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Determination of Selected Antiepileptic Drugs in Mouse Brain Homogenates by HPLC–DAD

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Summary. Three independent reversed phase high-performance liquid chromatography (HPLC) procedures with diode array detection (DAD) for the analysis of carbamazepine (CBZ), topiramate (TPM), and valproic acid (VPA) have been developed in order to determine drug penetration of the blood–brain barrier. Determination of CBZ was performed on C18 column with mobile phases containing methanol (55%, *v*/*v*), acetate buffer at pH 3.5 (20%, *v*/*v*), double distilled water (25%, *v*/*v*), and 0.025 M L^{-1} diethylamine (DEA). The mobile phase containing acetonitrile and water (8:2, *v*/*v*) or acetonitrile and phosphate–citrate buffer at pH 2.6 (1:1), respectively, for analysis of VPA and TPM was applied. Quantification of carbamazepine was performed at 285 nm without extraction procedure before the analysis. Determination of topiramate and valproic acid was performed using precolumn derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl). FMOC-Cl is a suitable agent, which reacts with both primary and secondary amines and also with acidic groups. Topiramate was determined at 263 nm and valproic acid at 300 nm. The proposed procedures are simple, not time-consuming, and suitable for the determination of investigated compounds in mouse brain homogenates.

Key Words: carbamazepine, topiramate, valproic acid, RP-HPLC, DAD, FMOC–Cl derivatization, mouse brain homogenates

Introduction

Carbamazepine inhibits the voltage-dependent sodium channels of the central nervous system and reduces neuronal excitability of the epileptic focus. It is used in the treatment of epilepsy, bipolar disorder, trigeminal neuralgia, and, potentially, in impulsive disorder, personality disorders, and addiction. Topiramate is indicated for treatment of epilepsy in adults and children, migraine prophylaxis in patients, bulimia with overweight, alcoholism, and binge disorder as well as bipolar disorder [1]. Valproic acid is

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an antiepileptic agent which is used in the treatment of epilepsy bipolar disorders, major depression, migraine headaches, schizophrenia, and infantile febrile convulsions [1, 2].

A number of analytical methods such as high-performance liquid chromatography (HPLC), high-performance thin-layer liquid chromatography (HPTLC), gas chromatography (GC), and micellar electrokinetic chromatography have been reported for the determination of carbamazepine (CBZ) and its metabolites in biological samples [3, 4]. Previously described procedures of CBZ determination in plasma, rats or mice brain, etc. usually required an isolation procedure prior to the chromatographic step, e.g., liquid–liquid extraction (LLE), solid-phase extraction (SPE), and protein precipitation [3, 4].

There are a lot of methods including HPLC, liquid chromatography–mass spectrometry (LC–MS) or liquid chromatography–tandem mass spectrometry (LC–MS/MS), GC, GC–MS, and capillary electrophoresis for the analysis of topiramate (TPM) in biological samples [5–9]. Berry and Patsalos determined TPM in plasma and serum by fluorescence polarization immunoassay [10].

TPM does not exhibit ultraviolet–visible (UV–vis) absorption or fluorescence emission. For this reason, analysis of the drug by HPLC with UV–vis or fluorescence detections is possible only after derivatization with appropriate reagents. Derivatization of TPM by 4-chloro-7-nitrobenzofurazan (NBD-Cl) [6] or 9-fluorenylmethyl chloroformate (FMOC-Cl) [7, 8, 11, 12] and its analysis in human serum or pharmaceutical formulations by HPLC coupled with UV or fluorescence detections have been reported. HPLC analysis was performed on Zorbax SB-C18 column with eluent containing acetonitrile and aqueous solution of NaH_2PO_4 with triethylammonium (pH 2.8) (52:48). The derivatization of TPM with FMOC-Cl depends on reaction conditions, e.g., pH of the buffer solution, concentration of the FMOC-Cl solution, reaction time, and reaction temperature [12]. There is only one report on derivatization with FMOC-Cl to detect TPM in the brain homogenate samples [9], but authors did not describe any details of the derivatization procedure. Various TPM isolation procedures such as SPE, LLE, and protein precipitation were also applied [5–9].

Several HPLC methods with UV [13, 14], fluorescence [15–17] or electrochemical detection [18], LC–MS/MS [19–22], gas chromatography with flame ionization detector (GC–FID) [23] or GC–MS [24], and capillary electrophoresis [25] for analysis of valproic acid (VPA) have been reported.

Direct determination of VPA by HPLC with UV detection has been reported [13], but the signal intensity was very low, because of the weak UV

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absorption of the drug. To improve the intensity of the VPA signal, derivatization techniques have been used. Various derivatization agents, e.g., *N*-(1-naphthyl) ethylenediamine [14], 2-bromo-4-nitroacetophenone, 2-(2-naphthoxy)ethyl2-(piperidino) ethanesulfonate [15], 4-bromomethyl-6,7-dimethoxycoumarin [16], 4-dimethylaminobenzylamine dihydrochloride [21], and 2-picolylamine [22] were applied. However, some disadvantages appeared such as multistep extraction procedure, long reaction time, light sensitivity, or toxicity of reagents for a lot of applied methods.

