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DETERMINATION OF ANTAZOLINE IN DOSAGE FORMS AND WITH NAPHAZOLINE IN COMBINATION

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ABSTRACT

A simple and rapid spectrophotometric method is described for the determination of antazoline and naphazoline either singly or in combination. The method is based on the interaction of both drugs in the form of base with chloranil in acetonitrile at room temperature. The interaction products of antazoline and naphazoline exhibit two λmax, one at about 300 nm for both drugs and the other at 448 and 510 nm for antazoline and naphazoline respectively. Different variables have been studied to optimize the reaction conditions for both drugs. Beer's law is obeyed for both drugs over a range of 5-45 µg/ml. Interference studies are carried out and the application of this procedure for the determination of antazoline in single component tablets and in combination with naphazoline in nasal drops is given with good recovery range(99.4-99.9%). Synthetic mixtures of antazoline and naphazoline are satisfactorily analyzed simultaneously with good recoveries.

INTRODUCTION

The frequent and popular utility of antazoline and naphazoline as effective antihistamines and/or vasoconstrictors led to extensive research to develop methods of analysis for these compounds. Among these are spectrophotometric methods for simultaneous determination of both drugs [1-3], or for the determination of naphazoline [4].

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Several colourimetric methods based on the interaction of both drugs with different reagents have been developed. Among these are oxidation with ceric ammonium sulphate[5,6], and by interaction with dimethylaminobenzaldehyde[7]. Antazoline gives colour by interaction with cobaltithiocyanate[8], nitrous acid[9] and p-nitrophenyldiazonium salts[9]. Naphazoline was determined colorimetrically by interaction with sodium nitroprusside[11], ammonium reinikate[12], and 3,5-dinitrobenzoyl chloride[13].

Non-aqueous titration methods have been recommended for the determination of antazoline[13,14] and naphazoline[15,16]. Complexometric[17,18], potentiometric[19] and gravimetric[20,21] methods have been also reported for the analysis of either antazoline or naphazoline.

Since binary mixtures of antazoline and naphazoline are frequently indicated for the treatment of histamine induced nasal and occular congestions, quantitative analysis of such mixture is an important subject for many pharmaceutical analysts and cannot be considered yet ideal. Consequently, the present investigations have been made for the analysis of antazoline and naphazoline either singly or in the presence of each other using chloranil as a chromogenic reagent.

EXPERIMENTAL

Apparatus

Zeiss spectrophotometer PM2 DL(Zeiss, Oberkochen, West Germany).

Antazoline hydrochloride, antazoline sulphate, and naphazoline nitrate were used as working standards. Chloranil was crystallized twice from benzene(charcoal) and had

a melting point of 289°. All chemicals used throughout this work were of analytical-reagent grade. 0.1% w/v Chloranil in acetonitrile was freshly prepared. 20% w/v Sodium hydoxide was prepared in distilled water.

Standard preparations

For antazoline and naphazoline salts: Weigh accurately about 10 mg of the salt (antazoline hydrochloride, antazoline sulphate or naphazoline nitrate) and transfer quantitatively into a small separating funnel. Dissolve in 10 ml distilled water, then add 2 ml of 20% w/v sodium hydroxide solution. Extract the liberated base with five quantities each of 25 ml of solvent ether. Wash the combined extracts with successive quantities, each of 5 ml of distilled water until the last washings are neutral to litmus paper. Evaporate the ether extract till dryness. Dissolve the residue in acetonitrile and dilute quantitatively so as to obtain a stock of 0.2 mg/ml of antazoline or naphazoline. Prepare working solutions by appropriate dilution with acetonitrile.

Sample preparations

For lab-made mixtures: Weigh accurately calculated quantities of antazoline sulphate and naphazoline nitrate so as to obtain mixtures of both drugs in ratios of 1:1, 10:1, and 20:1. Extract the powdered salt of each mixture as under standard preparation for antazoline and naphazoline beginning with (add 2 ml 20% w/v sodium hydroxide...). Dissolve the residue in acetonitrile and dilute quantitatively so as to obtain three synthetic mixtures in ratios of 1:1, 10:1, and 20:1 of antazoline sulphate :naphazoline nitrate.

For tablets: Weigh and powder twenty tablets. To an accurately weighed quantity of the powder, equivalent to

about 10 mg of antazoline base, add 50 ml of water, shake continuously for fifteen minutes, and filter. Proceed as under standard preparation for antazoline and naphazoline salts beginning with (add 2 ml of 20% w/v sodium hydroxide solution.....).

For eye drops: Measure accurately a quantity of eye drops equivalent to about 10 mg of antazoline base and mix with 10 ml of water. Proceed as under antazoline preparation for antazoline and naphazoline salts beginning with (add 2 ml of 20% w/v sodium hydroxide solution.....).

