Development of Direct Reversed-Phase High Performance Liquid Chromatographic Method for Quantitative Determination of Gabapentin in Pharmaceutical Dosage

WASEEM HASSAN*, BAKHT ZAMAN, SHAKILLA RAHMAN, ATA UR RAHMAN, NAUMAN ALI AND IMDAD ULLAH MOHAMMADZAI

Institute of Chemical Sciences, University of Peshawar, Peshawar -25120, Khyber Pakhtunkhwa, Pakistan

waseem_anw@yahoo.com*

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Summary: The objective of the present work was to develop and validate a rapid analytical method for quantitative determination of Gabapentin in pharmaceutical dosage tablets and capsules. An accurate, simple, and sensitive reversed-phase high performance liquid chromatographic (HPLC) method, UV detection at 215 nm and flow rate at 1.0 ml/min has been developed. Isocratic elution was used instead of gradient elution to reduce the time and cost of serial analysis. The mobile phase was a mixture of water and methanol (HPLC grade). The retention time (Rt) of Gabapentin was 4.681 ± 0.013 minutes. Recovery, Precision, accuracy, and linearity were determined for the stated method. The calibration curve was linear and the correlation coefficient was 0.9996. There was no chromatographic interference from other excipients present in dosage form. The method was validated appropriately and successfully used for determination of Gabapentin in Pharmaceutical formulations.

Keywords: Gabapentin, HPLC, UV Detection, Dosage Form.

Introduction

Gabapentin (GBP) 1- (amino methyl) cyclohexaneacetic acid, with a structure formula shown in (Fig. 1) is a white to off white crystalline solid having free solubility in water and both basic and acidic aqueous solutions. GBP has a melting point in the range of 162-166 C° and dissociation constant with a value of pKa_1 = 3.68; pKa_2 = 10.70 [1]. It is an antiepileptic drug (AED) used for treatment of partial seizures and for adjunctive treatment of partial epilepsy and management of post herpetic neuralgia. GBP is structurally related to the neurotransmitter, gamma-amino butyric acid (GABA) but does not interact with GABA receptors, and its mechanism of action is not known [2].

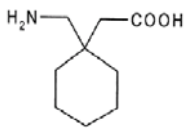


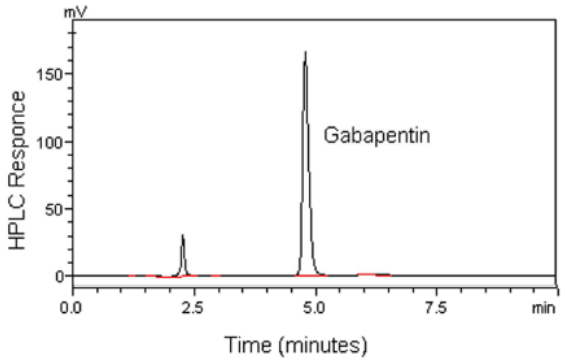
Fig. 1: Chemical structure of gabapentin.

Different methods are available in literature for the detection of GBP using different derivatizing reagents on HPLC-UV detection [3-11], liquid chromatography mass spectrometry (LCMS)[12], spectrofluorometry (SF) [13-14], capillary electrophoresis (CE) [15], gas chromatography (GC) [16], gas chromatography-mass spectrometry (GCMS) [17] and colorimetric detection [18]. Direct HPLC methods are also available in literature for Gabapentin bulk material [19], dissolution [20] and GBP related substance in drug product [21-23]. Most of the methods have limitations like complicated process for sample preparation, high sample concentration, lengthy run time, special reaction conditions, stability of derivative and possibility of a side reaction with other excipients present in tablets dosage, which is not be suitable for routine analysis.

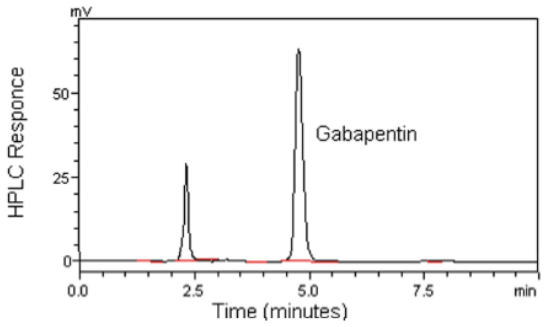
The aim of this work is to develop a sensitive and accurate method for the quantification of GBP contents in pharmaceutical formulation for routine analysis. To this end we report an isocratic HPLC method with UV detection at 215 nm using a simple mobile phase combination of water and methanol (3:2 v/v) and a total analysis run time less than 10 minutes. The validity of the proposed method is checked for different samples.

