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SPECTROPHOTOMETRIC DETERMINATION OF DAPSONE AND TRANEXAMIC ACID USING HANTZSCH REACTION AND ITS APPLICATION IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT:

A simple, rapid, sensitive and specific spectrophotometric method has been developed for the determination of two primary amines of pharmaceutical interest; dapsone and tranexamic acid. The method is based on condensation of the cited drugs with acetylacetone - formaldehyde mixture in acetic acid - sodium acetate buffer media by aid of heating at (97± 0.5°C) for a fixed time to yield a yellow colored condensation product "dihydrolutidine derivative" and possess a characteristic absorption maxima at 330 -345 nm.The variables affecting color development and the stability of the reaction product have been investigated and the conditions optimized. Beer's law is obeyed in the concentration range of 1 - 11 μg ml^{-1} with molar absorpitivities ranging from 1.475×10^4–2.015×10^4 $L.mol^{-1}cm^{-1}$ and Sandell sensitivities of 8.11×10^{-3} – 9.38×10^{-3} μg cm^{-2}. The proposed spectrophotometric procedure was applied successfully for the determination of the cited drugs in some of their pharmaceutical formulations.

INTRODUCTION

Dapsone is the drug of choice against leprosy[1,2]. It has been determined by spectrophotometry,[3-5] fluorometry,[6] HPLC,[7-9] capillary electrophoresis,[10] nuclear magnetic resonance (NMR)[11] and polarography[12].

Tranexamic acid is an antifibrinolytic drug which is used in treatment of haemorrhage and heridatry angioedema[1,2]. Several procedures were reported for its determination e.g. spectrophotometry,[13-16] fluorometry[17] and HPLC[18-20].

The aim of the present work is to investigate the analytical use of "Hantzsch reaction" in the assay of the cited drugs which provides a simple, rapid and sensitive method for their determination in pure forms and in pharmaceutical preparations. This work describes a spectrophotometric method that can be used in laboratories where modern and expensive apparatus such as that required for GLC or HPLC is not available.

EXPERIMENTAL

Apparatus

SHIMADZU UV–260 recording spectrophotometer with matched 10 mm quartz – cells and a Chemocadet pH-meter were used for all absorbance and pH measurements respectively.

Materials and Reagents

All reagents were of analytical grade. Double distilled water was used throughout all absorbance measurements.

1- Dapsone; The Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, A.R.E.

2- Tranexamic acid; Amoun Pharmaceutical Co., S.A.E.,El-Obour city, Cairo, Egypt.

3- Acetylacetone and Formaldehyde(28-32%); El-Nasr Pharmaceutical Chemicals, Egypt.

4- Acetic acid-sodium acetate buffer of pH (2 - 6) was prepared by mixing equal volumes of 0.2 M acetic acid and 0.2 M sodium acetate and adjusting pH to the desired value.

5- Reagent used for quantitative deteminations was prepared by mixing 10 ml of acetic acid - sodium acetate buffer (pH 2 - 6) with 2.1 ml freshly distilled acetylacetone and 5 ml formaldehyde (32%), complete to 25 ml with distilled water with shaking.[21,22] The reagent should be freshly prepared.

Standard drug solutions

Standard stock solutions of 1 mg ml^{-1} were prepared by dissolving the requisite drug amounts in methanol and water for dapsone and tranexamic acid respectively. Working solutions of 100 μg ml^{-1} were prepared by dilutions of standard stock solutions.

Formulations

The following commercial formulations were subjected to the analytical procedures:

1- Dapsone® tablets; The Nile Co. , A.R.E., (50 mg dapsone /tablet).

2- Dapson® tablets, The Arab Drug Co. ADCO., Egypt, (50 mg dapsone / tablet).

3- Kapron® tablets; Amoun pharmaceutical Co., Cairo, Egypt, (500 mg tranexamic acid / tablet).

4- Cyclokapron® ampoules; Pharmacia and Upjohn AB, Sweden, (500 mg tranexamic acid / 5 ml).

