysis of a real sample.

ASE with in-cell cleanup method was applied for the validation extraction of multiresidue pesticides comprising endosulfan, lindane, dieldrin, chlorpyrifos and bifenthrin. Method validation parameters such as the linearity, limits of detection and quantification, recovery, precision and accuracy of the method for all of the pesticides studied were carried out. Linear correlation coefficients for all of the tested pesticides were better than 0.995. The overall average recoveries using this method at three spiked concentration of 0.04, 2.0 and 3.5 µg g-1 ranged from 90 to 98%, and the relative standard deviations (RSDs) were less than 10% for all of the analytes. The limits of detections (LODs) ranged from 0.001 to 0.007 µg g-1, and the limits of quantifications (LOQs) ranged from 0.009 to 0.021 µg g-1. The results obtained from the validation parameters suggested that the ASE with in-cell cleanup method has good purification effect and therefore, resulting in a better detection and quantification by the GC-MS/MS.

This method was subsequently applied to ten different brands of tea samples obtained from a local supermarket. The concentration of pesticide residues in all samples were detected to be lie in the range of 0.008 to 0.161 µg g-1. The uncertainty in the measurement of each pesticide was also evaluated. The measurement uncertainty (MU) was used to decide whether the results indicate compliance or non-compliance with the maximum residual limits (MRLs) value. Two out of ten samples were found non-compliance with European Union’s regulation. The relative expanded uncertainty for all pesticides were ranged from 24 to 34% and were considered as satisfactory for routine analysis.

The incurred pesticide in a complex matrix such as tea makes it difficult to obtain complete extraction of pesticides. However, the combination of high temperature and pressure of ASE with in-cell cleanup method results in better extraction efficiency. The built-in features of this method allow short time sample extraction and less solvent used compared with the QuEChERS and other methods developed for pesticide analysis. The convenience, simplicity and reliability of the ASE with in-cell cleanup method is undoubtedly makes it a valuable tool for analysis of multiresidue pesticides in other agricultural products and can be applied as a routine method.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS PELBAGAI JENIS KELAS SISA RACUN MAKHLUK PEROSAK DIDALAM TEH DENGAN MENGGUNAKAN TEKNIK PENGEKSTRAKAN PELARUT DIPERCEPAT DENGAN PEMBERSIHAN DIDALAM SEL DAN GAS KROMATOGRAFI BERGANDINGAN SPEKTROMETER JISIM

Oleh

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Penggunaan racun perosak di dalam penanaman teh adalah amalan biasa untuk mengawal perosak dan penyakit terhadap tumbuhan. Racun perosak kimia yang berlebihan digunakan oleh peladang di dalam usaha untuk memenuhi permintaan makanan yang semakin meningkat. Oleh kerana kekurangan pemantauan yang betul, berterusan dan berkesan, petani menghadapi masalah apabila mengeksport produk pertanian disebabkan oleh kualiti yang teruk dan pelbagai masalah berkaitan dengan kesihatan manusia dilaporkan. Keadaan ini menuntut pelaksanaan kaedah analisis yang terbaik untuk memantau dengan tepat kandungan sisa racun perosak dalam produk pertanian dan bagi menguruskan masalah racun perosak yang sedia ada. Oleh itu, objektif utama kajian ini adalah untuk membangunkan satu kaedah yang berkesan untuk menganalisisa sisa racun perosak dalam teh. Tren semasa dalam analisa sisa racun perosak ialah penggunaan kaedah yang bukan sahaja menyediakan penentuan serentak bagi berbagai jenis racun perosak tetapi juga terpakai untuk analisa sampel yang banyak. Walaupun kemajuan dalam pembangunan peralatan analisis yang berprestasi tinggi bagi kaedah penentuan, kaedah penyediaan sampel masih merupakan bahagian penting untuk mendapatkan keputusan yang tepat.

