



Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Cefepime and Enmetazobactam in Combination

Pasupula Tulasi¹, Gunturu Jaya Syamala^{2*}, Y Ankamma Chowdary³,
Inturi Venkata Rama Rao⁴ and Gunturu Uma Soundarya⁵

¹Master of Pharmacy, Department of Pharmaceutical Quality Assurance, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

²Assistant Professor, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

³Dean, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

⁴Professor, Department of Pharmaceutics, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

⁵Research Scholar, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

***Corresponding Author:** Gunturu Jaya Syamala, Assistant Professor, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India.

DOI: 10.31080/ASPS.2026.10.1286

Received: September 08, 2026

Published: October 13, 2026

© All rights are reserved by **Gunturu Jaya Syamala, et al.**

Abstract

Introduction: Cefepime, a fourth-generation cephalosporin, is combined with enmetazobactam, a novel extended-spectrum β -lactamase (ESBL) inhibitor, to restore antibacterial activity against ESBL-producing Gram-negative organisms in complicated urinary tract infection. A simple, specific, stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of cefepime and enmetazobactam in combination.

Materials and Methods: Chromatographic separation was performed on a Waters Alliance e2695 system with a photodiode array (PDA) detector, using a Symmetry Shield RP-18 column (250 $\times$ 4.6 mm, 5 μ m) and a mobile phase of methanol and potassium dihydrogen phosphate buffer (pH 2.5, adjusted with ortho-phosphoric acid) in the ratio 30:70 v/v, isocratically delivered at 1.0 mL/min. Detection was carried out at 281 nm, the isosbestic wavelength identified by PDA scanning. The method was validated for system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantitation (LOQ) and forced-degradation behaviour as per ICH guidelines.

Results: Cefepime and enmetazobactam eluted at 2.122 minutes and 3.858 minutes, respectively, with a resolution of 8.15, tailing factors of 1.08 and 0.95, and theoretical plate counts of 9825 and 7531. Linearity was established over 50 - 300 µg/mL for cefepime ($R^2 = 0.99989$) and 12.5 - 75 µg/mL for enmetazobactam ($R^2 = 0.99969$). Mean recoveries were 99.5% (cefepime) and 98.9% (enmetazobactam), with %RSD below 1% for system precision and below 2% for method and intermediate precision. LOD/LOQ were 0.6/2.0 µg/mL for cefepime and 0.15/0.5 µg/mL for enmetazobactam. The method remained robust to $\pm 10\%$ variation in flow rate and $\pm 3\%$ variation in the organic-phase ratio. Under ICH-recommended stress conditions, both analytes showed pronounced degradation under acidic, alkaline, oxidative and hydrolytic conditions (10 - 16%) and comparatively limited degradation under reductive, thermal and photolytic stress ($< 4\%$), with peak-purity angle remaining below the purity threshold throughout.

Discussion: All validation parameters met ICH acceptance criteria, and the degradation profile confirms the expected susceptibility of the shared β -lactam pharmacophore to hydrolytic stress while demonstrating that the method can resolve the parent drugs from their degradation products.

Conclusion: The developed RP-HPLC-PDA method is simple, specific, accurate, precise, robust and stability-indicating, and is suitable for the routine simultaneous quality-control estimation of cefepime and enmetazobactam.

Keywords: Cefepime; Enmetazobactam; RP-HPLC; Method Development; Method Validation; Stability-Indicating; ICH Guidelines

Introduction

Extended-spectrum β -lactamase (ESBL)-producing Gram-negative pathogens are a major cause of complicated urinary tract infection (cUTI) and increasingly compromise the efficacy of cephalosporin therapy [1]. Cefepime, a fourth-generation cephalosporin, retains broad-spectrum activity against Gram-positive and Gram-negative organisms by inhibiting penicillin-binding proteins involved in bacterial cell-wall synthesis, but is itself susceptible to hydrolysis by ESBLs. Enmetazobactam is a novel, non- β -lactam ESBL inhibitor that protects cefepime from this enzymatic degradation, restoring bactericidal activity against resistant strains. The fixed-dose intravenous combination of cefepime and enmetazobactam was evaluated in a multicentre, randomised, double-blind phase 3 trial in which it demonstrated superiority over piperacillin-tazobactam for clinical cure and microbiological eradication in cUTI and acute pyelonephritis [2] and was subsequently approved by the United States Food and Drug Administration in February 2024 for this indication [3].

