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Validated Stability Indicating RP-HPLC Method for Simultaneous Determination of Cefixime and Acetylcysteine in Pharmaceutical Dosage Form

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Abstract

A simple stability indicating RP-HPLC method has been developed for the simultaneous determination of Cefixime in combination with Acetylcysteine using ODS C18 column (250 × 4.6 mm, 5 µm) with UV detection at 274 nm. The mobile phase consisting of 0.1% Ortho Phosphoric Acid (OPA) and acetonitrile in a ratio of 58:42, v/v and at a flow rate of 1.0 mL/min. The method was linear over the concentration range for Cefixime 50-375 µg/mL and for Acetylcysteine 75-400 µg/mL. The retention times for Cefixime and Acetylcysteine were found to be 2.018 and 5.141 min respectively. The average percentage recoveries of Active Pharmaceutical Ingredient (API) Cefixime and Acetylcysteine were found to be in the range of 99.23% and 100.13% respectively. %RSD of the Cefixime and Acetylcysteine were found to be 0.9 and 0.8 respectively. %Assay obtained as 99.23% and 100.13% for Cefixime and Acetylcysteine respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Cefixime and Acetylcysteine in combined tablet dosage form.

Keywords: Acetylcysteine; Cefixime; HPLC; Validation

Introduction

Cefixime (Figure 1), an antibiotic, is a third-generation oral bactericidal cephalosporin. Cefixime is chemically known as (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy) imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclooct-2-ene-2-carboxylic acid [1]. The antibacterial effect of Cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall. Cefixime is extremely stable in presence of β-lactamase enzymes. Cefixime is used in the treatment of uncomplicated urinary tract infections caused by *Escherichia coli* and *Proteus mirabilis*, otitis media caused by *Haemophilus influenzae,* pharyngitis and tonsillitis caused by *S. pyogenes* and uncomplicated gonorrhea (cervical/urethral) caused by *Neisseria gonorrhoeae* etc.

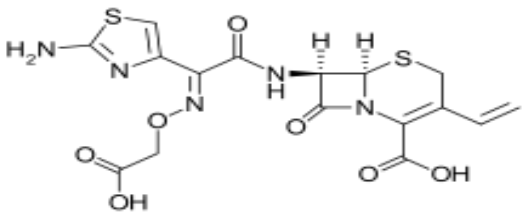

Figure 1: Structure of Cefixime.

Acetylcysteine (Figure 2), is primarily used as a mucolytic agent and in the management of acetaminophen poisoning. It is chemically known as (2R)-2-acetamido-3-sulfanylpropanoic acid [2]. It is a derivative of cysteine with an acetyl group attached to the amino group of cysteine. NAC is essentially a prodrug that is converted to cysteine (in the intestine by the enzyme aminoacylase 1) and absorbed in the intestine into the blood stream. Acetylcysteine

can also be used as a general antioxidant which can help mitigate symptoms for a variety of diseases exacerbated by Reactive Oxygen Species (ROS). N-acetylcysteine is now widely used in the treatment of HIV. Acetylcysteine is also being successfully used to treat a variety of neuropsychiatric and neurodegenerative disorders including cocaine, cannabis, and smoking addictions, Alzheimer's and Parkinson's diseases, autism, compulsive and grooming disorders, schizophrenia, depression, and bipolar disorder.

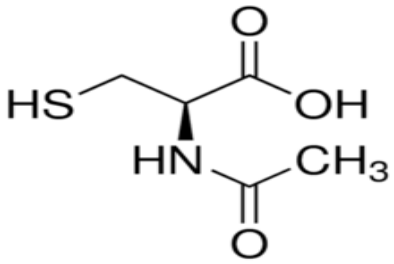

Figure 2: Structure of Acetylcysteine.

