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UTILITY OF 5-DIAZO-1,2,4-TRIAZOL-3-CARBOXYLIC ACID FOR COLORIMETRIC DETERMINATION OF SOME DIBENZAZEPINES

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قدمت طريقة بسيطة ، وسريعة ودقيقة لتعيين أربعة من الداى بنزازيبينات. تعتمد الطريقة على تفاعل ٥-ديازو-١،٢،٤-ترايازول-٣-حمض كربوكسيليك (د ت ك أ) مع الداى بنزازيبينات فى حمض نيتريك ١٠٪. وقد تم قياس النواتج الملونة عند ٤٧٥-٤٩٥ ن.م. وتم تحديد مدى التركيزات التى يمكن تطبيق هذه الطريقة بدقه فيه طبقا لقانون بيير. كذلك عينت الخواص الطيفية للمتراكبات الناتجة. طبقت الطريقة بنجاح لتعيين العقارات المدروسة فى صورها النقية أو مستحضراتها الصيدلية ووجد أنها متوافقة تماما مع الطرق الدستورية.

A simple, rapid, and accurate spectrophotometric method for determination of four dibenzazepines has been developed. The method is based on the interaction of 5-diazo-1,2,4-triazole-3-carboxylic acid (DTCA) with dibenzazepine drugs in 10% nitric acid media. The resulting colored products possess 2 absorption maxima, one in the range of 475-495 nm with higher molar absorptivity and the other in the range of 570-575 nm with lower molar absorptivity. Measurements were performed in the range of 475-495 nm. Beer's law is obeyed in the concentration range of 2-10 μg/ml for both imipramine HCl and desipramine HCl and 2-14 μg/ml for both clomipramine HCl and trimipramine maleate. The molar absorptivities of the studied drugs are 2.877 x 10⁴ for imipramine HCl at 487 nm., 3.164 x 10⁴ for desipramine HCl at 495 nm., 2.002 x 10⁴ for clomipramine HCl at 475 nm. and 2.350 x 10⁴ for trimipramine maleate at 475 nm. The proposed method is applied successfully for the determination of studied drugs in pure form and in commercial and laboratory prepared tablets. The obtained results are in good agreement with those obtained from official methods.

INTRODUCTION

Dibenzazepine drugs are widely used in the treatment of emotional and psychiatric disorders in which the major symptom is depression, particularly endogenous depression.[1] Many methods have been reported for determination of dibenzazepines, these include titrimetry,[2-4] voltametry,[5-7] ultraviolet and visible spectrophotometry,[2,3,8-14] fluorimetry,[15,16] atomic absorption spectroscopy,[17] thin layer chromatography,[18] gas chromatography,[19,20] high-performance liquid chromatography[21-25] and radioimmunoassay.[26] The official methods normally involve a non aqueous or spectrophotometric procedures.[2,3]

Diazotriazole carboxylic acid (DTCA) is used in our laboratory as a chromogenic reagent for many drug classes.[27] The present work involves the use of DTCA for the determination of four dibenzazepines in bulk and in tablets. The proposed method is simple, rapid, accurate and precise.

EXPERIMENTAL

Apparatus

Measurements were performed with a Uvidec 320 spectrophotometer (Jassco, Tokyo, Japan).

Materials and reagents

Pharmaceutical grade imipramine HCl desipramine HCl, clomipramine HCl and

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trimipramine maleate were obtained as gifts from Ciba-Geigy and specia and are used as working standards without further treatments. All other reagents and solvents were of analytical grade. Commercial dosage forms were purchased from local sources.

Preparation of DTCA

DTCA is prepared according to a reported method.[28] After purification a yellowish white crystalline diazonium salt was obtained. It is checked for purity by TLC and spectrophotometric method.[29]

DTCA solution

0.5% w/v in methanol (protected from light). The solution is stable for at least 12 hours.

Nitric acid

10% aqueous solution.

