Pharmaceutical Sciences
December 2018, 24, 298-303
doi: 10.15171/PS.2018.43
http://ps.tbzmed.ac.ir/

Research Article

Development of a Micellar Electrokinetic Chromatographic Method with Indirect UV Detection for Pregabalin Determination in Serum Samples

Hamid Reza Sargazi[1], Elnaz Tamizi[2], Elaheh Rahimpour[3], Abolghasem Jouyban[4,5*]

[1]*Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Science, Tabriz, Iran.*
[2]*Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
[3]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
[4]*Pharmaceutical Analysis Research Center, Faculty of Pharmacy, Tabriz University of Medical Science, Tabriz, Iran.*
[5]*Kimia Idea Pardaz Azarbayjan (KIPA) Science Based Company, Tabriz University of Medical Sciences, Tabriz, Iran.*

Article Info

Article History:
Received: 8 June 2018
Revised: 23 June 2018
Accepted: 5 July 2018
ePublished: 30 December 2018

Keywords:
-Field-amplified sample injection
-Micellar electrokinetic chromatography
-Capillary electrophorese
-Pregabalin

ABSTRACT

Background: A micellar electrokinetic chromatographic (MEKC)/ indirect UV detection method with hydrodynamic and electrokinetic injection has been developed for the determination of pregabalin in the serum samples.
Methods: Separation of the drug was achieved on Agilent capillary electrophorese in less than 5 min using a 50 cm × 75 µm i.d. uncoated fused-silica capillary and a background electrolyte (BGE) consisting of 5-aminosalicylic acid (5-ASA, 10 mmol L^{-1}), cetyl trimethylammonium bromide (CTAB, 1 mmol L^{-1}) and tri-sodium citrate (4% w/v). The influence of various parameters on the separation such as separation voltage, injection time, cassette temperature, pH of BGE and organic modifier was investigated.
Results: Method validation shown good linearity (R^2> 0.999) in the range of 1.5-100 µg mL^{-1} of pregabalin. A limit of detection (LOD) of 0.8 µg mL^{-1} and a limit of quantitation (LOQ) of 2.6 µg mL^{-1} were reported for pregabalin.
Conclusion: The proposed method was found to be suitable and accurate for the determination of pregabalin in serum samples.

Introduction

Pregabalin (the S-enantiomer of gamma-aminobutyric acid, Figure 1) is a beta amino acid used for treatment of epileptic patients, central nervous system disorders and neuropathic pains.[1] Mechanism of action of pregabalin is based on structure similarity to endogenous inhibitory neurotransmitter gamma-aminobutyric acid.[2] This drug might be abused by patients with opiate addiction.[3] Therefore, its determination in biological fluids, by a simple and sensitive analytical method, can help in dose adjustments and toxicity prevention. Different analytical methods have been employed for determination of pregabalin in a variety of matrices such as spectrophotometry,[4,5] spectrofluorimetry,[6] gas chromatography,[7] high-performance liquid chromatography-mass spectrometry (HPLC-MS),[8-10] ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS),[11-13] electrospray ionisation–tandem mass spectrometry (ESI-MS/MS),[14] and nuclear magnetic resonance spectroscopy (NMR).[15] However, only a few studies were performed based on capillary electrophoresis (CE) separation for quantification of pregabalin.[15,16] Béni et al[15] have reported a CE-NMR method for the chiral separation of pregabalin isomers. They employed a derivatization step with tosyl- and dansyl-chloride to introduce strong UV chromophore of different size. Rodríguez et al[16] have reported a nonaqueous CE-TOF-MS for determination of pregabalin in urine samples. Although the advantages of these methods are undeniable; however, they have important limitations and problems that are related to need a sample preparation system, time consuming, expensive and/or complicated analysis systems and hard operation. In the last decades, the use of CE method as an alternative or complementary approach to LC methods has increased in pharmaceutical analysis due to its good separation efficiency, selectivity, specificity and extremely low sample used and very short analyze time.[17] However, CE methods present relatively low sensitivity because of the small volume of sample injected (< 10 µL) and the short optical path. An alternative method to improve detection limit for compounds with no suitable chromophore or fluorophore is indirect UV detection.[18] The indirect UV detection is based upon the using a high chromophoric probe as a background electrolyte (BGE) without any covalent bond and detection of desired analyte by hypsochromic shift observed in electropherogram of probe proportional with analyte concentration.[19] The molecular structure of probe must be similar to the analyte with the same electrophoretic mobility for the analyte. Some compounds which can be used as a probe in indirect UV detection method are benzoate, sodium dodecyl sulfate (SDS), 5-

