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COLORIMETRIC DETERMINATION OF CERTAIN SYMPATHOMIMETIC DRUGS

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A rapid, simple and highly sensitive spectrophotometric method for the determination of oxiderine tartrate, etilefrine hydrochloride, metaraminol bitartrate, orciprenaline sulphate and isoprenaline sulphate is described. The method depends on the reaction of aqueous solution of these drugs with the diazotized 5-chloro-2,4-dinitroaniline in alkaline medium to give a highly coloured product with characteristic λ_{max} for each drug in the range of 420-550 nm. The colour produced in all cases obeyes Beer's law. This method is suitable for the analysis of these drugs in pure forms and in pharmaceutical preparations without prior separation.

Numerous methods have been described for the determination of the sympathomimetic drugs oxiderine tartrate, etilefrine hydrochloride, metaraminol bitartrate, orciprenaline sulphate, and isoprenaline sulphate, among these are titrimetric[1-6], chromatographic[7,8] gas chromatography[8], flurometric[9,10], and spectrophotometric methods[11-13]. Although all these methods are suitable for the determination of these drugs, colorimetric methods are generally preferred as they involve less expensive instrumentation and afford greater sensitivity when appropriate chromogenic reagents are employed.

Diazotized 5-chloro-2,4-dinitroaniline has been successfully employed in these laboratories as a good reagent for the colorimetric determination of certain phenolic compounds[14-15]. As all the aforementioned sympathomimetic drugs contain one or more typical phenolic group, it was decided to investigate the coupling of the reagent with them in order to develop a colormetric method for their determinations.

As a result of this investigation, a simple, rapid, accurate, and selective method is established for the determination of each of these drugs in pure form and in pharmaceutical preparations.

EXPERINENTAL

Apparatus :

Spectrophotometer (Spectromom 203) MOM, Budapest, Hungary.

Materials :

All chemicals and reagents used were analytical grade. 5-5-Chloro-2,4-dinitroaniline was prepared by a reported procedure[16]. Several crystallization from ethanol yielded an analytical sample m.p. 174°. Etilefrine hydrochloride (effortial hydrochloride(R) [1]), oxiderine tartrate (sympatol tartrate(R) [1]) metaraminol bitartrate[2], orciprenaline sulphate[1], and isoprenaline sulphate[1], were used as working standard without further treatment. Commercial preparations including tablets and drops containing sympathomimetic drugs are purchased from the market.

Diazotized 5-chloro-2,4-dinitroaniline :

Weigh accurately about 10 mg of 5-chloro-2,4-dinitroaniline into 10 ml volumetric flask, dissolve in 2 ml sulphuric acid. Cool in ice bath, add 0.5 ml 2% (W/V) sodium nitrite solution. After 2 minutes add 0.2 ml 10% (W/V) sulphamic acid and complete to volume with cold distilled water. Mix well and keep in ice bath This reagent solution is stable for five hours.

Colour development :

Into 10-ml volumetric flask containing 0.2 ml diazotized solution, pipet successively 1.0 ml aqueous solution of sympathomimetic drug (50 mcg/ml), 2.0 ml N/1 NaOH, mix well dilute to volume with distilled water. Measure absorbance of the final diluted solution at the specific λ_{max} for each drug against a blank treated concurrently .

Analysis of pharmaceutical preparations:

1- **Liquid preparations :**

Transfer appropriate, accurately weighed amount of the liquid preparation equivalent to specific amount of the sympathomimetic drug, dilute with distilled water to give a final concentration of 50 mcg/ml and proceed as under colour development .

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[1] Boehringer Ingelheim .
[2] Merk Sharp and Dohme. (MSD).

2- Tablets :

Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder equivalent to about 25 mg of sympathomimetic drug to 25 ml volumetric flask. Dissolve and dilute with distilled water. Either filter and discard the first portion of the filtrate or transfer the content of the flask to the centrifuge tube and centrifuge for 20 minutes. The clear solution obtained is the stock assay solution, from which a suitable dilution is made and proceed as under colour development.

