A selective HPLC method for determination of lercanidipine in tablets

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Abstract

An HPLC reversed phase method using both UV (356 nm) and electrochemical (1000 mV) detection was developed in order to determine lercanidipine in commercial tablets. Repeatability and reproducibility were adequate. For quantification we have used the calibration plot method for lercanidipine concentration ranging between 1×10^{-5} and 1×10^{-4} M. Also, the proposed method is sufficiently selective to distinguish the parent drug and the degradation products after hydrolysis, photolysis or chemical oxidation. Furthermore, the typical excipients included in the drug formulation (talc, lactose, cornstarch, microcrystalline cellulose, carboxymethylcellulose and magnesium stearate) do not interfere with the selectivity of the method. Finally, the proposed chromatographic method was successfully applied to the quantitative determination of lercanidipine in commercial tablets.

Keywords: Lercanidipine; HPLC; Tablet assay; 1,4-Dihydropyridine

1. Introduction

Lercanidipine, 2-[(3,3-diphenylpropyl)methyla-mine]-1,1-dimethylethylmethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridinedicarboxylic ester (Fig. 1), is a new drug which belongs to the well-known pharmacological active compound series classified as 1,4-dihydropyridine calcium channel blockers. This drug is used in hypertension treatments [1,2], based on its selectivity and specificity on the smooth vascular cells [3].

This molecule corresponds to a new molecular design in which its liposolubility has been increased to obtain a long action. It is an amphypatic drug which is transported quickly across the cellular barrier, arriving inside to both hydrophilic and hydrophobic sites in spite of its highest solubility in the lipophyllic bilayer. This fact explains the differences observed in both the clinic and the pharmacokinetic profiles compared with other type of drugs. For example, a long action of amlodipine in connection with a long plasma half-life, in contrast lercanidipine exhibits a short plasma half-life compared with a long pharmacological effect [4].

The drug is orally administered in dose of 10–20 mg daily as its hydrochloride salt [1,5] reducing
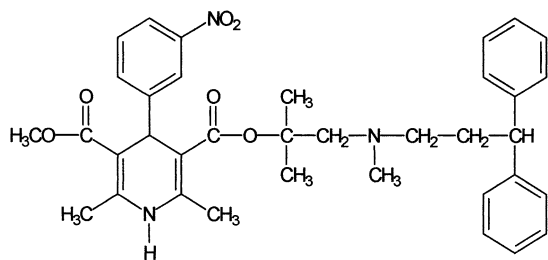
Fig. 1. Chemical structure of lercanidipine.

significantly the blood diastolic pressure after a single dose. It is rapidly absorbed from gastrointestinal tract, widely distributed and undergoes an extensive first pass metabolism [1,6] generating mainly inactive metabolites. Its half-life of elimination ranges from 2 to 5 h, but the therapeutic action is increased about 24 h due to it high liposolubility [1,5].

Considering that lercanidipine is a novel drug, few analytical methods for its determination have been described. Among them, a capillary electrophoresis method has been devoted to assay both the enantiomer and diasteromers purity [7] and recently the enantioseparation of dihydropyridine derivatives by means of neutral and negatively charged β-cyclodextrin derivatives using capillary electrophoresis has been described [8]. A clinical pharmacokinetic study on lercanidipine which was based on data obtained by HPLC-UV detection has been also published [9].

In our knowledge and after an exhaustive revision of the literature there is no evidence about the determination of this drug in pharmaceutical forms. Moreover, an official method for its determination has not been yet described in any Pharmacopoeia. Consequently, the implementation of an analytic methodology to determine lercanidipine in pharmaceutical forms is a pending challenge of the pharmaceutical analysis. In the present work, an HPLC method using both UV and electrochemical (amperometric type) detection was developed in order to determine lercanidipine in commercial tablets.

2. Experimental

2.1. Reagents and drugs

All reagents were of analytical grade unless indicated otherwise. Deionized water was obtained in the laboratory, using ionic interchanged columns Milli-Q (Millipore). Sodium hydrogen phosphate, phosphoric acid and acetonitrile HPLC grade (Mallinckrodt) were used.

