Simultaneous Determination of 16 Sulfonamides in Animal Feeds by UHPLC–MS–MS

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Abstract

A fast and sensitive method was developed for the simutaneous quantitative determination of 16 sulfonamides in animal feeds using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS–MS). With the developed method, a feed sample can be analyzed in less than 2 h. A solid phase extraction method using acetonitrile and basic alumina column was developed to extract and purify sulfonamides from animal feeds. The analysis time can be greatly reduced with this method compared with previous reports. A linear range of 0.2~40 ng/mL (R^2 > 0.996) were obtianed for most of the compounds. The limits of quantification for all sulfonamides were in the range of 0.5~20 µg/kg. The high precision and accuracy of this method were represented by average recoveries ranging from 80% to 120% and coefficients of variation of less than 10% for spiked animal feed samples. The method is suitable for fast determination of sulfonamides in concentrates, premixes, and complete feeds.

Introduction

Sulfonamides (SAs) are derivatives of sulfanilamide (p-aminobenzenesulfonamide), which were widely used in veterinary medicine for the therapy of bacterial infections. There are thousands of species in the SAs category, and dozens of them are commonly used in veterinary drugs and animal feeds (1). Food derived from animals treated with SAs can potentially be contaminated with these drugs. There is concern about the widespread usage of these drugs which may pose serious threats to human health [e.g., resistance to drugs or toxic reactions (2,3)]. Maximum residue limits (MRL) for SAs in food products from animal origin are being continually reduced with increasing concern of food safety, For example, Codex Alimentarius Commission (CAC) and most countries in Europe and America have required the total amount of SAs or individual SA such as sulfamethazine in foods and feeds not exceeding 0.1 mg/kg (4), while Japan completely banned SAs in foods (5).

SAs enter into foods mainly through animal feedstuffs. Therefore, the most effective and direct means of controlling SAs in foods is to monitor the SAs residues in feeds of edible animals. However, trace analysis of these drugs is difficult due to interference from complex additives in feeds. Several analytical methods have been developed for the determination of SAs in animal feeds. These methods included microbiological methods (6), thin-layer chromatography (TLC) (7), capillary electrophoresis (CE) (8), enzyme-linked immunosorbant assay (ELISA) (9), and high-performance liquid chromatography (HPLC) (10–13). Microbiological methods which were based on bacteriological growth inhibition tests usually requires 2–3 days for microbe growth and may be nonspecific or not sensitive enough to meet the MRL requirements. TLC screening has been replaced by high-performance thin-layer chromatography (HPTLC), which combined with automated sample application and densitometric scanning. Although the modern HPTLC technique, coupled with MS or MALDI-MS has been proven to be sensitive and reliable for the qualitative and quantitative analysis of various samples (14,15). It has not been reported to be used for the detection of sulfonamides. CE showed good separation capability for SAs, but suffered from low precision issues. To date, this technique has not been widely applied to routine analysis (16). Immunoassay is a competitive method for SAs screening because samples can be analyzed directly without extraction or cleaning up. For example, 18 different SAs can be detected in meat, milk, and serum below the MRL concentration level using a broad-selectivity sulfonamide antibody which was used as the binder of a competitive sulfonamide screening assay utilizing lanthanide fluoroimmunoassay technology (17). However, the immunoassays are not suitable for SA screening in animal feeds because of interferences from the complex matrix in feed and thus result in false positive results and nonspecific information (18,19). Chromatographic methods based only on ultraviolet or photodiode array detection are not the proper confirmatory method as the matrix interference from higher concentration additives and lipophilic compounds in feedstuffs is a serious hindrance to the accurate quantitation of SA residues in feedstuffs. Thus, the methods mentioned above can only be used for screening purposes because of their low sensitivity or selectivity.

Liquid chromatograph tandem mass spectrometry (LC–MS–MS) methods have been widely used for the validation of multi-component quantification and microanalysis with the

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increasing demand for mass spectrometric confirmation of drug residues in foods. LC–MS–MS methods provide both high sensitivity and selectivity, and have been applied to the analysis of SAs in foodstuffs, such as tissues (20–22), egg (23), milk (24), fish (25), and honey (26). A major shortcoming when developing a multi-residue method using LC–MS–MS, is the long chromatographic analysis time, more than 20 min, in most cases (27). The introduction of ultra-high performance liquid chromatography (UHPLC) has greatly reduced analysis time (28,29). By reducing the particle size of the stationary phase to less than 2 µm, the resolution can be increased by up to 60% and the analysis time can be reduced by more than 20 times compared to conventional LC (30). Here, we report for the first time the determination of SAs in animal feedstuffs using ultra-high liquid chromatography tandem mass spectrometry (UHPLC–MS–MS). The proposed method was validated by evaluating recovery, selectivity, linearity, accuracy, and repeatability, and could be applied for the analysis of real samples.

