Validated chiral high-performance liquid chromatography method for a novel anti-methicillin-resistant staphylococcus aureus fluoroquinolone WCK 771


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Abstract

A sensitive, simple, specific, precise, accurate and rugged method for the assay and determination of enantiomeric purity of S–(−)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid l-arginine salt tetrahydrate (WCK 771) in bulk drug has been developed. The method is RP-HPLC using endcapped C-18 stationary phase and chiral mobile phase. Chirality to the mobile phase was imparted with addition of /H9252\-cyclodextrin. The UV–vis detector was operated at 290 nm. The flow rate of mobile phase was 2 ml/min.

The method offers excellent separation of two enantiomers with resolution more than 2 and tailing factor less than 1.5. The method was validated for the assay of WCK 771 and quantification of R–(+)–enantiomer impurity in bulk drug. The precision (RSD) of the assay was 0.23%. The limit of detection and limit of quantitation of the method for WCK 771 were 0.015 and 0.06 g/ml, respectively. The limit of detection and limit of quantitation for R–(+)–enantiomer were 0.025 and 0.09 g/ml, respectively. The average recovery of the R–(+)–enantiomer was 100.5%. Same method was applied for the assay and determination of enantiomeric purity of WCK 771 in the intravenous formulation.

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Keywords: WCK 771; Enantiomeric purity; Chiral mobile phase additive

1. Introduction

A broad group of fluoroquinolones and quinolones is used as antibacterial agents in clinics. Many of these compounds are chiral in nature. Chirality to these compounds is either imparted by presence of stereogenic center in the core part (e.g. flumequin, ofloxacin, WCK 771) or side chain part (e.g. lomefloxacin, sparfloxacin, clinafloxacin, gatifloxacin, moxifloxacin, etc.). Several methods have been reported for enantiomeric separation of these compounds [1]. These methods include derivatization to diastereomers [2–5], chiral mobile phase based on ligand exchange [6–8] and chiral stationary phase (CSP) methods. Predominantly use of protein based CSPs and crown ether based CSPs have been reported [9–13].

Chemically WCK 771 is a S–(−)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid l-arginine salt tetrahydrate (WCK 771) [14,15]. Chemical structure is shown in Fig. 1.

WCK 771 is a novel anti-methicillin-resistant Staphylococcus aureus (MRSA)/anti-vancomycin-resistant S. aureus (VRSA) agent and is being developed for several clinical indications.

Chemically R–(+)–enantiomer is a R–(+)–9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid. R–(+)–enantiomer of WCK 771 is 64–256 times less active against Gram +ve and Gram −ve bacteria [16–19]. Therefore, it was necessary to control presence R–(+)–enantiomer impurity in WCK 771. This paper describes development and validation of a new analytical method, which can detect and quantify the R–(+)–enantiomer in WCK 771.

2. Experimental

2.1. Chemicals

WCK 771 and its R–(+)–enantiomer were prepared and provided by Chemistry group, Drug Discovery, Wockhardt Research Center. Both the compounds were characterized for...
their identity and purity. HPLC grade acetonitrile (Ranbaxy Fine Chemicals, India), ethylene diamine tetraacetic acid disodium salt, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium hydroxide and triethyl amine (all from Qualigens Fine Chemicals, India) and double distilled water passed through Purelab classic (US Filters) were used during studies.

2.2. Equipment

HPLC system used was either Shimadzu or Agilent. Shimadzu system comprised of degasser (DGU-14A), quaternary pump (LC-10ATvp), autoinjector (SIL-10ADvp), column oven (CTO-10ASvp) and UV–vis detector (SPD-10Avp). The signal was acquired and processed using Class LC10 software. The Agilent-1100 series system comprised of degasser, quaternary pump, autoinjector, and column compartment and variable wavelength detector. The system was controlled through Chemstation software.

2.3. Sample preparation

Solutions of WCK 771 and its opposite enantiomer were prepared by dissolving weighed quantities in 0.1 M sodium hydroxide and further diluting with mobile phase. For WCK 771 assay determination solution of 0.1 mg/ml was used. For quantification of \( R^+ \)-(+) enantiomer in WCK 771 a solution of 0.5 mg/ml was used.

2.4. Chromatographic conditions

The chromatographic column used was a 250 mm × 4.6 mm i.D. Discovery C18 (Supelco) with 5 μm particles. The mobile phase consists of a mixture of buffer and acetonitrile in proportion of 88:12 with final pH adjusted to 7.3 with triethylamine was used. The buffer was prepared by dissolving potassium dihydrogen phosphate (0.910 g), disodium hydrogen phosphate (0.38 g), β-cyclodextrin (11.35 g) and ethylenediamine tetracetic acid disodium salt (50 mg) in 1000 ml of water. The flow rate of mobile phase was 2.0 ml/min. The column was maintained at 50 °C and column eluent was monitored at 290 nm. Injection volume was 20 μL.

