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Extractive spectrophotometric methods for determination of lercanidipine

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Received March 17, 2003, accepted April 14, 2003

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Pharmazie 58: 801–803 (2003)

Extractive spectrophotometric methods are described for the determination of lercanidipine (LER) either in pure form or in pharmaceutical formulations. The methods involve formation of coloured chloroform extractable ion-pair complexes with bromothymol blue (BTB) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 417 and 416 nm for BTB and BCG, respectively. The optimization of the reaction conditions was investigated. Beer's law is obeyed in the concentration ranges 6.0–42.0 $\mu g \cdot ml^{-1}$ or 7.1–43.8 $\mu g \cdot ml^{-1}$ with BTB or BCG, respectively. The composition of the ion-pairs was found to be 1 : 1 by Job's method. The specific absorptivities, molar absorptivities, Sandell sensitivities, standard deviations and percent recoveries were evaluated. Also, LER was determined by measurement of its first derivative signals at 245 nm. Calibration graph was established for 4.2–58.0 $\mu g \cdot ml^{-1}$ of LER. The methods have been applied to the determination of drug in commercial tablets. Results of analysis were validated. No interferences were observed from common pharmaceutical adjuvants.

1. Introduction

Lercanidipine, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 2-[(3,3-diphenylpropyl)methylamino]-1,1-dimethylethyl methyl ester, a new calcium-channel blocker, is used in the treatment of hypertension. Until now only a HPLC and an electrochemical method have been described for the determination in pharmaceutical formulations [1, 2]. Except for these, no other analytical methods have been reported for the determination of LER.

Extractive spectrophotometric procedures are popular for their sensitivity: for diltiazem [3], for maprotiline hydrochloride [4], for some antiallergic drugs [5], for sparfloxacin [6], for ranitidine [7], for ofloxacin and lomefloxacin [8] and for trimethoprim [9]. Therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds. Derivative UV-Vis spectrophotometry involves calculating and plotting one of the mathematical derivatives of a spectral curve, which offers an alternative approach to drug analysis. Although the derivative transformation does not increase the information content of a given spectrum, this method shows good sensitivity and specificity and permits discrimination in the face of the broad band interference arising from turbidity or non-specific matrix absorption.

LER is not yet official in any pharmacopoeia. The goal of the present work was to develop new analytical methods for the quality control of pharmaceutical formulations containing LER.

2. Investigations, results and discussion

Lercanidipine can be transferred from an aqueous phase into an organic phase as an ion-pair formed with the anionic form of an acid dye. The extraction equilibria can be described as follows:

$$RH^{+}_{(aq)} + D^{-}_{(aq)} \leftrightarrow RH^{+}D^{-}_{(aq)} \leftrightarrow RH^{+}D^{-}_{(org)}$$

where RH^+ and D^- represent the protonated LER and the anion of the dye, respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively.

The dyes studied for LER which form ion-pair complexes in acidic buffer were BTB and BCG. These complexes are quantitatively extracted into chloroform. They absorbed maximally at 417 nm for BTB method and at 416 nm for BCG. The spectra and the maxima did not vary with pH. These results indicate that only one type of complex is formed.

Absorption spectra of LER in water were determined by first derivative spectrophotometry. In Fig. 1, the absorption (zero-order) (a) UV spectra and first derivative spectra (b) for the three different concentrations of LER in water are shown. For the determination of LER in bulk and dosage forms by measuring the peak-zero amplitude in the first derivative spectrum at 245 nm was used.

The effect of the extracting solvent on extraction efficiency and color intensity was examined. Chloroform, dichloromethane and 1,2-dichloroethane proved useful solvents; chloroform was selected because of its slightly higher efficiency and considerably lower extraction ability for the reagent blank.

The optimum reaction time was investigated by following the colour development at ambient temperature

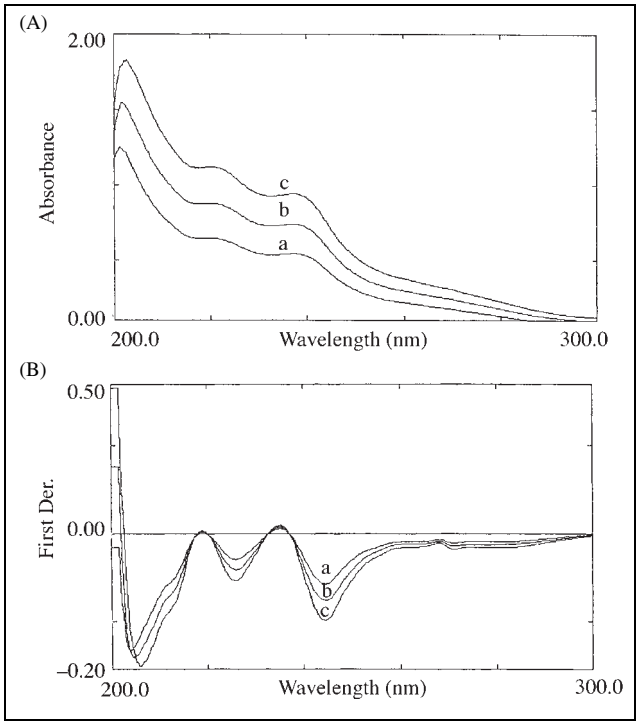

Fig. 1. Zero-order spectra of (A) and first derivative spectra (B) of a) 4.2 $\mu g \cdot ml^{-1}$; b) 31.6 $\mu g \cdot ml^{-1}$ and c) 58.0 $\mu g \cdot ml^{-1}$ LER in water

