Improvement of Development and Validation of an RP-HPLC Method for The Fluvastatin Sodium Using QbD Approach and Its Application to Forced Degradation Studies

Dhamdhere Rupali Balasaheb*1, Vijayalakshmi A2
1Research Scholar, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai – 600 117, Tamil Nadu, India
2Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai – 600 117, Tamil Nadu, India

ABSTRACT

The current examination portrays the improvement of the QbD way to deal with Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) framework utilizing Design of Experiments. Each of the three principle parts of the RP-HPLC measure (Buffer pH, Organic Step-percent acetonitrile, Organic Modifier-Methanol) are introduced in a successful test configuration zeroed in on methodical exploring. Through measurable investigation devices, for example, Analysis of Variance (ANOVA) and plots that uncovered the last chromatographic states of the strategy, the criticalness and communication impacts of these boundaries on the reaction factors (maintenance time and following component) were assessed. The chromatographic detachment was accomplished on Thermo Hypersil BDS RP C18 (250 × 4.6 mm, 5μ) section utilizing Buffer (pH 6.8): Acetonitrile (60:40v/v) as portable stage and discovery was finished utilizing Photo-Diode Array (PDA) identifier at 288 nm. The created fluvastatin sodium technique is direct with coefficients of relationship over a scope of 10-80 μg/ml. The (R2) estimation of the connection coefficient is 0.999. The percent RSD for the strategy’s exactness and accuracy was discovered to be under 2 percent. Investigations of Forced Degradation uncovered that the method was found to show security. The outcomes indicated that the strategy proposed is proper for the exact and precise assurance and detailing of fluvastatin sodium in mass.

INTRODUCTION

Fluvastatin sodium (Maryadele J. O’Neil , 2013) (FVS), chemically is (E,3R,5S)-7-[3-(4-fluorophenyl)-1-propan-2-ylindol-2-yl]-3,5-dihydroxyhept-6-enoic acid monosodium salt (Figure 1).

Lipid reduction agent and total cholesterol reduction. Fluvastatin sodium is a water-soluble cholesterol-lowering agent which acts through the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. (United states pharmacopeial convention , 2004) After its implementation by the United State Food and Drug Administration
As of late, the idea of Quality by Design has picked up conspicuousness in the field of the advancement of scientific techniques through the use of trial configuration draws near. QbD requires perception through an ideal arrangement of examinations of the basic factors and their collaboration effects. This article clarifies how the ideas of QbD can be incorporated factually to build up streamlined chromatographic conditions for the HPLC cycle. (Gupta and Kumar, 2015; Pohl et al., 2010; Yu, 2008) As per the Box-Behnken statistical screening design, the experimental runs were conducted. Factors including Buffer pH, Organic Step (percent acetonitrile) and Organic Modifier (methanol) were screened and optimized under this design. Literature research revealed that few analytical methods had been documented such as UV spectrophotometry and HPLC strategies have been accounted for the assessment of fluvastatin sodium. However, since the current methods do not indicate stability, have less sensitivity, specificity and specificity, the target of the current work was to develop a quick, rapid, reliable, precise and economical method of RP-HPLC using the QbD approach for bulk and tablet estimation of Fluvastatin sodium 1.

With the aid of architecture expert 9.0., the chromatographic conditions for the proposed technique have been optimized in addition; (Kalafsky et al., 1993; ICH Harmonised Tripartite Guideline, 1994; ICH, 2003) the established RP-HPLC approach was used for Fluvastatin sodium forced degradation study Blessy et al. (2014); Singh and Rehman (2012) in different stress condition in order to establish inherent stability of the drug. (Bakshi and Singh, 2002) The approach was further tested, and statistically and by rehabilitation trials, the findings of the study were tested. (Qiu and Norwood, 2007) The strategy created was discovered to be quick, reliable, precise and economical and can therefore be used for Fluvastatin sodium in vitro analysis.

MATERIALS AND METHODS

Instrumentation

The medication investigation was performed on a Thermo Hypersil BDS RP C18 (250 × 4.6 mm, 5μ) LC framework outfitted with a Quaternary Gradient HPLC Pump and a Photo Diode Array Detector utilizing an HPLC segment in the converse stage. The yield of the sign was noticed and incorporated utilizing Chrom NAV Chromatogram Software.

