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Application of Oxidative Coupling Reactions for Estimation of Lercanidipine Hydrochloride in Formulations

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Two simple and sensitive visible spectrophotometic methods (**A** and **B**) have been developed for the assay of lercanidipine hydrochloride in bulk and formulations. Methods **A** and **B** are based on the formation of colour species by means of forming oxidative coupling reactions with 4-aminophenazone/IO_4 (λ_{max} = 520) and N,N′-dimethylamino-*p*-phenylenediamine/chloramine-T (λ_{max} = 550). The results of analysis have been validated statistically and recovery studies range from 99.4 to 100.5 % for the routine assay of lercanidipine hydrochloride formulations.

Key Words: Spectrophotometry, Lercanidipine hydrochloride, 4-Aminophenazone, N,N-Dimethylamino-*p*-phenylenediamine, Chloramine-T.

INTRODUCTION

Lercanidipine hydrochloride (LER) chemically is 2-{(3,3-diphenylpropyl)-methylamine}-1,1-dimethylethyl-methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine carboxylic ester hydrochloride (Fig. 1) This drug is used as a calcium channel blocker in the treatment of hypertension[1]. The drug is official in Merck Index[2] and Martindale[3]. In literature, a number of analytical methods have been described for estimation of lercanidipine hydrochloride. These include HPLC[4,5], electrophoresis[6], LC-MS[7-11], Extractive spectrophotometric[12] and few visible spectrophotometric[13-22] methods. With an aim to develop relatively cheap, sensitive and useful for the laboratories with modest infra structure, the authors developed a simple, sensitive, accurate, reproducible, reliable and economical analytical method for estimation of lercanidipine hydrochloride in bulk drug and formulations.

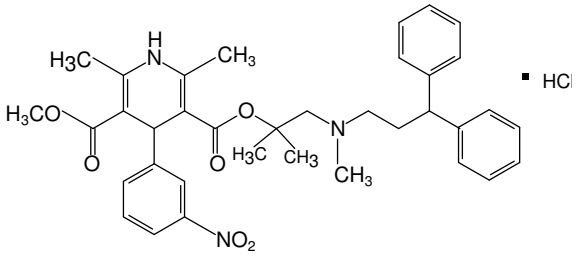

Fig. 1. Chemical structure of lercanidipine

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EXPERIMENTAL

All spectral and absorbance measurements were made on a Elico SL-177 model visible spectrophotometer with 1 cm matched glass cells and on UNICAM UV 500 spectrophotometer made by Thermo Electron Corporation. All pH measurements were made on a Elico LI 120 digital pH meter.

All the reagents were of analytical grade and all solutions were prepared in double distilled water. Aqueous solutions of 4-aminophenazone (4-AP) (Ferak, 0.5 %, 2.46×10^{-2} M), $NaIO_4$ (BDH; 0.2 %, 9.35×10^{-3} M); N,N-dimethylamino-*p*-phenylenediamine (DMPD) (E. Merck; 0.025 %, 1.19×10^{-3} M), chloramine-T (CAT) (Loba; 0.4 %, 1.42×10^{-2} M).

Standard drug solution: About 100 mg of bulk drug was dissolved in 10.0 mL methanol and reduced as per literature method[23]. 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 µg/mL (method **A**) and 5 µg/mL (method **B**).

Recommended procedures

Method A: Aliquots of standard drug solution (1.0-3.0 mL, 5 µg/mL) were transferred into a series of calibrated tubes. Then 2 mL of 2.46×10^{-2} M 4-AP was added to each tube and kept aside for 5 min. After that, to each tube 5.0 mL of 9.35×10^{-3} M $NaIO_4$ solution was added and the total volume was made up to 20.0 mL with distilled water. The absorbance was measured at 520 nm against a similar reagent blank. The stability of coloured species was found to be 5 min. The amount of LER was computed from its calibration graph.

Method B: Aliquots of LER solution (0.5-2.5 mL, 100 µg/mL) were transferred into a series of calibrated tubes. Then 2.0 mL 1.19×10^{-3} M of DMPD solution, 1.0 mL of 1.42×10^{-2} M CAT solution and the solution was made up to 15.0 mL with distilled water. The absorbance was measured immediately at 550 nm against a reagent blank prepared in a similar way. The stability of coloured species were found to be 5 mins. The amount of the drug was estimated from a calibrated graph.