***Corresponding Author:** Abolghasem Jouyban, E-mail: ajouyban@hotmail.com

aminosalicylic acid (5-ASA), sulphosalicylic acid, tryptophan and so on.[20] Indirect UV detection without any chemical derivation is a simple and robust method for drug analysis in clinical and industrial applications.[21]

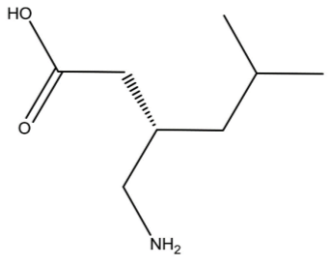

Figure 1. Structure of pregabalin.

On-line pre-concentration of analytes in CE technique is another way which could cover the low sensitivity of CE systems.[22] Among the various on-line pre-concentration techniques,[23,24] field-amplified sample injection (FASI) is very popular and simple method which takes advantage of the higher amount of analytes introduced into the capillary when electrokinetic injections are applied.

The objective of this work was to develop a micellar electrokinetic chromatographic (MEKC)/ indirect UV detection procedure with hydrodynamic and electrokinetic sample injection mode which would serve as reliable and rapid methods for the determination of pregabalin in the biological samples. Quality factors including limits of detection (LODs), limits of quantification (LOQs), linearity, run-to-run precision, and accuracy were established for the developed method.

Materials and Methods

Reagents and solutions

Pregabalin powder was purchased from sobhan darou company (Iran). 5- ASA, (Sigma, USA), tri-sodium citrate dihydrate (Scharlau, Spain), sodium hydroxide (NaOH, Merck, Germany), hydrochloric acid 36% (Carlo Erba, Italy), cetyl trimethylammonium bromide (CTAB, Merck, Germany), methanol (Merck, Germany), acetone (Scharlau, Spain), and acetonitrile (Scharlau, Spain) were used materials in this work. Deionized water (Shahid Ghazi Pharmaceutical Company, Iran) was used throughout this study. Standard stock (400 mg L^{-1}) solution of pregabalin was prepared by dissolving appropriate amount of it in deionized water and stored in 4 ^{o}C. Different concentrations of pregabalin were obtained by diluting standard stock solutions. BGE solution composition was 10 mmol L^{-1} 5-ASA, 1 mmol L^{-1} CTAB and 4 % (w/v) tri-sodium citrate and was prepared freshly every day.

Instrumentation and CE condition

A CE instrument (Agilent technologies 7100, Germany) coupled with a diode array detector (DAD 190-600 nm) was used for analysis. Agilent Chemstation (Germany) software was applied for instrument controlling and data mining. Separation was performed on a bare fused silica capillaries (50 cm length, 41.5 cm effective length and 75 µm ID.) purchased from Agilent Technology. Each new capillary have been rinsed with NaOH (1.0 mol L^{-1}) for 30 min, deionized water for 20 min and BGE for 30 min, sequentially. Each run started with washing the capillary sequentially with 0.1 mol L^{-1} NaOH and deionized water for 3 min. Detection was recorded at 215 nm. pH was adjusted using a Metrohm® pH meter (Herisau, Switzerland).

Sample preparation

Drug free serum samples for method development and validation purposes were provided by healthy volunteers who had not received any medical therapy and stored in –20 ^{o}C until analysis. Serum samples were thawed at room temperature and then spiked with an appropriate amount of pregabalin stock solution. To prepare serum samples, they were treated with 1 mL acetone. After vortex-mixing, these samples were centrifuged at 14,000 rpm for 10 min. The supernatant was collected at a glass vial and the pH of the solution was adjusted to nearly 11 and an appropriate aliquot of this solution was taken for analysis.

