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# AN IMPROVED COLORIMETRIC DETERMINATION OF ANTAZOLINE

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Abstract- A simple , precise and accurate spectrophotometric determination of antazoline salts is developed by improvement of the previously reported ceric sulfate procedure. Replacement of glacial acetic acid for water in preparing all assay solutions enabled the reproducible measurement of the chromogen absorbing at 505 nm. An appreciable increase in color stability was attained by the controlled addition of perchloric acid to the ceric sulfate solution, prior to interaction with antazoline at room temperature. Evidence is provided to account for the oxidation of antazoline-aniline moiety on the expense of an anionic ceric species. No interference with the investigated color reaction was offered by other 2-imidazolines or phenylephrine. Added to the considerably high value of chromogen molar absorptivity, ideal adherence of color absorption to Beer's law permitted the accurate and reproducible estimation of antazoline over the 1-10 mcg/ml concentration range. Application of the improved procedure to the satisfactory analysis of different antazoline dosage forms was also effected.

## I N T R O D U C T I O N

The frequent prescription of antazoline (2-N-phenyl-benzylaminomethyl-2-imidazoline) with other pharmaceutical amines and 2-imidazoline congeners has motivated the development of selective methods for the determination of this extensively utilized antihistamine. Since color reactions are oftenly of much value in this concern, several reports on antazoline analysis are mainly based on spectrophotometric measures (1-8). Out of the diverse chromogenic reagents adopted, only the sodium nitrite (5-7)

and ceric sulfate (8) procedures are claimed with pronounced selectivity. The ammonium reineckate (1,2) ,sodium nitroprusside (3) and dipicrylamine (4) methods are not specific ; other pharmaceutical amines and 2-imidazoline derivatives-likely to be present along with antazoline- do interfere (9).Moreover, the nitroprusside method while apparently specific for the intact imidazoline ring is subject to experimental buffer concentration and species, the age of reagents and the intrinsic color of the sample (10). However, criticism can also be raised against the selective procedures adopted for antazoline analysis. Whereas the utility of one of the nitrite methods is handicaped by interference of phenylephrine with color production (5), the suitability of the ceric sulfate procedure as an accurate tool for the estimation of antazoline is questionable. Discrepancies in recovery studies attained by this method reveal a considerable nonreproducibility, especially when compared with the nitrite methods (11). In addition, no quantitative data could be supplied when the ceric reagent was not properly cooled prior to interaction with antazoline or when much water was present during this interaction. These findings would attest for a significant thermolabile nature of the chromogen formed as well as for its marked sensitivity to pH variations.

Such shortcomings in the available methods for the

estimation of antazoline prompted this laboratory to develop a more accurate and precise procedure for such an estimation by improvement of the ceric sulfate method. Interpretation of the investigated antazoline-ceric color reaction as well as its application to the analysis of pure antazoline salts and some of its pharmaceutical formulations present the basis of the current contribution.

## E X P E R I M E N T A L

Materials-Pharmaceutical grade antazoline hydrochloride and antazoline methanesulfonate were utilized as the working standards.The following commercially available formulations were analyzed : antazoline tablets[1], antazoline injection[2] and antazoline-naphazoline solution[3].Other chemiclas were analytically pure.

Reagents-Ceric Sulfate Solution : by gentle warming dissolve 50 mg of the dried salt in 50 ml of 98 % (w/w) sulfuric acid, cool efficiently and complete quantitatively and stepwise to 100 ml with formate-free glacial acetic acid. The clear bright yellow-colored solution is quite stable at room temperature.

Instrumentation-A double beam UV-Vis spectrophotometer[4] with 1-cm glass cells was employed.

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[1]Antistine (CIBA-Geigy,Switzerland) contains 100 mg of antazoline hydrochloride per tablet.

[2]Histazine (CID,Egypt) contains 100 mg of antazoline methanesulfonate per 2-ml ampul.

[3]Antistine-Privine (CIBA-Geigy) contains 5 mg of antazoline sulfate and 0.25 mg of naphazoline nitrate per 1-ml solution.

[4]Spektromom 203 (MOM,Hungary).

