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ANTIBIOTICS RESIDUES IN HONEY: VALIDATION PROCEDURE

HONEY ANALYTICAL METHODS VALIDATION

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ABSTRACT

An uncontrolled used of antibiotics and sulphonamides is responsible for the presence of residues in beehive products and contributes to the problem of food safety. The optimisation of analytical methods for the detection of residues of antibacterial drugs (sulphonamides, SPs; tylosin, TL; streptomycin, ST) in honey was pursued and the methods applied to routine samples. ST was investigated by liquid chromatographic technique with a post column reaction and fluorimetric detection. SPs were quantified by HPLC equipped with fluorimetric detector and C8 column after a precolumn derivatisation by fluorescamine. The presence of TL residues in honey was confirmed by liquid chromatography – tandem mass spectrometric detection. Chromatographic separation of TL was performed on a C18 column. The validation parameters obtained with these methods were: 88%, 101% and 95-102% for recovery and 10 ng/g, 5 ng/g and 1 ng/g as quantification limits for ST, TL and SPs, respectively.

Keywords: *antibiotics, honey, residue, sulphonamide, validation*

INTRODUCTION

Sulphonamides and antibiotics are worldwide used in apicultural practices for the control of honeybee diseases, particularly American and European foulbrood (Hansen et al., 1999; Williams, 2000).

Their use can be responsible for the presence of residues in beehive products. In this paper we propose three analytical methods for the quantitative determination of seven sulphonamides (SPs), (Barbieri et al., 1995; Schwaigher et al., 2000), streptomycin (ST) (Gerhardt et al., 1994; Suhren et al., 1998) and tylosin (TL) (Dubois et al., 2001) in honey.

In SPs determination, a pre-column derivatisation and fluorimetric detection was used. A simple liquid-liquid cleanup procedure was applied to extract and concentrate SPs.

The ST determination was performed by ion-pair liquid chromatography with β-naphthoquinone-4-sulfonate (NQS) post-column derivatisation and fluorescence

detection. The clean-up of the extract is done by solid-phase extraction (SPE) with an styrene/divinylbenzene (SDB) cartridge.

TL residues was extracted and concentrated with a SPE cleanup procedure using octadecyl cartridge and analysed with a HPLC – MS/MS system.

The use of HPLC technique with fluorimetric and mass detection, together with an appropriate separation/concentration step, allowed to obtain good validation parameters such as recovery, quantification limits, linearity response and repeatability.

MATERIALS AND METHODS

Streptomycin extraction and analysis

A honey sample (5 g) was dissolved with 20 ml extraction solution (eptansulphonate – phosphate buffer, pH 2). The ST was separated in a SPE cartridge (500 mg styrene/divinylbenezene), conditionated with methanol and extraction solution and eluted with methanol. A millilitre of sodium dodecylsulphate 70 mM was added, after solvent dry evaporation.

The instrumental analysis was performed with a HPLC system equipped with a Supelcosil ABZ+Plus columns (150 x 4.6 mm 5 µm) and a fluorimetric detector ($\lambda_{EXCITATION}$ 263 nm, $\lambda_{EMMISSION}$ 435). The mobile phase was: 35% aqueous solution (sodium dodecylsulfate 0.1 M/ Sodium 1.2-Naphthochinon 4 Sulphonate) and 65% acetonitrile in isocratic mode. Flow: 0.8 ml/min. Injection volume: 100 µl. The post column derivatization was obtained with sodium hydroxide 0.3 M at 0.4 ml/min. The reaction temperature was 55°C with 1ml reaction coil. Sample concentration was calculated using the external standards.

Sulphonamides extraction and analysis

A honey sample (5 g), added with 5 ml hydrocloridric acid 2M, was shacken for 30 min. Then 10 ml of acetone/dichloromethane (80:20) and 2 g of sodium sulphate were added. After shaking for 10 min, the organic phase (5 ml) was taken and evaporated to dryness, the SPs (sulfaniline, sulfadiazine, sulfathiazole, sulfamethazine, sulfadoxin, sulfamethoxazole, sulfadimethoxine) were dissolved in 1 ml mobile phase. Successively they were quantified by HPLC with a pre-column derivatization with fluorescamine (100 µl of sample + 100 µl of fluorescamine + 200 µl of citric buffer pH 3).

