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QUANTITATIVE DETERMINATION OF LACOSAMIDE IN HUMAN PLASMA USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY AND ITS APPLICATION TO PHARMACOKINETIC STUDY

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ABSTRACT: A simple, reliable, and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the determination of lacosamide, an antiepileptic drug, in human plasma. The lacosamide and internal standard (IS) have been extracted by a simple liquid-liquid extraction method and separated on C_{18} (50 × 4.6 mm, 5µm) column with the mobile phase of 5mM ammonium formate and methanol (50:50, v/v), pumped at flow-rate of 0.5 mL/min. The ion transitions were monitored in positive ion mode $[M+H]^+$ at m/z 251.20 >91.10 for lacosamide and m/z 256.10>211.10 for IS. Method validation revealed excellent linearity over the concentration range of 9 - 9000 ng/mL together with satisfactory intra and inter-assay precision, accuracy, and extraction recoveries. Lacosamide was found stable throughout the various sample handling and processing conditions. It is proposed to evaluate the performance of the developed method by measurement of a plasma concentration of lacosamide versus time in four epileptic patients to monitor pharmacokinetic behavior of it. The variation in estimated pharmacokinetic parameters shows the need for individual monitoring of lacosamide concentration profile in epileptic patients by the sensitive, accurate and specific method.

INTRODUCTION: Epilepsy is a chronic non-communicable disorder of the brain that affects people of all ages. Approximately 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally [1]. Lacosamide ((2R)- 2- (acetylamino)- N- benzyl- 3- methoxy-propanamide) is a third-generation antiepileptic drug widely used as an adjunctive treatment of partial-onset seizures in most adult patients [2].

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Lacosamide has a different mechanism of action than other anticonvulsants, and hence, the patient who have failed to respond other anti-epileptic drugs (AEDs) may respond to lacosamide [3]. Furthermore, the selective action of lacosamide on sodium channel slow inactivation may results in improved tolerability compared to other AEDs. The reported pharmacokinetic parameter of lacosamide suggest that it has low plasma protein binding (<15 %), the maximum concentration in blood plasma was reached approximately 1 - 3 h after oral administration, having a half-life of about 12-16 hours, and it is mainly excreted unchanged through urine (95%) and by different metabolic pathways [4].

The monitoring of blood plasma levels of lacosamide has been playing an important role in the treatment of epilepsy to determine the optimum

therapeutic dose for an individual patient. Few methods were reported for the estimation of lacosamide from biological fluids. The efforts have been done to quantify lacosamide by high-performance liquid chromatography (HPLC) method from rat plasma [5], canine serum [6], human plasma [7], and human serum [8]. The hyphenated techniques were also available to quantify lacosamide, such as liquid chromatography-mass spectrometry (LC-MS) from human plasma, saliva, and urine sample [9], gas chromatography-mass spectrometry (GC-MS) from human plasma [10], and liquid chromatography-tandem mass spectrometry (LC-MS/MS) from rat plasma [11], human serum [12-13], post-mortem blood, serum and plasma [14], *In-vitro* metabolism study in human liver microsomes [15] as well as in aqueous samples [16-18].

In light of the aforementioned methods, a single LC-MS/MS method was available for quantification of lacosamide from human plasma [14]. However, the method required a long analytical run time (17 min) and less sensitivity level as the lower limit of quantification for lacosamide (0.5 µg/mL). **Table 1** summarized reported analytical methods for the measurement of lacosamide from biological matrices and set many reported methods to have relatively time-consuming analytical run time, insufficient sensitivity, large sample volumes, and complex sample preparation. Mainly, stated methods were used protein precipitation extraction (PPE) and solid-phase extraction (SPE) techniques to recover lacosamide from biological fluids. Studies have shown that PPE could not remove phospholipid as compared to liquid-liquid extraction (LLE) presented in plasma, which may be affecting the results.

Hence, sometimes PPE was considered as less clean extraction or crude extraction technology from plasma [19]. Moreover, the SPE technique is more complex, and it requires a high cost per sample than other extraction techniques. Hitherto, no LLE method was available for the extraction of lacosamide from the human biological matrices.

In this article, a new LC-MS/MS method was demonstrated to provide sensitive, precise, and accurate quantification of lacosamide from human plasma. This method allows simple and fast sample preparation using the LLE technique and a shorter runtime. Furthermore, the method has been effectively applied to epileptic patients to observe the lacosamide plasma concentration with respect to time and measured the pharmacokinetic parameters.

