Research Article

# Development and Validation of Stability Indicating UFLC Method for the Estimation of Cefotaxime Sodium and Diclofenac Sodium in Bulk and Pharmaceutical Dosage Forms

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**ABSTRACT**

A simple, responsive and stability indicating method has been developed for the simultaneous determination of Cefotaxime sodium and Diclofenac sodium using Ultra fast liquid chromatographic method (UFLC). The analysis was performed on Kromasil $C_{18}$ (250 × 4.6mm, 5µm) column using 1% formic acid in methanol and acetonitrile (80: 20 v/v) as mobile phase at flow rate 1 mL/min. The analytes were monitored with PDA detector at 260nm. In this developed method Cefotaxime sodium and Diclofenac sodium elutes at a retention time of 2.20 and 2.91 min respectively. The proposed method is having linearity in the concentration range from 5 to 50µg/mL of Cefotaxime sodium and Diclofenac sodium. The current method was validated with respect to system suitability, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery) and robustness according to the ICH guidelines. Forced degradation studies were also performed to check out the stability of the drugs under acidic, oxidation, alkaline, thermal, and UV degradation conditions. The proposed method can be readily utilized for determination of Cefotaxime sodium and Diclofenac sodium.

**Keywords:** Cefotaxime sodium, Diclofenac sodium, UFLC, Stability Indicating method.

## INTRODUCTION

Cefotaxime sodium is (6R,7R)-3-[(acetyloxy)methyl]-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is a beta lactam, third generation cephalosporin antibiotic.[1] Cefotaxime sodium is used for the treatment of infections caused by various gram-positive and gram negative bacteria like meningitis, septicaemia, biliary-tract infections, pneumonia, peritonitis and urinary-tract infections. The pharmacology of the Cefotaxime sodium is similar to that of the penicillins, It cause bacterial cell death (bactericidal) by acting on the bacterial cell wall and hinders with synthesis of peptidoglycan layer in cell wall, there by eventually causing cell lysis, it also binds and inhibits the activity of enzymes responsible for peptidoglycan synthesis.[2-4]

Diclofenac sodium is 2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetic acid, it is a phenyl acetic acid derivative having a effective cycloxygenase inhibition activity. It is a non-steroidal anti-inflammatory drug (NSAID) that is in general prescribed for the treatment of musculoskeletal injuries, degenerative arthritis, rheumatism and post-surgery analgesia in human and veterinary medicine.[5-7]

The combination of Cefotaxime sodium and Diclofenac sodium was selected for the current study. The wide literature survey carried out revealed that there is no method reported for the simultaneous estimation of these drugs, so the aim of present study was to develop a cost effective stability indicating UFLC method which is sensitive, simple, linear, precise, accurate, rapid, validated and cost effective method for the simultaneous estimation of these drugs in dosage forms. [8,9]

## MATERIALS AND METHODS

### Chemicals and reagents

Pure sample of Cefotaxime sodium and Diclofenac sodium was received from Micro Labs, Bangalore. Cefotaxime sodium and Diclofenac sodium formulation was obtained from local pharmacy. HPLC grade water, methanol and acetonitrile was obtained from Merck Pvt. Ltd, Mumbai. The chemicals used are of analytical reagent grade (AR grade) like orthophosphoric acid procured from Loba Chemie., Mumbai.

### Instrumentation

The current research was carried out on UFLC (SHIMADZU) equipped with PDA detector with LC solution software. Separation was attained using $C_{18}$ column. The mobile phase was a mixture of 1% formic acid in methanol and acetonitrile (80: 20 v/v). The contents of mobile phase were filtered before use through membrane filter (0.45 µ). The optimized chromatographic conditions are shown in Table 1.

### Preparation of Mobile Phase

Mobile phase is prepared by adding 1ml of formic acid in 99ml of methanol (1% formic acid in methanol) and acetonitrile were used in the ratio of 80: 20 (v/v).

