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RP HPLC Method Development of PREGABLIN in Bulk, Dosage Form and Validation Parameters

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Research Article

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ABSTRACT

This article explains about the method development process of pregabalin and the finalized optimized method and also gives a detailed note on parameters used for the validation of pregabalin. Validation parameters are the key things to be checked to access the method quality and liability. In this research we have validated 11 parameters and the statistical analysis.

INTRODUCTION

Only a few HPLC estimations are reportable within the literature for the determination of pregablin in bulk & indefinite quantity forms. The objective of this experiment was to optimize the assay methodology for pregablin supported the literature survey created [1-4]. There's no official pharmacopeias for pregablin, thus here the trial mentioned describes however the improvement was done.

Instrumentation used for the study: Agilent HPLC instrument

Reagents used for the study:

- Ortho phosphoric acid : AR grade
- Acetonitrile : HPLC grade
- Water : Milli-Q grade
- Methanol : HPLC grade
- Diammonium hydrogen phosphate: ARgrade

METHOD DEVELOPMENT

After 4 serial trails by changing the chromatographic conditions and mobile phase compositions one optimized method was finalized for the development of pregabalin [5-10].

The 4 trails include the following:

- Trial: 1

Mobile phase-A : Methanol (50%)
Mobile phase-B : Water (50%)

Chromatographic conditions:

Flow rate : 1.0 ml/min
Column : Develosil C18, 250 × 4.6 mm, 5 µ
Detector wave length : 210 nm
Column temperature : Ambient
Injection volume : 20 µl
Run time : 5 min
Diluent:buffer : methanol:water

There is no appearance of pregabalin peak.

- Trial: 2

Buffer preparation: Accurately take 5.28 g diammonium hydrogen phosphate to a 1000 ml volumetric flask containg water, make up to volume with water and mix , filter through 0.45 µm nylon membrane filter and degas. Adjust its pH to 6.5 ± 0.05 with phosphoric acid (85%) [6-15].

Mobile phase-A : Buffer (80%)
Mobile phase-B : Methanol (10%)
Mobile phase-C : Acetonitrile (10%)

Chromatographic conditions:

Flow rate : 0.8 ml/min
Column : Develosil C18, 250 × 4.6 mm, 5 µ
Detector wave length : 210 nm
Column temperature : Ambient
Injection volume : 20 µl
Run time : 5 min
Diluent:buffer : methanol:acetonitrile

Retention time was found to be 4.12

The retention time was too long for pregablin and the peak is non-symmetric

- Trial: 3

The buffer preparation is similar to that of Trial: 1.
Mobile phase-A : Buffer (80%)
Mobile phase-B : Methanol (10%)
Mobile phase-C : Acetonitrile (10%) [15-19]

Chromatographic conditions:

Flow rate : 1 ml/min
Column : Develosil C18, 250 × 4.6 mm, 5 µ
Detector wave length : 210 nm
Column temperature : Ambient
Injection volume : 20 µl
Run time : 5 min
Diluent:buffer : methanol:acetonitrile

The retention time was found to be 3.31
The peaks lost their symmetry and they do not pass tailing factor.

- Trial: 4

The buffer preparation is similar to that of Trial: 1.

Mobile phase-A : Buffer (60%)
Mobile phase-B : Methonol (20%)
Mobile phase-C : Acetonitrile (20%)

Chromatographic conditions:

Flow rate : 1.0 ml/min
Column : inertsil ODS 3 V, 250 × 4.6 mm, 5 µ
Detector wave length : 210 nm
Column temperature : Ambient
Injection volume : 20 µl
Run time : 5 min
Diluent:buffer : Methanol:acetonitrile

The retention was found to be 3.23
The retention time was good for pregabalin but the peak is non-symmetric.

Final optimized method include the following

Chromatographic conditions:
Flow rate : 1.2 ml/min
Column : inertsil ODS 3 V, 250 × 4.6 mm, 5 µ
Detector wave length : 210 nm
Column temperature : Ambient
Injection volume : 20 µl
Run time : 5 min
Diluent: Buffer : Methanol: acetonitrile

Mobile phase-A : Buffer (60%)
Mobile phase-B : Methanol (20%)
Mobile phase-C : Acetonitrile (20%)

The retention time was found to be 2.73

It shows the good peak with good symmetry. Hence this method was finalized for the development of pregablin.

