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Rapid multiresidue and multi-class screening for antibiotics and benzimidazoles in feed by ultra high performance liquid chromatography coupled to tandem mass spectrometry

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Amoxicillin (PubChem CID: 33613)
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Carbadox (PubChem CID: 5353472)
Trimethoprim (PubChem CID: 5578)
Chlortetracycline (PubChem CID: 54737570)
Flubendazole (PubChem CID: 35802)
Tiamulin (PubChem CID: 656958)
Lincomycin (PubChem CID: 3000540)
Tylvalosin (PubChem CID: 70685113)
Tilmicosin (PubChem CID: 6436128)

ABSTRACT

An analytical strategy was developed for high-throughput screening of multiple antibiotics and two benzimidazoles in feed. Generic sample processing was applied without any purification step. After methanol extraction, the samples were centrifuged, concentrated, and analysed by ultra-high-performance liquid chromatography hyphenated to tandem mass spectrometry in the multiple reaction monitoring mode. Qualitative validation was carried out for more than 50 antibacterials of various classes, including aminocoumarin, amphenicols, beta-lactams, lincosamide, macrolides, diaminopyrimidine, quinolones, sulfonamides, streptogramin, pleuromutilin, polypeptide, quinoxaline, and tetracyclines, and also some benzimidazoles in feed at μg/kg level. Validation was done in accordance with the guidelines laid down in European Commission Decision 2002/657/CE for qualitative screening methods.

This convenient, reliable, and sensitive method has been used successfully to monitor antibiotic residues in feeds.

1. Introduction

Conventional livestock production systems use antibiotics therapeutically, prophylactically, and as growth enhancers. The presence of antibiotics in feed is either authorized (for therapeutic and prophylactic purposes), unauthorized (antibiotics as growth promoters), or unintentional (due to cross-contamination).

The authorized antimicrobials most broadly used in medicated feed are tetracyclines, sulfonamides, trimethoprim, macrolides, β-lactams, aminoglycosides, pleuromutilins, and lincosamide. The use of medicated feeds is most common in intensive production, especially of pig and chicken (European Commission, 2010a). Although these antimicrobials are authorized, traces are undesirable in non-medicated feed. As medicated and non-medicated feeds are often manufactured in the same production line, carry-over of antimicrobials can occur when a feed miller switches from producing one feed to the next (Stolker et al., 2013) or later in the production line. To decrease the level of cross-contamination in feed in Belgium, the FASFC, in agreement with Belgian feed producers, has decided to impose replacing the principal mixer with an end-of-line mixer or a precision dose system as of January 2014 for the production of medicated feed excepted for deworming feed and pellets for rabbit.

Since 1997, the European Union has introduced bans on the use of antibiotic growth promoters such as avoparcin, ardacin, bacitracin, virginiamycin, tylosin, spiramycin, carbadox, olaquindox,

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monensin, salinomycin, avilamycin, and flavophospholipol in food animal production. Given the potential human health risk, the use of chloramphenicol is also prohibited in food-producing animals in many countries, including the EU and the USA.

It is very important to pay attention to feed contamination with such agents, because health hazards (allergies or toxic effects are associated with the persistence of antibiotics in foods of animal origin, such as muscle and liver tissue (Martínez, 2009; Vandenberge et al., 2012). Furthermore, antibiotic resistance due to an inappropriate use of therapeutic antimicrobials in human and veterinary medicine is increasingly recognized worldwide as a human and animal health issue (WHO, 2014).

Monitoring feed to ensure the absence of an increasing number of undesirable drugs at very low levels requires highly sensitive and selective methods. Recent reviews summarize the analytical methods reported over the past few years for the analysis of antimicrobial agents in animal feed (Borràs, Companyó, Granados, et al., 2011; Kantiani, Farré, Grases, & Barceló, 2009). The main difficulties arise from the complexity and variability of the animal feed matrix and from the frequently low levels of the compounds to be detected. The strategies developed for sample preparation and extraction of drug residues from such matrices usually involve extensive handling and clean-up to improve sensitivity and selectivity (Mol et al., 2008), but although extraction, clean-up, and matrix analyte concentration are key steps in determining antimicrobials in complex samples, one should bear in mind that such drugs have very different physicochemical properties. It is advisable to perform sample extractions as generic as possible, without extensive clean-up, so as to screen for as many analytes as possible.

