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Pharmanalytix: High-Performance Liquid Chromatography Profiling of Antibiotic Agents and their Chromatographic Parameters

Hemant Marathe[1], Pankaj Mali[2], Rohit Mistri[3], Gunjan Marathe[4], Shivani Mahale[5], Tejas Magare[6], Mr. Harshjeet Sisode[7]

[1,2,3,4,5,6] ***UG Students at Kisan Vidya Prasarak Sanstha's Institute of Pharmaceutical Education,*** *(Approved by PCI New Delhi, Govt. Maharastra, DTE & Affillated to KBC NMU, Jalgoan & MSBTE Mumbai) NAAC Accredited B+ (CGPA 2.68), An ISO 9001:2015 Certified*

[7]***Under Guide, M. Pharmacy in Pharmaceutical Quality Assurance***

A B S T R A C T

High-performance liquid chromatography (HPLC) has emerged as a powerful analytical technique for the precise quantification and qualitative analysis of various pharmaceutical compounds, including antibiotics. This review article provides a comprehensive overview of HPLC methods developed for the analysis of three widely used antibiotics: metronidazole, amoxicillin, and azithromycin. The review encompasses the principles of HPLC, including instrumentation, chromatographic columns, mobile phases, and detection techniques commonly employed in antibiotic analysis. It highlights the significance of HPLC in pharmaceutical research and quality control due to its high sensitivity, specificity, and reproducibility. Furthermore, the article discusses in detail the HPLC methods developed for the analysis of metronidazole, amoxicillin, and azithromycin, covering aspects such as sample preparation, chromatographic conditions (including column type, stationary phase, mobile phase composition, flow rate, and temperature), calibration, and validation parameters. Emphasis is placed on the optimization strategies used to enhance the separation, resolution, and sensitivity of these antibiotics in various pharmaceutical formulations and biological samples. The comparative analysis of different HPLC methods for each antibiotic provides insights into the advancements, challenges, and future prospects in antibiotic analysis using HPLC. The review also discusses the application of HPLC in pharmacokinetic studies, stability testing, and bioequivalence assessments of antibiotic formulations. Overall, this review article serves as a valuable resource for researchers, pharmacists, and pharmaceutical analysts interested in the HPLC analysis of metronidazole, amoxicillin, and azithromycin, offering a comprehensive understanding of the analytical techniques and methodologies employed in antibiotic drug analysis.

Keywords: hplc, antibiotics, chromatographic condition, metronidazole, azithromycin, amoxicillin.

INTRODUCTION

Chromatography, pioneered by Russian botanist Mikhail Tswett in 1931, is a fundamental technique in separation science extensively employed across research laboratories and pharmaceutical industries worldwide. It involves the mass-transfer separation between a stationary and mobile phase. High-performance liquid chromatography (HPLC) emerges as a predominant analytical method within this realm, utilizing a liquid mobile phase to segregate mixture components. In HPLC, the stationary phase can be either liquid or solid, with the mobile phase mechanically pumped through a column containing the stationary phase.(Yandamuri & Nagabattula, n.d.)

INSTRUMENTATION

- **Solvent reservoir**.

Holds the mobile phase comprising polar and non-polar liquid components, crucial for sample composition.(Abdu Hussen, 2022)

- **Pump**.

Functions akin to the human heart, continuously delivering the mobile phase at a constant pressure and flow rate, ensuring reproducible flow characteristics essential for accurate results.

- **Sample injector**

Delivers the mixture to the injection valve in HPLC. (Schafer et al., 2007)

- **Column**.

Varies columns are used in HPLC analysis some of them are listed below

- Silica–based ultra-sphere C-18 column.
- µ Bondapak phenyl column.
- Zorbax SB-CN column.
- C18 column.
- Eclipse XDB-phenyl column.
- Varian C18 Microssorb.
- m-Bondapak C18 reverse phase column

❖ **Detector**

- **elemental detectors**

a) atomic absorption/emission

b) inductively coupled plasma–mass spectrometry

c) microwave-induced plasma

- **optical detectors**

a) UV/visible

b) IR/Raman

c) optical activity

d) evaporative light scattering

e) refractive index

- **luminescent detectors**

a) fluorescence/phosphorescence

b) chemiluminescence/bioluminescence

- **electrochemical detectors**

a) potentiometry

b) novel material /modified electrodes

c) array electrodes and pulsed

d) oscillometric techniques

- **mass spectrometric detectors**

a) time-of-flight/MALDI

b) Fourier transform ion cyclotron resonance

c) mass spectrometry

d) electrospray/ thermosspray

e) atmospheric pressure ionization and particle beam(Zhang et al., 2008)

❖ **DATA collector**

Signals from the detector are collected on chart recorders with varying complexity and ability to process and store chromatographic data. Computers integrate detector responses to each component, presenting them in an easily interpretable chromatograph.

