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Validation of a 'multi-residue' analytical method for pesticide residue analysis in fruits

(Pengesahan kaedah analisis multi-residu bagi analisis residu pestisid dalam buah-buahan)

C.K. Ngan*, A.M. Khairatul*, S. Mohammad Shahid* and B.S. Ismail**

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Abstract

A simple, rapid and reliable multi-residue analytical method was validated by carrying out pesticide residue determination in fruits such as mango and carambola. The analytes determined were α-endosulfan, β-endosulfan and endosulfan sulphate (organochlorine pesticides); chlorpyrifos, fenitrothion and prothiofos (organophosphorus pesticides); bifenthrin, cypermethrin, deltamethrin and fenpropathrin (synthetic pyrethroid pesticides). The method involved a process whereby the sample was chopped and homogenized, followed by blending with ethyl acetate using the Ultraturax blender. The extract was then subjected to a clean-up process in a PSA (Primary-Secondary Amines) powder prior to GC determination. The method was validated by conducting recovery studies where homogenised mango and carambola portions were fortified with known amounts of the pesticides, followed by extraction using the above mentioned method and subsequent residue quantification by GC. The recovery studies using 10 pesticides in the two crops showed acceptable range of recovery levels from 70 – 130% with the exception of deltamethrin (57.1% recovery at the spike rate of 0.1 mg/kg). The repeatability of the method was shown to be consistent with standard deviation of less than 20% for all the tested pesticides. The limits of quantification of pesticides observed were at a range of 0.02 – 0.5 mg/kg.

Introduction

Analysis of pesticide residues in food is undertaken in order to address the need for safety and health problems caused by ingestion of food containing traces of pesticide residues. These analyses are mainly for the purpose of enforcement of the food safety regulation (in compliance with the Maximum Residue Limits or MRLs of the pesticide residues), health impact assessment and for establishment of the MRLs for food or agricultural commodities. Currently, there are more than 1,100 active ingredients (Wood 2012) used in pesticides since the introduction of the first modern pesticide, dichlorodiphenyltrichloroethane (DDT), during World War II. The vastness of the pesticide types presents a challenge to chemists in the development of any single analytical method that could encompass the various pesticides used.

*Strategic Resources Research Centre, MARDI Headquarters, Serdang, P. O. Box 12301, 50774 Kuala Lumpur, Malaysia
**School of Environmental Sciences and Natural Resources, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43650 UKM Bangi, Selangor, Malaysia
Authors' full names: Ngan Chai Keong, Khairatul Azmah Mohamed, Mohammad Shahid Shahrun and Ismail Sahid
E-mail: ckngan@mardi.my

Analytical methods have been developed to determine the various pesticide residues found in food. Some of the methods are only capable of analyzing certain chemicals group of pesticides. The current trend in pesticide residue analysis for food regulatory purposes shows a shift towards a simultaneous multi-residue method that is able to analyse more than one group of pesticides (organochlorines, organophosphorus, synthethic pyrethroids, etc.). The multi-residue method is preferred by the authorities concerned because of time and cost saving factors.

Most pesticide analytical methods for food samples involve more than a single stage of extraction where the first stage involves blending of the sample in an organic solvent followed by the second stage of partitioning with another organic solvent. This process is laborious and time consuming. In a scenario where residue analysis in compliance with the MRLs is needed for clearance of a large volume of perishable crops at port entry points, the method has to produce results fast so that the produce can be released as soon as possible. Currently, there are simple and rapid techniques developed to detect pesticides in food, namely, the use of fibre optic sensors (Grey 1992) and the rapid test kit employing the immunoassay principle such as ELISA (Hammock et al. 1987; Mumma and Brady 1987; Vanderlaan et al. 1987). A new rapid test method using a fibre optic measuring device to analyse organophosporus residues in vegetables, has been developed by MARDI (Zamri et al. 2004). However, these methods cannot rival the GC or HPLC-based method in terms of accuracy and/or sensitivity at low concentrations (< 0.1 parts per million). Nevertheless the rapid methods mentioned above are useful as screening tools for pesticide residues in food products.

