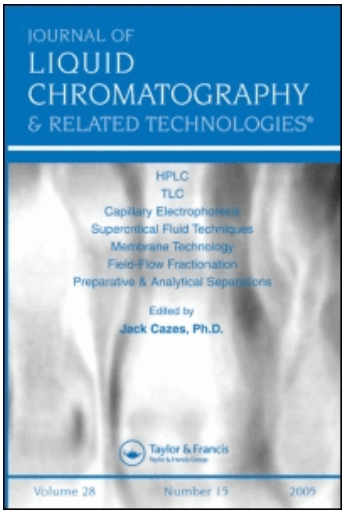

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A Validated HPLC Method For the Determination of Carbamazepine and Carbamazepine 10,11-Epoxide in Human Plasma

R. Brent Miller[a]; Manon Vranderick[a]
[a] Analytical Research Center, L.A.B. Bio-Research International Inc., Montréal, Québec, Canada

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A VALIDATED HPLC METHOD FOR THE DETERMINATION OF CARBAMAZEPINE AND CARBAMAZEPINE 10,11-EPOXIDE IN HUMAN PLASMA

R. BRENT MILLER AND MANON VRANDERICK
Analytical Research Center
L.A.B. Bio-Research International Inc.
87 Senneville Road
Senneville, Montréal, Québec H9X 3R3, Canada

ABSTRACT

A validated reversed-phase high-performance liquid chromatographic (HPLC) procedure employing ultra-violet (UV) detection for the analysis of carbamazepine (Tegretol®, CBZ) and its predominant metabolite, carbamzepine 10,11-epoxide (CBZ-E), in human plasma is reported. The method is rapid and coupled with standard HPLC procedures leads to a sensitive, accurate, and reproducible assay. The retention times of CBZ-E, CBZ, and internal standard, are 3.3, 7.0 and 10.8 minutes, respectively, with an overall chromatographic run time of 12.0 minutes. The peak height ratio versus plasma concentration is linear over the range of 0.10 to 10.0 μg/mL for each analyte and exhibits correlation coefficients of 0.9968 or better (n=7). The mean absolute recoveries of CBZ and CBZ-E using the described assay are 89.8 and 86.8%, respectively. The inter- and intra-day accuracy and precision is within 11.6% of the actual values for all concentrations investigated. Furthermore, the assay is suitable for obtaining the AUC, C_{max} and $t_{\frac{1}{2}}$ of CBZ and CBZ-E in human plasma after a single 400 mg oral dose of Tegretol®.

INTRODUCTION

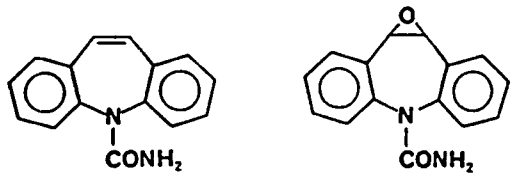
Carbamazepine (5H-dibenz [b,f] azepine-5-carboxamide) Figure 1, is a primary drug in the treatment of psychomotor epilepsy and trigeminal neuralgia [1]. A major active metabolite of CBZ is carbamazepine 10,11-epoxide. The parent drug is absorbed slowly and peak plasma concentrations are obtained within 4 to 24 hours following oral injestion [2]. Thus, it is desirable to have an analytical method to quantify both analytes simultaneously. Several methods have been used for the determination of CBZ including gas chromatography [3,4], enzyme-multiplied immunoassay technique [5], and HPLC [6-14]. The purpose of this work was to develop and validate an analytical method for both CBZ and CBZ-E, which could be readily applied to analyze several clinical samples daily.

The method reported herein for the determination of CBZ and CBZ-E is linear over the range of 0.10 to 10.0 μg/mL in human plasma. This range was selected on the basis of a C_{max} of 3.4 μg/mL for CBZ in plasma [15], and extrapolating more than 5 half-lives yields a limit of quantification (LOQ) of 0.10 μg/mL. Furthermore, this procedure was applied to ascertain the pharmacokinetics of a single 400 mg oral dose of Tegretol® in humans.

