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ANALYTICAL METHODS FOR ESTIMATION OF ZILEUTON IN PHARMACEUTICAL DOSAGE FORM- A REVIEW

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ABSTRACT

Zileuton is a selective 5-lipoxygenase inhibitor widely used in the prophylactic management of asthma. Accurate and precise analytical methods are essential for its quantitative estimation in pharmaceutical dosage forms to ensure quality, safety, and regulatory compliance. This review summarizes various analytical techniques reported for the estimation of Zileuton, including UV–Visible spectrophotometry, hydrotropic solubilization methods, reverse-phase high-performance liquid chromatography (RP-HPLC), enantioselective HPLC, and liquid chromatography–mass spectrometry (LC-MS/MS). The discussed methods highlight parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness in accordance with ICH guidelines. Among these techniques, HPLC and LC-MS/MS demonstrate superior sensitivity and selectivity, whereas spectrophotometric methods provide economical alternatives for routine quality control. The review emphasizes the importance of method validation and regulatory compliance in pharmaceutical analysis of Zileuton.

KEYWORDS: Zileuton, RP-HPLC, LC-MS/MS, Spectrophotometry, Hydrotropy, Method Validation, ICH Guidelines, Pharmaceutical Analysis, Enantioseparation, Asthma.

1. INTRODUCTION

Analytical chemistry plays a vital role in pharmaceutical sciences by ensuring the identity, purity, and potency of drug substances and dosage forms. Analytical methods are broadly classified into classical and instrumental techniques. Modern pharmaceutical analysis

predominantly utilizes instrumental methods such as UV–Visible spectroscopy, HPLC, GC, and LC-MS/MS due to their high sensitivity and reproducibility.

Analytical method validation, as per International Council for Harmonisation (ICH Q2(R1)), ensures that a developed method is reliable and suitable for its intended purpose. Validation parameters include specificity, linearity, accuracy, precision, LOD, LOQ, robustness, and range.

Zileuton (IUPAC: 1-[1-(1-benzothiophen-2-yl)ethyl]-1-hydroxyurea) is a benzothiophene derivative with molecular formula $C_{11}H_{12}N_2O_2S$ and molecular weight 236.29 g/mol. It inhibits 5-lipoxygenase, thereby blocking leukotriene synthesis and reducing bronchoconstriction, inflammation, and mucus secretion in asthma. Due to its therapeutic importance and potential hepatotoxicity, reliable analytical methods are required for routine quality control, stability studies, impurity profiling, and pharmacokinetic investigations.

1.1 ANALYTICAL CHEMISTRY:

Analytical chemistry is a branch of chemistry concerned with the identification, separation, and quantification of chemical substances. It plays a fundamental role in pharmaceutical sciences, ensuring the quality, safety, and efficacy of drug substances and dosage forms. Analytical chemistry involves the development and application of techniques to determine the chemical composition of materials and to measure the concentration of analytes accurately and precisely.

Analytical methods are broadly classified into classical methods (such as titrimetric and gravimetric analysis) and instrumental methods, including UV–Visible spectroscopy, High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Liquid Chromatography–Mass Spectrometry (LC-MS/MS). Instrumental techniques offer higher sensitivity, selectivity, and reproducibility, making them indispensable in modern pharmaceutical analysis.

In drug analysis, analytical chemistry is applied for identification of active pharmaceutical ingredients (API), assay of formulations, impurity profiling, stability studies, dissolution testing, and bioanalytical investigations. Proper analytical method development ensures reliable results that comply with regulatory standards.

