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Identification of incurred sulfonamide residues in eggs: methods for confirmation by liquid chromatography–tandem mass spectrometry and quantitation by liquid chromatography with ultraviolet detection

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Abstract

Two complementary methods for identifying and measuring sulfonamide residues in eggs were developed for use in surveying eggs for potential drug residues. The first method uses liquid chromatography–tandem mass spectrometry (LC–MS–MS) to confirm the presence of sulfonamide residues in eggs. During its validation the limit of confirmation was estimated to be 5–10 ng/g (ppb) depending on the drug. Also, a method for measuring residue level by liquid chromatography with ultraviolet detection (LC–UV) was validated using the same extraction procedure as the confirmatory method. The determinative method was validated over the 50–200 ppb range. Samples were prepared by homogenizing whole egg, extracting with acetonitrile, and cleaning up with a C_{18} solid-phase extraction cartridge. For confirmation, analytes were separated by gradient LC on a C_{18} column, ionized by electrospray ionization (ESI), and detected by MS–MS with an ion trap mass spectrometer. For determination, analytes were separated by a different gradient LC procedure and detected by UV at 287 nm. Fifteen drugs were dosed individually in laying hens, and residues of parent drug and/or metabolites were found in eggs for all the drugs. Validation was based on repetitive analyses of control samples, control samples fortified at 100 ppb sulfonamides, and samples of blended incurred eggs.

Keywords: Sulfonamides

1. Introduction

Liquid chromatography–tandem mass spectrometry (LC–MS–MS) can be an important surveillance tool in monitoring food tissues of animal origin for residues of animal drugs. Most antibiotics can be readily detected by LC–MS techniques such as electrospray ionization (ESI), while MS–MS provides the high specificity needed for target analytes in complex tissue extracts. We have developed an LC–MS–MS method for sulfonamide residues in preparation for conducting a survey of eggs for potential drug residues. As a corollary to validation of this method, it was demonstrated that residues from fifteen orally-dosed sulfonamides were transferred to eggs.

There are no sulfonamides approved for use in

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laying hens. The use of veterinary drugs for medicinal purposes in laying hens could result in violative drug residues in food meant for human consumption. Of all sulfonamides that are marketed, only sulfamethazine and sulfadimethoxine are approved for use in chickens. Furthermore, this approval extends only to broilers, not laying hens. The US Food and Drug Administration Center for Veterinary Medicine (CVM) has set tolerances for these two sulfonamide residues at 100 ng/g (ppb) in broiler muscle. Violative residues in eggs could result from giving medicated feed intended for broilers to laying hens.

A variety of LC–MS methods have been reported for the mass spectral analysis of sulfonamides in kidney [1] meat [2,3], fish [4] or milk [5,6]. Other methods have been based on gas chromatography–mass spectrometry (GC–MS) [7–13]. Methodology applied to the confirmation and/or assay of sulfonamide residues in eggs includes LC–MS [14], GC–MS [15], LC–UV [16–18], and LC with post-column derivatization [19]. There have been several methods reported recently for determination of sulfonamides in kidney or urine using LC–MS–MS [20–22], and these new methods exemplify the trend towards LC–MS–MS for residue analysis. A wide range of compounds can be detected by LC–MS ionization techniques without derivatization and without exhaustive clean-up procedures. It was our goal to combine these advantages of LC–MS ionization with the scan MS–MS capability of ion trap tandem mass spectrometry to produce a highly-specific yet broadly-applicable surveillance method.

The occurrence and depletion profile of some sulfonamides in eggs have been studied [23–26], although not all methods reported for sulfonamide detection in eggs have include analysis of residue-incurred eggs. The drugs selected for the present study covered a wide range of polarity, from sulfaguanidine (highest polarity) to sulfaquinoxaline (lowest polarity).

2. Experimental

2.1. Dosing

White leghorn hens (Dekalb Delta) were used in this study. Birds were provided a standard ration and environmentally housed to maintain a normothermic environment and lighting regime. Control eggs were obtained from laying hens prior to dosing. Individual hens were dosed orally with single drugs in capsules at 10 mg/day for 2 days, approximately 24 h apart. Eggs were collected for 10 days after the initiation of dosing.

2.2. Standards

Sulfonamide standards were obtained from Sigma (St. Louis, MO, USA). Drug names and structures are shown in Fig. 1. Stock standards (SS) were prepared at 1000 μg/ml in LC grade methanol (Burdick & Jackson). A mixed intermediate standard (IS) was prepared at 10 μg/ml by combining 1 ml

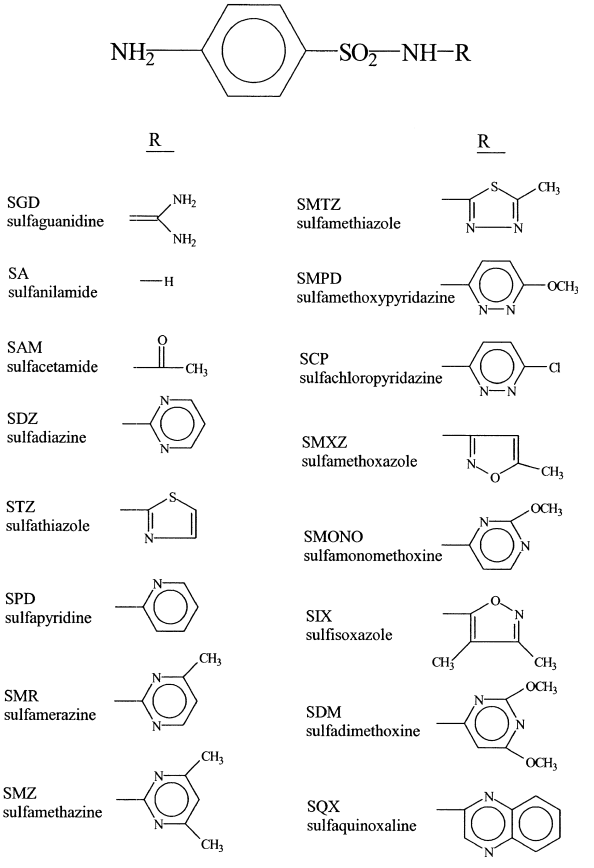


Fig. 1. Structures for the 16 sulfonamides evaluated in this study.

each SS and diluting to 100 ml with water purified through the Milli-Q system (Millipore). Working standards (WS) were prepared by serial dilution of the IS with water to yield solutions of 1.0, 0.5, 0.25 and 0.1 μg/ml. Based on a five-fold concentration during the extraction, the working standards were equivalent to 200, 100, 50 and 20 ng/g (ppb), respectively. WS standards were stored at <-20 °C.

