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Development and Validation of Analytical Method for Simultaneous estimation of Gabapentin and Nortriptyline Hydrochloride in Pharmaceutical Dosage Form

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ABSTRACT:

Based on the literature review, it was found that a number of studies involving method development for estimation of GABAPENTIN and NORTRIPTYLINE have been carried out in formulations/biological fluid. Thus, a number of analytical methods have been developed for estimation of both drugs individually and in combination with other drugs. Review of literature reveals that no chromatographic and Spectroscopic methods have been reported for simultaneous estimation of Gabapentin and Nortriptyline. In the Bulk drug and Pharmaceutical dosage form. Therefore it is found that there is a need to develop an analytical method for simultaneous estimation of Gabapentin and Nortriptyline. in formulations. So the present work is aimed for Development of simple and reproducible chromatographic (RP-HPLC) and spectrophotometric (UV Spectroscopic method) method for simultaneous estimation of Gabapentin and Nortriptyline. Validation of the developed chromatographic and spectroscopic method as per ICH guidelines.

KEY WORDS: Gabapentin And Nortriptyline RP-HPLC, Mobile phase, UV Spectroscope, Validation

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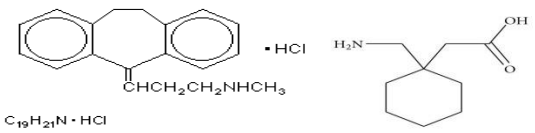
INTRODUCTION:

1. INTRODUCTION

The IUPAC name of the Gabapentin and Nortriptyline is 2-[1-(amino methyl)cyclohexyl] acetic acid and 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine respectively, with molecular formula $C_9H_{17}NO_2$ and $C_{19}H_{21}N.HCL$ respectively and molecular weight 171.24and 299.8respectively. The molecular structure of the drug is given in Fig.1.



Nortriptyline Gabapentin

Figure 1: Chemical structure

In this combination the Gabapentin used as a anticonvulsant to treat the convulsion which cause depression as a side effect. so, to treat the depression Nortriptyline used as antidepressant to treat the depression.

Gabapentin Official in BP and USP.and Serratiopeptidase is official. Nortriptyline Official in IP, BP and USP .However no analytical method has been reported till date for the estimation of Gabapentin and Nortriptyline

using UV-Spectroscopy and RP-HPLC method. The present paper describes the analytical method development and validation of estimation of Gabapentin And Nortriptyline in Pharmaceutical dosage form using RP-HPLC and UV-Spectroscopy. The proposed method are optimized and validated as per ICH guidelines.

MTERIALS AND METHODS

Materials

HPLC Thermo separation Product TSP UV 2000. UV-visible Spectrophotometer SHIMADZU 1800 double beam UV - Visible spectrophotometer with software UV probe 2.0 (Shimadzu Corporation, Kyoto, Japan).Gabapentin and Nortriptyline was purchased from Intas Pharmaceutical Pvt.Ltd. The commercial fixed dose GABAPIN-NT was procured from local market. All solvents (HPLC grade) were obtained from Merck Chemicals.

Working Standard preparation:

UV Spectroscopy:-

Preparation of standard stock solution

A. Standard GAB stock solution (1000 µg/mL):

Standard GAB powder (50 mg) was weighed accurately and transferred in to 50 ml volumetric flask and dissolved in and diluted to 50 ml with 0.5 N NaOH to prepare standard Stock solution having Concentration 1000 µg/ mL.c. Use 0.2 micron filter membrane to filter. pH should be around 8.2.

B. Standard NOR stock solution (100µg/ml):

Standard NOR powder (10 mg) was weighed accurately and transferred in to 10 ml volumetric flask and dissolved in and diluted to 10 ml with 0.5 N NaOH to prepare standard Stock solution having Concentration 1000 µg/ mL. From this solution 1 ml was taken and diluted to 10 ml with 0.5 N NaOH to prepare working standard solution having concentration 100 µg/ ml.

Sample preparation:

Preparation of sample solution

Tablets(20) was accurately weighed, average weight was calculated and powdered. A tablet powder equivalent to 400 mg of Gabapentin and 10 mg of Nortriptyline Hydrochloride was taken into 100 ml volumetric flask. Add sufficient volume of 0.5 N NaOH to dissolved and sonicate for 25 min. make up the volume up to mark with 0.5 N NaOH and filter through whatman filter paper. From this above solution, suitable aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with 0.5 NaOH to get final concentration of 400 µg/mL and 10 µg/mL for Gabapentin and Nortriptyline Hydrochloride respectively.

