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SENSITIVE AND SPECIFIC ASSAY OF TERTIARY AMINE DRUGS WITH PHTHALIC ANHYDRIDE

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The reaction of tertiary amines with phthalic anhydride/acetic anhydride mixture was studied and developed into a sensitive assay for the amines. The method is based on the condensation of the two anhydrides under the catalytic effect of the tertiary amine function, either free or in the form of a salt. The condensation product had a λ_{max} at 302 nm for all the drugs tested with apparent molar absorptivitis in the range 5.79 x 10^4 - 1.08 x 10^5. Absorption versus concentration was found linear up to about 6 mcg/ml for all drugs studied. The method has been applied for the analysis of certain tertiary amine drugs belonging to different pharmacological groups e.g. pilocarpine nitrate , butethamat citrate ,atropine sulphate , bietamiverine dihydrochloride, codeine phosphate and toclase citrate in pure form as well as in pharmaceutical preparations without prior separation and with good accuracy (100 38%) , recoveries and precision (SD ± 0.41).

Several methods are available in the literature for the analysis of amines e.g. potentiometric[1,2], chromatographic[3,4], volumetric[5], high frequency titration[6] , complexometric[77], colorimetric [8, 9] , and spectrophotometric [10 , 11] , methods. However specific methods for tertiary amines are fewer. Thus Feigl[12] and a number of others[13,14] have reported that the reaction between a mixture of acetic anhydride with malonic acid or citric acid and tertiary amines or their chloride salts, yields a highly coloured product suitable for the use as a spot test for tertiary amines. Pesez and Barots [15] developed the aconitic acid/acetic anhydride system for the quantitative analysis of tertiary amines.

In the present investigation. it has been found that phthalic anhydride/acetic anhydride mixture condenses with tertiary amines or their salts to give a colourless product but with high absorbance in the *uv* range. The experimental variables of the assay have been optimized to yield a simple, rapid, sensitive and specific spectrophotometric procedure for the determination of tertiary amines in pure form and in pharmaceutical preparations . The method is particulrly suitable to assay amines of originally low ultravioelet absorption such as pilocarpine , atropine sulphate , bietamiverine dihydrochloride, butethamat citrate, hexahydroadephenine hydrochloride, toclase citrate, codeine phosphate and similar compounds .

EXPERIMENTAL

Materials :

(a) Pharmaceutical compounds : Pharmaceutical grade pilocarpine nitrate, atropine sulphate, bietamiverine dihydrochloride , butethamat citrate, hexahydroadephenine hydrochloride, toclase citrate and codeine phosphate were obtained as gifts from various manufacturers and were utilized as working standards without further treatment.

(b) Formulations : The following commercial formulations were subjected to the analytical procedure .

(1) Toclase tablets (CID Chemical Industries Development , Cairo,Egypt) contain 25 mg toclase citrate per tablet.

(2) Priscophen tablets : (CIBA, Cairo, Egypt). Each tablet contains 2-benzylimidazoline hydrochloride (tolazoline hydrochloride) 0.025 g , nexahydroadiphenylacetyl-aminoethanol ester hydrochloride 0.01g and phenylethyl-barbituric acid (phenobarbitone) 0.02 g

(3) Atropine ampoules : (MISR Pharmaceutical Industries) : Each ampoule contains 1 mg atropine sulphate per 1 ml.

(4) Codacetine tablets : (Kahira Pharmaceutical and Chemical Industries) ; Each tablet contains salicylamide 0.3 g , p-acetamol 0.25 g , and codeine phosphate 0.01 g.

(5) Pilocarpine eye drops ; (Alexanderia Pharmaceutical & Chemical Industries) : Pilocarpine nitrate 3% in an isotonic solution .

Reagents and Chemicals :

(a) Chemicals : All chemicals and reagents used were analytical grade.

(b) Phthalic anhydride/acetic anhydride reagent : 5% solution of phthalic anhydride in acetic anhydride was prepared by heating at 100°C for 2 minutes. The reagent is stable for at least 5 hours .

Equipment :

Spectrophotometer, Spectromom 203 (MOM, Budapestm Hungary).

