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Development of an ultra-high-pressure liquid chromatography–tandem mass spectrometry multi-residue sulfonamide method and its application to water, manure slurry, and soils from swine rearing facilities

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ABSTRACT

An analytical method was developed using ultra-high-pressure liquid chromatography–triple quadrupole-tandem mass spectrometry (UHPLC–TQ-MS/MS) to simultaneously analyze 14 sulfonamides (SA) in 6 min. Despite the rapidity of the assay the system was properly re-equilibrated in this time. No carryover was observed even after high analyte concentrations. The instrumental detection limit based on signal-to-noise ratio (S/N) > 3, was below 1 pg/μL (5 pg on column) for all SAs except sulfachloropyridazine. Surface water, ground water, soil, and slurry manure contained in storage ponds in and around swine [*Sus scrofa domesticus*] rearing facilities were analyzed. Sample cleanup for ground water and surface water included using solid phase extraction (SPE) using Oasis® hydrophilic–lipophilic balance (HLB) cartridges. The soil and slurry manure required tandem strong anion exchange (SAX) and HLB solid phase extraction cartridges for sample cleanup. With few exceptions, the recoveries ranged from 60 to 100% for all matrices. The minimum detectable levels were below 2.0 ng/L for water, 30 ng/L for slurry manure, and 45 ng/kg for soil except for sulfachloropyridazine. The coefficient of variation (CV) was within 20% for most of the compounds analyzed. Using this method, sulfamethazine concentrations of 2250–5060 ng/L, sulfamethoxazole concentrations of 108–1.47×10^6 ng/L, and sulfathiazole concentrations of 785–1700 ng/L were found in the slurry manure. Sulfadimethoxine (2.0–32 ng/L), sulfamethazine (2.0–5.1 ng/L), and sulfamethoxazole (20.5–43.0 ng/L) were found in surface water and ground water. In top soil (0–15 cm), sulfamethazine ranged 34.5–663 ng/kg dry weight in those locations that received slurry manure as a nutrient; no SAs were found in the soil depths between 46 and 61 cm. The speed makes the method practical for medium to high throughput applications. The sensitivity and positive analyte identification make the method suitable for the demanding requirements for real world applications.

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1. Introduction

Pharmaceuticals have been widely detected in a variety of environmental matrices. In a 2002 report, the U.S. Geological Survey had identified 95 emerging pollutants in U.S. waterways, and found that surface water contained anti-microbials in concentrations ranging from 20 to 710 ng/L with a detection frequency as high as 27% [1]. Among the anti-microbials, a number of compounds belonging to the SA group were often found. There is an intense interest in developing and applying appropriate analytical methods to the proper identification and measurement of multiple members of the SA group because of their widespread use, environmental persistence, and their potential for causing the development of resistant strains of bacteria. The existing and proposed methods generally lack the speed, sensitivity or specificity required for modern day environmental monitoring.

Some 20–30 sulfonamides have become commercial products [2], requiring any practical analysis to be capable of simultaneously measuring multiple related compounds as well as determining the specific compound(s) being measured. Detection of SAs utilized in humans in a variety of environmentally important matrices is frequently reported in the literature, and the most commonly used SA, sulfamethoxazole, often with other SAs employed in human medicine, has been found in drinking water, surface water, wastewater, and bio-solids [3–5]. Sulfonamides have also been widely used in veterinary applications to control, treat, and prevent infectious diseases. The amount of SA used for both human and agricultural purposes is very large and increasing [6,7]. Of particu-

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lar concern is the appearance of SAs within or surrounding animal rearing facilities [8–10].

Sulfonamides are excreted from both humans and animals either unchanged or mostly as the *N*-acetylated metabolite (with acetylation occurring at the primary aromatic amino group), although other metabolites can be also present and metabolism can be dependent on diet [11]. The *N*-acetyl-metabolites can revert back to the parent compound under relatively mild conditions. Sulfonamides persist in a variety of matrices for prolonged periods. For example, one study reports no SA degradation during swine manure composting [12]. Slurry manure that contains SAs and other veterinary medicines is applied to the land as a source of nutrients for crop growth [13]. This manure application can be a source of SA from animal agriculture entering into the environment or even into crops and the food supply.

It was reported that SAs have relatively high polarity and water solubility, which results in weak sorption affinity to the soil particles and high mobility in the soil [14]. Holm et al. [10] detected up to 6.47 mg/L of SAs in ground water samples down-gradient from a landfill that was used for the disposal of household and pharmaceutical wastes. Another study [9] showed that the concentration of sulfamethazine and sulfadimethoxine was 0.076–0.22 and 0.046–0.068 μg/L, respectively, in the ground water near an animal feeding operation. Furthermore, SAs have been shown to be taken up by plants after liquid manure application [15].

Sophisticated multi-residue analysis methods such as LC-MS or LC-MS/MS allow unequivocal confirmation of the analytes' identity and are the focus of recent studies [16–24]. These methods involve different types of MS formats and have been increasingly sophisticated as new instruments and software have become available. Unfortunately, these methods are often time consuming, limiting the number of assays that may practically processed during a day.

Sample cleanup is critical for a method, particularly when analyzing samples from a complex matrix needed to be analyzed in an agriculture environment (liquid manure, soil). High sensitivity is required in addition to the ability to resolve the mixture of analytes.

Our objective was to develop a method meeting all the requirements of unambiguous analyte determination, sensitivity, good resolution, and still be completed in a short period of time to permit medium to high throughput analysis. An analytical method that can provide these measurements will advance environmental studies and source tracking methodologies. In this paper, a UHPLC-TQ-MS/MS method is proposed that can simultaneously analyze multiple SAs in a short time (6 min per injection, compared with 50 min per injection [22]), positively identify the analyte and, with adequate resolution and sensitivity, to deal with this complex problem. Coupled with the described sample cleanup this method was demonstrated to be able to make environmental measurements using samples taken near and in two swine rearing facilities confirming its ability to handle real world samples.

