tions are shown in Fig. 3. Total analysis time was ~15 min. Spiked samples of a placebo syrup were analyzed by this method, giving quantitative recovery from 2.0 to 14.0 mg/5 ml (Table II). Replicate analyses of a single lot of syrup (n = 6) on 2 days gave precision (expressed as the relative standard deviation) of 0.3% (Table II).

Other commercially available syrups containing I were obtained and analyzed by this method. The results are summarized in Table III. Excellent agreement with the labeled contents was obtained. One syrup tested showed a rather large excipient peak that was not resolved from the I peak. To solve this problem, the percentage of acetonitrile in the mobile phase was reduced to 45% (v/v). Under these conditions, the excipient peak was resolved completely from the I peak (Fig. 4) but the internal standard peak did not elute in a reasonable time. Using peak height measurements, a value of 9.4 mg/5 ml (label claim was 10.0 mg/5 ml) was obtained for this syrup. This approach demonstrates that the acetonitrile content, as well as the carbon chain length of the pairing ion, can be manipulated to produce resolution of the I peak from interfering components in the mixture.

The method also was applied to the analysis of bulk drug samples. In this case, consideration must be given to the resolution of I and potential impurities arising from the synthetic process. Compound I is synthesized by the methylation of (+)-3-hydroxy-17-methyl-9α,13α,14α-morphinan (III) with phenyltrimethylammonium bromide. The by-product of the reaction is dimethylaniline. Compendial assays for I include tests for dimethylaniline and phenolic compounds (*e.g.*, III). Since dimethylaniline and III both are amines, trace amounts would be expected to be carried over in any acid–base extraction of I used for purification. A chromatographic tracing of a mixture of I, III, and dimethylaniline is shown in Fig. 5. The two potential impurities are well resolved from I. The results of the analyses of bulk I are shown in Table IV. Quantitative recovery and excellent precision are observed.

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High-Performance Liquid Chromatographic Assay for Benzocaine and *p*-Aminobenzoic Acid Including Preliminary Stability Data

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Abstract □ A high-performance liquid chromatographic assay was developed that separates and quantitates benzocaine and its primary degradation product, *p*-aminobenzoic acid. This method is rapid, sensitive, and specific. Preliminary stability data obtained with this method demonstrate its utility for this purpose.

Keyphrases □ Benzocaine—high-performance liquid chromatographic analysis with *p*-aminobenzoic acid, effects of pH, temperature, and phosphate ion on benzocaine stability □ *p*-Aminobenzoic acid—degradation product of benzocaine, high-performance liquid chromatographic analysis with benzocaine □ High-performance liquid chromatography—analysis, benzocaine and *p*-aminobenzoic acid, stability studies of benzocaine □ Degradation—benzocaine, effect of pH, temperature, and phosphate ion

In spite of the long history of benzocaine as a therapeutic agent, little information has appeared regarding its stability. It has been reported to be relatively unstable under certain conditions, and attempts have been made to decrease its degradation through the use of complexing agents (1) and surfactants (2). Benzocaine also may be unstable in the presence of pharmaceutical excipients such as citric acid, glucose, and cherry flavoring (3). This lack of information is due in part to the absence of a rapid, specific, stability-indicating assay for benzocaine in the presence of its degradation products.

Over the years, benzocaine has been assayed quantitatively by titration (4), diazotization (5), colorimetry (6), potentiometry (7), and crystal formation (8), all of which take advantage of the basic and aromatic character imparted by the aryl amino group. Some initial benzocaine stability work was undertaken by Higuchi and Lachman (1) using a partition spectroscopic method of analysis. GLC was used later for the analysis of benzocaine (9–11). However, recent data (12, 13) showed interference by *p*-hydroxybenzoates in the UV, GLC, and column chromatographic methods for benzocaine, thereby raising questions about the specificity of these commonly employed methods.

