Differential Pulse Voltammetric Assay of Lercanidipine in Tablets

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Lercanidipine in ethanol–0.04M Britton-Robinson buffer (20 + 80) gives an irreversible anodic response on a glassy carbon electrode in a broad pH range (2–12) that depends on pH. This signal can be attributed to oxidation of the 1,4-dihydropyridine ring to give the corresponding pyridine derivative. For analytical purposes, differential pulse voltammetry at pH 4 was selected. Under these conditions, good values of both within- and interday reproducibility were obtained, with coefficient of variation (CV) values of 1.56 and 1.70%, respectively, for 10 successive runs. For quantitation, the calibration curve method was used for lercanidipine concentrations ranging from 1×10^{-5} to 1×10^{-4}M. The detection and quantitation limits were 1.39×10^{-5} and 1.49×10^{-5}, respectively. A liquid chromatographic method with electrochemical detection was used for comparison. The voltammetric method showed good selectivity with respect to both excipients and degradation products. The recovery study exhibited a CV of 0.94% and an average recovery of 98.3%, and it was not necessary to treat the sample before the analysis. The method was successfully applied to the individual tablet assay of lercanidipine in commercial tablets.

Drugs belonging to the 1,4-dihydropyridine group of compounds, such as nifedipine or nitrendipine, reversibly block voltage-dependent Ca^{+2} influx through L-type channels in cell membranes, generating peripheral vasodilation and reduction in blood pressure. Lercanidipine, 2-[(3,3-diphenylpropyl)methylamine]-1,1-dimethylethylmethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitro phenyl)-3,5-pyridinedicarboxylic ester (Figure 1), is a new derivative with potent, long-lasting and vascular-selective calcium entry-blocking activity. The drug is a third-generation dihydropyridine calcium antagonist with a bulky bis-phenylalkylamine side chain, which makes it more lipophilic than related compounds such as amlodipine, nitrendipine, isradipine, and nimodipine. Lercanidipine is used in the treatment of hypertension (1–4).

The drug is administered orally in a daily dose of 10–20 mg as the hydrochloride (1, 5). It is quickly absorbed from the gastrointestinal tract, is 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 extended to about 24 h because of its high liposolubility (1, 5).

Few analytical methods for lercanidipine determination have been described. Among them, a capillary electrophoresis (CE) method to assay both enantiomeric and diasteromeric purity was developed (7). The enantiomeric separation of dihydropyridine derivatives by means of neutral and negatively charged β-cyclodextrin derivatives by using CE has been described (8). The results of a clinical pharmacokinetic study of lercanidipine, based on data obtained by liquid chromatography (LC) with UV detection, have also been reported (9).

As shown in Figure 1, lercanidipine contains a 1,4-dihydropyridine ring that can undergo electrochemical oxidation, like other compounds belonging to the same class, such as nitrendipine, nimodipine, isradipine, and nisoldipine (10).

To the best of our knowledge, the electrochemistry of lercanidipine has not been described in the scientific literature. Furthermore, an official method for the determination of this drug in its pharmaceutical forms has not yet been published in any pharmacopeia. Considering this lack of information, we studied the electrochemistry of lercanidipine in order to propose a method that uses differential pulse voltammetry (DPV) and is capable of measuring this drug in commercial tablets. Furthermore, for comparison we determined lercanidipine in tablets by LC with electrochemical detection (11).

METHOD

Reagents and Drugs

All reagents were analytical grade unless indicated otherwise.

(**a**) *Water*.—Deionized in the laboratory, by using ionic interchanged columns (Milli-Q, Millipore, France).

(**b**) *Sodium hydrogen phosphate, phosphoric acid, and acetonitrile*.—LC grade (Mallinckrodt Chemical Inc., Paris, KY).

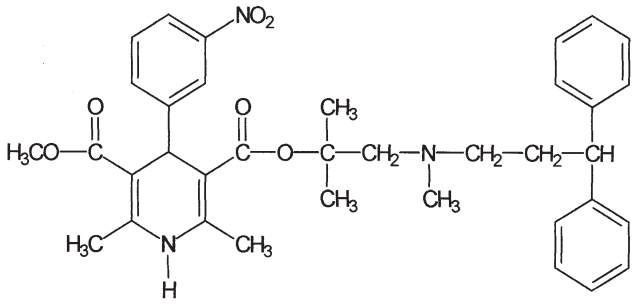

Figure 1. Chemical structure of lercanidipine.

