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Validated Spectrofluorimetric Method for the Determination of Lamotrigine in Tablets and Human Plasma Through Derivatization with *o*-phthalaldehyde

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Abstract A sensitive, simple and selective spectrofluorimetric method was developed for the determination of Lamotrigine (LMT) in pharmaceutical formulations and biological fluids. The method is based on reaction of LMT with *o*-phthalaldehyde in presence of 2-mercaptoethanol in borate buffer of pH 9.8 to yield a highly fluorescent derivative that is measured at 448 nm after excitation at 337 nm. The different experimental parameters affecting the development and stability of the reaction product were carefully studied and optimized. The fluorescence-concentration plot was rectilinear over the range of 0.1–1.0 µg ml^{-1} with lower limit of detection (LOD) 0.02 µg ml^{-1} and limit of quantification (LOQ) 0.06 µg ml^{-1} respectively. The proposed method was successfully applied to the the analysis of commercial tablets. Statistical comparison of the results obtained by the proposed and reference method revealed no significant difference in the performance of the two methods regarding the accuracy and precision respectively. The proposed method was further extended to the *in-vitro* and *in-vivo* determination of the drug in spiked and real human plasma. The mean percentage recoveries in spiked and real human plasma (n=3) were 95.78±1.37 and 90.93±2.34 respectively. Interference arising from co-administered drugs was also studied. A proposal for the reaction pathway with *o*-phthalaldehyde was postulated.

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Introduction

Lamotrigine, [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] (Fig. 1), a new-generation antiepileptic drug of the phenyltriazine class, is chemically unrelated to existing antiepileptic drugs.

Lamotrigine is effective in treating a wide spectrum of childhood epilepsies and it is being prescribed to a greater extent to treat children with severe epilepsy [1–3]. Plasma concentrations of lamotrigine were reduced ca. two to three folds during co-medication with oral contraceptives, known inducers of UGT [4]. Up till now no official monograph has been reported for the determination of LMT in pharmaceuticals. Therefore, it is very imperative to develop simple and suitable analytical method for the determination of lamotrigine in bulk and in formulations.

Few methods have been published for the analysis of LMT either in pharmaceuticals or in biological fluids including; spectrophotometry [5], adsorptive stripping voltammetry [6, 7], high-performance liquid chromatography [8–15] and capillary electrophoresis [16, 17]. For pharmacokinetic studies, suitable sensitive method that allows an accurate measurement of low concentration of lamotrigine in biological matrices is needed. For this reason our target was to develop a simple, sensitive and specific spectroflourimetric for the determination of lamotrigine in patient plasma treated with the drug.

O-Phthalaldehyde (OPA), in combination with a thiol compound such as 2-mercaptoethanol, is widely utilized as a fluoresorcent derivatizing agent for amino compounds.
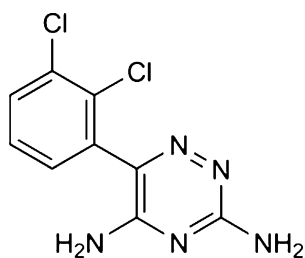
Fig. 1 Structural formula of lamotrigine

Several pharmaceutical compounds have been determined through this approach [18–22].

Fluorimetry, because of its great sensitivity find wide application in quantitative studies of rates of degradation, metabolism and excretion of drugs where other analytical techniques are not sufficiently sensitive [23]. In summary the two main advantages of fluorescence analysis are that it is capable of measuring much lower concentrations than spectrophotometric analysis, and it is potentially more selective because both the excitation and emission wavelength can be varied. Fluorimetric analysis is sensitive up to 10^{-8}–10^{-9} M.

In the present work, the specrofluorimetric method is based on the derivatization reaction of the amino group of the drug with o-phthalaldehyde. The high sensitivity, together with the recognized advantage of the relatively low cost of spectrofluorimetric instrumentation allowed the feasible application. The proposed method was applied for the determination of lamotrigine in its tablets without any interference from the excipients, and also applied in human plasma.

Experimental

Apparatus

The fluorescence spectra and measurements were performed on a Perkin-Elmer UK model LS 45 luminescence spectrometer, equipped with a 150 W Xenon arc lamp, grating excitation and emission monochromators for all measurements and a Perkin-Elmer recorder. Slit widths for both monochromators were set at 10 nm. A 1-cm quartz cell was used.

