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Analysis of Oral Suspensions Containing Sulfonamides in Combination with Erythromycin Ethylsuccinate

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Abstract □ The sulfonamides and erythromycin ethylsuccinate in combination oral suspensions were determined by high-performance liquid chromatography and automated turbidimetry, respectively. The chromatographic procedure was rapid, specific, and stability-indicating for sulfisoxazole acetyl and the trisulfapyrimidines using a reversed-phase system with UV detection at 254 nm. Erythromycin ethylsuccinate did not interfere with the sulfonamide analysis and these compounds were assayed with relative standard deviations (*RSD*) ranging from ±2.1 to ±3.1%. Erythromycin ethylsuccinate was determined as erythromycin with *RSD* values of ±1.3 or ±3.5% without interference by the sulfonamides present.

Keyphrases □ Sulfonamides—analysis of oral suspensions in combination with erythromycin ethylsuccinate, high-performance liquid chromatography □ Erythromycin ethylsuccinate—analysis of oral suspensions with sulfonamides, high-performance liquid chromatography □ High-performance liquid chromatography—analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate

Oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate have recently been developed for the treatment of acute otitis media. This paper presents the analysis of the sulfonamides and erythromycin ethylsuccinate present in two oral suspensions: erythromycin as erythromycin ethylsuccinate at 200 mg/5 ml and sulfisoxazole as sulfisoxazole acetyl at 600 mg/5 ml (I) and erythromycin as erythromycin ethylsuccinate at 200 mg/5 ml and trisulfapyrimidines: sulfadiazine, sulfamerazine, and sulfamethazine, each at 200 mg/5 ml (II).

Sulfonamide dosage forms are commonly assayed by nitrite titrations or colorimetric methods based on a previous (1) procedure. In mixtures containing more than one sulfonamide or in complex biological matrixes, paper and thin layer chromatography (TLC) have been used to quantitate the individual drugs (2–5). Often these chromatographic separations are followed by the Bratton and Marshall procedure (6–9). The current USP assay (10) of trisulfapyrimidine oral suspensions uses such a procedure and requires several hours to complete.

Use of gas-liquid chromatography (GLC) in the analysis of sulfonamides has been reported (11–14), but derivatization is generally required. High-performance liquid chromatography (HPLC) was used in this study to quantitate the individual sulfonamides. Separations of sulfonamides using ion exchange (15–17), reverse phase (18–24), normal phase (25–28), and ion pairing (29–30) are reported in the recent literature.

Numerous analytical techniques have been reported for the analysis of erythromycin and its various esters. Included are chemical methods based on ultraviolet–visible spectrophotometry (31–34) or fluorometry (35) in addition to GLC (36, 37), TLC (38–41), HPLC (42–45), and microbiological techniques (46–48). Since the microbiological assay is the official methodology required for the determination of erythromycin potency (49), an automated turbidimetric method was employed in this work.

EXPERIMENTAL

Reagents—Acetanilide[1], benzanilide[1], and potassium phosphate[2] (monobasic and dibasic) were obtained commercially and used without further purification. Sulfisoxazole acetyl[3,4], erythromycin base[3], erythromycin ethylsuccinate[5], sulfadiazine[3], sulfamerazine[3], and sulfamethazine[3] were of pharmaceutical quality and were used as received. Acetonitrile[6], chloroform[6], and methanol[6] were HPLC grade. Oral suspensions I[5] and II[5] were prepared from granules prepared in house.

[1] Eastman Kodak Co., Rochester, N.Y.
[2] AR grade, Mallinckrodt, Inc., St. Louis, Mo.
[3] USP Reference Standards, U.S. Pharmacopeia, Rockville, Md.
[4] Hoffmann–LaRoche, Inc., Nutley, N.J.
[5] Abbott Laboratories, North Chicago, Ill.
[6] Burdick & Jackson Laboratories, Muskegon, Mich.

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Equipment—The chromatographic system consisted of a constant flow pump[7], a septumless injector[8], a UV detector[9] operated at 254 nm, a computing integrator[10] and a strip-chart recorder[11]. The analytical column (4-mm i.d. × 300 mm) was prepacked with fully porous 10-μm silica particles to which octadecylsilane was chemically bonded[12]. Turbidimetric measurements were made with a turbidimeter[13] system containing a dilutor module and a reader module operated at 600 nm.

