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SPECTROPHOTOMETRIC DETERMINATION OF PIPERIDINE, PIPERAZINE AND PHENMETRAZINE

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A spectrophotometric assay procedure was developed for the quantitative assay of piperazine and phenmetrazine. The method is based on the formation of enamines of these drugs by interaction with acetaldehyde. The resulting enamines interact with chloranil to form the corresponding blue aminovinylquinone which can be quantitated spectrophotometrically at λ max 320 nm for both drugs and at λ max 667 nm and 670 nm for piperazine and phenmetrazine respectively. The Beer-Lambert law adhered to over the 0.5-20 ug/ml range for both drugs at both λ max. The optimum reaction conditions and the effect of variables have been studied. The method has been applied successfully to the analysis of commercially available piperazine tablets, ampoules and effervescent granules.

Piperazine is used in gout and anthelmintic preparations and phenmetrazine is used as narcotic.

The official compendia describes a gravimetric[1,2] and non aqueous titration[1] methods for piperazine , a spectrophotometric[3] and non-aqueous titration[4] methods for phenmetrazine hydrochloride. Among the methods described for the assay of piperazine are colorimetric analysis employing ammonium reineckate[5] , bromothymol blue[6] , sodium

β-naphthoquinone-4-sulphonate[7], 3,5-dichloro-p-benzoquinonechlorimine[8] and dichlone[9], gravimetric[10,11] and several chromatographic techniques including paper[12], column[13] as well as gas liquid[14] chromatographic methods. Polarographic technique[15] was also reported for the analysis of piperazine.

Phenmetrazine was determined by non-aqueous titration[16] method.

Chloranil reacts with piperazine to give coloured charge transfer complex product with λ max at 590 nm [17] in chloroform and at λ max 540 nm in dioxane[18].

The present report describes a new utility of chloranil for the spectrophotometric determination of piperidine, piperazine and phenmetrazine after the formation of their enamines by interaction with acetaldehyde. The resulting enamines react with chloranil to give the corresponding aminovinylquinone.

The drugs used in this study possess cyclic secondary amines. Piperidine was selected as a simple representative example to interact with acetaldehyde and chloranil.

EXPERIMENTAL

Apparatus:

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

Reagents:

All chemicals used were of analytical-reagent grade unless otherwise specified.

Chloranil: (Merk,Darmstadt, West Germany) was crystallized twice from benzene (charcoal) and had a melting point of 280°. A 1% w/v chloranil in dioxane was freshly prepared.

Acetaldehyde: A 10% v/v acetaldehyde in isopropanol, and 20% v/v acetaldehyde in isopropanol were freshly prepared.

Sodium hydroxide: A 50% w/v sodium hydroxide in distilled water was freshly prepared.

Silver oxide: It was prepared by mixing equimolar quantities of silver nitrate and sodium hydroxide solutions. The formed brown precipitate was filtered, washed with distilled water and dried.

Cyclic secondary amines and dosage forms: The cyclic secondary amines used were piperidine, piperazine hydrate, piperazine monophosphate, piperazine citrate, and phenmetrazine hydrochloride. They were obtained from different manufactureres and were used as working standards. Different piperazine dosage forms were purchased from local market.

Standard amine solutions:

For amine base: Dissolve the calculated amount of amine base(piperidine or piperazine) in isopropanol or ethanol. Dilute quantitatively to obtain a stock of 1 mg/ml for piperidine and 0.5 mg/ml for piperazine.

For phenmetrazine hydrochloride:

Weight accurately the calculated amount of phenmetrazine hydrochloride, dissolve in isopropanol, and dilute quantitatively to obtain a stock of 1 mg/ml. For each 1.0 mg of amine hydrochloride add 2.0 mg of silver oxide to the sample preparation, shake well continuously for three minutes, leave to settle and filter. Discard the first portion

of the filterate. The resulting solution is the assay solution.

