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ISOLATION AND CHARACTERIZATION OF PROCESS RELATED IMPURITY IN CEFUROXIME ACTIVE PHARMACEUTICAL INGREDIENT

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Abstract:
The impurity ranging from 0.08%-0.12% by peak area in Cefuroxime active pharmaceutical ingredient was detected by simple isocratic reverse-phase high performance liquid chromatography (HPLC). The impurity was isolated by prep-HPLC and characterized by LC-MS/MS and NMR experimental data. Based on the results obtained from different spectroscopic experiments, the impurity has been characterized as Anti Cefuroximic acid chemically known as [(6R, 7R)-3-[(Acetoxy) methyl]-7-[(2E) -2-furanyl (methoxyimino) acetyl] amino]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid].

Keywords: Cefuroxime, prep-HPLC, impurities, Mass Spectrometry, NMR, identification and characterization.

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INTRODUCTION

Cefuroxime axetil[1] is a semisynthetic, broad-spectrum cephalosporin antibiotic for oral administration. Chemically, cefuroxime axetil, the 1-(acetyloxy) ethyl ester of cefuroxime, is (RS)-1-hydroxyethyl (6R,7R)-7-[2-(2-furyl)glyoxyl-amido]-3-(hydroxyl methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate, 7[2]-(Z)-(O-methyl-oxime), 1-acetate 3-carbamate. Its molecular formula is $C_{20}H_{22}N_4O_{10}S$, and it has a molecular weight of 510.48. Cefuroxime is a well characterised and effective antibact erial agent which has bactericidal activity against a wide range of common pathogens, including β-lactamase producing strains. Cefuroxime has good stability to bacterial β-lactamase, and consequently is active against many ampicillin-resistant or amoxycillin-resistant strains. The bactericidal action of cefuroxime results from inhibition of cell wall synthesis by binding to essential target proteins.

Literature survey reveals Spectrophotometric [3] and HPTLC methods [4-6] for Cefuroxime axetil determination in combination with other drugs. Stability indicating [7] and bioanalytical chromatographic methods [8,9] for quantification of CA are also reported. CA is official in USP which dictates RPHPLC method for CA determination as single drug [10]. Impurities in active pharmaceutical ingredient (API) are highly undesirable and in some cases can prove to be harmful to the patient. The ICHQ-715 is a guidance [11] for API manufacturers, mentions that impurities be maintained below set limits. Thus it is pertinent to identify and characterize the impurities in API in order to develop suitable process where in their levels can be kept within permissible limits. The impurity profile study should be carried out for any bulk drug to identify and characterize all the unknown impurities that are present at a level of above 0.05%. A comprehensive study has been undertaken to isolate and characterize these impurities by spectroscopic techniques. This research article describes the separation, identification, isolation and characterization of Cefuroxime impurity present in the range of 1.0% by peak area in the bulk drug of Cefuroxime.

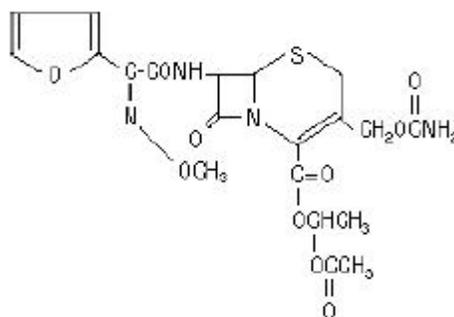Cefuroxime axetil is in the amorphous form and has the following structural formula:
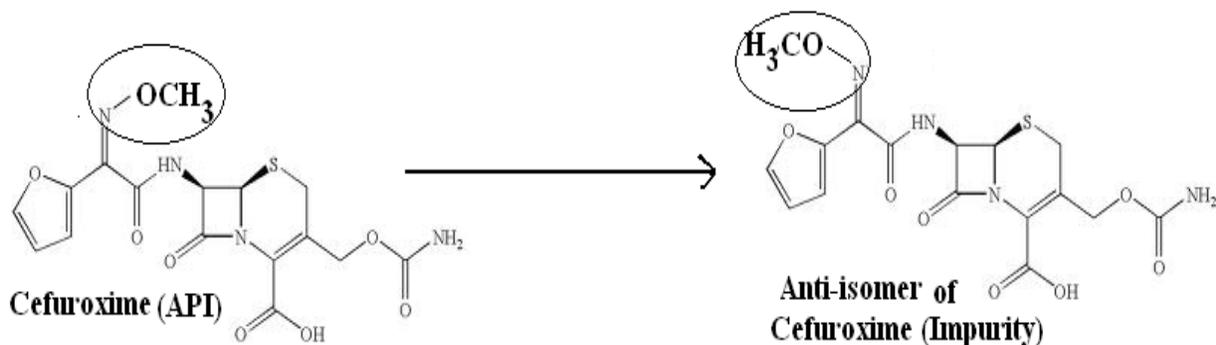

