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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NORTRIPTYLINE AND PREGABALIN PHARMACEUTICAL DOSAGE FORM BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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Abstract:

A simple and selective LC method is described for the determination of Pregabalin and Nortriptyline in tablet dosage forms. Chromatographic separation was achieved on a C18 column using mobile phase consisting of a mixture of 50 volumes of methanol and 50 volumes of phosphate buffer with detection of 255 nm. Linearity was observed in the range 60-140 µg /ml for Pregabalin (r2 =0.997) and 3-7µg /ml for Nortriptyline (r2 =0.995) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Pregabalin and Nortriptyline drugs was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

KEYWORDS: Pregabalin , Nortriptyline , Chromatographic separation , Drug development.

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INTRODUCTION:

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities ,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.

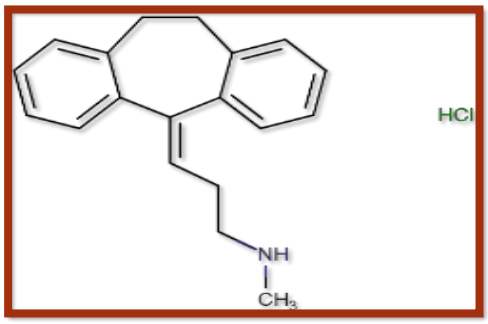
By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product meet two critical requirements.

1. Established identity and purity.
2. Established bio availability/dissolution.

DRUG PROFILE-NORTRIPTYLINE

- **NAME**: Nortriptyline
- **CATEGORY**: Tricyclic antidepressant(TCA)
- **CHEMICAL FORMULA**: $C_{29}H_{32}O_{13}$
- **IUPAC NAME:** methyl(3-{tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaen-2- ylidene}propyl) amine hydrochloride
- **USES**: It is useful in treatment of major depression

STRUCTURE:



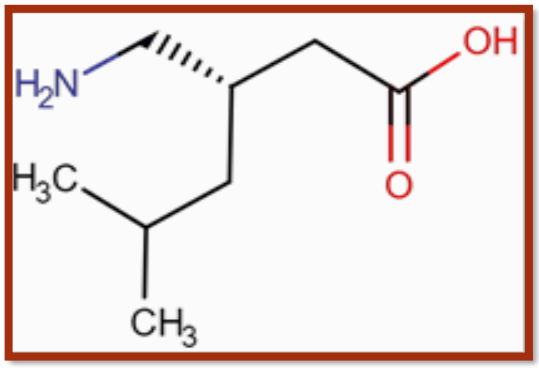
- It is also used for chronic pain
- It is used to treat bedwetting in children's
- It is used to treat nerve pain.
- **WEIGHT:**
- **Average**: 299.838 g/mol
- **Monoisotopic:** 299.144077416 Da
- **SOLUBILITY:**
- Soluble in organic solvents such as methanol
- Very slightly soluble in phosphate buffer.

DRUG PROFILE- PREGABALIN

NAME: Pregabalin

- CATEGORY: Anticonvulsants

STRUCTURE:



CHEMICAL FORMULA:C8H17NO2

- IUPAC NAME: (3S)-3-(aminomethyl)-5-methylhexanoic acid
- WEIGHT:
- Average: 159.2261 g/mol
- Monoisotopic: 159.125928793 Da
- SOLUBILITY:
- Soluble in acetonitrile,
- Sparingly Soluble in methanol.
- USES:It is used to relive neuropathic pain
- It is also used to treat epilepsy and anxiety
- It is used to treat neuropathic pain condition and fibromyalgia
- It is used for the partial onset seizures in combination with other anticonvulsants

AIM & PLAN OFWORK
AIM

To develop a new HPLC method for the simultaneous estimation of Pregabalin and Nortriptyline in

pharmaceutical dosage form.

PLAN OF WORK

Solubility determination of Pregabalin and Nortriptyline various solvent and buffers.
Determine the absorption maxima of both the drug in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
Optimize the mobile phase and flow rates for proper resolution and retention times.
Validate the developed method as per ICH guidelines.