2.5. Method development

The objective of this work was to develop a rugged method for assay and to determine enantiomeric purity of WCK 771 by quantification of \( R^+ \) enantiomer. In the method development use of HPLC columns filled with different chiral stationary phases were attempted. Chiral stationary phases used were protein based Chiral AGP (Sumika), a Pirkle type DNPBG (ChiraSep, E. Merck), an amylose based Chiralpak AD-H and Chiralpak AS (Daicel). WCK 771 and \( R^+ \)-(+) enantiomer could not be separated on Chiral-AGP, DNPBG and Chiralpak AD-H. A little resolution (Rs ≤ 2) was obtained on Chiralpak AS but the peak shape and tailing factor (T > 2) were of concern. During pre-formulation studies, (to enhance the solubility of WCK 771), it was observed that WCK 771 forms a complex with cyclodextrin and the complexes of two enantiomers have different solubility pattern. Therefore, an attempt was made to add β-cyclodextrin in mobile phase as chiral additive and to separate two enantiomers. Various combinations of buffers and organic solvent proportions were used as mobile phase. A better resolution was obtained with combination of phosphate buffer and acetonitrile at neutral pH. Peak shape was further improved by addition of triethyl amine and ethylenediamine tetracetic acid disodium salt. Separation was evaluated on different C18 phases with and without endcapping. Separation performance of different phases is shown as Discovery C18 (Rs = 2.33, T = 1.04), YMC Pack AM (Rs = 2.91, T = 1.02), Altima C18 (Rs = 1.76, T = 1.09), Ace C18 (Rs = 2.06, T = 1.12), Zorbax Extend C18 (Rs = 2.22, T = 1.16), Zorbax SB C18 (Rs = 1.90, T = 1.86) and Apollo C18 (Rs = 2.25, T = 1.48). It was observed that best resolution (Rs > 2, T < 1.5) between two isomers was obtained only on highly or doubly endcapped C18 columns e.g. Discovery C18 (Supelco), YMC Pack AM (YMC Co.), Altima C18 (Alttech Associates Inc.), Ace C18 (Advanced Chromatography Technologies) and Zorbax Extend C18 (Agilent technologies).

2.6. System suitability

Performance of the method was determined by injecting resolution mixture (equal quantities of WCK 771 and \( R^+ \)-(+) enantiomer, 0.1 mg/ml each). The enantiomers under analysis form a critical band pair in chromatogram. Therefore qualification criteria were resolution between two enantiomers should be not less than 2.0 and tailing factor not more than 1.5, which ensures baseline separation of two enantiomers and symmetrical peak shape.

2.7. Linearity

Linearity of WCK 771 was determined in the range of 0.05–0.15 mg/ml (50–150% of the assay solution concentration i.e. 0.1 mg/ml).
Linearity of R-(+)-enantiomer was determined in the range of 0.5–7.5 μg/ml (10–150% of the specified limit i.e. 1% of R-(+)-enantiomer in WCK 771).

2.8. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ of WCK 771 and R-(+)-enantiomer were determined by calibration curve method [20]. Solutions of both enantiomers were prepared in the range of 0.05–0.75 μg/ml and injected in triplicate. Average peak area of three analyses was plotted against concentration.

LOD and LOQ were calculated by using following equations.

\[
\text{LOD} = \frac{C_d \times \text{Syx}}{b}
\]

\[
\text{LOQ} = \frac{C_q \times \text{Syx}}{b}
\]

where \(C_d/C_q\) is coefficient for LOD/LOQ; Syx is residual variance due to regression; \(b\) is slope.

2.9. Precision

Precision of the method was determined for injection repeatability at assay concentration 0.1 mg/ml of WCK 771.

2.10. Accuracy/recovery

Accuracy of the method was ensured by determining recovery of the spiked amounts of R-(+)-enantiomer in the pre-analyzed sample of WCK 771.

2.11. Ruggedness

Ruggedness of the method was determined by performing assay and quantification of R-(+)-enantiomer on two different HPLC systems and columns by two chemists.

3. Results and discussions

The CSP in Chiralpak AS is an amylose tris-(S)-α-methylbenzyl carbamate coated on 10 μm silica gel. On this stationary phase resolution between two enantiomers was insufficient to quantify presence of ≤1% of R-(+)-enantiomer in WCK 771.