2.3. Sample preparation

Whole eggs (yolk and albumen combined) were blended with a Polytron homogenizer (Brinkmann) while immersed in an ice bath. Blended samples were extracted immediately or stored at <-60 °C.

2.4. Extraction

A 5-g amount of blended egg was fortified by adding appropriate volumes of the 1 μg/ml WS (for example, 0.5 ml=100 ppb). Fifteen ml LC grade acetonitrile (B&J) were added. The mixture was homogenized while immersed in an ice bath. The tubes were centrifuged at 0–4 °C for 10 min at 3000 *g*. After the supernatant was removed, 5 ml acetonitrile was added to the pellet and the homogenization and centrifugation steps were repeated. Water (3 ml) was added to the combined acetonitrile fractions.

The volume was reduced to about 1 ml under nitrogen stream at 40–45 °C. This step was critical to remove acetonitrile before solid-phase extraction (SPE). The SPE cartridge was a C_{18} Sep-Pak (6 ml, 1 g sorbent, cat. no. WAT036905, Waters, Millipore). Approximately 1 ml water was left in the SPE reservoir after conditioning with acetonitrile and water. The extract was loaded on the cartridge after vortex-mixing. The flow-rate was 1–2 drops/s. The cartridge was drained of liquid under vacuum but air was not drawn through it. Sulfonamides were eluted using 3 ml acetonitrile. A vacuum was applied to drain the cartridge into the receiving tube.

A 1-ml volume of water was added and mixed with the eluate by vortexing. The volume was reduced to about 0.5 ml under nitrogen stream at 40–45 °C. It was critical to not let the extract go to dryness. Water was added to the extract to yield a final volume of 1.0 ml. After vortex mixing, the sample was passed through a 0.2-μm PVDF acrodisc filter (Whatman PVDF syringe filter, 13 mm, 0.2 μm) using a disposable polypropylene syringe. During the extraction procedure, normal safety precautions were observed for protective clothing, solvent handling, and waste disposal.

2.5. Liquid chromatography–tandem mass spectrometry

The LC–MS–MS system consisted of a model HP 1050 LC pump and model HP 1100 autosampler (Agilent Technologies) and a Finnigan LCQ classic operated in positive ion electrospray ionisation (ESI) mode. The LC column was a hydrophilic-modified C_{18} silica-based column (ODS-AQ, YMC), 50×4 mm, with 3 μm silica. The mobile phase consisted of a multistep gradient combining (A) 0.1% formic acid in water and (B) methanol. The column was first equilibrated at 97% A and 3% B. The gradient was developed to elute only one or two sulfonamides at a time using a flow-rate of 450 μl/min. After injecting 50 μl at 0 min, the mixture was held at 97:3 for 0.5 min; stepped to 76:24 at 0.6 min; ramped to 60:40 at 10 min; stepped to 45:55 at 10.1 min; held at 45:55 until 14 min; ramped to 10:90 at 15 min; held at 10:90 until 20 min, and reequilibrated at 97:3 for 9 min.

Two ESI tunes files were used to compensate for different elution conditions throughout the gradient. Source conditions were optimized while infusing standard into 0.1% formic acid–methanol at either 80:20 or 97:3 ratio. The LCQ autotune program was used for daily optimization of voltages on the capillary, tube lens, octapoles, and interoctapole lens. For both tunes, the ESI needle voltage was set at 5 kV, automatic gain control was on, maximum isolation time was 500 ms, and one microscan per scan was acquired. For the 97:3 composition, a typical tune optimization based on SGD was as follows: sheath gas pressure, 90 p.s.i. (1 p.s.i.=6894.76 Pa); auxiliary gas pressure, 10 p.s.i.; capillary temperature, 250 °C. For the 80:20 composition, a typical tune optimization with STZ was as follows: sheath gas pressure, 80 p.s.i.; auxiliary gas pressure, 20 p.s.i.; capillary temperature, 225 °C.

Ion trap tandem mass spectral acquisition parameters are presented in Table 1. MH^+ ions were isolated and dissociated to produce full scan product ion mass spectra. MS–MS isolation width was 1.5 Da and collision energy was 24% (relative units) in all cases, except SA, where only MS data were acquired.

Data were processed by creating reconstructed ion chromatograms (RICs) for each analyte using up to three prominent ions. Product ion spectra were then averaged across each peak at about 10% full height and above. If no peak was evident, spectra were averaged across the expected time period. The product ion mass spectra were inspected to determine if the following confirmation criteria were met: (1) the RIC peak signal-to-noise (S/N) ratio was >3; (2) the retention time matched within 2% of standards; (3) the structurally-specific product ions were $>2\%$ relative abundance; and (4) the sample's product ion spectrum visually matched a contemporaneous standard spectrum, with a general correspondence between relative abundances. Since full scan data may include hundreds of significant data points for comparison, strict numerical criteria were not applied. Because protonated molecular ions were isolated before being completely dissociated, the appearance of at least two structurally-specific product ions in the MS^n spectrum was considered sufficient.

2.6. LC–UV quantitative method

Extracts prepared by the LC–MS procedure were also used to measure sulfonamide concentration in eggs by LC–UV. The LC–UV system consisted of a Series 410 quaternary pump, LC-95 UV–Vis spectrophotometric detector set at 287 nm, ISS-200 autosampler equipped with a 150-μl loop (PE Biosystems), and the TURBOCHROM 4 data system (PE Nelson). The LC column was a Symmetry C_8 (Waters) 25×4.6 cm, with 5 μm silica. The column was heated to 40 °C and flow-rate was 1.5 ml/min. Gradient elution was achieved with a ternary gradient combining (A) 0.1% formic acid–methanol (90:10); (B) methanol; (C) acetonitrile. The column was equilibrated at 95:0:5 for 10 min. After injecting 150 μl, the mixture was held at 95:0:5 for 18 min;

Table 1
Sulfonamide data acquisition parameters, LC–MS–MS[a]

