Food Control 48 (2015) 12–24

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Multiclass determination of 27 antibiotics in honey

Roberta Galarini*, Giorgio Saluti, Danilo Giusepponi, Rosanna Rossi, Simone Moretti

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Via Salvemini, 1, 06126 Perugia, Italy

ARTICLE INFO

Article history:
Received 29 November 2013
Accepted 28 March 2014
Available online 5 April 2014

Keywords:
Nitroimidazoles
Quinolones
Sulfonamides
Honey
LC–MS/MS
2002/657/EC

ABSTRACT

A multiclass approach for the screening and confirmation of antimicrobial substances in honey has been developed and validated according to Commission Decision 2002/657/EC. A total of 27 basic drugs belonging to sulfonamide, nitroimidazole and quinolone families were determined. Sample preparation consisted in an acidic hydrolysis of honey followed by a double purification step (defatting and strong cation exchange solid-phase extraction). Instrumental determination was performed by liquid chromatography tandem mass spectrometry (LC–MS/MS) operating in positive electrospray ionization mode. Chromatographic separation was performed on a Poroshell 120 EC-C18 column (100 × 3.0 mm, 2.7 μm) using a gradient with acetonitrile and water both containing 0.1% of formic acid. The method was validated in the range 0.1–10 μg kg^{-1} evaluating selectivity, linearity, precision, trueness, matrix effect, decision limits and detection capabilities. Satisfactory performances were obtained for all the analytes, although important differences were observed in function of the honey type.

The procedure was applied to the analysis of 74 honey samples of different botanical origins and geographical provenience collected from the Italian market. In nine honeys (12%) trace levels of sulfonamides were confirmed. The found levels (lower than 2 μg kg^{-1}) do not raise concerns with respect to the public health.

1. Introduction

The most widespread and deleterious honey bee diseases are American Foulbrood, European Foulbrood and varroatosis, caused by the spore-forming bacterial pathogens, *Paenibacillus larvae* ssp. larvae, *Melissococcus plutonius* and *mite Varroa destructor*, respectively (Reybroeck, Daeseleire, De Brabander, & Herman, 2012). Antibiotics have widely been used as a preventive and therapeutic way to counter these diseases, however they are not authorized for the treatment of honey bees in the European Union. Thus, there are no Maximum Residues Limits established in Regulation 37/2010 (Commission Regulation, 2010). However no harmonization has been defined worldwide and in many third countries some antimicrobials are authorized for the treatment of honey bees. Moreover illicit treatments are well documented both in the EU and in third countries. For example, in the last five years (2009–2013) most notifications of the Rapid Alarm System for Food and Feed from the Directorate-General for Health and Consumers (RASFF Portal) in honey bee products involved the presence of antimicrobial residues (71%). The found compounds were: sulfonamides (35%), tetracyclines (15%), nitrofurans (13%), lincomycin (13%), aminoglycosides (10%), nitroimidazoles (8%), macrolides (5%) and quinolones (3%). If in the global market era the availability of suitable analytical methods is fundamental, the increasing use of multiclass procedures also in veterinary drugs field largely improves the cost-effectiveness of the residue controls. The cost-effectiveness of analytical procedures is a key issue for any laboratory involved in residue analysis. Accordingly multi-methods and especially multi-class methods are of growing importance in residue control of food of animal origin. The "ideal" method for antimicrobial in honey should include all the compounds legally or illegally used in apiculture to permit a complete control with only one procedure. However in honey prior to sample treatment an acidic hydrolysis is necessary to break the bond of sulfonamides with sugars (Schwaiger & Schuch, 2000). As a consequence it is not possible to conceive a comprehensive method because some important drug families such as beta-lactams and macrolides are acid sensitive (Martínez Vidal, Aguilera-Luiz, Romero-González, & Frenich, 2009; Mastovska & Lightfield, 2008) and more than one procedure must necessarily be applied to control the presence of antimicrobials in honey.

