WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 12, 936-952. Research Article ISSN 2277–7105

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF GABAPENTIN AND NORTRIPTYLINE HYDROCHLORIDE BY UHPLC IN ORAL SOLID DOSAGE FORMS

Dr. C. N. Nalini*, R. Vijayakumar[1] and J. Premlal[2]

*M. PHARM, Ph.D., Principal and Head of The Department, Department of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai – 600097, India.

[1]M.PHARM, Associate Professor, Department Of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai – 600097, India.

[2]Student, Department of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai – 600097, India.

Article Received on
25 April 2024,

Revised on 15 May 2024,
Accepted on 04 June 2024

DOI: 10.20959/wjpr202412-32839

***Corresponding Author**
Dr. C. N. Nalini
M. PHARM, Ph.D., Principal and Head of The Department, Department of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai - 600097, India.

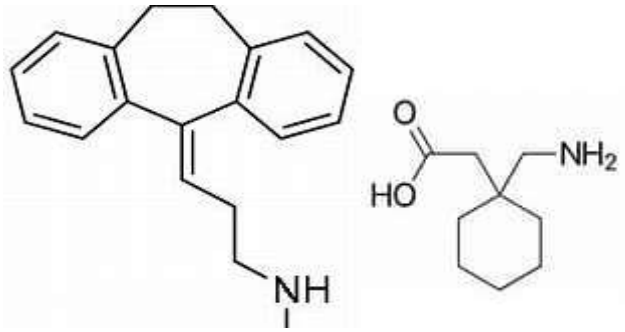
ABSTRACT

A new simple, precise, fast and accurate UHPLC method has been developed and validated for simultaneous determination of gabapentin and nortriptyline hydrochloride in oral solid dosage forms. The method was carried out on the chromatographic separation achieved by C_{18}; 3.9mm X 50 mm; 5 microns column, using mobile phase composition of buffer; methanol in the ratio of 300;700 ml, buffer; o.2% of triethylamine, adjusted the PH 4.5 with orthophosphoric acid with a flow rate of 0.5ml/min. Retention time of gabapentin was found 1.845 min & nortriptyline hydrochloride was found 3.345 min. %RSD of gabapentin was found 0.25% & nortriptyline hydrochloride was found 0.31%. Regression Equation of gabapentin Y = 0.0106 X – 0.105, R^2 = 0.9993 & Nortriptyline hydrochloride is Y = 3.0378 X + 158.8271, R^2 = 0.9998. The method was developed simple and economical because retention time were decreased and the run time was decreased, that can be adopted in regular quality control tests in industries.

KEYWORDS: Gabapentin and Nortriptyline Hydrochloride, UHPLC Validation, C_{18} column ICH guidelines.

INTRODUCTION

Gabapentin (GBP) 2- [1- (amino methyl) cyclo hexyl] acetic acid, is an anticonvulsant medication used in the management of peripheral neuropathic pains, post therapeutic neuralgia and partial – onset seisures. It has less protein binding (< 3%) and half life is about 511/ - 7 hrs. Freely soluble in water and alkaline and acidic solutions. Nortriptyline hydrochloride (NTH) [N- methyl-3 –(2 – tricyclo [$9.4.0.0^{3,8}$] pentadeca – 1 (15),3,5,7,11,13-hexa enylidene) propan-1- amine; hydrochloride] is a medication used for treating nerve pain. It is a tricyclicneuroleptic agent. Moderately soluble in water at room temperature. Moderately soluble in ethanol.



MATERIALS AND METHODS

Chemicals

Gabapentin & Nortriptyline Hydrochloride was prepared in house, HPLC water, Acetonitrile. All the above chemicals and solvents are from Rankem.

Instrumentation

Analysis was performed on Agilent UHPLC software: Agilent chemstation, ultra sonicator - Bvk enterprises, p^4 meter-Hanna, 5 point calibration, Electronics balance – Mettler.

Chromatographic Conditions

C_{18}; 3.9 mm x 50 mm; 5 Microns column were used for chromatographic separation using Moblie phase composition of Buffer : Method in the ration of 300 ml : 700 ml with a flow rate of 0.5 ml / min, run time of about 5 mins, detector wavelength is 210 nm and the retention time and peak shape is good.

Method Development

Preparation of Standard stock solution A: Weigh precisely and put about 40 mg of Gabapentin standard into a 10 mL volumetric standard flask. Dissolve and adjust the volume with the mobile phase.

Preparation of Standard stock solution B: Weigh precisely and transfer around 25 mg of Nortriptyline Hydrochloride standard to a 250 ml volumetric standard flask. With the mobile phase, dissolve and make up to the desired volume.

Preparation of Standard solution: Five milliliters of standard stock solutions A and B should be transferred into a fifty milliliter volumetric flask to be made up to the desired volume using the mobile phase.

Preparation of Sample solution: Weigh precisely enough powdered gabapentin sample to equal 400 mg into a 100 ml volumetric flask. Use the mobile phase to dissolve and make up the volume. Filter the mixture, then dilute five milliliters (5 milliliters) into a fifty milliliter volumetric flask and add mobile phase to get the desired volume.

