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Spectrophotometric estimation of cefixime and ofloxacin from tablet dosage form
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Abstract
Two simple, rapid, accurate and precise spectrophotometric methods have been developed for simultaneous estimation of Cefixime and Ofloxacin from tablet dosage form. Method І involves formation of ‘simultaneous equations’ at 234 nm (λ max of Cefixime) and 296 nm (λ max of Ofloxacin); while Method ІІ involves formation of ‘Absorbance ratio equation’ at 275(isoabsorptive point) and 296 nm (λ max of Ofloxacin) using methanol as a solvent. The linearity was observed in the concentration range of 4 - 20 µg/ml for Cefixime and 2 - 10 µg/ml for Ofloxacin. The results of analysis have been validated statistically and by recovery studies and were found satisfactory.

Key-Words: Cefixime(CEF), Ofloxacin(OFL), Simultaneous equation method, Absorbance ratio method

Introduction
Cefixime (CEF) is an oral third generationcephalosporin antibiotic. Chemically, it is (6R,7R)-7-[(2-(2-amino-1,3-thiazol-4-yl)-2 (carboxymethoxyimino) acetyl]amino]-3-ethenyl 8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid, clinically used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and lower urinary-tract infections. Ofloxacin (OFL) is a fluoroquinolone derivative. Chemically, it is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine -6-carboxylic acid. Literature review reveals that Cefixime can be estimated spectrophotometrically and Ofloxacin can be estimated spectrophotometrically in combination with other drugs only one method is reported for estimation of Cefixime and Ofloxacin in combination by RP-HPLC. So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for simultaneous estimation of Cefixime and Ofloxacin from tablet dosage forms using UV – Visible spectroscopy.

Material and Methods
Reagents and Chemicals
AR-grade methanol was used as solvent which was purchased from Loba Chem Pvt Ltd, Mumbai. Analytical pure standard sample of CEF and OFL were supplied as gift sample by Vapicare Pharma Pvt Ltd, Vapi, Gujarat and used without further purification. The pharmaceutical dosage form used in study was CEFIO 200 (label claim CEF 200 mg, OFL 200 mg) manufactured in India by Accent Pharma, India.

Instrumentation
The instrument used for the entire analysis was SIMADZU UV 1700 UV-VIS recording spectrophotometer. It is a double beam high speed scanning spectrophotometer with advanced quantitative software and provides full facilities for monochromators, a CRT display and a parallel head printer.

Preparation of standard stock solution
100 mg of Cefixime (CEF) and 100 mg of Ofloxacin (OFL) were weighed separately and transferred to two separate 100 ml volumetric flasks. Each drug was dissolved in 40 ml of methanol and shaken gently for 10 min. The volume was made up to the mark with same solvent and the final strength obtained was 1000 µg/ml.

Methods
Method I: Simultaneous Equation Method
In simultaneous equation method, when no region can be found free from overlapping spectra of two
chromophores, it is still possible to device a method based on measurements at two wavelengths. Two dissimilar chromophores must necessarily have different powers of light absorption at some point or in linear absorption spectra.

If samples contain two absorbing drugs (X and Y), each of which absorbs at the \( \lambda_{\text{max}} \) of the other, it may be possible to determine both drugs by the technique of simultaneous equations. From overlain spectra (Fig 1.) 234 nm \( \lambda_{\text{max}} \) for Cefixime and 296 nm \( \lambda_{\text{max}} \) for Ofloxacin were selected for formation of simultaneous equation of two drugs. The absorbance at 234 nm and 296 nm for CEF and OFL were measured. The absorptivity values of each drug at both wavelengths were determined. The absorbance and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

\[
C_x = \frac{A_2a_{y1} - A_1a_{y2}}{a_2a_{y1} - a_1a_{y2}} \quad \text{(I)}
\]
\[
C_y = \frac{A_1a_{x2} - A_2a_{x1}}{a_2a_{x1} - a_1a_{x2}} \quad \text{(II)}
\]

Where, \( A_1 \) and \( A_2 \) were absorbance of sample at 234 nm and 296 nm respectively, \( a_{x1} \) and \( a_{x2} \) are absorptivity of Cefixime at 234 nm and 296 nm, \( a_{y1} \) and \( a_{y2} \) are absorptivity of Ofloxacin at 234 nm and 296 nm.

Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components. Results of which are reported in Table 1.

**Method II: Absorbance Ratio Method**

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that, for a substance which obeys Beer’s Law at all wavelengths is a constant value independent of concentration or Path length. In the quantitative assay of two components in admixture by the absorbance by the absorbance ratio method, absorbance’s are measured at two wavelengths one being the \( \lambda_{\text{max}} \) of one of the components (\( \lambda_x \)) and the other being a wavelength of equal absorptivity of the two components (\( \lambda_y \)), that is , an isosbestic point.

