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Reserch Article

LIQUID CHROMATOGRAPHIC ASSAY OF LACOSAMIDE IN HUMAN PLASMA USING LIQUID-LIQUID EXTRACTION

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ABSTRACT

The main objective of the study was to develop and validate a simple, rapid, selective, accurate, sensitive, robust and reproducible HPLC method for the determination of lacosamide an anti-epileptic agent in human plasma. The analyte and internal standard, ranolazine were extracted by liquid – liquid extraction using diethyl ether–dichloromethane (70:30, v/v) using a Glas-Col Multi –pulse vortexer. The chromatographic separation was done on reverse phase chromatographic column Hypersil BDS C18 column with a mobile phase of orthophosphoric acid pH 2 –methanol (60:40, v/v) at a flow rate of 1ml/min and the eluent was monitored at 225 nm. The chromatograms showed good resolution and no interference from plasma. The retention time (min) of lacosamide and internal standard were approximately 3.0±0.05 and 4.5±0.05 respectively. The method was linear over the range of 0.201 µg/ml to 20.080 µg/ml with regression coefficient (r^2) 0.999. The mean recovery from the human plasma was found to be 95% ± 0.3%. Both accuracy and precision data showed good reproducibility.

Keywords: Lacosamide, Liquid-liquid extraction, HPLC, Method Validation.

INTRODUCTION

Lacosamide (fig.1) is anti epileptic agent used as adjuvant therapy for partial onset of seizures. Lacosamide is chemically R-2-acetamido-N-benzyl-3methoxypropionamide[1]. It occurs as a white to slight yellow crystalline powder with a molecular weight of 250.3 g/mol. It is soluble in organic solvents such as ethanol, DMSO and dimethyl formamide, slightly soluble in acetonitrile, soluble in phosphate buffered saline at pH 7.2[2,3]. To our best knowledge, there is only one published chromatographic technique on lacosamide determination in human plasma (C Kestelyn *et al.,* 2011[4]. Although simple and rapid, the said method suffers from incomplete protein removal as it involves protein precipitation. Problems can arise from LC column fouling, occasional trapping of analyte in the pelleted protein after centrifugation. Further, the method has narrow range of linearity.

The proposed LC method utilizes liquid – liquid extraction technique for sample preparation as it provides efficient sample clean-up and enrichment. This results in high selectivity and sensitivity for target analytes. It is well known that liquid -liquid extraction yields much cleaner samples than protein precipitation method. The study involves optimization of solvent system and adjustment of sample pH to obtain very clean extracts by excluding interferences from the matrix materials.

The purpose of this method is to develop and validate highly selective, sensitive, accurate and precise HPLC method for the quantification of Lacosamide in human plasma over a broad linearity range. The results of analysis were validated by statistical method and recovery studies.

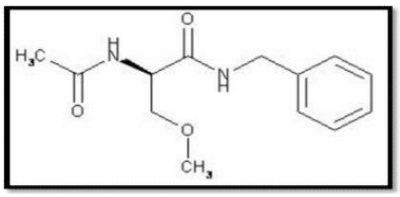

Fig. 1: Structure of Lacosamide

MATERIALS AND METHODS

Materials

Lacosamide standard (99.5% pure) and internal standard Ranolazine (99.7% pure) were obtained from Rainbow pharma lab, Hyderabad. Drug free human plasma containing EDTA was obtained from approved blood bank. HPLC grade solvents such as methanol, diethyl ether, Water, dichloromethane were obtained from Merck. All other chemicals were of analytical grade.

Method - Chromatographic conditions

HPLC system Waters (e2695) with PDA detector (Waters 2996) were used. Separation was carried out on Hypersil BDS C18 column (250 mm×4.6mm,5µ) using mobile phase orthophosphoric acid buffer pH 2 and methanol (60:40) at a flow rate 1ml/min with detector wave length set at 225 nm. The mobile phase was filtered through nylon Millipore (0.2µm) membrane filter ,and degassed using bath sonicator prior to use. Column temperature was maintained at 30^0c.

Stock solution of Lacosamide (5.0425mg/ml) and IS (10 µg/ml) were separately prepared separately in 10 ml and 100ml volumetric flasks respectively, using methanol as the solvent.

