



# **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**



Ain Shams University  
Faculty of Science  
Chemistry Department

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

A Thesis Submitted By

**Mahmoud Hamdy Ahmed Abdelwahed**

**For Ph. D. Degree in chemistry**

**Thesis Advisors**

**Thesis Approval**

- **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,  
Agricultural Research Center (ARC)

**Head of Chemistry Department**

**Prof. Dr. Ayman Ayoub Abdel-Shafi**

## *Acknowledgment*

*The author expresses his thanks to graceful and merciful God for helping him in making this manuscript comes to the truth.*

*The author wishes to express his deep gratitude and thanks to **Prof Dr. Eglal Myriam Raymond Souaya**, Professor Emeritus of Inorganic and Analytical chemistry, Faculty of Science, Ain Shams University for his supervision, continues encouragement and valuable comments throughout the work and for his revising the manuscript.*

*The author also wishes to express deep thanks to **Prof Dr. Mona Abd El Aziz Khorshed** Chief Researcher and Technical Manager, Central Lab of Residue Analysis of Pesticides and Heavy Metals in Food, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, for his great effort, suggestion of the subject (problem) of this theses, and assistance for his kind help and assistance through the course of the work and for his revising the manuscript.*

*Thanks and appreciation to all the staff members and technicians of Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods, Agricultural Research Center, Ministry of Agriculture and Land Reclamation.*

*My special thanks to my father, my mother, my brothers, my wife and my kids for giving me inspiration, confidence and patience throughout the period of investigation.*

*Mahmoud Hamdy Ahmed Abdelwahed*

---

# Abstract

---



---

---

## **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 ( $r^2$ )  $\geq 0.99$  for all analytes. The average recoveries for all the target pesticide residues were in the range of 70-120% with relative standard deviations RSDs  $\leq 20$  %. 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<sub>18</sub>-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<sub>18</sub>-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 ( $r^2$ )  $\geq 0.99$ . The average recoveries ranged from 84 to 110% with relative standard deviations RSDs  $\leq 17$ %. 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**

---

# **Aim of the Study**

---



## **AIM OF THE STUDY**

- ✓ Development and validation of two high performance liquid chromatography coupled with triple quadrupole mass spectrometer (LC-MS/MS) method for determination of twenty-one pesticide residues in some agriculture products from fruits, vegetables, dry herbal seeds and cereals to update the testing scope of the Egyptian reference laboratories.

### **Development of the two methods was done by the following criteria:**

- ✓ Dissolving the target pesticides in suitable solvents and preparing individual stock solutions, working mixture and calibration levels from these pesticides.
- ✓ Optimization of LC-MS/MS instrument parameters which depend on each tested pesticide.
- ✓ Studying different extraction procedures with different solvents to get suitable and optimum conditions for the extraction methods to fit our purpose depending on its cost, effectiveness, safety, time consumption and accuracy.

### **Validation of the developed method was done according to the international Protocols e.g. codex, EU standards and ISO 17025 by testing the following parameters:**

- a) Limits of Detection (LOD).
- b) Limits of Quantification (LOQ).
- c) Recovery Test.
- d) Method Linearity.
- e) Method Repeatability.
- f) Method Reproducibility.
- g) Method Accuracy and precision.
- h) Studying the matrix effect on pesticide residues determination and ways to decrease this effect.

## TABLE OF CONTENTS

	<b>Page</b>
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.....	-
<b>Chapter 1: Introduction and Literatures Review</b>	
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
<b>Chapter 2: Material and Methods</b>	
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

	<b>Page</b>
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
<b>Chapter 3: Results and Discussion</b>	
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
<b>3.6. Method Validation</b>	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 ABBREVIATIONS

### Abbreviations

### Synonyms

APC	Agriculture Pesticides Committee In Egyptian
CAC	Codex Alimentarius Commission
CAD	Collision Gas
CE	Collision Energy
CE-PDAD	Capillary Electrophoresis Equipped With A Photodiode Array Detector
CUR	Curtain Gas
CXP	Cell Exit Potential
DIW	Ultra-Pure Deionized Water
DP	Declustering Potential
EA	Ethyl Acetate
EFSA	European Food Safety Authority
EP	Entrance Potential
ESI (-)	Negative Electrospray Ionization Mode
ESI (+)	Positive Electrospray Ionization Mode
EU	European Union.
EURLs	European Reference Laboratories
FAO	Food And Agriculture Organization.
FAPAS	Food Analysis Performance Assessment Scheme
GAP	Good Agricultural Practice
GCB	Graphitized Carbon Black
GC-FID	Gas Chromatography Flame Ionization Detector

## Abbreviations

## Synonyms

IARC	International Agency For Research On Cancer
IC	Ion Chromatographic
IE-HPLC-UV	Developed Ionic Exchange High-Performance Liquid Chromatography And Ultraviolet Detection
IPM	Integrated Pest Management
IQC	Internal Quality Control
IS	Ion Spray
JECFA	Joint Fao/Who Expert Committee On Food Additives.
L/min	Litter Per Minute.
LC-APCI MS	Atmospheric Pressure Chemical Ionization Mass Spectrometric Detection
LCL	Lowest Calibration Level
LC-MS/MS	Liquid Chromatography Coupled With Triple Quadrupole Mass Spectrometry
LOD	Limit Of Detection.
LOQ	Limit Of Quantification.
MAE	Microwave-Assisted Extraction
ME	Matrix Eect
Me	Methanol
MH	Maleic Hydrazide
MRLs	Maximum Residues Limits
MRM	Multiple Reactions Monitoring Mode
n	Number Of Replicates
PGRs	Plant Growth Regulators
p <sup>Ka</sup>	Dissociation Constant

**Abbreviations****Synonyms**

POTW	Publicly Owned Treatment Works
PTFE	Polytetrafluoroethylene
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, And Safe
$r^2$	Correlation Coefficients
RP	Reversed-Phase
RSD or CV	Relative Standard Deviation.
SD	Standard Deviation.
TEM	Source Temperature
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organization.
WHO/GEMS	Who/Global Environment Monitoring System.

## 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