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COLORIMETRIC DETERMINATION OF CERTAIN TERTIARY AMINE DRUGS

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ABSTRACT

A colorimetric method is developed for the determination of four tertiary amine salts; atropine sulphate, carbetapentane citrate, strychnine hydrochloride and climazole hydrochloride, both in pure forms and in pharmaceutical preparations. The method is based on the reaction of trinitrobenzene with ethyl acetoacetate in presence of each of the studied drugs as the free base. The absorption spectra for the developed color exhibit two maxima; one at 460 nm with higher molar absorptivity, and the other at 565 nm with lower molar absorbtivity for all the studied drugs. The color formed obeys Beer's law at either wavelengths over a range of about 2-20 µg of the tertiary amine. Measurements are achived at λmax 460 nm which is the most intense peak. Optimun conditions for the color formation have been studied and the application of this procedure to the corresponding pharmaceutical preparations is given.

INTRODUCTION

Numerous methods have been described for determination of atropine sulphate, carpetapentane citrate, strychnine hydrochloride and climazole hydrochloride. These include non-aqueous titration[1], gravimetric[2], polarographic[3], spectrophotometric[4], colorimetric[5,6], coulometric[7], amperometric[8], and radiochemical techniques[9].

A Meisenheimer-like σ-complex is formed when 1,3,5-trinitrobenzene is allowed to react with an excess of active methylene compounds in alkaline medium[10]. This reaction was applied for the determination of certain basic compounds by using nitromethane as the source of the active methylene group[11]. In this report a new method has been developed for the determination of the aformentioned amine drugs in pure forms and in pharmaceutical preparations. The method is based on the reaction of 1,3,5,-trinitrobenzene with ethyl acetoacetate in the presence of the studied drugs in the form of its base.

EXPERIMENTAL

Equipment

A PM_2DL spectrophotometer (Zeiss, West Germany) was used in this study using 1-cm matched glass cells.

Materials

Pharmaceutical grade tropine base, atropine sulphate, carbetapentane citrate, strychnine hydrochloride and climazole hydrochloride were obtained as gifts from various manufacturers and were utilized as working standards without further treatment.

Formulations

a- Atropine ampoules: Each ampoule contains 1 mg of atropine sulphate.

b- Toclase tablets: each tablet contains 25 mg of carbetapentane hydrochloride.

c- Toclase syrup: each 5 ml contains 7.5 mg of carbetapentane hydrochloride.

d- Strychnine hydrochloride ampoules: each ampoule contains 10 mg of strychnine hydrochloride.

e- Allercur ampoules: each ampoule contains 10 mg of climzole hydrochloride.

Chemicals:

1,3,5-Trinitrobenzene (TNB) (Carlo Erba, Milano, Italy) was recrystallized from ethanol, mp 121-122°C.

Dimethyl sulphoxide (DMSO) was spectrograde (MERCK). Ethyl acetate, acetone, diethyl malonate, and ethyl acetoacetate (EAA) were of analytical grade.

Reagents

1,3,5-Trinitrobenzene (TNB) solution; 0.02% w/v in EAA.

Preparation of Assay solutions

1- For tropine base: 0.001-0.005% w/v in DMSO

2- For amine salts

An accurately weighed amount of the individual amine salts(10 mg) is transferred to a 30 ml separating funnel and dissolved in about 10 ml of distilled water. One milliliter of 1 N NaOH solution is added, the solution is extracted with three 5-ml portions of chloroform. The chloroform extracts are passed through the same 2g of anhydrous sodium sulphate supported by glass wool in a small funnel, then washed with 2 ml of chloroform. The filtrate and washing are collected in a small beaker (30 ml). The chloroform is evaporated to dryness in a stream of air. The residue is dissolved in DMSO and quantitatively transferred to 5 ml volumetric flask and completed to volume with the same solvent. This solution is stepwise and properly diluted by DMSO to give a final concentration of 0.01 mg/ml.

3- For ampoules

The contents of about 10 ampoules were mixed in a small conical flask. The calculated volume equivalent to 5 mg of atropine sulphate or strychnine hydrochloride or climazole hydrochloride was transferred to a 10-ml volumetric flask and completed to volume with distilled water. The procedure is continued as described under amine salts starting with "One milliliter of 1 N NaOH is added, ...) .

