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A NEW SIMPLE AND SENSITIVE RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NORTRIPTYLINE AND PREGABALIN IN BULK AND TABLET DOSAGE FORM

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Abstract:

A rapid, precise, accurate, specific and simple RP-HPLC method was developed for the simultaneous estimation of Nortriptyline and Pregabalin in bulk and its combined pharmaceutical dosage form. A High performance liquidchromatograph WATERS, software: Empower 2, 2695 separation module, 996 PDA detector, using Phenomenex Luna C18 (4.6mm×250mm) 5 µm or equivalent column, with mobile phase composition of Methanol: Phosphate Buffer pH-3.0 (70:30v/v) was used. The flow rate of 1.0 ml min-1 and effluent was detected at 230 nm. The retention time of Nortriptyline and Pregabalin was found to be 1.870min and 2.499minutes respectively.Linearity was observed over concentration range of 10-50µg ml-1 for Nortriptyline and 16-80µg ml-1 for Pregabalin respectively. The accuracy of the proposed method was determined by recovery studies and the Nortriptyline was found to be 99.1% and Pregabalin was found to be 98.8% respectively. The proposed method isapplicable to routine analysis of Nortriptyline and Pregabalin in bulk and pharmaceutical formulations. The proposed method was validated for various ICHparameters like linearity, limit of detection, limits of quantification, accuracy, precision, range and specificity.

Key Words: *Nortriptyline, Pregabalin, RP-HPLC, Robustness and ICH Guidelines.*

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INTRODUCTION:

Strategy of method development:

Method development ought to be supported many issues. It's desirable to possess most sample data to form development quick and desired for meant analytical technique application, physical and chemical properties area unit most desirable as primary data. Moreover, separation goal has to outline at starting so; acceptable technique is developed for the aim. AN LC technique development is extremely vast space for even prescribed drugs with restrictive demand of international standards. So, before technique validation and usage at internal control several aspects have to be compelled to focus as per ICH tips. Method development is supported a sample and goals moreover as offered resources for action however few basic steps for technique development area unit is mentioned as given below.

Steps in technique development:

1. Sample data ,define separation goals
2. Sample pre-treatment, want of special HPLC procedure
3. choice of detector and detector settings
4. choice of LC method; preliminary run; estimate best separation conditions
5. Optimize separation conditions
6. Check for issues or demand for special procedure
7. technique validation

Sample information:

1. variety of compounds gift
2. Chemical structure of compounds
3. Chemical nature
4. relative molecular mass of compounds
5. pKa Value(s) of compounds
6. Sample solubility
7. Sample stability and storage
8. Concentration vary of compounds in sample
9. Ultraviolet illumination spectra of compounds or properties for detection of compounds

RP-HPLC continues to be comparatively new technique, and literature isn't invariably offered on operative conditions for a selected application. The primary step in developing AN RP-HPLC analysis, or the other variety of natural process analysis, is to outline the matter and state the aim of study. So as to outline the matter, the subsequent question ought to be asked:

1. Is that the analysis aiming to be used habitually for an oversized variety of samples? Is case of operation and ease of nice importance?
2. May be a qualitative and / or qualitative analysis required?
3. Is it necessary to separate all the constituents within the sample or solely a tiny low cluster of constituents?
4. Area unit the constituents similar in structure or wide diverse?
5. Area unit the constituents gift in similar concentrations, or is one constituent presenting an oversized quantity and alternative solely in trace amounts?
6. Will sample be simply ready for RP-HPLC analysis?
7. Area unit there compounds gift that will interfere with the analysis of constituents of interest?
8. Will peaks within the recording be promptly identified?

The next step may be a literature search to find-out if these compounds are separated mistreatment alternative natural process techniques.

For example: The conditions utilized in thin-layer action (TLC) or open chromatography usually are adopted for HPLC; this is a place to begin and saves a valuable time.

A total RP-HPLC technique involves the subsequent steps:

1. Sample assortment
2. Sample preparation
3. Chromatography
4. Peak identification
5. Quantification
6. Information analysis and interpretation of results (Validation)

1. Sample assortment

The primary step within the analysis of biological and a few alternative samples sometimes needs sample filtration. Since RP-HPLC columns use 3-5 um packing materials, the column water is sometimes protected with a 2um frit or screen. Sample filtration is performed mistreatment membrane kind filters with zero.2 - 0.5 um pore sizes.

