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Short communication

Development and validation of a new HPLC method for determination of lamotrigine and related compounds in tablet formulations

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Abstract

A simple HPLC method was developed and validated for quantitation of lamotrigine and its related substances which may coexist in solid pharmaceutical dosage forms. The HPLC separation was achieved on a C18 μ-Bondapack column (250 mm × 4.6 mm) using a mobile phase of acetonitrile–monobasic potassium phosphate solution (35:65, v/v) containing orthophosphoric acid to adjust pH to 3.5 at a flow rate of 1.5 ml/min. The UV detector was operated at 210 nm, and column temperature was adjusted at 40 °C. The method was validated for specificity, linearity, precision, accuracy, robustness and limit of quantitation. The degree of linearity of the calibration curves, the percent recoveries of lamotrigine and related substances, the limit of detection and quantitation, for the HPLC method were determined. The method was found to be simple, specific, precise, accurate, and reproducible. The method was applied for the quality control of commercial lamotrigine tablets to quantify the drug and its related substances and to check the formulation content uniformity.

Keywords: Lamotrigine; Impurity; Pharmaceutical preparation; HPLC; Validation

1. Introduction

Lamotrigine, 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, is a novel antiepileptic drug chemically unrelated to other anticonvalsants (Fig. 1, compound A) used as an add-on therapy of seizure in children and adults [1]. It has been shown that lamotrigine is effective against partial and secondarily generalized tonic–clonic seizures as monotherapy or adjunctive treatment [2]. It's mechanism of action seems to be the inhibition of the release of excitatory neurotransmitters like aspartate and glutamate and also involvement in the blocking of voltage-dependent sodium channels [1–3].

Several methods for determination of lamotrigine and its metabolites in biological matrices have been developed including reversed-phase HPLC [2–10], gas chromatography with nitrogen phosphorus detector [11], capillary electrophoresis [12,13], chromatography–thermospray mass spectrometry [14], immuno fluorometric assay [15] and radioimmunoassay [16]. An analytical method for the detection of trace amount of the principal synthetic route indicative impurity in lamotrigine including preconcentration sample extract by normal-phase HPLC which then was analysed with a reversed-phase HPLC TSP-MS has also been reported [1].

A literature review revealed that at this time the HPLC method has been considered as the technique of choice for the separation and determination of lamotrigine. An official monograph of lamotrigine does not exist in any pharmacopoeia and determination of lamotrigine and related substances (Fig. 1A–C) in pharmaceutical formulations has not been yet described. Therefore, it is very imperative to develop a simple and suitable analytical method for the measurement of lamotrigine and related compounds in bulk and in formulations. Such methods could be easily adapted for routine and in-process quality control analysis, dissolution or similar studies. Due to the importance of bioequivalence of the dosage forms and limitations of UV spectrophotometric methods in detection of impurities, our purpose was to develop a simple, sensitive, and reliable method for simultaneous determination of the drug and respective impurities which can be applied in quality control laboratories. To achieve this aim, a liquid chromatographic assay was developed in this study. This work also describes the validation parameters stated by USP 25, to achieve an analytical method with acceptable

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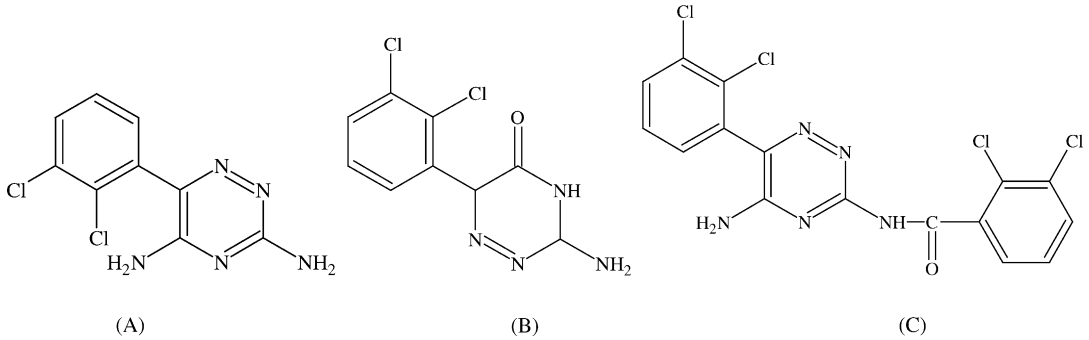

