

ORIGINAL ARTICLE

Comparative Solvent Extraction Methods for Determination of Pesticide Residues in Different Food Matrices and Its Analysis by GC- FID

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ABSTRACT

CONTEXT: Pesticides are often used in agricultural sector to protect crops before and after their harvest. Pesticide residues are deposits of active components, metabolites, or breakdown products of pesticides discovered in some component of the environment. **AIM:** Pesticide residues in fruits and vegetables were determined by gas chromatography/flame ionization detector. **MATERIALS AND METHOD:** Brinjal, tomato and grapes were purchased from the local market. Extraction of the samples was carried out using ethyl acetate/methanol and acetonitrile/toluene extraction procedures to determine the percentage recovery of pesticides chlorpyrifos—an organophosphate and cypermethrin—a synthetic pyrethroids from fruits and vegetables from both the extraction procedures. The extracts were cleaned using graphitized carbon black, magnesium sulphate and primary, secondary amine (PSA).

ANALYSIS: The analysis was done using gas chromatography with flame ionization detector.

RESULTS AND CONCLUSION: The samples of brinjal, tomato and grape were spiked with known concentrations of pesticide samples. Most of the pesticides recovered 60-70% of their concentrations at 0.01-0.10mg/kg range under ideal extraction and clean-up procedures. The recovery of different pesticides was dependent on the types of extraction procedure used. For chlorpyrifos, ethyl acetate/methanol recover more of the pesticides and in case of cypermethrin, acetonitrile/toluene recover more of the pesticides. The lesser recovery of pesticides on 5 and 7 days was due to the conversion of pesticides in their metabolites that was not detected in GC-FID.

KEYWORDS | residues, GC-FID, chlorpyrifos, cypermethrin, ethyl acetate, acetonitrile

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INTRODUCTION

THE MAJORITY OF FRUITS AND VEGETABLES available in market are either artificially grown or sprayed with pesticides. Pesticides are often used to protect crops before and after their harvest. Pesticides of various types are currently used to manage pests and infections that wreak havoc on crops. Pesticidal residues are deposits of pesticides' active components, metabolites, or breakdown products discovered in some component of the environment following pesticide application, spillage, or dumping.¹ Residues exist due to

overuse of a pesticide when it was applied just before harvest, even if it was a permitted pesticide; pesticides that are not authorized for illegitimate pesticide use, and inappropriate pesticide application during storage and transportation. Pesticide residue analysis is a critical step in determining the safety of specific pesticides.¹The persistence of various pesticides left residual amounts in fruits and vegetables from a variety of areas with varying residual levels. Pesticide residue analysis is routinely performed using multi-residue methods

that include homogenization of the sample with an appropriate solvent, separation of the liquid portion of the sample from insoluble material, purification and clean up, and chromatographic determination.²

The Gas Chromatography (GC) analytical technique is commonly used to detect the presence of these compounds in fruits and vegetables.² Pesticide residues in different food matrices have been assessed using a range of analytical techniques. Most of them used gas chromatography with electron capture detection (ECD), nitrogen-phosphorus detection (NPD), or flame photometric detection (FPD). Pesticide residues in fruits and vegetables are evaluated in two steps: 1) extraction and clean-up of the target analytes from the matrices, and 2) determination of the target analytes. The first step involves the use of different techniques such as liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), and QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction.³

Liquid-liquid extraction (LLE), also known as partitioning, is a separation process consisting of the transfer of a solute from one solvent to another, the two solvents being immiscible or partially miscible with each other. Organic solvents such as acetonitrile, ethyl acetate, chloroform, hexane, 1, 2-dichloromethane, etc., are usually used in Liquid-liquid extraction methods for the determination of pesticide residues in food and the environment, due to their good solubility in several immiscible liquids, such as in water and organic solvents.⁴

Ethyl acetate is shown to be an almost universal solvent, having the ability to extract many different classes of pesticides from various commodities, resulting in thousands of analyses over the years. The loss of basic pesticides in acidic crops is one of the method's drawbacks. Extraction with ethyl acetate yielded higher recoveries for polar pesticides and was somewhat faster, easier, and less expensive to perform.¹ Other method is extraction with acetonitrile and purification with dispersive solid-phase extraction (d-SPE). Acetonitrile method consumes few amounts of

sample and toxic solvents. There is no need for blending, filtration, large volume quantitative transfers, evaporation/condensation steps, or solvent exchanges. This is critical because each additional step complicates the procedure and therefore causes systematic and random errors. When using acetonitrile method of extraction to determine pesticides in fruits and vegetables, matrix effects are eliminated, allowing for high recoveries of target analytes.⁵

