Available online on 15.06.2024 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access **Research Article**

Development and Validation of UV Spectroscopic Method for the Quantification of Lamotrigine in Bulk and Marketed Formulations

Madhusri Chagi, Neeladri Srinivasulu, Yegnoor Anand Kumar*, Pooja Polkal, Keerthana Shetty

Department of Pharmaceutical chemistry, V.L. College of Pharmacy, Raichur, Karnataka
Department of Pharmaceutics, V.L. College of Pharmacy, Raichur, Karnataka

A B S T R A C T

Two Simple and precise UV spectroscopic methods were developed for the estimation of lamotrigine in bulk and marketed tablets. The methods developed by using two solvent systems viz., Methanol: Distilled water (1:1) and Acetonitrile: 0.1N HCl (1:1) were validated as per ICH guidelines. The two proposed solvent systems validated for linearity, accuracy, precision, robustness, ruggedness and solution stability. The percent recovery in the marketed tablet formulationswere found to be good agreement with the label claim. The methods validated statistically and the results suggest these methods can employed for the routine analysis of lamotrigine in bulk and marketed tablet formulations.

Keywords: Lamotrigine; UV spectroscopy; Validation; Accuracy; Precision

A R T I C L E I N F O: Received 18 Feb. 2024; Review Complete 19 April 2024; Accepted. 24 May 2024; Available online 15 June. 2024

Cite this article as:
Chagi M, Srinivasulu N, Yegnoor AK, Polkal P, Shetty K, Development and Validation of UV Spectroscopic Method for the Quantification of Lamotrigine in Bulk and Marketed Formulations, Asian Journal of Pharmaceutical Research and Development. 2024; 12(3):16-22
DOI: http://dx.doi.org/10.22270/ajprd.v12i3.1388

*Address for Correspondence:
Dr. Y. Anand Kumar Professor and Head ,Department of Pharmaceutics ,V.L. College of Pharmacy ,Raichur-584103 ,Karnataka, INDIA

INTRODUCTION

Lamotrigine is class of drug used to treat epilepsy and stabilize mood in bipolar disorder[1]. Lamotrigine (figure 1) chemically a phenyl triazine, making it different from other anticonvulsants, appears to inhibit release of excitatory neurotransmitters via voltagesensitive sodium channels and voltagegated calcium channels in neurons[2,3]. Several methods for determination of lamotrigine and its metabolites in bulk and biological matrices have been developed viz., reversed phase HPLC[4-12], gas chromatography with nitrogen phosphorus detector[13], capillary electrophoresis[14,15], chromatography thermos spray mass spectrometry[16], immune fluorometric assay[17] and radioimmunoassay[18]. Reported methods were found to expensive and tedious, so an attempt is made to develop two solvent blends and validated for the estimation of lamotrigine by UV spectroscopic method in bulk and marketed tablets.

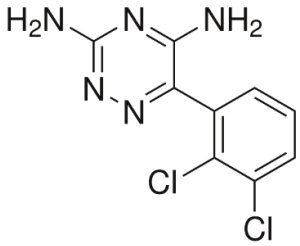

Figure 1: Chemical structure of Lamotrigine

MATERIAL AND METHODS

MATERIALS

Lamotrigine (LM) obtained as gift sample (Magnus Pharma Ltd, Nepal). Lamez-25 (Intas pharmaceuticals ltd, south Sikkim, India) and Lamosyn-25(Sun Pharma Laboratories ltd, Jammu, India) tablets procured from local retail community pharmacy. Solvent blends viz., Methanol: Distilled water (1:1) and Acetonitrile: 0.1N HCl (1:1) considered as Medium 1 and Medium 2 respectively. All reagents, solvents used were of

analytical grade (SD Fine-Chemicals, Bangalore, India). UV-1900 UV-VIS Spectrophotometer-Shimadzu Corp/Japan;Ultraviolet/Visible recording spectrophotometers connected to a compatible computer and supported with UV Probe software used for spectrophotometric measurements.

