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# RAPID, SENSITIVE COLORIMETRIC ASSAY FOR ISOPRENALINE

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*A simple, rapid, selective and highly sensitive colorimetric method for the determination of isoprenaline (isoproterenol) is described. The method depends on the interaction of dimethoxydiquinone with isoprenaline sulphate under specified conditions with the formation of a highly coloured product which has a* $\lambda_{max}$ *at 510 nm and apparent molar absorptivity of* $3.8 \times 10^6$. *Absorbance versus concentration is linear up to 6 mcg/ml. No interference is observed in presence of common pharmaceutical adjuvants. The developed method is applicable with almost completer recovery for assaying isoprenaline in commercial pharmaceutical dosage forms without prior separation. The proposed method is also recommended as stability indicating assay for oxidative degradation involving the Catecholic function of isoprenaline.*

The official USP XIX method[1], for the analysis of isoproterenol HCl involves its quantitive extraction from pharmaceutical solutions or tablets matrix by ion-pair formation with bis 2-ethylhexylphosphoric acid in ether, followed by partition chromatography of the solution through a suitably buffered siliceous earth column. The assay procedure may lack precision because many important variables such as tighteness of the column and rate of elution are not described. Further, the method is lengthy and time consuming .

Both BP 1973[2] and NF XIV[3] employ non specific colorimetric method for the analysis of the title drug in tablets and aerosol spray preparations.

The literature reveals a few methods for the analysis of isoprenaline, among these are colorimetric method based on the chelate formation between the catecholamines and molybdate anion [4], another methods based on the reduction

of 2, 3, 5-triphanyltetrazolium chloride and subsequent measurement of the formazan at 485 nm[5]. Welsh and Sammul[6] reported on the analysis of isoproterenol solutions using a modification of Helberg's[7] fluromoetric assay for epinephrine. However, no details of the procedure were given. Prasad et al[8] reported a flurometric method based on the fact that isoprenaline in an acidic buffer solution can be selectively oxidized to its "chrome" derivative, which subsequently cyclyzed in strong alkaline solution to the fluorescent "lutin" derivative. Watson et al[9] developed a GLC method for the quantitation of isoprenaline in pharmaceutical dosage forms which depends on the reaction of the dried residue of extract with an appropriate trimethylsilylating agent, and the derivatives are eluted from a methyl silicone column using temperature programing.

Dimethoxydiquinone (DMDQ) was observed in this laboratory to be an excellent analytical reagent for the readily oxidisable drug, ascorbic acid[10]. It was thought that such a reagent could be similarly valuable for the analysis of other readily oxidizable drugs. Owing to the presence of catechol function. isoprenaline is one of these drugs. So, in the presented work the applicability of DMDQ as an analytical reagent for isoprenaline is investigated. As a result of this investigation, a rapid , accurate and selective colorimetric method for the determination of isoprenaline is presented which is applicable to commercial pharmaceutical dosage form without prior separation.

## EXPERIMENTAL

**Instrumentation :**

Spectrophotometer, Spektromom 203, MOM , Budapest, Hungary.

**Materials :** Isoprenaline sulphate, British Pharmaceopoeal grade was used as the working standard. DMDQ was prepared according to a reported procedure[11], several crystallizations from dioxane yielded an analytical sample. All other chemicals used were either pharmaceutical or reagent grades. Distilled water was used throughout.

**Solutions :**

Buffer solution ; pH 7 McIlvaine's citric acid-phosphate buffer[12] diluted with water , one volume to ten volumes.

DMDQ solution : 0.05% of DMDQ in dimethylsulphoxide is prepared. This solution is suitable for use within 5 hours.

Isoprenaline solution : 50 mg isoprenaline sulphate are accurately weighed and transferred quantitively into 50 ml volumetric flask, dissolved and diluted to volume with distilled water. From this stock solution appropriate dilution is made .

Assay procdure : Into 10-ml volumetric flask, pipet successivelly 0.5 ml of isoprenaline sulphate solution (100 mcg/ml), 2 ml DMDQ solution, 1 ml diluted pH 7 McIlvaine's citric acid-phosphate buffer. Mix well after each addition and leave to stand for exactly 1 minute. Add 1 ml isopropanol and dilute to volume with dilute pH 7 McIlvaine's citric acid-phosphate buffer Measure the absorbance at 510 nm against a blank prepared similarly using 0.5 ml distilled water instead of isoprenaline solutions.

