3.1 GEMIFLOXACIN MESYLATE AND AMBROXOL HYDROCHLORIDE COMBINATION DRUG PRODUCT (G – CIN – A TABLET):[17]

G – CIN – A is a combination tablet of gemifloxacin mesylate 320mg and ambroxol hydrochloride 75mg, manufactured by Lupin Pharmaceuticals. It is used in the treatment of pulmonary obstructive acute bacterial exacerbation of chronic bronchitis and community-acquired pneumonia.

Chronic bronchitis brings about cough every day with sputum production that lasts for at least 3 months to two years in a row. Many of the bronchi develop chronic inflammation with swelling and excess mucus production. The inflammation, swelling, and mucus frequently and significantly inhibit the airflow to and from the lung alveoli by narrowing and partially obstructing the bronchi and bronchioles.

Pneumonia is an infection in one or both of your lungs in which small germs, such as bacteria, viruses, and fungi, can cause pneumonia. Germs sometimes find a way to enter the lungs and cause infections. The lungs air sacs (alveoli) become inflamed and fill up with fluid causing the symptoms of pneumonia, such as a cough, fever, chills, and trouble breathing.

G – CIN – A tablet acts by inhibits DNA gyrase and DNA topoisomerase IV. While other drug from combination that is Ambroxol Hydrochloride is a mucoactive drug and produces secretolytic actions that restore the physiological clearance mechanisms of the respiratory tract, which play an important role in the body’s natural defense mechanisms. It promotes mucus clearance, facilitates expectoration and eases productive cough, allowing patients to breathe freely and deeply.

**Side effects:** Common side effects are Nausea, diarrhea, dizziness, lightheadedness, headache, or trouble sleeping may occur.

**Storage Conditions:** Store tablets at room temperature.
3.1.1 GEMIFLOXACIN MESYLATE.\textsuperscript{[18–20]}

Introduction.

Gemifloxacin mesylate  broad-spectrum quinolone antibacterial agent used in the treatment of acute bacterial exacerbation of chronic bronchitis and mild-to-moderate pneumonia.

Gemifloxacin is indicated for the treatment of infections caused by susceptible strains of the designated microorganisms in the conditions listed below.

- Acute bacterial exacerbation of chronic bronchitis caused by \textit{S. pneumoniae}, \textit{Haemophilus influenzae}, \textit{Haemophilus parainfluenzae}, or \textit{Moraxella catarrhalis}.

- Community-acquired pneumonia (of mild to moderate severity) caused by \textit{S. pneumoniae} (including multi-drug resistant strains, \textit{Haemophilus influenzae}, \textit{Moraxella catarrhalis}, \textit{Mycoplasma pneumoniae}, \textit{Chlamydia pneumoniae}, or \textit{Klebsiella pneumonia}.

Description.

Gemifloxacin mesylate chemically is (R, S)-\textit{n}-\textit{(Z)}-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid (Figure 10).

![Chemical Structure of Gemifloxacin](image-url)

\textbf{Figure 10. Chemical Structure of Gemifloxacin.}
It is a compound related to the fluoroquinolone class of antibiotics, and is available as the mesylate salt in the sesquihydrate form. The mesylate salt is a white to light brown solid with a molecular weight of 485.49. Its empirical formula is $C_{18}H_{20}FN_{5}O_{4} \cdot CH_{4}O_{3}S$. Gemifloxacin is considered freely soluble at neutral pH (350 $\mu$g/mL at $37^\circ$C, pH 7.0). Its CAS number is 175463 – 14 – 6.

**Mechanism of Action**

Gemifloxacin inhibits DNA gyrase and DNA topoisomerase IV. DNA gyrase is needed for DNA replication and transcription, DNA repair, recombination and transposition. Topoisomerase IV facilitates separation of intertwined, replicated DNA before cell division occurs. Gemifloxacin forms a ternary complex with gyrase and topoisomerase IV, which blocks DNA replication, thus resulting in DNA release, chromosomal disruption and cell death.

**Pharmacokinetics.**

**Absorption and Bioavailability.**

Gemifloxacin, given as an oral tablet, is rapidly absorbed from the gastrointestinal tract. Peak plasma concentrations of gemifloxacin were observed between 0.5 and 2 hours following oral tablet administration. The pharmacokinetics of gemifloxacin were not significantly altered when it is administered with a high-fat meal.

**Distribution.**

*In vitro* binding of gemifloxacin to plasma proteins in healthy subjects is approximately 60 to 70% and is concentration independent. It is widely distributed throughout the body after oral administration. Concentrations of gemifloxacin in bronchoalveolar lavage fluid exceed those in the plasma. Gemifloxacin penetrates well into lung tissue and fluids.
**Metabolism.**

Gemifloxacin is metabolized to a limited extent by the liver. The unchanged compound is the predominant drug-related component detected in plasma (approximately 65%) up to 4 hours after dosing. All metabolites formed are minor (<10% of the administered oral dose); the principal ones are N-acetyl gemifloxacin, the E-isomer of gemifloxacin and the carbamyl glucuronide of gemifloxacin. Cytochrome P450 enzymes do not play an important role in gemifloxacin metabolism, and the metabolic activity of these enzymes is not significantly inhibited by gemifloxacin.

**Excretion**

Gemifloxacin and its metabolites are excreted via dual routes of excretion. Following oral administration in healthy subjects, a mean (± SD) of 61 ± 9.5% of the dose was excreted in the feces and 36 ± 9.3% in the urine as unchanged drug and metabolites.

**Adverse effects.**

Fluoroquinolones, including GEM are associated with an increased risk of tendinitis and tendon rupture in all ages. This risk is further increased in older patients usually over 60 years of age, in patients taking corticosteroid drugs, and in patients with kidney, heart or lung transplants. It may exacerbate muscle weakness in persons with myasthenia gravis, so it is better avoid it in patients with known history of myasthenia gravis. Common side effects are Nausea, diarrhea, dizziness, lightheadedness, headache, or trouble sleeping may occur.

**Storage / Stability.**

Store tablets at room temperature. Protect from light.
3.1.2 AMBROXOL HYDROCHLORIDE. [21]

Introduction.

Ambroxol hydrochloride is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. It is effective in all forms of tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma. During acute exacerbations of bronchitis it is given with the appropriate antibiotics. Ambroxol also provides pain relief in acute sore throat. Pain in sore throat is the hallmark of acute Pharyngitis. The main property of ambroxol hydrochloride for treating sore throat is the local anesthetic effect.

Description.

Ambroxol, chemically is 4 - [(2 – amino – 3,5 – dibromophenyl) methylamino] cyclohexan – 1 – ol (Figure 11).

![Chemical Structure of Ambroxol](image)

**Figure 11. Chemical Structure of Ambroxol.**

Ambroxol is an active N-desmethyl metabolite of the mucolytic, bromhexine. It is a white to yellowish crystalline powder; slightly soluble in water, ethanol; soluble in dimethylformamide, methanol; insoluble in chloroform and benzene; melting point
Ambroxol is a mucolytic agent. It produces inhibition of NO-dependent activation of soluble guanylate cyclase, which can suppress excessive mucus secretion, thereby lowering phlegm viscosity and improving mucociliary transport of bronchial secretions. Excessive nitric oxide (NO) is associated with inflammatory and some other disturbances of airways function. NO enhances the activation of soluble guanylate cyclase and cGMP accumulation.

It also stimulates synthesis and release of surfactant by type II pneumocytes. Surfactant acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, improving its transport and providing protection against infection and irritating agents.

**Pharmacokinetics.**

**Absorption.**

Ambroxol formulations which are not sustained release are absorbed swiftly and almost completely after oral administration. Oral bioavailability is approx. 60% owing to the first-pass effect. Plasma concentrations are in a linear relationship to the dose. Peak plasma levels are attained after 0.5 to 3 hours. An ambroxol modified-release capsule, on the other hand, has delayed absorption (Tmax 6.5 ± 2.2 h) and a relative bioavailability of 95% compared with the tablets.

**Distribution**

Plasma protein binding is around 90% in the therapeutic range. After oral, intravenous and intramuscular administration ambroxol is distributed swiftly and extensively from the blood into the tissues. The highest active ingredient concentrations are measured in the lung.
Metabolism.
Studies in human liver microsomes showed that CYP3A4 is the predominant isoform for ambroxol metabolism. Otherwise ambroxol is metabolised in the liver mainly by conjugation.

Elimination.
Around 30% of an oral dose is eliminated via the first-pass effect. The terminal half-life is about 10 hours. Total clearance is in the region of 660 ml/min, and renal clearance is 8% of total clearance.

Adverse effects.
At the recommended doses, ambroxol is well tolerated. Nausea, headache and gastrointestinal disorders have been observed rarely. Other toxic effects include skin irritation, eye irritation and respiratory tract irritation. Ingestion of large doses may cause gastrointestinal tract irritation with decreased motility or constipation, ulceration or bleeding from the stomach or duodenum, and peritonitis. It may affect behaviour/CNS (tremor, convulsions, ataxia and somnolence), respiration (dyspnoea, respiratory stimulation), the liver, blood (changes in white blood cell count) and the urinary system. Occasional gastrointestinal side effects may occur but these are normally mild.

Precaution.
Ambroxol hydrochloride has not been shown to have any teratogenic or toxic effects on the foetus. It is advisable to avoid use during the first trimester of pregnancy.

Storage Condition.
Store at temperatures not exceeding 30°C. Protect from light.
3.1.3 Reported Stability Indicating Methods for Gemifloxacin Mesylate Single and in Combination Products.

C.S. Paim et al., have reported a stability-indicating liquid chromatographic (LC) method they developed and validated for quantitative determination of gemifloxacin mesylate in coated tablets. The chromatographic method employed the Agilent Eclipse® XDB RP-18 (150 x 4.6 mm; 5 µm) with a mobile phase consisting of 0.3% triethylamine (pH 3.0) and acetonitrile (80:20, v/v). Drug and drug product solutions were exposed to direct UV-A and UV-C radiation, alkaline and acid hydrolysis, thermal stress and an oxidation effect by hydrogen peroxide to evaluate method stability-indication.\textsuperscript{[22]}

Clésio S. Paim et al., developed stability indicating HPLC method for gemifloxacin, solutions (from the drug product) exposed to direct UVA radiation (352 nm), alkaline hydrolysis, acid hydrolysis, thermal stress, hydrogen peroxide causing oxidation, and a synthetic impurity were used to evaluate the specificity of the bioassay. The drug reference standard (RS), photo degraded, and synthetic impurity samples were also studied in order to determine the preliminary in vitro cytotoxicity to peripheral blood mononuclear cells.\textsuperscript{[23]}

Ramzia I. EL-Bagary et al., observed that the first method depends on RP-HPLC, separation of drug and its degradation products was successfully achieved on a Hypersil BDS C18 column using mobile phase consisted of citrate buffer (adjusted to 2.5 pH by citric acid):Acetonitrile (70:30, v/v) at 1 ml/min flow rate and 267 nm wavelength of detection. The second method which achieved successful separation of drug and its degradation products depends on TLC densitometry using a developing system consisted of chloroform : methanol : toluene : diethylamine : water (33.6:33.6:16.8:10.8:6, v/v) with 20 µl spotting volume and 260 nm wavelength of detection.\textsuperscript{[24]}
Tahir Ali Sheikh et al., in their work reported a stability indicating RP-HPLC method that was developed and validated for the determination of gemifloxacin in tablet formulation. The drug was subjected to forced degradation study in terms of acidic, thermal, oxidative, photo and basic stresses. Degradation products produced as a result of stress testing were successfully separated through C18 column (250 x 4.6 mm, 5 μm) using ammonium acetate buffer (pH 2.7; 0.05 M) and acetonitrile (70:30, v/v) as a mobile phase at a flow rate of 0.7 mL/min. The diode array detection was performed at 272 nm.\[25\]

Lakshmana Rao A. et al., in their work reported a simple, rapid, accurate and precise stability indicating RP-HPLC method was developed for the determination of gemifloxacin in pure and tablet dosage forms. Separation of the drug was achieved on a reverse phase Symmetry C18 column. The method showed a linear response for concentration in the range of 10-50 μg/ml using acetate buffer: methanol as the mobile phase in the ratio of 38:62 v/v with detection at 273 nm with a flow rate of 1 ml/min and retention time was 2.044 min.\[26\]

Panda SS et al., in their work reported a novel, accurate and precise reverse phase ultra fast liquid chromatographic method for determination of gemifloxacin mesylate has been developed and validated. Separation was achieved on an Enable C18G column (250mm x 4.6mm i.d., 5μm) using methanol: 10mM TBAHS (70:30, v/v) as mobile phase at a flow rate of 1.0ml/min and PDA detection at 271nm. Linearity was observed in the concentration range of 1.0- 200 μg/ml (r²=1). Forced degradation was performed by using HCl, NaOH, oxidation, thermal and UV radiation.\[27\]

