# Determination of Pesticide Residues Using QuEChERS Extraction with Inert GC-MSD Analytical Technique and Application on Seasonal Fruits and Vegetables in Pakistan

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## Abstract

Pesticide residues cause different health hazards when they are present in primary as well as secondary agricultural products. In this manuscript, an analytical method was developed and validated for the assessment of pesticide residues present in various vegetables and fruits most commonly consumed in Pakistan. Seven samples of various fresh fruits and vegetables were processed and analyzed for the detection and identification of different pesticides by using the quick easy cheap effective rugged and safe (QuEChERS) extraction technique, which was followed by quantification on gas chromatography-mass spectrometer (GC-MS). The established internal standard method for determination of pesticides residues in vegetables and fruits, met the international validation requirements. Excellent results were obtained, within globally accepted validation reference values, particularly considering the low 18 concentration levels (0.01 μg/g) investigated. The method was sensitive, linear, accurate and precise. Out of seven tested fruit and vegetable samples, the amount of pesticide residues in five samples (guava, oranges, bitter guard, potatoes, lettuce) were higher than the permissible limits by European Commission and only in two samples (grapes, brinjal), the pesticide residues were below MRL. Continuous consumption of food products even with moderate pesticide contamination may have negative consequences on human health in the long term. To minimize this risk, therefore, it is highly recommended for the farmers to adhere to universal pesticide safety practices. It is also the need of hour for continuous pesticide residue monitoring. Moreover, this validated method can also be applied to determine pesticide residues in other vegetables, fruits and cereals.

## Subject Areas

Analytical Chemistry, Biological Chemistry

## Keywords

Pesticide Residues, Negative Consequences, MRL, Internal Standard Method, QuEChERS

## 1. Introduction

Pesticides play an essential role in maintenance of high productivity in agricultural industry, therefore, considering as major vital component of modern farming. Nowadays, the widespread use of pesticides in order to manage pests is dominating in high-input intensive agricultural production systems [1]. However, despite their many merits pesticides are considered to be the most toxic, stable and mobile substances in the environment. Their excessive use has a deleterious effect on humans and the environment, therefore, reliance on pesticides has become difficult to sustain [2]. Environmental stability, bioaccumulation and toxicity of pesticides have placed the human body at potential risk of disease and poisoning [3]. World health organization (WHO) has reported that roughly three million pesticide poisonings occur annually and result in 220,000 deaths worldwide. Various human health related concerns are associated with pesticides, ranging from short-term impacts such as headaches and nausea, to chronic impacts, such as various cancers, birth defects, infertility, and endocrine disruption [2] [4]. Children, in particular, are more endangered by short-term and chronic exposure to pesticides [5]. Commonly used pesticides for vegetables and include fruits in Pakistan include deltamethrin, Permethrin, Sulfotep, Brucine, Triazophos, Phorate, Pyriproxyfen, Profenofos, Carbofurane, acetamiprid, methoml, imidacloprid, lambda cyhalothrin, endosulfan, chlorfenapyr and bromoxynil. In order to ensure food safety for consumers and protect human health, many organizations and countries around the world have established maximum residue limits (MRLs) for pesticides in food commodities [6] [7] [8]. The maximum quantity of a pesticide residue is defined as MRL. It can be measured in mg·kg$^{-1}$ [9]. The governments have made different types of regulatory as well as monitoring mechanisms to check the compliance of MLRs in various food items [9].

Many methods are available in literature for the detection of pesticides with some limitations of their own, for example, use of expensive instruments like LC/MS/MS, LC-QTOF, derivatization techniques, low recoveries from matrices, high limits of detection and quantification, much time consuming and laborious extraction procedure, etc. Furthermore, the recovery rates ranged from 50% - 85% only which showed that those methods had limited applications and accuracy decreased with change in matrix or limited number of pesticides could be

quantified with accuracy. Moreover, amount of pesticides below their MRL were not accurately detected using those methods. Last but not the least, the total run times by these methods were high that further prolonged the analysis time as well as running cost. Matrix effect is a major problem in detection and quantification of pesticide residues in agricultural products due to co-extraction and interference of compounds like pigments, sugar, fatty acids. Clean up at the final step plays an important role in sample analysis. At present, a unique extraction technique and cleanup method that can completely eliminate the matrix effect is unavailable. Nevertheless, the "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) methodologies have been applied by many researchers with good results and high recoveries for several complex matrices extractions such as honey, soil, biological samples and food [7] [10] [11] [12] [13]. Therefore, authors of this research developed an internal standard method using QuEChERS extraction and an inert GC-MS (SCAN mode for detection and SIM mode for quantification) analytical technique for determination of pesticide residues in seasonal fruits and vegetables. The validated method was then applied to seven seasonal fruits and vegetables available in Pakistan.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

Pesticide certified reference standards including sulfotep, triazophos, carbofurane, phorate, profenofos, pyriproxyfen and permethrin were purchased from Fluka (Switzerland) with 99.9% purity. 8-hydroxyquinolone, used as internal standard in this research, was obtained from Cerilliant Corporation (Texas, USA) with certified purity of 99.9%. Acetonitrile (99.6% pure), magnesium sulphate and sodium acetate were obtained from Fisher scientific (Atlanta, Georgia). Dispersive Fruits and Vegetables (AOAC) SPE (PSA + C-18 sorbent) sample clean up packs were purchased from Agilent Technologies (USA).

