Research Paper

Identification and Characterization of Hydrolytic Degradation Products of Cefditoren Pivoxil using LC and LC-MS/TOF

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The present research work was carried out to determine stability of cefditoren pivoxil, an orally absorbed prodrug that is rapidly hydrolysed by intestinal esterases to the active cephalosporin cefditoren. Cefditoren was subjected to stress conditions recommended by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guideline Q1A (R2). Cefditoren pivoxil was susceptible for degradation under acidic, alkaline and neutral hydrolytic conditions while it was stable under photolytic and thermal stress conditions. Separation of cefditoren and degradation products were carried out by using HPLC. The unknown degradation products were characterized by liquid chromatography-mass spectrometry/time of flight studies. Structures were proposed for each fragment based on best possible molecular formula and complete degradation pathways were reported for cefditoren and its degradants.

Key words: Cefditoren pivoxil, Degradation pathways, HPLC-DAD, LC-MS-TOF, Stress studies

Cephalosporins are β-lactam antibiotics clinically useful for treatment of variety of infectious conditions. Compared to penicillins, these are hydrolytically more stable, but undergo different chemical and enzymatic transformations owing to substitution at C-3 and side chain at C-7. Degradation studies of cephalosporins have helped in isolation, purification and discovery of new agents[1]. Discovery of new cephalosporins was also triggered due to of multidrug resistances among microorganisms[2]. Severe biological interactions[3], immunological reactions[4] and sometimes fatal conditions[5] have been reported by degradation products of cephalosporins.

Cefditoren pivoxil (CEFP) is a third generation oral cephalosporin active against respiratory tract pathogens, hence used for treatment of acute exacerbations of chronic bronchitis (AECB) and community-acquired pneumonia[6]. Chemically it is (-)-(6R,7R)-2,2-dimethylpropionyloxymethyl7-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoaceta-mido]-3-[(Z)-2-(4-methylthiazol-5-yl)ethenyl]-8-oxo-5-thia-1-azabi-cyclo [4.2.0] oct-2-ene-2-carboxylate (fig. 1).

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Till date the drug is official in Japanese Pharmacopoeia and Martindale: the extra pharmacopoeia[7,8]. Literature survey showed various methods reported for estimation of CEFP from pharmaceutical formulations and from plasma, such as spectrophotometric[9-14], UPLC[15], HPLC[16-21], HPTLC[22,23], electroanalytical[24] and thermal[25] methods. Comparatively few reports were published on stress degradation study and development of stability-indicating method of CEFP[26-29] according to ICH guidelines ICH Q1A (R2)[30]. Till date, there is no report with regard to characterization of degradation products of CEFP; hence present research work is undertaken considering general interest.

MATERIALS AND METHODS

CEFP was obtained as a gift sample from Maxim Pharmaceuticals (Pune, India) along with certificate of analysis. Analytical reagent (AR) grade hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), ammonium formate (NH_4HCO_2) and formic acid (HCOOH) were purchased from Qualigens Fine Chemicals (Mumbai, India). HPLC grade acetonitrile (ACN) and methanol (CH_3OH)

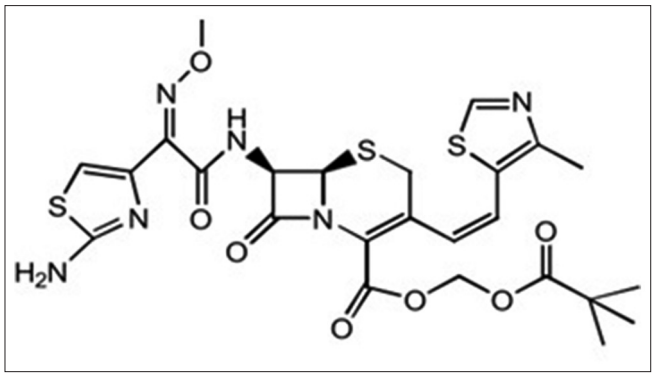

Fig. 1: Chemical structure of CEFP.
CEFP is cefditoren pivoxil.

was purchased from S. D. Fine-Chem Ltd. (Mumbai, India). HPLC grade water was prepared by using double distillation assembly of Lab-Sil Instruments (Bangalore, India).

Chromatographic studies were performed on a HPLC system (Shimadzu, Japan) equipped with Shimadzu SPD-M20A binary pump (LC-20AD), on-line degasser, sample injector fitted with 20 µl injection loop and prominence diode-array detector (DAD). Data was monitored and processed with LC solution software on a Dell computer.

All degradation studies were performed on precision water bath (Meta-Lab Ltd., Mumbai, India) equipped with thermostat for temperature control. Solid state thermal stress studies were carried out in hot air oven (Scientico Ltd., Mumbai, India). Photo stability studies were performed in photo stability chamber (Thermolab Scientific Equipments Pvt. Ltd., India). Calibrated lux and UV meter were used to measure visible illumination and near UV energy, respectively. The data was recorded and processed using Stability v7.2T software on Dell computer.