$(20 \pm 4\ ^{\circ}C)$. Maximal colour intensity was attained after 2 min of mixing for all complexes. Raising the temperature up to 26 °C had no effect on the absorbance, whereas above 26 °C the absorbance started to decay. The absorbance remained stable for at least 2 h.

The effect of pH of buffer solutions on the ion-pairs was studied. Different pH (2.0–9.0) values were tested and the absorbance of the drug-dye complexes was examined. Potassium hydrogen phthalate buffer solution pH 4.0 was found to be the optimum buffer for BTB or BCG complexes.

The effect of the dyes concentrations on the intensity of the color developed at selected wavelengths was tested using different volumes of the reagent. The results show, that, an excess does not affect the absorbance.

In order to apply the proposed method to pharmaceutical formulations, the influence of commonly used excipients (starch, lactose, glucose, sugar, talc, sodium chloride, titanium dioxide, and magnesium stearate) and additives was studied by preparing solutions containing 1.0×10^{-4} M of LER and increasing concentrations of the potential interfer up to 1.0×10^{-3} M. The tolerance of each excipient was conveyed as the largest amount yielding an error of less than $\pm 2.0\%$ of the analytical signal of LER. The maximum tolerable mole ratio was found to be 20–200.

The molar ratio of the reagents (drug: BTB or drug: BCG) in the ion-pair complex was determined by the

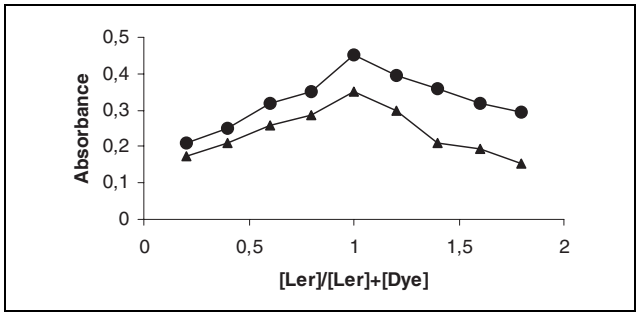

Fig. 2. Continuous variation plots for (a) ● ler-BTB; and (b) ▲ ler-BCG in chloroform (1.0×10^{-4} M)

method of continuous variations (Job's method), and found to be about 1 : 1 (Fig. 2).

The conditional stability constants (K_f) of the ion-pair associates of LER were calculated from the continuous variation data using the following equation:

$$K_f = \frac{A/A_m}{[1 - A/Am]^{n+2} C_M (n)^n} , \quad (1)$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated. C_M is the molar concentration of drug at the maximum absorbance and n is the stoichiometry with which the dye ion associates with the drug. The log K_f values for LER-BTB or LER-BCG complexes were 5.08 ± 0.02, or 4.99 ± 0.11, respectively.

The Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, and correlation coefficient determined for each method are given in Table 1. A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the range 6.0–42.0 $\mu g \cdot ml^{-1}$ with BTB, 7.1–43.8 $\mu g \cdot ml^{-1}$ with BCG and 4.2–58.0 $\mu g \cdot ml^{-1}$ for the first derivative spectrophotometry. Regression analysis of the Beer's law plots at λ_{max} reveals a good correlation. The high molar absorptivities of the resulting colored complexes indicate the high sensitivity of the proposed methods.

The procedures were validated evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability and recovery. The LOD and LOQ values were calculated from the calibration curves as kSD/b where $k = 3$ for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve. The values of LOD were found to be 0.083 $\mu g \cdot ml^{-1}$ for the LER-BTB method, 0.092 $\mu g \cdot ml^{-1}$ for the LER-BCG method and 0.057 $\mu g \cdot ml^{-1}$ for the first derivative spectrophotometric method, while the LOQ were found to be 1.78 $\mu g \cdot ml^{-1}$ for the LER-BTB method, 1.95 $\mu g \cdot ml^{-1}$ for the LER-BCG method and 0.96 $\mu g \cdot ml^{-1}$ for the first derivative spectrophotometric method.