Cefixime is an antibiotic which kills bacteria by preventing them from forming their own protective covering which is required for their survival in the human body. Acetylcysteine is a mucolytic which thins and loosens mucus (phlegm) making it easier to cough out. The combination of these two drugs can be used effectively to treat respiratory tract infection. In literature there are various methods were reported for individual estimation of Cefixime and Acetylcysteine in pharmaceutical dosage forms using UV Spectrophotometry [3-5], HPTLC [6,7] and HPLC [8,9], but very few HPLC methods [10,11] were reported for simultaneous estimation of these drugs in combined dosage form. The main objective of the present work is to develop, and valid a simple, sensitive, rapid, accurate and precise method developed for determination of Cefixime and Acetylcysteine in combined dosage form.

Materials and Methods

Materials

Cefixime and Acetylcysteine Pure Drugs (API) are procured from Spectram Labs, Hyderabad, Combination of Cefixime and Acetylcysteine tablets (Mucomelt Forte bearing the label claim Cefixime 200 mg, Acetylcysteine 300 mg) were procured from local market. Distilled water, Acetonitrile, Methanol, Potassium dihydrogen ortho phosphate, OPA is purchased from Rankem Chemicals Ltd., Mumbai, India.

Instrumentation

The analysis of drugs was carried out on a Waters HPLC system on an ODS C18 column (250 x 4.6 mm, 5 µm). The instrument is equipped with a 2695 pump with inbuilt degasser, 2998 photodiode array detector and a Rheodyne injector with 20 µL sample loop. A 20 µL Hamilton syringe was used for injecting the samples. Data was analysed by using Waters Empower 2 software. A double-beam Shimadzu UV-Visible 2450 spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Mobile Phase

A mobile phase consisting of mixture of 0.1% OPA and acetonitrile in the ratio of 58:42, v/v was prepared.

Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50, v/v.

Preparation of Standard Stock Solutions

Accurately weighed 20 mg of Cefixime, 30 mg of Acetylcysteine and transferred to 10 mL volumetric flask. Add 3/4thof diluent and sonicated for 10 minutes. Flasks were made up with diluent and labeled as standard stock solution (2000 µg/mL of Cefixime and 3000 µg/mL of Acetylcysteine).

Preparation of Standard Working Solution (100% solution)

1 mL from each stock solution was pipetted out and taken into a 10mL volumetric flask and made up with diluent (200 µg/mL of Cefixime and 300 µg/mL of Acetylcysteine).

Preparation of Sample Stock Solutions

5 tablets were weighed, and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 50 mL of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2000 µg/mL of Cefixime and 3000 µg/mL of Acetylcysteine).

Preparation of Sample Working Solutions (100% solution): 1mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent (200 µg/mL of Cefixime and 300 µg/mL of Acetylcysteine).

Results and Discussion

Determination of λ_{max} and Optimized wavelength

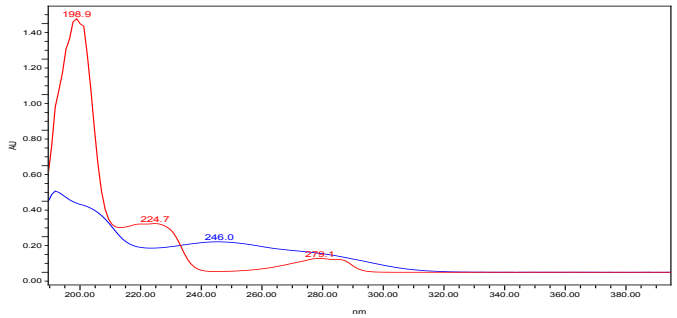

Figure 3: Overlay spectra of Cefixime and Acetylcysteine.

λ_{max} of Cefixime and Acetylcysteine was 279.1 nm and 246.0 nm respectively. Overlay spectra (Figure 3) gave the 274 nm as the optimized wavelength for these two drugs.

Method development

Various trails were performed by using different mobile phases and based on peak parameters the following chromatographic conditions (Table 1) were optimized and optimal chromatogram was shown in (Figure 4).