Method Development:

Determination of wavelength having maximum absorbance.

Standard solution of GAB (400 µg/mL) and NOR (10 µg/mL) was scanned in the range of 200 to 400 nm for the determination of wavelength having maximum absorbance. GAB shows 214 nm and NOR shows 238.7 nm as the wavelength having maximum absorbance.

Preparation of Calibration Curve

Aliquots of Standard solution of GAB (2,3,4,5,6,7 ml) and NOR (,0.7, 0.8,0.9,1, 1.1,1.2 ml) were transferred in a series of 10 ml volumetric flask. The volume was adjusted to the mark with 0.5 N NaOH and mixed.

The absorbance of all the solutions was measured at 214 and 238.7 nm against 0.5 N NaOH as blank.

Method Validation

Linearity & Range.

Calibration curve were plotted over a concentration range of 200 - 700 µg/mL and 7- 12 µg/mL for GAB and NOR, respectively. Accurately measured standard stock solution of GAB (2,3,4,5,6,7 ml) and NOR (0.7, 0.8,0.9,1, 1.1,1.2 ml) were pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with 0.5 N NaOH and the absorbencies of the solutions were measured at 214 and 238.7 nm against 0.5 N NaOH as a blank. The calibration curve was constructed by plotting the graph of absorbance Vs concentration for method-A and method-B.

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of

an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Repeatability : The precision of the proposed method was determined by analyzing standard solution of GAB and NOR for 6 times solutions of (400 µg/mL GAB & 10 µg/mL forNOR) (n=6) without changing the parameters of measurement. The absorbance of both were measures at 214 nm & 238.7 nm for both GAB and NOR. The results are reported in terms of relative standard deviation (%RSD).

Intermediate Precision (Reproducibility) It expresses within laboratory variations as on different days analysis or equipment within the laboratory. Variation of results within same day is called Intra-day precision and variation of results amongst days called Inter-day precision. The Intra-day precision (% R.S.D.) was determined for standard solution of GAB (400 µg/mL) and NOR (10 µg/mL) for six times at the same day. The Inter-day precision (% R.S.D.) was determined for standard solution of GAB (400 µg/mL) and NOR (10 µg/mL) for six times at different six days.

Limit of detection (LOD) & limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were calculated by using the following equations.

LOD = 3.3*σ/S

LOQ = 10*σ/S

Where, σ = the standard deviation of the response

S = slope of the calibration curv

Accuracy (% Recovery study)

The accuracy of the methods was determined by calculating recoveries of GAB and NOR by the standard addition method. Known amounts of standard solution of GAB and NOR were added at 80%, 100% and 120% levels to prequantified sample solutions of GAB (400 µg/mL) and NOR (10 µg/mL).

Assay (Analysis of GAB & NOR in combined tablet dosage form) The absorbance of final sample solution was measured against 0.5 N NaOH as blank at 214 and 238.7 nm for quantitation of GAB and NOR, respectively.

The amount GAB and NOR present in the sample solutions were determined by solving the following simultaneous equations for methodA.

C x = (A1 aY2 - A2 Ay1) / (aX1 aY2 - aX2 aY1)
C y = (aX1 A2 - aX2 A1) / (aX1 aY2 - aX2 ay1)
Where, A1, A2 =Abs. of components,
aX1 =Absorbitivity of first drug at λ1,
aX2 = Absorbitivity of first drug at λ2,
aY1=Absorbitivity of second drug at λ1,
aY2 = Absorbitivity of second drug at λ2.
 The amount GAB and NOR present in the sample solutions were determined by solving the following equations for method-B

$Cx = {}_1/\ \mathbf{ax_1}$

$Cy= \mathbf{A_2} -(\mathbf{ax_2}* \mathbf{C_x})/\ \mathbf{ay_2}$

Where, A1 and A2 are absorbance of sample solution at λ1 and λ2 respectively,
ax1 and ax2 are absorptivity value of drugs at λ1 and λ2 respectively,
ay2 is absorptivity value of Y at λ2 .