Several ways of macromolecule removal is used: immoderate filtration, precipitation of proteins with robust acid or organic solvents, ammonia sulphate precipitation, denaturation by heat etc.

2. Sample preparation

Usually within the analysis of complicated samples, solely variety of compounds area unit of interest. Therefore, it's not necessary to realize separation of

all sample constituents, however rather to optimize the conditions for speedy analysis of many elite compounds. In these cases, it's advantageous to polarity and solubility of the solutes.

Most typically used extraction procedures area unit solid-phase extraction, solid-phase small extraction, liquid-liquid extraction, liquid-phase small extraction, membrane based mostly extraction and critical fluid extraction.

Just in case of pure samples or bulk samples, merely dissolve within the mobile part consistent with their solubility and polarity.

3. Chromatography:

The bulk of study will currently be carried mistreatment RP-HPLC; so RP-HPLC is that the technique of selection unless the required separations can't be achieved, or unless another mode, like gel permeation, is clearly indicated. at the present industrial convenience of RP-HPLC column over ninetieth of all RP-HPLC separation is being dispensed mistreatment C18 as a bond part on 3-5 um oxide particles. In RP-HPLC, or in any separation, there area unit several parameters that may influence each resolution of compounds in mixture and also the potency of separation.

When the mobile part and stationary part area unit chosen, the optimum flows ridge and extraction mode should then be determined.

3.1. Stationary Phase:

The four necessary parameters concerned within the stationary part that may be varied in RP-HPLC separation area unit as follows:

1. Column length & internal diameter
2. Partical size
3. Variety of guaranteed phases
4. Surface coverage

Solely when exhausting the various potentialities of mobile part or mobile part mixtures ought to differ sorts of columns tried. However, in cases wherever stationary separations can't be obtained with these columns, stationary part parameters are modified individually or together. For example: Shorter columns (3-5 µm) gave higher resolution and shorter retention times for the determination of some compounds in humor. So in RP-HPLC, numerous stationary part moreover because the mobile part is altered to cive the required separation.

3.2. Mobile Phase:

One in all the nice benefits of HPLC is that the skillfulness afforded by a liquid mobile part. Currently solely will completely different parameters be varied once the mobile part is liquid, however the matter can even move with the mobile part should be ensured so as to stop precipitation.

For the mobile part, the variable to be set is whether or not AN organic or binary compound eluent ought to be used. With RP-HPLC analysis, either a binary compound eluent or sort of organic solvent like methyl alcohol or Acetonitrile is tried 1st. If the k1 values area unit overlarge with a binary compound solvent, then the separation ought to be tried by employing a mixture, the 2 in numerous proportions. Several straightforward Analyses is dispensed with isocratic extraction mistreatment AN binary compound eluent to that an organic modifier is extra. If sample to be analyzed contains a awfully complicated mixtures or mixture of compounds of numerous structure and retention behavior, then either a ternary mixture of solvents is used isocratically or gradient extraction could also be necessary.

3.3. Mode of elution & flow rate:

Whenever potential, isocratic extraction ought to be used as a result of it eliminates "turnaround time" on the column and so shortens overall analysis time. Also, retention duplicability is additional sure with isocratic extraction as a result of re-equilibration of the column when gradient extraction should be rigorously controlled. However, once adequate resolution can't be achieved at intervals cheap length of your time, thanks to the range of compounds during a mixture or once there's general extraction drawback, gradient extraction is judicious. Gradients are stepwise or continuous.

3.4. Optimization of RP-HPLC:

Optimization of latest technique development is extremely necessary. For these some equations area unit offered, however those area unit very little facilitate for unknown samples.

To optimize the retention time, several operative parameters ought to be thought of composition of eluent; extraction mode and rate if gradient extraction is being employed. To decrease k1 values, the strength of the initial or final eluent or the slope of the gradient ought to be redoubled. If the isocratic doesn't give the required resolution, the foremost obvious thanks to improve resolution are either by isocratic extraction with mixed solvents or by gradient extraction.