For the reference HPLC method: separation was performed with a Perkin Elmer™ Series 200 chromatograph equipped with a Rheodyne injector valve with a 20 μL loop and a UV/VIS detector. Total Chrom Workstation was applied for data collecting and processing (MA, USA). A C18 column (250 mm × 4.6 mm, 5 μm particle size) was used as a stationary phase and a mixture of phosphate buffer pH 4.5-acetonitrile (60:40, v/v) was used as a mobile

phase, at flow rate of 1 ml min^{-1}. The detection was carried out at 260 nm.

Materials and reagents

All chemicals were of Analytical Reagent Grade. Lamotrigine (LMT) reference standard was kindly provided by Glaxo SmithKline Company.

- Lamotrine tablets labeled to contain 25 mg LMT per tablet, Batch # 50607, product of S.P.I. for Apex Pharma S.A.E. Lamical tablets™ labeled to contain 50 mg LMT per tablet, Batch #R227153 and Lamical tablets™ each contains 100 mg LMT per tablet Batch # R307632. Both are products of Glaxo SmithKline Company. All pharmaceutical preparations were purchased from commercial sources in the local Pharmacy.
- Orthophthalaldehyde was purchased from Sigma (St. Louis, MO, USA). A stock solution containing 1 mg ml^{-1} was freshly prepared in methanol.
- 2-mer-captoethanol was also purchased from Sigma (St. Louis, MO, USA). A stock solution of 2-mercaptoethanol containing 0.05% (v/v) was freshly prepared in ethanol.
- Borate buffer solutions (0.2 M) was prepared by mixing appropriate volumes of 0.2 M boric acid and 0.2 M NaOH and adjusting pH to 9.8 using pH Meter.
- Methanol and diethylether (Merck, Darmstadt, Germany).
- Sodium hydroxide (BDH, UK) 1 M aqueous solution.
- Plasma samples were obtained from Mansoura University Hospital, Mansoura, Egypt, and were kept frozen until use after gentle thawing.

Preparation of stock and standard solutions

A stock solution of LMT was prepared by dissolving 20.0 mg of LMT in 100 ml methanol. This solution was further diluted with the same solvent as appropriate to obtain the working concentrations. The standard solution was stable for at least 7 days when kept in the refrigerator.

General procedure

Aliquots of LMT standard solutions covering the working concentration ranges (0.1–1.0 μg ml^{-1}); final concentration were quantitatively transferred into a series of 10 ml volumetric flasks. To each flask 2 ml of borate buffer (pH 9.8) followed by 0.3±0.1 ml of 2-mercaptoethanol solution (0.05%) were added and mixed well. The solutions were allowed to stand for 10 min. Then, 0.4 ml±0.1 ml of o-phthalaldehyde was added to each flask. The reaction

mixture was allowed to stand for 35±5 min. Then, each flask was completed to the volume with methanol. The fluorescence intensity of the reaction product was measured at 448 nm after excitation at 337 nm. The corrected fluorescence intensity was plotted *vs* the final concentration of the drug (µg ml^{-1}) to get the calibration graph. Alternatively, the corresponding regression equation was derived.

Applications

Procedure for commercial tablets

Twenty tablets were weighed and pulverized well. A weighed quantity of the powder equivalent to 20.0 mg of LMT was transferred into a small conical flask, and extracted three successive times each with 30 ml of methanol. The extracts were filtered into 100 ml volumetric flask; the conical flask was washed with few milliliters of methanol. The washing was passed into the same volumetric flask, and then completed to volume with same solvent. Aliquots covering the working concentration range were transferred into 10 ml volumetric flasks. The procedures described under "General procedure" were performed. The nominal content of the tablets was calculated either from the calibration curve or using the corresponding regression equation.

Procedure for spiked human plasma

One ml aliquots of plasma were transferred into a series of centrifuge tubes. Aliquots of LMT working solution were added so that its final concentration is in the range of 0.6–1 µg ml^{-1} and 0.5 ml of 1 M NaOH was added. The samples were mixed well using a vortex mixer, then extracted with 3×5 ml of diethylether for 2 min, then centrifuged at 3,500 rpm for 10 min. The resulting supernatant was evaporated to dryness under nitrogen stream at ambient temperature. The residue was reconstituted in 2 ml of methanol. The "General procedure" was performed. The nominal content of the drug was determined using the corresponding regression equations.