Sample processing

A 500-µL volume of plasma sample was transferred to 15-mL glass test tube and then 25µL of IS working solution (1.0 µg/ml) was spiked. After vortexing for 30s 4mL aliquot of extraction solvent, diethyl ether–dichloro methane (70:30)[5,6,7,8]was used using dispenser. The sample was vortex mixed for 3 mins using Multi-pulse vortexer at 3000 RPM (Glas-Col.Terre Haute, USA). The organic layer (3ml) was transferred to a 5 mL glass tube and evaporated to dryness using Turbo Vap LV Evaporator (Zymark, Hopkinton, MA,USA) at 40^0c under the stream of nitrogen .Then the dried extract was reconstituted in 200 µL of diluent (water-methanol,20:80,v/v) and 20µL aliquot was injected into the chromatographic system.

Bioanalytical method validation

Working solutions for calibration and controls were prepared from the stock solution by dilution using water–methanol (8:2). Working

solutions (0.2 ml) were added to 10ml drug free plasma to obtain lacosamide concentration levels of 0.201 ,0.402 ,1.004 ,2.008 ,4.016 ,8.032 ,16.064 and 20.080 µg/ml. Quality control (QC) samples were prepared in bulk ,at concentrations of 0.204µg/ml (LLOQ), 0.599 µg/ml (LQC) ,8.090 µg/ml (MQC) and 15.265µg/ml (HQC). A calibration curve was constructed from eight non- zero samples covering the total range (0.201 µg/ml-20.080 µg/ml), including lower limit of (LLOQ). Such calibration curves were generated on four consecutive days. The calibration curve should have a correlation coefficient (r^2) of 0.99 or better. The acceptance criterion for each back-calculated standard concentration was 15% deviation from nominal value except LLOQ, which was set at 20%. At least 75% of non zero standards should meet the above criteria, including acceptable LLOQ and upper limit of quantification. The within batch precision and accuracy were determined by analyzing six sets of quality control samples in batch. The between-batch determined by analyzing six sets of quality control samples on four different batches. The quality control samples were randomized daily, processed and analyzed in position either (a) immediately following the standard curve, (b) in the middle of batch or (c) at the end of the batch. The acceptance criteria of within batch and between batch precision were 20% or better for LLOQ and 15% or better for the rest of concentrations and the accuracy was 20% or better for LLOQ and 15% or better for rest of concentrations .Recovery of lacosamide from extraction was determined by a comparison of peak area of lacosamide in spiked plasma samples (six low and high quality controls) to the peak area of lacosamide in samples prepared by spiking extracted drug free plasma samples with the same amounts of lacosamide at the step immediately prior to chromatography. Similarly, recovery of IS was determined by comparing the mean peak areas of extracted quality control samples to mean peak area if IS in samples prepared by spiking extracted drug free plasma samples with the same amounts of IS at step immediately prior to chromatography.

RESULTS AND DISCUSSION

Under the chromatographic condition employed, the sample showed sharp peaks of drug and internal standard with good resolution. The retention time of drug was found to be 3.0±0.09 min and retention time of internal standard was 4.5± 0.05 min (fig.2). The method developed was validated for specificity, accuracy & precision, linearity and stability. The results of validation are given below.

Specificity

Specificity of method was proven by absence of the peaks in plasma near retention time of drug as well as internal standard(fig.3,4).

Linearity

The calibration function (peak area ratio vs concentration) was linear over working range of 0.201 µg/ml to 20.080 µg/ml used for quantification by linear regression. The regression equation for analysis was y=0.556x-0.012 and r^2=0.999. (fig5)

Recovery

The % mean recovery lacosamide for LQC ,MQC ,HQC levels were 95.3% ,96.4% ,94 .2% and IS mean recovery at MQC level was found to be 94.7% (Table .1,2).

