Multi-Residue Analysis of Pesticides in Pistachio Using Gas Chromatography-Mass Spectrometry (GC/MS)

*Azadeh Emami [1], Hossein Rastegar [2], Maryam Amirahmadi [3], Shahram Shoeibi [4], Zahra Mousavi *[5]*

ABSTRACT

Background: Analysis of pesticide residues in food and other environmental commodities have become an essential requirement for consumers, producers, food inspectors and authorities. This study is focused on validation of an accurate, rapid and reliable method for multi-residual analysis of pesticides in pistachio as a strategic crop for export and one of the main nuts in Iranian food basket.

Methods: We developed a "Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method based on spiking blank samples and used the data for drawing calibration curves instead of standard solutions. Sample preparations were developed for determination of 12 pesticide residues in pistachio by gas chromatography-mass spectrometry (GC/MS).

Results: Recovery of pesticides at 5 concentration levels (n=3) was in the range of 81.40% - 93.08 %. The method proved to be repeatable in the majority of samples with relative standard deviation (RSD) of lower than 20%. The limits of detection and quantification for all pesticides were 2 ppb and 10 ppb, respectively.

Conclusion: The calibration curves of pesticides were linear in the range of 10-500 (ng/g) and correlation coefficient of entire pesticides was higher than 0.994. The recovery of pesticides at 5 concentration levels (n=3) was in range of 81.41- 91.80 %. The method was proved to be repeatable with the majority of RSDs being lower than 20%. The limits of detection and quantification for all pesticides were 2 and 10 ppb, respectively. The recoveries and repeatabilities were in accordance with the criteria set by SANCO Guideline (Commission of the European Communities, 2006).

Keywords: Gas Chromatography-Mass Spectrometry, Pesticide Residues, Pistacia, Triphenylmethane.

INTRODUCTION

Pistachio nut, Pistacia vera L., is commercially grown in Iran, Turkey and USA. Iran is one of the largest producers and exporters of pistachio nuts in the world [1]. Pistachio is a rich source of antioxidant compounds, particularly phytoestrogens. Phytoestrogens protect the body against cardiovascular diseases and somehow against cancers. The phytosterols in pistachios can lower cholesterol level, especially that of LDL [2, 3]. The amino acid arginine found in pistachio nut increases arterial flexibility and strengthens blood circulation. Pesticides are chemicals used to prevent, destroy or reduce pests in farming crops [4,5]. Pesticide residues in agricultural products and environmental components [6] have irreparable effects on consumers. These effects are classified into acute and chronic [7]. Lack of information about the presence of pesticide residues in dry fruits has affected their export. Because pesticides might be used in cultivation of pistachio nuts, their control is necessary to protect consumers from harmful effects. It is necessary for the authorities in charge of food and agricultural products to manage optimal consumption of pesticides and monitor them according to provided limitations.

There have been just a few investigations concerning the evaluation of pesticides in

1. MS.c of Toxicology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) Tehran, Iran.
2. PhD of Pharmacology,Food and Drug Laboratory Research Center, Food and Drug Organization, MOH & ME, Tehran, Iran.
3. PhD of toxicology, Food and Drug Laboratory Research Center, Food and Drug Organization, MOH & ME, Tehran, Iran.
4. PhD of Food Chemistry, Food and Drug Laboratory Research Center, Food and Drug Organization, MOH & ME, Tehran, Iran.
5. Department of Toxicology & Pharmacology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) Tehran, Iran.
*Corresponding Author: E-mail: moosavi.z@iaups.ac.ir