The HPLC system was equipped with a Spherisorb C8 Columns (150 x 4.6 mm 3 µm) and a fluorimetric detector operating ($\lambda_{EXCITATION}$ 400 nm, $\lambda_{EMMISSION}$ 490). The mobile phase acetic buffer 0.125 M (pH 4.7), MeOH, ACN in gradient from 72:3:25 to 65:3:32 in 25 min was used. Sample concentration was calculated using the external standards. Injection volume: 100 µl.

Tylosin extraction and analysis

200 µl of roxytromycin (internal standard: 1 ppm) and 25 ml of TRIS buffer (pH 10.5) were added to 5 g of honey. This solution was shaken for 10 min. A SPE cleanup procedure with a Oasys HLB 60 mg cartridge was applied. TL was eluted with methanol/ammonia solution (95:5), which successively was evaporated completely. Sample was dissolved with 1 ml of mobile phase.

The HPLC system was equipped with a Quattro Ultima II column (100 x 2,1 mm 5 µm) and an ESI mass detector. The mobile phase was composed by ammonium

acetate 0,01 M (pH 3.5) and acetonitrile (50:50). Flow: 0.25 ml/min. Injection volume: 100 µl. Signal acquisition type: Multiple Reaction Monitoring for 916,2 ► 598,2, 916,2 ► 772,1 and 916,2 ► 174 transitions.

RESULTS AND DISCUSSION

These analytical methods are easy to perform with minimum sample manipulation and high selective detection (Figures 1, 2 and 3).

The performance data of the three methods are presented in Table I.

The sulphonamides method was introduced in our laboratory in 2000 in the frame of the national residues programme and applied to domestic and foreign honey samples. The streptomycin and tylosin methods were introduced at the end of 2003. The results obtained confirmed that antibacterial drugs are very often used by beekeepers for the control of certain honeybee diseases. Accordingly, in 2000-2003 period, 35 samples out of 212 analysed for SPs, ST and TL were found positive. This appears particularly relevant since no MRLs have been established for these drugs in honey.

Table I. Summary of the performance data

Compounds	*Streptomycin*	*Sulphonamides*	*Tylosin*
Recovery	85%	95-102%	101%
LOD	10 ng/g	1 ng/g	5 ng/g

