Colorimetric Assay of Benzocaine and Some Dosage Forms

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Abstract □ A colorimetric procedure for benzocaine and a number of its dosage forms was developed; it offers improvement in ease, speed, and sensitivity over the official method. The method is based on the formation of a red Schiff base between benzocaine and *p*-dimethylaminocinnamaldehyde in a nonaqueous acidic medium. At the maximum absorption of 544 nm, the Beer–Lambert law was adhered to over the 0.025–2.5-μg/ml range. Best accuracy can be obtained for solutions containing 0.25–1.25 μg/ml. The color was stable for at least 2 hr. Analysis of benzocaine in the dosage forms studied can be directly performed without prior drug extraction.

Keyphrases □ Benzocaine—colorimetric analysis, bulk drug and commercial dosage forms □ Colorimetry—analysis, benzocaine bulk drug and commercial dosage forms □ Anesthetics, topical—benzocaine, colorimetric analysis, bulk drug and commercial dosage forms

Benzocaine (ethyl *p*-aminobenzoate) is an extensively used local anesthetic in various products for the relief of pain and pruritis. A significantly large number of products are readily available as nonprescription drugs, varying in benzocaine concentration from 0.5 to 20.0%.

The NF XIV (1) method for benzocaine is based on the known diazotization or nitritometric titration (2–7). This tedious and time-consuming titration utilizes standard sodium nitrite at low temperatures with starch iodide paper as an external indicator. The official assay procedures (1) for benzocaine creams and ointments also are based on the same diazotization titration where the endpoint is determined potentiometrically. In ointments having water-insoluble bases, the drug must be isolated by solvent extraction prior to the diazotization titration. Furthermore, in formulations with antipyrine in glycerin or propylene glycol bases, the benzocaine is analyzed by UV spectrophotometry after it is separated from antipyrine by column partition chromatography using three different columns assembled serially (8, 9).

Colorimetric methods for the analysis of benzocaine based on the formation of Schiff bases were performed using *p*-dimethylaminobenzaldehyde (10) and vanillin (11) in acidic hydroalcoholic solutions, and the products obtained were yellow. *p*-Dimethylaminocinnamaldehyde was utilized as a colorimetric agent for primary aromatic amines (12–14). This reagent is advantageously employed in place of *p*-dimethylaminobenzaldehyde and vanillin, since the resulting Schiff bases are red. This paper presents a simple, sensitive, and accurate method for the analysis of benzocaine and some of its dosage forms.

EXPERIMENTAL

Instruments—A UV–visible double-beam spectrophotometer[1] with 1-cm cells and a dynode voltage of 500 v (slit width 0.35–0.55 mm) and an analytical balance[2] were used.

Materials and Reagents—The following were used: benzocaine[3], acetone-free absolute methanol containing 0.05% water[4], a 0.1% (w/v) solution of *p*-dimethylaminocinnamaldehyde[5] (mp 137–139°) in methanol (stable for at least 3 weeks if refrigerated), and a 50% (w/v) solution of trichloroacetic acid (ACS grade) in methanol.

Standard Curve—A stock solution of benzocaine was prepared by dissolving 50.0 mg of the drug in methanol. Further dilutions were made to prepare standard solutions containing 0.25–25.0 μg of benzocaine/ml. One milliliter of each solution was utilized for color development as described under *Assay Procedure for Benzocaine*. The stock and standard solutions were freshly prepared.

Assay Procedure for Benzocaine—Pipet 1.0 ml of a methanolic solution containing 2.5–12.5 μg of benzocaine into a 10-ml volumetric flask. To this solution, add exactly 2.00 ml of *p*-dimethylaminocinnamaldehyde reagent followed by 1.0 ml of trichloroacetic acid reagent and wait for 10 min. Dilute to volume with methanol, and measure the absorbance at 544 nm against a blank prepared similarly but omitting the benzocaine.

Assay Procedure for Benzocaine Dosage Forms—*Creams, Ointments with Water-Soluble Bases, and Otic Drops*—Weigh or pipet accurately an amount equivalent to 15–50 mg of benzocaine, transfer into a 100-ml volumetric flask, and dissolve or suspend in methanol. Dilute to volume with methanol. Filter through dry filter paper, if necessary, and discard the first 5 ml of filtrate. Pipet 1.0 ml of the filtrate into a 50-ml volumetric flask, and dilute to volume with methanol. Pipet 1.0 ml of the resulting solution into a 10-ml volumetric flask and proceed as described under *Assay Procedure for Benzocaine*, beginning with: "To this solution, add exactly . . . ."

