Voltammetric Behavior of Lercanidipine and Its Differential Pulse Polarographic Determination in Tablets

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Abstract
Lercanidipine is a new third generation 1,4-dihydropyridine calcium antagonist derivative used in hypertension treatments. From the structural point of view lercanidipine contains a nitroaromatic moiety which can be electrochemically reduced. Lercanidipine in ethanol/0.04 M Britton Robinson buffer solution (20/80) presents a well-defined cathodic response, studied by both differential pulse and Tast polarography. This response was due to the irreversible, diffusion controlled, four-electron and four-proton reduction of the nitro aromatic moiety producing the corresponding hydroxylamine derivative. The DPP peak was adequately well-resolved, reproducible and linear dependent with the lercanidipine concentration. For quantification the calibration plot method for lercanidipine concentrations ranging between $3 \times 10^{-5}$ M and $9 \times 10^{-5}$ M at pH 4.0 was selected. A recovery of 98.3 ± 0.9%, with a variation coefficient of 0.94, reveal adequate precision and accuracy for the developed method. The proposed DPP method was successfully applied to the individual tablet assay in order to verify the uniformity content of lercanidipine in commercial tablets. For comparative purposes a HPLC with UV detection determination also was developed.

Keywords: Lercanidipine, Differential pulse polarography, Tablet assay

1. Introduction
Lercanidipine, 2-[(3,3-diphenylpropyl)methylamine]-1,1-dimethylethylmethyl 1,4-di-hydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridinedicarboxylic ester (Figure 1), is a new 1,4-dihydropyridine 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 most other drugs of this class, including amlodipine, nitrendipine, isradipine and nimodipine. Lercanidipine reversibly blocks voltage-dependent Ca$^{2+}$ influx through L-type channels in cell membranes, and the subsequent peripheral vasodilation leads to a reduction in blood pressure. This drug is used in hypertension treatments [1 – 4].

The drug is orally administered in dose of 10 – 20 mg daily as its hydrochloride salt [1, 5] reducing 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 ranging from 2 – 5 h, but the therapeutic action is enlarged about to 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].

From the structural point of view lercanidipine contains a nitroaromatic moiety which can be electrochemically reduced. There are a lot of other compounds belonging to the same class, as nifedipine, nitrendipine, nicardipine, nimodipine, isradipine or nisoldipine, wherein the electrochemical approach in order to develop a quantitative method was a valuable contribution to the pharmaceutical analysis [10 – 16].

In our best knowledge, there are no reports about the electroreduction of lercanidipine in the scientific literature. Furthermore, an official method for the determination of
this drug in pharmaceutical forms has not been yet described in any pharmacopoeia. Considering this lack of knowledge we were interested in investigating about the electrochemistry of lercanidipine in order to develop a differential pulse polarographic method capable of determining this drug in commercial tablets. Furthermore, for comparative purposes the application of the proposed differential pulse polarographic method to the uniformity content of lercanidipine in tablets was also carried out by means of HPLC with UV detection [17].

2. Experimental

2.1. Reagents and Drugs

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

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

2.2. Solutions Preparation

2.2.1. Buffer Solutions

0.04 M Britton-Robinson buffers (acetic acid/boric acid/phosphoric acid) for polarographic experiments was used. A 0.1 M ionic strength was adjusted with KCl, and the desired pH was adjusted with concentrate solutions of NaOH or HCl. For HPLC a 0.05 M buffer phosphate solution (disodium hydrogen phosphate anhydrous salt) adjusted at pH 4 with phosphoric acid was used.

2.2.2. Stock Drug Solution

6.25 mg lercanidipine hydrochloride was dissolved and diluted up to 10 mL with ethanol, to obtain a final concentration of 1 × 10⁻³ M lercanidipine. The solution was protected from light by using amber glass material.

2.2.3. Work 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 or 0.04 M Britton-Robinson buffer, for HPLC or differential pulse polarography, respectively.

2.3. Apparatus

2.3.1. Voltammetric Analyzer

Experiments were performed with a Metrohm Model 693 VA-Processor, equipped with a Model 694 VA-stand. A 25-mL thermostated Metrohm measuring cell, with a dropping mercury electrode as the working electrode, a platinum wire counter electrode and an Ag/AgCl 3 M KCl reference electrode were employed. The operating conditions were: sensitivity 5–10 μA; drop time 1 s; potential range –200 to –1200 mV; ∆Eₚ = 5 mV; pulse retard: 40 ms; pulse height: 50 mV.

