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Research Article

DEVELOPMENT AND VALIDATION OF REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR GABAPENTIN AND ITS RELATED SUBSTANCES IN CAPSULE DOSAGE FORM AND EXCIPIENT COMPATIBILITY STUDIES

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ABSTRACT

Objective: A simple, accurate, precise, and reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for gabapentin (GBP) and its related substances in the capsule dosage form and excipient compatibility studies.

Methods: The review of literature indicates that various methods have been reported for the estimation of GBP. When some excipients were used for GBP, it produced degradation product called lactam due to the presence of more water content. Hence, a novel RP-HPLC method has been developed for studying excipient compatibility and related substances of GBP in capsule dosage form using excipients such as lactose anhydrous and dried maize starch which is having less water activity. Waters Alliance e2695 separation module with ultraviolet/photodiode array (UV/PDA) detector with Inertsil C8 (250 mm×4.6 mm); 5 µm with an injection volume of 50 µl is injected and eluted with the (gradient program) mobile Phase A buffer: acetonitrile (940:60) and mobile phase B buffer: acetonitrile (700:300) pH 6.9 with 5 N potassium hydroxide which is pumped at a speed of 1.5 ml/min and detected by UV/PDA detector at 210 nm. The peaks of GBP and GBP-related compound A are well separated at 6.7 min and 34.5 min, respectively.

Results: The method developed was approved for various parameters such as accuracy, specificity, precision, intermediate precision, range, linearity, robustness, limit of detection, limit of quantification, steadiness, and system suitability according to the International Conference on Harmonization guidelines. The results got were according to the acceptance criteria.

Conclusion: The technique proposed was assured for detection of related substances in the marketed formulation and could be used for the routine analysis of GBP and GBP-related compound A in the capsule dosage form.

Keywords: Gabapentin, Gabapentin-related compound A, Reversed-phase high-performance liquid chromatography, Validation and capsule dosage forms.

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INTRODUCTION

Gabapentin (GBP) is chemically 2-[1-(aminomethyl)cyclohexyl]acetic acid as shown in Fig. 1. By expansion of a cyclohexyl group to gamma-aminobutyric acid (GABA) GBP was formed, which allowed this form of GABA to cross the blood–brain barrier.

GBP was first incorporated to mimic the chemical structure of the neurotransmitter GABA, but it was not able to act on the similar cerebrum receptors.

GBP stops the formation of new synapses. GBP has been found to decrease calcium flow after constant application by binding to the α2δ subunit (1 and 2) but not by acute application through an impact on trafficking of voltage-dependent calcium flow in central nervous system. The calcium channel trafficking is another possible mechanism, yet the exact mechanism of action of GBP stays in dispute.

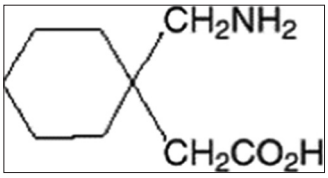

Fig. 1: Structure of gabapentin

GBP obviously has a novel mechanism of action, most likely including potentiation of GABA - mediated inhibition and conceivably inactivation of sodium channels also.

The literature survey [1-21] indicates that when lactose was used as an excipient for GBP, it produced an impurity called lactam, due to which stability was affected. Hence, according to the International Conference on Harmonization (ICH) guidelines, an attempt was made to create and validate a basic simple, accurate, precise, and efficient reversed-phase high-performance liquid chromatography (RP-HPLC) technique for the excipient compatibility studies and related substances of GBP using lactose monohydrate and maize starch as the excipients.

METHODS

Instrumentation

High-performance liquid chromatography (waters) with quaternary pump, with Inertsil C8 (250 mm×4.6 mm); 5 µm column detection of drug carried by ultraviolet (UV) detector data processing was carried out by Empower - 2 Software, weighing balance (Sartorius), sonicator (spectra lab), and pH meter (polmon).

Reagents and chemicals

GBP working standard, GBP-related compound A, acetonitrile (HPLC grade), HPLC water, potassium dihydrogen orthophosphate, potassium hydroxide; reference standards: GBP - USP certified reference standard

Bengaluru, GBP-related compound A - USP comprehensive ranking system, and excipients: Lactose monohydrate, lactose anhydrous, maize starch, and dried maize starch, talc were used.

