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Validation of Modified QuEChERS method using Low Cost Adsorbent and Screening of Selected Organochlorine and Organophosphorus Insecticides in Fruits and Vegetables

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Abstract

A highly economical and simple sample preparation technique for the analysis of insecticide residues in fruits and vegetables was developed by modifying the existing Quick Easy Cheap Efficient Rugged and Safe (QuEChERS) technique and a full in-house validation was carried out for different pesticides belonging to organochloride and organophosphorus groups. In this newly developed sample preparation technique, easily available low cost fly ash which is a sugarcane industry waste was used as an adsorbent in place of expensive Primary Secondary Amine (PSA). Efficiency of the modified method was compared with that of original QuEChERS method by conducting parallel experiments. Insecticides were investigated at levels ≥0.01µg/g in fruits (apple, grape and orange) and vegetables (cabbage, cauliflower, tomato and brinjal) using low cost adsorbent. Validation study fulfilled the requirement of SANCO guideline 2012. Experiments proved that the modified method can be adopted for routine monitoring of insecticide residues (dimethoate, malathion, alfa and beta-endosulfan) in fruits and vegetables. Out of seventy samples of fruits and vegetables collected from different outlets, Eleven samples were found to be contaminated with insecticides. Though residues were determined in tomato, cabbage, grape, orange and apple, they did not exceed the Maximum Residue Limit (MRL) prescribed under Food Safety Standard Authority of India. However, out of ten samples of cabbage analyzed, only one sample found to contain dimethoate residue, three times more the permitted level specified under Codex.

Keywords: Gas chromatography, QuEChERS, insecticides, fruits, vegetables, in-house method validation.

Introduction

Intake of fruits and vegetables promote health and may also help in preventing certain chronic diseases such as heart diseases and certain types of cancer[1-4]. The health benefits of fruits and vegetables seen in epidemiological studies are the main reasons for the recommended intake of at least 400 g of fruit and vegetables per day[5-6]. Pesticides and chemical fertilizers have played a significant role in making India the second largest producer of fruits and vegetables after China[7-8]. In the process of development of agriculture, insecticides have become an important tool as a plant protection agent for boosting food production. Further, insecticides play an important role by keeping away many dreadful diseases. But the indiscriminate and injudicious use of pesticides has resulted in widespread contamination of food, feed and water[7]. Exposure to insecticides both occupationally and environmentally can lead to serious human health problems. It has been observed that the pesticides exposures are increasingly linked to immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer[8]. The insecticide residues find their way into the human/animal body through food, water, and environment. But contaminated cereal, grains, fruits and vegetables are the major contributors of exposure of mankind to insecticides. The presence of insecticide residues in fruits, vegetables and other foods has become a global phenomenon. Many authors earlier have reported the presence of insecticide residues in fruits and vegetables from India[9-12] and abroad[13-14]. Because of wide spread use of insecticides, the presence of their toxic residues[15] have been reported in various environmental component/commodities[16-21]. This revelation has posed a major health concerns among consumers and also has affected international trade.

National and international regulatory bodies are working on minimizing the exposure of insecticides to mankind by bringing in strict and stringent regulations. Screening of food commodities for insecticide residues has become a routine exercise and many analytical methods have been developed and tested for the purpose. In recent times, the laboratories involved in monitoring of insecticide residues are looking for insecticide residue screening techniques which are less time consuming and cheap. Even though QuEChERS is meeting all the requirements, there is a need for replacement of expensive PSA (adsorbent) with a cheaper material. In this direction, a successful attempt was made for the replacement of PSA with cheaply available industrial waste material i.e. fly ash of sugar cane industry.

Methodology

Fruit and vegetable samples: During the period 2013-2014, seventy samples of fruits and vegetables (two kg each of apple, orange, grape, cabbage, cauliflower, tomato and brinjal) were collected from local vendors of Mysore city, Karnataka state, India. The samples were kept in a refrigerator (4-5^{o}C) till analysis. Only the edible parts of each fruit and vegetables were processed for insecticide residue analysis.

Reagents: All reagents were of analytical grade unless otherwise stated. Insecticide reference standards of dimethoate, malathion, alfa and beta-endosulfan were procured from Sigma-Aldrich and Laborchemikallen, GmbH. Ethyl acetate, toluene, acetone, n-hexane and anhydrous sodium sulfate were purchased from Merck (India). Primary Secondary Amine (PSA) and magnesium sulfate ($MgSO_4$) were procured from Agilent Technology (U.S). Sodium bi carbonate obtained from s.d. Chem Pvt ltd (India).

Sugarcane ash: Sugarcane ash was collected from Kisansahkari sugar mill, Sampurnanagar, Kheeri (U.P, India). The material was pulverized and sieved using 20 -200 mesh. Glass column (45 cm x 2cm) was plugged with cotton, over which 10 g of sugarcane ash was packed. The prepared column was washed with 200 ml acetone and n-hexane (1:1) and dried first at room temperature and later in an electric oven at 110°C for 24 h.

