Journal of Chromatography B, 962 (2014) 20–29

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Development and validation of an ultra high performance liquid chromatography tandem mass spectrometry method for simultaneous determination of sulfonamides, quinolones and benzimidazoles in bovine milk

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ARTICLE INFO

Article history:
Received 23 December 2013
Accepted 2 May 2014
Available online 14 May 2014

Keywords:
Sulfonamides
Quinolones
Benzimidazoles
Milk
LC–MS/MS

ABSTRACT

A simple, sensitive and reliable analytical method was developed for the simultaneous determination of 38 veterinary drugs (18 sulfonamides, 11 quinolones and 9 benzimidazoles) and 8 metabolites of benzimidazoles in bovine milk by ultra high performance liquid chromatography-positive electrospray ionization tandem mass spectrometry (UHPLC–ESI-MS/MS). Samples were extracted with acidified acetonitrile, cleaned up with Oasis® MCX cartridges, and analyzed by LC–MS/MS on an Acquity UPLC® BEH C_{18} column with gradient elution. The method allows such multi-analyte measurements within a 13 min runtime while the specificity is ensured through the MRM acquisition mode. The method was validated according to the European Commission Decision 2002/657/EC determining specificity, decision limit (CCα), detection capability (CCβ), recovery, precision, linearity and stability. For compounds which have MRLs in bovine milk, the CCα values fall into a range from 11 to 115 μg/kg, and the CCβ values fall within a range of 12–125 μg/kg. For compounds which have not MRLs in bovine milk, the CCα values fall into a range from 0.01 to 0.08 μg/kg, and the CCβ values fall within a range of 0.02–0.11 μg/kg. The mean recoveries of the 46 analytes were between 87 and 119%. The calculated RSD values of repeatability and within-laboratory reproducibility experiments were below 11% and 15% for the 46 compounds, respectively. The method was demonstrated to be suitable for the simultaneous determination of sulfonamides, quinolones and benzimidazoles in bovine milk.

1. Introduction

Veterinary drugs are widely used for the treatment and prevention of diseases in livestock. However, the use of veterinary drugs may result in residues of these drugs in animal products like milk, eggs and meat, which can lead to health problems [1–3]. Sulfonamides (SAs) and quinolones (QNs) are two important class of antibacterial compounds widely used in veterinary practice. The public health hazards related to their use in livestock involve several problems such as the increased risk of developing allergies in some hypersensitive individuals and the development of resistant strains of bacteria, and their potential carcinogenicity [4–6]. Benzimidazoles (BZs) are safe, broad-spectrum anthelmintic drugs and are widely used for prevention and treatment of parasitic infections in food-producing animals. However, the extensive use of BZs will leave residues in edible animal products, which are harmful to humans owing to their teratogenic and embryotoxic properties [7,8]. So, the maximum residue limits (MRLs) of SAs, QNs and BZs in bovine milk (100 μg/kg for SAs, ciprofloxacin, enrofloxacin, albendazole and thiabendazole, 30 μg/kg for danofloxacin and sarafloxacin and 10 μg/kg for oxfendazole, febantel and fenbendazole) have been established by the European Union (EU) and China to protect consumer health [9,10].

A large number of analytical methods including biological screening methods, quantitative and confirmatory methods have already been developed for the determination of veterinary drug residues in food [11–25]. For quantitative and confirmatory methods, gas chromatography (GC) or liquid chromatography (LC) followed mass spectrometry (MS) or tandem MS (MS/MS) have been frequently performed. In the last few years, LC–MS/MS has

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http://dx.doi.org/10.1016/j.jchromb.2014.05.005
1570-0232/

become one of the most promising techniques for the analysis of veterinary drug residues in food due to its versatility, specificity and selectivity. The LC–MS/MS method also allows for the analysis of a number of compounds in a single operation, as well as decreases the cost of analysis. So, a large number of multi-residue analytical LC–MS/MS methods have recently been established for the determination of veterinary drugs in food [12,14,15,20–22,25]. Nevertheless, the LC–MS/MS methods for the simultaneous determination of SAs, QNs and BZs are still very scarce.

