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High-Throughput Determination of 30 Veterinary Drug Residues in Milk Powder by Dispersive Solid-Phase Extraction Coupled with Ultra-High Performance Liquid Chromatography Tandem Mass Spectrometry

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Abstract A high-throughput method was established for the simultaneous determination of 30 β-agonists, fluoroquinolones, and cephalosporins in milk powder by dispersive solid-phase extraction (dSPE) and ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Samples were firstly dissolved in sodium acetate buffer, then extracted with formic acid-acetonitrile, and subsequently purified by dSPE. Thirty target analytes were separated on a C18 column by gradient elution, detected through electrospray ionization (ESI) source in the positive mode with multi-reaction monitoring (MRM) conditions. The developed method was validated in terms of linearity, accuracy, and precision. Results indicated that 30 target analytes displayed excellent linearity in their corresponding concentration ranges and that their correlation coefficients were all higher than 0.995. The limits of quantitation (LOQs) for these targets were in the range of 0.7–7.0 μg/kg. The mean recoveries for negative sample spiked at three concentration levels were calculated between 80.0 and 97.5% with the relative standard deviation (RSD, $n = 6$) values ranging from 2.8 to 10.8%. In addition, the inter-day precision ($n = 5$) of the second spiked concentration was less than 13% (4.4–12.4%). The established and validated method is accurate and rapid and suitable for the high-throughput analysis of β-agonist, fluoroquinolone, and cephalosporin multi-residues in milk powder.

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Introduction

Veterinary drugs are commonly used at therapeutic levels in livestock breeding for disease resistance and growth promotion (Chen et al. 2016; Dasenaki and Thomaidis 2015). The widespread use of drugs in animal husbandry may result in the presence of drug residues in animal-derived foods as one of the key issues for food safety, which has aroused great public concern. As a consequence of the veterinary drug residues in food, the human health is highly threatened (Mauro et al. 2014; Rico and Van den Brink 2014). Therefore, sensitive and reliable analytical methods to determine the veterinary drug and pharmaceutical residues in animal's original food are extremely needed to ensure consumers' safety.

The analytical methods of β-agonist (Chu, Zheng, Qu, Geng, and Kang 2017; Wang, Liu, Su, and Zhu 2015), fluoroquinolone (Aufartová, Brabcová, Torres-Padrón, Solich, Sosa-Ferrera, and Santana-Rodríguez 2017; de Oliveira et al. 2016; Denadai and Cass 2015), and cephalosporin (Bousova, Senyuva, and Mittendorf 2013; Chiesa et al. 2015; Li, Shen, Hong, Zhang, Yuan, and Zhang 2016; Liu, Yu, Zhao, Zhang, Li, and Duan 2014) residues in animal-derived foods have been well-established. However, these methods normally focus on the specific groups of residue determination which are not suitable for the extensive multi-residue analysis. In addition, to the best of our knowledge, few research studies (Zhu et al. 2016) have been reported to investigate the concentrations of these veterinary drug residues in the matrix of dairy products such as milk powder. Therefore, establishing a

sensitive and high-throughput method for the simultaneous determination of β-agonist, fluoroquinolone, and cephalosporin residues in milk powder is beneficial to improve the detection efficiency and reduce the detection cost and can provide technical support for the monitoring and control of veterinary drug residues in milk powder even in other dairy products.

Liquid chromatography–mass spectrometry (LC-MS) technique provides a universal approach applicable to determine the extensive veterinary drugs. Especially, liquid chromatography tandem mass spectrometry (LC-MS/MS) plays a dominant role in the field of veterinary drug analysis in food stuffs because it could provide an unambiguous identification and a reliable confirmation of target substances (Jia, Chu, Chang, Wang, Chen, and Zhang 2017; Jin et al. 2017; Masiá, Suarez-Varela, Llopis-Gonzalez, and Picó 2016; Zhan et al. 2013). It has been reported that MS/MS is widely used in the detection field than MS as it has better selectivity, qualitative ability, and anti-interference ability (Dong, Guo, Xian, Luo, Wang, and Wu 2015; Dong and Xiao 2017). Hence, ultra-high performance liquid chromatography tandem mass spectrometry was adopted in the present work for the determination of veterinary drug residues.

It has been reported that milk powder is a very complex matrix which is abundant in protein and lipids (Guo, Mu, Xian, Luo, and Wang 2016; Xian, Dong, Wu, Guo, Hou, and Wang 2016). In order to precipitate protein and remove lipids so as to reduce the interference of impurities in the UPLC-MS/MS analysis, appropriate extraction and purification processes are extremely required. In previous literatures, microwave-assisted extraction (Aufartová, Brabcová, Torres-Padrón, Solich, Sosa-Ferrera, and Santana-Rodríguez 2017) and solid-phase extraction (Masiá, Suarez-Varela, Llopis-Gonzalez, and Picó 2016) are the commonly used purification processes in the pretreatment of sample. However, these methods have disadvantages such as complicated operation, consumption of large amount of organic solvent, high running cost, and long analyzing time. Recently, dispersive solid-phase extraction (dSPE), which is also known as QuEChERS, has drawn more and more attention due to its quick, easy, cheap, effective, rugged, and safe advantages (Khezeli and Daneshfar 2017). The solid sorbent used in dSPE is added directly to a sample solution without processes of sample manipulation such as conditioning, so this procedure relies only on the shaking and centrifugation (Anastassiades et al. 2003). QuEChERS has firstly been developed for analyzing the pesticide residues in fruits and vegetables and has now been widely used for composition analysis in dairy products, soy sauce, and other complex food matrix (Anastassiades et al. 2003; Dong et al. 2016; Dong and Xiao 2017; Luo, Dong, Luo, Xian, Guo, and Wu 2016; Luo et al. 2015; Wu et al. 2015; Xian, Dong, Wu, Guo, Hou, and Wang 2016; Zeng, Bai, Xian, Dong, and Luo 2016). Hence, dSPE procedure was employed in the clean-up process for milk powder samples before UPLC-MS/MS analysis.

