RJC

RASĀYAN *J. Chem.*
Vol. 8 | No.4 |477 - 486 | October - December | 2015
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF CEFIXIME AND ORNIDAZOLE IN COMBINED PHARMACEUTICAL DOSAGE FORM

M. Nageswara Rao[1], P. R. K.Veni[2] and B. Haribabu[1*]

[1] Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.
[2]Department of Chemistry, Sasi institute of Engineering, Tadepalligudem, AP-India
*E-mail: drharibabuanu2015@gmail.com

ABSTRACT

A simple, precise and accurate isocratic high-performance liquid chromatography method is developed for the simultaneous estimation of Cefixime and Ornidazole in bulk drug and pharmaceutical dosage form. The separation and quantification is carried out using YMC Pack Pro C18, 250X4.6, 5 um analytical columns. The mobile phase comprises of 0.1M NaH_2PO_4 : Methanol (500: 500 v/v). The flow rate is 1.2 mL/min. The eluent is monitored at 270 nm. The retention time of Cefixime and Ornidazole are 3.275 min and 4.450 min, respectively. The method is validated in terms of linearity, sensitivity, precision, accuracy, specificity, selectivity and robustness. The stress testing is carried out under acidic, alkaline, oxidation, thermal degradation and photolytic conditions. The degradation products are well resolved from the Cefixime and Ornidazole peaks.

Keywords: Simultaneous estimation, HPLC, YMC Pack Pro, Cefixime, Ornidazole

INTRODUCTION

Cefixime[1-3] (CEF) chemically (6R,7R)-7-[[(Z)-2-(2-aminothiazol-4-yl)- 2-[(carboxymethoxy) imino] acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid trihydrate (Figure -1) is anti bacterial drug a third generation cephalosporin given by oral route in the treatment of susceptible infections which contains gonorrhea, urinary tract, pharynsitis and otitis media infection.

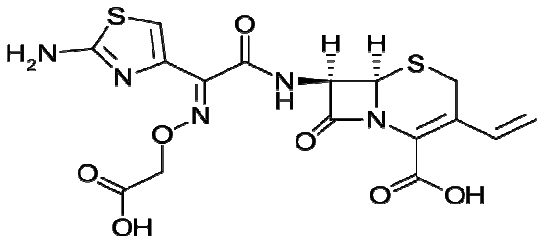

Fig.-1: Structure of Cefixime

Ornidazole[4-17] (OZ) is chemically 1-chloro-3-(2-methyl-5-nitroimidazole-1yl) -propan-2-ol (Figure -2). Used as anti-infective agent and it is not official in all types of Pharmacopoeia. The result analysis survey revealed HPLC and UV methods for the analysis of CEF and OZ either single component system or in combination with other drugs.

So far to our knowledge no stability indicating RP-HPLC assay method has been developed in the combined dosage form. So the aim of this work is to develop simple stability indicating RP-HPLC assay method for diagnosis of CEFI and ORNI in bulk drug and combined dosage form tablets as well as for different application for dissolution testing of tablet formulations[18-19].

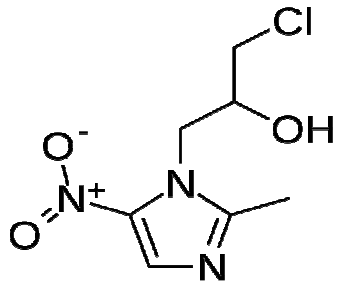

Fig.-2: Structure of Ornidazole

EXPERIMENTAL

Apparatus
The analysis of Cefixime and Ornidazole was carried out using a Waters 2695 alliance HPLC system which contains the Waters 2998 PDA detector with binary pump. For recording data, Waters Empower 2 software was used. YMC Pack Pro C18, 250X4.6, 5 um analytical columns was used to achieve chromatographic separation.

Commercial capsule dosage forms
Dixim-OZ tablet manufactured by Vaishnavi Pharmaceuticals LTD., India was purchased from local market. Each capsule claimed to contain 200 mg of Cefixime and 500 mg of Ornidazole.