METHODS

Preparation of Lamotrigine standard stock solutions: Accurately weighed 25 mg of LM was dissolved in 25 ml of Medium 1 in 25 ml volumetric flask by shaking for 5 min followed by 5 min sonication to get 1mg/ml solution (Stock 1). Similarly, 1mg/ml solution was prepared in Medium 2 (Stock 2) by same procedure.

Preparation of Lamotrigine working standard solution:2.5 ml of Stock 1 solution was diluted with Medium 1 in 25 ml volumetric flask to obtain working standard solution of 0.1 mg/ml (Stock 3). Similarly, 2.5 ml of Stock 2 solution was diluted with Medium 2 in 25 ml volumetric flask to obtain working standard solution of 0.1 mg/ml (Stock 4).

Preparation of marketed sample solution (LAMOSYN-25;LAMEZ-25): In each case 10 tablets were used for the study. Ten tablets were weighed accurately and triturated in a mortar to get fine powder. For sample preparation, powder equivalent to 25 mg of LM was shaken with 25 ml of Medium 1 in 25 ml volumetric flask, followed by 10 minutes sonication. The filtrate was collected and dilute appropriately with medium 1 for further studies. Similarly, sample solution was prepared in Medium 2. Sample solutions were prepared for both marketed tablets.

Method development

Determination of absorption maxima (λ max): To determine the absorption maxima of LM in Medium 1 and Medium 2 were done by scanning appropriately diluted Stock 3 and Stock 4 solutions (10µg/ml) in the range of 200 to 400 nm using double beam UV spectrophotometer.

Range: Stock 3wasappropriately diluted with Medium 1, Stock 4 with Medium 2 separately in a series of 10ml volumetric flask to get 2-40 µg/ml solutions. Absorbances of these solutions were measured to determine the beers range at stated wave length.

Linearity: The linearity is the ability of analytical procedure to produce test results, which are proportional to the concentration (amount) of analyte in samples within a given concentration range. For the study Stock 3wasappropriately diluted with Medium 1in a series of 10ml volumetric flask to get 5-30 µg/ml and similarly Stock 4 wasappropriately diluted with Medium 1in a series of 10ml volumetric flask to get 2-12 µg/ml. Absorbances of these solutions were measured and concentration vs. absorbance were plotted for Medium 1 and Medium 2 separately.

Limit of detection (LOD) and Limit of quantitation (LOQ):LODis the lowest amount of an analyte detected in a sample and LOQis the lowest amount of an analyte quantified in a sample with a suitable precision and accuracy. For the study Stock 3 was diluted appropriately with Medium 1 in a series of 10 ml volumetric flask to obtain lowest concentrations keeping Medium 1 as blank. Similarly, Stock 4 was appropriately diluted with Medium 2in a series of 10 ml volumetric flask to obtain lowest concentrations. The LOD and LOQ were determined based on standard deviation (SD) of response and slope (S) by using the following equations

LOD=3.3xσ/S; LOQ=10xσ/S Where, σ is standard deviation; S is slope

VALIDATION

The validation of proposed methods carried out as per ICH guideline.

Precision: Precision in terms of reproducibility, inter-day and intra-day of Medium 1 and Medium 2 were carried out at different concentrations. For the study Stock 3was diluted with Medium 1 in a series of 10 ml volumetric flasks to obtain 5 and 10 µg/ml. Similarly,Stock 4was diluted with Medium 2 in a series of 10 ml volumetric flasks to obtain 8 and 12 µg/ml. % RSD was used for interpretation of the precision.

Accuracy: Accuracy in terms of recovery was studied for two marketed LM tablets. The percent drug content was determined in Medium 1 and Medium 2. Further standard addition method was applied for recovery studied, in which a sample assayed with known amount of LM (40%, 80% and 120%) added to the prequantified marketed samples under the study. The results were interpreted through % recovered and % RSD.

Robustness: Robustness of the Medium 1 and Medium 2 were studied by checking the influence of intentionally changed method parameters. In these studies, the influence of change in actual wave length to ±5 nm on absorbance values were studied. For the studies Stock 3 was diluted appropriately with Medium 1 and Stock 4 with Medium 2in a series of 10 ml volumetric flask to obtain 4, 8 and 12 µg/ml solutions separately. Absorbance of these solutions were measured and compare with absorbance values with actual wave length, % RSD was used to interpret the results.

