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ChemTech

International Journal of ChemTech Research
CODEN( USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 908-911, April-June 2010

# Determination of Cypermethrin and Chlorpyrifos in Vegetables by GC-ECD

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**Abstract:** Extensive use of pesticides on vegetables started gaining momentum and continued its up-trend in India .The use of pesticides in agriculture concern of residue accumulation, which may remain in the food and agricultural environment causing human health and damaging ecological balance. This study reported is based on the determination of pesticide residue in cauliflower and brinjal. Cauliflower and brinjal were purchased from local market and analysed for their residual contents of chlorpyrifos and cypermethrin. The pesticide residues were extracted from the cauliflower and brinjal with ethyl acetate and cleaned-up with ethyl acetate and hexane (3:7 v/v) mixture using florisil and charcoal column and determination was carried out on GC-ECD. Recoveries of these residues are over 90 % with coefficient of variation below 5 %. The method is suitable for the analysis of in cauliflower and brinjal with high sensitivity and accuracy.

**Key Words**: Chlorpyrifos, Cypermethrin, GC-ECD.

## 1. Introduction

In modern day agricultural practices, the use of pesticides provides unquestionable benefits by increasing the production of crops. However, it has the drawback of pesticide residues which remain on the vegetables, constituting potential health risks to consumers. This leads on the one hand to the establishment of legal directives to control their levels through the maximum residue levels (MRLs) and on the other to continue search for pesticides, which are less persistent and less toxic for human beings (1). The cauliflower is low in fat, high in dietary fiber, contains water and vitamin C, possessing a very high nutritional density. In India context, the cauliflower and brinjal growers have been using pesticides frequently to have the higher yield. But the overdose of pesticides makes the residue problem, which might pollute our food and be harmful for our health. It has been reported that some of the pesticides are being used in the country where no pre-harvest time frame after application is maintained. What is most alarming is that pesticides use is very indiscriminate in India. There are areas where pesticides are used in excessive quantities, in such situation make monitoring and assessment of pesticides contamination very difficult. Therefore, pesticides residue is becoming a major food safety concern of consumers and government. Analytical instrument are needed to determine, quantify and confirm pesticide residues in vegetables for both research and regulatory purposes. The pesticides are generally analysed by spectrophotometry [2,3,], thin layer chromatography (TLC) [4,5,6], high performance liquid chromatography (HPLC) and high performance liquid chromatography-mass spectrophotometry (HPLC-MS) [7,8,9], gas chromatography (GC) [10,11,12,13] and GC-MS [14]. In the present study, a method employing GC equipped with ECD detector for the separation, identification and quantification of two widely used pesticides on cauliflower and brinjal were developed and validated. The compounds studied were cypermethrin and chlorpyrifos. Finally, the method was applied to the determination of these pesticides in commercial samples collected from the

local markets. Therefore, the purpose of this study was to develop an analysis scheme for determination of these pesticides in cauliflower and brinjal by GC-ECD.

## 2. Experimental

### 2.1 Extraction

The fresh cauliflower and brinjal samples were taken for the extraction of pesticide residues. Each vegetable was chopped into small pieces; a representative sample (50gm) was macerated with 5-10gm anhydrous sodium sulphate in blending machine to make fine paste. The macerated sample was extracted with 100 ml of ethyl acetate on mechanical shaker for 1 h; extract was filtered, concentrated up to 5 ml on rotary evaporator and finally injected into GC-ECD.

### 2.2 Sample clean up

The clean-up of cypermethrin and chlorpyrifos was carried out by using column chromatography. Column (60cm x 22mm) was packed with, Florisil and activated charcoal (5:1 w/w) in between the two layers of anhydrous sodium sulphate. Extract was eluted with 125 ml mixture of ethyl acetate and hexane (3:7 v/v). Elute was concentrated to 5 ml on rotary evaporator.

### 2.3 Chemical and reagents

The organic solvent ethyl acetate and hexane used were HPLC grade and purchased from E Merck. Technical grade pesticide standards were used for standardisations. The standards were stored in a freezer at -5$^{o}$C. Anhydrous sodium sulphate (AR) from E Merck used for residue extraction was maintained at 300$^{o}$C overnight.

### 2.4 Standard preparation

For preparation of stock solution, standards were dissolved in ethyl acetate and four levels of intermediate standard solution of each pesticide were prepared maintaining the same matrix concentration for the preparation of calibration curve and stored at 4$^{o}$C in the dark .Working solutions were prepared daily by appropriate dilution with ethyl acetate.