This study used a gas chromatography-flame ionization detector to assess two extraction techniques for evaluating and determining pesticides in different food matrices. One extraction method is ethyl acetate/methanol and the other is acetonitrile/toluene. The comparison of extraction methods was done on brinjal, tomato and grapes spiked with known concentrations of pesticides. The purpose of this study was to determine the percentage recovery of pesticides from fruits and vegetables and to find out which extraction method extracts more of pesticides, is recovery will be similar to both extraction methods for both of pesticides or will be specific to pesticides. Recovery have been studied on known concentration level in three selected matrixes.

METHOD AND MATERIALS

Chemicals: The chemical reagents and stock solutions that were employed (acetic acid glacial, acetonitrile, ethyl acetate, toluene, magnesium sulphate, methanol, sodium sulphate, and sodium chloride) were obtained from codon biotech. Pvt. Ltd. The pesticides studied were - Chlorpyrifos and Cypermethrin. Codon Biotech Pvt. Ltd. provided the pesticides' standards, which were verified to be >90 percent genuine. Individual stock standard solutions were prepared in hexane at a concentration of 2g/L and kept hidden in the dark. The functional solutions were made with a 20mg/L concentration.

Specimen

The fruits and vegetables used in this study (brinjal, tomatoes, and grapes) were purchased from the local markets. The samples were stored and packaged in a fresh brown bag. To protect

the bag from humidity, a little packet of silica gel was placed inside. A known volume (100ul) from working solution of pesticides were spiked into the individual samples and the samples were collected on 1,3,5 and 7 days after spiking of pesticides.

Gas Chromatography/Flame Ionization Detector

Samples were analyzed using a NUCON Model 5890 Series GC with a capillary column and a Flame ionizing detector (FID). Samples were analyzed by direct injection of 1 μ L into an injection port maintained at 250°C. The FID was maintained at 230°C.

The GC oven temperature was initially held at 50°C for 2 minutes and then maintained at 250-50°C where it was maintained for the remainder of the run with a column having head pressure of 20 psi. Nitrogen gas was used as the carrier gas. Calibration curves were created for each pesticide using standard dilutions of the spiking solution.

Sample Preparation and Ethyl Acetate Extraction

Fruits and vegetable samples were cut into small bits and blended. In a mortar and pestle, a 5g amount of homogenate material was measured and grinded with 1gm of sodium sulfate and 1gm of sodium chloride to produce a fine paste after being injected with 100uL of test solution. The macerated sample was mixed and homogenized with 10ml of ethyl acetate on mechanical shaker for 1hr. After that, the mixture was then centrifuged for 30 minutes at 5000rpm. By adding a pinch of activated charcoal to the supernatant and letting it overnight, the complete organic phase was cleaned out. Filter the solution and then evaporated at 50°C in oven. The residue was then redissolved in 1mL of methanol or acetonitrile. The solution was then introduced into the GC/FID apparatus in a volume of 1uL.

Sample Preparation and Acetonitrile Extraction

Fruit and vegetable samples were cut into small bits and blended. A 7.5 gm of homogenized sample of fruits and vegetables injected with 100 μ L of test solution was weighed and

macerated with mortar and pestle into fine paste. The macerated sample was mixed with 7.5ml of acetonitrile containing 0.75 μ L acetic acid in a centrifuge tube. After that, 3g of MgSO₄ and 0.75gm of sodium acetate were also added. The tube was shaken forcefully for 4 mins and then centrifuged at 5000 rpm for 5 mins. Clean up procedure was done by adding 300mg of MgSO₄ and 50mg primary-secondary amine into the supernatant liquid extract. The extract was shaken for 20sec and centrifuged at 3000rpm for 5mins. The solution was then filter and evaporated at 40°C in oven. After that, the residue was redissolved in 1mL of toluene. The solution was then introduced into the GC/FID apparatus in a volume of 1uL. Extraction and analysis were done on the same day in fruits and vegetables samples to determine the percentage recovery for each sort of matrices under investigation at 0.01mg/kg concentration level.

