The need for the separation of caffeine from carisoprodol can be understood by studying the IR-absorbing properties of both molecules. The near-IR spectrum of caffeine in the 3500–3200-nm region is characterized by a strong intensity absorption maximum at 3390 nm, attributable to the asymmetric CH-stretching mode of its three methyl groups, and a weaker maximum at 3340 nm, attributable to the corresponding symmetric CH-stretching mode (21). The carisoprodol molecule, incorporating four methyl groups and four CH_2 groups, absorbs strongly at three contiguous wavelengths (3390, 3420, and 3500 nm) due to asymmetric CH-stretching and moderately at 3340 nm due to symmetric CH-stretching. These spectral similarities and, more important, the much greater absorption intensities of the carisoprodol demonstrate the need for an isolation procedure for caffeine.

A series of caffeine standard solutions in carbon tetrachloride was studied spectrophotometrically, and the Beer's law plot exhibited linearity (Fig. 5, absorptivity 0.193). The data in Table II obtained from the analysis of spiked solutions, which shows an excellent solute recovery in terms of the mean and a relative standard deviation of 0.74%, verify the validity of the quantitative procedure for caffeine.

Four different commercial lots of tablets were assayed for carisoprodol, phenacetin, and caffeine. The results (Table III) indicate that the near-IR procedures described are reproducible with standard deviations of 1.38, 1.40, and 1.07 mg for carisoprodol, phenacetin, and caffeine, respectively. Since each sample lot is a true independent sample, the uncertainties experienced reflect both the variations inherent in the manufacturing process and the statistical perturbations possessed by the proposed near-IR spectrophotometric procedure. When the recovery data in Tables I and II are compared with those of Table III, the agreement obtained is reasonable. The results presented in Table III are considered satisfactory.

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Colorimetric Analysis of Procaine Hydrochloride

HENRY S. I. TAN[x] and D. SHELTON

Abstract □ A colorimetric assay procedure was developed for the quantitative analysis of procaine hydrochloride. The method is based on the interaction of procaine hydrochloride with *p*-dimethylaminocinnamaldehyde in the presence of trichloroacetic acid in absolute methanol to form a red Schiff base which can be quantitated spectrophotometrically at 547.5 nm. The Beer–Lambert law was adhered to over the 0.1–7-μg/ml range. Best accuracy was attained for solutions containing 0.4–4 μg/ml. The color was stable for at least 70 min. The method was applied to procaine hydrochloride injections, both with and without epinephrine, without prior separation of the drug. The results were comparable to those obtained by the official procedures.

Keyphrases □ Procaine hydrochloride—colorimetric analysis using *p*-dimethylaminocinnamaldehyde □ *p*-Dimethylaminocinnamaldehyde—colorimetric reagent for determination of procaine hydrochloride □ Colorimetry—determination, procaine hydrochloride, using *p*-dimethylaminocinnamaldehyde

The USP XVIII (1) assay procedure for procaine hydrochloride is time consuming, since it is based on a diazotization titration of the compound with standard sodium nitrite at low temperatures using starch iodide paper as the external indicator. This method is subject to variations between individuals in terms of determining the end-point. The official assay method (2) for procaine hydrochloride injection is also lengthy, since a prior isolation of the compound by solvent extraction is required before the compound can be determined. These reasons prompted this laboratory to develop an assay method offering

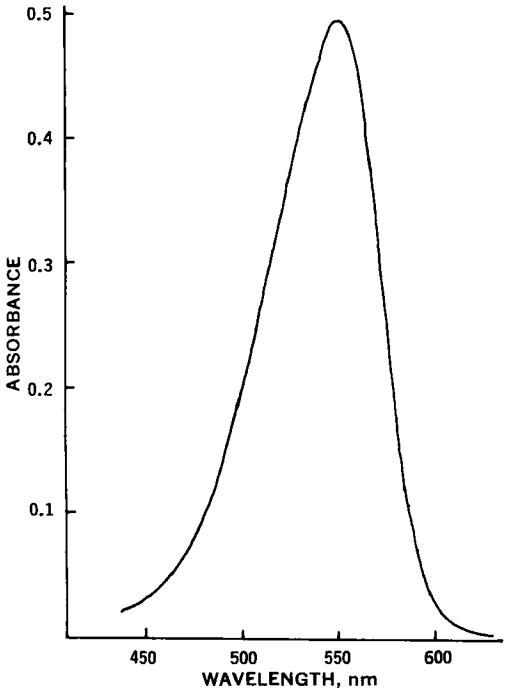

Figure 1—*Absorption spectrum of procaine hydrochloride–p-dimethylaminocinnamaldehyde reaction product at 2 μg procaine hydrochloride/ml.*