A recently developed high-performance liquid chromatographic (HPLC) assay was reported which measures intact benzocaine (14), but the report did not mention the separation or identification of the primary degradation product of benzocaine, *p*-aminobenzoic acid. This paper describes a rapid, precise, and sensitive method for the simultaneous analysis of benzocaine and *p*-aminobenzoic

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Table I—Degradation of Benzocaine in Aqueous Solutions at Room Temperature [a]

Minutes	Benzocaine Remaining[b], %			*p*-Aminobenzoic Acid Formed[c], %		
	pH 2	pH 7	pH 11	pH 2	pH 7	pH 11
0	100.0	100.0	100.0	—	—	—
5	100.1	100.4	97.5	—	—	2.20
10	100.3	—	90.7	—	—	9.00
15	99.2	100.4	88.3	—	—	11.82
30	98.0	100.8	83.6	2.40	—	16.21
45	96.5	99.2	77.7	3.70	—	22.10
60	95.5	99.5	73.1	6.20	—	27.92
120	92.4	98.8	52.0	9.51	—	49.25
180	87.6	99.2	40.6	13.60	—	62.32
240	85.1	98.3	28.9	16.32	1.25	69.05
300	78.5	98.0	20.0	24.0	2.20	79.93

[a] 25 ± 1°. [b] Average of two determinations. [c] Percent of amount formed from complete hydrolysis of benzocaine.

acid and reports preliminary stability data using this assay method.

EXPERIMENTAL

Apparatus—A high-performance liquid chromatograph equipped with a multiwavelength detector[1] was employed in conjunction with a microparticulate C_{18} column[2] for separation.

Reagents—All chemicals and reagents were analytical grade unless otherwise indicated. Benzocaine[3], *p*-aminobenzoic acid[4], monobasic potassium phosphate[5], and glass-distilled methanol[6] were used as received. Glass-distilled water was used throughout the study.

Analytical Procedure—All standard solutions of benzocaine and *p*-aminobenzoic acid were prepared in water. The concentrations of benzocaine and *p*-aminobenzoic acid were determined directly from their respective peak heights by comparison to a standard curve. The mobile phase was methanol–acetic acid–water (33:4:63). At ambient temperature and a solvent flow rate of 2 ml/min, the retention times for *p*-aminobenzoic acid and benzocaine were 2 min 28 sec and 7 min 10 sec, respectively.

The sensitivity of *p*-aminobenzoic acid and benzocaine at an injection volume of 10 μl was 0.3 and 0.7 μg/ml, respectively, which was equivalent to 3 and 7 ng injected on column. The assay sensitivity can be increased by increasing the injection volume or decreasing the dilution. Similarly, the assay sensitivity can be increased about fourfold by using 294 nm rather than the more conventional 254 nm as the detector wavelength, although this capability may not be available in all laboratories. Injections of benzocaine and *p*-aminobenzoic acid solutions prepared in nonbuffered and buffered solutions of pH 2, 7, and 11 are chromatographically similar. The solutions to be chromatographed are primarily aqueous, and there is little risk of evaporation at room temperature. Adequate reproducibility can be obtained without the use of an internal standard.

Stability Studies—*Effect of pH and Phosphate Ion*—A methanolic stock solution of benzocaine (5 mg/ml) was diluted to ~40 μg/ml with water and adjusted to pH 2, 7, or 11 with 0.1 *N* NaOH or 0.1 *N* HCl. These solutions were placed in glass vessels at room temperature (25 ± 2°). Each experiment was conducted in duplicate. Aliquots (10 μl) were withdrawn periodically and assayed for benzocaine and *p*-aminobenzoic acid.

In a similar experiment, phosphate (adjusted to 0.1 *M*) was used in place of sodium hydroxide or hydrochloric acid to adjust the solution pH. Only phosphate-ion-containing salts and their corresponding acids were used in preparing these solutions, as calculated by the Henderson–Hasselbach equation (15).

Effect of Temperature—Benzocaine solutions prepared in phosphate buffer (pH 7.0) were placed in sealed glass containers at 40, 60, and 70 ± 1.0°. Aliquots (10 μl) were assayed periodically for benzocaine and *p*-aminobenzoic acid over a 24-hr period.

Treatment of Data—Semilogarithmic plots of the benzocaine concentration as a function of time were constructed to evaluate the order and rate of benzocaine degradation. All plots were consistent with first-order kinetics, *i.e.*, plots of log benzocaine concentration *versus* time were linear. An Arrhenius plot (15) was constructed from the data obtained for the experiment on the effect of temperature. The line of best fit was determined by linear regression.

