Qualitative/Quantitative Determination of Sulfamethazine in Swine Tissue by Gas Chromatographic/Electron Impact Mass Spectrometry Using a Stable Isotope Labeled Internal Standard

Francis B. Suhre,* Randy M. Simpson, and Jerry W. Shafer

A qualitative/quantitative gas chromatographic/mass spectrometric assay has been developed for the determination of sulfamethazine in swine liver and muscle tissue. Qualitative identification is obtained by use of an internal standard and multiple ion monitoring. Quantitation is obtained by determining the ratio of the M – 65 fragmentation ion (molecular ion less 65 amu) of the labeled and unlabeled drug. Data are presented to show that the method can assay sulfamethazine with a high degree of specificity and precision at the tolerance level of 0.10 ppm in swine liver and muscle tissue.

Current government regulations (Code of Federal Regulations, 1977) permit the treatment of food producing animals with sulfamethazine for the control of bacterial infections. The established tolerance (Code of Federal Regulations, 1977) for sulfamethazine in uncooked edible tissue of swine and cattle is 0.10 ppm. For several years the Food Safety and Quality Service (FSQS) of the U.S. Department of Agriculture (USDA) has monitored swine liver and muscle tissue for sulfamethazine. The analytical procedure used by FSQS laboratories involves quantitation by a spectrophotometric procedure (Tishler et al., 1968) preceded by TLC/GLC screening (Goodspeed et al., 1978). The GC/EI-MS method described in this paper was developed in order to provide FSQS laboratories with a highly specific and accurate alternative to the Tishler procedure. A similar paper describing a gas chromatographic/chemical ionization mass spectrometric (GC/EI-MS) assay for sulfadimethoxine in swine and cattle tissue has been published (Garland et al., 1980). Those individuals interested in existing sulfonamide methodology should also refer to a recently published review article on this topic (Horwitz, 1981).

EXPERIMENTAL SECTION

Materials. Sulfamethazine, ^{13}C labeled, was purchased from KOR Isotopes, Cambridge, MA 02142. Isotopic purity was stated to be 90.5% at each of the six positions on the phenyl moiety of the molecule (Figure 1). Assuming a binomial distribution, the isotopically enriched sulfamethazine would be labeled as follows: six carbon-13's, 54.94%; five carbon-13's, 34.60%; four carbon-13's, 9.08%; three carbon-13's, 1.27%; two carbon-13's, 0.10%; one carbon-13, 0.004%; zero carbon-13, 0.0001%. Sulfamethazine (unlabeled) was purchased from Pfaltz and Bauer, Inc., Stamford, CT 06902. Diazald, used for generation of diazomethane, was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI 53233. All solvents were UV grade distilled in glass products of Burdick and Jackson Laboratories, Muskegon, MI 49442.

Extraction. All samples were fortified prior to extraction with a predetermined amount of labeled and unlabeled sulfamethazine from stock solutions containing 50 μg/mL in methanol. A modified version of Tishler's "Method A" (Tishler et al., 1968) was used for extraction purposes; i.e., a 25-g sample is placed in a 500-mL virtis flask, 100 mL of (1:1 v/v) chloroform–acetone is added, and the mixture is homogenized for 1 min at low speed. The liquid is decanted and filtered into a 1000-mL round-bottom flask. This extraction is repeated an additional 2 times. The combined extract is rotary evaporated to an oily residue. The residue is quantitatively transferred to a 250-mL separatory funnel by using, in order, four 25-mL portions of hexane, two 30-mL portions of acetone, and two 25-mL portions of hexane. Ten milliliters of 1 N HCl is added and shaken gently for 2 min. The phases are allowed to separate and the aqueous phase is filtered into a 125-mL separatory funnel. The organic phase is reextracted 2 times with 5-mL portions of 1 N HCl. The combined aqueous filtrate are made basic with 3 mL of 10 N sodium hydroxide and washed 2 times with 25-mL portions of chloroform. The chloroform is discarded after each wash. Upon completion of the modified version of Tishler's "Method A" extraction, the aqueous phase is buffered with 25 mL of saturated sodium citrate and the pH is adjusted to 5.55–5.65. The aqueous phase is then extracted 3 times with 15-mL portions of methylene chloride. The combined methylene chloride extract is placed in a water bath and evaporated to dryness under a stream of nitrogen and reconstituted in 1 mL of anhydrous methanol. One milliliter of freshly prepared saturated ethereal solution of diazomethane is added. The solution is mixed and allowed to stand for 5 min. The derivatized extract is transferred to a 15-mL or less concentrator tube and evaporated to dryness under a stream of nitrogen. The residue is reconstituted in 200 mL of anhydrous methanol and analyzed by GC/MS.

