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A SYSTEMATICHPLC METHODDEVELOPMENTFOR LUCOSAMIDE USING QUALITY BY DESIGN (QBD) APPROACH

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Abstract:

A simple and robust high-performance liquid chromatographic (HPLC) method was developed and validated for the quantitative estimation of Lucosamide in bulk and formulation. The systematic approach, one of the parts of QbD was use for the analytical method development. Chromatographic separation was carried out with Grace-C18 column (4.6×250 mm, 5-µm particle size), mobile phasewas used phosphate buffer and acetonitrile of pH 4.1 adjusted with acetic acid and thenfiltered through 0.45µ membrane filter and degassed in a sonicate for 10 min before use.Peak was obtained at retention time of 4.16 min flow rate of 0.6 ml/min,Detection was done using UV detector at 215nm.Optimization was done by response surface methodology, applying a three level BoxBehnken design with three centre points.The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines for linearity, range, accuracy, precision and robustness.

Introduction

Qualities by design refers to the achievement of certain predictable quality with desired and Predetermine specification. A very useful component of the quality by design is the Understanding of the factors and their interaction effects by a desired set of experiments. The Presentstudy describes the development of a comprehensive science and risk based HPLC Method and subsequent validation for the analysis of active pharmaceutical ingredient. Quality by design is a systemic process to build into a product from the inception of final Output.

There are many Qbd techniques, but we have used the systematic approach for The HPLC method development for the determination of lacosamide in Bulk and in pharmaceutical dosage

form-box behnken. Significance of factors were Analysed by their respective coefficient.Lacosamide anticonvulsant compound Used for treatment of partial onset seizures and neuropathic pain.the chemical Name forlacosamiden^{2}-acetyl-n-benzyl-d-homoserinamide.chemical structure For lacosamideis shown in fig-1 .mechanism of action lacosamide enhances The slow inactivation of voltage gated sodium channels without affecting the Fast inactivation of voltage gated channel. This inactivation prevents the channel From opening end the action potential.

Reference standard of Lacosamide was obtained from Glenmark pharmaceutical limited Mumbai. HPLC grade acetonitrile, acetic acid and phosphate buffer of merc were used. All aqueous solutions were prepared with HPLC grade ready water obtained in-house, Milli-Q water purification system (Millipore, USA).

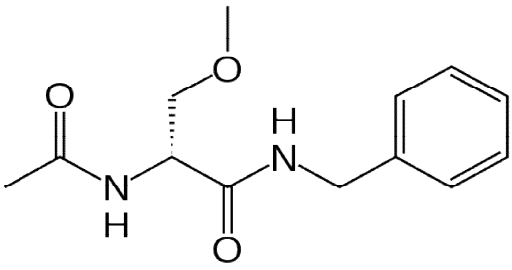

Fig1.chemical structure of Lacosamide

HPLC instrumentation

The HPLC system used was of JASCO HPLC LC-2000 plus Series consisted of Pump PU-2080. It has the Jasco Model UV-2075 Plus and Redone injector with 20 µl loop. HPLC system has Isocratic mode JascoBrowin version 1.2 software. The column used was ZORBAX SB- C18 (150x4.6, 5µ). UV visible spectrometer (Double Beam), JASCO 630V and wavelength range of 200-400 nm

Lacosamide sample preparation

Lacosamide stock solution for optimization of experiments was prepared by accurately weighing 10mg of Lacosamideand dissolving in 100ml volumetric flask, sonicate to dissolve. Transfer 1.0 ml of the above solution into a 50 ml volumetric flask and dilute to volume with diluent.

Mobile phase preparation

Phosphate buffer was prepared by dissolving 5.04 gm. of Disodium hydrogen phosphate and 3.01 gm. of PotassiumDihydrogen phosphate in HPLC grade water to make up to 1000ml. prepared buffer solution has pH of 4.1. Buffer solution was filtered and used for HPLC analysis. Appropriate mixture of Acetonitrile and buffer was made and pH was set by acetic acid and according to method design.

Wavelength selection for analysis

Appropriate dilutions of lacosamide were prepared and samples were scanned using UV spectrometer in the range of 200nm to 400nm. An absorbance maximum was obtained at 215nm.

