Isolation and Characterisation of Degradation Impurities in the Cefazolin Sodium Drug Substance

Balasubramanian SIVAKUMAR *, Kannabiran PARTHASARATHY, Raman MURUGAN, Ramajeyabalan JEYASUDHA, Saravanan MURUGAN, Rajendira JANARDHAN SARANGHDAR

Orchid Chemicals and Pharmaceuticals Limited, Research and Development Centre, Sozhanganallur, Chennai 600 119, Tamilnadu, India.

* Corresponding author. E-mails: sivakumarb@orchidpharma.com or sivaorchid@gmail.com (B. Sivakumar)

Sci Pharm. 2013; 81: 933–950    doi:10.3797/scipharm.1304-14
Published:  June 4th 2013    Received:  April 15th 2013
Accepted:  June 4th 2013

This article is available from: http://dx.doi.org/10.3797/scipharm.1304-14
© Sivakumar et al.; licensee Österreichische Apotheker-Verlagsgesellschaft m. b. H., Vienna, Austria.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Two unknown impurities were detected in the cefazolin sodium bulk drug substance using gradient reversed-phase high-performance liquid chromatography (HPLC). These impurities were isolated by preparative HPLC and characterized by using spectroscopic techniques like LC-MS, LC-MS/MS, 1D, 2D NMR, and FT-IR. Based on the spectral data, the impurities have been characterized as \( N\)-\((2,2\)-dihydroxyethyl\)-\(2\)-(\(1\)-tetrazol-1-yl)acetamide (Impurity-I) and \( 2\)-\{(carboxy\[(\(1\)-tetrazol-1-ylacetyl)amino\]methyl\)-\(5\)\)-methylidene-\(5,6\)-dihydro-\(2\)-\(H\)-1,3-thiazine-4-carboxylic acid (Impurity-II). The structures of these impurities were also established unambiguously by co-injection into HPLC to confirm the retention time. To the best of our knowledge, these two impurities were not reported elsewhere.

Keywords

Cefazolin sodium • Degradation Impurities • LC-MS/MS • NMR • \(\beta\)-Lactam

Introduction

Cephalosporin antibiotics are substitution products of 7-Aminocephalosporinic acid (7-ACA). Cefazolin sodium (CZS) is a first-generation cephalosporin antibiotic, has a tetrazolylacetyl side chain on the amino group and a 5-methyl-thiadiazolyl-thiomethyl
group on the 3-position of 7-ACA. CZS is a broad-spectrum antibiotic, active in vitro against most Gram-positive and Gram-negative bacteria. Chemically, CZS is sodium (6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanyl]methyl]-8-oxo-7-[(1H-tetrazol-1-yl-acetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [1–4]. CZS is used in the form of intramuscular and intravenous injections for the treatment of infections of the respiratory system, urogenital system, infections of skin and soft tissues, biliary tract, bones, joints, and in endocarditis and septicemia. CZS exhibits significant efficiency in the treatment of bacterial eye infections caused by staphylococci, pneumococci, and Gram-negative bacilli (Escherichia coli, Klebsiella spp., Proteus spp.) [5].

A literature search revealed that a number of high-performance liquid chromatographic (HPLC) methods have been developed to determine CZS in biological samples [6, 7]; CZS in buffered eye drops [8]; stress degradation [9]; stability of CZS sodium in polypropylene syringes and polyvinylchloride mini bags [10], and in heparinized and non-heparinized dialysis solution [11]. Photocatalytic degradation of CZS was reported along with the possible pathways of degradation [12]. However, the reported HPLC methods do not provide information about the unidentified impurities other than the ones listed in the European Pharmacopeia [13].

In CZS drug substances manufactured at Orchid Chemicals & Pharmaceuticals Ltd, two unknown impurities were observed at the relative retention time (RRT) of 0.08 and 0.20 by HPLC at a level of around 0.10%. These impurities were named as Impurity-I (RRT 0.08) and Impurity-II (RRT 0.20), found to be increasing to the level of 0.15% during stability studies. It is a mandatory requirement of regulatory authorities to identify and characterize any unknown impurity present or formed during stability studies with more than 0.1% in the drug substance and drug product [14–17]. A comprehensive study has been undertaken to isolate and characterize these two impurities by spectroscopic techniques [18–20]. This research paper describes the preparative HPLC isolation, identification, and characterization of these two unknown impurities of the CZS drug substance.

