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

HAROLD KADIN

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.

titrations were then examined. The remedies included treatment or testing of the acetic acid. One alternative titration involves addition of the aromatic amine, benzocaine, to the acetic acid. The benzocaine then reacts with traces of acetic anhydride and acetaldehyde to remove these interferences. Excess benzocaine is neutralized followed by the addition and titration of the aromatic amine one wishes to measure. A highly preferable alternative is a nitrite titration with an internal indicator (3).

The frequent recurrence of marginal nonaqueous titration values suggested that the problem of trace acetic anhydride or acetaldehyde in glacial acetic acids might be widespread. Thus, the implications of these studies should be of general interest.

EXPERIMENTAL

Equipment—A conventional 50-ml buret was used for the nitrite titrations, and an automatic 10-ml buret with reservoir and drying tubes[2] was used for the nonaqueous titrations. The air vent at the top of the buret and the U-shaped drying tube on the air-inlet side arm contained dehydrating silica gel[3].

Sample dissolution was accomplished in an 80-w ultrasonic cleaner[4].

Potentiometry with the pH meter coupled to calomel and glass electrodes was used to verify the visual end-point of the nonaqueous titration. The aqueous salt bridge of the calomel electrode was replaced with 1% lithium perchlorate in glacial acetic acid. When not in use, the calomel electrode was kept in glacial acetic acid. The glass electrode was kept in distilled water and was rehydrated in distilled water for about 15 min between titrations in glacial acetic acid. Prior to its immersion in the titration mixture, excess water was removed from the glass electrode by a quick immersion in glacial acetic acid.

Reagents—For the nonaqueous titrations, the reagents included: 0.1 *N* acetous perchloric acid, USP XVIII (p. 1035); benzocaine NF; glacial acetic acid *without* adjustment of water content (by addition of acetic anhydride) as specified for USP XVIII nonaqueous titration of procainamide hydrochloride; crystal violet T.S. indicator; and mercuric acetate, 6 g in 100 ml of glacial acetic acid.

The nitrite titration required: 6 *M* hydrochloric acid; ferrocyphen [dicyanobis(1,10-phenanthroline)-iron (II)] indicator, 0.50 g dissolved *without warming* in 50 ml of concentrated sulfuric acid; 0.1 *N* sodium nitrite, and USP sulfanilamide reference standard.

Colorimetry for total carbonyls required: 2 *N* hydrochloric acid, a freshly prepared mixture of chloroform–isooctane (40:60 v/v), 0.25% 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid, and acetaldehyde.

Analysis for Carbonyls (Expressed as Acetaldehyde) in Glacial Acetic Acid—*Sample*—Transfer 20.0 ml of glacial acetic acid into a 100-ml volumetric flask. Dilute to volume with distilled water and mix.

Standards—One milliliter of refrigerated acetaldehyde (density was 0.788 g/ml) is initially diluted to 100 ml with distilled water. A secondary dilution of 2 ml of the stock standard to 100 ml of distilled water is followed by tertiary dilutions in distilled water of 5 ml to 100, 10 ml to 100, and 20 ml to 100, yielding working dilutions of 8, 16, and 32 μg acetaldehyde/ml, respectively.

Procedure—Transfer 3.0 ml of the final samples and standard dilutions into individual 60-ml separators. Use 3.0 ml of distilled water for the blank. Add 1 ml of the dinitrophenylhydrazine reagent. Swirl the mixtures and let them stand for 5 min. Add 10 ml of the 2 *N* hydrochloric acid and then 10.0 ml of the freshly prepared chloroform–isooctane mixture. Shake for 2 min. Allow layers to separate. Transfer the upper, organic solvent layers to glass-stoppered centrifuge tubes. Clarify the extracts by centrifugation. Determine absorbances in 1-cm cells *versus* the blank in a spectrophotometer at 420 nm.

Beer's Law Conformity—The standards read 0.214, 0.425, and 0.860. When these absorbances were plotted *versus* their concentrations, a straight line was obtained which intersected the origin, indicating conformity to Beer's law.

Calculations—Calculate the individual standards as ratios of micrograms of acetaldehyde to absorbance. Since Beer's law is followed, the average of the three ratios as the standard factor is used in the following calculation, in which A = absorbance of acetic acid sample and K = standard factor. Then micrograms of carbonyls (as acetaldehyde) per milliliter = $(AK \times 100)/(3 \times 20)$, which simplifies to $(AK \times 1.67)$.