Principle

The evolution of chromatography principles is governed by the van Deemter equation, relating linear velocity (flow rate) and plate height (HETP or 1/column efficiency), expressed as**,**

H=A+B/ u + Cu

Were,

A- Eddy's diffusion

B- Longitudinal diffusion

C- Concentration

u- Linear Velocity

APPLICATION

- Pharmaceutical analysis (drug purity, stability, and impurity profiling)
- Environmental monitoring (pesticides, pollutants, and contaminants)
- Food and beverage analysis (additives, toxins, and flavours)
- Biochemical research (protein purification, amino acid analysis)
- Widely utilized in clinical chemistry, pharmacological and forensic institutes, occupational health mass spectrometric core facilities.
- HPLC, along with NMR, serves as the primary analytical tool for reaction progress analysis among organic process research chemists.

Advantages:

- **High Sensitivity**: Capable of detecting low concentrations of analytes.
- **High Resolution:** Provides excellent separation of complex mixtures.
- **Wide Applicability:** Suitable for a broad range of compounds, including polar, non-polar, and ionic species.
- **Automation:** Can be automated for high-throughput analysis.
- **Quantitative Analysis:** Allows accurate quantification of analytes.

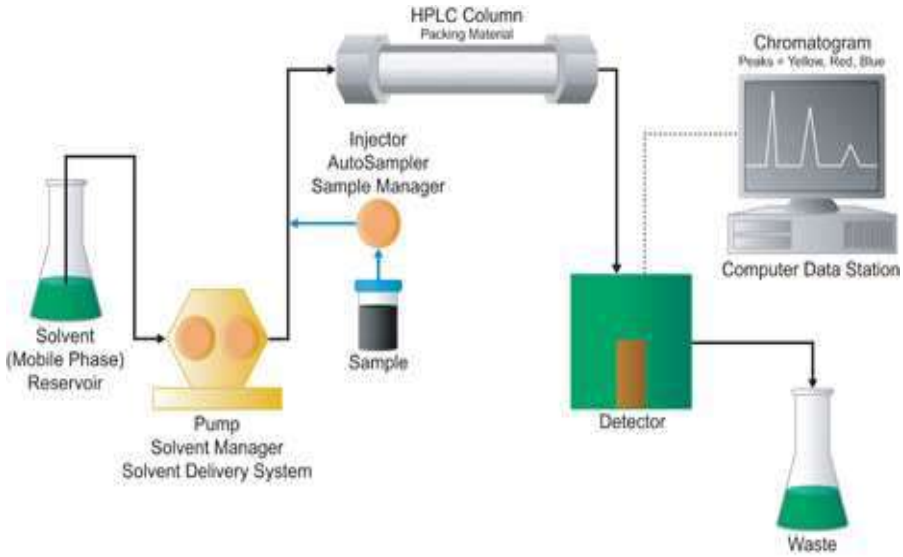
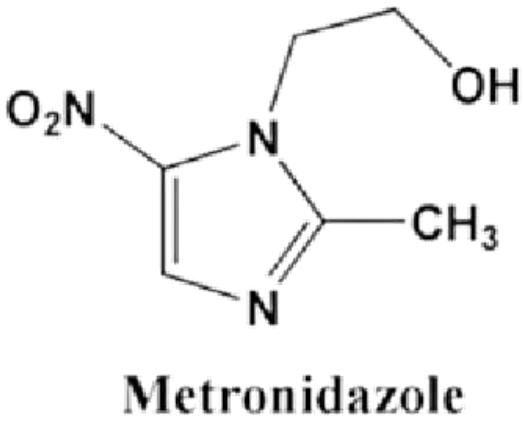


Diagram [1] HPLC instrumentation

INTRODUCTION TO DRUG PROFILE: [METRONIDAZOLE]

Metronidazole, a widely-used antibiotic, belongs to the 5-nitronimidazole class of drugs and is renowned for its effectiveness against anaerobic protozoa and bacteria. Its oral administration facilitates rapid absorption, allowing it to swiftly reach therapeutic concentrations in the bloodstream. Once absorbed, metronidazole exhibits excellent tissue penetration, permeating diverse bodily compartments including but not limited to bile, bone, breast milk, and cerebral abscesses. Its ability to achieve concentrations akin to those in plasma underscores its broad distribution and clinical utility in treating various infections. [6]

CHEMICAL STRUCTURE:



Metronidazole

Figure[1]

Metronidazole [2 -(2- methyl-5- nitroimidazole-1-yl) ethanol, Fig. [1] is an antimicrobial drug that is used to treat protozoal and anaerobic bacterial Infections.[7]

PHARMACODYNAMICS

The pharmacodynamics of metronidazole are not fully understood; however, its likely mechanism of action involves several steps. Metronidazole enters the cell through passive diffusion, where its nitro group is subsequently reduced to nitro radicals with the assistance of ferredoxin or flavodoxin. This reduction process is facilitated by the redox potential of electron transport components in anaerobic or microaerophilic microorganisms. The resulting toxic metabolites contribute to the drug's selectivity against these types of microbes.(Ceruelos et al., n.d.)