Experimental

Reagents and chemicals

Sulfadiazine (99.0%, SDZ), sulfapyridine (99.0%, SP), sulfamerazine (99.0%, SMR), sulfameter (99.0%, SMDZ), sulfamethazine (99.0%, SMZ), sulfamethoxypyridazine (99.2%, SMP), sulfamethoxazole (99.0%, SMX), sulfadimethoxine (99.0%, SDM), sulfaphenazole (99.0%, SPZ), sulfadoxine (98.0%, SD), sulfachloropyrazine (99.0%, SCR), sulfamonomethoxine (98.0%, SMM), sulfathiazole (99.5%, STZ), sulfacetamide (99.5%, SAA), sulfanilamide (99.0%, SA), and sulfaquinoxaline, (97.5%, SQ), were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and used without further purification (Table I). Stock standard solution of each SA (2 mg/mL) was prepared by dissolving in acetonitrile and stored in darkness at –20°C. Mixed stock standard solution was prepared by mixing the stock standard solution of each SA to a final concentration of 20 µg/mL. Working standard solutions were prepared daily by diluting the mixed stock standard solution with acetonitrile in appropriate proportions.

HPLC grade methanol and acetonitrile were obtained from Fisher Scientific International (Hampton, NH). HPLC grade formic acid was purchased from Dima Technology (Richmond Hill, Canada). All other chemicals were analytical grade. Water used was double-deionized water (Milli-Q, Millipore Corp., Billerica, MA) with 18.2 MΩ cm resistivity.

Formic acid (0.4%) was prepared by adding 2 mL formic acid in 500 mL water and mixed with different amounts of acetonitrile to obtain 5%, 10%, and 25% acetonitrile-formic acid mixtures (v/v), which were used as eluent in the solid phase extraction procedure.

Apparatus and procedures

UHPLC–MS–MS analysises were performed on an Agilent 1200 UHPLC system coupled with a 6460 Mass Selective Detector (Agilent Technologies, Fermont, CA). OASIS MCX and HLB solid phase extraction columns (both 3 mL, 60 mg), used for sample purification, were purchased from Waters Corporation (Milford, MA). Basic alumina columns (1000 mg, 6 mL) were purchased from Agilent Technologies. Desktop Constant Temperature Oscillator (Jing Hong, Shanghai, China) was used to promote sample dissolution. The solid phase extraction (SPE) system was a vacuum manifold processing station obtained from Agilent Technologies.

Sample extraction and purification

Three types of commercially prepared pig feeds without SAs were made at the pilot plant of the Ministry of Agricultural Feed Industry Center (Beijing, China) and used as blank samples. Before spiking or extraction, the feed samples were ground according to national standard (BG/T 20195-2006) (31). Briefly, 100 g feed sample was ground using grinding mill. The ground samples were passed through a griddle with 0.25 mm-diameter pore. Finally, the samples were mixed completely and stored in sealed container until needed.

Table I. The Chemical Structures of 16 Sulfonamides

Compound	Structure
Sulfanilami-de (SA)	
Sulfacetami-de (SAA)	
Sulfachloro-pyrazine (SCR)	
Sufadoxine (SD)	
Sulfadimeth-oxine (DSM)	
Sulfadiazine (DSZ)	
Sulfameter (SMDZ)	
Sulfamono-methoxine (SMM)	
Sulfamethoxy-pyridazine (SMP)	
Sulfamerazine (SMR)	
Sulfamethoxa-zole (SMX)	
Sulfamethaz-ine (SMZ)	
Sulfapyridine (SP)	
Sulfaphenaz-ole (SPZ)	
Sulfaquinox-aline (SQ)	
Sulfathiazol (STZ)	

SAs-spiked samples were prepared by mixing a certain amount of blank feed sample (complete feed, 5 g; concentrated feed, 2 g; premix feed, 2 g) with a certain amount of SAs mixed standard soltuion in a 150 mL conical flask. Sample extraction was achieved by adding 50 mL acetonitrile into the SAs spiked sample and shaking for 25 min followed by centrifuging at 8000 rpm (RCF 7012 × g) at 4°C for 10 min. The supernatant was taken to solid phase extraction (SPE) for further purificaiton.

The basic alumina column was preconditioned by passing through 5 mL of acetonitrile. Then, 1~10 mL of the above supernatant (5 or 10 mL for complete feed, 1 mL for premix and concentrated feeds) was slowly passed through the column. After washing with 5 mL acetonitrile, the analytes were eluted with 4 mL acetonitrile–0.4% formic acid eluent (10%, v/v). Finally, the sample was filtered via 0.22 µm microporous film and 10 µL of the solution was injected into the UHPLC–MS–MS system.

