Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 863 (2008) 177–180
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of lamotrigine in whole blood with on line solid phase extraction

Stefano Bompadre [a,*], Adriano Tagliabracci [b], Maurizio Battino [c], Raffaele Giorgetti [b]

[a] *Institute of Microbiology and Biomedical Science, Università Politecnica delle Marche, Via Ranieri 67, 60121 Ancona, Italy*
[b] *Institute of Legal Medicine, Università Politecnica delle Marche, Via Conca 71, 60126 Ancona, Italy*
[c] *Institute of Biochemistry, Università Politecnica delle Marche, Via Ranieri 67, 60121 Ancona, Italy*

Received 15 May 2007; accepted 27 December 2007
Available online 4 January 2008

Abstract

A simple, sensitive and reproducible method was developed for the determination of lamotrigine in whole blood with on-line solid phase extraction followed by HPLC separation with UV detection. Whole blood samples were diluted 1:1 with water and then injected directly on a clean-up column dry-packed with 40 μm C8 silica and separated on a C18 reversed-phase column (150 × 4.6 mm) at room temperature. The extraction column was activated with methanol and conditioned with phosphate buffer of pH 4.5. Mobile phases consisted of phosphate buffer of pH 4.5 for the extraction column and of phosphate buffer of pH 4.5 – acetonitrile (60:40, v/v) for the analytical column. At a flow rate of 1.0 ml/min and a connection time of 1.0 min, the complete cycle time was 10.0 min. Detection was carried out at 260 nm. No internal standard was necessary. The method was linear over concentration range 0.2–20.0 μg/ml for lamotrigine. Recovery was 98%. Within-day and between-day coefficients of variation ranged from 1.8 to 6.7%.

Keywords: Lamotrigine; HPLC; Solid phase extraction

1. Introduction

Lamotrigine is an anticonvulsant drug used in the treatment of partial and generalized epilepsy.

Although some analytical methods to estimate lamotrigine content in biological fluids have been reported in the peer-reviewed literature an HPLC method for the determination of lamotrigine in whole blood is still lacking [1–4]. Determination of drugs in whole blood is often necessary in forensic analysis because of the difficulty in obtaining serum or plasma. HPLC analysis of drugs in complex matrices such as whole blood usually involves time consuming liquid–liquid extraction. Such conventional procedure may involve tedious, time consuming, expensive, and complex steps, and finally even sample loss and contamination problems are not unusual. In the HPLC analysis, the preceding on-line solid phase extraction may solve these problems [5,6]. The sample is diluted with water or mobile phase to avoid clogging of the column filters and then directly injected onto a primary column which results in a preliminary sample clean-up by SPE. The sample dilution does not reduce the sensitivity, because the sample loop volume does not affect the peak width in a on-line solid phase extraction. After the SPE step, a small fraction of the effluent from the extraction column is selectively transferred to the analytical column for the final separation. Because of minimal sample manipulation no internal standard is necessary. In this paper we describe a rapid, accurate, precise, and inexpensive method to determine lamotrigine in whole blood using an on-line solid phase extraction procedure followed by analysis by HPLC.

2. Materials and methods

2.1. Chemicals and reagents

Lamotrigine (P.N. L3791-10MG), Acetonitrile HPLC grade (CHROMASOLV® Plus, P.N. 34998-2.5L), Sodium phosphate

* Corresponding author. Fax: +39 071 2204622.
E-mail address: s.bompadre@univpm.it (S. Bompadre).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.12.020

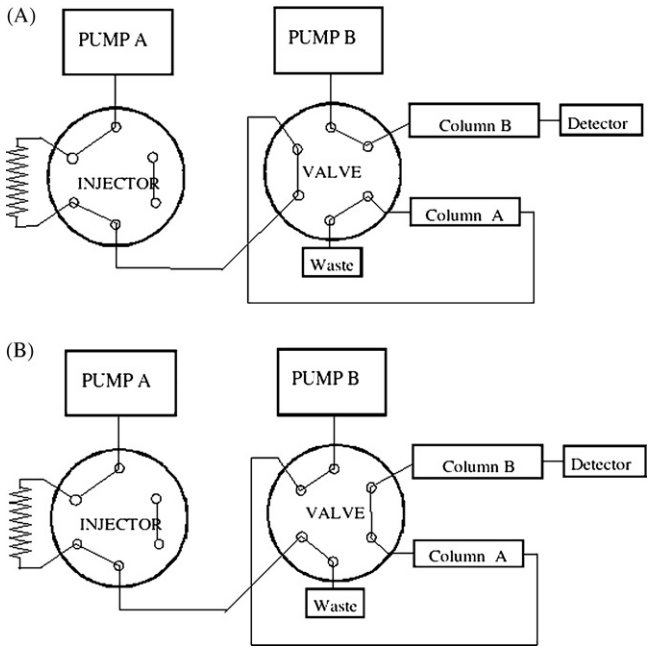

Fig. 1. The column-switching system.

