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Assay of Lercanidipine Hydrochloride with Azocaramine-G, Fe(III)/$K_3[Fe(CN)_6]$ and Folin Ciocalteu Reagent

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Three simple and sensitive visible spectrophotometic methods (A-C) have been developed for the assay of lercanidipine hydrochloride in bulk and formulations. Method **A** is based on the formation of ion-association complex with azocaramine-G (ACG) which was extracted into chloroform (λ_{max} = 540 nm). Method **B** is based on the formation of coloured complex with $K_3[Fe(CN)_6]$ in presence of Fe(III) (λ_{max} = 730 nm). Method **C** is based on the oxidation of drug with Folin-Ciocalteu reagent (λ_{max} = 750 nm). The results of analysis have been validated statistically. Recoveries range from 99.4-100.5 % for the routine assay of lercanidipine hydrochloride formulations.

Key Words: Lercanidipinehydrochloride, Azocaramine-G, Fe(III)/$K_3[Fe(CN)_6]$, Folin Ciocalteu reagent.

INTRODUCTION

Lercanidipine hydrochloride (LER) is chemically 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 cited in Merck Index[2] and Martindale[3]. In literature, a number of analytical methods have been described for estimation of LER. These include HPLC[4,5], electrophoresis[6], LC-MS[7,8-11], 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 LER in bulk drug and formulations.

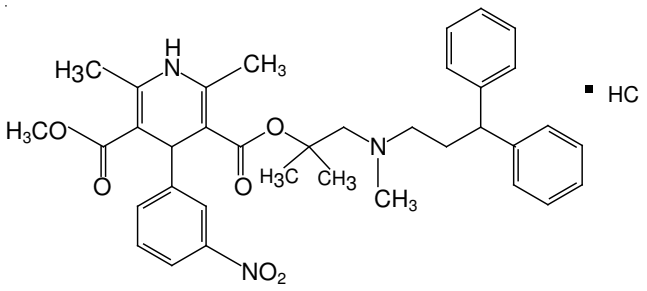


Fig. 1. Chemical structure of lercanidipine

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 ACG (Gurr; 0.05 %, 5.50×10^{-4} M), Fe(III) (Wilson; 0.054 %, 3.32×10^{-3} M), $K_3Fe(CN)_6$ (BDH; 0.1 %, 3.03×10^{-3} M), Na_2CO_3 (BDH; 10 %, 9.43×10^{-1} M), were prepared by dissolving requisite quantities in double distilled water. Folin-Ciocalteu (FC) (Loba; 2N) solution was used as it is. Buffer solution (pH 1.5) was prepared by mixing appropriate concentrations of glycine and HCl (Qualigen, AR Grade).

Standard drug solution: The stock solution (mg/mL) was prepared by dissolving 100 mg of drug in 100 mL of chloroform. Aliquot portion of (25.0 mL) the above stock solution was diluted stepwise with same solvent to obtain working standard solution of 40 µg/mL (method **A**). About 100 mg of bulk drug was dissolved in 10.0 mL methanol and reduced using standard literature method[23]. The reduced drug solution was 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 **B** and **C**).

Recommended procedures

Method A: Into a series of 125.0 mL separating funnels containing aliquots of standard drug solution [LER: 1.0-3.0 mL, 40 µg/mL], 6.0 mL of buffer solution (pH 1.5) and 2.0 mL of 5.50×10^{-4} M dye solution were added. The total volume in separating funnel was adjusted to 15.0 mL with distilled water. To each separating funnel 10.0 mL of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 540 nm against blank. The coloured species were stable for 1 h. The amount of drug present (LER) was deduced from the calibration curve.

