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ASSAY OF BIOACTIVE COMPOUND: LERCANIDIPINE HYDROCHLORIDE BY OXIDATIVE COUPLING REACTIONS IN DOSAGE FORMS

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ABSTRACT

Two simple and sensitive spectrophotometric methods have been developed for the assay of bioactive compound: lercanidipine hydrochloride (LER) in bulk and in formulations. The methods A and B are based on the formation of coloured species between the drug and brucine in the presence of sodium periodate in acid medium, or 2, 6-dichloroquinone-4-chlorimide (DCQC) by means of oxidative coupling reactions. For both the methods absorbance was measured at λ_{max} = 520. The methods have been analyzed statistically by applying Student's t- test- and F-test. The systems obeyed the Beer's law in the range 4-20, and 40-120 for method A, and B, respectively. Molar absorptivity values were found to be 2.18 × 10^4, and 2.99 × 10^3 L mol^{-1} cm^{-1}, respectively. Precision (RSD 0.4- 0.5 %) and accuracy (recoveries range from 98.7±1.4 to 101.2±0.4 %) of the developed methods were evaluated.

Keywords: Spectrophotometry, Lercanidipine Hydrochloride, Brucine, Sodium periodate Isopropylalcohol (IPA), 2,6-Dichloroquinone-4-chlorimide (DCQC),

I. INTRODUCTION

Bioactve compound: Lercanidipine hydrochloride (LER) is chemically 2[(3,3-diphenylpropyl)(methyl)amino]-1,1-dimethylethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. This drug is used as a calcium channel blocker in the treatment of hypertension [1]. In literature, a number of analytical methods have been reported for estimation of LER. These methods include HPLC[2-7] , TLC[8] ,Voltammetry[9,10] LC-MS[11], UPLC-MS[12] and few spectrophotometric methods[13-27]. The authors have developed simple and sensitive, spectrophotometric for estimation of LER in bulk drug and formulations.

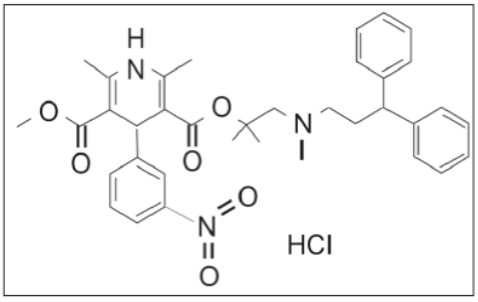

Fig 1: Structure formula of lercanidipine hydrochloride

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II. EXPERIMENTAL

2.1 Apparatus

The measurements were made on a SL-177 model (Elico, India) visible spectrophotometer with 1 cm glass cells and on UNICAM UV 500 spectrophotometer (Thermo Electron Corporation, UK). All pH measurements were made on a LI 120 digital pH meter (Elico, India).

2.2 Reagents and materials

All the reagents and solvents were of analytical grade and all solutions were prepared in deionised water. Aqueous solutions of brucine (BCN) (Loba, India, 0.2%, 5.06×10^{-3} mol L^{-1}), $NaIO_4$ (BDH, India, 0.2%, 9.35×10^{-3} mol L^{-1}), H_2SO_4 (Qualigens, India,1.15 mol L^{-1}), DCQC (Loba, India,0.2%, 9.52×10^{-3} mol L^{-1}), NQS (Loba, India , 0.5%, 1.92×10^{-2} mol L^{-1}), NaOH (Merck, India, 20%, 5 mol L^{-1}), Isopropylalcohol (Merck, India) were used. The bulk drug lercanidipine hydrochloride (Sun Pharmaceutical Ind. Ltd., India) was selected for the study. Two formulations, Lerka (Sun Pharmaceutical Ind. ltd., India) and Lerz (Glenmark, India) containing lercanidipine hydrochloride were purchased from local commercial sources. Tablets equivalent to 10 mg of different batches of two formulations were selected for this study.

