GLC Determination of Erythromycin in Enteric-Coated Tablets

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Abstract □ A GLC assay method for erythromycin in tablets is described. This method involves the extraction, silylation, and chromatography of erythromycin from tablets, and it yields an average recovery of 100.6% with a coefficient of variation of about 2%. The method is also useful in monitoring the stability of enteric-coated erythromycin base tablets in simulated gastric fluid.

Keyphrases □ Erythromycin enteric-coated tablets—GLC analysis □ Tablets, enteric coated, erythromycin—GLC analysis □ GLC—analysis, erythromycin enteric-coated tablets

Erythromycin, available as the base, salts, or esters, is perhaps one of the most widely used antibiotics. However, when given orally, erythromycin is susceptible to inactivation by acid in the stomach (1). For this reason, oral preparations of erythromycin are formulated with acid-resistant coatings or derivatized as esters.

The present procedure for the determination of erythromycin in tablets is a microbiological assay using the agar diffusion method (2). The method is, however, far from ideal due to the inherent variability associated with a microbiological system. The GLC assay method developed by Tsuji and Robertson (3) is perhaps the best practical method available to date for the qualitative and quantitative analysis of erythromycin, its esters, and its fractions. Erythralosamine and anhydroerythromycin, which normally predominate upon acid treatment of erythromycin, can also be quantitated by the GLC method.

The purposes of this paper are to demonstrate the applicability of the GLC method to the analysis of erythromycin in enteric-coated tablets and to explore the feasibility of the method to monitor the stability of enteric-coated erythromycin tablets in simulated gastric fluid.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] with flame-ionization detector was used. The gas flow rates were: hydrogen, 40 ml./min.; air, 600 ml./min.; and carrier gas (helium), 55 ml./min. A chart speed of 6.4 mm./min. and an isothermal oven temperature of 280° were used.

Column—A glass column, 3 × 1830 mm. (6 ft.) packed with 3% OV-225 on Gas Chrom Q, 100–120 mesh[2], was used. The column was no-flow conditioned at 330° for 45 min., followed by an injection of a mixture of three trimethylsilyl donors[3] and silylated erythromycin. The column thus prepared had 1319 theoretical plates per meter for silylated erythromycin A. If the column is used daily, its stability is about 3 weeks because of the high operating temperature.

Internal Standard Solution—Five milliliters of trimethylchlorosilane[4] was added to 5 ml. of *N,O*-bis(trimethylsilyl)acetamide[4] followed by 2 ml. of *N*-trimethylsilylimidazole[4]. This silylation reagent mixture was added to 12 ml. of pyridine containing approximately 48 mg. of 1,3-dimyristin[2].

Reference Standard—About 10 mg. of erythromycin reference standard was accurately weighed into a 1-dram vial[5].

Sample Preparation—To minimize tablet weight variation, 10 erythromycin tablets were accurately weighed and then finely

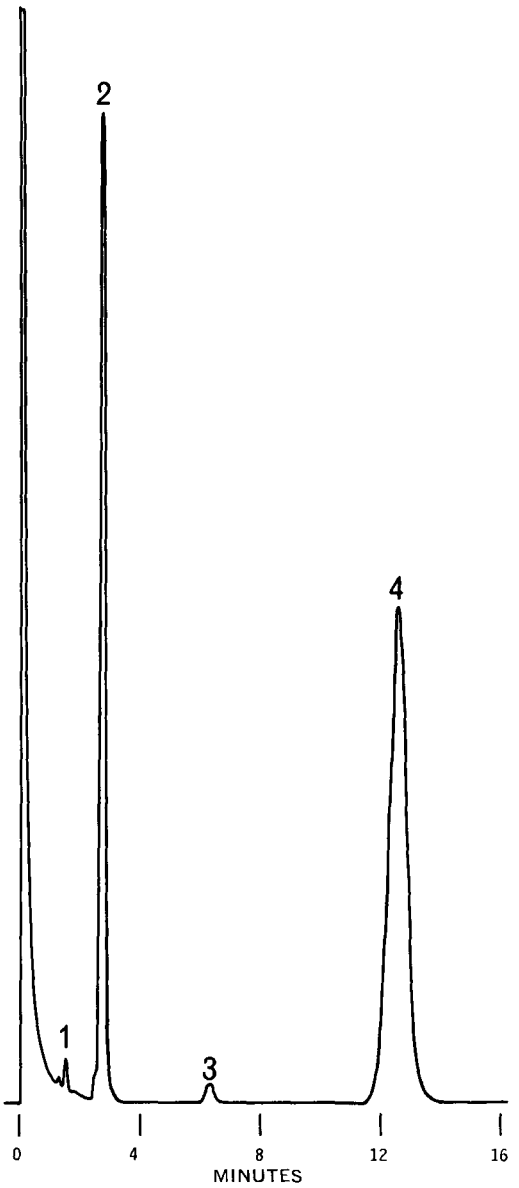

Figure 1—*Chromatogram of uncoated compressed erythromycin tablet after simulated gastric fluid treatment. Key: 1, erythralosamine; 2, internal standard; 3, acid-hydrolyzed erythromycin; and 4, anhydroerythromycin.*

[1] F & M model 400.
[2] Applied Science Laboratory, Inc., State College, Pa.
[3] Silyl-8, Pierce Chemical Co., Rockford, Ill.
[4] Pierce Chemical Co.
[5] Opticlear Stopper Vial, Owens-Illinois, Toledo, Ohio.