**Experimental**

**Samples and Chemicals**

The CZS sample manufactured at Orchid Chemicals and Pharmaceuticals Ltd, Alathur, Chennai, India was used for this study. Solvents were HPLC grade procured from Merck Specialities India Ltd. Potassium dihydrogen phosphate, sodium hydroxide, and orthophosphoric acid were HPLC grade supplied by Merck. Deuterated solvents for the NMR experiments were purchased from Euriso-top SA, France. The HPLC column, YMC Pack Pro, and preparative HPLC column YMC ODS-A were purchased from YMC, Japan and Thermo Electron Corporation. The water used for preparing the mobile phase was purified using the Millipore Milli-Q Plus (Milford, MA, USA) purification system.

**Analytical HPLC**

The chromatographic separation was achieved on the YMC Pack Pro C18-RP column (250 X 4.6 mm and 5 μm as particle size) using the Waters Alliance 2695 model. The gradient LC method employed 6.8 gm of KH2PO4 in 1000 mL water (pH-adjusted to 6.80 with sodium hydroxide solution) as mobile phase A and a mixture of 500 mL of acetonitrile and 3.4 gm of KH2PO4 in 500 mL of water as mobile phase B. The LC gradient program
was set as (T/%B) = 0/2, 7/15, 30/20, 35/20, 45/50, 50/50, and 55/2 with a post run-time of 10 min. The column temperature was maintained at 30 °C and the detection wavelength was set at 210 nm up to 4 minutes and after that 254 nm. The injection volume used was 20 μL and mobile phase A was used as a diluent.

**Forced Degradation of Cefazolin Sodium**

The forced degradation study was performed on the CZS drug substance with the intention to ensure the degradation products’ separation from the analyte peak. Accordingly, a degradation study was conducted separately by treating CZS with acid, base, aqueous, peroxide, light, and heat.

**Preparation of Sample Solution**

About 125 mg of the CZS sample was dissolved in 50 mL of buffer (3.53 gm of potassium dihydrogen orthophosphate and 5.76 gm of anhydrous disodium hydrogen orthophosphate in 1000 mL of water) solution.

**Acid Stressed Degradation**

To 10 mL of the sample solution, 5 mL of 0.1 N HCl solution was added and kept for about 2.5 hours at room temperature and then analysed by HPLC as per the method.

**Base Stressed Degradation**

To 10 mL of the sample solution, 10 mL of 0.2 N sodium hydroxide solution was added and analysed immediately by HPLC as per the method.

**Peroxide Stressed Degradation**

To 10 mL of the sample solution, 5 mL of 30% hydrogen peroxide solution was added and analysed immediately by HPLC as per the method.

**Thermal Stressed Degradation**

The sample solution was heated at 105°C for about 28 hours and analysed by HPLC as per the method.

**Photolytic Degradation**

The sample solution was exposed to UV radiation for about 33 hours and analyzed by HPLC.

**Humidity Degradation**

The sample solution was subjected to humidity degradation by keeping it at 25°C and 97% RH for about 34 hours and analyzed as per the HPLC method.

**Results of the Forced Degradation Study**

Impurity-I was observed at a level of 0.3% from acidic and photolytic degradation. The degradation conditions were optimised in such a way to increase Impurity-I to a level of 3%. The optimized condition was 1 gm of the CZS sample in 100 ml of water adjusted to a pH of 3.5 with orthophosphoric acid, and then exposed to UV light for about 12 hours.
Impurity-II was observed at a level of 2% from base degradation. The degradation condition was optimised to increase the Impurity-II content to 10% by heating the base degradation sample for about 10 minutes.

The other major impurities observed from stress degradation were 7-epimer of cefazolin, 5-methyl-1,3,4-thiadiazol-2-thiol (MMTD), cefazolin lactone, and cefazoloic acid. These impurities were listed in the European Pharmacopeia [13].

