Research Article

Stress Degradation Assessment of Lamotrigine Using a Validated Stability-Indicating HPTLC Method

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In this work, a sensitive and stability-indicating HPTLC method for the determination of lamotrigine is presented. According to the International Conference on Harmonization guidelines Q1A, lamotrigine was exposed to a variety of stress conditions; these include heating in acidic, basic and neutral media. Its stability towards oxidative stress, humidity, high temperature and direct sunlight was also examined. Separation of the drug from its forced degradation impurities was achieved using TLC silica gel plates and a mobile phase composed of ethyl acetate: methanol: ammonia. The linear regression analysis of the data obtained for the correlation plots showed good linearity over the concentration range of 10–300 ng/spot. The forced degradation studies showed that lamotrigine is susceptible to degradation under acidic, basic, neutral and oxidative conditions, among which alkaline-induced hydrolysis had the highest degradative potential. Alternatively, the drug was stable under heat, humidity, and daylight stress factors. In order to assess the purity and stability of the drug in tablet formulations, the developed method was applied to the analysis of commercial tablets in brand and generic products. The obtained results showed that the degradation of the drug has not occurred in the marketed formulations that were analyzed by the described methodology.

1. Introduction

Lamotrigine (LAM) [6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine] is an antiepileptic drug approved as a mono-therapy for partial seizures and for the adjunctive treatment of partial seizures, primary and secondary generalized tonic-clonic seizures, and in patients with Lennox-Gastaut syndrome. It is also approved for use in the maintenance treatment of bipolar disorder to delay the time to occurrence of mood episodes [1–3].

The USP describes an HPLC assay with UV detection for the assay of LAM in pure and tablet form [4]. On the other hand, the BP describes a nonaqueous acid-base titration for the assay of the pure drug [5]. Moreover, techniques as HPLC [6, 7] and TLC [7] were used to quantify LAM in the presence of its related impurities. The drug was also determined in

tablet form using a TLC method [8]. The Literature also contains a spectrophotometric direct UV method [9] as well as a spectrofluorimetric one utilizing the reaction with o-phthalaldehyde [10] for its determination in tablet form. Pertaining to stability-indicating studies, 2 HPLC methods for the separation of the investigated drug from its forced-degradation products were recently reported [9, 11]. To the best of our knowledge, HPTLC has not been previously reported for the stress testing of LAM and its determination in the presence of its forced degradation products. It is note-worthy that HPTLC has become an essential tool of modern analytical chemistry to assess the stability of a wide range of pharmaceuticals under a variety of stress conditions [12–14]. From another perspective, the International Conference on Harmonisation (ICH) guidelines Q1A [15] emphasize on the stability testing of new drug substances and products. This

testing aims at examining the features which are vulnerable to change under the influence of different environmental conditions during storage such as temperature, humidity, and light and thus impacting the quality, safety, and/or efficacy of the drug substance. In these guidelines, it is mentioned that forced decomposition studies should be carried out at temperatures rising in 10°C increments, humidity (e.g., 75% RH or greater), and under oxidative and photolytic conditions. In addition, the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension should be evaluated. Therefore, this work was directed towards the development of a simple and sensitive stability-indicating HPTLC that can be used to establish a stability profile of the drug and consequently assess its purity in bulk and tablet form.

2. Experimental

2.1. Materials.
All reagents and solvents used were of analytical grade. LAM was kindly supplied as a gift sample by Delta Pharm (Cairo, Egypt). 1 mg/mL standard stock solution was prepared in methanol and was further diluted by the same solvent to produce 0.1 and 0.5 mg/mL standard working solutions.

2.2. HPTLC Instrumentation.
The samples were spotted in the form of bands of 5 mm width with a Camag microlitre syringe under nitrogen stream using a Camag Linomat IV sample applicator (Switzerland). Precoated TLC silica gel aluminum plates $60F_{254}$ (20×10 cm, $200 \mu m$ thickness) purchased from Fluka (Buchs, Switzerland) were used. A constant application rate of $0.15 \mu L/s$ was employed with the distance between 2 bands adjusted at 4 mm. The slit dimensions were kept at 4×0.45 mm, and a scanning speed of 20 mm/s was utilized. The mobile phase consisted of ethyl acetate-methanol-ammonia ($17 : 2 : 1$, v/v/v), and 15 mL of which was used per chromatographic run. Linear ascending development was performed in a Camag twin trough glass chamber (20×20 cm) presaturated with the mobile phase for 15 min at room temperature. The length of the chromatogram run was 89 ± 2 mm. Subsequent to the development, the plates were air dried and scanned densitometrically in the absorbance mode at 310 nm using Camag TLC scanner III operated by CATS software (V 3.15). The source of the radiation was the deuterium lamp.