Sulfonamide Analysis by HPLC—*Mobile Phase*—The mobile phase for I and II consisted of water/acetonitrile (60:40) and water/acetonitrile (80:20), respectively. In preparing the mobile phases, appropriate volumes of acetonitrile were diluted to 1 liter with distilled water. The solutions were filtered through 0.45-μm polycarbonate membranes[14] and degassed under vacuum.

Chromatographic Conditions—The flow rate for I was 1.5 ml/min with a column pressure of ~1800 psi and the detector was operated at 0.32 aufs. The flow rate for II was 1.0 ml/min with a column pressure of ~700 psi and the detector operated at 0.10 aufs. The analytical column was at ambient temperature for all separations.

Internal Standard Solution—A 0.33 mg/ml solution of benzanilide in acetonitrile was prepared as the internal standard for I. A 0.20 mg/ml solution of acetanilide in acetonitrile was prepared as the internal standard for II.

Standard Solution—Approximately 50 mg of sulfisoxazole acetyl was accurately weighed into a 50-ml volumetric flask for I. The drug was dissolved and diluted to volume with the internal standard solution giving a concentration of 1.0 mg/ml. For II, ~20 mg each of sulfadiazine, sulfamerazine, and sulfamethazine were accurately weighed into a 100-ml volumetric flask. The mixture was dissolved and diluted to volume with the internal standard solution giving concentrations of 0.20 mg/ml for each drug.

Sample Preparation—A 1-ml aliquot of I was withdrawn with a disposable syringe and extracted with three 15-ml portions of chloroform. The bottom layers were combined and diluted to 50 ml with chloroform. A portion of the chloroform solution was filtered through a 0.45-μm silver membrane[15] and a 2-ml aliquot of the filtrate was evaporated to dryness under dry nitrogen. The residue was dissolved in 5 ml of the internal standard solution giving a drug concentration of ~0.96 mg/ml.

For II, a 1-ml aliquot of the suspension was withdrawn with a disposable syringe and transferred to a 200-ml volumetric flask containing ~100 ml of methanol/water (60:40). The suspension was mixed for 15 min on a mechanical shaker and diluted to volume with methanol/water (60:40). A portion of the solution was filtered through a 0.45-μm silver membrane[15] and a 1-ml aliquot of the filtrate was evaporated to dryness under nitrogen. The residue was dissolved in 1 ml of the internal standard solution giving concentrations for each drug of ~0.20 mg/ml.

Analysis and Calculation—For I and II, respectively, 5- and 4-μl injections of both the standard and sample preparations were made. Peak area ratios for duplicate injections were calculated and averaged as follows:

$$ST \text{ or } SL = \frac{PA_1}{PA_2} \qquad \text{(Eq. 1)}$$

where ST is the standard peak area ratio, SL is the sample peak area ratio, PA_1 is the peak area of the sulfonamide, and PA_2 is the peak area of the internal standard.

The concentrations of the sulfonamides in 5 ml of suspension were calculated by:

$$\text{sulfonamide content per 5 ml} = \frac{SL}{ST} \times \text{conc} \times 5 \text{ ml} \times DF \qquad \text{(Eq. 2)}$$

where conc is the concentration of sulfonamide standard (mg/ml) and DF is the appropriate dilution factor.

Erythromycin Ethylsuccinate Analysis with Turbidimetric Finish—*Dilution Buffer*—A 20% phosphate buffer was prepared by dissolving 192.6 g of dibasic potassium phosphate and 7.4 g of monobasic potassium phosphate in 1 liter of distilled water. The solution was further diluted 100 ml to 2 liter with distilled water (solution pH 8.0 ± 0.1).