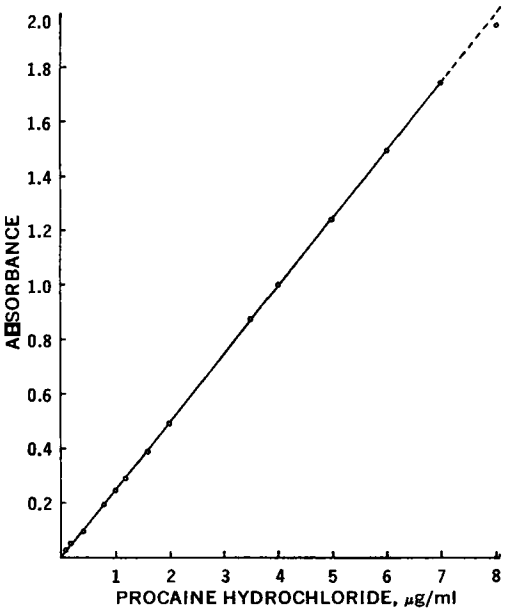

Figure 2—*Relationship between absorbance and concentration of procaine hydrochloride.*

improvements in sensitivity, speed, and ease over the official methods.

The use of *p*-dimethylaminocinnamaldehyde as a colorimetric agent for primary aromatic amines in acidic solutions was reported previously (3, 4). This reagent is advantageously employed in place of the usual aldehydes such as *p*-dimethylaminobenzaldehyde and vanillin, since the resulting Schiff bases are red. This report presents a sensitive, simple, and accurate method for the analysis of procaine hydrochloride with *p*-dimethylaminocinnamaldehyde in a nonaqueous acidic medium. The proposed procedure can be automated, which is important from the standpoint of industrial application.

EXPERIMENTAL

Instruments—The following were used: a UV-visible double-beam spectrophotometer[1] with 1-cm cells[2] and a slit width of 0.22 mm and an analytical balance[3].

Materials and Reagents—The following were used: procaine hydrochloride (ACS grade)[4]; acetone-free absolute methanol containing 0.008% water[5]; a 0.10% solution of *p*-dimethylaminocinnamaldehyde[4], mp 132–136°, in methanol (stable for at least 3 weeks if refrigerated); and a 50% solution of trichloroacetic acid[6] (ACS grade) in methanol.

Preparation of Standard Curve—A stock solution of procaine hydrochloride was prepared by dissolving 80.0 mg of procaine hydrochloride in 100 ml of methanol. Further dilutions were made to obtain standard solutions, each containing 1.0, 2.0, 4.0, 8.0, 10.0, 12.0, 16.0, 20.0, 35.0, 40.0, 50.0, 60.0, 70.0, and 80.0 μg procaine hydrochloride/ml, respectively. One milliliter of each solution was utilized for color development as described under *Assay Procedure for Procaine Hydrochloride*. The stock and standard solutions should be freshly prepared.

Assay Procedure for Procaine Hydrochloride—Pipet 1.0 ml of a methanolic solution containing 1–70 μg of procaine hydrochloride into a 10-ml volumetric flask. To this solution, add exactly 1.00 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 determine the absorbance at 547.5 nm against a blank prepared similarly but omitting the procaine hydrochloride. Read the concentration of procaine hydrochloride present from a calibration curve.

Assay Procedure for Procaine Hydrochloride Injection with or without Epinephrine—Pipet 5.0 ml of a 1% procaine hydrochloride injection *or* 2.0 ml of a 2% procaine hydrochloride injection into a 100-ml volumetric flask and dilute to volume with methanol. Transfer 1.0 ml of the resulting solution into a 50-ml volumetric flask and dilute to volume with methanol. Pipet 1.0 ml of the latter solution into a 10-ml volumetric flask and proceed as described in the *Assay Procedure for Procaine Hydrochloride* beginning with "To this solution add. . . ."

RESULTS AND DISCUSSION

The interaction of procaine hydrochloride and *p*-dimethylaminocinnamaldehyde in acidic medium produced a red Schiff base showing an absorption peak at 547.5 nm in its absorption spectrum (Fig. 1). Therefore, a more sensitive and deep coloration was obtained than the yellow color resulting from the reaction of procaine hydrochloride with the commonly used aldehydes such as *p*-dimethylaminobenzaldehyde and vanillin.

As shown in Fig. 2, at 547.5 nm a linear relationship exists between concentration and absorbance throughout the region practical for absorbance measurements. Under the proposed experimental conditions the Beer–Lambert law is followed over the con-

Table I—Effect of Time on Absorbance of Procaine Hydrochloride–*p*-Dimethylaminocinnamaldehyde Color Complex[a]

Minutes	Absorbance at 547.5 nm
20	0.653
30	0.652
40	0.651
50	0.649
60	0.651
70	0.651

[a] Concentration about 2.8 μg procaine hydrochloride/ml.