Lercanidipine hydrochloride (100% chromatographically pure) was supplied by Andrómaco Laboratories (Santiago, Chile) and commercial tablets of Zanidip® (declared amount per tablet 10.0 mg lercanidipine hydrochloride, Andrómaco Laboratories. Santiago, Chile) were obtained commercially.

2.2. Solution preparations

2.2.1. 0.01 M phosphate buffer solution at pH 4.0
Anhydrous (4.44 g) Na_2HPO_4 were dissolved in 500 ml deionized water (Milli-Q), adjusted at pH 4.0 with phosphoric acid and then diluted to 1 l with deionized water.

2.2.2. Stock drug solution
Lercanidipine hydrochloride (6.25 mg) were dissolved and diluted in ethanol to 10 ml, to obtain a final concentration of 1×10^{-3} M lercanidipine and protected from light by using amber glass material.

2.2.3. Working solution
An aliquot of the stock solution was taken and diluted to 10 ml with acetonitrile/0.01 M phosphate buffer solution (45/55), pH 4. All the solutions were protected from light by using amber glass material.

2.3. Apparatus and chromatographic conditions

HPLC measurements were carried out by using a Waters assembly (Waters, Milford, MA, USA) equipped with a model 600 Controller pump. Detection was carried out with a model 996 Photodiode Array detector (PDA) and a model 464 electrochemical detector. The acquisition and

treatment of data were made with MILLENIUM version 3.1 software in a Pentium pro II. The chromatographic column used was a Symmetry C-18 (3.9 × 150 mm I.D., Waters, Milford, MA, USA), 5 μm particle size, with a C18 Bondapak (30 × 4.6 mm I.D., Waters, Milford, MA, USA) guard column. The injector was a 20 μl Rheodyne valve. The column was kept at 25 ± 1 °C using a Waters column heater cartridge, model 600. Separation was accomplished using isocratic elution with a mobile phase consisting of acetonitrile/0.01 M phosphate buffer pH 4.0 (45/55). The flow rate was 1.0 ml/min.

The UV detection was carried out at 356 nm. The electrochemical detector was equipped with a glassy carbon working electrode, an Ag/AgCl/NaCl 3 M reference electrode, and a platinum rod as the auxiliary electrode. The detector was operated at 1000 mV (d.c.) (working vs. reference) with a 0.5 s time constant slow pass filter and a current range of 5 μA.

2.4. Calibration curve

By diluting the lercanidipine stock solution with mobile phase, nine working solutions ranging between 1×10^{-5} and 1×10^{-4} M were prepared. The solutions were injected and chromatographed according to the working conditions previously given and using both UV and electrochemical detection.

2.5. Recovery studies

Ten independent synthetic samples containing lercanidipine hydrochloride plus excipients, according to the manufacturer's batch formula were prepared. The tested excipients were talc, lactose, cornstarch, microcrystalline cellulose, carboxymethylcellulose and magnesium stearate. The synthetic samples were a powder mix for compression but they were not tableted.

Each sample was mix in 1 ml ethanol and diluted to 10 ml with mobile phase. The obtained solution was centrifuged for 10 min at $2700 \times g$, then a 0.5 ml aliquot was taken and diluted to 10 ml volume by adding mobile phase to obtain solutions concentration around 5×10^{-5} M lerca-

nidipine. The testing solutions were analyzed as above.

2.6. Content uniformity

For this study ten commercial tablets of lercanidipine were used. Each tablet was independently suspended in 5 ml ethanol with sonication to assure the complete dissolution of the drug and diluted to a final volume of 10 ml with mobile phase. Each mixture was centrifuged for 10 min at $2700 \times g$, and then a 0.5 ml aliquot of supernatant was taken and diluted to a 10 ml volume with mobile phase, obtaining solutions around 5×10^{-5} M lercanidipine, which was measured according to the method.

