

RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF THE DRUG CEFDINIR USING TINIDAZOLE AS INTERNAL STANDARD IN BULK AND PHARMACEUTICAL DOSAGE FORMS

ABSTRACT

A rapid and reproducible HPLC method has been developed and validated for the estimation of the drug Cefdinir using Tinidazole as internal standard in bulk drug and pharmaceutical dosage forms. The estimation was carried out using 5u C18(2) 100A column (100mm x 4.6mm, 5µm); mobile phase consisting of buffer at pH 2.5 and Methanol; the flow rate of 1.8 mL/min and ultraviolet detection at 290 nm. The retention time of Cefdinir was reported to be 2.9 min, the lowest Retention Time ever reported. The method was validated with respect to Precision, Linearity, Accuracy, Ruggedness and Robustness as per ICH Guidelines. The validated method was successfully applied to the commercially available pharmaceutical dosage forms, yielding very good and reproducible result.

Keywords: Cefdinir • Tinidazole • HPLC • LC Method development and validation.

1. INTRODUCTION

We are in the field of Analytical Chemistry for quite a few years. We have developed analytical methods for the analysis of various drugs[1-4]. The results were quite encouraging and forced us to continue with the development of methods for other drugs. In the present work sincere efforts are made to develop a very fast and user-friendly method for the quantification of the drug Cefdinir and we were very much successful in our attempt. This time we have tried to use Tinidazole as an internal standard, which will help the analyst in providing the error free analytical results.

Cefdinir is a semi-synthetic (partially man-made) oral antibiotic in the cephalosporin family of antibiotics. Like other cephalosporins, cefdinir stops bacteria from multiplying by preventing bacteria from forming the walls that surround them. The walls are necessary to protect bacteria from their environment and to keep the contents of the bacterial cell together. Bacteria cannot survive without a cell wall. Cefdinir is active against a very wide spectrum of bacteria, including *Staphylococcus aureus*; *Streptococcus pneumoniae*; *Streptococcus pyogenes* (the cause of strep throat); *Hemophilus influenzae*; *Moraxella catarrhalis*; *E. coli*; *Klebsiella*; and *Proteus mirabilis*. It is not active against *Pseudomonas*. Therapeutic uses of cefdinir include otitis media (infections of the middle ear), infections of soft tissues, and respiratory tract infections. The FDA approved cefdinir in December 1997. Therapeutic uses of cefdinir include otitis media, soft tissue infections, and respiratory tract infections, including sinusitis, strep throat, community-acquired pneumonia, *E. coli*, staph infections, streptococcus infections, middle ear infections, tonsillitis and acute exacerbations of bronchitis. The IUPAC name of Cefdinir is 8-[2-(2-amino-1,3-thiazol-4-yl)-1-hydroxy-2-nitroso- ethenyl]amino-4-ethenyl-7-oxo-2-thia-6- azabicyclo[4.2.0]oct-4-ene-5-carboxylic acid and the structure of the same is represented in Fig.1.

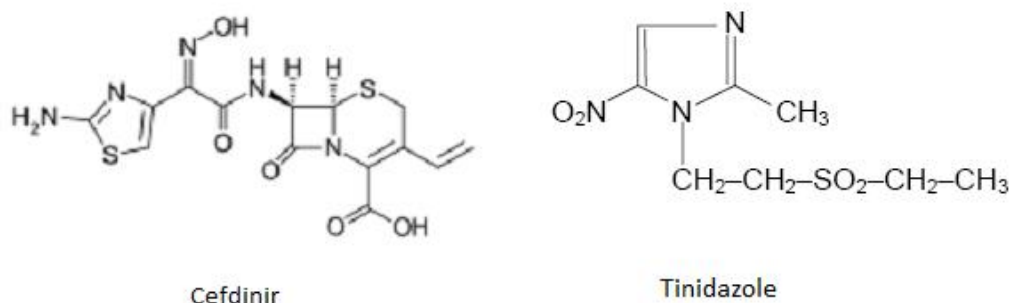


Fig. 1. Chemical structures of Cefdinir and Tinidazole

For the estimation of the drug Cefdinir, several methods are available in the literature[5-9]. But our aim is to develop a faster method compared to all, below 3 minutes, which is a highly challenging task. Cefdinir is a highly sensitive molecule and great efforts were made to develop the method. The present method is a rapid and user- friendly method for the estimation of Cefdinir in the presence of an internal standard, Tinidazole.

