



Revolutionizing Cephalosporin Analysis: Uniting All Generations with RP-HPLC Methods

¹Qumar Negar, ¹Shivam Kumar*, ¹Arvind Kumar Patel, ¹Dharmendra Kumar

¹Narayan Institute of Pharmacy, Gopal Narayan Singh University, Jamuhar, Sasaram, Rohtas- 821305 (Bihar), India

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Abstract

Antimicrobial resistance and the expanding therapeutic use of cephalosporins have increased the importance of reliable analytical procedures for identity, assay, purity, stability, and bioanalytical measurement. Cephalosporins comprise five generations with progressively modified structures and activity profiles, and these chemical differences can influence chromatographic retention, stability, and detection. Reversed-phase high-performance liquid chromatography (RP-HPLC) remains one of the most broadly applied techniques for cephalosporin analysis because it can accommodate pharmaceutical products, multicomponent formulations, and biological matrices while providing quantitative separation with conventional instrumentation. This review organizes the reported RP-HPLC approaches for cephalosporins across the first to fifth generations and critically compares stationary phases, mobile-phase composition, pH, flow rate, detection wavelength, retention behavior, sample preparation, and validation characteristics. Particular attention is given to simultaneous determination, bioanalytical applications, and stability-indicating methods. The compiled literature shows a strong preference for C18 stationary phases and UV/PDA detection, although C8 phases, ion-pair approaches, and HPLC-MS/MS have also been applied when matrix complexity or analyte selectivity requires additional control. Across generations, method selection should not rely on retention time alone; analytical performance must be considered in relation to selectivity, sensitivity, robustness, recovery, matrix effects, stability, and practical implementation. The review also identifies opportunities for more standardized, rapid, robust, and environmentally responsible chromatographic strategies for routine cephalosporin quality control and bioanalysis.

*Corresponding Author:

Mr. Shivam Kumar (shiv15gulshan@gmail.com)

ORCID ID: 0009-0008-2232-7203

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1. Introduction

Cephalosporins are β -lactam antibacterial agents that remain important in the management of infections caused by susceptible Gram-positive and Gram-

negative organisms. The evolution from early cephalosporins to later generations involved structural modification of the cephem nucleus and its

substituents to alter antibacterial spectrum, stability, pharmacokinetic behavior, and resistance characteristics. Because these modifications also influence physicochemical behavior, cephalosporins present a diverse analytical class rather than a uniform set of chromatographic analytes [1].

The analytical control of cephalosporin medicines is therefore closely linked to therapeutic reliability. Pharmaceutical products must be assessed for identity, assay, related substances, degradation products, and, where required, stability. In biological samples, the analytical problem becomes more demanding because cephalosporins may occur at lower concentrations and in the presence of endogenous components [2]. The literature compiled in this review includes studies describing identification, assay, impurity or degradation assessment, simultaneous estimation, bioanalytical measurement, and stability evaluation by HPLC or RP-HPLC in bulk drugs, finished dosage forms, and biological matrices. Studies were considered relevant when sufficient chromatographic information was available to compare practical variables such as stationary phase, mobile-phase composition, flow rate, detection wavelength, retention time, and validation characteristics [3].

Although spectrophotometry, electroanalytical methods, thin-layer chromatography, and NMR have all been used for cephalosporins, RP-HPLC has value when selective separation is needed in complex formulations or biological matrices. The present review therefore places RP-HPLC at the center of the discussion and uses the reported methods to identify cross-generational trends, analytical trade-offs, and gaps in current practice rather than treating the literature as a simple catalogue of methods [4].

2. Historical Development and Analytical Classification of Cephalosporins

2.1. Historical Background

The discovery of cephalosporins is linked to the work of Giuseppe Brotzu, who isolated antibacterial substances from the mold now recognized as *Acremonium*. Cephalosporin C was subsequently characterized and used as the parent structure from which semisynthetic derivatives were developed. The historical development of the class is important analytically because changes introduced at the cephem nucleus and side-chain positions generated compounds with different polarity, ionization, stability, and chromatographic behavior [5].

The cephalosporin scaffold combines a β -lactam ring

with a dihydrothiazine ring, while substitution at the principal side-chain positions contributes substantially to antibacterial and physicochemical differences. These structural variations explain why apparently similar compounds can require different mobile-phase ratios, column chemistries, detection wavelengths, or sample-treatment procedures. The historical expansion of the class thus also represents an expansion of the analytical design space [6].

2.2. Generational Classification and Structural Progression

The five-generation framework is primarily pharmacological, but it is also useful for organizing analytical literature. First-generation agents such as cefadroxil, cephalexin, cefazolin, and cefatrizine are well represented in conventional pharmaceutical assays [7]. Second-generation agents include cefaclor, cefprozil, and cefuroxime, for which both pharmaceutical and plasma methods are reported. Third-generation cephalosporins constitute the largest analytical group in the present compilation and include ceftriaxone, cefotaxime, ceftazidime, cefixime, cefdinir, cefpodoxime, cefoperazone, and related agents. Fourth-generation analysis is represented principally by cefepime and ceftipime, whereas fifth-generation analysis includes ceftaroline fosamil [8].

From an analytical perspective, progression across generations should not be interpreted as a simple increase in chromatographic difficulty. Instead, each generation adds compounds with distinct ionization and matrix-behavior profiles. Some later-generation agents also appear in combination with β -lactamase inhibitors or unrelated drugs, increasing the need for selective simultaneous separation. This is reflected in the expanded use of C18 columns, optimized buffered mobile phases, PDA detection, and in some bioanalytical applications, HPLC-MS/MS [9].

2.3. Structural and Pharmacological Relevance to Analysis

Cephalosporins exert antibacterial activity primarily by interacting with penicillin-binding proteins and inhibiting peptidoglycan cross-linking. Structural modifications that alter affinity, permeability, stability, and activity also modify the molecular environment presented to a chromatographic system. The analytical consequence is that retention and detection must be optimized for each chemical structure rather than assumed from its generation alone [10]. Representative structures of selected first-generation agents are shown in Figure 1, followed by second-, third-, fourth-, and fifth-generation examples in Figures 2-5.

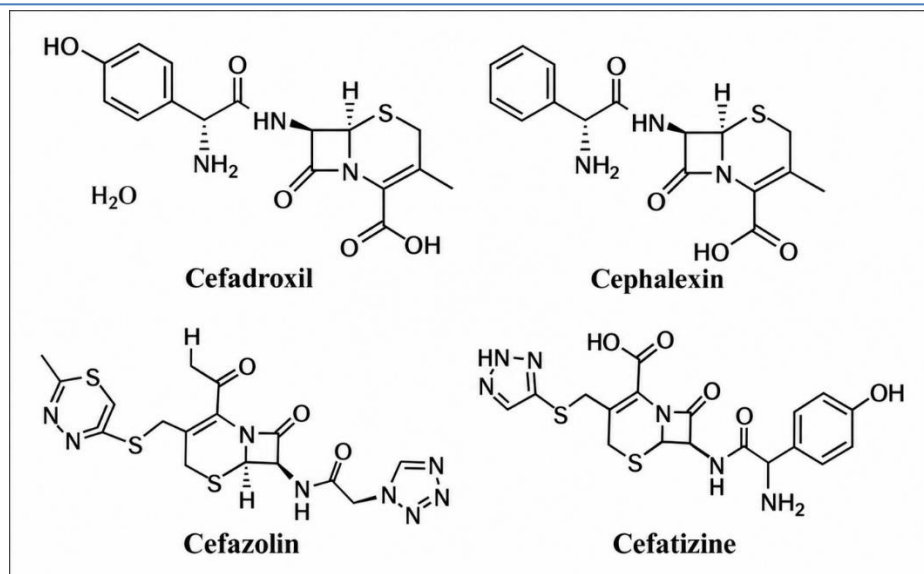


Figure 1: Representative structures of selected first-generation cephalosporins.

2.4. Second-generation Structural Characteristics

Second-generation cephalosporins extend activity toward a broader range of Gram-negative organisms while retaining useful activity against Gram-positive organisms. The compounds represented in the source

literature, including cefaclor, cefprozil, and cefuroxime, show substantial structural diversity, which is reflected in the range of reported C18 and C8 methods [11]. Their representative structures are shown in Figure 2.

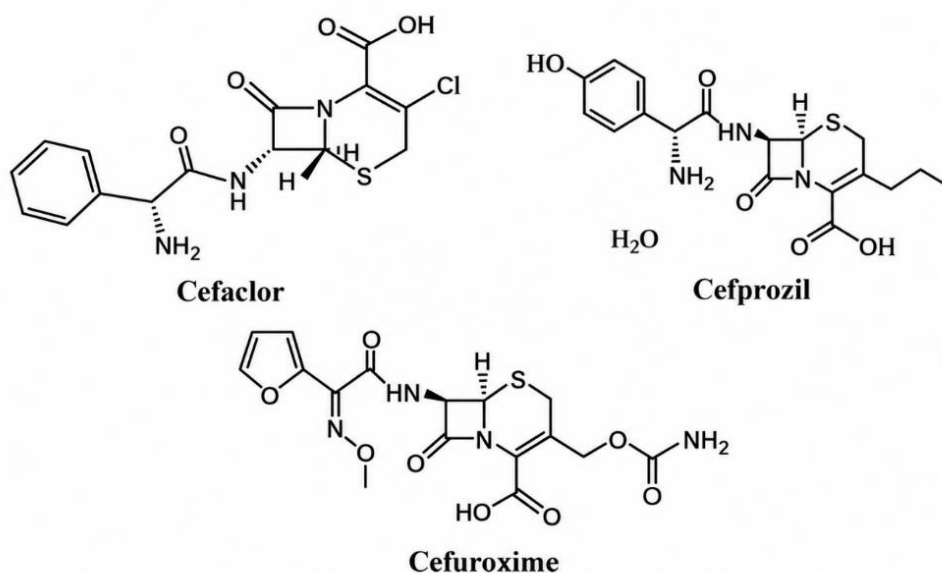


Figure 2: Representative structures of selected second-generation cephalosporins.

2.5. Third-generation Structural Characteristics

Third-generation compounds form the largest analytical group in the reviewed literature. Ceftriaxone, cefotaxime, ceftazidime, cefixime, cefdinir, cefpodoxime, cefoperazone, cefditoren, and related agents have been examined in bulk drugs,

dosage forms, dissolution studies, plasma, serum, urine, and multicomponent products. Their more extensive analytical coverage makes this generation particularly useful for identifying method-development trends [12]. Representative structures are shown in Figure 3.