### Preparation of Standard Solutions

Stock solution of Cefotaxime sodium and Diclofenac sodium was prepared by dissolving 100 mg of Cefotaxime sodium and Diclofenac sodium drugs in 50 mL of methanol in 100mL volumetric flask dissolved and volume

was made up to 100 mL using the methanol to get the standard stock solutions of concentration 1 mg/mL (1000 µg/mL) for both Cefotaxime sodium and Diclofenac sodium. Different working standard solutions were prepared from the above solution.

**Table 1:** Optimized chromatographic conditions

| Chromatoghaphic Conditions | |
|---|---|
| Column | $C_{18}$ (250 x 4.6 mm. 5 µ) Kromosil |
| Flow rate | 1.0 mL/min |
| Run time | 10 min |
| Wavelength | 260nm |
| Injection Volume | 10µL |
| Detector | PDA Detector |
| Elution | Isocratic |
| Mobile Phase | 1.0 % formic acid in methanol and Acetonitrile was used in the ratio of 80 : 20 (v/v) |
| Column oven temperature | 25 ± 5ºC |

### Preparation of Calibration Curve

From the stock solution (1000 µg/mL) aliquots of Cefotaxime sodium and Diclofenac sodium were pipetted into a series of 10 mL volumetric flask. The final volume was made up to the mark by using HPLC grade methanol. 10µL solution was injected to the column and peak areas were measured and the calibration curve was obtained. Linear correlations were found between peak ratios of Cefotaxime sodium and Diclofenac sodium and are described by regression equation. The Beer's law was obeyed in the concentration range of 5 – 50 µg/mL. (Figure 1)

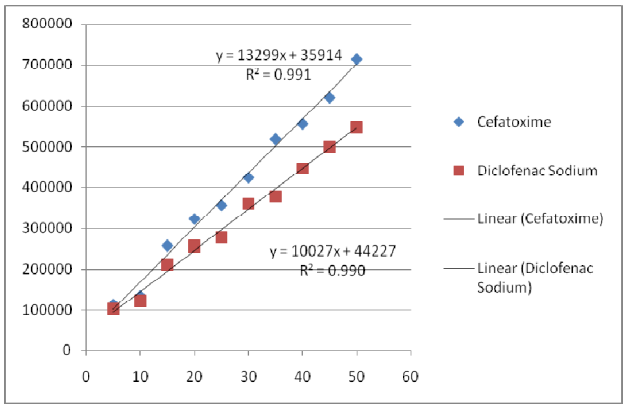


**Figure 1:** Standard calibration graph of Cefotaxime sodium and Diclofenac sodium

### Preparation of sample solution of formulation

Sample powder corresponding to 100 mg of Cefotaxime sodium and Diclofenac sodium from their respective formulation was weighed and transferred to 100 ml volumetric flask dissolved in 50ml of methanol and volume was made up to the mark with HPLC grade methanol such that the final concentration equal to 1000 µg/ ml. The prepared sample was mixed well and filtered through membrane filter of 0.45 µ pore size, the clear filtrate was than diluted to required concentrations.

### Assay procedure

From the fine powder of marketed formulations, an accurate amount of 100mg of powder was transferred into a 100ml volumetric flask and diluted with methanol. Resulting stock solution was diluted further with methanol, such that the concentration of Cefotaxime sodium and Diclofenac sodium was found to be each 20µg/ml. The column was equilibrated 1hr before use with the mobile phase flowing through the system with a flow rate of 1.0 ml/min and detector was set at a wavelength of 260nm. The retention time of Cefotaxime sodium and Diclofenac sodium in bulk drug were found to be 2.20 and 2.91 (Figure 2A) and the retention time of Cefotaxime sodium and Diclofenac sodium in its pharmaceutical formulation were found to be 2.26 and 3.0 (Figure 2B) respectively and blank (diluent) chromatogram is shown in (Figure 2C).