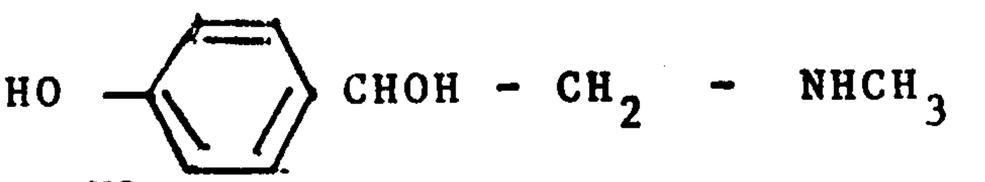
3- Recovery experiments :

Add accurately weighed amount of sympathomimetic drug to an accurately weighed amount of the liquid preparation or the powdered tablets equivalent to known weight of sympathomimetic drug in 50 ml volumetric flask, dissolve and complete to volume with distilled water. Continue as under liquid preparations or tablets.

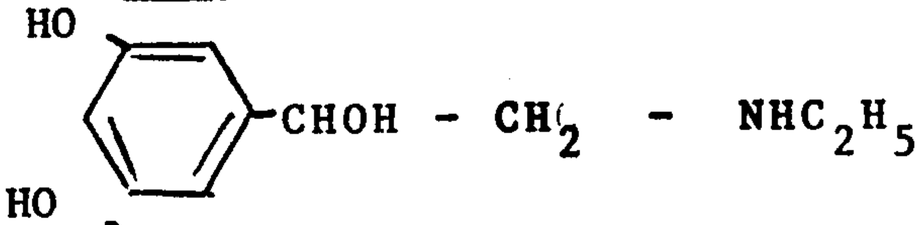
RESULTS AND DISCUSSION

The previous experience in these laboratories in the use of 5-chloro-2,4-dinitroaniline as an analytical reagent through coupling with certain phenolic compounds[14,15], calls for extending the applicability of this reagent for estimation of certain sympathomimetic drugs containing one or more typical phenolic group. The investigated drugs were chosen to represent various postion of phenolic group with respect to the side chain as shown in Scheme I .

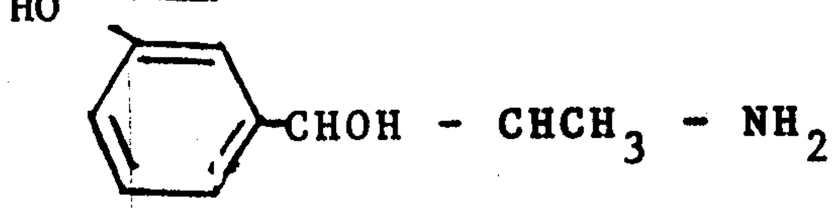
Oxiderine



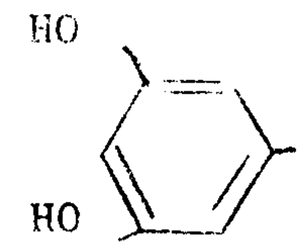
Etilefrine



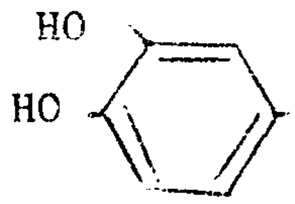
Metaraminol



Orciprenaline



Isoprenaline



Scheme I

Investigations were carried out using the previous condition applied in the estimation of ethinyl estradiol [14] and pyridoxine HCl [15], but no reproducible results were obtained. Accordingly, it was decided to carry out rigorous investigations to study different variables to determine the optimal reaction condition for the formation of the coupling colour product with each of the studied drugs.

Trials have been made for the preparation of separate diazonium salt solution and then using aliquot of this solution in the process of coupling. It was found that the reagent is soluble in sulphuric acid rather than hydrochloric acid, the removal of excess sodium nitrite is essential since it interferes with the colour product and this was done by 10% sulphamic acid.