Carbon (%) in sugarcane ash: Activated carbon in the fly ash was determined by following the one step pyrolysis method[22]. For this, the test samples were divided into three parts; the first part mixed with 10% phosphoric acid (100 g sample + 100 mL of H_3PO_4, wt/v) and the second part mixed with 10% potassium hydroxide (100 g sample + 100 mL of KOH, wt/v) and the third part was used as control without any addition. Both the treated and control samples were pyrolyzed at 400°C for 1 h in an electric muffle furnace. After activation, the mixture was removed from the furnace and allowed to cool to room temperature. The pyrolysed carbons were leached with 2% HCl (v/v) for 2 h and washed several times with de-ionized hot water until a neutral pH was achieved. Later the carbon paste was dried in an electric oven at 110°C for 24 h. The activated carbon yield was calculated by applying the formula[23].

$$X\ (\%) = m/mo \times 100,$$

Where: X is activated carbon yield (%), m is the activated carbon mass (g) and mo is the raw sample mass (g).

Standard solutions: The stock solutions of dimethoate, malathion, alfa and beta endosulfan were prepared in n-hexane and toluene (1:1). The standard solutions were stored in a refrigerator at 4 ^{o}C.

Working standard solution: A working standard was prepared by dilution of stock solution with n-hexane. For each insecticide working standard, one ml of primary stock solution (1000 µg/ml) was taken in a 10 mL volumetric flask and volume was made up with n-hexane to give a standard solution of 100 µg/mL concentrations. Other working solutions of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 µg/ml concentrations were prepared by serial dilution with n-hexane.

Apparatus, GC instrument: Gas Chromatograph-Shimadzu 2010 (Shimadzu, Kyoto, Japan) equipped with split/split less auto-injector model AOC-20i was used for the analysis. The non-polar stationary phase used was a fused silica capillary column of 30 m, 0.25 mm i.d., and 0.25 µm film thickness by Supelco, USA which were equivalent to DB-1 and DB-5 (1 % phenyl polysiloxane and 5 % phenyl polysiloxane). For the control of instrument and data analysis, 'GC Solution' software was used.

Turbo-Vap: Zymark Turbo Vap (R), LV evaporator (Caliper Life Sciences, USA) was used to concentrate the sample.

Homogenizer: Ultra turrax t18 basic homogenizer (6500 – 24000 rpm) was used to crush and grind the samples.

Centrifuge: Remi Laboratory Centrifuge Model R-24 (maximum speed 10,000 rpm) for 50 mL and 15 mL tubes capacity were used for centrifugation of sample.

Analytical balance: An electronic weighing balance (Mettler Toledo) with digital display was used to weigh the samples and reagents.

Vials and vessel: Centrifuge tubes 50 mL and 15 mL (Tarson) with screw cap were used for the extraction of sample and Dispersive SPE cleanup. GC auto sampler (1.5 mL) vials with septa were used for the final extracts.

Sample preparation: Vegetables (tomato, cauliflower, brinjal and cabbage) and fruits (apple, grape and orange) were used for the validation/screening experiments. For the extraction of insecticide residues, 0.5 kg of each sample was chopped and ground. For analysis, about 10 ± 0.03 g homogenized sub-samples were weighed and transferred to a 50 mL centrifuge tube and 2.0 ± 0.01 g sodium hydrogen carbonate ($NaHCO_3$) was added to each tube followed by 20 mL of ethyl acetate. The contents of the tubes were homogenized using high speed Ultra turrax t18 basic homogenizer (6500 – 24000rpm) for 5-6 min at 14000-15000 rpm. Sodium sulfate (Na_2SO_4) 4.0 ± 0.2 was added to the homogenized sample and mixed by shaking vigorously by hand to ensure that the solvent interacts well with the entire sample and then centrifuged at 3500-4000 rpm for 5 minutes to separate the organic layer. An aliquot of 10.0 mL ethyl acetate extract (upper layer) was transferred to the 15 mL dispersive-SPE tubes containing 0.20 ± 0.001 g (PSA) and 2.00 ± 0.01 g anhydrous $MgSO_4$ and also tubes with 10.00 mg (sugarcane ash) sorbent and 2.00 ± 0.01 g anhydrous $MgSO_4$ for cleanup (dispersive solid phase extraction, DSPE). The tubes

were tightly capped and shaken vigorously for one min and later centrifuged at 5,000 rpm for 2 min. Two mL of the supernatant ethyl acetate extract was transferred to a clean dry test tube and completely evaporated using turbo-vap nitrogen concentrator, with the water bath temperature maintained at 50°C and nitrogen flow rate at 15 psi. The residue was reconstituted in 1 mL n-hexane: toluene (1:1) and analyzed by Gas Chromatograph (GC) with Electron Capture Detector (ECD). The GC separation was conducted at following conditions: N_2 gas flow, 0.79 mL/min; Make up, 30 mL/min; inlet temperature, 280°C; injection volume, 1 µl; Spilt ratio, 1:10; Detector (ECD) temperature, 300°C; initial oven temperature, 170°C, held for 5 min, then a 1.5°C/min ramp to 220°C, held for 10 min followed by a 4°C/min ramp to 280°C (held for 7 min).