**Preparative HPLC**

Isolation of the impurities was carried out using the Waters 2000 Prep HPLC equipped with a UV detector monitored at 210 nm and a YMC-ODS-A C18 (250 x 50 mm), 10 µm column was used. The impurities were eluted by using ratio of 99 parts water to 1 part acetonitrile at a flow rate of 30 mL/minute. The impurity fractions were collected from several injections and then pooled. These pooled fractions were concentrated separately by using the Rotavapor (Heidolph Laboratory 4002 control) under high vacuum. The aqueous solutions were subjected to lyophilization to obtain the impurity. The relative retention time of the isolated impurities were further confirmed by using HPLC spiking studies, and the molecular mass was confirmed by LC-MS. The purity of the isolated impurities was at around 90%.

**Liquid Chromatography Mass Spectrometry (LCMS)**

LCMS experiments were carried out on a PE SCIEX API3000 LC-MS/MS equipped with a triple quadrupole mass analyzer and a TurbolonSpray sample introduction system. The chromatographic separation was achieved by the gradient HPLC systems Agilent 1100 G1311A(0) pump with Agilent 1100 G1329A(0) autosampler and Agilent 1100 DAD(0) detector. Analysis was carried out using the Hypersil ODS C18 (125 X 4 mm), 3µm column. Owing to the non-volatile nature of the phosphate-buffer mobile phase used in the HPLC related substances method, the chromatographic system was modified with 10 mM ammonium acetate, pH=7.5-adjusted with ammonia as mobile phase A and 100% acetonitrile was used as mobile phase B at a flow rate of 1.2 mL/min. The column oven temperature was maintained at 45 °C with an injection volume of 50µL with a 7 min injection delay. The UV detector was set to 254 nm. Gradient elution was performed by using mobile phase A and B. Gradient compositions were employed as (T/%B) = 0/2, 2/2, 4/15, 10/40, 11.5/65, 12 /65, 15/2, and 21/2. Mass analysis was performed in both positive and negative electrospray ionization modes. The capillary voltage was 5.5 kV. The interface temperature was 450°C. The impurity was subjected to a MS/MS study, wherein fragmentation of the various product ions was achieved by using different collision energies.

**NMR Spectroscopy**

The $^1$H NMR, $^{13}$C NMR, and DEPT (Distortionless Enhancement by Polarisation Transfer) experiments were performed with the Bruker Avance 400 MHz FT NMR spectrometer with a multinuclear BBO probe. DMSO-$d_6$ and D$_2$O were used as solvents. The NMR spectra of Impurity-I are recorded in D$_2$O, whereas the CZS and Impurity-II NMR spectra are recorded in DMSO-$d_6$. The $^1$H chemical shift values were reported on the $\delta$ scale in ppm, relative to TMS/DSS ($\delta = 0.0$ ppm) and in the $^{13}$C NMR, the chemical shift values were reported relative to DSS ($\delta = 0.0$ ppm) for Impurity-I and DMSO-$d_6$ ($\delta = 39.50$ ppm) as a reference in the case of Impurity-II. The DEPT spectra revealed the presence of methyl
Fig. 1. Structures of Cefazolin Sodium, Impurities-I+II, and other known Impurities
and methine carbons as positive peaks and methylene carbons as negative peaks. Homo- and heteronuclear chemical shift correlations were determined by the COSY and HSQC 2D NMR methods. Standard Bruker pulse sequences were applied by running Topspin 1.3 software. Typical samples were prepared for the $^{13}$C NMR experiments, which were 25 mg per 0.6 mL and 3–6 mg per 0.6 mL for $^1$H NMR measurements. The COSY and HSQC spectra were acquired in the magnitude and phase-sensitive mode. The HSQC spectrum was recorded with 64 scans, a relaxation delay of 1.5 s, a spectral width of 5341 and 16667 Hz in both dimensions, and 256 increments in t1 and 1K data points in t2.

**IR & UV Spectroscopy**

The IR spectrum was recorded in the solid state as a KBr dispersion medium using the FT-IR (Perkin Elmer, Spectrum 65 & JASCO-FT-IR-430) spectrophotometer. The UV spectrum was recorded on a Shimadzu UV-Visible spectrophotometer 2550 using water as a medium.