2. Materials and methods

2.1. Materials

Sulfamethoxazole (SMX) was purchased from US Pharmacopoeia (Rockville, Maryland). Sulfaguanidine (SG), sulfisoxazole (SSX), sulfathiazole (STZ), sulfadiazine (SDZ), sulfamethoxypyridazine (SMPD), sulfamerazine (SMR), sulfamethizole (SMTZ), sulfamethazine (SMZ), sulfachloropyridazine (SCP), sulfadimethoxine (SDM), sulfabenzamide (SB), sulfaphenazole (SPZ), and sulfaquinoxaline (SQX) were obtained from Sigma–Aldrich (St. Louis, MO). Sulfamethoxazole-d_4 and sulfamethazine-d_4 were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). [^{13}C]SMZ was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA). The chemical structures and properties of the above compounds can be found in Table 1. G4 Glass fiber filters were obtained from Fisher Scientific (Pittsburgh, PA). Solid phase extraction Oasis HLB cartridges (3 mL with 200 mg sorbent) and Oasis HLB Plus (225 mg sorbent) were purchased from Waters (Milford, MA). Supelclean^{TM} LC-SAX SPE (3 mL with 500 mg sorbent) was obtained from Supelco Analytical (Bellefonte, PA).

2.2. Sample collection

Samples were collected from two hog farms (Figs. 1 and 2), which accommodate different growing stages of swine at any given time with approximately 4000 total animals per farm. Sample types included well water, pond water, and liquid slurry manure from manure storage ponds at both facilities. Water samples from lysimeters and soils were also collected from the first research location. The collection intervals were approximately 4 weeks apart from May to December in 2008 for different bodies of water.

Thompson et al. [27] provides a description of the lysimeter installation at the first research location (Fig. 1). The first location was located on glacial outwash derived soil. In brief, lysimeters were installed 60 cm below the surface to collect soil water leachate on a plot site that received manure treatment. A lysimeter was also installed 30 cm below a hoop barn, which was constructed on compacted earth and covered with straw bedding. The bedding and manure mixture is scraped from the hoop barns approximately twice a year and placed uncovered on the soil surface in a static manure pile. A lysimeter was also located beneath this manure pile. The nursery contains pens with slotted flooring that allows urine and feces to drop through. Below the pens are collection pits that are emptied on a monthly basis into a manure storage pond. Liquid slurry manure from the storage pond is applied to nearby fields as fertilizer. The slurry manure in the storage pond was sampled from sample ports located at the surface and at approximate depths of 100, 200, and 300 cm. The second study site (Fig. 2) was similar in operation type and size as the first; however, the soil was derived from glacial till, which contained more fine textures (clay and silt) compared to the first location. Additionally, pipes from nursery and farrowing barns leading to the manure storage pond were included as samples (Fig. 2).

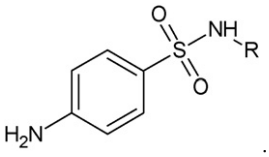
Surface (0–15 cm) and subsurface (46–61 cm deep) soil samples from study site one were collected before and after the application of slurry manure to the soil and from near the wells and lysimeters that have no known history of manure application [28]. Soil types at this study site were Hecla (sandy, mixed, frigid oxyaquic hapludolls), Garborg (sandy, mixed, frigid typic endoaquolls), and Ulen (sandy, mixed, frigid aeric calciaquolls) with % organic carbon values ranging from 0.82 to 2.09 for topsoil (0–15 cm) and 0.22 to 0.86 for subsurface (46–61 cm) samples. Samples were collected and transferred to the laboratory the same day and upon arrival at the laboratory the samples were immediately stored at −20 °C until analyzed.

2.3. Sample treatment

2.3.1. Lysimeter, surface, and well water

Samples were filtered through a 1.2 μm filter prior to SPE cleanup. One hundred mL of the filtered water were adjusted to pH 4, 100 μL of a mixture of 100 ng/mL of SMZ-d_4 and SMX-d_4 was added as internal standards, and the sample was applied to the pre-conditioned SPE columns. A North Dakota state well water that showed no sulfonamide activities was used as a control water. For each set of SPE cleanup, a negative control and a positive spike with 100 μL of 40 ng/mL sulfonamide mixture were included.

Table 1
Sulfonamide structures and their physicochemical properties used in this study.



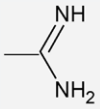
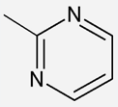
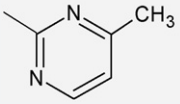
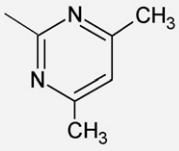
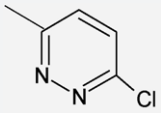
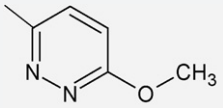
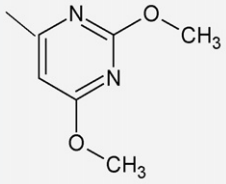
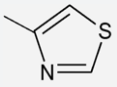
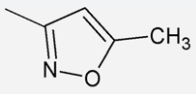
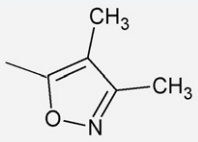
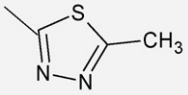
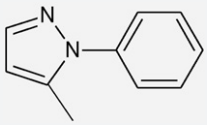
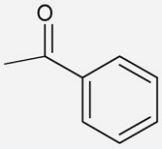
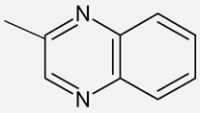
Name	Abbreviation	–R	pK_a[a]	Measured log Kow[a]
Sulfaguanidine	SG		2.75[b] 12.05	
Sulfadiazine	SDZ		1.57[c] 6.50	−0.092
Sulfamerazine	SMR		1.58[c] 6.98	0.14
Sulfamethazine	SMZ		2.79 7.59	0.89
Sulfachlorpyridazine	SCP		1.76[d] 5.71	0.31
Sulfamethoxypyridazine	SMPD		2.18[c] 7.19	0.32
Sulfadimethoxine	SDM		2.90 8.43	1.63
Sulfathiazole	STZ		2.36[b] 7.12	0.05
Sulfamethoxazole	SMX		1.57 6.40	0.89
Sulfisoxazole	SSX			1.01

