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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION USED IN PHARMACEUTICAL FORMULATIONS FOR THE TREATMENT OF PNEUMONIA- A REVIEW.

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Abstract **:** Pneumonia is a form of acute respiratory infection that affects the lungs. Symptoms include a cough with phlegm or pus, fever, chills and difficulty breathing. Amoxicillin, azithromycin, ciprofloxacin, erythromycin, etc. are the drug that can be used to treat the pneumonia. In this review we have to study Analytical method such as HPLC, UV, LC-MC-MC, Spectrophotometric, HPTLC for this various drug used in Pneumonia. In addition with this the best hospitals and doctors are given in the list format. The main approaches of this review is to explore all the possible analytical methodology for this drug. The review is also helpful to choose better option for analytical method development for this drug. This article introduces a simple, specific, precise and robust HPLC method which has been developed and validated based on FDA guidance and the ICH guidelines Q2A and Q2B to measure the number of drugs in order to assess their release profiles from new floating-sustained release tablet formulations

Key Terms - Pneumonia, Amoxicillin, Ciprofloxacin, HPLC, UV.

1. Introduction:

Pneumonia is a Lung illness caused by an acute respiratory infection. When a healthy person breathes, tiny sacs called alveoli in the lungs fill up with air. When a person develops pneumonia, the alveoli get clogged with pus and fluid, making breathing difficult and restricting oxygen intake. Infection that causes the air sacs in one or both lungs to become inflamed and fill with fluid. Anyone, but especially newborns, children, and adults over 65, is at risk of contracting the illness, which can be fatal.[1]

1.1 Symptoms:

Coughing up phlegm or pus, fever, chills, and trouble breathing are all symptoms. Many types of pneumonia can be treated with antibiotics. Vaccines can help prevent some types of pneumonia.[1]

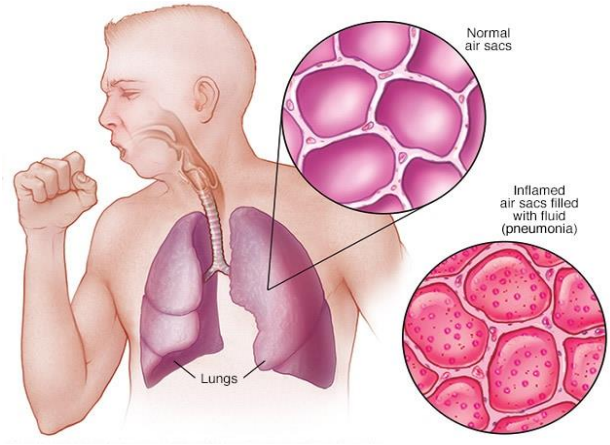


Fig. no.1 lungs with pneumonia

1.2 Pneumonia and your lungs Open pop-up dialog box:

Pneumonia is a lung infection that causes inflammation of the air sacs in one or both lungs. In these situations, various symptoms appear. When the air sacs fill with fluid or pus, it can cause a cough with phlegm or pus, a fever, chills, and difficulty breathing (purulent material). Pneumonia can be caused by bacteria, viruses, or fungi, among other things. Pneumonia can be mild or severe, and it can even be fatal. The most vulnerable are infants and young children, persons over 65, and people with health problems or weakened immune systems. .[2]

1.3 Sign and Symptoms:

The severity of pneumonia symptoms varies based on the type of bacteria that caused the sickness, as well as your age and overall health. Perplexity or changes in mental awareness are two examples of mental awareness shifts (in adults age 65 and older) A cough that generates phlegm is known as phlegm cough. Fatigue, Fever, perspiration, and shivering chills A lower-than-average body temperature (in adults older than age 65 and people with weak immune systems), Vomiting, nausea, or diarrhoea are all symptoms of a stomach bug. Breathing difficulties. In babies and infants, the signs of infection may go undiagnosed. They may also vomit, have a fever and cough, appear restless or tired and devoid of energy, or have breathing and feeding problems. [2]

1.4 Cause :

pneumonia can be caused by a variety of bacteria. Bacteria and viruses in the air we breathe are the most prevalent. These bacteria are typically prevented from invading your lungs by your body. Even if your health is typically strong, these bacteria can occasionally overwhelm your immune system.[2][3]

Table no. 1.1 Pneumonia is classified [3]

Sr. no.	Types of pneumonia	Causative agents
1.	Bacterial pneumonia.	*Streptococcus pneumoniae*
2.	Viral pneumonia	*Influenza (flu) A and B viruses*
3.	Mycoplasma pneumonia.	*Mycoplasma genitalium*
4.	Other pneumonias	*Pneumocytic pneumonia* *Legionella pneumonia* *Aspiration pneumonia* *Hypostatic pneumonia* *Lipid pneumonia*

Community-acquired pneumonia is the most common type of pneumonia. It occurs outside of hospitals or other health care facilities. It may be caused by:

Community-acquired respiratory disease is that the most common form of respiratory disorder. It happens outside of hospitals or alternative health care facilities. It's going to be caused by:

Bacteria: The foremost common reason for microorganism respiratory disease. The streptococcus pneumoniae this kind of respiratory disease will occur on its own or when you have a cold or the respiratory disease. It's going to have an effect on one part (lobe) of the respiratory organ, a condition known as lobar pneumonia.

Bacteria-like organisms: Mycoplasma pneumoniae can also cause pneumonia. It's usually producing milder symptoms than do different varieties of pneumonia. Walking pneumonia is an off-the-cuff name given to the present from of pneumonia, which generally is not sever enough to need bed rest.

Fungi: This kind of pneumonia is commonest in individual with chronic health issues or weakened immune systems, and in those who have inhaled massive doses of the organisms. The fungi that cause it is found in soil or birds dropping and vary depending upon geographical location.

Viruses: Are the foremost common reason behind respiratory disease in kids younger than five years. Viral infection is typically varied. However, in some because it will become terribly serious. Coronavirus 2019 (COVID-19) could cause respiratory disease, which might become severe.