The aim of the present study was to develop a sensitive and high-throughput dSPE-UPLC-MS/MS method for the simultaneous determination of 30 β-agonists, fluoroquinolones, and cephalosporins in milk powder. The method was also validated in terms of linearity, accuracy, and precision. Moreover, this developed and validated method was successfully applied to detect 30 veterinary drug residues in real commercial milk powder, confirming its applicability in veterinary drug residue detection. The method is beneficial for monitoring veterinary drug residues in milk powder and even other dairy products.

Materials and Methods

Chemicals and Reagents

Methanol, acetonitrile, and formic acid of chromatographically pure grade were purchased from Merck (Darmstadt, Germany). C18 and PSA were obtained from CNW Technologies GmbH (Kolner Landstrasse, Germany). Acetic acid, sodium acetate, and anhydrous magnesium sulfate were of analytical grade and purchased from Guangzhou Chemical Reagent Factory. Ultra-pure water (18.2 MΩ cm) was laboratory made.

Veterinary drugs and pharmaceuticals used in this study consisted of 20 kinds of β-agonists (Phenylethanolamine A, Terbutaline, Orciprenaline, Dopamine, Salbutamol, Ractopamine, Isoxsuprine, Clorprenaline, Carbuterol, Tulobuterol, Cimaterol, Clenproperol, Cimbuterol, Bromchlorbuterol, Bromobuterol, Mabuterol, Mapentrol, Procaterol, Fenoterol, and Penbutolol), five fluoroquinolones (Norfloxacin, Ciprofloxacin, Marbofloxacin, Orbifloxacin, and Enrofloxacin), and five cephalosporins (Cephapiriin, Cefazolin, Cefalonium, Ceftiofur, and Cefoperazone). All veterinary drug and pharmaceutical standards were of high-purity grade (>97%) and purchased from Dr. Ehrenstorfer Company, Germany.

Thirty standards were accurately weighed and dissolved in methanol respectively to obtain the individual standard stock solution and subsequently preserved at −18 °C. Afterwards, the mixed standard solution of 2.0 mg/L was prepared by diluting these standard stock solutions with methanol and then stored at 4 °C. It was diluted with the initial mobile phase to the required concentration of mixed standard working solution before use.

Instrumentation

The ACQUITY™ ultra-high performance liquid chromatography and Waters Xevo™ TQ tandem triple quadrupole mass spectrometer (UPLC-MS/MS, Waters Co., USA) were used for sample analysis. The dairy product samples were vortex-

mixed with a MS3 basic vortex mixer (IKA GmbH, Germany). The 5418 high speed centrifuge (Eppendorf Corp., Germany) was applied to centrifuged sample solution. Milli-Q Gradient A10 system used in the present work was purchased from Millipore Corp., Bedford, USA.

Sample Preparation

Approximately 1.00 g of milk powder sample was accurately weighed into a 50-mL plastic centrifuge tube. A volume of 8 mL 0.2 mol/L acetic acid-sodium acetate buffer solution (pH = 5.2, 40–50 °C) was then added into the tube, and the powder was uniformly dissolved by vortexing. After cooling to room temperature, 15 mL of 0.1% formic acid-acetonitrile (*v*/*v*) was added into the tube and mixed by vortex shaking for 3 min. The mixed sample solution was left to stand for 5–10 min at −18 °C and subsequently centrifuged at 10,000 r/min in the condition of 10 °C for 3 min. After that, the liquid supernatant was transferred into another 50-mL plastic centrifuge tube. Afterwards, 200 mg C18, 50 mg PSA, and 300 mg $MgSO_4$ were added into the latter tube and mixed by vortex shaking for 3 min. After standing for 5 min and centrifuging at 10,000 r/min for 3 min, the liquid supernatant was dried to near dryness by a mild nitrogen stream in a 40 °C water bath. After that, it was fixed to a volume of 1.0 mL with the initial mobile phase. Finally, the liquid was mixed by vortex shaking and filtered through a 0.22-μm membrane before UPLC-MS/MS analysis.

UPLC-MS/MS Conditions

UPLC conditions were described as follows: A BEH C18 chromatographic column (100 mm × 2.1 mm, 1.7 μm) was used at 30 °C for the chromatographic separation of target analytes. The column was used at a constant flow rate of 0.3 mL/min. The mobile phase consisted of acetonitrile (A) and 0.1% (*v*/*v*) formic acid water (B) was finally used. The gradient elution program was performed as follows: 0.0–1.0 min, 95% B; 1.0–3.0 min, 95–70% B; 3.0–6.5 min, 70–5% B; 6.5–8.0 min, 5% B; 8. 0–8.1 min, 5–95% B; 8.1–10.0 min, 95% B. The injection volume was 5.0 μL.

MS/MS conditions included the following: Electrospray ionization (ESI) source in the positive mode with multi-reaction monitoring (MRM) conditions was used for detection. The capillary voltage was 1.0 kV. The ion source temperature and desolvation gas temperature were 150 and 400 °C, respectively. The flow rates of the desolvation gas (nitrogen), cone gas (nitrogen), and collision gas (high-purity argon) were set at 800 L/H, 50 L/H, and 0.2 mL/min, respectively. The specific parameters for the 30 target analytes including the MRM confirmation transitions, the cone voltage, and collision energy were shown in Table 1. The dwell time of each ion pair was 0.01 s.

Statistical Analysis

Statistical analysis was performed by using the SPSS (SPSS Inc., Chicago, IL, USA). Significance was determined at $p < 0.05$ by analysis of variance (ANOVA) followed by Duncan's least significant test.

Results and Discussion

Optimization of Extraction Conditions

Metabolic studies of β-agonists have shown that β-agonists have a high proportion of conjugates in animal urine, bile, liver, and kidney. Generally, β-agonists give priority to prototypical drugs in muscle and milk (Masiá, Suarez-Varela, Llopis-Gonzalez, and Picó 2016). Yang et al. determined endogenous hormones in muscle and milk under the condition of enzymatic hydrolysis and non-enzymatic hydrolysis, respectively and results indicated no difference among them (Yang, Shao, Zhang, Wu, and Duan 2009). In the present study, a milk powder sample containing clenbuterol was determined in both enzymatic and non-enzymatic conditions. Results indicated that the relative deviation ($n = 6$) of the two conditions was less than 10% with no significant difference. Hence, non-enzymatic hydrolysis was applied in the sample pretreatment of this method in order to simplify the pretreatment step and achieve rapid determination.