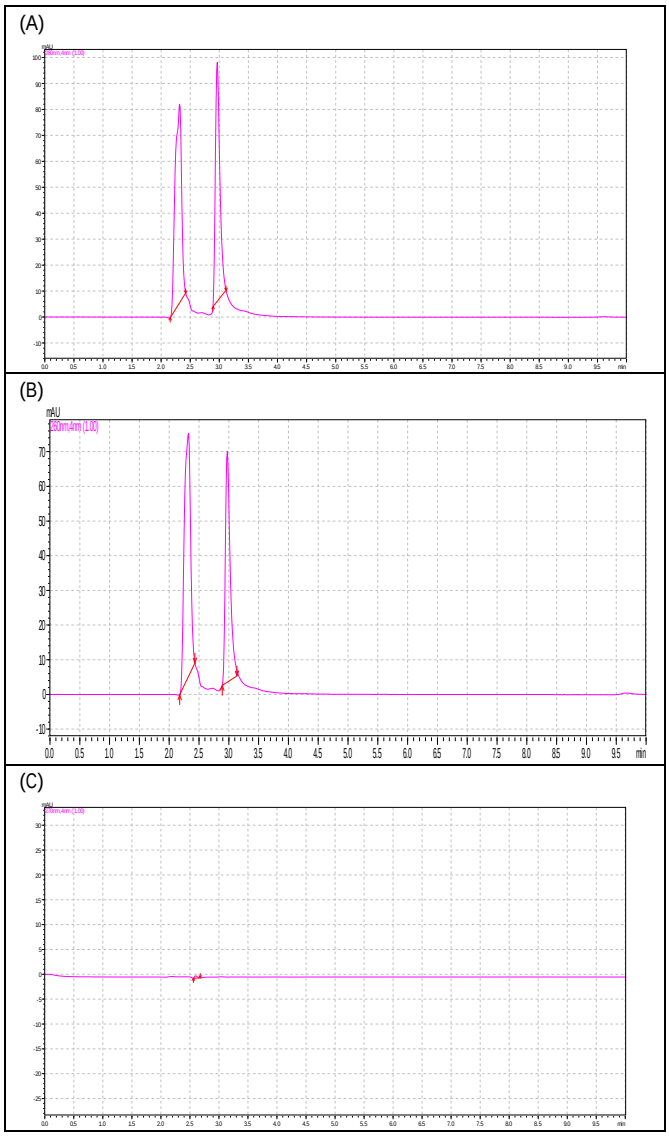


**Figure 2:** Chromatogram of **(A)** Standard Cefotaxime sodium and Diclofenac sodium (50µg/ml), **(B)** Sample chromatogram of Cefotaxime sodium and Diclofenac sodium, **(C)** Chromatogram of Blank

## RESULTS AND DISCUSSION

### Method validation[10-12]

The method was validated for different parameters like linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ), robustness.

### Linearity

From the experimental conditions described above, linear calibration curves of Cefotaxime sodium and Diclofenac sodium were obtained for ten different concentrations level for both. The $r^2$ for Cefotaxime sodium was 0.991 and for Diclofenac sodium was 0.990. Linear correlations were found between peak area of Cefotaxime sodium and Diclofenac sodium concentration and are described by the regression equation. The linearity range for Cefotaxime sodium and Diclofenac sodium is 5-50 µg/ml. Results are specified in Table 2.

### Precision

The intra-day and inter-day precision of the assay method was evaluated at three concentration levels (5µg, 15µg and 50µg) for each analyte and the RSD% of three obtained assay values were calculated on both Intraday and interday results are summarized in the Table 2.

### Accuracy

To set up the accuracy of the optimized method, triplicates of sample solutions were spiked with the test solutions of Cefotaxime sodium and Diclofenac sodium at 80%, 100% and 120% of the specification were prepared separately and injected into UFLC system according to the test procedure. The 'amount of drug added', 'amount of drug found' and average % recovery for Cefotaxime sodium and Diclofenac sodium spiked levels were calculated and the results are summarized in the Table 3.

