UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE CLUJ-NAPOCA

DOCTORAL SCHOOL

FACULTY OF AGRICULTURAL SCIENCES AND BIOTECHNOLOGIES

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CHROMATOGRAPHIC TECHNIQUES FOR CONTAMINANTS DETERMINATIONS IN HONEY

SUMMARY OF PhD THESIS

Scientific coordinator

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Cluj-Napoca

2011
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INTRODUCTION

Honey is a natural product with high biological and caloric value. Beekeeping offers products with great prophylactic and therapeutically values, which contributes to human health maintaining and economically it brings contribution to population welfare.

Over the last few years, the problem of honey and other bee products contamination was mentioned regarding the antibiotics and pesticides residues, substances with toxic acute and chronic effects on human health.

It is required that research structures to develop new and performing methodologies for antibiotics and pesticides residues control in order to promote free international circulation of Romanian bee products and their acknowledgement as authentic natural products.

Present thesis « Chromatographic techniques for contaminants determinations in honey » contains the following parts: introduction, bibliographic study, objectives, original research, conclusions, bibliographic references and appendix.

In the present thesis it was followed the development of high precision and sensitivity chromatographic analytical methods, which can ensure detection and quantification of antibiotics and pesticides residues from honey, in order to protect the consumers. It was also studied the possibility to reduce the damage of pesticides on honey by ultraviolet light treatment.

Bibliographic study, structured in 3 chapters, contains information regarding the contamination ways of bee products, structure and properties of main contaminants and also modern chromatographic techniques use for determination of mentioned compounds.

Personal research part, systematized in 4 chapters, describes the analytical proposed methods for tetracycline, chloramphenicol, organochloride and organophosphorous pesticides residues in honey, validation algorithm of the techniques and obtained results.

A study which followed the influence of UV radiations against pesticides residues in contaminated honey and also against honey quality parameters was employed.
Chapter 1. THE CONTAMINATION SOURCES OF BEE PRODUCTS

Bee products contamination is due to polluted environment and also to substances used by beekeepers for diseases control of honeybee colonies.

Main contaminates from environment are heavy metals, radioactive isotopes, organic pollutants, pesticides, photogene bacteria and genetic modified organisms. Bee products contamination is mainly due to beekeeping practices by using acaricides and antibiotics against hive diseases: Varroa, European and American Foulbrood.

Chapter 3. METHODS FOR IDENTIFICATION AND QUANTIFICATION OF ANTIBIOTICS AND PESTICIDES IN HONEY

Development and improvement of analytical methods for antibiotics and pesticides control levels is required, having detection and quantification limits as low as possible.

Honey samples testing in order to establish possible contaminations with antibiotics and pesticides residues are realized by the following:

• Screening methods, used identification of positive samples: ELISA, Charm II and ROSA tests;
• Quantitative determinations of positive samples by HPLC, LC-MS and GC techniques.

High performance liquid chromatography (HPLC) is the most used chromatographic technique for antibiotics analysis. Using the liquid chromatography coupled with mass spectrometer was achieved a performing instrument able to separate, identify and quantify compounds from complex samples. Over the years, LC-MS systems were subjected to significant transformations, starting with simple analysis as a replacement of traditional UV and achieving very precise qualitative and quantitative analysis.

Pesticide residues are generally analyzed by gas chromatography coupled with different detectors, such as ECD (electron capture), NPD (nitrogen – phosphor) or MS (mass spectrometer).
Chapter 4. AIM AND OBJECTIVES

Due to negative effects that bee products contaminated with antibiotics and pesticides residues have on consumers, quality control is necessary by secure methods which offer confident results.

Main objective was the development and validation of performing and modern liquid and gas chromatographic methods for antibiotics and pesticides residues determination in honey.

Established objectives are:

- Tetracycline residues determination by HPLC-PDA (chapter 5)
- Chloramphenicol residues determination by LC-MS (chapter 6)
- Organochloride and organophosphorous pesticides determination by GC-MS technique (chapter 7)
- Study of possible diminishes of pesticides content from contaminated honey, by UV irradiation (chapter 8).