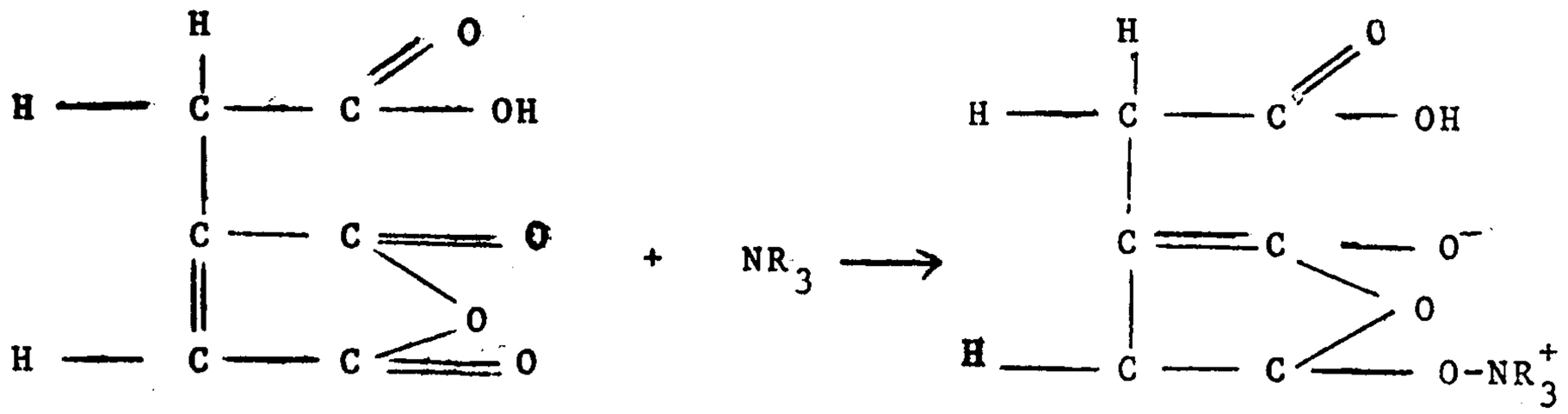
General procedure applicable to liquid preparations :

Pipet 1 ml (0.1%) ethanolic solution of the tertiary amine drug in the form of base or their salts into 25 ml volumetric flask, evaporate till dryness on a water bath. Add to the residue 2 ml phthalic anhydride/acetic anhydride reagent and place the flask in boiling water bath for 20 minutes. Cool, dilute the reaction mixture to volume with ethanol. A suitable aliquot is diluted to 25 ml with ethanol to contain in the final solution 1-6 mcg/ml of the amine. Measure absorbance of the final diluted solution at 302 nm against a blank, treated concurrently.

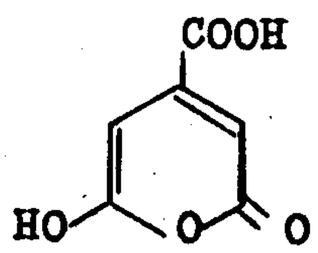
(a) Tablets : Weigh and powder 20 tablets. Extract an accurately weighed amount of the powder equivalent to 50 mg of the tertiary amine drug with three 10-ml portions of ethanol . Combine the extracts in 50 ml volumetric flask, dilute to volume with ethanol. Continue as in general procedure.

RESULTS AND DISCUSSION

The reaction of aliphatic organic acids with tertiary amines was applied previously as qualitative test for the amine[16]. The reaction has been applied afterwards for quantitative estimation of tertiary amines either by spectrophotometry or fluorometry[17-19]. The mechanism of the reaction is not precisely established. However, Sass et al[18] explain the reaction of aconitic anhydride with tertiary amines, amine salts or quaternized amines as the formation of internal salts of aconitic anhydride as shown below.



Groth and Dahlen[20] refer the colour or flurescence produced from the reaction of aconitic acid with tertiary amines to the action of acetic anhydride on aconitic acid to give α,γ - anhydroaconitic acid:



In the present work the phthalic anhydride/acetic anhydride is used for the first time to react with tertairy amines or amine salts, in presence of primary, secondary and quaternary ammonium salts without any interference , to give a condensation product with higher molar absorptivity compared to the original lower molar absorptivity for each drug, Table I.

