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Determination of Pesticides in Fruits by Gas Chromatography/Mass Spectrometry after High Performance Gel Permeation Clean up

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An analytical method was used for the determination of 115 multiclass pesticides in fruits by gas chromatography/mass spectrometry. The occurance of 115 pesticides were assessed in 30 samples (melon, maskmelon and watermelon) collected in Tehran during year 2005. Extraction of the pesticides were carried out with ethyl acetate and the extract was cleaned up and concentrated by high performance gel permeation chromatography (HPGPC). Pesticide residues were identified and quantified using ion trap gas chromatography mass spectrometry detector. One of these pesticides was detected in samples at level of 0.02-0.07 mg/kg. The recovery levels of 115 pesticides from melon, maskmelon and watermelon were in the range of 60-140 % and the relative standard deviation values of the pesticides were less than 5 %. The recovery indicated that the described multiresidue procedure is an efficient and reliable tool for monitoring pesticide residues in fruits and HPGPC clean up technique effectively removed sample matrices without pesticide loss. The data demonstrated that the samples analyzed did not contain residues of the monitored pesticides above the accepted maximum residue limits (MRLs) as adapted by the FAO/WHO Codex Alimentarius Commission.

Key Words: Fruits, Pesticides, GC/Mass, GPC, Multiresidue method.

INTRODUCTION

Pesticides are used on a wide variety of crops and residues in foods are commonly found. Over 4 billion pounds of pesticides are used each year in worldwide. Increasing public concern in recent years about possible health risks from pesticide residues in the diet has deeply modified the strategy for crop protection, with emphasis on food quality and safety and the widespread

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concern for the health of society has led to strict regulation of maximum residue limits (MRLs) and total dietary intakes of pesticide residues in food commodities. Akiyama *et al.*[1] studied on 107-204 pesticide residues in 765 agricultural products of Taiwan. The results show that 51 % of domestic and 32 % of imported samples contained no detectable residue and only 2.4 % of them contained more than 5 different residues. Vongbuddhapetak *et al.*[2] had also determined dietary exposure of Thais to pesticides. The results of investigation in 8 years (1989-1996) indicated that among 24 pesticides, DDT, dimethoate, methamidophos and parathion methyl were found every year. However dietary intakes of all pesticides were far below the established daily intake.

Recent surveys[3-7] of consumers have indicated that more than 80 % pesticide residues as a serious hazard. The health effects from pesticide exposure will depend on the type of pesticide involved of each major class of pesticide in terms of two types of exposure acute, high-level exposure and chronic, low level exposure.

Screening methods for pesticide multiresidues in fruits are necessary for the surveilance and identification of samples containing residue levels higher than maximum allowed values. The analysis of such samples must be rapid and accurate. Due to the complexity of the matrices involved, extraction is usually followed by clean up before gas chromatographic analysis[8]. Special care must also be observe for the extraction and clean up steps. A broad variety of solvent extraction and partitioning systems have been proposed for crop sample extractions. The acetone, methanol, acetonitrile and ethyl acetate are commonly used in solvent-based extraction methods[9]. Due to the broad range of physico-chemical properties of target analytes[10], many procedures are based on the use of extensive clean up of extracts *e.g.*, liquid-liquid extraction (LLE) or supercritical extraction, solid phase extraction (SPE), gel permeation chromatography (GPC), followed by an analytical separation method, typically liquid chromatography (LC) with fluorscence detection, gas chromatography (GC) with electron capture detection (ECD) or capillary electrophoresis or mass spectrometry (MS)[4,10-14]. Moreover, occurrence of interfering co-extractives from sample matrix requires extensive clean up. GPC is a widley used efficient technique for purification and large molecular removal from sample extracts[10].