Method B: In to a series of calibrated tubes, aliquots of standard drug solution (LER: 1.0-3.0 mL, 100 µg/mL) was transferred and 1.0 mL of 3.32×10^{-3} M $FeCl_3$ solution was added. The tubes were stoppered immediately and shaken well for 5 min. Then 0.5 mL of 3.03×10^{-3} M potassium ferricyanide solution was added into each tube and was closed with lids immediately. After 5 min, 1.0 mL of 1 N HCl was added and the final volume was made upto 20.0 mL with distilled water. The absorbance of the solution in each tube was measured immediately at 730 nm for (LER). The stability of coloured species was found to be 1 h. The amount of the drug (LER) was calculated from its calibration graph.

Method C: Aliquots of drug solutions (LER: 0.5-2.5 mL, 100 µg mL^{-1}) were delivered into a series of calibrated tubes. To each tube, 1.5 mL of Folin-Ciocalteu reagent (2.0 N) was added. After 3 min, 5.0 mL of 9.43×10^{-1} M Na_2CO_3 (10 %) solution was added and kept aside for 15 min (LER) and then diluted up to the mark (20.0 mL) with distilled water. The absorbance of the blue coloured solution was measured at 750 nm (LER) against corresponding reagent blank. The stability of coloured species was found to be 45 min. The amount of drug was computed from its appropriate calibration graph.

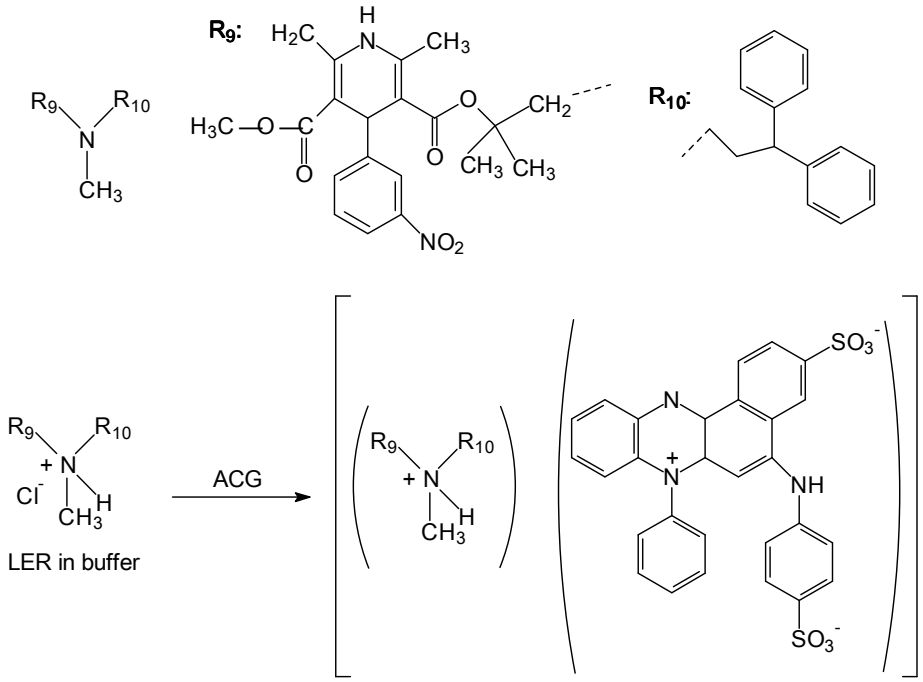
Pharmaceutical formulations: Since only two formulations (Larez and Lerka) are available for LER (tablets), these formulations of different batches were collected and analyzed as 4 sets to verify the validity of proposed methods. Accurately weighed quantity of tablet powder equivalent to 100 mg of LER 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. Working standard solutions of concentrations 40 µg/mL (method **A**) and 100 µg/mL (method **B** and **C**) were prepared to test the validity of methods developed. Further, the UV spectrophotometric method which 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 conditions 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 chemistry of coloured species

