Review Article

Determination of Cefixime in Pharmaceutical Dosage Forms and Biological Samples: Current Analytical Strategies

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Abstract

Cefixime is an orally administered, third-generation cephalosporin antibiotic belonging to the β-lactam class, widely used for its broad-spectrum activity against numerous bacterial pathogens. Its mechanism of action involves binding to bacterial penicillin-binding proteins (PBPs), thereby disrupting the final steps of peptidoglycan cross-linking in cell wall synthesis. This inhibition weakens the bacterial cell wall, leading to lysis and death. Cefixime is active against gram-positive bacteria such as Streptococcus pneumoniae and gram-negative pathogens, including Escherichia coli, Klebsiella pneumoniae, Haemophilus influenzae, and Neisseria gonorrhoeae. Clinically, it is employed in treating respiratory and urinary tract infections, otitis media, and uncomplicated gonorrhea. Its oral availability, favorable safety profile, and once- or twice-daily dosing make it a preferred choice for outpatient therapy. This review consolidates current analytical methods for detecting and quantifying Cefixime in raw drug materials, pharmaceutical formulations, and biological samples. Bioanalytical techniques are crucial for accurately assessing drug concentration and metabolism, essential for pharmacokinetic and therapeutic monitoring studies. Various laboratory-based techniques have been utilized, including spectrophotometry, spectrofluorimetry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and ultra-performance liquid chromatography (UPLC). The review aims to provide a comprehensive overview of these methods, facilitating advancements in drug analysis, formulation development, and clinical applications of Cefixime.

Keywords: Cefixime, Cephalosporin antibiotic, Analytical methodologies

Introduction

Cefixime (CEF) is a third-generation cephalosporin antibiotic within the β-lactam class, widely recognized for its potent activity against numerous gram-positive and gram-negative bacteria, including Enterobacteriaceae, Haemophilus influenzae, Streptococcus pyogenes, Streptococcus pneumoniae, and Moraxella catarrhalis. Its therapeutic advantage lies in its ability to withstand hydrolysis by several β-lactamase enzymes, making it an effective option where resistance to other β-lactams is present. Clinically, it is prescribed for respiratory tract infections, urinary tract infections, otitis media, and uncomplicated gonorrhea. The drug's bactericidal mechanism is mediated through high-affinity binding to penicillin-binding proteins (PBPs), disrupting bacterial cell wall synthesis and causing cell lysis via activation of endogenous autolytic enzymes. These biological properties, combined with its oral bioavailability and convenient dosing, make Cefixime a preferred antibiotic in outpatient therapy. (Brogden & Campoli-Richards, 1989; Castle, 2007; Page, 2012) Beyond its pharmacological action, accurate quantification and monitoring of Cefixime in pharmaceutical preparations and biological fluids are of significant importance for quality control, pharmacokinetic studies, and therapeutic drug monitoring. Analytical methods play a crucial role in ensuring drug purity, stability, dosage accuracy, and patient safety. Various techniques have been developed and validated for this purpose, including spectrophotometry, spectrofluorimetry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and ultra-performance liquid chromatography (UPLC). while derivative methods use 307 nm to enhance specificity and reduce interference.

These finished dosage forms, and biological samples such as plasma or urine. The advancement of these analytical techniques is essential not only for pharmaceutical manufacturing and regulatory compliance but also for improving drug formulation, optimizing therapeutic efficacy, and supporting ongoing clinical and pharmacological research.

This review, therefore, aims to provide a comprehensive and critical overview of the current analytical methodologies available for the determination of Cefixime in pharmaceutical and biological matrices, highlighting their principles, applications, advantages, and limitations to guide future research and enhance drug quality assessment and clinical monitoring practices.

