DETERMINATION OF SELECTED PESTICIDE RESIDUES IN FRUITS USING QUECHERS APPROACH AND REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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Abstract- This paper presents the application of reverse phase high performance liquid chromatographic (RP-HPLC) method equipped with UV detector for the determination of five pesticides, in common fruit samples. The reverse phase liquid Chromatography were used for the monitoring of pesticide residues employing QUEChERS approach of selected organophosphate pesticide Monocrotophos, 2.4 D, Chlorpyrifos, Malaxon, Isopruton, and Phorate sulphoxide. Proposed method was successfully applied for the determination of pesticide residues in orange, apple, pomegranate and grape fruits. Average recoveries achieved for all the pesticides at fortification levels of 1, 0.5, 0.1 mg/l in analyzed fruits were above 90% with relative standard deviations (RSD) less than 6%. The correlation coefficients (r) were higher than 0.999 indicating a high level of linear relationship between the concentration of monochrotophos and peak area.

Index Terms- Reversed-Phase High Performance Liquid Chromatography (RP-HPLC), Pesticides Residue, Quechers Approach, Correlation Coefficient.

I. INTRODUCTION

A high intake of fruit has been encouraged not only to prevent consequences due to vitamin deficiency but also to reduce the incidence of major diseases such as cancer [1], cardiovascular diseases and obesity. But fresh fruit could also be a potential source of harmful and toxic substances. Many researchers have estimated the pesticide residues (PRs) using RP-HPLC in various fruits including banana, mango, apple, peach, watermelon, melon, grape, orange, lemon, pear, pineapple, strawberry, raspberry, kiwi fruit, beet, papaya and litchi, etc. [2-7] 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 apple, orange, grapes, guava and pomegranate. The analysis of pesticide residues represents a basic instrument not only for the protection of human health, but also for trade and official control purposes [8]. Although multiresidue analysis of these compounds at trace levels has been carried out since decades, analysis of pesticides still remains a challenge as different chemicals are present at low concentrations in complex matrices. Therefore, it is necessary to continue developing multi-residue analytical methods with higher recoveries and lower limits of detection [9]. Reversed-phase HPLC (RP-HPLC) covers more than 95% of applications. Although new extraction methods based on micro extraction techniques and some conventional procedures are published time-to-time, they are not implemented in routine analysis. Hence, monitoring studies to know the actual status of contamination due to toxic pesticide residues in various food commodities for the formation of legal guidance and for consumer satisfaction is necessary. The QuEChERS approach has been extensively validated for hundreds of pesticide residues in many types of foods, and has become Association of Analytical Communities (AOAC) Official Method 2007.01 (10). In this study, QuEChERS approach was applied for the determination of pesticide residues namely Monocrotophos, 2, 4 D, Chlorpyrifos, Malaxon and Isopruton. The QuEChERS method has several advantages over most traditional methods of analysis. High recoveries (greater than 85%) are achieved for a wide polarity and volatility range of pesticides, including notoriously difficult analytes.

II. MATERIALS AND METHODS

A. Chemicals and reagents

The organic solvents, acetonitrile, ethyl acetate used were HPLC grade and were purchased from E. Merck with a purity of 95-99%. The standards were stored in a freezer at -5°C. Water and acetonitrile were degassed by vacuum suction. All samples and solvents were filtered through Millipore membrane filters (0.45 µm pore size) before injection on the column.

B. Sampling

Two kilogram sample of each commodity was purchased as per standard procedure of FAO/WHO (Anon., 1993). All the samples were sliced or peeled off and homogenized.

C. Extraction

Sub-samples were taken for extraction that was undertaken on the same day of sub-sampling.

Extraction was carried out on the same day as the sampling was done. Samples were analyzed in accordance with described procedures (Parveen & Masud, 2002). Each sample (15g) was extracted with 75ml of extraction mixture (Toulene + nHexane + Ethylacetate) in the ratio of 3:1:1. The decanted extract was concentrated to approximately 2-3ml and poured on a mini column of Florisil plus activated charcoal and eluate was collected for each sample. The cleaned up extract was evaporated to dryness in a rotary vacuum evaporator and taken up in 2ml acetone for Gas Chromatographic determination. For High Performance Liquid Chromatographic determination, evaporated dry residues were taken up in 2ml methanol (HPLC grade). The final extract was made up to 1 ml with acetonitrile. The clean samples were taken and were analyzed by a high performance liquid chromatography having UV/Visible detector.