VALIDATION

System suitability parameters: The preparation of a standard solution consisting gabapentin and nortriptyline hydrochloride injection was used for determining the system suitability characteristics. The features, including peak tailing, resolution, and USP plate count, were ascertained five times. It is recommended that the percentage RSD for the area of five standard injection results not exceed 2%.

Specificity: Checking for interference in the best approach. When using this technique to measure the retention durations of these medications, we shouldn't observe any conflicting peaks in the blank and placebo. It was stated that this approach was specific.

Precision

Preparation of Standard stock solution A: Accurately weigh and transfer about 40 milligrams of Gabapentin sample into a 10-milliliter volumetric standard flask. Use the mobile phase to dissolve and make up to the volume.

Preparation of Standard stock solution B: Accurately weigh and transfer about 25 mg of the Nortriptyline Hydrochloride standard into a 250 ml volumetric standard flask. Use the mobile phase to dissolve and make up the volume.

Preparation of Standard solution: Fill a 50 ml volumetric flask halfway full with mobile phase by transferring 5 ml each of standard stock solutions A and B.

Preparation of Sample solution: Fill a 100 ml volumetric flask with precisely 400 mg of powdered gabapentin. Mix with the mobile phase to dissolve and adjust the volume. Filter, then use mobile phase to make up the volume by diluting 5 ml of the aforementioned solution into a 50 ml volumetric flask.

Linearity (for Gabapentin & Nortriptyline Hydrochloride)

Preparation of Standard stock solution A: Precisely weigh and move about 400 mg of the gabapentin standard into a 100 ml volumetric standard flask. Utilizing the mobile phase, dissolve and modify to the volume.

Preparation of Standard stock solution B: The Nortriptyline Hydrochloride standard should be precisely weighed and then transferred into a 250 ml volumetric standard flask containing about 25 mg. Mix with the mobile phase to dissolve and adjust the volume.

50% Gabapentin & Nortriptyline Hydrochloride Standard solution: Using a pipette, 2.5 milliliters of standard stock A and 2.5 milliliters of standard stock B were combined to make 50 milliliters.

75% Gabapentin & Nortriptyline Hydrochloride Standard solution: A 100 ml volume was created by pipetting out 7.5 ml of standard stock A and 7.5 ml of standard stock B solutions.

100% Gabapentin & Nortriptyline Hydrochloride Standard solution: Pipetted out were 5.0 ml of standard stock A and 5.0 ml of standard stock B solutions, totaling 50 ml.

125% Gabapentin & Nortriptyline Hydrochloride Standard solution: Using a pipette, 12.5 ml of standard stock A and 12.5 ml of standard stock B solutions were combined to create 100 ml.

150% Gabapentin & Nortriptyline Hydrochloride Standard solution: 50 ml of solutions were prepared by pipetting out 7.5 ml of standard stock A and 7.5 ml of standard stock B.

Accuracy (for Gabapentin & Nortriptyline Hydrochloride)

Preparation of Standard stock solution A: Precisely weigh and transfer about 40 milligrams of Gabapentin standard into a 10-milliliter volumetric standard flask. Apply the mobile phase to dissolve and make up to the volume.

Preparation of Standard stock solution B: Transfer around 25 mg of the nortriptyline hydrochloride standard into a 250 ml volumetric standard flask after accurately weighing it. Employing the mobile phase, dissolve and adjust to the volume.

Preparation of Standard solution: Five milliliters of standard stock solutions A and B should be transferred into a fifty milliliter volumetric flask to be filled up to the desired volume using the mobile phase.

For preparation of 80% solution: Weigh properly 320mg of Gabapentin powdered sample into a 100 mL volumetric flask. Dissolve and adjust the volume with the mobile phase. Filter, then dilute 5ml of the above solution into a 50ml volumetric flask and build up to volume with mobile phase.

For preparation of 100% solution: Fill a 100 ml volumetric flask with precisely 400 mg of powdered gabapentin. Mix with the mobile phase to dissolve and adjust the volume. Filter, then use mobile phase to make up the volume by diluting 5 ml of the aforementioned solution into a 50 ml volumetric flask.

For preparation of 120% solution: In a 100 ml volumetric flask, precisely weigh the equivalent of 480 mg of powdered gabapentin. Mix with the mobile phase to dissolve and adjust the volume. Filter, then use mobile phase to make up the volume by diluting 5 ml of the aforementioned solution into a 50 ml volumetric flask.

Procedure

The injections included a standard solution, the accuracy -80%, the accuracy -100%, and the accuracy -120% solutions. The amounts added and discovered for the mean recovery values of nortriptyline hydrochloride and gabapentin have been calculated and the results were compiled.

Acceptance Criteria

For every stage, the percentage recovery should range from 98.0% to 102.0%.

Robustness: There was no discernible difference in the outcome and the small, intentional procedure adjustments (such as flow rate and wavelength) were within the acceptable range as per ICH guidelines.

Conditions of robustness, such as flow minus, flow plus, wavelength dropping, and wavelength raising, were upheld, and samples were injected twice. Every parameter was passed, and there was no impact on the system suitability parameters. %RSD was not over the threshold.

