Application of Response Surface Methodology in Development and Optimization of Stability Indicating RP-HPLC Method for Determination of Tolvaptan in Bulk and Formulation

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Authors’ contributions

This work was carried out in collaboration between both authors. Author ASS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CSM have corrected and supervised study. Both authors read and approved the final manuscript.

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ABSTRACT

Design of Experiment assisted stability indicating RP-HPLC was developed and optimized using response surface methodology for determination of Tolvaptan. Mobile phase was developed and optimized using Design of Experiment with response surface methodology. Acetonitrile and phosphate buffer with pH 5.5 (70:30% V/V) was optimized as mobile phase. The flow rate was maintained at 1ml/min. Stress studies were performed as per guidelines. Method was validated in accordance with regulatory requirements and results were within specified limits of regulatory guidelines. Tolvaptan was eluted at 3.24 min. It shows linearity from 2.5-15 µg/ml. Coefficient of correlation was 0.999, LOD and LOQ values were 1.0871 (µg/ml) and 3.2942 (µg/ml). Precision was determined with % RSD of 0.8669 and 0.9709%, mean percentage Recovery value was found to be 99.88 ±1.2. All stress degradation products are very well resolved from drug peak which indicate suitability indicating nature of the developed method. Design of Experiment technique can

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help in fast and economical optimization of mobile phase which in turn will save time for method development. The developed method is simple, accurate, sensitive which can be utilised as stability indicating method for identification of degradation products in routine analysis of the drug.

Keywords: Tolvaptan; response surface; validation; RP-HPLC; degradation.

1. INTRODUCTION

Chemically tolvaptan is N-{4-[(5R)-7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1H-1-benzazepine-1-carbonyl]-3-methylphenyl}-2-methylenzamide with molecular formula of C_{28}H_{32}ClN_{2}O_{3} [1]. Tolvaptan is a Vasopressin receptor antagonists [2,3] . Tolvaptan is mainly useful for patient with heart failure due to their high serum vasopressin levels [4].

Literature survey showed that different analytical methods like UV spectroscopy [5-7], HPLC [8-13] UPLC [14], LC-MS/MS [15-17] are available for determination of Tolvaptan in bulk in human and rat plasma, a stability indicating RP-HPLC was reported for Tolvaptan, but all these methods are developed using conventional technique of literature survey and trial and error technique. The objective of present research work was development of a simple, fast, stability-indicating RP-HPLC method with help of DoE technique for the determination of Tolvaptan with the aid of chemometric tool design of experiment technology with response surface methodology as a technique for optimizing the mobile phase in order to save time and chemicals for the development of new method [18-21].

HPLC is a versatile separation technique with wide range of applications but it has certain drawbacks the method development process is curtail and critical due to different variables like pH of mobile phase, Organic Solvent, concentration and type of buffer, flow rate, operating temperature etc. which may affect some important parameters hence they should be properly adjusted and controlled before every chromatographic run to attain desired separation [22]. Deeper understanding of these variables and their interactions is very important in development of new HPLC method. Chemometric is one of the best solutions to understand these interactions and important variables which can affect different parameters in method development. Chemometric tools and techniques can help in reducing number of experiments, reagent utilization and time required for laboratory work. Development of a mathematical model along with statistically significant parameters help to understand influence of various variables on expected chromatographic responses [23,24]. For many years, HPLC methods were developed through trial-and-error approach, improved with expertise, experience and knowledge, of the analyst. The approach involves study of one factor at a time while other factors are constant, but this traditional method development model leads to more expenses in terms of time, money, efforts, and labor [25].

A chemometric experimental design approach can solve this problem to some extend by systematically planning the experiments in order to model the data effectively and efficiently with a smaller number of experimental runs. This model can help in understanding influence of different variables and effect of their interactions on chromatographic behavior. Shah U et. Al have reported Development and validation of stability-indicating RP-HPLC method for estimation of pamabrom in tablets and another stability indicating RP-HPLC method for simultaneous estimation of rifampicin and piperine in pharmaceutical dosage form. Different stress conditions were used in study which includes, Hydrolytic decomposition: Acidic and alkaline hydrolysis were carried out in 0.1M HCl and 0.1M NaOH for 50°C for 15 min, Neutral decomposition: Neutral hydrolysis was carried out in distilled water at a 55°C for 30 min, Solutions for oxidative stress studies were prepared using 3% H2O2 at 40°C for 30 min, Photo degradation studies were carried out by exposing the drug to UV light in the UV chamber at the wavelength (λ = 254nm) for 120 min, Thermal studies were also conducted on solid drug, which was heated at 80°C for 30 min in hot air oven [26,27].

