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OPTIMIZING GEMIFLOXACIN MESYLATE ANALYSIS: HPLC METHOD REVIEW

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Abstract: A review article on the development and validation of HPLC (High-Performance Liquid Chromatography) methods for Gemifloxacin mesylate serves various significant functions in the realm of pharmaceutical analysis. Because of its durability, accuracy, and precision, HPLC is the preferred method for measuring medicines. Examining numerous HPLC techniques in detail, the review concentrates on important factors such the composition of the mobile phase, flow rate, detection wavelength, and column selection. These parameters have a major effect on the reproducibility, sensitivity, and selectivity of the procedure. The paper also covers new developments and methods that have increased the effectiveness and dependability of Gemifloxacin mesylate analysis, such as the use of sophisticated detectors and improved sample preparation procedures. Validation of methods and the significance of strict quality control in pharmaceutical applications are emphasized. The scope of this review is comprehensive, covering different elements of technique development, optimization, validation, and application for Gemifloxacin mesylate, an antibiotic medication.

Index Terms - Gemifloxacin mesylate, method development, validation, HPLC.

I. INTRODUCTION

Analytical chemistry uses modern technology to determine the composition of substances. We have the ability to give both qualitative and quantitative results. Analytical devices serve an important role in producing precise and reliable analytical results. Therefore, all personnel in the analytical laboratory must prioritise the assurance of equipment quality (1). It involves practical application of chemical knowledge, particularly in the areas of spectroscopy, chromatography, electrochemistry and more. It also involves in enhancing the existing techniques, developing newer analytical methods to quantify the chemical and to therefore expand its application (2).

Developing precise and reliable analytical methodologies for drug formulation analysis is critical in pharmaceutical development and manufacture. The reasons involved are the following:

To ensure Drug efficacy and safety by quantitative analysis, Impurity detection and stability testing. Because accurate methods ensure the correct dosage of api's (Active Pharmaceutical Ingredients) in final formulations and intermediate products. Impurities which may be present also has to be detected and quantified which ensures the purity and safety of the drug product and Stability testing ensures that the drug remains effective throughout its shelf life (3,4,5).

Regulatory Compliance- Compliance with regulatory bodies (Like FDA, CDSCO, MHRA), its regulatory guidelines require validated and accurate analytical methods and Good Manufacturing Practice to guarantee consistent product quality (6,7).

Cost Efficiency and Time Saving- Reliable and accurate methods reduce the need for rework due to inaccurate results, saving time and resources and speed up formulation development, to get the drugs to market faster. (8,9)

Quality control- accurate methods aid in optimizing the manufacture processes for efficiency and therefore to ensure quality from batch to batch (10,11).

HPLC- High Performance Liquid Chromatography

High-pressure liquid chromatography (HPLC) separates mixture ingredients into individual components by passing them from a mobile phase (flowing liquid) to a stationary phase (sorbents packed inside a column). Separation occurs due to component affinity differences between the mobile and stationary phases. The fundamental components include a solvent delivery unit, sample injector, column, and detector.

High performance liquid chromatography (HPLC) is a separation technique that takes advantage of differences in the distribution of substances between two phases known as the stationary phase and mobile phase. The stationary phase refers to the thin layer formed on the surface of small particles, while the mobile phase refers to the liquid that flows over them. Under specific dynamic conditions, each component in a sample has a different distribution equilibrium based on solubility in the phase and/or molecule size. As a result, components move at different speeds over the stationary phase and become separated from one another. The column is a stainless steel or resin tube filled with spherical solid particles. A liquid pump delivers a steady amount of mobile phase into the column inlet. A sample is injected via the sample injector, which is placed near the column inlet. The injected sample enters the column with mobile phase, and the sample's components move across it, switching between stationary and mobile phases. Compounds move in the column only when they are in mobile phase. Compounds that are typically dispersed in the stationary phase move more slowly. Each component is separated on the column and then eluted successively from the outlet. Each component eluting from the column is detected by a detector at the column's exit. When the separation process is monitored by recorders beginning with the injection of the sample, a graph is obtained. This graph is referred to as a chromatogram. The duration of retention and relationship between compound concentration and peak area are determined by the compound's characteristics.