Procedure

Pipette 0.3 ml of the drug solution (antazoline or naphazoline base) into 2 ml volumetric flask, add 0.2 ml of chloranil. Complete to volume with acetonitrile and mix well. Leave to stand at room temperature for thirty to forty minutes then measure absorbance against a blank treated simultaneously at λmax 448 nm for antazoline and 510 nm for naphazoline.

For synthetic mixtures or pharmaceutical preparations containing antazoline and naphazoline, measure absorbance at λmax 448 and 510 nm. Calculate the concentration of either compound from the following aquations[22]:

$$C_x = A_1 / \alpha_1 X \{ (b-m)/(b-a) \}$$

$$C_y = A_2 / \beta_2 X \{ b X (m-a) / m X (b-a) \}$$

Where C_x and C_y are the concentrations of antazoline and naphazoline, respectively, calculated as g/100 ml; A denotes the absorbance of 1 cm layer of the measured solution; α and β represent the values of A (1%, 1 cm) for antazoline and naphazoline respectively; the subscripts 1 and 2 refer to 448, and 510 nm respectively; $m=A_2/A_1$; $a=\alpha_2/\alpha_1$; $b=\beta_2/\beta_1$.

RESULTS AND DISCUSSION

When chloranil in dioxane was interacted with antazoline or naphazoline in various solvents namely ethanol, isopropanol, dioxane and benzene, it gave the same absorption spectrum with the same wavelength at λmax 550 nm.

Using acetonitrile solvent for all the interaction products, particular attention was focused on the corresponding absorption spectra which are quite different.

The absorption spectra (Fig.1) exhibit two maxima for each drug: at λmax 320 and 448 nm for antazoline and at λmax 298 and 510 nm for naphazoline. The 52 nm difference in the visible region seemed to be the ideal experimental set-up for the quantitative determination of antazoline and naphazoline in binary mixture by simultaneous equations.

In order to determine the optimal reaction conditions, investigations were carried out to study the different parameters affecting the formation of the interaction products of chloranil with both antazoline and naphazoline in acetonitrile solvent.

The effect of chloranil concentration on the intensity of the coloured product was studied at different time intervals.

Figure 2 illustrates, that the reaction of 1 mg/ml chloranil with antazoline in acetonitrile shows maximum absorption value after thirty minutes. It then remained stable for further thirty minutes. With higher chloranil concentration (2 mg/ml and 3 mg/ml), the intensity of absorption decreased.

Figure 3 indicates, that there is no appreciable change in the absorption intensity of the coloured product of naphazoline at λmax 510 nm with different chloranil concentration at certain time intervals . The colour of the

chromogen was developed after ten minutes and remained stable for at least ninety minutes. Accordingly, 1 mg/ml chloranil was selected as the suitable concentration with both antazoline and naphazoline in acetonitrile.

Stability of the coloured chromogen of both antazoline and naphazoline with chloranil was studied at different time intervals.

Figure 4 illustrates that, the spectrum of the yellow antazoline chloranil chromogen at the ultra violet band exhibits a hyperchromic effect by time, while at λ_{max} 448 nm, maximum absorption intensity was attained after thirty minutes, then decreased by time with the colour disappearance after ten hours.

On the contrary, the red chromogen of naphazoline chloranil (Fig. 5) shows higher stability at λ_{max} 510 nm even after two days, with slight increase in the absorption intensity at λ_{max} 298 nm.

Consequently, the naphazoline chloranil chromogen in acetonitrile seems to be of higher stability compared with antazoline chloranil chromogen.

The effect of temperature and heating time on the absorption intensity and stability of the interaction products of both drugs was studied. It was found that maximum absorbance for the coloured reaction of antazoline chloranil system in acetonitrile was attained by leaving the reactant mixture at room temperature for thirty minutes. It remained unchanged for at least further twenty five minutes, (Fig. 6).

In order to increase the reaction rate, the reactants were heated at three different temperatures in a thermostatic water bath. Figure 6 indicates that, on heating at

45±2°, the yellow colour developed after ten minutes and remained stable for further ten minutes. A slight increase in absorption occured during the further twenty minutes, followed by an abrupt decrease in the colour intensity . For the other two temperatures 60° and 80°, the colour was developed after ten minutes but with lower absorption value, and decreased gradually afterwards. These findings indicate,that the absorbance measurement after thirty minutes at room temperature is the optimum for estimating the developed yellow coloured chromogen at λ_{max} 448 nm.

For antazoline-chloranil chromogen, Table 1,indicates that ,heating in a thermostated water bath at 45, 60, and 80± 2° at different time intervals has a hypochromic effect.

At room temperature, the chromogen at λ_{max} 510 nm developed after ten minutes and remained stable for at least one hour. So the reaction of naphazoline with chloranil in acetonitrile was carried at room temperature and measured within one hour.