EXPERIMENTAL

Materials

Carbamazepine and the internal standard, lorazepam, were purchased from Sigma (St. Louis, MO, USA). Carbamazepine 10,11-epoxide was obtained from Ciba-Geigy (Mississauga, ON, Canada). Sodium hydroxide and sodium phosphate monobasic were purchased from Fisher Scientific (Montréal, QC, Canada). HPLC grade methanol was purchased from BDH (Ville St. Pierre, QC, Canada). HPLC grade chloroform was purchased from Caledon (Georgetown, ON, Canada). The water was deionized Type 1,



(I) (II)

FIGURE 1. Chemical structures of CBZ (I) and CBZ-E (II).

reagent grade (Millipore, Ville St. Laurent, QC, Canada). All reagents were used without further purification.

Instrumentation and Chromatographic Conditions

The chromatographic system consisted of a Waters Model 590 pump, a WISP 710B autosampler, and a Waters Lambda Max Model 481 UV detector (Waters Associates, Milford, MA, USA). A stainless-steel column (15 cm x 4.6 mm I.D.) was packed with Nucleosil C-18, particle size 5 micron (prepared in-house). The column was maintained at ambient temperature. The UV detector was set at 225 nm (0.02 aufs) to monitor the analytes. The mobile phase consisted of sodium phosphate monobasic (0.10 **M**): methanol (49:51, v/v), and was delivered at a flow rate of 1.4 mL/min. Under these conditions, the retention times for CBZ-E, CBZ and internal standard were 3.3, 7.0 and 10.8 minutes, respectively.

Preparation of Standards

Stock solutions of CBZ and CBZ-E were prepared at 1.00 mg/mL in methanol. Appropriate dilutions of the stock were made with methanol to prepare plasma standards at concentrations of 0.10, 0.20, 0.80, 1.25, 5.0, 7.5 and 10.0 μg/mL. Spiked plasma quality control samples (QCs) were prepared in pools of 20.0 mL at final concentrations of 0.30, 3.5 and 8.0

μg/mL. Individual aliquots of 600 μL were stored in 16x100 mm screw cap borosilicate tubes. A stock internal standard solution of lorazepam was prepared at 1.00 mg/mL in methanol and diluted to 3.0 μg/mL with sodium phosphate monobasic (0.1 **M**, pH 7.4). All solutions were stored at -20°C and were stable for at least one month except the internal standard, which was stable for only one week.

Sample Preparation

Aliquots of plasma (600 μL) were added to 16x100 mm screw cap borosilicate tubes. Samples were treated with 1.00 mL of the internal standard spiking solution (3.0 μg/mL lorazepam) and vortexed. To each tube 6 mL of chloroform was added. Samples were extracted at low speed (180 ± 20 oscillations/minute) on a reciprocating shaker for 10 minutes. After centrifugation for 10 minutes at **ca.** 1500 g, the organic layer was transferred into a 13x100 mm disposable borosilicate tube and evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was reconstituted in 300 μL of mobile phase, and 40 μL was injected onto the liquid chromatograph under the previously stated conditions. The reconstituted samples were stable at room temperature for at least 24 hours.

Data Acquisition

Peak heights of CBZ-E, CBE and internal standard were measured with a Spectra-Physics model 4270 integrator and down-loaded to Chrom-Station (Spectra-Physics Inc, Mountain View, CA, USA). The chromatographic data were automatically processed for peak height ratios for each drug and fitted to a weighted (1/C) linear regression.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms obtained from extracted plasma samples are illustrated in Figures 2(a-d). Figure 2(a) shows a representative chromato-

