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ESTIMATION OF LAMOTRIGINE IN HUMAN PLASMA BY LCMS/MS

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ABSTRACT

A robust, selective and sensitive LCMS/MS method was developed for quantification of lamotrigine in human plasma. Lamotrigine is an anticonvulsant drug used for seizures. The drug as well as labeled lamotrigene used as an internal standard was extracted from human plasma by liquid – liquid extraction method. Chromatographic separation was performed on a SCX cation exchange column using an isocratic mobile phase (5mM ammonium acetate in 0.05% formic acid: acetonitrile:: 30:70, v/v) at a flow rate of 1.0ml/min. It was detected in LCMS/MS in positive ion mode with electrospray ionization (ESI) interface. The method was validated in the concentration range of 10ng/ml to 8000ng/ml as per FDA guidelines. Mean recovery of lamotrigine was 72%. The assay accuracy was 99.68 – 110.15% of the nominal values. No matrix effect was noticed in this method.

KEYWORDS: Lamotrigine, Cation –exchange column, LCMS/MS, Human Plasma.

INTRODUCTION

Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a phenyl-triazine derivative with broad spectrum antiepileptic activity.[1] It is an anticonvulsant drug which is effective as adjunctive therapy and also as monotherapy for partial and generalized seizures.[2, 3] Its use for treatment of neurological lesions and as a tranquilizer has also been studied.[4]

It is rapidly and completely absorbed after oral administration (absolute bioavailability = 98%) with first-order linear pharmacokinetics and has a mean plasma half-life of approximately 24h. Lamotrigine is a lipophilic weak base with plasma protein binding of approximately 55%. Extensive metabolism of lamotrigine molecule takes place in the liver, predominantly via *N*-glucuronidation by uridine-glucuronyl-transferase isoenzymes. The metabolites are not thought to be pharmacologically active.

The actual plasma concentration of a drug is of major interest in pharmacokinetic studies. Several liquid chromatography/tandem mass spectrometry methods for determination of lamotrigine in different biological samples were reported.[5-9] Shah et al.[6] reported a methodology that has limit of quantification of 25ng/mL achieved while utilizing multiplexing technique involving more than one LC unit. Although the multiple inlets in the mass spectrometer allow increase in throughput, sensitivity loss and potential cross-inlets contamination are major drawbacks. Hotha et al.[7] achieved a limit of quantification of 0.1ng/mL using a LCMS/MS method. A new validated method for quantification of lamotrigine in human plasma has also been reported; however, this includes a solid-phase extraction (SPE) technique with a drying and reconstitution step.[9] In industry a simple but sensitive, robust method is preferable. The purpose of this study was to develop a fast, sensitive and robust method to quantify lamotrigine using isotopically labeled internal standard (ISTD) which will be suitable in routine quantification of lamotrigine in a pharmacokinetic study.

MATERIALS AND METHODS

Reagents

Lamotrigine (USP) and deuterated lamotrigine, used as an internal standard were purchased from Vivian Life Science, India. HPLC grade acetonitrile were obtained from Merck (India). All other chemicals of highest purity grade were locally purchased. Milli Q water (Millipore, USA) was used throughout the procedure.

K_2EDTA containing human blood was collected in-house from healthy volunteers. Plasma was separated by centrifuging at 3000 rpm for 10 min at 4ºC.

Preparation of standard samples

Stock solution (1000µg/ml) of lamotrigine was prepared in methanol. Concentration was then corrected using the potency and actual amount weighed. The stock solution was further diluted to the concentration range 525ng/ml to 400000ng/ml using methanol: Milli-Q-water:: 50:50, v/v as diluent.

For internal standard (IS), stock solution (200µg/ml) of

lamotrigine.[13]C D3 was prepared in methanol and corrected final concentration is obtained using the potency and amount weighed. Working solution (10 µg/mL) was then prepared from this stock solution using methanol: Milli-Q-water:: 50:50, v/v as diluent.
All stock and diluted solutions were stored in refrigerator at 2-8ºC until analysis.

Preparation of calibration standards
To prepare calibration curve standards, 980 µL of K2EDTA pooled plasma was spiked with 20 µL of respective diluted standard solutions of lamotrigine to obtain a concentration range of about 10ng/ml to 8000ng/ml. 0.2ml of each concentration was aliquoted into RIA vials. The bulk spiked samples were stored at about -70°C until use.

