RESEARCH ARTICLE

Chemometric Assisted UV-Spectrophotometric Quantification of Cefaclor in Suspension Dosage Form

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ABSTRACT

In this current study, the quality by design (QbD) concept is used for creating and validating a unique, resilient, accurate, and reliable spectrophotometric approach to quantify cefaclor (CEF) in injections. Fractional factorial design (FFD) was a design implemented to screen the initial parameters. Moreover, the variables went through the central composite design (CCD) to assess the dependency and optimize the design. Several measures were analyzed statistically to determine the appropriateness of the data obtained from the experiments. At 265 nm, by the use of ethanol, cefaclor displays an absorption maximum. Variables like screening, slit-width, and sampling interval were recognized as critical methods and again, evaluation was done by a CCD. A good linearity was produced for cefaclor in the range of 2 to 12 µg/mL, with R2>0.9993. The process was determined for being perfect, having a good average percent recovery (greater than 100%). According to ICH guidelines, validation of the developed method was performed. By implementing QbD principles, the spectrophotometric was created and designed to integrate the quality into the method. The process was manifested for being flexible and appropriate for identifying CEF in pharmaceuticals.

Keywords: Cefaclor, Factor screening, Spectrophotometric technique, Validation.

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INTRODUCTION

The IUPAC name of cefaclor (CEF), (6R,7R)-7-[(2R)-2-amino-2-phenylacetyl]amino-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Figure 1) belongs to category of cephalosporin antibiotics.[1-3] It is being functionalized inhibiting bacterial growth. CEF and other antibiotics will not treat a cold, flu, or other viral infection.[4] Meningitis (infection of the membranes that surround the brain and spinal cord) and skin infections brought on by bacteria both are treated with CEF.[5-7]

CEF in suspension formulation and original samples are evaluated by implementing LC-MS, UV-visible spectroscopy, and HPLC methods.[8-11] Although the recorded method of UV spectrophotometry has some limitations such as the absence of Sandell's sensitivity, narrow linearity range, and inability to satisfy molar extinction coefficient (ε), etc. Hence, many efforts were performed to develop an advanced and unique method of UV spectroscopy to quantify CEF in suspension dosage form through QbD approaches.

The QbD is combined access, ensuring standard integration throughout the procedure to get the planned report. As per ICH-Q8-(R2), QbD is methodical access to the final product advancement that starts along with a predetermined purpose and prioritizes understanding the elements and procedure as well as process control, built on trustworthy scientific principles in addition to control of risks.[12] The USFDA established Pharmaceutical Current Good Manufacturing Practices (cGMPs) over 21st century in 2002, which led to the discovery of QbD.[13] Analytical Quality by Design (AQbD) contains six stages of comprehensive development of an analytical method with improved performance and strong resilience.[14]

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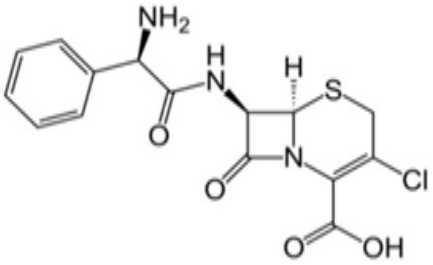


Figure 1: Structure of CEF

Utilizing the QbD methodology reduces the duration necessary for creating an effective analytical technique and is also deemed an economical manner of guaranteeing quality among outset belonging to the method development perspective. The design of experiments (DoE), which is an essential component of, QbD supplies a stable model area of the best performance of the method. This current investigation focuses on utilizing rational experimental designs to decrease variability in the spectrophotometric measurement of CEF. The goal is the identification of optimal solutions. In the beginning, a factor screening research utilizing FFD was done to identify crucial technique parameters that affect performance. Method optimization, utilizing the CCD, was done afterward to ensure robustness and accomplish predetermined goals. The goal of the research is to build a novel, appropriate, and exact UV spectrophotometry method for the quantification of CEF available injectables while validating existing procedures related to ICH requirements.[15]

MATERIAL AND METHOD

Standard and Reagent

The unmixed or clear medicament, CEF (purity > 99.5%) had received like a specimen in form of gift from Gokul Eximp, Mumbai, Maharastra, India. Ethanol was acquired from Merck Ltd., Jamshedpur, India and utilized for drug and reagent solutions preparation. The retailed suspension dosage form of CEF (250 mg/5 mL) bought from domestic market was procured and examined using a currently advanced procedure.

