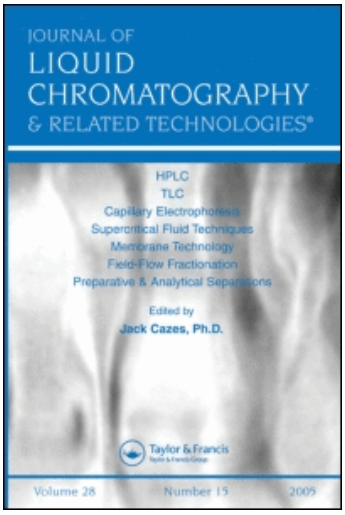

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Pattana Sripalakit[ab]; Arunee Srichaiya[b]; Ratchanok Kandee[b]

[a] Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand [b] Bioequivalent Test Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand

To cite this Article Sripalakit, Pattana , Srichaiya, Arunee and Kandee, Ratchanok(2008) 'Development and Validation of a HPLC Method for a Dissolution Test of Lamotrigine Tablets and its Application to Drug Quality Control Studies', Journal of Liquid Chromatography & Related Technologies, 31: 6, 926 — 939

To link to this Article: DOI: 10.1080/10826070801893615

URL: http://dx.doi.org/10.1080/10826070801893615

Journal of Liquid Chromatography & Related Technologies®, 31: 926–939, 2008

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070801893615

Development and Validation of a HPLC Method for a Dissolution Test of Lamotrigine Tablets and its Application to Drug Quality Control Studies

Pattana Sripalakit,[1,2] Arunee Srichaiya,[2] and Ratchanok Kandee[2]

[1]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand
[2]Bioequivalent Test Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand

Abstract: A high performance liquid chromatographic (HPLC) method suitable for routine determination of lamotrigine in a dissolution medium and tablet formulation has been developed. Chromatographic separation was performed on a Gemini C18 (250 mm × 4.6 mm i.d., 5 μm) column using a mobile phase of 0.05 M $(NH_4)H_2PO_4$-acetonitrile (68:32, v/v, pH 2.68), delivered at a flow rate of 1.2 mL/min and detected by ultraviolet at 265 nm. The method was validated for specificity, linearity, accuracy, and precision. Additionally, the conditions of the dissolution test for lamotrigine tablets were presented by using: paddle at 50 rpm stirring speed; medium volume of 900 mL; temperature at 37 ± 0.5°C; and pH 1.2 HCl solution, pH 4.5 acetate buffer and pH 6.8 phosphate buffer as dissolution media. The proposed analytical and dissolution methods were applied successfully for the quality control of commercial lamotrigine tablets and the comparison of *in vitro* performances of their products.

Keywords: Lamotrigine, HPLC, Validation, Dissolution test, Quality control

Correspondence: Pattana Sripalakit, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand. E-mail: pattana9@excite.com

INTRODUCTION

Lamotrigine [6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine] (Figure 1) is a broad spectrum anti-epileptic drug, chemically different from other anti-convalsants.[1–3] It's mechanism of action seems to be the inhibition of the release of excitatory neurotransmitters (aspartate and glutamate) and also involvement of the blocking of voltage dependent sodium channels.[4] It has been shown that lamotrigine is effective for treatment of partial and generalized tonic clonic seizures as a monotherapy or an adjuvant with other anti-epileptic drugs.[5]

Most methods for lamotrigine analysis in biological samples utilized a high performance liquid chromatographic (HPLC) technique with UV detection.[6–9] Other methods for determination of this drug in human plasma, including gas chromatography-mass spectrometry,[10] radioimmunoassay,[11] and capillary electrophoresis[12] have also been studied. There have been only a few articles published on the analysis of lamotrigine in pharmaceutical formulations using spectrophotometric,[13] thin layer chromatographic (TLC),[13] and HPLC methods.[13,14] In the pharmaceutical industry, dissolution testing is a very important tool in drug development and quality control. However, no analytical methods for dissolution study were found in literature.