4. Peak Identification:

The measuring of peak height or peak space is an element of the quantitative measuring. Many ways area unit offered to the analyst for the aim of confirmation of peak identity, as well as recurrent analysis on a column of a unique kind, various chemical treatment of the sample, and eventually the employment of multiple detection techniques so as to characterize any the solutes as they rinse from the column.

The main detectors in use nowadays for HPLC area unit the ratio (RI), ultraviolet illumination absorption, light detectors, and chemical science detector. The ultraviolet illumination absorption detector is out and away the foremost common detector in use in HPLC detection nowadays. Light-weight is directed through the sample stream eluting from finish of the column and also the quantity of sunshine absorbed by matter within the eluent is monitored. This light-weight is either a hard and fast wavelength or broadband wavelength. Not all analyte species absorb ultraviolet illumination radiation and thus the ultraviolet illumination detector isn't nearly as universal because the Ocean State detector.

5. Quantification:

Qualitative analysis mistreatment action is predicated on activity curves obtained from every of the substances analyzed. Activity is required altogether cases during which a sign associated with mass or concentration of a element in mixture, is obtained. Natural process take a look at technique use either external or internal standards for quantification.

5.1. External standard method

AN external commonplace technique is employed once the quality is analyzed on a separate recording from the sample. Quantification is predicated on a comparison of the height space or height (HPLC / GC) or spot intensity (TLC) of the sample thereto of a reference commonplace of the analyte of interest.

5.2. Internal standard method

With an indoor commonplace technique, compound of familiar purity that doesn't cause interference within the analysis is extra to the sample mixture. Quantification is predicated on the response quantitative relation of compound of interest to the inner commonplace vs. the response quantitative relation of an identical preparation of the reference commonplace (HPLC / GC). {This technique this system|this technique} is never used for TLC method.

5.3. Standard addition method

Once matrix interactions area unit found to be necessary, a regular addition technique might prove helpful. During this technique, a familiar amount of normal is extra to unknown compound. However it's not a lot of correct.

Though CDER doesn't specify whether or not the strategy use an indoor or external commonplace for quantification, it's usually discovered that HPLC technique for unleash and stability.

MATERIALS AND METHODS:

Nortriptyline-Sura labs, Pregabalin-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Anhydrous di hydrogen phosphate-Finar chemicals, Phosphate Buffer-Finar chemicals, Citric Acid-Finar chemicals.

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization:

Initially the mobile phase tried was Water: Methanol andACN: Phosphate BufferACN:Methanol with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 3), Methanol in proportion 30:70 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column ODS column, Zodiac column, and Xterra C18 column. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDAdetector 996 model.
Temperature	:	Ambient
Column	:	Phenomenex Luna C18 (4.6mm×250mm) 5 µm
Buffer	:	Phosphate buffer (pH-3)-Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000ml. Adjust the pH-3 by using ortho phosphoric acid.
pH	:	3.0
Mobile phase	:	Methanol: Phosphate Buffer pH-3 (70:30v/v)
Flow rate	:	1 ml per min
Wavelength	:	230 nm
Injection volume	:	10 µl
Run time	:	6 min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Phosphate buffer (pH-3.0):

Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust the pH-3 by using ortho phosphoric acid.

Preparation of mobile phase:

Accurately measured 700 ml (70%) of Methanol and 300 ml of Phosphate buffer pH-3 (30%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Mobile phase : Methanol: Phosphate Buffer pH-3 (70:30v/v)
Column : Phenomenex Luna C18 (4.6mm×250mm) 5 µm
Flow rate: 1 ml/min
Wavelength : 230 nm
Column temp: Ambient
Sample Temp: Ambient
Injection Volume: 10 µl
Run time : 4 minutes