Ointments and Suppositories with Water-Insoluble Bases—Weigh accurately an amount equivalent to 15–50 mg of benzocaine in a beaker, dissolve in 14 ml of cyclohexane, and transfer quantitatively into a 100-ml volumetric flask. Rinse the beaker three times with 2-ml portions of cyclohexane, and combine the washings with the cyclohexane solution in the volumetric flask. Shake the solution vigorously, and slowly add methanol to the mark. Proceed as described for creams, beginning with: "Filter through dry filter paper . . . ."

Lozenges—Weigh and grind 20 lozenges to a very fine powder. Add slowly an accurately weighed amount of the powder, equivalent to 15–50 mg of benzocaine, to about 75 ml of methanol in a 100-ml volumetric flask with shaking. Place the stoppered volumetric flask in an ultrasonic bath for 30 min to enhance dissolution of the benzocaine, and dilute to volume with methanol. Proceed as described for creams, beginning with: "Filter through dry filter paper . . . ."

Table I—Effect of Time on Absorbance of Benzocaine–*p*-Dimethylaminocinnamaldehyde Colored Product [a]

Minutes after Reaction	Absorbance at 544 nm
2	0.433
5	0.443
10	0.445
20	0.445
50	0.446
70	0.445
90	0.447
100	0.445
120	0.446

[a] Concentration of 0.75 μg of benzocaine/ml.

RESULTS AND DISCUSSION

The red Schiff base produced by the interaction of benzocaine and *p*-dimethylaminocinnamaldehyde in an acidic anhydrous medium exhibited a maximum absorption at 544 nm (Fig. 1). This reaction resulted in a more sensitive and deep coloration than the yellow color obtained

[1] Acta V, Beckman Instruments, Fullerton, Calif.
[2] Mettler H-18, Mettler Instruments Corp., Princeton, N.J.
[3] Eastman Kodak Co., Rochester, N.Y.
[4] Fisher Scientific Co., Pittsburgh, Pa.
[5] Aldrich Chemical Co., Milwaukee, Wis.

Table II—Reproducibility of Color Development of Replicate 0.5-μg/ml Samples of Benzocaine

Sample	Absorbance at 544 nm
1	0.301
2	0.303
3	0.302
4	0.302
5	0.300
6	0.300
7	0.303
8	0.295
9	0.295
Average	0.300
SD	0.0031
RSD	0.0103

from the reaction of benzocaine with the commonly used aldehydes such as *p*-dimethylaminobenzaldehyde (10) and vanillin (11). This bathochromic shift was expected since the structure of the Schiff base (I) formed with *p*-dimethylaminocinnamaldehyde showed greater conjugation than, for example, II, that formed with *p*-dimethylaminobenzaldehyde. The red color of the product was stable for at least 120 min (Table I).

The effect of reagents on color development was investigated by adding varying volumes of 0.1% *p*-dimethylaminocinnamaldehyde in methanol to 10-ml volumetric flasks, each containing 1.0 ml of 16 μg of benzocaine/ml and 1.0 ml of 50% trichloroacetic acid in methanol. The absorbance of each solution was measured at 544 nm against a blank[6] after each solution was diluted to volume with methanol. The absorbance readings increased with increasing amounts of *p*-dimethylaminocinnamaldehyde up to a concentration of about 250 μg/ml in the final solution.

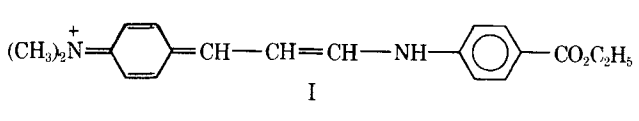
However, blank solutions containing more than 200 μg of *p*-dimethylaminocinnamaldehyde/ml were dark colored. This condition limits the range of usable concentrations and makes the method less sensitive, because wider slit widths must be used to maintain the dynode voltage on the spectrophotometer at 500 v. Based on these considerations, exactly 2.0 ml of the 0.1% *p*-dimethylaminocinnamaldehyde reagent was used for color development. Changing the volumes of 50% trichloroacetic acid did not appreciably affect absorbance readings; 1.0 ml of this acid reagent was used for the assay.

As in a previous study (14), the color formed was instable in the presence of water. Therefore, the methanol used must be as anhydrous as possible. The use of methanol containing 0.05% water appeared to be satisfactory for the benzocaine assay.