Instrumentation. All mass spectral analyses were conducted on a Hewlett-Packard (HP) 5992 quadrupole GC/MS operated in the multiple ion monitoring under the following conditions: electron energy, 70 eV; electron multiplier, 2400–2800 eV; source temperature, 140 °C; integration time, 200 ms/mass monitored.

A 2 mm i.d. × 3 ft glass column packed with 3% OV-17 on 80–100-mesh Gas-Chrom Q was used to affect separation. Columns and packing materials were purchased from Supelco, Inc., Bellefonte, PA 16826. GC conditions were as follows: injection port temperature, 230 °C; column temperature, 220 °C isothermal for 12 min and then increased 16 °C/min to 250 °C and held for 5 min; helium flow rate: 30 cm^3/min; GC/MS interface: silicone membrane separator. By use of the above conditions N^1-methylsulfamethazine elutes in ~10 min.

RESULTS AND DISCUSSION

The electron impact (EI) fragmentation pattern for the N^1-methyl derivative of sulfamethazine is shown in Figure 2. Although no molecular ion (m/e 292) is present in the EI spectrum, intense peaks are found at m/e 227 and m/e 228. These ions correspond to the well documented expulsion of SO_2, from the molecular ion, followed by loss of a hydrogen radical (Davis et al., 1977). These ionic

Chemistry Division Laboratory Branch, Science, Food Safety and Quality Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

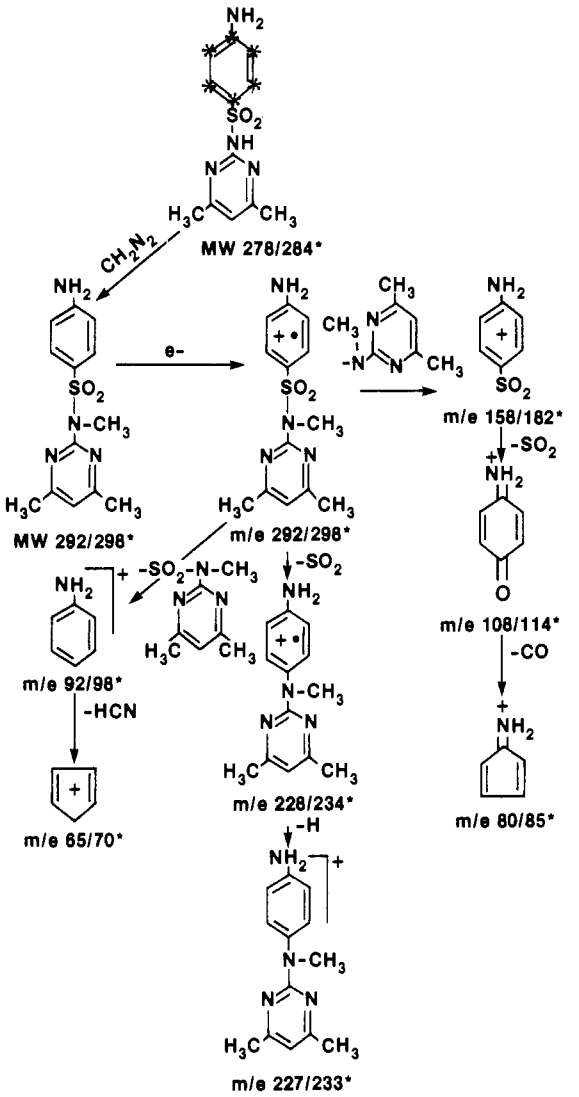

Figure 1. Derivatization and electron impact fragmentation pattern for sulfamethazine. The asterisk implies position of ^{13}C labeling and corresponding m/e ratio for the ^{13}C-labeled internal standard.

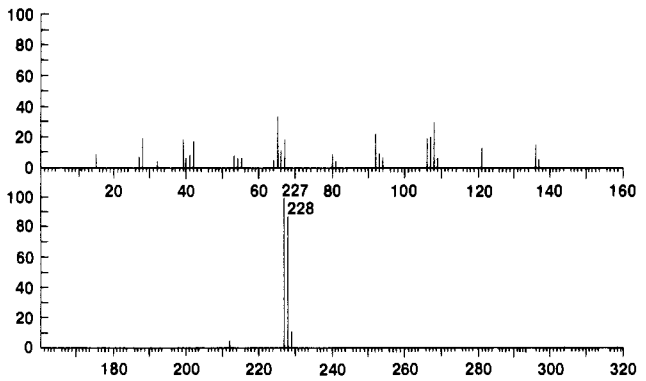

Figure 2. Mass spectrum of [^{12}C]-N^1-methylsulfamethazine. m/e 227 (M - 65); m/e 228 (M - 64).