2. MATERIALS AND METHODS

Tolvaptan gift sample was obtained from a reputed pharmaceutical industry. Methanol and Acetonitrile (HPLC grade) were procured from Thomas Baker Pvt. Ltd. and Merk Specialties Pvt. Ltd., Mumbai.
2.1 Instrument and Software

An Waters HPLC System with 515-pumps with column oven, PDA detector, Auto sampler (717-waters plus) with Empower 2 software were used for obtaining the chromatograms, Kromasil C18 (250 mm x 4.6 mm, 5µm) with Guard column was used as the stationary phase. Design of Experiment (trial version) was used to design and optimize the suitable mobile phase.

2.2 Preparation of Standard Stock Solution

Standard stock solution of Tolvaptan was prepared by transferring 100 mg pure tolvaptan to a 100 ml volumetric flask, it was dissolved in sufficient amount of methanol and finally the volume was made up to 100 ml mark using methanol.

The concentration of the standard drug solution was 1mg/ml. This solution was suitably diluted, and desired concentration range was obtained.

2.3 Preparation of Formulation Sample Solution

Twenty tablets of the marketed formulation were weighed accurately and powder equivalent to 100 mg of Tolvaptan was taken in 100 mL volumetric flask, methanol was added to flask and solution was sonicated. After the sonication volume of the flask was made up to 100 ml mark using methanol.

The resulting solution was filtered, and filtrate was diluted to get desired concentration of drug.

2.4 Optimization of Chromatographic Conditions

Initial goal of method development was to find out suitable mobile phase for determination of tolvaptan. The same mobile phase was used for separation of degradation products from pure tolvaptan. But it was found that proper resolution is not obtained with the help of same mobile phase. In order to solve this problem a new technique known as design of experiments was applied. This study was designed based on previous experimental runs and literature survey.

Initial experimental runs shows that percentage of organic phase, mobile phase pH and mobile phase flow rate are three important factors which should be considered for designing experiments. Alteration in these factors affects retention time, Tailing factor and resolution of degradation peak and drug peak. These three factors were designated as response. Response surface method with central composite design which uses 3 factors, and three responses was applied. The central composite design (CCD) gave 20 experimental conditions. These experiments were performed, and values of response variables were entered in the Design of Experiment software these values were statistically evaluated and possible optimized solutions were suggested by the software.

2.5 Stress Degradation Study

Methanolic solution of the drug (1 mg/ml) was prepared and was used to check the effect of stress degradation of the tolvaptan at various conditions. Stress samples were subjected to chromatographic runs after neutralization and dilution.

2.5.1 Acid hydrolysis

To check the effect of acidic environment on tolvaptan, 25 ml drug solution (1 mg/ml) was added to 25 ml 0.1 M Hydrochloric acid, and it was refluxed at 80°C till 8 hours. Stress sample was diluted before chromatographic run.

2.5.2 Alkaline hydrolysis

Effect of alkaline condition on tolvaptan was studied by mixing 25 ml methanolic drug solution with 25 ml 0.1 M sodium hydroxide this solution was refluxed at 50°C for eight hours and resulting stress sample was diluted and then chromatogram was acquired.

2.5.3 Oxidative stress studies

About 25 ml of drug solution was treated with 3% V/V Hydrogen peroxide it was refluxed at 50°C for 8 hours and then after suitable dilution subjected to chromatographic run.

2.5.4 Thermal stress studies

Thermal stability of tolvaptan was checked by keeping drug in oven at 100°C for 8 hours and then after suitable dilution subjected to chromatographic run.

2.5.5 Neutral hydrolysis

Neutral degradation was studied by refluxing 25 ml of drug solution with 25 ml distilled water for 8
hours; resulting solution was diluted before chromatographic run.

2.6 Validation of Method

2.6.1 Accuracy

Recovery studies were performed to evaluate accuracy of the developed method, standard drug solution was spiked in pre analyzed formulation at three levels of 80, 100 and 120%. The procedure was repeated in triplicate and mean percentage recovery of three levels was determined.

2.6.2 Precision

Method precision was evaluated for inter day and intra-day. Inter-day precision was evaluated by injecting six samples of same concentration of standard drug solution and six samples were injected on 2 continuous days for intra-day precision; % RSD was used as evaluation parameter for precision.

2.6.3 Linearity

Method linearity was studied by plotting a calibration curve for peak area against concentration. Coefficient of correlation was calculated using MS-Office Excel 2007.

