Colorimetric Determination of Procaine Hydrochloride in Pharmaceutical Preparations

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Abstract □ A new rapid, specific, and convenient colorimetric method for the determination of procaine hydrochloride in pharmaceutical preparations is described. This method is based on measurement of the intensity of the orange-red color developed when procaine hydrochloride is allowed to react with 1,2-naphthoquinone-4-sulfonic acid sodium salt in aqueous solution. Beer's law is followed over 6.0–20.0 μg/ml. The method was applied to the determination of procaine hydrochloride in procaine hydrochloride, procaine hydrochloride injection with or without epinephrine, penicillin G procaine, penicillin G procaine injection, and fortified procaine penicillin injection (BP). The results were comparable to those obtained by official procedures. Because of its simplicity, sensitivity, and accuracy, this method is particularly suited for routine analysis of official preparations of procaine hydrochloride.

Keyphrases □ Procaine hydrochloride—colorimetric assay using 1,2-naphthoquinone-4-sulfonic acid sodium salt □ Colorimetry—analysis, procaine hydrochloride reaction with 1,2-naphthoquinone-4-sulfonic acid sodium salt

The USP (1) and BP (2) methods for the determination of procaine hydrochloride depend on diazotization and titration of the compound with standard sodium nitrite solution. For procaine hydrochloride injection, the USP assay requires prior isolation of the procaine by solvent extraction and then determination of the procaine spectrophotometrically (3). The BP (4) method for the determination of procaine hydrochloride in procaine hydrochloride injection depends on solvent extraction of the procaine from alkaline solution followed by acid–base titration. Both methods are lengthy and involve solvent extractions.

The BP (5) methods for the determination of procaine in penicillin G procaine, penicillin G procaine injection, and fortified procaine penicillin injection are all similar, depending on solvent extraction followed by acid–base titration. The method is lengthy and lacks sensitivity.

A colorimetric method for the determination of procaine hydrochloride was developed recently (6) based on the interaction of procaine hydrochloride with *p*-dimethylaminocinnamaldehyde in the presence of trichloroacetic acid in absolute methanol. This method requires a nonaqueous acidic medium.

A simple, time-saving, and sensitive method for the determination of procaine in pharmaceutical preparations is needed. The proposed method depends on the reaction between procaine hydrochloride and 1,2-naphthoquinone-4-sulfonic acid sodium salt in aqueous solution. 1,2-Naphthoquinone-4-sulfonic acid sodium salt was reported previously (7) to be a detecting agent for aromatic amines on TLC plates.

EXPERIMENTAL

Instruments—A visible UV spectrophotometer[1] was used.

Materials and Reagents—The following were used: procaine hydrochloride[2]; a 0.1% solution of 1,2-naphthoquinone-4-sulfonic acid sodium salt in water[3]; a 2% procaine hydrochloride injection; a 2% procaine hydrochloride injection containing 0.002% epinephrine; a procaine and epinephrine injection[4] containing 2.0% procaine hydrochloride, 0.002% epinephrine, 0.2% sodium chloride, 0.3% potassium sulfate, and 0.002% phenylmercuric nitrate; penicillin G procaine[5]; a penicillin G procaine injection containing 300 mg of penicillin G procaine; fortified penicillin and penicillin G (sodium salt), 60 mg; and fortified penicillin G injection BP[6].

Calibration Curve Preparation—Several aliquots of exactly 2.0–10.0 ml of procaine hydrochloride solution (100 μg/ml) were pipetted into 50-ml volumetric flasks. Then 20 ml of water and 2.0 ml of 1,2-naphthoquinone-4-sulfonic acid sodium salt were added. Each flask was mixed well, let stand for 60 min, and diluted to volume with water. The absorbance was measured at 482 nm in a 1-cm cell against a blank prepared under the same conditions but without procaine hydrochloride.

Table I—Effect of Time on Absorbance of Procaine Hydrochloride [a]–1,2-Naphthoquinone-4-sulfonic Acid Sodium Salt Color Complex

Minutes	Absorbance at 482 nm
10	0.202
20	0.222
30	0.240
40	0.250
50	0.260
60	0.260
120	0.262
180	0.260

[a] Concentration of 16 μg of procaine hydrochloride/ml.

Table II—Effect of pH on Absorbance of Procaine Hydrochloride–1,2-Naphthoquinone-4-sulfonic Acid Sodium Salt Color Complex [a]

pH	Absorbance at 482 nm
4	0.198
5	0.200
6	0.198
7	0.200
8	0.201
9	0.200
10	0.105

[a] Concentration of 12 μg of procaine hydrochloride/ml.