Sample Preparation—A 5-ml aliquot of I or II was transferred to a

[7] Model M6000, Waters Associates, Milford, Mass.
[8] Model U6K, Waters Associates, Milford, Mass.
[9] Model SF 770, Schoeffel Instrument Corp., Westwood, N.J.
[10] Model System I, Spectra-Physics Corp., Santa Clara, Calif.
[11] Recordall Series 5000, Fisher Scientific Co., Pittsburgh, Pa.
[12] μBondapak C_{18}, Waters Associates, Milford, Mass.
[13] Autoturb, Elanco Instruments, Eli Lilly and Co., Indianapolis, Ind.
[14] Nuclepore Corp., Pleasanton, Pa.
[15] Selas Corp. of America, Huntingdon Valley, Pa.

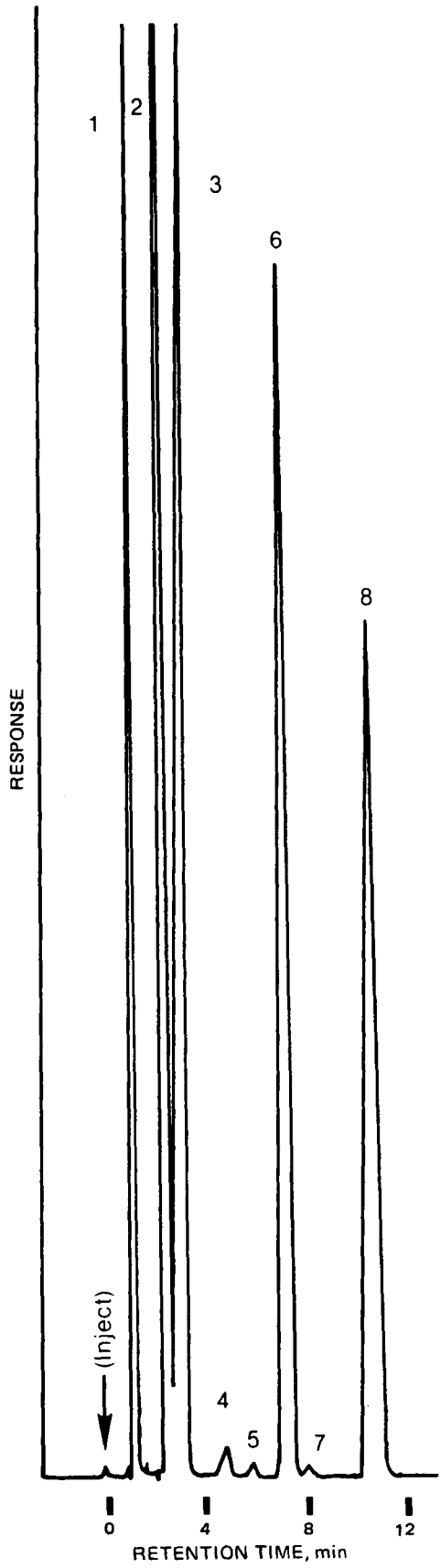

Figure 1—*Chromatogram of a synthetic mixture of sulfisoxazole acetyl (1.0 mg/ml) and three possible degradation products. Key: 1, sulfanilic acid; 2, sulfanilamide; 3, sulfisoxazole; 4, 5, 7, impurities from sulfisoxazole standard; 6, sulfisoxazole acetyl; 8, benzanilide (internal standard).*