Result and Discussion

Composition of BGE

As pregabalin lack of a strong UV chromophore, a MEKC method with indirect UV detection was used for the analysis. For this purpose, 5-ASA was used as probe since it provided excellent capacity for inorganic compounds by indirect UV detection. A decrease proportional with the concentration of pregabalin in the background absorbance at a wavelength of 215 nm corresponds to 5-ASA as a chromophore was observed which can be used to the quantification of pregabalin in sample solutions. The effect of of 5-ASA concentration on signal intensity was investigated in the range of 8-14 mmol L^{-1} and 10 mmol L^{-1} was chosen as the optimum concentration (Figure 2a). In an oxygenated basic solution, 5-ASA is rapidly decomposed. Two degradation pathways are considered for 5-ASA: decarboxylation and oxidation of aromatic ring.[25] The initial studies show that 5-ASA can be stabilized in alkaline solution in the presence of tri-sodium citrate. Citrate salt acts as an antioxidant agent and protects 5-ASA from oxidation reactions.[26] Tri-sodium citrate concentration in the BGE was studied between 2-6% w/v, and the results (Figure 2b) shown that the highest signal intensity was achieved when 4% w/v tri-sodium citrate was added in to the BGE.

MEKC mode was obtained with the addition of CTAB to the BGE. The effect of surfactant concentration on the analytical signal was examined and the results are shown in Figure 2c. As can be seen the signal intensity increased and continued to increase until 1 mmol L^{-1} later, when a plateau was reached. 1 mmol L^{-1} is higher than CMC value of CTAB (0.97 mmol L^{-1}) and the its molecules tend to form micelles.[27] This cationic surfactant is used for inverse electroosmotic flow (EOF) direction due to creating positively charged at the internal surface of the capillary. Beside reversed EOF, a negative polarity was applied for fast mobility of the negative compounds

existing in alkaline pH of BGE (i.e. pregabalin as analyte and 5–ASA as a probe). In this case, EOF and electrophoretic mobility of anionic compound have the same direction toward the detector (anode), whereas, cationic micelle migration direct is in opposite direction of EOF. So, a sweeping and pre-concentration of analyte in the narrow boundary was occurred in the short analysis time (5 min).

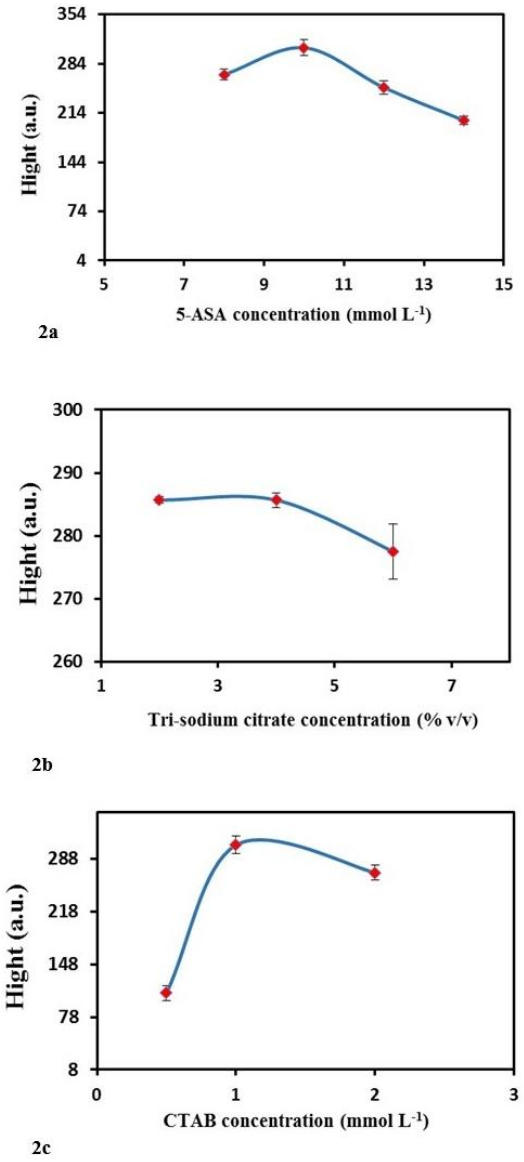

Figure 2. Effect of (a) 5-ASA concentration; [Conditions: voltage -25 kV, BGE pH=10, [tri-sodium citrate]=4% w/v, [CTAB]= 1 mmol L^{-1}, *T*=20 °C.] (b) tri-sodium citrate concentration; [Conditions: voltage -25 kV, BGE pH=10, [5-ASA]= 10 mmol L^{-1}, [CTAB]= 1 mmol L^{-1}, *T*=20 °C] and (c) CTAB concentration; [Conditions: voltage -25 kV, BGE pH=10, [5-ASA]= 10 mmol L^{-1}, [tri-sodium citrate]= 4% w/v, *T*=20 °C] on CE response in the presence of 100 µg mL^{-1} pregabalin.