Importance of Analytical Determination of Cefixime:

Role in Pharmaceutical Quality Control

Accurate analytical determination of cefixime is indispensable in pharmaceutical quality control, serving multiple essential functions. First, it ensures drug purity, enabling the reliable detection and quantification of active pharmaceutical ingredients and related impurities or degradation products. Stability-indicating methods are critical in characterizing how cefixime degrades under stress conditions such as heat, humidity, or light exposure, since degradation products may compromise both efficacy and safety (Bajwa et al., 2020; Talebpour et al., 2013). Second, regulatory bodies, including the International Council for Harmonisation (ICH), the European Pharmacopoeia, and national agencies, require validated analytical protocols that demonstrate accuracy, precision, specificity, and robustness to comply with standards for impurity profiling and shelf-life determination.(Ajmal et al., 2023)

Significance in Bioanalytical and Clinical Studies

Beyond manufacturing, analytical techniques play a pivotal role in bioanalytical and clinical investigations involving cefixime. In therapeutic drug monitoring, quantification of cefixime concentrations in biological fluids (e.g. plasma, urine) supports individualized dosing, particularly in populations with altered pharmacokinetics (e.g. renal impairment or pediatric patients).(Ajmal et al., 2023) In pharmacokinetic and bioequivalence studies, validated RP-HPLC and LC-MS/MS methods have been employed to derive key parameters such as C_{max}, AUC, clearance, and half-life, ensuring accurate comparison across formulations and under different dosing conditions. (Talebpour et al., 2013) Finally, these methods also facilitate investigations into drug–drug interactions, enabling detection of potential co-administered compounds and interpretation of their impact on cefixime's pharmacokinetics and therapeutic effectiveness(Ibrahim, Wahba, & Magdy, 2018).

Analytical Techniques for Cefixime Determination

Pharmacopeial Methods

According to the British Pharmacopoeia ("The British Pharmacopoeia, The Stationary Office on the behalf of the Medicines and Healthcare Products Regulatory Agency, 2019 London, UK.,"), a validated high-performance liquid chromatography (HPLC) method is officially recommended for the quantitative determination of cefixime (CEF) in pharmaceutical formulations. This method utilizes a reversed-phase C18 analytical column, measuring 125 mm in length and 4 mm in internal diameter, packed with 5 µm-sized particles to achieve adequate resolution and separation efficiency for the analyte and its potential impurities. The chromatographic system operates with a mobile phase composed of acetonitrile and an aqueous solution of tetrabutylammonium hydroxide, mixed in a volumetric ratio of 250:750 (v/v), with the pH carefully adjusted to 6.5 to optimize analyte ionization and retention characteristics. The mobile phase is delivered at a flow rate of 1.0 mL/min, ensuring consistent elution and peak sharpness throughout the analytical run. Detection of cefixime is accomplished using a UV detector set at a wavelength of 254 nm, where the compound exhibits strong absorbance, thereby facilitating sensitive and selective quantification. This pharmacopeial method is widely adopted in quality control laboratories as it adheres to internationally recognized validation criteria for accuracy, precision, linearity, and robustness, making it suitable for routine analysis of both raw active pharmaceutical ingredients and finished dosage forms of cefixime.

Spectrophotometric Methods

UV-Spectrophotometric Methods

Various UV–visible spectrophotometric techniques have been employed for the quantitative determination of cefixime (CEF) in tablet formulations. These include zero-order, first-order, and second-order derivative spectrophotometry, and area under the curve (AUC) analysis, all based on measuring the drug's UV light absorption. Methanol is commonly used as the solvent to ensure complete dissolution of the active ingredient. Zero-order analysis records absorbance at 287 nm, The

AUC method integrates absorbance between 281–295 nm, providing a cumulative estimate of concentration. (Mali, 2015)

A straightforward yet highly reliable UV–visible spectrophotometric method has been developed and rigorously validated following the standard guidelines for the quantitative determination of cefixime in its pure, unformulated state. During method optimization, the salt form of cefixime exhibited a pronounced absorption maximum (λmax) at 287 nm, making this wavelength ideal for analytical measurements. The proposed method demonstrated excellent linearity over a defined concentration range of 1–5 µg/mL, with a high correlation coefficient ($R^2 = 0.9980$), confirming its strong linear relationship between absorbance and concentration. (Babita, 2019)