2.7. Selectivity studies [10]Degradation trials

2.7.1. Hydrolysis
Individually 6.48 mg lercanidipine hydrochloride were dissolved in 5 ml ethanol in a 10 ml distillation flask and boiled for 1 h at reflux after adding: (a) 5 ml water for neutral hydrolysis, (b) 5 ml 0.1 M HCl for acid hydrolysis or (c) 5 ml 0.1 M NaOH for basic hydrolysis.

2.7.2. Chemical oxidation
6.48 mg lercanidipine hydrochloride were dissolved in 5 ml 0.01 M phosphate buffer/ethanol solution (80/20) and 100 μl 20% H_2O_2 solution (v/v) were added.

2.7.3. Photolysis
10 ml of 1×10^{-3} M lercanidipine ethanol solution were bubbled for 2 min with nitrogen and transferred to a black box and irradiated with UV light ($\lambda = 366$ nm) at a distance of 15 cm for 8 h (1.2×10^{19} quanta/s, determined by using the potassium ferrioxalate chemical actinometer [11]).

2.7.4. Thermolysis
Lercanidipine hydrochloride (3.8 mg) were heated at 105 °C for 5 h.

Each obtained solution from the degradation trials was diluted with mobile phase to obtain a theoretical concentration of 6×10^{-5} M lercanidipine. Samples from these studies were stored at

−20 °C and protected from light prior to HPLC analysis. Each sample was analyzed in duplicate.

3. Results and discussion

In the selected optimal experimental conditions (acetonitrile/0.01 M phosphate buffer pH 4.0 (45/55), 1.0 ml/min, 25 °C) lercanidipine exhibited a well-defined chromatographic peak with a retention time of 5.0 ± 0.7 min. In Fig. 2 a typical chromatogram obtained under these conditions is shown. As can be seen, the chromatograms obtained using both electrochemical or UV detection show an adequate retention for lercanidipine

standard. In the same figure the UV-spectra of the drug shows absorption bands at: 219 (shoulder), 237 and 356 nm. For analytical purposes the wavelength for the photodiode array detector was set at 356 nm, which presents better reproducibility than the other UV-bands. For the electrochemical detection the potential was set at 1000 mV.

The within-day and inter-day assays were determined by injecting ten replicate samples of lercanidipine standard at a 5×10^{-5} M level and expressed as the relative standard deviation (R.S.D.), calculated by the formula R.S.D. (%) = (standard deviation/mean of the peak areas) × 100 (Table 1). Results from these experi-
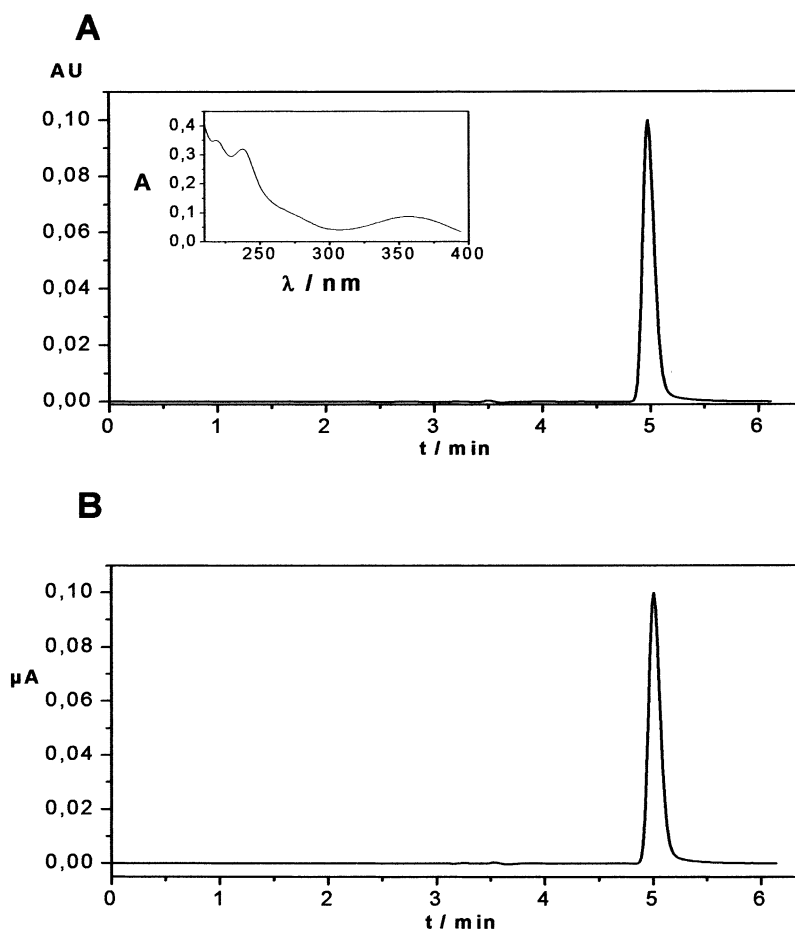