LC–MS–MS conditions

Chromatographic separation of SAs was achieved on an Eclipse Plus C18 analytical column (2.1 mm × 100 mm; 1.8 µm). The column temperature was 35°C. The mobile phase was delivered at a flow rate of 0.3 mL/min, it consisted of A: 0.1% aqueous formic acid solution and B: acetonitrile. A gradient program was used for the mobile phase: 5% B (initial), 5–23% B (0–6 min), 23% B (6–9 min), 23–90% B (9–9.01 min), 90–5% B (9.01–12 min). A 5-min equilibration was needed before the next injection, which resulted in a total run time of 17 min. The SAs in feed samples were quantified by external standard methods using calibration curve. In addition, if the concentration of SAs in feed sample exceeded the linear range of calibration curve, the sample solution was diluted before instrumental analysis such that the final concentration was in the range of calibration curve.

The optimized ESI condition was: gas temperature 350°C, gas flow 5 L/min, sheath gas temperature 400°C, sheath gas flow 12 L/min, capillary voltage 3500 V, and Delta EMV 300 V. High-purity nitrogen was used as the ESI nebulizing gas. Positive ions were monitored. Multiple reaction monitor (MRM) mode was applied for quantification and qualitative analysis.

Results and Discussion

Optimization of UHPLC–MS–MS conditions

To achieve the best selectivity and sensitivity, the mass spectrometry parameters including ionization mode, capillary voltage, source temperature, sheath gas flow, nebulizer pressure, especially fragmentor voltage and collision energy were optimized by direct flow injection of SA standards individually. Firstly, ESI+ mode was selected because all SAs have amino groups, which favor the formation of positive ions. In most cases, the precursor ions of SAs were their molecular ions $[M + H]^+$. Secondly, the ion source parameters and precursor ion masses were optimized for each SA by full scans. Thirdly, using SIM scan, the fragmentor voltage of precursor ions of each SA was optimized by setting up multiple selected ion-monitoring experiments with fragmentor voltages varying from 80 to 140 V. The voltage setting that produced the highest response was selected. Lastly, using product ion scan, the product ion masses and collision energy were optimized. Among those, two transitions were chosen on the basis of the best chromatographic S/N with minimum interference from matrix components. By examining the spectra and comparing peak intensities, the optimal collision energy settings for the SAs were determined. The characteristic ion and optimal MS–MS parameters including fragmentor voltage and collision energy for each SA were listed in Table II.

The chromatographic parameters, such as column selection, mobile phase composition, flow rate, and column temperature

Table II. MS–MS Parameters on the Precursor and Product Ion (*m/z*) of 16 Sulfonamides

Compound	Retention time (min)	Molecular weight	Quantitative transition	Qualitative transition	Fragmentor voltage (V)	Collision energy (V)
SA	1.7	172.2	172.9/92.9	172.9/156.0	100	25
				172.9/92.9	100	20
SAA	3.8	214.2	214.9/155.5	214.9/155.5	80	10
				214.9/107.8	80	15
SDZ	4.7	250	251.0/156.0	251.0/156.0	120	10
				251.0/185.0	120	10
STZ	5.6	255.3	255.7/155.5	255.7/155.5	130	10
				255.7/107.9	130	15
SP	5.8	249	250.0/156.0	250.0/156.0	110	10
				250.0/184.0	110	10
SMR	6.1	264	265.0/156.0	265.0/156.0	120	15
				265.0/172.0	120	15
SMZ	7.2	278	279.0/156.0	279.0/156.0	140	15
				279.0/186.0	140	15
SMP	7.6	280.3	281.1/155.9	281.1/155.9	110	10
				281.1/125.9	110	20
SMDZ	8.4	280	281.0/156.0	281.0/156.0	120	10
				281.0/126.0	120	20
SMM	8.4	280	280.7/155.7	280.7/155.7	110	10
				280.7/215.0	110	15
SCR	8.7	284	285.0/156.0	285.0/156.0	100	15
				285.0/108.0	100	20
SD	9.3	310.3	310.7/155.8	310.7/155.8	130	10
				310.7/139.0	130	15
SMX	9.4	253	254.0/156.0	254.0/156.0	120	15
				254.0/147.0	120	20
SQ	11.3	300	301.0/156.0	301.0/156.0	140	15
				301.0/208.0	140	15
SDM	11.3	310.3	311.0/156.0	311.0/156.0	130	15
				311.0/218.0	130	15
SPZ	11.3	314.4	314.8/155.9	314.8/159.6	140	15
				314.8/155.9	140	15