Optimization of CE separation condition

To accomplish the optimum conditions for CE separation, the effects of several parameters such as separation voltage, injection time, cassette temperature, pH of BGE and organic modifier on the analytical signal were investigated by one-at-a-time method as a logical approach to evaluate the set of factors involved in separation. All measurements were repeated three times.

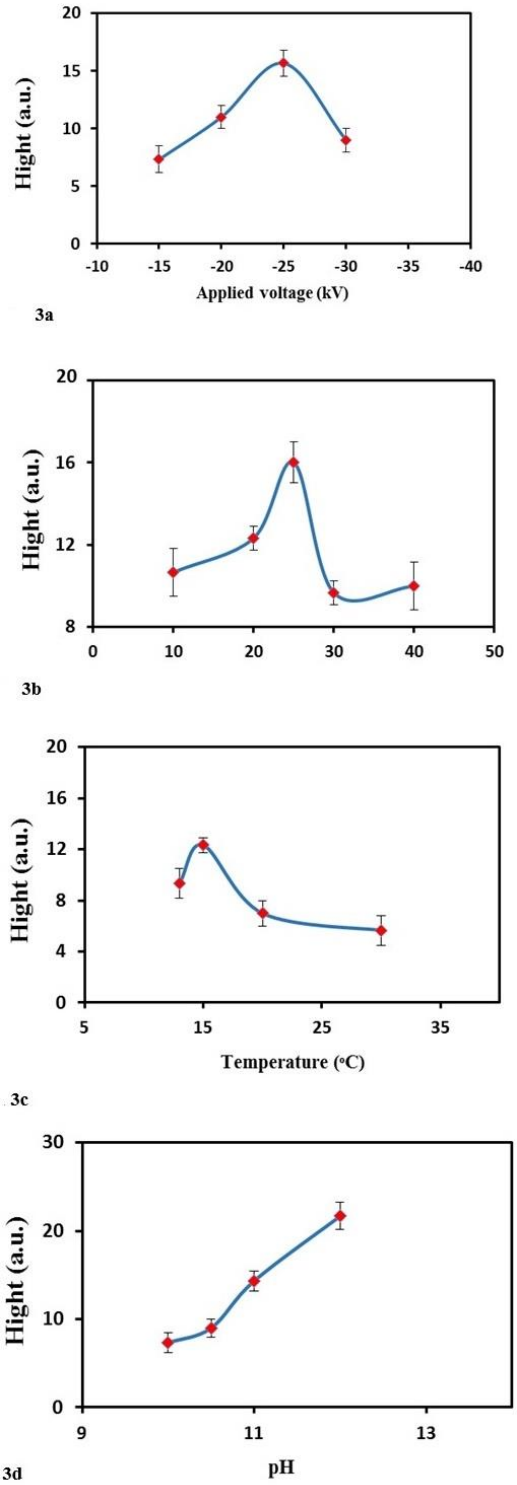

Figure 3. Optimization of condition of hydrodynamic injection mode. (a) Applied voltage; (b) time; (c) temperature; and (d) pH in the presence of 5 µg mL^{-1} pregabalin. Conditions: [5-ASA]=10 mmol L^{-1}, [tri-sodium citrate]=4% w/v, [CTAB]= 1 mmol L^{-1}.