Tammam Marwa Hosny has worked on photostability of gemifloxacin mesylate and lomefloxacin hydrochloride antibacterial agents in dilute aqueous solutions (bulk powder and dosage form) was studied by applying the ICH recommended conditions. The photodegradation processes were monitored by UV-Vis spectrophotometry and quantified by HPLC. The structures of degradation products in the aqueous solution have been deduced from LC-MS/MS.\[28\]
Rao RN et al., developed a validated stability indicating RP-HPLC assay of gemifloxacin mesylate by separating its related substances on an Inertsil-ODS3V-C18 (4.6 × 250 mm; 5 μm) column using 0.1% trifluoroacetic acid (pH 2.5) and methanol as a mobile phase in a gradient elution mode at a flow rate of 1.0 mL/min at 27°C. The column effluents were monitored by a photodiode array detector set at 287 nm.\(^{[29]}\)

Ravisankar Panchumarthy et al., has developed RP-HPLC method by using Welchrom C18 Column (4.6 X 250mm, 5μm), SHIMADZU LC-20AT prominence liquid chromatography. The mobile phase used was phosphate buffer (pH-3.2): acetonitrile (60:40 v/v) with a flow rate of 1mL/min. The responses are measured at 280 nm using SHIMADZU SPD-20A prominence UV-Vis detector. The retention time was found to be 5.663 min.\(^{[30]}\)

Ashraf M. Mahmoud, has developed a stability indicating HPTLC method for gemifloxacin. The chromatographic separation was performed on HPTLC precoated silica gel plate 60F254 as stationary phase. The mobile phase consisted of a mixture of ethyl acetate: methanol: 25% ammonia, (8:4.5:3, v/v/v). The detection was performed using fluorescence mode and the emission intensity was measured using optical filter K400 after excitation at 342 nm. The Rf value was 0.47 ± 0.03.\(^{[31]}\)

3.1.4 Reported in – vitro dissolution methods for gemifloxacin mesylate single and in combination products.

S. Poongothai et al., establish a correlation between in vitro dissolution and in vivo absorption data of prepared immediate release gemifloxacin tablets (Zagam) and compare with conventional tablets of gemifloxacin (Factive). In vitro release data were obtained for test and reference tablets by using the USP apparatus II, 0.01N HCl of pH 2.0 at 50 rpm. A group of six healthy, male human subjects participated for in vivo study. Serial blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hr. Gemifloxacin was measured by Ultra performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.\(^{[32]}\)
Khan Hajera *et al*., has work to develop and validate a dissolution test for gemifloxacin mesylate tablets using spectrophotometric method. The dissolution established conditions were: 900 mL of 0.01M HCl pH 2.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 271 nm for gemifloxacin mesylate.\(^{[33]}\)

Zahid Zaheer *et al*., has work to develop and validate a dissolution test for gemifloxacin mesylate and ambroxol hydrochloride tablets using spectrophotometric method. The dissolution established conditions were: 900 ml of 0.01M HCl pH 2.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 271 nm for gemifloxacin mesylate and 243.5 nm for ambroxol hydrochloride.\(^{[34]}\)

V. Balaji. S. *et al*., has work to develop and validate a dissolution test for gemifloxacin mesylate tablets using spectrophotometric method. The dissolution established conditions were: 900 ml of 0.01N HCl pH 2.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 271 nm.\(^{[35]}\)

Paim CS. *et al*., has reported in vitro dissolution profile obtained using 900 ml of pH 6.0 phosphate buffer as a dissolution medium at 37\(^{0}\)C±0.5\(^{0}\)C and paddles at 50 rpm. The in vitro dissolution samples were analyzed using a liquid chromatography method, and the validation was performed according to USP 34 (2011). The method showed specificity, precision, accuracy, robustness and linearity.\(^{[36]}\)
3.1.5 Reported stability indicating methods for Ambroxol hydrochloride single and in combination products.

P.S. Jain, has developed stability-indicating HPTLC method for the analysis of ambroxol hydrochloride both as a bulk drug and in formulations. The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of methanol–triethylamine (4:6 v/v). The system was found to give a compact spot for ambroxol hydrochloride (Rf value of 0.53 ± 0.02). Densitometric analysis of ambroxol hydrochloride was carried out in the absorbance mode at 254 nm.[37]

Dhiraj S.Nikam et al., has developed stability-indicating RP-HPLC method for ambroxol hydrochloride and roxithromycin in bulk and pharmaceutical formulation. The separation is made by phenomenex Gemini C18 column (250 cm × 4.6 mm, 5 µm) using water: acetonitrile: orthophosphoric acid (50:50:0.1) as a mobile phase. Detection is carried out at 210 nm. The retention time of ambroxol hydrochloride and roxithromycin is 2.33 and 4.22 min, respectively.[38]

Bapatu, Hanimi Reddy et al., develop a single RP-HPLC method for determination of Terbutaline sulphate, Guaifenesin, Ambroxol hydrochloride and preservatives (methyl paraben and propyl paraben) contents in liquid formulation. Chromatographic separation was achieved on a Sunfire C18, 250 x 4.6 mm, 5µm column. Mobile phase composed of Sol-A: phosphate buffer (0.01M Potassium dihydrogen orthophosphate buffer pH 6.0 ± 0.1) and Sol-B: Acetonitrile with a simple gradient program, 1.0 ml per min flow rate and detection was at 214 nm.[39]

Rakshit Kanubhai Trivedi et al., developed a stability indicating RP-UPLC method for simultaneous determination of ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben in liquid pharmaceutical formulation. The desired chromatographic separation was achieved on an Agilent Eclipse plus C18, 1.8 µm (50 x 2.1 mm) column using gradient elution at 237 nm detector wavelength. The optimized mobile phase consists of a mixture of 0.01 M phosphate buffer and 0.1 % triethylamine as a solvent-A and acetonitrile as a solvent-B.[40]
M. Sudheer et al., has developed method consisting the mobile phase K$_2$HPO$_4$ – pH 6.5 : Acetonitrile (68 : 32) with isocratic programming, Hypersil, BDS, C 8, column (150 mm x 4.6 mm i.d., 5μm particle size) as stationary phase with a flow rate of 1.5 mL/minute by using λmax 215nm and PDA detector. Ambroxol HCl and Azithromycin were subjected to stress condition and products were analyzed by using photo diode array detector. It was found to be stable in milder condition of stress (0.1 M HCl, 0.1 M NaOH, 3% H$_2$O$_2$, at 60°C/10 minutes). The analyte peaks were well resolved from the degraded impurities.[41]

M. Gnana Raja et al., developed a stability indicating HPLC determination of Cetirizine Hydrochloride and Ambroxol hydrochloride in pharmaceutical liquid oral dosage form syrup. Separation was achieved in Hypersil BDS C18 column, 5 μ, and 250 mm x 4.60 mm id with flow rate of 1.5 ml/minute and the detection at 230nm. 0.2 molar Ammonium phosphate with the pH of 4.0 with dilute orthophosphoric acid of about 65 percentage in acetonitrile combination was used as a mobile phase.[42]

G.V.S.Kumar et al., developed a simple, precise and accurate method has been for simultaneous estimation of Ambroxol hydrochloride and Levocetirizine dihydrochloride. The proposed RP-HPLC method utilizes Enable C18 G column (250 x 4.6mm, 5μm), mobile phase consisting of Phosphate buffer pH 3.0: Methanol in the ratio of 20:80 (v/v) and UV detection at 236nm using a photodiode array detector. The stability indicating capability of the method was proven by subjecting the drugs to stress conditions such as alkaline and acid hydrolysis, oxidation, photolysis, thermal degradation as per ICH guidelines.[43]

Sharada Musty et al., has developed a simple, sensitive, accurate, precise and selective stability indicating reverse phase high performance liquid chromatographic method and was validated for the determination of ambroxol and cefadroxil in bulk drug and pharmaceutical dosage form. Separation and quantification were achieved on a Phenomenex C18, 5μm, 150 x 4.6 mm i. d. column using PDA detector. The mobile phase was 0.1% OPA: methanol (50:50 v/v), at a flow rate of 1 ml/min and injection volume was 10μL. Detection was carried out at a wavelength of 249 nm.[44]
Sagar B.Wankhede et al., developed a RP-HPLC for simultaneous estimation gemifloxacin mesylate and ambroxol hydrochloride in tablets. Chromatographic separation was performed on Agilent ODS C18 (250 × 4.6 mm i.d., 5µm) column with a mobile phase comprising mixture of 25 mM potassium dihydrogen orthophosphate buffer (pH 3.5, adjusted with orthophosphoric acid) : acetonitrile (75:25 v/v) at a flow rate 1 ml/min, with UV-detection at 246 nm. To establish stability indicating nature of the LC method, forced degradation of drug substances was performed under different stress conditions viz. acid and base hydrolysis, dry heat degradation and oxidation.\cite{45}

Sohan S Chitlange et al., developed a rapid, precise, selective and sensitive HPTLC method for simultaneous determination of Amoxicillin trihydrate and ambroxol hydrochloride in pharmaceutical dosage form and was validated in the present work. The chromatographic separation was performed on precoated silica gel 60 F254 plates with Ethyl acetate: methanol: toluene: water: glacial acetic acid (6.0: 3.0: 2.0: 1.0: 0.5 v/v) as the mobile phase with UV detection at 237nm. Retention factor for AMOX and AMBRO were found to be 0.32 ± 0.04 and 0.70 ± 0.05 respectively. Drugs were subjected to oxidation, acid hydrolysis, base hydrolysis and sun light to apply stress condition for degradation studies as per ICH guidelines.\cite{46}

K. Lakshmi Narasimha Rao et al., has developed a stability-indicating RP-HPLC method for terbutaline sulfate, guaifenesin and ambroxol HCl, for its potential impurities in drug substances and drug products. Efficient chromatographic separation was achieved on X-Terra RP-18 column with a simple mobile phase combination containing a gradient mixture of solvents A and B at a flow rate of 1.0 mL min\(^{-1}\) and quantitation was carried out using ultraviolet detection at 222 nm with column temperature of 35\(^{0}\)C. The drugs were subjected to stress conditions as prescribed by international conference on harmonization (ICH).\cite{47}

Sameena Mehveen et al., has developed a stability indicating RP-HPLC method for simultaneous estimation of Loratidine, Ambroxol Hydrochloride and Guaifenesin in bulk and pharmaceutical liquid dosage forms. The method was carried out on a HYPERSIL, ODS (C18 250x4.6 ID) 5µm column with a mobile phase of mixed phosphate buffer :
Methanol : Acetonitrile (20:50:30 v/v) at a flow rate of 1.0 mL/min & PH of 5.5. Detection was carried out at 245 nm. The drug was subjected to stress conditions as per ICH guidelines & was found to be stable.\textsuperscript{[48]}

Sravya Neeli \textit{et al.}, reported in their work an RP-HPLC method for the simultaneous determination of Paracetamol, Guaifenesin, Ambroxol hydrochloride, Phenylephrine hydrochloride and Chlorpheniramine maleate in tablets was developed and validated \textsuperscript{®} as per ICH and FDA guidelines. The method was carried out on a with Zodiac C18 (50mm x 4.6mm, 5\textmu m) column. Ortho phosphoric acid buffer (1M) and Acetonitrile in the ratio (50:50v/v) used as mobile phase and flow rate of 1.5ml/min. The detection was carried out at 225nm and ambient column temperature was maintained. The drugs were subjected to stress conditions of acid, base, water hydrolysis, oxidation, photolysis and thermal degradation, as prescribed by international conference on harmonization (ICH).\textsuperscript{[49]}

Sreedhar Lade \textit{et al.}, has developed Stability indicating RP-HPLC for Ambroxol, Salbutamol and Theophylline in bulk and pharmaceutical dosage form. The chromatographic separation was achieved with Inertsil ODS C-18, (250×4.6 mm) and 5\textmu m particle size column. The optimized mobile phase consisting of phosphate buffer: Acetonitrile (55:45%v/v) and adjusted the mobile phase to pH 3.0 with o-phosphoric acid. The flow rate was 1.0 mL/min and eluents were detected at 225 nm using PDA detector Degradation studies were carried for Ambroxol, Salbutamol and Theophylline under various stress conditions such as acid hydrolysis, base hydrolysis, oxidation, thermal, photochemical and UV.\textsuperscript{[50]}