Risk factors: Mainly pneumonia can be divided into two age group in high risk

- Children who are 2 years old or younger
- People who are age 65 or older

Several other factors includes :

- Being admitted to the hospital, having a chronic illness
- Being admitted to the hospital. If you're in a hospital intensive care unit, you're more likely to have pneumonia, especially if you're on a breathing machine (a ventilator).
- Long-term illness. If you have asthma, chronic obstructive pulmonary disease (COPD), or heart problems, you're more likely to have pneumonia.
- Smoking. Smoking weakens your body's natural defences against pneumonia-causing germs and viruses.
- Immune system that is weakened or inhibited. People with HIV/AIDS, organ transplant recipients, and those who get chemotherapy or long-term steroids are also at risk.[3]

Complications: Despite therapy, some persons with pneumonia, particularly those in high-risk categories, may develop complications such as:

- Bacteria in the bloodstream (bacteraemia). Bacteria from your lungs can enter your circulation and spread to other organs, potentially causing organ failure.
- Breathing problems. You may have problems breathing in adequate oxygen if your pneumonia is severe or if you have persistent underlying lung illnesses. While your lung recovers, you may need to be hospitalised and use a breathing machine (ventilator).
- A build-up of fluid around the lungs (pleural effusion). Fluid may accumulate in the small area between the layers of tissue that border the lungs and chest cavity as a result of pneumonia (pleura). If the fluid gets contaminated, it may be drained using a chest tube or surgically removed.
- Abscess in the lungs. When pus grows in a hollow in the lung, it is called an abscess. Antibiotics are frequently used to treat an abscess. To remove the pus, surgery or drainage with a long needle or tube inserted into the abscess may be required.

1.5 Prevention

To help prevent pneumonia:

Vaccinate yourself. Some kinds of pneumonia and the flu can be prevented with vaccines. Make an appointment with your doctor to obtain these injections. Immunization requirements have changed over time, so check with your doctor about your vaccination status, even if you recall having a pneumonia vaccine earlier. Ascertain that your children are immunised. Children under the age of 2 and children aged 2 to 5 years who are at high risk of pneumococcal illness should receive a separate pneumonia vaccination, according to doctors. Vaccination is also recommended for children who attend a group child care centre. Children above the age of six months should also get flu vaccines, according to doctors.Maintain a healthy level of hygiene. Wash your hands often or use an alcohol-based hand sanitizer to protect yourself from respiratory infections that can lead to pneumonia. Please don't smoke. The natural defenses of your lungs against respiratory infections are harmed by smoking. Maintain a healthy immune system. Get enough of rest, exercise regularly, and eat a nutritious diet.[4]

Drugs available for pneumonia

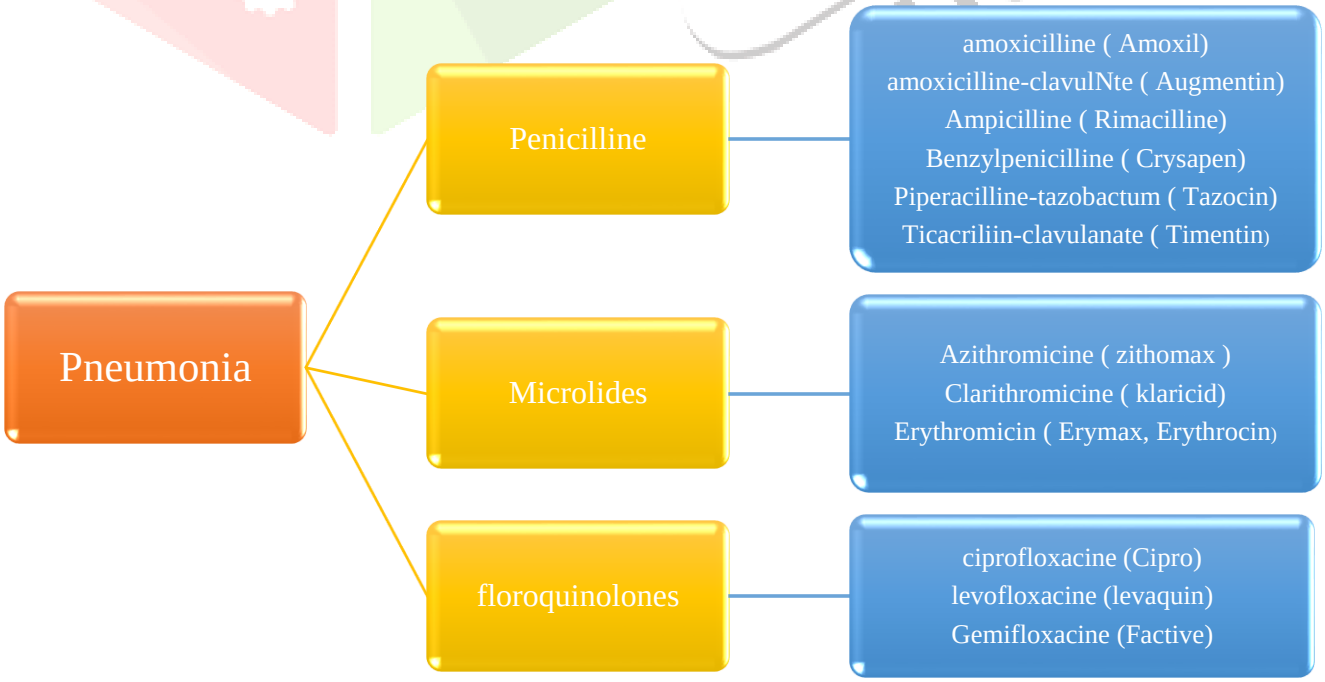


Fig no. 2 Classification of drugs used in pneumonia [5]

Table no. 2 Diagnosis and Treatment available for pneumonia [4]

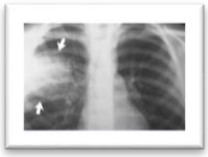
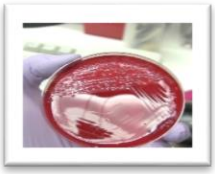
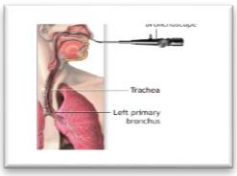
Sr. no.	DIAGNOSIS	Image
1	**Chest X-rays :** This helps your doctor diagnose pneumonia and determine the extent and location of the infection. However, it can't tell your doctor what kind of germ is causing the pneumonia.[6]	
2	**Blood tests :** Blood tests are used to confirm an infection and to try to identify the type of organism causing the infection. However, precise identification isn't always possible.[6]	
3	**Sputum test:** A sample of fluid from your lungs (sputum) is taken after a deep cough and analysed to help pinpoint the cause of the infection. [7]	
4	**Bronchoscopy:** Bronchoscopy is a procedure to **look directly at** the airways in the lungs using a thin, lighted tube (bronchoscope). The bronchoscope is put in the nose or mouth. It is moved down the throat and windpipe (trachea), and into the airways[8]	

Table no. 3 list of Best Hospitals available for transplant of lungs[9]