(**c**) *Lercanidipine hydrochloride.*—100% chromatographically pure (Andrómaco Laboratory, Santiago, Chile).

(**d**) *Zanidip® tablets.*—Declared amount per tablet, 10.0 mg lercanidipine hydrochloride (Andrómaco Laboratory); obtained commercially.

Preparation of Solutions

(**a**) *Buffer solutions.*—0.04M Britton-Robinson buffer (acetic acid–boric acid–phosphoric acid) was used for voltammetric experiments. Ionic strength was adjusted to 0.1M with KCl, and pH was adjusted with concentrated solutions of NaOH or HCl. For LC the solutions under study were buffered by using a 0.01M phosphate buffer solution (disodium hydrogen phosphate anhydrous salt) adjusted to pH 4.0 with phosphoric acid.

(**b**) *Drug stock solution.*—Dissolve 6.25 mg lercanidipine hydrochloride in ethanol, and dilute to 10 mL with ethanol, to obtain a final concentration of 1×10^{-3}M lercanidipine. Protect the solution from light by using amber glassware for storage.

(**c**) *Working solution.*—Dilute an aliquot of the stock solution to 10 mL with acetonitrile–0.01M phosphate buffer solution, pH 4.0 (45 + 55), or ethanol–0.04M Britton-Robinson buffer (20 + 80), for LC or DPV, respectively.

Voltammetric System

Experiments were performed with a Metrohm 693 VA processor, a 694 VA stand, and a 25 mL thermostat-controlled Metrohm measuring cell, with a glassy carbon electrode as the working electrode, a platinum wire counter electrode and an Ag/AgCl 3M KCl reference electrode. The operating conditions were as follows: sensitivity, 5–10 μA; potential range, 200–1500 mV; ΔEp, 5 mV; pulse retard, 40 ms; and pulse height, 50 mV. After each recording, the working electrode was cleaned with a chromic acid–sulfuric acid mixture to heavily oxidize the surface and was then thoroughly rinsed according to the recommendations of Adams (12).

Liquid Chromatography

Measurements were made by using a Waters assembly equipped with a Model 600 controller pump and a Model 996 photodiode array detector. Data were acquired and treated with Millenium Version 2.1 software. A Bondapak/Porasil C_{18} chromatographic column, 3.9 × 150 mm, and a C_{18} Bondapak guard column, 4.6 × 30 mm, were used. The injector was a 20 μL Rheodyne valve. Electrochemical detection at 1000 mV was used, and the column was kept at constant temperature by a Waters column heater cartridge Model 600.

Isocratic elution with a mobile phase of acetonitrile–0.01M phosphate buffer, pH 4.0 (45 + 55), was used. The flow rate was 1.0 mL/min, and the working temperature was kept constant at 25 ± 1°C. Under these conditions, the retention time of lercanidipine was 5.0 ± 0.7 min (11).

Preparation of the Calibration Curve

(**a**) *Voltammetry.*—Dilute an aliquot of the lercanidipine stock solution with ethanol–0.04M Britton-Robinson buffer, pH 4.0, to obtain working solutions ranging between 1×10^{-5} and 1×10^{-4}M and with a final ethanol content of 20%.

(**b**) *LC.*—Dilute an aliquot of the lercanidipine stock solution with mobile phase to obtain working solutions ranging between 3×10^{-5} and 3×10^{-4}M (dynamic range: 1×10^{-6} to 1×10^{-3}M). Inject and chromatograph the solutions according to the working conditions previously given, using the electrochemical detector operated at E = 1000 mV.

