



Published in final edited form as:

Biomed Chromatogr. 2007 March ; 21(3): 225–228.

Simultaneous determination of lamotrigine, zonisamide, and carbamazepine in human plasma by high-performance liquid chromatography

Elizabeth Greiner-Sosanko, Darla R. Lower, Mohamed A. Virji, and Matthew D. Krasowski*

Toxicology and Therapeutic Drug Monitoring Laboratory, Division of Clinical Chemistry, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA USA

Abstract

A high-performance liquid chromatography assay with ultraviolet detection was developed for the simultaneous determination of the anti-epileptic drugs lamotrigine, carbamazepine, and zonisamide in human plasma and serum. Lamotrigine, carbamazepine, zonisamide, and the internal standard chloramphenicol were extracted from serum or plasma using liquid-liquid extraction under alkaline conditions into an organic solvent. The method was linear in the range 1–30 µg/mL for lamotrigine, 2–20 µg/mL for carbamazepine, and 1–40 µg/mL for zonisamide. Within- and between-run precision studies demonstrated coefficient of variation < 10% at all tested concentrations. Other anti-epileptic medications tested did not interfere with the assay. The method is appropriate for determining lamotrigine, carbamazepine, and zonisamide serum or plasma concentrations for therapeutic monitoring.

Keywords

anticonvulsants; lamotrigine; zonisamide; carbamazepine; HPLC; UV detection

INTRODUCTION

Lamotrigine (Lamictal®) is a broad-spectrum anti-epileptic drug of the phenyltriazine class chemically unrelated to other anti-convulsants (LaRoche and Helmers, 2004a,b). Lamotrigine has an average elimination half-life of 33 hours, although this can be influenced by concomitant therapy with other medications such as valproic acid. Lamotrigine is predominantly metabolized in the liver by glucuronidation (Bialer, 2005). Therapeutic drug monitoring of lamotrigine targets the parent drug as little clinical benefit has been found for monitoring the glucuronide metabolite (Bialer, 2005).

Zonisamide (Zonegran®) is an anti-epileptic drug licensed in the United States for the treatment of partial seizures in adults (LaRoche and Helmers, 2004a,b). Zonisamide is also used in West's syndrome (a form of infantile epilepsy), Lennox-Gestaut syndrome (a refractory type of epilepsy), bipolar disorder, and migraine headaches. Zonisamide has an elimination half-life of approximately 50–70 hours in adults and is extensively metabolized by acetylation and conjugation. Zonisamide is a substrate of cytochrome P450 2C19 and 3A4 and is subject to drug-drug interactions with inhibitors or inducers of these hepatic enzymes (Bialer, 2005). Although several metabolites of zonisamide are generated following administration to humans,

* Correspondence to Matthew Krasowski, MD, PhD, Department of Pathology, Division of Clinical Chemistry, Children's Hospital Main Tower 5812, Pittsburgh, PA 15213 USA; e-mail: krasowskim@upmc.edu.

therapeutic drug monitoring of zonisamide has involved determination of serum/plasma concentrations of only the parent compound (Bialer, 2005).

Measuring lamotrigine and zonisamide concentrations in plasma is recommended for monitoring treatment adherence and for dose adjustment in patients with liver dysfunction or who are receiving other medications (Tomson and Johannessen, 2000; Bialer, 2005). Several methods have been used for determination of lamotrigine including high-performance liquid chromatography (HPLC) (Fraser *et al.*, 1995; Forssblad *et al.*, 1996; Lensmeyer *et al.*, 1997; Torra *et al.*, 2000; Contin *et al.*, 2005), gas chromatography with a nitrogen-phosphorus detector (Wattle *et al.*, 1997), and radioimmunoassay (Biddlecombe *et al.*, 1990). Several HPLC methods have been reported for zonisamide (Shimoyama *et al.*, 1999; Nakamura *et al.*, 2001; Juenke *et al.*, 2006). As yet, there are no commercially available assays for either drug, in contrast to multiple immunoassays available for the quantitation of more traditional anti-epileptic drugs such as carbamazepine, phenobarbital, phenytoin, and valproic acid. Given the expanding use of lamotrigine and zonisamide in our medical center, including co-administration of lamotrigine and zonisamide in some patients, we developed a rapid, simple HPLC method to measure the two drugs simultaneously. With a slightly longer run time, the assay can also be used to measure the concentrations of carbamazepine.