\textbf{3.1.6 Reported \textit{in – vitro} dissolution methods for Ambroxol hydrochloride single and in combination products.}

T. Alighieri \textit{et al.}, reported in their work in vitro dissolution of two ambroxol-HCl containing sustained release preparations (75 mg) and the effect of pH of the dissolution medium on the dissolution rats were investigated. The studies were carried out using the USP XXI paddle method.\textsuperscript{[51]}

\textsuperscript{®}
Khan Hajera et al., reported a developed dissolution test for Gemifloxacin mesylate and Ambroxol hydrochloride tablets using spectrophotometric method. The dissolution established conditions were 900 ml of 0.01M HCl pH 2.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 271 nm for Gemifloxacin mesylate and 243.5 nm for Ambroxol hydrochloride.\[52]\n
Dewan Taslima Akhter et al., reported a in vitro dissolution study for ambroxol hydrochloride for 12 hours; in 0.1 N hydrochloric acid (pH 1.2) for first 2hrs followed by phosphate buffer at pH 6.8 ±0.2. Hydrophilic and hydrophobic matrix tablets (F-3, F-6 and F-9) showed no change in physical appearance, drug content or dissolution pattern after storage at 40°C temperature and relative humidity 75% for 6 months.\[53]\n
Prabha Singh et al., reported a in vitro dissolution studies for Ambroxol hydrochloride by pH change method. A suitable HPLC method to determine the drug plasma concentration was developed. Also optimized formulation was subjected to stability study. The results of dissolution studies indicated that formulations containing a combination of matrix showed the desired drug release profile. The optimized formulation showed no change in their in vitro dissolution profiles after storage at 25°C/60%RH and 40°C/75%RH for a period of six months indicating good stability. Thus the developed formulation showed stability and release profile.\[54]\n
Table 7. Reported degradation products during Stress degradation studies of Ambroxol Hydrochloride.\[55\]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradation Products</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(2-amino-3,5-dibromophenyl)methanol</td>
<td>280.94</td>
</tr>
<tr>
<td>2</td>
<td>trans-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2H)-yl)cyclohexan-1-ol</td>
<td>390.11</td>
</tr>
<tr>
<td>3</td>
<td>trans-4-{((E)-[(2-amino-3,5-dibromophenyl)methyldene] amino}cyclohexan-1-ol</td>
<td>376.08</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Degradation Products</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>4</td>
<td>cis-4-{{(2-amino-3,5-dibromophenyl) methyl} amino} cyclohexan-1-ol</td>
<td>378.10</td>
</tr>
<tr>
<td>5</td>
<td>2-amino-3,5-dibromobenzaldehyde</td>
<td>278.92</td>
</tr>
</tbody>
</table>
Table 8. Reported degradation products during Stress degradation studies of Gemifloxacin Mesylate.\textsuperscript{[56]}

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradation Products</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Chemical Structure" /> 7-{(3\text{-(aminomethyl)}\text{-}4\text{-}hydroxy}\text{pyrrolidin-1\text{-}yl}}-1\text{-}cyclopropyl-6\text{-}fluoro-4\text{-}oxo-1,4\text{-}dihydro-1,8\text{-}naphthyridine-3\text{-}carboxylic acid</td>
<td>362.35</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Chemical Structure" /> 7-{(3S\text{-})3\text{-(aminomethyl)}\text{-}4\text{-}oxoppyrrolidin-1\text{-}yl}-1\text{-}cyclopropyl-6\text{-}fluoro-4\text{-}oxo-1,4\text{-}dihydro-1,8\text{-}naphthyridine-3\text{-}carboxylic acid</td>
<td>360.33</td>
</tr>
</tbody>
</table>
3.2 ABACAVIR SULPHATE AND LAMIVUDINE COMBINATION DRUG PRODUCT (ABAMUNE – L TABLET).[^57]

Abamune – L tablets, manufacture by Cipla Pharmaceuticals contain the active ingredients, abacavir 600mg and lamivudine 300mg, which are nucleoside reverse transcriptase inhibitors. Reverse transcriptase is a component of HIV required to infect cells so that more virus can be produced, however abacavir and lamivudine prevent transcriptase from functioning properly. Abamune – L tablets also contain the following inactive ingredients: magnesium stearate, microcrystalline cellulose, sodium starch glycollate.

The two medications abacavir and lamivudine combined together help prevent HIV from reproducing. The HIV virus is responsible for acquired immune deficiency syndrome as HIV infection destroys CD4 (T) cells, which are important to the body’s immune system. They increase CD4 cell counts, a type of white blood cell that plays an important role in maintaining a healthy immune system to help fight infection. Abamune – L tablets do not cure AIDS or destroy the HIV virus, but rather delay further damage to the immune system by stopping production of new viruses. It is important that you continue to take precautions to prevent this from happening as the HIV virus can still be passed on by sexual activity or by contamination with infected blood.

**Side effects:**

Abamune – L tablets can cause some side effects like nausea, vomiting, diarrhoea, upper abdominal pain, headache, high temperature, lethargy, fatigue, loss of appetite, hair loss, joint and muscle pain, abacavir hypersensitivity and skin rash. Less common side effects which could affect less than 1 in every 100 people include increased liver enzyme production, anaemia (low red blood cell count), neutropenia (low white blood cell count), and reduced number of blood cells important for blood clotting.

**Storage Conditions:**

Abamune-L tablets are stored in a cool, dry place below 25°C out of reach of children.
3.2.1 **ABACAVIR SULPHATE.**[^58–60]

**Introduction.**

Abacavir belongs to the class of medications called nucleoside reverse transcriptase inhibitors (NRTIs). It works by decreasing the amount of human immunodeficiency virus (HIV) in the blood. Although abacavir does not cure HIV, it may decrease your chance of developing acquired immunodeficiency syndrome (AIDS) and HIV related illnesses such as serious infections or cancer. Abacavir is used along with other medications to treat HIV infection. Use of this drug decreases the risk of transmitting (spreading) the HIV virus to other people.

**Description.**

Abacavir, chemically is \([(1S,4R)-4-[2\text{-amino}-6-(cyclopropylamino)-9H\text{-purin-9-yl}]\text{cyclopent-2-en-1-yl}]\text{methanol} \) (Figure 12).

![Figure 12. Chemical Structure of Abacavir Sulphate.](image-url)

[^58–60]: This is a citation or reference to a range of pages or works.
Abacavir sulfate, a synthetic carbocyclic nucleoside analogue with inhibitory activity against HIV-1. It is the enantiomer with 1S, 4R absolute configuration on the cyclopentene ring. It has a molecular formula of \((C_{14}H_{18}N_{6}O)_{2}\cdot H_{2}SO_{4}\) and a molecular weight of 670.76 daltons. It is a white to off-white solid with a solubility of approximately 77 mg/mL in distilled water at 25°C. It has an octanol/water (pH 7.1 to 7.3), partition coefficient (log P) of approximately 1.20 at 25°C. \textit{In vivo}, abacavir sulfate dissociates to its free base, abacavir.

**Mechanism of Action.**

Intracellularly, abacavir is converted by cellular enzymes to the active metabolite carbovir triphosphate, an analogue of deoxyguanosine-5′-triphosphate (dGTP). Carbovir triphosphate inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA. Viral DNA growth is terminated because the incorporated nucleotide lacks a 3′-OH group, which is needed to form the 5′ to 3′ phosphodiester linkage essential for DNA chain elongation and continued DNA synthesis.

**Pharmacokinetics.**

**Absorption.**

Rapidly absorbed after oral administration with an oral bioavailability of about 80%.

**Distribution.**

The apparent volume of distribution after IV administration of abacavir was 0.86 ± 0.15 l/kg, suggesting that abacavir distributes into extravascular space. Binding of abacavir to human plasma proteins is approximately 50% and is independent of concentration. Total blood and plasma drug-related radioactivity concentrations are identical, demonstrating that abacavir readily distributes into erythrocytes. It is capable of crossing the blood–brain barrier.
Metabolism.

It is metabolised primarily through alcohol dehydrogenase or glucuronyl transferase to a 5’- carboxylic acid metabolite and 5’-glucuronide metabolite, respectively. These metabolites have no antiviral activity. Abacavir is not significantly metabolized by cytochrome P450 enzymes. It also undergoes intracellular metabolism to carbovir triphosphate which is the active metabolite.

Route of Elimination.

Single oral dose of abacavir is elimination as the 5’-carboxylic acid metabolite, 5’-glucuronide metabolite, and unidentified minor metabolites in the urine. Fecal elimination accounted for 16% of the dose. Renal excretion of unchanged abacavir is a minor route of elimination in humans.

Adverse effects.

The most commonly reported adverse reactions of at least moderate intensity (incidence ≥10%) in adult HIV-1 clinical trials were nausea, vomiting, headache, malaise, fatigue, and dreams/sleep disorders. Serious hypersensitivity reactions have been associated with abacavir which has been strongly linked to the presence of the HLA-B*5701 allele.

Storage Conditions.

Store it at controlled room temperature (20°C to 25°C). Do not freeze.
3.2.2 LAMIVUDINE.^[61 – 63]^  

**Introduction.**  

Lamivudine (2’, 3’-dideoxy-3’-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (NRTIs).  

- Lamivudine (Epivir) is used along with other medications to treat human immunodeficiency virus (HIV) infection.  
- Lamivudine (Epivir-HBV) is used to treat hepatitis B infection.  

It works by decreasing the amount of HIV and hepatitis B in the blood. Although lamivudine does not cure HIV, it may decrease your chance of developing acquired immunodeficiency syndrome (AIDS) and HIV-related illnesses such as serious infections or cancer. Taking these medications along with practicing safer sex and making other life-style changes may decrease the risk of transmitting (spreading) the HIV virus to other people.  

**Description.**  

Lamivudine, chemically is 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one (Figure 13).  

![Chemical Structure of Lamivudine](image)  

**Figure 13. Chemical Structure of Lamivudine.**
Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine. It has a molecular formula of C₈H₁₁N₃O₃S and a molecular weight of 229.3. It is a white to off-white crystalline solid with a solubility of approximately 70 mg/mL in water at 20°C. Its CAS number is 134678 – 17 – 4.

**Mechanism of Action**

Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B virus. It is phosphorylated intracellularly to its active 5’-triphosphate metabolite, lamivudine triphosphate (L-TP) which competes with deoxycytidine triphosphate for binding to reverse transcriptase, and incorporation into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination and discontinues DNA synthesis. The lack of a 3’-OH group in the incorporated nucleoside analogue prevents the formation of the 5’ to 3’ phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated.

**Pharmacokinetics.**

**Absorption.**

Lamivudine was rapidly absorbed after oral administration in HIV-infected patients. The peak serum lamivudine concentration (Cmax) was 1.5 ± 0.5 mcg/mL when an oral dose of 2 mg/kg twice a day was given to HIV-1 patients. When given with food, absorption is slower, compared to the fasted state.

**Distribution.**

It get distributed in the whole body with crossing the blood-brain barrier and placenta; enters breast milk. Protein binding up to 36%. Apparent volume of distribution, IV administration (1.3 ± 0.4 L/kg). Volume of distribution was independent of dose and did not correlate with body weight.
Metabolism.

Metabolism of lamivudine occurs intracellularly and low hepatic metabolism is a minor route of elimination. In man, the only known metabolite of lamivudine is the trans-sulfoxide metabolite. This biotransformation is catalyzed by sulfotransferases.

Route of Elimination.

The majority of lamivudine is eliminated unchanged in urine by active organic cationic secretion. 5.2% ± 1.4% (mean ± SD) of the dose was excreted as the trans-sulfoxide metabolite in the urine. Lamivudine is excreted in human breast milk and into the milk of lactating rats.

Adverse effects.

Lamivudine produce lactic acidosis and severe hepatomegaly with steatosis, hepatic decompensation in patients co-infected with HIV-1 and Hepatitis C, pancreatitis. The most common adverse reactions are headache, nausea, fatigue, malaise diarrhea and cough signs and symptoms.

Storage Conditions.

Store at 25°C (77°F), Store away from heat and direct light.
3.2.3 Reported stability indicating methods for abacavir sulphate single and in combination products.