Fig. 2. Typical chromatograms for 5×10^{-5} M lercanidipine standard solution in the experimental selected conditions. A, UV detector at 356 nm; B, electrochemical detector at 1000 mV. Insert in (A) is the UV spectrum of lercanidipine standard.

Table 1
Regression data and their corresponding analytical parameters for lercanidipine pure drug

Parameter	HPLC$_{UV}$	HPLC$_{EC}$
Regression data		
Slope (area/M)	1.00201×10^{10}	1.16689×10^{11}
Intercept (area)	129921.5	48505.7
Number of data points	9	9
Residual sum of squares	6.23143	1.52087
Correlation coefficient (r)	0.9952	0.9993
Detection limit (M)	9.3×10^{-7}	7.5×10^{-7}
Quantitation limit (M)	1.2×10^{-6}	3.2×10^{-6}
Within-day reproducibility (R.S.D.,%)	1.52	1.93
Inter-day reproducibility (R.S.D.,%)	1.93	2.60

ments demonstrated that both signals were adequately reproducible to develop analytical applications. Furthermore, the chromatographic signal shows a linear dependence with the lercanidipine concentration enabling the use of this signal for lercanidipine quantification. For quantification the calibration plot method for lercanidipine concentration ranging between 1×10^{-5} and 1×10^{-4} M was used. The detection (LOD) and quantification limits (LOQ) of the method, were calculated by using the average (Yb) and standard deviation (Sb) of the blank estimated response, calibration curves slope (*m*) and a signal/noise ratio of 3 and 10, respectively, according to the following expressions [10]:

$$LOD = \frac{Yb + 3Sb}{m} \quad and \quad LOQ = \frac{Yb + 10Sb}{m}$$

Both, detection and quantification limits and the regression parameters of the calibration curve are shown in Table 1.

In order to check our proposed method for selectivity, different degradation pathways for lercanidipine were tried, due to that the degradation products were not available. Selectivity is a parameter that determines the ability of the method of producing a signal due only to the presence of the analyte (lercanidipine) and consequently free of interference of other components, such as degradation products, metabolites or pollutants. This study was carried out by using the following trials: hydrolysis (acidic, basic and neutral), photolysis, thermolysis and chemical oxidation [10].

When a lercanidipine solution was exposed to both neutral or acidic hydrolysis, no changes in the chromatograms were observed, evidencing that the drug was not affected in such conditions (1 h at reflux). But after the basic hydrolysis procedure, the chromatographic peak corresponding to the parent drug diminishes about 30% in 1 h. As can be seen in Fig. 3A, the UV-chromatogram reveals that lercanidipine produced three new small peaks. After the analyses of chromatograms (Fig. 3B), the UV spectra corresponding to the degradation product I and III differed from the parent drug. However, product II does not exhibit a significant difference from the original. On the other hand, by using electrochemical detection the three new signals also appeared at the same retention times than the UV-chromatogram (Fig. 3C), indicating that the basic hydrolysis does not affect the electro oxidizable 1,4-dihydropyridine ring [12]. Consequently, the initial signal at retention time of 5.0 min. was not interfered by the presence of the hydrolytic degradation products. The peak purity was checked by using PDA detector.