Mobile phase	OPA (0.1%): Acetonitrile 58:42(v/v)
Flow rate	1 mL/min
Column	ODS C18 (250 x 4.6 mm, 5 µm)
Detector wave length	274 nm
Column temperature	30°C
Injection volume	10 µL
Run time	8 min
Diluent	Water: acetonitrile in the ratio 50:50(v/v)
Retention time	**Cefixime-----2.018 min Acetylcysteine-----5.141 min**

Table 1: Optimized Chromatographic conditions.

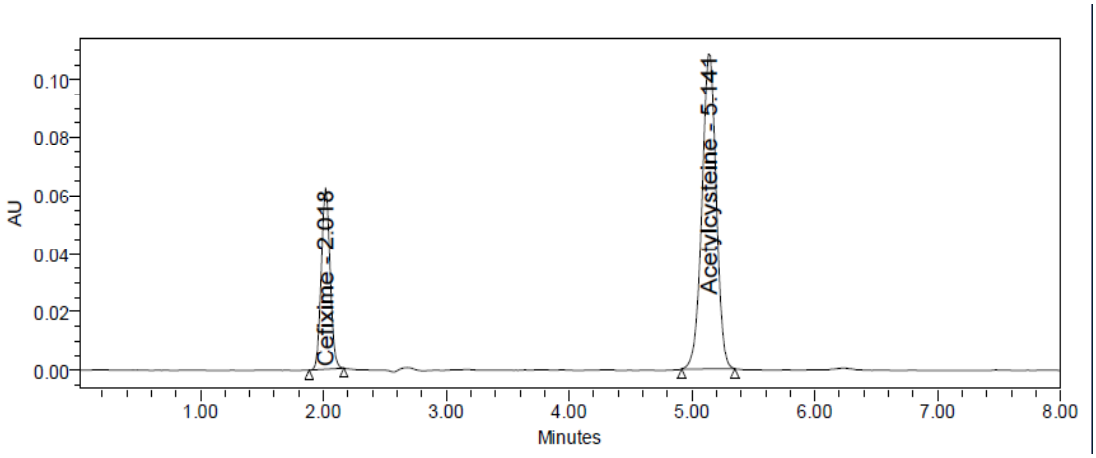

Figure 4: Optimized Chromatogram of Cefixime and Acetylcysteine.

Validation

System Suitability Parameters

The system suitability parameters were determined by preparing standard solutions of Cefixime (200 µg/mL) and Acetylcysteine (300 µg/mL) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. All the system suitability parameters (Table 2) were within the range and satisfactory as per ICH guidelines [12].

S.No.	Cefixime			Acetylcysteine			Resolution
	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	
1	2.017	3478	1.04	5.096	9415	0.98	17.6
2	2.017	3486	1.04	5.106	9656	0.97	17.6
3	2.018	3555	1.04	5.123	9725	0.98	17.7
4	2.02	3641	1.05	5.126	9677	0.97	17.6
5	2.021	3590	1.06	5.135	9512	0.97	18

6	2.028	3735	1.04	5.141	9650	0.97	17.7

Table 2: System suitability parameters for Cefixime and Acetylcysteine.

1.1 Specificity

Specificity is the parameter used to check the interference in the optimized method. We should not find interfering peaks in blank (Figure 5), placebo (Figure 6), standard (Figure 7) and sample (Figure 8) at retention times of these drugs in this method. So, this method was said to be specific.