Ruggedness: Robustness of the Medium 1 and Medium 2 were studied by checking the influence of change in the instrument and change of analyst. For the studies Stock 3 was diluted appropriately with Medium 1 and Stock 4 with Medium 2 in a series of 10 ml volumetric flask to obtain 4, 8 and 12 µg/ml solutions separately.Absorbance of these solutions were measured in two UV spectrophotometers; two different analysts and compare with absorbance values with chief analyst.% RSD was used to interpret the results.

Thermal degradation studies: The influence of elevated temperature on degradation of LM in Medium 1 and Medium 2 was studied by keeping Stock 1 and Stock 2 solutions at 40°C, 60°C and 80°C for 4 hr. The % loss of LM was calculated and interpret in terms of % RSD.

Solution stability: The stability of Medium 1 and Medium 2 were studied at different storage temperature for 24 to 48 hr.

For the studies Stock 1 was diluted appropriately with Medium 1 and Stock 2 with Medium 2 in a series of 10 ml volumetric flask to obtain different concentrations separately and were stored at room (25°C), refrigerated temperature (2-8°C) and accelerated temperature (45°C) in a tightly sealed glass containers protected from light. Measure the absorbance of these solutions % RSD was used to interpret the results.

RESULTS AND DISCUSSION

Method development

The absorption maxima were found to be 308 nm for Medium 1 and 293 nm for Medium 2 withcharacteristic peaks as shown in figure 2 (a) and (b). These absorption maxima were further used for the determination of range, linearity, LOD and LOQ for LM in Medium 1 and Medium 2, the results were shown in table 1,2, figure 3 (a) and (b). A linear relationship was observed in the concentration range of 1-40 µg for Medium 1 and Medium 2, within this range linearity curve was plotted. The goodness of fit study was used explain good correlation coefficient in terms of R^2 values (0.9989 and 0.9994 for Medium 1 and Medium 2) and validate Beer's law with intercept response < 2%, which was calculated by least square method indicate functional linearity between the concentration of analyte and the absorbance.

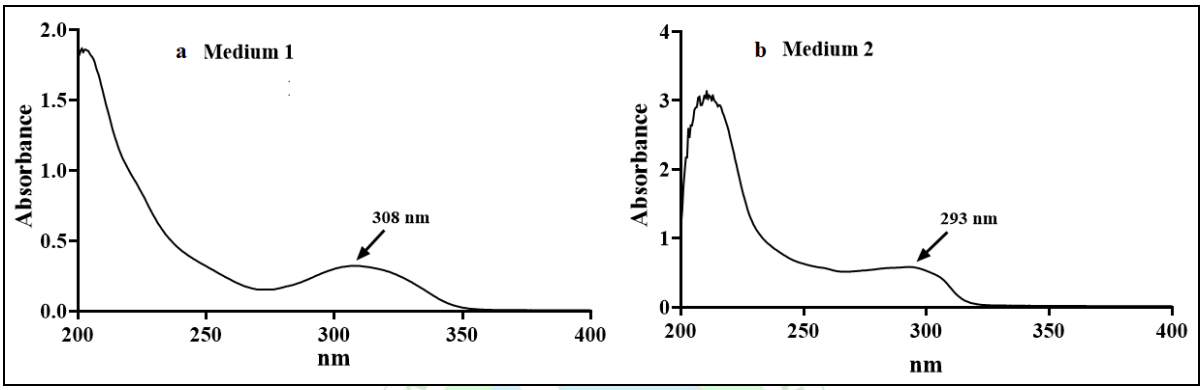

Figure 2: Absorption maxima of LM in Medium1 (a) and Medium 2 (b)

Table 1: Linearity curve of LM in Medium1 and Medium 2

Medium 1		Medium 2	
Concentration µg/ml	Mean ±SD n=6	Concentration µg/ml	Mean ±SD n=6
2	0.040±0.0005	5	0.093±0.000577
4	0.080±0.001	10	0.192±0.00378
6	0.120±0.0011	15	0.285±0.00152
8	0.160±0.0011	20	0.383±0.00199
10	0.2013±0.0020	25	0.481±0.0001
12	0.240±0.0014	30	0.576± 0.00152

