

**9-KLOROAKRIDİN İLE FLUOROMETRİK OLARAK  
PROKAINAMİD HİDROKLORÜR  
TAYİN YÖNTEMİ VE UYGULAMALARI**

**DETERMINATION OF PROCAINAMIDE HYDROCHLORIDE AND  
SOME DOSAGE FORMS WITH 9-CHLOROACRIDINE BY  
QUENCHING FLUOROMETRY.**

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**SUMMARY**

A fluorometric method was developed for the analysis of procainamide hydrochloride and some dosage forms. The method is based on the reaction of procainamide hydrochloride with 9-chloroacridine at pH 3. This interaction results in quenching of native fluorescence of the 9-chloroacridine reagent solution. The fluorescence measurements were performed at activation and emission wavelengths of 9-chloroacridine (400 and 437 nm respectively). The fluorescence intensity is linear over the concentration range of 40-800 ng/ml procainamide hydrochloride. The method was applied to the marketed dosage forms and the results were compared statistically with those of USP XVIII, TF 1974, and p-dimethylaminocinnamaldehyde methods in respect to t and F tests of significance.

**Ö Z E T**

Farmasötik preparatlarda prokainamid hidroklorür miktar tayini için fluorometrik bir yöntem geliştirildi. Yöntem prokainamid hidroklorürünün 9-kloroakridin ile pH = 3 deki reaksiyonuna dayanmakta, bu reaksiyon sonunda 9-kloroakridin belirteç çözeltisinin doğal floresansında bir sönme oluşmaktadır.

Fluoresans ölçmeleri 9-kloroakridinin aktivasyon ve emisyon dalga boylarında yapıldı (400 ve 437 nm). Floresans şiddetinin 40-800

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ng/ml prokainamid hidroklorür konsantrasyonu aralığında doğrusal olduğu saptandı. Yöntem, piyasada satılan dozaj şekillerine uygulandı ve sonuçlar USP XVIII, TF 1974, ve p-dimetilaminosinnamaldehid yöntemleriyle elde edilen sonuçlarla t ve F testleri yönünden kıyaslandı.

## INTRODUCTION

Dosage forms of procainamide hydrochloride, 4-amino-N-(2-diethylaminoethyl) benzamide hydrochloride, are used for prevention or treatment of cardiac arrhythmias and several assay methods covering spectrophotometric, fluorometric, and chromatographic procedures have been reported for the drug (1). The USP XVIII method (2) for the assay of the powder and capsules, and the TF 1974 method (3) for injectables are based on a nitritometric titration using starch as an external indicator. No compendial method is available for the analysis of the tablets.

Fluorometric methods for the analysis of procainamide are based on the measurement of its native fluorescence at pH 11 (4) or the formation of a fluorophore with fluorescamine at pH 5.5 (5) or pH 7.5 (6). More recently a fluorometric method based on a schiff base formation with o-phthalaldehyde in acidic methanol (7) was reported.

Interaction of the solutions containing primary aromatic amines with 9-chloroacridine gives orange coloured 9-aminoacridines, and a decrease in the native fluorescence of the 9-chloroacridine solution is observed. This observation forms the basis for the analysis of the amines by quenching fluorometry (8). This paper represents a method for the determination of procainamide hydrochloride and its dosage forms by quenching fluorometry using 9-chloroacridine reagent in acidic medium.

## EXPERIMENTAL

### Instruments

The following were used: A Zeiss PMQ II spectrophotometer equipped with a ZF M 4 fluorescence attachment and a St 41 mercury vapour lamp for quantitative measurements; a Perkin-Elmer 204 A spectrofluorometer with a xenon arc lamp for recording the excitation and emission spectra; 10x10x45 mm glass cells; a 100  $\mu$ l hamilton syringe.

### Reagents

- a) Procainamide hydrochloride (Siegfried Zofingen, Switzerland).
- b) 9-chloroacridine (Synthesized in the laboratory from acridon and phosphorus oxychloride (9) and recrystallized from ethanol. Mp 119-120°C, lit (9) mp 119-120°C). 0.025 % and 0.0062 % solutions of 9-chloroacridine in 96 % ethanol. The solutions were stable for approximately eight hours when kept refrigerated).
- c) 5 % Aqueous solution of hydrochloric acid.
- d) Reference standart (a solution of quinine sulphate in 0.1 N sulphuric acid prepared at a concentration that it shows 0.4-2.5 fold fluorescence intensity of that of the sample. All aqueous solutions were prepared using distilled deionized water.

### Assay Procedure

Powder- 0.1 ml of the aqueous stock solution containing 16-80  $\mu\text{g/ml}$  (and 4-20  $\mu\text{g/ml}$ ) procainamide hydrochloride was pipetted into a 10 ml volumetric flask. 0.1 ml of 0.025 % (and 0.0062 %) 9 chloroacridine and 0.01 ml of 5 % hydrochloric acid solutions were added, respectively. The mixture was heated at 50°C for 30 min. After cooling to room temperature and dilution to the volume with 96 % ethanol, the decrease in fluorescence of the reaction mixture compared with the corresponding 9-chloroacridine reagent blank was measured using activation and emission wavelength settings of 400 and 437 nm, respectively. The standardized fluorescence intensity  $I_Q$  was then calculated relative to the intensity of the appropriate reference standard solution.

A calibration graph was plotted between  $I_Q$  and procainamide hydrochloride concentration (ng/ml).

Dosage Forms (Tablets, capsules and injectables) — 0.1 ml of the aqueous stock solution of the dosage form containing 5  $\mu\text{g}$  procainamide hydrochloride was subjected to the procedure as described under «powder». The concentration was calculated by reference to previously determined calibration curve.