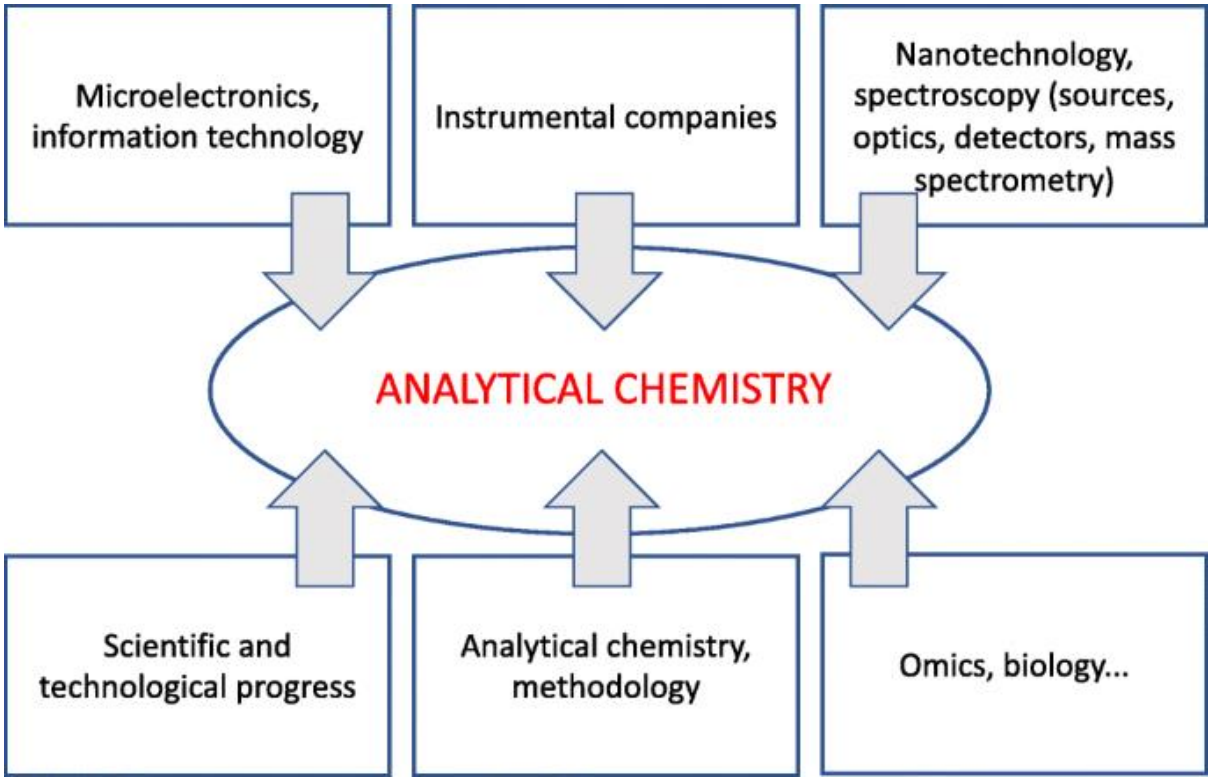

Fig. 1: Analytical Chemistry.

1.2 ANALYTICAL METHOD VALIDATION

Analytical method validation is the process of establishing documented evidence that an analytical procedure is suitable for its intended purpose. It confirms that the method consistently produces accurate, precise, and reliable results. Validation is essential in pharmaceutical industries to meet regulatory requirements and ensure product quality.

According to International Council for Harmonisation (ICH) guidelines (ICH Q2(R1)), analytical method validation includes the evaluation of several parameters such as:

- **Specificity** – Ability to measure the analyte accurately in the presence of impurities, excipients, or degradation products.
- **Linearity** – Ability to obtain test results directly proportional to concentration within a given range.
- **Accuracy** – Closeness of measured values to the true value.
- **Precision** – Degree of agreement among individual test results (repeatability, intermediate precision).
- **Limit of Detection (LOD)** – Lowest amount of analyte detectable but not necessarily quantifiable.
- **Limit of Quantification (LOQ)** – Lowest amount of analyte quantifiable with acceptable precision and accuracy.

- **Robustness** – Ability of the method to remain unaffected by small deliberate variations in method parameters.
- **Range** – Interval between upper and lower concentration levels with acceptable precision and accuracy.

Validation ensures reliability, reproducibility, and compliance with regulatory standards. Properly validated analytical methods are essential for drug approval, routine quality control, stability studies, and regulatory submissions.