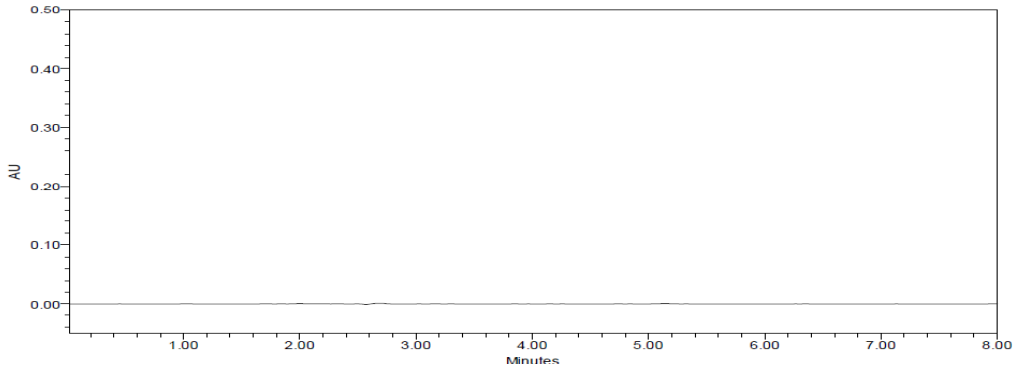

Figure 5: Chromatogram of blank.

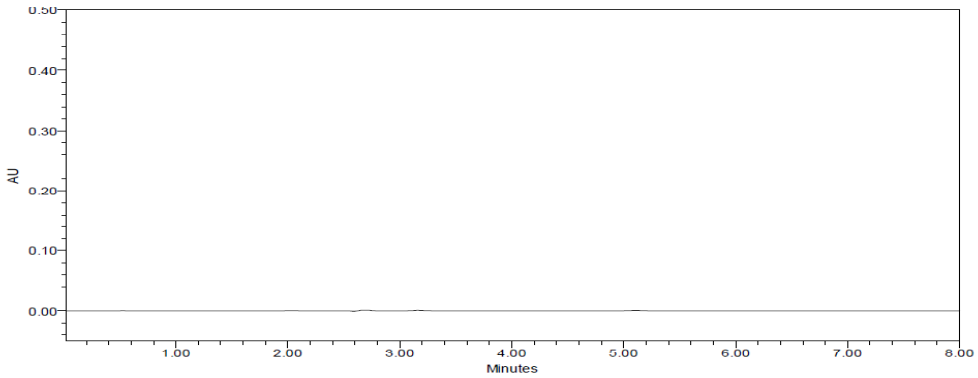

Figure 6: Chromatogram of placebo.

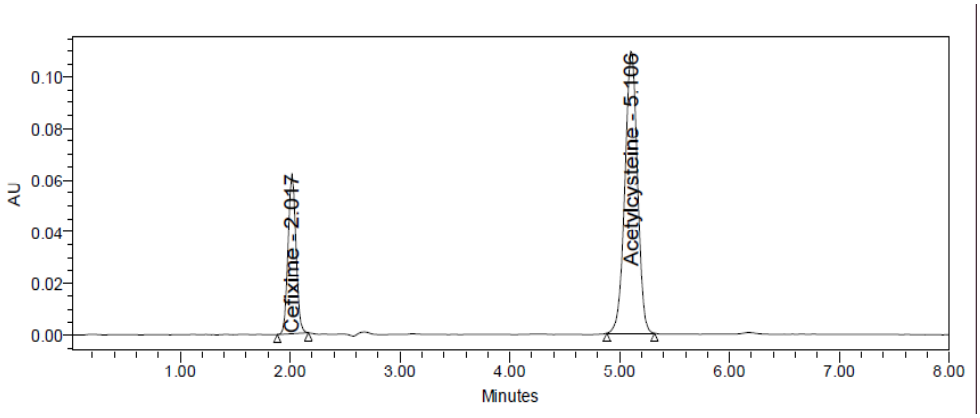

Figure 7: Chromatogram of working standard solution.