Molecular Formula	C6H9N3O3
Molecular Weight	171.16
Chemical Name	[2 -(2- methyl-5- nitroimidazole-1-yl) ethanol
Description	White to pale yellow crystalline powder with slight odour
Melting Point	316 to 320 f
Solubility	Less than 1mg/ml at 68 f
Adverse Effects	Hepatotoxicity
Bioavailability	95-100 %
Category	ANTIBIOTIC
Pka	2.57
BSC Class	Class 1
Half life	8 hours.

ANTIBIOTIC ACTION:

- **Amoebiasis**

Amoebiasis is an infection caused by the protozoan *Entamoeba histolytica*.

- **Trichomoniasis**

Trichomoniasis is infection caused by *Trichomonas vaginalis.*

- **Giardiasis**

Giardiasis is infection caused by *Giardia lamblia.*

- **peptic ulcers**

Is generally caused by the bacteria called *Helicobacter Pylori.*

Adverse Effects

- Nausea.
- Abdominal pain.
- Diarrhoea.
- Serious neurotoxicity.
- Optic neuropathy.
- Peripheral neuropathy.

Encephalopathy. No	Method	Short-term introduction	Ref
1	**RP_HPLC Development and Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. methanol: water in a ratio of 25:75. **Column.** silica–based ultra-sphere C-18 column (5 mm, 2.0 mm x 25 cm). **Detector.** Ultraviolet detector **Flow rate.** 1.0 ml/min−1 **Wavelength.** 276nm. **Retention Time** 4.5 min	(Mustapha et al)
2	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. 5% acetonitrile in 0.1 M of KH2PO4 buffer (pH = 4.5) **Column.** µ Bondapak phenyl (300 × 3.9mm, Waters, Ireland) column **Detector.** SPD-6AV UV detector. **Flow rate.** 1.5 ml/min−1 **Wavelength. 324**nm. **Retention Time** 4.9 min	(Emami et al., n.d.)

3	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. Buffer: acetonitrile (65: 35) **Column.** Zorbax SB-CN column (250 mm × 4.6 mm, 5 μm) **Detector.** UV detector **Flow rate.** 1.0 ml/min−1 **Wavelength. 319**nm. **Retention Time** 3.5 min	(Sahoo & Jain, 2016)
4	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. monobasic potassium phosphate (50 mM, pH 3, adjusted with phosphoric acid) and acetonitrile (65:35, v/v) containing sodium octane sulphonate (50 mM). **Column.** C18 column (250 mm 9 4.6 mm, 10 lm) **Detector.** UV–visible detector (SPD-20A, Tokyo, Japan) **Flow rate.** 1.2 ml/min−1 **Wavelength.** 280nm. **Retention Time** 4.49 min	(Elkady & Mahrouse, 2011)
5	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. Triethylamine: 0.02 M Potassium Dihydrogen phosphates (pH 3.5): Methanol (0.01:70:30) **Column.** C18 column (250mm × 4.6mm, 5μ) **Detector.** YL 9100 with PDA detector **Flow rate.** 1.2 ml/min−1 **Wavelength.** 292nm. **Retention Time** 6.10 min	(Trivedi et al., 2013)
6	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. 0.05M sodium acetate: acetonitrile: glacial acetic acid (75:25:1, v/v/v) with the pH adjusted to 4.0 with phosphoric acid. **Column.** *Eclipse XDB-phenyl column* **Detector.** UV detector **Flow rate.** ---- **Wavelength. 320**nm. **Retention Time** 4.06 min	(Ezzeldin & El-Nahhas, 2013)

7	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. Acetonitrile: 0.5 M potassium dihydrogen orthophosphate buffer pH 4.5 with triethylamine 30:70 (v/v) **Column.** C18 column (250x 4.6 mm, 5µm) **Detector.** UV detector UV-2075 plus **Flow rate.** 0.9 ml/min−1 **Wavelength. 289**nm. **Retention Time** 7.5 min	(Ghante et al., n.d.)
8	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase** Acetonitrile and 10 mM ammonium acetate (80:20, v/v) and 0.1% formic acid **Column.** Varian C18 Microssorb (150 mm × 4.6 mm i.d., 5 µm particle size) protected with a Phenomenex AJO-4287 C18 guard cartridge (5 mm × 4.6 mm i.d., 5 µm particle size) (Torrance, CA) **Detector.** UV detection (2–6,14–16) usually set at 313–318 nm, mass spectrometry (MS/MS) **Flow rate.** 1.0ml/min−1 **Wavelength.** 313–318 nm **Retention Time** 3.00 min	(Silva et al., 2009)
9	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. methanol_/water (40_/ 60, v/v) **Column.** m-Bondapak C18 reverse phase column packed with 10 mm dimethyl octadecyl silyl bonded amorphous silica (300 mm_/3.9 mm) **Detector.** Waters 996 photodiode array detector **Flow rate.** 1.0ml/min−1 **Wavelength.** nm. **Retention Time** 4.06 min	(Akay et al., 2002)