**Assay of isoprenaline in pharmaceutical dosage forms**

(a) Liquid preparations : Accurately weigh an aliquot of the liquid preparation into a suitable volumetric flask. Dilute with distilled water to obtain about 100 mcg of claimed isoprenaline sulphate per ml of the prepared solution, and continue as under assay procedure.

(b) Tablets : Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 25 mg of isprenaline sulphate to 25-ml volumetric flask. Dissolve and dilute to volume with distilled water. Either filter and discard the filtrate or centrifuge in a centrifuge tube for 10 minutes. From the resulting clear solution a suitable dilution is made and continue as under assay procedure.

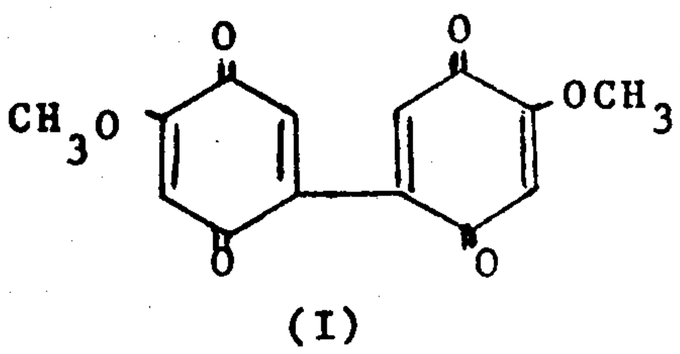
Recovery experiment : Add accurately weighed amout of isoprenaline sulphate to an accurately weighed amount of the liquid preparation or the powdered tablets equivalent to known weight of isoprenaline sulphate in 50-ml volumetric flask, dissolve and dilute to volume with distilled water. Continue as under liquid preparations or tablets.

**Interferences :** The possibility of interference from pharmaceutical adjuvant namely glucose, sucrose, lactose, starch , acaccia, sodium metabisulphite, sodium sulphite, glycerol , and ethanol was studied through preparation of synthetic mixture of these materials with isoprenaline and subjected to analysis according to the proposed method.

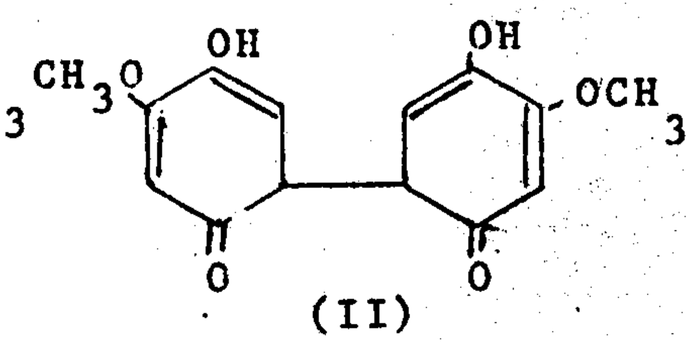
**Specificity to catecholic function :** The proposed procedure was applied to solutions of phenylephrine, orciprenaline and sample of isoprenaline oxidized with iodine[13] to N-isopropyl noradrenochrome[14]

## RESULTS AND DISCUSSION

Previous experiencee with DMDQ (I) showed the suitability of this reagent as a sensitive colorimetric reagent through an oxiuation reduction reaction in which the reagent is partially reduced to the highly coloured "indigoid" form (II) , on the expense of the oxidation of a susceptible molecule as in the case of ascorbic acid[10]. Catecholamine drugs (III) owing to their catechol function are very susceptible to oxidation with formation of the open chain quinone (IV) as an intermediate followed by the formtion of the respective aminochrome (V) as a final oxidation product[15]. It is assumed that interacting DMDQ and such type of drugs could result in a stoichiometric oxidation of the catechol function to form the quinonoid structure and reduction of DMDQ to form the highly coloured indigoid which can form the basis for a colorimetric determination of such drugs To prove this, isoprenaline was chosen as a model of these catecholamine drugs.