The pesticide recoveries were evaluated by dividing the area under the peak of analyte from the spike samples to that of the standard solutions. Figure 1 shows chromatogram of a standard solution of chlorpyrifos pesticides (1 μ L), showing the retention time and area under peak of standard solutions. Recoveries were determined for spiked brinjal, tomato and grapes samples (100 μ L) obtained after extraction by ethyl acetate and acetonitrile methods. Recoveries (Table 1, Table 2 and Table 3) were above 50% for chlorpyrifos using ethyl acetate extraction method and above 30% in acetonitrile extraction method.

DISCUSSION

Extraction Technique Comparison

When pesticide-containing foods are extracted, the percentage transfer of residues into the solvent is determined by the polarity and solubility of pesticide compounds. A single clean solvent solution cannot yield satisfactory recoveries due to the enormous varieties in polarity and solubility presented in the chemicals examined. The best extraction method was chosen based on three criteria: recovery, extract purity, and the number of

pesticides extracted. Extracting solvents with greater polarities, such as acetonitrile, acetone, ethyl acetate, and others, are often used for extraction of pesticide residues with significant polarity variations from agricultural products of fruits and vegetables, according to a review of multiresidue pesticide methods.⁵ Therefore, a comparative study was conducted to determine the recovery rate and extraction efficiency of pesticides from these solvents. Ethyl acetate was chosen because it is both polar and miscible with water, allowing for good penetration into plant cells. Furthermore, unlike halogenated solvents, ethyl acetate is anti-hazardous and has lower disposal costs. Ethyl acetate has proven to be a nearly universal solvent, with thousands of analyses demonstrating its capacity to remove many distinct kinds of pesticides from numerous commodities. Pesticides are extracted from fruit and vegetable samples using the acetonitrile extraction method, involves shaking with acetic acid-acetonitrile and salting out with sodium acetate and magnesium sulphate.⁵ Because magnesium sulphate hydrate is extremely soluble in water, not only it binds water but also promotes the partitioning of pesticides into the organic phase. The approach involves combining the acetonitrile extract with PSA SPE sorbent and magnesium sulphate in a dispersive SPE cleanup.⁶

Ethyl acetate has the benefit of being partly immiscible with water, which eliminates the need for the addition of other nonpolar solvents to separate water from the extract. Sodium sulphate (Na_2SO_4) is commonly used in multi-residue method processes to improve polar component recovery. Chlorpyrifos was removed during the extraction and cleaning steps, yielding overall results of 50, 40, and 48 percent in brinjal, tomato, and grapes, respectively by ethyl acetate method rather than of acetonitrile method. The lesser recovery of chlorpyrifos in matrices was due to loss of pesticides in clean up stage of acetonitrile method.⁸ So, it is concluded that the average recovery of chlorpyrifos is above 50% by ethyl acetate extraction method among the different matrices.

Acetonitrile isolates less lipophilic co-extractives than acetone and ethyl acetate, the

acetonitrile method yielded cleaner extracts. Product coextractants, like photosynthetic pigments, was discovered to be the least abundant in the acetonitrile extract.⁹ The recovery efficiency for non-polar compound (cypermethrin) extracted using two different methods and analysed using GC-FID was as follows: acetonitrile are superior to ethyl acetate.¹⁰ The average recovery percentage of cypermethrin in spiked samples of brinjal, tomato and grapes were 66, 55 and 51 respectively with acetonitrile extraction method rather than of ethyl acetate method. The average recovery of cypermethrin is between 60-50% in different matrices by acetonitrile extraction method. Between the extraction method ethyl acetate extraction was found time consuming and least suitable for isolation of multiclass pesticide residues from samples whereas acetonitrile offers advantages in extraction selectivity and compatibility with more diverse analytical techniques. Different components with a larger molecular size such as triglycerides and pigments are often found and must be removed to enable for a more precise measurement of specific threshold residues and to avoid adverse effects on detecting equipment, irrespective of the extraction method employed. Polarity-based extraction separation is used in many clean-up processes, such as liquid-liquid partitioning and column chromatography. PSA columns, according to this study, provided the most effective clean-up, removing the greatest number of sample matrices interferences.¹⁰

The lesser extraction of pesticides into their main compounds was because their residues also contains metabolites or their degradation products having different physicochemical properties. Besides of this, the climatic conditions, nature of chemicals, and the application methods also influence the degradation behavior of pesticides. Variations in recovery of pesticides residues were also shown in different matrices. Among all the matrices, brinjal and tomato have the higher recovery rate than that of grapes samples. The overall lesser recovery of pesticides by both the extraction methods was due to the lower concentration of spiked pesticides solution. This research underlined the need of further

