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# Method Development, Validation, and Analysis of Bifenthrin Residues in Fresh and Dry Cilantro Foliages and Cilantro Seeds Using GC-ECD

W. Jiang, R. T. Kon, R. A. Othoudt, R. A. Leavitt, S. Kumar, L. D. Geissel, E. A. Gomaa

National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824-1302, USA

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Pesticides of various chemical structures are used worldwide in agriculture industry to attack pests which result in better crops, increased yields and lower input costs. Although these chemicals are considered to be important to agricultural development, there is a growing public concern about the residues of these chemicals on health, environmental contamination, and ecological conditions. Bifenthrin, CAS number 82657-04-3, formula $C_{23}H_{22}ClF_3O_2$, IUPAC name [2-methyl-(1,1'-biphenyl)-3-y1]-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate, is a member of the pyrethroid pesticide chemical class which affects the nervous system and causes paralysis in insects (Briggs 1992, Walker et al. 1992). Bifenthrin is non-polar and has a high octanol-water coefficient ($K_{ow}$ = 1.0 × $10^6$), indicating bifenthrin has a low solubility and a correspondingly strong tendency to bind to crops (FMC 1983). Pure bifenthrin is an off-white to pale tan waxy solid with faint, slightly sweet smell (Meister 2002). Typical chemical structures of bifenthrin are given in Figure 1. In the United States, bifenthrin has been registered for used on greenhouse ornamentals and cotton (US EPA 1997).

Cilantro is the coriander plant, known for its pungent flavor, and is often used in Latino, Asian, and Caribbean cooking. With the development of analytical technology, gas chromatography (GC) equipped with an electron capture detector (ECD) is still an adequate method of choice for analysis of bifenthrin because it is rapid, inexpensive, convenient, and highly sensitive to halide-rich compounds (López-López et al. 2001, Pang et al. 1999). This work has assayed cilantro for bifenthrin residues in three cilantro matrices, i.e., fresh, dry foliage, and seeds. This paper presents the method development, validation and analysis.

## MATERIALS AND METHODS

The standard, bifenthrin (purity 98%) was obtained from the manufacturer, FMC Corporation (Princeton, NJ, U.S.A.). In this work, calibration standard solutions were prepared in our laboratory and the concentration range was typically between 0.01 and 5 mg/L. The calibration standards were stored in a refrigerator at 4 °C. The organic solvents used in this study were pesticide grade (J.T. Baker, NJ, U.S.A.).

*Correspondence to:* W. Jiang

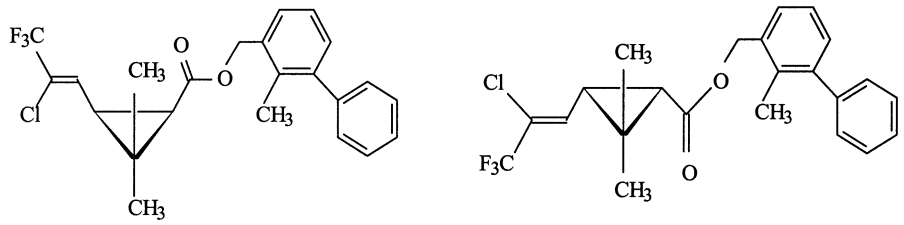


**Figure 1.** Typical structures of bifenthrin, (A) Z-(1R)-cis and (B) Z-(1R)-cis structures.

Cilantro samples were obtained from the Interregional Research Project Number 4 (IR-4). These samples were collected in California (CA), Texas (TX), and Georgia (GA) in the U.S.A., kept frozen in freezers (-25 °C), and shipped to our laboratory. The samples were maintained frozen before they were ground and subsampled. Grinding field samples was in a similar manner to the method recommended by the US FDA (1977). A representative sample was chopped in a Hobart food chopper with pulverized dry ice. Control samples untreated with bifenthrin were chopped followed by treated samples. After the samples were ground, spike samples were prepared by taking known amounts of the ground control samples and adding known amounts of bifenthrin. The solvent was allowed to evaporate. The subsamples were stored in a separate freezer (-20 °C).

The extraction was carried out in an automated accelerated extractor, ASE-200 (Dinex, Sunnyvale, CA, U.S.A.). A sample (5.00 g) was mixed with drying agent Hydromatrix® (Varian, Palo Alto, CA, U.S.A.) and then the mixture was transferred into an extraction cell. This sample cell was placed on ASE-200 and extracted with hexane at 2000 lbs/in$^2$, 90 °C, heat time 5 min, extraction time 20 min, and purge time 60 s. The extract was obtained in a collection vial. The extract was transferred to a round-bottom flask and was concentrated to approximately 5 mL on a rotary evaporator. The method of the sample cleanup process was similar to the FMC methods (FMC 1991, 1993). After cleanup, the extracts were concentrated on the rotary evaporator until approximately 1.5 mL was obtained. Finally, the extract was transferred to a 2-mL volumetric flask and the flask was filled to the 2-mL mark with hexane. The sample was ready for GC-ECD analysis.

