9-KLOROAKRIDİN İLE FLUOROMETRİK OLARAK PROKAINAMİD HIDROKLOORİR TAYIN YÖNTEMI VE UYGULAMALARI

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

Sezen İSLİMİYELİ

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 1874, and p-dimethylaminocinnamaldehyde methods in respect to t and F tests of significance.

ÖZET

Farmasötik preparatlarda prokainamid hidroklorür miktar tayini için fluorometrik bir yöntem geliştirildi. Yöntem prokainamid hidroklorürünün 9-kloroakridinin ile pH = 3 deki reaksiyonuna dayanmaktadır, bu reaksiyon sonunda 9-kloroakridin belirtec çözeltisinin doğal fluoresansında bir sönüme oluşmaktadır.

Fluoresans ölçmeleri 9-kloroakridinin aktivasyon ve emisyon dalga boyalarında yapıldı (400 ve 437 nm). Fluoresans siddetinin 40-800

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

**INTRODUCTION**

Dosage forms of procinamide 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 procinamide 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-phthaldialdehyde 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 procinamide 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; an Eppendorf 204 A spectrophotometer with a xenon arc lamp for recording the excitation and emission spectra; 10x10x45 mm glass cells; a 100 µl Hamilton syringe.

**Assay Procedure**

Powder - 0.1 ml of µg/ml (and 4-20 µg/ml into a 10 ml volumet 9 chloroacridine and were added, respecti 30 min. After coolin volume with 96 % reation mixture cor dina reagent blank w wavelength settings a rdized fluorescence the intensity of the A calibration gra hydrochloride concen

Dosage Forms (1 of the aqueous stock procinamide hydrod described under «pow reference to previous
Reagents

a) Procainamide hydrochloride (Siegfried Zofingen, Switzerland).

b) 9-chloroacridine (Synthesized in the laboratory from acridone 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 approximatively 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 μg/ml (and 4-20 μg/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 I₀ was then calculated relative to the intensity of the appropriate reference standard solution.

A calibration graph was plotted between I₀ 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 μg procainamide hydrochloride was subjected to the procedure as described under «powder». The concentration was calculated by reference to previously determined calibration curve.
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. I₀ value was found to be stable for at least 8 hours at 4°C when kept away from the daylight and for 10 min when exposed to the mercury vapour lamp.

Maximum concentration of 9-chloroacridine was found to be 2.5 µg/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 experiments graphs were plotted in procainamide hydrochloride straight lines as plotted are I₀ = 0.0539 C + range and I₀ = 0.2216 C + range, respectively. The 0.220, of the inte: (n = 5).

Precision of the n reproducibility at 480
The coefficient of variation

The proposed method was available capsules hydrochloride. The dos to USP XVIII, TF 197 (DACA) methods for co dial methods are titr
is not listed in any p methos was employed results were compared t and F tests of signifi

The proposed method TF 1974 methods in that more, the compendial r individuals in terms of dardization titration of be performed daily as v solutions. The DACA m tages of using nonaque chloroacetic acid. The quires no solvent other t
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 \( I_0 = 0.0539 \times 10^{-3} + 3.036 \) (r = 0.99987) for 800-160 ng/ml range and \( I_0 = 0.2216 \times 10^{-3} + 2.93 \) (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 methos 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>
<thead>
<tr>
<th>Dosage Form</th>
<th>Label Claim</th>
<th>Proposed Method</th>
<th>USP XVIII Method</th>
<th>DACA Method</th>
<th>% Found (recovery)</th>
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<tbody>
<tr>
<td>Tablets</td>
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<td>222.21</td>
<td>232.22</td>
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<tr>
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<td>112.24</td>
<td>112.24</td>
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<td>Injectables</td>
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<td>8.240</td>
<td>8.240</td>
<td>8.240</td>
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<td>0.053</td>
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<td>99.61</td>
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<tr>
<td>SD for capsules</td>
<td>0.053</td>
<td>0.053</td>
<td>0.053</td>
<td>0.053</td>
<td>99.61</td>
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<td>SD for injectables</td>
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<td>0.053</td>
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<td>0.053</td>
<td>99.61</td>
</tr>
</tbody>
</table>

\*According to TF 1974

\*Average of six assays

\*Significant differences.

2. The United States Pharmacopoeia
7. Tan, H.S.I., Mc Enaney, S.S., Procaimamide Hydrochloride, Mi
8. Stewart, J.T., Wilkin, R.E.: *De 9-Chloroacridine by Quenching I*
Table II: Statistical comparisons of the proposed and comparison methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Statistical Values</th>
<th>t test of significance</th>
<th>F test of significance</th>
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<tr>
<td>Proposed-DACA</td>
<td>0.913</td>
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<td>(Tablets)</td>
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<td>Proposed-USP XVIII</td>
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* Significant differences.

REFERENCES


