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Stability Indicating Method for the Quantitation of Trazodone Hydrochloride in Tablet Form

Naglaa M. El Kousy
National Organization of Drug Control and Research, Giza, Egypt.

A THIN layer chromatographic separation followed by dye-salt partition technique was employed to estimate the antidepressant drug trazodone hydrochloride in its tablet preparations. The chromatographic separation was carried out on silica gel plates using cyclohexane-benzene-diethylamine (5 : 4 : 1) as a mobile phase. The separated drug is determined colorimetrically using two acid dyes, tropeolin OO and bromocresol green. Chloroform was used as a solvent for the extraction of the formed ionpair. Absorbances were measured at 540 nm and 620 nm after libration of the free dye from the formed dye-salt. Beer's low was obeyed over the concentration ranges of 2-8 ug ml^{-1} and 8-24 ug ml^{-1} respectively.

Trazodone hydrochloride is an antidepressant drug. It was estimated in biological fluids in presence of its metabolite 1(3-chlorophenyl) piperazine by gas liquid chromatographic technique[1]. Several high performance liquid chromatographic methods have been published in the literature using different columns and internal standards[2–6]. Nunez Vergara evaluated trazodone hydrochloride by differential pulse voltammetry using vitreous carbon paste electrode[7]. The drug was also analysed potentiometrically in tablet form using liquid membrane electrode and silver-silver chloride reference electrode[8].

The aim of this work was to employ sensitive and accurate method which indicates the stability of the drug both in pure and tablet forms.

Experimental

Apparatus and Equipments

1. pH meter (Philips)
2. Spectrophotometer (prolabo).

3. Chromatographic glass jar.

4. Silica gel GF_{254} plates.

Materials and Reagents :

1. Standard trazodone hydrochloride and trittico tablets (100 mg) supplied by Eipico.

2. Mcllvaine's buffer solutions pH 3 and 7.

3. A 0.1% aqueous solution of tropeolin OO (prolabo).

4. A 0.05% solution of bromocresol green (Reanal) : 50 mg of bromocresol green was dissolved in 8 ml of 0.05 N sodium hydroxide solution and volume was completed to 100 ml with distilled water.

5. Acidifild methanol solution : 1.85 g sulfuric acid was diluted to 100 ml with methanol.

6. Cyclohexane-benzene-diethylamine : 5 : 4 : 1.

7. Chloroform (Merck).

8. Methanol (Merck).

Preparation of standard solution

An accurately weighed 250 mg of trazodone hydrochloride was transferred to 10 ml volumetric flask and dissolved in 7 ml methanol, volume was completed with the same solvent.

Procedure

Calibration curves

This layer chromatographic separation

To an activated silica gel plate five 50 ul aliquots of the standard solution were applied in the form of band of 5 cm length taking into consideration the complete drying of the solvent after each application. The plate was inserted into the chromatographic chamber containing 100 ml of the mobile phase, cyclohexane-benzene-diethylamine, which was allowed to run for 17 cm from the starting line. The plate was dried and examined under short ultraviolet light at 254 nm. The devlloped band of trazodone hydrochloride (R_f 0.3) was located, scraped into a beaker and shaken with 10 ml methanol for 15 minutes. Filtration was carried out into 25 ml volumetric flask followed by

washing with methanol. Volume was made up using the same solvent and the resulting solution is the working standard solution.

Procedure A

Different aliquots of the working standard solution equivalent to 40-200 ug trazodone hydrochloride were transferred to a series of 125 ml separating funnels containing 3 ml tropeolin 00 solution and 5 ml McIlvaine's buffer solution pH 3. Extraction was carried out with 10, 5 and 5 ml chloroform followed by filtration through a small piece of cotton wool saturated with chloroform into 25 ml volumetric flask containing 3 ml acidified methanol solution. Volume was made up with chloroform and absorbances were measured at 540 nm against blank experiment.

Procedure B

Different aliquots of the working standard solution equivalent to 0.3-1.3 mg trazodone hydrochloride were used applying the same method mentioned under procedure A using 4 ml bromocresol green solution instead of tropeolin 00 solution.

Extraction was carried out with 20, 20 and 10 ml chloroform from which the free dye was liberated by shaking with 20, 20 and 10 ml MCllvaine's buffer solution pH 7. The combined aqueous extracts were collected into 50 ml volumetric flask and volume was made up using the same buffer solution. Absorbances were measured at 620 nm against a blank experiment.

Pharmaceutical Preparations

General procedure for trazodone tablets

Twenty tablets were weighed and finely powdered. A known weight of standard trazodone hydrochloride was added to the powdered tablets. An accurately weighed quantity of the mixed powder equivalent to 250 mg trazodone hydrochloride was transferred to a 10 ml volumetric flask, shaken with 7 ml methanol for 15 min. Volume was made up using the same solvent and filtration was carried out. Five 50 ul aliquots of the filtrate was used for applying the same procedure mentioned under the thin

layer chromatographic separation to obtain the working sample solution. Procedures A and B were carried out using different aliquots of the working sample solution equivalent to from 90-200 ug and from 0.751.2 mg trazodone hydrochloride respectivelv.

