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Pesticide Residue Determination In Shahr-E-Rey Tomatoes Using Quechers Method

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Mahboubeh Yadolahi, Mehran Babri, Amir Abdolah Mehrdad Sharif, Amin Mousavi Khaneghah; Pesticide Residue Determination In Shahr-E-Rey Tomatoes Using Quechers Method

ABSTRACT

In this study, pesticide residues in tomato products from Shahr-e-Rey farms were measured and investigated by QuEChERS modified method. This method was on the base of the sample preparation in few steps and high speed. Measurement of pesticide residues was carried out by gas chromatograph with electron capture detector. In this study, most of the pesticides showed a recovery in the range of 70% – 120%. Limit of detection (LOD) and limit of quantification (LOQ) for all pesticides were lower than 0.50 and 1.90 µg/kg respectively. chlorpyrifos was detected in studied samples at average concentration 3.22 µg/kg, which was lower than the Maximum Residue Limits (MRLs) of national standard. Results showed that mentioned method is a very useful method for studying pesticides residues in tomato.

Key words: Gas chromatograph equipped with electron capture detector, pesticide residues, QuEChERS method, Shahr-e-Rey, Tomato.

Introduction

Application of chemical materials include pesticides had ever-increasing deployment in order to preserve and increase agricultural products withdrawal value, and caused to some anxieties on the subject of effect of pesticides residue in agricultural products. Fresh tomato usually is in Iranian diet in whole year. Since tomato plantation in fields and greenhouses is usually accompanied with excessive use of some pesticides, pesticides residue measurement in this product is very important in order to consumer's healthcare. Several analyses are done to pesticide residues in fruits and vegetables among tomato yet [1-7].

Results obtained from studying pesticides dispersion in tomato's skin and pulp showed that; organic pesticides were not found in tomato seeds and pesticides are most accumulated in skin region. Therefore samples with more thin skin had lower pesticide residues than tomatoes with thicker skin [8]. Also study on effect of tomato washing on pesticide residues showed that washing with water or detergents caused in decreasing the levels of pesticide residues. Also studies on effect of freezing and heating on tomato showed that both of them caused in decreasing the levels of pesticide residues [8-9].

QuEChERS method had recently defined to determination of pesticides in agricultural products. QuEChERS, an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe, is a sample preparation and clean-up technique for the analysis of multiple pesticide residues in high moisture food samples. [10-14]. In accordance with mentioned benefits, this method provides higher ability to control agricultural products for hygienic control structures.

Materials and methods

Chemical materials and solvents used in this work were:

Acetone, acetonitrile, anhydrous sodium acetate, anhydrous magnesium sulphate, dichlorobenzene, glacial acetic acid and n-hexane all analytical grade from Merck co. Germany, and Primary secondary amine (PSA) from Sigma-Aldrich co. England, and pesticide standards all from Dr.Ehrenstorfer Germany with purity of 95%.

Pesticides and their Maximum Residue Limit (MRL) in tomato are listed in table 1 [18].

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Table 1: Maximum residue limit for some pesticides in tomato (national standard N.12581).

pesticide	MRL[a] (µg/kg)
Quintozene	50
Diazinon	50
Chlorpyrifos-Methyl	50
Chlorpyrifos	100
Endosulfan	500
Penconazole	50
Oxadiazon	50
Captan	50
Edifenphos	50
Propiconazole	50
Fipronil	50
Bifenthrin	50
Bromopropylate	1000
Iprodione	50
Phosalone	50
Permethrin	1000
Cypermethrin	50
Fenvalerate	50
Deltamethrin	50
Indoxacarb	500

a: Maximum Residue Limit

Apparatus:

A Trace GC Ultra gas chromatograph (Thermo, US) equipped with electron-capture detector (GC-ECD) was used for the analysis of pesticides. A fused-silica capillary column, TR-5 (30 m×0.25 mm I.D.) and 0.25 µm film thickness was employed, with helium as carrier gas at 1.3 ml/min. Column thermal program started from 50 ^{0}C and after 0.2 min was increased to 130 ^{0}C with a rate of 12 ^{0}C/min, and finally to 280 ^{0}C with a rate of 5 ^{0}C/min and hold at this temperature for 15 min. The injector port was hold at 250 ^{0}C and the detector temperature was 300 ^{0}C. A 0.5 µl volume was injected in the splitless mode.

Materials retention time in gas chromatography column is provided in table 2. Surely, some of pesticides have several stereo isomers in accordance with their spatial structure, which gas chromatograph column can separate these isomers, and so several retention times are reported for them. A Universal-320 Centrifuge (Hettich Germany) and a MS-3 Basic Vortex mixer (IKA, Germany) were used for the sample preparation.

