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DEVELOPMENT OF SIAM FOR NORTRIPTYLINE AND PREGABALIN BY RP-HPLC : RESEARCH ARTICLE

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Abstract:

Pregabalin (Preg) and Nortriptyline hydrochloride (Nor) were determined in tablet dosage form using simple, rapid, specific, accurate, economical, and precise RP-HPLC techniques that were developed and validated as per ICH guidelines. BDS Hypersil C18 column (150mm, 4.6mm, and 2.5µm) and buffer: methanol pH4.2 0.1% (80:20v/v) as mobile phase was utilized to separate the samples using chromatography, which was detected by UV light at 235 nm. Pregabalin and Nortriptyline hydrochloride were exposed to acid-base hydrolytic, photolytic, and oxidative stress conditions as part of trials to force degradation of the drugs. Linearity, accuracy, precision, LOD, and LOQ of the method have all been validated. The retention time for Pregabalin and Nortriptyline hydrochloride were found to be 2.281 min and 7.073 min respectively.

Keywords : Nortriptyline, Pregabalin, Method development, Validation, Quality by design, Force degradation

Introduction:

Analysis is essential for every product or service, but it's extremely vital for drugs because they influence people's health.[1] Analytical chemistry is the study of the separation, quantification, and identification of chemical additives in synthetic and herbal materials comprised of one or more chemicals or components. The two main categories of analytical chemistry are qualitative evaluation and quantitative evaluation. Qualitative evaluation refers to the identification of the chemical additives present in the sample. Quantitative evaluation determines the quantity of positive detail or compound modern in the substance, i.e. the sample.[2] Annually, there are more drugs introduced to the market. These pharmaceuticals might also be label things or slight structural improvements made to the ones we already have. Medicines should be available in a way that guarantees their quality, bioavailability, adequate plasma concentration, desired period, commencement of action, optimum dose, safety, effectiveness, and stability during product storage.[3]

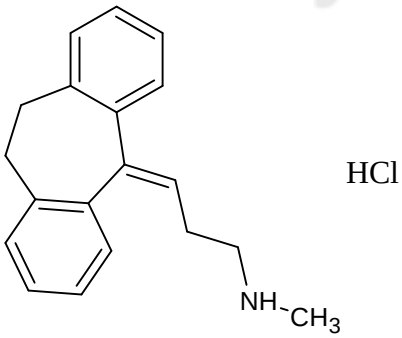
The components of a mixture are separated, rendered separate from one another, and measured using a technical method called high liquid chromatography (HPLC; formerly known as high-weight liquid chromatography). When a pressurized liquid dissolvable having the example mixture is present, pumps are needed to move a segment containing a powerful adsorbent component. (4) In the example, each component has a slightly different interaction with the adsorbent compound, which leads to differential stream rates for the individual segments and the partition of the components as they depart the section.(5)

Because HPLC works at significantly higher pressures (50–350 bar) than traditional (or "low pressure") liquid chromatography, which usually rely on gravity to move the mobile phase along the column, HPLC differs from conventional (or "low pressure") liquid chromatography. (6) Typical column dimensions range from 2.1 to 4.6 mm in diameter and 30 to 250 mm in length while analytical HPLC generally separates a small amount of substance. Reversed phase HPLC (RP-HPLC) uses a watery, tolerably polar stationary phase and a non-polar non-polar stationary phase. For example, silica that has been surface-adjusted with RMe2SiCl, where R is a straight chain alkyl group, as C18H37 or C8H17, is one basic stationary phase. (7)

Nortriptyline Hydrochloride

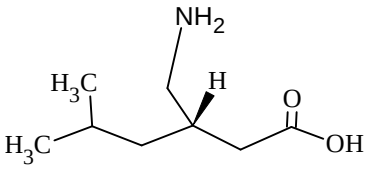
Nortriptyline Hydrochloride which is chemically known as 3-(10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5-

ylidene)-N-methyl-1-propanamine hydrochloride belongs to a general class of tricyclic antidepressant drugs. (8)



Pregabalin

Pregabalin (PRE) comes under the class of anticonvulsant in medical terminology. It decreases the number of pain signals that are sent by damaged nerves in the human body thereby relieving the pain. It is chemically (S)-3- (amino methyl)-5-methylhexanoic acid.[9]



The tablet containing the combination of NOR and PRE is used effectively as an antidepressant, anticonvulsant and to overcome neuropathic pain. [6]