2. MATERIAL AND METHODS

2.1. Method Development

Both the drugs, Cefdinir and Tinidazole were scanned by UV, individually, in a wavelength range of 200-400 nm and maxima for each drug was measured. The maxima for Cefdinir was found to be 283.4nm whereas, for Tinidazole maxima were found at 315.2nm. The corresponding UV spectrum graphs of Cefdinir and Tinidazole are as shown in Fig.2. To optimise the UV maxima, various HPLC experiments were performed at various wavelengths starting from 230nm to 350nm. The best response has been observed at 290nm. The average of the UV maxima was done and keeping in mind the main interest Cefdinir, 290 nm was selected for carrying out the experimental work. The respective chromatogram is shown in Fig. 2.

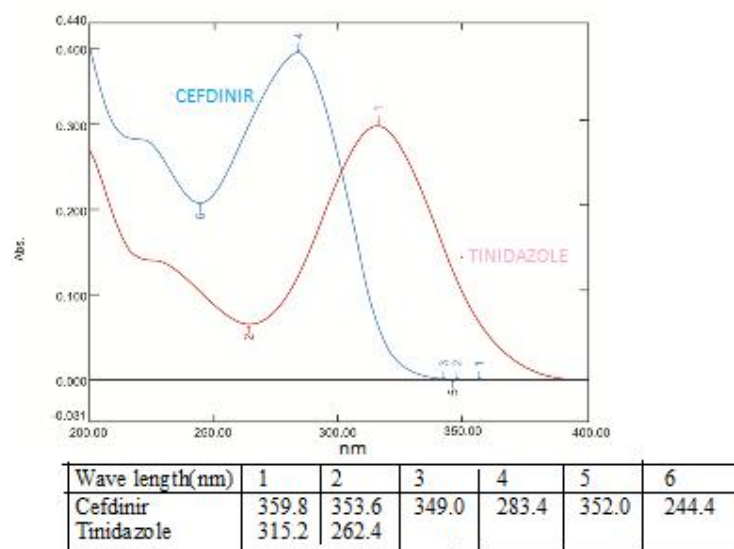


Fig. 2. UV spectrum of Cefdinir and Tinidazole

While optimising the mobile phase composition, experiments were done with different mobile phase compositions with an intention to develop a method comprising of mobile phase which is user-friendly and environmental-friendly. After many trials Potassium dihydrogen orthophosphate and Methanol mixture was selected as the mobile phase to be used for the experiment. Different compositions of this mixture was tried. Initially 90:10 ratio of Potassium dihydrogen orthophosphate and Methanol mixture was selected and analysis was carried out, but very high retention time of Cefdinir, 17 minutes was recorded. Then 82:18 ratio of Potassium dihydrogen orthophosphate and Methanol mixture was used for the experimental work, but no separation of cefdinir and Tinidazole peaks were obtained. Then 85:15 ratio of Potassium dihydrogen orthophosphate and Methanol mixture was used for the experimental work. The Retention time of Cefdinir was found to be 4.795 min. In order to fulfil our aim to get the lowest possible Retention time mobile phase composition 80:20 ratio of Potassium dihydrogen orthophosphate and Methanol mixture was used for the experimental work. The Retention time of Cefdinir was found to be below 3 min with a very good resolution of 12.754. The respective chromatograms are shown in Fig. 3. Hence, mobile phase composition of 80:20 ratio of Potassium dihydrogen orthophosphate and Methanol mixture was selected for the experimental work.