As mentioned earlier, our preformulation studies confirmed that WCK 771 forms complex with cyclodextrins, preferably β-cyclodextrin. β-Cyclodextrin is a cyclic oligosaccharide containing 7 units of α-glucopyranosyl units linked with α-(1–4)-glycosidic linkage. The analyte forms inclusion complex with β-cyclodextrin and stabilized by Van der Waals, hydrogen bonding and hydrophobic interactions. The spatial differences between isomers results in interactive selectivity. Therefore, β-cyclodextrin was used as chiral mobile phase additive (CMPA). The described method has an advantage that the R-(+)-enantiomer impurity elutes first and avoids “smearing” under main compound peak.

A representative chromatogram of resolution mixture is shown in Fig. 2. An excellent resolution (Rs = 2.33) between two enantiomers and ideal peak shape with tailing factor 1.04 was obtained.

The described method was found to be linear in the range of 50–150% of the assay solution concentration (0.1 mg/ml) of WCK771 with correlation coefficient of 1.0. The linearity curve is shown in Fig. 3. The method was also found to be linear in the range of 0.5–7.5 μg/ml of R-(+)-enantiomer with correlation coefficient of 1.0. The linearity curve is shown in Fig. 4.
Fig. 4. Linearity curve of R-(+)-enantiomer.

Table 1
Recovery results of R-(+)-enantiomer

<table>
<thead>
<tr>
<th>Amount spiked (mcg)</th>
<th>Amount found (mcg)</th>
<th>% RSD</th>
<th>% Recovery</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.580</td>
<td>0.599</td>
<td>0.27</td>
<td>103.3</td>
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<td></td>
</tr>
<tr>
<td>0.800</td>
<td>0.796</td>
<td>0.20</td>
<td>99.5</td>
<td></td>
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<tr>
<td>1.010</td>
<td>0.997</td>
<td>0.08</td>
<td>98.7</td>
<td></td>
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</tbody>
</table>

LOD and LOQ for both enantiomers were determined by calibration curve method. LOD and LOQ for WCK 771 were found to be 0.015 and 0.06 µg/ml, respectively. LOD and LOQ for R-(+)-enantiomer were found to be 0.025 and 0.09 µg/ml, respectively. Calculations were performed by considering values 3.3 and 10 for Cd and Cq, respectively.

Precision (RSD) of injection repeatability was found to be 0.23. Percentage recovery of R-(+)-enantiomer was found to be 100.55% with standard deviation of 2.44. Amount spiked and amount found of R-(+)-enantiomer are shown in Table 1.

The method was found to be rugged as assay values of WCK 771 and R-(+)-enantiomer content did not deviate significantly with overall relative standard deviation 0.003 and 0.21%, respectively. Ruggedness data for assay of WCK 771 is shown in Table 2. Ruggedness data for R-(+)-enantiomer content is shown in Table 3.

Table 2
Ruggedness data for assay of WCK 771

<table>
<thead>
<tr>
<th>WCK 771 assay (%)</th>
<th>System-1</th>
<th>System-2</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>99.28</td>
<td>99.31</td>
</tr>
<tr>
<td>2</td>
<td>99.30</td>
<td>99.30</td>
</tr>
<tr>
<td>3</td>
<td>99.33</td>
<td>99.32</td>
</tr>
<tr>
<td>4</td>
<td>99.35</td>
<td>99.30</td>
</tr>
<tr>
<td>5</td>
<td>99.27</td>
<td>99.32</td>
</tr>
<tr>
<td>Mean</td>
<td>99.306</td>
<td>99.310</td>
</tr>
<tr>
<td>SD</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.034</td>
<td>0.000</td>
</tr>
<tr>
<td>Overall mean</td>
<td>99.308</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.034</td>
<td></td>
</tr>
</tbody>
</table>

4. Conclusions

A simple, specific, linear, precise, accurate and rugged RP-HPLC method containing chiral mobile phase additive has been developed and validated for quantitative determination of WCK 771 and R-(+)-enantiomer in bulk drug. Same method was used for quantitative determination of WCK 771 and R-(+)-enantiomer in parenteral formulation. The method has two major advantages. Firstly, it is very simple, as it does not involve any derivatization to diastereomers. Secondly, it is very economical, as it does not involve use of any chiral stationary phase, it uses very inexpensive β-cyclodextrin as chiral mobile phase additive and RP-HPLC C18 column, which are available in every chromatography laboratories. The method could also separate enantiomers of 6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid with resolution 1.66 and tailing factor of 1.29.

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References