RESULTS

A typical chromatogram for benzocaine and *p*-aminobenzoic acid is shown in Fig. 1. Retention times varied slightly from day to day, but no interfering peaks were encountered. Standard curves for both benzocaine and *p*-aminobenzoic acid were linear over the concentration range of 0–100 mg/liter, using peak height or peak area as a function of concen-

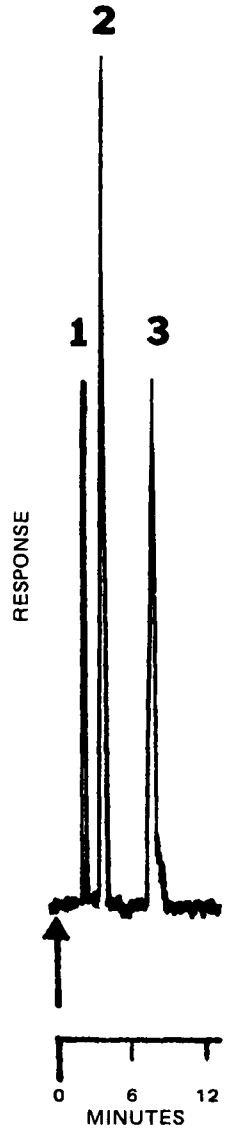

Figure 1—*Representative chromatogram for benzocaine and p-aminobenzoic acid. Key: 1, solvent peak; 2, p-aminobenzoic acid; and 3, benzocaine. The arrow indicates the time when the injection was made.*

[1] Model 1202 with model 711 solvent delivery system, Laboratory Data Control, Riviera Beach, Fla.
[2] Catalog No. 27324, Waters Associates, Milford, Mass.
[3] Amend Drugs and Chemical Co., Irvington, N.J.
[4] Ruger Chemical Co., Irvington, N.Y.
[5] Fisher Scientific Co., Fair Lawn, N.J.
[6] Burdick & Jackson Laboratories, Muskegon, Mich.

Table II—Degradation of Benzocaine in 0.1 *M* Phosphate Buffer Solutions at Room Temperature [a]

Minutes	Benzocaine Remaining[b], %			*p*-Aminobenzoic Acid Formed[c], %		
	pH 2	pH 7	pH 11	pH 2	pH 7	pH 11
0	100.0	100.0	100.0	—	—	—
5	99.4	99.76	99.7	—	—	—
10	99.1	99.97	99.5	—	—	—
15	99.5	99.5	98.7	—	—	—
30	98.3	99.8	97.8	—	—	0.84
45	99.1	99.8	96.6	—	—	1.50
60	98.8	100.0	94.6	—	—	2.02
120	96.9	99.0	93.3	0.82	—	3.20
180	95.7	99.0	88.2	1.10	0.42	6.20
240	93.8	98.2	85.5	2.50	0.96	9.60
300	92.3	98.2	83.6	6.53	0.80	11.35

[a] 25 ± 1°. [b] Average of two determinations. [c] Percent of total amount available for formation.

Table III—Apparent First-Order Rate Constants for Benzocaine Degradation at 25°

pH	Rate Constant, hr^{-1}	
	Nonbuffered Solution	Buffered Solution
2	0.0446	0.00604
7	0.0050	0.00385
11	0.314	0.03920

tration. The correlation coefficient for the standard curves over the concentration range used in this study, 0–100 mg/liter, was ≥0.99 (n = 8) in all cases. Standard solutions assayed repetitively over 15 weeks showed a coefficient of variation of 2% for benzocaine and 1.5% for *p*-aminobenzoic acid. No significant differences were observed (p = 0.05) between the mean peak height obtained from repeated injections (n = 10) on 2 days. The coefficient of variation in the slope obtained from eight standard curves over a 2-month period for benzocaine and *p*-aminobenzoic acid was <3%. The pH of the injected solutions did not affect the retention times.

Table I shows the percent of benzocaine remaining and the percent of *p*-aminobenzoic acid formed (based on the total amount available for formation) at three pH values. Benzocaine degradation was most rapid at pH 11, intermediate at pH 2, and slowest at pH 7. Good agreement was found between the amount of benzocaine lost and the amount of *p*-aminobenzoic acid formed, suggesting that *p*-aminobenzoic acid is the only major degradation product of benzocaine under these conditions. This conclusion is supported by the fact that no other peaks are seen in the chromatograms of degraded benzocaine solutions. A plot of log benzocaine concentration as a function of time for various pH values is shown in Fig. 2. The apparent first-order half-lives determined from these data were 2.2 hr at pH 11, 15.5 hr at pH 2, and 139 hr at pH 7.

