Development and Validation of an Analytical Method for Tetracycline Antibiotic Residues in Honey Using OPT-SPE and LC-MS/MS Detection

Saranya Wutthiosot[1,*] and Jamras Lerdsri[1]

Abstract

Background: The use of tetracycline antibiotics to control bee diseases can lead to residues in honey, posing risks to consumer safety and creating trade barriers, particularly with the European Union market, which enforces a zero-tolerance policy for such residues. Therefore, it is necessary to develop and validate an analytical method for detecting residues of oxytetracycline, tetracycline, chlortetracycline, and doxycycline in honey using liquid chromatography–tandem mass spectrometry (LC–MS/MS), a technique known for its high sensitivity, specificity, and reliability.

Objectives: To develop and validate an analytical method for determination of tetracycline residues in honey for routine implementation at the Veterinary Research and Development Center, Upper Northern Region.

Methods: The sample preparation method was developed by comparing the extraction efficiency of three solid-phase extraction (SPE) techniques, namely C18, OPT, and C8/SCX cartridges, using sulfaphenazole as the internal standard. Method validation was conducted in accordance with EURACHEM guidelines, covering the following parameters: matrix effects, analytical range and linearity, accuracy, precision, measurement uncertainty, decision limit (CCα), specificity, and method robustness.

Results: The OPT cartridge exhibited the highest extraction efficiency with percentage recoveries ranging from 92.5 to 103.2%. The developed method showed no significant matrix effects and demonstrated a linear analytical range of 2.0–40.0 ng/g with a coefficient of determination (R^2) ≥ 0.9978. The method exhibited good accuracy, with recoveries between 94.00 and 102.95%, and satisfactory precision, with %CV values ranging from 1.42 to 6.50. The measurement uncertainty was estimated in the range of ±6.4% to ±19.3%, and the decision limits (CCα) were between 2.10 and 5.17 ng/g, which are below the minimum required performance limit (10 ng/g) established by the European Union. The analytical method complied with the criteria of European Commission Implementing Regulation (EU) 2021/808. Furthermore, the developed method demonstrated adequate specificity and robustness against variations in sample preparation conditions.

Conclusion: The developed LC-MS/MS method coupled with OPT-SPE is suitable, sensitive, accurate, and reliable for routine determination of tetracycline residues in honey.

Keywords: Method development, Honey, Antibiotic residue, LC-MS/MS, Tetracycline

[1] Veterinary Research and Development Center (Upper Northern Region), Hang Chat, Lampang 52190 Thailand
[*] Corresponding author; E-mail: w_arinya@hotmail.com