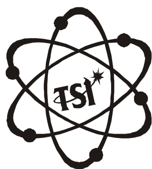

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Identification of E and Z isomers of some cephalosporins by NMR

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ABSTRACT

This article deals with the identification of geometrical isomers (E and Z) of some cephalosporin drugs by NMR. ^{1}H and ^{13}C 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, ceftiofur, ceftriaxone, cefpodoxime proxetil, cefuroxime and cefuroxime axetil samples were taken for the study.

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KEYWORDS

NMR;
Chemical shift;
^{1}H NMR;
^{13}C NMR;
Cephalosporin;
E-isomer;
Z-isomer.

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 generally 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 cephem nucleus under controlled reaction conditions in this and the subsequent steps to avoid isomerisation to the anti-isomer figure 2.

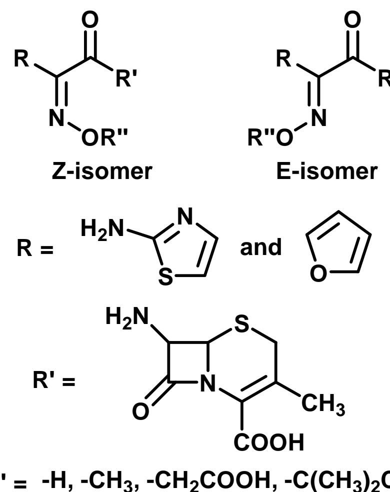

Figure 1 : E and Z isomer in cephalosporin drugs

Note

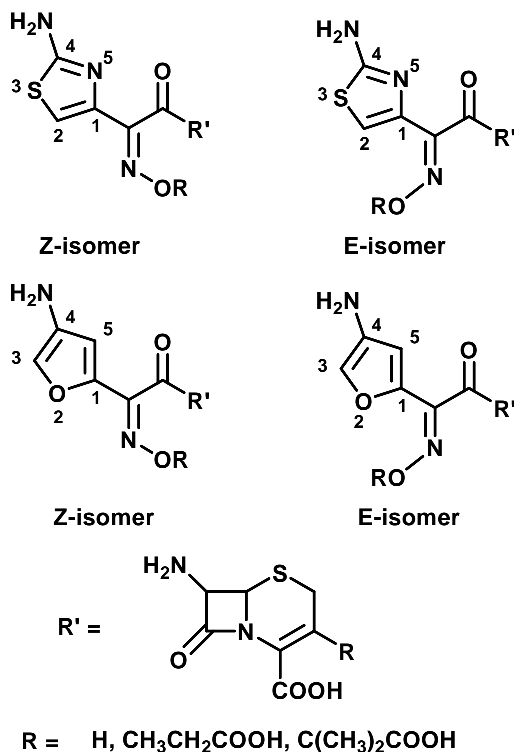

Figure 2 : E and Z orientation of oxime substituent

E-isomers are the process related impurities as well as possible degradation products. E-isomers are formed during the manufacturing process or by conversion of Z-isomer under the acidic conditions or on exposure to light and hence the necessity to separate and identify them. A process for the separation of syn and anti oxime isomers of Cephalosporin compounds by using macro reticular adsorption resin has been reported earlier[2].

The Z-isomer is the predominant isomer over the E-isomer and is used in the preparation of dosage forms. The antibacterial activity and oral absorbability of both isomers have been studied in details. The E-isomer is reported to be 2-32 times less active than the Z-isomer against gram-negative bacteria, although both isomers show appreciable oral absorbability regardless of the configuration of the oxime[3]. It is interesting to note that Z-isomer of cefotaxime (Compound 1) is upto 100 times more active against certain organisms than the E-isomer[4].

NMR analysis of some cephalosporin's have been reported earlier[3-10]. The present study describes the identification of E and Z isomers of nine cephalosporins of pharmaceutical interest viz, cefotaxime, cefixime, ceftazidime, cefdinir, ceftiofur, ceftriaxone, cefpodoxime proxetil, cefuroxime and cefuroxime axetil by using ^{1}H and ^{13}C NMR chemical shift values. Structural details of

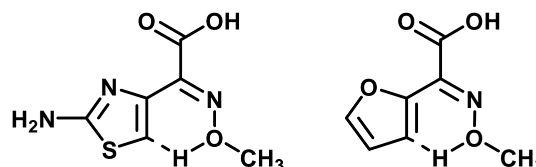

Figure 3 : Hydrogen bonding between the aminothiazole/furyl Hydrogen and oxygen of the oxime

the above cephalosporins were shown in TABLE 1. The E-isomers were isolated by a simple reverse phase preparative HPLC and were characterized using spectral data.

