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Carbamazepine and carbamazepine-10, 11-epoxide therapeutic drug monitoring and biochemical and hematological evaluation in bipolar disorder outpatients

Carbamazepina e carbamazepina-10, 11-epóxido de monitoramento de drogas terapêuticas e avaliação bioquímica e hematológica em pacientes ambulatoriais com transtorno bipolar

Monitoreo de fármacos terapéuticos con carbamazepina y carbamazepina-10, 11-epóxido y evaluación bioquímica y hematológica en pacientes ambulatorios con trastorno bipolar

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Abstract

The objective of this study was to validate an analytical technique with HPLC-PDA for plasma measurement of carbamazepine (CBZ) and its metabolite carbamazepine-10, 11-epoxide (CBZ-E) for therapeutic monitoring (TM) of patients diagnosed with bipolar disorder (BD), as well as evaluating hematological and biochemical parameters of patients using CBZ. Sixteen patients registered with the Public Service of Psychiatry were selected. CBZ and CBZ-E measurements were performed with HPLC-PDA shimadzu LC-20 AT Prominence, under concentration gradient. Validation criteria: selectivity, linearity, precision, accuracy, recovery, stability. Hematological and biochemical tests were performed using conventional methods. As a result, it was obtained: accuracy >85% and precision <15%, good selectivity, robustness and stability, LOQ = 250 $ng.mL^{-1}$ and HQL = 60,000 $ng.mL^{-1}$. Among patients, 25% and 6.25% had CBZ and CBZ-E levels within the therapeutic range, respectively. There were hematological and biochemical changes related to the drug. The validated method is reliable for its intended purpose. TM proved to be extremely useful for detecting therapeutic failure.

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Keywords: Therapeutic monitoring of drugs; Carbamazepine; Carbamazepine-10, 11-Epoxide; Hematological and biochemical parameters; HPLC-PDA validation; Bipolar disorder.

Resumo

O objetivo deste estudo foi validar uma técnica analítica com HPLC-PDA para dosagem plasmática de carbamazepina (CBZ) e seu metabólito carbamazepina-10, 11-epóxido (CBZ-E) para monitoramento terapêutico (TM) de pacientes com diagnóstico de transtorno bipolar (TB), bem como avaliar parâmetros hematológicos e bioquímicos de pacientes em uso de CBZ. Dezesseis pacientes cadastrados no Serviço Público de Psiquiatria foram selecionados. As dosagens de CBZ e CBZ-E foram feitas com HPLC-PDA Shimadzu LC-20 AT Prominence, sob gradiente de concentração. Critérios de validação: seletividade, linearidade, precisão, exatidão, recuperação, estabilidade. Os exames hematológicos e bioquímicos foram realizados com métodos convencionais. Como resultado, foi obtido: exatidão >85% e precisão <15%, boa seletividade, robustez e estabilidade, LOQ = 250 ng / mL e HQL = 60.000 ng / mL. Entre os pacientes, 25% e 6,25% apresentavam níveis de CBZ e CBZ-E dentro da faixa terapêutica, respectivamente. Houve alterações hematológicas e bioquímicas relacionadas ao medicamento. O método validado é confiável para a finalidade pretendida. TM se mostrou extremamente útil para detectar falha terapêutica.
Palavras-chave: Monitoramento terapêutico de medicamentos; Carbamazepina; Carbamazepina-10, 11-Epóxido; Parâmetros hematológicos e bioquímicos; Validação HPLC-PDA; Transtorno bipolar.

Resumen

El objetivo de este estudio fue validar una técnica analítica con HPLC-PDA para la medición plasmática de carbamazepina (CBZ) y su metabolito carbamazepina-10, 11-epóxido (CBZ-E) para la monitorización terapéutica (MT) de pacientes diagnosticados de trastorno bipolar (TB), así como evaluar los parámetros hematológicos y bioquímicos de los pacientes que utilizan CBZ. Se seleccionaron 16 pacientes registrados en el Servicio Público de Psiquiatría. Las mediciones de CBZ y CBZ-E se realizaron con HPLC-PDA shimadzu LC-20 AT Prominence, en gradiente de concentración. Criterios de validación: selectividad, linealidad, precisión, exactitud, recuperación, estabilidad. Las pruebas hematológicas y bioquímicas se realizaron mediante métodos convencionales. Como resultado se obtuvo: precisión> 85% y precisión <15%, buena selectividad, robustez y estabilidad, LOQ = 250 ng / mL y HQL = 60.000 ng / mL. Entre los pacientes, el 25% y el 6,25% tenían niveles de CBZ y CBZ-E dentro del rango terapéutico, respectivamente. Hubo cambios hematológicos y bioquímicos relacionados con el fármaco. El método validado es confiable para su propósito previsto. La MT demostró ser de gran utilidad para detectar fallos terapéuticos.
Palabra clave: Monitorización terapéutica de fármacos; Carbamazepina; Carbamazepina-10,11-Epóxido; Parámetros hematológicos y bioquímicos; Validación HPLC-PDA; Trastorno bipolar.

