Original Article

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Application of rapid and simple liquid chromatography method for determination of bioequivalence of generic lamotrigine tablets in healthy Iranian volunteers

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Abstract

A simple and rapid chromatography method was developed for determination of lamotrigine in human plasma. The method was used to compare the pharmacokinetic (PK) parameters of 50 mg generic and the reference lamotrigine (Lamictal) tablets in healthy Iranian volunteers. High performance liquid chromatography - ultraviolet method was developed and validated to determine lamotrigine concentration in plasma samples. The method was linear over the range of 0.1 to 15 µg/ml. The accuracy and precision were within the acceptable range. Limits of detection and quantification were calculated 0.06 and 0.10 µg/mL, respectively. A randomized, single-dose, two-period, two-sequence crossover study was carried out in healthy subjects receiving either the test or the reference products in each period. Pharmacokinetic parameters were determined using non-compartmental calculations. *In vivo* bioequivalency between the generic and the reference product was investigated according to the guidance for industry issued by US Food Drug Administration. AUC_{0-t}, $AUC_{0-\infty}$ and Cmax were calculated for the generic product 12.50±2.76 µg.h/mL, 15.04±3.66 µg.h/mL and 0.38±0.08 µg/mL, respectively. The 90% confidence interval for the test/reference ratios were laid in the range of 0.80-1.25 for the log-transformed PK parameters. The generic product is bioequivalent and can be prescribed by practitioners while indicated, however the AUC and C_{max} were lower in Iranian population if compared to the literature, which requires further investigations.

Keywords: Bioequivalence, lamotrigine, HPLC, UV detector, pharmacokinetic, Iranian.

1. Introduction

Lamotrigine, 3,5-diamino-6-(2,3-dichlorophenyl)-as-triazine, is a phenyltriazine antiepileptic drug which is indicated for use in the treatment of epilepsy and bipolar disorders (1). It is a very slightly soluble basic compound (solubility≈0.017%) with pKa value of 5.7 at 25 °C. The drug is rapidly and completely absorbed from GI tract after oral administration (absolute bioavailability≈98%). The drug is widely distributed in the body and binds to the plasma protein for 56%. Estimate of the mean apparent volume of distribution ranges from 0.9-1.3 L/Kg. Lamotrigine is metabolized predominantly by glucoronic acid conjugation. Its major metabolite is an inactive 2-N glucoronide conjugate. Roughly 94% of the drug is recovered in urine (t1/2≈32 hours) of which is 76% as glucoronide (2). Lamotrigine pharmacokinetic is independent of dose and similar following single and multiple doses in both epileptic patients and health volunteers; however, it might be time-dependent that happens by auto-induction mechanism (2).

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The clinical importance of therapeutic drug monitoring of lamotrigine plasma level has been addressed previously (3), hence we aimed to develop a rapid and simple HPLC method with UV detection for determination of the plasma level of lamotrigine in a bioequivalence study setting. Pharmacokinetic parameters of lamotrigine were determined in Iranian population and bioequivalency of the generic product was demonstrated in comparison to the reference product (50 mg Lamictal tablet, GSK, UK).

2. Materials and methods

2.1. Chemicals

Lamotrigine (α=99.5%) and carbamazepine working standards were supplied by Bakhtar Biochimie. Liquid chromatography grade solvents (acetonitrile and methanol) were purchased from Caledon (Canada). Dibasic potassium phosphate was obtained from Merck Co. (Germany). All other reagents were of analytical purity.

2.2. Clinical Protocol

The clinical protocol for human study was reviewed and accepted by the Ethics Committee of the Shiraz University of Medical Sciences. A randomized, 50 mg single-dose, fasting, 2-period, 2-sequence crossover study with 10 days washout period was used in this study.

Twelve healthy male volunteers aged between 18-40 years with body mass index from 18-30 Kg/m2 were enrolled in this study. All volunteers provided written informed consent prior to study initiation. The details and purpose of the study were explained clearly to the volunteers. The subjects recruited were non-smokers with no history of alcohol or drug abuse. Subjects were excluded from the study if they had a history of morbidity within last 3 weeks, drug use especially antiepileptic agents within last month or experiencing drug hypersensitivity. All subjects were screened for suitability by a review of their medical history. The subjects had a routine physical examination and they were required to have normal routine laboratory results with no history of hepatitis C virus and HIV infections.