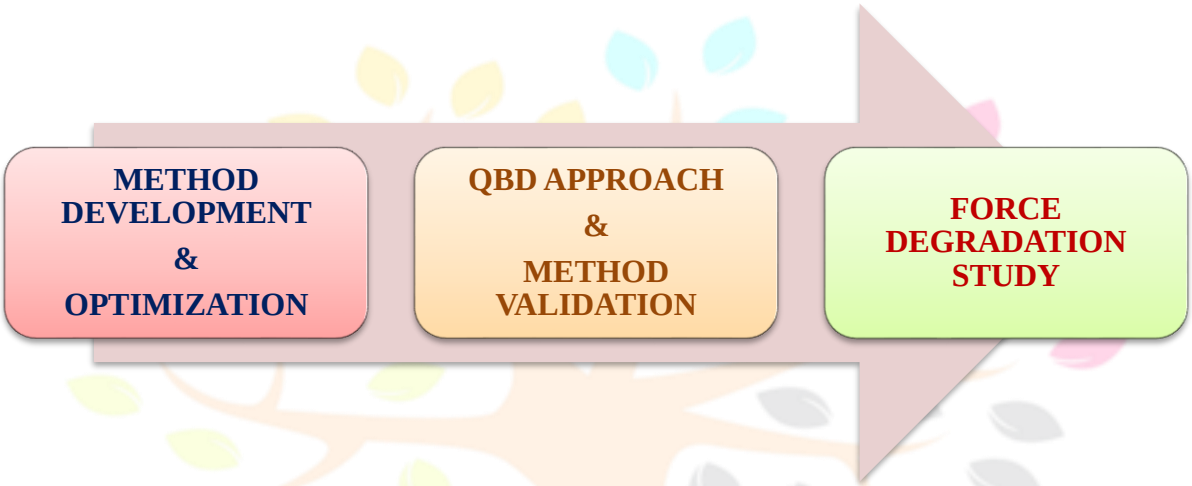


Figure.1 Analytical method development

A. METHOD DEVELOPMENT AND OPTIMIZATION [10]:

In the initial stages of method development, preparations of the solutions in amber flasks should be composed until it is proven that the active component is stable at room temperature and does not degrade under conventional laboratory conditions. Filtration of the sample is necessary; it is typically proposed to use filters with pore sizes of 0.22 or 0.45 m to remove particles.

➢ Materials and Instruments

Table.no 01

Sr.no.	Materials	Suppliers & version
1	Nortriptyline & Pregabalin	Swapnroop Drugs & Pharmaceuticals, Aurangabad
2	Acetonitrile (HPLC grade)	Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai
3	Methanol	Merck
4	Water	Merck

➢ **Instruments & Version**

Table.no02

Sr.no.	Name of Instrument	Brands/ Version
1	HPLC	Agilent 1100with auto sampler (Chemstation software 10.04)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C18)	Agilent C18 (150mmX 4.6mm,2.5µm)
4	pH meter	VSI pH meter (VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance.
6	Sonication	Ultrasonic electronic instrument

➢ **Preparation of Stock Solution**

5mg of Nor & 37mg of Preg were dissolved in 10ml of methanol which gives concentration of stock solution was 500:3700 respectively.

➢ **Preparation of Standard Solution**

Pipette out 0.2ml from stock solution and dilute with 90ml methanol & 10ml water to give 100ml solution.

Method optimization – After achieving the required separations and sensitivity, the experimental conditions should be optimised. Stability indicating assay experimental conditions will be accomplished through planned or systematic assessment of factors such as ph (if ionic), mobile phase components and ratio, gradient, flow rate, temperature, sample amounts, injection volume, & diluents solvent type.

B. QBD APPROACH & METHOD VALIDATION:[(11)]

Quality by design (QbD) has been an important concept in the pharmaceutical industry since it was endorsed by the US Food and Drug Administration. The concept of quality by design (QbD) has become more significant recently owing to the application of the design of experiments technique. The object of QbD is to identify the key variables and the results of their interactions through a desired set of tests.