Preparation of Quality Control Samples
Lamotrigine stock solution (1000µg/ml) was diluted with methanol:Milli-Q-water:: 50:50, v/v to obtain a concentration range of 531.43ng/ml to 305000.00ng/ml. A concentration range about 10ng/ml to 6220ng/ml was prepared by spiking 20 µL of each diluted standard of lamotrigine into 980 µL of K_2EDTA pooled plasma and stored at -70°C.

Methods
Sample extraction
50 µL of IS (Lamotrigine.[13] C D3) was added to each of the labeled RIA vial except blank. Sample was thawed at room temperature and then vortexed for complete mixing of the contents. 100 µL of thawed sample was then transferred to the respective RIA vial and vortexed again. 100µl of 100mM Di-Sodium hydrogen phosphate dihydrate was added to each vial and vortexed. 2 ml of TBME was then added to all vials. All samples were kept in vibramax for 10 min at 2500 RPM. This denaturation of protein during sample preparation helps in the uniform recovery. The samples were then centrifuged at 4000 RPM for 5 min at 4°C. 1.6 ml of upper organic phase was transferred into a new labeled RIA vial. The samples were dried at about 40°C in nitrogen evaporator and reconstituted using 1.0ml of mobile phase followed by vortexing. The samples were transferred to labeled vials and loaded into auto sampler.

Chromatography
10µL of sample was injected on a cation exchange column (Biobasic SCX, 50 × 4.6 cm, 5µm). An isocratic mobile phase (5mM ammonium acetate in 0.05% formic acid: acetonitrile::30:70, v/v) was used at a flow rate of 1.0ml/min with splitter in Shimadzu HPLC attached to API 4000 Mass spectrometer (Applied Biosystems, USA). The column was maintained at 50°C in the column oven. The run time was 3.5minutes.

Mass spectrometry
Electrospray ionization (ESI) interface operated in positive ionization mode was used for the multiple reaction monitoring (MRM). By infusing diluted stock solutions of analyte/IS the operational conditions were optimized as follows (Table 1).

Table – 1: Optimized mass parameters for analytes and internal standards

Analyte/IS	Dwell time (ms)	Declustering Potential (DP) (V)	Entrance Potential (EP) (V)	Collision Energy (CE) (V)	Collision Cell Exit Potential (CXP) (V)	Collision Activated Dissociation (CAD) (psi)	Ion Source Voltage (V)	Curtain Gas Flow (CUR) (psi)
Lamotrigine/ IS	300	100	10	37	12	08	4500	30

Source temperature was set at 450°C. Nebulizer gas (GS1) and auxiliary gas (GS2) flows were 45 and 55psi, respectively. Quadrupoles Q1 and Q3 were set on unit resolution. Acidic mobile phase (due to addition of formic acid) has improved the protonation of all compounds and has shown a positive impact on signal intensities. MRM transitions monitored were: m/z 256.0 → 211.0 (Lamotrigine) and m/z 262.1 → 217.0 (IS).
Sample concentrations were calculated by linear regression analysis using the analyst software 1.5.1. Data was processed by peak area ratio. The concentration of unknown was calculated from the equation (Y= mx+ c) using regression analysis of spiked plasma calibration standards with reciprocal of the square of the drug concentration ($1/X^2$).

RESULTS AND DISCUSSION

Method development
Three methods e.g. protein precipitation (PPT), liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are commonly used as specific and effective sample clean-up procedures which are required for sensitive and selective LC–MS/MS assays for determination of very low concentration levels of pharmaceutical targets present in biological samples. Unlike the relatively costlier SPE technique used by others.[6, 9] we used a simple and cost effective LLE method. By using a multiple port nitrogen evaporator the time consumption was substantially reduced. This technique was shown to be robust, provided clean samples and gave good and reproducible recoveries of analyte and IS. The extraction recovery of analyte/IS was determined by comparing peak areas from plasma samples (n = 6) spiked before extraction with those from aqueous samples. The mean overall recoveries and coefficient of variation across QC

levels of lamotrigine were 72% and 1.88%, respectively [Table – 2].

