

# Spiked Calibration Curve: A Valid Method for Simultaneous Analysis of Pesticides in Melon Using Gas Chromatography Mass Spectrometry (GC/MS)

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**ABSTRACT:** Recently, with increasing public awareness of food safety, developments of some valid and rapidly analytical methods are required for the determination of residual pesticides in crops. This study was conducted on validation of a reliable, accurate and precise method for multi analysis of pesticides residue in melon as a main fruit in Iranian food basket. A reliable, and valid method based on spiked calibration curves and modified QuEChERS sample preparation was developed for determination of 12 pesticide residues in melon by Gas Chromatography Mass Spectrometry (GC/MS). The interested pesticides are, diazinon, dimethoate, chlorothalonil, pirimicarb, chlorpyrifos-methyl, metalaxyl,  $\alpha$ -endosulfan,  $\beta$ -endosulfan, permethrin 1, 2 and fenvalerate 1, 2 that are belonged to different chemical groups of pesticides. Triphenylmethane (TPM) is used as internal standard. Use of spiked samples for constructing the calibration curve substantially reduced adverse matrix-related effects. The recovery of pesticides at 3 concentration levels ( $n=3$ ) was in range of 79.8-97.6%. The method was proved to be repeatable with RSD lower than 20%. The limits of quantification for all pesticides were <25 ng/g. The recoveries and repeatabilities were in accordance with the criteria set by SANCO Guideline (Commission of the European Communities, 2006). Using spiked samples for preparation of calibration curves is a sensitive, selective, rugged, rapid and accurate method in pesticides analysis.

**KEY WORDS:** Spiked calibration curve, GC/MS-SIM, Pesticides residue, Melon, Triphenylmethane.

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**Table 1: Physicochemical properties of the selected pesticides.**

Compound	Structural group	M.F.	M.W.	M.P. (°C)
Dimethoate	Organophosphorus	$C_5H_{12}NO_3PS_2$	229.28	51
Diazinon	Organophosphorus	$C_{12}H_{21}N_2O_3PS$	304.35	<25
Chlorpyrifos-methyl	Organophosphorus	$C_7H_7Cl_3NO_3PS$	322.5	46.5
$\beta$ -Endosulfan	Organochlorine	$C_9H_6Cl_6O_3S$	406.92	207-209°C
$\alpha$ -Endosulfan	Organochlorine	$C_9H_6Cl_6O_3S$	406.92	106°C
Fenvalerate 1,2	Pyrethroid	$C_{25}H_{22}ClNO_3$	419.92	<25°C
Primicarb	Carbamate	$C_{11}H_{18}N_4O_2$	238	91
Permethrin 1,2	Pyrethroid	$C_{21}H_{20}Cl_2O_3$	391.28	35
Metalaxyl	Acylalanin	$C_{15}H_{21}NO_4$	279.34	72
Chlorothalonil	Chlorophenyl	$C_8Cl_4N_3$	265.92	250

## INTRODUCTION

Fruits and vegetables are very important part of food intake to meet the balanced diet for humans, for healthy life [1]. To improve the growing demand of fruits and vegetables, farmers utilize pesticides to increase production, preventing pests and diseases which cause problems in vegetable and fruit production [2].

Recently claim for pesticide monitoring has increased in developed country. Therefore, it is necessary to develop quick, reliable and effective analytical methods for the simultaneous determination of the pesticides residue in order to obtain accurate data about the types and quantity of the pesticides used, to protect the consumers and the safety of agricultural products [3].

Analytical advances are leading to development of more specific and more sensitive methods to separate, identify, and quantify pesticide residue from foods [4, 5].

Over the past decades, approaches to trace level determination of pesticides have moved away from the use of GC with selective detectors including Electron Capture Detection (ECD) and Nitrogen Phosphorus Detection (NPD), to Mass Spectrometer (MS) detectors which are more sensitive and selective. Using of mass spectrometry techniques, with its information-rich content and explicit confirmation, is recommended for monitoring pesticides residue in the entire world [6-13].

The accumulation of co-extractives in a GC inlet may cause to successive adverse changes in the performance of the chromatographic system such as the loss of analytes and peak tailing due to undesirable interactions

with active sites in the inlet and column. For vulnerable analytes, significant improvements in peak quality are obtained when matrix components are present because they fill active sites of injector, thus decreasing analyte interactions. However, this can lead to problems with quantification. These matrix effects can produce an overestimation of the analyte concentration if calibration has been performed with standard solutions. The presence of matrix effects should be estimated for all tested analytes [14].