Table III—Reproducibility of Color Development of Replicate Samples of Procaine Hydrochloride Containing 6, 12, or 18 μg/ml (n = 10)

Concentration, μg/ml	Mean Absorbance at 482 nm	*CV*, %
6	0.0986	1.190
12	0.2001	0.684
18	0.2995	0.452

[1] DU-2, Beckman Instruments, Fullerton, Calif.

[2] BDH Laboratory, Poole, England.
[3] Fluka AG, Buchs SG, Switzerland.
[4] Planocaine, May and Baker Ltd., Dagenham, England.
[5] WHO, Geneva, Switzerland.
[6] Scurocilline, Specia, Paris, France.

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Table IV—Comparative Analysis of Preparations Containing Procaine Hydrochloride

Preparation	Labeled Amount, mg/ml	Amount Found, mg/ml[a] USP Method	BP Method	Proposed Method
Procaine hydrochloride, 2.0%	20.0	19.90 (CV = 0.34%)	19.93 (CV = 0.45%)	20.00 (CV = 0.25%)
Procaine hydrochloride, 2%, + 0.002% epinephrine	20.0	20.10 (CV = 0.66%)	19.91 (CV = 1.07%)	19.95 (CV = 0.66%)
Procaine hydrochloride, 2%, + 0.002% epinephrine + 0.2% sodium chloride + 0.3% potassium sulfate + 0.002% phenylmercuric nitrate	20.0	20.20 (CV = 0.74%)	19.81 (CV = 1.06%)	20.10 (CV = 0.20%)
Procaine hydrochloride, 2%, + 0.002% epinephrine + 5% sodium chloride + 0.1% chlorocresol	20.0	20.10 (CV = 1.79%)	19.70 (CV = 0.62%)	20.10 (CV = 0.50%)
Penicillin G procaine injection, 300,000 IU	139		130.00 (CV = 1.38%)	135.00 (CV = 0.74%)

[a] Mean of three results.

Table V—Recovery of Procaine Hydrochloride

Preparation	Procaine Hydrochloride, mg	Found, mg	Added, mg	Found after Addition, mg	Recovery, %
Injection (without epinephrine)	20	20.05	10.0	30.1	100.5
Injection (with epinephrine)	20	19.95	20.0	39.9	99.75
Solution	50	50.15	50.0	100.4	100.5

Procaine Hydrochloride—One hundred milligrams, accurately weighed, of procaine hydrochloride was dissolved in water to produce 100 ml. Then 0.8 ml of this solution was pipetted into a 50-ml volumetric flask, and the procedure described for the calibration curve was followed. The amount of procaine hydrochloride was calculated from the calibration curve.

Procaine Hydrochloride Injection with or without Epinephrine—Five milliliters of a 2% procaine hydrochloride injection was pipetted into a 100-ml volumetric flask and diluted to volume with water. Then 0.8 ml of this solution was pipetted into a 50-ml volumetric flask, and the procedure described for the calibration curve was followed. The amount of procaine hydrochloride was calculated from the calibration curve.

Penicillin G Procaine—Three hundred milligrams[7] of penicillin G procaine was dissolved in water to produce 100 ml. Then 0.5 ml of this solution was pipetted into a 50-ml volumetric flask, and the procedure described for the calibration curve was followed. The amount of procaine hydrochloride was calculated from the calibration curve.

Penicillin G Procaine Injection—An accurately measured volume of a well-shaken suspension, equivalent to 300 mg of penicillin G procaine, was diluted to 100 ml with water. Then 0.5 ml of this solution was pipetted into a 50-ml volumetric flask, and the procedure described for the calibration curve was followed. The amount of procaine hydrochloride was calculated from the calibration curve.

Fortified Procaine Penicillin Injection (BP)—The contents of the vial (containing 300 mg of penicillin procaine and 60 mg of penicillin G) were dissolved in water to produce 100 ml. Then 0.5 ml of this solution was pipetted into a 50-ml volumetric flask, and the procedure described for the calibration curve was followed. The amount of procaine hydrochloride was calculated from the calibration curve.

Interferences—The effect of epinephrine, sodium chloride, potassium sulfate, phenylmercuric nitrate, chlorocresol, and sodium metabisulfite on the determination of procaine hydrochloride was determined by preparing solutions containing these substances plus a known amount of procaine hydrochloride and performing the analysis according to the procedure for procaine hydrochloride. The effect of penicillin G on the determination of procaine in penicillin G procaine preparation was studied similarly.