4- For tablets

Twenty tablets are weighed and finely powdered. A sample of the powder equivalent to 25 mg of carbetapentane hydrochloride is weighed into a 25-ml volumetric flask, dissolved and completed to volume with distilled water. The solution is filtered and the procedure is continued as described under amine salts starting with "One milliliter of 1 N NaOH is added,.....".

5- For syrup

The calculated volume of syrup (6.7 ml of toclase syrup equivalent to about 10 mg carbetapentane HCl) is pipetted into 10-ml volumetric flask and completed to volume with distilled water. The solution is transferred into 30 ml separating funnel. The procedure is continued as described under amine salts starting with "One milliliter of 1 N NaOH is added,.....".

Development of the color

One milliliter of TNB solution in EAA is pipetted into 2-ml volumetric flask, then 1 ml of the tertiary amine solution in DMSO is added to it and mixed well. The absorbance of the developed color is measured at 460 nm against a blank treated similarly.

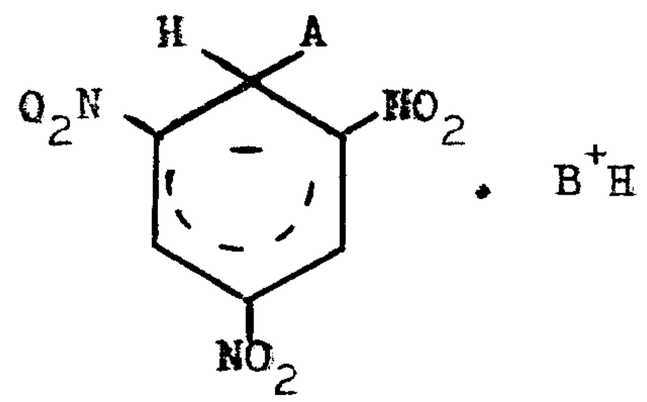
Reported procedure[12]

Pipette 1 or 2 ml (0.1%) ethanolic solution of the standard or sample tertiary amine drug in the form of base or its salt into 25 ml

volumetric flask, evaporate till dryness on a water bath, add to the residue 2 ml of malonic acid/acetic anhydride reagent[X] and place the flask in boiling water bath for 10 minutes. Cool, dilute the reaction mixture to the volume with ethanol. A suitable aliquot is diluted to 10 ml with ethanol to contain in the final solution 1 µg tertiary amine/ml. Measure the absorbance of the final diluted solution at λ max 333 nm against a blank treated concurently.

RESULTS AND DISCUSSION

The method presented here involves the interaction of three systems namely the amine base, active methylene ketoester and TNB. The amine base (the tertiary amine drug in this report(B in I)generates an anion from the ketonic or other active methylene compounds (A in I) which subsequently forms Meisenheimer-like σ-complex with TNB; the species which is measured spectrophotometrically. The reaction occurs in presence of certain solvents. Accordingly, investigations were carried out to determine the optimal reaction conditions. Formula I, represents the proposed reaction product.



I

X 10% solution of malonic acid in acetic anhydride prepared by gentle heating at 60°C with continuous shaking for 5 minutes.

Effect of Solvents

Initial trials have been made by using DMSO as a solvent for the interaction of the amine drug with the TNB-EAA system. It was found that pure 100% DMSO suffers from its high viscosity and high blank, under the proposed experimental conditions. Equally effective interaction was effected by reducing the amount of DMSO by dissolving the amine base in DMSO and dissolving TNB in ethyl acetate, acetone, diethyl malonate or EAA. The use of these mixed solvents served the dual purpose of being a source of the active methylene component as well as diluting DMSO.

Table 1 indicates that the color formed in the presence of all the studied solvents exhibits two absorption maxima; one at 460 nm and the other at 565 nm with different absorption intensities and that EAA is the best solvent. Table 2 shows that maximum absorption intensity was obtained by using a solvent mixture ratio of DMSO:EAA (1:1) which is recommended throughout this work.

Effect of concentration of TNB

The effect of the concentration of TNB on the absorption intensity of the interaction product of each tertiary amine drug in DMSO:EAA (1:1) was studied. It was found that there is an increase in the color intensity with increasing the amount of TNB. However, the concentration of 100 µg was selected because it offers a clear blank and hence a better reproducibility of the results.