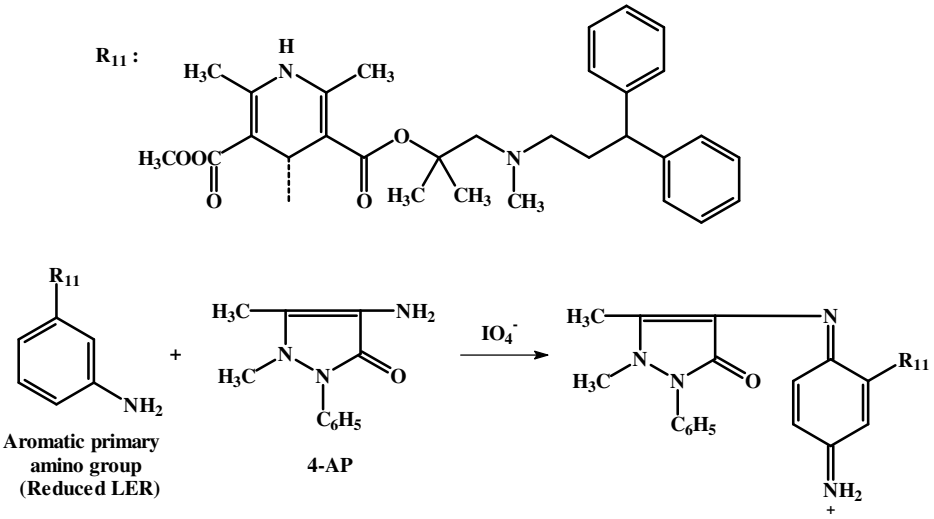
Pharmaceutical formulations: Since only two formulations are available for LER (tablets), these formulations of different batches were collected and analyzed as 4 sets to verify the validity of proposed methods Accurately weighed quantity of tablet powder equivalent to 100 mg of LER was extracted with warm chloroform (3×25.0 mL portions) and filtered. The volume of combined extract was evaporated to dryness, reduced as described in the preparation of standard drug solution and working standard solutions of concentrations 100 µg/mL (method **A**) and 5 µg/mL (method **B**) were prepared to test the validity of methods developed. Further, the UV spectrophotometric method was suggested for the identification of LER has been moulded for its assay and chosen as the reference method for ascertaining the accuracy of the proposed methods.

RESULTS AND DISCUSSION

The optimum conductions for each method were established by varying one parameter at a time[24] and keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

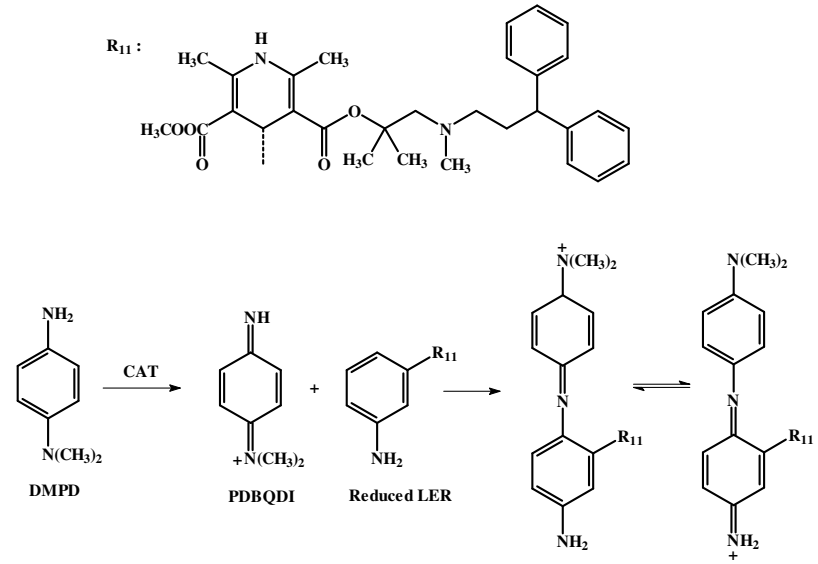
Optimum conditions and mechanism of reaction

Method A: The optimum conditions fixed as: 2.0 mL of 4-AP and 5.0 mL of $NaIO_4$. The addition of drug, 4-aminophenazone (4-AP) and oxidant in that order was found to give maximum absorbance. The coloured species were found to be stable for 15 min and was measured at 520 nm. In the present investigation, the oxidant sodium periodate has been used in combination with 4-AP for the determination of LER which possess reduced aromatic primary amino group. The formation of oxidative coupling product is represented in **Scheme-I**.