Retention is therefore used as an index for qualitative determination and peak surface area as index for quantitative determination. There are two modes of elution process

a) Isocratic elution

b) Gradient elution (12)

CHEMISTRY OF GEMIFLOXACIN MESYLATE

Fluoroquinolones are getting a lot of attention since their inception in later years of the 20th century. Due to the presence of fluorine and piperazine moieties in the structure, it provides activity against both Gram-positive and Gram-negative micro-organisms with less side effects. In April 2003, the US Food and Drug Administration (FDA) approved Gemifloxacin (GFX) as a fluorinated quinolone, fourth generation synthetic broad-spectrum antibacterial agent. It is present in two forms- one as free Gemifloxacin base and the other as Gemifloxacin mesylate salt.[13,14]

GFX has demonstrated strong effectiveness against the main pathogens that cause both respiratory and urinary tract infections. These pathogens include *Staphylococcus saprophyticus, Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila, Chlamydia species, Mycoplasma species, Escherichia coli,* and *Klebsiella.*[15-18]

The IUPAC name of Gemifloxacin is 7-[(4*Z*)-3-(Aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid.[1] Empirical formula: Gemifloxacin mesylate- $C_{18}H_{20}FN_5O_4$. CH_4O_3S

Structure-

The extensive review of the literature revealed that a wide range of analytical techniques have been developed and validated for the identification and quantification of pharmaceuticals and other compounds of interest in pharmaceutical matrices. Further, the amount of Gemifloxacin in pharmaceutical formulations and biological fluids was measured using sophisticated analytical techniques. Various analytical methods have been developed for the determination of the drug Gemifloxacin in tablet dosage form, human plasma and urine like spectrophotometry, HPLC, HPTLC, and LC-MS-MS.

MECHANISM OF ACTION

Gemifloxacin's methods of action are complicated and changing, similar to other fluoroquinolones. Quinolones kill bacteria by inhibiting key enzymes, including DNA gyrase and topoisomerase IV. DNA gyrase, a tetramer of two GyrA and two GyrB proteins encoded by the gyrA and gyrB genes, initiates DNA replication. (21,22)

Gemifloxacin has 2 mechanism of action- firstly, bind to DNA gyrase enzyme (Topoisomerase II), separates the strands of duplex bacterial DNA and inserting another strand of DNA through the break resealing the original strands therefore blocks bacterial DNA replication. Secondly, by inhibition of Topoisomerase IV required for replication, transcription, repair and recombination- leading to bactericidal action.[20] (protocol)

PHARMACOKINETICS AND PHARMACODYNAMICS-

Gemifloxacin is rapidly absorbed from the gastrointestinal tract, having an absolute bioavailability of around 71%. (95% confidence interval [CI] 60-84) 320 mg of gemifloxacin tablet.1 Time to attain Maximum gemifloxacin plasma concentration (Tmax) is roughly one hour in fasting healthy. participants, with the maximum plasma concentration (Cmax) decreasing in a biexponential manner. Gemifloxacin's C_{max} and $AUC_{0-\infty}$ increased linearly with dosages ranging from 20-800 mg and 160-640 mg. Multiple-dose experiments (once/day for 7 days) with gemifloxacin dosages ranging from 160-640 mg reached steady state by at least the fourth treatment.54 The terminal phase half-life (average 6-8 hours) was consistent across dosages and did not alter between single and subsequent administrations.29,30
Quinolone antibacterial drugs are most effective at eliminating germs at concentrations significantly higher than their MIC. The targeted microorganism exhibits a moderate-to-prolonged persistent killing impact.31,32. As a result, these medicines are classed as concentration-dependent killing antibacterials.