The aim of the present study was to develop and validate a simple HPLC assay capable of analyzing samples from dissolution experiments of lamotrigine tablets. The optimization of a dissolution protocol for lamotrigine containing formulations has also been studied since an official monograph on the dissolution of lamotrigine tablets does not exist in any pharmacopoeia. This method was applied to the comparison of *in vitro* dissolution between comparator and generic products. The monograph for lamotrigine assay in tablet formulation has also been described.

EXPERIMENTAL

Chemicals and Reagents

Lamotrigine (99% purity) as the reference standard was provided by Sigma (St. Louis, MO). The chemicals were of analytical reagent grade purchased

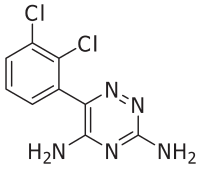

Figure 1. Chemical structures of lamotrigine.

from various sources. All solvents for analysis were of HPLC grade obtained from Lab-scan (Dublin, Ireland). All experiments used purified water obtained from TKA ROS 300 (Niederelbert, Germany).

Apparatus and Analytical Conditions

The HPLC system consisted of a dual plunger pump (LC-10ATVP, Shimadzu, Kyoto, Japan), a UV-Vis detector (SPD-10AVP, Shimadzu) equipped with system controller (SCL-10AVP, Shimadzu), and a Rheodyne (7725) sample injector (Rohnert Park, CA) fitted with a 20-μL sample loop. The chromatographic separations were carried out on Gemini C18 (250 mm ×4.6 mm i.d., 5 μm, 250Å) column (Phenomenex, Torrance, CA) fitted with a refillable guard cartridge packed with C18 (4.0 mm × 3.0 mm i.d., Phenomenex). The mobile phase was 0.05 M $(NH_4)H_2PO_4$-acetonitrile (68:32, v/v) adjusted to pH 2.68 with 40% *ortho*-phosphoric acid. All separations were performed isocratically at a flow rate of 1.2 mL/min and column temperature was maintained at room temperature. The peak areas were determined using a UV detector set at wavelength 265 nm.

Preparation of Standard Stock Solutions and Calibration Curves

The stock solution of lamotrigine was prepared in mobile phase to yield the primary standard solution with a concentration of 1 mg/mL as the base. Secondary standard solutions were prepared by dilution with mobile phase to yield concentrations of 25, 50, 75, 100, 125, and 150 μg/mL. Working standard solutions of lamotrigine at 2.5, 5, 7.5, 10, 12.5, and 15 μg/mL were prepared by dilution of each 100 μL of the secondary standard solution with 800 μL of mobile phase and 100 μL of dissolution medium.

Analytical Method Validation

Specificity

Specificity was assessed by examining the peak interference from the dissolution medium. This was assessed by inspecting chromatograms between blank and spiked medium samples.

Linearity

Calibration curves of six concentrations of lamotrigine (2.5, 5, 7.5, 10, 12.5, and 15 μg/mL) were constructed by linear least squares regression analysis plotting of peak area of lamotrigine versus the lamotrigine concentrations.

Accuracy and Precision

Accuracy and precision were determined from six replicates of each lamotrigine concentration (2.5, 5, 7.5, 10, 12.5, and 15 μg/mL) within the range of the calibration curve.

In Vitro Dissolution Test

Optimization studies were performed using Lamictal® (lamotrigine 100 mg tablet manufactured by Glaxo Wellcome Operations, Hertfordshire, UK). In each experiment, twelve tablets of the product were randomly selected. The dissolution test was performed on a tablet dissolution tester (VK 10–1500, Vankel Industries Inc., Cary, NC) by dissolving each lamotrigine tablets in a rotating vessel consisting of 900 mL of medium. The temperature of the medium was controlled at 37 ± 0.5°C and the vessel was rotated at a speed of 50 rpm for 60 min. Five milliliters of the medium were sampled after 5, 10, 15, 30, 45, and 60 min ($n = 6$), filtered through a 0.45 μm porosity nitrocellulose membrane (Millipore, Bedford, MA). Five milliliters of fresh medium were replaced into each vessel after sampling. A 100 μL of filtered solution was diluted with 900 μL of mobile phase in an Eppendrof tube, before analyzing by HPLC. A 20 μL of each sample was injected into the HPLC system for analysis. The quantity of lamotrigine in the dissolution medium was calculated from a calibration curve obtained from linearity. The results were computed to the % labeled amount of the dissolved active ingredient.

