

Development And Validation of A Stability-Indicating UHPLC Method For Simultaneous Estimation of Cefoperazone And Sulbactam in Powder For Injection Dosage Form

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Abstract

Background: Cefoperazone and sulbactam are commonly combined in pharmaceutical preparations, requiring reliable analytical methods for simultaneous estimation and quality control. **Objectives:** The present study aimed to develop and validate a rapid and reliable ultra-high performance liquid chromatography (UHPLC) method for simultaneous estimation of cefoperazone and sulbactam in powder for injection dosage form. **Materials and Methods:** Chromatographic separation was achieved using a C18 column (50 mm × 2 mm, 4 µm) under isocratic conditions. The mobile phase consisted of 10 mM phosphate buffer adjusted to pH 3.2 with orthophosphoric acid and acetonitrile in the ratio of 84:16 (v/v). The flow rate was 0.5 mL/min, injection volume was 2 µL, detection wavelength was 230 nm and total run time was 5 min. The method was evaluated for system suitability, specificity, assay, linearity, precision, accuracy and robustness. **Results:** Cefoperazone and sulbactam showed retention times of approximately 2.452 and 1.132 min, respectively. The assay results were 99.73% for cefoperazone and 100.51% for sulbactam. The method demonstrated excellent linearity with correlation coefficients (R^2) of 0.9999 and 0.9995 for cefoperazone and sulbactam, respectively. Repeatability % RSD values were 0.12% and 0.25%, while intermediate precision values were 0.23% and 0.33%, respectively. Accuracy results ranged from 99.29–100.47% for cefoperazone and 99.84–100.97% for sulbactam. Robustness studies showed % RSD values below 2%. **Conclusion:** The developed UHPLC method was found to be rapid, precise, accurate and robust for simultaneous estimation of cefoperazone and sulbactam in powder for injection dosage form and may be suitable for routine pharmaceutical quality-control analysis.

Keywords: Accuracy, Cefoperazone, Method validation, Sulbactam, UHPLC, Precision.

INTRODUCTION

Pharmaceutical analysis plays an important role in establishing the identity, purity, strength, quality and safety of pharmaceutical substances and finished dosage forms. Analytical methods are essential during pharmaceutical development and manufacturing because they enable quantitative estimation of active pharmaceutical ingredients and assessment of impurities and degradation products. Chromatographic techniques, particularly high-performance liquid chromatography (HPLC), are extensively used because of their selectivity, accuracy, precision and suitability for complex pharmaceutical matrices.

Ultra-high-performance liquid chromatography (UHPLC) is an advanced form of liquid chromatographic analysis that uses smaller particle-size stationary phases and optimized system components to provide efficient separation with reduced analysis time.

Cefoperazone is a third-generation cephalosporin antibiotic with broad-spectrum antibacterial activity. Sulbactam is a β -lactamase inhibitor that is combined with β -lactam antibiotics to enhance their antibacterial effectiveness. The combination is available as a sterile powder for injection and requires appropriate analytical control to ensure the quality and consistency of the dosage form. Several analytical methods have been reported for cefoperazone and sulbactam, including spectrophotometric, HPLC, HPTLC, LC-MS/MS and UPLC-based methods. The present study focused on development of a rapid UHPLC method for simultaneous estimation of the two drugs in powder for injection dosage form, followed by validation for system suitability, specificity, assay, linearity, precision, accuracy and robustness.

MATERIALS AND METHODS

Chemicals and Reagents

Cefoperazone and sulbactam were used as the active pharmaceutical ingredients for analytical method development and validation. Phosphate buffer, orthophosphoric acid, acetonitrile and water were used during chromatographic analysis. The diluent used for the optimized method was water.

Instrumentation and Chromatographic Conditions

Chromatographic separation was carried out using UHPLC instrumentation equipped with a UV detector. Separation was achieved on a C18 column measuring 50 mm × 2 mm with a particle size of 4 µm.

Parameter	Optimized condition
Chromatographic mode	Isocratic
Column	C18, 50 mm × 2 mm, 4 µm
Mobile phase	10 mM phosphate buffer: acetonitrile (84:16, v/v)
Buffer pH	3.2
pH adjustment	Orthophosphoric acid
Flow rate	0.5 mL/min
Detection wavelength	230 nm
Injection volume	2 µL
Runtime	5 min
Diluent	Water
Cefoperazone retention time	2.452 min
Sulbactam retention time	1.132 min

Method Development

Several chromatographic trials were performed by varying column, stationary phase, buffer pH and mobile-phase composition. Initial trials produced inadequate separation or peak shape. The optimized conditions using a short C18 column and phosphate buffer at pH 3.2 provided satisfactory chromatographic performance, with both analytes separated within a short analytical run.

Method Validation

The developed analytical method was evaluated for system suitability, specificity, assay, linearity, precision, accuracy and robustness.

System Suitability

System suitability was evaluated using chromatographic parameters including theoretical plate count, tailing factor and resolution. The predefined criteria were more than 2000 theoretical plates, tailing factor below 2 and resolution greater than 2.

Specificity

Specificity was evaluated by comparing blank, standard and sample chromatograms. The absence of interfering peaks at the retention times of cefoperazone and sulbactam was assessed.

Assay

The optimized method was applied for assay determination of cefoperazone and sulbactam in the powder for injection dosage form.