CHROMATOGRAPHIC PARAMETERS CONSIDERED-

A validated a method was developed for indication of stability by quantitative determination of the drug Gemifloxacin mesylate, its degradation products and Ambroxol hydrochloride by HPTLC assay. The aim of their study was to develop a stability indicating HPTLC assay using a fluorescence detection for the quantitation of the drug in dosage form. As the stationary phase, an HPTLC precoated silica gel plate 60F254 was used for the chromatographic separation and the mobile phase was a mixture of Ethyl acetate: methanol: 25% ammonia. Following excitation at 342 nm, the detection was carried out in fluorescence mode, and the emission intensity was evaluated using 8 optical filter K400. It was evaluated and validated as a GFX stability-indicating assay in the presence of its degradation products. The method stability indicating kinetic studies involved the drug GFX being subjected to different stress condition based on ICH guidelines like- Acidic and basic degradation, oxidative, neutral hydrolysis and photodegradation. The results showed that GFX was stable while kept at room temperature and in the refrigerator with light protection. Therefore, this method can be used for regular GFX quality control in the pharmaceutical and research industries. 33
Quantitative UFLC was carried out using a binary gradient UFLC system with two Shimadzu Prominence UFLC LC-20AD pumps, a manual sample injection loop, and an SPD M20A PDA detector. Shimadzu LC Solution Software was used for monitoring and integrating the output signal. The Enable C18G column (250 mm × 4.6 mm i.d., particle size 5 µm) was utilized for separation. The chromatographic analysis was performed at ambient temperatures on a column with methanol: 10mM TBAHS (70:30, v/v) as the mobile phase. The flow rate was 1.0 ml/min in isocratic mode. e. The PDA detection was set at 271nm. Water bath (Thermolab, India) and UV Chamber (Jain Scientific Glass Works, Ambala, India) were used for forced degradation study of the drugs. (35)
Shimadzu LC-20AB High Performance Liquid Chromatography System (HPLC) with Liquid Pumps (Shimadzu UFLC). DGU-20A5. The CBM 20A Communications Bus Module has a prominent degasser (Sil-20AC). Auto Sampler 20 MPa (maximum pressure) CTO-20AC column oven with Shimadzu RF-10AXL fluorescence detector. LC is solution software. The analytical column utilised was Hichrom packed Kromasil-100-C18 (250 mm×4.6 mm) with 5µm particle size. The mobile phase contains 10 mM sodium dihydrogen orthophosphate, which is adjusted to pH 3 using orthophosphoric acid and acetonitrile in an 85:15 ratio. The flow rate was 1.0 mL/min, the injection volume was 20 µL, and the column oven temperature was 25 °C. Fluorescence detections were performed at $\lambda_{ex}/\lambda_{em}$ 268/390 nm. (36)

Chromatographic analysis was conducted using an RP-HPLC system with a pump, manual sampler, and UV detector. The chromatographic column used was a Develosil Rp Aqueous AR-5 C18 column (250 mm × 4.6 mm i.d., 5 µm). The column temperature was kept at 25 °C. The mobile phase was made up of acetonitrile/acetate buffer (pH 4.5) at a ratio of 70:30% by volume. Separation was done using an isocratic mode at ambient temperature. The flow rate was 1.0 ml/min with an injection volume of 20 µl. The run time was 5.0 minutes. The UV detection wavelength was set to 244 nm. (34)

a simple and sensitive HPTLC method was developed for direct determination of Gemifloxacin mesylate with fluorescence detection in human plasma without any prior treatment. Montelukast was used as the internal standard as different 9 fluroquinolones (Levofloxacin, Gatifloxacin, Ciprofloxacin, Sparafloxacin and Norfloxacin) were testes but did not show any reliability. Ethyl acetate: methanol: 25% ammonia (8:4.5:3 v/v/v) was used as the mobile phase. The HPTLC plate was scanned and detected by fluorescence and the emission intensity was measured after excitation at 342 nm. This method was further validated in accordance with ICH guidelines based on the following parameters- linearity (correlation coefficient r=0.9965, n=6 at the concentration range of 3- 180ng/ml), LOD (0.45 ng/band), LOQ (1.5 ng/band), accuracy (recovery % was found to be 94.21–101.85%) and precision, selectivity and stability. Application of the suggested method on patient samples of human plasma provided evidence of its effectiveness. (37)

Mobile phase composition- Initially, other solvents such as methanol, hexane, chloroform, and ethyl acetate were investigated. The data indicate that GFX was created using methanol and ethyl acetate, but with an inadequate Rf value (less than 0.2) (37). Generally used solvent system for HPLC as mobile for the method development and validation of Gemifloxacin mesylate was based mainly on the its chemical properties like solubility. Optimized Solvents systems used were Acetonitrile: Ammonium acetate buffer (pH 4.5) (7:3)(33), Ethyl acetate: methanol: 25% ammonia (8:4.5:3, v/v/v)(37), Acetonitrile: Phosphate buffer pH 3 (15:85)(36) and methanol: 10mM TBAHS (70:30, v/v)(35).

METHOD VALIDATION PARAMETERS-

The International Council for Harmonization (ICH) of technical requirements for pharmaceuticals for human use provides guidelines for the validation of analytical procedures, including High-Performance Liquid Chromatography (HPLC) methods, which involves several critical steps to ensure the method's reliability, accuracy, and suitability for its intended use. The ICH guidelines specifically cover the validation of analytical procedures in the following categories:

i. Selectivity- Analyzing the target analyte in the presence of possible impurities, degradation products, and other matrix elements reveals the specificity of the approach. It guarantees that the method can accurately detect and measure the analyte in a complex sample.

ii. ii. Linearity- Determining the range of analyte concentrations for which the method may reliably yield a linear response. Typically, this is accomplished by evaluating standard analyte solutions at various concentrations and plotting a calibration curve. The calibration graph was created using a 20 µl injection loop. The study examined six GEMI concentrations (10 ng/mL, 30 ng/mL, 50 ng/mL, 70 ng/mL, 100 ng/mL, and 150 ng/mL). According to the experimental conditions. The calibration curve was created based on the response (peak area) and GEMI concentrations in standard solutions. Linearity was then measured.

