Development and Validation of a Rapid Column-Switching High-Performance Liquid Chromatographic Method for the Determination of Lamotrigine in Human Serum

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Abstract

This study describes a simple and sensitive column-switching high-performance liquid chromatographic method with UV detection for the determination of Lamotrigine in 50 µL of serum. After solid-phase extraction of Lamotrigine on an Oasis HLB extraction precolumn (20 × 3.9 mm; dp: 25 µm), chromatographic separation was achieved at 30°C on a Chromolith RP-18e column (50 mm × 4.6 mm i.d.) using a solution of 20% acetonitrile in 15 mM phosphate buffer (pH 7.0) as the mobile phase, at a flow-rate of 2.0 mL/min. The eluant was detected at 215 nm. The retention time for Lamotrigine was 1.28 min. The total analysis time was ca. 5 min. However, the overlap of sample preparation, analysis, and reconditioning of the precolumn increased the overall sample throughput to one injection every 3 min. The method was validated for system suitability, linearity, precision, accuracy, robustness, and limit of quantitation. The linearity of the calibration lines, expressed by the linear correlation coefficient, was better than 0.9996. Recovery studies achieved from Lamotrigine spiked plasma samples showed values greater than 93%, demonstrating the excellent extraction efficiency of the precolumn. Intra- and inter-day precision were generally acceptable; the coefficient of variation was < 2.3% in all cases. The detection limits for Lamotrigine at a signal-to-noise ratio of 3 was 0.002 µg/mL when a sample volume of 50 µL was injected. However, it was possible to enhance the sensitivity further by injecting larger volumes, up to 200 µL. The method was shown to be robust and the results were within the acceptable range. The method was successfully applied to the determination of Lamotrigine in human serum samples of patients submitted to Lamotrigine therapy.

Introduction

Epilepsy is a common chronic neurological disorder that is characterized by recurrent unprovoked seizures. In epilepsy treatment, anticonvulsant drugs are used to inhibit seizures. Among these, Lamotrigine is an oral antiepileptic drug effective in the treatment of focal epilepsies with or without secondary generalization, and has also been suggested to be efficacious in the treatment and prophylaxis of affective disorders (1–3). However, there is still limited information on therapeutic ranges for drug monitoring of Lamotrigine. It is well known that therapeutic drug monitoring is an established tool for adjusting the patient drug dosage, avoiding side effects, and assessing patient compliance to achieve an optimum clinical response (4).

Thus, for the optimal drug treatment of epilepsy, the monitoring of Lamotrigine concentrations in human serum samples is required.

Several methods have been reported for the determination of Lamotrigine in plasma and serum, including: radioimmunoassay (5), capillary electrophoresis (6,7), and high-performance liquid chromatography (HPLC) (8–18).

However, HPLC has been the method of choice. In general, the determination of Lamotrigine in biological fluids usually involves an isolation procedure prior to the chromatographic step. The most frequently used procedures are liquid–liquid extraction (8,9,11,13,14) protein precipitation (11,15,18) using an automated sequential trace enrichment of dialysates system (16), off-line solid-phase microextraction (17), off-line solid-phase extraction with Isolute C_8 cartridges (18), and using vacuum processors for extraction columns with a disposable extraction Oasis HLB extraction column (10,15). These methods are labor-intensive and time-consuming, and the addition of an internal standard is necessary. An alternative sample extraction method that generates a lot of interest is the direct injection of serum using an on-line flow extraction method.

In the reviewed literature, an HPLC automatic method with direct-injection of serum samples is still unavailable for the determination of Lamotrigine.

However, a direct injection technique is generally preferable,

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because the problems involved in off-line sample pretreatments, such as time-consuming procedures, errors, and the risk of low recoveries can be readily avoided (19–21). On the other hand, no internal standard is necessary because there is a minimal sample manipulation.

In this way, on-line column switching devices combined with an extraction precolumn have been shown to provide a powerful and reliable solution to the on-line sample treatment of complex biological matrixes such as human blood serum.