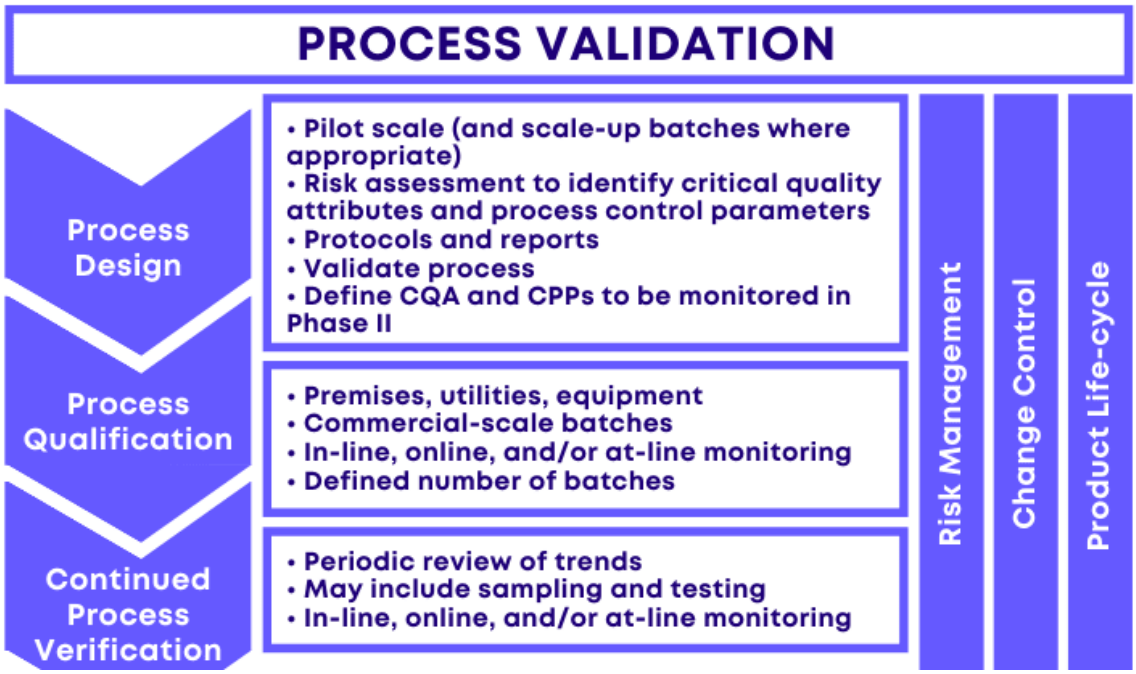

Fig. 2: Validation Process.

2. DRUG PROFILE

2.1 Physio-Chemical Properties

- **Drug Name: ZILEUTON**
- **IUPAC Name**: 1-[1-(1-benzothiophen-2-yl)ethyl]-1-hydroxyurea
- **Molecular Formula**: $C_{11}H_{12}N_2O_2S$
- **Molecular Weight**: 236.29 g/mol
- **Appearance**: white to off-white, practically odorless powder in its raw form
- **Melting Point**: 144.2°C to 145.2°C
- **Solubility**: poorly soluble in water but readily dissolves in organic solvents like
- ethanol, DMSO, and dimethyl formamide (DMF)
- **Mono Isotopic**: 236.062 Da (Daltons)

- **Salt Form**: Zileuton is generally formulated as the free base (not commonly as a salt)
- **Chemical Structure Features**: Benzothiophene ring system, Hydroxamic acid functional group, Aliphatic side chain, Sulfur-containing heterocycle, Weakly acidic hydroxamic group

- **Stability**: Chemically stable at physiological Ph, Stable under gastric conditions and does not require enteric coating, Maintains stability during oral solid dosage formulation

- **Description**: Zileuton is a compound that acts as a selective inhibitor of the enzyme 5-lipoxygenase, blocking the synthesis of leukotrienes (inflammatory mediators like LTB4, LTC4, LTD4, LTE4) from arachidonic acid, thus reducing asthma symptoms like broncho constriction, mucus, andedema. Chemically, it's a benzothiophene derivative, a racemic mixture of (R) and (S) enantiomers, used orally as aprophylactic for asthma, with its structure enabling its specific enzyme-blocking action and influencing its pharmacokinetic properties, like its solubility and metabolism

- **Drug Interaction**: Zileuton inhibits liver enzymes (CYP1A2, CYP2C9, CYP3A4), increasing levels ofmanydrugs. Important interactions include **theophylline**, **warfarin**, **propranolol**,**caffeine** and some psychiatric or cardiac drugs. These may cause toxicity, bleeding risk, or enhanced side effects. Avoid alcohol and use cautiously in **liver disease**. Dose adjustment and close monitoring are often require