10	**RP_HPLC Development & Validation.** **Drug name.** METRONIDAZOLE	**Mobile phase**. 50mM phosphate buffer and adjusted with 1M HCl to a pH of (4.27 ± 0.01) as mobile phase A—methanol as mobile phase B (50:50 v/v) **Column.** *250 × 4.6 mm2 (id.)* *5 μm ODS column (Inertsil, Tokyo, Japan)* **Detector.** UV detector **Flow rate.** 0.8 ml/min−1 **Wavelength.** **242** nm. **Retention Time** 4.5 min	(Elkhoudary et al., 2016)

Drug Detail [AMOXICILLIN]

A Broad-Spectrum Antibiotic

Mechanism of Action:

Inhibits synthesis of bacterial cell walls by disrupting peptidoglycan biosynthesis.

β-Lactam Ring Binding: Amoxicillin's antibacterial activity relies on its β-lactam ring, which binds irreversibly to penicillin-binding proteins (PBPs) inside bacterial cells. Specifically, it targets PBPs involved in transpeptidation during peptidoglycan synthesis.

Inhibition of Transpeptidation: PBPs are integral for cross-linking peptidoglycan strands in bacterial cell wall formation. Amoxicillin disrupts this process by inhibiting the transpeptidase activity of PBPs, hindering the formation of cross-links in the peptidoglycan layer.

Weakening of Cell Wall Integrity: This interference with transpeptidation compromises the structural integrity of the bacterial cell wall, rendering it vulnerable to osmotic pressure changes. Consequently, the compromised cell wall leads to bacterial cell lysis and eventual death.

Selective Toxicity: Amoxicillin exhibits selective toxicity towards bacterial cells while sparing mammalian cells, owing to disparities in the structure and composition of bacterial versus mammalian cell walls.

Synergy with Autolytic Enzymes: Additionally, amoxicillin amplifies the activity of bacterial autolytic enzymes, such as autolysins, further contributing to cell wall degradation and eventual bacterial cell death.

Resistance Mechanisms: Despite its efficacy, bacterial resistance to amoxicillin arises through various mechanisms, including the production of β-lactamases, alteration of PBPs, and efflux pumps. Ongoing research aims to develop strategies to combat or circumvent these resistance mechanisms.

Crucial for maintaining cell integrity.

Spectrum of Activity

Effective against a wide range of Gram-positive and some Gram-negative organisms.

Administration

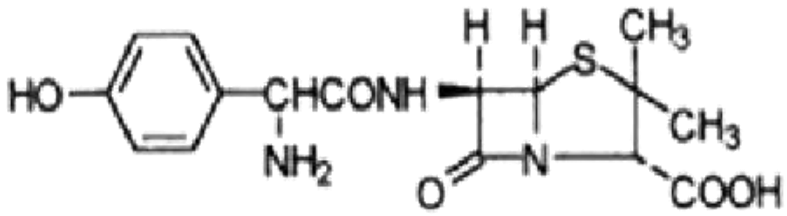
Superior absorption via oral administration compared to other β-lactam antibiotics.

Combination Therapy

Often co-formulated with potassium clavulanate, a β-lactamase inhibitor, to broaden its spectrum of activity.

Clinical Applications:

- Treatment of upper respiratory tract infections: tonsil, throat, larynx, and pharynx infections.
- First-line treatment for maxillary sinusitis and otitis media.
- Effective against Streptococcus pneumoniae and Haemophilus influenzae.
- Used for uncomplicated urinary tract infections caused by Escherichia coli.
- Prophylaxis against bacterial endocarditis.(De Marco et al., 2017)



Amoxicillin

Figure[2]

Pharmacokinetics and Pharmacodynamics:

Pharmacokinetics and pharmacodynamics: Amoxicillin boasts high oral bioavailability (70–90%), unaffected by food in the gastrointestinal tract. Plasma concentration peaks typically occur 1–2 hours post-dose. Approximately 20% of amoxicillin binds to plasma proteins.