Optimization of condition of hydrodynamic injection mode

In order to ensure that the maximum amount of sample is introduced into the capillary, the injection parameters, including the applied voltage and injection time, should be optimized. Optimization of applied voltage is crucial step in method development of CE. Applying high voltage produce a joule heating and a peak broadening was observed for analytical signals. The influence of applied voltage on the analytical signal was investigated in the range from -15 to -30 kV (Figure 3a) and potential of -25 kV is chosen which obtained the best compromise in terms of run time, current generated and linearity between voltage and current. The influence of the injection time on the analytical signal was also studied as another important factor (Figure 3b). For higher injection times (>25 s), a peak tailing and broadening were observed due to sample overloading in capillary; so injection time of 25 s was selected for further experiments. The effect of temperature on CE separation can be interpreted with different mechanisms, e.g. viscosity, joule heating and migration time.[28] Different capillary column temperatures were checked in the range of 13–30 °C. As can be seen from Figure 3c, the best signal was achieved at 15 °C. Higher temperatures of column produce noisy background and signal intensity was decreased at low temperatures (<15 °C).

As pH affects the ionization state of the analyte and the capillary wall, which in turn influences the magnitude of the EOF, pH optimization is considered as an important step in CE analysis. The effect of pH of BGE was investigated in the range of 10–12 (Figure 3d). pK_a value of pregabalin was 4.2 and 10.6, thus it is in anionic form at pH > 11. As has been already described, pregabalin in its anionic form has a fast mobility in the applied negative polarity which gave good resolution, peak shapes and short run time for analysis. Thus, pH of 12 was selected as optimum value for further experiments. The migration of compounds under study (pregabalin and 5-ASA) was decreased with decrease of pH.

Optimization of condition of FASI mode

To reach the high detection sensitivity and improvement of LOD, FASI was employed as an on-line pre-concentration method. Injection in FASI mode is based on electrokinetic mode. The following equation was utilized to estimate the sensitivity enhancement factor (SEF) of the FASI in terms of the peak heights:[29]

$$SEF = \frac{Hight\ \ with\ \ field\ \ amplified\ \ injection}{Hight\ \ with\ \ conventional\ \ injection} \times dilution \qquad \text{Eq. (1)}$$

Injection conditions in FASI mode were also optimized. The samples were processed at different injection times, applied voltage and pH to obtain maximal sensitivity. The injection time was evaluated in the range of 30–180 s and the best results were obtained for 150 s (Figure 4a).

The applied injection voltage was varied over the range of -5 to -12 kV and the highest peak intensity and symmetry were achieved for -10 kV (Figure 4b). Using of low conductivity solvent prior to sample injection has been proven to enhance the sensitivity for FASI mode.