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 ^{1}H and ^{13}C chemical shift values were reported on the δ scale in ppm, relative to DSS (δ = 0.00 ppm) in the case of D_2O solvent. In the case of DMSO-d_6 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). ^{1}H and ^{13}C 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

S.No.	Compound	R	R'	R''	Molecular formula
1	Cefotaxime			H	$C_{16}H_{17}N_5O_7S_2$
2	Cefixime			H	$C_{16}H_{15}N_5O_7S_2$
3	Cefdinir			H	$C_{14}H_{15}N_5O_5S_2$
4	Ceftiofur			H	$C_{18}H_{15}N_5O_7S_3$
5	Ceftriaxone			H	$C_{18}H_{17}N_8O_7S_3$
6	Ceftazidime			*	$C_{22}H_{22}N_6O_7S_2$
7	Cefpodoxime Proxetil				$C_{21}H_{27}N_5O_9S_2$
8	Cefuroxime			H	$C_{16}H_{17}N_5O_7S_2$
9	Cefuroxime axetil				$C_{20}H_{22}N_4O_{10}S$

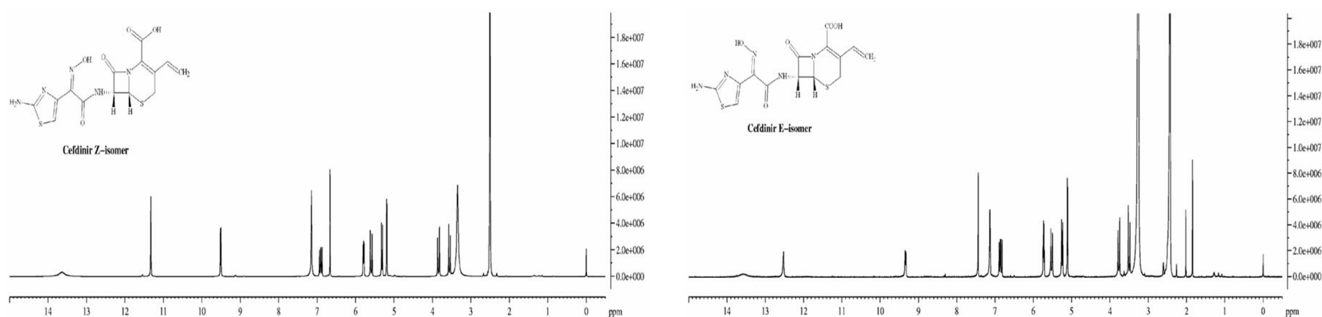

Figure 4 : ^{1}H NMR spectra of E and Z isomers of Cefdinir

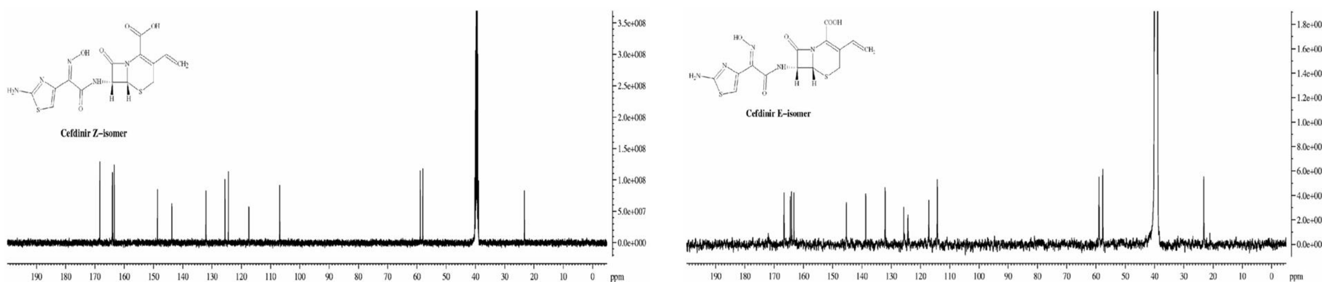

Figure 5 : ^{13}C NMR spectra of E and Z isomers of Cefdinir

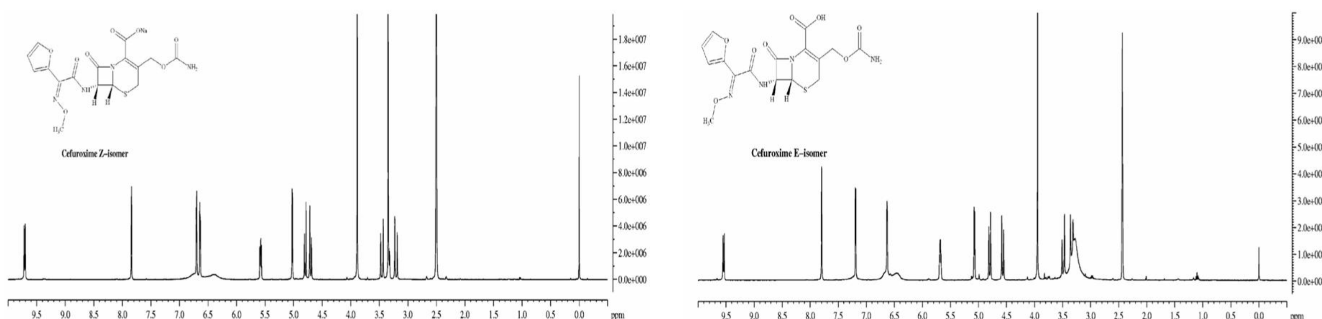

Figure 6 : ^{1}H NMR spectra of E and Z isomers of Cefuroxime

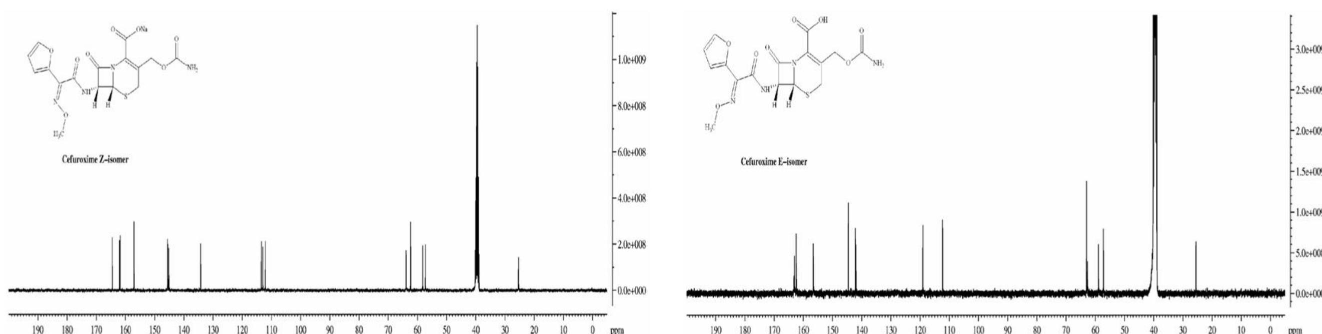

Figure 7 : ^{13}C NMR spectra of E and Z isomers of Cefuroxime

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 δ 6.70 to 6.90 ppm and δ 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

TABLE 2 : ^{1}H and ^{13}C NMR chemical shift values of aminothiazole/furyl proton and carbon of Z and E-isomer of some selected cephalosporins