This manuscript presents a new a column-switching HPLC method with UV detection for the determination of Lamotrigine in human serum samples. The extraction of Lamotrigine was performed on an Oasis HLB extraction precolumn. This precolumn was packed with a polymeric reversed-phase sorbent based on divinylbenzene-*N*-vinylpyrrolidone, which allows faster processing of plasma samples and the development of simple, rugged methods to assay drugs. It offers major advantages over other solid-phase extraction sorbents, and it is a universal sorbent with high, reproducible recoveries (22).

The separation of Lamotrigine from other endogenous compounds was achieved on a Chromolith RP-18e monolithic column. This kind of column consists of a single rod of silica-based material that can operate at a high flow-rate, thereby considerably reducing the analysis time and contributing to an improved signal-to-noise ratio. Additionally, it has a very large active surface area for high-efficiency separations (23,24)

Finally, when compared with the majority of methods described in the literature, our method offers several advantages, which include simplicity and short analysis time, low cost, and high sample throughput.

Experimental

Chemicals, reagents, and standards

Lamotrigine was kindly provided by Inversiones Dayamar C.A. (Venezuela). Potassium dihydrogen phosphate, potassium hydrogen phosphate, and ortho-phosphoric acid, and Suprapur 85% forming the buffer solution, were obtained from Merck KGaA (Darmstadt, Germany). HPLC-grade methanol and acetonitrile were obtained from J.T. Baker (Phillipsburg, NJ). Pharmaceuticals tablets of Lamictal were acquired in local pharmacies of Mérida City (Venezuela).

All aqueous solutions were prepared with double de-ionized water of 18 MΩ/cm specific resistivity, obtained in a Milli-Q (Millipore, Bedford, MA) ultra-filtration system.

An individual stock standard solution of Lamotrigine (1000 µg/mL) was prepared in acetonitrile and stored at –20ºC. Working solutions were prepared every day by an appropriate dilution of concentrated stock standard solutions in the extraction mobile phase. These solutions were stored at 4ºC. All standard solutions and samples for HPLC analysis were filtrated through 0.22 µm PVDF syringe filters (Millipore).

Serum samples and preparation

For the development of this work, a pool of 100 mL of normal human serum from healthy adult volunteers was used. In all of the cases, an informed consent was obtained from each person. Blood samples (10 mL) were drawn from a forearm vein into polyethylene tubes. All tubes were protected from light and centrifuged at 4800 rpm for 10 min. The serum was then separated and analyzed immediately, or stored at –20ºC until analysis.

To 200 µL of serum sample in an eppendorf tube, 300 µL of extraction mobile phase were added. The solution was vortex-mixed for 20 s, diluted to 1 mL, and centrifuged at 12,000 × *g* for 5 min. The supernatant was separated and thereafter was filtered through 0.22 µm PVDF syringe filters (Millipore). A volume of 50 µL of filtrate was then ready to be injected into the column-switching HPLC system.

Instrumentation

The details of the column-switching HPLC–UV system used here were as follows: a quaternary pump, column oven, autosampler, and UV–vis diode array detector (DAD); all components of a Series 200 HPLC System (PerkinElmer; Norwalk, CT). An additional Knauer 64 pump (Berlin, Germany) to deliver the extraction mobile phase, and a digital workstation with TotalChrom software Version 6.3 that served as both a controller and data manager for the overall system were also used. Injections were made with a Rheodyne type 7125 six-port valve equipped with a 50-µL loop. For the column-switching purposes, a column-switching six-port valve (Supelco, Bellefonte, PA) was attached to the system, controlled by the workstation. An Oasis HLB precolumn (20 × 3.9 mm; dp: 25 µm)