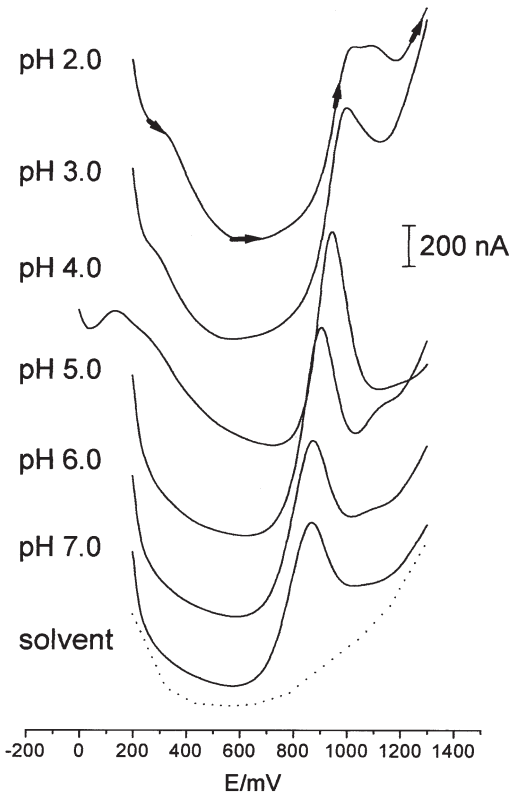

Figure 2. Differential pulse voltammograms of 1×10^{-4}M lercanidipine in ethanol–0.04M Britton-Robinson buffer solution (20 + 80) at different pH values. (• • •) = Ethanol–0.04M Britton-Robinson buffer (20 + 80).

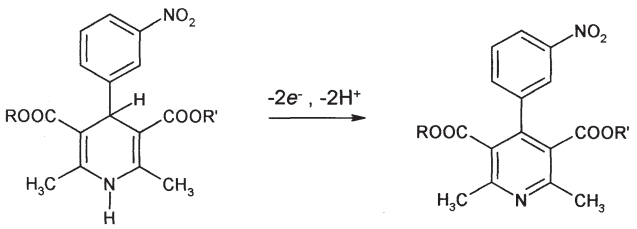

Figure 3. Overall electrochemical reaction of the 1,4-dihydropyridine ring oxidation.

Synthetic Samples

Add excipients (cornstarch, magnesium stearate, lactose, sodium lauryl sulfate, polyethylene glycol 6000, titanium dioxide, carboxymethylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose, and talc) to the drug for recovery studies, according to the manufacturer's batch formulas for 10.0 mg lercanidipine hydrochloride per tablet. Recovery experiments were performed with 10 synthetic samples, which were analyzed in duplicate.

Assay Procedure for Tablets

(**a**) *Voltammetry*.—Suspend 1 Zanidip tablet (amount declared, 10.0 mg lercanidipine hydrochloride per tablet), in a series of 10 tablets, in 5 mL ethanol; sonicate; and dilute to 10 mL with 0.04M Britton-Robinson buffer, pH 4.0. Take a 1 mL aliquot of each solution, and dilute each to 50 mL with 0.04M Britton-Robinson buffer, pH 4.0, to obtain a lercanidipine concentration of 6.2×10^{-5}M and final solvent proportions of ethanol–buffer (20 + 80). Transfer each sample solution to a polarographic cell, degas with nitrogen for 5 min, and record at least twice from 800 to 1200 mV. Calculate the amount of lercanidipine hydrochloride in the sample solution from the standard calibration curve.

(**b**) *LC*.—For this study no fewer than 10 commercial tablets of lercanidipine were used. Suspend each tablet independently in 5 mL ethanol with sonication to ensure complete dissolution of the drug, and dilute to a final volume of 10 mL with mobile phase. Centrifuge each of the solutions for 10 min at 4000 rpm; then take an aliquot of 0.5 mL supernatant, and dilute to 10 mL with mobile phase, obtaining solutions of around 7×10^{-5}M lercanidipine; measure according to the general procedure described above (11).

Selectivity Studies (13)

(**a**) *Degradation by hydrolysis*.—Dissolve 6.48 mg lercanidipine hydrochloride in 5 mL ethanol in a 10 mL distillation flask, and add (*1*) 5 mL water for neutral hydrolysis, (*2*) 5 mL 0.1M HCl for acid hydrolysis, or (*3*) 5 mL 0.1M NaOH for basic hydrolysis. Then boil for 1 h at reflux.

(**b**) *Degradation by chemical oxidation*.—Dissolve 6.48 mg lercanidipine hydrochloride in 5 mL 0.04M Britton-Robinson buffer, pH 4.0–ethanol (80 + 20). Add 100 µL 20% (v/v) H_2O_2 solution for oxidation.

(**c**) *Degradation by photolysis*.—Bubble 10 mL 1×10^{-3}M lercanidipine ethanol solution for 2 min with nitrogen; transfer to a black box, and irradiate with UV light (UV Black-Ray long-wave UV lamp, UVP Model B 100 AP, 50 Hz, 2.0 A, with a 100 W par 38 mercury lamp equipped with a 366 nm filter) at a distance of 15 cm for 8 h (1.2×10^{19} quanta/s, determined by using the potassium ferrioxalate chemical actinometer; 14).