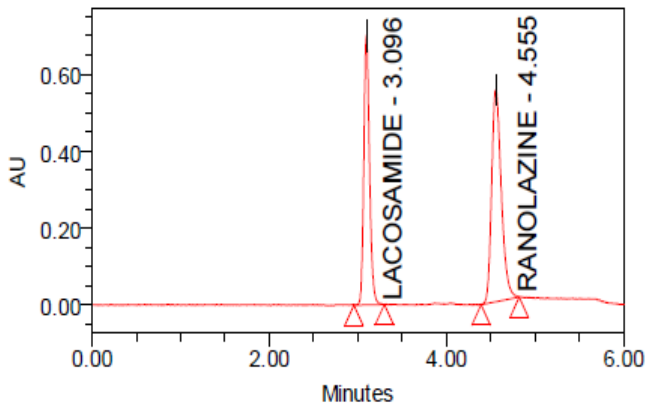

Fig. 2: Representative chromatogram of showing retention times of Lacosamide and IS at LLOQ

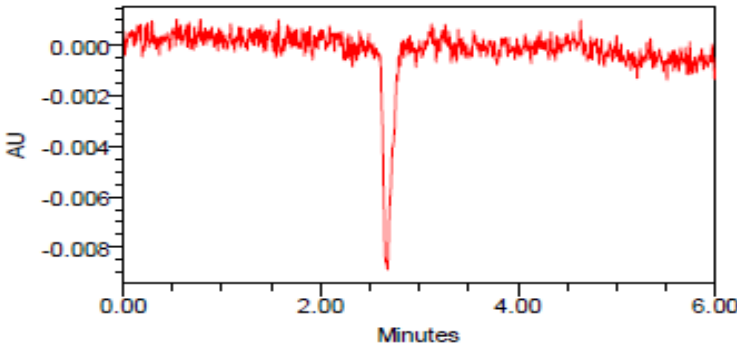

Fig. 3: Blank plasma showing no interference at RT of lacosamide and ranolazine

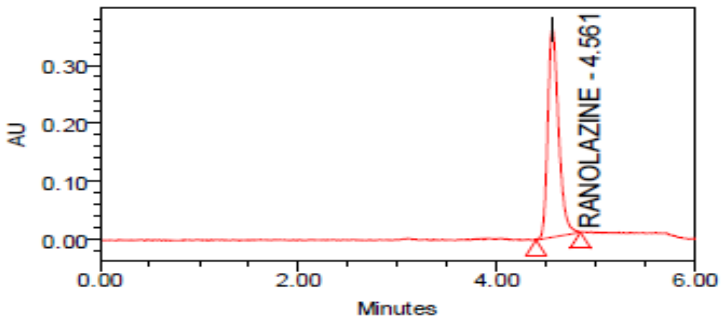

Fig. 4: Chromatogram showing IS peak

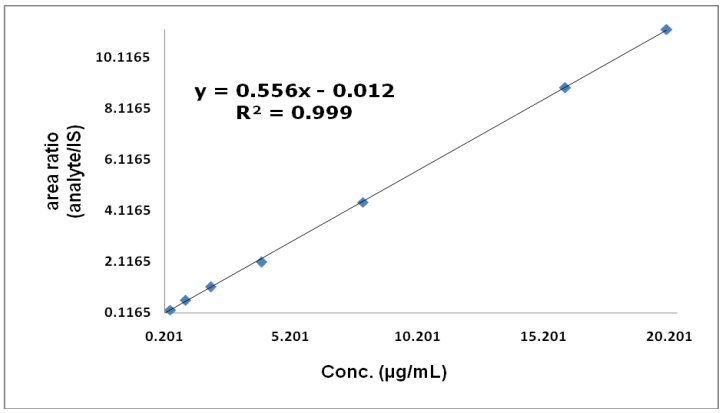

Fig. 5: Showing Linearity curve of lacosamide (0.201µg/ml to 20.080µg/ml)

Table 1: Showing Recovery of Lacosamide at LQC, MQC, HQC levels

Replicate	LQC		MQC		HQC	
	Extracted Area	Post extracted Area	Extracted Area	Post extracted area	Extracted Area	Post extracted Area
1	57944	60789	798566	812349	1523451	1608901
2	59640	61125	771234	809899	1512349	1612348
3	60088	61976	809899	810245	1509987	1599972
4	55123	60989	768234	807654	1509921	1618998
5	59234	61024	787954	821324	1499898	1596789
6	56890	60256	758234	809876	1443212	1518981
Mean	58153.2	61026.5	782353.5	811891.2	1499803	1592664.9
SD	1894	559	19787	4856	28724	36998
%RSD	3	1	3	1	2	2
% Recovery	95.3		96.4		94.2	