Optical Characteristics and Instrumentation

Single beam microprocessor UV-visible spectrophotometer LI-285 (Lasany, India) with ten millimeter matched quartz cuvettes were employed to measure the spectrums. The reagents were weighed using a chemical balance with high precision. The suspension formulation's ability to dissolve was influenced by ultrasonication (Enertech, India).

Setup of an Analytical Target Profile

Properly reviewing the current literature surveys and medication profiles (physicochemical properties) were conducted in order to develop a targeted configuration for assessment, providing an advanced overview of classification attributes a procedure analytically. These essentially entailed the creation of a quick, dependable, and profitable analytical procedure to estimate CEF in suspension dosage form. Resulting, a UV spectrophotometric approach for quick examination of CEF was chosen depending on the primary goal of this novel research. The reason for choosing the UV spectrophotometric methods was because of uncomplicated and speedy drug analysis compared with other complex techniques analytically.

Cause-Effect Relationship Establishment as Well as Risk Management

The diagram of Ishikawa fish-bone, is the simplest tool for understanding the cause-effect relationship between probable method factors, which might influence the presentation of the procedure. Regarding the above-mentioned matter, Ishikawa diagram was depicted (undepicted of the illustration) through accentuating various procedural factors that could potentially impact the method characteristics of CEF UV spectrophotometry. In the ongoing investigations, the needy factors influencing analytical qualities were found using a cause-effective relationship, risk assessment matrix, and CNX (Control-Noise-Experimentation) technique. Variations in the solvent utilized, scan speed, detection wavelength, sampling interval, slit width, and sample integrity were identified, being critical method variables (CMVs), linked to high final scores and high-risk variables. The most required method parameters for the CMVs were also assessed with the assistance of a screening model before being submitted utilising an appropriate experiment method to have response surface optimization.

Analysis of Essential Method Variables Through FFD

This model (Design Expert 11, Version-11.0.4.0, USA) was used for analyzing the essential parameters to find the high-risk variables. A few factors were chosen as essential method variables by analyzing spectrum structure, accuracy, and absorbance. Furthermore, the CMVs were estimated with a design screened for building up the critical method parameters (CMPs), and an appropriate experimental methodology optimized the response surface. Speed of scanning (X1), Width of the slit (X2), and sample interval (X3), otherwise, were analyzed using Design expert software through FFD consisting of no fewer than 5 trials (1 being a centre point). The parameters were examined at both their maximum and minimum values, and the programme was implemented to discover the crucial parameter values that influence the absorbance response variable (Y). Examining the actual against expected values plot, fitting summary plot, Pareto chart, and prediction equation yielded the significant parameters.

Method Optimization and Robustness Study by Using Ccd

The usage of CCD ensured the potency of the procedure for determining optimal method conditions.[16] Ten trial runs were acquired having at least two centre points depending on CCD for optimization of CMVs, such as slit width (A) and sample interval (B), as determined by investigations screened. Observations of the experimentation were analyzed in the assistance of absorbance at 265 nm as the response variable. A standard CEF of 10 µg/mL was employed for all of the experiments.

Reported results of experiment were fixed with a preferable model mathematically using multiple linear regression analysis (MLRA) via Design Expert software. The advanced models were permitted to research every important impacts and impact of interactions. Single coefficients of design terminologies reported being remarkable *P* value less than 0.05, according to ANOVA analysis, were determined in formatting the polynomial equation, as well as analyzing the modeling factors, such as comparison between the actual and predicted plot, fit summary, ANOVA following estimation of factors such as coefficient of correlation (R^2), predicted and adjusted R^2, Predicted Residual Sum of Squares (PRESS), in sequence manner. In addition, remaining critical factors such as interaction profiler, prediction profiler, and 3-D response surface profiler were utilized for the determination of the suitability of the design. The most effective resolution was examined by utilizing a numerical desirability function which involved balancing the analyzed variables to achieve the desired outcomes. This was then marked within the designated space of the design region.[17]

A Strategic Planning for the Method Control

Method control planned strategically, were established depending on the space, produced by DoE assess, where little changes during performing the method were permitted for maintaining the robustness of the method.

