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Development and validation of a quantitative confirmatory method for 30 β-lactam antibiotics in bovine muscle using liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

A method was developed for the confirmatory and quantitative analysis of 30 β-lactam antibiotic residues in bovine muscle. The method includes 12 penicillins (amoxicillin, ampicillin, cloxacillin, dicloxacillin, mecillinam, methicillin, nafcillin, oxacillin, penicillin G, penicillin V, piperacillin, ticarcillin), 12 cephalosporins (cefacetrile, cefadroxil, cephalexin, cefalonium, cefazolin, cefoperazone, cefotaxime, cefquinome, cefuroxime, desacetyl cephapirin, desfuroylceftiofur cysteine disulfide, desfuroylceftiofur dimer), five carbapenems (biapenem, doripenem, ertapenem, imipenem, meropenem) and faropenem. Samples were extracted using a simple solvent extraction with acetonitrile:water (80:20, v/v) and C_{18} dispersive solid-phase extraction (d-SPE) clean-up, followed by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) detection. Chromatography was performed on a reversed phase CSH C_{18} column, using a binary gradient separation comprising of 0.01% formic acid and 0.2 mM ammonium acetate in water (mobile phase A) and 0.01% formic acid in acetonitrile (mobile phase B). The mass spectrometer was operated in the positive electrospray ionisation mode (ESI(+)). Validation was performed following the 2002/657/EC guidelines. Trueness ranged between 69% and 143% and precision ranged between 2.0% and 29.9% under within-laboratory reproducibility conditions. The developed method uses minimal sample preparation and 30 test samples can be analysed by a single analyst in a single day. To the best of our knowledge, this is the first method for carbapenems in foodstuff that does not require derivatisation.

1. Introduction

The β-lactams are key antibiotics used in both human and veterinary medicine, which have a common four-membered ring in their molecular structure (Fig. 1) [1]. The penicillins, cephalosporins and carbapenems represent the most important β-lactam drug groups. In the penicillin and cephalosporin structures, the four-membered ring is fused to a five-membered thiazolidine or a six-membered dihydrothiazine ring, respectively. The carbapenems are similar to the penicillins, with a sulphur atom replaced by a carbon atom. Faropenem, a carbapenem-related compound, is another important member of the β-lactam class [2].

The β-lactams act by inhibiting the enzymes involved in the biosynthesis of the peptidoglycan in the cell wall, which can cause lysis in actively multiplying bacteria [3]. Penicillins are classified as broad- or narrow-spectrum antimicrobials, based on their activity, or according to their susceptibility to bacterial β-lactamases [1], while cephalosporin drugs can be categorised into different generations, based on their effectiveness against Gram-positive and Gram-negative organisms [4].

Penicillins and cephalosporins are often administered parenterally and orally to food-producing animals to prevent or treat bacterial infections. An inappropriate use of antibiotics may lead to residues in food, which can cause health hazards, such as allergic reactions in sensitive individuals. In addition, concerns have been raised over the excessive usage of antibiotics, which is linked to the increase of antimicrobial resistance in livestock and the potential transfer of resistant bacteria and resistant genes to humans and human pathogens, respectively [3]. This is particularly impor-

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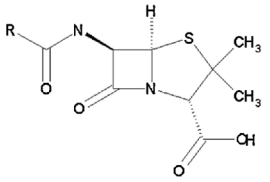
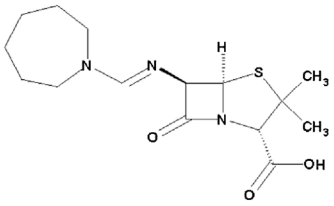
Penicillins

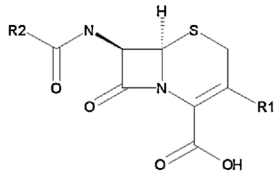


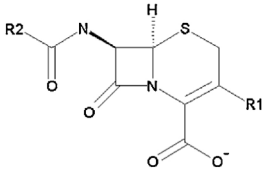


Mecillinam

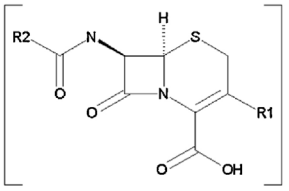
Penicillin	*R*	*Penicillin*	*R*
Amoxicillin	-C_7H_8NO	Oxacillin	-$C_{10}H_8NO$
Ampicillin	-C_7H_8N	Penicillin G	-C_7H_7
Cloxacillin	-$C_{10}H_7ClNO$	Penicillin V	-C_7H_7O
Dicloxacillin	-$C_{10}H_6Cl_2NO$	Piperacillin	-$C_{14}H_{16}N_3O_3$
Methicillin	-$C_8H_9O_2$	Ticarcillin	-$C_6H_5O_2S$
Nafcillin	-$C_{12}H_{11}O$		

Cephalosporins





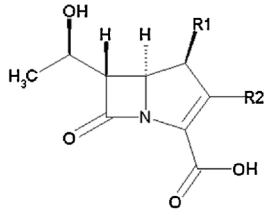
Cefalonium and Cefquinome

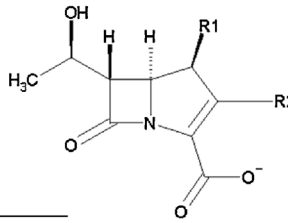


DCD

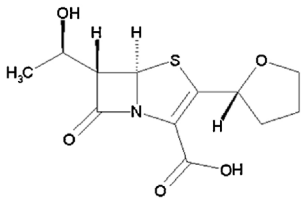
Cephalosporin	*R1*	*R2*	*Cephalosporin*	*R1*	*R2*
Cefacetrile	-$C_3H_5O_2$	-C_2H_2N	Cefotaxime	-$C_3H_5O_2$	-$C_5H_6N_3OS$
Cefadroxil	-CH_3	-C_7H_8NO	Cefquinome	-$C_{10}H_{13}N$	-$C_5H_6N_3OS$
Cephalexin	-CH_3	-C_7H_8N	Cefuroxime	-$C_2H_4NO_2$	-$C_6H_6NO_2$
Cefalonium	-$C_7H_8N_2O$	-C_5H_5S	DAC	-CH_3O	-C_6H_6NS
Cefazolin	-$C_4H_5N_2S_2$	-$C_2H_3N_4$	DCD	-CH_2S	-$C_5H_6N_3OS$
Cefoperazone	-$C_3H_5N_4S$	-$C_{14}H_{16}N_3O_4$	DCCD	-$C_4H_8NO_2S_2$	-$C_5H_6N_3OS$

Carbapenems and faropenem





Biapenem



Faropenem

Carbapenem	*R1*	*R2*
Biapenem	-CH_3	-$C_5H_7N_3S$
Doripenem	-CH_3	-$C_5H_{12}N_3O_2S_2$
Ertapenem	-CH_3	-$C_{12}H_{13}N_2O_3S$
Imipenem	-H	-$C_3H_7N_2S$
Meropenem	-CH_3	-$C_7H_{13}N_2OS$

Fig. 1. Chemical structures of the β-lactams included in the proposed UHPLC–MS/MS method.

tant among the third- and fourth-generation cephalosporins, due to the reliance of these drugs in human medicine as broad-spectrum antibiotics [5,6]. Consequently, Maximum Residue Limits (MRLs) are established for different species and target tissues [7] and accurate methods become essential to measure the presence of non-compliant antibiotic residues in food.

There are nine penicillin and eight cephalosporin active ingredients listed under Commission Regulation (EU) 37/2010 [7]. Many