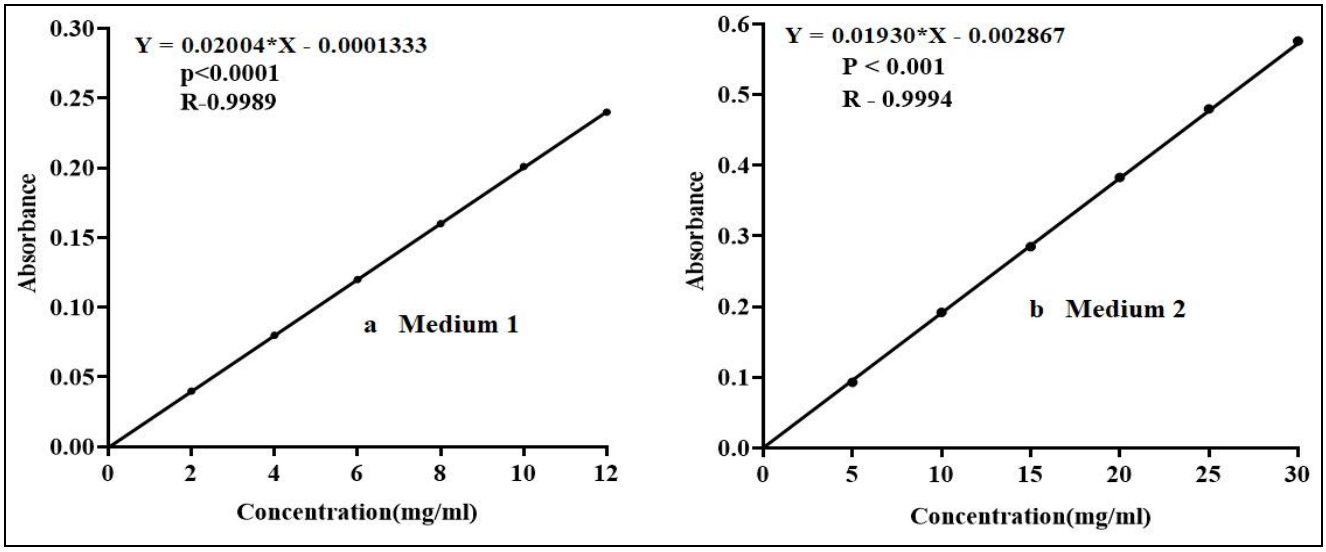

Figure 3: Linearity curve of LM in Medium 1 (a) and Medium 2 (b)

Table 2: Linearity statistical data of LM in Medium1 and Medium 2

Parameter	**Medium 1**	**Medium 2**
Absorption Maxima	308	293
Molar absorptivity	$2.12\times10^{2}/(m^{-cm})$	$1.86\times10^{2}/(m^{-cm})$
Best-fit values		
Slope	0.02004	0.0193
y-intercept	-0.0001333	-0.0028
x-intercept	0.006652	0.1486
1/Slope	49.89	51.82
95% Confidence Intervals		
Slope	0.01990 to 0.02018	0.01913 to 0.01947
x-intercept	-0.001218 to 0.0009516	-0.006213 to 0.0004797
y-intercept	-0.04778 to 0.06041	-0.02506 to 0.3194
Goodness of fit		
R Square	0.9989	0.9994
Equation	Y=0.02004*X-0.0001333	Y=0.01930*X-0.002867

The LOD and LOQ values was calculated based on standard deviation of the response and the slope of the solutions, it was found to be 0.00165 ± 0.019 µg/ml, 0.00188 ± 0.020 /ml for LOQ; 0.0057 ± 0.019µg/ml, 0.0057 ± 0.020 µg/ml for LOD with % RSD values less than 2.