Table –2: Recovery of lamotrigine from Biological Matrix

LQC (30 ng/mL)			MQC (3732ng/mL)			HQC (6220ng/mL)		
Unextracted area (n=6)	Extracted area (n=6)	Mean Percentage recovery	Unextracted area (n=6)	Extracted area (n=6)	Mean percentage recovery	Unextracted area (n=6)	Extracted Area (n=6)	Mean percentage recovery
9811	7173	73	1198352	850799	71	1859810	1367721	73

A number of methods were reported for estimation of lamotrigine using various techniques.[10 - 14]. However, most of these methods provide insufficient sensitivity, longer chromatographic run time and a labor intensive work-up procedure which presently are less favored in industries to support clinical studies. Shah et al.[6] reported the limit of quantification of 25 ng/mL utilizing a complex multiplexing technique. Hotha et al..[7] could achieve lower limit of quantification 0.1ng/mL in a relatively simpler method but they used flucanozole as an internal standard instead of labeled IS. Ghatol et. Al.[9] described a validated LCMS/MS method for estimation of lamotrigine in human plasma using reverse phase column after SPE technique for sample extraction. In fact, all these methods used reversed phase column chromatography. However, lamotrigine contains amino group which can be easily protonated and is the basis of its detection in LCMS/MS in positive ion mode. Because of that it was prudent to separate it on ion-exchange column instead of reverse phase chromatography as ion-exchange chromatography has specific advantages over reversed phase one. One of the main advantages of ion exchange is that there is only one interaction involved in the separation: the ionic analytical species interacting with the charged stationary phase resulting in a better separation.[15] We used a cation exchange column. The run time remains 3.5 min which is also ideally suited for being considered in high throughput analysis. The retention times for lamotrigine and IS were 2.1min and 2.1min respectively.

Method validation

The current LC–MS/MS assay was validated as per FDA Guidelines.[16] for specificity, linearity, intra- and inter-day precision and accuracy, and stability.

Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. Selectivity of this method was evaluated in eight individual human K_2 EDTA plasma lots including one lipemic and one hemolytic lot. Peak responses in blank lots were compared against the response of spiked LLOQ containing IS mixtures. No interference peak from endogenous compounds was observed at the retention time of the analyte and IS in the representative chromatograms in Fig. 1 (a and b) (Blank plasma spiked with analytes/IS) demonstrated the selectivity of the method.

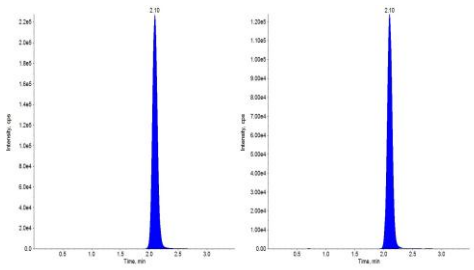

Figure 1 a & b: Chromatograms of lamotrigine and IS

Linearity and sensitivity

Eight-point calibration curve containing lamotrigine was made over the concentration range of 10.50ng/ml to 8000.00ng/ml. The peak-area ratio (y) of analytes to internal standards was plotted against the nominal concentration (x) of analytes to determine the linearity of each calibration curve. Excellent linearity was achieved with correlation coefficients greater than 0.999 for all validation batches.

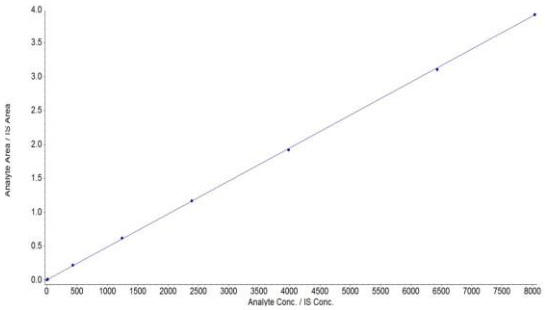

Fig. – 2: Calibration curve for lamotrigine

The concentrations of calibration standards were back calculated to obtain the accuracy of each calibration point and the resulting calculated parameters were used to determine concentrations of analyte in quality control or unknown samples. The correlation coefficient $r > 0.99$ was desirable for all the calibration curves. Calibration curves from accepted six precision and accuracy batches

were used to establish linearity. The accuracy of the calibration points for Lamotrigine was within 98.4–100.5%.

If the analyte response for the lowest standard on the calibration curve was at least five times more than that of drug free (blank) extracted plasma then it can be accepted as the LLOQ. In addition, the analyte peak of LLOQ sample should be identifiable, discrete, and reproducible with accuracy within ± 20.0% and a precision ≤ 20.0%. The deviation of standards other than LLOQ from the nominal concentration should not be more than ±15.0%. Precision and accuracy at the LLOQs were respectively 1.73% and 99.93% for lamotrigine. The LLOQs of the method is 10g/ml for lamotrigine,. Limit of detection was found to be 2.504ng/ml for lamotrigine; signal to noise ratio was > 31.314. This indicates that this method is sensitive enough for a pharmacokinetic study. Moreover, the good signal-to-noise obtained at this concentration indicates that the LLOQ of the method can be lowered further or the volume of plasma can be decreased. This further widens the scope of this method even to the pediatric patients.