General procedure for pure pharmaceuticals

Appropriate volumes of the standard stock solution ranging from 10 - 110 μg ml^{-1} (Table 2) were pipetted into series of test tubes followed by 0.4 – 0.5 ml of the reagent prepared in acetate buffer of pH 3.2 (Table 1). the contents of each tube were mixed well then the tubes were sealed and heated gently in boiling water bath maintained at (97±0.5C°) for the specified time (Table 1). After completion of heat treatment, the solution was cooled to room temperature, transferred carefully and quantitively to 10 ml calibrated flasks, the volume was made up to the mark with distilled water and the absorbance was measured at the specified wavelength (Table 1) against a reagent blank similarly treated . Concentration is then calculated from the corresponding calibration graphs or regression equations.

Procedure for dosage forms

For tablets

Ten tablets were accurately weighed and ground to fine powder, an amount equivalent to 100 mg of dapsone was extracted with 3 x 25 ml portions of methanol, the portions were filtered into 100 ml volumetric flasks, the filter was washed with methanol and the solution diluted to the mark with methanol. For kapron® tablets, a similar procedure was followed but water was used instead of methanol. The drug content in the obtained extract was determined as described under the general procedure.

For ampoules

The requisite volume equivalent to 100 mg of the active ingredient was transferred to 100 ml volumetric flasks and diluted to the mark with distilled water. Drug content was determined following the general procedure.

RESULTS AND DISCUSSION

The drugs under study, as primary amines, under go Hantzsch condensation reaction with acetylacetone – formaldehyde mixture in acetate buffer by aid of heating to form a yellow colored condensation product, thought to be a dihydrolutidine derivative was obtained as shown in Scheme 1[22].

Absorption spectra of the reaction product were measured against a reagent blank in the range of 200 - 400 nm. A characteristic absorption maxima at 330 - 345 nm were observed where the yellow colored condensation product was fully developed, (Table1), (Fig.1). Trials were performed to determine the optimum reaction conditions which were established based on the developement of maximum color intensity with maximum reaction product stability on variation of parameters such as reaction temperature, heating time, pH-values, reagent volume and the diluting solvent.

Samples prepared following the general procedures were subjected to the effect of heat over temperature range of 25 – 100°C, maximum absorbance and sensitivity were obtained by heating the reactants gently in water bath maintained at (97 ± 0.5°C). On the other hand, heating times in boiling water bath lead to variation in absorbance data, Optimum times which yield maximum absorbance and sensitivity were listed in (Table 1). It was observed that at temperatures below 97C° the rate of color development becomes progressively slower and it proceeds very slowly at room temperature (Fig. 2&3).

Acetate buffer of different pH values ranging from 2 - 6 was used to prepare the reagent in order to investigate the maximum color intensity and stability, optimum pH values were listed in (Table 1). Reagents prepared in lower or higher pH values than the recommended ones show deviating results (Fig. 4).

Another study on the volume of the reagent prepared in the recommended pH values was performed using various volumes of the reagent ranging from 0.1 - 3 ml, adequate reagent volumes are listed in (Table 1), (Fig.5). Lower or higher volumes produced a marked decrease in absorbance.

On the other hand different solvents were tested as diluting solvents e.g. water, methanol, ethanol, acetonitrile and acetone. Among the tesed solvents water gave the best results so water was recommended as the diluting solvent throughout the experiments.

The color obtained was found to be stable for about 2 - 3 hours at room temperature (Table 1). The effects of common additives and excepients were studied and it was found that there were no interferences thus the examined drugs can be assayed directly in their dosage forms without prior separation.

Calibration graphs

Calibration graphs were constructed by plotting drug concentration (μg ml^{-1}) against the corresponding absorbance values. The resulting graphs were linear from 2 - 9 μg ml^{-1}dapsone and 1-11 μg ml^{-1} tranexamic acid. The linearity of calibration graphs is apparant from the correlation coefficient, r, and the intercepts which are nearly close to zero. All calibration data are calculated and listed in (Table 2).

Sensitivity, accuracy and precision

The mean Sandell sensitivity for each drug was calculated and its value is given in (Table 2).

The precision and accuracy of the proposed method was tested by means of replicate measurements of the tested drugs within Beer's law limits. The %S.D. and %range of S.E. at 95% confidence limits are given in (Tables 3 and 4).

The utility of this method was verified by means of replicate measurements of pharmaceutical formulations and recovery experiments. Recoveries were determined either by adding standard drug to a preanalysed mixture of pharmaceutical preparation "standard addition method" or by calibration method.