monobasic dihydrate (BioChemika Ultra, P.N. 71502-1KG) and phosphoric acid (BioChemika Ultra, P.N. 438081-500ML) were of analytical grade and purchased from Sigma Aldrich (Sigma-Aldrich, St. Louis, MO, USA). The water was reagent grade (18.2 MΩ cm at 25 °C of resistivity) obtained from a Milli-Q system (Millipore, Billerica, Massachusetts, USA).

2.2. HPLC instrument

The HPLC system consisted of a Varian Vista 5500 Solvent Delivery System (Pump A) from Varian (Varian Inc., Walnut Creek, CA, USA), a Beckman System Gold 126 Programmable HPLC pump (Pump B) from Beckman (Beckman Coulter Inc., Fullerton, CA, USA) and a Shimadzu SPD-20AV UV/vis detector (Shimadzu corporation, Kyoto, Japan) set at 260 nm. The injector was a Rheodyne Model 7125 manual injection valve equipped with a 50 μl sample loop. The coupled-column system was operated by a six-port, automated, switching valve (Valco, Schencon, Switzerland) controlled by the Vista 5500 HPLC pump (Fig. 1).

The analytical column was a C18 3.0 μm reversed-phase column (150 × 4.6 mm) from Supelco (Sigma-Aldrich, St. Louis, MO, USA). The extraction column was an empty 50 × 4 mm column, dry packed with 40 μm C8 silica obtained from Supelclean LC-8 SPE, tube bed wt. 500 mg, volume 3.0 ml (Sigma-Aldrich, St. Louis, MO, USA). The chromatograms were integrated with a Star 5.5 software from Varian.

2.3. Extraction-analysis switching procedure

The extraction was performed with an in-house made 50 × 4 mm column (column A), consisting of an emptied old column, dry-packed with 40 μm C8 silica obtained from a Supelclean LC-8 SPE Tube. The extraction column was activated with methanol at a flow rate of 1.0 ml/min for 30.0 min in order to eliminate gas bubbles and then conditioned with a mobile phase constituted of a phosphate buffer of pH 4.5 (mobile phase 1), at a flow rate of 1.0 ml/min for 30.0 min. Separation was performed on a C18 reversed-phase column (150 × 4.6 mm) (column B) using a mobile phase constituted of 40% acetonitrile in phosphate buffer of pH 4.5 (mobile phase 2), at a flow rate of 1.0 ml/min.

The coupled-column system was operated by a six-port, automated, switching valve controlled by pump A (Fig. 1). Extraction was performed on the extraction column (A) using a mobile phase constituted of phosphate buffer of pH 4.5 (mobile phase 1), at a flow rate of 0.5 ml/min. Separation was performed on the analytical column (B) using a mobile phase constituted of 40% acetonitrile in phosphate buffer of pH 4.5 (mobile phase 2). at a flow rate of 1.0 ml/min. Extraction and analysis proceeded as follows:

1. After sample dilution (1:1, with water), 50 μl of the solution were injected onto the extraction column (A) where the analytes were retained while the matrix, passing through the column, was directed to waste, at a flow rate of 0.5 ml/min. At the same time, the analytical column (B) was conditioned with mobile phase 2 (switching valve at the initial position: Fig. 1A).
2. After a period of 1.0 min, the valve was switched and mobile phase 2 from pump B eluted the analytes trapped on the extraction column (column A) to the analytical column (column B) (Fig. 1B).
3. After a period of 1.0 min, the valve was switched to the initial position and extraction column (column A) was conditioned again with mobile phase 1 in order to prepare it for the next sample. Simultaneously, pump B maintained the flow of mobile phase 2 through the analytical column where the analytes were separated and detected.
4. The complete cycle time (extraction, elution, injection, analysis) was 10.0 min.

2.4. Preparation of standards

A stock solution of lamotrigine was prepared in MeOH/water 90/10 (v/v). The stock solution was stored at −80 °C. Working solutions of 20.0, 10.0, 5.0, 2.5, 1.0, 0.5, and 0.25 μg/ml of lamotrigine were prepared by dilutions of the stock solution with phosphate buffer of pH 4.5. All working solutions were stored at 4 °C. The calibration curve consisted of seven blank samples (whole blood, lamotrigine-free) spiked with lamotrigine.