**Table 2:** System suitability parameters of the proposed analytical method of Cefotaxime sodium and Diclofenac sodium

| Parameters | | Cefotaxime sodium | Diclofenac sodium |
|---|---|---|---|
| Linearity range (µg/ml) | | 5-50µg/ml | 5-50µg/ml |
| Regression equation | | Y = 13299x + 35914 | Y = 10027 x + 44227 |
| Slope | | 13299 | 10027 |
| Intercept | | 35914 | 44227 |
| Correlation coefficient | | 0.991 | 0.990 |
| Retention Time (Rt) min | | 2.26 | 2.95 |
| LOD (µg/ml) | | 0.820 | 0.627 |
| LOQ (µg/ml) | | 2.69 | 1.313 |
| Resolution | | 3.169 | -- |
| Tailing factor | | 0.853 | 1.609 |
| Theoretical plates | | 2907.385 | 5521.762 |
| Precision | Intraday % RSD | 0.80 | 0.62 |
| | Interday % RSD | 0.82 | 1.2 |

**Table 3:** Recovery results for Cefotaxime sodium and Diclofenac sodium.

| Level of % Recovery | Amount of std drug taken (µg/mL) | Amount of drug added (µg/mL) | Total amount of drug (µg/mL) | Total amount of drug found | %Recovery |
|---|---|---|---|---|---|
| 80 | 20 | 16 | 36 | 35.3 | 98.2 |
| | | | | 35.7 | 99.4 |
| | | | | 36.4 | 101.3 |
| | | | | ***Mean*** | ***99.6*** |
| 100 | 20 | 20 | 40 | 40.96 | 102.4 |
| | | | | 39.9 | 99.8 |
| | | | | 40.16 | 40.16 |
| | | | | ***Mean*** | ***100.8*** |
| 120 | 20 | 24 | 44 | 43.6 | 99.1 |
| | | | | 43.7 | 99.4 |
| | | | | 44.7 | 101.6 |
| | | | | ***Mean*** | ***100.03*** |

### Robustness

According to the ICH, robustness for an analytical procedure is a "measure of its capability to remain unaffected by minor, but deliberate variations in method optimized conditions. The most important aspect of robustness is to develop method that allows predictable variations in the optimized method parameters. ICH guidelines states that robustness must be considered early in the development phase of a method. The characteristic variations studied under this parameter are mobile phase composition, pH, flow rate, temperature, wavelength and the results are shown in Table 4 respectively.

### Forced degradation studies (Stress testing)

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Stock solution of the drug 50µg/ml for Cefotaxime sodium and Diclofenac sodium was prepared and subjected to following stress conditions at 0min, 30min, 1hr, 2hrs, 4hrs, 8hrs, 6hrs and 32hrs and results

are summarized in Table 5. For all the stability study, the percentage recovery of the sample after degradation was evaluated by calculating the percentage assay and by comparing the assay results with the assay of unstressed sample (Figure 3A).

**Acid hydrolysis:** Cefotaxime sodium and Diclofenac sodium of 50 µg/ ml was treated with 1ml of acid (0.1N HCl) and kept heating for 1 hr. After 1 hr the solution was neutralized with 0.1N NaOH analyzed using UFLC. (Figure 3B)

**Oxidation:** Cefotaxime sodium and Diclofenac sodium of 50 µg/ ml was mixed with 5 mL of 20% aqueous hydrogen peroxide solution and heating for 60 min. (Figure 3C)

**Alkali hydrolysis:** Cefotaxime sodium and Diclofenac sodium of 50 µg/ ml was treated with 1ml of alkali (1N NaOH) and kept heating for 50min. After heating the solution was neutralized with 1N HCl. (Figure 3D)