The performance of the proposed method was assessed by calculation of "t" and F values compared with the official and the reported methods. Results showed that no significant differnce in accuracy and precision between both methods (Tables 3 and 4).

CONCLUSION

The proposed method is advantageous when compared to many of the reported spectrophotometric methods in having higher sensitivity. The data given before reveal that the proposed method is simple, accurate and sensitive with a good accuracy and precision. It can be used directly to determine the cited drugs without prior separation as common additives and do not interfere.

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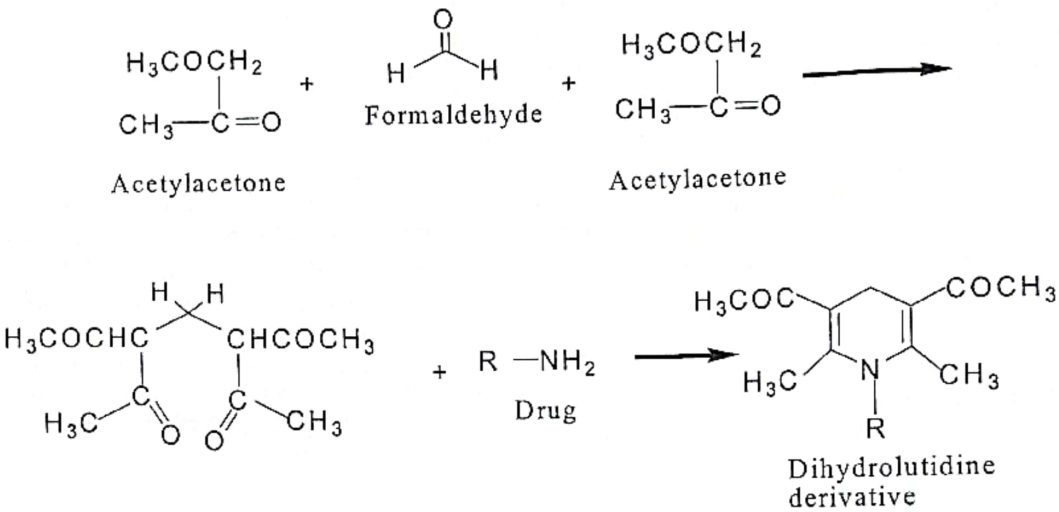

Scheme 1: Formation of chromogen due to reaction of acetylacetone and formaldehyde with the cited drugs (RNH_2)

Table 1: Analytical data for the determination of investigated drugs

Drug	Wavelength (nm)	Heating time (min.)	Volume of reagent	Reagent pH	Color stability (Hr)
1-Dapsone 2-Tranexamic acid	345 330	15 15	0.4 0.5	3.2 3.2	2 3

Table 2: Spectral characteristics for the reaction of cited drugs with acetylacetone – formaldehyde

Drug	Range ($\mu g ml^{-1}$)	Molar absorpitivity ($L.mol^{-1}cm^{-1}$)	Sandell senstivity ($\mu g\ cm^{-2}$)	r	b	a
1. Dapsone 2.Tranexamic acid	2-9 1-11	2.015×10^4 1.475×10^4	0.00811 0.00938	0.99997 0.99999	0.08574 0.0777	-0.0191 0.0516

**Regression equation: $A = a + bc$*

a = intercept b = slope c = concentration ($\mu g\ ml^{-1}$) A = absorbance unit

Table 3: Comparative analytical results of the proposed and the reported methods for the tested drugs in pure forms

Statisical	Dapsone		Tranexamic acid	
	Proposed method	Official method[1]	Proposed method	Official method[1]
Mean % recovery	99.96	100.29	100.04	100.09
N	7	3	7	3
Variance	0.168	0.511	0.229	0.193
S.D.	0.410	0.715	0.479	0.439
S.E.	0.155	0.413	0.181	0.253
"t"	0.748 (2.306)		0.161 (2.306)	
F	3.04 (5.14)		1.187 (5.14)	

N = Number of experiments S.D. = Standard Deviation S.E. = Standard Error t = "t" test of unpaired data F = Variance test

Table 4: Comparative analytical results of the proposed and the reported methods for the tested drugs in pure forms in some pharmaceutical formulations