The instrument used in this work was a Hewlett Packard GC 5890 (Palo Alto, CA, U.S.A.) equipped with an ECD and an HP 7673 autosampler. One (1.00) μL of the sample was injected into the GC and the amount of the bifenthrin residue was quantitated by the ECD. A calibration curve of bifenthrin standards was prepared by plotting peak area versus standard concentration. The instrument conditions are given as follows:

| | |
|---|---|
| Injector temp. (°C): | 275 °C |
| Column type: | DB-1, 0.25 mm bore and 0.25 μm film thickness |
| Column length: | 30 m |
| Temp. program (°C): | 250 (5 min),<br>250-300 (ramp at 5 °C/min),<br>300 (1.2 min) |
| Detector temp. (°C): | 325 |
| Carrier gas: | Helium |

## RESULTS AND DISCUSSION

Electron capture detectors (ECD's) have been proven to be good detectors for analyzing halide containing compounds (De Paoli 1997, López-López 2001). Since bifenthrin has three fluorides and one chloride, an ECD detector is assumed to be suitable for analysis of the bifenthrin residues. In this study, it was found that, under above experimental conditions, bifenthrin showed a retention time of 7.10 ± 0.10 min. Major peaks of impurities were found to be between 2.40 and 5.00 min retention time (Figure 2), allowing a separation of the signal of bifenthrin from those of contaminants present in the matrices, i.e., fresh, dry cilantro foliages and cilantro seeds. Typical chromatograms of control samples of fresh, dry foliage, seeds, and bifenthrin (0.02 ppm) are given in Figure 2. The chromatogram of the dry foliage control sample showed that background interference might be presented at retention time of bifenthrin (7.10 min).

The method validation developed for determination of bifenthrin in cilantro (fresh, and dry foliages and seeds) is described in the work. The topics of calibration, limits of detection and quantitation (LOD and LOQ), precision (repeatability and reproducibility), and accuracy (recovery of spike) are discussed in this work.

In this study, a standard quadratic relation curve between the peak area and the analyte concentration was used. This can be expressed as $y = ax^2 + bx + c$, where $x$ is the concentration (mg/L) of bifenthrin and $y$ is the peak area. The peak area ($y$) and the concentration ($x$) of bifenthrin were entered into a spreadsheet to calculate the values of a, b, c, and the correlation coefficient, $R^2$. In this case, the corresponding calibration equation can be expressed as $y = -663579\,x^2 + 140925\,x - 328$, where $R^2 = 0.9970$. A typical calibration curve is given in Figure 3. Although for most chromatographic procedures a linear relation is observed within a certain concentration range, as can be seen, a quadratic curve fits better throughout the concentration range in this study.

The limit of detection (LOD) is the lowest amount of analyte that is detectable using the instrument and method. It can be expressed in typically a concentration unit. The limit of quantitation (LOQ) is the lowest amount of analyte that can be quantitated with acceptable accuracy using the experimental conditions. LOD and LOQ can be obtained using various methods of experiments (Francotte et al.