Application to Drug Quality Controls

Dissolution Profiles Comparison

Lamictal® as the reference product and Brand A (generic lamotrigine 100 mg tablet) as the test product have been studied. The procedure for dissolution was done as previously described in the above section. The similarity of the dissolution profiles was determined by the difference factor (f_1) and similarity factor (f_2) calculated as follow:

$$f_1 = \{[\Sigma_{t=1}^{n}|R_t - T_t|]/[\Sigma_{t=1}^{n}R_t]\} \times 100$$

$$f_2 = 50 \times \log\{[1 + (1/n)\Sigma_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100\}$$

in which R_t is the percentage of drug dissolved at each time point of the reference, T_t is the percentage of drug dissolved at each time point of the product, and n is the number of sampling time points.

Assay in Tablet Formulation

Standard Preparation

An accurately weighed quantity of lamotrigine standard was dissolved in mobile phase to obtain a solution having a concentration of 0.010 mg/mL.

Assay Preparation

Each 20 tablets of test and reference product were weighed and then finely powdered. An accurately weighed portion of the powder, equivalent to about 100 mg of lamotrigine, was transferred to a 100 mL volumetric flask. A 50 mL of mobile phase was added. The volumetric flask was shaken by mechanical means for 5 min, sonicated for 10 min, diluted to volume, and mixed. A 100 mL of this solution was transferred to a 10 mL volumetric flask and diluted with mobile phase to volume. A portion of this solution was filtered through a 0.45 μm porosity filter membrane.

Procedure

A 20 μL of each standard and sample preparation (test and reference products) was injected into the HPLC, using the chromatographic system as described in section above. The quantity, in mg, of lamotrigine in the portion of tablets was taken by the formula:

$$10{,}000\, C(r_U/r_S)$$

in which C is the concentration, in mg, of lamotrigine in the standard preparation, and r_U and r_S were the lamotrigine peak areas obtained from the assay and standard preparations, respectively. The results were then computed to %labeled amount.

For the determination of dosage unit uniformity by the weight variation method,[15] 30 units of each test and reference product were selected. Each tablet from 10 units was weighed accurately. Calculation of the content of lamotrigine in each of the 10 tablets, assuming homogenous distribution of the active ingredient, is based on the result of the assay for percentage of labeled amount.

RESULTS AND DISCUSSION

Method Development and Validation

The pH value of the buffer solution or mobile phase had an effect on the UV absorption spectrum of lamotrigine. Lamotrigine has a high molar