Scheme-I

Method B: 2.0 mL of DMPD and 1.0 mL of CAT were found to be optimum conditions. The addition of drug, DMPD and oxidant in that order was found to give maximum absorbance. The coloured species were found to be stable for 15 min and was measured at 550 nm. The course of reaction in the formation of coloured species was explained based on analogy[25]. In the present investigation, DMPD hydrochloride undergoes oxidation in the presence of chloramines-T (CAT) with a two electron transfer to the less stable and highly reactive *p*-N,N-dimethylbenzoquinone diamine (PDBQDI). This species reacts with the coupler (reduced aromatic primary amine) under experimental conditions by electrophilic attack on the most nucleophilc site of the coupler (*i.e.* the *p*-position to the amino group, if the *p*-position is blocked, the *o*-position to the amine).The resulting lecuo-dye is oxidized to the indo-dye (**Scheme-II**).



Scheme-II

Analytical data: The optical characteristics such as Beer's law limits, molar absorptivity and sandell's sensitivity, regression characteristics, the relative standard deviation and percentage of error at 95 % confidence level are given in Table-1. The accuracy of the method for the drug was ascertained by comparing the result by proposed methods and reference method (UV), statistically[24] by t- and F- tests (Table-2). An additional check of accuracy of the proposed method, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulation and results are given in Table-2. This comparison show that there is no significant difference between the results obtained by the proposed methods and those of reference.

Interference studies: The ingredients usually present in the preparation of formulations such as, yellow oxide of iron and titanium dioxide did not interfere with the assay of LER by proposed methods. Commercial formulations(tablets) containing LER were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with t-and F- tests and found not to differ significantly. The results are summarized in Table-2.

Conclusion

The proposed methods exploit the various functional groups in lercanidipine hydrochloride (LER). The ingredients usually present in pharmaceutical formulations did not interfere in the colour development by proposed methods. All the proposed methods are simple, economical and does not require much instrumentation over the literature methods and hence useful for the determination of LER in pure and pharmaceutical formulations.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION, ACCURACY
OF THE PROPOSED METHODS

Optical characteristics	4-AP-IO_4^-	DMPD-CAT
	Method **A**	Method **B**
λ_{max} (nm)	520	550
Beer's Law limits (µg/mL)	0.25-0.75	3.3-16.7
Detection limit (µg/mL)	1.12×10^{-2}	1.21×10^{-1}
Molar absorptivity (l $mol^{-1}cm^{-1}$)	5.159×10^5	3.08×10^4
Sandell's sensitivity (µg/cm^2/0.001 Absorbance unit)	1.3×10^{-3}	2.11×10^{-2}
Regression equation (y = a + bC)		
Slope (b)	7.96×10^{-1}	4.73×10^{-2}
Standard Deviation on slope (S_b)	5.56×10^{-3}	1.73×10^{-4}
Intercept (a)	-1.32×10^{-3}	4.6×10^{-4}
Standard Deviation on intercept (S_a)	2.96×10^{-3}	1.91×10^{-3}
Standard error of estimation (S_e)	2.2×10^{-3}	1.82×10^{-3}
Correlation coefficient (r)	0.9999	0.9999
Optimum photometric range (µg/mL)	0.32-.068	3.5-15.4
Relative Standard Deviation*	0.66	0.73
% of range error (confidence limit)		
0.05 level	0.70	0.77
0.01 level	1.10	1.21
% Error in bulk sample***	0.42	-0.42

*y = a + bC where C is the concentraion of analyte in µg/mL and y is the absorbance unit
**Calculated from six determinations; ***Average error of three determinations.