Procedure

1-For Standard Antazoline Solutions : dissolve an accurately weighed amount of the appropriately well dried antazoline salt in glacial acetic acid so as to afford a concentration of 50 mcg of the working standard per 1-ml of the solution. Transfer 1 ml of the ceric sulfate reagent into a 10-ml volumetric flask, add 2 ml of 70 % (w/w) perchloric acid, mix well, release 1.0 ml of the standard antazoline solution, complete to volume with glacial acetic acid, mix well and directly transfer the solution into a 1-cm glass cell.Determine the absorbance of the color at 505 nm versus a blank prepared from 1.0 ml of glacial acetic acid and similarly treated.

2-For Assay Samples :

a-Tablets : place a single powdered tablet, or its equivalent (150 mg) from a composite of 20 tablets, in a 100-ml volumetric flask, add ca. 25 ml of glacial acetic acid and allow to satnd for 30 minutes with occaisonal shaking. Complete to volume with glacial acetic acid, mix well and filter through a dry filter into a dry flask, the first portions of the filterate being rejected. An aliquot of this sample is properly diluted with glacial acetic acid to afford a concentration of ca. 50 mcg of the claimed antazoline salt per 1-ml of the assay solution.

b-Injections and Solutions : pipet 1.0 ml of the assay solution, or the measured content of a single dose container,

into a suitable volumetric flask and dilute with glacial acetic acid to obtain ca. 50 mcg of the declared antazoline salt content per 1-ml of the assay solution. Determine the absorbance of the assayed antazoline solutions by carrying out the procedure mentioned as for the standard antazoline solution.

## RESULTS AND DISCUSSION

Antazoline-Cerium(IV) Interaction- The original application of ceric sulfate by Pitrowska as a color reagent for the identification of medicinal imidazoline derivatives revealed the high sensitivity of this reagent for the detection of antazoline (12). However, this reaction could not be quantified owing to the considerable instability of the red color formed in presence of 15 % aqueous sulfuric acid at room temperature. Though enhancing color stability for not more than 30 minutes, raising the acid concentration to 50 %-with substantial cooling before interaction-could not bring about the accurate estimation of antazoline, the range of recovery of which approximated 94-99 % (11). Water was utilized in this interaction for the preparation of antazoline and ceric sulfate solutions. As instability of the chromogen formed is appreciably enhanced on addition of water to the reaction mixture (11,12), our attention was paid to repeat the 50 % sulfuric acid-ceric interaction

in presence of organic solvents, with a rationale of stabilizing the chromogen once it forms through spontaneous solubilization.However, this direction was abandoned because of the insolubility of the chromogen in chloroform,ether, petroleum ethers and benzene.Investigation of the behaviour of this chromogen on addition of different non-aqueous solvents revealed its complete instability in absolute ethanol, methanol, acetonitrile, dioxane, dimethylformamide and formic acid. Neverthless, a marked stability was monitored when acetic acid was utilized as the reaction solvent, a finding that put forward the use of this solvent for the preparation of all solutions undertaken throughout this work. Relevant utility of glacial acetic acid for ceric sulfate oxidation of organic compounds is not uncommon(13).

Preliminary interaction at room temperature of antazoline hydrochloride with the ceric reagent, prepared in 50 % (v/v) sulfuric-glacial acetic acid mixture, resulted in the instantaneous production of a purple red color,with maximum light absorption at 505 nm, Figure 1. Maximum color intensity was manifested directly after mixing and measurement, with a steady rate of absorption over 45 minutes.Replication of this interaction using lower concentrations of sulfuric acid brought about a measurable decrease in the stability-time of the chromogen, with a negligible lowering of its maximum absorption, Figure 2.

These data obviously reflex the significance of protonation for the stabilization of the chromogen formed. Further investigation of the effect of higher sulfuric acid concentrations on such a stabilization was handicaped by the syrupy consistency, that was met with during preparation of the corresponding mixtures in glacial acetic acid. In contrast to sulfuric acid perchloric acid offered no such difficulties.
As revealed from the data given in Table I maximum color stability was achieved for 90 minutes by interaction of 1.0 ml of the working antazoline hydrochloride solution with equivalent volume of the ceric reagent, that was already mixed with 2.0 ml of 70 % w/w perchloric acid.

Under optimum conditions for color production, interaction of antazoline with ceric sulfate was seriously affected by rise of temperature, Table II, thus confirming the thermolabile nature of the chromogen initially postulated.It can be easily recognized,that discrepancies in antazoline estimation by the "aqueous" ceric sulfate method pertain to inaccuracies in controlling the local exothermic effect, encountered during water-acid mixing.

Investigation of the effect of the mode of addition on the sensitivity of color production recommeneded the addition of antazoline solution to the ceric reagent. Slightly lowered absorption values were obtained when the reverse order of addition was followed.