Kaedah QuEChERS (cepat, mudah, murah, berkesan, lasak dan selamat) dan pengekstrak pelarut terpecut (PPT) dengan pembersihan didalam sel adalah diantara kaedah penyediaan sampel moden yang telah dibangunkan untuk mengatasi kelemahan kaedah tradisional di dalam analisa racun perosak. Terdapat kajian yang dilaporkan untuk analisis pelbagai sisa racun perosak menggunakan kaedah QuEChERS dan versi asal kaedah ini juga telah diubah suai dalam usaha untuk mewujudkan pengekstrakan yang cekap dan prosedur pembersihan bagi penentuan sisa racun perosak dalam teh. Sebaliknya, PPT dengan kaedah pembersihan dalam sel telah berjaya digunakan dalam analisis racun perosak tetapi ia belum digunakan untuk pengekstrakan bagi sisa racun perosak dalam teh. Walaupun kedua-dua kaedah telah menunjukkan prestasi yang baik untuk analisa pelbagai sisa racun perosak dalam teh, tetapi prestasi pengektrakan mereka adalah berdasarkan nilai pemulihan melalui kaedah sampel diperkaya. Tidak seperti sampel yang tersedia ada kandungan sisa racun perosak didalamnya, bahan didalam sampel diperkaya hanya menyelaputi permukaan matrik dan tidak melekat pada struktur sel teh. Bahagian pertama kajian ini adalah untuk membandingkan kecekapan pengekstrakan QuEChERS dan PPT dengan pembersihan didalam sel dengan menggunakan bahan rujukan sisa racun perosak di dalam teh. Larutan bahan rujukan teh yang terekstrak dengan menggunakan kedua-dua jenis kaedah pengekstrakan itu kemudiannya dianalisis dengan menggunakan gas kromatografi bergandingan spektrometer jisim (GC/MS/MS). Kemudian, kaedah yang dipilih bagi analisa pelbagai sisa racun perosak di dalam teh dibuat pengesahan dan seterusnya di applikasikan bagi penentuan sisa racun perosak dalam sampel teh. Di samping itu, ketidakpastian pengukuran (MU) bagi sisa racun perosak menggunakan PPT dengan kaedah pembersihan dalam sel juga diukur dan dianggarkan berdasarkan kaedah bawah ke atas.

Peratus bagi perolehan semula untuk kaedah pengekstarakan pelarut terpecut (PPT) dengan pembersihan didalam sel adalah sebanyak 91% dengan sisihan piawai relatif 17% manakala kaedah QuEChERS memberikan peratus perolehan semula sebanyak 64% dengan sisihan piawai relatif 18%. Nilai perolehan semula bagi kaedah QuEChERS adalah diluar julat yang di terima berdasarkan kepada garis panduan kaedah pengesahan. Oleh itu, kaedah PPT dengan pembersihan dalam sel telah dipilih bagi penentuan pelbagai sisa racun perosak yang terdapat didalam sampel teh sebenar.

Applikasi kaedah ini terhadap analisis sampel teh sebenar bagi mengektrak pelbagai jenis sisa racun perosak seperti endosulfan, lindane, dieldrin, chlorpyrifos dan bifenthrin di sahkan. Parameter kaedah pengesahan yang meliputi kelinearan, had pengesanan dan kuantitatif, perolehan semula, kejituan dan ketepatan bagi semua sisa racun perosak yang dikaji telah dijalankan. Korelasi koefisen bagi semua racun perosak adalah lebih daripada 0.995. Purata keseluruhan perolehan kembali pada tiga kepekatan (0.04, 2.0 dan 3.5 $\mu g\ g^{-1}$) adalah daripada 90 ke 98% dengan sisihan piawai relatif kurang daripada 15% bagi semua racun perosak yang dikaji. Had pengesanan yang diperolehi adalah antara 0.001 hingga 0.007 $\mu g\ g^{-1}$ manakala had kuantitatif adalah diantara 0.003 hingga 0.021 $\mu g\ g^{-1}$. Hasil daripada parameter pengesahan ini menunjukkan bahawa kaedah yang dipilih ini adalah sesuai digunakan bagi mengekstrak pelbagai sisa racun perosak.

Kaedah ini seterusnya diapplikasikan terhadap sepuluh sampel teh yang dijual dipasaran Malaysia. Kepekatan bagi sisa racun perosak yang dikesan didalam sampel teh adalah diantara 0.008 hingga 0.161 $\mu g\ g^{-1}$. Ketidakpastian pengukuran untuk analisa setiap racun perosak juga dinilai. Ketidakpastian pengukuran adalah digunakan untuk membuat keputusan sama ada nilai pengukuran yang diperolehi itu mematuhi atau tidak dengan nilai had sisa maksimum (MRLs) yang dibenarkan. Dua daripada sepuluh sampel didapati tidak mematuhi peraturan yng ditetapkan oleh Kesatuan Eropah. Dengan mengambil kira MU, didapati bahawa kepekatan dieldrin dalam Sampel S6 dan S8 adalah melebihi nilai MRLs. Dua daripada sepuluh sampel didapati tidak mematuhi peraturan yang telah ditetapkan oleh Kesatuan Eropah. Ketidakpastian relatif berkembang untuk semua racun perosak adalah di antara 24-34 % dan ia dianggap sebagai memuaskan untuk analisa rutin.