Table 1(*Continued*)

Name	Abbreviation	–R	pK_a[a]	Measured log Kow[a]
Sulfamethizole	SMTZ		1.86 5.25	0.54
Sulfaphenazole	SPZ		1.9[b] 6.50	
Sulfabenzamide	SB		1.11[c] 4.18	
Sulfaquinoxaline	SQX		2.34[e] 5.97[e]	

[a] Unless noted, pK_a and log Kow value were from literature citation [19].
[b] From literature citation [25].
[c] From literature citation [13].
[d] From literature citation [9].
[e] From literature citation [26].

2.3.2. Liquid manure

Liquid manure was centrifuged at 15,000 × *g* for 30 min and the supernatant was filtered through a 1.2 μm filter. Ten mL of the filtered manure were mixed with 40 mL of nanopure water and adjusted to pH 7 using 1N HCl. To each sample 100 μL of a mixture of 100 ng/mL SMX-d_4 and SMZ-d_4 were added as the internal standard. A single batch of liquid manure from the second study site that was assayed and found to contain no sulfonamides was used as the control matrix. A negative control and a positive spike (100 μL of a 40 ng/mL sulfonamide mixture) were included in each sample cleanup set. The samples were then subjected to the SPE cleanup procedure.

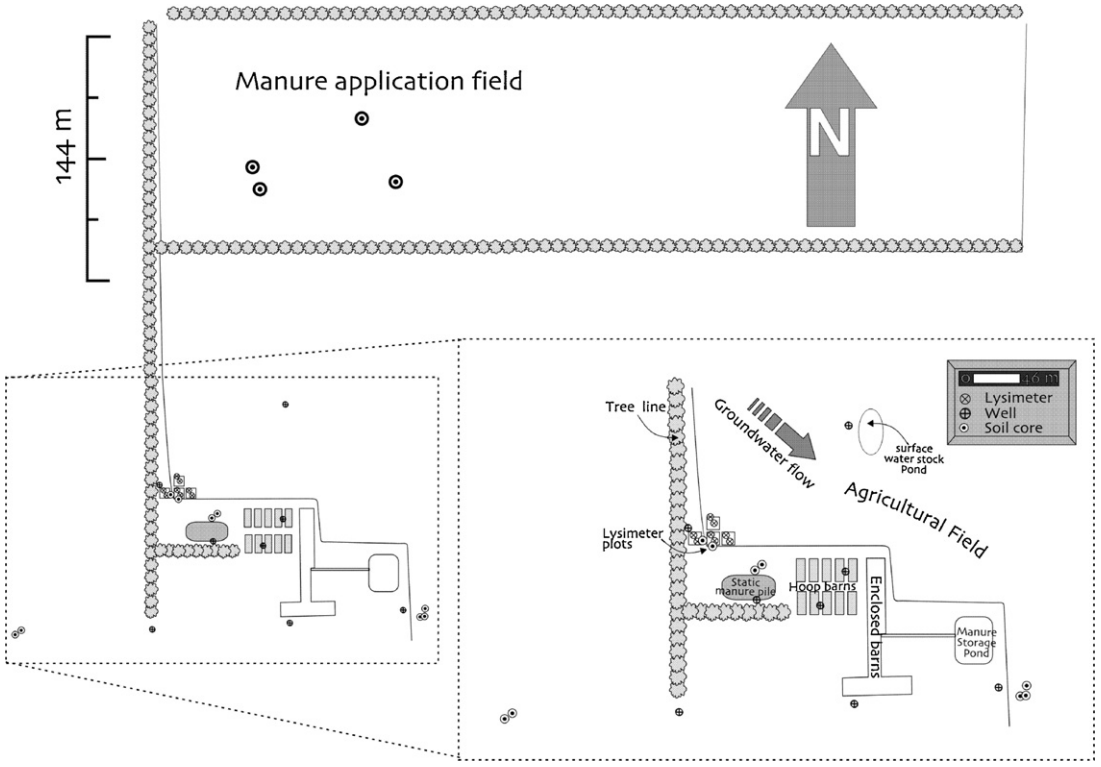


Fig. 1. Map of study site one.