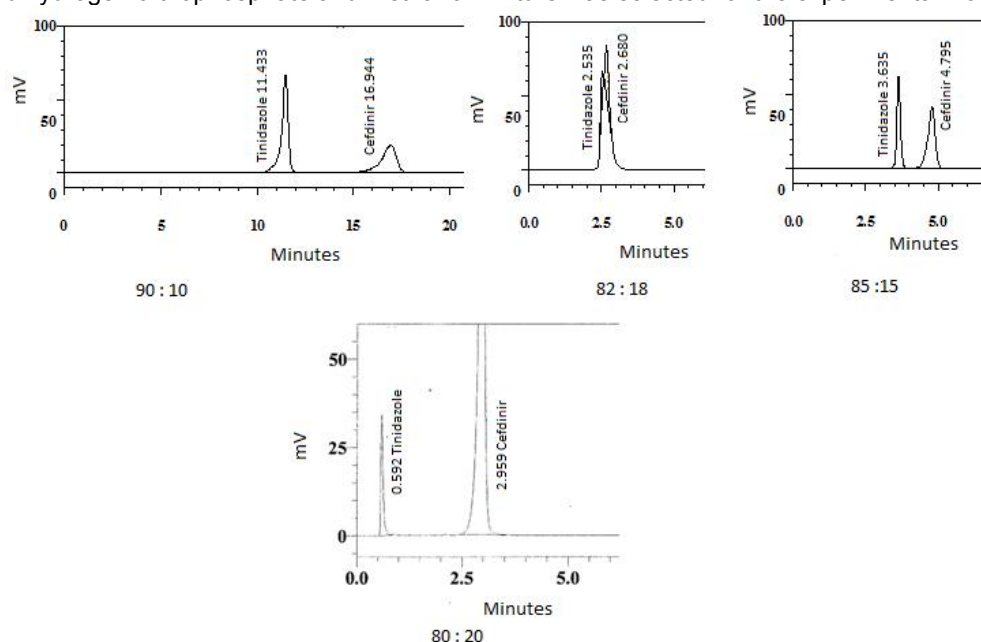


Fig.3. Different mobile phase composition chromatograms

While optimising the mobile phase pH, different pH values of mobile phase, 4.5, 3.5 and 2.5 were used. In case of pH values 3.5 and 4.5 the Retention time of cefdinir was below 2 min but the resolution was not satisfactory. With pH 2.5 the Retention time was 2.6 min with a very good resolution of 12. Hence pH 2.5 of mobile phase was selected for the experimental work. The respective chromatograms are shown in Fig.4.

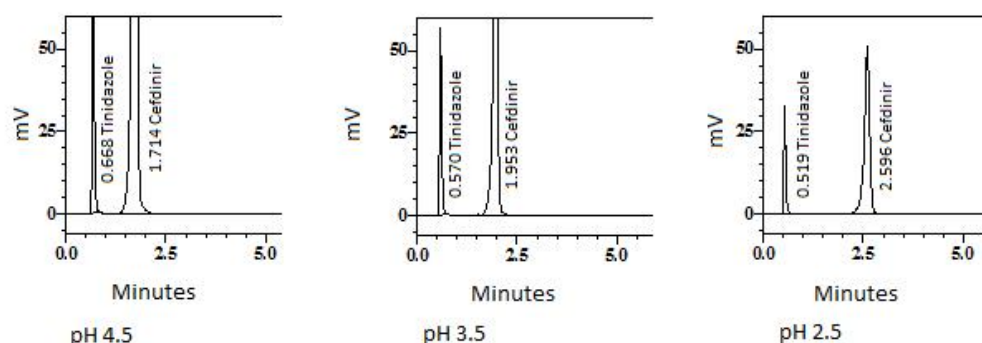


Fig.4. Different pH chromatograms

While optimising the Flow rate, initially flow rate of 1.2 mL/min was used and the Retention time of Cefdinir was 4.4 min. With an intention to reduce the Retention time of Cefdinir, the Flow rate was increased to 1.5 mL/min and the Retention time of Cefdinir was recorded to be 3.5 min. To still decrease the Retention time of Cefdinir, the flow rate was increased to 1.8 mL/min and the desired result of a very low Retention time of Cefdinir at 2.9 min was achieved with a good resolution of 12.754. The respective chromatograms are shown in Fig.5. Hence the Flow rate of 1.8 mL/min was selected for the experimental work. The ultimate method developed is summarised in the section 2.2 below.

