Comparative study of different sorbents for clean-up for pyrethroid analysis in cocoa powder by GC-ECD: A preliminary study

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Abstract. Three types of solvents and 13 combinations of sorbents were tested to extract pyrethroids (lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate, and deltamethrin) from cocoa powder and to remove interferences during clean-up step before being quantified by gas chromatography coupled with electron capture detector (GC-ECD). The result was analysed based on recovery study by spiking the cocoa powder with known amount of targeted pyrethroids. Acetonitrile, methanol, and n-hexane were compared as the solvents and the result showed that acetonitrile is relatively better in extracting pyrethroids. Moreover, 13 treatments, singly or combination of primary secondary amine (PSA), graphitized carbon black (GCB), C18, and florisil, were tested for clean-up step. The clean-up study shows that only combination of PSA and GCB; combination of C18 and Florisil; and combination of GCB and florisil comply the recovery guideline suggested by AOAC and EU, thus are suggested for clean-up for pyrethroid analysis in cocoa powder.

Keywords: d-SPE, clean up, GC-ECD, pyrethroid, cocoa powder

1. Introduction

The occurrence of pesticide residue in cocoa powder is possible due to the application of pesticides on cacao trees as the raw material of cocoa powder. Cacao trees are known to be vulnerable to pests and diseases [1-3] and pesticide application is commonly practiced to combat this issue. Pesticide applications on cacao trees are also needed to increase the global production of cacao beans. Cacao beans are the main product of cacao trees and used to produce cocoa powder and its global production is decreased by one-third due to pests and diseases [1,4]. In addition, pesticide is also applied to cacao beans during the storage [5] thus increase the possibility of pesticide contamination on cocoa powder.

Pyrethroid is one class of pesticide that commonly applied in cacao. It was an alternative to organochlorin and organophosphate that were banished due to their toxicity [6]. However, although pyrethroids were relatively safer compare to organochlorin and organophosphate, recent reports show that they are neurotoxic to mammals [5,7] thus raising concerns. Pyrethroids previously have been detected in cocoa beans [8] at concentrations above the maximum residue limit values [7]. These MRL values vary across countries. For comparisons, MRL values for lambda-cyhalothrin, permethrin,
cypermethrin, and fenvalerate, are higher in Europe than in Japan, although the values are still in tens or hundreds ppb range [9].

Although pesticide residue contamination in cocoa powder is possible due to their detection in cacao beans, there is no report on pesticide residue on cocoa powder. However, since the fat content of cacao beans and cocoa powder are very much different [10], it is needed to develop a method to analyze pyrethroids in cocoa powder.

Cocoa is a complex matrix and contains many compounds [11-14]. Its dried roasted beans may contain more than 300 compounds [11,15]. Pyrazines, thiazoles, oxazoles, pyrrole derivatives, pyridines and furans are major fundamental compounds in roasted cocoa. Moreover, flavonoids, polysaccharides, lignin are also common [12]. This complex matrix may interfere during sampling preparation and temper the result analysis.

Currently, dispersive solid phase extraction is applied for multi residue analysis of pesticide. This method is known as QuEChERS and proposed by Anastassiades et al. [16] in which dispersive solid phase extraction (d-SPE) sorbents such as PSA is used to remove interferences during clean-up step. This method was firstly applied in fruits and vegetables. However, since different matrices result different interferences, more investigation are needed to find the suitable sorbents for cocoa powder analysis. This paper reports the preliminary study of different sorbent application during clean-up step for pyrethroid analysis in cocoa powder.

2. Experimental

2.1. Chemicals and reagents
Acetonitrile for gas chromatography ECD and FID (SupraSolve®) and anhydrous magnesium sulfate were obtained from Merck. Pyrethroid standards of lambda-cyhalothrin (purity of 99.5 %), permethrin (purity of 99.6 %), cypermethrin (purity of 99.2 %), fenvalerate (purity of 99.5 %) and deltamethrin (purity of 99.3 %) were purchased from Chem Service, USA. Florisil, primary secondary amine (PSA), graphitized carbon black (GCB), and C18-encapped (C18) were used as d-SPE and obtained as bulk SPE sorbent. Florisil was obtained from Merck, while PSA, GCB, and C18 were obtained from Agilent, USA.

2.2. Apparatus
IKA Fortex Genius3 was used as a vortex, tube Rotary Agitator Model TRA 20 was used for extraction, and centrifuges EBA 12 was used for centrifugation. Sample extracts were analyzed on an Agilent 7890B coupled with a micro ECD. The GC-ECD analysis was performed according to Pitoi et al. [17] except for the detector temperature which was 300 °C in this analysis. Briefly, the injector temperature, oven initial temperature, and detector temperature are 250 °C, 200 °C, and 300 °C respectively. The auto sampler was operated in split mode (1:1) at 1 μL injection. Column HP-5 from Agilent was used for separation (length 30 m; id 0.320 mm; film 0.25 μm). The oven was held for 1 minute at the initial temperature and ramped at 20 °C/minute up to 280 °C and was then held for 8 minutes. Helium gas was used as the carrier gas at 2 mL/minute and nitrogen gas as the make-up gas at 30 mL/minute.

2.3. Cocoa powder samples
Cocoa powder samples used in this study are organic cocoa powder produced in South Sulawesi Indonesia. It was accepted as indicated in the label with 8-12 % fat and no added sugar.