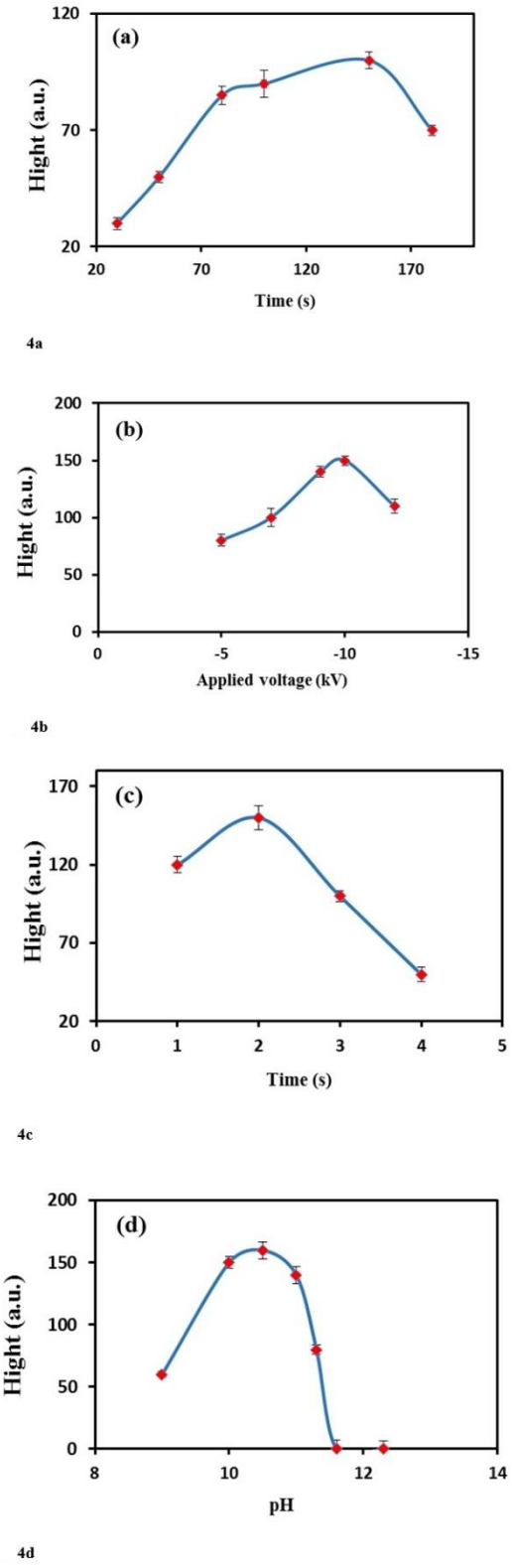

Figure 4. Optimization of condition of FASI mode. (a) Time; (b) applied voltage; (c) plug time of water; and (d) pH in the presence of 50 µg mL^{-1} pregabalin. Conditions: [5-ASA]= 10 mmol L^{-1}, [tri-sodium citrate]=4% w/v, [CTAB]= 1 mmol L^{-1}.

Therefore, a short plug of water was injected using hydrodynamic mode from 1 to 4 s. Increasing the injection time beyond 2 s disturbed the peak shape and intensity (Figure 4c), thus the water plug was injected at 50 mbar for 2 s.[30] Nevertheless, after one electrokinetic injection, peak height and thus repeatability dramatically decreased. Electrophoretic mobility of the analyte may be influenced by pH. The pH values were adjusted to a range of 9–12 and pH of 10.5 was selected as an optimum value (Figure 4d). Under the optimum conditions, a sensitivity enhancement of 10-fold (SEF=10) was achieved in serum samples.

Optimization of organic modifier

Effect of addition two organic modifiers i.e. methanol and acetonitrile on the peak shape and intensity was also investigated. The concentrations were varied from 10 to 20% (v/v) and the best signal was obtained in absence of organic modifiers.

Analytical figures of merit

The described MEKC/indirect UV detection method has been extensively validated for assay. Under optimum condition, linearity was checked by preparing standard solutions at seven different concentration levels ranging from 1.5 to 100 µg mL^{-1}. The average of three calibration curves constructed on three nonconsecutive days was used for linearity investigations. The equation for calibration curve is Y = 15.962X + 2.4954, where Y is the peak area of obtained electropherogram and X is the concentration of pregabalin in µg mL^{-1}. The correlation coefficient was found to be >0.999, indicating good linearity. The LOD and LOQ, defined as $3S_b/m$, and 10 S_b/m (where S_b is the standard deviation of the blank and m is the slope of the calibration curve), were 0.8 and 2.6 µg mL^{-1}, respectively.The precision of the proposed method, defined as the closeness of the calculated values to each other, was evaluated by repeated analysis of three concentration of pregabalin (i.e. 3, 12.5, 50 µg mL^{-1}) during the course of experimentation on the same day and on different days under the optimized experimental conditions. Overall relative standard deviation (%*RSD*) values of assay was found to be 4.6% and 8.0% for intra-day and inter-day analyses, respectively.

Table 1. Study of accuracy and recovery of spiked samples with different amounts of pregabalin.

Nominal concentration (µg mL^{-1})	Found concentration (µg mL^{-1})	RE (%)	RSD (%)	Recovery (%)
3.0	3.09 ± 0.10	+0.09	3.2	103.0
12.5	12.51 ± 0.31	+0.01	2.4	100.1
50	49.8 ± 2.75	-0.20	5.5	99.6

To verify the accuracy of the established procedure, recovery experiments were carried out by spiking the samples with different amounts of pregabalin. As can be seen from Table 1, the relative recoveries between 99.6 and 103.0% were obtained, confirming the accuracy of the presented method.

Conclusion

A MEKC/indirect UV detection method with hydrodynamic and electrokinetic injection mode have been developed and validated for the quantitative determination of pregabalin in serum samples. The developed method, as an alternative to existing LC methods, is suitable for routine use and offers advantage of simplicity of operation, flexibility and low cost. The proposed method could be applied to the determination of pregabalin in the serum samples.

Acknowledgments

H. R. Sargazi thanks Mr. Jafar Soleymani for his assistance in this project. This work is a part of M.Sc. thesis of H.R. Sargazi and a partial financial support of Tabriz University of Medical Sciences is gratefully acknowledged.