Effect of time on the absorption intensity

The interaction between all the studied drugs and TNB in DMSO:EAA (1:1) was affected by time. Maximum absorbance

is observed within 2-3 minutes and remained stable for about 10 minutes. Measurements were performed after 5 minutes for all the studied drugs.

Under the proposed conditions the absorption spectra of the colored products for all studied drugs are shown in Fig. 1. All the spectra are identical, showing the same λ max(s) at 460 and 565 nm but differ in the intensity of absorption. The calculated apparent molar absorptivities at λ max 565 nm were found to be $0.79X10^4$, $0.58X10^4$, $1.7X10^4$, $1.00X10^4$ and $0.78X10^4$ for tropine base, atropine sulphate, carbetapentane citrate, strychnine hydrochloride and clima-zole hydrochloride respectively.

The shorter wavelength peak (λ max 460 nm) is of higher absorption intensity compared to that of the longer one. Thus measurements were conducted at the shorter wavelength throughout this work. Beer's law was obeyed for all the studied tertiary amines at λ max 460 nm. Table 3 illustrates apparent molar absorptivities, limit of detection together with typical linear regression analysis for all the studied tertiary amines. Table 4 illustrates the suitable range which gives the best accuracy for each tertiary amine. Very low absorbances were obtained upon using concentrations less than 3 μg of strychnine hydrochloride and 2 μg of the other amine compounds. Unreproducible absorbances were given upon using concentration higher than those stated in Table 4 for each amine compound.