Recently, GC/MS has been generally accepted in the pesticide analysis field because it provides simultaneous determination and confirmation of a large number of compounds and low detection limits as a consequence of high selectivity by the use of the selected ion monitoring. However, the current observations have revealed that GC/ITMS with ion trap detector is suitable for rapid semiquantitative screening. Pesticides have a wide variety of properties such as solubility, volatility and stability, so that under the

sample preparation conditions, to prevent pesticides loss as mush as possible, a large amount of sample matrix components may be present in the test solution for GC/MS. Large amounts of matrices in test solutions for GC/MS often cause suppression or promotion of ionization of the analytes, thus interfering with the determination of pesticides in ion trap chromatograms. Furthermore,under the analytical conditions needed for over 100 pesticides, some pesticides peaks might overlap each other in the ion trap chromatograms. Therefore the analysis could lead to false positive and/or false negative detection. Moreover when a large number of test solutions are analyzed sequentially by GC/MS, lack of resolution and sensitivity are caused by a dirty injection port, separation column and ion source[15].

In present study, GC/ITMS method was used for analysis 115 pesticides in fruits, GPC clean up technique provided satisfactory separation of the pesticides from a large amount of the sample matrices. Thus by the use of these catridge column clean up, sample matrices which might take ionization energy and cause interference in ion trap chromatograms, were effectively removed.

EXPERIMENTAL

Ethyl acetate, sodium chloride, anhydrous sodium sulfate, acetone, cyclohexane and *n*-hexane were of pesticide-analytical grade (Fisher Scientific, UK). Water was obtained by using a puric-system. Phosphate buffer (1 M) was prepared by dissolving 105 g dipotassium hydrogen phosphate and 61 g potassium dihydrogen phosphate in water and dilution to 1 L.

Stock standard solution of each pesticide was prepared in ethyl acetate at concentration of 1 mg mL^{-1} and further dilutions were made with ethyl acetate. Applied pesticides included DDT-op, DDT-pp, HCH-γ, endosulfan (I), endosulfan (II), endosulfan-sulphate, hexachlorobenzen, quintozene and tecnazene (ThamesRestek, UK).

Sample preparation: Directive 2000/1/24/ CODEX (Codex Alimentarius, 2000) was followed for sampling and transporting of fresh fruits and vegetables[16]. A total 30 samples (10 watermelon, 10 maskmelon and 10 melon) were obtained from whole sale of fruit and vegetable in Tehran in October 2005. For residue analysis, 1-2 kg of each sample after freezing were stored at -18 °C before experiment. Frozen samples were placed in containers with dry ice and pasted barcode.

For analysis, 30 g of sample was dissolved in 60 mL of solvent including 5 g sodium bicarbonate and 40 g anhydrous sodium sulfate. The mixture was incubated for 30 s using an ultra turrax homogeniser. The rinse were filtered through the filter paper. The filtrate was evaporated to near dryness with a rotary vacuum evaporator.

HPGPC Clean up: Envirosep-ABC (Phenomenex J2, Scientific, Colombia, USA) 60 × 21.2 mm i.d. columns in series were used. A 2 mL aliquot of crude-extract (*i.e.*, equivalent of l g of original matrix) was loaded to HPGPC. The flow-rate of mobile phase (cyclohexan:ethyl acetate, 1:1, v/v) was 5 mL/min. Dump time 16 min, collect (14 mL) was evaporated next to the dryness using a rotary evaporator, remaining solvent was blown down by a gentle stream of nitrogen and the reminder was redissolved in l mL of toluene prior to GC analysis.

The pesticide fraction obtained by HPGPC clean up of crude extract, was evaporated to dryness using a mild stream of nitrogen. The residue was then dissolved in 10 mL ethyl acetate. To prepare "spiked sample", a 5 mL aliquot was evaporated carefully and the reminder was dissolved in a 0.5 mL toluene.