Table II shows the disappearance of benzocaine and the appearance of *p*-aminobenzoic acid in solutions containing 0.1 *M* phosphate buffer. As was observed in water, benzocaine degradation was most rapid at pH 11 and slowest at pH 7; good agreement existed between benzocaine loss

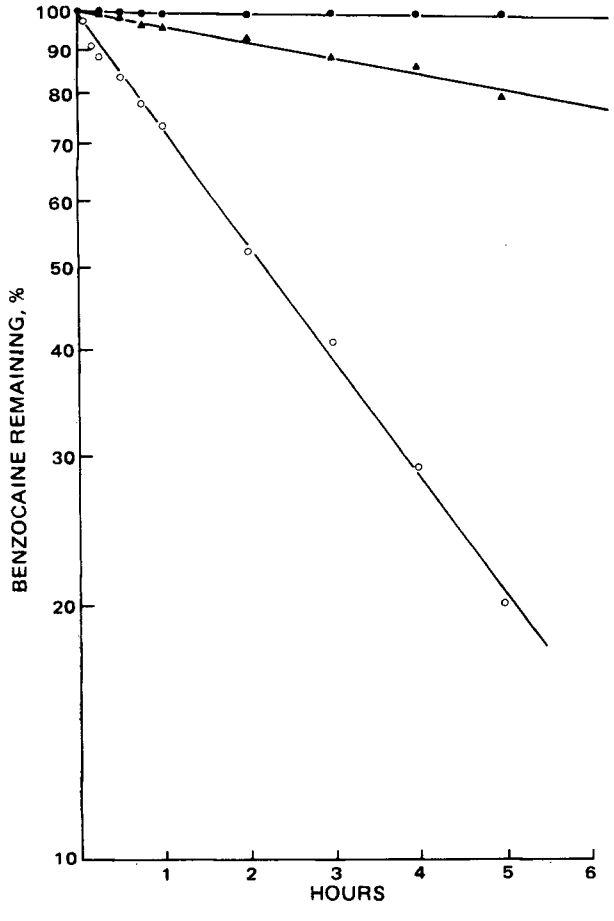

Figure 2—*Benzocaine degradation in aqueous solutions of three pH values at 25°. Key: ●, pH 7, ▲, pH 2; and ○, pH 11.*

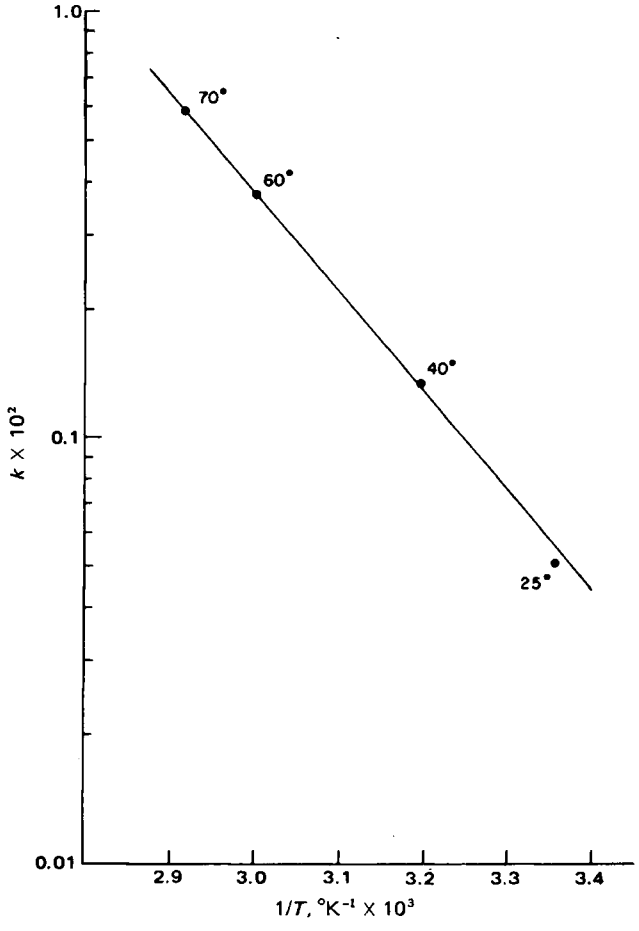

Figure 3—*Arrhenius plot of benzocaine stability in 0.1* M *phosphate buffer (pH 7).*