U. Seshachalam et al., has developed a stability indicating HPLC method for abacavir sulphate from its related substances and assay for the first time. This method involves the usage of C18 (Intertsil octadecyl silane-3V, 150 mm×4.6 mm, 5 μm) column. The method was validated over the range of 0.002–0.1 mg/mL for chloro impurity, 0.005–0.1 mg/mL for amino impurity and pyrimidine impurity, and 0.005–0.2 mg/mL for abacavir. The mobile phase consists of a mixture of 10 mM ammonium acetate buffer and ACN in the ratio of 90 : 10. The flow rate was set at 1.0 mL/min with UV detection monitored at 214 nm.\[64]\n
R. Nageswara Rao et al., reported a developed stability indicating HPLC method for Abacavir sulphate. The drug was subjected to forced degradation under the conditions of hydrolysis (acid, alkali and neutral), oxidation, photolysis and thermal stress as prescribed by ICH. Eight degradation products were formed and their separation was accomplished on Waters XTerra C18 (250mm×4.6mm, 5_m) column using 20mM ammonium acetate:acetonitrile as a mobile phase in gradient elution mode by LC.\[65]\n
Pallavi Vukkum et al., developed a novel, stability-indicating UHPLC method for the quantitative determination of Abacavir sulfate, its related substances, and forced degradation impurities in bulk drugs. The chromatographic separation was achieved on a Waters Acquity BEH C8, 50 mm × 2.1 mm, 1.7 μm particle size column with a mobile phase containing a gradient mixture of solution A (0.10 % v/v o-phosphoric acid in water) and solution B (0.10% v/v o-phosphoric acid in methanol). The flow rate was set at 0.40 mL/min and the run time was 6.0 min. The drug substance was subjected to the stress studies of hydrolysis, oxidation, photolysis, and thermal degradation.\[66]\n
CH Venkata Reddiah et al., developed a stability indicating UPLC method quantitative determination of Lamivudine, Zidovudine and Abacavir in active pharmaceutical ingredients and its dosage forms. Chromatographic separation of drugs from the possible impurities and the degradation products was achieved on an Inertsil ODS-3V 250 x 4.6 mm, 5.0μm column; the gradient elution achieved with in 90.0 min. Ammonium
dihydrogen phosphate and Diammonium hydrogen phosphate buffers pH 3.9 as mobile phase A and methanol as mobile phase B. The flow rate was 1.0 ml/min, column temperature 50°C and the detection was done at 270 nm. The above developed HPLC method was further subjected to hydrolytic, oxidative, photolytic and thermal stress conditions.\[67\]

Pradeep Kumar et al., developed a stability indicating RP-HPLC method for the estimation of Abacavir in bulk and in tablet dosage form. A High performance liquid chromatography 10AT SHIMADZU- SPD10A, using Phenomenex - Luna RP-18(2),250X4.6mm, 5 μm column, with a mobile phase composed from water: Acetonitrile [80:20 %(v/v)] were used. The flow rate of 1.0 ml/min and the effluent was detected at 285 nm by using a UV detector. The proposed method is applicable to stability studies and routine analysis of Abacavir in bulk and pharmaceutical formulations.\[68\]

Charushila H. Bhirud et al., developed a stability-indicating HPTLC method for densitometric determination of Abacavir, Lamivudine and zidovudine in both as a bulk drug and in formulation. The method employed TLC aluminium plates precoated with silica gel 60F 254 as the stationary phase. The solvent system consisted of Toluene: Ethyl acetate: methanol (8.0:1.0:1.0 v/v/v). Densitometric measurement of Abacavir, Lamivudine and zidovudine was performed in the absorbance mode at 279 nm. As the proposed method can effectively separate the drug from its degradation products, it can be employed as stability indicating method.\[69\]

Pramod Kumar Ragham et al., developed a stability indicating LC-MS method for the quantitative determination of Abacavir sulfate and its related substances. Significant degradation was observed during oxidation, and the major degradant was identified by LC–MS analysis. The chromatographic conditions were developed and optimized using an impurity-spiked solution and the forced degradation samples with a resolution of >2. The chromatographic separation was achieved on YMC Pack Pro C18, 150 mm x 4.6 mm, 3μ particle size column. Using 0.05% TFA in water as mobile phase A and the 0.05% TFA in Acetonitrile as mobile phase B with 1.0mL/min flow rate in gradient
mode. The column temperature was maintained at 25°C, detection wavelength was set at
220 nm and the injection volume was 10 μl. Water and Acetonitrile in the ratio
90:10(v/v) was used as a diluent.\[70\]

3.2.4 Reported in vitro dissolution methods for abacavir sulphate single and in
combination products.

Bahlul Zayed Awen et al., in their investigation develop the stability indicating
dissolution media for determination of abacavir sulphate in pharmaceutical dosage forms
during bioequivalence studies for first time. The stability of abacavir sulphate was tested
in various dissolution media, ie. 0.1M HCl, pH 1.2 KCl-HCl buffer and pH 5.8, 6.2, 6.6,
7.0, 7.4 and 7.8 phosphate buffers separately. The stability was tested at room
temperature and 37°C for 48 hrs. The samples were scanned for stability and the
optimized samples were selected for further study. Stability studies of the drug in various
media at room temperature and 37°C indicated that it was stable in 0.1M HCl and pH 5.8
buffer in the UV region for a period of 48 hr. The \( \lambda_{\text{max}} \) were found to be 297.6 for 0.1M
HCl and 286.3 nm for pH 5.8 phosphate buffer with observed low coefficient of variation
of <8.71%.\[71\]

Yalçın Özkan et al., in their work describes a new, fully validated, simple, rapid,
selective, and sensitive HPLC method with UV detection for the direct determination of
abacavir in pharmaceutical dosage forms, raw materials, spiked human serum, and drug
dissolution studies without any time-consuming extraction or evaporation steps prior to
drug assay. The mobile phase employed was methanol:acetonitrile:0.015 M KH₂PO₄
(36:2.6:61.4 v/v/v) adjusted to pH 6.9 with 5 N NaOH. The samples of 20 μL were
injected onto a Waters Spherisorb ODSI (250 × 4.6 mm, 5 μm particle size) column. The
samples were detected at 284 nm. It was successfully applied to the analysis of abacavir
pharmaceutical preparations, and human serum samples without any interference by the
excipients and endogenous substances. Moreover, the method can be used for the
determination of abacavir for monitoring its concentration for in vitro dissolution
studies.\[72\]
3.2.5 Reported stability indicating methods for lamivudine single and in combination products.

N. Kaul et al., developed a stability-indicating HPTLC method for analysis of lamivudine both as a bulk drug and in formulations. The solvent system consisted of carbon tetrachloride – methanol – chloroform - acetonitrile (7.0: 3.0: 2.0: 1.5, v/v/v/v). HPTLC analysis of lamivudine was carried out in the absorbance mode at 275 nm. This system was found to give compact spots for lamivudine ($R_F$ value of 0.36 ± 0.02) following double development of chromate plates with the same mobile phase. Lamivudine was subjected to acid and alkali hydrolysis, oxidation, dry heat and wet heat treatment and photo degradation. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one. Moreover, the proposed HPTLC method was utilized to investigate the kinetics of acid degradation process. Arrhenius plot was constructed and activation energy was calculated.[73]

Weerasak Samee et al., developed a rapid and reliable reverse-phase high performance liquid chromatographic method and validated for simultaneous determination of lamivudine, stavudine and nevirapine in presence of their acid-induced degradation products. Gradient chromatography using Thermo Hypersil Gold C18 column (150 mm x 4.6 mm, 5 µm) eluted with two mobile phase components: mobile phase A comprising of 20 mM sodium phosphate buffer with pH adjusted to 3.5 with phosphoric acid and mobile phase B (methanol) with a flow rate of 1.0 mL/min and a detection wavelength at 265 nm. The retention times for lamivudine, stavudine and nevirapine were 4.6, 7.8, and 14.8 min, respectively.[74]

Saranjit Singh et al., in their work reported lamivudine forced decomposition conditions of hydrolysis (neutral, acidic and alkaline), oxidation, photolysis and thermal stress, as suggested in the ICH guideline Q1A(R2). The drug showed instability in acid and alkali, while it remained stable in neutral conditions. It also degraded extensively under oxidative environment. It remained stable to light and thermal stress. In total, five degradation products were formed, which could be separated by LC on a C18 column using a gradient method. Subsequently, degradation pathway of the drug was laid down, along with mechanisms of formation of the degradation products.[75]
CH. Babu Rao et al., developed a stability indicating RP-HPLC method for the simultaneous estimation of Lamivudine and Stavudine in commercial tablets. The method has shown adequate separation for Lamivudine (RT-3.087) and Stavudine (RT-6.09) respectively. Separation was achieved on an YMC pack, C8, 150mmX4.6mm, 5µ column using a mobile phase consisting of buffer pH 3.5 and methanol in the ratio of 90:10v/v at a flow rate of 1.0ml/min. the detection was carried out by PDA detector at the wavelength maximum of 265 nm.\textsuperscript{[76]}

Umesh M. Patel et al., developed a stability indicating HPLC for simultaneous estimation of Lamivudine (3TC) and Stavudine (d4T) in bulk drugs and commercial tablets. Separation was achieved on a Kromasil C8 (250mm×4.6mm i.d; 5 µm) column at a detection wavelength of 265nm, using a mobile phase consisting of acetonitrile–0.02M ammonium acetate buffer (pH 4.5) in a gradient elution mode at a flow rate of 1 ml/min. Quantitation was achieved with UV detection at 265 nm.\textsuperscript{[77]}

Prashant S. Devrukhakar et al., developed a simple, rapid, and stability-indicating RP-HPLC method for a combination of tenofovir disoproxil fumarate (TDF), emtricitabine (FTC), and efavirenz (EFV) was developed and validated with the help of a suitable statistical software as an application tool for the quality by design. Successful separation of combined drugs from degradation products was achieved by gradient elution on a reverse-phase C18 column, using a mobile phase containing phosphate buffer (pH 3.5): acetonitrile at 1.5 mL min$^{-1}$ flow rate, detection wavelength 256 nm, column oven temperature 25$^\circ$C, and injection volume 10 µL.\textsuperscript{[78]}

Anjali Joshi et al., developed a simple reversed phase (RP) HPLC-UV method to determine 3TC, AZT, NVP with some of its degradation products cytosine and thymine that can be applied in monitoring the quality of such products. A RP gradient HPLC method was developed using following chromatographic conditions: LunaC18 150 x 4.6mm column; 50mM ammonium acetate buffer (pH = 6.8) and methanol as mobile phase; Gradient mode of elution with sample injection volume 50µl; detection wavelength 265nm. The method was used in dissolution studies of the fixed dose combination tablets of AZT/3TC/NVP using USP Type I method. No interference
between the analytes was observed. No matrix effect due to the presence of other excipients in the formulation was observed in the chromatograms of the dissolution samples.\[79\]

Lanka A. Rama Prasad et al., developed a new stability-indicating reverse phase LC method developed and validated for the simultaneous estimation of Lamivudine, Tenofovir DF and Nevirapine in extended release tablet dosage form. Tenofovir and Lamivudine are formulated into immediate release and Nevirapine into extended release. The chromatographic conditions were optimized using an impurity-spiked solution and the samples generated from forced degradation studies. The chromatographic separation was achieved on a core shell technology C18 stationary phase. The method employed a linear gradient elution and the detection wavelength was set at 260 nm. The mobile phases consists of buffer and acetonitrile delivered at a flow rate of 0.7 mL·min\(^{-1}\).\[80\]

María S. Gualdesi et al., Developed a stability indicating micellar liquid chromatography (MLC) method for lamivudine and its carbonate derivatives in simulated gastric (SGF) and intestinal (SIF) fluids samples. The optimized method involves a C18 column thermostated at 30\(^\circ\)C, UV detection at 272 nm, a flow rate of 1.0 mL min\(^{-1}\) and a micellar mobile phase composed by 0.15 M sodium dodecyl sulphate (SDS) – 4\% \((v/v)\) 1-butanol – 0.01 M KH\(_2\)PO\(_4\)–Na\(_2\)HPO\(_4\) (pH 7), using zidovudine (AZT) as internal standard. Finally, this chromatographic method was applied to stability studies which resulted in all the compounds following a pseudo-first-order kinetics, and in the determination of its kinetic constant and half-life time.\[81\]

Maheswari .G et al., developed a new, simple and sensitive spectrophotometric method has been developed for the determination of Lamivudine in bulk and in pharmaceutical formulations. Stability of Lamivudine was carried out in different PH Buffer solutions (acidic, neutral, basic) at zero, 24, 48 hours. Lamivudine was found to be stable in PH 9.7 Carbonate buffer, used as a solvent and analysed spectrophotometricaly. Lamivudine exhibits absorption maxima at 271.76 nm.\[82\]
Santosh Kumar. M et al., developed a new stability indicating reversed phase high-performance liquid chromatographic method and successfully validated for the simultaneous estimation of Lamivudine and Zidovudine. A Hypersil BDS C18 (250 x 4.6 mm, 5μ) analytical Column was used for chromatographic separation and column temperature was maintained at 30°C. Mobile phase used was a mixture of Buffer (pH 4.6): Acetonitrile (80:20) at a flow rate of 0.9 ml/min. The UV wavelength used for detection was 272 nm for Lamivudine and Zidovudine.\textsuperscript{[83]}