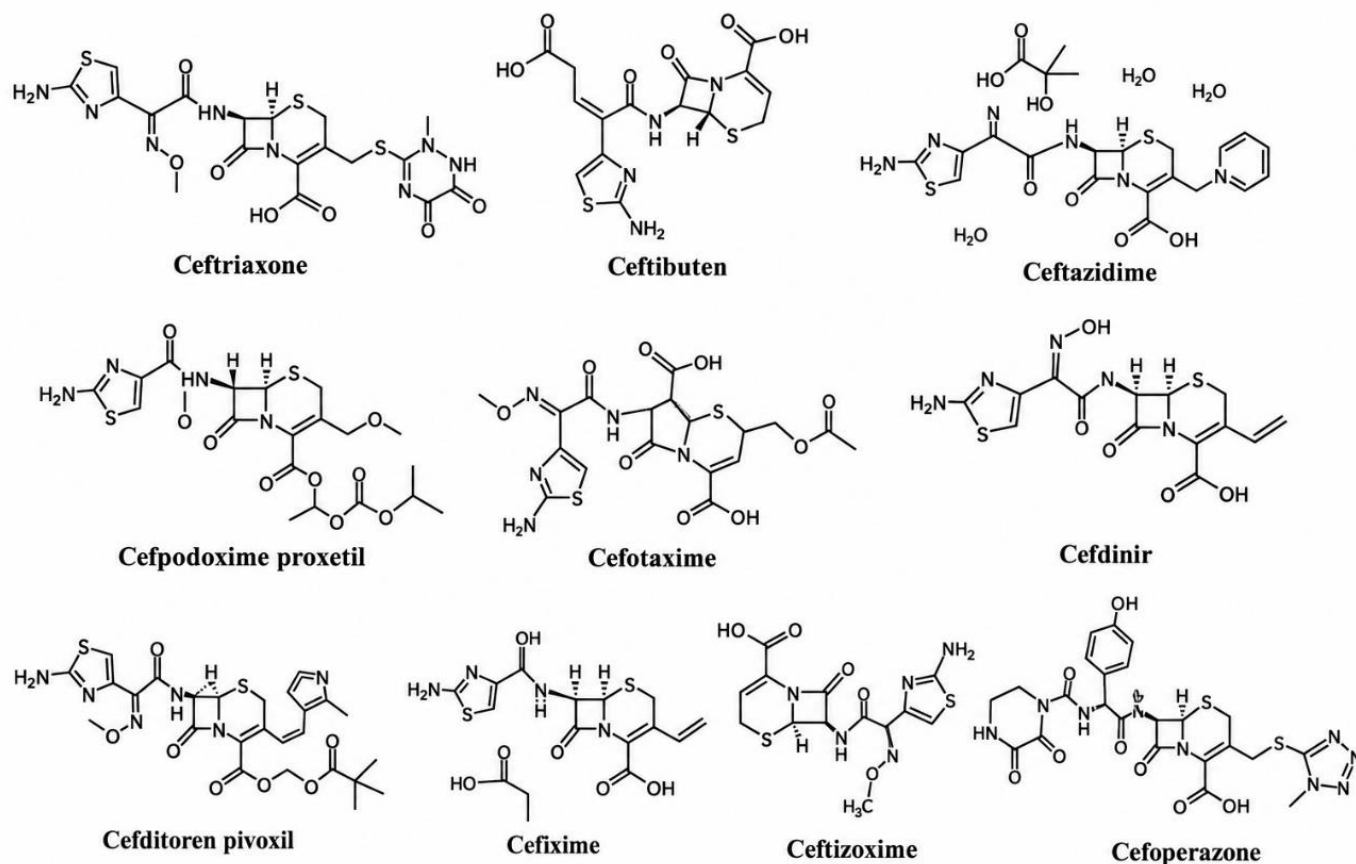


Figure 3: Representative structures of selected third-generation cephalosporins.

2.6. Fourth- and Fifth-generation Structural Characteristics

Cefepime represents the major fourth-generation analytical example, while ceftaroline represents the fifth-generation group in the compiled literature. Cefepime is structurally adapted for broad Gram-

negative activity and clinical use that may include central nervous system infections, whereas ceftaroline is distinguished by activity against methicillin-resistant *Staphylococcus aureus* and other susceptible Gram-positive organisms [13]. Representative structures are presented in Figures 4 and 5.

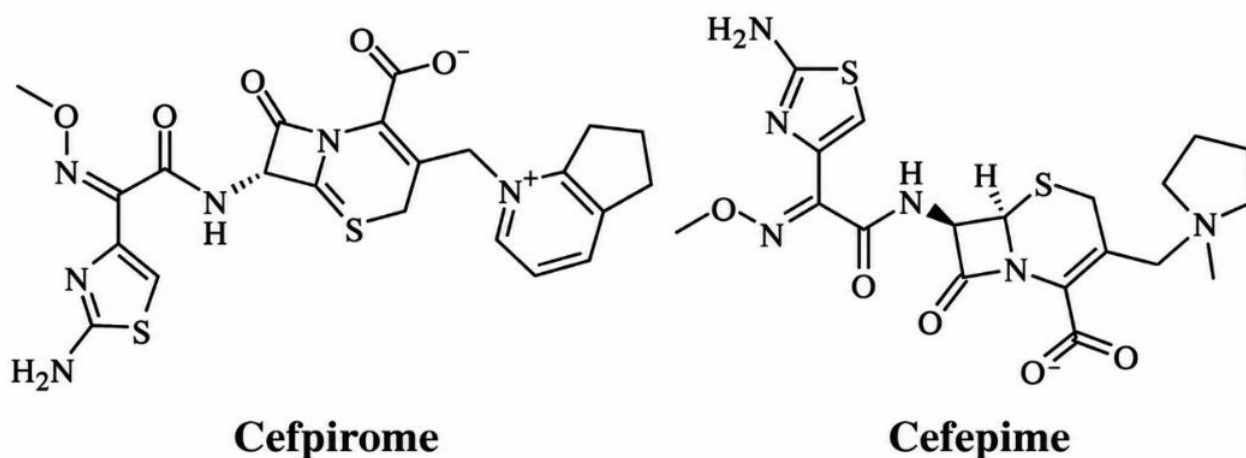
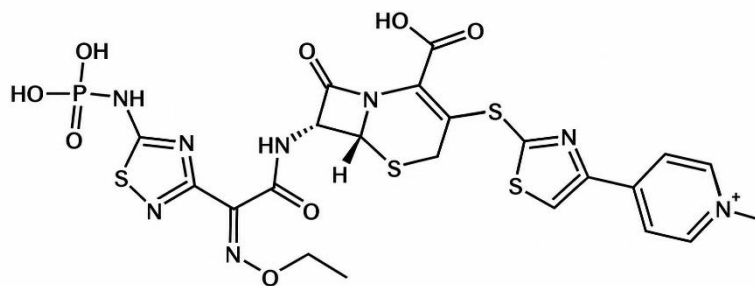


Figure 4: Representative structures of selected fourth-generation cephalosporins.



Ceftaroline fosamil

Figure 5: Representative structure of a fifth-generation cephalosporin.

3. Analytical Challenges in Cephalosporin Determination

3.1. Chemical Instability and Matrix Sensitivity

Cephalosporins can be sensitive to environmental and formulation conditions, and analytical methods therefore need to discriminate intact drug from degradation products when stability assessment is required [14]. The literature contains dedicated stability-indicating methods for cefazolin, cefuroxime axetil, cefdinir, cefixime, and combination products, demonstrating that chromatographic selectivity is central to meaningful assay results. A method that produces a short retention time but fails to resolve degradation products is not automatically superior to a longer method with demonstrable specificity [15].

3.2. Sample preparation and matrix effects

Sample preparation varies substantially with the matrix. For capsule and tablet formulations, the usual workflow involves quantitative transfer, dissolution, dilution, and filtration. For oral suspensions, representative portions are diluted before analysis [16]. Sterile powders for injection are reconstituted and diluted to the required working range. Biological samples require more rigorous treatment; the source literature describes protein precipitation, centrifugation, dilution, filtration, and solid-phase extraction for serum or plasma samples, while urine methods commonly employ buffered dilution and membrane filtration. These differences are analytical rather than merely procedural because recovery, matrix interference, and analyte stability can determine whether an otherwise suitable chromatographic method is fit for purpose [17].

3.3. Multicomponent formulation complexity

Simultaneous estimation creates an additional separation requirement because cephalosporins are often formulated with β -lactamase inhibitors, analgesics, antiemetics, fluoroquinolones, macrolides, mucolytics, or other co-administered agents. The compiled literature includes multiple simultaneous methods in which changes in stationary phase, mobile-phase composition, detection wavelength, and

flow rate were used to achieve adequate separation. Multicomponent analysis therefore provides a practical test of method selectivity and robustness beyond single-analyte assay [18].

4. RP-HPLC Principles and Method Development Considerations

4.1. Stationary phase selection

Reversed-phase separations reported in the source literature are dominated by octadecylsilane (C18) phases, with C8 phases used in selected biological or multicomponent methods. The preference for C18 reflects its broad applicability and the availability of conventional 4.6 mm internal-diameter analytical columns. C8 phases can provide different retention behavior for compounds or matrices in which excessive retention or selectivity is encountered on C18. The column should therefore be selected based on the analyte, matrix, desired run time, and separation requirement rather than on a generic preference for a particular phase [19].

4.2. Mobile-phase composition and pH

The reported methods use buffered aqueous phases combined with methanol, acetonitrile, or both. Phosphate buffers, ammonium acetate, acetate buffer, and related systems are common, while some methods use ion-pair reagents or triethylamine to modify retention and peak shape. The pH can be especially important for ionizable cephalosporins because small changes in ionization may alter retention, selectivity, and stability. The literature therefore illustrates the need to optimize pH together with organic-modifier proportion rather than adjusting either variable in isolation [20], [21].

4.3. Flow rate, detection, and retention behavior

Most conventional methods use flow rates around 0.7–1.5 mL/min, although biological or high-throughput methods may operate at lower or higher values. UV detection generally occurs between approximately 210 and 290 nm, with PDA or DAD used where spectral confirmation or multicomponent

analysis is beneficial [22]. Reported retention times vary from sub-minute measurements in some rapid methods to substantially longer separations such as the approximately 17 min retention reported for cefaclor in plasma. Retention time should be interpreted together with peak resolution, system suitability, sensitivity, and matrix selectivity; it should not be treated as a single surrogate for overall method quality [23].

