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QuEChERS METHOD VALIDATION AND ANALYSIS OF PESTICIDES IN GAS CHROMATOGRAPHY ELECTRON CAPTURE DETECTOR

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ABSTRACT

A gas chromatography (GC) electron capture detector (ECD) method was developed and validated for the determination of the residues of 33 pesticides in vegetables recovered from the developed method been validated for the extraction of pesticides of various chemical classes from vegetables such as tomato and brinjal. A mixture of 33 pesticides amenable to gas chromatography (GC) were quantitatively recovered from spiked tomato and brinjal, determined using gas chromatography-electron capture detector. The sample preparation approach is known as QuEChERS, which stands for "quick, easy, cheap, effective, rugged and safe". As expected, the results are excellent and showed overall average of 98% recoveries with 10% RSD. The method involved extraction with acetonitrile, liquid-liquid partition with addition of NaCl followed by $MgSO_4$ and primary secondary amine (PSA) and the analyses were carried out with GC–ECD equipment. It was a rapid, simple and cost effective procedure. The spiking levels for the recovery experiments were 0.1, 0.5 mg Kg^{-1} for GC–ECD analyses. Adequate pesticide quantification and identity confirmation were attained, even at the lowest concentration levels, considering the high signal-to-noise ratios, the very good accuracies and precisions, as well as the good matches between the observed ion ratios. Mean recoveries of Organo chlorines (OCs) in tomato varied from 80.50-92.20%, in brinjal varied from 78.8-89.9% and Organo phosphates (OPs) in mean recoveries of tomato varied from 75.30-92.50% and brinjal varied from 76.7-91.1%. Mean recoveries of SYNTHATIC pyrethroids (SPs) in tomato varied from 70.40-92.30% and brinjal varied from 73.3-90.5% and relative standard deviations (RSD) were generally below 10% (4.3% on average). Based on these results, the methodology has been proven to be highly efficient and robust and thus suitable for monitoring the MRL compliance of a wide range of commodity/pesticide combinations.

Key words: QuEChERS methodvalidation, Pesticides, GC–ECD, Vegetables.

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INTRODUCTION

India with about 4% of the world's cropped area shares around 1.7% of global pesticide consumption of the total 54,135 MT technical grade pesticide consumption in India in agriculture during 1999-2000, 60% were insecticides, 21% fungicides, 14% herbicides and 5% others. The percentage of organochlorines during this period has decreased from 40 to 14.5% accompanied by a sharp increase in consumption of organophosphates from 30 to 74%[1]. Consumption of pesticides in Haryana in agriculture during 1999-2000 was 5,030 MT. This followed Uttar Pradesh (7,400 MT), Punjab (7,100MT) and Andhra Pradesh (7,000 MT). However, the g/ha consumption in Haryana was 8,481 MT as compared to the average consumption of 288 g/ha in the country[1].

The use of pesticides is considered to be indispensable practice for the production of adequate food supply for growing population worldwide and for the control of insectborne diseases[2]. Thus, contamination of the environment with pesticides and their entry into the food chain is unavoidable especially in developing countries. Organo chlorines that enter the food chain bioaccumulate due to their lipophilicity and remain in ecosystem for a long period of time[3]. Monitoring studies from Asia revealed widespread contamination of foodstuff and animal feed with pesticide residues[4].

Consumers' perception of food quality has always been subject to change over time. In recent years, it has been observed that a substantial increase in the importance is placed on aspects related to pesticide residues and a growing demand is there for better agricultural practices, transparency and traceability in the production and marketing of conventional food. With increase in the violation rates of maximum residue limits (MRLs) and the detection of numerous pesticide misuse incidents in recent years (mainly through the use of more advanced analytical techniques), both the food industry and governments have been heavily criticized by consumer organizations and prompted to take action that will greatly improve the situation. Numerous countries are thus currently initiating programs to reduce pesticide usage in conventional agriculture, as well as promoting so called "farm to fork," "right to know" and "name and shame" laws.

Vegetables are the important ingredient of the human diet for the maintenance of the health and prevention of disease in the Indian sub-continent. The total Indian meal constitutes about 150-250 g of vegetables per day[5]. A wide range of pesticides are globally used for crop protection against pest infection during the cultivation of vegetables[6,7].