species, M - 64 and M - 65, cleanly shift to m/e 233 and m/e 234 in the [^{13}C]sulfamethazine spectrum (Figure 3). Furthermore, Figure 3 shows no interferences at m/e 227 and m/e 228 from either incomplete ^{13}C incorporation in the labeled material or some other previously unknown process. These results are consistent with the previously proposed fragmentation pathway (Davis et al., 1977) illustrated in Figure 1 and enabled the development of a

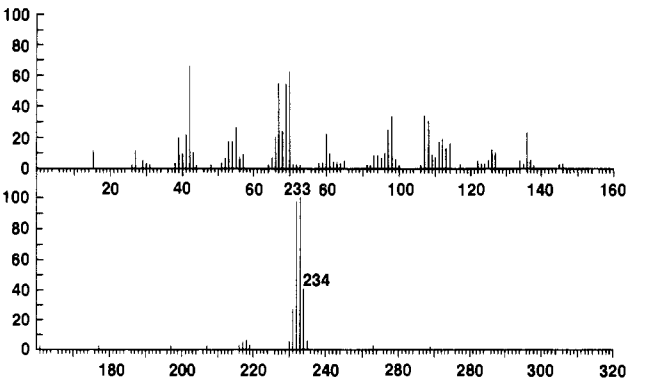

Figure 3. Mass spectrum of [^{13}C]-N^1-methylsulfamethazine. m/e 233 (M - 65); m/e 234 (M - 64).

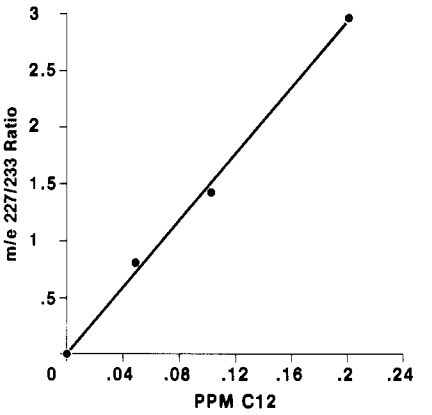

Figure 4. Standard curve.

multiple ion detection method using the M - 64 and M - 65 species.

Qualitative Identification. As the general use and acceptance of mass spectrometry has spread from biomedical quantitation into regulatory and forensic areas, the shortcomings of single ion monitoring have become more apparent. Computer searches of libraries of mass spectra indicate that at least three ions are necessary for the qualitative identification of an endogenous drug residue using low-resolution mass spectrometry (Sphon, 1978). In this work both an internal standard and GC/MS have been utilized. In addition, the relative ratios of two ions were monitored from both the endogenous (unlabeled) sulfamethazine and the isotopically enriched [^{13}C]sulfamethazine internal standard. The criteria for qualitative identification followed in this study were (a) coelution of the endogenous material with the internal standard, (b) the presence of all six ions monitored, three from the endogenous drug (m/e 92, 227, and 228) and three from the labeled standard (m/e 98, 233, and 234), and (c) the ratio of 228/227 and 234/233 ions from liver and muscle tissue samples should be within ±10% of the values determined in the standard curve, as discussed below.

Quantitation. The tissue concentration of sulfamethazine was calculated by linear regression. Standard solutions were prepared in such a manner as to represent tissue extracts containing 0.05, 0.10, and 0.20 ppm of [^{12}C]-sulfamethazine. In addition, each standard was fortified with the equivalent of 0.10 ppm of [^{13}C]sulfamethazine internal standard. The standards were analyzed in the multiple ion monitoring mode, and ion current profiles for m/e 92, 98, 227, 228, 233, and 234 were obtained. For quantitative purposes the 227/233 ion mass ratio was plotted against the amount of [^{12}C]sulfamethazine added to each standard. Figure 4 is representative of a standard curve produced in our laboratory. It is important to note

Table I. Liver

sulfamethazine added	N	av value calcd	SD	coeff of variation	qualitative identification requirements[a] a	b	c
0.00	12	ND[b]			–[c]	–	–
0.05	6	0.050	0.003	6.09	+[d]	+	+
0.10	9	0.104	0.005	4.58	+	+	+
0.20	6	0.211	0.009	4.19	+	+	+

[a] As stated under Results and Discussion. [b] ND, not detected. [c] (–) Implies requirements were not met. [d] (+) Implies requirements were met.