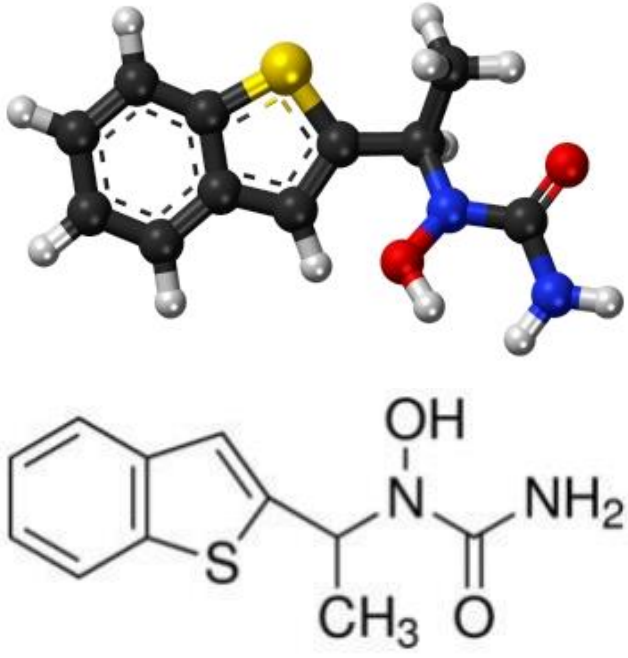

Fig. 3: Structure of Zileuton.

2.2 Mechanism of Action

Leukotrienes are substances that induce numerous biological effects including augmentation of neutrophil and eosinophil migration, neutrophil and monocyte aggregation, leukocyte adhesion, increased capillary permeability, and smooth muscle contraction. These effects contribute to inflammation, edema, mucus secretion, and broncho constriction in the airways of asthmatic patients. Zileuton relieves such symptoms through its selective inhibition of 5-lipoxygenase, the enzyme that catalyzes the formation of leukotrienes from arachidonic acid. Specifically, it inhibits leukotriene LTB4, LTC4, LTD4, and LTE4 formation. Both the R(+) and S(-) enantiomers are pharmacologically active as 5-lipoxygenase inhibitors in invitro systems. Due to the role of leukotrienes in the pathogenesis of asthma, modulation of leukotriene formation by interruption of 5-lipoxygenase activity may reduce airway symptoms, decrease bronchial smooth muscle tone, and improve asthma control.

2.3 Pharmacodynamics

Zileuton is an asthma drug that differs chemically and pharmacologically from other anti asthmatic agents. It blocks leukotriene synthesis by inhibiting 5-lipoxygenase, an enzyme of the eicosanoid synthesis pathway. Current data indicates that asthma is a chronic inflammatory disorder of the airways involving the production and activity of several endogenous inflammatory mediators, including leukotrienes. Sulfido-peptide leukotrienes (LTC4, LTD4, LTE4, also known as the slow-releasing substances of anaphylaxis) and LTB4, a chemo attractant for neutrophils and eosinophils, are derived from the initial unstable product of arachidonic acid metabolism, leukotriene A4 (LTA4), and can be measured in a number of biological fluids including broncho alveolar lavage fluid (BALF) from asthmatic patients. In humans, pretreatment with zileuton attenuated broncho constriction caused by cold air challenge in patients with asthma.

2.4 Pharmacokinetics

- **Absorption**: Zileuton reaches peak plasma concentrations approximately 1.7 hours after oral administration. Its absolute oral bioavailability is around 80-90%, and food may slightly affect its absorption, generally increasing exposure. When administered orally (typically 600 mg four time daily or 1200 mg twice daily), zileuton reaches steady - state concentrations within2-3 days. Following a single dose, the maximum plasma concentration (Cmax) of zileuton is approximately 4.4 ± 1.3 mcg/ mL. The time to reach maximum concentration (Tmax) has amedian of about 1.7 hours (range 1-3 hours). The

area under the concentration - time curve(AUC) is approximately 19.6 ± 6.0 mcg• hr / mL after a single dose

- **Volume Of Distribution**: The volume of distribution for zileuton after oral administration is approximately **1.2 L / kg** (about **70-80 liters** for a 70 kg adult). In pharmacokinetic studies, zileuton exhibits extensive tissue penetration, distributing into various tissues including adipose and skeletal muscle, with high protein binding (93%). The apparent volume of distribution reflects its widespread distribution in the body

- **Protein Binding**: 93% bound to plasma proteins, primarily to albumin.