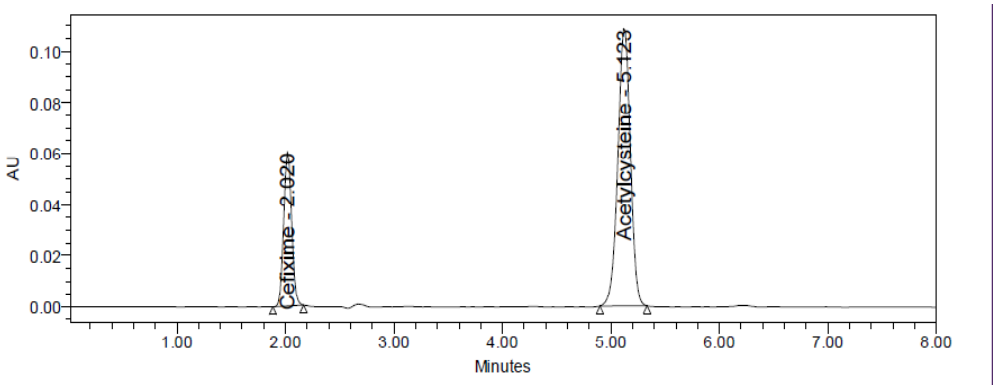

Figure 8: Chromatogram of working sample solution.

Linearity

Accurately weighed 20 mg of Cefixime, 30 mg of Acetylcysteine and transferred to 10 mL volumetric flask. Add 3/4th of diluent to these flasks and sonicated for 10 minutes. Flasks were made up with diluent and labeled as standard stock solution (2000 µg/mL of Cefixime and 3000 µg/mL of Acetylcysteine). From the above solutions transfer 0.25,0.5,0.75,1,1.25 and 1.5 mL to 10 mL volumetric flask and diluted to mark with diluent to prepare 50,100,150,200,250,300 µg/mL solutions of Cefixime and 75, 150, 225, 300, 375, 400 µg/mL solutions of Acetylcysteine. The results were furnished in (Table 3) and calibration curves were shown in (Figure 9&10).

Cefixime		Acetylcysteine	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
50	90458	75	243098
100	157126	150	427624
150	236651	225	645398
200	312067	300	872071
250	386173	375	1065936
300	462348	450	1284061
Slope	1523	Slope	2829.7
Intercept	6394	Intercept	11637
R^2	0.999	R^2	0.9994

Table 3: Linearity results for Cefixime and Acetylcysteine.

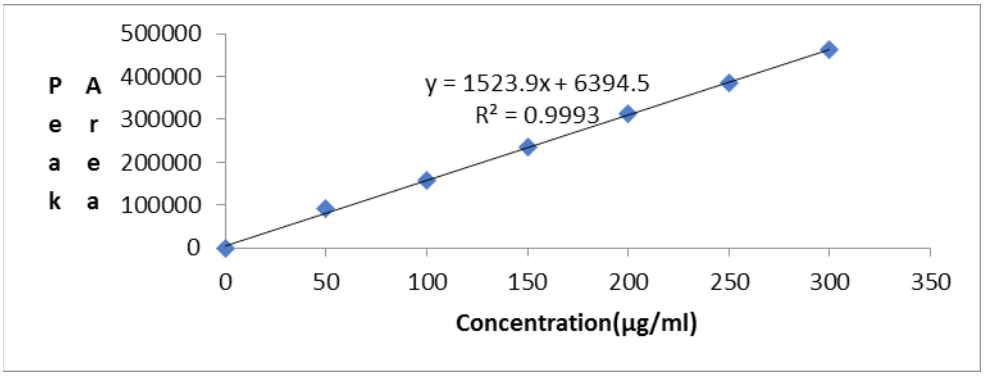

Figure 9: Calibration curve of Cefixime.