Standard Stock Solution Preparation

The standard stock solution of CEF (1000 µg/mL) was prepared, followed by dissolving exactly 10 mg of CEF with ethanol up to 10 mL. The prepared stock solution, 5 mL: of the mixture solution, was poured into a flask containing 50 mL volume and diluted up to 50 mL for producing standard solutions with a concentration of 100 µg/mL.[18]

Analysing Suspension Dosage Formulation

The labeled claim for CEF suspension is 250 mg/5 mL (Ceclor DS/Aspen, India). According to the instruction on the label, 60 mL of suspension is available, which contains 250 mg/5 mL. The mixture solution was formed and from this, 0.2 mL (weight equivalent to 10 mg) was introduced in a 10 mL of volumetric flask, making up to 10 mL using ethanol to produce 10 mg/10 mL solution. The content was ultrasonicated for 30 minutes. This above solution was again stained by the assistance of Whattmann filter paper for the separation of particulate matter, if present. The filtered mixture was again getting diluted using ethanol for investigation. The medicating ingredient available inside the standard solution was estimated via the calibration curve of standard CEF.

Specificity

The particularity of the procedure of UV spectrophotometry was estimated depending on the entity's assessment and its formulation excipients. Spectrum was estimated for possible interference as a reason of additives.

Linearity

Different tubes were considered using the CEF working standard solution in different 10 mL volumetric flasks and diluted using ethanol to produce a group of concentrated limiting from 2 to 12 µg per mL. At 265 nm, UV absorbance was determined. The calibration curve was scattered to evaluate the linearity by interpreting the absorbance on the Y-axis and the concentrated (µg per mL) on the X-axis.

Precision and Accuracy

Searching the clarity of the procedure, recovery studies were performed with 80,100 and 120% studies were established, in triplicate at each level. A calibration curve was calculated using a CEF standard drug added to the recovery solution. 6 replicants of a particular concentration of CEF (10 g/mL) were scanned on the exact same day to estimate intraday precision, and percent RSD values were determined.

RESULTS AND DISCUSSION

In the above research, a method of UV spectrophotometry has been established to assess the quantity of CEF available within suspension dosage form. QbD approaches were utilized for reporting variable factors in the advancement of ultimate spectrophotometric conditions. A standard diagram of Ishikawa fishbone was established to identify the variables in the method. Physical evaluation of the design variables was performed. Medication was being estimated to be insoluble in acetone or ether. However, CEF was dissolved in ethanol. Therefore, ethanol was chosen as an appropriate solvent system for future research. Standard CEF solution interpreted absorption maxima (λ_{max}) at 265 nm through ethanol (Figure 2) and was chosen such as detection wavelength.

The sample characteristics were satisfied according to the tested melting point. Although, the method variables such as sampling interval (SV), scanning speed (SS) and slit width (SW) required an investigation systematically to establish the impacts on the robustness of the method. Applying FFD approach assisted in CMVs scanning out of scanning speed, slit width, and sampling interval. The evaluation of design via predicted vs actual plots displayed the apt fitness of the preferred method. Model *p*-value (0.0275), R^2 (0.9173) and RMSE (0.0002) also preferred model aptness. Estimating the fit summary displayed predicted R^2 (0.4121) and adjusted R^2 (0.8140) values.

The design CCD was implemented for estimating the CMVs impression on response absorbance. A total of ten experiments were randomly carried out using a UV-visible spectrophotometer to acquire a bias-free response with two center points minimally. The responses acquired regarding every experiment and spectrophotometric range studied are listed below (Table 1).