2.3.2. HPLC

Measurements were carried out by using a Waters assembly equipped with a Model 600 controller pump and a Model 996 photodiode array detector. The acquisition and treatment of data were made with the Millenium version 2.1 software. As chromatographic column a Bondapak/Porasil C-18 column of 3.9 mm × 150 mm was used. As column guard a C18 Bondapak (30 mm × 4.6 mm) was employed. The injector was a 20 μL Rheodyne valve. An UV detector at 356 nm was employed and the column was kept at constant temperature using a Waters column heater cartridge Model 600.

An isocratic elution composed of a solution consisting of acetonitrile-0.01 M phosphate buffer pH 4.0 (45–55) mobile phase was used. The flow was 1.0 mL/min and the working temperature was kept constant at 25 °C ± 1 °C. In these conditions, lercanidipine exhibited a retention time of 5.0 ± 0.7 min [17].

2.4. Calibration Plot Preparation

2.4.1. Polarography

By diluting the lercanidipine stock solution with 0.04 M Britton-Robinson buffer pH 4.0, working solutions ranging between 3 × 10⁻⁴ M and 9 × 10⁻⁵ M were prepared.

2.4.2. HPLC

By diluting the lercanidipine stock solution with mobile phase, working solutions ranging between 3 × 10⁻⁴ M and 7 × 10⁻⁵ M were prepared. The solutions were injected and chromatographed according to the working conditions previously given. UV detector was operated at λ = 356 nm.

2.5. Synthetic Samples

Excipients (cornstarch, magnesium stearate, lactose, sodium lauryl sulfate, polyethylene glycol 6000, titanium dioxide, carboxymethylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose and talc) were added.
to the drug for recovery studies, according to manufacturer’s batch formulas for 10.0 mg lercanidipine hydrochloride per tablet.

2.6. Tablets Assay Procedure

2.6.1. Polarography

Ten series of one tablet of Zanidip (amount declared 10.0 mg lercanidipine hydrochloride per tablet) were suspended in 5 mL-ethanol, sonicated and diluted to 10 mL with 0.04 M Britton-Robinson buffer solution, pH 4.0. A 1 mL aliquot of each solution was taken and diluted to 50 mL with 0.04 M Britton-Robinson buffer solution, pH 4.0 to obtain a lercanidipine concentration of 6.2 × 10^-5 M. Each sample solution was transferred to a polarographic cell, degasses with nitrogen during 5 min and recorded at least twice from –300 mV to –500 mV. The mg amount of lercanidipine hydrochloride in the sample solution was calculated from the prepared standard calibration plot.

2.6.2. HPLC

For this study no less than 10 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 tablet of the above solution was centrifuged by 10 minutes at 4000 rpm, and then an aliquot of 0.5 mL supernatant was taken and diluted to a 10-mL volume with a mobile phase, obtaining solutions around 7 × 10^-5 M lercanidipine, which was measured according to the general methodology.

![Polarograms of 5 × 10^-3 M lercanidipine ethanol/0.04 M Britton Robinson buffer solution (50/50) at different pHs. A: Differential pulse polarography, B: Tast polarography.](image-url)
2.7. Selectivity Studies [18]

2.7.1. Degradation Trials

**Hydrolysis:** Individually 6.48 mg lercanidipine hydrochloride was dissolved in 5 mL ethanol in a 10 mL distillation flask and boiled for one hour at reflux and adding: a) 5 mL water for neutral hydrolysis, b) 5 mL 0.1 M HCl for acid hydrolysis and c) 5 mL 0.1 M NaOH for basic hydrolysis.

**Chemical oxidation:** 6.48 mg lercanidipine hydrochloride was dissolved in 5 mL 0.04 M Britton-Robinson buffer solution, pH 4.0-ethanol solution (80±20). For oxidation 100 μL 20% H₂O₂ solution (v/v) were added.

**Photolysis:** 10 mL of 1×10⁻³ M lercanidipine ethanol solution was bubbled by 2 minutes with nitrogen and transferred to a black box and then irradiated with UV light (UV Black-Ray long wave ultraviolet lamp, UVP ModelB100 AP (50 Hz, 2.0 A) with a 100 W Par38 mercury lamp equipped with a 366 nm filter) at a distance of 15 cm for 8 hours (1.2×10⁹ quanta/s, determined by using the potassium ferrioxalate chemical actinometer [19]).