9-Fluorenylmethyl chloroformate (FMOC-Cl) was usually used for derivatization of compounds with hydroxyl groups, and primary and secondary amines in alkaline conditions [26]. However, application of FMOC-Cl for derivatization of carboxylic acids has also been reported. For the first time, FMOC-Cl was applied for determination of sodium valproate in human plasma before the HPLC–MS analysis [20]. Application of FMOC-Cl for derivatization and determination of valproic acid in mouse brain homogenates by HPLC with diode array detection (DAD) has not been reported previously.

The aim of this study was to develop three simple and specific procedures for the quantitative determination of antiepileptic drugs CBZ, TPM, and VPA in the mouse brain homogenates by HPLC–DAD. Simple procedure for sample preparation of mice brain homogenates containing CBZ was applied. The derivatization with FMOC-Cl was modified and new simple procedures of determination of TPM and VPA in homogenates of mice brain were elaborated.

Experimental

Chemicals

Carbamazepine (CBZ), topiramate (TPM), and sodium valproate (VPA) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Methanol (MeOH) of chromatographic quality, diethylamine (DEA), acetic acid (99–100%), sodium hydroxide, phosphoric acid, and citric acid were obtained from Merck (Darmstadt, Germany). Water was double distilled.

The acetate buffer at pH 3.5 was prepared by mixing up 0.2 M L^{-1} acetic acid and 0.2 M L^{-1} sodium acetate in volume ratio 19:1. The borate buffer at pH 7.8 was prepared by dissolving 0.625 g of boric acid and 0.750 g of potassium chloride in 100 mL of double distilled water and adjusting to a pH of 7.8 using a 1 M L^{-1} potassium hydroxide solution. A borate buffer

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(0.05 M L^{-1}) at pH 9.3 was prepared in water and adjusted to target pH with 0.05 M L^{-1} sodium hydroxide solution.

A stock solution of glycine (4 mg mL^{-1}) was prepared by dissolving glycine in double distilled water. The FMOC-Cl solution (2.5 mg mL^{-1} and 0.5 mg mL^{-1}) was prepared in acetonitrile and stored at 4 °C until analysis.

Sample Preparation and Derivatization

(a) CBZ

Prior to analysis, 100 μL of mouse brain homogenate was diluted with double distilled water to 500 μL. The solution was filtered through a membrane filter (0.2 μm pore size), and 20 μL was injected directly into the HPLC column.

(b) VPA

Homogenized mouse brain samples were stored at −20 °C until assay, and frozen samples were thawed in water at 37 °C. Aliquots of samples (100 μL) were pipetted into the separate Eppendorf tubes. The samples were mixed with 10 μL of 5 M L^{-1} HCl solution and extracted with 1 mL of diethyl ether. After vortex mixing for 15 s, separation of the organic phase, and its evaporation at 40 °C, the residue was reconstituted in 500 μL of the FMOC-Cl solution (500 μg mL^{-1}). Then, the 100 μL of borate buffer (0.05 M; pH 9.3) was added and briefly mixed. The samples were kept at 60 °C for 10 min, and then, a volume of 20 μL of the post reaction mixture was injected onto the HPLC system.

(c) TPM

The 0.1 μL of borate buffer at pH 7.8 and 0.1 μL of FMOC-Cl solution (2.5 μg mL^{-1}) were added to 0.1 mL of homogenized samples and diluted to 400 μL with acetonitrile. Then, the solutions were mixed for 20 s and incubated at 50 °C for 15 min in a water bath. In order to terminate the reaction, 20 μL of glycine solution (4 mg mL^{-1}) was added to the solution and mixture was vortexed for 10 s. The solution was allowed to stand for 1 min prior to injection, and then, 20 μL aliquot of the resultant solution (after filtration through a membrane filter, 0.2 μm pore size) was injected onto the HPLC system.

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Apparatus and HPLC Conditions

Chromatographic analysis was performed using liquid chromatograph LaChrom Elite (Merck) equipped with an autosampler, column oven L-7350, solvent degasser L-7612, and diode array detector. The chromatographic separation was performed on Xbridge C18 column from Waters (150 mm × 4.6 mm, 5 μm). The samples at volume of 20 μL were injected onto HPLC system. The diode array detector was set in the 200–400 nm range. The chromatographic data was acquired and processed with EZChrom Elite HPLC software (Merck).

(a) HPLC conditions for analysis of CBZ

All chromatographic measurements were carried out at 22 °C with an eluent flow rate of 1.0 mL min^{-1}. The eluent consisted of methanol 55% *v*/*v*, acetate buffer (pH 3.5) 20% *v*/*v*, double distilled water 25% *v*/*v*, and 0.025 M L^{-1} diethylamine. Quantitative analysis was performed at 285 nm.

(b) HPLC conditions for analysis of VPA

The column oven temperature was set at 50 °C, and the eluent was pumped at a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of acetonitrile and double distilled water (8:2). Quantitative analysis was performed at 300 nm.