TABLE 1: THE SUMMARIZED REPORTED ANALYTICAL METHODS FOR THE MEASUREMENT OF LACOSAMIDE FROM BIOLOGICAL MATRICES

S. no.	Analytical Method	Biological Matrix	Sample Preparation	Linear range (µg/mL)	Plasma volume (µL)	Analysis Time (min)	Ref.
1	HPLC – UV	Rat Plasma	LLE	0.025 – 10	200	17	5
2	HPLC – UV	Canine Serum	PPE	0.5 – 25	100	12.5	6
3	HPLC – UV	Human Plasma	PPE	0.5 – 12.5	100	24	7
4	HPLC – UV	Human Serum	PPE	2.5 – 50	100	27	8
5	LC-MS	Plasma, Saliva and Urine	PPE	0.1 – 20	40	-	9
6	GC-MS	Human Plasma	SPE	0.20 – 20	200	11.5	10
7	LC-MS/MS	Rat plasma	PPE	0.0003 – 1	50	5	11
8	LC-MS/MS	Human Serum	SPE	0.5 – 50	50	1	12
9	LC-MS/MS	Human Serum	PPE	0.95 – 30.41	25	6.5	13
10	LC-MS/MS	Postmortem blood, Serum and plasma	PPE	0.5 – 50	100	17	14
11	LC-MS/MS	Human plasma	LLE	0.009 – 9	200	5	Present study

MATERIALS AND METHODS:

Chemical and Reagents: Lacosamide and Lamotrigine (IS) were generous gifts from Torrent Pharmaceutical Ltd., India. HPLC grade acetonitrile, methanol, diethyl ether, and dichloromethane were purchased from Spectrochem Pvt. Ltd., India. Ultrapure water was used throughout the study from the MilliQ-Elix system, Millipore Pvt. Ltd., India. All other chemicals used in this study were analytical grade and used without further purification. The control of human plasma was obtained from Rajkot voluntary blood bank and research center, Rajkot, Gujarat, India.

Instrumentation and Analytical Conditions: The LC-MS/MS system is equipped with a pulse-free solvent delivery system (LC-20AD), an efficient online degassing unit (DGU-20A5R), an auto-sampler with a built-in dehumidifier (SIL-20AC), and a column oven utilizing a Peltier effect (CTO-20AC). An LC-MS8030 triple quadrupole mass spectrometer (Shimadzu, Japan) interfaced with the HPLC via electrospray ionization (ESI) was used for the analysis. All the parameters of LC and MS were controlled by LabSolution software version 5.53 from Shimadzu, Japan.

The chromatographic separation was carried out on a Gemini C18 (50 × 4.6 mm, 5µm) analytical column placed in a column oven maintained at 40 °C. The mobile phase consisted of methanol: 5mM ammonium formate in the proportion of 50:50, *v/v*, and pass through the column at a flow rate of 0.5 mL/min. The autosampler temperature was kept at 4 °C, and 2 µL sample volume was injected into the system. The analytical run time was 5 min.

Mass spectrometric ionization and detection were performed by infusing a solution containing 500 ng/mL of lacosamide and IS. The optimized operating conditions were set as follows; the interface temperature and ion spray voltage were fixed at 350 °C and 4.5 kV, respectively. Nebulizing gas and drying gas were nitrogen at a flow rate of 3 L/min and 15 L/min, respectively. With argon as collision gas, multiple reaction monitoring (MRM) mode was applied to the ionic transition of m/z 251.20 → 91.10 for lacosamide and m/z 256.10 → 211.10 for IS in positive ionization mode. The optimized collision energies -20 eV and -25 eV was used for lacosamide and IS, respectively. The dwell time was set to 200 ms.

Preparation of Standard and Quality Control Samples (QCs): The stock solutions of lacosamide (1000 µg/mL) and IS (1000 µg/mL) were prepared in methanol. The stock solution was further serially diluted using methanol to get series of ten working standard solution having a concentration of 300000, 150000, 60000, 30000, 15000, 6000, 3000,1500, 600, and 300 ng/mL. From that, calibration curves were prepared by 3% spiking (30µL) of the standard working solutions of lacosamide into blank plasma (970 µL) to achieve a concentration of 9000, 4500, 1800, 900, 450, 180, 90, 45, 18, and 9ng/mL for lacosamide. The QCs were prepared in a similar manner at five concentration levels, *viz.* 8100 ng/mL (high, HQC), 4050 ng/mL (middle, MQC 2), 325 ng/mL (middle, MQC 1), 27ng/mL (low, LQC) and 9ng/mL (lower limit of Quantification, LLOQ QC) for lacosamide; A working IS solution was prepared in the same way to get a final concentration of 300 ng/mL. All samples were stored at 2 - 8 °C until analysis.

