Method validation for pesticide multi-residue analysis of pyrethroid on green beans of arabica gayo coffee using gas chromatography-electron capture detector (GC-ECD)

M Yusuf¹, R Idroes¹,², Saiful¹, Lelifajri¹, T K Bakri², M Satria¹, H Nufus¹, I Yuswandi¹, Z Helwani³, Muslem⁴ and Marlina¹

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
²Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
³Department of Chemical Engineering, Faculty of Engineering, Universitas Riau, Kampus Bina Widya Panam, Pekanbaru 28293, Indonesia
⁴Department of Chemistry, Faculty of Science and Technology, Universitas Islam Negeri Ar-Raniry, Banda Aceh 23111, Indonesia

*E-mail: rinaldi.idroes@unsyiah.ac.id

Abstract. The method validation for the pesticide multi-residue analysis of pyrethroid on green beans of Gayo Arabica coffee using the Gas Chromatography-Electron Capture Detector (GC-ECD) method was carried out. Coffee samples were taken at three locations in Bener Meriah Regency, namely Bandar, Permata, and Wih Pesam. The pyrethroid residues measured were cypermethrin, deltamethrin, and permethrin. The coffee samples were extracted by QuEChERS using a solvent of 1% acetic acid in acetonitrile. The validation parameters tested included selectivity, linearity, Limit of Detection (LoD), Limit of Quantification (LoQ), precision, and accuracy. The results showed that this method was selective, with only three pyrethroid peaks detected. The method was linear in the concentration ranges of 0.01-0.30 μg/mL, with the correlation coefficient of ≥0.99 for all samples. The sensitivity was excellent with LoD and LoQ of 0.0151-0.0420 μg/mL and 0.0504-0.1400 μg/mL. The accuracy and precision were very well with %recovery and % RSD of 83.85-105.19 and 1.5-8.3 for cypermethrin, 75.17-89.34, and 2.0-11.7 deltamethrin, and 83.16-109.43 and 3.1-3.6 for permethrin, respectively. The method application showed that no pyrethroid residues were found in all coffee samples. The result was confirmed by the obtained % recovery in ranges of 90-109 % for all samples.

1. Introduction
Gayo Arabica coffee is one of Indonesia’s leading export plantation commodities originated from Gayo Plateau in Aceh Province [1]. The plantation areas in Bener Meriah Regency reaches 46,264 hectares and produces 29,358 tons of coffee beans [2]. The effectiveness of Gayo Arabica coffee plantations is affected by various factors, one of which is pest control [3]. According to the Agricultural Technology Research Institute of Aceh [4], it found that climate change has increased the intensity of the Coffee Bean Borer (PBKo, Hypothenemus hampei Ferr.) and Root Fungus (Phellinus noxius). The use of synthetic pesticides is an option for farmers to overcome these pests. On the other hand, synthetic...
pesticides have a negative impact on consumers, so it is necessary to monitor pesticide residues on agricultural products [5]–[7].

A pyrethroid is an insecticide that is widely used by farmers to kill borer insects. Naturally, these compounds can be degraded by sunlight. However, if it has been associated with sediment or tissue, this compound will be resistant longer [8]. A pyrethroid is harmful to both animals and humans [9]. Therefore, it is essential to ensure agricultural products are free from these pesticide residues. Several instrumental methods such as Uv-Vis [10], laser [11], [12], FTIR [13]–[15] image processing [16], sensor [17], [18], and chromatography [19]–[22] can be used to measure the chemical composition on organic substance. This instrument can produce both qualitative and quantitative analysis well. However, the most important part of the analysis is the method validation involving the instrument starting from the preparation stages, analysis procedures, and verification of the obtained data [23]. Validation of analytical methods is essential in producing quality and accountable data [24]. This study reported the method validation of the pesticide multi-residue analysis of pyrethroid in Gayo Arabica coffee by adapting the SANTE guidelines [25]. The instrument used is Gas Chromatography (GC) equipped with an Electron Capture Detector (ECD). The extraction method was performed using QuEChERS (quick, easy, cheap, effective, rugged, and safe) adapted from AOAC 2007 [26]. The validation parameters tested were selectivity, linearity, Limit of Detection (LoD), Limit of Quantification (LoQ), precision, and accuracy.

2. Methods
The Samples were obtained from Gayo Arabica coffee plantations in 3 locations in the Bener Meriah district, namely Bandar, Permata, and Wih Pesam. The sampling point can be observed in Figure 1.