(c) HPLC conditions for analysis of TPM

The column oven temperature was set at 52 °C, and the eluent was pumped at a flow rate of 1.0 mL min^{-1}. A mixture of acetonitrile and phosphate–citrate buffer at pH 2.6 (1:1) was used as the mobile phase. Quantitative analysis was performed at 263 nm.

Preparation of Stock Solutions and Standard Working Solutions

The stock solution of CBZ was prepared at a concentration of 0.2 mg mL^{-1} by dissolving 10 mg drug standard in 50 mL of methanol. The stock solution was stored in darkness at 4 °C in glass vials. The standard working solutions were prepared from the stock solution immediately before the analysis.

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The stock solution of TPM was prepared at the concentration of 1 mg mL^{-1} by weighing 50 mg of TPM and into a 50-mL volumetric flask and making up to volume with acetonitrile.

The stock solution of sodium valproate was prepared at concentration of 0.2 mg mL^{-1} in methanol. The stock solution was protected from light and stored for 1 week at 4 °C. The stock solution was stable during this period.

Calibration Curves

(a) CBZ

Calibration curve was prepared by analyzing six concentrations ranging from 0.1 to 3 μg mL^{-1} in water.

(b) TPM

The mounts of the drug standard were evaporated to dryness, dissolved in 0.1 mL water, and submitted to derivatization procedure described earlier, obtaining the six concentrations ranging from 0.6 to 60 μg mL^{-1}.

(c) VPA

The amounts of the drug standard were evaporated to dryness and submitted to derivatization procedure described above, obtaining the six concentrations ranging from 0.5 to 20 μg mL^{-1}.

Method Validation

The proposed methods were validated by linearity, limit of detection (LOD), limit of quantification (LOQ), and accuracy.

Calibration curves were prepared by analyzing the six concentrations ranging from 0.1 to 3 μg mL^{-1}, from 0.6 to 60 μg mL^{-1}, and from 0.5 to 20 μg mL^{-1} for CBZ, TPM, and VPA, respectively. Before the HPLC analysis, the solutions were submitted to the pretreatment procedures including dilution and derivatization described earlier. The limits of detection (LOD) and quantification (LOQ) were calculated as the concentrations of drugs giving a signal-to-noise ratio of 3:1 and 10:1, respectively.

The accuracy of the method was tested by performing the recovery studies, and it was calculated according to formula:

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$$\left(\frac{\text{Calculated amount of drugs in homogenate of mouse brain}}{\text{Nominal amount of drugs added to homogenate of mouse brain}}\right)\times 100\%. \quad (1)$$

The amounts of the analytes added to the spiked mouse brain homogenates were calculated by interpolating the ratio of the peak areas on the calibration curves. The 0.1 mL of blank homogenate of mouse brain was spiked with drug standards to obtain the following concentration: 0.8 μg mL^{-1}, 5 μg mL^{-1}, and 12 μg mL^{-1} of CBZ, TPM, and VPA, respectively. The spiked blank samples were prepared and analyzed according to the described procedures. Blank samples were obtained from the mouse brain homogenates not treated by drugs. Samples were prepared by the same procedure as the samples of mouse brain homogenates spiked by drugs or the samples obtained from the mice treated by the investigated drugs. Spiked mouse brain homogenates were obtained by an addition of an appropriate amount of drugs to the mouse brain homogenates obtained from mice not treated by the drugs.

Application of the Proposed Procedures

The proposed procedures were applied for determination of investigated drugs in adult Swiss mice (weighing 22–26 g). Mice were kept in cages under standardized housing conditions temperature of 21 °C ± 1, relative humidity of 55% ± 5, and natural light–dark cycle. Animals have free access to food and tap water. Each of the experimental groups consisted of eight animals. The mice were administered with CBZ, TPM, or VPA at doses which corresponded to their ED_{50} values. One group, the controlled one, was not treated by any drug. The brain of mice was removed from skulls, weighed, and homogenized using Abbott buffer (1:2, *w*/*v*) in an Ultra-Turrax T8 homogenizer (IKA-Werke, Staufen, Germany). The homogenates were centrifuged at 10,000 *g* for 10 min at temperature of 4 °C. The supernatant samples (100 μL) were transferred to Eppendorf tubes and were then processed for determination of investigated drugs by HPLC using procedures described above.

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Results and Discussion

Drug standards *(Fig. 1)* were chromatographed on the C18 column by the use of three different aqueous mobile phases. The chromatographic systems were examined in terms of retention, separation selectivity, peak shape, and systems efficiency in respect of their usefulness for the analysis of investigated drugs in homogenates of mice brains. System efficiency, expressed as theoretical plate number (*N*), was calculated according to the US Pharmacopeia. Peak symmetry was expressed as asymmetry factor (As) (calculated at 10% of peak height).