[1] Beckman Acta V, Beckman Instruments, Fullerton, Calif.
[2] Bausch and Lomb.
[3] Mettler type H-18, Mettler Instrument Corp., Princeton, N.J.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Matheson, Coleman and Bell, Norwood, Ohio.
[6] Mallinckrodt Chemical Works, New York, N.Y.

Table II—Assay of Solutions of Known Concentrations of Procaine Hydrochloride at Different Levels

Amount Weighed, mg	Analyzed at Concentration Level, μg/ml	Amount Found[a], mg		Percent Recovery		
				Proposed Method		
		Proposed Method	USP XVIII Method	Individual Samples	Average	USP XVIII Method
50.0	1.0	49.9		99.8	100.7	
	4.0	50.8		101.6		
250.0	0.5	252.2		100.9	100.4	
	5.0	249.8		99.9		
300.0	0.6	305.0		101.7	101.8	
	6.0	305.5		101.8		
300.0			302.1			100.7
500.0			500.6			100.1
Overall percent recovery					101.0	100.4
Standard deviation					0.73	

[a] Each value is the average of two replicate assays.

centration range 0.1–7 μg procaine hydrochloride/ml. However, the Ringbom (5) plot indicates that best accuracy will be obtained for solutions containing approximately 0.4–4 μg procaine hydrochloride/ml (Fig. 3). The plot also shows an inflection at 37% transmittance, indicating that the system does indeed adhere to the Beer–Lambert law (6).

The concentration optimum of the color reagent was determined by adding varying volumes of 0.10% *p*-dimethylaminocinnamaldehyde in methanol to a series of 10-ml volumetric flasks, each containing 1.0 ml of 20 μg procaine hydrochloride/ml and 1.0 ml of 50% trichloroacetic acid in methanol, and measuring the absorbance of each solution at 547.5 nm against a blank[7] after diluting each solution to volume with methanol. Increasing amounts of *p*-dimethylaminocinnamaldehyde resulted in increased absorbance readings. Since this would result in shorter ranges of usable concentrations, it was decided to use 1.0 ml of 0.10% *p*-dimethylaminocinnamaldehyde as the optimum concentration for the assay. Changing the volumes of 50% trichloroacetic acid did not appreciably affect absorbance readings. In view of the results, 1 ml of 50% trichloroacetic acid was selected as the concentration for the assay.

Water adversely affected the interaction of procaine hydrochloride with *p*-dimethylaminocinnamaldehyde. The use of methanol containing 0.02% water caused the absorbance values to decrease slowly. Higher concentrations of water caused the red color to fade in 5–15 min. However, when 0.008% water-containing methanol was used, the color appeared to be stable for at least 70 min (Table I).

The stability of the *p*-dimethylaminocinnamaldehyde reagent was evaluated by recording its UV spectrum (λ_{max} 391 nm) over an extended period. The molar absorptivity remained unchanged for at least 3 weeks if the reagent was refrigerated.

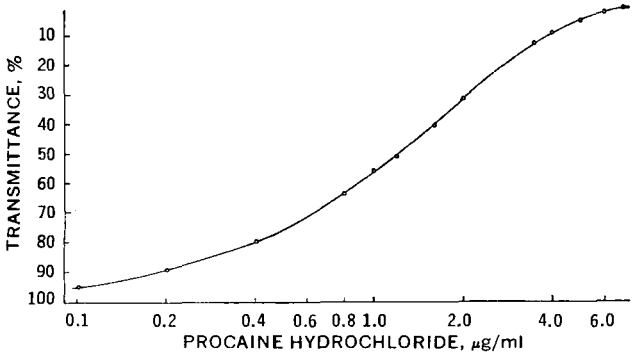

Figure 3—*Ringbom plot for the procaine hydrochloride–p-dimethylaminocinnamaldehyde reaction product.*

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

Recovery data for procaine hydrochloride in the concentration range studied proved to be accurate with the proposed procedure. Recovery studies were performed in three successive experiments using 50, 250, and 300 mg of procaine hydrochloride. In each case, the accurately weighed amount of procaine hydrochloride was dissolved in 100 ml methanol. Appropriate dilutions were then prepared to afford different concentrations (Table II) which were determined by the proposed method. All results were quantitative and reproducible. The overall percent recovery for the assay samples was 101.0% with a standard deviation of 0.73. In comparison, the average percent recovery for two samples analyzed by the USP XVIII method was 100.4% (Table II).