Chromatographic conditions
Column: Inertsil C8 (250 mm×4.6 mm); 5 µm or equivalent
Flow rate: 1.5 ml/min
Injection volume: 50 µl
Column (oven temp): Ambient
Wavelength: UV/PDA at 210 nm
Elutiontype: Gradient.

Gradient program

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0–4.0	100	0
4.0–45.0	0	100
45.0–45.1	100	0
45.1–50.0	100	0.

The peaks of GBP and GBP-related compound A are well separated at 6.7 min and 34.5 min, respectively.

Solution preparation

Preparation of 5N potassium hydroxide

Weigh about 28.06 g of potassium hydroxide transfer into 100 ml glass beaker, pour about 50 ml of purified water and dissolve it and makeup to 100 ml with water.

Diluent

Dissolve 1.2 g of dihydrogen potassium orthophosphate KH_2PO_4 in 1000 ml of water and maintain the pH at 6.9 using 5 N potassium hydroxide.

Preparation of mobile phase A

Dissolve 1.2 g potassium dihydrogen orthophosphate in 940 ml of water. Adjust the pH to 6.9 with 5 N potassium hydroxide and add 60 ml of acetonitrile and mix well. The solution was filtered through a membrane filter (0.45 µm) and degassed.

Preparation of mobile phase B

Dissolve 1.2 g potassium dihydrogen orthophosphate in 700 ml of water. Adjust the pH to 6.9 using 5 N potassium hydroxide and add 300 ml of acetonitrile and mixed well. The solution was filtered through a membrane filter (0.45 µm) and degassed.

GBP-related compound A stock solution

Accurately weigh about 10 mg of GBP-related compound A RS in a volumetric flask of 20 ml volume add diluent to solubilize the content and makeup to the volume by using diluent.

Standard preparation

Accurately weigh 0.025 g of GBP working standard into a volumetric flask of 50 ml, add diluent to solubilize the content by shaking for 1 min and make to volume with diluent. Pipette out 2 ml of this solution and 2 ml of GBP-related compound A stock solution into 25 ml and makeup to the volume with diluent and mixed well. The solution was filtered through a membrane filter (0.45 µm).

Calibration curves

The range and linearity of the analytical method for GBP was confirmed by injecting the various concentrations of GBP standard preparation prepared according to the range of limit of quantification (LOQ) covering six different concentrations up to 150% of specification level into the chromatograph, and the response was observed to be linear within that range. The linearity of GBP and each related substance was plotted by a graph between response factor and concentration. The relationship between the concentration and response was linear in the specified range, and the correlation coefficient was 0.99 for GBP and each related substance.

Sample preparation

Remove and weigh the contents of not fewer than 20 capsules. Transfer an accurate weighed portion of the powder, approximately to about 500 mg of GBP, to 25 ml volumetric flask and add about 15 ml of diluent. Shake for 1 min and makeup to the level using the diluent and then mix well. The solution was filtered through a membrane filter (0.45 µm). Collect the filtrate, after removing the certain amount of the filtrate.

Procedure
Equilibrate the column for not <30 min with an initial gradient and rate of flow was kept at 1.5 mL/min. Inject separately 50 µL of the above all solutions into the HPLC and measure the peak responses for the major peaks record the chromatograms. The typical chromatogram is shown in Figure 2.

RESULTS AND DISCUSSION

Method validation procedure
The objective of the method validation as stated in ICH guidelines is to exhibit that the technique is reasonable for its intended purpose.

Accuracy (recovery studies)
By adding known quantity of known impurity, the recovery studies were performed standards to test solution in the range of LOQ to 150% of specification limit specified in the method of analysis, with four different concentrations (LOQ, 50%, 100%, and 150% in triplicate) to represent the entire range. The percentage recovery and relative standard deviation (RSD) for all the values of percentage recovery were reported. The mean percentage recovery of all known related substance