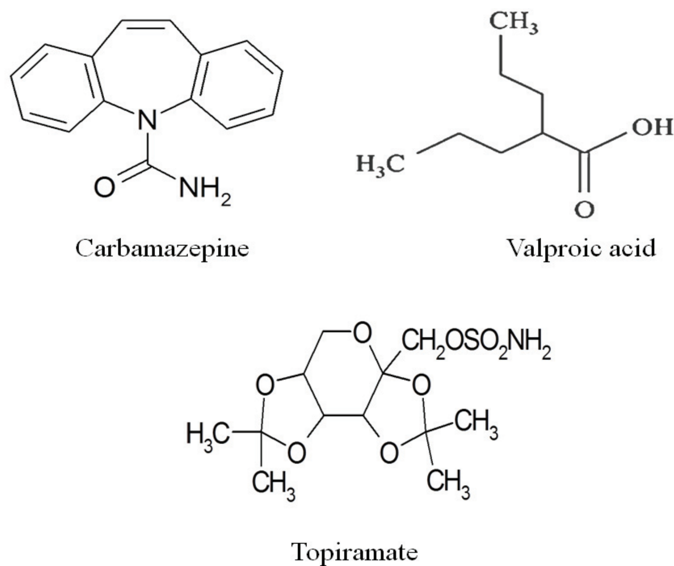

Fig. 1. Chemical structures of investigated drugs

The proposed procedure for determination of carbamazepine without the extraction step is simple and not time-consuming. Dilution and filtration for the sample pretreatment were only performed. Such simple sample pretreatment procedure was applied for the first time.

For analysis of CBZ, eluent containing MeOH, acetate buffer at pH 3.5, and addition of 0.025 M DEA was applied. CBZ is a basic compound and appears in aqueous solutions as ionized and unionized form. It is difficult for chromatographic determination due to tendency of peak tailing and

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poor system efficiency in aqueous conditions. Addition of DEA — strong base blocking free silanol groups on the surface of stationary phase, allows to obtain more symmetrical peak and higher system efficiency. The numbers of theoretical plates per meter (N/m) were from 14,429 to 32,171, and As values were from 1.34 to 2.0. The values were calculated for concentration of CBZ in the range from 0.1 to 3 μg mL^{-1} in water solutions. *Figure 2* shows chromatograms obtained for CBZ standard (A), for blank sample of brain homogenates (B) and of brain homogenates spiked with the drug (C), and for brain homogenates from mice treated with the drug (D). Retention time of peak of the CBZ was only 4.7 min. Endogenous material from mice brain homogenates did not impact on the quantification of CBZ.

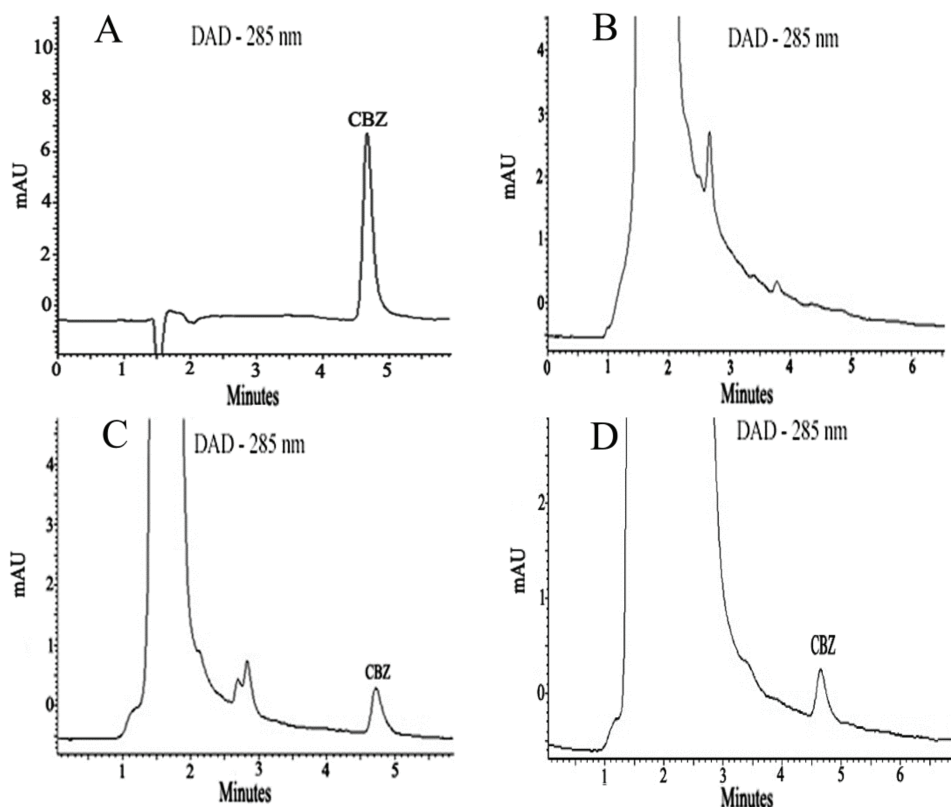

Fig. 2. Chromatograms obtained for carbamazepine standard (A); for blank samples of mouse brain homogenates (B); for brain homogenates spiked with the drug (C); and for samples of brain homogenates from mice treated with CBZ (D). System: C18/MeOH–buffer aqueous solution at pH 3.5–DEA aqueous solution 0.025 M L^{-1} (55:20:25)

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Chromatographic analysis of VPA and TPM was performed after the derivatization step. The derivatization methods using FMOC-Cl that were described previously [7, 8, 12, 20] were modified and adopted to new procedures of VPA and TPM determination. The proposed schemes of derivatization are presented in *Fig. 3*. Because of various properties (VPA is an acid; TPM is a base), derivatization procedures in various conditions were applied.