Solvents
Methanol of HPLC grade was purchased from Merck (India) Ltd., Mumbai. Ortho phosphoric acid of analytical reagent grade was obtained from SD Fine Chemicals Ltd., Mumbai. Mille Q water was used throughout the process.

Mobile Phase
The mobile phase consisting of 0.1M NaH_2PO_4 : Methanol was degassed and pumped from the solvent reservoir in the ratio of 500:500 (*v/v*).

Chromatographic conditions
The mobile phase was pumped from the reservoir into the column at a flow rate of 1.2 mL/min. The column temperature was set at 30 °C. The detection was monitored at 270 nm and the run time was 6 min. The volume of injection loop was 10 µL. Prior to injection of the drug solution, the column was equilibrated for at least 7 min. with the mobile phase.

Standard Solutions
Stock solutions of Cefixime and Ornidazole both with 1 mg/mL are dissolved to prepare the drugs in mobile phase then completing in 100 mL volumetric flasks. Series of working solutions of Cefixime and Ornidazole were prepared by the appropriate dilution of the stock solutions with mobile phase to reach the concentration ranges of 20- 60 µg/mL for Cefixime and 50-150 µg/mL for Ornidazole.

Procedure for Calibration Graph
10 µL injections were made for each working concentration and chromatographer under the condition mentioned above. The peak area for each concentration was plotted against the corresponding concentration to get the calibration graph and regression equation was derived.

Procedure for Pharmaceutical Dosage Sample
The contents of twenty capsules, labeled to contain 200 mg of Cefixime and 500 mg of Ornidazole, were weighed, mixed and finely powdered in a mortar. An amount of the powder equivalent to 200 mg of

Cefixime and 500 mg of Ornidazole was accurately weighed, shifted into 100 mL volumetric flask and make over with mobile phase. The sample solution was filtered using 0.45 µm filter. An aliquot of filtrate was pipette and diluted to obtain concentrations 40 µg/mL of Cefixime and 100 µg/mL of Ornidazole. The procedure was completed as mentioned above. The nominal concentration of Cefixime and Ornidazole was obtained either from calibration graph or from corresponding regression equation.

Specificity (Forced Degradation)

The specificity of the proposed method was assessed to prove the absence of interference from the degradants of Cefixime and Ornidazole. Degradation study was performed by subjecting the capsule powder to degradations such as acid, alkaline, oxidation, thermal and photolytic conditions to evaluate the interference of degradants. All forced degradation studies were analyzed at 40 µg/mL Cefixime and 100 µg/mL Ornidazole concentration levels. Thermal degradation was performed by keeping the sample in Petri dish and then placed them in an oven at 105^{o}C for half an hour. The photolytic study was carried out by placing the sample in Petri dish and exposed to sun light for 24 hours. Acid, base and oxidation degradations were performed by adding the 10 mL of 0.1N HCl, 10 ml of 0.1N NaOH and 10 mL of 30% peroxide solution, respectively to the sample and sonicate for half an hour. The acid degraded sample and base degraded sample are neutralized with 0.1 N NaOH and 0.1 N HCl, respectively.

RESULTS AND DISCUSSION

The main objective of the RP-HPLC method was to develop a validated stability indicating method for the estimation of Cefixime and Ornidazole simultaneously in bulk and capsule dosage form and to obtain well resolved peaks of Cefixime, Ornidazole and their degradants.

Method Development and Optimization

Chromatographic parameters such as mobile phase composition, wavelength of detection, column and column temperature were optimized to achieve better efficiency of the chromatographic system. For HPLC analysis we tested the YMC Pack Pro C18, 250X4.6, 5um column. The system suitability parameters like tailing factor, resolution, and plate count were taken into consideration. Based on the above said parameters YMC Pack Pro C18, 250X4.6, 5um column was finalized for simultaneous analysis. Different compositions of mobile phases containing a mixture (v/v) of 0.1 M NaH_2PO_4, 0.1M CH_3COONH_4 and methanol water were evaluated in order to obtain suitable composition of mobile phase. Finally the mixture of 10.1M NaH_2PO_4 : Methanol in the ratio of 500:500 (*v/v*) was selected as optimal as it produced well defined and well resolved peaks of Cefixime and Ornidazole at a flow rate of 1 mL/min and with column temperature of 30^{o}C.