Literature reveals that vegetables contain the residues of pesticides above their respective maximum residue limit[8] may pose health hazards to consumers[9,10]. Monitoring of pesticides is conducted globally to assess the environmental load of their residues. Currently pesticides (OPs, SPs and H) enjoy wide use in the world as an alternative pest control replaying persistent organochlorines[11-13]. Because of wide spread use of pesticides, the presence of their toxic residues[14] have been reported in various environmental component/ commodities[15-26]. These pesticide residues find their way into the human body through food, water, and environment. Thus, analysis of pesticide residues in food and other commodities like water, fruits, vegetables, and total diet has become essential requirement for consumers, producers, and food quality control authorities. In view of the above and to assess the present environmental load of the pesticide residues, it is imperative to determine the amount of pesticide residues in vegetable samples in India.

The increasing expectations and the overall tightening of quality control systems have also strongly affected governmental food surveillance and monitoring laboratories, which also feel pressure from the European Union (EU) as well as from consumer organizations to effectively and comprehensively control pesticide residues in food. Both governmental and private laboratories have been subjected to growing criticism over the last few years for not adjusting their scope of analysis to actual pesticide usage patterns, which is essential if the monitoring and surveillance system is to be efficient and effective, and is also part of the food industry's duties of self-control and care.

In order to address these growing expectations, many governmental and private laboratories are currently in the Process of modernizing their approaches to pesticide residue analysis. These efforts focus on both sample preparation- where there is a trend to shift from laborious traditional methods to new fast and simple approaches, such as the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) multiresidue method—and instrumental analysis, where there have fortunately been dramatic improvements in recent years in terms of separation technology [e.g., ultra pressure liquid chromatography (UPLC)] as well the sensitivity and selectivity of detection [e.g., tandem mass spectrometry, time of flight (TOF)].

QuEChERS is a novel sample preparation methodology for pesticide multiresidue analysis that was developed between 2000 and 2002 and first reported in 2003. It has already been widely accepted by the international community of pesticide residue analysts. The QuEChERS procedure involves an initial extraction with acetonitrile followed by an

extraction/partitioning step after the addition of a salt mixture. An aliquot of the raw extract is then cleaned up by adding $MgSO_4$ and PSA. The final extract in acetonitrile is directly amenable to determinative analysis based on LC and/or GC. The QuEChERS method effectively covers a very wide analytic scope, including highly polar pesticides as well as highly acidic and basic ones. Additional advantages of the method include the high sample through put and the low amounts of solvent, glassware, and bench space required. The method was primarily designed for low-fat commodities, but commodities with intermediate or high fat contents can also be analyzed when certain aspects are taken into account. This method is accurate and high recoveries will be achieved for many pesticides in many matrices even if different ratios and types of sample size, solvent, salts and sorbents are used in modifications. In multiclass, multiresidue pesticide analysis, the sample preparation method inherently necessitates broad analytical scope which makes it impossible to obtain a high degree of cleanup without reducing recoveries for some pesticides.

EXPERIMENTAL

Chemicals like *n*-hexane, acetone, and acetonitrile (HPLC grade) were purchased from Merck, USA, and were glassware distilled before use. Acetone was refluxed over potassium permanganate for 4 h and then distilled. Sodium chloride (NaCl), anhydrous sodium sulfate (Na_2SO_4), and anhydrous magnesium sulfate ($MgSO_4$*)* procured from Merck Pvt. Ltd. India. Before use, anhydrous sodium sulfate (Na_2SO_4*)* and anhydrous magnesium sulfate ($MgSO_4$) were purified with acetone and baked for 4 h at 600^oC in muffle furnace to remove possible phthalate impurities. Primary secondary amine (PSA) bondasil 40 μm part 12213024 were purchased from Agilent. Pesticide standards were procured from Supelco Sigma– Aldrich USA and Fluka Sigma–Aldrich, New Delhi, India.

Sample collection

Two different vegetables of tomato and brinjal were collected from the field of Student farm, College of Agriculture, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad, Andhra Pradesh, India.