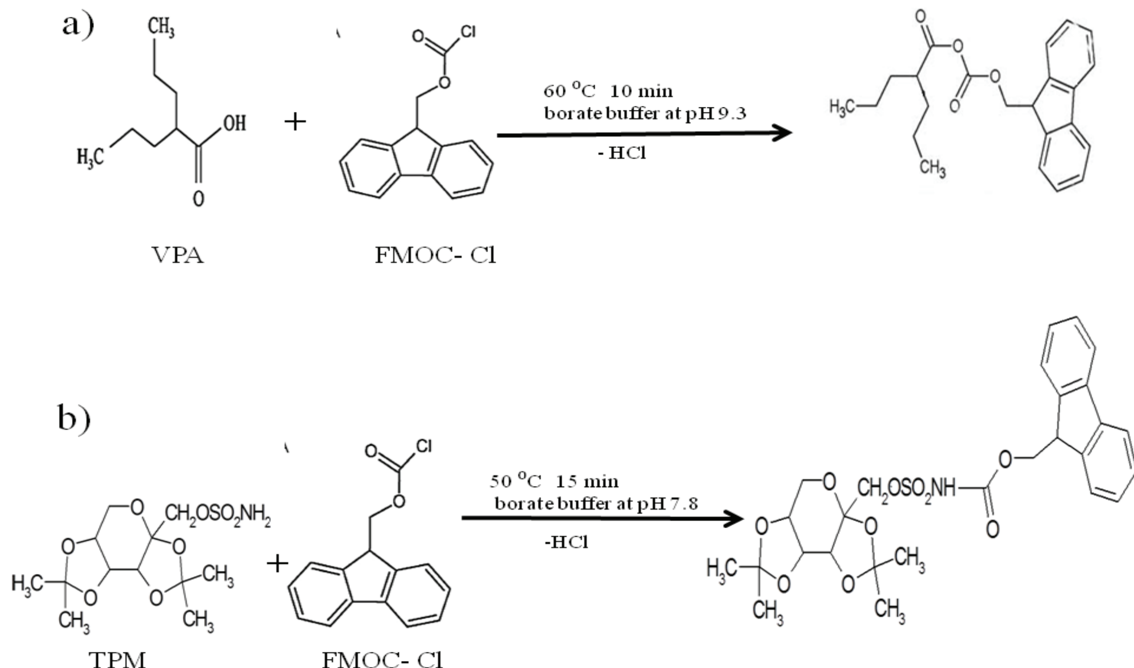

Fig. 3. Scheme of derivatization processes of VPA (A) and TPM (B)

The mobile phase for the analysis of valproic acid was performed in chromatographic system containing acetonitrile and water. *Figure 4* presents chromatograms obtained for the drug standard (A), for blank sample of mouse brain homogenates (B), for brain homogenates spiked with the drug (C), and for samples of brain homogenates from mice treated with VPA (D) (all samples after derivatization procedure). Peaks obtained for investigated drug are fully separated from derivatization reaction mixture components and symmetrical. The values of As were in the range of 0.97–1.3 for VPA, and N/m values from 22,907 to 46,436 were obtained. All values were calcu-

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lated for concentration range corresponding to calibration curve. The proposed high-performance chromatographic systems allows the separation of the analyte from the matrix components.

