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Bioequivalence of 250 mg lysine clonixinate tablets after a single oral dose in a healthy female Mexican population under fasting conditions

G. Marcelín-Jiménez[1], A.C.P. Ángeles[1], A. García[1], M. Morales[1], L. Rivera[1] and A. Martín-del-Campo[2]

[1]Clinical Pharmacology Research Service and [2]Mental Health Department, Hospital General de México, Mexico City, Mexico

Key words
lysine clonixinate – Chemical Abstracts Society (CAS) registry no. 55837-30-4 – bioequivalence and pharmacokinetics – ultra-performance liquid chromatography (UPLC) – tandem mass spectrometry

Abstract. Objective: To evaluate the bioequivalence between two 250 mg-tablets of lysine clonixinate, Dorixina Forte® (Siegfried Rhein, México) as reference product, and Prestodol® (Farmacéuticos Rayere, S.A., México) as test formulation. Methods: 26 healthy adult female Mexican volunteers received a single oral dose of 250-mg lysine clonixinate under fasting conditions. The drug was administered following a randomized, two-period, two-sequence, cross-over design. Twelve serial blood samples were collected up to 8 h after dosing, and clonixin (CLX) was measured by ultra-performance liquid chromatography (UPLC) coupled with tandem mass spectrometry. Decimal logarithm values of C_{max} and area under the curve (AUC) were used to construct a classic confidence interval at 90% (90% CI). Bioequivalence was established if 90% CI of mean ratios (test/reference) fall within the 0.8 – 1.25 range. Results: Volunteers formed a homogeneous population in terms of age (27.2 ± 6.3 years), weight (55.9 ± 6.5 kg), height (1.6 ± 0.04 m), and body mass index (BMI) (22.91 ± 2.03 kg/m^2). Reference formulation exhibited the following pharmacokinetics: C_{max} (32.39 ± 8.32 µg/ml); t_{max} (0.64 ± 0.2 h); $AUC_{0\ 8h}$ (48.92 ± 16.51 µg.h/ml); $t_{1/2}$ (1.3 ± 0.24 h); CL_{app} (5.64 ± 1.99 l/h), and Vd_{app} (10.22 ± 2.91 l). Concerning bioequivalence, 90% CI were: C_{max} (82.32 – 98.79), $AUC_{0\ t}$ (94.59 – 106.29), and $AUC_{0\ inf}$ (94.61 – 106.42), with a statistical power of > 0.90 at every tested interval. Conclusions: This single-dose study found that both 250-mg immediate-release tablets of lysine clonixinate met the Mexican regulatory criteria for bioequivalence in these volunteers.

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Correspondence to
G. Marcelín-Jiménez, PhD
Servicio de Investigación de Farmacología Clínica (405-E), Hospital General de México, Dr. Balmis No. 148, Col. Doctores, 06726 México, D.F., México
gabmarcelin@hotmail.com

Introduction

Clonixin (CLX) is an anti-inflammatory non-steroidal analgesic drug without narcotic effects (NSAIDs) that belongs to the fenamates family; its salt, lysine clonixinate (Chemical Abstracts Society (CAS) registry no. 55837-30-4) is an amorphous white powder soluble in organic solvents. It is used to relieve middle to severe episodes of dental pain [11], dysmenorrhea [3], post-operative pains [2], and migraine [10].

The mechanism of action of CLX relies on the blunting of 5-lipoxygenase with diminishing in the synthesis of the pro-inflammatory 5-HETE [6], and it has also been reported that CLX exerts an inhibitory effect on the expression of nitric oxide synthase (NOS) induced, which participate during inflammation [5]. This selectivity of CLX in 5-lipoxygenase over cyclooxygenases may explain the lack of effect on platelet number and function during its therapeutic use associated with common NSAIDs [9].

Pharmacokinetic data of CLX are succinct. The first attempt to determine the metabolic pathways of CLX in humans employed the tritium-labeled drug [8]. It showed three main metabolites both in plasma and urine: 5-OH-clonixin; 4′-OH-clonixin and 2′-ethoxyclonixin. Erratic values of plasma concentrations have been previously reported following an intravenous (IV) dose of lysine clonixinate solution in 10 children post-surgery [7]. These authors reported a distribution volume of ca. 1.3 l/kg, and a dose-dependent elimination half-life between 30 and 50 min. Serum concentrations were quite similar between IV and oral administration after 45 min post-dose; and areas under the curve were also similar, demonstrating high degree of bioavailability by oral route.

