Original Article

A validated stability indicating high-performance liquid chromatographic method for simultaneous estimation of cefuroxime sodium and sulbactam sodium in injection dosage form

Abstract

Background: A fixed dose combination of cefuroxime sodium (β lactam antibiotic) and sulbactam sodium (β Lactamase inhibitor) is used in ratio of 2:1 as powder for injection for the treatment of resistant lower respiratory tract and other infections. **Aims:** A simple, precise, and accurate ion-pair reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for determination of cefuroxime Na(CEF) and sulbactam Na(SUL) in injection. **Materials and Methods:** Isocratic RP-HPLC separation was achieved on an ACE C_{18} column (150×4.6 mm id, 5 µm particle size) using the mobile phase 0.002 M tetrabutylammonium hydroxide sulfate (TBAH) in 10 mm potassium di-hydrogen phosphate buffer–acetonitrile (86:14 v/v, pH 3.7) at a flow rate of 1.0 ml/min. **Results and Conclusion:** The retention time of sulbactam Na and cefuroxime Na were 3.2 min and 10.2 min, respectively. The ion-pairing reagent improved the retention of highly polar sulbactam Na on reverse-phase column. The detection was performed at 210 nm. The method was validated for linearity, precision, accuracy, robustness, solution stability, and specificity. The method was validated for linearity, precision, accuracy, robustness, solution stability, and specificity. The method was linear in the concentration range of 10–100 µg/ml for cefuroxime Na and 5–50 µg/ml for sulbactam Na, with a correlation coefficient of 0.9999 and 0.9998 for the respective drugs. The intraday precision was 0.13–0.21% and 0.48–0.65%, and the interday precision was 0.32–0.81% and 0.60–0.83% for cefuroxime Na and sulbactam Na, respectively. The accuracy (recovery) was found to be in the range of 98.76–100.61% and 98.99–100.30% for cefuroxime Na and sulbactam Na, respectively The drugs were found to degrade under hydrolytic and oxidative conditions. The drugs could be effectively separated from different degradation products, and hence the method can be used for stability analysis.

Key words:
Cefuroxime sodium, forced degradation, reverse-phase high-performance liquid chromatography, stability-indicating method, sulbactam sodium, validation

Introduction

Cefuroxime sodium is sodium(7R)-3-carbamoyloxymethyl-7-[(z)-furan-2-yl-2-methoxyiminoacetamido]-3-cephem-4-carboxylate. Cephalosporins are bactericidal and have the same mode of action as other β-lactam antibiotics (such as penicillin), but are less susceptible to hydrolysis of β-lactamase produced by microbes. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls.[1-3] Sulbactam sodium is sodium(7R)-3-carbamoyloxymethyl-7-[(z)-furan-2-yl-2-methoxyiminoacetamido]-3cephem-4-carboxylate. It is an irreversible inhibitor of β-lactamase; it binds the enzyme and does not allow it to interact with the antibiotic. Hydrolysis of the β-lactam rings either by enzymatic cleavage with β-lactamase or by acid destroys

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Falguni M. Patel, Jayant B. Dave, Pratik J. Vyas, Chhagan N. Patel

Department of Quality Assurance, Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India

Address for correspondence:
Ms. Falguni M. Patel,
Shri Sarvajanik Pharmacy College, Near Arvind Baugh, Mehsana, Gujarat, India.
E-mail: falgunipatel1486@gmail.com

the antibacterial activity of β-lactam antibiotic. Certain molecules can inactivate β-lactamase, thus preventing the destruction of β-lactam antibiotics.[1-10]

The chemical structures of cefuroxime Na and sulbactam Na are shown in Figures 1 and 2, respectively.