Fig 1: Chemical Structure of Cefuroxime

Fig 2: Conversion of Cefuroxime in to Respective Ante Isomer

EXPERIMENTAL

Samples and chemicals

The Cefuroxime bulk drug sample was received from Covalent Laboratories Ltd, India. HPLC grade acetonitrile was obtained from Merck Co., Mumbai, India. A. R. grade Sodium acetate, glacial acetic acid, Sodium hydroxide and acetone were obtained from Merck Co., Mumbai, India. Ultra pure water was collected from Elix Millipore water purification system. Nitrogen is used of ultra pure grade (99.999%).
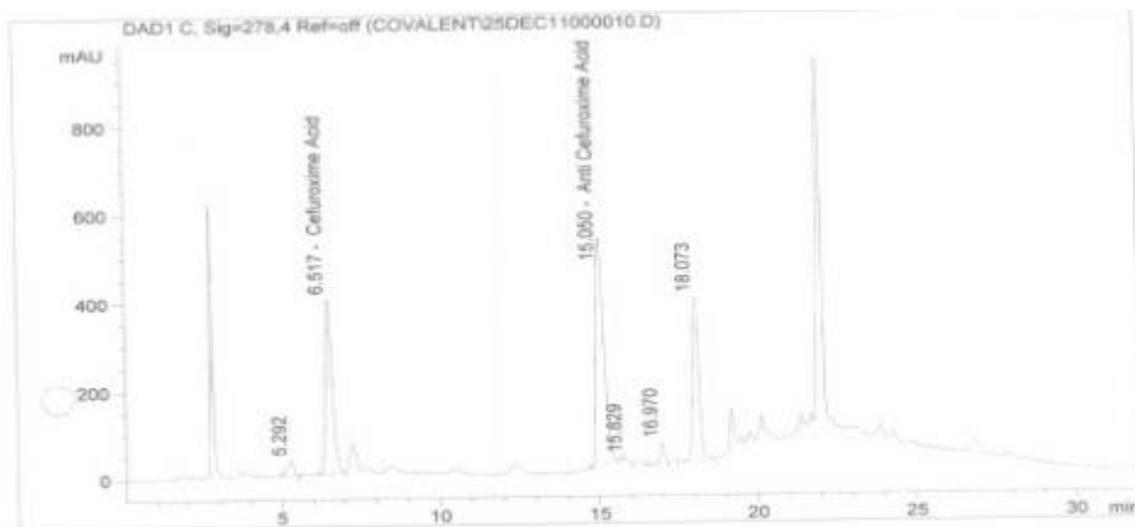
High Performance Liquid Chromatography (analytical)

An Agilent HPLC system equipped with low pressure quaternary gradient pump along with photo diode array detector and auto sampler has been used for the analysis of samples. The data was collected and processed using Chemstation software. An hypersil

MOS (250 * 4.6 mm, 5-Micron) column was employed for the separation of impurity from Cefuroxime. The column eluent was monitored at 278 nm. The reverse-phase HPLC method in gradient mode was optimized for the separation of impurity from Cefuroxime active pharmaceutical ingredient where the mobile phase composition was a mixture of sodium acetate buffer (pH 3.4) as mobile phase A and 50:50 v/v sodium acetate buffer and acetonitrile as mobile phase B. The gradient program is shown in table-1. Chromatography was performed at room temperature using at a flow rate of 1.5 mL min-1. The chromatographic run time was 30 min. The gradient program for analytical HPLC was shown in table 1. Typical analytical chromatogram representing the cefuroximic acid and impurity was shown in figure-3.

