



DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GABAPENTIN AND AMITRIPTYLINE HYDROCHLORIDE IN ITS PHARMACEUTICAL DOSAGE FORM

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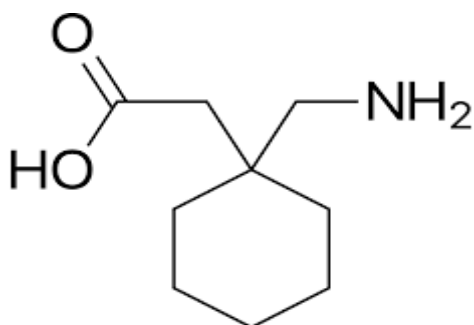
ABSTRACT

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Gabapentin and Amitriptyline Hydrochloride in Pharmaceutical Dosage Form. RP-HPLC method, Column used was Water Symmetry250 x 4.6mm 5 μ C18, with mobile phase containing 20 mM Ammonium Acetate (PH 6.0) : Acetonitrile (65:35) %v/v The flow rate (1.0 ml/min) and wavelength (272 nm). The retention time for Gabapentin and Amitriptyline Hydrochloride was found to be 2.793 $\pm$ 0.01 min. and 4.831 $\pm$ 0.01 min. respectively. Correlation coefficient for Gabapentin and Amitriptyline hydrochloride was found to be 0.999. Assay result of marketed formulation was found to be in 99.5 % and 98.7% for Gabapentin and Amitriptyline Hydrochloride. The proposed method was validated with respect to ICH Guideline. Percentage recovery for Gabapentin and Amitriptyline hydrochloride was found to be 99.0 – 100.0%. Analysis proves that the developed method can be successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

KEYWORDS: Gabapentin, Amitriptyline Hydrochloride, RP-HPLC, analytical method development, Validation, ICH, Chromatography.

INTRODUCTION

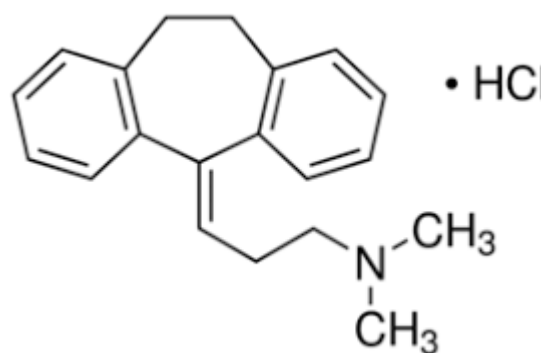
The IUPAC name of the Gabapentin 2-[1-(aminomethyl)cyclohexylacetic acid] and Amitriptyline hydrochloride is 3-(10,11-dihydro-5H-dibenzo[a,d]cycloheptene-5-ylidene)-n,n-dimethylpropan-1-amine. With molecular formula and molecular weight $C_9H_{17}NO_2$. And 171.24 g/mol and $C_{20}H_{23}N$ and 277.411 g/mol respectively.



The molecular structure of the drug is given in Fig.1

➤ Gabapentin use as antiseizures. The IUPAC name of the Gabapentin is 2-[1-(aminomethyl) cyclohexylacetic acid] With molecular

formula and molecular weight $C_9H_{17}NO_2$. and 171.24 g/mol respectively.



The molecular structure of the drug is given in Fig.2.

➤ Amitriptyline hydrochloride is antidepressant. However no RP-HPLC method has been reported till date for the estimation of Gabapentin and Amitriptyline Hydrochloride using the RP-HPLC method. The present paper describes the analytical method development and validation of estimation of Metformin Hydrochloride and Teneligliptin Hydrobromide Hydrate in Pharmaceutical dosage form using RP-HPLC. The proposed method are

optimized and validated as per ICH guidelines.

