



## Development of Method for Determination of Pesticide Residues from Chilly and Ladyfinger by Using HPLC and GC-MS

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### Abstract

*This article describes an efficient and effective analytical method to screen pesticide in fruits and vegetables samples using HPLC and GC-MS. Determining the pesticide residue of endosulfan, Carbendazim & Chlorpyrifos in chilly and ladyfinger samples in various area of Nashik, Maharashtra Nashik. A quick, easy, cheap, effective, rugged and safe (QuEChERS) method with acetate buffering was used for samples preparation. Sample is chopped and homogenize with acetonitrile, dried with sodium chloride, dehydrated using sodium sulphate and centrifuged at high speed. Pesticide is eluted using rotary evaporator.*

**Keywords:** Pesticide Residue, Pesticide, HPLC, GC-MS, QuEChERS

### Introduction

Chilly and ladyfinger (Bhindi) are the higher consumption in the Indian Market, These vegetables have agreeable flavor. There are many variables that influence the chemical composition of chilly and ladyfinger, such as methods of cultivation, use of fertilizers and pesticides, quality of water for irrigation or storage and commercialization.

Organophosphate and Carbamate are systemic pesticides widely used in agriculture for pre- and post-harvest treatment in the globe for control of wide range of pests and insects. Simultaneous and consecutive analytical method for pesticide residue in large number of food samples using acetonitrile extraction followed by GC<sup>8</sup>, LC-MS<sup>9</sup> was investigated.

In the present work a chromatographic methodology was used to identify pesticide residue in different samples of chilly and ladyfinger produced in various locations in Nashik district (Maharashtra). Three samples were collected of each from the field at nearby villages

Dindori, Satana and Wani, and were analyzed in laboratory. The simultaneous determination for endosulfan, Carbendazim, and Chlorpyrifos residue was carried out by HPLC-UV and GC-MS.

This is very unfortunate that all farmers do not follow legal pesticide. Due to tremendous number of pesticide and crops in production lots of analytical methods are designed to determine multiple pesticide residues (Fillion *et al.*, 2000; Sheridan and Meola 1999; Lehotay, 2000; General Inspectorate for Health Protection, 1996; Food and Drug Administration, 1999; Luke *et al.*, 1975; Specht and Tilkes, 1980; Lee *et al.*, 1991; Anderson and Palsheden, 1991; Cook *et al.*, 1999; Lehotay, 2000). In 2003, the QuEChERS



method for pesticide residue analysis was introduced (Anastassiades *et al.*, 2003), which provides good quality results in a fast, easy and inexpensive approach. The QuEChERS approach has been extensively validated for hundreds of pesticides residues in many types of foods, and has become Association of Analytical Communities (AOAC) official method 2007.01 (Lehotay *et al.*, 2007) and CEN (2008).

A critical review of literature showed that different solvents such as n-hexane, petroleum ether, methyl chloride and acetone or ethyl acetate have been used for extraction of pesticide residue from fruits (Coulston and Korte, 1975). Many workers used acetonitrile for extraction of pesticides from fruits instead of Methylene chloride, which is hazardous to the environment (Cook *et al.*, 1999; Krause and August, 1983).

## Methodology

### Sampling

Fresh samples of chilly and ladyfingers were collected from various fields in local area Dindori, Wani, Satana of Nashik. Samples were kept and wrapped in clean paper bag. Small sachet of silica gel helps to keep away it from moisture. A total of three different samples of each vegetable were collected for the analysis. Their detection and quantification by different analytical techniques are the major steps involved in pesticide residue analysis.

Samples are categorized as follows with respect to their area of growing.

	Chilly	Ladyfinger
Sample No.1	Satana	Wani
Sample No.2	Wani	Dindori
Sample No.3	Dindori	Satana

### Reagents and Materials

Water and Acetonitrile were HPLC grade; Analytical grade Dimethyl formamide; anhydrous sodium sulphate were obtained from Fischer-Scientific. Acetic acid and sodium acetate from Merck were used for sample preparation. Analytical grade pesticide standards were obtained from Sigma-Aldrich.

Crystalline standards were dissolved in acetonitrile for preparation of stock solution of standards. A standard mix solution was prepared from the individual stock solution to yield 10 mg/ml.

### Sample Preparation

The acetate buffered sample preparation method for pesticide was applied to all samples. 50g samples were chopped and crushed in household grinder equipped with stainless steel blades and homogenize with 100 ml acetonitrile. Then 10 g of sodium chloride is added to it. Then, 6 g NaSO<sub>4</sub> were added to absorb moisture and shaken well. The extract was centrifuged at 5000 rpm for six minutes. Then ~15ml of the



supernatant were filtered through a 0.45 mm PTFE filter. Pesticide was eluted with 20 ml acetonitrile. Sample was concentrated using a rotary evaporator.

#### HPLC Condition

Analytical Technologies 3000 series HPLC having UV/visible detector was used for identification and quantification of pesticides. Separation was performed on C18 (4.6ID x 250mm) column. Samples were injected manually through Rhyodyne injector. Detector was connected to the computer for data processing. The working condition of HPLC was binary gradient, mobile phase was acetonitrile: water (70:30), flow rate was 0.8ml/min, injection volume 20  $\mu$ l, pressure 6-7 MPa and the wavelength of the detector was fixed at 254 nm for the residual analysis of three pesticides endosulfan, Carbendazim & Chloropyrifos.

