Arabian Journal of Chemistry (2017) **10**, S355–S360

King Saud University

**Arabian Journal of Chemistry**

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

# RP-HPTLC method for determination of Voriconazole in bulk and in cream formulation

**Manali W. Jain [1], Atul A. Shirkhedkar [*], Sanjay J. Surana**

*R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule 425 405 (M.S), India*

Received 19 April 2012; accepted 19 September 2012
Available online 28 September 2012

**KEYWORDS**

Voriconazole;
RP-HPTLC;
Validation

**Abstract** Voriconazole is used as an antifungal agent. A new rapid, simple, economical and environmental friendly Reversed -Phase High-Performance Thin-Layer Chromatography (RP-HPTLC) has been developed and validated for quantitative determination of voriconazole in bulk and in cream formulation. RP-HPTLC separation was performed on aluminium plates precoated with silica gel 60RP-18F-$_{254}$S as the stationary phase using Acetonitrile: Water (60:40% *v*/*v*) as mobile phase. Quantification was achieved by densitometric analysis at 257 nm over the concentration range of 200–1200 ng/band. The method was found to give compact and well resolved band for Voriconazole at Retention factor ($R_f$) 0.48 ± 0.02. The linear regression analysis data for calibration graph showed good linear relationship with $r^2$ = 0.999. The method was validated for precision, recovery, robustness, ruggedness and sensitivity as per International conference on Harmonization (ICH) guidelines. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 19.99 ng and 60.60 ng, respectively. The proposed developed RP-HPTLC method can be applied for identification and quantitative determination of Voriconazole in bulk and in cream formulation.



## 1. Introduction

Voriconazole is designated as a (αR,βS)-α-(2,4-Difluorophenyl)-5-fluoro-β-methyl-α-(1*H*-1,2,4-triazol-1-yl-methyl)-4-pyrimideethanol (Fig. 1). It is used as an antifungal agent (The Merck Index 2006) and its primary mode of action is by inhibition of the fungal cytochrome P450-dependent 14α-sterol demethylase, an essential enzyme in ergosterol biosynthesis (Block and Beale, 2004).

In the literature, several methods have been described for determination of Voriconazole in biological fluids including HPLC assay of voriconazole in human plasma (Pennick et al. 2003), Simultaneous determination of Voriconazole and Itraconazole and its hydroxy metabolite in human serum using

* Corresponding author. Address: Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule 425 405 (M.S), India. Tel.: +91 9823691502.
E-mail addresses: manali.jn23@gmail.com (M.W. Jain), atulshirkhedkar@rediffmail.com (A.A. Shirkhedkar), sjsurana@yahoo.com (S.J. Surana).
[1] Department of Quality Assurance, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule 425 405 (M.S), India.

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2012.09.006
1878-5352 

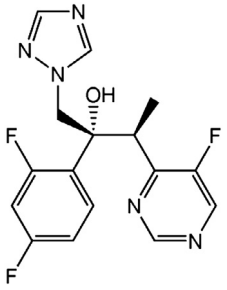

**Figure 1** Chemical structure of Voriconazole.

HPLC (Khoschosour et al. 2005), and HPTLC method for determination of voriconazole in human plasma using toluene : methanol : triethylamine (6:4:0.1 *v*/*v*/*v*) as mobile phase. (Dewani et al. 2011). Some methods are reported for determination of Voriconazole in pharmaceutical preparations such as Liquid chromatography using prominence diode array detector and mobile phase of water : acetonitrile (35:65 % *v*/*v*) (Patel et al. 2009), HPLC method for determination of Voriconazole using UV–visible detector and mobile phase of buffer : acetonitrile (50:50 % *v*/*v*) (Bharati et al., 2010), Stability indicating HPLC method (Wamorkar et al. 2010, Eldin et al. 2010), Polarography using a 0.01 M $KH_2PO_4$ water solution (pH 4.5) as supporting electrolyte (Gianfranco et al. 2009), Comparison of Microbiological and UV-Spectrophotometric Assays for Determination of Voriconazole in Tablets (Adams et al. 2006), and HPTLC determination in bulk and formulation (Khetre et al. 2008).