Fig. 1. Chemical structures of the compound investigated. (A): 3,5-diamino-6-(2,3-dichlorophenyl)–1,2,4-triazine (lamotrigine); (B): 3-amino-6-(2,3-dichlorophenyl)-3,6-dihydro-1,2,4-triazine-5(4H)-one (Impurity A); (C): *N*-[5-amino-6-(2,3-dichlorophenyl)-1,2,4-triazine-3yl]-2,3-dichlorobenzamide (impurity B).

characteristics of suitability, reliability and feasibility [17]. The proposed method has been validated and applied for controlling of commercially available lamotrigine tablets containing 50 mg active substance.

2. Experimental

2.1. Materials

Individual standards of lamotrigine, impurities A and B were provided by Jubilant Organosys Ltd. (India). Lamotrigine tablets containing 50 mg lamotrigine were supplied by Bakhtar Pharmaceutical Co. (Kermanshah, Iran). The inactive ingredient used as the drug–matrix including microcrystalline cellulose (FMC, Ireland), sodium starch glycolate (FMC, Ireland), and lactose were obtained from market. Analytical grade monobasic potassium phosphate, methanol and hydrochloric acid and HPLC grade acetonitrile were purchased from Merck (Germany).

2.2. Determination of appropriate UV wavelength

A suitable wavelength was required for simultaneous determination of lamotrigine and two related substances. The appropriate wavelength for the detection of drug and two impurities in mobile phase was determined by wavelength scanning over the range of 200–400 nm with a Shimadzu® UV-160 (Shimadzu, Japan) double beam spectrophotometer.

2.3. Chromatographic system and conditions

HPLC method was performed using a Waters 515 HPLC pump with a Rheodyne 7725I autoinjector, Waters 2487 Dual λ absorbance detector and Waters 746 Chromatopac integrator (Waters, USA). Separation was operated on a C_{18} μ-Bondapack column (250 mm × 4.6 mm). The mobile phase consisted of acetonitrile–monobasic potassium phosphate solution (35:65, v/v) at a flow rate of 1.5 ml/min. Monobasic potassium phosphate solution was prepared by dissolving 1400 mg KH_2PO_4 in 1 l double distilled water. Final pH of the mobile phase was adjusted to 3.5 by diluted orthophosphoric acid. Column temperature was set at 40 °C and 20 μl of samples was injected to the HPLC system.

2.4. Preparation of solutions

2.4.1. Standard solutions of lamotrigine and related substances

An accurately weighed quantity of lamotrigine (50 mg) or related substances (A and B) (0.5 mg, added as methanolic solution), in accordance with the limit accepted for each related substance, was transferred to a 50-ml volumetric flask, approximately 30 ml of the sample preparation solvent (methanol) was added, shaken, dissolved and brought to volume by methanol and properly mixed. Appropriate dilutions of this stock solution were prepared using methanol to obtain solutions of known concentrations to be used for linearity studies and assay purposes.

2.4.2. Standard solutions of lamotrigine and related substances in the drug–matrix

Samples of 50 mg lamotrigine or 0.5 mg related substances (weighed by aliquot method) mixed with appropriate proportions of the drug–matrix components. The mixtures were dispersed and diluted quantitatively to 50 ml using methanol. The solutions were sonicated for 15 min, centrifuged at 4000 rpm for 10 min and the supernatant were used to prepare methanolic solutions containing various quantities of lamotrigine or related substances.