(I)



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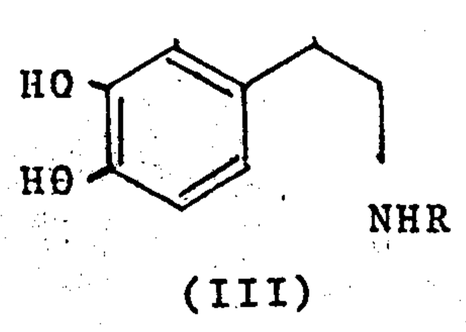

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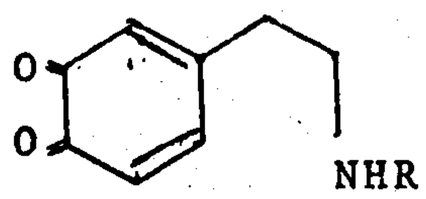

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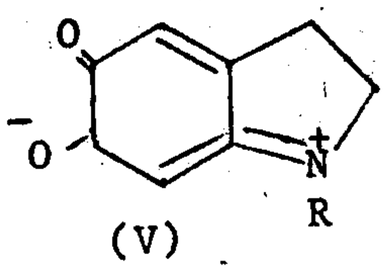

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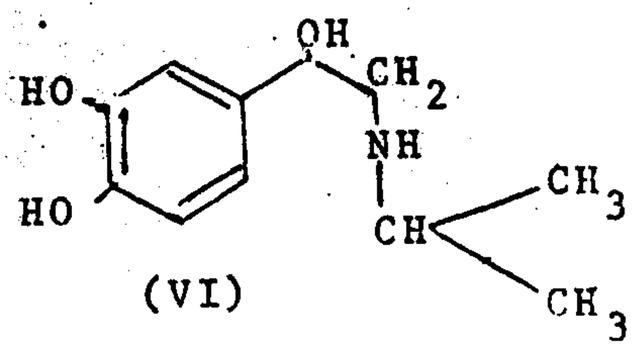

(VI)

Earlier trials to apply directly the condition established for colour formation on interaction of DMDQ with ascorbic acid[10] to isoprenaline proved unsatisfactory as a very weak reaction occured . Trials to potentiate the reaction through application heat failed. So, rigerous investigation to optimise reaction conditions through study of various variables, including solvent used in preparing DMDQ solution, pH of the reaction medium, type and concentration of the buffering system, and addition of the buffering system, and addition of water-miscible nonaqueous solvents was conducted.

Various solvents were used to make the DMDQ solution namely dioxane, dimethylformamide and dimethylsulphoxide. These solvents affect the extent and stability of the colour formed. Dimethylsulphoxide was found to be the best solvent for DMDQ where the resulting solution is stable for 5 hours at room temperature and the intensity of the developed colour is greater in presence of dimethylsulphoxide. 0.05% DMDQ in dimethylsulphoxide was found to be the most suitable concentration.

Prelimenary investigation showed that the rate and intensity of colour formation is optimal at pH 7. At pHs higher than this value the blank liquid is coloured rapidly, while below this value the reaction rate is adversely affected. Different buffers systems namely phosphate buffer[16], Sorensen's phosphate buffer[12] and McIlvaine's citric acid-phosphate buffer[12], were tried to fulfil the pH requirement. However, precipitate formation was associated with the use of all these buffering systems. So, various dilutions of these buffering system ranging from one in two to one in twenty were investigated. Most satisfactory results as high colour intensity, higher stability of the formed colour and absence of precipitate formation were attained upon using McIlvain's citric acid-phosphate buffer. It is obvious from Table I, that dilution of McIlvaine's citric acid-phosphate buffer one in ten produces the highest colour intensity, while Table II showed that pH 7 is the most appropriate pH for the reaction. However, it was found that the optimal volume required from the buffer is 1 ml and the time required for the reaction to reach its maximum is one minute and the reached condition is then preserved by adding one ml isopropanol

before completing the volume with the diluted buffer. The addition of isopropanol proved essential to keep the solution clear and to prevent blank coloration. Larger volumes of isopropanol resulted in a decrease of colour intensity. Solvents other than isopropanol like methanol, ethanol, and acetone were less satisfactory for this purpose.