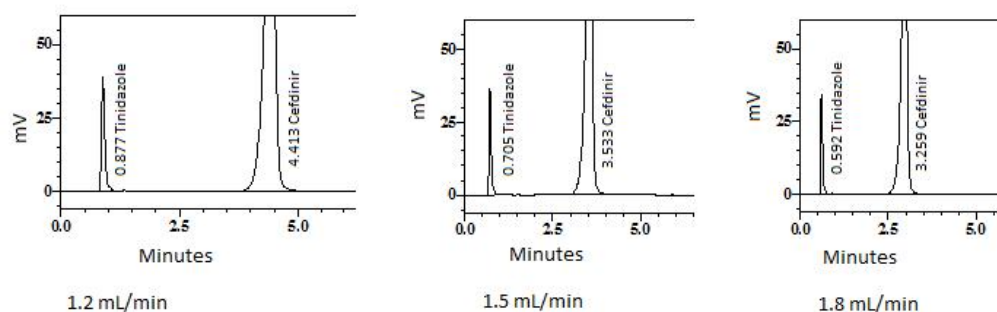


Fig.5. Different Flow rate chromatograms

2.2. Chromatographic conditions:

Various columns as well as mobile phase combinations have been tried to develop the desired method. Finally we have been able to work out an isocratic method which can be adopted very easily by any analyst. The chromatographic conditions which has been optimised was on stationary phase of 5u C18(2) column with dimension of 100mm L x 4.6mm D, 5µm. Mobile phase was prepared with Buffer and Methanol(80:20). Buffer was prepared by dissolving 0.025M of Potassium dihydrogen orthophosphate in 1000 ml of water. The pH was adjusted to 2.5 with Orthophosphoric acid. Injection volume was 10µL. The pump flow rate was 1.8 mL/min. The column temperature was maintained at room temperature (~ 27°C). The eluent was detected at 290 nm.

2.3. Materials, Reagents and Chemicals:

Cefdinir and Tinidazole Standards were obtained from Startech Labs. Tablets used for the experiment, Cefdiel was manufactured by Ranbaxy laboratories, Adcef DT was manufactured by Torrent pharmaceuticals. HPLC grade Methanol, Potassium dihydrogen Orthophosphate and Orthophosphoric acid were obtained from Merck, Darmstadt, Germany.

2.4. Equipments:

UV Visible spectrophotometer used was Shimadzu, model UV-2450. The HPLC instrument used was Shimadzu make, Model LC 2010 CHT. Class VP Software was used for data acquisition.

2.5. Preparation of Standard solution:

Stock solution of Cefdinir was prepared by taking 25 mg of Cefdinir in 25mL volumetric flask and diluted upto the mark with mobile phase. Standard solution of 0.1 mg/mL of Cefdinir (treat this as 100 % for various experimental purpose) was prepared by taking 1mL of Cefdinir stock solution in 10mL volumetric flask and dilute upto the mark with mobile phase. Stock solution of Tinidazole was prepared by taking 25mg of Tinidazole in 25mL volumetric flask and dilute upto the mark with mobile phase. Standard solution of 0.1 mg/mL of Tinidazole (treat this as 100 % for various experimental purpose) was prepared by taking 1mL of Tinidazole stock solution in 10mL volumetric flask and diluted upto the mark with mobile phase.