Although several clinical trials have been published whose results support the pharmacological use of CLX, there is very little regarding quantitative methodology for the measurement of this molecule in body fluids; thus, its dose-effect relationship has been established merely by observation.

Obviously, pharmacokinetic data are highly influenced by the analytical technique employed. To date, the few methods reported for CLX quantification in plasma are based on liquid chromatography with ultraviolet detection [1]. More recently, another method has been reported for lysine clonixinate measurement in pharmaceutical formulations [13], but not for bioanalytical purposes. Currently, HPLC coupled with tandem mass spectrometry (MS/MS) has become the gold standard for quantitative analysis of drugs in biological samples, due to its high degree of sensitivity and specificity.

Thus, the aim of present work was to evaluate bioequivalence between two 250-mg tablets of lysine clonixinate in a healthy adult female population under fasting conditions to fulfill the regulatory request and to report pharmacokinetic data obtained during the trial. Plasma CLX concentrations were obtained with a fast micro-assay based on ultra-performance liquid chromatography coupled with MS/MS (UPLC-MS/MS), developed in our laboratory for this purpose.

Materials and methods

Studied formulations

The reference product was Dorixina Forte® 250-mg lysine clonixinate tablets (Siegfried Rhein, S.A. de C.V., México; batch No. 606238; expiration date: July 2009), while the test product was Prestodol® 250-mg lysine clonixinate tablets (Farmacéuticos Rayere, S.A., México; batch No. 7779; expiration date: November 2010).

Volunteers

26 female volunteers participated in the study under the following inclusion criteria: age between 18 and 45 years; non-smokers; body mass index (BMI) between 20 and 28 kg/m^2; normal clinical history; normal values in laboratory tests (hematology, blood biochemistry, hepatic function, and urine analysis); negative results for HIV/AIDS, hepatitis types B and C, and pregnancy urine test (baseline of all laboratory examinations were performed 1 month prior to the beginning of the study); contraceptive treatments and no breast-feeding were documented prior the beginning of the study. Exclusion criteria included any disease state 4 weeks prior to study, history of alcohol or drug addiction, or the use of any drug 2 weeks before study initiation. Retirement criteria considered throughout the study comprised hypersensitivity to CLX, loss of three or more samples around C_{max} in any period due to blunted catheter, dietetic transgression, or vomiting during the lapse between drug administration and t_{max}. Signed informed-consent formats relative to screening and study procedures were obtained from each volunteer, and the study protocol was reviewed and approved by the Hospital General de México Ethics and Scientific Boards and carried out in accordance with the Helsinki declaration and related amendments.

Study design

Studies were carried out with a single 250-mg oral dose of lysine clonixinate (equivalent to 160.617 mg CLX); experimental design was two treatments, two periods, two sequences, double-blind, cross-over, and randomized. Treatment groups were balanced, and volunteers were randomly distributed into product administration sequences. Reference and test products were blinded by an identification code for both clinical and analytical phases of the study. Decode was carried out prior to statistical analysis.

Volunteers entered the study 12 h previous to Phase 1 initiation, having dinner at 8:00 pm and an overnight fasting period in our Clinical Unit facilities. The following morning, an indwelling catheter was put in the forearm vein, and a single dose (250-mg lysine clonixinate) of either tablet (reference or test) was taken with 250 ml of tap water at 8:00 am. Breakfast and lunch were served 2 and 6 h after dose administration. Volunteers left the Research Center after sample of 8 h post-dose and re-entered Phase 2 after a 6-day washout period (day prior to second administration) on the same schedule.

Side effects were identified by asking specific questions to determine the general state of volunteers. Vital signs (body temperature, blood pressure, heart rate, and respiratory frequency) were taken at regular predefined intervals throughout the study using non-invasive electronic devices. Volunteers were clinically monitored up to 7 days after the end of the second phase.

Sample collection

Blood samples of approximately 5 ml were taken through the catheter into heparinized vacuum tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ, USA) at 0 h (prior to drug administration) and at 0.16, 0.33, 0.5, 0.66, 1, 1.5, 2, 3, 4, 6, and 8 h after dosing. Before sampling collection, 700 µl of blood was discarded, and after sampling, the catheter was flushed with 1 ml of heparin solution (2 IU/ml in physiological saline solution). Blood samples were placed in crushed ice and plasma was immediately separated by centrifugation at 2,520 × g at 4 °C during 10 min, transferred into labeled siliconized cryogenic vials (Corning, Inc., Corning, NY, USA) and stored at -70 °C until CLX analysis.