For piperazine salts: Weight accurately 10 mg of piperazine salt (piperazine citrate or piperazine monophosphate) into small separating funnel, dissolve in 5 ml distilled water, then add 10 ml of 50% NaOH solution. Extract the liberated base with five quantities, each of 25 ml chloroform. Wash the combined chloroform extracts with successive quantities, each of 5 ml of distilled water until the last washings are neutral to litmus paper. Evaporate the chloroform extract till dryness. Dissolve the residue in ethanol, and dilute quantitatively to obtain a stock of 5 mg/ml piperazine base.

Sample preparations:

For piperazine tablets: Weigh and powder 20 tablets. Extract an accurately weighed amount of the powder equivalent to 5 mg of piperazine base with three 5 ml portions of distilled water, and proceed as under salts of piperazine beginning with "add 10 ml of 50% NaOH solution....."

For piperazine ampoules: Measure accurately a volume of ampoule solution equivalent to 10 mg of piperazine base, mix with 5 ml distilled water. Proceed as under piperazine salts beginning with "add 10 ml of 50% NaOH solution....."

For piperazine effervescent granules: Weigh accurately a quantity of effervescent granules equivalent to 5 mg of piperazine base and dissolve in 10 ml of distilled water . When effervescence ceases, proceed as under piperazine salts beginning with "add 10 ml of 50% NaOH solution..."

Procedure

Pipette 0.1 ml of the amine base (piperidine, piperazine, or phenmetrazine) into 10-ml volumetric flask, add 0.1 ml of acetaldehyde and 0.1 ml of chloranil.

I- For piperidine

Leave to stand at room temperature for ten minutes, complete to volume with isopropanol, then measure the absorbance at λ max 680 nm against a blank treated simultaneously.

II- For piperazine

Dilute to volume with ethanol, and leave to stand at room temperature for sevent to eighty minutes, then measure the absorbance at λ max 667 nm against a blank treated simultaneously.

III- For phenmetrazine

Heat for fifteen minutes in a thermostatic waterbath at 55 $\pm$ 2° room temperature, complete to volume with isopropanol, then measure the absorbance at λ max 670 nm against a blank treated simultaneously.

RESULTS AND DISCUSSION

Investigations were carried out to study different analytical parameters in order to determine the optimal reaction conditions for the development of the blue aminovinylquinone of the studied secondary amines.

Suitable concentration of acetaldehyde in isopropanol to obtain the maximum absorption intensity for each amine is 10% for piperidine and 20% for piperazine and phenmetrazine.

The need of higher concentration of acetaldehyde for piperazine suggests that enamine might be formed at the two nitrogen sides.

The absence of acetaldehyde (Fig. 1) results in the appearance of the violet aminoquinone band (λ max 510-560 nm) of much weaker intensity. Accordingly, the presence of acetaldehyde is essential to intensify absorbance by extraconjugation included due to enamine formation.

The effect of different solvents on absorption intensity of the developed coloured quinone of the studied cyclic secondary amines was investigated.

Figures 2-4 indicate that the absorption intensity as well as the position of λ max for the interaction products of the three studied amines were affected by using different solvents.

Maximum absorption intensity of the blue chromogen of piperidine and phenmetrazine were obtained in isopropanol as shown in Fig. 2 and 4.

For piperazine (Fig. 3), it was found that ethanol shows the higher absorption intensity. Other solvents namely methanol and dioxane show hypochromic effect.

Stability of the blue quinones of the obtained secondary amines at room temperature were carried out.

Piperidinovinylquinone exhibits maximum absorption intensity immediately in isopropanol, while phenmetrazine interaction coloured product was obtained after twenty minutes and remained stable for further fifty minutes.

Piperazinovinylquinone was obtained after seventy minutes from dilution with absolute ethanol and remained stable for further two hours approximately.

Th[illegible] and heating time on the absorptic[illegible] and stability of the blue interaction proc[illegible] of the studied cyclic secondary amines were investigated.