2.6.4 LOD and LOQ

Minimum amount of tolvaptan which can be detected (LOD) and minimum amount of tolvaptan which can be quantitated (LOQ) with sufficient accuracy were calculated using formula given by USP guidelines.

2.6.5 Robustness

Deliberate minor changes in flow rate, detection wavelength and temperature were used to evaluate Robustness and evaluation was based on % RSD values.

2.7 System Suitability Test

System suitability tests were studied by injecting standard drug solution in the column and Theoretical plates, tailing factor, resolution values were evaluated.

2.8 Assay

The tablet formulation sample was injected six times in the system and % assay was calculated using standard formula based on labeled claim area, dilution, and average weight of tablet.

3. RESULTS

3.1 Method Optimization

HPLC method was optimized by new fast and rapid chemometric tool design of experiment using response surface methodology with an objective to develop a method with stability indicating property and good resolution between drug and degradation product peaks, sufficient number of theoretical plates, and tailing factor within specific values of standard guidelines. Based on initial preliminary runs pH, Flow rate and percentage of organic phase are important factors which can influence chromatographic separation. Central Composite Design (CCD) suggested 20 runs with different values for these three variables (Table 1).

Anova test was performed, and F and P values were evaluated. Lower p values (0.0001) of model shows that model is significant along with A and C terms are significant in determining retention time and resolution between two peaks. Response surface plots for tailing and resolution are shown in Fig. 1a, b respectively.

The next step in Design of Experiment is Numerical optimization which was performed on the model. All the desired criteria required for clear separation of drug peak with degradation product peak were entered in the optimization after evaluation and optimization 8 solutions were derived by the systematic statistical study and based on desirability value. First solution with high desirability value was selected for experimental work and to check stability indicating nature of the method. Point prediction values for, resolution and tailing factor are shown in Fig. 2 and Fig. 3 respectively.

To check the prediction ability of the model mobile phase with Acetonitrile: Phosphate buffer (10 m mol) pH 5.5 (70:30 %V/V) was utilized for chromatographic analysis. The results obtained were much more superior than the predicted values given by the model, and it shows desired values for resolution and tailing factor for Tolvaptan and its related degradation products. Elution of Tolvaptan and degradation products were monitored using PDA detector. Chromatogram of pure Tolvaptan is shown in Fig. 4.
Table 1. Central composite design with all responses for tolvaptan

| Run | Factor1 | Factor2 | Factor3 | Response1 | Response2 | Response3 |
|-----|---------|---------|---------|-----------|-----------|-----------|
|     | A: ACN % | B: Flow Rate ml/min | C: pH | Retention Time | Tailing | Resolution |
| 1   | 69      | 1       | 6.3     | 5.95      | 1.12      | 1.84      |
| 2   | 69      | 1       | 4.2     | 5.75      | 1.54      | 1.67      |
| 3   | 69      | 1       | 4.2     | 5.5       | 1.62      | 1.55      |
| 4   | 69      | 1       | 2.1     | 6         | 1.95      | 1.32      |
| 5   | 69      | 1       | 4.2     | 5.62      | 1.49      | 1.59      |
| 6   | 69      | 1       | 4.2     | 5.68      | 1.5       | 1.62      |
| 7   | 50.5    | 1       | 4.2     | 9.21      | 3.5       | 0.5       |
| 8   | 80      | 1.2     | 3       | 3.91      | 2.14      | 2.51      |
| 9   | 80      | 0.8     | 5.5     | 4.2       | 1.95      | 2.65      |
| 10  | 69      | 1       | 4.2     | 5.74      | 1.41      | 1.62      |
| 11  | 87.5    | 1       | 4.2     | 1.24      | 1.66      | 3.1       |
| 12  | 80      | 0.8     | 3       | 4.95      | 2.55      | 2.48      |
| 13  | 58      | 0.8     | 5.5     | 5.81      | 3.1       | 0.8       |
| 14  | 80      | 1.2     | 5.5     | 3.42      | 2.02      | 2.6       |
| 15  | 58      | 1.2     | 5.5     | 7.18      | 3.24      | 0.68      |
| 16  | 58      | 0.8     | 3       | 7.84      | 3.65      | 0.75      |
| 17  | 58      | 1.2     | 3       | 7.65      | 3.59      | 0.66      |
| 18  | 69      | 1       | 4.2     | 5.68      | 1.62      | 1.49      |
| 19  | 69      | 0.66    | 4.2     | 6.105     | 1.57      | 1.55      |
| 20  | 69      | 1.3     | 4.2     | 5.34      | 1.39      | 1.22      |