To calculate the concentration of a sample its absorbance was compared with that of a standard similarly analysed.

Results and Discussion

In this work a stability indicating method is developed for the estimation of the antidepressant drug trazodone hydrochloride. A thin layer chromatographic separation had been carried out before applying the dye-salt formation method.

F. Angelini laboratories thin layer chromatographic method is used as a limit test for impurities and degradation products of trazodone hydrochloride. The procedure is carried out by spotting reference and sample solutions of trazodone hydrochloride,

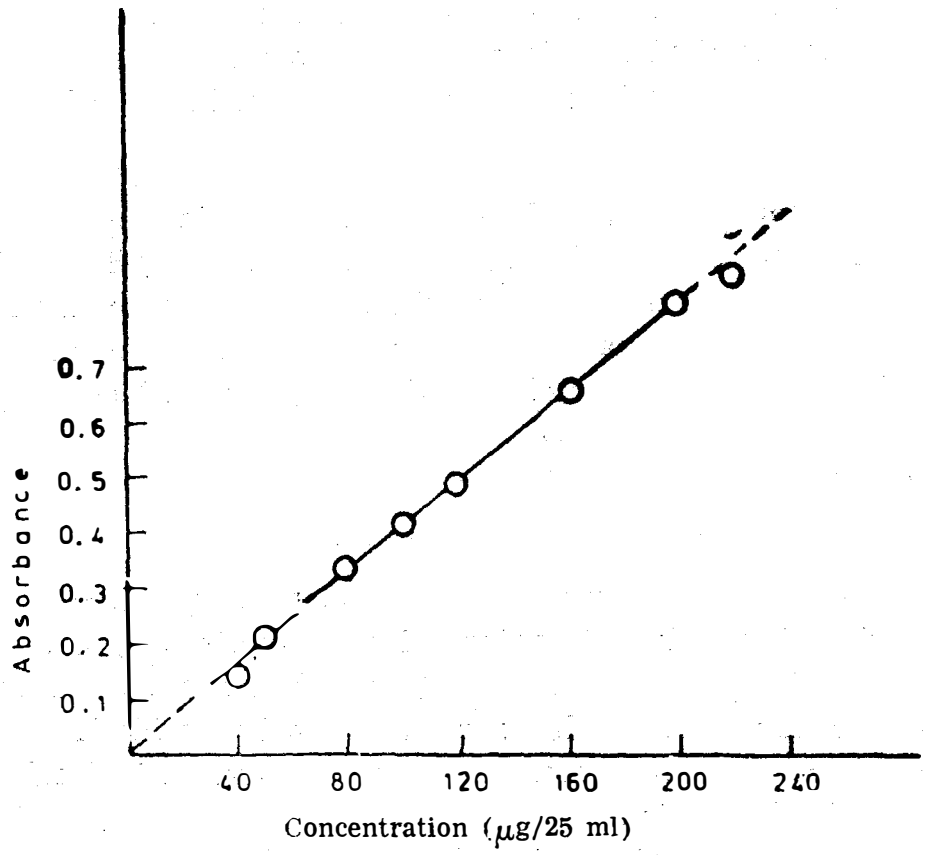

Fig. 1. Calibration curve for the determination of trazodone hydrochloride using tropeolin OO.