Analysis for Acetic Anhydride in Glacial Acetic Acid—Accurately weigh about 0.16 g of benzocaine NF. Transfer to a 250-ml conical flask containing 100 ml of the glacial acetic acid, and seal the flask with aluminum foil. Dissolve the benzocaine in the acid by placing the flask in the ultrasonic cleaner for 2–3 min. Let stand at room temperature for 45 min. Add 2 drops of the crystal violet indicator and titrate with the acetous perchloric acid to a green end-point with absence of blue shades. Perform sample and blank titrations in duplicate.

Again, accurately weigh, in duplicate, about 0.16 g of benzocaine from the same container of benzocaine used previously. Transfer each sample individually to one of the neutralized solutions obtained from the preceding titrations. Dissolve in the acid, as indicated previously, and again titrate to a green end-point with absence of blue shades. A blank is not required for the latter two titrations.

If A = milliliters titrated for the sample after 45 min contact with acid, B = milliliters titrated for the blank, A_t = milliliters titrated for the sample added to a previously neutralized 45-min sample, N = normality of acetous perchloric acid, W_1 = weight in milligrams of the benzocaine sample used for the first titration, W_2 = weight in milligrams of the benzocaine sample added to the neutralized sample of W_1, 165.19 = equivalent weight of benzocaine, and 102.09 = equivalent weight of acetic anhydride, then micrograms of acetic anhydride per milliliter of glacial acetic acid equals:

$$102.09\left[\frac{(165.19\,NA_t/W_2)W_1 - 165.19N(A-B)}{165.19}\right]1000/100$$

which simplifies to:

$$NW_1\left[\frac{A_t}{W_2} - \frac{(A-B)}{W_1}\right] \times 1020.9$$

Nonaqueous Titration for Procainamide Hydrochloride—Weigh approximately 150–170 mg of benzocaine into a 250-ml conical flask. Transfer 100 ml of acetic acid into the flask. Dissolve the benzocaine in the acid by placing the container in an ultrasonic bath at 20–25° for 2–3 min[5].

Add 10 ml of the mercuric acetate solution, and let the flask stand for 45 min. Add 2 drops (about 0.04 ml) of the crystal violet indicator. Titrate with the 0.1 *N* perchloric acid to a green end-point with absence of blue shades. Weigh accurately about 135 mg of the procainamide hydrochloride sample into the flask containing the neutralized benzocaine solution. Dissolve the procainamide hydrochloride by placing the flask in an ultrasonic bath at 20–25° for 2–3 min. Titrate with the standardized perchloric acid to a green end-point with absence of blue shades. Do not perform a blank titration.

The percent procainamide hydrochloride = [(ml × N × 135.9)/sample wt., mg] × 10^2, where ml = acetous perchloric acid to titrate procainamide hydrochloride sample, N = normality of acetous perchloric acid, and 135.9 = equivalent weight of procainamide hydrochloride.

Nitrite Titration for Procainamide Hydrochloride—*Standardization*—Transfer an accurately weighed 0.4-g sample of USP sulfanilamide reference standard into a 250-ml conical flask. Add 100 ml of 6 *M* hydrochloric acid. Place the flask in an ultrasonic cleaner for 3–5 min to dissolve the sulfanilamide. Add 1.0 ml of

[2] No. 10-8352, Ace Scientific Supply, Linden, N.J.
[3] Tel-Tale No. 42, W. R. Grace & Co., Baltimore, Md.
[4] Model 8845-3, Cole-Parmer, Chicago, Ill.

[5] If appreciable yellowing occurs (due to Schiff-base formation of the benzocaine with excessive contaminating acetaldehyde), begin again with a new lot of glacial acetic acid. Schiff-base formation is readily visible with 0.01% (v/v) of acetaldehyde. Yellow superimposed on the blue indicator may yield premature green end-points.

the ferrocyphen solution. Titrate with the 0.1 N sodium nitrite[6] to a violet end-point that is stable for at least 3 min. Perform a blank titration on the reagents. Standardize the sodium nitrite solution daily, as needed.

Procainamide Hydrochloride Assay—Weigh accurately about 500 mg of the procainamide hydrochloride sample into a 250-ml conical flask. Add 100 ml of 6 M hydrochloric acid and place the flask in an ultrasonic water bath at 20–25° for 2–3 min to dissolve the sample. Add 1.0 ml of the ferrocyphen indicator and titrate promptly[6] with the standardized sodium nitrite until a rose end-point is observed that is stable for at least 3 min. Do a blank titration.