RESULTS AND DISCUSSION

A characteristic orange-red color with an absorbance maximum at 482 nm developed when procaine hydrochloride was reacted with 1,2-naphthoquinone-4-sulfonic acid sodium salt in aqueous medium. When the observed absorbances were plotted against concentrations, a linear calibration curve with a slope of 0.01636 was obtained over the concentration range of 6.0–20.0 μg/ml, indicating obedience to Beer's law. This linearity was also confirmed when the correlation coefficient, r, was calculated from the values of absorbances and concentrations and was 0.9991.

The color reached maximum intensity after 60 min and remained stable for at least an additional 2 hr (Table I).

The effect of pH on the color intensity was studied. It was stable between pH 4 and 9 but dropped suddenly at pH 10 (Table II). Therefore, buffers were not used to adjust the pH since the method could be used at any pH between 4 and 9.

The concentration optimum of the color reagent was determined by adding various volumes of 0.1% 1,2-naphthoquinone-4-sulfonic acid sodium salt reagent to a series of 50-ml volumetric flasks, each containing 800 μg of procaine hydrochloride, diluting to volume with water, and measuring the absorbance of each solution at 482 nm against a blank[8]. Maximum color intensity was obtained with 1.5 ml of the reagent. Increasing the volume to 4.0 ml did not increase the absorbance. Therefore, it was decided to use 2.0 ml as the optimum concentration.

The precision study was performed at three different concentrations, 6, 12, and 18 μg/ml, by running 10 replicates of each. The coefficients of variation were 1.190, 0.684, and 0.452%, respectively (Table III).

The proposed method was compared to the official methods for prepared and commercially available procaine hydrochloride pharmaceutical preparations. There was a close relationship between the results obtained by the official procedures and those obtained by the suggested method (Table IV).

Samples of procaine hydrochloride preparation were analyzed before and after the addition of known quantities of procaine hydrochloride solution (Table V). The recovery data obtained proved that the suggested method is accurate. Temperature change had an insignificant effect on chromophore formation.

The presence of epinephrine, penicillin G, sodium chloride, potassium sulfate, phenylmercuric nitrate, chlorocresol, and sodium metabisulfite in the concentrations used produced no effect on the development, intensity, or stability of the color and, therefore, had no effect on the colorimetric assay.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 408.

[7] Equivalent to 139.0 mg of procaine hydrochloride.

[8] Each blank contained the same amount of 1,2-naphthoquinone-4-sulfonic acid sodium salt reagent as in the solution studied.

(2) "British Pharmacopoeia 1973," Her Majesty's Stationery Office, London, England, 1973, p. 385.
(3) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 409.
(4) "British Pharmacopoeia 1973," Her Majesty's Stationery Office, London, England, 1973, p. 386.
(5) *Ibid.*, p. 387.
(6) H. S. I. Tan and D. Shelton, *J. Pharm. Sci.*, **63**, 916 (1974).
(7) R. B. Smyth and G. G. McKeown, *J. Chromatogr.*, **16**, 454 (1964).

Quantitation of Acetazolamide in Plasma by High-Performance Liquid Chromatography

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Received August 20, 1979, from the *Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada.* Accepted for publication October 5, 1979.

Abstract □ A method for estimating acetazolamide concentrations in human plasma is described. Buffered plasma (pH 4.8) containing chlorothiazide as an internal standard is extracted twice with ethyl acetate. The extract is evaporated, redissolved, and chromatographed on silica gel with hexane–chloroform–methanol–acetic acid (65:25:10:0.25) as the mobile phase. The extraction efficiencies were >90%, the coefficients of variation at 1 and 30 μg/ml of plasma were 3.5 and 2.0%, respectively, and the calibration curves were linear and had an intercept of essentially zero. The suitability of the method for pharmacokinetic studies was verified in a normal volunteer dosed with 250-mg (solution) and 500-mg (sustained-release tablet) acetazolamide formulations.

Keyphrases □ Acetazolamide—analysis, high-performance liquid chromatography, plasma □ High-performance liquid chromatography—analysis, acetazolamide, plasma □ Carbonic anhydrase inhibitors—acetazolamide, high-performance liquid chromatography, plasma

Acetazolamide, a carbonic anhydrase inhibitor, is used to decrease the ocular aqueous humor secretion in certain types of glaucoma. Methods for the quantitation of this drug in biological fluids include measurement of the carbonic anhydrase inhibition (1, 2), colorimetry (3, 4), electron-capture GLC (5), and high-performance liquid chromatography (HPLC) (6). However, the GLC and HPLC procedures are time consuming (2), the colorimetric method is only partially successful (4, 6), and the modified enzymatic assay (2) lacks sufficient precision.