Standard solutions

Into 50 ml volumetric flask dissolve an accurately weighed 25 mg of dibenzazepine salt in 40 ml of water and complete to volume with the same solvent. Prepare the required working standard solutions by diluting the proper amount of stock solution with water to give final concentrations in the range of 20-150 μg/ml of studied dibenzazepine.

Preparation of sample solutions

Weigh accurately and finely powder 20 tablets. Into 50 ml volumetric flask, transfer a quantity of the powdered tablets equivalent to 25 mg of the studied drug quantitatively and dissolve in about 40 ml of water. Sonicate the solution for few minutes to complete dissolution and then dilute to the mark with water. Filter and discard the first portion of the filtrate. Dilute quantitatively an amount of the filtrate with water to obtain the required concentration for determinations.

General procedure

Transfer an accurately measured 1 ml of either standard or sample solution into 10 ml volumetric flask followed by 1 ml of DTCA solution. Mix well and then complete to mark with 10% nitric acid. Measure the absorbance at the given maximum for each drug in table 1 against a blank solution treated similarly using 1 ml distilled water instead of the drug solution.

Table 1: Comparative summary of some statistical data and spectral characteristics of studied dibenzazepines.

Drug	λ_{max} (nm.)	ε_{max} L.mole^{-1}.cm^{-1}	Detection limit μg/ml	Linearity range μg/ml	Intercept	Slope	r*
Imipramine HCl	487	2.877x10^4	0.031	2-10	-0.026	0.096	0.9996
Desipramine HCl	495	3.164x10^4	0.076	2-10	-0.066	0.106	0.9995
Trimipramine maleate	475	2.350x10^4	0.034	2-14	-0.017	0.061	0.9990
Clomipramine HCl	475	2.002x10^4	0.018	2-14	-0.018	0.060	0.9994

* Correlation coefficient.

RESULTS AND DISCUSSION

Absorption spectra

Diazotriazole carboxylic acid (DTCA) reacts with dibenzazepines in an acidic medium to produce highly colored products. Absorption spectra of the reaction products of studied drugs showed two absorption bands one in the range of 475-495 nm with higher absorption intensity and the other in the range of 570-575 nm with lower absorption intensity. The measurements were conducted at the shorter wavelength peaks throughout this work. Spectral characteristics of the studied drugs are summarized in table 1. Figure 1 shows the absorption spectrum of the colored product from imipramine HCl as a representative example of the studied dibenzazepines.