Plasma Sample Preparation: Sample preparation involved LLE to isolate lacosamide and IS from human plasma. The spiked plasma samples, including calibration standards and QC samples, were thawed at room temperature previously-stored in a deep freezer at -20 °C. These samples were properly vortexed and mixed before pipetting. These samples were (200 µL) were transferred into pre-labeled 2 mL polypropylene vials and mixed with 50 µL of an internal standard for 30 s using a vortex mixer except blank sample. To this 50 µL of extraction buffer (0.1N HCl) was added and vortex for 2 min. the extraction was followed by the addition of 1.3 mL of liquid extracting premixed solvent diethyl ether: dichloromethane (70:30, v/v). The tubes were vortexed for 5 min.

The resultant mixture afterward centrifuged at 4000 rpm at 10 °C for 15 min, the organic phase was transferred to a vial and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The dried samples were reconstituted with 100 µL of mobile phase as a reconstitution solvent and a 2 µL sample were injected into the chromatographic system.

Bioanalytical Method Validation: The bioanalytical method validation was performed and meet the acceptance criteria for the various fundamental parameters according to the industrial guidance of USFDA [20].

Specificity: The specificity of the method was evaluated by screening eight different batches of blank human plasma (six were K_3EDTA and one each of lipidemic and haemolysed plasma) to investigate the interferences at the signal of lacosamide and IS. The different plasma samples were customized to prepare LLOQ and blank samples. The percentage interference should be less than 20% of the response of lacosamide at the LLOQ level.

Linearity, Precision and Accuracy: The linear correlation between analyte response and concentration was determined by analysis of a ten point calibration curve over the concentration range of 9 – 9000 ng/mL of lacosamide. Each calibration curve (n = 3) was analyzed individually by least square weighted linear regression method ($1/x^2$) of peak area ratios of lacosamide to IS (y) versus actual concentration (x). Slope (m) and intercept (c), were calculated for each standard curve. The regression equation ($y = mx \pm c$) for the calibration curve was used to back-calculate the measured concentrations at each standard as well as QC level. A correlation coefficient (r^2) value of greater than 0.990 was desirable for all calibration curves.

The intra and inter-assay precision and accuracy were determined by analyzing a set of QC samples (n = 6) at LLOQ QC, LQC, MQC 1, MQC 2, and HQC level (9, 27, 324 and 4050 and 8100 ng/mL, respectively) on the same batch and on three different batches respectively. The relative standard deviation (RSD, %) and percentage deviation from the nominal concentration (% Bias) serve as a measure of precision and accuracy, respectively. The acceptable values of precision and accuracy should be felt within 15% of nominal value, except LLOQ where it should be allowed within 20%.

Recovery and Matrix Factor: The extraction recovery and matrix effect for lacosamide were performed in six replicates of LQC, MQC-2, and HQC samples (27, 4050, and 8100 ng/mL). The extraction recovery of the lacosamide processed by LLE was evaluated by comparing peak areas obtained from pre (C) and post (B) spiked plasma processed samples at equivalent concentrations. The percentage recovery was measured by (C/B) × 100. The matrix factor owes to plasma was evaluated by comparing the peak area of post spiked samples (B) to those of pure standard solutions in the mobile phase at the same concentrations (A).

The matrix factor was calculated by (B/A). The extraction recovery and matrix factor of IS were evaluated at the working concentration (300 ng/mL) in the same manner. The acceptable limits were fixed at 15% deviation from the mean recovery, and the matrix factor should be within 0.80 - 1.20 at each QC level.

Dilution Integrity: Dilution integrity was investigated to ensure that samples could be diluted with a blank matrix without affecting the final concentration in case if study samples go beyond the validated calibration range of the method. For this, the concentration of standard lacosamide solution (13500 ng/mL) was selected 1.5 times higher than the upper limit of quantification (9000 ng/mL, ULOQ). To demonstrate the dilution integrity of lacosamide, pre-determined aliquots were diluted with human plasma (1:2 and 1:10) to form diluted quality control samples (DQCs) having a concentration of DQC (1/2) 6750 ng/mL and DQC (1/10) 1350 ng/mL. The back-calculated standard concentrations had to comply with both precision of ≤15% and an accuracy of 100 ± 15% similar to other QC samples.

