

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

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Title: Automated Robotic Extraction / TLC Analysis for Sulfonamide Residues in Animal Tissues, Eggs, and Egg Products		
Revision: 04	Replaces: CLG-SUL2.03	Effective: 07/22/2009

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A. INTRODUCTION

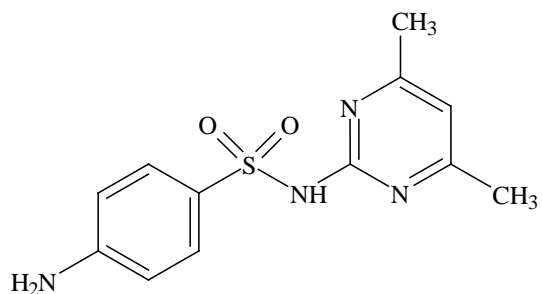
1. Theory

This automated procedure for sulfonamides uses thin layer chromatography with fluorometric scanning densitometry. After extraction, separation of the drugs in the extract is carried out on a silica gel plate containing a preadsorbent spotting area. Visualization is accomplished using UV light after dipping the plate in fluorescamine solution.

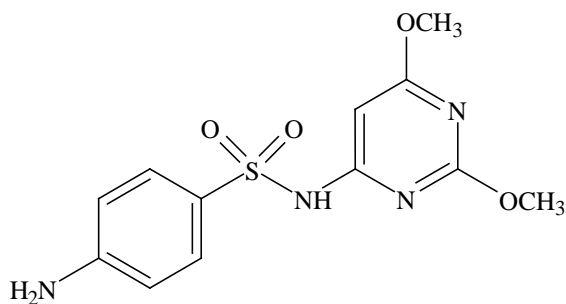
2. Applicability

This method is applicable for the analysis of sulfamethazine, sulfathiazole, sulfaquinoxaline, and sulfadimethoxine in muscle and liver tissues of porcine, bovine, and avian species at levels ≥ 0.05 ppm. It is also applicable for the analysis of sulfamethazine, sulfadimethoxine, and sulfaquinoxaline in eggs and egg products at levels ≥ 0.05 ppm.

3. Structures



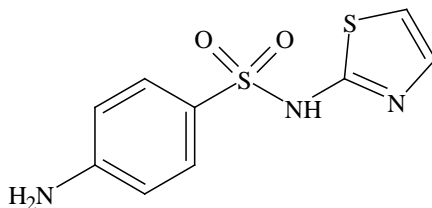
a. Sulfamethazine



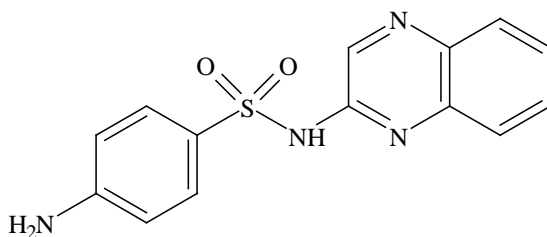
b. Sulfadimethoxine

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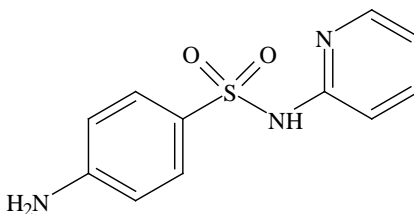
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c. Sulfathiazole



d. Sulfaquinolone



e. Sulfapyridine (internal standard)

B. EQUIPMENT

Note: Equivalent apparatus and instrumentation may be substituted for the following:

1. Apparatus

- a. Zymark Robotic System - Zymate Pytechnology System equipped with the following modules:
 - i. Zymate System V Core System with System V Operating System Version 2.62
 - ii. ZP-510 MLS station (2)
 - iii. ZP-830 Power and event controller
 - iv. ZP-900-1 General purpose gripping hand