Table I—Recovery of Erythromycin from 250-mg. Placebo Tablets Spiked at 80, 100, and 120% of the Manufacturing Theory

Erythromycin Spiked, mg.	Erythromycin Recovered, mg.	Percent Recovery
8.000	7.91	98.8
10.000	10.0	100.0
11.999	12.4	103.6
	Average recovery	100.6

ground using a Wiley mill with a 60-mesh screen. A portion of the powder equivalent to the weight of two tablets (about 500 mg. of erythromycin) was accurately weighed and placed in a ground-glass-stoppered, 50-ml., round-bottom centrifuge tube. Twenty-five milliliters of methylene chloride was added, and the tube was tightly stoppered and then shaken vigorously or rotated continuously for 45 min. on a shaker (Eberback) or tube rotator (BBL). The sample was then centrifuged at 3000 r.p.m. (10,000×*g*) for 15 min. The methylene chloride extract, 0.5 ml., was then pipeted into a 1-dram

Figure 2—*Chromatogram of enteric-coated erythromycin tablet after simulated gastric fluid treatment. Key: 1, internal standard; 2, acid-hydrolyzed erythromycin; 3, erythromycin C; 4, erythromycin A; 5, erythromycin A–isomer II; 6, erythromycin B and/or anhydroerythromycin A; and 7, erythromycin A–isomer III.*

Table II—Recovery of Erythromycin from Placebo Tablets Spiked at the Label Potency

Sample	Tablet Material[a], mg.	Erythromycin Spiked, mg.	Erythromycin Recovered, mg.	Percent Recovery
1	340	500.0	490	98.1
2	340	499.9	493	98.6
3	340	499.9	502	100.4
4	340	500.0	506	101.1
5	340	499.9	485	97.1
6	340	500.0	516	103.2
			Average recovery	99.8
			Coefficient of variation	2.3

[a] From E-Mycin tablets.

Table III—Precision of GC Assay for Erythromycin in Erythromycin 200-mg. Tablets[a]

Weight of Sample, g.	Area: Internal Standard	Area: Erythromycin A	Area: Erythromycin B	Area: Erythromycin C	GC Calculated Biopotency, mg./Tablet
1.56679	115.5	387.5	9.5	0	194
1.56699	113.5	381.5	10.0	0	195
1.56683	109.5	371.5	10.5	1.0	197
1.56685	108.0	377.0	12.0	4.5	204
1.56696	121.5	422.5	14.5	7.5	204
1.56702	127.0	429.5	10.5	1.5	196
			Average potency		198 mg./tablet
			Coefficient of variation		2.19%

[a] Erythromycin 200 mg., Japan Upjohn Ltd.

screw-cap vial[6] and evaporated to dryness under a stream of dry nitrogen. To ensure dryness, the sample was then dried for an additional 10 min. in a vacuum oven at 60° at <5 mm. Hg pressure.

Silylation Procedure—One milliliter of internal standard solution was added to the vial containing erythromycin by means of a glass tuberculin syringe. The cap, lined with a 2.6-mil polyethylene liner, was then tightly sealed and placed in an oil bath at 75° for 24 hr.

Calculation—As previously indicated (3), the microbiological responses of erythromycins B and C are 50 and 40%, respectively, that of erythromycin A. Therefore, the following formula was devised to make the GC data comparable with that of the microbiological assay.

$$\text{GC calculated biopotency (mg. erythromycin/tablet)} = [Ra/Rs] + 0.5\,[Rb/Rs] + 0.4\,[Rc/Rs] \times [Ws/Wt] \times F_1 \times F_2 \times W_1 \quad \text{(Eq. 1)}$$

where:

Rs = ratio of a reference standard erythromycin A peak area to its internal standard peak area
Ra = ratio of a sample erythromycin A peak area to its internal standard peak area
Rb = ratio of a sample erythromycin B peak area to its internal standard peak area
Rc = ratio of a sample erythromycin C peak area to its internal standard peak area
Ws = weight of erythromycin reference standard in milligrams
Wt = weight of the powder equivalent of 500 mg. erythromycin in milligrams
F_1 = assigned value of erythromycin reference standard expressed in micrograms of erythromycin base per milligram of standard
F_2 = dilution factor (50)
W_1 = weight of one tablet in milligrams

[6] Part No. 60910, Kimble Glass, Owens-Illinois, Toledo, Ohio.