CHLOROPYRIFOS

Recovery of Residue [%] Brinjal samples		
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	78	34.7
3 days	46	26.2
5 days	37	21.6
7 days	34	12.9

CYPERMETHRIN

Recovery of Residue [%] Brinjal samples		
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	41.5	90
3 days	27	78
5 days	12.5	56
7 days	12.0	40

CHLOROPYRIFOS

Recovery of Residue [%] Tomato samples		
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	50.6	45.6
3 days	46.2	22.9
5 days	11	20.3
7 days	3	13.3

CYPERMETHRIN

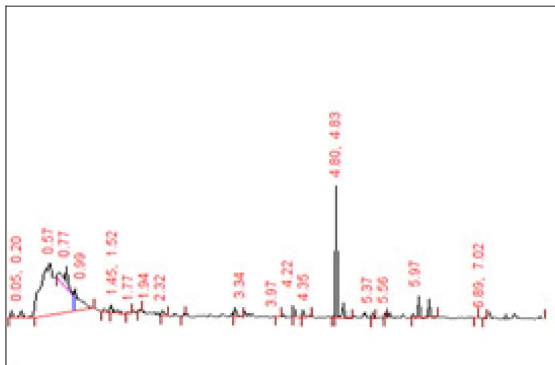
Recovery of Residue [%] Tomato samples		
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	37.9	86.8
3 days	18.1	78.1
5 days	16.3	24.4
7 days	10.4	24.0

CHLOROPYRIFOS

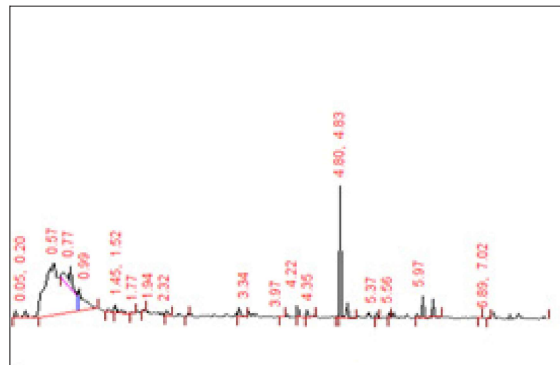
Recovery of Residue [%] Grape samples		
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	72	20.4
3 days	50	11.4
5 days	33	11.0
7 days	30	7.11

CYPERMETHRIN

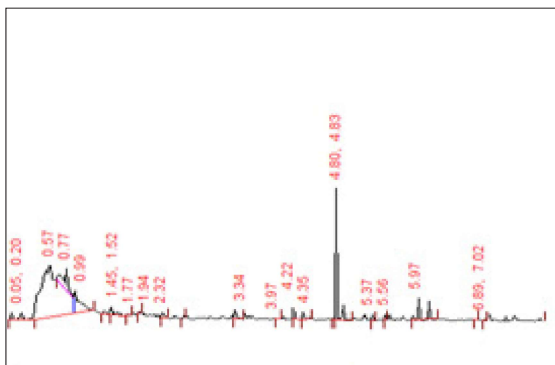
Recovery of Residue [%] Grape samples		
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	12.8	83.3
3 days	10.4	70.4
5 days	7.66	38.1
7 days	0.9	13.6



A



B



C

Figure 1: Chromatograms showing the recovery of pesticides using ethyl acetate extraction method
a) day 1 (Brinjal), b) day 3, c) day 5

