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Spectrophotometric Determination of Lisinopril Dihydrate Using Dye Bleaching Method in Pure and Pharmaceutical Drugs

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A B S T R A C T

Through oxidizing Lisinopril dihydrate (LIS) with known excess N-bromosuccinimide (NBS) as an oxidizing agent, a spectrophotometric method based on the bleaching of 5,5′ -indigo disulfonic acid sodium salt (IG) dye in an acidic medium is presented. This method is accurate, straightforward, and precise. The oxidizing agent residue functions as a bleach for the sodium salt (IG) color 5,5′ -indigodisulfonic acid. After the experimental conditions were optimized, the dye's intensity was measured at 610 nm. Beer's law was applied to the proposed method, and it was valid within a concentration range of 2.5–50 µg/mL, and the linear regression was R2 = 0.9968. The limit of quantitation was 2.5 µg/mL, and the molar absorptivity coefficient (ε) 1.8985×10^3 L/mol.cm, Sandell's sensitivity was 0.2325 µg/cm^2. The tablet's excipients don't interact with anything. The current method worked well for determining the amount of lisinopril (LIS) in tablet formulation, with mean recoveries ranging from 98.24 to 102.1 per cent. The student's t-and-F-test were used to statistically compare the data with those of a standard reference.

Keywords: *Lisinopril (LIS), N-bromosuccinimide (NBS), 5,5'-indigodisulfonic acid sodium salt (IG), redox reaction, spectrophotometric.*

التقدير الطيفي لليزونوبريل ثنائي الهيدرات باستخدام قصر الصبغة في شكله النقي ومستحضره الصيدلاني

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الملخص

من خلال أكسدة ليسينوبريل (LIS) بكمية زائدة معروفة من بروموسوكسينيميد (NBS) كعامل مؤكسد، تُقدم طريقة قياس طيفي فلوري تعتمد على تبييض صبغة ملح الصوديوم (IG) من حمض 5,5'-نيلي ثنائي السلفونيك في وسط حمضي. تتميز هذه الطريقة بالدقة والوضوح. يعمل بقايا عامل الأكسدة كمبيض للون ملح الصوديوم (IG) من حمض 5,5'-نيلي ثنائي السلفونيك. بعد تحسين الظروف التجريبية، قُيست شدة الصبغة عند طول موجي 610 نانومتر. طُبق قانون بير على الطريقة المقترحة، وكانت صالحة ضمن نطاق تركيز يتراوح بين 2.5 و50 ميكروغرام/مل، وكان الانحدار الخطي 0.9968 = R2. كان حد القياس 2.5 ميكروغرام/مل، ومعامل الامتصاص المولي(ε) 1.8985×10^3 لتر مول/سم. كان دالة ساندل 0.2325 ميكروغرام/سم^2. لا تتفاعل سواغات القرص مع أي شيء. نجحت الطريقة الحالية في تحديد كمية ليزينوبريل (LIS) في تركيبة القرص، حيث تراوح متوسط نسبة الاسترداد بين 98.24% و102.1% استُخدم اختبار (t-and-F) لمقارنة البيانات إحصائيًا مع بيانات مرجعية القياسية.

الكلمات المفتاحية: *ليزينوبريل (LIS)، N- بروموسوكسينيميد (NBS)، ملح الصوديوم 5,5'-حمض إنديجو ديسلفونيك (IG)، تفاعل الأكسدة والاختزال، مطيافية ضوئية.*

1. Introduction

Lisinopril dehydrate (LIS) {(S)-1-[N2-(1-carboxy-3-phenylpropyl)-L-proline] dihydrate}, is an angiotensin-converting enzyme inhibitor used to treat excessive blood pressure, heart failure, and heart attacks, as well as of problems renal and retinal complications of diabetic injured [1], [2], [3] Many methods of weight estimation LIS in pharmaceutical formulations have been published in the literature. High-performance liquid chromatography(HPLC) is one of them [4], Gas liquid chromatographic(GC) [5], liquid chromatography technique (LC) [6], spectrophotometry [7], capillary electrophoresis [8], Single-crystal X-ray structure [9], radioimmunoassay [10]. Many methods have been used for the simultaneous determination of LIS. Considered a UV-Visible spectrophotometric method for the determination of LIS in raw and pharmaceutical products is an obvious economical, rapid, and selective method, particularly for routine quality control analysis of pharmaceutical products [11], [12].