RESULT AND DISCUSSION

Method-A and Method-B

Method Development

The working standard solution of GAB and NOR were prepared separately. They were scanned in the wavelength range of 200-400 nm. Maximum absorbance was obtained at 214 nm and 238.7 nm for GAB and NOR, respectively. These two wavelengths were employed for the determination of GAB and NOR. Overlain spectra of both the drugs are shown in Figure

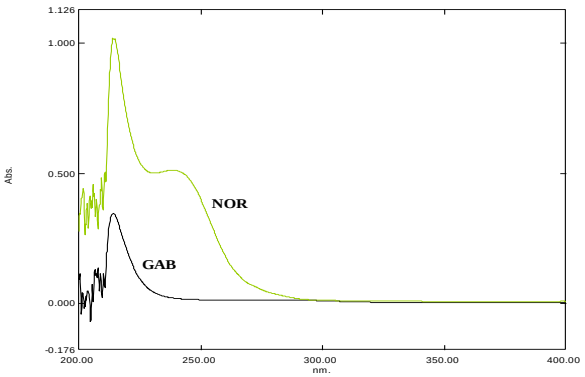


Figure 2 Absorption spectra of GAB (400 µg /mL) and NOR (10 µg /mL)

Method validation

Linearity & Range

The calibration curves were plotted over a concentration range of 200-700 µg/mL for GAB and 7-12 µg/mL for NOR. Accurately measured standard solutions of GAB (2,3,4,5,6,7 ml) and NOR (0.7, 0.8,0.9,1, 1.1,1.2 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with0.5 N NaOH. The absorbance of the solutions were measured at 214 and 238.7 nm against 0.5 N NaOH as blank. The calibration curves were constructed by plotting absorbencies versus concentrations and the regression equations were calculated. The high value of correlation coefficient indicated that method has linear value for response on the conc. Range of GAB 200700 µg /mL & for NOR 7-12 µg /mL.

Linearity for GAB

Sr No.	Conc. of GAB (µg/mL)	Abs. at 214 nm (for GAB) n=3 Mean±S.D.	Abs. at 238.67 nm (for GAB) n=3 Mean±S.D.
1	200	0.168 ± 0.0423	0.015 ± 0.0042
2	300	0.261 ± 0.0405	0.019 ± 0.0020
3	400	0.363 ± 0.0328	0.022 ± 0.0015
4	500	0.462 ± 0.0400	0.026 ± 0.0015
5	600	0.572 ± 0.0350	0.029 ± 0.0020
6	700	0.673 ± 0.0341	0.034 ± 0.0026

Linearity for NOR

Sr. No.	Conc. of NOR (µg/mL)	Abs. at 214 nm (for NOR) n=3 Mean±S.D.	Abs. at 238.67 nm (for NOR) n=3 Mean±S.D.
1	7	0.426 ± 0.0238	0.294 ± 0.0175
2	8	0.539 ± 0.0456	0.331 ± 0.0327
3	9	0.629 ± 0.0316	0.382 ± 0.0195
4	10	0.720 ± 0.0247	0.426 ± 0.0311
5	11	0.793 ± 0.0121	0.465 ± 0.0461
6	12	0.901 ± 0.0240	0.513 ± 0.0119

Repeatability

The % RSD values of repeatability study for GAB was found to be 1.25 % at 214 nm and for NOR it was found to be 1.77 % at 238.7 nm.

Sr. no	Absorbance of GAB (400 µg /mL)	Absorbance of NOR (10 µg /mL)
	At 214 nm	**At 238.7 nm**
1	0.392	0.421
2	0.378	0.426
3	0.384	0.405
4	0.383	0.422
5	0.383	0.424
6	0.384	0.418
Mean	0.383	0.419
S.D.	0.00480	0.00743
% RSD	1.25	1.77

Intra-day precision

For evaluation of Intra-day precision, standard solution of 400 µg/mL for GAB and 10 µg/mL for NOR were estimated for six times on the same day.

Sr. No.	Time (Hours)	Absorbance	
		GAB(400 µg/mL)	NOR(10 µg/mL)
		At 214 nm	At 238.7 nm
1	0 hr	0.392	0.421
2	1 hr	0.378	0.426
3	2 hr	0.384	0.405
4	3 hr	0.383	0.421
5	4 hr	0.382	0.418
6	6 hr	0.384	0.424
MEAN		0.383	0.419
S.D.		0.00480	0.00743
% R.S.D.		1.25	1.77

LOD and LOQ

Parameter	GAB		NOR	
	At 214 nm	**At 238.7 nm**	**At 214 nm**	**At 238.7 nm**
Detection Limit	29.7 µg/mL	31.18 µg/mL	2.05 µg/mL	0.97 µg/mL
Quantitation Limit	90 µg/mL	99.5 µg/mL	6.23 µg/mL	2.95 µg/mL

Accuracy

Accuracy was determined in terms of recovery study and the recoveries are done at three levels i.e. 80%, 100% and 120%. The data shows that the proposed method is accurate.