Commonly, it is important and necessary to choose an appropriate extraction solution to achieve the simultaneous extraction of target compounds in the complex matrix of dairy products (Xian, Dong, Wu, Guo, Hou, and Wang 2016). It is well-known that acetonitrile has good versatility and plays a key role in precipitating protein (Dang et al. 2017; Dong, Guo, Xian, Luo, Wang, and Wu 2015; Wu, Xu, Li, Guo, Xian, and Dong 2016b; Zeng, Bai, Xian, Dong, and Luo 2016). The impurities of lipid extraction are relatively few when acetonitrile is considered as extracting agent. Moreover, acetonitrile can be separated from the aqueous phase by salting-out process so that the strong polarity substances such as salt substances and pigments are retained in the aqueous phase (Dong, Guo, Xian, Luo, Wang, and Wu 2015; Xian, Dong, Wu, Guo, Hou, and Wang 2016). All these advantages are conductive to the follow-up purification and concentration. Therefore, acetonitrile was chosen as the extraction solvent and the sodium acetate buffer solution was selected to dissolve milk powder samples as salt solution is conducive to salting-out stratification with acetonitrile.

In the meantime, we also investigated the effect of acetonitrile and 0.1, 0.2, 0.5, and 1% (*v*/*v*) of formic acid-acetonitrile as extracting agent on the extraction efficiency of target analytes in negative milk powder spiked in the concentration of 50 μg/kg. The absolute recoveries were

Table 1 The specific parameters of 30 target compounds in MS/MS analysis

No.	Compound	Formula	Confirmation ion (m/z)	Cone voltage (V)	Collision energy (eV)
1	Dopamine	$C_8H_{11}NO_2$	154/91, 154/137[a]	15	20, 10
2	Salbutamol	$C_{13}H_{21}NO_3$	240/148[a], 240/222	20	20, 10
3	Terbutaline	$C_{12}H_{19}NO_3$	226/125, 226/152[a]	20	25, 15
4	Cimaterol	$C_{12}H_{17}N_3O$	220/160[a], 220/202	15	18, 10
5	Procaterol	$C_{16}H_{22}N_2O_3$	291/231, 291/273[a]	20	20, 15
6	Cimbuterol	$C_{13}H_{19}N_3O$	234/160[a], 234/216	15	15, 10
7	Fenoterol	$C_{17}H_{21}NO_4$	304/107[a], 304/135	25	35, 20
8	Clorprenaline	$C_{11}H_{16}ClNO$	214/154[a], 214/196	20	17, 15
9	Clenbuterol	$C_{12}H_{18}Cl_2N_2O$	277/203[a], 277/259	15	20, 15
10	Tulobuterol	$C_{12}H_{18}ClNO$	228/154[a], 228/172	20	15, 12
11	Bromchlorbuterol	$C_{12}H_{18}BrClN_2O$	323/249[a], 323/305	20	20, 15
12	Brombuterol	$C_{12}H_{18}Br_2N_2O$	367/293[a], 367/349	20	20, 15
13	Mabuterol	$C_{13}H_{18}ClF_3N_2O$	311/217, 311/237[a]	20	25, 15
14	Mapenterol	$C_{14}H_{20}ClF_3N_2O$	325/217, 325/237[a]	20	25, 15
15	Phenylethanolamine A	$C_{19}H_{24}N_2O_4$	345/150[a], 345/327	15	20, 15
16	Ractopamine	$C_{18}H_{23}NO_3$	302/107[a], 302/164	20	35, 20
17	Orciprenaline	$C_{11}H_{17}NO_3$	212/152[a], 212/194	20	17, 15
18	Isoxsuprine	$C_{18}H_{23}NO_3$	302/107[a], 302/284	20	15, 30
19	Carbuterol	$C_{13}H_{21}N_3O_3$	268/94, 268/134[a]	20	15, 25
20	Penbutolol	$C_{18}H_{29}NO_2$	292/133, 292/236[a]	25	25, 15
21	Norfloxacin	$C_{16}H_{18}FN_3O_3$	320/302[a], 320/276	30	20, 15
22	Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	332/231, 332/314[a]	25	20, 10
23	Marbofloxacin	$C_{17}H_{19}FN_4O_4$	363/72[a], 363/345	25	20, 20
24	Orbifloxacin	$C_{19}H_{20}F_3N_3O_3$	396/295[a], 396/352	30	25, 20
25	Enrofloxacin	$C_{19}H_{22}FN_3O_3$	360/245, 360/316[a]	20	20, 10
26	Cephapiriin	$C_{17}H_{17}N_3O_6S_2$	424/152[a], 424/292	20	25, 15
27	Cefazolin	$C_{14}H_{14}N_8O_4S_3$	455/156[a], 455/324	20	20, 10
28	Cefalonium	$C_{20}H_{18}N_4O_5S_2$	459/152[a], 459/337	15	20, 10
29	Ceftiofur	$C_{19}H_{17}N_5O_7S_3$	524/210, 524/241[a]	25	20, 15
30	Cefoperazone	$C_{25}H_{27}N_9O_8S_2$	646/143[a], 646/530	15	35, 15

[a] Transitions for quantification

calculated from the corresponding standard solutions. As displayed in Fig. 1, the extraction efficiency of β-agonists, fluoroquinolones, and cephalosporins was improved by adding formic acid into acetonitrile. Comprehensively, 0.1% of formic acid-acetonitrile was selected as extraction solvent for the next step, and the extraction efficiency of targets was ranging from 86 to 104%.