### 2.2. Sample Collection, Storage and Processing Prior to Analysis

The samples included four pesticide-intensive vegetables (brinjal, bitter gourd, lettuce, potatoes) and three fruits (guava, grapes, oranges) are representative of commonly consumed commodities in Pakistan. All vegetables and fruits were collected from local markets of Lahore and Sheikhupura districts of Pakistan. The sampling was performed in accordance with the general principles and methods of the European Commission (EC) directive 2002/63/EC [14] for establishing MRLs in food commodities. Each representative vegetable or fruit sample was a composite of five subsamples of the same commodity collected through random sampling. All the samples (1 - 2 kg each) were placed in sterile polythene bags, with ice packs, to avoid contamination and deterioration, labeled, and transported to the laboratory for processing. In laboratory, samples were kept at −20°C for storage prior to processing and analysis. A representative portion "20 - 25 g" of each sample was defrosted, chopped into small pieces and

blended using a food processor. The homogenized samples were either analyzed immediately or stored in sterile plastic jars at 4°C and analyzed within 24 h. Home-grown pumpkin vegetable (analyzed and was shown to be free of pesticides) was utilized for the preparation of the matrix-matched calibration standards and recovery studies.

## 2.3. Preparation of Standard Solutions

Multi-residue pesticide standard stock solution (0.1 mg/mL) was prepared by dissolving 1 mg of each pesticide standard in acetonitrile quantity sufficient to 10 mL in a volumetric flask. Multi-residue pesticide working standard solution (0.005 mg/mL) was then prepared by diluting 500 μL of stock standard solution with acetonitrile in a 10 mL volumetric flask. 1 mg/mL solution of 8-hydroxy quinolone was prepared in acetonitrile at 25°C to serve as an internal standard for GC-MS determination.

## 2.4. Preparation of Matrix-Matched Calibrators and Quality Control Samples

Pumpkin pulp, negative for pesticide residue, was used as blank matrix for preparation of different concentration levels used during analytical method validation, calibration levels, positive and negative quality control samples. Six calibration levels (0.01, 0.02, 0.04, 0.08, 0.16, 0.32 μg/g) were prepared by spiking appropriate volume of multi-residue pesticide working standard solution in six separately labeled 15 mL plastic tubes each containing 1 g pumpkin pulp. 0.06 μg/g positive quality control sample was prepared by spiking 15 μL of multi-residue pesticide working standard solution in a labeled 15 mL plastic tubes containing 1 g pumpkin pulp. 1 g of pumpkin pulp was added to a 15 ml plastic tube to be used as negative quality control sample.

## 2.5. QuEChERS Extraction and Dispersive SPE Clean-Up Method

The extraction and clean-up method used was based on QuEChERS (quick easy cheap effective rugged and safe) sample preparation method for pesticides [15]. An aliquot of 1 g of homogenized sample was placed in a 15 mL plastic centrifuge tube. 50 μL of 8-hydroxy quinoline (internal standard) was spiked in all tubes. 5 ml acetonitrile and 5 ml distilled water were then added, vortexed for 30 seconds after addition of two ceramic homogenizers in each tube. 2.5 g powder mixture (magnesium sulphate: sodium acetate, 4:1) was added into each tube, quickly vortexed for a minute and then centrifuged the tubes at 6000 rpm for 10 minutes. Supernatant from each tube was taken into separate labelled 15 ml plastic extraction tubes, added 1.5 g of Dispersive SPE Fruits and Vegetables AOAC into each tube, rotated for 10 minutes and then centrifuged at 4000 rpm for 3 minutes. Supernatant from each tube was then taken into separate labelled 15 ml plastic extraction tubes (Note: At this step, upper layer should be taken with great care because dispersive phase at bottom can be mixed up during pipetting). Tubes containing supernatant were completely dried at 45°C under ni-

trogen in turbo-evaporator, reconstituted with 25 μL of acetonitrile and then transferred into labeled GC-MS vials with inserts for analysis. The extraction method described above was used for analytical method validation also. All calibration levels, positive and negative quality control samples were extracted in a similar manner to avoid possibility of interferences (either from matrix or extraction technique) in determination of pesticide residue in fruits and vegetables.