Table I. Optimized Experimental Conditions of the Procedure

Step	Component system	Conditions
Sample injection (*t* = 0 min)	Rheodyne 7125 valve: Inject position Switching-valve: Load position	Injection volume: 50 µL
Sample extraction (*t* = 3 min)	Extraction precolumn: Oasis HLB (20 × 3.9 mm; dp: 25 µm)	Extraction mobile phase: 10% acetonitrile in 15 mM phosphate buffer, pH 7.0 Flow-rate: 0.8 mL/min
Transfer of analytes (*t* = 0.5 min)	Switching-valve: Inject position	Analytical mobile phase: 20% acetonitrile in 15 mM phosphate buffer, pH 7.0 Flow-rate: 2.0 mL/min
Analyte separation (t = 2 min)	Switching-valve: Load position Analytical column: Chromolith RP-18e column (50 mm × 4.6 mm i.d.)	Analytical mobile phase: 20% acetonitrile in 15 mM phosphate buffer, pH 7.0 Flow-rate: 2.0 mL/min Detection UV: 215 nm
Next injection (t = 3 min)		

was employed as the extraction precolumn in order to extract and concentrate Lamotrigine from the human serum sample. Chromatographic separation was achieved on a Chromolith monolithic column (50 mm × 4.6 mm i.d.; Merck) maintained at a temperature of 30ºC.

General analytical procedure

The coupled-column analysis was subdivided into three different steps as follows.

Sample loading and processing. With the switching valve in Load position, a volume of 50 µL of serum sample was injected into the extraction precolumn using the extraction mobile phase. While the endogenous compounds were flushed to waste, Lamotrigine was retained by the inner surface of the Oasis HLB precolumn. During the sample cleanup step (3 min), the analytical column was equilibrated with the analytical mobile phase.

Analyte transfer. After 3 min, the switching valve was switched to the Inject position, and the precolumn and analytical column were series-connected. Thus, the retained analyte was swept by the analytical mobile phase from the Oasis HLB precolumn to the top of the Chromolith monolithic column in a backflush mode during 0.5 min.

Analyte separation. Half a minute later, the switching valve was changed back to the Load position to recondition the Oasis HLB precolumn with the extraction mobile phase to be ready for the next injection. In the meantime, Lamotrigine was separated in the Chromolith monolithic column under isocratic mode elution at 30ºC and detected by UV-DAD at 215 nm.

The total analysis time was ca. 5 min. However, the overlap of sample preparation, analysis, and reconditioning of the precolumn increased the overall sample throughput to one injection every 3 min (see Table I).

Results and Discussion

Method development

The aim of our study was to develop a rapid and sensitive column-switching method for the routine analysis of human serum samples in pharmacokinetic Lamotrigine.

When developing a column-switching approach, the choice of a precolumn, washing solvent, analytical column, analytical mobile phase, and column-switching time are crucial in order to obtain complete analyte recovery and clean chromatograms.

In this way, for the sample preparation, a hydrophilic copolymeric sorbent (Oasis HLB) packed in a precolumn was used to extract and clean up Lamotrigine from the serum sample. It is a macroporous poly (*N*-vinylpyrrolidone-divinylbenzene) copolymer which allows a good retention of Lamotrigine during the clean-up of the sample.

In the separation step, a monolithic column was used, which has lower separation impedance compared to the particulate packings, therefore much faster separations are possible and the productivity of chromatographic processes can be increased by at least one order of magnitude, as compared to traditional chromatographic columns packed with porous particles.

To determine an adequate switching time and to optimize chromatographic conditions, a blank serum sample was injected onto the precolumn, and the elution profile of the sample matrix with an optimized extraction mobile phase was measured by direct connection of the precolumn with a UV detector. There are two major factors in selecting a suitable extraction mobile phase for the system: the choice and content of the organic modifier, and the pH of the mobile phase. To prevent the system peaks from interfering, miscibility of the mobile phases is also necessary. In this study, the best extraction mobile phase was 10% acetonitrile in 15 mM phosphate buffer, pH 7.0 at a flow-rate of 0.8 mL/min. At pH 7, the Lamotrigine molecule clearly existed in fully unionized (pK_a 5.7) form in the mobile phases, so that it was possible to enhance Lamotrigine recovery and improve the clean-up efficiency in the serum.