The subjects were admitted to Golestan Clinic at 7:30 AM. The prior day, they had a light meal before 8:00 PM and drink until 12:00 PM. The subjects took a single 50 mg lamotrigine test or reference coded tablet at 8:00-8:30 AM with a glassful of drinking water under overnight fasting conditions. They were provided a standard breakfast meal 2 hours after drug administration. They were served a low-fat launch after 6 hours and were prohibited to drink cola, tea or coffee. Subjects were asked if they suffered undesirable side effects. Five ml of blood samples were collected using A 20 gauge angiocath IV Catheter into heparinized tubes before administration and 1, 2, 4, 8, 12, 24, 36, 72 and 96 hours after lamotrigine administration. The blood samples were centrifuged at 2000 g for 10 min. The plasma samples were withdrawn and kept in poly propylene capped tubes and stored in at -20 °C freezer until drug analysis.

2.3.Plasma sample preparation

A protein precipitation method was developed with minor modification from the conventional method for the preparation of plasma samples (4). Briefly, a 500 µL plasma samples from the subjects or spiked with working standard solutions of lamotrigine at different concentrations was admixed with 500 µL acetonitrile containing 1 µg/mL carbamazepine as internal standard. After one min mixing using a vortex mixer, the mixture was centrifuged for 10min at 2000 g. The supernatant were injected into high-performance liquid chromatography (HPLC) system for analysis.

2.4. Preparation of stock and standard solutions

Stock solutions containing 0.6 mg/ml of lamotrigine were prepared in acetonitrile. The stock solutions were stored at -20 °C freezer. Each day, calibration standard solutions were prepared by serial dilution of the stock solutions with deionized water. Fresh plasma was spiked with the standard solutions in the volume ratio of 4 to 1 to prepare the final indicated lamotrigine concentrations. Calibration standard solutions prepared in triplicate freshly every day for 3 days.

2.5. Chromatography conditions

Chromatography method was based on the previously published methods (5-7)with some modifications. The HPLC system consisted of Smartline model 1000 pump (Knauer, Germany), Smartline 2500 UV-detector (Knauer, Germany) and manual sample injector (Rheodyne 7725, California) fitted with a 20 µL loop. The chromatographic separation was carried out on a Perfectsil

C18 column (250×4.6 mm id, 5 µm) supported with a C18 security guard cartridge. Mobile phase composed of acetonitrile/50 mM potassium phosphate buffer pH=7.5 (60:40 v/v) was pumped in isocratic mode. All separations were performed at a constant flow rate of 2 mL/min at room temperature. Drug concentration was determined at λ=307 nm and analyzed by ChromGate software.

2.6. HPLC method validation

HPLC method was validated according to US FDA guidance for analytical method validation. Spiked plasma samples at final concentrations of 0.10, 0.25, 0.75, 1.5, 3, 6 and 15 µg/mL were used for construction of the calibration curve. The ratios of the lamotrigine peak area to that of internal standard (1 mg/L carbamazepine) were plotted against lamotrigine concentrations for 3 consecutive days. The linearity of calibration curve was determined by calculation of squared correlation coefficient (r2).

Accuracy and precision of the method was determined by calculation of recovery and coefficient of variation percentages (CV %) for 3 replicate samples and in 3 consecutive days, respectively from the calibration curve. Limit of detection (LOD) of the method was determined from the curve slope and the variation of the results at the most diluted standard solutions. Limit of quantification (LOQ) was considered as the minimum concentration with relative standard deviation less than 10%.

2.7. Pharmacokinetic analysis

A non-compartmental pharmacokinetic approach was employed for determination of lamotrigine pharmacokinetic parameters. T_{max} and Cmax were obtained directly from the data. AUC_{0-t} was determined by trapezoidal method. $T_{1/2}$ and elimination rate were determined from terminal phase slope of the natural logarithm of concentration vs. time. $AUC_{0-\infty}$ was calculated

Table 1. Within and between run precision and accuracy of the assay method for determination of lamotrigine concentration in plasma samples.