Table 2: Showing Recovery of Ranolazine at MQC level

Replicate	Internal Standard	
	Extracted Area	Post extracted Area
1	185456	194347
2	185178	191346
3	175076	191896
4	175034	191134
5	184989	192154
6	185346	190897
Mean	**181846.5**	**191962.4**
SD	**5263**	**1259**
%RSD	**3**	**1**
% Recovery	**94.7**	

Lowest concentrations

The lower limit of quantification (LLOQ) of lacosamide in human plasma assay was found to be 0.201 µg/ml .The between – batch precision at LLOQ was found to be 7.8 % . The between batch accuracy was found to be 107.0% (Table 3). The within batch precision was 6.0% and the accuracy was 111.7% for lacosamide.

LQC, MQC, HQC concentrations

The LQC, MQC and HQC concentrations were 0.599, 8.090, 15.265 µg/ml. The between batch precision of LQC, MQC, HQC were 5.4%, 4.5%, 6.6% respectively. The between batch accuracy of LQC, MQC, HQC were 95.0%, 93.7%, 96.3% respectively. The within batch precision of LQC, MQC, HQC were 4.4%, 4.0%, 5.3% respectively. The within batch accuracy of LQC, MQC, HQC were 95.4%, 92.4%, 99.4% respectively (Table 3)

Stability

Freeze –thaw stability

The freeze- thaw stability of lacosamide in human plasma was assessed by analyzing six replicates of quality control at low and high levels (stability samples),previously frozen and thawed over 3 cycles . The freeze thaw stability of lacosamide after 3 cycles was 94.4%, 96.3% at low and high concentrations (Table 4) .

In-injector stability

In injector stability of Lacosamide in human plasma was assessed by storing the processed QC samples at low and high levels (stability samples) concentrations for 38.15 hrs at 10 ^{0}c in auto sampler and then analyzed by using freshly prepared calibration. The % stability at low and high QC level was calculated .The in- injector stability of low and high concentrations after 38.15 hrs was found to be 95.0% and 95.6% respectively (Table 4).

Table 3: Showing Precision and accuracy of HPLC method for determining Lacosamide concentration in plasma samples

Concentration added µg/ml	Within –batch precision (n=6)			Between batch precision (n=4)		
	Concentration found(mean ±SD;µg/ml)	Precision (%)	Accuracy (%)	Concentration found (mean ±SD;µg/ml)	Precision (%)	Accuracy (%)
0.201	0.2279±0.0136	6.0	111.7	0.2182±0.0170	7.8	107.0
0.599	0.5709±0.0250	4.4	95.4	0.5686±0.0308	5.4	95.0
8.090	7.4785±0.3002	4.0	92.4	7.5836±0.3390	4.5	93.7
15.265	15.1753±0.8024	5.3	99.4	14.6982±0.9630	6.6	96.3

Table 4: Showing Results of stability

Sample conc. µg/ml	Mean conc. µg/ml	Precision (%)	Accuracy (%)
Three freeze-thaw cycles			
0.599	0.565	**5.0**	**94.4**
15.265	14.707	**4.6**	**96.3**
In –injector stability			
0.599	0.569	**5.0**	**95.0**
15.265	14.597	**4.5**	**95.6**

CONCLUSION

In summary, liquid chromatographic assay of lacosamide in human plasma by liquid –liquid extraction method was developed and fully validated as per FDA guidelines[9,10] .This method offers significant advantages over the previously reported methods in terms of simple and cleaner sample preparation, improved sensitivity and selectivity .The desired sensitivity of lacosamide was achieved with an LLOQ of 0.201 µg/ml which has a within-batch and between-batch precision 6.0% and 7.8% respectively. The validated method allows quantification of lacosamide in the 0.201µg/ml to 20.080 µg/ml range.

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