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Determination of lamotrigine in small volumes of plasma by high-performance liquid chromatography

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Abstract

Lamotrigine is a broad-spectrum antiepileptic agent. This study describes a simple and sensitive high-performance liquid chromatographic method for the determination of lamotrigine in 50 μl of plasma. Lamotrigine and the internal standard guanabenz were extracted with 1.2 ml of diethyl ether, after the samples alkalinized with 10 μl of sodium hydroxide solution (1N). Chromatographic separation was achieved on a silica column with the mobile phase of acetonitrile–water containing 0.2% phosphoric acid and 0.3% triethylamine (pH 2.7) (84:16, v/v), at a flow-rate of 1 ml/min. The eluant was detected at 225 nm. The retention time was about 6 min for lamotrigine and 7 min for guanabenz. No endogenous substances and concomitant anticonvulsants were found to interfere. Calibration curves were linear from 0.1 to 5 μg/ml. The relative recovery of lamotrigine averaged about 80%. The limit of quantitation was 0.1 μg/ml. The intra- and inter-day precision (expressed as coefficient of variation, CV) was 8.1%, or less, and the accuracy was within 11.5% deviation of the nominal concentration. The method is suitable in pharmacokinetic investigation and monitoring lamotrigine concentration.

Keywords: Lamotrigine; Antiepileptive; HPLC; Pharmacokinetics

1. Introduction

Lamotrigine, [3,5-diamino-6-(2,3-dichloropheyl)-1,2,4-triazine] (Fig. 1), is a novel antiepileptic agent that is indicated for the adjunctive and monotherapy treatment of partial seizures both with and without secondary generalization [1,2]. Its mode of action involved the stabilization of the presynaptic membrane via blockade of voltage-dependent sodium channels and inhibition of glutamate and aspartate release [1,2]. The efficacy of lamotrigine as an add-on therapy has been well established in adults and children. 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].

Lamotrigine is being used increasingly for women with epilepsy during their reproductive years, however, little is known about its pharmacokinetic characteristics associated with pregnancy and childbirth [4,5]. Lamotrigine crossed the placenta rapidly and easily, suggesting that the maternal medication leads to a considerable foetal exposure [4]. Pregnancy increased the apparent clearance progressively by >330% of baseline and reverted quickly after delivery [5]. Thus, it is important to monitor lamotrigine concentrations before, during and after pregnancy in order to minimize fetal risk and to maximize seizure control.

The most frequent and potentially life-threatening adverse effect of lamotrigine is rash, which occurring more frequently in children and is greater when lamotrigine is concomitant with valproate [1–3]. Lamotrigine is a lipophilic weak base, and it is well absorbed after oral administration. It is extensively metabolized via hepatic glucuronidation by uridine 5′-diphosphate-glucuronosyl transferase (UGT1A4) [1,6]. To-

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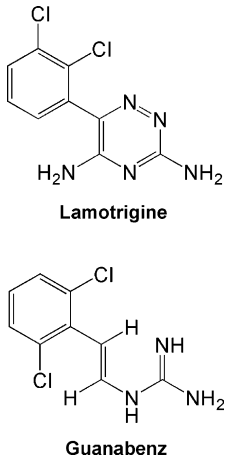

Fig. 1. Structures of lamotrigine and internal standard guanabenz.

tal body clearance of lamotrigine is reduced significantly by valproic acid through inhibition of hepatic metabolism [1]. Plasma concentrations of lamotrigine were reduced ca. two to three folds during co-medication with oral contraceptives, known inducers of UGT [6]. Therefore, it has been suggested that lamotrigine concentrations should be monitored during concomitant with agents that are enzyme inhibitors or inducers, in order to minimize its side effects [1,6]. To address all these issues, a suitable assay for quantitation of lamotrigine is necessary.

