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Analysis of Pesticide Residue in Vegetables Local Market Nanded, India

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Abstract: A study was conducted to analyse the residues of chlorpyrifos, cypermethrin and monocrotophos in locally grown vegetables. The vegetable samples of brinjal, capsicum, cauliflower and okra were collected from local market, Nanded, India. For this purpose, a total of 288 vegetable samples, were periodically collected and analyzed by gas chromatography equipped with mass detector. The results obtained showed that vegetable samples analyzed contained detectable level of the pesticides residues below the maximum residue limit (MRL).The vegetables samples were extracted by the application of a single-phase extraction of 15 g of sample with acetonitrile containing 1% of acetic acid, followed by a liquid-liquid partition formed by the addition of MgSO4 and NaOAc. Cleanup of the extract was carried out with primary secondary amine (PSA) and magnesium sulphate. The average recoveries of pesticide residues in brinjal, capsicum, cauliflower and okra samples were 75.0 to 105.0 %. The method offers cheaper and safer alternative to typical multi-residue analysis methods for the determination of pesticides residues in vegetables samples.

Keywords: Chlorpyrifos; Cypermethrin; Monocrotophos; GC-MS.

Introduction

Pesticides are used worldwide to protect crops before and after harvest in agriculture, gardening, homes and soil treatment. Variety of pesticides is used in current agricultural practice to manage pests and infections that spoil crops (1). A wide range of pesticides (13–14%) are used for the production of fruits and vegetables in India due to heavy pest infestation throughout the cropping season of horticultural crops whereas cropped area is only 3% (2). Pesticides have potentially adverse effects on vegetables, fruits, animal resources and human health (3). Because of the wide spread use, their toxic residues have been reported in various environmental matrices (4-9). There are many methods for determination of pesticides multiresidue in agricultural products and animal derived foods, but the key technique is: firstly, how several dozens of varieties or even hundreds of pesticides residues can be thoroughly extracted from the complex matrixes; secondly, how a great deal of interfering matters co-extracted with the pesticides can be cleaned up; thirdly, what analytical modes should be adopted for the pesticides requiring determination (10).

Liquid extraction is the fundamental method utilised for the isolation of pesticide residues from various food matrices. Many aspects such as ability to cover pesticides of a wide polarity range, selectivity involved in extraction and clean-up step and compatibility with separation techniques have to be considered. The choice of the solvent is one of the most important decisions to take in a multiresidual method (11). In the last few years acetone, acetone in combination with dichloromethane, ethyl acetate and acetonitrile are the extraction solvents most commonly used in extraction methods for the determination of pesticide residues in food (12-13). The original procedure consists of extracting the homogenised sample by hand-shake or Vortex with the same amount of acetonitrile in order to have a final extract concentrated enough without the need of a solvent evaporation step. The technique has attracted the attention of pesticide analysis studies worldwide (14-17). Gas chromatography coupled with mass spectrometry detection is considered a powerful technique for the quantitative determination of lower levels of contaminants in complex matrices (18). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid phase extraction for the determination of pesticides residues in produce was developed (19).

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 chlorpyriphos, cypermethrin and monocrotophos on brinjal, capsicum, cauliflower and okra, were developed and validated. Finally, the method was applied for the determination of chlorpyriphos, cypermethrin and monocrotophos in the vegetable samples collected from the local market, Nanded. Therefore, the purpose of this study was to develop an improved analytical method for the determination of the pesticide residues in brinjal, capsicum, cauliflower and okra by GC-MS.

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. Improved method for pesticide residues involves the extraction of the sample with acetonitrile (MeCN) containing 1% acetic acid (HAc) and simultaneous liquid liquid partitioning formed by adding anhydrous magnesium sulfate (MgSO4) plus sodium acetate (NaAc) followed by a simple cleanup step with primary secondary amine (PSA) and magnesium sulphate. This method has been extensively validated for hundreds of pesticide residues in many types of foods, and it described in Association of Analytical Communities (AOAC) Official Method 2007.01

Experimental

Chemical and reagents

The organic solvent acetic acid, acetonitrile HPLC grade, magnesium sulphate and sodium acetate AR grade purchased from E Merck and primary secondary amine purchased from Agilent Technologies. 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

Samples consisted of 1–2 kg of each vegetable comprising brinjal, capsicum, cauliflower and okra were collected from Itwara market, Nanded. In the laboratory, samples were packed in plastic bags and kept in refrigerated for analyses. The fresh vegetables samples cut into small pieces and homogenized 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). 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 samples were 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 30m 0.2mm 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.