Chapter 5. TETRACYCLINE RESIDUES DETERMINATION (TECTRACYCLINE AND OXYTETRACYCLINE) FROM HONEY BY HPLC-DAD

Antibiotics residues determination from honey was a major concern over the last years, because these compounds may reduce the efficacy of honey properties and if they are found in significant quantities could represent a serious treat on human health.

5.1. MATERIALS

Development and validation of identification and quantification chromatographic method of tetracycline residues from honey was based on determinations made on honey samples of beekeeping Department hives of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, production 2007.
5.2. METHOD

Tetracycline residues determination by HPLC-PDA was adjusted for “honey” matrix according to the method described AOAC Official Methods of Analysis (1995) for antibiotics determination in milk.

5.2.1. Method development for tetracycline residues determination

Extraction and purification of tetracycline residues from honey is done on MCAC columns (affinity chromatography using metal chelators).

The system used for tetracycline determination was a high performance liquid chromatograph, Shimadzu model, VP series coupled with UV-Vis detector and photo diode Array, which allows continuous registration of the spectrum on the whole spectral domain, in milliseconds time intervals offering a high quantity of information.

Chromatographic determination:

Different concentration gradients of mobile phases were tested. Optimum variant is showed in table 1, it ensures an efficient separation of tetracyclines, high resolution and short working time.

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%)</th>
<th>Mobile phase B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>11.5</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>17</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>19</td>
<td>74</td>
<td>26</td>
</tr>
</tbody>
</table>

Operational parameters of the chromatographic system:

- Flow rate of the mobile phase: 1ml/min
- Injection volume: 50µl
• Column temperature: 35°C
• Registration at λ 360nm (oxitetracycline) and λ 355nm (tetracycline)
• Separation time: 28 minutes

5.2.2. Method validation in tetracycline residues determination

Performance parameters determination of the used method for tetracycline determination by HPLC-PDA was realized according to the requirements of Eurachem Guide for analytical methods validation. Analytical method was validated through the evaluation of the following parameters: calibration curve, identity and specificity confirmation, precision, detection limit, quantification limit, recovery rate, robustness, stability.

5.3. RESULTS AND DISCUSSIONS

Performances of the developed method are shown in table 2. Linearity was achieved during the whole range of studied concentrations, as indicated by correlation coefficients R², very close to unit.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regression equation</th>
<th>Correlation coefficient R²</th>
<th>Linearity (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>Y=0.0135X+0.0</td>
<td>0.9994</td>
<td>5-75</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Y=0.0127X+0.0</td>
<td>0.9995</td>
<td>5-75</td>
</tr>
</tbody>
</table>

For tetracycline identification and potential interfering substances, following chromatograms were overlaid: standards mixture (a), uncontaminated honey extract (b) and honey extract supplemented with oxitetracycline and tetracycline (c) (figure 1). Retention times of oxitetracycline (7.1 min) and tetracycline (8.6 min) standards have
coincided with the ones from supplemented honey, while for uncontaminated honey no signal was registered. This fact indicates the absence of interference compounds.

Fig. 1. Chromatogram of a mixture tetracyclines (a), honey sample free of antibiotics (b) and honey sample spiked with mixture of antibiotics (c)

Method precision was evaluated by standard relative deviation measurement in conditions of repeatability and reproducibility; determinations were made on honey samples supplemented with tetracycline standard at 3 different concentrations. Due to the fact that RSD values were always situated below 10%, we may consider method precision being satisfactory.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean of concentrations ± SD (µg/kg)</th>
<th>LOD (µg/kg)</th>
<th>LOQ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>3.32 ± 0.39</td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3.08 ± 0.58</td>
<td>4.8</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Results presented in table 3, show that the analytical method developed for tetracycline determinations is able to detect and quantify antibiotic concentrations lower than the limit proposed by European Legislation (10µg/kg). This fact offers a high sensitivity method.

