Medycyna Pracy 2017;68(5):583–591
http://medpr.imp.lodz.pl/en
https://doi.org/10.13075/mp.5893.00622
ORIGINAL PAPER

Joanna Stragierowicz[1]
Adam Daragó[1]
Sławomir Brzeźnicki[2]
Anna Kilanowicz[1]

OPTIMIZATION OF ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) WITH FLUORESCENCE DETECTOR (FLD) METHOD FOR THE QUANTITATIVE DETERMINATION OF SELECTED NEUROTRANSMITTERS IN RAT BRAIN

[1] Medical University of Lodz, Łódź, Poland
Faculty of Pharmacy, Department of Toxicology
[2] Nofer Institute of Occupational Medicine, Łódź, Poland
Department of Biological and Environmental Monitoring

Abstract

Background: Glutamate (Glu) and γ-aminobutyric acid (GABA) are the main neurotransmitters in the central nervous system for excitatory and inhibitory processes, respectively. Monitoring these neurotransmitters is an essential tool in establishing pathological functions, among others in terms of occupational exposure to toxic substances. **Material and Methods:** We present modification of the HPLC (high-performance liquid chromatography) to the UPLC (ultra-performance liquid chromatography) method for the simultaneous determination of glutamate and γ-aminobutyric acid in a single injection. The isocratic separation of these neurotransmitter derivatives was performed on Waters Acquity BEH (ethylene bridged hybrid) C18 column with particle size of 1.7 μm at 35°C using a mobile phase consisting of 0.1 M acetate buffer (pH 6.0) and methanol (60:40, v/v) at a flow rate of 0.3 ml/min. The analytes were detected with the fluorescence detector (FLD) using derivatization with o-phthaldialdehyde (OPA), resulting in excitation at 340 nm and emission at 455 nm. **Results:** Several validation parameters including linearity (0.999), accuracy (101.1%), intra-day precision (1.52–1.84%), inter-day precision (2.47–3.12%), limit of detection (5–30 ng/ml) and quantification (100 ng/ml) were examined. The developed method was also used for the determination of these neurotransmitters in homogenates of selected rat brain structures. **Conclusions:** The presented UPLC-FLD is characterized by shorter separation time (3.5 min), which is an adaptation of the similar HPLC methods and is an alternative for more expensive references techniques such as liquid chromatography coupled with tandem mass-spectrometry (LC-MS/MS) methods. Med Pr 2017;68(5):583–591

Key words: brain, neurotransmitters, glutamate, fluorescence detector, ultra-performance liquid chromatography, γ-aminobutyric acid

Corresponding author: Joanna Stragierowicz, Medical University of Lodz, Faculty of Pharmacy, Department of Toxicology, Muszyńskiego 1, 90-151 Łódź, Poland, e-mail: Joanna.Stragierowicz@umed.lodz.pl
Received: March 3, 2017, accepted: June 1, 2017

INTRODUCTION

Two of the most important neurotransmitters in the brain are glutamate (Glu) and γ-aminobutyric acid (GABA). Thanks to the opposing functions, glutamate is excitatory while GABA is inhibitory, they play an important role in the central nervous system [1]. The disruption of the homeostasis of glutamate and GABA is not only connected to the neurological and psychiatric disorders such Alzheimer's disease [2], schizophrenia [3] and epilepsy [4], but it is also the result of exposure to some toxic substances: methoxychlor [5], 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [6] and polychlorinated biphenyls (PCBs) [7]. Moreover, in the case of occupational exposure to toxic substances such as organic solvents (e.g., toluene) [8] and some metals (e.g., manganese), abnormalities in the levels of neurotransmitters were observed [9].

Due to the diversity of physiological functions associated with these neurotransmitters, many researchers

Funding: the work is financially supported by the Medical University of Lodz, project No. 502-03/3-045-01/502-34-043 entitled "Evaluation of the neurotoxicity of hexachloronaphthalene in the subchronic study in rats." Project manager: Joanna Stragierowicz, M.Sc.

measure the levels of GABA and glutamate in microdialysis samples [10], brain homogenates from experimental animals [11], and human cerebrospinal fluid and serum [12]. In the analysis of neurotransmitters (especially in microdialysis samples and brain homogenates) mainly HPLC (high-performance liquid chromatography) with fluorescence detector is used [10,11].

Generally, in all matrices, chromatographic methods, mainly liquid chromatography coupled with detectors of different sensitivity and specificity, are preferred. In the quantification of monoamine neurotransmitters (MANT), like dopamine and serotonin, an electrochemical detector (ECD) is most often used [13]. However, in the analysis of amino acid neurotransmitters (AANT) the most popular technique is HPLC coupled with a UV (ultraviolet) detector [14] or fluorescence detector (FLD) [11,15,16]. The greatest advantage of these detectors (UV, ECD, FLD) is the ability to create simple, sensitive and relatively inexpensive methods for the analysis of a single group of neurotransmitters. However, most of them also possess some limitations: low sensitivity (UV), low repeatability (ECD) and often the need for derivatization (UV, ECD and FLD).