Calculations—

1. Standardization:

$$\text{normality of sodium nitrite solution} = \frac{\text{sulfanilamide wt., mg}}{(ml_1 - B_1) \times 172.21} \quad \text{(Eq. 1)}$$

where ml_1 = milliliters of sodium nitrite solution to titrate sulfanilamide, B_1 = milliliters to titrate blank, and 172.21 = equivalent weight of sulfanilamide.

2. Procainamide Hydrochloride Assay:

$$\text{percent procainamide hydrochloride} = \frac{(ml_2 - B_2) \times N \times 271.79}{\text{sample wt., mg}} \times 10^2 \quad \text{(Eq. 2)}$$

where ml_2 = milliliters of sodium nitrite solution to titrate procainamide hydrochloride, B_2 = average milliliters to titrate blank, N = normality of sodium nitrite, and 271.79 = equivalent weight of procainamide hydrochloride.

RESULTS AND DISCUSSION

Nonaqueous Titration—The lot (Sample 1) of acetic acid giving low procainamide hydrochloride assay values, as well as two other lots (Samples 2 and 3) of acetic acid, was analyzed by the procedures described for total carbonyls and acetic anhydride. Water was also determined with the Karl Fischer titration.

Table I summarizes the assay data and demonstrates, with experimental Modifications A–F, the effects of water, acetic anhydride, acetaldehyde, and three lots of acetic acid on the USP XVIII nonaqueous titration of procainamide hydrochloride. The three acids may be categorized as giving seriously low, slightly low, and satisfactory results in the order in which they appear in Table I from left to right.

Extremely low assay values can be obtained by adding very small amounts of acetaldehyde or acetic anhydride (Modifications D and E, respectively) to glacial acetic acids that previously gave slightly low or satisfactory titrations. Table I also compares assay results obtained with the benzocaine modification to those obtained with the USP XVIII assay, the USP assay after procainamide hydrochloride contact with the acids for 45 min, and the USP assay after the addition of water to the acid the evening prior to the assay. Again, these experimental modifications indicate that traces of acetic anhydride already present in the acetic acid titration solvent seriously lower the values obtained with the USP XVIII titration. The benzocaine method, Modification F, refers to the nonaqueous titration presented under *Experimental*. In this modification, benzocaine reacts with acetic anhydride and acetaldehyde, thus purifying the acetic acid prior to the addition of the procainamide hydrochloride.

Assays of the various glacial acetic acids (Table I) clearly implicate the acetic anhydride, rather than the acetaldehyde, as responsible for the low assay values. The evidence becomes more convincing if one calculates the theoretical effect on the titration of the concentration of acetic anhydride, 232 μg/ml, found in Sample 1. Assuming complete reaction of the acetic anhydride with the procainamide hydrochloride, a purity of 77.4% would result. This calculated value is in excellent agreement with the actual titration of 77.6% (Modification B).

Further confirmation is obtained by calculation of the effect of 300 ppm (v/v) of *added* acetic anhydride (Modification E). A theoretical value of 68.2% would be found, which is in excellent agreement with the actual value of 68.3%.

It was established that maximum acetylation of benzocaine in Sample 1 occurred after about 15 min of contact. Since no further acetylation was evident after 2 hr, it appeared that acetylation was complete. A 45-min contact period is recommended in the nonaqueous titration to provide a safety factor if the rate of acetylation should vary from acid to acid.

The low water content of 0.01% in Sample 1 (Table I) may enhance the formation and stability of the acetic anhydride. Marked, but incomplete, improvement in the assay of Sample 1 (to 91%) could be obtained by the addition of water (Modification C). However, Posgay (4) reported that trace amounts of water hydrolyze equivalent amounts of acetic anhydride in acetic acid, without a catalyst, in about 90 days. Even in the presence of 0.5% water, the hydrolysis time required would be 10–15 days (4). Therefore, the addition of water did not seem practical and was discontinued.

Glacial acetic acid T.S. is specified in USP XVIII as the titration medium for procainamide hydrochloride. The Test Solution section of USP XVIII (p. 1023) directs that if the acid contains more than 0.05% of water, one must add a few milliliters of acetic anhydride, mix, and allow to stand overnight before again determining the water content.

In view of both Posgay's (4) paper and the present report, addition of acetic anhydride to glacial acetic acid appears ill-advised.