Linearity

Linearity was evaluated at five concentration levels corresponding to 50%, 75%, 100%, 125% and 150% of the target concentration. Solutions were injected in duplicate and calibration curves were constructed.

Precision

Repeatability was evaluated using six working sample solutions of the same concentration. Intermediate precision was evaluated on a separate day. Mean, standard deviation and percentage relative standard deviation (%RSD) were determined.

Accuracy

Accuracy was evaluated at 80%, 100% and 120% concentration levels. Triplicate injections were performed at each level and percentage recovery was calculated.

Robustness

Robustness was investigated by deliberate variation of flow rate and detection wavelength. The effects of these changes on assay and %RSD were assessed.

RESULTS

The optimized UHPLC method produced well-defined peaks for both analytes with a short total analysis time. The principal validation results are summarized below.

Validationparameter	Cefoperazone	Sulbactam
Assay(%mean)	99.73%	100.51%
Linearity(R ²)	0.9999	0.9995
Repeatability(%RSD)	0.12%	0.25%
IntermediateprecisionDay2 (%RSD)	0.23%	0.33%
Accuracy(%recovery)	99.29–100.47%	99.84–100.97%
Specificity	Complies	Complies
Robustness	Complies	Complies

MethodDevelopment

The optimized chromatographic conditions resulted in cefoperazone elution at approximately 2.452 min and sulbactam at approximately 1.132 min. Plate count and tailing factor were satisfactory, and the method was selected for validation.

Specificity

No interfering peaks were observed in blank or placebo at the retention times of either drug, demonstrating specificity of the method.

Assay

The mean assay was 99.73% for cefoperazone and 100.51% for sulbactam.

Linearity

The regression equation for cefoperazone was $y = 3.1658x - 17.1084$, with $R^2 = 0.9999$. For sulbactam, the regression equation was $y = 1.9510x - 5.8960$, with $R^2 = 0.9995$.

Precision

Repeatability %RSD values were 0.12% for cefoperazone and 0.25% for sulbactam. Intermediate precision on Day 2 gave %RSD values of 0.23% and 0.33%, respectively.

Accuracy

Mean recovery ranged from 99.29% to 100.47% for cefoperazone and from 99.84% to 100.97% for sulbactam.

Robustness

Condition	Cefoperazone%RSD	Cefoperazoneassay	Sulbactam%RSD	Sulbactamassay
Flow rate – (0.3 mL/min)	0.13%	99.75–100.10%	0.27%	99.56–100.19%
Flow rate + (0.7 mL/min)	0.27%	99.63–100.40%	0.19%	99.73–100.20%
Wavelength – (228 nm)	0.24%	99.83–100.43%	0.56%	99.22–100.87%
Wavelength + (232 nm)	0.12%	99.87–100.19%	0.33%	99.52–100.52%

DISCUSSION

The present investigation was undertaken to develop a rapid UHPLC method for simultaneous

estimation of cefoperazone and sulbactam in powder for injection dosage form. Method development involved systematic changes in the stationary phase, buffer pH and organic-phase proportion. The final C18-based conditions produced satisfactory peak shape and chromatographic performance.

The short column and optimized mobile-phase composition enabled both analytes to be separated within a 5-min run. The absence of significant interference in blank and placebo chromatograms supported the specificity of the method.

The assay results of 99.73% for cefoperazone and 100.51% for sulbactam were satisfactory. The high correlation coefficients demonstrated a strong relationship between analyte concentration and chromatographic response.

The low %RSD values obtained during repeatability and intermediate precision studies demonstrated good reproducibility. Accuracy studies produced recoveries close to 100% for both analytes, supporting the trueness of the method.

Robustness studies showed that deliberate changes in flow rate and detection wavelength did not significantly affect analytical performance. All investigated %RSD values were below 2%, indicating that the method was sufficiently robust under the tested variations.

The thesis results document specificity, assay, linearity, precision, accuracy and robustness. Explicit forced-degradation experiments and degradation-product resolution data were not available in the reviewed results. Therefore, the validation data establish the analytical performance of the developed UHPLC method, while additional forced-degradation studies would be required to experimentally substantiate full stability-indicating capability.

CONCLUSION

A rapid and reliable UHPLC method was developed for simultaneous estimation of cefoperazone and sulbactam in powder for injection dosage form. The method employed a C18 column with an isocratic phosphate buffer-acetonitrile mobile phase and UV detection at 230 nm. Both analytes were successfully separated within a short 5-min run.

The method demonstrated satisfactory specificity, assay, linearity, precision, accuracy and robustness. Cefoperazone and sulbactam showed assay values of 99.73% and 100.51%, respectively, with excellent linearity and low variability during precision studies. The method therefore provides a simple, rapid and reproducible approach for routine quality-control analysis of cefoperazone and sulbactam in the investigated dosage form.

For a complete stability-indicating claim, forced-degradation studies under appropriate acidic, alkaline, oxidative, thermal and photolytic conditions, together with adequate separation of degradation products from the drug peaks, should be incorporated if such studies have not already been performed.

Acknowledgements

The authors acknowledge the management and faculty members of Excel College of Pharmacy, Komarapalayam, for providing the facilities and support required for completion of the research work. The authors sincerely thank Dr. R. Manivannan, Professor and Principal, Excel College of Pharmacy, for his guidance, encouragement and valuable suggestions throughout the study. The authors also acknowledge the Department of Pharmaceutical Analysis and other faculty members for their technical support and cooperation.

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