were optimized to obtain the best peak shape, resolution, and retention time. First, three types of LC columns with different inner diameters and particle sizes were compared for signal intensity, separation efficiency, and retention time of the 16 SAs. Typical LC conditions recommended by manufacturer were used for initial test, such as the flow rates is 0.3 mL/min for Eclipse Plus C_{18} column (2.1 mm × 100 mm; 1.8 µm) and Waters Symmetry C_{18} column (2.1 × 150 mm, 3.5 µm), and 1.0 mL/min for Agilent Eclipse XDB column C_{18} (4.6 × 150 mm, 5 µm). Column temperature was kept at 30°C for all columns and the composition of the mobile phase was also the same for the columns. The results show that the ultra high-resolution chromatography column with 1.8 µm stationary phase is the best in decreasing analysis time and increasing resolution compared with conventional LC columns (Figure 1). The run time was decreased by one third when UHPLC column was used. In addition, the LC signal intensity was 200 times stronger with the UHPLC column than the other two columns with the same injection volume. Thus, the application of UHPLC column to the analysis of SAs not only reduced analysis time, but also greatly enhanced sensitivity. A gradient program using 0.1% formic acid and acetonitrile mixture was employed to achieve satisfactory separation based on the characteristic pK_a values of SAs (22) and the electrospray ionization conditions. The mobile phase B concentration jumped from 23% to 90% within 0.01 min. Such rapid change in mobile phase to high proportion of organic solvent facilitated elution of the remaining SAs and completely washed the column, which did not influence the repeatability of the separation. Finally, the flow rate and column temperature were also optimized to achieve best efficiency. The flow rates were set at 0.2, 0.3, and 0.4 mL/min, and temperatures were set at room temperature, 30°C, and 40°C. The results indicate that the 0.3 mL/min and 40°C were the optimal flow rate and column temperature considering the resolution and total run time. Under the optimal UHPLC conditions, the typical quantitative daughter ion chromatograms of the 16 SAs were presented (Figure 2).

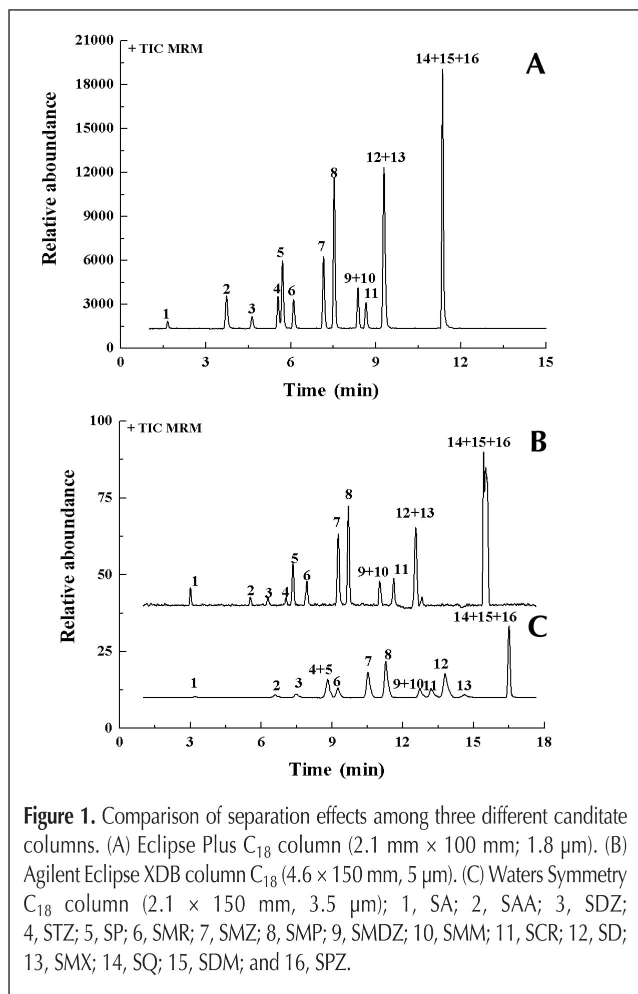

Figure 1. Comparison of separation effects among three different canditate columns. (A) Eclipse Plus C_{18} column (2.1 mm × 100 mm; 1.8 µm). (B) Agilent Eclipse XDB column C_{18} (4.6 × 150 mm, 5 µm). (C) Waters Symmetry C_{18} column (2.1 × 150 mm, 3.5 µm); 1, SA; 2, SAA; 3, SDZ; 4, STZ; 5, SP; 6, SMR; 7, SMZ; 8, SMP; 9, SMDZ; 10, SMM; 11, SCR; 12, SD; 13, SMX; 14, SQ; 15, SDM; and 16, SPZ.