RESULTS

The elute times for gabapentin and nortriptyline hydrochloride were 1.845 and 3.345 minutes, respectively. The plate count and tailing factor were highly satisfactory, leading to the optimization and validation of this approach. The chromatogram was shown in figure 1. According to ICH criteria, all of the system suitability data were sufficient and were within the acceptable range. The ICH criteria state that a plate count of more than 2000, a tailing factor of less than 2, and a resolution of more than 2 are required. Every system-appropriate parameter passed and remained within the range. It was shown in figure 2.

The elution times for gabapentin and nortriptyline hydrochloride were 1.849 and 3.395 minutes, respectively. Using this strategy, we were unable to discover any interfering peaks in the blank and placebo during the retention durations of these medications. It was stated that this approach was specific.

Gabapentin and nortriptyline hydrochloride injections were made in duplicate at five linear concentrations each. The linearity equations for gabapentin ($y = 0.0106x - 0.0105$) and nortriptyline hydrochloride ($y = 3.0378x + 158.8271$) were found, and average areas were previously described. The correlation coefficients for gabapentin and nortriptyline hydrochloride were found to be $R^2 = 0.9993$ and $R^2 = 0.9998$, respectively. It was shown in figure 3 and 4.

Six working sample solutions with identical concentrations were created, allowing for the preparation of several samples. Each injection from a working sample solution was administered, and the areas that were acquired are listed in the above table. For gabapentin

and nortriptyline hydrochloride, the average area, standard deviation, and percentage RSD were determined. % RSD was found to be 0.31% for nortriptyline hydrochloride and 0.25% for gabapentin. The system precision was passed using this technique because the precision limit was less than "2".

Using various sample weight techniques, three levels of accuracy samples were created. For every accuracy level, three injections were administered, and the mean percentage of recovery for gabapentin and nortriptyline hydrochloride was found to be 99.02% and 100.22%, respectively.

Samples were injected in duplicate under robustness settings such as Flow minus (0.3 ml/min), Flow plus (0.7 ml/min), Wavelength minus (208 nm), and Wavelength plus (212 nm). All of the system suitability parameters passed with little to no impact. %RSD was not over the upper limit. It was shown in table 3.

Average percentage Assay of Gabapentin for 99.49% & Nortriptyline Hydrochloride 99.30% respectively. It was shown in figure 6 and 7.

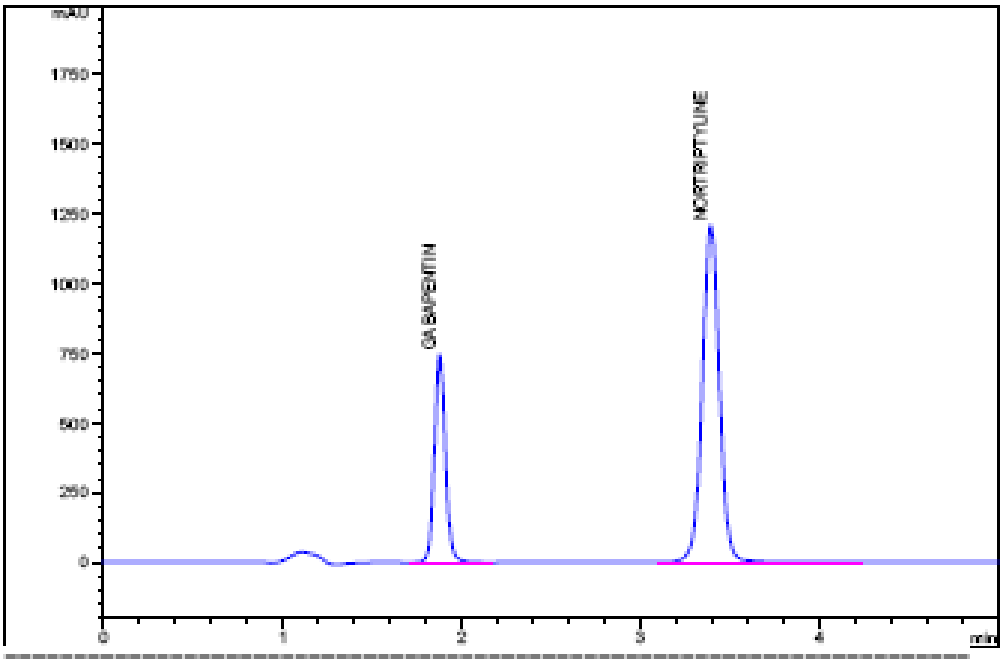


Figure 1: optimized chromatogram.