Table 2: Pesticide retention time and internal standard per min.

pesticide	Retention time (min)			
	1	2	3	4
Quintozene	19.60			
Diazinon	20.10			
Chlorpyrifos-Methyl	22.39			
Chlorpyrifos	23.96	29.84		
Endosulfan	26.10			
Penconazole	26.49			
Oxadiazon	27.78			
Captan	28.22			
Edifenphos	31.13	31.08		
Propiconazole	31.00			
Fipronil	31.31			
Bifenthrin	31.69			
Bromopropylate	32.58	33.74		
Iprodione	33.33			
Phosalone	35.16	35.68		
Permethrin	35.34	38.65		
Cypermethrin	38.24	41.47	38.72	39.02
Fenvalerate	40.74	43.57		
Deltamethrin	42.61			
Indoxacarb	44.52			
Internal standard	6.12			

Samples preparation:

Studied tomato samples in this work were collected from Shahr-e-Rey farms. Blank samples were collected for recovery test from a farm in Shahr-e-Rey which produces organic tomato, and then transferred to Kimia Shangarf Pars research laboratory in 1 kg plastic bags. Each tomato was cut into four equal pieces and two cross pieces were transferred to mill and two other pieces were omitted. Finally, 10 g of milled sample was transferred to a 50 ml polypropylene centrifuge tube and remained

samples for subsequent tests were placed in plastic containers, labelled and stored at -18 ^{0}C [18].

Extraction and cleanup procedure:

Pesticide residues were extracted and purified by QuEChERS modified method [15]. This method included extracting sample with acetonitrile in presence of salts such as Magnesium sulphate. In this method, after extraction, Dispersive Solid Phase Extraction (DSPE) was used to eliminate interruptions and purify sample. acetonitrile was selected because it can dissolve a wide range of organic compounds with very different polarity like pesticides and extract them from sample matrix [16-17].

In this method at first 10 g of sample (homogenized tomato) and 15 ml of acetonitrile, containing 1% (v/v) of acetic acid, was transferred to a polypropylene 50 ml centrifuge tube. acetonitrile was used for extraction process and acetic acid to control and adjusting PH value between 5 - 5.5. Sample was shaken manually for 1 min hardly and vortexed for 15 sec. Then extract powder include 6 g magnesium sulphate (to enhance extraction as well as the removal of water and the partitioning of residues into the solvent phase) and 1.5 g anhydrous sodium acetate (to control PH) were transferred to centrifuge tube contents, and after it completely blended manually for 1 min and vortexed for 15 sec, and then centrifuged for 5 minutes at 4000 rpm. 8 ml of solvent in 50 ml centrifuge tube were transferred to 15ml centrifuge tube by using a Pasteur pipette. This tube contains required materials for purifying; include 400 mg PSA (to eliminate fatty acids and sugars) and 1200 mg magnesium sulphate (for removing residual moisture). Then sample was shaken manually for 1 min immediately and after vortexing for 30 sec, it centrifuged for 5 minutes at 5000 rpm. Afterwards, 5 ml of upperlayer was separated by Pasteur pipette and poured in sample's clean container. Sample had concentrated by nitrogen gas light flow till its volume decreased to half. After this section, sample was passed through a Pasteur pipette with few pressed cotton in it, to purify. Then concentration process by exerting a light flow of nitrogen gas was continued till remaining about one drop of sample, to eliminate acetonitrile solvent for increasing analytical sensitivity, then 500 µl of hexane-acetone (1:1 v/v) was added to sample container. Finally 50 µl internal standards (1 mg/kg dichlorobenzene in acetone) were added to sample container and 0.5 µl of prepared sample was injected to the GC-ECD system [15]. Accuracy (recovery) and repeatability precision were assessed using spiked blank samples at the concentration levels of 0.5, 1 and 2.5 mg/kg.

Results and discussion

Obtained standard mixture of samples were injected to GC-ECD system in mentioned conditions with 0.5, 0.75, 1, 1.5 and 2 µg/ml concentration and calibration curve for each pesticide was prepared. Data for indicating point's coordinates on regression line for plotting calibration curve are provided in table 3. Also, in accordance with blank sample's standard deviation, Limit of detection (LOD) and Limit of Quantification (LOQ) calculated for each pesticide are shown in table 3.

Table 3: Calibration data and Limit of Detection (LOD) and Limit of Quantification (LOQ) of 20 pesticides in tomato samples.