## 2.6. Gas Chromatograph-Mass Spectrometry (GC-MS) Analysis

An Agilent (7890A) gas chromatograph equipped with inert mass selective detector (5975) and DB-35MS GC column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) was used for analysis. Sample injection was performed in the split-less mode, with an injector temperature of 250°C and an interface temperature of 280°C. The temperature of the oven was programed from an initial value of 100°C for 0.5 min, ramped to 340°C at 20°C/min for 1 min. Helium was used as a carrier gas and electron impact ionization was used at −70 eV in selective ion monitoring (SIM) and full-scan modes between 50 m/z and 500 m/z for the detection of different analytes. The following pesticides were analyzed with GC-MS: sulfotep, triazophos, carbofurane, phorate, profenofos, pyriproxyfen and permethrin.

## 3. Results

### 3.1. Method Validation Results

Specificity/selectivity, linearity/linear range, limit of detection/sensitivity, limit of quantification, bias/recovery, precision, ruggedness and robustness were evaluated as per International Conference on Harmonization (ICH) and European Commission guidelines [16]. Matrix-matched concentration levels were prepared in pumpkin blank and extracted. The total run time was 18.5 min. Areas under the peak versus concentrations were fitted using linear regression to obtain the equation for the standard curves for the tested pesticides. The proposed method had permitted the quantification of pesticides over linearity in the concentration range of 0.01 - 0.32 μg/g with $R^2$-value ≥ 0.9992 and 90% - 105% recovery. Linear curves for tested pesticides are shown in Figure 1. Relative standard deviation at six concentration levels (0.01, 0.02, 0.04, 0.08, 0.16, 0.32 μg/g) was in the range of 4% - 19% that indicated high degree of accuracy and precision of established method. Limit of detection (LOD) of this method was 0.005 μg/g with precision ranged 4% - 19% for tested pesticides. Limit of quantification (LOQ) was set to 0.01 μg/g having precision less than 20% and accuracy within ±20% range. All the pesticides LOD and LOQ values were lower than the MRLs established by Codex [17] for the fruits and vegetables sampled. For robustness study, flow rate of carrier gas (±2), initial GC temperature (±2), GC column cutting, liner and septum change were deliberately varied. Analysis was also performed by different analysts. All these changes did not affect the method efficiency and relative standard deviation (% CV) was found below 20%. The summarized results for validation study are shown in Table 1.

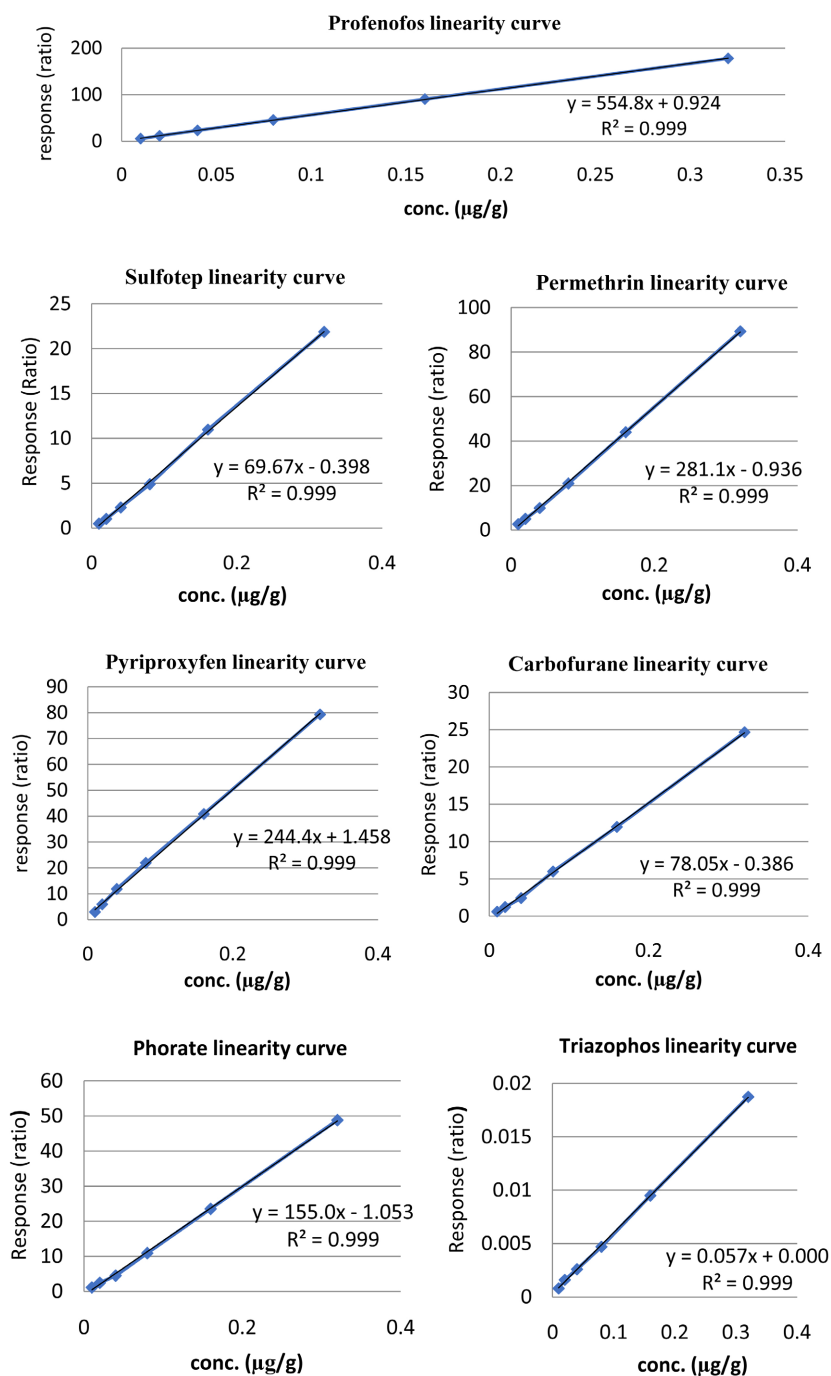Figure 1. Linearity curves for seven tested pesticides.