On the other hand, when lercanidipine solutions were exposed to a chemical oxidation with H_2O_2, the corresponding peak diminishes in 90% and no new signal in the chromatograms appeared (data not shown). Consequently, the degradation products from the oxidative procedure did not interfere the signal at retention time of 5.0 min. corresponding to the unaltered lercanidipine.

However, a rather different behavior can be appreciated in the photolysis test. Thus, when the lercanidipine standard solution was exposed to UV-366 nm light, the peak corresponding to parent drug practically disappeared after 1 h (Fig. 4A). Moreover, in the UV chromatogram extracted at 356 nm corresponding to lercanidipine solution test after 1 h photolysis at 366 nm, four new signals can be observed (insert in Fig. 4A), which do not interfere with the parent drug peak.
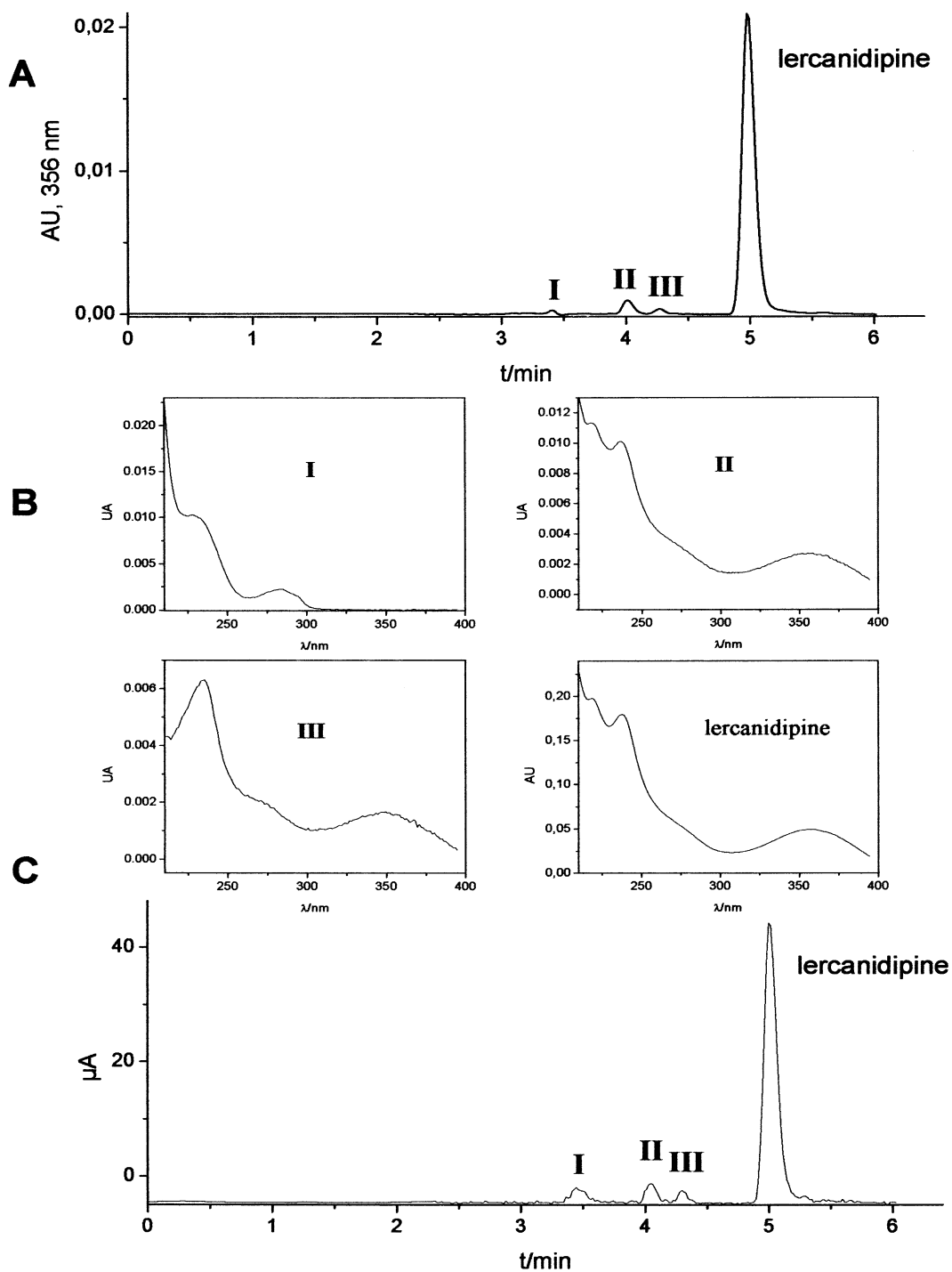
Fig. 3. A, UV chromatogram corresponding to lercanidipine standard solution test extracted at 356 nm after 1 h of basic hydrolysis; B, UV spectrograms of each chromatographic peak after 1 h of the hydrolysis trial; C, Electrochemical chromatogram of lercanidipine standard solution after 1 h of the hydrolysis trial.
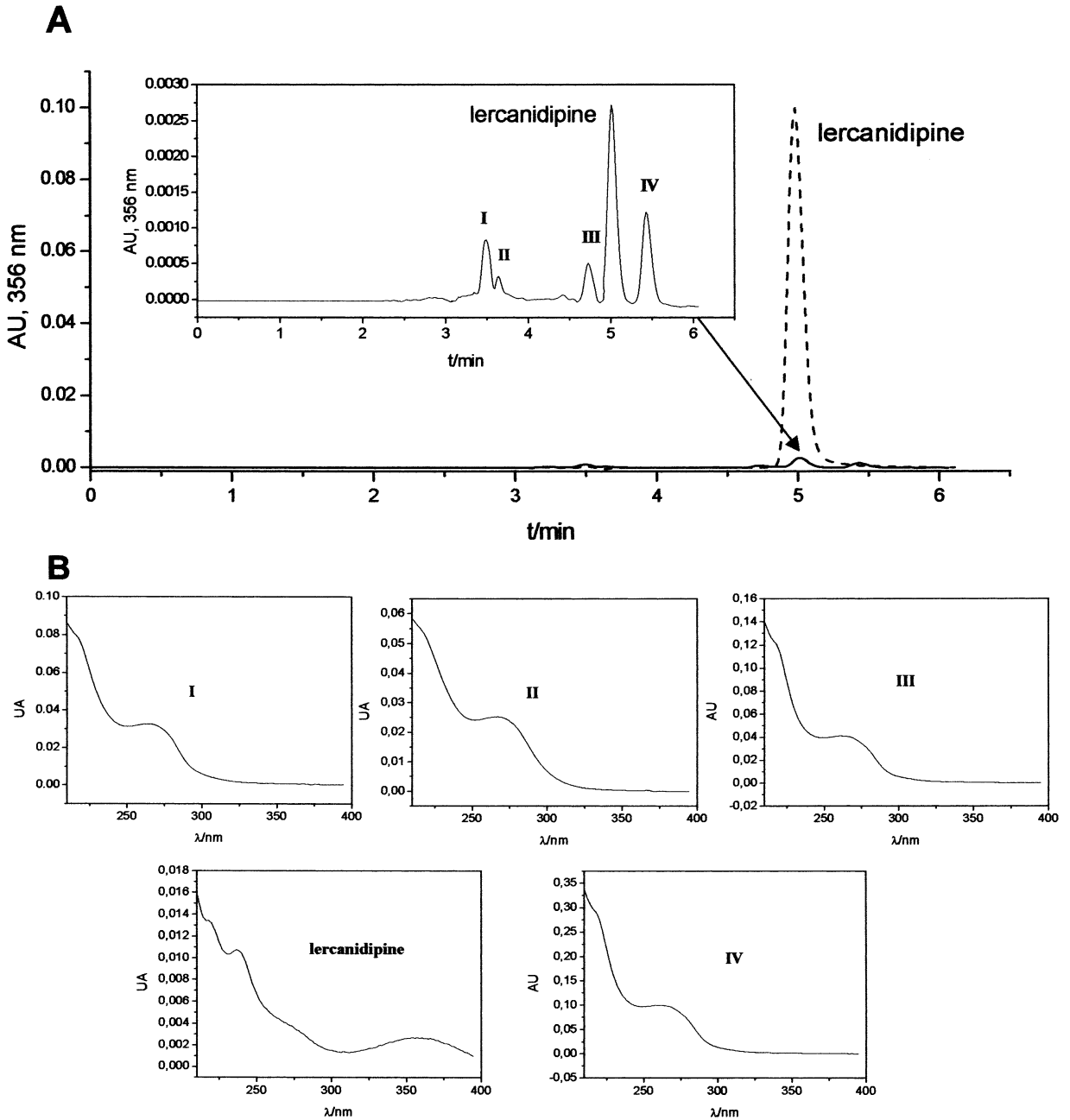
A

