

Gas Chromatography- Mass Spectrometry determination of Pesticide Residue in Fruits

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Abstract: Apple and grape samples were analysed for the detection of pesticide residues employing multiresidue analysis by gas chromatography mass –spectrometry. This method was successfully developed for the determination of pesticide residues namely monocrotophos, chlorpyrifos, endosulfan and cypermethrin in apple and grape samples. The fruits 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 grape and apple samples were 75.0 to 105.0 %. The method offers cheaper and safer alternative to typical multi-residue analysis methods for apple and grape fruits.

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

1.Introduction

Apples and grape is an important fruit crop in India. These fruits have a pivotal role in the diet for maintenance of health and prevention of disease. A wide range of pesticides are used for the production of fruits and vegetables in India, due to heavy pest infestation throughout the cropping season of horticultural crops¹. Grape (*Vitis vinifera*) and apple (*Malus domestica*) are one of the widely consumed fruits in the world, are rich in pesticides. Because of wide spread use, their toxic residues have been reported in various environmental matrices²⁻⁴. The number of organochlorine pesticides which have been banned by the government for their toxicity is still exists in the medium. People are directly exposed to these pesticides through dermal contact and inhalation and indirectly through the food chain. Although several million people are exposed, the health impact

of chronic pesticide exposure in the country is largely unknown. Currently India is the largest producer of pesticides in Asia and the third largest consumer of pesticides in the world. Thus the determination of pesticide residues in fruits and vegetables and other environmental components/ commodities like soil, water and total diet have become increasingly essential requirements for consumers, producers and authorities for food quality control.

Many researchers have estimated the pesticide residues (PRs) in various fruits including banana, mango, apple, peach, watermelon, melon, grape, orange, lemon, pear, pineapple, strawberry, raspberry, kiwi fruit, beet, papaya and litchi, etc⁵⁻¹⁴ and reported the occurrence of pesticide residues to be even more than maximum residue level (MRL) values recommended by European union (EU), world health organization (WHO) and food and agricultural organization (FAO). In India organochlorine pesticides i.e., HCH, DDT and endosulfan were detected in almost all the fruits samples of ber, grapes and guava¹⁵.

Different extraction and quantification methods are used by various researchers for estimation of multi class pesticide residues in several vegetables and fruits. The main criteria for opting any methodology is that analytical method should be fast, easy, inexpensive and applicable to different matrices. In recent years, gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS)^{16,18}, gas chromatography-ion trap mass spectrometry (GC-ITMS)¹⁷ (Savant R.H. et al 2010) and gas chromatography-tandem mass spectrometry (GC-MS/MS)¹⁹. The variety of sensitive detectors coupled with GC such as electron capture detector (ECD)²⁰⁻²² nitrogen.

phosphorus detector (NPD)²³⁻²⁶, flame ionization detector (FID)²⁷, pulsed flame photometric detector (GC-PFPD)²⁸, flame photometric detector (FPD)²⁹ improved the detection and quantification procedures of pesticide residues monitoring in different matrices. Besides GC-MS methods, there are other traditional quantification methods like high performance liquid chromatography (HPLC) (S. A. Baig et al, 2009), liquid chromatography-mass spectrometry (LC-MS)³⁰, liquid chromatography-tandem mass spectrometry (LC-MS/MS)³¹ and low-pressure gas chromatography-mass spectrometry (LP-GC/MS)³². Organochlorine, organophosphorus and pyrethroid pesticides are widely used in agriculture and animal production for the control of various insects. Most of them have been banned (endosulfan in India), yet their residues still appear as pollutants in food as well as in the environment therefore it is important to detect the pesticide for general public awareness.

In this study describe method of extraction, cleanup and determination of a pesticide residue by using gas chromatography (GC) equipped with mass detector (MS) for the separation, identification and quantification of monocrotophos, chlorpyrifos endosulfan and cypermethrin, on apples and grapes were developed and validated. Therefore, the purpose of this study is to develop an improved analytical method for the determination of these pesticides on apples and grapes by GC-MS. It is hoped that the data will establish a base line for determining changes in residue levels of different pesticides in future years.

2. Experimental

2.1 Chemical and Reagent

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°C. Anhydrous magnesium sulphate used during residue extraction was maintained at 300°C overnight and kept in air tight container. Polyethylene or PTFE 15 ml and 50 ml with screw cap tubes.

2.2 Sample Collection

A total of 20 samples of fruits namely apples and grapes were collected from the local market Vasco-Da-Gama, Goa, India during the period of August 2012 to March 2013 according to the fresh samples availability in the market on seasonal basis. The quantity of the samples was selected to purchase 2kg of each fruit for extraction and cleanup.

2.3 Sample Extraction And Cleanup

The fresh apple and grape 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). 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.

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°C in the dark. Working solutions were prepared daily by appropriate dilution with ethyl acetate.

2.5 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.