2.5. Method validation

The analytical procedure was validated in terms of linearity, repeatability, accuracy, recovery and sensitivity. Linearity was examined at concentrations within the range 0.2–20.0 μg/ml. Seven aliquotes of whole blood were spiked with a stock solution of lamotrigine to obtain seven calibration samples containing 0.2, 0.5, 1.25, 2.5, 5.0, 10.0, 20.0 μg/ml of lamotrigine. The samples were analyzed and the peak areas of the lamotrigine

Table 1
Within-day repeatability, accuracy and linearity for lamotrigine in whole blood

Nominal	Actual value (mean ± S.D., $n = 6$)	Repeatability (%)	Accuracy (%)
0.5	0.47 ± 0.02	4.6	94.4
1.0	1.03 ± 0.03	2.8	103.4
5.0	5.17 ± 0.13	2.6	103.3
10.0	9.82 ± 0.21	2.1	98.2
20.0	20.34 ± 0.24	1.2	101.7

fitted versus the sample concentration to obtain a calibration curve. Standard curves were linear with a correlation coefficient of $R^2 = 0.998$ (Fig. 2). Repeatability and accuracy, shown in Table 1, were determined by injecting the same sample six times, and then calculating the mean and the standard deviation of peak areas.

The recovery was determined by comparing the peak areas of standard solutions extracted with the procedure just described (i.e. extraction by SPE followed by analysis by HPLC), with the peak areas resulting from the same solutions injected directly onto the analytical column at three different concentration levels.

The specificity of the method was tested analyzing a blank sample without lamotrigine. No endogenous interferences were observed (Figs. 3–5).

The limit of detection (LOD), defined as three times the noise (S/N = 3), was 0.05 μg/ml. The limit of quantification (LOQ) defined as ten times the noise (S/N = 10), was 0.2 μg/ml, and interassay repeatability (%RSD, $n = 10$) was less than 7.0%.

3. Results and discussion

The method to determine the lamotrigine has been reveled to be rapid, accurate and precise; infact the standard curves obtained for the validation of the method were linear within the range 0.2–20.0 μg/ml with a correlation coefficient of $R^2 = 0.998$ and repeatability ranged between 1.2 and 4.5%, while accuracy was found in the range 94.4–103.4% (Table 1). Finally, between-day repeatability (%RSD $n = 10$) for a nominal concentration of 5.0 μg/ml of lamotrigine was 6.7%. The optimum connection time was estimated by multiple injections of a whole blood sample spiked with 5 μg/ml of lamotrigine and measuring the peak area. A time of 1.5 min for extraction (step 1) and a transfer

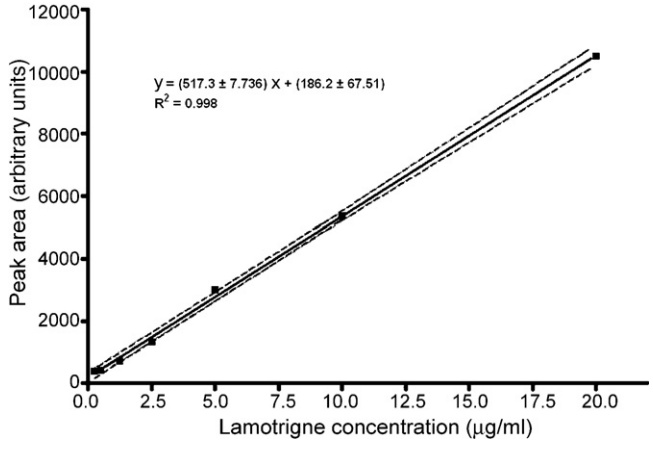

Fig. 2. Calibration curve with 95% confidence interval of lamotrigine in whole blood.

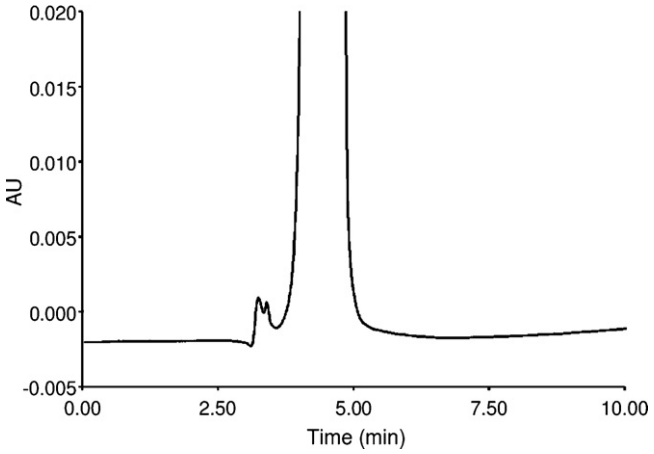

Fig. 3. HPLC chromatogram of whole blood sample without lamotrigine (blank sample).