The LC/MS/TOF studies were performed with series 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) and MicrOTOF-Q mass spectrometer (Bruker Daltonics, Bremen, Germany). The LC system was equipped with an on-line degasser (G1379A), binary pump (G131A), auto-injector (G1313A), column oven (G1316A) and diode-array detector (G1315B). Signals were recorded and processed by combination of Hyphenation Star (version 3.1) and MicrOTOF Control (version 2.0) software. The mass spectrometer was run in positive electron spray ionization (ESI) mode with mass to charge (*m/z*) ratio in the range of 100-1000 *m/z*.

Chromatographic separation was carried out on HiQSil C_{18} (250×4.6 mm, 5 µ) column. Linear gradient elution system was employed with the flow rate of 1.0 ml/min using methanol:ammonium acetate ($NH_4CH_3CO_2$, 25 mM, pH 3.5 adjusted with formic acid) as mobile phase. All the dilutions were done using methanol:ammonium acetate buffer (25 mM, pH 3.5) in the ratio 50:50 v/v as diluent.

The pH of mobile phase and other solutions were adjusted by using pH meter (Controlled Dynamics, Vadodara, India). Other equipments used were sonicator (Spectralab UCB 30, Mumbai, India) and analytical balance (Precissa XR 205 SMDR, Sweden).

Stress studies:

Stress studies were carried out as per the ICH guideline Q1A (R2)**.** The drug was exposed to different degradation conditions namely hydrolysis, oxidation, dry heat and photolysis. All the stress conditions were optimized to achieve 10-15% degradation of drug. Response of drug was monitored by HPLC using DAD detector set at 230 nm wavelength. Characterization of degradants generated during different stress condition was performed with LC-MS/TOF system in positive ESI mode for fragmentation pattern and accurate masses. All operating parameters optimized for LC-MS/TOF system are mentioned in Table 1.

CEFP was dissolved in methanol to obtain a stock solution with a concentration of 1000 µg/ml. Hydrolytic degradation of CEFP was carried out under acidic, alkaline and neutral conditions separately by taking 1ml each of HCl (0.1 N), NaOH (0.01 N) and water with 1 ml of 1000 µg/ml CEFP at ambient temperature for 3.0 h. Samples were neutralized with equal strength of acid or base after required exposure. Samples treated with acid were neutralized by using equal strength of base and vice versa. For oxidative stress 1 ml of CEFP stock solution was treated with 1 ml of 10, 15, and 30% H_2O_2 at room temperature for 24 h. Effect of dry heat (thermal degradation) was studied on solid state while effect of light (photo degradation) was studied on solid and solution state conditions. In case of thermal degradation, the solid drug contained in sealed glass ampoule was heated in an oven at 60° for a period of seven days. Control sample was maintained in the same way at room temperature. During photo degradation, solid drug powder was exposed to fluorescent light (1.25 million

lux hours) and UV light (200 Whm^{-2}) in a photo stability chamber along with control samples. All standard and control samples kept for stability study were covered with aluminum foil. Optimized stress conditions were shown in Table 2.

Preparation of samples for HPLC analysis:
All the stressed samples were diluted with the help of diluent (methanol:buffer, $NH_4CH_3CO_2$, pH=3.5, 50:50 v/v) to obtain concentration of original drug (100 μg/ml) and injected in HPLC system. Samples of thermal and photo degradation (solid state) were weighed accurately and diluted appropriately with diluent to obtain final concentration of 100 μg/ml of CEFP. All the stressed samples after mixing in equal volume were used for the development of stability indicating assay method.

TABLE 1: OPTIMIZED PARAMETERS FOR MS/TOF STUDIES IN POSITIVE ESI MODE

Mode	Parameters for fragmentation
Source	
Ion polarity	Positive
Capillary (V)	4500
End plate offset (V)	−500
Nebulizer (Bar)	1.2
Dry heater (^{0}C)	200
Dry gas (L/min)	6.0
Ion optics	
Hexapole storage (V)	47.0
Hexapole extraction (V)	38.0
Collision storage (V)	30.0
Collision extraction (V)	18.6
Funnel 1 RF (Vpp)	300.0
Funnel 2 RF (Vpp)	300.0
Hexapole RF (Vpp)	300.0
ISCID energy (eV)	0.0
Quadrupole	
Ion energy (eV)	5.0
Isolation mass (m/z)	250.0
Collision energy (eV)	12.0
Collision cell RF (Vpp)	500.0
Transfer time (μs)	50.0
Pre-pulse storage time (μs)	10.0
TOF	
Corrector fill (V)	48.0
Pulsar pull (V)	399.0
Pulsar push (V)	399.0
Reflector (V)	1300.0
Flight tube (V)	9000.0
Corrector extract (V)	910.0
Detector TOF (V)	2170.0