3. Results and Discussion

The occurrence of selected organochlorine and organophosphate and pyrethroid pesticides was studied in the apple and grapes collected from local market at Goa. The pesticides detected were monocrotophos, chlorpyrifos, endosulfan and cypermethrin. This study has shown the presence of organochlorine (endosulfan) pesticides despite the fact that it has been banned for use in India. This study therefore suggest the possibility of sporadic use of these pesticides for agriculture or mainly due to the past extensive use of these pesticides for agriculture in India as it has been banned for over a two year ago. Two fruits samples were analyzed in this study consist of the main part of dietary intakes of Indian people. The pesticides targeted, were related on basis of their frequency of application. Twenty samples of fruits were screened for four different pesticides during said period and it was found that most of the samples were contaminated with the one or more pesticides under study. Residue levels of these compounds in fruits and vegetables are listed in Tables 4 and 5.

3.1validation Of The Method

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

3.2 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.

3.3 Limit Of Detection and Limit Of Quantification

The limit of detection (LoD) was calculated from the peak intensity at 0.01 mg 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, chlorpyrifos, endosulfan and cypermethrin, was 0.005, 0.003, and 0.004, 0.003 mg kg^{-1} respectively. LoQ was obtained for monocrotophos, chlorpyrifos, endosulfan and cypermethrin was 0.015, 0.009, 0.012 and 0.009 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.

3.4 Recovery

Recovery studies were performed to examine the efficacy of extraction and clean up. Untreated apple and grape 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 apple and grape samples are 75.0 to 105.0 %.

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

Compound	Molecular formula	RT (min)	LoDs (mg kg^{-1})	LoQs (mg kg^{-1})
Monocrotophos	$\text{C}_7\text{H}_{14}\text{NO}_5\text{P}$	17.89	0.005	0.015
Chlorpyrifos	$\text{C}_9\text{H}_{11}\text{Cl}_3\text{NO}_3\text{PS}$	25.12	0.003	0.009
Endosulfan	$\text{C}_9\text{H}_6\text{Cl}_6\text{O}_3\text{S}$	26.72	0.004	0.012
Cypermethrin	$\text{C}_{22}\text{H}_{19}\text{Cl}_2\text{NO}_3$	31.32	0.003	0.009

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

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
Chlorpyrifos	97	314	0.02-1.00	0.988	5.4
Endosulfan	373	237	0.02-1.00	0.989	5.8
Cypermethrin	181	127	0.02-1.00	0.990	5.6

Table 3: Recovery of pesticides in the spiked samples.

Sample	Compound	Concentration (mg kg^{-1})	Recovery (%)	Coefficient of variation (n = 5) %
Apple	Monocrotophos	1.0	90.00	4.58
Apple	Chlorpyrifos	1.0	99.80	4.70
Apple	Endosulfan	1.0	95.40	4.40
Apple	Cypermethrin	1.0	75.80	4.80
Grape	Monocrotophos	1.0	99.60	4.40
Grape	Chlorpyrifos	1.0	100.30	4.56
Grape	Endosulfan	1.0	105.00	4.68
Grape	Cypermethrin	1.0	77.90	4.88

Table 4: Amounts of pesticides residue detected in apple samples collected during August 2012 to December2012.

Sample	Monocrotophos (mg kg ⁻¹)	Chlorpyrifos (mg kg ⁻¹)	Endosulfan (mg kg ⁻¹)	Cypermethrin (mg kg ⁻¹)
Apple	ND	ND	0.026	0.008
Apple	0.024	ND	0.012	ND
Apple	ND	0.008	0.008	0.032
Apple	0.045	ND	ND	0.025
Apple	0.015	0.042	0.022	0.012
Apple	ND	ND	0.105	ND
Apple	0.024	ND	0.044	ND
Apple	0.050	ND	ND	0.080
Apple	ND	0.085	0.052	ND
Apple	0.054	ND	0.022	ND

Table 5: Amounts of pesticides residue detected in grapes samples collected during December2012 to March2013.

Sample	Monocrotophos (mg kg ⁻¹)	Chlorpyrifos (mg kg ⁻¹)	Endosulfan (mg kg ⁻¹)	Cypermethrin (mg kg ⁻¹)
Grape	0.027	ND	ND	0.008
Grape	ND	0.088	0.012	0.040
Grape	0.018	ND	0.028	ND
Grape	0.022	ND	0.036	0.044
Grape	0.035	0.024	ND	0.028
Grape	0.027	ND	ND	0.008
Grapes	ND	ND	0.002	ND
Grape	0.045	0.084	ND	ND
Grape	ND	0.052	ND	0.074
Grape	ND	0.105	0.086	ND

ND = Not Detected

4. Conclusion

Contamination in the fruits with the pesticide residues poses a significant health risk to the public from consuming contaminated fruits hence it is important to develop effective method for the detection of the compounds. The 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. This method was useful for detection of pesticide residue present in the grape and apple. It is most effective and widely acceptable in terms of accuracy and reliability. The objective of this study was to create awareness among the fruits consumers who are consuming contaminated apple and grape.

5. References

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