## 3.2. Result for Matrix-Matched Quality Control Samples

The results of positive QC sample are described in **Table 2**. Gas chromatograms for positive and negative quality control samples are shown in **Figure 2(a)** and **Figure 2(b)**. Pesticide residues were not detected in the negative quality control sample (**Figure 3**). The concentration of spiked pesticides recovered was within acceptable range (±20% of target concentration = 0.048 - 0.072 µg/g), therefore, result of positive QC was accurate showing recovery between 81% - 106%.
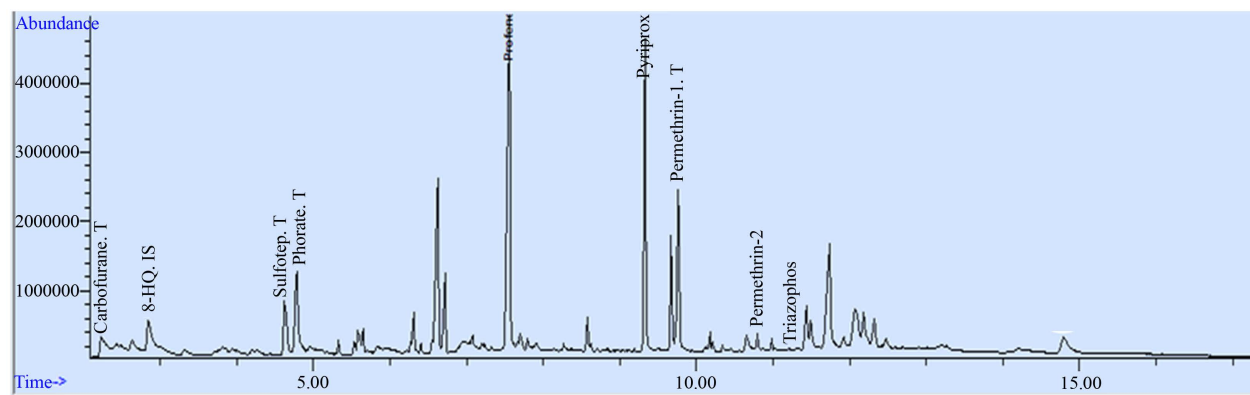
(a)
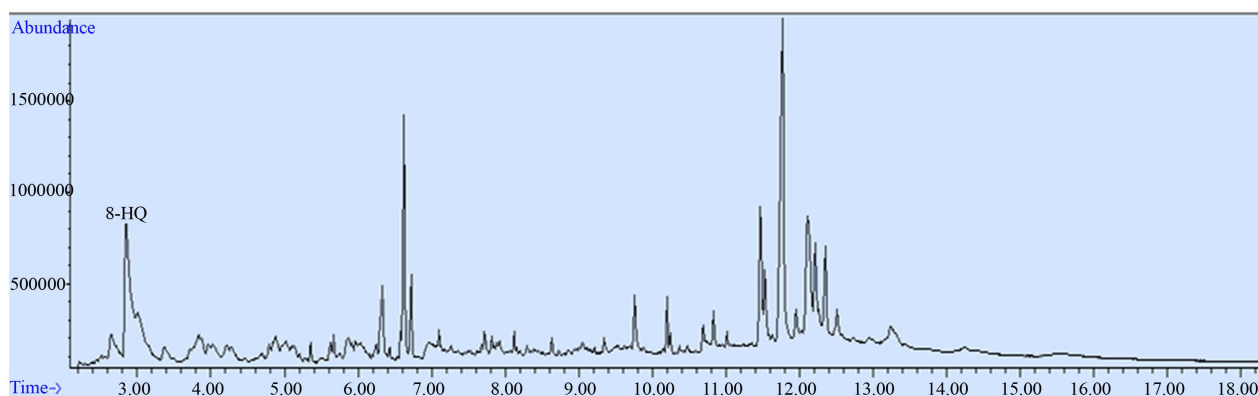


(b)