The technique that allows for the analysis of both MANT and AANT with the highest sensitivity and selectivity is HPLC coupled with tandem mass spectrometry (MS/MS) [17]. The widely used reversed phase liquid chromatography (RPLC) coupled with mass spectrometry is often associated with the need to add appropriate derivatization reagents [18] and/or the utilization of ion-pairing reagents [17]. These additives, especially an ion-pair reagent, may cause ion suppression in mass spectrometry [19]. Additionally, in the analysis of neurotransmitters containing more than one type of functional group, such as an amino group and a carboxylic acid group, it is necessary to switch ion polarity MS conditions during detection [20]. Finally, along with said inconveniences, these advanced techniques (HPLC-MS/MS, UPLC-MS/MS) require a qualified operator and therefore are uncommon in many laboratories.

A review of the literature shows that there are no available methods for the determination of GABA and glutamate using ultra performance liquid chromatography (UPLC) coupled with a fluorescence detector (FLD). This study aims at adaptation of the HPLC to UPLC method for quantification of these 2 neurotransmitters in selected structures of the rat brain and to compare obtained results with the most commonly used techniques (HPLC-FLD, and LC-MS/MS).

MATERIAL AND METHODS

Chemicals and reagents

High-performance liquid chromatography gradient grade methanol and perchloric acid was obtained from JT Baker (USA). Sodium acetate, acetic acid, sodium hydroxide, sodium tetraborate, β-mercaptoethanol (β-ME), ortho-phthaldialdehyde (OPA), and amino acid standards: L-glutamic acid (glutamate) γ-aminobutyric acid (GABA) were obtained from Sigma-Aldrich (USA). Water was Milli-Q deionized.

Animals

Ten female Wistar rats weighing 200–250 g were used in this study. The animals were fed a standard pelletized diet "Murigram" (Agropol, Polska) and had access to water. The rats were housed 5 per cage (cage size: 35×55×25 cm) at room temperature of 22±2°C and humidity of 55±5%. The animals were subjected to a constant light/dark cycle of 12/12 h. This investigation was performed with the permission of the Local Ethical Committee for Experimentation on Animals (No. 13/ŁB703/2014). All efforts were made to minimize animal suffering.

Preparation of brain tissue samples

Rats, in random order, were decapitated. The brain was rapidly removed from the cranium and the 3 areas were dissected out on an ice-cold plate. The cerebellum, brain stem and basal nucleus were separated into 2 ml microcentrifuge Eppendorf tubes. Each brain structure was homogenized in 4 equivalents of water, and again after the addition of 4 M perchloric acid before centrifugation (12 000×g, 10 min, 4°C). Aliquots of the supernatants were stored at –80°C until the analysis.

Protein measurement

The protein concentration of each sample homogenized in water (before addition of perchloric acid) was determined by the Folin protein method, as described by Lowry et al. [21].

Stock and working solutions

Stock solutions of GABA and glutamate standards (500 μg/ml) were prepared in Milli-deionized water, aliquoted out and stored at –20°C. Working standard solutions (0.5 μg/ml, 5 μg/ml and 50 μg/ml) were prepared daily by dilutions of stock solutions, aliquoted out and stored at 4°C until derivatization and the analysis.

Derivatization protocol

The procedure of derivatization was based on a modified method previously reported [10]. Borate buffer solution (pH = 9.9, 0.05 M) was prepared by dissolving sodium tetraborate in water and the pH was adjusted using 0.1 M sodium hydroxide. The derivatization was performed by mixing 100 µl of diluted sample or standard solution, 20 µl of daily prepared OPA (5 mg OPA/ml of methanol), 75 µl of 0.05 M borate buffer (pH = 9.9) and 5 µl β-ME. The resulting solution was vortexed and analyzed after 5 min room temperature incubation period that limited exposure to direct light. A 10 µl aliquot was injected into the UPLC system.

UPLC-FLD instrumentation and conditions

Chromatographic analyses were performed with an Acquity UPLC system (Waters, USA), using an Acquity UPLC BEH (ethylene bridged hybrid) C18 column (1.7 µm, 100×2.1 mm) from Waters.

The mobile phase consisted of 100 mM acetate buffer (pH = 6.0) and methanol (60:40, v/v). Chromatographic analyses were performed at the column temperature of 35°C. The flow rate was kept constant at 0.3 ml/min.

Fluorescence detection was performed using a Waters Acquity FLR (fluorescence) Detector (Waters, USA). The instrument was operated using an excitation wavelength of 340 nm and an emission wavelength of 455 nm.

RESULTS AND DISCUSSION

Optimization of derivatization

Aliphatic amino acids with a low molecular weight such as glutamate and GABA are not naturally fluorescent. Therefore, in the quantitative assessment of these neurotransmitters using a fluorescence detector, it is necessary to carry out the derivatization. A literature review discovered various methods using different reagents for derivatization. Zhao and Suo [16] synthesized their own reagent for derivatization – 1,2-benzo-3,4-dihydrocarbazole-9-ethyl chloroformate (BCEOC). Though there are advantages of using this reagent, such as mild conditions for the derivatization reaction, simple mixture preparation, and high sensitivity, it is not commercially available.