From overlain spectra (Fig 1.) 275 nm (Isobestic point) and 296 nm \( \lambda_{\text{max}} \) for Ofloxacin were selected for formation of Absorbance ratio equation of two drugs.

The absorbance at 275 nm and 296 nm for CEF and OFL were measured. The absorptivity values of each drug at both wavelengths were determined. The absorbance and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

\[
C_x = \frac{Q_{M} - Q_{Y}}{A_{1}} \quad \text{(III)}
\]
\[
C_y = \frac{Q_{M} - Q_{X}}{A_{2}} \quad \text{(IV)}
\]

\[
Q_{M} = \frac{A_2}{A_1}, \quad Q_{X} = \frac{ax_2}{ax_1}, \quad Q_{Y} = \frac{ay_2}{ay_1}
\]

Where, \( A_1 \) and \( A_2 \) were absorbance of sample at 275 nm and 296 nm respectively, \( a_{x1} \) and \( a_{x2} \) are absorptivity of Cefixime at 275 nm and 296 nm, \( a_{y1} \) and \( a_{y2} \) are absorptivity of Ofloxacin at 275 nm and 296 nm.

Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components. Results of which are reported in Table 1.

**Assay of tablet formulation**

Twenty tablets were weighed and crushed to obtain a fine powder. An accurately weighed sample equivalent to 100 mg of CEF and 100 mg of OFL was taken in a stoppered volumetric flask (100.0ml); 40ml of Methanol was added and sonicated for 10 min. The solution was filtered through Whatmann filter paper (No 41) and the volume was made up to the mark with the same solvent. The aliquot portions of above solutions were further diluted with solvent to get final concentration of about 6 \( \mu \)g/ml CEF and 6 \( \mu \)g/ml of OFL, respectively and absorbances were measured at 234.0 nm, 275 nm and 296.0 nm against blank. The concentrations of two drugs in sample were determined by using equations 1 and 2 and equation 3 and 4. The results are reported in the Table 2.

**Recovery studies**

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80%, 100%, and 120%) within range of linearity for both the drugs. Results are reported in table 3.
Results and Conclusion

Table 1: Result of analysis of mixed standard for method I and method II

<table>
<thead>
<tr>
<th>Conc Present (μg/ml)</th>
<th>Conc Found (%)</th>
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<tbody>
<tr>
<td></td>
<td>Method I</td>
</tr>
<tr>
<td></td>
<td>Method II</td>
</tr>
<tr>
<td>CEF</td>
<td>CEF</td>
</tr>
<tr>
<td></td>
<td>OFL</td>
</tr>
<tr>
<td>4</td>
<td>101</td>
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<td>99.67</td>
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<tr>
<td></td>
<td>100.66</td>
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<tr>
<td></td>
<td>104.66</td>
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Method I: Simultaneous Equation Method
UV-spectrophotometric method using simultaneous equation was developed. CEF showed absorbance maxima at 234 nm and OFL at 296.0 nm. Linearity was observed in the concentration range of 4 - 20 μg/ml for CEF and 2 - 10 μg/ml for OFL. Correlation coefficient was found to be 0.9996 and 0.9998 at 234 nm and 296 nm respectively. The proposed method was applied for pharmaceutical formulation and % label claim for CEF and OFL was found to be 97.00 and 102.20, respectively. The method is accurate and precise and can be used for routine pharmaceutical analysis.

Method II: Absorbance Ratio Method
UV-spectrophotometric method by using absorbance ratio method was developed. Absorbances selected were 275 nm (isosbestic point) and 296 nm (λ max of Ofloxacin). Linearity was observed in the concentration range of 4 - 20 μg/ml and 2 - 10 μg/ml. Correlation coefficient was found to be 0.9995 and 0.9998 respectively. The proposed method was applied for pharmaceutical formulation; % label claim for CEF and OFL was found to be 95.16 and 102.83, respectively. The low % RSD indicates method is accurate and precise.

References
Table 2: Result of analysis of tablet formulation

<table>
<thead>
<tr>
<th>Label Claimed mg/ml</th>
<th>% Label Estimated (Mean ± S.D)</th>
<th>% R.S.D</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Method I</td>
<td>Method II</td>
</tr>
<tr>
<td>CEF</td>
<td>CEF</td>
<td>OFL</td>
</tr>
<tr>
<td>200</td>
<td>97.00 (±0.016)</td>
<td>102.20 (±0.0024)</td>
</tr>
<tr>
<td>OFL</td>
<td>0.31</td>
<td>0.26</td>
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