1. Introduction

Bipolar disorder (BD) is a psychological, multifactorial condition that affects about 1% of the world population (Grande *et al*., 2016) and causes severe mood changes, which cause cognitive and behavioral damage to the individual, affecting their habits and its relationship with society (Martins, 2021). The disease has two classifications, type 1 and type 2, the first being related to more severe symptoms and lasting at least one week, characterized by at least one episode of fear, irritable mood, increased energy, decreased need for sleep, among others. Type 2 BD comprises recurrent episodes of hypomania, lasting four consecutive days and, necessarily, at least one episode of major depression throughout life, being responsible for frequently causing suicidal behavior (Jaworska-Andryszewska & Rybakowski, 2019; Martins, 2021).

BD is a chronic disease; its treatment is long-term. Aiming to minimize mood symptoms and maintain its stability. For the treatment, a combination of psychotropic drugs that generate good results is used, such as antidepressant drugs, mood stabilizers, anticonvulsants and antipsychotics (Vieta *et al*., 2018). CBZ is one of the drugs indicated for mood stabilization in patients with BD (Grande *et al*., 2016).

According to Resolution n. 899, May 29, 2003, from the Brazilian Health Regulatory Agency (ANVISA), carbamazepine is one of 21 drugs with a low therapeutic index and that produces an active biotransformation product, carbamezapine 10, 11-epoxide, CBZ-E119 (Korolkovas & França, 2010). Although CBZ has been considered one of the best tolerated antiepileptic drugs, several adverse and toxic effects are known and when they occur, they can negatively impact the quality of life of patients. Periodic hematology tests are necessary to assess serum CBZ levels. The adverse effects of CBZ are

reversible after dose reduction or withdrawal of the drug, however it is necessary to monitor bone marrow suppression, liver toxicity and hematological disorders such as agranulocytosis, pancytopenia and aplastic anemia (Asadi-Pooya & Sperling, 2009; Araújo *et al*., 2010; Verrotti *et al*., 2014).

Therefore, it is necessary to have a bioanalytical method capable of identify and quantify CBZ, to improve its monitorization in patients, to reduce the risks of toxicity. So, the aim of this study was to validate an analytical technique with a High-Performance Liquid Chromatography (HPLC) coupled with a photodiode array detector (DAD) for plasma quantification of CBZ and CBZ-E in patients diagnosed with bipolar disorder, in addition to evaluating hematological and biochemical parameters.

2. Methodology

This study is a quantitative and comparative study, which we quantify concentration of CBZ and CBZ-E, and compare results of biochemical analysis from pacients (Pereira *et al.,* 2018).

This study was approved by the Research Ethics Committee of the Federal University of Goiás (CEP-UFG), protocol n°241/09.

2.1 Sample acquired

Sixteen patients (5 male and 11 female) were selected, whose age was above 18 years, with BD, under CBZ treatment for at least 6 months with dosage prescription set by the Psychiatric Service of Goiânia's Municipal Department of Health. Participants were not taking part in another study and signed the free informed consent form.

Blood samples were drawn from 7:00 to 9:00 a.m. and divided in 2 tubes, with and without Ethylenediamine tetraacetic acid disodium salt (EDTA) for clot activator. the sample without clot activator for was used in biochemical assays and the sample with clot activator was used for hematological assay and CBZ and CBZ-E measurement.

2.2 Hematological and biochemical exams

Hematological and biochemical exams were performed by Rômulo Rocha Clinical Analyses Center - Centro de Análises Clínicas Rômulo Rocha - UFG. Hemogram, reticulocyte count, direct and indirect bilirubin measurement, Aspartate transaminase (AST), Alanine transaminase (ALT), urea, creatinine, gamma-glutamyl transferase (gamma-GT) and albumin parameters were evaluated.

Hemogram was performed by Abbott CELL-DYN Rub an integrated multiparameter hematology analyzer, CELL-DYN Ruby uses flow cytometric techniques to analyze the red blood cells (RBC), Platelets (PLT) and white blood cells (WBC) populations, for erythrocyte and leukocyte abnormalities blood smears were prepared on glass slides and stained with May-Grünwald-Giemsa dye (RIBEIRO, 1971). Manual reticulocyte count was performed by stained with cresol brilliant blue method. Albumin, AST, ALT, bilirubin, creatinine, gamma-GT and urea measure were assayed spectrophotometrically using a commercial kit (LABTEST, Diagnostica S.A., Minas Gerais, Brazil).

After hematological analysis blood samples without EDTA were centrifuged at 3000 × g for 10 min and frozen at -20 ºC, CBZ and CBZ-E were measured in the Núcleo de Estudos e Pesquisas Tóxico-Farmacológicas (NEPET-UFG) associated to Unidade de Pesquisa Clínica-UFG (UPC-UFG).

