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Hydrotropy – A Solubility Enhancement Tool for the Estimation of Cefdinir in its Suspension Dosage Form by UV-Spectroscopy

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Abstract : Present work describes development and validation of a simple, novel, accurate, precise, economical and reproducible spectrophotometric method in ultraviolet region for the assay of Cefdinir in suspension formulation using sodium bicarbonate and distilled water (1:9) as hydrotropic solvent. Cefdinir exhibits absorption maxima at 287nm in hydrotropic solvent. Beer's law was found to be obeyed in the concentration range of 2.5-17.5µg/ml. The developed method was validated as per the ICH guidelines. The calibration plot was linear over the concentration range investigated (2.5–17.5µg/ml) for Cefdinir in hydrotropic solvent with correlation coefficient, r^2, 0.99903. The method is accurate, precise and economical. In this proposed method, there was no interference from common pharmaceutical excipients. The proposed method is therefore successfully used for the routine analysis of the Cefdinir in its suspension dosage form.
Keywords : Cefdinir, Hydrotropy, UV-Spectroscopic method, Validation, ICH.

Introduction

Pharmaceutical analysis plays an important role right from testing of raw materials, in process quality checks to the analysis of finished products. Pharmaceutical analysis is considered to determine identity, strength, quality and purity of drug samples [1-2]. The aqueous solubility of insoluble and slightly soluble drugs has been increased by various methods to avoid the usage of organic solvents. Among those techniques Hydrotropy is the one used for enhancement in solubility of insoluble solute in water by adding the agent called as hydrotrope. Hydrotropes are micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. The formation of molecular structure in the form of complexes can be reason for the solubility enhancement [3-4].

Chemically Cefdinir is 5-thia-1-aza bicyclo (4.2.0) oct-2-ene-2-carboxylic acid (Fig. 1). It is a semi-synthetic, broad-spectrum, third-generation cephalosporin. The molecular formula of Cefdinir is ($C_{14}H_{13}N_5O_5S_2$) with a molecular weight of 395.42g/mole [5-6]. It has a broad spectrum of activity, good therapeutic action against susceptible Gram-positive and Gram- negative bacteria having positive microbial

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activity, excellent efficacy, convenient dosing and favourable tolerability correlated with other antimicrobial agents [7-10]. A simple inexpensive, selective, and rugged UV spectrophotometric method was developed. The methods reported in the literature for the analysis of cefdinir in its formulations areUV spectrophotometry (AUC method), Stability-Indicating Spectrophotometric Method, Difference Spectroscopic and Reverse Phase HPLC Method [11-14] and there was no method using hydrotropic solvents for the estimation of cefdinir. As cefdinir is poorly water soluble drug, its solubility is enhanced by applying one of the solubility enhancement techniques known as Hydrotropy. Hence, the present study aimed to develop a simple, precise and economical UV- Spectrophotometric method in its suspension dosage form by hydrotropy.

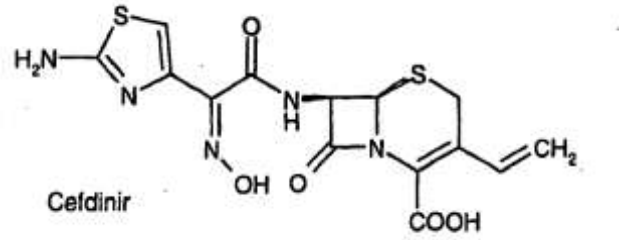

Fig. 1 Chemical Structure of Cefdinir

Experimental Work

Materials

Cefdinir working standard was obtained as gift sample from Aurobindo Pharma Limited, Hyderabad. A Suspension formulation (Sefdin) containing 125mg/5ml was purchased from local pharmacy. An analytical grade solvent Sodium bicarbonate -0.2M was used as Hydrotropic solvent for the present work.

Instruments

A Double beam UV-Visible spectrophotometer (UV-1800, Shimadzu) connected to computer loaded with spectra manager software i.e. UV Probe 2.34 with 1cm quartz cells were used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-350 nm; scan speed: medium; sampling interval: 1.0nm; band width (Δλ):10.0 nm; spectral slit width: 1 nm. All weights were taken on Electronic Weighing balance – single pan, Model Axis LC/GC.

UV Method Development:

The following steps were conducted to developUV method for Cefdinir.