B

Fig. 4. A, UV chromatograms corresponding to lercanidipine solution test extracted at 356 nm; ···, before photolysis; —, after 1 h photolysis at 366 nm. Insert: the amplified UV chromatogram of lercanidipine solution after 1 h photolysis; B, UV-spectrograms of each peak of chromatogram after 1 h photolysis trial.

Table 2
Recovery study for synthetic tablets of lercanidipine*

	UV ($\lambda = 356$ nm)		Electrochemical ($E = 1000$ mV)	
	mg per tablet	(%) recovery	mg per tablet	(%) recovery
Average	9.60	96.0	9.76	97.6
S.D.	0.10	0.99	0.24	2.37
CV (%)	1.03	1.03	2.43	2.43

* Each simulated tablet was prepared with excipients plus 10.0 mg lercanidipine.

The electrochemical detector did not show new signals. These results could mean that the electro oxidizable 1,4-dyhidropyridine ring was completely degraded, i.e. the aromatization of such moiety has occurred (data not shown). As can be seen from Fig. 4B, the UV absorption band at 350 nm, corresponding to the nitroaromatic moiety has disappeared in all the photoproducts [12,13].

Finally, when lercanidipine powder standard drug was submitted to thermolysis trial, no change in the original chromatogram was observed, i.e. diminish in the original signal or the appearance of new ones. Consequently, the drug is stable under these tested experimental conditions.

According to the above experiments, we can conclude that the proposed method results to be sufficiently selective to distinguish the parent drug and the degradations products after hydrolysis, photolysis or chemical oxidation.

In order to obtain precision and accuracy of the develop method, a recovery study was performed. The results are summarized in Table 2. As can be seen, the method exhibits an adequate precision and accuracy and consequently, can be applied to the determination of lercanidipine in tablets. Also, a placebo formulation (formulation without analyte) was analyzed, concluding from these experiments that the typical excipients included in the drug formulation (talc, lactose, cornstarch, microcrystalline cellulose, carboxymethylcellulose and magnesium stearate.) do not interfere with the selectivity of the method. When UV and electrochemical detection results are compared, we can conclude that the best precision was obtained with the electrochemical detector, but the results with the UV detector were most accurate, being both

Table 3
Content uniformity assay of lercanidipine tablets*

	UV ($\lambda = 356$ nm) mg per tablet	Electrochemical ($E = 1000$ mV) mg per tablet
Average	10.0	9.8
S.D.	0.2	1.2
CV (%)	2.12	1.97

* Each tablet contained 10.0 mg lercanidipine hydrochloride.

statistically equivalent when t-test was applied ($t_{exp} = 1.76 < t_{table} = 2.12$, df $= 18$, $P < 0.05$, $n = 10$).

Finally, the proposed chromatographic method (UV- and electrochemical detection) was successfully applied to the quantitative determination of lercanidipine in commercial tablets. In Table 3, the uniformity content assay for lercanidipine commercial tablets is presented. The content for all assayed tablets falls within the range of 85.0–115.0% of the label claim and the relative standard deviation is less than 6.0%, fulfilling the Pharmacopoeia criteria for content uniformity of tablets which the average of the limits in the potency of the active ingredient is 100.0% or less [14].

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