TOF: Time of flight analyzer

Development and validation of stability-indicating assay (SIAM) method:
CEFP is a weak acid with pKa value of 4.2 at 25° Most of the reported HPLC methods were developed on C18 column by using combination of acetonitrile/methanol with buffer (pH in the range of 2.0 to 6.0). Therefore it was aimed to develop simple and economic LC method by using combination of methanol and buffer ($NH_4CH_3CO_2$, pH=3.5, 25mM). Several trials were performed on HiQSil C18 (250×4.6 mm, 5 μ) column at 230 nm (wavelength maximum of CEFP) to achieve optimum separation of the drug and its degradation products (DPs). Initially individual stressed samples were analyzed followed by their mixture. Method was validated according to ICH guideline Q2 (R1)[31]. For linearity, test solutions were prepared from stock solution (1000 μg/ml in methanol) at five concentration levels in the range of 25 to 250 μg/ml. All dilutions were prepared in triplicate and peak area was measured. The peak area versus concentration data was processed by least-square linear regression analysis and correlation coefficient of curve was calculated. Standard addition method was used to determine accuracy (recovery) of the method. Mixture of stressed samples containing 100 μg/ml of remaining CEFP was spiked with three known concentrations of pure drug such as 50, 100 and 150 μg/ml. All recovery samples were prepared in triplicate and injected for analysis. Percent recovery of the added pure drug was calculated. The intraday and interday precision

TABLE 2: STRESS CONDITIONS FOR OPTIMUM DEGRADATION

Stress condition	Concentration of stressor	Exposure condition	Duration (hours)	% Drug degradation
Hydrolysis		RT		
Acid	HCl 0.1N		03.0	18.35
Base	NaOH 0.01N		03.0	25.84
Neutral	H_2O		03.0	16.85
Oxidation	H_2O_2 (10, 15 and 30%)	RT	24.0	13.89
Photolysis	Fluorescent light 1.2 million lux hours and uv light 200 Whm^{-2}	-	-	<2
Thermal	-	60°	0.5	<2

RT: Room temperature

was carried out by analyzing 50, 100 and 150 µg/ml drug solutions prepared in triplicate on the same day and on consecutive day. The specificity of the method was established by peak purity profiless and resolution between drug and amongst all the degradants.

LC-MS/TOF studies on drug and degradation products:

The fragmentation pattern of the drug was studied by LC-MS/TOF analysis. All mass spectra were recorded in positive electrospray ionization mode (ESI) in the range of 50–800 *m/z*. Drug solution (10 µg/ml diluted in methanol) was analyzed by injection into MS/TOF using syringe pump. Ionization conditions were optimized to obtain molecular ion peak and subsequent fragmentation pattern. The optimized parameters are depicted in Table 1. The masses of all peaks were recorded up to fourth decimal precision. The mixture of stressed sample was also subjected to LC-MS/TOF analysis using optimized parameters of drug (Table 1). Developed gradient elution method was used for analysis. Fragmentation pattern was established for each degradant from obtained mass spectrum and accurate *m/z* values.

RESULTS AND DISCUSSION

The degradation products generated under different stress conditions are indicated as I and II in accordance with the sequence in which peak appears from left to right on the HPLC chromatogram (fig. 2). Details of two degradation products generated under respective stress conditions are tabulated below HPLC chromatogram (fig. 2).

It was observed that CEFP was prone to hydrolytic (acidic, basic and neutral) and oxidative stress conditions, while it was stable under thermal and photolytic stress conditions. Desired separation was achieved by using proportion of methanol (A) and ammonium acetate buffer solution (B) (25 mM, pH adjusted to 3.5 by using formic acid) in gradient mode (T_{min}/A:B; T_0/50:50; T_{30}/70:30; T_{40}/50:50). The developed method was validated with respect to specificity selectivity, linearity, accuracy and precision. The mixture of degradants was analyzed by using developed HPLC method. The method was found to be specific and selective as peaks of degradants were well-resolved from the drug and from one another. Typical chromatogram is shown in fig. 2. The resolution amongst the peaks was observed to be >2. The peak purity of CEFP and degradants were recorded using DAD detector (Table 3) and found to be >0.999.

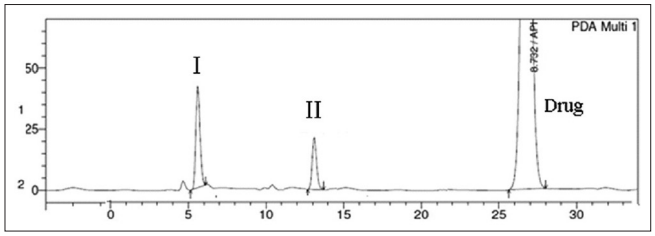

Fig. 2: Chromatograms of CEFP and two degradation products I and II
Chromatogram showing separation of cefditoren pivoxil (CEFP) and two degradation products (DPI and DPII) after injection of stress sample mixture in all the conditions A: acid; B: base; N: neutral; O: oxidation

Excellent correlation was observed between response for the drug (peak area) and concentration in the range of 25–250 µg/ml. Corresponding slope and correlation coefficient (r^2) were 84014 and 0.9996 (Table 4). Determination of intraday and interday precision was performed at three different concentrations (50, 100 and 150 µg/ml) in the range. Results are indicated in Table 5. The RSD (%) values for intraday and interday precision were found to be <2, which indicates that method is reproducible.