Pengekstrakan lengkap bagi sisa racun perosak terkandung di dalam matriks kompleks seperti teh adalah sukar untuk dieprolehi. Walau bagaimanapun, dengan gabungan suhu dan tekanan tinggi PPT dengan pembersihan dalam sel menghasilkan pembersihan dalam kecekapan pengeluaran yang lebih baik. Ciri-ciri yang terbina di dalam kaedah ini membolehkan pengekstrakan sampel mengambil masa yang singkat dan kurang menggunakan pelarut berbanding dengan kaedah QuEChERS dan kaedah-kaedah lain

yang dibangunkan untuk analisa sisa racun perosak. Kemudahan, dan kebolehpercayaan keadah PPT dengan pembersihan di dalam sel sudah pasti menjadikannya sebagai kaedah yang berguna untuk analisa pelbagai sisa racun perosak dalam produk pertanian yang lain dan boleh digunakan sebagai kaedah rutin

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ASE	Accelerated solvent extractor
APMP	Asia Pacific Metrology Programme
CODEX	Codex Alimentarius Commissions
CRM	Certified reference material
D-SPE	Dispersive solid phase extraction
ECD	Electron capture detector
EI	Electron impact
EPA	Environment Protection Agency
EPU	Economic Planner Unit
EU	European Union
ISO	International Standard Organisation
IUPAC	International union and pure applied chemistry
ISTD	Internal standard
FAO	Food and Agricultural Organizations
GCB	Graphitized carbon black
GC-MS	Gas chromatography mass spectrometry
HKGL	Hong Kong Government Laboratory
LC-MS	Liquid chromatography mass spectrometry
HPLC	High performance liquid chromatography
LOD	Limits of detection
LOQ	Limits of quantification
MAE	Microwave assisted extraction
MRM	Multi-residue method

MRL	Maximum Residual Limits
MSPD	Matrix solid phase dispersion
MU	Measurement uncertainty
OCP	Organochlorine pesticide
OPP	Organophosphate pesticide
PSA	Primary secondary amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RSD	Relative standard deviation
SPE	Solid phase extraction
SPME	Solid phase micro-extraction
UPLC	Ultra-performance liquid chromatography
VIM	Vocabulary of Metrology
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Background of study

Concern over food safety issues regarding pesticide residues in tea has increased among producers and consumers. As tea producers have strived to increase the yields of their crops, cultivation practices have become more extensive. The tea industry uses integrated pest management practices in which the use of pesticides is an essential part of protecting the plants and producing a large quantity of high quality tea (Gurusubramanian, 2008). Pesticides are applied during plant cultivation as well as during storage for post-harvest protection. Food and Agricultural Organization (FAO), in its documents, has stated that the use of pesticides resulted in pollution to the environment and threatened the ecological environment as well as human health.

There has been an increase in studies and investigation related to the occurrence of pesticide residues in tea. They are many methods developed for determination of pesticides in tea. One of the most important aspects is technique on how the pesticide residues can be thoroughly extracted from the complex matrices and how the co-extracted interfering compounds can be cleaned up. The current trend in pesticide residue analysis is the use of a multiresidue method that not only provides the simultaneous determination of multiple pesticides but is also applicable to a large number of samples.

Traditional sample preparation methods for pesticide analysis such as liquid-liquid extraction, Soxhlet extraction and the Luke method are laborious, time consuming, expensive, require large amounts of organic solvents and usually involve many steps leading to the loss of some quantity of analyte (Beyer and Biziuk, 2008). As a result, modern sample preparation procedures such as accelerated solvent extraction (ASE) (Feng et al., 2013), supercritical fluid extraction (SFE) (Cai et al., 2003), microwave assisted extraction (MAE) (Ning et al., 2006), solid phase extraction (SPE) (Lambropoulou and Albanis, 2007), solid phase micro-extraction (SPME) (Schurek et al., 2008), matrix solid phase dispersion (MSPD) extraction (Hu et al., 2005) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) (Anastassiades et al., 2003) have been developed to overcome the drawbacks of traditional approaches for the extraction of pesticides in tea.