Sr. no	Best Hospitals for Lung Transplant in India
1.	Fortis, Delhi
2.	Apollo Hospital, Delhi
3.	Sir Ganga Ram Hospital, Delhi
4.	Max Healthcare Hospital, Delhi
5.	Blk Super Speciality Hospital, Delhi
6.	Medanta Hospital, Gurgaon
7.	Kokilaben Dhirubhai Ambani Hospital, Mumbai
8.	Wockhard Hospital, Mumbai
9.	Jaslok Hospital, Mumbai
10.	Lilavati Hospital, Mumbai
11.	Jupiter Hospital, Thane, Maharashtra
12.	Sahyadri Hospital, Pune
13.	MaxCure Hospital, Hyderabad, Telangana
14.	KIMS Hospital, Hyderabad
15.	Continental Hospital, Hyderabad
16.	Narayana Health, Bangalore
17.	Manipal Hospital, Bangalore
18.	Paras Hospital, Patna
19.	Aster Medcity, Kochi Kerala
20.	Columbia Asia Hospital, Bangalore
21.	Amrita Hospital, Kochi
22.	Yashoda Hospital, Secunderabad
23.	Frontier Lifeline Hospital, Chennai, Tamil Nadu
24.	SIMS Hospital, Chennai, Tamil Nadu
25.	Global Hospital, Chennai, Tamil Nadu
26.	PSG Hospital, Tamil Nadu
27.	Kauvery Hospital, Chennai
28.	Billroth Hospital, Chennai, Tamil Nadu
29.	Sri Ramachandra Medical Centre, Chennai, Tamil Nadu

Table no.4 List of top transplant surgeon[9]

Sr no.	List of top transplant surgeon
1.	Dr. Sandeep Attawar
2.	Dr. P.V. Naresh Kumar
3.	Dr. Sunil Agarwal
4.	Dr. Nandkishore Kapadia
5.	Dr. Prasanna Ratnakar Salvi
6.	Dr. Manish Garg
7.	Dr. Alla Gopala Krishna Gokhale
8.	Dr. Thangaraj Paul Ramesh
9.	Dr. Kirun Gopal
10.	Dr. Sanjog Rawtani
11.	Dr. K R Balakrishnan
12.	Dr. Naresh Trehan

2. Analytical prospective of drugs used in pneumonia

Validation parameters or characterization of methods of analysis

Accuracy, specificity, selectivity, limit of detection, limit of quantification, linearity, recovery, repeatability, sensitivity.

Sensitivity: "The change in response on a measuring instrument divided by the corresponding change in stimulus."

Specificity: "The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components."

Limit of detection: "The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitatively determined as an exact value." Based on the standard deviation of the response and the slope, detection limit may be expressed as follow.

(LOD) may be expressed as:

LOD=3.3 x standard deviation of the regression line (σ) /Slope(S)

Limit of quantitation: "The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy." Based on the standard deviation of the response and the slope, the quantitation limit (LOQ) may be expressed as

LOD=10 S & where:

LOD=10 x standard deviation of the regression line (σ) /Slope(S)

Linearity and range:

Linearity: the linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Thus, in this section, "linearity" refers to the linearity of the relationship of concentration and assay measurement.

Range:

the range of an analytical procedure is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with a suitable level of precision, accuracy, and linearity using the procedure as written. The range is normally expressed in the same units as test results (e.g., percent, parts per million) obtained by the analytical procedure.

Determination of linearity and range:

linearity should be established across the range of the analytical procedure. It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods (e.g., by calculation of a regression line by the method of least squares). Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares.

Precision: "Degree of conformity between independent measurement results obtained under prescribed conditions."

Repeatability: "Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision."

Robustness/ruggedness: The degree of independence of the method of analysis from minor deviations in the experimental conditions of the method of analysis.

Selectivity: "The selectivity of a method of analysis refers to the degree to which the method of analysis is usable for determining the presence of specific analytical parameters in a complex mixture (matrix) without interference from other analytical parameters in the mix."

I. Amoxicillin :

AMO is an antibacterial medication that is semi-synthetic and generated from a fermentation product. In terms of chemistry, it is (2S,5R,6R) -6-[[(2R) - 2-Amino-2-(4-hydroxyphenyl) acetyl] amino] -3,3-dimethyl -7-oxo-4- thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid trihydrate having a molecular formula of C16H19N3 O5 S.3H2 O and its molecular weight is 419.4. . AMO is a crystalline powder that appears white or almost white. Water is marginally soluble, ethanol is very barely soluble (96 percent), and fatty oils are essentially insoluble. It dissolves in dilute acids and alkali hydroxide solutions.[10]

Structure :

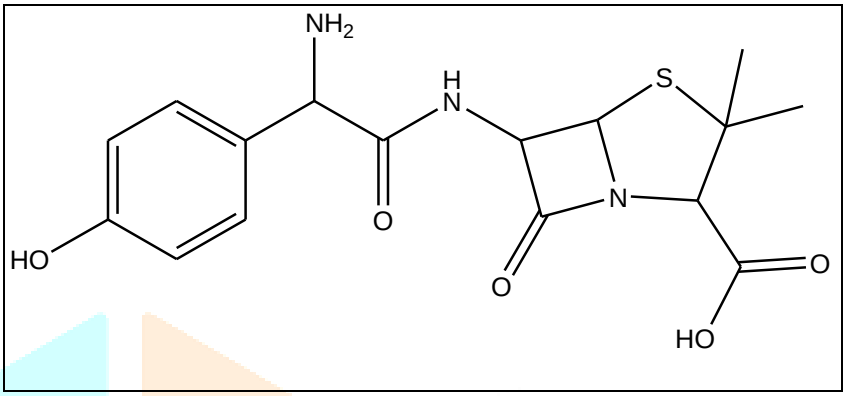


Fig. no. 2.1 Chemical structure of amoxicillin [11]