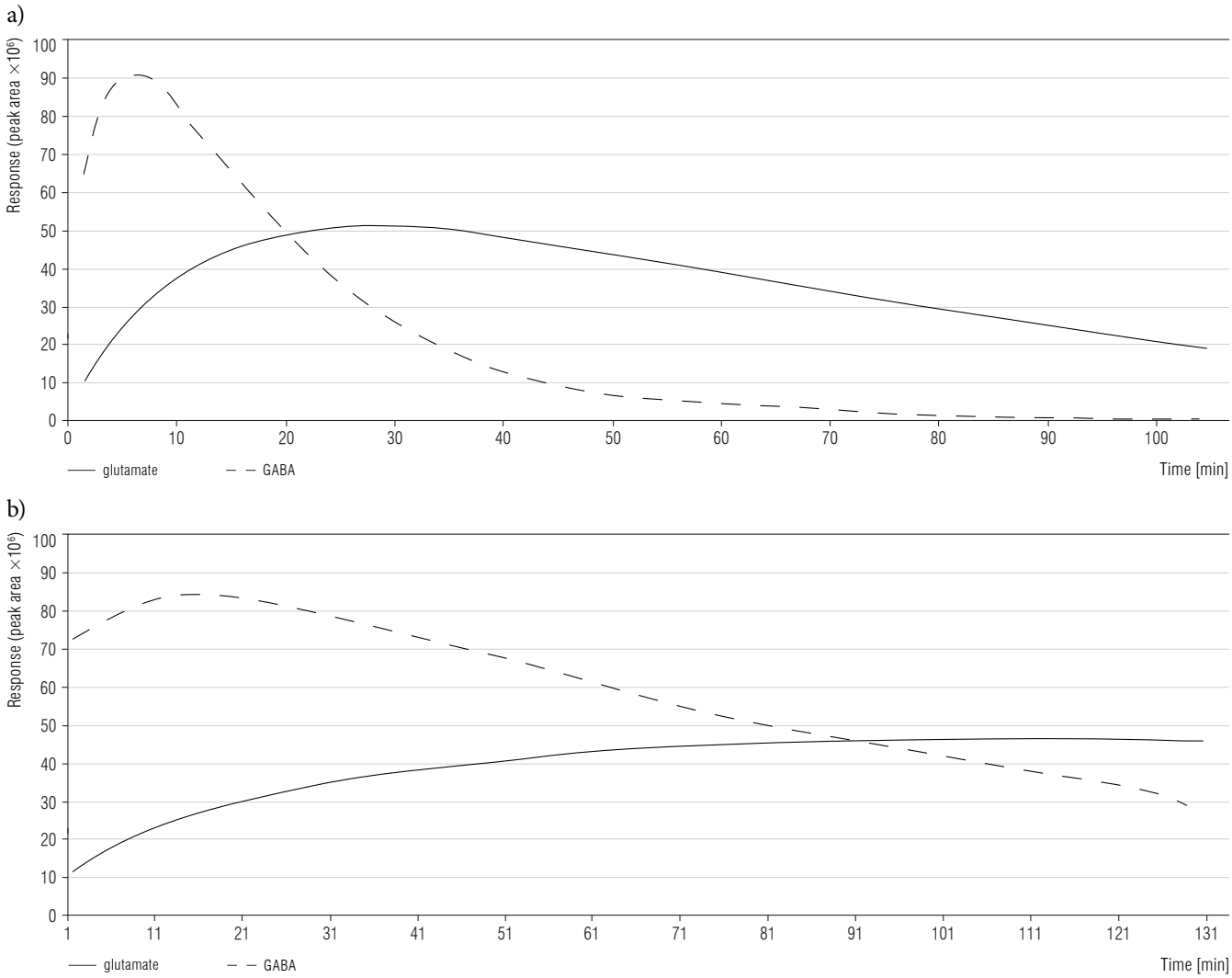
Dawson et al. [15] developed a method of derivatization using naphthalene-2,3-dicarboksyaldehyde (NDA). The reaction of this reagent with primary amines generated derivatives of 1-cyanobenz[f]isoindole (CBI) which are very stable over time, but toxic. Another reagent with wide application is phthalic dialdehyde (OPA) [5,10,11]. In this work we chose to use the OPA reagent due to its easy accessibility, relative inexpensiveness, and wide application. The stability of the derivatization products of GABA and glutamate were studied by calculating peak areas of standard solutions that had been kept absent from light at room temperature and in 4°C after OPA reaction (Figure 1).