2.4.3. Working standard solution

The working standard solution contained 25 μg/ml of lamotrigine, 0.25 μg/ml of each related substance and a known quantity of excipients (in accordance with qualitative and quantitative composition of the pharmaceutical product tested).

2.4.4. Assay sample preparation

Twenty tablets were weighed, finely powdered and portions equivalent to 50 mg lamotrigine were transferred into a 50 ml volumetric flask; 25 ml methanol was added, shaken throughly to dissolve, was brought to volume, mixed well and centrifuged. One millilitre of supernatant was diluted continually to 25 μg/ml. Five milliliters of this diluted solution was filtered through a millipore filter (0.45 μm) and 20 μl was injected into the HPLC column.

2.4.5. Content sample preparation

For the assessment of drug content uniformity in each tablet, 10 tablets were separately transferred to a 50 ml volumetric flask and 25 ml of methanol was added, shaken for about 1 h by mechanical means, brought to volume, centrifuged and the supernatant was used to prepare solutions of 25 μg/ml of lamotrigine using the methanol as the diluent.

2.5. Method validation

2.5.1. Quantification

Equal volumes (20 μl) of the working standard solution and the assay or content preparation containing lamotrigine or related substances in the methanol were injected into the HPLC column and the chromatograms and the peak areas were recorded. The quantity of lamotrigine or related substance in the assay as well as content solution was calculated from the equation C_S (A_t/A_s), where A_t and A_s are the areas under the test and standard peaks, respectively, and C_S is the concentration of lamotrigine or related substance in the working standard solution [18].

2.5.2. Specificity

The selectivity of the analytical method was confirmed by the analysis of a solution containing 20 μg/ml of lamotrigine and related substances and a known added quantity of excipients (in accordance with qualitative and quantitative composition of the pharmaceutical product tested). The ability to separate all the compounds (related substances, excipients) from lamotrigine in the sample was demonstrated by assessing the resolution between the peaks corresponding to various substances. The tailing factor for lamotrigine and the related compounds was also assessed. The identification was performed by comparing the retention time of major peaks in the chromatogram of the assay solution with those in the chromatogram of the standard solution [19].

2.5.3. Linearity

Calibration plots were constructed for lamotrigine and related substances in either standard solutions or synthetic mixtures of the drug product components by plotting the concentration of compounds versus peak area response. Standard solutions containing 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, and 35 μg/ml of lamotrigine and 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, and 0.35 μg/ml of related substances were prepared in triplicate and 100 μl was injected into the HPLC column. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method [18]. The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

2.5.4. Precision and repeatability

The precision of the HPLC method was determined by repeatability (intra-day) and intermediate precision (inter-day). Each level of precision was investigated by repeated analysis of standard solutions at concentration ranging from 0.1 to 35 μg/ml prepared in drug–matrix. The intra-day variability was performed by the same analyst over one day, while inter-day precision was carried out by another independent analyst over various days [18–20].

2.5.5. Accuracy

The accuracy of the method was tested by analyzing different samples of lamotrigine and related compounds at various

