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Determination of pesticide residues in vegetables by gas chromatography - Mass spectrometry

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ABSTRACT

Gas chromatography-mass spectrometry method is successfully developed for the determination of pesticide residues monocrotophos, chlorpyriphos, and endosulfan in cauliflower and capsicum. The samples were extracted with 1% acetic acid in acetonitrile mixture and cleaned up with primary secondary amine (PSA) and magnesium sulphate. Experiments on two fortification concentrations are carried out, and the limits of detection are 0.005, 0.003, and 0.004 mg kg^{-1} for monocrotophos, chlorpyriphos, and endosulfan respectively. The average recoveries of pesticide residues in cauliflower and capsicum samples are 90.0 to 105.0 %.

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KEYWORDS

Monocrotophos;
Chlorpyriphos;
Endosulfan;
GC-MS.

INTRODUCTION

India is produces about 109 million metric tones of vegetables and it is the second largest producer after China, and accounts for 13.4% of world production. Surveys carried out by institutions spread throughout the country indicate that 50-70% of vegetables are contaminated with insecticide residue (1). The use of pesticides to control pest and diseases are a common practice in the fields to increase crop yield. However, these chemicals can reach plant tissues, leaving residues that can be detected in the vegetables. This may become a significant route to human exposure to these toxic compounds. In order to protect consumer's health, maximum residue levels (MRLs) in these vegetables have been established in different countries and internationally by Codex Alimentarius. The high number of pesticides to be monitored in those matrices, along with the typically low concentrations of the MRLs, requires highly sensitive and selective methods. Consequently, sample preparation becomes a key step of the analytical procedure. In recent times, extensive efforts have been made to the development of new sample preparation techniques that save time, labor and solvent consumption to improve the analytical performance of the procedure. 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[2-4], thin layer chromatography (TLC)[5-7], high performance liquid chromatography (HPLC)[8-10], gas chromatography (GC)[11-16] and GC-MS[17-22]. The present study describe method of extraction, cleanup and determination of a pesticides by using gas chromatography (GC) equipped with mass detector (MS) for the separation, identification and quantification of monocrotophos, chlorpyriphos and endosulfan on cauliflower and capsicum were developed and validated. 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 the determination of these pesticides in cauliflower and capsicum by GC-MS.

EXPERIMENTAL

Chemical and reagents

The organic solvent acetic acid, magnesium sulphate HPLC grade and purchased from E Merck and primary secondary amine purchased from Agilent Techonlogies. The technical grade pesticide standards were used for standardizations. The standards were stored in a freezer at -5^{o}C. Anhydrous magnesium sulphate used during residue extraction was maintained at 300^{o}C overnight and kept in air tight container.

Sample preparation and clean up

The fresh cauliflower and capsicum 2 kg each samples were taken for the extraction of pesticide residues. After homogenization with a house-hold mill (equipped with stainless steel knives), a 15 g portion of the homogenized sample was weighed into a 50 ml polytetrafluoroethylene (PTFE) tube added 15 ml of acetonitrile containing 1% acetic acid (v/v not accounting for purity). Then, 6 g MgSO4 and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of anhydrous form) were added, and the sample was shaken forcefully for 4 min and kept in ice bath. The sample was then centrifuged at 4000 rpm for 5 min and 6 ml of the supernatant were transferred to a 15 ml PTFE tube to which 900 mg MgSO4 and 300 mg PSA were added. The extract was shaken using a vortex mixer for 20 s and centrifuged at 4000 rpm again for 5 min., approximately 2ml of the supernatant were taken in a vials. This extracts were evaporated to dryness under a stream of nitrogen and reconstituted in n-hexane in auto sampler tube for the GC-MS analysis.

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.

Instrumentation

GC–MS analysis was performed with a Varian 3800 gas chromatograph with electronic flow control (EFC) and fitted with a Saturn 2200 ion-trap mass spectrometer (Varian Instruments, Sunnyvale, CA, USA). Samples were injected into a Varian 8200 auto sampler SPI / 1079 split / splitless programmed-temperature injector using a 10µl syringe operated in the large volume injection technique. The glass liner was equipped with a plug of carbofrit (Resteck, Bellefonte, PA, USA). A fused-silica untreated capillary column 2 m 30.25 mm I.D from Supelco (Bellefonte, PA, USA) was used as a guard column connected to a Rapid-MS [wall-coated open tubular (WCOT) fused-silica CP-Sil 8 CB low bleed of 10m 30.53 mm I.D, 0.25 mm film thickness] analytical column from Varian Instruments (Sunnyvale, CA, USA) for high speed analysis. The mass spectrometer was operated in electron impact (EI) ionization mode. The computer that controlled the system also held a GC-MS library specially created for the target analytes under our experimental conditions. The mass spectrometer was calibrated weekly with perfluoro-tributylamine. Helium (99.999%) at a flow-rate of 1 ml min^{-1} was used as carrier and collision gas.