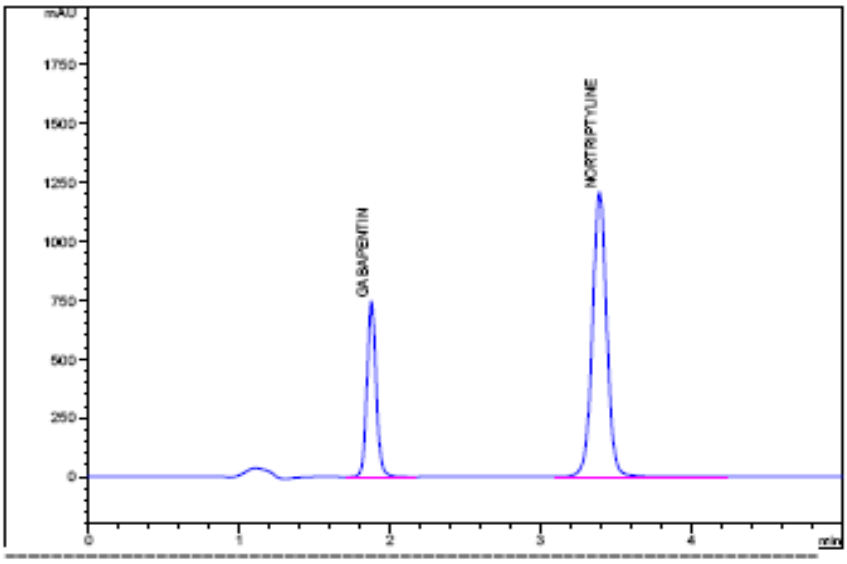


Figure 2: Chromatogram For System Suitability.

VALIDATION PARAMETER : LINEARITY

LABEL CLAIM:

GABAPENTIN	400	mg	Std purity :	99.65	%
			Factor :	1.0000	

GABAPENTIN linearity stock dilution:

400.02	mg	standard diluted to	100	ml	(Stock Soln.)

Soln. ID	*Linearity solution dilutions:*				*Concentrations*	
50.0% :	2.5	ml	Stock soln diluted to	50	ml	199.31 mcg/ml
75.0% :	7.5	ml	Stock soln diluted to	100	ml	298.96 mcg/ml
100.0% :	5.0	ml	Stock soln diluted to	50	ml	398.62 mcg/ml
125.0% :	12.5	ml	Stock soln diluted to	100	ml	498.27 mcg/ml
150.0% :	7.5	ml	Stock soln diluted to	50	ml	597.93 mcg/ml

Linearity Concentrations-->	17.219	25.091	34.012	41.932	51.132
Observed Area-->	1595.428	2391.253	3187.709	3984.922	4781.288

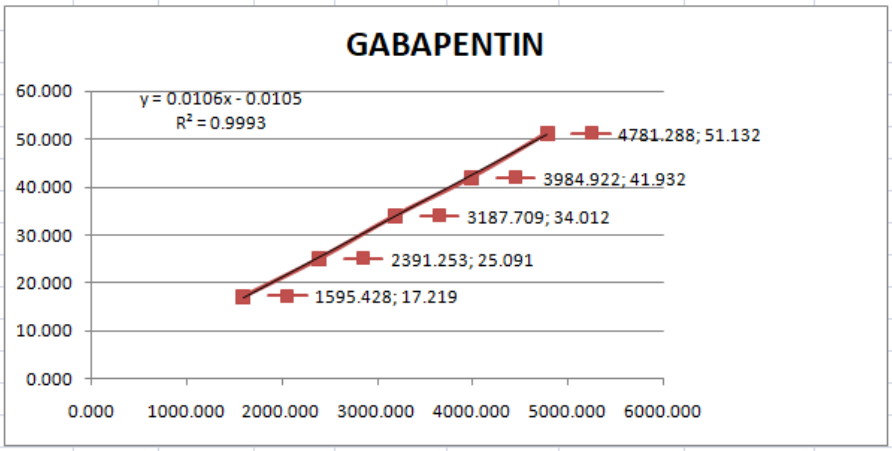


Figure 3: Linearity For Gabapentin.

VALIDATION PARAMETER : LINEARITY

LABEL CLAIM:

NORTRIPTYLINE HYDROCHLORIDE	10	mg	Std purity :	99.66	%
			Factor :	0.8785	

NORTRIPTYLINE HYDROCHLORIDE linearity stock dilution:

25.56 mg standard diluted to 250 ml (Stock Soln.)

Soln. ID	*Linearity solution dilutions:*		*Concentrations*
50.0% :	2.5 ml	Stock soln. diluted to 50 ml	4.48 mcg/ml
75.0% :	7.5 ml	Stock soln. diluted to 100 ml	6.71 mcg/ml
100.0% :	5.0 ml	Stock soln. diluted to 50 ml	8.95 mcg/ml
125.0% :	12.5 ml	Stock soln. diluted to 100 ml	11.19 mcg/ml
150.0% :	7.5 ml	Stock soln. diluted to 50 ml	13.43 mcg/ml

Linearity Concentrations-->	1284.628	1926.485	2568.457	3218.465	3849.625
Observed Area-->	4022.660	6025.896	8008.454	9951.861	11813.814

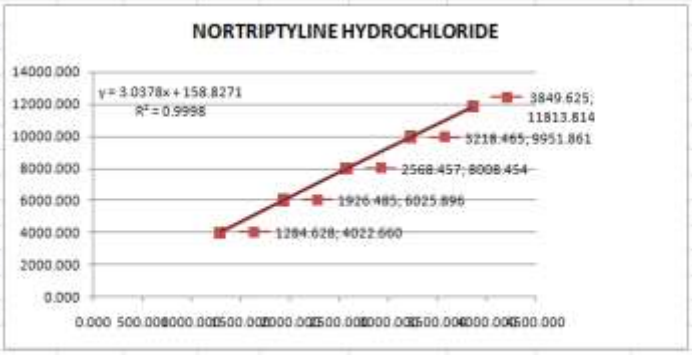


Figure 4: Linearity For Nortriptyline Hydrochloride.