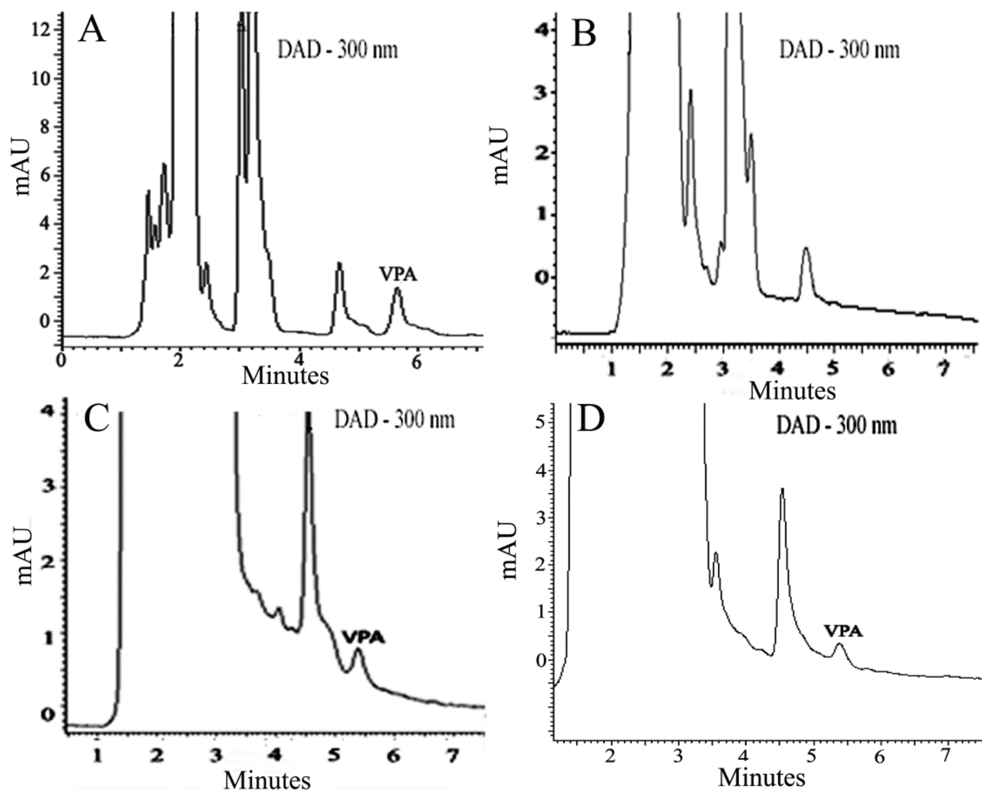

Fig. 4. Chromatograms obtained for valproic acid standard (A); for blank samples of mouse brain homogenates (B); for brain homogenates spiked with the drug (C); and for samples of brain homogenates from mice treated with VPA (D). All samples after derivatization procedure as in *Fig. 3A*. System: C18/MeCN–water (8:2)

Topiramate, as base, was chromatographed by use of buffered mobile phase containing acetonitrile and phosphate–citrate buffer at pH 2.6. Acidic eluent causes suppression of silanol ionization and makes analyte–silanol interactions hardly possible. Because of that, highly symmetric peaks of TPM (As in the range of 1.09–1.18) and high system efficiency (*N*/m from 55,882 to 71,893) were received. *Figure 5* presents chromatograms obtained

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for the TPM standard (A), for blank sample of mouse brain homogenates (B), for brain homogenates spiked with the drug (C), and for samples of brain homogenates from mice treated with TPM (D) (all samples after derivatization procedure).

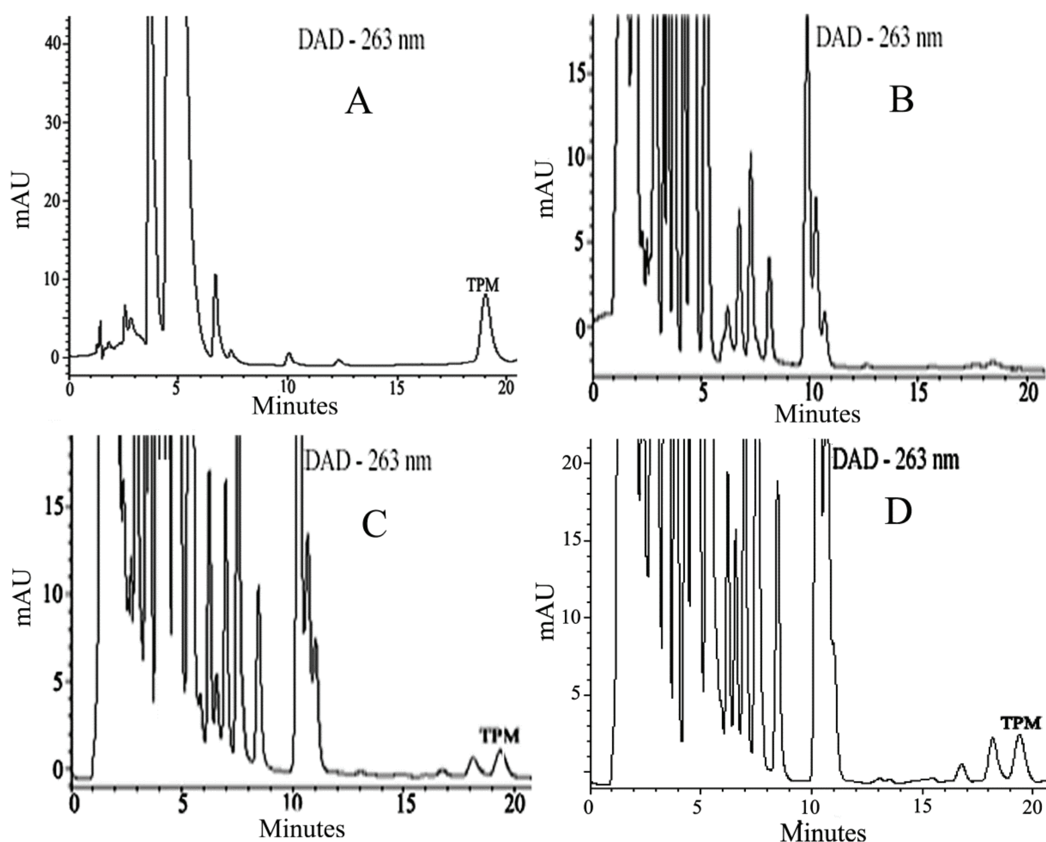

Fig. 5. Chromatograms obtained for topiramate standard A); for blank samples of mouse brain homogenates (B); for brain homogenates spiked with the drug (C); and for samples of brain homogenates from mice treated with TPM (D). All samples after derivatization procedure as in *Fig. 3B*. System: C18/MeCN–buffer aqueous solution at pH 2.6 (1:1)

Parameters of the calibration curves for quantitative analysis of investigated drugs are presented in *Table I*. Calibration curves were linear over the concentration ranges. The obtained values of LOD and LOQ allow the identification and determination of investigated drugs in applied experimental conditions.

