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Development and validation of an RP-HPLC method for estimation of cefuroxime axetil and its degradation products in tablets

Abstract

A simple precise specific sensitive and accurate stability indicating RP-HPLC method for determination of cefuroxime Axetil and its degradation products in tablets. The separation was performed on Teknokroma, tracer excel C8 column (15cm x 0.46cm.5µm) using mobile phase consisting of 0.02M potassium dihydrogen phosphate: methanol: Acetonitrile (60:35:5 v/v). The flow rate was 1mL/min and detection was monitored at 278nm. The column temperature was set at 35°C. The retention times were 2.4, 11.09, 12.6, 12.6, 14.13, 20.76 and 24.24minutes for cefuroxime Axetil B, cefuroximeA A, Δ^3-isomers, E-isomer 1 and E-isomer 2, respectively. The calibration curves were found to be linear in the concentration range of 120-312µg/mL (r2 =0.99964) and 0.24-7.2µg/mL (r2=0.9997) at assay level and low-level of cefuroxime Axetil. The percentage recoveries of cefuroxime Axetil at assay level were found to be in the range of 98.54-99.35% and 101.4-103.69% at low-level at 95% confidence limit. The intraday precision was 0.517% and 2.019% at to assay level and low-level of CFA, respectively. The intermediate precisions were 1.102% and 2.273% at assay level and low-level of CFA, respectively.

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Introduction

Cefuroxime Axetil (CFA) is a mixture of the 2diastereoisomers of (1RS)-1-(acetyloxy) ethyl (6R,7R)-3-[(carbamoyloxy)methyl]-7-[[(Z)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thial-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.[1]

Cefuroxime Axetil is the orally absorbed ester prodrug of the cefuroxime sodium. Since cefuroxime sodium is not absorbed orally, the 1-acetyloxyethyl ether was substituted for sodium on the cefuroxime molecule to increase its lipid solubility and improve its gastrointestinal absorption.[2]

The absorbed ester is hydrolyzed in the intestinal mucosa and in portal circulation.[3] Estimation of cefuroxime Axetil has been described by spectrophotometry,[3–5] HPTLC[6–8] and HPLC.[9–18] HPLC methods are available in United State Pharmacopoeia[19] and in British pharmacopoeia.[1] The column in the official methods is Trimethylsilane bonded-phase. The mobile phase consisting of 0.2M ammonium dihyrogen phosphate (23gm/litre). In the official methods the buffer concentration is very high and that may be lead to adversely affect on the operation of HPLC system. The buffer may be precipitate in the column and LC system when it is exposed to the highest organic concentration. The backpressure is very high.

The column in the B.P and USP methods is trimethylsilane which is less stable than longer-chain alkyl bonded phase packing (e.g., C_8 and C_{18}). The proposed method was carried out on C_8 column which more stable than C1 column and the operation backpressure was very low because the buffer concentration was low (0.02M KH_2PO_4).

Experimental

Instrumentation

An HPLC system from Shimadzu Corporation consisting of an LC-20 AT pump, SPD-20 A UV/vis-detector, (CT-20A oven, DGU 20A3, a rheodyne injector with 20µL loop, teknokroma, tracer excel. C_8 analytical column (15cmx0.46cm,µm) and C_1column (25cm x 0.46cm, 5µm).

Reference substances, reagents and chemicals

Cefuroxime Axetil was obtained from archid chemical (India), methylparaben, sodium benzoate was obtained from shiba pharma (Yemen). Monobasic ammonium potassium phosphate was obtained from fluka (Switzerland). Methanol HPL grade, acetonitrile HPL grade and tetrahydrofuran HPLC grade were obtained from sigma-aldrich (Germany). Water was obtained from arium-sartorius water purified system (Germany).

Mobile phase preparation

Mobile phase was prepared by mixing, 0.02M potassium dihydrogen phosphate, methanol and acetonitrile in the ratio of 60:35:5.

Chromatographic conditions

Column: Teknokroma,

C8 (150mm×4.6mm×5µm) column temperature: 35°C

Flow rate: 1mL/minute

Wavelength: 278nm

Injection volume: 20µL

Run time: 25minutes

Standard preparation

Standard stock solution of cefuroxime Axetil was prepared by dissolving 30mg of CFA in methanol to get a concentration of 1.2mg/

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mL. Ten mL of this solution was transferred to a 50mL volumetric flask and diluted with mobile phase.