Sulfa	Retention time (min)	MH^+	Segment	Segment duration (min)	Scan event	MS–MS scan range
SGD[a]	2.80	215	1	3.10		60–230
SA[a]	3.40	173	2	0.90		100–230[b]
SAM[a]	4.90	215	3	1.10		60–230
SDZ	5.40	251	4	0.40		70–270
STZ	5.80	256	5	0.60	1	70–270
SPD	5.90	250			2	70–270
SMR	6.40	265	6	0.65		70–270
SMZ	7.40	279	7	1.00	1	75–290
SMTZ	7.60	271			2	75–290
SMPD	8.00	281	8	0.80		75–290
SCP	8.90	285	9	0.95	1	75–290
SMXZ	9.20	254			2	70–270
SMONO	9.80	281	10	2.00	1	75–290
SIX	10.20	268			2	75–290
SDM	13.40	311	11	3.65	1	85–320
SQX	13.80	301			2	80–320

[a] For SGD, SA, and SAM a tune file optimized at 97:3 mobile phase was used. For the others, a tune file optimized at 80:20 mobile phase was used.
[b] Full scan MS.

ramped to 55:20:25 at 32 min; and held at 55:20:25 until 37 min. The column was flushed after each sample set for 30 min each with water, acetonitrile, and acetonitrile–water (25:75). Back-flushing with acetonitrile and methanol restored performance after analyzing 100–150 samples.

2.7. System suitability

Working standard solutions were prepared to be equivalent to 200, 100, 50 and 20 ppb, in eggs. Resolution between closely eluting pairs (SCP–SMONO and SDM–SQX) had to be at least 0.6, where

$$\text{Resolution} = 2(t_2 - t_1)/(W_1 + W_2)$$

t is the retention time and W is the peak width at 5% height. The correlation coefficient had to be at least 0.99 for the standard curve. The relative standard deviation of peak height for repetitive injections of the 0.5 μg/ml standard had to be no greater than 5%. The S/N ratio for 0.50 μg/ml working standard had to be greater than 25:1.

3. Results and discussion

3.1. Extraction

The amino-propyl SPE procedure [1] was tested at 100 ppb fortification, but apparent recoveries were unsatisfactory (well below 60%) for some of the drugs. A C_{18} SPE procedure was tested and found to be satisfactory. This method had been developed in our laboratory for determination of cephalosporin drugs in milk [27], and was used with only minor modifications.

3.2. LC–MS–MS method

Mass spectral specificity was found to be acceptable for confirmating 14 of the 16 parent drugs studied. The exceptions were SA and SAM. Product ion complexity for the other 14 compounds was sufficiently diagnostic to meet the confirmation criteria described above. Fig. 2 shows a general scheme for predicting product ion structures resulting

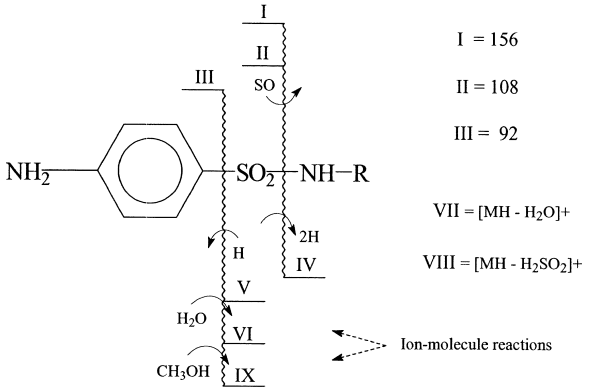


Fig. 2. Proposed scheme for product ion formation from sulfonamide MH^+ ions via ion trap MS–MS.

from collision-induced dissociation of MH^+ ions in the ion trap. Figs. 3–6 show the product ion spectra from all compounds tested. Table 2 compares the MS–MS product ions among the various drugs according to the fragmentation scheme of Fig. 2. For the fourteen drugs validated for this method, the mass spectra were very reproducible over many injections and multiple days.

All sulfonamides shared a common product ion at m/z 156. Fig. 7 shows an extracted ion chromatogram of m/z 156 from a working standard injection using the gradient described above and the time-scheduled MS–MS conditions in Table 1. Although the amount of each drug was the same, the relative abundances of m/z 156 varied widely. This variation is possibly attributable to the relative basicities of the R groups (Fig. 1).

The elution times of all sulfonamides were very consistent during the development of this method. It was possible to schedule fairly narrow time segments for acquisition (as in Table 1) without significant time drift, even when runs of some forty injections were made overnight. This suggests that the extracts were free of coextractants that could have affected chromatographic performance.

Two sulfonamides shared the same molecular mass, SMPD and SMONO. Although the product ion spectra contain the same ions, both the the relative abundances and retention times varied significantly from the other, so they would not be mistaken for