Method validation as per SANCO guideline 2012: The method must be tested to assess for mean recovery, sensitivity (as a measure of trueness), precision, and limit of quantification (LOQ). This effectively means that spiked experiments to check the accuracy of the method should be undertaken. A minimum of 5 replicates is required to check the precision and sensitivity of the method. The LOQ is defined as the lowest validated spike level meeting the method performance acceptability criteria (mean recoveries) for each representative commodity in the range 70-120%, with an RSD (≤ 20%). Other approaches to demonstrate that the analytical method complies with performance criteria may be used, provided that they achieve the same level and quality of SANCO information[24].

Data Processing: The chromatograms were acquired using computer-based software. The concentration of the unknown was calculated from the equation using regression analysis of the reciprocal of the pesticide residue concentration as weighting factor (1/x).

$y = mx + c$

Where: y = Analyte area, x = Concentration of analyte, m = Slope of the calibration curve, c = Intercept

Concentration (µg/g) = (concentration found in µg per gram x final volume)/(weight of sample taken (g)

Results and Discussion

Chromatographic analysis of the sample extracts were carried out using GC coupled with ECD which is known for its high sensitivity for halogenated pesticides even all kinds of electron-attracting functional groups such as nitro groups and aromatic structures also give a response on this detector[25,26]. The sugarcane ash contain 35 % carbon was used for clean up in parallel with PSA. It was found to absorb the pigments and other co-extracts in fruits/vegetables as presented in figure-1.

Method Validation: The test method for the simultaneous determination of the four insecticides in apple, orange, grape, tomato, cauliflower, cabbage and brinjal using low cost adsorbent was validated according to the SANCO guidelines 2012. Validation of the developed method covered the parameters such as accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, linearity, range, specificity and system suitability.

Accuracy (Preliminary Test): The accuracy of an analytical method is the closeness of results obtained by the method being validated to the true value. The accuracy of the method was estimated through recovery experiment. For this purpose, samples (apple, orange, grape, cabbage, tomato, cauliflower and brinjal) were spiked with a mixture of insecticides at five levels 0.05, 0.1, 0.2, 0.5, 2.0 µg/g in triplicate and processed separately as per the methodology described above. The recoveries and reproducibility (RSD) of the developed method varied from of 80.36% – 108.69% and 3.00 % – 4.50%, respectively, and were considered acceptable, indicating satisfactory accuracy as given in table-1. According to SANCO requirements recovery values are deemed acceptable if lies between 70–120 %. These values are similar to the recoveries reported by other author for the Matrix Solid Phase Dispersion (MSPD) extraction of several insecticides from fruits and vegetables[27].

