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Short communication

Indirect photometric assay determination of gabapentin in bulk drug and capsules by capillary electrophoresis

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Abstract

A capillary electrophoresis (CE) method was developed for the assay determination of gabapentin (GBP) in bulk drug and capsules. Separation was carried out on 74 cm (62.5 cm effective length) × 75 μm i.d. fused silica capillary by applying a potential of −20 kV at ambient temperature. Background electrolyte (BGE) consisting of 5 mM 5-sulphosalicylic acid and 0.5 mM cetyltrimethylammonium bromide (CTAB), pH 11.0 was employed for the separation. Indirect method of UV detection was performed at a wavelength of 215 nm using sulphosalicylate ion as a chromophore. A linear calibration curve was obtained over a concentration range 20−200 μg/ml of GBP in deionized water with a correlation coefficient (r) of 0.9998. Recoveries were shown to be ≥98% both in bulk drug and capsules with standard deviation (S.D.) ≤2.3%. No placebo (matrix) peaks were observed at the migration times of GBP and L-glutamic acid (internal standard, I.S.).
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1. Introduction

Gabapentin [1-(aminomethyl)cyclohexaneacetic acid, GBP] is a new generation antiepileptic drug in treatment of intractable pain of reflex sympathetic dystrophy. It is structurally (Fig. 1) related to γ-aminobutyric acid (GABA), an inhibitory neurotransmitter [1]. GBP is also found to be more effective for patients who are resistant to conventional antidepressants, although its mechanism of action is not fully understood. It has very low toxicity in humans, and it is well absorbed and excreted completely [2].

A variety of separation techniques are available for assay determination of GBP in biological fluids. These include gas chromatography (GC) [3], gas chromatography–mass spectrometry (GC–MS) [4,5], liquid chromatography–mass spectrometry (LC–MS) [6], high performance liquid chromatography (HPLC) [7–15] and capillary electrophoresis (CE) [16,17]. Due to the very low UV absorption of GBP, all the earlier reported methods, the UV or fluorometric detections were performed by precolumn derivatization of GBP with a suitable reagent. To the best of our knowledge, only two methods are reported in the literature for assay determination of GBP in pharmaceutical formulations. One of the methods is derivatization of GBP with fluorescamine followed by spectrofluorimetric determination [18] and other one by colorimetric determination [19] based on the reaction of GBP with vanillin and ninhydrin. The derivatization is often time consuming and the stability of the reaction products depend on the various experimental conditions such as pH, temperature and time. Despite wide use of GBP, a simple and reliable analytical technique is required for its assay in bulk drug and pharmaceutical formulations.

Capillary electrophoresis (CE) has been extensively utilized in pharmaceutical analysis [20]. Fast analysis, small volume of sample and low running cost are some of the advantages of CE. It is often employed for indirect detection of compounds that lacks of suitable chromophores [21] (and reference cited therein). In this form of detection, a suitable

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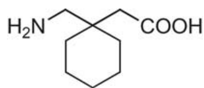

Fig. 1. Structure of gabapentin.

UV absorbing co-ion is added to the background electrolyte (BGE), a quantifiable decrease of chromophore ion in the background absorbance corresponds to the concentration of compound present in the sample. A few reports for the indirect detection and determination of active ingredients in pharmaceutical formulations by CE [22–24] are also available in the literature. Soga and Imaizumi [25] have developed an indirect CE method for the analysis of inorganic anions, organic acids, amino acids, nucleotides, and other anionic compounds. GBP has similar functional group as amino acids and this prompted us to develop a simple indirect CE method for assay determination of GBP in bulk drug and pharmaceutical capsules.

2. Experimental

2.1. Chemicals and reagents

All chemicals used in the preparation of BGE and solutions were of analytical reagent grade. Sodium hydroxide and cetyltrimethylammonium bromide (CTAB) were supplied by s.d. fine chem. Ltd. (Mumbai, India). Gabapentin was a gift from Matrix Labs Ltd. (Hyderabad, India). The 5-sulphosalicylic acid dihydrate and L-glutamic acid were purchased from Fluka (Buchs, Switzerland). Neurontin capsules (300 and 400 mg) were obtained from local pharmacies. Deionized water was obtained using a Milli-Q water purification system (Millipore, Molsheim, France). All the standards and samples were prepared in deionized water.

