Original Article

Sensitive spectroscopic method for content analysis of cefixime in solid dosage form using hydrotropy phenomenon

Abstract

Aim: The main aim of this present investigation is to apply the hydrotropic solubilization phenomenon for spectroscopic analysis of poorly water-soluble drugs to avoid the use organic solvents which may be costlier, toxic to environment, volatile, and pollutant. **Materials and Methods:** A simple ultra violet spectroscopic method was used for the content analysis by diluting the drug cefixime with various hydrotropic agents. In this study, 20% solutions of sodium salicylate (SS), sodium citrate (SC), sodium acetate (SA), and sodium benzoate (SB) were used as hydrotropes for the analysis of cefixime. **Results and Discussion:** The drug cefixime showed the linearity of 0.5–2 μg/mL in SS, 5–30 μg/mL in SC, 5–50 μg/mL in SA, and 0.05–0.30 μg/mL in SB solution. Then the proposed methods were validated with respect to accuracy and precision as per International Conference of Harmonization guidelines Q2 (R1), November 2005 (Validation of Analytical Procedures: Text and Methodology). The drug showed less limit of detection (LOD) and limit of quantification (LOQ) values (LOD = 0.0225471 μg/mL and LOQ 0.0683246 μg/mL) to SB solution and it obeyed the Beer's law at very low concentration range (0.05–0.30 μg/mL) which proved that the drug has high sensitivity with SB solution. **Conclusions:** Finally, it was concluded that the all proposed methods were simple, cost-effective, safe to environment, rapid, reproducible, and highly sensitive with SB solution.

Key words:
Economic, environment-friendly, hydrotropy, sensitive

Introduction

Cefixime, an antibiotic, is a third-generation cephalosporin like ceftriaxone and cefotaxime. Like all beta-lactam antibiotics, cefixime binds to specific penicillin-binding proteins located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cefixime is highly stable in the presence of beta-lactamase enzymes. As a result, many organisms resistant to penicillins and some cephalosporins due to the presence of beta-lactamases may be susceptible to cefixime. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall. It is chemically (6R, 7R)-7-[2-(2-Amino-1, 3-thiazol-4-yl)-2-[(carboxymethoxy) imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [Figure 1].

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To avoid the use of organic solvents to solubilize the water-insoluble drugs or poorly water soluble drugs, the hydrotropic solubilization method is mainly used.[1-3] Many high-performance liquid chromatographic methods are reported for estimation of cefixime in formulation and biological samples.[4-8] A high-performance thin layer chromatographic method also reported for estimation of ceftriaxone, cefixime, and cefotaxime in dosage forms.[9] From the extensive literature review, the spectro fluorimetric,[10] voltametric,[11] and kinetic spectrophotometric method[12] of estimation also reported.

Many literatures revealed that the concentrated solutions of a large number of hydrotropic agents have been used to enhance the aqueous solubility of many poorly water-soluble drugs and also used for its estimation in bulk

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and formulation.[13,14] Quantitative spectrophotometric determination of cefixime tablet formulation using sodium tartarate as hydtrotropic solubilizing agent is done by Maheshwari RK *et al*.[15] In this work, the sensitivity of the methods was compared using different hydrotropic solubilizing agents and the developed methods were validated as per International Conference of Harmonization guidelines.[16]

Hydrotropic solubilization is superior to other solubilization techniques, because the solvent character is independent of pH and does not require emulsification process to increase the miscibility. It does not require any chemical modification of hydrophobic drugs, but it requires only the mixing of drug with hydrotropes in water. The hydrotropes have both hydrophilic and hydrophobic parts, but the hydrotropic part is too small. So it does not cause self aggregation and also with hydrophobic drug.[17]

Materials and Methods

Preliminary solubility studies of reference standard (Cefixime) in different solvents

Solubility of cefixime was determined at room temperature. About 100 mg of drug was taken and solubility was determined in different aqueous system such as distilled water, 20% sodium salicylate (SS) solution, 20% sodium citrate (SC) solution, 20% sodium acetate (SA) solution, and 20% sodium benzoate (SB) solution.

Construction of standard calibration curve

The standard stock solution of cefixime (1 mg/mL) was prepared by dissolving in 20 mL solutions of 20% SS, 20% SC, 20% SA, and 20% SB individually and made up to 100 mL with distilled water. They were labelled as sodium salicylate stock solution (SS stock), sodium citrate stock (SC stock), sodium acetate stock solution (SA stock), and sodium benzoate stock solution (SB stock).