The absorption spectrum for the condensation product of the studied tertiary amines with phthalic anhydride/acetic anhydride reagent is shown in Fig. 1. All curves are nearly identical with the same λ_{max} at 392 nm but differ in the intensity of the absorption, Table I. Beer's law was obeyed for all drugs in the range of 1-6 mcg/ml .

The condensation product resulting from interaction between the tertiary amine and phthlic anhydride/acetic anhydride seems to involve acetic anhydride as active ingredient, since no condensation occured when the tertiary amine was reacted with phthalic anhydride alone. This finding might recall a Perkin type condensation[21-23]. This explanation which seems to have escaped the attention of early investigatores, was supported by running the condensation using sodium acetate as the base catalyst as in the original Perkin reaction[21], whereby an identical

λ_{max} for the product was obtained .

Investigations were carried out to study different veriables to determine the optimal reaction conditions for the formation of the condensation product of the mixed anhydride system (phthalic anhydride/acetic anhydride) with certain tertiary amines and amine salts.

Effect of heating time : It was found that heating in a boiling water bath for 20 minutes gives a condensation reaction with stable λ_{max}. Further heating gives no change of the intensity of absorbance, Table II. Heating at lower temperature required a long time more than one hour to complete the condensation reaction.

Effect of phthalic anhydride concentration : Table III indicates that the absorbance of toclase citrate or hexahydroadephenine HCl as representative example for the tertiary amine studied, is affected by the concentration of phthalic anhydride or phthalic acid in acetic anhydride. The intensity of absorbance is gradually increased till the concentration of either phthalic anhydride or phthalic acid reaches 4% and then remains constant up to 10%. A 5% concentration is chosen as a suitable concentration for phthalic anhydride or phthalic acid in this procedure .

Effect of dilution by different solvents: : The dilution of the condensation product by different solvents shows no effect on the position of the λ_{max}, while the intensity of absorbance is affected. Ethanol gives high absorbance and so it was chosen as the most suitable solvent for dilution as shown in Table 4.

Stability of the condensation product : The concentrated condensation products (ca. 40 mcg/ml) of the tested tertiary amines are stable for at least 3 hours. Dilute solutions (5 mcg/ml) show a very slight decrease in absorbance after 20 or 30 minutes (Table V).

Interferences and analysis of pharmaceutical preparations: Primary, secondary and quaternized amine do not interfere in the determination of these drugs, However, other teriary amines

will interfere with them and these required prior separation from each other.

Application of this procedure to the analysis of the pharmaceutical preparations present in the market gives a good result as shown in Table VI. Recovery experiments indicate that there is no interference from the frequently encountered excipients or additives.

Table I : Absorption Characteristics of Some Teriary Amine Drugs

Teriary amine drug	*Proposed method at* λ_{max} 302	ξ	*solvent*	*Reported method, UV* λ_{max}	ξ
Toclase citrate	1.08×10^5		methanol	255	421
Hexahydroadephenine HCl	5.79×10^4		0.1N H_2SO_4	248	428
Butethamat citrate	8.01×10^4		0.1N H_2SO_4	247	182
Bietamiverine dihydrochloride	6.76×10^4		ethanol	259	888
Pilocarpine nitrate	6.05×10^4		0.1N H_2SO_4	255	5954
Atropine sulphate	1.04×10^5		0.1N HCl	252	478
Codeine phosphate	7.06×10^4		0.1N HCl	211	2160

Table II: Effect Of Heating Time

Reaction time, minutes	*Absorbance*+	
	Toclase citrate	*Hexahydroadephenine HCl* ×
5	0.635	0.51
10	0.795	0.638
15	0.823	0.654
20	0.826	0.654
25	0.826	0.654
30	0.826	0.654
60	0.826	0.654

+ Final concentration 4 mcg/ml .

× Average of 6 determinations .

Table III: Effect of Phthalic Anhydride Concentration

Phthalic Anhydride in Acetic anhydrice , g%	*Absorbance*[+] *Toclase citrate*[x]	*Hexahydroadephenine HCl*[x]
1	0.440	0.395
2	0.710	0.590
3	0.820	0.648
4	0.825	0.654
5	0.825	0.654
7	0.825	0.654
10	0.826	0.654
100	no reaction	no reaction

[+] Final Concentration 4 mcg/ml

[x] Average of 6 determination.