Method A: 6.0 mL buffer (pH = 1.5), 2.0 mL of azocaramine-G solution (5.50×10^{-4} M), chloroform as solvent for extraction of coloured species (3:2 ratio of aqueous to organic phase) were identified as optimum conditions. The absorbance of coloured species was measured at 540 nm after 5 min. The drug (LER) possess tertiary amino group in the side chain and this group was involved in ion-association complex formation with azocaramine-G which is extractable into chloroform. The colour formation by azocaramine-G reagent with LER may be explained in the following manner based on the analogy reports[25] (**Scheme-I**). The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be



Scheme-I

interpretable in terms of electronic, steric and other effect of complexing. The protonated nitrogen (positive charge) of the drug (LER) under appropriate experimental conditions is expected to attract the oppositively charged part of dye (negative charge) and behaves as a single unit being held together by electrostatic attraction. This was further confirmed by the mole-ratio of drug to dye (1:1) obtained by slope-ratio studies.

Method B: The optimum conditions in this method were fixed as: 1.0 mL of Fe(III) solution (3.32×10^{-3} M), 0.5 mL of potassium ferricyanide (3.03×10^{-3} M), 1.0 mL of 1 N HCl. The order of addition of reagents is: drug, Fe(III), potassium ferricyanide and 1 N HCl. The role of HCl is for the maintenance of acidity prior to dilution.The absorbance of coloured species was measured at 730 nm after 10 min. In this method, the formation of the coloured complex obtained is due to formation of ferrous ferricyanide complex. Fe(II) produced by the reduction of Fe(III) in the oxidation of drug (LER). This Fe(II) subsequently reacts with potassium ferricyanide to give coloured ferrous ferricyanide complex (**Scheme-II**).

Step 1

$$\underset{\text{(LER)}}{\text{Drug}} + \text{Fe(III)} \longrightarrow \text{Oxidation products of drug} + \text{Fe(II)} + \underset{\text{(Unreacted)}}{\text{Fe(III)}}$$

Step 2

$$3Fe^{2+} + 2Fe(CN)_6^{3-} \longrightarrow Fe_3[Fe(CN)_6]_2$$

Scheme-II

Method C: The method involves the reduction of Folin-Ciocalteu reagent by the drug in the alkaline medium. The optimum conditions found in this method were 1.5 mL of Folin-Ciocalteu reagent (2 N), 5.0 mL of 10 % Na_2CO_3 (9.43×10^{-1} M) and order of addition of reagents are: drug, Folin-Ciocalteu reagent and Na_2CO_3. The absorbance of coloured species was measured at 750 nm after 15 min. The colour formation by Folin-Ciocalteu reagent with LER may be explained in the following manner based on the analogy with the reports of the earlier workers[26]. The mixed acids in the Folin-Ciocalteu preparation are the final chromogen and involve the following chemical species

$3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$ and $3H_2O{\cdot}P_2O_5{\cdot}14WO_3{\cdot}4MoO_3{\cdot}10H_2O$

Lercanidipine hydrochloride probably effects a reduction of 1, 2 or 3 oxygen atoms from tungstate and/or molybdate in Folin-Ciocalteu reagent, thereby producing one or more of the possible reduced species which have a characteristic intense blue colour.

Analytical data: The optical characteristics such as Beer's law limits, molar absorptivity, Sandell's sensitivity and regression characteristics, the relative standard deviation and percentange 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 reference method.

Interference studies: The ingredients usually present in the preparation of formulations such as, yellow oxide of iron and titanium dioxide anticipated in pharmaceutical formulations did not interfere with the assay of LER by proposed methods. Commercial formulations such as Lerez and Lerka (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

Unlike in GLC and HPLC procedures, spectrophotometer involved in present procedure is cheap and simple to operate. The high sensitivity, selectivity and simplicity of the methods

