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Rapid Fluorometric Determination of Procainamide Hydrochloride Dosage Forms

HENRY S. I. TAN [x] and CHERYL BEISER

Abstract □ A fluorometric procedure for procainamide hydrochloride was developed, and it offers improvements in ease, speed, and sensitivity over the official method. The new procedure is based on the reaction with fluorescamine in aqueous medium at pH 7.5 to form a fluorophore, with activation and emission wavelengths of 400 and 485 nm, respectively. The fluorescence is linear (r = 0.999) over the 0.04–1-μg/ml concentration range and is stable for at least 2 hr. Recovery data appeared to be accurate, quantitative, and reproducible. The overall recovery was 99.8% with a standard deviation of ±1.14 (n = 5). The method was successfully applied to commercially available dosage forms.

Keyphrases □ Procainamide hydrochloride—rapid fluorometric determination, bulk and dosage forms, fluorescamine reagent □ Fluorometry—analysis, procainamide hydrochloride dosage forms □ Fluorescamine—reagent in fluorometric analysis of procainamide hydrochloride dosage forms

The USP XVIII (1) assay procedure for procainamide hydrochloride capsules is time consuming, since it is based on a prior separation of the drug from the dosage form followed by a diazotization titration of the compound with standard sodium nitrite at low temperatures and starch iodide paper as the external indicator. This method is subject to variations between individuals in determination of the end-point. The assay method (2) for procainamide hydrochloride injections is also lengthy, since the sample must be evaporated and dried before the drug can be analyzed by nonaqueous titrimetry with standard perchloric acid.

Recently, it was reported (3–5)[1] that 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (fluorescamine) is a reagent for the detection of primary amines in the picomole range. Its reactions with amines is almost instantaneous at room temperature in aqueous solutions. The resulting fluorophores are fluorescent, whereas the reagent and its degradation products are nonfluorescent. This report presents a sensitive, simple, and accurate method for the assay of procainamide hydrochloride and its dosage forms with fluorescamine in an aqueous medium.

EXPERIMENTAL

Instruments—The following were used: a fluorescence spectrophotometer[2] with 1-cm cells, a mixer[3], and an analytical balance[4]. The response of the fluorescence spectrophotometer was calibrated daily, using a 0.5-μg/ml quinine sulfate solution in 0.1 *N* sulfuric acid, at activation and emission wavelengths of 350 and 445 nm, respectively.

Materials and Reagents—The following were used: procainamide hydrochloride[5], acetone[6], a 0.15% (w/v) solution of fluorescamine[7] in acetone, and pH 7.5 phosphate buffer. This buffer solution was prepared by adding 40.8 ml of 0.2 *M* sodium hydroxide to 50.0 ml of 0.2 *M* monobasic potassium phosphate in a 200-ml volumetric flask and diluting to volume with water. The pH of the resulting buffer solution was checked with a pH meter. All chemicals were analytical grade.

Preparation of Standard Curve—A stock solution of procainamide hydrochloride was prepared by dissolving 50.0 mg of the compound in 1000 ml of water. Further dilutions were made to obtain procainamide hydrochloride standard solutions containing 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 μg/ml. One milliliter of each solution was utilized for fluorescence development as described under *Assay Procedure for Procainamide Hydrochloride*. The stock and standard solutions were stable for at least 3 weeks.

Assay Procedure for Procainamide Hydrochloride—Pipet 1.0 ml of an aqueous solution containing 0.25–2.5 μg of procainamide into a 25 × 200-mm test tube. To this solution, add 1.0 ml of phosphate buffer (pH 7.5), and place the test tube on a vortex mixer. While shaking the tube vigorously, add 0.5 ml of the fluo-

[1] After completion of this work, while this article was being prepared, a paper entitled "Spectrophotofluorometric Analysis of Procainamide and Sulfadiazine in Presence of Primary Aliphatic Amines Based on Reaction with Fluorescamine," by J. M. Sterling and W. G. Haney [*J. Pharm. Sci.*, **63**, 1448(1974)] was published.

