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Multi-residue determination of 115 veterinary drugs and pharmaceutical residues in milk powder, butter, fish tissue and eggs using liquid chromatography–tandem mass spectrometry

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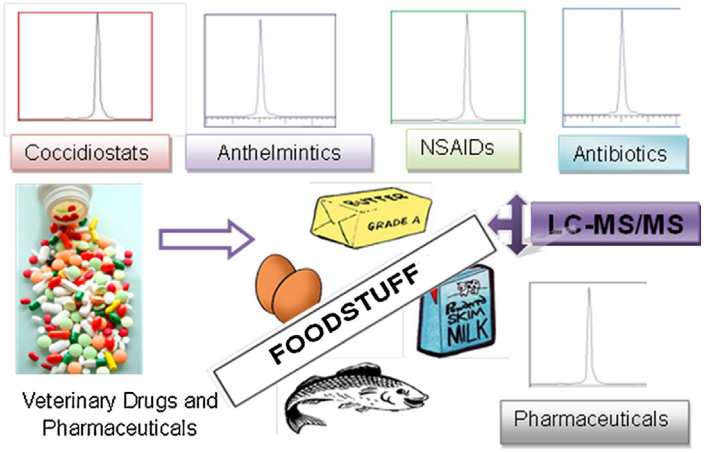
HIGHLIGHTS

- A novel multi-residue determination of veterinary drugs in food is presented.
- A thorough sample preparation optimization for all the compounds was performed.
- Validation data and applications in milk, egg, butter and fish samples are provided.
- Successful proficiency testing verified the applicability of the developed method.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple and sensitive multi-residue method for the determination of 115 veterinary drugs and pharmaceuticals, belonging in more than 20 different classes, in butter, milk powder, egg and fish tissue has been developed. The method involves a simple generic solid–liquid extraction step (solvent extraction, SE) with 0.1% formic acid in aqueous solution of EDTA 0.1% (w/v)–acetonitrile (ACN)–methanol (MeOH) (1:1:1, v/v) with additional ultrasonic-assisted extraction. Precipitation of lipids and proteins was promoted by subjecting the extracts at very low temperature (−23 °C) for 12 h. Further cleanup with hexane ensures fat removal from the matrix. Analysis was performed by liquid chromatography coupled with electrospray ionization and tandem mass spectrometry (LC-ESI-MS/MS). Two separate runs were performed for positive and negative ionization in multiple reaction monitoring mode (MRM). Particular attention was devoted to extraction optimization: different sample-to-extracting volume ratios, different concentrations of formic acid in the extraction solvent and different ultrasonic extraction temperatures were tested in butter, egg and milk powder samples. The method was also applied in fish tissue samples. It was validated, on the basis of international guidelines, for all four matrices. Quantitative analysis was performed by means of standard addition calibration. For over 80% of the analytes, the recoveries were between 50% and 120% in all matrices studied, with RSD values in the

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range of 1–18%. Limits of detection (LODs) and quantification (LOQs) ranged from 0.008 μg kg^{-1} (oxfendazole in butter) to 3.15 μg kg^{-1} (hydrochlorthiazide in egg). The evaluated method provides reliable screening, quantification, and identification of 115 veterinary drug and pharmaceutical residues in foods of animal origin and has been successfully applied in real samples.

1. Introduction

Veterinary drugs are widely used at therapeutic levels in the systems of livestock breeding for treating different diseases as well as food additives to promote animal growth. Antibacterials are widely used by farmers to fight against bacterial infections [1]. Furthermore, other families of veterinary drugs, such as anthelmintics and coccidiostats, are used for the treatment of parasitic diseases and coccidiosis (an infectious disease caused by a microscopic protozoan parasite), respectively [2,3]. Some of these drugs show also growth-promoting effects and are commonly misused for this reason. The possible presence of veterinary drug residues and other contaminants in edible tissues and even food products is one of the key issues for food safety which arouses great public concern. As a consequence of these residues of veterinary drugs in food, the health of consumers is jeopardized, since they can be responsible for toxic effects, allergic reactions in individuals with hypersensitivity, and can result in the development of resistant strains of bacteria [4–6]. Moreover, general pharmaceuticals for human medication are considered as widespread emerging pollutants with potential to enter the food chain.

In order to combat this problem and protect the human consumers, the use of veterinary drugs is tightly regulated. The use of veterinary drugs was regulated through EU Council Regulation 2377/90/EC [7], which has been repealed by Council Regulation 470/2009/EC [8], Council Directive 96/23/EC [9] and most recently, Commission Regulation 37/2010/EC [10]. The requirements for performance and validation of analytical methods employed in the official residues control for screening and confirmatory purposes are described in European Decision 2002/657/EC [11].