Table no.2.1 Analytical method for Amoxicillin

Sr. no.	Title of paper	Journal name	Methods	Instrumental parameter	Result of validation	Author and year of publication
1.	Development and validation of a simple HPLC method for simultaneous in vitro determination of amoxicillin and metronidazole at single wavelength	Journal of Pharmaceutical and Biomedical Analysis	**RP-HPLC**	**COLUMN** : Whatman Partisil **5 ODS-3 column (100 mm × 4.6 mm)** 5µm particle size, plus Whatman guard cartridge (RP cartridge). **MOBILE PHASE:** The mobile phase was a degassed and filtered (0.45µm; Millipore) mixture of phosphate buffer solution (pH 4.7; 0.05 M)–methanol (95:5, v/v) with final pH adjusted to approximately 4.0. **FLOW RATE ;** 1.5 ml/min. **DETECTOR :** The UV detector wavelength was set at 254 nm. **SOFTWARE:** Millenium32® software.	**Retention time :** 3.2 min. **Linearity equation :** y = 1013.78 (±25.12)x + 700.82 (±46.08) (n = 54, R^2 = 0.9992) **RSD =** ≤2.7% **LOQ** = 0.15 µg/ml **LOD =** 0.05 µg/ml	Naser Tavakoli and et al. (2006) [11]
2.	Validation of HPLC-UV method for determination of amoxicillin Trihydrate in capsule	Annals of Advances in Chemistry	**RP-HPLC (HPLC cecil)**	**COLUMN:** column hypersil reverse phase, c-18 column 250 mm x 4.6 mm i.d. particle size 5µm **MOBILE PHASE:** potassium dihydrogen phosphate and methanol in the ratio (95:05v/v). **FLOW RATE:** 1.5ml/minute **DETECTOR :** UV detector wavelength at 254 nm **SOFTWARE :** power stream	**Retention time :** 3.5±0.02 min. **Linearity equation :** y = 1.6517x+5.8667 **r^2**=0.9998 **RSD** = 1.166442% **LOQ** = 4.784979 µg/ml **LOD =** 1.579043 µg/ml	Sendanyoye Marcel* and et al. (2018) [12]
3.	Development and Validation of Stability Indicating HPLC Method for Simultaneous Estimation of Amoxicillin and Clavulanic Acid in Injection A	American Journal of Analytical Chemistry	**HPLC**	**COLUMN**: Inertsil C18 column (250 × 4.0 mm, 4 µm) **MOBILE PHASE:** sodium dihydrogen phosphate monohydrate and methanol.(95:5 v/v) pH 5.0 buffer **FLOW RATE:** 1 ml/minute **DETECTOR :** UV detection at 220 nm, photodiode array detector **SOFTWARE :** Chem station	**Retention time:** 7.8 min **Linearity equation :** y = 39.323x + 40.197 **r^2**= 0.9998 **RSD =** 1.81 **LOQ** = 12.06 µg/ml **LOD =** 3.98 µg/ml	Durga Mallikarjuna Rao Tippa* and et al. (2010) [13]
4.	A simple, sensitive and green bienzymatic UV-	Enzyme and Microbial Technology	**UV**	UV–visible spectrophotometer (Agilent, USA) using a semi-micro quartz cuvette type (1cm path length)	**Linearity** (n=3; 0–100µM) **Slope±S.D. =**0.0185±0.0001 **r2 =**0.9998	Theerasak Rojanarataa and et.al.

	spectrophotometric assay of amoxicillin formulations			230nm.	**LOD=** 0.77µM **LOQ=** 2.55µM	(2009) [14]
5.	Simultaneous determination of Amoxicillin and Clavulanate in combined tablets by non-derivative and derivative UV spectrophotometric techniques	International Journal of PharmTech Research	**UV**	A UNICAM UV 300 double beam spectrophotometer (Thermo Spectronic, USA) with a fixed slit width (1.5 nm) 270.4 nm	**Linearity=** 60.0 – 160.0 **Slope±S.D. =** 0.0011 **Intercept±S.D.** Y = 0.0024CAMO + 0.0185 **r2 =**0.9999 **LOD=** 0.80um **LOQ=** 3.11um	Vu Dang Hoang and Vu Thi Huong [15]
6.	Comparative Study of RP-HPLC and UV Spectrophotometric Techniques for the Simultaneous Determination of Amoxicillin and Cloxacillin in Capsules	Pharmaceutical Analysis	**HPLC and UV derivative spectrophotometry**	**COLUMN**: Inertsil C18 column (250 × 4.0 mm, 4 µm) **MOBILE PHASE:** sodium dihydrogen phosphate monohydrate and methanol.(95:5 v/v) pH 5.0 buffer **FLOW RATE:** 1 ml/minute **DETECTOR :** UV detection at 220 nm, photodiode array detector **SOFTWARE** A UNICAM UV 300 double beam spectrophotometer (Thermo Spectronic, USA) Thermo Spectronic VISION32 software	**Linearity=** 60.0 – 140.0 **Slope±S.D. =** **Intercept** Y = 31.244CAMO – 32.250 **r2 =** 0.9995 **LOD=** 0.70um **LOQ=** 2.89um	Giang Do T, Hoang Vu D [16]

II. AZITHROMICIN

Azithromycin is a15-membered ring, semi-synthetic macrolide antibiotic with two deoxy-sugars, derived from erythromycin through a methyl-substituted nitrogen atom in the lactone ring. Its chemical name is 9-deoxy-9a-azo-9a-methyl-9ahomoerythromycin A, with molecular weight 748.88 and chemical formula C38H72N 2O12. Its chemical structure is shown below (USP 2012): Azithromycin is a bacteriostatic agent, which binds to the 50S ribosomal subunit of susceptible microorganisms and interferes with protein synthesis.

Structure :

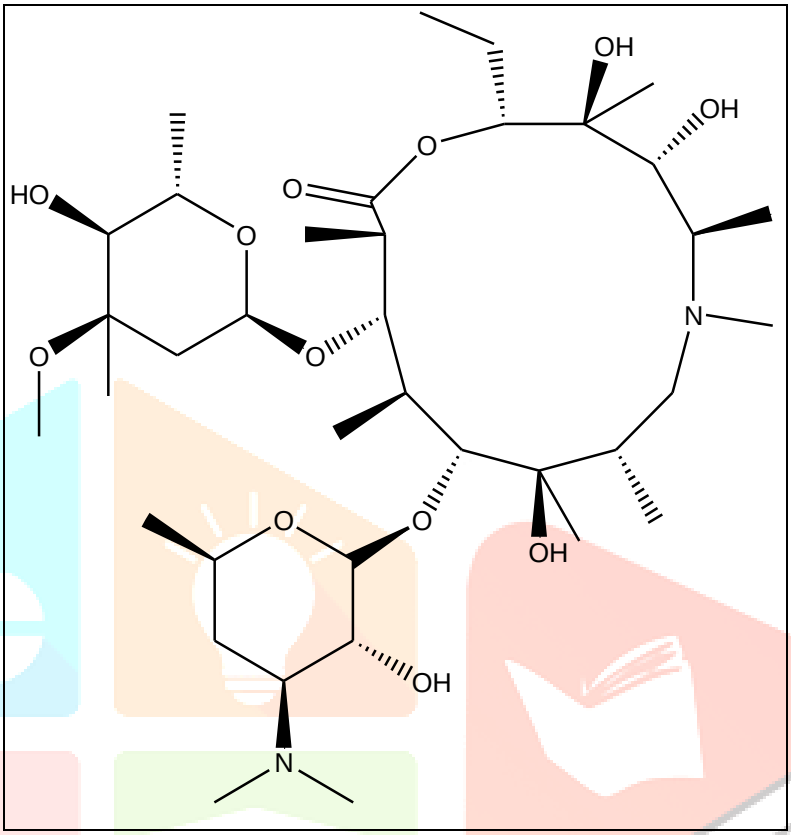


Fig. no. 2.2 Structure of azithromycin.