2.3. Calibration Standards.
Serial volumes of 0.1 mg/mL LAM standard working solution were transferred into a set of 10 mL volumetric flasks, and the volumes were completed to mark with methanol. Each calibration concentration was prepared in triplicates, and $5 \mu L$ aliquots of each were spotted onto the TLC plate to obtain final concentrations of 10–300 ng/spot. Plate spotting and development as well as spot scanning and quantitation were performed as mentioned in Section 2.2, and the calibration curve was constructed.

2.4. Assay of Tablets.
Ten tablets were weighed, powdered and thoroughly mixed. An accurately weighed quantity of the powdered tablets equivalent to 50 mg drug according to the label claim was sonicated for 2×5 min with 2×20 mL methanol portions. The combined extracts were filtered into a 50 mL volumetric flask, and the volume was finally completed to the mark with methanol. Ten mL of this solution was diluted to 100 mL with methanol to produce a 0.1 mg/mL LAM solution. Aliquots of this solution were treated as in Section 2.3. The nominal contents of the tablets were then calculated using the developed regression equation.

2.5. Accelerated Degradation of LAM.
The 0.5 mg/mL LAM standard working solution was used in the forced degradation studies to provide evidence of the stability-indicating capacity and the selectivity of the proposed method. With the exception of the photolytic degradation, all forced degradation studies were performed in the dark in order to exclude the possible degradative effect of light.

2.5.1. Acid and Base-Induced Degradation.
To 10 mL of the standard working solution, 10 mL of 5 M HCl or 2.5 M NaOH was added separately. These mixtures were refluxed in a thermostatically controlled water bath maintained at 80°C for 2 h. The samples were then cooled to room temperature and quantitatively transferred into 25 mL volumetric flasks. Finally, the volumes were completed to mark with methanol. One μL of each solution (200 ng/spot) was applied on the plate, and the chromatograms were run as previously described.

2.5.2. Neutral Hydrolysis-Induced Degradation.
Ten mL of the LAM standard working solution was refluxed with 10 mL water in a thermostatically controlled water bath maintained at 80°C for 72 h. The solution was then treated as in the Section 2.5.1.

2.5.3. Hydrogen Peroxide-Induced Degradation.
To 10 mL of the standard working solution, 10 mL of H_2O_2 (30% v/v) and 20 mL water were added, and the mixture was refluxed in a thermostatically controlled water bath maintained at 80°C for 4 h. The solution was then heated in a boiling water bath for 30 min to remove completely the excess of H_2O_2. Afterwards, the solution was cooled and quantitatively transferred to a 50 mL volumetric flask and then completed to volume with methanol. Finally, $2 \mu L$ solution (200 ng/spot) was applied on the plate, and the chromatograms were run as previously described.

2.5.4. Dry Heat-Induced Degradation.
Pure drug powder was spread as a thin layer on a Petri dish and stored in a hot air oven at 100°C for 72 h. A methanolic solution was then prepared using the stressed powder at a concentration of 0.2 mg/mL. One μL of this solution (200 ng/spot) was applied on the plate, and the chromatograms were run as previously described.

2.5.5. Humidity-Induced Degradation.

Pure drug powder was spread as a thin layer on a Petri dish and stored in a humidity chamber (relative humidity = 75%) maintained at 40°C for 72 h. The procedure described in Section 2.5.4 was then followed.

2.5.6. Photolytic Degradation.

Ten mL of the standard working solution was transferred into a 25 mL volumetric flask. The volume was completed to mark with methanol, and the solution was exposed to direct sunlight for 72 h (12 days, 9 : 00–15 : 00). Finally, 1 μL of this solution (200 ng/spot) was applied on the plate, and the chromatograms were run as previously described.

3. Results and Discussion

3.1. Optimization of the Mobile Phase.

In order to optimize the separation of intact drug from its degradation products, drug solutions were spotted and developed in different mobile phases. A system composed of n-hexane-methanol [16 : 4] was found to be of low polarity index (1.4); accordingly, none of the components ascended from baseline. On the other hand, the previous mixture at [4 : 16] was highly polar (polarity index: 5.3) causing the intact drug and its degradation products to coelute with the solvent front. The previous mixture was also tried at a ratio of [10 : 10]; however, this failed to resolve the drug from its degradation products. Acetone toluene [16 : 4] resulted in poor resolution as well. Ethyl acetate-methanol in ratios [17 : 2, 15 : 4, 11 : 8] resulted in satisfactory separation. Consequently, the ratio showing the most adequate retention factor (R_f value) [17 : 2] was chosen. The addition of ammonia was found to enhance peak shape and symmetry leading to compact spots. Therefore, the optimized mobile phase consisted of ethyl acetate : methanol : ammonia [17 : 2 : 1], and LAM was observed at an R_f value of 0.59 ± 0.1. The chamber was saturated with the mobile phase at room temperature for 15 min prior to development. The optimized procedure resulted in well-defined spots with reproducible R_f values (Figure 1).