Sample preparation and UPLC-MS/MS analysis

For unknown samples of volunteers, calibration curves, and quality control points, 100 µl of plasma were pipetted into 1.5-ml microcentrifuge tubes. Samples were added with 50 µl of a solution of sodium meclofenamate (MCLF 500 µg/ml in methanol: aqueous 0.1% formic acid (50 : 50 v/v)) as internal standard of the technique. Tubes were vortex-mixed briefly, and then plasma proteins were precipitated with 500 µl of cold acetonitrile (4 °C). Samples were vortex-mixed for 30 sec at high speed and centrifuged at 13,000 × g at 4 °C for 5 min. 300 µl of clear supernatants were transferred into 96-well "V-bottom" polypropylene plates (Corning, Inc.) and placed in the autosampler for further injection into the UPLC system. Quantification was achieved by using a 6-point calibration curve based on CLX/MCLF peak-area ratios vs. CLX concentrations in a 1/X weighted linear model.

CLX quantification was done in a Waters Acquity UPLC (Milford, MA, USA) coupled with a Waters-Micromass Quattro Micro tandem mass spectrometer (Manchester, U.K.) employing MassLynx version 4.1™ software. The analytical column was an Acquity Shield RP-18 (1.7-µm particle size, 100 mm × 2.1 mm i.d.), which was maintained at 40 °C. Mobile phase consisted of a mixture of acetonitrile and aqueous 0.1% formic acid (50 : 50 v/v) that was pumped at a 450-µl/min flow rate. The autosampler was maintained at 10 °C, and a volume of 5 µl per sample was injected into the system. The flow was split in a 1 : 1 ratio. The mass spectrometer was employed in positive electro-spray ionization mode (ESI+); CLX and MCLF were monitored through selected reaction monitoring mode using the transitions of m/z^{1+} 263.17 > 245.09 Th and m/z^{1+} 296.11 > 278.04 Th, respectively.

Assay validation

The analytical method was validated following criteria established in Mexican regulatory guidelines. CLX standard was 99.6% pure (YES-Pharma Ltd., Bet Shemesh, Israel) and MCLF was > 99% pure (Sigma-Aldrich Co., St. Louis, MO, USA).

Standard calibration curves were constructed by spiking drug-free human-pool plasma with a known amount of CLX at concentrations of 0.1, 10, 20, 30, 40, and 50 µg/ml. Quality control points at low, medium, and high levels (0.3, 15, and 35 µg/ml, respectively) were utilized to determine absolute recovery and within- and between-day precision and accuracy. Selectivity was evaluated by preparing lower limit of quantification (LOQ) in lipemic or hemolyzed plasma; drug-free plasma was also spiked with aspirin, caffeine, chlorphenamine, acetaminophen, and butylhyoscine. Finally, stability (biological matrix at -70 °C, bench-top at room temperature, freeze-and-thaw cycles, and processed samples inside the autosampler) was also evaluated. All statistical procedures during validation were performed using SPSS™ version 12.0 software (SPSS, Inc., Chicago, IL, USA).

Pharmacokinetics and statistical analysis

There was no formal sample-size calculation. The minimum requested by Mexican Regulatory Guidelines is 24; thus, and considering a minimum of dropouts, we decided to include 26 volunteers. Pharmacokinetic parameters were calculated by programming plasma data, a single extra-vascular dose, and a non-compartmental model utilizing WINNONLIN™ version 3.2 software (Pharsight Co., Mountain View, CA, USA). Area under the plasma concentration-time curve from time zero to 8 h (AUC_{0-t}), Area under the plasma concentration-time curve from time zero to infinity ($AUC_{0\ inf}$), apparent total clearance of the drug from plasma after oral administration (CL_{app}), apparent volume of distribution (Vd_{app}), and mean residence time (MRT) were software outputs. Maximum plasma drug concentration (C_{max}) and time to reach maximum plasma concentration following drug administration (t_{max}) were experimentally obtained by observation of the kinetic profiles of each volunteer. The algorithm employed by software to calculate elimination half-life ($t_{1/2}$), is through a lineal regression by least-squares from the terminal logarithm-normal decay part of the pharmacokinetic profile (elimination phase); the negative slope of the curve is the elimination constant (k_e); $t_{1/2} = (\ln 2)/k_e$.