Several high-performance liquid chromatography (HPLC) methods were used for the determination of lamotrigine in biological fluids [7–25]. In general, the previously described methods require the use of relatively large sample volume (0.2–1 ml), and have a limit of quantitation (LOQ) of 0.2–1 μg/ml. For pharmacokinetic studies, a suitable sensitive method that allows an accurate measurement of low concentration of lamotrigine in biological matrices is needed. For routine drug monitoring in children and pregnant women, and experimental research in small animals such as rats, assays that require small sample volumes are very useful. In this report, we present a simple, sensitive and specific HPLC method to determine lamotrigine concentration in plasma. The LOQ of this validated method was 0.1 μg/ml using 50 μl of plasma. The applicability of this assay was demonstrated in animal pharmacokinetic studies.

2. Experimental

2.1. Chemicals and reagents

Lamotrigine (purity, 99.75%, by HPLC) was kindly provided by U-Chu Pharmaceutical Co. Ltd. (Taoyuan, Taiwan). Guanabenz acetate (Lot 127H4650, purity > 99%, by HPLC) (Fig. 1) was purchased from Sigma (St. Louis, MO, USA). All chemicals were analytical grade reagents and used as received without further purification. HPLC grade acetonitrile, methanol, and phosphoric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Diethyl ether (GR for analysis) was from Merck (Germany). Triethylamine (Lot 72670) was from Riedel-deHaen (Germany). Milli-Q Reagent Water (Millipore, Bedford, MA, USA) was used in the preparation of mobile phase. Human plasma was obtained from the Blood Center of Tainan (Tainan, Taiwan).

2.2. Instrumentation and chromatography

The HPLC system consisted of a Hitachi L-7100 pump, a Hitachi L-7200 autosampler, a Hitachi L-7400 UV detector, and a Hitachi D-7000 Chromatography Data Station (Tokyo, Japan). The analytical column was a Hypersil HS Silica (5 μm, 25 cm × 4.6 mm i.d.) column. The mobile phase comprised acetonitrile and water (84:16, v/v) containing 0.2% phosphoric acid and 0.3% triethylamine (pH 2.7). The prepared mobile phase was filtered through a 0.45-μm Millipore filter and degassed ultrasonically before use. Analyses were run at a flow-rate of 1 ml/min at ambient temperature. The detector wavelength was set at 225 nm, and peak areas were measured.

2.3. UV spectrometry

The UV spectra of lamotrigine (5 μg/ml) in aqueous solutions with various pH values (1.2–10) were recorded with 10 mm quartz cell using a Hitachi U2010 spectrophotometer (Tokyo, Japan).

2.4. Standards and controls

Master stock solutions of lamotrigine (100 μg/ml in methanol) and the internal standard guanabenz (100 μg/ml in methanol) were prepared monthly and kept tightly sealed at −20 °C. The stock solution of lamotrigine was diluted with drug-free plasma to give the calibration standards at concentrations of 0.1, 0.2, 0.5, 1, 2 and 5 μg/ml lamotrigine. The quality controls were prepared independently at concentrations of 0.1, 1, and 5 μg/ml prior to the start of sample collection and stored at −20 °C until used. The working solution of guanabenz was obtained by diluting the stock solution in methanol to 20 μg/ml. A complete calibration curve was generated with each analytical run.

2.5. Sample preparation

The samples to be analysed were removed from the freezer and thawed. Calibration standards, controls, and unknown samples were pipetted into 1.5-ml microcentrifuge tubes and processed as a batch. To 50-μl aliquots of human plasma samples were added a 10-μl aliquot of the internal standard working solution, 10-μl of 1N sodium hydroxide solution, and 1.2 ml of diethyl ether. After vortex-mixed for 30 s and upon centrifugation at 15,850 × *g* for 10 min, the upper organic layer was transferred by pipetting to a 1.5-ml microcentrifuge

tubes and evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted with 400 μl of acetonitrile and a 20-μl of aliquot was injected onto the column for HPLC analysis.