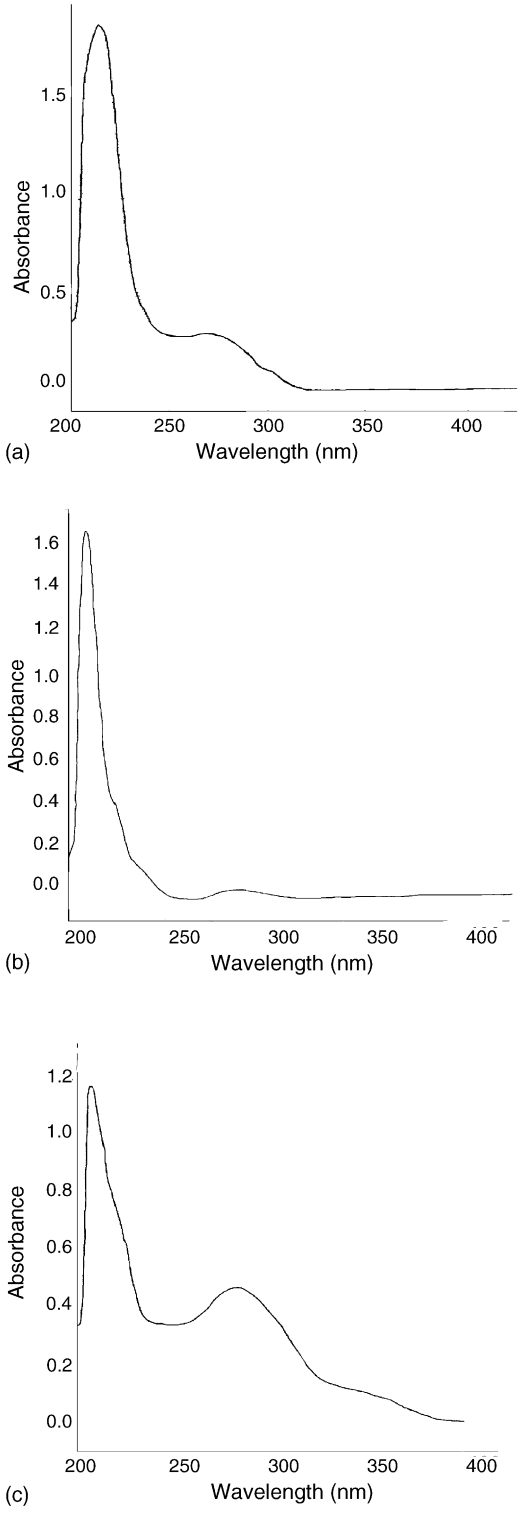

Fig. 2. UV spectrums of: (a) lamotrigine; (b) substance A; and (c) substance B.

concentration levels in either pure solutions or in solutions comprising the drug–matrix used in tablet formulation.

2.5.6. Robustness

The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the percentage of mobile phase acetonitrile, in the pH, in the mobile phase flow rate and in the temperature. The effect on retention time and peak parameters were studied [18–20].

3. Results and discussion

3.1.1. Determination of suitable wavelength

In order to investigate the appropriate wavelength for simultaneous determination of lamotrigine and two impurities, solutions of lamotrigine and related compounds in mobile phase were scanned by UV spectroscopy in the range 200–400 nm. The UV spectra of three substances are illustrated in Fig. 2. The maximum absorbance for three tested substances occurs at about 210 nm. Alternatively, solutions of each substance in mobile phase were also injected to HPLC directly and the responses (peak area) were recorded both at 210 and 281 nm. Greater peak areas were observed for drug and compounds A and B at 210 nm as compared with those of 281 nm. Therefore, it was concluded that 210 nm is the most appropriate wavelength for analysing three substances with suitable sensitivity (chromatograms are not shown).

3.2. Specificity

The HPLC chromatograms recorded for the mixture of the drug excipients revealed almost no peaks within a retention time range of 10 min. A representative chromatogram for a mixture of lamotrigine with its related compounds, A and B (containing 20 μg/ml of each component) is shown in Fig. 3. The figure shows that lamotrigine is clearly separated from its related compounds. The resolution factor obtained between lamotrigine and peaks adjacent to it was 3.33 and 4.8 and for impurity A and B was 5.72 and the tailing factor for lamotrigine and the related compounds was always in the range 1.03–1.1. Thus, the HPLC method presented in this study is selective for lamotrigine and the other two related compounds, which might coexist as impurities. The study of the absence of excipients and impurities showed that none of the peaks appears at the retention time of lamotrigine and it was concluded that the developed method is selective in relation to the excipients and impurities of the final preparation.