Extraction and cleanup

The collected fresh vegetable sample (100 g) was chopped, and grind in warring blander. 15 g sample of each vegetables in triplicate was taken for multi-pesticide residue analysis by QuEChERS method. Fifteen grams of macerated sample was mixed with 30 mL

acetonitrile and 3 g of NaCl was added and centrifuged at 2500 rpm. Then 9 g of sodium sulphate was added to remove water content, and vortexed for 10 min at 50 rpm on rotospin test tube mixture. The extract was centrifuged for 10 min at 10,000 rpm. One milliliter aliquot of supernatant extract was cleaned with the mixture of 0.4 g PSA, 1.2 g anhydrous. $MgSO_4$ and 10 mg activated charcoal. The extract was again shaken for 10 min at 50 rpm on rotospin and centrifuged for 10 min at 10,000 rpm. The 2 mL of supernatant was collected and evaporated with Turbo vap and finally made up to 1 mL with hexane. One microliter of clean extract was used for the multi pesticide (OCs, SPs, and OPs) residues analysis on gas chromatography (GC).

Analysis

GC-ECD

The final extracts were analyzed by Gas Chromatography (GC Schimadzu 2010) equipped with fused silica capillary column MR-1 (30 mt × 0.25 mm id) coated with 1% phenyl-methylpolysiloxane (0.25 μm film thickness) using 63Ni electron-capture detector (ECD) for the analysis of OCs, OPs and SPs. General operating condition were as follows: Column temperature program: initially 130°C for 5 min, increase at 3°C/min to 180°C hold for 5 min, then 240°C increase 2°C/min hold for 33.3 min, Total programme 90 min; injection volume: 1 μL nitrogen flow rate 0.79 mL/min and makeup 35 mL/min with split ratio 1 : 10; using carrier gas (N2) 99.5%; Injector port temperature 280°C; detector temperature 300°C (Figs. 1, 2, 3).