International Non-proprietary Names (INN): Amoxicillin, formerly Amoxycillin

Synonyms: Amox; AMC; Amoxicillin trihydrate; Amoxicillin anhydrous; Amoxycillin trihydrate; DAmoxicillin; p-Hydroxyampicillin

IUPAC Names: (2S,5R,6R)- 6-{[(2R)-2-amino- 2-(4-hydroxyphenyl)- acetyl]amino}- 3,3-dimethyl7-oxo- 4-thia- 1-azabicyclo[3.2.0]heptane- 2-carboxylic acid [2S – [2α,5α,6β(S*)]] – 6 – [[Amino (4 – hydroxyphenyl)acetyl]amino] – 3,3 – dimethyl – 7 – oxo – 4 – thia – 1 – azabicyclo [3.2.0] heptane – 2 – carboxylic acid

Pure Active Ingredient: Amoxicillin

Appearance: Powder/Crystalline solid

Melting Point: 194°C

pH: 4.4–4.9 (0.25% w/v solution)

Optical Rotation: +290°–315°

Solubility: 3430 mg/L water

UVmax: 272 nm (water

No	Method	Short-term introduction	Ref
1	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase**. Potassium dihydrogen phosphate: methanol in a ratio of 95:0.5 v/v. **Column.** hypersil reverse phase, C-18 column (250 mm x 4.6 mm). **Detector.** Ultraviolet detector **Flow rate.** 1.5 ml/min **Wavelength.** 254nm. **Retention Time**	(Sendanyoye, 2018)

		3.5+/- 0.002 min	
2	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase**. Sodium dihydrogen phosphate: methanol (95:5v/v) **Column.** Agilent 1260 Infinity Thermostated column compartment (G2260A) **Detector.** UV detector. **Flow rate.** 1.5 ml/min **Wavelength. 230**nm. **Retention Time** 6.292 min	(Mem & Ma, 2018)
3	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase**. Buffer solution of KH2PO4:CH3CN (24: 1v/v) **Column.** Knauer (Germany) Spherimage-80, ODS,2-5 μm C18 column **Detector.** UV detector **Flow rate.** 0.7 ml/min **Wavelength. 870**nm. **Retention Time** 3.058 min	(Ashnagar & Naseri, 2007)

4	**RP_HPLC Development & Validation.** **Drug name.** AMOXICLLIN	**Mobile phase**. Methanol: acetic acid (20:80) **Column.** Reverse phase column µBondapak C18 (30 cm by 3.9mm) **Detector.** UV–photo diode **Flow rate.** 1.5 ml/min−1 **Wavelength.** 254nm. **Retention Time** 3.8 min	(Hsu & Hsu, 1992)
6	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase**. 0.05M potassium dihydrogen orthophosphate buffer And acetonitrile **Column.** Agilent ZorbaxSB-C8, column (150mm ×4.6mm,5µ) **Detector.** Photodiode array detector **Flow rate.** 2.0 mL/Min **Wavelength. 230**nm. **Retention Time** 4.8 min	(Raju et al., 2009)
5	**RP_HPLC Development & Validation.** **Drug name.** AMOXILLIN	**Mobile phase**. Amoxicillin trihydrate: potassium clavulanate (2:1) **Column.** Thermo hypersil zorbax BDS C18 (250mm × 4.6mm, 3µm) column **Detector.** Photodiode array PDA detector **Flow rate.** 0.6 to 0.5 ml/min−1 **Wavelength.** 215nm. **Retention Time** min	(Bellur Atici et al., 2017)

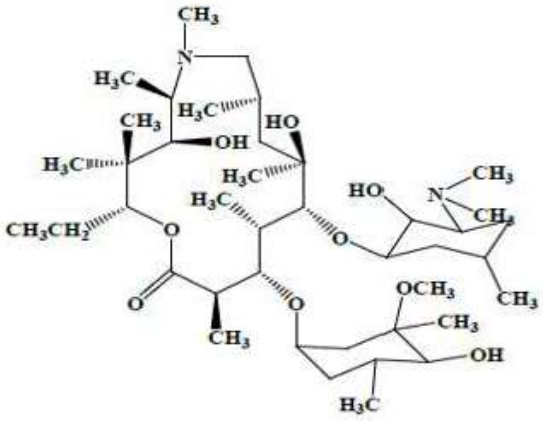
7	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase**. Ph 0.5 buffer: methanol (95:5 v/v) **Column.** Inertsil C18 column (250x 4.0 mm, 4µm) **Detector.** Photodiode array detector (PDA) **Flow rate.** 1 ml/min−1 **Wavelength. 220**nm. **Retention Time** 7.5 min	(Tippa & Singh, 2010)
8	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase** Acetonitrile and phosphate buffer containing methanol at ph 5.0 **Column.** Reverse phase C18e (250 mm × 4.0 mm, 5 µm) **Detector.** DAD-3000 UV-VIS diode array detector **Flow rate.** 0.8ml/min−1 **Wavelength.** 267nm **Retention Time** 4.00 min	(Batrawi et al., 2017)
9	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase**. 95% phosphate buffer (0.01mol/L), ph=4.8 and 5% acetonitrile mixture **Column.** Lichrosorb 10 µm C18 reverse phase column **Detector.** UV detector **Flow rate.** 1.3 ml/min−1 **Wavelength.** **229** nm. **Retention Time** 4.5 min	(deAbreu et al)