pistachios around the world. In a survey conducted in 2010 in India, Pandey et al. investigated the amount of organochloric pesticides (DDT, Endosulfan, HCH) in pistachios, peanuts, walnuts, coconuts and apricots. This analysis was carried out by gas chromatography and an electron capture (ECD). The results showed very low levels of HCH (0.007-1.328 mg/ kg), DDT (ND-0.140 mg/ kg) and Endosulfan (ND-0.091 mg/ kg) in the samples [8]. In another study by Cortes et al. (2008), a novel method of multi-analysis of pesticides in nuts was optimized and presented using reversed-phase liquid chromatography-gas chromatography (RPLC-GC). In this study, organophosphate pesticides (i.e. Diazinon, Methylchlorpyrifos, Fenitrothion, Malathion, Chlorpyrifos, Parathion, Phenthoate, Chlorfenvinphos, Methidathion and Ethion) were simultaneously analyzed by RPLC-GC and NPD detectors. Also, some Organochlorous pesticides such as Captan, Lindane, Alph- Endosulfan, Beta- Endosulfan, Oxyfluorfen were analyzed concurrently by an RPLC-GC and ECD detectors in nuts, peanuts, walnuts, hazelnuts and sunflower seeds [9]. Due to the fatty nature of these seeds, their extracts contained high amounts of fat that subsequently needed two stages of refinement. To avoid such procedures the analysis was carried out by RPLC-GC. In this analysis LC system was replaced by condensing and clearing processes. This method was based on rapid extraction by ethyl acetate and its analysis by RPLC-GC. The validation outcomes indicated that calibration curves of pesticides were linear in the range of 0.01 to 0.15 mg/kg for OPs and 0.01 to 0.25 mg/kg for CPs [9]. In one study by Husain, S.W. and et al. in Iran (2003), ethion and diazinon residues in pistachio were examined using matrix solid-phase dispersion (MSPD) method and application of lanthanum silicate column by gas chromatography via NPD detector. Recoveries for C_{18}-MSPD co-column lanthanum silicate were 95% for ethion and 94% for diazinon with relative standard deviations of 7-13% in the concentration range 0.5-4 mg/ kg. The limits of detection were 0.02 mg /kg for ethion and 0.035 mg/ kg for diazinon [10].

There is no data bank on pesticide residues in domestic pistachio in Iran. Therefore, it is necessary to establish valid and applicable analytical methods for simultaneous determination of pesticides residue in order to obtain correct and reliable information about the type and quantity of these residues in crops. Due to the wide range of physicochemical properties of foods and pesticides, researchers have used several methods for purification of extracts such as, liquid-liquid partitioning, solid-phase extraction (SPE), gel-permeation chromatography (GPC), matrix solid-phase dispersion (MSPD), etc [11]. Nowadays several new procedures have been developed in which acetonitrile is of great necessity and sodium chloride (NaCl) is used in order to improve the extraction. In this regards, Anastassiades et al. have set up a method for extraction of pesticides in a variety of foods that is known as QuECHERS which stands for (Quick), (Easy), (Cheap), (Effective), (Rugged) and (Safe). This approach is based on extraction by acetonitrile and dewatering by magnesium sulfate in the presence of salt, after which two phases of cleaning (Clean-up) by a PSA (Primary secondary amine) and C_{18} is followed [12,13].

Several other methods such as liquid chromatography along with fluorescence detector, UV, and gas chromatography by different detectors are also used for determination of pesticide residues in crops. Since 1970, conventional methods of determining pesticide residues in foods entered a new stage which was essentially based on the application of gas chromatography connected to a variety of detectors including mass detector (MS). Multi-residue Method (MRM) in the presence of Mass Spectrometer detector MRM methods that we use in our study are employed for simultaneous analysis of pesticides in crops. This method is based on spiking blank sample and the data is used for drawing calibration curves instead of standard solutions. QuEChERS sample preparations were developed for determination of 12 pesticide residues in pistachio by gas chromatography-mass spectrometry (GC/MS). The investigated pesticides included: diazinon, carbaryl, fenitrothion, fenthion, chlorpyrifos, alpha-endosulfan, beta endosulfan, cis- and trans-permethrin, ethion, propargite and phosalone that belong to different chemical groups of pesticides. Triphenylmethane (TPM) was used as an internal standard. A list of under study pesticides and some of their physicochemical characteristics along with the specified allowable limits by the Iranian Standards Institute are shown in Table 1.

1175

Table 1. List pesticides of interest and some of their physicochemical characteristics along with the MRL established by ISIRI.

