terium incorporation. Though the chemical shifts of the protons at C-6 and C-5 and their coupling constants changed due to the conversion of one isomer to the other, the protons themselves remained coupled to each other, and the intensities of the peaks remained unchanged relative to the peaks of the other protons in the spectrum. These observations appear to contradict the suggestion that the enamine form of penamaldic acid exists in equilibrium with penicilloic acid in alkaline media and, consequently, exclude the enamine as an intermediate in the conversion of 5*R*,6*R*-benzylpenicilloic acid to the 5*S*,6*R*-epimer. Hence, the imine tautomer of penamaldic acid remains as the only probable intermediate involved in the epimerization process (Scheme I, pathway B).

An analytical technique employed to determine the imine in the presence of other degradation products including the enamine at very low concentrations was differential pulse polarography. Unlike the other compounds that may be present in solution, the imine tautomer contains a C=N group which is reducible at an electrode surface. Consequently, a solution of disodium 5*R*,6*R*-benzylpenicilloate was prepared and polarographed in the differential-pulse mode of operation. A reduction wave with a peak potential at −1.1 V *versus* SCE was observed. When aliquots were withdrawn from the reaction vessel after 12, 24, and 36 h and polarographed, the wave remained constant in height. An oxidation wave was also observed in the sulfhydryl region of the polarogram (~ −0.6 V *versus* SCE). However, it was not utilized for unequivocal identification since it is subject to interference from other sulfhydryl-containing compounds. Based on these results, it is concluded that in alkaline aqueous solutions 5*R*,6*R*-benzylpenicilloic acid epimerizes to 5*S*,6*R*-benzylpenicilloic acid and involves the imine tautomer rather than the enamine form of penamaldic acid as an intermediate.

The kinetic transformation of 5*R*,6*R*-benzylpenicilloic acid to its 5*S*,6*R*-epimer was determined quantitatively using the ^{1}H-NMR data presented in Table II. Assuming that the intermediate attains a steady-state concentration during the epimerization process and that the reaction rates are first order, the rate constants for both the forward and reverse reactions were computed using the following equations:

$$R \underset{k_r}{\overset{k_f}{\rightleftharpoons}} S \qquad \text{(Eq. 1)}$$

$$\log \frac{R_0 - R_{eq}}{R - R_{eq}} = \frac{(k_f + k_r)}{2.303} t \qquad \text{(Eq. 2)}$$

and

$$K = \frac{k_f}{k_r} = \frac{S_{eq}}{R_{eq}} \qquad \text{(Eq. 3)}$$

where R_0 is the initial concentration of 5*R*,6*R*-benzylpenicilloic acid, R_{eq} is the equilibrium concentration of 5*R*,6*R*-benzylpenicilloic acid, R is the concentration of 5*R*,6*R*-benzylpenicilloic acid at time t, k_f is the forward rate constant, k_r is the reverse rate constant, K is the equilibrium constant of the reaction, S is the concentration of 5*S*,6*R*-benzylpenicilloic acid at time t, and S_{eq} is the equilibrium concentration of 5*S*,6*R*-benzylpenicilloic acid; k_f was found to be 7.4×10^{-2} h^{-1}, while k_r was 1.8×10^{-2} h^{-1}. These values indicate that, at equilibrium, the ratio of the concentration of 5*R*,6*R*-benzylpenicilloic acid was ~0.2 × that of the 5*S*,6*R*-epimer, a value that is in agreement with the HPLC analysis (Table I).

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Simultaneous Determination of Methyl, Ethyl, Propyl, and Butyl 4-Hydroxybenzoates and 4-Hydroxybenzoic Acid in Liquid Antacid Formulations by Gas Chromatography

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Abstract □ An isothermal chromatographic (GC) method employing an SE-30 column and flame-ionization detection has been developed for the simultaneous assay of methyl, ethyl, propyl, and butyl 4-hydroxybenzoates and 4-hydroxybenzoic acid in liquid antacid formulations. The method, which uses a silica column chromatographic cleanup step prior to GC, is specific for the compounds with respect to possible degradation products, impurities, and excipients.