Fig. 1a. Response surface plot for tailing

The retention time of Tolvaptan was 3.24 min all other system suitability parameters were found within regulatory limits. System suitability parameters (n=6) are shown in Table 2.
Fig. 1b. Response surface plot for resolution

Fig. 2. Point prediction for tailing factor
Table 2.

| Sr. No. | Parameter       | Tolvaptan     |
|---------|-----------------|---------------|
| 1       | Tailing Factor  | 0.9968 ±1.92  |
| 2       | Theoretical Plates | 7841.5 ±1.78 |
| 3       | Resolution      | 1.8952        |

3.2 Linearity and Sensitivity

Linearity was examined by plotting a graph of selected concentrations against peak area for tolvaptan. The value of correlation coefficient ($r^2$) was 0.9992 for the concentration range 2.5-15 μg/ mL. Calibration curve is shown in Fig. 5.

3.3 Accuracy

Results of Percentage recovery studies were obtained from the amount recovered and the actual added concentration (Table 4).
Fig. 5. Calibration curve for tolvaptan

Table 3. Linear regression analysis

| Sr.No. | Parameter               | Tolvaptan          |
|--------|-------------------------|--------------------|
| 1      | Linearity (µg/ml)       | 2.5-15.0           |
| 2      | Slope                   | 1555               |
| 3      | Correlation Coefficient($r^2$) | 0.9992           |
| 4      | LOD(µg/ml)              | 1.0871             |
| 5      | LOQ (µg/ml)             | 3.2942             |

Table 4. Results of recovery studies of tolvaptan

| Sr. No | Recovery Level (%) | Amount of pre analyzed Sample (µg/ ml) | Amount of standards spiked (µg/ ml) | Amount Recovered (µg/ ml) | % Recovery |
|--------|--------------------|----------------------------------------|------------------------------------|--------------------------|------------|
| 1      | 80                 | 5                                      | 4                                  | 8.95                     | 99.44      |
|        |                    |                                        |                                    | 8.89                     | 98.77      |
|        |                    |                                        |                                    | 8.91                     | 99         |
| 2      | 100                | 5                                      | 5                                  | 10.14                    | 101.4      |
|        |                    |                                        |                                    | 10.11                    | 101.1      |
|        |                    |                                        |                                    | 10.08                    | 100.8      |
| 3      | 120                | 5                                      | 6                                  | 10.8                     | 98.18      |
|        |                    |                                        |                                    | 11.14                    | 101.27     |
|        |                    |                                        |                                    | 10.89                    | 99         |
| Average|                   |                                        |                                    |                          | 99.88      |
| SD    |                    |                                        |                                    |                          | 1.246      |
| %RSD  |                    |                                        |                                    |                          | 1.247      |

3.4 Precision

Inter and Intra-day precision based on % RSD values for pure tolvaptan were less than 2%. The value for percentage relative standard deviation are mentioned in Table 5 a,b.

These results pointed that developed method shows good precision and reproducibility.

Assay value of Tolvaptan per tablet was found within the label claimed as mentioned in Table 6.
Table 5a. Inter-day precision

| Drug     | Conc (µg/ml) | R1    | R2    | R3    | R4    | R5    | R6    | % RSD |
|----------|--------------|-------|-------|-------|-------|-------|-------|-------|
| Tolvaptan| 5            | 7579  | 7629  | 7696  | 7586  | 7612  | 7679  | 0.6317|
| Tolvaptan| 10           | 16245 | 16147 | 15986 | 16141 | 16452 | 16357 | 1.029 |
| Tolvaptan| 15           | 23214 | 23658 | 23551 | 23727 | 23548 | 23214 | 0.940 |
|          | Mean         |       |       |       |       |       |       | 0.8669|

Table 5b. Intra-day precision

| Drug     | Conc (µg/ml) | R1    | R2    | R3    | R4    | R5    | R6    | % RSD |
|----------|--------------|-------|-------|-------|-------|-------|-------|-------|
| Tolvaptan| 5            | 7476  | 7598  | 7524  | 7621  | 7593  | 7611  | 0.7598|
| Tolvaptan| 10           | 16321 | 16117 | 16098 | 15986 | 16222 | 16547 | 1.2248|
| Tolvaptan| 15           | 24105 | 24311 | 23895 | 24174 | 23701 | 24184 | 0.9283|
|          | Mean         |       |       |       |       |       |       | 0.9709|