MATERIALS AND METHODS MATERIALS

a) Instruments

- Analytical Weighing Balance
- Sonicator
- FT-IR spectrophotometer
- HPLC system
- Millipore Filter Unit
- pH Meter
- UV Spectrophotometer

b) Glasswares

- Beaker
- Conical flask
- Measuring cylinder
- Petri dish
- Pipette
- Volumetric flask

c) Chemicals

- Standard Gabapentin and Amitriptyline hydrochloride chemdyes corporation and wockhardt Ltd.
- The commercial fixed dose combination **GABANTIP AT 100** Tablets 10's manufactured by Pure and cure health care pvt.ltd was procured from local market.

d) Method

- Chromatographic method

Methods

Working Standard preparation

- **Solution Preparation of Gabapentin: (100 µg/ml)**
About 5 mg of metformin HCl API was weight and dissolve in 50 ml of water.

System Suitability Parameters

Table 1: System Suitability Test Parameters for Gabapentin and Amitriptyline HCL.

Sr. No.	System suitability parameter	Gabapentin	Amitriptyline hydrochloride
1	Retention time (min)	2.788	4.835
2	Resolution (R)	-	8.5
3	Theoretical plate number (N)	35693	12583
4	Tailing factor (T)	1.0	1.1

Linearity and Range (n=3)

- The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in sample within a given range.
- The range of analytical method is the interval between the upper and lower levels of analytes that have been demonstrated to be determined within a

• Solution preparation of Amitriptyline hydrochloride: (10µg/mL)

10 mg of Amitriptyline hydrochloride API was dissolved in 100 ml of diluent, further diluted 1 ml to 10 ml with water to get 10µg/ml concentration of Amitriptyline hydrochloride in solution.

• Formulation Sample preparation of Gabapentin and Amitriptyline: (100 µg/mL & 10µg/mL respectively)

- Based on above label claim sample was prepared as per following procedure:
- Transferred 2 intact tablets in to 200mL volumetric flask, added about 170mL of diluent in to it, sonicated for 30 minutes with intermittent shaking, cooled to attain room temperature and made up to volume with diluent and mixed well. It was filtered through 0.45µ syringe filter. 5mL of this filtrate was further diluted to 50mL with diluent to attain the final concentration as 100µg/mL of Gabapentin and 10µg/mL of Amitriptyline. This sample was injected.

METHOD VALIDATION

Chromatographic conditions and System Suitability Parameters: Mode of chromatography: Reversed Phase Chromatography.

Mode of Elution: Isocratic **Flow Rate:** 1.0ml/min

Oven: Ambient: 25° ± 2°C **Detector:** Type: UV detector

Wavelength: 272 nm.

Column: 250 x 4.6mm C18, Water symmetry.

Sample Volume: 20µl.

Run time: 10min

Mobile Phase: 20mM ammonium acetate (pH 6.0, adjust with diluted glacial acetic acid): Acetonitrile (65:35).

suitable level of precision, accuracy and linearity.

- The linearity was determined at five levels over the range of 50-150 µg/ml for Gabapentin and 5.0-15.0 µg/ml Teneligliptin Amitriptyline hydrochloride. Peak area of above linearity solution preparations were taken at each concentration three times. Mean Peak Area at each concentration was calculated and Graph of Mean Peak Area (y axis) versus

Concentration (x-axis) was plotted.

Precision Repeatability

Six replicate of 100 µg/ml concentration of Gabapentin and 10 µg/ml concentration of Amitriptyline hydrochloride were prepared and chromatographic were recorded at the optimized condition. SD and RSD were calculated.

Intraday Precision and Interday Precision

Variations of results within the same day (intra-day), variation of results between days (inter-day) were analyzed. Intra-day precision was determined by analyzing both standard solutions for three times in the same day. Interday precision was determined by analyzing the drugs daily for three days. %RSD was calculated.