The present study was undertaken to develop an assay without the described deficiencies that could be used to quantitate acetazolamide in human or animal plasma following administration of single doses of 250 mg of a regular acetazolamide formulation or 500 mg of a sustained-release formulation.

EXPERIMENTAL

Materials—Hexane, chloroform, and methanol were HPLC grade[1,2]. Acetazolamide and chlorothiazide were USP reference standards. Sodium acetate, ethyl acetate, sodium bicarbonate, sodium hydroxide, and acetic acid were reagent grade.

One oral acetazolamide solution, used in the plasma profile experiments, was prepared by dissolving 250 mg of the USP standard in 20 ml of ethanol and diluting to 200 ml with fresh aqueous sodium bicarbonate (0.1 *M*). The final pH was 8. Another oral solution, used in determining the absence of interference, was prepared by dissolving a commercial parenteral preparation[3] in water.

Subjects—After an overnight fast, a healthy, 40-year-old male subject (90 kg) was administered 200 ml of the solution (250 mg) and separately one commercial 500-mg sustained-release formulation[3] with 150–250 ml of water at a 1-week interval.

Blood samples (10 ml) were collected in heparinized evacuated containers[4] at the various times (Figs. 1 and 2). The samples were centrifuged at 1000×*g*, and the separated plasma was stored at −18° until analysis.

Method—To tubes containing 1 ml of plasma were added 0.5 ml of internal standard (20 μg of chlorothiazide/ml of water), 2 ml of sodium acetate buffer (0.05 *M*, pH 4.8), and 10 ml of ethyl acetate. After mixing[5] for 10 min and centrifuging for 10 min at 1000×*g*, the organic layer was removed, and the extraction steps were repeated. The combined organic layers were evaporated at 60° to dryness using a nitrogen stream. After the residue was dissolved in 10 μl of 0.45 *N* NaOH by mixing[6], 1 ml of ethyl acetate was added, and the contents were mixed again. Aliquots (200 μl) of this solution were chromatographed.

The HPLC system consisted of a pump, an injector, and a detector[7] set at 280 nm. The silica gel column[8] (5 μm, 3.2 × 250 mm) was packed using a balanced density slurry method. The mobile phase was hexane–chloroform–methanol–acetic acid (65:25:10:0.25) at a flow rate of 3 ml/min (2900 psi). Quantitation was achieved by measuring the peak height ratio of the drug to the internal standard.

Calibration Curve—The linearity of the calibration curve was determined by adding 0.5, 2, 4, 8, and 12 μg of acetazolamide/ml to blank plasma samples. Two aliquots of each concentration were assayed as described. The coefficients of variation were determined using replicate samples of plasma standards containing 1 and 30 μg of acetazolamide/ml.

Determination of Extraction Efficiency—The extraction efficiency for the drug was estimated from the change in the peak height ratio when the drug was added to the plasma while the internal standard was added to the final extract compared to the peak height ratio when both were added to the final extract. The extraction efficiency for the internal standard was estimated from the change in the peak height ratio when the internal standard was added to the plasma while the drug was added to the final extract compared to the peak height ratio when both were added to the final extract.

Absence of Interferences—To verify the absence of metabolites and naturally occurring plasma constituents interfering with the internal standard or drug, plasma samples obtained from another volunteer 2 and 2.5 hr after ingestion of 500 mg of acetazolamide solution were pooled and extracted without the internal standard and chromatographed.

The effluents containing acetazolamide were combined and evaporated. One aliquot was rechromatographed on the same column using hexane–chloroform–methanol–acetic acid (70:25:5:0.25) as the mobile phase at a flow rate of 3 ml/min. Another aliquot was injected onto a bonded amino-silica gel column[9] (10 μm, 4.0 × 300 mm) using a mobile

[1] Burdick & Jackson Laboratories, Muskegon, Mich.
[2] Fisher Scientific, Ottawa, Canada.
[3] Diamox, Lederle, Canadian Cyanamide Ltd., Montreal, Canada.
[4] Vacutainers, Becton Dickinson, purchased through Canlab, Ottawa, Canada.
[5] Roto-rack, Fisher Scientific.
[6] Vortex, Fisher Scientific.
[7] Models 6000A, U6K, and 440, Waters Associates, Mississauga, Ontario, Canada.
[8] Li-Chrosorb Si-60, 5 μm, Brinkmann Instruments, Rexdale, Ontario, Canada.
[9] μBondapak-NH_2, Waters Associates.

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