Reaction Interpretation :

Substitution of phenylephrine or certain 2-imidazoline derivatives for antazoline in the given interaction failed to induce color formation.The list of the imidazolines investigated included naphazoline[5], oxymetazoline[6],tolazoline[7],xylometazoline[8],tetrahydrozoline[9] and 2-methyl-2-imidazoline.With phentolamine[10] a transient blue color dominated only for few seconds, turning after into a pale yellow tint.Interaction of

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[5]Privine

[6]Nasivine

[7]Priscol

[8]Otrivine

[9]Tyzine

[10]Regitine.

different aniline derivatives with ceric sulfate afforded an instantaneous red-brown color.These derivatives included N-ethylaniline, N,N-dimethylaniline,o-toluidine and aniline itself.Making use of these data and of the fact, that different aniline derivatives are frequently utilized as redox indicators for cerium (IV) titrations (14), a suggestion can be safely made,to account for the antazoline-cerium interaction in terms of an oxidation of the aniline moiety of antazoline.That the imidazoline ring is not vulnerable to this effect is also in accord to the considerable stability of this heterocycle towards oxidations (15).This stability is further augmented in antazoline salts in view of the resonance-stabilized guanidinium structure.

The observation, that the antazoline-cerium chromogen is appreciably stabilized in the presence of strong acids is also in agreement with the properties of other N-disubstituted aniline chromogens (16).Establishment of the exact nature of this chromogen was not attempted on account of its thermolabile character, sensitivity to pH variations and insolubility in most organic solvents.

Noteworthy can be the fact, that addition of excess perchloric acid to the ceric sulfate reagent prior to interaction with antazoline lowered reaction sensitivity, Table I.Meanwhile,such lowering was not observed when

this excess was added after interaction of antazoline with the reagent alone. This paradox in the effect of perchloric acid would attest to the significance of the role exhibited by an anionic complex cerium species, mostly as $Ce(SO_4)_3^{2-}$, for an effective oxidation of antazoline. The formation of this complex in the presence of higher perchloric acid concentrations is doubtful (18). In support of this view, no color response could be evidenced up to a sensitivity limit of ca. 100 mcg antazoline/ml, when 0.05 % solution of ceric perchlorate in 50 % perchloric-glacial acetic acid mixture was reacted under the optimum conditions afore mentioned.

Quantitative Analysis- For the investigated antazoline-cerium(IV) interaction, the intensity of color absorption was a function of the concentration of antazoline interacted. A linear regression analysis of Beer's plot of antazoline hydrochloride revealed an excellent correlation , ($r = 0.9996$) over a concentration range of 1-10 mcg/ml, with a slope value of 0.082 and an apparent molar absorptivity of $2.47 \times 10^4$. For antazoline methanesulfonate, the corresponding parameters were : 0.9995, 2-12 mcg/ml, 0.066 and $2.05 \times 10^4$ respectively. This permitted the development of the investigated color reaction into a sensitive spectrophotometric analysis of antazoline salts. Replicate analysis of these salts by the improved method, Table III, proved to be highly precise, with a relative standard deviation of $4.098 \times 10^{-3}$. Recovery studies of antazoline

hydrochloride working solutions analyzed at different concentration levels,Table IV, afforded a mean percent recovery of 100.03 ± 0.85 in comparison to 102.6 ± 0.4 attained by the sodium nitrite methods (5-7).No discrepancies in the recovery data of antazoline assayed by the presented method could be observed, in contrast to the previously reported ceric sulfate procedure (8,11). Application of the improved color interaction to the analysis of different commercially availabe antazoline dosage forms,Table V, proved to be quite satisfactory.

## C O N C L U S I O N S

The presented improved ceric sulfate colorimetric determination of antazoline salts offers several advantages over the old method in terms of accuracy, precision and convenience. It also surpasses one of the sodium nitrite methods on account of its high selectivity, and stability of the color reagent. These considerations would recommend the utilization of the presented procedure for the automated analysis of anatzoline formulations.