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- v. ZP-900-2 General purpose gripping hand
 - vi. ZP-013-7 50 mL centrifuge tube rack (2)
 - vii. ZP-013-3 16x 100 mm test tube rack
 - viii. ZP-913-1 Syringe hand, 1 - 4 mL
 - ix. ZP-620-6 Dilute and dissolve station (50 mL tubes)
 - x. Custom nozzle parking station - fabricated in house
 - xi. ZP-620-2 Dilute and dissolve station (15 mL tubes)
 - xii. Custom rack for pouring station - fabricated in house
 - xiii. Custom rack for sodium acetate solution - fabricated in house
 - xiv. ZP-77413 1 mL syringe hand
 - b. Thelco Model 6M incubator - GCA Corp.
 - c. Convection oven - Model 20, GCA Corp.
 - d. Balance - PB3002-S, Mettler.
 - e. N-EVAP - Model 112 solvent evaporation system, Organomation Associates, Inc.
 - f. TLC developing tank - glass w/glass top, Cat. No. 7645, Alltech Associates, Inc.
 - g. Stainless steel dipping tank
 - h. Vortex mixer - Thermolyne Maxi Mix model M-16715, Thermolyne Corp.
 - i. Polypropylene centrifuge tubes - 50 mL, Falcon Blue Max, Cat. No. 2098, and 15 mL, Falcon Blue Max, Cat. No. 2097, Falcon.
 - j. Saturation pads - 20 x 20-cm, Cat. No. 7623, Alltech Associates, Inc.
 - k. Open tip fast flow filter CC-09-m - Whale Scientific Inc.
 - l. Pipet tip - 5 mL, Cat. No. ZA7306, Zymark Corp.
 - m. Blender - Waring Products Division.
 - n. Robot Coupe processor - Robot Coupe U.S.A., Inc.
 - o. Silica gel thin layer plates w/pre-absorbant layer - Whatman LK6D, Cat. No. 4865821, Alltech Associates, Inc.
2. Instrumentation
- a. CAMAG TLC Scanner III scanning densitometer with data system and printer.
 - b. Spectroline Model C-3F UV light box - Black Light Eastern Corp.

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C. REAGENTS AND SOLUTIONS

Note: Equivalent weights/volumes may be used.

1. Reagents

Note: All solvents are HPLC grade unless otherwise specified.

- a. Hexane
- b. Ethyl Acetate
- c. Chloroform - No ethanol preservative added.
- d. Methanol
- e. Acetone
- f. Tert-butanol - Reagent grade.
- g. Concentrated hydrochloric acid (HCl) - Reagent grade.
- h. Diethylamine - Reagent grade, Cat. No. 9216-01, Baker.

Note: If doing the method robotically then dilute 5 mL of diethylamine to a volume of 20 mL with ethyl acetate.

- i. Sodium Acetate - Anhydrous, Certified ACS Reagent grade.
- j. Deionized Water

2. Solutions

- a. Fluorescamine dipping solution (Pierce Chemical)

Dissolve 30 mg in 250 mL acetone. Solution is stable for 1 month when stored in the dark.

- b. TLC solvent system: chloroform/t-butanol (80:20 v/v)

Add 100 mL solvent (80 mL chloroform and 20 mL t-butanol) and 20 mL deionized water to a 250 mL separatory funnel. Equilibrate by shaking for 30 seconds and allow phases to separate.

Note: TLC developing solvent is equilibrated with water prior to use. Discard chloroform/tert-butanol in the developing chamber daily.

- c. 0.2M phosphate buffer

Weigh 45.65 g potassium phosphate dibasic crystals ($K_2HPO_4 \cdot 3 H_2O$) and dissolve in 1000 mL distilled water in a volumetric flask (Solution 1). Dissolve 27.22 g KH_2PO_4 , (potassium phosphate monobasic) in 1000 mL distilled water in a volumetric flask (Solution 2). Adjust solution 1 to pH 7.55 ± 0.05 with Solution 2. (Use all of solution 1 and adjust with solution 2 at an approximately 80:20

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ratio.)

- d. 3.5M sodium acetate

Add 287.12 g to a 1000 mL volumetric flask. Dissolve with deionized water and bring to volume.