Optimization of Purification Conditions

Milk powder is a kind of complex matrix which is rich in protein, fat, carbohydrates, minerals and vitamins, and other nutrients (Xian, Dong, Wu, Guo, Hou, and Wang 2016). A large amount of impurities in extraction solution could not only contaminate the instrument but also cause a strong matrix effect and further affect the accurate quantification. Dispersive solid-phase extraction (dSPE) method is a kind of purification process that possesses rapid, simple, and efficient advantages. It was proposed by the Department of Agriculture of the United States in 2003 and used for pretreatment (Anastassiades et al. 2003). The dSPE process has been widely applied for recent years due to its advantages. Commonly, C18 as one solid-phase dispersant could adsorb lipophilic and non-polar impurities. PSA can adsorb organic acid impurities, while $MgSO_4$ can absorb water and reduce the solubility of water-soluble impurities in organic extracts (Xian, Dong, Wu, Guo, Hou, and Wang 2016). Based on dSPE purification, we investigated the effect of different combinations of C18, PSA, and $MgSO_4$ on the purification of the matrix standard solutions (25 μg/L) prepared with the negative milk powder extraction solution. The concrete combinations were as follows: A 100 mg C18 and 50 mg PSA; B 100 mg C18, 50 mg PSA, and 200 mg $MgSO_4$; C 100 mg C18, 50 mg

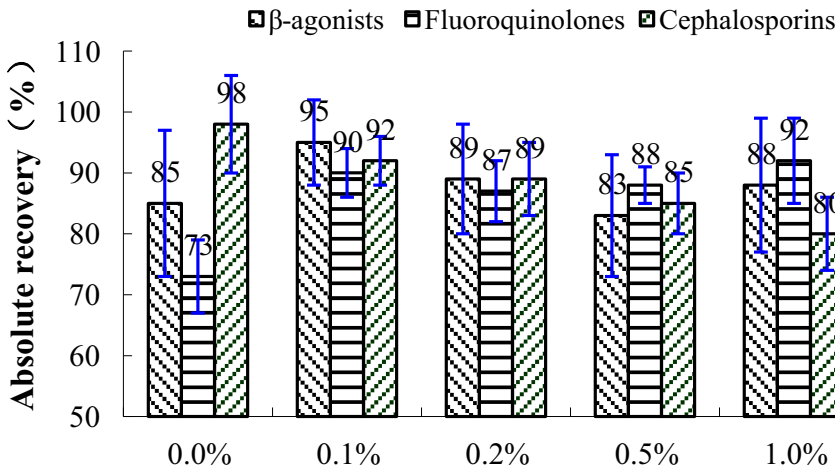

Fig. 1 The effect of different extraction solvents on the extraction efficiency of target compounds

PSA, and 300 mg $MgSO_4$; D 200 mg C18, 50 mg PSA, and 200 mg $MgSO_4$; E 200 mg C18, 50 mg PSA, and 300 mg $MgSO_4$. The absolute recoveries were calculated as the comparison of the purified standard solution and the 25 μg/L pure solvent standard solution. As depicted in Fig. 2, E group (200 mg C18, 50 mg PSA, and 300 mg $MgSO_4$) showed the best purification effect with the absolute recoveries ranging from 89 to 93%. Therefore, the dispersive solid-phase extraction packings of the E group were selected for the purification in the study.

Optimization of Instrumental Analysis Conditions

According to the chemical structures of the target compounds, ESI-positive switching mode was adopted to analyze the target compounds in the present work. The MS was adopted to scan the target compounds, and the capillary voltage and cone voltage were optimized in order to obtain the highest $[M + H]^+$ peak for each compound. Afterwards, MS/MS was applied to the precursor ions in order to obtain the product ions for each compound. The MS/MS spectra showed that the fragmentation pattern of the cephalosporin included the neutral loss of NH_2COOH and CH_3COOH which is caused by the C–O bond breakage, as well as the absence of thiazine ring and side chain in the β-lactam ring. Fluoroquinolones mainly contained dehydration peak, decarboxylation, and the ions produced by the C_2H_4NR absence in the rearrangement of piperazine ring fracture after the decarboxylation. Meantime, MS/MS spectra of the β-agonists displayed the neutral lost fragments in the groups associated with hydroxyl, tert-butyl, isopropyl, and isopropylamino, resulting in the fragment ions such as $[M + H\text{-}18]^+$, $[M + H\text{-}56]^+$, and $[M + H\text{-}74]^+$. Two MRM confirmation transitions were selected as the qualitative and quantitative transitions for each target compound. The collision voltage was optimized for each product ion and was also shown in Table 1.

The elution effect of acetonitrile is stronger than that of methanol, and adding the appropriate concentration of formic acid in the water phase in the ESI^+ mode could enhance the efficiency of ionization. In the present work, the BEH C18 column (100 mm × 2.1 mm, 1.7 μm) was used and 0.1% acetonitrile-formic acid was selected as the mobile phase. Symmetrical peak shapes of the target compounds and favorable response values and retention time of the 30 target compounds were obtained by optimizing the elution gradient. The MRM chromatograms of the 30 target compounds under the optimized instrumental conditions are displayed in Fig. 3. The representative substance of each peak is listed in Table 1.

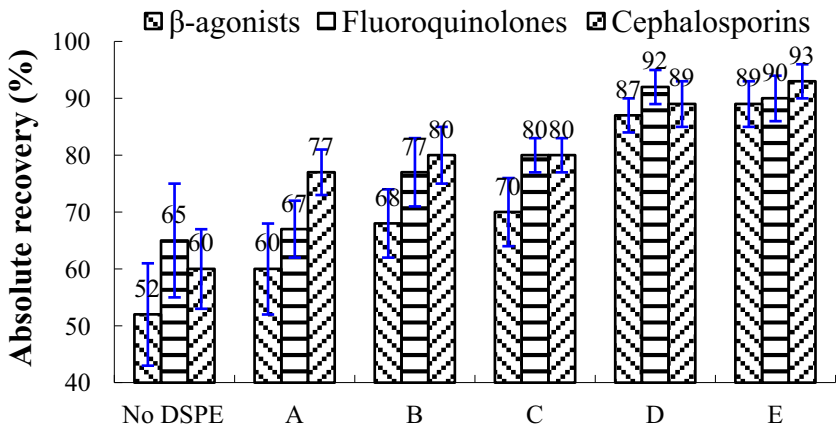

Fig. 2 The effect of different dispersive solid-phase extraction packings on the purification of targets