10	**RP_HPLC Development & Validation.** **Drug name.** AMOXICILLIN	**Mobile phase**. Methanol and glacial acetic acid (1%w/w) In the proportion of 50:50 **Column.** Intersil column (250 mm × 4.6 mm) **Detector.** U.V.975, Borwin software **Flow rate.** 1.0ml/min−1 **Wavelength.** **254** nm. **Retention Time** 3.04 min	(Sonawane & B. Bari, 2010)

DRUG DETAIL: [AZYTHROMYCIN]

Azithromycin is an antibiotic used to treat various types of infections caused by bacteria. It is effective in treating respiratory tract infections, skin infections, ear infections, eye infections, and some sexually transmitted diseases like gonorrhea12. Here are some key points about Azithromycin:

CHEMICAL STRUCTURE:



Figure[3]

Azithromycin [9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin] is a part of the azalide subclass of macrolides, and contains a 15-membered ring, with a methyl-substituted nitrogen instead of a carbonyl group at the 9a position on the aglycone ring, which allows for the prevention of its metabolism.

Mechanism of Action:

Azithromycin is a macrolide antibiotic that inhibits bacterial growth by interfering with protein synthesis and translation. This action helps treat various bacterial infections1.

Additionally, Azithromycin has immunomodulatory effects, making it useful in managing chronic respiratory inflammatory diseases1.

USE:

Azithromycin is primarily used for treating:

Respiratory Infections: Such as acute bacterial exacerbations of chronic obstructive pulmonary disease (COPD), acute bacterial sinusitis, and community-acquired pneumonia.

Enteric Infections: Including infections caused by Chlamydophila pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, and Streptococcus pneumoniae.

Genitourinary Infections: It may be used instead of other macrolides for some sexually transmitted infections1.

Adverse effects -:

Feeling sick (nausea) Stick to simple meals and do not eat rich or spicy food while you're taking this medicine.

Diarrhoea. Drink lots of fluids such as water or squash to avoid dehydration.

Being sick (vomiting)

Losing your appetite.

Headaches.

Feeling dizzy or tired.

Changes to your sense of taste.

No	Method	Short-term introduction	Ref
1	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase**. 0.0335 M Phosphate Buffer (pH 7.5) and methanol in the proportion 20:80 **Column.** C-8, 250 mm X 4.6 mm, 5 µm **Detector.** Ultraviolet detector **Flow rate.** 1.2 ml/min **Wavelength.** --- **Retention Time** 8.35 min	(Afzal et al,2016)
2	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase**. 90:10 v/v isocratic methanol/buffer **Column.** C18 (25 cm × 4.6 mm) **Detector.** UV detector. **Flow rate.** 2.0 ml/min−1 **Wavelength. 210**nm. **Retention Time**	(Waqar et al., 2022)

		7.614 min	
3	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase**. 30nm potassium dihydrogen phosphate buffer: acetonitrile **Column.** Welchrome C18 (4.6 × 250 mm, 5 µm) **Detector.** Photodiode ARRAY detector **Flow rate.** 1.1 ml/min−1 **Wavelength.** **235**nm. **Retention Time** 22.0 min	(Singh et al., n.d.)
4	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase**. 80:20Na2HPO4: methanol PH adjusted to 8 **Column.** SUPLECO C18 column (25 × 4.6 mm, 5 µm) **Detector.** PDA detector **Flow rate.** 1.0 ml/min−1 **Wavelength. 273**nm. **Retention Time** 2.77 min	(Nagaraju & Chowdary, 2018)

5	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase**. Ammonium dihydrogen phosphate conc. – 0.067 mol/l with triethylamine; PH 6.5 **Column.** Kromasil C18 (250 mm × 4.6 mm, 5.0 μm) **Detector.** **...** **Flow rate.** 1.0 ml/min−1 **Wavelength. 210**nm. **Retention Time** 6.10 min	(Zhuo, 2021)
6	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase**. 0.01 m dibasic sodium phosphate buffer 750: 250 (v/v). acetonitrile & methanol **Column.** Shim pack XR ODS 75 x 3.0 mm 2.2 μm **Detector.** UV detector **Flow rate.** 1.2 ml/min−1 **Wavelength.** **210** nm. **Retention Time** 7.656 min	(Bhardwaj et al., 2016)

7	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase**. Acetonitrile & mono basic potassium phosphate Buffer ph 8.5 ratio 65:35 v/v. **Column.** C18 Phenomenex Gemini 5u 250 x 4.6 **Detector.** PDA detector **Flow rate.** 2 ml/min−1 **Wavelength.** **220** nm. **Retention Time** 6.1 min	(Raja, n.d.)
8	**RP_HPLC Development & Validation.** **Drug name.** AZITHROMYCIN	**Mobile phase** 0.02M potassium dihydrogen phosphate Acetonitrile in ratio 65:35 (v/v) **Column.** Phenomenex C18 column (250x4.6mm 5um) **Detector.** UV detector **Flow rate.** 1.0ml/min−1	(Nyola & Jeyabalan, n.d.)