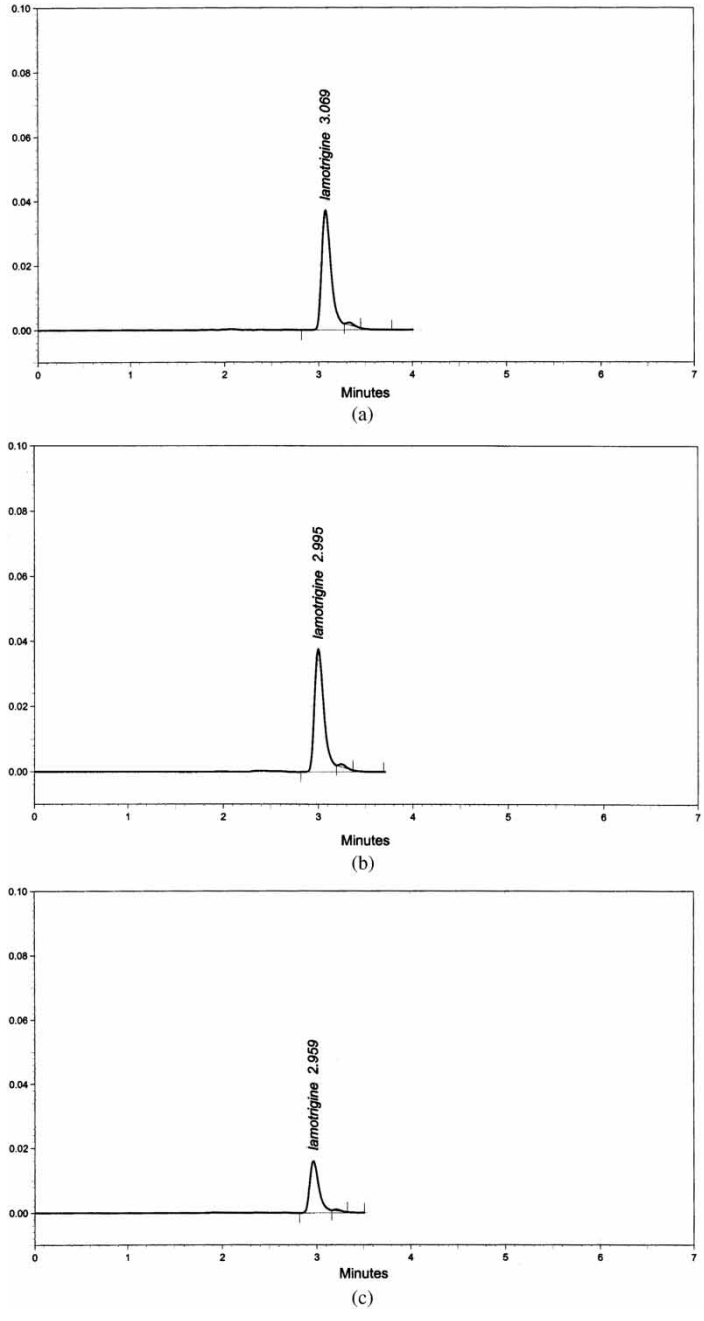

Figure 2. Representative HPLC chromatograms for the analysis of lamotrigine in dissolution medium by the same concentration: (a) pH 1.2 HCl solution, (b) pH 4.5 acetate buffer, and (c) pH 6.8 phosphate buffer.

Table 1. The slope, intercept and regression coefficient of calibration curves obtained from three different dissolution media ($n = 6$)

Media	Slope	Intercept	Regression coefficient
pH 1.2 HCl solution	29278.73	−732.62	0.9991
pH 4.5 Acetate buffer	28953.10	−17.33	0.9996
pH 6.8 Phosphate buffer	29156.71	−4279.54	0.9998

absorbance at 268 and 308 nm in acidic (pH $<$ 4.5) and basic (pH $>$ 6.8) medium, respectively. These phenomena were explained by the degree of ionization and species distribution of lamotrigine under various pH values.[9] The UV wavelength for analysis of lamotrigine in plasma was fluctuation,[6–9] depending on the pH of the mobile phase and the internal standard used. In this study, there are three following dissolution media: (i) pH 1.2 HCl solution; (ii) pH 4.5 acetate buffer; and (iii) pH 6.8 phosphate buffer carried out according to the drug release guidelines.[16]

Table 2. Accuracy and precision of the method for determining the concentration of lamotrigine in three dissolution medium samples ($n = 6$)