2.6. Preparation of Linearity solutions:

For Linearity 150%, 125%, 100%, 75%, 50% & 25% solutions were prepared. 150% Solution was prepared by dissolving 15 mL of each of Cefdinir and Tinidazole stock solution in 100mL volumetric flask and diluted upto the mark with mobile phase. 20.83 mL of 150% solution was taken in 25 mL volumetric flask and diluted upto the mark with mobile phase for preparing 125% solution. 16.7mL of 150% solution was taken in 25 mL volumetric flask and diluted upto the mark with mobile phase for

preparing 100% solution. 12.5mL of 150% solution was taken in 25 mL volumetric flask and diluted upto the mark with mobile phase for preparing 75% solution. 8.3mL of 150% solution was taken in 25 mL volumetric flask and diluted upto the mark with mobile phase for preparing 50% solution. 4.2mL of 150% solution was taken in 25 mL volumetric flask and diluted upto the mark with mobile phase for preparing 25% solution.

2.7. Preparation of Accuracy Solution:

Solution was prepared by taking 25mg of Cefdinir standard in 25mL volumetric flask and diluted upto the mark with mobile phase. Further 1mL of the above solution and 1mL Tinidazole standard stock solution was taken in 25mL volumetric flask and diluted upto the mark with 50%, 100% & 150 % linearity solutions for getting the respective spiked solution.

2.8. Preparation of Sample solution for Batch Analysis:

The method was applied to the analysis of two commercial samples available in the market, Cefdiel, Adcef DT. The average weight of ten tablets was calculated and found to be 415.86mg and 259.58mg of Cefdiel and Adcef DT respectively. The tablet was crushed to a homogeneous mixture and equivalent to 10 mg of Cefdinir in 100mL volumetric flask and diluted upto the mark with mobile phase and 10 mg of Tinidazole in 100mL volumetric flask and diluted upto the mark with mobile phase. 14.36mg and 22.28mg of the tablets Cefdiel, and Adcef DT respectively were dissolved in 100mL of the mobile phase. The solutions were sonicated for 5 minutes followed by cyclomixing for 5 minutes to extract the drug in solution. The resulting solutions were filtered by using Millipore syringe filter (0.42µ). The resulting clear solutions were injected in HPLC in duplicate as per the developed method.

2.9. Analytical Method Validation

2.9.1. Specificity of the method:

The parameter, specificity of the method was performed to know the Retention Time of Cefdinir in the presence of internal standard Tinidazole, in standard and in the sample.

2.9.2. System Precision:

System Precision (System Suitability) test is used to verify if the resolution and reproducibility of the chromatographic systems are adequate for the analysis to be done. The tests are based on the fact that the equipment, electronics, samples to be analyzed constitutes an integral system that can be evaluated as such. The limits for system suitability were set for Theoretical plates, Resolution, Asymmetry.

2.9.3. Linearity:

To measure linearity of the method, concentrations of the standard mixture, 25%, 50%, 75%, 100%, 125% and 150% were prepared and injected and chromatogram was recorded. A graph was plotted for the concentration of the corresponding drug versus Area. The Correlation coefficient(r) for Cefdinir and internal standard, Tinidazole was calculated.

2.9.4. Accuracy:

It is very important that the developed method is accurate, which is evaluated by the recovery of the drugs. To determine the recovery in sample preparation method of standard additions was made for measuring the recovery of the drugs. To the known standard concentrations (0.04mg/ml) of the drug, 50%, 100% and 150% of Cefdinir was added. Three different solutions were prepared as mentioned above under sample preparation for Accuracy. The accuracy was expressed as the percentage of Cefdinir recovery.

2.9.5. Robustness:

Robustness of the method has been checked by obtaining chromatograms with slight changes in the method. Changes were made in the Flow rate, Temperature and mobile phase composition and the analysis was done. The flow rate as per the developed method is 1.8mL/min. This has been purposely changed to 1.6mL/min and 2mL/min and the chromatogram was obtained. The temperature as per the developed method is 27°C. This was changed purposely to 24°C and 30°C and the

chromatogram was obtained. The mobile phase composition as per the developed method is 80:20. This has been purposely changed to 75:25 and 85:15 and the chromatogram was obtained.

2.9.6. Ruggedness:

In order to demonstrate the Ruggedness, analysis was performed on different days and different chemists and the chromatograms were obtained and checked for any variation in the results. The percentage RSD for the retention time and area was calculated.