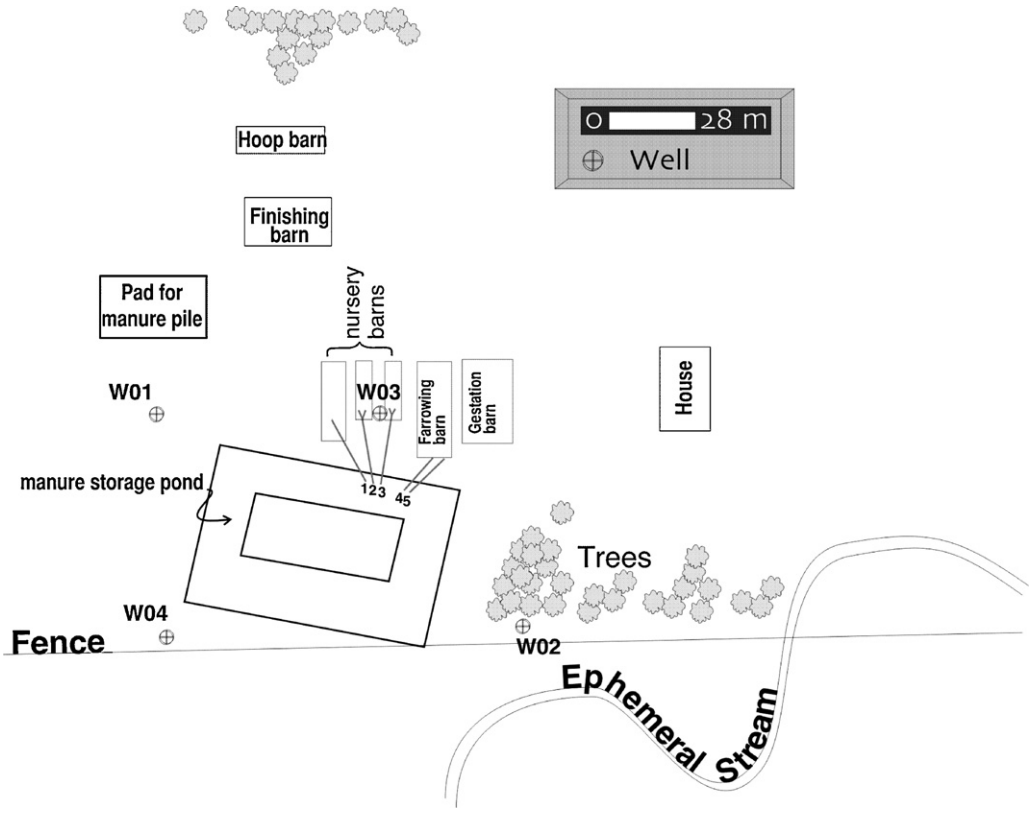


Fig. 2. Map of study site two.

2.3.3. Soil

The soils were first homogenized and 5 g subsamples were weighed and used for the SA analysis. A control soil that was obtained at least 150 km away from both study sites and has no recent history of agricultural activity was used as a negative control sample. Each set contained a negative and a positive control. For the positive control 100 μL of a 40 ng/mL SA mixture were spiked into a 5 g control sample. To each sample was added 100 μL of a mixture of 100 ng/mL SMX-d_4 and SMZ-d_4 as the internal standard. The soils were then extracted three times with 30 mL of water. The supernatant was adjusted to pH 7 with 1N HCl and filtered through a 1.2 μm filter followed by SPE cleanup.

2.4. SPE procedure for ground, surface, and lysimeter waters

The SPE cleanup procedures were similar to those reported by Shelver et al. [29]. Briefly, samples were passed through preconditioned Oasis HLB cartridges (200 mg sorbent) at <5 mL/min. Conditioning consisted of 5 mL each of 50% MeOH/ethyl acetate, 2.5% ammonium hydroxide water/MeOH, and H_2O pH 4. Once samples were loaded, cartridges were washed with 4 mL each of 5% MeOH/H_2O and 5% MeOH/2% acetic acid, dried under vacuum for 30 min, and eluted with 5 mL each of 50% MeOH/ethyl acetate and 2.5% ammonium hydroxide water/MeOH. The combined eluant was removed with a stream of nitrogen gas and the sample was reconstituted with 50 μL of [^{13}C]SMZ at 200 ng/mL and 150 μL of 50% MeOH/H_2O containing 0.2% formic acid. The sample was centrifuged at 10,000 × *g* for 10 min, filtered through a 0.45 μm syringe filter, and stored in amber LC glass vials at −20 °C until analyzed.

2.5. SPE procedure for slurry manure and soils

Diluted liquid manure or soil extract was applied to a preconditioned SAX column (500 mg sorbent) on top of an Oasis HLB-Plus (225 mg sorbent) SPE cartridge. The pre-conditioning involved 10 mL each of 50% MeOH/ethyl acetate, 2.5% ammonium hydroxide water/MeOH, and H_2O (pH 7). Once the samples were loaded, the cartridges were washed with 10 mL of H_2O then the SAX cartridge was removed and discarded. The HLB-Plus cartridge was further washed with 10 mL each of 5% MeOH and 5% MeOH/2% acetic acid. The elution and re-constitution conditions were the same as described in the water section.

2.6. LC-MS/MS analysis

The LC-MS/MS consisted of a Waters Acquity pump in conjunction with a Waters Acquity triple quadrupole mass spectrometer using MassLynx 4.1 with TargetLynx™ to acquire, process, and quantify the data. Using TargetLynx™ allows simultaneous quantitation of three product ions, which permitted simultaneous measurement of two transition ion ratios for the analytes. Chromatography was done using an ACQUITY UPLC™ BEH C_{18} column (1.7 μm, 2.1 mm × 50 mm; Waters) and VanGuard pre-columns (1.7 μm, 2.1 mm × 5 mm) with a flow rate of 0.6 mL/min and an injection volume of 5 μL/sample. The UHPLC column was maintained at 40 °C and autosampler at 4 °C. The solutions for the binary gradient system were solvent A, 5% MeOH/H_2O with 0.2% formic acid, and solvent B, 100% MeOH with 0.2% formic acid. The solution transitions for the gradient system were as follows: at time 0 min 10% B; 0.5–4 min 10% B → 30% B; 4–5 min 30% B → 100% B; 5–5.5 min 100% B; 5.5–5.52 min 100% B → 10% B, and 5.52–6 min 10% B. For mass spectrometry, initially each SA to be analyzed was directly infused using electro-spray ionization in positive mode to identify the precursor ion, product ions, and optimum cone voltage and collision energies using Autotune. For each SA, the sum from three product ions was used for quantitation as summarized in Table 2. Using the three product ions for quantitation improves sensitivity over quantitation of a single ion. This also offers greater

Table 2
UPLC-MS/MS parameters and performance for the sulfonamides used in this study.