A detailed survey of analytical literature for cefuroxime Na revealed several methods based on various techniques, viz. high-performance liquid chromatography (HPLC),[11-13] spectrophotometry,[14-16] spectrofluorimetry,[17] and specific stability-indicating method by UV–visible method.[18] Similarly, a survey of the analytical literature for sulbactam Na revealed several methods based on various techniques, viz. HPLC,[19-22] spectrophotometry,[23-25] and high-performance thin layer chromatography (HPTLC).[26] According to detailed survey of analytical literature, none of the reported analytical procedures describes a simple and satisfactory HPLC method for simultaneous determination of cefuroxime Na and sulbactam Na in their combined dosage forms. Hence, the objective of this work was to develop suitable stability-indicating HPLC and method for combination drug product containing cefuroxime Na and sulbactam Na.

Materials and Methods

Instrumentation

Liquid chromatographic Shimadzu (LC-2010C_{HT}) system manufactured by Shimadzu, Kyoto, Japan, equipped with auto-sampler, UV and Photodiode Array (PDA) detector, and Rheodyne injector with 20 µl loop, and ACE C_{18} column (150 × 4.6 mm id, 5 µm particle size) was used. An analytical balance (Acculab ALC-210.4, Huntingdon Valley, PA, USA), pH meter (Thermo Electron Corp., Pune, India), and sonicator (EN 30 US Enertech Fast Clean, Mumbai, India) were used.

Materials

Cefuroxime Na and sulbactam Na bulk powder were gifted by Zydus Cadila Health Care Ltd., Ahmedabad, India, and Bharat Parentral Ltd., Baroda, India, respectively. The commercial injectable product was procured from the local market. Acetonitrile (HPLC Grade, Finar Chemicals Pvt. Ltd., Ahmedabad, India), tetrabutylammonium hydroxide (Loba Chemine Pvt. Ltd., Mumbai, India), water (HPLC Grade, Finar Chemicals Pvt. Ltd., Ahmedabad, India), and nylon filter (Millipore Pvt. Ltd., Bangalore, India) were used.

Preparation of stock solution

Accurately weighed CEF and SUL (100 mg and 50 mg, respectively) were transferred into a 100 ml volumetric flask and dissolved in and diluted to the mark with water to obtain the standard stock solutions, 1000 µg/ml CEF and 500 µg/ml SUL. The stock solutions were serially diluted with water to obtain solutions in the linearity range of 10–100 µg/ml for CEF and 5–50 µg/ml for SUL.

Preparation of sample solution

Ten market preparations, FASTGARD 2.25 (1500 mg CEF and 750 mg SUL), were taken and the weight of average content was determined. Powder weight equivalent to 20 mg cefuroxime and 10 mg sulbactam was transferred to 100 ml volumetric flask and dissolved in water with sonication. This was further diluted with water to obtain 20 µg/ml CEF and 10 µg/ml SUL. This was filtered through 0.45 µm filter and used for analysis.

Optimized chromatographic condition

- Stationary phase: ACE C18 (150 mm × 4.6 mm, 5 µm particle size)
- Mobile phase: 0.002 M tetrabutylammonium hydroxide sulfate (TBAH) in 10 mM potassium di-hydrogen phosphate buffer–acetonitrile (86:14 v/v)
- pH: pH of buffer was adjusted to 3.7 with dilute ortho-phosphoric acid
- Flow rate: 1 ml/min
- Detection wavelength: 210 nm
- Column temperature: 25°C
- Diluent: water

Method validation

This optimized HPLC method was validated for the parameters listed in ICH guidelines.[27]