Analytical target profile

"QbD is systematic approach to product, process design and development[4]. Hence it begins with determination of goal or method intent. In aphesis given on the product and process understanding [1]. Here method intent was to develop HPLC method of Lacosamide which is robust, accurate, precise and USP tailing less than.12, number of theoretical period as per requirement and short analysis time i.e. less than 10 min. as per QbD norms a robust method should be developed with help of visualized a design space.

Initial Chromatographic condition

Chromatographic separation was carried out with Grace-C18 column (4.6×250 mm, 5-µm particle size), mobile phasewas used phosphate buffer and acetonitrile of pH 4.1 adjusted with acetic acid and thenfiltered through 0.45µ membrane filter and degassed in a sonicate for 10 min before use. Peak was obtained at retention time of 4.16 min flow rate of 0.6 ml/min, prior to the injection of drug solution: column was equilibrated with mobile phase flowing through the system. Detection was done using UV detector at 215nm. Further changes were done according to optimization model. pH was change by using acetic acid.

Various tools for risk assessment are [17],

1. Ishkawa or fishbone diagram,
2. Failure mode effect analysis(FMEA),
3. Pareto analysis

Critical Quality Attribute (CQA)

Factors that directly affect the quality and safety of the product are sorted out and its possible effect on method development is studied. Understanding of product and method will help to sort the CQA [38].

From Software generate results parameters which affect the tailing were determined. Factor such as flow rate, Acetonitrile concentration in mobile phase and Column temp.were found to be critical. Selection of stationary phase was also critical parameter. The nature of the drug is more retentive on C18. But for HPLC method to be effective it should have lesser retention time.

Method design

Optimization was done by response surface methodology, applying a three level Box Behnken design with three centre points . Three factors selected were flow rate. Column temperature and Acetonitrile concentration in mobile phase. Evaluation of main factor, their interaction and quadric effect on peak USP tailing factor were done. Injection volume of 10µl, column oven temperature was kept constant as their effect on tailing was less significant. Experiments were conducted by making triplicate injections (total 51 runs) of standard Lacosamide solution and the average of USP tailing was analysed using Design Expert 8 software. Application of multivariate regression analysis resulted in a fitted full quadrate model for the average responses for peak USP tailing given by the equation 1

$$Y= \beta_0+\beta_1X_1+\beta_2X_2+ \beta_3X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{33} X_3^2 + \beta_{12} X_1 X_2+ \beta_{13} X_1X_3+ \beta_{23}X_2X_3$$

Where Y is the response, β_0 is the arithmetic mean response. B_1 β_2 and β_3 are regression coefficients of the factor X_1, X_2 and X_3 respectively. B_{11}, β_{22} β_{33} are squared coefficients β_{12}, $\beta_{13}$and β_{23} are interaction coefficients.[38,39]

Method validation

The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) [15] guidelines for linearity, range, accuracy, precision and robustness. For system suitability, standard solution of 25µg/ml of Lacosamide was prepared by diluting and mixing drug with acetonitrile. Five replicate injection of the system standard solution were analysed before sample analysis. The acceptance criteria for Lacosamide were less

than 2% relative standard deviation (RSD)for peak area, retention time, symmetry USP tailing factor less than 2 and number of theoretical plates greater than 2000 for all peaks.

Results and discussion for lacosamide

Preliminary studies

Lacosamide is chemically N^2-acetyl-N-benzyl-D-homoserinamide. It contains amino group in its structure hence it may be more retained on C18 column hence flow rate has to be increase in order to carry drug substance with mobile phase also retention time has to be consider while optimization. Different mobile phases tried but the separation was carried out on C18 column with mobile phase of phosphate buffer: Acetonitrile having p^H of 4.1 (80:20 v/v). Peak was obtained at retention time of 4.16 min, at flow rate of 0.6 ml/min, column oven temperature of 25^{o}C. Further optimization was done by carrying runs by Box-Behnken model.

Method design

Multivariate regression analysis was applied and fitted full quadratic model was obtained for the USP tailing factor of peak. Regression analysis and p-values obtained from software generated report are given in **(Table1**)

Table1: Regression coefficients and

associated probability value

(p-values) USP tailing of Lacosamide

Analysis of variance(ANOVA) was perform for findings are 'statistically significant' by convention, it is $p<0.05$[54].

A value of Probe > F was found to be less than 0.05, hence model was found to be significant for prediction of response , Significant factors found were flow rate(p-value 0.0372) and interaction of Flow rate x temperature (p-value 0.0084).