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Table I- Effect of perchloric acid addition to antazoline reaction medium on color development.

| Mls added/10 mls assay solution [a] | | Absorbance, 505 nm | Stability, minutes |
|---|---|---|---|
| 0.05% Ceric reagent | perchloric acid | | |
| 0.5 | 1.0 | 0.400 | 60 |
| 0.5 | 2.0 | 0.360 | 70 |
| 0.5 | 3.0 | 0.310 | 80 |
| 1.0 | 1.0 | 0.395 | 70 |
| 1.0 | 2.0 | 0.410 | 90 |
| 1.0 | 3.0 | 0.405 | 90 |
| 2.0 | 1.0 | 0.385 | 70 |
| 2.0 | 2.0 | 0.390 | 80 |
| 2.0 | 3.0 | 0.378 | 80 |

[a]Contains 5 mcg of antazoline hydrochloride/ml.

Table II- Effect of Temperature rise on antazoline-ceric interaction.

| Temperature[a], C° | Absorbance[b], 505 nm |
|---|---|
| 25 | 0.408 |
| 40 | 0.383 |
| 75 | 0.255 |
| 95 | 0.125 |

[a]of a thermostated water bath.
[b]measured after 10 minutes interaction at (a).

Table III- Replicate analysis of antazoline hydrochloride working solutions ( 5 mcg/ml )

| Replication | Absorbance |
|---|---|
| 1 | 0.410 |
| 2 | 0.408 |
| 3 | 0.408 |
| 4 | 0.409 |
| 5 | 0.410 |
| 6 | 0.412 |
| 7 | 0.404 |
| Average = | 0.409 |
| Standard Deviation = | $\pm$ $1.676 \times 10^{-3}$ |

Table IV- Recovery-analysis of antazoline hydrochloride working solutions.

| Sample | Antazoline hydrochloride | | | |
|---|---|---|---|---|
| | Weight taken (mgm) | analyzed as (mcg/ml) | found[a] ( mg ) | recovery,% |
| 1 | 25.0 | 4.0 | 24.9 | 99.6 |
| 2 | 25.0 | 8.0 | 25.1 | 100.4 |
| 3 | 50.0 | 3.0 | 49.9 | 99.8 |
| 4 | 50.0 | 6.0 | 50.8 | 101.6 |
| 5 | 100.0 | 2.0 | 99.5 | 99.5 |
| 6 | 100.0 | 10.0 | 99.3 | 99.3 |
| Mean percent recovery = | | | 100.03 | |
| Relative SD = | | | $\pm$ $8.545 \times 10^{-3}$ | |

[a] Average of five determinations.

Table V- Analysis of antazoline commercial formulations

| Preparation[a] | Antazoline salt content, mg/unit | | | |
|---|---|---|---|---|
| | labelled | found[b] | added | recovery |
| Tablets | 100 | 101.1 | 50 | 151.2 |
| Injection[c] | 50 | 49.3 | 10 | 59.4 |
| Solution[d] | 5.0 | 5.1 | 100 | 105.5 |

[a]Detailed composition, cf. Experimental.

[b]Average of three determinations.

[c]Antazoline methanesulfonate per 1-ml.

[d]Calculated as antazoline hydrochloride and multiplied by 1.0415 to provide the corresponding sulfate.