![Figure 1. Map of sampling points.](image)

2.1. Tools and chemicals
The tools used in this study were Gas Chromatography (GC-2010 Plus) equipped with Electron Capture Detector (ECD) installed with GC-Solutions software and autosampler AOC-5000 Plus, syringe 10 μL (Shimadzu), vortex-homogenizer (IKA), micropipette (Eppendorf), centrifuge (Thermo Scientific), shaker (IKA), grinder (IKA), glassware set, 2 mL vial, 50 mL and 15 mL polyethylene (PE) tube.
The materials used were green beans of Gayo Arabica coffee, standard pyrethroids (cypermethrin, deltamethrin, and permethrin), acetonitrile for chromatography grade (Merck), acetic acid glacial (Merck), Kit-QuEChERS (6 g MgSO$_4$ and 1.5 g sodium acetate), and Kit-Clean Up (1200 mg MgSO$_4$, 400 mg PSA, and 400 mg GCB).

2.2. Extraction of green beans
Green beans were grinded until smooth and homogeneous. 5±0.2 grams of coffee powder was put in a polyethylene tube. Into the tube, it was spiked 150 µL each of the standard pyrethroids 10 µg/mL and let stand for 30 minutes. The Kit-QuEChERS powder, the ceramic homogenizer, and 15 mL of acetic acid 1% in acetonitrile was added into the tube. The solution then was vortexed until homogeneous for 2 minutes and centrifuged for 5 minutes at 4000 rpm to obtain two layers of the upper layer (organic phase) and the lower layer (waste) [26].

6 mL of the organic phase was put into a 15 mL PE tube filled with absorbent (150 mg MgSO$_4$, 50 mg PSA, and 50 mg GCB). The mixture was vortexed for 30 seconds until homogeneous and centrifuged for 2 minutes at 4000 rpm. 1000 µL of the organic phase were taken and put into the vial. Then the vial was put in an autosampler and injected as much as 1 µL of the solution into GC [26].

2.3. Gas chromatography conditions
The Gas Chromatography conditions used in this study can be seen in table 1.

| Parameters             | Details                                           |
|------------------------|---------------------------------------------------|
| Capillary column       | RTX-5 (cross bond, 95% dimethyl polysiloxane - 5% diphenyl) |
| Detector               | ECD (Electron Capture Detector)                   |
| Column dimension       | 30 m × 0.25 mmID × 0.25 µmDF                      |
| Mobile phase           | Helium (99.9995% purities)                        |
| Flow rate              | 0.97 mL/minutes                                   |
| Make up speed          | 30 mL/minutes                                     |
| Detector temperature   | 320 ºC                                            |
| Injector temperature   | 250 ºC                                            |
| Injection mode         | Splitless                                         |
| Oven temperature       | 210–280 ºC (temperature programming 10 ºC/minutes, hold 5 minutes) |

2.4. Method validation
The method validation was carried out at the Food Safety Laboratory, Department of Food, Aceh Province. The validation stage is carried out based on the SANTE guidelines [25]. The validation parameters tested were selectivity, linearity, LoD, LoQ, precision, and accuracy.

3. Results and discussion
3.1. Selectivity
The selectivity of the method is determined by comparing the spiked chromatogram with the blank chromatogram. The spiked sample was an analytical sample added with standard pyrethroids (cypermethrin, deltamethrin, and permethrin), while the blank was not added with the standards. The results showed that the method used was selective [27]. There were only three peaks representing
cypermethrin, deltamethrin, and permethrin that appeared in the spiked chromatogram. Besides, the blank chromatogram did not show the same retention peaks as the peaks belonging to the standards.

3.2. Linearity
The linearity test was performed using the standard concentration series of 0.01, 0.05, 0.10, 0.20, 0.30 μg/mL. Each standard's peak area was plotted to the concentration to produce a linear regression equation and a correlation coefficient [28]. The correlation coefficient value of ≥0.98 (table 2) stated that the method's linearity had met the requirements to be acceptable [29].

| Standards    | linearity               | R²     | LoD (μg/mL) | LoQ (μg/mL) |
|--------------|-------------------------|--------|-------------|-------------|
| Cypermethrin | y = 205130x – 287.58    | 0.9989 | 0.0151      | 0.0504      |
| Deltamethrin | y = 268821x – 578.64    | 0.9965 | 0.0259      | 0.0866      |
| Permethrin   | y = 164379x + 1011.7    | 0.9989 | 0.0420      | 0.1400      |

3.3. LoD and LoQ
LoD is the minimum concentration of analyte that can be detected, while LoQ is the minimum concentration of analyte that can be measured by the instrument [29]. LoD and LoQ were defined as the signal-to-noise ratio of 2:1-3:1 for LoD and 10:1 for LoQ [30]. This method showed high sensitivity with LoD and LoQ of 0.0151-0.0420 μg/mL and 0.0504-0.1400 μg/mL.

