NEW APPROACHES TO QUANTITATING AND CONFIRMING INCURRED SULFONAMIDE RESIDUES IN SWINE TISSUES

R. L. Ellis, R. L. Epstein, R. M. Simpson, F. B. Suhre, M. H. Thomas

U.S. Department of Agriculture, Food Safety and Quality Service, Science, Chemistry Division Laboratory Branch, Beltsville, Maryland U.S.A.

INTRODUCTION

Currently FSQS laboratories use the Tishler Bratton-Marshall (Method A) procedure for quantitation and confirmation of sulfonamide residues in swine tissue. At the tolerance level (0.1 ppm) the reliability of this method is limited by a lack of sensitivity and significant background response. Quantitative thin layer chromatography and gas chromatography/mass spectrometry are two techniques that provide significant improvements in sensitivity and accuracy as well as providing needed selectivity. The high sample throughput of TLC coupled with fluorescence detection makes it an ideal choice for sensitive screening procedures whereas GC/MS provides unambiguous confirmation. The use of a stable isotope internal standard in conjunction with GC/MS analysis provides the additional benefit of extremely accurate quantitation. Both techniques allow quantitation below 0.02 ppm of sulfamethazine.

MATERIAL AND METHODS

Sulfamethazine was obtained from Pfaltz and Bauer, Stamford, Ct. and sulfapyridine was obtained from Sigma Chemical Co., St. Louis, Mo. The isotopically enriched internal standard, ^{13}C labeled sulfamethazine, was obtained from KOR Isotope, Cambridge, Ma. Thin layer chromatography was carried out on silica gel with a preadsorbent spotting layer (LK6D, Whatman, Inc., Clifton, N.J.) and visualized with fluorescamine (Pierce Chemical Co., Rockford, Il.)

An Amico-Bowman scanning spectrofluorimeter and a Hewlett-Packard 5992 quadrupole GC/EI/MS were used for this study.

For initial screening, small samples of swine tissue (2.5 g) were homogenized with ethyl acetate. The ethyl acetate was partitioned against 1 N hydrochloric acid. The acid phase was separated and the pH adjusted to 6.5. The sulfonamides were then back extracted into methylene chloride and concentrated prior to TLC. For GC/MS analysis all samples were initially fortified with ^{13}C labeled sulfamethazine internal standard equivalent to 0.10 ppm. Sample workup was conducted according to Method A of Tishler's procedure (1). Specifically, either muscle or liver tissue was extracted with 1:1 chloroform/acetone. Solvent was removed by evaporation. Residue was partitioned between 1 N HCl and hexane. The hexane phase was discarded and the aqueous phase adjusted to pH 6.25. Sulfamethazine was extracted with methylene chloride and evaporated to dryness.
Extracts were derivatized with diazomethane prior to analysis by GC/MS (Figure 1).

RESULTS

Both techniques have been evaluated in swine liver and muscle over the range 0.05-0.20 ppm sulfamethazine. The accuracy and precision of the TLC results are presented in Table 1. The excellent precision, both within-day and day-to-day, as well as the accuracy of the method are largely a function of the use of an internal standard, sulfapyridine, and preadsorbent TLC plates for reproducible chromatography (Figure 2). In addition the confidence interval associated with the screening method was evaluated by constructing over 350 standard curves in each tissue using fortified control tissue and measuring the standard error to estimate (S y·x) for each curve. Based on this data, for a 95 percent confidence interval, a confirmation threshold of 0.07 ppm could be established 90 percent of the time in liver and 100 percent of the time in muscle. This should ensure that (with 95 percent probability) all violative but relatively few non-violative samples will be carried through the GC/MS confirmation procedure. An example of this approach is illustrated in Figure 3.

The accuracy and precision of the GC/MS quantitation/confirmation procedure for both muscle and liver tissue is presented in Tables 2 and 3. A major feature of this procedure is that the data necessary to both accurately quantitate and confirm the sulfamethazine is generated in a single analysis. In our procedure both the presence of certain specific ions and the relative ratios of certain ion pairs are required for confirmation. In addition the absence of an additional ion is required to demonstrate the lack of interference from methyl esters of fatty acids. Figure 4 lists the necessary conditions which must be met to accomplish confirmation.

Reference

(1) Tishler, F., Sutter, J. L., Bathish, J. N. and Hagman, H. E.; J. Agri. Food Chem. 16, 50-53 (1968).