2.6. Assay validation

The model for the calibration curve of lamotrigine used the peak area ratio of lamotrigine to guanabenz (PAR) and the lamotrigine concentration (C), as given in the following equation: PAR = slope × C + (y intercept). The slope and y intercept were determined by a nonlinear least-squares program (*WinNonlin*, Professional Version 2.1, Pharsight Inc., Mountain View, CA, USA), using nominal concentrations and measured PARs from calibration standards with a weighting scheme of 1/C. Lamotrigine concentrations were estimated from PARs using the formula: C = (PAR − (y intercept))/slope.

Intra-day precision was evaluated by analysing the spiked controls six times over one day in random order, while inter-day precision was evaluated from the analysis of each control once on each of six different days. Assay precision (coefficient of variation, CV) was assessed by expressing the standard deviation of the measurements as a percentage of the mean value. The accuracy was estimated for each spiked control by comparing the nominal concentration with the assayed concentration. The lower LOQ was the lowest non-zero concentration level, which could be accurately (relative error < 20%) and reproducibly (CV < 20%) determined. Two additional 10 and 20 μg/ml QC samples were spiked at concentrations higher that the upper limit of quantitation (ULOQ, 5 μg/ml) and are known as dilution QCs. They were diluted 10-fold with blank plasma prior to analysis.

Assay specificity was examined in relation to interference from endogenous substances in six independent drug-free human plasma. In order to evaluate the selectivity, several potential co-administered medicines were examined under the assay conditions.

Absolute recoveries of 0.1, 1 and 5 μg/ml concentrations of lamotrigine in plasma were determined by assaying the samples as described above and comparing the peak areas of both lamotrigine and guanabenz with those obtained from direct injection of the standard solutions.

Freeze–thaw stability of lamotrigine (0.1 and 5 μg/ml) in plasma samples was determined for two freeze–thaw cycles. The samples were thawed at the room temperature without any assistance, and then kept in the freezer (−20 °C) for 4 h before taking out for the next thawing. The bench-top stability of lamotrigine in plasma at ambient temperature (ca. 20 °C) was studied for 4 h. The post-preparative stability of lamotrigine in processed samples left at ambient temperature was followed for 24 h.

2.7. Pharmacokinetic application

The assay was applied to a single dose (4 mg/kg) pharmacokinetic study in rats. Male Sprague–Dawley rats were obtained from the Animal Breeding Center of National Cheng Kung University. The study protocol complied with the Institutional Guidelines on Animal Experimentation of National Cheng Kung University. After intravenous bolus administration via the left femoral vein, 0.2 ml of blood samples for analytical determinations were collected via the right femoral vein at specific time intervals for 8 h. Plasma samples were stored at −20 °C until analysis.

3. Results

3.1. UV absorption spectra

As lamotrigine is a lipophilic base with a pK_a value of 5.7, the potential effect of pH value on its UV spectrum deserves a systematic investigation (Fig. 2). In the aqueous solution with pH less than 4.5, the UV spectra of lamotrigine were similar and showed absorption maximum (λ_{max}) at about 268 nm. The λ_{max} gradually shifted to 299 nm as the pH value increased to 5.7. At pH values greater than 6.8, the spectra of lamotrigine were similar and showed a distinct λ_{max} at about 308 nm. These phenomena could be explained by the degree of ionization and species distribution of lamotrigine under various pH values. The adjustment of solution to pH less than 4.5 resulted in lamotrigine existing predominantly as the ionized cation with a λ_{max} of 268 nm, whereas adjustment of solution to pH greater than 6.8 resulted in lamotrigine existing mainly as non-ionic form with a λ_{max} of 308 nm. For pH values between 4.5 and 6.8, lamotrigine existed as a mixture of non-ionized and cation forms, accordingly the shape and intensity of its UV spectrum changed as a function of the extent of species existed.