When coupling was conducted in sodium acetate medium followed by addition of certain volume of sodium hydroxide solution no reproducible results were obtained. It was found that the direct addition of certain volume of sodium hydroxide solution to the mixed sympathomimetic drug and the diazotized reagent solutions gives immediately intense reddish colour miscible with water.

The volume of sodium hydroxide solution required in the coupling process depends upon its normality. 2 ml N/1 sodium hydroxide solution proved to be the suitable concentration (Table I). The intensity of the developed colour and the position of the λ_{max} for all the studied drugs is significantly affected by the type of diluent (Table II). However , distilled

water was used for the development of analytical procedure as diluent for availability.

Under these conditions, the absorption spectra of the resulting coloured coupling product for each of the studied drugs with the diazotized reagent solution showed a specific absorption peak at 545, 530 , 550 and 420 nm for, etilefrine hydrochloride, oxiderine tartrate, metaraminol bitartrate, orciprenaline sulphate and isoprenaline sulphate respectively (Fig. I and Table III). The calculated apparent molar absorptivity are 2.0×10^4 , 1.9×10^4 , 1.5×10^4 , 5.8×10^4 and 2.6×10^4 for etilefrine hydrochloride, oxiderine tartrate, metaraminol bitartrate, orciprenaline sulphate and isoprenaline sulphate respectively (Table III). A linear relationship between the concentration of the drug in the sample solution and the absorbance of the coloured product at the specific λ_{max} was proved in the range of, 1-8 mcg/ml for etilefrine hydrochloride and metaraminol bitartrate, 1-10 mcg/ml for oxiderine tartrate, 0.5-6 mcg/ml for orciprenaline sulphate, and 1-6 mcg/ml for isoprenaline sulphate. The lower limit of sensitivity is 1 mcg/ml for each of etilefrine hydrochloride, oxiderine tartrate, metaraminol bitartrate and isoprenaline sulphate, and 0.5 mcg/ml for orciprenaline sulphate.

It is quite evident from Table III that the position and number of hydroxyl group in the phenyl moiety of the molecule of these drugs greately affects the position of the λ_{max} and the ϵ value of the coloured product formed with diazotized 5-chloro-2,4-dinitroaniline. Etilefrine and metaraminol with only one hydroxy group located at position 3 in the phenyl moiety produced a coloured product with more or less the same λ_{max}, 545 nm and 550 respectively. However a significant difference in the ϵ of the produced coloured product is observable which could be assumed to a contribution of the side chain which is not identical in the two drugs. When the hydroxyl group is located in position 4 as in oxiderine rather than in position 3 a significant hypsochromic shift is observed (λ_{max} 530 nm)

On the other hand, when an additional hydroxyl group to that located at position 3, is present in the phenyl moiety of the molecule a hypsochromic shift is resulted which is very sharp, when the additional group is located at position 4 as in the case of isoprenaline(λ_{max} 420 nm), and only appreciaple when this group is located in position 5 as in the case of orciprenaline(λ_{max} 500 nm). This additional hydroxyl groups also affects the ε value of the colour product but in opposite pattern to that observed aith λ_{max}. When this hydroxyl group is located at position 4 an appreciable increase in ξ value results while approximatelv three fold for ξ results when the hydroxyl group is located at position 5 as in the case of orciprenaline.