About 100 mg of bulk drug was dissolved in 10.0 mL methanol and reduced using standard literature method [28]. The reduced drug solution in methanol was evaporated to dryness. The residue was dissolved and diluted stepwise with distilled water to obtain working standard solutions of concentrations 100 0g mL^{-1} (method A), 400 0g mL^{-1} (for methods B). All the stock and working standard solutions were protected from light by using amber glass material.

2.3 Analytical procedures

2.3.1. Method A (brucine-periodate method). - Aliquots of the working standard solution of the drug (LER : 1.0 - 5.0 mL,100 0g mL^{-1}), 3.0 mL of 5.06×10^{-3} mol L^{-1} brucine, 1.5 ml of 9.35×10^{-3} mol L^{-1} $NaIO_4$ solution and 2.0 mL of 1.15 mol L^{-1} sulphuric acid were added successively into series of calibrated tubes. The volume was brought up to 10.0 mL with distilled water and kept in boiling water bath for 20 min. The solutions were cooled to room temperature and the volume was made up to 25 mL with distilled water. The absorbance of colored species was measured at 520 nm against blank solution within 10 min. The colored species was found to be stable for 40 min. The concentration of LER was computed from the calibration graph.

2.3.2. Method B (DCQC method). - Aliquots of standard drug solution (LER: 1.0 – 3.0 mL, 400 0g mL^{-1}) were transferred into series of calibrated tubes. Then 1.0 mL of 9.52×10^{-3} mol L^{-1} DCQC was added and volume made up to 10.0 mL with isopropanol and kept in hot water bath for 20 min. It was cooled to room temperature and the volume again made up to 10.0 mL with isopropanol. The absorbance of the colored species was measured at 520 nm against blank solution. The colored species was stable for 30 min. The concentration of drug was computed from the calibration curve.

2.4 Pharmaceutical Formulations

Since only two formulations are available for LER (tablets), these formulations of different batches were collected and analyzed as 4 sets to verify the validity of proposed methods. Accurately weighed quantity of tablet powder equivalent to 100 mg of LER was extracted with warm chloroform (3 × 25.0 mL) and filtered. The volume of combined extract was evaporated to dryness, reduced as described in the preparation of standard drug solution (mg mL^{-1}) and working standard solutions of concentrations 100 0g mL^{-1} (method A), 400 0g mL^{-1}

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(method B) were prepared to test the validity of methods developed. The UV spectrophotometric method has been chosen as the reference method [29] for ascertaining the accuracy of the proposed methods.

III. RESULTS &DISCUSSION

The optimum conditions for each method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored species.

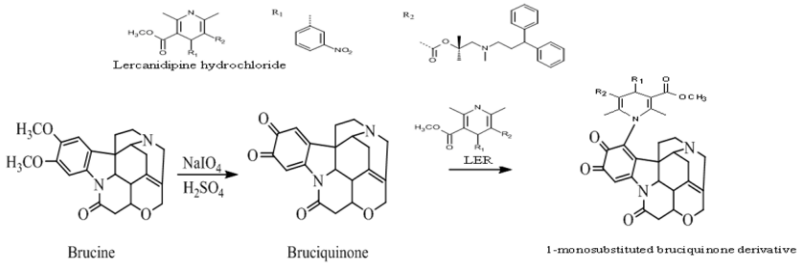
3.1 Optimization of reaction conditions

For brucine-periodate method, the effect of various parameters such as concentration of oxidant and brucine, the order of addition of reagents, effects of solvents on color development and stability of the final colored product were studied. The optimum conditions developed for color development are as follows. 2.5- 3.5 mL (5.06 -7.08 × 10^{-4} mol L^{-1}) of brucine solution, 1.0 -2.0 mL (3.74 -7.48 × 10^{-4} mol L^{-1}) of $NaIO_4$ solution, 1.5 - 2.5 mL (0.069 - 0.115 mol L^{-1}) of H_2SO_4 solution were found to be optimum. In the procedure 3.0 mL (6.07× 10^{-4} mol L^{-1}) of brucine solution, 1.5 mL (5.61 × 10^{-4} mol L^{-1}) of $NaIO_4$ solution, and 2.0 mL (0.092 mol L^{-1}) of H_2SO_4 solutions were found to be optimum conditions. The order of addition which gives maximum absorbance (λ_{max} = 520 nm) is identified as: drug, brucine and $NaIO_4$.