Results and discussion

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 by injecting a blank brinjal, capsicum, cauliflower and okra 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 chlorpyriphos, and cypermethrin and monocrotophos, was 0.005, 0.003, 0.003 and 0.004 mg kg^{-1} respectively. LoQ was obtained for chlorpyriphos, cypermethrin and monocrotophos, was 0.015, 0.009, 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.

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 brinjal, capsicum, cauliflower and okra samples were 75.0 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 (brinjal, capsicum, cauliflower and okra) were analysed for the target compounds. The concentrations of each pesticide were obtained and calculated in the final extracts of the vegetables samples collected from local market (table:4 to table:10).

The present survey of market vegetables revealed about 75% contamination. Residues of the pesticides were not exceeded above MRL. The results obtained in present investigations are in accordance with those carried out earlier in India (5-7,20).

Table 1: Molecular formula, retention time, LODs and LOQs of chlorpyrifos, cypermethrin and monocrotophos

Compound	**Molecular formula**	**RT (min)**	**LoDs (mg kg^{-1})**	**LoQs (mg kg^{-1})**
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	25.12	0.003	0.009
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	31.32	0.003	0.009
Monocrotophos	$C_7H_{14}NO_5P$	17.89	0.004	0.015

Table 2: Quantitation ion, conformation ion and calibration range of chlorpyriphos, cypermethrin and monocrotophos

Compound	**Quantitation ion**	**Confirmation ion**	**Calibration range (mg kg^{-1})**	**Correlation coefficient**	**Coefficient of variation (n = 5) %**
Chlorpyriphos	97	314	0.02-1.00	0.988	5.5
Cypermethrin	181	127	0.02-1.00	0990	5.6
Monocrotophos	127	98	0.02-1.00	0.991	5.8

Table 3: Recovery of pesticides in the spiked samples.

Sample	Compound	Concentration (mg kg^{-1})	Recovery %	Coefficient of variation (n = 5) %
Brinjal	Chlorpyrifos	1.0	90.00	4.58
Brinjal	Cypermethrin	1.0	89.80	4.70
Brinjal	Monocrotophos	1.0	104.20	4.40
Capsicum	Chlorpyrifos	1.0	93.20	4.25
Capsicum	Cypermethrin	1.0	100.30	4.56
Capsicum	Monocrotophos	1.0	105.00	4.68
Cauliflower	Chlorpyrifos	1.0	88.50	4.75
Cauliflower	Cypermethrin	1.0	91.05	4.60
Cauliflower	Monocrotophos	1.0	89.10	4.78
Okra	Chlorpyrifos	1.0	90.50	4.85
Okra	Cypermethrin	1.0	75.20	4.80
Okra	Monocrotophos	1.0	102.30	4.90

Table 4: Recovery (%) of chlorpyrifos in vegetables samples collected from local market, Nanded, analysed by GC-MS using using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Matrix	Sample collection period	No. of sample collected	Residue level mgkg^{-1} in sample no.					
			1	2	3	4	5	6
Brinjal	January 2010 to March 2010	6	0.025	ND	0.020	ND	0.008	ND
Capsicum	January 2010 to March 2010	6	ND	0.008	ND	0.014	ND	ND
Cauliflower	January 2010 to March 2010	6	ND	0.004	0.018	ND	ND	0.096
Okra	January 2010 to March 2010	6	0.012	0.008	ND	0.020	0.022	0.014
Brinjal	April 2010 to June 2010	6	ND	0.012	0.040	ND	0.008	0.014
Capsicum	April 2010 to June 2010	6	0.010	ND	ND	0.040	0.012	ND
Cauliflower	April 2010 to June 2010	6	ND	ND	0.032	ND	ND	0.012
Okra	April 2010 to June 2010	6	0.024	0.026	ND	0.022	0.006	ND

Table 5: Recovery (%) of chlorpyrifos in vegetables samples collected from local market, Nanded, analysed by GC-MS using using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Matrix	Sample collection period	No. of sample collected	Residue level mgkg^{-1} in sample no.					
			1	2	3	4	5	6
Brinjal	July 2010 to September 2010	6	0.020	ND	ND	0.008	0.028	0.008
Capsicum	July 2010 to September 2010	6	0.018	ND	ND	0.024	0.018	ND
Cauliflower	July 2010 to September 2010	6	0.022	ND	0.016	0.008	ND	0.096
Okra	July 2010 to September 2010	6	ND	0.032	0.010	ND	0.012	ND
Brinjal	October 2010 to December 2010	6	0.015	0.012	ND	0.008	ND	0.014
Capsicum	October 2010 to December 2010	6	0.022	0.022	ND	0.016	ND	0.022
Cauliflower	October 2010 to December 2010	6	0.012	ND	0.032	0.012	0.062	ND
Okra	October 2010 to December 2010	6	0.024	0.032	ND	0.040	0.038	0.022