Type of Pesticides	MRL (ppm)	M.P. (°C)	M.W.	M.F.	Structural group	Compound
Insecticide	1	164	384.48	C9H22O4P2S4	Organophosphorus	Ethion
Insecticide	0.05	145	201.224	C12H11NO	Carbamat	Carbaryl
Insecticide	0.05	3.4	277.2	C9H12NO5PS	Organophosphorus	Fenitrothion
Insecticide	1	7.5	278.33	C10H15O3PS2	Organophosphorus	Fenthion
Insecticide	0.2	41.5	350.59	C9H11Cl3NO3PS	Organophosphorus	Chlorpyrifos
Insecticide	0.1	120>	304.35	C12H21N2O3PS	Organophosphorus	Diazinon
Insecticide	0.3	106	406.93	C9H6Cl6O3S	Organochlorous	Alpha- Endosulfan
Insecticide	0.3	207	406.93	C9H6Cl6O3S	Organochlorous	Beta –Endosulfan
Insecticide	2	34	391.29	C21H20Cl2O3	Pyrethroid	Permethrin1
Insecticide	2	34	391.29	C21H20Cl2O3	Pyrethroid	Permethrin2
Insecticide	0.1	46	367.81	C12H15ClNO4PS2	Organophosphorus	Phosalone
Acaricide	0.3	47	350.5	C19H26O4S	Sulfite ester	Propargite

MATERIALS AND METHODS

Chemicals

The solvents used in extraction and cleanup processes were all HPLC grade and were purchased from Merck (Darmstadt, Germany). The Primary Secondary Amine (PSA) was provided from Supelco (Bellefonte, USA), NaCl was from Merck (Darmstadt, Germany), and anhydrous $MgSO_4$ was obtained from SIGMA Aldrich CO (Japan). Also, all the employed pesticide standards were purchased from Dr. Ehrenstorfer Co. (Augsburg, Germany).

In order to activate and remove residual water before using, magnesium sulfate was put into the furnace for 5 hours at the temperature of 500 °C.

GC-SQ/MS

An Agilent Technologies 6890 N Network GC System chromatograph (Wilmington, USA) with a SQ detector and equipped with an Agilent 7683B autosampler (Agilent technologies, USA) was used and an HP-5 capillary column (30 m × 0.25 mm I.D., 1 μm film thickness) was established for separation.

Calibration

Ethylacetatic solution (1 mg/mL) of each standard and ethylacetatic solution of Triphenyl methane (TPM) (1 mg/mL) as internal standard were prepared and after encoding and registration, were stored at -20° C. Mixed standard solutions of 12 pesticides with concentration of 10 μg/mL were prepared in ethyl acetate.

In order to overcome matrix effect, calibration curve was depicted in the presence of internal standard, using spiked samples with the selected pesticides.

Ten grams of milled and homogenized blank pistachios was treated with a mixture of 12 pesticides at 10 ng/g, 25 ng/g, 50 ng/g, 100 ng/g, 200 ng/g, 300 ng/g and 500 ng/g concetrations obtained by adding 10 μL, 25 μL, 50 μL, 100 μL, 200 μL, 300 μL and 500 of the standard mixture of pesticides respectively and concentrations of 10 μg/mL and 5 μL of the internal standard of the primary solution with the concentration of 1 mg/mL at the level of 500 ng/g. Then, for better attachment of pesticides and the matrix, the samples were kept in a cool and dark place for one hour. The results of the analyzed samples, obtained in accordance with the sample preparation method described in the next section, were used for drawing the calibration curve.

This process was repeated on three different days and, finally, the calibration curve was drawn for each pesticide with an average of 18 to 21 points using Excel program. (The curves were drawn according to LOQ of each pesticide).

