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High performance liquid chromatographic analysis of lercanidipine in human breast milk

Evrim Kepekçi Tekkeli[1], Işıl Gazioğlu[1*], Gizem Tırıs[1] and Cem Önal[2]

[1]*Faculty of Pharmacy, Department of Analytical Chemistry, Bezmialem Vakif University, Fatih, Istanbul, Türkiye*
[2]*Abdi İbrahim Pharmaceuticals Hadimkoy/Istanbul, Türkiye*

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Abstract: A simple, rapid, precise and accurate isocratic reversed phase HPLC method was developed and validated for the determination of lercanidipine hydrochloride in pharmaceutical tablets and spiked human breast milk. The chromatographic separation was achieved on C18 (250×4.6 mm×5µm) column using a mobile phase consisting of acetonitrile and phospate buffer (pH=4) (55:45, v/v) at a flow rate of 1.1 mL/min and UV detection at 237 nm. The linearity of the proposed method was investigated in the range of 1.0-40 µg/mL (r^2=0.9990). The method was validated in terms of accuracy, precision, reproducibility, specificity, robustness, and detection and quantification limits, in accordance with ICH guidelines. The proposed method is found as suitable for routine quantification of lercanidipine in human breast milk.

Keywords:. Antihypertensive; lercanidipine hydrochloride; HPLC-UV; human breast milk; method validation © 2019 ACG Publications. All rights reserved.

1. Introduction

Lercanidipine (LER), (±)2-[(3,3-diphenylpropyl) methylamino]-1,1dimethylethyl methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1-4-dihydropyridine-3,5-dicarboxylate (Figure 1), is a third-generation lipophilic and vaso-selective dihydropyridine calcium-channel blocker developed for an oral administration and is used in hypertension treatments to effectively and safely reduce high levels of blood pressure, with a low adverse effect [1-3]. Lercanidipine hydrochloride in pharmaceutical tablets and spiked human breast milk was used.

The drug is orally administered as its hydrochloride salt in dose of 10-20 mg daily. It is rapidly absorbed from gastrointestinal tract, widely distributed and undergoes an extensive first pass metabolism [4, 5], generating mainly inactive metabolites. LER exhibits a short plasma half-life (ranges from 2 to 5 h) but the therapeutic action is increased about 24 h due to its high liposolubility [1-3]. So, the analysis of LER is important for therapeutic drug monitoring.

Several high performance liquid chromatographic method (HPLC) analysis of LER in biological fluids or pharmaceutical preparations which involved HPLC-Ultraviole detection (HPLC-UV) [6] HPLC–tandem mass spectrometry (MS/MS) [7-12], ultra-performance liquid chromatography

* Corresponding author: E-Mail: isilhacibekiroglu@gmail.com

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(UPLC)– MS/MS [13,14], high performance thin layer chromtagraphy (HPTLC) [15], voltammetry [16] and capillary electrophoresis [17] are used.

Human breast milk is the optimum source of nutrition during the first six months of life with several health and social benefits [18]. In our best knowledge and after an exhaustive revision of the literature there is no method about the determination of LER in human breast milk. Although breast milk is the most suitable nutrient for the infant, the breastfed child may be exposed to drugs during maternal drug therapy and for this reason it is important to determine the extent of medication that can be transferred to the milk, and how this may affect the development of the infant [19].

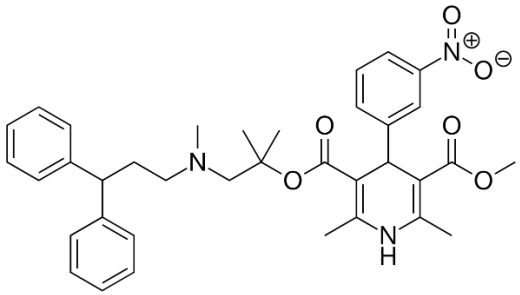

Figure 1. Chemical structure of Lercanidipine

2. Experimental

In the present work, an HPLC method using UV detection was developed and validated according to ICH criteria [20] in order to determine LER in spiked human breast milk samples.

2.1. Chemicals and Reagents

LER was obtained from Shanghai Yingxuan Pharmaceutical Science & Technology (China). Acetonitrile, monobasic dihydrogen phosphate and dibasic monohydrogen phosphate, hydrochloric acid (HPLC grade) and hexane (analytical grade) were supplied from Merck (Darmstadt, Germany). Water was purified by Human (Japan) ultrawater purification system.