Recovery studies were performed by adding pure drug to the degradation samples. Good recoveries were obtained when a mixture of stressed samples were spiked with the drug at three given concentration levels. (mean recovery=99.30%). The results are shown in Table 6. CEFP was subjected for LC-MS/TOF study. The data obtained from mass spectra is

TABLE 3: RETENTION TIME, RELATIVE RETENTION TIME AND PEAK PURITY

Drug/degradation products	Retention time (min)	Relative retention time	Peak purity (>0.999)
DP-I	6.37 min	0.23	0.9985
DP-II	13.39 min	0.68	0.9998
Drug (CEFP)	26.8 min	1.00	0.9999

CEFP: Cefditoren pivoxil, RRT: relative retention time, RT: retention time

TABLE 4: LINEARITY STUDY

Conc (µg/ml)	Peak area			
	Injection 1	Injection 2	Injection 3	Average±SD, RSD (%)
25	1001874	1008459	1002357	1004230±3670, 0.36
50	4615683	4690456	4689567	4665235±42915, 0.91
100	8739075	8712985	8783902	8745321±35868, 0.41
150	13984572	13740985	13798486	13841348±127324, 0.91
250	21895675	21398054	21502169	21598633±262460, 1.21

SD: Standard deviation, RSD: relative standard deviation

tabulated (Table 7). The presence of molecular ion peak (*m/z*=621.1265) was confirmed as it closely matches with exact mass of CEFP which is 621.1260, also there was presence of peak next to molecular ion peak at *m/z* value of 643.1070 which corresponds to $(M+Na)^+$.

Fragmentation of drug led to formation of total eight fragments. The most probable molecular formula is calculated for each fragment from experimental accurate mass values with the help of elemental composition calculator. This data was helpful to establish origin of each fragment and in understanding fragmentation pathway of the drug. The major fragments of drug had *m/z* values 591.109, 507.0468, 491.0615, 461.0429, 447.0714, 350.0660, 282.0474, and 240.0702. From available mass data structures were proposed for each fragment. The complete fragmentation pathway of drug is shown in fig. 3. The structural elucidation of DP-I and DP-II were achieved with the help of their major fragments observed in MS/TOF studies and comparison with the fragmentation pattern of drug (fig. 3).

Presence of molecular ion peak (m/z 507.0499) of DP-I was confirmed from peak of sodium adduct (m/z 529.0369). It was observed that DP-I was formed by loss of pivoxil [$(CH_3)_3C$-CO-O-CH_2] moiety. Best possible molecular formula was generated for DP-I with the help of mass frontier software and elemental composition calculator. Fragmentation of DP-I led to formation of total four fragments having m/z values 477.0397, 350.0631, 282.0451 and 240.0754. From available mass spectral data structures were assigned for DP-I and successive fragments. The fragmentation pathway for DP-I is outlined in fig. 4.

The presence of molecular ion peak (m/z 521.0730) of DP-II was confirmed from sodium adduct peak (m/z 543.0599). It was observed that DP-II was formed by loss of pivaloyloxy [$(CH_3)_3C$-CO-O]

TABLE 5: INTRADAY AND INTERDAY PRECISION STUDIES

Actual concentration (µg/ml)	Intraday precision measured concentration (µg/ml)±SD, RSD (%)	Interday precision measured concentration (µg/ml)±SD, RSD (%)
50	50.11±0.36, 0.72	49.96±0.44, 0.88
100	100.13±0.38, 0.37	100.23±0.62, 0.67
150	149.89±0.31, 0.20	149.65±0.77, 0.51

SD: Standard deviation, RSD: relative standard deviation

TABLE 6: RECOVERY STUDY OF CEFDITOREN PIVOXIL

Spiked concentration (µg/ml)	Calculated spiked concentration (µg/ml) mean±SD, RSD (%)	Recovery (%)
50	50.86±0.85, 1.67	101.72
100	99.93±1.26, 1.26	99.93
150	149.72±1.059, 0.707	99.81