INSTRUMENTS USED

REAGENTS USED

UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer Software	Vision Pro
HPLC SOFTWARE	Spin chrome (LC SOLUTIONS)
HPLC	Shimadzu LC Solutions
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	INERTSILcolumn,C18(150x4.6 ID) 5µm

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Phosphate	AR Grade
Acetonitrile	HPLC Grade
Disodium hydrogen phosphate	AR Grade

DRUGS USED

Nortriptyline and pregabalin	Chandra labs, Prashnathi nagar, kukatpally, Hyd
Nortriptyline and pregabalin (10mg/75mg)	Obtained from local pharmacy (PREGABID-NT)

RESULTS:

Solubility Studies

These studies are carried out at 25 0 C

Nortriptyline: Soluble in organic solvents such as methanol.Very slightly soluble in phosphate buffer.
Pregabalin: Soluble in acetonitrile,sparingly soluble in methanol

Determination of Working Wavelength (λmax)

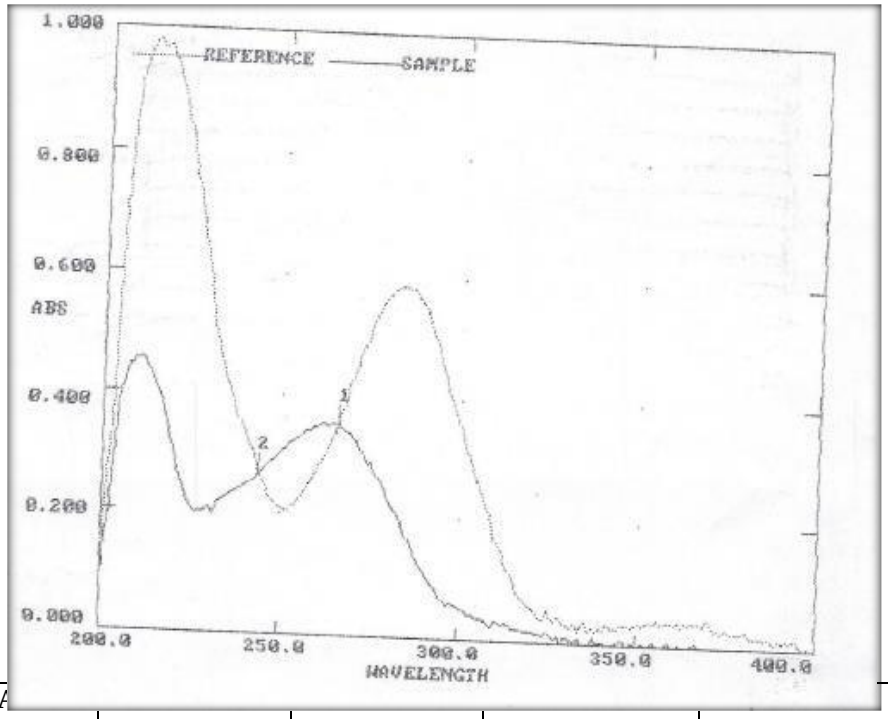
In estimation of drug wavelength maxima is used.

Preparation of mixed standard solution. :. Weighed accurately 100 mg of PREGABALIN and 5 mg of NORTRIPTYLINE in 100 ml of volumetric flask and dissolved in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 5µg/ml of NORTRIPTYLINE and 100 µg/ml of PREGABALIN is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram

Results:

The wavelength of maximum absorption (λmax) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig and the absorption curve shows characteristic absorption maxima at 248 nm for NORTRIPTYLINE, 229 nm for PREGABALIN and 255nm for the combination.

Observation: λmax was found to be 248 nm for Nortriptyline and 229nm Pregabalin.