1. The solubility studies are performed to dissolve the drug in various Hydrotropic Reagents
2. λ_{max} determination
3. Optimization of concentration of drug

The solubility studies of drug

10mg of Cefdinir working standard was weighed and solubility waschecked in 10 ml water, 1M potassium acetate, 1M sodium acetate, 1M urea, 1M potassium citrate, 1M sodium citrate, 1M sodium benzoate and 0.2M sodium bicarbonate. The drug was found to be freely soluble in 0.2M sodium bicarbonate, but poorly soluble in water, and other selected solvents. Therefore sodium bicarbonate and water (10:90 % v/v) was selected as diluent as they are freely available and drug was also found to be stable for 24 hours in stability studies.

λ_{max} determination and Optimization of concentration of drug

Preparation of standard stock solution:

Weighed accurately about 100mg of Cefdinir working standard in a 100 ml volumetric flask. Then added 10ml of 0.2M sodium bicarbonate and 90ml of distilled water to get a concentration of 1mg/ml. Initial dilution was made by pipetting 1 ml of primary stock solution into 10 ml volumetric flask and make up to the volume with distilled water. Further dilution was made by pipetting 1ml from second solution to obtain a concentration of 10µg/ml. The optimized concentration of Cefdinir standard was 10µg/ml. The solution was scanned in UV region in the wavelength range from 200 to 350 nm and λ_{max} was optimized at 287 nm.

Standard calibration curve was constructed for Cefdinir in hydrotropic solvent. A series of aliquots were prepared from the above stock solutions using diluent to get the concentration 2.5–17.5µg/ml and analysed 6 times in UV-1800. Calibration curves are constructing as by taking average absorbance on Y-axis and concentration on X-axis. Regression equation was calculated from the calibration curves, and it is used to calculate drug substance in formulation.

Estimation of Cefdinir in suspension dosage forms:

Commercially available Cefdinirsuspension equivalent to 100mg of cefdinir was taken in 100ml volumetric flask. To it, 10ml of 0.2M sodium bicarbonate was added. Mixed it well and left the solution for 15 min, then 90ml of distilled water added and filtered through Whatsmann filter paper #41. From the above stock solutioninitial dilution was made by pipetting 1 ml into 10 ml volumetric flask and made up to the volume with distilled water. Further dilution was made by pipetting 1ml from second solution to obtain a concentration of 10µg/ml. The optimized conc. of Cefdinir Suspension was 10µg/ml. The solution was scanned in UV region in the wavelength range from 200 to 350 nm and λ_{max} was optimized at 287 nm.

UV Method Validation:

The method was validated according to ICH guidelines. [15-17]

Precision:

System precision:

The system precision was performed by analyzing a standard solution of Cefdinir (10µg/ml) at working concentration level for 6 times.

Acceptance criteria:

The percentage relative standard deviation values should be less than 2.0

Method precision:

The method precision was performed by analysing a sample solution of Cefdinir at working concentration level for 6 times.

Acceptance criteria:

The percentage relative standard deviation values should be less than 2.0

Linearity and range:

The linearity of an analytical method is its ability (within a given range) to produce the test results which are directly proportional to the concentration (amount) of analyte in the samples within a given range.A calibration curve was plotted between concentration and absorbance. The linearity was performed in various

concentrations ranging from 2.5, 5.0, 7.5, 10, 12.5, 15 and 17.5µg/ml.Cefdinir was linear with the concentration range of 2.5-17.5 µg/ml in Distilled water at 287 nm.

Acceptance criteria : Regression coefficient is not less than 0.995.

LOD and LOQ:

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or on an accepted reference value and the value found.

The determination of content of Cefdinir was performed at three levels by adding the calculated amount of Cefdinir. The sample was prepared in triplicate (9 determinations) i.e. 50%, 100%, 150% of the working concentrations of the method.

Concentration-50%:

A requisite volume of the drug equivalent to 100mg was transferred into a 100 ml volumetric flask (1mg/ml). Initial dilution was made by pipetting 1 ml of mother liquor into 10 ml volumetric flask and make up to the volume with distilled water. Further dilution was made by taking 5 ml of mother liquor into 100 ml volumetric flask and make up to the volume with solvent. The final conc. of Cefdinir sample was 5µg/ml. The solutions were scanned in UV region in the wavelength range from 200 to 350 nm.

Concentration-100%:

A requisite volume of the drug equivalent to 100mg was transferred into a 100 ml volumetric flask (1mg/ml). Initial dilution was made by pipetting 1 ml of mother liquor into 10 ml volumetric flask and make up to the volume with distilled water. Further dilution was made by taking10ml of mother liquor into 100 ml volumetric flask and make up to the volume with solvent. The final conc. of Cefdinir sample was 10µg/ml. The solutions were scanned in UV region in the wavelength range from 200 to 350 nm.