Standard preparation
Weigh accurately regarding 30 mg of pregablin normal in to 20 ml meter flask, add 15 ml of dilutant and sonicate to dissolve and additional frame volume with dilutant [19,20].

Test preparation
Weigh and transfer capsule powder adequate 150 mg of pregablin into 100 ml meter flask add 60 ml of thinner and sonicate to dissolve for concerning 15 min and conjure the quantity with thinner. Additional filter the answer through 0.45 metric linear unit filter [21-26].

Buffer preparation
Accurately take five 0.28 g diammonium H phosphate to a 1000 ml volumetrical flask containg water, compose to volume with water and blend, filter through zero.45µm nylon membrane filter and take away. Modify its hydrogen ion concentration to six. 5 ± 0.05 with orthophosphoric acid (85%) [26-30].

Procedure
Flush the HPLC system completely with water followed by wood alcohol [31].
Equilibrate column for not less than 30 min with initial mobile part at a rate of one 2 ml/min.
Inject 20 µl of blank, 5 times of normal preparation, and every sample preparation in duplicate into the action system. Record the chromatograms and live the height responses [32-35].

Calculation

Calculate the amount of each drug by using the following formula

$$\text{Pregablin (mg/tablet)} = \frac{Ap}{ASp} \times \frac{DS}{DT} \times \frac{P}{100}$$

Where
AT=Average area counts of injections for pregablin peak in the chromatogram of sample solution.
ASp=Average area count of five replicate injections for pregablin.

Peak in the chromatogram of standard solution

DS=Dilution factor of standard solution (weight dilution).
DT=Dilution factor of sample solution.
P=Percentage purity of working standard used.

$$\% \text{ Labeled Amount} = \frac{\text{Content of each drug (mg/tablet)}}{\text{Label claim, in mg}} \times 100$$

METHOD VALIDATION

Method validation is often outlined as (ICH) "Establishing documented proof that provides a high degree of assurance that a selected activity can systematically manufacture a desired result or product meeting its preset specifications and quality characteristics" [36-38]. Technique validation is AN integral a part of the tactic development; it's the method of demonstrating that analytical procedures are appropriate for his or her supposed use which they support the identity, quality, purity, and efficiency of the drug substances and drug product. Simply, technique validation is that the method of proving that AN analytical technique is appropriate for its supposed purpose [39,40].

Technique Validation, however, is usually a one-time method performed when the tactic has been developed to demonstrate that the tactic is scientifically sound which it serves the supposed analytical purpose [19,36,41-43]. All the variables of the tactic ought to be thought of, as well as sampling procedure, sample preparation, activity separation and detection and information analysis. For activity ways utilized in analytical applications there's additional consistency in validation apply with key analytical parameters as well as [44-49].

- Recovery
- Response function
- Sensitivity
- Precision
- Accuracy
- Limit of detection
- Limit of quantization
- Ruggedness
- Robustness
- Stability
- System suitability

Recovery

The absolute recovery of analytical methodology is measured because the response of a processed spiked matrix commonplace expressed as a proportion of the response of pure commonplace that has not been subjected

to sample pre-treatment and indicates whether or not the tactic provides a response for the whole quantity of analyte that's gift within the sample [50,51]. It's best established by comparison the responses of extracted samples at low, medium and high concentrations in replicates of a minimum of half-dozen with those non-extracted standards, that represent 100 percent recovery [41].

$$\text{Absolute recovery} = \frac{\text{Response of an analyte spike into matrix (processed)}}{\text{Response of analyte of pure standard (unprocessed)}} \times 100$$

If an interior customary is employed, its recovery ought to be determined severally at the concentration levels utilized in the strategy [52].

Response of function

In activity ways of study, peak space or peak height could also be used as response operates to outline the linear relationship with concentration called the activity model. It's essential to verify the activity model selected to make sure that it adequately describes the connection between response operate (Y) and concentration (X) [52-58].

Sensitivity

The method is claimed to be sensitive if little changes in concentration cause giant changes in response operate. The sensitivity of associate degree analytical technique is set from the slope of the activity line [58-62]. The boundaries of quantification (LOQ) or operating dynamic vary of bio analytical technique are outlined because the highest and lowest concentrations, which might determine with acceptable accuracy. It's steered that, this be set at ± 15% for each the higher and lower limit of quantisation severally [36,49]. Any sample concentration that falls outside the activity vary can't be interpolated from the activity line and extrapolation of the activity curve is discouraged. If the concentration is over varying, the sample ought to be diluted in sober matrix and re-assayed [63-65].