Chemical and Reagents

The working standard of Fluvastatin sodium was provided as a gift sample from Kachhela Medex Pvt. Ltd., Nagpur. Maharashtra. The marketed formulation, i.e. Lescol XL tablets containing 80 mg Fluvastatin sodium, were procured from a local market. Phosphate buffer pH 6.8, Potassium dihydrogen orthophosphate (AR Grade), o-phosphoric acid (GR Grade), HPLC-grade methanol and acetonitrile were purchased from E. Merck, Mumbai, India. HPLC grade water was obtained by double distillation and purification through the milli-Q water purification system. The choice of column and selection of mobile phase for performing an experiment is mentioned in Table 1 and Table 2 respectively.

Preparation of standard solution

Precisely gauge and move 24 mg Fluvastatin into a 50 ml volumetric flask. Add around 30 ml of methanol and sonicate to break up it totally and make volume sufficient with a similar dissolvable (stock arrangement). Further pipette out 5 ml of stock arrangement into a 10 ml volumetric flask and weaken sufficiently with methanol to get an answer of Fluvastatin (0.12 mg/ml). Sonicate for 30 minutes and makeup with methanol (stock solution). Final chromatographic condition mentioned in Table 3.

INITIAL METHOD DEVELOPMENT

Choice of column

In order to choose the appropriate column, initial experimental runs were carried out, as shown in Table 1 and Table 2. According to the observations of the above initial trials and its chromatograms, C18 column was selected for further trials.

Software aided method development

A new Reverse Phase-HPLC technique was created for the assurance of fluvastatin sodium by utilizing a QbD approach. A Quality by Design with Design of Experiments approach to the development of an analytical method mainly involves two phases as follows:

1. Screening Phase
2. Statistical Analysis and Final Optimization

Screening Phase

Another Reverse Phase-HPLC strategy was created for Fluvastatin sodium utilizing Design Expert 9 programming. In this product, Box-Behnken factual screening configuration was utilized to enhance the Critical Process Parameters (CPP) or Critical Method Parameters (CMPs) and to assess cooperation impacts of these boundaries on the Critical Quality Attributes (CQAs).
This Box-Behnken factal screening configuration is a 3 factor-2 level plan which was explicitly chosen since it requires less exploratory runs than other screening plans.

**Selection of Critical Quality Attributes (CQAs)**

Basic Quality Attributes are the reactions that are estimated to pass judgment on the nature of the created explanatory techniques. Along these lines, the Critical Quality Attributes chose for the examination are Retention time and Tailing Factor. These reactions were observed during the trial preliminaries.

**Experimental Trials**

As per the Box-Behnken statistical screening design, low, medium and high levels of the critical method parameters were selected based on the preliminary experimentation. So, the Design summary for Box-Behnken screening design is given in Table 4. Evaluation of all of the above critical method parameters with a Box-Behnken design leads to 12 experimental trials due to permutation and combination of the three parameters. These 12 experimental trials were carried out using the aforementioned chromatographic conditions using the previously selected Thermo Hypersil RP C18 column mentioned in Table 5.

**Statistical Analysis and Final Optimization**

The investigation of enhancement information and approval of the model was done utilizing Design Expert version 11 Software 2 to fit the trial information alongside the examined factors into the second-degree quadratic polynomial models for assessing various reactions. The coefficient of correlation (R) and lack of fit analysis was selected as model evaluation parameters from different parameters. Subsequently, coefficients of p < 0.001 were taken into account for the assessment of the variance analysis (ANOVA) model. In addition, factor-response relationship analysis using 3-D response and 2-D contour plots was carried out. 3D response surface graphs for mobile phase ratio and flow rate mentioned in Figure 2.

**Validation of the optimized method**

Validation of the analytical procedure was performed for fluvastatin sodium using the following parameter.

**System Suitability**

Framework reasonableness testing is a basic piece of any diagnostic methodology. Framework reasonableness testing was done by infusing 4 repeats of 10µg/ml standard fluvastatin calcium arrangement. In this test, maintenance time, a number of hypothetical plates and following element were assessed.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

Using the developed HPLC process, the LOD and LOQ of the developed method were calculated by injecting increasingly low concentrations of the standard solution of fluvastatin calcium.