Each obtained solution from the degradation trials was made to the final volume with 0.04 M Britton-Robinson buffer solution, pH 4.0 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 polarographic analysis. Each sample was analyzed in duplicate.

2.7.2. Statistic Analysis

Comparison between different techniques, as well as the comparison with standard deviations, was carried out by means of the t-Student test, and using significance limits between 95% and 99% of confidence [20, 21].

3. Results and Discussion

Lercanidipine in ethanol/0.04 M Britton Robinson buffer solution (20/80) presents a well-defined cathodic response, studied by both differential pulse and tast polarography. In Figure 2 typical polarograms of lercanidipine at different pH are shown. As can be seen at strong acidic pH (pH 2.0) a well-resolved peak or wave near −200 mV appears, which is shifted towards more cathodic potentials as the pH increases. This signal can be assigned to the nitroaromatic reduction, as occurred in the related members of 4-nitroaryl-1,4-dihydropyridine family [10–16], according to the following overall reaction [10–16, 22]:

\[
\text{ArNO}_2 + 4\text{H}^+ + 4e^- \rightarrow \text{ArNHOH} + \text{H}_2\text{O}
\]

In Figure 3 the peak potential vs. pH plot is presented. Peak potential is linear with pH, and three different zones can be observed, with breaks at pH 4 and 10 (slopes of 64.2 mV/pH; 49.3 mV/pH and 13.7 mV/pH). This break presumably is due to a change in protonation-deprotonation process of the electroactive molecule. On the other hand, limiting current remains practically constant between pH 1 and 8 (Insert, Figure 3) but beyond this pH the drug suffers a loss of solubility distorting the real limiting current. However, the loss of solubility did not affect the peak potential versus pH behavior. In order to obtain a maximum pH range wherein the loss of solubility of lercanidipine does not affect the limiting current we selected a solution containing 5×10⁻⁵ M of lercanidipine in 50/50: ethanol/0.04 M Britton-Robinson buffer. The solubility of lercanidipine was strongly dependent on pH, ethanol content and lercanidipine concentrations. At pH 4 the solubility of lercanidipine was complete at concentrations below 1×10⁻³ M and 20/80: ethanol/buffer ratio.

By using linear cyclic voltammetry at pH 4, an irreversible wave at all sweep rates is observed (Figure 4). Peak potentials shifting cathodically with the sweep rate increases. Furthermore, a linear relationship between peak current and sweep rate was found with a slope value of 0.508 that indicates that the electrode process is diffusion-controlled [23].

From the above-obtained results, differential pulse polarography at pH 4 was selected for analytical purposes. For quantification we have selected the calibration plot method for lercanidipine concentrations ranging between 3×10⁻⁵ M and 9×10⁻⁵ M at pH 4.0. Within-day and inter-day reproducibility’s were adequate with RSD values lower than 2%. In Table 1 the analytical parameters are summarized.

In order to check our proposed method for selectivity, we tried different degradation pathways for lercanidipine. Selectivity is a parameter that gives account of the capacity of the method of producing a signal due only to the presence of the analyte (lercanidipine) and consequently free of interference of other components, as degradation products,
metabolites or pollutants. Using the following trials carried out in this study: hydrolysis (acidic, basic and neutral), photolysis and chemical oxidation [18].

When a lercanidipine solution was exposed to either basic or acidic hydrolysis, the polarographic peak changes dramatically. After 1 hour of basic hydrolysis the drug’s peak disappears (Figure 5A), on the other hand, after 1 hour of acid hydrolysis the signal decreases around 90% (Figure 5B). In both cases any new signal appearing in the polarograms affected the main signal. On the other hand, chemical oxidation of lercanidipine solutions with H$_2$O$_2$ (Figure 5C), produces an increase in the peak current at least twice and the shape of polarograms changes dramatically. Furthermore, in order to check the selectivity of possible photodecomposition products, lercanidipine was exposed to 366 nm-UV for 8 hours. The lercanidipine’s peak disappeared and a new signal at approximately 150 mV before the drug’s peak appeared. Probably this fact is due to the reduction of the nitroso-pyridine degradation product as has been previously informed for related compounds (Figure 5D) [12, 15, 24–26].