The endogenous components from serum were completely removed from the precolumn within 3 min, according to a visible analysis of the elution profile when the detector signal reached the baseline without any detectable interfering signal; the detector signal corresponding to Lamotrigine did

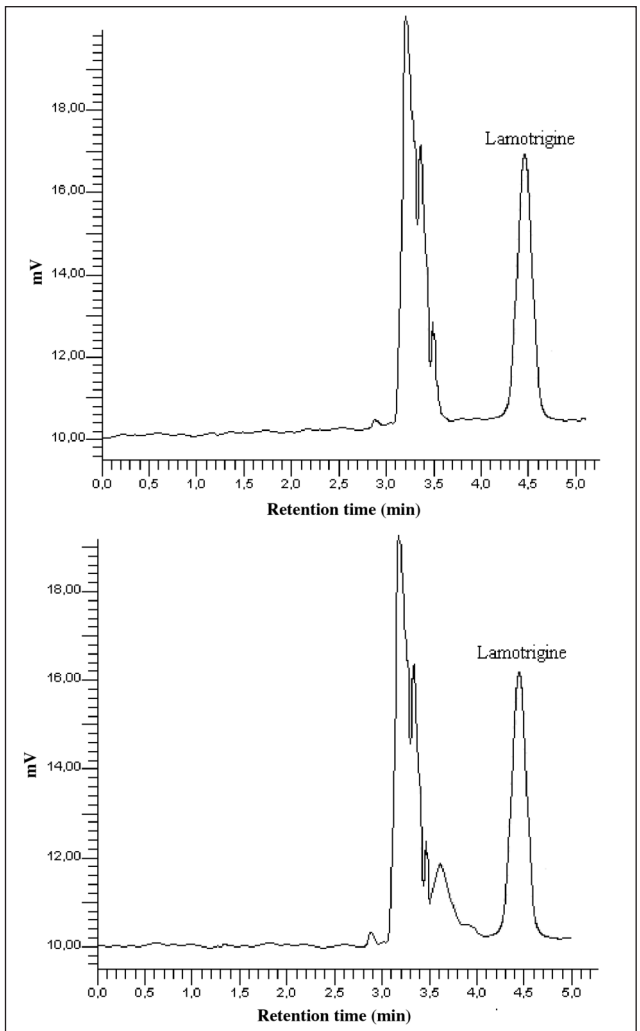

Figure 1. Chromatograms obtained with the optimized procedure: standard solution of Lamotrigine at a concentration of 0.5 µg/mL (A); serum sample spiked with Lamotrigine at a concentration of 0.5 µg/mL (B) (experimental conditions in Table I).

not emerge for at least 5 min. We, therefore, chose to make the first valve switching at 3 min.

Lamotrigine isolated from the precolumn by the valve-switching step was focused on the top of analytical column (transfer step), and then was separated and detected by UV at 215 nm. The wavelength of 215 nm was used here because high absorbance was obtained at this wavelength.

In the transfer step, the adsorbed Lamotrigine was transferred from the precolumn to the analytical column by the analytical mobile phase. Peak compression of the analytes eluting in backflush mode from the precolumn can be achieved by ensuring that the percent of the organic modifier used for transfer and separation is higher than in the extraction mobile phase. The composition of the analytical mobile phase was also chosen to ensure peak compression of the analytes eluting in backflush mode from the precolumn and that there was enough selectivity in the analytical column. A mobile phase of 20% acetonitrile in 15 mM phosphate buffer, pH 7.0 at a flow-rate of 2.0 mL/min under isocratic conditions gave a good resolution (resolution factor = 1.6) between Lamotrigine and other endogenous compounds in a short analysis time. On the other hand, it was found that the analytes adsorbed in the extraction precolumn were completely eluted out in 0.5 min by this analytical mobile phase. After 0.5 min, the valve was switched back to position to recondition the extraction precolumn with the extraction mobile phase during 2 min for the next sample injection.

Under the chromatographic conditions described (Table I), Lamotrigine and the endogenous compound's peaks were well resolved in a short time (less than 2 min). The endogenous serum components did not give any interfering peaks. Figure 1 shows typical chromatograms of blank serum in comparison to spiked samples analyzed. The average retention time of Lamotrigine was 1.28 min.