Under the optimal reaction condition, Beer's law is obeyed for antazoline and naphazoline at λ_{max} 448 and 510nm respectively (Table 2).

Analysis of synthetic mixtures of antazoline sulphate: naphazoline mitrate in the ratios of 1:1,10:1,and20:1 calculated as g/100 ml, was solved by simultaneous equations. Table 3 shows ,that when antazoline sulphate and naphazoline nitrate are present in binary mixture of ratio 1:1 it can be analyzed simultaneously with good recoveries for each drug. For mixtures of ratios 10:1 and 20:1 only antazoline sulphate, the major component ,can be analysed without any interference from naphazoline nitrate.

The proposed method gave accurate and reproducible results when applied for the analysis of pharmaceutical preparations containing antazoline either singly or in presence of naphazoline(Table 4) without interference from the commonly encountered excipients or additives.

Table 1: Effect of temperature and heating time on the intensity and stability of the interaction product of naphazoline chloranil chromogen in acetonitrile at λ_{max} 510 nm.

Time of reaction (minutes)	*Room temperature*	*Absorbance at**		
	25°	*45°*	*60°*	*80°*
10	0.320	0.310	0.300	0.283
20	0.320	0.307	0.306	0.278
30	0.320	0.307	0.286	0.270
40	0.320	0.290	0.275	0.252
50	0.320	0.284	0.260	0.240
60	0.320	0.260	0.245	0.210

* Average of three determinations
The above mentioned temperatures were kept unchanged within ± 2°

Table 2: Linearity data for the reaction of antazoline in acetonitrile.

Drug	λmax *nm*	*Apparent molar absorptivity*	*Calibration linearity range ug/ml*	*Intercept* *a*	*Slope* *b*	*Correlation coefficient* *r*
Antazoline	448	4.457×10^3	5-45	-0.0105	0.0173	0.9993
Naphazoline	510	2.243×10^3	5-50	-0.0199	0.0114	0.9996

Table 3: Determination of synthetic mixtures of antazoline[x] and naphzaoline[y] salts using simaltaneous equations.

Synthetic mixture antazoline: naphazoline	*Added g/100 ml* antazoline	naphazoline	*Recovery %* antazoline	naphazoline
1 : 1	0.0015	0.0015	100.78 SD=±0.462 (CV= 0.458)	99.84 SD=±0.777 (CV= 0.778)
10 : 1	0.0030	0.0003	101.25 SD=±0.598 (CV= 0.590)	not determinable
20 : 1	0.0030	0.00015	99.940 SD=±.474 (CV= .474)	not determinable

x Antazoline was calculated as antazoline sulphate.
y Naphazoline was calculated as naphazoline nitrate.

Table 4: Application of the proposed method for the analysis of antazoline in pharmaceutical preparations

Formulation	*Claimed* mg	*Found* mg	%	*Added* mg	*Recovery** %
Antistine tablets	100/tab.	99.05	99.05 SD=±0.159 (CV= 0.168)	15	99.9 SD=±0.403 (CV= 0.407)
Antistine – privine drops	5/ml	4.94	98.80 SD=±0.370 (CV= 0.431)	15	99.4 SD=±0.530 (CV= 0.534)

Antistine tablets: (Swisspharma, Cairo, Egypt) Each tablet contains 100 mg of antazoline hydrochloride.
Antistine privine drops: (Swisspharma, Cairo, Egypt) Each ml contains 5 mg antazoline sulphate and 0.25mg naphazoline nitrate.