Most of the established methods use highly toxic solvents such as benzene, dichloromethane, hexane and toluene to extract the pesticide residues. The Steinwandter method (Steinwandter 1985) is one of the methods used by the Department of Agriculture and Ministry of Health to routinely analyse pesticide residues in food. In this method, dichloromethane and cylohexane are used as the extracting solvents. Disposal of these solvents has created a dilemma of potential environmental problems (Steinwandter 1992). Proper treatment of solvent wastes will incur additional cost to the analysis. The safety and long term health problems of the analyst handling the above solvents are another concern. It is rather ironic that the purpose of pesticide residue analysis is to determine residue levels, so as to protect human health and environment, but in doing so, this has inadvertantly introduced potentially undesirable chemicals and products that could cause environmental and health safety problems.

This paper details the validation of a multi-residue method for determination of 10 pesticides in carambola and mango. This method is modified from the original method published by Anastassiades et al. (2003). The modified method involves single stage extraction, and the GC-based method was developed from the need to address the health and environmental problems mentioned above. The modified method takes into account method simplicity in terms of performance (to ensure fast execution and reduces error in analysis), time constraint and at the same time, maintains the most important aspect of the analytical analysis, namely, reliability in terms of sensitivity and accuracy. The method uses less volume of solvent (which lowers the material cost per sample and generates less wastage) and a less toxic organic solvent (ethyl acetate compared to other more toxic solvents such as benzene, dichloromethane and hexane which are the main solvents in the other methods). The usage of the less toxic ethyl acetate as the extracting solvent, will have a lower impact on the environment and human health.

Two types of locally grown fruits, carambola and mango, were selected for the validation study. These 2 types of fruits have become important export commodities to the nation. Chlorpyrifos, cypermethrin and deltamethrin are among the pesticides commonly used in farms for the commercial production of carambola and mango, based on the authors' working experience. Traces of pesticide residues remained on the surface of the fruits as a result of direct spraying of pesticides onto the fruits and leaves (although some farmers practice fruit wrapping to reduce pest attack).

Materials and methods

Standard pesticides

All standard pesticides were purchased from the pesticide supplier, Reidel-de Haën. Standard pesticides were used for fortification (recovery studies) and calibration (residue quantification by GC) purposes.

Description of the analytical multi-residue method

The work flow of the method is outlined in *Figure 1*. The raw samples were chopped and further homogenised in a food processor. An analytical portion of homogenised sample (30 g) was weighed into a 250 ml borosilicate bottle. In the same bottle, 60 ml of ethyl acetate, 5 g of sodium hydrogen carbonate and 30 g of sodium sulphate were added. The mixture was blended for 2 min in an Ultraturax blender. After blending, the extract was shaken in an orbital shaker for 2 h at 150 rpm. Then, 20 ml of the supernatant was decanted into a 25 ml graduated tube and 5 g of magnesium sulphate was added. The tube was vortexed for one min. About 10 ml of the vortexed extract was decanted and subjected to a clean-up process using Primary-Secondary Amines (PSA) powder. In the clean-up method with PSA powder, 10 ml of the supernatant was decanted into a 10 ml centrifuge tube, followed by addition of 0.25 g of PSA powder and

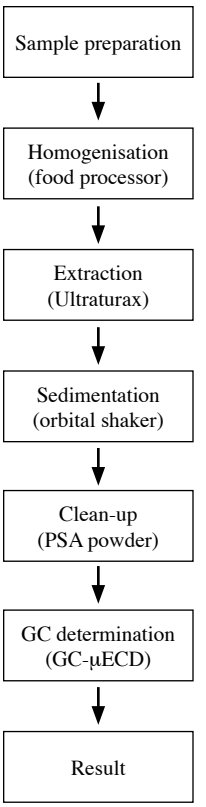

Figure 1. General diagram flow of the 'multi-residue' method of analysis

1.5 g of magnesium sulphate. The tube was centrifuged at 2,000 rpm for 2 min. Then, 5 ml of supernatant was decanted into a 10 ml graduated tube. The volume was concentrated using a gentle stream of nitrogen until a final volume of 2 ml was achieved. The sample extract was injected into a GC-μECD for quantification.