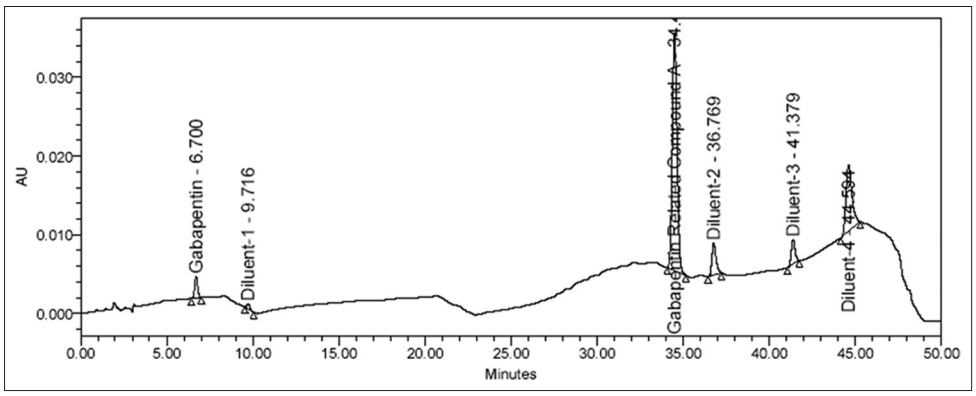

Fig. 2: Typical graph of gabapentin

Table 1: Recovery of GBP-related compound A

% Level/sample ID	% of impurity added with respect sample concentration	% total impurity found in spiked sample	% mean impurity in control sample	% of impurity found with respect to spiked quantity	% of impurity recovered with respect to spiked quantity	Average recovery
LOQ level sample-1	0.0025	0.0176	0.0148	0.0028	112.00	112.0
LOQ level sample-2	0.0025	0.0176	0.0148	0.0028	112.00	
LOQ level sample-3	0.0025	0.0176	0.0148	0.0028	112.00	
50% level sample-1	0.1006	0.1154	0.0148	0.1006	100.00	100.0
50% level sample-2	0.1005	0. 1155	0.0148	0.1007	100.20	
50% level sample-3	0.1005	0.1151	0.0148	0.1003	99.80	
100% level sample-1	0.2011	0.2213	0.0148	0.2065	102.69	102.7
100% level sample-2	0.2011	0.2213	0.0148	0.2065	102.69	
100% level sample-3	0.2011	0.2216	0.0148	0.2068	102.83	
150% level sample-1	0.3017	0.3264	0.0148	0.3116	103.28	103.6
150% level sample-2	0.3017	0.3277	0.0148	0.3129	103.71	
150% level sample-3	0.3016	0.3275	0.0148	0.3127	103.68	
Overall statistical analysis		For LOQ level		Mean	112.0	
				SD	0.00	
				% RSD	0.0	
		For other levels		Mean	102.1	
				SD	1.62	
				% RSD	1.6	

GBP: Gabapentin, SD: Standard deviation, LOQ: Limit of quantification, RSD: Relative standard deviation

Table 2: Percentage of GBP-related compound A at RT~34.4 for six samples of the same batch

Sample ID	Weight of sample taken (mg)	GBP-related compound A at RT~34.4	% of GBP-related compound A at RT~34.4 with respect to GBP-related compound A (standard solution)
Sample-1	662.52	41,642	0.01
Sample-2	661.30	44,841	0.01
Sample-3	661.83	45,046	0.01
Sample-4	662.92	44,172	0.01
Sample-5	661.62	43,863	0.01
Sample-6	662.04	43,504	0.01
Mean			0.01
SD			0.00
% RSD			0.0

GBP: Gabapentin, SD: Standard deviation, RSD: Relative standard deviation

Table 3: Percentage of total impurities for six samples of the same batch

Sample ID	Total impurities (%)
Sample-1	0.01
Sample-2	0.01
Sample-3	0.01
Sample-4	0.01
Sample-5	0.01
Sample-6	0.01
Mean	0.01
SD	0.00
% RSD	0.0

SD: Standard deviation, RSD: Relative standard deviation

GBP-related compound A of nine determinations over three different concentrations and three determinations at LOQ levels were within the acceptance limit is shown in Table 1.

Precision

Repeatability of analytical technique was established by assessing the related substances for six different test solutions of the same batch. The percentage of related substances for all six test solutions was calculated, and the percentage (RSD) for the same was reported. The evaluation of related substances, i.e., GBP-related Compound A, for all six determinations has been presented in Tables 2 and 3. Unknown impurity is not detected in the all six sample preparations of the sample batch.