A standard solution of neurotransmitter derivatives was analyzed every 1 min (in first 10 min) and then every 5 min. The results presented in the Figure 1 show that the concentration of derivatives of GABA and glutamate change differently over time. Orthophthaldialdehyde-γ-aminobutyric acid (GABA-OPA) complex, both at room temperature and at 4°C rapidly reaches its peak value and is less stable over time than the Glu-OPA complex. At room temperature, both of neurotransmitters derivatives achieved their peak values faster than at 4°C. Since the reagents, samples, and standards of GABA and Glu were delivered to room temperature immediately prior to the derivatization reaction, we selected room temperature conditions to carry out this reaction. This experiment was optimized for 5 min. After that time, the maximum concentration of GABA-OPA complex was reported. Since the literature data suggests that glutamate is present in higher concentrations in the brain tissue homogenates, the incubation time after the addition of OPA was adjusted to GABA. However, in the analysis of neurotransmitters using OPA it is very important to maintain an identical time and temperature regime between the addition of the OPA reagent to the sample and the injection into the column.

The authors of the various reported methods of OPA derivatization used different incubation times. For example Sizemore et al. [22] conducted incubation for 4 min (no data on temperature). The most commonly used incubation time was 1 min in room temperature [5,10].

Moreover, in this study we also examined the potential interferences from the OPA reagent in the GABA and glutamate elution. The typical chromatograms of blank, standard, and tissue homogenate samples have been shown in the Figure 2. Peaks derived from the OPA reagent do not interfere with the peaks of analyzed neurotransmitters.

The only disadvantage of OPA is the regulated timing of its addition to the sample prior to the sample's injection into the column. However, modern liquid chromatographs allow for complete automation of this process.



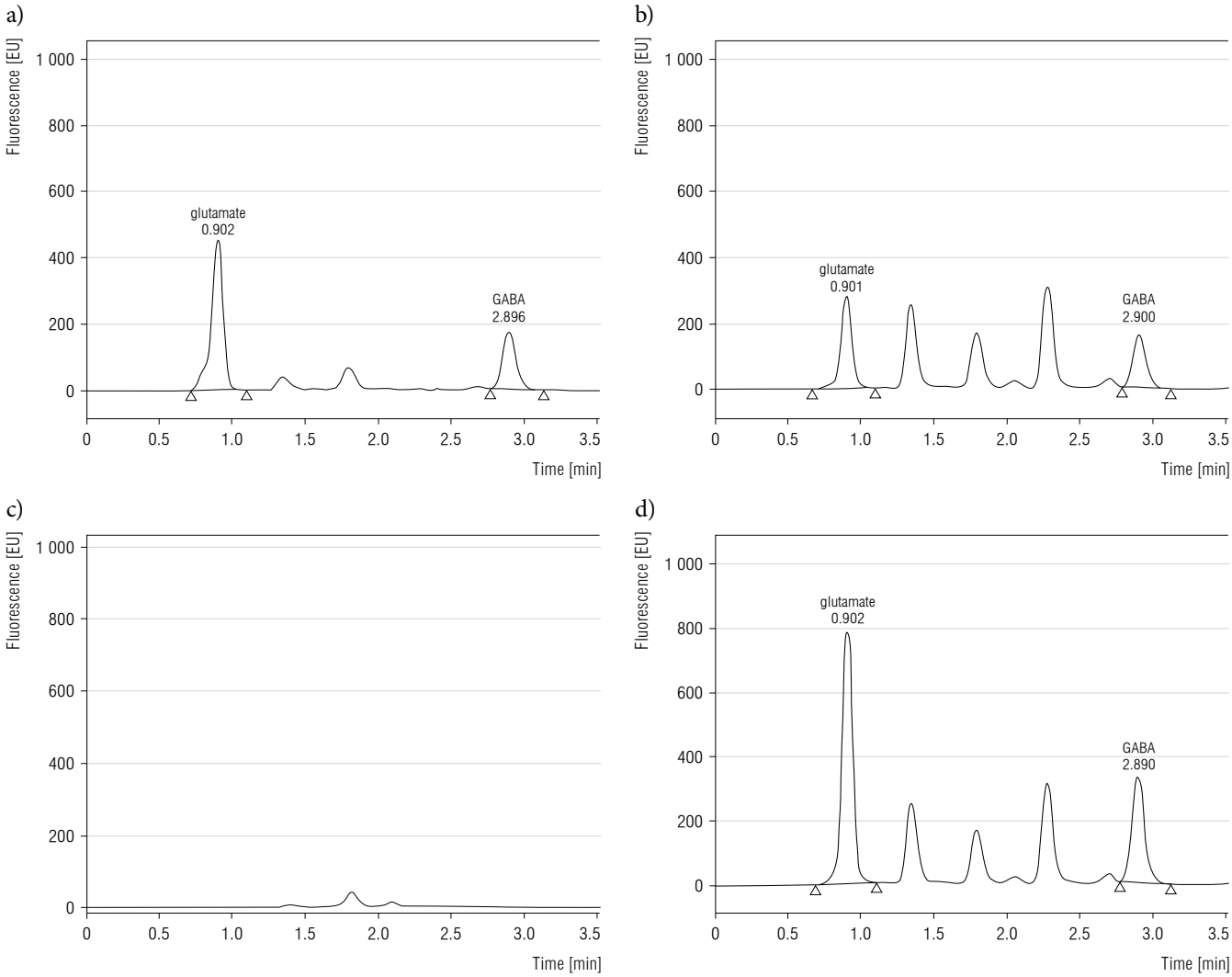
GABA – γ-aminobutyric acid.