3.2. Chromatography

Fig. 3 shows chromatograms of a pooled blank human plasma (A) and blank human plasma fortified with 0.1 μg/ml

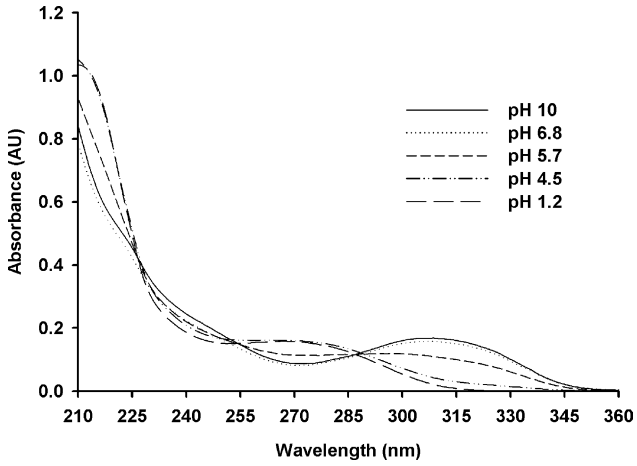

Fig. 2. The UV spectra of lamotrigine (5 μg/ml) in aqueous solutions with various pH values.

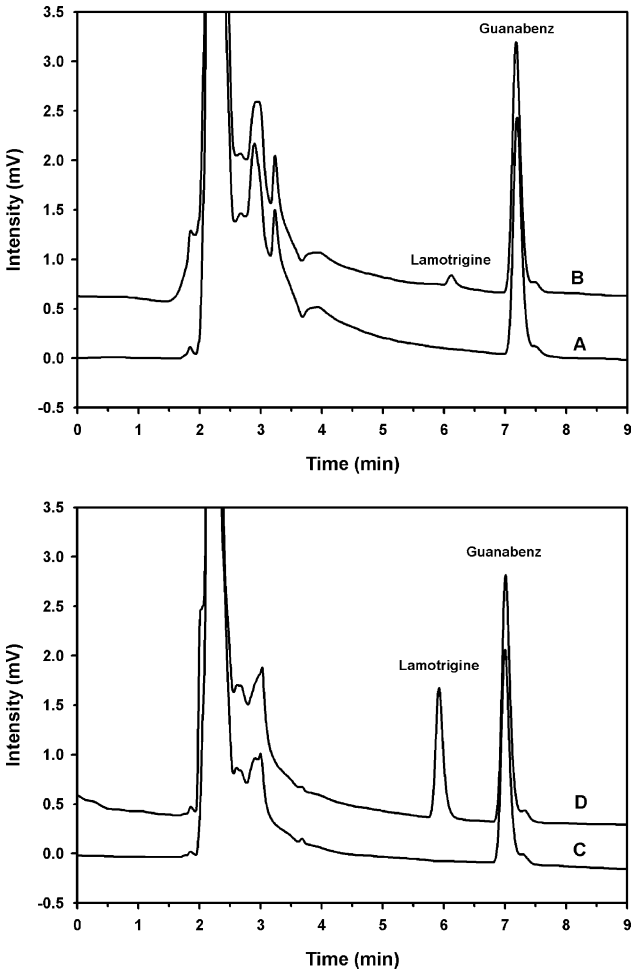

Fig. 3. HPLC chromatograms of a pooled blank human plasma sample spiked with the internal standard (A) and blank human plasma fortified with 0.1 μg/ml lamotrigine and the internal standard (B). The chromatograms of extracts from pre-dosing (C) and 8 h (D) plasma samples from a rat after intravenous bolus of 4 mg/kg lamotrigine were also shown.

lamotrigine and the internal standard (B). Fig. 3C and D represent chromatograms of extracts from pre-dose and 8 h, respectively, after administration of 4 mg/kg lamotrigine to a rat. The concentration of lamotrigine was 1.24 μg/ml at 8 h post-dose. Lamotrigine and guanabenz were eluted after 6 and 7 min, respectively. The total run time was less than 8 min. As can be seen in Fig. 3, a good separation of lamotrigine and the internal standard was achieved under the chromatographic conditions specified in Section 2.