Concentration-150%:

A requisite volume of the drug equivalent to 100mg was transferred into a 100 ml volumetric flask (1mg/ml). Initial dilution was made by pipetting 1 ml of mother liquor into 10 ml volumetric flask and make up to the volume with distilled water. Further dilution was made by taking 15 ml of mother liquor into 100 ml volumetric flask and make up to the volume with solvent. The final conc. of Cefdinir sample was 15µg/ml. The solutions were scanned in UV region in the wavelength range from 200 to 350 nm.

Acceptance criteria:98% to 102% recovery.

Specificity:

Ability of the method to measure accurately and specifically the analyte of interest in presence of matrix and other components likely to be present in the sample matrix and impurities, degradation products and other related substances. For this, one may compare the test results of analysis of samples containing other ingredients/ impurities / degradation products / related substances/placebo ingredients with those obtained from analysis of sample without these, i.e., the method must allow distinct analytical measurement of analyte of interest and exclusion of all other relevant interferences.

The specificity of the method is established by known concentration of Cefdinir is taken in different solvents and estimated as per analytical method.

Acceptance criteria:

The percentage relative standard deviation values should be less than 2.0

Robustness:

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters.

λ max variation:

Actual: 287 for Cefdinir in $NaHCO_3$ and distilled water (1:9)
Low : 285 for Cefdinir in $NaHCO_3$ and distilled water (1:9)
High : 289 for Cefdinir in $NaHCO_3$ and distilled water (1:9)

Acceptance criteria:

The percentage relative standard deviation values should be less than 2.0

Results and Discussion

The sample and standard solutions of 10µg/ml of Cefdinir in Sodium bicarbonate (0.2M) and Distilled water in the ratio of 1:9 was prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 350 nm by using Distilled Water as blank and the standard and sample spectrum of Cefdinir was shown in Fig. 2 and 3. From the spectrum 287nm was selected for the estimation of Cefdinir without any interference. The optimum concentration of the Cefdinir was found to be 10µg/ml. This concentration of Cefdinir was shown good absorbance valve at 287nm wavelength was found to be 0.5087.

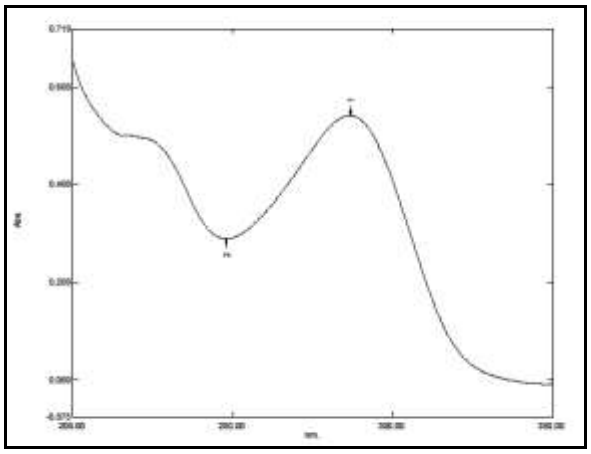

Fig. 2 UV Spectrum of Cefdinir Standard

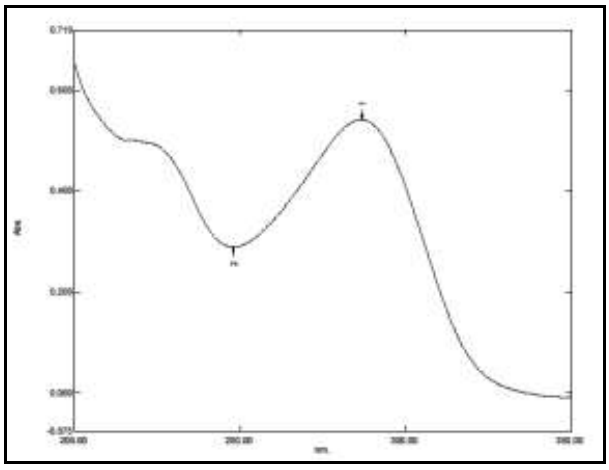

Fig. 3 UV Spectrum of Cefdinir Sample (Suspension)

Method Validation

Precision:

The precision was performed by analyzing standard and sample solutions of Cefdinir (10µg/ml) at working concentration level for 6 times. The % RSD value of system precision and method precision were found to be 0.00023 and 0.000294 respectively. The results were shown in Table No. 1 and 2.The results showed that the precision of the method was confirmed.

