A REVIEW ON DIFFERENT HPLC ANALYSIS METHOD OF VORICONAZOLE

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Abstract: This review article was prepared with an aim to review different methods developed for Voriconazole drug by HPLC analytical method and strategies developed and optimized for efficient method development. There is a continuous research in this field and different researchers are exploiting different mobile phase combinations for their research work. Voriconazole is generally used as an antifungal drug under the brand name Vfend. The use of the Voriconazole as a drug essential in pharmaceutical formulations highlights the requirement for its determination and quantification with appropriate analytical methods.

Keywords: HPLC, Voriconazole

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INTRODUCTION

Literature survey revealed that method development by HPLC has been taken in plasma samples, solid, liquid and semisolid dosage formulations for estimation of antifungal drug voriconazole. Till now few methods have been developed for estimation of antifungal drugs voriconazole. Thus the aim of this article is to be to discuss various high performance liquid chromatographic methods developed by various researchers for determination of these antifungal drugs with different mobile phase combinations.

M. Vamsi Krishna et al. developed an RP-HPLC method for evaluation of in vitro permeability of voriconazole in the presence of enhancers through rat skin. In this method an isocratic RP-HPLC–UV method for the analysis of voriconazole in skin diffusate samples has been developed and validated. In this method experimental design was employed to optimize the method. The method was validated as per ICH guidelines. Linearity was observed over the concentration range of 2–100 lg mL_1 (r² = 0.999). Limits of detection and quantification were 0.6 and 2 lg mL_1, respectively. Intra-day and inter-day precision (% RSD) was within the ICH limits (62%). The method was successfully used to analyze skin diffusate samples, and the effectiveness of different permeation enhancers was compared with respect to flux and permeability coefficient.

The main purpose of this research was to develop and validate an isocratic RP-HPLC method for the quantitative analysis of VCZ in skin diffusate samples as well as to evaluate the enhancing effects of three penetration enhancers, i.e., DMSO, 1% SLS, and eucalyptus oil on the percutaneous absorption of VCZ through rat skin in vitro.1-6

B. Sridhar et al. validated RP-HPLC method for the Estimation of Voriconazole in Bulk and tablet dosage form. In this a simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Voriconazole in bulk and tablet dosage form. Isocratic elution was done at a flow rate of 1 mL/min on a Inertsil C8 column (250 x 4.6 mm; 5μ) at ambient temperature. The mobile phase consisted of Buffer (0.01M sodium dihydrogen orthophosphate, pH was adjusted to 5.0):Acetonitrile (50:50 v/v) the UV detection wavelength was 254 nm and 20μl of sample was injected. The retention time for Voriconazole was found to be 6.905 min. The % recovery was within the range between 99.73% and 99.85%. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Voriconazole in bulk samples and its formulations.

In this method important parameters found are given in the Table 1 and Table 2 and the chromatogram obtained is given in Fig. 1

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Table 1: Statistical analysis of calibration curves in the HPLC determination of Voriconazole (n=6) Parameters Values

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>λ max (nm)</td>
<td>1.11</td>
</tr>
<tr>
<td>2</td>
<td>Beer’s law limit (μg/ml)</td>
<td>25-75</td>
</tr>
<tr>
<td>3</td>
<td>Correlation coefficient</td>
<td>0.9996</td>
</tr>
<tr>
<td>4</td>
<td>Regression equation</td>
<td>Y = 24.875X+1.65</td>
</tr>
<tr>
<td>5</td>
<td>Limit of detection (μg/ml)</td>
<td>0.65</td>
</tr>
<tr>
<td>6</td>
<td>Limit of quantification (μg/ml)</td>
<td>1.98</td>
</tr>
</tbody>
</table>

Table 2: System suitability and study of Voriconazole

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tailing factor</td>
<td>1.11</td>
</tr>
<tr>
<td>2</td>
<td>Asymmetric factor</td>
<td>1.20</td>
</tr>
<tr>
<td>3</td>
<td>Theoretical plates</td>
<td>5622</td>
</tr>
<tr>
<td>4</td>
<td>Capacity factor</td>
<td>1.50</td>
</tr>
<tr>
<td>5</td>
<td>HETP</td>
<td>0.0366</td>
</tr>
</tbody>
</table>

Fig.1 Typical chromatogram obtained from the analysis of voriconazole standard solution