VALIDATION PARAMETER - SYSTEM PRECISION

LABEL CLAIM: **Avg. of capsule: 556.89 mg**

GABAPENTIN 400 mg

Factor : 1.0000

STANDARD DILUTIONS : *Purity of std.* : 99.65 %

40.02 mg diluted to 10 ml. further 5 ml diluted to 50 ml.

STANDARD VALUES :

3194.688	3200.124	3198.577	3195.458	3199.025

Average : 3197.574

Standard Deviation : 2.368

% RSD : 0.07

SAMPLE DILUTIONS :

Sample diluted to 100 ml. further 5 ml diluted to 50 ml.

Content in mcg

$$\frac{\text{Spl. Area}}{3197.574} \times \frac{40.02}{10} \times \frac{5}{50} \times \frac{100}{\text{Spl. Wt.}} \times \frac{50}{5} \times \frac{99.650}{100} \times 556.89 \times 1.000$$

Conc. level	Sample ID	Sample wt. (mg)	Sample Area	Calculated Assay (in mg)	Calculated Assay (in percentage)
MIDDLE LEVEL (100%)	Sample -01	557.63	3200.410	398.6233	99.66
	Sample -02	556.28	3208.964	400.6587	100.16
	Sample -03	557.98	3200.541	398.3895	99.60
	Sample -04	556.41	3207.874	400.4290	100.11
	Sample -05	556.66	3199.658	399.2240	99.81
	Sample -06	557.88	3200.689	398.4794	99.62
			Average :	**399.301**	**99.83**
			SD. :	**1.01**	**0.25**
			% RSD :	**0.25**	**0.25**

Figure 5: System precision of Gabapentin.

VALIDATION PARAMETER - SYSTEM PRECISION

LABEL CLAIM: Avg. weight of a tablet : 556.89 mg

NORTRIPTYLINE HYDROCHLORIDE 10 mg

Factor : 0.8785

STANDARD DILUTIONS : *Purity of std.* : 99.66 %

25.56 mg diluted to 250 ml, further 5 ml diluted to 50 ml.

STANDARD VALUES :

8034.266	8036.485	8030.254	8039.574	8036.477

Average : 8035.411

Standard Deviation : 3.447

% RSD : 0.04

SAMPLE DILUTIONS :

Sample diluted to 100 ml, further 5 ml diluted to 50 ml.

Content in mcg

$$\frac{\text{Spl. Area}}{8035.411} \times \frac{25.56}{250} \times \frac{5}{50} \times \frac{100}{\text{Spl. Wt.}} \times \frac{50}{5} \times \frac{99.660}{100} \times 556.89 \times 0.8785$$

Conc. level	Sample ID	Sample wt. (mg)	Sample Area	Calculated Assay (in mg)	Calculated Assay (in percentage)
MIDDLE LEVEL (100%)	Sample -01	557.63	8968.357	9.9773	99.77
	Sample -02	556.28	9002.145	10.0392	100.39
	Sample -03	557.98	8969.985	9.9728	99.73
	Sample -04	556.41	9005.632	10.0407	100.41
	Sample -05	556.66	9001.254	10.0313	100.31
	Sample -06	557.88	8999.015	10.0069	100.07
			Average :	**10.01**	**100.11**
			SD. :	**0.031**	**0.31**
			% RSD :	**0.31**	**0.31**

Figure 6: System precision of Nortriptyline Hydrochloride.

VALIDATION PARAMETER - ACCURACY

LABEL CLAIM: Avg. of net content : **556.89** mg

GABAPENTIN 400 mg Factor : **1.0000**

STANDARD DILUTIONS : *Purity of std.* : 99.65 %

40.02 mg diluted to 10 ml, further 5 ml diluted to 50 ml.

STANDARD VALUES :

3194.688	3200.124	3198.577	3195.458	3199.025

Average : 3197.574

Standard Deviation : 2.368

% RSD : 0.07

SAMPLE PREPARATIONS :

80% sample -01:	444.28	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
80% sample -02:	444.98	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
80% sample -03:	445.26	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
100% sample -01:	555.98	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
100% sample -02:	556.68	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
100% sample -03:	554.98	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
120% sample -01:	668.47	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
120% sample -02:	667.29	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
120% sample -03:	668.95	mg diluted to	100	ml, further	5	ml diluted to	50	ml.

Sample ID	Sample wt. (mg)	Sample Area	Calculated Content (in mg)	Calculated Content (in %)
80% sample -01:	444.28	2563.698	400.7867	100.20
80% sample -02:	444.98	2547.856	397.6835	99.42
80% sample -03:	445.26	2560.241	399.3653	99.84
100% sample -01:	554.98	3203.365	400.8965	100.22
100% sample -02:	556.68	3198.745	399.0958	99.77
100% sample -03:	555.98	3206.654	400.5863	100.15
120% sample -01:	668.47	3812.025	396.0746	99.02
120% sample -02:	667.29	3846.587	400.3724	100.09
120% sample -03:	668.95	3850.214	399.7554	99.94
			Average :	99.85
			SD :	0.402
			% RSD :	0.40

Figure 7: Accuracy of Gabapentin.

