Qualitative and Quantitative Analysis Of Some Pesticide Residues In Some Agricultural Products By Using High Chromatographic Technique

Thesis Submitted By

Mahmoud Hamdy Ahmed Abdel-Wahed

M. Sc. in chemistry / Faculty of Science / Ain Shams University
2017

For the Degree of Doctor of Philosophy in chemistry (Ph. D.)

Chemistry Department - Faculty of Science
Ain Shams University

Under Supervision of

Prof. Dr. Eglal Myriam Raymond Souaya

Professor Emeritus of Inorganic and Analytical Chemistry -
Faculty of Science - Ain Shams University

Prof. Dr. Mona Abd El Aziz Khorshed

Chief Researcher and Technical Manager - Central Lab of Residue Analysis of Pesticides and Heavy Metals in Food - Agriculture Research Center

2020

TABLE OF CONTENTS

	Page
Approval Sheet …….....	-
Acknowledgment ……..….....	-
Abstract ……..…….....	-
Aim of the Study……..	-
Table of contents …….....	-
List of abbreviations……	i
List of figures …….....	iv
List of tables ……..…….....	v
List of publications……..	-
Chapter 1: Introduction and Literatures Review	
1.1.Food and Food Safety	1
1.2.Pesticides and their Health Problems	2
1.3.Maximum Residues Limits (MRLs) For Pesticides	3
1.4.Pesticide Residues Analysis Methods	3
1.5.LC-MS/MS Technique	4
1.6.Egyptian Agricultural Pesticide Committee	5
1.7.Literatures Review	6
1.8.Maleic Hydrazide (MH)	6
1.9. Applications of Maleic Hydrazide	7
1.10. Toxicity of Maleic Hydrazide	8
1.11. Maximum residue limits (MRLs) for Maleic Hydrazide	8
1.12. Literatures Review in Maleic Hydrazide	9
Chapter 2: Material and Methods	
2.1. Apparatus	13
2.2. Reagents and Chemicals	13
2.3. Pesticide Reference Standard	15
2.4. Test Samples	19
2.5. Sample Processing and Homogenization	20

	Page
2.6. Sample extraction of 20 pesticides by QuEChERS	21
2.7. Sample extraction of Maleic Hydrazide	22
2.8. Instrumentation	22
2.9. LC ESI (-) MS/MS Analysis	23
2.10. LC ESI (+) MS/MS Analysis	24
Chapter 3: Results and Discussion	
3.1. Maleic Hydrazide Extraction Method Optimization	26
3.2.Optimization of 6500 QTrap MS/MS conditions	26
3.3.Optimization of 4000 QTrap MS/MS conditions	31
3.4.Optimization of LC-Column and Mobile phase for MH	36
3.5.Optimization of Extracting Solvent for MH	39
3.6.Method Validation	41
3.6.1. Selectivity and Specificity for 21 Pesticides	41
3.6.2. Limit Of Quantification (LOQ)	47
3.6.3. LOQ for MH	47
3.6.4. LOQs for 20 pesticides	54
3.6.5. Method Linearity for MH	57
3.6.6. Method Linearity for 20 Pesticides	58
3.6.7. Matrix Effect for 20 Pesticides	60
3.6.8. Matrix Effect for MH	62
3.6.9. Recovery, precision and Repeatability for 20 Pesticides	64
3.6.10. Recovery, precision and Repeatability for MH	77
3.7. Internal Quality Control (IQC)	78
4. Conclusion ……..	79
5. Summary……...……	80
References ……..……	83
Arabic Summary	--
Arabic Abstract	--