Table 1: The Gradient Program for Analytical HPLC

Time (min)	pH 3.4 Acetate Buffer (mobile phase A)	Mobile phase B
0	80	20
10	80	20
20	40	60
25	40	60
30	80	20

High Performance Liquid Chromatography (preparative)

Fig 3: Typical Analytical Chromatogram Representing the Cefuroximic Acid and Impurity

An Agilent prep-HPLC system equipped with 1200 series pump, photo diode array detector, auto sampler fitted with 5000 μL loop and 1200 series preparative fraction collector was used. The data was collected and processed using Agilent "Chemstation" software. An Kromasil C18 column (250 × 21.2 mm, 10-Micron,) was employed for loading the sample. An analytical method was developed in gradient mode separately to resolve the impurity, followed by scaling up the same method for prep-HPLC to collect the required impurity fractions. The mobile phase consisted of 0.1% Acetic acid in water as mobile phase A and acetonitrile as mobile phase B. The gradient programme for preparative HPLC were shown in table-2. The flow rate was set at 32 mL min-1. Detection was carried out at 278 nm. Approximately 50 mg mL-1 of sample was prepared using 1.5 mL acetonitrile, 1.5 mL of water and 1 mL acetone as diluent to load on to the column. The gradient programme shown in table -2. Typical preparative chromatogram was shown in figure-4.
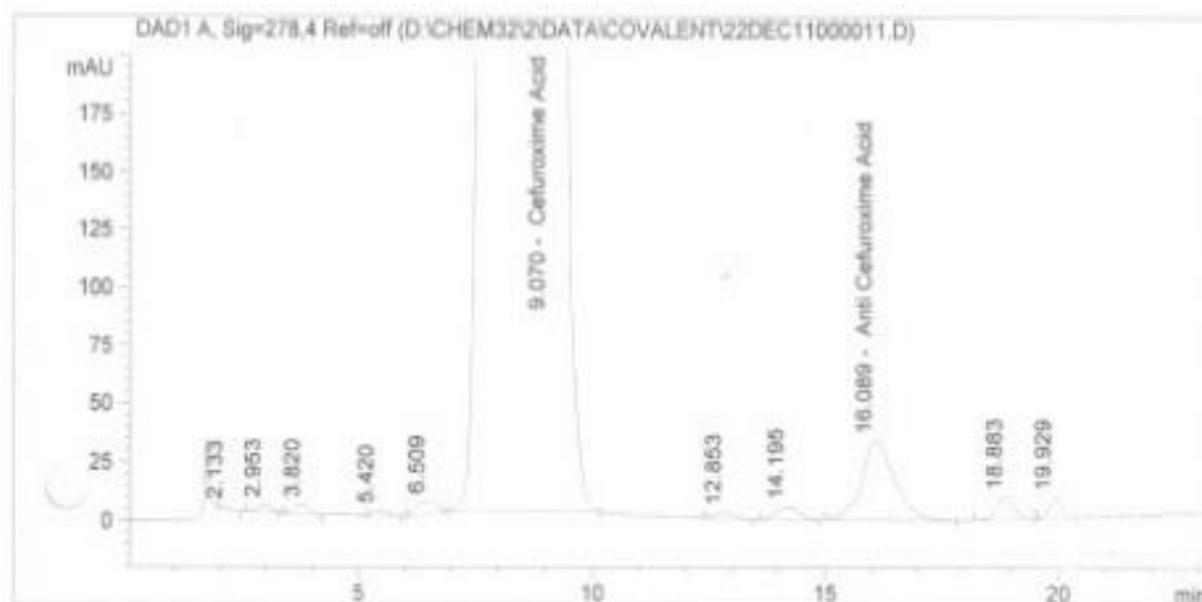