3.2.6 Reported in – vitro dissolution methods for lamivudine single and in combination products.

N. A. Ochekpe et al., Developed in vitro dissolution studies for three products which are chemically equivalent because they all contain lamivudine and zidovudine at the same label strength. However, Lazid and Virex-LZ differ from Combivir in their dissolution characteristics. Virex-LZ and Lazid showed greater than 85% dissolution of both APIs in 15 min or less in 0.1 N HCl (pH 1.2) and in pH 4.5 and 6.8 phosphate buffers and can be classified as very rapidly dissolving products.\textsuperscript{[84]}

K. Prakash et al., work on solubility and dissolution rate of three antiretroviral drugs such as lamivudine, zidovudine and stavudine was studied in four media having different pH. The samples were analyzed by using UV Visible spectrophotometer. lamivudine shows more solubility in 0.01 N HCl. All three drugs showing lower solubility in pH 6.8 phosphate buffer. The solubility and dissolution data in various media is helpful in predicting the bioavailability and also in dissolution method development.\textsuperscript{[85]}

Nayak Bhabani Shankar et al., The objectives of the present study were to select a formulation that has an ideal in vitro dissolution profile and to compare the sustaining/controlling efficacy of the selected formulation with that of the commercial conventional tablet in order to establish a good degree of in vitro–in vivo correlation. The in vitro release profiles obtained in 0.01N HCl as dissolution medium. The microspheres were subjected to characterization for particle size, encapsulation efficiency, loose crystal
study, stability study, *in vitro* release rate profile, release kinetics and *in vivo* study in New Zealand white rabbit species.[86]

D. K. Mandloi *et al.*, developed a new RP-HPLC method was developed for the determination of Lamivudine in the bulk drug and tablet dosage forms and it was applied for the *in-vitro* drug dissolution studies. Isocratic elution mode with a mixture of methanol and water in the ratio of (89:11) was selected as the mobile phase with a C18 column (250 x 4.6mm, 5μ) for separation. This mixture was found to be appropriate allowing good elution for the Lamivudine at retention time 2.72 minute at flow rate of 1 ml/min and detection wavelength at 272nm.[87]

Ashok Kumar. P *et al.*, worked on design of oral controlled release matrix tablets of Lamivudine using different proportion of Guar gum as the retardant polymer and to study the effect of formulation factor such as polymer proportion on the in vitro release of drug. *In vitro* drug release studies were carried out using USP XXII dissolution apparatus type II at 50 rpm. The dissolution medium consisted of 900 ml of pH 6.8 phosphate buffer, maintained at 37± 0.5°C. The release kinetics were analyzed using the zero-order, first-order model equation, Higuchi's square-root equation, and the Korsmeyer-peppas model.[88]

Sridhar. N. Y *et al.*, developed a sensitive, selective and rapid RP-UPLC method and validated for the simultaneous estimation of lamivudine, nevirapine and stavudine in their tablet dosage form and in vitro dissolution studies. The chromatographic separation was achieved on acquity X-Bridge column (50 cm 4.6 mm, 3.5 μm) using phosphate buffer of pH 2.5 and methanol with gradient elution at a flow rate of 1.0 ml min-1. The UV detection was performed at 266 nm, column oven temperature was 30°C and total run time was 5 minutes within which all three compounds were separated.[89]
Table 9. Reported degradation products formed during Stress degradation studies of Lamivudine.\textsuperscript{[90]}

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradation Products</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><img src="image1.png" alt="Degradation Product 1" /> 1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidine-2,4(1H,3H)-dione</td>
<td>230.24</td>
</tr>
<tr>
<td>2.</td>
<td><img src="image2.png" alt="Degradation Product 2" /> 4-aminopyrimidin-2(1H)-one</td>
<td>111.10</td>
</tr>
<tr>
<td>3.</td>
<td><img src="image3.png" alt="Degradation Product 3" /> pyrimidine-2,4(1H,3H)-dione</td>
<td>112.08</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Degradation Products</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>4.</td>
<td><img src="image1.png" alt="Degradation Product 4" /> 4-amino-1-[(3S)-2-(hydroxymethyl)-3-oxo-1,3-oxathiolan-5-yl]pyrimidin-2(1H)-one</td>
<td>245.25</td>
</tr>
<tr>
<td>5.</td>
<td><img src="image2.png" alt="Degradation Product 5" /> 4-amino-1-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]pyrimidin-2(1H)-one</td>
<td>213.19</td>
</tr>
</tbody>
</table>
### Table 10. Reported degradation products formed during Stress degradation studies of Abacavir Sulphate.\textsuperscript{[91]}

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradation Products</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>[(4\textit{R})-4-(2,6-diamino-9\textit{H}-purin-9-yl)cyclopent-2-en-1-yl] methanol</td>
<td>246.26</td>
</tr>
<tr>
<td>2.</td>
<td>9-[(1\textit{R})-4-(\textit{tert}-butoxymethyl)cyclopent-2-en-1-yl]-9\textit{H}-purine-2,6-diamine</td>
<td>302.27</td>
</tr>
<tr>
<td>3.</td>
<td>9-[(1\textit{R})-4-{[(2,5-diamino-6-chloropyrimidin-4-yl)oxy]methyl}cyclopent-2-en-1-yl]-9\textit{H}-purine-2,6-diamine</td>
<td>388.81</td>
</tr>
</tbody>
</table>
3.3 LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE

COMBINATION DRUG PRODUCT (TENVIR – L TABLET).[92]

TENVIR – L tablet manufactured by Cipla contains Tenofovir disoproxil fumarate 300 mg and Lamivudine 300 mg, given for the treatment of HIV infection which leads to AIDS. It belongs to a class of drugs called nucleoside reverse transcriptase inhibitors and helps to control the further infection of the HIV virus by lowering the risk of getting HIV complication (e.g. new infections, cancer, etc.) and thereby improving the quality of life. However, it does not properly cure the virus, hence care is taken while using this medication. It is not recommended to be used as a component of a triple nucleoside regimen and also it should not be co administered with lamivudine, Tenofovir disoproxil fumarate tablets and any other Tenofovir and lamivudine-containing products. It can be used in combination with other antiretroviral agents, for the treatment of HIV-1 infection in adults.

TENVIR – L can be taken with or without meal orally and careful should be taken while using this medication in patients who have creatinine clearance < 50 mL/min as it is a fixed dose tablet. There is no adjustment in dose in patients who have a mild renal impairment i.e. creatinine clearance 50-80 ml/min). It is not recommended to the pregnant women and breast feeding women, but it is given if the potential benefits far outweigh the potential risk to the fetus or the nursing baby.

Side effects.

TENVIR – L is not given without prescription because it can lead to lactic acidosis and other side effects like weakness, unusual muscle pain, trouble in breathing, nausea, stomach pain, vomiting and cold or feeling of numbness in legs or arms, and many other unwanted symptoms. It is contraindicated in patients who have hypersensitivity (e.g. anaphylaxis) to any of the components of this product.

Storage Conditions.

It should be stored very carefully in a place where children cannot reach at all.
3.3.1 LAMIVUDINE.

Lamivudine drug information is same as mentioned in previous combination.

3.3.2 TENOFOVIR DISOPROXIL FUMARATE.[93–95]

Introduction.

Tenofovir disoproxil fumarate belongs to the class of medications called nucleotide analog reverse-transcriptase inhibitors (NtARTIs or NtRTIs) and mainly used to treat human immunodeficiency virus (HIV) infection.

- HIV-1 infection: Tenofovir is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and pediatric patients 2 years of age and older.
- Tenofovir is indicated for the treatment of chronic hepatitis B in adults and pediatric patients 12 years of age and older.

It is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ.

Description.

Tenofovir, chemically is ([(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy)methyl) phosphonic acid (Figure 14).

Tenofovir disoproxil fumarate is a white to off-white crystalline powder with a solubility of 13.4 mg/ml in distilled water at 25°C. It has a molecular formula of C_{19}H_{30}N_{5}O_{10}P • C_{4}H_{4}O_{4} and a molecular weight of 635.51. It has melting point of 276 – 280°C. It has an octanol/phosphate buffer (pH 6.5) partition coefficient (log p) of 1.25 at 25°C. Its CAS number is 147127-20-6.
Mechanism of Action.

Tenofovir disoproxil fumarate is converted to tenofovir in the intestinal lumen and plasma by diester hydrolysis. Tenofovir is then internalized into cells, possibly by endocytosis, and subsequently phosphorylated in sequential steps to tenofovir monophosphate and to the active metabolite, tenofovir diphosphate. In a mechanism similar to that of NRTIs, tenofovir diphosphate competes with its natural nucleotide counterpart, deoxyadenosine 5′-triphosphate, for incorporation into newly forming HIV DNA. Once successfully incorporated, termination of the elongating DNA chain ensues, and DNA synthesis is interrupted.

Pharmacokinetics

Absorption.

Tenofovir disoproxil fumarate is the water soluble diester prodrug of the active ingredient tenofovir. The oral bioavailability in fasted patients is approximately 25%. Administration of food (high fat meal containing 40 to 50% fat) increases the oral bioavailability, with an increase in the AUC of approximately 40%.
Distribution.

Widely distributed into most tissues, especially kidneys, liver and intestinal contents. Protein binding: <0.7% to plasma proteins, 7% to serum protein.

Metabolism.

Tenofovir is metabolize in liver to a small extent were cytochrome P450 enzyme system is not involved with the metabolism of either tenofovir disoproxil or tenofovir.

Route of Elimination.

Following IV administration of tenofovir, approximately 70–80% of the dose is recovered in the urine as unchanged tenofovir within 72 hours of dosing. Tenofovir is eliminated by a combination of glomerular filtration and active tubular secretion. There may be competition for elimination with other compounds that are also eliminated by renal route.

Adverse effects.

The most common side effects associated with tenofovir include nausea, vomiting, diarrhea, and asthenia. Less frequent side effects include hepatotoxicity, abdominal pain, and flatulence. Tenofovir has also been implicated in causing renal toxicity, particularly at elevated concentrations. Tenofovir can cause acute renal failure, Fanconi syndrome, proteinuria, or tubular necrosis. These side effects are due to accumulation of the drug in proximal tubules.

Storage Conditions.

Store it at 25°C (77°F), excursions permitted to 15–30°C (59–86°F).
3.3.3 Reported stability indicating methods for lamivudine single and in combination products.

and

3.3.4 Reported in vitro dissolution methods for lamivudine single and in combination products.

Lamivudine literature survey and degradation products are same as mentioned in previous combination literature Section 3.2.5 and 3.2.6 and Table 9. Respectively.