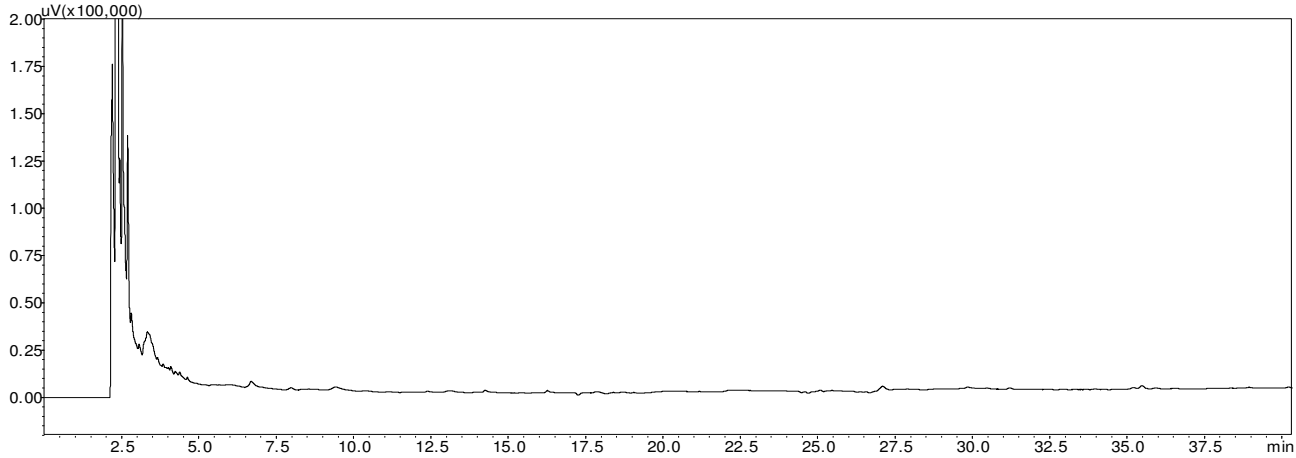

Figure-1
GC-ECD profile of grape extract after clean-up with sugar-cane ash

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ values for the determination of dimethoate, malathion, alfa and beta endosulfan by the proposed GC-ECD method were calculated by the signal-to-noise (S/N) ratio obtained from serial dilution of the standard solution and injection of the blank solution. LOD is the lowest concentration of analyte in a sample that can be detected, but not quantitated, under the stated experimental conditions. The analyte concentration that produced an S/N of >3 was accepted as the LOD. Typical limits of detection ranged from 0.005-0.01 µg/mL with the method. The lowest concentration which produced a S/N of >10was considered as the Limit of Quantification (LOQ) of the method. The LOQ obtained for dimethoate and malathion were 0.031 and 0.045 µg/g respectively, while they were 0.01 and 0.02 µg/g for alfa and beta endosulfan, respectively. The obtained LOD and LOQ values demonstrate that the method is sensitive enough to detect residues at the required lower level for 0.05µg/g.

Precision: Instrument injection precision was tested for both retention time and peak area for all target compounds by repeated injections (n=7) of low concentration of 0.01 µg/mL of matrix standard solutions. Instrument injection precision for retention time was below 0.5% for all compounds and between 1.50–1.75% for peak area without internal standard compensation indicating reliable instrument performance. Within-day and between-day precision values for the method were determined for each matrix at three different spiking levels (0.1, 0.2 and 0.5 µg/g) and expressed as %RSD over 5 days with individually prepared samples (n=5). Mean value for within day precision was determined by considering average of the 5 individual days mean precision, while between day precision was expressed as mean of the overall precision data. The % RSD values (3.65-9.80) indicate that method is sufficiently precise. According to SANCO requirements <20% was set as acceptance criteria for the target compounds and matrices. Measured values are shown in table-2 and 3.

Table-1
Preliminary recovery (%) study of dimethoate, malathion, alfa and beta-endosulfan at 0.05, 0.2, 0.4, 0.5 and 2 µg/g spiking levels using PSA and sugarcane ash as clean-up sorbent

Insecticide	Fruit/vegeta -ble	Recovery (%)*									
		Primary Secondary Amine					Sugarcane Ash				
		0.05 (µg/g)	0.2 (µg/g)	0.4 (µg/g)	0.5 (µg/g)	2.0 (µg/g)	0.05 (µg/g)	0.2 (µg/g)	0.4 (µg/g)	0.5 (µg/g)	2.0 (µg/g)
Dimethoate	Apple	91.02	89.68	84.52	99.99	105.65	83.65	90.56	85.65	92.65	89.99
	Orange	85.89	97.89	86.35	108.65	108.02	81.75	85.65	88.56	98.69	90.35
	Grape	82.25	90.25	84.05	99.25	99.65	87.89	94.25	89.65	104.52	102.69
	Cabbage	82.35	91.26	99.88	96.35	86.32	84.56	85.25	87.41	96.58	93.58
	Cauliflower	80.89	95.65	86.88	90.36	98.68	89.58	82.35	82.65	91.69	98.69
	Tomato	83.52	82.35	85.81	97.34	105.65	91.12	84.25	87.56	97.65	85.63
	Brinjal	80.36	88.36	83.25	87.65	109.36	96.36	90.36	84.65	99.65	101.25
Malathion	Apple	84.95	90.35	91.23	99.36	100.36	92.65	96.25	88.25	96.58	107.36
	Orange	83.65	91.35	90.65	89.59	105.65	94.65	96.35	86.65	98.69	98.69
	Grape	90.98	94.32	95.65	88.35	98.65	95.25	97.58	87.58	93.52	100.56
	Cabbage	81.58	92.58	98.58	98.65	97.85	96.35	92.36	99.65	97.36	92.36
	Cauliflower	80.90	90.58	91.05	95.65	105.68	93.05	98.58	107.85	89.65	94.58
	Tomato	81.21	93.65	93.65	90.25	108.74	94.96	94.25	87.65	98.69	90.68
	Brinjal	82.97	94.06	92.52	93.65	110.25	86.58	96.98	79.58	104.05	95.03
Alfa-Endosulfan	Apple	85.58	96.33	95.65	96.35	100.32	99.85	91.25	76.35	87.65	94.36
	Orange	86.58	99.02	90.58	87.58	99.02	90.89	93.62	87.96	89.69	90.25
	Grape	87.42	90.65	96.58	99.68	96.35	94.56	94.25	89.65	89.25	99.69
	Cabbage	81.25	91.65	89.65	97.55	91.25	97.05	98.62	99.68	94.65	95.65
	Cauliflower	82.65	93.65	96.52	102.33	93.65	92.65	86.62	89.36	92.65	90.36
	Tomato	84.25	84.65	97.58	96.58	97.58	91.25	94.52	97.58	90.02	105.58
	Brinjal	81.32	98.65	90.52	105.65	95.65	93.65	86.58	85.62	89.26	96.69
Beta-Endosulfan	Apple	83.65	96.58	87.58	99.65	93.65	94.35	82.35	86.35	85.08	93.65
	Orange	82.52	99.85	85.65	95.52	94.58	90.54	84.56	84.56	87.69	104.32
	Grape	84.23	90.65	93.56	103.65	93.65	91.85	94.56	102.35	92.15	102.39
	Cabbage	85.65	92.65	94.36	94.58	97.52	82.65	92.35	86.52	98.25	99.68
	Cauliflower	83.65	98.58	95.65	100.87	96.35	85.58	85.35	87.65	88.12	96.52
	Tomato	85.23	93.65	99.58	98.32	93.62	90.89	91.58	89.05	99.68	97.36
	Brinjal	86.23	91.58	94.03	91.52	90.25	91.54	88.25	100.36	108.69	99.65