Sample preparation

Twenty tablets were weighed. An accurately amount of the finely powdered tablet equivalent to 120mg of cefuroxime Axetil was taken and transferred into a 100mL volumetric flask fifty a mL of methanol was added, shaked by mechanical means for about 10minuts and diluted with methanol to volume. The resultant solution was filtered through 0.45µm syring filter. The first 10mL was discarded and 10mL of filtrate was transferred into a 50mL volumetric flask, diluted with mobile phase to the mark and twenty µL was injected. A portion of the standard solution was exposed to sunlight for 5hours to generate E-isomers. A portion of the standard was heated for 1 hour or more at 60 ℃ temperature to generate the Δ^3-isomers.

Related substances

Using the procedure described under assay. The percentage area content of related substances were calculated from the areas of the peaks in the chromatogram was obtained with test solution by normalization procedure. The sum of the areas of the pair of peaks in the chromatogram obtained with test solution corresponding to the E-isomers is not greater than 1.5% by normalization, the sum of the areas of any peaks corresponding to Δ^3-isomers is not greater than 2.0% by normalization. The area of any other secondary peak is not greater than 1.0% by normalization.[1] The relative retention times are about 0.8 for CFA- diastereisomer B, 0.9 for CFA A, and 1.0 for CFA delta-3-isomer; the resolution, R_s, between the CFA diastereisomer A and B peaks is not less than 1.5 and the resolution R_s, between the cefuroxime Axetil diastereisomer A and the cefuroxime axetil delta-3-isomers is not less than 1.5. The column efficiency is not less than 3000 theoretical plates when measured using the CFA A peak and the % RSD is not more than 2.0%.

Determination linearity for assay of CFA

Different aliquots of standard stock solution (1.2 mg/mL) were transferred into a series of volumetric flasks and diluted to the mark with mobile phase to obtain final concentrations of 120µg/mL, 168µg/mL, 204µg/mL, 240, 276µg/mL and 312µg/mL . TwentyµL of each of CFA (standard solution) was injected in triplicate.

Determination of linearity at low-level of CFA for degradation products

Different aliquots of standard solutions (0.24mg/mL) were transferred into a series of volumetric flasks and diluted to the mark with mobile phase to obtain final concentration of 0.24µg/mL, 1.2µg/mL, 2.4µg/mL, 3.6µg/mL, 4.8µg/mL and 7.2µg/mL. TwentyµL of each CFA standard solution was injected, in triplicate.

Determination of accuracy for assay of cefuroxime Axetil (accuracy was performed on 250mg CFA tablet)

Accurate weight of CFA (21mg, 30mg and 35mg) were introduced into three volumetric flask (25mL) and (61mg) of placebo for CFA was added to each volumetric flask, dissolved and completed to volume with methanol (Stock solution). A aliquot (10mL) of each stock solution was transferred into different (50mL) volumetric flask and diluted with mobile phase to obtain concentrations of 168µg/mL, 240µg/mL and 312µg/mL. The final solution was filtered through syring filter (0.45µm) and twentyµL was injected.

Three replicate were prepared for each level. The percent recovery was determined by an external standard at 100% level.

Determination of accuracy at low-level of CFA for determination of degradation products

Different aliquots from standard solutions (0.24mg) were transferred and mixed with 61mg of placebo into a series of volumetric flasks. The volumes were completed with mobile phase to obtain concentrations of 0.24µg/mL, 1.2µg/mL, 2.4µg/mL, 3.6µg/mL, 4.8µg/mL and 7.2µg/mL. Three replicate were prepared for each level .The final solution were twenty µL was injected. The percent recovery was determined by an external standard at 100% level (0.24mg/mL).

Precision

Determination of repeatability precision for assay of CFA: Spiked placebo solution was prepared by transferring 120mg of CFA and 61mg of placebo into a 100mL and diluted to the mark with methanol. Ten mL of spiked placebo solution was transferred into 50mL V.F and diluted with mobile phase to the mark. Solution was filtered through syring filter (0.45mg) and twenty µL was injected. Six replicate sample solutions was prepared and two injection of each sample were injected.

Determination of intermediate precision for assay: Intermediate precision was performed such as repeatability procedure on different days and different preparations.