D. Standard preparation

A stock solution of monochrotophos, copper oxychloride and Galaxy (100 mg kg-1) was prepared in acetonitrile. Standard calibration solutions (10 to 50 mg kg-1) for the assessment of linearity were prepared from this stock solution using the mobile phase. The solutions were filtered through a 0.45-µm nylon filter. Working solutions were prepared daily by appropriate dilution with acetonitrile. The filtered solution was then injected into the HPLC system.

E. HPLC systems

Agilent Technologies 1220 infinity High performance liquid chromatography having UV/visible detector was used for identification and quantification of pesticides. Separation was performed on reversed phase C-18 column (Water). Samples were injected manually. Detector was connected to the computer for data processing. In this study, a reversed-phase semi-micro column was used for simultaneous determination, reducing the solvent volume and the measurement time and obtaining high separativity. These pesticides, capable of determination by HPLC, were mainly selected among pesticides to set a regulated value in Japan. Recovery tests from samples were ultimately performed for 27 pesticides divided into 5 groups, because the retention times of some pesticides were very close. The HPLC chromatograms of standard mixtures are shown in Figure1.
The working condition of HPLC was binary gradient, mobile phase was acetonitrile: water; (60:40), flow rate was 0.3 mL min-1, injection volume was 25 µL and the wavelength of the UV/visible detector was fixed at 254 nm for the pesticides i.e., monochrotophos, copper oxychloride and Galaxy. Ultimately, the wavelength was set at 230 nm for greater sensitivity for pesticides.

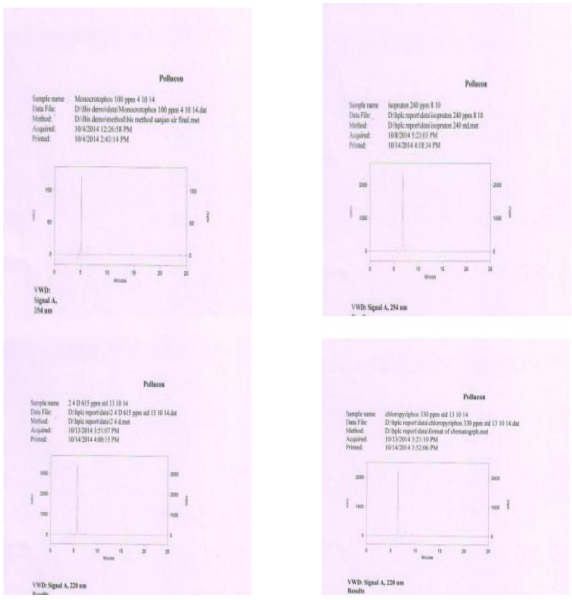


Figure 1: HPLC chromatogram of Pesticide standards

III. RESULTS AND DISCUSSION

HPLC determination of pesticide standards

Pesticide residues were identified by comparing their retention time Retention time (Rt). Monochrotophos (Rt-2.258), Copperoxychloride (Rt-2.908) and Galaxy (Rt-3.608) and their standards have been shown in Figure 1 and Table 1. The quantitative determination was carried out with the help of a calibration curve (Figure 2). A good linearity was established by a correlation coefficient (R2) value of 0.990 ± 0.0001 (Table 2). Correlation coefficient is a statistical tool used to measure the degree or strength of this type of relationship, and here, a high correlation coefficient value (a value very close to 1.0) indicates a high level of linear relationship between the concentration of monochrotophos and peak area. For quantification, an external calibration curve with four different concentrations of each pesticide, with matching matrix were made.

Table I: Pesticide Standards

Peak	Name of Pesticide and concentration(mg kg-1)	Rf Value	Peak Area	S/N ratio
1	Monocrotophos (10ppm)	5.10	17179590	1871
2	2,4 D, (10 ppm)	5.66	735718066	1992
3	Chloropyriphos,(10 ppm)	6.44	413792960	952
4	Malaxon(10 ppm)	6.19	26291890	11.47
5	Isopruton (10 ppm)	6.79	586587765	302
6	Phorate Sulphoxide (10 ppm)	6.18	1027724112	1012

