Optimization of Simultaneous Analysis of Cefixime and Dicloxacillin Sodium in Oral Tablets

Bharat G. Chaudhari and Heena J. Patel*

Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India.

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of cefixime and dicloxacillin sodium in their combined tablet dosage form. The method was based on additive property of absorbance and correction of absorbance for the analysis of two drugs using methanol as solvent. The two wavelengths were selected from the UV spectra of both the drugs, which 218.4 nm and 289 nm. Cefixime was determined directly at 289 nm (λ\textsubscript{max} of cefixime) without any interference of dicloxacillin sodium in binary mixture. At 218.4 nm, both drugs have reasonable absorbance. So to remove interference of cefixime, its absorbance was calculated by using its standard absorptivity values at this wavelength. Finally, corrected absorbance of dicloxacillin sodium at 218.4 nm was found by subtracting the absorbance of cefixime from the total absorbance at 218.4 nm. The linearity was found in the concentration range of 3-16 μg/ml for both the drugs. The % recovery was found in the range of 100.22 ± 0.43 and 100.46 ± 0.32 for cefixime and dicloxacillin sodium respectively. The intermediate precision data obtained under different experimental setup, the calculated value of % coefficient of variation (% CV) was found to be less than critical value. The method was successfully applied for the simultaneous determination of these two drugs from their combined tablet dosage form without any interference.

KEYWORDS: Cefixime; Dicloxacillin sodium; Spectrophotometric method; Absorbance correction; Validation; Tablet dosage form.

Introduction

Cefixime is chemically (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(carboxymethoxy)iminooacetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo-oct-2-ene-2-carboxylic acid (O’Neill MJ, 2001) (Figure-1). Cefixime (CEE), an antibiotic, is a third generation cephalosporin. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall (Tripathi, 2009). Cefixime is official in Indian Pharmacopoeia (Indian Pharmacopoeia, 2010), British Pharmacopoeia (British Pharmacopoeia, 2010), and United States Pharmacopoeia (The United States Pharmacopoeia, USP27-NF22, 2009). These three pharmacopoeias describe liquid chromatography method for its estimation. Literature survey reveals UV spectrophotometry (Dey et al., 2012; Uzochukwu et al., 2013; Azmi et al., 2013; Omkar, 2013), High Performance Liquid Chromatography (Nahata et al., 1991; Khaja et al., 2010; Hafiz et al., 2009; Kandikonda et al., 2010) methods for determination of cefixime alone. Literature survey also reveals UV (Attimarad et al., 2011a; 2012b; Gadiya et al., 2013; Rajendran et al., 2011; Shah et al., 2012; Patel M et al., 2013; Patel D et al., 2013; Sharma et al., 2012; Chaudhari et al., 2012; Patel DP et al., 2012; Kumar et al., 2011), High Performance Liquid Chromatography (Wankhede et al., 2010; Rathinavel et al., 2008; Rao VJ et al., 2010a; 2011b; Shah CK et al., 2012; Patel SA et al., 2011a; 2012b; Dhoka et al., 2010; Natesan et al., 2011; Patel JV et al., 2013; Kher et al., 2012; Trivedi et al., 2012; Malothu et al., 2012; Sheth et al., 2012; Rao et al., 2013) methods for the determination of cefixime with other drugs combination.

Finally, corrected absorbance of dicloxacillin sodium at 218.4 nm was found by subtracting the absorbance of cefixime from the total absorbance at 218.4 nm. The linearity was found in the concentration range of 3-16 μg/ml for both the drugs. The % recovery was found in the range of 100.22 ± 0.43 and 100.46 ± 0.32 for cefixime and dicloxacillin sodium respectively. The intermediate precision data obtained under different experimental setup, the calculated value of % coefficient of variation (% CV) was found to be less than critical value. The method was successfully applied for the simultaneous determination of these two drugs from their combined tablet dosage form without any interference.

KEYWORDS: Cefixime; Dicloxacillin sodium; Spectrophotometric method; Absorbance correction; Validation; Tablet dosage form.