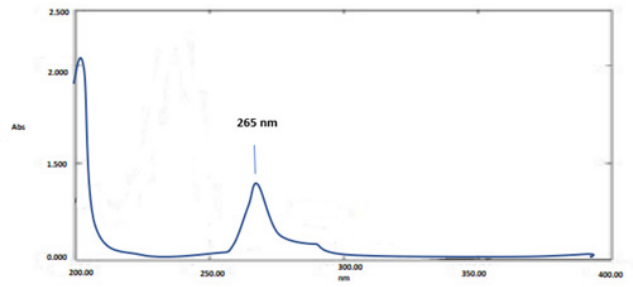


Figure 2: CEF - Standard UV absorption spectrum

Table 1: Experimental design-matrix showing spectrophotometric range studied for robustness study as well as resulted responses

Run No	*Slit Width (SW) (A)*	*Sampling Interval (SI) (B)*	*Absorbance (Y)*
1	0.18934	1.25	0.261
2	2.31066	1.25	0.274
3	2	2	0.29
4	1.25	1.25	0.35
5	0.5	0.5	0.241
6	0.5	2	0.264
7	1.25	1.25	0 .35
8	1.25	2.31066	0.271
9	2	0.5	0.31
10	1.25	0.18934	0.25

Range	*Low*	*High*
	0.5	2
	0.5	2

Proper evaluation of CCD model implementing varieties of analytical tools statistically were performed and observations were considered by ANOVA, a factor estimating the prediction profiler.

In Figure 3A, perturbation plots for projected models are shown to get result of the influence of distinct components on a provided response while maintaining every factor fixed at an initial point of reference. The steepest inclination or curve shows the affectability of the feedback to a particular factor, in Figure 3A it was being obtained the sampling interval (factor B) had the most required effect on absorbance, followed by slit width. Figure 3B accompanies baseline design (blue Points) among the actual vs predicted plot, where the line, for the data attained from the experiment was recorded being good within the range or confines the assurance interims. It refuses the H_0, as the variation in data described by the model effectively, at which the assumed and attained report were reported to be quite equivalent.

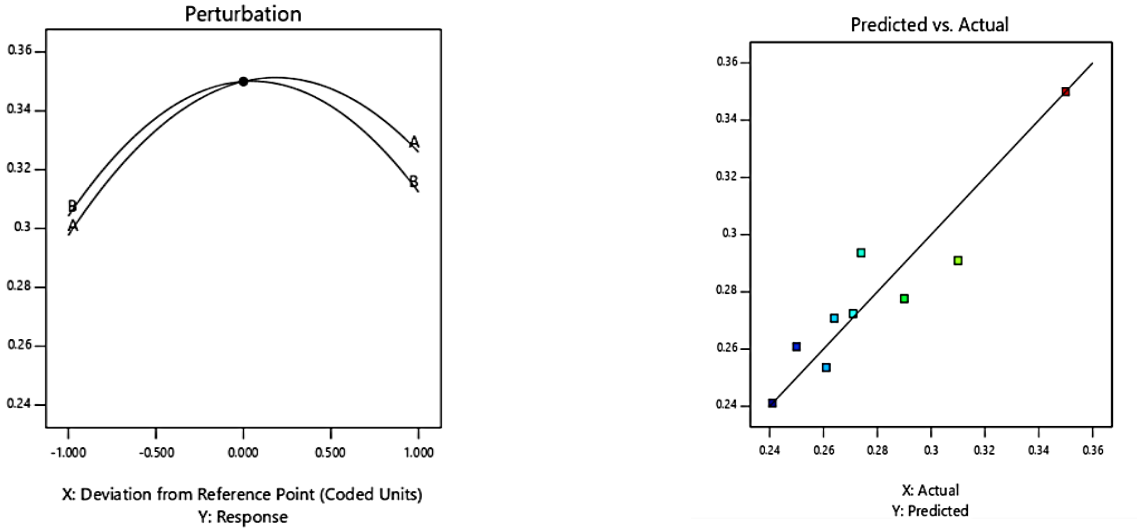
Response surfaces plots for slit width and sampling interval are interpreted in Figure 4 (slit width is plotted against the sampling interval). Analyzing optimized models' response plots and perturbation plots uncovered that factor had a huge response on the analyte absorbance. Further, ANOVA recommended that the probability value is smaller than 0.0275, indicating the perfectness of the dummy addressing the variability and suggesting rejection of the H_0. Aside from, the smaller values for PRESS also ratified the perfectability of the dummy. Factors evaluation assessment is critical for estimating the variable risk among various variables. An obtained probability value smaller than 0.05, prefer a non-zero value obtained by the slope.