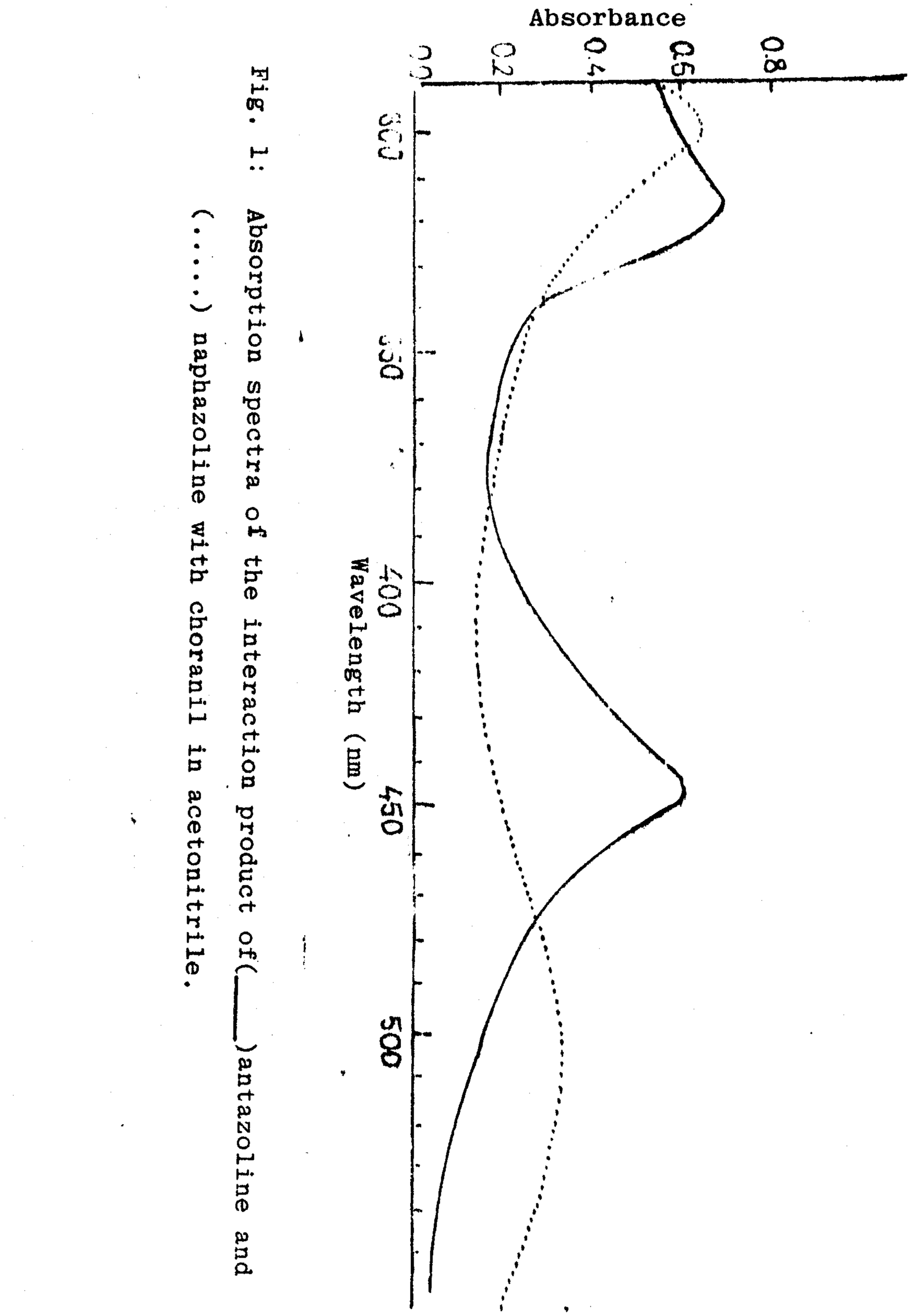

Fig. 1: Absorption spectra of the interaction product of(____)antazoline and (.....) naphazoline with choranil in acetonitrile.