Validation

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the Medium 1 and Medium 2 were justified from the absorbance values obtained in repeatability studies, intra and inter day studies of a fixed amount of LM. The SD and % RSD wascalculated in Medium 1 and Medium 2 and were given in table 3. The percentage RSD values for repeatability studies, intraday and inter day studies is less than 2 % indicate mediums under the study were precise and reproducible.

Table 3: Repeatability precision data.

Precision parameters	**Labelled Claim (µg)**	**Amount recovered**	**% Recovered Mean*± SD**	**% RSD**
Medium 1				
Repeatability	8	8.05	100.6±1.25	1.24
	12	12.01	100.1±0.230	0.92
Intra day	8	8.06	100.8±1.443	1.13
	12	12.01	100.1±0.230	0.82
Inter day	8	8.01	100.2±0.346	0.34
	12	12.01	100.1±0.230	0.72
Medium 2				
Repeatability	5	5.13	102.7±0.51	0.50
	10	9.84	98.00±1.00	1.02
Intra day	5	5.13	102.7±1.15	1.12
	10	9.83	98.37±0.92	0.95
Inter day	5	5.06	101.4±1.53	1.11
	10	9.86	98.63±1.22	1.23
* In each case six replicates were studied n=6				

Accuracy: Assay and accuracy of two marketed tablet formulations were done in Medium 1 and Medium 2 and data was given in table 4,5. Accuracy study was performed for Medium 1 and Medium 2 by standard addition method. The drug content was within the permissible limit with RSD values less than 2% and percentage recovery in standard addition method found within the permissible limits with RSD values less than 2% indicate non-interference of the excipients in the formulations. The accuracy results suggest LAM content in two marketed products determined by the Mediums under the study was in good agreement with the label claimwith % RSD values less than 2.

Table 4: Assay data of LM marketed formulations in Medium 1 and Medium 2

Brand name	Labelled claim	Amount Recovered (µg)	% Recovery Mean* ± SD	% RSD
Medium 1				
LAMOSYN-25	8	7.9	99.1±0.96	0.96
	12	12.35	102.9±1.25	1.21
LAMEZ-25	8	8.03	100.4±0.34	0.33
	12	12.03	100.2±0.34	0.33
Medium 2				
LAMOSYN-25	5	5.07	101.4±1.67	1.64
	10	10.2	102.6±1.05	1.02
LAMEZ-25	5	5	100.1±0.298	0.14
	10	10.2	102.1±0.55	0.53
* In each case six replicates were studied n=6				

Table 5: Recovery data of LM by standard addition method in Medium 1 and Medium 2

Brand name	Labelled Claim µg	% Added	Pure drug Added µg	Amount Recovered µg	% Recovery Mean ± SD	% RSD
Medium 1						
LAMOSYN-25	8	40	3.2	11.21	100.08±0.66	0.65
	8	80	6.4	14.22	98.75±0.73	0.73
	8	120	9.6	17.71	100.62±0.45	0.44
	12	40	4.8	16.71	99.6±0.75	0.75
	12	80	9.6	21.62	100.09±0.20	0.19
	12	120	14.4	24.30	101.25±0.20	0.20
LAMEZ-25	8	40	3.2	11.21	100.08±0.23	0.22
	8	80	6.4	14.32	99.33±0.72	0.72
	8	120	9.6	17.62	100.11±0.25	0.25
	12	40	4.8	16.71	99.46±0.34	0.34
	12	80	9.6	21.62	100.09±0.25	0.25
	12	120	14.4	26.31	99.65±0.11	0.11
Medium 2						
LAMOSYN-25	5	40	2	6.90	98.7±0.86	0.87
	5	80	4	9.07	100.8±1.45	1.44
	5	120	6	10.91	99.83±1.43	1.43
	10	40	4	14.08	100.57±1.28	1.27
	10	80	8	18.03	100.16±0.60	0.60
	10	120	12	21.96	99.82±1.12	1.12
LAMEZ-25	5	40	2	7.01	100.14±1.16	1.16
	5	80	4	9.01	100.11±0.85	0.85
	5	120	6	11.07	100.63±0.99	0.98
	10	40	4	14.08	100.57±1.12	1.11
	10	80	8	17.90	99.44±0.60	0.60
	10	120	12	21.98	99.90±0.46	0.46

Robustness and Ruggedness: Change in λ max of ± 5 nm to the actual λ max in robust analysis results significant different in the percentage recovery in both mediums indicates the methods were not robust. In ruggedness, analysis by different analyst and change of instrument indicates the proposed solvent systems were significantly rugged. The robustness and ruggedness data given in tables 6, 7.

