American Journal of Biochemistry and Biotechnology 8 (1): 1-6, 2012
ISSN 1553-3468

Determination of Pesticide Residues in Banana by Using High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry

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Abstract: Problem statement: The occurrence of endosulfan, carbendazim, chloropyripos in 10 banana samples in southern area of Tamilnadu, India (hill banana, karpuravalli, monthan, nendran, ney poovan, pachanadan, poovan, rasthali, red banana, robusta) was investigated. **Approach:** In 7 samples, Carbendazim was found at concentrations ranging from 0.002-0.11 mg kg^{-1}. In three samples, carbendazim was not found, whereas endosulfan, chloropyripos was not detected in any sample. **Results:** Analysis was carried out using HPLC-UV and samples were confirmed by GC-MS. The seven samples contained carbendazim that not exceeded the FAO/WHO codex alimentarius standards for MRLs (Maximum Residue Limit) values of carbendazim pesticide on banana (whole) is 1.0 mg kg^{-1}. **Conclusion/Recommendations:** Based on the HPLC results carbendazim is finding in Hill banana (0.007 mg kg^{-1}), Monthan (0.019 mg kg^{-1}), Nendran (0.002 mg kg^{-1}), Pachanadan (0.007 mg kg^{-1}), Poovan (0.016 mg kg^{-1}), Rasthali (0.017 mg kg^{-1}) and Robusta (0.11 mg kg^{-1}) and carbendazim is not finding Karpuravalli, Ney poovan and Red banana. Endosulfan, Chloropyrifos and Carbendazim in Robusta Banana sample are identified by matching their retention times and characteristic ion. TIC chromatogram for a positive Robusta Banana sample.

Key words: Gas Chromatography-Mass Spectrometry (GC-MS), commercialization conditions, important components, Gel Permeation Chromatography (GPC)

INTRODUCTION

Banana (*Musa paradisiacal L.*) is the fruit with the highest consumption in the Indian market. It is an excellent tropical fruit, has an agreeable flavour and a high nutritional value. The contribution to the intake of sugars, fibre, vitamins and minerals from the consumption of bananas is high, with a very low contribution to the intake of fat. It is one of the most important components of the human diet in different countries, where it is consumed in its raw form, home-cooked or processed as juice or paste. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease. There are many variables that influence the chemical composition of bananas, such as methods of cultivation, fertilizers and pesticides used quality of water for irrigation, or storage and commercialization conditions. Benzimidazole fungicides are systemic pesticides, widely used in agriculture for pre- and post-harvest treatment for the control of a wide range of fruit and vegetable pathogens (Papadopoulou-Mourkidou, 1991).

Simultaneous and consecutive analytical methods for pesticide residues in large numbers of food samples using acetonitrile extraction, followed by Gel Permeation Chromatography (GPC) and mini cartridge column cleanup and then dual-column GC equipped with ECD was investigated by (Ueno *et al*., 2004). In the present work, a chromatographic methodology was used to identify pesticide residues in different banana samples produced in Tamilnadu. Ten banana samples were collected market, Trichy, Tamilnadu, India and were analyzed in our laboratory. The simultaneous determination of Endosulfan, carbendazim and Chlorpyrifos residues was carried out by HPLC-UV and GC-MS.

MATERIALS AND METHODS

Sampling: Fresh samples of banana fruits were collected from market (Gandhi market, trichy). A total of 10 varieties of banana samples were collected for the analysis.

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Methodology employed for pesticide residue analysis: Extraction of pesticide residues from variety of bananas with different solvents and their detection and quantification by different analytical techniques are the major steps involved in pesticide residue analysis.

HPLC analysis: 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) in the present study, for the extraction of pesticide residue from banana fruit samples the *et al* method was followed with little modification.

Sample preparation: Homogenize 50 g chopped sample with 100 mL acetonitrile. Add 10 g sodium chloride (= 8mL in a graduated cylinder). Homogenize 5 min. Transfer ~13 mL of acetonitrile (top) layer to 15mL graduated centrifuge tube. Add ~3g sodium sulfate (liquid level to 15 mL mark), cap, shake well to remove water. Centrifuge at high speed for 5 min. Transfer 10 mL aliquot (= 5g of sample) to a clean 15 mL tube. Evaporate to 0.5mL under clean nitrogen. Elute pesticides with 20 mL acetonitrile/toluene (3:1). Using a rotary evaporator, concentrate sample to ~2 mL. Add 2×10 mL acetone, concentrating the material to ~2mL after each addition, to make a solvent exchange to acetone. Transfer quantitatively to a clean 15 mL tube.