Accuracy (% Recovery)

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 5 tablet powder were weighed and analysis was carried out as per assay. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into

consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameter

- Flow rate: ± 0.2 ml/min
- Change in the ratio of component in the mobile phase: $\pm 2\%$.
- pH of mobile phase: ± 0.2

RESULT

VALIDATION PARAMETER

Linearity and Range

Linear correlation was obtained between peak area and concentration of Gabapentin in the range of 50-150 µg/ml and Amitriptyline hydrochloride in the range of 5-15 µg/ml. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r).

Table 2: Linearity data for Gabapentin.

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation Coefficient
50	50	3229389	0.999
75	75	4846389	
100	100	6466487	
125	125	8080712	
150	150	9741987	

Table 3: Linearity data for Amitriptyline hydrochloride.

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation Coefficient
50	5	1204965	0.999
75	7.5	1808062	
100	10	2415565	
125	12.5	3018554	
150	15	3633029	

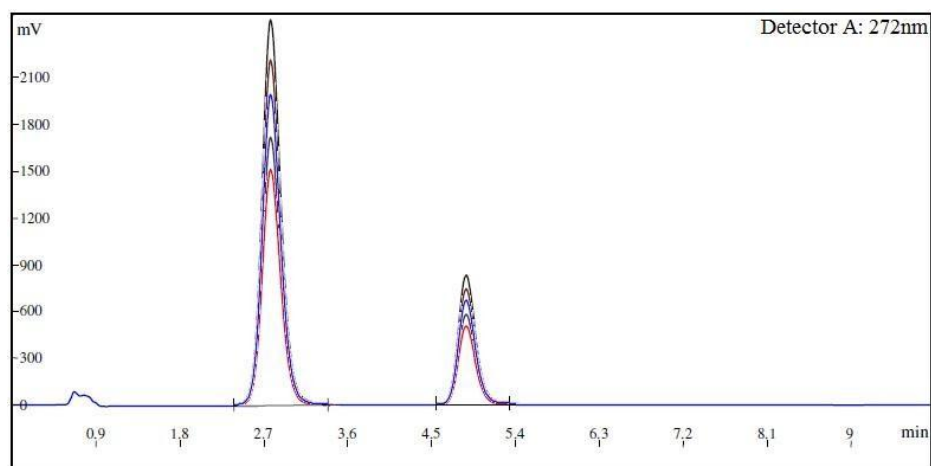


Figure 3: Overlay chromatogram of different concentration of gabapentin and amitriptyline hydrochloride.