Table 1

Accuracy and Reproducibility of Sulfamethazine Concentration as Determined by TLC

Tissue	ppm Added	ppm Found (N = 20)	Mean Within-Day COV (6 days)	Day-To-Day COV	ppm found / ppm added x 100
Muscle	0.10	0.101	3.86	5.56	101
Liver	0.10	0.101	5.87	3.63	101

TABLE 2

MUSCLE GC-MS QUANTITATION/CONFIRMATION RESULTS

Muscle

Sulfamethazine Added	N	Average Value Calculated	Std. Dev.	Coefficient of Variation	Confirmatory* Requirements (a)	(b)	(c)
0.00	12	Not Detected	—	—	—	—	—
0.05	6	0.053	0.007	12.29	+	+	+
0.10	9	0.098	0.005	4.65	+	+	+
0.20	6	0.208	0.011	5.21	+	+	+

*As stated in the Results and Discussion Section.
— Implies requirements were not met
+ Implies requirements were met

TABLE 3

LIVER GC-MS QUANTITATION/CONFIRMATION RESULTS

Liver

Sulfamethazine Added	N	Average Value Calculated	Std. Dev.	Coefficient of Variation	Confirmatory* Requirements (a)	(b)	(c)
0.00	12	Not Detected	—	—	—	—	—
0.05	6	0.050	0.003	6.09	+	+	+
0.10	9	0.104	0.005	4.58	+	+	+
0.20	6	0.211	0.009	4.19	+	+	+

*As stated in the Results and Discussion Section.
— Implies requirements were not met
+ Implies requirements were met

FIGURE 1

Methylation with CH_2N_2

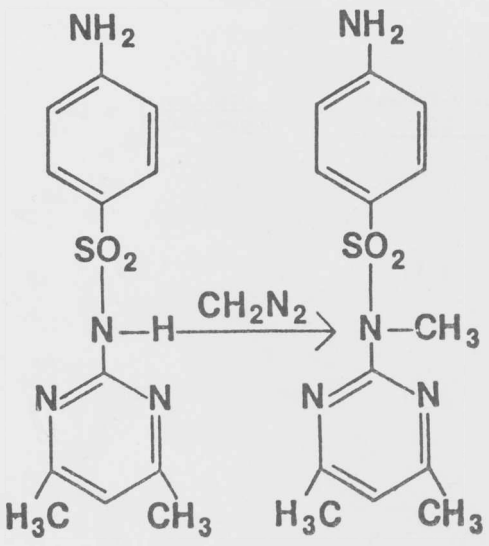
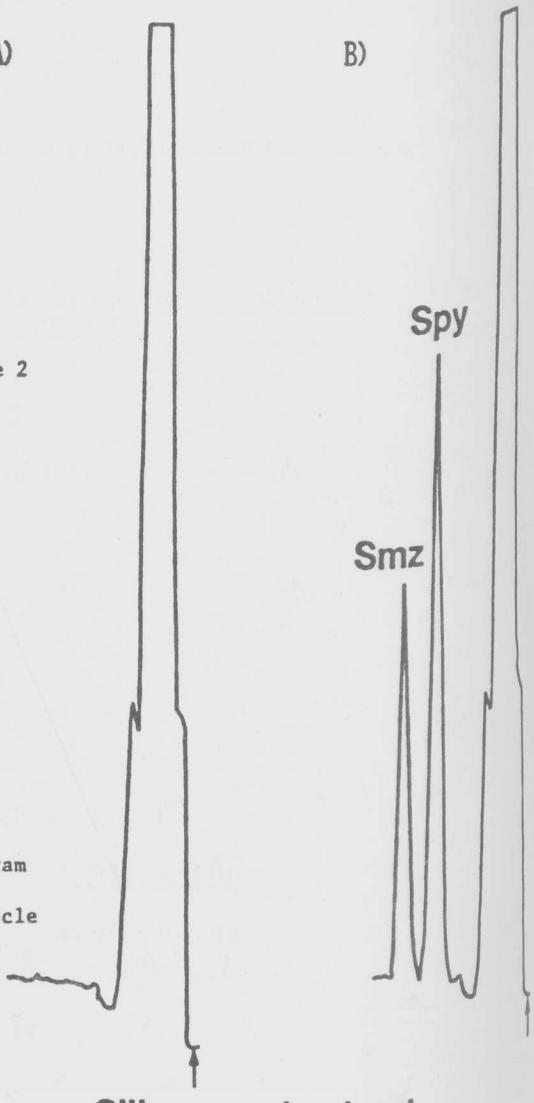