Sample preparation

The sample for monitoring pesticides was prepared following these steps: 5 mL of deionized water and 12.5 mL acetonitrile were added to 10 gr milled and homogenized pistachio sample in a 50 mL falcon tube and mixed for extraction. Then, 1 gr NaCl and 2 gr of magnesium sulfate was added into falcon tube and mixed for 1 minute by vortex. The samples were centrifuged at 3000rpm, for 5 minutes in-5°C. The upper layer was transferred to a 15 mL falcon tube containing 1gr magnesium sulfate and 0.1gr PSA and 0.1 gr C_{18}. The mixture was mixed by vertex for 1 minute and then

1176

Iranian Journal of Toxicology

Azadeh Emami et al

centrifuged at 3000 rpm, for 5 minutes in -5˚C. Four ml of the supernatant was next transferred to a vial and was concentrated under nitrogen gas to 0.3-0.5 ml. The residue was reconstituted by toluene to 1ml and finally, the vials were mixed by vortex for 3 minutes and then 2μl of the solution was injected into gas chromatograph.

Recovery studies

To determine the recovery, accuracy and precision of the method, blank pistachio samples were spiked using a standard mixture of pesticides with a concentration of 10 μg/mL at levels of 15, 45, 75, 150 and 250 ng/g respectively and in the presence of 5 μL of the internal standard of the primary solution with a concentration of 1 mg/mL at the level of 500 ng/g. Then, the samples were analyzed according to the method described in the previous section. This process was repeated on three different days, and the mean recovery and CV% were calculated.

GC-SQ-MS analysis

Helium was used as carrier gas at a constant flow of 1 ml/min. The injection port was adjusted at 250°C and splitless mode was used. The oven temperature program for separating compounds was as follows: Initial temperature was 75°C and remained so for 3 min; then it was increased to 120°C at 25°C/min ramp rate and finally, increased to 300°C at 5°C/min ramp, holding at 300°C for 10 min.

All of the current pesticides were injected into GC/ MS machine individually. This operation took place for several purposes including determination of retention time of each compound, identification of the relevant chromatograms, and detection of chromatograms and calculating the ion ratio for each compound. After attaining mass spectra of each compound in the scan mode, diagnostic ions were selected according to their magnitude and participation. Selected ions and retention time of pesticides and internal standard used in this analysis are shown in Table (2).

Quantitation

The standard curves were plotted by obtained values from division area under the curve of the pesticides in spike samples to the area under curve of the internal standard. This curve was used to determine the concentration of pesticide residues in spike samples.

Simultaneous analysis of current pesticides was performed in scan mode. After evaluation of appropriateness of isolation and identification of pesticides, to gain higher specificity and sensitivity, selected ion monitoring method (SIM) was used. Using this technique leads to decrease or elimination of matrix effects and increases sensitivity and specificity of the analysis.

Quantitative determination of pesticides was done by extract technique with regard to the quantification ion. The ion, selected according to the magnitude of m/z and intensity of the high presence after evaluation of appropriateness of validation parameters, was considered as certain quantification ion. The chromatogram from the simultaneous analysis of pesticides and internal standard in SIM mode is shown in Figure 1.

Table 2. The retention time, diagnostic ions and selected quantification ions obtained for the studied pesticides and the internal standard.

Quantification ions (m/z)	Diagnostic ions (m/z)	Retention time (min)	Compound
231.0	384.1,231,175	36.61	Ethion
144.0	144,115.1,125.9,116.1	20.64	Carbaryl
144.0	144,115.1,125.9,116.1	29.55	Carbaryl
277.0	277,260,214,276.1	30.37	Fenitrothion
278.0	278,262.9,169,115	31.12	Fenthion
314.0	314,257.8,197,153	31.20	Chlorpyrifos
304.0	304,276,179,199.1	27.012	Diazinon
236.9	236.9,264.9,338.9	34.45	Alpha- Endosulfan
236.9	339.1,264.9,236.9,266.9	36.66	Beta -Endosulfan
183.1	183.1,127,163.1,153	43.49	Permethrin$_1$
183.1	,127,163.1,153 183.1	43.79	Permethrin$_2$
182.0	367,182,154,183.9	41.27	Phosalone
350.2	350.2, 335.2 ,201.1	38.41	Propargite
244.1	244.1,165	30.81	Triphenylmethane(TPM)