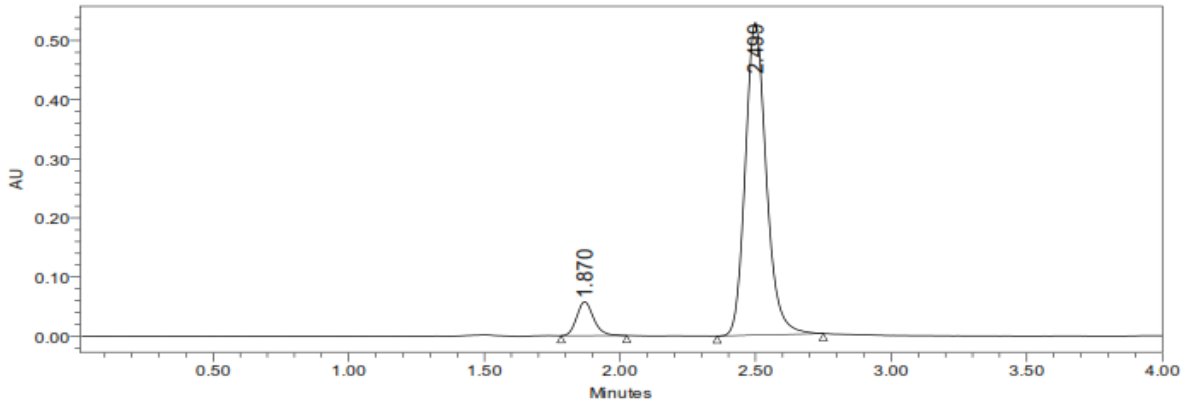

Figure-: Chromatogram for Trail-5

Table-: Peak Results for Trail-5

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Nortriptyline	1.870	5664027	299752			2314
2	Pregabalin	2.499	5033532	210321	4.6	1.3	2921

Observation:

From the above chromatogram it was observed that the Nortriptyline and Pregabalin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Assay (Standard):

Table-: Showing assay standard results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Nortriptyline	1.866	2762937	399854		1.3	2300.1	1
2	Pregabalin	2.496	2534375	210326	4.6	1.3	2937.7	1
3	Nortriptyline	1.866	2774613	386542		1.3	2344.7	2
4	Pregabalin	2.497	2526189	226741	4.7	1.3	3008.8	2

5	Nortriptyline	1.868	2776429	364121		1.3	2344.2	3
6	Pregabalin	2.498	2546248	231494	4.7	1.3	2990.7	3

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):

Table-: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Nortriptyline	1.870	2732203	294531		1.3	2314	1
2	Pregabalin	2.495	2507543	216321	4.6	1.3	2954	1
3	Nortriptyline	1.873	2751843	286473		1.3	2369	2
4	Pregabalin	2.499	2509101	216354	4.6	1.3	2944	2
5	Nortriptyline	1.874	2744776	312684		1.3	2329	3
6	Pregabalin	2.501	2515628	206571	4.6	1.3	2990	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Nortriptyline and Pregabalin in pharmaceutical dosage form was found to be 99.9% and 99.9% respectively.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY FOR Nortriptyline:

Linearity Results: (forNortriptyline)

S.No.	Linearity Level	Concentration(ppm)	Area
1	I	10	892464
2	II	20	1866364
3	III	30	2777423
4	IV	40	3709213
5	V	50	4601317
Correlation Coefficient			0.999