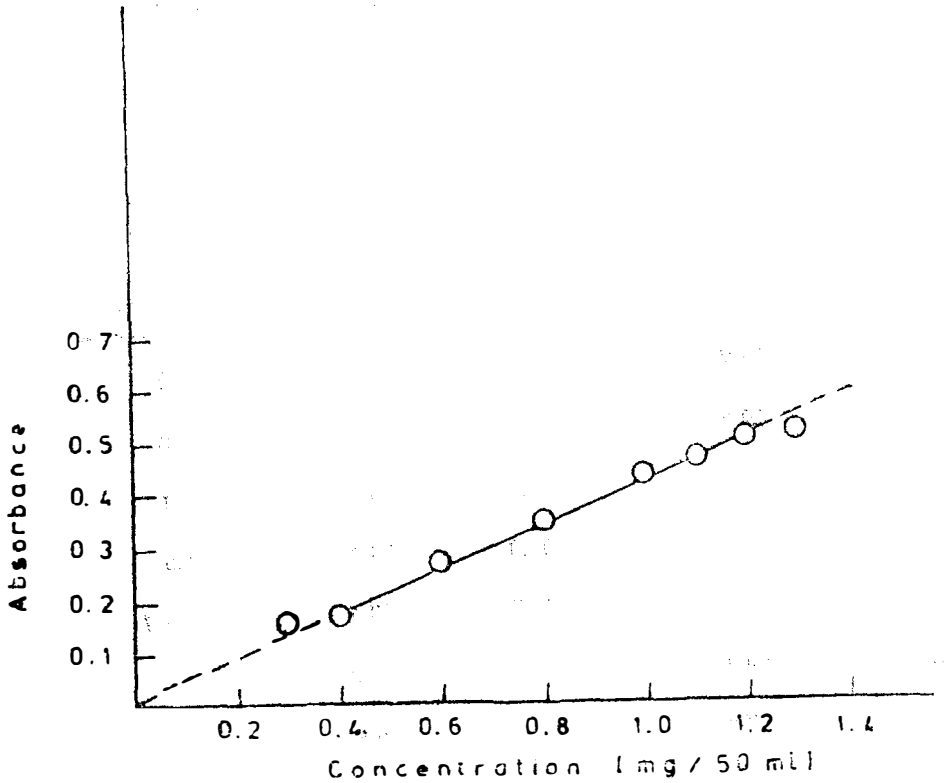

Fig. 2. Calibration curve for the determination of trazodone hydrochloride using bromocresol green.

1-(3-chloropropyl)-4-(3-chlorophenyl)-piperazine hydrochloride solution (AF 2335) and 1,3-bis-(4-(3-chlorophenyl)-1-piperazinyl) propane dihydrochloride solution (AF 1575). The chromatogram is developed using cyclohexane-benzene-diethylamine as mobile phase and the spots relative to the reference, the sample and the impurities are visually compared.

In the present work F. Angelini laboratories method is modified so as to be used for the separation of trazodone hydrochloride from any impurities present and the developed bands of the pure drug were scraped and quantification was carried out applying the acid dye technique. Trazodone hydrochloride shows R_f value of 0.3 while AF 2335 and AF 1575 have R_f values of 0.55 and 0.4 respectively.

A blank experiment was carried out simultaneously showing absorbance value of 0.001 at the wave lengths of maximum ab-

TABLE 1. Analysis of trazodone hydrochloride tablets using the proposed and the reference methods.

Proposed method				F. Angelini lab. method[a]	
Tropeolin OO		Bromocresolgreen			
Added mcg/25ml	Recovery %	Added mg/50ml	Recovery %	used mg/100ml	Recovery %
30	99.7	0.25	98.9	1.0	99.4
50	99.3	0.40	99.6	1.2	99.9
70	99.5	0.50	98.9	1.4	99.3
100	99.0	0.60	99.3	1.5	99.3
120	99.0	0.70	99.4	1.7	99.6
Mean	99.3		99.2		99.6
SD	0.31		0.31		0.25
F ratio[b]	1.48		1.48		
Student t test[c]	1.67		2.22		

(a) Spectrophotometric determination in ethanol, λmax = 250±1 nm.
(b) Theoretical F ratio value is 6. 4.
(c) Theoretical t value is 2.306.

sorption indicating that no interference occurs from the efflorescent indicator impregnated in the silica gel F_{254}.

Isolation and analysis of different dye-salts were carried out by Mukerjee and Coworkers(9) which showed that these are simple salts formed by normal stoichiometric reactions. Continuous variation curves showed relationship of 1 : 1 between trazodone hydrochloride and the acid dyes. The accuracy and sensitivity of the method depend on several factors which were carefully studied. Chloroform was selected as solvent for the extraction of the formed dye-salts and the most significant amount of the dye was found to be 3 mg of tropeolin 00 and 5 mg of bro-

mocresol green. The effect of pH had an important role in the extraction of the dye-salt and the optimum pH was found to be 3 and any increase in the pH leads to decrease in absorbance values.

Under the chosen favourable condition of reaction Beer's law was found to be valid over the concentration range of 2-8 ug ml^{-1} in the tropeolin 00 method and 8-24 ug ml^{-1} in the bromocresol green method (Fig. 1, 2). The validity of the method was tested by applying the standard addition technique of pure trazodone hydrochloride to Trittico tablets. A statistical comparison([10]) between the results of analysis of the proposed and reference methods was carried out using t-test and F ratio and no significant differences were observed between the results (Table 1).

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التقدير الكمى لاقراص الترازادون هيدروكلوريد بطريقة مؤكدة لثبات المستحضر

نجلاء محمود القوصى
الهيئة القومية للرقابة والبحوث الدوائية

تستخدم فى هذه الطريقة كروماتوجرافيا الطبقة الرقيقة على ألواح السليكاجيل لفصل المادة الفعالة فى صورة نقية من أى مواد أخرى قد تكون موجودة كشوائب من عملية التخليق أو من تكسير المادة أثناء تخزينها تقدر مادة الترازادون هيدروكلوريد بعد فصلها عن طريق تكوين أملاح صبغية بأستعمال صبغة التروبولين OO وصبغة البروموكريزل الاخضر . تستخلص الاملاح الصبغية بواسطة الكلوروفورم ثم يعاد فصل الصبغة من أملاحها وقياس اللون الناتج ضوئيا . وكذلك تم تطبيق الطريقة على أقراص تريتكو بنجاح . ولقد تم مقارنة هذه الطريقة بطريقة أخرى مرجعية وثبت مدى حساسيتها ودقتها .