Fig. 1. Chemical structure of cefixime.
Dicloxacillin sodium (DIC) is chemically (2S,5R,6R)-6-[3-(2,6-dichlorophenyl)-5-methyl-1,2-oxazole-4-amido]-3,3-dimethyl-7-oxo-1-thia-1-azabicycloheptane-2-carboxylic acid (O’Neill MJ, 2001) (Figure 2). Dicloxacillin sodium is an anti-bacterial agent. It is official in Indian Pharmacopoeia (Indian Pharmacopoeia (IP), 2010), British Pharmacopoeia (British Pharmacopoeia (BP), 2010), and United States Pharmacopeia. (The United States Pharmacopoeia (USP), USP27-NF22, 2009). IP, BP, USP describe High Performance Liquid Chromatography-method for its estimation. Literature survey reveals. High Performance Liquid Chromatography (Lauriault et al., 1984; Harale et al., 2013) methods for estimation of dicloxacillin sodium alone. Literature survey also reveals UV (Morelli et al., 1988; Acharya et al., 2012; Nour et al., 2006), High Performance Liquid Chromatography (Kathiresan et al., 2009; Walili et al., 1992; Patel HA et al., 2012; Samanidou et al., 2009; Barot et al., 2009; Acharya et al., 2013) methods for determination of dicloxacillin sodium with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of cefixime and dicloxacillin sodium in their combined synthetic mixture or dosage forms. Literature survey reveals only High Performance Liquid Chromatography (Kathiresan et al., 2009; Prabhudev et al., 2013; Kathiravan et al., 2010; Dhoka et al., 2010) and High Performance Thin Layer Chromatography (Tank et al., 2012) methods for cefixime and dicloxacillin sodium in combined dosage forms.

The present manuscript describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on absorbance correction method for simultaneous estimation of both drugs in tablet dosage form.

Materials and Methods

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.1 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and materials

Cefixime and Dicloxacillin sodium were kindly supplied as a gift samples from Brussels Laboratories Pvt. Ltd, Changodar, Ahmedabad, Gujarat, analytical grade Methanol (S.D. Fine Chemical Ltd., Mumbai, India.) as a solvent and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solution

An accurately weighed cefixime and dicloxacillin sodium powder (10 mg) were transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution having concentration 100 µg/ml for both of drugs.

Methodology

If the identify, concentration and absorptivity of the absorbing interferents are known, it is possible to calculate their contribution to the total absorbance of a mixture. The utility of absorbance correction data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. Overlain UV spectrum of working standard solutions (10 µg/ml) of cefixime and dicloxacillin sodium was examined for the selection of wavelengths for simultaneous estimation of these two drugs (Figure 3, 4). From the UV spectrum of cefixime, wavelength of 289 nm (λmax of cefixime) was selected at which dicloxacillin sodium shows zero absorbance. So it was used for the estimation of cefixime in the mixture of two drugs.

From overlain UV spectrum of both the drugs, 218.4 nm wavelengths selected at which two drug show reasonable absorbance. So in the mixture due to additive properties, absorbance at this wavelength will be sum of individual absorbances of two drugs. As per Beer's equation, it can be explained.

\[ A_{mix} = A_{1\%1cm}^C_x + A_{1\%1cm}^C_y \]

Where,

\[ C_x = \text{concentration of cefixime} \]
\[ C_y = \text{concentration of dicloxacillin sodium} \]

To remove interference of cefixime, its absorbance was calculated by using its standard absorptivity value (A1%1cm) at 218.4 nm using its working standards and concentration determined at 289 nm in binary mixture of two drugs.

Finally, calculated absorbance of cefixime at 218.4 nm was subtracted from total absorbance of two drugs at this wavelength to corrected absorbance of dicloxacillin sodium at 218.4 nm was used for the estimation of dicloxacillin sodium in the binary mixture of two drugs.
Fig. 3. Overlain absorption spectra of cefixime and dicloxacillin sodium showing $\lambda_{\text{max}}$ of cefixime at 289 nm in Methanol.

Fig. 4. Overlain absorption spectra of CEF and DIC showing wavelength (218.4 nm) for dicloxacillin sodium.
Validation of the Proposed Method

The proposed method was validated for linearity, range, accuracy, precision, LOD and LOQ. The validation was carried out as per International Conference on Harmonisation (ICH) guideline.