PRECISION AND ACCURACY

Precision and accuracy for intra- and inter-day batches for all analytes were determined by six replicate analyses of QC samples (n=6) at four different concentrations – lower limit of quantification (LLOQ), low quality control (LQC), middle quality control (MQC) and high quality control (HQC). The respective concentrations for lamotrigine were 10.03ng/ml for LLOQ, 29.86ng/ml for LQC, 3731.94ng/ml for MQC and 6219.90ng/ml for HQC. Results of precision and accuracy were presented in Table 3a and 3b. The intra-day and inter-day precision were within 4.25% for all analytes. The assay accuracy was 99.68 – 110.15% of the nominal values. The accuracy of the assay was expressed by [(mean observed concentration) / (spiked concentration)] x 100% and precision was evaluated by relative standard deviation (RSD).

Table – 3a: Precision & Accuracy of method for lamotrigine analysis for calibration standards

Sample ID	Mean	SD	Accuracy (%)	% CV
STD A	10.01	0.17	99.93	1.73
STD B	20.06	0.69	100.13	3.48
STD C	437.40	6.39	100.44	1.46
STD D	1239.75	12.96	99.63	1.05
STD E	2403.93	23.86	100.46	0.99
STD F	4000.01	34.64	100.30	0.87
STD G	6327.86	98.12	98.37	1.55
STD H	8100.75	124.41	100.75	1.54

Table – 3b: Precision & Accuracy of method for lamotrigine analysis for quality control samples

Analyte	LOQQC	LQC	MQC	HQC
Within run accuracy (%)	100.35 - 110.15	100.06 - 103.54	100.46 - 101.55	99.68 - 102.25
Within run precision (%)	1.91- 3.42	0.56 - 3.91	0.81- 1.36	0.64 - 1.74
Between batch accuracy (%)	104.39	101.16	101.02	101.21
Between batch precision (%)	4.25	2.88	1.09	1.47

Matrix effect

Matrix effect was investigated by extracting blank plasma from eight different sources, including one hemolytic and one lipemic lot. 100µL of blank plasma from each lot was processed as per the procedure mentioned in sample preparation. Aqueous solution of individual analyte either at LQC or HQC level was added to each of the final eluent. These samples were considered as post extracted samples (presence of matrix).

Similarly, the aqueous solution of individual analyte either at LQC or HQC level was prepared with the elution solvent and was considered as aqueous samples (absence of matrix). Six replicates each of aqueous samples was injected along with post extracted samples of LQC and HQC.

Individual analyte area response and IS area response of each post extracted sample were compared with the mean analyte area response and mean IS area response of the aqueous sample respectively. The matrix effect was calculated via the formula: Matrix effect (%) = A_2/ A_1 x 100 (%), where A_1= response of aqueous concentrations and A_2 is response of post-extracted concentrations.

Average (n=6) matrix factor was 99.5% with a CV of 2.4% at LQC level. At HQC level, it was 100.67% with a CV of 1.0% which is within the accepted limit (% CV ≤15) (Table 4).

Table -4: Matrix effect for estimation of lamotrigine

Matrix ID	LQC analyte area in absence of matrix	LQC analyte area in presence of matrix	LQC matrix factor for analyte	HQC analyte area in absence of matrix	HQC analyte area in presence of matrix	HQC matrix factor for analyte
PL_726	9003	8469	97.08	1699033	1685384	100.80
PL_738	8292	9082	104.11	1659633	1696895	101.49
PL_742	8528	8579	98.34	1670516	1694574	101.35
PL_745	8662	8729	100.06	1668708	1683889	100.71
PL_746	9022	8575	98.29	1647410	1679023	100.42
PL_748	8836	8492	97.34	1686498	1702169	101.81
LPL_643		8635	98.98		1674919	100.18
HPL_749		8879	101.78		1648303	98.58
Average	8723.83	8680.00	99.50	1671966.33	1683144.50	100.67
SD	285.68	209.33	2.40	18501.99	16842.92	1.01
%CV	3.27	2.41	2.41	1.11	1.00	1.00

Dilution integrity

Dilution integrity of the method was evaluated after diluting 2-fold and 4-fold with interference free human plasma. Six replicates of these samples were processed and analyzed against a set of freshly spiked calibration standards. Minimum 67% of the dilution integrity samples at each level should meet the acceptance criteria for accuracy and precision of ±15% and ≤15% respectively.