Procedure for real human plasma

Blood samples were withdrawn from an epileptic patient (male, 12– years old). The patient is treated with lamotrigine daily dose 100 mg divided into two equal doses. Five ml blood sample was withdrawn after 2 h of oral administration of Lamictal^{TR} tablets (50 mg/ tablet), 4 ml of citrate solution were added, and centrifuged at 3,500 rpm for 15 min. to get about 3.0 ml of plasma. The procedure described for spiked human plasma was applied. The nominal content of LMT was determined using the following equation [24]:

$$Recovery_{in\,vivo} = Delivery_{in\,vivo} \times Recovery_{in\,vitro}/Delivery_{in\,vitro}$$

This means that % recovery for LMT in real human plasma

$$= \frac{\text{Concentration of the drug in real plasma} \times \% \text{ recovery in spiked plasma}}{\text{Concentration of the drug in spiked plasma}}$$

Results and discussion

In the present study lamotrigine was found to react with *o*-phthalaldehyde in borate buffer of pH 9.8 via its amino group yielding a strong fluorescent reaction product at 448 nm after excitation at 337 nm (Fig. 2).

The present spectrofluorimetric method is highly specific for the determination of LMT in presence of its major metabolite (approx. 75 to 90%) which is 2-*N*-glucuronide conjugate, trace amounts is metabolized as the 2-*N*-methyl metabolite and less than 5% as the other metabolites [25], these metabolites are deprived from the primary amine that is essential for the reaction.

Optimization of the reaction condition

The different experimental parameters affecting the development of the reaction product and its stability were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors include: pH, volume of buffer, volume of the 2-mercaptoethanol, volume of *o*-phthalaldehyde and the reaction time.
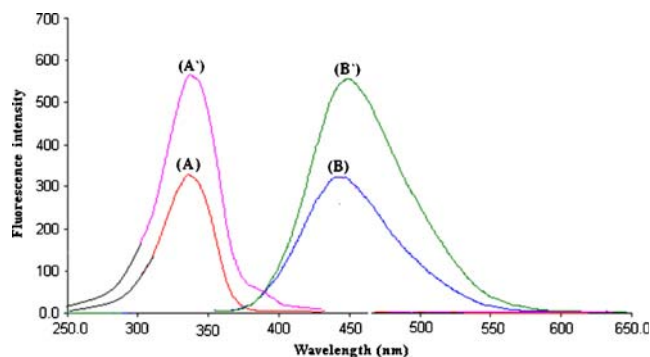
Fig. 2 Fluorescence spectra of the reaction product of: (A, B) Blank *o*-phthaldehyde at pH 9.8. (A`, B`) Lamotrigine (1.0 µg ml^{-1}) with *o*-phthalaldehyde at pH 9.8. A,A`) excitation spectra B,B`) emission spectra
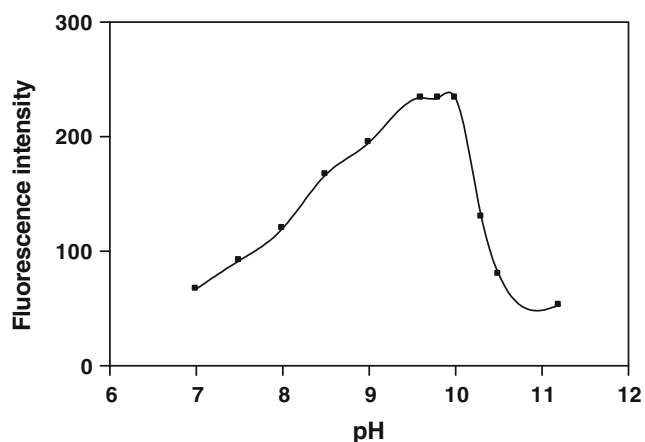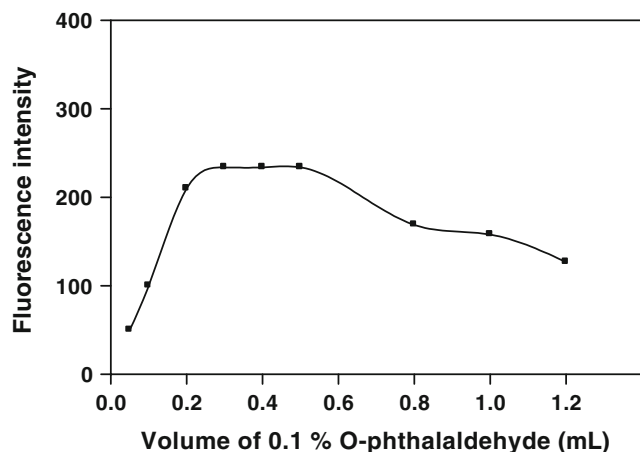
Fig. 3 Effect of pH on the fluorescence intensity of the reaction product of lamotrigine (1.0 μg ml^{-1}) with *o*-phthalaldehyde
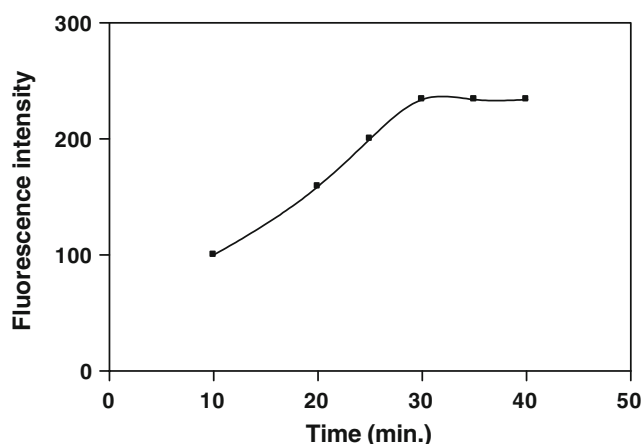
Effect of pH