Sr. No.	Compound	^{1}H Chemical shift for Aminothiazole/ Furan proton (δ in ppm)		^{13}C Chemical shift for Aminothiazole/Furan proton bearing carbon (δ in ppm)	
		Z-Isomer	E-Isomer	Z-Isomer	E-Isomer
1	Cefotaxime	6.74	7.55	109.8	116.6
2	Cefixime	6.81	7.60	111.5	117.3
3	Cefdnir	6.67	7.51	107.7	115.1
4	Ceftiofur	6.89	7.45	110.5	115.6
5	Ceftriaxone	6.73	7.44	113.6	116.5
6	Ceftazidime	6.70	7.62	110.7	124.8
7	Cefpodoxime proxetil*	6.73	7.54	109.9	117.9
8	Cefuroxime	6.70	7.23	113.0	119.9
9	Cefuroxime axetil*	6.69	7.26	113.8	120.0

***Mixture of diastereoisomers**

of other possible isomeric impurities like Δ^3-isomer and 7-epimer. Similarly in the ^{13}C 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 Eisomer, respectively. ^{1}H and ^{13}C 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}C spectra, E-isomer exhibit a downfield shift for furan carbon C2, when compared to the Z-isomer. ^{1}H and ^{13}C NMR spectrum of E and Z isomers of Cefuroxime is shown in figure 6 & 7.

Comparison of ^{1}H 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}C NMR spectra of E and Z isomers is about 7 ppm . Higher chemical shift difference (0.9 ppm in ^{1}H NMR and 15 ppm in ^{13}C NMR) observed for the Ceftazidime 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}H and ^{13}C NMR chemical shift values have been discussed. ^{1}H 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%.

ACKNOWLEDGEMENTS

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