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Research Article

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Formulation of immediate release lamotrigine tablets and bioequivalence study

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ABSTRACT

Lamotrigine tablets were compressed directly by means of Avicel PH102, sodium starch glycolate, magnesium stearate, Aerosil 200 and PVPK25. A rapid, sensitive and simple high-performance liquid chromatographic (HPLC) method for the determination of lamotrigine in plasma is described. The drug was extracted from one mL of each rabbits plasma sample was transferred into a 15 mL tube fitted with a polyethylene cap 1 mL acetonitrile were added to the sample. The supernatant was injected into the HPLC system. The drug and the internal standard (carbamazepine) were eluted from C18 Zorbax ODS(4.6 x 250mm, USA) column at ambient temperature with a mobile phase consisting of acetonitrile and 20mM potassium dihydrogen phosphate buffer (35:65,v/v) and adjusted to (pH7) using 1NNaOH, at a flow rate of 1.5 ml min^{-1} and the detector was monitored at 210 nm. Quantitation was achieved by measurement of the peak-area ratio of the drug to the internal standard and the lower limit of detection for lamotrigine in plasma was 0.491 µg ml^{1}. The intraday precision ranged from 0.801-7.692 % coefficient of variation (CV) and accuracy ranged from 0.048-4.9(relative error%) for samples. The relative recoveries of lamotrigine ranged from from 95.10 to 101.89%. The method was applied in studying the pharmacokinetics of lamotrigine administered orally to rabbits. This reliable micro-method would have application in pharmacokinetic studies of lamotrigine. The relative percentage bioavailability of prepared lamotrigine tablets with respect to the commercially available Lamictal® tablets was 134.68%.

Keywords: Lamotrigine; Liquid chromatography; Plasma; Pharmacokinetic studies.

INTRODUCTION

Lamotrigine [3,5-diamino-6(2,3-ichloroph-enyl)-1,2,4-triazine] is a novel antiepileptic drug, chemically unrelated to antiepileptic agents in current use. Its pharmacological action is similar to that of phenytoin and carbamazepine [1-25]. Lamotrigine is effective as an add-on therapy in the management of simple and complex partial seizures and secondarily generalized tonic-clonic seizures resistant to multiple-drug therapy [1,13]. In humans, lamotrigine is rapidly and completely absorbed with an oral bioavailability of about 98% [12]. The drug has an elimination half life of about 24 h [12] and a plasma protein binding of 55% of the administered dose, 70% can be recovered in the urine, 90% of which is in the form of a glucuronide conjugate [15,17].

A number of high-performance liquid chromatographic (HPLC) assays are presently available for the measurement of lamotrigine in biological fluids [3,8-15,21-25]. However, there are a number of disadvantages associated with some of these methods. These include lengthy extraction procedures, relatively large volumes of organic solvents and poor recovery [12].

This report presents a simple, and specific HPLC method for determination of lamotrigine in plasma was used for assessing the bioavailability of the drug from prepared lamotrigine tablets in comparison to the commercially available lamictal® tablets. A cross over design was carried out using rabbits as a model. Relative bioavailability and pharmacokinetic parameters were calculated.

EXPERIMENTAL SECTION

2.1. Materials:

2.1.1. Apparatus

The high-performance liquid chromatographic system consisted of: Isocratic pump L-7110, (Hitachi Ltd, Japan); UV/VS Detector L-7420, (Hitachi Ltd, Japan); C18 HPLC column, Zorbax ODS(4.6 x 250mm, USA). Single punch tablet press machine model TDP (Shanghai Tiane Pharmaceutical Machinery Factory, Shanghai, China).

2.1.2.Materials and Reagents

Lamotrigine (kindly Supplied by Delta Pharma S.A.E, Egypt) Batch no LM10110606. Lamictal® tablets batch no. R1-77991; (Wellcome, London). Carbamazepine (internal standard) was obtained from Novartis Pharma, (Cairo Egypt). Microcrystalline cellulose "Avicel PH102", sodium starch glycolate "Explotab" (FMC Co, Pennsylvania U.S.A). Colloidal silicon dioxide "Aerosil 200"(hydrophilic) (Degussa, U.S.A), magnesium stearate (ADWIC, Egypt). Solvents used were of HPLC grade and all other chemicals and reagents were of analytical grade.