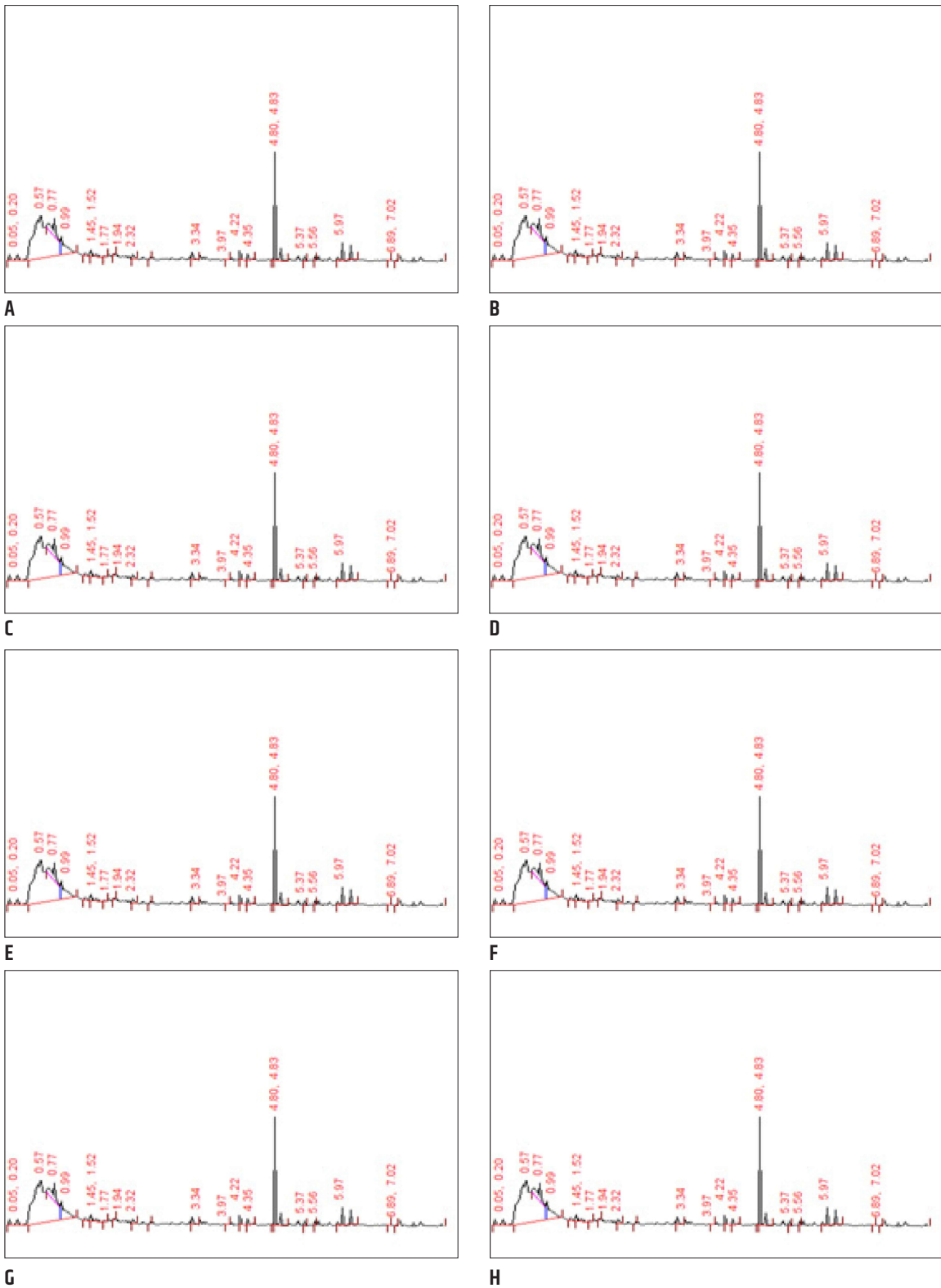


Figure 2: Chromatograms showing the recovery of pesticides using ethyl acetate extraction methods.