The one-step liquid–liquid extraction by diethyl ether was sufficient to isolate lamotrigine and guanabenz from plasma without any interfering endogenous peaks. The method is specific for lamotrigine. No interfering endogenous peaks were observed at the retention of lamotrigine when drug-free human plasma (Fig. 3A) and blank pre-dose rat plasma samples were analyzed (Fig. 3C). The potential for chromatographic interferences by concomitant anticonvulsants in this method was evaluated. These included acetazolamide (100 μg/ml), carbamazepine (40 μg/ml), clonzepam (50 μg/ml), garbapentin (100 μg/ml), oxcarbazepine (12 μg/ml), phenobarbital (100 μg/ml), phenytoin (30 μg/ml), topiramate (200 μg/ml), valproic acid (500 μg/ml), vigabatrin (200 μg/ml), diazepam (100 μg/ml). None of these drugs eluted before 10 min, therefore, do not cause interference in the assay.

3.3. Assay validation

The calibration curves were linear from 0.1 to 5 μg/ml. The mean (±S.D.) regression equation for eight replicated calibration curves constructed using 50 μl of plasma samples on different days was: $PAR = (3.84 \pm 0.16) \times 10^{-4} \times C + (-0.015 \pm 0.007)$, $r^2 = 0.997 \pm 0.004$.

Precision and accuracy (0.1–5 μg/ml) were investigated by replicated analyses of spiked controls (Table 1), and in all cases the intra- and inter-day precision was acceptable at a CV of 8.1% or less. In addition, accuracy was within 20% deviation when compared with nominal concentrations across this range. From this experiment, the LOQ of the method was determined to be 0.1 μg/ml, with the intra-day imprecision and error of 2.3% and 11.5%, and the inter-day imprecision and error of 4.9% and 9.6%. Nevertheless, lamotrigine concentration at least down to 0.025 μg/ml could be still detected while preserving the signal-to-noise ratio well above 3. The ULOQ of 5 μg/ml is the highest concentration in the calibration curve that was analyzed. Samples that contain lamotrigine concentration higher than the ULOQ can be assayed after dilution with blank plasma. When this occurs, two dilution QC samples (10 and 20 μg/ml) are assayed together with the study samples (Table 1).

Satisfactory assay sensitivity was assisted by adequate and reproducible recoveries for lamotrigine and guanabenz as shown in Table 2. The mean absolute recovery of lamotrigine from plasma in the conditions of the assay between 0.1 and

Table 1
Intra- and inter-day accuracy and precision for the determination of lamotrigine in 50-μl plasma aliquots ($n = 6$)

Intra-day				Inter-day		
$C_{nominal}$ (μg/ml)	C_{est} (μg/ml)	CV (%)	Error (%)	C_{est} (μg/ml)	CV (%)	Error (%)
0.1	0.11	2.3	11.5	0.11	4.9	9.6
1	0.90	8.1	−10.3	0.95	3.2	−4.7
5	5.24	3.5	4.8	5.16	4.5	3.3
10	10.0	1.2	−0.2	9.81	2.5	−1.9
20	19.4	5.5	2.8	18.5	5.0	−8.9

Table 2
Recovery of lamotrigine in spiked plasma in the presence of internal standard (mean ± S.D., $n = 3$)

Concentration (μg/ml)	Absolute recovery (%)		Relative recovery (%)
	Lamotrigine	Guanabenz	
0.1	70.7 ± 0.7	95.3 ± 1.8	74.2 ± 0.7
1	73.1 ± 0.2	89.0 ± 2.2	82.3 ± 2.2
5	82.1 ± 2.0	97.5 ± 1.8	84.3 ± 1.3
Mean	75.3 ± 5.3	93.9 ± 4.2	80.2 ± 4.8

Table 3
Stability of lamotrigine in spiked plasma and post-preparative samples (mean ± S.D., $n = 3$)

Conditions	%Remained	
	0.1 μg/ml	5 μg/ml
Freeze–thaw stability (−20 °C/20 °C)		
Cycle 1	102.6 ± 1.3	103.7 ± 7.5
Cycle 2	94.1 ± 2.2	95.2 ± 1.3
Bench-top stability in plasma (20 °C, 4 h)	100.3 ± 3.6	97.1 ± 2.0
Post-preparative stability (20 °C, 24 h)	100.1 ± 5.3	102.7 ± 1.7

5 μg/ml was 75.3 ± 5.3%. The mean absolute recovery of the internal standard was 93.9 ± 4.19%.