3.3. Linearity

Calibration curves for lamotrigine and two related substances were checked in pure solutions as well as in the drug–matrix solutions and it was found to be linear with correlation coefficients of greater than 0.999 in most cases. Table 1 lists the linearity parameters of the calibration curves for lamotrigine and related compounds in pure and drug–matrix solutions. The LOD and LOQ are also given in this table.

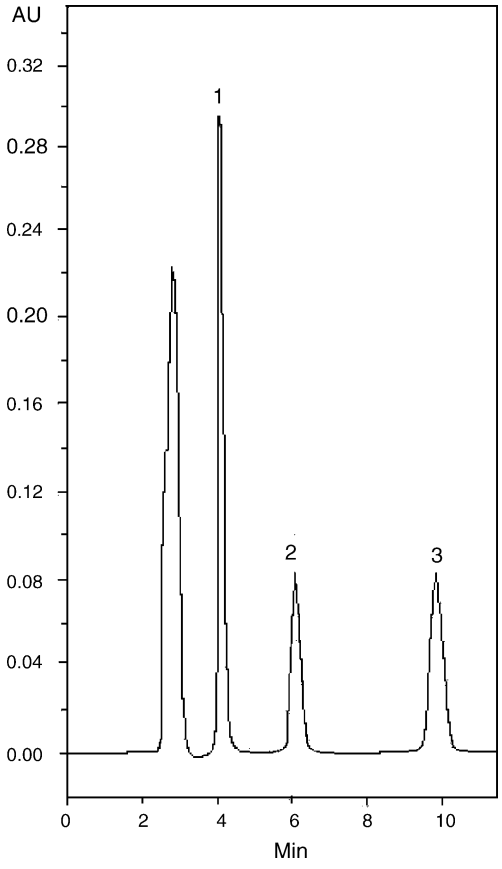

Fig. 3. Representative chromatogram of lamotrigine and related compounds in standard solution. (1) Lamotrigine; (2) compound A; and (3) compound B.

3.4. Precision and repeatability

The results obtained for repeatability studies and for intermediate precision are presented in Table 2. Method precision has a relative standard deviation (R.S.D.) below 1.9% for repeatability and 1.8% for intermediate precision, which comply with the acceptance criteria proposed (R.S.D.: not more 2.0%) [17].

3.5. Accuracy

The results were expressed as percent recoveries of the particular components in the samples. Table 3 shows that the overall percent recoveries of lamotrigine in pure and drug–matrix solutions were 100.7 (relative standard deviation, R.S.D. = 1.92%) and 98.02 (R.S.D. = 1.15%), respectively. However, the related compounds showed the overall percent recoveries ranging from 99.9 to 101.5 with R.S.Ds. ranging from 2.61 to 3.73%.

3.6. Robustness

The result of the robustness of the assay method is demonstrated in Table 4. Method robustness checked after deliberate

Table 1
Linearity of calibration curves for lamotrigine and its related compounds in standard solutions and in the drug–matrix preparation (each point in the regression line is the mean of five experiments)

Compound	Calibration range (μg/ml)	R^2	Slope	95% CI of the slope	Intercept	95% CI of the intercept	LOD (μg/ml)	LOQ (μg/ml)
Standard preparations								
Lamotrigine	0.1–35	0.9999	44.74	1.82	4.01	0.121	0.01	0.10
Impurity A	0.05–0.35	0.9997	31.50	1.23	1.78	0.049	0.01	0.05
Impurity B	0.05–0.35	0.9996	12.84	0.42	1.48	0.035	0.01	0.05
Drug–matrix preparations								
Lamotrigine	0.1–35	0.9996	44.92	1.86	4.01	0.121	0.01	0.10
Impurity A	0.05–0.35	0.9994	31.82	1.25	1.7892	0.049	0.01	0.05
Impurity B	0.05–0.35	0.9995	13.05	0.44	1.4812	0.035	0.01	0.05

Table 2
Intra and inter-day variations of the HPLC method for determination of lamotrigine