Tetracyclines recovery in percentage higher than 70% is acceptable, losses during extraction being reduced a lot for oxitetracycline, comparing to tetracycline (table 4).

Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Spiked level (µg/kg)</th>
<th>Recovery (mean ± SD) (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>10</td>
<td>93.81 ± 3.29</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>95.89 ± 5.39</td>
<td>5.63</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>98.96 ± 3.19</td>
<td>3.22</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10</td>
<td>75.18 ± 3.49</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>73.75 ± 5.65</td>
<td>7.67</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>73.67 ± 4.60</td>
<td>6.25</td>
</tr>
</tbody>
</table>

5.4. PRELIMINARY CONCLUSIONS

- Method used for tetracycline determination from honey involves selective extraction of the analytes by Cu²⁺ ions complexes, chromatographic separation and detection with UV-Vis detector with photodiode Array.
- Used extraction technique realizes in a simultaneous way isolation, cleaning and tetracycline concentration, without being necessary other operations.
- Studied performance parameters during method validation prove that developed method is characterized by high selectivity, specificity, accuracy, robustness and sensitivity.
- HPLC-PDA technique is efficient for identification and quantification of tetracyclines residues from different honey types, absence of interferences being independent of botanical origin of analyzed honey.
Chapter 6. DETERMINATION OF CHLORAMPHENICOL RESIDUES IN HONEY BY LC-MS TECHNIQUE

Liquid chromatography technique coupled with mass detector (LC-MS) is known for high selectivity and sensitivity ensured for analytical methods of chloramphenicol determination from complex matrices.

6.1. MATERIALS

Development and validation of chromatographic method of identification and quantification of chloramphenicol residue from honey was based on determinations made on honey samples of beekeeping Department hives of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, production 2009.

6.2. METHOD

Analytical method for chloramphenicol residues determination is based on the isolation of the antibiotic from honey using the technique described by Shen and Jiang (2005), with minor modifications and with the help of mass spectrometry for identification and quantification.

6.2.1. Development of the method for chloramphenicol residues determination

Sample preparation procedure is simple, chloramphenicol residues being extracted from honey using the PBS-ethyl acetate-Na$_2$SO$_4$ system, after that being separated by reverse phase liquid chromatography and detected with mass spectrometer equipped with electrospray ionization source and quadruple analyzer.

**Chromatographic determination:**

Zorbax Eclipse XDB-C$_{18}$ reverse phase column was used for separation, 2.1x150mm and granulation of 3.5µm. Concentration gradient used for mobile phases is shown in table 5.
Table 5

Linear gradient program for chloramphenicol determination

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%)</th>
<th>Mobile phase B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>14.01</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Operational parameters of the chromatographic system:

- Column temperature: 25°C
- Autosampler temperature: 10°C
- Injection volume: 50μl
- Flow rate: 0.3ml/min
- Wavelength: 278nm
- Separation time: 28 min
- Interface voltage: 4.5KV
- Interface temperature: 250°C
- Gas flow rate: 1.5l/min (N₂)
- Monitored ions selection: m/z 321, 323
- Ionization mode: ESI negative

6.2.2. Method validation in chloramphenicol residues determination

Validation protocol followed for chloramphenicol residues determination is according to European Commission Decision 2002/657/EC criteria. Evaluated performant parameters are: calibration curve and linearity, specificity, precision, detection limit and detection capability, recovery.
6.3. RESULTS AND DISCUSSIONS

Calibration curve obtained for chloramphenicol was realized through external standard method and presented linearity over the whole concentrations domain. Linear regression data shown in table 6, indicate a good correlation between detector answer and analytes concentration. Detection limit and detection capability are according to criteria established by Commission Decision 2002/657/EC, being below the concentration level of 0.3µg/kg.