[2] Model 204, Perkin-Elmer Corp., Norwalk, Conn.

[3] Vortex-Genie, Scientific Industries, Springfield, Mass.

[4] Mettler type H-18, Mettler Instrument Corp., Princeton, N.J.

[5] E. R. Squibb & Sons, Princeton, N.J.

[6] Spectranalyzed grade, Fisher Scientific Co., Pittsburgh, Pa.

[7] Roche Diagnostics, Nutley, N.J.

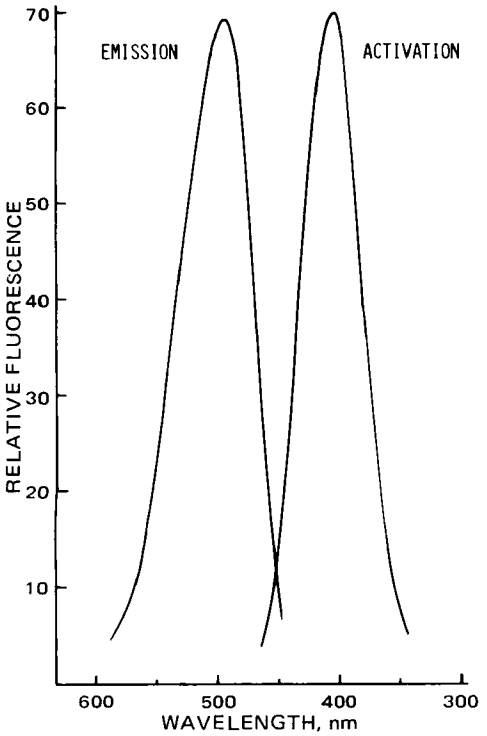
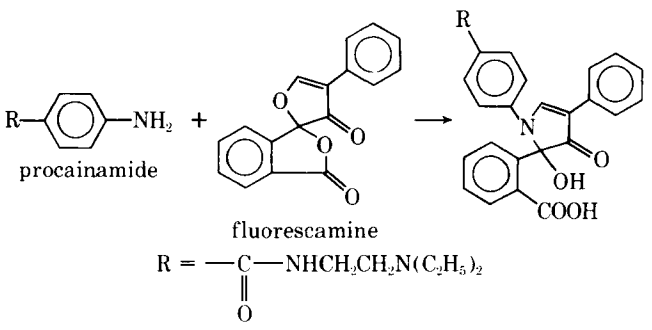


Figure 1—*Activation and emission spectra of procainamide hydrochloride–fluorescamine fluorophore.*

rescamine solution *directly into the whirling solution.* After 10 sec, measure the fluorescent intensity at activation and fluorescence wavelengths of 400 and 485 nm, respectively, against a blank prepared similarly but without the procainamide hydrochloride. Read the concentration of procainamide from a calibration curve.

Assay Procedure for Procainamide Hydrochloride Capsules—Weigh, as completely as possible, the contents of 20 capsules. Weigh accurately a portion of the powder, equivalent to about 100 mg of procainamide hydrochloride, and transfer to a 1000-ml volumetric flask[8]. Add water to the mark and shake well for a few minutes. Filter the mixture through fluted filter paper, rejecting the first 10 ml of filtrate. Pipet 1.0 ml of the filtrate into a 100-ml volumetric flask and dilute to volume with water. Pipet 1.0 ml of this solution into a 25 × 200-mm test tube and proceed as described under *Assay Procedure for Procainamide Hydrochloride*, beginning with "To this solution, add . . . ."

Assay Procedure for Procainamide Hydrochloride Injections—Pipet 1.0 ml of a 10% procainamide hydrochloride injection into a 1000-ml volumetric flask and dilute to volume with water. Transfer 1.0 ml of this solution into a 100-ml volumetric flask and dilute to volume with water. Pipet 1.0 ml of the resulting solution into a 25 × 200-mm test tube and proceed as described under *Assay Procedure for Procainamide Hydrochloride*, beginning with "To this solution, add . . . ."