Dilute each solution from the degradation trials to final volume with 0.04M Britton-Robinson buffer, pH 4.0 (maintaining a final ethanol content of 20%), to obtain a theoretical concentration of 6×10^{-5}M lercanidipine. Store the solutions from these studies at –20°C, and protect from light before voltammetric analysis. Analyze each solution in duplicate.

Statistical Analysis

The Student's *t*-test with significance limits between 95 and 99% confidence (15, 16) was used for comparison of the different techniques and the comparison with standard deviations.

Results and Discussion

Lercanidipine in ethanol–0.04M Britton-Robinson buffer (20 + 80) gives an anodic response in a broad pH range, between 2 and 12, when DPV on a glassy carbon electrode is used.

Typical voltammograms of lercanidipine at different pH values are shown in Figure 2. As can be seen, at strong acidic pH (pH 2.0) a poorly resolved peak near 1000 mV appears. As the pH increased, the resolution improved. This signal can be attributed to the oxidation of the 1,4-dihydropyridine ring to give the corresponding pyridine derivative, as occurs with

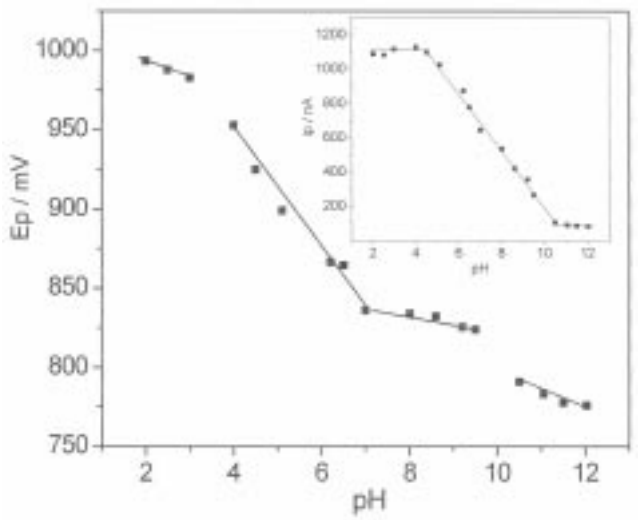

Figure 4. Evaluation of peak potential with change in pH of ethanol–0.04M Britton-Robinson buffer (20 + 80) solutions of lercanidipine. Inset: behavior of peak current with change in pH.

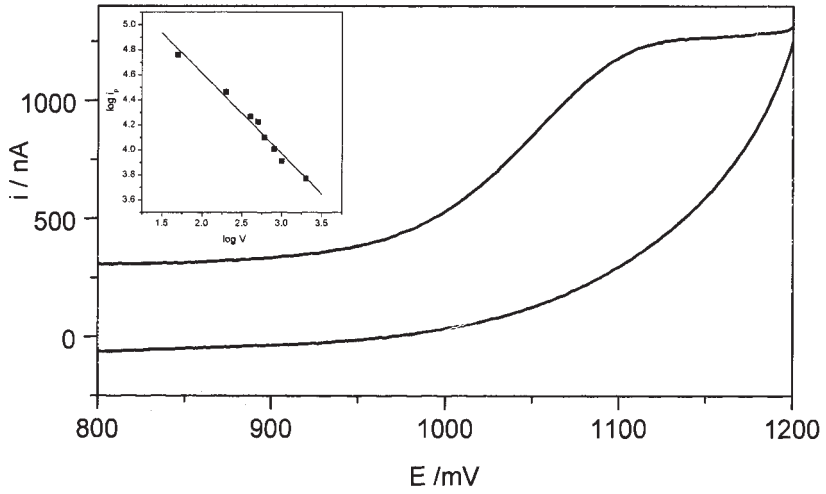

Figure 5. Cyclic voltammogram of a 1×10^{-3}M solution of lercanidipine in ethanol–0.04M Britton-Robinson buffer (20 + 80) at 1000 mV/s. Inset: plot of log i_p versus log V.

other members of this family (10), according to the well-known overall reaction shown in Figure 3.

Figure 4 shows a plot of peak potential versus pH. Peak potential has a linear relationship to pH, and 4 different zones can be observed, with breaks at pH 4, 7, and 10 and slopes of 10.5, 35.9, 7.2, and 10.0 mV/pH unit for each linear range, respectively. These breaks are presumably due to changes in protonation-deprotonation of the electroactive molecule. On the other hand, peak current is both maximal and stable between pH 3 and 5 and then decreases linearly to become independent of pH at 10 (inset, Figure 4).