- e. 3.2M HCl

Add approximately 500 mL deionized water to a 1000 mL volumetric flask. Add 277 mL conc. HCl. Dilute to volume with deionized water.

D. STANDARDS

Note: Equivalent standards and solutions may be substituted for any of the following.

1. Source

Reference standard materials are available from U.S. Pharmacopeia; Sigma Chemical Co; Fluka Chemical Corp., and Pfaltz and Bauer.

Compound	Cat. No.	Identifier
Sulfamethazine	# S-6256 Sigma	(SMZ)
Sulfaquinoxaline	# S11120 Pfaltz and Bauer	(SQX)
Sulfapyridine	# S-6252 Sigma	(SPY Internal Standard - IS)
Sulfadimethoxine	# S-7007 Sigma	(SDM)
Sulfathiazole	# S-9876 Sigma	(STZ)

2. Preparation

- a. Stock solution (1 mg/mL):

All sulfonamide standards, including the internal standard (Sulfapyridine) are prepared as follows:

Weigh 100 ± 0.1 mg of each sulfonamide into separate 100 mL volumetric flasks. Dissolve and bring to volume with acetone.

Note: If using a sodium salt of the sulfonamide then the weight must be corrected as needed. Dissolve the sodium salt of the sulfonamide with a few drops of distilled water and then bring to volume with acetone.

- b. Working standards (used for fortification):

All working standards are diluted with **0.2M phosphate buffer (C.2.c)**.

- i. 10 µg/mL standards:

- (a) Sulfonamide mixed standard solution.

Pipet 1 mL of stock solution of each sulfonamide of interest into a

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100 mL volumetric flask. Do not add internal standard. Bring to volume.

(b) Internal standard (IS) solution.

Pipet 1 mL of the 1 mg/mL internal standard (IS) solution into a 100 mL volumetric flask. Bring to volume.

ii. Solution D (2.50 µg/mL IS):

Pipet 25 mL of the 10 µg/mL IS solution into a 100 mL volumetric flask and bring to volume.

iii. Solution C (5.00 µg/mL sulfonamide of interest):

Pipet 50 mL of the 10 µg/mL sulfonamide mixed standard solution into a 100 mL volumetric flask and bring to volume.

iv. Solution B (2.5 µg/mL sulfonamide of interest):

Pipet 25 mL of solution C into a 50 mL volumetric flask and bring to volume.

v. Solution A (1.25 µg/mL sulfonamide of interest):

Pipet 25 mL of solution B into a 50 mL volumetric flask and bring to volume.

Note: Standard solutions C, B, and A may be used to prepare check samples.

3. Storage Conditions

All standards can be stored in polyethylene bottles and refrigerated at 2 - 8 °C, except for stock solutions which should be stored at < -10 °C.

4. Shelf Life Stability

a. Stock solution: 6 months.

b. Working solutions: 3 months.

Storage stability is based upon the storage conditions mentioned in D.3.

E. SAMPLE PREPARATION

Samples of liver are blended until homogeneous. Muscle samples are processed until homogeneous. Egg and egg products should be mixed or shaken before weighing to ensure homogeneity. All samples are stored refrigerated or frozen until analyzed.

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F. ANALYTICAL PROCEDURE

1. Preparation of positive controls, negative controls, and samples

a. Tissue and Liquid Eggs

i. Fortification Standard Curve and/or Negative Control Blank

Weigh four 2.5 ± 0.1 g portions of blank control matrix. Fortify three of the blanks as follows:

Volume Added(μL)	Solution Used	μg Added	ppm*
100	A	0.125	0.05
100	B	0.250	0.10
100	C	0.500	0.20

*ppm of sulfa of interest is calculated using 2.50 g as sample weight.

Add 100 μ L of standard solution D (2.50 μ g/mL) to the negative control (blank) three fortified controls, and the internal check sample, when used. This will yield 0.10 ppm IS in each control sample.

Note: The blank (negative control) is optional if using previously tested tissue. Prepare additional control matrix for use as check sample when needed.