2.2. Solutions

A stock solution of LER (0.1 mg/mL) was prepared and diluted with water to give standard solutions of from 1.0 to 40 μg/mL.

Phosphate buffer was prepared by 2.0209 g of sodium phosphate dibasic and 0.3394 g of sodium phosphate monobasic solution in 50 mL water. The pH level was adjusted to 4 with 0.1 M hydrochloric acid solution, and the volume was made up to 100 mL with water.

2.3. Sample Preparation

Breast milk samples were collected from a 35 years old volunteer mother (informed consent form was obtained according to ethical commitee approval) into polyethylene storage packs. The milk samples were stored at −20°C. To extract the drug from the milk samples different extraction techniques such as liquid-liquid extraction (LLE) with various extraction solvents and solid phase extraction (SPE) with various cartridge type and lengths were trialed.

2.4. Instrumentation

Spectrophotometric measurements were carried out using a Shimadzu UV-160 A spectrophotometer with 1 cm glass cells to obtain the spectrum of LER.

The HPLC analyses were conducted on a Shimadzu (Japan) LC 20 liquid chromatography which includes a LC-20AT pump, SIL AT-HT autosampler part, a SPD-20A HT UV spectrophotometric detector and CTO 10 AC column oven. Different mobile phase and column type and size combinations were trialed with different flow rates and column temperatures in order to get the most efficient chromatographic separation.

2.5. Data Evalaution and Discussion

2.5.1. Sample preparation Process

For the extraction of the lercanidipine from the human breast milk, 2 mL milk was alkalinized with 250 µL 0.1 M NaOH, and the solution was then extracted into 5 mL of hexane. The contents were mixed with vortex mixer at moderate speed for 5 min and centrifuged at 4500 × g for 3 min. The aqueous layer was discarded. The organic layer was evaporated to dryness under nitrogen at room temperature. The residue was dissolved in 300 µL acetonitrile. The solution was mixed with a vortex mixer for 30 sec. and 20 µL sample was injected into the HPLC-UV system.

2.5.2. Chromatographic Process

Maximum absorption of LER was observed at 237 nm by UV spectrophotometer and chromatographic separation was performed by isocratic elution at room temperature on a GL Sciences (Japan) C18 (ODS) column with acetonitrile and phosphate buffer (pH=4) (55:45, v/v) with a flow rate of 1.1 mL/ min. The chromatograms of standard LER solution and blank solution and breast milk sample solution including LER are given in Figure 2, Figure S1 and Figure S2.

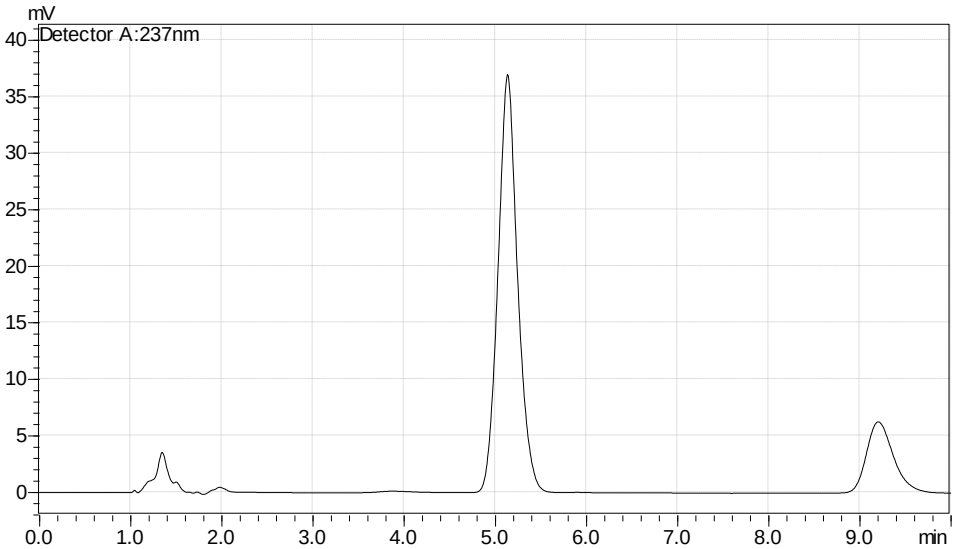

Figure 2. 10 µg/mL of LER

3. Results and Discussion

3.1. Validation of the Method

Validation of the method was applied according to the following guidelines given by the International Conference on Harmonization (ICH) (11).