It has been noticed from Table 1 that, the absorption intensity of piperidine interaction product decreased with increasing heating time and temperature, while that of piperazine in absolute ethanol was obtained after heating at 55 ± 2° for ten minutes, and decreased with further heating.

It was found that the reaction of piperazine developed at room temperature is more stable than that obtained after heating. Consequently, it was preferred to carry out the reaction at room temperature, inspite it takes longer time(seventy minutes) to reach maximum absorbance.

For phenmetrazine, the maximum absorption intensity was obtained after heating in a thermostatic water bath at 55 ± 2° for fifteen minutes and remained stable after dilution with isopropanol at room temerature for further twenty minutes. Accordingly, blue chromogen of phenmetrazine was measured after heating at 55 ± 2° for fifteen minutes.

Under the proposed conditions, the absorption spectrum of the resulting interaction product for each of the studied cyclic secondary amines showed two absorption bands, an intense ultraviolet band at λ max 320 nm, and a visible broad band at λ max 667 - 680 nm.

A linear relationship between the concentration of each amine and the absorbance of its chromogen at the two λ max was proved with small intercept and good correlation coefficients (Table 2).

Application of the proposed method for the analysis of different dosage forms of piperazine were carried out with good recoveries (Table 3). But since the pharmaceutical preparations of phenmetrazine hydrochloride are rarely present in the market, authentic mixtures of this drug with excipients and additives which are commonly present in tablets, were prepared and analyzed according to the proposed method. Good recoveries were obtained as shown in Table 4.

Table 1: Effect of temperature and heating time on the absorption intensity of the interaction products of the studied cyclic secondary amines, acetaldehyde and chloranil.

Time of reaction (minutes)	*Absorbance* at* 55°			70°			80°		
	1	2	3**	1	2	3**	1	2	3**
5	0.500	0.530	0.353	0.465	0.600	0.345	0.429	0.550	0.287
10	0.447	0.613	0.365	0.441	0.570	0.309	0.360	0.490	0.190
15	0.425	0.610	0.367	0.400	0.512	0.280	0.315	0.320	0.129
20	0.405	0.580	0.365	0.360	0.498	0.246	0.270	0.215	0.088

* Average of three determinations.

1- Piperidine. 10 ug/ml in isopropanol (λ max 680 nm)
2- Piperazine. 5 ug/ml in ethanol (λ max 667 nm)
3- Phenmetrazine 10 ug/ml in isopropanol (λ max 670 nm).

** Phenmetrazine was calculated as hydrochloride salt.

Table 2: Comparative summary of statistical data of the reaction of the studied cyclic secondary amines with acetaldehyde and chloranil

Secondary amine	*λmax nm*	*Apparent molar absorptivity*	*Linear calibration rang ug/ml*	*Intercept a*	*Slope b*	*Correlation Coefficient r*
Piperidine	320	12.45×10^3	0.5-6	0.0141	0.1419	0.9958
	680	4.22×10^3	1.0-15	0.0009	0.0467	0.9997
Piperazine	320	58.26×10^3	0.5-3	0.0185	0.2656	0.9946
	667	25.5×10^3	1.0-6	-0.0297	0.1359	0.9995
Phenmetrazine	320	20.41×10^3	0.5-8	-0.0287	0.1028	0.9995
	670	7.67×10^3	1.0-20	-0.0189	0.0358	0.9992

Phenmetrazine was calculated as hydrochloride salt.

Tabel 3: Application of the proposed method for the analysis of piperazine in different pharmaceutical preparations.

Formulation	*Claimed*	*Found* *		*Added*	*Recovery* *	
	mg	*mg*	*%*	*mg*	*mg*	*%*
Parazine tablets	300/tab.	299.19	99.73 SD=±0.548 CV= 0.567	5	4.9	98.80 SD=±0.163 CV= 0.167
Bilharcid ampoule	30/ml	29.65	98.82 SD=±0.380 CV= 0.391	5	4.97	99.52 SD=±0.249 CV= 0.254
Urolithine effervescent granules	50/5g	49.47	98.94 SD=±0.229 CV= 0.235	5	5.01	100.10 SD=±0.270 CV= 0.276

* Average of three determinations.