Nominal Concentration (µg/mL)	Day	Mean Measured Concentration (mg/L)	Recovery (%)	Between- and Within-Run Accuracy (Mean Recovery, %)	Between- and Within-Run Precision (RSD, %)
15.00	1	29.68	98.93	99.77, 99.26	0.84, 0.85
15.00	2	29.93	99.77		
15.00	3	30.18	100.60		
6.00	1	17.01	113.40	102.07, 101.49	10.02, 10.06
6.00	2	14.89	99.27		
6.00	3	14.03	93.53		
3.00	1	6.77	112.83	102.39, 104.32	9.26, 5.41
3.00	2	6.00	100.00		
3.00	3	5.66	94.33		
1.50	1	3.12	104.00	98.67, 97.25	7.94, 8.11
1.50	2	3.07	102.33		
1.50	3	2.69	89.67		
0.75	1	1.53	102.00	91.78, 89.41	10.01, 10.36
0.75	2	1.23	82.00		
0.75	3	1.37	91.33		
0.25	1	0.66	94.29	89.05, 89.25	5.02, 4.48
0.25	2	0.59	84.29		
0.25	3	0.62	88.57		
0.10	1	0.21	86.00	92.19, 86.10	8.26, 9.48
0.10	2	0.22	89.00		
0.10	3	0.25	101.57		

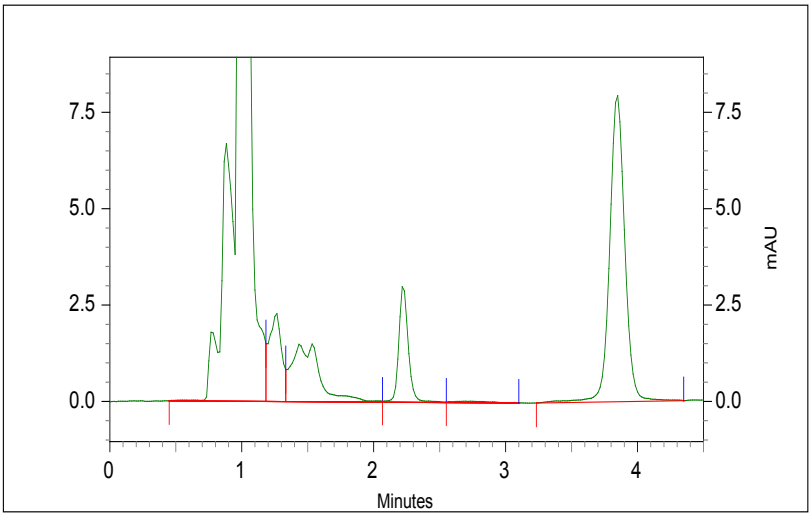

Figure. 1: HPLC chromatogram of 0.75 µg/ml lamotrigine spiked human plasma after extraction by 1µg/ml carbamazepine (internal standard) solution in acetonitrile.

from AUC_{0-t} and elimination rate constant.

2.8. Statistics

An analysis of variance (ANOVA) was performed with SPSS for Windows version 11 on pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and Cmax using GLM procedure in which the subject, sequence, period and formulation were considered as sources of variation. The 90% confidence interval (CI) of the test/reference ratio for PK parameters was determined for log-transformed data. The products were considered bioequivalent if 90% CI of the log-transformed PK parameters were within 0.80 and 1.25. Data were presented as mean±SD.

3. Results and discussion

3.1. Validation of chromatographic condition

There are several HPLC methods previously reported for determination of lamotrigine concentrations in human plasma using UV detection at 306-310 nm and C18 columns (8-11). A series of optimization experiments were conducted to obtain short elution time and high sensitivity assay by changing the mobile phase composition. A representative lamotrigine chromatogram at the optimum chromatography condition for spiked plasma samples are shown in fig. 1. The retention time for lamotrigine and carbamazepine was 2.2±0.1and 3.8±0.1 min respectively that is regarded as short time for in-vivo assay. The method produced linear responses (r2=0.999) over the wide range of concentrations (0.1-15 µg/mL). The regression equation was y=270x(µg/L)+0.11 (n=9) for determination of lamotrigine in plasma samples. The intercept was determined non-significant (P>0.05). The recovery percentages and CV% are shown in table 1. The recoveries varied between 89.2% to 104.32% and 89.05% to 102.39% for respective within and between-run experiments. Since within and between-run CV% were roughly less than 10% and the recoveries were within 15% of the nominal lamotrigine assay in plasma, the method shows acceptable accuracy and precision for lamotrigine assay in plasma. LOD and LOQ were determined 0.03 and 0.10 µg/mL that are comparable to those of the most sensitive lamotrigine assay methods reported in range of 0.1-0.5 µg/mL.

