

Quantitation of Sulfamethazine in Pork Tissue by Thin-Layer Chromatography

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Our earlier method to detect and quantitate sulfamethazine (SMZ) in milk at the 10 ppb level was modified to quantitate SMZ in pork tissue. Sulfabromomethazine (SBZ) is added to the tissue as an internal standard. SMZ and SBZ are extracted from the tissue into water as the supernatant of a centrifuged, aqueous homogenate and are cleaned up and concentrated by a series of solid-phase extractions. The sulfonamide-containing eluate is then separated on a silica gel thin-layer chromatographic plate. SBZ and SMZ are derivatized with fluorescamine, and their fluorescence is quantitated with a scanning densitometer. The limit of detection was estimated at 0.25 ppb (signal-to-noise ratio, 3:1). The average accuracy over the analysis range (0.54–21.8 ppb [$\mu\text{g}/\text{kg}$]) was 95.6% (standard deviation = 29.4%, $n = 54$).

Sulfamethazine (SMZ) is a commonly used sulfonamide that is effective for treating various bacterial infections in food-producing animals. The United States has a current tolerance of 0.1 ppm for SMZ residues in edible swine tissues (21 CFR 556.670) (1), but recent reports from the National Center for Toxicological Research on the possible carcinogenicity of SMZ (2, 3) prompted the U.S. Food and Drug Administration (FDA) to consider lower tolerance limits, possibly into the low ppb levels (4, 5).

Current AOAC official methods for quantitating SMZ in swine tissue (982.40, 982.41, and 982.31) (6) are applicable only down to the 50 ppb level. Moreover, these methods are time-consuming and produce an appreciable amount of waste solvents. Goals of new methodology are to decrease the analysis time and reduce the amount of solvents used.

Solid-phase extraction (SPE) is one way to reduce high solvent consumption. A recently reported multiresidue method for sulfonamides in swine tissue uses a type of SPE (7) that requires very small amounts of halogenated and nonhalogenated solvents per sample (8 mL each) in the analyte isolation phase.

However, the method uses liquid chromatography (LC) for analyte separations, which produces a substantial volume of waste solvent; the method also uses a photodiode array detection system for quantitation, which results in a minimal detectable limit between 31 and 62 ppb (7).

Aerts et al. reported a sensitive multiresidue LC method (reported limit of detection, 5 ppb) for sulfonamides in meat tissue (8, 9). A continuous flow system involving column switching is used to isolate and concentrate the analytes from an aqueous tissue extract before LC analysis. Postcolumn derivatization with dimethylaminobenzaldehyde enhances both the sensitivity and selectivity of this method. The use of aqueous saline to quantitatively extract sulfonamides and chloramphenicol from tissues is successful at trace levels (10).

We recently reported a very sensitive method (quantitative range of analysis, 0.5–15 ppb) for detecting SMZ in milk (11). The method uses a series of solid supports to extract, isolate, and concentrate the analyte. After thin-layer chromatographic (TLC) separation, the analyte is quantitated by fluorescence densitometry. The method uses a total of about 20 mL nonhalogenated organic solvents per sample. Small modifications to the method for SMZ in milk permitted the use of an aqueous tissue extract (8–12) in conjunction with the series of solid supports. These modifications resulted in the method we now report. Results obtained from assays of swine tissue fortified with SMZ in the 0.5–20 ppb range are presented.

Experimental

Reagents

- (a) *Solvents*.—LC grade.
- (b) *Water*.—LC grade, from Modulab Polisher I system (Continental Water Systems, San Antonio, TX).
- (c) *Reagents*.—Baker analyzed (J.T. Baker, Phillipsburg, NJ) except for fluorescamine, SMZ, *N*-acetylsulfanilyl chloride, and 2-amino-4,5-dimethylpyrimidine, which were obtained from Sigma Chemical Co. (St. Louis, MO).
- (d) *Solutions*.—Prepare stock solutions of SMZ (Sigma Chemical Co., S-6256) and SBZ (11) at 1 mg/mL in acetone, and store at -80°C . Prepare working solutions in water of 1.0 μg SBZ/mL and 2.0, 1.5, 1.0, 0.8, 0.75, 0.50, 0.40, 0.25, 0.20, 0.10, and 0.05 μg SMZ/mL by diluting stock solutions. Prepare fresh working solutions monthly, and store at $0-5^{\circ}\text{C}$.