Keyphrases □ 4-Hydroxybenzoic acid—simultaneous determination with its methyl, ethyl, propyl, and butyl esters, liquid antacid formulations, gas chromatography □ Antacid formulations—liquid, simultaneous determination of 4-hydroxybenzoic acid and its methyl, ethyl, propyl, and butyl esters, gas chromatography

The methyl (I), ethyl (II), propyl (III), and butyl (IV) esters of 4-hydroxybenzoic acid (V) in various combinations are commonly used as preservatives in liquid pharmaceutical preparations. Since the antimicrobial activity of various combinations of esters are generally more than additive (1), a method for simultaneously determining the

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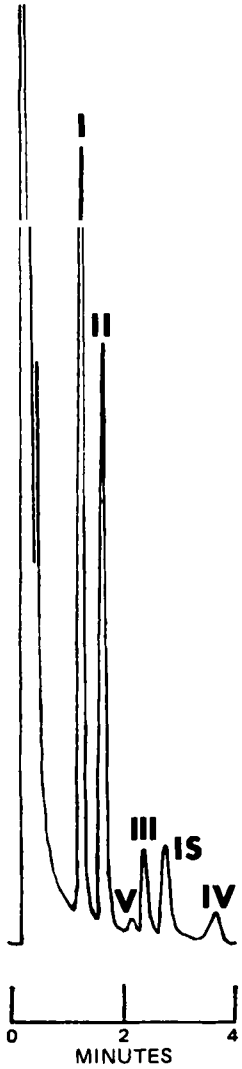

Figure 1—*Chromatogram of the trimethylsilyl derivatives of 1.5 μg of methyl 4-hydroxybenzoate (I), 1 μg of ethyl 4-hydroxybenzoate (II), 0.2 μg of propyl 4-hydroxybenzoate (III), 0.11 μg of butyl 4-hydroxybenzoate (IV), 0.03 μg of 4-hydroxybenzoic acid (V), and 0.75 μg of 3,4-dimethoxybenzoic acid internal standard (IS) using a 3% SE-30 column at 150°C.*

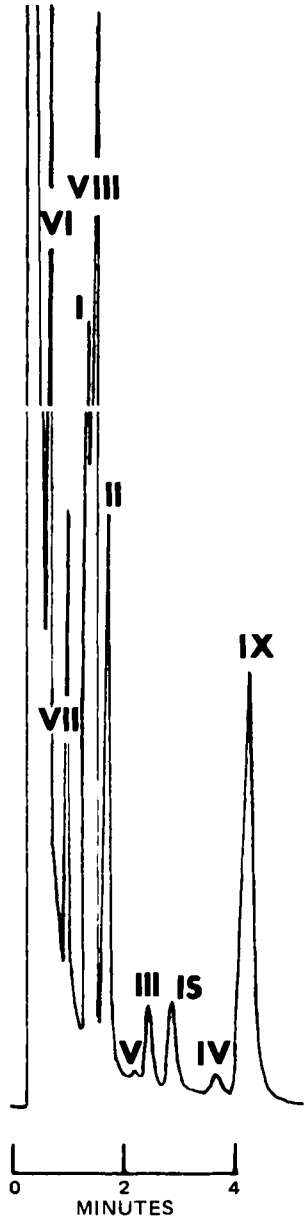

Figure 2—*Chromatogram of the trimethylsilyl derivatives of methyl (I), ethyl (II), propyl (III), and butyl (IV) 4-hydroxybenzoates, 4-hydroxybenzoic acid (V), 3,4-dimethoxybenzoic acid (IS), and possible impurities and degradation products: benzoic acid (VI), methyl salicylate (VII), salicylic acid (VIII), and 2,5-dihydroxybenzoic acid (IX) using a 3% SE-30 column at 150°C. A peak for phenol was not observed.*

concentrations of the four esters along with that of the hydrolytic degradation product (V) would be desirable. This would both aid the development of the optimum combination and concentration of the esters for maximum antibacterial effectiveness and assist in a study of the preservative stability in finished dosage forms.

Among the available assays, the HPLC assays of I–IV do not allow sufficient retention of V to separate it from the excipients. The weak eluant required for any significant retention on μ-phenyl or μ-C_{18} columns (2) makes elution of I–IV lengthy. The addition of a tetrabutylammonium ion pair does not significantly increase the retention of V on reverse-phase columns (3); however, this is not surprising since Doyle and Proctor have found that V yields low ion-pair extraction efficiency with tetrabutylammonium as the counter ion (4).