So far no RP-HPTLC method for the analysis of Voriconazole has been reported. Therefore, in the present research paper a simple, accurate, sensitive and precise RP-HPTLC method has been developed for determination of Voriconazole in the bulk and in cream formulation.

## 2. Experimental

### 2.1. Materials and reagents

Voriconazole was supplied as a gift sample from Glenmark Pharmaceutical LTD, Sinnar, and Nasik, India. All chemicals and reagents used were of Analytical grade and were purchased from Merck Chemicals, India.

### 2.2. Chromatographic conditions

The plates were prewashed with methanol and activated at 100 °C for 5 min prior to chromatography. The drug standard and samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on precoated silica gel aluminium plates 60 RP-18 $F_{254}$ S(20 × 10 cm with 200 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The slit dimension was kept at 6.00 × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of Acetonitrile: Water (60:40 *v*/*v*), and 10 mL of mobile phase was used. Linear ascending development was carried out in a 20 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25 °C ± 2). The length of the chromatogram run was approximately 80 mm. After development; the HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by winCATS software (Version 1.3.0).

### 2.3. Preparation of standard solution and linearity study

Stock standard solution was prepared by dissolving 10 mg of Voriconazole in 10 mL methanol to obtain concentration 1 mg/mL. Aliquots of standard solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL of Voriconazole were transferred into six separate 10 mL volumetric flasks and volumes were made up to the mark using same solvent. An appropriate volume 10 µL was applied with the help of microlitre syringe, using Linomat 5 applicator on RP-HPTLC plate to obtain concentrations of 200, 400, 600, 800, 1000 and 1200 ng/band. The standard curves were assessed for within day and day-to-day reproducibility. Each experiment was repeated for six times.

## 3. Method validation

### 3.1. Precision

Precision can be performed at two different levels i.e. repeatability and intermediate precision. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same band (600 ng/band of Voriconazole). The intermediate precision results from the variations such as different days, analysts and equipments. The intra-day variation experiments were studied using three different concentrations over the linearity range within same day. The inter-day variations in the methods were assessed by studying three different concentrations for three different days over a period of week. The intra and inter-day variation for the determination of Voriconazole was done at three different concentration levels of 400, 600, and 1000 ng/band.

### 3.2. Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine limit of detection and limit of quantification, Voriconazole concentrations in the lower part of the linear range of the calibration curve were used. Voriconazole solutions of 200, 240, 280, 320, 360 and 400 ng/band were prepared and applied on RP-HPTLC plate. The LOD and LOQ were calculated using equation LOD = 3.3 × N/B and LOQ = 10 × N/B, where, '*N*' is standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of noise, and '*B*' is the slope of the corresponding calibration curve.

### 3.3. Specificity

The specificity of the method was determined by examining Voriconazole standard and Voriconazole extracted from the cream formulation. The spot for Voriconazole in sample was confirmed by comparing the $R_f$ values and spectra. The peak-purity of Voriconazole was assessed by comparing the spectra at three different levels, i.e., peak- start (*S*), peak- apex (*M*) and peak- end (*E*) positions of the band.

### 3.4. Ruggedness

Ruggedness of the method was performed by spotting 600 ng/band of Voriconazole by two different analysts keeping same experimental and environmental conditions.

### 3.5. Accuracy

The pre- analysed samples were spotted with extra 80 %, 100% and 120% of the Voriconazole standard and the mixtures were re-analysed by the proposed method. This was performed to check the recovery of the drug at different levels in the formulations.

### 3.6. Robustness

Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate various in method parameters. By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different compositions of Acetonitrile: Water (58:42 *v*/*v*), Acetonitrile: Water (62:38 *v*/*v*) tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity varied in the range of ± 5%. The plates were prewashed with methanol and activated at 100 ± 5 °C for 2, 5 and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning varied from 20, 30 and 40 min.

