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Short communication

Assay of lercanidipine hydrochloride in dosage forms using nucleophilic substitution reaction

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A simple and sensitive spectrophotometric method has been developed for the assay of lercanidipine hydrochloride (LER) in bulk and in formulations. The method is based on the formation of coloured species between the drug and 1,2-naphthaquinone-4-sulphonic acid sodium salt (NQS) by means of nucleophilic substitution reaction. Absorbance was measured at λ_{max} = 460 nm. The method was analyzed statistically. The system obeyed the Beer's law in the range 20–100 μg mL^{-1}. Molar absorptivity value was found to be 4.79 × 10^3 L mol^{-1} cm^{-1}. Limits of detection and quantification were found to be as low as 0.04 and 0.13 μg mL^{-1}. Precision (RSD, 0.4 %) and accuracy (recovery 99.2 ± 0.6 to 101.1 ± 0.8 %) of the developed method were evaluated.

Keywords: spectrophotometry, lercanidipine hydrochloride, 1,2-naphthaquinone-4-sulphonic acid sodium salt (NQS)

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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 (Fig. 1). The drug is used as a calcium channel blocker in the treatment of hypertension (1). In literature, a number of analytical methods have been described for estimation of LER. These methods include HPLC (2, 3), TLC (4), voltammetry (5) and a few spectrophotometric methods (6–11). The authors have developed a simple, sensi-

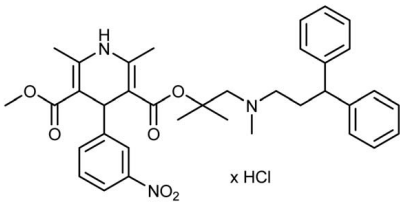

Fig. 1. Chemical structure of lercanidipine hydrochloride.

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tive, accurate, reproducible, reliable and economical analytical method for estimation of LER in bulk drug and in formulations, useful for the laboratories with modest infrastructure.

EXPERIMENTAL

Apparatus

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

Reagents and materials

All reagents and solvents were of analytical grade and all solutions were prepared in deionized water. Aqueous solutions of NQS (Loba, India, 0.5 %, 1.92×10^{-2} mol L^{-1}), NaOH (Merck, India, 20 %, 5 mol L^{-1}), 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.

About 100 mg of bulk drug was dissolved in 10.0 mL methanol and reduced using the standard literature method (12). The reduced drug solution was diluted stepwise with distilled water to obtain the working standard solution of 400 μg mL^{-1} for the proposed method.

Optimization of reaction conditions

Optimum conditions for the 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.

The effects of various parameters such as time, concentration of NQS and NaOH and solvent for final dilution on the stability and intensity of colored species were studied. Optimum conditions were as follows: $0.48 - 1.44 \times 10^{-3}$ mol L^{-1} NQS, 0.75–1.25 mol L^{-1} NaOH, temperature of 28 ± 3 °C and the time required for color development within 8–15 min were found to be optimal. In the procedure, 0.5 mL (0.96×10^{-3} mol L^{-1}) of NQS solution, 2.0 mL (1.0 mol L^{-1}) of NaOH solution, temperature of 28 ± 3 °C and 10 min required for maximum color development were found to be optimum conditions.

General procedure

Aliquots of the standard drug solution (0.5–2.5 mL, 400 μg mL^{-1}) were placed in a series of calibrated tubes. Then, 0.5 mL of 1.92×10^{-2} mol L^{-1} NQS and 2.0 mL of 5 mol L^{-1} sodium hydroxide solutions were added to each tube and made up to the mark with dis-

tilled water. The absorbance of colored species was measured at 460 nm against blank solution after keeping the solutions for 10 min at laboratory temperature. The stability of colored species was found to be 40 min. Drug concentration was computed from the calibration graph.