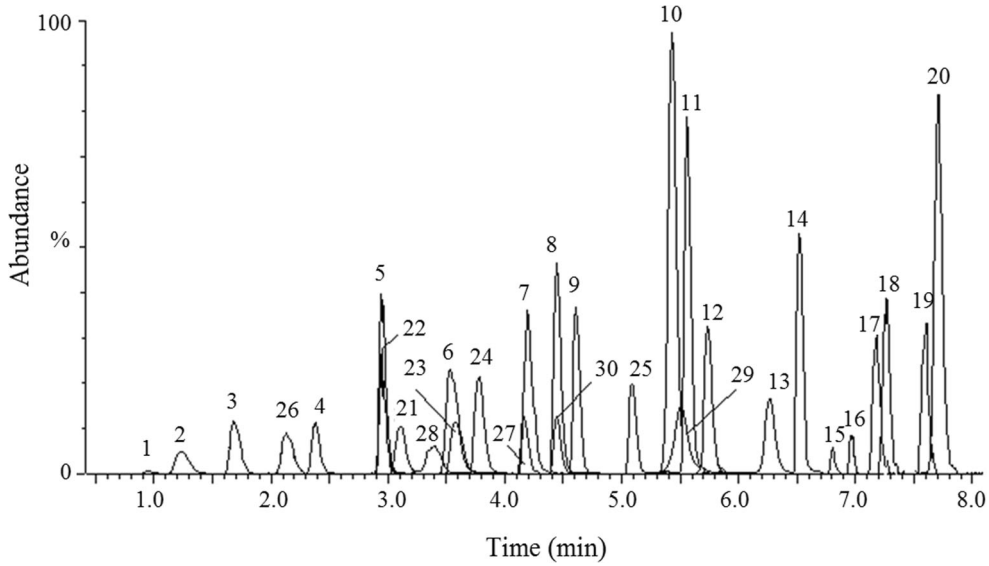

Fig. 3 The MRM chromatograms of 30 target compounds under the optimized instrumental conditions (the representative substance of each peak is listed in Table 1)

Method Validation

Method Specificity

To explore the presence of interference of the impurity compositions in the samples, 20 negative samples were pretreated and detected according to the pretreatment method and instrumental conditions of the developed method. The results demonstrated that impurity compositions in the samples had no interference effects on the quantitative and qualitative analyses of the target compounds because of the high selectivity of the triple quadrupole mass spectrometry, suggesting that the specificity of the established method was favorable.

Linearity, ILOQs, MLOQs, and Matrix Effects

Based on the matrix effect confirmation method established by Matuszewski and Chavez-Eng (2003), a series of concentrations (0.5, 1.0, 5.0, 20.0, 50.0, 100.0, 200.0, and 500.0 μg/L) of pure solvent standard working solutions and matrix calibration standard working solutions were prepared with the negative sample extraction solution and pure solvent. According to the instrumental condition in this work, the matrix calibration curve and standard curve were obtained by plotting the ratios of each target compound quantitative ion peak area (y) versus the corresponding mass concentrations (x, μg/L). The matrix effect (ME) was identified by the slope value of the matrix calibration working curve versus that of the pure solvent standard working curve. ME < 1 represents matrix suppression while ME > 1 indicates matrix enhancement (Dong, Guo, Xian, Luo, Wang, and Wu 2015; Wu, Xu, Li, Guo, Xian, and Dong 2016a, 2016b). The limit of detection (LOD) and limit of quantification (LOQ) of the established method refer to the triple signal-to-noise ratio ($S/N = 3$) and tenfold signal-to-noise ratio ($S/N = 10$), respectively (Wu, Xu, Li, Guo, Xian, and Dong 2016b). The instrument LOD (ILOD) and instrument LOQ (ILOQ) were measured by the standard solution with the pure solvent, while the method LOD (MLOD) and method LOQ (MLOQ) were determined by calibration matrix solution.

Results found that when the concentrations for dopamine and cephalosporins were in the range of 5.0–500.0 μg/L, the remaining β-agonists and fluoroquinolones were 0.5–200.0 μg/L, both the correlation coefficient of pure solvent standard curve and matrix calibration curve were more than 0.995 (Table 2), presenting a good linear relationship between the quantitative ion peak areas and analytic concentrations. The ILOQs and MLOQs for the 30 target compounds were in the range of 0.5–5.0 μg/L and 0.7–7.0 μg/kg, respectively (Table 2), indicating the high sensitivity of the established method. The values of ME ranged from 0.82 to 1.65. Some of β-agonists exhibited matrix-enhancing effects, while only individuals among fluoroquinolones and cephalosporins had a slight matrix effect. Hence, it is recommended to perform the primary screening in the practical test, and the matrix-matching calibration solution is suggested to use for quantification for the compounds with obvious matrix effect.

Accuracy and Precision

The methodological indicators including recovery, accuracy, and precision were investigated by the addition of the negative sample recovery test ($n = 6$). Concretely, three concentration levels of the mixed standard solutions were added into the negative samples. Subsequently, the samples were processed and measured under the experimental conditions. Each added level contained six parallel experiments, so as to investigate the recovery and intra-day precision. The recovery was calculated on the foundation of a matrix-matched calibration solution. As for the middle added level, the experiment was continuously conducted for 5 days to determine the inter-day precision ($n = 5$). As presented in Table 3, the results indicated that within the added

Table 2 Linear equations of pure solvent standard curve and matrix calibration curve, correlation coefficients, matrix effects, and ILODs and MLOQs for 30 target compounds