**Specificity**

The particularity of the technique was dictated by recording the chromatogram of a standard stock arrangement of fluvastatin calcium (10µg/ml) and clear chromatogram (just diluent). Specificity signifies the identification of analyte, interference from other peaks and peak purity.

**Linearity and Range**

The Linearity of reaction was dictated by getting ready various centralizations of standard arrangement for example 5µg/ml (half), 8µg/ml (80%), 10µg/ml (100%), 12µg/ml (120%) and 15µg/ml (150%). At that point each level was infused multiple times into HPLC, chromatograms were recorded and top territory was recorded for all the pinnacles. The alignment diagrams were plotted as pinnacle zone of the analyte against the grouping of the medication in µg/ml.

**Precision and Accuracy**

The Precision is accounted for as far as Relative Standard deviation (RSD) over the scope of quantitation for a solitary trial wherein norms are tested in recreating (Intraday) and for a progression of analyses wherein principles are examined in more than a few investigations (Interday). The exactness of the created expository technique was tried by infusing three reproduce infusions of fixation 8µg/ml, 10µg/ml and 12µg/ml (80%, 100% and 120% of the working level). Intraday and interday accuracy study was done by assessing the relating reactions for the arrangements of over 3 fixation levels around the same time and on 3 distinct days separately. Exactness was determined for similar arrangements which were infused for Intraday Precision.

**Analysis of marketed formulation**

Business tablets of Fluvastatin (Lescol 80mg tablet) were taken and their normal weight was resolved, they were squashed to a fine powder. Powder comparable to 10mg of Fluvastatin was taken in 100ml volumetric cup and disintegrated in 75ml of Methanol with shaking for 5-10 minutes and afterwards sonicated. The supernatant fluid was sifted through a 0.2µm layer channel and afterwards moved to 100 ml volumetric jar and volume were made up with Acetonitrile. After that 10 ml of the above arrangement was weakened up to 100 ml.
with the versatile stage.

**Stability Indicating Assay of Fluvastatin**

To prove the stability-indicating nature of the method, the stock solution of the drug Fluvastatin was stressed under different conditions as follows to promote degradation. The standard solution and the stressed solutions were prepared as follows:

**Preparation of standard drug solutions**

An initial concentration of Fluvastatin at 500\(\mu\)g/ml was used for all stress degradation studies.

**Acid Hydrolysis**

Two ml of a stock standard solution of Fluvastatin (2500 \(\mu\)g/ml) was transferred into a 10 ml volumetric flask. Then 1 M HCl, 0.1 M HCl, 0.1 M HCl, 1 M NaOH, and 0.01 M NaOH were added to each flask to reach the volume and the flasks were kept at room temperature or 35ºC to study the degradation of Fluvastatin. The resulted solutions were neutralized by appropriate amounts of NaOH or HCl and injected to the HPLC system after dilution to 25 \(\mu\)g/ml by the mobile phase. Finally, this solution was loaded into HPLC and the corresponding chromatogram was recorded. The chromatogram of acid and basic hydrolysis is shown in Figure 3 and Figure 4 respectively.

**Oxidative degradation**

2 ml of the standard stock arrangement of Fluvastatin and 8 ml of 3% hydrogen peroxide were moved to a 10 ml volumetric cup and kept at room temperature or 35ºC. Finally, this solution was loaded into HPLC and the corresponding chromatogram was recorded. The chromatogram obtained in Figure 5.

**Thermal and light degradation**

A solid sample of Fluvastatin was spread in a thin layer in a watch glass and exposed to heat (70ºC) and light (visible and UV) for 5 days. Then, a standard solution was prepared at the concentration level of 25 \(\mu\)g/ml in the mobile phase and injected to the HPLC system. Finally, this solution was loaded into HPLC and the corresponding chromatogram was recorded. Chromatogram shown in Figure 6.

In all degradation studies, % recovery of the drug and % degradation products was calculated.