Concentration (μg/ml)	Intra-day precision and accuracy				Inter-day precision and accuracy			
	Mean	S.D.	R.S.D.	%Recovered	Mean	S.D.	R.S.D.	%Recovered
0.1	0.104	0.002	1.9	104.0	0.103	0.0018	1.7	103.0
0.5	0.516	0.008	1.6	103.2	0.513	0.009	1.8	102.6
1	0.982	0.012	1.2	98.20	0.972	0.016	1.6	97.20
5	5.085	0.061	1.2	101.7	4.935	0.075	1.5	98.70
10	9.82	0.105	1.1	98.20	9.88	0.125	1.3	98.80
15	14.85	0.125	0.8	99.00	15.25	0.225	1.5	101.6
20	20.25	0.142	0.7	101.25	20.35	0.271	1.3	101.7
25	25.39	0.155	0.6	101.5	24.61	0.252	1.0	98.44
30	30.36	0.254	0.8	101.2	30.52	0.351	1.1	101.7
35	34.723	0.412	1.2	99.20	35.523	0.412	1.2	101.4

alterations of mobile phase composition, flow, pH and temperature shows that the changes of the operational parameters do not lead to essential changes of the performance of the chromatographic system. The tailing factor for lamotrigine and the related compounds always ranged from 1 to 1.1 and the components were well separated under all the changes carried out. The percent recoveries of lamotrigine and related compounds were good under most conditions and did not show a significant change when the critical parameters were modified. Considering the result of modifications in the system suitability parameters and the specificity of the method, it would be concluded that the method conditions are robust.

3.7. Assay of tablets

The method developed in the present study was applied for the determination of lamotrigine and the related substances content in tablets from the Iranian market. Fig. 4 shows an HPLC chromatogram of lamotrigine and related compounds in a pharmaceutical formulation tablet. One of the compounds related to lamotrigine (Substance A) appeared clearly on the chromatogram indicating that the proposed method can differentiate between the active moiety and its related impurities. The results showed a percent recovery of 100.1% and a R.S.D. of 1.5%. Impurity B was not detected in any significant amount while the content of impurity A was less than 1% of total amount of lamotrigine in each tablet.

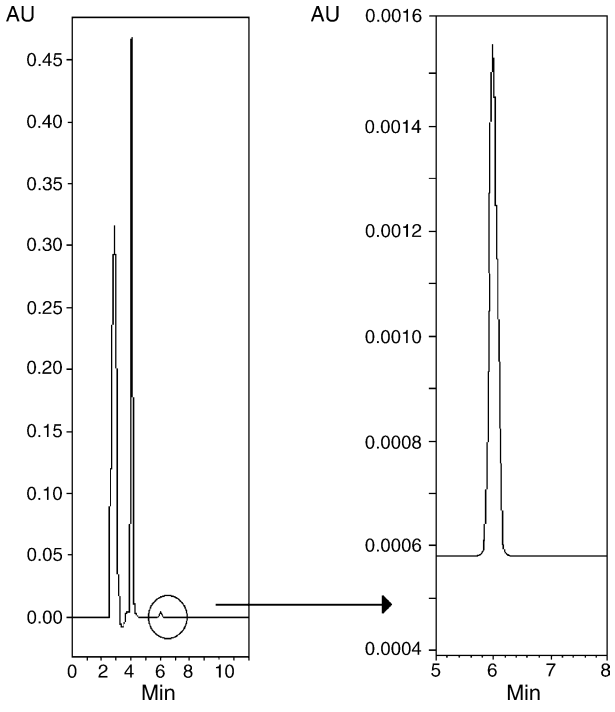

Fig. 4. Typical HPLC chromatogram of lamotrigine and related compounds in a pharmaceutical formulation tablet. Selected peak which corresponds to related compound A is magnified using Borwin software.