VALIDATION PARAMETER - ACCURACY

LABEL CLAIM: Avg. weight of a tablet : **556.89** mg

NORTRIPTYLINE 10 mg Factor : **0.8785**

STANDARD DILUTIONS : *Purity of std.* : 99.88 %

25.56 mg diluted to 250 ml, further 5 ml diluted to 50 ml.

STANDARD VALUES :

8034.266	8036.485	8030.254	8039.574	8036.477

Average : 8035.411

Standard Deviation : 3.447

% RSD : 0.04

SAMPLE PREPARATIONS :

80% sample -01:	444.28	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
80% sample -02:	444.98	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
80% sample -03:	445.26	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
100% sample -01:	554.98	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
100% sample -02:	556.68	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
100% sample -03:	554.98	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
120% sample -01:	668.47	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
120% sample -02:	667.29	mg diluted to	100	ml, further	5	ml diluted to	50	ml.
120% sample -03:	668.95	mg diluted to	100	ml, further	5	ml diluted to	50	ml.

Sample ID	Sample wt. (mg)	Sample Area	Calculated Content (in mg)	Calculated Content (in %)
80% sample -01:	444.28	7203.658	10.0809	100.81
80% sample -02:	444.98	7185.452	10.0396	100.40
80% sample -03:	445.26	7102.564	9.9175	99.18
100% sample -01:	554.98	8923.254	9.9965	99.97
100% sample -02:	556.68	9001.201	10.0530	100.53
100% sample -03:	554.98	9002.564	10.0854	100.85
120% sample -01:	668.47	10802.365	10.0471	100.47
120% sample -02:	667.29	10778.624	10.0427	100.43
120% sample -03:	668.95	10689.547	9.9350	99.35
			Average :	100.22
			SD :	0.600
			% RSD :	0.60

Figure 8: Accuracy of Nortriptyline Hydrochloride.

VALIDATION PARAMETER : ASSAY

LABEL CLAIM: Each tablet contains

GABAPENTIN	400	mg
Average of net content:	556.89	mg

STANDARD DILUTIONS :

Name of standard : **GABAPENTIN**

Purity of standard : *99.65* % *(as such)*

40.02 mg diluted to 10 ml, further 5 ml diluted to 50 ml.

SAMPLE DILUTIONS :

559.87 mg diluted to 100 ml, further 5 ml diluted to 50 ml.

STANDARD VALUES :

3194.688	3200.124	3198.577	3195.458	3199.025

Average : 3197.574

SD : 2.368

% RSD : 0.07

SAMPLE VALUES :

3206.264	3209.874

Average : 3208.069

SD : 2.55

% RSD : 0.08

Content in mg

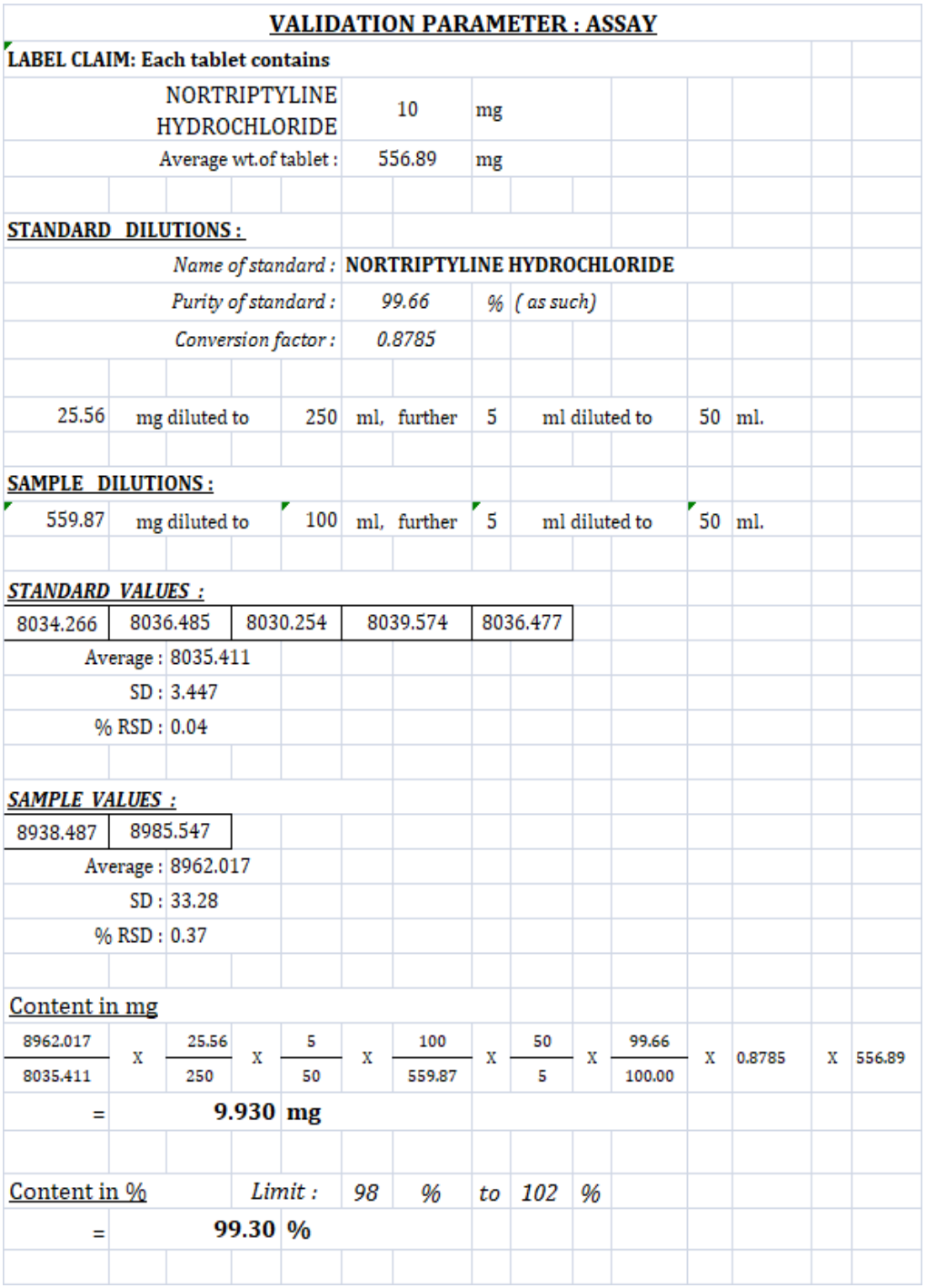
$$\frac{3208.069}{3197.574} \times \frac{40.02}{10} \times \frac{5}{50} \times \frac{100}{559.87} \times \frac{50}{5} \times \frac{99.65}{100.00} \times 556.89$$