3.2. Lamotrigine pharmacokinetic and bioequivalence analysis

HPLC chromatograms of the human plasma after oral administration of 50 mg lamotrigine tablet in one volunteer are presented in fig 2. No volunteer was withdrawn from the study and no serious adverse effects were observed during the study for the reference and the test products. Two subjects experienced headache and nausea that was irrelevant of the product type and disappeared after having breakfast.

The mean plasma concentration-time curve of lamotrigine after single-dose administration of 50 mg tablets of the generic and reference products are presented in fig.3. The multivariate analysis revealed the absence of any formulation effect with regard to AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}. The point estimates for the mean ratio of test/reference product of AUC_{0-t}, $AUC_{0-\infty}$ and Cmax were 0.90, 0.98 and 0.95, respectively. The 90% CI for

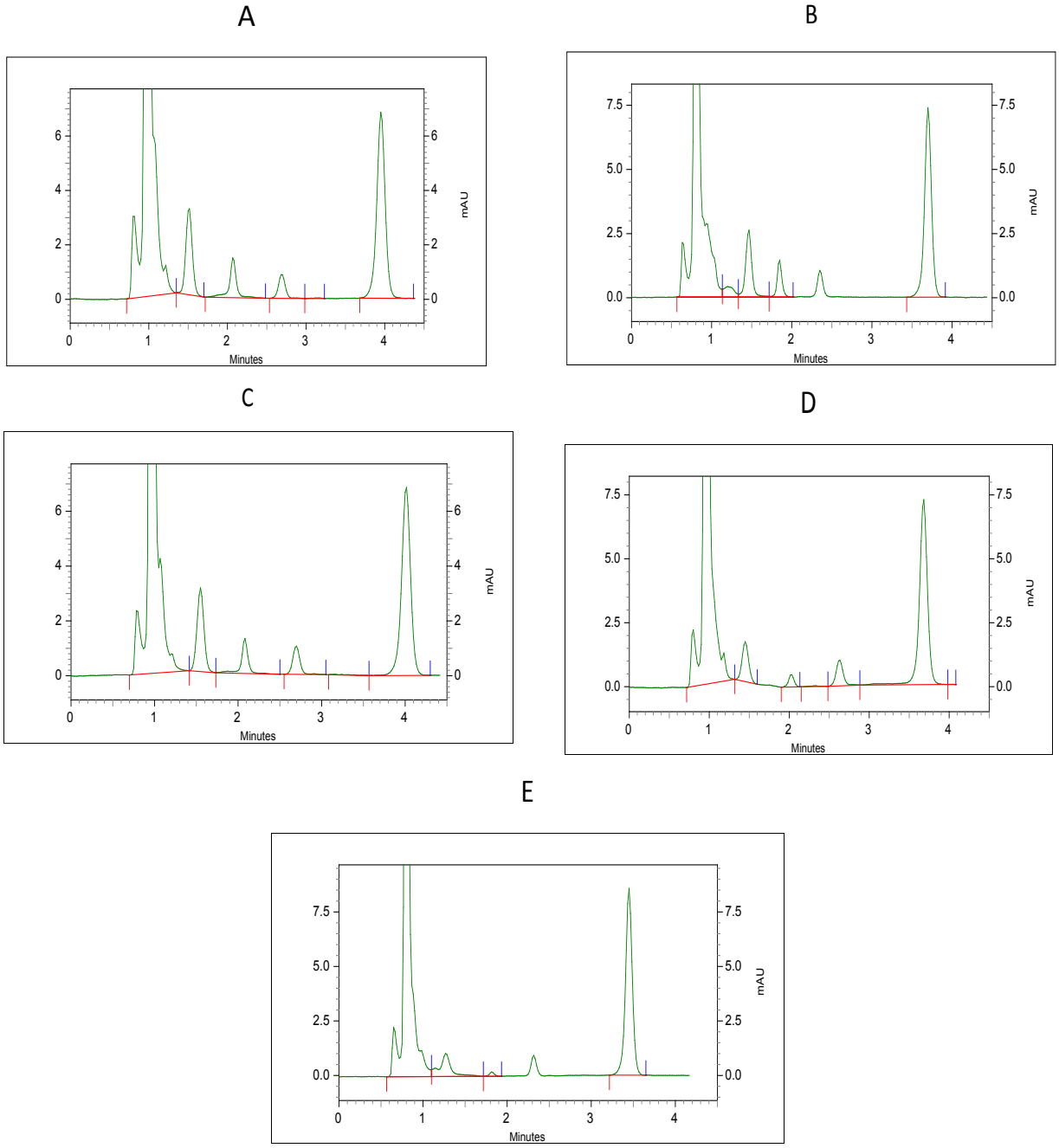

Figure 2: HPLC chromatogram of human plasma A (1h), B (4h), C (8h), D (36h) and E (72h) after oral administration in one volunteer.