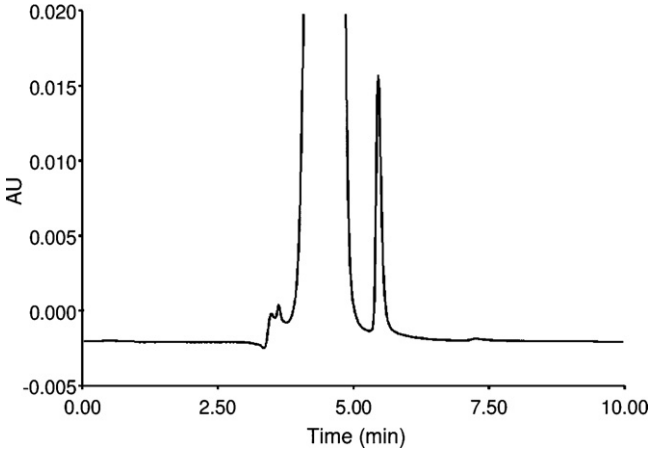

Fig. 4. HPLC chromatogram of whole blood spiked with 7.0 μg/ml of lamotrigine (spiked sample).

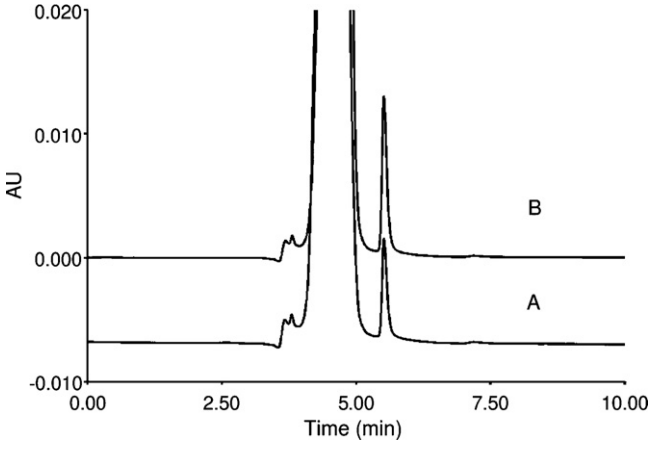

Fig. 5. HPLC chromatogram of whole blood from a subject treated with lamotrigine (sample) (A) and the same sample spiked with 2.5 μg/ml of lamotrigine (sample spiked with lamotrigine) (B).

time of 1.0 min (step 2) were found to be the best compromise for a complete recovery and a clean chromatogram. It should be taken into account that the peak width as well as the sensitivity in a solid phase extraction depend on the optimum choice of columns and phases, rather than on the connection time. Usually, the retentive capacity of the extraction column should be lower than that of the analytical column. This solution provokes a compression of analyte in a small volume during the transfer from the first to the second column. The connection time affects the recovery and the presence of interfering peaks. The real injection volume is the mobile phase volume transferred from the extraction column to the analytical column. Therefore, the loop volume does not affect the chromatogram quality.

4. Conclusion

Several HPLC methods used for the determination of lamotrigine have previously been reported, but none for lamotrigine present in complex matrices like whole blood. The proposed assay is sufficiently easy, specific, accurate, and free from interferences of endogenous components. One of the main advantages of this method is the easy and controlled procedure of sample pretreatment. By using the herein presented solid phase extraction, other time consuming extraction procedures can be avoided. This method has been successfully applied in forensic determination of lamotrigine in whole blood.

Acknowledgements

This work was partially supported by Università Politecnica delle Marche. Authors are indebted to M. Glebocki for extensive editing of the manuscript.

References

[1] L. Franceschi, M. Furlanut, Pharmacol. Res. 51 (2005) 297.
[2] M.P. Kuldeep, L.B. Subhash, J. Chromatogr. B 823 (2005) 152.
[3] M. Torra, M. Rodamilans, S. Arroyo, J. Corbella, Ther. Drug Monit. 22 (2000) 621.
[4] N.R. Barbosa, A.F. Midio, J. Chromatogr. B 741 (2000) 289.
[5] S. Bompadre, L. Ferrante, M. De Martinis, L. Leone, J. Chromatogr. A 812 (1998) 249.
[6] M. Battino, L. Leone, S. Bompadre, Methods Enzymol. 378 (2004) 156.