The precision study was performed by running replication studies on nine samples of procaine hydrochloride, each containing 1 μg/ml. Each sample was then analyzed by the proposed method. The coefficient of variation for the nine replicate samples was 0.53% (Table III).

The proposed analytical method was then compared to the official method in the analysis of commercially available preparations of procaine hydrochloride injection. There is a close relationship between the results obtained by the official procedure and those obtained with the proposed analytical method (Table IV).

A favorable characteristic of the proposed procedure is the speed and ease of performing the assay. Since the *p*-dimethylaminocinnamaldehyde reagent can be stored for several weeks without any noticeable deterioration, this reagent does not have to be prepared daily. In contrast, the USP XVIII method for procaine hydrochloride requires freshly prepared sodium nitrite solutions. In the analysis of parenteral preparations, no prior isolation of the procaine hydrochloride is necessary and the drug does not have to be separated from epinephrine in epinephrine-containing formulations. Thus, it eliminates the need for extracting both the parenteral solution and the blank required by the official assay procedure. In addition, due to its speed and ease, the proposed method can be modified for automation.

Table III—Reproducibility of Color Development of Replicate Samples of Procaine Hydrochloride Containing 1 μg/ml

Solution	Absorbance at 547.5 nm
1	0.270
2	0.267
3	0.268
4	0.269
5	0.267
6	0.268
7	0.267
8	0.269
9	0.271
Average	0.268
Standard deviation	0.0014
Coefficient of variation	0.53%

Table IV—Assay of Commercially Available Procaine Hydrochloride Parenteral Solutions

Preparation	Amount of Procaine Hydrochloride Claimed, mg/ml	Amount Found[a], mg		Percent of Claim	
		Proposed Method	USP XVIII Method	Proposed Method	USP XVIII Method
Procaine, 1%	10.0	10.08	10.15	100.8	101.5
Procaine, 2%	20.0	20.25	20.40	101.3	102.0
Procaine, 2%, with 0.002% epinephrine	20.0	20.50	20.70	102.5	103.5

[a] Average of duplicate assays of samples from three manufacturers.

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Interference by Traces of Acetic Anhydride in Nonaqueous Titrimetry of Primary Aromatic Amines: Improved Titrations for Procainamide Hydrochloride

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Abstract □ Traces of acetic anhydride and acetaldehyde in the acetic acid titration solvent depress the nonaqueous titrimetry of primary aromatic amines. Solutions to the problem include a modified nonaqueous titration and a nitrite titration with internal ferrocyphen indicator. These alternative methods for the titration of procainamide hydrochloride were compared with the USP XVIII nonaqueous titration of procainamide hydrochloride. The nitrite titration is the best procedure.

Keyphrases □ Procainamide hydrochloride—analysis, acetic anhydride interference in nonaqueous titration, improved procedure compared with alternative nitrite and compendial titrations □ Amines, primary aromatic—interference of trace acetic anhydride in nonaqueous titrimetry, improved procedure and alternative nitrite titration □ Titrimetry, nonaqueous and nitrite—analysis, primary aromatic amines (procainamide hydrochloride) □ Acetic acid—interference of trace acetic anhydride in nonaqueous titration of primary aromatic amines (procainamide hydrochloride)

Procainamide hydrochloride[1] is an efficacious, widely prescribed agent for the control of cardiac arrhythmias. Among its purity safeguards is a USP (1) requirement that assays of the drug by nonaqueous titrimetry fall within the narrow limits of 98.0–100.5%.

In these laboratories, titrimetry of procainamide hydrochloride by the USP method (1) repeatedly yielded assays that were close to the 98% USP minimum specification. However, other evidence, including melting ranges and TLC, suggested that the purity of the samples exceeded 99%. Suspicion centered on the titration solvent, glacial acetic acid, when the use of one lot of glacial acetic acid yielded an extremely low assay for procainamide hydrochloride of 81%. When other lots of glacial acetic acid were used as the titration medium, the same batch of procainamide hydrochloride gave assay values above 98%.

Posgay (2) reported that trace amounts of acetic anhydride in glacial acetic acid can acetylate certain amines, leading to low results when these amines are titrated in acetic acid with acetous perchloric acid. Thus, the lot of glacial acetic acid whose use led to low procainamide hydrochloride assay values might have contained traces of acetic anhydride and/or acetaldehyde, which could effect acetylation or Schiff-base formation of the primary aromatic amine function. This blocked amine, being unavailable for perchloric acid titration, could be responsible for the low assay values.

Examination of the acetic acids for acetic anhydride, total carbonyls, and water indicated that the cause of the low assay values was trace amounts of acetic anhydride. Possible remedies and alternative

[1] Pronestyl, Squibb.