HPLC conditions: A Shimadzu CLASS-VP V6.13 SP2 Area% Report, Version 5.22 High performance liquid chromatography having UV/visible detector was used for identification and quantification of pesticides. Separation was performed on Luna C18 Colum. Samples were injected manually through a Rheodyne 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 1 mL min-1, injection volume was 20 µL and the wavelength of the UV/visible detector was fixed at 254 nm for the residual analysis of three pesticides chlorpyrifos carbendazim and endosulfan.

GC-MS analysis:

Sample preparation: Before the extraction process and preconcentration, the samples were made into a concentrate juice (50% w/v) in distillated water. Then, fresh juice was centrifuged at 4000 r.p.m. for 15 m and the supernatant liquid portion was filtered through a 0.45 µm nylon filter. After filtration, the juice was diluted with a water-methanol mixture (1:1% v/v) and stirred by 2 h in a screw cap vial containing small magnetic stirrer bar. The combined extracts were transferred to a separating funnel.

The filtrate solution was percolated on cartridge C18 (at a descending flow rate of 2 mL min^{-1}). Elution was performed with 5 mL of ethyl acetate. Then, the sample was concentrated to dryness by evaporation under inert conditions at constant flow rate of the N_2 gas. The final residue was dissolved in 1 mL of acetonitrile water (4: 1% v/v) at pH 4. 1 µL of injection volume was used for the chromatographic system.

GC-MS conditions: GC analysis (Steven *et al*., 2005) was conducted on a GC Perkin-Elmer-Clarus-500 plus MS Perkin- Elmer-Clarus-500 (Column: Elite-1 (100% Dimethyl poly siloxane), 30×0.25 mm × 1µmdf, with the following conditions: He constant flow, 1.3 mL/min; initial inlet temperature, 80°C ramped to 280-200°C/min after a 30 s delay; injection volume, 5 mL (LVI) onto a Carbofrit plug in the liner with an open purge valve (30:1 split ratio) for 24 s, closed until 3.5 min and open again (30:1) until the end of the run; oven temperature program, 75°C for 3 min, then 25°C/min ramp to 180°C followed by a 5°C/min ramp to 300°C and held for 3 min (total run time: 34.2 min). Mass detector: Turbo mass gold-Perkin Elmer Software: Turbomass 5.4.2, The MS instrument transfer line temperature was 240°C, with 230°C ion trap and 120°C manifold temperatures. MS Programme: Library used: NIST Version-Year 2005, Inlet line temperature: 200°C, Source temperature: 200°C, Electron energy: 70 eV, Mass scan: (m/z): 45-450, Total MS running time: 36 min. The individual constituents showed by GC were identified by comparing their MS with standard compounds of NIST library.

RESULTS

Both the chromatographic techniques i.e., High Performance Liquid Chromatography (HPLC) for all samples and Gas chromatography-Mass Spectrometry (GC-MS) for one sample were used for the determination of pesticides in fruit samples in the present study.

Identification and quantification by HPLC: RP-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) and their standards have been shown in Fig. 1 and Table 1.

The pesticide residues present in the banana 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 procedures.

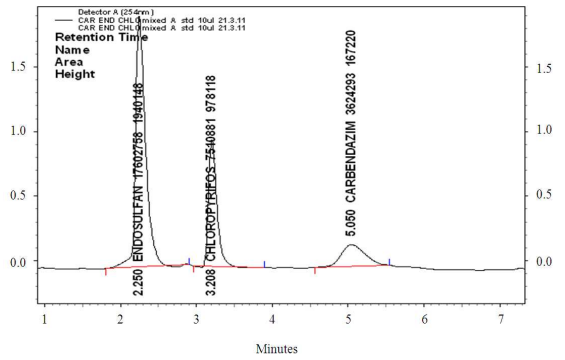


Fig. 1: Standard chromatogram of pesticides standard

Table 1: HPLC validation data for pesticides standard

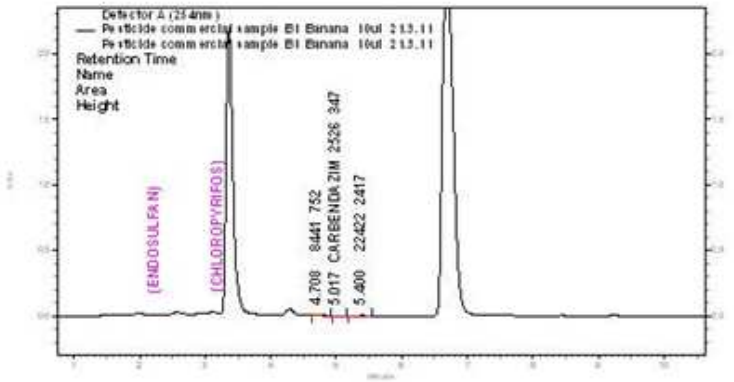
	Detector A (254nm)					
PK #	Retention Time	Area	Height	Name	ESTD Concentration	Units
1	2.250	17602758	1940148	ENDOSULFAN	10.000 CAL	µg/µL
2	3.208	7510881	978118	CHLOROPYRIFOS	10.000 CAL	µg/µL
3	5.050	3624293	167220	CARBENDAZIM	10.000 CAL	µg/µL