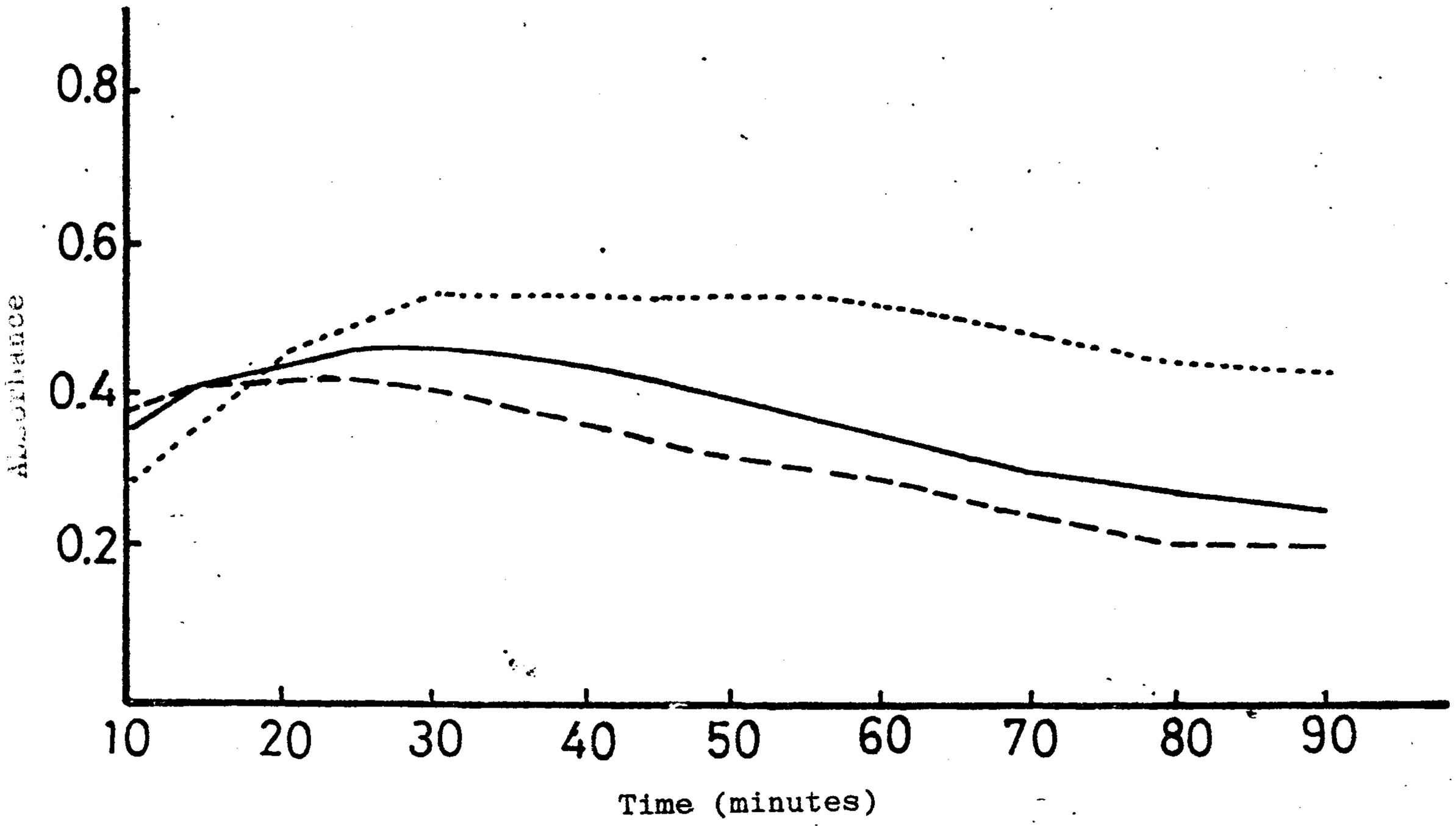

Fig. 2: Effect of time on the intensity of the interaction coloured product of antazoline with different chloranil concentrations in acetonitrile at λ max 448 nm.
Chloranil concentration,(.....) 1 mg/ml,(——) 2 mg/ml,
(------) 3 mg/ml.