Precision

The purpose of winding up a determination is to get a legitimate estimate of a 'true' price. once one considers the factors consistent with that Associate in Nursing analytical procedure is chosen, preciseness and accuracy square measure sometimes the primary time to come back to mind [65-67]. Preciseness and accuracy along confirm the error of a private determination. They're among the foremost necessary criteria for judgment analytical procedures by their results.

Preciseness refers to the duplicability of mensuration among a collection, that is, to the scatter of dispersion of a collection concerning its central price. The term 'set' is outlined as pertaining to variety (n) of freelance replicate measurements of some property [67-70]. One amongst the foremost common applied mathematics terms utilized is that the variance of a population of observation. Variance is that the root of the ad of squares of deviations of individual results for the mean, divided by one but the quantity of leads to the set. The quality deviation S, is given by:

$$S = \sqrt{\frac{1}{n-1}\sum_{i-1}^{n}\left(x_i - \bar{x}\right)^2}$$

Standard deviation has identical units because the property being measured [71-75]. The sq. of ordinary deviation is termed variance (S2). Relative variance is that the variance expressed as a fraction of the mean, i.e., S/x. it's sometimes increased by one hundred and expressed as a p.c relative variance. It becomes an additional reliable expression of preciseness. % Relative standard deviation =S × 100/x

Accuracy

Accuracy unremarkably refers to the distinction between the mean x****, of the set of results and also the true or correct worth for the number measured [75-77]. In step with IUPAC accuracy relates to the distinction between results (or mean) and also the true worth [78]. For analytical ways, there is a unit 2 potential ways in which of determinant the accuracy, absolute methodology and comparative methodology [79-81].
Accuracy is best according as proportion bias that is calculated from the expression:

$$\%Bias = \frac{(measured\ value - true\ value)}{true\ value} X\,100$$

Since for real samples truth worth isn't well-known, Associate in nursing approximation is obtained supported spiking drug–free matrix to a nominal concentration [82]. The accuracy of analytical methodology is then determined at every concentration by assessing the agreement between the measured and nominal concentrations of the analytes within the spiked drug–free matrix sampler [83-85].

Calibration

Calibration is that the most significant step in bioactive compound analysis. An honest exactitude and accuracy will solely be obtained once an honest standardisation procedure is adopted. Within the spectrophotometric ways, the concentration of a sample cannot be measured directly, however is set victimisation another physical activity amount 'y' (absorbance of a solution). AN unambiguous empirical or theoretical relationship will be shown between this amount and therefore the concentration of AN analyte. The standardisation between y=g(x) is directly helpful and yields by inversion of the analytical calculation perform [85-87].
The standardisation perform will be obtained by fitting AN adequate mathematical model through the experimental knowledge. The foremost convenient standardisation perform is linear, goes through the origin and is applicable over a good dynamic vary. In apply; however, several deviations from the perfect standardisation line might occur. For the bulk of analytical techniques the analyst uses the standardisation equation [88-91].

$$Y=a+bx.$$

In standardization, invariant regression is applied, which suggests that everyone observations square measure dependent upon one variable X.

Standard deviation of slope (Sb)

The standard deviation of slope is proportional to plain error of estimate and reciprocally proportional to the vary and root of the amount of knowledge points.

$$Sb = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{(n-2)}} \quad \sqrt{\frac{1}{\sum_{i=1}^{n}(x_i - \bar{x}_i)^2}}$$

Where, Xi is the arithmetic mean of Xi values.

Standard deviation of intercept (Sa)

Intercept values of statistical method fits of information area unit typically to judge additive errors between or among completely different ways.

$$Sa= \sqrt{\frac{\sum_{i=1}^{n}\left(y-\hat{y}_i\right)^2}{(n-2)}} \quad \sqrt{\frac{1}{\sum_{i=1}^{n}(x_i-\bar{x}_i)^2}} \quad \sqrt{\frac{\sum_{i=1}^{n}x_i^2}{n}}$$

Where, Xi denotes the arithmetic mean of xi, values.