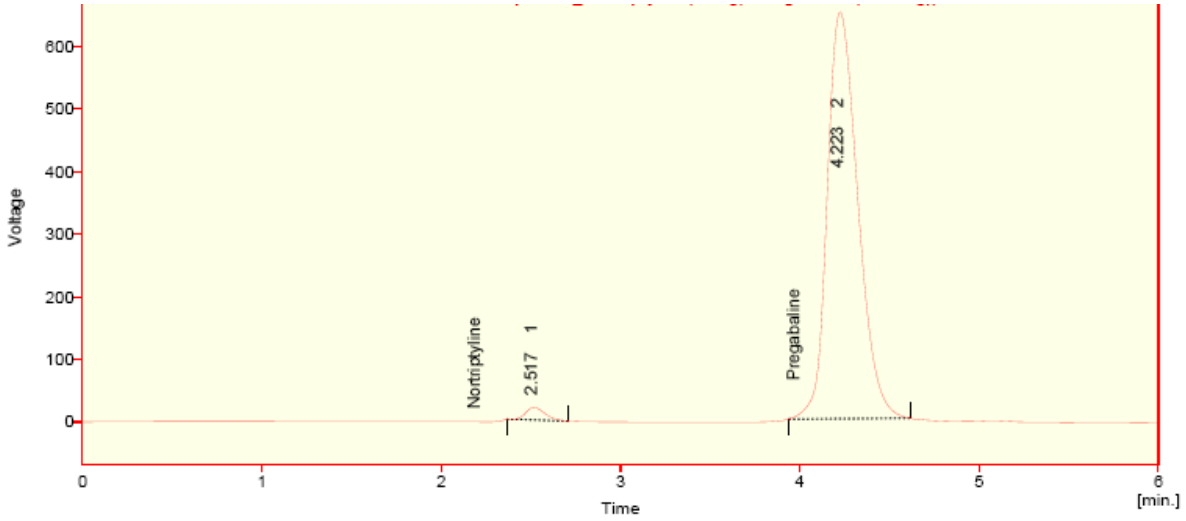
CHROMATORA CONDITIONS					TRIAL 5
MOBILE PHASE	Water: Methanol: CAN	OPA(pH3.5) :buffer: Methanol	Buffer: ACN: Mixed phosphate	KH2PO4buffer: methanol	Methanol: KH2PO4
RATIO	60:20:20	65:35	50:50	60:40	50:50
COLUMN	InertsilODS , 3V(250×4.6 ×5µ)	InertsilODS, 3V(250×4.6 ×5µ)	InertsilODS, 3V(250×4.6× 5µ)	InertsilODS, 3V (250×4.6× 5µ)	InertsilODS, 3V(250×4.6× 5µ)
WAVELENGTH	255nm	255nm	255nm	255nm	255nm
FLOW RATE	1ml/min	1 ml/min	1ml/min	1ml/min	1ml/min
pH	3.0	3.5	6.8	4.5	4.0
Run time	5 min	10 min	11 min	8 min	5 min
Injection volume	20µl	20µl	20µl	20µl	20µl
Temperature	30°C	30°C	30°C	30°C	30°C

OPTIMISED CHROMATOGRAPHIC CONDITIONS

Mobile phase	Methanol: KH2PO4
Ratio	50:50
pH	4.0
Column	INERTSIL ODS 3Vcolumn,C18(250x4.6x5µ)
Flow rate	1.0 ml/min
Column temperature	Room temperature
Sample temperature	Room temperature
Wavelength	255 nm
Injection volume	20 µl
Run time	5min
Retention time	About 2.517 min for PREGABALIN and 4.223 min for NORTRIPTYLINE

Therefore, trial 5 shows similar optimum conditions as required for the tailing of peak with retention time of 2.517 min for Pregabalin and 4.233 min for Nortriptyline at wavelength 255 nm.

Optimized chromatogram:



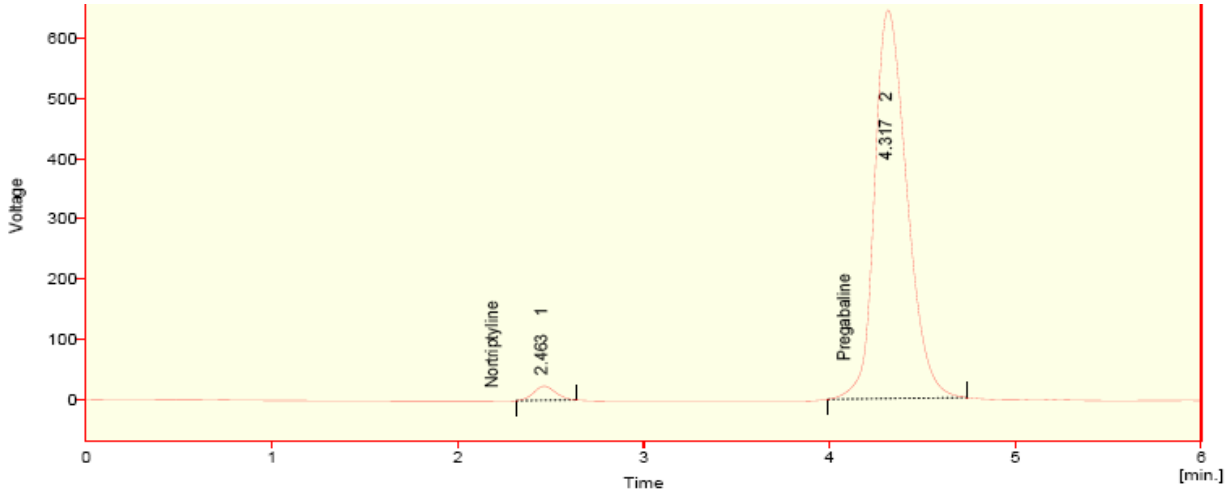
ASSAY:

Preparation of mixed standard solution

 Weigh accurately 100 mg of PREGABALIN and 5 mg of NORTRIPTYLINE in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100 µg/ml of PREGABALIN and 50 µg/ml of NORTRIPTYLINE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution:

 10 tablets (each tablet contains PREGABALIN-100 mg and NORTRIPTYLINE-5mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of PREGABALIN and NORTRIPTYLINE (150µg/ml) were prepared by dissolving weight equivalent to 100 mg of PREGABALIN and 5 mg of NORTRIPTYLINE and dissolved in sufficient mobile phase.