Scheme I

[8] The portion of the powder must come from a representative distribution of the contents of the 20 capsules.

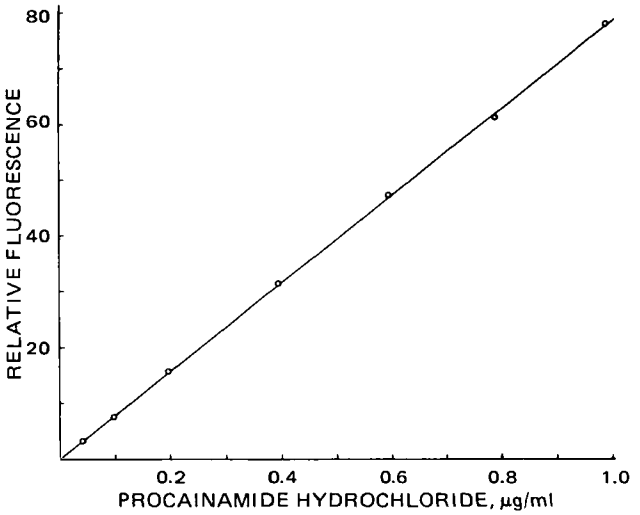


Figure 2—*Relationship between fluorescence and concentration of procainamide hydrochloride.*

Table I—pH Dependency of Fluorophore Using 0.3 μg/ml Procainamide Hydrochloride

pH	Relative Fluorescence Intensities
Borate buffer	
10.0	5.5
9.5	8.0
9.1	13.0
8.6	15.5
8.1	23.5
Phosphate buffer	
8.0	29.7
7.8	28.7
7.5	28.7
7.0	28.7

Table II—Effect of Time on Fluorescence Intensity of Fluorophore[a]

Minutes	Relative Intensity[b]
3	32.5
15	33.0
30	32.8
45	32.0
60	31.8
75	31.8
90	31.5
105	31.5
120	31.2

[a] Procainamide hydrochloride concentration about 0.1 μg/ml. [b] Instrument settings: $\lambda_{activation}$, 400 nm; $\lambda_{emission}$, 485 nm; selector, X1; and sensitivity, 4.

Table III—Effect of Fluorescamine Reagent Solution on Fluorescence Intensity of Fluorophore[a]

15% (w/v) Fluorescamine in Acetone, ml	Relative Intensity[b]
0.17	3.2
0.25	24.0
0.50	28.2
1.00	12.5

[a] Procainamide hydrochloride concentration about 0.08 μg/ml. [b] Instrument settings: $\lambda_{activation}$, 400 nm; $\lambda_{emission}$, 485 nm; selector, X1; and sensitivity, 4.

Table IV—Reproducibility of Fluorophore Development of Replicate Procainamide Hydrochloride Samples Containing 0.4 μg/ml

Solution	Relative Intensity[a]
1	54.2
2	56.5
3	54.4
4	54.8
5	55.1
6	55.9
7	56.0
8	54.6
9	54.5
Average	55.1
Standard deviation	0.822
Coefficient of variation	1.49%

[a] Instrument settings: $\lambda_{activation}$, 400 nm; $\lambda_{emission}$, 485 nm; selector, X1; and sensitivity, 4.

was $y = 0.161x - 0.643$ with a standard error of the estimate of y on x of 1.36 and standard errors of the estimate of the intercept and slope of 1.06 and 0.0018, respectively. The fluorophore produced appeared to be stable for at least 120 min (Table II).

The concentration optimum of the fluorescamine reagent was determined by adding varying volumes of 0.15% fluorescamine in acetone to a series of 25 × 200-mm test tubes, each containing 1.0 ml of 0.5 μg/ml procainamide hydrochloride, and measuring the fluorescence against a blank[10] after diluting each solution to a total volume of 2.5 ml with pH 7.5 phosphate buffer. The results (Table III) indicated that 0.5 ml of the fluorescamine reagent gave the highest response. It was also established that, at constant volume, within certain limits the amount of pH 7.5 phosphate buffer did not affect the fluorescence.