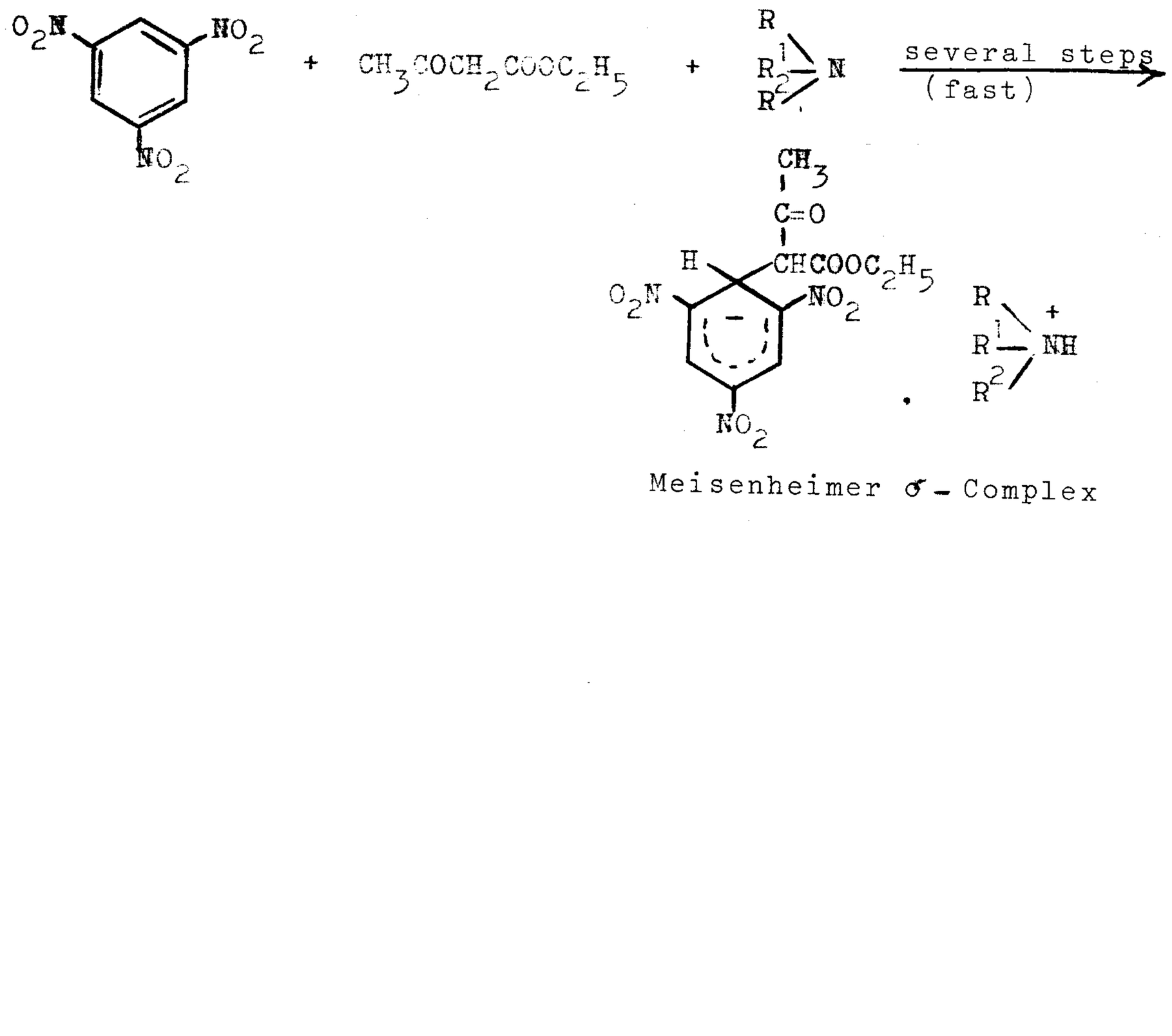
Application to dosage forms

The proposed method is applicable to the analysis of the tertiary amine content of the pharmaceutical formulations listed in Table 5. All the formulations gave percent recovery

ranging between 98.5-102. Table 5 gives also the results obtained by comparing the proposed procedure by a reported method[12]. According to t- and F- tests, there are no significant difference between calculated and theoretical values. This means that the proposed method is as accurate and precise as the reported method (Table 5).

Mechanism of the reaction

The mechanism of the reaction is suggested to be a Meisenheimer-like σ-complex. This suggestion is based on the previously reported mechanism for a similar reaction of dibenzyl ketone (EAA in this report) and TNB in DMSO in the presence of triethylamine[13]. The reaction is illustrated by the following scheme.



Meisenheimer σ - Complex

Table 1. Effect of Different Solvents on the Absorption Intensity of the Interaction Product of Tropine Base and Toclase Citrate With TNB.

	Absorbance[x] in DMSO:Ketone (1:1) at λmax							
	Solvent							
Compound	Ethyl acetate		Acetone		Diethyl malonate		EAA	
	460 nm	570nm	455nm	570nm	460nm	565nm	460nm	565nm
Tropine base[a]	0.046	0.026	0.316	0.125	0.165	0.076	0.560	0.298
Carbetapentane citrate	-----	-----	0.016	-----	-----	0.040	0.505	0.255

x Average of 3 experiments
a Final concentration 5 µg/ml

Table 2. Effect of DMSO:EAA Ratio on the Absorption Intensity of the Interaction Product of Some Tertiary Amine Compounds with TNB[x]

	Absorbance[a] in DMSO:EAA ratio at λmax					
	Ratio	1:3	3:1		1:1	
Compound[b]	460nm	570nm	465nm	570nm	460nm	565nm
Tropine base	0.350	0.180	0.480	0.250	0.560	0.278
Carbetapentane citrate	0.420	0.204	0.320	0.160	0.505	0.255
Strychnine HCl	0.276	0.124	0.237	0.117	0.320	0.194

a Average of 3 experiments
b Final concentration 5 µg/ml
x 100 µg/ml

Table 3. Comparative Summary of Statistical Data at λmax 460 nm

Tertiary amine compound	Linear calibration range µg/ml	Limit of detection, ε max µg/ml	Intercept	Slope	Correlation coefficient
Tropine base	2 - 8	1.0	$1.58X10^4$	0.0055	0.0355	0.9989
Atropine sulphate	2 - 16	1.0	$2.82X10^4$	0.0030	0.0955	0.9978
Carbetapentane citrate	2 - 8	1.5	$3.38X10^4$	0.0542	0.1006	0.8956
Strychnine HCl	3 - 12	210	$1.94X10^4$	0.0350	0.0735	0.9999
Climazole HCl	2 - 20	1.5	$1.89X10^4$	0.0107	0.0328	0.9999

Table 4. Determination of some Tertiary Amine Compounds by the Suggested Method (λ max 460 nm).