Media	Actual concentration (μg/mL)	Detected concentration (Mean ± S.D. μg/mL)	Accuracy (%)	Precision CV (%)
pH 1.2 HCl solution	2.5	2.54 ± 0.02	101.50	0.62
	5.0	5.00 ± 0.04	99.97	0.74
	7.5	7.41 ± 0.15	98.82	2.02
	10.0	9.98 ± 0.90	99.83	0.90
	12.5	12.62 ± 0.08	100.96	0.67
	15.0	14.95 ± 0.23	99.67	1.54
pH 4.5 Acetate uffer	2.5	2.54 ± 0.03	101.44	1.07
	5.0	5.00 ± 0.03	99.93	0.51
	7.5	7.43 ± 0.04	99.06	0.51
	10.0	9.97 ± 0.10	99.73	1.01
	12.5	12.60 ± 0.06	100.79	0.51
	15.0	14.97 ± 0.12	99.77	0.78
pH 6.8 Phosphate buffer	2.5	2.44 ± 0.01	97.48	0.36
	5.0	5.07 ± 0.01	101.39	0.19
	7.5	7.46 ± 0.02	99.41	0.25
	10.0	10.06 ± 0.04	100.58	0.40
	12.5	12.55 ± 0.03	100.43	0.25
	15.0	14.93 ± 0.03	99.51	0.21

It was concluded that $\leq$268 nm was appropriate for the detection of lamotrigine in two of the three media. The analytical performance characteristics have been performed as a minimal requirement, according to category III of the compendial assay procedures.[15] They have been validated in the three different media as follows.

Specificity and Optimization of Chromatographic Conditions

The method demonstrated excellent chromatographic specificity, with no interfering peaks from the mobile phase and dissolution medium observed at the retention time of lamotrigine. Representative chromatograms for the analysis of lamotrigine in pH 1.2 HCl solution, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer are shown in Figures 2A, and 2C, respectively. In the identical concentration, smaller peak areas of lamotrigine were observed in pH 6.8 phosphate buffer, as compared with those in pH 1.2 HCl solution and pH 4.5 acetate buffer. The peak of lamotrigine was at retention time of 3.0 min and analysis could be achieved within 4 min for a total chromatography run. No endogenous peaks from tablet excipients were found to interfere with the elution of the drug.

Linearity

The calibration curves for lamotrigine were linear within the range of 2.5–15 μg/mL. The regression coefficient for all calibration was greater than 0.99. The equations of linear regressions and regression coefficients of the calibration curve for each medium are presented in Table 1.

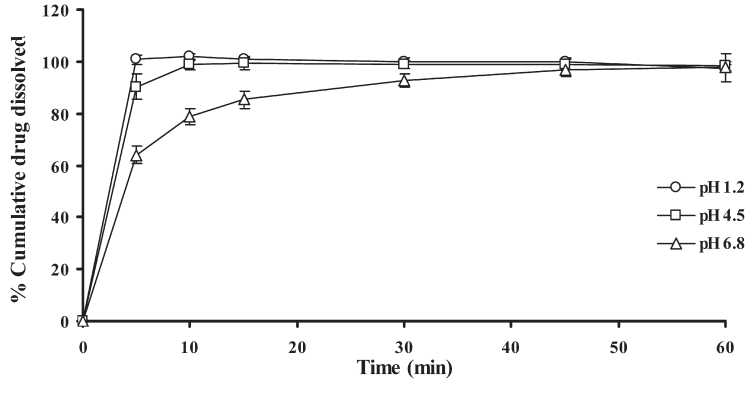

Figure 3. Effect of the pH of the medium on the dissolution rate of lamotrigine tablets (Lamictal®).

Accuracy and Precision

The results of the accuracy and precision determination are shown in Table 2. The accuracies were between 98 and 102% and the intra-day precisions expressed as coefficient of variation were less than 2.02% from the three various dissolution media.