Thermal degradation studies: The influence of elevated temperature on degradation of LM in Medium 1 and Medium 2 was given in table 8. In both the mediums at 60°C and 80°C significant loss of LM was observed whereas at 45°C non-significant loss LM was observed.

Stability studies: The results of stability study of LM in Medium 1 and Medium 2 were within the acceptable limit and indicate LM stability in Medium 1 and Medium 2 was stable over the period of 48 hr.

Table 6: Robustness data for proposed method

ƛmax	Concentration (µg)	Amount Recovered (µg)	% Recovery Mean ± SD	% RSD
Medium 1				
Actual (308nm)	8	8.03	100.4±0.34	0.33
	12	12.0	100.2±0.34	0.33
308 (+5nm)	8	8.6	102.1±0.75	0.73
	12	12.6	101.2±1.44	1.42
308 (-5nm)	8	7.6	95.6±1.36	1.42
	12	11.2	97.7±0.92	0.94
Medium 2				
Actual (293nm)	5	5	99.2±0.63	0.64
	10	10.1	101±0.50	0.49
298 (+5nm)	5	5.22	104.3±0.05	0.05
	10	10.18	101.8±0.66	0.65
288 (-5nm)	5	4.08	82.53±0.55	0.66
	10	9.38	93.73±0.40	0.43

Table 7: Ruggedness data for proposed methods

Parameter	Concentration (µg)	Amount Recovered (µg)	% Recovery Mean ± SD	%RSD
Medium 1				
Analyst 1	8	8.2	103.5±1.30	1.25
	12	12.0	100.5±0.61	0.60
Analyst 2	8	8.06	100.5±0.23	0.22
	12	12.1	100.9±0.61	0.60
Medium 2				
Analyst 1	5	5	99.2±0.63	0.64
	10	10.1	101±0.50	0.49
Analyst 2	5	5.17	103.5±0.63	0.61
	10	9.84	98.37±0.55	0.55

Table 8: Thermal degradation studies

Thermal degradation	Concentration (µg)	Amount Recovered (µg)	% Recovery Mean ± SD	%RSD
Medium 1				
AT 45^{O}C	8	8.1	101.38±1.59	1.58
	10	10.26	102.60±0.52	0.50
AT 60 ^{O}C	8	6.4	80.20±1.04	1.06
	10	8	80.03±0.79	0.78
AT 80 ^{O}C	8	2.02	25.38±0.60	0.59
	10	2.73	27.43±0.30	0.29
Medium 2				
AT 45^{O}C	5	5.11	102.78±0.60	0.58
	10	10.44	103.12±0.52	0.51
AT 60 ^{O}C	5	3.96	79.17±1.05	1.03
	10	7.76	77.60±0.52	0.54
AT 80 ^{O}C	5	1.26	25.35±0.60	0.59
	10	2.73	27.43±0.31	0.29

CONCLUSIONS

The present study was aimed to develop and validate simple UV spectroscopic method for the estimation of Lamotrigine in bulk and marketed tablets. The proposed method comprisingMethanol: Distilled water (1:1) - Medium 1; and Acetonitrile: 0.1N HCl (1:1) -Medium 2 were used and validated. The accuracy and precision results suggest the method was found to be reproducible.The proposed method conveniently applied to estimate the Lamotrigine in bulk and marketed formulations.The statistical parameters and the recovery data reveal high precision and accuracy of the methods besides being robust and rugged.Therefore, the validated method could be useful for routine quality control assay of the studied drug in pure form and pharmaceutical formulations.

ACKNOWLEDGEMENT

The authors are thankful to Sri. Sarvottam Giri, Technical director, Magnus Pharm Ltd, Nepal for providing gift sample of Lamotrigine and thanks to the principal and management of V.L. College of Pharmacy for providing the facilities to carry out the work.