Note: If the sample was previously analyzed and was outside the linear range of the curve it may be necessary to reweigh a smaller amount of the sample for reanalysis.

Note: High level samples are not concentrated during the procedure as are low level samples. It is possible, therefore, to evaluate both with this fortification curve.

ii. Samples:

Weigh 2.5 ± 0.1 g of sample into a 50 mL polypropylene centrifuge tube. Allow to thaw, if necessary.

(a) For low level:

Add 100 μ L of standard solution D (2.50 μ g/mL) to all low level samples. This will yield 0.10 ppm of IS.

(b) For high level (> 1 ppm):

Add 25 μ L of the internal standard stock solution (1 mg/mL) to all high-level samples. This will yield 10 ppm of IS.

b. Powdered Eggs

i. Fortification Standard Curve and/or Negative Control Blank

Weigh four 1.0 ± 0.1 g portions of blank powdered egg matrix. Add 1.0 mL distilled H₂O to each portion. Fortify three of the blanks as follows:

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<u>Volume Added(µL)</u>	<u>Solution Used</u>	<u>µg Added</u>	<u>ppm*</u>
40	A	0.125	0.05
40	B	0.250	0.10
40	C	0.500	0.20

*ppm of sulfa of interest is calculated using 1.0 g as sample weight.

Add 40 µL of standard solution D (2.50 µg/mL) to the negative control (blank), the three fortified controls, and the internal check sample, when used. This will yield 0.10 ppm IS in each control sample.

Note: The blank (negative control) is optional if using previously tested tissue. Prepare additional control matrix for use as check sample when needed.

Note: If the sample was previously analyzed and was outside the linear range of the curve it may be necessary to reweigh a smaller amount of the sample for reanalysis.

ii. Samples

Weigh 1.0 ± 0.1 g of sample into a 50 mL polypropylene centrifuge tube. Allow to thaw, if necessary. Add 1 mL distilled H₂O. Add 40 µL of standard solution D (2.50 µg/mL) to each sample. This will yield 0.1 ppm IS.

2. Extraction

Note: The following steps are performed manually or automatically by the robotic system.

a. Addition of extraction solvent

i. Tissue and Powdered Eggs

Add 6.0 mL ethyl acetate to tissue and powdered egg samples and vortex for 2 minutes. Let samples stand for at least 10 minutes.

Manual – Vortex or shake by hand to break up sample. Place samples on horizontal shaker for approximately 10 minutes on high or vortex for 2 minutes. Let stand at least 10 minutes or centrifuge for 5 minutes at 2500 rpm.

ii. Liquid Eggs

Add 10.0 mL ethyl acetate to liquid egg samples and vortex for 2 minutes. Let samples stand for at least 10 minutes.

Manual – Vortex or shake by hand to break up sample. Place samples on horizontal shaker for approximately 10 minutes on high or vortex for 2 minutes. Let stand at least 10 minutes or centrifuge for 5 minutes at 2500 rpm.

Note: Liquid egg samples sometimes form emulsions after shaking. Vortex the sample on the highest vortexer setting until the emulsion breaks up.

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- b. Filter ethyl acetate through a fast flow filter column and collect filtrate into a clean 15-mL centrifuge tube.

Note: Because the fast flow filters do not stop liquid egg from passing into the 15 mL centrifuge tube, care must be taken when pouring the samples through the filters. Liquid egg mixed into the ethyl acetate extract will cause emulsion problems later in the method.

- c. Add 1.0 mL 3.2M HCl.

- d. Vortex for 30 seconds and let stand for at least 5 minutes.

Manual – Shake approximately 5 minutes on high or vortex for 30 seconds. Let stand at least 5 minutes or centrifuge for 5 minutes at 2500 rpm.

- e. Aspirate ethyl acetate to waste.

- f. Add 5.0 mL hexane and vortex for 30 sec., let stand for 5 minutes.

Manual – Shake approximately 5 minutes on low or vortex for 30 sec., and let stand for at least 5 minutes or centrifuge for 5 minutes at 2500 rpm.