Parazine tablets: (CID, Chemical Industries Developments) Each tablet contains 300 mg piperazine monophosphate.
Bilharcid ampoule. (CID, Chemical Industries Developments) Each ampoule contains 60 mg piperazine di-antimonyl tartrate.
Urolithine effervescent granules: (Kahira Pharmaceutical and Chemical Industries) Each 5 g contains piperazine hexahydrate 0.05 g, lithium citrate 0.35 g, lactose 0.25 g, hexamine 0.25 g, sodium bicarbonate 2.69 g, citric acid 1.3 g and tartaric acid 1.3 g.

Table 4: Application of the proposed method for the analysis of synthetic mixtures of phenmetrazine hydrochloride in the presence of common excepients and additives

Substance added (per 25 mg phenmetrazine hydrochloride)	*mg*	*Recovery* * *mg*	*%*
Lactose	50	25.10	100.40 SD=±0.329 CV= 0.334
Fructose	50	24.67	98.69 SD=+0.262 CV= 0.265
Lactose	5	24.81	99.24 SD=+0.442 CV=±0.445
Magnesium stearate	5	24.52	98.09 SD=+0.285 CV=±0.291

Table 4: Cont.

Substance added (per 25 mg phenmetrazine hydrochloride)	*mg*	*mg*	*Recovery* %*
Acacia	5	24.65	98.59
			SD=±0.530
			CV= 0.539
Starch	5	24.95	99.80
			SD=±0.563
			CV= 0.566
Calcium phosphate tribasic	60	24.82	99.27
			SD=±0.278
			CV= 0.279

* Average of three determinations.