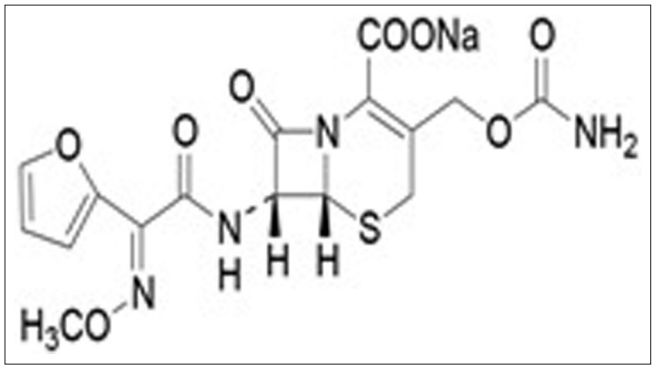

Figure 1: Chemical structure of cefuroxime sodium

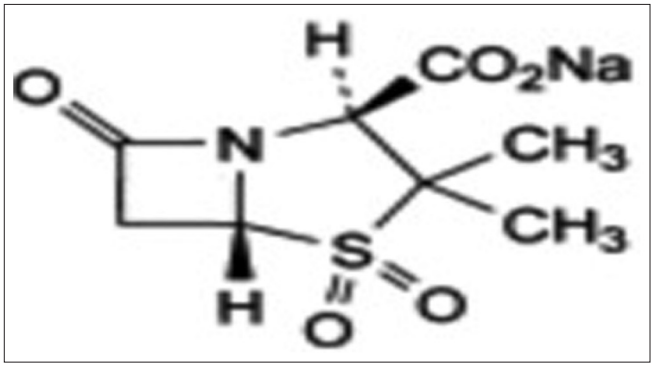

Figure 2: Chemical structure of sulbactam sodium

Linearity

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml of the stock solution of CEF and SUL were transferred into a series of 10 ml volumetric flasks and diluted to the mark with water. This yielded 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μg/ml of CEF and 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 μg/ml concentration of SUL, respectively. The calibration curve was constructed by plotting peak areas versus concentrations and the regression equation was calculated. Each response was an average of five determinations.

Precision

Intr- and inter-day precision were evaluated by determining the corresponding responses in triplicate on the same day and on different days for CEF (20, 30, and 40 μg/ml) and SUL (10, 15, and 20 μg/ml) standard solution. The repeatability was also performed using six replicate sample analyses. The results were reported in terms of relative standard deviation (% RSD).

Accuracy

Accuracy was determined by calculating recovery of CEF and SUL by the standard addition method. Known amounts of standard solutions of CEF (5, 10, 15 μg/ml) and SUL (2.5, 5, 7.5 μg/ml) were added to prequantified test solutions of CEF (20 μg/ml) and SUL (10 μg/ml). Each solution was injected in triplicate, and the recovery was calculated by measuring peak areas and fitting these values into the regression equation of the calibration curve.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the standard formula as per the ICH guidelines:

$$LOD = 3.3 \times (\sigma/S), LOQ = 10 \times (\sigma/S),$$

where σ is standard deviation of the response and S is slope of the calibration curve.

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variations in the chromatographic conditions. The robustness was checked by changing the mobile phase flow rate (±0.1 ml/min), composition (±5% in organic phase), pH (±0.2 units), and temperature (±5°C).

System suitability test parameters

System suitability parameters were verified with respect to number of theoretical plates, asymmetric factor, and RSD of six replicate of injection of CEF (20 μg/ml) and SUL (10 μg/ml).

Solution stability study

The stability of the test solution was evaluated. The solution was stored at ambient temperature and tested at intervals of 2, 6, 12, 18 and 24 h. The responses for the aged solution were evaluated using a freshly prepared standard solution.

Specificity

The specificity of the method was established through study of resolution factor of the drug peak from the nearest peak and the peak purity data of the analyte peaks in forced degradation samples.

Forced degradation studies

Acid degradation

Ten milliliters of a mixture of solution containing 2 mg/ml of CEF and 1 mg/ml of SUL in 0.1 N HCl was heated at 60°C for 1 h and then neutralized with 0.1 N NaOH. Further dilution was made with water to give CEF 400 μg/ml and SUL 200 μg/ml and analyzed under the optimized chromatographic conditions.