Analysis of variance (ANOVA) for a 2 × 2 cross-over design was performed on the decimal logarithm (log)-transformed parameters C_{max}, $AUC_{0\ t}$, and AUC_{0-inf} to evaluate fixed effects such as period, sequence, formulation, and carryover. Log-transformed values of these parameters were taken into consideration to construct a classic confidence interval at 90% (90% CI) with a significance level (α) of 0.05. The formulations were considered bioequivalent if 90% CI of log-transformed ratios (test/reference) of C_{max} (an index of the rate of absorption) and $AUC_{0\ 8h}$, and $AUC_{0\ inf}$ (indexes of extent of absorption) were within the predefined range of 0.8 – 1.25, and reached a statistical power of at least 0.8 [4, 12]. The Schuirmann's-(two one-sided)test was also performed as a complimentary statistical assay; bioequivalence is claimed when probability was < 0.05 for each side of the test.

Results

Volunteer 13 was excluded from the statistical analysis because she did not show CLX plasma concentration in second period. Regarding demographic data, the population considered for bioequivalence assay formed a homogeneous sample in terms of age (27.2 ± 6.3 years), weight (55.9 ± 6.5 kg), height (1.6 ± 0.04 m), and BMI (22.91 ± 2.03 kg/m^2). The most common side effects related with both formulations and reported by volunteers were sickness (10 volunteers during first period, and 4 of the same volunteers during second period), gastric pain (the same two volunteers in both periods), one volunteer with slight cephalea and another with somnolence, both in first period; all side effects were observed during the first 2 h post-dose.

In terms of analytical technique, the calibration curve was linear in the 0.1 – 50 µg/ml concentration range in a first-order model (area ratio = –0.057 + 2.172 (concentration); r^2= 0.995). Signal-to-noise ratio was 30 : 1 (CV = 3.03%) for low LOQ. Within-day assay accuracy expressed as % of recovery, and precision values, expressed as % of coefficient of variation, were the following: 0.3 µg/ml (98.1%, CV 5.11%); 15 µg/ml (98.9%; CV 6.67%), and 35 µg/ml (103.46%, CV 3.96%), with n = 6 for each level. Between-day accuracy and precision during 3 days were as follows: (102.16%, CV 6.2%); (100.27%, CV 4.87%), and (100.88, CV 4.04%) for the low, medium, and high levels, respectively, with n = 18 for each level. CLX proved stable in biological samples for at least two 24-h freeze-and-thaw cycles (final mean recovery of 105.61%; CV 5.8%) and for at least 4 h on the worktable at 24 °C and 48% relative humidity (98.71%, CV 5.1%). Moreover, processed samples were stable for at least 6 h in the autosampler (100.8%, CV 6.0%). CLX in plasma was stable for at least 90 days at –70 °C (93.13%, CV 7.7%). There were no endogenous compounds that might interfere with either CLX or with MCLF ionization in the plasma samples tested for the assay, nor did aspirin, butylhyoscine, caffeine, chlorphenamine, heparin, or acetaminophen exhibit ion suppression. However, lipemia and hemolysis suppress the CLX signal by 15% and 40%, respectively.

Mean plasma concentration-time profiles of the 25 female volunteers for the 2

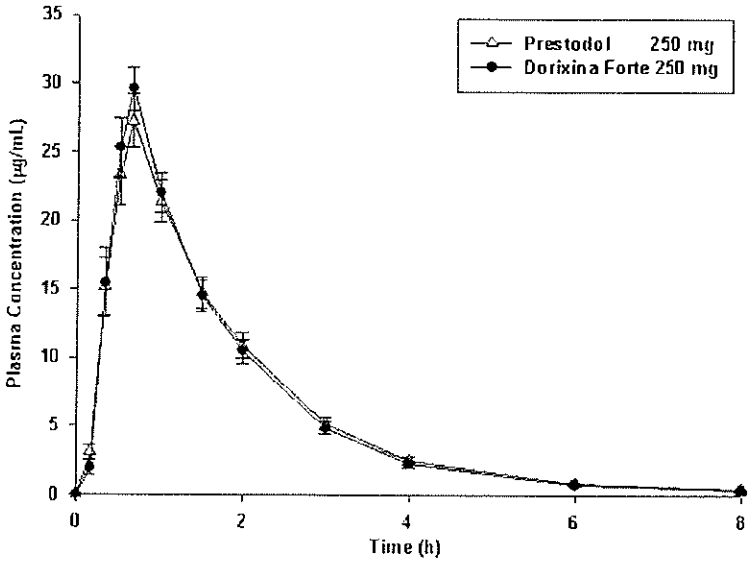

Figure 1. Plasma concentration profiles (mean ± standard error) of Clonixin, after a single oral administration of two brands of 250 mg lysine clonixinate tablets, in 25 young healthy female Mexican volunteers under fasting conditions.