Conventionally, coincident analysis of pesticide residues from different classes in foods is a time-consuming procedure that often entails several post-extraction clean up steps prior to the analysis. A simple, safe, cheap and high sample throughput method named QuEChERS has been recently proposed for the analysis of pesticide residues [15-17].

This paper presents a rapid multi-residue method of analysis based on a QuEChERS extraction procedure using spiked calibration curve to simultaneously determine and confirm 12 pesticide residues in melon. Melon is a main fruit in Iranian daily food basket [18]. The selected pesticides are twelve GC-amenable which lists of them along with some of their physico-chemical properties are presented in Table 1.

In order to overcome the adverse matrix-related effects, it was definite to prepare the calibration curves by spiking blank melon samples with certain amounts of pesticides and constructing the calibration curve using these spiked samples.

## EXPERIMENTAL SECTION

### Chemicals

All organic solvents, intended for extraction, were at least LC grade and purchased from Merck (Darmstadt, Germany). Primary Secondary Amine (PSA) was purchased from Supelco (Bellefonte, USA). Bulk quantities of NaCl and florisil were obtained from Merck (Darmstadt, Germany). Anhydrous MgSO<sub>4</sub> was obtained from SIGMA-Aldrich CO. (Japan). The MgSO<sub>4</sub> was baked for 5 h at 500 °C in a furnace to remove phthalates and residual water. All pesticides standards were purchased from Dr. Ehrenstorfer Co. (Augsburg, Germany).

### GC-SQ/MS

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

### Calibration curve

Individual stock standard solutions (1 mg/mL) were prepared in ethyl acetate and stored in the dark at -20 °C. Prior to their use, they were kept for 1h at ambient temperature. A mixed stock standard solution of pesticides was prepared in ethyl acetate at 15 μg/mL with respect to each pesticide. Spiked calibration curves at 7 levels of 10, 25, 50, 100, 200, 300 and 500 ng/g triplicate were prepared by addition of 10 μL, 25 μL, 50 μL, 100 μL, 200 μL, 300 μL and 500 μL of mixed standard stock solution, respectively, to 15 g portions of blank melon samples in each case.

A stock solution of triphenylmethane (TPM) in ethyl acetate at concentration of 1 mg/mL was used as internal standard and an aliquot of 10 μL of TPM solution in ethyl acetate was added to the spiked melon sample. The samples so obtained were treated as described in next section.

### Sample preparation

An aliquot of 10 μL of internal standard solution (1000 mg/L) was added to 15 g of blended blank melon sample in a 50 ml falcon tube and after being left for 1h at ambient temperature in dark, 15 mL acetonitrile (containing acetic acid 1% and sodium acetate

tri hydrate (0.45g) was added. The mixture was mixed at high speed with vortex mixer for 1 min. 4 grams of activated anhydrous MgSO<sub>4</sub> was added to the mixture, and mixing was continued for an additional 60 s. The mixture was centrifuged for 5 min at 3000 rpm at -5 °C. The supernatant was transferred to a 15 mL falcon tube containing 2 g MgSO<sub>4</sub>, 300 mg PSA and 100 mg florisil. After shaking for 1 min and centrifugation for 5 min at 3000 rpm at -5 °C, 4 mL of supernatant was transferred to a 5 mL vial and evaporated to dryness under a gentle stream of nitrogen gas. The residue was reconstituted by toluene to obtain 1 mL solution, and after shaking for 3 minutes, 2 μL of the solution was injected into gas chromatograph.

### Recovery studies

For recovery determination, spiked blank melon samples at concentration levels of 30 ng/g, 60 ng/g and 150 ng/g were prepared in triplicates and they were kept for 1 h at ambient temperature prior to their use and then treated according to the procedure described in sample preparation section. The recoveries were calculated using the calibration curves constructed using spiked samples.

### GC-SQ-MS analysis

The GC-SQ-MS was employed with helium as the carrier gas at a constant flow of 1 mL/min. The oven temperature started at 75 °C and remained at this temperature for 3 min increasing to 120 °C at 25 °C/min ramp rate and then increased to 300 °C at 5 °C/min ramp, holding at 300 °C for 11 minutes. Injection port was adjusted at 250 °C and splitless mode was used.