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	Cone (V)	r^2	Between run[a] (%CV)	Within run[b] (%CV)
Sulfaguanidine	0.29	215.0	155.9 107.9 91.9	14 22 14	30	0.9850	5.58	2.45
Sulfadiazine	0.54	251.0	155.9 107.9 91.9	16 23 28	35	0.9974	4.21	4.59
Sulfathiazole	0.63	255.9	155.9 107.9 91.9	14 24 27	35	0.9986	4.16	2.36
Sulfachloropyridazine	0.62	284.8	155.9 107.9 91.9	16 24 30	35	0.9932	9.95	7.91
Sulfamerazine	0.8	264.9	155.9 107.9 91.9	18 26 29	40	0.9988	4.15	3.65
Sulfamethizole	1.22	270.9	155.9 107.9 91.9	14 24 29	35	0.9983	9.91	3.05
Sulfamethazine	1.21	279.0	185.9 124.0 91.9	17 24 30	40	0.9989	2.43	2.76
Sulfamethoxypyridazine	1.4	280.9	155.9 107.9 91.9	18 24 32	35	0.9982	3.84	6.11
Sulfamethoxazole	1.77	253.9	155.9 107.9 91.9	17 27 31	35	0.9988	6.70	1.72
Sulfisoxazole	2.13	267.9	155.9 113.0 91.9	14 16 28	35	0.9981	5.68	3.74
Sulfabenzamide	2.37	276.9	155.9 107.9 91.9	14 26 26	30	0.9980	6.25	3.74
Sulfaphenazole	2.87	314.9	158.1 131.0 91.9	28 54 42	45	0.9988	4.78	3.35
Sulfadimethoxine	3.32	310.9	155.9 107.9 91.9	21 29 34	40	0.9982	5.10	3.11
Sulfaquinoxaline	3.63	300.9	155.9 107.9 91.9	16 26 31	40	0.9987	4.79	3.12

[a] Determined by injecting the check standard for 6 different runs with 4 injections per run.
[b] Determined by injecting the check standard from a single assay with 10 repeat injections.

confidence that the product ions originated from the precursor ion of interest. The source temperature was set at 150 °C and desolvation temperature was set at 500 °C. Capillary voltage was set at 3.5 kV. Cone gas flow of nitrogen was set at 50 L/h and desolvation gas flow was at 800 L/h while collision gas flow of argon was set at 0.15 mL/min. A calibration curve using duplicate injections of 0.5, 1, 2, 5, 10, 20, 100, 200, and 500 μg/L of each compound prepared in nanopure water/MeOH (1:1, v/v)/0.2% HCOOH and analyte area*(IS concentration/IS area) vs. concentration was analyzed using linear regression utilizing TargetLynx™ software that computes and displays the equation of the calibration curve, r^2, the response plot and the residual plot. Sulfamethazine-d_4 was used as the internal standard for SMZ and surrogate internal standard for SG, SDZ, STZ, SCP, SMR, SMTZ, and SMPD while SMX-d_4 was used as the internal standard for SMX and surrogate internal standard for SSX, SB, SPZ, SDM, and SQX. [^{13}C]Sulfamethazine was used as an injection standard to verify the reproducibility of the injection. The response and the residual of the response from the mean response for both the deuterated and the ^{13}C standards were monitored for consistency. The sample concentrations were computed from the standard curves using instrument software and are uncorrected for recovery. Fig. 3 shows total ion chromatograms of SA mixtures prepared in 50% nanopure water/MeOH containing 0.2% formic acid, the positive spikes from state well water, liquid manure, and soil at comparable concentrations (20 μg/L).

3. Results and discussion

3.1. Sample clean up optimization

Because of the diverse physicochemical properties of the SAs, purification of SAs using SPE can be problematic and requires careful selection of the SPE column and conditions. The conditions were selected by adjusting pH, wash, and elution solvent composition.