Linearity and Range

Calibration curves were constructed by plotting absorbance against concentrations. Linear correlation was obtained between absorbance versus concentrations of Cefixime and Dicloxacillin sodium in the concentration ranges of 3-16 \( \mu \text{g/ml} \) for both the drug. The calibration curves were constructed at selected wavelengths by plotting absorbance versus concentrations and the regression equations were calculated.

Precision (Repeatability)

The precision of the method was checked by repeated measurement of absorbance of solutions \((n = 6)\) of cefixime \((10 \mu \text{g/ml})\) and dicloxacillin sodium \((10 \mu \text{g/ml})\) by proposed method without changing any parameter. The results were reported in terms of %RSD.

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days for different concentrations of standard solutions of cefixime \((8, 10, 12 \mu \text{g/ml})\) and dicloxacillin sodium \((8, 10, 12 \mu \text{g/ml})\). The results were reported in terms of % RSD.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated by using the following equations:

\[
\text{LOD} = 3.3 \times \sigma/S \\
\text{LOQ} = 10 \times \sigma/S
\]

Where, \( \sigma \) = the standard deviation of the response \\
\( S \) = slope of the calibration curve

Accuracy

The accuracy of the method was determined by calculating recoveries of Cefixime and Dicloxacillin sodium by the standard addition method. Known amounts of standard solution of cefixime and dicloxacillin sodium were added at three levels \((50\%, 75\% \text{ and } 100\%)\) to pre-quantified sample solutions of cefixime and dicloxacillin sodium. The experiment was repeated for three times.

Assay

Twenty tablets were weighed individually and powdered. Labelled claim: 200 mg cefixime and 500 mg dicloxacillin sodium. Quantity of the powder equivalent to 10 mg cefixime was transferred to 100 ml volumetric flask containing 50 ml methanol, cork it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to mark with methanol to get a concentration of cefixime \((6.0 \mu \text{g/ml})\) and dicloxacillin sodium \((15.0 \mu \text{g/ml})\). The absorbance of sample solution was measured against methanol as blank at selected wavelengths for quantification of cefixime and dicloxacillin sodium. The amount of cefixime and dicloxacillin sodium present in the sample solutions were determined by solving respective regression equations. The procedure was repeated for six times to get reproducible results.

Results and Discussion

Linear correlation was obtained between absorbance versus concentrations of Cefixime and Dicloxacillin sodium in the concentration ranges of 3-16 \( \mu \text{g/ml} \) for both the drug (Table 1). Calibration curves with regression equation of cefixime and dicloxacillin sodium were plotted (Figure 5, 6, 7).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration of cefixime (( \mu \text{g/ml} ))</th>
<th>Absorbance at 289.0 nm</th>
<th>Concentration of dicloxacillin sodium (( \mu \text{g/ml} ))</th>
<th>Absorbance at 218.4 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0.14038</td>
<td>3</td>
<td>0.14294</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.19238</td>
<td>4</td>
<td>0.17627</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.29431</td>
<td>6</td>
<td>0.27173</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.37109</td>
<td>8</td>
<td>0.382203</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.46375</td>
<td>10</td>
<td>0.47559</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>0.54797</td>
<td>12</td>
<td>0.56335</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>0.63965</td>
<td>14</td>
<td>0.66895</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>0.70837</td>
<td>16</td>
<td>0.77051</td>
</tr>
</tbody>
</table>

Fig. 5. Calibration curve of cefixime at 289.0 nm.
For intermediate precision, the %RSD values of cefixime for interday (0.49-1.2%) and intraday (0.21-0.68%) at 289.0 nm and for dicloxacillin sodium, the %RSD values at 218.4 nm for interday (0.33-1.2%) and for intraday (0.25-0.61%) (Table 3).