System suitability: The parameters, retention time, resolution factor and trailing factor, were evaluated. The variation in retention time among 7 replicate injections of standard solution (0.01 – 5 µg/mL) containing all insecticides was very low, with %RSD values ranging from 0.245 – 0.365. The results obtained from the system suitability tests are in agreement with the requirement (≤2) of SANCO guideline 2012.

Linearity: Peak areas (average of 3 replicate injections) versus concentration, were plotted for dimethoate, malathion, alfa and beta endosulfan over the concentration range of 0.01 - 5 µg/mL of the target level. Good correlation coefficients (r^2) were obtained for all of the compounds ranging from 0.9965 to 0.9999. The correlation coefficient for all 4 insecticides (r^2 >0.996) suggest that the method has a broad linearity range.

Specificity: No interferences were observed in the different fruit and vegetable matrices taken for the study. As shown in Figure-1 and 2, all insecticides, i.e., dimethoate, malathion, alfa and beta endosulfan are separated with fairly good resolution without any interference.

Range: To demonstrate the working range of the proposed method, 7 samples each of the lowest concentrations (0.01-0.031 µg/g) and highest concentration (5 µg/g), similar to the accuracy sample were prepared for analysis. The mean recovery ranged between 81 and 110 % and the % relative standard deviation (% RSD) was below 10%.

Robustness: Robustness of the developed analytical method was studied by varying parameters like laboratory personnel, extraction and clean-up batches. Results were compared with the original method and significant differences were sought based on Analysis of Variance (ANOVA). None of the parameters which were varied led to significant differences in measured values, consequently indicating that the method is robust.

Table-2

Intra-assay (% recovery) of dimethoate, malathion, alfa-endosulfan and beta-endosulfan at 0.1, 0.2 and 0.5 µg/g spiking levels using PSA and sugarcane ash as clean-up sorbent

Insecticides	Fruits/vegetables	Recovery (%) and Relative Standard Deviation (n=5)					
		Primary Secondary Amine			Sugarcane Ash		
		0.1(µg/g)	0.2(µg/g)	0.5(µg/g)	0.1(µg/g)	0.2(µg/g)	0.5(µg/g)
Dimethoate	Apple	81.02±9.90	89.68±7.40	84.52±5.80	83.65±8.80	87.56±8.50	95.65±7.60
	Orange	85.89±8.50	87.89±6.50	86.35±6.10	80.65±9.95	85.65±7.00	98.56±6.30
	Grape	84.25±6.50	85.25±8.60	84.05±4.20	84.89±8.60	84.25±7.60	89.65±5.10
	Cabbage	82.35±9.25	86.26±5.60	89.88±5.60	84.56±8.70	84.25±8.10	97.41±4.20
	Cauliflower	82.89±8.95	85.65±6.10	86.88±6.30	86.58±9.30	82.35±5.60	89.65±3.90
	Tomato	82.52±7.60	82.35±5.90	85.81±4.60	82.12±9.90	84.25±6.10	99.56±4.10
	Brinjal	81.36±8.20	88.36±7.10	83.25±4.80	89.36±7.80	90.36±5.80	94.65±3.70
Malathion	Apple	84.95±7.00	88.35±6.50	81.23±4.10	82.65±8.60	86.25±6.30	91.25±5.60
	Orange	83.65±6.90	89.35±8.15	84.65±5.05	84.65±9.00	96.35±6.50	96.65±6.40
	Grape	88.98±9.00	84.32±5.90	85.65±4.60	85.25±9.80	97.58±6.70	97.58±5.30
	Cabbage	81.58±9.60	82.58±6.50	88.58±4.50	82.35±9.95	92.36±7.60	99.65±4.60
	Cauliflower	87.00±8.70	85.58±6.30	81.05±4.70	83.05±8.70	88.58±5.60	97.85±7.00
	Tomato	81.21±9.60	83.65±6.20	83.65±5.10	84.96±8.60	94.25±6.30	97.65±5.30
	Brinjal	82.97±8.50	84.06±5.80	82.52±4.90	86.58±8.50	96.98±5.00	99.58±5.60
Alfa-Endosulfan	Apple	85.58±7.60	86.33±5.70	85.65±4.00	89.85±8.65	91.25±5.20	96.35±4.30
	Orange	86.58±9.30	89.02±5.90	90.58±4.60	81.89±7.50	93.62±5.30	87.96±4.50
	Grape	87.42±7.50	87.65±5.50	96.58±4.90	84.56±9.00	94.25±5.10	89.65±4.60
	Cabbage	81.25±8.00	88.65±6.30	89.65±5.20	87.05±7.60	98.62±4.80	99.68±4.10
	Cauliflower	82.65±6.80	83.65±7.20	96.52±6.00	82.65±8.00	96.62±4.90	89.36±4.30
	Tomato	84.25±5.90	84.65±7.60	97.58±5.30	81.25±7.50	84.52±4.70	97.58±4.50
	Brinjal	81.32±9.00	88.65±7.10	90.52±4.70	83.65±6.50	86.58±4.00	95.62±4.00
Beta-Endosulfan	Apple	83.65±8.70	86.58±5.80	87.58±4.20	84.35±7.00	92.35±3.90	86.35±3.90
	Orange	82.52±7.60	89.85±4.70	85.65±4.00	85.54±8.60	94.56±4.55	94.56±4.20
	Grape	84.23±7.70	84.65±6.10	83.56±4.30	81.85±9.20	84.56±5.20	98.35±4.00
	Cabbage	85.65±8.50	82.65±5.00	84.36±4.40	82.65±8.60	92.35±4.60	86.52±4.20
	Cauliflower	83.65±9.80	88.58±4.80	95.65±5.00	85.58±9.30	95.35±3.90	97.65±3.65
	Tomato	85.23±8.30	83.65±4.30	90.58±6.20	83.89±9.80	91.58±4.80	89.05±4.15
	Brinjal	86.23±9.10	81.58±4.60	94.03±5.00	84.54±7.40	98.25±5.00	100.36±4.85