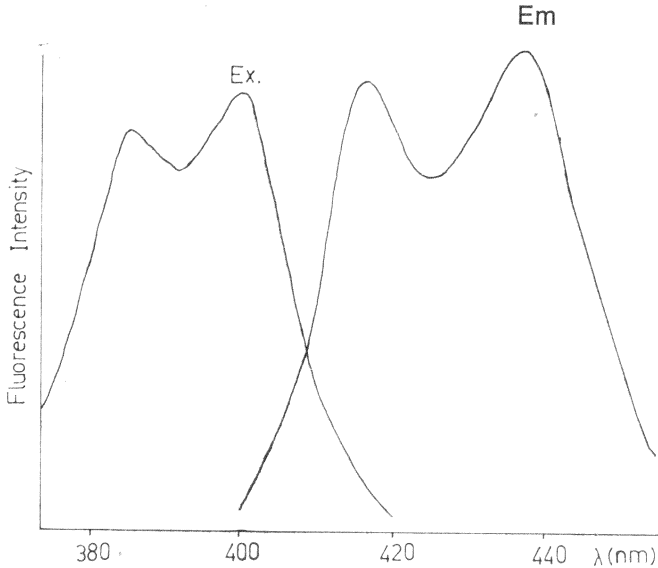


Figure — 1 : The fluorescence spectra of 9-chloroacridine recorded in ethanol.  
Ex. = Excitation, Em. = Emission.

## RESULTS AND DISCUSSION

The interaction of procainamide hydrochloride and 9-chloroacridine in acidic medium results in a quenching of the native fluorescence of 9-chloroacridine reagent solution. Monitoring this quenching at the activation and emission maxima (400/437 nm) of 9-chloroacridine (see figure 1) permits the fluorometric determination of procainamide hydrochloride described here.

Optimum conditions of the method were investigated. The best results were obtained at pH 3 in 30 min standing period at 50°C after addition of the reagent.

Stability of the reaction mixture was investigated by performing repeated readings of the same sample at different times.  $I_Q$  value was found to be stable for at least 8 hours at 4°C when kept away from the day light and for 10 min when exposed to the mercury vapour lamp.

Maximum concentration of 9-chloroacridine was found to be 2.5  $\mu\text{g/ml}$ , because its fluorescence showed a self quenching at higher concentrations. Fourfold molar excess quantity of the reagent to procainamide hydrochloride was found sufficient.

Under the experimental conditions described, two calibration graphs were plotted in two different concentration ranges of procainamide hydrochloride. The regression equations for the straight lines as plotted with Texas SR-52 electronic calculator are  $I_Q = 0.0539 C + 3.036$  ( $r = 0.99987$ ) for 800-160 ng/ml range and  $I_Q = 0.2216 C + 2.93$  ( $r = 0.99976$ ) for 200-40 ng/ml range, respectively. The standard deviations of the slopes are 0.054 and 0.220, of the intercepts are 3.036 and 3.158, respectively ( $n = 5$ ).

Precision of the method was calculated by means of its reproducibility at 480 ng/ml procainamide hydrochloride level. The coefficient of variation is 1.51% ( $n = 6$ ).

The proposed method was applied to the analysis of commercially available capsules, tablets, and injectables of procainamide hydrochloride. The dosage forms were also analysed according to USP XVIII, TF 1974 and p-dimethylaminocinnamaldehyde (DACA) methods for comparison (see table I). Since the compendial methods are titrimetric ones and analysis of the tablets is not listed in any pharmacopeia, spectrophotometric DACA method was employed as a second method of comparison. The results were compared statistically with each other in terms of *t* and *F* tests of significance at 95 % confidence level (see table II).

The proposed method is simpler than the USP XVIII and TF 1974 methods in that fewer manipulations are involved. Furthermore, the compendial methods are subject to variations between individuals in terms of the end-point determination of the standardization titration of the sodium nitrite titrant, which must be performed daily as well as the titrations of sample and blank solutions. The DACA method is precise, but it has the disadvantages of using nonaqueous methanol and highly corrosive trichloroacetic acid. The proposed method is precise, fast, and requires no solvent other than ethanol and water.

Table -- I : Analysis of procainamide hydrochloride in marketed dosage forms with three different methods.

Dosage Form	Label Claim <sup>a</sup>	Found <sup>a, b</sup>			% Found (recovery)		
		Proposed Method	USP XVIII Method	DACA Method	Proposed Method	USP XVIII Method	DACA Method
Tablets	250	233.21	—	233.22	93.28	—	93.29
Capsules	300	303.71	299.96	304.04	101.23	100.02	101.34
Injectables	100	99.82	99.41 <sup>c</sup>	99.61	99.82	99.41 <sup>c</sup>	99.61
SD for tablets		1.124	—	1.074	0.450	—	0.430
SD for capsules		1.505	8.240	1.705	0.503	2.743	0.569
SD for injectables		0.653	2.261	0.580	0.653	2.261	0.580

<sup>a</sup>mg/tablet, mg/capsule, or mg/ml<sup>b</sup>Average of six assays<sup>c</sup>According to TF 1974

Table — II : Statistical comparisons of the proposed and comparison methods

Methods	Statistical Values	
	t test of significance	F test of significance
	t = 2.23 for p = 0.05 $n_1 = n_2 = 6$	F = 0.05 for p = 0.05 $n_1 = n_2 = 6$
Proposed-DACA (Tablets)	0.913	1.09
Proposed-USP XVIII (Capsules)	1.090	29.96*
Proposed-DACA (Capsules)	0.360	1.28
Proposed-TF 1974 (Injectables)	0.440	12.01*
Proposed-DACA (Injectables)	0.610	1.26

\* Significant differences.

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