Development of a multi-residue method for β-lactam antibiotics in bovine muscle using UHPLC-MS/MS

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Introduction

The β-lactams are key antibiotics used in the treatment of bacterial infections in both humans and food producing animals. Inappropriate use of antibiotics may lead to residues in food and cause human health hazards, such as allergic reactions in sensitive individuals, or contribute to antimicrobial resistance. The objective of this work was to develop and validate a multi-residue method for quantitative confirmatory determination of β-lactam antibiotic residues in bovine muscle using UHPLC-MS/MS. Chromatography and mass spectrometry conditions were optimised in order to allow sensitive detection of 30 β-lactams (12 penicillins, 12 cephalosporins, 5 carbapenems and faropenem). A sample preparation procedure based on water/acetonitrile extraction and C_{18} dispersive solid-phase extraction (dSPE) clean-up was developed for isolating β-lactam residues from bovine muscle (**Figure 1**).

Sample preparation

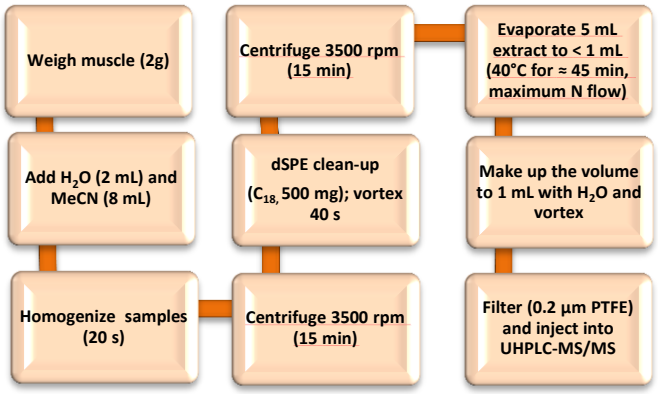

Figure 1. Sample preparation protocol for bovine muscle.

UHPLC-MS/MS conditions

Analysis was carried out using a Waters Acquity UHPLC system coupled to a Waters Quattro Premier UHPLC-MS/MS triple quadrupole mass spectrometer equipped with ESI interface.

UHPLC parameters:

Chromatography was performed on a Waters Acquity CSH C_{18} (2.1 x 100 mm, 1.7 µm) analytical column, using a binary gradient of **[A]** 0.01% HCOOH + 0.2 mM ammonium acetate in water and **[B]** 0.01% HCOOH in MeCN (**Figure 2**).

Time (min)	A%	B%
0.00	100	0
1.50	100	0
3.50	80	20
8.50	20	80
8.60	0	100
10.50	0	100
10.60	100	0
18.00	100	0

Figure 2. Gradient conditions.

Column temperature: 30°C
Flow rate: 0.4 mL min^{-1}
Injection volume: 10 µL
Run time: 18 min

MS parameters:

Ionisation mode: ESI+
Capillary voltage: 2.4 kV
Source temperature: 140°C
Desolvation temperature: 450°C

Results and Discussion

Method development

Mass spectrometry conditions were optimised to give at least two product ions and four identification points for each analyte. Different mobile phase additives were evaluated, showing that the best overall sensitivity and chromatography were obtained using 0.01% formic acid in both mobile phases A and B and 0.2 mM ammonium acetate in mobile phase A.

A number of dSPE sorbents were tested (C_{18}, C_8, PSA, NH_2, Z-Sep, Z-Sep+, Z-Sep/C_{18}). For each procedure, sensitivity, precision, recovery and ion suppression were investigated. The most suitable method included simple solvent extraction with water and acetonitrile and C_{18} dSPE clean-up.

Method validation

Validation was performed in accordance with Commission Decision 2002/657/EC. Linearity (R^2>0.988) was achieved for all the analytes over the calibration range of the method. Selectivity studies showed no matrix interferences. Overall absolute recoveries ranged from 61% to 89% for most analytes. Accuracy ranged between 86% and 108% and precision ranged from 1.5% to 17.3% in within-laboratory reproducibility study (**Figure 3**). Matrix effects were also evaluated, and both ion suppression and enhancement were observed. However, accuracy was significantly improved using the internal standards.

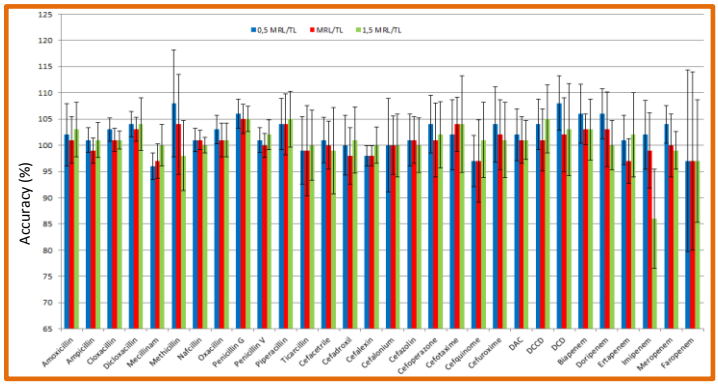

Figure 3. Accuracy and precision (shown by error bars) obtained in within-laboratory reproducibility study (n = 3). For the legislated compounds, validation was carried out at 0.5, 1 and 1.5 times the Maximum Residue Limits (MRLs), except for desfuroylceftiofur cysteine disulphide (DCCD) and desfuroylceftiofur dimer (DCD), which were validated at 250, 500 and 750 µg kg^{-1}. For the non-MRL substances, validation was carried out around a Target Level (TL) that was identified based on the sensitivity of the method. *DAC = Desacetyl cephapirin.*

Conclusions

- Our research showed that 30 β-lactam antibiotic residues can be easily extracted from fortified bovine muscle tissue samples and detected using UHPLC-MS/MS, with no need for derivatization or extensive steps.
- Different clean-up approaches were tested for dSPE and C_{18} was selected as the best compromise for all analytes.
- Validation results showed good accuracy and precision for all compounds.
- Further work is required to assess the method through the application to inter-laboratory studies.

Acknowledgements

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