Optimization of sample extraction and purification

Feed samples typically contain a significant amount of inorganic salts and proteins that can interfere with the detection of SAs. A viable extraction protocol is needed to recover as much SAs from the matrix with minimum interference. Four extract solvents together with 3 different extraction methods were tested. The solvents included: acetonitrile, 0.2 M phosphoric acid–methanol solution (20:80, v/v), 0.1 M hydrochloric acid–methanol solution (20:80, v/v), and 0.1 M hydrochloric acid-methanol solution (80:20, v/v). Acidic extract solvents made up the majority of the candidate list because the SAs have higher solubility in acidic solvents. The three extract approaches included: ultrasonic, stir, and shaking. Interestingly, acetonitrile and shaking yielded the best reproducibility and recovery of the 16 SAs. Moreover, most of the proteins present in feeds were precipitated in the presence of acetonitrile, and the following refrigerated centrifugation procedure can precipitate them efficiently.

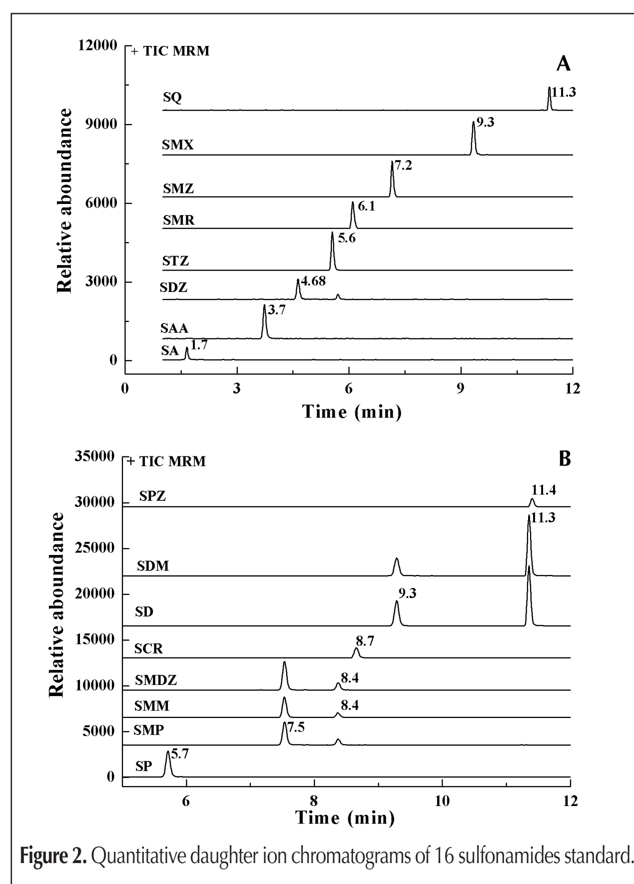

Figure 2. Quantitative daughter ion chromatograms of 16 sulfonamides standard.

Thus, acetonitrile was chosen as the extraction solvent. Any additional interfering compounds can be removed in the subsequent clean-up procedure using solid phase extraction (SPE). Several SPE columns, such as mixed cation-exchange (MCX, 3 mL), hydrophilic-lipophilic balance (HLB, 3 mL), and basic alumina columns (1000 mg, 6 mL), were tested. Supernatants from acetonitrile and 0.1 M hydrochloric acid-methanol (80:20, v/v) extraction were directly transferred into the preconditioned basic alumina and HLB columns, respectively. Supernatants from 0.2 M phosphoric acid-methanol (20:80, v/v) and 0.1 M hydrochloric acid-methanol (20:80, v/v) extraction were first dried under nitrogen, reconstituted in 2% formic acid and then introduced into MCX column. The HLB and MCX column were preconditioned by passing 3 mL of methanol, followed by 3 mL of double-deionized water through the column. Then, 5 mL of the extract solution was slowly passed through the column at a flow rate of 1 mL/min. For MCX column, after washing with 3 mL of double-deionized water and 3 mL of methanol, respectively, the analyte was eluted with 3 mL of ammonia-methanol solution (5%, v/v). For HLB column, after washing with 3 mL of double-deionized water and 3 mL of 0.1 M hydrochloric acid, respectively, the analyte was eluted with 3 mL methanol. Finally, the eluted solutions were evaporated in a 50°C water bath under nitrogen. The residue was reconstituted with initial mobile phase. Sample purification with basic alumina column was shown in the Experimental Section. When HLB or MCX was used, the recoveries of SAs were low and unstable. Only basic alumina columns could yield high and stable recoveries. For example, the recoveries of SAs for 1.0 mg/kg spiked level were ranged from 30% to 77% for HLB and MCX columns and 80–95% for basic alumina (Figure 3). The SPE experiments performed here were under optimal conditions recommended by their manufacturers. Thus, it is almost impossible to obtain higher recoveries by further optimizing the conditions of HLB and MCX. Thus the two columns are not suitable for the purification of SAs in feeds. In addition, the procedure of basic alumina extraction is extremely fast and simple compared with other SPE methods because the acetonitrile extractant could be directly added into the column and the eluting solution could be directly injected into the UHPLC–MS–MS system without drying and reconstitution.