Five simple, accurate, and selective UV–visible spectrophotometric methods have been successfully developed for the quantification of cefixime (CEF), even in the presence of its acidic and basic degradation products, without requiring prior physical separation of the analyte from its breakdown components. The first approach employed first-order (1D) and second-order (2D) derivative spectrophotometry, where measurements of derivative amplitudes at 289 nm and 308 nm, respectively, were used to estimate cefixime concentrations in complex mixtures. The second method, known as first-derivative ratio spectrophotometry (1DD), allowed simultaneous quantification of CEF and its degradation products, with absorbance readings taken at 311 nm for acidic degradants and at 273 nm and 306 nm for basic degradants. The third technique, termed ratio subtraction spectrophotometry, enabled selective determination of cefixime at 286 nm, even when degradation peaks overlapped with those of the intact drug. The fourth method was based on a dual-wavelength analysis, utilizing absorbance differences at 209 nm and 252 nm for acidic degradation mixtures, and 310 nm and 321 nm for basic degradation mixtures, thereby enhancing selectivity. Finally, a bivariate spectrophotometric calibration method was introduced, employing four simultaneous linear equations derived from paired measurements at 231 nm and 290 nm (acidic conditions) and 231 nm and 285 nm (basic conditions), facilitating accurate resolution and quantification of cefixime in multi-component mixtures. (Mostafa, Abdel-Fattah, Weshahy, Hassan, & Boltia, 2019)

A first-derivative UV–visible spectrophotometric method has been developed and validated for the simultaneous quantification of cefdinir and cefixime (CEF) in combined pharmaceutical formulations. The analytical procedure involved dissolving both cephalosporin antibiotics in a 0.1 M sodium hydrogen phosphate buffer solution, adjusted to pH 8.0, to ensure optimal solubility and spectral stability. Derivative absorbance values (dA/dλ) were subsequently recorded at 306.8 nm for cefdinir and 307.0 nm for cefixime, allowing for their selective determination despite their closely overlapping zero-order spectra. (Bas, Çağlayan, Palabiyik, & Onur, 2013) Similarly, two complementary spectrophotometric techniques were proposed for the simultaneous determination of moxifloxacin and cefixime in binary mixtures. The first method employed first-derivative spectrophotometry, with specific measurements taken at 287 nm for moxifloxacin and 317.9 nm for cefixime, ensuring minimal spectral interference. The second approach utilized the first derivative of ratio spectra, enabling enhanced resolution and improved analytical selectivity; in this case, the derivative amplitudes were measured at 359.3 nm for moxifloxacin and 269.6 nm for cefixime. (M. Attimarad et al., 2012)

For the simultaneous estimation of cefixime (CEF) and ofloxacin (OFX) in both bulk drug substances and finished pharmaceutical products, two UV–visible spectrophotometric approaches were established and validated to provide selective and interference-free analysis. The first technique applied first-derivative spectrophotometry, with absorbance readings taken at 282.8 nm for OFX and 318.6 nm for CEF, corresponding to their respective zero-crossing wavelengths, thus allowing accurate quantification in mixed samples. The second method, based on ratio derivative spectrophotometry, involved calculating the first derivative of ratio spectra and measuring amplitudes at 337.2 nm for OFX and 317.0 nm for CEF, thereby improving resolution and reducing overlap between the spectra of the two drugs. (M. Attimarad & Nair, 2011)

To enable the simultaneous quantification of cefixime (CEF) and erdosteine (ERD) in combined pharmaceutical formulations, four distinct and reliable spectrophotometric methods were designed, optimized, and rigorously validated without the need for prior physical separation of the two analytes. The first approach utilized first-derivative ratio spectrophotometry (1DD), where spectral amplitudes were recorded at 310 nm and 315 nm for CEF and 248 nm for ERD, allowing their independent determination despite overlapping absorption bands. The second method employed the ratio difference spectrophotometric method (RDSM), in which the difference in amplitudes between 325 nm and 326 nm on the ratio spectra was directly correlated with CEF concentration, unaffected by the presence of ERD; likewise, the difference between 236 nm and 249 nm was used to selectively quantify ERD. (Mahrouse, Elwy, & Salem, 2020)

The third analytical strategy applied chemometric modeling techniques, specifically classical least squares (CLS) and partial least squares (PLS) regression, to simultaneously analyze both drugs, enhancing accuracy in multi-component systems. The fourth method involved mean centering of ratio spectra (MCR), where absorbance values were measured at 313 nm for CEF and 237 nm for ERD, improving signal resolution in binary mixtures. All four methodologies were successfully evaluated using laboratory-prepared mixtures and commercial formulations, demonstrating high recovery rates and robustness. Validation of these methods was performed in compliance with the International Council for Harmonisation (ICH) guidelines, confirming their precision, accuracy, and suitability for routine quality control of CEF–ERD combination products. (Mahrouse et al., 2020)