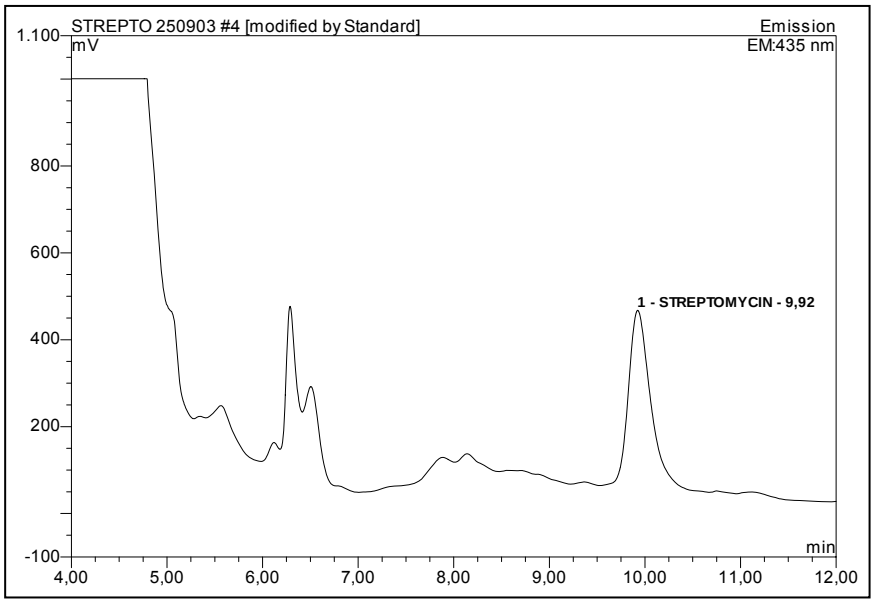

Fig. 1 Chromatogram of honey sample added with 100 ng/g of streptomycin

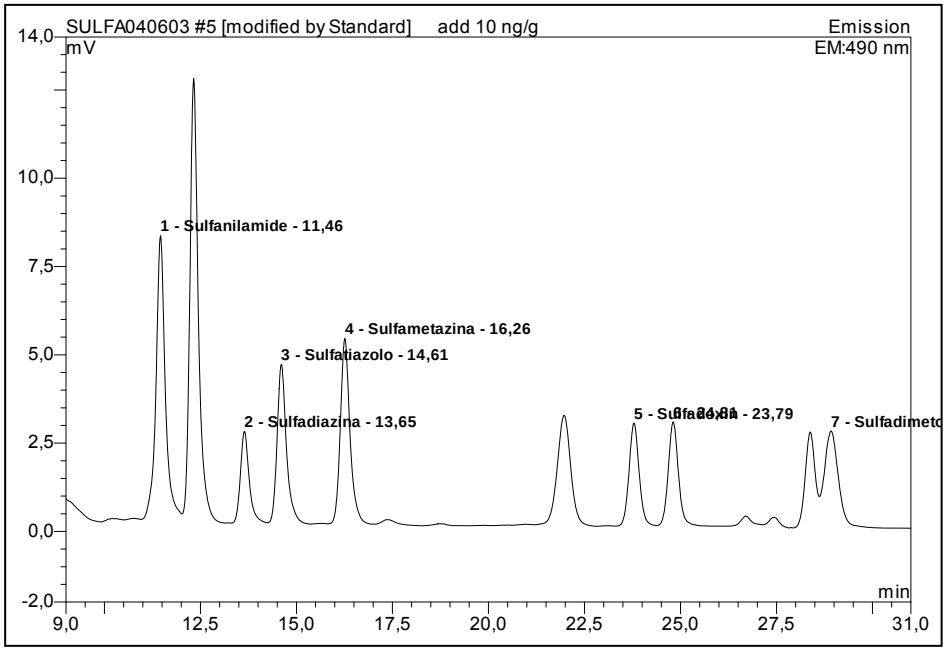

Fig. 2 Chromatogram of honey sample added with 10 ng/g of sulphonamides

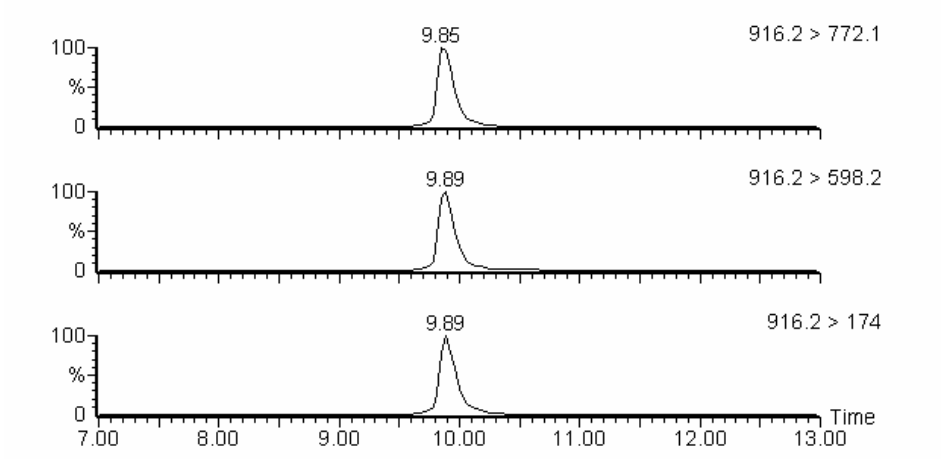

Fig. 3 Chromatogram of honey sample added with 25 ng/g of tylosin (3 transition considered)

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