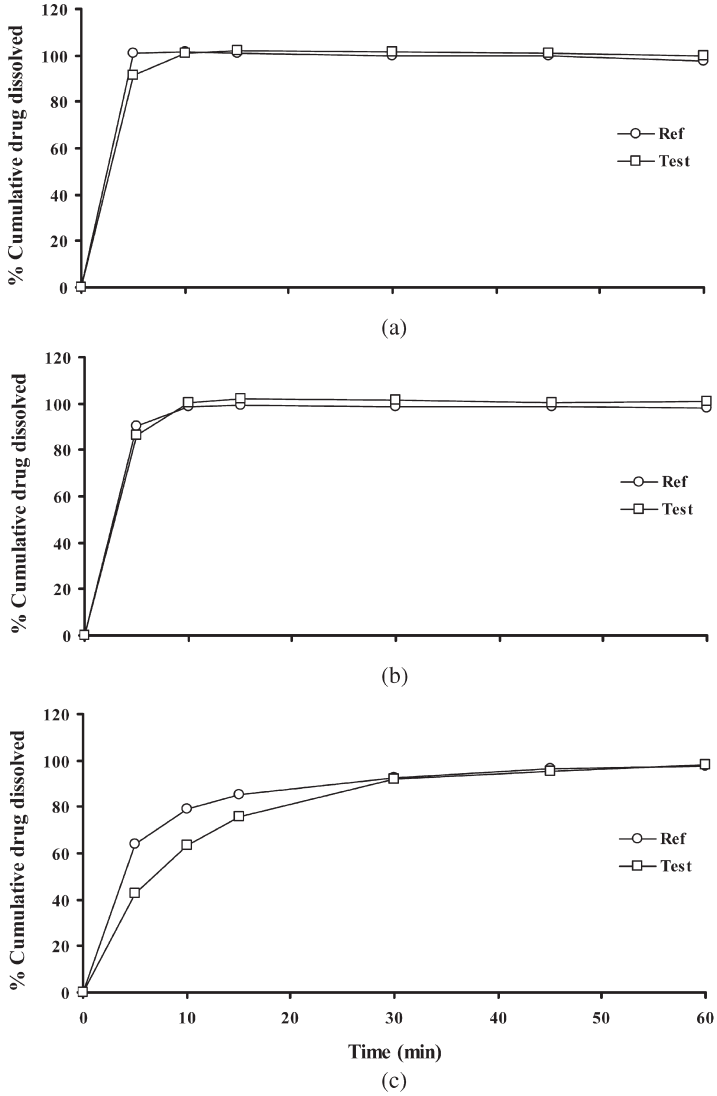

Figure 4. Dissolution profiles of Lamictal® (Ref) and Brand A (Test) in pH 1.2 HCl solution (a), pH 4.5 acetate buffer (b), and pH 6.8 phosphate buffer (c).

In Vitro Dissolution Study

Drug release was carried out in accordance with United States Pharmacopoeia general methods using Apparatus II.[15] After preliminary studies, the dissolution test was conducted at a irring speed of 50 rpm and medium temperature of 37 ± 0.5°C. The dissolution of lamotrigine tablets was evaluated in pH 1.2 HCl solution, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer. The optimal conditions of the dissolution test are present in Table 3, and the dissolution profiles of Lamictal® are summarized in Figure 2. The dissolution rate increases at the more acidic pH, due to higher solubility of the active ingredient. However, the dissolution limits set at ≥85% at 15 min by the pharmacopoeia[15] were fulfilled in all media. It should be noted that in three different media, 100% liberation of lamotrigine was achieved within 60 min of dissolution time.

Application to Drug Quality Controls

Comparison of Dissolution Profiles

Comparison of *in vitro* therapeutic performance of two medical products containing the same active substance is a critical means of assessing the possibility of alternative use of innovator and generic products. The dissolution of two 100 mg lamotrigine tablet formulations (Lamictal® as reference product and brand A as test product) were compared in 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8 (Figures 2, 3, and 4 respectively). Lamotrigine was very rapidly dissolved in all dissolution media, in which the percentages of the dissolved drug at 15 min were more than 85%, except in pH 6.8 phosphate buffer for Brand A (Table 4). The dissolution profiles in all

Table 3. Conditions for dissolution test of lamotrigine tablets

Conditions	Data/Unit
Apparatus	Apparatus II (Puddle)
Test media	i. pH 1.2 HCl solution ii. pH 4.5 Acetate buffer iii. pH 6.8 Phosphate buffer
Volume of the dissolution medium	900 mL
Temperature of the dissolution medium	37 ± 0.5°C
Revolution of the stirrer	50 rpm
Number of tablet in vessel	1 Tablet
Sampling time	5, 10, 15, 30, 45, and 60 min
Sampling volume	5 mL
Medium replacement	⊞ Yes □ No