Given the recent introduction of this combination into clinical use, robust analytical methods for its quality control are still being established. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been reported for the bioanalytical quantitation of cefepime and enmetazobactam in human plasma [4] and a stability-indicating ultra-performance

liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has been described for cefepime and its degradants in active pharmaceutical ingredient [5]. However, mass-spectrometric methods require specialised instrumentation that is not universally available in routine pharmaceutical quality-control laboratories, and a simple, specific RP-HPLC method with ultraviolet/photodiode-array (PDA) detection and demonstrated stability-indicating capability for the simultaneous estimation of this specific combination has not been widely reported [6].

Aim of the Study

The present study was therefore undertaken to develop and validate such a method, with the specific objectives of (i) establishing simple, rapid chromatographic conditions for the simultaneous resolution of cefepime and enmetazobactam, and (ii) validating the method for system suitability, specificity, linearity, accuracy, precision, robustness, LOD, LOQ and forced-degradation behaviour in accordance with the ICH Q2 (R1) guideline on validation of analytical procedures [7].

Materials and Methods

Chemicals and reagents

Cefepime and enmetazobactam working standards were used as received. HPLC-grade methanol and Milli-Q water were obtained from Merck; potassium dihydrogen phosphate (KH_2PO_4) and orthophosphoric acid (OPA), both of analytical-reagent grade, were also

obtained from Merck. Solutions were filtered through 0.45 µm membrane/injection filters before chromatographic analysis.

Instrumentation

Chromatographic analysis was performed on a Waters Alliance e2695 HPLC system fitted with a quaternary isocratic pump, an autosampler and a photodiode array (PDA) detector, with data acquisition using Empower™ 2 chromatography software. A digital pH meter (Eutech), an analytical balance (Sartorius), a UV-visible spectrophotometer (UV-1700, Shimadzu-type bench system) and an ultrasonic bath (Unichrome UCA 701) were also used.

Determination of working wavelength:

Because two analytes were to be estimated simultaneously, the detection wavelength was selected at the isosbestic point — the wavelength at which the molar absorptivities of the two compounds are equal — to allow accurate quantitation of both analytes on a single chromatographic run. Standard solutions of cefepime and enmetazobactam were scanned individually between 200 and 400 nm using the PDA detector, against a methanol-KH₂PO₄ buffer (pH 2.5, OPA-adjusted; 30:70 v/v) blank. The overlaid spectra intersected at an isosbestic point of 281 nm (Figure 1), and this wavelength was accordingly selected for detection.

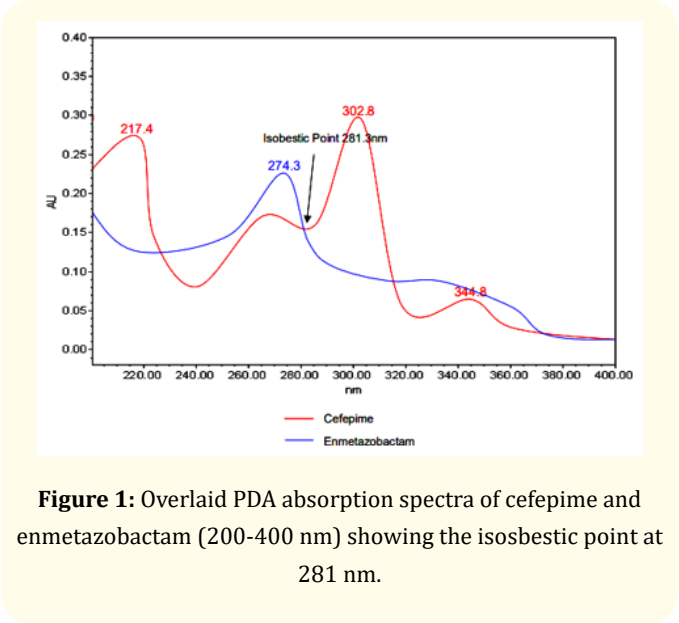


Figure 1: Overlaid PDA absorption spectra of cefepime and enmetazobactam (200-400 nm) showing the isosbestic point at 281 nm.