Amount taken µg/ml[x]	Recovery % Tropin base	Carbetapentane citrate	Strychnine HCl	Atropine sulphate	Climazole HCl
2	98.34	97.43	----	100.00	100.91
3	99.21	98.94	98.98	-----	----
4	100.21	99.83	101.90	98.83	98.61
5	99.92	98.90	100.23	-----	------
6	99.73	98.21	100.00	99.32	99.42
7	99.89	97.34	99.20	-----	-----
8	98.99	96.62	100.10	100.21	100.31
9	----	----	99.98	-----	----
10	----	----	99.24	100.19	100.99
11	----	----	101.20	----	----
12	----	----	98.72	99.03	101.12
13	-----	----	-----	-----	----
14	-----	----	----	101.10	100.20
15	----	----	----	----	----
16	----	----	----	99.40	100.93
17	----	----	----	----	----
18	----	----	----	----	99.32
19	----	----	----	----	----
20	----	----	----	----	98.20
Mean recovery ± fiducial limits% (P=0.05)	98.32±0.66%	98.22±1.05%	99.96±96.72%	99.76±0.72%	100.00±0.75

x In the final measured solution

Table 5. Determination of some Tertiary Amine Drugs in Commercial Formulations by the Proposed Method and a Reported Method[12].

Formulation[+]	Claimed	Found[x]		Added	Recovery[x]		Reported method Found%[x]
	mg	mg	%±SD	mg	mg	%±SD	
Atropine ampoules	1/ml	1.02	101.80±0.55 t=2.22,F=1.71	1/ml	1.02	101.20±0.62	101.30±0.42
Toclase tablets	25/tab	25.03	99.98±0.75 t=1.14,F=3.89	25/tab	24.80	99.80±0.32	100.33±0.38
Toclase Syrup	7.5/3ml	7.40	100.10±0.53	7.5/5ml	7.43	100.06±0.39	----------
Strychnine hydrochloride ampoules	4/ml	3.95	98.99±0.99 t=2.30,F=3.12	4/ml	3.94	99.67±0.53	99.92±0.56
Allercur ampoules	10/ml	10.28	101.50±0.68 t=0.85,F=2.44	10/ml	10.20	101.22±0.42	101.20±0.55

+ Details of composition, cf. experimental.
x Average of 6 experiments.
Theoretical value: t=2.57 at the 95% confidence level
Theoretical value: F=5.05 at the 95% confidence level