Alkali degradation

Ten milliliters of a mixture of solution containing 2 mg/ml of CEF and 1 mg/ml of SUL in 0.1 N NaOH was heated at 60°C for 1 h and then neutralized with 0.1 N HCl. Further dilution was made with water to give CEF 400 μg/ml and SUL 200 μg/ml and analyzed under the optimized chromatographic conditions.

Oxidative degradation

Ten milliliters of a mixture of solution containing 2 mg/ml of CEF and 1 mg/ml of SUL was prepared in 1% H_2O_2. The mixture was stored at room temperature for 30 min. Further dilution was made up with water to give CEF 400 μg/ml and SUL 200 μg/ml and analyzed under the optimized chromatographic conditions.

Neutral degradation

Ten milliliters of a mixture of solution containing 2 mg/ml of CEF and 1 mg/ml of SUL was prepared in water and heated at 60°C for 2 h. Further dilution was made up with water to give CEF 400 μg/ml and SUL 200 μg/ml and analyzed under the optimized chromatographic conditions.

Thermal degradation and photodegradation

For dry heat and photostability studies, the sample powder was placed in an oven at 60°C and in a photostability chamber (UV light) for 8 h. Appropriate dilutions of CEF 2000 μg/ml and SUL 1000 μg/ml were made in water to give CEF (400 μg/ml) and SUL (200 μg/ml) and analyzed under the optimized chromatographic conditions.

Results and Discussion

Optimization of the chromatographic conditions

The mobile phase was chosen after several trials with methanol, acetonitrile, water, and buffer solutions in various proportions and at different pH values. Mobile phase consisting of 0.002 M TBAH in 10 mM potassium

di-hydrogen phosphate buffer–acetonitrile (86:14 v/v, pH 3.7) was selected to achieve maximum separation and resolution. Ion-paring reagent was used to improve the retention of highly polar sulbactam sodium. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time for CEF (10.2±0.02) and SUL (3.2±0.03) [Figure 3].

Linearity

The response for the drugs was found to be linear in the concentration range of 10–10 μg/ml for CEF and 5–50 μg/ml for SUL, with correlation coefficient of 0.9999 and 0.9998, respectively. The linear regression equations obtained are: y = 10554x + 5270 and y = 5850x + 451.7 for CEF and SUL, respectively [Table 1].

Precision

The % RSD value for intraday precision study was found to be 0.13–0.21% for CEF and 0.48–0.65% for SUL, and the interday precision was found to be 0.32–0.81% and 0.60–0.83% for CEF and SUL, respectively, thus confirming precision of the method [Table 2].

Accuracy

Excellent recoveries were obtained at each level of added concentrations. The result obtained (n = 3 for each 25%, 50%, 75% level) indicated the mean recovery for CEF to be 99.12–100.61% and for SUL to be 98.99–100.30% [Table 3].

Limit of detection

The LOD was found to be 0.047 μg/ml and 0.053 μg/ml for CEF and SUL, respectively [Table 4].

Limit of quantitation

The LOQ calculated by standard formula as given in ICH guidelines was found to be 0.14 μg/ml and 0.16 μg/ml for CEF and SUL, respectively [Table 4].

Robustness

There were no significant differences in the test sample between the results obtained by applying the analytical condition established for the method and those obtained in experiments in which some of the conditions were varied slightly. Thus, the method was shown to be robust [Table 5].

Table 1: Linearity data for CEF and SUL

Cefuroxime sodium		Sulbactam sodium	
Concentration (μg/ml)	Area* ± SD	Concentration (μg/ml)	Area* ± SD
10	123,784 ± 152	5	30,492 ± 95
20	217,882 ± 421	10	59,534 ± 322
30	327,947 ± 288	15	89,681 ± 371
40	422,370 ± 905	20	114,804 ± 1033
50	517,784 ± 1657	25	147,647 ± 306
60	626,244 ± 1019	30	178,197 ± 868
70	737,066 ± 2585	35	198,828 ± 571
80	852,045 ± 869	40	234,376 ± 266
90	954,364 ± 155	45	263,794 ± 487
100	1,077,665 ± 4843	50	296,169 ± 489