Ruggedness

The ruggedness of the analytical method was confirmed by estimating the related substances for six different test solutions of the same batch by another analyst on a different HPLC system using another column on some another day. The percentage of related substances for all six test solutions was calculated, and the percentage RSD was reported for the same. The analytical method intermediate precision was established by estimating the related substances for six different samples of the same batch by another analyst on a different HPLC system using a column of another lot number on some another day. The evaluation of related substances, i.e., GBP-related Compound A, for all six determinations has been presented in Tables 4 and 5. Two unique analysts carried out the analysis of the same batch of GBP 400 mg capsules on two distinct instruments, with two different columns on distinct days. The details presented in Table 6.

Linearity

The linearity of the analytical method was demonstrated by injecting the various concentrations of all known impurities, and GBP prepared according to the range of LOQ covering at least six different concentration levels up to 150% of specification level into the chromatograph. Regression analysis results of the linearity data have been presented in Table 7 and Figure-3. The linearity and range of the analytical method for GBP-related Compound A was established by injecting the various concentrations of GBP-related Compound A standard preparation prepared according to a range of LOQ, covering six different concentrations up to 150% of specification level into the chromatograph. Regression analysis results of the linearity and range of the analytical method for GBP-related Compound A have been presented in Table 8 and linearity graph of GBP-related Compound A, presented in Figure-4.

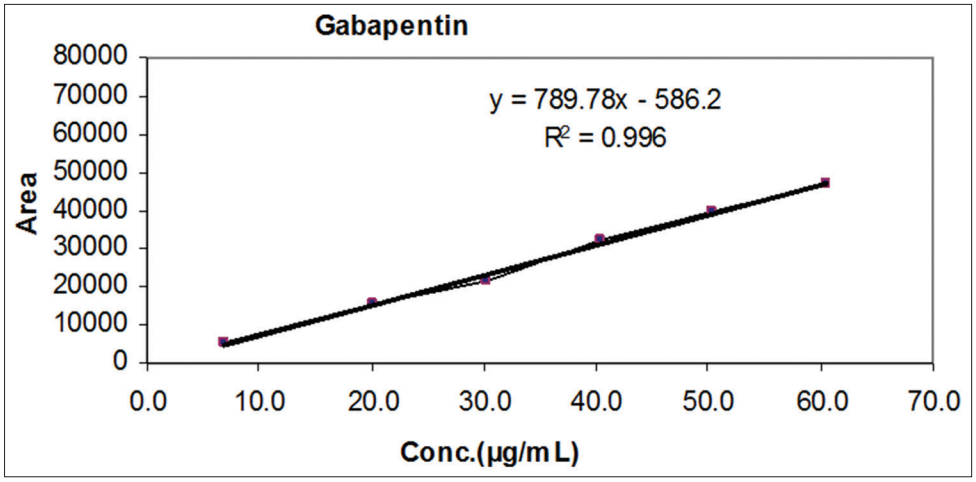

Fig. 3: Linearity graph of gabapentin

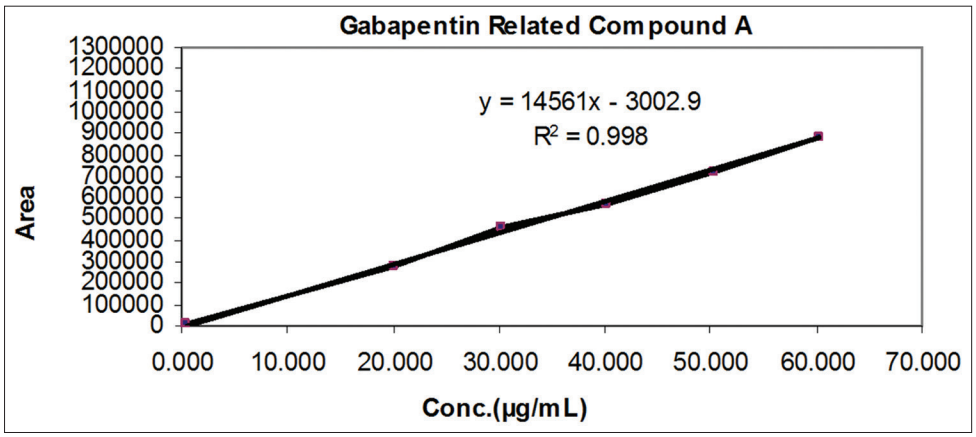

Fig. 4: Linearity graph of gabapentin related compound A

Table 4: Percentage of GBP-related compound A at RT~34.4 for six samples of the same batch