the above motioned ratios was calculated based on log-transformed data to be 0.86-0.97, 0.86-0.97 and 0.83-1.09, respectively that were within the regulatory range (12). Moreover, no significant sequence effect was found for the PK parameters ($P>0.05$, table 2). The nonparametric Wilcoxon signed rank test did not reveal a significant difference between the two products with regard to Tmax (median Tmax of 2.2±1.4 and 2.8±1.8 h for the test and the reference product, $P>0.05$).

Comparing the pharmacokinetic data with the previous reports on lamotrigine shows that the average AUC0-96 was lower in the present study ($P<0.05$) than the study conducted in Thai population (19.1±0.3 µg.h/mL) (13). Furthermore, with regard to Cmax if linearly extrapolated to 100 mg dose, it was less than the reported values in Thai population (1.1 or 1.7±0.3 µg/mL); however, median Tmax of 2h was not significantly different with the previously published data. Regarding that

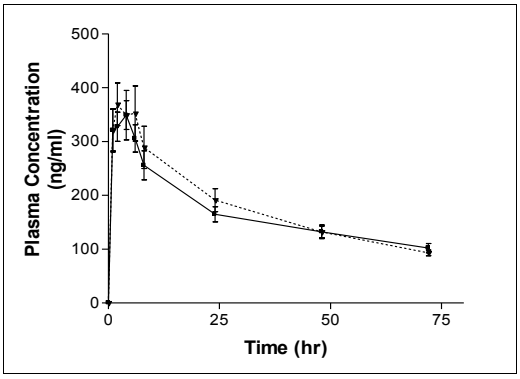

Figure 3. Lamotrigine mean plasma concentration versus time after single dose oral administration of 50 mg generic lamotrigine (solid line) or Lamictal® (dashed line) tablet.

the MRT for the test and the reference products did not differ from the previously reported data, it seems the possible differences between AUC of the populations may be due to a different drug disposition.

4. Conclusion

The validated rapid and simple HPLC method was suitable for lamotrigine pharmacokinetic studies. The results of bioequivalence study shows that the generic lamotrigine 50 mg tablet is bioequivalent in the healthy Iranian volunteers. Moreover, AUC and Cmax of a similar dose of lamotrigine tablet in healthy Iranian volunteers were estimated lower than some other reported populations. So, a population pharmacokinetic study is proposed to be performed in future in a larger sample of Iranian subjects to investigate demographic, environmental and genetic factors affecting drug disposition.

Conflict of interest

None declared.

Table 2 . Lamotrigine pharmacokinetic parameters in Iranian healthy subjects after oral administration of the test and the reference 50 mg tablets (n=12).

PK parameter	Product	Mean	SD	Mean Ratio (90% Confidence Interval)		P value
				Normal Scale	Logarithmic Scale	
AUC0-76 (µg.hr/mL)	Test	14.005	2.758	0.90 (0.85-0.95)	0.92 (0.86-0.97)	
	Reference	15.498	4.067			
$AUC_{0-\infty}$ (µg.hr/mL)	Test	16.743	3.657	0.98 (0.91-1.05)	0.96 (0.86-0.97)	
	Reference	17.037	4.497			
Cmax (µg/mL)	Test	0.424	0.085	0.95 (0.82-1.07)	0.96 (0.83-1.09)	
	Reference	0.447	0.125			
T_{max} (hr)	Test	2.2	1.4			
	Reference	2.8	1.8			
λz (hr-1)	Test	0.023	0.007			0.21
	Reference	0.020	0.004			
MRT (hr)	Test	45.7	11.5			0.52
	Reference	50.0	10.1			
Cl/F (mL/min)	Test	59	18			0.31
	Reference	52	15			
Vss/F (L)	Test	162	59			0.76
	Reference	156	41			

Abbreviations: AUC: area under the concentration curve, Cmax: maximum concentration observed, Tmax: time of maximum concentration observed, λZ: slope of the terminal disposition phase, MRT: mean residence time, Cl/F: clearance to bioavailability ratio, Vss/F: apparent steady-state volume of distribution to bioavailability ratio.

5. References

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