LIST OF FIGURES

Figure	Figure Title	Page
1	LC-MS/MS spectrum for MH……..…….	32
2	Optimization of DP on LC-MS/MS…….….	33
3	Optimization of CE on LC-MS/MS for transition 111/83…….	34
4	Optimization of CXP on LC-MS/MS for transition 111/83……..	35
5	LC-MS/MS Response expressed as Peak Areas for Different Mobile Phase compositions and LC-Columns at 1000 ng/ml Maleic Hydrazide in Solvent…..	37
6	Comparison of Spiked Grape Matrix By 4 different Extracting Solvents in 3 Replicates in 500 ng/g Expected Concentration….....	38
7	Comparison of Spiked Potatoes Matrix By 4 different Extracting Solvents in 3 Replicates in 500 ng/g Expected Concentration….....	39
8	LC-MS/MS Chromatogram of Blank Reagent……	42
9	LC-MS/MS Chromatograms of Blank Onion, Potatoes, Grape and Citrus Matrices…….....	43
10	The Selected and Total Ion Chromatograms of Blank Methanol in (+MRM) Mode……..	44
11	The Selected and Total Ion Chromatograms of Blank Apple Extract in (+MRM) Mode……...……..…….....	45
12	The Selected and Total Ion Chromatograms of Blank Dry Fennel Seeds Extract in (+MRM) Mode……...…….…..…	46
13	LC-MS/MS Chromatograms for MH at LOQs for Onion, Potatoes, Grape and Citrus Matrices……..	48
14	LC-MS/MS Calibration Curve of Malic Hydrazide in Solvent……	58
15	Calibration Curves for Flucarbazone Sodium which was the first appeared compound and Brodifacoum which was the last appeared compound…….	59
16	Matrix Effect % of All Targeted Pesticides by LC-MS-MS at 0.05 mg/kg Standard Addition Level in Green Beans Extracted Matrix.....……	61
17	Matrix Effect % of All Targeted Pesticides by LC-MS-MS at 0.05 mg/kg Standard Addition Level in Rice Extracted Matrix…….	62
18	LC-MS/MS Calibration Curves in Solvent, Onion Extract, Potatoes Extract, Grape Extract and Citrus Extract……	63

LIST OF TABLES

Table	Table Title	Page
1	The full list of twenty pesticides and their formula, CAS No., chemical class, toxicity class and Log K_{OW} that were analyzed……....	16
2	The list of some important properties for Maleic Hydrazide……..……	19
3	The LC Gradient Elution program……..	25
4	LC-MS/MS optimization parameters including molecular masses to charge ratio (m/z) in the quads (q1&q3), molecular ion, Decluster Potential (DP), Entrance Potential (EP), Collision Energy (CE) and Collision Cell Exit Potential (CXP) for all target MRM transitions….	28
5	LC-MS/MS optimization parameters including molecular masses to charge ratio (m/z) in the quads (q1&q3), molecular ion, Declustering potential (DP), Entrance Potential (EP), collision energy (CE) and Collision Cell Exit Potential (CXP) for the five targeted MRM transitions…….	31
6	List of Lowest calibration level (LCL) concentrations, correlation variation (r^2), retention time (Rt) by minutes and Europian Maximum Residue Limits (EU MRLs) by (ng/g) for onion, potatoes, grape and citrus…….....	47
7	The average recovery results of Maleic Hydrazide by LC-MS/MS that were spiked at 50, 100, 500 and 1000 ng/g (5 replicates, one analyst, at each level) in Onion Matrix…….	50
8	The average recovery results of Maleic Hydrazide by LC-MS/MS that were spiked at 50, 100, 500 and 1000 ng/g (5 replicates, one analyst, at each level) in Potatoes Matrix…….	51
9	The average recovery results of Maleic Hydrazide by LC-MS/MS that were spiked at 50, 100, 500 and 1000 ng/g (5 replicates, one analyst, at each level) in Grape Matrix…….	52
10	The average recovery results of Maleic Hydrazide by LC-MS/MS that were spiked at 50, 100, 500 and 1000 ng/g (5 replicates, one analyst, at each level) in Citrus Matrix.----------	53

Table	Table Title	Page
11	List of Lowest Calibration Level (LCL) concetrations, Limit Of Quantification (LOQ) for fresh and herbal samples, Correlation Variasion (r^2) and Europian Maximum Residue Limits (EU MRLs) by (mg/kg) for Green Beans, Apple, Rice and Fennel seeds……	55
12	The average pesticide recovery results by LC–MS/MS that were spiked at 0.01, 0.05, and 0.1 mg/kg (6 replicates, one analyst, at each level) in the Green Beans matrix…….	65
13	The average pesticide recovery results by LC–MS/MS that were spiked at 0.01, 0.05, and 0.1 mg/kg (6 replicates, one analyst, at each level) in the Apple matrix……	68
14	The average pesticide recovery results by LC–MS/MS that were spiked at 0.01, 0.05, and 0.1 mg/kg (6 replicates, one analyst, at each level) in the Rice matrix……..	71
15	The average pesticide recovery results by LC–MS/MS that were spiked at 0.01, 0.05, and 0.1 mg/kg (6 replicates, one analyst, at each level) in the Fennel seeds matrix…….	74