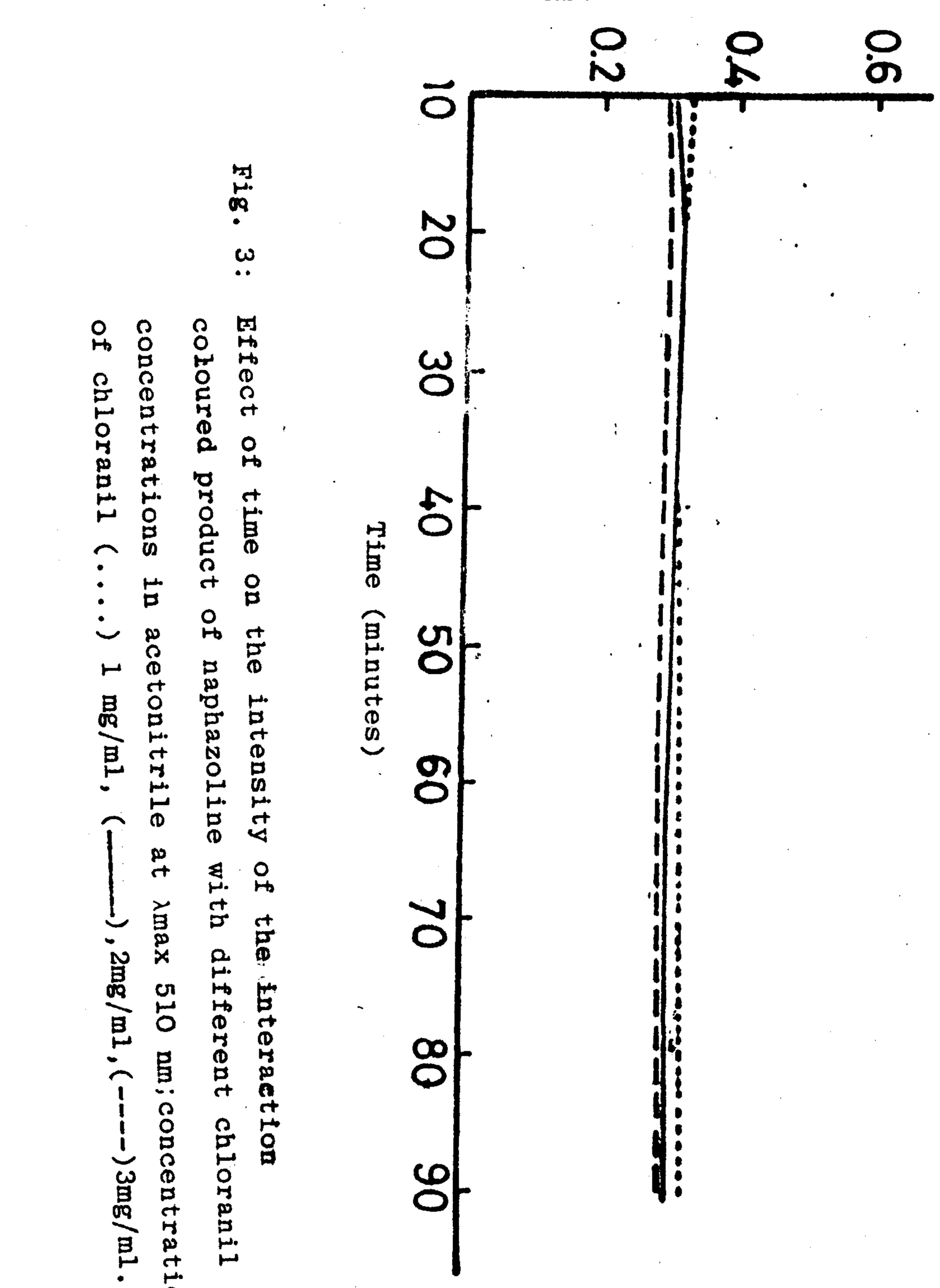

Fig. 3: Effect of time on the intensity of the interaction coloured product of naphazoline with different chloranil concentrations in acetonitrile at λmax 510 nm;concentration of chloranil (....) 1 mg/ml, (——),2mg/ml,(----)3mg/ml.

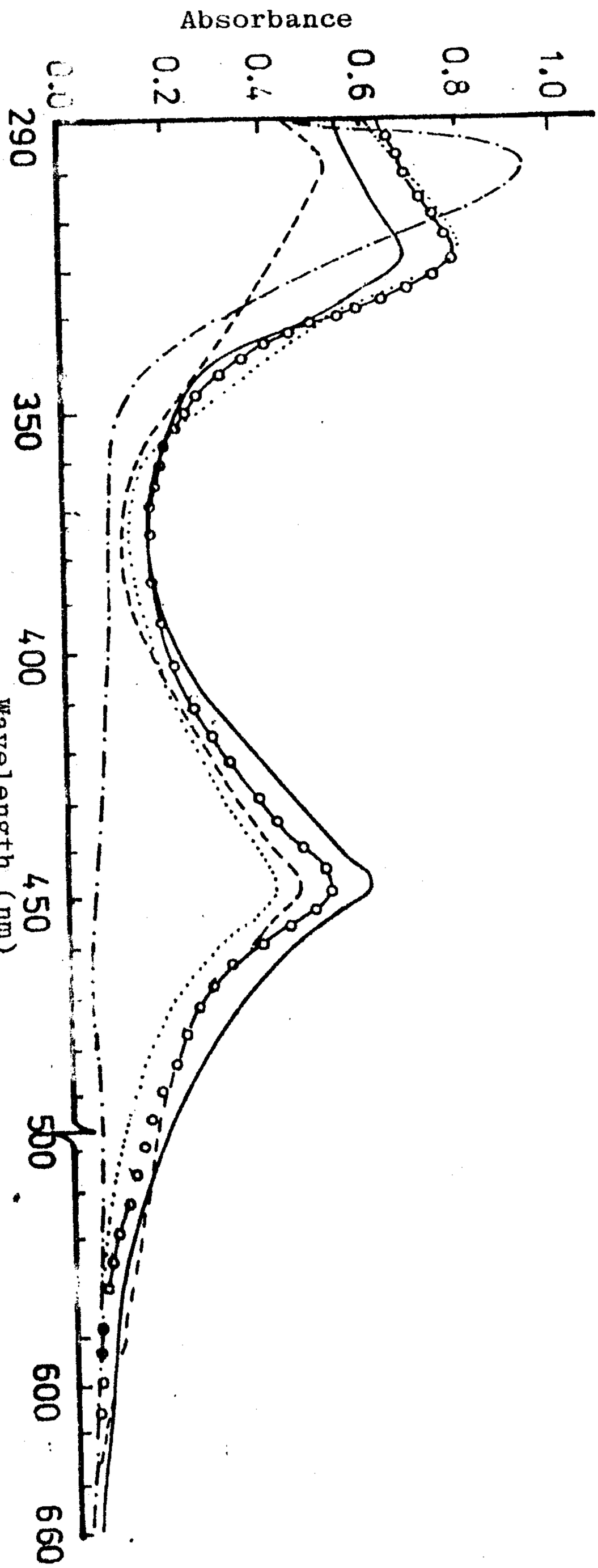

Fig. 4: Absorption spectra of the interaction product of antazoline with chloranil in acetonitrile.
Key: (——) 10-15 minutes, (- - - -) 30 minutes, (o—o—o)60 minutes, (.....)80 minutes, (-.-.-.-) 10 hours.