### 2.5 GC-ECD System

The cleaned extract were analysed on Hewlett Packard 5890A GC equipped with capillary column using $Ni^{63}$ electron capture detector (ECD). The separation of pesticides was done in a 30 meter length, 0.25 mm internal diameter and 0.25 µm film thickness coated with 5% diphenyl - 95% methylpolysiloxane HP-5MS column. Helium was used as the carrier gas at 9.6 psi pressure and 1 ml min$^{-1}$ flow. The injector was used at constant temperature and 280$^{0}$C, the detector temperature was 300$^{0}$C. The initial oven temperature was 110$^{0}$C (3 min isothermal) to 275$^{0}$C (at 10$^{0}$C min$^{-1}$) isothermal for 15 minutes. The injection volume was 1µl.

## 3. Results and Discussion

### 3.1. Identification and quantification

The compound was identified by comparing its retention time with respect to technical grade reference standard. The quantitative determination was carried out with the help of a calibration curve drawn from chromatographic experiments with standard solution. For quantification an external calibration curve with four different concentrations of each pesticide, with matrix matching were made. The standard solutions for the calibration curves were prepared in control matrix because samples may possess co-extractants in the matrix which may affect the peak area of the unknown samples.

### 3.2 Limit of detection and limit of quantification

The limit of detection (LoD) was calculated from the peak intensity at 0.01mg/kg and blank in recovery tests. LoD was defined as S/N>4 so that it is in the linear range of the standard calibration. The LoD of chlorpyrifos and cypermethrin are 0.005 mg/kg. LoQ was obtained for chlorpyrifos and cypermethrin was 0.02 mg/kg. Linear calibration curves were found between peak areas and analyte concentration in the whole range of studies. The linear regression ($y = a + bx$) parameters for method calibration were taken (table 1). The determination coefficients ($R^2$) of analytical curves were near 0.99, with linearity for each compound, which allows the quantitation of these compounds by the method external standardization.

### 3.3 Recovery

Recovery studies were performed to examine the efficacy of extraction and clean up. Untreated cauliflowers and brinjal were spiked with known concentration of the pure insecticides standard solution of each of type of pesticide and extraction and clean-up were performed as described earlier. The concentration of each pesticide in the final extracts was calculated (table 2).

## 4. Application to actual samples

Samples were obtained at a local market and residual pesticides were determined by the method proposed in this study and extraction and clean-up were performed as described earlier.

## Conclusion

The cauliflower and brinjal growers have been using the pesticides frequently to have the higher and insect free yield. But the overdoses of pesticides make the residue problem, which might pollute our food and environment. The pesticides residues can be decreased if recommended dose applied by the vegetable growers.

**Table 1 Retention time and recovery of chlorpyrifos and cypermethrin**

| Compound | RT (min) | Calibration range mg/kg | Correlation Coefficient | Coefficient of variation (n = 5) % |
|---|---|---|---|---|
| Chlorpyrifos | 12.969 | 0.02-1.00 | 0.991 | 5.8 |
| Cypermethrin | 19.728 | 0.02-1.00 | 0.988 | 6.0 |

**Table 2 Recovery of pesticides in the spiked samples**

| Sample | Compound | Concentration (mg/kg) | Recovery (%) | Coefficient of Variation (n = 5) % |
|---|---|---|---|---|
| Cauliflower | Chlorpyrifos | 50 | 90.80 | 4.58 |
| Cauliflower | Cypermethrin | 50 | 91.80 | 3.90 |
| Brinjal | Chlorpyrifos | 50 | 91.50 | 4.40 |
| Brinjal | Cypermethrin | 50 | 91.20 | 4.25 |

**Table3: Amounts of pesticides residue detected in cauliflower and brinjal samples.**

| Sample | Chlorpyrifos (mg/kg) | Cypermethrin (mg/kg) |
|---|---|---|
| Cauliflower | nd | nd |
| Cauliflower | 0.024 | nd |
| Cauliflower | nd | 0.002 |
| Cauliflower | 0.027 | nd |
| Cauliflower | nd | nd |
| Brinjal | nd | 0.012 |
| Brinjal | 0.021 | nd |
| Brinjal | nd | 0.008 |
| Brinjal | 0.018 | 0.003 |
| Brinjal | 0.020 | nd |

* nd = non detected

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