EXPERIMENTAL

Chemicals and apparatus

Lamotrigine was generously supplied by GlaxoSmithKline (Triangle Park, NC, USA). Carbamazepine, zonisamide, and the internal standard (IS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, and water were HPLC grade (Fisher, Pittsburgh, PA, USA). Ethylacetate, potassium phosphate, and phosphoric acid were analytical grade (Fisher). Drug-free human plasma was obtained from the University of Pittsburgh Medical Center Central Blood Bank. The HPLC system consisted of a Waters/Millipore 717 Plus Autosampler and Systems Controller.

Chromatographic conditions

The separation was performed at 22 °C with a μ Bondapak C-18 column. The mobile phase was a mixture of aqueous 30 mM potassium phosphate buffer (adjusted to pH 3.7 with 5% phosphoric acid) and acetonitrile (65:35) at a flow rate of 1.2 mL/min. Detection was monitored at 270 nm using a Waters 486 detector.

Sample preparation

Stock solutions of carbamazepine, lamotrigine, zonisamide, and the IS chloramphenicol were at 1000 μ g/mL in HPLC grade methanol. To 250 μ L of sample (calibrator, control, or patient sample) in a 16 \times 125 mm glass culture tube, 100 μ L of IS solution, 1.5 mL NaOH, and 4.0 mL ethylacetate were added. The samples were immediately vortexed for 1 min and centrifuged at 1,700 *g* for 5 min. The organic (upper) layer was transferred to a conical tube and dried completely at 40°C using a nitrogen evaporator. The sample was then reconstituted with 100 μ L mobile phase, vortexed, and transferred to an autosampler vial for HPLC analysis.

Determination of recovery, intra-day and inter-day precision and accuracy

The absolute recovery was estimated by comparison with direct injection of aqueous drug solutions of corresponding concentrations. Intra-day precision and accuracy were evaluated by the analysis of spiked samples. The precision and accuracy for inter-day comparisons were assessed at the same concentration and summarized as coefficient of variation (CV%) and relative deviation (RD%), respectively.

RESULTS AND DISCUSSION

Specificity

Under the described conditions, the retention times of zonisamide, IS, lamotrigine, and carbamazepine were 4.3, 4.7, 5.6, and 7.3 min, respectively (Fig. 1). A wide variety of therapeutic drugs were tested for interference, including other anti-epileptic medications (and in some cases their metabolites) such as ethosuximide, gabapentin, levetiracetam, oxcarbazepine, 10-hydroxycarbamazepine (active metabolite of oxcarbazepine), phenobarbital, phenytoin, primidone, topiramate, and valproic acid. None of the therapeutic drugs tested exhibited any interference. In addition, no peak interferences were found in any of the batches of drug-free plasma. The method has been used extensively in our clinical laboratory for therapeutic drug monitoring of lamotrigine and zonisamide. An example of a chromatogram from the plasma of a patient taking lamotrigine and zonisamide chronically is shown in Fig. 2.

Linearity, accuracy, precision, and detection limits

The method exhibited a good linearity over a concentration range of 1–30 µg/mL for lamotrigine, 2–20 µg/mL for carbamazepine, and 1–40 µg/mL for zonisamide. A representative regression line for lamotrigine was $y = 1.0014x - 0.024$ ($r^2=0.9992$), with correlation coefficient greater than 0.99 on five different days. Results of intra- and inter-day accuracy and precision are shown in Table 1. The lower limit of detection was 0.5 µg/mL for lamotrigine, 0.5 µg/mL for zonisamide, and 0.25 µg/mL for carbamazepine.

Recovery

The average absolute recovery values for lamotrigine were 96% for 2.5 µg/mL, 96% for 7 µg/mL, 97% for 12 µg/mL, and 97% for 20 µg/mL. The average absolute recovery values for zonisamide were 97% for 10 µg/mL, 94% for 15 µg/mL, 95% for 20 µg/mL, and 98% for 30 µg/mL. The mean percentage recovery of the IS was 94%. The results indicate that the extraction efficiency of the method is consistent and reproducible.

CONCLUSIONS

The HPLC method described here is simple, sensitive, and specific and allows for the simultaneous determination of three commonly prescribed anti-epileptic drugs. Using this methodology, we now analyze over 1000 patient plasma or serum samples per year for lamotrigine and/or zonisamide concentrations, drugs for which reliable immunoassays have yet to be made available commercially.