The influence of pH on the fluorescence intensity of the reaction product was investigated using 0.2 M borate buffer over the pH range 7–11.2. Maximum fluorescence intensity was obtained at pH 9.8±0.2, after which the fluorescence intensity of the reaction product gradually decreased. Therefore, pH 9.8 was chosen as the optimum pH for such study (Fig. 3).

Other buffers having the same pH value such as phosphate and hexamine were attempted and compared with 0.2 M borate buffer. Borate buffer was found to be superior to others as revealed by the high net fluorescence intensity.

Also, different volumes of borate buffer, pH 9.8 were investigated. It was found 1.6±0.4 ml was sufficient to produce maximum fluorescence intensity of the reaction product.

Fig. 5 Effect of time of on the fluorescence intensity of the reaction product of lamotrigine (1.0 μg ml^{-1}) with *o*-phthalaldehyde at pH 9.8

Effect of volume of 2-mercaptoethanol

The influence of volume of 2-mercaptoethanol was studied using different volumes of 0.05% solution of the reagent. It was found that, increasing the volume of the reagent produces a proportional increase in the fluorescence intensity of the reaction product up to 0.2 ml. However, no further increase in the fluorescence intensity was observed upon increasing the volume of the reagent up to 0.4 ml, after which further increase produces a gradual decrease in the fluorescence intensity. Therefore, 0.3±0.1 ml of 0.05% of 2-mercaptoethanol solution was chosen as the optimal volume of the reagent. 2-Mercaptoethanol was added to stabilize the reaction product of LMT with *o*-phthalaldehyde.

The reaction time after addition of 2-mercaptoethanol is an essential part of the experiment. It was found that after 10 min, the reaction product reaches the highest fluorescence intensity then remains constant.

Fig. 4 Effect of volume of *o*-phthalaldehyde (0.1%) on the fluorescence intensity of the reaction product of Lamotrigine (1.0 μg ml^{-1}) at pH 9.8

Table 1 Performance data of the proposed method

Parameter	Proposed method
Concentration range (μg ml^{-1})	0.1-1.0
Limit of detection (LOD) (μg ml^{-1})	0.02
Limit of Quantification (LOQ) (μg ml^{-1})	0.060
Correlation coefficient (r)	0.9997
Slope	232.33
Intercept	-0.77
$S_{y/x}$ = standard deviation of the residuals	2.305
S_a = standard deviation of the intercept of regression line.	1.422
S_b = standard deviation of the slope of regression line	2.974
% RSD	1.05
% Error = RSD% / $\sqrt{n}$	0.47

Table 2 Application of the proposed and reference methods to the determination of lamotrigine in pure form[a]

Parameters	Proposed method	Reference method [15]
No of experiments	5	3
Mean found, %	100.004	98.71
±SD	± 1.05	± 0.68
Variance	1.103	0.46
Student's t-value	1.89 (2.45)	
Variance ratio F-test	2.39 (6.94)	

N. B.:

[a] Values between parenthesis are the tabulated t and F values respectively, at $p=0.05$ [27]

Effect of volume of o-phthalaldehyde

The influence of the different volumes of o-phthalaldehyde was studied using different volumes of 0.1% solution of the reagent. It was found that, increasing the volume of the reagent produces a proportional increase in the fluorescence intensity of the reaction product up to 0.3 ml. However, no further increase in the fluorescence intensity was observed upon increasing the volume of the reagent up to 0.5 ml, after which further increase produces a gradual decrease in the fluorescence intensity. Therefore, 0.4±0.1 ml of 0.1% of o-phthalaldehyde solution was chosen as the optimal volume of the reagent (Fig. 4).