Therefore, sensitive and reliable analytical methods for the determination of veterinary drug and pharmaceutical residues in food of animal origin are needed to ensure consumers' safety. An emerging trend in drug residue analysis is the development of generic methods that are capable of monitoring a wide variety of compounds, belonging to different drug classes. This appears as a considerable challenge since the different chemical groups, the amphoteric properties of many compounds, and the large polarity range pose difficulties for extraction, clean up, and analytical separation. Although strongly required, multiclass methods for veterinary drugs are still limited.

LC–MS techniques provide a universal approach applicable to the widest number of veterinary drugs and especially LC-MS/MS has dominated in the field of veterinary drug analysis in food stuffs [12–27]. LC-MS/MS provides an unambiguous identification and a reliable confirmation of substances, because it combines analyte separation and structural information [28]. The versatility, high selectivity and sensitivity of LC-MS/MS have allowed the successful quantification of drug residues in animal products even in the low ng L^{-1} range. Applications of quantitative multi-analyte methods reported lately in the last 5 years (since 2010) and related to LC coupled to MS/MS are presented in Table 1.

Table 1
Quantitative applications in multi-residue analysis of veterinary drugs in food matrices.

Compounds	Matrix	Sample preparation technique	Stationary phase	Mobile phase	Detection-identification	Recoveries	Reference
TCs (4), MCs (4), Qs (5), SAs (4) and ANTHs (8)	Egg	Comparison of solvent extractions – QuEChERS – SPE – MSPD	Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 μm)	A: MeOH, B: 0.05% (v/v) formic acid in H_2O	LC-ESI-MS/MS (+)	SEs': 70.4% (tetracycline) to 94.4% (tilmicosin)	[12]
Qs (4), TCs (3), MCs (9), β-LACTs (4), SAs (9), AMPs (3), AMGs (6) and NSAIDs (1)	Amimal tissue	Solid–liquid extraction with ACN/H_2O (86:14, v/v) and defatting with hexane	Atlantis dC18 (20 × 3.9 mm, 3 μm) and ZIC–HILIC (50 × 2.1 mm, 5 μm) for AMGs	Reversed phase: A: 0.1% (v/v) formic acid in H_2O, B: ACN HILIC:A: 0.4% (v/v) formic acid in H_2O, B: ACN	LC-ESI-MS/MS (+) and (−)	–	[13]
SAs (14), TCs (4), Qs (9), β-LACTs (7), MCs (5), LINCs (1), and TRIM	Egg	PLE with 1:1 (v/v) mixture of ACN and 0.01 mol L^{-1} succinic acid buffer pH 6.0	Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 μm)	A: oxalic acid 2-hydrate 0.13 g L^{-1} in H_2O with 0.02% formic acid, B: 0.1% (v/v) formic acid in ACN	LC-ESI-MS/MS (+)	47% (amoxicillin) to 320% (danofloxacin)	[14]
β-LACTs (4), SAs (8), TCs (4), Qs (3), MCs (3), BAC, NSAIDs (1), PHARMs (1) and ANTHs (1)	Milk	Extraction with ACN, cleanup with SPE (HLB) and with an 30 kDa MW cutoff filter	YMC ODS-AQ (100 ×2 mm, 3 μm)	A: 0.1% (v/v) formic acid in H_2O, B: ACN	LC-ESI-MS/MS (+)	22% (ampicillin) to 143.3% (enrofloxacin)	[15]
Qs (11), SAs (20), TCs (4), MCs (9), NFs (7), β-LACTs (15), AMPs (1), QUINOXs (1), VIRG, NOV, ANTHs (12), β-AGONs (9), NSAIDs (6), CORTs (7), THYRs (5) and other contaminants (11)	Bovine kidney	Extraction ACN-H_2O (4:1, v/v), cleanup with hexane partitioning	Prodigy ODS-3 (150 × 3mm, 5 μm)	A: 0.1% (v/v) formic acid in H_2O, B: 0.1% (v/v) formic acid in ACN	LC-ESI-MS/MS (+)	53% (chlortetracycline) to 129% (semicarbazide)	[16]
TCs (3), β-LACTs (5), Qs (2), SAs (3) and other contaminants (8)	Egg	MeOH:H_2O:CH_3COOH 80:20:1 (v/v/v), 0.5 g CH_3COONa and 2.0 g	ACE C18 (150 × 2.1 mm, 3 μm)	A: 0.1% (v/v) formic acid in H_2O, B: 0.1%	LC-ESI-MS/MS (+)	56% (oxacillin) to 79% (doxycycline)	[17]