For the detection and quantification of Cefixime and Ornidazole, 270 nm was selected as the optimum wavelength. At this wavelength best detector response for both Cefixime and Ornidazole was obtained. The retention time for Cefixime and Ornidazole was found to be 3.275 min and 4.450 min, respectively. A typical chromatogram is given in Figure-3.

Method Validation

The developed method was validated as per the guidelines given by International Conference on Harmonization.

System Suitability

For system suitability testing, five replicates of Cefixime (40 µg/mL) and Ornidazole (100 µg/mL) standard solutions were injected. The retention time, peak area, USP plate count and USP tailing factor of each replicate were established. The results of system suitability in comparison with the required limits are shown in Table-1 and are found to be within the accepted limits.

Table-1: System suitability

Parameters	Cefixime	Ornidazole	Recommended limits
Retention time	3.275	4.450	-
Peak area	506339 (%RSD – 0.7)	777189 (%RSD – 0.6)	RSD ≤1
USP resolution	-	5.00	> 1.5
USP plate count	4303	5065	> 2000
USP tailing factor	1.74	1.52	≤ 2

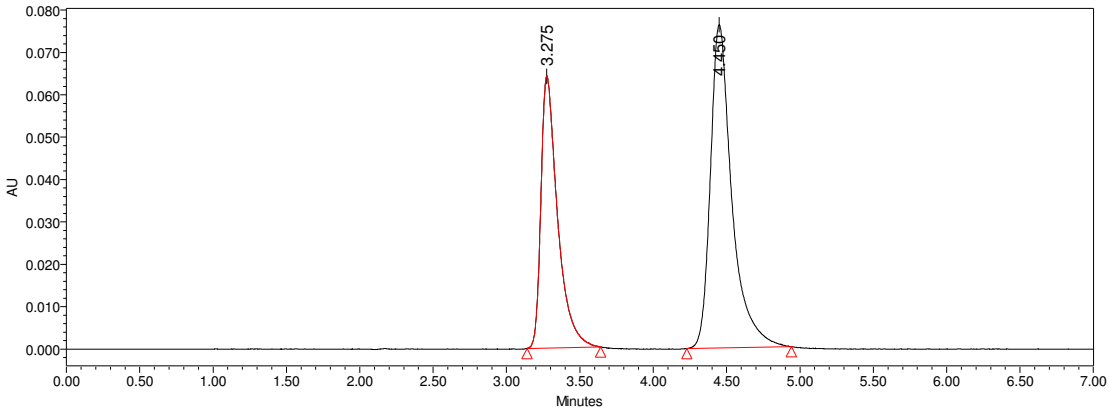

Fig.-3: Typical chromatogram of Cefixime (t_R- 3.275) and Ornidazole (t_R – 4.450)

Linearity

The proposed method was tested for linearity by plotting peak area against concentration of drug. The plot of peak area *vs.* the respective concentrations of Cefixime and Ornidazole were found to be linear in the concentration range of 20-60 µg/mL and 50-150 µg/mL respectively. The results of linearity and regression equations for Cefixime and Ornidazole were given in Figures-4 and 5. The results shows that an excellent correlation exists between area and drug concentration within the concentration range indicated above.