4.4. Isocratic and gradient strategies

Isocratic RP-HPLC is widely used for routine pharmaceutical assays because of its simplicity, reproducibility, and ease of transfer to quality-control laboratories. Gradient strategies become more useful when several analytes or degradation products have substantially different retention characteristics. The source literature also demonstrates that UPLC and HPLC-MS/MS can reduce analysis time or improve selectivity in selected applications, but these platforms require more specialized instrumentation and method-transfer considerations [24].

4.5. Sample preparation strategies for pharmaceutical products and biological samples

The source manuscript illustrates four recurring sample-preparation contexts: solid oral dosage forms, oral suspensions, sterile injectable powders, and biological fluids. For capsules and tablets, quantitative transfer, dissolution, dilution, and filtration are generally sufficient when excipients do not compromise the assay [25]. Suspension products require representative dispersion and dilution to obtain a working concentration suitable for chromatographic measurement. Injectable powders are typically reconstituted and quantitatively diluted, with the final solution adjusted to the validated working range. These apparently simple procedures nevertheless require attention to quantitative transfer and solution stability because an error introduced before injection cannot be corrected by chromatographic performance [26].

Biological matrices require an additional cleanup step because proteins and endogenous components can affect analyte recovery, column performance, and detector response. The source literature describes precipitation with acetone, centrifugation, dilution, membrane filtration, and solid-phase extraction for serum or plasma analysis [27]. The choice among these approaches represents an analytical trade-off. Protein precipitation is relatively simple and rapid, whereas solid-phase extraction can provide greater cleanup at the cost of additional handling and consumables. Urine methods may permit simpler buffered dilution and filtration when the analyte

concentration and matrix characteristics allow this approach. Consequently, sample preparation should be treated as an integrated component of method development rather than as a separate procedural step [28].

4.6. Method-selection logic for routine and advanced applications

A practical method-selection strategy can be inferred from the compiled literature. When the analytical target is a single active ingredient in a relatively simple dosage form, an isocratic C18 procedure with UV detection is often sufficient. When the formulation contains several active ingredients, selectivity becomes the primary development target and C8 or C18 screening, pH adjustment, mixed organic modifiers, or an internal standard may be required [29]. When the matrix is plasma or serum, sample preparation and matrix control become equally important, and the use of PDA or mass spectrometric detection may provide additional confidence in peak identity. When degradation products are part of the analytical objective, the development process must demonstrate that the parent drug and relevant degradation peaks are chromatographically resolved [30].

The same logic can be applied when transferring a reported method to a new laboratory. Column chemistry should be treated as a controlled variable rather than assumed to be interchangeable across manufacturers. Mobile-phase composition should be prepared accurately, with pH adjustment performed consistently because small differences may change retention for ionizable analytes. Flow rate, column temperature, and detector wavelength should be treated as method parameters rather than incidental instrument settings. This approach is particularly important for cephalosporins because the compiled methods show that apparently minor changes in chromatographic conditions can alter retention time and separation performance [31].

The source literature therefore supports a hierarchy of analytical priorities in which suitability for purpose precedes convenience. A fast assay is useful only when it remains selective and reproducible; a highly sensitive method is useful only when recovery and precision are acceptable; and a sophisticated hyphenated method is justified only when its additional selectivity or sensitivity is required. This framework provides a consistent basis for evaluating the large number of cephalosporin methods summarized in the following sections [32].

5. RP-HPLC Analysis of First-Generation Cephalosporins

First-generation methods in the compiled literature

are predominantly conventional RP-HPLC assays for cefadroxil, cephalexin, cefazolin, and related agents. C18 columns are frequently selected for dosage-form analysis, while C8 phases appear particularly useful for plasma or other complex matrices. The methods illustrate the importance of matching mobile-phase strength and pH to the analyte because closely related compounds can produce materially different retention times under otherwise similar conditions [33].

For cefadroxil, reported methods include a Hypersil ODS C18 column with phosphate buffer and acetonitrile and an alternative C18 method using phosphate buffer, methanol, and acetonitrile. The latter produced a shorter retention time of about 4.17 min under the reported conditions, illustrating how mobile-phase composition can materially alter chromatographic efficiency. For cefazolin, both pharmaceutical and serum methods are available. Lalitha et al. reported a stability-indicating C18 method, while Kunicki et al. used an RP-C8 phase for serum with a short retention time and validated recovery, precision, and accuracy [34].

The first-generation literature also demonstrates that method optimization is application specific. Cephalexin in human plasma was determined using a

C18-based method with diode-array detection, whereas cefazolin methods ranged from routine pharmaceutical assay to serum analysis and stability-indicating quality control [35]. The reported cefazolin studies are particularly informative because changes in stationary phase and mobile-phase composition altered retention while preserving quantitative performance. This reinforces the need to distinguish between a method designed for routine dosage-form assay and one designed for pharmacokinetic or stability applications [36].

The multicomponent studies further show how the analytical target changes when another drug or endogenous component is present. Simultaneous cefazolin or ceftizoxime determination in the presence of ascorbic acid, vancomycin-cephalexin determination in plasma, and cefazolin-metronidazole measurement all required chromatographic conditions capable of separating chemically different components. In these circumstances, selectivity and peak identity become as important as absolute retention time. The compiled chromatographic conditions are summarized in Tables 1 and 2, with the source studies cited in the accompanying text [37]. The detailed chromatographic conditions compiled from the source literature are presented in Tables 1 and 2.

Table 1: Reported RP-HPLC methods for single-drug formulations and selected biological analysis of first-generation cephalosporins.

S. No.	Drug	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	LOD/L OQ	Recovery (%)	References
1.	Cefadroxil	Hypersil ODS, 4.6 × 250 mm, 5 μm	Phosphate buffer (pH 5.0):acetonitrile (96:4, v/v)	1.0	20	254	8.1	0.4 mg	99.03–101.8	[38]
2.	Cefadroxil	ODS C18, 4.60 × 250 mm, 5 μm	Phosphate buffer (pH 4.8):methanol:acetonitrile (95:3:2, v/v/v)		20	230	4.17	NR	93.06–99.40	[39]
3.	Cephalexin	Atlantis dC18, 4.6 × 150 mm, 5 μm	Cetyltrimethylammonium bromide:dipotassium phosphate:acetonitrile (60:40:0.001, v/v/v)	1.0	10	260	5.3	NR	≥94	[40]
4.	Cefazolin	Wakosil II-C18, 250 × 4.6 mm, 5 μm	Phosphate buffer (pH 6.8):methanol (5:2, v/v)	1.0	100	254	5.7	0.1 μg/mL	96–105	[41]
5.	Cefazolin	LiChrosorb C8, 250 × 4.6 mm, 5 μm	CH ₃ CN:H ₂ O:K ₂ HPO ₄ (100:894:6, v/v)	1.5	10	272	3.6	NR	NR	[42]
6.	Cefazolin sodium	Hibar® μBondapak® C18, 4.6 × 75 mm, 3.5 μm	Acetonitrile:monobasic sodium phosphate buffer (17:83, v/v)	1.0	20	254	5.5	12.92 ng/mL	98.35–100.86	[43]

		μm								
7.	Cefadr oxil Monoh ydrate	Hypersil ODS C18, (250 x 4.6 mm, 5 μm)	KH ₂ PO ₄ : Acetonitrile (65:35, v/v)	1	10	220	3.2	NR	NR	[44]

Table 2: Reported RP-HPLC methods for multicomponent formulations and biological matrices involving first-generation cephalosporins.

S. No.	Sample	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	References
1.	Cephalexin and Bromhexine	Kromasil C8, (250 mm x 4.6 mm, 5 μm)	Water: Acetonitrile (50 :50, v/v)	1	10	215	2.2 and 2.8	[45]
2.	Vancomycin and Cephalexin	Zorbax Eclipse XDB C8, (125 x 4.0 mm, 5 μm)	Methanol and Disodium hydrogen phosphate (40/60, v/v)	1	10	200 and 380	2.1 and 3.5	[46]
3.	Cefazolin, Cefprozoximes and Ascorbic acid	C18, (25 x 0.46 cm, 5 μm)	Acetonitrile: Water (60:40, v/v)	0.75	20	265, 265 and 270	4.1, 4.3 and 2.9	[47]
4.	Cefazolin and Metronidazole	Nucleoshell C18, (100x 3 mm, 2.7 μm)	Sodium Phosphate: Acetonitrile 875:125, (v/v)	0.4	1-5	272 and 320	3.6 and 1.9	[48]
5.	Levofloxacin, Cefazolin, and Cefminox	Kromasil C18, (250 x 4.6 mm, 5 μm)	TBAB: Methanol, (75:25, v/v)	1	20	291, 300 and 274	1.81, 1.31, and 2.38	[49]
6.	Cefatrizine and Clavulanic acid	Crestpak C18, (150 mm x 4.6 μm)	Phosphate buffer: Acetonitrile, (91.5:8.5, v/v)	1	50	269	6 and 4.9	[50]

6. RP-HPLC Analysis of Second-Generation Cephalosporins

Second-generation cephalosporins show a broader methodological range than might be expected from their close structural relationship. Cefaclor, cefprozil, and cefuroxime have been analyzed in plasma and pharmaceutical matrices using C18 or C8 stationary phases and aqueous-organic mobile phases. The reported cefaclor plasma method used a C18 phase with a relatively long retention time, whereas cefprozil methods using C18 columns produced shorter retention and were evaluated using both HPLC-UV and HPLC-MS/MS, providing a useful comparison between conventional and hyphenated detection [51].

Cefuroxime methods further illustrate the diversity of analytical objectives. Conventional dosage-form assays use C8 or C18 columns and buffered organic mobile phases, while dedicated impurity analysis and experimental-design-supported methods emphasize separation of the parent compound from its degradation or impurity components [52]. Simultaneous determination of cefuroxime with cefprozil, cefixime, linezolid, potassium clavulanate, or

ornidazole required further adjustment of mobile-phase composition and detection conditions, demonstrating the increasing importance of selectivity in multicomponent formulations.

For cefaclor, the reported plasma method used a C18 column, an acidic mobile phase containing sodium 1-pentanesulfonate, triethylamine, water, and methanol, and a retention time of approximately 17 min [53]. The comparatively long retention time illustrates that a method intended for a biological matrix may prioritize selectivity and matrix control over maximum speed. Cefprozil studies provide a contrasting example because an HPLC-UV approach and an HPLC-MS/MS approach were directly compared for diastereoisomer determination in human plasma. The MS/MS strategy employed selected reaction monitoring transitions, whereas UV detection was performed at 292 nm, highlighting how detector choice can become central when isomeric or low-level measurement is required [54].