Fig. 1. Stability of ortho-phthaldialdehyde (OPA) derivatives dependent on temperature: a) room temperature, b) 4°C

Optimization of chromatographic and detection conditions

In regard to the conditions employed in the chromatographic analysis of fluorescents derivatives of GABA and glutamate, many studies report the time of implementing the method of using a concentration gradient for the elution of these compounds. The primary purpose of developing new conditions for the separation of these neurotransmitters was to reduce the retention times of elution, while maintaining good resolution and sensitivity. In addition, it was important to achieve proper separation of the tested amino acid derivatives and the components of the derivatizing agent. A literature review on the various quantification methods of GABA revealed that the composition of the mobile phase was often a sodium acetate buffer, varying in concentration (10–100 mM) and pH (5.5–6.5) [5,10]. Furthermore, methanol was most reported as the organic component of the mobile phase [5,10].

In addition, more procedures used gradient separation than isocratic elution. Our experiments aimed to investigate the chromatographic condition of flow rate, level of organic solvent (methanol) in the mobile phase, and buffer pH on resolution of GABA/glutamate derivatives. Compounds were eluted isocratically over a 3.5 min runtime. The time of the analysis was reduced by increasing both the pH of the buffer from 5.5–6.0 and the percentage of methanol in isocratic separation from 30–40%. The chromatographic conditions that resulted in satisfying peak shapes, good resolution, and short analysis time were 100 mM sodium acetate buffer (pH = 6.0) and methanol (60:40, v/v) at the flow rate



a) standard solution – GABA (250 ng/ml) and glutamate (2000 ng/ml), b) tissue homogenate samples (brain stem) – GABA (250 ng/ml) and glutamate (1180 ng/ml), c) blank sample, and d) tissue homogenate samples (brain stem) spiked with GABA (250 ng/ml) and glutamate (2000 ng/ml).
EU – emission units.
Other abbreviations as in Figure 1.

Fig. 2. Chromatograms of ortho-phthaldialdehyde (OPA) derivatives

of 0.3 ml/min and column temperature at 35°C. The fluorescence detection conditions were optimized by the observations made on the excitation and emission spectrum of GABA and glutamate derivatives. The optimal excitation and emission wavelength were established at 340 nm and 455 nm, respectively.

Calibration curve and linearity

The calibration curves with peak area (y) vs. neurotransmitter concentrations (x, ng/ml) were created. At the beginning of the analysis 7 points of calibration in the range of 25–10 000 ng/ml were performed. From this range we determined the equation of the calibration curve and the linearity expressed as a coefficient of correlation r^2. For each calibration point, the concentration value was calculated (calculated value) using the area and the equation of the calibration curve. Calibration accuracy refers to how close the measured value of a calibration is to the true value, therefore the relative error (%) was calculated between the expected value (X value) and the calculated value. The largest deviations, amounting to 200%, were characterized by the lowest points of calibration curve. Such large errors, despite a very good correlation coefficient $r^2 = 0.999$, eliminate the use of this curve.

Therefore a new calibration curve equation for the concentration range of 100–10 000 ng/ml with equally high linearity was designed. Calculated values from the

Table 1. Working range of calibration curve for γ-aminobutyric acid (GABA) and glutamate concentration in rats brain tissue homogenate samples

Neurotransmitter	Concentration [ng/ml]		Relative error [%]
	expected	calculated	
GABA[1]			
	100	96.8	–3.20
	250	252.2	0.90
	1 000	1 058.8	5.90
	2 500	2 610.6	4.40
	5 000	5 224.3	4.50
	10 000	9 967.4	–0.30
Glutamate[2]			
	100	101.5	1.50
	250	248.6	–0.56
	1 000	999.6	–0.04
	2 500	2 500.2	0.01
	5 000	5 118.0	2.36
	10 000	9 847.6	–1.52

[1] Range: 100–10 000 ng/ml, y = 43 340x+3 000 000, correlation coefficient (r^2) = 0.999.
[2] Range: 100–10 000 ng/ml, y = 13 156x+95 126, r^2 = 0.999.