- g. Aspirate hexane to waste.

- h. Add 2.0 mL 3.5 M sodium acetate.

- i. Add 3.0 mL ethyl acetate, vortex for 30 sec., let stand for at least 5 minutes.

Manual – Shake 5 minutes on low or vortex for 30 sec., and let stand for at least 5 minutes or centrifuge for 5 minutes at 2500 rpm..

- j. Transfer ethyl acetate to a clean centrifuge tube.

Note: For high-level sulfas (≥ 1 ppm) proceed to F.3.d.; otherwise, continue as follows.

3. Development procedure (continued, for all matrices)

Note: The spotting and developing steps may be optimized to account for changes in plate manufacture.

- a. Add either 10 μ L manually or an equivalent amount of a diluted solution of diethylamine using the robot to each sample tube.

Note: The remaining steps are carried out manually.

- b. Evaporate to dryness under nitrogen using an N-Evap with water bath temperature set to $40 \pm 5^\circ\text{C}$.

- c. Add 100 μ L methanol to each sample tube and re-dissolve residue by mixing briefly using a vortex mixer.

- d. Spot 20 μ L of each sample on the pre-absorbant layer of a LK6D thin layer plate. Spotting is facilitated by heating the pre-absorbant layer using a small hot plate

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or heat strip. The heat strip or hot plate should be hot enough to evaporate the spot within a few seconds. Position the sample spot approximately 10-15 mm from the silica gel interface. Do not use the outermost lanes. Accurate densitometry depends on careful spotting technique to achieve an even band. After spotting, dry plate for at least 10 minutes in a forced air drying oven set to 100 °C. This drying step is helpful in preventing band broadening when plate is developed.

- e. Develop the plate with methanol to a distance of 1 cm above the silica gel interface. Remove the plate and dry for at least 1 minute in a convection oven at 100 °C. Repeat this development cycle if necessary.
 - f. Develop the plate to a distance of 6 cm above the silica gel interface in chloroform/t-butanol (80:20) in a saturated developing tank. Dry plate for at least 1 minute in an oven at 100 °C. Redevelop the plate in chloroform/t-butanol (80:20) to a distance of 12 cm above the silica gel interface. (Development must be carried out at 25 - 30 °C to insure consistent RR1 values.)
 - g. Oven dry plate for at least 1 minute at 100 °C and dip in fluorecamine solution. Keep plate in solution 1 - 2 seconds after fully submerged (longer immersions will result in high background).
 - h. Place plate in the dark for at least 15 - 30 minutes to allow for development.
 - i. Scan plate using densitometer using 410 ± 10 nm excitation.
4. Instrumental Settings

Set up Scanning Densitometer according to manufacturer instructions. The following are conditions for a Camag Scanning Densitometer and should be used for reference only.

Scanner settings:

Plate size (width x height)...	20 x 20 cm
Application position Y	10.0 mm
Position of solvent front	145.0 mm
Scan start position Y	60 mm
Scan end position Y	145.0 mm
Scan start position X	20.0 mm
Distance between tracks	10.0 mm
Number of tracks	18
Lamp	Mercury
Monochromator bandwidth	20 nm
Wavelength	410 nm
Slit dimension	6.0 x 0.20 mm
Data step resolution	100 fm
Display scaling	700 AU
Measurement mode	Fluorescence / Reflection

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Scanning speed	10.0 mm/sec.
Optical filter	460 nm filter
Zeroing mode	Automatic
Y-position for 0 adjustment	60.0 mm, Track: 1
Quick scan from	60.0 mm to 145 mm, all tracks
Offset	10%
Sensitivity	Automatic
High voltage of PM	257 v

Integration Parameters

Video integration	no
Baseline correction	yes
Peak thresh. height	10 AU
Peak thresh. area	300
Peak thresh. slope	3
Filter factor	3
Data selection factor	1
Track start position	88.1mm
Track end position	136.8mm

G. CALCULATIONS

For each plate construct a standard curve from the three fortified samples applied to that plate as follows:

- a. Measure the peak heights of all sulfonamides of interest and internal standard peak for each track. Calculate peak height ratio by dividing sulfonamide peak height by internal standard peak height.

$$\text{Peak Height Ratio} = \frac{\text{Sulfonamide peak height}}{\text{Internal Standard Peak Height}}$$

- b. Using linear regression, construct a standard curve of each sulfonamide. The linear relationship is $Y = mX + b$ is used to calculate concentration in unknown samples where X is the sulfonamide concentration, Y is peak height ratio, m is the slope of the regression line and b is the intercept of the regression line.

Note: For processed products the internal standard peak heights may need to be monitored to ensure successful analyses.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — Eye protection, non-permeable gloves, and lab coats.

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2. Hazards

<i>Reagent</i>	<i>Hazard</i>	<i>Recommended Safe Procedures</i>
Ethyl acetate, hexane, diethylamine, chloroform, t-butanol, methanol, acetone	These solvents may be flammable and may produce toxic effects to skin, eyes, and the respiratory system.	Use reagents in an efficient fume hood away from all electrical devices and open flames. Use approved gloves and protect skin from exposure.

3. Disposal Procedures

<i>Procedure Step</i>	<i>Hazard</i>	<i>Recommended Safe Procedures</i>
Waste Organic Solvents	See above	Gather and store in an approved collection container until disposed of by a contractor or an in house specialist.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

<i>Analyte</i>	<i>Analytical Range (ppm)</i>	<i>Acceptable Recovery (%)</i>	<i>Acceptable Repeatability (%CV)</i>
All sulfonamides	0.05 - 0.2 ¹ 5 - 100 ²	80 - 120 ³	≤ 20 ⁴

¹Normal protocol.

²High level protocol.

³In all species/tissue combinations tested

⁴Calculated at 0.05ppm.

Regression coefficient ≥ 0.995

Standard error ≤ 0.075

2. Critical Control Points and Specifications

<i>Record</i>	<i>Acceptable Control</i>
a. Sample weight	2.5 g ± 0.10 g

3. Readiness To Perform

- a. Familiarization in **Meat and Meat Products**

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- i. Phase I: Standards, minimum criteria -TLC, densitometric standard curve preparation: Four sulfonamides (sulfamethazine, sulfadimethoxine, sulfaquinoxaline, sulfathiazole) at three levels:
 - (a) 0.05 ppm
 - (b) 0.1 ppm
 - (c) 0.2 ppm

The number of replicates is at the discretion of the supervisor.

- ii. Phase II: Sample fortification (self-provided tissues), minimum criteria. Three levels -- 0.05, 0.1, and 0.20 ppm ---- plus blanks.
 - (a) Turkey ---- liver and muscle ---- Sulfaquinoxaline.
 - (b) Turkey ---- liver and muscle ----Sulfamethazine, and Sulfadimethoxine
 - (c) Swine --- liver and muscle --- Sulfathiazole, Sulfadimethoxine, and Sulfamethazine.
 - (d) Beef --- liver and muscle --- Sulfadimethoxine, Sulfathiazole, and Sulfamethazine.

The number of replicates is at the discretion of the supervisor.

Note: Phase I and Phase II may be performed concurrently.

- iii. Phase III: Check samples for analyst accreditation.
 - (a) Analyze 14 samples furnished by Quality Assurance Manager (QAM) or Supervisor.
 - (b) Authorization from the QAM and supervisor is required to commence official analysis.

b. Familiarization in **Egg and Egg Products**

- i. Phase I: Standards, minimum criteria. TLC, densitometric standard curve preparation: Four sulfonamides (Sulfamethazine, Sulfadimethoxine, Sulfaquinoxaline, Sulfathiazole) at four levels on three consecutive days:
 - (a) 0 ppm
 - (b) 0.05 ppm
 - (c) 0.1 ppm
 - (d) 0.2 ppm

The number of replicates is at the discretion of the supervisor.