Determination of repeatability precision for degradation products at low- level of CFA: Spiked placebo solution was prepared by transferring 120mg of CFA into a 100mL volumetric flask and diluted to the mark with methanol. Ten mL of this solution was transferred into a 50mL and diluted with mobile phase to the mark. Two mL of second solution (0.24mg/mL) and 61mg of placebo were transferred into a 100mL volumetric flask and diluted with mobile phase to the mark. Twenty µL was injected. Six replicate samples were prepared and two injection of each sample were injected.

Determination of intermediate precision at low-level: Intermediate precision was performed such as repeatability procedure on different day and different preparations.

Determination of limit of detection (LOD) and limit of quantitation (LOQ)

Different aliquots of standard solution (0.024mg/mL) were transferred into a series volumetric flask and diluted with mobile phase to obtain concentrations of 0.0048µg/mL, 0.012µg/mL, 0.024µg/mL, 0.12µg/mL and 1.2µg/mL. Twenty µL was injected.

Forced degradation studies

Acid degradation: A 10mL aliquot of stock standard solution and 10mL of sample spiked placebo solution were pipetted into two 50mL volumetric flask and 5mL of 0.1N HCl was added to each volumetric flask. The two solutions were stored for 28hours in room temperature and then neutralized with 5mL of 0.1N NaOH. The solutions were diluted with mobile phase to the volume and twenty µL was injected.

Base degradation: A 10mL of the CFA stock solution and a 10mL of sample (spiked placebo) solution were transferred into two 50mL volumetric flask and 5mL of 0.1N NaOH was added to each flask.

The solution were stored in room temperature for twohours in room temperature and then neutralized with 5mL of 0.1N HCl. The solutions were diluted with mobile phase to volume and twenty µL was injected.

Hydrogen peroxide degradation

A 10mL of the CFA stock solution (1.2mg/mL) and a 10mL of sample (1.2mg/mL) were transferred into two 50mL volumetric flask and 5mL of 3% hydrogen peroxide was added to each flask. The solution were stored in room temperature for 24hours and then diluted with mobile phase to the mark and twenty µL was injected.

Light degradation

A 10mL of the CFA stock solution (1.2mg/mL) and a 10mL of the sample stock solution (1.2mg/mL) were transferred into two 50mL volumetric flask. The two solutions were exposed to sunlight for 5hours and then diluted with mobile phase to the mark and twenty µL was injected.

Heat degradation

A 10mL of the CFA stock solution (1.2mg/mL) and a 10mL of the sample solution were transferred into two 50mL volumetric flask. The two solutions were heated at 90℃ for 2hours then diluted into the mark with mobile phase and twenty µL was injected.

i. Determination of robustness for assay

ii. An assay standard solution was prepared as according in the analytical method.

iii. Acetonitrile (5%) was varied by ±10%

iv. Methanol (35%) was varied by ±10%

v. The flow rate 1mL/min was varied by ±10%

vi. The column temperature (35℃) was varied by ±5%

vii. The system suitability standard solution was injected twice

Determination solution stability

Fresh standard and spiked placebo were prepared and analyzed as per the test method. Time zero value for each were established. An aliquot of each solution was placed in clear glassware and exposed to ambient (benchtop) conditions and aliquot was place of in a refrigerator. The solutions were analyzed every 24hours for at least 2days (48hr) and two injections was injected of each solution.

Results and discussion

Several mobile phases were tried in different ratios. For 80% v/v methanol, the retention time was 1.8mintes and the peak was near t0, distorted and no peaks were observed after 20minutes-Since, successive reduction in% methanol by 20% v/v was required and a weaker mobile phase was increased. For 60% methanol, the retention time was 2.8min and the peaks CFAB/CFAA were overlapped. For 40% v/v methanol, the retention times were 1.81, 9.64, 11.08, 12.58, 18.09 and 21.57 minutes for cefuroxime (CF), CFAB, CFAA, Δ^3-isomers, E-isomer1 and E-isomer2,respectively. There was good separation but methylparaben was overlapped with CFAB. The retention times of CFAB and CFAA were no effected by pH 2.5, 3.5 because they were neutral. The retention time of cefuroxime was increased at pH 2.5 because the cefuroxime is acidic compound (Pk_a 2.5). Therefore the decrease in pH leads to increase the hydrophobicity of CF which lead to increase the retention time in RP-HPLC.