3.2. Method Validation

3.2.1. Linearity.

Six concentrations of the drug over a concentration range of 2–60 ng/μL corresponding to 10–300 ng/spot were prepared in triplicates and calibration curve for LAM was constructed by plotting the average peak area of the drug as a function of the corresponding concentration. The regression equation was obtained using the least squares regression model. The good linearity of the proposed method was validated by an acceptable correlation coefficient and an insignificant difference from zero for the intercept [16, 17] (Table 1).

3.2.2. Accuracy and Precision.

The accuracy and the intra- and interday reproducibilities for the determination of the drug among its degradation products were tested using 5 replicates at 3 different concentration levels, namely 30,
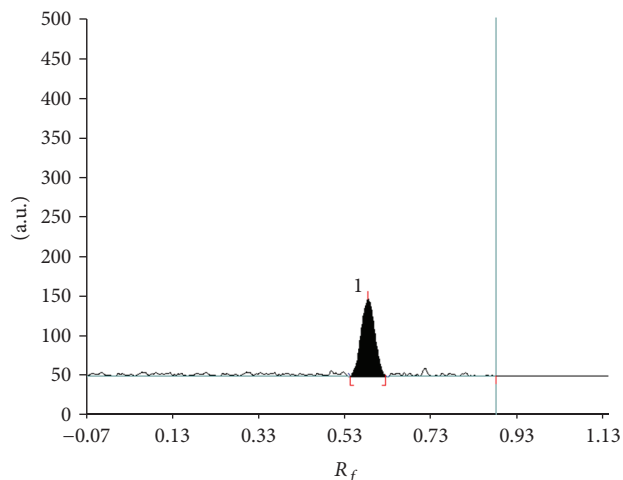

Figure 1: A typical HPTLC chromatogram of LAM scanned at 310 nm (100 ng/spot, R_f = 0.59).

Table 1: Regression and statistical parameters for the determination of LAM using the proposed HPTLC method.

Parameter	Value
Linearity range (ng/spot)	10–300
r	0.9998
a	26.75
b	126.31
S_a	32.77
S_b	1.08
$S_{y/x}$	56.27
t^*	0.82

*Statistical significance of the intercept $t = |a|/S_a$ [16, 17] theoretical value of $t = 2.78$ (at $P = 0.05$ level of significance, number of points = 6).

150, and 250 ng/spot over 3 consecutive days. The proposed method is deemed accurate as warranted by the low values of percentage relative error (Er%) gathered in Table 2. The percentage relative standard deviation (RSD%) values did not exceed 0.91% indicating acceptable intra- and interday precisions for the determination of LAM in presence of its degradants by the proposed method (Table 2).

3.2.3. Limits of Detection (LOD) and Quantification (LOQ).

For the estimation of LOD and LOQ, blank methanol was spotted 6 times following the same parameters described for the construction of the calibration curve, and the noise detected at the same R_f value of LAM was computed. The signal-to-noise ratio 3 : 1 [18] was considered as the LOD and was found to be 2.34 ng/spot. LOQ was calculated as 7.81 ng/spot using a 10 : 1 signal-to-noise ratio [18].

3.2.4. Selectivity.

The peak purity of LAM was assessed by comparing the spectra of the standard and sample solutions at 3 different levels, namely, peak start (S), peak apex (M), and peak end (E) positions of the spot, that is, r (S, M) and r

TABLE 2: Intra- and interday precisions and accuracy of the proposed HPTLC method for the determination of LAM.

Frequency of analysis	Concentration taken (ng/spot)	Concentration found $\pm$ SD* (ng/spot)	RSD (%)	Er (%)
	30	29.94 $\pm$ 0.27	0.91	−0.21
Intraday	150	150.05 $\pm$ 1.03	0.68	0.03
	250	250.26 $\pm$ 0.59	0.24	0.11
	30	30.06 $\pm$ 0.15	0.50	0.19
Interday	150	149.55 $\pm$ 1.15	0.77	−0.30
	250	250.32 $\pm$ 1.95	0.78	0.13

*Values are the mean of 5 experiments.