Effect of reaction time

Different time intervals were tested and it was found that increasing the reaction time resulted in a gradual increase in the fluorescence intensity of the reaction product up to 30 min after which fluorescence intensity remains constant up to 40 min. Therefore allowing the reaction mixture to stand for 35±5 min is adequate for maximum fluorescence intensity. (Fig. 5)

Different temperature settings were investigated with constant heating time. Increasing the temperature was found to produce a gradual decrease in the fluorescence intensity of the reaction product. Therefore, the reaction was carried out at room temperature. Regarding the stability of the produced derivative, it was found to be stable for at least 2 h.

Table 3 Application of the proposed and reference methods to the determination of lamotrigine in dosage forms

Preparation	Proposed method		Reference method [15]
	Amount taken (µg ml^{-1})	% recovery	
1- Lamotrine tablets^{TM} (25 mg LMT / tablet)	0.2	102.00	100.53
	0.4	100.25	100.95
	0.8	99.63	97.99
	1.0	101.40	
Mean found, %		100.82	99.82
±SD		± 1.08	± 1.6
Student's t t-value		0.99 (2.57)	
Variance ratio F-test		2.19 (9.55)	
2- Lamictal tablets^{TM} (50 mg LMT / tablet)	0.2	101.50	100.24
	0.4	98.75	97.91
	0.8	100.38	98.14
	1.0	100.00	
Mean found, %		100.16	98.76
±SD		± 1.13	± 1.28
Student's t t-value		1.54 (2.57)	
Variance ratio F-test		1.28 (9.55)	
3- Lamictal tablets^{TM} (100 mg LMT / tablet)	0.2	98.93	101.18
	0.4	100.08	99.86
	0.8	98.90	99.34
	1.0	98.37	
Mean found, %		99.07	100.13
±SD		± 0.72	± 0.95
Student's t t-value		1.69 (2.57)	
Variance ratio F-test		1.74 (9.55)	

N. B.:

Figures between parentheses are the tabulated t and F values respectively, at $p=0.05$. 27)

1 Product of S.P.I for Apex Pharma S.A.E. Batch # 50607

2 Product of GlaxoSmithKline Company. Batch # R227153

3 Product of GlaxoSmithKline Company. Batch #R307632

Effect of diluting solvent was also tested using different solvents, *viz* water, methanol, acetone, dimethylsulfoxide and dimethylformamide. Of all the solvents studied, the highest fluorescence intensity was obtained upon using methanol.

Effect of different surfactants

Influence of different surfactants on the fluorescence intensity of the reaction product was studied; hopefully a significant increase in the fluorescence intensity could be achieved. In order to investigate the influence of the surfactant nature on the fluorescence enhancement, various surfactant were used such as sodium dodecyl sulfate (SDS) (0.015 M) (anionic surfactant), cetrimide (0.01%) (cationic surfactant), myristyl ammonium propane sulphonate (MAPS) (0.01%), Tween 80 (0.02%), β-cyclodextrin (0.05%) methylcellulose (MC) (0.05%) and Triton X 100 (0.05%) were investigated as non ionic surfactants.

Different surfactants such as SDS, β-cyclodextrin and MAPS caused a significant decrease in the fluorescence intensity of LMT reaction product. On the other hand MC caused a slight decrease in the fluorescence intensity of

LMT reaction product. Moreover, increasing the concentrations of Triton X 100 resulted in a gradual decrease in the fluorescence intensity of LMT reaction product. Therefore, no surfactant was used in this study. However, the specific concentration of each surfactants was preliminary chosen, in an attempt to enhance the fluorescence characteristics of LMT reaction product without causing over background fluorescence of the blank reading.

Validation of the proposed methods

The validity of the methods was tested regarding; linearity, specificity, accuracy, repeatability and precision according to ICH Q2 (R1) recommendations [26].

Linearity

By using the above procedures, linear regression equation was obtained. The regression plot showed that there was a linear dependence of the fluorescence intensity on the concentration of the drug over the ranges cited in Table 1.