= **397.979 mg**

Content in % *Limit : 98 % to 102 %*

= **99.49 %**

Figure 6: Assay of Gabapentin.



VALIDATION PARAMETER : ASSAY

LABEL CLAIM: Each tablet contains

NORTRIPTYLINE HYDROCHLORIDE	10	mg
Average wt.of tablet :	556.89	mg

STANDARD DILUTIONS :

Name of standard : **NORTRIPTYLINE HYDROCHLORIDE**

Purity of standard : *99.66* % *(as such)*

Conversion factor : *0.8785*

25.56 mg diluted to 250 ml, further 5 ml diluted to 50 ml.

SAMPLE DILUTIONS :

559.87 mg diluted to 100 ml, further 5 ml diluted to 50 ml.

STANDARD VALUES :

8034.266	8036.485	8030.254	8039.574	8036.477

Average : 8035.411

SD : 3.447

% RSD : 0.04

SAMPLE VALUES :

8938.487	8985.547

Average : 8962.017

SD : 33.28

% RSD : 0.37

Content in mg

$$\frac{8962.017}{8035.411} \times \frac{25.56}{250} \times \frac{5}{50} \times \frac{100}{559.87} \times \frac{50}{5} \times \frac{99.66}{100.00} \times 0.8785 \times 556.89$$

= **9.930 mg**

Content in % *Limit : 98 % to 102 %*

= **99.30 %**

Figure 7: Assay of Nortriptyline Hydrochloride.

Table 1: System Suitability For Gabapentin And Nortriptyline Hydrochloride.

DETAILS	GABAPENTIN			
Injection No.	**Retention Time**	**Area**	**Theoretical Plates**	**Tailing Factor**
Injection - 01	1.878	3194.688	4760	1.109
Injection - 02	1.875	3200.124	4765	1.11
Injection - 03	1.881	3198.577	4762	1.102
Injection - 04	1.875	3195.458	4768	1.108
Injection - 05	1.882	3199.025	4763	1.113

Average :	**1.878**	**3197.574**	**4764**	**1.108**
SD :	0.00	2.368	3.05	0.00
% RSD :	**0.17**	**0.07**	**0.06**	**0.36**

DETAILS	**NORTRIPTYLINE HYDROCHLORIDE**			
Injection No.	**Retention Time**	**Area**	**Theoretical Plates**	**Tailing Factor**
Injection - 01	3.393	8034.266	6352	1.012
Injection - 02	3.392	8036.485	6353	1.015
Injection - 03	3.395	8030.254	6359	1.019
Injection - 04	3.393	8039.574	6358	1.015
Injection - 05	3.395	8036.477	6352	1.023
Average :	**3.394**	**8035.411**	**6355**	**1.0168**
SD :	0.00	3.447	3.42	0.00
% RSD :	**0.04**	**0.04**	**0.05**	**0.42**

Table 2: Specificity For Gabapentin And Nortriptyline Hydrochloride.

Sample ID	**GABAPENTIN**	
	RT	**AREA**
BLANK	1.849	No peak observed
STANDARD	1.849	3198.647
PLACEBO	1.849	No peak observed

Sample ID	**NORTRIPTYLINE HYDROCHLORIDE**	
	RT	**AREA**
BLANK	3.395	No peak observed
STANDARD	3.395	8034.548
PLACEBO	3.395	No peak observed

Table 3: Robustness For Gabapentin and Nortriptyline Hydrochloride.

S. No	**Condition**	**%RSD of Gabapentin Assay**	**%RSD of Nortriptyline Hydrochloride Assay**
1	Flow rate (-) _0.3	0.58%	1.05%
2	Flow rate (+) _0.7	0.38%	0.68%
3	Wavelength (-) _208	0.21%	0.40%
4	Wavelength (+) _212	0.50%	0.40%

DISCUSSION

Table 4: Summary of Gabapentin and Nortriptyline Hydrochloride.