Chromatographic optimization and final conditions

Chromatographic conditions were optimized over six trials, varying the column, mobile-phase composition and buffer system. Initial trials using a Waters X-Bridge Phenyl column with acetonitrile-0.1% trifluoroacetic acid mobile phases gave retention times or plate counts outside the acceptable range, and a subsequent methanol-phosphate combination on the same column produced unresolved, unidentified peaks. Switching to a Symmetry Shield RP-18 column with a methanol-KH₂PO₄ (pH 2.5) mobile phase progressively improved peak shape and response; a mobile-phase ratio of 30:70 v/v (methanol:buffer) at a flow rate of 1.0 mL/min gave the highest, most symmetrical peak response with a short (6 min) run time and was therefore selected as the final, optimized condition (Table 1).

Parameter	Condition
Instrument	Waters Alliance e2695 HPLC with PDA detector
Column	Symmetry Shield RP-18 (250 × 4.6 mm, 5 µm)
Mobile phase	Methanol : KH ₂ PO ₄ buffer (pH 2.5, OPA-adjusted) = 30:70 v/v
Elution mode	Isocratic
Flow rate	1.0 mL/min
Detection wavelength	281 nm (PDA)
Injection volume	10 µL
Column temperature	Ambient (25°C)
Run time	6 min

Table 1: Optimized chromatographic conditions.

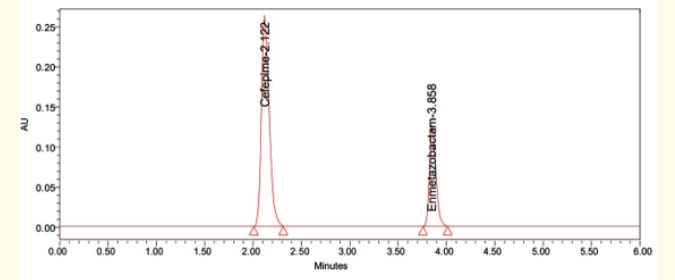


Figure 2: Representative optimized chromatogram showing baseline resolution of cefepime (2.122 minutes) and enmetazobactam (3.858 minutes).

Preparation of mobile phase and diluent

KH_2PO_4 buffer was prepared by dissolving 1.36g KH_2PO_4 in 1L of HPLC-grade water, adjusting the pH to 2.5 with OPA and filtering through a 0.45 μm membrane filter. The mobile phase was prepared by mixing methanol and this buffer in a 30:70 v/v ratio and filtering through a 0.45 μm membrane filter before use. Methanol was used as the diluent.

Preparation of standard solution

Cefepime (20 mg) and enmetazobactam (5 mg) were accurately weighed into a 10 mL volumetric flask, dissolved in diluent by sonication and made up to volume to obtain a stock solution. An aliquot (1 mL) of this stock was further diluted to 10 mL with diluent to give a working standard solution containing 200 $\mu\text{g/mL}$ cefepime and 50 $\mu\text{g/mL}$ enmetazobactam.

Preparation of sample solution

Cefepime and enmetazobactam sample (25 mg of the combined bulk drug) was accurately weighed into a 10 mL volumetric flask, dissolved in diluent by sonication for 30 minutes, centrifuged, and made up to volume. An aliquot (1 mL) of this solution was diluted to 10 mL with diluent and filtered through a 0.45 μm injection filter before analysis, to give final concentrations equivalent to the working standard.

Statistical analysis

All chromatographic determinations were performed with a minimum of three to six replicate injections per condition, as specified for each validation parameter below, and results are expressed as mean $\pm$ standard deviation (SD), with precision reported as percentage relative standard deviation (%RSD = $\text{SD}/\text{mean} \times 100$). Calibration data were fitted by unweighted least-squares linear regression of peak area (y) against nominal analyte concentration (x, $\mu\text{g/mL}$) using Empower™ 2 chromatography software, and the correlation coefficient (R^2), slope and y-intercept were recorded for each analyte. Acceptance of each validation parameter was based on the numerical criteria specified in the ICH Q2 (R1) guideline (e.g. %RSD $\leq$ 2.0% for precision, tailing factor $\leq$ 2, theoretical plates $\geq$ 2000, resolution $\geq$ 2, recovery within 98 - 102%).