The evidence in Table I demonstrates that the benzocaine method is a marked improvement over the USP XVIII method (1). Whereas the USP XVIII method yielded unacceptable assay variations with three different glacial acetic acids (81.0, 97.8, and 99.0%), the benzocaine modification precisely elevated these assay results to 99.5, 99.7, and 99.5%, respectively, in the same three acids.

Again, viewing Table I, it is evident that the benzocaine modification improved the nonaqueous titration in glacial acetic acid, Samples 1 and 2, but no significant advantage was observed with Sample 3. This finding suggests that, in lieu of the benzocaine modification, a simpler solution might be to set maximum specifications for acetic anhydride and acetaldehyde in acetic acids. There is presently no American Chemical Society (ACS) specification for acetic anhydride in glacial acetic acid (5). Rosin (6) described a test for acetic anhydride in acetic acid. However, this test is a qualitative color test without an expressed sensitivity limit. The frequent recurrence of the problem of low or margin-

Table I—Effects of Water, Acetic Anhydride, Acetaldehyde, and Various Acetic Acids on the Nonaqueous Titration of Procainamide Hydrochloride

	Glacial Acetic Acid		
	Sample 1[a]	Sample 2[b]	Sample 3[c]
Water content, %	0.01	—[d]	0.06
Acetic anhydride, μg/ml	232	—[d]	8
Carbonyl (as acetaldehyde), μg/ml	0.6	—[d]	None detected
Procainamide assay (percent), by experimental modification:			
A. USP XVIII method, no modification	81.0	97.8	99.0
B. USP, after 45 min contact with acid	77.6	96.9	99.4
C. USP, 0.2% water added to acid previous night	91.0	—[d]	—[d]
D. USP, 0.01% acetaldehyde added, 45 min contact	—[d]	—[d]	75.0
E. USP, 0.03% acetic anhydride added, 45 min contact	—[d]	68.3	—[d]
F. Benzocaine method, nonaqueous titration	99.5	99.7	99.5

[a] Mallinckrodt WXXK. [b] Baker 9507. [c] Mallinckrodt VMK. [d] Not tested.

[6] Prolonged interruptions during the titration or excessively slow titrations lead to overconsumption of nitrite. For example, when the titration rate was excessively slow at 0.5 ml/min, a 2% increase in nitrite consumption was obtained over that obtained when titration rates were at normal speeds (3–7 ml/min).

Table II—Percent Procainamide Hydrochloride by Nitrite, USP XVIII, and Benzocaine Modified Titrations

Lot Number	Nitrite	Nonaqueous[a]	
		USP XVIII (1)	Benzocaine
MBA97521	98.9, 98.9, 98.9	96.9, 96.7, 96.8	98.0, 98.0, 98.0
35438-029	100.0, 99.7, 99.9	99.0, 99.0 —	99.0, 99.3, 99.7
	100.2, 100.3, 99.4	— — —	99.6, 99.6, 99.4
	99.7, 99.8 —	— — —	99.4, 99.5, 99.4
	Avg. 99.9 ± *SD* 0.29		Avg. 99.4 ± *SD* 0.21
35438-028	100.5, 100.5, 100.5	98.7, 98.6 —	99.4, 99.5, 99.6
91970-543	100.3, 100.5 —	98.8, 98.8, 98.8	99.2, 99.2, 99.3
91970-506	99.1, 100.1 —	99.0, 99.0 —	— — —
91970-540	99.6, 99.4 —	99.2, 99.0 —	— — —
91970-541	99.3, 99.5, 100.2	98.5, 99.0 —	— — —
91970-542	99.4, 98.8, 99.4	98.2, 98.9 —	— — —
38974-548	99.5 — —	— — —	99.7 — —
92107-002	99.0 — —	— — —	98.5, 99.2 —
92104-001	98.9 — —	— — —	98.3 — —
40613-530	99.6 — —	— — —	99.8, 99.7 —
40613-529	99.4 — —	— — —	99.3, 99.7 —
40250-524	98.3 — —	— — —	99.7, 99.1 —
40250-523	98.3 — —	— — —	99.1, 99.5 —
39036-508	99.0 — —	— — —	99.0, 98.6 —
39035-507	98.9 — —	— — —	98.7, 99.2 —
39121-551	99.1 — —	— — —	99.0 — —

[a] These titrations were performed in various lots of glacial acetic acid other than Sample 1.

al titrations in these laboratories suggests that a quantitative specification for acetic anhydride might be desirable.