For DCQC method, the effect of various parameters such as concentration of DCQC, solvent for final dilution and stability of the colored species were studied. The optimum conditions developed for the color development are as follows.0.75 -1.5 mL (0.714 – 1.43 × 10^{-3} mol L^{-1}) of DCQC solution, a temperature of 70-80 ^{0}C and the time required for color development is 5-30 min. The solvent isopropanol was used for final dilution and the absorbance of colored species since other solvents like methanol, ethanol, 1, 4-dioxane and DMF ensured lower values of absorbances. The addition of 1.0 mL (0.952 × 10^{-3} mol L^{-1}) of DCQC solution was found necessary for maximum color development. Increasing the volume of DCQC further has no added advantage. Less than 0.75 mL (0.714 × 10^{-3} mol L^{-1}) of DCQC resulted in low absorbance. In the procedure, 1.0 mL (0.952 × 10^{-3} mol L^{-1}) of DCQC solution, a temperature of 70^{0}C and the time required for color development are 10 min. were found to be the optimum conditions. The absorbance of the colored species was measured at 520 nm.

3.2 Mechanism of reactions

Sodium metaperiodate is used as an effective oxidant and it also functions as a color stabilizer. Sastry et.al[30] used brucine-periodate reagent for the spectrophotometric determination of drugs. In the present study, the bruciquinone (formed from brucine and periodate) undergoes nucleophilic attack on the most electron-rich position of coupler (-NH-) in LER to give 1-monosubstituted bruciquinone derivative (Scheme 1).



Scheme--1: Oxidative coupling reaction of LER with brucine -periodate

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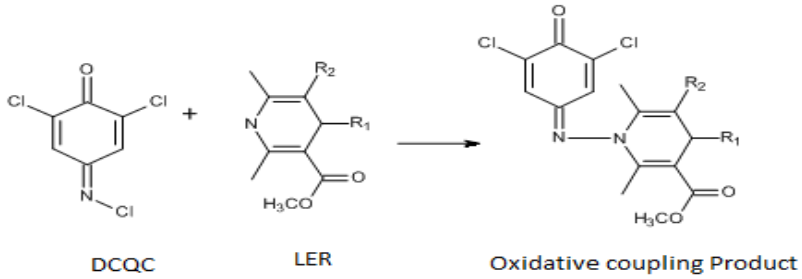
S M Hussan et.al[31] used 2,6 Dichloroquuinone-4-Chloroamide(DCQC) as coupling reagent for the determination of amines. DCQC method involves the reaction of LER with DCQC in the presence of isopropyl alcohol. The imino (-NH-) group in LER couples directly with N-Cl in DCQC to get the colored product (Scheme 2).



Scheme-2: Oxidative coupling reaction of LER with DCQC

3.3 Validation of methods

The developed methods were validated as per ICH guidelines [32]. The optical characteristics of proposed methods were studied whose results are presented in table-1. Regression analysis using the method of least square was made to evaluate the slope (b), intercept (a), and correlation coefficient(R). The results are given in table-2. The results of linearity, precision, and accuracy of the proposed methods are as follows.

3.3.1. Linearity

The linearity was found to be in concentration range of 4 - 20, 40 - 120 0g mL^{-1} with correlation coefficient (R) values 0.9992 and 0.9995 for methods A and B respectively (Table-2)

3.3.2. Precision

Precision is expressed as the relative standard deviation (%RSD) (n=6).The precision of the proposed methods was estimated in terms of inter-day precision and intra-day precision wherein the methods were repeated on six different days and repeated for six different time periods in the same day respectively. The results are presented in table-3

3.3.3. Accuracy

The accuracy of the methods was determined in terms of % recovery of standard LER. Recovery studies were carried out by addition of standard drug solution at three different levels (8, 10, 12 0g mL^{-1}) was added to previously analyzed sample (tablet) solution. Values of recovery ± SD were found to be in the range of 98.7 - 101.2 %. (n=3) indicate that proposed methods are accurate for the analysis of the drug. The results are presented in table-4

The results obtained by the proposed methods and UV spectrometric reference method for the formulations were compared by means of Student's *t*-test and *F*- test and were found that these proposed methods do not differ significantly in precision and accuracy from the reference method.