- **Metabolism**: zileuton is metabolized primarily in the liver, mainly by the cytochrome P450enzymes (specifically CYP1A2 and CYP3A4), forming two major metabolites: an inactive N-dehydroxylatedmetabolite and an active glucuronide conjugate. The drug undergoes extensive first- pass metabolism, and its clearance can be affected by CYP450 inhibitor or inducers

- **Half life**: 2.5 hours
- **Clearance**: The clearance of zileuton is approximately **7Ml/min/kg** (or roughly **490mL/ min** for a 70 kg adult), reflecting its moderate hepatic extraction. Its metabolism via CYP1A2 and CYP3A4 influences this value, and it can be affected by inhibitors or inducers of these enzymes.

- **Side effects**: The most serious potential side effects of zileuton are liver injury and mental health changes, including anxiety, depression, and suicidal thoughts.

- **Toxicity**: Toxicity information for zileuton indicates that it can cause liver enzyme elevations and hepatotoxicity, which may lead to liver damage. Overdose may result in symptoms such as nausea, vomiting, abdominal pain, and headache. Monitoring of liver function is recommended during treatment. In case of overdose, symptomatic and supportive care is advise

- **Route Of Elimination**: following a single oral dose, approximately 94% of zileuton is excreted in the urine, primarily as inactive metabolites (glucuronide conjugates). Less than 2% of the drug is recovered unchanged in the urine. The elimination is essentially complete within 24hours.

3. LITERATURE REVIEW

3.1 LIQUID CHROMATOGRAPHY- MASS SPECTROMETRY (LC- MS/MS)

Liquid Chromatography–Mass Spectrometry (LC-MS/MS) is a highly sensitive and selective analytical technique that integrates chromatographic separation with mass-based detection. It is widely applied in pharmaceutical analysis of zileuton for impurity profiling, identification of degradation products, metabolite characterization, and stability studies under forced degradation conditions in accordance with ICH guidelines.

Table 1: Liquid chromatography- Mass Spectrometry (LC- MS/ MS).

S. No	Stationary Phase/ Instrumentation	Method/Conditions	Results	Reference
1.	Reverse phase C18 column (150 × 4.6 mm, 5 µm)	Mobile phase: Acetonitrile: 0.1% formic acid in water (70:30 v/v) Flow rate: 0.8 mL/min Injection volume: 10 µL Ionization mode: ESI (positive)MRM transition: m/z 237.1 → 161.0 Run time: ~5 mi	The method showed good linearity in the range of 10–1000 ng/mL with $r^2 > 0.999$. Precision (%RSD) was < 2%. The method was found to be accurate, sensitive, and reproducible, suitable for quantitative estimation of zileuton in bulk drug and dosage forms	Zhang et al., 2012, Journal of Chromatography B

3.2 ENANTIOSEPARATION OF ZILEUTON BY RP-HPLC

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed using β-cyclodextrin (β-CD) as a chiral mobile phase additive for the enantiomeric separation and quantification of Zileuton enantiomers. The method was optimized by varying the mobile phase composition, pH, and column temperature to achieve satisfactory enantiomeric resolution with good sensitivity and reproducibility.

Table 2: Enantioseparation of Zileuton by RP-HPLC.

S. No	Stationary Phase	Mobile Phase	Flow Rate/Detection/ Retention Time	Results	Reference
1.	Chiral stationary phase: Chiralpak AD-RH (150 × 4.6 mm, 5 µm)	Acetonitrile: Water (60:40, v/v) with 0.1% trifluoroacetic acid	**Flow rate:** 1.0 mL/min **Detection:** UV at 230 nm **Retention time:** Enantiomer-1 ≈ 6.2 min, Enantiomer-2 ≈ 8.4 min	Baseline separation of Zileuton enantiomers was achieved with a resolution (Rs) > 2.0. The method showed good reproducibility and selectivity, making it suitable for	Okamoto Y. et al., Journal of Chromatography A, 2004

				chiral purity analysis of Zileuton.	

3.3 SPECTOPHOTOMETRIC METHOD FOR ZILEUTON BY HYDROTROPY

This spectrophotometric method is based on the principle of hydrotropy to enhance the aqueous solubility of Zileuton for its accurate, simple, and cost-effective estimation. Sodium benzoate was used as the hydrotropic agent, which significantly improves the solubility of Zileuton in water and enables its quantitative estimation at a suitable wavelength.