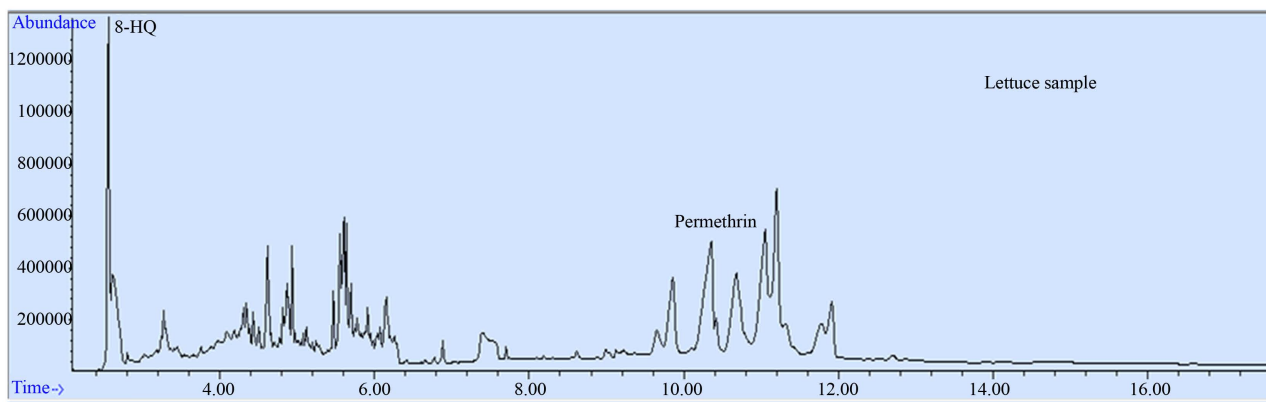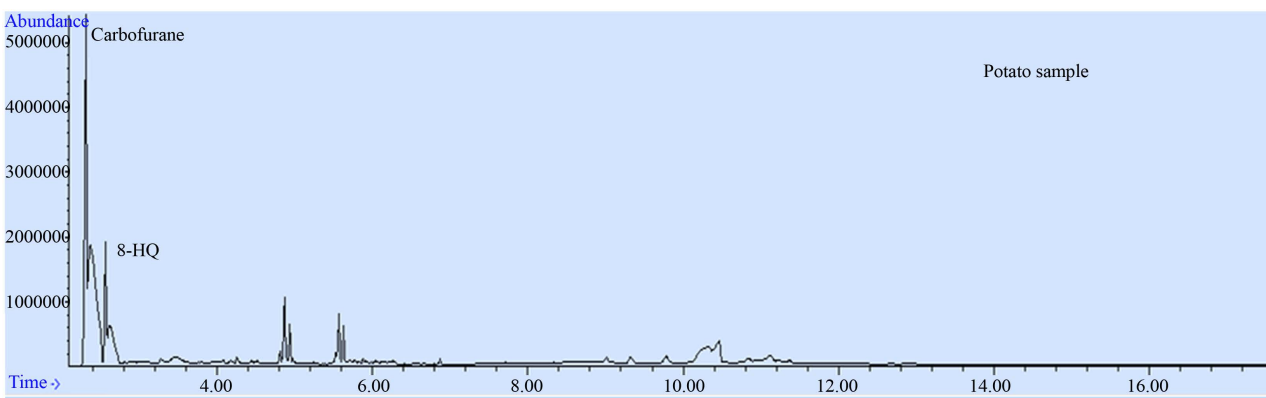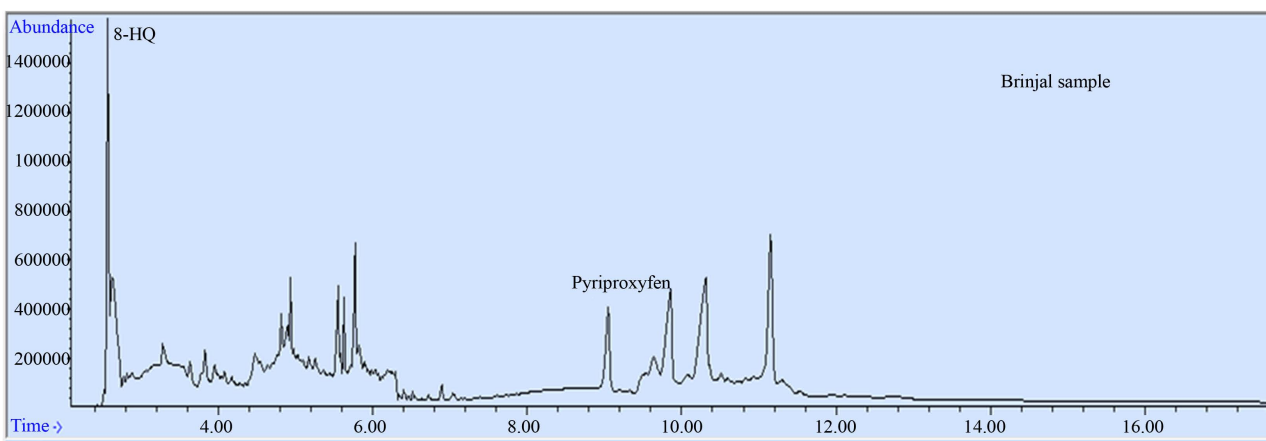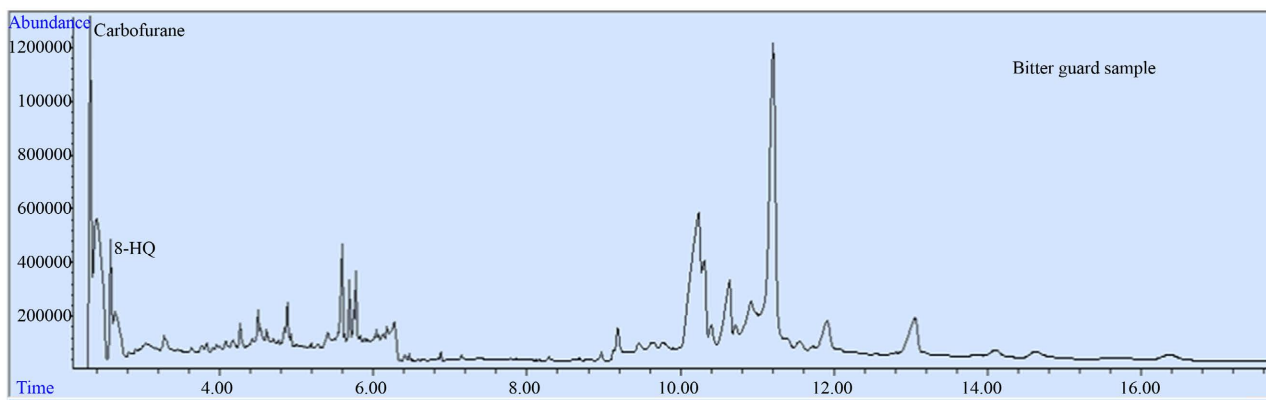**Figure 2.** (a) Gas chromatogram for matrix-matched positive quality control sample (0.06 μg/g) showing seven pesticides recovered along with internal standard (8-HQ); (b) Gas chromatogram for matrix-matched negative quality control sample showing only recovered internal standard (8-HQ). No pesticide residue detected.