Cefuroxime has been studied in injections, pharmaceutical dosage forms, and impurity-focused

applications. An RP-HPLC method for cefuroxime injection used a C8 column with acetonitrile and acetate buffer and produced a short retention time, while an experimental-design-supported method for cefuroxime axetil and its impurities employed a C18 column and a methanol-water system [55]. The simultaneous methods involving cefuroxime axetil with cefprozil, cefixime, linezolid, potassium clavulanate, or ornidazole further illustrate that chromatographic conditions change when co-analytes are introduced. These examples provide a consistent message: method development should begin with the

analytical question and matrix rather than with a fixed chromatographic recipe [56].

Taken together, the second-generation literature suggests that the analytical method is driven primarily by matrix and assay objective rather than generation label. Single-analyte dosage-form methods can remain relatively simple, whereas plasma analysis and impurity or combination-product work require greater control of selectivity and sample preparation. The compiled methods are summarized in Tables 3 and 4.

Table 3: Reported RP-HPLC methods for single-analyte analysis and bioanalysis of second-generation cephalosporins.

S. No.	Sample	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	References
1.	Cefaclor	Supelcosil C18, (25 cm x 4.6mm, 5 μm)	Water: Methanol, (78:22, v/v)	1.5	25	265	17	[57]
2.	Cefprozil	XbridgeC18, (4.6 x 150 mm, 5 μm)	Acetonitrile: Potassium phosphate, (13:87, v/v)	1	100	292	4.7	[58]
3.	Cefprozil	Hypersil BDS C18, (200 mm x 4.6 mm, 5 μm)	Acetonitrile: Acetic acid, (92:8, v/v)	1	20	282	8.8	[59]
4.	Cefuroxime	Zodiac C8, (150 x 4.6 mm, 5 μm)	Acetate buffer: Acetonitrile, (10:1, v/v)	2	10	254	3.08	[60]
5.	Cefuroxime Axetil	Luna C18, (4.6 x 150 mm, 5 μm)	Methanol: Water, (39:61, v/v)	1	5	278	10.1 and 11 7	[61]

Table 4: Reported RP-HPLC methods for multicomponent analysis of second-generation cephalosporins.

S. No.	Sample	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	References
1.	Cefaclor and Methylparaben	Agilent Eclipse XDB C18, (250 mm x 4.6 mm, 5 μm)	Acetonitrile: Methanol: Ammonium dihydrogen phosphate (25:10:65, v/v)	1	20	265	0.12 and 0.18	[62]
2.	Cefoxitin, Cefuroxime, Cephalixin and Cephaloridine	Corasil RP-C18, (37-50 μm)	Acetonitrile: Acetate buffer (85:15 v/v)	1	10	254	12.2, 10.3, 6.2 and 17.3	[63]
3.	Cefuroxime Axetil and Cefprozil	C18, (125 mm x 4.6 mm, 5.0 μm)	Triethylamine : Methanol: Acetonitrile: Ultra-Pure	1.5	10	262	2.8 and 4.1	[64]

			Water (0.1: 5: 25: 69.9, v/v/v/v)					
4.	Cefuroxime axetil and Cefiximetrihydrate	C18, (250 mm x 4.6 mm, 5.0 μ m)	Triethylamine : Methanol: Acetonitrile: Ultra-Pure Water (2:10:20:68, v/v)	1	20	265	3.9 and 3.6	[65],[66]
5.	Cefuroxime axetil and Linezolid	C18, (250 mm x 4.6 μ m)	Potassium phosphate: Acetonitrile (60:40, v/v)	1	20	240	3.7 and 6.0	[67]
6.	Cefuroxime axetil and Potassium clavulanate	Microsor b-MV 100-5 C18, (250 x 4.6 mm, 5 μ m)	Methanol: water, (90:10, v/v)	1	20	230	2.4 and 3.3	[68]
7.	Cefuroxime axetil and Linezolid	Hypersil Gold C18, (250 mm x 4.6 μ m)	Potassium dihydrogen phosphate: Methanol (60:40, v/v)	1	10	255	8.2 and 2.5	[69]
8.	Cefuroxime axetil and Ornidazole	HiQ-SiL C18, (250 mm x 4.6 μ m)	Potassium dihydrogen orthophosphate: methanol, (56:44, v/v)	1.5	20	281	8 and 4.5	[70]

7. RP-HPLC Analysis of Third-Generation Cephalosporins

Third-generation cephalosporins account for the largest body of analytical data in the source manuscript. The reported methods cover ceftriaxone, ceftibuten, ceftazidime, cefpodoxime proxetil, cefotaxime, cefdinir, cefditoren pivoxil, cefixime, and numerous combination products. This breadth makes the third generation particularly informative for comparing routine dosage-form assays with stability-indicating, dissolution, bioanalytical, and simultaneous methods [71].

7.1. Individual Pharmaceutical and Bioanalytical Determination

Ceftriaxone methods demonstrate the typical balance between simple routine assay and matrix-specific bioanalysis. A conventional C18 method for pharmaceutical products used a buffered aqueous phase with methanol, whereas simultaneous methods involving ceftriaxone with vancomycin, cefpodoxime proxetil, or sulbactam required different mobile-phase compositions and detection conditions. Ceftibuten was quantified in bulk and capsule material by a YMC ODS-A C18 method using ammonium acetate buffer and acetonitrile, while ceftazidime has also been

determined in plasma by RP-HPLC with UV detection [72].

Cefpodoxime proxetil has been analyzed using C18 columns with buffered ammonium acetate-acetonitrile systems and has also been studied using isomer-specific RP-HPLC, showing that stereochemical or isomeric control can require specialized chromatographic optimization. Cefotaxime sodium was evaluated by a conventional C18 method for raw materials and parenteral products, while cefdinir has been examined in bulk material, tablets, and serum using different C18-based conditions. These reports collectively show that the same drug can require different method-development strategies depending on whether the analytical purpose is routine assay, pharmacokinetic measurement, or stability assessment [73].

7.2. Cefixime-Focused Analytical Development

Cefixime is especially prominent in the third-generation literature. Reported methods include routine HPLC-UV assay of capsules and raw materials, economic stability-indicating methods, analysis of powder-for-suspension products, and simultaneous determination with several co-formulated or co-

administered drugs. Stress studies incorporating acid, alkali, water, peroxide, and ultraviolet exposure demonstrate the importance of separating the intact drug from degradation products when the analytical objective extends beyond assay [74].

Individual cefixime methods in the source manuscript range from raw-material and capsule assays to stability-indicating procedures and analysis of oral suspension products. An HPLC-UV method for capsules used a Bondapak C18 column with methanol and phosphate buffer at acidic pH, whereas a stability-indicating method used a C18 column with sodium dihydrogen phosphate and methanol and demonstrated separation after ultraviolet stress [75]. Another reported method combined cefixime with stress testing under acid, alkali, water, hydrogen peroxide, and ultraviolet exposure, emphasizing the need to distinguish the parent peak from degradation products rather than simply measure loss of assay.

The oral suspension study is also analytically informative because the formulation matrix and product presentation influence both sample preparation and chromatographic conditions. A C18 phase with tetra-butylammonium hydroxide and acetonitrile was used at a higher flow rate, with a relatively short retention time and short total run. Such a method is attractive for routine product testing, but the source literature does not support the conclusion that it is universally superior; suitability depends on the product matrix, validation scope, and required selectivity [76].

The extensive simultaneous-estimation literature for cefixime demonstrates how far the analytical design space can expand within a single drug. Methods are reported with fluoroquinolones, macrolides, β -lactamase inhibitors, cloxacillin or dicloxacillin, analgesics, and other agents. The stationary phase is usually C18 or C8, while the mobile phase ranges from relatively simple phosphate-buffered organic mixtures to systems containing triethylamine or tetra-butylammonium reagents [77]. Detection is commonly performed by UV or PDA, and reported retention times vary according to the number and chemical diversity of analytes. These studies collectively support the use of chromatographic selectivity as the primary design criterion for combination products.

7.3. Multicomponent, Dissolution, and Stress-Analysis Applications

A substantial part of the third-generation literature concerns simultaneous determination rather than single-drug assay. Ceftriaxone has been combined with vancomycin, cefpodoxime proxetil, or sulbactam, and the reported conditions demonstrate how changes in buffer strength, organic modifier, pH, and detection

wavelength are used to maintain separation [78]. A ceftriaxone-cefpodoxime method for pharmaceutical preparations and spiked plasma, for example, used a C18 column, a triethylamine-containing aqueous-acetonitrile phase, and UV detection at 240 nm, with reported retention times of approximately 2 and 4 min for the two analytes. The short retention times are analytically useful, but their value depends on adequate selectivity in the spiked biological matrix [79].

Combination methods involving cefpodoxime proxetil show similar variation. Cefpodoxime has been analyzed with azithromycin using a C18 phase and a mixed acetonitrile-methanol-phosphate mobile phase, with different retention times for the two components. A C18 method for cefpodoxime and levofloxacin used a phosphate-buffered mobile phase and provided close retention behavior for the two analytes, whereas a C8 method for cefpodoxime with ambroxol used an internal standard and a different pH to obtain resolution [80]. The literature further includes C18 and C8 approaches for cefpodoxime with ofloxacin, levofloxacin, clavulanic acid, dicloxacillin, and azithromycin. These examples suggest that simultaneous analysis is often governed by relative selectivity between co-analytes rather than by the absolute retention of the cephalosporin alone.

The chromatographic challenge becomes more pronounced when stereoisomers or chemically diverse components are present. A cefpodoxime proxetil-levofloxacin method reported different retention for the two cefpodoxime isomers in addition to the co-analyte, demonstrating that a method may need to resolve more than one form of the same active ingredient. Similarly, simultaneous cefdinir-cefixime analysis in human plasma required a C18 guard cartridge, a mixed methanol-acetonitrile mobile phase, elevated column temperature, and UV detection at 285 nm. Such methods highlight the interaction between column efficiency, temperature, solvent strength, and matrix-specific requirements [81].

Cefdinir has also been integrated into dissolution and degradation testing. A comparative study applied RP-HPLC and UPLC for simultaneous determination of cefdinir and sodium benzoate in dosage forms and in-vitro dissolution samples. The UPLC configuration used a short C18 column at lower flow, illustrating how reduced-scale chromatography can be used to shorten analysis time or reduce solvent usage [82]. In another study, cefdinir and sodium benzoate were measured together with degradation products using gradient HPLC, showing that dissolution or stability applications can require different chromatographic

strategies from routine assay. The analytical lesson is that method performance should always be evaluated against the test purpose, because dissolution monitoring and stability testing impose different selectivity requirements from a simple content-uniformity assay [83].