**RESULTS AND DISCUSSION**

Methanol and water in the proportion of 75:25 percent \(\nu/\nu\) (portable stage 1) and methanol (versatile stage 2) 0.02 M phosphate cradle pH 6.8 and methanol in the proportion of 60:40 \(\nu/\nu\) (portable stage 3) were portable stages. In the course of the method development, primarily initial studies were carried out with the following mobile phases. A mobile phase consisting of methanol and water with a ratio of 75:25 percent \(\nu/\nu\) gave the best results during these studies. The injection volume, mobile phase flow rate and analytical wavelength were kept constant (20\(\mu\)L, 1.0 ml/min and 304 nm) for the above-selected injection volumes during the course of these studies. Result shown in Table 1 and Table 2.

![Figure 1: Structure of Fluvastatin sodium](image1)

![Figure 2: 3D response surface graphs for mobile phase ratio and flow rate](image2)

**Risk assessment studies**

With reference to the literature survey and after critical exercise, a risk assessment estimate was prepared where various input factors were considered and different levels of risks associated with each parameter of the system were illustrated (Figure 2). Among the many variables examined during risk evaluation, such as peak optimization, parameters such as flow rate, mobile phase ratio, injection length, column oven temperature, etc. were tested to be correlated with high risk(s) and were considered to be cMPs for screening studies by maintaining constant buffer \(P^H\), mobile phase ratio, and acceptable column specifications.

**Response surface mapping**

Answer surface mapping was initiated using a central composite design (CCD) optimization method and second-order quadratic equation data analysis was performed, including the key and interaction effects for each of the important analytical attributes (CAA) studied. Table 2.
Table 1: Experimental trials for a choice of column

| Column | Observation | Inference      |
|--------|-------------|----------------|
| C8     | Poor retention of Analyte | Broad and poor peak shape |
| C18    | Improved retention of Analyte | Better peak shape |

Table 2: Experimental trials for a choice of mobilephase

| Mobile Phase composition | Observation | Inference |
|--------------------------|-------------|-----------|
| Water: Acetonitrile      | No precision in Retention time. Broad Peak with tailing | Use of buffer required and use of methanol to improve peak shape. |
| Water: Methanol          | No precision in Retention time. Better peak shape | Use of buffer and Methanol required. |
| Water: Acetonitrile: Methanol | No precision in Retention time. Good Peak shape | Use of buffer, acetonitrile and methanol required. |

Table 3: Chromatographic condition

| Parameters                  | Description                                           |
|-----------------------------|-------------------------------------------------------|
| Column C18                  | Thermo Hypersil BDS (250 x 4.6 x 5 mm)               |
| Mobile phase                | pH 6.0 phosphate buffer and acetonitrile (60:40v/v)   |
| Injection volume            | 20 μl                                                |
| Flow rate                   | 1 ml/min                                              |
| Detector                    | Wavelength PDA at 288 nm                              |
| Column Temperature          | 40°C                                                  |
| Auto Sampler Temperature    | 25°C                                                  |
| Run Time                    | 20 min                                                |

Table 4: Design matrix as per central composite design for optimization of the liquid chromatographic analytical method of Fluvastatin Sodium

| Selected parameter | Decoded factor levels |
|--------------------|-----------------------|
| Mobile phase ratio (%) | Low (-1) | Intermediate (0) | High (+1) |
| Flow rate (mL/min)  | 0.8 | 1.0 | 1.2 |
| Mobile phase ratio (%) | 40:60 | 50:50 | 75:25 |

Table 5: Design matrix as per central composite design for optimization of the liquid chromatographic analytical method of fluvastatin sodium

| Run | Coded factor levels |
|-----|---------------------|
|     | Factor 1 (Mobile phase ratio) | Factor 2 (Flow rate) |
| 1   | +1                   | 0                     |
| 2   | +1                   | -1                    |
| 3   | +1                   | +1                    |
| 4   | +1                   | -1                    |
| 5   | +1                   | +1                    |
| 6   | 0                    | 0                     |
| 7   | 0                    | +1                    |
| 8   | 0                    | -1                    |
| 9   | 0                    | -1                    |
| 10  | 0                    | +1                    |
| 11  | 0                    | -1                    |
| 12  | +1                   | 0                     |
Table 6: System suitability parameters from chromatogram (10 μg/ml)