Stability: All stabilities in human plasma were carried out by analyzing six replicates of LQC and HQC samples (27, 8100 ng/mL) in different conditions. The bench-top stability (6 h at ambient temperature), freeze-thaw stability (-20 ± 5 °C), auto-sampler (wet extract) stability (at 4 °C for 24 h), dry extract stability (2 - 8 °C for 24 h.), and long term stability (-20 ± 5 °C for 28 days) were determined. The effect of various stability conditions was subsequently calculated based on the % mean stability changed of the concentrations determined to that spiked in the QC samples. If the RSD of mean stability changed was within 15%, the sample was considered stable.

Application of the Method: The main objective of this clinical study was to evaluate the performance of the bioanalytical technique in real samples. Prior to the start, the study protocol and statement of informed consent were approved by the human ethics committee of the Department of Pharmaceutical Sciences affiliated to Saurashtra University (Rajkot, Gujarat, India). Meanwhile, the clinical study was performed according to the ethical values of the Declaration of Helsinki. An experienced specialist neuro-physicians of Wellcare hospital (Rajkot, Gujarat, India) carried out the diagnosis of the disease and prescribed appropriate drug therapy for his patients. In this manner, a total of four patients already under concomitant lacosamide therapy were recommended by the physician for the experimental monitoring.

The patients were prescribed 100 mg/day of lacosamide in which the routine dose of 50 mg of lacosamide was set for examination day in the morning. Under the supervision of a physician, one dose of lacosamide has been stopped on the day before an examination day to offer a short washout period (~ 24h). There was no restriction on a diet to the patients between the samplings. Approximately 3 ml of the blood sample was drawn into an EDTA tube at the interval of 0, 0.5, 1, 2, 3, 4, 12, and 24 h after oral administration of 50 mg lacosamide tablet. The plasma samples were prepared by centrifuging the blood samples at 3000 rpm, for 10 min at Wellcare Pathology Laboratory (Rajkot, Gujarat, India). The plasma samples were stored at -20 °C prior to sample processing.

RESULTS AND DISCUSSION:

Optimization of Mass Spectrometric Condition: The lacosamide and IS were analyzed firstly by direct infusion of the individual standard solution. Both positive and negative ionization modes were inspected for the detection of lacosamide and IS, and the positive ionization mode gave more efficiently ionized and therefore employed. The MS/MS conditions were optimized to obtain the best possible sensitivity. MRM was used to monitor precursor and production, which could reduce interference and enhance selectivity.

In this manner, the molecular ion $[M+H]^-$ of lacosamide **Fig. 1a** and IS was detected at 251.20 m/z, and 256.10 m/z, respectively. The collision-induced (-20 eV for lacosamide and -25 eV for IS) dissociation at 91.10 m/z due to the stabilized product ion of $[C_7H_7]^+$ from lacosamide **Fig. 1b**, whereas 211.10 m/z due to loss of rearrangement fragmentation of CH_5N_2 moiety $[M^+ - CH_5N_2]$ from the parent structure of IS **Fig. 1c**. The corresponding mass spectrum as shown in **Fig. 1**.