3.3.5 Reported stability indicating methods for tenofovir disoproxil fumarate single and in combination products.

Pradeep Kumar et al., developed a rapid, precise, accurate, specific and simple RP-HPLC method for the estimation of Tenofovir in its tablet form. A High performance liquid chromatography 10AT SHIMADZU- SPD10A, using Phenomenex - Luna RP-18(2), 250 mm x 4.6mm, 5 µm column, with mobile phase composition of Acetonitrile: water [78:22 %(v/v)] was used. The flow rate of 1.0 mL min-1 and effluent was detected at 260 nm. The retention time of Tenofovir was 5.541 minutes. The proposed method is applicable to stability studies and routine analysis of Tenofovir in bulk and pharmaceutical formulations. [96]

Sunil R. Dhaneshwar et al., developed a stability-indicating high-performance thin-layer chromatographic (HPTLC) method for determination of tenofovir disoproxil fumarate in bulk drug and in tablet and validated. The mobile phase selected was chloroform: methanol (9.0 : 1.0, v/v) with ultraviolet (UV) detection at 260 nm. The retention factor was found to be 0.49±0.03. Method had the potential to determine tenofovir disoproxil fumarate from tablet without any interference, and it was a stability-indicating one. [97]
Sunil R. Dhaneshwar et al., in their study describes the degradation of tenofovir disoproxil fumarate under different prescribed stress conditions (hydrolysis, oxidation, dry and wet heat and photolysis) following the ICH and application of a specific and selective stability-indicating HPLC assay. Separation of drug and degradation products was successfully achieved on C18 analytical column using methanol: water (60:40, v/v) at a flow rate of 1.0 ml/min and detection at 260 nm, the mass balance was found to be close to 100.4%.\[98\]

Syed Sajjad Hussen et al., developed a novel stability-indicating high performance liquid chromatographic (HPLC) method for Tenofovir Disproxil fumarate (TEN) with photodiode array (PDA) detection and validated as per ICH guidelines. The developed method was successfully applied for assay of Tenofovir Disproxil fumarate to nanoparticle formulation. A Lichrocart (C18) (250mm × 4.6mm, 5 μm particle size) column and a mobile phase composed of acetonitrile and 0.025M potassium di hydrogen phosphate buffer (pH 3.0 adjusted by using 10% v/v Orthophosphoric acid) in the ratio 35:65 (v/v) was used, and the detection wavelength of 260 nm. Forced degradation studies under different stress conditions like Acid, Base and Oxidation was successfully achieved, TEN was found to degrade significantly in alkaline and acidic conditions.\[99\]

Prashant S. Devrukhakar et al., developed a stability-indicating RP-HPLC method for a combination of tenofovir disoproxil fumarate, emtricitabine, and efavirenz. The drugs individually, and in combination, were subjected to forced degradation (thermal, photolytic, hydrolytic, and oxidative stress conditions) and accelerated stability studies (40 ± 1°C/75 ± 3% RH for three months). Successful separation of combined drugs from degradation products was achieved by gradient elution on a reverse-phase C18 column, using a mobile phase containing phosphate buffer (pH 3.5): acetonitrile at 1.5 mL min−1 flow rate, detection wavelength 256 nm, column oven temperature 25°C, and injection volume 10 μL.\[100\]
Battula Srinivasan Rao et al., developed a stability-indicating RP-HPLC method for Emtricitabine and Tenofovir disoproxil fumarate in pharmaceutical Tablet dosage form. The mobile phase consisted of 65:35 % (v/v) of Methanol and 0.1M of potassium dihydrogen ortho phosphate and pH adjusted to 3.2 with ortho phosphoric acid. The method developed is operated on isocratic mode. The flow rate is 1.0 ml/min. Chromatographic determination of Emtricitabine and Tenofovir disoproxil fumarate was performed on Phenomenex C18 column (150 X 4.6 mm Id, ODS-2, 5μm). The wavelength of detection is 260 nm.[101]

Shubhangi Sutar et al., describes the development and validation of stability indicating RP-HPLC method for Tenofovir disoproxil fumarate (TDF), an antiviral drug. In the present study, the drug was subjected to acid (0.1N HCl and 0.01N HCl), and alkaline (0.1N NaOH and 0.01N NaOH) hydrolysis. The degraded products formed under different stress conditions were eluted by HPLC. The chromatographic separation of Tenofovir disoproxil fumarate and its degradation products was done on C18 column. The mobile phase containing ACN (Acetonitrile) and Water pH 4.5 with OPA (Ortho phosphoric acid)in 70:30v/v proportions was found to be most satisfactory at a flow rate of 1ml/min. Detection was carried out using single wavelength detector at 260nm.[102]

S. S. Chitlange et al., developed a stability indicating HPTLC method for concurrent estimation of Rilpivirin, Emtricitabine and Tenofovir as the bulk drug and in combined tablet dosage form. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of Methanol: Toluene: Ethylacetate: Ammonia (1.5:5.5:1.5:0.1 v/v/v/v). Densitometric evaluation of the separated zones was performed at 272 nm. The drugs are subjected to stress degradation studies as per ICH guidelines.[103]

Sivaram Manavarthi et al., developed stability indicating RP-HPLC method for analysis of Tenofovir. Successful separation of drug from degradation products formed under stress conditions was achieved by a gradient HPLC method, which was performed by Perkin Elmer HPLC using C18 (Phenomenex 100 x 4.6 mm x 5 micron) Column and optimized mobile phase consists of Methanol as Solvent-A,10 mM potassium di
hydrogen ortho phosphate buffer of pH -3 as a Solvent-B in the ratio of 30:70 % v/v and UV detection was carried out at 260 nm.\textsuperscript{[104]}

A Lakshmana Rao \textit{et al.}, developed a stability indicating HPLC method for the simultaneous determination of Efavirenz, Tenofovir and Emtricitabine in pharmaceutical dosage form. The method was carried out using Zorbax C8 column (150 mm x 4.6 mm, 5 μm) and mobile phase comprised of mixture of dilute orthophosphoric acid solution pH 2.4 ± 0.02 as buffer and acetonitrile in the ratio of 70:30 v/v and degassed under ultrasonication. The flow rate was 1.0 ml/min and the effluent was monitored at 252 nm.\textsuperscript{[105]}

Sriveena G S \textit{et al.}, developed a simple, economic, accurate stability indicating RP-HPLC method and validated for the estimation of tenofovir disoproxil fumarate in tablet dosage form. The method was carried out using HYPERSIL BDS C18 (150 x 4.6 mm, 5μm column). The detection was carried out at the wavelength of 254nm. The elution was achieved isocratically with a mobile phase comprising a mixture of potassium dihydrogen phosphate buffer pH 6.5 and Acetonitrile in the ration of 60:40 (%v/v). The flow rate was 1.0 ml/min.\textsuperscript{[106]}

Bi-Botti C. Youan \textit{et al.}, developed a stability-indicating LCMS method for the determination of tenofovir. A reversed phase C18 column was used as the stationary phase. TFV exhibited degradation under acidic and alkaline hydrolytic conditions. No significant degradation was observed at pH 4.5 (normal cervicovaginal pH) and oxidative stress conditions of 3% and 30% v/v hydrogen peroxide solutions. Stability analyses revealed that the TFV was stable in various stress conditions. However, formulation strategies should be implemented to protect it in strong acidic and alkaline environments.\textsuperscript{[107]}

Kalyan Donthineni \textit{et al.}, developed a stability-indicating HPLC method for the determination of tenofovir. Reversed-phase chromatography was performed on Hitachi Elite Lachrome, equipped with UV detector using Develosil C18 column (150 mm × 4.6 mm, 5 μm). Isocratic elution was performed using methanol and water (55:45, v/v). The overall run time was 10 min. and the flow rate was 1.0 ml/min Detection wavelength was
240 nm and ambient temperature. Tenofovir was subjected to stress conditions (acidic, alkaline, oxidation, UV and thermal degradation) and validated as per ICH guidelines.\cite{108}

### 3.3.6 Reported in–vitro dissolution methods for tenofovir disoproxil fumarate single and in combination products.

Vishnu. P. Chaudhari et al., reported three simple, economical, precise, and accurate methods are described for the simultaneous determination of Tenofovir disoproxil fumarate (TE) and Emtricitabine (EM) in combined tablet dosage form. The first method is ratio derivative spectra, second is first-order derivative spectrophotometry and third is absorption corrected method. Absorption corrected method was successfully applied to carry out dissolution study of commercial tablet formulation by using USP II dissolution test apparatus.\cite{109}

Divya Sree et al., developed a reverse phase-high performance liquid chromatographic method and validated for the simultaneous determination of tenofovir disoproxil fumarate and efavirenz in tenofovir and efavirenz finished formulation product and in vitro dissolution studies. An isocratic elution mode with a mixture of acetonitrile and water in the ratio of (55:45 % v/v) was selected for the mobile phase with a C18 (4.6 mm x 250 mm x 5μm) column as stationary phase for simultaneous separation of tenofovir disoproxil fumarate and efavirenz. The separation was achieved at a flow rate of 1 mL/min and detection wavelength of 252 nm at room temperature.\cite{110}

Kalpana Jayapalu et al., developed a rapid, economic and robust stability indicating HPLC method and validated to quantify Tenofovir disoproxil Fumarate (TDF), Emtricitabine (EMT) and Nevirapine (NVP) simultaneously at single wavelength (254 nm) in order to assess the in vitro drug release profile from tablet formulations. Chromatographic separation was performed with a gradient elution of samples on a 4.6 mm x 150 mm, 5 μm, Inertsil ODS-2 column with buffered mobile phase containing solvent A (10 Mm ammonium acetate buffer, pH 4.6) and solvent B (acetonitrile) at a flow rate of 1.0 ml/min). In dissolution studies, the sink condition was optimized based on quantitative solubility of TDF, EMT and NVP standards in different dissolution medium as recommended by USP.\cite{111}
Table 11. Reported degradation products formed during Stress degradation studies of Tenofovir disoproxil fumarate.\textsuperscript{[112]}

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradation Products</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\begin{align*} 9-[(1E)-prop-1-en-1-yl]-9\textit{H}-purin-6-amine \end{align*}</td>
<td>175.19</td>
</tr>
<tr>
<td>2</td>
<td>N-Hydroxylated Tenofovir derivative</td>
<td>549.46</td>
</tr>
<tr>
<td>3</td>
<td>\begin{align*} {[(\text{propan-2-yl})\text{oxy}\text{carbonyl}]\text{oxy}\text{methyl hydrogen} {[(2S)-1-(6\text{-amino-9H-purin-9-yl})\text{propan-2-yl}]\text{oxy}\text{methyl} \text{ phosphonate} \end{align*}</td>
<td>403.32</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Degradation Products</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>4</td>
<td><img src="image1" alt="Degradation Product 1" /></td>
<td>287.21</td>
</tr>
<tr>
<td></td>
<td>(((2S)-1-(6\text{-amino}-9H\text{-purin-9-yl})\text{propan-2-yl}oxy)\text{methyl})\text{phosphonic acid}</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><img src="image2" alt="Degradation Product 2" /></td>
<td>213.19</td>
</tr>
<tr>
<td></td>
<td>Tenfovir 6-Isopropyl Carbamate degradation product</td>
<td></td>
</tr>
</tbody>
</table>
3.4 MOXIFLOXACIN AND CEFIXIME COMBINATION DRUG PRODUCT (AELXIM – M TABLET)\textsuperscript{[113]}

AELXIM – M tablet consist of 400 mg moxifloxacin and 400 mg cefixime. It is used to treat lower respiratory tract infections include lungs abscess, pneumonia and acute bronchitis etc, which is having symptoms like shortness of breath, weakness, high fever, coughing and fatigue. Antibiotics are often thought to be the first line treatment in lower respiratory tract infections.

AELXIM – M is useful for the treatment of diseases, in particular of infectious diseases by bacteria, like Gram-negative and Gram-positive pathogens. This combination drug product posses synergistic effect against both type of pathogens and there is no drug interaction among both the drugs. The combination is especially useful for the treatment of bacterial diseases caused by Pseudomonas aeruginosa, Streptococcus pneumoniae and Staphylococcus aureus.

Both antibiotics can be given safely to impaired renal and liver patients and there will be no dosage adjustment required. There is no photosensitive reaction with moxifloxacin unlike other quinolones. The combination is once a day which will help to accept by the physicians and patients coverage safely with convenient dosage schedule in an economical rate.

**Side effects.**

Gastrointestinal effects such as decreased activity, somnolence, tremor, convulsions, vomiting, and diarrhea. It may also exacerbate muscle weakness in persons with myasthenia gravis.

**Storage Conditions.**

Store it at room temperature (25\(^0\)C).
3.4.1 MOXIFLOXACIN HYDROCHLORIDE.\textsuperscript{[114–116]}

Introduction.

Moxifloxacin is a synthetic fluoroquinolone antibiotic agent, used to treat certain infections such as pneumonia, bronchitis, and sinus, skin, bacterial conjunctivitis and abdominal (stomach area) infections caused by bacteria. It is also sometimes used to treat tuberculosis (TB) and endocarditis (infection of the heart lining and valves) when other medications cannot be used.

Its antibacterial spectrum includes enteric Gram – negative rods (\textit{Escherichia coli}, \textit{Proteus} species, \textit{Klebsiella} species), \textit{Haemophilus influenzae}, atypical bacteria (\textit{Mycoplasma}, \textit{Chlamydia}, \textit{Legionella}), \textit{Streptococcus pneumoniae}, and anaerobic bacteria. It has greater antibacterial activity than other fluoroquinolone class of drugs such as levofloxacin and ciprofloxacin against Gram – positive bacteria and anaerobes. Because of its potent activity against the common respiratory pathogen \textit{Streptococcus pneumoniae}, it is considered a "\textit{respiratory quinolone}."  

Description.

Moxifloxacin, chemically is l-Cyclopropyl-6-fluoro-l,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolol[3,4-b] pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid, mono hydrochloride (Figure 15).