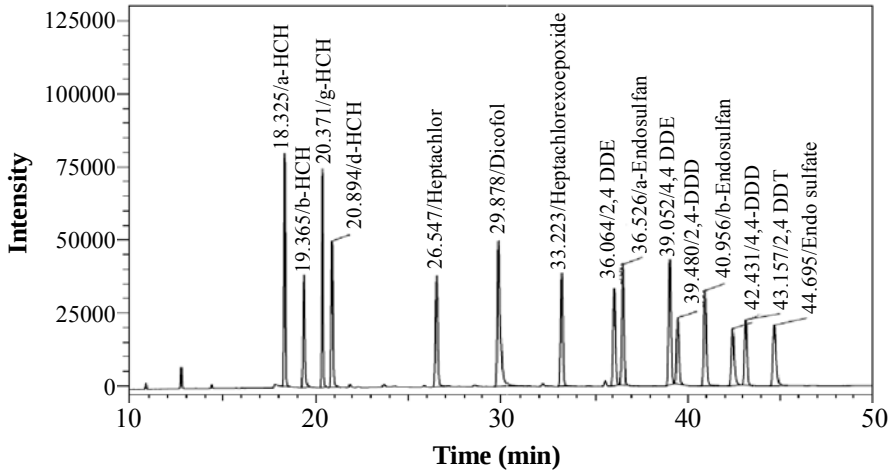

Fig. 1: GC ECD Chromatogram for organo chlorinated pesticides

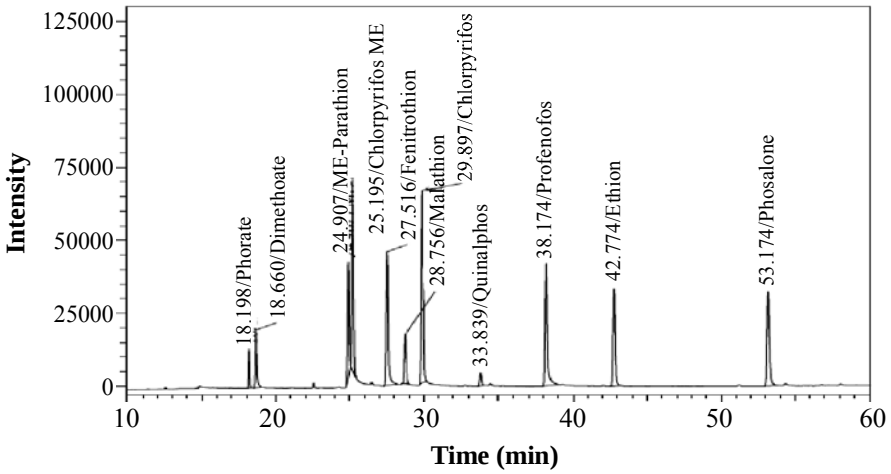

Fig. 2: GC ECD Chromatogram for organo phosphate pesticides

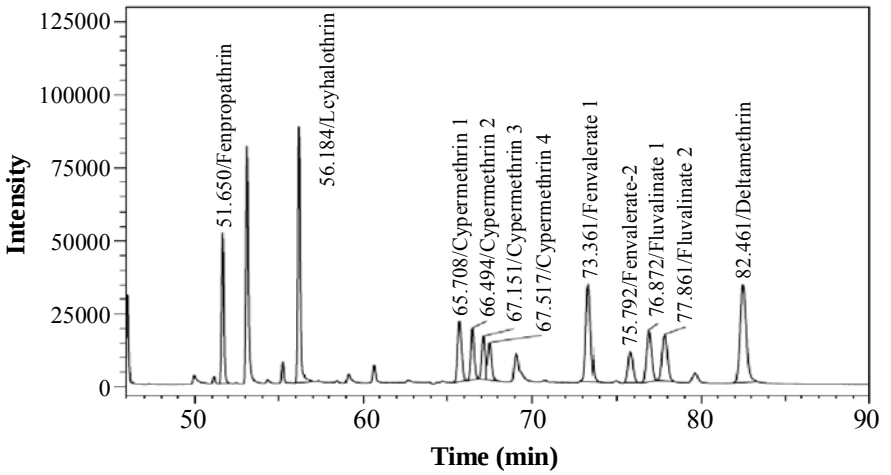

Fig. 3: GC ECD Chromatogram for synthetic pyrethroid pesticides

RESULTS AND DISCUSSION

The percent recovery, limit of detection and retention time of 15 OC analyzed pesticides in two different vegetables are shown in Table 1. The analyzed pesticides are α-HCH, β-HCH, γ-HCH, δ-HCH, Heptachlor, Dicofol, Heptachlor exo epoxide, *o*,*p*-DDE, *p*,*p*-DDE, *o*,*p*-DDD, *p*,*p*- DDD, *p*,*p*-DDT, α-Endosulfan, β-Endosulfan and Endosulfan sulphate. LOD of OP pesticides varied in the range of 0.001-0.002 mg Kg^{-1}. Similarly, the percent recovery of OCs is in the range of 80.5-92.2% and 78.8-89.9% in tomato and brinjal respectively with fortification level of 0.1 mg Kg^{-1}.

Table 1: The percent recoveries and retention time of OCs in fortified vegetable samples

Pesticides	Fortification level (mg Kg^{-1})	Recovery (%) in Tomato	Recovery (%) in Brinjal	Limit of detection (mg Kg^{-1})	Retention time
α-HCH	0.1	85.1	82.1	0.001	18.33
β-HCH	0.1	88.2	83.5	0.001	19.36
γ-HCH	0.1	91.0	84.7	0.001	20.37
δ-HCH	0.1	83.5	79.9	0.001	20.89
Heptachlor	0.1	88.3	80.8	0.001	26.55
Dicofol	0.1	88.0	81.9	0.001	29.88
Heptachlorexo-epoxide	0.1	87.1	78.3	0.001	33.22
o,p DDE	0.1	92.2	89.9	0.001	36.06
α-Endosulfan	0.1	83.0	79.0	0.001	36.53
p,p DDE	0.1	88.6	82.2	0.001	39.06
o,p DDD	0.1	89.4	84.9	0.002	39.48
β-endosulfan	0.1	83.3	78.7	0.001	40.96
p,p DDD	0.1	80.5	79.6	0.002	42.44
o,p DDT	0.1	87.6	80.0	0.001	43.17
Endosulfan sulphate	0.1	89.9	83.0	0.001	44.70

The percent recovery, limit of detection and retention time of 12 OP analyzed pesticides in tomato and brinjal are shown in Table 2. The analyzed pesticides are Dichlorvos, Phorate, Dimethoate, Methyl-parathion, Chlorpyrifos-methyl, Fenitrothion, Malathion, Chlorpyrifos, Profenofos, Ethion and Phosalone. LOD of pesticides is in the range of 0.001-0.009 mg Kg^{-1}. Similarly, the percent recovery of OPs is in the range of 75.3-92.5% and 76.7-91.1% in tomato and brinjal, respectively with fortification level of 0.5 mg Kg^{-1}.