The SS stock was diluted further with distilled water to obtain the concentration range of 0.5–2 µg/mL. The SC stock was diluted appropriately with distilled water to obtain the linearity range of 5–30 µg/mL. The SA stock was diluted with distilled water to get the final concentration ranging from 5 to 50 µg/mL. The SB stock also diluted with distilled water to produce the concentration range from 0.05 to 0.30 µg/mL. One of the solutions of each batch was scanned between 200 and 400 nm individually. The recorded graphs were shown in Figures 2–5. The absorbances of the other diluted solutions were measured at their corresponding λ-max.

Analysis of cefixime tablet formulation by proposed method

Twenty tablets of cefixime marketed formulation were weighed and ground to fine powder. An accurately weighed powder sample equivalent to 100 mg of cefixime was transferred into four individual 100 mL volumetric flask. About 20 ml of 20% solutions of SS, SA, SC, and SB were added individually to above four flasks.

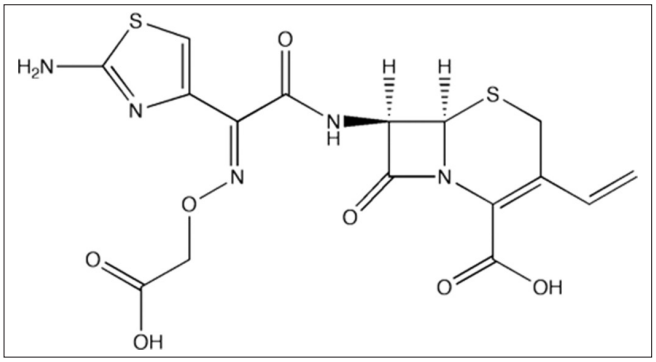

Figure 1: Chemical structure of cefixime

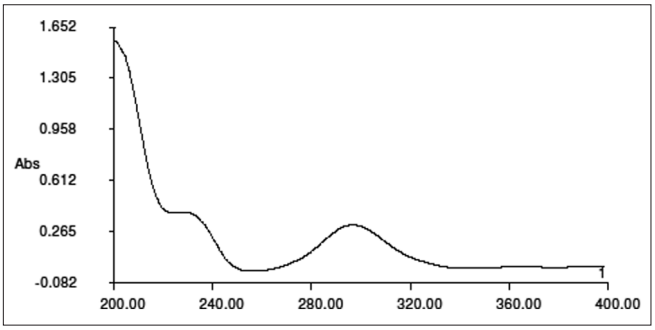

Figure 2: λ-max graph for cefixime in 20% SS solution, λ-max is obtained at 296 nm

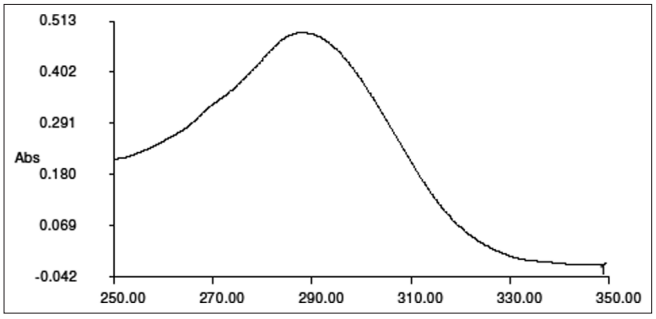

Figure 3: λ-max graph for cefixime in 20% SC solution, λ-max is obtained at 288 nm

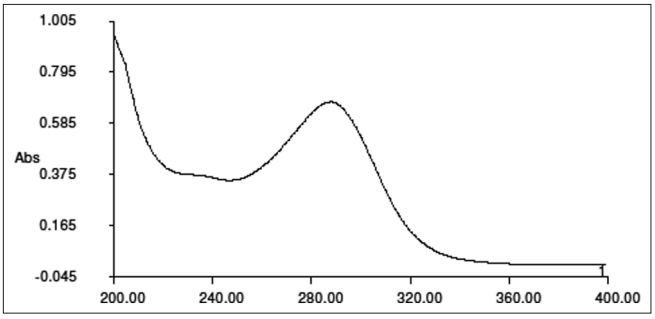

Figure 4: λ-max graph for cefixime in 20% SA solution, λ-max is obtained at 287 nm

All the flasks were shaken for about 20 minutes to solubilize the drug. Then they were kept in the sonicator for about 10 minutes to complete the dissolution. The volume was made up to the mark with distilled water and was filtered through Whatman filter paper No. 44. The above sample stock solutions were diluted individually with distilled water to get the concentration of 1 µg/mL for SS sample stock, 15 µg/mL for SC sample stock, 20 µg/mL for SA sample stock, and 0.2 µg/mL for SB sample stock. The absorbances were measured at respective λ-max and the drug contents of the tablet formulation were then calculated.

Validation of proposed method

Accuracy (Recovery studies)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed as trueness. Accuracy of the proposed method was determined by the recovery study experiments using standard addition method. To the accurately weighed tablet powder, the pure reference standards of respective drug were added at different levels (10%, 20%, and 30% level). These were further analyzed by the proposed method and the amount of drugs recovered from each level has been calculated.

