Simultaneous determination of deltamethrin and 4 other pyrethroids residues in infusion tea: Preliminary study

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Abstract. Deltamethrin and other pyrethroids, which were thought to be relatively safe pesticides, recently are gaining concerns due to their toxicity. Residues of deltamethrin and other pyrethroids incur in tea-related products, such as tea-based functional beverage, due to the pesticide application at tea plantations, thus their concentrations need to be monitored. This preliminary study was aimed to develop a method for simultaneous analysis of deltamethrin and 4 other pyrethroids in infusion tea implementing low volume liquid-liquid extraction to extract the target compounds, followed by quantification by gas chromatography coupled with micro electron capture detector (GC-μECD). The GC-μECD was firstly optimized for the quantification of deltamethrin and 4 other pyrethroids. The optimization of the instrument was able to detect the pyrethroids at 0.1 μg/L with good repeatability showed by %RSD of 1.97-10.21%, which was very much lower compared to the AOAC guideline at 1 ppb level (30%). Simultaneous analysis of mix standard solutions at concentration ranges of 1 to 10 μg/L showed very good linearity (r>0.997). In addition, the low volume liquid-liquid extraction using n-hexane was able to detect the target pyrethroids from the spike experiment of infusion tea samples as low as 0.67 μg/L.

1. Introduction

Pyrethroids are synthetic pesticides that have a similar structure to the natural pesticide pyrethrum [1]. They were synthesized by modifying the structure of the natural pesticide to result in more persistent structures with similar insecticide properties [2]. The properties of some pyrethroids are given in Table 1 and the chemical structures are given in Figure 1.

Pyrethroids including deltamethrin were used to know as relatively safe pesticides. IARC listed deltamethrin, fenvalerate, and permethrin in group 3 as “is not classifiable as to its carcinogenicity to humans” [3] and not listed cypermethrin and lambda-cyhalothrin. Meanwhile, USEPA [4] classified cypermethrin (in 1988) as group C, a possible human carcinogen; deltamethrin (in 2003) and permethrin (in 2002) as not likely to be carcinogenic to humans; lambda-cyhalothrin (in 2002) as group D, a not classifiable as to human carcinogenicity; and fenvalerate (in 2003) as group E, evidence of non-carcinogenicity for humans. Similarly, WHO [5] classified lambda-cyhalothrin, cypermethrin, deltamethrin, fenvalerate and permethrin as moderately hazardous pesticides (class II).

However, recent studies show the dangerous effects of pyrethroids. For example, pyrethroids were shown to be very toxic to fish and invertebrate with low values of LC50 [6]. Deltamethrin has been proven to exhibit neurotoxicity and hepatotoxicity in mammals [7-9], while fenvalerate was suggested as human sperm genotoxic agent [10].
Table 1. Chemical properties of pyrethroids [11,12].

| Pesticide       | Lambda-cyhalothrin | Permethrin | Cypermethrin | Fenvalerate | Deltamethrin |
|-----------------|--------------------|------------|--------------|-------------|--------------|
| Formula         | $C_{23}H_{19}ClF_3NO_3$ | $C_{23}H_{20}Cl_2O_3$ | $C_{22}H_{18}Cl_2NO_3$ | $C_{25}H_{22}ClNO_3$ | $C_{22}H_{19}Br_2NO_3$ |
| Molecular weight| 449.850            | 391.288    | 416.297      | 419.900     | 505.20       |
| Log $K_{OW}$    | 6.8                | 6.50       | 6.60         | 6.20        | 6.20         |
| Solubility      | $5.0 \times 10^{-3}$ mg/L | $0.0111$ mg/L | $4 \times 10^{-3}$ mg/L | $2.4 \times 10^{-2}$ mg/L | $<0.002$ mg/L |

Figure 1. Chemical structures of several pyrethroids [12].

Pyrethroids residues in tea were sourced from the application of pesticides at tea plantations. Moreover, the short time interval between pesticides application and the harvest causes the pyrethroids residues to stay in the tea. Furthermore, the pesticides were also applied during the storage time directly to the tea leaves to prevent pests and prolong the storage, therefore increases the pesticides residue level in tea. Deltamethrin contamination in tea has been reported in India until 50 mg/kg [13,14]. The minimum regulated limit (MRL) for pyrethroids is given in Table 2.