2.2. Preparation of background electrolyte

BGE was prepared by dissolving 5 mM 5-sulpho salicylic acid (1.271 g) and 0.5 mM CTAB (0.182 g) in water and made up to 1000 ml with water. The pH of the buffer was adjusted to 11.0 with 1 M NaOH. Due to the diffusion of carbon dioxide in the buffer solution, the pH changes slowly to the lower values, hence pH readjustment is necessary before performing the analysis.

2.3. Preparation of standard solutions

Stock standard solution of gabapentin and L-glutamic acid (internal standard, I.S.) of each 1.0 mg/ml were prepared separately in water. Before analysis, the required concentrations of GBP (20–200 μg/ml) and I.S. (52 μg/ml) were prepared by mixing appropriate volume of stock standard solutions, and diluting with water.

2.4. Capsules

Twenty capsules (Neurontin 300 or 400 mg) of each were separately emptied out and homogenized. A portion of the powder equivalent to 10.0 mg of GBP was taken in a 10 ml calibrated standard flask, added 5 ml of water, shaken for 3 min and made up to the volume with the same solvent. Each capsule contains around 16% of inactive ingredients such as lactose, cornstarch and talc, which are less water-soluble and they can be separated from the active ingredients by simple filtration. An appropriate concentration of sample and I.S. were prepared in water at the time of analysis. Sample and standard solutions were stored in a refrigerator at 4 °C when it is not used. They are stable for more than 1 month under this storage condition. Both BGE and samples were filtered through 0.45 μm membrane prior to use.

2.5. Apparatus

CE experiments were performed using a Prince CE system (Prince Technologies, Model No. 460, The Netherlands) equipped with a Lambda 1010 UV–vis detector, an auto sampler and a power supply to deliver up to 30 kV. An uncoated fused silica capillary of 75 μm ID (Polymicro, Phoenix, AZ, USA) with a total length of 74 cm (62.5 cm effective length) was used for separation. The capillary was thermo stated at 25 °C. Samples were kept at ambient temperature in the auto sampler and injected by applying a pressure of 50 mbar for 6 s. A constant voltage of −20 kV was applied throughout the analysis. Indirect UV detection was performed using a wavelength of 215 nm. The acquisition and analysis of data were carried out with the DAx software supplied by the instrument manufacturer.

A new capillary was conditioned by rinsing with 0.1 M sodium hydroxide for 20 min, water for 5 min, and finally the BGE for 10 min. Between each run, the capillary was rinsed with water for 2 min, 0.1 M sodium hydroxide for 2 min, water for 1 min, and the BGE for 3 min, successively.

3. Results and discussion

Glutamic acid was selected as an internal standard for the assay of gabapentin. Initially, analytical conditions such as concentration of chromophore ion, pH and voltage were optimized for the better separation and peak shape of GBP and I.S. In order to maximize the negative charge of analyte and I.S., the pH of the BGE was adjusted to 11.0. GBP exhibits a very low UV absorption and hence, sulphosalicylic acid (chromophore ion) with the same charge as the GBP was added to the BGE. When GBP ion displaces the chromophore ion, it will decrease the background absorbance and appears as a negative peak. By using the system software, the negative peak was inverted (positive peak) and the peak area measured was corresponding to the concentration of the analyte. CTAB (0.5 mM) was added to the BGE in order to reverse

Table 1
Reproducibility of migration time and peak area of internal standard

Concentration (μg/ml)	% R.S.D. ($n = 3$)	
	Migration time	Peak area
52	0.84	1.02
78	1.21	1.44
117	1.32	1.52

the direction of the electroosmotic flow (EOF) [26]. And the separation was performed with reversed polarity (applying a negative voltage). 5 mM sulphosalicylic acid and 0.5 mM CTAB (pH 11.0) was found to be suitable for separation of GBP and I.S. At separation voltage of −20 kV, the I.S. and GBP were migrated at 6.28 and 12.10 min, respectively. Analyte and I.S. have sufficient response at a wavelength of 215 nm for the indirect photometric detection. The relative response of internal standard was twice that of analyte and hence the concentration of sample was taken approximately twice the concentration of the internal standard to minimize the integral errors.