Instrumental conditions

Sample aliquots of 1.0 µl were injected into the GC operating at a syringe injection flow-rate of 10 ml s^{-1}. The initial injector temperature of 70^{0}C was held for 0.5 min and then increased at 100^{0}C min^{-1} to 310^{0}C, which was held for 10 min. After injection the column temperature, initially 70^{0}C, was held for 3.5 min, then increased at 50^{0}C min^{-1} to 150^{0}C, then increased at 3^{0}C min^{-1} to 235^{0}C and finally raised to 300^{0}C at 50^{0}C min^{-1} and held for 3 min. The ion-trap mass spectrometer was operated in EI-MS mode. The transfer line, manifold and trap temperatures were 280, 50 and 200^{0}C, respectively. The analysis was performed with a filament-multiplier delay of 4.75 min to prevent instrument damage. The automatic gain control (AGC) was activated with an AGC-target of 5000 counts. The emission current for the ionisation filament was set at 80 mA, generating electrons with an energy of 70 eV. The axial modulation amplitude voltage was 4.0 V.

RESULTS AND DISCUSSION

Validation of the method

In order to check the feasibility of the GC-MS method for the analysis of pesticide residues in fresh vegetable sample extracts, it was validated using cauliflower and capsicum extracts.

Identification and confirmation of target analytes

The identification of the pesticides was based on the retention time windows (RTW) that are defined as the retention time average 63 S.Ds of the retention time when 10 blank samples spiked at the second calibration level of each compound were analysed. The confirmation of a previously identified compound was done by comparing the GC–MS spectra obtained in the sample with another stored as reference spectrum in the same experimental conditions. The reference spectra were obtained daily by injecting a blank cauliflower and capsicum sample spiked at the concentration of the second calibration point.

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.

Limit of detection and limit of quantification

The limit of detection (LoD) was calculated from the peak intensity at 0.01mg kg^{-1} 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 monocrotophos, chlorpyriphos, and endosulfan was 0.005, 0.003, and 0.004 mg kg^{-1} respectively. LoQ was obtained for monocrotophos, chlorpyriphos, and endosulfan was 0.015, 0.009 and 0.012 mg kg^{-1} respectively (TABLE 1). 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 2). The correlation coefficients of analytical curves were near 0.99, with linearity for each compound, which allows the quantitation of these compounds by the method external standardization.

TABLE 1 : Molecular formula, retention time, LODs and LOQs of monocrotophos, Chlorpyriphos and endosulfan.

Compound	Molecular formula	RT (min)	LoDs (mg kg^{-1})	LoQs (mg kg^{-1})
Monocrotophos	$C_7H_{14}NO_5P$	17.89	0.005	0.015
Chlorpyriphos	$C_9H_{11}Cl_3NO_3PS$	25.12	0.003	0.009
Endosulfan	$C_9H_6Cl_6O_3S$	26.72	0.004	0.012

TABLE 2 : Quantitation ion, conformation ion and calibration range of monocrotophos, chlorpyriphos and endosulfan.

Compound	Quantitation ion	Confirmation ion	Calibration range (mg kg^{-1})	Correlation coefficient	Coefficient of variation (n = 5) %
Monocrotophos	127	98	0.02-1.00	0.991	5.8
Chlorpyriphos	97	314	0.02-1.00	0.988	6.0
Endosulfan	373	237	0.02-1.00	0.989	5.8

Recovery

Recovery studies were performed to examine the efficacy of extraction and clean up. Untreated cauliflowers and capsicum samples were spiked with known concentration of the pure pesticides standard solution and extraction and clean-up were performed as described earlier. The concentration of each pesticide in the final extracts was calculated (TABLE 3). The average recoveries of pesticide residues in cauliflower and capsicum samples are 90.0 to 105.0 %.

APPLICATION TO THE ANALYSIS OF MARKET SAMPLES

In order to test the feasibility of the GC–MS approach for routine analysis of pesticide residues in the market samples of vegetables (cauliflower and capsicum) were analysed for the target compounds. The concentrations of each pesticide in the final extracts of the market were obtained and calculated (TABLE 4).

TABLE 3 : Recovery of pesticides in the spiked samples.

Sample	Compound	Concentration (mg kg^{-1})	Recovery (%)	Coefficient of variation (n = 5) %
Cauliflower	Monocrotophos	1.0	90.00	4.58
Cauliflower	Chlorpyrifos	1.0	99.80	4.70
Cauliflower	Endosulfan	1.0	104.20	4.40
Capsicum	Monocrotophos	1.0	93.20	4.25
Capsicum	Chlorpyrifos	1.0	100.30	4.56
Capsicum	Endosulfan	1.0	105.00	4.68

TABLE 4 : Amounts of pesticides residue detected in cauliflower and capsicum samples.