SD: Standard deviation, RSD: relative standard deviation

TABLE 7: SUMMARY OF LC-MS/TOF DATA OF DRUG AND DEGRADANTS

Compound	Parameter	Value	Major fragments		Error in mmu	Molecular formula for best possible fragments	RDB
			EM	TM			
CEFP $(M+H)^+$	EM	621.1265	591.1090	591.1149	−5.9	$C_{24}H_{27}N_6O_6S_3^+$	14.5
	TM	621.126	507.0468	507.0574	−10.6	$C_{19}H_{19}N_6O_5S_3^+$	13.5
			491.0615	491.0624	−0.9	$C_{19}H_{19}N_6O_4S_3^+$	13.5
	Error in mmu	0.5	461.0429	461.0519	−9	$C_{18}H_{17}N_6O_3S_3^+$	13.5
			447.07145	447.0726	−1.15	$C_{18}H_{19}N_6O_2S_3^+$	12.5
	Molecular formula	$C_{25}H_{29}N_6O_7S_3^+$	350.0660	350.0740	−8	$C_{14}H_{18}N_5O_2S_2^+$	08.5
			282.0474	282.0543	−6.9	$C_{11}H_{12}N_3O_4S^+$	07.5
	RDB	14.5	240.0702	240.0801	−9.9	$C_{10}H_{14}N_3O_2S^+$	05.5
DP I $(M+H)^+$	EM	507.0499	477.0397	477.0468	−7.1	$C_{18}H_{17}N_6O_4S_3^+$	13.5
	TM	507.0574	350.0631	350.0740	−10.9	$C_{14}H_{18}N_5O_2S_2^+$	09.5
	Error in mmu	-7.5	282.0451	282.0543	−9.2	$C_{11}H_{12}N_3O_4S^+$	07.5
	Molecular formula	$C_{19}H_{19}N_6O_5S_3^+$	240.0754	240.0801	−4.7	$C_{10}H_{14}N_3O_2S^+$	05.5
	RDB	13.5					
DP II $(M+H)^+$	EM	521.0699	506.0392	506.0501	−10.9	$C_{19}H_{18}N_6O_5S_3^+$	14.0
	TM	521.073	477.0376	477.0468	−9.2	$C_{18}H_{17}N_6O_4S_3^+$	13.5
	Error in mmu	-3.1	350.0667	350.0740	−7.3	$C_{14}H_{18}N_5O_2S_2^+$	09.5
	Molecular formula	$C_{20}H_{21}N_6O_5S_3^+$	282.0444	282.0543	−9.9	$C_{11}H_{12}N_3O_4S^+$	07.5
	RDB	13.1	240.0891	240.0801	9	$C_{10}H_{14}N_3O_2S^+$	05.5

CEFP: Cefditoren pivoxil, DP: Drug cefditoren, LC-MS/TOF data of drug and degradants (DP I and II) with molecular formulae and major fragments. EM is experimental mass, TM is theoretical mass and RDB is ring plus double bond

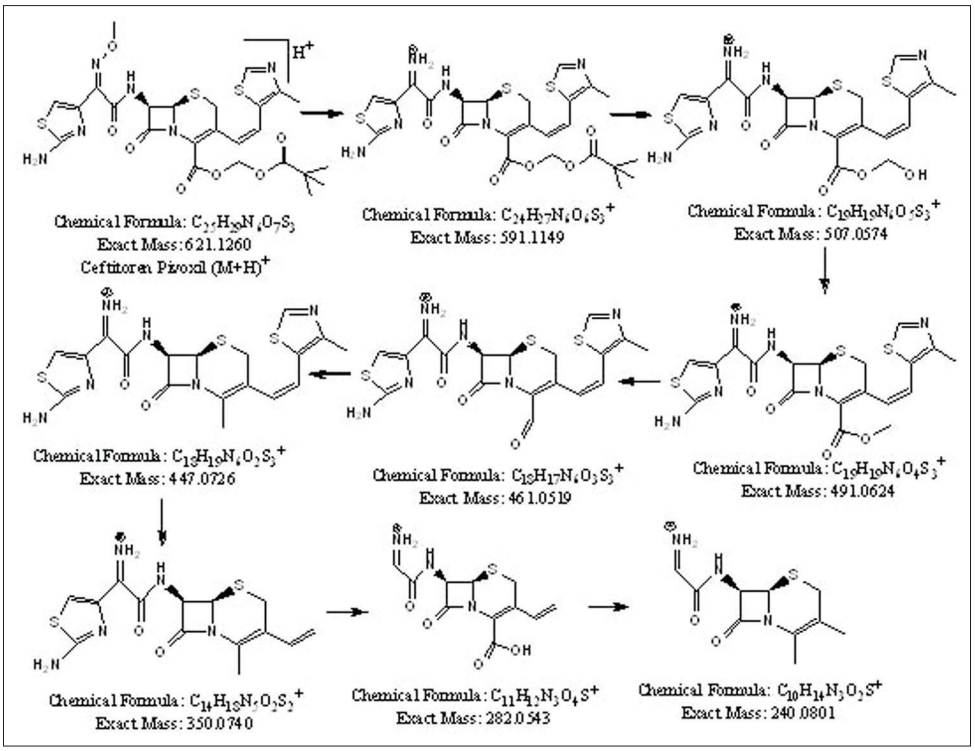

Fig. 3: Fragmentation pathway of CEFP.
Fragmentation pathway of cefditoren pivoxil (CEFP) along with molecular formula and exact masses of the fragments.