Table IV: Effect of Solvents

Solvent	*Absorbance*[+] *Toclase citrate*[x]	*Hexahydroadephenine HCl*[x]
Ethanol	0.825	0.655
Methanol	0.805	0.638
Isopropanol	0.795	0.627
Dioxane	0.780	0.621

[+] Final Concentration: 4 mcg/ml

[x] Average of 8 determinations.

Table V : Effect of Time on the Stability of Chromogen

Time (minutes)	*Toclase citrate*[+]		*Hexahydroadephenine HCl*[+]	
	Absorbance[x]	*Decrease %*	*Absorbance*[x]	*Decrease %*
Immediately	0.825	0.00	0.655	0.00
5	0.825	0.00	0.655	0.00
10	0.824	0.10	0.624	0.10
15	0.824	0.10	0.654	0.10
20	0.820	0.60	0.651	0.10
30	0.820	0.60	0.651	0.61
40	0.817	0.98	0.648	1.0
60	0.812	0.60	0.642	1.9
overnight	0.562	33.00	0.460	30.00

[+] Final concentration 4 mcg/ml

[x] Average of 4 determinations .

Table VI: Analysis Of Formulations

Formulation[x]	*Claimed*	*Found*[+]		*Added*	*Recovered*[+]	
	mg	*mg*	*%*	*mg*	*mg*	*%*
Toclase citrate	25	24.53	98.12 (SD=1.7)	25	24.85	99.4 (SD=1.47)
Prisccphen Tablets	10	9.90	99.00 (SD=1.73)	10	9.94	99.4
Atropine sulphate ampoules	1	0.985	98.50 (SD=0.675)	1	0.995	99.50 (SD=0.66)
Pilocarpine nitrate eye drops	30/ml	29.32	97.70 (SD=1.1)	30	29.40	98.00 (SD=1.2)
Codoine phosphate tablets	10/tab	9.45	94.5% (SD=1.72)	10	9.785	97.85 (SD=1.225)

[+] Average of 8 deter inations

[x] Detailed composition under experimental.

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طريقة حساسة ودقيقة لتحليل الامينات الثلاثية بواسطة انهيدريد الفساليك

على محمود طه ، سلوى رزق الشابورى ، عبدالمعبود اسماعيل راجح

قسم الكيمياء الصيدلية ـ كلية الصيدلة ـ جامعة اسيوط

فى هذا البحث تم دراسة واستحداث طريقة جديدة وحساسة لتفاعل الامينات الثلاثية مع مزيج من أندريد الفساليك وأندريد حمض الخليك . وهذه الطريقة تعتمد على تكثيف أندريد الفساليك وأندريد حمض الخليك تحت تاثير منشط من تاثير مجموعة الامينات الثلاثية أما فى حالتها الحرة أو فى حالتها على هيئة ملح .

وقد وجد أن نتيجة هذا التكثيف نشوء مركب جديد له درجة امتصاص قصوى عند ٣٠٢ ن . م . لجميع الأدوية المدروسة ودرجة امتصاص جزئى تتراوح ما بين ٥ر٧٩ × ١٠ ـ ١ر٠٨ × ١٠^{٥} .

ووجد أن العلاقة بين درجة التركيز والامتصاص تكون خط مستقيم ص ٦ ميكروجرام لكل ملليمتر لجميع الأدوية التى درست .

وقد طبقت هذه الطريقة لتحليل بعض الادوية التى تحتوى على الامينات الثلاثية والتى تنتمى الى مجموعات فارماكولوجية مختلفة ومن هذه الادوية على سبيل المثال نترات البيلوكاروبين ، سلفات الاتروبين ، ثنائى كلوريد البيتاميفيرين ، سترات البثتامات ، كلوريد الهيكساهيدروافينين ، فوسفات الكودايين وسترات التركلازين فى حالتها النقية وفى المستحضرات الطبية بدون فصل سبق مع دقة فائقة (كيفية الارتجاع ٣٨ر١٠٠٪) وانحراف (حيود قياسى ± ٤١ر٠)