Pesticide	Slope	Intercept	LOD[a] (µg/kg)	LOQ[b] (µg/kg)	R^{2c}
Quintozene	0.702	0.188	0.38	1.50	0.9986
Diazinon	0.053	0.014	0.48	1.93	0.9905
Chlorpyrifos-Methyl	0.401	0.072	0.02	0.08	0.9953
Chlorpyrifos	0.570	0.206	0.05	0.18	0.9985
Endosulfan	0.493	0.194	0.07	0.27	0.9943
Penconazole	0.369	0.012	0.15	0.58	0.9965
Oxadiazon	0.057	0.008	0.28	1.13	0.9978
Captan	0.548	0.198	0.03	0.13	0.9981
Edifenphos	0.205	0.097	0.02	0.08	0.9936
Propiconazole	0.798	0.373	0.01	0.03	0.9995
Fipronil	0.208	0.095	0.02	0.07	0.9921
Bifenthrin	0.388	0.180	0.06	0.23	0.9942
Bromopropylate	0.526	0.227	0.01	0.05	0.9972
Iprodione	0.043	0.141	0.43	1.74	0.9988
Phosalone	0.107	0.005	0.29	1.15	0.9974
Permethrin	0.032	0.003	0.28	1.12	0.99780
Cypermethrin	0.037	0.019	0.34	1.37	0.9988
Fenvalerate	0.067	0.006	0.38	1.53	0.9907
Deltamethrin	0.006	0.039	0.48	1.91	0.9980
Indoxacarb	0.021	0.014	0.36	1.44	0.9982

a: Limit of detection
b: Limit of Quantification
c: Correlation coefficient
n=5

Recovery test results showed that average recovery for most of studied pesticides, at the concentration levels of 0.5, 1 and 2.5 mg/kg in tomato samples, is acceptable, between 70% and 120% (table 4). These results indicate the suitability of defined method in extracting and purifying sample in mentioned concentration limits [18].

Table 4: Mean percent recovery ± RSD of 20 pesticides in studying 10 g contaminated tomato, in three levels (mg/kg).

Pesticide	% Recovery 0.5 (mg/kg)	% Recovery 1 (mg/kg)	% Recovery 2.5 (mg/kg)
Quintozene	71.95±4	109.15±2	88.05±4
Diazinon	102.80±3	107.44±1	82.75±7
Chlorpyrifos-Methyl	80.07±7	113.05±6	109.95±3
Chlorpyrifos	96.59±4	122.75±5	106.14±6
Endosulfan	115.72±8	90.63±5	101.04±2
Penconazole	108.59±5	112.68±7	119.16±9
Oxadiazon	117.46±5	120.93±6	118.62±8
Captan	85.89±9	98.99±1	100.65±6
Edifenphos	87.79±4	73.89±7	75.40±5
Propiconazole	105.71±4	125.57±6	121.16±2
Fipronil	108.04±6	80.07±3	76.44±8
Bifenthrin	96.50±2	79.73±5	109.942±
Bromopropylate	105.94±8	88.75±4	121.40±4
Iprodione	82.10±3	92.04±6	78.26±6
Phosalone	95.49±6	84.86±7	117.58±3
Permethrin	121.63±2	91.33±3	99.46±1
Cypermethrin	88.26±6	81.22±9	120.46±7
Fenvalerate	87.76±7	8±80.97±	112.33±3
Deltamethrin	78.70±9	84.07±8	85.69±4
Indoxacarb	90.88±11	74.96±5	117.40±5

n=3

This study demonstrated that chlorpyrifos was detected in collected samples from Shahr-e-Rey farms at average concentration 3.22 µg/kg, which this value was lower than the Maximum Residue Limits (MRLs) of national standard. However; determined pesticide residue values in all studied samples were lower than the Maximum Residue Limits (MRLs) of national standard [19]. Since tomato commonly freshly and in a high quantity is in Iranian peoples diet whole the year, consuming contaminated foodstuffs for a long time, can cause in human chronic poisoning. This kind of poisoning is caused by consuming of pesticide residues in low quantities but consecutive in foodstuffs and accumulating contaminants occurs in special tissues of poisoned one's body. In accordance with mentioned cases and the fact that many of chemical compounds of pesticides are canserogen, and most of scientists believe that, nowadays outbreaking new different diseases in modern communities, can be affected by foodstuffs preparing methods. Out of them, pesticides and other chemical compounds which are used in food technology are highly suspected; therefore application of suitable methods in controlling pests and disease, like using biological factors can be a useful method to decreasing pesticide residues values in agricultural products. Finally instructing farmers and producers can certainly effect on decreasing foodstuffs contamination.

In this study a simple and fast method for determining residues for 20 pesticides in tomato, with high sensitivity and power to detecting and determining pesticide residue values in lower quantities than defined MRL was used. QuEChERS method is a quick, easy, cheap, effective, rugged and safe method for preparing and purifying samples for analyzing pesticide residues in foodstuffs. Otherwise electron capture detector is a selected, suitable and very sensitive detector to pesticides. Recommended method either allows to contemporary assessment and determining the quantity of many pesticides with high recovery rate and low detection limit, more it's a simple and fast method and so is recommended and useful for daily analyzing.

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