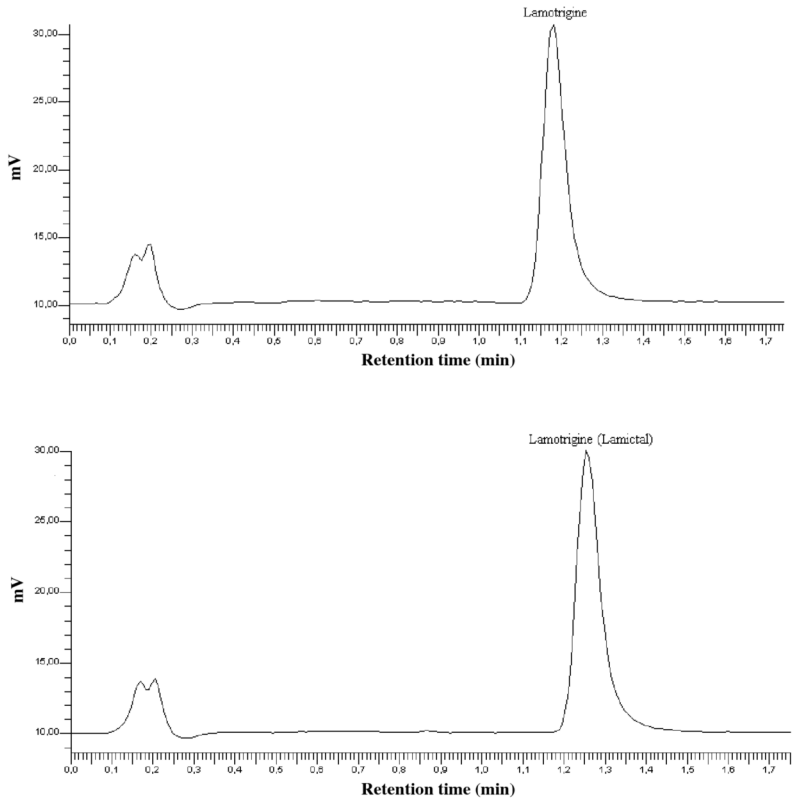

Figure 2. Chromatograms obtained in the system suitability study: standard solution of Lamotrigine at a concentration of 1.0 µg/mL (A); commercial sample of Lamotrigine at a concentration of 1.0 mg/mL (B).

Table II. The System Suitability Test Results of the Developed Method for the Determination of Lamotrigine

Compound	Lamotrigine concentration (µg/mL)	Retention time (min) (Mean ± %RSD)	Capacity factor (k′)	Tailing factor	Peak area (Mean ± %RSD)	Plate count/ column meter
Lamotrigine	1.0	1.19 ± 0.56	5.08	1.24	841.0 ± 0.42	42288
Lamictal 25 mg	1.0	1.24 ± 0.27	6.46	1.24	839.5 ± 0.61	43359
Lamictal 50 mg	1.0	1.25 ± 0.76	6.34	1.24	833.0 ± 0.53	43906
Lamictal 100 mg	1.0	1.23 ± 0.64	5.01	1.24	840.0 ± 0.37	43190

Method validation

To meet current pharmaceutical regulatory guidelines [i.e., ICH (25,26) and USP (27,28)], a number of parameters must be investigated in order to validate analytical methods such as precision, accuracy, specificity, linearity, system suitability, and robustness study.

System suitability test limits. System suitability test parameters must be checked to ensure that the system is working correctly during the analysis. The following parameters were used for the system suitability evaluation (27,28): capacity factor (k'), column efficiency (number of theoretical plates), tailing factor (T), and relative standard deviation (RSD) of peak area of six injections of 1 µg/mL of Lamotrigine standards and Lamictal. A typical chromatogram is shown in Figure 2, and the system suitability test results are presented in Table II. The capacity factor obtained was within the accepted values ($0.5 < k' < 10$): greater than 2 for the endogenous compound peak and less than 10 (6.5) for the Lamotrigine peak. T refers to peak asymmetry. A T of 1 refers to a symmetric peak. The calculated value for the T for Lamotrigine (1.24) was in the acceptable range of $0.8 \leq T \leq 1.5$. Column efficiency (number of theoretical plates) refers to the performance of the stationary phase. It means how well the column is packed. There are several methods to measure the column efficiency, which may or may not be affected by chromatographic anomalies, such as tailing or fronting. In the present study, the number of theoretical plates was calculated using the