Sample ID	Weight of sample taken (mg)	GBP-related compound A at RT~34.4	% of GBP-related compound A at RT~34.4 with respect to GBP-related compound A (standard solution)
Sample-1	661.53	40,925	0.01
Sample-2	660.85	36,944	0.01
Sample-3	661.36	39,602	0.01
Sample-4	661.40	39,902	0.01
Sample-5	661.62	39,154	0.01
Sample-6	661.92	40,986	0.01
Mean			0.01
SD			0.00
% RSD			0.0

GBP: Gabapentin, SD: Standard deviation, RSD: Relative standard deviation

Table 5: Percentage of total impurities for six samples of the same batch

Sample ID	Total impurities (%)
Sample-1	0.01
Sample-2	0.01
Sample-3	0.01
Sample-4	0.01
Sample-5	0.01
Sample-6	0.01
Mean	0.01
SD	0.00
% RSD	0.0

SD: Standard deviation, RSD: Relative standard deviation

Limit of detection (LOD) and LOQ

LOD and LOQ values of related substance and GBP can be predicted based on S/N ratio method. For this, the 100% linearity solutions of each related substance and GBP were considered from the respective linearity experiment. The signal-to-noise ratio should be 10 and 3.3 for LOQ and LOD, respectively. Each anticipated concentration was checked by setting the solutions at about anticipated concentrations and infusion every solution 6 times into the chromatograph independently. Percentage RSD for the areas obtained for the six replicate injections of GBP and each related substance has been given in Tables 9-12.

Specificity/selectivity

This method proves the specificity of the analytical technique for the estimation of related substances in GBP 400 mg capsules by HPLC.

Table 6: Intermediate precision details

Experiment name	Method precision		Ruggedness
Analyst	Analyst 1		Analyst 2
Equipment ID	FD-20		FD-21
Column ID	LC004		LC005
Day	November 27, 2010		December 04, 2010
Impurity name	**Overall impurity summary**		
	Mean	**SD**	**% RSD**
GBP-related compound A	0.01	0.00	0.0
Any unknown impurity	Not applicable	Not applicable	Not applicable
Total impurities	0.01	0.00	0.0

GBP: Gabapentin, SD: Standard deviation, RSD: Relative standard deviation

Table 7: Linearity of GBP

Percent solution with respect to specification limit	Concentration (µg/ml)	Area
LOQ	6.804	5534
50	20.160	15160
75	30.240	21557
100	40.320	31977
125	50.400	39727
150	60.480	47121
Slope		790
Intercept		−586
Correlation coefficient		0.998
R^2		0.996

GBP: Gabapentin, LOQ: Limit of quantification

Table 8: Linearity of GBP-related compound A

Percent solution with respect to specification limit	Concentration (µg/ml)	Area
LOQ	0.503	6478
50	20.120	281,328
75	30.180	456,939
100	40.240	566,144
125	50.300	723,277
150	60.360	884,844
Slope		14,561
Intercept		−3003
Correlation coefficient		0.999
R^2		0.998

GBP: Gabapentin, LOQ: Limit of quantification

Table 9: Range

Impurity name	Range (% of specification)
GBP-relate compound A	OQ to 150

GBP: Gabapentin, LOQ: Limit of quantification

Table 10: LOD and LOQ for GBP

Injection ID	Area of GBP	
	LOD	**LOQ**
1	1648	6025
2	1717	5850
3	1677	5847
4	1569	5851
5	1543	6199
6	1623	5984
Mean	1630	5959
SD	65.5	140.5
% RSD	4.0	2.4
Con. (µg/mL)	2.244	6.801

LOD: Limit of detection, LOQ: Limit of quantification, GBP: Gabapentin, SD: Standard deviation, RSD: Relative standard deviation

Table 11: LOD and LOQ for GBP-related compound A

Injection ID	Area of GBP-related compound A	
	LOD	**LOQ**
1	1548	6789
2	1812	6989
3	1831	6812
4	1852	6748
5	1923	7136
6	1622	7193
Mean	1765	6945
SD	146.0	190.1
% RSD	8.3	2.7
Con. (µg/mL)	0.166	0.503

LOD: Limit of detection, LOQ: Limit of quantification, GBP: Gabapentin, SD: Standard deviation, RSD: Relative standard deviation