Parameters	**Limit**	**Gabapentin**	**Nortriptyline Hydrochloride**
Linearity: Regression equation (Y=mx+c)	R^2 not less than 0.999	R^2 = 0.9993	R^2 = 0.9998
Assay (% mean assay)	90.0% -110.0%	99.49%	99.30%
Specificity	No interference of any peak	Complies	Complies
System Precision %RSD	RSD NMT 2.0%	0.25%	0.31%

Intermediate Precision Day-01 %RSD		RSD NMT 2.0%	0.28%	0.16%
Intermediate Precision Day-02 %RSD		RSD NMT 2.0%	0.85%	0.30%
Accuracy %		98-102%	99.02% to 100.22%	99.18% to 100.85%
Robustness	**FM**	RSD NMT 2.0%	0.58%	1.05%
	FP	RSD NMT 2.0%	0.38%	0.68%
	WM	RSD NMT 2.0%	0.21%	0.40%
	WP	RSD NMT 2.0%	0.50%	0.40%

It was discovered that the established UHPLC technique was appropriate for measuring gabapentin and nortriptyline simultaneously with little tailing, acceptable peak morphologies, and good resolution.

This method was validated as per ICH guidelines. %RSD of gabapentin and Nortriptyline Hydrochloride having less than 2% indicates this method was robust. % Mean assay of gabapentin and nortriptyline hydrochloride are in the acceptable range. No interferences were observed and this method exhibited good selectivity and specificity. Using various sample weight techniques, three tiers of accuracy samples were created. For every accuracy level, three injections were administered, and the mean percentage of recovery for gabapentin and nortriptyline hydrochloride was found to be 99.02% and 100.22%, respectively.

CONCLUSION

For the simultaneous measurement of gabapentin and nortriptyline hydrochloride in solid dose form, a straightforward, accurate, and exact approach was created. The retention times for gabapentin and nortriptyline hydrochloride were determined to be 1.845 and 3.345 minutes, respectively. % The RSD of nortriptyline hydrochloride was determined to be 0.31% and that of gabapentin to be 0.25%. % 99.49% of gabapentin and 99.30% of nortriptyline hydrochloride were recovered from the assay. The regression equations for nortriptyline hydrochloride are $y = 3.0378x + 158.8271$ $R^2 = 0.9998$ and gabapentin are $y = 0.0106x - 0.0105$ $R^2 = 0.9993$. The method that was created was easy to use and cost-effective, making it suitable for routine quality control testing in industries. Both the retention periods and the run time were reduced.

ACKNOWLEDGEMENT

I can never adequately convey the deep respect and appreciation I have for my parents, without whom I could not have accomplished anything in my life. The facilities required to

conduct the research were provided by C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai, Tamilnadu, India, for which the authors are grateful.

REFERENCES

website

https://en.wikipedia.org/wiki/Gabapentin

https://go.drugbank.com/drugs/DB00996

https://en.wikipedia.org/wiki/Nortriptyline

https://www.drugs.com/nortriptyline.html

JOURNALS

1. Gilron I, Bailey JM, Tu D, Holden RR, Weaver DF, Houlden RL.N Engl J Med., Mar. 31, 2005; 352(13): 1324-34. doi: 10.1056/NEJMoa042580.
2. Derry S, Wiffen PJ, Aldington D, Moore RA.Cochrane Database Syst Rev., Jan. 8, 2015; 1(1): CD011209. doi: 10.1002/14651858.CD011209.pub2.
3. Chaparro LE, Wiffen PJ, Moore RA, Gilron I.Cochrane Database Syst Rev., Jul. 11, 2012; 2012(7): CD008943. doi: 10.1002/14651858.CD008943.pub2.
4. Chandra K, Shafiq N, Pandhi P, Gupta S, Malhotra S.Int J Clin Pharmacol Ther., Aug. 2006; 44(8): 358-63. doi: 10.5414/cpp44358.
5. Irving G, Jensen M, Cramer M, Wu J, Chiang YK, Tark M, Wallace M.Clin J Pain., Mar-Apr, 2009; 25(3): 185-92. doi: 10.1097/AJP.0b013e3181934276.
6. Basnet A, Butler S, Honoré PH, Butler M, Gordh TE, Kristensen K, Bjerrum OJ. Acta Anaesthesiol Scand, Jan. 2014; 58(1): 61-73. doi: 10.1111/aas.12218. Epub 2013 Nov 22.
7. Haviv Y, Rettman A, Aframian D, Sharav Y, Benoliel R.J Oral Facial Pain Headache, 2015 Spring; 29(2): 144-51. doi: 10.11607/ofph.1408.
8. Moore RA, Wiffen PJ, Derry S, Toelle T, Rice AS.Cochrane Database Syst Rev., Apr. 27, 2014; 2014(4): CD007938. doi: 10.1002/14651858.CD007938.pub3.
9. Jensen TS, Finnerup NB. Lancet, Oct. 10, 2009; 374(9697): 1218-9. doi: 10.1016/S0140-6736(09)61205-8. Epub 2009 Sep 30.