Here has been used a method which is based on the color bleaching of 5,5′-indigodisulfonic acid sodium salt (IG) is one of the most commonly used dyes worldwide as a dye. It has another name is indigo carmine, which enters into reduction–oxidation (redox) processes required for dyeing [13]. The development of quantitative spectrophotometric methods was the primary objective for the determination of LIS Figure 1 [14], 5,5′-indigodisulfonic acid sodium salt is used for medical diagnostic purposes [15], and N-bromosuccinimide (NBS) acts as an oxidizing agent, especially in an acid medium [16], [17].

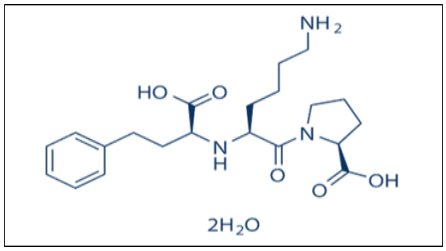

Figure 1. Chemical structure of LIS

The research paper describes a sensitive and accurate spectrophotometric method for the determination of LIS in pharmaceutical preparations. This is confirmed by comparative results with data from the British Pharmacopoeia Commission.

2. Experimental

2.1. Apparatus

The JASCOV-360 digital spectrophotometer, which has 1-cm glass cells, was used to record all spectrophotometric observations. Samples were added using a Gilson micropipette with disposable tips.

2.2. Chemicals and Reagents

LIS was supplied by Meryer (Shanghai) Chemical Technology, China. LIS tablets were 5,10 mg (Accord, UK) and 5,10mg (Bristol, UK), and N-bromosuccinimide (NBS) and Hydrochloric acid HCl, nitric acid HNO_3, acetic acid CH_3COOH and sulfuric acid H_2SO_4 (Fluka, UK), 5,5′-indigodisulfonic acid sodium salt ((IG) (FLINN scientific, Canada).

2.3. Preparation of Standard Solution

0.1 g of pure LIS powder was dissolved in an adequate amount of distilled water to create a stock solution of pure LIS (1000 µg/mL), which was then transferred to a 100 mL volumetric flask using the same solvent used to prepare standard solutions (500 µg/mL) every day. All of LIS's pharmaceutical products are precisely weighed for seven tablets, and the average weight of one tablet is determined. Pure LIS is made with the same concentration by dissolving it in the proper amount of distilled water, stirring it for fifteen minutes, and then filtering it to remove any insoluble excipients using Whatman filter paper. Using the same solvent, transfer the solution to a 100 mL volumetric flask.

2.4. Preparation of the reagent

NBS was prepared (1×10^{-3}M) in a 100 mL volumetric flask by dissolving 0.0177 g, and 100 µg/mL of IG dye was prepared by dissolving 0.01 g in the appropriate amount of distilled water. The mixture was then transferred to a 100 mL volumetric flask, and 8.4 mL of HCl was added to the same volume of the flask. At 610 nm, the absorbance of LIS was measured.

3. Results and Discussion

Due to the presence of an amine group (NH_2), LISacts as a reducing agent. The suggested mechanism of reaction is shown in Figure 2.

3.1. Preliminary study and absorption spectrum

To assess LIS in pure medicines and tablets, reaction conditions, as well as other experimental

parameters, influence dye stability and color development. As a result, it is vital to choose

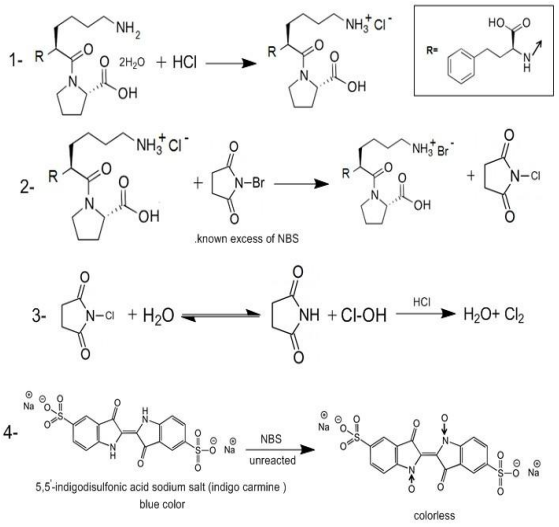

Figure 2. The suggested mechanism of reaction of LIS with reagents

circumstances that produce the highest absorption value with the best arithmetic yield, while also considering laboratory safety.

3.1.1. Selection of IG dye concentrations

A series of varying quantities of IG dye in the range of 1–14 µg/mL was taken in an acidic medium and then finished with distilled water in 10 mL volumetric flasks. At wavelength 610, absorbance was observed in comparison to a blank that contained only distilled water Figure 3.