2.3 Chemicals and reagents

CBZ reference standard and internal standard 5-(p-methylphenyl)-5-phenylhydantoin (MPPH), were acquired from Sigma-Aldrich® of Brazil. However, CBZ-E reference standard, from PGS Labs Analytical®, was granted by the Pharmaceutical Sciences Institute - Instituto de Ciências Farmacêuticas - ICF. It was also used 0.01 mol. mL^{-1} monobasic potassium phosphate buffer pH 2.3, acetonitrile (ACN), methanol and methyl tert-butyl ether (MTBE) degree HPLC (Tedia® Brazil Produtos para Laboratórios Ltda, Rio de Janeiro, RJ, Brazil). Ultrapure water was obtained daily with the water ultra-purifying system Gehaka Master A&D TOC (São Paulo, SP, Brazil).

2.4 Chromatographic conditions

The plasmatic concentrations of CBZ and CBZ-E was measure by HPLC-PDA with concentration gradient system. The development conditions was performed using a C18 5µm 100 x 4.6mm id ACE®, mobile phase constituted of the mixture of 0.01 $mol.L^{-1}$ (pH 2.3) monobasic potassium phosphate buffer and ACN (70:30 v/v). Wavelength used for measure was 212 nm, 20 µL sample volume injected in equipment and the HPLC flow rate was 1.0 $mL.min^{-1}$. All the measurements were performed at room temperature.

2.5 Sample preparation

The stock solutions were prepared by dissolving the reference standard in methanol to a final concentration of 2500 $\mu g.mL^{-1}$ for CBZ, 2500 $\mu g.mL^{-1}$ for CBZ-E and 100 $\mu g.mL^{-1}$ for MPPH respectively, and kept at 4 °C in amber glass vials.

Blood samples were prepared diluting 125 µL of plasma and 25 µL of MPPH to 1 mL with MTBE and vortex-mixed for 1 min and centrifuged for 10 min at 10,000 rpm to complete phase separation. 800 µL of organic phase was carefully removed and transfer to an Eppendorf and air-dried, and then were dissolved in 100 µL of ACN 50% and transfer to a vial.

2.6 Method validation

This method was validated according to Brazilian Surveillance Agency, ANVISA (Brasil, 2003) and these following parameters were calculated: selectivity, linearity, precision, accuracy, recovery and stability. However, the chromatographic system suitability was calculated based on United States Food and Drug Administration Guidance for Industry (FDA, 2000).

The performance of the chosen chromatographic system was evaluated according to the FDA (Food and Drug Administration) parameters: capacity factor (‘K), resolution and asymmetry factor not less than 2 and theoretical plates number not less than 2000 (FDA, 2000).

The matrix effect was used to evaluate selectivity and recovery comparing both samples with blank, lipemic and hemolyzed plasma samples. The recovery test was performed as an indicator of trueness, 18 blank plasma samples were spiked with 3 different concentrations (6 HQC, 6 MQC e 6 LQC), and extracted conform sample preparation description. Recovery was calculated by ratio of spiked plasma by solutions I the same concentrations.

For linearity and sensibility, 6 Calibration curves were generated by covering the working concentration ranges. For each curve, 6 different calibration points were used: 150; 1.000; 3.000; 5.000; 10.000; 20.000 $ng.mL^{-1}$. Linear calibration curves were constructed by plotting the ratios of peak analyte heights, divided by the corresponding internal standard against the concentration of each compound. The data from the standard curves were analyzed using regression analysis to obtain the slopes, the intercepts and correlation coefficients. The calculation of limit of detection (LOD) and limit of quantification (LOQ) was based on the signal-to-noise approach (signal: noise ratio 10:1 and 3:1, respectively).

. Precision and accuracy assay were performed using six experimental replicates at different levels concentrations: LOQ, low- (LQC), medium- (MQC) and high-quality control (HQC). Coefficient of variation was obtained by expression:

$$CV\% = [SD/(ECA)]x100.$$

Were CV being for coefficient of variation, SD - Standard deviation, ECA - Experimental concentration average. Accuracy was expressed in percentage by relation between experimental and nominal concentrations (± 15 %), except for LOQ which accepts ± 20 %
obtained

For stability test, the control pooled plasma was aliquoted and stored at three different conditions: at room temperature (20–25 °C) with daylight exposure, in a refrigerator (2–8 °C), at −20 °C, then analyzed after time intervals of 4 to 90 hours and the difference with the baseline value calculated.