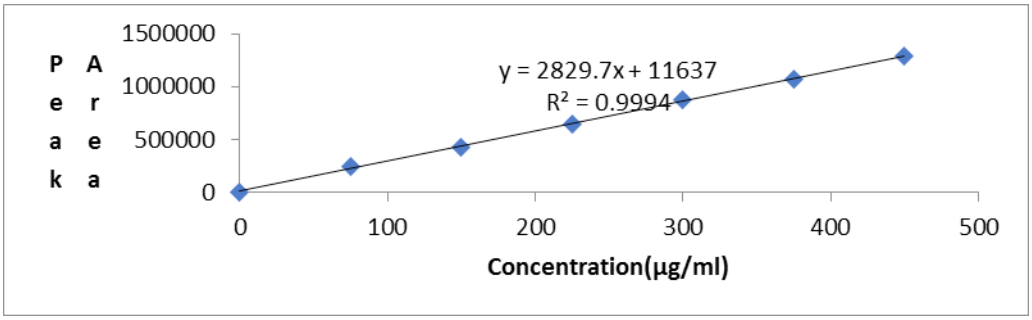

Figure 10: Calibration curve of Acetylcysteine.

Precision

Precision of method was studied by performing intra-day and inter-day precision. Intra-day precision (Table 4) and inter-day precision (Table 5) was studied by injecting the 6 replicates of standard solution in a single day and six days. Calculate the %RSD and it should not be more than 2.0.

S. No.	Area of Cefixime	Area of Acetylcysteine
1	308617	871018
2	307253	866153
3	309971	878547
4	304898	868728
5	309887	872930
6	309719	877729
Mean	308391	872518
S. D	2002.3	4915.3
%RSD	0.6	0.6

Table 4: Intra-day Precision results of Cefixime and Acetylcysteine.

S. No.	Area of Cefixime	Area of Acetylcysteine
1	325919	924692
2	326311	929501
3	328279	923486
4	326918	934770
5	323091	915533
6	331546	934992
Mean	327011	927162
S.D	2801.1	7476.8
%RSD	0.9	0.8

Table 5: Inter-day Precision results of Cefixime and Acetylcysteine.

Accuracy

The accuracy of the method was established by calculating percentage recovery of Cefixime and Acetylcysteine by the method of addition. Known amount of Cefixime and Acetylcysteine at 50%, 100%, and 150% was added to a pre-quantified sample solution. The recovery studies (Tables 6,7) were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery at each level was not less than 99% and not more than 101.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	100	100.38	100.38	99.67%
	100	100.17	100.17	
	100	99.58	99.58	
100%	200	198.86	99.43	
	200	199.07	99.53	
	200	200.1	100.05	
150%	300	300.11	100.04	
	300	296.2	98.73	
	300	297.27	99.09	

Table 6: Accuracy results of Cefixime.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	150	150.08	100.05	99.66%
	150	151.14	100.76	
	150	148.01	98.67	
100%	300	297.8904	99.3	
	300	297.7296	99.24	
	300	297.275	99.09	
150%	450	448.6034	99.69	
	450	447.1729	99.37	
	450	453.4871	100.77	

Table 7: Accuracy results of Acetylcysteine.

Sensitivity

Limit of Detection was calculated by intercept method and LOD for Cefixime and Acetylcysteine were found to be 0.50 and 1.53 µg/ml respectively. Limit of Quantification was calculated by intercept method and LOQ for Cefixime and Acetylcysteine were found to be 0.49 and 1.47 µg/ml respectively.

Robustness

Small deliberate changes in method like flow rate, mobile phase ratio and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like flow minus (0.9 mL/min), flow plus (1.1 mL/min), mobile phase minus, mobile phase plus, temperature minus (25˚C) and temperature plus (35˚C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected, and all the parameters were passed. %RSD was within the limit. The results were shown in (Table 8).

S. No.	Condition	%RSD of Cefixime	%RSD of Acetylcysteine
1	Flow rate (-) 1.1mL/min	1	0.7
2	Flow rate (+) 1.3mL/min	0.6	0.3
3	Mobile phase (-) 35B:65A	1	0.6
4	Mobile phase (+) 45B:55A	0.9	0.6
5	Temperature (-) 25˚C	0.8	0.5
6	Temperature (+) 35˚C	0.5	0.5

Table 8: Robustness data for Cefixime and Acetylcysteine

Assay

Mucomelt Forte, bearing the label claim Cefixime 200 mg, Acetylcysteine 300 mg. Assay was performed with the above formulation. Average % Assay for Cefixime (Table 9) and Acetylcysteine (Table 10) obtained was 99.23 and 100.13%respectively.