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Tassone DM, Boyce E, Guyer J, Nuzum D. Pregabalin: A novel γ-aminobutyric acid analogue in the treatment of neuropathic pain, partial-onset seizures, and anxiety disorders. Clin Ther. 2007;29(1):26-48. doi:10.1016/j.clinthera.2007.01.013
2. Taylor CP, Angelotti T, Fauman E. Pharmacology and mechanism of action of pregabalin: the calcium channel α2–δ (alpha2–delta) subunit as a target for antiepileptic drug discovery. Epilepsy Res. 2007;73(2):137-50. doi:10.1016/j.eplepsyres.2006.09.008
3. Grosshans M, Lemenager T, Vollmert C, Kaemmerer N, Schreiner R, Mutschler J, et al. Pregabalin abuse among opiate addicted patients. Eur J Clin Pharmacol. 2013;69(12):2021-5. doi:10.1007/s00228-013-1578-5
4. Gujral RS, Haque SM, Shanker P. Development and validation of pregabalin in bulk, pharmaceutical formulations and in human urine samples by UV spectrophotometry. Int J Biomed Sci. 2009;5(2):175-80.
5. Önal A, Sagirli O. Spectrophotometric and spectrofluorimetric methods for the determination of pregabalin in bulk and pharmaceutical preparation. Spectrochim Acta A Mol Biomol Spectrosc. 2009;72(1):68-71. doi:10.1016/j.saa.2008.08.009
6. Shaalan RAA. Spectrofluorimetric and spectrophotometric determination of pregabalin in capsules and urine samples. Int J of Biomed Sci. 2010;6(3):260-7.
7. Mudiam MKR, Chauhan A, Jain R, Ch R, Fatima G, Malhotra E, et al. Development, validation and comparison of two microextraction techniques for the rapid and sensitive determination of pregabalin in urine and pharmaceutical formulations after ethyl chloroformate derivatization followed by gas chromatography–mass spectrometric analysis. J