Table 2. Comparison of minimum regulated limits for pyrethroids in tea (mg/kg) [15].

| Pesticide         | Codex | EU | China | Australia | Japan | Indonesia |
|-------------------|-------|----|-------|-----------|-------|-----------|
| Lambda-cyhalothrin| 1     | 15 | 15 [16]| 20        |       |           |
| Permethrin        | 20    | 0.1| 20 [16]|           | 20    |           |
| Cypermethrin      | 10    | 0.1|       |           |       |           |
| Fenvalerate       | 5     | 0.1|       |           |       |           |
| Deltamethrin      | 5     | 5  | 10 [16]| 5         | 10    | 10 [18]   |
Similar to other pesticides, pyrethroids analysis formerly involved liquid-liquid or solid-liquid extraction which used a big volume of samples and solvents. Further sample preparation was developed to lower the volume of samples and solvent and utilized some combinations of several extraction techniques such as vortex, ultrasonication, centrifugation, rotary evaporation [19-21]. Some of the sample preparation utilized dichloromethane as a solvent during extraction [21]; dichloromethane is listed in Group 2A (probably carcinogenic to human) in IARC classification [22].

Sample preparation by QuEChERS has been known in pyrethroids analysis in vegetables [23], olive [24], paprika [25], rice [26] and even for tea [20, 27-30]. Nevertheless, these are still combined with the above-mentioned steps. Moreover, some still applied relatively dangerous solvents such as carbon tetrachloride [30], which has been classified in group 2B [31]. Therefore, method development for the analysis is still needed especially in order to minimize the use of samples and solvents and to use a “safer” solvent according to the principles of green analytical chemistry.

This paper reports the preliminary study for simultaneous analysis of deltamethrin and 4 other pyrethroids in an infusion of tea-based functional beverage by implementing low volume liquid-liquid extraction to extract the pyrethroids followed by analysis by gas chromatography coupled with micro electron capture detector.

2. Experimental setup

2.1. Chemicals and reagents
Lambda-cyhalothrin was sourced from Chem Service, USA, while fenvalerate, deltamethrin, permethrin and cypermethrin were sourced from Sigma Aldrich, Germany. Stock solutions were prepared separately for each chemical in acetonitrile at 100 mg/L (ppm). Standard solutions were prepared as mix standards from the stock solutions at different concentrations. Solvents and other chemicals were sourced from Merck unless otherwise stated. The tea samples were collected from the market around Bandung, West Java, Indonesia.

2.2. Apparatus
An Agilent 7890B gas chromatography coupled with a micro electron capture detector from Agilent was used for the GC-µECD analysis.

2.3. Optimization of GC-µECD
An HP-5 Agilent column was utilized for GC-µECD separation for the pyrethroids. Helium was used as the carrier gas while nitrogen as the make-up gas. An automatic liquid autosampler injected the samples at a ratio of 1:1. The temperature of the injector and detector temperature were set at 250°C and 350°C, respectively. The oven was programmed as these: the initial temperature was 280°C and hold for 1 minute, then was ramped up at 20°C/min to 280°C and hold for 8 minutes.

2.4. Analysis of spiked tea samples
The optimum condition of the GC-µECD was also used to analyse tea samples that have been spiked with pyrethroids and prepared by low volume liquid extraction. Infusion tea sample was prepared by dissolving 5 g of tea in 150 mL of boiling water as described elsewhere [32]. A spike experiment was conducted by spiking mix standard of pyrethroids at a certain volume to the 30 mL of the infusion tea aliquot. The infusion tea was then extracted by the low volume of n-hexane (3 mL) using a rotary agitator. The extraction was done twice and the collected organic phase was filtered and analysed by GC-µECD. A control experiment was also conducted in a similar procedure as the spike experiment but without spiking of pyrethroids.
3. Result and discussion

3.1. Instrument optimization

The GC-μECD was able to detect the pyrethroids with the total running time of 13 minutes. The resulting chromatograms for the individual standards at 100 mg/L and the mixed standard of pyrethroids at 1 mg/L are given in Figure 2 and Figure 3, respectively. It is noted from both figures that all pyrethroids standards used in this study were mixtures of two isomers except for cypermethrin, which was a mixture of 3 isomers. This result confirmed the certificate of analysis for each pyrethroid. For further analysis, the total area from all isomers for each pyrethroid was used for calculation.