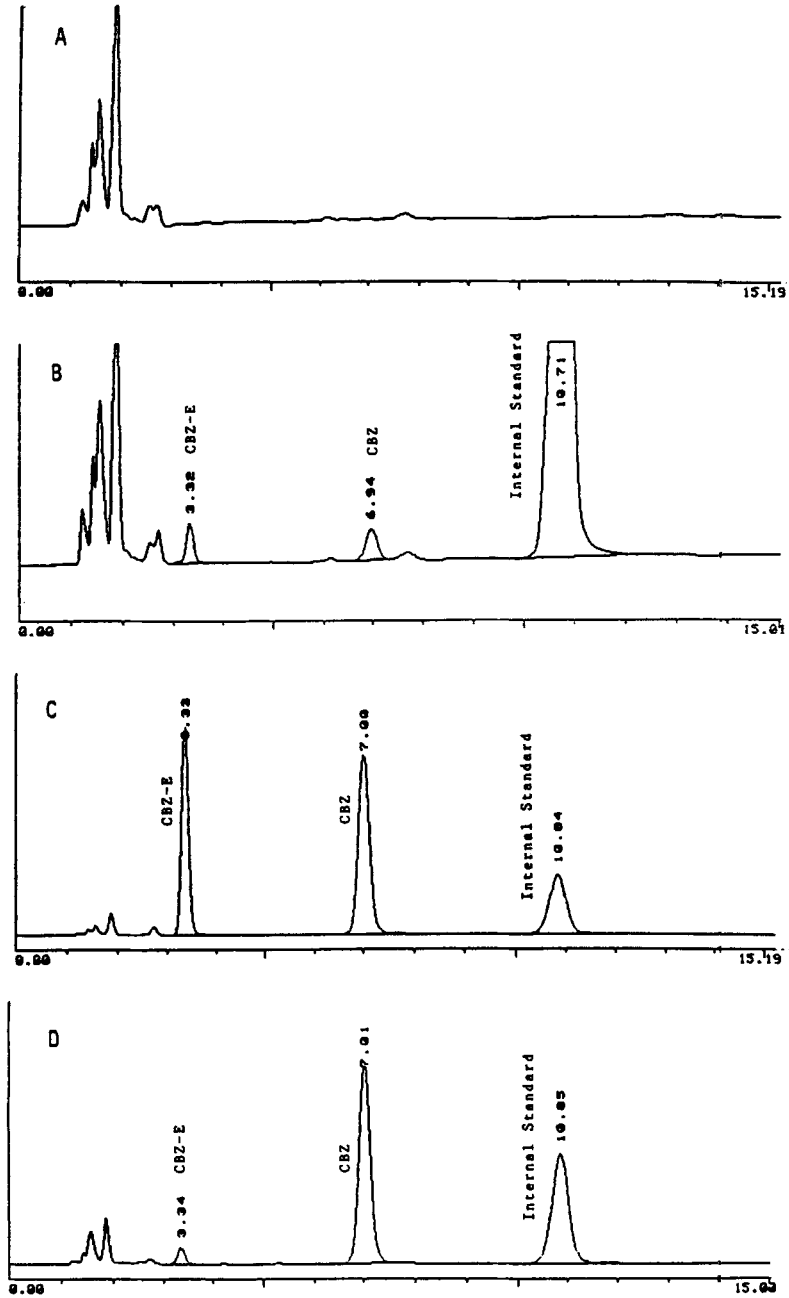


FIGURE 2. Chromatograms of (A) blank human plasma, attenuation = 8, (B) human plasma spiked at 0.10 μg/mL for CBZ and CBZ-E, attenuation = 8, (C) human plasma spiked at 10.0 μg/mL for CBZ and CBZ-E, attenuation = 128, and (D) a subject 24 hr after a single 400 mg oral dose of Tegretol®, attenuation = 64

gram of a processed plasma blank. This chromatogram indicates that no endogenous compounds exist at the retention times of CBZ, CBZ-E and internal standard. Figure 2(b) is a chromatogram amplified to the same degree as the blank showing the limit of quantification (0.10 μg/mL). Figure 2(c) is a plasma sample at the upper limit (10.0 μg/mL) of the calibration range. Figure 2(d) is a plasma sample taken from a subject 24 hr after a single 400 mg oral dose of Tegretol®. The retention times of CBZ-E, CBZ and internal standard are 3.3, 7.0 and 10.8 minutes, respectively. The overall chromatographic run time was 12.0 minutes.

Linearity and Quantification Limit

A linear response in peak height ratios of both CBZ and CBZ-E to internal standard over the range of 0.10 to 10.0 μg/mL was observed with a minimum signal-to-noise ratio of 10:1. The correlation coefficients for either compound were 0.9968 or better (n=7).

Recovery

The absolute recoveries of CBZ and CBZ-E were evaluated by comparing the concentrations found in plasma samples spiked with known amounts of each analyte to the concentrations found in solution (adjusted for sample concentration due to extraction and reconstitution). Spiked human plasma at two concentrations; one at four times the LOQ and the other at the upper limit of the assay, in replicates of eight, were extracted as previously described except the internal standard was not added. The absolute peak heights from the extracted samples were compared to unextracted standard solutions prepared in mobile phase. Similarily, the recovery of the internal standard was determined at the final recommended concentration. These results are provided in Table 1.