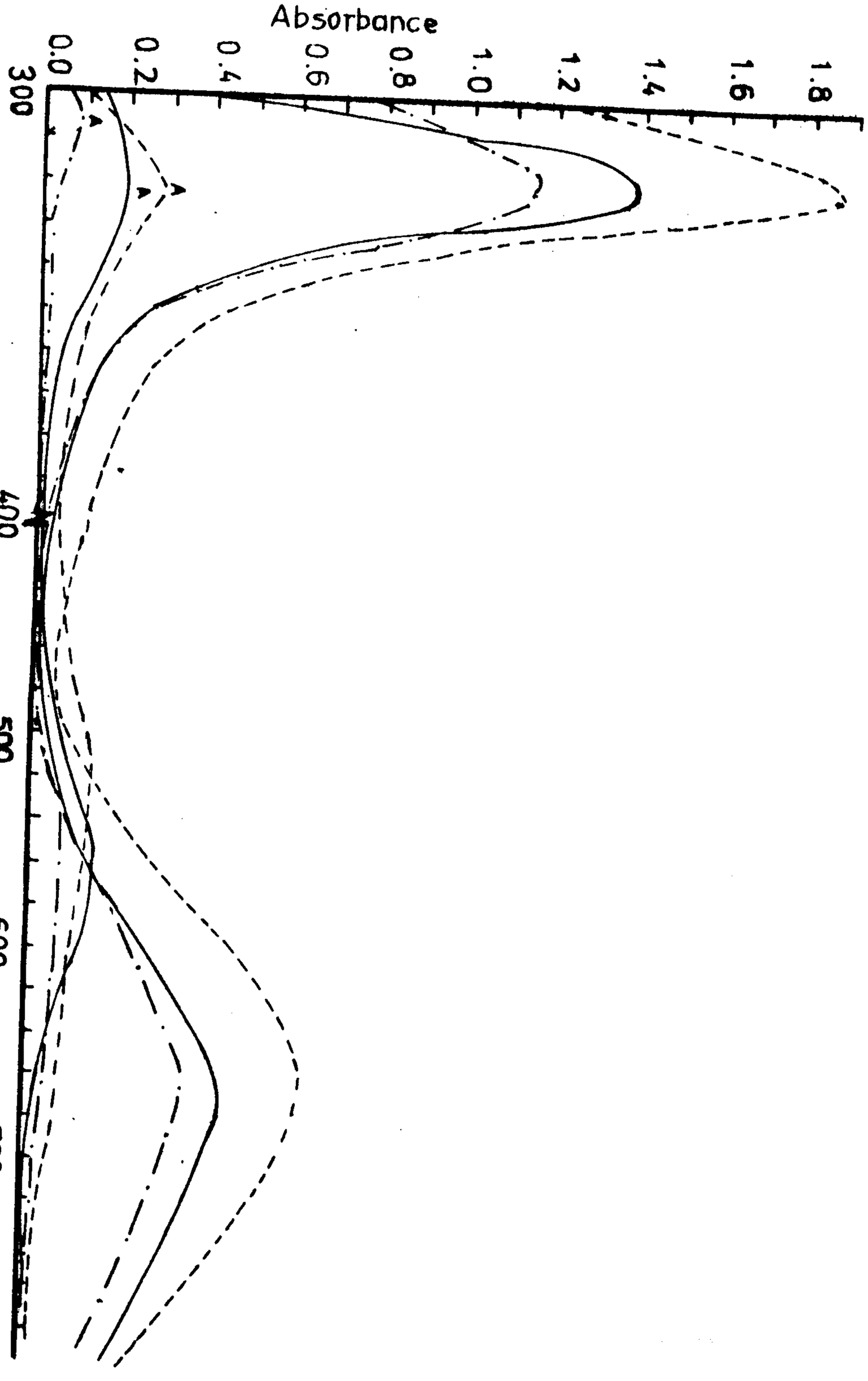

Fig.1: Absorption spectra of the interaction products of the studied amines with chloranil in presence and in absence of acetaldehyde.
Key: (———) piperidine, (---) piperazine, (-·-·-·-·-)phenmetrazine
A. in absence of acetaldehyde.