The observed difference in λ_{max} and ξ of the coupling products of these drugs and the diazotized 5-chloro-2,4-dinitroaniline in relation to the specific location and the number of hydroxyl group in the phenyl moiety of the molecule of these drugs can be interpreted on the basis that; the position of the phenolic group relative to the side chain and relative to each other, if more than one hydroxyl group are present per phenyl moiety, determines the most active and accessible centers for coupling with the diazotized reagent. Thus in the case of 3-hydroxyphenyl-drugs, etilefrine and metaraminol, coupling is assumed to occur preferentially at position 6 on the phenyl moiety which is para to the hydroxyl group and ortho to the side chain, while in the case of 4-hydroxvphenyl-drug, oxiderine, position 3 and 5 which are ortho to the hydroxyl group and meta to the side chain are similarly activated and accessible for coupling thus forming a product in which the diazo residue is adjacent in position to the hydroxyl group. In the case of 3,4 dihydroxyphenyl-drug, isoprenaline, coupling is assumed to occur preferentially at position 6 which is para to the 3-hydroxyl group, meta to the 4-hydroxyl group and ortho with respect to side chain. In orciprenaline with 3,5-dihydroxy moiety, position 1 and 6 could assumed to be highly reactive for coupling as each is being ortho to one hydroxyl group and in the same time para to the other hydroxyl group in addition to being ortho to the side chain and it is probable that coupling could occur at the same time at these two reactive positions producing a product with two diazo residue and this could explain the three fold increase in ξ of the coloured product with orciprenaline compared to the other drugs. However,

investigations are still required to determined the exact structure of the coupling products formed under the reaction condition which is beyond the scope of the presented communication.

Table IV shows the results obtained upon carrying out the analysis of sympathomimetic drugs in pharmaceutical dosage forms. All formulations gave percentage assay close to the label claim Neverthless, different amounts of sympathomimetic drugs were added to each of the formulations and the analysis was performed. Percentage recoveries of the added drugs ranged from 98.5-101.6% and these results validate the suitability of the developed method for the determination of these drugs in pharmaceutical preparations

Table I : Effect of Addition of Different Volumes of 1 N Sodium Hydroxide Solution on the Intensity of the Developed Colour .

Volume of N/1 sodium hydroxid, solution added (ml)	Absorbance+			
	Etilefrine HCl x λ_{max} 545 nm	Metaraminol bitartrate+ λ_{max} 550 nm	Orciprepaline sulphate+ λ_{max} 500 nm	Isoprenaline sulphate+ λ_{max} 420 nm
1.5	--	--	--	--
1.6	0.460	0.249	--	--
1.8	0.460	0.249	0.530	0.225
2.0	0.460	0.249	0.530	0.225
2.2	0.460	0.249	0.530	0.225
2.4	0.460	0.249	0.530	0.225
2.6	0.460	0.249	0.530	0.225
2.8	0.440	0.240	0.530	0.220
3.0	0.400	0.220	0.500	0.200

\+ Average of 5 determinations.

x Concentration of sympathomimetic drug in the final solution is 5 mcg/ml

Table II: Effect of Dilution by Different solvents on the intensity of the Developed Colour and on the Position of the λ max.

Solvent Added	*Etilefrine HCL*		*Oxiderine tartrate*		*Metaraminol bitartrate*		*Orciprenaline sulphate*		*Isoprenaline sulphete*	
	A^+	λmax	A^+	λ max	A^+	λmax	A	λ max	A^+	max
Water	0.46	545	0.302	530	0.25	550	0.530	500	0.225	420
Methanol[+]	0.54	580	0.335	550	0.85	350	0.800	460	0.000	000
Ethanol[+]	0.635	596	0.350	580	0.395	586	0.820	470	0.203	420
Acetone	0.860	560	0.450	600	0.550	608	0.345	470	0.105	430
Propanol	0.740	596	0.580	580	0.450	590	0.646	460	1.165	420
Isopropanol	0.675	606	0.465	592	0.430	600	0.561	460	0.569	386
Dioxane	0.675	610	0.630	570	0.000	000	0.432	450	0.105	420

[+] Average of 4 determinations

* Precipitation occur and so centrifuge for 10 minutes before measuring the absorbance.
Concentration of sympathomimetic drug in the final sample solution is 5 mcg/ml.

Table III : Absorption Characteristics of Certain Sympathomimetic Drugs by the Proposed Method.