According with the above results obtained in the selectivity trials, it can be concluded that the proposed differential pulse polarographic method is sufficiently selective in order to be applied to lercanidipine quantification.

In order to obtain the precision and accuracy of the developed method, a recovery study was performed. These results reveal that the method has an adequate precision and accuracy (percentage recovery = 98.3 $\pm$ 0.9, with a C.V. = 0.94%) and consequently, can be applied to the determination of lercanidipine in tablets. Also, from these experiments we can conclude that typical excipients included in the drug formulation (talc, lactose, cornstarch, microcrystalline cellulose, carboxymethylcellulose and magnesium stearate.) did not interfere with the selectivity of the method and previous separation or extractions are not necessary.

Finally, the proposed DPP method was applied successfully to the individual tablet assay in order to verify the uniformity content of lercanidipine in tablets. With comparative purposes, also a HPLC analysis was carried out [17], whose results are shown in Table 2. As can be seen, the content for all assayed tablets falls within $\pm$2.5% of the claimed amount, fulfilling the pharmacopoeia requirement for uniformity content of tablets that permits a $\pm$15% tolerance in this type of dosage forms [27].

From the statistical analysis of each applied method it could be concluded that no significant differences among them was found and that they were statistically equivalent, by comparing the results obtained in the uniformity content.

### Table 1. Regression data and their corresponding analytical parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Slope (nA/M)</td>
<td>$9.710 \times 10^6$</td>
</tr>
<tr>
<td>Intercept</td>
<td>$-117.78957$</td>
</tr>
<tr>
<td>Regression coefficient (r), $n=7$</td>
<td>0.9980</td>
</tr>
<tr>
<td>Detection limit (M)</td>
<td>$9.339 \times 10^{-6}$</td>
</tr>
<tr>
<td>Quantification limit (M)</td>
<td>$1.223 \times 10^{-5}$</td>
</tr>
<tr>
<td>Within-day reproducibility (RSD, %)</td>
<td>0.148</td>
</tr>
<tr>
<td>Inter-day reproducibility (RSD, %)</td>
<td>1.157</td>
</tr>
</tbody>
</table>

### Table 2. Uniformity content assay of lercanidipine tablets. Declared amount/tablet: 10.0 mg lercanidipine hydrochloride.

<table>
<thead>
<tr>
<th></th>
<th>Differential pulse polarography</th>
<th>HPLC UV ($\lambda = 356$ nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/tablet</td>
<td>10.15</td>
<td>10.02</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>CV %</td>
<td>1.07</td>
<td>2.12</td>
</tr>
</tbody>
</table>

In order to obtain the precision and accuracy of the developed method, a recovery study was performed. These results reveal that the method has an adequate precision and accuracy (percentage recovery = 98.3 $\pm$ 0.9, with a C.V. = 0.94%) and consequently, can be applied to the determination of lercanidipine in tablets. Also, from these experiments we can conclude that typical excipients included in the drug formulation (talc, lactose, cornstarch, microcrystalline cellulose, carboxymethylcellulose and magnesium stearate.) did not interfere with the selectivity of the method and previous separation or extractions are not necessary.

Finally, the proposed DPP method was applied successfully to the individual tablet assay in order to verify the uniformity content of lercanidipine in tablets. With comparative purposes, also a HPLC analysis was carried out [17], whose results are shown in Table 2. As can be seen, the content for all assayed tablets falls within $\pm$2.5% of the claimed amount, fulfilling the pharmacopoeia requirement for uniformity content of tablets that permits a $\pm$15% tolerance in this type of dosage forms [27].

From the statistical analysis of each applied method it could be concluded that no significant differences among them was found and that they were statistically equivalent, by comparing the results obtained in the uniformity content.
test, by applying the Snedecor F test (variance proportion) and then the t-Student test ($p < 0.05, n = 10$).

In addition, we can conclude that the differential pulse polarographic developed method is an adequate tool for the lercanidipine determination in pharmaceutical forms in that it exhibits adequate accuracy, reproducibility and selectivity. Furthermore, treatment of the sample is not required, the method is not time-consuming and less expensive than the HPLC ones.

4. Acknowledgements

Authors are very grateful with the support of FONDECYT Grant N° 8000016.

5. References


Fig. 5. 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)