Drug	Amt taken (µg/mL) (n=3)	Amt of standard added (µg/mL) (n=3)	Abs.	Recovered concentration (µg/mL)	% Recovery ± SD (n=3)
GAB	400	320	0.643	708.91	98.46 ± 0.635
	400	400	0.715	782.48	97.81 ± 0.357
	400	480	0.786	864.07	98.19 ± 0.743
MEAN ± S.D.					98.15 ± 0.323
NOR	10	8	1.336	17.59	98.17 ± 0.515
	10	10	1.485	19.67	98.35 ± 0.664
	10	12	1.633	21.6	98.20 ± 0.412
MEAN ± S.D.					98.24 ± 0.530

Assay of the pharmaceutical formulation

The proposed method was applied successfully to the tablet dosage form and results obtained are shown in Table.

Brand Name	Drug	Amount taken(µg/ml)	Amount found(µg/ml)	% Assay ± SD
Gabapin-NT	Gabapentin	400	393.12	98.28 ± 0.39
	Nortriptyline	10	9.811	98.11 ± 0.23

Assay results of GAB and NOR by Absorption correction method (n=6)

Brand Name	Drug	Amount taken(µg/ml)	Amount found(µg/ml)	% Assay ± SD
Gabapin-NT	Gabapentin	400	392.12	98.03 ± 0.512
	Nortriptyline	10	9.864	98.64 ± 0.425

CONCLUSION

A simultaneous estimation, absorption correction and Area under curve method have been developed for simultaneous estimation of GAB and NOR in their combined dosage form.

The methods are simple, linear, precise, accurate & suitable for simultaneous estimation of GAB and NOR in their combined dosage form.

RP-HPLC

1.Pump

Mode of chromatography: Reversed Phase Chromatography

Mode of Elution: Isocratic

Flow Rate: 1.0 ml/min

2. Oven:

Oven Temperature: 30° ± 2°C

3. Detector:

Type: DAD detector

Lamp: D2 lamp

Wavelength: 215 nm

4. Auto sampler Configuration:

Rinsing Volume: 1000 µl

Sampling speed: 20 µl/sec

5. Other parameters:

Column: C8 (150 µm i.d 5 µm)

Run time: 5 min

Mobile Phase: MeOH : ACN : 0.028 M phosphate Buffer water (35:40:25 v/

v/v) pH 4.5 with O- Phosphoric acid

System Suitability Parameters:

System suitability parameter	GAB	NOR
Retention time	1.61 min	2.6 min
Tailing factor ± RSD	1.38 ± 1.32	1.55 ± 0.84
Theoretical Plate ± RSD	3849 ± 0.81	3209.03 ± 1.4
Resolution ± RSD	4.92 ± 1.27	

Method

Preparation of Buffer

The buffer was prepared by taking 1.92 g Potassium dihydrogen phosphate in 500 ml volumetric flask, dislove it and make up volume with double distilled Water. Set the pH 4.5 with O-Phosphoric acid and then that buffer was filtered through 0.45 µmWhatman Filter paper. Ultrasonic for 15 min. Note that each day freshly prepared and degassed buffer solutions were used.

NOTE: Potassium dihydrogen phosphate (KH2PO4) molecular weight was 136.17gm

Preparation of mobile phase

Freshly Prepared buffer solution, Acetonitrile and Methanol were taken and sonicate for 25 minute for degassing. Then Fill all three solution in reservoir and set the volumes to 35:40:25 v/ v/v for Methanol: Acetonitrile: 0.028 M Phosphate Buffer(pH-4.5).