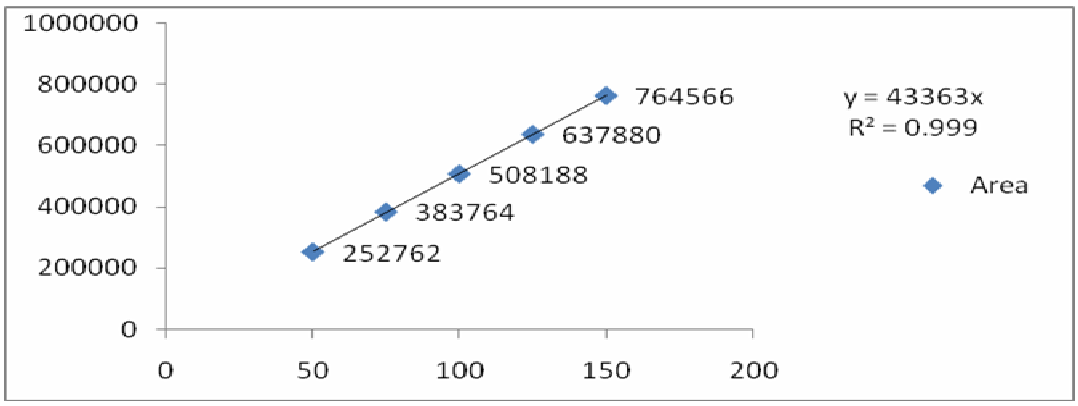

Fig.-4: Linearity curve and regression equation of Cefixime

Limit of quantification and detection

Limit of quantification (LOQ) and detection (LOD) were predicted by plotting linearity curve for different nominal concentrations of Cefixime and Ornidazole. The LOQ and LOD values were predicted using following formulae (a) and (b):

(a) LOQ = 10 σ / S (b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response; S = slope of the calibration curve.

The LOD and LOQ for Cefixime were found to be 0.021 µg/mL and 0.070 µg/mL, respectively. The LOD and LOQ for Ornidazole were found to be 0.0461 µg/mL, 0.1536 µg/mL, respectively.

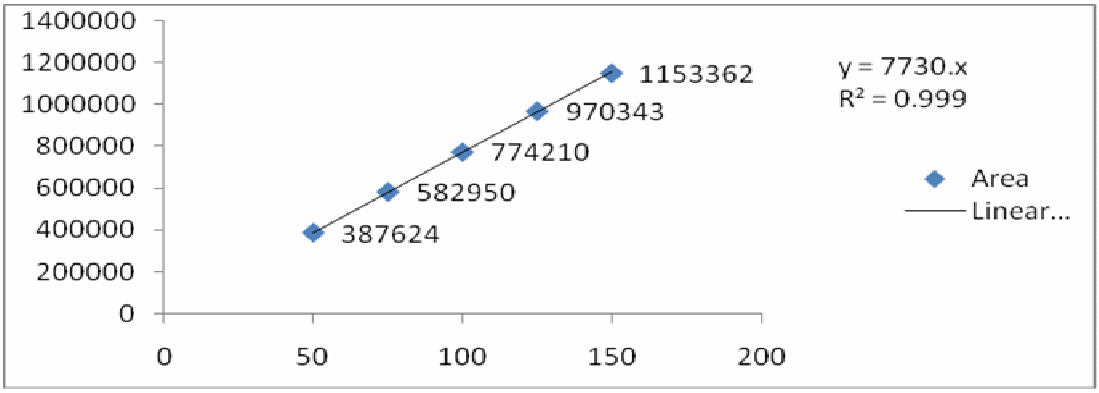

Fig.-5: Linearity curve and regression equation of Ornidazole

Precision

The precision of the proposed method was determined by the analysis of a fixed concentration of the selected drugs (Cefixime: 40 µg/mL and Ornidazole: 100 µg/mL), within the linearity range, by six replicate analyses. The precision was expressed as percent standard deviation. The results were illustrated in Table-2.

Accuracy

To study the accuracy of the proposed method, recovery studies were conducted at three concentrations of 50%, 100%, and 150% levels by standard addition method. The accuracy expressed as percentage recoveries was shown in Table-3. The results indicated that the method is accurate.

Table-2: Precision

Cefixime		Ornidazole	
Peak area	%RSD	Peak area	%RSD
509883	0.40	777008	0.18
505765		778666	
509929		775797	
508657		776711	
505853		774699	
506039		775518	

Table-3: Accuracy

Drug	Spiked Level	µg/mL added	µg/mL found	% Recovery	% Mean
Cefixime	50%	19.800	19.88	100	100
	50%	19.800	19.74	100	
	50%	19.800	19.85	100	
	100%	39.600	39.54	100	100
	100%	39.600	39.62	100	
	100%	39.600	39.40	100	
	150%	59.400	59.59	100	100
	150%	59.400	59.61	100	