Specificity

Human plasma was collected from 10 healthy donors and screened for interference at the retention times of CBZ, CBZ-E and internal standard.

TABLE 1

Recovery of CBZ, CBZ-E and Internal Standard From Human Plasma*

Drug	Concentration (μg/mL)	% Recovery
CBZ	0.10	94.3
	10.0	89.5
CBZ-E	0.10	90.8
	10.0	83.9
Internal Standard	3.0	85.5

*n=8

No significant interference had been observed in drug free plasma samples. Furthermore, the anticonvulsant drugs valproic acid, phenytoin, and ethosuximide were also tested to establish specificity along with the following over-the-counter (OTC) drugs caffeine, ibuprofen, aspirin, nicotine, acetaminophen, and theophylline. These anticonvulsant and OTC drugs did not interfere with the analysis of CBZ and CBZ-E.

Precision and Accuracy

The inter-day precision and accuracy was assessed by the repeated analyses of plasma specimens containing different concentrations of CBZ and CBZ-E (Table 2). The precision was based on the calculation of the relative standard deviation (% RSD). The accuracy was based on the calculation of the relative error (% RE) of the mean found concentration compared to the nominal concentration. Two samples at each QC concentration (low, medium and high) together with a calibration curve were run as

TABLE 2

(a) Inter-day Precision and Accuracy of CBZ in Human Plasma

Nominal Conc. (μg/mL)		n	Mean Measured Conc. (μg/mL)	SD	% RSD	% RE
STD	0.10	7	0.105	0.008	8.1	4.7
STD	0.20	7	0.206	0.013	6.3	2.8
STD	0.80	7	0.783	0.061	7.8	-2.0
STD	1.25	7	1.215	0.047	3.9	-2.7
STD	2.50	7	2.403	0.154	6.4	-3.9
STD	5.00	7	4.969	0.398	8.0	-0.6
STD	7.50	7	7.560	0.447	5.9	0.8
STD	10.00	7	10.108	0.529	5.2	1.1
QC	0.30	14	0.307	0.017	5.5	2.4
QC	3.50	14	3.907	0.233	6.0	11.6
QC	8.00	14	8.492	0.540	6.4	6.2

b) Inter-day Precision and Accuracy of CBZ-E in Human Plasma

Nominal Conc. (μg/mL)		n	Mean Measured Conc. (μg/mL)	SD	% RSD	% RE
STD	0.10	7	0.104	0.005	5.0	-4.0
STD	0.20	7	0.201	0.014	4.1	1.1
STD	0.80	7	0.793	0.061	2.1	1.1
STD	1.25	7	1.214	0.051	1.6	1.4
STD	2.50	7	2.440	0.099	1.2	0.8
STD	5.00	7	5.045	0.251	2.3	0.2
STD	7.50	7	7.588	0.361	1.1	-0.8
STD	10.00	7	9.965	0.419	2.3	0.0
QC	0.30	14	0.326	0.017	5.3	8.8
QC	3.50	14	3.819	0.200	5.2	9.1
QC	8.00	14	8.544	0.467	5.5	6.8

a single batch. To be regarded as a separate batch, the entire sample processing must take place in a time domain completely separate from one another. At spiked plasma concentrations of 0.30, 3.5 and 8.0 μg/mL for CBZ, the method yields relative standard deviations (% RSD) of 5.5, 6.0 and 6.4%, respectively. The % relative error (% RE) of the mean concentrations obtained from the calibration curve ranged from -3.9 to 4.7% of the nominal concentrations for CBZ. At spiked plasma concentrations of 0.30, 3.5 and 8.0 μg/mL for CBZ-E, the method yields % RSD of 5.3, 5.2 and 5.5%, respectively. The % RE of the mean concentrations obtained from the calibration curve ranged from -4.0 to 1.4% of the nominal concentrations for CBZ-E.

The intra-day precision and accuracy was determined by the evaluation of a typical production run consisting of CBZ and CBZ-E at concentrations of 0.30, 3.5 and 8.0 μg/mL. The % RSD of these samples were within 7.5%, while the % RE of the mean measured concentrations ranged from -7.3 to 8.7% of the nominal concentrations. These results are provided in Table 3.