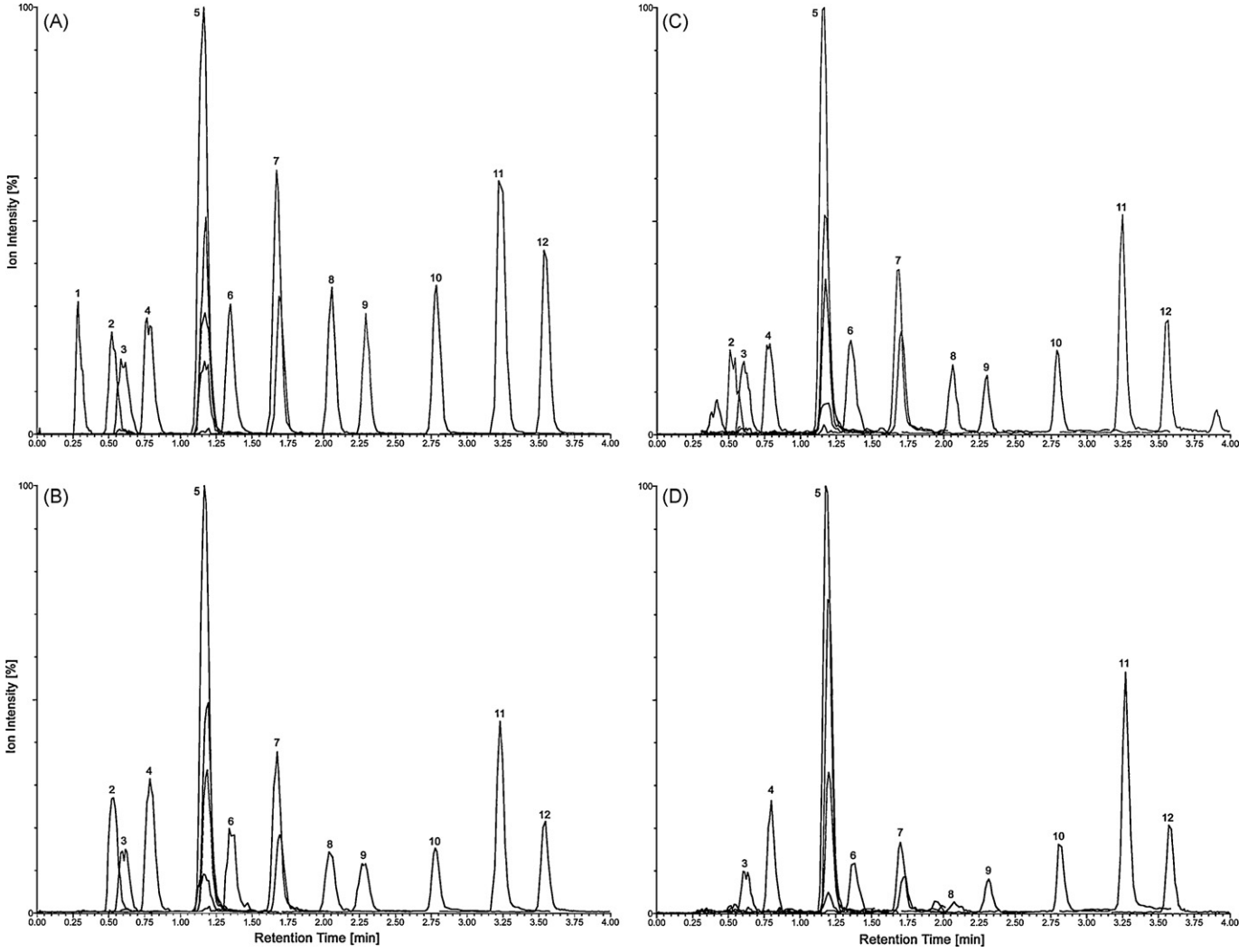


Fig. 3. Representative total ion chromatograms of SA mixtures prepared in nanopure water (A), the positive spikes from state well water (B), liquid manure (C), and soil (D) at comparable final concentrations of 20 μg/L reconstituted in 50% MeOH/H_2O containing 0.2% formic acid. Peak 1, SG; peak 2, SDZ; peak 3, STZ/SCP; peak 4, SMR; peak 5, SMTZ/SMZ/[^{13}C]SMZ/SMZ-d4; peak 6, SMP; peak 7, SMX/SMX-d4; peak 8, SSX; peak 9, SB; peak 10, SPZ; peak 11, SDMX; peak 12, SQ.

Tests of several types of SPE columns indicated that the HLB column had the best retention for the broadest range of SAs. Furthermore, several other studies have reported successful applications for a variety of chemicals, including SAs, using HLB columns [8,9,24,30].

For slurry manure and soil samples, tandem SPE columns (SAX/HLB) were used. Initially, liquid extraction with different solvents at different pHs (2–9) followed by application to HLB (pH 4–7 for loading, 5–30% methanol for washing, and different elution conditions) was explored. Different types of SPE cartridges, and combinations of HLB with other types of SPE cartridges were tested. The use of the SAX and HLB cartridge combination was chosen because it provided the most satisfactory recoveries from the spiked samples. The SAs could pass through the SAX column while the unwanted organic materials were retained. The HLB column, located below the SAX, retained the desired SAs, which could then be eluted with an appropriate solvent.

3.2. Method evaluation

3.2.1. Quality assurance

A number of methods were utilized to provide quality assurance including independent standards to assure proper system performance, sample blanks to check for carryover, and rigorous adherence to ion ratios assuring proper identification of the analyte. To provide validation of the process, an independent check standard (50 μg/L in nanopure water/MeOH (1:1, v/v)/0.2% HCOOH) was injected approximately every twenty injections and the concentration computed which was required to be within 30% of the expected value. The within day variations were much smaller <10% ($n > 3$ for each set). This provided assurance the standard curve functioned correctly to compute accurate concentrations. A sample blank was injected after the check standard as well as after the highest concentration of the calibration standard to assure that there was no carry over. Three transition ions were summed and two ion ratios were simultaneously monitored that were within 30% of the target value. Earlier exploratory work indicated the sum from the three transition ions improved the sensitivity as well as selectivity because of the additional transition that was monitored. Our ability to be able to monitor three transitions is due to the greater capabilities of Targetlynx™ software over Quanlynx software which can only monitor 2 transition ions simultaneously. Because concentrations lower than 2 μg/L did not consistently meet ion ratios, they were not included in the calculation of the ratio average or sample quantitation. The reported quantitative values were required to have the same retention time as the calibration standard (±20%), the same ion ratios (±30%), and S/N > 10.

Table 3
Instrument detection limits (IDL), instrument quantitation limits (IQL), method detection limits (MDL), mean spiked recoveries and coefficient of variation from ground water, liquid manure, and soil.

Compound	IDL[a] (pg)	IQL[a] (pg)	MDL[b] water (ng/L)	MDL[b] slurry manure (ng/L)	MDL[b] soil (ng/kg)	Water		Slurry manure		Soil	
						% rec[c]	% CV	% rec[d]	% CV	% rec[e]	% CV
Sulfaguanidine	4.2	8.6	1.9	15.6	32.4	4.7	13.8	4.1	24.4	3.2	36.9
Sulfadiazine	1.9	6.0	0.9	9.2	16.8	157.3	14.9	82.4	18.8	13.6	59.7
Sulfathiazole	2.2	5.5	0.9	10.2	26.0	78.8	7.4	80.6	10.5	50.8	8.2
Sulfachloropyridazine	27.9	88.8	9.1	264.0	343.6	80.5	14.9	64.4	41.6	59.7	30.7
Sulfamerazine	1.7	5.1	0.5	6.0	13.2	112.5	22.2	85.6	7.0	110.6	7.7
Sulfamethizole	2.9	6.5	1.3	16.6	44.4	61.4	5.9	44.0	10.9	29.4	22.6
Sulfamethazine	1.8	5.6	0.5	7.8	12.8	95.2	5.0	101.0	5.8	96.0	3.3
Sulfamethoxypyridazine	3.7	8.6	1.7	17.6	36.4	73.4	6.4	70.8	10.1	45.4	18.3
Sulfamethoxazole	1.7	5.3	0.6	11.8	41.6	97.8	2.9	109.2	9.0	107.9	7.2
Sulfisoxazole	1.1	3.0	0.6	8.8	26.8	77.3	5.8	63.1	10.5	27.8	38.3
Sulfabenzamide	1.3	3.1	0.6	10.0	33.6	90.1	13.1	80.8	9.6	115.8	7.3
Sulfaphenazole	0.8	2.4	0.4	6.2	26.4	63.9	10.5	67.7	11.1	115.6	23.3
Sulfadimethoxine	0.3	1.0	0.1	2.6	10.4	99.6	14.9	105.3	6.5	188.2	23.3
Sulfaquinoxaline	1.1	3.1	0.5	27.4	25.6	78.0	12.4	88.6	10.2	118.9	25.2