Method validation

The optimized method was validated as per ICH guidelines for system suitability, specificity, linearity, accuracy, precision,

robustness, LOD, LOQ and forced-degradation (stability-indicating) behaviour [7].

System suitability: Six replicate injections of the standard solution were analyzed, and retention time, peak area, tailing factor, theoretical plate count, resolution and %RSD were evaluated against ICH/USP-recommended limits.

Specificity: Blank (diluent), placebo and standard/sample chromatograms were compared to confirm the absence of any interfering response at the retention times of cefepime and enmetazobactam.

Linearity: Six-point calibration curves were prepared by serial dilution of stock solution to give concentrations of 50 - 300 $\mu\text{g/mL}$ for cefepime and 12.5 - 75 $\mu\text{g/mL}$ for enmetazobactam (corresponding to 25 - 150% of the target assay concentration), and each level was injected and linearity evaluated by linear regression of peak area versus concentration; a correlation coefficient $\geq$ 0.999 was set as the acceptance criterion.

Limit of detection and limit of quantitation: LOD and LOQ were calculated from the standard deviation of the response (σ) and the slope of the calibration curve (S) using the equations $\text{LOD} = 3.3\sigma/S$ and $\text{LOQ} = 10\sigma/S$.

Accuracy: Recovery studies were performed by the standard-addition method at three levels (80%, 100% and 120% of the target assay concentration), with three replicate preparations at each level; a mean recovery of 98-102% was set as the acceptance criterion.

Precision: System precision (six replicate injections of standard solution), method precision/repeatability (six replicate sample preparations) and intermediate precision (repeated on a second day) were evaluated and expressed as %RSD, with an acceptance criterion of %RSD $\leq$ 2.0%.

Robustness: Robustness was evaluated by making small, deliberate changes to the flow rate (0.9, 1.0 and 1.1 mL/min) and to the organic-phase proportion of the mobile phase (27:73, 30:70 and 33:67, methanol:buffer), and observing the effect on retention time, peak area, resolution, tailing factor, theoretical plates and %RSD.

Forced degradation: The combined sample was subjected to acid hydrolysis (1N HCl, 60°C, 30 minutes, neutralized with 1N NaOH), alkaline hydrolysis (1N NaOH, 60°C, 30 minutes, neutralized with 1 N HCl), oxidative stress (10% w/v hydrogen peroxide, 60°C, 30 minutes), reductive stress (10% w/v sodium bisulphite, 60°C, 30 minutes), neutral hydrolysis (HPLC-grade water, 60°C), thermal stress (105°C, 3h, solid state) and photolytic stress (photostability chamber, 3h), and the stressed samples were analyzed under the optimized chromatographic conditions. Percentage assay remaining, percentage degradation, and PDA peak-purity angle and purity threshold were recorded for each condition to confirm specificity in the presence of degradation products.

Results

System suitability

System suitability parameters obtained from six replicate injections ($n = 6$) of the standard solution were within ICH/ USP-recommended limits for both analytes — tailing factor ≤ 2 , theoretical plates ≥ 2000 , resolution ≥ 2 and %RSD $\leq 2.0\%$ — confirming that the chromatographic system performed reproducibly (Table 2). Cefepime and enmetazobactam eluted at 2.122 minutes and 3.858 minutes, respectively, and were baseline-separated, with a resolution of 8.15.

Parameter	Cefepime	Enmetazobactam	ICH/USP acceptance limit
Retention time (min)	2.122	3.858	—
Peak area	2,736,891	1,328,271	—
Tailing factor	1.08	0.95	≤ 2.0
Theoretical plates (N)	9825	7531	≥ 2000
Resolution (Rs)	—	8.15	≥ 2.0
%RSD (peak area, $n = 6$)	0.46	0.12	$\leq 2.0\%$

Table 2: System suitability parameters for cefepime and enmetazobactam ($n = 6$).