	150%	59.400	59.43	100	
Ornidazole	50%	50.000	49.84	100	100
	50%	50.000	49.98	100	
	50%	50.000	49.89	100	
	100%	100.000	99.82	100	100
	100%	100.000	99.97	100	
	100%	100.000	99.59	100	
	150%	150.000	150.43	100	100
	150%	150.000	149.61	100	
	150%	150.000	150.19	100	

Specificity

The degradation study was carried out using the capsule powder containing Cefixime and Ornidazole. Specificity of the method was performed by injecting the stressed degradation samples into the HPLC system. The chromatograms of the samples after forced degradation treatment are shown in Figures-6-10. The samples submitted to all degradation conditions showed significant alteration in the peak areas. In all the degradation conditions two peaks, in addition to the Cefixime and Ornidazole peaks, were observed expect in photolytic degradation where three additional peaks were observed. The degradation peaks were well resolved from that of Cefixime and Ornidazole peaks. The degradation results of various stress conditions were shown in Table-4.

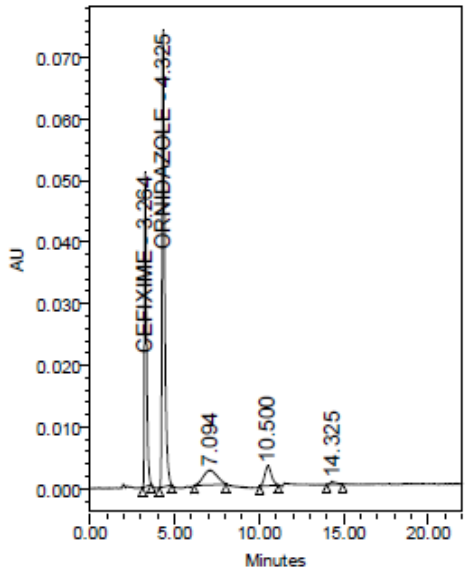

Fig.-6: Chromatogram of acid treated Cefixime and Ornidazole

Table-4: Forced degradation studies

Type of degradation	Cefixime			Ornidazole		
	Peak area	% Assay	% Degradation	Peak area	% Assay	% Degradation
Undegraded	508822	100	-	776128	100	-
Acid	484474	94	6	740285	95	5
Base	489765	95	5	749029	96	4
Peroxide	483151	94	6	742554	95	5
Heat	489499	95	5	749530	96	4
Sunlight	488118	95	5	748959	96	4