Table no.2.2 Analytical method for Azithromycin

Sr no.	Title of paper	Journal name	Method	Instrumental parameter	Result of validation	Author and year of publication
1.	A rapid, developed and validated RP-HPLC method for determination of azithromycin	SN Applied Sciences	**HPLC**	**COLUMN:** reversed phase column ODS-3 (250 mm × 4.6 mm x 5 µm) **MOBILE PHASE:** Methanol: Phosphate buffer (9:1, v/v) **FLOW RATE**: 1.2 ml/min **DETECTOR :** PDA detector 210 nm((Shimadzu)	**Retention time:** 5.804 min. **Linearity equation :** Y= 1138.1x+9.0828. **r^2**= 0.9996 **RSD =** 0.66% **LOQ** = 28.7 µg/ml **LOD =** 86.9 µg/ml	Mostafa F. Al-Hakkani (2019) [17]
2.	Analysis of Azithromycin and its Related Compounds by RP-HPLC with UV Detection	Journal of chromatographic science	**RP-HPLC with UV detection**	**COLUMN:** Reverse phase C18, 5mm, 25 cm length, 4.6 mm diameter, column temperature 50°C . **MOBILE PHASE:** Methanol- Phosphate buffer, pH 7.5 (80:20,v/v) **FLOW RATE**: 2.0 mL/min **DETECTOR : UV** detector 210 nm	**Retention time:** 6.8 min. **Linearity over the range :** 0.3- 2.0 mg/ml **r^2**= 0.9999 **std error:** 23,798 **RSD** = 0.2 % **LOQ** = 0.0005 mg/ml **LOD** = 0.0008 g/ml **Accuracy of the method :** 100.5%	Fuad AL-Rimawi and Maher Kharoaf (2010) [18]
3.	Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form	Journal of Pharmaceutical and Biomedical Analysis	**reversed-phase HPLC**	**COLUMN:** Xterra RP18 (250mm x 4.6mm, 5 um) Analytical column. **MOBILE PHASE :** acetonitrile–dipotassium phosphate (30 mM) (50:50, v/v) (pH 9.0) **FLOW RATE**: 1.7 ml/min **DETECTOR :** photodiode array detector **DETECTOR WAVELENGH :** detector wavelength at 215 nm **SOFTWARE :** Empower Software.	**Retention time:** 11.5 min **Linearity equation :** y = 504.51x − 5017.3 **Linearity over the range :** 250–1500 mg/ml **r^2**= 0.99 **% Recovery** : 99.90 % **RSD =** 1.51 % **LOQ** = 0.01um/ml **LOD =** 2.3 um/ml **Accuracy of the method :**	K.A. Shaik, S.D. Patil and A.B. Devkhile (2008) [19]
4.	A new HPLC method for azithromycin quantitation	Journal of Pharmaceutical and Biomedical Analysis	**HPLC**	**COLUMN:** LiChroCART® 125×4.6 mm HPLC Cartridge LiChrospher® 100 RPS select B (5 um) Merck Darmstadt column **MOBILE PHASE :** buffer, acetonitrile and methanol (60:20:20) adjusted to pH 8.0&0.1 with phosphoric acid, **FLOW RATE**: 1.0 ml/min **DETECTOR :** UV-Vis detector (model KNK-029-757) **Model SP 4600**	**Retention time:** 10.3 min. **Linearity equation :** Y=−1.17×103+1.13×104 X **Linearity over the range :** mg/ml **r^2**= 0.9994 **% Recovery** : 99.4 % **RSD =** 1.51 % **LOQ** = 0.01um/ml **LOD =** 2.3 um/ml	Patricia Zubata and et al. (2001) [20]

5.	Analysis of Azithromycin in Human Plasma by LC–MS–MS	Chromatographic Supplement Vol. 66, 2007	**LC–MS–MS**	**COLUMN:** SunFire C18, 50 mm · 2.1, 3.5 um column **MOBILE PHASE :** 1.54 g ammonium acetate, 250 mL water, 570 mL acetonitrile, 180 mL methanol and 0.6 mL glacial acetic acid **FLOW RATE**: 0.2 mL min)1 **DETECTOR :** UV detector **DESOLVATION GAS FLOW :** 450 L/hr **CONE GAS FLOW :** 50 L /hr **COLLISION ENERGY :** 43 **GAS PRESSOR (Argon) :** 3.8 e^{-3} mbar **Black out temp. :** 120 ^{o}C **DISOLVAATION TEMP. :** 350^{o}C	**Concentration range :** 2-1,000ng/ml **Retention time :** 0.9 min **Accuracy :** 91.83% **Precision :** 2.71- 7.25% **r^2**= 0.98941 **% Recovery** : 99.4 % **RSD =** 1.51 % **LOQ** = 2 ng mL^{-1} **LOD =** 2.3 um/ml **Absolute recovery :** 81.97 %	N. Yuzuak and et al. (2007) [21]
6.	Azithromycin assay in drug formulations: Validation of a HPTLC method with a quadratic polynomial calibration model using the accuracy profile approach	Elseview Masson France	**HPTLC**	Precoated silica gel 60 F254 TLC plates (20 × 10 cm) (Merck, Darmstad, Germany) were used as stationary phase. **Mobile Phase:** mobile phase consisting of chloroform — ethanol — 25% ammonia (6:14:0.2; v/v/v). **Visualization :** Visualization reagent was concentrated sulfuric acid (18 M) — ethanol (1:17; v/v) **Software :** TLC scanner 3 with winCATS software	**RF value :** 0.53- 0.54 **Linearity :** y = 8197x + 613.3. **R^2 :** 0.9937 **RSD% :** 0.72% **LOD :** 0.0463ug/ml **LOQ :** 0.141 ug/ml	Bouklouze A. & et al. (2016) [22]
7.	New Spectrophotometric Method for Azithromycin Determination	Analytical Letters	**SPECTROPHOTOMETER**	VARIAN Cary 50 spectrophotometer **Software :** Cary WinUV software	**Linearity equation :** Y = 0.1276x + 0.01449. **R^2 =** 0.9996 **LOD :** 2.54 x 10^{-7}M **LOQ :** 8.91x 10^{-7}M **ANOVA DATA:** P =0.943 at 5% level and F = 0.01	Rachadi M. and et al. (2007) [23]
8.	RP-HPLC method development and validation for the simultaneous estimation of azithromycin and ambroxol hydrochloride in tablets	International Journal of PharmTech Research	**RP-HPLC**	**COLUMN:** The column used was C18 phenomenex Gemini, 5m, 250cm x 4.6mm, **MOBILE PHASE:** acetonitrile: monobasic potassium phosphate buffer pH: 8.5 at the ratio of 65:35v/v. pH **FLOW RATE**: 2ml/min **DETECTOR :** PDA detection at 220nm.	**Linearity range** = 80-125 mg/ml Correlation Co-efficient (r^2) = 0.9998 % RSD= 0.318 Accuracy= 99.58±0.895 Resolution factor (RS)= 11.314 No.of theoretical plates (N)= 101000 Tailing factor= 1.314	Venkatesh, V &et al (2011) [24]