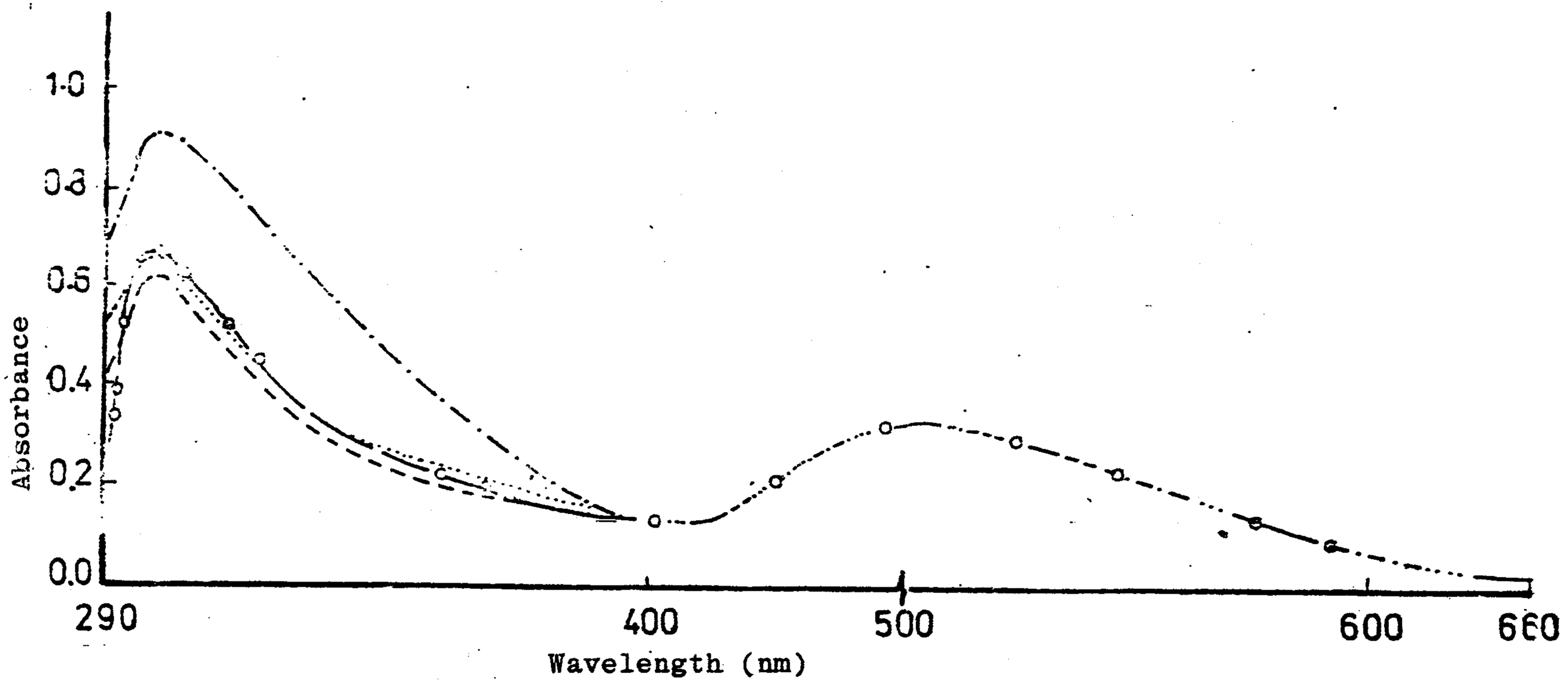

Fig. 5: Absorption spectra of the interaction product of naphazoline with chloranil in acetonitrile at room temperature(25°).

Key : (------)10-15 minutes, (o—o—o) 60 minutes, (. . . .)80 minutes, (.-.-.) 48 hours.