The benzocaine modification or revised ACS specifications for acetic acids may be generally applicable to titration of aromatic amines and other bases that acetylate or combine as Schiff bases with the acetic anhydride or acetaldehyde in the glacial acetic acids. However, a preferable solution to the problem is the nitrite titration with internal ferrocyphen indicator (3).

Nitrite Titration—The satisfactory precision of this titration is evident in Table II. The accuracy of the visual end-point was validated potentiometrically with a pH meter equipped with glass and platinum electrodes. Two potentiometric titrations gave purity figures of 100.4 and 100.2%, comparing favorably with the average for the visual titrations of the same lot, 99.9 ± 0.29 *SD* (Table II).

Determination of the end-point in nitrite titrations has generally been amperometric or visual, with starch iodide paper used as the external indicator (7). Although these methods have yielded satisfactory results, visual determination of the end-point by an internal indicator does, of course, offer greater convenience. Although several internal indicators had been proposed for use (7), none had been generally suitable until the advent of the stable, reversible ferrocyphen indicator (3). This investigation extends the evidence for the general suitability of ferrocyphen as an internal indicator for nitrite titrations.

A statistical comparison of the three titrations in Table II by an analysis of variance procedure (8) was made possible by averaging the replicate titrations for the single lots of procainamide hydrochloride. The means for each of the three methods were as follows: nitrite, 99.32; USP XVIII, 98.36; and benzocaine, 99.23.

The means for the nitrite titration and the benzocaine modification were very similar. This suggests that the nitrite titration is suitable as a substitute for the nonaqueous titration. Using various lots of glacial acetic acid which were not obviously suspected (as was Sample 1 in Table I), the statistical evaluation of Table II indicates that the mean for the USP XVIII titration was significantly lower than the means for both the benzocaine ($p < 0.01$) and the nitrite ($p < 0.001$) titrations. This suggests that acetic anhydride and/or possibly acetaldehyde contamination of acetic acids might be the cause of a recurrent and a not always readily apparent problem in the nonaqueous titration of primary aromatic amines.

Evaluation of Alternative Solutions—In consequence of this investigation, three solutions to this problem are evident:

1. Maintain the present USP method with a requirement that acetic acids must pass maximum acetic anhydride–acetaldehyde specifications. Abolish the present requirement for glacial acetic acid T.S. that a few milliliters of acetic anhydride be added to glacial acetic acids having water contents greater than 0.05%.
2. Substitute the benzocaine modified titration for the USP XVIII method.
3. Utilize the nitrite titration instead of the USP XVIII titration.

The first of these alternatives requires additional testing of acetic acids, which can be avoided by utilizing the second or third alternative. However, the second solution is more cumbersome and prolonged than the third. Consequently, the comparatively simple nitrite titration offers the best solution to the problem.

The nitrite titration in the presence of crushed ice and with external, visual starch iodide end-point indication is presently required by USP XVIII for procainamide hydrochloride capsules. Undoubtedly, the nitrite rather than the nonaqueous titration is used because of its greater selectivity. Indeed, the excellent selectivity of the nitrite titration has been amply demonstrated in these laboratories with simple direct titrations of procainamide hydrochloride in several experimental tablets containing such diverse ingredients as povidone (polyvinylpyrrolidone), ethylcellulose, magnesium stearate, talc, lactose, calcium silicate, stearic acid, and carboxyvinyl polymer. Since some of these excipients are titrated in glacial acetic acid with perchloric acid (9), accurate titration of procainamide hydrochloride would require time-consuming separations.

The present USP XVIII nitrite titration in the presence of crushed ice and with external, visual starch iodide end-point for procainamide hydrochloride capsules might, with advantage, be replaced by the nitrite titration at room temperature with internal ferrocyphen indicator.

The simplicity of the nitrite titration with an internal indicator likewise is recommended over the present USP XVIII analysis of procainamide hydrochloride injection. The latter requires time-consuming evaporation and heating prior to nonaqueous titration. With the nitrite titration, it is only necessary to boil a suitable aliquot of the injectable in 6 *N* hydrochloric acid for 1 min, followed by cooling to room temperature and then titrating. The boiling is required to remove the excipient bisulfite as sulfurous acid.

These considerations invalidate the present USP XVIII titration for procainamide hydrochloride and recommend its substitution by the nitrite titration at room temperature with internal ferrocyphen indicator. The latter might, for convenience and economy supplant the present nitrite titration with external indicator for procainamide hydrochloride capsules and the nonaqueous titration for procainamide hydrochloride injection.