2.9.7. Performance of the method on Commercial Samples:

As a final verification of the method, the method was applied to the analysis of drug in commercial tablets. For this purpose, performance test of the method has been conducted on the market samples, Cefdiel, Batch No.2417457, manufactured by Ranbaxy Laboratories, Adcef DT, Batch No.Y6771001, manufactured by Torrent pharmaceuticals.

3. RESULTS AND DISCUSSION

The present work involves estimation of Cefdinir in bulk and dosage forms using Reverse Phase HPLC in the presence of an internal standard Tinidazole. The method was developed and optimised in such a way that the product can be analysed rapidly using a simple methodology, which results in saving sophisticated instruments and chemist's valuable time. This is the reason that currently UFLC (Ultra-Fast Liquid Chromatography) technique [10,11] is gaining a lot of importance due to its high speed of analysis, though most of the Pharmacopeia still have the HPLC methods in their monographs. This time, we have tried to use Tinidazole as an internal standard. The benefit of using internal standard in the analytical method is that it helps the analyst in attaining the consistency in the end result. The current analytical method is rapid and encouraging. The developed method has been validated as per the ICH guidelines. Findings of Validation parameters are expressed in following lines.

3.1. Specificity of the method:

The Retention times of the drug Cefdinir and the internal standard Tinidazole were measured individually and found to be 2.975 min and 0.592 min, respectively. The retention time of a mix solution of Cefdinir and internal standard Tinidazole was measured and found to be 2.915 min and 0.592 min, respectively. The retention time of Cefdinir and Tinidazole was found to be 2.695 min and 0.592 min, respectively for the sample solution (market sample in the form of Tablet spiked with the internal standard). This shows that there is no specific change in the retention time of the drug individually or in the presence of an internal standard in bulk and dosage forms. This indicates that there is no any interference of the internal standard or the excipient and hence the method can be used for the determination of Cefdinir in the presence of an internal standard; both in bulk and pharmaceutical dosage forms. Respective HPLC chromatograms are represented in the Fig. 6, 7 and 8.

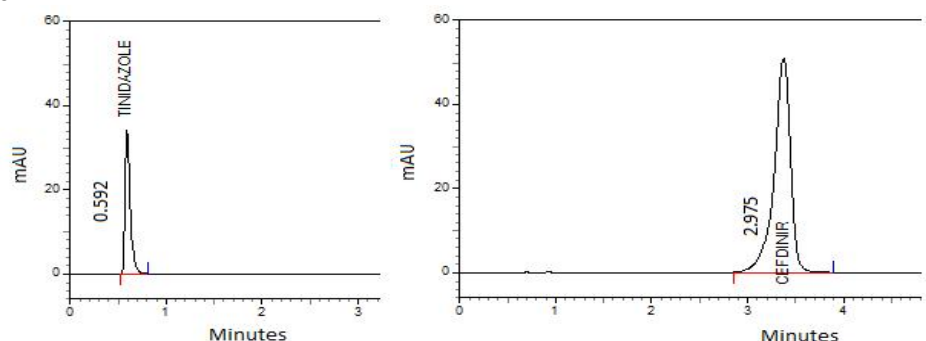


Fig.6. Chromatograms of Cefdinir and Tinidazole individually

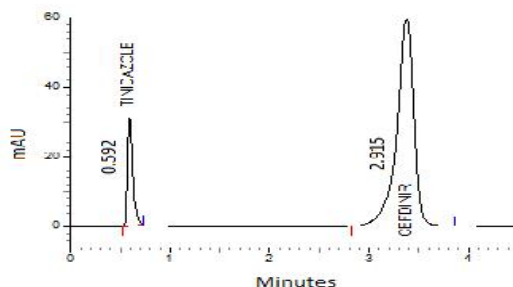


Fig.7. Chromatogram of Standard Cefdinir solution containing Tinidazole as an internal standard