Saidulu Goli et al. developed and validated voriconazole for Injection by RP-HPLC Method. In this a new simple, precise, accurate and selective RP-HPLC method has been developed and validated for voriconazole in parental dosage form. The method was carried out on an Intersil ODS-C18 (150X4.6X5μ) column with a mobile phase consisting of mixed phosphate buffer, ACN and Methanol (65:30:5) and flow rate of 2.0 mL min⁻¹. Detection was carried out at 257 nm.
The retention time for VCZ was found to be 6.413 min. The VCZ % recovery was within the range between 99.55-99.63%. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per ICH guidelines. The developed method was validated for precision, accuracy, sensitivity and robustness. The developed method can be used for routine analysis of titled drug in formulation.

In this method important parameters found are given in the table 3 and table 4 and the chromatogram obtained is given in Fig.2 and Fig.313-18

Table 3 Accuracy data

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc.(ppm)</th>
<th>Standard concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80%</td>
<td>7.94</td>
</tr>
<tr>
<td>2</td>
<td>90%</td>
<td>8.93</td>
</tr>
<tr>
<td>3</td>
<td>100%</td>
<td>9.92</td>
</tr>
<tr>
<td>4</td>
<td>110%</td>
<td>10.92</td>
</tr>
<tr>
<td>5</td>
<td>120%</td>
<td>11.91</td>
</tr>
</tbody>
</table>

Table 4 System suitability parameters

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>%R.S.D</td>
<td>0.12</td>
</tr>
<tr>
<td>2</td>
<td>%Tailing factor</td>
<td>1.04</td>
</tr>
<tr>
<td>3</td>
<td>%No of theoretical plates</td>
<td>8.022</td>
</tr>
</tbody>
</table>

Fig.2 Chromatographic conditions
F. Péhourcq et al. developed direct injection HPLC micro method for the determination of voriconazole in plasma using an internal surface reversed-phase column. The method was found to be easy to perform and requires 10 µL of a plasma sample. The chromatographic run time was less than 9 min using a mobile phase of 17:83 v/v acetonitrile–potassium dihydrogen phosphate buffer, 100 mm, pH 6.0 and UV detection at 255 nm. The flow rate was 1 mL/min. A linear response was observed over the concentration range 0.5–10 µg/mL. A good accuracy (bias ≤7.5%) was achieved for all quality controls, with intra-day and inter-day variation coefficients inferior to 6.7%. The lower limit of quantitation was 0.2 µg/mL, without interference of endogenous components. In this method stability of voriconazole in plasma stored at different temperatures was checked. Finally, the possibility of direct injection of plasma samples into the column permits a reduction in reagent consumption and in analytical steps, and hence in analytical error. The chromatogram obtained during the study is given in figure 4.

Figure 4. Chromatogram obtained from the study
Yuru Li et al. developed and validated a stability-indicating HPLC Method for Determination of Voriconazole and its Related Substances. An isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination of voriconazole and its related substances. The drug substance was subjected to stress conditions of UV light, water hydrolysis, acid, base, oxidation, and deoxidization to observe the degradation products. The successful separation of voriconazole from its synthetic impurities and degradation products formed under stress conditions was achieved using an Agilent Zorbax SB-C18 (250mm × 4.6 mm i.d., 5 μm) column maintained at 25°C with a mobile phase of a mixture of ammonium phosphate dibasic buffer (pH adjusted to 6.0 using diluted orthophosphoric acid; 50 mM)–acetonitrile (52:48, v/v). The mobile phase flow rate was 1.0 mL/min, and the detection wavelength was 250 nm. The stress sample solutions were assayed against the qualified reference standard of voriconazole and the mass balance in each case was close to 99.7%, confirming its stability-indication capacity. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The developed HPLC method to determine the related substances and assay determination of voriconazole can be used to evaluate the quality of regular production samples. It can be also used to test the stability samples of voriconazole. Important parameters found in the study are given in table 5 and table 6.