Recovery study

The recovery study was conducted to validate the method used. Fruits (carambola and mango) with no history of pesticide treatment were used in this study. The fruits were chopped into small pieces and homogenized in a food homogenizer. Analytical portions of the homogenised samples were fortified with known amounts of standard pesticides at 2 levels of concentrations (*Tables* 2 and *3*). There were 5 replicates of recovery samples

for the recovery study of organochlorine, organophosphate and synthetic pyrethroid pesticides. The fortified analytical portions were subjected to extraction using the method described above. The final extracts were injected into the GC to determine the residue levels. Blank samples were also analysed to determine the background levels. The percentage analytes recovered indicated the effectiveness of the method in extracting the pesticides from the sample matrices. The pesticides studied were α-endosulfan, β-endosulfan and endosulfan sulphate (organochlorine pesticides); chlorpyrifos, fenitrothion and prothiofos (organophosphorus pesticides); bifenthrin, cypermethrin, deltamethrin and fenpropathrin (synthetic pyrethroid pesticides). However for recovery study in mango, only endosulfan sulphate was excluded.

Residue determinations

Sample extracts from the recovery study were injected into the Gas Chromatograph-Electron Capture Detector (GC-μECD) for quantification of residues. The GC, Hewlett-Packard model 6890, equipped with a micro Electron Capture Detector (μECD) and DB-5MS UI column (30 m long, 0.32 mm internal diameter and 0.25 μm film thickness) was used to determine the organochlorine, organophosphorus and synthetic pyrethroid residues. The flow of the carrier gas, helium, was set at 2 ml/min at constant flow mode. The injection mode was splitless. The injection volume of the sample was set at 1 μl. The injector and detector temperature levels were set at 250 °C and 320 °C respectively. The oven was set at an initial temperature of 60 °C, which was maintained for 1 min and then raised to 320 °C at a rate of 30 °C/min. The latter temperature was maintained for 3 min.

Before sample extracts were injected, a series of standard pesticides at 4 (minimum 4) different concentrations (within a range of concentrations) were injected into the GC to obtain the calibration curve, which was used to calculate the residue level in the samples. The parameters of each pesticide's calibration curve are shown in *Table 1*.

The described GC column in analytical method was used for the quantitative purpose. In practice, positive identification of pesticide residues in samples should be confirmed either by mass-spectrometry based detector with 2 different types of GC detectors or by the same instrument analysis (GC/LC) using 2 columns of different polarity. Therefore, the very same sample can be re-injected again with the same analytical instrument but with different columns of different polarity such as DB-XLB (30 m long, 0.32 mm internal diameter and 0.25 μm film thickness) column for GC-μECD.

Results and discussion

All the pesticides showed good linearity in the concentration range (0.02 – 0.5 mg/kg) studied with a R^2 of 1. *Table 1* shows parameters related to the calibration curve of pesticide analysis in the GC-μECD. *Figure 2* shows an example of a calibration curve of α–endosulfan analysis in GC-μECD. Similar calibration curves were determined for all the pesticides studied.

The Limit of Quantification (LOQ) is defined as the lowest spiked concentration that gives recovery within 70 – 120% with precision <20% (EU 2009). The recovery rates of all the pesticides are summarised in *Tables 2* and *3*. The recovery levels of the 10 pesticides (except for deltamethrin, 129.2% and cypermethrin, 127.5%) were within the 70 – 120% range, which is an acceptable range, according to Hänel et al. (2000). Recovery of deltamethrin was 57.1% at the spike rate of 0.1 mg/kg (*Table 3*). Low recovery is acceptable if the precision parameter is small (European Commission 2000). In this case, the precision of 3.3% corresponded to 57.1% recovery of deltamethrin indicating that the analytical method can be accepted for analysis of deltamethrin at a level of 0.1 mg/kg. For the

Table 1. Concentration range of pesticide standards, calibration curve equations and R^2 for pesticide residue analysis in GC-μECD