					Slope = 9.52 Intercept = 20.42 Lod = 31.91 ug/ml LOQ = 96.7 ug/ml	
9.	Spectrophotometric estimation of Azithromycin in tablets	ijps	**Spectrophotometry**	UV/VIS Spectrophotometer with 10 mm matched quartz cells were used for absorbance measurement	**ƛmax** = 547 nm **beers limit** =2-2- ug/ml **molar absorptivity** = $2.1994x10^4$(l/mol/cm) **r^2**= 0.99343 **%RSD** = 0.612	Jayanna, et al (2012) [25]

III. CIPROFLOXACIN

Ciprofloxacin (CPX) is a fluorinated quinolone antibacterial which is chemically 1-cyclopropyl-6- fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid. Ciprofloxacin is a broad spectrum antibiotic active against both Gram-positive and gram-negative bacteria. It functions by inhibiting DNA Gyrase, a type-II Topoisomerase, and topoisomerase IV enzymes necessary to separate bacterial DNA, thereby inhibiting cell division

Structure:

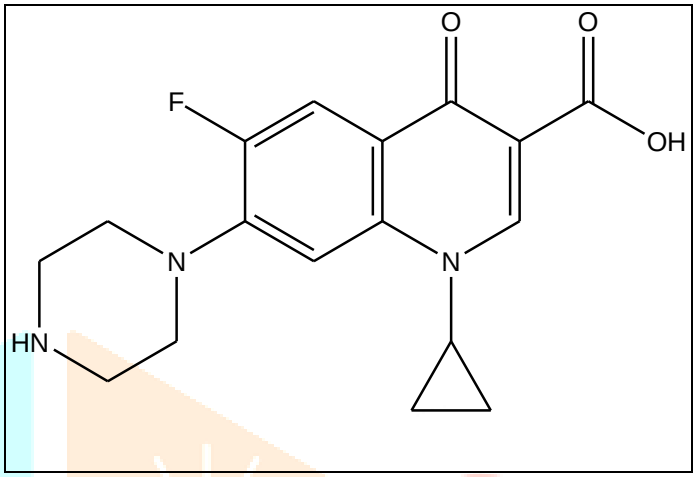


Fig. no.2.3 Structure of ciprofloxacin

Table no. 2.3 Analytical method for ciprofloxacin.

Sr no.	Title of paper	Journal name	Method	Instrumental parameter	Result of validation	Author and year of publication
1.	A First-Derivative Spectrophotometric Method for the Determination of Ciprofloxacin Hydrochloride in Ophthalmic Solution	Article *in* Physical Chemistry · December 2013	**UV**	A Shimadzu UV-1601 PC, UV-visible, double beam spectrophotometer with matched quartz cells with path-length of 10 mm (Shimadzu Corp., Kyoto, Japan) UVPC version 3.1 personal spectroscopy software	**Linearity range = 50.0-100ug/lml** **Slope±S.D. =** - 0.0002x-0.0009 **r2** =0.9999 **RSD %** = 2.92% **LOD=** 0.18mg/l **LOQ=** 0.60mg/l	Edith C. L. Cazedey and et al. (2013) [26]
2.	A simple HPLC-UV method for the determination of ciprofloxacin in human plasma	Journal of Chromatography	**HPLC-UV**	**COLUMN:** ACE® 5 C18 column (250 x 4.6 mm, 5 µm ; Agilent Pursuit 5 C18 Meta Guard® column (10 x 4.6 mm, 5 µm; Agilent Technologies, Amstelveen, Netherlands). **MOBILE PHASE :** A 0.02 M phosphate buffer and acetonitrile 77:23 (v/v) at pH 2.7 **FLOW RATE**: 1.5 ml/min. **DETECTOR :** UV detector was set at 277 nm.	**Retention time** = 3.26 min. **Linearity conc. Range** = 0.05-8 um/ml. **RF value :** 0.53- 0.54 **Linearity :** y = 32.6508x + 0.0337. **R^2 :** 0.999 **RSD% :** 0.72% **LOD :** 0.01 ug/ml **LOQ :** 0.05 ug/ml	Janis Vella and et al. (2015) [27]
3.	Analysis of Ciprofloxacin by a Simple High-Performance Liquid Chromatography Method	Journal of Chromatographic Science	**HPLC**	**Column :** Alltima C_{18} (4.6 × 150 mm; 5 µm) analytical column and a Nova-Pak C_{18} (4 µm) guard column (Waters) **Mobile phase :** mixture of 2% acetic acid aqueous solution and ACN (84:16, v/v) **Flow rate :** 1.0 mL/min. **Injection volume :** 10 uL. **Detector :** UV detector seet at 280nm.	**Retention time** = 6.5 min. **Linearity conc. Range** = 0.51-130 uM. **R^2 :** 0.999 **RSD% :** 3.39 % **LOD :** 0.25 uM **LOQ :** 0.009 uMß	Shihn-sheng Wu and et al. (2008) [28]
4.	Development and Validation of a Stability- Indicating HPLC Method for Determination of Ciprofloxacin Hydrochloride	Chromatographia Supplement Vol. 66, 2007	**HPLC**	**Column :** reversed phase Inertsil ODS3 C8 column (250 · 4.6 mm; 5 mL particle size). **Mobile phase :** The optimized mobile phase consisted of phos- phoric acid solution: acetonitril. **Flow rate :** 1.5 mL/min.	**Retention time** = 3.26 min. **Linearity conc. Range** = 250-750 um/ml. **Slope** = 40.999 **Intercept = 105.18** . **R^2 :** 0.999	B. Aksoy and s. rollas and et al. (2007).[29]