Method of analysis: Identification and determination of the pesticide was performed using a Varian Model 3800 gas chromatograph (GC) fitted with an ion trap mass spectrometric (ITMS varian 2200). A DB-5 (Folsom, CA, USA) capillary column (25 m × 0.25 mm I.D., 0.25 mm film thickness) was employed. Injections were performed with the column oven at 100 °C in the splitless mode. This temperature was maintained for 1 min and then programmed at 10 °C/min to 200 °C and at 4 °C/min to 300 °C and held for 3 min. The carried gas was helium in system, at flow rate of 1.0 mL/min and gas pressure program was 70 Kpa (l min hold), to 110 Kpa at 8 Kpa/min, to 150 Kpa at 1 Kpa/min and then to 174 Kpa at 4 Kpa/min (5 min hold). The Varian Saturn 2200 GC/MS begins with the CP-3800 gas chromatograph. For ITMS the temperature of the injector was 250 °C. Quantification matched calibrants were run with each batch at 5 levels.

RESULTS AND DISCUSSION

A total of 30 samples of fruits were examined for 115 pesticide residues listed in (Table-1). The results obtained showed that 73.4 % of the fruit samples meanwhile no level of pesticides, 26.6 % of samples gave results with trace levels of pesticide residues (< MRLS). The finding of detectable residue indicate that 5 maskmelon (Varamin & Torbatjam) and 2 melon contained endosulfan II and endosulfan-sulphate. The results of the current study, showed that no residues of restricted or banned pesticides such as DDT, HCH-γ and their metabolites were present in any of the analyzed samples. Limit of detection and recovery of each pesticide have been shown in Table-2. Recoveries of pesticides from melon, maskmelon and watermelon were determined 5 times. The recoveries values obtained for 2 pesticides were for endosulfan (II) 107 % and endosulfan-sulphate 118 %. The relative standard deviation (RSD) values of the pesticides were < 5 %, which were estimated by the version 5.51 Software workstation. In comparison,

TABLE-1
PESTICIDE RESIDUES IN SAMPLES WITH GC/ITMS METHOD

	Pesticide (mg kg^{-1})			
	Endosulfan sulphate		Endosulfan (II)	
	Residue	MRL	Residue	MRL
Maskmelon Torbatjam*				
1	0.02	0.5	-	-
2	0.02	0.5	-	-
5	0.02	0.5	-	-
Maskmelon Varamin*				
2	-	-	0.04	0.5
3	-	-	0.03	0.5
5	-	-	0.04	0.5
Melon Saveh*				
5	0.05	0.5	-	-
8	0.07	0.5	-	-

*Cultivation area.

TABLE-2
METHOD RECOVERIES AND DETECTION LIMITS OF PESTICIDES IN SAMPLES

Sample	Melon (mg kg^{-1})		Watermelon (mg kg^{-1})	
	LOD	Recovery	LOD	Recovery
DDT-op	0.05	0.01	0.05	0.01
DDT-pp	0.05	0.01	0.05	0.01
HCH-γ	0.05	0.01	0.05	0.01
Endosulfan (I)	0.05	0.02	0.05	0.05
Endosulfan (II)	0.05	0.05	0.05	0.02
Endosulfansulphate	0.05	0.05	0.05	0.02
Hexachlorobenzene	0.05	0.01	0.05	0.01
Quintozene	0.05	0.01	0.05	0.01
Tecnazene	0.05	0.01	0.05	0.01

in a monitoring program of the U.S. Food and Drug Administration the rate of contamination were 47.4 and 65.3 %, for fruit and vegetable samples[17].

The result obtained from a study conducted in Mauritius in year 1997 showed that 61.5 % of the vegetable and fruit samples analyzed by gas liquid chromatography contained no detectable levels of insecticide residues, all below the MRL, while 2.3 % of the sample showed results above the MRL[1]. Montoring of 151 pesticide residues in products of plant origin in E.U, Norway, Iceland, Liechtenstein in year 2000 showed that 2.9 % samples

contained residues above EC-MRLs[2]. The rate of contamination of 149 pesticides in fruits and vegetables in the annual report of pesticide residues committee of FAD[18] were 2 % above the MRL and 38 % of the samples residues below the MRL.