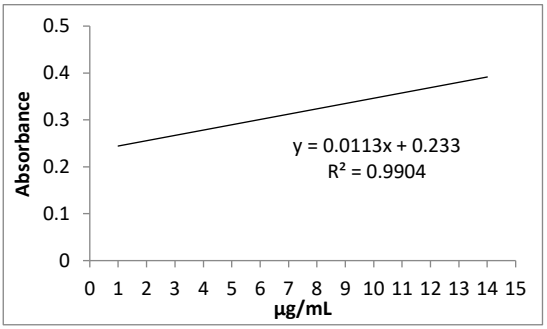

Figure 3. Absorbance vs. solutions that contain different volumes of IG dye

1.0 ml was chosen because it represents the last volume within the linear range, after which the deviation occurs at a value of (R2 = 0.9983), and the volume was adopted as a fixed value in subsequent experiments.

3.1.2. Selection of type and acid volume

Taken 1mL from 1M HCl, nitric acid, acetic acid and sulfuric acid individually and added to four volumetric flasks, 10mL contains 1mL (LIS), dye, NBS, and completed to 10mL with distilled water, giving Hydrochloric acid highly absorbance Figure 4., and the highest absorbance was observed with 0.5 mL of 1M HCl Figure 5., Adopted this volume and concentration in subsequent experiments.

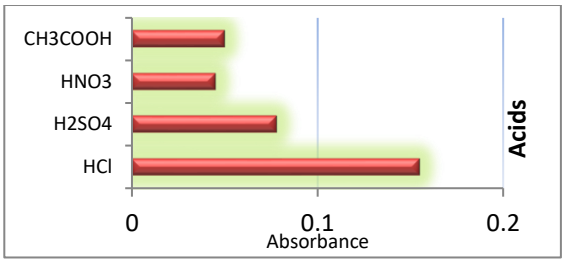

Figure 4. Absorbance vs. different acids

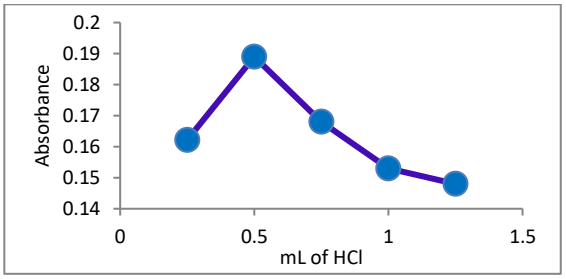

Figure 5. Absorbance vs. different volumes of HCl

3.1.3. Selection of type and volume oxidizing agent

Taking 0.5mL from N-bromosuccinimide (NBS), N-chlorosuccinimide (NCS), sodium periodate ($NaIO_4$), and potassium periodate (KIO_4), gave (NBS) high absorbance. Figure 6, after that prepared different volumes (0.1-1.3mL) in 10mL volumetric flasks, completed the volume with distilled water and 0.5mL IM HCl,1mL IG dye, and the results obtained in Figure 7., The appropriate volume of the oxidizing agent is 0.6mL. Subsequent experiments were performed with this volume of NBS.

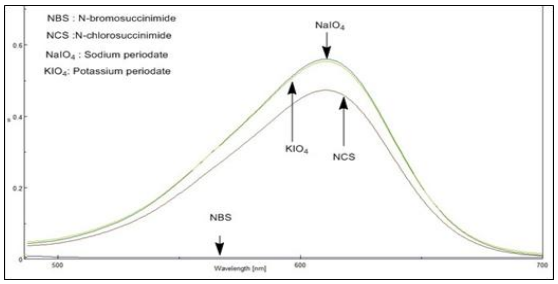

Figure 6. Absorption spectra of oxidation agents

3.1.4. Time of reaction and stability dye color

Time is a factor in the completion of the oxidation-reduction reaction. 1mL (500 µg/mL) LIS(with 0.6 mL (1× 10^{-3}M) NBS solution in 1 mL (1M) of HCl

acidic medium and bleaching of 1 mL (100 µg/mL) IG dye in 10 mL volumetric flasks and completed the volume with DW. The absorbance was attained at different times at room temperature 25 ± 2°C Table 1. IG dye was added at different times after the mixture

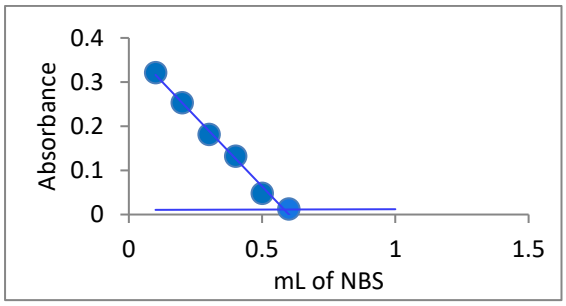

Figure 7**.** Calibration curve of the spectrophotometric indirect volume of the NBS

of reagents with LIS, then the appropriate times to mix the reaction mixture [18].

