ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'UMBRIA E DELLE MARCHE-PERUGIA

6th International Symposium on RECENT ADVANCES IN FOOD ANALYSIS

November 5-8, 2013 - Prague, Czech Republic

RAFA 2013

MULTICLASS DETERMINATION OF ANTIBIOTICS IN HONEY

R. Galarini, S. Moretti, D. Giusepponi, R. Rossi, G. Saluti

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Via G. Salvemini, 1 - 06126 - Perugia (PG) (Italy) - www.izsum.it

Overview

A reliable confirmatory multiclass method for the determination of twenty-seven antibiotics in honey by liquid chromatography tandem mass spectrometry (LC-MS/MS) has been successfully developed and validated. The procedure demonstrated satisfactory performance characteristics. A survey on fifty honeys of different botanical and geographical origins evidenced the presence of sulfonamides at trace levels in six of the analysed samples (12 %).

Introduction

Antibiotic drugs are not authorized for the treatment of honey bees in the European Union, however no harmonization has been defined worldwide. Moreover illicit treatments with antibiotics are well documented both in EU and in third countries. Among the most used substances by the beekeepers, there are sulfonamides, nitroimidazoles and quinolones. Therefore if in the global market era the availability of suitable analytical methods is fundamental, the increasing application of multiclass procedures also in veterinary drugs field largely improves the cost-effectiveness of the controls. For this purpose a multiclass approach for the confirmation of 27 antimicrobial substances belonging to sulfonamide, quinolone and nitroimidazole groups has been developed and validated in honey.

LC-MS / MS Method

- Instrument: Thermo Electron Corporation HPLC Surveyor - TSQ Quantum ULTRA
- Column: Agilent Technologies Poroshell 120 EC-C18 (100 x 3.0 mm; 2.7 µm)
- Flow - rate: 0.3 mL/min
- Mobile phases: acetonitrile + 0.1 % formic acid [A] - 0.1 % formic acid [B]
- Acquisition mode: Single Reaction Monitoring (SRM)

Results

Method validation - Summarized validation data (4 replicates x 3 days on five levels = 60 observations) are reported in Table 1. For all tested compounds the trueness and the precision ranged from 59 to 100 % (recoveries) and from 11 to 24 % (coefficient of variation obtained in within-lab reproducibility conditions, CV_R, %). Decision limits (CCα) and detection capabilities (CCβ) were estimated for all analytes starting from the first reliable spiking level (0.1, 0.33 or 1 µg/kg).

Real samples - In six honeys (12 %) trace levels of sulfonamides were found. In three samples the presence of sulfathiazole was confirmed (0.3, 0.6 and 2 µg/kg), as well as that of sulfadimethoxine in three cases (0.2, 0.4 and 0.7 µg/kg).