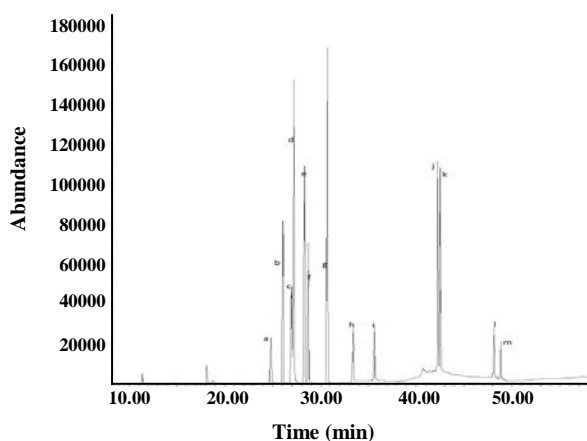
After acquisition of the total ion chromatogram for the mixed stock standard solutions in scan mode, peaks were identified by their retention time and mass spectra. The most abundant ion that showed no evidence of chromatographic interference and had the highest signal-to-noise ratio was selected for quantification purposes.

### Quantitation

The concentrations of pesticides were determined by interpolation of the relative peak areas for each pesticide to internal standard peak area in the sample on the spiked calibration curve. In order to compensate for losses during sample processing and instrumental analysis, internal standard (TPM) was used.

**Table 2: The retention time, diagnostic ions and selected quantification ion for the target pesticides and internal standard.**

No.	Compound	Diagnostic ions	Quantification ion	Retention time (min)
1	Dimethoate	228.9, 143, 125	125	24.85
2	Diazinon	304.1, 199.1, 179.1, 227.1	304.1	26.11
3	Chlorothalonil	265.9, 263.9, 267.9	265.9	27.01
4	Pirimicarb	238.2, 166.1, 138,	166.1	27.25
5	Chlorpyrifos-methyl	285.9, 287.9, 289.9	285.9	28.35
6	Metalaxyl	206.1, 220.1, 249.1	206.1	28.77
7	Triphenylmethane	244.1, 165	244.1	30.74
8	$\alpha$ -Endosulfan	240.9, 264.9, 338.9	240.9	33.44
9	$\beta$ -Endosulfan	339.1, 266.9, 238.9	238.9	35.62
10	Permethrin 1	183.1, 163.1, 165	183.1	42.29
11	Permethrin 2	183.1, 163.1, 165	183.1	42.56
12	Fenvalerate 1	419.2, 225.1, 207	225.1	47.74
13	Fenvalerate 2	419.2, 225.1, 207	225.1	48.47



**Fig. 1:** A representative chromatogram obtained for 12 pesticides and internal standard a) Dimethoate; b) Diazinon; c) Chlorothalonil; d) Pirimicarb e) Chlorpyrifos-methyl; f) Metalaxyl g) Triphenyl methane (Istd); h)  $\alpha$ -endosulfan; i)  $\beta$ -endosulfan; j) Permethrin 1; k) Permethrin 2; l) Fenvalerate 1 and m) Fenvalerate 2, in spiked melon sample at 300 ng/g in SIM mode.

## RESULTS AND DISCUSSION

### Gas chromatographic determination

Analysis was performed in the SIM mode based on the use of one target and two or three qualifier ions. Pesticides were identified according to their retention times and target and qualifier ions. The quantitation

was based on the peak area ratio of the targets to that of internal standard. Table 2 summarizes studied pesticides with their diagnostic and qualifier ions used in SIM mode. A GC-SQ-MS chromatogram of 12 pesticides and internal standard (TPM) analyzed in spiked melon is shown in Fig. 1.

### Method Validation

#### Linearity of the calibration curves

The twelve pesticides showed linearity in SIM mode. Linear spiked calibration curves for all the interest pesticides were obtained with correlation factors  $>0.997$ . The Calibration data (equation and regression coefficient) of 12 pesticides in spiked melon calibration curves is showed in Table 3.

#### Limits of detection and limits of quantification

Limit Of Detections (LODs) and Limit Of Quantifications (LOQs) of the proposed method were measured in spiked samples and calculated by considering a value 3 and 10 times that of background noise, respectively. The LODs and LOQs for all the pesticides were  $\leq 10$  ng/g and  $\leq 25$  ng/g.