- ii. Phase II: Sample fortification (self-provided tissues), minimum criteria.

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Fortified recovery samples using blanks of the appropriate egg matrix fortified at 0, 0.05, 0.1, and 0.2 ppm. Duplicates on three days.

- (a) Liquid Whole Eggs - Sulfaquinoxaline
- (b) Liquid Whole Eggs - Sulfamethazine and Sulfadimethoxine
- (c) Liquid Egg Whites - Sulfaquinoxaline
- (d) Liquid Egg Whites - Sulfadimethoxine and Sulfamethazine
- (e) Liquid Egg Yolks - Sulfaquinoxaline
- (f) Liquid Egg Yolks - Sulfadimethoxine and Sulfamethazine
- (g) Powdered Whole Eggs - Sulfaquinoxaline
- (h) Powdered Whole Eggs - Sulfadimethoxine and Sulfamethazine
- (i) Powdered Egg Whites - Sulfaquinoxaline
- (j) Powdered Egg Whites - Sulfadimethoxine and Sulfamethazine
- (k) Powdered Egg Yolks - Sulfaquinoxaline
- (l) Powdered Egg Yolks - Sulfadimethoxine and Sulfamethazine

The number of replicates is at the discretion of the supervisor.

Note: Phase I and Phase II may be performed concurrently.

- iii. Phase III: Check samples for analyst accreditation.

Unknown Samples - 1 blank, 1 recovery at the 0.05 ppm level, and 5 fortified samples ranging from 0.05 - 0.20 ppm (excluding the blank and 0.05 ppm recovery).

Authorization from the QAM and supervisor is required to commence official analysis.

- c. Acceptability criteria.

Refer to I. 1.

4. Intralaboratory Check Samples

- a. System, minimum contents.

- i. Frequency: minimum of 1 per week per analyst.

- b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

- i. Stop all official analyses by that analyst.

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ii. Take corrective action.

5. Sample Acceptability and Stability

- a. Matrices: Liver, kidney, muscle, processed products, eggs.
- b. Species: avian, bovine, porcine.
- c. Sample receipt size, Meat minimum: 1 lb. Eggs 6 - 8 oz.
- d. Condition upon receipt: Chilled or frozen.
- e. Sample storage:
 - i. Time: Not more than 90 days (3 months).
 - ii. Condition: Frozen

6. Sample Set

Each sample set must contain:

- a. standard curve of fortified tissues at 0.05, 0.10, and 0.20 ppm,
- b. recovery spiked at tolerance (0.10 ppm),
- c. samples of the same tissue.

Note: The blank is optional if using previously analyzed tissue

For samples requiring quantitation, a standard curve consisting of the same tissue shall be analyzed.

7. Sensitivity

- a. Minimum proficiency level (MPL): 0.05 ppm.

J. WORKSHEET

[RESERVED]

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K. APPENDIX

1. Relative Retention Factor (Rf) Values for Sulfonamides of Interest

Sulfonamides	Identifier	RR₁ value
Sulfapyridine	SPY	1.00
Sulfadiazine	SDZ	1.12
Sulfathiazole	STZ	0.88
Sulfamethazine	SMZ	1.27
Sulfachloropyridazine	SCP	1.24
Sulfamethoxypyridazine	SMP	1.25
Sulfaquinoxaline	SQX	1.34
Sulfadimethoxine	SDM	1.43
Sulfaethoxypyridazine	SEP	1.36
Sulfaphenazole	SPZ	1.39
Sulfatroxazole	STX	1.38
Sulfisoxazole	SFX	1.32
Sulfadoxine	SDX	1.48

Note: RR₁ = Relative retention factor normalized to, the internal standard, sulfapyridine.

2. Reference

¹ Chemistry Chemical Guidebook method 5.018 (Revised June, 1987).

² N. Takeda and Y. Akiyama, Journal of Chromatography, 558,175-180 (1991)

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L. APPROVALS AND AUTHORITIES

1. Approvals on file.
2. Issuing Authority: Director, Laboratory Quality Assurance Division.