Veterinary Drug Analysis in Animal Origin Food and Feed and Their Relevant Products: A Modern Multi-Class, Multi-Residue Method Using UHPLC-MS/MS

Hui Zhao, John Zulkoski, Azeem Hasan and Katerina Mastovska; Covance Food Solutions, Madison, WI, USA  hui.zhao@covance.com

Introduction
Veterinary drugs are a complex group of different chemical classes and therapeutic agents. They are used within animal husbandry to treat and prevent disease and ensure animal health and growth. Residues of such drugs in animal edible tissues are not desirable because they could pose a potential threat to consumer health and promote antibiotic resistant bacteria strains. Therefore, these substances are strictly regulated and monitored in foods to ensure food safety and prevent the unnecessary exposure of consumers to veterinary drugs. For that purpose multi-class, multi-residue methods are becoming increasingly popular in regulatory monitoring programs globally because of their extended analytical scope and laboratory efficiency.

Modern Multi-Class, Multi-Residue Method Using LC-MS

Benefits
► Cost-effective
► Time-effective
► Selective detection of individual analytes
► Improved sensitivity for low LODs/LODs
► Identification/confirmation

Challenges
► A large spectrum of drug classes
► Parent drugs and metabolites
► Different physical/chemical properties
   – Hydrophobic to hydrophilic
   – Acidic, neutral and basic
   – Stability
   – Interaction with matrix components
► Compromise between analyte scope and performance characteristics
► Matrix effects and potential interference from co-extractives

Analytes in Positive Mode (~150) Divided into 9 Groups

LC-MS/MS Analysis

UHPLC: Agilent Fast LC 1290
Column: Agilent C18 Zorbax Eclipse Plus, 2.1x100 mm, 1.8 µm
Column Oven Temperature: 40°C
Injection Volume: 5 µL
Flow Rate: 0.5 mL/min
Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in Methanol
Gradient: Time [min] %A %B
0.0 98 2
0.75 98 2
7.0 60 40
11.0 0 100
13.0 0 100
13.1 98 2
10.0 98 2

Mass Spectrometer: Agilent Triplet Quadrupole
MS/MS: 6495A
MS Acquisition: Dynamic MRM
Cycle Time: 600 ms
Ion Source Type: ESI+
Collision Energy: Optimized for individual MRM
Cell Accelerator Voltage: Optimized for individual MRM

Results and Discussion

Sample Preparation Optimization

► Extraction
   - Ratio between aqueous and organic solvents: The 50:50 acetonitrile:water (v/v) mixture was selected over 75:25 acetonitrile:water (v/v) mixture to improve the recoveries of beta-lactams.
   - Addition of EDTA buffer: EDTA (0.05M) was added to the water to prevent chelation of tetracyclines and quinolones with metals.
   - Clean-up - evaluated options
     - Addition of EDTA buffer: EDTA (0.05M) was added to the water to prevent chelation of tetracyclines and quinolones
     - Ratio between aqueous and organic solvents: The 50:50 acetonitrile:water (v/v) mixture was selected over 75:25 acetonitrile:water (v/v) mixture to improve the recoveries of beta-lactams.

► Clean-up - evaluated options
   - EMR (disperse): enhanced matrix removal-lipid
   - SLE supported liquid extraction
   - C18 SPE cartridge format
   - PPL+: phosphotidyl removal
   - Hexane:diol + C18 cartridge SPE

► Factors used to evaluate clean-up efficiency:
   - Recovery and precision
   - Matrix co-extractive removal efficiency by a gravimetric test
   - Matrix suppression/enhancement evaluation using post-column infusion
   - From gravimetric test and post-column infusion, EMR and SLE provided the best co-extractive removal efficiency.
   - Considering the lower recoveries observed for some critical compounds (e.g. tetracyclines, beta-lactams) when applying the various clean-up procedures, clean-up was omitted from the final method.

Conclusions
► The developed method was validated in infant formula powder, showing satisfactory validation results, including identification, selectivity, matrix effects, linearity, LOQs, accuracy and precision.
► This method can be used in routine analysis for the simultaneous detection and quantification of a large number of veterinary drug residues in infant formula.
► In the future, the method will also be validated for screening/quantitation of veterinary drugs in other relevant matrices, such as dairy products, seafood, meat, eggs, honey, pet food and animal feed.

Method Validation Process
► Evaluation of accuracy and precision at 0.5, 1, 5, 10, 50 and 100 ng/g (n = 5) in the infant formula powder and intermediate precision (ruggedness) at 1, 5, and 10 ng/g (n = 10: 2 different analysts on 2 different days).
► To test the linearity of the method and compare quantification results, the following three sets of standards were prepared:
   - Extracted matrix match standards to quantify the potential veterinary drug residues in the samples (mimicking the standard addition procedure) and determine corrected analyte recoveries presented in Table 1.
   - Post-extracted matrix matched standards to determine absolute analyte recoveries.
   - Standards in solvent to monitor matrix effects.

Method Validation Results
► Method performance was evaluated according to CAC/GL 71-2009 guideline

<table>
<thead>
<tr>
<th>Concentration, ng/g</th>
<th>CV within the lab, %</th>
<th>Accuracy, range of mean recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 to 10</td>
<td>5 to 100</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>50 to 120</td>
</tr>
<tr>
<td>10</td>
<td>50 to 120</td>
<td></td>
</tr>
</tbody>
</table>

► Acceptable analyte corrected recoveries (CR, within the 70-120% range) and CVs (±30%) were obtained for all analytes at and above their LOQs, except for 4-epioxytetracycline, doxycycline, dimethacin and colistine A and B, which were excluded from the final method used for the routine analysis.

► The method validated LOQ was determined for each analyte as the lowest spiking concentration (scoring from 2 days) in Infant Formula Powder

Presented at EuroResidue VIII 2016