The upper concentration limits were shown to be extendable upto 12620.09ng/mL for lamotrigine by dilution with blank plasma. The mean accuracy for 2-fold and 4-fold dilution samples were 94% and 95% respectively. The precision for 2T and 4T were 0.65% and 1.35 % respectively (data not shown).

Carry over effect

Carry over effect was performed in order to evaluate the cleaning ability of rinsing solution used for the injection needle and port. This avoids any carry over of injected sample in subsequent runs. The experiment was carried out by placing samples in following order: LLOQ of individual analyte, blank plasma, upper limit of quantitation (ULOQ) of individual analyte and blank plasma. No carry over was observed during the experiment.

Stability

Stability evaluations were performed in both aqueous and matrix based samples. For aqueous solution both short-term and long-term stabilities were determined as follows:

Stability in aqueous solution

I. Short term stock solution stability (STSS)

MQC concentration of each analyte was prepared by dilution of respective stock solution and stored at 25°C for 24hrs. Six replicate injections were given for MQC sample. No significant differences were noticed when these results were compared with those obtained from the freshly prepared MQC samples indicating that all analytes were stable at 25°C (Table 5). Accepted criteria for the ratio of mean response for stability samples should be between 90-110%.

II. Long term stock solution stability (LTSS)

Aqueous MQC sample of each analyte, prepared by dilution from respective stock solution which was stored at 2-8°C for 26 days was injected. Mean area response of MQC of stored stock solution was then compared against MQC from freshly prepared stock solution. Percent stabilities for lamotrigine (99.98) and IS (100.37) were well within accepted limit (90 – 110%). This indicated the stability of lamotrigine and IS solutions for 26 days at 2-8°C (Table 5).

Table – 5: Stability studies of aqueous stock solutions of lamotrigine

Stability Check	Samples	Area for stored Solution	Area for Fresh Solution	% Stability
STSS (24hrs)	MQC (n=6)	620076.50	617983.00	100.59
LTSS (26 days)	MQC (n=6)	856312.50	858138.83	99.98

II. Stability in human plasma

I. Bench-top stability

Six aliquots of each analyte in human plasma (at LQC and HQC concentrations) from -70°C were allowed to thaw unassisted in wet ice for 8hrs and processed along with a set of freshly prepared calibration standards as well as LQC and HQC samples. The stability for LQC and HQC samples of lamotrigine were found to be 101.30% and 97.41% respectively.

II. Freeze thaw stability

Six aliquots each of HQC and LQC were frozen at about -70°C temperature. The first freeze thaw cycle was carried out after a minimum of 24hrs of freezing and

subsequent cycles were carried out after minimum of 12hrs. After 4 freeze thaw cycles the stability for LQC and HQC samples of lamotrigine were 102.08% and 98.22% respectively.

III. In-injector stability

LQC, MQC and HQC samples retained in the auto sampler at 10°C for 24hrs were analyzed against a set of freshly spiked calibration standards and six aliquots of comparison samples of each HQC, MQC and LQC. The stability for LQC and HQC samples of lamotrigine were 101.64% and 100.25% respectively. In-injector stability of internal standard was calculated by comparing the area ratio (IS/analyte) of MQC retained in the auto sampler against comparison MQC samples. IS stability was found to be 96.43%.

III. Wet extract stability

HQC and LQC samples (Six sets each) were processed and stored at room temperature for 05hrs. These samples were analyzed against freshly spiked samples. The stability for LQC and HQC samples of lamotrigine after 5hrs at 25°C were 102.01% and 99.28% respectively.

Accepted range for all the above mentioned stability studies is that the mean concentration for stability samples should be 85-115% of the mean concentration of freshly prepared samples. Thus the analyte, lamotrigine, was stable during the analysis process. Results of the stability evaluations were presented in Table 6.

Table – 6: Stability studies of lamotrigine

Stability Check	Samples	Nominal Conc.(ng/ml)	Calculated Conc. (ng/ml)	%cv	% Stability
Bench Top for (8hr)	LQC (n=6)	29.86	27.19	3.26	101.30
	HQC(n=6)	6219.90	5685.06	3.03	97.41
Freeze Thaw (4 cycles)	LQC (n=6)	29.86	27.39	3.63	102.08
	HQC(n=6)	6219.90	5732.14	1.19	98.22
In-Auto-sampler(24hr)	LQC (n=6)	29.86	27.28	2.25	101.64
	HQC(n=6)	6219.90	5850.60	0.85	100.25
Wet Extract (5 hr)	LQC (n=6)	29.86	27.38	3.79	102.01
	HQC(n=6)	6219.90	5793.87	0.85	99.28

Extended precision and accuracy run

Extended precision and accuracy run was performed by processing and analyzing one set of CC and 65 sets of LQC and HQC as a batch (total 140 samples). Results of precision and accuracy were presented in Table 7. The precision for lamotrigine were 2.60% for LQC and 1.77% for HQC. The accuracies for LQC and HQC were 100.62% and 99.25% respectively.

