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### Extraction and Analysis of Carbamate and Pyrethroid Pesticides in Tomatoes and Rice by Gas Chromatography Mass Spectrometry

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#### 1. Abstract

This work aimed to explore common methods for the extraction of pesticide and to perform qualitative and quantitative analysis by gas chromatography coupled with electron impact mass spectrometry (GC-EI-MS). Extraction was conducted using QuEChERS and Liquid-Liquid (L-L). Calibration curve and standard addition curve were both plotted for different concentration of mixtures. Additionally, the efficiency of the QuEChERS extraction methods was examined by spiking the organic rice and tomato with standard mixture of pyrethroid and carbamate and applying the extraction. Results showed that, in general, carbamate species (especially the aliphatic types) were eluted at earlier times compared to pyrethroids species. Carbamates were more susceptible to degradation during GC separation compared to pyrethroids. A chromatographic resolution of 3.24 was obtained for the two permethrin isomers. Good linearity of the three quantitative methods ( $R^2 > 0.99$ ) were obtained for most compounds. Based on using the standard addition curve, the recovery for the different pyrethroid and carbamate compounds were determined.

#### 2. Introduction

Pesticides poisoning of farmers are largely attributed to the inappropriate pesticide handling, improper use of personal protective equipment (e.g., gloves, respirators, and masks) and lack of knowledge about the toxicity of chemicals that they contain. Common misuses of pesticides in farming include the use of large volumes or

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concentration of pesticides than indicated on labels, ineffective use of protective equipment while mixing or applying the pesticides, improper management and disposal of pesticides, and lack of awareness of pre-harvest intervals following application. This causes high level of pesticides residues in fruits and vegetables. Therefore, pesticide management is of great necessity. Common methods used to extract pesticides can be classified into three types: liquid-liquid extraction, solid phase extraction (SPE), and Soxhlet extraction. One important and recently developed extraction method is QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) [1]. QuEChERS offers simple simultaneous extraction and clean-up steps of a wide range of analytes, in both polar and non-polar samples. It is a faster, convenient and cost-effective method than conventional liquid-liquid extraction producing premium results in a few steps with minimum solvent amount used. Two types of pesticides (carbamates and pyrethroids) were the focal points of this research. Carbamates are a group of pesticides which contain carbamic acid backbone. The high solubility of carbamate derivatives allows plants to absorb them by the root and the leaves [2]. Pyrethroids are synthetic insecticides derived from the naturally found pyrethrins. Although pyrethroids are more specific to insects, with less impact on human and environment, they are not widely used because of their short life-time compared to other synthetic pesticides [3–4].

### 3. Objectives

The primary aim of the research is to explore the use of common methods for the extraction of pesticides from foodstuffs and to qualitatively and quantitatively analyze them by GC-MS method. Calibration curve and standard addition were utilized as quantitative methods. The recoveries of QuEChERS extraction of carbamates and pyrethroids from spiked rice and tomato were evaluated. This work was also aimed to improve the skill of mass spectra interpretation and conducting scientific literature surveys.

### 4. Experimental

#### 4.1. Instrumentation and operational conditions

GC-MS (Shimadzu QP-2010) was used for the analysis of the pesticides. DB5 (30 m, 0.25 mm, 0.25  $\mu$ m) column was used for the separation of pesticides. Scanning mode was used to identify the pesticides, while selective ion monitoring mode was used to determine their concentrations. The operational condition of the GC and MS are shown in Table 1 and 2.

#### 4.2. Materials

The standards (Carbamate & Pyrethroids) pesticides were purchases from Dr. Ehrenstorfer GmbH, Germany. The contents of each mixture are shown in Table 3. The QuEChERS kit was purchased from Agilent Technology (U.S.A). The QuEChERS kit (Agilent, U.S.A), consisted of premeasured packet of  $\text{MgSO}_4/\text{Na}$ -acetate mixture, and three types of DSP cleanup kit, which came in three different led-colors (red, blue and green), containing  $\text{MgSO}_4/\text{PSA}$  DSP in different ratios.

#### 4.3. Preparation of standards for quantitative analysis

Four standard samples containing mixture of both pyrethroids and carbamates were prepared. The standards only differed in concentration (0.125, 0.25, 0.50, and 1.0 ppm). These standards were analyzed by the GC-MS to obtain the calibration curve. The standards were also used in standard addition analysis.

#### 4.4. QuEChERS extraction

About 15 ml of tomato juice was transferred into 50 mL tubes. 15 mL of acetonitrile was added to the tube. The extraction of rice is performed following similar steps, but prior to the addition of acetonitrile, the rice was mixed with equal amount of distilled water. Then the 50 mL tube was shaken for 30 seconds. After that, premeasured packet of mixture ( $\text{MgSO}_4/\text{Na}$ -acetate) was added to each tube and shaken again vigorously for 1 min. The tubes were placed in ice for five minutes to allow the liquids to separate in two layers. The upper liquid layer was collected into another 50 mL tube.