Formulation& lable claim	Statistical parameters	Proposed method		Reference method
		Calibration method	Standard addition method	
1- Dapsone® tablets (ADCO)	Mean % recovery	98.79	100.57	99.65 [1]
	N	8	6	3
	Variance	0.176	0.111	0.555
	S.D.	0.420	0.333	0.745
	S.E.	0.148	0.136	0.430
	"t"	1.891 (2.262)	2.04 (2.365)	
	F	3.153 (4.74)	5.00 (5.79)	
2- Dapsone® tablets (Nile Co.)	Mean %recovery	98.94	100.12	99.55 [1]
	N	8	6	3
	Variance	0.125	0.179	0.557
	S.D.	0.353	0.423	0.746
	S.E.	0.125	0.173	0.431
	"t"	1.359 (2.262)	1.227 (2.365)	
	F	4.45 (4.74)	3.11 (5.79)	
3- Kapron® tablets	Mean %recovery	98.87	99.51	99.4 [1]
	N	8	7	3
	Variance	0.403	0.503	0.865
	S.D.	0.635	0.709	0.930
	S.E.	0.225	0.268	0.537
	"t"	0.910 (2.262)	0.183 (2.306)	
	F	2.146 (4.74)	1.720 (5.14)	
4- Cycklocapron® ampoules	Mean %recovery	97.96	99.13	98.5 [1]
	N	8	7	3
	Variance	0.576	0.441	0.163
	S.D.	0.759	0.664	0.404
	S.E.	0.268	0.251	0.233
	"t"	1.521 (2.262)	1.840 (2.306)	
	F	3.534 (4.74)	2.706 (5.14)	

N = Number of experiments S.D. = Standard Deviation S.E. = Standard Error t = "t" test of unpaired data F = Variance test

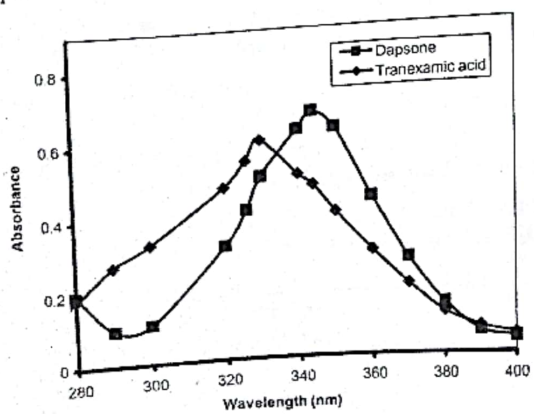

Fig.1: Absorption spectra of reaction products:
1. 8 µg / ml Dapsone. 2. 7µg / ml Tranexamic acid.

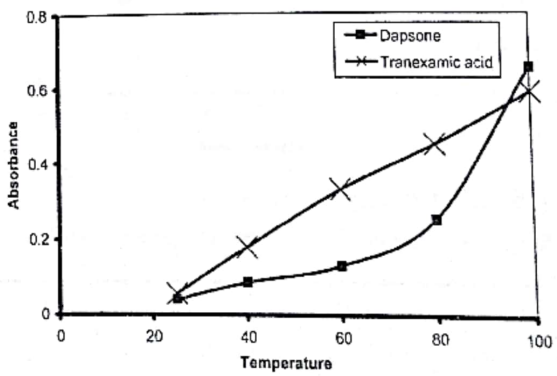

Fig. 2: Effect of the reaction temperature:
1. 8 µg / ml Dapsone + 0.5 ml reagent (pH 3.2) for 15 minutes.
2. 7 µg / ml Tranexamic acid + 0.5 ml reagent (pH 3.2) for 15 minutes.

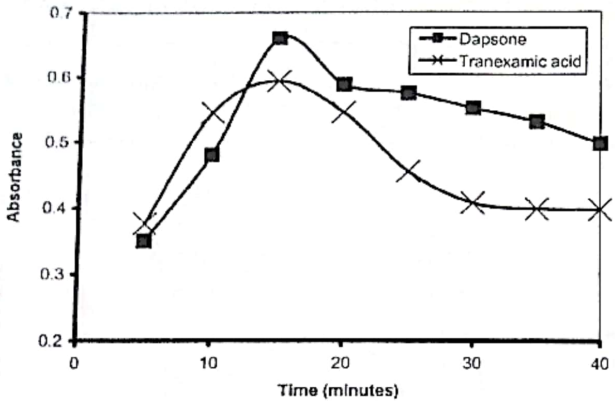

Fig. 3: Effect of reaction time:

1. 8 µg / ml Dapsone + 0.5 ml reagent (pH 3.2).
2. 7 µg / ml Tranexamic acid + 0.5 ml reagent (pH 3.2).