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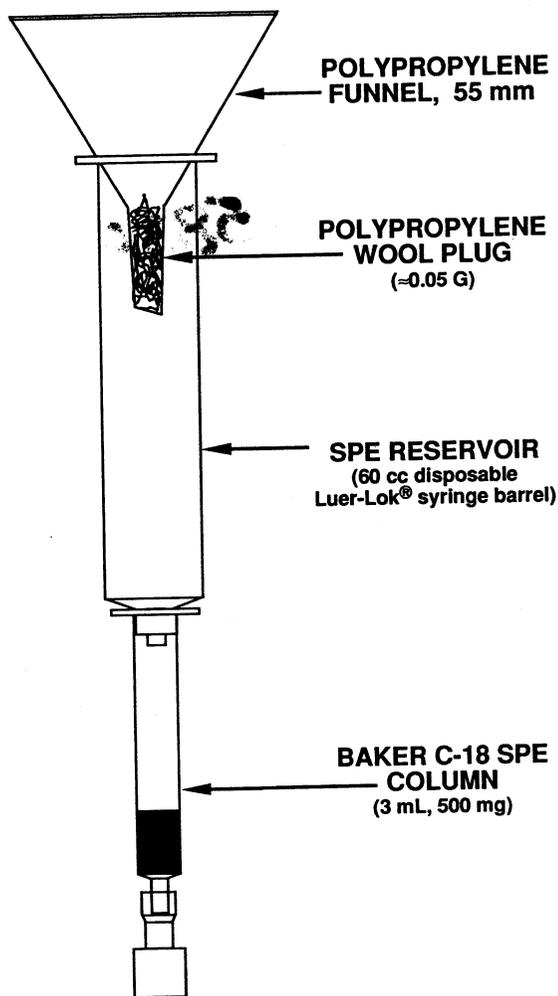


Figure 1. Filtration and extraction column setup. The Luer-Lok fits tightly into the 500 mg C₁₈ SPE column without the need for an adapter. The poly(propylene) wool plug is loosely packed to fill the stem of the funnel and is only intended to retain the fat that floats on the supernatant.

(e) *Acidic alumina.*—Purchase as activated and use as received, 95+%, -60 mesh (Alfa Products, Danvers, MA).

(f) *Cation-exchange resin.*—AG MP-1, 100–200 mesh, chloride form (Bio-Rad Labs, Richmond, CA).

Apparatus

(a) *Homogenizer.*—Polytron Model PT 10/35 (Brinkmann Instruments, Inc., Westbury, NY) equipped with Model PTA-20S generator, and operated at a setting of ca 4½ for 20 s.

(b) *Centrifuge.*—Refrigerated centrifuge (IEC Centra-7R, International Equipment Co., Needham Heights, MA) with 12 × 50 mL tube capacity rotor (Model 822A). Low-density poly(ethylene) centrifuge tubes with closures (Nalge No. 3112-0050, Fisher Scientific, Pittsburgh, PA). Mark tubes at 25 mL level before analysis. Centrifuge samples 15 min at 15°C and 4800 rpm (ca 4000 × g).

(c) *SPE manifold.*—Visiprep™, equipped with vacuum gauge and optional Teflon solvent guide needles (Supelco, Inc.,

Bellefonte, PA). Fit 1/4 in. piece of 3/8 in. rubber tubing around sample control valves of manifold to increase fingertip control of sample flow. Collect eluates in 10 × 75 mm disposable culture tubes (VWR, San Francisco, CA).

(d) *SPE setup.*—A reservoir, ≥50 mL, attached to extraction column (Bakerbond C₁₈, 3 mL, Baker) is required [we used 60 mL Luer-Lok poly(propylene) syringe barrel (Becton Dickinson & Co., Rutherford, NJ) with end cut off at 60 mL graduation mark; presence of Luer-Lok permits attachment without need of connecting adaptor]. Place 55 mm disposable poly(propylene) funnel (Fisher Scientific) in top of reservoir. Pack funnel stem loosely with ca 0.05 g teased poly(propylene) wool (Aldrich Chemical Co., Inc., Milwaukee, WI) to pre-filter sample (see Figure 1). Attach C₁₈ columns to the vacuum manifold and wash with two 3 mL volumes each of methanol then water; place an additional volume of 1.5 mL water above the bed.