Table 2. Recovery and accuracy of γ-aminobutyric acid (GABA) and glutamate concentration in rats brain tissue homogenate samples

Neurotransmitter	Concentration [ng/ml]	Recovery [%]	CV[a] [%]	Accuracy [%] (M±SD)
GABA				101.1±5.1
	250	106.4	1.01	
	500	103.9	2.06	
	1 000	94.9	1.26	
	2 000	99.3	0.23	
Glutamate				101.1±3.5
	500	102.0	2.01	
	1 000	102.5	1.55	
	2 000	96.0	1.34	
	5 000	103.9	1.74	

CV – coefficient of variations, M – mean, SD – standard deviation.
[a] Obtained by repeated injections of tissue homogenate samples with spiked known concentrations of neurotransmitter.

new curve were closer to the expected one and characterized by lower relative errors. The Table 1 shows the most optimal range of the calibration curve, linear regression equation and correlation coefficient. Both neurotransmitters reported good linear responses over the optimized range with correlation coefficients of 0.999. The limit of determinations (LODs) were calculated at signal to noise (S/N) ≥ 3 (5 and 30 ng/ml for glutamate and GABA, respectively). The limit of quantification (LOQ) (100 ng/ml for glutamate and GABA) was calculated according to the European Union Guideline and was defined as the lowest concentration on the calibration curve at which the coefficient of the variation was lower than 20%.

Table 3. Precision of analytes determination in rats brain tissue homogenate samples

Analyte	Intra-day precision [%] (M±SD)	Inter-day precision [%]
GABA	1.52±0.62	2.47
Glutamate	1.84±0.54	3.12

Abbreviations as in Tables 1 and 2.

Recovery and accuracy

The Table 2 summarizes the recovery and accuracy at 4 concentrations of GABA and glutamate. The precision determined at each concentration level (N = 5) does not exceed 2.06% of the coefficient of variation (CV). The recovery (%) was calculated as follows:

$$\text{recovery} = 100\times(c_1 - c_2)/s \quad (1)$$

where:
c_1 – tissue homogenate solution spiked with standard,
c_2 – tissue homogenate solution (matrix),
s – the known concentration of the standard added to the matrix.

The accuracy expressed as a mean recovery and for both neurotransmitters is 101.1%.

Precision

Precision of the method was established for inter-day and intra-day reproducibility (Table 3). The intra-day precision was expressed as the relative standard deviation (RSD) of 5 replicates of the same calibration standard within the same day. For the evaluation of the inter-day reproducibility a set of calibration standard samples was analyzed each day during 3 consecutive days. According to the European Medicines Agency (EMA) guidelines [23] the intra-day and inter-day precision values, expressed as RSD, should be lower

than 15%. The precision obtained in our study ranged from 1.52–3.12%.

Application of the method to rat brain tissue homogenate

The effectiveness of the proposed method was tested by analyzing brain tissue samples (cerebellum, basal ganglia and brain stem). The derivatization reaction for GABA and glutamate in brain tissue homogenates were carried out under the same conditions described for the preparation of the standards. The mean concentrations of GABA and glutamate registered in the 3 rat brain regions are shown in the Table 4. It may be observed that the basal ganglia region presented the highest (almost double) value of GABA concentration, in comparison with other regions. Whereas the lowest concentration of glutamate was detected in the brain stem. According to the literature data, similar concentrations of GABA and glutamate in rat brain samples were detected. The Table 4 shows that the authors indicated various methods of quantification of neurotransmitters in the brain structures at different levels. This differentiation may be caused by the use of different rat species, their gender and age, and different analytical techniques. In this work, neurotransmitters were also standardized at the level of protein to give concentrations of several mg GABA or Glu per 1 g protein. The results of other researchers show a very wide variation in the levels, from several μg/g to several mg/g protein [5].

Table 4. γ-Aminobutyric acid (GABA) and glutamate levels in different rats brain areas in the selected studies

Brain region and used method	Rat sex and species	Concentration [μg/g tissue] (M±SEM)		References
		GABA	glutamate	
Brain stem				
HPLC-ECD	male Sprague-Dawley	197.6±13.3	n.d.	24
HPLC-ECD, HPLC-FLD	male Sprague-Dawley	173.4±4.253	709.7±20.81	25
HPLC-FLD	male Wistar	~1 031[a]	~662[a]	26
HPLC-MS	male Sprague-Dawley	145	1 150	27
HPLC-MS	male Sprague-Dawley	175	950	27
HPLC-FLD	female Wistar	159.3±6.3 (0.9±0.04[b])	1 267.2±39.3 (7.3±0.60[b])	present study
Cerebellum				
HPLC-FLD	male Sprague-Dawley	~687[a]	~378[a]	28
HPLC-FLD	male Wistar	~229[a]	5 219.31±378.35	29
UPLC-FLD	female Wistar	183.5±9.0 (1.3±0.05[b])	1 828.1±55.9 (9.2±0.46[b])	present study
Basal ganglia (*striatum*)				
HPLC-PDA	male Wistar	1 076.6±378.5	n.d.	30
Basal ganglia (*globus pallidus*)				
HPLC-PDA	male Wistar	966.2±304.2	n.d.	30
Basal ganglia (*nucleus accumbens*)				
UHPLC-MS/MS	female Wistar	69.3±5.5	1 032±46.7	17
Basal ganglia				
UPLC-FLD	female Wistar	345.7±16.8 (2.3±0.06[b])	1 746.5±27.3 (7.8±0.43[b])	present study

HPLC – high pressure liquid chromatography, ECD – electrochemical detector, FLD – fluorescence detector, MS – mass spectrometry, UPLC – ultra-performance liquid chromatography, PDA – photodiode array detector, UHPLC – ultrahigh-pressure liquid chromatography, MS/MS – tandem mass spectrometry.
n.d. – no data.
[a] Values taken from graph, SEM data not available.
[b] In mg/g protein.