Dilution of the reaction mixture before spectrophotometric measurmets has been tried with water methanol, propanol, isopropanol, dimethylsulphoxide, and dimethylformamide instead of the diluted McIlvaine's citric acid-phosphate buffer pH 7 , but it was found that the latter gives most satisfactory results while the formers yield non reproducible results and/or lower colour intensity .

Under the established optimal condition, the interaction of isoprenaline with DMDQ results in the formation of a reddish violet coloured product which have absorption peak at 510 nm (Fig. 1) and apparent molar absorptivity of $3.89 \times 10^6$ . The absorbance versus concentration is linear up to 6 mcg isoprenaline sulphate per ml, The lower limit of detection is 1 mcg/ml. The color is stable for at least 30 minutes. This established conditions fulfil the requirement for a rapid and sensitive colorimetric method for isoprenaline.

The suitability of the developed colorimetric method for the detemination of isoprenaline in the various pharmaceutical dosage forms as well as in synthetic mixture was further evaluated. Table III reveals that the application of the developed colorimetric method for the assay of isoprenaline in commercial dosage forms without prior separation gives results closely adher to that claimed and no interference was observed from the compounding ingredients in these preparations as indicated by almost complete recovery of added isoprenaline to samples of these preparations. Further evidence for the absence of interference from compounding ingredients namely, glucose, sucrose, lactose, starch, acacia, sodium metabisulphite, sodium sulphite, glycerol and ethanol which are of potential use in the preparation of solid and liquid dosage form of isoprenaline was proved experimentally. Analysis of synthetic mixtures of these materials

with isoprenaline by the developed method showed almost complete recovery of the added isoprenaline.

It is important to note that the quite similarity in the absorption peak of the formed coloured product from the interaction between DMDQ and isoprenaline and that formed from the interaction between DMDQ and ascorbic acid previously reported[10], gives an evidence for the suggestion that the "indigoid" chromogenic reduction product, is the responsible for the formed colour in both cases. The responsibility of the catechol function of isoprenaline for the transformation of DMDQ into the indigoid is evidenced by the experimental result that no colour is formed when the reaction condition was applied to phenylephrine which contains only one phenolic group in the benzene ring and orciprenaline which is quite similar to isoprenaline with the exception that the two phenolic groups are meta to each other. Further when isoprenaline was oxidized to N-isopropyl noradrenochrome [14] in which the phenolic groups are already transformed to the quinonoid structure, it failed to develop the colour reaction upon interaction with DMDQ .

So, in addition to the clearly obvious advantages offered by the proposed method for determination of isoprenaline, as rapidity , simplicity, high sensitivity and selectivity, this method can be recommended safely as stability indicating assay for oxidative degradation involving the catecholic function of isoprenaline .

**Table III : Analysis of Isoprenaline in pharmaceutical preparations**

| *Formulation* | *Claimed* | *Found* | | *Added* | *Recovery* x | |
|---|---|---|---|---|---|---|
| | *mg* | *mg* | *%* | | *mg* | *%* |
| Tablets (A) | 20/tabl. | 19.5 | 96.5 (SD=0.54) | 10 | 9.95 | 99.5 (SD=1.05) |
| Tablets (B) | 10/tabl. | 9.64 | 96.4 (SD=0.62) | 10 | 9.80 | 98.00 (SD=0.75) |
| Solution | 10/g | 9.90 | 99.0 (SD=0.75) | 10 | 9.87 | 98.7 (SD=0.17) |

x Average of 5 determinations .

**Table I : Effect of different dilutions of pH 7 McIlvaine's citric acid-phosphate buffer on the intensity of the developed colour at $\lambda_{max}$ 510 nm .**

| *Dilution ratio* | *Absorbance* $\bar{x}_i$ |
|---|---|
| 1 in 2 | 0.120 |
| 1 in 5 | 0.201 |
| 1 in 10 | 0.280 |
| 1 in 15 | 0.250 |
| 1 in 20 | 0.200 |

x Average of 4 determinations for a final concentration of 4 mcg/ml.