Method validation – The process by which it is verified, by laboratory studies, that the performance characteristics of the procedure fulfill the criteria for its intended objectives is known as validation of an analytical procedure. According to ICH guidelines, all analytical techniques intended for use in the analysis of clinical samples must be validated. [(12)]

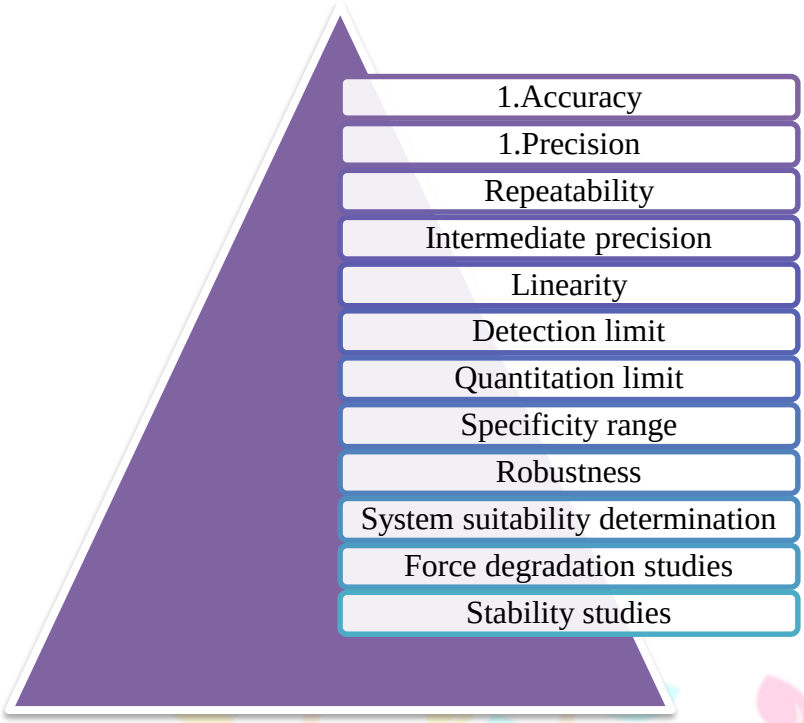


Figure.2 Analytical Validation parameters

➢ **Standard Sample Preparation**

I. Stock solution – Weighed Std Pregabalin of 37mg & 5mg of Nortriptyline and dissolved in 10ml of Methanol. Stock solution contains 3700µg/ml of Pregabalin & 500µg/ml of Nortriptyline.

a) Take 0.1ml from stock solution and volume make up with mobile phase (79ml water + 21ml methanol). Solution contains 37µg/ml of Pregabalin & 5µg/ml of Nortriptyline.

b) Take 0.2ml from stock solution and volume make up with mobile phase (79ml water + 21ml methanol). Solution contains 74µg/ml of Pregabalin & 10µg/ml of Nortriptyline.

c) Take 0.3ml from stock solution and volume make up with mobile phase (79ml water + 21ml methanol). Solution contains 111µg/ml of Pregabalin & 15µg/ml of Nortriptyline.

d) Take 0.4ml from stock solution and volume make up with mobile phase (79ml water + 21ml methanol). Solution contains 148µg/ml of Pregabalin & 20µg/ml of Nortriptyline.

e) Take 0.ml from stock solution and volume make up with mobile phase (79ml water + 21ml methanol). Solution contains 185µg/ml of Pregabalin & 25µg/ml of Nortriptyline.

C. FORCE DEGRADATION STUDY:

Drug substances and pharmaceuticals can degrade under controlled circumstances as part of a forced degradation technique. In the interest of assessing how stable a molecule is, degradation produces byproducts. According to the ICH guidelines, stress testing is intended to identify possible degradation products that can be used to analyze a molecule's inherent stability, establish probable degradation pathways, and validate the reliability of the stability indicating techniques. [13]

➢ **Preparation of solutions for force degradation study**

1) 0.1N HCL- Take 0.2 ml Sample from Stock (API) and add 05 ml 0.1N HCL and make up volume with diluent, after 1 - 2 Hr take HPLC reading (Before inject neutral sample).

2) 0.1 N NaOH - Take 0.2 ml Sample from Stock (API) and add 05 ml 0.1N NaOH and make up volume with diluent, after 1 - 2 Hr take HPLC reading (Before inject neutral sample).

3) 3 % H2O2 - Take 0.2 ml Sample from Stock (API) and add 05 ml 3%H2O2 and make up volume with diluent, after 1-2 Hr take HPLC reading (Before inject neutral sample).