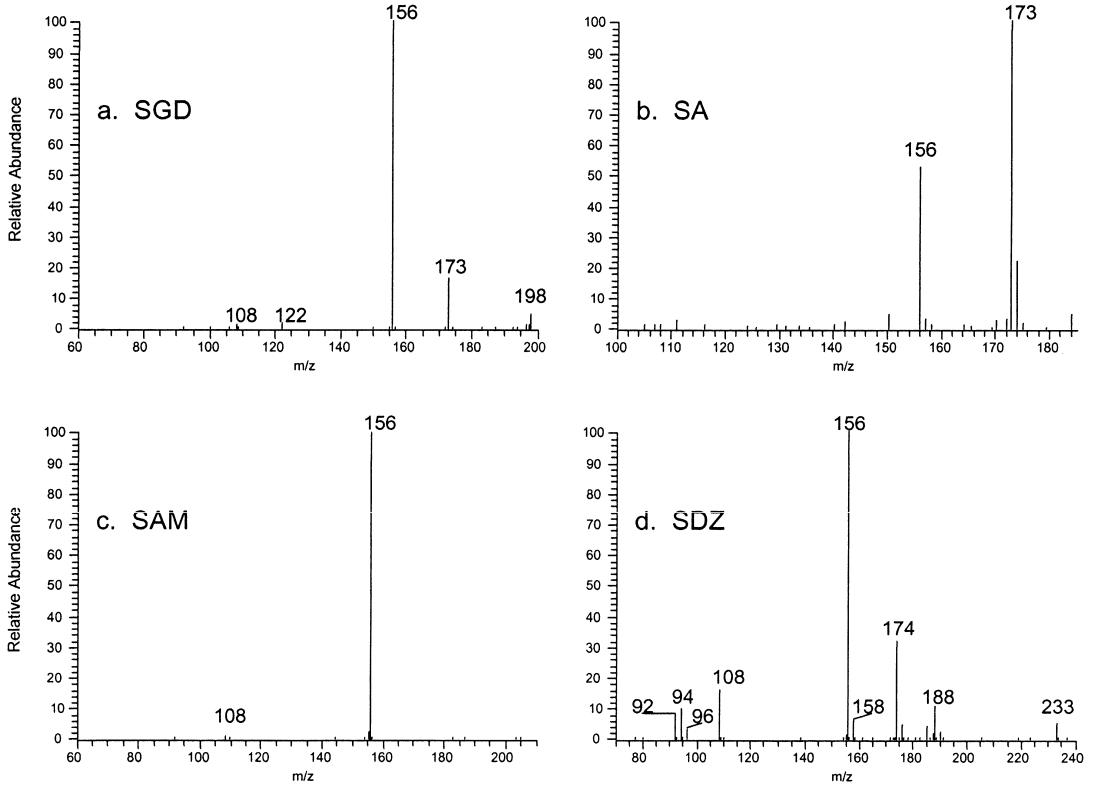


Fig. 3. Ion trap MS–MS spectrum from sulfaguanidine (a); MS spectrum from sulfanilamide (b); MS–MS spectrum from sulfacetamide (c); MS–MS spectrum from sulfadiazine (d).

one another (Figs. 5b and 6a). The product ion spectra of SMTZ and SCP (Fig. 5a and c) are nearly identical, but the compounds differ in molecular mass and retention time, eliminating the possibility of misidentification.

SA yielded only one very weak MS–MS product ion at m/z 156, and with this limited diagnostic information, confirmation was not possible. The SAM MS–MS spectrum contained only two product ions, with one at such low intensity that it sometimes did not appear above 2% relative abundance. Analysis of both compounds was complicated by losses on extraction. Although confirmation of SA was not possible in many cases, hens were dosed with SAM. Hens were not dosed with SA. Even though problems confirming SA and SAM were noted, fortified samples were prepared which included these two compounds.

In some cases product ions occurred due to ion-molecule reactions during CID (Fig. 2, type VI and IX). This is a characteristic of ion trap MS–MS, resulting from the presence of neutral vapor molecules in the collision region. The structure assigned for the type IX ion from SMZ (m/z 218) was verified by an infusion study where acetonitrile was substituted for methanol. The ion at m/z 218 was virtually eliminated as a result.

Method validation consisted of analyzing a set of seven control samples, eight samples fortified at 100 ppb, and sixteen samples consisting of blended residue-incurred eggs, incorporating three to seven drugs in each. To prepare the blended samples, three to seven individually incurred eggs were combined. The incurred samples were blinded to the analyst. Controls, fortified controls (quality assurance samples) and comparison standards were analyzed with each sample set to verify method performance. The presence or absence of drug was identified correctly

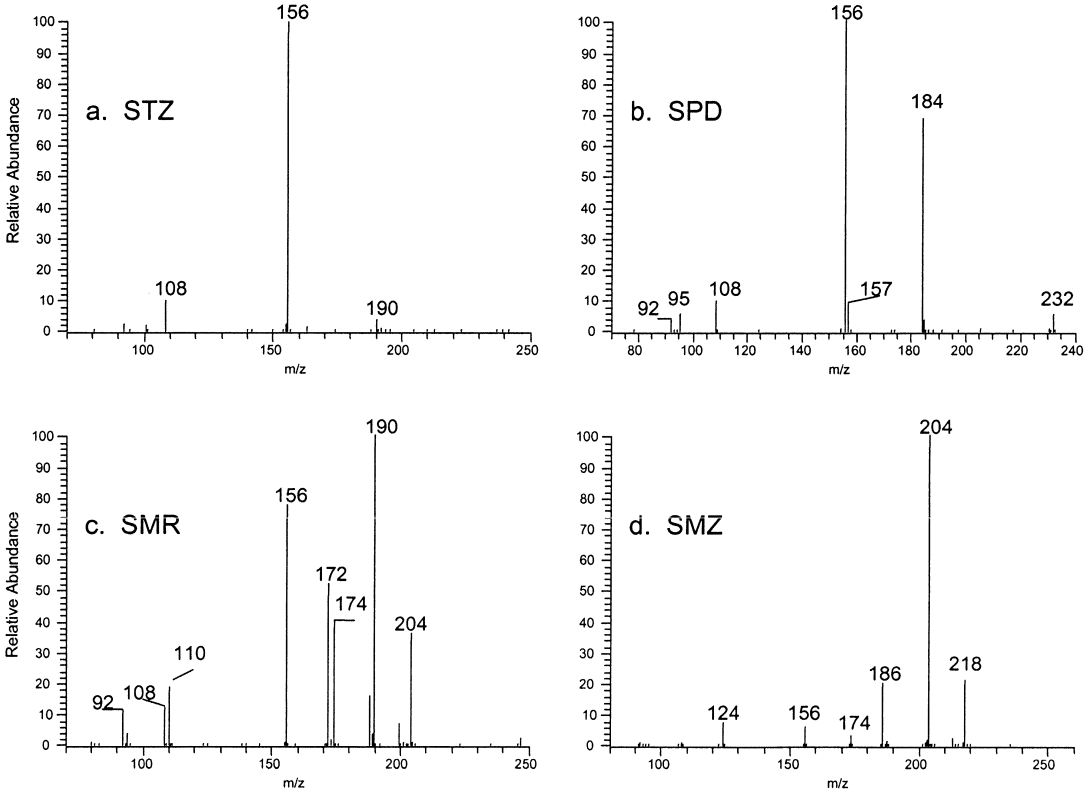


Fig. 4. Ion trap MS–MS spectra from sulfathiazole (a); sulfapyridine (b); sulfamerazine (c); sulfamethazine (d).

in virtually all cases for 14 drugs. As described above, SA and SAM did not perform well in this method.

SIX was confirmed in all samples of fortified eggs but only in three of five samples of residue-incurred eggs. Comparison with the working standards suggested that SIX was present below 10 ppb in the residue-incurred sample, which suggests the incurred level was below the method's limit of confirmation for SIX.

Control eggs showed no false positive identification for any drug, with one exception. In one of two replicate analyses of a control egg, SPD signals appeared at a level just sufficient to pass confirmation criteria. Comparison with standards suggested that SPD was present below 10 ppb. We believe this sample represented isolated low-level carryover during extraction at the method's limit of confirmation. (A second injection showed SPD signals, but confirmation criteria were not met.) To avoid false positives arising from such cases, it would be advisable to apply a more stringent S/N criterion, such as requiring that a 3:1 S/N be observed on the weakest diagnostic ion, rather than on the RIC.