Table 3
Accuracy of the HPLC method for the determination of lamotrigine and its related compounds in standard or drug–matrix solutions

Compound	Standard concentrations (μg/ml)	Standard solutions		Drug–matrix solutions	
		Observed concentrations (μg/ml)	Recovery (%)	Observed concentrations (μg/ml)	Recovery (%)
Lamotrigine	0.1	0.104	104.0	0.096	96.00
	0.5	0.516	103.2	0.485	97.00
	1	0.982	98.20	0.972	97.20
	5	5.085	101.7	4.881	97.60
	10	9.820	98.20	9.782	97.80
	15	14.85	99.00	14.75	98.33
	20	20.25	101.2	19.64	98.20
	25	25.39	101.5	24.52	98.08
	30	30.36	101.2	29.48	98.27
	35	34.72	99.20	34.88	99.66
Overall recovery			100.7		98.02
%R.S.D.			1.92		1.15
Impurity A	0.05	0.052	104.0	0.048	96.00
	0.10	0.103	103.0	0.104	104.0
	0.15	0.156	104.0	0.153	102.0
	0.20	0.194	97.00	0.197	98.50
	0.25	0.256	102.4	0.256	102.4
	0.30	0.292	97.33	0.305	101.7
	0.35	0.345	98.57	0.354	101.1
Overall recovery			101.5		100.81
%R.S.D.			3.73		2.67
Impurity B	0.05	0.052	104.0	0.049	98.00
	0.10	0.105	105.0	0.102	102.0
	0.15	0.144	96.20	0.145	96.66
	0.20	0.196	98.00	0.205	102.5
	0.25	0.242	96.80	0.245	98.00
	0.30	0.294	98.00	0.308	102.7
	0.35	0.355	101.4	0.358	102.3
Overall recovery			99.88		100.3
%R.S.D.			3.59		2.61

Table 4
Influence of changes in experimental parameters on the performance of chromatographic system

Parameter	Modification	Lamotrigine (% recovery)	Impurity A (% recovery)	Impurity B (% recovery)
Mobile phase ratio (v/v) acetonitrile: buffer	40:60	101.8	102.2	98.9
	37.5:62.5	99.6	98.7	92.9
	35:65	101.4	102.4	97.9
	32.5:67.5	99.5	101.0	104.9
	30:70	99.1	98.5	103.6
pH	4	99.8	94.6	103.6
	3.5	101.4	102.4	97.90
	3	101.5	103.0	104.2
Flow rate (ml/min)	1.25	98.9	101.7	97.3
	1.5	101.4	102.4	97.9
	1.75	102.2	103.9	103.4
Temperature (°C)	45	98.8	102.2	106.1
	40	101.4	102.4	97.9
	35	101.7	101.3	105.9

3.8. Content uniformity

Results of content uniformity experiment exhibited that lamotrigine and impurity A content in 10 tablets examined was in the range of 95.6–107.8% and 0.68–0.74% and the R.S.D. value was 3.75 and 4.8%, respectively. This indicates uniform distribution of drug in tablets without any significant variation. According to USP pharmacopoeia the acceptance limit for drug content uniformity and the R.S.D. is 85–115% and less than 6%, respectively. The acceptance criteria set forth by the company for related substances is less than 1%.

4. Discussion and conclusion

The present method differs only slightly from those reported previously in terms of mobile phase and chromatographic conditions [3,4,7,8]. All the HPLC methods previously reported have been developed for determination of lamotrigine in biological fluids [2–10]. In the present study, however, a simple validated HPLC method was developed for the analysis of lamotrigine and related substances in tablet formulations. The main novelty, therefore, exist in the use of different matrices as the aim of this work was totally different from previous studies. The developed method showed no interference with the formulation excipients and good resolution between drug and impurities. The method was also linear and precise. Because there is no pharmacopoeial method for detection of lamotrigine and its related compounds, this method is recommended for quality control of drug content in pharmaceutical preparations.

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