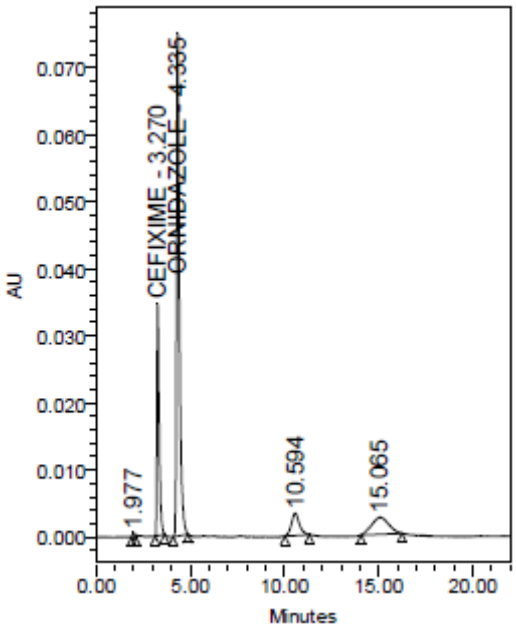

Fig.-7: Chromatogram of base treated Cefixime and Ornidazole

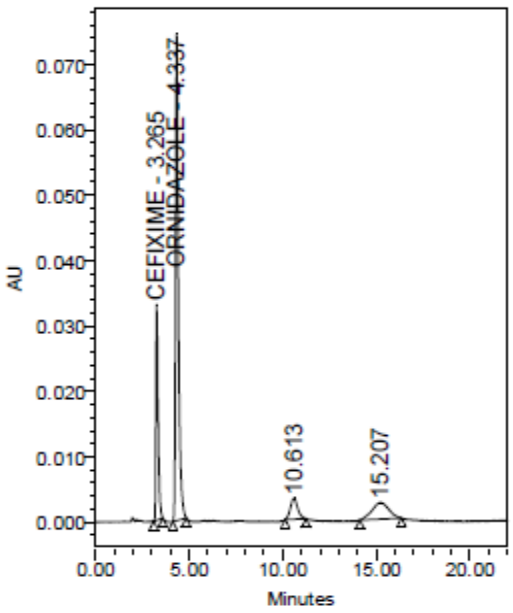

Fig.-8: Chromatogram of peroxide treated Cefixime and Ornidazole

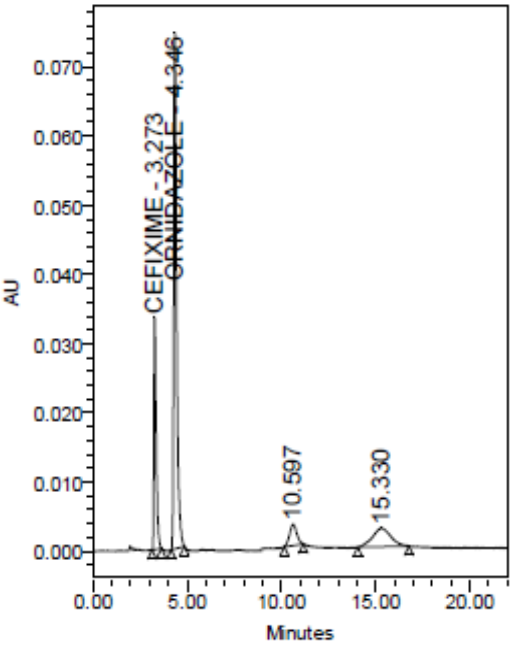

Fig.-9: Chromatogram of thermal treated Cefixime and Ornidazole

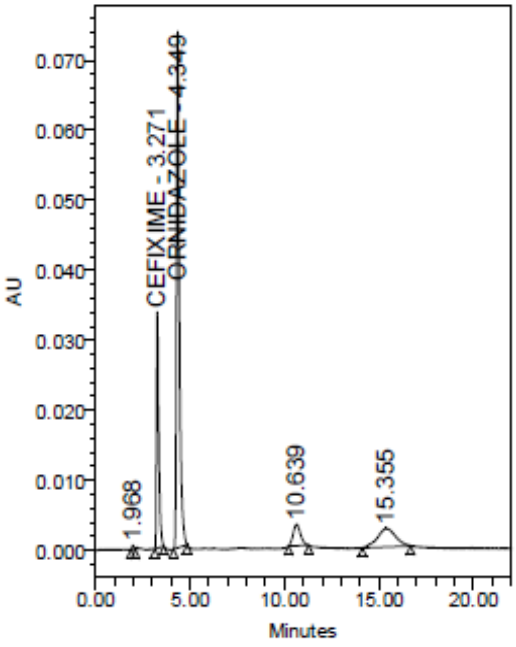

Fig.-10: Chromatogram of sunlight treated Cefixime and Ornidazole

Spectral homogeneity of Cefixime and Ornidazole in the presence of their stress degradation products was checked. Peak purity passed for both the Cefixime and Ornidazole. The results are presented in Table 5. Purity angle value was less than the purity threshold for Cefixime and Ornidazole peaks indicating both the peaks are spectrally homogeneous.

Table-5: Spectral homogeneity of Cefixime and Ornidazole

Type of Degradation	Cefixime		Ornidazole	
	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
Acid	0.409	0.875	0.206	0.544
Base	0.441	0.942	0.199	0.443
Peroxide	0.452	0.854	0.196	0.453
Heat	0.437	0.968	0.197	0.446
Sunlight	0.443	0.764	0.203	0.546

Selectivity

To confirm the noninterference of placebo, placebo solution was prepared in the same way of the capsule sample solution in the presence of all excipients of the capsule dosage form but without Cefixime and Ornidazole. The chromatograms of blank, placebo, test sample and standard were compared to give reason for the selectivity of method. The method was selective since excipients in the formulation and components of the mobile phase did not interfere in the simultaneous analysis of Cefixime and Ornidazole (Figures-11and 12).