Reference

1.Abdu Hussen, A. (2022). High-Performance Liquid Chromatography (HPLC): A review. *Annals of Advances in Chemistry*, *6*(1), 010–020. https://doi.org/10.29328/journal.aac.1001026

2.Akay, C., Özkan, S. A., Şentürk, Z., & Cevheroğlu, Ş. (2002). Simultaneous determination of metronidazole and miconazole in pharmaceutical dosage forms by RP-HPLC. *Il Farmaco*, *57*(11), 953–957. https://doi.org/10.1016/S0014-827X(02)01296-X

3.Ashnagar, A., & Naseri, N. G. (2007). Analysis of Three Penicillin Antibiotics (Ampicillin, Amoxicillin and Cloxacillin) of Several Iranian Pharmaceutical Companies by HPLC. *E-Journal of Chemistry*, *4*(4), 536–545. https://doi.org/10.1155/2007/480907

4.Batrawi, N., Wahdan, S., & Al-Rimawi, F. (2017). A Validated Stability-Indicating HPLC Method for Simultaneous Determination of Amoxicillin and Enrofloxacin Combination in an Injectable Suspension. *Scientia Pharmaceutica*, *85*(1), 6. https://doi.org/10.3390/scipharm85010006

5.Bellur Atici, E., Yazar, Y., Ağtaş, Ç., Ridvanoğlu, N., & Karlığa, B. (2017). Development and validation of stability indicating HPLC methods for related substances and assay analyses of amoxicillin and potassium clavulanate mixtures. *Journal of Pharmaceutical and Biomedical Analysis*, *136*, 1–9. https://doi.org/10.1016/j.jpba.2016.12.032

6.Bhardwaj, S. K., Agarwal, D. D., Sharma, V., & Budakoti, S. K. (2016). *Development and Validation of RP- UHPLC Method for Azithromycin and Its Related Compounds in Tablet Dosage Form.*

7.Ceruelos, A. H., Romero-Quezada, L. C., Ruvalcaba, J. C., & Contreras, L. L. (n.d.). *Therapeutic uses of metronidazole and its side effects: An update*.

8.De Marco, B. A., Natori, J. S. H., Fanelli, S., Tótoli, E. G., & Salgado, H. R. N. (2017). Characteristics, Properties and Analytical Methods of Amoxicillin: A Review with Green Approach. *Critical Reviews in Analytical Chemistry*, *47*(3), 267–277. https://doi.org/10.1080/10408347.2017.1281097

9.Elkady, E. F., & Mahrouse, M. A. (2011). Reversed-Phase Ion-Pair HPLC and TLC-Densitometric Methods for the Simultaneous Determination of Ciprofloxacin Hydrochloride and Metronidazole in Tablets. *Chromatographia*, *73*(3–4), 297–305. https://doi.org/10.1007/s10337-010-1898-x

10.Elkhoudary, M. M., Abdel Salam, R. A., & Hadad, G. M. (2016). Development and Optimization of HPLC Analysis of Metronidazole, Diloxanide, Spiramycin and Cliquinol in Pharmaceutical Dosage Forms Using Experimental Design. *Journal of Chromatographic Science*, chromsci;bmw126v1. https://doi.org/10.1093/chromsci/bmw126

11.Emami, J., Ghassami, N., & Hamishehkar, H. (n.d.). *A RAPID AND SENSITIVE HPLC METHOD FOR THE ANALYSIS OF METRONIDAZOLE IN HUMAN PLASMA: APPLICATION TO SINGLE DOSE PHARMACOKINETIC AND BIOEQUIVALENCE STUDIES*.

12.Ezzeldin, E., & El-Nahhas, T. (2013). New Analytical Method for the Determination of Metronidazole in Human Plasma: Application to Bioequivalence Study. *Tropical Journal of Pharmaceutical Research*, *11*(5), 799–805. https://doi.org/10.4314/tjpr.v11i5.14

13.Ghante, M. R., Pannu, H. K., Loni, A., & Shivsharan, T. (n.d.). *Development and Validation of a RP- HPLC Method for Simultaneous Estimation of Metronidazole and Norfloxacin in Bulk and Table*. *4*.