Figures of merit for detection (*LOD*) and limit of quantification (*LOQ*) were established according to ICH guidelines (14) using the formula: $LOD = k\ SD_a / b$, where k = 3.3, for *LOD* and 10 for *LOQ*. SD_a is the standard deviation of the intercept and b is the slope of the calibration line.

The repeatability of the method was studied by repeating the method six times (n = 6). To study intra-day precision, the method was repeated six times a day. Similarly, the method was repeated on six consecutive days to determine inter-day precision.

The accuracy of the method was determined in terms of % recovery of LER standard. Recovery studies were carried out by addition of standard drug solution at three different levels (8, 10, 12 μg mL^{-1}) to previously analyzed sample (tablet) solution.

Results obtained for *LOD*, *LOQ*, RSD and confidence interval are given in Table 1.

Table I. Optical characteristics, precision and accuracy of the proposed method

Parameter	Value
λ_{max} (nm)	460
Beer's law limits (μg mL^{-1})	20–100
Limit of detection (μg mL^{-1})	0.04
Limit of quantification (μg mL^{-1})	0.13
Molar absorptivity (L mol^{-1} cm^{-1})	4.79×10^3
Regression equation ($y = bc + a$)	
Slope ± SD	$7.4 \times 10^{-3} \pm 1.4 \times 10^{-5}$
Intercept ± SD	$6.0 \times 10^{-4} \pm 9.4 \times 10^{-5}$
Correlation coefficient (R)	0.9999
Presicion (RSD, %)[a]	
Intra-day	0.4
Inter-day	0.5
Range of error[a,b]	
CL (95 %) (μg mL$^{-1)}$)	± 0.44
CL (99 %) (μg mL$^{-1)}$)	± 0.69

[a] n = 6.
[b] LER concentration: 60 μg mL^{-1}.
CL – confidence limits.

Assay of pharmaceutical formulations

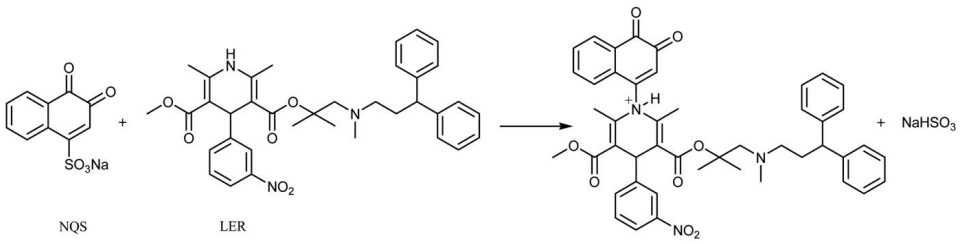
Tablets. – Since only two formulations, Lerka 10 mg and Lerez 10 mg, were available as LER tablets, these formulations were collected from different batches and analyzed as 4 sets to verify the validity of the proposed method. Accurately weighed quantity of tablet

powder equivalent to 100 mg of LER was extracted with warm chloroform (3 × 25.0 mL) and filtered. The combined extract was reduced using the standard literature method (12). The reduced drug solution was diluted stepwise with distilled water to obtain the working standard solution of concentration 400 μg mL^{-1} and appropriate aliquots of the solution were treated as mentioned above in the general procedure to test the validity of the method developed. The UV spectrophotometric method (13) was chosen as the reference method for ascertaining the accuracy of the proposed method.

RESULTS AND DISCUSSION

Reaction mechanism

In the NQS method, imino group of the drug replaces the sulphonic acid group of NQS. The most probable mechanism of nucleophilic substitution reaction of LER with NQS is presented in Scheme 1.



Scheme 1. Scheme of the proposed reaction mechanism

Method validation

The developed method was validated as per ICH guidelines (14) for its linearity, precision, accuracy, and limit of detection.

Linearity and limit of detection/quantification. – Linearity was found in the concentration range 20–100 μg mL^{-1}. Beer's law plots (n = 6) were linear with a correlation coefficient of 0.9999. *LOD* and *LOQ* were found to be as low as 0.04 and 0.13 μg mL^{-1}, respectively (Table I).

