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Multiclass and multi-residue determination of antibiotics in bovine milk by liquid chromatography–tandem mass spectrometry: Combining efficiency of milk control and simplicity of routine analysis

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ABSTRACT

Antibiotics are widely used in veterinary medicine and incorrect practices may lead to drug residues in food, including milk. Fast and simple sample preparation methods have been developed for determination of quinolones, fluoroquinolones, tetracyclines, sulphonamides, trimethoprim and bromexine using liquid chromatography–tandem mass spectrometry. Small volumes of sample (0.5 mL) were extracted using organic solvent followed by centrifugation and evaporation and/or dilution. Validation took into account the maximum residue limits (MRLs). Recoveries of 62–108% were obtained. Linearity (r^2) above 0.96 was achieved for all compounds using concentrations in the range 0.25–2.0 × MRL. Intraday precision with coefficients of variation (n = 6) lower than 15.0% and inter-day precision lower than 17.3% were obtained for three concentration levels in the range 0.5–1.5 × MRL. Accuracy was 87–108%. Limits of detection and quantitation, as well as decision limit, detection capability, robustness and applicability, were evaluated using >600 real milk samples.

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1. Introduction

Antibiotics are extensively administered to dairy cattle for disease treatment and prophylaxis purposes. When good veterinary practices are not observed, residues of the drugs can remain in animal tissues or fluids (KuKanich, Gehring, Webb, Craigmill, & Riviere, 2005; Woodward, 2004). The possible presence of antibacterial residues in milk is a public health concern, since milk is consumed worldwide. European Commission has set maximum residue limits (MRLs) for several veterinary drugs in milk, including different classes of antibiotics (European Commission, 2010). An important tool to closely monitor and assure this compliance in Brazil is the National Residue Control Plan (NRCP) (de Queiroz Mauricio & Lins, 2012). A laboratory network operating under NRCP is crucial to provide valuable data for the evaluation of the potential exposure of milk consumers to drug residues. In Brazil, the laboratory network of the Ministry of Agriculture, Livestock and Food Supply (MAPA) performs this survey for veterinary drugs residues in milk, meat, honey, fisheries, eggs and other products of animal origin (Lins, Conceição, & Mauricio, 2012; Ministério da Agricultura, Pecuária e Abastecimento, 2014).

To determine antibiotics drugs residues in milk, analytical methods with high sensitivity and specificity have been developed (Bogialli & Di, 2009). These methods mainly use liquid chromatography coupled to mass spectrometry in tandem mode (LC–MS/MS) to achieve the needed criteria proposed by the regulatory authorities (Brasil Ministério da Agricultura Pecuária e Abastecimento, 2011; European Commission, 2002). Milk is a complex matrix; therefore sample preparation protocols for bovine milk samples are a frequent concern for researches. Many extraction procedures have been described in literature, including protein precipitation using trichloroacetic acid (TCA) (Bohm, Stachel, & Gowik, 2009; Ruiz-Viceo et al., 2012) and the use of hot water as extraction solvent (Bogialli et al., 2005; Bogialli, D'Ascenzo, Di, Laganà, & Nicolardi, 2008). Nevertheless, one of the most frequent methods for milk sample preparation is based on solid phase extraction (SPE) (Hermo,

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Nemutlu, Kir, Barrón, & Barbosa, 2008; Junza, Amatya, Barrón, & Barbosa, 2011; Tang, Yang, Tan, & Luo, 2009; Turnipseed, Andersen, Karbiwnyk, Madson, & Miller, 2008).

Ortelli, Cognard, Jan, and Edder (2009) proposed an interesting method for screening 150 veterinary drugs using Liquid Chromatography time-of-flight mass spectrometry (LCqTOF). The method is easy and quick, using acidified acetonitrile in low volumes followed by centrifugation and concentration; this method was proposed just for screening purposes. In the present study, a simple, quick and cheap method for screening and quantitative and confirmatory analysis for antibiotics belonging to the classes of quinolones (Qs), fluoroquinolones (FQs), tetracyclines (TCs), sulphonamides (SAs), trimethoprim and bromexine was developed and validated.

The authors have previously reported simple and innovative sample preparation protocols, without use of SPE, and focused on reducing solvent volume and replacing, when possible, hazardous organic solvents with less harmful products, as ethanol (Bittencourt, Martins, de Albuquerque, Barreto, & Hoff, 2012; Martins et al., 2014, 2015). Herein, we applied the same experimental design for milk. The goal of this work was to determine antibacterial substances commonly used in dairy cow treatment in Brazil and several other countries using a simple and environmental-friendly extraction procedure. The main objective was to develop a feasible high-throughput method for routine analysis.