To facilitate the simultaneous quantification of azithromycin (AZI) and cefixime trihydrate (CEF) in combined tablet formulations, a simple, accurate, and highly precise UV–visible spectrophotometric method was developed and thoroughly validated. This analytical approach is based on the simultaneous equation technique, which enables concurrent estimation of both active pharmaceutical ingredients without prior separation. When dissolved in methanol, AZI and CEF exhibited distinct absorption maxima (λmax) at 222 nm and 289 nm, respectively, allowing their selective measurement within a linear concentration range of 10–50 µg/mL for both drugs. The method demonstrated excellent linearity, with correlation coefficients (r^2) of 0.999, indicating a strong relationship between absorbance and concentration. The limits of detection (LOD) were determined to be 0.81 µg/mL for AZI and 1.52 µg/mL for CEF, while the limits of quantification (LOQ) were 2.40 µg/mL and 4.60 µg/mL, respectively, confirming the sensitivity of the technique for trace-level measurements. (Jagdish V. Manwar, 2017)

In a related approach, a UV–spectrophotometric method utilizing the isobestic point was optimized for the simultaneous estimation of cefixime trihydrate and cloxacillin sodium in fixed-dose tablet combinations. The selected wavelengths were 241.8 nm, representing the isobestic point where both drugs exhibit identical absorbance, and 290.0 nm, corresponding to the individual absorption maximum of cefixime trihydrate. This method was rigorously validated following established analytical guidelines, demonstrating high accuracy, precision, ruggedness, and a well-defined linear response range, confirming its suitability for routine quality control and stability testing of multi-component pharmaceutical preparations. (Jadhav & 2020)

Colorimetric Methods

A colorimetric spectrophotometric method has been devised and validated for the quantitative determination of cefixime (CEF) in both its pure drug form and pharmaceutical formulations. The analytical approach is based on a redox reaction followed by complex formation, resulting in the development of a chromophoric species suitable for UV–visible detection. Specifically, cefixime is allowed to react with ferric ions (Fe^{3+}) in an acidic medium, where it acts as a reducing agent, converting Fe^{3+} to ferrous ions (Fe^{2+}). The generated Fe^{2+} ions subsequently form a stable, orange-red colored complex with 1,10-phenanthroline, commonly referred to as ferroin. The intensity of the resulting chromogen is directly proportional to the cefixime concentration and is measured spectrophotometrically at a wavelength of 510 nm. This technique offers a simple, cost-effective, and sensitive alternative to chromatographic methods, making it particularly suitable for quality control testing in settings with limited analytical resources. (M. Khan, Qayum, Rehman, Gulab, & Idrees, 2015)

A simple, reproducible, and selective spectrophotometric methodology has been developed for the quantitative determination of cefixime (CEF) in both bulk drug substances and pharmaceutical formulations. The analytical principle is based on complex formation between cefixime and bromophenol blue, resulting in the production of a bluish-green ion-pair complex in a mixed solvent system of dimethyl sulfoxide (DMSO) and acetonitrile. The reaction proceeds in a 2:1 stoichiometric ratio (CEF:dye), producing a stable chromophore with a characteristic maximum absorbance (λmax) at 610 nm. The intensity of the resulting complex is directly proportional to the drug concentration, enabling accurate and sensitive spectrophotometric quantification. (Keskar & Jugade, 2015) In a similar approach, another spectrophotometric assay was developed based on the interaction of cefixime with palladium (II) ions in an acidic buffer medium adjusted to pH 3.0. The resulting metal–

drug complex exhibited a strong absorption band at 352 nm, allowing precise quantification of cefixime in pharmaceutical preparations and providing cost-effective, straightforward, and reliable alternatives to chromatographic techniques. (Azmi et al., 2013)