(e) *Preparation of anion-exchange resin.*—Shake 10 g AG MP-1 (ca 1 min) with 300 mL 10% acetic acid in acetone, let settle 15 min, and decant. Shake resin with 300 mL water, let settle 15 min, and decant. Shake resin with 300 mL 2M HCl, let settle 5 min, and decant. After rinsing with water in a coarse-fritted funnel until water is neutral, shake resin 1 h, using a mechanical shaker, with 300 mL 0.2M K₂HPO₂ buffer, pH 7.9. Filter resin through a coarse-fritted funnel, wash with water until water is neutral, and dry in the funnel (vacuum 5 min). Store the 10 g of resin, refrigerated, in 200 mL ethanol-water (1 + 1), and use 0.5 mL suspension for column B.

(f) *Concentration column (column B).*—Use the end of a 1 mL disposable pipet tip with a 70 μm porous poly(propylene) disc (2.5 mm punched from 1.59 mm sheet 70 μm Fritware® [BEL-ART, Pequannock, NJ]), and add 0.5 mL anion-exchange resin suspension and let drain to waste.

(g) *Clean-up column (column A).*—Fill a Quik-Snap column (Isolab, Akron, OH) to the reservoir with methanol, and slowly pour 0.50 ± 0.02 g acidic alumina into the column. Place a bed of coarse sand (ca 5 mm) on top of the alumina after it has settled. Snap the bottom closure off, and place the column above the concentration column (column B, Figure 2), letting methanol drain through column B to waste.

(h) *TLC development and application.*—Use ascending one-dimensional development in a twin trough chamber, 10 × 10 cm (Camag, Muttenz, Switzerland) with chamber saturation for 10 min. TLC plates (10 × 10 cm) precoated with Silica Gel 60 were obtained from Merck (Darmstadt, Germany). Wash plates by immersing in methanol 5 min and then dry at 80°C for 30 min. Apply samples to TLC plate with Camag Linomat IV (Camag, Wrightsville Beach, NC). Use N₂ to spray samples onto TLC plate 10 mm from bottom edge at rate of 6 s/μL. Starting 10 mm from plate edge, apply samples in 6 mm bands separated by 4 mm. This arrangement permits 8 lanes per plate, 3 of which will always be standards.

(i) *TLC detection and quantitation.*—Use ethyl acetate-toluene (1 + 1) as solvent; split 10 mL evenly between troughs. Running time and distance are 11 min and 63.0 ± 0.4 mm from plate bottom, respectively. To allow detection, mechanically dip the dried chromatogram (5 min under flow of nitrogen at

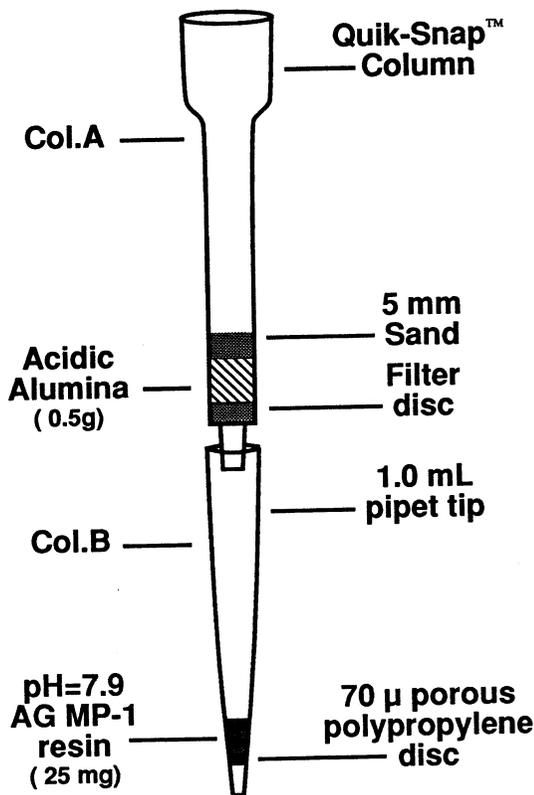


Figure 2. Cleanup and isolation column setup.