Specificity

Blank and placebo chromatograms showed no response at the retention times of cefepime (2.122 min) or enmetazobactam (3.858 min), confirming that the chromatographic response of both analytes was specific and free from interference by the sample matrix.

Linearity

Calibration curves constructed from six concentration levels were linear over 50 - 300 $\mu\text{g/mL}$ for cefepime and 12.5 - 75 $\mu\text{g/mL}$ for enmetazobactam, with correlation coefficients of 0.99989 and 0.99969 and linear regression equations $y = 13380.20x + 12650.79$ and $y = 25817.39x + 16825.71$, respectively (Table 3, Figure 3), both exceeding the ICH acceptance criterion of $R^2 \geq 0.999$.

Analyte	Concentration ($\mu\text{g/mL}$)	Peak area	Regression equation
Cefepime	50	670946	$y = 13380.20x + 12650.79$ $R^2 = 0.99989$
	100	1355893	
	150	2052366	
	200	2709712	
	250	3326185	
	300	4022659	
Enmetazobactam	12.5	338694	$y = 25817.39x + 16825.71$ $R^2 = 0.99969$
	25.0	662942	
	37.5	1004715	
	50.0	1326318	
	62.5	1636431	
	75.0	1925746	

Table 3: Linearity data for cefepime and enmetazobactam.

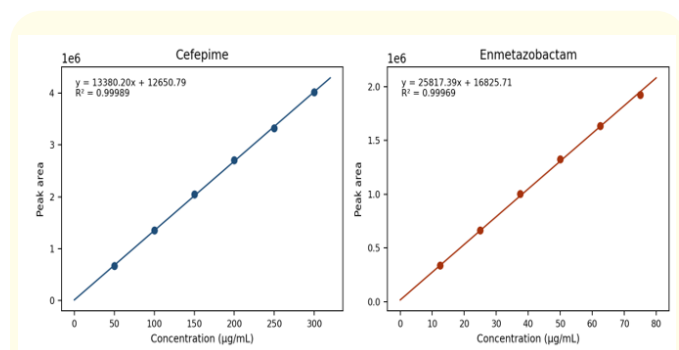


Figure 3: Calibration curves for cefepime and enmetazobactam.

Limit of detection and limit of quantitation:

The LOD (S/N = 3.3:1) and LOQ (S/N = 10:1) were 0.6 µg/mL and 2.0 µg/mL for cefepime, and 0.15 µg/mL and 0.5 µg/mL for enmetazobactam, respectively (Table 4), indicating that the method is sufficiently sensitive for the intended application.

Analyte	LOD (µg/mL)	S/N (LOD)	LOQ (µg/mL)	S/N (LOQ)
Cefepime	0.6	3.3	2.0	10
Enmetazobactam	0.15	3.3	0.5	10

Table 4: Sensitivity parameters (LOD and LOQ).

Accuracy

Recovery studies performed by standard addition at 80%, 100% and 120% of the target concentration gave mean percentage recoveries of 98.8 - 100.1% for cefepime and 98.7-99.3% for enmetazobactam (Table 5), with overall mean recoveries of 99.5% and 98.9%, respectively — both within the ICH-recommended range of 98 - 102%.

Analyte	Spiking level (%)	Mean recovery (%)
Cefepime	80	98.8
	100	99.5
	120	100.1
Enmetazobactam	80	98.7
	100	99.3
	120	98.8

Table 5: Accuracy (recovery) data for cefepime and enmetazobactam.

Precision

System precision (n = 6) gave %RSD of 0.46% (cefepime) and 0.12% (enmetazobactam). Method precision (repeatability, n = 6) gave %RSD of 0.94% and 0.59%, respectively, and intermediate precision (day 1 / day 2) gave %RSD of 0.97%/0.42% for cefepime and 0.84%/0.63% for enmetazobactam (Table 6) — all well within the ICH acceptance limit of %RSD ≤ 2.0%.

Analyte	System precision (n = 6)	Method precision / repeatability (n = 6)	Inter-mediate precision — Day 1	Inter-mediate precision — Day 2
Cefepime	0.46	0.94	0.97	0.42
En-metazo-bactam	0.12	0.59	0.84	0.63

Table 6: Precision data (%RSD) of the proposed method.