2.7 Statistical Analysis

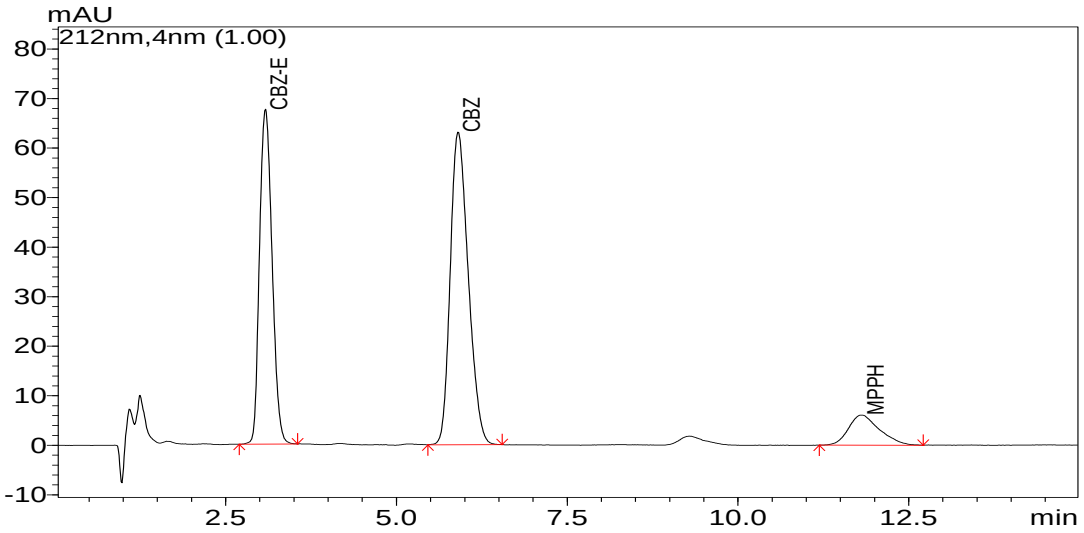
Frequency distribution was obtained through the application of the Chi-square test (χ^2) with 95% confidence interval, assuming that there is a correlation between the blood concentration of the drug, which is directly proportional to the concentration of this drug. Data dispersions were obtained for a determined parameter measured through range, mean standard-deviation and variance. The analytical technique the same descriptive statistic parameters were applied, adding the application of linear regression to the calibration curve and, consequently, straight-line equation obtaining, in addition to the CV: Coefficient of variation ≤ 15% for drug concentration measures, both of standard and of biological samples, for the complete validation of the analytical technique (Centeno, 1990).

3. Results

3.1 Chromatographic validation

A representative chromatogram of HPLC-PDA analytical validation for the plasmatic quantitation of carbamazepine and carbamazepine-10, 11-epoxide is shown in Figure 1 below.

Figure 1: Representative chromatogram of the HPLC-PDA analytical method for carbamazepine, CBZ (7500 ng.mL^{-1}) and carbamazepine-10, 11-epoxide, CBZ-E (7500 ng/mL) quantification with internal standard, MPPH (7500 ng.mL^{-1}). Chromatographic conditions: C18 column, mobile phase pH 2.3 potassium phosphate buffer: ACN (70:30), flow rate 1.0 mL min and injection volume 20 µL.



Source: Authors' data (2012).

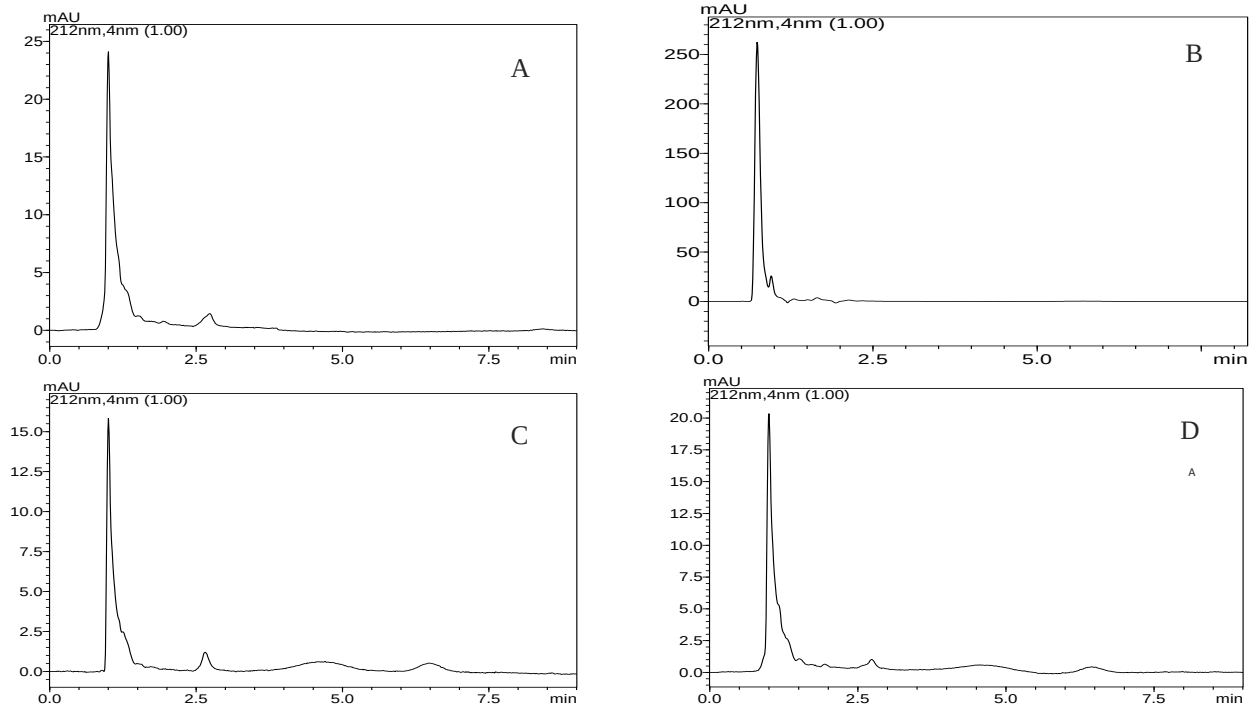
This chromatogram represented in figure 1 shows that this method could separate all peaks without overlay. Thus, it's possible to say that CBZ can be well separated of his metabolite and his concentration isn't affected by the presence of CBZ-E or MPPH.