room temperature) (Camag Immersion Device II) at low speed for 2 s in 100 mL fluorescamine solution (25 mg in 10 mL acetone, to which 90 mL hexane is added). Dry the dipped plate 5 min with nitrogen then spray with 0.2M H_3BO_3 (adjust pH to 8.0 with 1M NaOH). After spraying with borate buffer, immediately place plate in $-20^\circ C$ freezer for ≥ 5 min before densitometry. Place positioning plate in $-20^\circ C$ freezer for ≥ 15 min before scanning. Place TLC plate prepared for chilling on positioning plate and chill parts together. For densitometry, scan lanes with a 0.025×5 mm band at 0.5 mm/s in the fluorescence mode using a Camag TLC Scanner II. After placing positioning plate (with respective TLC plate) back in densitometer, place similarly chilled Ace flexible cold compress (purchased at local drug store) on positioning plate to help maintain low-temperature environment during densitometry. Measure excitation at 366 nm (Hg lamp), and measure emission after a 400 nm cut-off filter. Record the densitogram, and measure peak heights on a Camag SP4290 integrator.

Sample Preparation

Intact pork loin roast purchased from local market was used for all work reported here. Pork tissue was manually deboned, and all grossly exposed connective tissue and fat were manually removed. Muscle tissue was cut into ca 1 in. cubes and ground twice through 2 mm hole plate (household model meat grinder; Sears Roebuck & Co., Chicago, IL). Ground meat was divided into ca 140 g lots and stored at $-80^\circ C$ in Whirl-Pak bags until analyzed.

Determination

Place frozen tissue (still in closed bag) under running cold water until meat is thawed. Weigh 5.00 g samples into aluminum weighing dishes, and fortify each analytical sample with 50 μL SBZ internal standard working solution. Up to 12 samples can be analyzed concurrently on equipment described (centrifuge and vacuum manifold). Designate 3 samples as calibration standards, and fortify 1 each at 0, 7.5, and 15.0 ppb by adding 50 μL water or respective working solution (0.75 or 1.50 μg SMZ/mL). For recovery studies, fortify each of the other 9 samples with 50 μL SMZ working solutions containing 2.0, 1.0, 0.8, 0.5, 0.4, 0.25, 0.2, 0.1, and 0.05 μg SMZ/mL. Let tissue sample stand at room temperature for 15 min to allow incorporation of drug into tissue. Transfer tissue sample into 50 mL centrifuge tube, dilute to mark (25 mL) with water (use wash bottle), and then homogenize sample. After homogenization, carefully use water wash bottle to rinse generator probe (direct rinse into centrifuge tube). A total of 40–45 mL homogenate should now be in centrifuge tube. After all samples are homogenized, cap tubes, shake briefly (5 s), and centrifuge. While samples are centrifuging, prepare C_{18} SPE setup as described in *Apparatus*, (d). After centrifuging, decant supernatants into their respective reservoirs through poly(propylene) wool plug in funnels. After all samples are filtered into their reservoirs, open each sample valve on vacuum manifold one full turn. Vacuum should be off at this point. Samples will begin to flow slowly by gravity through columns. Carefully apply vacuum to manifold until pressure is 20–30 kPa (ca 7 in. Hg). This should result in flow of 6–8 mL sample solution/min through column. Close sample valve of each tube when sample solution level reaches top of C_{18} column bed. Wait until all samples reach this point. Remove reservoirs, and wash all columns with 3 mL water at full vacuum (ca 90 kPa, 27 in. Hg), followed by 3 mL hexane (use wash bottles to apply wash water and hexane, and fill column barrel to top). Air-dry columns 10 min by applying full vacuum. While C_{18} columns are drying, prepare cleanup and isolation setup (Figure 2) as described in *Apparatus*, (f) and (g). After columns are dry, attach Teflon solvent guide needles inside manifold chamber and setup to collect column eluants. Discard previous column eluants from samples and washings to waste.