Preparation of Standard/Working stock solution

To Weighed accurately 200 mg of standard API of Gabapentin and transferred in 50 ml volumetric flask, dissolved and diluted up to the mark with Mobilephase, to get final concentration 4000 µg/mL. To Weighed accurately 10 mg of standard API of Nortriptyline Hydrovhloride and transferred in 10 ml volumetric flask, dissolved and diluted up to the mark with Mobilephase, to get final concentration 1000 µg/mL. Solution of 100 µg/mL of NOR was prepared from 1000 µg/mL by diluting 1 ml up to 10 ml with Mobilephase.Suitable aliquots were taken and transferred into 10 ml volumetric flask and volume was made up to the mark with mobile phase**.**

Preparation of test sample solution

Weigh 20 tablets accurately and powdered. A tablet powder equivalent to 400 mg of Gabapentin and 10 mg of Nortriptyline Hydrochloride was taken into 100 ml volumetric flask. Add sufficient volume of mobilephase to dissolved and sonicate for 25 min. make up the volume up to mark with mobilephase and filter through whatman filter paper. From this above solution, suitable aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with mobile phase to get final concentration of 400 µg/mL and 10 µg/mL for Gabapentin and Nortriptyline Hydrochloride respectively.

Calibration curve of standard Gabapentin and Nortriptyline

A calibration curve was plotted over a concentration range of 200-1000 µg/mL for Gabapentin and 4-12 µg/mL for Nortriptyline Hydrochloride by taking a suitable aliquots of WSS of both drugs and transferred into 10 ml of volumetric flask and diluted up to mark with mobile phase. The resulting solution was injected into the column. Calibration curve was constructed by plotting peak area versus concentration..

Method Validation

Linearity

The linear response of GAB and NOR were determined by analyzing five independent levels of the calibration curve in the range of 200-1000 µg/mL for both GAB and 4-12 µg/mL for NOR. Result should be expressed in terms of correlation co-efficient.

Precision

Repeatability

Repeatability experiment was performed by preparing the standard solution of 400 µg/mL for GAB and 10 µg/mL for NOR six times and analyzed as per the proposed method. Percentage relative standard deviation (% RSD) should be less than 2%.

Intermediate Precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory**.**

Intra-day precision

Variation of results within same day is called intra-day precision. The intra-day precision was determined for standard solution of 400 µg/mL for both GAB and 10 µg/mL for NOR for the six times on the same day.

Inter-day precision

Variation of results amongst days called inter-day precision. The inter-day precision was determined for standard solution of 400 µg/mL for both GAB and 10 µg/mL for NOR for three times at different six days.

Accuracy (% Recovery)

Accuracy may often be expressed as % recovery by the assay of known, added amount of analyte. It's measure of the exactness of the analytical method. The recovery

experiments were carried out in triplicate by sparking previously analyzed samples of the injection (GAB 400 µg/mL and NOR 10 µg/mL) with three different concentrations of standards at 80%, 100% and 120% GAB (320, 400 and 480 µg/mL) and NOR (8, 10 and 12 µg/mL).

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 x N/S

Where,
N is the standard deviation of the intercept of the calibration curve of the drug
And S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 x N/S

Where,
N is the standard deviation of the intercept of the calibration curve of the drug and
S is the slope of the corresponding calibration curve.

Robustness

Robustness of the proposed method was checked by changing the mobile phase composition, flow rate and wavelength of determination. Robustness was checked at the concentration of 400 µg/mL for both GAB and 10 µg/mL for NOR.

System Suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment and different analyzer. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

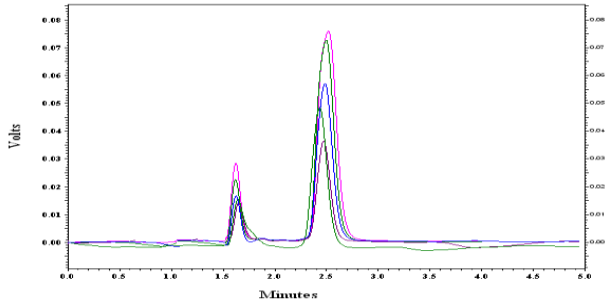
Application of proposed Method to the pharmaceutical dosage form

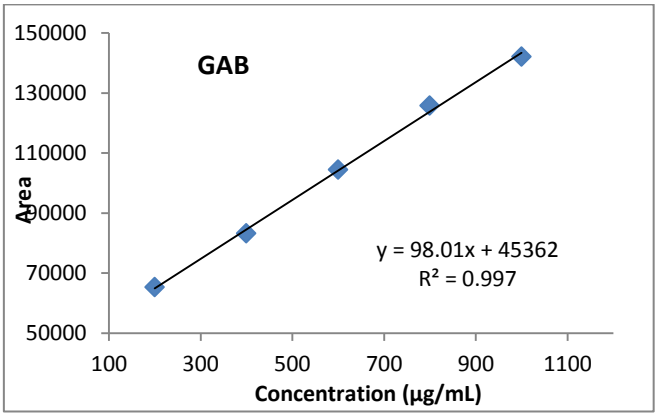
The method was applied to the pharmaceutical dosage form for determination of GAB and NOR. 400 µg/mL GAB and 10 µg/mL NOR was evaluated using the proposed method and area was calculated. The proposed validated method was successfully applied for the routine simultaneous estimation of GAB and NOR in bulk powder and in injection dosage form.