Robustness

Small, deliberate changes in flow rate (± 0.1 mL/min) and in the organic-phase proportion of the mobile phase (± 3%) produced only minor shifts in retention time and did not compromise resolution (R_s remained ≥ 6.8), tailing factor or theoretical plate count for either analyte; peak-area %RSD remained below 1% in all conditions except the higher-organic-phase trial for enmetazobactam, which nonetheless remained below the 2% acceptance limit (Table 7), confirming that the method is robust.

Forced degradation and specificity under stress

Both analytes showed pronounced degradation under acidic, alkaline, oxidative and neutral-hydrolytic conditions (10.3 - 15.6% for cefepime; 9.4 - 13.7% for enmetazobactam), and comparatively limited degradation under reductive, thermal and photolytic stress (0.6 - 3.5% for cefepime; 1.2 - 4.0% for enmetazobactam) (Table 8). Across all seven stress conditions, the PDA peak-purity angle for both analytes remained below the corresponding purity threshold, confirming that the cefepime and enmetazobactam peaks remained homogeneous and were resolved from their degradation products, and that the method is stability-indicating.

Variable	Level	Cefepime RT (min) / %RSD	Enmetazobactam RT (min) / %RSD
Flow rate (mL/min)	0.9	2.346 / 0.27	3.919 / 0.36
	1.0 (original)	2.122 / 0.46	3.858 / 0.12
	1.1	1.917 / 0.52	3.665 / 0.45
Organic phase (MeOH:buffer)	27:73	2.562 / 0.60	4.143 / 0.49
	30:70 (original)	2.124 / 0.46	3.851 / 0.12
	33:67	1.803 / 0.89	3.578 / 0.83

Table 7: Robustness data for cefepime and enmetazobactam.

Stress condition	Cefepime: % assay remaining	Cefepime: % degradation	Enmetazobactam: % assay remaining	Enmetazobactam: % degradation
Control (unstressed)	100.0	0.0	100.0	0.0
Acid (1N HCl, 60°C)	87.3	12.7	89.7	10.3
Alkali (1N NaOH, 60°C)	86.6	13.4	87.9	12.1
Peroxide (10% H ₂ O ₂ , 60°C)	84.4	15.6	86.3	13.7
Reduction (10% NaHSO ₃ , 60°C)	97.9	2.1	98.8	1.2
Thermal (105°C, 3h)	99.4	0.6	97.4	2.6
Photolytic (3h)	96.5	3.5	96.0	4.0
Neutral hydrolysis (H ₂ O, 60°C)	89.7	10.3	90.6	9.4

Table 8: Forced-degradation results for cefepime and enmetazobactam.

Note: Peak-purity angle remained below purity threshold for both analytes under all stress conditions (data recorded during analysis), confirming absence of co-eluting degradation products.

Discussion

The chromatographic behaviour observed during method development is consistent with the acid-base character of the two analytes. Cefepime carries a permanently cationic N-methylpyrrolidinium group, while enmetazobactam possesses a zwitterionic triazolium-carboxylate moiety; operating the mobile phase at pH 2.5, well below the carboxylate pK_a of both compounds, suppressed carboxylate ionization and favoured a consistent, protonated cationic form for both analytes, which minimized peak tailing (tailing factor ≤ 1.1) and produced sharp, symmetrical peaks on the Symmetry Shield RP-18 stationary phase. This mechanistic picture is consistent with the empirical observation that the earlier acetonitrile-trifluoroacetic acid mobile phases (Trials 1-2) gave poor plate counts or out-of-range retention, and that the initial methanol-phosphate trial on a phenyl-bonded column (Trial 3) produced unidentified peaks, whereas transferring to a base-deactivated, shielded C18 phase with a methanol-phosphate mobile phase (Trials 4-6) progressively resolved these issues as the methanol proportion was optimized to 30% v/v.