following equation: $N = 5.54\ (t_R/w_{0.5})^2$, in which $w_{0.5}$ is the width of the peak at half-height, and t_R is the retention time along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component. The N values per column meter for each compound are represented in Table II. The United States Pharmacopeia defines the RSD of peak area for repetitive injections as one of the parameters that can determine system suitability prior to analysis. For an assay method, the RSD typically should be less than 1% for these repetitive injections. The mean of peak area ± RSD of six injections of 1 µg/mL of Lamotrigine standards and Lamictal are shown in Table II; in all cases, the RSD was < 0.67. It was concluded that the developed method is optimum according to the studied parameters; all obtained results were within the acceptable range established by these guidelines (25–28). Therefore, this method can be applied to its intended purpose with no problems, its suitability being proved.

Calibration, accuracy, and precision. Calibration curves for Lamotrigine were linear within the range examined (from 0.006 to 8.00 µg/mL). Table III shows the regression equations of the peak area as a function of Lamotrigine concentration obtained for aqueous standard and for spiked human serum samples. These data were obtained by running calibration curves using the method of standard additions. Each calibration set included seven data points and each point was run at least three times. For each of the regression lines, the correlation coefficients (r^2) were larger than 0.9996. On the other hand, the slopes of the calibration curves using aqueous standard solutions were not statistically different ($P < 0.05$) from those for Lamotrigine additions to real serum; hence, the standard calibration technique with aqueous standards could be used for the determination of the analyte in the human serum samples investigated in this work.

The absolute recoveries of Lamotrigine from human serum at five different concentrations were estimated by comparing the peak area obtained from the injections of the standards with those obtained from serum samples spiked with the analyte at the same concentration levels. The results are summarized in Table IV. Recovery values for all cases were superior to 92.8% [% coefficient variation (CV) < 3.15; $n = 5$]. These values are quantitative and demonstrate the extraction efficiency of the Oasis HLB precolumn.

The intra-day assay precision of the method was examined by a replicate analysis ($n = 5$) of aqueous standards and human serum spiked with known amounts of Lamotrigine. The precision, expressed by CV, was < 1.60% for all tested concentrations, showing that the method provided good reproducibility. The inter-day assay precision was also determined by assaying aqueous standards and spiked serum samples on five different days. The CV did not exceed 2.34% in any concentrations, demonstrating the good stability and repeatability of this described system.

Limits of detection and quantitation. The limit of detection (LOD) was determined using the signal-to-noise of 3:1 by comparing test results from samples with known concentrations of the analyte with blank samples. The results showed that the LOD was 0.002 µg Lamotrigine/mL when a sample volume of 50 µL was injected. However, it was possible to enhance the sensitivity further by injecting larger volumes, up to 200 µL (LOD: 0.0004 µg Lamotrigine/mL).

The limit of quantitation (LOQ) is defined as the lowest concentration of analyte that can be determined with acceptable precision and accuracy. The LOQ of the method was 0.006

Table III. Calibration Curves

Compound	Matrix	Dynamic range (µg/mL)		Intercept	Slope	Correlation (r^2)
Lamotrigine ($n = 7$)	Aqueous standard	0.006–8.0	Mean	0.274	5315.4	0.9996
			SD	0.001	67.74	0.0007
			% CV	0.36	1.270	0.0700
	Serum	0.006–8.0	Mean	171.5	5317.7	0.9999
			SD	4.67	32.78	0.0012
			% CV	2.72	0.616	0.1200

Table IV. Recoveries of Lamotrigine from Spiked Human Serum

Compound	Added (µg/mL)	Found (µg/mL)	Recovery (%)	CV (%) ($n = 5$)*
Lamotrigine	0.25	0.24	98.8	3.15
	0.5	0.50	100.1	0.27
	1.0	0.99	99.0	0.94
	2.0	1.86	92.8	1.60
	4.0	4.21	105.2	0.47

* CV: coefficients of variation (n = 5).

