

Available online on 15.06.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open  Access

Research Article

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Gabapentin and Amitriptyline Hydrochloride in Pharmaceutical Dosage Forms

Vhanmane Amol*, Tamboli Ashpak, More Sunil

Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India

ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Gabapentin and Amitriptyline hydrochloride in bulk and tablet dosage form. A C8 Column (250mm x 4.6 mm, 5µm. particle size) was used in this method. The mobile phase comprises of Acetonitrile: 0.1% Orthophosphoric acid (60:40v/v) with flow rate 1ml/min (UV detection at 210 nm). The retention time for Gabapentin was 1.86min and Amitriptyline was 2.18min. The detection concentration was linear over 300-1500 µg/mL for Gabapentin and 2.5-12.5 µg/mL for amitriptyline hydrochloride. Regression equation of Gabapentin and Amitriptyline hydrochloride were found to be $y = 73.22x - 7921$ and $y = 8748x + 16611$ respectively with regression co-efficient of Gabapentin and Amitriptyline is 0.995 and 0.999. So the present work is aimed for Development of simple and reproducible chromatographic (RP-HPLC) method for simultaneous estimation of Gabapentin and Amitriptyline hydrochloride. The developed method was successfully validated in accordance to ICH guidelines. Hence, this method can be conveniently adopted for the routine analysis in quality control laboratories.

Keywords: Gabapentin (GABA) And Amitriptyline hydrochloride (AMTR), RP-HPLC, Mobile phase, Validation

Article Info: Received 13 April 2019; Review Completed 20 May 2019; Accepted 24 May 2019; Available online 15 June 2019



Cite this article as:

Vhanmane A, Tamboli A, More S, RP-HPLC Method Development and Validation for the Simultaneous Estimation of Gabapentin and Amitriptyline Hydrochloride in Pharmaceutical Dosage Forms, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):62-68 <http://dx.doi.org/10.22270/jddt.v9i3-s.2756>

*Address for Correspondence:

Mr. Vhanmane Amol, Research Scholar, Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade- 413307, Maharashtra, India

1. INTRODUCTION

It is an antiepileptic medication. It decreases pain and seizures by modulating calcium channel activity of the nerve cells and amitriptyline is used in the treatment of depression and neuropathic pain. The IUPAC name of the gabapentin is 2-[1-(aminomethyl) cyclohexyl] (fig.1)^[1] acetic acid with molecular formula : C₉H₁₇NO₂ and molecular weight 207.70. Gabapentin is freely soluble in water, alkaline and acidic solution, sparingly soluble in methanol^[1] Gabapentin is well absorbed orally and excreted unchanged in urine with t_{1/2} of 6 hrs^[2] Gabapentine and pregabalin have very little liability for causing metabolic based drug drug interaction, particularly when used in combination with other antiepileptic drugs (AEDs) because they are not metabolized in humans, and 90% drugs are excreted through kidney^[3,8]

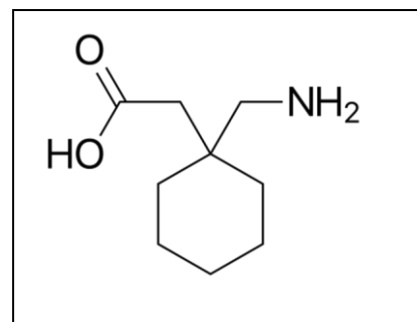


Fig.1. Gabapentin

Molecular Formula: C₉H₁₇NO₂

Molecular Weight: 207.70 g/mol

For the treatment of depression and neuropathic pain Amitriptyline HCL is used. Amitriptyline HCL increases the levels of chemical messengers in the brain that help in regulating the disposition and treat depression. IUPAC name of amitriptyline is 3-(5, 6- dihydrodibenzo [2,-b: 2', 1'-f] 7-annulen-11-ylidene)- N, dimethyl propan (fig.2) with molecular formula: $C_{20}H_{23}N$, HCL and molecular weight is 313.87. It is freely soluble in water and alcohol [1]. It is a colourless crystals or a white or almost white powder, odourless. It have less anticholinergic, less sedative and more stimulant action than amitriptyline[4]. It is tricyclic antidepressants which can elevate mood in depressive illness [5, 6]. Amitriptyline increases the levels of chemical messengers in the brain that help in regulating the mood and treat depression [7, 8].