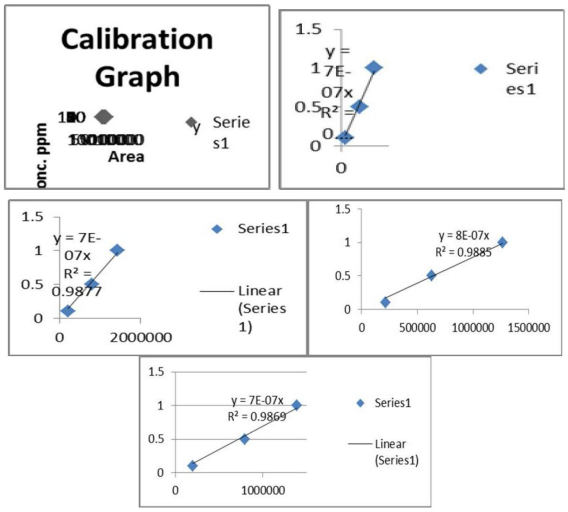


Figure 2: Calibration curve of Monocrotophos, 2,4 D, Chlorpyrifos, Malaxon, Isopruton, and phorate sulphoxide standards

Table II: Validation data of Monocrotophos, 2.4 D, Chlorpyrifos, Malaxon, Isopruton, and Phorate sulphoxide standards obtained from HPLC chromatogram

S.No	Retention time	Peak area	S/N ratio	Monocrotophos (mg/l)
1	5.10	1310630	1019	1
2	5.12	632668	393	0.5
3	5.12	210985	160	0.1

S.No	Retention time	Peak area	S/N ratio	2,4 D (mg/l)
1	5.52	1589327	196	1
2	5.52	794650	56	0.5
3	5.53	200192	75	0.1

S.No	Retention time	Peak area	S/N ratio	Chloropyriphos (mg/l)
1	6.44	41379296	952	1
2	6.45	4552743	201	0.5
3	6.44	959625	117	0.1

S.No	Retention time	Peak area	S/N ratio	Malaxon (mg/l)
1	5.52	1512297	1315	1
2	5.56	820010	222	0.5
3	5.54	204024	214	0.1

S.No	Retention time	Peak area	S/N ratio	Isopruton (mg/l)
1	5.92	5401767	1501	1
2	5.93	2700468	513	0.5
3	5.92	919599	969	0.1

S.No	Retention time	Peak area	S/N ratio	Phorate Sulphoxide (mg/l)
1	6.18	102772411	947	1
2	6.18	46214206	208	0.5
3	6.20	23107103	197	0.1

HPLC determination of pesticide residue in Fruit samples

HPLC result based on the Retention time (Rt) of monochrotophos (Rt-2.25) content in tender coconut samples is given in Table 3. An amount of 5.847 ppm of monochrotophos was detected which was more than MRL value in Malayan orange sample and the amount was below the MRL value (0.5-1 mg kg-1) as per the EU standards in 9 samples. As per all India network project on pesticide residue survey (2009-10), a total of 2114 samples of different fruits namely apple, orange, grapes, guava, mango and pomegranate, the maximum number of sample have been reported to have presence of persistent organochlorine pesticide such as DDT. Dicofol, isomer of HCH and endosulfan and chloropyriphos was most prominent pesticide detected in all fruit samples. As per report, the most contaminated samples were apple, orange, grape and pomegranate. Results of our study reveals same pattern of contamination in all type of selected fruit samples but the concentration of pesticide residue was below the MRL value given in Table 3. Isoproton and malaxon pesticides were not detected in all the samples.

Table III: Amounts of pesticides residue detected in apple samples collected during Nov 2014 to December2014.

Sample	Monocrotophos	2,4 D	Chloropyriphos	Malaxon	Isoproton	DDT	Endosulfan
Apple	0.021	ND	0.04	ND	ND	ND	0.01
Orange	0.20	0.01	0.01	ND	ND	ND	ND
Grape	0.18	ND	0.03	ND	ND	0.01	0.03
Pomegranate	0.04	0.01	ND	ND	ND	ND	ND

CONCLUSION

In this study, the RP-HPLC method was used to determine pesticides in tender coconut water samples. The results indicate that, among 10 samples of tender coconut water samples that were examined, only one two sample contained monochrotophos and copper oxychloride. The malayan orange sample that exceeded the FAO/WHO Codex Alimentarius Maximum Residue Limits (MRLs) (Codex Alimentarius Commission, 1996). Monocrotophos is a low cost and many possible applications have kept up demand in India despite growing evidence of its negative impact on human health. The results demonstrate the accuracy of the method used for multi residue analyses of pesticides. With regards to the 90% of the tender coconut water samples from a local market, no detectable residues of the pesticides were found.

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