Table 4 Validation of the proposed method for the determination of lamotrigine in dosage forms

Preparation	% recovery (repeatability)		% Recovery intermediate precision	
	$(0.8 \mu g ml^{-1})$	$(1.0 \mu g ml^{-1})$	$(0.8 \mu g ml^{-1})$	$(1.0 \mu g ml^{-1})$
1- Lamotrine tablets[TM] (25 mg LMT / tablet)	100.36	98.91	98.58	101.15
	99.64	100.24	98.93	100.58
	100.91	99.63	99.61	99.71
Mean found, %	100.30	99.59	99.04	100.48
±SD	±0.64	±0.67	±0.52	±0.73
% RSD	0.64	0.67	0.53	0.73
% Error	0.37	0.39	0.31	0.42
2- Lamictal tablets[TM] (50 mg LMT / tablet)	101.10	99.73	100.82	99.63
	100.69	100.85	101.09	100.37
	99.73	98.52	100.91	99.01
Mean found, %	100.21	99.70	100.94	99.67
±SD	±0.68	±1.16	±0.14	±0.68
% RSD	0.68	1.16	0.14	0.68
% Error	0.39	0.67	0.08	0.39
3- Lamictal tablets[TM] (100 mg LMT / tablet)	100.20	98.37	101.09	98.55
	98.83	99.68	100.93	100.18
	98.35	100.14	99.61	99.82
Mean found, %	99.13	99.40	100.54	99.52
±SD	±0.96	±0.92	±0.81	±0.86
% RSD	0.96	0.92	0.81	0.86
% Error	0.55	0.53	0.47	0.50

[a] Each result is the average of three separate determinations

Linear regression analysis of the data gave the following equations:

$$F = -0.77 + 232.33 \ C \qquad (r = 0.9997)$$

Where F is fluorescence intensity, C is the concentration of the drug ($\mu g \ ml^{-1}$), and r is correlation coefficient.

The limit of quantification (LOQ) and the limit of detection (LOD) were calculated according to ICH Q2 (R1) [26]. The results are abridged in Table 1.

LOQ and LOD were calculated according to the following equations [26]:

$$LOQ = 10\sigma/S$$

$$LOD = 3.3\sigma/S$$

Where

σ   is the standard deviation of the intercept of regression line.
S   is the slope of the calibration curve.

The proposed methods were evaluated for the accuracy as percent relative error (% Er) and the precision as percent relative standard deviation (% RSD) (Tables 1).

Table 5 Application of the proposed method for the determination of LMT in spiked and real human plasma[a]

Sample	Amount added ($\mu g \ ml^{-1}$)	Amount found ($\mu g \ ml^{-1}$)	% recovery
1-Spiked plasma	0.6	0.5750	95.83
	0.80	0.7770	97.13
Intra-day precision	1.00	0.9439	94.39
Mean found, %			95.78
±SD			±1.37
%RSD			1.43
%Error			0.83
Inter-day precision	1.0	0.9800	98.00
	1.0	1.0230	102.30
	1.0	1.029	102.90
Mean found, %			99.65
±SD			±3.05
%RSD			3.06
%Error			1.77
2-Real plasma	0.50	0.4522	90.44
	0.50	0.4435	88.87
	0.50	0.4674	93.48
Mean found, %			90.93
±SD			±2.34
%RSD			2.57
%Error			1.49

[a] Each result is the average of three separate determinations
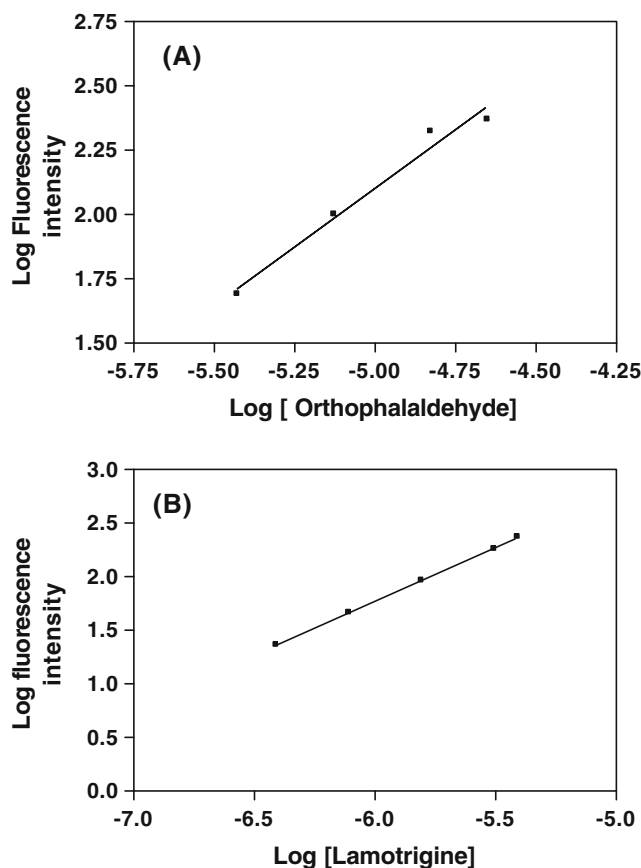
Fig. 6 Limiting logarithmic plots for the molar ratio. a Log fluorescence intensity *vs*. Log [*o*-phthalaldehyde]. b Log fluorescence intensity *vs*. log [Lamotrigine]