Table 1. Comparison of pharmacokinetic parameters of Clonixin, after a single oral dose of two brands of 250-mg lysine clonixinate (equivalent to 160.617 mg of clonixin) in a young healthy female Mexican population.

Parameters	Prestodol® 250 mg tablet	Dorixina Forte® 250 mg tablet
C_{max} (µg/ml)	29.4 ± 1.72	32.3 ± 1.66
t_{max} (h)	0.70 ± 0.04	0.64 ± 0.04
AUC_{0-t} (µg × h/ml)	49.12 ± 3.5	48.92 ± 3.3
AUC_{0-inf} (µg × h/ml)	49.88 ± 3.6	49.64 ± 3.4
$t_{1/2}$ (h)	1.3 ± 0.056	1.3 ± 0.047
CL_{app} (l/h)	3.62 ± 0.25	3.62 ± 0.25
Vd_{app} (l)	6.59 ± 0.41	6.57 ± 0.37
MRT (h)	1.73 ± 0.056	1.66 ± 0.045

Values are given as mean ± standard error (SE). N = 25.

Table 2. Statistical results of bioequivalence between Prestodol® (250 mg lysine clonixinate tablets) and Dorixina Forte® (250 mg lysine clonixinate tablets) in 25 young healthy female Mexican volunteers after a single oral dose of 250 mg under fasting conditions.

Parameter	90% CI		Statistical power (%)	Schuirmann's test	
	Lower	Upper		P_1	P_2
Log (C_{max})	82.32	98.79	97.7	0.0171	0.000
Log (AUC_{0-t})	94.59	106.29	99.9	0.000	0.000
Log (AUC_{0-inf})	94.61	106.42	99.9	0.000	0.000

Statistics were applied to decimal logarithm transformed data. 90% CI, Classic confidence interval at 90%. *Bioequivalence is assumed when 90% CI is between 80 and 125 limits with a statistical power ≥ 80%, and P_1 and P_2 < 0.05 for Schuirmann's-test.

250-mg tablets (test and reference) are shown in Figure 1. Sampling time was sufficient to calculate up to 98% of AUC_{0-inf}. All calculated pharmacokinetic parameters for both CLX formulations are summarized in Table 1.

Due to the exclusion of one volunteer, ANOVA was performed specifying square sum type III for unbalanced groups in WINNONLIN software. During statistical analysis, ANOVA revealed the absence of any sequence, period, or formulation effects with regard to C_{max}, $AUC_{0\ t}$, and $AUC_{0\ inf}$. The intra-subject variability observed were: 25.09% for C_{max}, 17.17% for $AUC_{0\ t}$, and 17.44% for $AUC_{0\ inf}$. With respect to bioavailability comparisons between Dorixina Forte® and Prestodol®, 250-mg tablets, 90% CI for all ratios (log-transformed values of C_{max}, $AUC_{0\ t}$, and $AUC_{0\ inf}$) fell within the 0.80 – 1.25 interval with a statistical power > 0.98 in each tested parameter. Moreover, a probability of $p < 0.05$ for each side of the Schuirmann's-test was obtained. Results are shown in Table 2.

Discussion

To our knowledge, this is the first UPLC-MS/MS that is fully validated for CLX quantification in plasma. The present technique measures lysine clonixinate as CLX; although anion clonixinate is the free form in plasma, the most intense and stable signal was obtained in positive electro-spray mode for the protonated molecule. The advantages of this analytical coupling are that more specific and precise results are obtained, allowing the use of small amounts of plasma and having no time-consuming and clean extractive procedures, which can be translated into high throughput.

Regarding clinical trials, the most frequent side effects (gastric pain and dizziness) were reported between drug administration and breakfast. These are common symptoms associated with the administration of any NSAID drug, such as lysine clonixinate, under fasting conditions.