As following in the paragraphs below, the chromatographic validation parameters for carbamazepine and carbamazepine-10, 11-epoxide quantitation are presented, according to analytical and bioanalytical validation guides (Brasil, 2003; FDA, 2000; Ribani *et al*., 2004).

System suitability: Resolution between CBZ and CBZ-E peaks was 6.72 and between CBZ and MPPH was 9.00 indicating efficient separation. Asymmetry factor was 1.22 for CBZ-E; 1.28 for CBZ and 1.28 for MPPH, indication acceptable values. Theoretical plate numbers were 2,667.14; 2,353.21; 3,508.37 for CBZ-E, CBZ and MPPH, respectively, indicating column efficiency. For capacity factor (k') values found were 2.71 for CBZ-E, 6.10 for CBZ and 13.23 for MPPH, within acceptable limits (FDA, 2000; Ribani *et al*., 2004).

Selectivity: it was observed that there was no interference of blank (A), hemolyzed plasma (C) and lipemic (D) components (Figure 2).

Figure 2: Representative chromatograms of blank plasma analysis with EDTA (A), heparin (B), hemolyzed plasma (C) and lipemic plasma (D). Mobile phase: monobasic potassium phosphate buffer (0.01M and pH 2.3), ACN (70:30 v/v); Column: C18 100mm x 4.6 mm d.i.; injection volume: 20 µL; Flow: 1.0 mL/min.



Source: Authors' data (2012).

All blank samples shown that no interference was detected, so, all concentrations detected by patient and standard samples wasn't affected by them.

Linearity (Table 1): the technique proved to be linear in the interval from 150 to 20,000 ng. mL^{-1} for CBZ/CBZ-E, obtaining correlation coefficients (R^2) higher than 0.998.

Precision and accuracy (Table 1 Intra-day accuracy presented variations from 0.75% to 12.1% for CBZ and 1.26% to 4.21% for CBZ-E. Inter-day accuracy presented variations of 2.40% to 6.62% for CBZ and 2.02% to 3.94% for CBZ-E. These results indicate the precision and accuracy of the method.

Recovery: percentage recovery for CBZ and CBZ-E were in accordance with literature data, presenting variations from 63 to 110% (Matar *et al*., 1999; Patil & Bodhankar, 2005; Queiroz *et al*., 2008; Ribani *et al*., 2004).

Stability: precision varied from 0.72% to 5.64% for CBZ controls and from 1.26% to 3.37%, for CBZ-E. Accuracy was between 95% and 105% for CBZ controls and between 96% and 107%, for CBZ-E, according to ANVISA requirements (Brasil, 2003; Ribani *et al*., 2004).

Table 1: Validation data for HPLC-PDA analytical technique for plasmatic quantification of carbamazepine and carbamazepine-10, 11-epoxide.

	Carbamazepine	**Carbamazepine-10, 11-epoxide**
Rt[1] (min)	6.10	2.71
r^2 (n = 3)	0.9998	0.9998
CV[3] (%)	0.02774	0.01296
Intra-day ($ng.mL^{-1}$; n = 6)	150 a 15,000	150 a 15,000
CV (%)	0.75 a 12.1	1.26 a 4.21
Accuracy (%)	87 a 106	92 a 110
Inter-day ($ng.mL^{-1}$; n = 6)	150 a 15,000	150 a 15,000
CV (%)	2.40 a 6.62	2.02 a 3.94
Accuracy (%)	80.3 a 101.6	89.0 a 101.6
LOQ[4] (n = 5)	150 $ng.mL^{-1}$	150 $ng.mL^{-1}$
CV (%)	6.62	2.63
Accuracy (%)	80.3	89
Absolute recovery (%)	**97± 5**	**95± 3**

Rt: retention time; r^2: regression slope; CV: coefficient of variation; LOQ: limit of quantification (<20%).

Source: authors' data (2012).