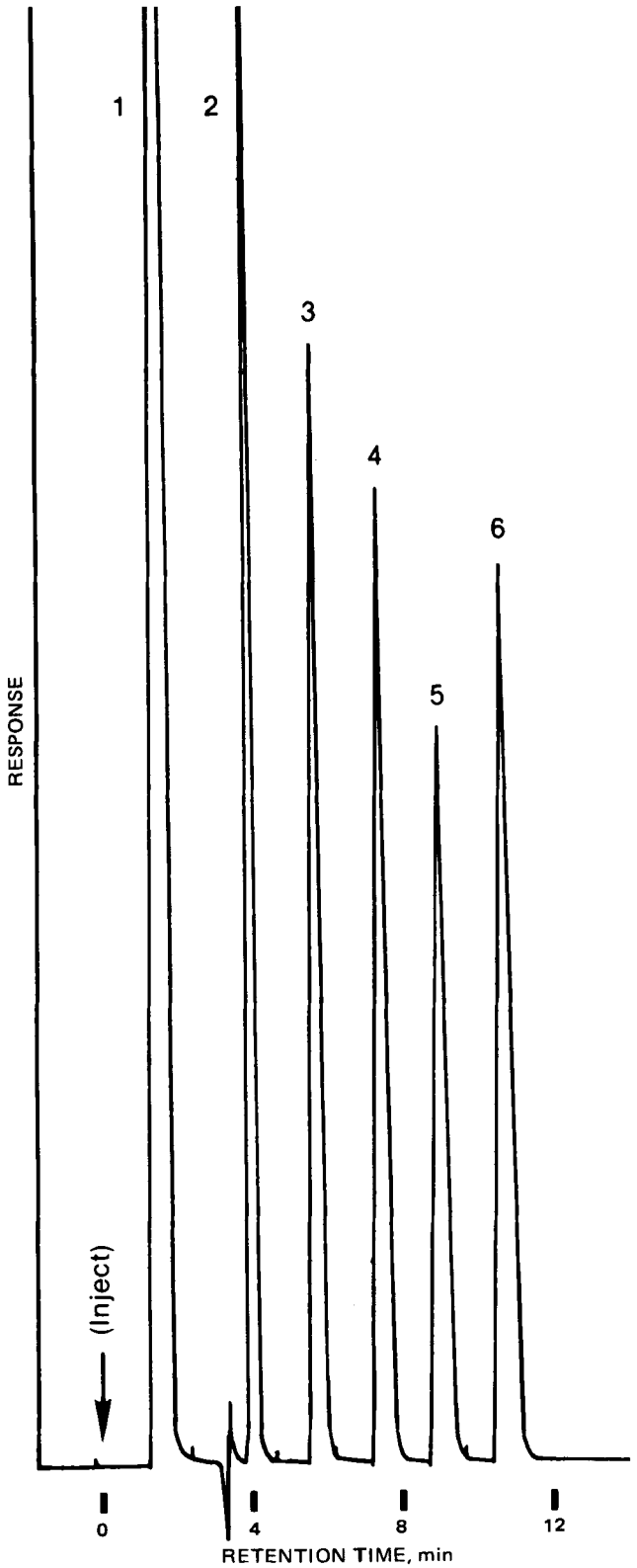

Figure 2—*Chromatogram of a synthetic mixture of trisulfapyrimidines (0.20 mg/ml) and two possible degradation products. Key: 1, sulfanilic acid; 2, sulfanilamide; 3, sulfadiazine; 4, sulfamethazine; 5, sulfamerazine; 6, acetanilide (internal standard).*

high-speed blender containing 195 ml of methanol and was blended for 3 min. The methanolic solution was diluted with the dilution buffer to give a solution containing 100 μg of erythromycin base/ml. This solution was allowed to hydrolyze 16–18 hr at room temperature and diluted to a concentration of 4 μg of erythromycin base/ml with dilution buffer. Sample preparations were performed in quadruplicate.

Table I—Precision Data for Sulfisoxazole Acetyl in I

Sample	Label Claim[a], %
1	105.5
2	110.4
3	110.8
4	107.3
5	110.1
6	106.5
7	106.0
Mean	108.1
SD	±2.3
RSD, %	±2.1

[a] Label claim for I was 600 mg of sulfisoxazole as sulfisoxazole acetyl/5 ml.

Table II—Precision Data for Trisulfapyrimidines in II [a]

Sample	Theoretical Percentage[b]		
	Sulfadiazine	Sulfamethazine	Sulfamerazine
1	105.0	103.5	103.8
2	105.6	104.0	103.5
3	106.6	104.1	103.8
4	100.6	99.6	102.1
5	107.6	109.6	112.2
6	106.7	103.4	107.3
7	104.7	105.3	106.4
8	106.6	105.7	108.0
Mean	105.4	104.4	105.9
SD	±2.2	±2.8	±3.3
RSD, %	±2.1	±2.7	±3.1

[a] Label claim for II was 200 mg of each drug/5 ml. [b] Calculated on a weight basis by accurately weighing suspension sampled and total suspension prepared from granules containing 65.8 mg of each drug/g.