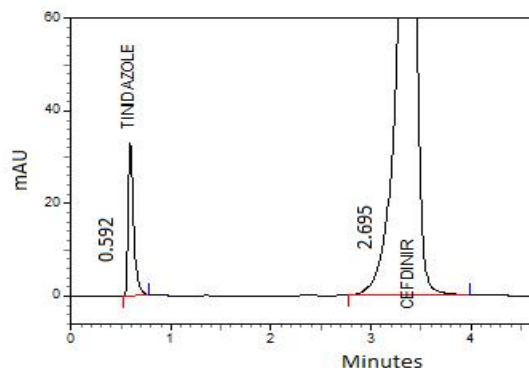


Fig.8. Chromatogram of market Sample of Cefdinir spiked with Tinidazole as an internal standard

3.2. System Precision:

For measuring system precision, five injections of the standard mix were injected and the respective chromatograms were obtained. The Retention time, Areas, Resolution, Theoretical plates and peak Asymmetry were calculated. Percentage RSD value was calculated. All the values have been found to be well within the acceptable range. The results obtained are given in following Table 1.

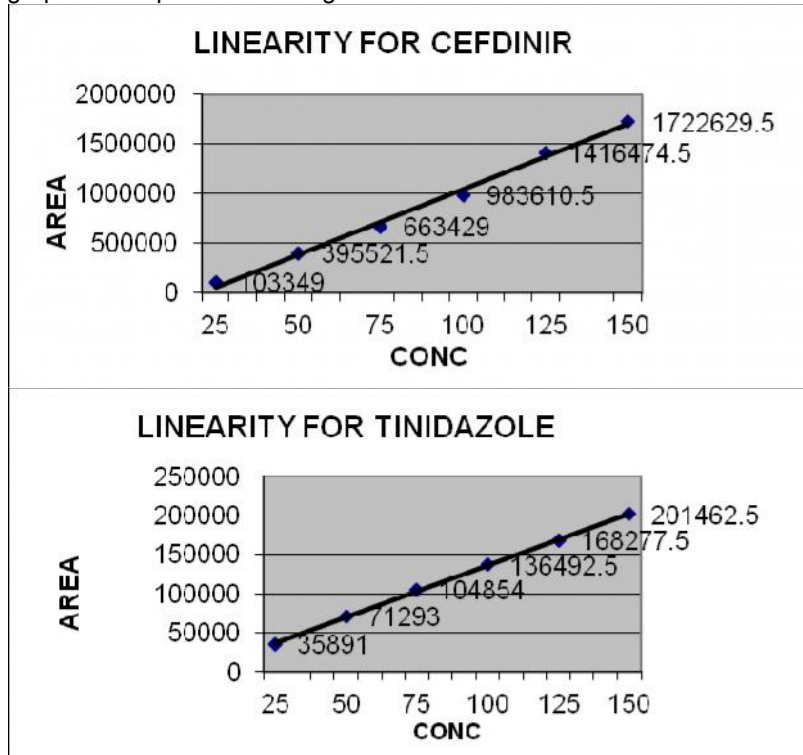
Table 1. System Precision Results

		Average	SD	%RSD
Cefdinir	Retention Time	3.3686	0.004	0.11
	Area	670419	3440.19	0.51
	Theoretical plates	2157.43	15.96	0.74
	Resolution	13.948	0.06	0.43
	Asymmetry	0.798	0.004	0.56
Tinidazole	Retention Time	0.59	0.004	0.76
	Area	120369	51.93	0.04
	Theoretical plates	1480.09	7.024	0.47
	Resolution	0	0	0
	Asymmetry	1.556	0.03	1.74

3.3. Linearity:

Linearity of an analytical method is the ability of the method to elicit test results that are directly proportional to the concentration of the analyte. The concentration of both the analytes were found to be proportional to the area and the response of the detector. The correlation coefficient (r) obtained was calculated and it was found to be greater than 0.99 for Cefdinir and Tinidazole, which is well

257 within the acceptance range. The response was found to be linear over the range of 0.025mg/mL to
 258 0.15mg/mL for Cefdinir and Tinidazole. The results are shown in Table 2 and respective linearity
 259 graphs are represented in Fig.9.