**Table 1.** Validation study results for tested pesticides.

| Pesticide | Retention time (min. ±0.1) | LOD (μg/g) | LOQ (μg/g) | Linearity ($R^2$-value) | Recovery at six concentration levels 0.01 - 0.32 μg/g (%) | Precision at three concentration levels 0.01, 0.08 and 0.32 μg/g (% CV or RSD) |
|---|---|---|---|---|---|---|
| Sulfotep | 4.6 | 0.005 | 0.01 | 0.9995 | 90 - 105 | 14 - 19 |
| Triazophos | 11.2 | 0.005 | 0.01 | 0.9997 | 90 - 105 | 10 - 17 |
| Phorate | 4.8 | 0.005 | 0.01 | 0.9992 | 90 - 105 | 4 - 17 |
| Profenofos | 7.5 | 0.005 | 0.01 | 0.9999 | 90 - 105 | 13 - 19 |
| Permethrin | 9.8 | 0.005 | 0.01 | 0.9998 | 90 - 105 | 18 - 19 |
| Pyriproxyfen | 9.3 | 0.005 | 0.01 | 0.9994 | 90 - 105 | 2 - 17 |
| Carbofurane | 2.3 | 0.005 | 0.01 | 0.9996 | 90 - 105 | 13 - 19 |