iii. Accuracy- The closeness of the measured value to the true values are assessed by compassion of the results obtained from the HPLC method with respect to a reference standard. The method's accuracy was determined by testing GFX recovery at three concentration levels across the prescribed range (six replicates of each concentration). Good accuracy and recovery percentage ranged from 94.21-101.85%, indicating that the suggested approach for GFX extraction without pretreatment is highly efficient.

iv. Precision- The method's repeatability (intra-day precision) and intermediate precision (inter-day precision) is evaluated by performing multiple analysis of the sample with varying chromatographic conditions.

v. Limit of Detection (LOD) and Limit of Quantitation (LOQ): The lowest detectable and quantifiable levels of the analyte are established that can be reliably measured by the developed HPLC method.

vi. Robustness- The methods capacity is evaluated to remain unaffected by small, deliberate variations in method parameters and asses its reliability. (39)

Validation methods demonstrated that the devised analytical approach was linear, accurate, precise, and specific according to the International Conference of Harmonization (ICH) guidelines for validation of analytical procedures. The approaches remained reliable even when flow rate, detection wavelength, and organic phase composition were altered intentionally. The methods were successful in determining gemifloxacin dosage in tablet form. Gemifloxacin can be estimated without interference from routinely used excipients. This approach can be used for routine analysis of gemifloxacin mesylate in API, pharmaceutical formulations, dissolving medium, and biological fluids.

APPLICATIONS-

Gemifloxacin is a fluroquinolone antibiotic with a wide range of activity. It works well for treating both acute community-acquired pneumonia and aggravation of persistent bronchitis. When treating these kinds of infections, it doesn't seem to provide any advantages over the other fluoroquinolone antibiotics on the market, and it might be more likely to cause rash reactions. Gemifloxacin generally has a lower minimum inhibitory concentration (MIC) against a range of pathogens, according to in vitro experiments. It is yet unclear, however, if these lower MICs against *S. pneumoniae* and the other species translate into a therapeutic advantage.

IV. CONCLUSION

4.1 Results of Descriptive Statics of Study Variables

The overview of maximising the analysis of Gemifloxacin Mesylate using High-Performance Liquid Chromatography (HPLC) concludes by highlighting the significant developments and techniques in the sector. Because of its reliability, accuracy, and precision in measuring Gemifloxacin Mesylate in a variety of matrices, HPLC is still a top analytical method. The article presents a number of optimised techniques while highlighting the significance of variables including the composition of the mobile phase, flow velocity, detection wavelength, and column selection. The results' repeatability, sensitivity, and selectivity are all greatly impacted by these variables. The review also covers the application of cutting-edge methods and technological developments, such as the use of sophisticated detectors and improved sample preparation procedures, which have significantly increased the accuracy and dependability of HPLC analysis. For pharmaceutical applications to ensure quality control and therapeutic efficacy, these techniques must be continuously developed and improved. The investigation of green chemistry strategies to lessen environmental impact, the integration of HPLC with other analytical techniques for thorough profiling, and a sustained emphasis on method validation are recommended as future research areas. In general, the enhanced HPLC techniques for Gemifloxacin Mesylate offer a sturdy structure for its evaluation, guaranteeing elevated standards in pharmaceutical quality assurance and enhancing therapeutic outcomes.