The cleaning up process using basic alumina cartridge was further optimized using three acetonitrile–0.4% formic acid (5:95, 10:90, and 25:75, v/v) eluents. Three SA spiked (1.0, 0.1, and 0.05 mg/kg) feeds were tested to compare the recoveries of different eluents. The recoveries obtained were shown in Figure 4. Analysis of variance (ANOVA) was used to analysis the data and a P-value less than 0.05 was considered statistically significant. The statistics results showed that with 10% and 25% eluents, the recoveries for the 1.0 and 0.1 mg/kg spiked feeds had no statistical differences. However, the recoveries obtained by 10% eluent was better than the 25% eluent at 0.05 mg/kg spiked level for several SAs, such as STZ, SDZ, and SA. Thus, 10% acetonitrile-formic acid solution was selected as eluent for the basic alumina cartridge extraction process. The MRM total ion chromatogram (TIC) of SA-spiked animal feed (Figure 5B) resembled that from SA standard solutions (Figure 1A). The results indicated that the matrix effects were not significant after SPE purification and did not interfere with the detection of the analytes.

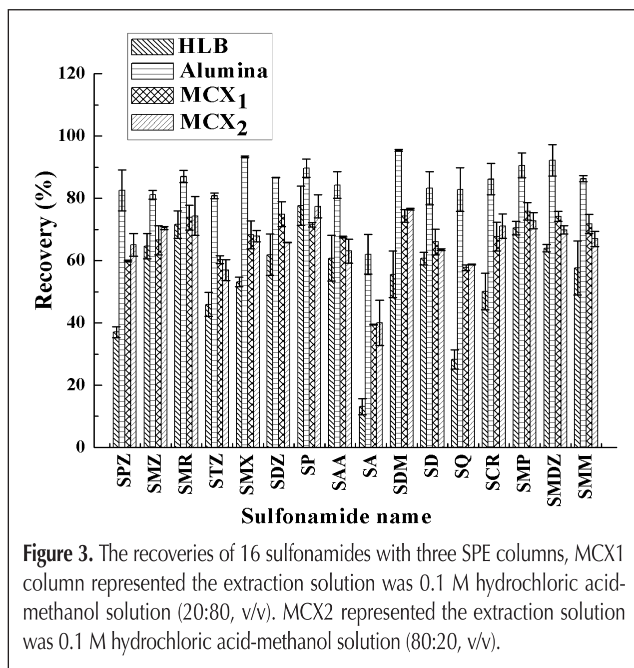

Figure 3. The recoveries of 16 sulfonamides with three SPE columns, MCX1 column represented the extraction solution was 0.1 M hydrochloric acid-methanol solution (20:80, v/v). MCX2 represented the extraction solution was 0.1 M hydrochloric acid-methanol solution (80:20, v/v).

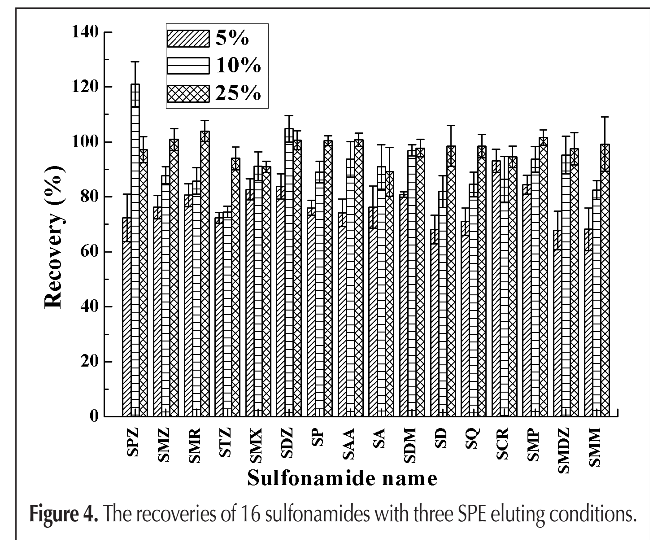

Figure 4. The recoveries of 16 sulfonamides with three SPE eluting conditions.