Method Validation

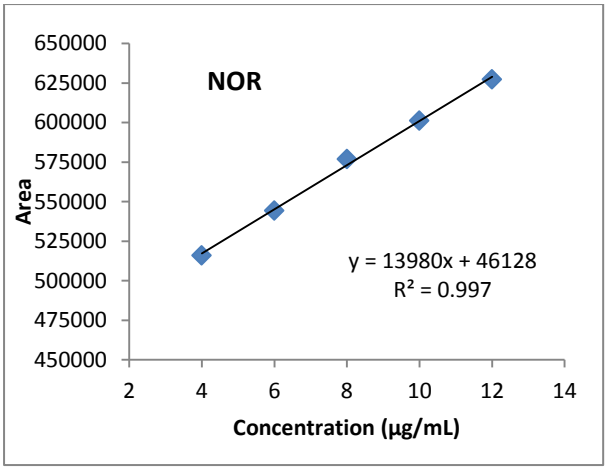
Linearity

Calibration curve was found to be linear in the range of 200-1000 µg/mL for GAB and 4-12 µg/mL for NOR





Sr. No.	Concentration (µg/ml)	Area Mean ± SD (n=3)	%RSD
1	200	65345 ± 421.729	0.64%
2	400	83191 ± 323.505	0.38%
3	600	10442.7 ± 1205.79	1.15%
4	800	125823 ± 950.039	0.75%
5	1000	142040.3 ± 1602.848	1.12%



Sr. No.	Concentration	Area Mean ± SD (n=3)		%RSD
1	4	515931.3 1506.434	±	0.29 %
2	6	544342.7 4013.111	±	0.7 %
3	8	576856 1320.802	±	0.22 %
4	10	601190 1249.158	±	0.21 %
5	12	627311.7 1606.411	±	0.25 %

Repeatability

Repeatability of the results for a concentration of 400 µg/mL for GAB and 10 µg/mL for NOR was evaluated by 6 replicate determinations.

Sr. No.	Concentration (µg/mL)		Area	
	GAB	**NOR**	**GAB**	**NOR**
1	400	10	82191	589874
2	400	10	81547	605412
3	400	10	83945	601478
4	400	10	83095	589874
5	400	10	82843	596563
6	400	10	83512	601112
MEAN			82855.5	597385.5
S.D.			875.128	6458.82
%R.S.D.			1.05%	1.08%

Intra-day precision

For evaluation of intra-day precision, standard solution of 400 µg/mL for GAB and 10 µg/mL for NOR were estimated for six times on the same day.

Sr. No.	Time (Hours)	Area	
		GAB(400 µg/mL)	**NOR(10 µg/mL)**
1	0 hr	82191	589874
2	1 hr	81547	605412
3	2 hr	83945	601478
4	3 hr	83095	589874
5	4 hr	82843	596563
6	6 hr	83512	601112
MEAN		82855.5	597385.5
S.D.		875.128	6458.821
% R.S.D.		1.05 %	1.08 %

Inter-day precision

For evaluation of inter-day precision, standard solution of 400 µg/mL for GAB and 10 µg/mL for NOR were estimated for six times on the different day.

Sr. No.	Days	Area	
		GAB (400 µg/mL)	**NOR(10 µg/mL)**
1	Day - 1	82154	585541
2	Day - 2	83471	584112
3	Day - 3	82971	594487
4	Day - 4	80147	604785
5	Day - 5	81444	596632
6	Day - 6	82623	582254
MEAN		82037.4	591301.8
S.D.		1195.987	8798.817
% R.S.D.		1.45%	1.48 %

Accuracy

Accuracy was determined in terms of recovery study and the recoveries are done at three levels i.e. 80%, 100% and 120%. The data shows that the proposed method is accurate.