The validity of the methods was proved by statistical evaluation of the regression lines, using the standard deviation of the residuals ($S_{y/x}$), the standard deviation of the intercept (S_a) and standard deviation of the slope (S_b). The results are abridged in Table 1. The small values of the figures point out to the low scattering of the calibration points around the calibration line and high precision.

Accuracy

To test the validity of the proposed methods they were applied to the determination of authentic sample of LMT over the working concentration range. The results obtained were in good agreement with those obtained using comparison method [15]. Statistical analysis using the Student's t-test and the variance ratio F-test [27] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 2). An HPLC reference method was adopted using a mixture of phosphate buffer pH 4.5-acetonitrile (60:40, v/v) as a mobile phase, at flow rate of 1 ml min^{-1}. The detection was carried at 260 nm [15].

Pharmaceutical applications

The proposed methods were then applied to the determination of LMT in its tablets. The methods were tested for selectivity, accuracy, precision and robustness according to ICH Q2 (R1) recommendations.
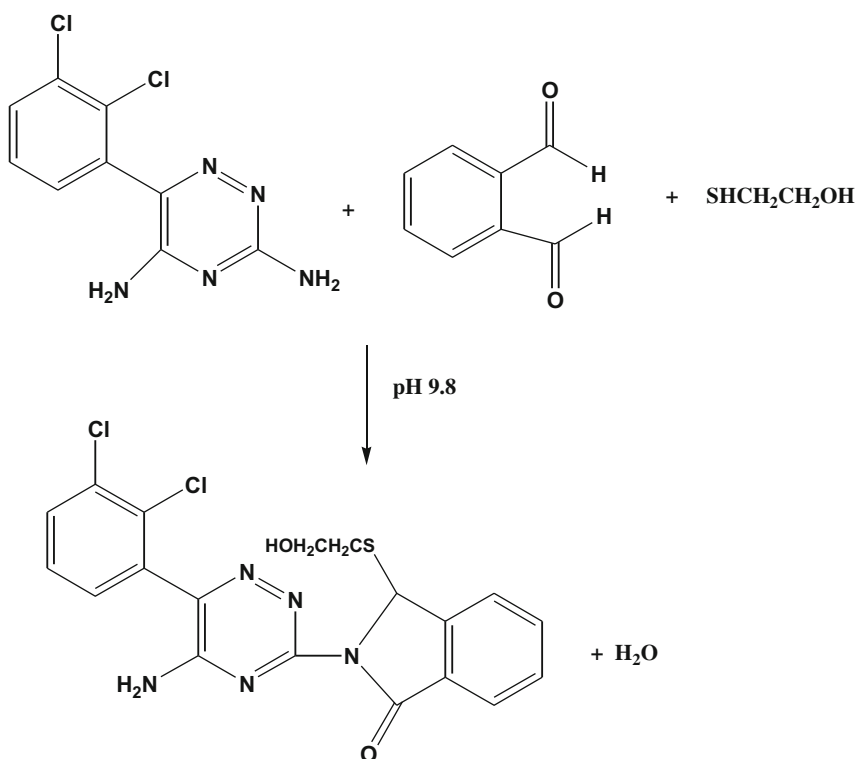
Selectivity

Lamictal™ and Lamotrine™ Tablets contain lamotrigine and the following non-medicinal ingredients: cellulose, lactose, magnesium stearate, povidone, sodium starch glycolate. The selectivity of the method was investigated by observing any interference encountered from these tablet excepients, which did not interfere with the proposed methods.