Sympathomimetic Drug	λ *max*	ε	*Beer's law obeyed in the range mcg/ml*
Etilefrine hydrochloride	545	2.0×10^4	1 - 8
Oxiderine tartrate	530	1.9×10^4	1 - 10
Metaraminol bitartrate	550	1.5×10^4	1 - 8
Orciprenaline sulphate	500	5.8×10^4	0.5 - 6
Isoprenaline sulphate	420	2.6×10^4	1 - 6

Table IV : Determination of Sympathomimetic Drugs in Pharmaceutical Formulations

Drug	Dosage form	Claimed	Found +		Added mg	Recovered +	
			mg	%		mg	%
Etilefrine	tablets[1]	5/tab	4.96	99.20 SD=0.46	5	5.08	101.6 SD=0.4
Etilefrine	drops[2]	7.5/g	7.478	99.70 SD= 0.6	10	9.95	99.46 SD=0.94
Oxiderine	drops[3]	10/tab	10.003	100.03 SD=0.403	10	9.97	99.7 SD=0.75
Orciprinaline	tablets[4]	20/tab.	20.50	102.50 SD=0.64	10	9.962	99.62 SD=1.7
Isoprenaline	tablets[5]	10/tab.	9.528	95.28 SD=0.84	10	9.85	98.50 SD=0.43
Isoprenaline	solution[6]	10/g	9.45	94.50 SD=0.35	10	9.91	99.1 SD=0.69

+ Average of 5 determinations .

1- Effortil tablets : Each tablet contains 2-methylamino-1-(-3-hydroxyphenyl) ethanol hydrochloride 5 mg.

2- Effortil dropss ; Each g. contains 2-ethylamino-1-(3-hydroxyphenyl)ethanol hydrochloride 7.5 mg.

3- Sympatol drops : Each g. contains 1-(4-hydroxyphenyl)-2-methylaminoethanol tartrate 100 mg .

4- Alupent tablets : Each tablet contains 1-(3,5-dihydroxyphenyl)2-isopropylaminoethanol sulphate 20 mg .

5- Isoprine Tablets : Each tablet contains 1-(3,5-dihydroxyphenyl)-2-isopropylaminoethanol sulphate 10 mg .

6- Aleudrin solution: Each g. contains 1-(3,4-dihydroxyphenyl)2-isopropylaminoethanol sulphate 10 mg .