The precision of the assay procedure was determined by running replicate studies on nine 1.00-ml aliquots of a standard procainamide hydrochloride solution containing 1 μg/ml. Each solution was assayed by the proposed procedure. The coefficient of variation for the nine replicate samples was 1.49% (Table IV).

Table V—Results of Analysis of Known Concentrations of Procainamide Hydrochloride

Amount Weighed, mg	Analyzed at Concentration Level, ng/ml	Amount Found[a], mg		Recovery, %	
		Proposed Method	USP XVIII Method	Proposed Method	USP XVIII Method
40.6	162	40.0		98.5	
52.4	210	51.8		98.9	
123.8	495	123.2		99.5	
189.5	758	191.3		100.9	
221.8	887	224.0		101.0	
205.9			200.4		97.3
203.1			198.6		97.8
Overall recovery, %				99.8	97.6
Standard deviation				1.14	

[a] Average of duplicate assays.

Table VI—Results of Analysis of Commercially Available Procainamide Hydrochloride Dosage Forms

Dosage Form	Amount of Procainamide Hydrochloride Claimed, mg/Capsule or mg/ml	Amount Found[a], mg		Percent of Claim	
		Proposed Method	USP XVIII Method	Proposed Method	USP XVIII Method
Capsule	250	241.2	237.9	96.5	95.2
Capsule	500	494.6	492.8	98.9	98.6
Injection	100	99.4	97.8	99.4	97.8

[a] Average of duplicate assays.

RESULTS AND DISCUSSION

The reaction between procainamide and fluorescamine (Scheme I) presumably proceeds in several steps. There is a Michael addition of the amine to the acrylic function of fluorescamine, followed by ring closure and opening of the lactone ring.

The fluorophore obtained from the interaction between procainamide hydrochloride and fluorescamine shows activation and fluorescence wavelengths of 400 and 485 nm, respectively (Fig. 1), between pH 7.0 and 10.0. However, the fluorescence intensity is a function of the pH when alkaline borate buffer (pH 8.0–10.0) is used. In this case, the intensities decrease with increasing pH (Table I). When the phosphate buffer (pH 7.0–8.0) is used, the fluorescent intensities appear to be constant and are of greater magnitude than those in borate buffer (Table I). Based on these findings, the reaction was carried out in pH 7.5 phosphate buffer.

Under the experimental conditions, a linear relationship existed between fluorescent intensity and concentration over the 0.04–1-μg/ml concentration range (Fig. 2), with a correlation coefficient of 0.999. Regression analysis[9] showed that the regression equation

As little as 0.043 μg of procainamide hydrochloride can be assayed by the proposed method. The method, however, is reliable only for concentrations above 0.05 μg. Data presented in Table V indicate the accuracy of the procedure. The overall recovery for the six samples was 99.8% with a standard deviation of ±1.14. In comparison, the average recovery for two samples analyzed by the USP XVIII method was 97.6%.

Results obtained by applying the proposed fluorescence procedure to commercially available procainamide hydrochloride dosage forms are presented in Table VI. Comparison of the experimental data with those obtained by the compendial method shows a good correlation. Particularly in the analysis of the parenteral solution, the proposed procedure offers considerable improvement in speed over the USP XVIII method. Evaporation of the sample, which requires at least 60–75 min, and drying of the residue as directed by USP XVIII are completely eliminated.

It was also established that in the assay of the capsules the emptied capsules need not be soaked and agitated for 15 min as directed by the USP procedure. As long as care was taken to empty the

[9] Programa 101 Minicomputer, Olivetti Underwood Corp.

[10] Each blank contained the same amount of fluorescamine as in the solution studied but contained no procainamide hydrochloride.

250- or 500-mg capsules as completely as possible, the recovery appeared to be satisfactory (Table VI). Another advantage of the present method is the ease of performing the assay. The fluorescamine reagent is stable for several weeks. In contrast, the USP XVIII method for the capsules requires freshly prepared sodium nitrite solutions.