Three of the factor was found to affect the peak response from their respective coefficients. Temperature.And Acetonitrile interaction and column temperature x column temperature.isshowing inverse relationship with tailing. Flow rate also has shown effect on response.Response surface and contour plot were studied to visualize effect of factor and their interaction so as to develop design space for robust method 3D graph are given below in **fig.2** (A.B.C)

From **(Fig.2 A)** showing effect of flow rate and Column Temperature.(where Acetonitrile concentration is constant at 20%), it can be observed that between flow rate of 0.7-0.8 ml tailing was found to be more than 1.2, tailing was in specified limit between lower flow rate of 0.5-0.6., Flow rate is not showing much effect but when flow rate was increased throughout

Term	**Coeffi-Cient**	**p-value**
Intercept	1.27	‹0.0001
Flow rate	1.00	0.0372
Column Temperature.	2.500	0.5415
Acetonitrile	5.000	0.2402
Flowrate x Column Temperature.	0.020	0.0084
Flow rate x Acetonitrile	0.000	1.0000
Column Temperature.x Acetonitrile	0.000	1.0000
Flow rate x Flow rate	7.500	0.2052
ColumnTemperature. xColumn Temperature.	7.500	0.2052
Acetonitrile x Acetonitrile	0.097	‹0.0001

the pH range tailing was increased with increased in the flow rate. When column temperature was kept constant at 25^{o}C **(fig.2 B)** and effect of flow rate and Acetonitrile concentration was observed.,flow rate of around 0.7 ml and Acetonitrile concentration in between 25-30 tailing factor exceeded the limit. But at flow rate 0.6 ml it was within the limit.When flow rate was kept constant and column temperature and Acetonitrile concentration was studied Acetonitrile concentration is not showing much effect but when column temperature was at lower limit peak tailing was specified limit 1.2**(fig.2 C**).

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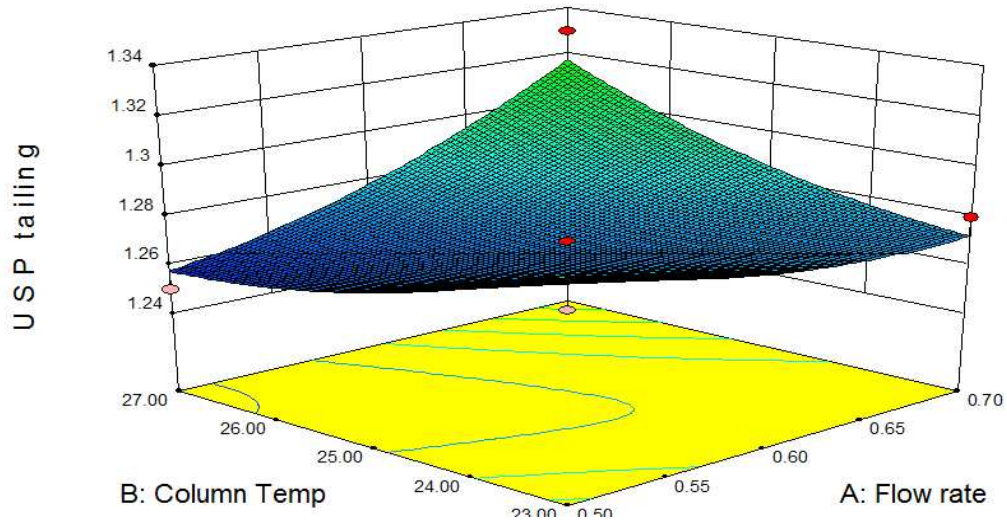