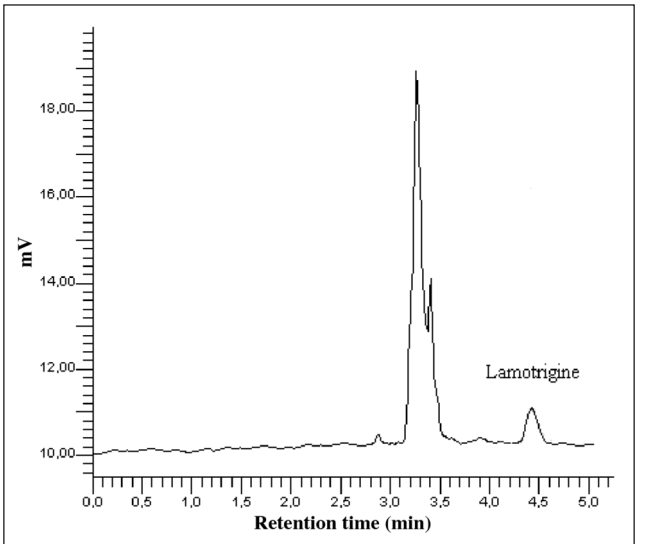

Figure 3. Chromatogram obtained for a serum sample enriched with Lamotrigine to a concentration near the LOQ method (0.009 µg Lamotrigine/mL).

µg Lamotrigine/mL based on 50 µL of serum sample. Figure 3 shows a chromatogram obtained for a serum sample spiked with a Lamotrigine concentration close to the limit of quantification.

Robustness test. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, which provides an indication of its reliability during normal usage (29). In order to study the robustness of the proposed method, deliberate modifications in column temperature, mobile phase flow-rate, and mobile phase composition were made. These results are summarized in Table V. A change of ± 0.2 units around 2.0 mL/min mobile phase flow-rate, ± 3 units on the recommended operating temperature of 30ºC, and 2.5% on the mobile phase composition had no impact on chromatographic performance. The percent recoveries of Lamotrigine were good under most conditions and did not show a significant change when the critical parameters were modified. These results demonstrated that the Lamotrigine method conditions were found to be very robust.

Table V. Summary of Results for Robustness

Parameter	Retention time (min) Mean ± SD	% Change	Lamotrigine (% recovery) Mean ± SD	% Change
Mobile phase flow-rate (mL/min)				
1.8 mL/min (–8%)	1.36 ± 0.04	6.3	104.49	3.0
1.9 mL/min (–4%)	1.33 ± 0.04	3.9	103.67	2.2
2.0 mL/min (0%)	1.28 ± 0.02	0.0	101.40	0.0
2.1 mL/min (4%)	1.20 ± 0.04	–6.3	97.19	–4.2
2.2 mL/min (8%)	1.17 ± 0.05	–8.6	99.74	–1.6
Mobile phase composition				
Acetonitrile–5 mM phosphate buffer pH = 7 (v/v)				
22.5%–77.5%	0.90 ± 0.01	–30.2	99.15	–2.2
20%–80%	1.29 ± 0.01	0.0	101.40	0.0
17.5%–82.5%	1.84 ± 0.03	42.6	99.10	–2.3
Column temperature (°C)				
27°C (–10%)	1.31 ± 0.01	1.6	98.25	–3.1
30°C (0%)	1.29 ± 0.01	0.0	101.40	0.0
33°C (10%)	1.24 ± 0.04	–3.8	98.73	–2.6

Application to patient serum. The proposed method was applied to the determination of Lamotrigine in serum samples from 3 subjects receiving a Lamictal treatment, as the first step towards a pharmacokinetic study which was approved by the local bioethics committee. The serum samples were collected after oral doses of 100, 150, and 200 mg Lamotrigine (Lamictal). Table VI shows the obtained results. It should be noted that these values are within the therapeutic range for Lamotrigine, proposed by different authors (30). Therefore, the method developed here is suitable to perform the routine monitoring of the drug in patients with epilepsy, in order to individualize patient therapy.