| Peak No | Ret time (min) | Height (μm) | Area (mV) | Conc (%) |
|---------|----------------|-------------|-----------|----------|
| 1       | 2.073          | 2039.872    | 40141.801 | 17.7147  |
| 2       | 2.932          | 198.966     | 17799.400 | 7.8549   |
| 3       | 4.348          | 9660.032    | 167502.172| 73.9194  |
| 4       | 5.198          | 58.106      | 1157.733  | 0.5109   |
| **Total** |               | **11956.976** | **226601.106** | **100.0000** |

|                      | Half peak width (cm) | Theoretical levels | Resolution | Tail factor | Asymmetry |
|----------------------|----------------------|-------------------|------------|-------------|-----------|
| 1                    | 0.298                | 267.569           | 0.000      | 1.327       | 1.664     |
| 2                    | 0.283                | 593.100           | 1.476      | 1.741       | 2.507     |
| 3                    | 0.202                | 2575.652          | 2.921      | 0.719       | 0.478     |
| 4                    | 0.333                | 1347.350          | 1.589      | 1.593       | 1.971     |

Table 7: LOD and LOQ

| Parameters | Conc (μg/ml) |
|------------|--------------|
| LOD        | 0.00246      |
| LOQ        | 1.755        |

Table 8: Linearity Studies & Curve of Fluvastatin

| Conc. μg/ml | Area |
|-------------|------|
| 2           | 34154|
| 4           | 65436|
| 6           | 97105|
| 8           | 12874|
| 10          | 161369|
| 12          | 191432|
| slope       | y=16016x|
| RSQ(r²)     | 0.999|
| LOD         | 0.00231|

Table 9: Precision and accuracy of the method for determination of Fluvastatin

| (3 sets for 3 days) Concentration added (μg/ml) | Concentration found (μg/ml) | CV (%) | Error (%) |
|-------------------------------------------------|-----------------------------|--------|-----------|
| **Within day (n = 3)**                           |                             |        |           |
| 5.00                                             | 5.10±0.4                    | 1.26   | 0.46      |
| 30.00                                            | 29.936±0.42                 | 1.49   | -0.11     |
| 60.00                                            | 59.94±0.21                  | 0.38   | -0.04     |
| **Between day (n = 9)**                          |                             |        |           |
| 5.00                                             | 5.21±0.04                   | 1.14   | 0.14      |
| 30.00                                            | 29.90±0.31                  | 1.10   | -0.17     |
| 60.00                                            | 60.12±0.48                  | 0.80   | 0.22      |

6.1.7 Accuracy: result mentioned in Table 10
Table 10: Recovery data of Fluvastatin

| Sr. No | Level of addition (%) | Peak area of standard Flu (2 µg/ml) | Peak area theoretical (mV) | % Recovery |
|--------|------------------------|------------------------------------|-----------------------------|------------|
|        |                        | 1                                  | 2                           | 3          |
| 1      | 80                     | 35479.801                          | 32951.098                   | 34333.949  | 98.72      |
| 2      | 100                    |                                    |                             |            | 99.62      |
| 3      | 120                    |                                    |                             |            | 99.24      |

Table 11: The results of the stress degradation tests on Fluvastatin bulk powder using different conditions

| Stress test condition | Solvent       | Temperature | Time   | % of Fluvastatin |
|-----------------------|---------------|-------------|--------|------------------|
| Acidic                | 1 M HCl       | Room temp   | 30 min | 81.1             |
|                       | 1 M HCl       | 35ºC        | 30 min | 24.5             |
|                       | 0.1 M HCl     | 35ºC        | 1 h    | 66.4             |
| Basic                 | 1 M NaOH      | Room temp   | 30 min | 20.4             |
|                       | 0.01 M NaOH   | Room temp   | 15 min | 44.1             |
| Oxidative             | 3% H₂O₂      | Room temp   | 30 min | 93.8             |
|                       | 3% H₂O₂      | 35ºC        | 30 min | 18.9             |
|                       | 1% H₂O₂      | 35ºC        | 1 h    | 81.1             |
| Photolytic            | UV light      | Solid form  | Room temp | 5 days | 100.3      |
|                       | Visible light | Solid form  | Room temp | 5 days | 99.9       |
| Heat                  |               | Solid form  | 90ºC    | 5 days | 100.1      |
|                       |               |            | 70ºC    |        |            |
|                       |               |            | 60ºC    |        |            |

