



ISSN: 2349-5448

Intercontinental Journal of Pharmaceutical Investigations and Research (ICJPIR)

ICJPIR | Vol.12 | Issue 2 | Apr - Jun -2025

www.icjpir.com

DOI: <https://doi.org/10.61096/icjpir.v12.iss2.2025.63-68>

Research

Method Designing And Its Assessment To Determine Cefotaxime And Sulfamethoxazole In Combined Formulation By Hplc

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	Abstract
Published on: 07 May 2024	A simple, accurate method is used to determine Cefotaxime (CFT) and Sulfamethoxazole (SMZ) in bulk and their combination formulation. Chromatogram was produced on Thermofischer BDS Hypersil C18, 250 x 4.6 mm, 5mm column, with 0.1%formic acid and methanol in 80:20v/v mobile phase, 0.8ml/min flow rate, 265nm, 30°C column CFT and SMZ retention times are 3.453 and 7.991min. We found 0.9957 and 0.9933 regression coefficients for CFT and SMZ. % Recovery was 100.08% for CFT and 100.06% for SMZ. The CFT and SMZ regression models yield LOD values of 0.08, 0.25 and LOQ values of 0.04, 0.12. Simple and cost-effective quality analysis of this combination during clinical trials was suggested.
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	Keywords: Cefotaxime, Sulfamethoxazole, RP-HPLC

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for separating, identifying, and quantifying components in a mixture. It operates on the principle of liquid-phase chromatography, where a liquid mobile phase carries the analytes through a stationary phase (column), leading to differential retention and separation based on chemical properties such as polarity, molecular weight, and interaction with the stationary phase.¹

Cefotaxime is a third-generation cephalosporin used to treat Gram-positive and Gram-negative bacterial infections. It has a molecular formula of C₁₆H₁₇N₅O₇S₂ and appears as a solid powder with a solubility of 50 mg/mL in water at 25°C. The compound should be stored at 25°C. Its IUPAC name is (6R,7R,Z)-3-(Acetoxymethyl)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. The mechanism of action involves binding to penicillin-binding proteins (PBPs), inhibiting the transpeptidation step of peptidoglycan synthesis, leading to bacterial cell wall disruption. It is

resistant to beta-lactamase degradation, making it effective against resistant bacterial strains. The appropriate dosage and administration depend on the extent of infection and microorganism susceptibility. Cefotaxime is marketed under the brand name Claforan.²

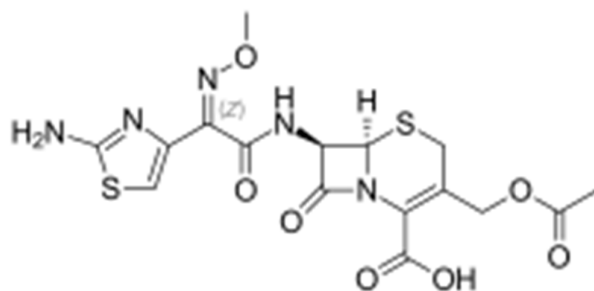


Fig 1: Structure of CFT

Sulfamethoxazole (SMZ) is a sulfonamide antibiotic belonging to the isoxazole class, with a molecular formula of $C_{10}H_{11}N_3O_3S$ and a molecular weight of 253.28 g/mol. It appears as a solid powder, with an aqueous solubility of 3942 mg/mL at 25°C and should be stored at 25°C. Its IUPAC name is *4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide*. SMZ inhibits dihydrofolic acid synthesis by mimicking para-aminobenzoic acid (PABA), disrupting bacterial growth. It functions as an antibacterial, anti-infective, P450 inhibitor, and dihydropteroate synthase inhibitor, and is also classified as an environmental contaminant and xenobiotic.^{3,4}

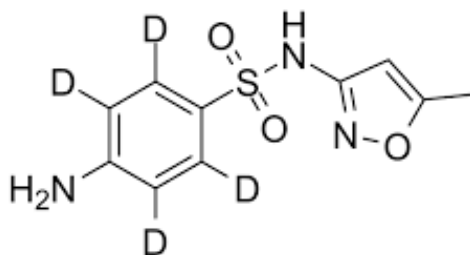


Fig. 2: SMZ structure

Several studies have explored analytical methods and clinical efficacy of Cefotaxime (CFT). Talib developed a spectrophotometric method to determine CFT levels using Fe (III) reduction, confirming its applicability in pharmaceutical formulations.⁵ Latha Saranya et al. established an LC-MS method for simultaneous estimation of CFT and paracetamol with a linearity range of 1-40 ppm and precision $\leq 2\%$.⁶