Table No.1 Data of System Precision Study

S. No	Absorbance Cefdinir Standard
1	0.5087
2	0.5086
3	0.5087
4	0.5085
5	0.5084
6	0.5086
Mean	0.50858
SD	0.000117
% RSD	0.00023

Table No.2 Data of Method Precision Study

S. No	Sample Absorbance values at 287 nm	Percentage label claim (%w/w)
1	0.542	102.38
2	0.5423	102.43
3	0.5424	102.45
4	0.5421	102.4
5	0.542	102.38
6	0.542	102.38
Mean	0.5421	102.4
SD	0.00018	0.03011
% RSD	0.00032	0.00029

Table No.3 Intra-Day precision data for Cefdinir (%w/w)

S. No	Analyst-1	Analyst-2
1.	102.38	102.43
2.	102.4	102.4
3.	102.41	102.38
4.	102.38	102.41
5.	102.45	102.57
6.	102.38	102.41
Mean	102.4	102.43
SD	0.027568	0.068896
%RSD	0.000269	0.000673

Table No.4 Inter-Day precision data for Cefdinir (%w/w)

S. No	Day-1	Day-2
1.	102.38	102.45
2.	102.49	102.4
3.	102.43	102.41
4.	102.4	102.43
5.	102.43	102.45
6.	102.41	102.43
Mean	102.42	102.42
SD	0.037771	0.020412
%RSD	0.000369	0.000199

Intermediate Precision:

Intermediate precision of the method was confirmed by intra-day and inter-day analysis. The analysis of formulation was carried out for Six times in the same day by two different analysts and Six times in the two consecutive days respectively. The % RSD value of intraday analysis were found to be 0.000269 (Analyst-1) and 0.000673 (Analyst-2). The % RSD value of inter-day analysis were found to be 0.000369 (Day-1), and 0.000199 (Day-2). The results were shown in Table No.3 and 4.

Linearity and Range:

Different aliquots of Cefdinir in sodium bicarbonate (0.2M) and distilled water in the ratio of 1:9 were prepared in the concentration range of 2.5-17.5µg/ml. The absorbance values of solutions were measured at 287 nm and shown in Table No 5. Overlay of standard spectrum of Cefdinir for linearity study shown in Fig. 4. The calibration curve was plotted using concentration against absorbance. The calibration graph at 287 nm for Cefdinir was shown in Fig.5. The preparation of calibration curve was repeated for six times for each solution at their selective wavelengths. The correlation coefficient for the drug solutions of different solvents were found to be above 0.99903. This indicates that all the drug solutions obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear.

Table No.5 Standard Calibration curve data for Cefdinir

Concentration (µg/ml)	Absorbance
0	0.000
2.5	0.160
5.0	0.272
7.5	0.392
10	0.509
12.5	0.654
15.0	0.755
17.5	0.884

Table No.6 Evaluation data of Accuracy study of Cefdinir

% Recovery Level	Actual amount (mcg/ml)	Amount added (mcg/ml)	Total Amount (mcg/ml)	Amount recovered	% Recovery
50%	10	2.5	12.5	12.3	98.4
	10	2.5	12.5	12.4	99.2
	10	2.5	12.5	12.4	99.2
100%	10	5.0	15.0	14.7	98
	10	5.0	15.0	14.8	98.6
	10	5.0	15.0	14.9	99.3
150%	10	7.5	17.5	17.3	98.8
	10	7.5	17.5	17.4	99.4
	10	7.5	17.5	17.4	99.4