Table 6: Recovery (%) of cypermethrin in vegetables samples collected from local market, Nanded, analysed by GC-MS using using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Matrix	Sample collection period	No. of sample collected	Residue level mgkg^{-1} in sample no.					
			1	2	3	4	5	6
Brinjal	January 2010 to March 2010	6	0.035	0.042	0.058	ND	ND	0.035
Capsicum	January 2010 to March 2010	6	ND	0.045	ND	ND	0.021	ND
Cauliflower	January 2010 to March 2010	6	0.035	ND	0.015	0.072	ND	0.016
Okra	January 2010 to March 2010	6	0.012	0.078	0.084	ND	0.022	0.014
Brinjal	April 2010 to June 2010	6	ND	0.012	ND	0.044	0.050	ND
Capsicum	April 2010 to June 2010	6	ND	ND	0.054	0.012	ND	0.040
Cauliflower	April 2010 to June 2010	6	ND	0.012	ND	ND	0.038	0.062
Okra	April 2010 to June 2010	6	0.065	0.026	ND	0.010	ND	0.012

Table 7: Recovery (%) of cypermethrin in vegetables samples collected from local market, Nanded, analysed by GC-MS using using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Matrix	Sample collection period	No. of sample collected	Residue level mgkg^{-1} in sample no.					
			1	2	3	4	5	6
Brinjal	July 2010 to September 2010	6	0.025	ND	ND	0.014	ND	0.035
Capsicum	July 2010 to September 2010	6	ND	ND	0.052	ND	0.021	0.32
Cauliflower	July 2010 to September 2010	6	0.065	ND	ND	0.080	ND	ND
Okra	July 2010 to September 2010	6	0.012	0.008	0.086	0.180	0.022	ND
Brinjal	October 2010 to December 2010	6	0.015	0.012	ND	0.084	0.050	0.014
Capsicum	October 2010 to December 2010	6	0.050	ND	0.024	ND	0.012	ND
Cauliflower	October 2010 to December 2010	6	0.065	ND	0.135	ND	ND	0.096
Okra	October 2010 to December 2010	6	0.012	0.008	0.86	ND	0.022	0.014

Table 8: Recovery (%) of monocrotophos in vegetables samples collected from local market, Nanded, analysed by GC-MS using using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Matrix	Sample collection period	No. of sample collected	Residue level mgkg^{-1} in sample no.					
			1	2	3	4	5	6
Brinjal	January 2010 to March 2010	6	0.025	0.042	0.050	ND	0.080	0.031
Capsicum	January 2010 to March 2010	6	ND	0.045	ND	0.014	ND	0.032
Cauliflower	January 2010 to March 2010	6	0.065	ND	0.035	ND	ND	0.056
Okra	January 2010 to March 2010	6	0.012	0.151	ND	0.061	0.022	ND
Brinjal	April 2010 to June 2010	6	0.015	0.032	0.040	ND	0.050	0.014
Capsicum	April 2010 to June 2010	6	0.050	ND	0.054	ND	ND	ND
Cauliflower	April 2010 to June 2010	6	0.012	0.032	ND	ND	0.086	0.012
Okra	April 2010 to June 2010	6	0.066	ND	ND	0.022	0.006	0.012

Table 9: Recovery (%) of monocrotophos in vegetables samples collected from local market, Nanded, , analysed by GC-MS using using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Matrix	Sample collection period	No. of sample collected	Residue level mgkg^{-1} in sample no.					
			1	2	3	4	5	6
Brinjal	July 2010 to September 2010	6	0.025	ND	0.050	0.014	ND	0.031
Capsicum	July 2010 to September 2010	6	ND	0.045	ND	0.014	0.021	ND
Cauliflower	July 2010 to September 2010	6	ND	0.035	0.035	0.044	ND	0.056
Okra	July 2010 to September 2010	6	0.012	0.008	ND	0.018	0.022	0.014
Brinjal	October 2010 to December 2010	6	0.015	0.032	0.040	ND	0.050	0.014
Capsicum	October 2010 to December 2010	6	0.050	ND	ND	0.040	0.012	ND
Cauliflower	October 2010 to December 2010	6	0.012	0.002	ND	0.038	0.006	0.012
Okra	October 2010 to December 2010	6	0.065	0.026	ND	0.022	0.006	0.012

Conclusion

This method has several advantages over most traditional methods of analysis in the following ways: (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, (iv) high recoveries are achieved for a wide polarity and volatility range of pesticides, (v) solvent usage and waste is very small, and no chlorinated solvents are used. 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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