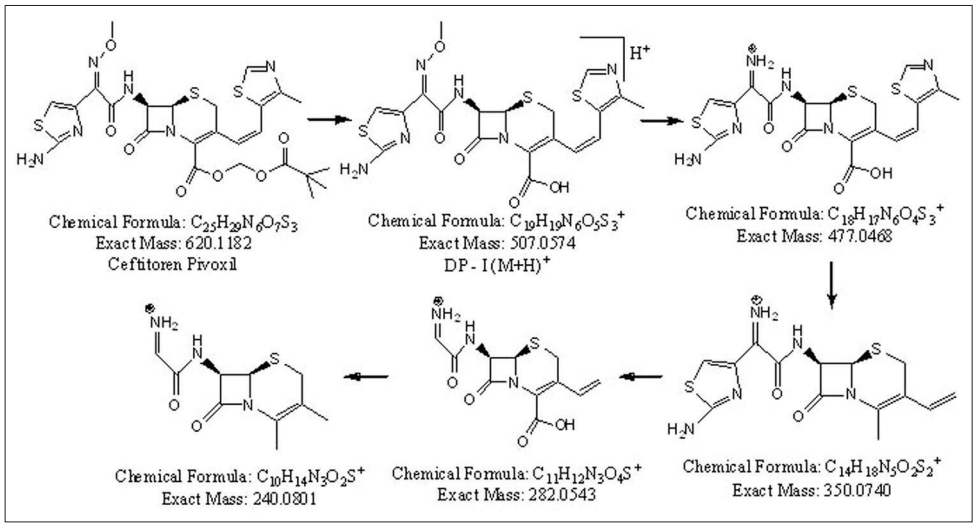

Fig. 4: Fragmentation pathway of DP-I.
Fragmentation pathway of DP-I along with molecular formula and exact masses of the fragments.

moiety from drug CEFP. Fragmentation of DP-II led to formation of five fragments having m/z values 506.0392, 477.0376, 350.0667, 282.0444 and 240.0891. Best possible molecular formula was generated for DP-II with the help of mass frontier software and elemental composition calculator. From available mass spectral data structures were assigned for DP-II and successive fragments. The fragmentation pathway for DP-II is outlined in fig. 5.

Degradation pattern of CEFP was thus studied by exposing drug to ICH recommended stress conditions. The drug was found more susceptible towards hydrolytic degradation while it is resistant to thermal and photolytic degradation. Drug and degradant peaks were well separated from each other by RP-HPLC. DP-I and DP-II was formed under acidic, basic and neutral hydrolytic stress. For identification and characterization of unknown degradants, drug and

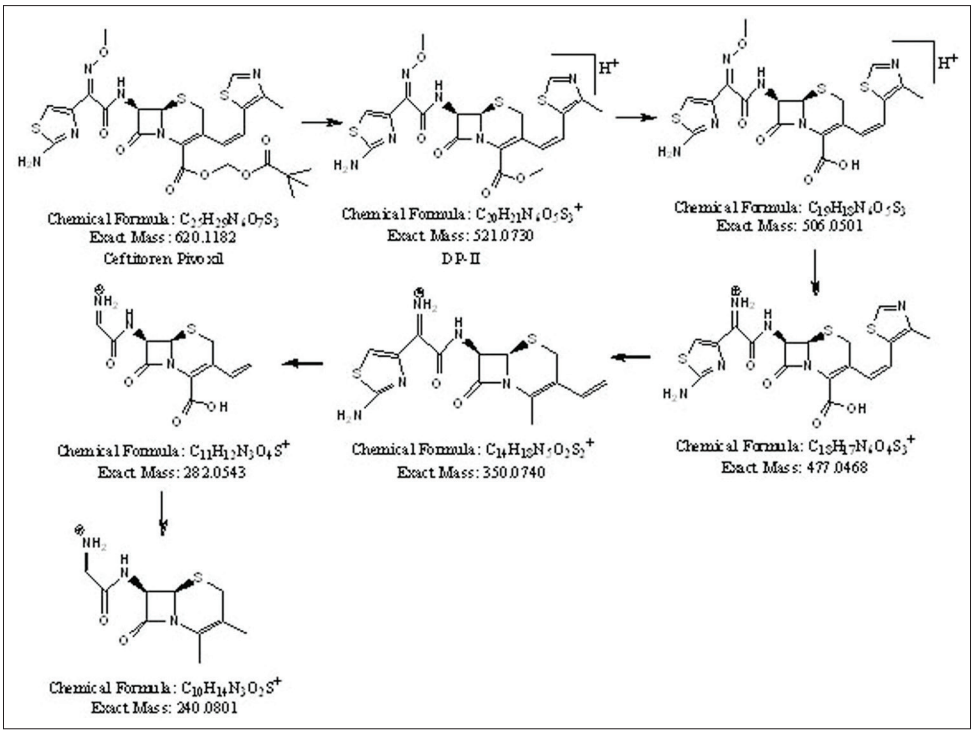

Fig. 5: Fragmentation pathway of DP-II.
Fragmentation pathway of DP-II along with molecular formula and exact masses the fragments.