Application

Eight human volunteers (all male) took a 400 mg (2 x 200 mg) oral dose of Tegretol®. The volunteers did not use any concomitant medication. Blood samples were collected in 10 mL EDTA Vacutainers prior to dosing and at 17 subsequent time points following administration for a period of 120 hours. Following collection, the the blood samples were centrifuged at 1500 g for 7 minutes at 8°C and the plasma was stored at -20°C until analyzed. All samples were analyzed by the method presented here.

Estimates of the AUC, C_{max} and $t_{½}$ for both CBZ and CBZ-E were obtained. The AUC was calculated using the trapeziodal rule. Peak plasma concentrations (C_{max}) were read from the concentration-time profiles, while the $t_{½}$ values were calculated from the slope of the β phase by linear

TABLE 3

(a) Intra-day Precision and Accuracy of CBZ in Human Plasma

Nominal Conc. (μg/mL)		n	Mean Measured Conc. (μg/mL)	SD	% RSD	% RE
QC	0.30	6	0.278	0.0160	5.8	-7.3
QC	3.50	6	3.806	0.2840	7.5	8.7
QC	8.00	6	7.832	0.4252	5.4	-2.1

b) Intra-day Precision and Accuracy of CBZ-E in Human Plasma

Nominal Conc. (μg/mL)		n	Mean Measured Conc. (μg/mL)	SD	% RSD	% RE
QC	0.30	6	0.316	0.0146	4.6	5.3
QC	3.50	6	3.506	0.1982	5.7	0.2
QC	8.00	6	7.572	0.4427	5.8	-5.3

TABLE 4

Estimates of Pharmacokinetic Paramters Following a 400 mg Oral Dose of Tegretol®*

Parameters	CBZ	CBZ-E
AUC (μg hr/mL)	208.67 ± 53.72	11.05 ± 2.09
$t_{½\beta}$ (hours)	31.1 ± 8.7	11.3 ± 4.8
C_{max} (μg/mL)	3.22 ± 0.48	0.17 ± 0.02

* mean ± SD

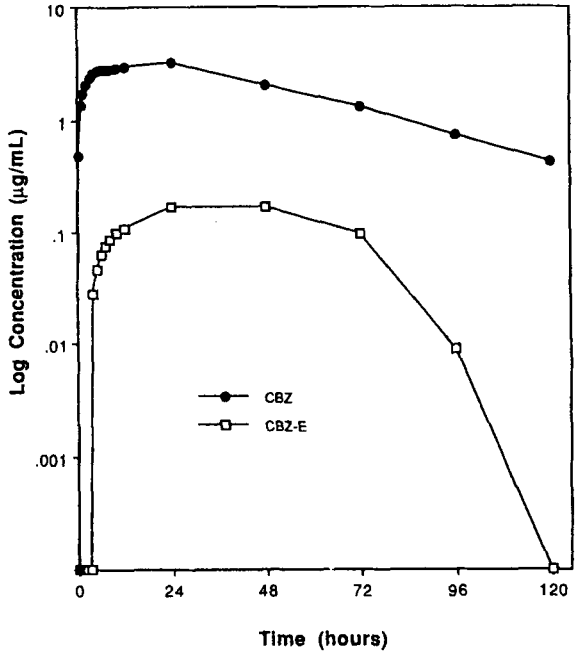


FIGURE 3. Representative plasma concentration-time profile of CBZ (●) and CBZ-E (□) of a subject after a single 400 mg oral dose of Tegretol®.

regression analysis. The time course of a typical plasma concentration profile of CBZ and CBZ-E is depicted in Figure 3. Table 4 summarizes the pharmacokinetic data of the eight volunteers.

Conclusion

The described method for the analysis of CBZ and CBZ-E in human plasma is specific, sensitive, and robust. The intra- and inter-assay precision of the method was below 8.1%, while the accuracy of the method was within 11.6% even at the lowest concentration for both drugs. Furthermore, the method is fast and requires a relatively simple sample preparation, resulting in **ca.** 90 samples being processed daily.

This procedure allows the quantification of CBZ and CBZ-E plasma levels for at least 96 hours following a single 400 mg oral dose of Tegretol®, and permits the complete characterization of the resulting plasma profiles.

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