Sampling interval × sampling interval (B2), as well as slit width (A) were found as the most influencing method variables.

$$Absorbance\,(Y) = 0.3500 + 0.0142A + 0.0041B - 0.0107AB - 0.0382A2 - 0.0417B2$$

where, A = Slit width, B = Sampling interval.

The characteristics of the optical spectrophotometric methods has tabulated in Table 2. The methods established were reported particularly as selective as the generally applied dosage form, additives available within the suspension formulation were observed non interfere the estimation procedure. Pharmaceutical entities were linear, directing a concentrated limit within 2 to 12 µg per mL. Regression analysis of linearity results displayed perfect fit overall. The results acquired for factors statistically like R^2, adjusted R^2 and predicted R^2 were observed to be 0.9173, 0.8140 and 0.4121, sequentially. ANOVA preferred the perfectness of the procedure ($p < 0.05$), regarding linearity data. The percent recovery or improvement of the suspension dosage formulation were reported and observed to be 99.83% (S.D = ± 0.049, n = 6). Mean recovery, for accuracy study, limited from 99.9 to



A B

Figure 3: A: Pertubation plot, B: Predicted vs. Actual plot

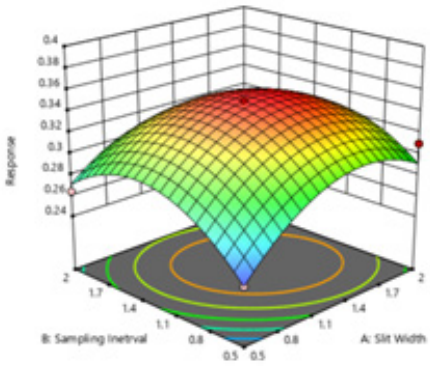


Figure 4: 3D - Response surface plot (RSP), for absorbance against slit width vs sampling interval

Table 2: Optical characteristics as well as summary of validation parameters

Parameters	*Obtained Values*
Wavelength (λmax)	265 nm
Linearity Range - (µg/ml)	2–12
Sandell's sensitivity - (µg/cm^2 / 0.001AU)	0.0416
Molar extinction coefficient (ltr/ mol.cm)	0.88272 * 10^{-5}
Regression equation(Y = ax+b)*	0.0199x + 0.0304
Correlation coefficient(R^2)	0.9993
Precision (% R.S.D., n = 6)	0.60726397
Accuracy (% Recovery ± S.D.)	
80%	0.189004 ± 0.000186
100%	0.170103 ± 0.000186
120%	0.155227 ± 0.000186
% Range of error	
95% confidence limits	± 0.039
99% confidence limits	± 0.052

100.3%. The percent RSD was achieved below 2%, in case of intra-day determination displaying a great extent of exactness of the predicted procedure. Report of the procedure occured between the limit that was prescribed, displaying that the process is having no interference of additives.

R.S.D–Relative standard deviation; S.D–Standard deviation; A.U - Absorbance units,* is Y = ax+b, where Y = absorbance, a = slope, b = intercept and x is the conc., ± is average of three determinations at each level

CONCLUSION

A QbD methodology was used to establish a reliable method of UV spectrophotometry method for CEF quantification. Using the QbD procedure the analytical quality of the procedure is being confirmed. There were two influential CMVs, Slitwidth and sampling interval, which require specific consideration by the analyst while performing the method controls strategically and further experiments regarding continuous development in the performance of the method. The reports prefer the research is unique, specific, exact and to-the-point. Statistically, investigations of validating the procedure reports prefer the advanced methods' perfect implementation in the quality control laboratory. These methods are appropriate for the determination of CEF in suspension formulation without any obstructions from generally known additives. Henceforth, the design must be employed in regular evaluation purposes.

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