Pesticide	Concentration range of pesticide standards (μg/ml)	Calibration curve equations	R^2
α-endosulfan	0.005 – 0.03	y = 1114599x + 366	1
β-endosulfan	0.005 – 0.03	y = 1088902x + 877	1
Endosulfan sulphate	0.005 – 0.03	y = 831965x + 572	1
Chlorpyrifos	0.0125 – 0.075	y = 506925x + 2534	1
Fenitrothion	0.0125 – 0.075	y = 385948x + 1914	1
Prothiofos	0.005 – 0.03	y = 710308x + 725	1
Bifenthrin	0.005 – 0.03	y = 231063x + 496	1
Cypermethrin	0.025 – 0.15	y = 133468x + 118	1
Deltamethrin	0.025 – 0.15	y = 456937x – 403	1
Fenpropathrin	0.005 – 0.03	y = 255839x + 500	1

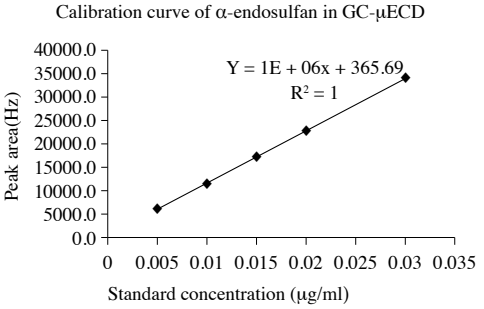

Figure 2. Example of calibration curve of α-endosulfan (in ethyl acetate) in GC-μECD

pesticides that had less than 100% recovery percentage, the difference could be attributed to losses of the analyte during various stages of the analysis.

The recovery of the analytes above 100% was due to several factors. One of the main causes was the co-extractive effects, also known as matrix-induced chromatographic effects (Soboleva et al. 2000). Compounds in the fruit matrix may enhance the response of a chromatographic peak to an artificially high level, thus leading to higher estimation of the recovery value. Some pesticides have shown enhancement as high as 1000% (Wylie and Uchiyama 1996). A clean-up of sample extracts can eliminate or reduce this effect. In the method used, the PSA sorbent was the clean-up material. The PSA sorbent is a very effective medium to purify extracts in fruit and vegetable analysis (Schenck and Lehotay 2000; Schenck et al. 2002). Another way to counter the high recovery effect is the use of matrix-matched calibration standards. In the method used, calibration standards were prepared in solutions containing the fruit matrix instead of the pure solvent. In the present study, only the pure solvent of the calibration standard was used and no significant matrix enhancement effect was found in most of the pesticides. This was probably due to the PSA clean-up which reduced this effect significantly or that mango and starfruit do not produce significant matrix enhancement effects (very high recovery) when analysed using the above mentioned method. However, prothiofos exhibited recovery rates of >120% in 1 or 2 replicates but not in all the replicates. The recovery value could fall within the 70 – 120% range if the matrix-matched calibration standard is applied.

Precision of the method is described by the relative standard deviation (RSD) which is also shown in *Tables 2* and *3*. The precision in the context of the present study, can be regarded as repeatability of the method. Repeatability is defined as closeness of agreement of independent test results under the same method on replicated analytical portions in the same laboratory by the same operator using the same equipment within short intervals of time (Hänel et al.

Table 2. Mean recovery (%) and repeatability, RSD_r (%) of pesticides in carambola

Pesticide	Spike concentration* (mg/kg)	Number of replicates	Mean recovery (%)	Repeatability, RSD_r (%)
α-endosulfan	0.5	5	82.8	3.6
	0.02	5	95.3	3.4
β-endosulfan	0.5	5	92.4	2.2
	0.02	5	107.3	2.8
Endosulfan sulphate	0.5	5	87.2	13.4
	0.02	5	90.6	6.7
Chlorpyrifos	0.5	5	90.6	1.6
	0.05	5	111.1	2.3
Fenitrothion	0.5	5	82.3	4.4
	0.05	5	88.4	6.6
Prothiofos	0.5	5	88.4	1.0
	0.05	5	114.8	2.9
Bifenthrin	0.5	5	82.2	8.1
	0.05	5	110.0	2.5
Cypermethrin	0.5	5	93.0	2.7
	0.1	5	127.5	4.3
Deltamethrin	0.5	5	100.3	4.8
	0.1	5	129.2	3.7
Fenpropathrin	0.5	5	79.9	4.4
	0.05	5	108.6	2.2