After extraction of drug residues with organic solvents, various authors have used a purification step involving solid-phase extraction (SPE) (Aguilera-Luiz, Romero-González, Plaza-Bolaños, Martinez Vidal, & Garrido Frenich, 2013; Ardsoongnearn, Boonbanlu, Kittijaruwattana, & Suntornsuk, 2014; Kantiani, Farré, Grases, & Barceló, 2010; Van Poucke, De Keyser, Baltusnikiene, McEvoy, & Van Peteghem, 2003; Vincent, Chedin, Yasar, & von Holst, 2008; Wang et al., 2014), liquid–liquid clean-up (Cronly et al., 2010), filtration (Kaklamanos, Vincent, & Von Holst, 2013), or QuEChERS (Boscher, Guignard, Pellet, Hoffmann, & Bohn, 2010; Lopes et al., 2012). Others have used simpler means: dilution of the extract before analysis (Boix et al., 2014; Cháfer-Pericás, Maquieira, Puchades, Miralles, & Moreno, 2011) or protein precipitation by freezing the extract (Nácher-Mestre, Ibáñez, Serrano, Pérez-Sánchez, & Hernández, 2013).

The aim of the present work was to develop a multi-class screening method for extracting and analysing in feed a wide range of antibiotic families simultaneously. We describe here the development, optimization, and validation of a convenient, reliable, and sensitive method involving feed sample extraction with methanol (without any tedious purification step) followed by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Identification of contaminants is done in the MRM mode with at least one transition per substance (positive and negative ionisation modes). We also report on the successful routine use of this method over a three-year period, notably in the context of proficiency testing. The method shows good performances for ppb-level determination of most of the tested compounds.

Table 1
MS/MS transitions for each antimicrobial and benzimidazole.

Name	1st Transition	2nd Transition	Name	1st Transition	2nd Transition
Aminocoumarin			**Phenicoles**		
Novobiocin	613 > 188.9	613 > 217.9	Thiamphenicol	354.1 > 290	354.1 > 184.8
β–Lactams			Florfenicol	356 > 336	356 > 185
Amoxicillin	366 > 114	366 > 349	Chloramphenicol	321 > 152	321 > 257
Ampicillin	350 > 160	350 > 192	Chloramphenicol d5 (I.S.)	328 > 157	
BenzylPenicillin	335 > 160	335 > 176.1	**Polypeptide**		
Cloxacillin	435.8 > 276.8	435.8 > 159.8	Bacitracin	712 > 869.4	712 > 669.5
Dicloxacillin	469.7 > 159.8	469.7 > 310.8	**Quinolones**		
Nafcillin	437 > 319	437 > 278	Danofloxacin	357.9 > 254.9	357.9 > 82
Oxacillin	402 > 242.7	402 > 160	Difloxacin	400 > 356	400 > 298.9
Cefalexin	348 > 158		Cirprofloxacin	332 > 245	332 > 230.8
Cefapirin	424 > 152	424 > 292	Enrofloxacin	360.0 > 316.0	360.0 > 244.9
Cefquinome	529.2 > 324	529.2 > 133.9	Flumequine	262 > 243.9	
Ceftiofur	523.9 > 240.9	523.9 > 125	Marbofloxacin	362.9 > 319.9	362.9 > 72
Cefalonium	458.7 > 123	458.7 > 337.2	Oxolinic acid	261.9 > 243.9	261.9 > 215.8
Cefazolin	455.1 > 155.6	455.1 > 322.8	Sarafloxacin	385.9 > 299	385.9 > 342
Cefoperazone	646 > 530	646 > 142.9	Cinoxacin	262.8 > 244.9	262.8 > 160.9
Phenoxymethylpenicillin	351 > 160	351 > 114	Lomefloxacin (I.S.)	352.2 > 265.2	
Benzimidazoles			**Quinoxaline**		
Fenbendazole	300 > 268.01		Carbadox	263 > 229	263 > 231
Flubendazole	314 > 282		**Sulfonamides**		
Triclabendazole-d3 (I.S.)	364.2 > 346		Sulfadimethoxine	311 > 155.8	311 > 91.9
Diamino–pyrimidine derivative			Sulfamethoxazole	254 > 156	254 > 108
Trimethoprim	291.1 > 230.1		Sulfathiazole	256.1 > 156	
Trimethoprim-d9 (I.S.)	300 > 234.1		Sulfadimidine	278.9 > 92	278.9 > 124
Lincosamides			Sulfadoxine	311 > 155.8	311 > 107.9
Lincomycin	407 > 126		Streptogramin		
Macrolides			Virginiamycin M1	548.2 > 287	548.2 > 243
Erythromycin	734.4 > 158	734.4 > 115.9	**Tetracyclines**		
Spiramycin	843.4 > 174	843.4 > 101	Chlortetracycline	478.9 > 443.9	478.9 > 97.9
Tilmicosin	869.5 > 174	869.5 > 87.9	Doxycycline	444.9 > 427.9	
Tylosin	916.5 > 174	916.5 > 773	Oxytetracycline	460.9 > 426	
Tylvalosin	1042.6 > 174	1042.6 > 229	Tetracycline	444.9 > 409.9	
Tiamulin	494.2 > 192				
Valnemulin	565.4 > 263.2				