After selection of reaction, the stability of IG dye and the appropriate temperature for reaction, which gives maximum absorbance, the maximum absorbance was at room temperature 25 ± 2°C Table 2.

3.1.5. Interferences and surfactants studies

Under optimum conditions, the effects of common excipients added to pharmaceutical drugs of LIS tablets as possible interferences were studied [17]. Arabian gum, fructose, starch, sucrose, and glucose were individually mixed with 50µg/mL LIS pure in the final volume of 10 mL. The error E% is less than ±5%, the level of interference is considered acceptable [19] as well as The following surfactants (CTAB, CBC, SDS) were also studied. Under the established conditions, no significant positive change was observed due to the addition of common excipients and surfactants when determining LIS Table 3A and B.

3.1.6. Order of Addition

A series of 10mL volumetric flasks contains of 1mL (500) LIS pure, 0.5mL (1M) HCl, 0.5mL (1× 10-3M) NBS addition in different sequences. Studied the effect through investigated by bleaching of the color of IG dye and measuring its absorbance at 610 nm. Best absorbance was achieved in the order of drug, HCl, NBS and dye, Figure 8.

Table 1. Time of oxidation-reduction reaction for IG dye

Time oxidant	Time added of dye (min)				
(min)	5	10	15	20	25
5	0.102	0.088	0.096	0.103	0.096
10	0.196	0.185	0.169	0.221	0.199
15	0.289	0.291	0.295	0.296	0.301
20	0.35	0.331	0.364	0.364	0.364
25	0.362	0.362	0.361	0.362	0.362

Table 2. Stability dye color reaction for IG dye

Temp.(Ċ)	Time to stay the sample in temperatures (min)									
	5	10	15	20	25	30	35	40	50	60
10	0.337	0.337	0.334	0.332	0.332	0.325	0.325	0.325	0.320	0.320
RT	0.361	0.362	0.364	0.364	0.362	0.362	0.362	0.360	0.350	0.360
30	0.353	0.353	0.354	0.353	0.352	0.352	0.352	0.351	0.350	0.349
40	0.342	0.342	0.342	0.341	0.341	0.341	0.341	0.340	0.339	0.339

Table 3a. The effect of Interference from common excipients on LIS

Excipients	concentrations (µg/mL)	E%
Arabian gum	100	-1.9
fructose	100	1.2
Starch	100	-1.7
Glucose	100	0.9
Sucrose	100	2

Table 3b. Effect of surfactants on LIS

surfactants	concentrations (µg/mL)	E%
CTAB	100	-0.6
CBC	100	-1.02
SDS	100	-0.17
With out	0	0

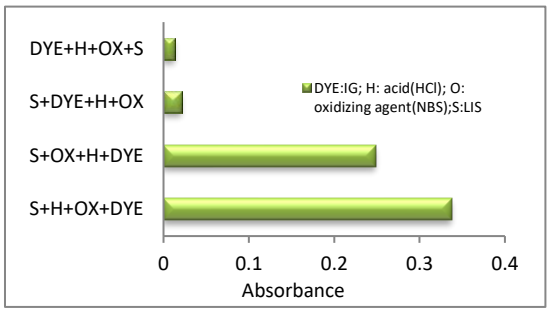

Figure 8. Absorbance of different additions of mixed reaction

3.2. Statistical computations and the calibration curve

In 10 ml volumetric flasks, pure LIS was progressively added in doses ranging from 2.5 to 60 µg/ml. Separately, 0.5 ml (1 mol) of hydrochloric acid, 0.5 ml (1×10-3 mol) of NBS, and 1 ml (100 µg/ml) of IG dye were added. After that measured the absorbance at 610 nm in comparison to the blank, Figure 9. The calibration curve was computed using the regression equation derived from Beer's law.[20].

3.3. Accuracy and precision of the method

Four ascending concentrations (repeated three times) were taken from the calibration curve of LIS. These concentrations were within the linearity of the calibration curve and measured at the selected wavelength under the ideal conditions. The results are shown in Table 4.