This LC–MS procedure differed in several ways from that of Tarbin et al. [14] which was based on ion-exchange SPE, atmospheric pressure chemical ionization, selected ion monitoring with a single quadrupole instrument, and quantitation by comparison to deuterated SMR or SMZ. In contrast, our use of ion trap MS–MS provides very high specificity as required for regulatory confirmation.

3.3. Evaluation of LC–UV quantititative performance

The LC–UV method was evaluated by analysis of five replicates of control samples fortified at 50, 100, and 200 ppb samples, respectively, and four replicates of control samples. Examples of standard,

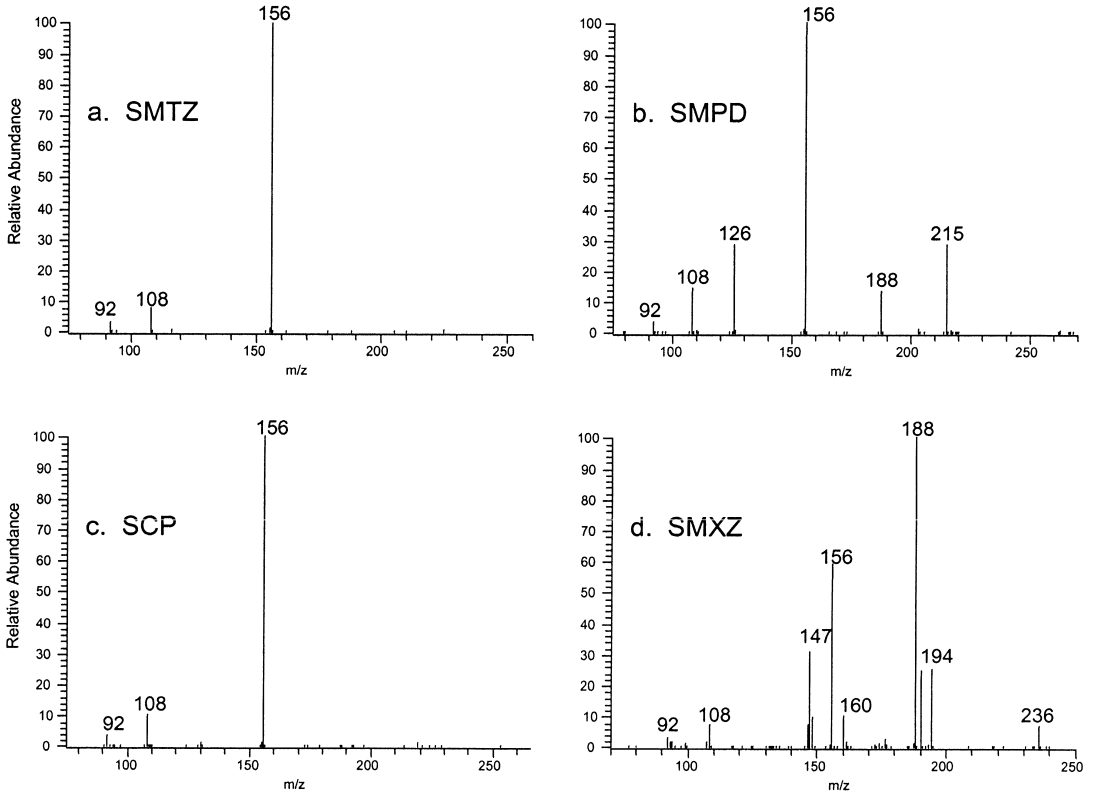


Fig. 5. Ion trap MS–MS spectra from sulfamethizole (a); sulfamethoxypyridazine (b); sulfachloropyridazine (c); sulfamethoxazole (d).

control, and fortified chromatograms are shown in Fig. 8. Average percent recoveries and relative standard deviations (RSD) are reported in Table 3. Also, five replicates of residue-incurred eggs were analyzed, but not all of the residues identified by LC–MS–MS fell within the quantitative range of the LC–UV system (50–200 ppb). Only five incurred drug residues could be assayed for parent drug content. Five replicates of these residue-incurred eggs were analyzed, and ppb levels found (with RSD, %) were as follows: SQX, 68 ppb (6%), SDM, 165 ppb (6%), SMZ, 99 ppb (15%), SMR, 62 ppb (6%), SDZ, 117 ppb (6%). The recovery and RSD values found meet CVM guidelines for determination of drug level in the 50–200 ppb range, except for SAM, whose recovery was about 55% with excessive variability, and SA, which was not distinguishable from interferences. It was necessary to manually integrate SGD and SMZ, due to matrix peaks eluting very close to their retention times. If SMZ was present above 200 ppb, the interference peaks merged with the SMZ peak.

3.4. Evaluation of LC–MS–MS quantitative performance

An attempt was made to measure drug level using LC–MS–MS data acquired in the course of the confirmatory analysis, for comparison to LC–UV results. RICs from 2 to 3 prominent ions were integrated. External standard curves were calculated using all standards from each sample set. Standard curves ranged from 20 to 200 ppb (2 days) or 2 to 200 ppb (1 day). Calibration parameters were: quadratic fit, weighting factor $1/x$, ignore origin. Sample concentrations were calculated in ng/g in egg (ppb), assuming 5.0 g of egg and a 5-fold concentration factor.

Eight control samples fortified at 100 ppb were measured over 3 days. Absolute response gradually