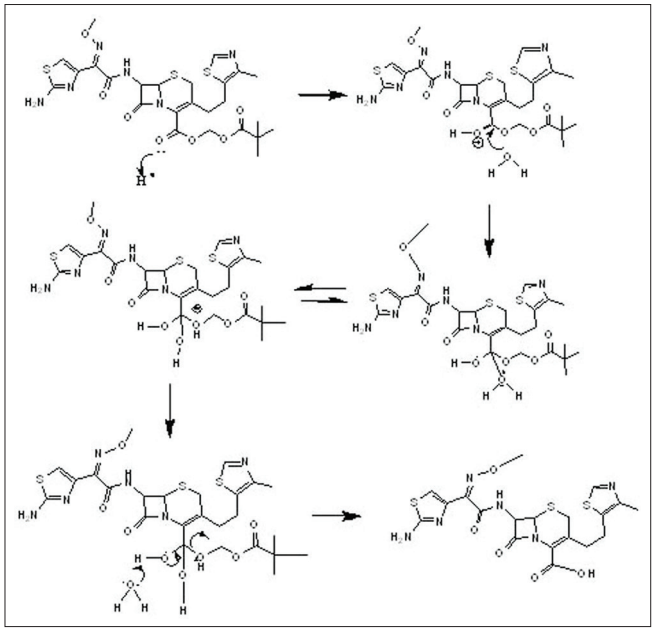

Fig. 6: Mechanistic approach towards acid catalyzed hydrolysis of CEFP to DP I.
CEFP is cefditoren pivoxil and DP I degradation product I.

all the degradants were subjected for LC-MS/TOF study. Two unknown degradants (DPs I and II) were characterized. From available mass spectral data complete degradation pathway for drug (fig. 3) and degradation products were sketched (figs. 4 and 5).

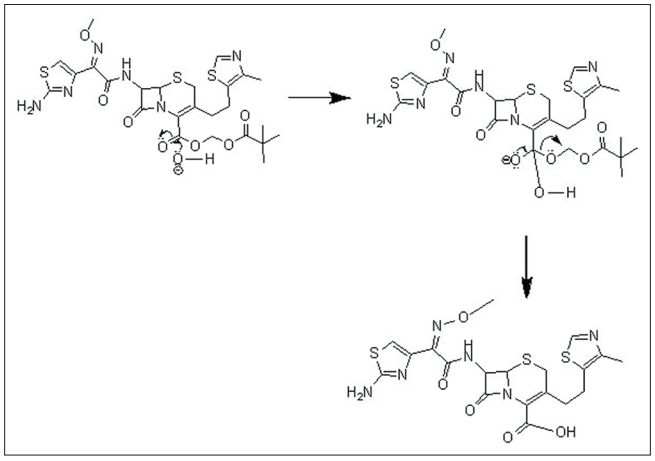

Fig. 7: Mechanistic approach towards base catalyzed hydrolysis of CEFP to DP I.
CEFP is cefditoren pivoxil and DP I degradation product I.

The mechanistic approach is provided for hydrolytic degradation of CEFP (figs. 6 to 8), it was found hydrolyzed to original drug cefditoren (DP I). This information is being reported for the first time.

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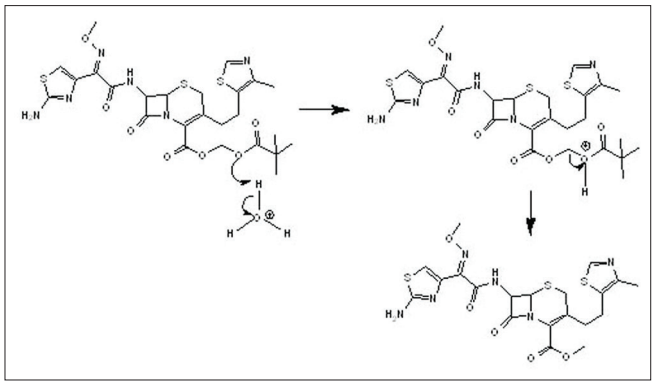

Fig. 8: Mechanistic approach towards hydrolysis of CEFP to DP II. CEFP is cefditoren pivoxil and DP II degradation product II.