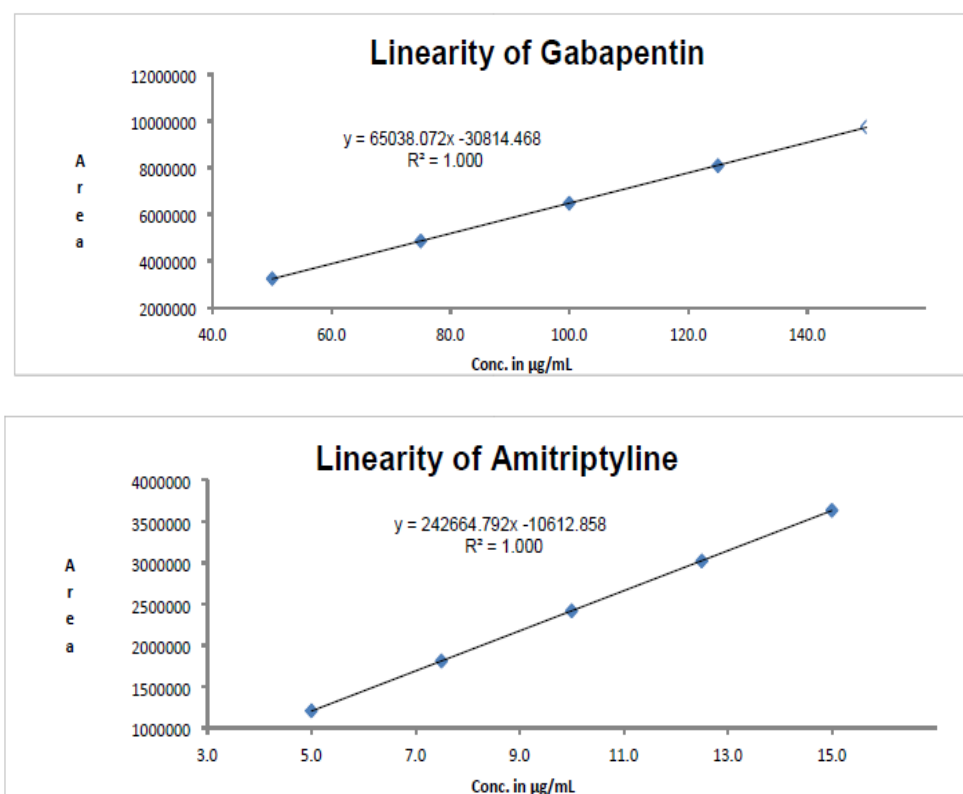


Figure 4: Calibration curve of gabapentin and amitriptyline hydrochloride.

ACCURACY

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of

standard addition. Percentage recovery for gabapentin and amitriptyline hydrochloride was found to be 99.0 – 100.0 %.

Table 4: Recovery Data of Gabapentin.

Accuracy Level %	Set no.	Amount Added (mg)	Amount Recovery (mg)	% Recovery	Mean	% RSD
80	1	160	158.10	98.8	99.6	0.7
	2	161	161.18	100.1		
	3	159.20	159.00	99.9		
100	1	201	202.14	100.6	99.6	0.9
	2	200	197.84	98.8		
	3	199	197.55	99.3		
120	1	241	241.78	100.3	99.5	0.9
	2	241.40	240.39	99.6		
	3	239.60	236.31	98.6		

Table 5: Recovery Data of Amitriptyline hydrochloride.

Accuracy Level %	Set no.	Amount Added (mg)	Amount Recovery (mg)	% Recovery	Mean	% RSD
80	1	16.00	15.99	99.9	99.6	1.2
	2	15.90	15.99	100.6		
	3	16.14	15.87	98.3		
100	1	20.00	19.88	99.4	99.3	0.5
	2	20.10	19.85	98.8		
	3	19.96	19.93	99.8		
120	1	24.00	24.22	100.9	99.8	1.0
	2	24.12	24.04	99.5		
	3	24.02	23.78	99		

PRECISION**Repeatability (Method precision, n=6)****Table 6: Repeatability of Gabapentin.**

Sr. no	Area	Mean	SD	%RSD
1	6415278	6416293	19763.1	0.3
2	6405281			
3	6395622			
4	6432115			
5	6447210			
6	6402253			

Table 7: Repeatability of amitriptyline hydrochloride.

Sr. no	Area	Mean	SD	%RSD
1	2415236	2408161	10411.6	0.4
2	2401569			
3	2395562			
4	2421145			
5	2399956			
6	2415500			

7. The % RSD for Repeatability data was found to be 0.3% and 0.4% respectively.

Intraday precision

The data for intraday precision for Gabapentin is shown in Table-8. The % RSD For intraday precision was found to be 0.23%. The data for intraday precision for Amitriptyline hydrochloride is shown in Table-9. The % RSD for intraday precision was found to be 0.44%.

Repeatability

The data for repeatability of Gabapentin and Amitriptyline hydrochloride is shown in Table 6 & Table

Table 8: Intraday precision for gabapentin (n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	50	3205109±7382.5	0.23
2	100	6411926±13325.3	0.21
3	150	9659931±25520.1	0.26
Mean			0.2333

Table 9: Intraday precision for amitriptyline hydrochloride (n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	5	1194648±1386.6	0.12
2	10	2394890±3659.5	0.15
3	15	3902001±18477.8	0.51
Mean			0.44

Interday precision

The data for interday precision for Gabapentin is shown in Table-10. The % RSD For intraday precision was found to be 0.82%. The data for interday precision for amitriptyline hydrochloride is shown in Table-11. The % RSD for intraday precision was found to be 0.70%.