Results and Discussion

In order to achieve good sensitivity, separation and suitable retention time at ambient temperature, UV detection at 215 nm was chosen. Under the described chromatographic parameters a mixture of water and methanol (3:2 v/v) was used as mobile phase. Chromatograms shown in Fig.2 (A), (B) and (C), illustrate the separation of GBP in bulk material, capsule and tablets respectively.



(2A)



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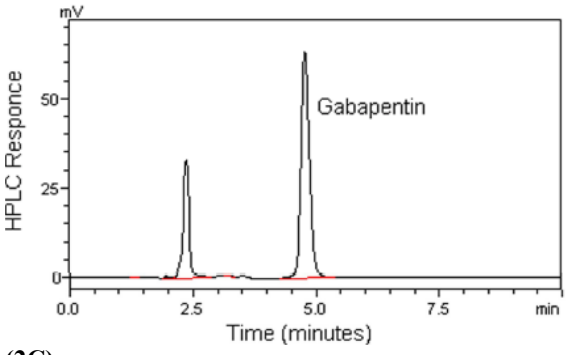

(2C)

Fig. 2: Representative chromatogram for separation of gabapentin in (A) bulk material, (B) Capsule and (C).Tablets

Validation of the analytical method was performed according to the requirements of USP for quantification of major component of bulk drug or active ingredients. System suitability was performed before analysis of sample and the results for all critical parameters were found in the required limits during the whole work. The results showed 100 ± 1 % recoveries and accuracy in the range of 0.25 to 2mg/ml for the proposed analytical method. RSD for the intra day and inter day precision studies were found less than 2 %. A linear detector response was obtained for concentration ranging 0.8 to 1.2mg/ml, LOD and LOQ were 0.014 and 0.042 mg/ml respectively. The application of the method was checked by analyzing different dosage forms of different manufacturer (Table-1) available in the market and results for assay were found according to the label claim ranged from 99.71 to 101.91.

Table-1: Results of GBP dosage form collected from market.

Product	Dosage	% Assay (Mean ± SD)	% RSD
GBP bulk material		**100.07 ± 0.43**	**0.43**
Capsule A	**100 mg**	**99.71 ± 0.66**	**0.66**
Capsule B	**300 mg**	**100.85 ± 0.96**	**0.95**
Capsule C	**400 mg**	**100.46 ± 0.52**	**0.52**
Tablets A	**600 mg**	**100.21 ± 0.67**	**0.67**
Tablets B	**800 mg**	**101.91 ± 0.49**	**0.48**

The results of the tests carried out for the validation of the method were in complete agreement with the required limits and criteria. We proved from our data that the method is accurate, precise, linear and specific for its intended purpose i.e. Quantification of GBP bulk material and pharmaceutical formulations tablets and capsules containing GBP as active ingredient.

Experimental

Materials and Methods

GBP (United State Pharmacopeia) reference standard having purity 99.78 % was obtained from (SUPELCO) and GBP bulk material (Matrix Laboratories Ltd, India and Hangzhou Chiral Medicen Chem Co. Ltd. China) was provided by Shaigan pharmaceuticals. GBP dosage form GABIX 100 mg and 400 mg Capsule (Getz Pharma, Pakistan), NEOGAB 300 mg Capsule (Hilton Pharma, Pakistan) and Neurontin 600 and 800 mg Tablets (Pfizer Canada Inc.) were purchased from local Pharmacy. Excipients of capsules (lactose, corn starch, and talc), Tablets (poloxamer 407 NF, copolyvidone, corn starch, magnesium stearate, hydroxypropylcellulose, talc and candelilla wax) and methanol, gradient grade for liquid chromatography, were obtained from Merck KGaA, Germany.