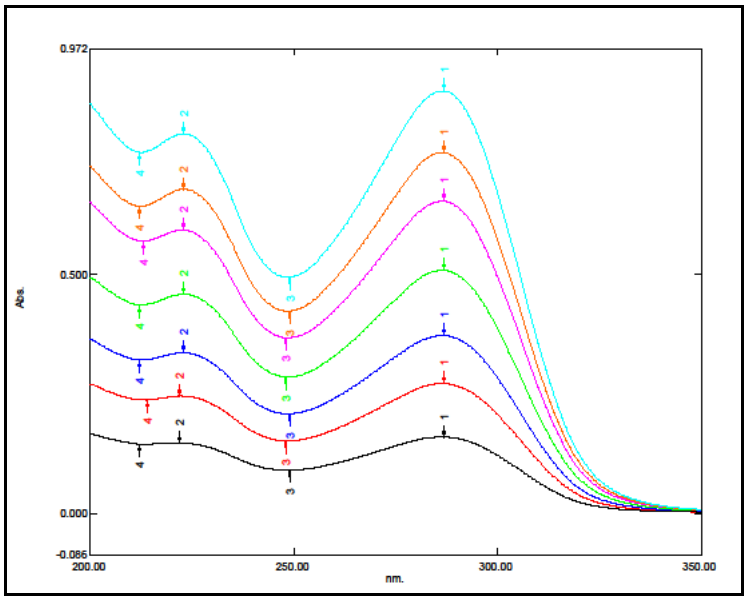

Fig.4 Overlay of standard Spectrum of Cefdinir for Linearity Study

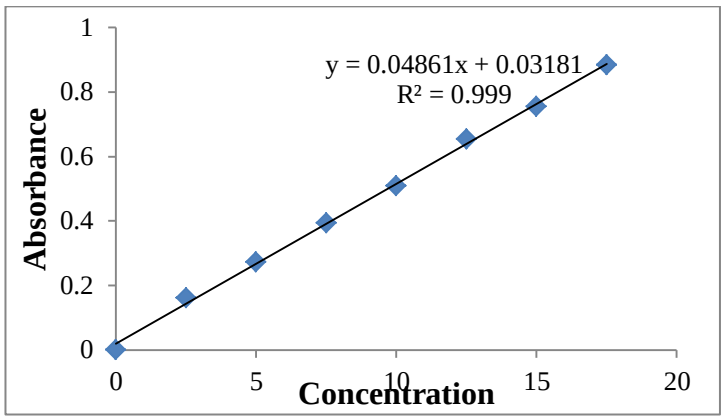

Fig. No. 5 Calibration curve for Cefdinir

Accuracy:

The accuracy of the method was performed by recovery studies. A known quantity of Cefdinir raw material solutions were added at three different levels (50, 100 and 150%). The absorbance of the solutions

were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 98 – 99.4%w/w. The low % RSD value of drug solutions indicates that this method is very accurate. The recovery data was shown in Table No 6.

Robustness:

The Robustness was performed at different wavelength by using sample solutions of Cefdinir. The % RSD values for wavelength variation were found to be 0.000149, 0.000149 and 0.000739 respectively. The low % RSD values indicate that the developed method was more rugged. The results were shown in Table No 7.

Table No.7 Robustness data for wavelength variation

S. No	Actual (287nm)	Lower (285nm)	Upper (289nm)
1.	102.4	102.43	102.45
2.	102.38	102.4	102.43
3.	102.41	102.41	102.57
Mean	102.39	102.41	102.48
SD	0.015275	0.015275	0.075719
% RSD	0.000149	0.000149	0.000739

Table No. 8 Optical Characteristics of Cefdinir

Parameters	Cefdinir in Hydrotropic Solvent (1:9)
Beers law limit (µg/ml)	2.5-17.5
Molar absorptivity (L mol^{-1} $cm^{-1)}$	2.1431x 10^4
Correlation coefficient (r^2)	0.99903
Regressionequation	y = 0.04861 x + 0.03181
Slope (m)	0.04861
Intercept (c)	0.03181
LOD (µg/ml)	0.01188
LOQ (µg/ml)	0.0360

The optical parameters like molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and standard error were calculated and results were shown in Table No 8.

Conclusion

The developed method for the quantitation of cefdinir in its suspension dosage form by using 0.2M sodium bicarbonate and distilled Water (1:9) as the hydrotropic agent was found to be the best alternative for estimation of poorly water-soluble drugs and to minimize the use of organic solvents. The proposed method utilized solution of non-toxic, non-volatile hydrotropic agent, which gives a novel, cost-effective, and environment-friendly method for the estimation of cefdinir in its Suspension dosage form.The present analytical method was validated as per ICH Q2 (R1) guideline and it meets to specific acceptance criteria. The statistical parameters and the recovery test data indicated the high reproducibility and accuracy of the proposed method. Analysis of authentic samples containing the studied drug showed no interference from common additives and auxiliary substances in general. It is concluded that the developed analytical method was simple, precise, linear,

accurate, robust, economic and having stability indicating characteristics. The proposed method was successfully used for the routine quality control analysis of the Cefdinir in marketed Suspension dosage forms.

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