Table II. Muscle

sulfamethazine added	N	av value calcd	SD	coeff of variation	qualitative identification requirements[a] a	b	c
0.00	12	ND[b]			–[c]	–	–
0.05	6	0.053	0.007	12.29	+[d]	+	+
0.10	9	0.098	0.005	4.65	+	+	+
0.20	6	0.208	0.011	5.21	+	+	+

[a] As stated under Results and Discussion. [b] ND, not detected. [c] (–) Implies requirements were not met. [d] (+) Implies requirements were met.

that the 1:1 ratio of [^{12}C]sulfamethazine to [^{13}C]sulfamethazine as measured by the 227/233 ion mass ratio does not yield the theoretically calculated value of 1.82 (1/0.5494). As previously stated (see Materials) 54.94% of the isotopically enriched sulfamethazine internal standard is labeled at all six positions of the phenyl moiety while 34.60% is labeled at only five positions of the phenyl moiety. It is obvious that a positive contribution to m/e 233 (M – 65 fragment labeled at all six positions with ^{13}C) will occur when m/e 228 (M – 64 fragment) is labeled with ^{13}C at only five positions. Furthermore, less obvious positive contributions also occur. These positive contributions to the m/e 233 ion affect the 227/233 quantitative ion ratio. A typical 227/233 ion mass ratio from a 1:1 [^{12}C]sulfamethazine/[^{13}C]sulfamethazine standard when determined experimentally in our laboratory was 1.37.

Swine liver and muscle tissue previously determined to contain less than 0.01 ppm of sulfamethazine by the method of Tishler (Tishler et al., 1968) were fortified with 0.05, 0.10, and 0.20 ppm of [^{12}C]sulfamethazine. In addition, each sample was fortified with 0.10 ppm of [^{13}C]-sulfamethazine internal standard. Tables I and II present qualitative and quantitative data collected by using the conditions as stated under Instrumentation. It is apparent from the data presented that GC/MS in conjunction with an internal standard provides a very powerful technique for the detection and quantitation of sulfamethazine in swine liver and muscle tissue.

LITERATURE CITED

Code of Federal Regulations, 1977, Title 21, Parts 558.128, 546.110(e), 546.113(b), 520.2260(b), 556.670.
Davis, R.; Hurst, D. T.; Taylor, A. R. *J. Appl. Chem. Biotechnol.* **1977**, *27*, 543–548.
Garland, W.; Mirva, B.; Weiss, G.; Chen, G.; Saperstein, R.; MacDonald, A. *Anal. Chem.* **1980**, *52*, 842–846.
Goodspeed, D. P.; Simpson, R. M.; Ashworth, R. B.; Shafer, J. W.; Cook. H. R. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 1050–1053.
Horwitz, W. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 104–130.
Sphon, J. A. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 1247–1252.
Tishler, F.; Sutter, J. L.; Bathish, J. N.; Hagman, H. E. *J. Agric. Food Chem.* **1968**, *16*, 50–53.

Received for review August 18, 1980. Accepted March 16, 1981.

High-Pressure Liquid Chromatographic Determination of Fungicidal Dithiocarbamates

K. Håkan Gustafsson*[1] and Richard A. Thompson[2]

A specific method for determination of thiram, salts of alkylenebis(dithiocarbamic acids), and *N,N*-dimethyldithiocarbamic acid is presented. Iron, zinc, and manganese salts were transformed into water-soluble sodium salts with an alkaline EDTA solution. The extract was subjected to ion-pair methylation at pH 6.5–8.5 in chloroform–hexane. The organic phase was concentrated and analyzed by HPLC and UV detection at 272 nm. Thiram was reduced by nabam into *N,N*-dimethyldithiocarbamate, a reaction which was avoided by extracting with chloroform. However, extraction with chloroform of apple samples drastically reduced the recovery of ethylenebis(dithiocarbamates). Thiram was preferably determined after extracting with chloroform and purifying the extract on a silica gel column. The limit of detection in water solutions for zineb, ziram, and thiram was 0.05, 0.01, and 0.01 ppm, respectively, and the recovery from apple samples fortified at the 1.0-ppm level was 61, 88, and 88% in the order mentioned.

Salts and disulfides of mono- and dialkyldithiocarbamic acids are widely used as pharmaceuticals, rubber vulcanizers, and fungicides (Thorn and Ludwig, 1962). The polymeric salts of ethylenebis(dithiocarbamic acid) (the EBDC's zineb, maneb, and mancozeb, VII–IX; Figure 1) form the most important class of pesticides for broad spectrum control of a variety of fungal diseases on growing crops (Engst, 1977).

Many different types of methods have been developed for the analysis of the dithiocarbamates. Most of these are based on degradation of the dithiocarbamates prior to detection. These methods include hydrolysis to carbon disulfide and amines (Cullen, 1964; McLeod and McCully, 1969; Newsome, 1974; Greve and Hogendoorn, 1978; Uno et al., 1979) reduction (Domar et al., 1949; Rangaswamy

Food Research Department, The National Food Administration, S-751 26 Uppsala, Sweden.
[1]Present address: Högskolan i Kalmar, S-391 29 Kalmar, Sweden.
[2]Present address: University of Hawaii at Manoa, Department of Chemistry, Honolulu, HI 96822.

0021-8561/81/1429-0729$01.25/0