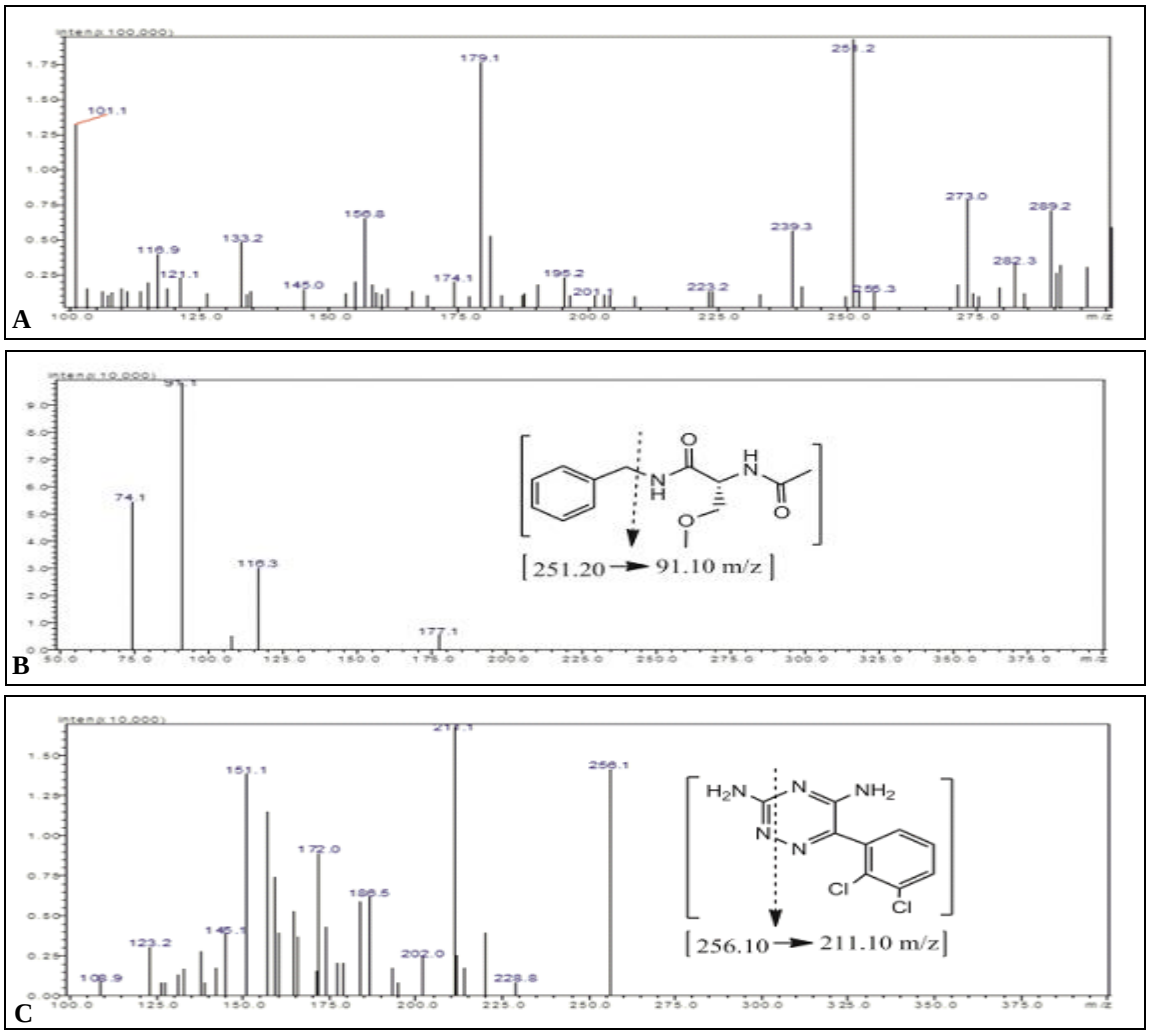

FIG. 1: MASS SPECTRUM OF (A) PRECURSOR ION OF LACOSAMIDE (B) PRODUCTION OF LACOSAMIDE, (C) PRECURSOR TO PRODUCTION OF IS, WITH ITS POSSIBLE FRAGMENTATION PATTERN

Optimization of Sample Preparation: The sample preparation is the most critical and difficult parameter in the development of the bioanalytical method to remove the interfering matrix from biological fluids with maximum recovery of analytes. It should be rapid, easy, and should require the least amount of reagents. With aimed at this, we established the LLE method in such a way that the sample preparation took shorten processing time, and it presented high recoveries. Different trials were taken with an organic solvent such as tert-butyl methyl ether, n-hexane, dichloromethane, ethyl acetate, diethyl ether, and several buffers such as 0.1 N HCl, 0.1 N NaOH & water. Among them, a combination of diethyl ether: dichloromethane (70: 30) and 0.1 N HCl showed maximum and reproducible recovery. To the best of our knowledge, the developed LLE technique for the extraction of lacosamide from the human plasma is the first time employed with LC-MS/MS method, which was more effective, faster and less expensive than reported methods **Table 1**.

Optimization of Chromatographic Condition: The selection of the mobile phase was preformed, taking into account the symmetric peak shape with a shorter run time that further leads to low consumption of organic solvent altogether, making the method cost-effective. We tried different brands of C18 columns with various organic solvents such as acetonitrile, methanol, and ammonium formate, ammonium acetate as a buffer solution for the development of the mobile phase. The final results were obtained in Gemini C18 (50 × 4.6 mm, 5µm) column with the several combinations showed that methanol: 5mM ammonium formate buffer (50:50, *v/v*) serves the desired purpose with utmost effectiveness. The proposed chromatographic conditions were designed for improved sensitivity with shorter chromatographic run time than the aforementioned reported methods **Table 1**.