Abbas and Mohamed introduced a spectrophotometric method involving CFT and 3,5-dimethyl phenol, forming a stable dye with maximum absorbance at 497 nm.⁷ Al Hakkani (2020) developed a RP-HPLC method for CFT analysis, proving its robustness, selectivity, and cost-effectiveness.⁸ Lalitha et al. validated an RP-HPLC assay for injection formulations, demonstrating high sensitivity with detection limits of 0.3 $\mu\text{g/mL}$.⁹ Chaudhari devised an HPLC method using dried blood spots (DBS) and plasma, proving clinically valid for pharmacokinetic studies in neonates.¹⁰

While there is no existing literature on the combination of CFT and SMZ, studies confirm no drug-drug interactions. Since they have distinct mechanisms of action, their combination reduces antibiotic resistance, supporting the development of a novel antibiotic formulation and an RP-HPLC method for its analysis.

MATERIALS AND METHODS

Chemicals and Solvents

HPLC grade Water and Methanol, Formic Acid AR Grade, CFT and SMZ API Standards and combined formulation. Tablets with 500mg of CFT and 400mg of SMZ were punched in our laboratory. SMZ (working standard) was obtained as a gift sample from Emcure Pharmaceuticals, India and CFT from GMT Pharma International.

Instrumentation

HPLC analysis was performed using Shimadzu LC 2030C 3D Plus HPLC (Prominence-i series) with Empower-2 software. The Agilent Zobrax C18 column (250 $\times$ 4.6 mm, 5 μm) was used for separation.

Preparation of Solutions

The standard solution consists of 500 mg of CFT and 400 mg of SMZ standards. APIs and diluent were accurately measured and placed into a 10 ml volumetric vessel, sonicated for 10 minutes, and subsequently adjusted to the desired volume. 1 ml of the primary solution is transferred to a 10 ml volumetric flask and the volume is adjusted with diluent. Following the measurement of 1 ml, it was transferred to a 10 ml volumetric vessel, and the volume was adjusted with diluent to achieve concentrations of 50 µg/ml CFT and 40 µg/ml SMZ solutions.

Standard Graph Construction

Volumes of 1, 2, 3, 4, and 5 ml were extracted from primary solutions of 500 µg/ml CFT and 400 µg/ml SMZ, and transferred to a 10 ml volumetric vessel. The final volume was adjusted with diluent to achieve concentrations of 10 - 100 µg/ml for both CFT and SMZ. For each step, 10 µl of each individual sample was loaded three times, and a calibration curve was constructed using peak area versus target molecule concentration.

Assay

Tablets labelled CFT 500mg and SMZ 400mg were manufactured in our laboratory. The punched formulation was utilised for the experiment.

Method Validation

System suitability was assessed by injecting standard solutions of 50 µg/ml CEF and 40 µg/ml SMZ five times to evaluate the tailing factor, area, and USP plate count, with an RSD of less than 2%. Specificity was confirmed as no interfering peaks were observed at the drug retention times. Accuracy and linearity were evaluated by analyzing ALB and CLS at concentrations ranging from 10–50 µg/ml, with a calibration curve confirming linearity and a recovery rate of 98–102%. Precision was assessed using spiked solutions at 50%, 100%, and 150% levels. Robustness was tested by varying the flow rate and mobile phase composition, with %RSD remaining within acceptable limits. Sensitivity was confirmed through LOD and LOQ studies, with sample dilutions of 0.25 ml and 0.3 ml demonstrating reliable detection.

RESULTS AND DISCUSSION

The optimized chromatographic conditions included a mobile phase of 0.1% formic acid and methanol (80:20 v/v) with a flow rate of 0.8 mL/min. Separation was carried out using a Thermofisher BDS Hypersil C18 column (4.6 × 250 mm, 5 µm) at ambient temperature. The detector operated at a maximum absorption of 265 nm, with an injection volume of 10 µL and a run time of 11 minutes. Methanol was used as the diluent, ensuring effective sample preparation and analysis. The retention times were found 3.453 for CFT and 7.991 for SMZ.

