A conventional HPLC-MS method for the simultaneous determination of Ofloxacin and Cefixime in plasma: Development and validation

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Introduction
A combination of ofloxacin and cefixime is available in the market, which is highly active against many bacterial infections such as typhoid fever, urinary and respiratory tract infections, nosocomial infections, soft tissue and intra abdominal infections.

High performance liquid chromatographic (HPLC) and high performance thin layer chromatography (HPTLC) methods were reported for the simultaneous determination of ofloxacin and cefixime in formulations, but no method has been reported so far for simultaneous estimation of ofloxacin and cefixime in human plasma.

Some of the reported methods for determination of ofloxacin and cefixime were in single or with other drugs in pharmaceutical preparations and biological fluids were spectrophotometry, fluorometry, HPLC, LC/MS/MS and capillary electrophoresis.

Most of the reported methods for the determination of ofloxacin and cefixime in biological fluids involve tedious sample preparation procedures (liquid/liquid or solid phase extraction), low extraction yields and low sensitivity.

To bypass these difficulties, we have develop more conventional procedures for determining ofloxacin and cefixime using liquid chromatography coupled with mass spectrometry (LC-MS). This assay is simple and robust, as well as sufficiently sensitive for the pharmacokinetic studies.

Objectives
The aim of this study was to develop and validate simple analytical method for simultaneous determination ofloxacin and cefixime in human plasma. Newly develop method could be used for pharmacokinetic and therapeutic drug monitoring.

Results and Discussion

![Figure 3: LCMS chromatograms (SIM mode) of blank human plasma](image1)

![Figure 4: LCMS chromatograms (SIM mode) of human plasma spiked with OFL (500 ng/mL), MOX (500 ng/mL) and CEF (4000 ng/mL)](image2)

Table 1: Intraday and Interday Precision and Accuracy for ofloxacin and cefixime

<table>
<thead>
<tr>
<th></th>
<th>Intraday (n=5)</th>
<th>Interday (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected</td>
<td>Measured (ng/mL)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.81±0.53</td>
<td>5.40</td>
</tr>
<tr>
<td>100</td>
<td>99.82±2.8</td>
<td>2.81</td>
</tr>
<tr>
<td>400</td>
<td>392.69±12.95</td>
<td>3.30</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.6±5.9</td>
<td>93.1±5.4</td>
</tr>
<tr>
<td>Cefixime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>98.38±3.02</td>
<td>3.07</td>
</tr>
<tr>
<td>2000</td>
<td>1962.39±80.23</td>
<td>4.09</td>
</tr>
<tr>
<td>4000</td>
<td>3942.73±171.09</td>
<td>4.34</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.9±4.4</td>
<td>92.6±4.8</td>
</tr>
</tbody>
</table>

Table 2. Stability of ofloxacin and cefixime trihydrate in human plasma

<table>
<thead>
<tr>
<th></th>
<th>Ofloxacin Spiked concentration (ng mL⁻¹)</th>
<th>Measured concentration (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze and thaw stability</td>
<td>Mean ± SD: 9.88 ± 0.3</td>
<td>98.02 ± 22.9</td>
</tr>
<tr>
<td></td>
<td>RE (%): -2.2</td>
<td>-1.2</td>
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<tr>
<td></td>
<td>Post-preparative stability (24 h at room temperature)</td>
<td>Mean ± SD: 10.27 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>RE (%): 2.7</td>
<td>-1.9</td>
</tr>
<tr>
<td>Stability for 15 days at -20°C</td>
<td>Mean ± SD: 9.75 ± 0.3</td>
<td>97.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>RE (%): -2.5</td>
<td>-2.7</td>
</tr>
</tbody>
</table>

Table 3: Comparison of the results of the developed method with the reported method

<table>
<thead>
<tr>
<th></th>
<th>Developed Method</th>
<th>Reported Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>98.6±5.9</td>
<td>98.6±5.5</td>
</tr>
<tr>
<td>Cefixime</td>
<td>93.1±5.4</td>
<td>93.1±5.3</td>
</tr>
</tbody>
</table>

Conclusion

- We have developed a specific, rapid, sensitive, and inexpensive LCMS method for determination of OFL and CEF in plasma and validated.
- The assay involves a simple sample preparation procedure followed by separation on a reversed phase column with MS detection.
- The specificity test showed that no additional peaks due to endogenous substances were observed that would interfere with the OFL and CEF.
- The accuracy, precision, recovery, and quantitation and detection limits enable use of the procedure in pharmacokinetic, clinical and residues studies.
- Because of the short chromatographic run time (5 min) and simple sample preparation procedure, the reported method is suitable for processing of many samples on a daily basis.

Acknowledgement

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