[a] Obtained from calibration curves using analyte dissolved in MeOH:H_2O 1:1 + 0.2% Formic acid to produce S/N > 3 (IDL) or S/N > 10 (IQL).
[b] Obtained from spiked matrices that went through the sample cleanup procedures.
[c] Mean of 6 different sets of samples spiked at 40 ng/L with duplicate injections.
[d] Mean of 8 different sets of samples spiked at 400 ng/L with duplicate injections.
[e] Mean of 8 different sets of samples spiked at 800 ng/kg with duplicate injections.

3.2.2. Calibration range and linearity

The calibration range used for all SAs was 0.5–500 μg/L and was linear with a coefficient of determination (r^2) > 0.99 except sulfaguanidine (r^2 > 0.98) (Table 2). When sample concentrations exceeded the highest calibration point, an expanded calibration curve (0, 1, 5, 10, 50, 100, 500, 1000, 5000, 10,000, and 20,000 μg/L) was made and samples were re-analyzed. The expanded calibration curve had r^2 > 0.99. We elected to use the expanded standard curve to avoid potential problems in diluting the sample affecting recovery or changing ion suppression/enhancement potential. The dynamic range of the instrument easily accommodates the expanded standard curve. Sample concentrations were within 15% of the original value using the expanded calibration curve.

3.2.3. Instrumental variation

The within run and between run variations were evaluated using a check standard at a concentration of 50 μg/L for each SA. For all the SAs, the within run CV determined from 10 replicate injections of the check standard were <10% (Table 2) with all but 2 < 5%. The between run variations using the average of duplicate determinations for 6 seperate runs had CV values <10% (Table 2) with all but 2 at <7%.

3.2.4. Sensitivity

Instrumental detection limits were defined as S/N value >3 and ranged from 0.25 pg on column for SDM to 27.9 pg on column for SCP (Table 3). Instrumental quantitation limit was defined as S/N > 10 and ranged from 1 pg on column for SDM to 88.8 pg on column for SCP. For ground water the method detection limits (MDL) ranged from 0.14 ng/L for SDM to 9.1 ng/L for SCP (mean of six different sets). For liquid manure the MDL ranged from 2.6 ng/L for SDM to 264 ng/L for SCP. For soil the MDL ranged from 10.4 ng/kg for SDM to 344 ng/kg for SCP.

3.2.5. Specificity

Each individual SA was analyzed based on the retention time as well as the precursor and product ions as shown in Table 2. No carry over was observed from sample to sample despite running blank samples after both check samples and high calibration samples.

3.2.6. Precision and accuracy

Precision and accuracy were determined using control matrices (described above) known to be free from sulfonamides, spiking them with the SAs and processing them as samples (including any SPE processes). The accuracy (recovery) was computed from the amount measured in this sample and the amount prepared in nanopure water/MeOH (1:1, v/v)/0.2% HCOOH. Precision was evaluated from repetition of the analysis. These repetitions were separate preparations from the beginning, including all processing such as SPE and thus provides an accurate measure of variation. For the spiked ground water samples, all the SAs showed satisfactory recoveries and CVs with few exceptions. Sulfadiazine had >150% recovery from ground water and its recovery from soil was approximately 14% with a 60% CV, whereas the recovery from slurry manure was an acceptable 82% with a CV of 19% (Table 3). Since this compound was not found in the current study sites from the slurry manure, the reason for the matrix dependency was not explored. All other SAs, except SG and SMR, had acceptable recoveries from ground water that ranged from 60% to 110% and CV values of less than 15%. Sulfaguanidine's structure contains a strongly basic group (pK_a 11) that will be positively charged under most conditions, which makes recovery potentially difficult. Recoveries for this compound were <10% and so this compound was dropped from further study.

The slurry manure samples had recoveries that ranged from 60% to 110% with the exception of SMTZ (44%). The CVs were below 15% with the exception of SDZ (~20%) and SCP (~40%).

The soil samples showed greater variations in recovery compared to slurry manure samples. The difference may be attributed to the complex sorption behavior of the SAs to the soil particles. Samples spiked after the soil extraction showed slightly higher recoveries (data not shown), which confirmed that variations in recovery were caused by the soil matrix. The complex sorption behavior of SA could be due to a number of properties of SAs and their interaction to different soil compositions. Because of the two pK_a's, SAs can exist as an anion, a neutral compound, or a cation and the charged molecule can bind to various soil components through ion exchange mechanisms or potentially hydrophobic sorption processes. In addition, the primary aromatic amine is capable of forming covalent bonds with a number of electrophiles. A portion of the SA is not extracted by normal solvent treatment, but that portion is believed to be irreversibly bound to soil so that it is of little environmental concern. Since the objective was to identify water

Table 4
Summary of sulfonamides found in different water bodies (ng/L) and soils (ng/kg) from study site one.