By using linear cyclic voltammetry at pH 4, a characteristic irreversible peak was obtained (Figure 5). We studied the irreversible peak in a wide range (50–2000 mV/s) of sweep rates. Peak current increases concomitantly as the sweep rate increases. Specifically, we found a linear relationship between the log of peak current and the log of sweep rate with a slope of 0.6444, which indicates that the electrodic process is diffusion controlled (17).

For analytical purposes, DPV at pH 4.0 was selected. Under these conditions, good values of within- and interday reproducibility were obtained, with coefficient of variation (CV) values of 1.56 and 1.70%, respectively, for 10 successive runs. For quantitation we selected the calibration curve method for lercanidipine concentrations ranging from 1×10^{-5} to 1×10^{-4}M at pH 4.0. The analytical parameters are summarized in Table 1.

To check our proposed method for selectivity, we tried various degradation pathways for lercanidipine. Selectivity is a parameter that describes the capacity of the method to produce a signal that is due only to the presence of the analyte (lercanidipine) with no interference from other components, such as degradation products, metabolites, or pollutants. In our selectivity studies, we evaluated degradation by hydrolysis (acidic, basic, and neutral), photolysis, and chemical oxidation (13).

In both the basic and the acidic hydrolysis of the lercanidipine solution, the voltammetric peak changed dramatically. In 1 h of basic or acid hydrolysis, the drug peak decreased around 90% (Figure 6, A and B), and a new signal appeared in the voltammograms, interfering the main signal. In parallel, the degradation was investigated by LC with electrochemical detection, but no new signals were found in the chromatograms after hydrolysis. On the other hand, chemical oxidation of lercanidipine solutions with H_2O_2 (Figure 6C) produced an increase in the peak current and a change in the shape of the polarograms. Similarly, a decrease in the drug peak area with any new signals was observed when the chemical degradation was investigated by LC.

Furthermore, when lercanidipine was exposed to UV radiation at 366 nm for 8 h, the peak current was nearly totally diminished, and a new signal appeared (Figure 6D). The same behavior was observed when the photolysis degradation was investigated by LC with electrochemical detection at 1000 mV.

Table 1. Regression data and the corresponding analytical parameters for the DPV determination of lercanidipine

Parameter	Value
Calibration curve	
Slope (nA/M)	9.35×10^6
Intercept	93.668
Regression coefficient (r), $n = 7$	0.9983
Detection limit (M)	1.40×10^{-5}
Quantitation limit (M)	1.50×10^{-5}
Within-day reproducibility (RSD, %)[a]	1.56
Interday reproducibility (RSD, %)[a]	1.70

[a] RSD = relative standard deviation.