**Results and Discussion**

**Detection of Impurity-I and II**

The structures of Impurity-I, Impurity-II, and CZS are shown in Figure 1 along with the other known isolated/synthesized impurities, EP impurity-A, EP impurity-B, EP impurity-C, EP impurity-D, EP impurity-E, EP impurity-F, EP impurity-G, EP impurity-H, EP impurity-I, EP impurity-K, and EP impurity-L. A typical analytical HPLC overlaid chromatogram of the CZS sample and Impurities-I and -II spiked with the sample and the blank were analyzed using the method as described and shown in Figure 2. The target impurities under study were marked as Impurity-I and Impurity-II. The Relative Retention Times (RRT) of Impurity-I and -II are 0.084, and 0.202, respectively. These retention times indicate that Impurity-I and Impurity-II are more polar in nature than CZS.

![Fig. 2. Overlaid chromatograms of blank and spiked impurities along with sample](image-url)
Characterization of Impurity-I

The ESI mass spectrum of Impurity-I (Figure 3a) exhibits two distinct ions, m/z at 186 [M-H]− and m/z 168 ([M-H-H2O]-). The molecular mass of 187 supports the absence of the cepham nucleus in the impurity. The major product ions (Figure 4a) in MS/MS were observed at m/z 168, 140, and 112.

The 1H NMR and 13C NMR spectra of CZS and Impurity-I are shown in Figures 5(a), 6(a), and Figure 5(b), 6(b), respectively. The NMR and mass spectroscopic data of the isolated impurity was compared with those of the CZS data. In the 1H NMR spectrum of Impurity-I, hydrogen belongs to the cepham nucleus and the methylmercaptothiadiazole moiety at C-3 was not observed, and the methylene hydrogens and a methine hydrogen were newly observed while the hydrogen for the tetrazole was present at δ 9.25 ppm. The methylene group attached to tetrazole appeared as a singlet at δ 5.44 ppm (in CZS, the corresponding tetrazole and methylene hydrogen signals were observed at δ 9.15 and 5.42 ppm, respectively). The appearance of a characteristic doublet and triplet at δ 3.37 and 5.13 ppm, respectively, with a coupling constant J1,3 = 5.26 Hz indicate the presence of a –CH2-CH-moiety. A triplet at δ 5.13 ppm shows that the -CH- is attached to a more electronegative atom like oxygen.

In the 13C NMR spectrum, the signals appear at δ 145.6 and 50.2 ppm due to the tetrazole hydrogen-bearing carbon and the attached methylene carbon (in CZS, the corresponding carbon signals were observed at δ 145.7 and 50.1 ppm). A signal appeared at δ 170.0 ppm indicating the presence of a carbonyl group. The deshielding of the methine carbon (δ 90.0 ppm) indicates the attachment of two hydroxyl groups to that carbon. The DEPT results indicate that Impurity-I has two methylene carbons and two methine carbons.

The IR spectrum shows the characteristic stretching band at 3364 cm⁻¹ due to the NH stretching vibration and 1689 cm⁻¹ & 1552 cm⁻¹ due to the Amide-I and Amide-II bond, respectively. One broad band around 3300 cm⁻¹ indicates the presence of a hydroxyl group. No β-lactam carbonyl stretching was observed in the IR spectrum. The UV spectrum of the impurity shows the λmax at 210 nm.

From the above NMR, MS, and FT-IR spectral results, the structure of Impurity-I was assigned as N-(2,2-dihydroxyethyl)-2-(1H-tetrazol-1-yl)acetamide having the molecular formula C₅H₉N₅O₃, an aldehyde hydrate derivative involving the 7-acyl moiety of CZS and tetrazole acetic acid.

An LCMS/MS study was performed in negative ionization mode. The major fragment ions at m/z 168, 140, 112 & 69 are showed in Figure 4(a). The product ion at m/z 168 corresponds to the dehydrated product of Impurity-I. The elimination of carbon monoxide followed by rearrangement yields the base peak at m/z 112. The fragment ion at m/z 69 was due to the tetrazol-1-ide moiety. These mass spectral fragments further confirm the elucidated structure.

The degradation pathway for the formation of the impurity is shown in Figure 9(a). Upon acid hydrolysis, cefazolin degraded to cefazolocoic acid and subsequently formed lactone, then was decarboxylated with resulting bond migration, and expelled the cepham nuclues to give Impurity-I. A similar mechanism was reported in the literature for one of the
cephalosporin drug substances, cefdinir [21, 22]. The $^1$H and $^{13}$C NMR signal assignments of CZS and Impurities-I are shown in Table 1.