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 262 **Fig. 9. Graphs Showing Linearity of Cefdinir and Tinidazole**

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 265 **Table 2. Linearity results**

	Linearity Range	Correlation Coefficient
Cefdinir	0.025-0.15mg/mL	0.9998
Tinidazole	0.025-0.15mg/mL	0.9971

3.4. Accuracy:
For measuring the accuracy of the method, 3 spiked solutions have been prepared as mentioned in Materials and method section under the heading Preparation for accuracy solution. Chromatograms were obtained for all the three spiked solutions and recoveries have been calculated. As per the ICH guidelines, the acceptable range of recovery should be between 80-120%. The results obtained were in the range of 99.02 and 104.77, which indicates that the recoveries are well within the acceptance range. Therefore, the method is accurate and it can be used for the estimation of Cefdinir. Details are presented in Table 3.

Table 3. Results for Accuracy of the method

Accuracy at---→	50 %	100 %	150 %
Area (Known Conc _n + spiked)(a)	2056132	2530138	3522350
Area Cefdinir Known Conc (b)	1674528	1674528	1674528
Area Cefdinir (a-b)	381604	855610	1847822
Cefdinir Area (expected)	364792	864055	1763705
% Recovery (a-b)x100/c	104.61	99.02	104.77

3.5. Robustness:
Due to deliberate change in the flow rate, Temperature and mobile phase composition excellent performance of the method was observed. This indicates that the method is Robust. The results obtained are shown in Table 4.

Table 4. Robustness results

			Retention time %RSD	Area %RSD
Different Flow Rates	1.6mL/min	Cefdinir	0.04	0.09
		Tinidazole	0.68	0.05
	2.0mL/min	Cefdinir	0.03	0.34
		Tinidazole	0.08	0.21
Different Temperatures	24 ⁰ C	Cefdinir	0.15	0.1
		Tinidazole	0.6	0.9
	30 ⁰ C	Cefdinir	0.01	0.34
		Tinidazole	0.08	0.21
Different Mobile Phase Compositions	75:25	Cefdinir	0.07	0.99
		Tinidazole	0.88	0.2
	85:15	Cefdinir	0.05	0.24
		Tinidazole	0.29	0.14

3.6. Ruggedness:
Analysis performed on different days and by different analysts . Data evaluated and % RSD of Area and Retention time were calculated for various trials and data obtained are tabulated in Table 5. Based on the data it is evident that the method is Rugged.

292 **Table 5. % RSD on different days and different Analysts**
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		Standard %RSD	
		Retention Time	Area
DAY 1	Cefdinir	0.17	0.81
	Tinidazole	0.87	0.9
DAY 2	Cefdinir	0.51	0.02
	Tinidazole	1.16	0.4
ANALYST 1	Cefdinir	0.25	0.97
	Tinidazole	0.77	0.72
ANALYST 2	Cefdinir	0.28	0.08
	Tinidazole	0.66	1.12

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3.7. Performance of the Method on Commercial Formulations:

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For evaluating the performance of the method, two market samples have been taken namely Cefdiel, Adcef DT. As per the label claim, Cefdiel capsules contain 300mg of Cefdinir and Adcef DT tablet contain 125 mg of Cefdinir. The quantification of the drug was done in the two formulations and the results have been found to be in the range of 99.8 to 100.3 %. This indicates that the method developed by us can be used for the estimation of Cefdinir in any of the pharmaceutical dosage forms. The results obtained are shown in Table 6.

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Table 6. Estimation of Cefdinir in commercial samples

	Label claim	Acquired data	% Recovery
Cefdiel	300mg/cap	309.43mg/cap	103.14%
Adcef DT	125mg/tab	124.29mg/tab	99.43%

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4. CONCLUSION

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The HPLC method developed in the present work is sensitive, accurate, unique, rapid, precise, user friendly and reproducible for the estimation of Cefdinir using Tinidazole as an internal standard in bulk mix and Pharmaceutical dosage forms. The method was validated as per ICH Guidelines. Hence, it is conclude that, this method which is very fast can be used by the industries and academic institutions for the estimation of Cefdinir.

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