*The lowest spiked concentration is the Limit of Quantification (LOQ)

Table 3. Mean recovery (%) and repeatability, RSD_r (%) of pesticides in mango

Pesticide	Spike concentration* (mg/kg)	Number of replicates	Mean recovery (%)	Repeatability, RSD_r (%)
α -endosulfan	0.5	5	87.8	3.6
	0.02	5	81.0	3.0
ß -endosulfan	0.5	5	95.7	5.9
	0.02	5	94.7	3.6
Chlorpyrifos	0.5	5	110.8	4.9
	0.05	5	92.2	2.9
Fenitrothion	0.5	5	106.2	2.7
	0.05	5	100.5	7.9
Prothiofos	0.5	5	101.0	4.0
	0.05	5	117.4	1.5
Bifenthrin	0.5	5	97.2	4.9
	0.05	5	101.5	14.6
Cypermethrin	0.5	5	93.9	2.7
	0.1	5	97.2	2.4
Deltamethrin	0.5	5	98.8	4.9
	0.1	5	57.1	3.3
Fenpropathrin	0.5	5	103.1	4.4
	0.05	5	83.5	10.5

*The lowest spiked concentration is the Limit of Quantification (LOQ)

2000). The repeatability of all the pesticides reported in the study is acceptable as the standard deviation was <20%. This indicated that the method did not produce results with high variation.

The list of the 10 pesticides can be further expanded to include other pesticides from the same group which have similar chemical properties. However, pesticides from groups other than the organochlorines, organophosphorus and synthetic pyrethroids can also be determined by the method. The scope of commodities can be extended to other commodities with different matrix properties (e.g. fruits with high fat content such as durian). Further work is needed to examine the scope of pesticides and commodities that can be covered by the method used.

Conclusion

Overall, the method is effective in qualitative and quantitative determination of 10 pesticide residues from organochlorine, organophosphorus and synthetic pyrethroids groups in fruits such as carambola and mango.

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Abstrak

Satu kaedah analisis multi-residu yang mudah, cepat dan dipercayai telah disahkan dengan menjalankan penentuan residu pestisid dalam buah-buahan seperti mangga dan belimbing. Analit yang dikaji ialah α-endosulfan, β-endosulfan dan endosulfan sulfat, (pestisid organoklorin); chlorpyrifos, fenitrothion dan prothiofos (pestisid organofosforus); bifenthrin, cypermethrin, deltamethrin dan fenpropathrin (pestisid pirethroid sintetik). Dalam kaedah ini, sampel dipotong dan dihomogen, kemudian dicampur dengan etil asetat menggunakan pengisar Ultraturax. Ekstrak dibersih di dalam serbuk PSA (*Primary-Secondary Amines*) sebelum penentuan GC. Kaedah disahkan dengan pelaksanaan kajian perolehan semula. Pestisid yang dikaji dalam kuantiti tertentu diperkuatkan pada sampel buah-buahan yang telah dihomogen. Sampel tersebut kemudian diekstrak dengan kaedah tersebut di atas dan seterusnya residu di dalam sampel ditentukan dengan GC. Kajian perolehan semula terhadap 10 pestisid dalam mangga dan belimbing menunjukkan peratusan perolehan semula antara 70 – 130% kecuali deltamethrin (perolehan semula 57.1% pada kepekatan perakuan 0.1 mg/kg). Pengulangan kaedah ini adalah konsisten dengan sisihan piawai kurang daripada 20% bagi semua pestisid yang dikaji. Had kuantifikasi pestisid adalah dalam julat 0.02 – 0.5 mg/kg.

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