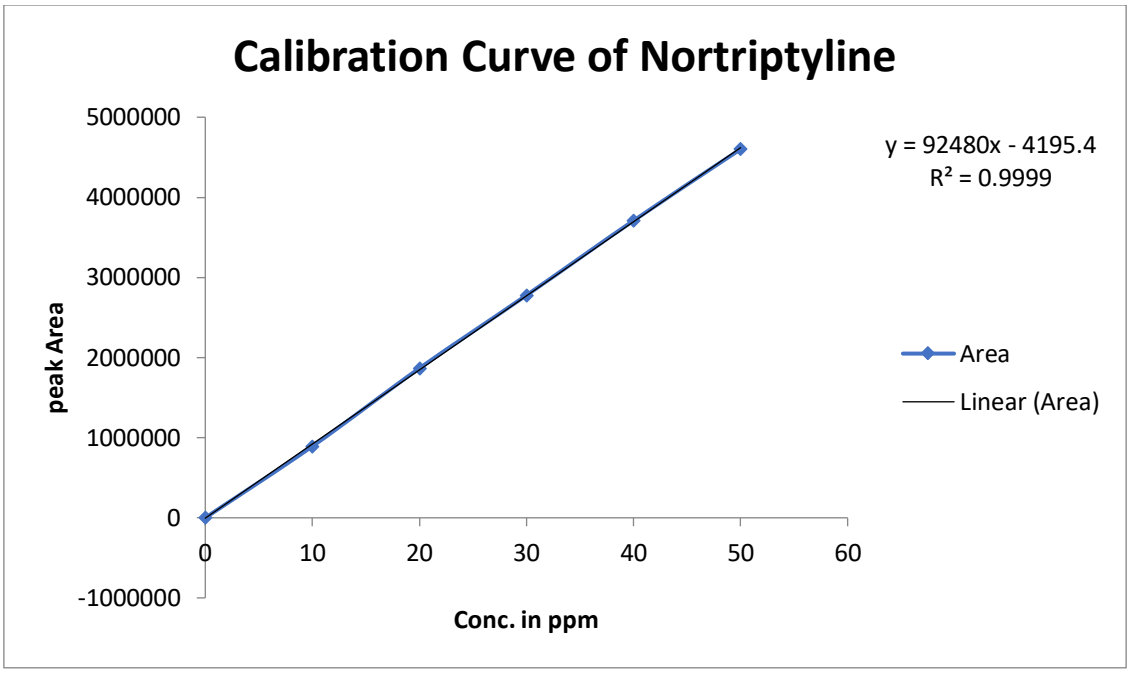

Fig: Chromatogram showing linearity level

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY FOR PREGABALIN:

S.No.	Linearity Level	Concentration(ppm)	Area
1	I	16	920032
2	II	32	1752782
3	III	48	2521426
4	IV	64	3326009
5	V	80	4217393
Correlation Coefficient			0.999

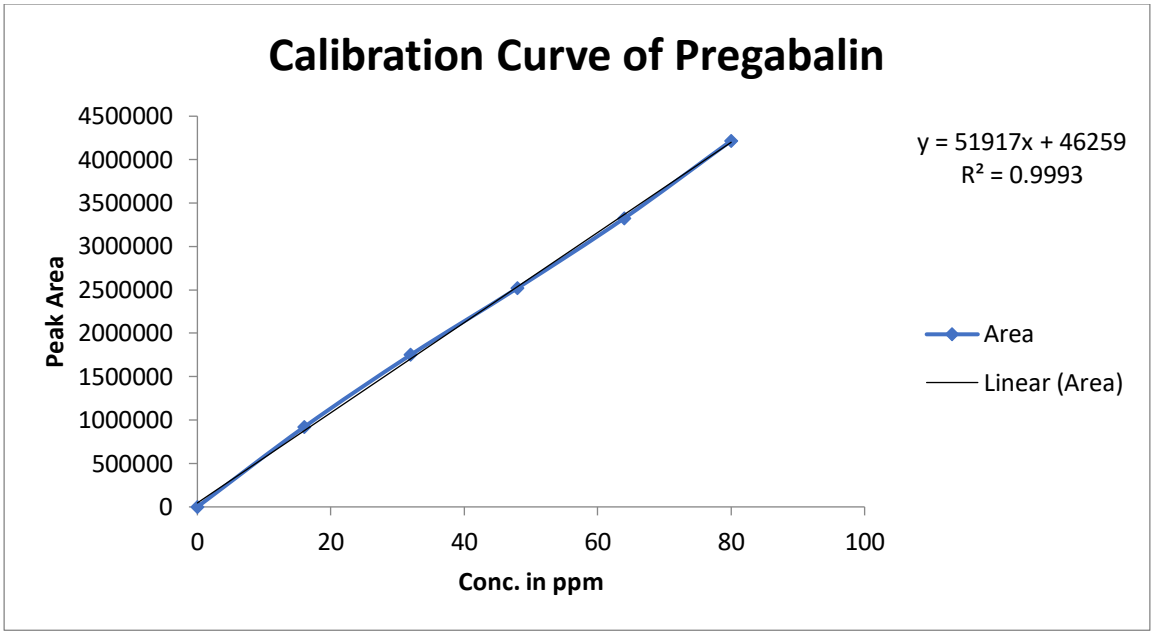

Fig:Chromatogram showing linearity level

Precision:

Table-: Results of method precession for Nortriptyline:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Nortriptyline	1.861	2766870	294578	2684	1.3
2	Nortriptyline	1.862	2771971	286541	2347	1.3
3	Nortriptyline	1.862	2771958	302657	2674	1.3
4	Nortriptyline	1.866	2780299	293412	2451	1.3
5	Nortriptyline	1.868	2789695	283154	2678	1.3
6	Nortriptyline	1.866	2766870	296759	2861	1.3
Mean			2774611			
Std. Dev			8873.946			
% RSD			0.3			

Table-: Results of method precession for Pregabalin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Pregabalin	2.490	2534539	193240	2761	1.3	4.7
2	Pregabalin	2.491	2539247	201647	2489	1.3	4.6
3	Pregabalin	2.492	2544661	193472	2367	1.3	4.6
4	Pregabalin	2.497	2548839	196475	2845	1.3	4.6
5	Pregabalin	2.498	2558822	201394	2347	1.3	4.7
6	Pregabalin	2.498	2534539	182641	2647	1.3	4.6
Mean			2543441				
Std. Dev			9415.761				
% RSD			0.3				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision/Ruggedness:

Table-: Results of Intermediate precision for Nortriptyline:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Nortriptyline	1.869	2781856	294651	2647	1.3
2	Nortriptyline	1.872	2761510	284123	2781	1.3
3	Nortriptyline	1.872	2748811	274561	2984	1.3
4	Nortriptyline	1.873	2790831	281241	2475	1.3
5	Nortriptyline	1.874	2785112	286471	2647	1.3
6	Nortriptyline	1.872	2781932	294512	2489	1.3
Mean			2775009			
Std. Dev			16222.05			
% RSD			0.5			

Table-: Results of Intermediate precision for Pregabalin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Pregabalin	2.497	2536301	211541	2495	1.4	4.6
2	Pregabalin	2.499	2541972	206141	2694	1.4	4.6
3	Pregabalin	2.500	2521259	198641	2785	1.4	4.7
4	Pregabalin	2.500	2537081	206741	2947	1.4	4.6
5	Pregabalin	2.500	2549869	209487	2742	1.4	4.6
6	Pregabalin	2.500	2536301	193481	2914	1.4	4.6
Mean			2537131				
Std. Dev			9370.087				
% RSD			0.3				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Table-: Accuracy (recovery) data for Nortriptyline:

%Concentration (at specification Level)	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	2771991	15	14.9	98%	99.1%
100%	5664027	30	29.99	99.9%	
150%	8337191	45	44.95	99.6%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

Table-: Accuracy (recovery) data for Pregabalin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2426681	24	23.9	98%	98.8%
100%	5033532	48	47.92	99.2%	
150%	7419721	72	71.9	99.3%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table-: System suitability results forNortriptyline:

S.No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2231.8	1.3
2	1.0	2344.7	1.3
3	1.1	2071.6	1.3

***** Results for actual flow (1.0 ml/min) have been considered from Assay standard.

Table-: System suitability results forPregabalin:

S.No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2953.6	1.3
2	1.0	3008.8	1.3
3	1.1	2704.0	1.3

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

Variation of mobile phase organic composition:

Table-: System suitability results for Nortriptyline

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing

1	10% less	2867.2	1.2
2	*Actual	2344.7	1.2
3	10% more	2347.8	1.2

Table-: System suitability results for Pregabalin:

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3336.1	1.2
2	*Actual	3008.8	1.2
3	10% more	3010.3	1.2

***** Results for actual mobile phase have been considered from Assay standard.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Nortriptyline and Pregabalin was done by RP-HPLC. The Phosphate buffer was pH-3 and the mobile phase was optimized with consists of Methanol: Phosphate buffer (pH-3) mixed in the ratio of 70:30% v/v. A Phenomenex Lunacolumn C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1 ml/min. The linearity range of Nortriptyline and Pregabalin were found to be from 10-50µg/ml, 16-80µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 99.9-99.9% of Nortriptyline and Pregabalin.LOD and LOQ were found to be within limits.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method was found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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