Concerning pharmacokinetic data, lysine clonixinate appears to have age- and dose-dependency. While previous work [7] reported a $t_{1/2}$ of 38.6 ± 10.9 min after an IV dose of 4 mg/kg in 10 children (aged between 4 and 10

years) and a $t_{1/2}$ of 44.3 ± 6.3 min after an IV dose of 6 mg/kg in the same children, we can observe in the present results a $t_{1/2}$ of 1.3 ± 0.2 h (90 ± 12 min) after a single oral dose of approximately 4.47 mg/kg (250-mg normalized with respect to the average weight of population employed) in females aged between 18 and 45 years. There are no data regarding the effect of concomitant food intake on lysine clonixinate pharmacokinetics, although its administration is recommended with meals, which represents a limitation of the present work. Nor are there data on gender-associated differences; however, during therapeutics the prescribed dose for children aged > 10 years and male and female adults is indistinct.

Regarding the bioavailability comparison, it can be concluded that after a single oral dose of 250-mg lysine clonixinate, differences among test and reference tablets in the fasting state met regulatory definitions to assume bioequivalence in these healthy female volunteers.

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References

[1] *Bica A, Farinha A, Blume H, Barbosa CM.* Determination of clonixin in plasma and urine by reversed-phase high-performance liquid chromatography. J Chromatogr A. 2000; *889:* 135-141.

[2] *De los Santos AR, Di Girolamo G, Martí ML.* Efficacy and tolerance of lysine clonixinate versus paracetamol-codeine following inguinal herniaplasty. Int J Tissue React. 1998; *20:* 71-81.

[3] *Di Girolamo G, Zmijanovich R, de los Santos AR, Martí ML, Terragno A.* Lysine clonixinate in the treatment of primary dysmenorrhea. Acta Physiol Pharmacol Ther Latinoam. 1996; *46:* 223-232.

[4] *Food and Drug Administration.* Guidance for Industry: Statistical Approaches to Establishing Bioequivalence. U.S. Department of Health and Human Services. FDA-CDER, USA 2001.

[5] *Franchi AM, Di Girolamo G, Farina M, De los Santos AR, Martí ML, Gimeno MA.* In vivo and in vitro effects of lysine clonixinate on nitric oxide synthase in LPS-treated and untreated rat lung preparations. Nitric Oxide. 2001; *5:* 150-157.

[6] *Franchi AM, Di Girolamo G, Farina M, De los Santos AR, Martí ML, Gimeno MA.* Efecto del clonixinato de lisina y la indometacina sobre la actividad de la lipooxigenasa y la ciclooxigenasa de colon aislado de pacientes con neoplasia. Medicina (Buenos Aires). 1998; *58:* 291-294.

[7] *González-Martín G, Cattan C, Zúñiga S.* Pharmacokinetics of lysine clonixinate in children in post operative care. Int J Clin Pharmacol Ther. 1996; *34:* 396-399.

[8] *Katchen B, Buxbaum S, Ning J.* Disposition of clonixin in humans. J Pharmacol Exp Ther. 1973; *187:* 152-157.

[9] *Kramer EH, Sassetti B, Kaminker AJ, De los Santos AR, Martí ML, Di Girolamo G.* Accion del clonixinato de lisina sobre la function plaquetaria: comparación con otras drogas antiinflamatorias no esteroideas. Medicina (Buenos Aires). 2000; *60:* 310-307.

[10] *Krymchantowski AV, Barbosa JS, Cheim C, Alves LA.* Oral lysine clonixinate in the acute treatment of migraine: a double-blind placebo-controlled study. Arq Neuropsiquiatr. 2001; *59:* 46-49.

[11] *Martí ML, De los Santos AR, Di Girolamo G, Gil M, Manero EO, Fraga C.* Lysine clonixinate in minor dental surgery: double-blind randomized parallel study versus paracetamol. Int J Tissue React. 1993; *15:* 207-213.

[12] *Mexican Health Ministry.* NOM-177-SSA1-1998: Tests and procedures to demonstrate interchangeability between pharmaceutical formulations. Mexico 1998. http://www.ssa.gob.mx unidades/cdi/nom 177ssa18.html (Accessed July 24, 2009).

[13] *Pineros I, Ballesteros P, Lastres JL.* Extraction and liquid chromatography determination of lysine clonixinate salt in water/oil microemulsions. J Pharm Biomed Anal. 2002; *27:* 747-754.