### Table 5. Recovery Results of Voriconazole Sample

<table>
<thead>
<tr>
<th>Added (μg) (n = 3)*</th>
<th>Recovered (μg)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.3</td>
<td>25</td>
<td>98.8</td>
<td>0.9</td>
</tr>
<tr>
<td>50.6</td>
<td>50.8</td>
<td>100.4</td>
<td>0.6</td>
</tr>
<tr>
<td>7.5</td>
<td>74.8</td>
<td>99.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* n = number of determinations

### Table 6 Summary of Forced Degradation Results

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Time</th>
<th>% Assay of active substance</th>
<th>% Mass balance (% assay + impurities)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid hydrolysis (0.5N HCl)</td>
<td>48 h</td>
<td>87.9</td>
<td>99.7</td>
<td>Degraded to imp-A and imp-D</td>
</tr>
<tr>
<td>Base hydrolysis (0.5N NaOH)</td>
<td>48 h</td>
<td>0</td>
<td>99.8</td>
<td>Degraded to imp-A, imp-D, and other unknown degradants</td>
</tr>
<tr>
<td>Oxidation (3% H2O2)</td>
<td>48 h</td>
<td>91.9</td>
<td>99.6</td>
<td>Degraded to imp-D and other unknown degradants</td>
</tr>
<tr>
<td>Deoxidization (3.0% NaHSO3)</td>
<td>10 days</td>
<td>99.6</td>
<td>99.6</td>
<td>No degradation products formed</td>
</tr>
<tr>
<td>Water hydrolysis at 60°C</td>
<td>48 h</td>
<td>77.5</td>
<td>99.7</td>
<td>Degraded to imp-A and imp-D</td>
</tr>
<tr>
<td>UV (254 nm)</td>
<td>48 days</td>
<td>99.6</td>
<td>99.6</td>
<td>No degradation products formed</td>
</tr>
</tbody>
</table>
Gennethel J. Pennick et al. developed and validated a High-Performance Liquid Chromatography Assay for Voriconazole. In this analytical method for the determination of voriconazole (UK-109,496; Pfizer) in plasma was developed and validated. The method utilizes solid-phase extraction technology and high-performance liquid chromatography. The lower limit of quantitation is 0.2 µg/ml, and the range of linearity tested was 0.2 to 10 µg/ml.

Important parameters found in the study are given in figure 5.

![Figure 5](image)

**Figure 5** Representative chromatograms of VRC and the internal standard

Rafael Linden et al. developed Ultra-Performance Liquid Chromatographic Method for Measurement of Voriconazole in Human Plasma and Oral Fluid. In this a simple, sensitive and selective ultra-performance liquid chromatography method for the determination of voriconazole in plasma and oral fluid was developed and validated. After a liquid-liquid extraction with methyl-tert-butyl ether, the analyte and internal standard were separated on a Hypersil Gold C18 column (2.1 × 100 mm, p.d. 1.9 μm), eluted with a mobile phase composed of thietylammonium phosphate buffer and acetonitrile (70:30, v/v). Total run time was found to be 4 min, total mobile phase consumption of 2.2 mL. Detection was performed with a photodiode array detector with quantitation at 256 nm. Voriconazole concentrations in oral fluid were on average 57.5% (± 5.3) of those measured in paired plasma samples.

Important parameters found in the study are given in figure 6.
Michael Vogeser et al. quantified voriconazole in plasma by liquid chromatography-tandem mass spectrometry. A convenient liquid chromatography-tandem mass spectrometry method for the quantification of the triazole antifungal agent voriconazole in plasma samples is described. Fenbuconazole is used as an internal standard. After protein precipitation, automated solid-phase extraction is applied. Electrospray ionization in the positive mode is used. The analytical run time is 4 min. The response was linear from 78 to 5000 mg/L. The total coefficient of variation (ns16) was 12.6% for a low-concentration pool (143 mg/L), 4.7% for a medium-concentration pool (419 mg/L), and 5.0% for a high-concentration pool (4304 mg/L). The method is proposed for future investigations that should be performed to test the hypothesis that therapeutic drug monitoring of voriconazole is clinically useful. Important parameters found in the study are given in figure 7.
Figure 7 Representative chromatogram of voriconazole (350)126.6) and fenbuconazole (337)125) used as the internal standard (concentration of voriconazole, 143 mg/L; the Y-axis represents the relative intensity of the signal with respect to the base peak of the chromatogram).33-37