14.Hsu, M. C., & Hsu, P. W. (1992). High-performance liquid chromatographic method for potency determination of amoxicillin in commercial preparations and for stability studies. *Antimicrobial Agents and Chemotherapy*, *36*(6), 1276–1279. https://doi.org/10.1128/AAC.36.6.1276

15.Mem, H., & Ma, M. (2018). Development and validation of RP-HPLC method for determination of amoxicillin residues and application to NICOMAC coating machine. *Journal of Analytical & Pharmaceutical Research*, *7*(5). https://doi.org/10.15406/japlr.2018.07.00287

16.Nagaraju, K., & Chowdary, Y. A. (2018). *Analytical Method Development and Validation for The Simultaneous Estimation of Azithromycin and Cefixime by Rp- Hplc Method in Bulk and Pharmaceutical Formulations*.

17.Nyola, N., & Jeyabalan, G. (n.d.). *SIMULTANEOUS ESTIMATION OF CEFIXIME AND AZITHROMYCIN IN API'S AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC*. *2*(2231).

18.Raja, M. S. (n.d.). *RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AZITHROMYCIN AND AMBROXOL HYDROCHLORIDE IN TABLETS*.

19.Raju, Ch. B. V. N., Sharma, H. K., Rao, Ch. S., & Rao, G. N. (2009). RP-HPLC method for analysis of related substances in amoxicillin drug substance. *Acta Chromatographica*, *21*(1), 57–70. https://doi.org/10.1556/AChrom.21.2009.1.5

20.Sahoo, D. R., & Jain, S. (2016). A Rapid and Validated RP-HPLC Method for the Simultaneous Quantification of Benzoic Acid, Metronidazole and Miconazole Nitrate in Vaginal Formulations. *Journal of Chromatographic Science*, *54*(9), 1613–1618. https://doi.org/10.1093/chromsci/bmw113

21.Schafer, W. A., Hobbs, S., Rehm, J., Rakestraw, D. A., Orella, C., McLaughlin, M., Ge, Z., & Welch, C. J. (2007). Mobile Tool for HPLC Reaction Monitoring. *Organic Process Research & Development*, *11*(5), 870–876. https://doi.org/10.1021/op7000854

22.Sendanyoye, M. (2018). Validation of HPLC-UV method for determination of amoxicillin Trihydrate in capsule. *Annals of Advances in Chemistry*, 055–072. https://doi.org/10.29328/journal.aac.1001014

23.Silva, M., Schramm, S., Kano, E., Koono, E., Porta, V., & Serra, C. (2009). Development and Validation of a HPLC-MS-MS Method for Quantification of Metronidazole in Human Plasma. *Journal of Chromatographic Science*, *47*(9), 781–784. https://doi.org/10.1093/chromsci/47.9.781

24.Singh, N., Singh, Y., Bhadauria, R. S., & Govindasamy, J. (n.d.). *ANALYTICAL METHOD DEVELOPMENT, OPTIMIZATION AND VALIDATION OF COMBINATION OF AZITHROMYCIN AND BENZOYL PEROXIDE BY RP-HPLC USING DESIGN OF EXPERIMENT AS PER ICH GUIDELINE*.

25.Sonawane, L. V., & B. Bari, S. (2010). Development and Validation of RP-HPLC Method for The Simultaneous Estimation of Amoxicillin Trihydrate and Bromhexine Hydrochloride from Oily Suspension. *Pharmaceutica Analytica Acta*, *01*(02). https://doi.org/10.4172/2153-2435.1000107

26.Tippa, D. M. R., & Singh, N. (2010). Development and Validation of Stability Indicating HPLC Method for Simultaneous Estimation of Amoxicillin and Clavulanic Acid in Injection. *American Journal of Analytical Chemistry*, *01*(03), 95–101. https://doi.org/10.4236/ajac.2010.13013

27.Trivedi, K. D., Chaudhary, A. B., & Mohan, S. (2013). *Devlopment and validation of RP-HPLC Method for estimation of Metronidazole and Norfloxacin in suspension form*.

28.Waqar, A., Hasnain, S., Butt, M. T., Hafeez, Z., Raza, A., Ishaq, M., & Iqbal, S. (2022). Development of a Simple RP-HPLC, UV-VIS, TGA, XRD Method for Validation and Azithromycin in Bulk and Pharmaceutical Dosage forms as an Alternative to the USP Method. *International Journal of Scientific and Research Publications (IJSRP)*, *12*(06), 320–330. https://doi.org/10.29322/IJSRP.12.06.2022.p12640

29.Yandamuri, N., & Nagabattula, K. R. S. (n.d.). *Comparative Study of New Trends in HPLC: A Review*.

30.Zhang, B., Li, X., & Yan, B. (2008). Advances in HPLC detection—Towards universal detection. *Analytical and Bioanalytical Chemistry*, *390*(1), 299–301. https://doi.org/10.1007/s00216-007-1633-0

31.Zhuo, Y. (2021). A Study on the Determination of Azithromycin by High-Performance Liquid Chromatography. *Proceedings of Anticancer Research*, *5*(4), 125–130. https://doi.org/10.26689/par.v5i4.2368