1177

http://www.ijt.ir; Volume 8, No 27, Winter 2015
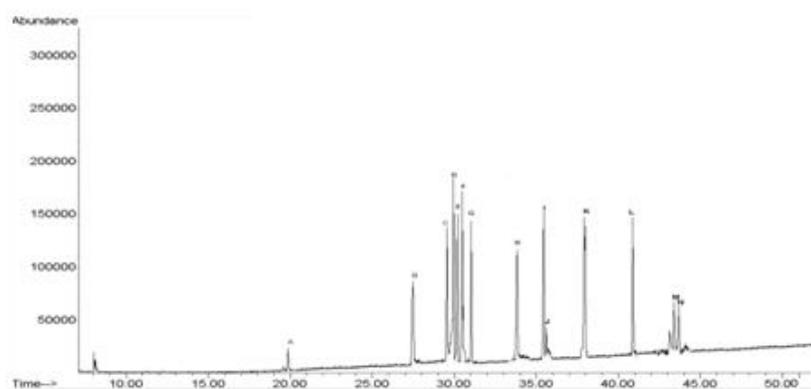
Figure 1. The representative chromatogram obtained for the 12 pesticides and the internal standard.
A) Carbaryl$_1$, B) Diazinon, C) Carbaryl$_2$, D) Fenitrothion,
E) Triphenylmethan (TPM), F) Fenthion, G) Chlorpyrifos
H) Alpha- Endosulfan, I) Ethion, J) Beta-Endosulfan, K) Propargite
L) Phosalone, M) Permethrin$_1$ and N) Permethrin$_2$ in SIM mode

RESULTS

Method validation

The applied method has been devised by considering the scientific and practical criteria of a valid process. In this regard, validation parameters including evaluation of linearity range, determination of the recovery percentage, accuracy study, as well as detection and quantification limits have been taken into account [14].

Linearity of the calibration curves

The results showed acceptable linearity of the pesticides in SIM mode. (Table.3) The correlation coefficient for all the pesticides were more than 0.994.

Limits of detection and limits of quantification

The limits of detection and quantification for all pesticides were 2 ppb and10 ppb, respectively. Limits of quantification were measured in spiked samples considering Signal/Noise =9/1 for LOQ and Signal/Noise =3/1for LOD.

Recovery

The recovery of pesticides at 5 concentration levels (n=3) was in the range of 81.41- 91.80 %. The method was proved to be repeatable with the majority relative standard deviations (RSD) being lower than 20%. The recoveries and repeatabilities were in accordance with the criteria set by SANCO guideline (Commission of the European Communities, 2006). Table 4 presents the recovery and repeatability of 5 concentration levels of the spiked samples.

Table 3. The Calibration data (equation and regression coefficient) of the studied pesticides in spiked pistachio calibration curves.

Regression Coefficient	Equation	Compound
0.9966	y = 0.001x + 0.001	Ethion
0.9966	y = 0.002x - 0.005	Carbaryl
0.9990	y = 0.001x - 0.004	Fenitrothion
0.9975	y = 0.004x - 0.033	Fenthion
0.9943	y = 0.0006x + 0.004	Chlorpyrifos
0.9961	y = 0.00017x + 0.005	Diazinon
0.9989	y= 0.00007 x - 0.0003	Alpha- Endosulfan
0.9986	y = 0.00009 x - 0.0002	Beta -Endosulfan
0.9975	y = 0.001x + 0.008	Permethrin1, 2

Table 4. The Average recoveries (%) and range of relative standard deviations (RSD) (%) obtained by GC-MS analysis of pistachio samples at 5 spiking levels (n=3).