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Colorimetric Determination of Cephradine, a Cephalosporin Antibiotic

JOEL KIRSCHBAUM

Abstract □ The quantitative colorimetric determination of microgram quantities of cephradine is detailed. An aqueous solution of this compound is reacted with sodium hydroxide, partially neutralized, and subsequently reacted with 5,5′-dithiobis(2-nitrobenzoic acid); the absorbance of the resulting yellow solution is then measured. Other cephalosporins tested give a similar color. Studies with various cephalosporins showed that the development of maximum color intensity was attributable to the presence of the R—$CHNH_2$—CO—cephalosporin nucleus, in which R is a mono-, di-, or trienyl cyclohexyl ring. Molar absorptivities of penicillin solutions tested by the same procedure were found to be 5–15% of those of the cephalosporins.

Keyphrases □ Cephradine—colorimetric analysis □ Cephalosporins and penicillins—colorimetric determination □ Penicillins and cephalosporins—colorimetric determination □ 5,5′-Dithiobis(2-nitrobenzoic acid)—reagent for colorimetric determination of cephalosporins and penicillins □ Colorimetry—analysis, cephalosporins and penicillins

This report describes a colorimetric method for the quantitative determination of cephradine (1), a cephalosporin antibiotic, utilizing treatment with base followed by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (I), a compound known to react with sulfhydryl groups. The procedure appears generally applicable to cephalosporins and penicillins.

EXPERIMENTAL

Reagents—The following were used: sodium hydroxide–sodium carbonate solution (dilute 20 ml of 0.5 *M* sodium carbonate and 8 ml of 10 *M* sodium hydroxide to 100 ml); 2 *N* sulfuric acid; 0.5 *M* sodium carbonate buffer, pH 9.2 ± 0.05; and 5,5′-dithiobis(2-nitrobenzoic acid)[1] (I) color reagent, 15.5 mg/50 ml of 95% ethanol (prepared fresh daily).

Procedure—Pipet 5 ml of an aqueous solution containing 30–600 μg of cephradine into a test tube. Then pipet 5 ml of sodium hydroxide–sodium carbonate solution. Cover the tube loosely and place it in a boiling water bath for 75 min. Rapidly cool the test tube to room temperature by immersing it in an ice bath, and then add 2 *N* sulfuric acid to adjust the contents to pH 9.2 ± 0.1 (pH meter). Transfer the solution to a 25-ml volumetric flask. Into the same flask, pipet first 10 ml of 0.5 *M* sodium carbonate buffer and then 3 ml of I color reagent. Dilute to volume with water and mix. Determine spectrophotometrically[2] the absorbance of the 412-nm peak between 5 and 20 min after addition of the color reagent, using a reagent blank for comparison.

RESULTS AND DISCUSSION

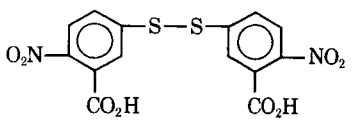
Beer's law is followed by solutions containing 1–30 μg of cephradine/ml (25–750 μg/25-ml flask). The sensitivity of this assay of 0.2 μg/ml indicates its utility, although the error of quantitation is inversely proportional to cephradine concentration. Base treatment with acetone, as described by Marrelli (2) for the estimation of cephalexin, another cephalosporin antibiotic, has a sensitivity of 0.1–2 mg/ml and does not follow Beer's law. Under the experimental conditions employed here, I is as sensitive to sulfide and sulfite (3) and to such mercaptans as cysteine and mercaptoethanol as it is to base-degraded cephradine. The thioether methionine does not interfere with the assay.

Related reagents that react with base-degraded and partially neutralized cephradine are 2,2′-dithiodipyridine and 4,4′-dithiodipyridine, but their maximal absorbance is in the same UV region of the spectrum as are the absorbance peaks of possible interfering substances, and the color development cannot be followed visually.

The results of the investigation of several variables of this procedure are discussed here.

Effect of Heating Time—Samples heated for 60–90 min and then processed as described had absorbances that did not differ from each other by more than 1%. Heating the sample for 120 min or longer resulted in a reduction of absorbance.

Effect of pH—Base-degraded solutions adjusted to pH < 9.2



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[1] Aldrich Chemical Co.

[2] A Beckman DU spectrophotometer was used.