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Liquid chromatography with ultraviolet absorbance detection for the analysis of tetracycline residues in honey

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Abstract

The separation of tetracyclines (TCs) using reversed-phase liquid chromatography (LC) is proposed. The use of an amide-based stationary phase prevents the interaction of tetracyclines with the residual silanol groups and thus avoids the appearance of tailed peaks. Detection was based on using an UV spectrophotometer and gradient elution with acetonitrile–oxalic acid as mobile phase permitted good separation of all the peaks. Specificity was demonstrated by the retention characteristics, UV spectra and peak purity index. Linearity, precision, recovery and sensitivity were satisfactory. The procedure was applied to the analysis of tetracycline residues (tetracycline, oxytetracycline (OTC), chlortetracycline (CTC), doxycycline (DC), minocycline (MINO) and methacycline (MTC)) in honey of different types. Extraction involved using a mild acidic solvent containing EDTA to release protein-bound or sugar-bound tetracyclines. For the clean-up step, solid phase extraction using phenyl cartridges was applied. Detection limits in the honey using the proposed procedure are between 15 and 30 ng g^{-1}, depending on the tetracycline.

Keywords: Food analysis; Honey; Tetracycline; Antibiotics

1. Introduction

Tetracyclines (TCs) are broad-spectrum antibiotics which show activity against Gram-positive and Gram-negative bacteria, including some anaerobes [1] and have been widely used for the treatment of infectious diseases and as an additive in animal foodstuffs. In 1990, the Commission of the European Union laid down the procedure for establishing maximum residue limits (MRLs) of veterinary drugs in foodstuffs of animal origin. However, no MRLs have been fixed for use with bee products. Antibiotics such as tetracyclines have had MRLs imposed for their use in large animals but are illegal for use with bees. Honey is composed primarily of sugars and water [2] being a natural healthy food. At present, application of the law in relation to these antibiotics is not harmonised across all member states. Some countries do not permit honey to contain drugs above the corresponding detection limit, while others apply an action level such as 50 ng g^{-1}. However, it must be stressed that the presence of these drugs in honey is illegal at any level [3]. The Spanish plan for residue control and healthy food (Plan CREHA) of 2002 has established maximum limits for the concentration of residual substances [4]. As regards the group of tetracyclines, the analytes included are tetracycline, oxytetracycline (OTC) and chlortetracycline (CTC), which have been analyzed in meat, milk, honey and eggs. The detection limit obtained using liquid chromatography (LC) is 20 ng g^{-1} or 20 ng ml^{-1} and the maximum acceptable limit has been set at 100 ng g^{-1} or 100 ng ml^{-1}. These limits are similar to those marked by the Belgian Agency for Safe Foods.

Tetracyclines can be successfully determined using liquid chromatography (LC) in the reversed-phase mode [5–7] and with different detection modes, such as spectrophotometry [8–13], fluorescence [14,15], fast-atom-bombardment mass spectrometry (FAB-MS) [16] and MS–MS [17,18].

In the present study, the separation of tetracycline, oxytetracycline, chlortetracycline, doxycycline (DC), minocycline (MINO) and methacycline (MTC) was optimized using an UV detector and a stationary phase involving a ligand with amide groups and the endcapping of trimethylsilyl. This phase also proved satisfactory for the determination of fluoroquinolone antibacterial agents [19]. These tetracyclines

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