#### 4.5. Liquid–Liquid (L-L) extraction

About 200 g of blended tomato was placed in an Erlenmeyer flask. 150 mL of solvent mixture (3:2:1) (n-hexan:DCM:ACN) was added to the sample. The mixture was shaken for about one hour. The two immiscible phases were left overnight to separate into two layers. The upper layer was transferred into graduated cylinders.

#### 4.6. The cleanup steps

Extracts (each one mL) from the two previous extraction procedures (L-L and QuEChERS) were transferred into two dispersive cleanup tubes, containing 150 mg of  $\text{MgSO}_4$  and 25 mg PSA. The extracts in the tubes were shaken for one minute and centrifuged for 5 min. The extracts were then transferred into vials and stored in the refrigerator, ready to be analyzed by the GC-MS.

#### 4.7. Recovery study of QuEChERS method

For the recovery study of QuEChERS extraction method, the organic tomato and rice were spiked before the extraction with known amount of mixture consisting of carbamates and pyrethroids. The mixture was prepared by mixing 15 mL of carbamates (20 ppm) with 15 mL of pyrethroids (20 ppm) to produce a concentration of 10 ppm for each compound in the mixture. Then, 200 g of organic tomato and organic rice were spiked with 15 mL of the prepared mixture. Based on this spiking, the concentration of each pesticide compound in the tomato and rice before extraction should be  $\sim 0.70$  ppm. To determine the concentration of pesticides after extraction, standard addition method was used. The QuEChERS extract was first diluted to half its original concentration. Then the extract was divided into three 1.0 mL portions. In the first, second, and third portion, 0.50 mL of standards mixture with concentration of 0.125 ppm, 0.250 ppm, 0.50 ppm were added, respectively.

### 5. Results and discussion

#### 5.1. Qualitative and Quantitative Analysis of Pesticides

The chromatograms of carbamates and pyrethroids are shown in Fig 1 and Fig 2, respectively. Generally carbamates species were eluted at lower retention time (tr) compared to pyrethroids. Aliphatic carbamate derivatives were even observed at lower tr compared to the aromatic species, and they were more susceptible to degradation. Isomers of both resmethrin and permethrin were very well resolved with resolution  $> 3$ . The peaks in the chromatogram can be identified based on their fragmentation and isotopic pattern in the mass spectra by matching the patterns with those available in the GC-MS software library. Two peaks, at 7.08 & 8.85 minutes belong to promecarb. The first peak was attributed to the degradation while the second was attributed to the fragmentation of promecarb as shown in their mass spectra in Fig 3 (a, b). Observing the parent ion peak at  $m/z = 207$  Da confirm that the mass spectrum in Fig 3 (b) belong to the fragmentation (not the degradation) of promecarb. Also, logically, the degradation product of any compound is expected to be observed at earlier retention time. It was noticed that the C-O bond of the carbamate backbone is more likely to break down. According to Wang and Schnuta [5], carbamates are polar and/or thermally labile and not suitable for GC analysis. Aromatic compounds (resmethrin and permethrin) were less susceptible to degradation as observed, for instant, in the mass spectrum of permethrin (Fig 4). According to the isotopic pattern, two chloride atoms are present in the fragment at  $m/z = 163$  Da. The proposed fragments for permethrin are shown in the spectrum in Fig 4.

#### 5.2. Quantitative analysis and recovery results

Calibration curves for all standard pesticides were plotted. Also, standard addition method was used to determine the recovery of QuEChERS extraction method for these pesticides. The unknown concentration of carbamate residues in the tomato extract (after extraction) was determined by adding different concentrations of standards and plotting the curve as shown for 3-Hydroxycarbofuran (3-HCF) in Fig. 5. The lines are extrapolated to obtain the concentrations of the extracts before the addition of standards. The recovery was calculated as following, taking 3-HCF as example. Since the extract before addition of

standard was diluted to half of its original concentration, the concentration of 3-HCF in the original extract is calculated as:  $0.28 \text{ ppm} \times 2 = 0.56 \text{ ppm}$ , where 0.28 is the value intercepted by extrapolation in the x-axis. Then, the recovery was calculated as follows:  $\text{Recovery} = (\text{Extracted conc}/\text{original conc}) \times 100 = (0.56/0.70) \times 100 = 80\%$ . The linearity of the standard addition curves was acceptable, with correlation coefficient ( $R^2$ ) higher than 0.99. The recoveries of the other pesticides were calculated similarly and they are shown in the Table 4.

## 6. Conclusions

Fragmentation of compounds by GC-MS allowed identification of peaks in the chromatograms. Most of the carbamate compounds degrade in the GC-column, especially the non-aromatic species. The chromatograms illustrate that the non-aromatic carbamate compounds are found at low retention time unlike the aromatic ones. The C-O bond of the carbamate backbone is more likely to break down by either degradation in the GC column prior MS ionization, or fragmentation in the MS via the ionization source. The quantitative analysis is performed chiefly by three methods, which are standard calibration curve, standard addition. All these methods gave acceptable linearity with  $R^2 > 90\%$ . Standard addition is an alternative to the calibration curve technique that is useful to determine compounds in complex matrices and to measure the recovery of extraction methods.

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