Suitability of glutamic acid as an internal standard was tested in terms of migration time and peak area reproducibility. Three concentrations (52, 78 and 117 μg/ml) were prepared and each concentration was injected in triplicate by applying 50 mbar pressure for 6 s. The percent relative standard deviations (% R.S.D.s) of migration time and peak area were calculated and given in Table 1. It can be seen from the table that the % R.S.D. of migration time and peak area were 0.84 and 1.02, respectively at a concentration of 52.0 μg/ml, which was relatively lower than other concentrations. Therefore, internal standard around 52.0 μg/ml was preferred for obtaining better results. Further, any peak due to the matrix (placebo) or system peaks in the region of I.S. and GBP was tested by running a placebo blank. Typical electropherogram from deionized water blank, excipients blank (placebo), and standard mixture containing GBP and I.S. in deionized water are depicted in Fig. 2A–C. From these figures, it can be observed that no placebo or systemic peaks occurred in the region of I.S. and GBP. Both the analyte and I.S. migrated within 12.5 minutes and the peaks are symmetric.

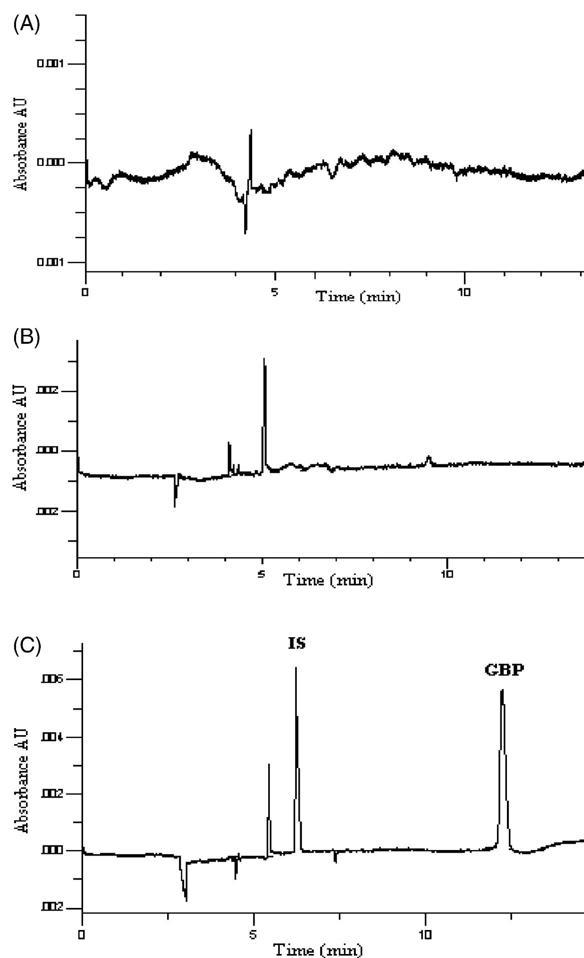

Fig. 2. Electropherograms of: (A) water blank; (B) excipients blank, and; (C) standard GBP (102.0 μg/ml) and I.S. (52.0 μg/ml) spiked with excipients. CE conditions: capillary length 74 cm (62.5 cm effective length) × 75 μm; applied voltage −20 kV; indirect detection at 215 nm; hydrodynamic injection for 6 s at 50 mbar; BGE: 5 mM 5-sulphosalicylic acid and 0.5 mM CTAB; pH of the buffer was adjusted to 11.0 with 1 M NaOH.

3.1. Method Validation

After the optimization of the method parameters, the method was tested for linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ).

3.1.1. Linearity range

Six different concentration of GBP in the range 20–200 μg/ml were prepared from standard stock solution by diluting suitably in deionized water and the concentration of I.S. (52 μg/ml) was kept constant. Each solution was injected twice using a 50 mbar injection pressure for a period of 6 s. In CE, peak areas were directly proportional to both the sample concentration and migration time. Therefore, the peak area was divided by the migration time to obtain corrected peak area. A linear detector response with peak area ratios to the concentration of GBP was obtained with a correlation coefficient (r) of 0.9998 (Table 2).