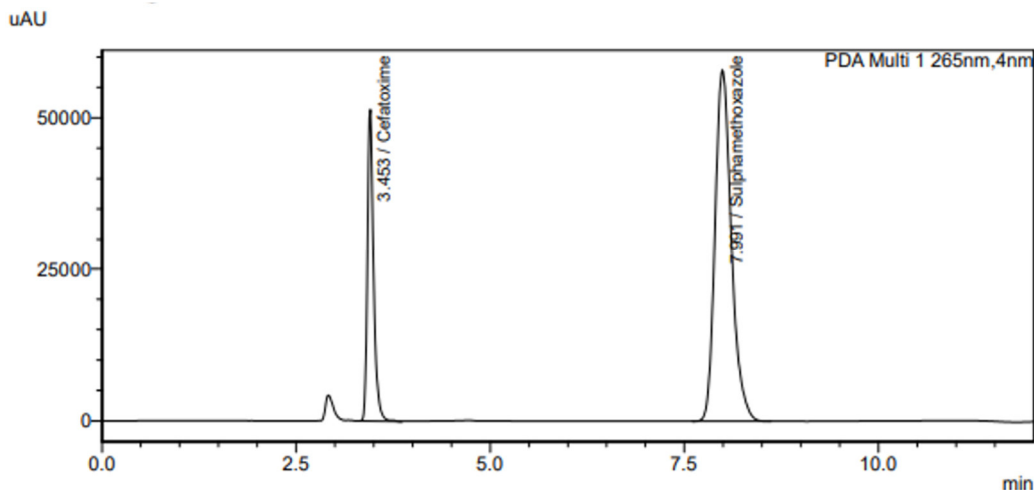
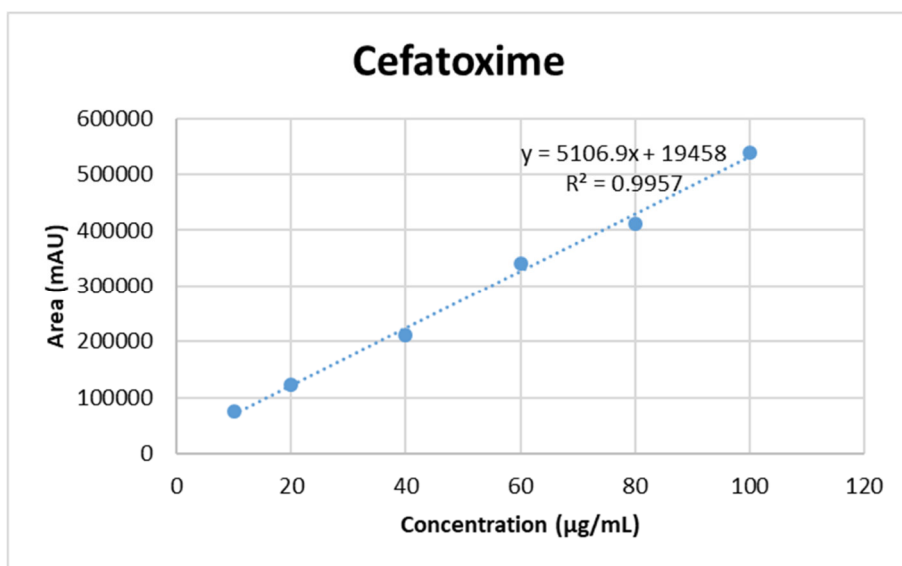
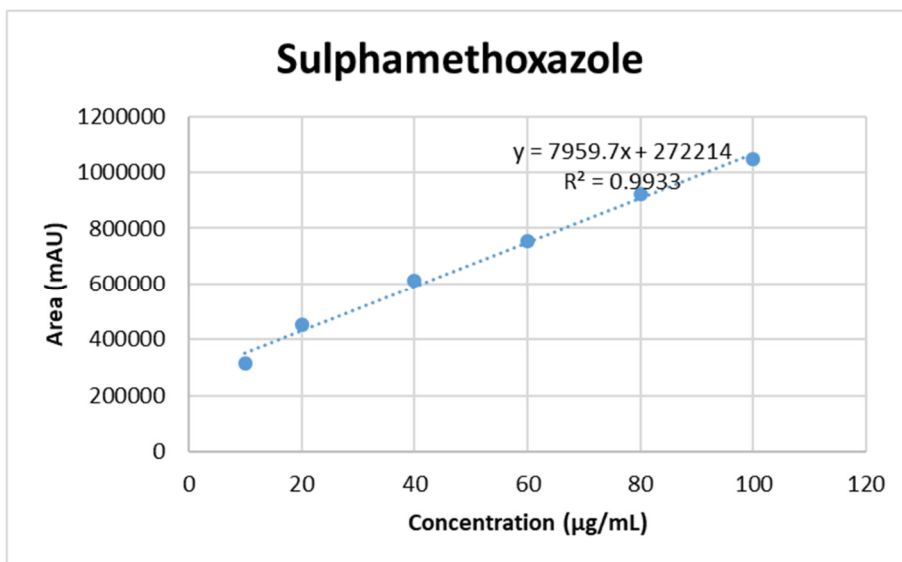


Fig 3: Chromatogram (Optimised)

The following table 1 and Figs. 4 and 5 shows the calibration data of CFT and SMZ. Correlation coefficients obtained were 0.994 for CFT and 0.996 for SMZ.