2.4. Stock standard solution
The stock standard solutions were prepared separately for each pyrethroid at around 100 mg/L and the mix pyrethroid solution of 5 mg/L was prepared by mixing the standard solutions. The mix pyrethroid solution 5 mg/L was used to fortify the cocoa powder samples and also for preparing the mix standard solutions at different concentrations.
2.5. Sample extraction
For each extraction, cocoa powder sample of 2.0 g was transferred into a 50-mL screw cap centrifuge tube. For the recovery experiment, each sample was fortified with mix pyrethroid solution 5 mg/L so the final concentration in the powder is 50 μg/kg. The fortified samples were mixed using a vortex and allowed to stand for 1 hour to let the pyrethroids interact with the samples. Three solvents: acetonitrile, methanol, and n-hexane were used as solvents during the extraction. For the extraction, 10 mL of solvent was added into the fortified samples in each tube and the mixtures were shaken using a vortex. Extraction was performed on a rotary agitator for 30 minutes at 22.5 rpm, followed by centrifugation for 1 minute at 4000 rpm.

2.6. Dispersive solid-phase extraction (d-SPE) clean up
Various sorbents, singly or in combination, were investigated in d-SPE study for cocoa powder clean-up step at 50 μg/kg fortification concentration and in duplicate for each sorbent treatment. After the extraction, an aliquot (2 mL) of extract was transferred to a 15-mL of screw cap centrifuge tube containing one these following sorbent combinations: (1) PSA; (2) C18; (3) GCB; (4) Florisil; (5) PSA/C18; (6) PSA/GCB; (7) PSA/Florisil; (8) C18/GCB; (9) C18/Florisil; (10) GCB/Florisil; (11) PSA/C18/GCB; (12) PSA/C18/Florisil; (13) C18/GCB/Florisil. Each sorbents was weighed 50 mg for each treatment and 150 mg of anhydrous MgSO4 was added for all treatment. The tubes were then capped tightly and shaken for 1 min before being centrifuged at 4000 rpm for 1 min. Finally, a 2.0-mL aliquot of the extract from each tube was filtered by a syringe filter of 0.45 μm and transferred to a 1.5-mL vial for GC-ECD analysis. All the sorbent combination was done in duplicate.

3. Results and Discussions

3.1. Qualitative analysis
Figure 1 showed the GC-ECD separation for mix pyrethroids standard solution. It can be seen that lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate, and deltamethrin are well separated.
3.2. Solvent extraction

The recovery of the extraction for each solvent without any clean-up is given in Table 1. Although the average recovery of acetonitrile is not as high as methanol and n-hexane, its extract is clear and its recovery was not fluctuated as others, showed by the lowest standard deviation. Moreover, although methanol gave the highest average recovery, its recovery for each pyrethroid is fluctuated. In addition, though n-hexane gave a relative similar result to acetonitrile, its extract is murkier then methanol and acetonitrile. Therefore, acetonitrile was chosen in preference for the extraction for the clean-up experiment.

| Pyrethroid     | Solvent       | Acetonitrile | Methanol | n-Hexane |
|----------------|---------------|--------------|----------|----------|
| \(\lambda\)-Cyhalothrin | 18.34         | 26.16        | 25.85    |
| Permethrin     | 92.78         | 260.75       | 106.66   |
| Cypermethrin   | 30.89         | 52.41        | 50.52    |
| Fenvalerate    | 6.19          | 6.76         | 9.94     |
| Deltamethrin   | 17.42         | 141.68       | 22.02    |
| Average        | 33.12         | 97.55        | 43.00    |
| SD             | 34.48         | 104.83       | 38.52    |

3.3. Dispersive solid-phase extraction (d-SPE) clean up

The recovery for each sorbent combination is given in Figure 2. The recovery values for all sorbent combinations fall in between 62.63 to 119.74 %. For comparison, the AOAC expected recovery values for 10 ppb is between 60 to 105 % [18] while the recovery range for residue analysis suggested by European Union is higher, accounted for 70-120 % [19]. It can be seen that, except for deltamethrin, the recovery values for all pyrethroids fall in the range suggested by AOAC. For deltamethrin, except for sorbent combinations 5; 6; 9; and 10, other sorbent combinations are above the range suggested by AOAC. Therefore, in term of AOAC recovery value, sorbent combination 5, 6, 9 and 10 are suggested to be used for clean-up. However, the recovery values for sorbent combination 5 for permethrin and fenvalerate are below the recovery guideline from European Union. Thus, to comply with both AOAC and EU guidelines, sorbent combinations 6, 9, 10 are suggested.

PSA, GCB, C18, and Florisil are commonly used in clean-up. During clean-up, each sorbent interacts with the pyrethroids, which are the target analytes, and the co-extracts, which are the interferences. Sometime the interaction does not only remove the interferences, which is good for the analysis, but also the target analytes. PSA is known to remove polar organic acids, polar pigments, sugar, and fatty acids [20-23] while GCB removes sterols and pigments [21, 24]. Moreover, C18 is used to remove non polar compounds such as lipids [21] and florisil is used for polar interferences [25].

In this study, combination of PSA and GCB; combination of C18 and Florisil; and combination of GCB and florisil result recovery values that comply with AOAC and EU guidelines.
Figure 2. Recovery of pyrethroids for fortified sample at 50 μg/kg for different sorbent combinations.

4. Conclusion
This study shows that, in term of recovery values and repeatability, acetonitrile is a relatively better solvent compare to methanol and n-hexane. Moreover, the recovery study suggests that the combination of PSA and GCB; combination of C18 and Florisil; and combination of GCB and florisil comply the recovery guideline suggested by AOAC and EU thus are suggested for clean-up of the extract in cocoa powder analysis.

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