2. Material and methods

2.1. Analytical standards

Standards of ciprofloxacin (CIPRO), enrofloxacin (ENRO), difloxacin (DIFLO), sarafloxacin (SARA), norfloxacin (NORFLO), danofloxacin (DANO), nalidixic acid (NALI), oxolinic acid (OXO), flumequine (FLU), sulphadiazine (SDZ), sulphatiazole (STZ), sulphamerazine (SMR), sulphamethazine (SMZ), sulphadoxine (SDX), sulphadimethoxine (SDMX), sulphachlorpyridazine (SCP), sulphamethoxazole (SMA), sulphisoxazole (SFZ), sulphaquinoxaline (SQX), trimethoprim (TMP), bromexine (BMX), tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), doxycycline (DOXI) and the internal standards enrofloxacin-D_5 (ENRO-D_5), sulphapyridine (SPY) and demeclocycline (DEMO) were purchased from Riedel–de-Haen (Buchs, Switzerland) or Sigma–Aldrich (St. Louis, MO, USA). Stock standard solutions were prepared in methanol at concentrations of 1.0 mg mL^{-1}, except for bromexine, which was prepared in ultrapure water. For fluoroquinolone stock solutions, it was necessary to add some drops of acetic acid (1 M) for complete solubilisation. For sulphachlorpyridazine, it was necessary to use acetone for solubilisation prior to completing the volume with methanol. Finally, for sulphadiazine it was necessary to use sodium bicarbonate (100 mM). Intermediate solutions were prepared in methanol using different amounts of each analyte to achieve their respective MRLs, obtaining solutions varying from 1 to 10 μg mL^{-1}. The intermediate solution was diluted in methanol to obtain a working solution at concentrations of 0.1–1 μg mL^{-1}. A separate working solution was prepared in methanol for internal standards to achieve a final concentration of 1 μg mL^{-1}. Stock solutions were stored at −20 °C and intermediate and working solutions were stored at 5 °C.

2.2. Reagents and chemicals

Except when indicated, all reagents were of high performance liquid chromatography (HPLC) grade. Acetonitrile (ACN) was purchased from J.T.Baker (Phillipsburg, NJ, USA) and methanol was purchased from Merck (Darmstadt, Germany). Acetic acid, ethanol and formic acid were of HPLC grade J.T.Baker (Phillipsburg, NJ, USA). Ultrapure deionised water was produced by a Milli-Q apparatus (Millipore, Bedford, MA, US). Disodium ethylenediaminetetracetate (EDTA) was obtained from Sigma.

2.3. Liquid chromatography–tandem mass spectrometry

The LC analysis were performed on a Waters Alliance Separations Module 2695 with a SYMMETRY® C_{18} column

Table 1
Optimised values for each analyte.[a]

Compound	Abbreviation	Precursor ion (*m/z*) > quantification ion	Precursor ion > confirmation ion	Cone voltage	Collision energy	Retention time (min)
Enrofloxacin	ENRO	360.3 > 245.2	360.3 > 316.2	35	25, 20	6.30
Ciprofloxacin	CIPRO	332.2 > 288.2	332.2 > 314.0	40	17, 20	6.20
Difloxacin	DIFLO	400.2 > 299.2	400.2 > 356.3	45	28, 20	6.70
Danofloxacin	DANO	358.2 > 95.8	358.2 > 340.1	40	25, 20	6.20
Sarafloxacin	SARA	386.3 > 342.0	386.3 > 368.2	40	20, 25	6.70
Norfloxacin	NORFLO	320.2 > 276.2	320.2 > 302.2	40	17, 20	6.10
Nalidixic acid	NALI	233.1 > 187.0	233.1 > 215.2	22	25, 15	10.9
Oxolinic acid	OXO	262.1 > 216.1	262.1 > 244.3	30	25, 20	9.60
Flumequine	FLU	262.2 > 202.2	262.2 > 244.1	30	30, 20	11.2
Enrofloxacin-D5	ENRO_D5 (IS)	365.3 > 245.2	365.3 > 321.2	45	20, 20	6.30
Sulphadiazine	SDZ	251.0 > 108.0	251.0 > 108.0	25	25, 15	7.04
Sulphamethazine	SMZ	279.2 > 123.8	279.2 > 123.8	35	20, 20	8.06
Sulphamethoxazole	SMA	254.0 > 155.9	254.0 > 155.9	30	15, 25	9.51
Sulphaquinoxaline	SQX	301.1 > 156.0	301.1 > 156.0	40	15, 25	10.08
Sulphadimethoxine	SDMX	311.0 > 140.0	311.0 > 140.0	35	20, 25	10.18
Sulphachlorpyridazine	SCP	285.0 > 107.8	285.0 > 107.8	35	25, 20	9.13
Sulphatiazole	STZ	256.0 > 155.9	256.0 > 155.9	30	15, 25	7.21
Sulphamerazine	SMR	265.2 > 156.0	265.2 > 156.0	35	15, 25	7.64
Sulphadoxine	SDX	311.3 > 155.9	311.3 > 155.9	35	20, 25	9.18
Sulphisoxazole	SFZ	268.1 > 156.0	268.1 > 156.0	25	15, 15	9.74
Trimethoprim	TMP	291.0 > 275.2	291.0 > 230.2	45	25, 22	5.38
Bromexine	BMX	376.9 > 113.9	376.9 > 113.9	16	25, 35	8.17
Sulphapyridine	SPY (IS)	250.1 > 155.9	250.1 > 155.9	35	15, 25	7.39
Tetracycline	TC	445.2 > 410.2	445.2 > 153.9	30	20, 25	7.1
Oxytetracycline	OTC	461.2 > 426.2	461.2 > 444.2	35	20, 12	6.9
Doxycycline	DOXI	445.4 > 428.2	445.4 > 153.9	35	20, 30	8.5
Chlortetracycline	CTC	479.2 > 153.9	479.2 > 97.60	30	30, 40	8.2
Demeclocycline	DEMO (IS)	465.1 > 153.9	465.1 > 448.2	35	30, 18	7.6

[a] Collision energies shown refer to quantification ion and confirmation ion, respectively.

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