Lamotrigine is stable when stored in the refrigerator and freezer. Short-term (4 °C) and long-term (−20 °C) stability of lamotrigine in plasma have been investigated previously [8,10,11,15,19,23]. It has been reported that lamotrigine in plasma seemed to be unstable at room temperature [15]. In this study, lamotrigine was stable in plasma at 20 °C for up to 4 h, and the mean concentration for the quality control samples (0.1 and 5 μg/ml) was within ±5.9% of nominals for lamotrigine following the second freeze–thaw cycle (Table 3). The present study also showed that lamotrigine was stable in processed samples left at ambient temperature for up to 24 h (Table 3).

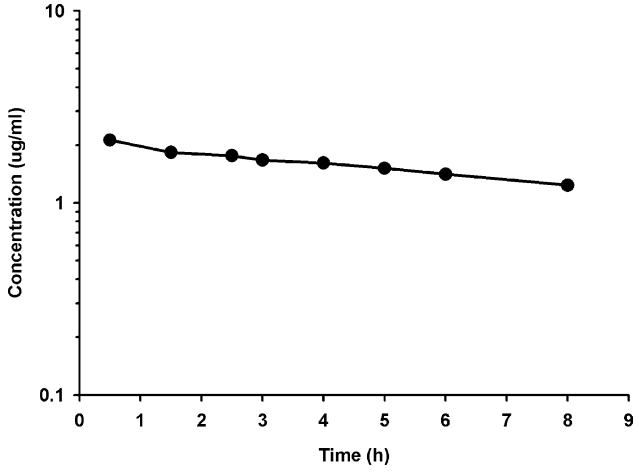

Fig. 4. The plasma concentration–time profile of lamotrigine after intravenous bolus of 4 mg/kg lamotrigine to a male rat.

3.4. Pharmacokinetic application

The assay was applied to a preliminary pharmacokinetic experiment in rats. A single bolus dose of 4 mg/kg of lamotrigine was administered intravenously to a male rat. The plasma concentration–time profile is illustrated in Fig. 4. The results showed that this simple and rapid method is sufficiently sensitive to follow blood level of lamotrigine.

4. Discussion

4.1. Choice of column and mobile phase

Concentration of lamotrigine in biological fluids has been analyzed by HPLC mainly on reversed-phase column of C18, C8, cyano or diphenyl bond phases (Table 4). An internal surface reversed-phase column was employed recently for direct injection of serum samples to quantitate lamotrigine [7]. Lamotrigine can also be eluted using silica gel column with polar or non-polar mobile phases [13,16,19,25]. In fact, the initial HPLC method for the determination of lamotrigine in serum was developed using a silica column with an ammonium hydroxide/ethanol/hexane mixture as mobile phase [25]. Silica column with aqueous–organic mobile phases were increasingly used in the analysis of polar ionic compounds, because such applications can provide better retention, sensitivity and versatility than those with reversed-phase columns [26]. Previously we have used silica columns with aqueous–organic mobile phases to quantitate plasma concentrations of several basic drugs, such as buformin, metformin and mitoguazone [27–29]. These compounds all have a guanidine group and show some structure similarity with lamotrigine. Therefore, in the present study, we used a silica column with a simple phosphoric acid–acetonitrile system. The basic additive triethylamine was used to improve peak shapes. Both phosphoric acid and triethylamine are soluble in acetonitrile; therefore, mobile phase with various ratios of acetonitrile and aqueous phase can be easily prepared.