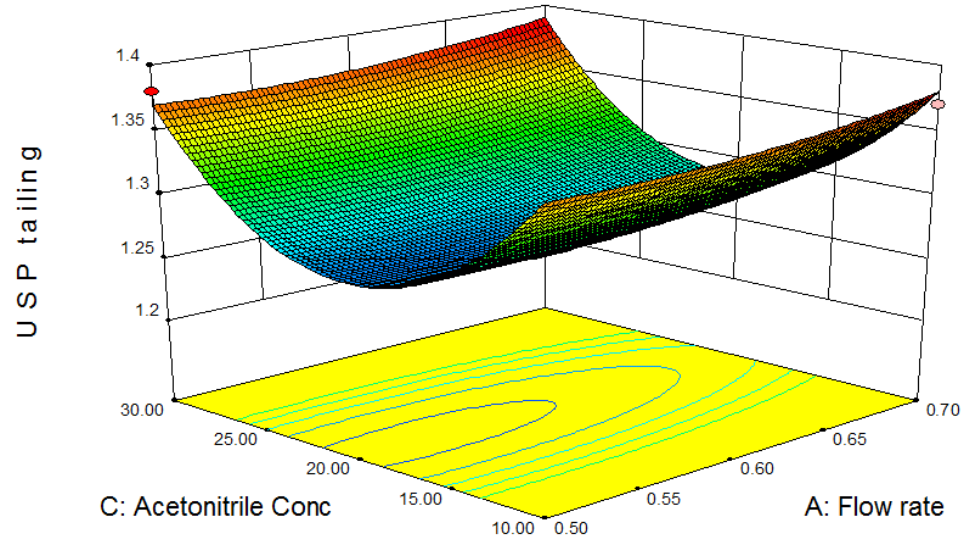

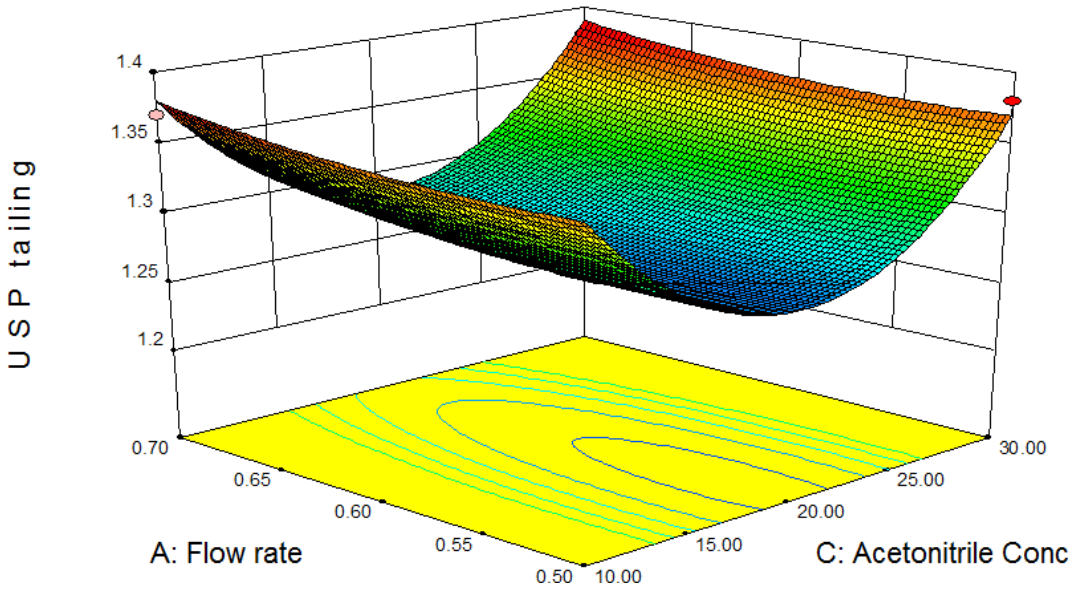

Figure 2: Response surface (3D) and contour plots showing effect of flow rate, Temperature and CAN concentration on USP tailing of Lacosamide.

A) Effect of flow rate and temperature B) Effect of flow rate and ACN
C) Effect of temperature and ACN.