S. No.	Standard area	Sample area	% Assay
1	309272	308617	99.3
2	309026	307253	98.86
3	309782	309971	99.74
4	310161	304898	98.11
5	311398	309887	99.71
6	311342	309719	99.66
Avg	310164	308391	99.23
St.dev	1014.6	2002.3	0.644
%RSD	0.3	0.6	0.65

Table 9: Assay Data of Cefixime.

S. No.	Standard area	Sample area	% Assay
1	867368	871018	99.95
2	869486	866153	99.4
3	871302	878547	100.82
4	874116	868728	99.69
5	870462	872930	100.17
6	870588	877729	100.72
Avg	870554	872518	100.13
St.dev	2215.8	4915.3	0.564
%RSD	0.3	0.6	0.56

Table 10: Assay Data of Acetylcysteine.

Degradation Studies

Acid Degradation Studies

To 1 mL of stock solution Cefixime and Acetylcysteine 1 mL of 2N Hydrochloric acid was added and refluxed for 30 mins at 60˚C. The resultant solution was diluted to obtain 200 µg/mL & 300 µg/mL solution and 10µL solutions into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 mL of stock solution Cefixime and Acetylcysteine, 1 mL of 2N sodium hydroxide was added and refluxed for 30 min at 60˚C. The resultant solution was diluted to obtain 200 µg/mL & 300 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidation

To 1 mL of stock solution of Cefixime and Acetylcysteine, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60˚C. For HPLC study, there resultant solution was diluted to obtain 200 µg/mL & 300 µg/mL solution and 10µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105˚C for 1h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 200 µg/mL & 300 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability Studies

The photochemical stability of the drug was also studied by exposing the 2000 µg/mL & 3000 µg/mL solution to UV Light by keeping the beaker in UV Chamber for 1day or 200-Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 200 µg/mL & 300 µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1 hr at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 200 µg/mL & 300 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Degradation studies results were computed in (Table 11,12).

S. No.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.34	0.832	1.398
2	Alkali	2.97	1.041	1.492
3	Oxidation	2.31	0.877	1.404
4	Thermal	1.68	0.041	0.492
5	UV	0.83	0.819	1.384
6	Neutral	0.05	0.169	0.094

Table 11: Degradation Data of Cefixime.

S. No.	Degradation Condition	%Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.72	0.115	0.286
2	Alkali	3.45	0.079	0.295
3	Oxidation	1.67	0.112	0.294
4	Thermal	1.16	0.079	0.295
5	UV	0.95	0.1	0.275
6	Neutral	0.59	0.094	0.279

Table 12: Degradation Data of Acetylcysteine.

Discussion

A stability indicating RP-HPLC method was developed for the simultaneous estimation of Cefixime and Acetylcysteine by using mobile phase consisting of 0.1% OPA and Acetonitrile in the ratio of 58:42, v/v. Retention times for Cefixime and Acetylcysteine were found to be 2.018 and 5.141 min respectively. The method was validated as per ICH guidelines. Linearity range was found to be 50-300 µg/mL for Cefixime and 75-400 µg/mL for Acetylcysteine. Mean recoveries were found to be satisfactory. This method also passed the test for repeatability, intermediate precision and robustness for both drugs.

Degradation studies are also carried out in acid, base, peroxide, heat, UV light and neutral stressed conditions. The results revealed that both the drugs are stable in described conditions. Thus, it is evident that the described method can be adopted for routine estimation of Cefixime and Acetylcysteine in combined dosage form.

Conclusion

The present method was proposed for the simultaneous estimation of the Cefixime and Acetylcysteine by using RP-HPLC in tablet dosage form is found to be simple, accurate, rapid and precise. Retention times were decreased, and the run time was decreased. The method developed was simple and economical therefore can be applied in regular quality control tests in industries.

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