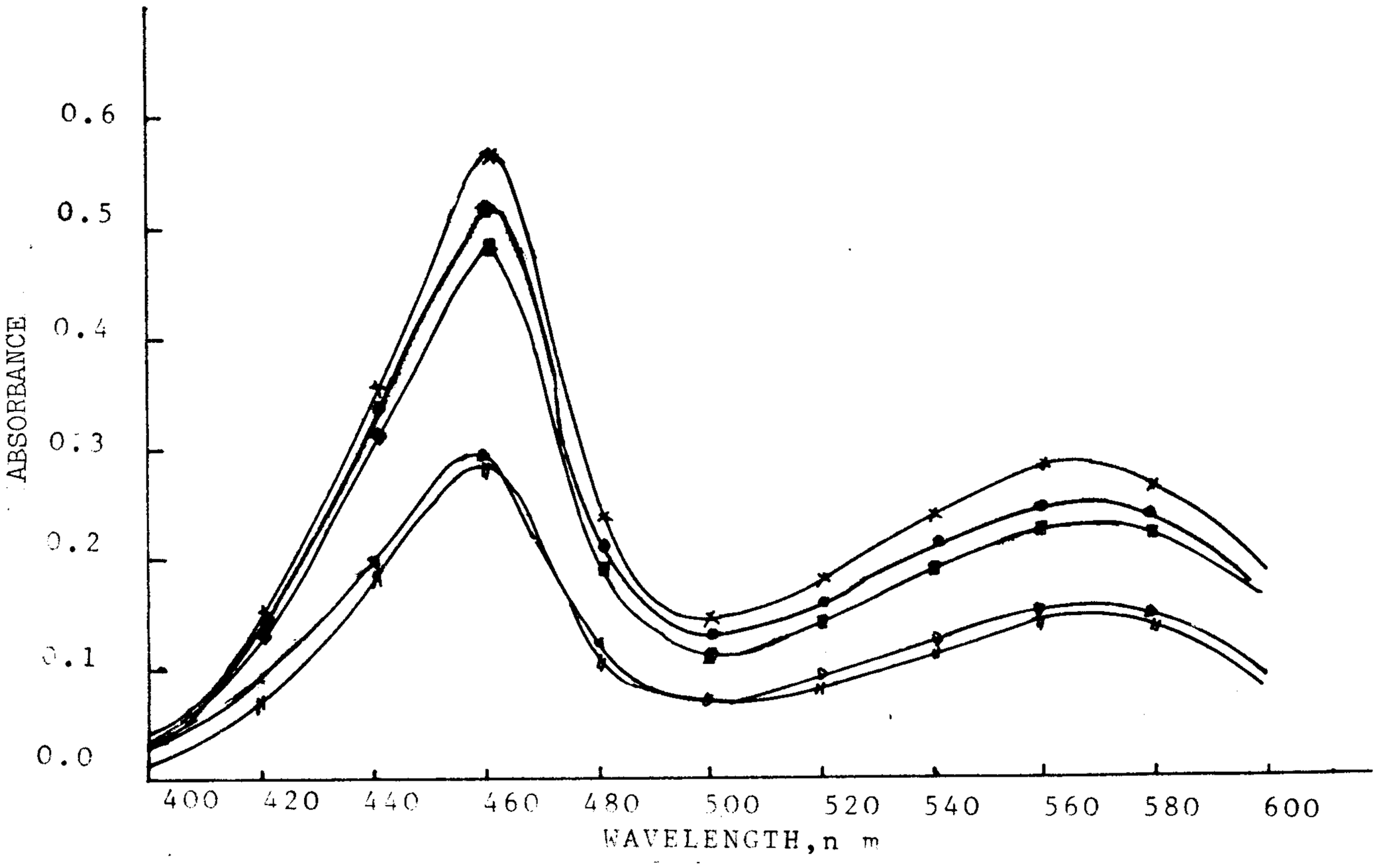


Fig.1-Absorption Spectra of The Interaction Product of Tertiary Amine Compounds with TNB -EAA.

Key: (x—x—x)tropine base,(o—o—o)toclase citrate, (■—■—■)atropine sulphate,(▶——▶)climazole.HCl and(//——//)strychnine.HCl.

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طريقة لونية لتحليل بعض العقاير الثلاثية الامين

سلوى رزق الشابورى ـ سميحة عبد الرحمن حسين ـ على محموط طه ـ كاملة عمارة

قسم الكيمياء الصيدلية ـ كلية الصيدلة ـ جامعة اسيوط

فى هذا البحث تم استحداث طريقة جديدة لتحليل اربعة عقاقير ثلاثية الامين وهى : كبريتات الاتروبين ، وسيترات الكاربيتابنتان وهيدروكلوريد الاستركينين وهيدروكلوريد الكليمازول فى حالتهم النقية وفى المستحضرات الطبية .

وتعتمد الطريقة على تفاعل ثلاثى النيتروبنزين مع اثيل اسيتواسيتات فى وجود كل عقار فى حالته القاعدية . وقد وجد ان المنحنى الطيفى للون الناتج لجميع العقاقير التى درست له درجتى امتصاص قويتين ، واحدة عند ٤٦٠ نم مع درجة امتصاص جزيئى عالية ، والاخرى عند ٥٦٥ نم مع درجة امتصاص جزيئى منخفضة .

وقد وجد ان اللون الناتج يتبع قانون بيير فى حدود ٢ـ٢٠ ميكروجرام من الامين الثلاثى عند كلا ذروتى الامتصاص ـ وقد تمت القياسات عند الموجة التى طولها ٤٦٠ نم حيث انها تعطى درجة امتصاص اقوى .

وقد تمت دراسة افضل الطرق المناسبة للحصول على اللون وتطبيق الطريقة المقترحة لتحليل بعض المركبات الصيدلية التى تحتوى هذه الامينات الثلاثية .

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