Table – 7: Batch size test of lamotrigine

Sample id	Nominal conc	Calculated conc	Accuracy (n= 65)	Precision (%CV)
LQC	29.86	30.04	100.62	2.60
HQC	6219.90	6173.56	99.25	1.77

Haemolysis and Lipemic Effect

Six replicates each of LQC and HQC spiked in haemolysed and lipemic plasma are processed and injected. The mean percent accuracy should be within ±15% of the nominal value for each QC. The precision (%CV) should be less than or equal to 15%. Results were presented in Table 8 (a & b).

Table -8a: Lipemic effect on lamotrigine

LQC (Nominal conc. 29.856ng/ml)			HQC(Nominal conc. 6219.904ng/ml)		
Sample ID	**Calculated conc.(ng/ml)**	**Accuracy (%)**	**Sample ID**	**Calculated conc.(ng/ml)**	**Accuracy (%)**
LPL_LQC_01	29.81	99.85	LPL_HQC_01	6129.10	98.54
LPL_LQC_02	30.34	101.62	LPL_HQC_02	6328.15	101.74
LPL_LQC_03	28.91	96.84	LPL_HQC_03	6302.86	101.33
LPL_LQC_04	29.37	98.37	LPL_HQC_04	6137.27	98.67
LPL_LQC_05	29.36	98.32	LPL_HQC_05	6305.31	101.37
LPL_LQC_06	28.50	95.47	LPL_HQC_06	6199.18	99.67
Mean conc.	29.38			6233.64	
SD	0.65			89.74	
Mean Accuracy	98.41			100.22	
%CV	2.20			1.44	

Table -8b: Hemolysis effect on lamotrigine

LQC (Nominal conc. 29.856ng/ml)			HQC(Nominal conc. 6219.904ng/ml)		
Sample ID			6219.904		
HPL_LQC_01	Calculated conc.(ng/ml)	Accuracy (%)	Sample ID	Calculated conc.(ng/ml)	Accuracy (%)
HPL_LQC_02	29.99	100.47	HPL_HQC_01	6381.38	102.60
HPL_LQC_03	29.96	100.33	HPL_HQC_02	6215.22	99.92
HPL_LQC_04	30.04	100.63	HPL_HQC_03	6281.87	100.10
HPL_LQC_05	30.11	100.84	HPL_HQC_04	6179.87	99.36
HPL_LQC_06	29.28	98.09	HPL_HQC_05	6181.97	99.39
	29.63	99.26	HPL_HQC_06	6110.04	98.23
Mean conc.	29.84			6225.06	
SD	0.32			94.75	
Mean Accuracy	99.94			100.08	
%CV	1.06			1.52	

CONCLUSION

A fast, sensitive and specific LC–MS/MS method for estimation of lamotrigine in human plasma was developed and validated. In this method cation exchanging column was used instead of reverse phase column because of poor retention of analyte in this column. Moreover, LLE was used to obtain consistent and reproducible recoveries for both analyte and IS with insignificant interference and matrix effect. As per FDA guidelines, internal standard should preferably be identical to the analyte [17] and hence labeled internal standards were used. The method was validated in the concentration range of 10ng/ml to 8000ng/ml. However, dilution integrity study clearly demonstrated that the upper level can be easily changed to 12620.095ng/ml. Moreover, by using 100µL plasma samples, the lower limits of quantification were achieved. Limit of detection was found to be 2.50ng/ml for lamotrigine with a signal to noise ratio > 31.314. This suggests that the LLOQ of the method can be lowered further or the volume of plasma can be decreased. The possibility of lowering of plasma volume permits the applicability of this method even to the pediatric patients where blood volume is always a constraint. Lamotrigine was found stable during storage, processing, and analysis in human plasma samples. The method is reproducible, sensitive and suitable for high-throughput sample analysis. This method also has the potential to be useful for bioequivalence studies and routine therapeutic drug monitoring.

COMPETING INTERESTS

All authors hereby declare that no competing of interests is associated with the publication of this manuscript.

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