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GLC Determination of 17α-Ethynylestriol 3-Cyclopentyl Ether

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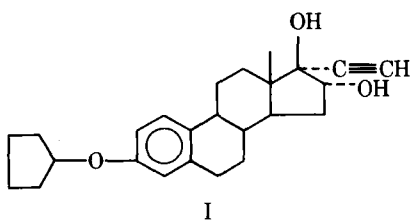
Abstract □ A rapid, sensitive, and accurate GLC method of analysis of a new estrogenic drug, 17α-ethynylestriol 3-cyclopentyl ether, was developed. The drug and the internal standard, tetratriacontane, are dissolved in chloroform, and an aliquot is heated with *N*-trimethylsilylimidazole at 80° for 30 min. The silylated sample is chromatographed using a column packed with 1% methyl vinyl silicone gum on Gas Chrom Q. Quantitation is achieved by computer calculation of the peak area ratios. The observed peak is the 16α,17β-bistrimethylsilyl derivative of the new drug substance. The GLC method was applied to the quantitative determination of the estrogenic compound in a tablet formulation containing 25 μg/tablet.

Keyphrases □ 17α-Ethynylestriol 3-cyclopentyl ether—GLC analysis as trimethylsilyl derivative □ Estrogens—GLC analysis of 17α-ethynylestriol 3-cyclopentyl ether □ GLC—analysis, 17α-ethynylestriol 3-cyclopentyl ether

A new potent estrogenic hormone, 17α-ethynylestriol 3-cyclopentyl ether (I), useful in the treatment of spontaneous or induced menopausal syndrome, estrogen deficiency, or conditions when estrogen may be used therapeutically, has been synthesized (1, 2).

In the process of developing a GLC method for analyzing I, the literature concerning the GLC determination of estrogens was screened. Numerous publications deal with the GLC determination of estriol, both as the pure compound (3–6) and in biological materials (7–29). Although there are several reports of the GLC of free estriol (4, 5, 15, 17, 27), the use of estriol derivatives is preferred to prevent irreversible adsorption on the column and/or thermal decomposition of the underivatized hormone (30, 31). Higher volatility of the steroid derivative results in a shorter retention time, sharper peak, and improved sensitivity (31, 32).



I

The trimethylsilyl (21, 22, 25, 28, 33), acetate (19, 23, 24, 29, 33), heptafluorobutyrate (14, 34), and trifluoroacetate (6, 35, 36) derivatives are the most frequently used. The advantages of the trimethylsilyl derivatives relative to the acetate derivatives were discussed (37, 38). Other derivatives that were also reported for the GLC determination of estriol include the 3-methyl ether (39, 41), monochloroacetate (42), chloromethyldimethyl ether (43), chlorodifluoroacetate (43), 3-methyl-16,17-diacetate (44–46), 3-methyl-16,17-diheptafluorobutyrate (7), and 3-methyl-16,17-ditrimethylsilyl (13, 20, 39, 44) and iodomethyldimethylsilyl ethers (47). Several reported procedures were tried with no success for the analysis of I in this study. The purpose of this paper is to report the trimethylsilyl derivatization and GLC conditions for the quantitation of I.

EXPERIMENTAL

Equipment—A gas chromatograph[1] equipped with a flame-ionization detector was used. The detected signals were fed to a computer[2] for peak area integration and to a potentiometric recorder[3] with a chart speed of 38.1 cm (15 in.)/hr and a 1-sec full-scale response. Helium, with a flow rate of 60 ml/min, was the carrier gas. The flow rates of hydrogen and oxygen were adjusted to optimum sensitivity and low noise background in the electronic integrator and were found to be 40 and 300 ml/min, respectively.

[1] F&M model 402, Hewlett-Packard, Avondale, Pa.
[2] Hewlett-Packard 2100.
[3] Honeywell Electronic 16, Honeywell, Philadelphia, Pa.