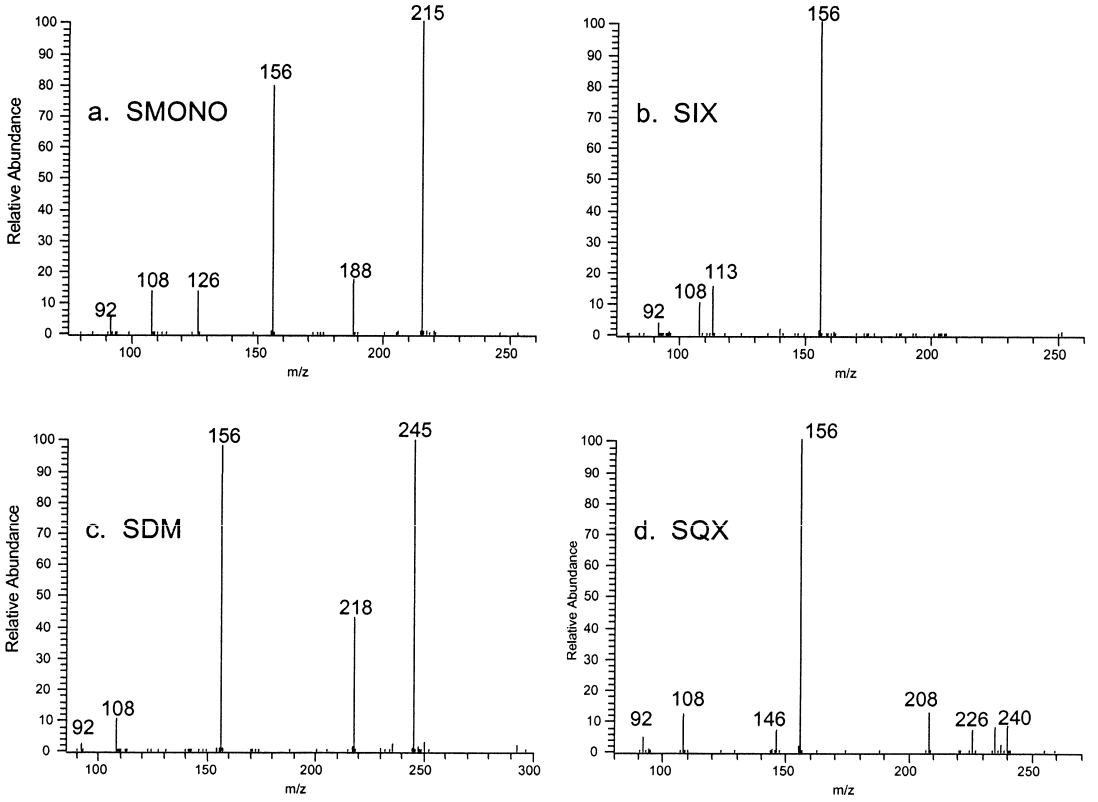


Fig. 6. Ion trap MS–MS spectra from sulfamonomethoxine (a); sulfisoxazole (b); sulfadimethoxine (c) sulfaquinoxaline (d).

Table 2
Sulfonamide product ions: ion trap MS–MS

Sulfa	MH^+	I	II	III	IV	V	VI	VII	VIII	IX
SGD	215	156	108			122		197		
SA	173	156								
SAM	215	156								
SDZ	251	156	108	92	96	158	176			
STZ	256	156	108	92		163			190	
SPD	250	156	108	92		157			184	
SMR	265	156	108	92	110	172	190		199	204
SMZ	279	156			124	186	204			218
SMTZ	271	156	108	92						
SMPD	281	156	108	92	126	188		263	215	
SCP	285	156	108	92						
SMXZ	254	156	108	92				236	188	
SMONO	281	156	108	92	126	188	206	263	215	
SIX	268	156	108	92	113					
SDM	311	156	108	92	156	218	236	293	245	250
SQX	301	156	108	92	146	208	226		235	240

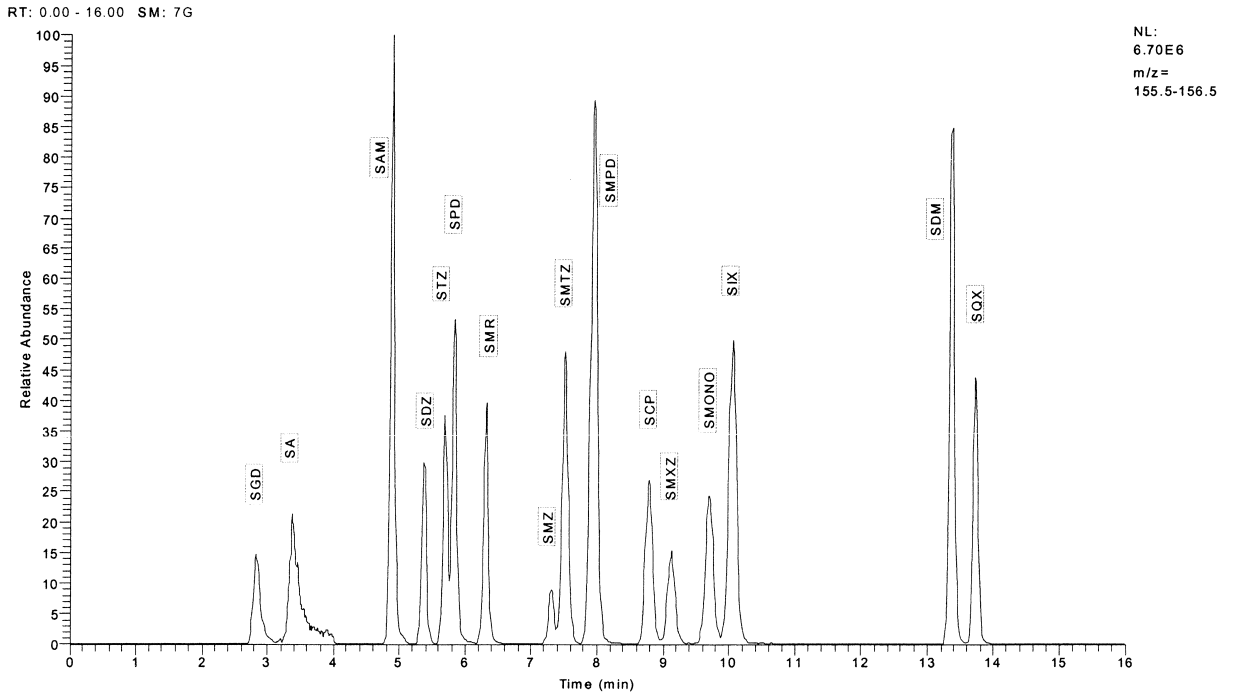


Fig. 7. Example of chromatography from time-scheduled MS–MS, showing the ion chromatogram for m/z 156 common to all sulfonamides.