Cefixime combination methods form the broadest part of the third-generation data set. Simultaneous RP-HPLC procedures have been described for cefixime with moxifloxacin, ornidazole, azithromycin, ofloxacin, cloxacillin, clavulanic acid, paracetamol, dicloxacillin, levofloxacin, and linezolid, as well as several three-component systems[84]. The source studies use C18 or C8 columns, with phosphate buffers and aqueous methanol or acetonitrile as the principal mobile-phase systems. Detection wavelengths vary with the analyte combination, generally remaining within the UV range. These methods demonstrate the flexibility of RP-HPLC, but they also expose a limitation of direct comparison: the reported validation ranges and concentration units are not uniform. Consequently, a numerical value such as an apparently high correlation coefficient cannot be used on its own to rank methods across studies.

Several reported methods also incorporate forced degradation. Cefixime has been challenged with acidic, alkaline, oxidative, aqueous, and ultraviolet stress, and the resulting chromatograms are intended to distinguish the parent peak from degradation products. Cefoperazone-tazobactam methods likewise include stability-indicating objectives and forced-degradation evaluation. These studies are important because they move the analytical objective from simple quantification toward lifecycle control of the active pharmaceutical ingredient. For a stability-indicating method, chromatographic resolution of the parent compound and degradation products is more important than maximizing speed at the expense of selectivity [85].

An additional feature of the third-generation literature is the coexistence of conventional isocratic methods with procedures designed for dissolution or degradation studies. A cefdinir-sodium benzoate application compared RP-HPLC and UPLC for dosage-form and dissolution testing, with the UPLC configuration using a lower flow rate and a shorter C18 column. The comparison is analytically meaningful because it demonstrates that chromatographic miniaturization can change throughput and solvent use while retaining the same general reversed-phase separation principle [86]. At the same time, the source literature shows that degradation studies may require gradient or more selective conditions, as in the simultaneous analysis of

cefdinir and sodium benzoate with degradation products.

The large collection of cefixime combination methods also permits a useful methodological comparison. Procedures using C18 columns and phosphate-buffered methanol or acetonitrile systems are generally straightforward, whereas methods incorporating triethylamine, tetra-butylammonium hydroxide, or multiple co-analytes require more deliberate optimization. The reported retention times demonstrate that chemically different drug pairs can sometimes be separated within a few minutes, but three-component systems and degradation studies may require longer runs. This variation should be considered a normal consequence of selectivity requirements rather than a failure of method standardization [87].

The source data also contain several examples of simultaneous methods in which an internal standard is used to improve quantitative control. Diclofenac sodium, aspirin, hydrochlorothiazide, and other compounds are employed in selected procedures, while other methods rely on direct external calibration. The choice of internal standard can be particularly important in complex sample preparation or bioanalytical workflows, but it also adds another peak that must be chromatographically separated and validated. Thus, the analytical benefit of an internal standard should be weighed against the additional separation requirement [88].

Third-generation cephalosporin analysis demonstrates that RP-HPLC can accommodate a broad spectrum of analytical objectives without requiring a fundamentally different separation platform. The principal changes occur in sample preparation, stationary-phase selection, mobile-phase chemistry, detection strategy, and validation scope. The breadth of this literature makes the third generation a useful benchmark for method development in newer cephalosporins, provided that the analyst avoids if conditions optimized for one compound can be transferred unchanged to another [89].

7.4. Comparative Interpretation of Third-Generation Methods

Across the third-generation methods, C18 phases are the dominant stationary phase, with UV or PDA detection commonly used in the low- to mid-200 nm range. Flow rates around 1 mL/min are frequent in routine pharmaceutical analysis, whereas some methods use higher or lower values to achieve improved selectivity or shortened run time. Reported retention times generally fall within a few minutes for many routine assays, but the range becomes wider in

multicomponent and bioanalytical methods. The practical implication is that method transfer should be based on a complete set of chromatographic parameters rather than on retention time alone [90]. The third-generation literature also reveals that simultaneous estimation has become an important design driver. In many combination products, the analytical target is not simply the cephalosporin but

the complete formulation, including an inhibitor or another active pharmaceutical ingredient. This requires deliberate control of selectivity and peak identity, and in degradation studies it requires additional confidence that degradation products do not interfere with quantification. The detailed individual and combination methods are presented in Tables 5 and 6.

Table 5: Reported RP-HPLC methods for individual and dosage-form determination of third-generation cephalosporins.

S. No.	Sample	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	References
1.	Ceftriaxone Sodium	Sunfire TM C18, (4.6 x 100 mm, 3.5 μm)	Buffer de-ionized water: Methanol, (80:20, v/v)	0.7	20	241	4.69	[91]
2.	Ceftibuten	YMCODS C18, (150 mm x 4.6 mm, 5μm)	Acetonitrile: Ammonium acetate (10:90, v/v)	1	20	262	8.3	[92]
3.	Ceftazidime	Symmetry C18, (4.6 x 250 mm, 5 μm)	Potassium phosphate: Acetonitrile, (90:10, v/v)	1	100	260	6.2	[93]
4.	Cefpodoximeproxetil	LiChroSpher® 100 C18, (250mm x 4.6 mm, 5μm)	Ammonium acetate buffer: Acetonitrile, (62:38, v/v)	1	40	235	12.5 and 14.5	[94]
5.	Cefotaxime sodium	Hypersil C18, (250 mm x 4.6 mm, 5 μm)	Sodium dihydrogen phosphate: Acetonitrile, (37: 63, v/v)	1	10	277	8.7	[95]
6.	Cefdinir	Welchrom C18, (4.6 x 250 mm, 5 μm)	Phosphate buffer: Acetonitrile, (50:50, v/v)	1	20	235	2.5	[96]
7.	Cefdinir	Nucleosil C18, (4.6 x 250 mm, 5 μm)	Sodium dihydrogen phosphate buffer: Methanol, (70:30, v/v)	1	20	254	3.1	[97]
8.	Cefdinir	Develosil C18, (4.6 x 150 mm, 5 μm)	Acetonitrile: phosphate buffer, (40:60, v/v)	1	20	254	6.3	[98]
9.	Cefdinir	Waters RP-Spherisorb C18, (250 mm x 4.6 mm, 5μm)	Water: acetonitrile: Methanol, 13:5:2, (v/v/v)	1	20	286	7.6	[99]
10.	Cefixime trihydrate	Waters Spherisorb C18, (25 cm x 4.6 mm, 5	Sodium dihydrogen phosphate monohydrate	1	10	254	9.7	[100]

		μm)	: Methanol, (50:50, v/v)					
11.	Cefixime	Shim-pack CLC-ODS C18, (6 mm x 15 cm)	Methanol: buffer solution Sodium dihydrogen phosphate, (35:65, v/v)	1	20	288	6	[101]
12.	Cefixime trihydrate	Phenomenex (250 mm length, 4.6 mm internal diameter, 5μm)	Disodium hydrogen phosphate: Methanol, (75:25, v/v)	1	20	289	6.8	[102]
13.	Cefixime	Supleco L C18, (4.6 mm x 250 mm, 5μm)	Tetra butyl ammonium hydroxide: Acetonitrile, (72.5:27.5, v/v)	2	10	254	3.1	[103]

Table 6: Reported RP-HPLC methods for combination, simultaneous, and multianalyte determination of third-generation cephalosporins.

S. No.	Sample	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	References
1.	Ceftriaxone and Vancomycin	Hypersil C18, (250 mm x 4.6 mm, 5 μm)	Acetonitrile: Methanol, (90:10, v/v)	1	20	241	3.8 and 4.4	[104]
2.	Ceftriaxone and Cefpodoximeproxetil	C18, (4.6 mm x 250 mm)	Acetonitrile: Triethylamine, (3:1 v/v)	0.9	20	240	2.1 and 4	[105]
3.	Ceftriaxone and Sulbactam	Intersil ODS C18, (250 x 4.6 mm, 5 μm)	Acetonitrile: phosphate buffer, (45:55, v/v)	1	20	210	2.8 and 4.2	[106]
4.	Cefazolin and Ceftizoxime	Kromasil 100 C18, (25 x 0.46 cm, 5 μm)	Acetonitrile: Water (60:40, v/v)	0.75	20	265 and 270	4.1 and 4.3	[107]
5.	Ceftazidime pentahydrate and Tazobactam sodium	BDS C18, (150 x 4.6 mm, 5 μm)	Phosphate buffer: Acetonitrile: Tetrahydrofuran (60:30:10, v/v/v)	1	20	205	3.4	[108]
6.	Cefpodoximeproxetil and Azithromycin dihydrate	C18, (150 x 4.6 mm, 5 μm)	Acetonitrile: Methanol: phosphate buffer, (40:40:20, v/v)	1	20	235	6.1 and 2.9	[109]
7.	Cefpodoximeproxetil and Levofloxacin	C18, (250 x 4.6 mm x 5 μ)	Phosphate buffer: Acetonitrile, (50:50, v/v)	1	20	240	5.6, 5.9 and 2.7	[110]
8.	CefpodoximeProxetil and Ambroxol hydrochloride	Qualisil C8, (250 mm x 4.6 mm, 5 μm)	Acetonitrile: Potassium dihydrogen phosphate, (70:30 v/v)	1	10	248	3.8 and 2.6	[111]

9.	Levofloxacin and Cefpodoximeproxetil	C18, (250 mm x 4.6mm, 5µm)	Orthophosphoric acid: Methanol, (70:30, v/v)	1	10	230	3.7 and 4.8	[112]
10.	Levofloxacin aemihydrate and Cefpodoximeproxetil	YMC C18, (250 mm x 4.6 mm, 5 µm)	K ₂ HPO ₄ : Methanol, (40:60, v/v)	1	20	275	2.8 and 3.9	[113]
11.	Cefpodoximeproxetil and Ofloxacin	X-terra C8, (4.6 x 250 mm, 5 µm)	Triethyl amine buffer: Acetonitrile, (30:70, v/v)	1.2	20	227	2.7 and 2.0	[114]
12.	Cefpodoximeproxetil and Levofloxacin	C18, (100 mm x 4.6 mm, 3.5 µm)	Ammonium acetate buffer: Acetonitrile: triethylamine (60:40:0.2, v/v/v)	1	10	258	5.7 and 6.2	[115]
13.	Cefpodoxime proxetil and Clavulanic acid	Zorbax Eclipse XDB C18, (150 x 4.6 mm)	Acetonitrile: Potassium dihydrogen phosphate buffer (70:30, v/v)	1	20	228	6.4 and 44	[116]
14.	Cefpodoximeproxetil and Dicloxacillin sodium	Kromasil C18, (250 x 4.6 mm, 5 µm)	Acetonitrile: Methanol: Trifluoroacetic acid, (30:50:20, v/v/v)	1	20	235	3.3 and 1.9	[117]
15.	Azithromycin and Cefpodoxime	Hypurity C18 (100 mm x 4.6 mm, 5 µm)	Methanol: Toluene: potassium dihydrogen phosphate buffer (60:30:10, v/v/v)	1	10	218	5.2 and 2.9	[118]
16.	Cefdinir and CefditorenPovoxil	Kromasil C18, (250 x 4.6 mm, 5µm)	Methanol: Potassium dihydrogen phosphate buffer, (75:25, v/v)	1	20	285	2.9	[119]
17.	Cefdinir and Sodium benzoate	Agilent Eclipse XDB C8, (250 mm x 4.6 mm, 5 µm)	Acetonitrile: Phosphate buffer, (50:50, v/v)	1	10	254	2.3	[120]
18.	Cefdinir and Cefixime	Spheriosorb Discovery HS C18, (150 mm x 4.6 mm, 5 µm)	Methanol: Acetonitrile, (50:50, v/v)	2	20	285	4.5	[121]
19.	Cefdinir and Sodium benzoate	Hichrom C18 (15 x 0.46 cm, 5µm)	Solution A- Tetramethyl ammonium hydroxide solution: EDTA (1000:0.4 v/v) and solution B- Tetramethyl ammonium hydroxide solution: Acetonitrile: Methanol: EDTA (500:300:200:0.	1	10	254	2.8	[122]