4.2. Wavelength selection

In the previous assays, phosphate buffers were commonly employed in the mobile phase and the pH value of the buffer solution/mobile phase varied from 2.2 to 7.5 (Table 4). Lamotrigine has high molar absorbance at 268 and 308 nm in

Table 4
Analytical characteristics of reported HPLC methods for the determination of lamotrigine in plasma/serum

Reference	Column	Mobile phase	Internal standard	Flow-rate (ml/min)	UV (nm)	Volume (ml)	LOQ (μg/ml)	Pretreatment
This study	Si	CH_3CN/[0.2% H_3PO_4 + 0.3% TEA (pH 2.7)] = 84/16	Guanabenz	1	225	0.05	0.1	Diethyl ether
Croci et al. [7]	ISRP	CH_3CN/0.01 M phosphate (pH 6) = 18/82	BWA725C	1	330	0.1	0.1[a]	No
Castel-Branco et al. [8]	C18	CH_3OH/0.1 M KH_2PO_4/TEA = 35/64.7/0.3	BW725C78	1	306	1	0.1[a]	Ethyl acetate
Torra et al. [9]	C18	CH_3CN/0.25 M phosphate (pH 3.8) = 45/55	Butalbital	0.8	210	0.1	0.2[a]	SPE
Barbosa and Midio [10]	C18	CH_3OH/0.1 M KH_2PO_4/TEA = 43.5/56/0.01	BW725C	0.8	306	1	0.15	Ethyl acetate
Matar et al. [11]	C18	CH_3CN/CH_3OH/0.01 M phosphate (pH 7.5) = 17/18/65	HMCA	1	220	0.1	0.5[a]	Diethyl acetate
Böttiger et al. [12]	C18	CH_3CN/CH_3OH/0.01 M phosphate (pH 7.1) = 10/30/60	–	1.5	310	0.05	0.256	Acetonitrile
Vidal et al. [13]	Si	CH_3OH/H_2O/1 M $NH_4H_2PO_4$ (pH 5) = 94/5.96/0.04	Protriptyline	1	280	0.3	0.35	Dichloromethane
Angelis-Stoforidis et al. [14]	C8	CH_3CN/H_2O/0.5 M phosphate (pH 6.5) = 20/79/1	BWA725C	1.6	306	0.2	1	Acetonitrile
Ren et al. [15]	C18	CH_3CN/CH_3OH/0.0175 M phosphate (pH 6.8) = 16/14/70	–	1	310	0.5	0.1[a]	Acetonitrile
Sallustio and Morris [16]	Si	CH_3OH/H_2O/1 M $NH_4H_2PO_4$ (pH 4) = 94/5.92/0.08	BWA725C	1	280	0.25	0.5	Ethyl acetate
Hart et al. [17]	C18	[CH_3OH/H_2O/KH_2PO_4 = 25/32/43] + 0.058% TEA	Thiopental	3	306	0.5	0.5	Chloroform
Lensmeyer et al. [18]	Cyano	CH_3CN/CH_3OH/H_2O/HOAc/TEA = 15/12.5/72.5/0.06	Cyheptamide	1.2	214	0.2	0.3[a]	SPE
Bartoli et al. [19]	Si	CH_3OH/heptane/CH_2Cl_2/NH_4OH = 20/40/40/0.3	Nortriptyline	1	240	0.275	0.4	Dichloromethane
Londero and Greco [20]	Diphenyl	CH_3CN/0.3% H_3PO_4 (pH 3.5) = 30/70	BWA725C	1.2	265	0.05–0.2	0.2	Chloroform/iso-propanol; H_3PO_4
Yamashita et al. [21]	C18	[CH_3CN/0.01 M phosphate (pH 3.5) = 27/73] + 0.05 M SOS	Acetanilide	1	265	0.1	0.2	SPE
Fraser et al. [22]	C18	CH_3CN/H_2O/0.5 M phosphate (pH 6.5) = 20/79/1	BW725C78	1.6	306	0.2	0.51[a]	Ethyl acetate
Cociglio et al. [23]	Cyano	CH_3CN/0.01 M NH_4OAc (pH 3.5) = 55/45	A725C	1.5	280	0.2–1	–	Acetonitrile
Sinz and Remmel [24]	C8	[CH_3CN/0.05 M H_3PO_4 (pH 2.2) = 33/67] + 0.05 M SDS	BWA725C	1.5	277	0.25[b]	0.1	SPE
Cohen et al. [25]	Si	Hexane/C_2H_5OH/NH_4OH = 80/20/0.25	BWA725C	2	306	0.2	–	Ethyl acetate