Under the proposed experimental conditions, a linear response between absorbance and concentration was demonstrated over the concentration range of 0.025–2.5 μg/ml (r = 0.9997). The linear regression line[7] was $A_{544} = 0.5974C - 0.0059$, where A_{544} is the absorbance at 544 nm and C is the concentration expressed in micrograms per milliliter in the final test solution. The standard deviations of the slope and y intercept were 0.0121 and 0.0076, respectively (n = 4). The calculated molar absorptivity, ϵ, was 9.87×10^4 liter mole^{-1} cm^{-1}.

Determination of the concentration range for maximum accuracy was done by constructing the Ringbom plot (15, 16). The plot (Fig. 2) indicates an optimum range for solutions containing 0.25–1.25 μg of benzocaine/ml in the final test solution. The curve also shows an inflection at 37% transmittance, indicating that the system follows the Beer–Lambert law.

The limit of detection, as determined on 20 samples by the procedure of Kaiser (17), at the 95% confidence level is 5.5×10^{-7} M or 0.090 μg of benzocaine/ml in the test solution.



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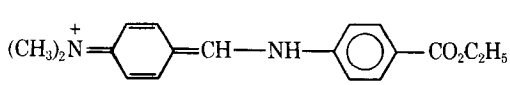

II

[6] Each blank contained the same amount of *p*-dimethylaminocinnamaldehyde and trichloroacetic acid as in the solution studied but contained no benzocaine.

[7] Wang computer 700 C and plotting output writer 2202.

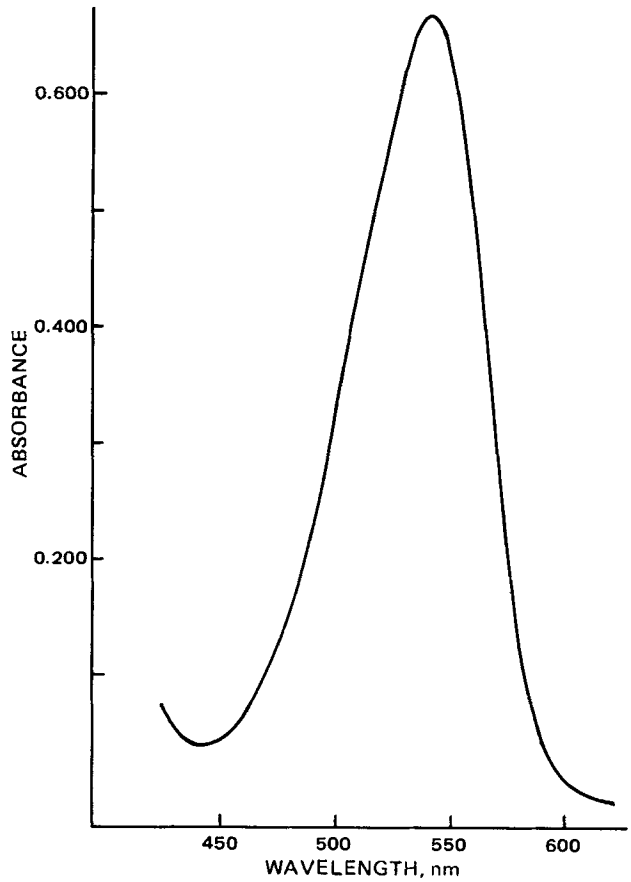

Figure 1—*Absorption spectrum of the benzocaine–*p*-dimethylaminocinnamaldehyde reaction product.*

The reproducibility of the proposed color development was determined by analyzing nine replicate samples of benzocaine solutions in methanol, each containing 0.5 μg/ml. The precision at this concentration level showed a relative standard deviation of 0.0103 (Table II).

The data in Table III show the accuracy of the procedure. These studies were performed on four different, but accurately known, amounts of benzocaine. Each sample was dissolved in 100.0 ml of methanol. Appropriate dilutions were then made to give different concentration levels (Table III), which were analyzed by the proposed method. The results

Table III—Assay of Solutions of Known Concentrations of Benzocaine at Different Levels

Amount Weighed, mg	Analyzed at Concentration Level, μg/ml	Amount Found, mg (Proposed Method)	Percent Found (Proposed Method)	
			Individual Samples	Average
54.0	0.5	53.7	99.4	
	1.6	53.0	98.1	98.5
	2.2	53.0	98.1	
54.5	0.5	54.9	100.7	
	1.6	54.7	100.4	100.4
	2.2	54.6	100.2	
150.0	0.6	149.8	99.9	
	1.2	152.1	101.4	100.8
	1.8	151.6	101.1	
154.5	0.6	154.0	99.7	
	1.2	153.1	99.1	98.8
	1.9	150.7	97.5	
300.0		302.0[a]	100.7[a]	
300.0		301.5[a]	100.5[a]	
Overall percent found				99.6
SD				1.14

[a] Analyzed by NF XIV method.