All validation parameters — system suitability, specificity, linearity, accuracy, precision, robustness, LOD/LOQ and forced degradation — met the acceptance criteria recommended in the ICH Q2 (R1) guideline [7] with correlation coefficients exceeding 0.999, recoveries within the 98 - 102% range, and %RSD values below 2% throughout. The method's tolerance to small, deliberate variation in flow rate and organic-phase ratio indicates adequate ruggedness for routine use. The forced-degradation profile is consistent with the well-documented susceptibility of the shared β-lactam (cephem) ring system to acid-, base- and peroxide-catalysed hydrolytic ring-opening [5] which explains the comparatively higher degradation observed under acidic, alkaline, oxidative and neutral-hydrolytic stress relative to the milder reductive, thermal and photolytic conditions. Critically, the peak-purity angle remained below the purity threshold for both analytes under every stress condition, demonstrating that the method can resolve cefepime and enmetazobactam from their degradation products and therefore qualifies as stability-indicating.

Relative to the previously reported LC-MS/MS bioanalytical method for this combination [4] and the UPLC-MS/MS stability-indicating method reported for cefepime alone [5] the present RP-HPLC-PDA method uses conventional, widely available instrumentation and a short (6 minutes) isocratic run, which is advantageous for routine pharmaceutical quality-control laboratories that may not have ready access to mass-spectrometric detection [8,9].

Limitations of the Study

This validation was performed on bulk drug (active pharmaceutical ingredient) rather than a specific marketed finished formulation, and quantitative assay data for a commercial combined dosage form are not reported here; application of the method to a specific marketed formulation, together with multi-laboratory reproducibility assessment and characterization of the individual degradation products by hyphenated techniques (e.g. LC-MS), represent appropriate directions for future work.

Conclusion

A simple, specific, accurate, precise, robust and stability-indicating RP-HPLC-PDA method was developed and validated for the simultaneous estimation of cefepime and enmetazobactam in combination, in accordance with the ICH Q2 (R1) guideline. Mechanistically, adequate peak shape and resolution were achieved by operating the mobile phase near pH 2.5 to control the ionization state of both analytes on a base-deactivated C18 stationary phase. The method demonstrated linearity ($R^2 > 0.999$), accuracy (recovery 98 - 102%), precision (%RSD < 2%), robustness and a stability-indicating capacity confirmed by peak-purity analysis under seven ICH-recommended stress conditions. On this basis, the method is recommended for adoption in the routine quality-control estimation of cefepime and enmetazobactam, and as a platform for future stability and forced-degradation studies of this combination.

Acknowledgement

The authors thank the management and Principal, School of Pharmacy, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu, Agiripalli, for providing the necessary facilities to carry out this work.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Bibliography

1. Bassetti M and Peghin M. "How to manage KPC infections". *Therapeutic Advances in Infectious Disease* 7 (2020): 2049936120912049.
2. Kaye KS, et al. "Effect of cefepime/enmetazobactam vs piperacillin/tazobactam on clinical cure and microbiological eradication in patients with complicated urinary tract infection or acute pyelonephritis: a randomized clinical trial". *Journal of the American Medical Association* 328.13 (2022): 1304-1314.
3. US Food and Drug Administration. "FDA approves new antibacterial drug for complicated urinary tract infections as part of ongoing efforts to address antimicrobial resistance [cefepime and enmetazobactam, Exblifep]". Silver Spring: FDA (2024).
4. Mameli M., et al. "Liquid chromatography-tandem mass spectrometry for the simultaneous quantitation of enmetazobactam and cefepime in human plasma". *Journal of Pharmaceutical and Biomedical Analysis* 174 (2019): 655-662.
5. Jabeen S and Venkatappa S. "Stability indicating UPLC-MS/MS method for quantification and identification of cefepime and its degradants in API". *Journal of Applied Pharmaceutical Science* 13.5 (2023): 199-207.
6. Shah CU and Satavara JR. "The review article on analytical method development and validation of antibiotic drugs cefepime and enmetazobactam for urinary tract infection". *International Journal of Research Publication and Reviews* 6.3 (2025): 10234-10240.
7. International Council for Harmonisation. ICH Q2 (R1): Validation of analytical procedures: text and methodology. Geneva: ICH (2005).
8. Snyder LR., et al. "Practical HPLC method development". 2nd edition. New York: John Wiley & Sons (1997).
9. Skoog DA., et al. "Fundamentals of analytical chemistry". 9th edition. Belmont: Brooks/Cole, Cengage Learning (2014).