Identification of E and Z isomers of some cephalosporins by NMR

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ABSTRACT
This article deals with the identification of geometrical isomers (E and Z) of some cephalosporin drugs by NMR. 1H and 13C NMR chemical shift values are used to distinguish between the Z isomer of cephalosporins having superior antibacterial activity from E isomer having less antibacterial activity. Nine cephalosporins of pharmaceutical interest viz, cefotaxime, cefixime, ceftazidime, cefdinir, cefitiofur, ceftriaxone, cefpodoxime proxetil, cefuroxime and cefuroxime axetil samples were taken for the study.

INTRODUCTION
Cephalosporins, first isolated and identified by Brontzen 1948, are still an interesting class of β-lactam antibiotics because of their therapeutic action against a large number of both Gram-positive and Gram-negative microorganisms. Cephalosporin structures are based on the 7-aminocephalosporanic acid nucleus (with a condensed dihydrothiazole ring in its skeleton) and are generally stable in the acid media and in the presence of penicillase. Many efforts have been made to synthesize cephalosporins with various physico-chemical properties (mainly liposolubility) by varying the substituents[1].

Cephalosporin compounds possessing an oxime group in the 7α-sidechain have generally been found to exhibit high stability to β-lactamases produced by many pathological organisms. However, it has been found that Z-isomers (syn-isomers) of these cephalosporin compounds exhibit superior antibacterial activity to the corresponding E-isomers (anti-isomers), so that the oxime group containing cephalosporin antibiotics are gener-
ally obtained and used in the form of their syn isomers figure 1. The Z-isomers of the cephalosporin compounds may be prepared by employing an acid that is substantially in the syn-isomer form (Z-isomer), or a derivative thereof, in the 7-position of the cephen nucleus under controlled reaction conditions in this and the subsequent steps to avoid isomerisation to the anti-isomer figure 2.

Figure 1 : E and Z isomer in cephalosporin drugs
E and Z isomers of some Cephalosporins by NMR

EXPERIMENTAL

High performance liquid chromatography (Preparative)

The isomers were separated by Preparative HPLC using Preparative HPLC system (Waters LC2000 and Waters Delta Prep4000 (Waters, Milford, US) and using UV detector (Waters 2487 detector). The data were collected and processed using Waters Millenium32 software. The fractions were collected separately, the collected fractions were confirmed by analytical HPLC method. The fractions of impurity were pooled together and lyophilized using Virtis Freezemobile 35 EL.

NMR spectroscopy

Deuterated solvents were obtained from Aldrich and Euriso-top. The following suite of NMR spectra were collected at 298 K using a 5mm BBO gradient probe on a Bruker Avance 400 MHz NMR spectrometer (Bruker Biospin, Faellanden, Switzerland) proton, and carbon. The 1H and 13C chemical shift values were reported on the δ scale in ppm, relative to DSS (δ = 0.00 ppm) in the case of D2O solvent. In the case of DMSO-d6 solvent, 1H chemical shift values were reported relative to TMS (δ = 0.00 ppm). The carbon spectrum was referenced using the residual DMSO-d6 signal as reference, and set equal to δ 39.5 ppm.

RESULTS AND DISCUSSION

Identification of E and Z isomers present in the Cephalosporins[1-9] have been made based on the chemical shift values of aminothiazole/furyl proton and carbon at C-2 (Figure 2). 1H and 13C NMR chemical shift values of aminothiazole/furyl proton and carbon of Z and E-isomer of some selected cephalosporins were shown in Table 2.
TABLE 1: Structural details of cephalosporins studied