Acknowledgements

M.D.K. is supported by a Clinical-Scientist development award K08-GM074238-01A1 from the National Institutes of Health and a Competitive Medical Research Fund (CMRF) grant from University of Pittsburgh.

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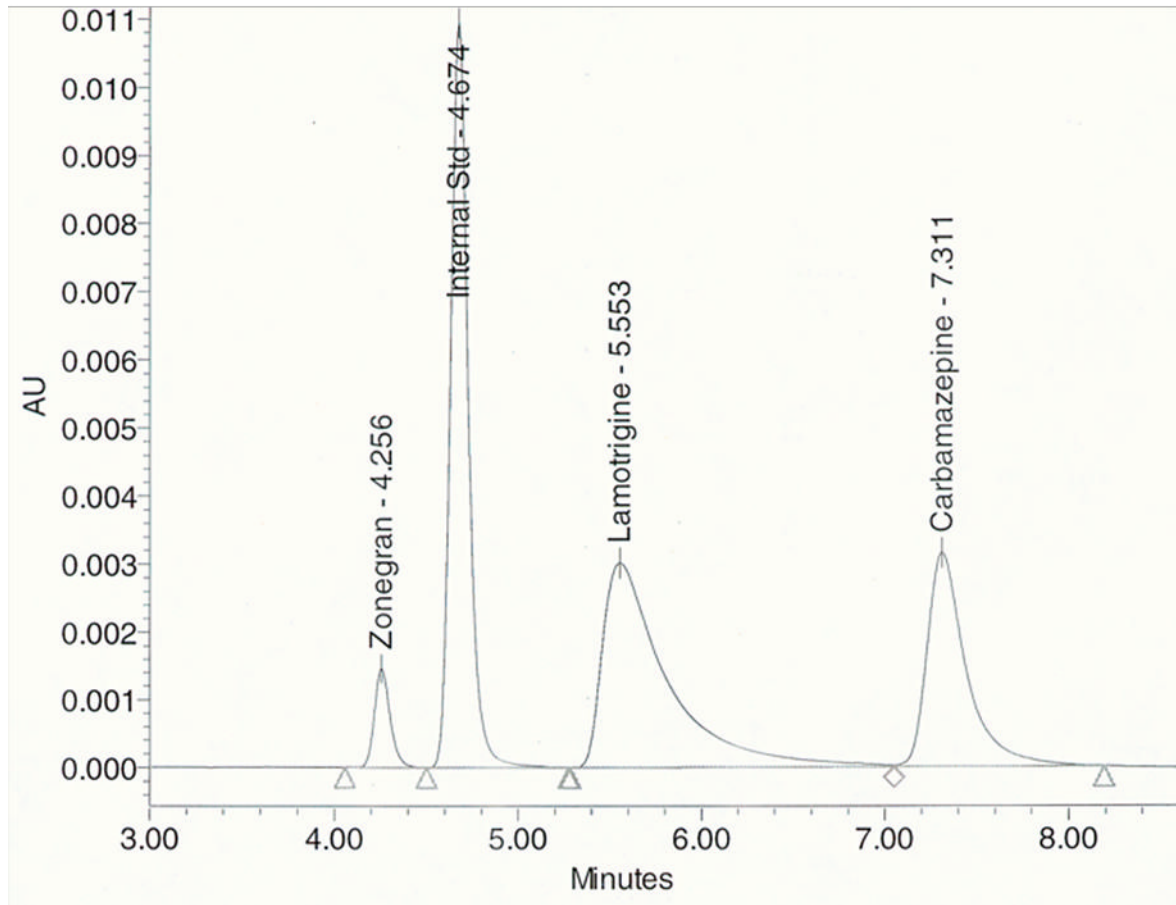


Figure 1.