**Thermal:** Samples were heated at 80°C for 1 hr. (Figure 3E)

**Photolysis:** Samples were exposed to UV light for 1 hr and observed by UFLC. (Figure 3F)

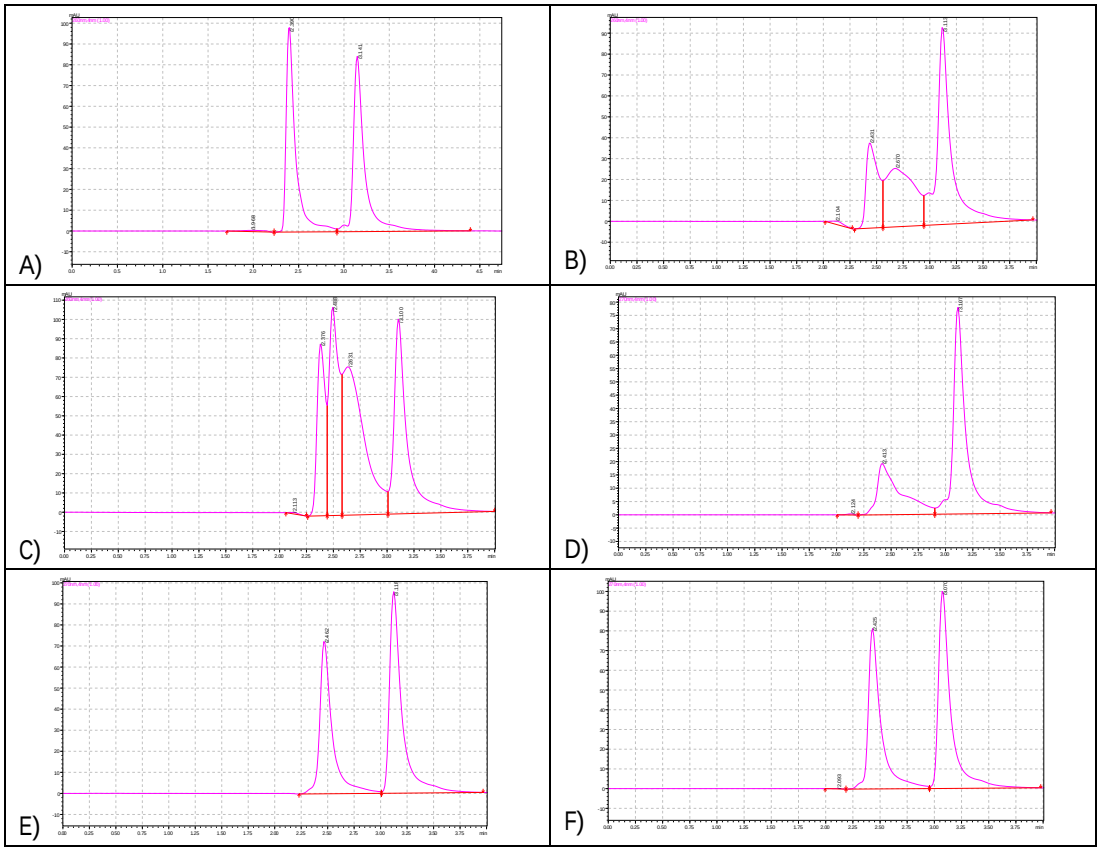


**Figure 3:** Chromatogram of **(A)** Unstressed Sample [at normal conditions], **(B)** 0.1N HCl Stressed Sample [Acid Stress degradation], **(C)** Peroxide Stressed Sample [Oxidation Degradation], **(D)** 0.1N NaOH Stressed Sample [Alkali Stress Degradation], **(E)** Thermal Stressed Sample [Thermal Stress degradation], **(F)** UV-Light Exposed Sample [Photo Stress degradation].