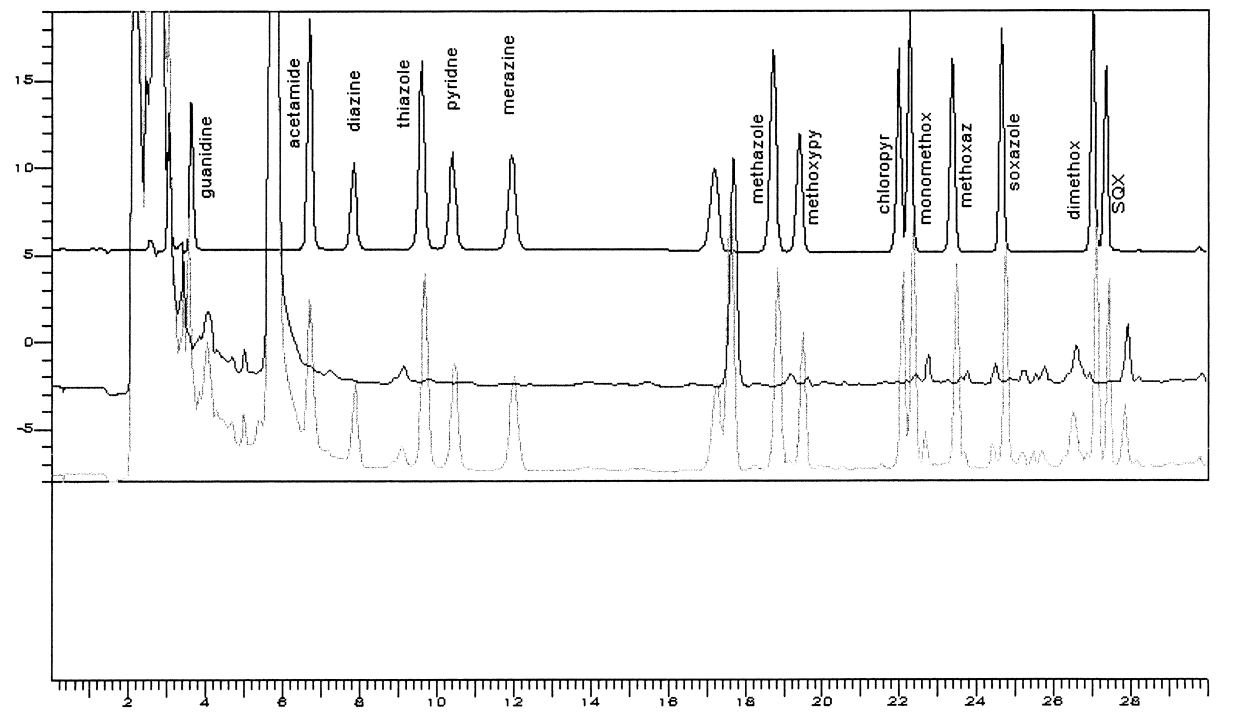


Fig. 8. LC–UV chromatograms showing sulfonamide standard, 0.5 μg/ml, equivalent to 100 ppb (top trace); control egg (middle trace); and extract of control egg fortified at 100 ppb sulfonamides (bottom trace).

Table 3
Quantitative performance, validation of LC–UV method with fortified samples

Drug	Recovery (%) (RSD, %)		
	50 ppb Fortified	100 ppb Fortified	200 ppb Fortified
SGD	90 (10)	92 (11)	95 (9)
SAM	58 (29)	54 (13)	51 (33)
SDZ	87 (14)	87 (8)	84 (10
STZ	92 (15)	93 (9)	93 (4)
SPD	100 (12)	100 (10)	101 (7)
SMR	93 (12)	93 (8)	92 (4)
SMZ	96 (10)	95 (8)	95 (6)
SMTZ	87 (14)	88 (8)	87 (3)
SMPD	103 (12)	98 (10)	96 (5)
SCP	86 (15)	86 (9)	86 (4)
SMXZ	95 (13)	91 (7)	88 (3)
SMONO	91 (15)	89 (9)	87 (3)
SIX	93 (12)	92 (9)	91 (5)
SDM	87 (13)	89 (9)	90 (5)
SQX	83 (15)	84 (10)	85 (5)

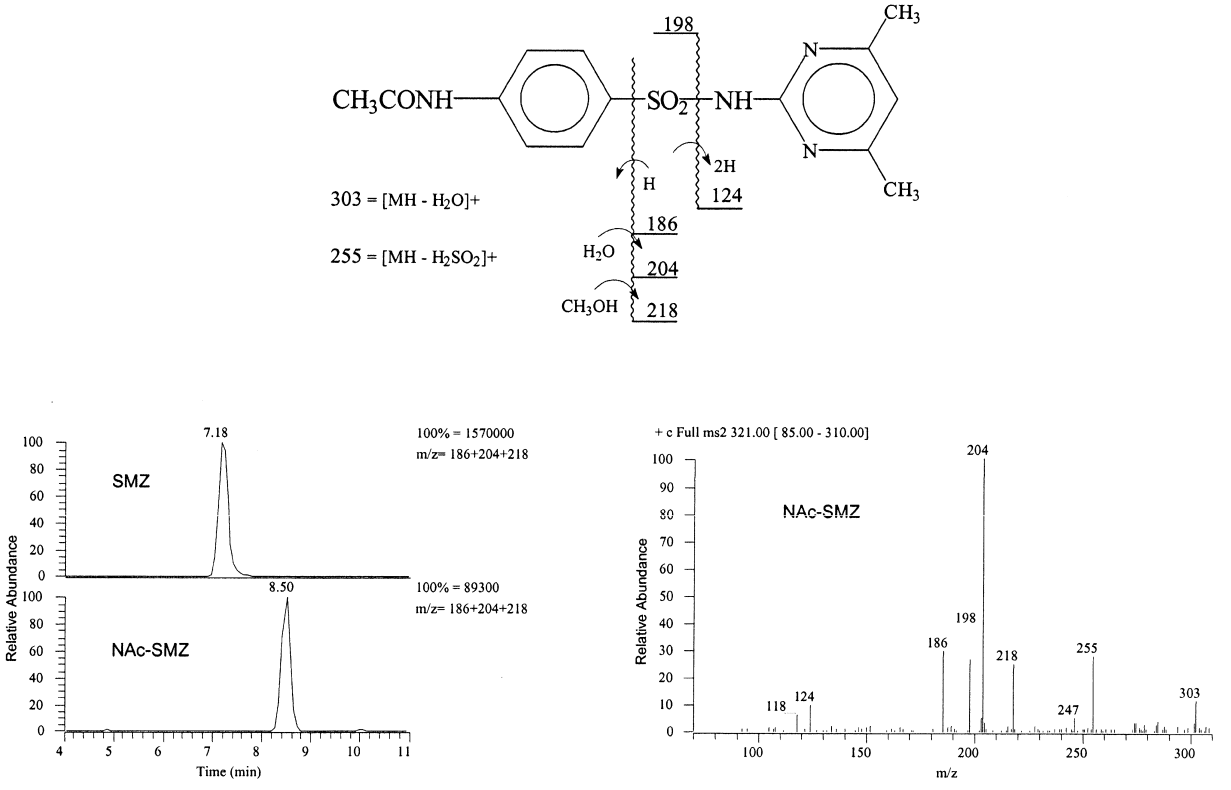
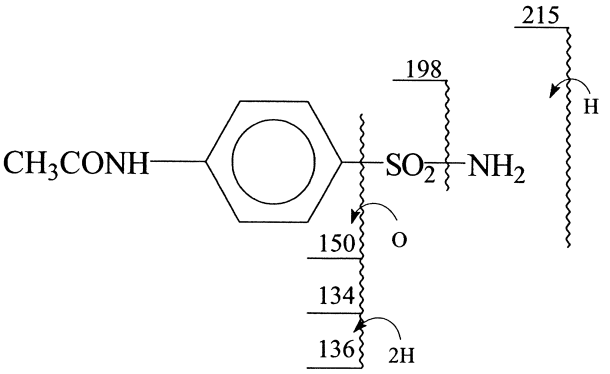