Table IV—Assay of Some Commerically Available Benzocaine Formulations

Formulation	Amount Benzocaine Claimed, mg/g or mg/ml	Amount Found, mg/g or mg/ml		Average Percent Label Claim	
		Proposed Method	NF XIV Method	Proposed Method	NF XIV Method
Ointment (water-insoluble base	20.0	19.9 20.2	19.7 20.0	100.3	99.3
Ointment (water-soluble base)[a]	200.0	199.6 202.2	199.4 202.0	100.5	100.4
Cream[b]	10.0	9.67 9.65	10.8 11.1	96.6	109.5
Suppository[c]	130.0[d]	126.0 126.8	124.5 127.3	97.3	96.2
Otic drops[a]	200.0	229.0 228.0	230.4 229.5	114.3	115.0
Otic drops[e]	14.0	13.9 14.1	14.2 14.4	100.0	102.2
Lozenges	10.0[f]	9.80 9.88	9.72 9.89	98.4	98.1

[a] Contains benzethonium chloride. [b] Contains menthol, camphor, and triclosan. [c] Contains oxyquinoline sulfate, zinc oxide, menthol, and Peru balsam. [d] Milligrams per suppository. [e] Contains antipyrine. [f] Milligrams per lozenge.

were in agreement with those obtained by the compendial method. An average of 99.6% of the original amount weighed was found. In comparison, an average of 100.6% was obtained for two benzocaine samples analyzed by the NF XIV method.

Results of analyses of different benzocaine dosage forms by the proposed method are presented in Table IV. The recovery data showed good correlation with those of the NF XIV procedure. The results also indicated that benzocaine in ointments or suppositories with water-insoluble bases need not be isolated by solvent extraction prior to analysis as required by the official method. This difference shortens the analysis time considerably.

The concentration of cyclohexane used to dissolve the ointment or suppository in the final test solution was 0.04% (w/v). Increasing the cyclohexane concentration to 0.06% (w/v), which corresponded to an initial volume of 30 ml, did not affect color intensity or stability. Furthermore, active ingredients normally present in the benzocaine formulations studied, such as antipyrine, menthol, camphor, triclosan, benzethonium chloride, and oxyquinoline sulfate, do not interfere with the reaction and do not have to be removed; these compounds lack the primary aromatic amino functional group necessary for the reaction with *p*-dimethylaminocinnamaldehyde to form a red Schiff base. On the other hand, *p*-aminobenzoic acid, a degradation product of benzocaine, reacts under the proposed experimental conditions, producing a red color with λ_{max} of 541 nm.

In the analysis of lozenges, the lozenges must be ground to a very fine powder, and care must be taken when suspending the powder in methanol. The powder must be added slowly with frequent shaking to prevent the formation of lumps. The undissolved particles must flow freely upon shaking. The use of an ultrasonic bath facilitates the disintegration of lumps and enhances the dissolution of benzocaine. The pink dye present in the lozenges did not interfere with the analysis.

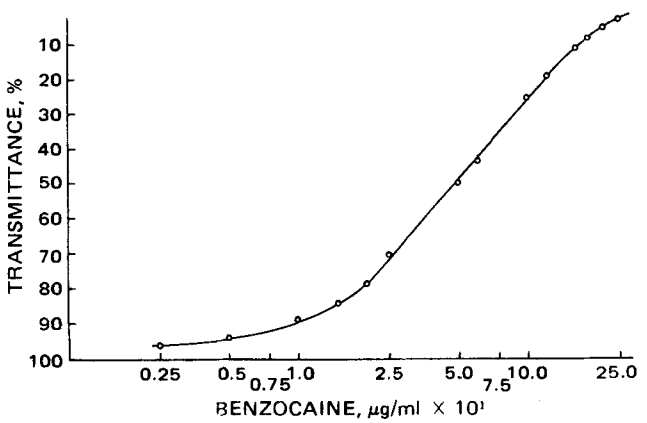

Figure 2—*Ringbom plot for the benzocaine–p-dimethylaminocinnamaldehyde reaction product.*

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