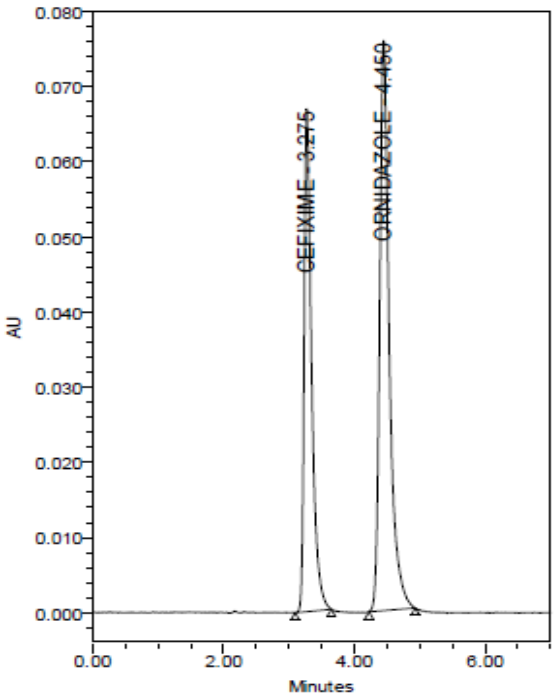

Fig.-11: Chromatogram of test sample

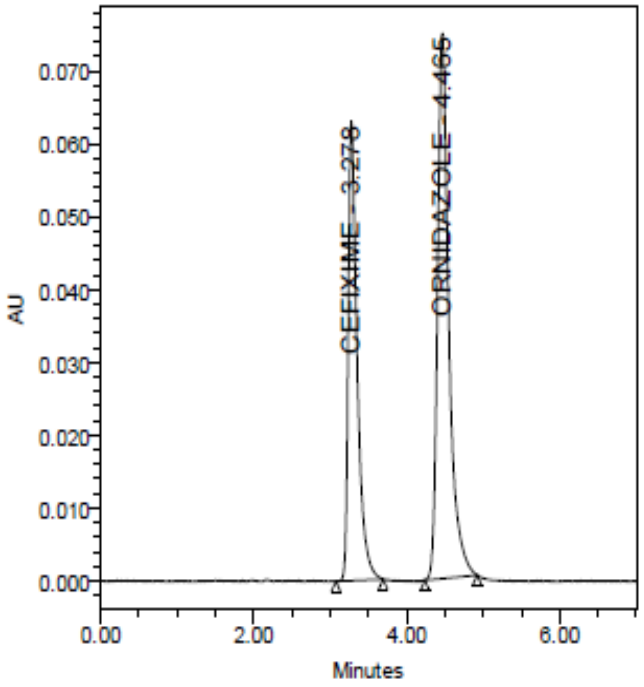

Fig.-12: Chromatogram of standard

Table-6: Robustness for Cefixime

Sample. No	Sample Name	RT	Area	Theoretical plates	USP Tailing
1	Temp-1(25°C)	4.366	660632	3554	1.25
2	Temp-2(35°C)	2.638	396354	4452	1.23
3	Flow-1(0.8ml/min)	4.370	657275	3600	1.17
4	Flow-2(1.2ml/min)	2.637	394589	4354	1.27

Robustness

Robustness of the method was determined by making little (very less) changes in the chromatographic conditions (Flow rate ±0.2 mL/min, column temperature ±5°C) Tables-6 and 7. It was observed that there were no marked changes in System suitability / parameters of the standard chromatograms which explained that the RP-HPLC method developed is robust.