*Average of five determinations and SD is standard deviation; CEF – Cefuroxime Na; SUL – Sulbactam Na

Table 2: Result from determination of precision of CEF and SUL

Concentration (μg/ml)		Intraday precision area* ± % RSD		Interday precision area* ± % RSD	
CEF	SUL	CEF	SUL	CEF	SUL
20	10	214,330 ± 0.21	58,687.7 ± 0.65	205,533 ± 0.40	54,718.0 ± 0.70
30	15	321,790 ± 0.13	88,126.3 ± 0.48	314,061 ± 0.32	82,958.7 ± 0.83
40	20	423,672 ± 0.11	115,545 ± 0.52	417,780 ± 0.51	111,175 ± 0.60

*Average of three determinations and % RSD is relative standard deviation; CEF – Cefuroxime Na; SUL – Sulbactam Na

Table 3: Accuracy data for analysis of CEF and SUL

% Addition	Amount of test solution		Amount of std. added		Conc. found		Amount recovered* ± SD		% Recovery	
	CEF	SUL	CEF	SUL	CEF	SUL	CEF	SUL	CEF	SUL
0	20	10	0	0	19.68	9.78				
25	20	10	5	2.5	24.71	12.26	5.03 ± 0.0011	2.47 ± 0.0017	100.61	98.99
50	20	10	10	5	29.55	14.71	9.87 ± 0.0032	4.92 ± 0.0045	98.76	98.58
75	20	10	15	7.5	34.55	17.30	14.86 ± 0.0085	7.52 ± 0.0078	99.12	100.30

*Average of three determinations; CEF – Cefuroxime Na; SUL – Sulbactam Na

System suitability test parameters

The system suitability test parameters are listed in Table 6.

Solution stability study

The solution stability study at different time intervals showed that the CEF and SUL solutions were stable up to 24 h at ambient temperature as no significant difference was found in the results for CEF and SUL.

Formulation analysis

CEF and SUL injection content was found to be 99.01±0.49% and 98.20±0.12%, respectively [Table 7].

Stability-indicating study

Acid degradation

Acid degradation study showed one additional peak for SUL at relative retention time (RRT) of 0.8 and three additional peaks at RRT of 0.7, 0.8, and 1.6. The peak purity of the analyte peaks was 1.0 for CEF and SUL, and resolution from the nearest peak was 3.3 for CEF and 4.2 for SUL [Figure 4].

Alkali degradation

Base degradation study showed one additional peak for SUL at RRT of 0.8 and three additional peaks at RRT of 0.7, 1.0, and 1.3. The peak purity of the analyte peaks was 1.0 for CEF and 0.9999 for SUL, and resolution from the nearest peak was 3.1 for CEF and 3.8 for SUL [Figure 5].

Oxidative degradation

Oxidative degradation study showed no additional peak for SUL and three additional peaks for CEF at RRT of 0.4, 0.5, and 0.9. The peak purity of the analyte peaks was 1.0 for CEF and SUL, and resolution from the nearest peak was 2.0 for CEF and 5.2 for SUL [Figure 6].

Neutral degradation

Neutral degradation study showed no additional peak for

Table 4: Summary of validation parameters

Validation parameter	CEF	SUL
Regression equation	y=10554x+5270	y=5850x+451.7
Linearity	0.999	0.999
Precision (% RSD)		
Intraday (%)	0.13–0.21	0.48–0.65
Interday (%)	0.32–0.81	0.60–0.83
Recovery (%)	98.76–100.61	98.99–100.30
LOD (µg/ml)	0.04	0.05
LOQ (µg/ml)	0.14	0.16