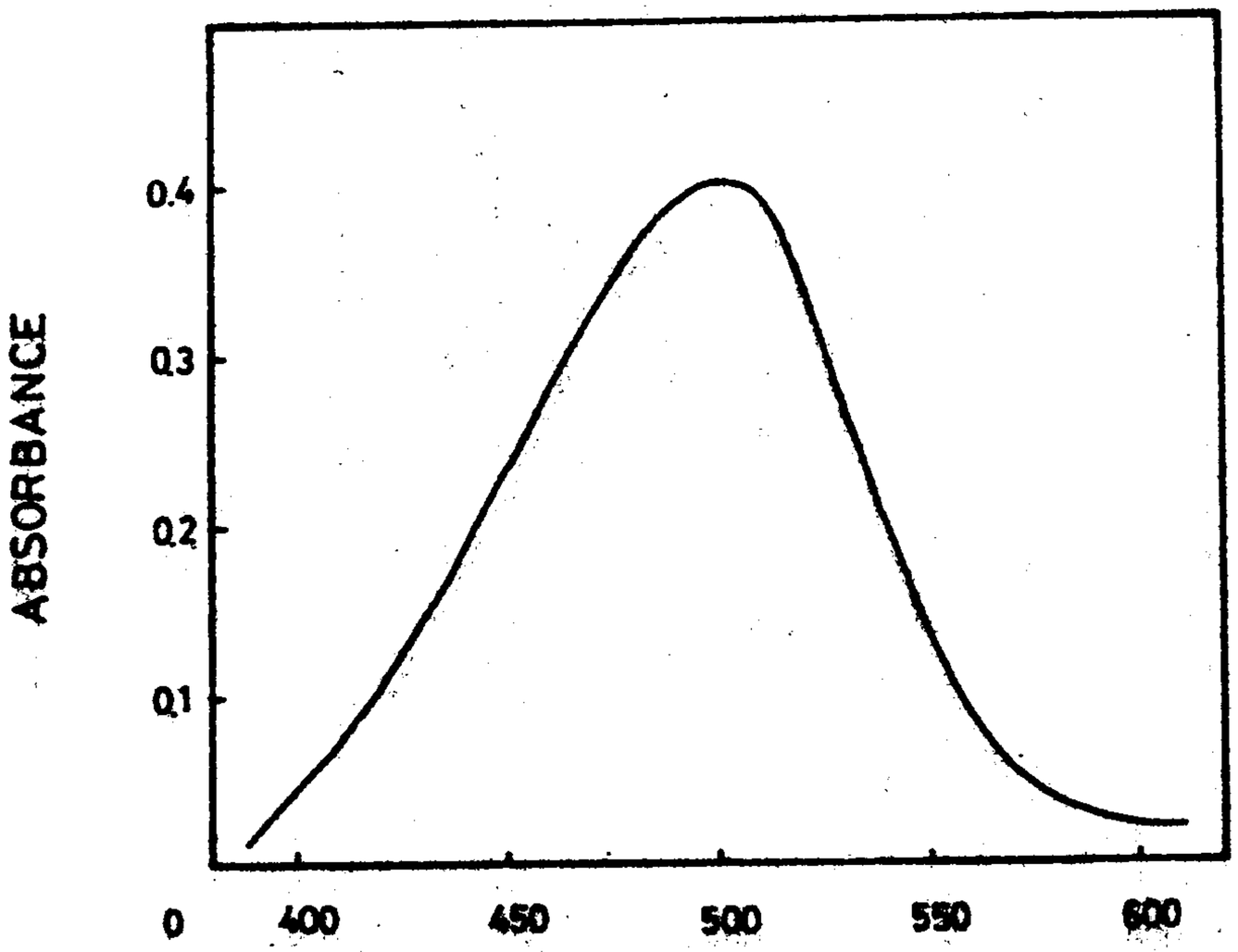


Figure 1-Light absorption spectrum of antazoline-Ce(IV) chromogen in glacial acetic acid, c,5 mcg/ml.