**Table II : Effect of different pH values of McIlvaine's citric acid-phosphate buffer (1 in 10) on the intensity of the colour and absorption $\lambda_{max}$**

| *pH* | $\lambda_{max}$ *nm* | *Absorbance* x |
|---|---|---|
| 6.6 | -- | -- |
| 6.8 | 520 | 0.175 |
| 7.0 | 510 | 0.280 |
| 7.2 | 450 | 0.200 |
| 7.4 | 450 | 0.032 |

x Average of 4 determinations for a final concentration of 4 mcg/ml .

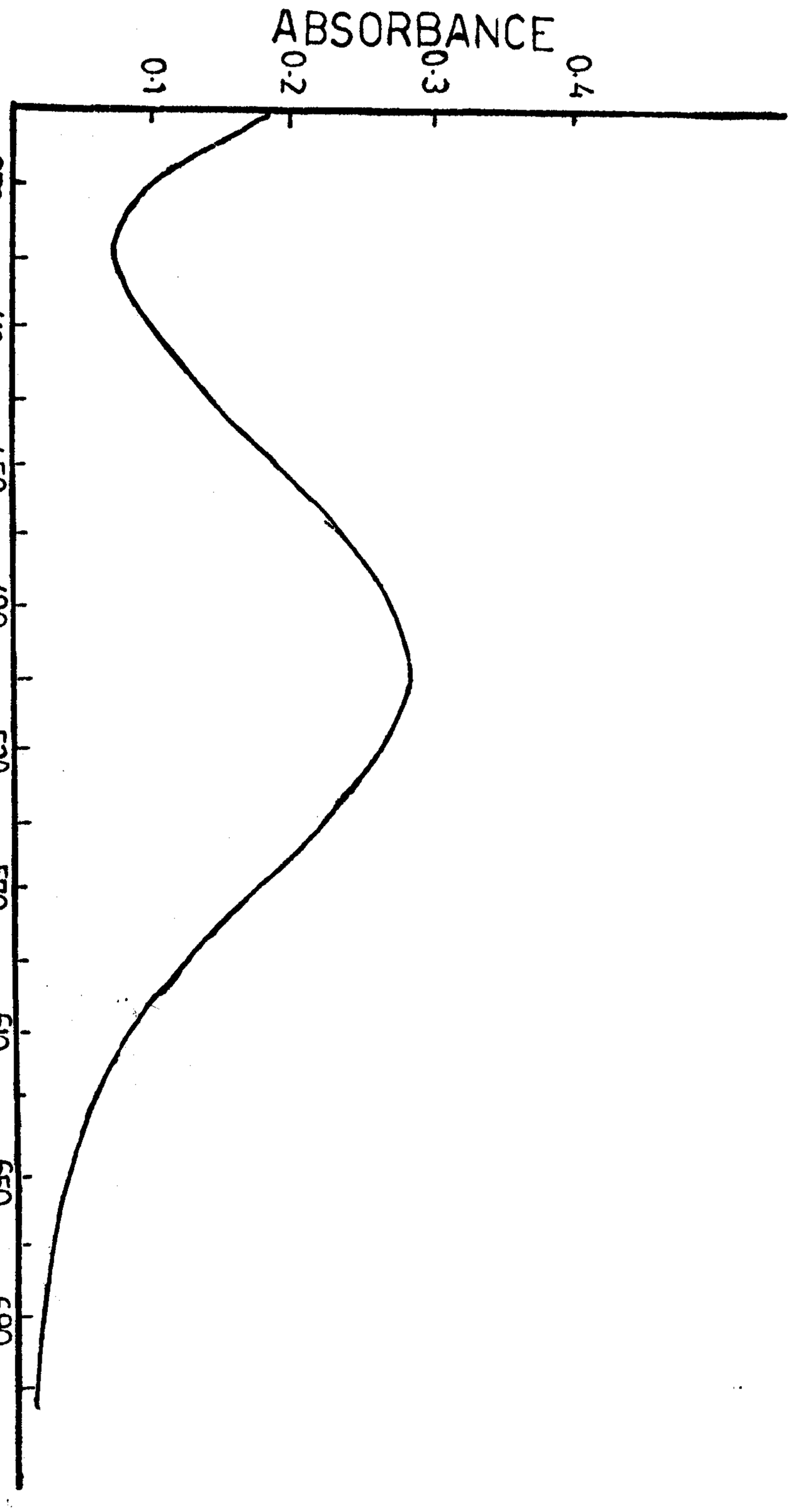


Fig. 1: Absorption spectrum of the coloured product of interaction of isoprenaline with DMDQ.