### 3.7. Application of proposed method to cream formulation

To determine the concentration of Voriconazole in cream (Labelled claim: 1% voriconazole), 3 g of cream formulation was accurately weighed and transferred into 50 mL volumetric flask, to it 5 mL of tetrahydrofuran was added and heated on a water bath at 60 °C, cool and volume was made up to the 50 mL mark with solvent (Acetonitrile: Water 50:50 *v*/*v*). The resulting solution was filtered using 0.41 µm filter (Millifilter, Milford, MA). From it, appropriate volume 2 mL was diluted to 10 mL with methanol. The appropriate volume 5 µL was applied on RP-HPTLC plate. The experiment was repeated six times.

## 4. Results and discussion

### 4.1. Development of optimum mobile phase

Different compositions of the mobile phase for RP-HPTLC analysis were experimented with an objective to obtain high resolution and reproducible peaks. The required objective was achieved using Acetonitrile: Water (60:40 % *v*/*v*) as the mobile phase. The wavelength of 257 nm was found to be optimal for the highest sensitivity. Sharp and well defined peaks for the Voriconazole were obtained at $R_f$ 0.48 ± 0.02 when the chamber was saturated with mobile phase for 25 min at room temperature. The chromatogram of Voriconazole standard is shown in Fig. 2.

### 4.2. Calibration curve

The acceptability of linearity data is often judged by examining the correlation coefficient and intercept of the linear regression line for the response versus concentration plot. The linear regression data for the calibration curves showed good linear relationship over the concentration range 200–1200 ng/band.

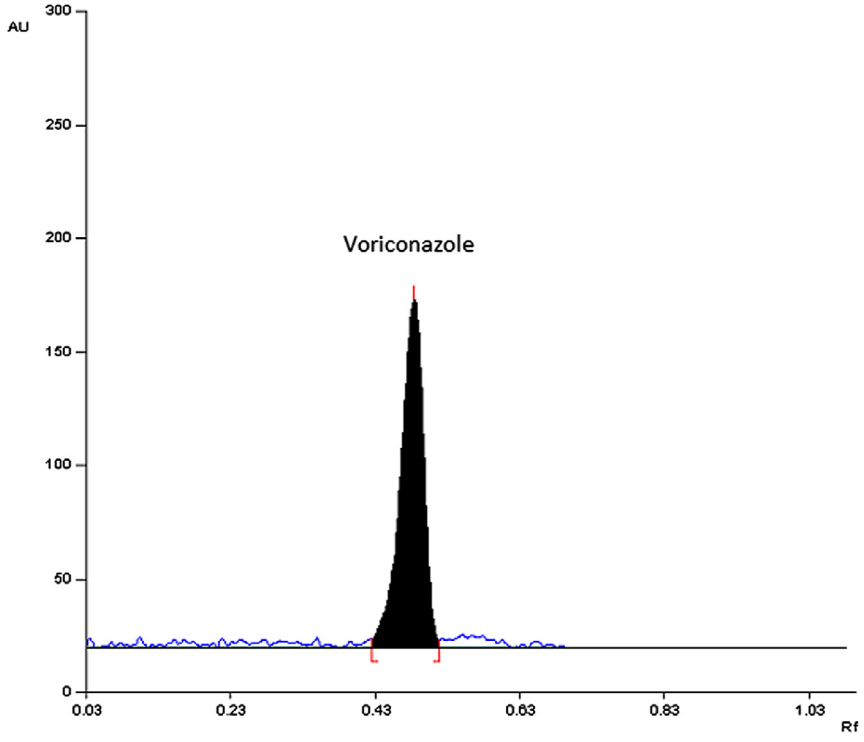


**Figure 2** Chromatogram of Voriconazole standard 1000 ng/band ($R_f$ 0.48 ± 0.02) in Acetonitrile: Water (60:40 *v*/*v*) as mobile phase scanned at UV detection at 257 nm.

**Table 1** Intra-day and Inter-day precision of RP-HPTLC method.

| Drug | Conc. ng/band | Intra-day | | Inter-day | |
|---|---|---|---|---|---|
| | | %Amount found[a] | % R.S.D. | % Amount found[a] | % R.S.D |
| Voriconazole | 400 | 98.82 | 0.93 | 98.99 | 1.03 |
| | 600 | 101.34 | 0.53 | 101.03 | 0.80 |
| | 1000 | 100.46 | 0.60 | 100.30 | 0.90 |

[a] Mean of three estimations.

