



بسم الله الرحمن الرحيم

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كلية العلوم – قسم الكيمياء

**Development of multi-residue methods for analysis
of pesticide in beeswax and honey using tandem
mass spectrometry techniques**

Thesis Submitted by

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B.Sc. (Chemistry) 2008

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**For the requirement of Ph.D.Degree of Science in
Chemistry**

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To

Department of Chemistry

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Muhammad Mahmoud Hassan Mahmoud Issa

ABSTRACT



Abstract

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Name: Muhammad Mahmoud Hassan Mahmoud Issa

Title of the thesis: Development of multi-residue methods for analysis of pesticide in beeswax and honey using tandem mass spectrometry techniques.

Position: Researcher Assistant

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

The rising potential for honey bees to be exposed to complex mixes of pesticides is one of the possibilities that may help explain the loss of honey bee colonies around the world. Two multi-residue methods based on distinct extraction and clean up protocols were developed and validated for the measurement of 373 pesticides in beeswax (including 306 pesticides using LC-MS/ MS and 160 pesticides using GC-MS/ MS) and 313 pesticides using LC-MS/ MS to better understand this phenomenon. For beeswax, a mixture of acetonitrile and ethyl acetate (1:3, v/v) was used to extract pesticide residues from beeswax. This combination of solvents not only allows the melting of beeswax samples at a lower temperature than when alone acetonitrile is used, but it also creates a one-phase solution. The beeswax extract was directly injected into both GC-MS/MS and into LC-MS/MS. On the other hand, a mixture of acetonitrile and ethyl acetate (1:1, v/v) was used to extract pesticide residues from honey followed by cleanup using a dispersive solid-phase extraction (d-SPE) with primary second amine (PSA) then directed to LC-MS/ MS for analysis. The developed methods were validated according to SANTE/ 11813/2017 European Union guidelines. For all studied compounds, it was found that the limit of quantifications (LOQ) ranged from (20 to 100) µg/Kg and (4 to 20) µg/Kg for beeswax and honey, respectively. For beeswax, there are 265 and 139 pesticides of LOQ equal 20

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µg/kg using LC-MS/MS and GC-MS/MS, respectively. On the other hand, there are 270 pesticides having LOQ equal 4 µg/kg using LC-MS/MS in honey. The developed assay was linear over concentration range of 2 – 100 µg/L, with correlation coefficient of more than 0.995 in both beeswax and honey. Most of the studied pesticides have acceptable recovery between 75 and 120% with good reproducibility (most of them have RSD below 12%) for both beeswax and honey. Finally, the developed methods were successfully applied for the analysis of real beeswax and honey samples. Chlorpyrifos, malathion, and cypermethrin were detected in all analyzed beeswax samples, indicting uncontrolled pesticide practices around beehives. Tau-fluvalinate, the commonly used insecticides/acaricides, was detected in 60% of the tested samples of beeswax at concentration range between <LOQ-1730 µg/kg. On the other hand, results revealed that only two honey samples out of the fifteen analyzed samples was contaminated with DMF at concentration levels of 20 and 26 µg/kg. This wide scope assay protocol is applicable for monitoring pesticide residues in honey and beeswax by national regulatory authorities and accredited labs; that should help ensure safety of such widely used products.

Keywords: Beeswax, Honey, Pesticide residues, Analytical method, LC-MS/MS, GC-MS/MS, Egypt.

Abstract

Supervisors' approval:

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AIM OF STUDY

- ✓ Using a proper solvent (or mixture of solvents) that not only enable analysis of higher number of pesticides but also enables direct injection to LC MS/ MS and GC MS/ MS.
- ✓ Decreasing the co-extracted matrix components for the developed method by applying different clean up conditions, to protect the used analytical instruments.
- ✓ Development a reliable method for pesticides residue analysis in beeswax that enable the detection of a high number of pesticides (about 180 pesticides using GC MS/ MS and about 300 pesticides using LC MS/ MS).
- ✓ Development a reliable method for pesticides residue analysis in honey that enables the detection of high number of pesticide about 300 pesticides using LC MS/ MS.
- ✓ The developed method will be validated through determination the following parameters:
 - Linearity, which will be determined by analysis a set of calibration points that were prepared in beeswax and honey matrices.
 - Intra-day precision, which will be determined through analysis of five repeated fortified beeswax and honey samples at different spiking concentrations in the same day.

Aim of study

- Inter-day precision, which will be determined through analysis of five repeated fortified beeswax and honey samples at a specified spiking concentration in repeated five days.
 - Matrix effects calculations, a set of calibration points were prepared in pure solvent for LC MS/ MS and in an alternative sample extract for GC MS/ MS together with that prepared in beeswax and honey extracts will be used for calculation of matrix effects.
- ✓ Applying the developed method for analysis of different collected real samples (beeswax and honey).