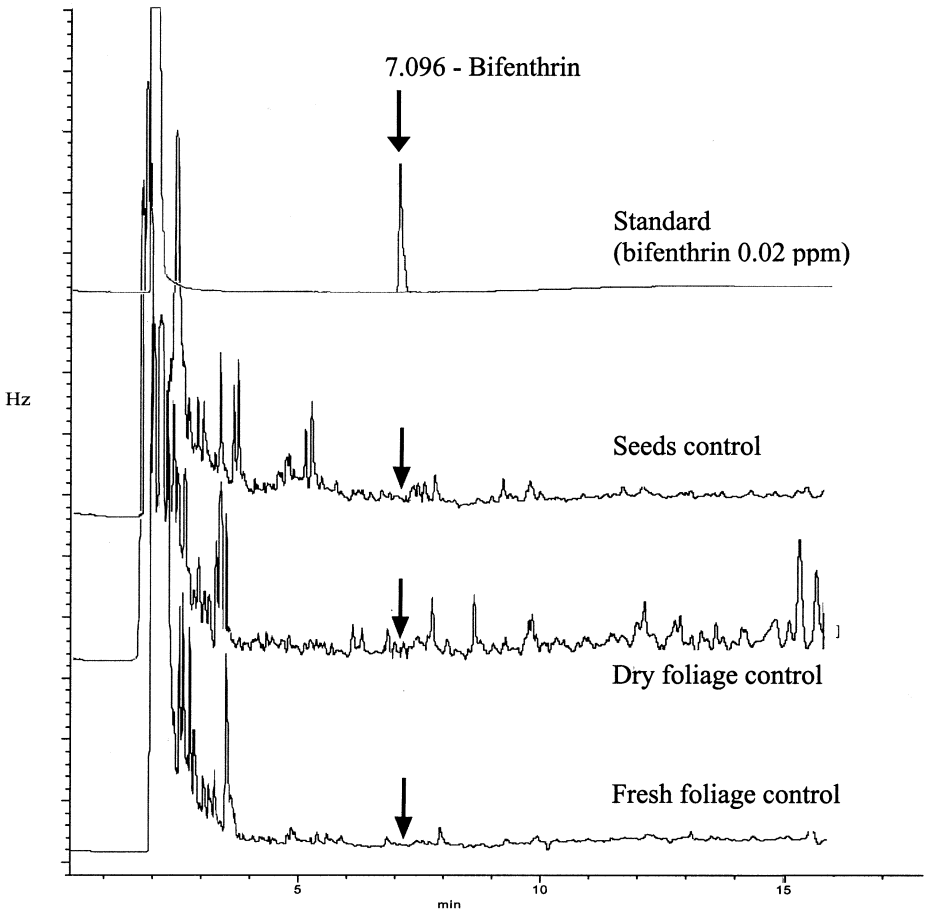


**Figure 2.** Chromatograms of control samples of fresh foliage, dry foliage, and seeds, and the standard (bifenthrin,0.02 ppm). Bifenthrin peak's retention time is at 7.096 min.

1996, Smith 1999, Zanella et al. 2000). In this study, the method of calculation of statistics LOD and LOQ is in the same manner described by Smith (1999). Thus, LOD and LOQ are calculated by

$$\text{LOD (ppm)} = (\text{standard deviation, ppm}) \times (\text{one-tailed t-statistic})$$
$$\text{LOQ (ppm)} = 3 \times \text{LOQ (ppm)}$$

The results of LODs and LOQs for the three matrices are given in Table 1. The recovery of spike was 92, 82 and 99%, and the LOQ was 0.026, 0.11, and 0.071 ppm for fresh, dry foliages and seeds, respectively. The LODs of dry foliage and seeds were higher than that of fresh foliage. This was due to the interferences presented in dry foliage and seeds samples. For fresh foliage, results were found to be similar under the same conditions after three years, i.e., from 2000 to 2003,

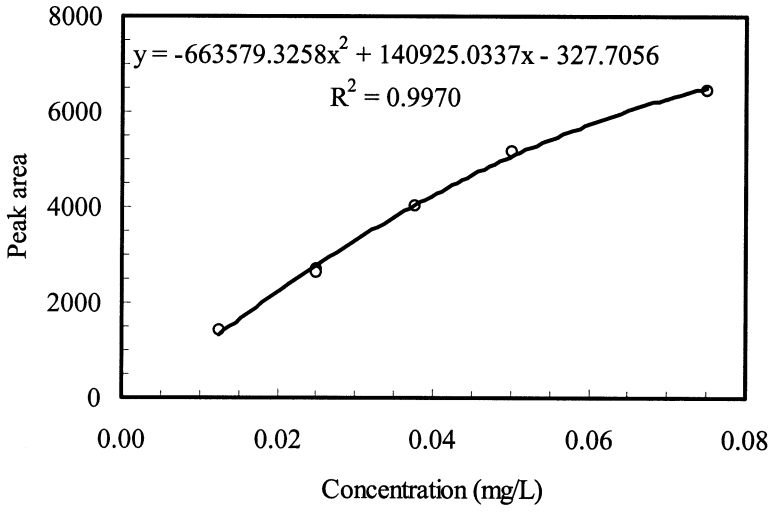


**Figure 3.** Bifenthrin calibration curve.

indicating that this method was stable, robust, and reliable. The lowest levels of method validation (LLMV) were set at 0.05 ppm for fresh foliage, 0.10 ppm for dry foliage, and 0.05 ppm for seeds. Precision (repeatability and reproducibility) is one of the important parameters in the method validation. It determines the analysis deviation and is often used to evaluate the performance of the method in method validation. Repeatability is usually expressed as the relative standard deviation (RSD), calculated by (Flaschka 1969).