![Chemical Structure of Moxifloxacin Hydrochloride](image)

\textbf{Figure 15. Chemical Structure of Moxifloxacin Hydrochloride.}
Moxifloxacin hydrochloride is an 8-methoxy fluoroquinolone anti-infective, with a diazabicyclononyl ring at the C7 position. It is a slightly yellow to yellow crystalline powder, with a molecular weight of 437.9. Its empirical formula is $\text{C}_{21}\text{H}_{24}\text{FN}_3\text{O}_4$.HCl. Its CAS number is 354812-41-2

**Mechanism of Action.**

Moxifloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. The bactericidal action of moxifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. DNA gyrase is an essential enzyme that is involved in the replication, transcription and repair of bacterial DNA. Topoisomerase IV is an enzyme known to play a key role in the partitioning of the chromosomal DNA during bacterial cell division. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian.

**Pharmacokinetics.**

**Absorption.**

Well absorbed from the gastrointestinal tract. Absolute oral bioavailability is approximately 90%. Co-administration with a high fat meal (that is, 500 calories from fat) does not affect the absorption of moxifloxacin.

**Distribution.**

Moxifloxacin is approximately 30-50% bound to serum proteins, independent of drug concentration. The volume of distribution of moxifloxacin ranges from 1.7 to 2.7 L/kg. Moxifloxacin is widely distributed throughout the body, with tissue concentrations often exceeding plasma concentrations. Moxifloxacin has been detected in the saliva, nasal and bronchial secretions, mucosa of the sinuses, skin blister fluid, subcutaneous tissue, skeletal muscle, and abdominal tissues and fluids following oral or intravenous administration of 400 mg.
Metabolism.

Approximately 52% of an oral or intravenous dose of moxifloxacin is metabolized via glucuronide and sulfate conjugation. The cytochrome P450 system is not involved in moxifloxacin metabolism, and is not affected by moxifloxacin. The sulfate conjugates (M1) accounts for approximately 38% of the dose, and is eliminated primarily in the feces. Approximately 14% of an oral or intravenous dose is converted to a glucuronide conjugate (M2), which is excreted exclusively in the urine. Peak plasma concentrations of M2 are approximately 40% those of the parent drug, while plasma concentrations of M1 are, in general, less than 10% those of moxifloxacin.

Route of Elimination.

Approximately 45% of an oral or intravenous dose of moxifloxacin is excreted as unchanged drug (~20% in urine and ~25% in feces).

Adverse effects.

Symptoms of overdose include CNS and gastrointestinal effects such as decreased activity, somnolence, tremor, convulsions, vomiting, and diarrhea. Rare but serious adverse effects include irreversible peripheral neuropathy, spontaneous tendon rupture and tendonitis, hepatitis, psychiatric effects (hallucinations, depression), torsades de pointes, Stevens-Johnson syndrome and clostridium difficile-associated disease (CDAD), and photosensitivity/phototoxicity reactions. It may also exacerbate muscle weakness in persons with myasthenia gravis. Therefore it should be avoided in patients with known history of myasthenia gravis.

Storage Conditions.

Store it at room temperature (25°C).
3.4.2 CEFIXIME TRIHYDRATE\textsuperscript{[117-119]}

Introduction.

Cefixime is a third-generation cephalosporin antibiotic which is highly stable in the presence of beta-lactamase enzymes. As a result, many organisms resistant to penicillin’s and some cephalosporin’s due to the presence of beta-lactamases may be susceptible to cefixime.

It is very effective in the treatment of the following infections when caused by susceptible strains of the designated microorganisms.

- Uncomplicated urinary tract infections caused by \textit{E. coli} and \textit{Proteus mirabilis}.
- Otitis media caused by \textit{Haemophilus influenza} and \textit{Moraxella catarrhalis}.
- Pharyngitis and tonsillitis caused by \textit{S. pyogenes}.
- Acute bronchitis and acute exacerbations of chronic bronchitis caused by \textit{Streptococcus pneumoniae} and \textit{Haemophilus influenzae}.
- uncomplicated gonorrhea (cervical/urethral) caused by \textit{Neisseria gonorrhoeae}.

Antibiotics will not work for colds, flu, or other viral infections.

Description

Cefixime, chemically is (6R, 7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy) imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Figure 16).

![Figure 16. Chemical Structure of Cefixime.](image-url)
Cefixime is a semi synthetic, cephalosporin antibacterial for oral administration. It is pale yellow solid with molecular weight of 507.50 as the trihydrate and Chemical Formula is C_{16}H_{15}N_{5}O_{7}S_{2}•3H_{2}O. It is hygroscopic with meting point of 218-225°C. Its CAS number is 79350-37-1.

Mechanism of Action.

The bactericidal action of Cefixime is due to the inhibition of cell wall synthesis. It binds to one of the penicillin binding proteins (PBPs) located inside the bacterial cell wall, which inhibits the final transpeptidation step of the peptidoglycan synthesis in the bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death. Moreover, osmotic drive from the outside isotonic environment of the host cell to the inside of the hypertonic bacterial cytoplasm and finally activation of the autolysin enzyme leading to the lysis of bacteria.

Pharmacokinetics.

Absorption.

Only 40-50% is absorbed from the GI tract (oral), the rate may be decreased if taken with food. Absorption from oral suspensions is more than that of tablet.

Distribution.

Serum protein binding is concentration independent with a bound fraction of approximately 65%. there was little accumulation of drug in serum or urine.

Metabolism.

There is no evidence of cefixime in vivo metabolism. Approximately 50% of the dose absorbed is excreted unchanged in the urine in 24 hours. In animal studies, it was noted that cefixime is also excreted in bile in excess of 10% of the administered dose.
**Route of Elimination.**

20% of an oral dose excreted via urine unchanged; 60% nonrenal elimination; some is excreted via bile into faeces. Substantially removed by dialysis.

**Adverse effects.**

Adverse reactions included diarrhea, loose or frequent stools, abdominal pain, nausea, flatulence, and dyspepsia. The incidence of gastrointestinal adverse reactions, including diarrhea and loose stools, in pediatric patients receiving the suspension was comparable to the incidence seen in adult patients receiving tablets.

**Storage Conditions.**

Store it at room temperature (25°C).
3.4.3 Reported stability indicating methods for moxifloxacin single and in combination products.

Motwani. S. et al., developed a stability indicating HPTLC method for densitometric determination of moxifloxacin both as a bulk drug and from pharmaceutical formulation. The method employed TLC aluminium plates pre-coated with silica gel 60F-254 as the stationary phase and the mobile phase consisted of n-propanol-ethanol-6Mammonia solution (4:1:2, v/v/v). As the method could effectively separate the drug from its degradation products, it can be employed as stability-indicating one.\textsuperscript{[120]}

M. Lalitha Devi et al., developed a stability-indicating RP-HPLC method for quantitative analysis of moxifloxacin and its related substances in bulk samples and pharmaceutical dosage forms in the presence of degradation products and process-related impurities. Method uses the C\textsubscript{18} column with a simple linear mobile phase gradient prepared from aqueous sodium dihydrogen orthophosphate dihydrate containing triethylamine, pH adjusted to 3.0 with orthophosphoric acid, and methanol. Detection was performed at 240 nm.\textsuperscript{[121]}

Predrag Djurdjevic et al., developed a RP-HPLC method for the separation and determination of impurities of moxifloxacin, in its pharmaceutical forms as well as moxifloxacin degradation products, with the aid of DryLab\textregistered{} software and chemometric (response surface) approach. The separation of four synthesis-related Impurities was achieved on a Waters C18 XTerra column using a mobile phase of (water + triethylamine (2%, v/v): acetonitrile = 90:10 (v/v %)); the pH of water phase being adjusted with phosphoric acid to 6.0. Flow rate of the mobile phase was 1.5 ml/min and UV detection at 290nm was employed. The column was thermostated at 45\textdegree{}C. The impurity content in the tablets and infusion was quantified as 0.1\% of total drug. Two degradation products were noted under hydrolytic conditions.\textsuperscript{[122]}
Mamdouh R. Rezk et al., developed a simple stability indicating HPLC method and validated for determination of moxifloxacin hydrochloride in the presence of its induced degradation products. The developed method utilized Symmetry C18 column (250 × 4.6 mm, 5 μm) in an isocratic separation mode. The mobile phase consisted of methanol: 0.2% triethylamine (pH 2.5 with orthophosphoric acid), (35: 65, v/v) at a flow rate 1.5 mL/min with UV-detection at 290 nm.\cite{123}

A.P. Dewani et al., developed a simple, specific and isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method with UV detection at 294 nm and validated for analysis of Moxifloxacin Hydrochloride (MOXI) in presence of its degradation products. Retention time of the MOXI was found to be 7.8 min. A mobile phase consisting of 10mM sodium phosphate buffer and methanol (60:40 v/v) pH 4.4 at flow rate of 1mL/min was employed in this study.\cite{124}

G. Naveen Kumar Reddy et al., developed a stability indicating UPLC method for Moxifloxacin. Chromatography was performed with mobile phase containing potassium dihydrogen ortho phosphate (adjusted to pH 1.8 with orthophosphoric acid), Methanol & acetonitrile in the ratio of 60:20:20, with a flow rate of 0.3mL/min, C-18 column & UV detection at 296nm. Moxifloxacin tablets were subjected to different stress conditions like acid, alkali, peroxide, thermal, water & UV studies and checked for its specificity, degradation & stability.\cite{125}

Syed Naeem Razzaq et al., developed a stability indicating RP-HPLC method for Moxifloxacin moxifloxacin and prednisolone in bulk drugs and pharmaceutical formulations. Optimum chromatographic separations among the moxifloxacin, prednisolone and stress-induced degradation products were achieved within 10 minutes by use of BDS Hypersil C8 column (250 X 4.6 mm, 5 μm) as stationary phase with mobile phase consisted of a mixture of phosphate buffer (18 mM) containing 0.1% (v/v) triethylamine, at pH 2.8 (adjusted with dilute phosphoric acid) and methanol (38:62 v/v) at a flow rate of 1.5 mL min-1. Detection was performed at 254 nm using diode array detector.\cite{126}
Syed Naeem Razzaq et al., developed a simple, RP-HPLC method for determining moxifloxacin and ketorolac in pharmaceutical formulations. Moxifloxacin, ketorolac and their degradation products were separated using C8 column with methanol and phosphate buffer pH 3.0 (55:45 v/v) as the mobile phase. Detection was performed at 243 nm using a diode array detector.\[127\]

V. Suman Kunagu et al., developed a rapid RP-HPLC method for determination of Moxifloxacin Hydrochloride in bulk and pharmaceutical dosage forms. Moxifloxacin Hydrochloride was found to be degraded under different set of conditions as followed according to ICH guidelines. And the degradants so formed along with Moxifloxacin Hydrochloride are separated by using Zorbax eclipse XDB C18, 250mm × 4.6mm × 5μm using Buffer: Methanol (68:32) as mobile phase, with a flow rate of 1.1ml/min with a detection wavelength of 293nm with a injection volume of 10μl.\[128\]

Urszula Hubicka et al., developed a stability indicating HPTLC method for Moxifloxacin in the presence of products of acidic hydrolysis. It was proved that decomposition of moxifloxacin proceeds according to kinetics of the first-order reaction and is dependent on temperature, incubation time and the type of the metal ion. Based on the calculated kinetic (k, t0.1 and t0.5) and thermodynamic (Ea) parameters, it was observed that among studied ions the highest effect on decomposition process of moxifloxacin had Cu(II) ions.\[129\]

Jayant B. Dave, et al., developed a stability indicating RP-HPLC method for moxifloxacin hydrochloride and ketorolac tromethamine in eye drops. Isocratic HPLC separation was achieved on a ACE C18 column (C18 (5 μm, 150 mm × 4.6 mm, i.d.)) using the mobile phase 10 mM potassium dihydrogen phosphate buffer pH 4.6 Acetonitrile (75:25 v/v) at a flow rate of 1.0 ml/min. The detection was performed at 307 nm.\[130\]

Munib-ur-Rehman et al., developed a stability indicating reversed phase HPLC method and validated for the simultaneous quantitation of antitubercular drugs, ethionamide and moxifloxacin (MOX) with commonly coprescribed vitamin, pyridoxine in tablet dosage form. The method was found rapid, precise and accurate. The separation was performed
in Hibar 150-4.6, Purospher STAR, RP- C18 (5 µm) column, using mobile phase A (0.03M sodium citrate adjusted to pH 5 with glacial acetic acid) and mobile phase B (100% methanol), ran at variable proportions at flow rate of 1.0 ml/min. The detection was carried out at 320 nm.\textsuperscript{[131]}

Raghabendra Narayan Singh \textit{et al.}, developed a stability indicating high performance liquid chromatographic method for the determination of moxifloxacin. Optimum separation was achieved in less than 10 minutes using Phenomenex ODS C18 (250x 4.6 mm packed with 5µ) column. The analyte was resolved by using a mobile phase 20 mmol/liter ammonium formate and acetonitrile (70:30) pH adjusted to 4.0 with formic acid at flow rate 1 ml/min on a isocratic high performance liquid chromatographic system at a wavelength of 295 nm. For stress studies the drug was subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat and photolytic degradation. The degradation studies indicated the drug to be susceptible to acid, alkali hydrolysis and oxidative degradation.\textsuperscript{[132]}

Syed Naeem Razzaq \textit{et al.}, developed a stability indicating RP-HPLC method for moxifloxacin hydrochloride and dexamethasone in bulk drugs and pharmaceutical formulations. Method used BDS Hypersil C8 column (250 · 4.6 mm, 5 lm) as stationary phase with mobile phase consisting of a mixture of phosphate buffer (20 mM) containing 0.1% (v/v) triethylamine, at pH 2.8 (adjusted with dilute phosphoric acid) and methanol (38.5:61.5 v/v) at a flow rate of 1.5 mL min\textsuperscript{-1}. Detection of the analytes and degradation products was performed at 254 nm using a diode array detector.\textsuperscript{[133]}

Mohamed R. Elghobashy \textit{et al.}, Three novel moxifloxacin selective electrodes were investigated with 2-nitrophenyl octyl ether as a plasticizer in a polymeric matrix of polyvinyl chloride (PVC). Sensor 1 was fabricated using tetrakis (4-chlorophenyl) borate (TpClPB) as an anionic exchanger without incorporation of an ionophore. The proposed sensors displayed useful analytical characteristics for the determination in bulk powder, pharmaceutical formulation, and biological fluids (plasma) and in the presence of its alkaline degradation product and thus could be used for stability-indicating studies.\textsuperscript{[134]}
3.4.4 Reported *in–vitro* dissolution methods for moxifloxacin single and in combination products.