Expanding on previously reported colorimetric and metal-complexation approaches for cefixime (CEF) analysis, two additional colorimetric methods have been established for determining CEF in pure drug form and pharmaceutical formulations. Method A utilizes a reaction between 3-methyl-2-benzothiazolinone hydrazone (MBTH) and ferric chloride, producing a green-colored complex with a maximum absorbance at 620 nm, allowing proportional quantification of the drug. Method B involves the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by cefixime, followed by complexation with 1,10-phenanthroline, generating a chromophore absorbing strongly at 510 nm. Both techniques offer simple, selective, and cost-effective alternatives for routine quality control applications. (Rajendran, 2001) Complementing these assays, a spectrophotometric method based on complex formation with copper (II) ions (Cu^{2+}) has also been developed for cefixime analysis in bulk and tablet formulations produced in Syria. Conducted in an acetate buffer at pH 7.8, the reaction yields a pink-colored complex exhibiting a maximum absorbance at 546 nm and a molar absorptivity of 3.28×10^3 $L \cdot mol^{-1} \cdot cm^{-1}$. (Abdul aziz ramadan, 2013)

Further complementing the previously described colorimetric and complexation-based spectrophotometric assays for cefixime (CEF), two additional colorimetric methods have been developed for its determination in bulk drug material and pharmaceutical formulations. The first approach incorporates cloud point extraction (CPE) using Triton X-114 as a non-ionic surfactant, enabling preconcentration of the analyte and improving detection sensitivity. This method demonstrates good linearity according to Beer's law in the concentration range of 10–160 µg/mL, making it suitable for routine analysis. The second approach, which does not involve CPE, relies on a diazotization reaction where CEF is treated with sodium nitrite at 5 °C, forming a diazonium salt that subsequently reacts with ortho-nitrophenol in an alkaline medium to produce a stable, orange-colored chromogen. The absorbance of this complex is measured at 400 nm, exhibiting excellent linearity within the same concentration range and offering high sensitivity, with a limit of detection (LOD) of 0.07896 µg/mL and a limit of quantification (LOQ) of 0.085389 µg/mL. Collectively, these two techniques, along with the previously described bromophenol blue, palladium (II), and iron-based reactions, expand the analytical options available for accurate, sensitive, and cost-effective determination of cefixime in pharmaceutical quality control. (Kadhim, Hamed, Mubdir, Abdullah, & Taher, 2020)

Spectrofluorimetric Methods:

Spectrofluorimetric techniques have been explored as highly sensitive and selective approaches for the quantification of cefixime (CEF) in pharmaceutical and biological samples, owing to their capability of detecting low drug concentrations with high accuracy. One reported method utilizes the quenching effect of cefixime on the fluorescence intensity of the terbium–danofloxacin (Tb^{3+}–Dano) complex, providing an indirect but reliable means of drug estimation. In this system, the terbium ion forms a fluorescent coordination complex with danofloxacin, which exhibits strong luminescence when excited at 347 nm and emits a characteristic fluorescence signal at 545 nm. The introduction of cefixime into the system results in a progressive reduction (quenching) of fluorescence intensity, and this quenching is proportional to the concentration of cefixime, thereby enabling quantitative analysis within a defined linear range. (Manzoori, Amjadi, Soltani, & Jouyban, 2014)

Spectrofluorimetric methods are particularly advantageous compared to conventional UV–visible spectrophotometry due to their higher sensitivity, lower detection limits, and reduced interference from excipients or degradation products. Such methods have been successfully applied in pharmaceutical quality control and bioanalytical studies, including drug dissolution testing, pharmacokinetic profiling, and trace-level determination in biological fluids. Furthermore, spectrofluorimetry is a cost-effective and rapid alternative to chromatographic techniques, making it a valuable tool in settings with limited analytical infrastructure. Ongoing research aims to enhance the selectivity of these assays by exploring novel fluorophore-metal complexes and advanced quenching mechanisms, which could further expand their application for routine cefixime analysis in multi-component drug formulations and clinical samples (M. N. Khan, Irum, & Mursaleen, 2021).

Chromatographic Methods

HPLC Methods Using UV Detectors

Various high-performance liquid chromatography (HPLC) methodologies have been developed and optimized for the quantitative determination of cefixime (CEF), both as a single active pharmaceutical ingredient and in combination with other therapeutic agents. A summary of these chromatographic approaches, including their experimental conditions and analytical performance characteristics, is presented in **Table 1**.