Precision. – The RSD values for both intra-day and inter-day precision are 0.4 and 0.5 %, indicating that the proposed method is precise enough for the analysis of LER (Table 1).

Interference studies. – The extents of interference by various excipients that often accompany pharmaceutical formulations are tabulated in Table II. The high percentage of recovery mean value of 99.9% showed that excipients did not interfere with the proposed method.

Table II. Assay of LER in the presence of excipients[a]

Excipient	Concentration (mg mL^{-1})	Recovery (%)[b]
Talc	3.0	99.8 ± 0.2
Macrogol	4.0	100.3 ± 0.4
Polyvinyl alcohol	5.0	99.9 ± 0.6
Yellow iron oxide	3.0	99.7 ± 0.7
Titanium dioxide	4.0	99.8 ± 0.5

[a] Concentration of drug 40 μg mL^{-1}.
[b] Mean ± SD, n = 5.

Table III. Assay of LER in pharmaceutical formulations

Formulations Tablet	Mass per tablet (mg)	Drug found (mg)[a,b]	Literature method (13)[c]	Recovery (%)[d]
Lerka (Batch I)	10	9.93 ± 0.13 F = 1.60 t = 0.33	9.96 ± 0.10	99.3 ± 1.36
Lerka (Batch II)	10	9.98 ± 0.07 F = 3.06 t = 2.09	10.05 ± 0.13	99.9 ± 0.70
Lerez (Batch I)	10	9.93 ± 0.06 F = 3.87 t = 0.33	9.92 ± 0.12	99.3 ± 0.6
Lerez (Batch II)	10	10.11 ± 0.08 F = 1.94 t = 1.68	9.92 ± 0.12	101.1 ± 0.8

[a] Mean ± SD, n = 6.
[b] Theoretical values at 95 % confidence limit: F = 5.05, t = 2.57.
[c] Mean ± SD, n = 3.
[d] n = 3.

Accuracy and application of the proposed method. – Values of recovery ± SD were found to be in the range of 99.2–101.1 % (n = 3), indicating that the proposed method is accurate for the analysis of the drug (Table III).

Application of the proposed method for the assay of pharmaceutical formulations was examined for tablets and the results were statistically compared with those obtained by the literature method (13). The results obtained by the proposed and UV spectrophotometric literature method for the formulations were compared by means of Student's t-test and F-test and it was found that both methods do not to differ significantly in precision and accuracy. The results are summarized in Table III.

CONCLUSIONS

The proposed method is simple, sensitive, accurate and precise enough to be successfully adopted as an alternative to the existing spectrophotometric methods.

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S A Ž E T A K

Određivanje lerkanidipin hidroklorida u ljekovitim oblicima reakcijom nukleofilne supstitucije

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Razvijena je osjetljiva spektrofotometrijska metoda za određivanje lerkanidipin hidroklorida (LER) u čistoj tvari i ljekovitim oblicima. Metoda se temelji na stvaranju obojenih produkata između ljekovite tvari i natrijeve soli 1,2-naftakinon-4-sulfonske kiseline (NQS) reakcijom nukleofilne supstitucije. Apsorbancija je mjerena pri λ_{max} = 460 nm. Metoda je obrađena statistički. Sistem je slijedio Beerov zakon u koncentracijskom području 20–100 mg mL^{-1}. Molarni apsorpcijski koeficijent iznosio je 4,79 × 10^3 L mol^{-1} cm^{-1}, granice detekcije i kvantifikacije 0,04, odnosno 0,13 mg mL^{-1}. Nepreciznost i ispravnost metode iznosile su 0,4 %, odnosno 99,2 ± 0,6 do 101,1 ± 0,8 %.

Ključne riječi: spektrofotometrija, lerkanidipin hidroklorid, natrijeva sol 1,2-naftakinon-4-sulfonske kiseline (NQS)

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