Motwani. S. K *et al.*, developed a *in vitro* dissolution studies for Moxifloxacin by spectrophotometric method. Moxifloxacin was estimated at 296 nm in 0.1N hydrochloric acid (pH 1.2) and at 289 nm in phosphate buffer (pH 7.4). Beer's law was obeyed in the concentration range of 1-12 µg ml\(^{-1}\) \((r^2=0.9999)\) in hydrochloric acid and 1-14 µg ml\(^{-1}\) \((r^2=0.9998)\) in the phosphate buffer medium.\[135\]

Alija Uzunović *et al.*, reported the dissolution rate of two fluoroquinolone antibiotics (ciprofloxacin and moxifloxacin) was analysed in presence/absence of three antacid formulations. Disintegration time and neutralization capacity of antacid tablets were also checked. Variation in disintegration time indicated the importance of this parameter, and allowed evaluation of the influence of postponed antacid-fluoroquinolone contact. The results obtained in this study showed decreased dissolution rate of fluoroquinolone antibiotics from tablets in simultaneous presence of antacids, regardless of their type and neutralization capacity.\[136\]

Muruganatha. G. *et al.*, report the interaction of moxifloxacin and lomefloxacin at neutral, acidic and basic conditions both at room temperature and 37\(^0\)C. The effect of dissolution medium simulating various body environments with response to pH has been examined in order to elucidate the interactions. The response of moxifloxacin and lomefloxacin after interaction with co-administered drugs at different conditions and temperature were noted using a Shimadzu HPLC system with PDA detector. It was seen that interaction of these fluoroquinolones was more at 37\(^0\)C than at room temperature.\[137\]

Arya. K. *et al.*, report in their study the *in vitro* release of Moxifloxacin Hydrochloride and ketorolac tromethamine from the formulations was studied through cellophane membrane using an open end cylinder. The dissolution medium used was simulated tear fluid freshly prepared (pH 7.4). The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37± 1°C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1 ml volume, were withdrawn at hourly intervals.
and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 289 nm and 323.5 nm.\[138\]

### 3.4.5 Reported stability indicating methods for cefixime trihydrate single and in combination products.

S. P. Gandhi \textit{et al.}, developed a stability indicating spectrophotometric assay methods for cefixime trihydrate (CEF) degradation behavior study stress degradation recommended by International Conference of Harmonization (ICH). The stress conditions include effect of temperature, humidity, light, oxidizing agents and susceptibility across a wide range of pH values.\[139\]

Babita Singh \textit{et al.}, developed a thin-layer chromatographic method for the analysis of cefixime using precoated silica gel 60F$_{254}$ TLC aluminum sheets as stationary phase. The mobile phase was optimized to ensure that no other drug from cephalosporin class and degradation products (formed under stress condition) of the drug interfered with the estimation. By using toluene-ethyl acetate-formic acid-water (10:58:22:10, v/v) as mobile phase, a sharp, well-defined, and symmetrical peak was obtained in the chromatogram with an $R_F$ value 0.54 ± 0.02. Densitometric measurement was made in the reflectance/absorbance mode at 293 nm.\[140\]

Ahmed E. M. Saeed \textit{et al.}, developed a stability indicating RP-HPLC method for cefixime. Two major decomposed products were successfully resolved on a C18 column (waters spherisorb 25 cm × 4.6 mm, 5μm), utilizing mobile phase of tetra butyl ammonium hydroxide solution (0.03M aqueous) pH adjusted to 6.5 with diluted ortho phosphoric acid (10 % aqueous) and acetonitrile in a ratio of 3:1 respectively. Mobile phase was delivered at the flow rate of 1.0 ml/min. Ultra violet detection was carried out at 254 nm.\[141\]
Sutar S. V. et al., developed a Spectrophotometric method for degradation study of cefixime trihydrate. The ultra violet spectrum of both acidic and basic degraded product was found to substantial difference from pure drug. The extent of degradation can be calculated by comparing the decrease in absorbance at selective wavelength.\textsuperscript{142}

Zahra Talebpour et al., developed a stability indicating HPLC method for Cefixime in preparation and storage steps. The chromatographic conditions were comprised of a reversed-phase C18 column (4.6 x 250 mm, 5 pm) with a mobile phase composed of water: acetonitrile (85:15 v/v, with 0.5% formic acid) and ultraviolet detection (UV).\textsuperscript{143}

Mittal P. Joshi. et al., developed a stability indicating RP-HPLC method for the estimation of Cefixime trihydrate and Potassium clavulanate in tablet and suspension dosage form. This method was developed using Phenomenex C18 Column (250 mm x 4.6 mm id, 5\(\mu\)m) and 25mM NaH\(_2\)PO\(_4\).2H\(_2\)O pH 6.5 buffer (20:80 v/v) as the mobile phase at 1ml/min flow rate with detection at 220nm. The method is deemed to be stability indicating as it separates both the drugs from likely degradation products.\textsuperscript{144}

Satyanarayana Battu et al., developed a new stability indicating HPLC method and validated for the determination of Cefixime and Linezolid in tablet dosage form and spiked plasma sample. The chromatographic separation was achieved on a X-Terra RP-18 (150mm x 4.6mm, 5\(\mu\)m) stationary phase maintained at a temperature of 30\(^\circ\)C with a mobile phase combination of 1% Orthophosphoric acid and Methanol (60:40) at a flow rate of 1.0 mL min\(^{-1}\) and the detection was carried out by using UV detector at 250 nm.\textsuperscript{145}

Prateek Jain et al., developed a stability indicating RP-HPLC method for the estimation of Ofloxacin and Cefixime in pharmaceutical formulation in presence of degradation products. The chromatographic separation of Ofloxacin and Cefixime was achieved on Shimadzu LC-20AT series HPLC having C18-ODS bonded column (250 x 4.6 mm, 40 \(^\circ\)C, 10 \(\mu\)L) using UV/Visible detector at 276 nm.\textsuperscript{146}
Badmanaban. R. *et al.*, developed a stability indicating RP-HPLC method for the estimation of Cefixime trihydrate and Ornidazole in bulk drug and pharmaceutical dosage form. Separation and quantification were achieved on an ACE C18, 5μm, 150 x 4.6 mm i. d. column. The mobile phase was Acetonitrile: 20mM KH2PO4 buffer (20:80) and buffer pH 5.5 adjusted using ortho phosphoric acids after adding 0.1% triethylamine, at a flow rate of 0.8 ml/min and injection volume was 20μL. Detection was carried out at a wavelength of 310 nm. The stressed sample chromatograms demonstrate the specificity of the proposed method for the determination of target analytes in presence of degradants.[147]

3.4.6 Reported *in – vitro* dissolution methods for cefixime trihydrate single and in combination products.

Madhura Vishal Dhoka *et al.*, developed and validate a dissolution test for Cefixime and Erdosteine capsules using spectrophotometric method. The dissolution test conditions established were: 900 ml of 0.05 M Potassium phosphate buffer pH 7.2 adjusted with1 N NaOH as dissolution medium for 45 minutes, using a basket apparatus at a stirring rate of 100 rpm at temperature 37°C. UV spectrophotometric method was developed for evaluating drug release namely absorption correction method (295 nm is wavelength max of CEF ,where ERDO has practically nil absorbance, detection of ERDO was carried out at its wavelength max 237 nm).[148]

Vishnu P. Choudhari *et al.*, developed a simple, precise and accurate methods for simultaneous determination of Cefixime (CEFI) and Ofloxacin in combined tablet dosage form. The method is based on Ratio spectra derivative and Area under curve spectrophotometry using methanol and 0.1 N HCl, respectively as solvents. The method was successfully applied to carry out dissolution study of commercial tablet formulation by using USP II dissolution test apparatus.[149]
Tariq Ali et al., developed in vitro dissolution studies in buffer pH 7.4 at 37°C. Later cefixime along with each non steroidal anti-inflammatory drugs was individually subjected to dissolution test in buffer pH 7.4 and availability of each drug was determined using UV-Visible spectrophotometer. The results obtained from the two sets of experiment were compared to determine the change in drug availabilities after interaction and analyzed statistically by t test. The results indicate highly statistically significant interaction between cefixime and diclofenac sodium, where the availability of cefixime was significantly increased and that of diclofenac sodium was significantly decreased. Cefixime was found to greatly decrease the availability of mefenamic acid in the dissolution medium, where as the availability of tiaprofenic acid was increased. No significant interaction could be identified between cefixime and flurbiprofen. Analysis of the overall results clearly indicates that there is a potential for interaction between cefixime and non steroidal anti-inflammatory.\textsuperscript{150}
### Table 12. Reported degradation products of stress degradation studies of Cefixime Trihydrate.\[151\]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradation Products</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>([2E]-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino)(2S)-5-methyl-7-oxo-1,2,5,7-tetrahydro-4H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid</td>
<td>471.46</td>
</tr>
<tr>
<td>2</td>
<td>((E)-[1-(2-amino-1,3-thiazol-4-yl)-2-(([(2S)-5-methyl-7-oxo-1,2,5,7-tetrahydro-4H-furo[3,4-d][1,3]thiazin-2-yl]methyl]amino)-2-oxoethylidene]amino)oxy]acetic acid</td>
<td>427.45</td>
</tr>
<tr>
<td>3</td>
<td>(6S,7S)-7-((2E)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid</td>
<td>441.43</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Degradation Products</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>4</td>
<td><img src="" alt="Chemical Structure" /> (6S,7S)-7-((2E)-2-(2-amino-1,3-thiazol-4-yl)-2-[(2-ethoxy-2-oxoethoxy)imino]acetyl)amino)-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid</td>
<td>481.50</td>
</tr>
</tbody>
</table>
Table 13. Reported degradation products of stress degradation studies of Moxifloxacin Hydrochloride.\cite{152}

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradation Products</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(4aS,7aS)-6-(3-carboxy-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydroquinolin-7-yl)octahydro-1H-pyrrolo[3,4-b] pyridin-1-ium</td>
<td>390.40</td>
</tr>
<tr>
<td>2</td>
<td>(4aS,7aS)-6-(3-carboxy-1-cyclopropyl-6,8-dimethoxy-4-oxo-1,4-dihydroquinolin-7-yl)octahydro-1H-pyrrolo[3,4-b] pyridin-1-ium</td>
<td>414.47</td>
</tr>
<tr>
<td>3</td>
<td>(4aS,7aS)-6-(3-carboxy-1-cyclopropyl-6-fluoro-8-ethoxy-4-oxo-1,4-dihydroquinolin-7-yl)octahydro-1H-pyrrolo[3,4-b] pyridin-1-ium</td>
<td>416.46</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Degradation Products</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>4</td>
<td>(4aS, 7aS)-6-(3-carboxy-1-cyclopropyl-6-fuoro-8-ethoxy-4-oxo-1,4-dihydroquinolin-7-yl)octahydro-1H-pyrrolo[3,4-b]pyridin-1-ium</td>
<td>388.14</td>
</tr>
</tbody>
</table>