Fig. 3. Mass Spectra of (a) Impurity-I and (b) Impurity-II
Fig. 4. MS/MS spectra of (a) Impurity-I and (b) Impurity-II
Characterization of Impurity-II

The ESI mass spectrum of Impurity-II (Figure 3(b)) shows a deprotonated molecular ion peak at m/z 339, which is 137 amu less than that of CZS, which indicates the absence of the MMTD moiety in the molecule. The distinct ions at m/z 399 and m/z 679 are due to [M+CH₃COOH-H]⁻ and [2M-H]⁻. The MS/MS fragmentation pattern is shown in Figure 4(b). The major product ions were observed at m/z 298, 185, and 100.

Tab. 1. ¹H and ¹³C NMR assignment for Cefzolin Sodium, Impurity-I and II

| Atom | Cefazolin sodium | In-house Impurity-I | In-house Impurity-II |
|------|------------------|---------------------|---------------------|
|      | ¹H ppm/J ¹³C DEPT | ¹H ppm/J ¹³C DEPT | ¹H ppm/J ¹³C DEPT |
| 2    | 134.6            | 134.6               | 168.1               |
| 3    | 113.9            |                     | 131.3               |
| 4    | 26.5 CH₂         | 1 9.26/s 148.1 CH   | 2 3.31&3.60/2d, 14.2|
| 1    | 1 4.98/d, 4.8    | 2 5.44/s 52.7 CH₂   | 1 5.13/s 63.7 CH    |
| 6    | 56.9 CH          | 170                 | 4.44/dd, 9.2, 2.6   |
| 7    | 58.4 CH          |                     | 58.5 CH             |
| 8    | 165.4            |                     | 172.7               |
| 9    | 9.45/d, 8.4      | 2 3.37/d, 5.3 48 CH₂| NH 9.13/bs          |
| 10   | 165.6            | 1 5.12/t, 5.3 90.9 CH|                     |
| 11   | 49.1 CH₂         | 2 5.41&5.68/2d, 16.4|                     |
| 16   | 145.2 CH         | 1 9.40/s 144.9 CH   |                     |
| 17   | 37.2 CH₂         | 2 5.49&5.93/2s 122.6|                     |
| 19   | 163.6            |                     |                     |
| 22   | 166.1            |                     |                     |
| 24   | 15.3 CH₃         | 162.4               |                     |
| 25   | 172.7            |                     |                     |

The ¹H NMR and ¹³C NMR spectra of Impurity-II are shown in Figure 5(c) and 6(c), respectively. From the ¹H NMR spectrum, it is observed that two singlets at δ 5.49 ppm and δ 5.93 ppm correspond to two geminal olefinic protons, which is further confirmed by the intense off-diagonal contours in the ¹H-¹H COSY data (Figure 7(a)). A comparison of the ¹H NMR spectra of the impurity and CZS shows an upfield shift for the 7-CH proton suggests the cleavage of the β-lactam ring. Also, the absence of the methyl signal in the aliphatic region in ¹H/¹³C NMR data eliminates the presence of the methylmercapto-thiadiazole (MMTD) moiety at C-3 position of the cephem nucleus in the molecule. The DEPT spectrum shows the presence of three methylene carbons. The negative signal at δ 122.6 ppm ascertains that the olefinic carbon was present in the molecule. The HSQC data (Figure 7(b)) showed an intense contour between the hydrogen signals at δ 5.49 & 5.93 ppm and the corresponding carbon signal at δ 122.6 ppm, which confirms the presence of the =CH₂ moiety in the molecule. The ¹H and ¹³C NMR results indicate that there is no considerable change in the chemical shifts of hydrogen/carbon of the acyl.
Fig. 5. $^1$H NMR spectra of (a) Cefazolin Sodium (b) Impurity-I and (c) Impurity-II
Fig. 6. $^{13}$C NMR spectra of (a) Cefazolin Sodium; (b) Impurity-I; and (c) Impurity-II

substituent at C-7 position, which confirms that the tetrazole acetic acid moiety is present in Impurity-II. The FT-IR spectral data of Impurity-II show the absence of the band at ~1776 cm$^{-1}$, which confirmed the cleavage of the $\beta$-lactam ring which was present in the FT-IR spectra of CZS. The UV spectrum of the impurity shows the $\lambda_{\text{max}}$ at 226 nm, which matches that of the degradation impurity having $=\text{CH}_2$ at C-3 position [24].
Based on the above spectroscopic results, the structure of Impurity-II was elucidated as 2-{carboxy[(1H-tetrazol-1-ylacetyl)amino]methyl}-5-methylidene-5,6-dihydro-2H-1,3-thiazine-4-carboxylic acid. This structure was well-supported by the MS/MS fragmentation pattern as shown in Figure 8(b). The $^1$H and $^{13}$C NMR signal assignments are shown in Table 1.