Table 6

<table>
<thead>
<tr>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation coefficient R²</th>
<th>Linearity (µg/kg)</th>
<th>CCα (µg/kg)</th>
<th>CCβ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.142</td>
<td>0.035</td>
<td>0.9999</td>
<td>0.3-25</td>
<td>0.11</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Chloramphenicol identification was made according to chromatographic retention time of the etalon (13.1min). Compound identity confirmation was realized by comparing with absorption and mass spectra of the etalon, which were registered in the spectra library and also by comparing ions intensity ratio.

Obtained chromatograms were compared for chloramphenicol standard, honey sample supplemented with CAP and an antibiotic free sample (blank) (figure 2). In the blank chromatogram no signal was registered for chloramphenicol retention time, which proves that no other chemical or matrix compound was co-extracted. Absence of a fake positive signal indicates a good analytical method performance.

Relative standard deviation (RSD) obtained were below the limits established by EU: 10%, and 2% respectively in conditions of repeatability and reproducibility made for method precision evaluation.

Recovery rate obtained higher than 85% indicate the efficiency of sample preparation. Results are shown in table 7 and indicate high method accuracy.
Fig. 2. LC-MS-SIM chromatograms obtained for (a) CAP standard, (b) honey sample spiked with CAP and (c) blank honey sample

Table 7

<table>
<thead>
<tr>
<th>Theoretical concentration (µg/kg)</th>
<th>Within-day</th>
<th>Between-day</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean concentration±SD (µg/kg)</td>
<td>RSD (%)</td>
<td>Mean concentration±SD (µg/kg)</td>
</tr>
<tr>
<td>0.6</td>
<td>0.48 ± 0.021</td>
<td>3.8</td>
<td>0.51 ± 0.042</td>
</tr>
<tr>
<td>2</td>
<td>1.68 ± 0.080</td>
<td>5.6</td>
<td>1.74 ± 0.098</td>
</tr>
<tr>
<td>5</td>
<td>4.35 ± 0.211</td>
<td>7.7</td>
<td>4.55 ± 0.401</td>
</tr>
</tbody>
</table>

6.4. PRELIMINARY CONCLUSIONS

- LC-MS technique for chloramphenicol residues determinations from honey combines simple extraction, but efficient with adequate chromatographic separation and mass spectrometry detection which ensures certainty to compound identification.
- Analytical technique developed shows high sensitivity, is able to detect chloramphenicol concentrations below the limit established by European Legislation.
• Validation procedure followed all necessary steps to ensure correct identification and quantification of chloramphenicol residues.

• Chloramphenicol contamination level is essential to be checked for autochthonous honey and also imported honey, in order to ensure human health.

Chapter 7. DETERMINATION OF ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDE RESIDUES IN HONEY BY GC-MS TECHNIQUE

Pesticides, being used on high scale, are part of the ecosystems circuit, and theirs negative effects are amplified at the end of the trophic chain (human organism). Multi-residual performing methodologies are necessary to be implemented, able to detect a high number of pesticides through one determination.

7.1. MATERIALS

Development and validation of the identification and quantification chromatographic method for pesticides from honey was based on analysis made on honey samples of Beekeeping Department hives of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, production 2010.

7.2. METHOD

Pesticides residues determination through GC-MS technique is a multi-residual chromatographic method which identifies and quantifies 37 compounds simultaneously.

7.2.1. Method development for pesticide residues determination

OCL and OP pesticides residues from honey are transferred through acetonitrile extraction in the presence of high salts quantities (magnesium sulphate, sodium chloride, sodium citrate). Purification of the extract is realized by solid phase dispersive extraction
(dSPE) followed by sample concentration in order to improve the quantity of inters analytes.

Compounds are separated through gas chromatography and are detected through mass spectrometry.

**Chromatographic determination:**

Compounds separation was realized by active layer of the DB-5MS capillary column (30m length, 0.25mm internal diameter, 0.25µm film thickness) and by gradient temperature program shown in table 8.