$$\text{RDS (\%)} = 100 \times (\text{Standard deviation}) \div (\text{Mean})$$

RSD indicates the variation in repetitive analyses under same experimental conditions. The relative standard deviation for reproducibility ($RSD_R$) reflects the variations under various conditions and indicating the degree of agreement with individual experimental results. In this study, the $RSD_R$ of the method was obtained by analyzing at least three replicates of MVSs that were prepared by spiking the control sample with various amounts of bifenthrin. The calculation of $RSD_R$ is in the same manner as that of RSD using the above equation. The results are given in Table 2. It was observed again that the $RSD_R$ for dry foliage (11 ± 6 %) and seeds (11 ± 5 %) were higher than that of fresh foliage (3.5 ± 1.9 %) at similar concentration levels due to the interferences of matrices. This meant that the deviations in results for dry foliage and seeds were higher. This shows the similar pattern to the results of LODs and LOQs (Table 1).

The recovery of spike (accuracy) is obtained by dividing the observed value by the true value of the analyte. The true value was the amount of bifenthrin added to matrix blank. The recovery tests were carried out on at least three replicates at

**Table 1**. Limit of detection (LOD) and limit of quantitation (LOQ).

| Matrix | Analysis Date | Spike conc. (ppm) | Observed conc. (ppm) | Recovery of spike (%) | LOD [b] (ppm) | LOQ [c] (ppm) |
|---|---|---|---|---|---|---|
| Fresh | 11-Apr-00 | 0.050 | 0.04558 | 90 | | |
| Foliage | 11-Apr-00 | 0.050 | 0.04607 | 91 | | |
| | 11-Apr-00 | 0.050 | 0.04179 | 83 | | |
| | 22-Apr-03 | 0.050 | 0.04941 | 98 | | |
| | 22-Apr-03 | 0.050 | 0.04775 | 95 | | |
| | 22-Apr-03 | 0.050 | 0.04706 | 93 | | |
| | | Mean ± SD [a]: | 0.0463 ± 0.0026 | 92 ± 5 | 0.0087 | 0.026 |
| Dry | 03-Jun-03 | 0.100 | 0.07774 | 77 | | |
| Foliage | 03-Jun-03 | 0.100 | 0.09812 | 98 | | |
| | 03-Jun-03 | 0.100 | 0.08468 | 85 | | |
| | 13-Jun-03 | 0.100 | 0.06571 | 66 | | |
| | 13-Jun-03 | 0.100 | 0.08350 | 83 | | |
| | 13-Jun-03 | 0.100 | 0.08077 | 81 | | |
| | | Mean ± SD [a]: | 0.0818 ± 0.0105 | 82 ± 11 | 0.0353 | 0.11 |
| Seeds | 23-Mar-03 | 0.049 | 0.05320 | 108 | | |
| | 23-Mar-03 | 0.049 | 0.03886 | 79 | | |
| | 23-Mar-03 | 0.049 | 0.05675 | 116 | | |
| | 02-Apr-03 | 0.049 | 0.04545 | 92 | | |
| | 02-Apr-03 | 0.049 | 0.05432 | 111 | | |
| | 02-Apr-03 | 0.049 | 0.04385 | 89 | | |
| | | Mean ± SD [a]: | 0.0487 ± 0.0070 | 99 ± 14 | 0.0236 | 0.071 |

[a] SD stands for standard deviation.

[b] Number of samples = 6, one-tailed t-statistic = 3.365; LOD = 3.365 × SD.

[c] LOQ = 3 × LOD.

each spike level. The average recovery of spike was 92%, 82%, and 99% (Table 1) and 93%, 84%, and 89% (Table 2) for fresh, dry foliages and seeds, respectively.

Each field trial set contained a control sample and a field trial sample. A concurrent quality assurance sample (QAS) was prepared per field set in the laboratory by adding a known amount of bifenthrin to a known amount of control sample of that field trial set. Finally, the control, field trial, and concurrent quality assurance were extracted in the same extraction batch and analyzed in the same GC sequence.