GC appears more promising for a simultaneous assay of I–V mainly because the large difference in the polarity of V from that of I–IV can be negated by the formation of derivatives of both the phenolic and carboxylic functions. SE-30 and OV-17 columns have been used to analyze plant extracts for trimethylsilyl derivatives of V and other phenolic acids (5). Similarly, OV-11 and OV-1 columns have been used to assay derivatized V and other aromatic acids in urine (6). A cyclohexane–dimethanol succinate column was used to assay trimethylsilyl derivatives of I–IV in an injection preservative (7), and a sucrose acetate isobutyrate column was used in the separation of the four esters in cosmetics (8). The present work involves similar approaches and the development of a column chromatographic cleanup step to yield a GC assay for simultaneously determining I–V in liquid antacid formulations.

EXPERIMENTAL

Reagents and Chemicals—All reagents and chemicals were ACS, USP, or NF quality and were used without further purification. Silyla-

Table I—Linearity of the Peak Height Ratios

Parameter	4-Hydroxybenzoate Esters				4-Hydroxybenzoic Acid
	Methyl	Ethyl	Propyl	Butyl	
Correlation coefficient	0.9997	0.9998	0.9998	0.9997	0.9996
Standard error of estimate ($SE_{y/x}$)	0.2	0.09	0.016	0.007	0.02
Intercept[a], %	3.7	−0.6	−3.6	−8.0	2.1
Variation[b], %	1.9	1.5	1.6	1.8	2.9

[a] (y-intercept/$\bar{y}$) × 100, where $\bar{y}$ is the average of y values (9). [b] ($SE_{y/x}/\bar{y}$) × 100.

tion-grade pyridine[1] and *N,O*-bis(trimethylsilyl)trifluoroacetamide[1] were used as received.

Apparatus—A chromatograph[2] equipped with a flame-ionization detector was used with a 1.8-m × 2-mm i.d. glass column[3] containing 3% SE-30 on 100–200 mesh support[4]. The gas flow rates were adjusted as follows: helium carrier gas, 30 mL/min; hydrogen, 50 mL/min; air, 400 mL/min. The detector and injector temperatures were both set at 250°C. The column temperature was set at 150°C for isothermal runs. Additional work was done with a 5% OV-1 column with temperature programming, as described below. Peak heights were determined either manually or with a computerized data acquisition system[5].

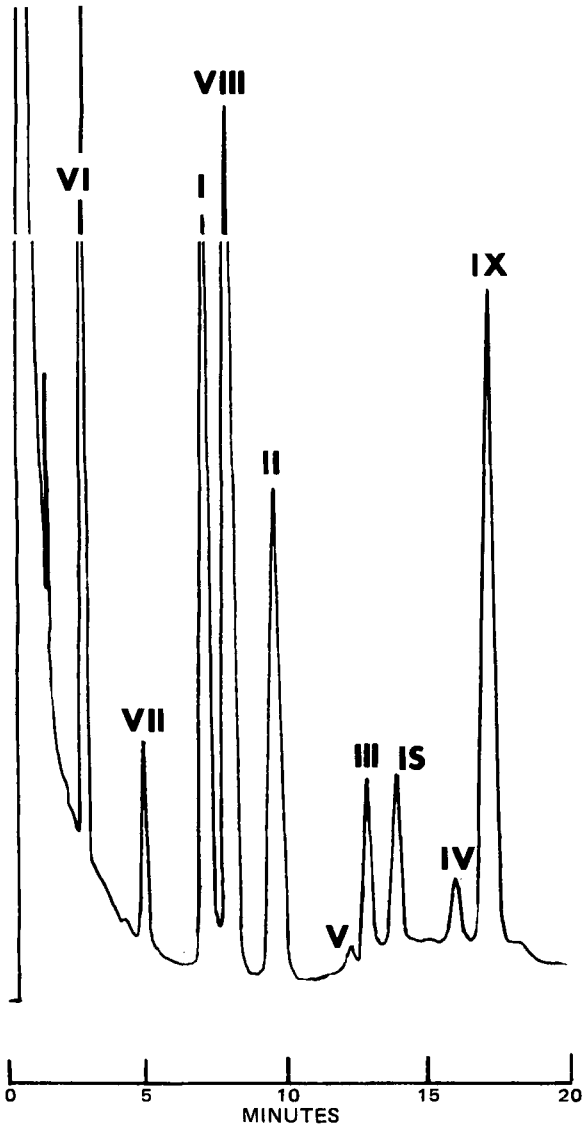

Figure 3—*Chromatogram of the trimethylsilyl 4-hydroxybenzoates, 4-hydroxybenzoic acid, impurities, and degradation products using a 5% OV-1 column, 150°C for 10 min, 32°C/min to 175°C. See Fig. 2 for peak identification.*