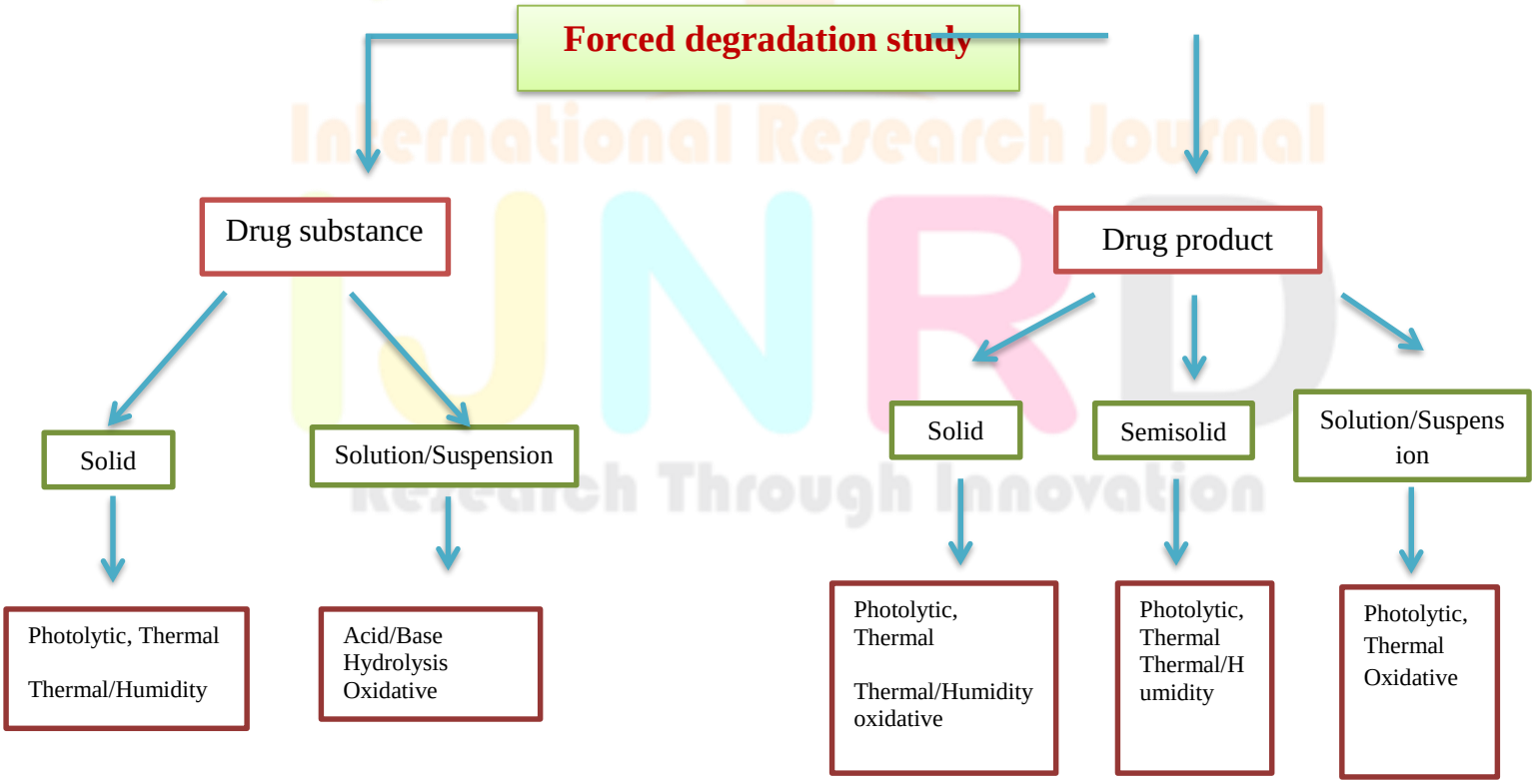
4) NEUTRAL - Take 0.2 ml Sample from Stock (API)and add 05 ml Water and make up volume with diluent, after 1-2 Hr take HPLC reading.

➢ **Strategy for selection of degradation conditions** [14,15]

The stability indicating method, a precise and validated method, has been established to identify the precise concentration of the drug's active ingredient without interference and to accomplish this after degradation. Stability testing reveals an array of degradation mechanisms, probable degradation products, pathways defining likely drug degradation, and interactions between the drug and excipients in the drug product.

➢ **Stability Testing of New Drug** [16]

A general protocol of degradation conditions used for drug substance and drug product is shown in Scheme 1.



