VETERINARY DRUG RESIDUES

Determination of Erythromycin and Tylosin Residues in Honey by LC/MS/MS

RODRIGO GRANJA, ALFREDO MONTES NIÑO, ROBERTO ZUCCHETTI, and ROSARIO MONTES NIÑO
Microbioticos Laboratories, Av. Santa Isabel 2120, Campinas, SP, 13084-471, Brazil
RAJ PATEL
University of London, Centre for Chemical and Bioanalytical Sciences, School of Biological Sciences, Royal Holloway, Egham, Surrey TW20 OEX, United Kingdom
ALESSANDRO GONZALEZ SALERNO
Microbioticos Laboratories, Av. Santa Isabel 2120, Campinas, SP, 13084-471, Brazil

Antibiotics are used in apiculture to protect bees against a variety of brood diseases. As a result of the development of resistance to oxytetracycline, erythromycin and tylosin are increasingly used for the prevention and treatment of these diseases. Therefore, Brazilian authorities have added these antibiotics to the National Regulatory Monitoring Program for the control of residues in honey. An analytical method has been developed for the determination of residues of erythromycin and tylosin in honey. The procedure involves solid-phase extraction of diluted honey samples with Bond Elut cartridges, followed by LC/MS with electrospray positive ionization in the multiple reaction monitoring mode. Two characteristic transitions were monitored for both drugs. Average analyte recoveries of erythromycin and tylosin ranged from 99 to 109% from sets of replicate honey samples fortified with drug concentrations of 5, 10, 15, and 20 μg/kg. The method decision limits were determined to be 1.27 and 0.59 μg/kg for erythromycin and tylosin, respectively. The detection capabilities were 5 and 5.2 μg/kg for erythromycin and tylosin, respectively.

Antibiotics have been used in many countries to control American foulbrood (AFB), considered to be the most serious disease afflicting apiculture today. Oxytetracycline was found to be an effective treatment for AFB (1), and has been used extensively in many parts of the world since the 1950s. As a result of the development of resistance to oxytetracycline, erythromycin (ERY) and tylosin (TYL) are now increasingly used for the prevention and treatment of AFB (2, 3).

ERY (Figure 1) and TYL (Figure 2) are macrolide antibiotics that are active against Gram-positive and some Gram-negative bacteria. Incorrect use of these drugs or insufficient withdrawal periods after treatment can possibly lead to the presence of macrolide residues in foods and increase the potential risk to consumers because of allergic reactions to these antibiotics (4, 5). To avoid these consequences, residues of these bactericides in honey matrix have been banned in many countries, including Brazil.

In the absence of maximum residue limits for honey, the aim of the present work was development of a method for the simultaneous detection and confirmation of ERY and TYL in this matrix at trace levels. LC/MS/MS is a very accurate technique for this type of analysis and has been used to identify macrolides in animal tissues, eggs, milk, and honey (6, 7).

Currently, there are no analytical methods reported or available for the determination of macrolides in Brazilian honey, which seems to have peculiar characteristics, such as sugar content and formation of sugar clusters around the analyte molecule. On the other hand, the wide diversity in the composition of Brazilian honey—which is derived from many different floral species, and from specific and unique environmental conditions—accounts for the distinct characteristics of the compounds present in this honey. Six great Brazilian biomes are responsible for 175 native and cultivated honey floral species (8): Amazônia, Caatinga, Pantanal, Pampa Gaúcho, Mata Atlântica, and Cerrado.

In this paper, we describe a simple, rapid, and reliable LC/MS/MS method for the determination of ERY and TYL in Brazilian honey by using solid-phase extraction (SPE) for sample cleanup. The performance of the method was evaluated in accordance with European Union (EU) Directive 2002/657 (9) and is being considered for use for surveillance purposes in Brazil.

Experimental

Apparatus

Analyses were carried out with an Alliance 2695 HPLC quaternary gradient pump system coupled to a Micromass Quattro Micro tandem mass spectrometer with an electrospray ionization (ESI) source and MassLynx 4.0 software (Waters, Milford, MA). A Gemini C18 110A HPLC column

Received August 11, 2008. Accepted by JB October 14, 2008.
Corresponding author's e-mail: rodrigo@microbioticos.com