Since, change in organic solvent from methanol to acetonitrile was required by using the solvent-monograph to an estimate the required value of % ACN, (33.3%ACN), that give similar run time as for 40% methanol. For 33.33% ACN, the retention time was 10.78 and 11.81 for CFAB and CFAA and the resolution between CFAB/CFAA was 2.0 the retention time was 7.33 for methylparaben. When 33.3% ACN in water was used, the peaks of methylparaben and CFAB were resolved, but the resolution between CFAB and CFAA was decreased. Since, mixtures of ACN and methanol were tried by blending equal volume of the 40% methanol and 33.3% ACN.

Different ratios of methanol: Acetonitrile: phosphate buffer were tried (23.4:16.6:60),(32:8:60) and (35:5:60) . Different temperatures were tried at 30℃, 35℃ and 40℃.

The best mobile phase was 0.02M KH2PO4: ACN: Me OH methanol in ratio (60:5%:35%) which showed good and complete separation of cefuroxime Axtile and its degradation products. The run time for cefuroxime Axetil and its degradation products was 35minutes in USP method while. In the present work, the run time was 25minutes as shown in Figure 1. Forced degradation studies were performed to demonstrate specificity of stability-indicating method. The stress test solution were performed by exposing the standard and spiked placebo solution to acidic, basic, peroxide, light and warmth conditions. The chromatograms of the stress tests were shown that, there were no interference between the degradation products and peaks of the CFA. The loss of CFA content was a corresponding to increase in the level of decomposition products as shown in Table 1.

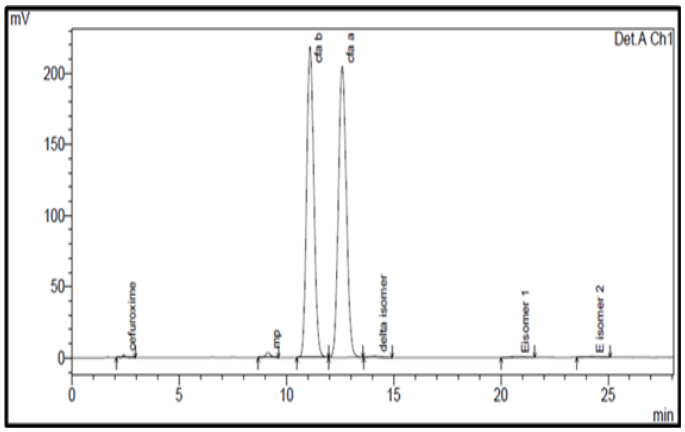

Figure 1 Separation of CFA and its degradation products.

The CFA was disappeared of in basic condition this indicates that there were not co eluent in the position of CFA peaks. The unaffected assay of CFA in the presence of decomposition products confirms the stability-indicating power of the method.

Robustness

The concept of robustness of an analytical procedure has been defined by ICH[20] as a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters. The method parameters were varied according to Table 2 and the figures of merit were measured . The results in Table 3 showed that acceptable resolution, N, tailing factor, %RSD and R.T for CFAA were obtained. All system suitability requirements were met except that

R.T was obtained of 10% methanol variation was outside the system suitability requirement (±2minutes). The method was robustness. The system suitability tests are used to verify that the resolution and reproducibility of the HPLC system and analysis procedure are adequate for the analysis, the results obtained were shown in Table 2 & Table 3.

Table 1 Results obtained by the proposed RP-HPLC for determination CFA and its degradation products

Item		CFA at assay level	CFA at low-level
Retention time		11.9	11.9
Wavelength of detection (nm)		278	278
Range of linearity (µg/mL)		120-312	0.24-7.2
Calibration point		6	6
Regression equation		Y=44221x +48106	Y=44773x +22002
Regression coefficient(r2)		0.999619	0.999974
SDa		48021	55.63
SDb		209	221.5
95% Confidence limit of the slope		43777-44665	44655-44891
95% Confidence limit of the intercept		-1503602	-939.1
Standard error of the estimation		57318	546.1
LOD(µg/mL)		3.58	0.0041
LOQ(µg/mL)		10.9	0.00124
Intraday %RSD		0.517	2.019
Interday %RSD		1.102	2.273
%Recovery	%mean	98.95	102.55
	95%Confidence limit of mean	98.54-99.35	101.4-103.69

Table 2 System suitability test for the proposed RP-HPLC method for the determination of cefuroxime axetil

System suitability parameters	Cefuroxime axetil-A
N≥2000	7189
T≤2	1.19
R≥1.5 Resolution between CFA and Δ3-isomer	2.155
k ≥ 2	7.2
%RSD ≤ 2.0%	0.17
R.T	11.961

Stability of solution sample and standard solution which were stored at room temperature for 48hours were not stable because ; % difference from initial time point was 2.92% and 3% for standard and sample solutions respectively. The acceptance criteria for stability of solution for assay is not more than 2% from initial point time, while, the sample and standard solutions which were stored in refrigerator for 48hours were stable .