Method Validation:

Selectivity: Fig. 2 shows the representative mass chromatograms of blank plasma for lacosamide, standard zero (IS), LLOQ, and ULOQ sample were presented. No significant interference in the blank plasma traces was observed from endogenous substances in drug-free human plasma at the retention time of lacosamide and/or IS. The retention times were 2.34 and 2.51 min for lacosamide and IS, respectively.

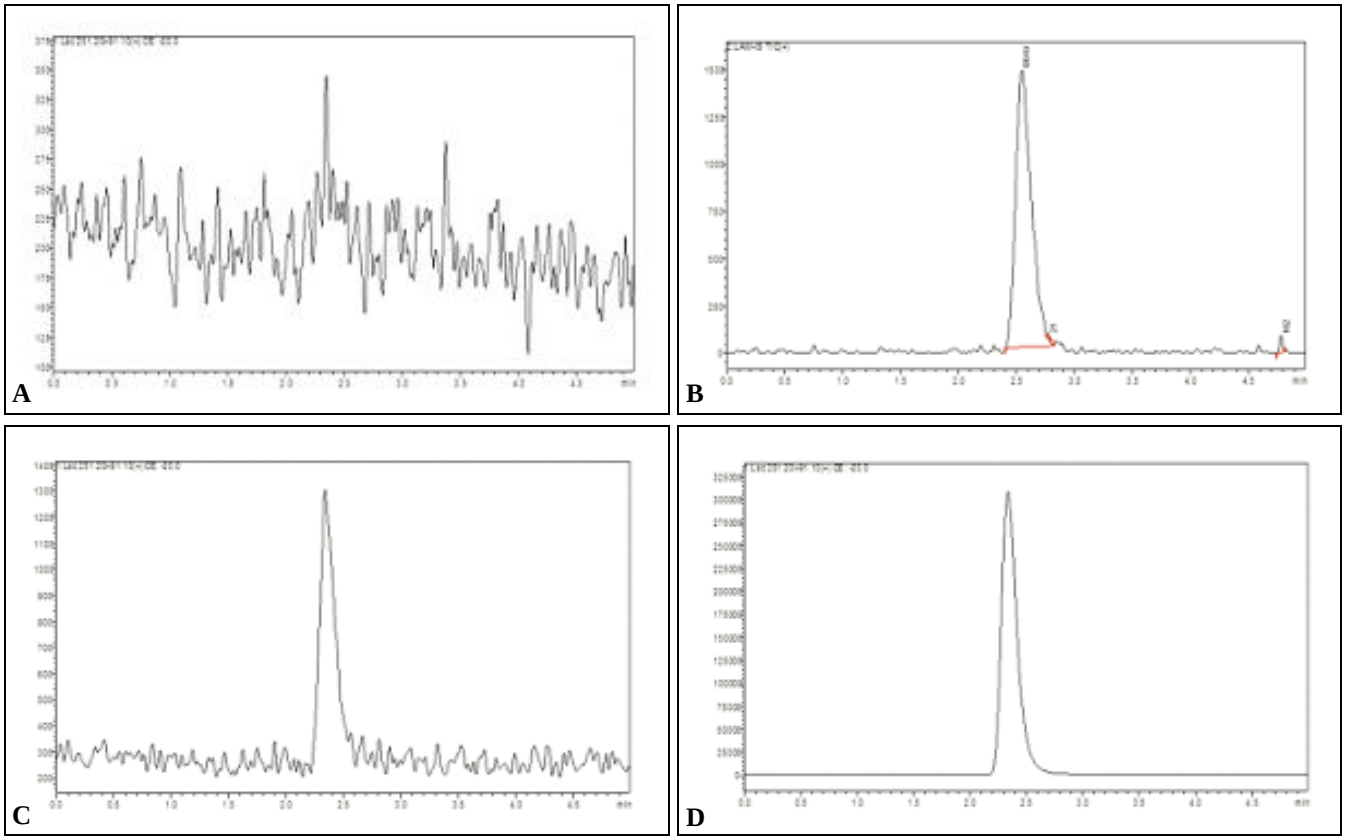

FIG. 2: MASS CHROMATOGRAM OF (A) BLANK PLASMA FOR LACOSAMIDE (B) STANDARD ZERO (IS) (C) LLQC SAMPLE OF LACOSAMIDE (D) HQC SAMPLE OF LACOSAMIDE

Linearity, Accuracy, and Precision: The chromatographic responses were found linear over the concentration range of 9 - 9000 ng/mL of lacosamide in human plasma. The correlation coefficient, r^2 was consistently equal to or greater than 0.995 during the course of validation. The calibration curve samples were within ± 15% of the theoretical value. The intra and inter-assay precision and accuracy were assessed by analyzing six replicate at each QC level. Concentrations were back-calculated from the calibration curve. The method was reliable and reproducible since the intra and inter-assay precision (RSD, %) values were less than 12.86 % and 10.78 %, respectively. On the other hand, intra and inter-assay accuracy (% Bias) values were within 4.63% and 3.96%, respectively. **Table 2** showed that all values of accuracy and precision fell within limits as acceptable.