CBZ measurement in patients' plasma verified that only 25% presented concentration within the therapeutical range. The other presented concentration higher than 18.75%, lower with 25% and 31.25% with zero plasma concentration, suggesting lack of treatment adhesion. Otherwise, it was observed for CBZ-E measurement only 6.25% of the patients presented metabolite concentration within the estimated therapeutical range; 12.50% and 68.75%of the patients presented metabolite plasma concentration higher and lower, respectively; 31.25% presented zero concentration (Table 2).

Table 2: Plasmatic concentration of carbamazepine and carbamazepine-10, 11-epoxide for bipolar disorder patients, under carbamazepine treatment with the Psychiatric Service of Goiânia's Municipal Department of Health and Rômulo Rocha Clinical Analyses Center of UFG.

Patient	Age (years)	Gender	Dosage ($mg.day^{-1}$)	CBZ ($ng.ml^{-1}$) $.10^3$	CBZ-E ($\mu g.ml^{-1}$)
Therapeutical range				8.00 - 12.00	
P1	36	M[1]	400	15.88	2.78
P2	60	M	200	1.73	0.00
P3	40	M	400	8.62	0.89
P4	54	F[2]	200	0.00	0.00
P5	49	F	800	0.00	0.00
P6	48	F	1,000	13.07	
P7	50	M	400	0.86	0.11
P8	51	F	600	7.58	0.32
P9	51	F	200	9.35	0.79
P10	54	M	200	9.22	0.59
P11	31	F	400	14.47	1.23
P12	19	F	400	10.95	4.33
P13	18	F	200	0.00	0.00
P14	72	F	400	6.10	1.43
P15	53	F	200	0.00	0.00
P16	72	F	200	0.00	0.00
Mean±SD	47.63±15.65			6.12±5.77	1.68±1.59
CV%	32.86			94.28	94.64

M: male; F: female; [CBZ]: carbamazepine concentration; [CBZ-E]: carbamazepine-10, 11-epoxide concentration. SD: standard deviation; CV: CV: Coefficient of variation

Source: Authors' data (2012).

The mean values of analyzed blood parameters remained within reference limits except for the increased gamma-GT and glucose dosage and low sodium dosage (Tables 3, 4 and 5). Among analyzed patients 12.5% were anemic, 6.25% presented neutropenia, 18.75% macrocytosis, 6.25% thrombocytopenia, 37.5% reticulocytosis (Table 4). Increased levels of AST and gamma-GT were observed in one patient.

Table 3: Leucogram evaluation of bipolar disorder patients, under carbamazepine treatment with the Psychiatric Service of Goiânia's Municipal Department of Health and Rômulo Rocha Clinical Analyses Center of UFG (2012-Goiânia).

Patient	Leuc (. μl^{-1})	Rod (. μl^{-1})	Seg (. μl^{-1})	Eos (. μl^{-1})	Bas (. μl^{-1})	Lim (. μl^{-1})	Mon (. μl^{-1})	Plat (10^3. μl^{-1})
RV	3500-10,000	0-500	1500-7,000	35-500	0-100	800-4,000	100-1,000	150-400
P1	6,980	344	4616	69	0	1,377	482	238
P2	9,210	276	7,737	92	0	553	553	362
P3	3,810	190	2,248	0	0	1,143	228	157
P4	7,790	156	3,739	312	0	2,337	389	216
P5	6,650	133	4,057	399	0	1,795	266	440
P6	5,000	150	2,500	150	50	1,700	450	179
P7	13,100	786	9,170	262	0	2,227	655	306
P8	3,860	76	1,930	266	38	1,958	386	210
P9	4,700	184	1,927	47	94	2,585	329	241
P10	5,820	116	3,725	116	58	1,339	466	216
P11	4,370	175	2,185	13	44	1,661	306	196
P12	3,800	114	836	456	38	1,824	532	267
P13	4,950	99	2,425	99	50	1,931	296	116
P14	8,580	172	6,692	172	86	944	515	208
P15	8,290	415	5,140	83	83	2,155	415	258
P16	7,980	239	4,708	479	80	1,835	638	347
Mean±SD	6,5±2,6	225.7±180	3,67±1194.8	169.1±139.6	36.1±34.6	101.4±272.9	417.9±119.7	240.7±80.8
CV%	40.3	80.0	325.5	82.6	96.0	269.1	28.6	33.6

RV: reference values, LEUC: leucocytes, ROD: rod neutrophils, SEG: segmented neutrophils, EOS: eosinophils, BAS: basophils, LIM: lymphocytes, MON: monocytes, PLAT: platelet counts, SD: standard deviation, CV: Coefficient of variation

Source: Authors' data (2012).

Table 4. Erythrogram evaluation of bipolar disorder patients, under carbamazepine treatment with the Psychiatric Service of Goiânia's Municipal Department of Health and Rômulo Rocha Clinical Analyses Center of UFG.