Since, assay preparation promptly, or refrigerate and use on the day prepared. The intraday precisions were 0.51% and 2.019% at assay and low level, respectively. The inter day precisions were 1.102% and low-level, respectively as show in Table 3.

The results for the evaluation the linearity of cefuroxime Axetil at assay level were given in Table 4. The correlation coefficient of 0.99962 which was acceptable –the slope was 44221 and the Y-intercept was 48106. The regression equation for the graph was computed and found to be: y=44221x+48106→ (1), where x is concentration in µg/mL the regression statistics was performed by Excel and SPSS. The Y-intercept was 0.45% compared to the calculated Y-value at 100% standard (240µg/mL) and the acceptance criteria was ≤2.0%. The residual standard deviation was 0.53% (standard error compared to calculate Y-value at 100% level) and an acceptance criterion was ≤2.0%. These results clearly demonstrate a linear relationship between the peak areas and the concentrations of CFA in the ranges of 120µg/mL to 312µg/mL.

The results for the evaluation of the linearity of CFA at low-level were given in Table 4. In the ranges of 0.24µg/mL to 7.2µg/mL shown that the correlation coefficient was 0.9999 and which was acceptable .The slope was 44773 and intercept was 22.02 the regression equation for the graph was computed and found to be: y=44773x + 22.02→(2), where x is the concentration at low-level. The regression statistics were performed by Excel and SPSS. Y-intercept value was 0.02% and acceptance criteria of ≤5% based on 1%level (2.4µg/mL). The residual standard deviation was 0.51% and the acceptance criterion of 10%.

The results clearly demonstrate a linearity relationship between the peak areas and the concentrations of the range of 0.24µg/mL to 7.2µg/mL. The difference in slope of upper range and slope of lower range in linearity curves was 1.25% and the acceptance criterion of ≤4.0%.[21] Therefore, the 100% standard or area percent normalization could be used to quantitate the levels of degradation products. The results in Table 4 shown that the percent recovery for assay of CFA was inside of 100±2% limit for the average of each level. Each individual sample recovery was inside of 98-102% limit. The recovery of the RP-HPLC method for the assay of CFA was demonstrated by a mean of 98.95%.

The results in Table 4 shown that, the recovery of the individual and average of each degradants level was within the range of 75% - 125% at each concentration level. The LOD was 0.0024µg/mL (0.002%) and the LOQ was 0.2µg/mL (0.01%). Statistical comparison between the results was obtained by the proposed RP-HPLC and the official HPLC reference method (USP30) for assay of CFA tablet. The calculated value of t was 2.34 which was less than the tabulated t value of 2.78 with 4 degrees of freedom (df) at the 5% level, the difference was not significant at 5% level between the two methods, concerning accuracy and precision as shown in Table 5. Comparison between the results of determination of the degradants of CFA tablet was obtained by the proposed RP-HPLC method and the official HPLC reference method.[1] The British pharmacopoeia (2010) has published a monograph for tablets in which the sum of the areas of the pair of peaks corresponding to the E-isomers is not greater than 1.5%

by normalization, the sum of the areas of Δ^3-isomers is not greater than 2% by normalization and the area of any other secondary peak is not greater than 1.0% by normalization.

The result in Table 6 & Figure 3 that, the levels of cefuroxime, unknown1, Δ^3-isomers and E-isomers did not more than 1%, 2% and 1.5% respectively which were in acceptable level in both methods .