Analysis in real sample: The concentrations of dimethoate, malathion, alfa-endosulfan and beta-endosulfan residues in selected fruits and vegetables collected from different regions in Mysore, Karnataka are shown in table-4. Among different fruits and vegetables analyzed under the present study, contamination was noticed in significant numbers of samples analyzed. About 16% of the tested fruit and vegetable samples (70) were found to be contaminated with low but measurable amounts of pesticide residues and it ranged from 0.10 to 0.25 µg/g. Among 13 samples of tomato analyzed, three samples (23 %) showed the presence of malathion, alfa-endosulfan and beta-endosulfan. The detected residues were in the range of 0.10-0.23 µg/g in case of malathion, 0.15-0.20 µg/g for alfa-endosulfan and 0.11-0.18 µg/g in case of beta-endosulfan. Out of 10 apple samples analyzed, malathion and alfa-endosulfan were present in only two samples (20%) which ranged 0.11-0.15 µg/g and 0.13 – 0.20 µg/g respectively. Only three orange samples (12.50 %) out of 12 orange samples were contaminated with dimethoate and residues ranged 0.10-0.16 µg/g. Only one cabbage sample (10 %) was contaminated with dimethoate and residue was found to be present at 0.15 µg/g. Of the 13 grape samples tested, about 15 % were found to contain malathion residues which ranged from 0.10 – 0.25 µg/g. None of the twelve cauliflower and brinjal tested samples showed the presence of pesticides tested under this study.

Table-3
Inter assay (% recovery) of dimethoate, malathion, alfa-endosulfanand beta-endosulfan at 0.1, 0.2 and 0.5 µg/g spiking level using PSA and sugarcane ash as clean-up adsorbent