Sr. No	**Amt. taken**	**Amt. added**	**Total Amt.**	**Total Area**	**Area of std. recovered**	**Amt. Re-covered**	**% Recovery**	**Mean % Re-covery**
1	400	0	400	83191	-	-	-	-
2	400	320	720	157259.53 157539.84 157618.25	74068.53 74348.84 74427.24	313.3 316.1 316.9	97.91 98.81 99.05	98.58
3	40	40	80	1669	8374	391.	97.9	98.

	0	0	0	33.71 1670 90.53 1672 47.34	2.71 8389 9.53 8405 6.34	6 393. 2 394. 8	98.3 98.7	3
4	40 0	48 0	88 0	1728 92.12 1722 80.54 1726 61.60	8970 1.12 8908 9.54 8947 0.60	472. 8 466. 5 470. 4	98.5 97.8 98.0 1	98. 1
MEAN								98. 32
S.D.								0.7 61
% R.S.D.								% 0.7 9

Data recovery study for NOR

Sr. No	***Amt. taken***	***Amt. added***	***Total Amt.***	***Total Area***	***Area of std. recovered***	***Amt. Re-covered***	***% Recovery***	***Mean % Re-covery***
1	*10*	*0*	*10*	*6011 90.7*	*-*	*-*	*-*	*-*
2	*10*	*8*	*18*	*7560 83.1 7572 01.5 7553 84.1*	*1548 92.4 1560 10.8 1541 93.4*	*7.78 7.86 7.73*	*97.3 1 98.2 5 96.6 9*	*97. 41*
3	*10*	*10*	*20*	*7841 82.9 7855 80.9 7868 39.1*	*1829 92.2 1843 90.2 1856 48.4*	*9.79 9.89 9.98*	*97.9 8 98.9 1 99.8 1*	*98. 9*
4	*10*	*12*	*22*	*8125 62.3 8142 06.3 8143 57.3*	*2113 71.6 2130 15.6 2131 66.6*	*11.8 2 11.9 3 11.9 4*	*98.5 99.4 8 99.5 7*	*99. 1*
MEAN								*98. 47*
S.D.								*0.9 23*
% R.S.D								*% 0.9 3*

LOD and LOQ

Parameter	GAB	NOR
Standard deviation of the Y-intercepts of the three calibration curves.	1828.163	126.824
Mean slope of the three calibration curves.	98.01	13980
Detection Limit	61.55 µg/mL	0.029 µg/mL
Quantitation Limit	186.53 µg/mL	0.09 µg/mL

Robustness

Robustness data clearly shows that the proposed method is robust at small but deliberate change. Robustness was performed by changing the mobile phase composition, detection wavelength and flow rate.

Change in Flow rate:

Sr No.	**0.8 ml/min**		**1.2 ml/min**	
	GAB	NOR	GAB	NOR
1	83547	601254	83191	601190
2	82915	602457	83528	599765
3	83111	599861	82876	602348
MEAN	83191	601190.7	83198.33	601101
SD	323.5058	1299.158	326.0619	1293.798
% RSD	0.388	0.216	0.391	0.215

Change in Mobile phase ratio(MeOH : ACN : Phosphate Buffer)

	34:39:27		**36:41:23**	
	GAB	**NOR**	**GAB**	**NOR**
1	83154	604251	832225	604576
2	82931	593479	824758	596398
3	83257	602145	831942	600997
MEAN	83114	599958.3	829641.7	600657
SD	166.6403	5709.214	4231.746	4099.588
% RSD	0.2	0.95	0.51	0.68
Change in wavelength:				
	214		216	
	GAB	NOR	GAB	NOR
1	83256	593745	83456	587456
2	82472	586458	83337	596632
3	83987	592459	82745	585545
MEAN	83238.33	590887.3	83179.33	589877.7
SD	757.6545	3889.434	380.8206	5926.951
% RSD	0.91	0.65	0.45	1.0

Application of proposed method to the Pharmaceutical dosage form (tablet)

No interference of the excipients with the peaks of interest appeared; hence the proposed method is applicable for the routine estimation of Gabapentin and Nortriptyline in pharmaceutical dosage forms.

Brand Name	Drug	Amount taken (µg/ml)	Amount found (µg/ml)	% Assay ± SD
Gabapin-NT	Gabapentin	400	394.72	98.68 ± 0.33
	Nortriptyline	10	9.88	98.88 ± 0.23

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