Table 3 Robustnes of proposed RP-HPLC method for assay of cefuroxime axetil

Parameter change		RT	N	Tailing factor (T)	Capacity factor	Rs
Method conditions		11.098	5400	1.093	4	1.9
Flow rate	1.1mL/min	10.23	5485	1.087	5.82	1.87
	0.9mL/min	12.418	6206	1.1	7	1.89
Columns Temperature	30℃	12.77	5797	1.085	7.85	1.924
	40℃	10.743	6112	1.093	5.83	2
Mobile phase	-10%ACN	12.306	6137	1.088	7.5	1.9
	+10%ACN	9.85	5734	1.097	5.6	1.8
	- 10% Methanol	15.02	7236	1.12	9	2.089
	+10% Methanol	7.87	5361	1.11	4.24	1.63

Table 4 Results of forced degradation for cefuroxime axetil

Treatment		% Degradation	% CFA	R.RT to CFAA-RT(CFAA)=11.56
Acid Degradation for (0.25hr)	API	24.3	73	0.21(2.7%), 0.299, 0.44, 0.531(1.7%), 1.115, 1.21, 1.36, 1.52, 1.619, 1.72, 1.87,
	Spiked placebo	24	75	0.12(19.2%), 0.299, 0.44, 0.53(1.5%), 1.115, 1.21, 1.36, 1.52, 1.619, 1.72, 1.87
Base Degradation for (1hr)	API	75	1.58	0.126, 0.17, 0.21(26.9%), 0.23(9.8%), 0.31(20.7%), 1.37, 1.53(29.9%)
	Spiked placebo	75.45	1.2	0.2(28.7%), 0.23(7.8%), 0.3(20.3%), 0.4, 1.24, 1.31, 1.58, 1.53(35.5%)
Peroxide Degradation for	API	25.36	68.3	0.2(6.3%), 0.299, 0.39, 0.44, 0.53(12.1%), 0.611, 0.69, 1.116(3.8%), 1.2, 1.6, 1.72, 1.88
	Spiked placebo	31	63.2	0.2(7.7%), 0.299, 0.39, 0.44, 0.53(16.2%), 0.611, 0.69, 1.116(3.55%), 1.21, 1.52, 1.60, 1.72, 1.88
Light Degradation for (2hr)	API	19.2	73	0.2(1.3%), 1.17, 1.63(8.9%), 1.89(9.1%)
	Spiked placebo	20	73	0.2(1.44%), 1.63(12.2%), 1.89(12.5%)
Heated Degradation for (2hr at 60℃)	API	13.3	89.7	0.2(8.87%), 0.22, 0.53, 1.116(1.2%), 1.209, 1.628, 1.89
	Spiked placebo	17.87	77	0.2(11.9%), 0.217, 0.527, 1.117(2%), 1.205, 1.63, 1.89

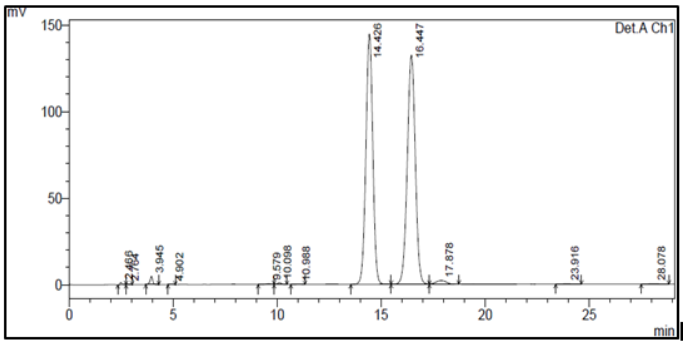

Figure 2 Chromatogram of degradation products CFA sample analyzed by USP and B.P method.

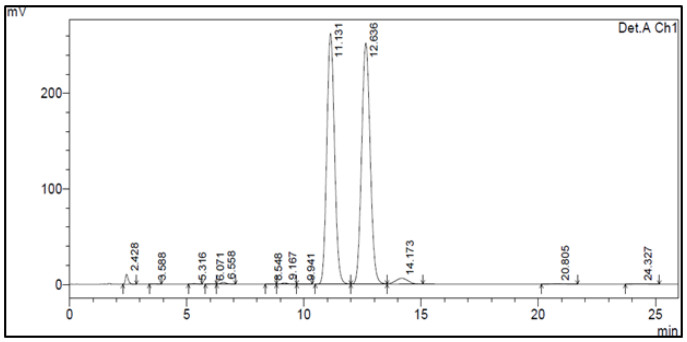

Figure 3 Chromatogram of degradation products CFA sample analyzed by the proposed method.