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

Optical characteristics	Azocaramine-G	Fe(III)/$K_3[Fe(CN)_6]$	Folin-Ciocalteu reagent
	Method **A**	Method **B**	Method **C**
λ_{max} (nm)	540	730	750
Beer's law limits (µg/mL)	4-12	5-15	2.5-12.5
Detection limit (µg/mL)	$7.55\text{-}10^{-2}$	$1.05\text{-}10^{-1}$	$5.14\text{-}10^{-2}$
Molar absorptivity (l mol^{-1} cm^{-1})	$3.27\text{-}10^{4}$	$2.94\text{-}10^{4}$	$3.13\text{-}10^{4}$
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	$1.98\text{-}10^{-2}$	$2.2\text{-}10^{-2}$	$2.07\text{-}10^{-2}$
Regression equation (y = a + bC)*			
Slope (b)	$5.06\text{-}10^{-2}$	$4.58\text{-}10^{-2}$	$4.88\text{-}10^{-2}$
Standard deviation on slope (S_b)	$1.5\text{-}10^{-4}$	$1.5\text{-}10^{-4}$	$1.01\text{-}10^{-4}$
Intercept (a)	$-2.0\text{-}10^{-4}$	$-1.2\text{-}10^{-3}$	$-7.0\text{-}10^{-4}$
Standard deviation on intercept (S_a)	$1.27\text{-}10^{-3}$	$1.606\text{-}10^{-3}$	$8.35\text{-}10^{-4}$
Standard error of estimation (S_e)	$9.49\text{-}10^{-4}$	$1.197\text{-}10^{-3}$	$7.96\text{-}10^{-4}$
Correlation coefficient (r)	0.9992	0.9994	0.9997
Optimum photometric range (µg/mL)	4.2-11.5	4.8-14.4	5.2-12.2
Relative standard deviation**	0.46	0.62	0.65
Percentage range of error (confidence limit)			
0.05 level	0.48	0.66	0.68
0.01 level	0.76	1.03	1.07
Percentage error in bulk sample***	-0.24	0.21	-0.27

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

TABLE-2
ASSAY OF LERCANIDIPINE HYDROCHLORIDE PHARMACEUTICAL FORMULATIONS

Formulations*	Labelled amount (mg)	Amount found by proposed method (mg)**			Reference method	Recovery by proposed methods*** (%)		
		M-A (ACG)	M-B (Fe(III)/ $K_3(Fe(CN)_6)$	M-C (F-C)		M-A (ACG)	M-B (Fe(III)/K_3 $(Fe(CN)_6)$	M-C (F-C)
Tablet I	10	10.02 ± 0.08 F = 1.39 t = 0.67	9.98 ± 0.07 F = 2.26 t = 0.32	10.13 ± 0.11 F = 1.12 t = 1.49	9.96 ± 0.1	100.3 ± 0.8	99.76 ± 0.66	101.3 ± 1.1
II	10	10.03 ± 0.11 F = 1.27 t = 0.55	9.90 ± 0.07 F = 2.60 t = 1.53	9.88 ± 0.14 F = 1.39 t =1.27	10.07 ± 0.12	100.3 ± 1.1	99.02 ± 0.74	98.83 ± 1.4
III	10	9.91 ± 0.09 F = 1.76 t = 0.26	9.98 ± 0.13 F = 1.25 t = 1.18	9.87 ± 0.10 F = 1.42 t = 2.15	9.92 ± 0.12	99.07 ± 0.9	99.78 ± 1.34	98.65 ± 1.0
IV	10	10.10 ± 0.16 F = 1.89 t = 1.31	10.00 ± 0.11 F = 1.04 t = 0.61	9.98 ± 0.09 F = 1.60 t = 0.46	9.92 ± 0.12	101.0 ± 1.6	100.0 ± 1.1	99.78 ± 0.9

*Four different samples of tablets. **Average ± standard deviation of six determinations; 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.

proposed make them ideally suitable for the assay of LER in pharmaceutical formulations. The proposed methods exploit the various functional groups in LER. The ingredients usually present in pharmaceutical formulations do not interfere in the colour development by proposed methods. All the proposed methods are simple, economical and do not require much of instrumentation.

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