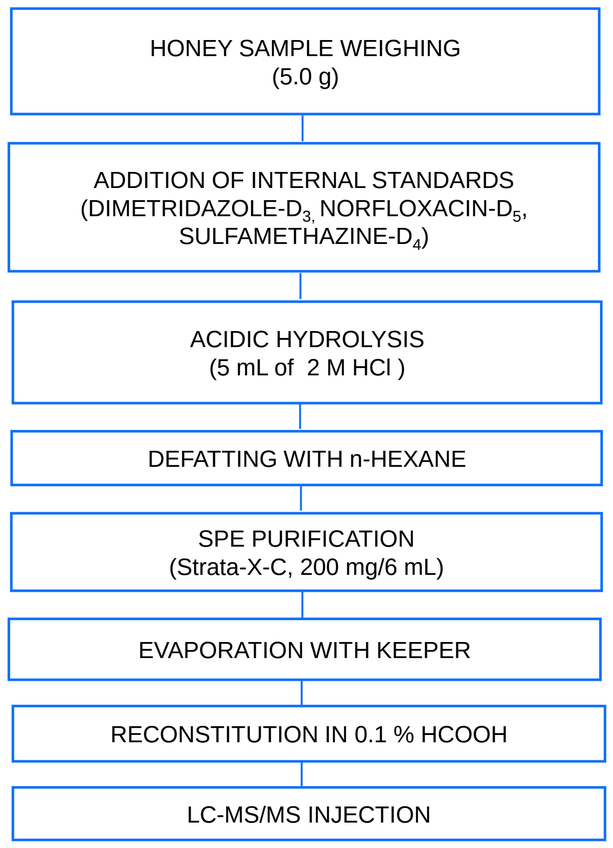

Figure 1. Flow diagram of sample preparation

Validation study

The method was validated in the range 0.1-10 µg/kg (0.1, 0.33, 1.0, 3.3 and 10 µg/kg) according to Commission Decision 2002/657/EC. Method selectivity, linearity, precision, trueness, decision limits and detection capabilities were evaluated.

Real samples analyses

The procedure was applied to analysis of 50 honey samples of different botanical and geographical origins collected from Italian market.

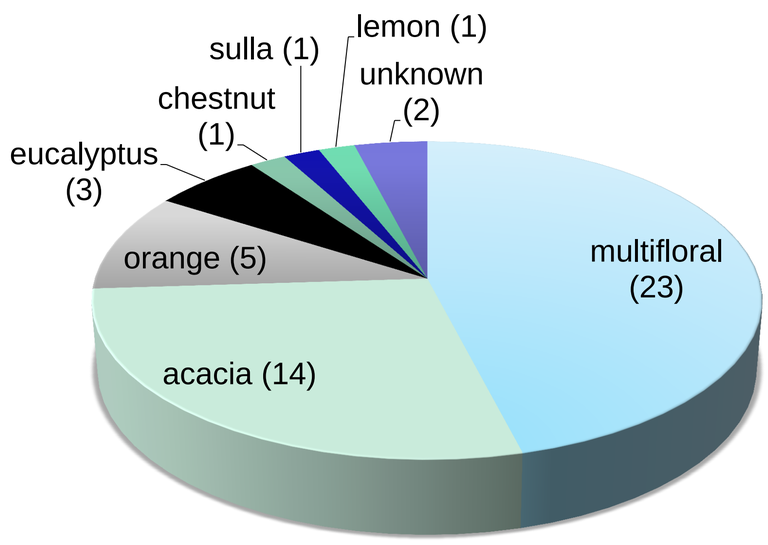

Figure 2. Analysed honeys

Table 1 - Validation data

Group	Analyte	Recovery (%)	CV_R (%)	CCα (µg/kg)	CCβ (µg/kg)
Quinolones	Oxolinic Acid	86	19	0.16	0.24
	Norfloxacin	91	14	0.47	0.60
	Ciprofloxacin	91	14	0.51	0.70
	Danofloxacin	93	19	1.5	2.0
	Enrofloxacin	100	17	0.16	0.23
	Marbofloxacin	82	24	0.59	0.91
	Sarafloxacin	91	14	0.48	0.63
	Difloxacin	97	17	0.15	0.21
Nitroimidazoles	Dimetridazole	71	20	0.49	0.65
	HMMNI	66	21	1.5	2.0
	Ipronidazole	62	16	0.15	0.21
	Metronidazole	75	12	0.43	0.52
	Ternidazole	76	13	0.39	0.43
	Secnidazole	81	15	0.16	0.24
	Ipronidazole-OH	80	17	0.15	0.21
	Metronidazole-OH	62	24	1.7	2.5
	Ronidazole	59	23	0.55	0.80
Sulfonamides	Sulfapyridine	82	11	0.44	0.54
	Sulfadiazine	72	20	0.55	0.82
	Sulfamethoxazole	80	20	0.16	0.23
	Sulfathiazole	80	15	0.15	0.21
	Sulfamerazine	86	16	0.48	0.62
	Sulfamethazine	78	20	0.18	0.27
	Sulfamonomethoxine	83	20	0.52	0.72
	Sulfachloropyridazine	74	16	0.51	0.71
	Sulfaquinoxaline	68	23	0.57	0.85
	Sulfadimethoxine	76	15	0.14	0.19

