HIGH SENSITIVITY LC-MS/MS METHODS FOR ANTIBIOTIC AND PESTICIDE RESIDUE QUANTIFICATION IN HONEY, PROPOLIS, ROYAL JELLY AND POLLEN PRODUCTS.

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Introduction

A high sensitivity method has been developed and validated for the analysis of different types of antibiotics (tetracyclines, sulfamides, macrolides) and pesticides (acaricides and fungicides). A simple preparation procedure was developed on the basis of different L/L, SPE (Liquid/Liquid, Solid Phase Extraction) extraction according to matrix type.

Final extract were injected into LC-MS/MS (liquid chromatograpy tandem mass spectrometry) instrument programmed in MRM (Multiple Reaction Monitor) mode (two transition / molecule).

Method validation was carried out according to European Union Directive 2002/657/CE at concentration level of 0.5-1-5 µg/kg depending on sensitivity of molecules and matrix.

Limit of detection and limit of quantification have been calculated on the basis of report limit fixed at 5 µg/kg; the limits of detection (LODs) were lower than 0.5 µg/kg for honey, 1 µg/kg for royal jelly, 5 µg/kg for propolis and 5 µg/kg for pollen.

Experiments

Extraction/Clean Up of Antibiotics
10 g Sample
Acetonitrile/Water Extraction
SPE Purification
Concentration
LC-MS/MS Analysis MRM Mode

Extraction/Clean Up of Chloramphenicol
10 g Sample
Ethyl Acetate Extraction
Water/Hexane Purification
Concentration
LC-MS/MS Analysis MRM Mode

Extraction/Clean Up of Pesticides
10 g Sample
Acetonitrile Extraction
PSA Purification
Concentration
LC-MS/MS Analysis MRM Mode

Validation Data and Proficiency testing

To confirm the developed methods we participate to proficiency testing for all compounds, we report for example the result of Tetracycline (see figure with Z score results). (FAPAS 2011 Nr.02162 Matrix: Honey, Floramo Corp. Lab Nr.29.)

<table>
<thead>
<tr>
<th>Compound (representative analytes)</th>
<th>LOD µg/kg</th>
<th>LOQ µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>0.07</td>
<td>0.21</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>Tylosine</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Chlorfenvinphos</td>
<td>0.32</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Validation data are calculated on the basis of ISO 11843, ISO 17025 and European Union Directive 2002/657/CE. Recovery rates range for all analytes: 78-104%

Conclusion

A highly sensitive mass spectroscopic methods for determination of many xenobiotic compound residues has been validated and accredited in according to ISO17025 Standard. Many bee hive product samples (years 2010 – 2011) were analyzed by this method and many positivity both for antibiotics and pesticides were found at low level concentration (about 1 µg/kg) in all studied matrix.

High sensitivity methods has been very useful to our clients in order to better estimate their honeybee product purchasing.