Fig. 7.  (a) HSQC and (b) COSY Spectrum of Impurity-II
Fig. 8. LC-MS/MS Fragmentation pattern of (a) Impurity-I and (b) Impurity-II
An LCMS/MS study was performed in positive ionization mode. The major fragment ions at m/z 298, 185 & 100 are shown in Figure 4(b). The expulsion of the azide moiety from the tetrazolyl side chain yielded the daughter ion at m/z 298. Decarboxylation followed by elimination of the methyldiene amino acetaldehyde moiety resulted in the fragment ion at m/z 185. Successive elimination and rearrangement resulted in the fragment ion at m/z 100. These MS/MS fragmentation data further confirm the elucidated structure.

The degradation pathway for the formation of the impurity is also shown in Figure 9(b). Upon base degradation, the hydroxyl group attacked the carbonyl carbon of the β-lactam ring. Subsequent bond migration expelled the MMTD moiety and resulted in the formation of the impurity. Similar compounds and their 1H NMR and UV absorption spectra were reported earlier as products of aminolysis and enzymatic hydrolysis of the cephalosporins [24, 25].

**Fig. 9.** Degradation pathway for (a) Impurity-I and (b) Impurity-II
Conclusion

Two of the degradation impurities of cefazolin sodium were detected in the HPLC analysis. These impurities were isolated by preparative HPLC and their structures were elucidated using the 1D NMR, 2D NMR, LC-MS, LC-MS/MS, and FT-IR spectral techniques. The LC-MS/MS fragmentation pattern and a possible degradation pathway were reported.

Acknowledgement

Authors would like to thank Orchid Chemicals and Pharmaceuticals Ltd Management for giving the permission to publish this work. Authors wish to acknowledge Dr. U. P. Senthilkumar, Head-Beta lactam Process Development Group & IPM for his input for the degradation mechanism for the Impurity formation. Also thanks to Dr. N. K. Ramasamy, Quality Control for providing the stability study samples and also to our colleagues of Analytical Research and Development and IPM for their support.

Authors’ Statement

Competing Interests

The authors declare no conflict of interest.

References

[1] Andreotti D, Biondi S, Modugno ED. β-Lactam Antibiotics. Abraham DJ; ed. John Wiley & Sons, New Jersey. Burger's Medicinal Chemistry, Drug Discovery and Development. 2010; 5: 638–639. http://dx.doi.org/10.1002/0471266949.bmc087

[2] Mine Y, Nishida M, Goto S, Kuwahara S. Cefazolin, a new semisynthetic cephalosporin antibiotic. J Antibiot. 1970; 23: 195–203. http://dx.doi.org/10.7164/antibiotics.23.195

[3] Kariyone K, Harada H, Kurita M, Takano T. Synthesis and chemical properties of Cefazolin. J Antibiot. 1970; 23: 131–136. http://dx.doi.org/10.7164/antibiotics.23.131

[4] Zappala AF, Holl WW, Post A. Cefazolin. Florey K; ed. Academic Press, New York. Analytical Profile of Drug Substances. 1975; 4: 1–20. http://dx.doi.org/10.1016/S0099-5428(08)60006-4

[5] Kodym A, Bilski P, Domaniśka A, Helminiak Ł, Jabłońska M, Jachymska A. Physical and chemical properties and stability of sodium cefazolin in buffered eye drops determined with HPLC method. Acta Pol Pharm. 2012; 69: 95–105. http://www.ncbi.nlm.nih.gov/pubmed/22574512
[6] Al-Rawithi S, Hussein R, Raines DA, AlShowaier I, Kurdi W. Sensitive assay for the determination of cefazolin or ceftriaxone in plasma utilizing LC. J Pharm Biomed Anal. 2000; 22: 281–286. http://dx.doi.org/10.1016/S0731-7085(99)00273-3