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>R</th>
<th>R'</th>
<th>R''</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cefotaxime</td>
<td></td>
<td><img src="image" alt="Diagram" /></td>
<td>NOCH₃</td>
<td>C₁₆H₁₇N₉O₈S₂</td>
</tr>
<tr>
<td>2</td>
<td>Cefixime</td>
<td></td>
<td><img src="image" alt="Diagram" /></td>
<td>NOCH₂⁻COOH</td>
<td>C₁₆H₁₅N₉O₈S₂</td>
</tr>
<tr>
<td>3</td>
<td>Cefdinir</td>
<td></td>
<td><img src="image" alt="Diagram" /></td>
<td>NOH</td>
<td>C₁₆H₁₅N₉O₈S₂</td>
</tr>
<tr>
<td>4</td>
<td>Ceftiofur</td>
<td></td>
<td><img src="image" alt="Diagram" /></td>
<td>NOCH₃</td>
<td>C₁₆H₁₅N₉O₈S₃</td>
</tr>
<tr>
<td>5</td>
<td>Ceftriaxone</td>
<td></td>
<td><img src="image" alt="Diagram" /></td>
<td>NOCH₃</td>
<td>C₁₆H₁₇N₈O₇S₃</td>
</tr>
<tr>
<td>6</td>
<td>Ceftazidime</td>
<td><img src="image" alt="Diagram" /></td>
<td><img src="image" alt="Diagram" /></td>
<td>NO(CH₃)₂COOH</td>
<td>C₂₂H₂₂N₈O₈S₂</td>
</tr>
<tr>
<td>7</td>
<td>Cefpodoxime Proxetil</td>
<td><img src="image" alt="Diagram" /></td>
<td><img src="image" alt="Diagram" /></td>
<td><img src="image" alt="Diagram" /></td>
<td>C₂₁H₂₁N₉O₈S₂</td>
</tr>
<tr>
<td>8</td>
<td>Cefuroxime</td>
<td><img src="image" alt="Diagram" /></td>
<td><img src="image" alt="Diagram" /></td>
<td>NOCH₃</td>
<td>C₁₆H₁₇N₉O₈S₂</td>
</tr>
<tr>
<td>9</td>
<td>Cefuroxime axetil</td>
<td><img src="image" alt="Diagram" /></td>
<td><img src="image" alt="Diagram" /></td>
<td>NOCH₃</td>
<td>C₂₀H₂₂N₈O₁₀S</td>
</tr>
</tbody>
</table>
Interpretation of $^1$H NMR spectra of the Z and corresponding E isomer (compound 1-7), indicated that the aminothiazole proton in the E-isomer appears at downfield than the Z-isomer. The deshielding of the aminothiazole proton in E-isomer may be due to the hydrogen bonding type of interaction between this proton and the oxime oxygen (Figure 3). In $^1$H NMR, the aminothiazole proton appears as a singlet around $\delta$ 6.70 to 6.90 ppm and $\delta$ 7.2 to 7.6 ppm in Z and E isomer, respectively.

As the aminothiazole proton appears as a singlet and is well separated from the other signals, it is very easy to detect the presence of the E-isomer in lower limits also.

In this way $^1$H NMR is the best choice for the determination of E-isomer presence even in trace amounts. Being an isomeric impurity, the E-isomer cannot be determined by LCMS due to the presence
of other possible isomeric impurities like Δ<sup>3</sup>-isomer and 7-epimer. Similarly in the 13<sup>C</sup> NMR spectra, E-isomer exhibits a downfield shift for aminothiazole carbon C5 when compared to the Z-isomer. The aminothiazole carbon bearing the proton appears around δ 107-109 ppm and δ 115 to 120 ppm in Z and E-isomer, respectively. 1<sup>H</sup> and 13<sup>C</sup> NMR spectrum of E and Z isomers of Cefdinir is shown in figure 4 & 5 as an example.

In the case of Cefuroxime and Cefuroxime axetil (8 and 9), where, R is furyl group, characterization of E and Z isomers have been made based on the chemical shift values of furan Hydrogen at C-2 position as discussed above. Similarly in the 13<sup>C</sup> spectra, E-isomer exhibit a downfield shift for furan carbon C2, when compared to the Z-isomer. 1<sup>H</sup> and 13<sup>C</sup> NMR spectrum of E and Z isomers of Cefuroxime is shown in figure 6 & 7.

Comparison of 1<sup>H</sup> NMR data of the selected Cephalosporins indicates that, approximately about 0.7 ppm chemical shift difference observed between the E and Z isomers. The approximate chemical shift difference in 13<sup>C</sup> NMR spectra of E and Z isomers is about 7 ppm. Higher chemical shift difference (0.9 ppm in 1<sup>H</sup> NMR and 15 ppm in 13<sup>C</sup> NMR) observed for the Cefazidime drug substance. Observation of these data shows that the difference is due to the attachment of 2-methylpropionic acid moiety attached to the oxime oxygen.

This NMR data can be used for the quantitative determination of unwanted E-isomer in some cephalosporins in drug substances and various dosage forms. The determination is based on the integration of the aminothiazole/furyl proton in E-isomer relative to the Z-isomer.

### CONCLUSION

Qualitative and quantitative determination of E and Z isomers of nine pharmaceutically important cephalosporins in bulk drugs by 1<sup>H</sup> and 13<sup>C</sup> NMR chemical shift values have been discussed. 1<sup>H</sup> NMR is found to be a better tool in terms of time compared to HPLC and LCMS techniques for the detection of the presence of E-isomer at levels around 1.0%.

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### REFERENCES