![Figure 2. Chromatograms for individual pyrethroids standards at 100 mg/L.](image)

The mix standard solutions were then used to analyse the instrument detection limit (IDL). The instrument detection limit was measured by analysing the mix standard solution and lowering the concentration until the detector could not detect the pyrethroids. The IDL was a concentration above the concentration where the detector could not detect the pyrethroids. It was found that the instrument detection limit was 0.1 μg/L (ppb level) for the simultaneous analysis. For the reference (Table 2), MRLs of pyrethroids are ranging from 0.1 to 20 mg/kg (ppm level). Therefore, analysis of pyrethroids by GC-μECD was applicable in order to meet the MRLs.

The linearity of the standard solutions was evaluated by injecting a series of concentration in μg/L level and the result was given in Figure 4. It was shown from Figure 4 that the coefficient of correlation (r) values for all pyrethroids were all above 0.997, suggesting very good linearity for a simultaneous determination.

To evaluate the precision of the area, a standard solution of 0.1 μg/L were injected for seven times and the relative standard deviation (RSD) was calculated based on the resulted area (Table 3). It was found that RSD (%) values for all tested pyrethroids were all below the AOAC guideline (30% at 1 ppb) [28] and the 2/3 CV Horwitz (42.67% at 0.1 ppb) suggesting good repeatability of the analysis.
Figure 3. Chromatogram of mix standards of pyrethroids at 1 mg/L.

Figure 4. The linearity of standard solutions at 1 to 10 µg/L.

Table 3. The precision of the retention time and the area at 0.1 µg/L (n=7).

| Pyrethroids       | Average area | Standard deviation | Relative standard deviation |
|-------------------|--------------|--------------------|-----------------------------|
| Lambda cyhalothrin| 33.818       | 2.256              | 6.672                       |
| Permethrin        | 19.403       | 1.982              | 10.214                      |
| Cypermethrin      | 111.655      | 3.648              | 3.267                       |
| Fenvalerate       | 40.120       | 2.350              | 5.858                       |
| Deltamethrin      | 192.531      | 3.791              | 1.969                       |
Figure 5. Chromatogram for no spike/control (blue) and spike (red) experiment at 0.67 µg/L.

Figure 6. Chromatogram of spike experiment at several concentrations.
3.2. Analysis of infusion tea samples
A spike experiment coupled with control was done to see if the low volume liquid-liquid extraction was able to extract pyrethroids from the infusion tea at a low concentration (0.67 μg/L). The result is given in Figure 5.

Figure 5 shows that pyrethroids peaks appeared in the spike experiment but not in the no-spike/control experiment. This suggested that the low volume liquid-liquid extraction using n-hexane as a solvent was able to extract the pyrethroids from the infusion tea. The relatively high log Kow values of pyrethroids (Table 1) suggesting a high affinity to organic solvents such as n-hexane, thus n-hexane was able to extract pyrethroids from the infusion tea.

However, one peak of lambda-cyhalothrin and one peak of cypermethrin also appeared in the chromatogram of the control experiment. This may suggest that the tea samples used in this experiment already has lambda-cyhalothrin and cypermethrin in it.

Moreover, the spike experiment was also conducted at several different concentrations which were 0.01 mg/L, 0.1 mg/L, and 0.5 mg/L and the result is given in Figure 6. It was shown from Figure 6 that higher spike concentrations resulted in bigger peaks. This implies that the low-volume liquid-liquid extraction might be applied at those ranged concentrations.

However, Figure 5 and 6 also show some unidentified peaks, suggesting that many compounds may interfere with the analysis. Compounds found in tea such as polyphenols, chlorophylls, tannins, and caffeine [33-37] may interact and/or co-extracted with pyrethroids, thus interfering with the analysis. Therefore, further investigation and modification of the extraction are still needed to eliminate the analytes that may interfere with the analysis.

4. Conclusion
The GC-μECD can be used to analyze pyrethroids in infusion tea samples with a low IDL, good linearity and repeatability. The low volume liquid-liquid extraction was able to extract pyrethroids from the infusion tea as low as 0.67 μg/L, although some improvement and/or modification must be done to eliminate interferences in the tea samples and to validate the method.

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