Table-7: Robustness for Ornidazole

Sample. No	Sample Name	RT	Area	Theoretical plates	USP Tailing
1	Temp-1(25°C)	5.977	1023373	4518	1.20
2	Temp-2(35°C)	3.638	611498	4727	1.29
3	Flow-1(0.8ml/min)	5.983	1016927	4410	1.21
4	Flow-2(1.2ml/min)	3.643	616549	4717	1.22

CONCLUSION

A simple, rapid, economical stability indicating RP-HPLC method was developed for the separation and simultaneous quantification of Cefixime and Ornidazole in the presence of its stress degradation products in bulk and in its pharmaceutical dosage forms. Degradation behavior of Cefixime and Ornidazole was studied under various degradation conditions like acid, base, peroxide, thermal and sunlight. Degradation peaks were observed in all stress conditions. All the stress degradation products were well separated from Cefixime and Ornidazole revealing the stability-indicating capability of this method. The developed method can be used for the simultaneous quantification of Cefixime and Ornidazole in routine analysis.

ACKNOWLEDGEMENTS

One of the authors Mr. M. Nageswara Rao thankful to Vaishnavi Pharmaceuticals LTD Hyderabad India for providing the gift sample of Cefixime and Ornidazole reference drug. Also thankful to Acharya Nagarjuna University, Nagarjuna Nagar, Guntur for support and encouragement.

REFERENCES

1. The United State Pharmacopoeia (The United state pharmacopoeia convection. Rockville; USP23) (1995), National formulary, pp.291-293.
2. Jaime N. Delgado and William A. 10th ed.(1998), Remer's Lippincott – Raven Philadelphia, 287, 3.
3. G Roche, *Presse Med.*, **18(32)**, 1541(1989)
4. S. Budhavari The Merck Index, 13th Edition (1997), Merck & Co Inc, White house Station NJ, pp.1229.
5. Nimila, P. Balan, *Asian Journal of Research in Chemistry*, **4(2)**, 227(2011).
6. B. Dhandapani, N. Thirumoorthy, Shaik Harun Rasheed, M. Rama Kotaiah and N. Anjaneyalu, *International Journal of Pharma Sciences and Research*,**1(2)**,78 (2010).
7. S. Paramane, Kothapalli , A. Thomas and A.D. Despandey, *Indian Journal of Pharmaceutical Sciences*, **69**, 525 (2007).
8. P. Patel, K. Patel, K.K. Bhatt, S. Patel, *International Journal of Research in Pharmaceutical and Biomedical Sciences,***2(2)**, 710 (2011).
9. P.N. Ranjane, S.V. Gandhi, S.S. Kadukar. and S.S. Ranher, *Hin. J . Chromatogr*., **26**,763(2008).
10. M. Sudhakar, J. Venkateshwara Rao, and G.S. Devika, P.R .Ramesh, *International Journal of Chemical and Pharmaceutical Sciences*, **1(2)**, 34 (2010).
11. C.M. Nahata, *Journal of Liquid Chromatography*, **14(20)**, 3755 (1991).
12. P.B. Shah, K. Pundarikakshudu, *Ind. J. Pharm. Sci.*,**68(1)**, 90(2006).
13. Khaja Pasha, C.S. Patil, K. Vijay Kumar, Sadath Ali and V.B. Chimkod, *Research J. Pharma. Bio. Chem. Sci.*, **3**, 226(2010).
14. S.M. Soma, V. Vidyasagar, N. Narsaiah, R. Anand Kumar and Krishna, *Indian J. Pharm.Sci.*,**67**, 302(2005).

15. M. Gandhimathi, T.K. Ravi and Nilima Shukla, *Ind. J. of Pharm. Sci.*, **68(6)**, 838 (2006).
16. S. Natarajan and B. Raman, *Indian Pharmacist.,* **4**, 79 (2005).
17. S.E. Jovanovic, D. Agbaba, Zivanov- Stakie and S. Vladimirov, *J. Pharm. Biomed. Analysis*, **18(4-5)**, 893(1998).
18. Shahnaz Gauhar, Hafiz Muhammad Arshad, Raheela Bano and Iyad Naeem Md., *Journal of Pharmaceutical Research,* **8(2)**,98(2009)
19. ICH, Q2B, Validation of Analytical Procedures: Methadology, In proceedings of the International Conference on Hormonisation: Geneva, November 1996, pp.1-8.

[RJC-1353]