Table III—Standard Addition and Recovery Data for Sulfisoxazole Acetyl in I

Assay Level	Sulfisoxazole Acetyl, mg		
	Added[a]	Recovered	Recovery, %
75%	104.6	106.4	101.7
90%	124.9	125.9	100.8
100%	138.4	138.1	99.8
110%	152.5	151.6	99.4
125%	173.5	172.1	99.2
		Mean	100.2

[a] Weight of drug added per ml with a 1 ml sample preparation.

Assay Procedure and Calculation—Erythromycin determinations were performed turbidimetrically as described for a variety of antibiotics (49).

RESULTS AND DISCUSSION

Sulfonamide Analysis—In the sulfonamide analyses for I and II, high-performance liquid chromatography (HPLC) offered the desired accuracy, sensitivity, and specificity. By operating the UV detector at 254 nm, erythromycin ethylsuccinate carried through with the sample work-up does not interfere in the sulfonamide assay.

In Figs. 1 and 2, respectively, synthetic solutions of sulfisoxazole acetyl (1.0 mg/ml) and trisulfapyrimidines (0.20 mg/ml) are spiked with several possible degradation products and chromatographed. As shown in the figures, the degradation products are well resolved from the drug and internal standard peaks, making the HPLC analyses stability indicating. Figures 3 and 4, respectively, show authentic samples of I and II carried through the HPLC assay procedure. Total elution time for both I and II was ~11 min under the chromatographic conditions chosen.

Linearity of response for the sulfonamides contained in I and II was demonstrated by plotting the peak area ratio of the drugs to the internal standards *versus* drug concentrations (mg/ml). For sulfisoxazole acetyl, detector response was linear to at least 1.5 mg/ml. For the trisulfapyrimidines, detector response was linear to at least 0.30 mg/ml for each compound. The linearity curve for each sulfonamide assayed essentially intersected the origin and the correlation coefficient was >0.999 for each curve.

Precision data for the sulfonamide assay in I and II are presented in Tables I and II, respectively. Analyses for I were performed over a 5-day