The method was also applied to determine a profile of the drug into a female patient aged 29, a pregnant woman who had been treated with a dose of 100 mg of Lamictal at 10 P.M. and a 50 mg dose at 8 A.M. The first serum sample was taken at 8.15 min and then at 1, 3, 7, and 8 h of the dose ingested. Figure 4 shows the mean plasma concentrations of Lamotrigine. The serum levels reached a maximum 7 h after the administration, and thereafter the serum level begins to decline. These results are preliminary because it is necessary to make profiles for 24 h and for several days. However, this study showed that the dose provided to the patient should be changed because the patient was having some morning seizures.

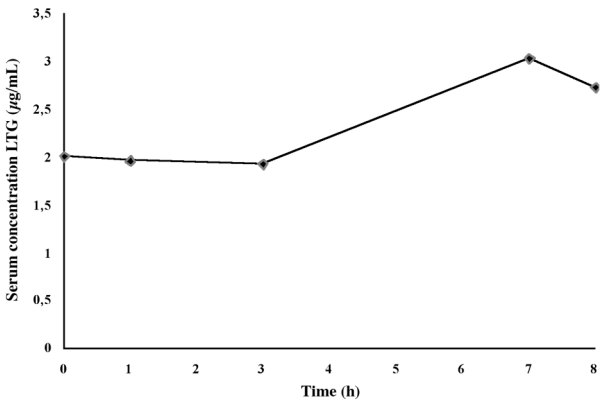

Figure 4. Serum concentrations of Lamotrigine after a dual oral dose of 100 mg and 50 mg of Lamictal ingested by a pregnant female patient at 10 P.M. and 8 A.M., respectively.

Table VI. Analysis of Real Samples

Patient	Age	Daily dose of Lamictal (mg)	Serum concentration of Lamotrigine (µg/mL)
1	42	100	1.40
2	21	200	3.50
3	29	150	3.05

This study was useful in order to identify the treatment of a specific patient and the difficulty of adjusting the dose. It is known that the initial proposed therapeutic range for Lamotrigine was from 1 to 4 mg/L (31), but it is clear that many patients require higher concentrations. There are researchers who reported an optimum 5 to 10 mg/L, but there are particularly sensitive patients who can respond to levels of 2 to 5 mg/L and others that may require levels of 10 to 15 mg/L. In this sense, it is important to better define the optimal monitoring intervals of new antiepileptic drugs such as Lamotrigine to be able to adjust the initial dose before disposing of a clinical criterion, and to help resolve doubts about the ineffectiveness or toxicity of the treatment.

Finally, it is important to note that the serum concentrations of almost all antiepileptic drugs decrease during pregnancy, particularly those which are metabolised by

glucuronidation. The interindividual variability is pronounced. In highly protein-bound drugs, such as phenytoin and valproate, the unbound drug is less affected than total concentrations. Lamotrigine and Levetiracetam concentrations may decrease by more than 50% in the course of pregnancy; monohydroxyoxcarbazepine by up to 30–40%.

An appropriate clinical follow-up tailored to individual needs and supported by therapeutic drug monitoring should be performed in pregnant women with epilepsy. Education concerning reproductive issues is an essential part of the epilepsy service to fertile women (32).

Conclusion

An HPLC method has been developed and validated and found to be a very good alternative for the determination of Lamotrigine in human serum samples.

By using an Oasis HLB extraction precolumn, the serum samples could be injected directly into the chromatographic system, after dilution and centrifugation, with a sampling throughput of 20 samples/h. Enhancement of the detection sensitivity is technically feasible by increasing the volume of the serum introduced onto the Oasis precolumn.

The proposed method was further compared with those published and proved to be more rapid and selective, and requires a simple sample preparation procedure. Moreover, the lower solvent consumption, along with the short analytical run time of 2.0 min, leads to a cost-effective and environmentally friendly chromatographic procedure.

Finally, the method proved to be simple, useful, and appropriate for clinical and experimental research and routine monitoring of Lamotrigine serum concentrations in patients, with the aim of defining the optimum intervals that are used in different types of epilepsy: in children, adults, and the elderly.

Acknowledgments

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