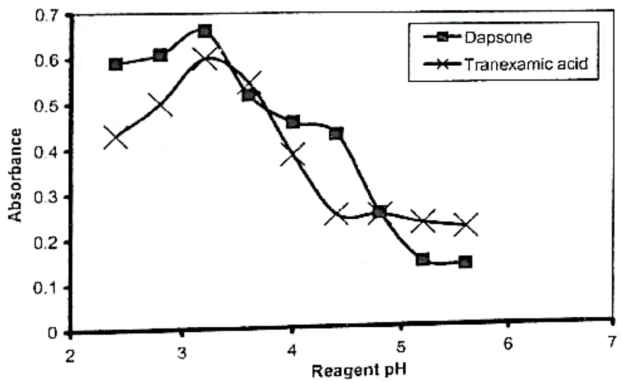

Fig. 4: Effect of reaction pH:

1. 8 µg / ml Dapsone + 0.5 ml reagent for 15 minutes.
2. 7 µg / ml Tranexamic acid + 0.5 ml reagent for 15 minutes.

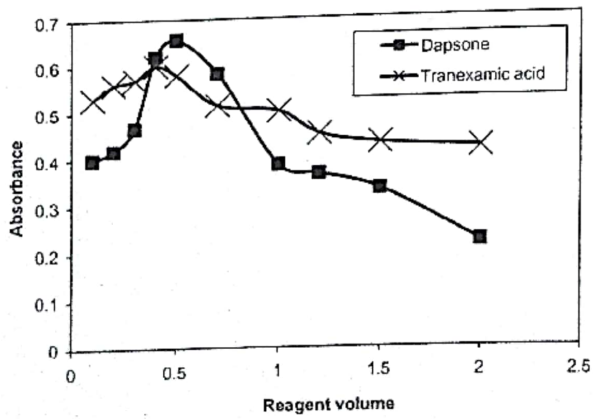

Fig. 5: Effect of reagent volume:

1. 8 µg / ml Dapsone 2. 7 µg / ml Tranexamic acid.

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طريقة طيفية لتعيين الدابسون و حامض الترانسكميك بطريقة تفاعل هانش

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في هذا البحث تم استحداث طريقة طيفية عن طريق:

تفاعل خليط من الاسيتيل اسيتون والفور مالدهيد مع مركبات الدابسون , حامض الترانكسميك عند درجة حامضية ٣,٢ وبمساعدة السخين في حمام مائي . وقد تم قياس اللون الناتج عند أطوال موجية تتراوح ما بين ٣٣٠– ٣٤٥ نانو متر وقد تمت دراسة بعض العوامل المؤثرة على التفاعل مثل : درجة الحرارة والوقت ودرجة الحامضية وحجم المحلول المستخدم

هذا وقد كان اللون الناتج ثابتاً لمدة لا تقل عن ساعتين في درجة حرارة الغرفة وقد وجد أن هناك علاقة طردية بين الامتصاص والتركيز عند قيم تتراوح ما بين ٢–٩ , ١ – ١١ ميكرو غرام / مل لمركبات الدابسون , حامض الترانكسميك على الترتيب وتتراوح معامل الامتصاص المولاري ما بين ١,٤٧٥٦ * ١٠^{٣} –١,٤٧٥٦ * ١٠^{٣} لتر . مول$^{-١}$ سم$^{-١}$ ومعامل ساندل ما بين ٠,٠٠٨١١ – ٠,٠٠٩٣٨ ميكرو غرام سم$^{-٢}$ وقد تم استخدام هذه الطريقة للتقدير الكمي للمستحضرات الصيدلية المختلفة .

وقد أعطت هذه الطريقة نتائج عالية الدقة وبمقارنتها مع نتائج الطرق الدستورية والمرجعية لم تظهر أي فروق جوهرية بينهما.