CEF – Cefuroxime Na; SUL – Sulbactam Na

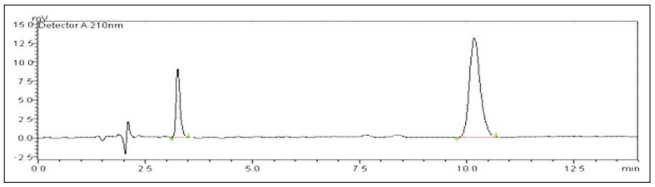

Figure 3: HPLC chromatogram of cefuroxime sodium (CEF) and sulbactam sodium (SUL)

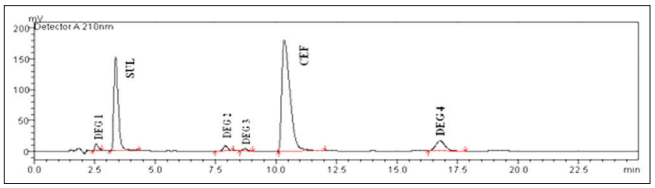

Figure 4: Acid degradation of cefuroxime sodium and sulbactam sodium

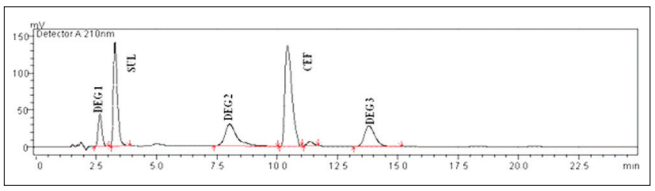

Figure 5: Alkali degradation of cefuroxime sodium and sulbactam sodium

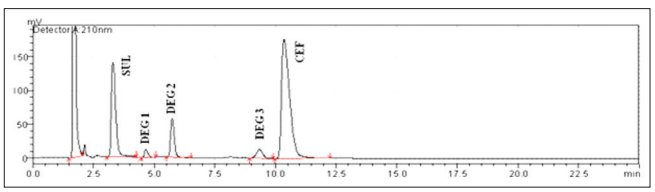

Figure 6: Oxidative degradation of cefuroxime sodium and sulbactam sodium

Table 5: Robustness of result

Condition	Variation	CEF			SUL		
		% Assay	SD	% RSD	% Assay	SD	% RSD
Temp. (30±5°C)	35°C	99.64	0.35	0.15	98.18	0.46	0.32
	25°C	99.44	0.65	0.45	98.35	0.52	0.26
Flow rate (1±0.1 ml/min)	1.1 ml/min	99.22	0.48	0.32	98.01	0.91	0.85
	0.9 ml/min	99.34	0.95	0.75	98.18	0.85	0.56
Organic phase (14±5%)	13+87 (v/v)	98.73	1.054	1.014	98.52	0.42	0.34
ACN:buffer, 14:86 (v/v)	15+85 (v/v)	98.90	1.012	0.95	98.67	0.75	0.62
pH (3.7±0.2)	pH 3.5	99.20	0.85	0.76	98.10	0.32	0.24
	pH 3.9	99.10	0.96	0.85	98.80	0.78	0.92

CEF – Cefuroxime Na; SUL – Sulbactam Na

Table 6: System suitability parameter

Parameter	CEF	SUL
Retention time (min)	10.2±0.026	3.2±0.023
Theoretical plate±SD	7633±25.24	5244±26.88
Asymmetry±SD	1.1±0.08	1.3±0.03

CEF – Cefuroxime Na; SUL – Sulbactam Na

Table 7: Analysis of market formulation

Parameter	CEF	SUL
Label claim (% w/w)	1500 mg	750 mg
Drug content (%)±SD	99.01±0.43	98.22±0.25
% RSD	0.31	0.18