	and its Related Compounds in Film-Coated Tablets			**Injection volume :** 10 uL. **Detector :** Inertsil ODS3 column using UV detection.	**SD :** 497.04 +-1.69 mg **RSD% :** 0.34% **LOD :** 5.159 ug/ml **LOQ :** 15.632 ug/ml	
5.	Determination of ciprofloxacin in plasma and urine by HPLC with ultraviolet detection	Drug Monitoring and Toxicology	**HPLC**	**Column :** A stainless steel column packed with ymc pack A-312 (octadecylsilane; beaad size, 5um; 150mm x 6mm i.d., Yamamura chemical laboratory) was used. Column was protected with a pre-column (Guard-pak™) filled with uBondapak™ C_{18} cartridge. **Mobile phase :** consisted of a mixture of 900 mL of 50 mL/L acetic acid, 50 mL of acetonitrile, and 50 mL of methanol per liter **Flow rate :** 1 ml/min. **Detector :** The UV detector was set at 280 nm. **Sensitivity :** set at 0.02 absorbance unit full scale.	**Retention time :** 12 min. **Linearity conc.** Range : 0.01-2.5 mg/L (plasma) **0**.5 - 500 mg/L (urine). **R^2 :** 0.999 **LOQ :** 0.01 mg/L (plasma) 0.5 mg/L (urine)	Marika Kamberi and et al. (1998) [30]
6.	Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, and ciprofloxacin, in pharmaceuticals and blood serum by HPLC	© Springer-Verlag 2003	**HPLC**	**Column :** A kromasil 10 c18 250mm x 4mm, 5um particle, purchased by MZ Analysentchnik (Mainz, Germany). **Injector :** Rheodyne (Cotati California, USA 7125 ijection valve with a 20 uL loop. **Mobile phase :** The isocratic eluent system consisted of CH_3CN–CH_3OH–citric acid 0.4 mol L^{-1} (7:15:78, % v/v). **Flow rate :** 1.2 mL/min. **Injection volume :** 20 uL. **Detector :** SSI 500UV-Vis detector at wavelength of 275nm **Sensitivity :** 0.002 AUFS	**Retention time** = 8.566 min. **Linearity conc. Range** = 0.01-8 ng/uL. **Linearity :** Y=(0.03439±0.04023)+(0.54483±0.01728)X **R^2 :** 0.998 **LOD :** 0.01 ng/uL. **LOQ :** 0.03 ng/uL.	V. F. Samanidou and et al. (2003)[31]
7.	**High Performance Liquid Chromatography (HPLC) Method Development and**	Journal of Applied Pharmaceutical science	**HPLC**	**Column :** A column oven L- 2300, packed with silica C18, 5µm particle	**Retention time** = 1.750-1.753 min.	**Sani A. Ali** and et al. (2011).[32]

	Validation Indicating Assay for Ciprofloxacin Hydrochloride			size, an organizer and diode Arrary Detector L-2455 **Pump :** HITACHI L- 2130 pump **Injector :** autosampler L-2200 syringe loading sample injector valve fitted with a 20µl sample loop of 200vials **Mobile phase :** Methanol: Buffer (Orthophosphoric acid + Triethylamine) 40:60 **Flow rate :** 2.0 mL/min. **Injection volume :** 20µl. **Detector :** UV-VIS detector L-2420 at 278nm.	**Linearity conc. Range** = 10-50µg/ml **Linearity equation :** y=211063x – 6934.9 **R^2 :** 0.9993 (n=5) **SD :** 5108.665 **RSD% :** 0.12 %(n=3)	
8.	Pharmacokinetics of fluoroquinolones in critical care patients: A bio-analytical HPLC method for the simultaneous quantification of ofloxacin, ciprofloxacin and moxifloxacin in human plasma	Journal of Chromatography B	**HPLC**	**Column :** A Waters 2695 HPLC system water Xbridge™ C18 HPLC (3.5um particle size, 150mm x 2.1mm) (Milford, Massachusetts, USA) with an Alltima C18 guard column (5um particle sixe, 7.5 mm x 2.1 mm) (Grace, Columbia, MD, USA). **Column temp.** : 30°c **Flow rate :** 1 mL/min. **Injection volume :** 20µl. **Detector :** Water 470 Scanning Fluorescence detector. **Software :** Water Millenium [32] (ver.3.2)	**Retention time =** 6.567 min. **Linearity conc. Range** = 0.02-7.50 ug/ml. **SD :** 0.022 ug/mL. **RSD% :** 2.8 % **R^2 :** 0.998 **Accuracy :** 109.4 % **LOD :** 3.4 ng/ml **LOQ :** 0.02 ug/ml	Julie De Smet and et al. (2009). [33]
9.	Development and validation of UV spectroscopic methods for simultaneous estimation of ciprofloxacin and tinidazole in tablet formulation	International Current Pharmaceutical Journal	**UV spectroscopy**	Absorbance measurements were made on Shimadzu 1800 UV/Visible spectrophotometer with a pair of 10 mm matched quartz cells, Shimadzu digital balance for weighing and Cintex sonicatorwere used.	**Absorbtion wavelength :** 271 nm . **Absorptivity:** (1%, 1cm) A = 924bC + 108bC **Simultaneous equation method** **Molar Absorptivity** : 30614 l/mol./cm. **Shandell's sensitivity** : 0.01 um/cm^2/0.001 **Intercept (c)** : 0.0418 **Slope(m)** : 0.0895	Sowjanya Gummadi and et al. (2012)[34]

					Correlation coefficient (r^2) : 0.9992 **Q-Analysis** **Molar Absorptivity :** 6779 **Shandell's sensitivity :** 0.046 um/cm^2/0.001 **Intercept (c) :** 0.004 **Slope(m) :** 0.02 **Correlation coefficient (r^2) :** 0.999	

IV. ERYTHROMYCIN :

Erythromycin is an macrolide antibiotic that has an antimicrobial spectrum similar to slightly wider than that of penicillin. Erythromycin was discovered in 1952 by Mc Guire and coworkers from a strain of *streptomyces erythreus*. It is often used for people who have allergy to penicillin. Its is poorly soluble in water, bactericidal, particularly at higher concentrations. The trade name for Erythromycin was E-Mycin or Erythrocin. Erythromycin contain three characteristics part in the molecule, A highly substituted macrocyclic lactone – Aglycone , A ketone group , and An amino desoxysugar . chemically it is $C_{37}H_{67}N_1O_{13}$.