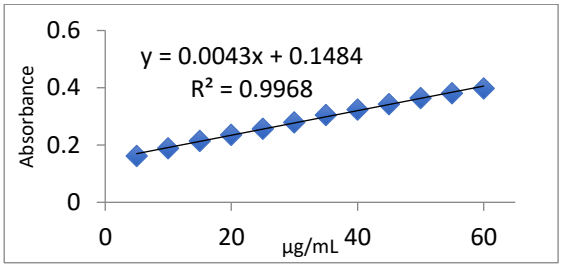

Figure 9. Calibration curve of LIS pure with NBS and dye

3.4. Application to Pharmaceutical Preparations and Statistical Operations

After establishing optimal conditions, the method was applied to two pharmaceutical companies. Statistical calculations were performed to demonstrate the method's success and results by comparing the standard LIS analysis with the British Pharmacopoeia methods [21]. The F-test and T-test values were calculated, at 95% confidence interval was used for three degrees of freedom. Table 5. shows that the results obtained showed acceptable recovery, confirming the success of the method [22], [23].

Table 4. Accuracy of the proposed method and its precision

The concentration	Found from the proposed method	SD	RSD%	E%	REC%
10	10.25	0.00325	1.01900	1.7600	101.7
30	30.16	0.00676	2.07851	0.4216	100.4
50	50.45	0.00359	0.97770	0.90609	100.9
60	59.41	0.005068	1.28577	-0.97965	99.02

SD: Standerd deviation; RSD: Relative Standerd deviation; E; relative error; RSD:RELATIVE Recovery

Table 5. Application of the proposed approach to the determination of the studied compounds in drugs

tablets	Observed values (mg)	Values from the suggested approach	RSD%*	E%*	REC%*	B.Ph	t and F test value**
Accord UK	5	4.95±0.05	2.188	2.95	97.05	99.7	t= -1.47;F= 0.15
	10	9.8±0.17	2.063	-0.43	100.4		t= 0.35;F= 0.16
Bristol UK	5	4.88±0.12	2.364	0.57	99.43		t= -0.18;F= 0.14
	10	10.27±0.27	1.318	0.56	99.44		t= -0.17;F= 0.26

*Average of three determinations, ** 95% Confidence Interval of the Difference

3.5. Comparison of the Method

The results of the current method were compared with previous research papers, The results of the current method were compared with previous research papers, comparing the reaction medium, which represents the most important part of the experiment, the more non-toxic or hazardous solvents the reaction medium contains, the better the method is. In addition to the reaction range, which provides an intermediate area for stimation without interference in concentrations, the closest concentration that the method can sense can be included, which represents one of the important parameters from which the accuracy of the method is measured. One of the important pillars is the wavelength used in the estimation, as the more it moves towards the visible region, the higher the selectivity of the method, as it moves away from wavelengths that overlap with other compounds. It was noted that the wavelength was outside the interference limits compared to other methods, and the sensitivity was higher, in addition to the safer working medium (water) compared to other methods Table 6.

Table 6. Comparison of the method with other methods in previous research papers

Reagent	λ_{max} (nm)	Linear Range µg/mL	Molar absorptivity L/mol.cm	LOD	medium	Ref
(IG)	610	2.5–50	1.899×10^3	0.005	H_2O	This work
Alizarine	432	4.415-300.23	1.619×10^3	NA	1:2H_2O:C_2H_5OH	[24]
1-fluoro-2,4-dinitrobenzene	405.5	8.0–120	0.323×10^{-4}	0.023	acetonitrile	[25]
N-bromosuccinimide	520	25-600	7.28×10^2	0.94	acetone	[26]
p-chloranilic acid	525	25–300	1.192×10^3	0.277	methanol	[27]

NA: Not Available.

Conclusions

Despite the difficulty of the chemical structure of the pharmaceutical preparation Lisinopril in entering color reactions, the indirect assay method used in the estimation process succeeded, and its accuracy was good and safer when compared to previously published methods. This provides a good method for estimating pharmaceutical preparations that are difficult to estimate, as they can be estimated using indirect methods. It gives a good range (2.5-50 µg/mL), the molar absorptivity coefficient (ε) 1.8985×10^3 L/mol.cm. Sandell's sensitivity was 0.2325 µg/cm^2, Average RSD was 1.53, Limit of detection and Limit of quantitation were 0.521, 1.739 µg/mL, respectively.

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