CEF – Cefuroxime Na; SUL – Sulbactam Na

Table 8: Result of degradation

Type of degradation	Condition	No. of peaks	% Degradation	Peak purity
Acid degradation	0.1 M HCl, 60°C,1 h	4	CEF: 18.43 SUL: 11.70	CEF: 1.0 SUL: 1.0
Basic degradation	0.1 N NaOH, 60°C, 30 min	4	CEF: 38 SUL: 28	CEF: 1.0 SUL: 0.99998
Neutral condition	60°C, 2 h	2	CEF: 38.14 SUL: 11.70	CEF: 1.0 SUL: 1.0
Photolytic degradation	1.2 million lux, 8 h	–	CEF: 0.43 SUL: 0.26	CEF: 1.0 SUL: 1.0
Oxidative degradation	1% H_2O_2, RT 30 min	3	CEF: 21.79 SUL: 24.98	CEF: 1.0 SUL: 1.0
Thermal degradation	60°C, 8 h	–	CEF: 3.5 SUL: 10	CEF: 1.0 SUL: 1.0

CEF – Cefuroxime Na; SUL – Sulbactam Na

SUL and two additional peaks for CEF at RRT of 0.7 and 0.8. The peak purity of the analyte peaks was 1.0 for CEF and SUL, and resolution from the nearest peak was 3.6 for CEF and 2.7 for SUL [Figure 7].

Thermal study

Thermal degradation study showed negligible degradation and no additional peaks [Figure 8].

Photodegradation study

Thermal degradation study showed negligible degradation and no additional peaks [Figure 9].

The developed method successfully separated cefuroxime and sulbactam from degradation products formed under stressed conditions. CEF was found to degrade significantly under alkaline condition, followed by acidic and neutral conditions, whereas SUL was found for degrade to a lower extent under these conditions. Both the drugs were found to degrade significantly under oxidative condition, whereas they were not found to degrade under uv light exposure. Sulbactam was found to be more susceptible to thermal degradation compared to cefuroxime [Table 8].

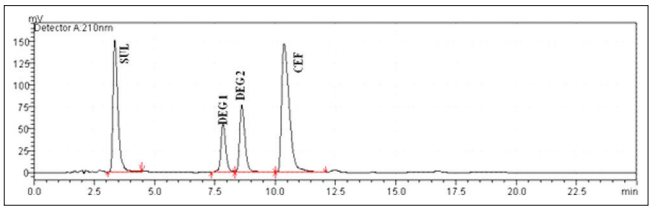

Figure 7: Wet degradation of cefuroxime sodium and sulbactam sodium

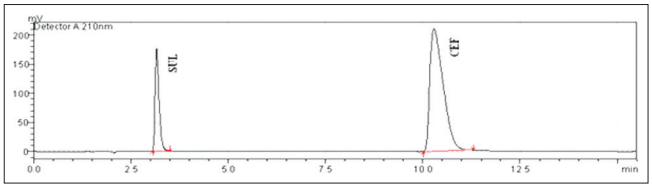

Figure 8: Dry degradation of cefuroxime sodium and sulbactam sodium

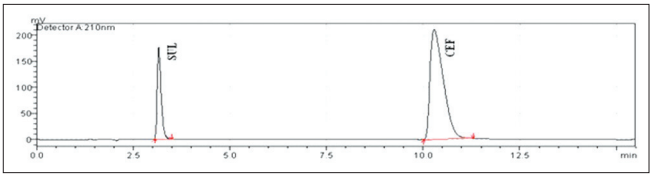

Figure 9: Photodegradation of cefuroxime sodium and sulbactam sodium

Conclusion

A new analytical method has been developed for the estimation of CEF and SUL mixture in injection dosage form. Forced decomposing study was performed to reveal the degradation pattern and establish stability-indicating assay method. Both the drugs were found to degrade significantly under alkaline, acidic, neutral, and oxidative conditions, and were comparatively stable under thermal and photolytic conditions. There was no interference of degradation products in the determination of CEF and SUL, confirming the stability-indicating property. So, developed method applied as stability indicating assay method for CEF and SUL.

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