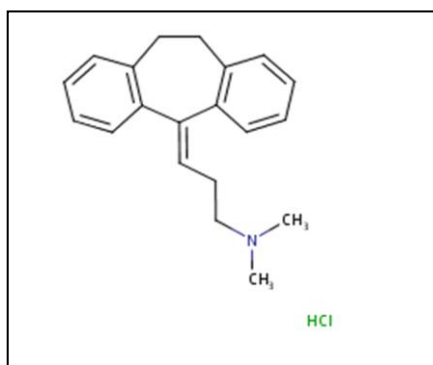


Fig.2. Amitriptyline Hydrochloride

Molecular Formula: $C_{20}H_{24}ClN$

Molecular Weight: 313.864 g/mol

2. EXPERIMENTAL:

Instrumentation:

Chromatography was performed with Systronic HPLC system provided with Hamilton Syringe, auto sampler and UV detector.

Reagents and Materials:

Gabapentin and Amitriptyline Hydrochloride were obtained as gift sample from Sun pharma laboratories Ltd, Pharmaceutical Company in Goa Industrial Estate, Goa, India. HPLC grade Acetonitrile, Water and O-Phosphoric acid procured from Research lab fine chem Industries Mumbai.

Preparation of mobile phase:

1000 ml of mobile phase was prepared by mixing 600 ml acetonitrile and 400 ml 0.1 % Orthophosphoric acid.

Degassing of mobile phase:

The prepared mobile phase was degassed by ultrasonication for 15 min, so to avoid the disturbances caused by dissolved gases.

Filtration of mobile phase:

The degassed mobile phase was filtered through 0.45 μ m filters to avoid the column clogging due to smaller particles.

Preparation of standard stock solution:

Standard stock solution of Gabapentin and Amitriptyline Hydrochloride were prepared separately by dissolving 10 mg of drug in 10 ml of mobile phase to get concentration of 1000 μ g/ml. From the respective standard stock solution, working standard solution was prepared containing 300 μ g/ml of GABA and 10 μ g/ml of AMTR separately in mobile phase.

Selection of Detection Wavelength:

From the standard stock solution further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that both the drug showed considerable absorbance at 210nm.

Loading of standard solution:

Standard solution of Gabapentin and Amitriptyline Hydrochloride were loaded in to the HPLC

Vial stand placed in sample tray & were injected.