### Table 8

**Column program**

<table>
<thead>
<tr>
<th>Growth rate (°C/min)</th>
<th>Temperature (°C)</th>
<th>Stagnation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>45</td>
<td>1.00</td>
</tr>
<tr>
<td>20</td>
<td>150</td>
<td>5.00</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>225</td>
<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>260</td>
<td>0.00</td>
</tr>
<tr>
<td>20</td>
<td>300</td>
<td>8.25</td>
</tr>
</tbody>
</table>

**Operational parameters of the chromatographic system:**

- Injector temperature: 250°C
- GC-MS interface temperature: 280°C
- Used gas: He, total flow rate: 9 ml/min
- Column flow rate: 0.99 ml/min
- Linear velocity: 36cm/s
- Column temperature: 45°C
- Ionization mode: electrons impact (70eV)
- Detection system: SIM (selected ions monitoring)
- Ions source temperature: 230°C
- Detector tension: 1.56kV
- Separation time: 50min

SIM quantitative detection technique was employed (figure 3).
7.2.2. Method validation for pesticide residues determination

Method validation of organochloride and organophosphorous pesticides residues determinations is according to European Commission Decision 2002/657/EC criteria. Evaluated performance parameters are: calibration curve and linearity, identity and specificity confirmation, precision, detection and quantification limit, recovery rate.

7.3. RESULTS AND DISCUSSIONS

MS detector response was linear through concentration range specified in table 9 for each pesticide. Method sensitivity was good for most compounds, 30 pesticides detection being possible at concentrations below 10µg/kg concentrations. Quantification limits ranged between 3.44-47.22µg/kg, while RSD values were between 1.09 and 13.01% (table 9).
Table 9

Calibration parameters and analytical limits (limit of detection and limit of quantitation) for organochlorine and organophosphorus pesticides

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Correlation coefficient $R^2$</th>
<th>Linearity (µg/kg)</th>
<th>Concentration ± SD (µg/kg)</th>
<th>RSD %</th>
<th>LOD (µg/kg)</th>
<th>LOQ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorvos</td>
<td>0.9974</td>
<td>10-500</td>
<td>2.98±0.12</td>
<td>4.06</td>
<td>3.34</td>
<td>3.65</td>
</tr>
<tr>
<td>Mevinphos</td>
<td>0.9986</td>
<td>25-500</td>
<td>19.63±0.39</td>
<td>7.08</td>
<td>20.8</td>
<td>21.82</td>
</tr>
<tr>
<td>Demeton O</td>
<td>0.9984</td>
<td>10-500</td>
<td>3.91±0.51</td>
<td>13.01</td>
<td>5.44</td>
<td>6.76</td>
</tr>
<tr>
<td>Ethoprop</td>
<td>0.9966</td>
<td>10-500</td>
<td>3.33±0.24</td>
<td>7.10</td>
<td>4.03</td>
<td>4.65</td>
</tr>
<tr>
<td>Naled</td>
<td>0.9881</td>
<td>50-500</td>
<td>38.57±0.40</td>
<td>10.44</td>
<td>39.78</td>
<td>40.82</td>
</tr>
<tr>
<td>Sulfotep</td>
<td>0.9987</td>
<td>10-500</td>
<td>5.46±0.37</td>
<td>6.87</td>
<td>6.59</td>
<td>7.56</td>
</tr>
<tr>
<td>Phorate</td>
<td>0.9988</td>
<td>10-500</td>
<td>4.49±0.32</td>
<td>7.24</td>
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<td>10-500</td>
<td>5.57±0.66</td>
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<td>7.56</td>
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<td>25-500</td>
<td>23.43±0.04</td>
<td>1.68</td>
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<td>4.23±0.38</td>
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<td>γ HCH</td>
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<td>10-500</td>
<td>6.56±0.38</td>
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<td>δ HCH</td>
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<td>10-500</td>
<td>4.82±0.51</td>
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<td>6.34</td>
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<td>9.06</td>
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<td>Endosulfan sulfate</td>
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<td>4,4’ DDT</td>
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<td>10-500</td>
<td>3.51±0.28</td>
<td>7.84</td>
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<td>Endrin ketone</td>
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<td>6.53±0.41</td>
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<td>Methoxychlor</td>
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<td>10-500</td>
<td>3.04±0.17</td>
<td>5.62</td>
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<td>Coumaphos</td>
<td>0.9939</td>
<td>50-500</td>
<td>45.90±0.24</td>
<td>8.33</td>
<td>46.61</td>
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Compounds identity confirmation is based on comparing mass spectrum and reference ions abundance ratios of each compound identified in the sample, with standards using spectra library.