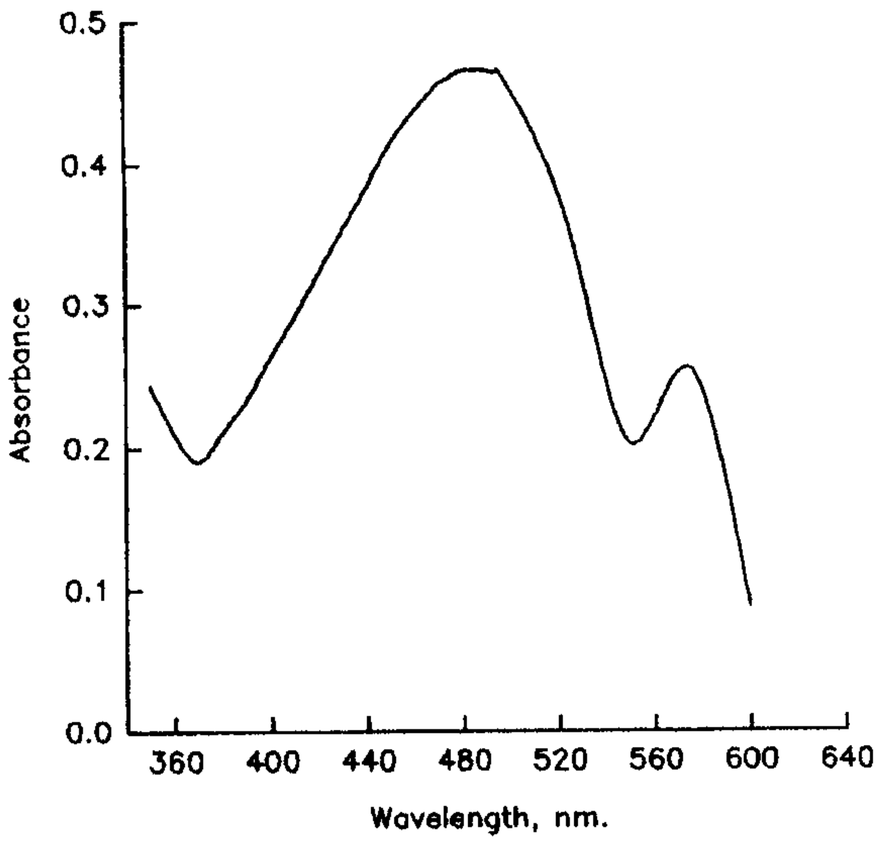


Fig. 1: Absorption spectrum of imipramine HCl (5 μg/ml)-DTCA reaction product.

Effect of reagent concentration

Several solutions of DTCA in concentration range of 0.1-1.0% w/v were prepared and tested with the studied drugs. Maximum color intensity and stability was obtained by using DTCA solutions in concentration ranging from 0.4-0.7%. Therefore, 0.5% DTCA solution was used in all the subsequent work. Figure 2 shows that 1 ml of 0.5% w/v DTCA solution was suitable for determinations.

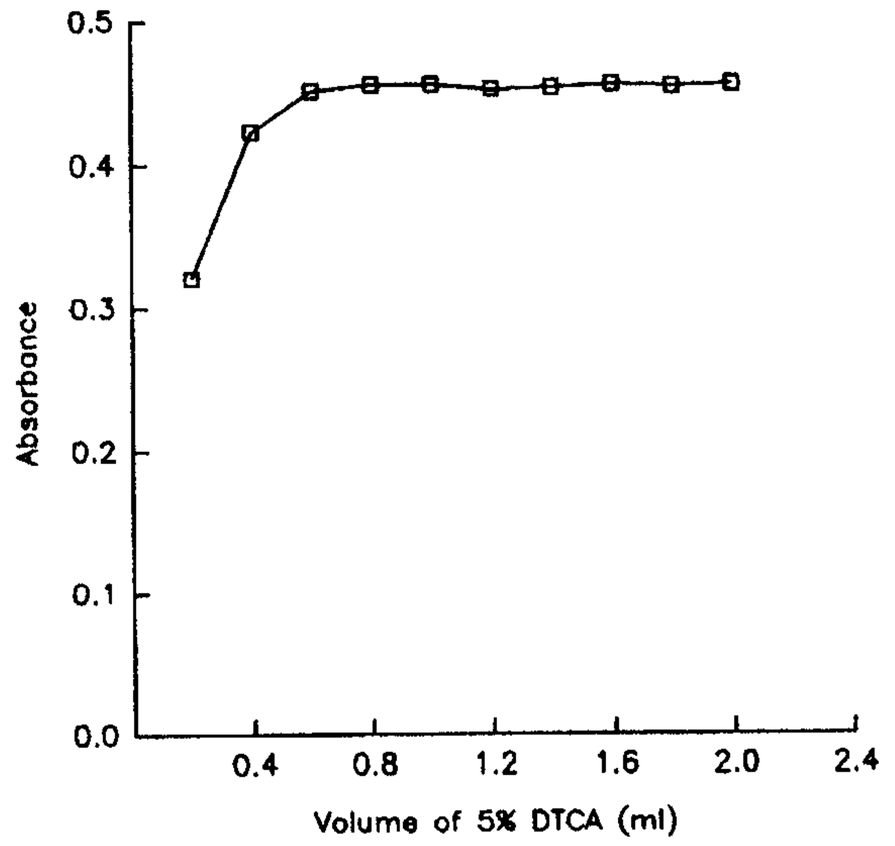


Fig. 2: Effect of DTCA volume (0.5% w/v) on the absorption intensity of colored product from imipramine HCl (5 μg/ml) with diazonium salt.