Elute C_{18} columns with three 1 mL portions of methanol (use 1000 μL automatic pipet for this and subsequent solvent applications). Apply vacuum at 20–30 kPa (ca 7 in. Hg) to assist this elution only after first milliliter has completely wetted column by gravity percolation. Remove culture tubes from manifold, and pour eluates into Col. A of tandem setups (Figure 2). Flow from this point on in method is controlled by gravity. Rinse culture tubes with two 1 mL portions of methanol, and apply rinses to tandem setup only after previously applied methanol has completely passed through both columns. Rinse walls of Col. A with 1 mL methanol. After methanol has passed through, discard Col. A. Rinse walls of Col. B with 1 mL methanol. Discard methanol eluates from tandem column setups to waste.

Pass 250 μL acetone–acetic acid–methanol (94 + 5 + 1) through Col. B, and collect in 5 cc Reacti-Vials (Pierce Chem-

Table 1. Effect of scanner temperature^a

SMZ added, ng/g	Chilled		Unchilled	
	SMZ found, ng/g \pm SD ^b	Av. % accuracy ^c	SMZ found, ng/g \pm SD ^b	Av. % accuracy ^c
0.51	1.12 \pm 1.2	218.21	0.27 \pm 2.4	52.54
1.02	2.67 \pm 1.5	260.88	2.13 \pm 0.56	208.19
5.11	4.58 \pm 0.9	89.49	4.38 \pm 1.8	85.77
7.67	7.35 \pm 1.4	95.80	7.25 \pm 2.5	94.50
10.22	9.21 \pm 1.2	90.06	10.01 \pm 2.4	97.97
15.34	14.99 \pm 2.1	97.77	16.59 \pm 0.8	108.15

^a Results obtained during method development, not using the final procedure presented.

^b $n = 3$; one analysis at each concentration on each of 3 days.

^c Relative % accuracy based on SMZ found by using the internal standard calculation and the SMZ added.

ical Co., Rockford, IL), or similar conical vials. SMZ and SBZ are contained in this eluant. Cap vials and mix by vortexing 10 s. Apply 50 μ L sample to TLC plate as described in *Apparatus*, (h). If 12 tissue samples (vacuum manifold and centrifuge capacity) are analyzed, 2 TLC plates are needed for analysis, and 3 calibration standards each must be applied to both plates. Calibration standards are routinely applied to tracks 2, 4, and 6 to space standards over plate. When sample application is completed, develop each plate for 11 min in toluene-ethyl acetate (1 + 1). Dry plate 5 min with N₂ before fluorescamine dipping, and dry after dipping ca 2 min with N₂ before spraying with borate buffer. Do not dry TLC plate after spraying with buffer, but immediately place on positioning plate of TLC scanner, already in -20°C freezer, for 5 min before scanning. Scan plate and record data as described above in *Apparatus and Reagents*, (g). Calculate linear calibration equations as follows for each plate from standards by using least-squares fit option in Cricket Graph software:

$$[\text{ng SMZ/g} = m \frac{\text{SMZ height}}{\text{SBZ height}} + b] \quad (1)$$

Calculate SMZ found for each fortified sample by substituting observed SMZ/SBZ height ratios into calibration equation (Eq. 1) obtained for plate on which sample was developed.

Results and Discussion

Three parameters of the milk method were changed to permit tissue analysis: (1) the sample size was reduced to 5 g, (2) the fluorescence densitogram was obtained at a reduced temperature, and (3) the AG MP-1 resin elution volume was reduced to 250 μ L.

The sample size was reduced, because we could not reliably homogenize 10 g tissue with 15 mL water. Mixing 5 g tissue with 20 mL water changed the liquid/solid ratio enough to allow complete and reliable homogenization and extraction, and the homogenate volume was kept within the capacity of a 50 mL centrifuge tube.