57% of total studied pesticides showed recovery rates within the interval 83%-99%, 40% of them were between the limits of 102% and 117%. Lowest recovery rate was for endrin aldehyde, 72%, accepted value.

7.4. PRELIMINARY CONCLUSIONS

• QuEchERS extraction technique is highly competitive for GC-MS analysis, being obtained low interferences.
• Mass detector has high selectivity and offers important spectral information, which ensures identification and quantification of pesticides residues.
• Evaluated validation parameters indicate that the method offers high accuracy and precision results with very high recovery rates.
• It is necessary to control even pesticides with prohibited use, especially organochloride ones, because there is the possibility of these compounds to be found in honey, due to remanence.

Chapter 8. EXTERNAL FACTORS WHICH INFLUENCE THE STABILITY OF ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDES

In the environment there are processes which influence pesticides stability, transforming them into inactive compounds, less toxic. There are three types of pesticides degradation: microbial, chemical and photo-chemical (destruction under light).

8.1. UV RADIATIONS INFLUENCE AGAINST PESTICIDES RESIDUES IN CONTAMINATED HONEY

We aimed to study the UV radiation influence against organochloride and organophosphorous pesticides stability from contaminated honey. It was also made a
comparison with the inactivity and concentration diminish of some pesticides from the environment under the influence of certain processes, of which is played by photo-chemical reactions.

8.1.1. Materials

Two types of honey were analyzed: acacia (MS) and multiflower (MP). After the OCL and OP pesticides were added in the honeys at 200μg/kg (MSA and MPA) concentration, these were subjected to UV radiation action for different time periods.

8.1.2. Method

Artificial irradiation source of UV light was a UV VL-204.G (Vilber Lourmat) lamp with λ=254nm wavelength. Irradiation times were: 5, 12, 24, 36, 48, 72 hours.

8.1.3. Results and discussions

Pesticides with the lowest stability, removed from the samples in the shortest time period (36 hours), were: stirofos, bolstar, coumaphos and methoxychlor (figure 4).

![Degradation dynamics](image)

Fig. 4. Degradation dynamics, expressed as percentage (%), for stirofos, bolstar, coumaphos and methoxychlor after irradiation
After 48 hours exposure time, other 5 pesticides were destroyed, 3 organophosphorous compounds (naled, methyl parathion and fenthion), as shown in figure 5 and 2 organochloride compounds (4,4'-DDE and 4,4'-DDT).

Fig. 5. Degradation dynamics, expressed as percentage (%), for naled, methyl parathion and fenthion after irradiation

Fig. 6. Degradation dynamics, expressed as percentage (%), for 4,4'-DDT and its metabolites after irradiation
While 4,4′ DDT and 4,4′ DDE were not present in the samples after 48 hours of treatment, 4,4′ DDD was only partially destroyed, 34.9% of its concentration being present even after maximum irradiation time applied (figure 6).

Other 4 organophosphorous pesticides (dichlorvos, mevinphos, phorate, chlorpyriphos) (fig. 7) and 3 organochloride pesticides (aldrin, dieldrin, endrin) were no longer present in the samples at GC-MS analysis, when irradiation time was extended to 72 hours.

Fig. 7. Degradation dynamics, expressed as percentage (%), for dichlorvos, mevinphos, phorate and chlorpyriphos after irradiation

Aldrin and dieldrin were both eliminated from the samples, after 72 hours of UV irradiation time, but in a different degradation rate (figure 8).

