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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 chromatography 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

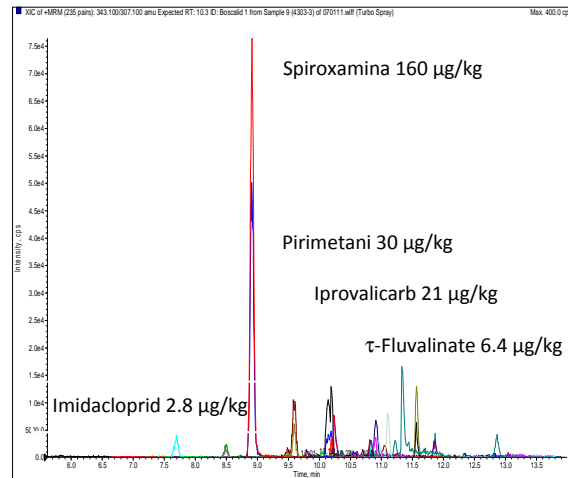
10 g Sample
Acetonitrile/Water Extraction
SPE Purification
Concentration
LC-MS/MS Analysis MRM Mode

Extraction/Clean Up of Chloramphenicol

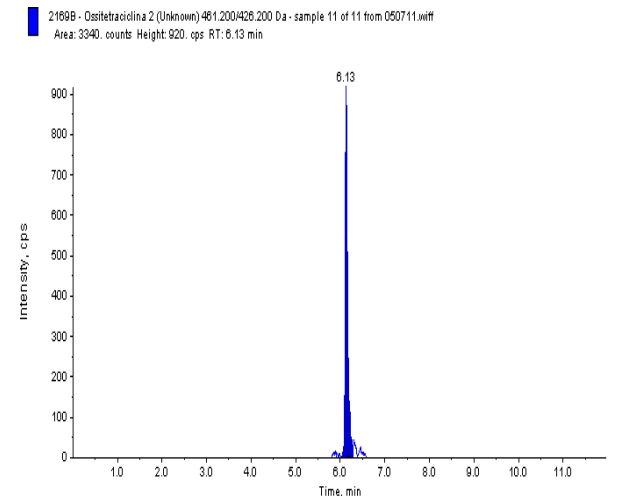
10 g Sample
Ethyl Acetate Extraction
Water/Hexane Purification
Concentration
LC-MS/MS Analysis MRM Mode

Extraction/Clean Up of Pesticides

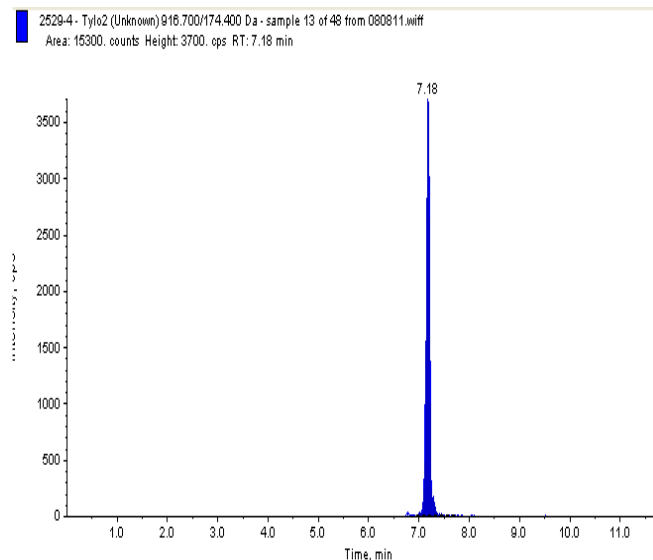
10 g Sample
Acetonitrile Extraction
PSA Purification
Concentration
LC-MS/MS Analysis MRM Mode



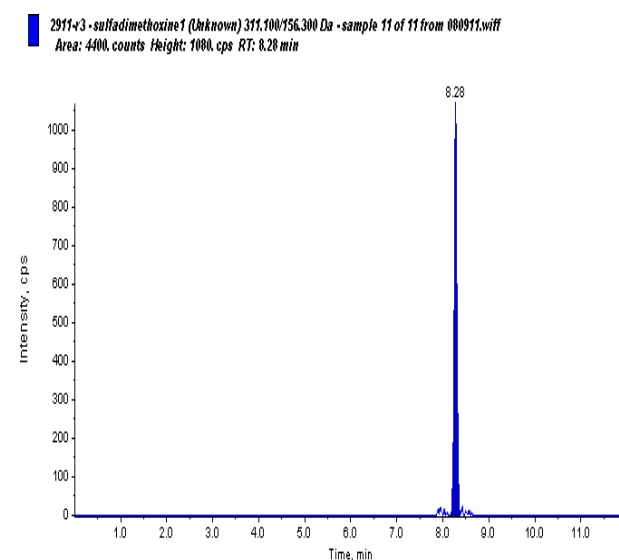
Different Pesticides in real Pollen sample.