The internal temperature of the TLC scanner often reached 34°C after the Hg lamp was turned on and permitted to warm up. Surface temperature measurements of the positioning plate in the scanner yielded similar temperatures. Because fluores-

camine derivatives are not heat-stable, increased temperature (>10°C above room temperature) probably is adversely influencing the results. To assess this effect, 6 fortification levels were evaluated each day for 3 days, with cooling and then without cooling (Table 1). The standard error of the estimate was calculated for each condition according to the following formula:

$$S_{xy} = \sqrt{\sum (X - \hat{Y})^2 / (n - 2)} \quad (2)$$

where Y is the amount of SMZ found and \hat{Y} is the amount of SMZ added. $S_{xy} = 1.41$ when the plate is scanned chilled, and $S_{xy} = 1.83$ when the plate is scanned at the densitometer's ambient operating temperature (about 30–34°C). In addition to the reduced standard error, an increase in the signal-to-noise ratio was also observed when scanning was done at the reduced temperatures. Therefore, with a reduction in the S_{xy} and an increase in the signal-to-noise ratio, reduced temperature scanning was incorporated into the method. The effect of reduced temperature scanning is being investigated further, and we devised an alternative positioning plate that conveniently maintains the reduced temperature for enough time (13) without the need of the Ace cold pack.

The precision and accuracy values obtained at the 0.5 and 1.0 ppb levels (Table 1) were not as good as those we obtained with the method for SMZ in milk (11). We reduced the sample size from 10 mL (about 10 g) to 5 g tissue and applied a 100 μ L sample to the TLC plate to compensate for this reduction. However, when 100 μ L was applied to the plate, the chromatographed bands were not as narrow as when 50 μ L was applied. We believe that this is because of an excess of acetic acid in the sample band that interferes with the chromatography. To correct the problem, the elution volume of the AG MP-1 column was reduced to 250 μ L from 500 μ L. This change permitted a smaller volume (50 μ L) to be applied to the plate. The 50 μ L volume did not affect the chromatography, and the reduction to 250 μ L did not affect the recoveries.

Once the described procedure was developed, SMZ was successfully extracted with water and then cleaned up from pork tissues fortified with SMZ at 1.1 and 2.2 ppb (Figure 3). A good signal-to-noise ratio was obtained even at the 1.1 ppb