* Corresponding author. Tel.: +39 075 343272; fax: +39 075 3433060.
E-mail address: r.galarini@izsum.it (R. Galarini).

http://dx.doi.org/10.1016/j.foodcont.2014.03.048
0956-7135/

To date some multi-class methods for antimicrobials in honey are described in literature (Bohm, Stachel, & Gowik, 2012; Debayle, Dessalces, & Grenier-Loustalot, 2008; Gómez-Pérez, Plaza-Bolaños, Romero-González, Martínez-Vidal & Garrido-Frenich, 2012; Hammel, Mohamed, Gremaud, LeBreton, & Guy, 2008; Lopez, Pettis, Smith, & Chu, 2008; Martínez Vidal et al., 2009; Wang & Leung, 2012) and, although most of them include sulfonamides, the acidic hydrolysis prior to sample treatment is not always reported. The most applied strategy for sample purification was reverse phase solid phase extraction (Bohm et al., 2012; Debayle et al., 2008; Lopez et al., 2008; Martínez Vidal et al., 2009). However Hammel et al. (2008) implemented four subsequent liquid/liquid extraction steps to determine 42 antibiotics and Wang and Leung (2012) applied the QuEChERS method to quantify 31 veterinary drugs.

In the best of our knowledge in this work for the first time the use of an SPE purification based on the strong cationic exchange is reported for multiclass determination of basic veterinary drugs in honey. The goal of this research was to develop a selective and rapid procedure for screening and confirmatory purposes of a set of twenty-seven antimicrobial drugs belonging to nitroimidazole, quinolone and sulfonamide families widely used in apiculture.

Table 1
Summary of the selected reactions transitions (SRM) monitored for the twenty-seven targeted analytes.

Analyte	Retention time (min)	Precursorion (*m*/*z*)	Tube lens offset	Productions (*m*/*z*)	Collision energy (eV)
Metronidazole-OH	4.17	188.1	65	68.1	24
				123.1	15
				126.0	19
				144.1	12
HMMNI	5.46	158	85	55.2	23
				140.1	10
Metronidazole	5.51	172.1	70	82.1	25
				128	15
Dimetridazole-d_3	6.20	145	90	99.1	19
Dimetridazole	6.29	142.1	60	81.1	30
				95.1	30
Ronidazole	6.95	201.1	70	110.1	21
				140	12
Sulfadiazine	6.99	251.1	95	108	26
				156	16
Ternidazole	7.17	186.1	110	82.1	28
				128	15
Sulfapyridine	8.11	250.1	90	108	26
				156	17
Secnidazole	8.03	186.1	110	82.1	28
				128	15
Sulfathiazole	8.07	256	100	92.1	28
				156	15
Sulfamerazine	8.52	265.1	90	108	27
				156	17
Marbofloxacin	8.96	363.2	90	276.1	14
				320.1	14
Norfloxacin	9.28	320.2	90	233.1	24
				276.1	17
Norfloxacin-d_5	9.26	325.2	80	281.2	17
Ciprofloxacin	9.49	332.2	90	245.1	23
				288.1	17
Sulfamethazine-d_4	9.53	283.1	80	96.1	30
Sulfamethazine	9.59	279.1	90	108.0	30
				124.1	28
Danofloxacin	9.82	358.2	110	283.1	24
				340.1	22
Ipronidazole-OH	9.90	186.1	70	106.1	41
				121.1	29
Enrofloxacin	10.07	360.2	110	245.0	26
				316.1	19
Sarafloxacin	10.91	386.2	90	299.1	26
				342.1	18
Difloxacin	11.06	400.2	90	299.1	28
				356.1	18
Sulfamonomethoxine	11.18	281.1	90	108.0	28
				156.0	16
Sulfachloropyridazine	11.61	285	90	92.1	29
				156.0	16
Ipronidazole	12.20	170.1	80	109.1	27
				123.1	25
Sulfamethoxazole	12.43	254.1	95	108.0	26
				156.0	16
Oxolinic acid	13.35	262.1	90	216.0	29
				244.0	18
Sulfadimethoxine	14.33	311.1	95	108.0	29
				156.0	21
Sulfaquinoxaline	14.38	301.1	100	108.0	27
				156.0	21