Instrumentation and Chromatographic Parameters

All the instruments used in this work were pre-calibrated and were traceable to international bureau of weight and measures (BIPM), Asia pacific metrology program (APMP), National Physical Standard Laboratory (NPSL) Pakistan and National Metrological Institute (NMI) Pakistan. SHIMADZU

UV-1700 double beam UV–visible spectrophotometer with 1-cm quartz cell (Shimadzu, Kyoto, Japan), Shimadzu Electronic Balance AW220 of 0.1mg readability, HPLC system, Shimadzu LC20AT Pump, Shimadzu SPD 20AT UV-VIS detector, equipped with Shimadzu LC solution software for integration, and Shimadzu manual injector 20µL were used in this work. The chromatographic separation was achieved by C-18 stainless steel column (25cm x 4.6mm) packed with octadecylsilyl silica gel (ODS). A mixture of water and methanol (3:2 v/v) was used as mobile phase with a flow rate of 1ml/min, at ambient temperature. The injection volume was 20µl and detection wavelength was 215nm.

Mobile Phase Preparation

300 ml Water and 200 ml Methanol was mixed with a mechanical shaking at 100 rpm for 30 minutes. The solution was sonicated to remove any air bubbles and filtered through nylon 0.45µm (47 mm) filter paper of Sartorius, Germany using vacuumed filtration.

Standard Preparation

50 mg of Gabapentin reference standard containing known quantity of GBP was accurately weighed and transferred into 50 ml volumetric flask and was dissolved in mobile phase. The volume was adjusted up to mark (50 ml) with same solvent and mixed.

The solution was filtered through nylon 0.2 µm injection filter paper before injecting to the column and used as quality control standard solution for system suitability and assay determination.

Sample Preparation

Samples of GBP bulk material, capsules and tablets were prepared separately.

Bulk Material

Sample of GBP bulk martial was prepared in the same manner as discussed in section 3.4.

Capsule

Twenty (20) capsule samples containing 50 mg of GBP according to the labeled amount was accurately weighed and dissolved in mobile phase. The final volume was adjusted to 50ml.

Tablets

Twenty (20) tablets were weighed, crushed and powered. A portion of powder containing 50 mg of GBP according to the labeled amount on the base of average weight per tablet was dissolved in mobile phase. The final volume was adjusted to 50ml.

Procedures

Sample and standard solutions were filtered through nylon 0.2µm injection filter paper, separately injected to the column and chromatograms were recorded. Results were calculated using formula,
(% assay = AU /AS x P)

Where:

AU = Peak area of sample.
AS = Peak area of standard and,
P = purity of reference standard.

Method Validation

Different Performance parameters such as precision, recovery, accuracy, linearity and range of the analytical method were checked according to the United State Pharmacopoeia (USP), and International conference on Harmonization (ICH) guidelines for validation of analytical method as discussed below in detail.

System Suitability

System suitability was performed before proceeding analysis using standard solution. The statistical data Theoretical plates (N), Symmetry factor (As), and mass distribution ratio (capacity factor k) for replicate chromatograms (n =6) were calculated according to USP guidelines and the results are presented in Table-2.

Table-2: Results of system suitability.

Peak area: (Mean± SD)	**704893 ± 5261.78**
Relative standard deviation (RSD)	**0.75 %**
Retention time Rt: (Mean ± SD)	**4.681 ± 0.013 min**
Theoretical plats N: (Mean ± SD)	**648962 ± 15459**
Symmetry Factor As:	**1.32 ± 0.02**
Capacity factor K:	**1.51 ± 0.07**

Accuracy and Recovery

For the study of accuracy and recovery, known amount of sample of GBP bulk material corresponding to five concentrations levels, covering rang from 0.25 to 2mg/ml were analyzed. Results were obtained using (n= 6) replicate of each concentration. The relative standard deviation was found below 2 %. The results of accuracy and recovery studies are presented in Table-3 and representative chromatograms are shown in Fig. 3.

Table-3: Results of recovery studies.

Concentration injected (mg/ml)	Concentration recovered mg/ml (mean± SD)	% Assay (Mean ± SD)	% RSD
0.25	0.249 ±0.003	99.60 ± 1.36	1.37
0.5	0.498 ± 0.005	99.50 ± 1.05	1.05
1	0.997 ± 0.004	99.65 ± 0.39	0.39
1.5	1.496 ± 0.013	99.7 ± 0.86	0.86
2	2.001 ± 0.016	100.05 ± 0.81	0.81

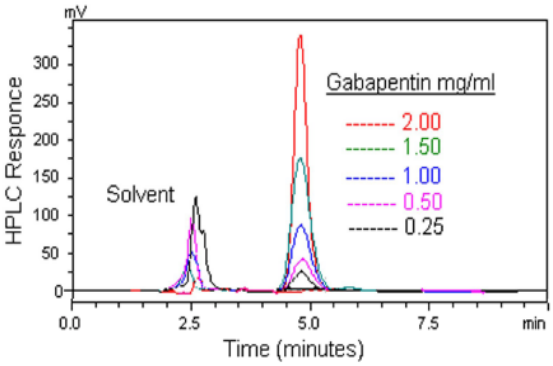

Fig. 3: Representative chromatogram for the study of recovery and accuracy.