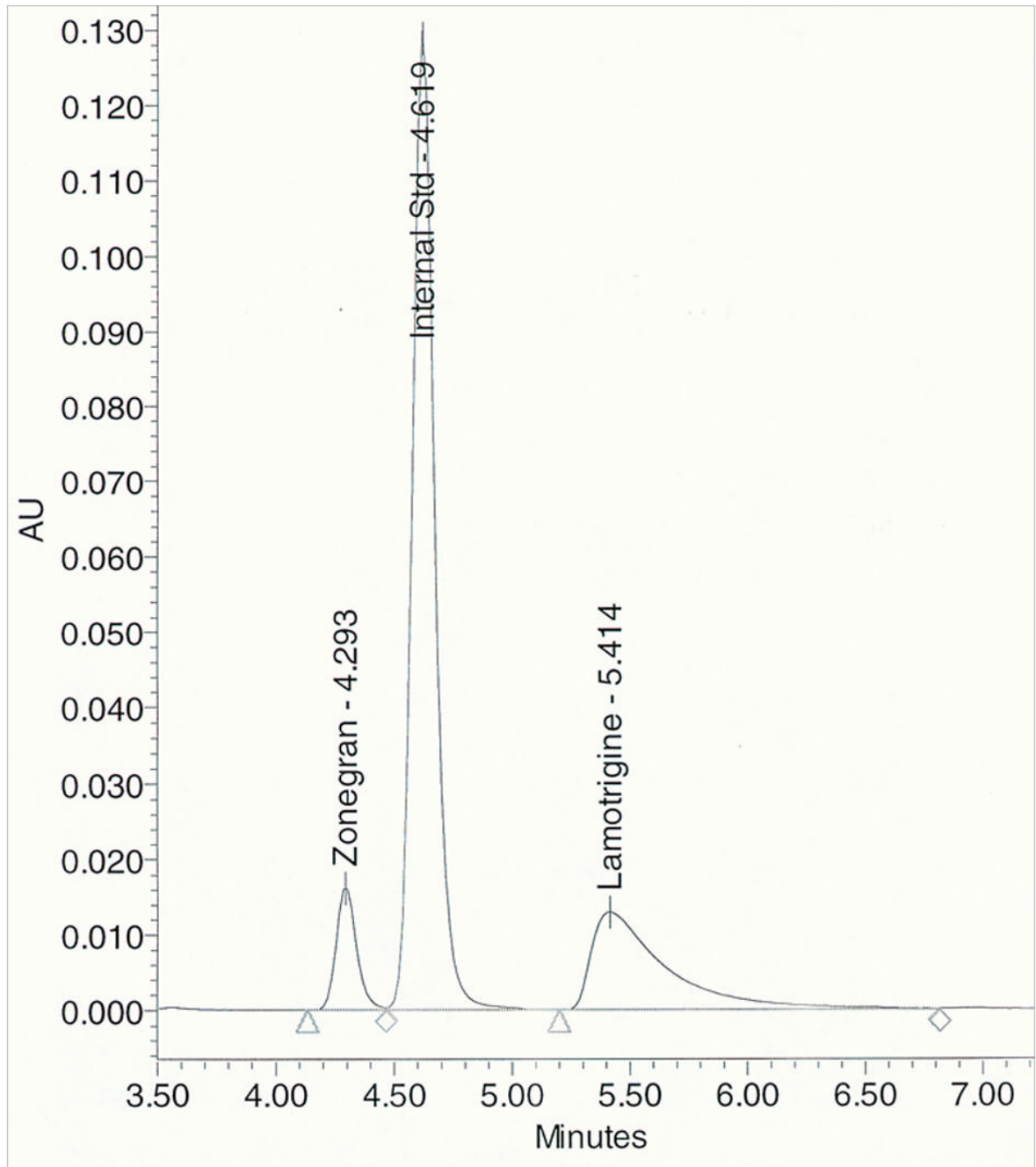


Figure 2.

Table 1

Intra-day and inter-day accuracy and precision for lamotrigine and zonisamide determination in human plasma/serum (all concentrations in $\mu\text{g/mL}$)

Lamotrigine			
Intra-day (n=5)			
Nominal concentration	10.5		
Mean	10.66 ± 0.14^a		
Accuracy as RD (%)	1.6		
Precision as CV (%)	1.3		
Inter-day (n=10)			
Nominal concentration	7.0	12	
Mean	7.57 ± 0.55	12.25 ± 0.91	
Accuracy as RD (%)	8.1	2.1	
Precision as CV (%)	7.3	7.5	
Zonisamide			
Intra-day (n=5)			
Nominal concentration	21.5		
Mean	21.74 ± 0.14		
Accuracy as RD (%)	1.1		
Precision as CV (%)	0.6		
Inter-day (n=10)			
Nominal concentration	20	30	
Mean	20.94 ± 1.85	32.72 ± 2.38	
Accuracy as RD (%)	4.7	9.1	
Precision as CV (%)	8.8	7.3	

^aValues are reported as mean \pm SD.