[7] Wold JS. Rapid Analysis of Cefazolin in Serum by High-Pressure Liquid Chromatography. Antimicrob Agents Chemother. 1977; 11: 105–109. http://dx.doi.org/10.1128/AAC.11.1.105

[8] Baranowska I, Markowski P, Baranowski, J. Simultaneous determination of 11 drugs belonging to four different groups in human urine samples by reversed-phase high-performance liquid chromatography method. Anal Chim Acta. 2006; 570: 46–58. http://dx.doi.org/10.1016/j.aca.2006.04.002

[9] Lalitha N, Sanjav PPN, Vcshak MG, Kadri, U. Stability Indicating Reverse Phase HPLC Method for the determination of Cefazolin. Trop J Pharm Res. 2010; 9: 45–50.

[10] Donnelly RF. Stability of Cefazolin Sodium in Polypropylene Syringes and Polyvinylchloride Minibags. Can J Hosp Pharm. 2011; 64: 241–245. http://www.ncbi.nlm.nih.gov/pubmed/22479065

[11] Robinson RF, Morasco RS, Smith CV, Mahan JD. Stability of Cefazolin Sodium in Four Heparinised and Non-Heparinised Dialysate Solutions at 38°C. Perit Dial Int. 2006; 26: 593–597. http://www.ncbi.nlm.nih.gov/pubmed/16973516

[12] Gurkan YY, Turkten N, Hatipoglu A, Cinar Z. Photocatalytic degradation of Cefazolin over N-doped TiO2 under UV and sunlight irradiation: Prediction of the reaction paths via conceptual DFT. Chem Eng J. 2012; 184: 113–124. http://dx.doi.org/10.1016/j.cej.2012.01.011

[13] Cefazolin Sodium. European Pharmacopeia monograph 7.7, 2013.

[14] ICH harmonized tripartite guideline. Q3A (R2). Current step 4 version dated 25 October 2006.

[15] ICH harmonized tripartite guideline. Stability testing of new drug substances and Products Q1A (R2). Current step 4 version dated 6 February 2003.

[16] US Food and Drug Administration. Guidance for Industry: ANDAs: Stability testing of Drug Substances and Products, September 1987.

[17] United States Pharmacopeia. 25, US Pharmacopeial Convention, Rockville, MD 2000, 7, General Notices.

[18] Ahuja S, Alsante KM. Handbook of isolation and characterization of impurities in Pharmaceuticals. Academic Press, California, 2003.

[19] Smith RJ, Webb LW; eds. Analysis of Drug Impurities. Blackwell Publishers, Oxford, UK. 2007. http://dx.doi.org/10.1002/9780470988749.fmatter

[20] Ahuja S. Impurities Evaluation of Pharmaceuticals. Marcel Dekker, NY, 1998.
[21] Okamoto Y, Kiriyama K, Kiriyama Y, Namiki Y, Matsushita J, Fujioka, Yasuda T. Degradation Kinetics and Isomerization of Cefdinir, a New Oral Cephalosporin in Aqueous solution 1. J Pharm Sci. 1996; 85: 976–983. http://dx.doi.org/10.1021/js950446r

[22] Okamoto Y, Kiriyama K, Kiriyama Y, Namiki Y, Matsushita J, Fujioka, Yasuda T. Degradation Kinetics and Isomerization of Cefdinir, a New Oral Cephalosporin in Aqueous solution 2. Hydrolytic degradation pathway and mechanism for Beta lactam ring opened Lactones. J Pharm Sci. 1996; 85: 984–989. http://dx.doi.org/10.1021/js950447j

[23] Hamilton-Miller JMT, Newton GFF, Abraham EP. Products of Aminolysis and Enzymic Hydrolysis of the Cephalosporins. Biochem J. 1970; 116: 371–384. http://www.ncbi.nlm.nih.gov/pubmed/5435685

[24] Hamilton-Miller JMT, Richards E, Abraham, EP. Changes in Proton Magnetic Resonance Spectra during Aminolysis and Enzymatic Hydrolysis of Cephalosporins. Biochem J. 1970; 116: 385–395. http://www.ncbi.nlm.nih.gov/pubmed/5435686