Insecticides	Fruits/vegetables	Recovery (%) and Relative Standard Deviation (n=5)					
		Primary Secondary Amine			Sugarcane Ash		
		0.1 (µg/g)	0.2 (µg/g)	0.5 (µg/g)	0.1 (µg/g)	0.2 (µg/g)	0.5 (µg/g)
Dimethoate	Apple	81.25±11.25	83.65±13.52	88.65±10.20	83.65±11.25	95.65±9.85	99.58±10.55
	Orange	85.35±9.50	82.58±9.90	87.58±11.50	82.65±12.30	84.52±10.55	92.35±9.55
	Grape	83.52±12.50	84.65±10.30	89.60±9.60	83.52±14.55	86.50±12.55	96.65±10.55
	Cabbage	81.58±11.60	83.65±12.50	84.80±10.25	83.65±13.60	82.10±13.02	97.58±12.60
	Cauliflower	82.36±8.96	85.25±10.60	109.25±13.30	81.65±12.55	87.98±14.10	99.68±11.50
	Tomato	84.58±13.50	84.25±8.60	85.60±11.60	83.62±9.95	91.25±12.30	97.52±14.20
	Brinjal	88.56±14.80	82.98±10.30	86.05±14.20	82.58±9.20	90.00±13.40	99.68±13.60
Malathion	Apple	85.25±10.25	83.62±12.50	82.52±15.00	92.36±10.10	94.58±14.20	98.65±14.65
	Orange	81.05±11.25	84.25±13.60	93.05±12.30	85.25±10.65	85.65±13.20	93.52±13.55
	Grape	84.65±10.20	83.65±8.90	101.58±9.60	84.65±12.55	90.25±11.30	86.25±14.20
	Cabbage	86.05±8.50	84.08±10.50	103.05±9.60	83.65±12.30	91.36±14.50	87.52±14.90
	Cauliflower	82.25±7.65	85.65±14.20	94.52±10.25	85.05±12.90	85.65±12.30	89.25±14.70
	Tomato	81.25±14.50	84.58±13.50	91.25±11.60	91.25±13.50	93.65±11.25	96.35±14.60
	Brinjal	83.05±15.00	87.65±14.10	96.35±12.60	90.95±12.40	92.15±13.60	108.25±13.60
Alfa-Endosulfan	Apple	84.58±13.20	89.65±13.60	86.52±14.33	85.69±13.20	84.68±14.20	107.99±12.90
	Orange	86.35±8.90	86.69±11.25	84.78±14.55	82.15±11.20	97.58±12.60	96.09±11.50
	Grape	82.25±8.50	87.25±13.00	88.35±15.00	82.25±13.20	96.35±12.20	99.65±11.90
	Cabbage	83.65±10.90	84.65±14.50	89.59±10.20	83.12±14.50	92.35±13.20	101.25±11.60
	Cauliflower	81.25±12.50	86.65±13.20	90.36±12.30	83.68±13.25	85.65±14.20	98.35±13.10
	Tomato	88.95±11.60	89.02±15.00	89.65±12.60	84.69±14.20	82.65±13.20	99.00±12.50
	Brinjal	86.35±10.20	87.69±14.60	88.36±13.30	84.56±13.30	86.35±14.00	105.36±12.70
Beta-Endosulfan	Apple	83.25±9.65	88.63±12.30	89.99±13.50	83.00±12.55	88.58±13.60	102.98±14.60
	Orange	81.25±7.50	90.35±11.60	90.35±14.80	89.58±13.80	87.58±14.20	95.00±14.60
	Grape	82.62±10.90	98.69±13.30	82.69±14.40	88.80±13.60	91.36±13.20	99.65±10.25
	Cabbage	83.32±10.50	88.26±14.50	93.58±12.20	91.65±14.20	85.69±14.50	98.52±12.60
	Cauliflower	84.25±9.60	83.65±12.00	98.69±11.90	93.62±14.90	88.25±12.25	94.50±8.95
	Tomato	83.62±12.30	86.25±11.80	85.63±12.65	95.65±12.60	99.65±11.20	89.25±11.65
	Brinjal	87.58±14.00	88.97±10.50	91.25±13.55	88.5±13.65	103.99±9.20	85.65±14.85

Table-4
Organochlorine and Organophosphorous insecticides concentrations in different vegetables and fruits of Mysore district

Fruit/vegetable	No of sample Analyzed	No of sample contaminated	Conc (µg/g)			
			Dimethoate	Malathion	Alfa-Endosulfan	Beta-Endosulfan
Apple	10	2	BDL	0.11 – 0.15	0.13 - 0.20	BDL
Orange	12	3	0.10 -0.16	BDL	BDL	BDL
Grape	13	2	BDL	0.10 -0.25	BDL	BDL
Cabbage	10	1	0.15	BDL	BDL	BDL
Cauliflower	12	0	BDL	BDL	BDL	BDL
Tomato	13	3	BDL	0.10 -0.23	0.15 – 0.20	0.11 -0.18
Brinjal	12	0	BDL	BDL	BDL	BDL

Table-5
The maximum residue limits of organochlorine and organophosphorous insecticides in fruits/vegetables

Fruit/vegetable	Insecticide	Codex MRL (µg/g)	FSSAI* MRL (µg/g)
Tomato andApple	Malathion	0.50	5.00
	Endosulfan		
Orange	Dimethoate	5.00	
Grape	Malathion		
Cabbage	Dimethoate	0.05	

*Food Safety Standard Authority of India

In no sample exceeded the Maximum Residue Level (MRL) prescribed under Food Safety Standards Act, 2011. All detected pesticide residues samples were also compared with MRL of codex which presented in table-5.

Only one sample of cabbage was found to contain dimethoate residues three times more than the limit specified under codex as given in table-4. From the study, it is evident that the contamination level of dimethoate, malathion, alfa-endosulfan and beta-endosulfan in the tested varieties of fruits and vegetables is very low which could be probably due to its smaller usage dose. The results obtained in the present study are similar to the reported in previous studies where low levels of pesticide residues were determined in different types of fruit and vegetables[28].

Judicious use of pesticides with proper Good Agricultural Practice (GAP) adopted by farmers in the cultivation of vegetable and fruits crops may be one of the major reasons for detecting residues at a very low level. Pesticides, in particular, highly persisting organochlorines (OCs) enters food chain and gets accumulated in the human and animal body through the consumption of contaminated food commodities and may produce toxicological hazards[29-30].