Patient	RED[1]	HB[2]	HT[3]	MGV[4]	MGH[5]	MGHC[6]	RDW[7]	RET[8]
Units	tera/l	g/dL	%	fl	pg	%	%	%
RV (male)	4.0-5.0	11.5-15.5	36-45	80-95	26-32	32-36	11.6-14.8	0.5-2.0
RV (female)	4.1-6.0	13.5-17.5	41-50	80-95	26-32	32-36	11.6-14.8	0.5-2.0
P1	4.76	14.4	43.0	90.4	30.2	33.5	11.8	1.6
P2	4.63	13.6	40.2	86.8	29.3	33.8	11.6	2.4
P3	4.46	13.6	38.4	86.2	30.6	35.5	11.2	0.9
P4	4.12	12.5	39.0	84.7	30.4	32.1	11.1	1.3
P5	4.17	9.81	31.9	76.5	23.5	30.8	15.0	2.0
P6	3.63	12.2	35.7	98.4	33.7	34.3	10.5	1.8
P7	4.58	15.2	45.3	98.9	33.3	33.6	21.4	1.8
P8	3.85	11.3	33.8	88.0	29.3	33.3	11.4	2.7
P9	4.43	13.7	42.6	96.0	30.9	32.1	11.9	2.5
P11	4.60	13.5	40.0	87.0	29.3	33.8	11.6	1.8
P12	4.20	12.3	35.9	85.5	29.3	34.3	10.0	1.4
P13	4.85	14.0	41.3	85.1	28.8	33.9	12.2	1.7
P14	4.70	12.1	37.5	79.3	25.6	32.3	15.1	2.4
P15	4.50	14.3	42.7	94.7	31.7	33.5	12.2	1.5
P16	5.50	14.5	44.4	81.0	26.5	32.7	13.2	2.2
Mean±SD[10]	4.5±0.4	13.1±1.4	39.4±3.9	87.9±6.7	29.5±2.7	33.3±1.1	12.7±2.8	1.9±0.5
CV%	10	10.8	10	7.6	9.2	3.4	22.1	26.8

RED: red cell count, HB: hemoglobin dosage, HT: hematocrit, MGV: mean globular volume, MGH: mean globular hemoglobin, MGHC: mean globular hemoglobin concentration, RDW: red cell distribution width, RET: reticulocytes, RV: reference value, SD: standard deviation, CV: Coefficient of variation.

Source: Authors' data (2012).

Table 5. Biochemical dosage evaluation of selected bipolar disorder patients, under carbamazepine treatment with the Psychiatric Service of Goiânia's Municipal Department of Health and Rômulo Rocha Clinical Analyses Center of UFG.

Patient	AST	ALT	TB	DB	IB	γ-GT (m)	γ -GT (f)	UREA	CREAT	K^+	Na^+	GLUC	ALB
Units	UI,L^{-1}	UI,L^{-1}	$mg.dL^{-1}$	$mg.dL^{-1}$	$mg.dL^{-1}$	$UI.L^{-1}$	$UI.L^{-1}$	$mg.dL^{-1}$	$mg.dL^{-1}$	$mEq.L^{-1}$	$mEq.L^{-1}$	$mg.dL^{-1}$	$g.dL^{-1}$
RV	11.0-39.0	11.0-45.0	≤1.20	≤0.40	≤0.80	7-58	5-39	15-45	0.7-1.2	3.6-5.0	135-148	65-99	3.5-5.5
P1	19	20	0.30	0.17	0.13	75	-	16	0.70	4.2	132	86	4.32
P2	27	32	0.15	0.11	0.04	60	-	23	0.66	5.0	125	71	3.98
P3	19	16	0.30	0.11	0.19	35	-	27	0.91	4.6	134	118	4.04
P4	23	12	0.48	0.20	0.28	-	27	22	0.68	4.5	131	297	4.58
P5	25	13	0.39	0.23	0.16	-	118	22	0.61	4.4	139	125	2.91
P6	16	14	0.33	0.12	0.21	-	39	29	0.61	4.5	127	137	4.34
P7	42	20	0.55	0.20	0.35	416	-	13	0.90	3.9	140	73	3.88
P8	17	20	0.34	0.10	0.24	-	210	20	0.52	4.0	139	96	3.80
P9	19	30	0.24	0.18	0.09	-	33	23	0.67	4.6	132	91	2.26
P10	25	30	0.12	0.06	0.06	29	-	42	0.84	5.5	131	100	4.32
P11	24	20	0.18	0.12	0.06	-	31	30	0.89	4.6	123	91	3.71
P12	18	17	0.70	0.25	0.45	-	28	34	0.80	4.3	132	80	3.56
P13	28	26	0.69	0.32	0.37	28	-	26	0.54	5.0	137	74	4.07
P14	23	17	0.15	0.07	0.08	-	29	35	0.97	4.8	136	94	3.36
P15	21	20	0.25	0.15	0.10	-	33	31	0.83	4.7	133	97	4.08
P16	30	29	0.75	0.43	0.32	-	30	28	0.70	4.7	135	123	3.93
Mean± SD	23.50 ±6.4	21.00 ±6.4	0.37 ±0.2	0.18 ±0.1	0.20 ±0.1	107.2 ±152.4	57.8 ±60.1	26.3 ±7.3	0.74 ±0.14	4.6 ±0.4	132.9 ±4.91	109.7 ±53.7	3.8 ±0.6
CV%	27.32	30.81	56.75	55.55	65.00	142.26	104.01	27.93	18.92	8.73	3.69	49.01	15.18