3.4. Accuracy and precision
Accuracy and precision were determined based on %recovery and percentage of relative standard deviation (%RSD) through standard measurements with various concentrations of 0.01, 0.05, and 0.1 μg/mL in 6 repetitions. The results showed %recovery and %RSD of 83.85-105.19 and 1.5-8.3 for cypermethrin, 75.17-89.34, and 2.0-11.7 for deltamethrin 83.16-109.43 and 3.1-3.6 for permethrin, respectively (Table 3). This showed the accuracy and precision of measurement had met the requirements by SANTE [25,31]. The pesticide residue analysis method is valid if %recovery and %RSD are in the ranges of 70-120% and ≤20%, respectively [32]. Thus, the GC-ECD method is precise and accurate for analyzing the pesticide multi-residue of pyrethroids in coffee samples' green beans.

| No | Standards    | Spiked concentrations (μg/mL) | Recovery (%) | RSD (%) |
|----|--------------|-------------------------------|--------------|---------|
|    |              | 0.01                          | 105.19 ± 9.27| 8.3     |
|    |              | 0.05                          | 96.87 ± 4.22 | 5.8     |
|    |              | 0.10                          | 83.85 ± 2.50 | 1.5     |
|    |              | 0.01                          | 89.34 ± 13.63| 11.7    |
|    |              | 0.05                          | 84.81 ± 3.40 | 3.4     |
|    |              | 0.10                          | 75.17 ± 1.67 | 2.0     |
|    |              | 0.01                          | 83.16 ± 7.04 | 3.6     |
|    |              | 0.05                          | 80.33 ± 2.78 | 2.8     |
|    |              | 0.10                          | 109.43 ± 4.0 | 3.1     |

3.5. Pesticide multiresidue analysis of pyrethroids in green beans of arabica gayo coffee
The validated method was then used for the multi-residue analysis of pyrethroids on three samples of Gayo Arabica coffee obtained from 3 locations in Bener Meriah Regency, namely Bandar, Permata, and Wih Pesam Districts. The results showed no peak of cypermethrin, deltamethrin, and permethrin
pesticides that appeared on the chromatogram. This showed that Gayo Arabica coffee did not contain pesticide residues of pyrethroids.

Table 4. Pesticide multi-residue analysis of pyrethroids in green beans of arabica gayo coffee.

| No | Standards  | Sample locations | Spiked concentrations (µg/mL) | Recovery (%) |
|----|------------|------------------|------------------------------|--------------|
| 1  | Cypermethrin | Bandar           | 0.01                         | 99.53        |
|    |            | Permata          | 0.01                         | 105.58       |
|    |            | Wih Pesam        | 0.01                         | 109.29       |
| 2  | Deltamethrin | Bandar           | 0.01                         | 99.10        |
|    |            | Permata          | 0.01                         | 90.55        |
|    |            | Wih Pesam        | 0.01                         | 101.80       |
| 3  | Permethrin  | Bandar           | 0.01                         | 95.02        |
|    |            | Permata          | 0.01                         | 99.82        |
|    |            | Wih Pesam        | 0.01                         | 99.70        |

To verify the analysis results of coffee samples, the peak area recovery for both the spiked and blank chromatogram from all samples was identified. The results showed a well %recovery in the ranges of 90-109. This showed that the results of the analysis were accurate.

4. Conclusion
The validation of the GC-ECD method for multi-residue analysis of pyrethroids in green beans of Gayo Arabica coffee showed precise, accurate, sensitive, and selective results. The validation data obtained included the selectivity by producing only three pyrethroid peaks, the linearity in the concentration range of 0.01-0.30 µg/mL with the correlation coefficient ≥0.99, the sensitivity with LoD and LoQ of 0.0151-0.0420 µg/mL and 0.0504-0.1400 µg/mL, respectively, and the accuracy and the precision with %recovery and %RSD of 83.85-105.19 and 1.5-8.3 for cypermethrin, 75.17-89.34 and 2.0-11.7 for deltamethrin, and 83.16-109.43 and 3.1-3.6 for permethrin, respectively. This validated method's application showed that green beans of Gayo Arabica Coffee from Bener Meriah Regency are free from pyrethroid pesticide residues.

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