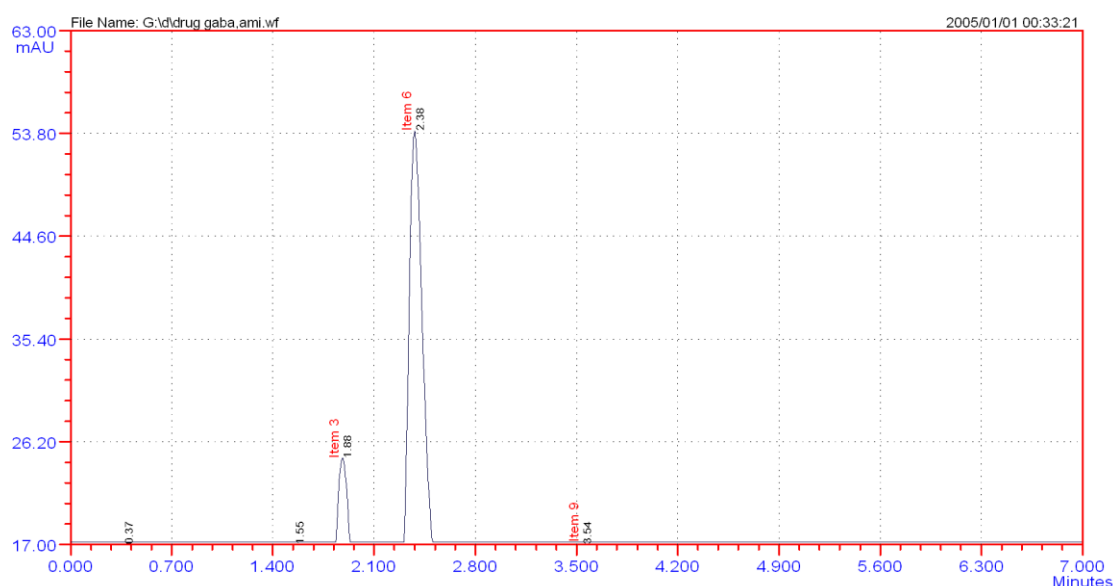


Fig. 3: Chromatogram of standard mixture of Gabapentin and Amitriptyline Hydrochloride.

Preparation of sample solution (Tablet Formulation Analysis):

Ten tablets each containing 300 mg of GABA and 10 mg of AMTR and was weighed and powdered (Each uncoated tablet contains Gabapentin IP 300 mg and Amitriptyline Hydrochloride IP 10 mg). Powder equivalent to 300 mg of Gabapentin (10 mg of AMTR) was transferred to 10 ml volumetric flask and was diluted with mobile phase then ultrasonication was done and volume made to 10 ml (3000

µg/ml of GABA and 1000 µg/ml of AMTR) with mobile phase then further dilutions were made with mobile phase to get the final concentration of 300 µg/ml of GABA and 10 µg/ml of AMTR. Solution was filtered by 0.45 µm nylon membrane filters by using vacuum filter. Tablet formulation analysis was carried out as mentioned under section Tablet Formulation Analysis. Procedure was repeated for six times. Sample solution was injected and area was recorded for each drug. Concentration and % purity was determined from linear equation shown in (Table 1)

Table 1: Assay results for GABA and AMTR

Sr. no.	GABA			AMTR		
	Peak area	Amount recovered (µg/ml)	% recovery	Peak area	Amount recovered (µg/ml)	% recovery
1	14113.9	300.941	100.314	103260.4	9.905053	99.051
2	14015	299.590	99.863	103014.7	9.876966	98.770
3	13956.2	298.787	99.596	105017.8	10.10594	101.059
4	13794.8	296.583	98.861	103960.4	9.985071	99.851
5	14313.6	303.668	101.223	104196.7	10.01208	100.121
6	13993.2	299.293	99.764	104087.3	9.999577	99.996
Mean	14031.117	299.810	99.937	103890.0	9.981	99.808
% RSD	1.233	0.789	0.788	0.689	0.820	0.820

3. METHOD VALIDATION: [9, 10, 11, 12, 13]

Linearity:

The linearity for GABA and AMTR were assessed by analysis of combined standard solution in the range of 300-1500 µg/ml and 2.5-12.5 µg/ml respectively. Correlation

coefficient for calibration curve GABA and AMTR was found to be 0.995 and 0.999. The results obtained are shown in (Table 2 and Fig.3) for GABA and in (Table 3 and Fig.4) for AMTR. Regression equation of GABA and AMTR were found to be $y = 73.22x - 7921$ (Figure 3) and $y = 8748.x + 16611$ (Figure 4).

Table 2: Linearity study of GABA

Replicates	Concentrations of GABA (µg/ml)				
	300	600	900	1200	1500
	Peak Area				
1	16131.9	33181.3	56733.9	82633.2	101217.8
2	16123.5	33191.4	56758.3	82642.3	101214.9
3	16124.9	33167.8	56747.9	82660.5	101208.3
4	16049.6	33185.9	56731.4	82687.1	101226.5
5	16123.7	33169.4	56768.8	82649.2	101219.9
6	16129.9	33192.8	56721.9	82649.1	101219.4
Mean	16113.92	33181.43	56743.70	82653.57	101217.80
Std.dev.	31.70	10.76	17.80	18.73	6.02
%RSD	0.20	0.03	0.03	0.02	0.01