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Table I. Parameters of calibration curves for quantitative analysis of selected psychotropic drugs in mice brain homogenates: calibration curves' equations, concentration range, regression coefficient (r), limit of detection (LOD), and limit of quantitation (LOQ)

Name of compounds	Concentrations range ($\mu g\ mL^{-1}$)	Equation of calibration curve	r	LOD	LOQ
Carbamazepine	0.1–3	$y = 197{,}304x + 3851.9$	0.9997	0.02	0.07
Topiramate	0.6–60	$y = 206{,}607x - 50{,}421$	0.9999	0.25	0.82

The wavelengths 285 nm, 263 nm, and 300 nm for determination of CBZ, TPM, and VPA, respectively, were chosen according to possibly lowest matrix effect with good peaks visibility and good selectivity. The wavelength 285 nm corresponds to λ_{max} of CBZ, and about 263 nm is λ_{max} of TPM–FMOC-Cl complex.

Accuracy of the procedures was tested by performing recovery studies. The obtained recoveries for CBZ, TPM, and VPA were 100.97%, 96.00%, and 80.55%, respectively.

The proposed procedures were applied for determination of CBZ, TPM, and VPA in samples of mice brain homogenates from mice treated by investigated drugs. Doses of drugs administrated to mice and average concentrations determined in homogenates of mice brains are presented in *Table II*. The identities of the analyte peaks in mice brain samples were confirmed by comparison of their retention times and UV spectra with the retention times and spectra of drug standards *(Fig. 6)*.

Table II. Doses of drugs used in study and average concentrations determined in homogenates of mice brains (n = 8)

Name of drugs	Drug dose ($mg\ kg^{-1}$)	Average concentration found ($\mu g\ mL^{-1}$)
Carbamazepine	8.4	1.07
Topiramate	155.9	4.25
Valproic acid	89.7	7.88

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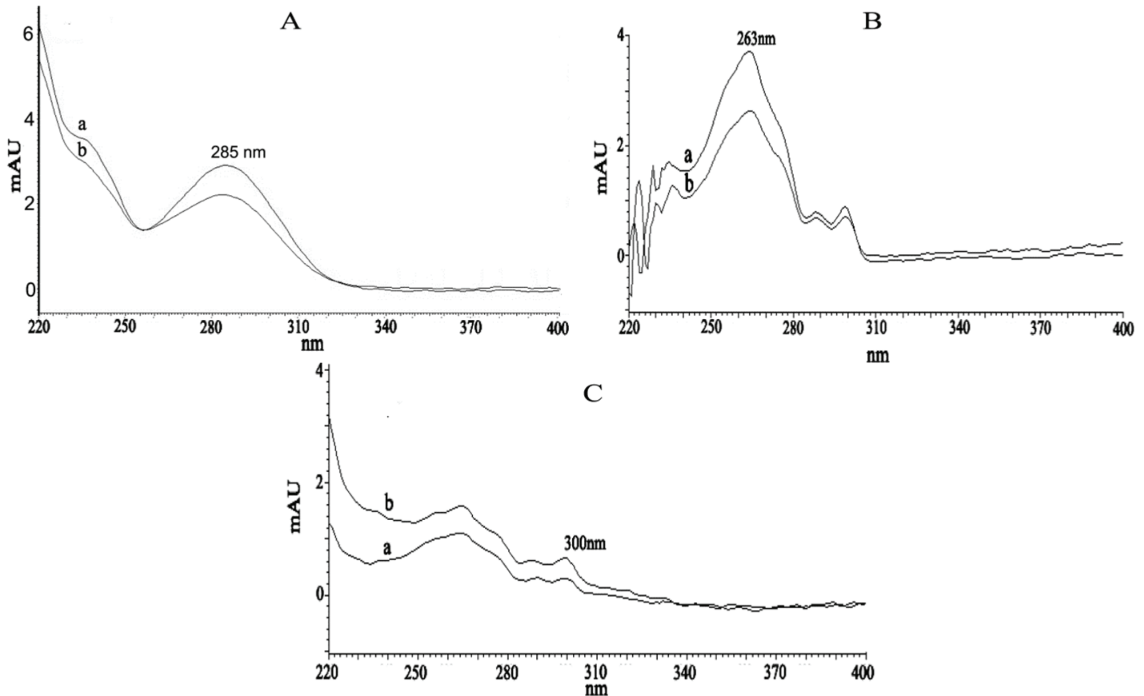

Fig. 6. UV spectra of (a) drug standards and (b) drugs detected in mouse brain homogenates obtained for (A) CBZ, (B) TPM, and (C) VPA

Conclusions

Simple HPLC–DAD procedures for the determination of CBZ, TPM, and VPA in the mice brain homogenates were developed and validated.

Quantification of CBZ was performed without prior extraction procedure which greatly simplified the procedure and shortened the time of sample preparation.