Compound	Average recovery (%) (n=3)					Total Recovery (%) (n=15)	Rang of RSD
	15 ng/g	45 ng/g	75 ng/g	150 ng/g	250 ng/g		
Ethion	81.52	78.11	93.44	94.47	82.07	85. 92	6.9-17.8
Carbaryl	90.01	81.20	95.01	93.19	77.95	87.48	8.1-24.5
Fenitrothion	85.44	71. 89	98.04	74.81	76.80	81.40	1.7-18.6
Fenthion	112.84	77.58	81.77	77.72	72.43	84.47	4.3-20 .9
Chlorpyrifos	93.15	88.17	86.34	88.91	81.72	87.66	9.4-28.6
Diazinon	87.24	75.34	95.15	76.45	80.43	82.92	18.9-2.7
Alpha- Endosulfan	88.09	81.57	93.71	86.13	73.87	84.67	3.1-16.6
Beta -Endosulfan	100.59	95.97	86.24	90.57	73.59	89.39	3.7-18.1
Permethrin$_{1,2}$	97.57	89.49	94.22	94.92	83.25	91.89	3.1-19.8
Phosalone	119.98	87.75	96.98	84.89	74.86	93.08	4.8-19.1
Propargite	91.41	75.32	88.71	106.52	77.21	87.83	1.8-14.7

DISCUSSION

The present study is considered as a multiresidue method based on spiking blank samples for overcoming matrix effects and QuEChERS sample preparation that was developed for determination of 12 pesticide residues in pistachio by gas chromatography-mass spectrometry (GC/MS).

Optimizing the multi analysis method of pesticides and delivering innovation in this method as well as its establishment in country's food and drug control laboratories is the current study's scope. Establishment of a valid method is an important step in   monitoring and control of pesticides residues in agricultural products. The novel and updated articles and studies presented in the world in the field of analysis of pesticides mainly focus on improving the methods of concurrent monitoring of this substance. The most important aspects of these optimizations include: improvement of methods in the field of simplicity and reduction of analysis time, decreasing consumption of hazardous solutions, increasing the percentage of recovery and reduction of matrix effects.

The major reason for the lack of accuracy in analysis of pesticides by (GC/ MS), especially in food, is related to the compounds injected into the machine together with the analyte. This process is called the matrix effect and impairs the accuracy of obtained results. Matrix is able to increase or decrease chromatographic responses and complete elimination of this effect by cleaning is not possible [15,16] Different strategies such as matrix-matched standards, standard addition method, isotopically labeled internal standards and spiked calibration curves, have been applied by investigators to decrease or eliminate the matrix effects [17,18].

In the present study spiked calibration standard method was used. After spiking pistachio samples and their analysis, we plotted the standard curve by the values from divisions of areas under the curve of pesticides in spike sample to the area under the curve of the internal standard. The resulted curves were used to determine the concentration of residual pesticides in unknown samples.

This study was the first attempt for optimizing a multiresidue analysis method for pesticide residues from different chemical groups in pistachio samples and a reliable and accurate method based on spiked calibration curve and QuEChERS sample preparation was developed for determination of pesticide residues in pistachio by gas chromatography–mass spectrometry (GC/MS). Due to the identical circumstances of the samples and curves, this approach, in terms of matrix presence, is effective in reducing matrix effects. On the other hand, possible errors during

1179

extraction, clearing and analysis of the calibration curve led to improvement of the recovery rates and increased the accuracy and precision of the analysis.

In QuEChERS technique, to remove fatty compounds in the cleanup step, samples were stored for more than 12 hours in the refrigerator and cold water was added to them. This procedure led to extra time and difficulty for removing the water from extraction solvent. Therefore, in order to improve the process and decrease the analysis time for removal of the hindering compounds, the samples were centrifuged at a temperature of -5°C.

CONCLUSION

The results of validation indicated that calibration curves of pesticides were linear in the range of 10-500 (ng/g) and correlation coefficient of entire pesticides was higher than 0.994.

The recovery of pesticides at 5 concentration levels (n=3) was in range of 81.41- 91.80 %. The method was proved to be repeatable with the majority of RSDs being lower than 20%. The limits of detection and quantification for all pesticides were 2 and 10 ppb, respectively.

The recoveries and repeatabilities were in accordance with the criteria set by SANCO Guideline (Commission of the European Communities, 2006).