CONFLICT OF INTERESTS

No conflict of interest

REFERENCES

1. Calabrese JR, Bowden CL, Sachs GS, et al. Double-blind placebo-controlled study of lamotrigine monotherapy in outpatients with bipolar I depression. J Clin Psychiatry1999;60:79-88.
2. Grof P, Robbins W, Alda M, Berghoefer A, Vojtechovsky M, Nilsson A, et al. Protective effect of pregnancy in women with lithium-responsive bipolar disorder. J Affect Disord2000; 61:31-39.
3. Lamotrigine. PubChem Open Chemistry Database. US: National Institutes of Health. Retrieved 2016.
4. Lamotrigine analysis in blood and brain by high-performance liquid chromatography. JChromatography B 2000; 755(1-2): 119-127.
5. BarbosaNR, MdioAF.Validated high-performance liquid chromatographic method for the determination of lamotrigine in human plasma, JChromatography B 2000; 741(2): 289-293.
6. CrociD, SalmaggiA, Grazia UD, BernardiG.New High-performance Liquid Chromatographic Method for Plasma/Serum Analysis of Lamotrigine. Therapeutic Drug Monitoring 2001; 23(6): 665-668.
7. VidalE, PascualC, PouL.Determination of lamotrigine in human serum by liquid chromatography. JChromatography B 1999; 736(1-2): 295-298.
8. Angelis-StoforidisP, MorganDJ, O'BrienTJ, Vajda FJE. (1999). Determination of lamotrigine in human plasma by high-performance liquid chromatography.JChromatography B 1999; 727(1-2): 113-118.
9. MatarKM, NichollsPJ, BawazirSA, Al-HassanMI, Tekle A. (1998). A rapid liquid chromatographic method for the determination of Lamotrigine in plasma. JPharmBiomedAna 1998;17(3): 525-531.
10. TorraM, RodamilansM, ArroyoS, CorbellaJ.Optimized procedure for lamotrigine analysis in serum by high-performance liquid chromatography without interferences from other frequently co-administered anticonvulsants. TheraDrug Monit 2000;22: 621-625.
11. RenS, ScheuerML, Zheng W.Determination of Lamotrigine in Biologic Materials by a Simple and Rapid Liquid Chromatographic Method. TheraDrug Monit 1998;20(2): 209-214.
12. CooperJDH, ShearsbyNJ, Taylor JE, FookSheung CTC.Simultaneous determination of lamotrigine and its glucuronide and methylated metabolites in human plasma by automated sequential trace enrichment of dialysates and gradient high-performance liquid chromatography, JChromatography B 1997; 702(1-2): 227-233.
13. WatelleM, DemedtsP, FranckF, De DeynPP, WautersA, NeelsH.Analysis of the Antiepileptic Phenyl triazine Compound Lamotrigine Using Gas Chromatography with Nitrogen Phosphorus Detection, TheraDrug Monit 1997; 19(4):460-464.
14. TheurillatR, KuhnM, ThormannW.Therapeutic drug monitoring of lamotrigine using capillary electrophoresis: Evaluation of assay performance and quality assurance over a 4-year period in the routine arena. JChromatography A 2002; 979(1-2): 353-368.
15. ShihabiZK, Oles KS.Serum lamotrigine analysis by capillary electrophoresis. JChromatography B 1996; 68(1): 119-123.
16. DoigMV, ClareRA.Use of thermo sprays liquid chromatographymass spectrometry to aid in the identification of urinary metabolites of a novel antiepileptic drug, Lamotrigine. JChromatography B 1991; 554(1-2):181-189.
17. SailstadJM, FindlayJW.Immunofluorometric assay for lamotriginein Huron plasma. TheraDrug Monit 1991; 13:433-442.
18. BiddlecombeRA,DeanKL, SmithCD, Jeal SC.Validation of a radioimmunoassay for the determination of human plasma concentrations of Lamotrigine. JPharmBiomedAnal1990; 8(8-12):691- 694.