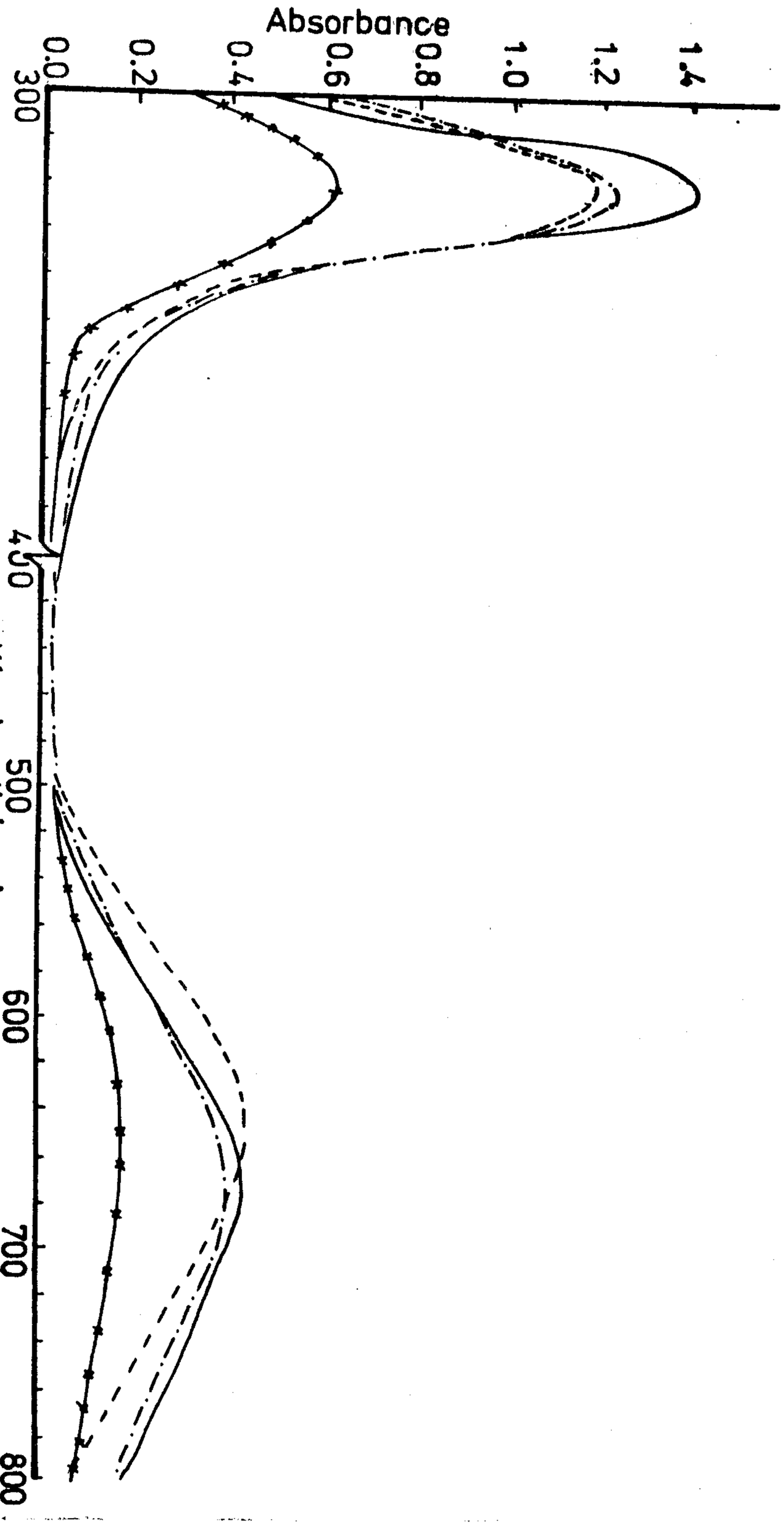

Fig.2: Effect of solvents on the absorption intensity of the interaction product of piperidine acetaldehyde chloranil system. Key: (———) isopropanol, (-·-·-·-) ethanol, (x—x—x) methanol and (----) dioxane.