A few LC–MS/MS methods have been developed for the simultaneous determination of SAs, QNs and BZs residues in animal-derived food [12,25–29]. However, some of them often were chosen as a kind of screening method [12]. Lopes et al., Clark et al., Frenich et al., and Aguilera-Luiz et al. have developed four LC–MS/MS confirmatory methods for the simultaneous determination of multi-class veterinary drugs involved SAs, QNs and BZs in animal-derived food with good sensitivity and accuracy [25–28]. However, the varieties of drugs involved for the four methods (the largest numbers of SAs, QNs and BZs in the four methods are 8, 5 and 5, respectively) are too few and the metabolites of BZs have not been included. Recently, Li et al. have developed a LC–MS/MS confirmatory method in chicken liver with 39 drugs (12 SAs, 19 QNs and 8 BZs), but the sensitivity (the limits of quantification were 10 μg/kg for 39 drugs) is poor for determination of some of BZs (0.50 MRL of febantel, fenbendazole and oxfendazole is 5.0 μg/kg) in bovine milk [29]. So, it is a challenge to develop an LC–MS/MS confirmatory method, which can simultaneously determine SAs, QNs and BZs over 40 drugs with high sensitivity.

The aim of the method reported here was to overcome the disadvantages mentioned above and develops a confirmative and quantitative method for the determination of 46 drugs from SAs, QNs and BZs in bovine milk. For the clean-up of milk extracts, a simple solid phase extraction (SPE) had been established based on the SPE method described by Xia et al. for BZs [15]. After optimization, the method can simultaneous determine 18 SAs, 11 QNs, 9 BZs and 8 metabolites of BZs in bovine milk. Finally, the method was validated according to Commission Decision 2002/657/EC. Validation parameters tested were specificity, CCα, CCβ, recovery, precision, linearity and stability.

Table 1
LC gradient elution program.

Time (min)	*A* (%)	*B* (%)
0	90	10
1.0	90	10
2.5	80	20
8.0	60	40
10	10	90
11	10	90
11.1	90	10
13	90	10

2. Materials and methods

2.1. Materials and reagents

Methanol (LC grade) and acetonitrile (LC grade) were obtained from Fisher Chemicals (Fairlawn, NJ, USA). Formic acid (LC grade) was obtained from Tedia Company Inc (Fairfield, CA, USA). Hydrochloric acid, ammonium hydroxide and ammonium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sulfacetamide (SA), sulfapyridine (SPY), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamerazine (SMR), sulfamethoxazole (SMZ), sulfamoxole (SMX), sulfisoxazole (SIX), sulfamethizole (SMT), sulfamethazine (SM_2), sulfamethoxypyridazine (SMP), sulfamonomethoxine (SMM), sulfameter (SM), sulfachloropyridazine (SCP), sulfadoxine (SDX), sulfaquinoxaline (SQX), sulfadimethoxine (SDM), sulfaphenazole (SPZ), ofloxacin (OFL), norfloxacin (NOR), enoxacin (ENO), ciprofloxacin (CIF), enrofloxacin (ENR), lomefloxacin (LOM), danofloxacin (DAN), sarafloxacin (SAR), sparfloxacin (SPA), pefloxacin (PEF), difloxacin (DIF), 5-hydroxythiabendazole (5-OH-THI), albendazole

Table 2
LC–ESI-MS/MS parameters for 46 analytes and isotope internal standards.