Fig 4: Typical Preparative Chromatogram of Anticefuroximic Acid

Table 2: Gradient Program for the Preparation of Anticefuroximc Acid from Cefuroximic acid

Time (min)	0.1% Acetic acid in water (mobile phase A)	Acetonitrile (Mobile phase B)
0.01	80	20
15	80	20
30	40	60

Mass Spectrometry
Mass analysis has been performed on micromass Waters. The analysis was performed in positive ionization mode with turbo ion spray interface. The parameters for ion source voltage IS = 5500 V, declustering potential, DP = 70 V, focusing potential, FP = 400 V, entrance potential, EP = 10 V were set with nebuliser gas as air at a pressure of 40 psi and curtain gas as nitrogen at a pressure of 25 psi. An Inertsil ODS-3V (250 * 4.6 mm, 5-Micron, GL Sciences, Japan) column was used for the separation. The mobile phase is a mixture of 2 mmol L-1 ammonium acetate and acetonitrile in a ratio of 50:50 (v/v). The analysis was performed at a flow rate of 1.0 mL min -1 with splitting.

NMR spectroscopy
The 1H, 13C NMR and DEPT experiments were carried out at precessional frequencies of 399.939 MHz and 100.574 MHz respectively, in CDCl3 at 25 °C temperature on a Varian-400 FT NMR spectrometer. 1H and 13C chemical shifts are reported on the d scale in ppm, relative to tetra methyl silane (TMS) d 0.00 and CDCl3 at 77.0 ppm in 13C NMR respectively. NMR spectra of cefuroximic acid and its impurity are shown in figure-6.

Anti-isomer A:
^{1}H NMR : (400 MHz, DMSO): δ 7.85 (s, 1H), 7-25-7-24 (d ,1H), 6.69-6.88 (m, 2H), 6.56(m, 1H), 5.71-5-68 (m, 2H), 5.10 (s, 2H), 4.88-4.66 (m, 2H,), 4.01 (s, 3H), 3.54-3.30 (m, 5H), 2.51 (s, 3H).
^{13}C NMR: (75 MHz, CDCl₃): δ 170.8, 164.1, 163.1, 158.0, 143.0, 141.6, 129.4, 122.0, 109.1, 108.4, 92.0, 63.4, 62.1, 59.1, 58.4, 54.2, 24.0, 20.1,18.8.
Mass: ESI-MS = 511 [M+ 1]⁺

Cefuroxime axetil:
^{1}H NMR : (400 MHz, DMSO): δ 7.83 (s, 1H), 7-24 (d ,1H), 6.29-6.22 (d, 1H), 6.21-6.19 (d, 1H), 6.50(m, 1H), 5.64-5-53 (m, 2H), 5.00 (s, 2H), 4.76-4.67 (m, 2H,), 3.98 (s, 3H) 3.71-3.43 (m, 5H), 2.32 (s, 3H).
^{13}C NMR: (75 MHz, CDCl₃): δ 169.88, 168.41, 163.1, 162.8, 158.9, 144.8, 142.2, 130.4, 121.2, 108.7, 108.1, 91.3, 62.4, 61.4, 59.0, 58.1, 53.9, 24.9, 20.7, 18.7.
Mass: ESI-MS = 511 [M+ 1]⁺

Results and Discussion
Detection of impurities by HPLC
Typical analytical HPLC chromatogram of Cefuroxime bulk drug and its impurity obtained by using the HPLC method discussed under the heading 'High Performance Liquid Chromatography (analytical)' is shown in Figure 1. The targeted impurity under study is eluted at retention time of about 15.4 min, while Cefuroxime eluted at about 5.5 min.