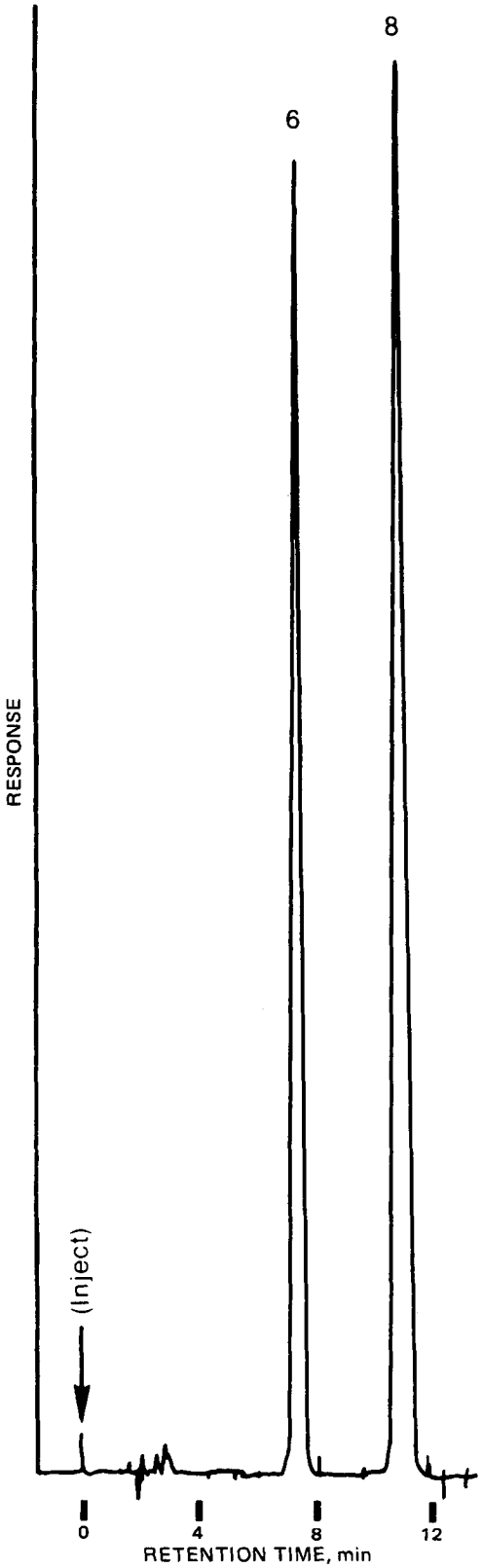

Figure 3—*Chromatogram of an authentic assay preparation of I. Key: 6, sulfisoxazole acetyl; 8, benzanilide (internal standard).*

period by two different analysts, preparing fresh suspensions each day. As shown in Table I, the average assay was 108.1% of the label claim amount, and the relative standard deviation of the procedure was ±2.1%. For II, analyses were performed by two different analysts over a 2-day period, and results were calculated as percent theory on a weight basis. Each sample shown in Table II represents a freshly prepared suspension and as shown, the relative standard deviations ranged from ±2.1 to ±3.1%.

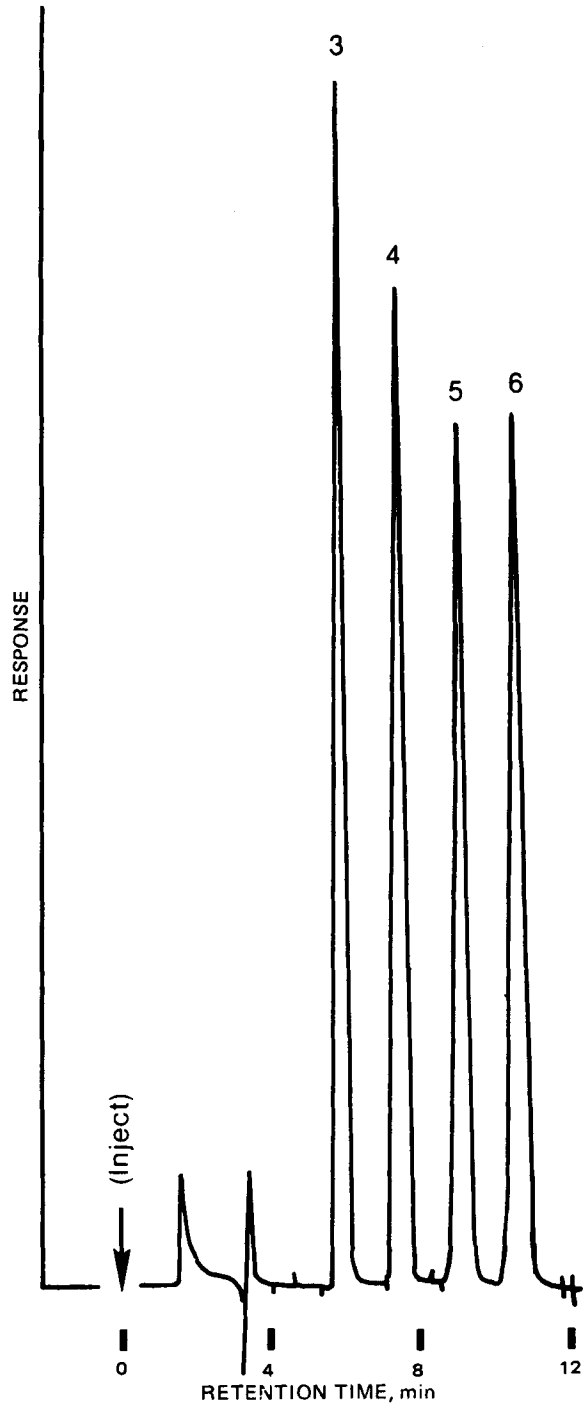

Figure 4—*Chromatogram of an authentic assay preparation of II. Key: 3, sulfadiazine; 4, sulfamethazine; 5, sulfamerazine; 6, acetanilide (internal standard).*

To determine if sulfisoxazole acetyl was recovered quantitatively in the sample preparation of I, portions of the drug were added at 75–125% of the formulation level to a suspension placebo. The resulting mixtures were extracted and assayed as described. The erythromycin ethylsuccinate and excipients were maintained at the same level as for I. The standard addition–recovery results are summarized in Table III and as shown, an average recovery of 100.2% was obtained.