ACKNOWLEDGMENTS

REFERENCES

1. Federico D, Magdalena M, Mortaz M. A Critical Analysis of the Competitiveness of the Iranian Pistachio Industry'. International Journal of Business and Social Science. 2011; 2: 31-8.
2. Gentile C, Tesoriere L, Butera D, Fazzari M, Monastero M, Allegra M, et al. Antioxidant activity of Sicilian pistachio (Pistacia vera L. var. Bronte) nut extract and its bioactive components. Journal of Agricultural and Food Chemistry. 2007;55(3):643-8.
3. Kocyigit A, Koylu A, Keles H. Effects of pistachio nuts consumption on plasma lipid profile and oxidative status in healthy volunteers. Nutrition, metabolism and cardiovascular diseases. 2006;16(3):202-9.
4. Whylie P, Albaiges J, Barra R, Bouwman H, Dyke D, Wania F, et al. Global Report 2003. Regionally Based Assessment of Persistent Toxic Substances. United Nations Environment Programme (UNEP), Geneva, Switzerland. 2003:207.
5. O'Dwyer T. Tracking the distribution of persistent organic pollutants. Applied Catalysis B: Environmental. 1996;11(1):N2-N3.
6. Babu Rajendran R, Venugopalan V, Ramesh R. Pesticide residues in air from coastal environment, South India. Chemosphere. 1999;39(10):1699-706.
7. Owens K, Feldman J, Kepner J. Wide Range of Diseases Linked to Pesticides. Pesticide-Induced Diseases Database 2010; 30: 13-21.
8. Pandey P, Raizada R, Srivastava L. Level of organochlorine pesticide residues in dry fruit nuts. 2010; 31:705-7.
9. Cortés JM, Toledano RM, Villén Js, Vazquez A. Analysis of Pesticides in Nuts by Online Reversed-Phase Liquid Chromatography− Gas Chromatography Using the Through-Oven Transfer Adsorption/Desorption Interface. Journal of Agricultural and Food Chemistry. 2008;56(14):5544-9.
10. Husain S, Kiarostami V, Morrovati M, Tagebakhsh M. Multiresidue determination of diazinon and ethion in pistachio nuts by use of matrix solid phase dispersion with a lanthanum silicate co-column and gas chromatography. Acta Chromatographica. 2003:208-14.
11. Bogialli S, Di Corcia A. Matrix solid-phase dispersion as a valuable tool for extracting contaminants from foodstuffs. Journal of biochemical and biophysical methods. 2007;70(2):163-79.
12. Gilbert-López B, García-Reyes JF, Molina-Díaz A. Sample treatment and determination of pesticide residues in fatty vegetable matrices: A review. Talanta. 2009;79(2):109-28.
13. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC international. 2003;86(2):412-31.
14. Chandran S, Singh R. Comparison of various international guidelines for analytical method

validation. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2007;62(1):4-14.

15. Shoeibi S, Amirahmadi M, Yazdanpanah H, Pirali-Hamedani M, Pakzad SR, Kobarfard F. Effect of cooking process on the residues of three carbamate pesticides in rice. Iranian journal of pharmaceutical research: IJPR. 2011;10(1):119-20.

16. Amirahmadi M, Yazdanpanah H, Shoeibi S, Pirali-Hamedani M, Gholami MO, Mohseninia MF, et al. Simultaneous Determination of 17 Pesticide Residues in Rice by GC/MS using a Direct Sample Introduction Procedure and Spiked Calibration Curves. Iranian journal of pharmaceutical research: IJPR. 2013;12(2):295-6.

17. Hajšlová J, Zrostlı́ková J. Matrix effects in (ultra) trace analysis of pesticide residues in food and biotic matrices. Journal of Chromatography A. 2003;1000(1):181-97.

18. Soboleva E, Rathor N, Mageto A, Ambrus A. Estimation of Significance of'Matrix-induced'Chromatographic Effects. Special Publications of the Royal Society of Chemistry. 2000;256:138-56.