2.2. Methods:

2.2.1. Preparation of Conventional Immediate Release Lamotrigine Tablets

Lamotrigine tablets were compressed directly by means of Avicel PH102, as diluent. As disintegrant sodium starch glycolate. 1% magnesium stearate as a lubricant. Tablets weighing 200mg and containing (Lamotrigine 50mg + 63.5% Avicel PH102 + 5% Explotab + 0.5% Aerosil 200 + 5% PVPK25 + 1% Magnesium stearate). Using single punch machine and concave 9mm punch and die set, batch of tablets was prepared.

2.2.2. Preparation of standard solutions

Lamotrigine stock solution was made up in methanol to a concentration of 100 µg ml^{-1}. This solution was further diluted in methanol to give a working standard solution of 10 µg ml $^{-1}$. A stock solution of carbamazepine as internal standard was made up in methanol to a concentration of 100 µg ml^{-1}. Further dilution was made in methanol to give a working internal standard solution of 10 µg ml^{-1}.

2.2.3. Chromatographic conditions

A mobile phase consisting of acetonitrile and 20mM potassium dihydrogen phosphate buffer (35:65,v/v) and adjusted to (pH7) using 1NNaOH was used. It was freshly prepared and degassed daily by passing it through a 0.22 mm Millipore membrane filter (Millipore, Bedford, MA, USA). Chromatography was performed at ambient temperature using a flow rate of 1.5 ml min^{-1}. The column elute was monitored at 210 nm with a sensitivity of 0.01 absorbance units full scale (AUFS) and a chart speed of 0.5 cm min^{-1}.

2.2.4. Extraction procedure

To 1 ml of plasma were applied in the preparation of plasma samples and standards, where one mL of each rabbits plasma sample was transferred into a 15 mL tube fitted with a polyethylene cap; 1 mL of carbamazepine internal standard working solution (10µgml^{-1}) and 1 mL acetonitrile were added to the sample. After vortex mixing for 30 seconds and centrifugation for 10 minutes at 3000rpm. 25µL of the supernatant was injected into the HPLC system.

2.2.5. Application

The study was performed for formulae, namely; lamictal® tablets (market product) and the prepared lamotrigine tablets. Six rabbits were randomly divided into two groups, each containing three rabbits. A simple cross over design was applied on two phases, so that each group received a single oral dose of one of the tested formula in each phase.

Six healthy male New Zealand rabbits, weighing between 2 and 2.5kg were used in the study. The animals were fasted overnight (water given ad libitum) and then given a single oral dose of (20mgkg^{-1}). Blood samples were collected into small plastic centrifuge tubes through the marginal ear vein just before dosing and at 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12 and 24h after lamotrigine administration. The blood samples were withdrawn into tubes washed with diluted heparin to guard against coagulation of blood. The blood samples were then centrifuged at 3000rpm for 10 minutes and the clear plasma was then collected in polyethylene capped tubes and deep frozen at-20ºC till required for extraction and analysis.

2.2.6. Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and time needed to attain this concentration (T_{max}) were observed directly from the plasma concentration–time profiles. The first order disposition rate constant (Kd) was determined from the best log-linear fit of the terminal phase by least-squares linear regression analysis and then the half-life was calculated as 0.693/Kd. The area under the plasma concentration–time curve (AUC) and the area under the first

moment of plasma concentration–time curve (AUMC) were calculated by the trapezoidal method. Mean residence time (MRT) of the drug in the body was estimated as MRT:$AUMC_{0-\infty}/AUC_{0-\infty}$.

RESULTS AND DISCUSSION

The mobile phase at pH 7 and the flow rate 1.5 ml min^{-1} used for the assay achieved optimum resolution of lamotrigine and the internal standard with no interference. It was also observed that adjusting the detector wavelength at 210nm gave maximum sensitivity of lamotrigine compared to that of 305 nm (Fig. 1).