Results & Discussion:

Table.no 03: Optimized Chromatographic Conditions

Parameters	Conditions
Mobile Phase	Buffer (pH 4.2 0.1%): Methanol (80:20v/v)
Stationary Phase	BDS hypersil C18 column (150mm, 4.6mm, and 2.5µm)
Flow rate (ml/min)	0.7 ml/min
Run Time (min)	8 min
Injection Volume (µl)	20 µl
Detection Wavelength(nm)	235nm
Retension Time (min)	Pregabalin: 2.281min Nortriptyline: 7.073min

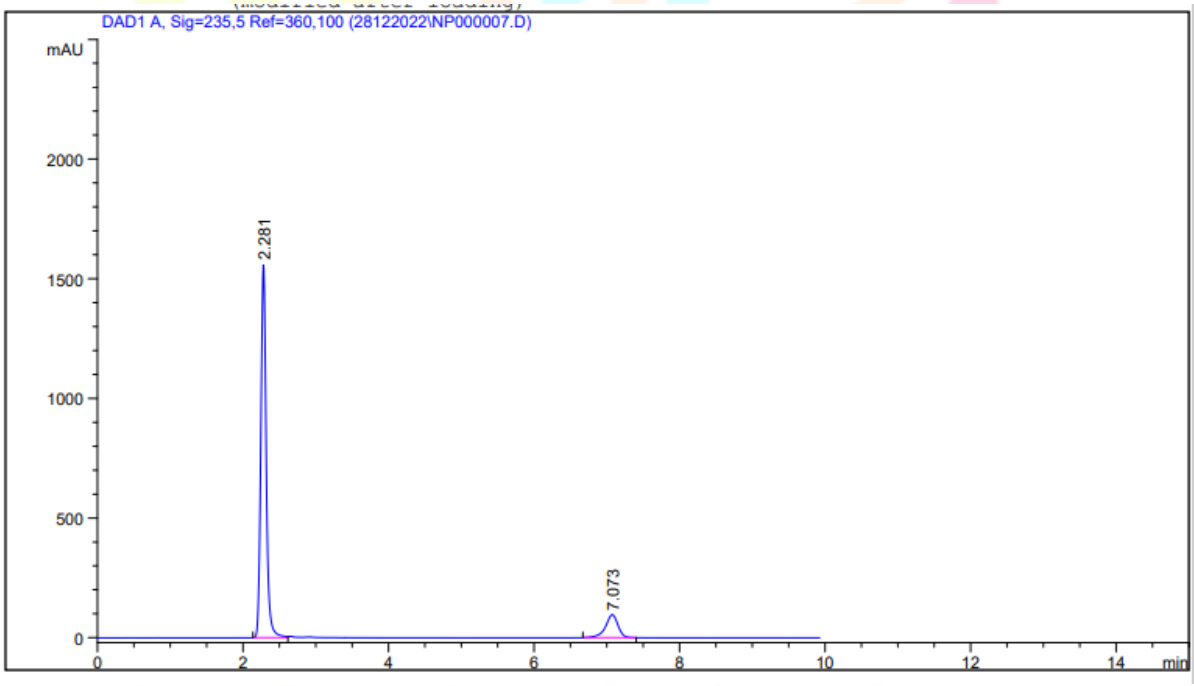


Figure:3 Chromatogram of Preg and Nor HCl in Buffer (pH 4.2 0.1%): Methanol (80:20 v/v) (Flow rate-0.7 ml/min)

Table.no 04: Analytical validation parameters (system suitability and linearity)

Parameter	NOR	PREG
Linearity	5-25 µg/ml	37-185 µg/ml
Slope	32	48.05
Intercept	28.33	242.4
Coefficient of correlation	0.9999	0.9999
LOD	0.0408 µg/ml	0.8790 µg/ml
LOQ	0.1236 µg/ml	2.6663 µg/ml
Theoretical Plate	9225	5048
Tailing Factor	0.89	1.19

Table. no 05 Results & Statistical data for Forced Degradation for PREG

Sr. no,	Degradation	Area of degrades sample	Degrade up to %	Actual % Degradation
1	Acid	4843.46	86.15	13.85
2	Base	4754.95	84.57	15.43
3	H2O2	5279.91	93.91	6.09
4	Neutral	5595.17	99.52	0.48

Table. no 06 Results & Statistical data for Forced Degradation for NOR

Sr. no,	Degradation	Area of degrades sample	Degrade up to %	Actual % Degradation
1	Acid	444.7177	87.51	12.49
2	Base	464.7919	91.46	8.54
3	H2O2	502.5424	98.89	1.11
4	Neutral	504.8736	99.35	0.65

Conclusion:

The method has been validated and it has been proven that it is reliable, linear, accurate, and precise, and that it is robust with small variations in chromatographic parameters. The current research study provides a simple and validated HPLC stability indicating method for the simultaneous assessment of NOR and PRE in the presence of degradation products. The fact that the developed method was specific and reliability indicating is shown by the well-separated peaks of the degradation products that are produced when a drug is exposed to stress conditions. Hence, the described method can be used to accurately identify marketed formulations that contain NOR and PRE.

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