Sample	Monocrotophos (mg kg^{-1})	Chlorpyriphos (mg kg^{-1})	Endosulfan (mg kg^{-1})
Cauliflower	nd	nd	0.002
Cauliflower	0.024	nd	0.003
Cauliflower	nd	0.002	0.001
Cauliflower	0.027	nd	nd
Cauliflower	nd	nd	0.002
Capsicum	nd	0.012	0.008
Capsicum	0.021	nd	0.021
Capsicum	0.007	0.008	0.027
Capsicum	0.018	0.023	Nd
Capsicum	0.020	nd	0.026

nd = non detected

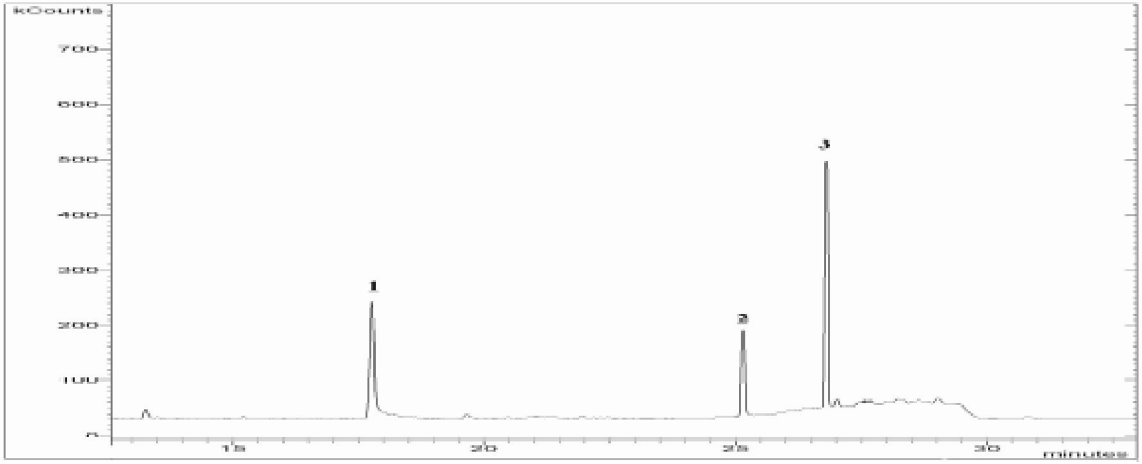

Figure 1 : Chromatopgram of monocrotophos[1], chlorpyriphos[2], and endosulfan[3] in actual sample of capsicum.

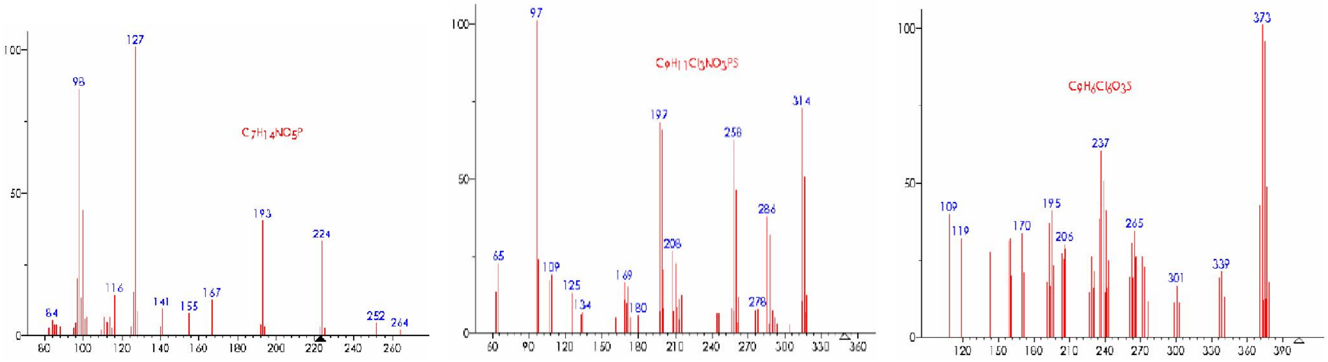

Figure 2 : Mass spectra of monocrotophos, chlorpyriphos, and endosulfan.

chlorpyriphos, and endosulfan in actual sample of capsicum and figure 2 shows mass spectra of monocrotophos, chlorpyriphos, and endosulfan.

CONCLUSION

In this study, the operating parameters of GC-MS for the analysis of 3 representative pesticides in cauliflower and capsicum were optimized, and sample preparation method was evaluated. The main conclusions of the study can be summarised as follows: (i) a good separation and high sensitivity was achieved by GC-MS method for all pesticides using a capillary column, (ii) the classical procedure that involves extraction with 1% acetic acid in acetonitrile cleanup with PSA and magnesium sulphate, showed an efficient removal of interferences, providing a simple, rapid and reliable analysis of pesticides in all matrices; (iii) for most of the pesticides assayed the performance characteristics obtained within validation study were acceptable, within the quality control requirements. Applying this method, analysis time is shorter compared to other methods. Thus, high sample throughput can, therefore, be achieved, which is useful in pesticide monitoring programs with a large number of samples to analyse.

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