TABLE 3: Statistical evaluation of the results obtained by the proposed and reference methods for the determination of LAM in tablets.

Pharmaceutical preparation	Proposed method			Reference method[d]				
	Mean $\pm$ SD[a]	RSD%	$S^{2\,b}$	Mean $\pm$ SD[a]	RSD%	$S^{2\,b}$	t^c	F^c
Lamictal 100 tablets	100.08 $\pm$ 0.82	0.82	0.67	99.62 $\pm$ 0.74	0.74	0.55	0.94	1.22
Lamictal 50 tablets	99.58 $\pm$ 0.69	0.70	0.47	100.14 $\pm$ 0.50	0.50	0.25	1.47	1.88
Lamictal 25 tablets	99.68 $\pm$ 0.62	0.62	0.38	99.85 $\pm$ 0.83	0.83	0.69	1.89	1.82
Lamotrigine 100 tablets	99.80 $\pm$ 0.58	0.58	0.34	99.85 $\pm$ 0.46	0.46	0.21	0.13	1.62
Lamotrigine 50 tablets	99.91 $\pm$ 0.74	0.74	0.55	100.01 $\pm$ 1.15	1.15	1.32	0.17	2.40
Lamotrigine 25 tablets	99.63 $\pm$ 0.76	0.76	0.58	99.41 $\pm$ 0.96	0.97	0.93	0.39	1.60
Leptrogine 200 tablets	99.77 $\pm$ 0.58	0.58	0.34	99.39 $\pm$ 1.04	1.05	1.08	0.73	3.20
Leptrogine 25 tablets	99.62 $\pm$ 0.82	0.82	0.67	99.45 $\pm$ 0.80	0.80	0.64	0.36	1.05
Larogen 100 tablets	99.52 $\pm$ 1.38	1.39	1.90	99.76 $\pm$ 0.85	0.85	0.73	0.34	2.60

[a]Mean %R $\pm$ SD of 5 experiments.
[b]Variance (S^2).
[c]The theoretical t and F values, 2.31 and 6.39, respectively at $P = 0.05$.
[d][9].

(M, E) were also not less than 0.999 in all cases. In addition, LAM was detected at the same R_f value in both solutions.

3.3. Assay of Tablets. A single spot at $R_f = 0.59 \pm 0.1$ was observed in the chromatogram of the drug samples extracted from 4 different commercially available products including the brand and 3 generic products. There was no interference from the excipients commonly present in the tablet matrix. Results obtained were precise as seen by the low values of RSD%, and they were also in good agreement with the label claim as seen by the satisfactory % recoveries. It may therefore be inferred that degradation of the drug has not occurred in the marketed formulations that were analyzed by this method. Results obtained by the proposed method were statistically compared with those of a reference method [9]. The developed assay revealed no significant difference in terms of accuracy (t-test) and precision (F-test) at the 95% confidence level (Table 3). The good performance of the method suggests its suitability for routine stability testing and assay of LAM in tablet form.

3.4. Accelerated Degradation of LAM. The ICH Q1A guidelines provide useful definitions and general comments about forced degradation studies. However, details concerning the scope, timing, and best practice are either general or mainly lacking [15, 19, 20]. Moreover, the information on the basic steps to be followed for the development of stability-indicating methods is neither provided in other regulatory guidelines nor in pharmacopoeias [19, 20]. Generally, 5–20% degradation is a common consensus for stress studies because overstressing may result in the complete destruction of the drug or may lead to further degradation of the relevant primary degradants [19]. As a result, the stress conditions were varied and finally adjusted in order to degrade approximately 5–20% of LAM. A stability-indicating HPLC method for its determination was recently published [11]. In that work, LAM did not undergo significant degradation under photolytic or thermal stress. Thus, attempts in this work were directed towards studying its behavior under modulated experimental stress conditions. The proposed method offers substantial advantages over previously published HPTLC methods since it represents a more sensitive, stability-indicating assay, where detection was achieved at a more selective wavelength. However, though HPTLC offers a more economic, easily handled substitute to HPLC, the latter technique has a wider scope of applications and is generally more appealing to several laboratories. The chromatogram of the base-degraded sample showed 2 additional peaks besides the analyte's peak (Figure 2), while that of the acid-degraded sample showed 2 extra peaks besides those produced by alkaline hydrolysis (Figure 3). In addition, the drug's peak area in the treated samples was less than that of the standard's peak (200 ng/spot) indicating that LAM undergoes partial degradation under both acidic and basic

TABLE 4: % Recoveries of LAM and R_f values of detected peaks under different stress conditions.