#### GC Condition

Analytical Technologies GC 2012 model having FID detector was used for identification and quantification of pesticides. Separation was performed on Carbowax Packed column (SS 2meters). Samples were injected manually through Rhyodyne injector. Detector was connected to the computer for data processing. Following temperature conditions were maintained: Constant flow rate 1.0 ml/min, injection volume 1.0  $\mu$ l, injector temperature 100 ° C, Oven temperature 110 ° C, Detector temperature 100 ° C. Total MS running time 20 min. The individual constituents showed by GC were identified by comparing their MS with standard compounds of NIST library.

#### Results and Discussion

The QuEChERS method is believed to give best results. Advance chromatographic techniques i.e. HPLC and GC-MS are supposed to be best for determination of pesticide residue, The least effort give excellent qualitative and quantitative results.

**Identification and quantification by HPLC:** HPLC method for the identification of pesticide residues and the chromatographic separations of Retention time (Rt), Endosulfan (Rt-2.250), Chloropyrifos (Rt-3.208) and Carbendazim (Rt-5.050). (Paranthumet *al.* 2012). Their standards are shown in fig.-1 and Table-1.

The pesticide residues present in the chilli and ladyfinger samples were identified and quantified with reference to standard pesticides. The calculation of the amount of the pesticides present was carried out by comparing the peak areas for unknown samples with the corresponding peaks for standards, according to established procedure.

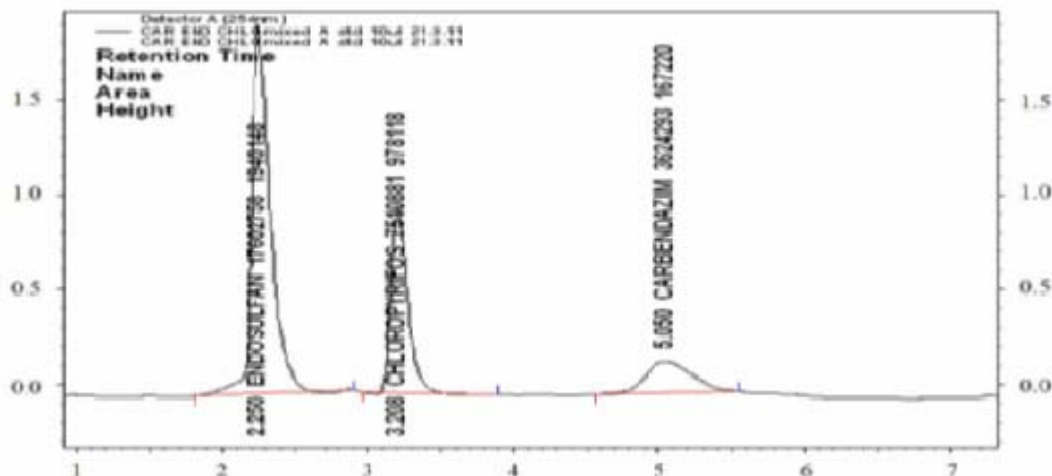


Fig. 1: Standard chromatogram of pesticides standard

Table 1: HPLC validation data for pesticides standard

Peak#	Time	Conc. ( $\mu\text{g}/\mu\text{l}$ )	Area
1	2.250	10.000	17602758
2	3.208	10.000	7510881
3	5.050	10.000	3624293

Based on the HPLC results endosulfan is finding in Chilli of satana ( $0.4 \mu\text{g}/\mu\text{l}$ ), wani ( $0.09 \mu\text{g}/\mu\text{l}$ ).Chloropyrifos is finding in wani ( $0.08 \mu\text{g}/\mu\text{l}$ ) and in Dindori ( $0.003 \mu\text{g}/\mu\text{l}$ ).The carbendazim is finding in chilli of Dindori ( $0.79 \mu\text{g}/\mu\text{l}$ ). Endosulfan in Ladyfinger of Dindori is ( $0.6 \mu\text{g}/\mu\text{l}$ ) and in Satana ( $0.95 \mu\text{g}/\mu\text{l}$ ).Carbendazim is finding in dindori ( $0.074 \mu\text{g}/\mu\text{l}$ ), in Wani ( $0.6 \mu\text{g}/\mu\text{l}$ ) and satana ( $0.036 \mu\text{g}/\mu\text{l}$ ). Chloropyrifos is finding in only one sample of ladyfinger in Dindori( $0.16 \mu\text{g}/\mu\text{l}$ ).

## Conclusion

In this study the HPLC and GC-MS multiresidue method used to determine pesticides in chilly and ladyfinger samples in less time and low detection limit. The results of our monitoring indicate that, among 3 samples of each chilli and ladyfinger that were examined; not even single sample contained Chloropyrifos or endosulfan that not exceeded the FAO/WHO Codex Alimentarius Maximum Residue Limits (MRLs)<sup>9</sup> (Codex Alimentarius Commission, 1996). In addition, the obtained results clearly indicate the actual situation of the misuse of insecticides which may affect in turn at long period the consumers health.

For determination and analysis using HPLC and GC-MS the solvent used acetonitrile is best solvent among the ethyl acetate, DMF, toluene and acetone as almost all organic compound are soluble in it and it is good mobile phase hence generally used as mobile phase in HPLC and GC or GC-MS.



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