Table 2
Method validation regarding linearity and limits of detection and quantification

Validation parameters	Results
Correlation coefficient (r)	0.9998
Concentration range[a] (μg/ml)	20–200
Intercept (a)	0.0001
Slope (b)	3.0090×10^{-3}
Standard deviation (intercept)	0.0002
Standard deviation (slope)	3.0275×10^{-6}
Standard error estimate	0.0005
Limit of detection (μg/ml)	4.98
Limit of quantification (μg/ml)	16.60

[a] At each concentration level duplicate injections, six data points.

3.1.2. LOD and LOQ

The limit of detection and quantification were calculated based on the slope (S) of the calibration curve and the standard deviation of response (S.D.) using the formula for LOD = 3.0 S.D./S and for LOQ = 10 S.D./S) [27]. The LOD and LOQ were 4.98 and 16.60 μg/ml, respectively.

3.1.3. Accuracy

Accuracy of the method was tested by adding GBP of three different concentrations ranging from 85−120% to a constant mixture of excipients. The solutions were injected twice and the recoveries determined were compared to the theoretical values. The recoveries ranged from 98.8 to 100.6% with S.D. <1.8%, which means that the method gives sufficient accuracy.

3.1.4. Precision as repeatability

Repeatability of migration times and peak areas were tested using a standard solution of GBP (102 μg/ml) and I.S. (52 μg/ml). The relative standard deviation of peak areas and migration times (n = 6) of GBP were <3.0 and <2.4%, respectively. When the relative migration time and peak area calculated with respect to I.S. (n = 6) were <2.1 and <1.8%, respectively.

3.1.5. Robustness

The robustness of the method was examined during the development of the method. The influence of significant changes of buffer pH (10.8–11.2), concentration of sulphosalicylic acid (4.8–5.2 mM) and applied voltage (−19 to −21 kV) were investigated in the presence of I.S. The absolute migration times and peak areas of GBP and I.S. varied when each variable was changed. However, the relative migration times and the peak area ratios of the drugs versus I.S. did not change significantly. With reference to I.S., deviations less than 2.8% were found to be relative to the optimum value of each variable.

3.1.6. Analysis of GBP in bulk drug and capsules

The applicability of the proposed method was examined by the assay determination of GBP in bulk drug and capsules (other dosage forms are not available in India). Table 3 shows, the percentage recovery along with standard deviation. Recoveries were shown to be ≥98% both in bulk drug and capsules with S.D. <2.3%. Typical electropherogram of gabapentin (Neurontin) capsule 300 mg analyzed according to the proposed method is shown in Fig. 3. The real tablet formulation of GBP is not available locally; the applicability of the proposed method was tested by adding known amount of GBP to a common tablet excipients (talc, lactose, starch and magnesium stearate) and analyzed for the content. Recoveries of GBP from the excipients were ≥98.8% with S.D. <2.1% (data not shown) indicated that this method could be utilized for the analysis of GBP in tablet formulation.

Table 3
Assay results of gabapentin in bulk drugs and capsules

Sample	% Recoveries ± S.D.*
Bulk drug A	98.5 ± 1.3
Bulk drug B	99.4 ± 2.2
Bulk drug C	100.2 ± 1.8
Neurontin capsule 300 mg A	101.8 ± 1.7
Neurontin capsule 300 mg B	99.6 ± 2.1
Neurontin capsule 400 mg C	101.1 ± 1.3

* Mean of three samples ± standard deviation.

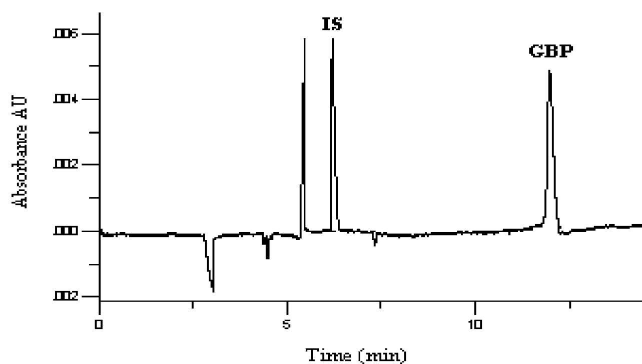

Fig. 3. Typical electropherogram of neurontin capsule formulation under conditions proposed as given in Fig. 1.