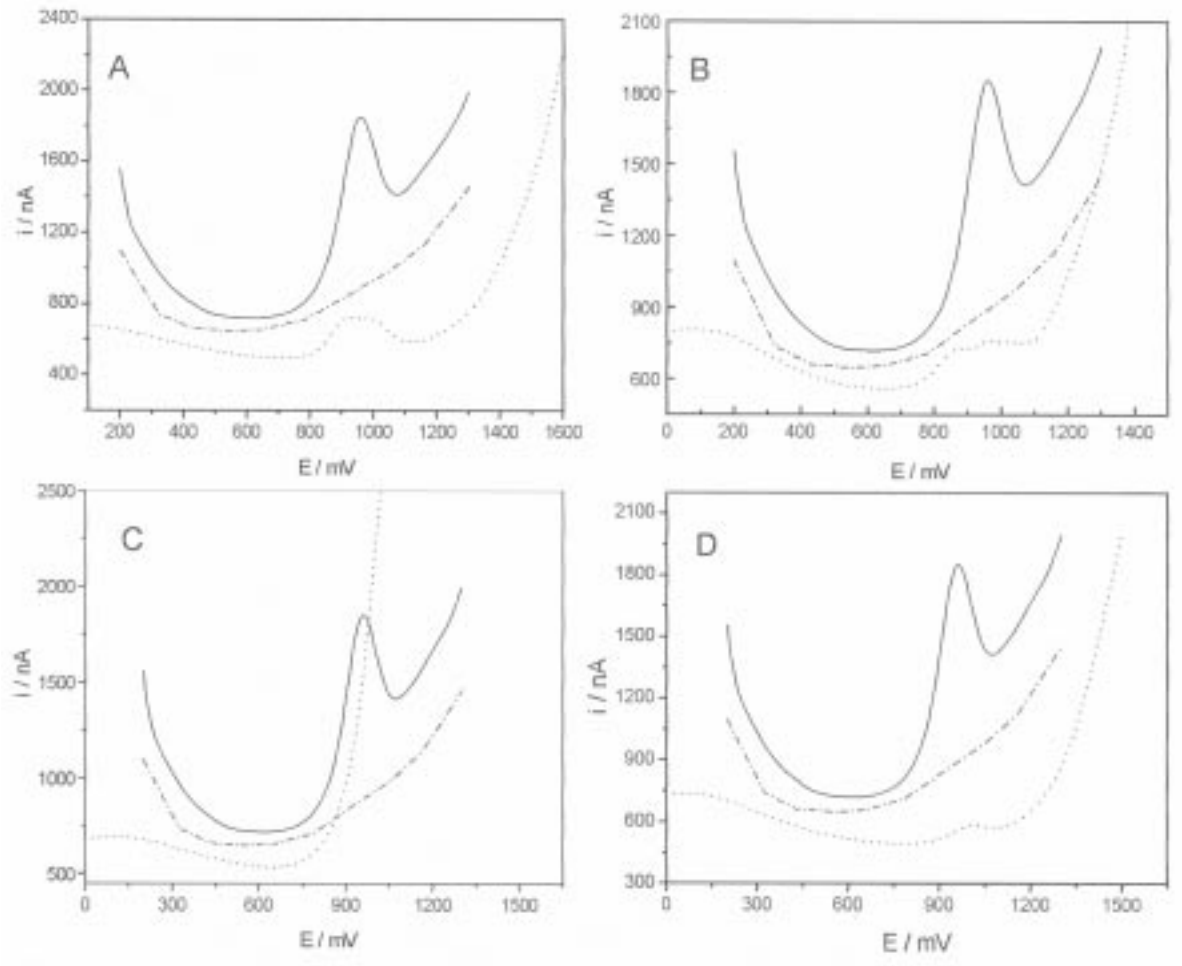

Figure 6. Degradation trials of lercanidipine. (A) Basic hydrolysis, (B) acid hydrolysis, (C) chemical oxidation (___ = initial time; • • • = after 1 h), and (D) photolysis (___ = initial time; • • • = after 8 h). Solvent-blank = – • – • –.

On the basis of the above-mentioned selectivity trials, we found the proposed DPV method to be sufficiently selective to be applied to the determination of lercanidipine.

To evaluate the precision and accuracy of the developed method, we performed a recovery study. The results reveal that the precision and accuracy of the voltammetric method are adequate, with an average recovery of 98.3 ± 0.92% and a CV of 0.94%. (The average recovery obtained by LC was 97.59 ± 1.23% with a CV of 2.31%.) Consequently, the voltammetric method can be applied to the determination of lercanidipine in tablets. Also, from these experiments we can conclude 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, and that previous separation or extraction is not necessary.

Finally, the proposed DPV method was applied successfully to individual tablet assay in order to verify content uniformity of the drug product. For comparison, an LC analysis with electrochemical detection at 1000 mV was performed (11). Table 2 summarizes the results obtained by both methods. The lercanidipine content of all assayed tablets fell within ±15% of the claimed amount, fulfilling the requirement of the *United States Pharmacopeia* for uniformity content of tablets (18).

From the statistical analysis of each applied method, we concluded that there were no significant differences between them, and that they were statistically equivalent; we compared the results obtained in the uniformity content test by applying

Table 2. Results from the uniformity content assay of lercanidipine tablets[a]

Parameter[b]	DPV, mg/tablet	LC, mg/tablet
Avg.	10.15	9.93
SD	0.11	1.19
CV, %	1.07	1.97

[a] Each tablet contained 10.0 mg lercanidipine hydrochloride. Each value is the average result from 10 tablets assayed in duplicate.

[b] SD = standard deviation; CV = coefficient of variation.

the Snedecor *F*-test (variance proportion) and then the Student's *t*-test ($p < 0.05$, $n = 10$).

In addition, we can conclude that the DPV developed method is an adequate tool for the routine determination of lercanidipine in pharmaceutical forms, because the method exhibits an adequate selectivity for the typical excipients tested (talc, lactose, cornstarch, microcrystalline cellulose, carboxymethylcellulose, and magnesium stearate), and acceptable accuracy and reproducibility. Furthermore, treatment of the sample is not required, and the DPV method is not time-consuming and is less expensive than an LC method.

Acknowledgments

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