Precision studies

The precision of an analytical procedure expresses the closeness of agreement (results) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. For repeatability, four determinations were made within 1 day at 100% level. For intermediate precision, same experiment was performed in 4 consecutive days at same concentration level. The mean, standard deviation, and percentage relative standard deviation (% RSD) were calculated.

Limit of detection and limit of quantification

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Results and Discussion

The results of solubility studies indicated that the solubility of cefixime in 20% SS solution, 20% SC solution, 20% SA solution, and 20% of SB solution were more than 65, 200, 140, and 75 times, respectively, when compared with its solubility in distilled water.

The calibration curve was found to be linear in the below mentioned concentration [Table 1] along with correlation coefficient (r^2) of 0.995–0.998. Regression equations and correlation coefficient of the curve were displayed on each graph [Figures 6-9]. Marketed cefixime tablet formulations were analyzed for percentage content using the above said hydrotropic solutions by proposed method. The results are tabulated on Table 2.

Recovery studies were performed by adding different level

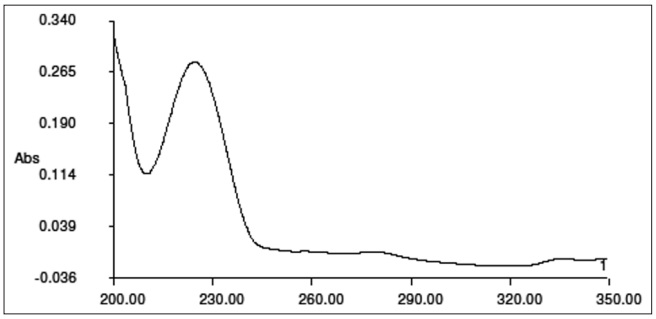

Figure 5: λ-max graph for cefixime in 20% SB solution, λ-max is obtained at 224 nm

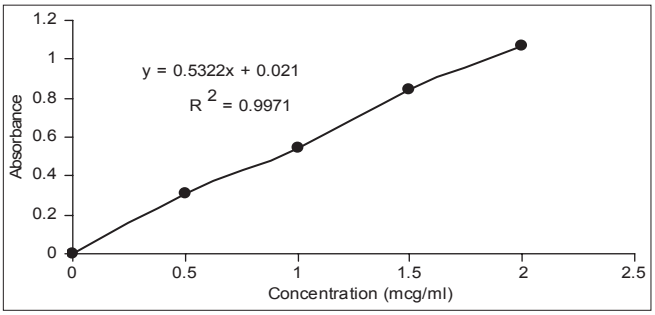

Figure 6: Calibration curve for cefixime in 20% SS solution

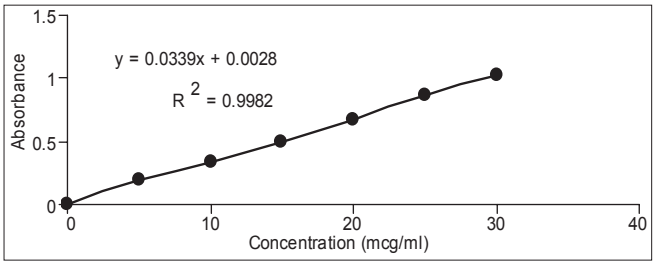

Figure 7: Calibration curve for cefixime in 20% SC solution

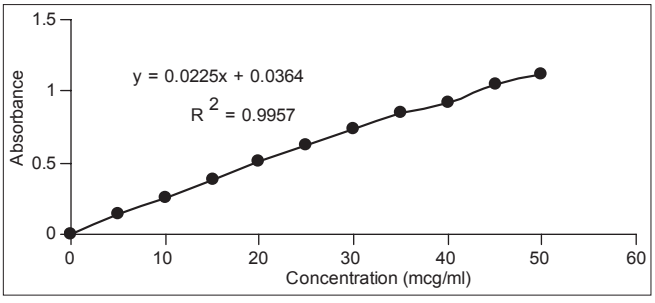

Figure 8: Calibration curve for cefixime in 20% SA solution

of pure reference standard (10%, 20%, and 30%) to the pre-analyzed tablet powder and followed the same method of analysis. All types of analysis were performed in triplicate, and the results are tabulated in Table 3.

For repeatability (*intraday* precision) study, cefixime reference standard and formulations were analyzed as per proposed method at short interval of time (i.e., four times within the day). For intermediate (*interday)* precision study, analysis was carried out with same cefixime reference standard and tablet formulations for 4 conservative days. The results were tabulated [Table 4] and percentages RSD were calculated.