### Recovery

Table 4 presents the recovery and repeatability for three concentration levels of pesticides. The recovery

**Table 3: Calibration data (equation and regression coefficient) of 12 pesticides in spiked melon calibration curves.**

Compound	Equation	Regression Coefficient
Dimethoate	$y = 0.3603x - 0.0012$	0.999
Diazinon	$y = 0.3014x + 0.0011$	0.999
Pirimicarb	$y = 1.08502x - 0.0102$	0.999
Metalaxyl	$y = 0.5769x - 0.0019$	0.998
Chlorpyrifos-methyl	$y = 1.0817x - 0.0061$	0.999
Chlorothalonil	$y = 0.5225x - 0.0137$	0.997
$\alpha$ -Endosulfan	$y = 0.134x - 0.0002$	0.999
$\beta$ -Endosulfan	$y = 0.0937x + 0.0002$	0.999
Permethrin 1,2	$y = 1.5486x - 0.001$	0.998
Fenvalerate 1,2	$y = 0.1639x - 0.0003$	0.997

**Table 4: Average recoveries (%) and relative standard deviations (%) of pesticides obtained by GC-MS analysis of melon samples at 3 spiking levels (n=3).**

Compound	Average recovery (%) (n=3)			Total recovery (%) (n=9)	Range of RSDr (%)
	30 ng/g	60 ng/g	150 ng/g		
Dimethoate	98.6	92.1	90.3	93.6	14.7-20.4
Diazinon	104.4	104.8	70.8	93.3	3.5-16.8
Pirimicarb	102.1	105.2	82.9	96.8	10.1-20.7
Metalaxyl	106.4	102.9	79.6	96.3	7.0-11.1
Chlorpyrifos-methyl	107.8	97.3	87.8	97.6	2.5-20.6
Chlorothalonil	89.9	70.5	78.1	79.5	0.43-10.0
$\beta$ -Endosulfan	92.3	82.2	92.7	89.0	9.4-21.1
$\alpha$ -Endosulfan	77.4	93.7	70.1	80.4	3.1-13.9
Permethrin 1, 2	80.8	101.9	71.2	84.7	1.7-21.7
Fenvalerate1, 2	87.9	83.6	80.5	84.0	9.2-14.0

of pesticides at 3 concentration levels triplicates was in the range of 79.5-97.6%. In terms of repeatability, the majority of the pesticides gave  $RSD < 20\%$ . The recoveries and repeatabilities are in accordance with the criteria set by SANCO Guideline [19].

### Discussion

The main source of inaccuracy in pesticide residue analysis by GC-MS, especially in food, is related to the injection of interfering components from the sample, so-called "matrix effect". In conventional gas chromatographic analysis, such as the analysis of pesticide

residues in foods, co-extracted matrix components may be problematic in obtaining the true information. [14].

Theoretically, elimination of matrix components or active sites would surmount the matrix-induced enhancement effect; but, complete and permanent GC system deactivation or comprehensive sample clean up in practice is impossible [20].

There are some approaches for preventing, decreasing, or compensating for the occurrence of matrix effects including the application of alternative calibration methods including the use of (A) matrix-matched calibration method, (B) standard addition method, (C) isotopically

labeled internal standards (not feasible in multiresidue pesticide analysis due to their unavailability or high price) and (D) usage of analyte protectants [14, 21-23].

In the present study, we used spiked calibration curve approach to overcome the problems caused by the matrix. In this approach, calibration curve are prepared by the addition of standard solution to blank melon samples and these samples subjected to the same sample preparation procedure which is intended to be used for unknown samples. This way, the standard sample matrices will have the same composition as the unknown samples and therefore the effect of matrix is reflected in both standards and unknown samples. The calibration curve is constructed using these spiked samples and it is easily used to calculate the concentration of analyte(s) in unknown sample without being concerned about the matrix effects. Other applied methods for overcoming matrix effect in pesticides residue analysis with GC/MS, use from matrix match calibration, Standard addition and isotope dilution techniques. The last two techniques have some disadvantage like difficulty, long time spending and difficulties of isotope usage, but in the applied spike calibration curve technique not only have the advantages of the first technique but also because of the most analytical unwanted effects have normalized in calibration curve, the results are closer to the real amounts.

In this study the recoveries and repeatabilities were in accordance with the criteria set by SANCO Guideline [19].

It is concluded that spiked calibration curve along with matrix-matched standards, standard addition method, isotopically labeled internal standards and usage of analyte protectants are proper approach for elimination of matrix effects in pesticide residues analysis. The method proposed for determination of various classes of pesticide residues is rapid, simple, sensitive, selective and rugged.

## CONCLUSIONS

An accurate and precision method was developed to determine 12 pesticide residues in melon; a main fruit in Iranian food basket. The method which consists of a QuEChERS sample preparation and GC-SQ-MS-SIM analysis showed a high sensitivity and confirmatory power necessary for the determination of pesticide

residues at the low levels. The developed method has the advantage of using spiked calibration curves that minimizes the matrix interferences leading to higher accuracy for pesticides analyses.

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