The accuracy, precision, and repeatability of the methods were tested by means of recovery test. Five replicate de-

Table 1: Analytical data for lercanidipine ion-pair complexes

Method					Linearregression			
	λ_{max}	Conc. range ($\mu g \cdot ml^{-1}$)	Molar absorptivity ($l \cdot mol^{-1} \cdot cm^{-1}$)	Sandell sensitivity ($\mu g \cdot cm^{-2}$)	Intercept (a)	Slope (b)	Corr. coeff. (r)	RSD* (%)
BTB	417.6	6.0–42.0	8.17×10^6	1.72	0.81	1.13	0.9992	0.92
BCG	415.9	7.1–43.8	5.05×10^6	1.05	0.63	1.82	0.9990	0.90
First Der. Spec.	244.8	4.2–58.0	8.00×10^6	–	0.05	0.006	0.9999	0.75

* Average of ten determinations

Table 2: Spectrophotometric determination of lercanidipine in pure drug and in pharmaceutical formulations

Sample	Added ($\mu g \cdot ml^{-1}$)	% Found ± SD (n = 5)		
		First Der. Spec.	BTB	BCG
Lercanidipine (Pure drug)	5.0	99.5 ± 1.20	97.8 ± 1.32	98.5 ± 1.06
	10.0	99.2 ± 0.54	101.2 ± 1.16	98.8 ± 1.08
	15.0	98.9 ± 0.92	98.5 ± 1.22	98.2 ± 1.52
Commercial formulations[a]		98.7 ± 0.49	98.9 ± 1.07 t: 0.98 (2.26)[b]	97.9 ± 0.92 t:1.12

[a] Labelled to contain 10.0 mg lercanidipine in one tablet of Lercadip® tablet
[b] Values in parentheses are the theoretical values at p = 0.95. Theoretical values at % 95 confidence limit t = 2.26

terminations at three different concentrations were carried out to test of the methods (Table 1). The standard deviations were found to be less than 0.92% indicating good accuracy, precision, and repeatability of the proposed methods. The applicability of the proposed methods was tested by the determination of LER in pharmaceutical formulations. The results obtained are shown in Table 2.

The first derivative method was chosen as the analytical reference method. Statistical analysis of the results obtained by the proposed methods and first derivative spectrophotometry was done using the Student's t-test. The calculated values did not exceed the theoretical ones, indicating a good agreement between the proposed methods. The proposed procedures are simple, sensitive, rapid and accurate, and can be used for routine determination of lercanidipine in its dosage forms.

3. Experimental

3.1. Apparatus

A Shimadzu 1601 double beam spectrophotometer with 1 cm matched cells was used for all absorbance measurements. pH measurements were made with a NEL model 890 pH meter digital equipped with a combined glass-calomel electrode and an ultrasound generator.

3.2. Reagents

Bromothymol blue (BTB) or bromocresol green (BCG) were used without further purification. A stock solution (1.0×10^{-4} M) was prepared by dissolving the appropriate weight of BTB or BCG in doubly distilled water.

Lercanidipine was supplied by Fako Pharmaceutical Industries, Turkey, and used without further purification. Stock solutions were prepared by dissolving 50 mg of the drug in 250 ml water. A 1.0×10^{-4} M solution was also prepared by dissolving an appropriate weight in 100 ml water. Potassium hydrogen phthalate buffers were prepared by dissolving 1.020 g potassium hydrogen phthalate in water and completed to 50 ml with water and adjusting pH by addition of 0.1 M hydrochloric acid. Freshly prepared solutions were always employed.

3.3. Procedure for the assay of bulk sample

Into a series of 50 ml separating funnels, 5.0 ml of buffer solutions of pH 4.0 and 3.0 ml of BTB solution or 5.0 ml of BCG solution were placed. An appropriate 5.0 ml drug solution (6.0–42.0 $\mu g \cdot ml^{-1}$ for BTB method or 7.1–43.8 $\mu g \cdot ml^{-1}$ for BCG method) was added to each funnel and mixed well. The funnels were shaken vigorously with 10 ml chloroform for 2 min, then allowed to stand for clear separation of the two phases. The absorbances of the organic phase at 417 or 416 nm were measured in each case against a reagent blank similarly prepared. All measurements were made at room temperature 20 ± 2 °C . The procedures were repeated for other analyte aliquots. Calibration plots were drawn to calculate the amount of drugs in unknown analyte samples. On the other hand, the standard solutions were prepared by dilution of stock solutions in water to reach concentration ranges of 4.2–58.0 $\mu g \cdot ml^{-1}$ of lercanidipine for first derivative spectrophotometry.

3.4. Procedure for pharmaceutical formulations

Ten tablets (Lercadip®) were weighed, ground into a fine powder and mixed. An accurately weighed portion of the powder equivalent to one tablet was transferred into a 50 ml volumetric flask. The volume was made up to the mark with water. After 30 min of mechanically shaking, the solution was filtrated in a 50 ml calibrated flask through Whatman no 42 filter paper. Necessary amounts of filtrate were diluted to a 100 ml water and the same procedure was applied as described under the procedure for bulk samples in section 3.3.

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