No.	Compound	Solvent, *r*	Matrix, *r*	Matrix effect	ILOQ (μg/L)	MLOQ (μg/kg)
1	Dopamine	$y = 307.4x + 118.2$, 0.9951	$y = 482.6x + 103.0$, 0.9964	1.57	5.0	7.0
2	Salbutamol	$y = 1718.2x + 470.1$, 0.9985	$y = 2250.8x + 398.5$, 0.9980	1.31	0.5	0.7
3	Terbutaline	$y = 1005.3x + 378.7$, 0.9990	$y = 975.3x + 346.8$, 0.9981	0.97	0.5	0.7
4	Cimaterol	$y = 1257.8x + 390.6$, 0.9993	$y = 1698.0x + 409.2$, 0.9989	1.35	0.5	0.7
5	Procaterol	$y = 475.3x + 202.7$, 0.9987	$y = 708.2x + 231.9$, 0.9992	1.49	0.5	0.8
6	Cimbuterol	$y = 1411.6x + 568.8$, 0.9989	$y = 1764.5x + 503.0$, 0.9983	1.25	0.5	0.7
7	Fenoterol	$y = 428.8x + 180.8$, 0.9972	$y = 707.8x + 112.7$, 0.9984	1.65	0.5	0.8
8	Clorprenaline	$y = 3042.3x + 711.4$, 0.9994	$y = 3650.7x + 651.0$, 0.9988	1.20	0.5	0.7
9	Clenbuterol	$y = 893.5x + 290.1$, 0.9993	$y = 1295.6x + 300.8$, 0.9990	1.45	0.5	0.7
10	Tulobuterol	$y = 3771.1x + 882.4$, 0.9995	$y = 3394.0x + 801.7$, 0.9987	0.90	0.5	0.7
11	Bromchlorbuterol	$y = 1801.2x + 526.7$, 0.9975	$y = 2107.4x + 498.7$, 0.9979	1.17	0.5	0.7
12	Brombuterol	$y = 1925.6x + 601.8$, 0.9981	$y = 2464.8x + 643.0$, 0.9977	1.28	0.5	0.7
13	Mabuterol	$y = 2173.5x + 700.5$, 0.9985	$y = 2890.7x + 614.6$, 0.9980	1.33	0.5	0.7
14	Mapenterol	$y = 3634.3x + 817.7$, 0.9990	$y = 3706.9x + 764.8$, 0.9986	1.02	0.5	0.7
15	Phenylethanolamine A	$y = 567.1x + 201.1$, 0.9980	$y = 612.5x + 241.0$, 0.9983	1.08	0.5	0.8
16	Ractopamine	$y = 942.3x + 281.3$, 0.9989	$y = 1206.1x + 309.6$, 0.9980	1.28	0.5	0.7
17	Orciprenaline	$y = 464.5x + 281.4$, 0.9983	$y = 664.7x + 207.1$, 0.9990	1.43	0.5	0.8
18	Isoxsuprine	$y = 1402.3x + 779.2$, 0.9992	$y = 1879.1x + 689.5$, 0.9995	1.34	0.5	0.7
19	Carbuterol	$y = 851.2x + 471.0$, 0.9987	$y = 1234.2x + 411.8$, 0.9991	1.45	0.5	0.7
20	Penbutolol	$y = 3266.8x + 860.7$, 0.9995	$y = 3103.5x + 784.2$, 0.9991	0.95	0.5	0.7
21	Norfloxacin	$y = 618.5x + 170.2$, 0.9977	$y = 760.8x + 256.9$, 0.9968	1.23	0.5	0.8
22	Ciprofloxacin	$y = 575.6x + 251.3$, 0.9984	$y = 788.6x + 349.7$, 0.9980	1.37	0.5	0.8
23	Marbofloxacin	$y = 891.6x + 250.3$, 0.9993	$y = 1150.2x + 338.4$, 0.9985	1.29	0.5	0.7
24	Orbifloxacin	$y = 1509.1x + 468.2$, 0.9990	$y = 1735.4x + 561.7$, 0.9993	1.15	0.5	0.7
25	Enrofloxacin	$y = 986.4x + 251.3$, 0.9995	$y = 1302.0x + 343.9$, 0.9978	1.32	0.5	0.7
26	Cephapiriin	$y = 377.4x + 118.5$, 0.9978	$y = 308.7x + 102.3$, 0.9962	0.82	5.0	6.5
27	Cefazolin	$y = 422.7x + 174.5$, 0.9984	$y = 393.1x + 128.0$, 0.9973	0.93	5.0	6.5
28	Cefalonium	$y = 407.1x + 165.2$, 0.9993	$y = 366.4x + 131.5$, 0.9990	0.90	5.0	7.0
29	Ceftiofur	$y = 523.4x + 211.3$, 0.9986	$y = 580.5x + 261.7$, 0.9992	1.11	5.0	6.5
30	Cefoperazone	$y = 457.8x + 226.1$, 0.9982	$y = 430.3x + 201.0$, 0.9987	0.94	5.0	7.0