Source	Sulfathiazole	Sulfamethazine	Sulfamethoxazole	Sulfadimethoxine
Slurry manure	785–1702	2250–5060	108–1,470,000	
Mean (median)	1138 (1009)	3495 (3708)	138,000 (14,560)	
Quantifiable/*n*	12/12	12/12	12/12	
Surface water			43	2
Quantifiable/*n*	0/1	0/1	1/1	1/1
Wells		5.1	20.5	8.3–32
Mean (median)				21.5 (24.3)
Quantifiable/*n*	0/23	1/23	1/23	3/23
Lysimeters		2-2.3		
Quantifiable/*n*	0/5	2/5	0/5	0/5
Soils		34.5–663		
Mean (median)		170 (112)		
Quantifiable/*n*		14/43		

extractable SAs, the recoveries were based on SAs spiked to soil prior to water extraction. The recovery of SDM was >150%, which may have indicated some sort of interference/recovery problem, and so SDM levels in soil were not quantitated, even though some samples had matched retention time, ion ratios, and exceeded the limit of quantitation. The analysis demonstrated that SMZ, which was consistently found in the slurry manure, was quantifiable in the soil samples.

3.3. Sulfonamide detected at the study sites

3.3.1. Ground water, lysimeter water, and surface water

For the first farm location, the SAs most often detected in the ground water, lysimeter water, and surface water were SDM (4 out of 29 collections; concentration range 1.6–32 ng/L) and SMZ (3 out of 29 collections; concentration range 2.0–5.1 ng/L). Sulfamethoxazole was found in 2 of 29 collections of ground water at 20.5 ng/L and surface water at 43 ng/L (Table 4). These concentrations were lower than those found by Batt et al. [9] where SMZ was found in concentrations of 76–220 ng/L and SDM was found in concentrations of 46–68 ng/L and those found by Stoob et al. [31] where SMZ concentrations were 150–330 ng/L. This result may be due to differences in the number of hogs in the facilities, the time of collections relative to SA use, amount of SA use, differences in soils types (e.g. clay charges, organic matter content), and/or other factors.

3.3.2. Slurry manure

At the first farm, STZ, SMZ, and SMX were found in the slurry manure samples. Sulfathiazole concentrations ranged from 785 to 1700 ng/L and was found in all twelve collection samples. Sulfamethazine was also found in all twelve collections and concentrations ranged from 2250 to 5060 ng/L. These results were consistent with those reported by others in the USA [9,32] as well as other countries [22,31,33]. Sulfamethoxazole had the widest variation in concentration and was found in all 12 collections, and ranged from 108 to 1,470,000 ng/L (Table 4). The December samples yielded the highest concentrations. The reason for this was not identified but could be due to greater use during the winter months or greater persistence (less mobility or less degradation). When temperature is lower, the biological activity in the manure storage pond is also low, which may lead to lower degradation rates. Varying the sampling depth in the lagoon (surface, 100 cm, or 200 cm below the surface) produced little difference in concentration (data not shown). Different possible modes of degradation such as sunlight at the surface or anaerobic biodegradation deeper in the lagoon appears to either give little cumulative effect, or produced parallel degradation showing no difference between the layers.

The second farm demonstrated relatively few detections of SAs compared to the first farm. Sulfamethoxazole was the only SA found, and was present in only two of seventeen sample collections. A sample from pipe 3 (Fig. 2) that leads into the manure storage pond had a concentration of 138 ng/L, and the slurry manure at the bottom of the storage pond had a concentration of 38 ng/L. Pipe 2 sample results indicated there was sulfamethoxazole present but because the ion ratios did not meet the set criteria it was treated as not quantifiable. No SAs were found in the ground water or surface water from this location (out of 17 collections). The difference between the two farms most probably reflects differences in SAs antimicrobial usage (farm 2 engaged in organic farming practices), and demonstrates how the analytical procedure can detect the presence or absence of SAs.

3.3.3. Soil

The soil samples collected from plots that received slurry manure contained sulfamethazine (34.5–663 ng/kg, dry weight), and were only detected after the slurry manure had been applied. Sulfamethazine was detectable 1 year after manure application similar to the result reported by Christain et al. where SMZ was detectable 7 months after manure application [34]. We only found SMZ in the top layer of soil (0–15 cm) and not at the 46–61 cm depth (data not shown) and in general, the concentration decreased with time. Sulfamethazine was not detected in samples collected prior to the manure application. This method was suitable for analyses in various matrices and demonstrated the persistence of sulfamethazine in soil. Soil samples collected adjacent to the lysimeters or wells contained no detectable SA concentrations, and was consistent with the management of these sites, in which manure had not been applied in at least 2 years.

4. Conclusions

A rapid multi-residue method for the determination of commonly used antibacterial sulfonamides was developed. The method included development of sample clean up procedures using HLB or SAX coupled with HLB solid phase extraction that provided adequate purification prior to UHPLC-MS/MS. The chromatography required only 6 min total analysis time including column re-equilibration and permitted up to 80 samples to be measured per day. This allowed time for calibration curves and check samples to be run providing adequate quality control and validation of the operation. Blank samples after high calibration points and check samples demonstrated that there was no carry over, so any analyte found was unequivocally from the sample. By measuring three daughter ions, two ion ratios provided positive identification and assured the validity of the assay. Sensitivity was high, permitting quantitation of the low amounts in environmental samples. Application to multiple matrices in two farm settings verified the assay's utility in real world situations. The results indicated that SMZ present in slurry manure was transferred to soil after appli-

cation of the manure to the soil. The SMZ persisted for at least 1 year in the surface 0–15 cm soil. Very few surface or ground water samples contained SMZ, which indicated either high sorption to the soil, dilution to below detectable amounts, or low usage at current site. The method is clearly applicable to monitoring various matrices surrounding hog rearing operations and should facilitate developing guidelines to minimize environmental pollution.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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