### 3.3. Pesticide Residues in Analyzed Samples

The levels of pesticide residues in seven vegetable and fruit samples were determined using this validated method. The quantity of pesticides detected in tested vegetable and fruit samples are shown in Table 3 and Figure 3.
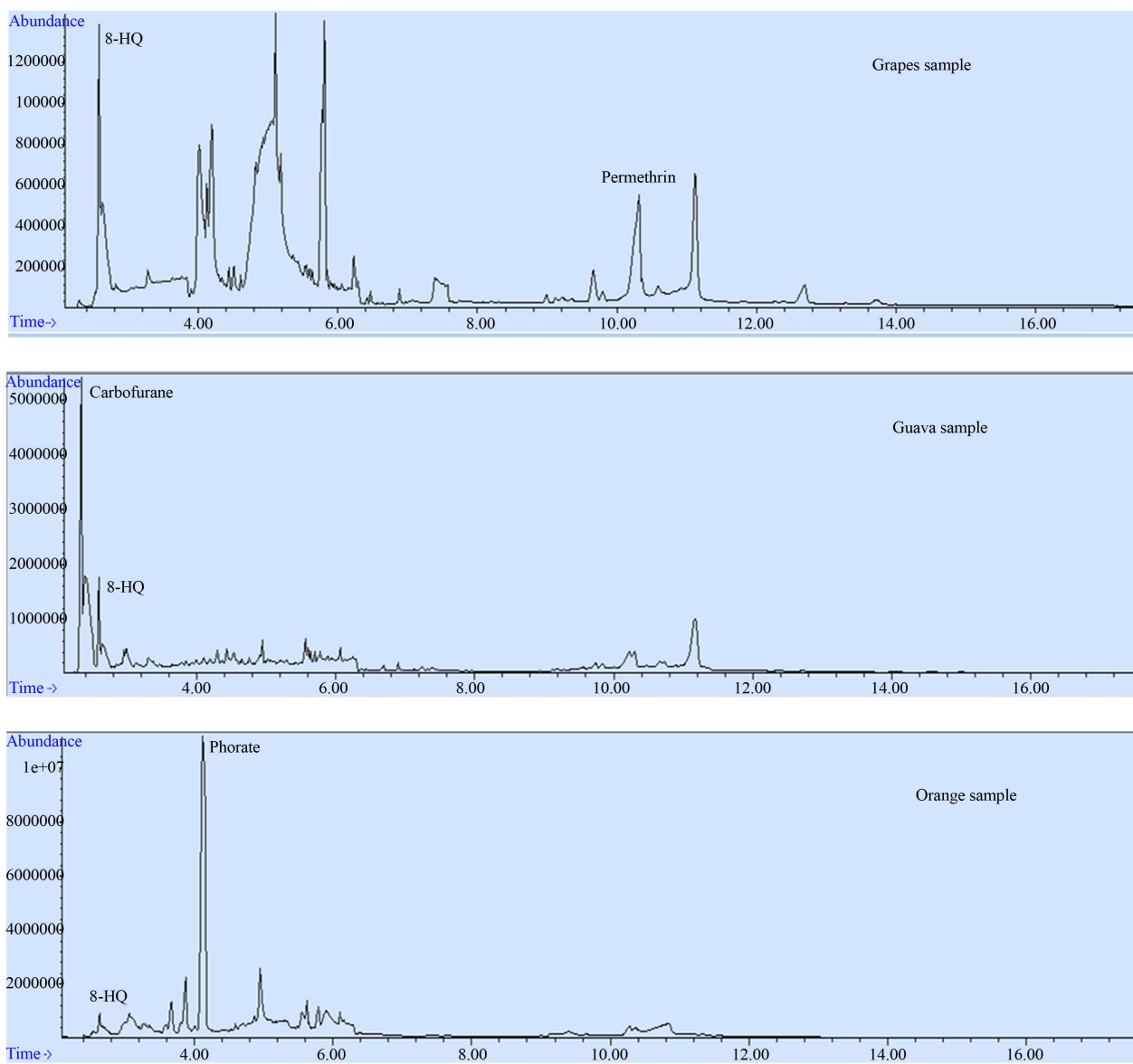
**Figure 3.** Gas chromatograms for analyzed vegetable and fruit samples showing recovered pesticide residue and internal standard (8-HQ).

**Table 2.** Results for matrix-matched positive quality control sample (0.06 μg/g).

| Analyte | Retention time (min) | Target conc. (μg/g) | Response y (ratio) | Slope (m) | Intercept (b) | Recovered conc. in μg/g (x = y − b/m) | Pesticide recovery (%) |
|---|---|---|---|---|---|---|---|
| Sulfotep | 4.64 | 0.06 | 0.236176 | 3.95 | 0.0040 | 0.05878 | 98 |
| Triazophos | 11.20 | 0.06 | 1.768890 | 29.64 | 0.0476 | 0.05806 | 97 |
| Phorate | 4.26 | 0.06 | 0.713321 | 11.11 | 0.0059 | 0.06369 | 106 |
| Profenofos | 7.12 | 0.06 | 2.361011 | 41.73 | 0.3403 | 0.04842 | 81 |
| Permethrin | 10.3 | 0.06 | 0.957505 | 14.67 | 0.1318 | 0.05627 | 94 |
| Pyriproxyfen | 9.10 | 0.06 | 1.810100 | 32.57 | 0.1634 | 0.05056 | 84 |
| Carbofurane | 2.34 | 0.06 | 0.362097 | 5.24 | 0.0451 | 0.06046 | 101 |

Table 3. Results for pesticide residues determination in vegetables and fruits.

| Vegetable/Fruit analyzed | Pesticide detected | Response y (ratio) | Slope (m) | Intercept (b) | Recovered conc. in μg/g (x = y − b/m) | MRL by EU (μg/g) |
|---|---|---|---|---|---|---|
| Guava | Carbofurane | 2.81250 | 5.243 | 0.0451 | 0.528 | 0.010 |
| Grapes | Permethrin | 0.29166 | 14.674 | 0.1318 | 0.011 | 0.050 |
| Oranges | Phorate | 5.83300 | 11.106 | 0.0059 | 0.525 | 0.010 |
| Brinjal | Pyriproxyfen | 0.82000 | 32.566 | 0.1634 | 0.020 | 1.000 |
| Bitter guard | Carbofurane | 0.68800 | 5.243 | 0.0451 | 0.123 | 0.002 |
| Potatoes | Carbofurane | 2.86900 | 5.243 | 0.0451 | 0.539 | 0.002 |
| Lettuce | Permethrin | 1.01000 | 14.674 | 0.1318 | 0.060 | 0.050 |