AST: Aspartate transaminase; ALT: Alanine transaminase; TB: Total bilirubin; DB: direct bilirubin; IB: indirect bilirubin; γ-GT: gamma-glutamyl transferase; CREAT: creatinine; K+: potassium; Na+: sodium; GLUC ALB: albumin; RV: Reference values; m: male; f: female: SD: Standard deviation: CV: Coefficient of variation

Source: Authors' data (2012).

It was show in Tables 3, 4 and 5, that, nonstatistical difference was found of Biochemical, Leucogram and Erythrogram evaluation from patients that was presented concentration within the therapeutical range of CBZ.

4. Discussion

Plasma quantification of CBZ and CBZ-E performed with HPLC-PDA is useful for monitoring therapeutic drugs in the treatment of chronic diseases. Bipolar disorder, due to its repercussions, is considered one of the most serious and prevalent psychiatric disorders. The main treatments aimed at bipolar disorder focus on pharmacological interventions to control acute episodes in the long term and to maintain the clinical picture. In this sense, pharmacological treatment with CBZ is continuous and considered essential for the reduction of manic symptoms, frequency of acute episodes and mood swings (Bojic; Becerra, 2017).

In addition, plasma quantification of CBZ and CBZ-E is useful in controlling the drug's tolerability profile during treatment initiation. In the present study it was verified that only 25% of the patients presented CBZ plasma concentrations within the therapeutic range (8 – 12 $\mu g.ml^{-1}$) demonstrating the importance of therapeutic monitoring.

The patients treated with CBZ may present blood dyscrasias such as agranulocytosis, leucopenia, eosinophilia, thrombocytopenia, leukocytosis, pancytopenia and aplastic anemia. Aplastic anemia generally occurs in the first three months of treatment, with morbidity index between 33 and 50% (Leis *et al.*, 2018). In this study, patients did not have pancytopenia, as well as reticulocytopenia, hematological data that may suggest aplasia. According to Stefano, Truini and Cruccu (2018), due to the frequent use of CBZ for long periods, aplasia may be an infrequent effect, however, users of this drug must be monitored periodically.

In this study, it was observed that 37.5% of the patients presented reticulocytotic, however only one of these patients presented anemia. When there is anemia and hypoxemia stimulating erythropoietin synthesis, this accelerates erythroblast proliferation and maturation and there is increased reticulocyte release (Failace; Fernandes, 2009).

Biochemical alterations in serum levels of gamma-GT were also observed. According to Pedre *et al*., (2020), the serum levels of CBZ above normal matches with the increase in the concentrations of gamma-GT in the blood. Furthermore, in their study on the efficacy and tolerability of CBZ for the treatment of pain in trigeminal neuralgia, 42.11% of patients presented toxicity to the use of CBZ, showing that there is a positive correlation between CBZ and gamma-GT.

In the present study, neutrophil levels remained normal and, according to Navya; Sujatha and Ashok (2017), periodic monitoring and hematological examinations should be performed to rule out the possibility of neutropenia, as this is an adverse effect observed in the prolonged use of CBZ.

Increase of gamma-GT and hyponatremia are one of the main adverse reactions observed during CBZ treatment (Pedre *et al*., 2020; Narwat; Sharma; BALA, 2018). According to Navya; Sujatha and Ashok (2017), CBZ stimulates the release of antidiuretic hormone, which can cause hyponatremia and hypoosmolality in user patients.

Dominguez *et al.*, (2010) described the case of a man diagnosed with trigeminal neuralgia, who presented hyponatremia secondary to CBZ treatment. The authors consider moderate hyponatremia with sodium serum <130 $mmol.L^{-1}$ severe hyponatremia with plasmatic sodium < 125 mmol/L. Patients with chronic hyponatremia may be asymptomatic. For Barrons and Robert (2010), CBZ does not produce hematological, hepatic or hyponatremic side effects in short-term clinical trials.

5. Conclusion

The analytical technique validated for CBZ and its metabolite's plasmatic dosage is reliable for the intended finality and was proven to be extremely useful to detect therapeutic non-compliance since the studied patients presented high prevalence of sub-therapeutic and toxic levels diagnosis, being also observed hematological and biochemical alterations associated with the use of the drugs such as the increase of gamma-GT, hyponatremia, anemia, neutropenia, macrocytosis and thrombocytopenia.

The proposed analytical technique is useful to simplify the detection of CBZ and its metabolites, which can be employed for the quality control analysis of CBZ in human plasma. Thus, it is able to evaluate if the treatment is being followed by the patient or if CBZ-E is highly concentrated.

Conflict of interests

The authors declared no conflicts of interest.

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