			4, v/v)					
20.	Cefixime and Moxifloxacin	Purospher BDS C18, (250mm x 4.6 mm, 5µm)	Acetonitrile: KH ₂ PO ₄ , (40:60, v/v)	1	20	276	3.1	[123]
21.	Cefixime and Ornidazole	YMC Pack Pro C18, (250 x 4.6, 5 µm)	NaH ₂ PO ₄ : Methanol (500: 500, v/v)	1.2	10	270	3.2	[124]
22.	Cefixime and Azithromycin	Phenomne x C18, (250 x 4.6 mm, 5 µm)	Potassium dihydrogen phosphate: Acetonitrile, (65:35, v/v)	1	20	227	5.22	[125]
23.	Cefixime, Ornidazole and Moxifloxacin	Agilent Zorbax SB-C18, (100 x 4.6 mm, 5 µm)	Di-potassium hydrogen phosphate: Acetonitrile, (50:50, v/v)	1	20	280	10.1	[126]
24.	Cefixime and Ofloxacin	HiQ-SiLNeosphere C18, (150 x 4.6 mm)	Methanol: Dihydrogen phosphate buffer, (70:30, v/v)	1	20	290	3.6	[127]
25.	Cefixime and Azithromycin	Supleco C18, (25cm x 4.6 mm, 5µm)	Na ₂ HPO ₄ : Methanol, (80:20, v/v)	1	10	273	4.93	[128]
26.	Cefixime and Paracetamol	ODS C18, (250 mm x 4.6 mm, 5 µm)	Phosphate buffer: Acetonitrile, (80:20, v/v)	1	10	245	5.9	[129]
27.	Cifixime and Ofloxacin	ODS C18, (250 x 4.6 mm, 10 µm)	Methanol: Phosphate buffer (50:50, v/v)	1	10	276	1.6	[130]
28.	Cefixime and Cloxacillin	C18, (25 cm x 4.6 mm, 5 µm)	Phosphate buffer: Acetonitrile: Methanol, (80:17:3, v/v)	2	20	225	5.6	[131]
29.	Cefixime and Clavulanic acid	C18, (4.6 mm x 25 cm, 5 µm)	Methanol: Water containing disodium hydrogen phosphate, (20:80, v/v)	1	20	220	3.2	[132]
30.	Cefixime trihydrate and Ofloxacin	Teknokroma (Kromosil) C8, (150 x 0.6 mm)	Triethylaminephosphate buffer: Acetonitrile, (80:20, v/v)	0.8	20	285	2.8	[133]

The relative scarcity of fourth- and fifth-generation studies should be interpreted cautiously. It does not necessarily indicate lower analytical importance; rather, the source compilation contains fewer published methods for these newer agents than for long-established third-generation compounds. Where methods are available, they frequently address clinical or multianalyte questions, making them valuable for understanding how analytical requirements evolve

with application complexity.

8. RP-HPLC Analysis of Fourth- and Fifth-Generation Cephalosporins

8.1. Fourth-Generation Analysis

The fourth-generation literature in the source manuscript is centered on cefepime and ceftipime. Ceftaroline is treated separately as a fifth-generation compound. Cefepime has been measured in

pharmaceutical products, human serum, urine, and complex plasma samples, demonstrating that a common drug can require substantially different analytical configurations depending on the matrix. A validated serum method for cefepime used a LiChrospher 100 RP-C18 column with phosphate buffer and methanol and a short retention time, while simultaneous plasma or urine methods incorporated cefepime with quinolones, vancomycin, imipenem, or cefazolin. These studies illustrate how selectivity requirements increase when cefepime is measured with coexisting anti-infective agents, particularly in clinical matrices. Cefpirome has similarly been incorporated into simultaneous methods with cefaclor, ceftazidime, cephradine, and cetirizine or levocetirizine [134].

8.2. Fifth-Generation Analysis

Ceftaroline fosamil is the main fifth-generation

cephalosporin represented in the compiled analytical literature. A stability-indicating RP-HPLC method for the drug in bulk and parenteral dosage forms used a C18 stationary phase with an aqueous-organic mobile phase and UV detection. The fifth-generation data set is smaller than that for the third generation, which limits broad statistical comparison but also highlights the relative analytical maturity of older cephalosporin generations [135]. A further development is the use of chromatographic separation coupled to mass spectrometric detection for simultaneous quantification of ceftaroline with other anti-infective drugs in plasma. This approach demonstrates the analytical transition that becomes relevant when conventional UV detection no longer provides sufficient sensitivity or selectivity in a complex biological matrix [136]. The fourth- and fifth-generation methods are compiled in Tables 7 and 8.

Table 7: Reported RP-HPLC methods for individual determination of fourth- and fifth-generation cephalosporins.

S. No.	Sample	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	References
1.	Ceftarolinefosamil	X-terra C18, (250 mm x 4.6mm, 5μm)	Buffer: Acetonitrile: Water, (40:30:30, v/v/v)	1.0	10	242	3.53	[137]
2.	Cefepime	LiChrospher 100 C18, (250 mm x 4 mm, 5 μm)	Phosphate buffer: Methanol, (75:25, v/v)	1.0	20	256	2.8	[138]

Table 8: Reported RP-HPLC methods for combination and multianalyte determination of fourth- and fifth-generation cephalosporins.

S. No.	Sample	Column	Mobile phase	Flow rate (mL/min)	Injection volume (μL)	Detection (nm)	Retention time (min)	References
1.	Ceftaroline, Daptomycin, Linezolid, and Rifampicin	Short phenyl-hexyl column (2 mm × 50 mm, 5 μm)	Water: Acetic acid, (99.9:0.1, v/v)	300	10	250	0.96, 0.96, 0.95 and 0.95	[139]
2.	Cefepime Hydrochloride and Amikacin sulphate	C18, (250 x 25 mm, 25 μm)	Acetonitrile: Water, (90:10, v/v)	1.0	10	212	2.51 and 6.23	[140]
3.	Cefepime, Garenoxacin, Moxifloxacin and Levofloxacin	LiChrospher 100 RP-C18, (4 mm x 250, 5 μm)	Acetonitrile: Phosphoric acid: N-octylamine solution, (50:40:10)	1.0	20	256, 292, 294 and 282	4.9, 7.5, 8.9 and 10.7	[141]
4.	Cefepime, Vancomycin and Imipenem	Supelcosil™ C18, (25 cm x 4.6mm x 5 μm)	Acetate buffer: Acetonitrile, (92:8, v/v)	0.8	5	230	9.3, 17.4, and 22.7	[142]
5.	Cefepime	LiChrospher	Acetonitrile:	1.3	20	259 and 278	4.03 and	[143]

	and Grepafloxacin	100 RP-C18, (4 mm x 250, 5 µm)	Phosphoric acid: N- octylamine, (50:40:10, v/v/v)				8.85	
6.	Cefepime and Cefazoline	Onyx monolithic C18, (20 cm x 4.6 mm, 5 µm)	Dibasic potassium hydrogen phosphate: Methanol, (10:90, v/v)	1.0	2	254	3.4 and 5.8	[144]
7.	Cefepime and Ceftazidime	µBondapak C18, (30 cm x 3.9 mm, 10 µm)	Acetate buffer: Acetonitrile, (90:10, v/v)	2.0	50	257 and 280	5.25 and 6.85	[145]
8.	Cefpirome, Cefaclor, Ceftazidime and Cephadrine	Mediterranea C18, (25 x 0.46 µm)	Methanol: Water, (30:70, v/v)	1.0	20	265	3.13, 4.86, 7.93 and 9.73	[146]
9.	Cefpirome and Cetirizine or Levocetirizine	Nucleosil 120, C18, (12.5 x 0.46, 5 µm)	Acetonitrile: Water, (75:25, v/v)	1.0	20	232	0.95 and 1.3	[147]

9. Bioanalytical Applications of RP-HPLC

9.1. Plasma and Serum Analysis

Bioanalytical cephalosporin methods impose stricter requirements than routine pharmaceutical assays because target concentrations can be lower and matrix components can interfere with extraction, retention, or UV response. The literature includes validated methods for cephalixin, cefazolin, cefaclor, cefprozil, cefdinir, cefepime, ceftazidime, ceftriaxone, cefpodoxime proxetil, and ceftaroline-containing combinations in plasma or serum.

The dominant preparation strategies include protein precipitation with organic solvent, centrifugation, dilution, filtration, and, when greater matrix cleanup is required, solid-phase extraction. The chromatographic conditions then have to compensate for the increased matrix complexity. Shorter columns, optimized C18 or C8 phases, ion-pair reagents, and in some cases mass spectrometric detection have been used to preserve selectivity and sensitivity [148].

9.2. Urine and Other Biological Matrices

Urine methods illustrate the usefulness of simpler sample treatment when the matrix and analyte concentration permit direct buffered dilution and filtration. Simultaneous determination of cefazolin with other agents and cefepime with quinolones or related drugs demonstrates the ability of RP-HPLC to support multi-analyte monitoring without necessarily requiring mass spectrometric detection. More specialized matrices, including dialysate or ultrafiltrate collected during hemodiafiltration,

require additional consideration of matrix composition and recovery [149].