CONCLUSIONS

We have successfully developed a simple and fast UPLC method which allows isocratic separation of the OPA derivatives of GABA and glutamate with fluorescence detection. This method provides satisfactory values of linearity, recovery, precision (intra- and inter-day precision) and sensitivity. The developed and validated method is characterized by faster separation (3.5 min) in comparison with other HPLC methodologies and could be an alternative method for an advance technique such as HPLC-MS/MS. Reducing the time of quantification enables the efficient management of a large number of samples. Moreover, this method may be easily adopted to other biological samples due to the commercial availability of reagents and instrumentation.

REFERENCES

1. Hassel B, Dingledine R. Glutamate and glutamate receptors. In: Brady ST, Siegel GJ, Albers RW, Proce DL, editors. Basic neurochemistry: Principles of molecular, cellular, and medical neurobiology. New York: Academic Press; 2012. p. 342–66, https://doi.org/10.1016/B978-0-12-374947-5.00017-1.
2. Burbaeva GS, Boksha IS, Tereshkina EB, Savushkina OK, Prokhorova TA, Vorobyeva EA. Glutamate and GABA-metabolizing enzymes in post-mortem cerebellum in Alzheimer's disease: Phosphate-activated glutaminase and glutamic acid decarboxylase. Cerebellum. 2014;13(5):607–15, https://doi.org/10.1007/s12311-014-0573-4.
3. Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in schizophrenia: New evidence. Annu Rev Pharmacol Toxicol. 2001;41:237–60, https://doi.org/10.1146/annurev.pharmtox.41.1.237.
4. Bradford HF. Glutamate, GABA and epilepsy. Prog Neurobiol. 1995;47(6):477–511, https://doi.org/10.1016/0301-0082(95)00030-5.
5. Lafuente A, Cabaleiro T, Caride A, Gutierrez A, Esquifino AI. Toxic effects of methoxychlor in rat striatum: Modifications in several neurotransmitters. J Physiol Biochem. 2007;63(2):171–8, https://doi.org/10.1007/BF03168228.
6. Ahmed RG. Perinatal TCDD exposure alters developmental neuroendocrine system. Food Chem Toxicol. 2011;49(6):1276–84, https://doi.org/10.1016/j.fct.2011.03.008.
7. Boix J, Cauli O, Felipo V. Developmental exposure to polychlorinated biphenyls 52, 138 or 180 affects differentially learning or motor coordination in adult rats. Mechanisms involved. Neuroscience. 2010;167(4):994–1003, https://doi.org/10.1016/j.neuroscience.2010.02.068.
8. Cruz SL, Rivera-Garcia MT, Woodward JJ. Review of toluene action: Clinical evidence, animal studies and molecular targets. J Drug Alcohol Res. 2014;3:1–15, https://doi.org/10.4303/jdar/235840.
9. Long Z, Li XR, Xu J, Edden RA, Qin WP, Long LL, et al. Thalamic GABA predicts fine motor performance in manganese-exposed smelter workers. PLoS One. 2014;9(2):e88220, https://doi.org/10.1371/journal.pone.0088220.
10. Yang ZZ, Li J, Li SX, Feng W, Wang H. Effect of ginkgolide B on striatal extracellular amino acids in middle cerebral artery occluded rats. J Ethnopharmacol. 2011;136(1):117–22, https://doi.org/10.1016/j.jep.2011.04.027.
11. Silva DM, de Freitas, Ferraz VP, Ribeiro ÂM. Improved high-performance liquid chromatographic method for GABA and glutamate determination in regions of the rodent brain. J Neurosci Methods. 2009;177(2):289–93, https://doi.org/10.1016/j.jneumeth.2008.10.011.
12. Pålsson E, Jakobsson J, Södersten K, Fujita Y, Sellgren C, Ekman CJ, et al. Markers of glutamate signaling in cerebrospinal fluid and serum from patients with bipolar disorder and healthy controls. Eur Neuropsychopharmacol. 2015;25(1):133–40, https://doi.org/10.1016/j.euroneuro.2014.11.001.
13. Ferry B, Gifu EP, Sandu I, Denoroy L, Parrot S. Analysis of microdialysate monoamines, including noradrenaline, dopamine and serotonin, using capillary ultra-high performance liquid chromatography and electrochemical detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2014;951–2:52–7, https://doi.org/10.1016/j.jchromb.2014.01.023.
14. Kang X, Xiao J, Huang X, Gu Z. Optimization of dansyl derivatization and chromatographic conditions in the determination of neuroactive amino acids of biological samples. Clin Chimica Acta. 2006;366(1–2):352–6, https://doi.org/10.1016/j.cca.2005.11.011.
15. Dawson LA, Organ AJ, Winter P, Lacroix LP, Shilliam CS, Heidbreder C, et al. Rapid high-throughput assay for the measurement of amino acids from microdialysates and brain tissue using monolithic C_{18}-bonded reversed-phase columns. J Chromatogr B Analyt Technol Biomed Life Sci. 2004;807(2):235–41, http://dx.doi.org/10.1016/j.jchromb.2004.04.012.
16. Zhao XE, Suo YR. Simultaneous determination of monoamine and amino acid neurotransmitters in rat endbrain tissues by pre-column derivatization with high-performance liquid chromatographic fluorescence detection and mass spectrometric identification. Talanta. 2008;76(3):690–7, https://doi.org/10.1016/j.talanta.2008.04.032.