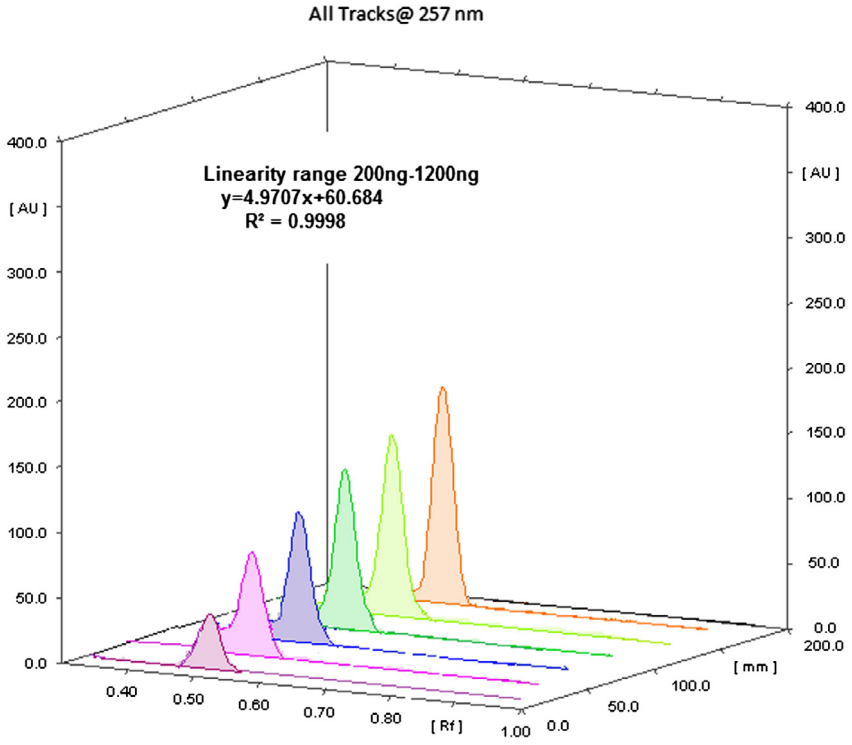


**Figure 3** 3D Calibration linearity graph of Voriconazole (200–1200 ng/band).

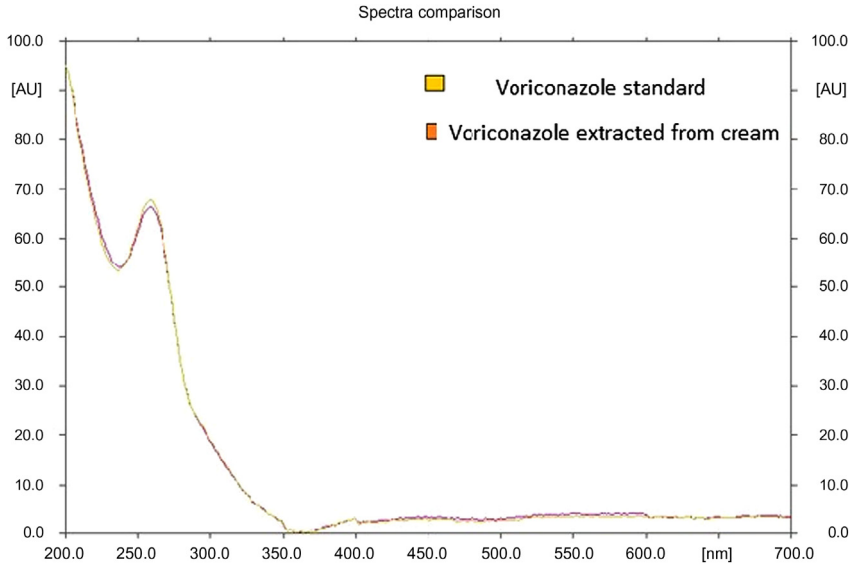


**Figure 4** Comparison of Voriconazole standard and Voriconazole extracted from Cream.

**Table 2** Recovery study.

| Drug/Label claim | Initial amount of drug (ng/band) | Amount of standard drug added (%) | % Recovery[a] | %RSD |
|---|---|---|---|---|
| Voriconazole 1%(*w*/*w*) | 500 | 80 | 98.47 | 1.06 |
| | | 100 | 101.10 | 0.85 |
| | | 120 | 100.31 | 0.61 |

[a] Mean of three estimations at each level.