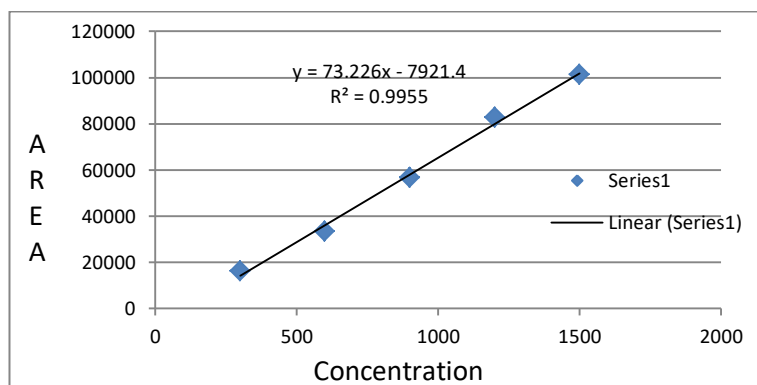


Fig. 3: Calibration curve for AMTR

Table 3: Linearity study of AMTR

Replicates	Concentrations of AMTR (µg/ml)				
	2.5	5	7.5	10	12.5
	Peak Area				
1	38168	61397.1	81479.2	103981.9	126199.4
2	38159.8	61348.8	81429.4	103977.1	126184.8
3	38128.9	61358.3	81489.1	103933.9	126198.4
4	38159.2	61349.9	81465.0	103961.1	126178.2
5	38109.3	61356.8	81482.1	103959.5	126198.5
6	38141.6	61318.9	81462.1	103948.5	126189.0
Mean	38144.47	61354.97	81467.82	103960.33	126196.70
Std.dev.	22.33	25.12	21.46	17.81	8.80
%RSD	0.06	0.04	0.03	0.02	0.01

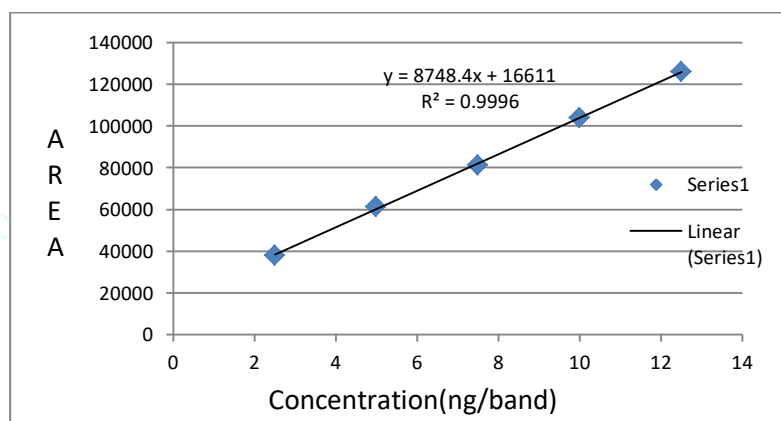


Fig. 4: Calibration curve for AMTR

Precision:**Intra-day:**

The intraday precision for Gabapentin and Amitriptyline Hydrochloride is shown in table (4 & 5). The % RSD for intraday precision was found to be 0.773 for Gabapentin and 0.500 for Amitriptyline Hydrochloride

Table 4: Intra-day precision study GABA

Concentration (µg/ml)	Area	% Recovery	Mean % Recovery ± SD	Mean % Recovery ± % RSD
300	14001.2	99.801	100.010± 0.302	99.508 ±0.773
	14123.3	100.356		
	14017.1	99.873		
600	35134.2	98.004	98.898± 1.019	
	35432.2	98.683		
	36014.1	100.007		
900	57423.8	99.161	99.617± 0.531	
	58108.3	100.199		
	57642	99.492		

Table 5: Intra-day precision study AMTR

Concentration (µg/ml)	Area	% Recovery	Mean % Recovery ± SD	Mean% Recovery± % RSD
2.5	38424.6	99.742	99.118 ±0.582	99.169 ±0.500
	38172.6	98.590		
	38267.4	99.023		
5	60124.8	99.483	99.503 ±0.045	
	60156.2	99.555		
	60119.4	99.471		
7.5	81320.2	98.627	98.886 ±0.594	
	81213.6	98.465		
	81935.8	99.565		

Inter-day:

The interday precision for Gabapentin and Amitriptyline Hydrochloride is shown in table (6 & 7). The % RSD for interday precision was found to be 1.114 for Gabapentin and 0.926 for Amitriptyline.