Accuracy

The results of the proposed methods were statistically compared with those obtained using the reference method [15]. Statistical analysis of the results, using Student's t-test and variance ratio F-test [27] revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (Table 3).

Precision

a. Repeatability

The repeatability was performed by applying the proposed method for the determination of two concentrations of lamotrigine in their dosage forms on three successive times, and the results are listed in Table 4.

b. Intermediate precision

It was performed through repeated analysis of lamotrigine in their dosage forms for a period of three successive days. The results are summarized in Table 4.

Robustness of the method

The robustness of the spectrofluorimetric method adopted is demonstrated by the constancy of the fluorescence intensity with the deliberated minor changes in the experimental parameters such as pH 9.8 ±0.2, change in the volume of 2-mercaptoethanol (0.05%), 0.3±0.1 mL, change in the volume of o-phthalaldehyde (0.1%), 0.4± 0.1 mL, and the reaction time 35±5 min. These minor changes that may take place during the experimental operation, didn't affect the fluorescence intensity of the reaction product.

Scheme 1 Proposal reaction pathway between LMT and O-Phthalaldehyde in presence of 2-mercaptoethanol

Application to human plasma

The high sensitivity of the proposed methods allowed the determination of lamotrigine in biological fluids. Hence, the proposed method was further applied to the *in-vitro* and *in vivo* determination of LMT in spiked and real human plasma.

Following oral ingestion of a 50 mg dose of lamotrigine a mean peak plasma concentration of 2–5 μg ml^{-1} is attained after 3.1 h [25]. Extraction procedure described by Cheng *et al* was applied [14].

Validation of the proposed method for the determination of LMT in human plasma

The proposed method was tested for specificity, accuracy and precision in the analysis of human plasma.

Accuracy

The propsed method was evaluated by determining the acuuracy as the precent error and precision as % RSD, the results are shown in Table 5.

Precision

The within-day precision was evaluated through replicate analysis of plasma samples spiked with different concentrations of the drug as cited in Table 5. The mean percentage recoveries based on the average of three separate determinations were 95.78±1.37 (Table 5).

The inter-day precision was also evaluated through replicate analysis of plasma samples spiked with 1 μg ml^{-1} of the drug on three successive days. The percentage recoveries based on the average of three separate determinations were 99.65±3.05. The results shown in Table 5 are satisfactorily accurate and precise. On the other hand, the % recoveries of lamotrigine in real human plasma were 90.93±2.34 (Table 5).

Effect of co-administered drugs

Interference likely to be introduced from common co-administered drugs was studied. Co-administered drugs such as valproic acid, clonazepam, sertraline, diazepam and acetazolamide did not interfere with the spectrofluorimetric assay since they did not contain primary amines which is essential for the reaction with *o*-pthalaldehyde for yielding a fluorescent reaction product.

Mechanism of the reaction with *o*-pthalaldehyde

The stoichiometry of the reaction between LMT and *o*-pthalaldehyde was studied adopting the limiting logarithmic method. The fluorescence of the reaction product was measured in presence of excess of the drug and the reagent [28]. Plots of log fluorescence versus log [*o*-pthalaldehyde] and log [Lamotrigine] gave two straight lines, the values of their slopes were 0.91 / 1.00 for *o*-pthalaldehyde and LMT, respectively (Fig. 6). Hence, it is concluded that, the reaction proceeds in the ratio of 1:1, confirming that one molecule of the drug condenses with one molecule of *o*-phthalaldehyde. As illustrated in Scheme 1 and by analogy to similar previous report [21]. It is clear that only one amino group of LMT molecule is involved in the reaction with *o*-pthalaldehyde. and hence the molar ratio is 1:1. However, this could be attributed to inactivation of the other amino group by the inductive effect of the two electron withdrawing chlorine atoms.

Conclusion

A sensitive and simple spectrofluorimetric method was developed for the determination of LMT in pharmaceutical preparations, spiked and real human plasma. By virtue of high sensitivity of the proposed fluorimetric method, it could be applied to the determination of LMT in spiked and real human plasma with good accuracy and precision. The proposed method do not require elaborate treatment for the sample or tedious procedure for the extraction. As well as, the method is sensitive enough for the analysis of lower concentration of LMT as low as 0.02 μg ml^{-1}. Furthermore, the proposed method is a good analytical tool for the determination and monitoring of LMT in spiked and real human plasma. Also the proposed method is specific for the determination of lamotrigine in the presence of other co-administered drugs which are devoted from primary amines that is essential for the reaction.

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