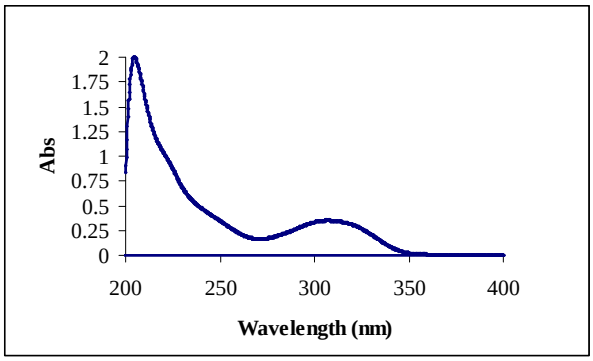

Fig.1. UV absorption spectrum of lamotrigine

A variety of extraction solvents, including the extraction solvent of methanol and acetonitrile was selected, because it gave cleaner chromatograms and better recovery of lamotrigine in our HPLC assay it eluted very quickly with the endogenous plasma components at a retention time of 3.07 min.

Fig. 2 shows representative chromatograms of drug-free rabbit plasma, and a plasma sample taken at 24 h from a rabbit taking lamotrigine (20 $mgkg^{-1}$,PO) using the described procedure. Retention times of lamotrigine and the internal standard were 3.07 and 6.24min, respectively.

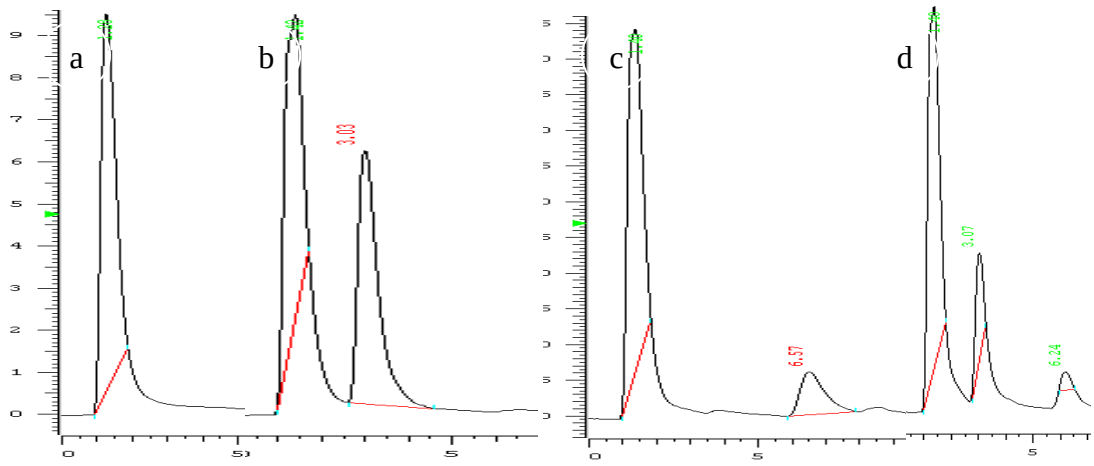

Fig.2.HPLC chromatograms of: (a) blank rabbit plasma. (b) rabbit plasma spiked with lamotrigine. (c) rabbit plasma spiked with carbamazepine(internal standard). (d) rabbit plasma spiked with lamotrigine and carbamazepine (internal standard).*Retention time of: lamotrigine =3.07 min. carbamazepine = 6.24 min

3.1. Quantitation

The quantitation of the chromatograms was achieved by the peak-area ratios of the drug to the internal standard. To determine the linearity of the assay, various rabbit plasma standards were prepared by spiking drug-free rabbit plasma samples with known quantities of the drug at eight non-zero concentrations over the range of 0.5– 40 µg ml^{-1}. Standards were analysed in replicates of three, analysed at concentrations 0.5, 1, 2, 3, 5, 10, 20 and 40 µg ml^{-1}.