Table 2 - Positive results survey

Botanical origin	Geographical origin	Analyte found	Concentration (µg/kg)
Multifloral	Italy	Sulfathiazole	2.0
Multifloral	Italy, Hungary, Argentine	Sulfadimethoxine	0.2
Sulla	Italy	Sulfathiazole	0.6
Multifloral	Argentine, Hungary	Sulfadimethoxine	0.7
Multifloral	Italy	Sulfathiazole	0.3
Multifloral	Argentine, Hungary	Sulfadimethoxine	0.4

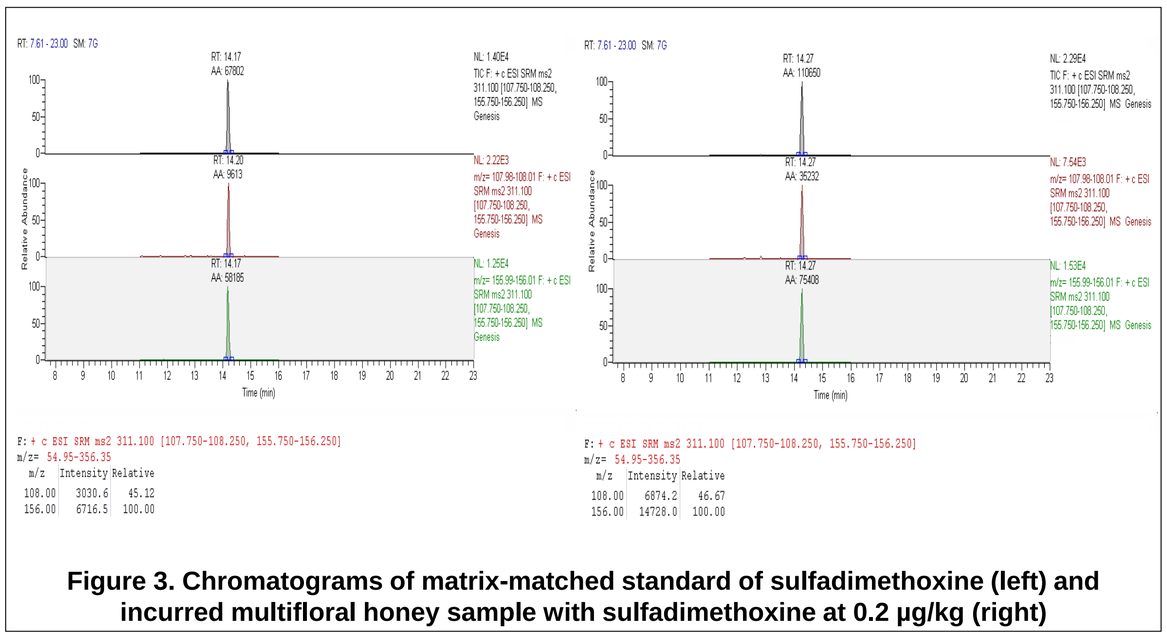

Figure 3. Chromatograms of matrix-matched standard of sulfadimethoxine (left) and incurred multifloral honey sample with sulfadimethoxine at 0.2 µg/kg (right)

Conclusions

The developed procedure is fit for purpose with low decision limits and satisfactory accuracies. The application of the method to analysis of 50 honey samples showed that sulfathiazole was detected only in honeys of Italian origin and sulfadimethoxine in samples also containing honeys of foreign origin (Argentine and Hungary). Considering the found levels there are no concerns about public health.

Contact:
Dr. Roberta Galarini
r.galarini@izsum.it

Stampato a cura dell'Unità Operativa di Supporto Biblioteca, Informazione, Editoria (2011).