Table 2: HPLC Validation data for Pesticides residues in Banana sample

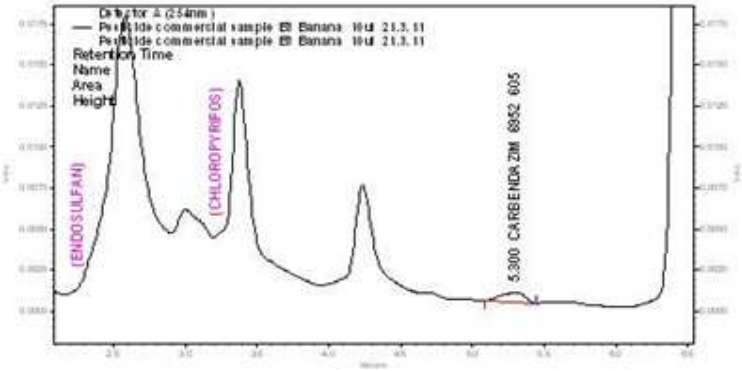
	Detector A (254 nm)			
Sample	Retention time	Area	Height	Concentration (mg/kg)
Hill banana	5.017	2526	347	0.007
Karpuravalli	Not detected	Not detected	Not detected	Not detected
Monthan	5.300	6952	605	0.019
Nendran	5.250	750	100	0.002
Ney poovan	Not detected	Not detected	Not detected	Not detected
Pachanadan	5.125	2628	227	0.007
Poovan	4.992	5717	523	0.016
Rasthali	4.992	6196	511	0.017
Red banana	Not detected	Not detected	Not detected	Not detected
Robusta	5.158	3914	317	0.11

Table 3: GC-MS Validation data for pesticides residues in robusta banana sample

Sample analyzed	Compound name	Mass number referred	Result
Robusta Banana	Endosulfan	195, 241, 265, 339, 406	Absent
	Carbendazim	105, 132, 146, 159, 191	Present
	Chloropyripos	97, 125, 1 97, 314, 349	Absent



(a)



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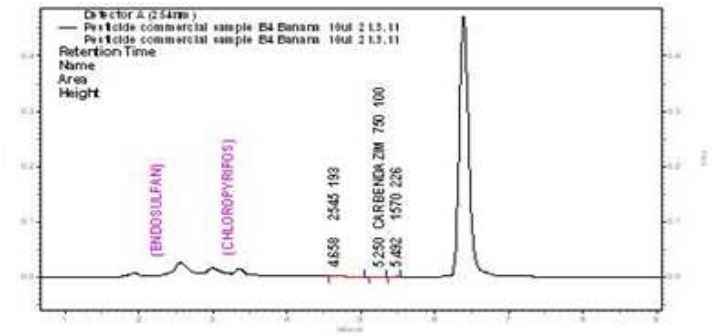

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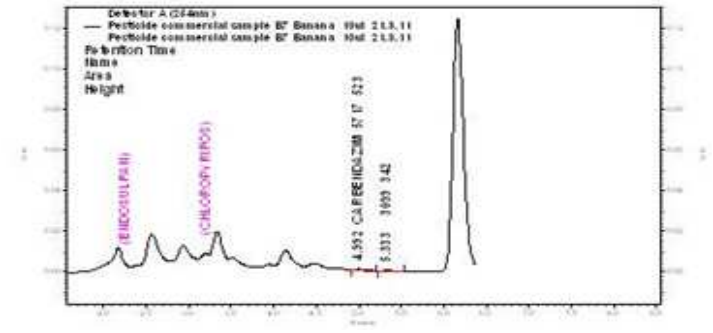

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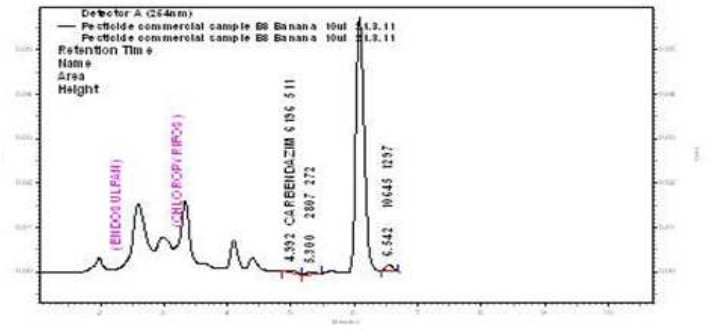