Stationary Phase	Mobile Phase	Detection wavelength	Application	Reference number
LiChrospher 100 RP C18 column (250 × 4 mm), 5 µm	Phosphate buffer: acetonitrile, (pH 7), (93:7, v/v)	287 nm	Determination of CEF in tablets	(González-Hernández, Nuevas, Soto-Mulet, López-López, & Hoogmartens, 2001)
µ-Bondapak C18	Methanol:water : phosphate buffer (22:28:50, v/v)	286 nm	Bioanalytical Assessment of CEF in Plasma and Bronchoalveolar Lavage Fluid	(Liu, Dai, Ma, Lin, & Tang, 1998)
Waters Spherisorb C18 column (250 × 4.6 mm), 5 µm	Sodium dihydrogen phosphate monohydrate: methanol, (pH 2.5), (3:1, v/v)	254 nm	Stability-Indicating Determination of CEF and Its Photodegradation Products	(Adam, Saeed, & Barakat, 2012)
ODS C18 column (250 × 4.6 mm), 5 µm	0.1% Ortho-Phosphoric acid: acetonitrile, (58:42, v/v)	274 nm	Quantitative Analysis of Cefixime and Acetylcysteine in Pharmaceutical Tablets	(A. L. Rao, Prasanthi, & Aswini, 2018)
YMC Pack Pro C18, (250 × 4.6 mm), 5 µm	0.1M NaH_2PO_4: methanol, (500:500, v/v)	270 nm	Validated Method for the Simultaneous Analysis of Cefixime and Ornidazole in Capsules	(M. N. Rao, Veni, & Haribabu, 2015)
Phenomenex Luna C18 column (250 × 4.6 mm), 5 µm	phosphate buffer: methanol, (pH 7), (60:40, v/v)	276 nm	Estimation of CEF and linezolid in dosage form	(Patel, Tandel, Patel, & Thakkar, 2014)
Phenomenex C18 column (250 × 4.6 mm), 5 µm	0.02M Potassium dihydrogen phosphate (KH 2 PO 4): Acetonitrile (65:35, v/v)	227 nm	Simultaneous Determination of Cefixime and Azithromycin in Pharmaceutical Dosage Forms	(Nyola & Govindasamy, 2013)
Hypersil C18	Methanol:Buffer (85:15, v/v)	275 nm	Estimation of Cefixime and	(Nagaraju, 2016)

column (250 ×4.6mm), 5µm			Azithromycin in tablet dosage form	
HiQ sil C-8 (4.6×250 mm), 5 µm	acetonitrile: tetra-butyl ammonium hydroxide buffer (pH 4), (45:55,v/v)	225 nm	Analytical Assessment of Cefixime and Cloxacillin in Solid Oral Dosage Form	(Wankhede, Mali, Karne, Khale, & Magdum, 2010)

Table (1): Summary of reported methods for the determination of CEF by HPLC using UV detection

HPLC Methods Using Mass Spectrometry Detectors

A high-performance liquid chromatography–mass spectrometry (HPLC–MS) method was developed and validated for the simultaneous determination of cefixime (CEF) and ofloxacin in human plasma, using moxifloxacin as an internal standard to ensure analytical precision and compensate for variability in sample processing. Separation was achieved on an Agilent LC–MS system equipped with a Zorbax Eclipse XBD C18 column (150 mm × 4.6 mm, 5 µm), providing effective resolution of both analytes. The mobile phase consisted of acetonitrile, methanol, and 0.5% formic acid in a 23:10:67 (v/v/v) ratio, delivered at a flow rate of 0.6 mL/min. (M. V. Attimarad & Alnajjar, 2013)

This method offers high selectivity and sensitivity, enabling accurate quantification of CEF and ofloxacin at trace levels in complex biological matrices. It is especially suitable for pharmacokinetic, bioequivalence, and therapeutic drug monitoring studies, where precise measurement in plasma is critical. The use of moxifloxacin as an internal standard enhances method robustness and reproducibility, supporting reliable application in clinical and research settings.