Table 3 Recovery, accuracy, and precision of 30 target compounds

No.	Compound	Added (μg/kg)	Recovery (%, $n = 6$)	Intra-day precision (%, $n = 6$)	Inter-day precision (%, $n = 5$)
1	Dopamine	7.0, 14.0, 70.0	84.1, 85.9, 89.6	10.3, 7.9, 5.2	11.2
2	Salbutamol	1.0, 2.0, 10.0	86.0, 83.2, 87.7	8.3, 7.0, 5.5	9.5
3	Terbutaline	1.0, 2.0, 10.0	80.9, 93.3, 89.2	6.9, 4.4, 3.9	8.9
4	Cimaterol	1.0, 2.0, 10.0	81.2, 81.9, 84.2	8.3, 6.0, 4.9	10.0
5	Procaterol	1.0, 2.0, 10.0	90.0, 88.6, 94.1	6.3, 7.0, 5.2	9.8
6	Cimbuterol	1.0, 2.0, 10.0	82.2, 84.8, 85.9	5.4, 4.8, 6.2	7.5
7	Fenoterol	1.0, 2.0, 10.0	93.2, 85.9, 93.1	5.9, 6.1, 4.8	8.1
8	Clorprenaline	1.0, 2.0, 10.0	83.8, 82.6, 85.9	8.3, 5.9, 5.0	10.5
9	Clenbuterol	1.0, 2.0, 10.0	89.8, 87.0, 87.8	7.8, 8.1, 5.7	9.2
10	Tulobuterol	1.0, 2.0, 10.0	88.9, 95.4, 97.1	6.7, 5.9, 5.3	7.8
11	Bromchlorbuterol	1.0, 2.0, 10.0	90.8, 84.6, 88.0	5.0, 4.9, 4.1	6.5
12	Brombuterol	1.0, 2.0, 10.0	84.1, 85.3, 92.7	7.2, 6.5, 6.0	8.3
13	Mabuterol	1.0, 2.0, 10.0	93.3, 87.0, 92.2	4.0, 3.9, 4.2	5.8
14	Mapenterol	1.0, 2.0, 10.0	83.0, 87.2, 86.9	10.8, 9.2, 6.8	12.4
15	Phenylethanolamine A	1.0, 2.0, 10.0	92.7, 88.6, 87.3	6.1, 3.7, 2.8	7.1
16	Ractopamine	1.0, 2.0, 10.0	91.5, 96.0, 92.1	4.9, 4.3, 4.1	5.7
17	Orciprenaline	1.0, 2.0, 10.0	96.7, 85.5, 90.1	5.3, 5.0, 4.2	6.6
18	Isoxsuprine	1.0, 2.0, 10.0	91.3, 88.4, 87.5	5.0, 4.9, 5.3	6.2
19	Carbuterol	1.0, 2.0, 10.0	88.2, 90.4, 91.8	4.2, 3.8, 3.0	4.4
20	Penbutolol	1.0, 2.0, 10.0	85.5, 84.9, 86.4	5.2, 5.9, 4.4	8.2
21	Norfloxacin	1.0, 2.0, 10.0	95.0, 90.2, 93.3	9.8, 10.4, 6.5	11.3
22	Ciprofloxacin	1.0, 2.0, 10.0	87.1, 91.3, 94.5	5.1, 4.7, 3.2	6.7
23	Marbofloxacin	1.0, 2.0, 10.0	81.1, 83.5, 84.6	7.7, 5.7, 6.5	9.5
24	Orbifloxacin	1.0, 2.0, 10.0	85.0, 85.4, 88.9	8.8, 9.2, 6.5	10.4
25	Enrofloxacin	1.0, 2.0, 10.0	89.3, 92.2, 95.0	4.3, 7.8, 5.9	9.2
26	Cephapiriin	7.0, 14.0, 70.0	86.1, 84.7, 90.4	6.2, 5.5, 5.1	8.8
27	Cefazolin	7.0, 14.0, 70.0	82.0, 80.4, 85.7	5.8, 7.1, 4.9	8.4
28	Cefalonium	7.0, 14.0, 70.0	80.0, 81.7, 89.3	4.0, 4.9, 5.2	6.5
29	Ceftiofur	7.0, 14.0, 70.0	84.2, 89.0, 97.5	3.8, 5.3, 4.8	6.1
30	Cefoperazone	7.0, 14.0, 70.0	93.3, 85.8, 89.6	5.5, 4.5, 3.9	7.5

concentration range, the average recoveries, intra-day precision (n = 6), and inter-day precision (n = 5) of the 30 target compounds were respectively in the ranges of 80.0–97.5, 2.8–10.8, and 4.4–12.4%, suggesting this method had excellent recovery, accuracy, and precision.

Analysis of Practical Samples

The method established in this work was adopted to determine a total of 30 commercially available milk powder samples. Detection results found that none of the 30 target compounds were detected in these samples.

Conclusions

Based on the dispersive solid-phase extraction (dSPE) technology, a simple and sensitive analytical method, using the UPLC-MS/MS, was established for the simultaneous determination of 30 kinds of β-agonists, fluoroquinolones, and cephalosporins in milk powder. The optimal conditions were obtained by optimizing the pretreatment conditions and the parameters of the instrument. According to methodological indicators, the results indicated that this established method has good specificity, accuracy, intra-day precision, and inter-day precision. Moreover, the results obtained conform the suitability of the method proposed for the high-throughput quantitative and qualitative analyses of β-agonist, fluoroquinolone, and cephalosporin multi-residues in milk powder to improve the actual detection efficiency.

Compliance with Ethical Standards

Conflict of Interest Ruijiao Li declares that she has no conflict of interest. Moucheng Wu declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with animals performed by any of the authors.

Informed Consent Not applicable.

References

Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86(2):412–431

Aufartová J, Brabcová I, Torres-Padrón ME, Solich P, Sosa-Ferrera Z, Santana-Rodríguez JJ (2017) Determination of fluoroquinolones in fishes using microwave-assisted extraction combined with ultra-high performance liquid chromatography and fluorescence detection. J Food Compos Anal 56:140–146

Bousova K, Senyuva H, Mittendorf K (2013) Quantitative multi-residue method for determination antibiotics in chicken meat using turbulent flow chromatography coupled to liquid chromatography–tandem mass spectrometry. J Chromatogr A 1274:19–27

Chen D, Yu J, Tao Y, Pan Y, Xie S, Huang L, Peng D, Wang X, Wang Y, Liu Z, Yuan Z (2016) Qualitative screening of veterinary antimicrobial agents in tissues, milk, and eggs of food-producing animals using liquid chromatography coupled with tandem mass spectrometry. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 1017-1018:82–88

Chiesa L, Nobile M, Arioli F, Britti D, Trutic N, Pavlovic R, Panseri S (2015) Determination of veterinary antibiotics in bovine urine by liquid chromatography–tandem mass spectrometry. Food Chem 185:7–15

Chu L, Zheng S, Qu B, Geng S, Kang X (2017) Detection of β-agonists in pork tissue with novel electrospun nanofibers-based solid-phase extraction followed ultra-high performance liquid chromatography/tandem mass spectrometry. Food Chem 227:315–321

Dang H, Liu D, Hou X, Wu Y, Wang B, Dong H, Xian Y (2017) One-step extraction prior to two-step detection by ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) to determine different groups of antibacterial agents in infant disposable hygiene products. Anal Methods 9(3):482–489

Dasenaki ME, Thomaidis NS (2015) Multi-residue determination of 115 veterinary drugs and pharmaceutical residues in milk powder, butter, fish tissue and eggs using liquid chromatography–tandem mass spectrometry. Anal Chim Acta 880:103–121

de Oliveira HL, da Silva Anacleto S, da Silva ATM, Pereira AC, de Souza Borges W, Figueiredo EC, Borges KB (2016) Molecularly imprinted pipette-tip solid phase extraction for selective determination of fluoroquinolones in human urine using HPLC-DAD. J Chromatogr B 1033–1034:27–39