Linear regression equation was found to be Y = 4.9707X + 60.684 (Fig. 3.) The regression coefficient ($r^2$ = 0.999) is generally considered as evidence of acceptable fit.

### 4.3. Validation of method

#### 4.3.1. Precision

The precision of the developed RP-HPTLC method was expressed in terms of % relative standard deviation (%RSD). The % RSD value for repeatability of sample application and amount of Voriconazole was estimated and was found to be less than 2. The results depicted revealed high precision of the method and are presented in Table 1.

#### 4.3.2. LOD and LOQ

Detection limit and quantification limit were calculated by the method as described above. The LOD and LOQ were found to be 19.99 ng and 60.60 ng respectively. This indicates that the sensitivity of the method is adequate.

**Table 3** Robustness of the method.

| Parameters | %RSD of peak area |
|---|---|
| Mobile phase composition (±2 mL) | 0.44 |
| Development distance (±0.5 cm) | 0.62 |
| Duration of saturation (±2 min) | 1.18 |
| Time from spotting to chromatography (±10 min) | 0.52 |
| Time from chromatography to scanning (±10 min) | 0.45 |

#### 4.3.3. Specificity

The peak purity of Voriconazole was assessed by comparing the spectra at peak-start, peak -apex and peak- end positions of the spot, i.e., $r^2$ (*S*, *M*) = 0.996 and $r^2$ (*M*, *E*) = 0.998. Excellent correlation ($r^2$ = 0.999) was also obtained between standard and sample spectra of Voriconazole Fig. 4.

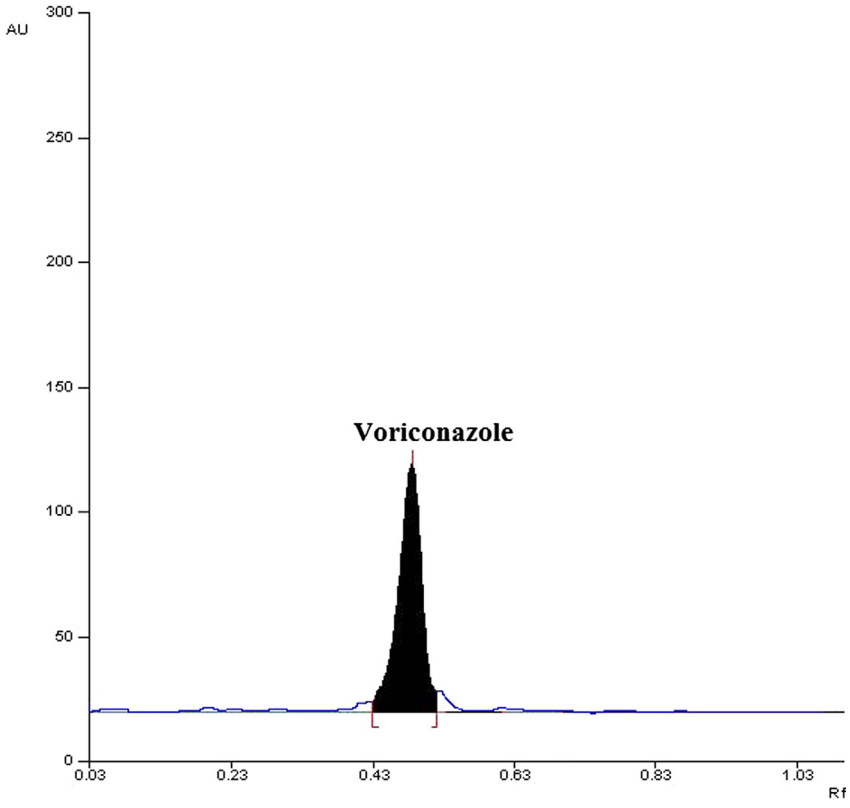


**Figure 5** Chromatogram of Voriconazole extracted from cream 600 ng/band ($R_f$ 0.48 ± 0.02) in Acetonitrile: Water (60:40 *v*/*v*) as mobile phase scanned at UV detection at 257 nm.