Conclusion

It is evident from the results of the study that the proposed method is suitable for the routine monitoring of dimethoate, malathion, alfa and beta endosulfan residues in fruits/vegetables. And also, the proposed method is simple, economical, and precise and can be extended to analyze traces of insecticide residues in other fruits and vegetable samples too by carrying out minimum method validation studies. From the studies, it is clear that the fruits and vegetables collected from in and around Mysore city, India are comparatively safe with respect to dimethoate, malathion, alfa-endosulfan and beta-endosulfan pesticide residues. A regular monitoring of insecticide residues in food commodities, soil and water by the regulatory authorities of food quality control and safety is the need of the hour to safe guard the health of the consumers. Use of insecticides in appropriate dose and restricting the use of insecticides just before harvesting the crop and during transportation and storage will reduce the concentration levels of insecticide residues in fruits and vegetables. By providing proper education and awareness among farming community, the risk of occurrence of high level insecticide residues in foods may be avoided.

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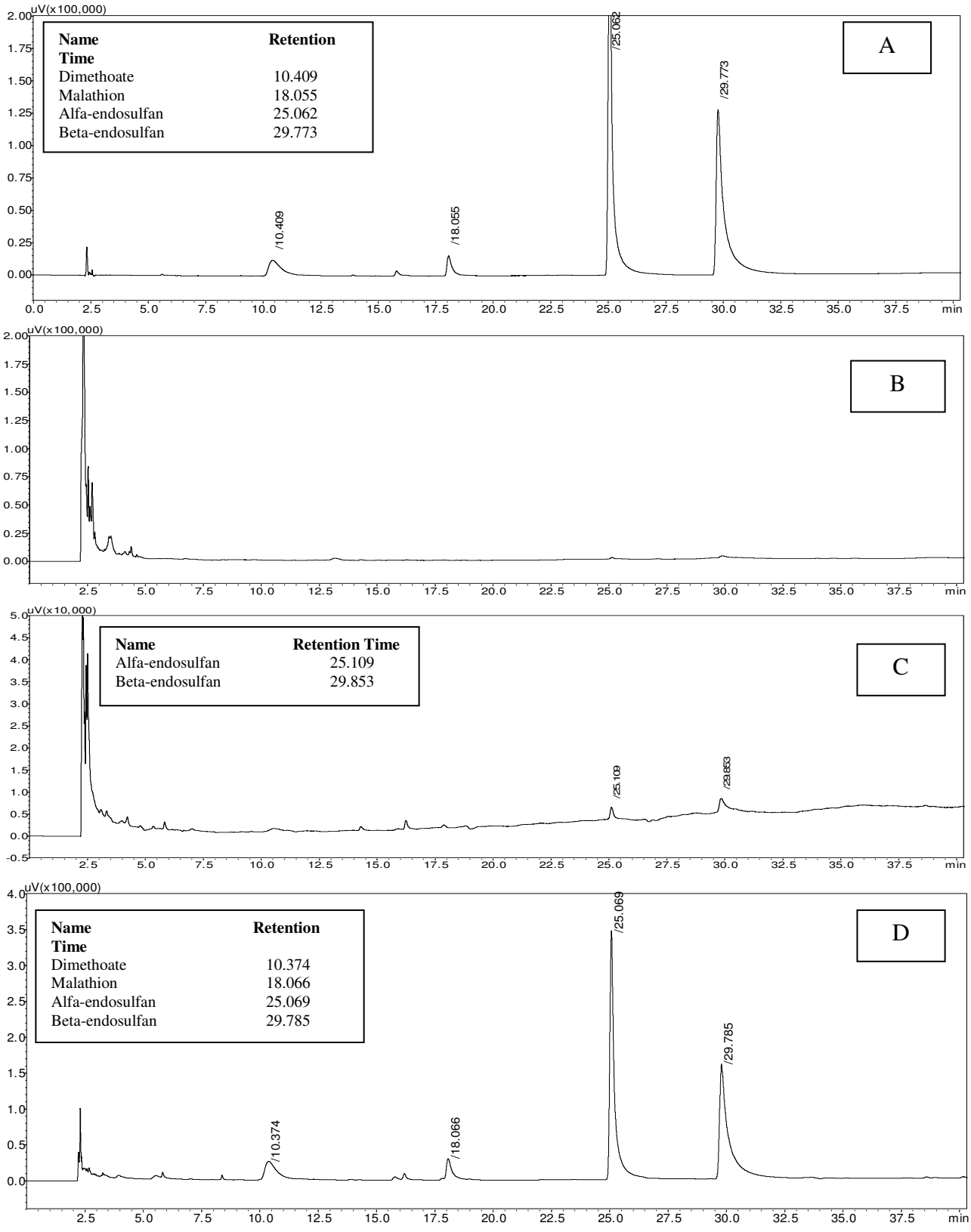

Figure-2

GC-ECD Chromatograms: (A) Standard (B) A negative tomato sample (C) A Positive tomato sample contaminated with alfa-endosulfan and beta-endosulfan (D) Spiked tomato sample at 0.25 µg/g level

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