Table 12: Drug Evaluated from Tablet

| Concentration in 10 µg/ml | Peak area of std. | Peak area of Sample | % Assay |
|---------------------------|-------------------|---------------------|--------|
| 10                        | 9660.032          | 9460.010            | 97.9   |

Figure 3: Chromatogram of acid hydrolysis
Figure 4: Chromatogram of Basic hydrolysis

Figure 5: Chromatogram oxidative hydrolysis

Figure 6: Chromatogram Thermal degradation

Figure 7: Supplementary Data of Drug

Figure 8: Coordinates for Design Space

Analysis of Variance (ANOVA)

According to the ANOVA parameters, different coefficients, along with p-value and R2 value, of different quadratic polynomial models. Where β0 is the intercept, β1 and β2 are the coefficients of factor X1 and X2, and β3 is the coefficient of the term of interaction between factor X1 and X2, and the coefficients of the quadratic terms are β4 and β5. In addition, the 3-D response surface and 2-D con-
tour plots were used. Figure 7 and Figure 8 were used. The umbrella-like 3-D reaction surface was seen in Figure 8, speaking to the impact of portable stage proportion or stream rate on Tailing Factor (TF). In the present investigation, the stream rate was contrarily corresponding to versatile stage proportion and starting linearity of bend ruin. The following element noticed expanding from midpoint to down point. The base estimation of TF was seen at the midpoint for X1 and X2. Comparing 2-D form plot Figure 9 and Figure 10 strengthens the above understandings of a 3-D surface, i.e., the least qualities were seen at a mid estimation of portable stage and stream rate. Figure 10 However, at lower level stream rate is legitimately corresponding to versatile stage proportion and they are by rate content.

Search for an optimum chromatographic solution

Quest for an ideal chromatographic arrangement was completed with the assistance of mathematical streamlining by various CAAs to acquire the ideal objectives, including minimization of TF and boost % measure and TPC. The upgraded chromatographic arrangement was seen at versatile stage creation containing 75:25 % v/v combination of phosphate cradle pH 6.8 and Methanol at ideal stove temperature, 10 μl infusion volume and stream pace of 1 mL/min, which yielded attractive quality estimation of 1 with TF of 0.979, %. Further, the graphical streamlining was completed by the outline of the ideal arrangement inside the diagnostic plan locale, as appeared in Figure 11.

Validation of the optimized method

When the chromatographic conditions were set, technique approval was done on fluvastatin sodium for System suitability, Specificity, Limit of Detection, Limit of Quantitation, Linearity, Range, Accuracy and Precision.

Evaluation of System Suitability

The standard solution (10 μg/ml) was injected four times. The % RSD obtained from four replicate injections was found to be less than 2.0%. Tailing fac-
The parameter was less than 2.0. Theoretical plates were also found to be above 2000. Thus, all the parameters evaluated for system suitability were found to be within the acceptance criteria the chromatogram mentioned in Figure 12 and the system was suitable for analysis of fluvastatin sodium. Result mentioned in Table 6

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ were obtained by successively reducing the fluvastatin sodium concentration as long as a signal-to-noise ratio of not less than 3:1 and 10:1 is preserved. The LOD was found to be 0.00246ng/ml of fluvastatin sodium. It was found that the LOQ for fluvastatin sodium was 1.755ng/ml. These values suggest that the evolved approach is sensitive. LOD and LOQ mentioned in Table 7

Specificity
The method demonstrated good separation between the peaks and was found to be free of interference. For demonstrating the specificity of the method for drug formulation, the drug was spiked, wherein the excipients used in different formulation products did not interfere with the drug peak and thus the method was specific for FVS.

Linearity and Range
A standard curve was obtained in the concentration range of 2-12μg/ml for Fluvastatin. The linearity of this method was evaluated by linear regression analysis. The slope, intercept and correlation coefficient \( r^2 \) of the standard curve were plotted and calculated and are given in Figure 13 & Table 8 demonstrating the linearity of the proposed RP-HPLC method.

Accuracy and precision
The exactness and accuracy of the strategy were assessed by the assurance of Fluvastatin standard arrangements in the versatile stage at three distinctive fixation levels (20, 60, and 80μg/ml). This examination was acted in three-fold in one day and three continuous days.