Condition	(%) Recovery	R_f value of detected peaks
Acid hydrolysis (5 N, 80°C, 2 h)	85.25	0.01, 0.02, 0.06, 0.1
Base hydrolysis (2.5 N, 80°C, 2 h)	77.84	0.01, 0.1
Neutral hydrolysis (80°C, 72 h)	95.91	—
Hydrogen peroxide (30%, 80°C, 4 h)	87.20	0.03, 0.5
Dry heat (100°C, 72 h)	99.22	—
Humidity (75% RH, 40°C, 72 h)	99.14	—
Daylight (72 h)	99.92	—

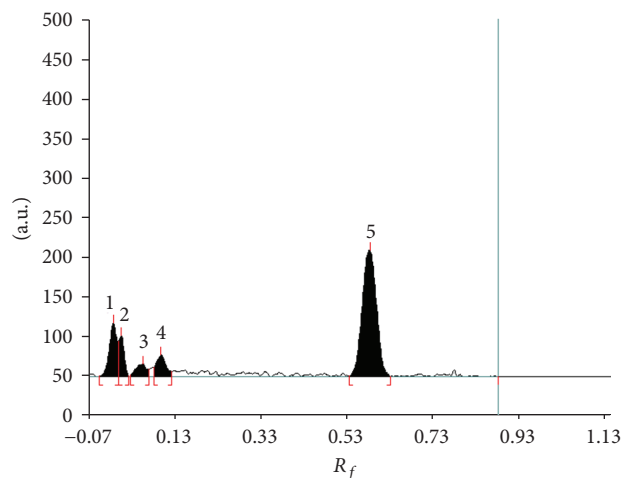FIGURE 3: HPTLC chromatogram of the acid-induced degradation of LAM (200 ng/spot).

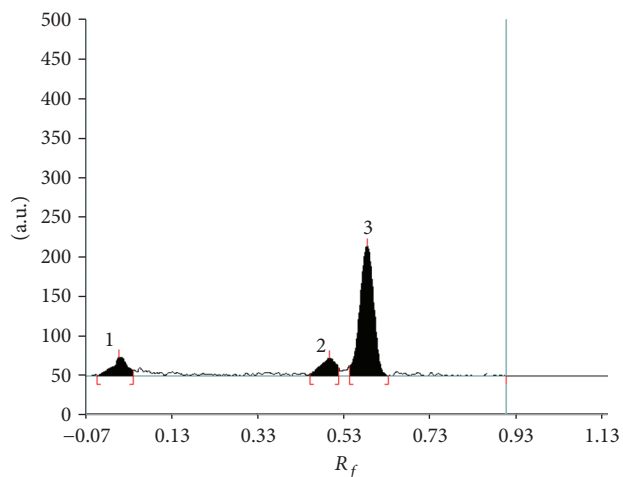FIGURE 2: HPTLC chromatogram of the base-induced degradation of LAM (200 ng/spot).

FIGURE 4: HPTLC chromatogram of the hydrogen peroxide-induced degradation of LAM (200 ng/spot).

4. Conclusion

According to the ICH guidelines, the safety, quality, and/or efficacy of a drug substance is liable to inadequate storage conditions. Therefore, the need for stability-indicating methods appears indispensable to establish the stability and purity profiles of a drug substance. In this work, LAM was exposed to 7 different stress conditions among which considerable degradation occurred in acidic, basic, and oxidative media. LAM appears more sensitive to alkaline degradation. Whereas heat, humidity, and light had no effect on LAM. The proposed HPTLC method was capable of quantifying low levels of LAM and effectively resolving it from forced degradation impurities. The developed HPTLC assay was also applied to the analysis of tablets, and the ensuing results showed no statistically significance differences from those obtained by a reference method.

conditions. R_f values of the detected peaks and % recoveries of LAM remaining after different degradation processes were calculated and listed in Table 4. According to the results complied in that table, the drug is more susceptible towards hydrolysis in a basic medium than in an acidic one. Oxidative degradation resulted seemingly in the generation of degradation products different from those obtained after acid or alkaline-induced hydrolysis (Figure 4). Other degradation pathways had no significant effect on LAM stability except for neutral hydrolysis which showed a subtle potential. In all forced degradation studies, the purity of the drug's spot was confirmed by the high values of r (S, M) and r (M, E) obtained through the application of the chromatographic peak purity tool.

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