Table 4. Percentage of mean of drug dissolved in Lamictal® (reference product) and Brand A (test product) in three different media ($n = 12$)[a]

	Dissolution medium					
	pH 1.2 HCl solution		pH 4.5 Acetate buffer		pH 6.8 Phosphate buffer	
Time (min)	Lamictal®	Brand A	Lamictal®	Brand A	Lamictal®	Brand A
5	100.71 (1.95)	91.36 (3.53)	90.35 (5.60)	86.63 (7.43)	64.09 (4.90)	42.47 (9.66)
10	101.75 (1.25)	100.74 (1.91)	98.72 (2.02)	100.60 (2.33)	78.94 (3.80)	63.47 (7.13)
15	100.76 (1.32)	101.88 (2.00)	99.38 (2.25)	101.78 (1.70)	85.34 (3.99)	75.58 (6.60)
30	100.08 (1.15)	101.73 (1.23)	98.92 (1.68)	101.22 (1.62)	92.72 (2.70)	91.82 (5.80)
45	100.01 (1.24)	101.17 (2.10)	98.79 (2.07)	100.64 (2.47)	96.59 (2.57)	95.54 (5.60)
60	97.57 (5.55)	99.93 (2.38)	98.12 (1.67)	100.68 (2.56)	97.64 (1.38)	97.93 (5.81)

[a]Numbers in parentheses represent RSD (%).

Table 5. The difference factor and similarity factors between Lamictal® (reference product) and Brand A (test product) in three different dissolution media

Dissolution Medium	Difference factor (f_1)	Similarity factor (f_2)
pH 1.2 HCl solution	2.78	68.87
pH 4.5 Acetate buffer	2.52	78.26
pH 6.8 Phosphate buffer	9.53	46.74

dissolution media show the difference factors within the range of 0–15 (Table 5). A similarity factor between 50 and 100 suggests that the two dissolution profiles are similar.[16] In this study, the similarity factors were more than 50, except in pH 6.8 phosphate buffer. Therefore, these results did not reflect the similarity of the two curves and, thus, the inequivalence of the *in vitro* performance of the two products.

Assay in Tablet Formulation

Since lamotrigine is not officially available in the pharmacopoeia, we have developed the monograph for analysis of this drug in tablet dosage form. The validated HPLC assay was applied to the production quality control of two products (Lamictal® and Brand A). Tablet content and recovery are presented in Table 6. Recovery close to 100% proves the suitability and accuracy of the proposed method. The content in 10 tablets examined was in the range of 97.78–100.40% and 102.02–103.86%, and the RSD value was 0.96% and 0.70% for Lamictal® and Brand A, respectively. According to the pharmacopoeia the acceptance limit for drug content uniformity is 85–115% with the RSD less than 6%. Uniform distribution of the drug in both formulations was indicated.

Table 6. Content and recovery for analysis of lamotrigine in Lamictal® and Brand A

	Lamictal®	Brand A
Actual content (mg)	100	100
Found content (as %labeled amount)[a]	99.67 ± 1.68	103.09 ± 1.13
Recovery (%)	99.67	103.09

[a]Mean of three replicates ± SD.

CONCLUSION

The presented method has been developed for quantitative determination of lamotrigine content in tablet dosage form and for the samples contained in the *in vitro* dissolution studies. In addition, the dissolution monograph for lamotrigine tablets was also established in three media (pH 1.2 HCl solution, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer) using the paddle (Apparatus II), 50 rpm stirring speed, and 37 $\pm$ 0.5°C. This method demonstrated to be adequate for the quality control of lamotrigine dosage form, since there is no official monograph.

ACKNOWLEDGMENTS

The authors wish to thank the Bioequivalence Test Center, Faculty of Pharmaceutical Sciences, Naresuan University (Phitsanulok, Thailand) for financial support and to all staff of the Bioequivalence Test Center.

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Received October 12, 2007
Accepted November 18, 2007
Manuscript 6216