17. González RR, Fernández RF, Vidal JL, Frenich AG, Pérez ML. Development and validation of an ultra-high performance liquid chromatography-tandem mass-spectrometry (UHPLC-MS/MS) method for the simultaneous determination of neurotransmitters in rat brain samples. J Neurosci Methods. 2011;198(2):187–94, https://doi.org/10.1016/j.jneumeth.2011.03.023.
18. Song P, Mabrouk OS, Hershey ND, Kennedy RT. *In vivo* neurochemical monitoring using benzoyl chloride derivatization and liquid chromatography-mass spectrometry. Anal Chem. 2012;84(1):412–9, https://doi.org/10.1021/ac202794q.
19. Shi S, Zhao B, Yagnik G, Zhou F. An interface for sensitive analysis of monoamine neurotransmitters by ion-pair chromatography-electrospray ionization-mass spectrometry with continuous online elimination of ion-pair reagents. Anal Chem. 2013;85(14):6598–602, https://doi.org/10.1021/ac401396j.
20. Park JY, Myung SW, Kim IS, Choi DK, Kwon SJ, Yoon SH. Simultaneous measurement of serotonin, dopamine and their metabolites in mouse brain extracts by high-performance liquid chromatography with mass spectrometry following derivatization with ethyl chloroformate. Biol Pharm Bull. 2013;36(2):252–8, https://doi.org/10.1248/bpb.b12-00689.
21. Lowry OH, Rosenbrough J, Farr AL, Randall RJ. Protein measurement with the follin phenol reagent. J Biol Chem. 1951;193(1):265–5.
22. Sizemore GM, Co C, Smith JE. Ventral pallidal extracellular fluid levels of dopamine, serotonin, gamma amino butyric acid, and glutamate during cocaine self-administration in rats. Psychopharmacology (Berl). 2000;150(4):391–8, https://doi.org/10.1007/s002130000456.
23. European Medicines Agency. Guideline on bioanalytical method validation [Internet]. London: The Agency; 2012 [cited 2017 Jan 17]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf.
24. Li Q, Jin CL, Xu LS, Zhu-Ge ZB, Yang LX, Liu LY, et al. Histidine enhances carbamazepine action against seizures and improves spatial memory deficits induced by chronic transauricular kindling in rats. Acta Pharmacol Sin. 2005;26(11):1297–302, https://doi.org/10.1111/j.1745-7254.2005.00220.x.
25. Clarke G, O'Mahony S, Malone G, Dinan TG. An isocratic high performance liquid chromatography method for the determination of GABA and glutamate indiscrete regions of the rodent brain. J Neurosci Methods. 2007;160(2):223–30, https://doi.org/10.1016/j.jneumeth.2006.09.006.
26. Bikjdaouene L, Escames G, León J, Ferrer JM, Khaldy H, Vives F, et al. Changes in brain amino acids and nitric oxide after melatonin administration in rats with pentylenetetrazole-induced seizures. J Pineal Res. 2003;35(1):54–60, https://doi.org/10.1034/j.1600-079X.2003.00055.x.
27. Jing Y, Fleete MS, Collie ND, Zhang H, Liu P. Regional variations and age-related changes in arginine metabolism in the rat brain stem and spinal cord. Neuroscience. 2013;252:98–108, https://doi.org/10.1016/j.neuroscience.2013.08.002.
28. Jiang YY, Cao BB, Wang XQ, Peng YP, Qiu YH. Cerebellar ataxia induced by 3-AP affects immunological function. Neuro Endocrinol Lett. 2015;36(3):246–56.
29. Ferreira-Vieira TH, de Freitas-Silva DM, Ribeiro AF, Pereira SR, Ribeiro ÂM. Perinatal thiamine restriction affects central GABA and glutamate concentrations and motor behavior of adult rat offspring. Neurosci Lett. 2016;617:182–7, https://doi.org/10.1016/j.neulet.2016.01.060.
30. Akula KK, Chandrasekaran B, Kaur M, Kulkarni SK. Development and validation of a specific RP-HPLC method for the estimation of γ-aminobutyric acid in rat brain tissue samples using benzoyl chloride Derivatization and PDA detection. Acta Chromatogr. 2015;27(3):413–33, https://doi.org/10.1556/AChrom.27.2015.3.2.

Publisher / Wydawca: Nofer Institute of Occupational Medicine, Łódź, Poland