Table 6: Inter-day precision study GABA

Concentration (µg/ml)	Area	% Recovery	Avg % Recovery ± SD	Mean % Recovery ± % RSD
300	14033.4	99.947	100.503 ± 0.604	99.888 ± 1.114
	14296.7	101.146		
	14136.2	100.415		
600	35181.4	98.112	99.410± 1.133	
	35975.6	99.919		
	36098.4	100.199		
900	58943.7	101.467	99.752 ± 1.538	
	57510.3	99.292		

Table 7: Inter-day precision of AMTR

Concentration (µg/ml)	Area	% Recovery	Avg % Recovery± SD	Mean% Recovery± % RSD
2.5	38724.6	101.114	100.490 ± 0.582	100.414 ± 0.926
	38472.6	99.962		
	38567.4	100.395		
5	61054.8	101.609	100.549 ± 0.999	
	60187.2	99.626		
	60530.8	100.411		
7.5	81467.2	98.851	100.202± 1.421	
	83325.9	101.684		
	82267.4	100.071		

Accuracy:

The accuracy of the method was confirmed by recovery study from the marketed formulation at three level of standard addition. The results are shown in table (8 & 9)

Table 8: Recovery studies of GABA

Level	Conc. (µg/ml)		Area	% Recovery	Mean % Recovery ± RSD
	Sample	Std.			
50 %	300	150	24886.5	99.571	99.571 ± 0.633
			25094.1	100.201	
			24678.7	98.940	
100 %	300	300	35281.4	98.339	100.001 ± 1.525
			36154.7	100.327	
			36154.7	101.337	
150 %	300	450	47486.4	100.532	100.678 ± 0.863
			47878.5	101.611	
			46934.1	99.891	

Table 9: Recovery studies of AMTR

Level	Conc. (µg/ml)		Area	% Recovery	Mean % Recovery ± RSD
	Sample	Std.			
50 %	5	2.5	83467.2	101.899	101.066 ± 0.863
			82325.9	100.160	
			82967.4	101.138	
100 %	5	5	104260.4	100.194	100.469 ± 0.760
			105256.7	101.333	
			103987.1	99.881	
150 %	5	7.5	126196.7	100.216	100.590 ± 0.816
			127634.5	101.530	
			125986.3	100.023	

Table 10: System suitability parameters

Parameters	GABA	AMTR
Retention time (min)	1.88	2.38
Area	32361.3	73401.5
Plates	475.98	665.02
Resolution	0.44	1.42

Limit of detection (LOD) and Limit of Quantification (LOQ):

In the present study, the LOD and LOQ were calculated according to the standard deviation of the response and the slope of the calibration curve i.e., $3.3\sigma / S$ and $10\sigma / S$ criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve. The LOD of GABA and AMTR was found to be 121.032ng/band and 0.289/band and LOQ was 366.765ng/ band and 0.876ng/band for GABA and AMTR, respectively.

Specificity:

Specificity of the RP-HPLC method was determined by entire separation of GABA and AMTR with parameter like retention time (tR), resolution (Rs), and tailing factor (T). The peak obtained for GABA and AMTR were sharp and have clear baseline separation. The results are shown in table (11).

Table 11: Specificity parameters

Parameters	GABA	AMTR
Tailing factor (T)	0.93	1.74
Resolution (Rs)	0.44	1.42
Retention time (tR)	1.88	2.38

Robustness:

This was done by minute change in the chromatographic conditions and found to be unchanged by minute change like $\pm 2\%$ change in volume of organic solution of mobile phase.