Table 5 Significance test for proposed-RP-HPLC method for the assay of cefuroxime axetil tablets compared with reference method

Statistical term	Reference method (USP)2	Proposed RP-HPLC method 2
	100.0818	99.1935
	99.5883	99.07506
	99.62778	99.04545
	98.7987	98.7
	99.63765	99.0948
Mean	99.546846	99.02176
S.D±	0.4643308	0.188256
%RSD	0.4664445	0.190116
n		
F test(6.38)	6.083562	
Calculated t(2.78)	2.343362	

Table 6 Test for significance for the proposed RP-HPLC method for degradation products of cefuroxime axetil tablet compared with reference method (B.P)

Name	Reference method		Proposed method	
	RRT	%	RRT	%
	0.23	0.459		0.653
		0.517		0.676
Cefuroxime		0.561		0.708
		0.607		0.723
		0.66		0.743
X¯		0.5608		0.7006
SD		0.077873		0.036143
%RSD		13.88605		5.158831
F test(6.388)	4.642272			
t (2.446)	3.641193			
	0.58	0.107	0.52	0.269
		0.126	0.52	0.252
Unknown 1		0.092		0.269
		0.108		0.259
X¯		0.10825		0.26225
SD		0.013913		0.008302
%RSD		12.85305		3.165531
F test(9.276)	2.808948			
t (2.57)	19.01017			
	1.09	0.834	1.12	1.09
		0.905		1.208
Δ^3-isomer		0.966		1.252
		1.03		1.33
		1.05		1.34
X¯		0.957		1.244
SD		0.089292		0.102088
%RSD		9.330372		8.206447
F test(0.156)	0.765016			
t (2.306)	4.731698			
	1.45	0.113	1.65	0.136
		0.116		0.137
E-isomer 1		0.118		0.137
		0.119		0.138
		0.112		0.141
X¯		0.1156		0.1378
SD		0.00305		0.001924
%RSD		2.638054		1.395891
F test(6.388)	2.513514			
t 2.36)	13.76786			
	1.71	0.096		0.118
		0.097		0.122
E-isomer 2		0.01		0.123
		0.101		0.121
		0.106		0.124
X¯		0.082		0.1216
SD		0.040441		0.002302
%RSD		49.31868		1.893234
F test(6.388)	308.5849			
t (2.776)	2.186011			

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Statistical comparison between degradants results obtained by two methods shown that, the difference between the content of all degradants product contents were significant difference. The % level of the all degradants obtained by new method were more than the % level of degradants, obtained by official method that may be due to the new method was more sensitive or more extracted than the official method. The method has been validated for the assay and determination of degradants of CFA in tablets. The method was shown to be selective, precise, linear and accurate within the range of 50% to 15% of target concentration 100% level and within the range of 0.05% to 3% of the target concentration at low-level . The method was shown to be robust with methanol volume established as critical parameter. The standard and sample solution was shown to be stable for 48hour under refrigerated condition.

Conclusion

The proposed method was found to be linear at assay level of CFA in the range of 120µ/mL to 312µ/mL and linear at low-level of CFA UN the range of 0.24-7.2µ/mL. The proposed method was found to be precise, intermediate precise and accurate at assay level of CFA and at low-level of CFA. All degradation products formed during forced degradation studies were well separated from the CFA, demonstrating that the developed method was specific and stability-indicating. The method was shown to be robust with methanol volume established as critical parameter. The 100% standard solution or area percent normalization could be used to quantitate the levels of degradation products of CFA. The statistical test between results was obtained by proposed method for determination of CFA at assay level compared to reference method was not significant difference. The test ascertained that the new method was as the reference method. The % levels of the all degradants obtained by the proposed method were more than the reference methods, which may be due to the new method, were more sensitive or more extracted than the official methods.

The proposed method was found to be simple sensitive, specific, rapid and economic for determination of CFA and its degradation products in tablets. The LOD and LOQ were found to be 0.0024µg/mL 0.024µg/mL, respectively. The standard and sample solution were not stable for 48hours at room temperature, since solutions of CFA must be promptly used or stored in refrigerator and use on the day prepared. The RP-HPLC method for determination of CFA and its degradation products can be used for stability studies for CFA in tablets. The RP-HPLC method for CFA and its degradants is suitable for routine analysis and quality control of pharmaceutical dosage form.

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Conflict of interest

Author declares that there is no conflict of interest.

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