10. Stability-Indicating RP-HPLC Methods

10.1. Forced Degradation and Degradation Separation

Stability-indicating methods are particularly important for cephalosporins because chemical instability can compromise assay results. The source literature includes forced degradation or degradation-product separation for cefazolin, cefuroxime axetil, cefdinir, cefixime, and cefoperazone-tazobactam combinations. Stress conditions reported across these studies include acid, alkali, oxidative, aqueous, and ultraviolet challenges. The analytical purpose is not merely to produce a lower assay value after stress but to demonstrate that the parent peak can be distinguished from products formed during degradation [150].

10.2. Implications for Method Specificity

Specificity in a stability-indicating method should therefore be judged by demonstrated separation and peak integrity rather than by nominal retention alone. PDA-based approaches may provide additional spectral information, whereas MS-based approaches can offer molecular selectivity in suitable laboratories. However, conventional RP-HPLC remains attractive for routine stability programs because it can combine acceptable selectivity with widely available instrumentation and comparatively simple operation [151].

11. Method Validation and Regulatory Considerations

The source literature repeatedly reports validation in terms of linearity, accuracy, precision, recovery, sensitivity, and, in selected studies, robustness or system suitability. A method intended for routine quality control should demonstrate not only an acceptable correlation coefficient but also performance across the intended concentration range, adequate accuracy and precision, and freedom from relevant interference. Bioanalytical procedures additionally require attention to extraction recovery, matrix effects, and analyte stability during sample handling.

11.1. Specificity, Linearity, and Range

Specificity is the foundation of a cephalosporin method, particularly when degradation products, formulation excipients, or co-administered drugs are present. Linearity should cover the operational range required by the analytical application rather than being selected solely to maximize the reported correlation coefficient. The compiled studies include wide variation in concentration ranges, reflecting the difference between dosage-form assays and trace-level biological measurements [152].

11.2. Accuracy, Precision, Recovery, LOD, and LOQ

Accuracy and precision are central to method suitability, and reported recovery values in pharmaceutical methods are often close to the nominal value while plasma methods place greater emphasis on variability at low concentrations. LOD and LOQ become especially important when the method is intended for pharmacokinetic sampling, trace impurity measurement, or multicomponent analysis. Direct numerical comparison remains difficult when studies use different concentration units, matrices, sample-preparation procedures, or definitions; therefore, validation parameters should be interpreted within the complete experimental context [153].

11.3. Robustness and System Suitability

Robustness evaluates the stability of method performance when controllable chromatographic variables vary within reasonable limits. Changes in pH, organic-phase composition, flow rate, temperature, or detector conditions can influence retention and peak shape for cephalosporins. A robust method is more likely to transfer successfully between analysts, instruments, or laboratories. The ability to meet system-suitability requirements consistently is consequently an important practical criterion for routine implementation.

11.4. Relationship Between Validation and Chromatographic Design

Another important validation consideration is consistency between the validation design and the intended routine workload. A method developed for occasional investigative analysis may tolerate longer preparation and more complex chromatography, whereas a quality-control method must remain reproducible over repeated sequences and multiple analysts. The source literature contains examples of simple isocratic procedures that are attractive for routine use and more elaborate stability or bioanalytical procedures that prioritize selectivity. These should not be judged by a single universal performance criterion [154].

The reported literature shows that validation parameters cannot be separated from chromatographic design. A method with excellent linearity but insufficient resolution from an impurity or co-formulated drug is not fit for the intended analytical purpose. Likewise, a highly sensitive method may be impractical if sample preparation causes poor recovery or unacceptable variability. The most useful validation strategy is therefore one in which selectivity, calibration, accuracy, precision, recovery, sensitivity, and robustness are interpreted together and against the intended matrix.

The compiled methods also illustrate the importance of reporting units correctly. The source manuscript contains examples in which concentration ranges, LOD and LOQ values, or injection volumes were reported with units that require careful interpretation. Such inconsistencies make otherwise valuable studies difficult to reproduce. Standardized reporting should distinguish mass concentration from molar concentration, state whether flow is expressed in mL/min or $\mu\text{L}/\text{min}$, and identify the detector and wavelength explicitly. It should also make clear whether a reported retention time refers to the parent analyte, an internal standard, or a component of a multianalyte system.

For routine implementation, system-suitability criteria should be linked to the separation objective. Retention time reproducibility, peak symmetry, theoretical plate count, resolution, and detector response can collectively indicate whether the chromatographic system is behaving as expected. The source literature includes methods reporting theoretical plates, tailing factor, precision, and recovery, but not all studies provide the same breadth of evidence. More uniform reporting would improve the ability to compare methods and select procedures for transfer to another laboratory.

12. Comparative Evaluation of RP-HPLC Methods Across Cephalosporin Generations

The compiled evidence demonstrates a strong cross-generational preference for conventional reversed-phase columns, particularly C18, combined with buffered aqueous-organic mobile phases and UV/PDA detection. This configuration offers an effective compromise between selectivity, instrument availability, throughput, and cost. C8 phases are used when altered retention characteristics are beneficial, especially in biological or multicomponent analysis, while ion-pair systems and hyphenated detection appear when the chemical or matrix environment becomes more demanding. The first generation is characterized mainly by routine pharmaceutical assays and selected bioanalytical applications. The second generation shows greater emphasis on plasma determination, impurity analysis, and simultaneous estimation. The third generation has the broadest methodological landscape because many compounds are marketed in combination products and have been extensively studied for both pharmaceutical and biological applications. The fourth and fifth

generations have fewer reported methods in the source compilation, but their literature includes important clinical-matrix and multianalyte applications in which selectivity is more demanding [155].

Retention time is one of the most frequently reported method-development outputs, but the comparison also shows why it should not be used as the sole definition of a “best” method. A rapid method is useful when it preserves resolution, sensitivity, acceptable peak shape, and repeatability. Conversely, a somewhat longer run can be preferable when it provides reliable separation of parent drug, impurities, or multiple analytes. The source manuscript explicitly compared methods on this basis and identified examples in which changes in column, mobile phase, and detection wavelength shortened analysis time without necessarily defining overall analytical superiority. Representative chromatograms that illustrate these multicomponent and comparative separation issues are presented in Figures 6 and 7.

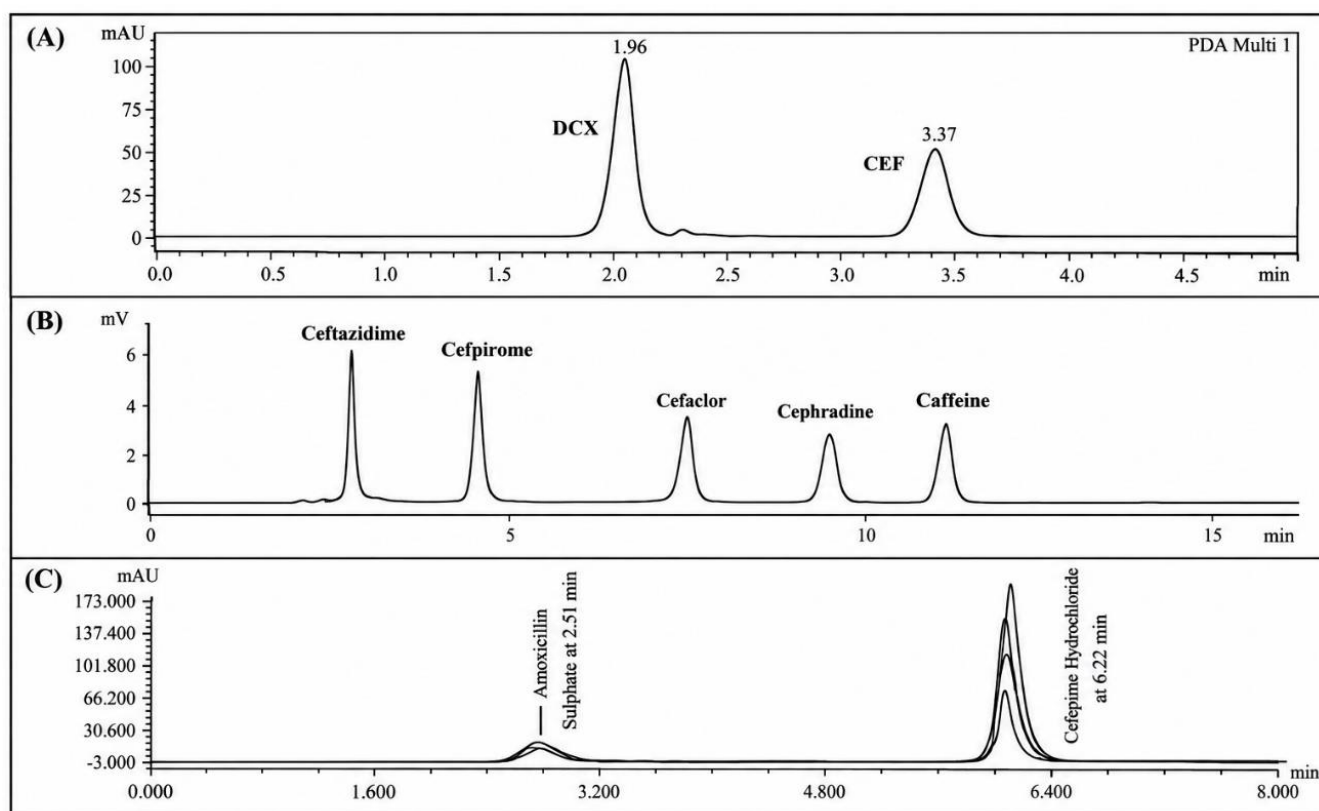


Figure 6: Representative HPLC chromatograms reported for multicomponent cephalosporin analysis: cefpodoxime proxetil with dicloxacillin sodium (A), cefpirome with cefaclor, ceftazidime, and cephadrine (B), and cefepime hydrochloride with amikacin sulphate (C).