A correlation of the sulfisoxazole acetyl analysis by HPLC and sodium nitrite titration (USP methodology) was made for I to determine if the presence of erythromycin ethylsuccinate or excipients interfered in the titration procedure. Suspensions containing placebo, placebo plus sulfisoxazole acetyl, and placebo plus acetyl sulfisoxazole and erythromycin ethylsuccinate were titrated and simultaneously analyzed for sulfisoxazole acetyl by HPLC. The suspensions were prepared by accurately weighing quantities of each drug substance that would be present in I,

Table IV—Percent Sulfisoxazole Acetyl Recovered by Sodium Nitrite Titration and HPLC in I

Sample	HPLC Analysis	$NaNO_2$ Titration with Potentiometric End-Point[b]	$NaNO_2$ Titrated with Amperometric End-Point[c]
Sulfisoxazole acetyl			
Suspension 1	102.1[a]	102.1[a]	104.6[a]
Suspension 2	102.7[a]	101.1[a]	101.7[a]
I	101.6	108.1	108.5
Placebo	0	0	0
	0	0	—
Placebo + sulfisoxazole acetyl	97.6	98.0	100.2
	98.4	99.2	—
Placebo + sulfisoxazole acetyl + erythromycin ethylsuccinate	98.2	103.6	105.2
	99.0	105.4	—

[a] Percent manufacturer's label claim. [b] Platinum–calomel electrode. [c] Platinum–platinum electrode.

Table V—Erythromycin Precision Data for I and II

Day	Label Claim in I[a,b], %	Label Claim in II[a,b], %
1	96.4	105.0
2	98.9	104.3
2	99.4	—
3	98.2	97.1
4	98.2	98.3
5	100.1	102.6
Mean	98.5	101.5
SD	±1.3	±3.6
RSD, %	±1.3	±3.5

[a] Percent label claim entries are averages of quadruplicate assays. [b] Label claim for I and II was 200 mg of erythromycin as erythromycin ethylsuccinate per 5 ml.

Table VI—Standard Addition and Recovery Data for Erythromycin Ethylsuccinate in I

Erythromycin Added[a], mg	Erythromycin Recovered[a,b], mg	Recovery, %
200.0	198.8	99.4
200.1	193.5	96.7
200.2	192.8	96.3
200.3	193.8	96.8
199.9	195.8	97.9
200.0	198.0	99.0
	Mean	97.7

[a] Milligrams of erythromycin activity (as erythromycin ethylsuccinate) per 5 ml. [b] Obtained from quadruplicate dilutions of a stock methanol sample preparation.

and these suspensions were sampled by weighing an appropriate volume accurately. From the analysis, the weight of suspension sampled and the weight of total suspension prepared, the percent recovery of sulfisoxazole acetyl was calculated. In addition, two commercial sulfisoxazole acetyl suspensions were titrated and simultaneously assayed by HPLC.

In Table IV the results of the sodium nitrite titrations and the HPLC analysis are summarized. For those suspensions containing sulfisoxazole acetyl but no erythromycin ethylsuccinate, the HPLC results and titrations agreed with experimental error. However, for those suspensions containing erythromycin ethylsuccinate, the titration results were invariably higher than the HPLC results. The extent of this high bias ranged from 5.4–7.0% in the sodium nitrite titrations. Placebo alone titrated identically to a solvent blank and no response was observed from the placebo preparations by HPLC. A placebo suspension containing erythromycin ethylsuccinate gave a higher sodium nitrite titer than the placebo alone. A possible explanation for the higher titer in this preparation is the reversible reaction of nitrous acid with the tertiary aliphatic amine group of erythromycin ethylsuccinate (50).

Erythromycin Ethylsuccinate Analysis—Automated turbidimetric analysis for a variety of antibiotics are in wide use. To determine the precision of this technique for I and II, freshly prepared suspensions of each were assayed over 5 days. The precision data are summarized in Table V as percent label claim in terms of erythromycin activity/5 ml of suspension. For I the percent label claim averaged 98.5% with a relative standard deviation of ±1.3%. For II the percent label claim averaged 101.5% with a relative standard deviation of ±3.5%.