Fig. 9. N_4-Acetyl sulfamethazine: structure and product ion formation; comparison of retention time with SMZ, and MS–MS spectrum.

drifted lower, which caused poor precision when standard curves were extrapolated over time. RSD values for measurement of the fortified samples ranged from ~15–30%. Average 'apparent' recoveries ranged from ~80 to 110%, except for SDZ and especially STZ, which were significantly lower due to matrix suppression vs. pure standard (LC–UV results listed in Table 3 established that these two drugs were actually recovered fairly well). Overall, this approach to quantitation did not meet CVM guidelines for recovery, relative standard deviation, or linearity [28]. Nevertheless, comparison with standards enabled absolute limits of confirmation for 14 drugs to be estimated at 2–5 ppb in eggs, depending on the compound. Method limits of confirmation using more stringent S/N criteria (i.e. single ion chromatograms of weaker diagnostic ions) would be about 5–10 ppb, depending on drug.

3.5. Metabolite identification

N_4-Acetyl metabolites of sulfonamides have been reported in eggs [20]. Such metabolites would show MH^+ ions 42 Da higher than the parent drug. Using LC–MS–MS parameters that isolated the predicted metabolite masses and produced product ion spectra with 24% collision energy, we screened those individual residue-incurred eggs that had showed the highest parent drug response (SMZ, SDM, SQX, SDZ, STZ and SMR) for the predicted N_4-acetyl and



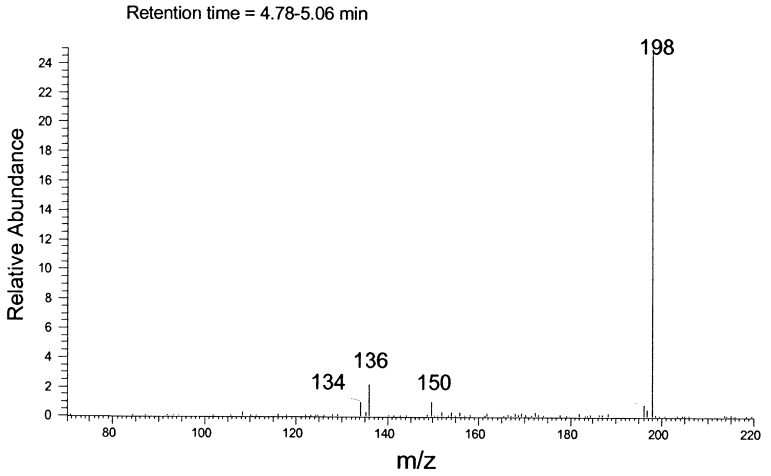


Fig. 10. N_4-Acetyl sulfanilamide metabolite of sulfacetamide: structure and product ion formation, and MS–MS spectrum.

des-amino metabolites (i.e. MH+42 Da, MH−15 Da, respectively).

The N_4-acetyl metabolite of SMZ was readily identifiable (Fig. 9). Also, evidence for N_4-acetyl metabolites of SDM and SQX was observed, in the form of type I ions (Fig. 2) +42 Da at m/z 198, but these MS–MS spectra did not include other corroborating details due to the low level observed. The apparent response level of these metabolites was quite low relative to the parent drug responses. The peak area responses differed by a factor of approximately 100, although no quantitative inferences can be drawn from this ratio, due to the possibility of differing ionization efficiencies or matrix suppression effects for each compound. All three N_4-acetyl metabolites eluted about 1–1.5 min after the corresponding parent drug. No evidence for the N_4-acetyl metabolite of SDZ, STZ or SMR was observed, and no evidence for the des-amino metabolite of the six sulfonamides was observed.

The LC–MS–MS method did not perform acceptably for SAM at 100 ppb fortification, so it was not surprising when SAM could not be confirmed in the incurred eggs. However, during screening analyses of the SAM-incurred eggs, a signal corresponding to the same molecular mass as SAM (MH+ at m/z 215) appeared at a later retention time. Examination of the MS–MS spectrum was suggestive of an N_4-acetyl sulfacetamide (N_4-SA) structure (Fig. 10).

Furthermore, no ions indicative of the N_4-acetyl SAM at m/z 257 were observed in scouting runs of the entire elution profile. We infer that SAM is heavily metabolized to the N_4-acetyl derivative, with concurrent loss of the acetamide moiety. Blom reported high rate of sulfanilamide metabolism to N_4-acetyl SA, in contrast to other sulfonamides which followed this metabolic route only slightly [20]. From this information, we conclude that N_4-acetyl sulfanilamide should be used as a marker for the dosing of either SA or SAM in laying hens, and should be included in survey methodology for such residues. For all other sulfonamides, the presence of the parent drug is the most appropriate marker for identifying cases of sulfonamide dosing.

The major advantages of this method are the application of a generic SPE procedure to the extraction of a wide variety of compounds, combined with a highly specific, full scan LC–MS–MS confirmation scheme. The method specificity is based on the capabilities offered by ion trap tandem mass spectrometry. This project will enable CVM to survey eggs for a wide range of possible drug residues. The effort to develop and validate this method gives us confidence that such methodology can be applied to more drug residues from other compound classes. Future work will be based on an attempt to expand this methodology to include drug classes other than the sulfonamides.

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