IV. CONCLUSIONS

The sensitivity and simplicity of the methods proposed together with low cost make them suitable for the assay of bioactive compound : lercandipine hydrochloride (LER) in pharmaceutical formulations. The proposed method

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exploits the functional group in LER. The proposed methods are precise enough to be successfully adopted as an alternative method of GLC or HPLC technique for routine analysis and in formulations.

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Table 1. Optical characteristics, of the proposed methods M_1 & M_2

Optical characteristics	Brucine-IO_4^-	DCQC
λ_{max} (nm)	520	520
Beer's Law limits (µg mL^{-1})	4-20	40-120
Limit of detection (µg mL^{-1})	7.41×10^{-2}	5.51×10^{-1}
Molar absorptivity (l $mol^{-1}cm^{-1}$)	2.18×10^{4}	2.99×10^{3}
Sandell's sensitivity (µg/cm^2/0.001Absorbance unit)	2.96×10^{-2}	2.16×10^{-1}

Table2. Regression parameters of the proposed methods M_1 & M_2

Regression parameters	Brucine-IO_4^- M_1	DCQC M_2
Regression equation ($y = a + bC$)*		
Slope (b)	3.38×10^{-2}	4.6×10^{-3}
Standard deviation on slope (SD_b)	6.29×10^{5}	1.0×10^{-5}
Intercept (a)	7.0×10^{-4}	-8.0×10^{-4}
Standard deviation on Intercept (SD_a)	8.35×10^{-4}	8.49×10^{-4}
Correlation coefficient (r)	0.9992	0.9995

$y = a + bC^*$ where C is the concentration of analyte in µg mL^- and y is the absorbance unit

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Table3. Evaluation of precision and accuracy of the proposed methods M_1 & M_2

Regression parameters	Brucine-IO_4^- M_1	DCQC M_2
Precision (Relative Standard Deviation)*		
Iinter-day precision	0.4629	0.5050
Intra-day precision	0.4625	0.5049
% range of error (confidence limit)		
0.05 level	0.48	0.53
0.01 level	0.76	0.83

Relative standard deviation* : Average of six determinations

Table-4. Assay of LER in pharmaceutical formulations by proposed methods

Formu-Lations	Mass per tablet (mg)	Mass per tablet (mg)[a, b]		Reference method[6]
		Brucine- IO_4^-	DCQC	
Tablet Lerka (Batch-I)	10	9.92 ± 0.13 *F* = 1.81 *t* =1.52	10.05 ± 0.12 *F* = 1.41 *t* = 0.74	9.96 ± 0.10
Lerka (Batch-II)	10	10.09 ± 0.12 *F* = 1.09 *t* =0.30	10.07 ±0 .03 *F* = 4.15 *t* =0.005	10.07 ± 0 .12
Lerez (Batch-I)	10	9.87 ± 0.14 *F* = 1.44 *t* =0.95	10.12 ± 0.04 *F* = 4.56 *t* =2.28	9.92 ± 0.12
Lerez (Batch-II)	10	10.07 ± 0.07 *F* = 2.68 *t* =1.37	9.95 ± 0.11 *F* = 1.09 *t* =0.78	9.92 ± 0.12

a: Average ± standard deviation of six determinations.

b: Theoretical values at 95% confidence limit. *F* = 5.05, *t* = 2.57

Table-5 Recovery studies of LER by proposed methods

Formulations	Amount of the drug in mg	Recovery of proposed methods [a]	
		Brucine-IO_4^-	DCQC
Lerka (Batch-I)	10	99.16□ 1.34	100.5□ 1.2
Lerka (Batch-II)	10	100.9□ 1.2	100.7□ 0.3
Lerez (Batch-I)	10	98.7□ 1.44	101.2□ 0.4
Lerez (Batch-II)	10	100.6□ 0.7	99.55□ 1.1

a: Average value of 3 determinations