Denadai M, Cass QB (2015) Simultaneous determination of fluoroquinolones in environmental water by liquid chromatography–tandem mass spectrometry with direct injection: a green approach. J Chromatogr A 1418:177–184

Dong H, Guo X, Xian Y, Luo H, Wang B, Wu Y (2015) A salting out-acetonitrile homogeneous extraction coupled with gas chromatography–mass spectrometry method for the simultaneous determination of thirteen N-nitrosamines in skin care cosmetics. J Chromatogr A 1422:82–88

Dong H, Luo D, Xian Y, Luo H, Guo X, Li C, Zhao M (2016) Adulteration identification of commercial honey with the C-4 sugar content of negative values by an elemental analyzer and liquid chromatography coupled to isotope ratio mass spectroscopy. J Agric Food Chem 64(16):3258–3265

Dong H, Xiao K (2017) Modified QuEChERS combined with ultra high performance liquid chromatography tandem mass spectrometry to determine seven biogenic amines in Chinese traditional condiment soy sauce. Food Chem 229:502–508

Guo X, Mu T, Xian Y, Luo D, Wang C (2016) Ultra-performance liquid chromatography tandem mass spectrometry for the rapid simultaneous analysis of nine organophosphate esters in milk powder. Food Chem 196:673–681

Jia W, Chu X, Chang J, Wang PG, Chen Y, Zhang F (2017) High-throughput untargeted screening of veterinary drug residues and metabolites in tilapia using high resolution orbitrap mass spectrometry. Anal Chim Acta 957:29–39

Jin Y, Zhang J, Zhao W, Zhang W, Wang L, Zhou J, Li Y (2017) Development and validation of a multiclass method for the quantification of veterinary drug residues in honey and royal jelly by liquid chromatography–tandem mass spectrometry. Food Chem 221:1298–1307

Khezeli T, Daneshfar A (2017) Development of dispersive micro-solid phase extraction based on micro and nano sorbents. TrAC Trends Anal Chem 89:99–118

Li W, Shen H, Hong Y, Zhang Y, Yuan F, Zhang F (2016) Simultaneous determination of 22 cephalosporins drug residues in pork muscle using liquid chromatography–tandem mass spectrometry. J Chromatogr B 1022:298–307

Liu X, Yu Y, Zhao M, Zhang H, Li Y, Duan G (2014) Solid phase extraction using magnetic core mesoporous shell microspheres with C18-modified interior pore-walls for residue analysis of cephalosporins in milk by LC–MS/MS. Food Chem 150:206–212

Luo D, Dong H, Luo H, Xian Y, Guo X, Wu Y (2016) Multi-element (C, N, H, O) stable isotope ratio analysis for determining the geographical origin of pure milk from different regions. Food Anal Methods 9(2):437–442

Luo D, Dong H, Luo H, Xian Y, Wan J, Guo X, Wu Y (2015) The application of stable isotope ratio analysis to determine the geographical origin of wheat. Food Chem 174:197–201

Masiá A, Suarez-Varela MM, Llopis-Gonzalez A, Picó Y (2016) Determination of pesticides and veterinary drug residues in food by liquid chromatography-mass spectrometry: a review. Anal Chim Acta 936:40–61

Matuszewski BK, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 75(13):3019–3030

Mauro D, Ciardullo S, Civitareale C, Fiori M, Pastorelli AA, Stacchini P, Palleschi G (2014) Development and validation of a multi-residue method for determination of 18 β-agonists in bovine urine by UPLC–MS/MS. Microchem J 115:70–77

Rico A, Van den Brink PJ (2014) Probabilistic risk assessment of veterinary medicines applied to four major aquaculture species produced in Asia. Sci Total Environ 468–469:630–641

Wang P, Liu X, Su X, Zhu R (2015) Sensitive detection of β-agonists in pork tissue with novel molecularly imprinted polymer extraction followed liquid chromatography coupled tandem mass spectrometry detection. Food Chem 184:72–79

Wu Y, Luo D, Dong H, Wan J, Luo H, Xian Y, Guo X, Qin F, Han W, Wang L, Wang B (2015) Geographical origin of cereal grains based on element analyser-stable isotope ratio mass spectrometry (EA-SIRMS). Food Chem 174:553–557

Wu Z, Xu Y, Li M, Guo X, Xian Y, Dong H (2016a) Determination of three fluorescent whitening agents (FWAs) and their migration research in food contact plastic packaging containers and food simulants by UPLC-MS/MS method. RSC Adv 6(22):17941–17946

Wu Z, Xu Y, Li M, Guo X, Xian Y, Dong H (2016b) Simultaneous determination of fluorescent whitening agents (FWAs) and photoinitiators (PIs) in food packaging coated paper products by the UPLC-MS/MS method using ESI positive and negative switching modes. Anal Methods 8(5):1052–1059

Xian Y, Dong H, Wu Y, Guo X, Hou X, Wang B (2016) QuEChERS-based purification method coupled to ultrahigh performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) to determine six quaternary ammonium compounds (QACs) in dairy products. Food Chem 212:96–103

Yang Y, Shao B, Zhang J, Wu Y, Duan H (2009) Determination of the residues of 50 anabolic hormones in muscle, milk and liver by very-high-pressure liquid chromatography–electrospray ionization tandem mass spectrometry. J Chromatogr B 877(5–6):489–496

Zeng X, Bai W, Xian Y, Dong H, Luo D (2016) Application of QuEChERS-based purification coupled with isotope dilution gas chromatography-mass spectrometry method for the determination of N-nitrosamines in soy sauce. Anal Methods 8(26):5248–5254

Zhan J, Zhong YY, Yu XJ, Peng JF, Chen S, Yin JY, Zhang JJ, Zhu Y (2013) Multi-class method for determination of veterinary drug residues and other contaminants in infant formula by ultra performance liquid chromatography–tandem mass spectrometry. Food Chem 138(2–3):827–834

Zhu WX, Yang JZ, Wang Z-X, Wang CJ, Liu YF, Zhang L (2016) Rapid determination of 88 veterinary drug residues in milk using automated TurborFlow online clean-up mode coupled to liquid chromatography-tandem mass spectrometry. Talanta 148:401–411