To determine if erythromycin ethylsuccinate was recovered quantitatively in the sample preparation of I, accurately weighed quantities of the drug were added to a placebo suspension. Sulfisoxazole acetyl and excipients were at the same levels as in the actual product. The usual sample work-up and analysis was performed using quadruplicate dilutions of the methanol solution. In Table VI, the standard addition–recovery data collected over 5 days by two analysts are presented. As shown, a mean recovery of 97.7% was obtained.

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Gas Chromatographic Analysis of Meperidine and Normeperidine: Determination in Blood after a Single Dose of Meperidine

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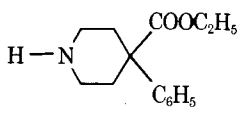
Abstract □ A method is described for the determination of meperidine and its pharmacologically active metabolite, normeperidine, in blood, plasma, and urine using gas chromatography with nitrogen–phosphorus detection. Structural analogs of both meperidine and normeperidine were used as internal standards. Unlike previously reported assays, this procedure was sensitive and convenient enough for use in pharmacokinetic studies of both meperidine and normeperidine following single doses of meperidine. The assay was sensitive to 5 ng of meperidine/ml and 2.5 ng of normeperidine/ml extracted from a 1-ml biological sample. The between-assay coefficients of variation at these concentrations were 9.4 and 10.4%, respectively.

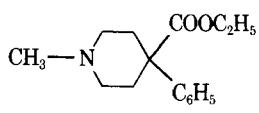
Keyphrases □ Meperidine—gas chromatographic analysis in blood □ Normeperidine—gas chromatographic analysis in blood after single dose of meperidine □ Gas chromatography—analysis of meperidine and normeperidine in blood □ Analgesics—meperidine, gas chromatographic analysis in blood

Normeperidine (I), the *N*-demethylated metabolite of the analgesic drug meperidine (II), is pharmacologically active (1) and may cause seizures in humans (2). Therefore, blood levels of I should be determined when delineating the side effects and drug interactions that occur with II. To carry out such studies after single doses of II, a sensitive and specific gas chromatographic (GC) assay for I and II in biological fluids was developed. The assay allows measurement of II up to 24 hr and I up to 48 hr after a single dose of II.

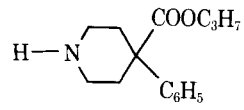
Previously published methods with adequate sensitivity for the measurement of I in plasma after a single dose have involved GC–mass spectrometry with selected ion monitoring (3, 4), radioimmunoassay (5), or GC using electron-capture detection (6). GC methods with flame-ionization detection (7–10) may be satisfactory for determining therapeutic concentrations of II, but have marginal sensitivity for the determination of plasma I concentrations (7–9) following single doses of the parent drug.



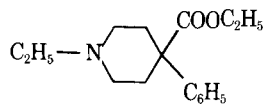
I



II



III



IV

This report describes the GC determination of I and II using a nitrogen–phosphorus detector, which is highly selective toward nitrogen-containing compounds such as II and its derivatives. The assay is significantly faster and more convenient than previously reported GC methods, and is applicable to whole blood, plasma, or urine.

EXPERIMENTAL

Materials—Meperidine hydrochloride and normeperidine hydrochloride were obtained[1]. Heptafluorobutyric anhydride and sodium cyanoborohydride were obtained commercially[2]. Normeperidinic acid *n*-propyl ester (III) was synthesized by a previous method (6). *N*-ethylnormeperidine (IV) was synthesized as will be described. All other chemicals and solvents were analytical reagent grade.

Synthesis of *N*-Ethylnormeperidine Hydrochloride—Sodium cyanoborohydride (100 mg) was added to a solution of normeperidine hydrochloride (250 mg) and acetaldehyde (0.5 ml) in 50 ml of 50% aqueous

[1] Kindly supplied by Sterling-Winthrop Research Institute, Rensselaer, N.Y.
[2] Aldrich Chemical Co., Milwaukee, Wis.

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