Table II—GC Assay of Commercial Liquid Antacids

Sample	Sample Weight, g	Amount Recovered, mg/mL I	II	III	IV	V
1[a]	0.7053	1.52	0.97	0.179	0.121	0.062
	0.7148	1.51	0.94	—	—	—
	0.6740	1.43	0.93	0.172	0.116	0.060
	0.9192	1.53	0.98	0.172	0.122	0.056
	0.9285	1.48	0.95	0.171	0.116	0.058
	0.8125	1.44	0.94	0.174	0.117	0.058
	1.038	1.45	0.95	0.178	0.120	0.060
	0.9910	1.48	0.93	0.168	0.117	0.057
	0.9424	1.45	0.93	0.173	0.118	0.061
	1.2885	1.50	0.96	0.170	0.116	0.060
	1.3027	1.52	0.98	—	—	—
	1.2816	1.48	0.96	0.174	0.121	0.061
Mean		1.48	0.95	0.173	0.118	0.059
RSD (1σ)		2.3%	1.8%	2.0%	1.9%	3.5%
2[b]	1.191	—	—	0.05	0.05	0.20
3[b]	1.001	0.12	—	0.21	—	0.81

[a] Sample 1, Experimental liquid antacid from Norwich Eaton; sample 2, Mylanta II; sample 3, Maalox. Label is 1.75 mg/mL for I, 1.02 mg/mL for II, 0.20 mg/mL for III, and 0.12 mg/mL for IV. [b] Label values not known.

Sample and Standard Preparation—One gram of liquid antacid, 0.4 mL of 1 M HCl, and 2.5 g of silica gel were mixed in a beaker until a fine, free-flowing powder formed. The powder was transferred to a 1-cm i.d. glass tube containing a glass wool pledget and eluted with 25–30 mL of ether at ~2 mL/min. Exactly 2.0 mL of a 0.2-mg/mL ether solution of 3,4-dimethoxybenzoic acid (internal standard) was added to the eluate, and the solution was evaporated to dryness under a nitrogen stream at room temperature. (Insufficient aqueous solubility precluded adding the internal standard prior to this step.) Similarly, 2.0–4.0 mL of an ether standard solution (depending on the expected 4-hydroxybenzoate content of the sample) containing 440 μg/mL of I, 225 μg/mL of II, 60 μg/mL of III, 60 μg/mL of IV, and 7.5 μg/mL of V was added to a test tube. Exactly 2.0 mL of the internal standard solution was added, and the solution was evaporated to dryness as for the sample. (Since studies have shown that no sample losses occur if an aqueous standard solution was eluted with ether through a silica column, a "method standard" was not necessary.) Trimethylsilyl derivatives of the compounds were formed by adding 0.4 ml of pyridine and 0.4 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide to the sample and standard residues. After briefly mixing, 1 μL of the solution was injected into the chromatograph.

Forced Degradation Sample—A fresh liquid antacid sample containing I and III (with no V initially present) was raised to a pH of ~10 by the addition of 2 M NaOH and heated in a stoppered container at 65°C for 48 h. The sample was extracted, derivatized, and chromatographed as described above.

RESULTS AND DISCUSSION

Initial attempts at solvent extraction of I–V from synthetic liquid antacid samples (placebo spiked with standard) yielded 100% recovery for I–IV but only ~50% recovery for V. Ion-pair extraction with tetraethylammonium ion also proved fruitless since V does not readily pair with other ions (4). A silica column chromatographic cleanup step, as described above, was found to be successful, yielding (after derivatization) the chromatogram shown in Fig. 1.

The compounds are resolved from possible degradation products (phenol, benzoic acid), impurities (methyl salicylate, 2,5-dihydroxybenzoic acid), and excipients. Salicylic acid is only partially resolved from

[1] Pierce Chemical Co., Rockford, Ill.
[2] Perkin-Elmer Model 900.
[3] Supelco, Bellefonte, Pa.
[4] Supelcort, Supelco.
[5] Hewlett-Packard Model 3356.

I; however, it was not present in the antacid formulations studied (Fig. 2). It was found that salicyclic acid could be better resolved, if necessary, by utilizing a 5% OV-1 column and temperature programming (150°C for 10 min, then 32°C/min to 175°C) (Fig. 3). However, chromatography time increased from ~5 min (SE-30 column) to >16 min (OV-1 column) per injection.

Assay of the forced degradation antacid sample revealed that ~10% of I had been converted to V. Compound III had not appreciably hydrolyzed during this short interval. No additional degradation peaks were observed.