TABLE-2
ASSAY OF LERCANIDIPINE HYDROCHLORIDE (LER) IN
PHARMACEUTICAL FORMULATIONS

Formulation tablet*	Labelled amount (mg)	Amount found by proposed methods (mg)**		Reference method	% Recovery by	
		(4-AP-IO_4^-) Method **A**	(DMPD-CAT) Method **B**		(4-AP-IO_4^-) Method **A**	(DMPD-CAT) Method **B**
I	10	10.04 ± 0.06 F = 2.5 t = 1.03	9.96 ± 0.09 F = 1.26 t = 0.40	9.96 ± 0.10	100.4 ± 0.6	99.61 ± 0.89
II	10	10.12 ± 0.07 F = 2.92 t = 1.61	9.87 ± 0.16 F = 1.76 t = 1.32	10.07 ± 0.12	101.2 ± 0.7	98.65 ± 1.58
III	10	9.88 ± 0.08 F = 2.44 t = 1.12	10.11 ± 0.07 F = 2.68 t = 1.78	9.92 ± 0.12	98.8 ± 0.77	101.1 ± 0.73
IV	10	10.10 ± 0.09 F = 1.48 t = 1.55	9.91 ± 0.09 F = 1.76 t = 0.42	9.92 ± 0.12	101.0 ± 0.9	99.07 ± 0.86

*Four different samples of tablets; **Average ± standard deviation of six determination, the t and F-test values refer to comparison of the proposed method with the reference method. Theoretical values of 95 % confidence limit, F = 5.05, t = 2.57; ***After adding 3 different amounts of the pure labeled to the pharmaceutical formulations, each value is an average of 3 determinations.

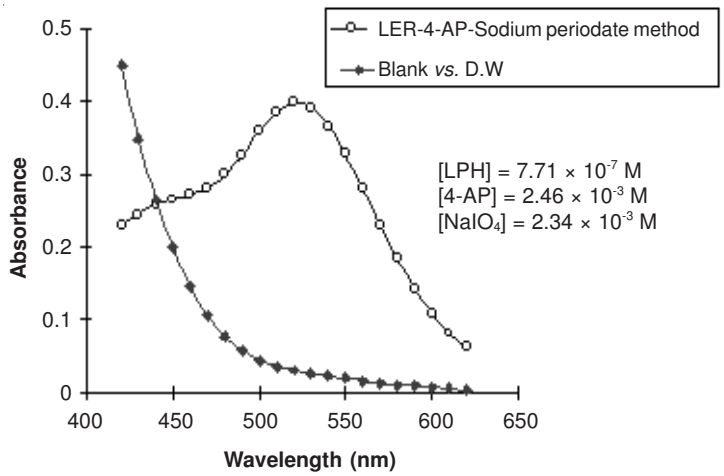


Fig. 2. Absorption spectrum of LER-4-AP-$NaIO_4$ (method **A**)

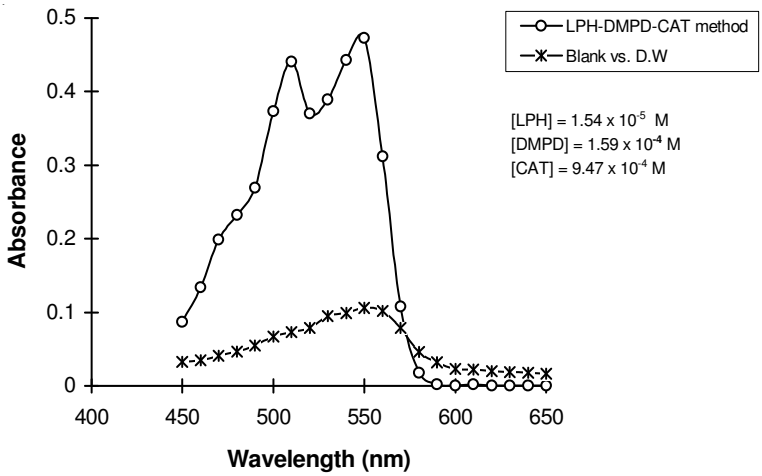


Fig. 3. Absorption spectrum of LER-DMPD-CAT (method **B**)

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