The analytical results of bifenthrin residue are given in Table 3. Three matrices of the CA trials and one matrix (fresh foliage only) of the TX and GA trials were analyzed in this study. Totally, 5 concurrent quality assurance samples were analyzed. The IR-4 protocol of application of bifenthrin in the fields was identical. It was observed that bifenthrin was found to be at various concentrations in field samples. This is probably due to the non-polar structures

**Table 2.** Relative standard deviations for reproducibility ($RSD_R$).

| Matrix | Replicate No. | Spike conc. (ppm) | Observed conc. (ppm) | Spike recovery (%) | $RSD_R$ (%) |
|---|---|---|---|---|---|
| | | | **Fresh foliage** | | |
| Control [a] | 3 | 0 | < LOD | n. a. | n. a. |
| MVS [b] | 3 | 0.050 | 0.0463 ± 0.0026 | 93 ± 5 | 5.6 |
| MVS [b] | 3 | 0.509 | 0.4790 ± 0.0153 | 94 ± 3 | 3.2 |
| MVS [b] | 3 | 4.87 | 4.4492 ± 0.0814 | 91 ± 2 | 1.8 |
| | | | Mean ± SD [c]: | 93 ± 2 | 3.5 ± 1.9 |
| | | | **Dry foliage** | | |
| Control [a] | 5 | 0 | < LOD | n. a. | n. a. |
| MVS [b] | 6 | 0.10 | 0.0818 ± 0.0105 | 81 ± 10 | 13 |
| MVS [b] | 3 | 1.00 | 0.7073 ± 0.1060 | 71 ± 10 | 15 |
| MVS [b] | 3 | 4.97 | 4.611 ± 0.640 | 99 ± 13 | 14 |
| | 3 | 19.9 | 15.36 ± 0.32 | 77 ± 2 | 2 |
| | | | Mean ± SD [c]: | 84 ± 14 | 11 ± 6 |
| | | | **Seeds** | | |
| Control [a] | 4 | 0 | < LOD | n. a. | n. a. |
| MVS [b] | 6 | 0.049 | 0.0488 ± 0.0071 | 99 ± 14 | 15 |
| MVS [b] | 3 | 0.409 | 0.3767 ± 0.0215 | 92 ± 5 | 5.7 |
| MVS [b] | 3 | 4.91 | 3.767 ± 0.521 | 77 ± 11 | 14 |
| | | | Mean ± SD [c]: | 89 ± 11 | 11 ± 5 |

[a] Control samples.

[b] MVS stands for method validation sample. An MVS was were prepared by adding known amount of bifenthrin to the control sample.

[c] SD standards for standard deviation.

resulting in low solubility in water and tendency to bind to crops. This is consistent to the FMC's report (1983) that bifenthrin is relatively stable to abiotic hydrolysis at pH 5-9 at 25 °C over a 30-day period. It was also observed that, for the CA field trials, the amount of bifenthrin residue in dry foliage was significantly higher than those in seeds and fresh foliage, i.e., dry (15.6 ppm) >> seeds (3.68 ppm) > fresh (2.73 ppm). This indicates that the matrix significantly affects the residue of bifenthrin in cilantro. This is not surprising because of the carboxylate ester linkage in bifenthrin which is usually a point of hydrolysis when water is present. In this case, dry foliage has the least water content and fresh foliage has the most water content. For the same matrix (fresh foliage), the amount of bifenthrin residue in the 3 field trial samples was slightly different from field to field, i.e., GA (3.96 ppm) > CA (2.73 ppm) > TX (2.21 ppm), probably due to the change in weather and/or geography. The average bifenthrin residue in fresh foliage was 3.0 ± 0.9 ppm, suggesting that the field trial results are reproducible in the study. For the concurrent QA samples, recoveries of spike were in satisfactory ranges indicating that results in this study be acceptable. Based on the results obtained in this study, it can be concluded that the method presented in this work is a rapid, efficient, and accurate method for measuring the pesticide, bifenthrin in the fresh, dry cilantro foliages and cilantro seeds.

**Table 3.** Residues in control, field trial, and concurrent QA samples. [a]

| Field | Sample | Bifenthrin concentration (ppm) | | |
|---|---|---|---|---|
| | | Matrix | | |
| | | Fresh foliage | Dry foliage | Seeds |
| CA | Control: Observed | < LOD | < LOD | < LOD |
| | Field: Observed | 2.73 | 15.6 | 3.68 |
| | QAS: Spiked | 0.50 | 4.95 | 0.41 |
| | spike recovery | 81% | 76% | 71% |
| TX | Control: Observed | < LOD | - | - |
| | Field: Observed | 2.21 | - | - |
| | QAS: Spiked | 0.50 | - | - |
| | spike recovery | 69% | - | - |
| GA | Control: Observed | < LOD | - | - |
| | Field: Observed | 3.96 | - | - |
| | QAS: Spiked | 0.50 | - | - |
| | spike recovery | 86% | - | - |

[a] At least 2 parallel runs per sample were performed. Mean values are given.

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