Table 10: Interday precision for Gabapentin(n=3).

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	50	3175676±24563.6	0.77
2	100	6353044±48847.8	0.77
3	150	9571307±90104.6	0.94
Mean			0.82

Table 11: Interday precision for amitriptyline hydrochloride (n=3)

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	5	1198658±9019.0	0.75
2	10	2402906±12766.0	0.53
3	15	3614079±30442.5	0.84
Mean			0.70

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The Limit of detection (LOD) and Limit of quantitation (LOQ) Gabapentin and Amitriptyline hydrochloride as mention below table 12.

Table 12: Results of LOD and LOQ.

Drug	Gabapentin	Amitriptyline hydrochloride
LOD	0.79	0.019
LOQ	2.40	0.058

ROBUSTNESS**Robustness**

The method is found to be robust as the results were not significantly affected by slight variation in composition of mobile phase, Mobile phase pH and flow rate of the mobile phase.

Table 13: Change the flow rate

Standard repetitions (n=6)	0.8ml/min		1.2ml/min	
	Gaba	AMI	Gaba	AMI
Mean Area	7041947	2648977	5828107	2171380
% RSD	0.4	0.4	0.4	0.8

Table 14: Change the mobile phase composition.

Standard repetitions (n=6)	63:37		67:33	
	GABA	AMI	GABA	AMI
Mean Area	6443052	2411952	6444624	0.7
% RSD	0.6	0.5	2392103	0.3

Table 15: Change the mobile phase pH.

Standard repetitions (n=6)	6.1		5.9	
	GABA	AMI	GABA	AMI
Mean Area	6424310	2411952	6422815	2394103
% RSD	0.3	0.5	0.3	0.4

System Suitability tests

Table 16: System Suitability Test Parameters for Gabapentin and Amitriptyline hydrochloride.

Sr. No.	System suitability Parameter	GABA	AMI
1	Retention time (min)	2.788	4.835
2	Resolution (R)	-	8.5
3	Theoretical plate number (N)	35693	12589
4	Tailing factor (T)	1.0	1.1

Assay preparation (Marketed formulation)

Label claim: GABA-100mg and AMI-10mg.

Sample stock solution:**Formulation Sample preparation of Gabapentin and Amitriptyline**

(100 µg/mL & 10µg/mL respectively)

Based on above label claim sample was prepared as per following procedure.

Transferred 2 intact tablets in to 200mL volumetric flask, added about 170mL of diluent in to it, sonicated for 30

minutes with intermittent shaking, cooled to attain room temperature and made up to volume with diluent and mixed well. It was filtered through 0.45µ syringe filter. 5mL of this filtrate was further diluted to 50mL with diluent to attain the final concentration as 100µg/mL of Gabapentin and 10µg/mL of Amitriptyline. This sample was injected.

Working sample preparation

Take 5ml from sample stock solution into a 50ml and make up with mobile phase. (GABA- 100mcg/ml, AMI- 10mcg/ml)

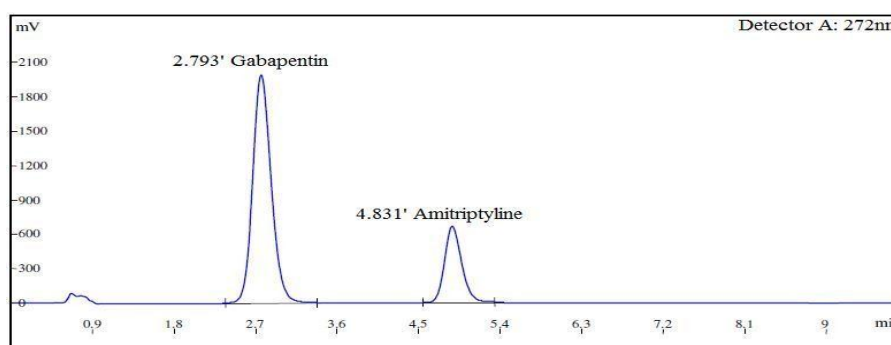


Figure 16: Injection of marketed formulation.