Table 12: LOD and LOQ for GBP,GBP-related compound A

S. No.	Impurity name	LOD (µg/mL)	LOQ (µg/mL)
1.	GBP-related compound A	0.166	0.503
2.	GBP	2.244	6.801

LOD: Limit of detection, LOQ: Limit of quantification, GBP: Gabapentin

Table 13: Data from spiked sample

Name	RT	Peak purity	
		Purity angle	**Purity threshold**
GBP	6.685	0.645	0.663
GBP-related compound A	34.503	0.055	0.213

GBP: Gabapentin

Table 14: Peak purity of GBP data from control sample

Sample ID	RT	Peak purity	
		Purity angle	**Purity threshold**
GBP	6.682	0.583	0.665
GBP-related compound A	34.512	0.560	0.636

GBP: Gabapentin

Selectivity of the method has been established by injecting the following:

a. Standard and test solutions, prepared as per test method as a part of identification.
b. Blank and placebo solutions to check any interference of peaks from these solutions with that of the analyte peaks.
c. All the related substances solutions individually to confirm the retention times.
d. Control Sample (capsule sample) and Spiked Sample (Sample spiked with all known related substances at specification level).
e. GBP to confirm the retention time.

The data are represented in Tables 13 and 14.

Table 15: Stability of GBP-related compound A (standard)

Sample ID	Area of GBP-related compound A	
	Area	% Difference
Initial	651,914	-
After 2 h	651,434	0.1
After 4 h	650,035	0.3
After 6 h	651,434	0.1
After 8 h	648,785	0.5
After 10 h	648,152	0.6
After 12 h	648,162	0.6
After 14 h	648,386	0.5
After 16 h	646,013	0.9
After 18 h	645,712	1.0
After 20 h	651,501	0.1
After 22 h	648,538	0.5
After 24 h	648,302	0.6

GBP: Gabapentin

Table 16: Stability of GBP (standard)

Sample ID	Area of GBP	
	Area	% Difference
Initial	34,690	-
After 2 h	35,056	-1.1
After 4 h	35,141	-1.3
After 6 h	35,069	-1.1
After 8 h	35,008	-0.9
After 10 h	35,047	-1.0
After 12 h	35,164	-1.4
After 14 h	35,273	-1.7
After 16 h	35,513	-2.4
After 18 h	35,187	-1.4
After 20 h	35,417	-2.1
After 22 h	35,435	-2.1
After 24 h	35,433	-2.1

GBP: Gabapentin

Table 17: Stability of GBP-related compound A (sample)

Sample ID	Area of GBP-related compound A	
	Area	% Difference
Initial	45,116	-
After 2 h	45,100	0.0
After 4 h	44,934	0.4
After 6 h	45,703	−1.3
After 8 h	45,029	0.2
After 10 h	45,645	−1.2
After 12 h	45,065	0.1
After 14 h	45,669	−1.2
After 16 h	45,944	−1.8
After 18 h	46,084	−2.1
After 20 h	44,823	0.6
After 22 h	46,532	−3.1
After 24 h	46,518	−3.1

GBP: Gabapentin

Stability of analytical solutions

Stability of standard and test solutions at room temperature (25°C) was established by injecting the standard, and test solutions were made according to test technique at various time intervals by keeping all the standard and test solutions at room temperature (~25°C). GBP area in standard solution was measured, and the difference in the area was calculated. The areas of known and unknown related substances in sample solution were measured, and the percentage differences of areas were calculated. The values for the stability of standard and samples solutions have been presented in Tables 15-18.

Table 18: Stability of GBP (sample)

Sample ID	Area of GBP	
	Area	% Difference
Initial	16,706,661	-
After 2 h	16,715,428	−0.1
After 4 h	16,723,944	−0.1
After 6 h	16,718,998	−0.1
After 8 h	16,707,811	0.0
After 10 h	16,705,271	0.0
After 12 h	16,716,568	−0.1
After 14 h	16,720,348	−0.1
After 16 h	16,730,682	−0.1
After 18 h	16,792,874	−0.5
After 20 h	16,818,753	−0.7
After 22 h	16,795,617	−0.5
After 24 h	16,728,026	−0.1