Correlation coefficient, (r)

The coefficient of correlation r (x,y) is a lot of helpful to specific the link of the chosen scales. To get a coefficient of correlation the variance is split by the merchandise of the quality deviation of x and y

$$r= \frac{\left[\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})\right]\Big/(n-1)}{\left[\sum_{i=1}^{n}(x_i-\bar{x})^2(y_i-\bar{y})^2\right]\Big/(n-1)^2}$$

Linearity and sensitivity of the method

Knowledge of the sensitivity of the colour is vital and therefore the following terms square measure unremarkably used for expressing sensitivity. In line with Bouger-Lambert–Beer's law, log intensity of incident radiations [92-94].

$$A = \text{Log}\frac{\text{Intensity of incident light}}{\text{Intensity of transmitted light}} = \in ct$$

The absorbance (A) is proportional to the concentration (c) of the engrossing species, if absorption factor and thickness of the medium (t) are constant. Once c is in moles per cubic decimeter, the constant is named molar absorption factor. Beer's law limits and μ_{max} values are expressed as μg ml^{-1} and $mole^{-1}$ cm^{-1} severally.
Sand ell's sensitivity refers to the amount of μg of the drug to be determinant, regenerate to the coloured product, that associate degree exceedingly column answers of cross section 1 cm^2 shows and absorbance of 0.001 (expressed as μg cm^{-2}).

Limit of detection (LOD)

The limit of detection (LOD) of AN analytical methodology is also outlined because the concentration, which provides rise to AN instrument signal that's considerably completely different from the blank [95]. For spectroscopical techniques or alternative strategies that depend on a standardization curve for quantitative measurements, the IUPAC approach employs the quality deviation of the intercept (Sa), which can be associated with LOD and therefore the slope of the standardization curve, b, by

$$LOD=3\ Sa/b$$

Limit of quantization (LOQ)

The LOQ is that the concentration which will be quantitate faithfully with a mere level of accuracy and exactitude. The LOQ represent the concentration of analyte that may yield a ratio of ten.

$$LOQ=10\ Sa/b$$

Where, Sa- the estimate is that the variance of the height space quantitative relation of analyte to IS (5 injections) of the medication. b- is slope of the corresponding standardization curve.

Ruggedness

Method toughness is outlined because the reliability of results once the tactic is performed beneath actual use conditions. This includes totally different analysts, laboratories, columns, instruments, supply of reagents, chemicals, solvents etc. methodology toughness might not be known once a technique is initial developed, however insight is obtained throughout subsequent use of that methodology [96].

Robustness

The construct of strength of associate degree analytical procedure has been outlined by the ICH as "alive of its capability to stay unaffected by little however deliberate variations in methodology parameters" [97,98]. The strength of a technique is that the ability to stay unaffected by little changes in parameters like pH scale of the mobile part, temperature, organic fertilizer solvent strength and buffer concentration etc. to see the strength of the tactic experimental conditions were by choice altered and chromatographical characters were evaluated [7].

Stability

To generate consistent and reliable results, the samples, standards and reagents used for the HPLC methodology should be stable for an inexpensive time (e.g. one day, one week, and one month relying upon need). For example, the analysis of even one sample might need 10 or additional chromatographical runs to see the system quality, as well as commonplace concentrations to form an operating analytical curve and duplicate or triplicate injections of the sample to be assayed [15,26].

System suitability

System quality experiments are outlined as tests to confirm that the tactic will generate results of acceptable accuracy and exactitude **(Table 1)**. The necessities for system quality square measure sometimes developed when methodology development and validation are completed. (Or) The USP (2000) defines parameters which will be accustomed verify system quality before analysis [99,100]. The criteria hand-picked are going to be supported the particular performance of the tactic as determined throughout its validation. For instance, if sample retention times type a part of the system quality criteria, their variation (SD) throughout validation is determined system quality would possibly then need that retention times fall among a 3 Mount Rushmore State vary throughout routine performance of the tactic [37].

Table 1. The parameters needed for assay validation of various classes as per USP square measure.

Parameters	Assay Category 1	Assay Category 2		Assay Category 3	Assay Category 4
		Quantitative Tests	Limit tests		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
LOD	No	No	Yes	*	No
LOQ	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

STATISTICAL ANALYSIS

To Calculate

- Average
  Average=Sum of the values/Number of items
- Standard Deviation
  Standard Deviation=$\sum\sqrt{}$ (Xi-X)2/n-1
  Xi=no of observation
  X=Arithmetic mean
  N=Total no of observation

- Relative Standard deviation
  Relative standard deviation=Standard deviation × 100/Average Value.

CONCLUSION

The article gives a detailed note on the best suited method for the best suited method for the development of Pregabalin drug as such and in dosage for too. It also gives clear cut explanation of the parameters used for the validation of the pregabalin drug along with statistical analysis.

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