ABSTRACT

Name: Mahmoud Hamdy Ahmed Abdelwahed

Title of the thesis: Qualitative and Quantitative Analysis Of Some Pesticide Residues In Some Agricultural Products By Using High Chromatographic Technique

Position: Researcher Assistant

Degree: Ph.D., Faculty of science, Ain Shams University

Food is expected to contain pesticide residues that might have many problems due to their toxicities for human and animals. So, it is very important to detect and quantify the pesticides contamination levels to increase food safety for the human. It is important to update the testing scope of the Egyptian laboratories that deal with pesticide residues analysis by introducing new pesticides used by farmers in their scope. The target of our study is to analyse a twenty-one new pesticides including different pesticide classes such as 1 acaricide, 3 fungicides, 3 plant growth regulators, 11 herbicides, 1 insecticide, 1 rodenticide, and 1 metabolite which were selected according to their toxicity for human and animals, their modern application in the Egyptian agriculture as well as the recommendation of the Egyptian Agriculture Pesticides Committee (APC). The research is focused on the method validation for the routine analysis of the targeted pesticide residues according to the European SANTE/11813/2017 international standard guideline. The validation was carried out by fortifying of three levels at 0.01, 0.05 and 0.1 mg/kg for 20 analytes in different agriculture products from vegetables (Green Beans), fruits (Strawberry), dried herbs (Fennel) and rice which represent different classes of food. The most common citrate buffered QuEChERS extraction method and liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) device were used for all studied analytes except Maleic Hydrazide that has a single developed method. The mass spectrometer was operated in the positive electrospray ionization ESI (+) mode and the non-scheduled multiple reactions monitoring (MRM) method in a short run time of 16.0 minutes.

The limits of quantifications (LOQs) for all pesticides ranged between 0.01 and 0.05 mg/kg. Good linearity of the method was in the concentration range 0.001-0.5 µg/ml with acceptable correlation coefficients (r^2) ≥ 0.99 for all analytes. The average recoveries for all the target pesticide residues were in the range of 70-120% with relative standard deviations RSDs ≤ 20 %. The matrix effect was compensated by using the standard addition method.

Maleic Hydrazide (MH) is used as a plant growth regulator, herbicide and sprouting inhibitor for some fruits and vegetables thus, MH residues should be analyzed in food. Most of the chromatographic analysis methods for MH residues were operated by using ion chromatographic (IC) columns which need complicated and extra washing and conditioning steps. The developed method has overcome this problem by using reversed-phase (RP) polar C_{18}-column. Liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometer (LC-ESI-MS/MS) method was developed and validated at four fortification levels in four food matrices. The method optimization was carried out by using different extracting solvents, LC-columns and mobile phase ratios. The LC-MS/MS separation was developed by using polar C_{18}-column, negative electrospray ionization mode and non-scheduled multiple-reactions monitoring (MRM) method. The limits of quantification (LOQ) for MH was in the range 50-100 ppb that was below the EU maximum residue level, set for onion, potatoes, citrus and grape (15, 50, 0.2 and 0.2 ppm) respectively. Good method linearity was obtained in the concentration range 10-2000 ng/ml with correlation coefficients (r^2) ≥ 0.99. The average recoveries ranged from 84 to 110% with relative standard deviations RSDs ≤ 17%. The matrix effects on the MH signals were studied and compensated. The two short run time LC ESI MS/MS Methods was developed to help laboratories which deal with the routine pesticide residues analysis in different food samples.

Keywords: Food Analysis; Pesticide Residues; Maleic Hydrazide; Liquid Chromatography Triple Mass Spectrometer; Plant Growth Regulator; Method Validation

Supervisors' approval:

- **Prof Dr. Eglal Myriam Raymond Souaya**

  Professor Emeritus of Inorganic and Analytical Chemistry, Faculty of Science, Ain Shams University

  Signature: ……..……

- **Prof Dr. Mona Abd El Aziz Khorshed**

  Chief Researcher and Technical Manager, Central Lab of Residue Analysis of Pesticides and Heavy Metals in Food, Agriculture Research Centre (ARC)

  Signature: ……

Head of Chemistry Department

Prof. Dr. Ayman Ayoub Abdel-Shafi