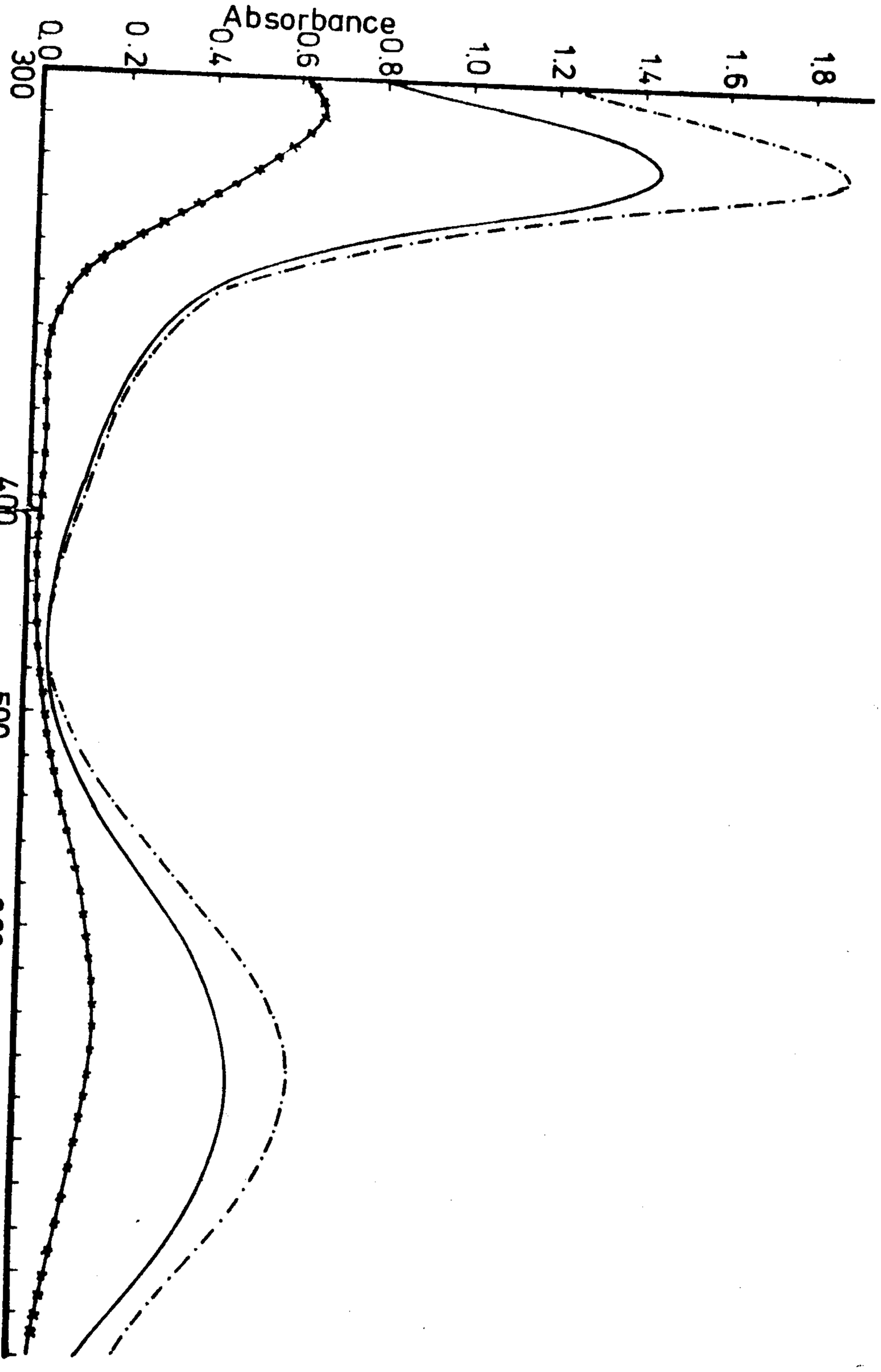

Fig. 3: Effect of solvents on the absorption intersity of the interaction product of piperazine acetaldehyde chloranil system.
Key: (-·-·-) ethanol, (———) isopropanol, and (x-x-x)methanol