Recovery and Matrix Factor: The lacosamide and IS were recovered by LLE from human plasma. The % mean recoveries of lacosamide were ranged from 78.78 - 83.7%, whereas the mean value of the matrix factor was varied from 0.87 - 0.93 with an acceptable RSD value. The recovery and matrix factor of IS was achieved to nearly 88.54% and 0.91, respectively.

Table 3 showed recovery of lacosamide and IS were high and consistently precise and reproducible and there was no any endogenous substance significantly influenced ion suppression of lacosamide and IS as well in this analytical method and allowed us to conclude, our method is efficiently recovered lacosamide and IS in human plasma.

Dilution Integrity: Precision (RSD, %) values for reliability for 1/2nd and 1/10th dilutions were 8.51 and 10.55%, while the accuracy was 97.22 and 95.75% for lacosamide, respectively. Results were within the acceptance limit of 15% for precision and 85 - 115% for accuracy, as shown in **Table 4**.

TABLE 2: ACCURACY AND PRECISION VALUES OF LACOSAMIDE IN HUMAN PLASMA

Nominal conc. (ng/mL)	Intra assay (n=6)			Inter assay (n=18)		
	Mean ± SD	% RSD	% Bias	Mean ± SD	% RSD	% Bias
9	8.58 ± 1.10	12.86	4.63	8.68 ± 0.94	10.78	3.59
27	26.14 ± 2.64	10.11	3.19	25.93 ± 2.39	9.20	3.96
324	335.62 ± 26.93	8.02	-3.59	323.57 ± 25.28	7.81	0.13
4050	3890.10 ± 356.67	9.17	3.95	3927.64 ± 263.88	6.72	3.02
8100	8285.98 ± 391.01	4.72	-2.30	8228.61 ± 376.69	4.58	-1.59

TABLE 3: EXTRACTION RECOVERY AND MATRIX EFFECT OF LACOSAMIDE IN HUMAN PLASMA

Analytes	Spiked conc. (ng/mL)	Recovery		Matrix effect	
		% Mean ± SD	% RSD	% Mean ± SD	% RSD
Lacosamide	27	83.77 ± 6.06	7.24	0.93 ± 0.03	3.21
	4050	78.78 ± 4.16	5.28	0.91 ± 0.04	4.88
	8100	82.48 ± 5.34	6.47	0.87 ± 0.04	5.03
IS	300	88.54 ± 3.41	3.85	0.91 ± 0.08	8.32

TABLE 4: DILUTION INTEGRITY OF LACOSAMIDE IN HUMAN PLASMA

	DQC (1/2)	DQC (1/10)
Dilution concentration (ng/mL)	6750	1350
Mean ± SD (n=6) (ng/mL)	6562.17 ± 558.57	1292.62 ± 136.34
% Mean Accuracy	97.22	95.75
% RSD	8.51	10.55

TABLE 5: STABILITY DATA OF LACOSAMIDE UNDER DIFFERENT STORAGE CONDITIONS

Stability conditions	LQC (27 ng/mL)			HQC (8100 ng/mL)		
	Mean ±SD (n = 6)	% RSD	% Mean stability changed	Mean ±SD (n = 6)	% RSD	% Mean stability changed
Bench top stability for 6 h (at ambient temperature)	23.43 ±1.33	5.67	9.19	7675.76 ±314.86	4.10	2.54
Freeze and Thaw stability at -20 °C (5 cycle)	23.68 ±1.94	8.21	5.96	7347.40 ±389.77	5.30	8.12
Dry extract stability at -20 °C	24.80	4.42	2.04	7592.45	6.88	3.19

for 24 h	±1.10			±522.18		
Auto sampler stability 4 °C for 24 h	25.80 ±1.74	6.76	-6.99	7398.90 ±373.28	5.05	-0.34
Long term stability at -20 °C for 28 days	24.97 ±0.85	3.41	2.09	7354.82 ±516.66	7.02	4.87

Stability: Table 5 summarizes the results of the bench-top stability, freeze-thaw stability, dry extract stability, auto-sampler stability, and long term stability in human plasma as the percentage mean stability of the calculated vs. theoretical concentration. No significant deviations were observed compared to theoretical concentration, indicating that lacosamide was stable under all tested conditions. Therefore, the method has been proved to be applicable for routine analysis.