The peak area ratios of D/I (drug/internal standard) were plotted against the concentrations. The slope, intercept and correlation coefficient were determined by the method of least-squares linear regression analysis. Standard curves of lamotrigine in rabbit plasma were constructed on three different days to determine the variability of the slopes and

intercepts. Table 1 shows the results from the linearity study. The linear regression analysis of the data was characterized as having a slope of 0.053 and an intercept of -0.0032 (correlation coefficient-0.9999). The results showed little day-to-day variability of slopes and intercepts, as well as good linearity over the plasma concentration range studied.

Table 1. Lamotrigine standard curve summery

Concentration (µg/mL)	Peak Area Ratio (PAR)±SD	C.V.%
0.00	0.00	----
0.5	0.026±0.002	7.692
1	0.052±0.003	5.769
2	0.101±0.006	5.940
3	0.155±0.008	5.161
5	0.252±0.012	4.761
10	0.540±0.015	2.777
20	1.041±0.045	4.322
40	2.121±0.017	0.801

C.V. % Percentage Coefficient of Variation (precision)

3.2. Sensitivity

The lower limit of quantitation (LOQ) for lamotrigine was established by injecting three different rabbit plasma samples containing 0.5µg ml^{-1} (the lowest concentration on the standard curve). The CV was 7.7%. Therefore, the LOQ for lamotrigine was 0.5µg ml^{-1}.

3.3. Specificity

The specificity of the method was established by analyzing six independent sources of the drug-free rabbit plasma. All the tested blanks were free from endogenous plasma components at the retention times of the drug and the internal standard.

3.4. Precision

The intraday precision was determined from replicate analysis of pooled rabbit plasma samples containing lamotrigine at eight different concentrations (0.5, 1, 2, 3, 5, 10, 20 and 40 µg ml^{-1}) covering the low, medium and high ranges of the calibration curve.

Precision is expressed as the percent coefficient of variation (%CV) for the concentrations back-calculated from the regression analysis. Accuracy is expressed as a percentage the intraday precision ranged from 0.801-7.692(C.V%) and accuracy ranged from 0.048-4.9(relative error%) for samples(tables 1& 2).

Table 2. Intraday accuracy of lamotrigine in rabbit plasma

$C_{nominal}$(µg/mL)	C_{est}(µg/mL)	SD	Relative Error %
0.5	0.491	0.012	-1.8
1	0.981	0.016	-1.9
2	1.906	0.011	-4.7
3	2.925	0.003	-2.5
5	4.755	0.025	-4.9
10	10.189	0.002	1.89
20	19.642	0.020	-1.79
40	40.019	0.019	0.048

$C_{nominal}$: Nominal (added) Concentration.
C_{est}: Estimated (found) Concentration.
SD: Standard Deviation.
Relative Error%: Relative Deviation from the Nominal Concentration (Accuracy)

3.5. Recovery

Relative recoveries for lamotrigine and the internal standard were determined by spiking drug free rabbit plasma with known amounts of the drug and the internal standard to achieve the lamotrigine concentrations of 0.5, 1, 2, 3, 5, 10, 20 and 40µg ml^{-1}. The samples were extracted and analyzed with the developed procedure.

The absolute recoveries were calculated by comparing the resultant peak areas with those obtained from pure standards, in mobile phase, of the drug and the internal standard at the same concentrations. The relative recovery of lamotrigine was calculated by comparing the concentrations of the drug-spiked plasma with the actual added concentrations. The relative recoveries of the lamotrigine ranged from 95.10 to 101.89% (Table 3).