*4.3.4. Ruggedness*

The method was executed out by two different analysts under the same experimental and environmental conditions; the results were calculated in terms of % RSD of amount found. The % RSD was found to be less than 2 which indicate that the method is rugged.

*4.3.5. Recovery study*

The accuracy of the method is studied to measure that other components in the pharmaceutical formulation do not interfere with analytical method.

The proposed method when used for extraction and subsequent quantification of Voriconazole from the cream formulation after over spotting with 80%, 100% and 120% of additional drug, gives excellent recovery of Voriconazole. The amounts of drug added were determined and the % recovery is shown in Table 2. The results obtained indicate that other components do not interfere with the analytical method.

*4.3.6. Robustness of the method*

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The low value of % RSD, Table 3 indicates the reliability of analytical method during normal usage.

*4.3.7. Assay of Cream formulation*

The chromatogram of Voriconazole after extraction from cream formulation showed as single spot at $R_f$ 0.48 ± 0.02. It was further observed that there was no interference from the excipients commonly present in the cream formulation. (Fig. 5) The % drug content ± S.D. was found to be 99.88 ± 0.91. The amount of drug estimated was found to be in close agreement with label claim which indicates the suitability of this method for routine analysis of Voriconazole in pharmaceutical dosage forms.

## 5. Conclusion

The present RP-HPTLC method is precise, specific, sensitive and accurate. Statistical analysis proved the method is reproducible and selective for analysis of voriconazole in the bulk drug and in cream formulations. The method can be used to determine the purity of the commercially available drug. The additives usually present in the cream formulations of the assayed samples did not interfere with determination voriconazole.

## Acknowledgements

Authors are thankful to the Glenmark Pharmaceutical Ltd. Nasik (MS) India for providing Voriconazole as gift sample and R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur Dist: Dhule (MS) for providing necessary facilities to carry out this work.

## References

Adams, A.L., Steppe, M., Froehlich, P.E., Bergold, A.M., 2006. J. AOAC Int. 89 (4), 960–965.

Bharathi, J., Sridhar, B., Jitendra, P., Upendra, U., Nagarju, P., Rao, H.K., 2010. International Journal of Research in Pharmaceutical and Biomedical. Sciences 1 (1), 14–18.

Block, J.H., Beale, J.M., 2004. Wilson and Gisvold's, Textbook of organic Medicinal and Pharmaceutical Chemistry, eleventh ed. Lippincott Williams &Wilkins, Philadelphia PA.

Dewani, M.G., Borole, T.C., Gandhi, S.P., Madgulkar, A.R., Damle, M.C., 2011. Der Pharma chemica 3 (4), 201–209.

Eledin, A.B., Shallaby, A.A., Magda, Y.M., 2010. Acta Pharmaceutica Sciencia 52, 229–238.

Gianfranco, C., Assunta, Z., Caterina, R., D'Arpino, L., Racheleb, A., Elena, M.D., 2009. Curr. Anal. Chem. 5 (3), 238–243.

Khetare, A.B., Darekar, R.S., Snha, P.K., Jeswani, R.M., DamLe, M.C., 2008. Rasayan J. Chem. 1 (3), 542–547.

Khoschosour, G.A., Friwirth, F., Zelzer, S., 2005. Antimicrob. agent chemother. 49 (8), 35–69.

Patel, C.N., Dave, J.B., Patel, J.V., Panigrahi, B., 2009. Indian J. Pharm. Sci. 6 (71), 699–702.

Pennick, G.J., Clark, M., Sutton, D.A., Rinaldi, M., 2003. Antimicrob. Agents Chemother. 47 (7), 2348–2350.

The Merck index., 2006 an Encyclopedia of chemicals, fourteenth ed. White house station, NJ, USA.

Wamorkar, V.V., Ramma, C.S., Manjuanth, S.Y., Reddy, V.M., 2010. Int. J. Pharm. Sci. 3 (1), 978–982.