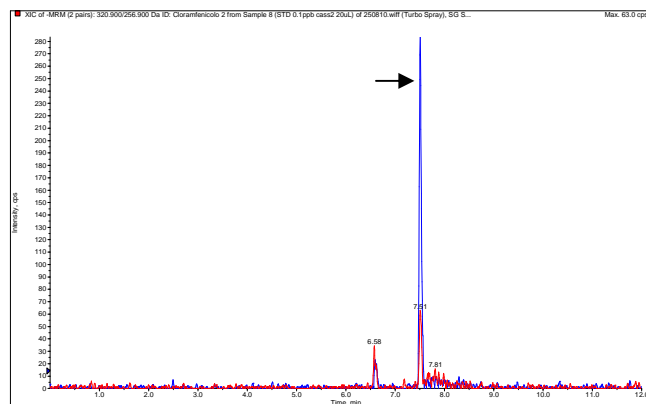
Ossitetracycline in real Honey sample at 0.79 µg/kg



Tylosin in real Honey sample at 0.62 µg/kg



Sulfadimethoxine in real Propolis sample at 0.24 µg/kg



Chloramphenicol in Royal Jelly sample spiked 0.1 µg/kg

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,)

Analyte: Tetracycline - Matrix: Honey

Floramo Corp. Lab.number:29, Z score= -0.3 (31.9 µg/kg vs. 34.1 µg/kg)

FAPAS® Report 02162
CERTIFIED DOCUMENT

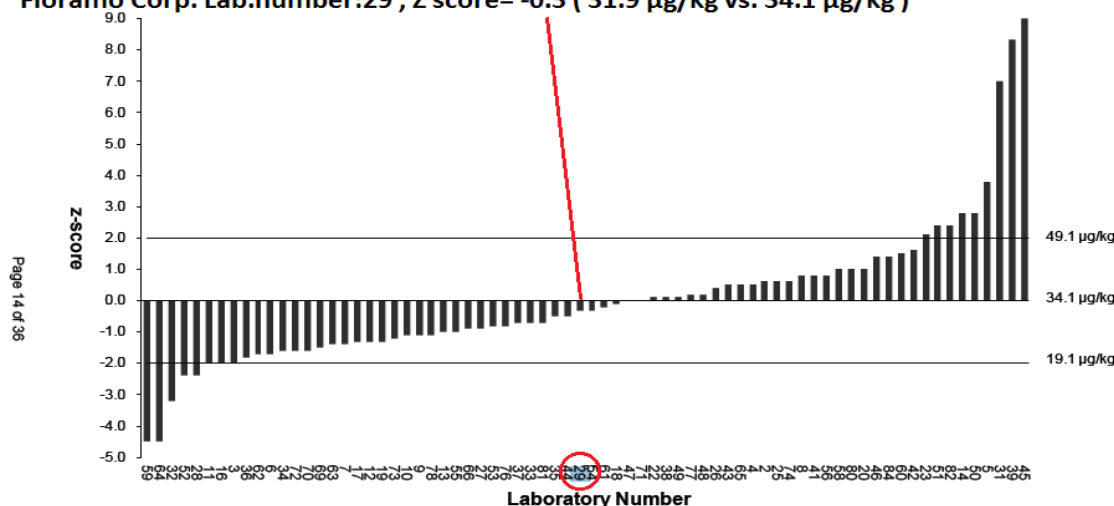


Figure 2: z-Scores for Tetracycline (Total) participants assigned a result of 0 for tetracycline (total) obtain a z-score of -4.5

Validation Data		
Compound (representative analytes)	LOD µg/kg	LOQ µg/kg
Oxytetracycline	0,07	0,21
Tetracycline	0,04	0,13
Sulfathiazole	0,06	0,17
Tylosine	0,05	0,14
Chloramphenicol	0,02	0,05
Chlorfenvinphos	0.32	0.97

LOD: Limit Of Detection
LOQ: Limit Of Quantification

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 beehive 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.