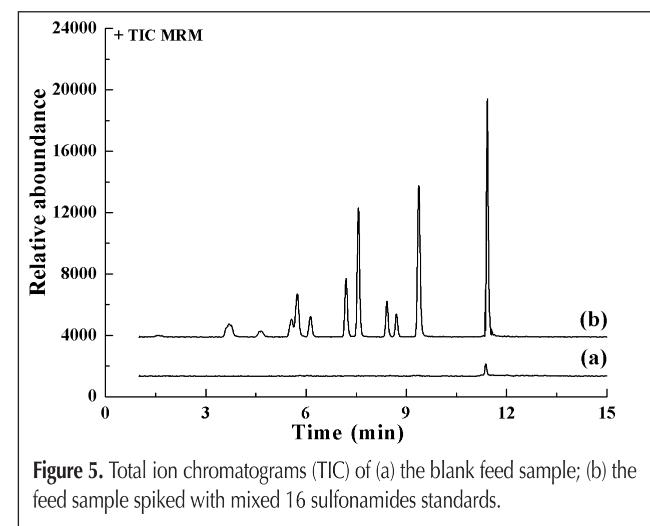

Figure 5. Total ion chromatograms (TIC) of (a) the blank feed sample; (b) the feed sample spiked with mixed 16 sulfonamides standards.

Method validation

The linearity, sensitivity, as well as precision and accuracy of the method were validated by a series of experiments described below. Linearity was studied by analyzing mixed standard solutions of SAs at seven different concentrations: 0.2, 1.0, 5.0, 10, 20, 40, and 80 ng/mL. Most of the SAs showed satisfactory linearity within the concentration range of 0.2~40 ng/mL except sulfanilamide and sulfamonomethoxine (Table III). The limit of quantitation (LOQ), which was defined as the concentration at 10 times the value of noise, was determined by examining UHPLC–MS spectra of SAs spiked animal feeds. The LOQ values were in the range of 0.5~20 µg/kg (Table III). To evaluate the repeatability and stability of the method, the inter- and intra-assay repeatability was tested. For example, the recoveries of SAs spiked in complete feed were determined by six replications at intermediate concentration (0.1 mg/kg). The results indicated that the recoveries of SAs ranged from 90% to 110% and coefficients of variation were less than 6% for both intra-assay (within a day) and inter-assay(over a period of five consecutive days) measurements.

Analysis of spiked feed samples

Complete pig feeds spiked with SAs were analyzed using the optimized method mentioned above. Three concentrations of SAs (0.05, 0.1, and 1.0 mg/kg) were spiked in complete feeds with six replicates for each concentration. The presence of SAs was confirmed by comparing the peak intensity ratio of two product ions of each SA with the ratio obtained from standard samples. The peak ratios show good correlations between the spikes and the standards with variations less than 10%. Thus, the UHPLC–MS–MS method can act as a confirmatory procedure to verify the existence of SAs in animal feed.

The product ion *m/z* 156, which is a common fragment ion of most of the SAs, is the most abundant ion in the mass spectra (32). The peak area of *m/z* 156 was used for quantification and to determine the recoveries and coefficients of variation for all SAs except for SA. For SA, ion at *m/z* 93 was used for such purpose (22). The recoveries of SAs spiked at different concentrations were shown in Table IV. They ranged from 80% to 112% and the coefficients of variation were less than 10%.

The UHPLC–MS–MS methods established above were also applied to the detection of SA-spiked premixes and concentrate feed samples. SAs was spiked in premixes with 5.0 mg/kg and concentrates with 2.0 and 5.0 mg/kg. Six replicates were tested for each concentration. Spike levels, spike recoveries and correlation coefficients were also evaluated. The actual spiked quantity and the measured concentration in the premixes and concentrates matrice showed good consistency (Table V). The recoveries ranged from 80–120% and coefficients of variation were less than 10%.

Table III. Linear Relationships and Sensitivity of Sulfonamides

Compound	Linear range (ng/mL)	Linear equation	R^2	LOQ (µg/kg)
SPZ	0.2~40	y = 187.1x – 43.48	0.999	2.0
SMZ	0.2~40	y = 346.7x – 147.1	0.998	2.0
SMR	0.2~40	y = 280.9x – 79.8	0.998	4.0
STZ	0.2~40	y = 387.9x – 160.6	0.999	5.0
SMX	0.2~40	y = 361.2x – 176.7	0.999	1.0
SDZ	0.2~40	y = 222.2x – 70.5	0.998	5.0
SP	0.2~40	y = 693.5x – 156.3	0.999	0.5
SAA	0.2~40	y = 380.3x – 133.7	0.999	10.0
SA	1.0~40	y = 111.7x – 91.3	0.997	20.0
SDM	0.2~40	y = 2515.9x – 382.0	0.999	0.5
SD	0.2~40	y = 712.3x – 334.7	0.999	0.2
SQ	0.2~40	y = 170.7x – 9.0	0.999	2.0
SCR	0.2~40	y = 273.8x – 54.8	0.999	2.0
SMP	0.2~40	y = 605.3x – 227.1	0.999	1.0
SMDZ	0.2~40	y = 192.8x – 41.7	0.999	2.0
SMM	1.0~40	y = 124.6x – 69.4	0.996	2.0