**Table 4:** Results for Robustness for Cefotaxime sodium & Diclofenac sodium

| | | Cefotaxime sodium | | | Diclofenac sodium | | |
|---|---|---|---|---|---|---|---|
| **Condition** | | **Tailing** | **Theoretical plates** | **% RSD** | **Tailing** | **Theoretical plates** | **% RSD** |
| As such condition (optimized method) | | 0.853 | 2907.385 | | 1.609 | 5521.762 | |
| Mobile phase ratio As such (80:20) | 75:25 | 1.098 | 3456.98 | 0.57 | 1.688 | 4887.37 | 0.98 |
| | 85:15 | 0.642 | 2698.65 | 1.87 | 1.243 | 4998.9 | 1.09 |
| % of formic acid | Decreased (-0.2 units) | 1.234 | 2457.98 | 1.37 | 1.28 | 5772.42 | 0.69 |
| | Increased (+0.2 units) | 0.988 | 3475.2 | 1.98 | 0.99 | 5839.8 | 1.26 |
| Flow rate | Decreased (-0.2 mL/ min) | 0.879 | 2908.32 | 0.39 | 1.567 | 4982.79 | 1.58 |
| | Increased (+0.2 mL/ min) | 1.38 | 3019.8 | 0.53 | 1.383 | 5883.94 | 1.98 |
| Column temperature | Decreased (-5°C) | 0.834 | 3098.1 | 0.98 | 0.845 | 6097.12 | 1.45 |
| | Increased (+5°C) | 1.00 | 2987.3 | 1.09 | 1.859 | 5048.22 | 1.87 |
| Wave length | Decreased (1nm) | 1.223 | 3762.5 | 0.98 | 1.465 | 5267.42 | 1.37 |
| | Decreased (2nm) | 1.008 | 2698.65 | 0.78 | 1.84 | 5362.27 | 0.48 |
| | Increased (1nm) | 0.897 | 3089.1 | 1.58 | 0.98 | 4872.1 | 1.47 |
| | Increased (2nm) | 1.098 | 3475.2 | 1.95 | 1.370 | 5733.1 | 1.85 |

**Table 5**: Results for Recovery studies of Cefotaxime sodium & Diclofenac sodium after the stress conditions

| Time | Drug | UV | Heat | 0.1N HCl | 0.1N NaOH | 3% $H_2O_2$ |
|---|---|---|---|---|---|---|
| 0 min | Cefotaxime sodium | 82.24% | 73.11% | 71.65% | 72.34% | 56.47% |
| | Diclofenac Sodium | 84.23% | 76.76% | 87.79% | 89.35% | 81.34% |
| 30 min | Cefotaxime sodium | 77.34% | 60.76% | 57.29% | 61.34% | 44.19% |
| | Diclofenac Sodium | 80.34% | 67.31% | 84.14% | 87.34% | 74.34% |
| 1 hr | Cefotaxime sodium | 69.32% | 47.86% | 52.3% | 54.34% | 32.47% |
| | Diclofenac Sodium | 72.43% | 50.16% | 78.86% | 80.34% | 68.23% |
| 2 hr | Cefotaxime sodium | 61.73% | 28.66% | 37.47% | 42.34% | 25.19% |
| | Diclofenac Sodium | 67.34% | 37.14% | 74.78% | 78.38% | 60.87% |
| 4 hr | Cefotaxime sodium | 54.22% | 19.81% | 28.07% | 30.87% | 15.47% |
| | Diclofenac Sodium | 59.34% | 21.69% | 67.27% | 70.34% | 44.34% |
| 8 hr | Cefotaxime sodium | 47.82% | 8.89% | 14.64% | 13.32% | 4.43% |
| | Diclofenac Sodium | 52.23% | 30.15% | 59.65% | 57.23% | 32.62% |
| 16 hr | Cefotaxime sodium | 39.22% | --- | 6.34 | --- | --- |
| | Diclofenac Sodium | 43.87% | --- | 44.64 | 43.24% | 22.23% |
| 32 hr | Cefotaxime sodium | 22.43% | 11% | --- | --- | --- |
| | Diclofenac Sodium | 44.24% | ---- | 33.11 | 32.23% | 11.33% |

## CONCLUSION

The study describes simple, rapid, sensitive, reliable, specific, accurate and precise stability indicating UFLC method was developed and validated for the estimation of Cefotaxime sodium & Diclofenac sodium. The method has a good resolution for the determination of Cefotaxime sodium & Diclofenac sodium making it a suitable choice for quality control laboratories, industries and research laboratories for routine and biological sample analysis.

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