Pharm Biomed Anal. 2012;70:310-9. doi:10.1016/j.jpba.2012.05.001
8. Collins JA, Janis GC. Analysis of Selected Anticonvulsants by High Performance Liquid Chromatography-Tandem Mass Spectrometry. In: Methods in Molecular Biology. Totowa: Humana Press; 2012. p. 201-9.
9. Vaidya VV, Yetal SM, Roy SMN, Gomes NA, Joshi SS. LC-MS-MS determination of pregabalin in human plasma. Chromatographia. 2007;66(11-12):925-8. doi:10.1365/s10337-007-0430-4
10. Mandal U, Sarkar AK, Gowda KV, Agarwal S, Bose A, Bhaumik U, et al. Determination of pregabalin in human plasma using LC-MS-MS. Chromatographia. 2008;67(3-4):237-43. doi:10.1365/s10337-007-0440-2
11. Dahl SR, Olsen KM, Strand DH. Determination of gamma-hydroxybutyrate (GHB), beta-hydroxybutyrate (BHB), pregabalin, 1, 4-butane-diol (1, 4BD) and gamma-butyrolactone (GBL) in whole blood and urine samples by UPLC–MSMS. J Chromatogr B. 2012;885:37-42. doi:10.1016/j.jchromb.2011.12.009
12. Pauly C, Yegles M, Schneider S. Pregabalin determination in hair by ultra-high-performance liquid chromatography-tandem mass spectrometry. J Anal Toxicol. 2013;37(9):676-9. doi:10.1093/jat/bkt081
13. Chahbouni A, Sinjewel A, G den Burger JC, Vos RM, Wilhelm AJ, Veldkamp AI, et al. Rapid quantification of gabapentin, pregabalin, and vigabatrin in human serum by ultraperformance liquid chromatography with mass-spectrometric detection. Ther Drug Monit. 2013;35(1):48-53. doi:10.1097/ftd.0b013e31827788c0
14. Shah GR, Ghosh C, Thaker BT. Determination of pregabalin in human plasma by electrospray ionisation tandem mass spectroscopy. J Adv Pharm Technol Res. 2010;1(3):354-7. doi:10.4103/0110-5558.72423
15. Béni S, Sohajda T, Neumajer G, Iványi R, Szente L, Noszál B. Separation and characterization of modified pregabalins in terms of cyclodextrin complexation, using capillary electrophoresis and nuclear magnetic resonance. J Pharm Biomed Anal. 2010;51(4):842-52. doi:10.1016/j.jpba.2009.10.010
16. Rodríguez J, Castaneda G, Muñoz L. Direct determination of pregabalin in human urine by nonaqueous CE-TOF-MS. Electrophoresis. 2013;34(9-10):1429-36. doi:10.1002/elps.201200564
17. Boone CM, Waterval JCM, Lingeman H, Ensing K, Underberg WJM. Capillary electrophoresis as a versatile tool for the bioanalysis of drugs - A review. J Pharm Biomed Anal. 1999;20(6):831-63. doi:10.1016/s0731-7085(99)00088-6
18. Ackermans M, Everaerts F, Beckers J. Quantitative analysis in capillary zone electrophoresis with conductivity and indirect UV detection. J Chromatogr A. 1991;549:345-55. doi:10.1016/s0021-9673(00)91445-6
19. Tait RJ, Skanchy DJ, Thompson DP, Chetwyn NC, Dunshee DA, Rajewski RA, et al. Characterization of sulphoalkyl ether derivatives of β-cyclodextrin by capillary electrophoresis with indirect UV detection. J Pharm Biomed Anal. 1992;10(9):615-22. doi:10.1016/0731-7085(92)80088-5
20. Sekar R, Azhaguvel S. Indirect photometric assay determination of gabapentin in bulk drug and capsules by capillary electrophoresis. J Pharm Biomed Anal. 2004;36(3):663-7. doi:10.1016/j.jpba.2004.07.033
21. Soga T, Serwe M. Determination of carbohydrates in food samples by capillary electrophoresis with indirect UV detection. Food Chem. 2000;69(3):339-44. doi:10.1016/s0308-8146(00)00044-3
22. Osbourn DM, Weiss DJ, Lunte CE. On-line preconcentration methods for capillary electrophoresis. Electrophoresis. 2000;21(4):2768-79. doi:10.1002/1522-2683(20000801)21:14<2768::aid-elps2768>3.0.co;2-p
23. Núñez O. On-Line Electrophoretic-based Preconcentration Methods in Capillary Zone Electrophoresis: Principles and Relevant Applications. In: Capillary Electrophoresis (CE): Principles, Challenges and Applications. Reed C editor. USA: Nova Science Publishers; 2015.
24. Quirino JP, Terabe S. On-line concentration of neutral analytes for micellar electrokinetic chromatography. 5. Field-enhanced sample injection with reverse migrating micelles. Anal Chem. 1998;70(9):1893-1901. doi:10.1021/ac9711504
25. Palsmeier RK, Radzik DM, Lunte CE. Investigation of the degradation mechanism of 5-aminosalicylic acid in aqueous solution. Pharm Res. 1992;9(7):933-8. doi:10.1023/a:1015813302412
26. Frenkel A, Safadi M, Koltai TUS. Citrate salt of rasagiline. Patent No. 7855233. Washington, DC: USA Patent and Trademark Office; 2010.
27. Modaressi A, Sifaoui H, Grzesiak B, Solimando R, Domanska U, Rogalski M. CTAB aggregation in aqueous solutions of ammonium based ionic liquids; conductimetric studies. Colloids Surf A Physicochem Eng Asp. 2007;296(1-3):104-8. doi:10.1016/j.colsurfa.2006.09.031
28. Schutzner W, Fanali S. Enantiomers resolution in capillary zone electrophoresis by using cyclodextrins. Electrophoresis. 1992;13(1):687-90. doi:10.1002/elps.11501301146
29. Hamidi S, Khoubnasabjafari M, Ansarin K, Jouyban-Gharamaleki V, Jouyban A. Chiral separation of methadone in exhaled breath condensate using capillary electrophoresis. Anal Methods. 2017;9(15):2342-50. doi:10.1039/c7ay00110j
30. Simpson Jr SL, Quirno JP, Terabe S. On-line sample preconcentration in capillary electrophoresis. Fundamentals and applications. J Chromatogr A. 2008;1184(1-2):504-41. doi:10.1016/j.chroma.2007.11.001