3.1.1. Linearity

Calibration curve was prepared by the analysis of standard solutions of LER with various concentrations between 1.0-40 µg/mL. According to linear least-squares regression analysis, the linear concentration ranges (each concentration was studied as five replicates) of the method was determined and the equation of the calibration curve was calculated as y = 49981x + 7000,5, where y shows the peak areas and x indicates the concentrations of LER in 1.0-40 µg/mL.

3.1.2. The limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were determined using the formula: LOD or LOQ= kSDa/b, where k=3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The parameters for the analytical performance of the proposed method are summarized in Table 1.

Table 1. The analytical parameters of the method

Parameters	***Method***
Concentration range [a] (µg/mL)	1-40
Regression equation[b]	y=49981x+ 7000.5
Intercept±SD	7005±21.44
Slope±SD	49981±67.53
Correlation coefficient (r^2)	0.9990
LOD (µg/mL)	0.33
LOQ (µg/mL)	1

[a] Average of five determinations
[b] y=xC +b where C is the concentration in µg/mL and y is the peak area

3.1.3. Accuracy, Precision and Recovery

QC samples at three different concentrations (3, 15.0, and 35.0 µg/mL) that can be selected as low, medium and high concentrations (n = 5) in milk and aqueous sample were prepared. The accuracy was determined by recovery values and the precision of the recovery study were defined by RSD values of the recovery results in five replicate studies. The recovery of LER from human breast milk was examined by extraction of spiked milk samples and comparison with peak areas obtained from the same amounts of aqueous unextracted LER solutions. The mean absolute recovery of LER were of 97.3%. Five replicates of samples at each concentration were studied on the same day for intraday and on five different days for interday precision and accuracy. The RSD values of both intraday and interday assays were all less than 4.13%. According to all these results summarized in Table 2 good precision and accuracy were observed.

Table 2. Accuracy and precision of the method

Added concentration (µg/mL)	Found concentration (µg/mL) (Mean±SD[1])	Recovery (%)	RSD of recovery	RSD of intraday variation	RSD of interday variation
3.00	2.94 ± 0.06	98.0	2.57	2.26	3.15
15.00	14.63 ± 0.82	97.5	3.18	3.75	4.13
35.00	33.79 ± 2.12	96.5	3.45	2.67	3.92
Mean relative recovery =		97.3			

For each concentration (n=5)

3.1.4. Robustness

Robustness was assessed by determination of the QC samples at three concentration levels as described at validation section above (n=3). The parameters, that are changed to measure the robustness of the method, are flow-rate, column oven temperature, acetonitrile and aqueous phase contents of the mobile phase. The mobile phase proportions were changed from 55:45 (acetonitrile–buffer solution) to 50:50 and 60:40 and the flow rate was changed from 1.1 to 0.8 and 1.4 mL/ min. These changes had no significant effect on peak area and resolution.

3.1.5. Stability

The effects of freezing and thawing on LER concentrations were studied using spiked LER milk standards at 3, 15, 35 µg/mL, which were subjected to four freeze–thaw cycles before analysis. The stability of LER in spiked milk stored at room temperature for 24 h and –20ºC for 2 weeks was evaluated, as well. Stock solutions of LER were stable at least for 30 days when stored at −20ºC. After 20 days no decrease was observed in the concentration of LER in milk.

4. Conclusion

As a conclusion, this new method will fulfill the requirements for quantitation of LER in human breast milk. There is no method published for the determination of LER in this matrix. And the amount of LER is important in order to understand the effects of this drug on infants during breast feeding. In some situations mothers have to use different types of drugs during breast feeding. LER is a widely used vaso-selective dihydropyridine calcium-channel blocker that is preffered in hypertension theraphy. Its lipophilic structure may cause different accumulations in body and excretion by breast milk is possible. But there is no available data about this potential. This study will provide to quantify LER in human breast milk with a simple and cost reduced method. The sample pretreatment procedure depends on just one step LLE and immediately after HPLC with UV dedection provides sensitive analysis of LER. Preparation of the mobile phase is simple and nontoxic, no gradient elution is needed. Nearly in 5 min. selective and sensitive assay of LER can be carried out.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/journal-of-chemical-metrology

ORCID
Evrim Kepekçi Tekkeli: 0000-0002-1871-017X
Işıl Gazioğlu: 0000-0002-3283-1824
Gizem Tırıs: 0000-0002-2547-6086
Cem Önal: 0000-0002-5840-7386