QuEChERS and ASE with in-cell cleanup methods was a streamlined sample preparation method whereby the sample extraction and cleanup was performed in a single step. QuEChERS is a manual technique whereas ASE with in-cell cleanup is an automated technique. These methods have been successfully used for the determination of multi-residue pesticides in different food matrices (Lambropoulou and Albanis, 2007). Both methods offer the advantages of high recovery, accuracy and high sample throughput, as well as a low solvent and glassware usage. In addition, ASE has the potential to be easily automated for sequential extraction. In ASE, the extraction and cleanup processes can be integrated into a single step via the incorporation of an in-cell cleanup (Labarta et al., 2012).

In this study, the extraction efficiencies of QuEChERS and ASE with in-cell cleanup methods for analysis of pesticides in tea were compared in order to select the method that provides the highest possible recovery and good precision.

1.2 Problem statement

QuEChERS and ASE with in-cell cleanup were developed as an alternative to the traditional methods that employed large volumes of organic solvents and were lengthy as well as laborious (Lambropoulou and Albanis, 2007). In the last few years, QuEChERS method has been widely used for analysis of pesticides in tea (Steiniger et al., 2010; Chen et al., 2011; Guan et al., 2013; Chen et al., 2014). This method also has been modified from its original method for better selectivity of pesticides in tea.

Applications of ASE resulting in better extraction efficiencies and short analysis times for the simultaneous extraction of multiple pesticides in tea have been reported in the literature (Hu et al., 2008; Feng et al., 2013). Recent advances in these automated system with an in-cell cleanup have demonstrated the selective removal of interferences for matrices such as fish and fish oil, soil and mushroom (Haglund et al., 2007; Jia and Deng, 2008; Labarta et al., 2012). To the best of our knowledge, no procedures have been reported on the use ASE with in-cell cleanup for simultaneous extraction of multiresidue pesticides in tea.

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 sample. These spiked samples was prepared by adding known amounts of analyte into a matrix sample and the recovered spiked analyte from the sample matrix is calculated (Magnusson and Örnemark, 2014). 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 samples. This inaccuracy is observed because unlike the incurred sample, the pesticide in spiked samples only coats the surface of matrices and may not be bound up in the sample the same way that the incurred analyte is incorporated (Betterncourt et al., 2003). When the pesticides sprayed to the growing plant, they penetrate to the inside of a cell wall and travelling along its absorption path. Thus, the pesticide was incorporated and tightly bond into the tea cell structure (Guan et al., 2013). Studies on the analysis of pesticide residues in incurred tea samples are seldom reported. There is no published data regarding the extraction efficiency of QuEChERS and ASE with in-cell cleanup methods using the incurred pesticides in tea sample. This is due to a limited number of reference material available especially pesticides in tea (Sin et al., 2011).

In order to utilize a result to decide whether it indicates compliance or non-compliance with a specification, it is necessary to take into account the measurement uncertainty. Measurement uncertainties provide good evidence that the measurement value is well above or well below the limit. Previous studies on the analysis of multiresidue pesticides in tea often lacked information on the uncertainty of results. Therefore, the decision on compliance with regulatory for a measurement result is difficult especially when pesticides concentration are detected close to the MRLs value (Rodriguez et al., 2002).

1.3 Scope of the study

The results from the extraction of pesticides in tea using different extraction methods (QuEChERS and ASE with in-cell cleanup) were investigated on a reference material (RM) of tea containing pesticides. This RM contained incurred beta-endosulfan pesticides and comes with not only the assigned pesticide value but also an uncertainty value. The results were compared to determine the method with the highest extraction efficiency. The selected method was later applied for the multi-residual determination of pesticides in real tea samples. Due to the absence of reference material of multiple pesticides in tea, a spiked sample was used for the recovery study in method validation.

The uncertainty for each pesticide associated with the selected method was estimated to establish the comparability of the results. The estimation of the uncertainty takes into account either random or systematic errors and gives information about the range in which result can be expected. In this study, an adequate identification and estimation of each source of uncertainty was established to provide information on the accuracy and reliability of the measurement results.

1.4 Research objectives

The objectives of this study are as follows:

1. To compare the extraction efficiencies of QuEChERS and accelerated solvent extractor (ASE) with in-cell cleanup methods by using the reference material of pesticides in tea.
2. To validate the best method for the analysis of pesticide residues in tea.
3. To quantify the occurrence of pesticides in tea samples.
4. To quantify and estimate the uncertainty of the measurement for pesticides in tea using the selected method.

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