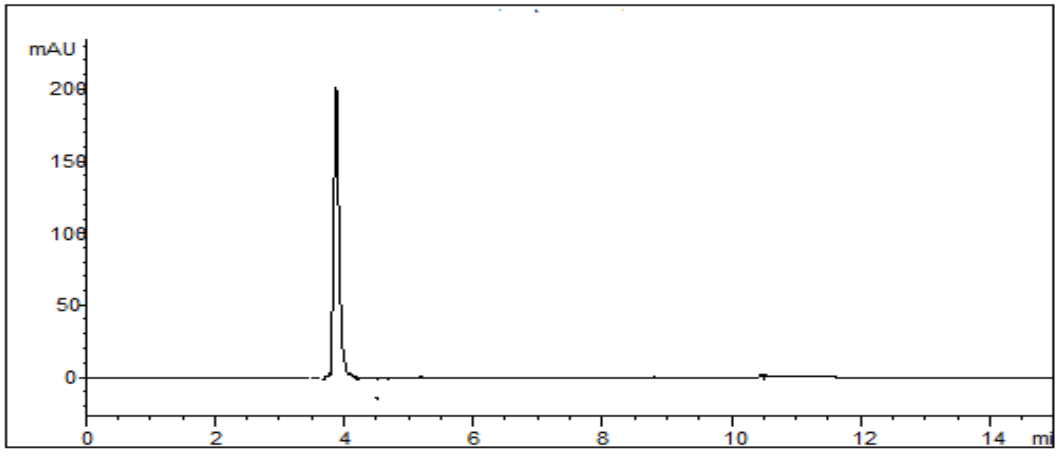

Fig 3: Chromatogram of Lacosamide

Method Validation

Method validation was done according to the ICH guideline Q2 [15]. Results were within the specified limit. Method was found to be accurate, precise and robust. Validation results are given below in**(Table 2)**

Linearity

A set of five solution of Lacosamide at concentration ranging from 40-120 µg/ml were prepared. Each sample was analysed in triplicate, calibration curve was constructed by plotting the peak area verses the concentration using linear regression analysis. The correlation coefficient was found to be 0.999 **(Table 3) (Fig. 4)**

Table 2: Validation of method in term of linearity, precision and accuracy of Lacosamide

Validation parameter	**Result**	**Acceptance criteria**
Linearity	Coefficient of	Coefficient of
(40-150 µg/ml)	Correlation-0.999	Correlation0>0.999
Accuracy	Recovery-99.5%	Recovery 98-102%
Precision		
Repeatability	RSD: 0.052%	RSD less than 2%
Intraday	0.69%	
Interday	0.03%	

Table 3: Linearity of Lacosamide

Standard Concentration(µg/ml)	**Peak area of Lacosamide**
40	6293
60	10154
80	13076
100	16452
120	19994
Regression equation	Y=165.32X-286.2
Regression coefficient	0.998

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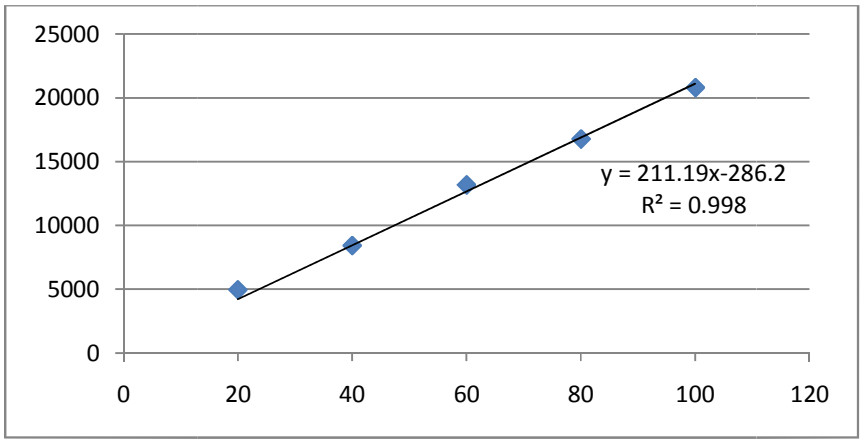

Figure 4: Linearity plot of Lacosamide

Repeatability

Repeatability was determined by running fivereplicates of samples and evaluating the average and %RSD for sample by comparing peak area.

Table 4: Repeatability of Lacosamide

Sr.no	Concentration (µg/ml)	Peak Area
1	40	10154
2	40	10150
3	40	10152
4	40	10161
5	40	10241
Average		10171.6
%RSD		0.3835%

Conclusion

From above it is concluded that,

A Quality by Design approach was successfully applied to HPLC method development of the Lacosamide and in bulk. All key aspect of QbD were tried to be implemented in said study. Systematic approach was utilized for method developments which include beginning with

determination of target profile characteristics, risk assessment, Critical Quality Attributes, Design of experiment and validation. Interaction and quadratic effect of the factors were studied with least possible runs by using Box Behnken model. Response surface diagrams and contour plots were studied for coming to conclusion which factor are affecting response and their limits were recorded.

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