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Supporting Information

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Evrim Kepekçi Tekkeli[1], Işıl Gazioğlu[11], Gizem Tırıs[1] and Cem Önal[2]

[1]*Faculty of Pharmacy, Department of Analytical Chemistry, Bezmialem Vakif University, Fatih, Istanbul, Türkiye*

[2]*Abdi İbrahim Pharmaceuticals Hadimkoy/Istanbul, Türkiye*

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[1] Corresponding author: E-Mail: isilhacibekiroglu@gmail.com

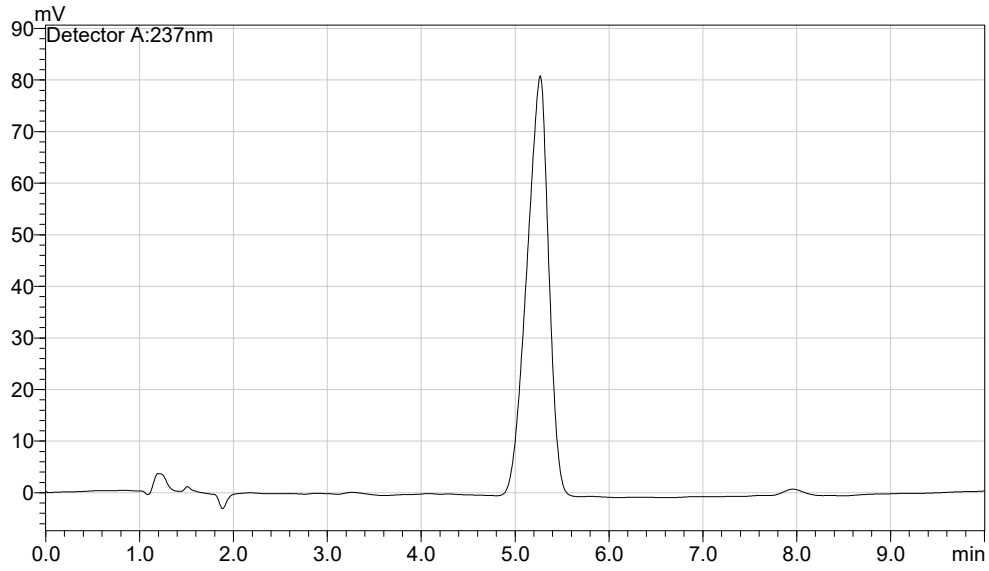

Figure S1. Chromatogram of the spiked (30 µL/mL of LER) milk sample

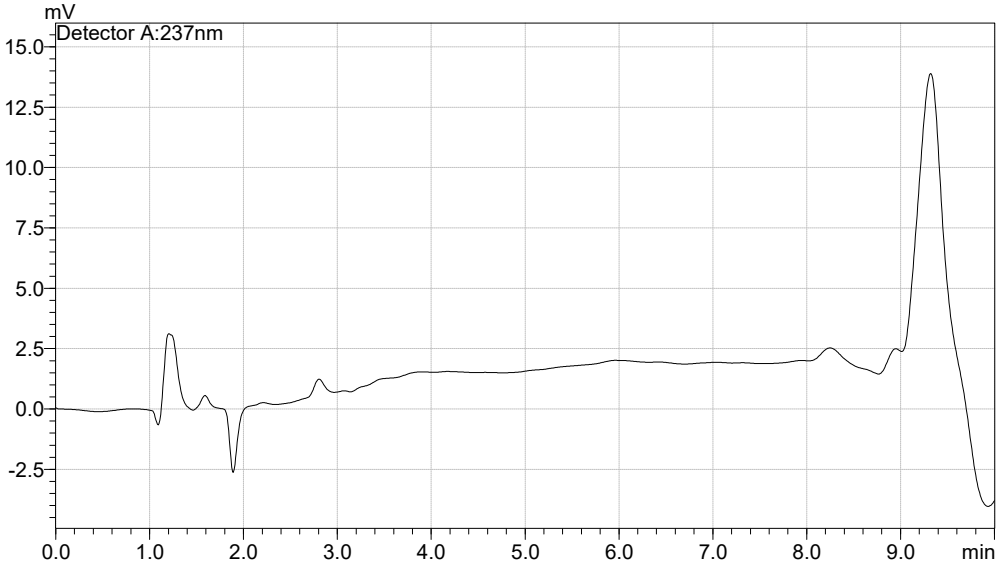

Figure S2. The chromatogram of the blank