TABLE 3
Intraday and interday precision data of cefixime and dicloxacillin sodium proposed method

<table>
<thead>
<tr>
<th>DRUG</th>
<th>Concentration (µg/ml)</th>
<th>Intraday Precision</th>
<th>Interday Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean absorbance ± RSD (n = 3)</td>
<td>Mean absorbance ± RSD (n = 3)</td>
<td></td>
</tr>
<tr>
<td>Cefixime</td>
<td>8 0.36891 ± 0.47</td>
<td>0.37213 ± 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 0.46372 ± 0.68</td>
<td>0.46247 ± 0.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 0.54854 ± 0.21</td>
<td>0.55016 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>Dicloxacillin sodium</td>
<td>8 0.3930 ± 0.61</td>
<td>0.3912 ± 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 0.4751 ± 0.44</td>
<td>0.4728 ± 0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 0.5691 ± 0.25</td>
<td>0.5649 ± 0.33</td>
<td></td>
</tr>
</tbody>
</table>

The recovery experiments were performed by the standard addition method. The mean recoveries were found to be 100.98 ± 0.54 and 100.85 ± 0.37 for cefixime and dicloxacillin sodium, respectively (Table 4).

TABLE 4
Recovery Data of CEF and DIC by proposed method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>Amount Taken (µg/ml)</th>
<th>Amount Added (%)</th>
<th>% Mean Recovery ± S.D. (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEF</td>
<td>I</td>
<td>6</td>
<td>50</td>
<td>99.48 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>6</td>
<td>75</td>
<td>100.77 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>6</td>
<td>100</td>
<td>100.41 ± 0.31</td>
</tr>
<tr>
<td>DIC</td>
<td>I</td>
<td>8</td>
<td>50</td>
<td>100.45 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>8</td>
<td>75</td>
<td>101.64 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>8</td>
<td>100</td>
<td>99.29 ± 0.15</td>
</tr>
</tbody>
</table>

The proposed method was applied for analysis of cefixime and dicloxacillin sodium in tablet dosage form and results were described in Table 5.

In absorbance correction method, the concentration of the absorbing component of interest is then calculated from the corrected absorbance (total absorbance – the absorbance of the interfering substance) in the usual way. Calibration curves were prepared at 289 nm (λmax of cefixime) to find out the concentration of cefixime and at 218.4 nm to find out the standard absorptivity of cefixime at this wavelength. For dicloxacillin sodium, calibration curve was prepared with corrected absorbance at 218.4 nm versus concentration. The responses were found to be linear in the concentration range 3 to 16 µg/ml for both the drugs. The linearity of the calibration curve was validated by the high values of correlation coefficient.

The %RSD values of cefixime and dicloxacillin sodium were found to be 0.57% and 0.64% at 289.0 nm and 218.4 nm respectively. Low RSD value means the repeatability of the proposed method is good. The low % RSD of intraday and interday indicates that the proposed method is precise. Limit of detection and limit of quantification values for cefixime were found to be 0.21 and 0.63 µg/ml, at 289.0 nm respectively. Where, Limit of detection and limit of quantification values at 218.4 nm...
were found to be 0.15 and 0.44 µg/ml. Low value of limit of detection and limit of quantification indicates that the method is sensitive.

The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 6. The recovery experiment was performed by the standard addition method. The results obtained (n = 3 for each level 50%, 75%, 100% level) indicated the mean recovery 100.22 ± 0.43 and 100.46 ± 0.32 for cefixime and dicloxacillin sodium respectively. These values of recovery experiment reveal that the proposed method is highly accurate.

The proposed validated method was successfully applied for determination of cefixime and dicloxacillin sodium in their tablet dosage form. No interference of the excipients with the absorbance of analytes of interest observed; hence the proposed method is applicable for the routine simultaneous analysis of cefixime and dicloxacillin sodium in tablet dosage form.

**Conclusions**

Based on the results which have been obtained from the analysis using proposed method, it can be concluded that the method has linear response in the range 3 to 16 µg/ml for both the drugs, cefixime and dicloxacillin sodium. The result of the analysis of three different marketed tablet dosage forms by the proposed method is highly reproducible, reliable, as well as in agreement with label claim of the drugs. The additives present in the synthetic mixture did not interfere in the analysis. Thus, this method can be used for the routine analysis of drugs in combination.

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**References**


Address correspondence to: Heena J. Patel, Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India.
E-mail: heena2206@yahoo.com