- a) Day 1 (tomato), b) Day 3 c) Day 5, d) Day 1 (Grapes)
- e) Day 3 f) Day 5

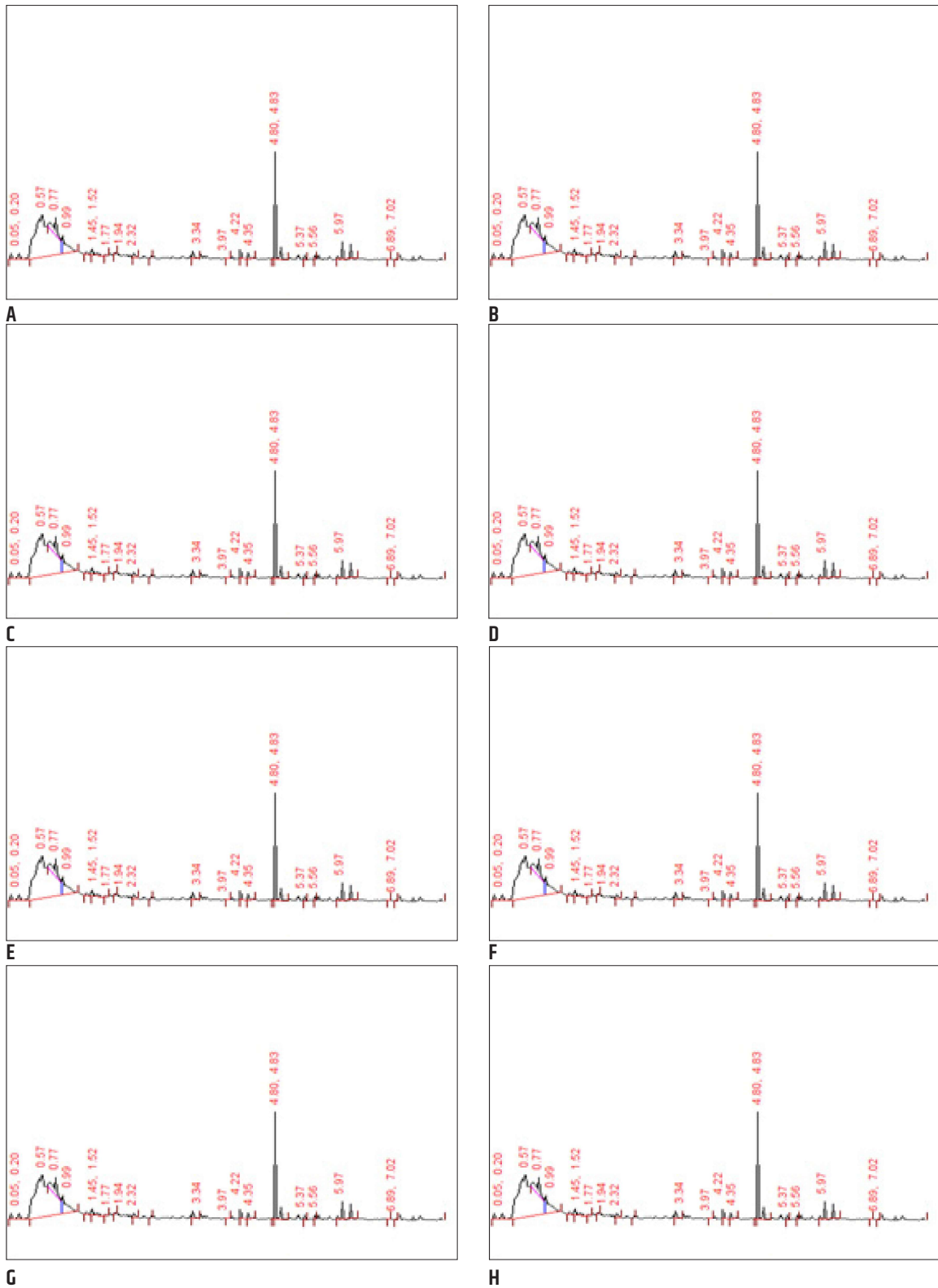


Figure 3: chromatograms showing the recovery of pesticides using acetonitrile extraction method. d) Day 1(Tomato), e) Day 3, f) Day 5

research such as: a) to assess the recovery rate of pesticides at higher concentrations, b) the validation of study by performing on more food matrices and with different analytical methods.

CONCLUSION

A multiresidue approach was used to undertake trace analysis of common pesticides that are commonly used in fruits and vegetables. Using ethyl acetate and acetonitrile, this approach uses a fast and non-selective extraction procedure. The pesticides under investigation were determined using gas chromatography with a flame ionization detector. This research also shows that this procedure is easy, quick, and adaptable to a variety of fruits, and vegetables with just modest amounts of solvent used each sample (10ml ethyl acetate, 7.5 ml acetonitrile). Brinjal, tomato, and grape samples were successfully extracted using the proposed multiresidue approach. Both acetonitrile and ethyl acetate are acceptable solvents for extracting pesticide residues from fruits and vegetables having a wide polarity range. It was proven clearly that Chlorpyrifos was extracted to a better extent by Ethyl acetate and Cypermethrin was extracted to a better extent by acetonitrile. There are numerous cleanup procedures. When compared to other standard multiresidue methods previously employed in the laboratory, the usage of multi-solvent is also a better and easier way. It can be concluded that extraction using acetonitrile gives more

recovery than ethyl acetate in specific pesticides and vice versa. The extraction and cleanup processes developed are suitable for a variety of plant materials and can be used with a wide range of multiclass pesticide concentrations.

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Conflict of Interest

The authors state that they have no known conflict financial interests or personal ties that could have influenced the research presented in this study.