Simulated Gastric Fluid Treatment—Samples of erythromycin coated with potassium acetate phthalate were treated in simulated gastric fluid according to the directions outlined in USP XVIII (4). After gastric fluid treatment, the simulated gastric fluid was filtered off using a Millipore filter with 0.45-μ HA Millipore membrane. The insoluble material was then rinsed with 100 ml. of water. After rinsing, suction was maintained until the solids were dry. Samples thus treated were then extracted and silylated as outlined in the standard tablet procedure.

RESULTS AND DISCUSSION

Placebo tablets (containing all tablet ingredients except erythromycin) were assayed with and without internal standard or erythromycin to determine if any interfering components in the GLC chromatogram are extracted with methylene chloride. No interference was observed.

Placebo 250-mg. tablets were spiked with erythromycin at 80, 100, and 120% of manufacturing theory. The average recovery for this three-level spiked study was 100.6% (Table I).When erythromycin was spiked at label potency using six replicates of individually weighed and extracted samples, the average recovery was 99.8% with a coefficient of variation of 2.3% (Table II).

The precision of the method was determined by assaying six replicates of individually weighed and extracted samples of one lot each of regular production lots of erythromycin tablets containing 200 mg.[7] and 250 mg.[8] of erythromycin, respectively. The regular production 200-mg. tablets assayed at an average potency of 198 mg./tablet with a coefficient of variation of 2.19% (Table III). Microbiological assay of this lot was 209 mg./tablet. The 250-mg. tablet (manufacturing theory 262 mg./tablet) gave an average potency of 258 mg./tablet with a coefficient of variation of 1.25% (Table IV). Microbiological assay of this lot was 255 mg./tablet.

Thirteen regular production lots of erythromycin 250-mg. tablets were assayed by GC and microbiologically. The average GC calculated biopotency (250 mg./tablet) compared favorably with the microbiological assay value (254 mg./tablet).

The effectiveness of enteric coating erythromycin tablets with potassium acetate phthalate was tested by treating with simulated gastric fluid and then by GC of the methylene chloride-extracted residue. Uncoated erythromycin tablets showed degradation to anhydroerythromycin A (97%), the prime product of acid degradation, and a trace of erythralosamine (Fig. 1). Effectively coated erythromycin tablets showed no degradation (Fig. 2). Thus, the GLC method for erythromycin has been successfully used to assay enteric-coated erythromycin tablets. Although the present study was confined to enteric-coated erythromycin base tablets, the GLC method may be applied to monitor the stability of various erythromycin esters in simulated gastric fluid, since erythromycin esters and acid-degradation compounds of erythromycin can be separated and quantitated by the GLC method (3).

[7] Erythromycin 200 mg., Japan Upjohn Ltd.
[8] E-Mycin 250 mg., The Upjohn Co.

Table IV—Precision of GC Assay for Erythromycin 250-mg. Tablets[a]

Weight of Sample, g.	Area: Internal Standard	Area: Erythromycin A	Area: Erythromycin B	Area: Erythromycin C	GC Calculated Biopotency, mg./Tablet
0.89976	101.0	343.0	46.0	11.0	257
0.89944	144.0	503.0	54.0	11.0	261
0.89966	118.5	414.5	34.5	9.5	258
0.89954	113.5	390.5	32.0	5.0	253
0.89951	115.0	408.5	32.0	9.0	261
0.89954	141.0	489.5	38.0	8.5	255
		Average potency			$\overline{258}$ mg./tablet
		Coefficient of variation			1.25%

[a] E-Mycin 250 mg., The Upjohn Co.

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Analysis of Cacodylate Injections by NMR Spectroscopy

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Abstract □ An NMR procedure was developed for the assay of sodium cacodylate injection. The results obtained by this technique compare favorably with those obtained by the NF X method and atomic absorption spectrophotometry. The advantages of the NMR procedure over the other procedures are that it is highly specific for the cacodylate and that it is less time consuming.

Keyphrases □ Cacodylate injections—NMR analysis, compared to NF X and atomic absorption spectrophotometry methods □ Sodium cacodylate injections—NMR analysis, compared to NF X and atomic absorption spectrophotometry methods □ NMR spectroscopy—analysis, cacodylate injections

The position of arsenic-containing compounds in therapeutics has gradually been displaced as more specific drugs have been developed. One of the organic arsenic compounds that still enjoys some use is sodium cacodylate. This drug releases arsenic(III) in the body slowly and is formulated as a solution for injection. In