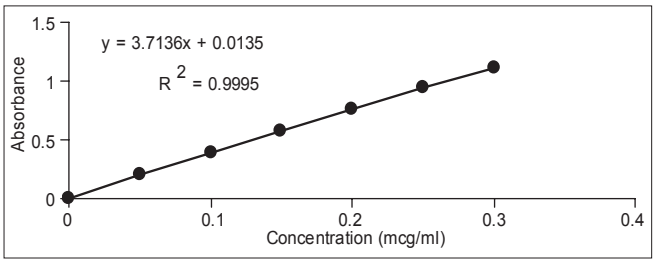

Figure 9: Calibration curve for cefixime in 20% SB solution, Y = Mx + C = regression equation, R^2 = correlation co-efficient

Conclusion

From the solubility determination, it was concluded that the aqueous solubility of cefixime was increased more than 50 times for 20% SS and 20% SB solution, and more than 100 times for 20% SC and SA solution when compared with distilled water. It seems from the results that the amount of drug estimated by the proposed method using various hydrotropic agents and in recovery studies is very close to 100%, indicating the accuracy of the proposed method. Low

Table 1: Parameters for the calibration curve

Parameters	SS solution	SC solution	SA solution	SB solution
λ-max (nm)	296	288	287	224
Linearity (μg/mL)	0.5–2	5–30	5–50	0.05–0.30
Regression equation	Y = 0.532x + 0.021	Y = 0.033x + 0.002	Y = 0.022x + 0.036	Y = 3.713x + 0.013
Correlation coefficient	0.997	0.998	0.995	0.999
Slope	0.5322	0.0339	0.0225	3.7136
Intercept	0.021	0.0028	0.0364	0.0135
LOD (μg/mL)	0.1302631	0.5000000	0.0475071	0.0225471
LOQ (μg/mL)	0.3947368	1.515151	0.087506	0.0683246

SS – Solution means the drug is in sodium salicylate solution; SC – Solution means the drug is in sodium citrate solution; SA – Solution means the drug is in sodium acetate solution; SB – Solution means the drug is in sodium benzoate solution

Table 2: Results for percentage drug content of cefixime marketed tablets

Hydrotropic agents used	Concentration for assay (μg/mL)	Percentage content* (%w/w)
Sodium salicylate	1	98.0
Sodium citrate	15	99.67
Sodium acetate	20	100
Sodium benzoate	0.2	99.80

%W/W is percentage weight/weight, *n*=3 is three observations (**n*=3)

Table 3: Recovery study data by spiking method

Hydrotropic agents used	Level of standard drug added	% Recovery*
Sodium salicylate	10%	99.05
	20%	99.34
	30%	99.87
Sodium citrate	10%	99.88
	20%	99.01
	30%	100.01
Sodium acetate	10%	100.57
	20%	99.78
	30%	101.0
Sodium benzoate	10%	99.56
	20%	99.78
	30%	100.5

n=3 is three observations (**n*=3)

Table 4: Precision study data

Parameters	Repeatability				Intermediate precision			
	SS	SC	SA	SB	SS	SC	SA	SB
Amount found (mg)*	49.62	49.42	50.08	49.50	49.19	49.61	50.07	49.46
% Drug content	99.4	98.8	100.1	99.01	98.39	99.23	100.1	98.92
% RSD	0.883	0.910	0.341	0.787	0.794	1.137	0.310	1.103

%Drug content is the percentage of drug content in tablet formulation, %RSD is percentage relative standard deviation, $n=3$ is three observations, SS = means the cefixime stock solution is prepared using sodium salicylate solution, SC = means the cefixime stock solution is prepared using sodium citrate solution, SA = means the cefixime stock solution is prepared using sodium acetate solution, SB = means the cefixime stock solution is prepared using sodium benzoate solution (*$n=3$)

values of %RSD are confirmed that the method is precise enough. It is concluded that the proposed methods are new, simple, environment-friendly, accurate, sensitive, and less cost. Among these four developed methods, the method done by using SB as the hydrotropic agent showed less LOD and LOQ values (LOD = 0.0225471 µg/mL and LOQ 0.0683246 µg/mL) and it is considered to be more sensitive method.

Simple spectroscopic determination of cefixime using various hydrotropic solubilizing agents can be successfully used in the routine analysis with precluding the use of costlier and unsafe organic solvents. There is a good scope for other poorly water-soluble drugs which may be tried to get solubilized by individual hydrotropic agent (or) by combining two (or) more agents in specific ratio (mixed-hydrotropy) to exert synergistic effect on solubility.

Key Messages

The usages of various hydrotropic agents minimises the drawbacks of organic solvents during analysis like volatility, cost and pollution. In future, the mixture of different proportions of two or three hydrotropic agents (mixed solvency) may increase the aqueous solubility of poorly water soluble drugs and its effectiveness synergistically.

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