GBP: Gabapentin

Table 19: Result from standard - system suitability solution

Condition	Variation	USP Tailing-GBP	%RSD	
			GBP	GBP-related compound A
STP	Actual	1.1	0.4	0.4
Flow rate (mL/min)	1.3	1.1	0.3	0.1
	1.7	1.1	0.3	0.1
% organic composition (solution: Acetonitrile)	720:280	1.1	0.3	0.2
	680:320	1.1	0.3	0.1
Column oven temperature (°C)	20	1.1	0.4	0.2
	30	1.1	0.3	0.1
Wavelength (nm)	Actual	1.1	0.6	0.5
	208	1.1	1.2	0.3
	212	1.1	1.0	0.9

GBP: Gabapentin, RSD: Relative standard deviation

Table 20: Result from spiked sample

Condition	Variation	GBP-related compound A retention time
STP	Actual	34.747
Flow rate (mL/min)	1.3	36.690
	1.7	33.067
% Organic composition (solution: Acetonitrile)	720:280	35.989
	680:320	33.513
Column oven temperature (°C)	20	35.285
	30	34.509
Wavelength (nm)	Actual	34.503
	208	34.503
	210	34.503

GBP: Gabapentin

Robustness

In analytical technique, robustness was established by infusing system suitability solution and sample solution added with other known related substances at particular specification level into HPLC under deliberately modified chromatographic conditions. The results from system suitability and spiked sample were meeting the acceptance criteria at each of the variable condition, and the results have been presented in Tables 19 and 20.

System suitability

To confirm the suitability of the chromatographic system described under the technique which is analyzed by establishing system suitability parameters such as percentage RSD and tailing factor. Standard was prepared and injected into chromatography as per methodology on a

Table 21: Result from system suitability on daily basis

S. No.	Parameters	GBP USP tailing factor	%RSD	
			GBP	GBP-related compound A
1	Repeatability	1.1	0.5	0.3
2	Solution stability	1.1	0.2	0.2
3	Robustness	1.1	0.4	0.4
4	LOD-LOQ prediction	1.1	0.6	0.1
5	LOD-LOQ precision (GABA)	1.1	0.6	0.5
6	LOD-LOQ precision (Imp-A)	1.1	0.5	0.5
7	Accuracy	1.1	0.5	0.4
8	Ruggedness	1.0	0.7	0.9
9	Specificity	1.1	0.6	0.5
10	Linearity	1.1	1.2	1.3

GABA: Gamma-aminobutyric acid, GBP: Gabapentin, RSD: Relative standard deviation, LOD-LOQ: Limit of detection-limit of quantification

daily basis. The tailing factor for GBP extent in the standard was not >2.0, and the results are shown in Table 21.

CONCLUSION

The literature survey indicates that several techniques have been already reported for the estimation of GBP in various dosage forms. However, very little information has been reported for excipient compatibility and related substances of GBP.

The literature survey indicates that when lactose monohydrate, maize starch was used as an excipient for GBP, it produced degradation product called lactam by Maillard-type condensation reaction due to the presence of more water content and hence water activity.

Hence, an effort has been made to find an RP-HPLC technique for studying excipient compatibility and related substances of GBP in capsule dosage form by using excipients such as lactose anhydrous and dried maize starch which is having less water activity.

A RP-HPLC technique is initiated for the estimation of related substances of GBP in the capsule dosage form. Waters Alliance e2695 separation module with UV/photodiode array (PDA) detector with Inertsil C8 (250 mm×4.6 mm); 5 µm with an injection volume of 50 µl is injected and eluted with the (Gradient program) mobile phase A buffer: acetonitrile (940:60) and mobile phase B buffer: Acetonitrile ACN (700:300) pH 6.9 with 5 N potassium hydroxide which is pumped at a flow rate of 1.5 mL/min and detected by UV/PDA detector at 210 nm. The peaks of GBP and GBP-related compound A are well separated at 6.7 min and 34.5 min, respectively.

The developed technique is validated for various parameters according to the ICH guidelines such as linearity, accuracy, precision, intermediate precision, specificity, range, robustness, LOD, LOQ, stability, and system suitability. The results got were according to the acceptance criteria.

The proposed technique is assured for detection of related substances in marketed formulation. Hence, the proposed technique observed to be agreeable and could be utilized for the routine analysis of GBP and GBP-related compound A in the capsule dosage form.

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