Peter H Tang et al. quantified Antifungal Drug Voriconazole in Serum and Plasma by HPLC-UV. In this a simple, sensitive and rapid high-performance liquid chromatographic (HPLC) method for the determination of voriconazole in human serum or plasma was developed. Voriconazole and internal standard clonazepam were extracted from plasma or serum with methanol and analyzed on a Microsorb-MV C18 column with ultraviolet (UV) detection set at wavelengths of 256 and 310 nm, respectively. The calibration curve was linear through the range of 0.1-10 mg/L using a 0.1 mL sample volume. The within-run and between-run precisions were all less than 6%. Accuracies ranged from 97 to 106%. Absolute recovery was 96.4 ± 1.3% for voriconazole. The method has been applied to monitor voriconazole use in order to ascertain clinical efficacy and minimize toxic effects.38-44

Reehana Shaik et al. developed an RP-HPLC method for quantitative estimation of voriconazole for injection in pharmaceutical dosage forms. In this a simple RP-HPLC method for the determination of voriconazole in pharmaceutical dosage forms. Numerous HPLC conditions were tested for determination of voriconazole. The best result was achieved by using inertsil ods-2, c-18 (150×4.6mm) 5μm column and a mobile phase consisting of OPA: acetonitrile: methanol(65:30:5 v/v/v), a flow rate of 2.0 ml/min with ultraviolet detection at 257nm. The retention time of the drug was 6.438 min. The method produced liner responses in the concentration range of 7 to 12 ppm of voriconazole. The method was found to be applicable for determination of the drug in injection. Important parameters found in the study are given in figure 8 and table7.45-48
Mrinalini C. Damle et al. determined Voriconazole in Human Plasma by High Performance Liquid Chromatography with Ultraviolet Detection. In this a simple, selective, and sensitive high performance liquid chromatography method for the determination of voriconazole in human plasma was developed. Fluconazole was used as an internal standard. The method utilizes liquid-liquid extraction with n-hexane:ethyl acetate (3:1 v/v) as the sample preparation technique. The samples were then analyzed on Neosphere C18 column with UV detection at 254 nm. The calibration curve was linear through the range of 0.2-12 μg/ml. The lower limit of quantification was found to be 0.2 μg/ml. % R.S.D. was less than 3% for intra- and inter-day precision. The mean recovery was found to be 93.09% for voriconazole. The method showed acceptable values for accuracy, precision, recovery, sensitivity and stability. The method is well...
suited for routine analysis of voriconazole in human plasma and can further be extended for pharmacokinetic studies.\textsuperscript{49-58}

Kabeer a. shaikh et al. validated Stability-Indicating Liquid Chromatographic Method for determination of degradation Impurities and Diastereomers in Voriconazole Tablets. In this a reversed-phase gradient liquid chromatographic method has been developed for the quantitative determination of Voriconazole, along with its degradation and diastereomeric impurities in tablet dosage form. Chromatographic separation has been achieved on an Inertsil ODS 3V, 150 x 4.6 mm, 5 \( \mu \)m column. The mobile phase consisting of solvent A 0.05 molar (M) potassium dihydrogen phosphate (pH 2.5 buffer) and solvent B (mixture of acetonitrile and methanol in the ratio 90:10 (v/v)), was delivered at a flow rate of 1.2 mL min\(^{-1}\) with the detection wavelength at 256 nm. Resolution of Voriconazole and all five potential impurities was achieved at greater than 2.0 for all pairs of compounds. The drug was subjected to stress conditions such as oxidative, acid and base hydrolysis, and thermal and photolytic degradation. Voriconazole was found to degrade significantly under base hydrolysis stress conditions compared to acid hydrolysis stress conditions. The degradation products were well-resolved from the main peak and its impurities, thus proving the stability-indicating power of the method. The stressed samples were assayed against a reference standard and the mass balance was found to be close to 99.0\%. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision, and robustness.\textsuperscript{58-60}

**CONCLUSION**

It can be concluded from the entire review that HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules.

HPLC method development has taken place of voriconazole drug in different drug formulations and there is huge potential for new methods to be developed. Research is a continuous process and several regulatory agencies require the submission of analytical data and hence method development gives an advantage for the analysis of drugs. Voriconazole is an antifungal drug. In this article different HPLC methods of voriconazole are presented with different parameters found after the research work, the parameters analysed are accuracy, precision, LOD, LOQ, system suitability, robustness parameters, if we compare these methods we came to the conclusion that these is huge potential of developing a HPLC method for the drug voriconazole in order to increase accuracy, system suitability and precision by overcoming the loopholes of the present methods and lower limits of LOD and LOQ can be achieved by choosing suitable mobile phase combinations and altering the flow rate and column parameters.
REFERENCES