Precision and Repeatability

Repeatability and precision of the method were assured by analyzing solution at three concentrations level 0.5, 1 and 1.5 mg/ml GBP reference standard having known amount of pure substance. Results for intra day and inter day precision were obtained using replicate analysis (n=6) and the result of precision was calculated as relative standard deviation. The results of precision studies are presented in Table-4.

Table-4: Results of studies of precision and repeatability.

Concentration mg /ml	Retention time (Mean ± SD)	Peak Area (Mean ± SD)	% RSD
0.5	4.641 ± 0.033	352970 ± 2231	0.317
1	4.647 ± 0.036	703704 ± 5685	0.540
1.5	4.621 ± 0.014	1053642 ± 6735	0.639

Linearity

For linearity determination, standard solutions having concentration of GBP from 0.8 to 1.2mg/ml were prepared. Based on results of five concentrations (replicate of each concentration (n=6)) the Peak area (mean ± SD) was plotted against concentration and was found linear. Slope and correlation coefficient (R2) were determined. Limit of detection (LOD) and Limit of quantification (LOQ) was calculated using expression (3.3δ/slope) and (10δ/slope) respectively. The value for standard deviation of peak area (δ = 3089.22) was calculated from linearity concentration levels and the value for slop (719745.8) was calculated from linearity graph. The results are shown in Table-5.

Table-5: Characteristics of the analytical method derived from the calibration curve.

Slop	R^2 Value	LOD (mg/ml)	LOQ (mg/ml)
719745.8	0.9996	0.014	0.043

Specificity and Placebo Interference

By applying the same sample preparation method, placebo was prepared using all excipients of tablets and capsules except active ingredient and was analyzed. No peak was obtained at Rt ± 2 minutes of GBP, representative chromatograms are shown in Fig. 4.

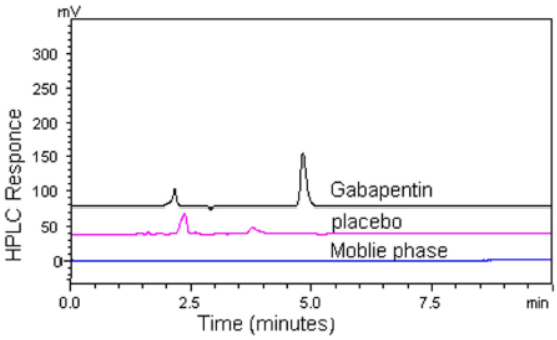

Fig. 4: Representative chromatogram for mobile phase, placebo and gabapentin.

Specificity was also determined by spiking the sample with appropriate level of excipients that showed no significant alteration in peak area. The results proved that there was no chromatographic interference of other excipients present in GBP dosage forms and the developed method was specific for the analysis of GBP in the tablets and capsules.

Robustness of the Analytical Method

Robustness of the method was checked by small changes in flow rate and mobile phase ratio. The peak area of GBP was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness are shown in Table-6.

Ruggedness of the Analytical Method

For ruggedness of the analytical method GBP Sample was analyzed by two different systems were used. Repeated results were obtained and the % RSD was found below 2 %. Results for the study of ruggedness were presented in Table-7.

Table 6: Robustness of analytical method.

Parameter	Changes	Retention time (Mean ± SD)	Peak area(Mean ± SD)	% RSD
Normal condition	**3 : 2, 1 ml / min**	**4.620 ± 0.011**	**704205 ± 5674**	**0.81**
Mobile phase ratio changes	**3:1 :1.9**	**4.866± 0.043**	**710865 ± 5569**	**0.78**
	2:9: 2.1	**4.551± 0.034**	**697761 ± 5259**	**0.75**
Flow rate changes	**1.1 ml/min**	**4.420 ± 0.004**	**690554 ± 5451**	**0.79**
	0.9 ml/min	**4.99566±0.067**	**727923 ± 4948**	**0.68**

Table-7: Ruggedness of analytical method.