Table IV—Observed and Predicted Values for Benzocaine Stability at pH 7 in 0.1 *M* Phosphate Buffer [a]

Temperature (t), °C	$1/T$, °K^{-1} × 10^3	k, hr^{-1}	log k	Time for 10% Degradation, hr	Time for 50% Degradation, hr
40	3.195	0.0136	−1.866	7.75	50.96
60	3.003	0.0376	−1.425	2.80	18.44
70	2.915	0.0586	−1.232	1.80	11.82
25	3.356	0.0051	−3.295	20.78	136.70
25		0.0057[b]	−2.444[b]	18.49[b]	121.60[b]

[a] Frequency factor (A) = 2.35 × 10^7 hr^{-1}. Activation energy (E_a) = 5.217 kcal/mole. [b] From Arrhenius plot, based on data at 40, 60, and 70°C.

and *p*-aminobenzoic acid appearance. However, at all three pH values, the degradation rate was slower in phosphate buffer than in water. Half-lives for the loss of benzocaine in the presence of 0.1 *M* phosphate were 17.7 hr at pH 11, 115 hr at pH 2, and 180 hr at pH 7. Thus, benzocaine was approximately 1.3 (pH 7), 7.3 (pH 2), and 8.0 (pH 11) times more stable in phosphate buffer than in nonbuffered solutions of equivalent pH (Table III). The reason for the protective effect of phosphate ion is unknown. However, increasing the phosphate buffer concentration from 0.1 to 0.5 *M* while maintaining a constant ionic strength with sodium chloride did not alter the degradation rate at room temperature and pH 7.

Benzocaine stability was studied at 25, 40, 60, and 70°C in 0.1 *M* phosphate buffer at pH 7.0. An Arrhenius plot (15) constructed by plotting the apparent first-order rate constants obtained as a function of the inverse of temperature (°K) is shown in Fig. 3. Extrapolation of the elevated temperature data to room temperature (25°C) results in a predicted first-order rate constant of 0.0057 hr^{-1}, which compares favorably with the rate constant of 0.0051 hr^{-1} observed at room temperature (Table IV).

DISCUSSION

Various analytical methods were employed in previous stability studies of benzocaine. These methods suffer from several deficiencies, including the inability to quantitate or separate the primary degradation product of benzocaine and the lack of a chromatographic step to separate benzocaine from other potentially interfering materials. The potential interference of *p*-aminobenzoic acid in the methods used in some previous stability studies (1, 11) has been discussed (13). The official assay (16) employs a diazotization titration and hence may not be specific for benzocaine alone. The assay described here is rapid and precise and allows simultaneous quantitation of benzocaine and its primary degradation product, *p*-aminobenzoic acid. This capability allows for the continuous monitoring of mass balance during stability studies and precludes nonspecific losses such as loss into packaging materials.

The new assay has been used to study several stability characteristics of benzocaine, including the effects of pH, temperature, and phosphate ion. Benzocaine degradation is both acid and base catalyzed but is much slower in the presence of phosphate ion. First-order kinetics are observed in all cases. Studies at elevated temperatures indicate that benzocaine obeys the Arrhenius equation. Degradation rates at room temperature agree with those predicted from elevated temperature studies.

Essentially all of the benzocaine lost due to degradation can be accounted for by the appearance of *p*-aminobenzoic acid; hence, *p*-aminobenzoic acid appears to be the only significant degradation product under the study conditions used.

The sensitivity of this assay is ~0.7 μg/ml for benzocaine and ~0.3 μg/ml for *p*-aminobenzoic acid. The sensitivity can be enhanced fourfold if a multiple-wavelength detector is used at 294 nm or if the injection volume is increased.

The method described here suffers from no known interference and is applicable for the content uniformity and quality control of products containing benzocaine. This rapid assay is good for the evaluation of raw materials and employs basic equipment found in most analytical laboratories.

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