Effect of type and concentration of acid

In alkaline and neutral media no or very slow development of color was observed. In acid medium the red colored products were developed so rapidly. Some acids in concentration range from 1-10% w/v were tested, namely; acetic, hydrochloric, nitric, phosphoric and sulphuric acid. From which nitric acid produced the most intense and stable colors. Increasing the concentration of nitric acid up to 5% resulted in increasing the intensity of the colored products. Higher concentrations produced virtually the same absorbance readings. Thus 10% nitric acid was preferred and used in the present work.

Effect of diluting solvents

Methanol, ethanol, isopropanol, dimethyl-formamide, dimethyl-sulphoxide and water were tested as diluting solvents. In all cases marked decrease in color intensity and stability was observed. When 10% nitric acid was used instead of solvents for dilution of the reaction mixture more stable and intense colors were obtained.

Reaction and stability times

In all cases, colors developed immediately and attained a maximum within one minute and

then remained stable for at least 40 minutes. In this work absorbance was measured 5 minutes after dilution with nitric acid.

Calibration graphs

Beer's law was obeyed for all compounds at their corresponding maxima. Table 1 shows typical linear regression correlation for all drugs studied. Separate determinations at different concentration levels of each drug gave coefficients of variation not exceeding 1.7%, which is adequate level for quality control analysis of pharmaceutical preparations.

Analysis of pharmaceutical preparations

Commercial tablets containing imipramine HCl and clomipramine HCl and laboratory-prepared tablets of trimipramine maleate were analyzed by both the proposed and official methods. Recovery experiments were carried out for each drug in its respective pharmaceutical preparation. As shown in Table 2, the results are in good agreement with those obtained by applying the official methods. The recovery experiments indicate the absence of interferences from frequently encountered excipients and additives.

Reaction mechanism

The dibenzazepines studied are susceptible to electrophylic attack at the 2 and 8 positions (at which the electron density is highest).[30] In addition, it is reported that dibenzazepines could be oxidized by many oxidants in acid media to form a reactive radical-cation. Fast coupling at the 2- or 8-position was suggested to be the next step of the process.[31] This may reflect the vital role of nitric acid in the reaction. From the other hand, application of Jop's method of continuous variation showed that all studied drugs reacted with DTCA in the molar ratio 1:1. Hence mono-substituted derivatives are the most probable products under the stated conditions. Scheme 1 shows the possible reaction pathway, as predicted from the previous reports.[30,31]

The good stability of the reagent at room temperature, if compared with the other diazonium salts which need specified conditions for preparation and dealing adds the advantage of simplicity to the method. In addition, the method is convenient and could be applied successfully for routine analysis of drugs in pure and in dosage forms.

Table 2: Determination of some dibenzazepines in commercial and laboratory-prepared tablets by the proposed and official methods*.