Analyst	System	Column	Peak area(Mean ± SD)	% RSD
Analyst A	**System A SHIMADZU**	**Kromasil ODS C18 5µm**	**708473 ± 4578**	**0.65**
		Hypersil ODS C18 5µm	**697761 ± 5117**	**0.73**
		Mediterranea ODS C18 5µm	**716843 ± 3031**	**0.43**
Analyst B	**System B WATERS 501**	**Kromasil ODS C18 5µm**	**712568 ± 3570**	**0.50**
		Hypersil ODS C18 5µm	**715091 ± 6153**	**0.86**
		Mediterranea ODS C18 5µm	**720844 ± 4447**	**0.62**

References

1. A. C.Moffat, M. D.Osselton, B. Widdop (Eds.), Clark's analysis of drugs and poisons, Pharmaceutical press, UK, 3rd edition, **2**, 1069 (2004).
2. Kahlen Parfill, Sean Sweetman (Eds), Martindale, Pharmaceutical press, UK, 32nd edition, **1**, 346 (1999).
3. Olcay Sagirli, Sevil Muge Cetin, Armagan Onal, *Journal of Pharmaceutical and Biomedical Analysis*, **42**, 618 (2006).
4. Hassan Jalalizadeha, EffatSouria, Maliheh Barazandeh Tehrani., *Journal of Chromatography*. B, **854**, 43 47 (2007)
5. Z. Zhu and L. Neirinck, *Journal of Chromatography*. B, **779**, 307 (2002).
6. Effat Souri, Hassan Jalalizadeh, and Abbas Shafiee., *Chemical Pharmaceutical Bulletin*. **5,** 1427 (2007).
7. T. A.Vermeij and P. M.Edelbroek, *Journal of Chromatography.* B, **810**, 297 (2004).
8. H. Hengy and E. U.Kolle, *Journal of Chromatography*. **341**, 473 (1985).
9. Q. Jiang and S. Li, *Journal of Chromatography*. B, **27**, 119 (1999).
10. G. H. Bahrami and B. Mohammadi, *Journal of Chromatography* B, **837**, 24 (2006).
11. D. F. Chollet, L. Goumaz, C. Juliano and G. Anderegg, *Journal of Chromatography* B, **746,** 311 (2003).
12. D. R. Ifa, M. Falci, M. E. Moraes, F. A. Bezerra, M. O.Moreas and G. Denucci, *Journal of Mass Spectrometry*. **36,** 188 (2001).
13. N. Wad, G. Kramer, G. *Journal of Chromatography* B, **705**, 154 (1998).
14. F. Belal, H. Abdine, H. Al-Majed and N. Y.Khalil, *Journal of Pharmaceutical and Biomedical Analysis.* **27**, 253 (2002).
15. R. Sekar and S. Azhaguvel, *Journal of Pharmaceutical and Biomedical Analysis*, **36**, 663 (2004).
16. W. D.Hooper, M. C. Kavanagh and R. G.Dickinson, *Journal of Chromatography*. **529**, 167 (1990).
17. M. M.Khusir, J. Crosset, P. I. Brown and F. M.Urry, *Journal of Analytical Toxicology*.**23**, 1 (1999).
18. H. E. Abdellatef and H. M.Khalil, *Journal of Pharmaceutical and Biomedical Analysis*. **31**, 209 (2003).
19. The United States Pharmacopeia, 30th revision, Asian Edition, United States Pharmacopeial Convention, Inc, p. 2200, (2007).
20. Abhay Gupta, Anthony B.Ciavarella, Vilayat A. Sayeed , Mansoor A. Khan, PatrickJ.Faustino. *Journal of Pharmaceutical and Biomedical Analysis*, **46**, 618 (2008).
21. Anthony B. Ciavarella, AbhayGupta, Vilayat A. Sayeed, Mansoor A.Khan, Patrick J. Faustino, *Journal of Pharmaceutical and Biomedical Analysis*, **43**, 1467 (2007).
22. A. Mumtaz, A. A. Kazi, R. Nazir, M. U. Sabri and M. N. Shahid, *Journal Of The Chemical Society Of Pakistan*, **33**, 351 (2011).
23. K. U. Abbasi, M. Y. Khuhawar, M. I. Bhamger, K. P. Mahar and A. H. Channar, *Journal of the Chemical Society of Pakistan*, **33**, 778 (2011).