	PREGABALIN		NORTRIPTYLINE	
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	3941.191	3933.444	378.411	372.761
Injection-2	3925.782	3930.759	67.951	371.408
Injection-3	3941.042	3936.783	375.523	370.373
Injection-4	3925.782	3920.484	550.591	541.451
Injection-5	3941.191	3945.931	384.450	378.411
Average Area	3937.618	3933.479	411.385	406.880
Standard deviation	0.824		0.752	
%RSD	2.0		0.9	
Assay(%purity)	**99.89**		**100.9**	

Observation :

The amount of PREGABALIN and NORTRIPTYLINE present in the taken dosage form was found to be 99.89% and 100.9% respectively.

VALIDATION

System suitability:

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated

Results for system suitability of PREGABALIN

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.463	3945.931	4476	1.765
2	2.453	3943.148	4439	1.611
3	2.460	3941.042	4828	1.611
4	2.450	3925.782	4789	1.556
5	2.443	3941.191	4763	1.706
Mean	2.4538	3939.419	-	-
SD	0.007981	0.78	-	-
%RSD	**0.02**	**1.00**	-	-

Results for system suitability of NORTRIPTYLINE

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	4.303	378.411	7125	1.481
2	4.257	367.951	6971	1.577
3	4.237	375.523	6906	1.393
4	4.427	550.591	3613	1.781
5	4.383	384.450	6998	1.556
Mean	4.3214	411.3852	-	-
SD	0.081479	0.078	-	-
%RSD	**0.2**	**1.00**	-	-

Observation:

The % RSD for the retention times and peak area of PREGABALIN and NORTRIPTYLINE were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Specificity:

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Observation:

It is observed from the above data, diluent or excipient peaks are not interfering with the PREGABALIN and NORTRIPTYLINE peaks

Linearity and range:

Linearity is the ability of the method to obtain results which are proportional to the concentration in the sample and Range is the concentration interval within the method perform suitable performance.

Preparations	Volume from standard Stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				PREGABALIN	NORTRIPTYLINE
Preparation 1	0.6	0.3	10	60	3
Preparation 2	0.8	0.4	10	80	4
Preparation 3	1.0	0.5	10	100	5
Preparation 4	1.2	0.6	10	120	6
Preparation 5	1.4	0.7	10	140	7

Acceptance criteria:

The relationship between the concentration of PREGABALIN and NORTRIPTYLINE and area of PREGABALIN and NORTRIPTYLINE should be linear in the specified range and the correlation should not be less than 0.99.

Observation:

The correlation coefficient for linear curve obtained between Concentration vs Area for standard preparations of PREGABALIN and NORTRIPTYLINE is 0.9997 and 0.9961. The relationship between the concentration of PREGABALIN and NORTRIPTYLINE and area of PREGABALIN and NORTRIPTYLINE is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

PARAMETER	OBSERVED VALUE	ACCEPTANCE CRITERIA
LINERAITY	Pregabalin : r2 =0.9997 Nortriptyline: r2 =0.9961	r2 should be < 1
RANGE	Pregabalin:60-140µg/ml Nortriptyline:3-7µg/ml	Linear in specified range
ACCURACY	% Recovery of Pregabalin:101.55% % Recovery of Nortriptyline:112.7%	%Recovery of Pregabalin and Nortriptyline should lie between 98% and 120%
PRECISION	%RSD of Pregabalin:0.14 %RSD of Nortriptyline:0.013549	%RSD not more than 2%
LIMIT OF DETECTION	LOD of Pregabalin:2.35µg/ml LOD of Nortriptyline:1.21µg/ml	< 20% of LOQ
LIMIT OF QUANTIFICATION	LOQ of Pregabalim:7.148µg/ml LOQ of Nortriptyline:3.68µg/ml	≤ 10%
RETENTION TIME	%RSD of Retention time(pregbalin): 0.02 %RSD of Retention time(Nortriptyline): 0.2	%RSD of Retention time not more than 2%
RUGGEDNESS	%RSD of Pregabalin:0.14 %RSD of Nortriptyline:0.013549	%RSD of Assay values between 2 analyst should not be more than 2%

CONCLUSION:

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Pregabalin and Nortriptyline drugs was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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