Abbreviations: ISRP: internal surface reverse phase; BW725C, BWA725C, BW725C78, A725C: 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine; HOAc: acetic acid; NH_4OAc: ammonium acetate; SOS: sodium octanesulfonate; SDS: sodium dodecyl sulfate; HMCA: 9-hydroxymethyl-10-carbamyl acridan; SPE: solid phase extraction.

[a] The lowest concentration of the calibration curve validated was taken as the limit of quantitation (LOQ), if it was not specified.

[b] Blood.

acidic (pH < 4.5) and basic (pH > 6.8) medium, respectively (Fig. 2). Therefore, most of the reported HPLC assays applied UV detection at the region of 265–280 nm or 306–310 nm, depending on the pH of the mobile phase used. Low wavelength (210–220 nm) can provide better sensitivity irrespective to the pH of the mobile phase. In the present study, the mobile phase components, phosphoric acid and triethylamine, have low UV-cutoffs and are, therefore, suitable for detection with low wavelength. Thus, UV 225 nm was employed for the detection of lamotrigine. Because of its lipophilicity and weak basicity, lamotrigine can be efficiently extracted after alkalinizing the sample into various organic solvents, such as diethyl ether, dichloromethane, ethyl acetate and chloroform. In this study, diethyl ether was used as the extraction solvent as it gave high extraction recoveries for both lamotrigine and guanabenz, and provided relative clean chromatograms as detected under UV 225 nm.

4.3. Choice of internal standard

The lamotrigine analog 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (BWA725C) was widely used as the internal standard, but it was not readily available. Matar et al. used 9-hydroxymethyl-10-carbamyl acridan as the internal standard for HPLC assay of lamotrigine and other antiepileptic agents [11]. However, it bears little structure similarity to lamotrigine and it is neither commercially available. Others have used commercially available agents such as acetanilide [21], butalbital [9], cyheptamide [18], nortriptyline [19], protriptyline [13] and thiopental [17] as the internal standards; nevertheless, the structures of all these compounds were not similar to that of lamotrigine. In the present study, the antihypertensive agent guanabenz was chosen as the internal standard because it is commercially available and its peak was sufficiently separated from that of lamotrigine. Guanabenz has a guanidine group and a dichlorophenyl group and its structure resembles that of lamotrigine (Fig. 1). The usual oral dose of guanabenz for antihypertensive is 4 mg twice a day initially, and it can be increased to the prescribing limit of 32 mg/day in adults. Following oral administration of 16 mg or 32 mg of guanabenz, the peak plasma concentrations of guanabenz was less than 5.2 ng/ml (ca. 0.26 ng in 50 μl plasma) in patient with essential hypertension [30]. In the present method, 200 ng of guanabenz was spiked into the samples during sample preparation. Thus, it should be alright even if the patient is on guanabenz.

4.4. Sample volume and quantitation limit

Due to the clinical status of patients with epilepsy, especially in young children and pregnant women, it might be difficult to obtain suitable amount of blood from these patients. Thus, efforts were made in this study to lower considerably the volume of plasma sample needed, from 0.2–1 to 0.05 ml (Table 4), and still providing good sensitivity for lamotrigine quantitation. This is very useful in reducing the blood collection, offering the possibility to make sufficient numbers of blood samples for pharmacokinetic study, and minimizing the amount of blood-derived biological waste.

In conclusion, a new HPLC method for the determination of lamotrigine has been developed. The method offers the sensitivity and selectivity for monitoring therapeutic concentration of lamotrigine. The method is flexible and required only 50 μl plasma, making it suitable for studying the pharmacokinetics of lamotrigine in children, pregnant women and small animals. The application of this method was demonstrated in a pharmacokinetic study in rats.

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