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Figures and Tables

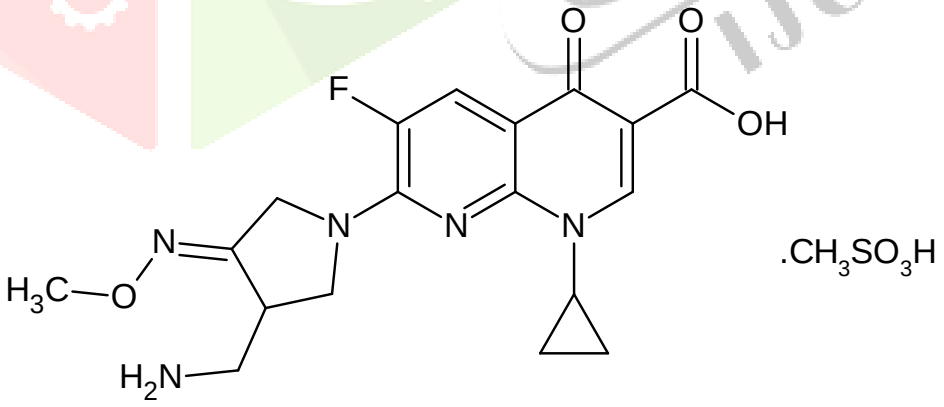

Fig.1 Structure of Gemifloxacin mesylate

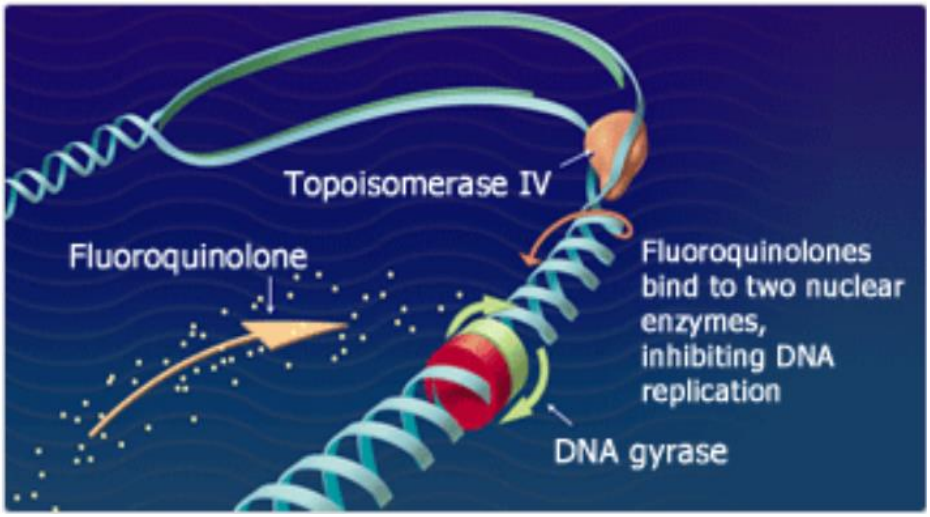

Fig.2 Mechanism of action of Fluroquinolones

Quinolone resistance is often caused by point mutations in specific areas, rather than active efflux pumps (23-25). The gyrA, gyrB, parC, and parE genes contain quinolone resistance-determining domains. Research on quinolone activity in Escherichia coli found that resistance-inducing mutations typically emerge in gyrA (or gyrB) initially, followed by parC (or parE) later. (26-28)

Table 1 : Chromatographic methods.

Sl.no	Instrument	Mobile phase	Column	Flow rate	Wavelength	Validation parameters
1	Shimadzu Prominence UFLC LC-20AD **(35)**	methanol: 10mM TBAHS (70:30, v/v)	C18G column (250 mm × 4.6 mm i.d., particle size 5 µm)	1.0 ml/min	271nm	Calibration curve linear (concentration range 1.0-200µg/ml) limit of detection and limit of quantification values were 0.33µg/ml and 0.98µg/ml, respectively.
2	Shimadzu LC-20AB **(36)**	orthophosphoric acid: acetonitrile (85:15 ratio)	Hichrom packed Kromasil-100-C18 (250 mm×4.6 mm) with 5µm	1.0 ml/min	268/390 nm.	Linear range- 10-150 limit of detection and limit of quantification values were 2.56ng/ml and

			particle size			7.77ng/ml respectively.
3	HPLC (Waters) **(34)**	Acetonitrile: ammonium acetate buffer (pH 4.5) 70:30%	Develosil Rp Aqueous AR-5 C18 column (250 mm × 4.6 mm i.d., 5 µm)	1.0 ml/min	244 nm	Accuracy- % recovery 99.77% Precision- less than 2% limit of detection and limit of quantification values were 12.678µg/ml and 14.261 µg/ml respectively.
4	Shimadzu (Kyoto Japan) LC-20A system **(38)**	70:30 (v/v) 0.05 M ammonium acetate buffer (pH 2.7, adjusted with 5% phosphoric acid)–acetonitrile	250 mm × 4.6 mm, 5-µm particle, C18 column (Merck)	0.7 mL min^{-1}	272nm	Linear range- 0.256- 128 µg/ml limit of detection and limit of quantification values were 3.3 δ/S and 10 δ/S respectively.
5	HPLC Cyber Lab LC-100 **(39)**	Buffer (KH2PO4 adjusted with pH 6.8): Acetonitrile in the ratio of 80:20 (%v/v)	Capcell Pak ODS C18 (250 × 4.6 mm with 5 µm particles) column	1.2 mL/min	265 nm	Linear range- 25-150 µg/ml Accuracy- 99.95% System Precision (n=6)- 0.82%RSD Method Precision (n=6)- 0.78%RSD

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