TABLE-3
PESTICIDES RESIDUE IN FRUITS TAKEN FROM WHOLE SALE OF FRUIT IN TEHRAN

Matrix	Sample no.	Pesticides (common name)	Residue found (mg/kg)	Coded MRL (mg/kg)	Recovery level (mg/kg)	Recovery (%)
Maskmelon	2	Endosulfan II	0.04	0.5	0.05	123
	3	Endosulfan II	0.03	0.5	0.05	123
	5	Endosulfan II	0.04	0.5	0.05	123
Maskmelon	1	Endosulfan sulfate	0.02	0.5	0.05	128
	2	Endosulfan sulfate	0.02	0.5	0.05	128
	5	Endosulfan sulfate	0.02	0.5	0.05	128
Melon	5	Endosulfan sulfate	0.05	0.5	0.05	106
	8	Endosulfan sulfate	0.07	0.5	0.05	106

In several studies, the effectiveness of GPC for removing pigments from food extracts have been demonstrated[19]. No pesticide gave noticeably reduced matrix enhancement after GPC clean up. Therefore GPC is very useful for removing pigment produced considerable amount of compounds such as food colours, chlorophyll and carotenoid (carotene) in the pesticide fraction. Because of the matrices in the pesticide fraction could not be retained on the sorbent in catridge column clean up, most matrices were present in the test solution for GC/MS.

Lack of resolution and breakdown of pesticides during GC/MS are always caused by high-molecular-weight, high-boiling and/or very polar matrix components. Most of the high molecular weight matrix components, such as lipids were removed by GPS. The size exclusion mechanism underlying this technique allows the compounds in the sample to be separated according to molecular weight. Under the GPC conditions described in the experimental section, tailing of the matrices produced considerable amount of compounds such as food colours, chlorophyll and carotenoid (carotene) in the pesticide fraction. Because of the matrices in the pesticide fraction could not be retained on the sorbent in catridge column cleanup, most matrices were present in the test solution for GC/MS. The occurrence of matrix-induced effects and their extent are simultaneously influenced by many factors *e.g.*, pesticide character, matrix type, matrix concentration

and the state of GC system. HPGPC applied in present experiments as a single clean up technique provided good separation of bulk plant coextracts (pigments and cuticular waxes) from matrices. GPC equipped with a 10 mm diametre column reduced solvent consumption and increase clean up efficiency. Ethyl acetate was used for extraction of 115 pesticides with different physico-chemical properties from fruits. In addition the sample cleanup technique effectively removed sample matrices without pesticide loss.

Conclusion

Using mass spectroscopy, quantification (through selective ion monitoring) and confirmation are achieved simultaneously. The recovery show that the described multiresidue method is an efficient and reliable tool for monitoring pesticide residues in fruits. GC-ITMS is not only an effective confirmation tool but also a more reliable quantification analysis method of multiresidue pesticides. The results indicates that the described multiresidue method is an efficient and reliable tool for monitoring pesticide residues in fruits.

Calculation of the estimated daily intakes of the 115 pesticides studied and their comparison with MRLs established by the FAO/WHO demonstrated the safety of fruits and vegetables consumption and showed the importance of the monitoring for pesticide residues. Furthermore, unambigiuous results for many compounds are demanded by producers and consumers to guarantee the safety of food. The results of the current study, showed that no residues of restricted or banned pesticides such as DDT, HCH-γ and their metabolites were present in any of the analyzed samples. HPGPC applied in our experiments as a single clean up technique provided good separation of bulk plant coextracts (pigments and cuticular waxes) from matrices.

Although there were not any significant residue in above mentioned Iranian friuts, but in developing countries, there is a very special emphasis on a continuous monitoring of pesticide residues in friuts and vegetables due to food security and safety. This monitoring of pesticide residues in vegetables is also very important due to lack GMP in agriculture crops from farm to fork and increased consumption of vegtables in Iranian food due to vegeterian diets. Our future work has been focused on this field of research to investigate validity of method for vegetable safety.

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