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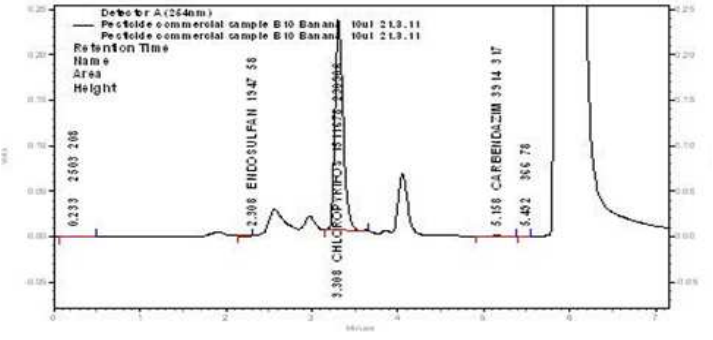

(f)

Fig. 2: HPLC Chromatogram of Pesticides residues in Banana sample (a) hill banana sample (b) monthan banana sample (c) pachanadan banana sample (d) Poovan banana sample (e) Rasthali banana sample (f) Robusta banana sample

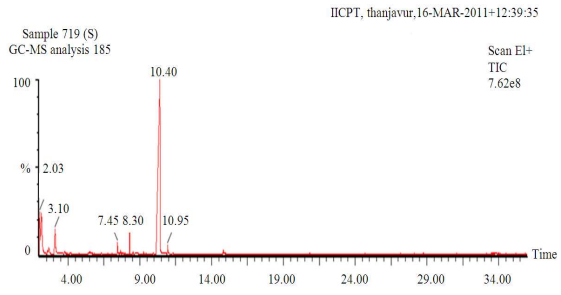

Fig. 3: GC-MS Chromatogram of Robusta Banana sample

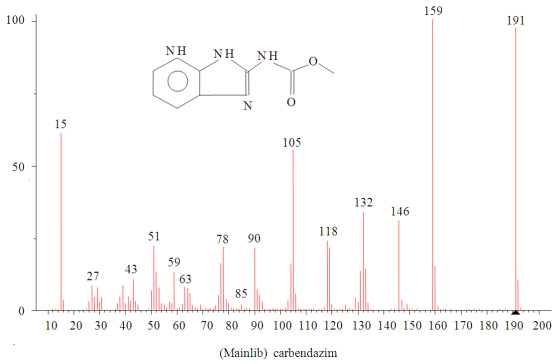

Fig. 4: GC-MS Spectrum of robusta banana sample

The content of each pesticide was calculated from the corresponding calibration curve and presented as the mean of five determinations as shown in Table 1. The HPLC Result based on the Retention time (Rt), Carbendazim (Rt-5.050) content in banana samples was given in Fig. 2a-f, Carbendazim was detected and the amount was below the MRL value (1 mg kg^{-1}) in the 10 samples. Based on the the HPLC results carbendazim was found in Hill banana (0.007 mg kg^{-1}), Monthan (0.019 mg kg^{-1}), Nendran (0.002 mg kg^{-1}), Pachanadan (0.007 mg kg^{-1}), Poovan (0.016 mg kg^{-1}), Rasthali (0.017 mg kg^{-1}) and Robusta (0.11 mg kg^{-1}) and carbendazim was not found Karpuravalli, Ney poovan Red banana given in Table 2.

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Gas chromatography-mass spectrometry (GC-MS): Endosulfan, Chloropyrifos and Carbendazim in Robusta Banana sample were identified by matching their retention times and characteristic ions with those of standards given in Table 3. A GC-MS-TIC chromatogram for a positive Robusta Banana sample is reported in Fig. 3 and 4.

DISCUSSION

The pesticides were identified by comparing its retention time with respect to technical grade reference standards. The quantitative determination was carried out with the help of a calibration curve drawn from chromatographic experiments with standard solution of pesticides. The main problem faced was resolving the pesticide peaks from possible interfering coextractives from sample matrices. For this reason, in the process of development of GC methods we were looking for high resolution of chromatographic peaks and to reach lower limits of detection. Selective and sensitive detectors, as in ECD, provided good responses even to very low concentrations. In many cases MS detection has been employed for quantitation in our work, however, we only performed MS for confirmation of peak identity.

CONCLUSION

In this study the HPLC and GCMS multiresidue method used to determine pesticides in banana samples less time and low detection limit. The results of our monitoring indicate that, among 10 samples of bananas that were examined, only seven samples contained carbendazim that not exceeded the FAO/WHO Codex Alimentarius Maximum Residue Limits (MRLs) (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.

ACKNOWLEDGMENT

The reaches deeply thanks the Dr.K. Alagusundaram, Director, Indian Institute of Crop Processing Technology, Ministry of Food Processing Industries, Government of India, Thanjavur for Providing all the facilities and support used to carry out the work.

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