Structure:

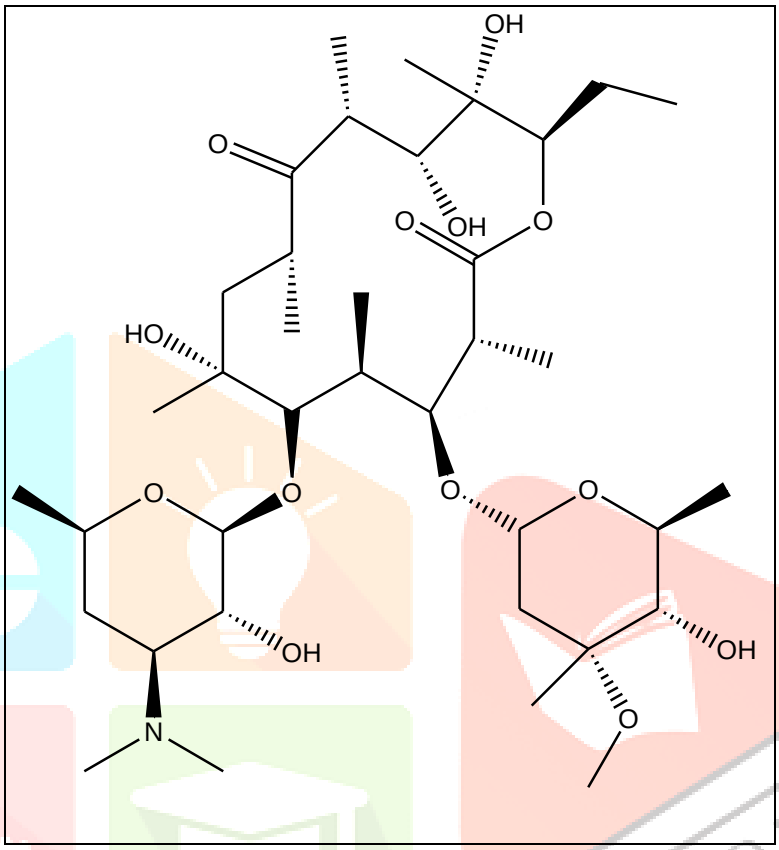


Fig. no. 2.4 structure of Erythromycin.

Table no. 2.4 Analytical method for Erythromycin

Sr no.	Title of paper	Journal name	Method	Instrumental parameter	Result of validation	Author and year of publication
1.	**Analysis of Erythromycin and Benzoyl peroxide in combined Dosage form by UV- visible spectrometry**	International Journal of Pharmacy and Pharmaceutical Sciences	**UV- visible spectrometry**	UV/Visible spectrophotometer with a pair of 10 mm matched quartz cells **SOLVENT** : Acetonitrile : water (1:1)	**Absorbtion wavelength :** 480 nm **Linearity range :** 5-25 ug/ml **LOD :** 1.4358 **LOQ :** 3.7341 **Regression equation :** y= 0.039X + 0.005 **R^2 :** 0.9991 **%RSD :** 0.94(intraday), 0.96 (interday) **% Accuracy : 101.01**	ROHINI WANKHADE and et al (2012) [35]
2.	Analysis of erythromycin and oleandomycin residues in food by high-performance liquid chromatography with fluorometric detection	Food Additives and Contaminants	**high-performance liquid chromatography with fluorometric**	**Column:** Inertsil 150-5 ODS-2(125 x4mm) analytical column with a Lichrospher 100-5 RP-18ec (8x4mm) precolumn. **Mobile Phase :**HPLC elunet A was 0.03mol/L Phosphate buffer pH 7.0/CAN 36:64 v/v and HPLC elunet B is CAN. **Flow rate :** 1.0ml/min. **Detector** : fluorometric detection at 260nm excitation and a 305nm emission wavelength **Precolumn derivatization** : 9-Fluromethylchloroformate (FMOC).	**Linearity range :** 5-50 ug/mL **Signal/noise ratio :** n= 3 **LOD :** 50 ug/kg **LOQ :** 100 ug/kg **R^2 :** 0.998	P. Edder and et al (2010) [36]
3.	High-Performance Liquid Chromatographic Determination of Erythromycin	Journal of Chromatography	**Isocratic RP-HPLC**	**Column :** Rbeo- dyne injector (Model 71-20, Berkeley, Calif., U.S.A.) with a lOO-~1 fixed loop. A reversed-phase column @Bondapak CL8. Waters Assoc., Milford, Mass., U.S.A.), 300 x 3.9 mm I.D., with a 50 x 2.1 mm I.D. stainless-steel pre-column packed with PBondapak C,,	**Linearity range :** 10-100ug/ml **% RSD :** 0.64% **Retention time :** 0.72 min. **R^2 :** 0.997	KlYOSHE TSUJI and et al. [37]

				Mobile phase: acetonitrile-methanol-O.2 M ammonium acetate-water (45:10:10:25). pH 7.0-7.8 **Flow rate :** 1 ml/min. **Detector :** a variable- wavelength detector (SpectroMonitor I) at 215 MI was used		
4 .	Development and validation of UV spectrophotometric method for estimation of erythromycin in bulk drug and pharmaceutical formulation	INTERNATIONAL JOURNAL OF RECENT ADVANCES IN PHARMACEUTICAL RESEARCH	**UV-spectrophotometric method**	A shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software.	**Amax: 285 nm** **Beer's-Lambert's range (µg/ml) :** (1-9) ug/m **Regression equation :** Y=0.0109x+0.3469 **Slope (m) : 0. 0.0109 0.3469** **Intercept (c) : 0.3469** **Correlation coefficient (r2) : 0.9873** **Recovery + S. D. (n = 3) : 99.59 + 0.13** **LOD (µg/ml) : 0.96** **LOQ (µg/ml) : 2.92** **Intermediate Precision(%RSD) Interday (n = 3): 0.7661 - 1.7811** **Intraday (n = 3): 0.1967 - 1.5121**	ANINDYA BAGCHI and et al. (2015) [38]

Conclusion :

Pneumonia is a common acute respiratory infection that affects the alveoli and distal airways; it is a major health problem and associated with high morbidity and short-term and long-term mortality in all age groups worldwide. In general, we have listed antibiotic drugs and their mechanism of action in the treatment of pneumonia. We have included epidemiology and drug used in the treatment of pneumonia one of the most important advantages of making this review article is that we have listed best hospitals and surgeons available for transportation of lungs in India. The above data is the collective data regarding analytical prospective for the drugs that can be used in Pneumonia. These drugs are majorly used in treatment of pneumonia. These analytical methods are validated as per ICH Q2 guidelines. We have enlisted HPLC method since 1998 to 2021 for the quantitative and qualitative analysis of Drugs used in Pneumonia, meanwhile we have also listed existing Analytical method named as UV, LC-MC-MC, Spectrophotometric, HPTLC for the same.

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