Table IV. Recoveries of Sulfonamides from the Spiked Complete Feed Samples Determined by LC–MS–MS (*n* = 6)

Compound	Spiked recovery of sulfonamides (%)		
	0.05 mg/kg*	0.1 mg/kg*	1.0 mg/kg*
SPZ	90.3 ± 7.9	105.6 ± 7.5	83.6 ± 4.8
SMZ	91.7 ± 4.2	94.8 ± 3.4	92.8 ± 3.3
SMR	93.9 ± 1.2	101.9 ± 5.6	95.3± 4.7
STZ	104.0 ± 6.0	101.6 ± 3.8	80.1 ± 1.1
SMX	99.1 ± 7.7	92.0 ± 4.5	85.9 ± 3.1
SDZ	92.6 ± 5.4	97.5 ± 4.6	88.1 ±2.7
SP	90.1 ± 2.9	93.9 ± 6.1	90.2 ± 4.0
SAA	91.2 ± 7.6	83.2 ± 6.4	88.5 ± 3.2
SDM	87.4 ± 5.4	93.4 ± 2.4	99.9 ± 2.5
SA	89.9 ± 5.9	93.5 ± 3.6	94.8 ± 3.8
SD	103.2 ± 7.5	95.0 ± 3.3	88.1 ± 3.0
SQ	93.0 ± 3.6	95.9 ± 6.6	95.3 ± 3.8
SCR	90.3 ± 5.6	91.3 ± 4.8	85.7 ± 2.8
SMP	91.2 ± 5.0	88.5 ± 3.7	87.7 ± 4.5
SMDZ	103.0 ± 7.8	88.2 ± 4.8	97.4 ± 6.2
SMM	111.7 ± 9.2	106.5 ± 9.3	91.0 ± 8.7

* spiked concentration.

Table V. Recoveries of Sulfonamides from the Spiked Premix and Concentrate Feed Samples Determined by LC–MS–MS (*n* = 6)

Compound	Premix feed (%)	Concentrate feed (%)	
	5.0 mg/kg*	2.0 mg/kg*	5.0 mg/kg*
SPZ	118.7 ± 8.2	92.1 ± 4.2	100.5 ± 7.6
SMZ	96.2 ± 4.2	98.7 ± 7.6	94.3 ± 7.6
SMR	92.9 ± 4.2	97.0 ± 9.0	94.9 ± 10.7
STZ	86.8 ± 2.0	87.3 ± 2.6	90.3 ± 6.2
SMX	104.3 ± 3.8	96.2 ± 2.6	91.1 ± 3.5
SDZ	101.2 ± 4.6	103.9 ± 2.7	102.7 ± 5.2
SP	96.6 ± 2.7	98.0 ± 4.1	94.7 ± 6.5
SAA	95.3 ± 5.0	96.3 ± 4.9	97.3 ± 7.2
SA	99.5 ± 7.6	76.3 ± 4.5	90.1 ± 7.7
SDM	99.1 ± 1.0	97.2 ± 3.2	97.3 ± 2.2
SD	99.2 ± 5.3	98.7 ± 4.3	89.9 ± 5.7
SQ	80.1 ± 5.1	95.9 ± 9.8	91.5 ± 8.2
SCR	99.0 ± 4.3	96.5 ± 5.5	90.5 ± 6.2
SMP	89.1 ± 3.4	100.3 ± 2.2	97.6 ± 6.0
SMDZ	106.4 ± 7.0	103.3 ± 3.0	96.4 ± 6.7
SMM	106.3 ± 10.9	90.5 ± 6.5	87.6 ± 9.7

* spiked concentration.

Conclusion

In summary, the challenges of analyzing SAs in animal feeds were mainly from the complex sample matrix that contains salts and proteins which interference with the detection using many modern analytical methods and long analytical time. Here, acetonitrile extraction together with basic alumina column solid phase extraction were used to meet the challenges. The basic alumina columns showed superior efficiency in that the acetonitrile extract can be injected into the column directly and the eluent from the column can directly be analyzed with LC, which greatly reduced sample preparation time. UHPLC–MS–MS, which was used for the subsequent confirmation and quantitation of the SAs, also showed faster analysis time compared with conventional LC and tremendously enhanced signal intensity. It took less than 2 h in our lab to finish analyzing a feed sample from scratch, which can match the ELISA method, however, the sensitivity and selectivity of the UHPLC–MS–MS method is unmatchable by ELISA. These results indicate that the method developed here is able to detect SAs from animal feeds at concentration level well below the MRL with unprecedented speed. Further researches are granted to expand this method to more SAs that may exist in all kinds of foodstuffs.

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