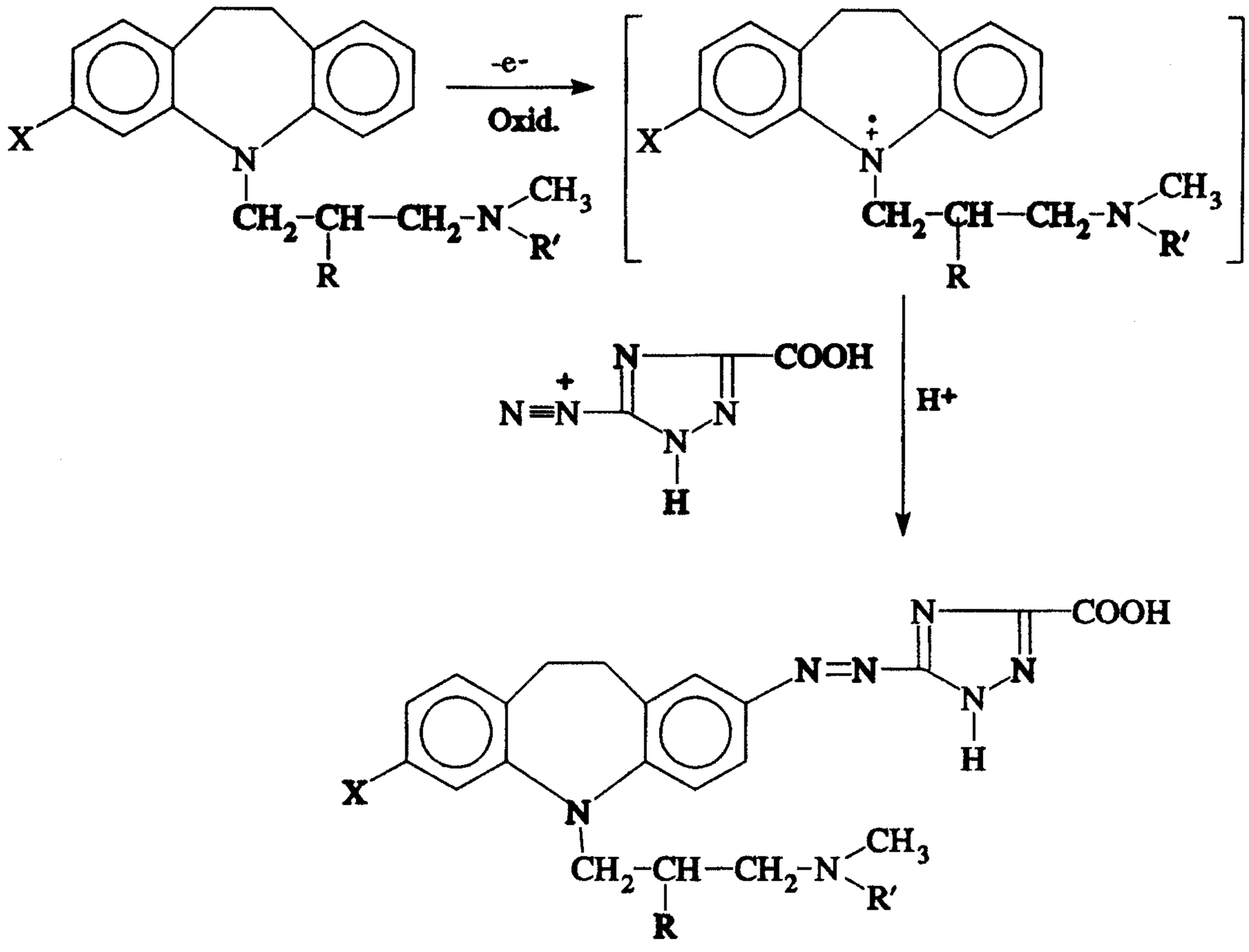
Product	Dibenzazepine	Nominal content mg/tablet	Proposed method			Found by official method+, %
			Found, %	Added mg	Recovery, %	
Tofranil Tablets	Imipramine HCl	25	102.7±1.4 t= 1.75. F= 2.42	25	101.9±1.2	101.4±0.9
Anafranil Tablets	Clomipramine HCl	25	101.8±0.7	25	101.1±0.9	---
Trimipramine Tablets#	Trimipramine maleate 50	50	100.6±0.5 t= 1.04, F= 2.00	50	100.4±0.8	100.2±0.7

* Average ± standard deviation of five determinations. + References 2, 3.

\# Laboratory prepared tablets containing 25 mg starch, 25 mg glucose, 25 mg lactose, 1 mg magnesium stearate, 4 mg gum acacia and 20 mg of talc/tablet as excipients.

Theoretical values of t- and F- at 95% confidence limit are t= 2.306, F= 6.39.

Scheme 1: Proposed mechanism for the reaction between dibenzazepines and DTCA.



R , R'= H, CH_3; X= H, Cl

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