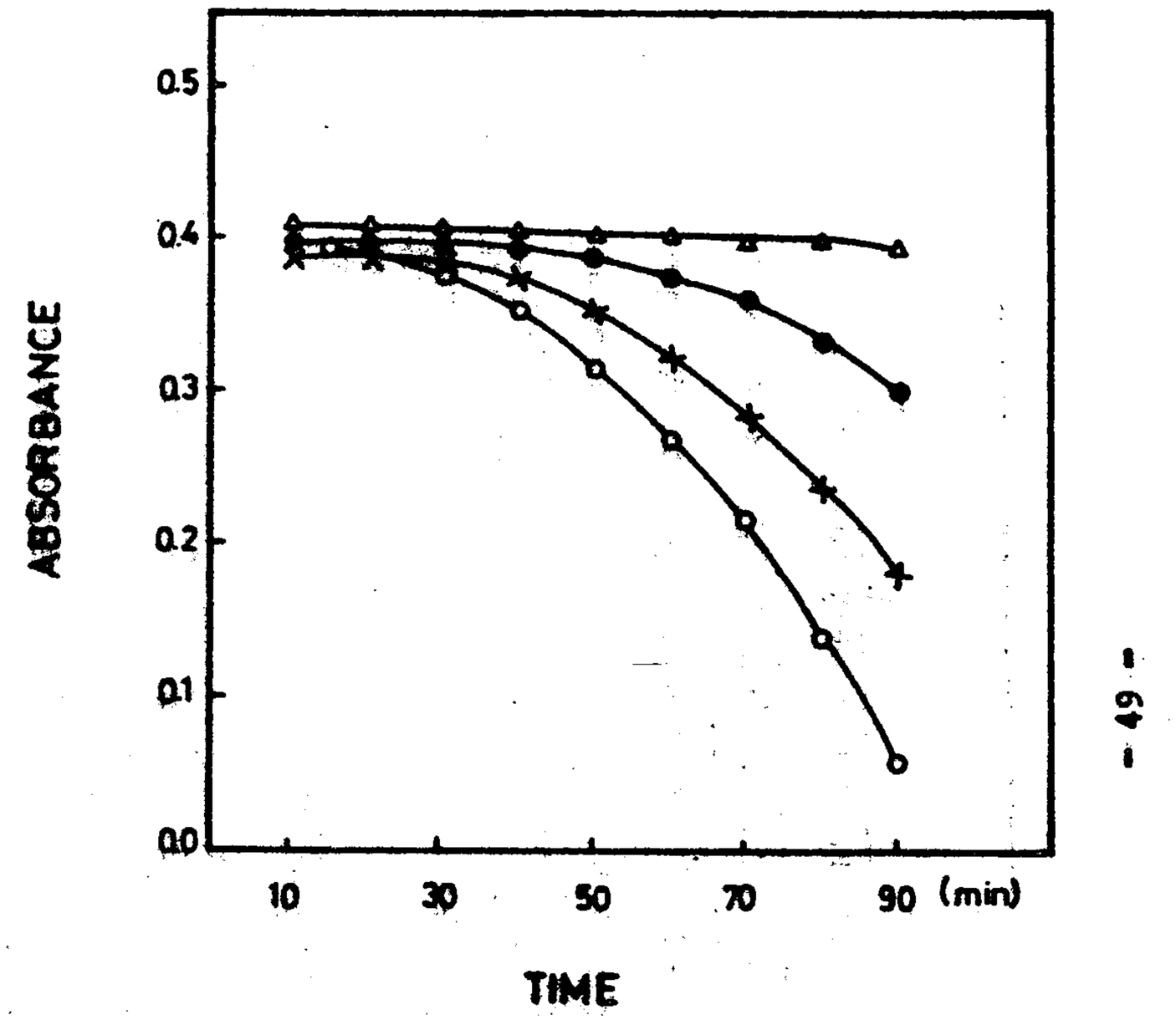


Figure 2- Absorbance-Stability behaviour of antazoline-Ce(IV) chromogen at different sulfuric acid concentrations

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# تقييم الأنتازولين بطريقة لونية محسنة

نبيل محمود عمر

قسم الكيمياء الصيدلية ـ كلية الصيدلة ـ جامعة اسيوط

استهدف هذا البحث استجلاء العوامل التى تسمح بالتحكم فى التفاعل اللونى للأنتازولين مع كبريتات السيريوم توطئة لتحويل هذا التفاعل الى طريقة طيفية دقيقة لتقييم هذا المشتق الايميدازولينى فى تراكيبه الصيدلية • ومن المعروف أن محاولة السيطرة على عدم ثبات المادة الملونة الناشئة عن هذا التفاعل باستخدام تراكيز مختلفة من حامض الكبريتيك لم تؤد الى الدقة والاسترجاعية المطلوبتين لاستغلال هذه الطريقة فى التعيين الكمى للأنتازولين•

ولقد أثبتت نتائج البحث أن استبدال الماء المستعمل كوسط مذيب فى الطرق القديمة بحامض الخليك الثلجى يؤدى الى تجنب الأثر الضار لكل من ارتفاع درجة الحرارة والحلمأة على ثباتية المادة الملونة ـ كما كان لاضافة قدر معين من حامض فوق الكلوريك المركز الى كبريتات السيريوم المذابه فى خليط من حامض الكبريتيك المركز والخليك الثلجى أثرة الملحوظ فى رفع حساسية التفاعل وثباتية المادة الملونة حيث أمكن تقدير ميكروجرام واحد من كلوريد الأنتازولين بالقياس الضوئى عند ٥٠٥ نم بدقة عالية •

ولقد كان لاقتصار هذا التفاعل على الأنتازولين وعدم امتداده الى مشتقات ايميدازولينية أخرى باع كبير فى ايجاز نشوء اللون الى تأكيد نواة الأثيلين الموجودة بجزئ الأنتازولين ـ كما أمكن ربط هذا التأكيد وأهمية وجود معقد كبريتاتى لعنصر السيريوم•

وجانب عناصر الدقة والحساسية الفائقة فلقد تميزت الطريقة المتقدمة بسهولة أدائها وامكانية تطبيقها المباشر لتحليل كثير من التراكيب الصيدلية لأملاح الأنتازولين دون تداخل من المواد الأخرى المصاحبة•