Table 1: Linearity data of CFT and SMZ

	CFT	SMZ
Conc (µg/ml)	Area	Area
10	74815	316954
20	122168	450929
40	212066	610224
60	340458	753188
80	412243	922112
100	538125	1047384

**Fig 4: Calibration graph for CFT****Fig 5: Calibration graph for SMZ**

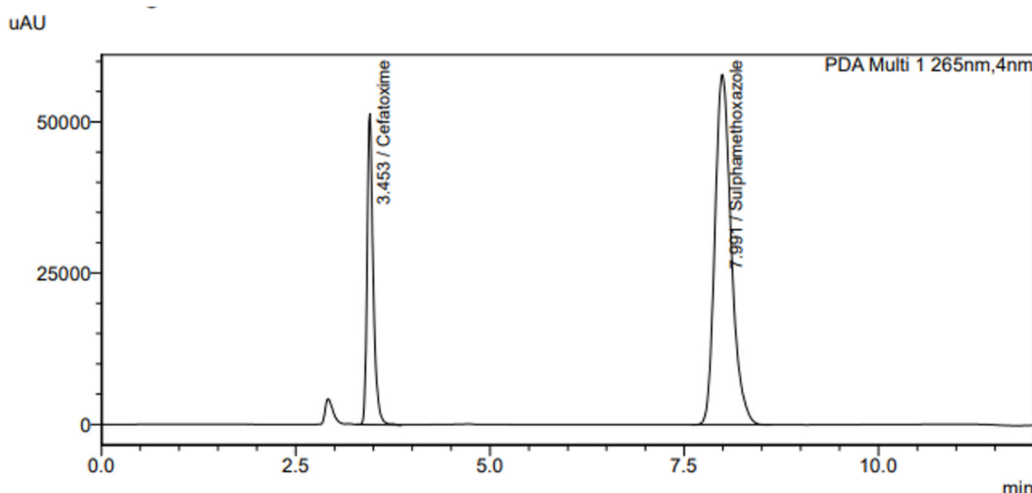


Fig 6: Chromatogram of Assay

The assay results for CFT showed an average assay value of 100.13%, with a %RSD of 0.6, indicating high accuracy and precision. Similarly, the assay results for SMZ demonstrated an average assay value of 99.96%, with a %RSD of 0.5, confirming consistency and reliability in drug quantification (Fig. 6). The standard and sample areas for both compounds were closely matched, ensuring the method's robustness. The low %RSD values ($\leq 1.0\%$) further validate the precision of the analytical method, making it suitable for routine quality control analysis.

The method validation for Cefotaxime (CFT) and Sulfamethoxazole (SMZ) demonstrated excellent linearity within the 10-100 $\mu\text{g/mL}$ range, with regression coefficients of 0.9957 for CFT and 0.9933 for SMZ, confirming a strong correlation between concentration and response.

The method was confirmed to be specific, as no interfering peaks were observed at the retention times of either drug. System precision was validated with a %RSD of 0.9% for both CFT and SMZ, while method precision showed 0.9% for CFT and 0.5% for SMZ, meeting the requirement of $\%RSD \leq 2.0$, demonstrating reproducibility. Accuracy values were 100.08% for CFT and 100.06% for SMZ, remaining within the 98-102% range, indicating high reliability.

The method showed high sensitivity, with limits of detection (LOD) of 0.07 $\mu\text{g/mL}$ for CFT and 0.04 $\mu\text{g/mL}$ for SMZ, and limits of quantification (LOQ) of 0.21 $\mu\text{g/mL}$ for CFT and 0.13 $\mu\text{g/mL}$ for SMZ, fulfilling the standard S/N ratio criteria (3:1 for LOD and 10:1 for LOQ).

Robustness testing, including variations in flow rate (FM), flow precision (FP), mobile phase composition (MM), and mobile phase precision (MP), resulted in %RSD values below 2.0, confirming the method's stability under minor modifications. These results indicate that the method is precise, accurate, sensitive, and robust, making it suitable for routine analysis of CFT and SMZ.

CONCLUSION

An accurate, precise, robust, and very cost-effective method was developed for the concurrency estimation of CFT and SMZ in tablets. CFT and SMZ were kept for 3.453 minutes and 7.991 minutes, respectively. The recovery rates for CFT and SMZ were 100.08% and 100.06%, respectively. LOD and LOQ values of 0.08 and 0.25, and 0.04 and 0.12, respectively, were determined using the regression equation. The regression coefficient for CFT is 0.9957, but for SMZ it is 0.9933. The reduction in retention and run lengths rendered the developed system both inexpensive and user-friendly, hence enhancing its efficacy for sample analysis in drug testing facilities.

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