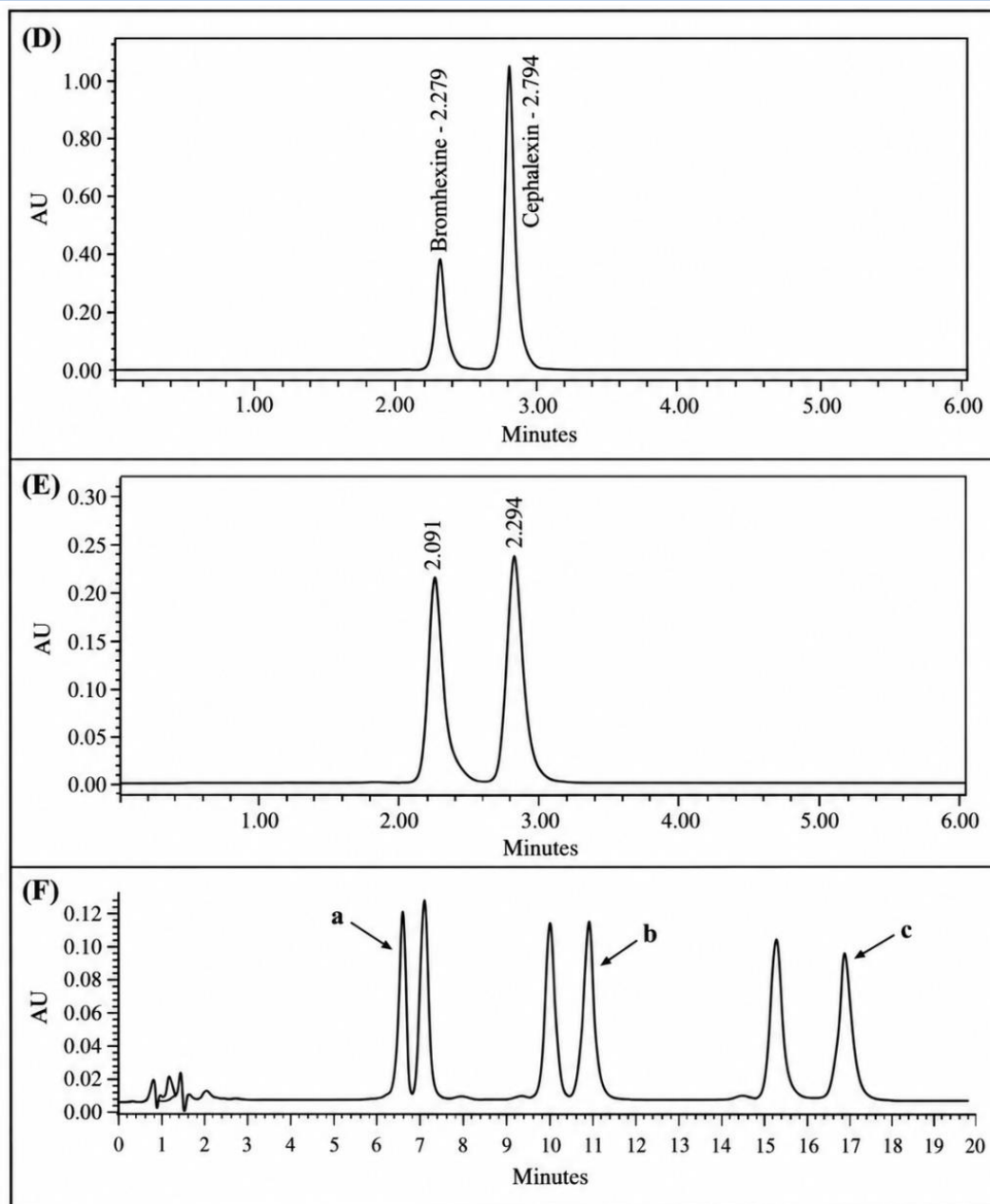


Figure 7: Representative HPLC chromatograms reported for multicomponent analysis of bromhexine with cephalixin (D), cefpodoxime proxetil with ofloxacin (E), and cefpodoxime proxetil (F).

Figures 6 and 7 illustrate the practical value of chromatographic resolution in multicomponent and single-analyte applications. The chromatograms also reinforce an important methodological principle: visual peak separation must be interpreted together with validation data and the intended analytical purpose. A visually attractive chromatogram is not, by itself, evidence of a validated method.

A further gap concerns harmonization of sample-preparation descriptions. Several source studies provide enough information to reproduce the procedure, whereas others summarize preparation only briefly. Reproducibility is particularly vulnerable when the analyst must decide how much sample to weigh, what solvent to use, how long to extract, or whether filtration should precede dilution. For

biologic matrices, the amount of sample, precipitating solvent, centrifugation conditions, and extraction cartridge can materially affect recovery. More complete reporting would reduce uncertainty during method transfer and validation.

Another limitation arises from the use of different detector technologies under the broad label of HPLC. UV, PDA, DAD, and MS/MS methods can all be described as HPLC-based, but their selectivity and sensitivity are not equivalent. The compiled literature illustrates this clearly in cefprozil bioanalysis and ceftaroline multianalyte measurement. Cross-study comparison should therefore state the detector configuration explicitly rather than treating all HPLC procedures as analytically interchangeable.

13. Critical Gaps and Methodological Limitations

The literature remains heterogeneous in how chromatographic and validation information is reported. Some studies provide complete information on column dimensions, mobile-phase composition, detection, linearity, recovery, and precision, whereas others omit important parameters or present units inconsistently. Such heterogeneity limits direct head-to-head comparison and complicates method transfer. The problem is particularly relevant when apparently similar compounds are assessed using different matrices or different definitions of sensitivity.

Another limitation is the uneven analytical coverage across generations. Third-generation cephalosporins are extensively represented, while fifth-generation analysis remains comparatively limited in the source literature. There is also a recurring dependence on UV/PDA detection even in applications where matrix complexity or very low concentrations may justify additional selectivity. However, the introduction of HPLC-MS/MS should be driven by analytical need rather than technological preference; conventional RP-HPLC remains appropriate when it delivers the required specificity, sensitivity, and robustness [156].

Future method-development studies should therefore emphasize transparent reporting, complete validation, and rational comparison of analytical trade-offs. A method should be considered superior only when it provides an appropriate balance of resolution, sensitivity, analysis time, reproducibility, sample preparation burden, solvent consumption, and operational cost for the intended use.

14. Potential for Routine Laboratory Implementation

General acceptance of an analytical method depends on accuracy, precision, sensitivity, selectivity, robustness, simplicity, cost, and compatibility with available instrumentation. Practical implementation also depends on reagent availability, analyst training, maintenance requirements, and the ease with which the procedure can be incorporated into standard operating procedures. For routine cephalosporin quality control, a well-validated C18 RP-HPLC method with a simple aqueous-organic mobile phase may offer a more sustainable laboratory solution than a more complex technique that provides only marginal analytical benefit.

The most transferable methods in the compiled literature are those that combine clear sample preparation, readily available stationary phases, moderate run times, well-defined detection conditions, and complete validation. In biological

laboratories, additional preparation or hyphenated detection may be justified when required concentration levels or matrix effects exceed the practical capability of conventional UV methods. Thus, routine implementation should be application-driven and supported by documented validation rather than selected only on the basis of novelty [157].

15. Emerging Analytical Technologies and Future Perspectives

The next generation of analytical development should also emphasize method lifecycle thinking. A procedure selected for a single development batch may later be expected to support release testing, stability studies, dissolution analysis, or clinical sample measurement. A method that is robust across these contexts is more valuable than a narrowly optimized assay that performs exceptionally under one set of conditions but requires substantial re-development for another application. The cross-generational evidence in this review supports designing methods around anticipated use rather than around a single reported chromatogram.

Automation and data handling can further improve consistency without changing the fundamental chromatographic platform. Standardized preparation, controlled mobile-phase formulation, sequence suitability checks, and electronic recording of chromatographic parameters can reduce analyst-to-analyst variability. These measures are particularly relevant for high-volume quality-control environments in which many cephalosporin products or combination formulations may be analyzed routinely.

The future of cephalosporin analysis is likely to involve incremental integration of conventional RP-HPLC with higher-selectivity detection rather than wholesale replacement of established methods. The source literature already contains examples of HPLC-MS/MS and HPLC-DAD approaches for plasma and multicomponent analysis, indicating a pathway toward more selective measurements when conventional UV detection becomes limiting.

Method development can also move toward more efficient use of chromatographic resources. Shorter columns, optimized flow rates, rational adjustment of organic modifiers, and better sample preparation can reduce analysis time while maintaining adequate separation. At the same time, environmental considerations increasingly favor methods that minimize solvent consumption and avoid unnecessary complexity. Such objectives should be integrated during method development rather than treated as an afterthought.

A further opportunity is greater harmonization of reporting across cephalosporin analytical studies. Consistent reporting of column chemistry, dimensions, particle size, buffer identity and pH, organic-phase ratio, flow rate, temperature, injection volume, detection wavelength, retention time, calibration range, recovery, precision, LOD, LOQ, robustness, and stability performance would substantially improve reproducibility and facilitate comparative method selection. The tables in this review illustrate both the value of these parameters and the problems created when they are incompletely reported.

For clinically relevant bioanalysis, the analytical platform should be selected according to concentration range and matrix complexity. Where UV detection is sufficient, RP-HPLC can remain attractive because of its availability and operational simplicity. Where multiple anti-infective agents must be measured simultaneously or concentrations approach the limits of conventional detection, mass-spectrometric detection can provide a stronger selectivity margin. The balance between analytical capability, cost, throughput, and laboratory infrastructure should remain central to technology selection.

An important implication of the compiled literature is that analytical development should be documented as a decision process rather than only as a final chromatographic recipe. The reported studies collectively show repeated adjustment of the same core variables, including stationary phase, aqueous-organic ratio, pH, flow rate, and detection wavelength. Reporting why a condition was selected, what competing conditions were considered, and which performance criteria determined the final choice would make future cephalosporin methods more transparent and easier to reproduce. Such documentation would also allow laboratories to distinguish genuine analytical improvement from simple changes in chromatographic conditions.

The review further indicates that comparison across generations is most meaningful when analytical purpose is held constant. A routine tablet assay, a stability-indicating assay, a dissolution method, and a plasma bioanalytical method have different priorities even when the target drug is the same. Cross-generational method evaluation should therefore compare like with like and should interpret chromatographic metrics within their intended

application. This perspective helps explain the diversity of reported methods while maintaining RP-HPLC as a common and transferable analytical framework.

Conclusion

The literature demonstrates that RP-HPLC remains a versatile and widely applicable platform for the analysis of cephalosporins across all five generations. C18-based methods with buffered aqueous-organic mobile phases and UV or PDA detection form the core of routine pharmaceutical analysis, while C8 phases, ion-pair strategies, and HPLC-MS/MS extend the analytical capability to selected biological and multicomponent applications. The comparative evidence also shows that no single chromatographic condition can be considered universally optimal across cephalosporins, matrices, and analytical objectives. Reliable method selection therefore requires integrated consideration of specificity, sensitivity, accuracy, precision, recovery, robustness, stability, analysis time, and practical laboratory requirements. Future development should focus on better standardization of validation and reporting, greater efficiency, and environmentally responsible chromatographic design while preserving the accessibility and robustness that make RP-HPLC useful for routine cephalosporin quality control.

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AI Declaration

No any AI tools are used for generating scientific data, results, or conclusions.

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