TLC-Densitometric Methods

A high-performance thin-layer chromatographic (HPTLC) method has been developed and optimized for the quantitative analysis of cefixime (CEF) in pharmaceutical preparations. The method utilized silica gel 60F254 aluminum-backed plates as the stationary phase, selected for their high adsorption capacity and reproducible separation performance. A carefully tailored mobile phase consisting of toluene, ethyl acetate, formic acid, and water in a 10:58:22:10 (v/v/v/v) ratio was employed, ensuring sharp resolution of cefixime from potential co-eluting cephalosporins or degradation products, particularly those formed under forced degradation or stress-testing conditions. Under these optimized chromatographic parameters, CEF produced a well-defined, symmetrical peak with a retention factor (Rf) of 0.54 ± 0.02, confirming method consistency and reliability. Detection was achieved through densitometric scanning in reflectance–absorbance mode at a wavelength of 293 nm, providing high sensitivity and quantitative accuracy. The method demonstrates the advantages of HPTLC, including cost-effectiveness, reduced solvent consumption, and simultaneous analysis of multiple samples, making it suitable for routine quality control, stability testing, and impurity profiling of cefixime-containing formulations. Additionally, its selectivity ensures minimal interference from related substances, supporting its application in pharmaceutical development and regulatory compliance studies. (Singh, Parwate, ivastava, & Shukla, 2011).

Electrochemical Methods

An advanced electrochemical sensor was developed for the sensitive and selective detection of cefixime (CEF) in biological matrices through the modification of a glassy carbon electrode (GCE). The electrode surface was initially enhanced with a hybrid nanocomposite comprising expanded graphene oxide and gold nanowires, providing a high surface area and excellent electrical conductivity. Subsequently, a molecularly imprinted polymer (MIP) layer of polyaniline was electropolymerized onto the modified electrode, creating specific recognition sites complementary to the cefixime molecule, thereby improving sensor selectivity. The morphology and structural features of the fabricated sensing layer were extensively characterized using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), confirming the successful formation of a uniform, nanostructured coating.

Analytical evaluation demonstrated that the developed sensor exhibited a linear and reproducible electrochemical response across a concentration range of 20.0–950.0 nM, with a remarkably low limit of detection (LOD) of 7.1 nM, highlighting its high sensitivity. When applied to complex biological samples such as blood and urine, the sensor achieved excellent recovery rates, indicating its strong potential for real-sample analysis. Compared to other modified GCE-based sensors, the MIP-integrated nanocomposite electrode demonstrated superior sensitivity, selectivity, and stability, making it a promising tool for therapeutic drug monitoring and bioanalytical applications of cefixime. (Dehghani, Nasirizadeh, & Yazdanshenas, 2019)

Capillary Electrophoresis

High-performance capillary electrophoresis (HPCE) has been employed as an efficient and highly selective analytical technique for the separation and quantification of cefixime (CEF) and its metabolites in the human gastrointestinal system. Among the various electrophoretic approaches evaluated, capillary zone electrophoresis (CZE) demonstrated the most effective separation performance, enabling clear resolution of the parent drug from all five of its major metabolites. Optimal results were achieved using a phosphate buffer solution at pH 6.8, supplemented with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS) as a zwitterionic surfactant. The addition of CHAPS improved analyte migration and minimized interactions with the capillary walls, enhancing both separation efficiency and peak symmetry. This optimized HPCE method provided rapid, reproducible, and high-resolution separation, allowing precise profiling of cefixime metabolism within the digestive tract. Such detailed separation is essential for pharmacokinetic and metabolic studies, as it facilitates the identification of active and inactive metabolites, supporting the assessment of drug absorption, distribution, and biotransformation. (Honda, Taga, Kakehi, Koda, & Okamoto, 1992).