## 4. Discussion

The validated method was successfully applied on seven vegetable and fruit samples (guava, grapes, oranges, brinjal, bitter guard, lettuce and potatoes) in real time to determine the amount of pesticide residue. This study shows the evidence of the presence of pesticide residues in fruits and vegetables in Pakistan. Pesticides determined in samples include phorate (in oranges), carbofurane (in bitter-guard, potato and guava), permethrin (in grapes and lettuce) and pyriproxyfen (in brinjal). Pyriproxyfen is a pyridine-based pesticide with mild toxicity. The quantity of pyriproxyfen detected in brinjal (0.020 μg/g) was below its MRL (1.0 μg/g). Phorate is an extremely toxic organophosphate (cholinesterase inhibitor) pesticide and its use is restricted. The MRL of phorate is 0.01 μg/g whereas in analyzed oranges the quantity determined was much above the MRL (0.525 μg/g). Carbofurane is one of the most toxic carbamate pesticides and its use is also restricted. It was detected in three samples (bitter-guard, guava and potatoes) in concentrations (0.123 μg/g, 0.528 μg/g and 0.539 μg/g respectively) much higher than the established MRLs (0.01 μg/g for grapes, 0.002 μg/g for bitter guard and potatoes). Permethrin is an insecticide in the pyrethroid family having mild toxicity. Permethrin was detected in two samples, grapes and lettuce, with recovered concentration of 0.011 μg/g and 0.06 μg/g respectively. In lettuce sample, the amount of permethrin determined was above its MRL (0.05 μg/g). Out of seven tested fruit and vegetable samples, the amount of pesticide residues in five samples (guava, oranges, bitter guard, potatoes, lettuce) was higher than the permissible limits by European Commission whereas in only two samples (grapes, brinjal), the pesticide residues were below MRL. Continuous consumption of food products even with moderate pesticide contamination may have negative consequences on human health in the long term. Pesticides can accumulate in the tissues of organisms as they are not aqueous soluble to be excreted easily. The current study showed that the percentage of samples contaminated with high levels of pesticides was 71% which further suggests that these pesticides have been used indiscriminately, which could lead to health problems

not only to the farmers but also to the general consumers. The widespread and overuse of pesticides in Pakistani agriculture, especially in greenhouse vegetable production, is a serious problem. Faced with several pest complexes, farmers simply rely on pesticides to address pest problems because of lack of viable alternative methods of pest control.

Greater priorities must be given to develop strategies for pesticide reduction in agriculture through farmer training in judicious and safe pesticide use, and promote alternatives to chemical pest control such as biological control. Intervention strategies by regulatory agencies to strengthen the enforcement mechanisms of current pesticide laws at the farm and retail level are a necessity in promoting safe pesticide use. Adherence to pesticide label instructions, especially pre-harvest intervals, needs to be ensured. It is also critical to raise awareness among the general public, who may be directly or indirectly be exposed to pesticides, about the risk of these chemicals and how to reduce this risk. Consumers should be aware of practical measures to reduce the contamination of pesticides in fresh agricultural products, especially fruits and vegetables that may be consumed raw. For example, washing, boiling, and especially peeling, have been demonstrated to reduce pesticide residues on fruits and vegetables [18] [19]. Consequently, a follow-up investigation is needed to determine whether peeling, in particular, could reduce the dietary intake of pesticide residues in Pakistan. Finally, due to increasing trend in pesticide use in Pakistan, routine monitoring of pesticide residues in agricultural products is a necessity to ensure the safety of consumers.

## 5. Conclusion

The established internal standard method for determination of pesticides residues (Sulfotep, Triazophos, Phorate, Permethrin, Pyriproxyfen, Profenofos and Carbofurane) in vegetables and fruits, met the validation requirements. Excellent results were obtained, within globally accepted validation reference values, particularly considering the low concentration levels (0.01 μg/g) investigated. The method was sensitive, linear, accurate and precise. Authors of this article investigated the levels of pesticide residues in commonly used fruits and vegetables in Pakistan. The results indicated that most of the fruit and vegetable samples were contaminated with pesticide residues, with concentrations above the MRL. From a public health perspective, the observed levels of pesticide residues pose a potential health risk to consumers. Therefore, to reduce this risk, sensitization of farmers to better pesticide safety practices and the need for continuous pesticide residue monitoring is highly recommended. Moreover, this validated method can also be applied to determine pesticide residues in other vegetables, fruits and cereals.

## Conflicts of Interest

The authors declare no conflicts of interest.

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