While endrin was no longer identified in sample MSA-72h, its isomers, endrin aldehyde and endrin ketone were found in 28% concentration level and 17.6% respectively (figure 9).
Fig. 8. Degradation dynamics, expressed as percentage (%), for aldrin and dieldrin after irradiation

Fig. 9. Degradation dynamics, expressed as percentage (%), for endrin, endrin aldehyde and endrin ketone after irradiation

Most stable compound was sulfotep, also a relatively high stability showing demeton S, diazinon and α HCH pesticides (figure 10).
Fig. 10. Degradation dynamics, expressed as percentage (%), for sulfo tep, demeton O, demeton S and diazinon after irradiation

Unlike demeton S, its isomer, demeton O has suffered a pronounced loss of concentration for irradiation times higher or equal to 36 hours.

HCH compounds variation is pointed out in figure 11.

Fig. 11. Degradation dynamics, expressed as percentage (%), for HCH isomers after irradiation
The rest of organophosphorous pesticides (ethoprop, disulfoton, ronnel, trichloronate, tokuthion) were destroyed in different proportions under the UV radiations action, percentages of elimination from samples being between 89.4% (tokuthion) and 63.4% (ethotrop).

Photochemical transformation product of endosulfan which is composed of isomers endosulfan I and endosulfan II, is endosulfan sulfate. These three compounds showed concentration variations almost equally after 72 hours of UV treatment (figure 12).

Fig. 12. Degradation dynamics, expressed as percentage (%), for endosulfan I, endosulfan II and endosulfan sulfate after irradiation

Organochloride compounds, heptachlor and heptachlor epoxide, were significantly destroyed under UV radiations influence, having lower than 8% concentrations in the sample after 72 hours of irradiation.
8.2. INFLUENCE OF IRRADIATION TREATMENTS AGAINST HONEY QUALITY PARAMETERS

8.2.1. Methods for determination of honey phisico-chemical parameters

Parameters followed for pesticides contaminated honey and UV irradiation were: HMF content, diastase index, acidity and sugars content.

8.2.2. Results and discussions

Regardless of irradiation time, UV light did not affect the pH of honey samples or sugars and HMF contents. Honey samples acidity was maintained at initial values, slightly increased values were registered for maximum irradiation times.

Out of all phisico-chemical followed parameters, the only one which has suffered significant modifications was diastase index. Amylase is sensitive to light, even after 5 hours of UV treatment the concentration dropped to half of the initial one. As irradiation time was increased, diastase index continued to drop, reaching values below the limit proposed by European Community.

8.3. PRELIMINARY CONCLUSIONS

• UV radiations have a beneficial effect on honey with pesticides residues by diminishing the contamination level.
• Photo-degradation of pesticides is dependent on irradiation time, radiations intensity and chemical structure of these compounds.
• For a percentage of 44% of organochloride and organophosphorous pesticides studied, total degradation was achieved, all others having the concentrations reduced in different proportions.
• Pesticides residues behavior to UV treatment was similar for the two honey types investigated.
• Total or partial remove of pesticides from honey by ultraviolet exposure does not involve honey adulteration.

Chapter 9. GENERAL CONCLUSIONS AND RECOMMENDATION

ORIGINAL ELEMENTS

1. Development, optimization and validation of analytical methods for honey contaminants determination, using modern techniques, out of which are pointed liquid chromatography coupled with mass spectrometer detector (LC-MS).
2. Use for the first time in our country of QuEChERS method, for pesticides residues isolation and purification from honey.
3. Experimental protocol for pesticides contaminated honey toxicity decrease, by UV radiations treatment, without significant honey quality adulteration.

RECOMMENDATIONS AND PERSPECTIVES

1. Expansion of control for different antibiotic classes and pesticides for the other bee products: pollen, bee bread, wax, propolis and royal jelly.
2. Multi-residual analytical methods development, with large applicability in order to detect high numbers of compounds in one analysis.
3. Study of an important research area: metabolites and degradation compounds determination of bee products contaminants, especially those considered toxic and cancer inducers.