Application of the Method: The validated method was applied to monitoring the plasma concentration of lacosamide from epileptic patients. The calibrations, QCs, and real samples were run and analyzed successfully on the same batch. The mean plasma concentration versus time profile was presented in **Fig. 3**. The pharmacokinetic parameters such as peak plasma concentration (C_{max}), the time required to achieve maximum plasma concentration (T_{max}), half-life ($t_{1/2}$), Area under curve (AUC), mean residence time (MRT), Elimination rate (Lz), and clearance (CL) of lacosamide were calculated by pksolver tool in Microsoft excel sheet and summarized in **Table 6**. All calculated pharmacokinetic parameters were comparable to the reported method available on lacosamide tablet in human plasma [9].

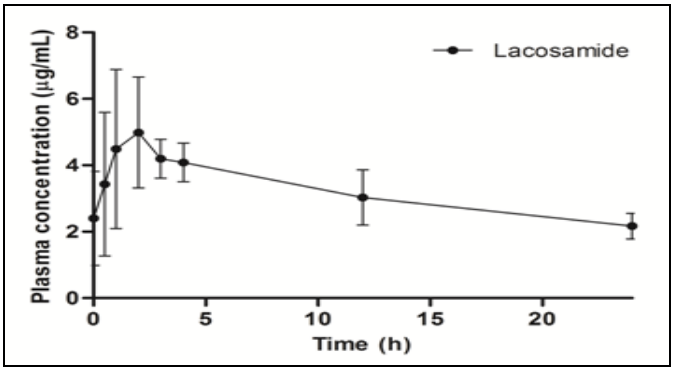

FIG. 3: PLASMA CONCENTRATION OF LACOSAMIDE VERSUS TIME PROFILE OF FOUR EPILEPTIC PATIENTS

TABLE 6: PHARMACOKINETIC PARAMETER OF LACOSAMIDE AFTER ORAL ADMINISTRATION 50 mg TO 4 EPILEPTIC PATIENTS

Subject	Pharmacokinetic parameter						
	T_{max} (h)	C_{max} (µg/mL)	$t_{1/2}$ (h)	$AUC_{0\rightarrow 24}$ (µg*h/mL)	$MRT_{0\rightarrow 24}$ (h)	Lz (h^{-1})	CL (L/h)
Patient A	1.00	2.18	17.52	41.76	9.81	0.04	1.20
Patient B	1.00	1.48	11.00	16.31	8.44	0.06	3.07
Patient C	1.00	2.03	9.85	32.38	8.12	0.07	1.54
Patient D	0.50	1.92	8.98	24.71	8.08	0.08	2.02

A – female, 35 year old, 50 kg., B – Male, 55 year old, 90 kg., C – Male, 61 year old, 80 kg., D – female, 54 year old, 50 kg.

CONCLUSION: A simple and rapid LC-MS/MS method for the quantification of lacosamide in human plasma was developed and fully validated. This method exhibited excellent performance over those previously reported methods in the biologic matrix, in terms of simple sample preparation with consistent recovery by LLE from human plasma, the short analytical run time (5 min), and improved sensitivity with an extensive range of concentration to cover sub-therapeutic to the toxic level of lacosamide in human plasma. The reliability of the method was proven by means of complete validation, the absence of matrix effect and stable under routine sample handling & processing conditions leads to a reproducible and rugged method for quantification of lacosamide in human plasma. The method was successfully applied to the real samples with effective monitoring of plasma drug concentration. The method was successfully used to determine the pharmacokinetic parameter of individual patients after oral administration of 50 mg of lacosamide tablets. The developed method can be suitable for bioavailability and bioequivalence studies and routine dose-dependence profile with the chosen precision and accuracy.

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Compliance with Ethical Standards: The study has been approved by the Institutional Ethics Committee, Department of Pharmaceutical Sciences, Saurashtra University, India. And it has been performed in accordance with the ethical standards of the guideline. (Approved protocol no. SUDPS/ETHICLIN/03/2014/14).

Informed Consent: Written consent was obtained from all the individuals involved in the study.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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