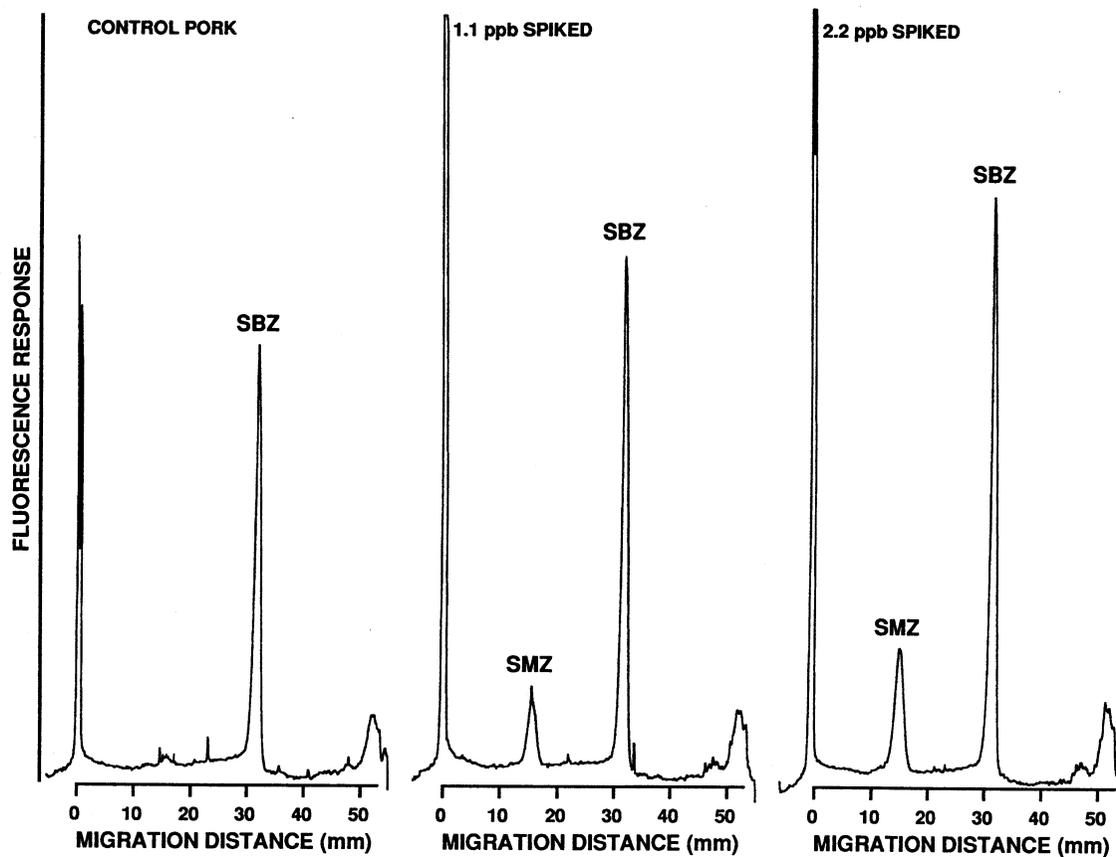


Figure 3. Sample densitograms: Tissue samples fortified with 10 ppb SBZ and either 0, 1.1, or 2.2 ppb SMZ are illustrated. Origin is at 0 mm migration, and solvent front is at 53 mm migration.

level (about 10:1). All samples were fortified with 10 ppb SBZ. The different response heights for the SBZ peak are evidence of the variability of the absolute percent recovery in the method and demonstrate the need for an internal standard correction. Calibration lines were calculated from samples fortified at 0, 7.5, and 15 ppb SMZ (as described above), with SBZ as an internal standard. The average squared correlation coefficient for the calibration curves was 0.995 ± 0.006 ($r^2 \pm SD$, $n = 12$). The high correlation for the curves over the 15 ppb range illustrates the suitability of SBZ for use as an internal standard. Table 2 shows the results obtained for pork tissue fortified at 9 levels and analyzed daily for 6 days. The average accuracy over the analysis range of 0.5–21 ppb was 95.65% (SD = 29.45%, $n = 54$).

Close examination of the control sample (Figure 3) reveals a small interference at 16 mm migration distance ($R_f = 0.30$). This coincides with the SMZ migration in the fortified samples. The level was undetectable by the integrator and was not subtracted from the results. Because pork used in these studies was obtained from a local meat market, samples may not have been completely sulfonamide-free. To determine whether the peak might be SMZ, three 5 g samples of unfortified pork (neither SMZ nor SBZ was added) were extracted. The methanol eluates from the 3 C_{18} SPE columns were combined and passed through the same aluminum oxide and AG MP-1 tandem setup; then, 50 μ L of the acidic acetone eluate of the AG MP-1 col-

umn was applied to a TLC plate and developed as described. The baseline interference at the 16 mm migration distance increased in height approximately 3 times, and the rest of the densitogram remained unchanged. This result indicates 2 conclusions. First, SBZ is a good choice as an internal standard, because no evidence was found of interferences at SBZ's migration distance of 31 mm ($R_f = 0.58$). Second, the pork tissue used for this study may have been contaminated with an SMZ residue of approximately 0.1 ppb. No attempt was made to confirm the identity of the interference by other means. Other sulfonamides could be the cause of the interference; sulfamerazine and sulfadiazine were both isolated by the method and both migrate with SMZ in the TLC system presented. SMZ could be confirmed by using a different TLC system (15) or one of several TLC (16, 17) or LC systems (8, 11, 18). Sulfathoxypyridazine and sulfadimethoxine are 2 other sulfonamides isolated by the method, but they are both resolved from SMZ. The only other sulfonamide to which the method was applied was sulfapyridine (SPD), which is not isolated. SPD's pK_a is 8.43 (19), and thus, it is not retained by the 7.9 AG-MP1 resin. Altering the pH of the AG-MP1 resin to 9 or above should isolate SPD. SPD is chromatographically resolved from SMZ, as are many other sulfonamides (15). The potential for a multisulfonamide method is present but has not been evaluated.

The identity of the interference, although important, does not greatly influence the determination of SMZ at the 5–10 ppb

Table 2. Accuracy results for sulfamethazine-fortified pork tissue

SMZ added, ng/g	SMZ found, ng/g \pm SD ^a	Av. % accuracy ^b	CV, %
0.54	0.56 \pm 0.39	102.19	71.51
1.09	1.16 \pm 0.51	106.08	43.98
2.18	1.84 \pm 0.42	84.51	22.50
2.73	2.31 \pm 0.27	84.64	11.93
4.36	3.72 \pm 0.33	85.16	8.97
5.45	4.75 \pm 0.27	87.18	5.61
8.72	8.19 \pm 0.29	93.88	3.62
10.91	11.06 \pm 0.63	101.40	5.72
21.81	24.93 \pm 3.73	114.31	14.95

^a $n = 6$; one analysis at each concentration on each of 6 days.