Table 2: The percent recoveries and retention time of OPs in fortified vegetable samples

Pesticides	Fortification level (mg Kg^{-1})	Recovery (%) in Tomato	Recovery (%) in Brinjal	Limit of detection (mg Kg^{-1})	Retention time
Dichlorvos	0.5	79.5	73.9	0.005	5.246
Phorate	0.5	80.5	77.8	0.006	18.19
Dimethoate	0.5	82.5	80.1	0.005	18.65
Methyl parathion	0.5	78.6	81.6	0.005	24.89
Chlorpyrifos methyl	0.5	77.9	78.5	0.005	25.19
Fenitrothion	0.5	79.8	82.1	0.005	27.51
Malathion	0.5	80.5	84.0	0.005	28.75
Chlorpyrifos	0.5	83.5	91.1	0.005	29.90
Quinolphos	0.5	75.3	76.7	0.009	33.84
Profenophos	0.5	90.5	88.8	0.005	38.16
Ethion	0.5	92.5	90.0	0.005	42.76
Phosalone	0.5	85.5	86.5	0.005	53.15

The percent recovery, limit of detection and retention time of 6 SP analyzed pesticides i,e Fenpropathrin, λ-Cyhalothrin, α-Cypermethrin, Fenvalerate and Fluvalinate in tomato and brinjal are shown in Table 3. LOD of pesticides varied in the range of 0.001-0.009 mg Kg^{-1}. Similarly, the percent recovery SPs is in the range of 70.40-92.30% and 73.3-90.5% in tomato and brinjal, respectively with fortification level of 0.50 mg Kg^{-1}.

Table 3: The percent recoveries and retention time of SPs in fortified vegetable samples

Pesticides	Fortification level (mg Kg^{-1})	Recovery (%) in Tomato	Recovery (%) in Brinjal	Limit of detection (mg Kg^{-1})	Retention time
Fenpropathrin	0.5	73.5	72.9	0.01	51.70
λ-cyhalothrin	0.5	76.2	80.8	0.01	56.23

Cont…

Pesticides	Fortification level (mg Kg^{-1})	Recovery (%) in Tomato	Recovery (%) in Brinjal	Limit of detection (mg Kg^{-1})	Retention time
Cypermethrin	0.5	70.4	73.3	0.01	65.78
Fenvelarate	0.5	74.5	76.7	0.01	73.48
Fluvalinate	0.5	80.5	79.0	0.01	77.06
Deltamethrin	0.5	92.3	90.5	0.01	82.68

The use of acetone in place of acetonitrile in QuEChERS method has many advantages, but it has low recovery compared to acetonitrile and also, it is difficult to analyse in LC. The use of acetonitrile in QuEChERS method has good recovery including its ability to separate from water upon the addition of salt without the addiction to nonpolar solvent and amenability with GC and LC applications.

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if the centrifuge tube is shaken vigorously for a few seconds immediately after the addition of the salt mixture. One-minute extractions of the entire batch can be performed in parallel after the salt has been added to all of the samples. PSA removes acidic components, certain pigments (e.g., anthocyanidines) and to some extent sugars. On the other hand, freezing-out removes most of the lipids, waxes and sugars as well as other components with low solubilities in acetonitrile that may negatively affect the robustness of the GC and LC analysis. Following contact with PSA, the pH of the extracts increases, reaching measured values of > 8. This compromises the stability of base-sensitive pesticides. By acidifying the extracts quickly to pH < 5, degradation is reduced sufficiently to allow extracts to be stored for days (at least two weeks) at room temperature without the occurrence unacceptable losses of most pesticides. At this pH, acid-labile pesticides are also sufficiently stable for several days at room temperature. All of the pesticides within this study were stable for more than two weeks in the final QuEChERS extracts when stored at room temperature rather than the freezer. The impact of cleanup on the removal of matrix components from various commodity extracts has been evaluated via gravimetric measurements of the evaporated extracts before and after cleanup, and the results are accurate.

CONCLUSIONS

The QuEChERS methodology proved to be rapid and highly effective when applied to the determination and surveillance of a wide range of pesticides in vegetables, with

validation results being highly satisfactory in most cases. In GC ECD analysis, matrix effects resulting from certain commodity/pesticide combinations cannot be neglected and should be addressed in order to avoid incorrect results.

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