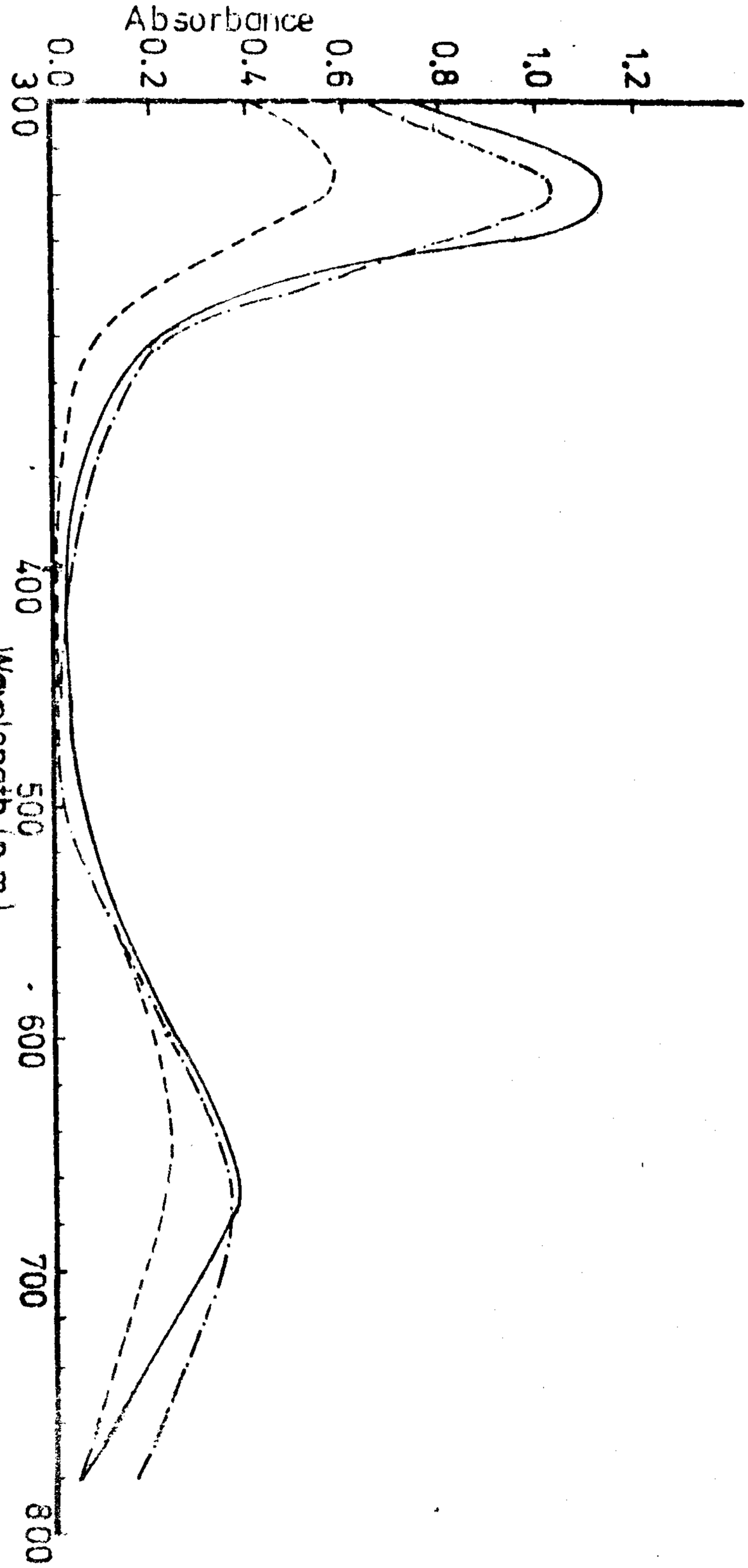

Fig.4: Effect of solvents on the absorption intensity of the interaction product of phenmetrazine acetaldehyde chloranil system.
Key: (———) isopropanol, (___.___) ethanol, and(----) dioxane

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تقييم طيفى لتحليل البيبريدين والبيبرازين وهيدروكلوريد الفينميرازين

سلوى رزق الشابورى ، محمدمحمد عامر، علىمحمودطه ، بيكينازيوسفخشبه

قسم الكيميـــاء الصيدليـــة ـ كلية الصيدلة ـ جامعـة أســـــــيوط

فـى هـذا البحث أستحدثت طريقة طيفية لتحليل أملاح البيبرازين والفينميرازين وتعتمدهذه الطريقة على تفاعل الكلورانيل مع الكيلفينيل الامين الناتج من تكاثف الامين الحلقى(بيبرازين وفينميرازين) فى الصورة القاعدية والاسيتالدهيد حيث ينتج لون أزرق ذو حساسيه عالية .

وبدراسة جميع تغيرات التفاعل الممكنة تم التوصل الى ضبط ظروف التفاعل

وقد وجد ان جميع نواتج تفاعل الامين الثنائى الحلقى تظهر موجبتى قصوى احداهما عند ٣٢٠ ن م لكلا العقارين والثانية عند ٦٦٧ ن م للبيبرازين والفينميرازين على التوالى.

وقد طبقت هذه الطريقة بنجاح لتحليل أملاح البيبرازين الموجودة فى صـــــورة اقـراص وحقن وأملاح فـواره .

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