Pharmacopeial methods	
HPLC (Acetonitrile : tetra butyl ammonium hydroxide 250:750)	("The British Pharmacopoeia, The Stationary Office on the behalf of the Medicines and Healthcare Products Regulatory Agency, 2019 London, UK.,")
UV Spectrophotometric methods	
Estimation of cefixime using first order derivative second order derivative	(Mali, 2015)
Direct spectrophotometric method for cefixime estimation	(Babita, 2019)
Estimation of cefixime in presence of degradation products using 1st derivative, 2nd derivative, first derivative ratio, ratio subtraction, dual wavelength and bivariate spectrophotometric calibration methods	(Mostafa et al., 2019)
Estimation of Cefixime and Cefdinir using first derivative	(Bas et al., 2013)
Estimation of cefixime and moxifloxacin using first derivative and first derivative of ratio spectra	(M. Attimarad et al., 2012)
Estimation of Cefixime and Oxofloxacin using first derivative and ratio derivative spectrophotometric methods	(M. Attimarad & Nair, 2011)
Estimation of cefixime and erdosteine using first derivative, ratio difference, chemometric and mean centering methods	(Mahrouse et al., 2020)
Estimation of cefixime and Azithromycin using simultaneous equation method	(Jagdish V. Manwar, 2017)
Estimation of cefixime and cloxacillin using isobestic point	(Rahul Jadhav 2020)
Colorimetric Methods	
Cefixime forms colored complex with iron	(Khan et al., 2015)

CEF forms bluish green complex with bromophenol blue	(Keskar & Jugade, 2015)
Cefixime and palladium ions forms colored complex	(Azmi et al., 2013)
Cefixime forms a green colored complex with 3-methyl-2-benzothiazolinone hydrazone and ferric chloride **Cefixime forms a colored complex with 1,10-phenanthroline**	(Rajendran, 2001)
Cefixime forms a pink colored compound with copper ions (Cu(II))	(Abdul aziz ramadan, 2013)
Orange colored complex is formed with ortho-nitrophenol	(Kadhim et al., 2020)
Spectrofluorimetric methods	
Cefixime was measured by looking at how much it reduced the glow of the Tb^{3+}-Dano complex.	(Manzoori et al., 2014)
HPLC Methods	Table 1
TLC methods	
Stationary phase: Silica gel 60F254 aluminum plates **Mobile phase : Toluene, ethyl acetate, formic acid, and water in the ratio of 10:58:22:10 by volume**	(Singh et al., 2011)
Electrochemical methods	
An electrochemical sensor was created by altering a glassy carbon electrode (GCE) with expanded graphene oxide and gold nanowires	(Dehghani et al., 2019)
Capillary electrophoresis	
Separation of cefixime and its metabolites using zone electrophoresis	(Honda et al., 1992)

Table (2): Summary of common analytical methods used for the determination of CEF.

Conclusions:

The determination of cefixime (CEF) across various matrices, including its pure bulk form, pharmaceutical formulations, and biological fluids, has been extensively investigated using a wide range of analytical techniques. These include spectrophotometric and spectrofluorimetric assays, high-performance liquid chromatography (HPLC) and its hyphenated variants (HPLC-DAD, HPLC-MS/MS), high-performance thin-layer chromatography (HPTLC), electrochemical sensors, and high-performance capillary electrophoresis (HPCE). Among these, HPLC remains the most widely adopted method, primarily due to its high sensitivity, selectivity, reproducibility, and compatibility with advanced detection systems, enabling precise quantification even in complex biological samples. Spectrophotometric techniques, while less sensitive, are widely favored for routine pharmaceutical quality control, offering cost-effectiveness, simplicity, and rapid execution. More sophisticated approaches, such as LC-MS/MS and electrochemical sensors, have demonstrated exceptional trace-level detection capabilities, making them highly valuable for pharmacokinetic profiling, bioequivalence studies, and therapeutic drug monitoring.

The selection of an appropriate analytical approach depends largely on the nature of the sample, required detection limits, analytical specificity, and intended application, whether for regulatory testing, clinical monitoring, or stability evaluation. Nonetheless, there remains a clear need for continued innovation in analytical methodologies for cefixime determination. Current challenges include the reliance on organic solvents with environmental impact, high operational costs, limited throughput, and the need for specialized instrumentation and expertise. Future developments should focus on adopting greener analytical techniques to minimize solvent and energy use, exploring miniaturized platforms to reduce sample and reagent consumption, and leveraging automation to enhance efficiency and reproducibility. Additionally, the integration of AI-driven data analysis offers significant promise for method optimization, predictive modeling, and rapid decision-making. Such advancements would not only improve the reliability and sustainability of cefixime analysis but also support optimized therapeutic use and ensure drug quality and safety across clinical and industrial settings.

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