^b Relative % accuracy, based on SMZ found using the internal standard calculation and the SMZ added.

range, but it should affect the method's detection limit. Without correcting for the interfering residue and using the signal-to-noise ratio of 3:1, we estimate the method's limit of detection at approximately 0.25 ppb; however, this noise level is not suitable for regulatory purposes. The FDA's general guidelines for methodology for residue analyses below 100 ppb require the background noise at the regulatory level to be less than 10% of the residue's signal and the coefficient of variation to be less than 20%. According to these guidelines, the presented method

could be suitable for regulatory purposes at and above the 2 ppb level (see Table 2 and Figure 3).

The upper limit of the method must also be considered. Figure 4 shows 2 plots of the residuals (SMZ found - SMZ added) for the values used in Table 2. The average residual and the upper and lower control limits (+3SD and -3SD, respectively) are indicated as lines across each plot. Plot A includes the values found for the 21.81 ppb fortification; Plot B does not. There is a larger control limit span in Plot A, and 2 values in the 21.81 ppb zone exceed the upper control limit. This indicates a problem with the method at this level of analysis. The standard error of analysis calculated as defined above (Eq. 2) with and without the 21.81 ppb data yields values of 1.66 and 0.58, respectively. An F-test on the variances indicates that the 2 sets of data are not of the same group. A possible cause is the saturation of the photomultiplier tube with the 21.8 ppb samples. Therefore, we suggest setting the upper quantitative limit of the method to approximately 15 ppb, which, from our experience with SMZ in milk (11), is within the linear range of the photomultiplier tube. If quantitation at a level higher than 15 ppb is desired, we suggest decreasing the sample size, lowering the sensitivity of the photomultiplier, or fortifying with SBZ at the target level desired and then either decreasing the volume applied to the TLC plate or increasing the elution volume of the AG MP-1 column.

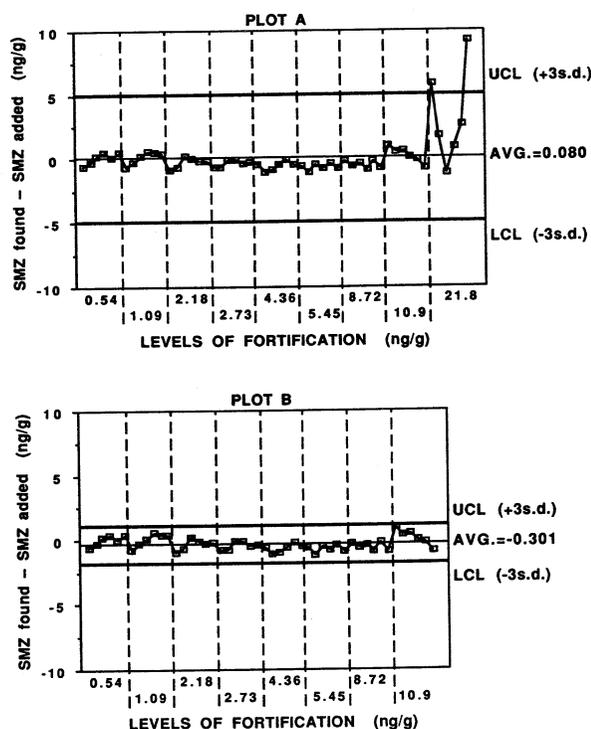


Figure 4. Quality control plots: Values for the difference between the SMZ found and SMZ added are plotted for each fortification level. The average difference, 99% upper control limit (UCL = +3SD) and 99% lower control limit (LCL = -3SD) were calculated and are illustrated.

Conclusions

The method is rapid (one analyst can manually complete 12 samples in 8 h), uses little solvent (about 20 mL solvent per sample for sample preparation and chromatography), and is sensitive (detection limit is approximately 0.25 ppb, with a linear range of quantitation of approximately 2-15 ppb).

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