In this study, the modified procedures for the determination of TPM and VPA in the mouse brain homogenates were developed. Determination of both drugs required precolumn derivatization and 9-fluorenylmethyl chloroformate was used as derivatization reagent in proposed procedure.

The described procedures are simple and adequate for the determination of investigated drugs in homogenates of mice brains without interferences from brain endogenous compounds. The procedures, which were applicable to in vivo evaluation of the concentration of analyzed drugs in

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mouse brain, may be suitable for the investigation of the contribution of altered transport of drugs across the blood–brain barrier in experimental mouse models.

References

[1] M.J. Martín-Vázquez, In: Dr. Farid Badria (Ed.), Psychiatric Drugs in Medical Practice, Pharmacotherapy, InTech, 2012, pp. 93–96. Available from: http://www.intechopen.com/books/pharmacotherapy/psychiatric-drugs-in medical-practice
[2] R.J. Porter and B.S. Meldrum, In: B.G. Katzung (Ed.), Basic and Clinical Pharmacology, McGraw-Hill, New York, NY, 2011, p. 399
[3] P.A. Datar, J. Pharm. Anal., **5**, 213 (2015)
[4] A. Owen, J.N. Tettey, P. Morgan, M. Pirmohamed, and B.K. Park, J. Pharm. Biomed. Anal., **26**, 573 (2001)
[5] E.C. Pinto, M.D. Dolzan, L.M. Cabral, D.W. Armstrong, and V.P. de Sousa, Biomed. Chromatogr., **29**, 1461 (2015)
[6] G. Bahrami and B. Mohammadi, J. Chromatogr. B, **850**, 400 (2007)
[7] G. Bahrami, S. Mirzaeei, and A. Kiani, J. Chromatogr. B, **813**, 175 (2004)
[8] G. Bahrami, S. Mirzaeei, B. Mohammadi, and A. Kiani, J. Chromatogr. B, **822**, 322 (2005)
[9] J.J. Luszczki, A. Wlaz, S. Karwan, M. Florek-Luszczki, and S.J. Czuczwar, Eur. J. Pharmacol., **720**, 247 (2013).
[10] D.J. Berry and P.N. Patsalos, Ther. Drug Monit., **22**, 460 (2000)
[11] A. Mohammadi, N. Rezanour, M. Ansari, and R.B. Walker, Asian J. Chem., **22**, 3856 (2010)
[12] B. Mohammadi, E. Tammari, and S. Fakhri, G. Bahrami, J. Chromatogr. B, **928**, 32 (2013)
[13] H. Amini, M. Javan, and A. Ahmadiani, J. Chromatogr. B, **830**, 368 (2006)
[14] G. Kamalinia, M.R. Rouini, and P. Ghaeli, Chromatographia, **70**, 569 (2009)
[15] M.C. Lin, H.S. Kou, C.C. Chen, S.M. Wu, and H.L. Wu, J. Chromatogr. B, **810**, 169 (2004)
[16] Y. Zhong, Z. Jiao, and Y. Yu, Biomed. Chromatogr., **2**, 319 (2006)
[17] S. Hara, M. Kamura, K. Inoue, M. Fukuzawa, N. Ono, and T. Kuroda, Biol. Pharm. Bull., **22**, 975 (1999)
[18] A. Kotani, T. Kotani, N. Ishii, H. Hakamata, and F. Kusu, J. Pharm. Biomed. Anal., **97**, 47 (2014)
[19] D.S. Jain, G. Subbaiah, M. Sanyal, and P. Shrivastav, Talanta, **72**, 80 (2007)
[20] B. Mohammadi, M.B. Majnooni, P.M. Khatabia, R. Jalili, and G. Bahrami, J. Chromatogr. B, **880**, 12 (2012)
[21] H. Cheng, Z. Liu, W. Blum, J.C. Byrd, R. Klisovic, M.R. Grever, G. Marcucci, and K.K. Chan, J. Chromatogr. B, **850**, 206 (2007)
[22] K.M. Jooa, D. Choib, Y.H. Parkd, C.G. Yia, H.J. Jeonga, J.C. Choa, and Kyung-Min Lim, J. Chromatogr. B, **938**, 35 (2013)
[23] P. Shahdousti, A. Mohammadi, and N. Alizadeh, J. Chromatogr. B, **850**, 128 (2007)

Unauthenticated | Downloaded 03/15/25 01:02 PM UTC

[24] E. Gaetani, C.F. Laureri, and M. Vitto, J. Pharm. Biomed. Anal., **10**, 193–197 (1992)
[25] T.T. Pham, H.H. See, R. Morand, S. Krahenbuhl, and P.C. Hauser, J. Chromatogr. B, **907**, 74 (2012)
[26] S. Einarsson, B. Josefsson, and S. Lagerkvist, J. Chromatogr., **282**, 609 (1983)
[27] G. Bahrami and B. Mohammadi, J. Chromatogr. B, **850**, 417 (2007)
[28] R.A. Cox, In: J. Ermer, J.H. McB. Miller (Eds.), Method Validation in Pharmaceutical Analysis: A Guide to Best Practice, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005, p. 338

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