## *REFERENCES*


(1) *"The United States Pharmacopeial" , 19th rev., Mack Publishing Co., Easton, Pa., 1975 p. 274.*

(2) *"The British Pharmacopoeia 1973" , Her Majesty's Stationary Office, London, England, 1973, p. 257 .*

(3) *"The National Fornulary" 14th ed., Mack Publishing Co., Easton, Pa., 1975, p. 368 .*

(4) *Halmekoski J., Kivinen A. , Farm. Aikak,, 75, 225 (1966) through Chem. Abstr, 57969 (1967).*

(5) *El-Rabbat Nawal A. and Omar N. M. J. Phar, Sci, 67, 779 (1978).*

(6) *Welsh L. H.and Sammul O. R. , J. Ass. Offic. Anal. Chem., 51 , 176 (1968) .*

(7) *Helloerg H. Sr. Farm. Tidskr., 64 493 (1960).*

(8) *Prasad V. K. and Ricci R. A. , B., C. Nunningm and A. P. Grantekm J. Pharm. Sci., 62, 1135 (1973).*

(9) *Watson J. R., and Lawrence R. C., J. Pharm. Sci., 66 , 561 (1977).*

(10) *Eldawy Mohamed A. , Tawfik A. S. and Elshabouri S. R., Anal Chem. 47 , 461 (1975).*

(11) *Erdtmanm Proc. Roy. Soc., Sec., 143, 211 (1934).*

(12) *Documenta Geigy "Scientific Tables". J. R. Geigy S. A., Basle Switzerland, p. 314.*

(13) *Reference (8) , p. 1130 .*

(14) *"Progress in Medicinal Chemistry" volum 9 , Ellis G. and West G. Eds., Elsevier Amestrdan. The Netherlandsm, 1973, p. 284 .*

(15) *Ibid , p. 291 .*

(16) *Reference (1), P. 939.*

" تقييم لونـى سـريع وحساس للايزوبرنالين "
سـلوى رزق الشابورى ـ السيد على ابراهيـم"
قسمى الكيمياء الصيدليـة والصيدلانيـات ـ كلية الصيدلة ـ جامعة اسيوط

يصـف هـذا البحث طريقـة لونيـة سهلة وتخصصية وذات حساسة فائقة وسريعة لعقار الايزوبرنالين • وتعتمـد هذه الطريقـة على التفاعـل بين ثنائى النيتروكسـى ثنائى الكيتون وكبريتات الايزوبرنالين تحـت ظروف معينة حيث يتكون ناتـج ذو لـون فائـق لـه ذروة امتصاص للضوء عنـد موجـه طولهـا ٥١٠ ن•م ومعامـل امتصـاص جزيئى ظاهـرى قـدره ٣٫٨ × ١٠^٦

وتوجد علاقـة خطيـة بين تركيـز الايزوبرنالين فى المحـلول ومقدار امتصاص الضوء للناتج الملون عنـد ذروة الامتصاص ٥١٠ ن م حتى تركيز ٦ ميكروجرام فى المليلتر• بحد ادنـى للحساسية قدره واحد ميكروجرام فى المليلتر•

والطريقة المقدمة صالحة للتطبيق فى تقييم الايزوبرنالين فى مستحضراته الصيدلية المسوقة مباشـرة دون الحاجة الى عمليـة فصل مسبق للايزوبرنالين من المكونات الاخرى الموجـودة فى هـذه المستحضرات•

اعطت هذه الطريقة نتائج تتفق مع ماذكره الصانع وكذلك ثبت علميا ان الصوغ والاضافات التى تستعمل فى تركيب المستحضرات المستحضرات الصيدلية السائلة والصلبة لاتتداخـل فى عمليـة التقييم المقترحـة

وكذلك فان الطريقة المقدمة يمكن الاعتماد عليها لقياس مدى التلف التأكسدى للعقار عندما يتضمن هذا التلف التأكسـدى مجموعة الكيتول بالجزئ•