Table 6. Analysis of marketed formulation of tolvaptan

| Sr. No. | Labeled Claim (µg/mL) | Amount Found (µg/mL) | % Label claim |
|---------|------------------------|----------------------|---------------|
| 1       | 15                     | 15.21                | 101.4         |
| 2       | 15                     | 14.95                | 99.66         |
| 3       | 15                     | 14.78                | 98.53         |
| 4       | 15                     | 15.15                | 101.0         |
| 5       | 15                     | 14.77                | 98.46         |
| 6       | 15                     | 14.91                | 99.40         |
| Average |                        |                      | 99.74         |
| SD      |                        |                      | 1.121         |
| %RSD    |                        |                      | 1.123         |

Table 7. Summary of validation Parameters

| Validation Parameter | Tolvaptan |
|----------------------|-----------|
| Linearity (µg/ml)    | 2.5 to 15 |
| Repeatability (% RSD)| 1.274     |
| Correlation coefficient $r^2$ | 0.9992 |
| Regression equation  | $y=1555.6x-136.61$ |
| Intraday precision (n=3) (% RSD) | 0.9709 |
| Interday precision(n=3) | 0.8669 |
| LOD (µg/ml)          | 1.0871    |
| LOQ (µg/ml)          | 3.2942    |
| Assay                | 99.74%    |
| Robustness           | Robust    |

Stability indicating behavior of developed method was checked by injecting stress samples in system. A chromatogram of acid degradation study shows additional degradation product peak along with drug peak at 2.25 min which is very well resolved from drug peak with 1.75% degradation in 0.1 M HCl solution. Chromatogram of alkaline degradation sample shows additional degradation product peak at 3.9 min with sufficient resolution from drug peak it shows 2.6% degradation in the drug peak. Neutral hydrolysis sample did not show any additional peak which indicates that Tolvaptan is stable in neutral conditions. Chromatogram of oxidative degradation sample shows additional degradation product peak at 3.0 min along with Tolvaptan peak with 4.42% degradation in Tolvaptan which shows that Tolvaptan is susceptible to oxidative hydrolysis in presence of hydrogen peroxide. Chromatogram of Thermal degradation shows two additional peaks at 1.2 and 1.3 min respectively with 5.42% degradation.
in drug peak suggest that Tolvaptan can undergo thermal degradation at elevated temperature. Chromatograms of stress conditions are shown in Fig. 6 and percent degradation of drug peak at different stress conditions are shown in Table 7.

4. DISCUSSION

A DoE assisted, simple, and fast stability indicating RP-HPLC method was developed with help of CCD and response Surface methodology. This systematic experimentation technique saves chemicals, time, cost for new method development. Developed method was validated as per ICH guidelines and all the parameters are within specified limits of regulatory requirements. Stability indicating behavior was confirmed from the fact that degradation peaks were very well separated from drug peak.

![Stress degradation chromatograms of tolvaptan](image)

**Fig. 6. Stress degradation chromatograms of tolvaptan**
- a. Acidic Condition
- b. Alkaline Condition
- c. Oxidative Stress
- d. Thermal degradation

**Table 8. Results of % degradation of tolvaptan at different stress conditions**

| Sr. No. | Stress Condition      | Number of Peaks | % Drug     | % DP  |
|---------|-----------------------|-----------------|------------|-------|
| 1       | Standard Control      | 01              | 100        | 0     |
| 2       | Acid Degradation      | 02              | 98.25      | 1.75  |
| 3       | Base Degradation      | 02              | 97.14      | 2.86  |
| 4       | Peroxide Degradation  | 02              | 95.58      | 4.42  |
| 5       | Thermal Degradation   | 02              | 94.58      | 5.42  |
Linearity- Found from 2.5-15 mcg/ml value of correlation coefficient was near to 1 which suggest linear relationship between concentration and response variable. Precision- % RSD values for Intera day and Intrada day were found below 2 which suggest precision of the method. Accuracy- Percentage Recovery value was found between 98-102% suggesting no interference of excipients in quantitation. LOD and LOQ- The value calculated of LOD & LOQ were very less which shows sensitivity of the method.

Specificity- The chromatogram showed no interfering peak at retention time.

5. CONCLUSION

The proposed approach is accurate, precise, fast, for designing and optimizing a suitable experimental condition and developed method is selective for the simultaneous estimation of Tolvaptan in bulk and solid dosage form. The method can be used as a stability indicating method as degradation products are resolved from the drug peaks. Hence it could be conveniently adapted for the routine quantitative estimation and studying stability of Tolvaptan.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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