Analyte	Precursor ion (*m*/*z*)	Daughter ion (*m*/*z*)	Dwell time (s)	Collision energy (eV)	Cone voltage (V)	Analyte	Precursor ion (*m*/*z*)	Daughter ion (*m*/*z*)	Dwell time (s)	Collision energy (eV)	Cone voltage (V)
SA	215.2	108.0, 156.0*	0.10, 0.10	20, 10	18	NOR	320.3	205.0, 233.1*	0.10, 0.10	32, 24	28
SPY	250.2	108.0, 156.0*	0.10, 0.10	24, 16	34	CIF	332.3	231.2*, 245.2	0.10, 0.10	30, 24	18
SDZ	251.2	108.0, 156.0*	0.10, 0.10	20, 14	28	LOM	352.3	237.1, 265.2*	0.10, 0.10	34, 22	18
STZ	256.2	108.0, 156.0*	0.10, 0.10	24, 14	28	ENR	360.3	203.4, 245.2*	0.10, 0.10	36, 26	12
SMR	265.2	156.0*, 172.0	0.10, 0.10	16, 14	30	SAR	386.3	299.2, 342.1	0.10, 0.10	28, 20	36
SMZ	254.2	108.0, 156.0*	0.10, 0.10	22, 16	28	DIF	400.4	299.2*, 256.1	0.10, 0.10	26, 46	38
SMX	268.2	156.0*, 113.0	0.10, 0.10	16, 18	30	ENO	321.3	234.1, 303.2*	0.10, 0.10	20, 25	34
SIX	268.2	113.1, 156.0*	0.10, 0.10	12, 14	26	PEF	334.3	204.9, 233.1*	0.10, 0.10	34, 24	30
SMT	271.2	108.0, 156.0*	0.10, 0.10	20, 14	26	OFL	362.3	205.0, 261.2*	0.10, 0.10	42, 27	16
SM_2	279.2	156.0*, 186.0	0.10, 0.10	18, 18	30	SPA	393.3	251.2*. 292.2	0.10, 0.10	30, 24	14
SMP SM SMM	281.2	126.0, 156.0*	0.10, 0.10	20, 18	30	DAN	358.3	255.1*, 283.2	0.10, 0.10	42, 24	26
SCP	285.1	108.0, 156.0*	0.10, 0.10	24, 14	22	NOR-D_5	325.4	238.2	0.10	24	32
SDX SDM	311.2	108.0, 156.0*	0.10, 0.10	28, 20	38	ABZ-SO_2	298.2	159.0, 224.0	0.10, 0.10	36, 26	36
SQX	301.2	108.0, 156.0*	0.25, 0.25	22, 14	34	PAR	248.3	173.0, 216.1	0.10, 0.10	32, 20	24
SPZ	315.2	156.0*, 158.0	0.10, 0.10	20, 32	40	ABZ	266.2	191.2, 234.1	0.10, 0.10	32, 18	38
SM_2-$^{13}C_6$	285.2	162.0	0.10	18	32	MEB	296.3	105.0, 264.1*	0.10, 0.10	34, 22	40
THI	202.1	131.1, 175.1*	0.10, 0.10	30, 24	48	FEN	300.3	159.0, 268.1*	0.10, 0.10	34, 22	40
THI-D4	206.1	179.1	0.10	24	48	FLU	314.3	123.0, 282.2*	0.10, 0.10	34, 22	38
5-OH-THI	218.2	147.0, 190.9*	0.10, 0.10	32, 24	48	FBT	447.4	383.1*, 415.1	0.10, 0.10	18, 12	30
ABZ-NH_2-SO_2	240.2	133.1*, 198.3	0.10, 0.10	28, 18	36	OXI	250.2	176.1, 218.1*	0.10, 0.10	28, 18	36
NH_2-MEB	238.2	105.0*, 132.9	0.10, 0.10	24, 34	48	FLU-NH_2	256.2	95.0, 123.0*	0.10, 0.10	36, 26	52
ABZ-SO	282.3	159.1*, 208.0	0.10, 0.10	36, 24	28	5-OH-MEB	298.3	220.1, 266.1*	0.10, 0.10	42, 22	38
OXF	316.3	191.1*, 284.1	0.10, 0.10	20, 18	42	OXF-SO	332.2	159.0*, 300.0	0.10, 0.10	38, 22	40
OXF-D_3	319.3	194.1	0.10	22	38	–	–	–	–	–	–

* Ion for quantification