4. RESULT AND DISCUSSION

In this method 1000 ml of mobile phase was prepared by mixing 600 ml acetonitrile and 400 ml 0.1 % Orthophosphoric acid. The degassed mobile phase was filtered through 0.45 µm filters to avoid the column clogging due to smaller particles. The flow rate was found to be optimum at 1ml/ min resulting in short retention time, good baseline stability with low noise level. In the present developed RP-HPLC method, the standard and sample preparation required less time and no tedious extraction was involved. By the use of the proposed method the retention time of GABA and AMTR was found to be 1.88 minutes and 2.38 minutes respectively. The assay of GABA and AMTR in bulk drug and in combined tablet dosage form was found to be 99.937% and 99.808%. A good linear relationship, $r^2 = 0.995$ for GABA and $r^2 = 0.999$ for AMTR was observed between the concentration range of 300-1500µg/ml and 2.5-12.5µg/ml respectively. The LOD values were found to be 121.032µg/ml and 0.289µg/ml and LOQ values were found to be 366.765µg/ml and 0.876µg/ml for both GABA and AMTR respectively. Low values of standard deviation of retention time and peak areas indicate high precision of the method. From the recovery studies data, it was found that the mean % recovery was within the limits, indicated high accuracy of the proposed method. There are no additional peaks observed in the chromatogram, which indicates non-interference of the common excipients used in the formulation. No marked changes were observed in % assay of the optimized conditions with that of the altered conditions in the robustness study indicating that the method is robust.

5. CONCLUSION

A simple and competent method was developed for the simultaneous estimation of Gabapentin and Amitriptyline hydrochloride within a short analysis time with no interference of the common additives present in the pharmaceutical formulation. The proposed method can be applied for routine quality control and analysis in bulk and in tablet dosage forms.

ACKNOWLEDGEMENT

I am very much thankful to Sahyadri college of Pharmacy, Methawde (Sangola), Maharashtra, for giving permission to carry out my work.

REFERENCES

1. Shaikh S., Jain V., Development and validation of novel RP-HPLC method for simultaneous estimation of Gabapentin and Amitriptyline hydrochloride in bulk and pharmaceutical dosage forms. *International Journal of Applied Pharmaceutics*, 2018; 10(2):97-104
2. Tripathi K.D., *Essential of medical pharmacology* 6th edition pp. 409, 410
3. Mathias C. LU, Wilson and Gisvold's *Textbook of Organic Medicinal and Pharmaceutical Chemistry* Twelfth Edition, 2015, 498.
4. Indian Pharmacopoeia 2014 "Government of India ministry of health and family welfare, published by Indian Pharmacopoeia commission", Government of India Ghaziabad, 2014, 1043-1044.
5. Faraat Ali, Singh G. N., et al. Application of an LC/HPLC method development and validation for the simultaneous estimation of Amitriptyline hydrochloride and chlorthalidopoxide in tablet dosage form by using a reverse phase technique. *Scholars Research Library*. 2015; 7 (10):172-177.
6. Tripathi, K. D., *Essential of Medical Pharmacology*, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 6th ed. 2008; 172.
7. Patel N., Kaur G., et al. Development and Validation of Analytical Methods for Simultaneous Estimation of Pregabalin and Amitriptyline Hydrochloride in their Combined Marketed Dosage form. *J. Pharm. Sci. Bioscientific Res.*, 2016; 6(4):547-551.
8. Vhanmane A. et al., HPTLC method development for the simultaneous determination of Gabapentin and Amitriptyline Hydrochloride in pharmaceutical dosage forms. *International journal of Research and Analytical review*. 2019; 6(2): 653-666.
9. Chinnalalaiah Runja, P. Ravikumar et al. A Validated Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Aliskiren Hemifumarate and Amlodipine Besylate in Pharmaceutical Dosage Form. *J Hindawi Publishing Corporation*. Volume 2014, Article ID 628319; 1-7.
10. Bekett AH, Stenlake JB: *Practical Pharmaceutical Chemistry*, CBS Publishers and Distributors, New Delhi, India, Part-2, 2002; 275-237.
11. Willard HH: *Instrumental Method of Analysis* 7th ed. CBS publisher and Distributors, New Delhi, India. 1986; 1-5.
12. ICH Q2A Text on validation of analytical procedure, International conference on harmonization. Tripartite guideline. 1994; 1-5.
13. ICH Q2B Validation of analytical procedure, methodology, International conference on harmonization. Tripartite guideline. 1994; 1-10.