The information acquired from exactness and accuracy tests appears in Table 9 the CV qualities for the inside day and between-day were under 1.4%, which affirms adequate reproducibility of the proposed strategy. Exactness was determined for similar arrangements which were infused for Intrady Precision. The outcomes for Accuracy appear in Table 10.

Stability Indicating Assay of fluvastatin sodium
In fundamental conditions, Fluvastatin was discovered to be more labile. The medication was corrupted about 80% after 30 min presentation to 1M NaOH at room temperature. The debasement was slower under presentation to 0.01 M NaOH and 55% corruption was seen after 15 min at room temperature. Another top at the maintenance season of about 2.4 was showed up in the chromatogram Fluvastatin was found to corrupt in 1% H₂O₂ to a degree of 18.9% after 1h. More debasement was seen by utilizing 3% H₂O₂ at room temperature or Fluvastatin mass powder was steady under presentation to warm, UV light, and noticeable light and no huge debasement was noticed.

Application on Marketed Formulation
The developed Stability Indicating RP-HPLC method was successfully applied for the estimation of fluvastatin from the marketed formulation of Lescol XL 80Mg Novartis. Tablets which was found to contain 97.9% of the Label Claim. Data are shown in Table 12.

CONCLUSION
The RP-HPLC assay method developed for fluvastatin by QbD approach is linear, accurate, precise, reproducible and specific as evident from the validation results. The developed method is also stability-indicating and can be conveniently used for quality control to determine the assay in regular fluvastatin product development, production and stability samples.

ACKNOWLEDGEMENT
The author thanks, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu for providing required facilities and support to carry out this research.

Conflict Support
The authors declare that they have no conflict of interest for this study.

Funding Support
The authors declare that they have no funding support for this study.

REFERENCES
Bakshi, M., Singh, S. 2002. Development of validated stability-indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis, 28(6):1011–1040.
Blessy, M., Patel, R. D., Prajapati, P. N., Agrwal, Y. K. 2014. Development of forced degradation and sta-
bility indicating studies of drugs—A review. *Journal of Pharmaceutical Analysis*, 4(3):159–165.

Dogrukol, A. K. D., Kircal, K., Tuncel, M., Aboul-Enein 2001. Validated analysis of fluvastatin in a pharmaceutical capsule formulation and serum by capillary electrophoresis. *Biomedical Chromatography*, 15(6):389–392.

Gupta, V., Kumar, P. V. 2015. A review quality by design approach (QBD) for pharmaceutical. *International journal of drug development and research*, 7(1):52–60.

ICH 2003. Guidance for Industry: Q1A (R2) stability testing of new drug substances and products. *International Conference on Harmonization (ICH)*, pages 24–24.

ICH Harmonised Tripartite Guideline 1994. Validation of Analytical Procedures: Text and Methodology Q2(R1). *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use*, pages 1–17.

Kalafsky, G., Smith, H. T., Choc, M. G. 1993. High-performance liquid chromatographic method for the determination of fluvastatin in human plasma. *Journal of Chromatography Biomedical Sciences and Applications*, 614(2):307–313.

Maryadele J. O’Neil 2013. The Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals. 15th Edition. RSC Publishing. ISBN 9781849736701 Pg. 4245.

Pohl, M., Schweitzer, M., Hansen, G. 2010. Implications and opportunities of applying the principles of QbD to analytical measurements. *Pharmaceutical Technology Europe*, 34(2):29–36.

Qiu, F., Norwood, D. L. 2007. Identification of Pharmaceutical Impurities. *Journal of Liquid Chromatography & Related Technologies*, 30(5-7):877–935.

Singh, R., Rehman, Z. 2012. Current trends in forced degradation study for pharmaceutical product development. *J. Pharm. Educ. Res*, 3(1):54–63.

United states pharmacopeial convection 2004. The United States Pharmacopoeia: USP 27: The National Formulary: NF 22, Washington, D.C. , pages 2222–2224.

Yu, L. X. 2008. Pharmaceutical Quality by Design: Product and Process Development, Understanding, and Control. *Pharmaceutical Research*, 25(4):781–791.