The linearity data for I–V, determined by plotting peak height ratios (sample/internal standard) *versus* weight ratios injected, are presented in Table I. The correlation coefficients and percent variations (9) all indicate good linearity over the range studied: 0.75–6 μg for I, 0.5–3.8 μg for II, 0.1–0.9 μg for III, 0.06–0.5 μg for IV, and 0.014–0.7 μg for V. The percent intercepts indicate no serious problem with single-point standard calculations, except for IV. The significant negative intercept for IV demands that standard and sample concentrations be matched within 10%. Alternatively, a standard curve analysis could be run.

Assays of eight synthetic liquid antacid samples made by spiking placebo with solutions containing known amounts of standard I–V at levels of 60–125% of the theoretical yielded average recoveries and relative standard deviations of 99.0 ± 2.0% for I, 99.3 ± 1.9% for II, 98.7 ± 1.8% for III, 100.3 ± 1.6% for IV, and 100.0 ± 1.4% for V.

Twelve replicate assays of an actual liquid antacid sample containing all four preservatives and their common hydrolysis product V yielded good precision, as shown in Table II. The assay results of two other commercial liquid antacids are also shown in Table II. Since these samples had been stored for ~2 years prior to assay, relatively high concentrations of V were encountered. Although only antacids were examined in the present work, the technique should be readily applicable to other pharmaceutical preparations, such as liquids, elixirs, parenterals, creams, and gels.

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Analysis of Ritodrine in Serum by High-Performance Liquid Chromatography with Electrochemical Detection

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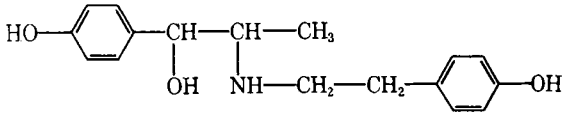
Abstract □ A sensitive and specific assay for ritodrine in serum was developed using high-performance liquid chromatography (HPLC) with electrochemical detection. Serum samples were alkalinized to pH 9.4 by the addition of a sodium carbonate buffer and extracted with ethyl acetate. The extracts were evaporated to dryness and the residues were reconstituted in the HPLC mobile phase and chromatographed on a octadecylsilane reverse-phase column. The detection of ritodrine was achieved by an electrochemical detector with a glassy carbon electrode. The sensitivity was 0.2 ng for on-column injection. The extraction efficiency was 80%.

Keyphrases □ Ritodrine—analysis in serum, high-performance liquid chromatography with electrochemical detection □ High-performance liquid chromatography—analysis of ritodrine in serum, electrochemical detection

Ritodrine hydrochloride, *erythro*-*p*-hydroxy-α-[1-[(*p*-hydroxyphenethyl)amino]ethyl]benzyl alcohol hydrochloride (I), is a β-adrenergic receptor stimulant developed specifically for obstetrical use in the management of premature labor. Pharmacological studies have shown that ritodrine is a potent inhibitor of myometrial contractility with only minor cardiovascular effects (1–4). Studies on experimental animals have demonstrated the uterine relaxant properties of ritodrine. Depending on the animal species and the route of administration, the effective dose ranges from 5 μg/kg to 15 mg/kg (5). In humans, the therapeutic dose varies from 50 to 200 μg/min. The infusion rate is maintained 24–48 h according to the response of the patient and side effects (6). Treatment is usually followed with an oral 10-mg dose four to eight times daily for several days or until term. Human disposition studies have been carried out on healthy nonpregnant volunteers using tritiated ritodrine. It was found that the majority of the drug was excreted in the urine with a maximal excretion rate attained within 1 h after drug administration. The data suggested a two-compartment open model with half-lives of 2 and 13 h (5).

Analytical assay of ritodrine had been limited to the use of radioactively labeled drug (5) until recently when a sensitive RIA (7) was reported. The RIA has a sensitivity of <1 ng and has been used to measure serum levels of ritodrine in humans after oral or parenteral administration. Whereas good sensitivity was achieved by this procedure, some unexplained variability was observed in the data, possibly due to cross-reactivity with some endogenous interferences in the samples. The RIA antiserum is cur-



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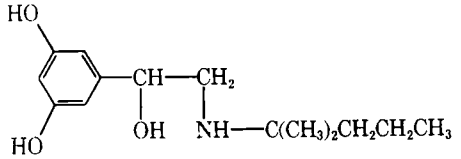

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