Table 3. Extraction recovery of lamotrigine from spiked rabbit plasma, (n=3)

Concentration Added (µg/mL)	Mean Concentration Found (µg/mL) ±SD	% Recovery ±SD
0.5	0.491±0.012	98.20±2.21
1	0.981±0.016	98.10±4.08
2	1.906±0.011	95.30±1.23
3	2.925±0.003	97.50±5.40
5	4.755±0.025	95.10±4.07
10	10.189±0.002	101.89±2.16
20	19.642±0.020	98.21±3.25
40	40.019±0.019	100.05±1.44
Mean % Recovery±SD		98.044±2.98

SD: Standard Deviation

3.6. Application

The mean plasma concentration–time profile after a single lamotrigine oral dose (20mg kg^{-1}) to six healthy male New Zealand rabbits is shown in Fig. 3. The absorption of lamotrigine in rabbits is rapid, reaching peak plasma concentration in about 1.0h. The computed pharmacokinetic parameters are shown in Table 4.

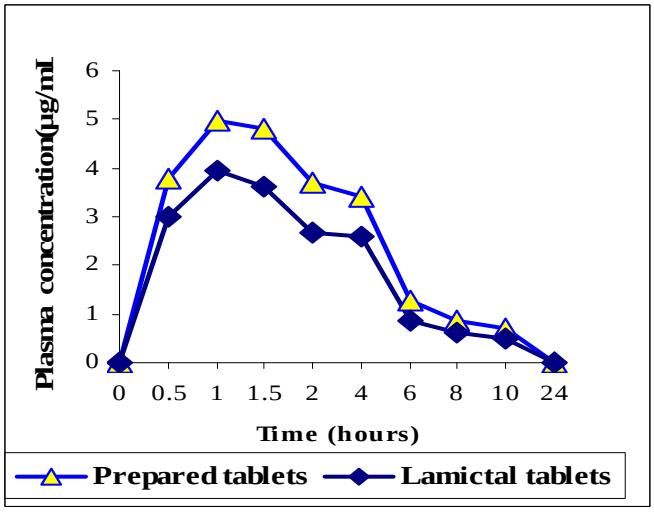

Fig.3. Mean plasma lamotrigine concentration following a single oral dose administration of the prepared lamotrigine and the Lamictal® tablets

Table 4.Pharmacokinetic parameters (mean± SD) of lamotrigine after an oral administration of Lamictal® tablets and prepared tablets (20mg kg^{-1}) to six rabbits

Parameter	Lamotrigine Formulations			
	Lamictal® Tablets		Prepared Tablets	
	Mean	SD	Mean	SD
Cp_{max}(µg/mL)	3.999	0.097	5.197	0.349
T_{max}(hour)	1 (Median)		1.25 (Median)	
$AUC_{(0-t)}$	17.10	1.145	23.03	1.319
$AUC_{(0-\infty)}$	20.52	1.218	27.63	2.485
$AUMC_{(0-t)}$	55.62	7.746	80.19	5.995
$AUMC_{(0-\infty)}$	117.31	19.313	157.41	30.022
MRT(hour)	5.70	0.745	5.65	0.615
$T_{1/2}$(hour)*	5.09	0.782	4.61	0.557
K_{el}($hour^{-1}$)**	0.139	0.023	0.152	0.018
Relative Bioavailability (%)	134.68%			

SD: Standard Deviation
**Elimination Half-Life*
***Elimination Rate Constant*

Plasma lamotrigine concentrations obtained following a single oral dose administration of 20mgkg^{-1} of the market product (Lamictal® tablets), and prepared lamotrigine tablets; to six rabbits. The mean plasma lamotrigine concentrations versus time are graphically illustrated in figure (3). The individual and the mean pharmacokinetics parameters calculated from lamotrigine plasma concentration time data of the six rabbit following the administration

of each the tested formula, in addition to the relative bioavailability of prepared lamotrigine tablets with respect to Lamictal® tablets are shown in table (4).

CONCLUSION

The HPLC method described for the measurement of lamotrigine in plasma is sensitive, simple, reproducible, rapid and precise, making it valuable in many applications, particularly in pharmacokinetic studies including bioequivalence studies. Moreover, this method can be adapted to simultaneously measure the plasma concentrations of other antiepileptics. The relative percentage bioavailability of prepared lamotrigine tablets with respect to the commercially available Lamictal® tablets was 134.68%.

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