4. Conclusion

A practical and good CE method for assay determination of GBP was developed and partly validated. The proposed method was found to be easy and practical to apply for the assay of GBP in bulk drug and capsules. Acceptable levels of precision (as repeatability) in terms of migration time (<2.4% R.S.D.) and peak areas (<3% R.S.D.) were obtained. The limit of detection and limit of quantification were found to be 4.98 and 16.60 μg/ml, respectively. Recovery studies of GBP in capsules were ≥99.6% with S.D. of <2.1%, which indicates accuracy of the method. As compared to the reported spectrofluorimetric [18] and colorimetric [19], present indirect photometric detection method does not require any derivatization and extraction for the assay of gabapentin. Further, no interferences due to excipients were observed while assaying of gabapentin.

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References

[1] D. Chadwick, Lancet 343 (1994) 89–91.
[2] J.P. Leach, M.J. Brodie, Seizure 4 (1994) 5–9.

[3] W.D. Hooper, M.C. Kavanagh, R.G. Dickinson, J. Chromatogr. 529 (1990) 167–174.
[4] F. Belal, H. Abidine, A. Al-Majed, N.Y. Khatil, J. Pharm. Biomed. Anal. 27 (2002) 253–260.
[5] M.M. Khusir, J. Crosset, P.I. Brown, F.M. Urry, J. Anal. Toxicol. 23 (1999) 1–6.
[6] D.R. Ifa, M. Falci, M.E. Moraes, F.A. Bezerra, M.O. Moreas, G. de Nucci, J. Mass Spectrom. 36 (2001) 188–194.
[7] H. Hengy, E.U. Kolle, J. Chromatogr. 341 (1985) 473–478.
[8] U.H. Juergens, T.M. May, B. Rambeck, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 1459–1471.
[9] G. Forrest, G. Sills, J.P. Leach, M.J. Brodie, J. Chromatogr. B 681 (1996) 421–425.
[10] N. Ratnaraj, P.N. Patsalos, Ther. Drug Monit. 20 (1998) 430–434.
[11] N. Wad, G. Kramer, J. Chromotogr. B 705 (1998) 154–158.
[12] Q. Jiang, S. Li, J. Chromatogr. B 727 (1999) 119–123.
[13] P.H. Tang, M.V. Miles, T.A. Glauser, T. DeGrauw, J. Chromatogr. B 727 (1999) 125–129.
[14] D.F. Chollet, L. Goumaz, C. Juliano, G. Anderegg, J. Chromatogr. B 746 (2003) 311–314.
[15] Z. Zhu, L. Neirinck, J. Chromatogr. B 779 (2002) 307–312.
[16] L.L. Garcia, Z.K. Oles, J. Chromatogr. B 669 (1995) 157–162.
[17] P. Rada, S. Tucci, J. Perez, L. Teneud, S. Chuecos, L. Hermandez, Electrophoresis 19 (1998) 2976–2980.
[18] F. Belal, H. Abdine, A. Al-Majed, N.Y. Khalil, J. Pharm. Biomed. Anal. 27 (2002) 253–260.
[19] H.E. Abdellatef, H.M. Khalil, J. Pharm. Biomed. Anal. 31 (2003) 209–214.
[20] K.D. Altria, M.A. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 204–225.
[21] P. Dobe, M. Macka, P.R. Haddad, Trends Anal. Chem. 19 (2000) 10–16.
[22] B. Jamali, H.M. Nielsen, J. Chromatogr. A 996 (2003) 213–223.
[23] M.T. Ackermans, F.M. Everaerts, J.L. Reckers, J. Chromatogr. 606 (1992) 228–235.
[24] H.Y. Chang, C.H. Kuo, S.W. Sun, J. Pharm. Biomed. Anal. 23 (2003) 949–956.
[25] T. Soga, M. Imaizumi, Electrophoresis 22 (2001) 3418–3425.
[26] T. Soga, G.A. Ross, J. Chromatogr. A 767 (1997) 223–230.
[27] B. Uslu, S.A. Ozkan, Anal. Chim. Acta 466 (2002) 175–185.