REFERENCES

1. Deshpande AD, Baheti KG, Chatterjee NR. Degradation of β-lactam antibiotics. Curr Sci 2004;87:1684-95.
2. Dancer SJ. The problem with cephalosporins. J Antimicrob Chemother 2001;48:463-78.
3. Fulias A, Bobric A, Vlase G, Vlase T, Doca N. Thermal stability and biological interactions of some cephalosporins. Rev Roum Chim 2011;56:959-66.
4. Romano A, Mayorga C, Torres MJ, Artesani MC, Suau R, Sánchez F, *et al*. Immediate allergic reactions to cephalosporins: Cross-reactivity and selective responses. J Allergy Clin Immunol 2000;106:1177-83.
5. Meyer O, Hackstein H, Hoppe B, Gobel F, Bein G, Salama A. Fatal immune haemolysis due to a degradation product of ceftriaxone. Br J Haematol 1999;105:1084-5.
6. Barberán J, Mensa J. Cefditoren and community-acquired lower respiratory tract infections. Rev Esp Quimioter 2009;22:144-50.
7. Japnese Pharmacopoeia. 14th ed. Japan: Society of Japnese pharmacopoeia; 2001. p. 325.
8. Sweetman SC. Martindale: The Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005. p. 172.
9. Raju SA, Karadi AB, Manjunath S. Visible Spectrophotometric Determination of Cefditoren Pivoxil in Pharmaceutical Formulations. J Indian Council Chem 2009;26;54-7.
10. Niraimathi V, Aruna A, Suresh AJ, Prema V. Spectrophotometric estimation of cefditorenpivoxil in pharmaceutical oral solid dosage form. Int J Chem Sci 2010;8:724-8.
11. Narala SR, Saraswathi K. A novel use of oxidative coupling reactions for determination of antibiotic and antiinflammatory drugs in pharmaceutical formulations. Int J Pharm Sci Res 2011;2:366-9.
12. Narala SR, Saraswathi K. Validated Spectrophotometric methods for the Determination of Cefditoren Pivoxil in Drug Formulations. Int J Chem Tech Res 2011;3:1025-7.
13. Karadi AB, Raju, SA, Manjunath S, Darak V. Development and Validation of Visible Spectrophotometric Methods for Determination of Cefditoren Pivoxil in Pharmaceutical Formulations. Res J Pharm Tech 2011;4:1269-73.
14. Narala SR, Saraswathi K. Application of Oxidants to the Spectrophotometric Determination of Cephalosporins (Cefditoren Pivoxil and Cefdinir) In Formulations. Asian J Res Chem 2011;4:270-1.
15. Garg R, Singh N, Srinivas KS, Deb B, Ahmed A. UPLC method development and validation for Cefditoren Pivoxil in active pharmaceutical ingredient. J Appl Pharm Sci 2011;1:149-53.
16. Vidhya S, Narayanan BL, Malairajan P, Sahayaraja RJ, Kumar EP, Mahibalan S. Analytical method development and Validation for the Quantitative estimation of Cefditoren Pivoxil in tablet formulation by RP-HPLC. Int J Drug Dev Res 2012;4:186-92.
17. Narala SR, Saraswathi K. RP-HPLC Methods for the Determination of Cephalosporins (Cefditoren Pivoxil and Cefdinir) in Pharmaceutical Dosage Forms. J Pharm Sci Res 2011;3(1):1002-4.
18. Albishri HM. Simple Stereoselective HPLC Method for Studying the Isomerisation of Cefditoren Pivoxil and Its Isomeric Purity in Pharmaceuticals. Chromatographia 2012;75:1327-33.
19. Liu Q, Yao JH, Su CY, Han GZ, Sun HJ, Wang K. Bioequivalence of Cefditoren in human and pharmacokinetics of absorption in rats. Asian J Phar Pharmacokinet 2006;6:214-8.
20. Sadaba B, Azanza JR, Quetglas EG, Campanero MA, Honorato J, Coronel P, *et al*. Pharmacokinetic/pharmacodynamic serum and urine profile of cefditoren following single-dose and multiple twice- and thrice-daily regimens in healthy volunteers: A phase I study. Rev Esp Quimioterap 2007;20:51-60.
21. Rieck W, Platt D. Determination of cefditoren (ME 1206) in the plasma of elderly patients with multiple diseases using high-performance liquid chromatography. Clin Lab 2000;46:477-82.
22. EI-Bagary RI, Ramzia TA, Nisreen F, Eldin M, Badawi N. Different validated methods for determination of cefditoren pivoxil. JPC-J Planer chromatogr - Mod TLC 2013;26:43-55.
23. Dhoka MV, Sonawane PA, Madgulkar AR, Nimbalkar U. Determination of CefditorenPivoxil in human plasma by high performance thin layer chromatographic method. Int J Res Ayurveda Pharm 2011;2:1582-4.
24. Al-Tamimi SA, Al-Mohaimeed AM, Alarfaj NA, Aly F. Ion Selective Electrodes for Determination of Cefditoren Pivoxil in Pharmaceutical Formulations and Biological Fluids. Int J Electrochem Sci 2013;8:3988-4001.
25. Ohta M, Tozuka Y, Oguchi T, Yamamoto BK. Comparison of Crystallinity of Cefditoren Pivoxil Determined by X-Ray, Differential scanning calorimetry and Microcalorimetry. Chem Pharm Bull 1999;47:1638-40.
26. Jayswal UP, Patel HU, Patel CN. A Validated Stability Indicating RP-HPLC Method for Cefditoren Pivoxil in Bulk Drug and Pharmaceutical Dosage Form. Res J Pharm Tech 2011;4:1461-5.
27. Annapurna MM, Goutam SV, Anusha S, Srinivas L. Development and validation of the stability-indicating LC–UV method for the determination of Cefditoren pivoxil. J Pharm Anal 2012;2:466–9.
28. Narayana PS, Reddy AS, Sekar R. Development and validation of a HPTLC Method for Stability Indicating Assay of Cefditoren Pivoxil, a Third Generation Cephalosporin Antibiotic in both Bulk Drugs and Pharmaceutical Dosage Forms. J Pharm Res 2012;5:1628-32.
29. Dewani AP, Kochar NI, Abooj HC, Bakal RL, Chandewar AV, Barik BB. Determination of Cefditoren Pivoxil in bulk by RP-HPLC in presence of its degradation products. J Pharm Res 2010;3:2588-91.
30. ICH Q1A (R2), Stability testing of new drug substances and products in: Proceedings of the International Conference on Harmonization, IFMPA, Geneva, 2003.
31. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology in: Proceedings of the International Conference on Harmonization IFMPA, Geneva, 2005.

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