Peak Table**Table 17: Injection of marketed formulation.**

Sr. No	Peak name	Retention time	Area	Tailing factor	Theoretical Plates	Resolution time
1	GABA	2.793	6393553	1.0	34526	-
2	AMI	4.831	2370303	1.0	12410	8.5

Observations

In formulation sample preparation, both peaks are found well separated with good peak shape.

% Assay Results from Formulation**Table 18.**

Sr. No.	Sample name	% Assay of GABA	% Assay of AMI
1	Formulation (Gabantip AT 100 TAB)	99.5%	98.7%

SUMMARY OF REGRESSION PARAMETERS**Table 19: Summary of Regression Parameters for GABA and AMI.**

Sr. No.	Parameters	GABA	AMI	REMARK
1	Linearity ($\mu\text{g/ml}$)	50-150 $\mu\text{g/ml}$	5-15 $\mu\text{g/ml}$	Linear
2	%Recovery	99.5-99.9	99.5-99.8	Accurate (98.0%-102%)
3	Precision (%RSD)	0.3%.	0.4%.	Precise (%RSD < 2)
	Repeatability (n=6)			
	Intra-day (n=3)			
	Inter-day (n=3)			
4	Specificity	Specific	Specific	Specific (No interference)
5	Robustness	Robust	Robust	(No difference in result)

DISCUSSION

A simple, accurate and precise RP-HPLC method for the simultaneous estimation of Gabapentin and Amitriptyline hydrochloride in Pharmaceutical Dosage form has been developed and validated. 20mM Ammonium acetate PH 6.0: Acetonitrile (65:35 % v/v) Separation of drugs was carried out using mobile phase at 10 min. run time and 272nm. The Rt value for Gabapentin and Amitriptyline hydrochloride were found to be 2.788 ± 0.01 min. and 4.835 ± 0.01 min. respectively.

The drug response with respect to peak area was linear over the concentration range 50-150 $\mu\text{g/ml}$ Gabapentin and 5-15 $\mu\text{g/ml}$ for Amitriptyline hydrochloride. The percentage recovery of Gabapentin and Amitriptyline hydrochloride was found to be 99.5-99.9% and 99.5-99.8% respectively.

The % RSD values for intra-day precision study and inter-day study were $\leq 2.0\%$, confirming that the method was sufficiently precise.

The %RSD values of Robustness study were $\leq 2.0\%$, confirming that the proposed method was found to be robust enough to withstand such deliberate changes and allow routine analysis of the sample. Interference studies

reveals that the common excipients and other additives usually present in the dosage form did not interfere in the proposed method.

So it is concluded that the developed method is specific. The system test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the simultaneous determination of Gabapentin and Amitriptyline hydrochloride in pharmaceutical dosage form.

CONCLUSION

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Gabapentin and Amitriptyline Hydrochloride in Pharmaceutical Dosage Form.

RP-HPLC method, Column used was Water Symmetry250 x 4.6mm 5 μ C18, with mobile phase containing 20 mM Ammonium Acetate (PH 6.0) : Acetonitrile (65:35) %v/v The flow rate (1.0 ml/min) and wavelength (272 nm). The retention time was found to Gabapentin and Amitriptyline Hydrochloride was found to be 2.793 ± 0.01 min. and 4.831 ± 0.01 min. respectively. Correlation co-efficient for Gabapentin and Amitriptyline hydrochloride was found to be 0.999.

Assay result of marketed formulation was found to be in 99.5 % and 98.7% for Gabapentin and Amitriptyline Hydrochloride. The proposed method was validated with respect to linearity, accuracy, precision and robustness. Percentage recovery for Gabapentin and Amitriptyline hydrochloride was found to be 99.0 – 100.0%. Analysis proves that the developed method was successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

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