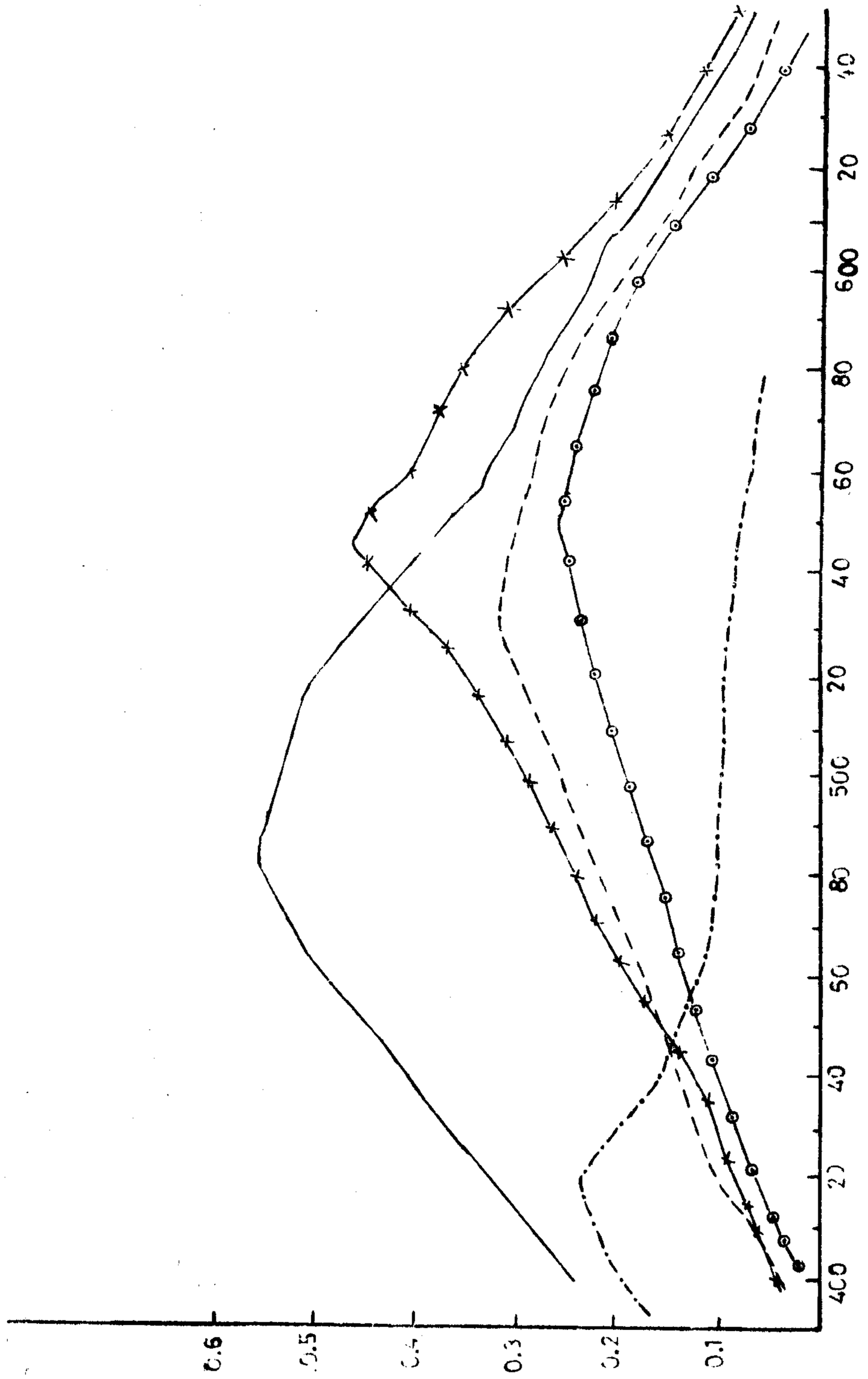


Fig. I: Absorption spectra of the colored p produced with sympathomimetic drugs.

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طريقـة لونيـة لتعيين بعض الادوية السمتاوية

سلوى رزق حسن الشـــابورى
قسم الكيمياء الصيدليـة ـ كليـــة الصيـــدلة ـ جامعـــة اسيوط

هـــذا البحث يحتـــوى على شرح لطريقة طيفية سريعـة وسهلة وطليـة الحســاسيـة لتعييـن ترتـرات الاكسيـديرين وهيدروكلوريـد الايتلفريـن ، وثنائـى ترتـرات الميتوانيول ،وسلفات الارسهرنيلين وسلفات الايزوبرينالين.

وهـــذه الطـــريقة تعتمـــد عـــلى تفاعـــل المحلول المائى لهـــذه الادويـــة مع ديهـــازوتيزد ـ هـ كلورو ـ ٢ ، ٤ ـ ثنائى النيتروانيليـــن فى وســـط قاعـــدى معطيـــه الوان واضحـة مع درجـة لون قصـــوى محـــددة لكـــل دواء وتتـــراوح هـــذه الدرجـة مابيـن ٤٢٠ ، ٥٥٠ ن.م

وهـــذا اللـــون الناتج فى جميـــع الحـــالات يتبع قانـــون بيـــر

وهذه الطـــريقة مناسبـــة لتحليـــل هـــذه الادويـــة فى حالتها النقية وفى المستحضرات الصيدليـــة بدون فصـــل مسبق.