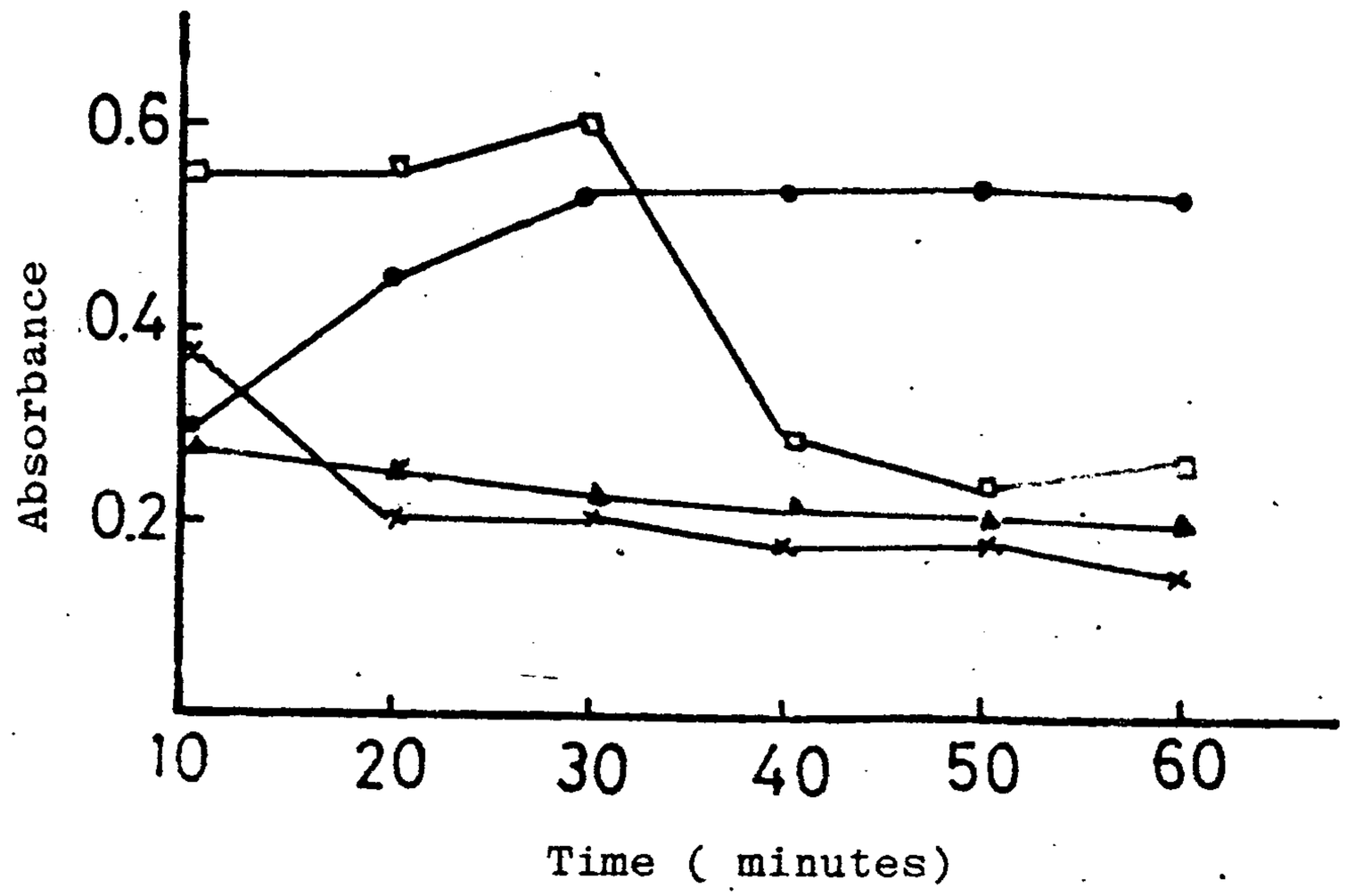

Fig. 6: Effect of temperature and heating time on the absorption intensity of the interaction product of antazoline with chloranil in acetonitrile at λ max 448 nm.

Key : (●—●—●) room temperature, (×—×—×)60°, (□—□—□)45°, and (▲—▲)80°

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طريقة لتقييم الاشتازولين فى الاشكال الصيدلية وفى مصاحبة النافازولين

سلوى رزق الشابورى – محمد محمد عامر – على محمود طه – بيكناز يوسف خشبة
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يبين هذا البحث طريقة سهلة وسريعة لتقييم الانتازولين والنفازولين سواء كانا منفردين او مخلوطين معا . وتعتمد الطريقة على تفاعل كل من العقارين فى صورتيهما القاعدية مع الكلورانيل فى الاسيتونيتريل عند درجة حرارة الغرفة . ويظهر ناتج التفاعل لكل العقارين موجتى امتصاص قصوى احداهما طولها ٣٠٠ نم لكلا العقارين بينما طول الاخرى ٥١٠،٤٤٨ نم لكل من الانتازولين والنفازولين على التوالى .

وقد تم دراسة متغيرات التفاعل المختلفة لتحديد الظروف المثلى للتفاعل. وقد وجد ان الامتصاص الطيفى الناتج عن التفاعل يتمشى مع قانون بير بين تركيز ٥– ٤٥ميكجم / مليلتر .

هذا وقد تم دراسة التداخل المحتمل مع المواد المختلفة وتم تطبيق هذه الطريقة المستحدثة لتقييم الانتازولين فى الاقراص ذات المركب الواحد وكذلك فى تركيباتة مع الانتازولين فى نقط الانف وكأن معدل الاستعادة يتراوح بين (٤و٩٩ – ٩و٩٩،٠/) كما تم تحليل مخاليط تخليقية لكل من الانتازولين والنفازولين آنيا بمعدل استعادة جيدة .

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