Isolation of the impurities by prep-HPLC
A simple reverse phase chromatographic system, discussed under the heading 'High Performance Liquid Chromatography (preparative)' was used for isolating the process related impurity. In this chromatographic system, Cefuroxime eluted at about 9.0 min whereas the impurity eluted at about 16.0 min. The impurity I fraction was collected between 15.0 min and 18.0 min. The impurity fractions were concentrated by evaporating acetonitrile portion at room temperature under high vacuum on a Buchii Rotavapour Model R124. The concentrated aqueous layer containing individual impurity was extracted into methylene chloride. The fraction was concentrated by evaporating at room temperature under high vacuum on a Rotavapour. Purity of the impurity was tested in analytical mode and was found to be 97.2%, (Figure 4) before carrying out spectroscopic experiments.
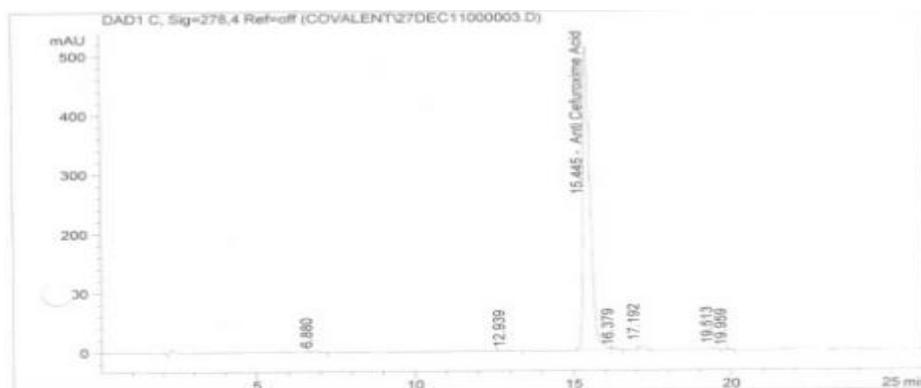

Fig 4: Fraction Chromatogram of Anti Cefuroximic Acid Isolated from Cefuroximic acid

LC-MS/MS analysis

LC-MS/MS analysis of Cefuroxime bulk drug sample and impurity I was performed using the chromatographic system as described under the heading 'Mass spectrometry (LC-MS/MS)'. Results of LC-MS/MS analysis revealed that impurity I exhibited molecular ion at m/z (M+1) 294 amu (Table 1) and fragmentation pattern is very similar to that of Cefuroxime (Figure 7). Based on this fact it was assumed that the first impurity was similar to that of Cefuroxime which might be a positional isomer. Impurity I and impurity II were analyzed comfortably by GC-MS.

Structural elucidation of impurity I

The spectral data of this impurity was compared with that of Cefuroxime spectral data. LC-MS/MS analysis exhibited molecular ion for this impurity at m/z 294(M+1). Fragmentation pattern of Cefuroxime and impurity were also exactly matching. The ^{1}H NMR spectrum of Cefuroxime exhibited two separate signals for the protons on the triazole ring which indicated that these two protons on the triazole ring were chemically non-equivalent. It was quite interesting to note that the ^{1}H NMR spectrum of this impurity exhibited a single signal integrating for two protons attached to the triazole ring. The ^{1}H NMR spectrum is shown in Figure 6. This observation indicated that the two protons attached to triazole ring were in chemically equivalent environment. ^{13}C NMR and DEPT-NMR experiments were also performed for this impurity. The two carbon atoms in the triazole ring were resonated at the same frequency as compared to that of Cefuroxime. This observation further confirmed that two carbon atoms of this impurity in the triazole ring were chemically equivalent (see Table 2). Based on these spectral results, it was confirmed that the impurity having the molecular formula $C_{17}H_{19}N_5$ and the same was characterized as 2,2'-(5-((4H-1,2,4-triazol-4-yl) methyl)-1,3- phenylene) bis (2-methyl propanenitrile). Chemical structures of Cefuroxime and impurities are shown in Figure 5. Fragmentation pathways for impurities are shown in Figure 4. Major fragments obtained in LC-MS/MS analysis for Cefuroxime and its impurities are given in Table 1.

CONCLUSIONS

Cefuroxime is a potent aromatase inhibitor drug used in the treatment of cancer diseases. The present research work describes a HPLC method for detection, separation of three process related impurities from Cefuroxime and prep-HPLC method for isolation of these impurities from the Cefuroxime bulk drug. All the three impurities detected were characterized using GC-MS, LC-MS/MS and NMR experimental data.

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