Figure 2



Scanning Curve of Thin Layer Chromatogram

of Control and Fortified (0.1 ppm) Muscle

Figure 3

Actual TLC Standard Curve and 95% Confidence Interval

$S_{y \cdot x}$ Equal 0.005 ppm

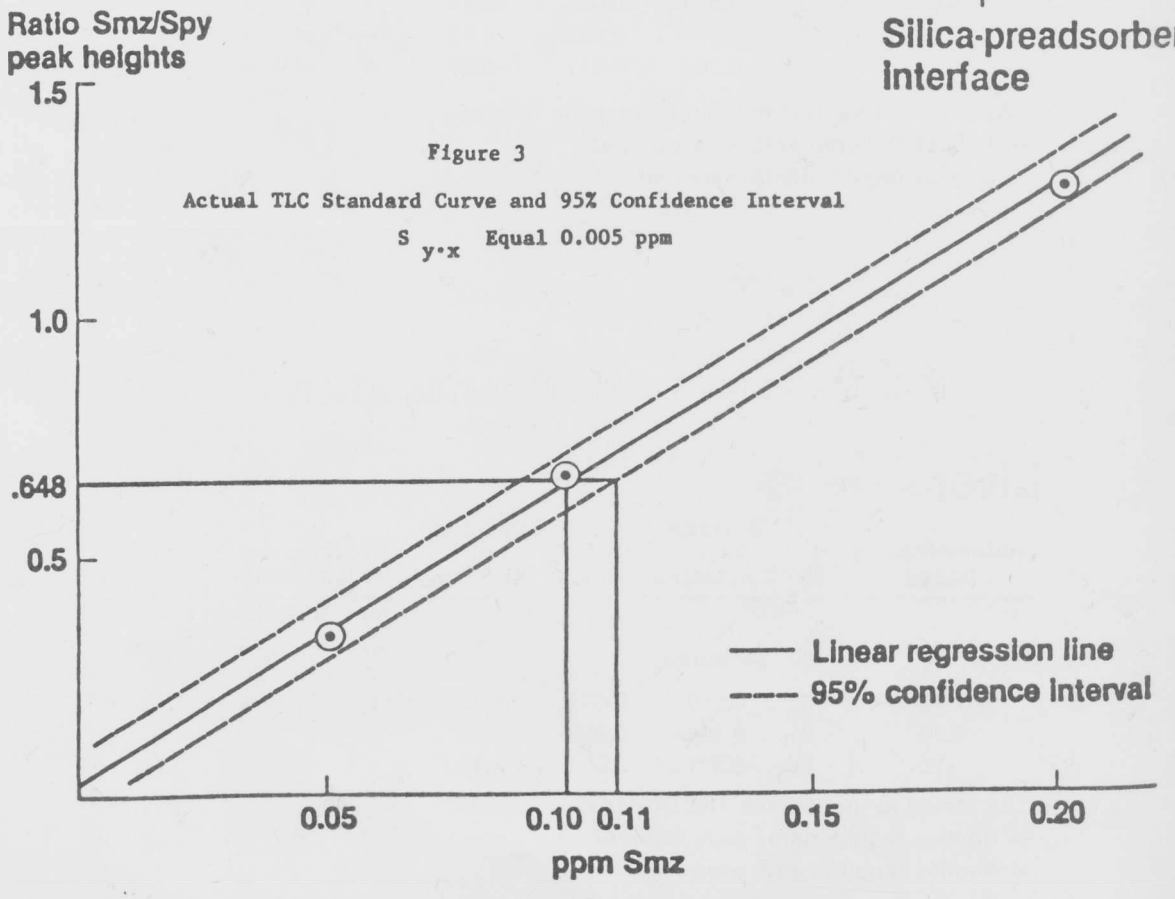


Figure 4

Criteria for Confirmation

a) Co-elution of endogenous material and internal standard.

b) Presence of ion fragments at m/e 92, 227, 228, 233 and 234. No ion fragment at m/e 74.

c) Ratio of 228/227 and 234/233 ions should be $\pm$ 10 percent of those values determined in the standard curve.