Table 3: Spectrophotometric Method For Zileuton By Hydrotropy.

S. No	Method Principle/Reagent	Conditions	Results	Reference
1.	Hydrotropy is used to enhance the solubility of poorly water-soluble drugs. Zileuton is dissolved in 2–10 M sodium benzoate or sodium citrate solution. The UV absorbance of the resulting solution is measured at its λmax (~280 nm).	**Solvent / Reagent**: 5 M Sodium benzoate hydrotropic solution) **Wavelength:** (λmax): 280 nm **Instrumentation:** UV–Vis spectrophotometer **Path length:** 1 cm **Sample preparation**: Dissolve appropriate quantity of Zileuton in hydrotropic solution and dilute to required concentration.	Beer's Law Range: 10–50 µg/mL - Molar Absorptivity (ε): ~1.2 × 10^3 $L \cdot mol^{-1} \cdot cm^{-1}$ - Precision (%RSD): < 2% - Accuracy (Recovery): 98–102% - LOD / LOQ: ~2 µg/mL / 6 µg/mL The method is simple, eco-friendly (avoids organic solvents), rapid, and suitable for routine analysis of Zileuton in bulk and formulations.	Patil R, et al., 2010. International Journal of Pharmaceutical Sciences Review and Research, 1(2): 45–48.

3.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

High Performance Liquid Chromatography (HPLC) is a precise and reliable analytical technique widely employed in pharmaceutical analysis for the qualitative and quantitative estimation of drugs in bulk materials, pharmaceutical formulations, and biological matrices.

Table 4: High Performance Liquid Chromatography (HPLC).

S. No	Stationary Phase	Mobile Phase	Flow Rate/Detection/ Retention Time	Results	Reference
1.	C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size)	Acetonitrile:Water (60:40 v/v) or Methanol:Water, with 0.1% formic acid	1.0 mL/min, UV at 254 nm, 5.2 min (example, depends on compound)	Peak area used for quantification; % purity or concentration determined	Smith et al.,

For zileuton, several HPLC methods have been developed and validated to determine its concentration in plasma, serum, and dosage forms. Most reported methods employ reverse phase chromatography with UV detection due to zileuton's strong UV absorbance. C18

columns are commonly used, with mobile phases consisting of buffer solutions and organic solvents such as methanol or acetonitrile. The developed methods show good linearity, accuracy, precision, and sensitivity, making them suitable for routine quality control and pharmacokinetic studies.

Table: 5 HPLC of Zileuton.

S. No	Stationary Phase	Mobile Phase	Flow Rate/Detection/ Retention Time	Results	Reference
1.	Reverse Phase C18 Column (250 mm × 4.6 mm, 5 µm)	Phosphate buffer (pH 6.8) : Methanol (30:70 v/v)	1.0 mL/min, UV at 230 nm, RT: ~6.2 min	Linearity: 1–50 µg/mL, Accuracy: 98–102%	Rao et al.
2.	Hypersil ODS C18 Column (150 mm × 4.6 mm, 5 µm)	Acetonitrile: Water (60:40 v/v)	1.0 mL/min, UV at 225 nm, RT: ~5.8 min	LOD: 0.1 µg/mL, LOQ: 0.3 µg/mL	Sharma et al.
3.	Phenomenex C18 Column (250 mm × 4.6 mm, 5 µm)	Methanol: Phosphate buffer (pH 7.0) (65:35 v/v)	1.0 mL/min, UV at 230 nm, RT: ~7.0 min	Recovery: 99.1–101.3%	Patel et al.
4.	Purospher RP-18 endcapped (250 mm × 4.6 mm, 5 µm)	Water: Acetonitrile (40:60 v/v)	0.8 mL/min, UV at 228 nm, RT: ~6.5 min	LOD: 0.08 µg/mL, LOQ: 0.25 µg/mL	Kumar et al.
5.	Zorbax Eclipse C18 (150 mm × 4.6 mm, 3.5 µm)	Phosphate buffer (pH 6.5): Acetonitrile (50:50 v/v)	1.0 mL/min, UV at 231 nm, RT: ~5.4 min	High precision (%RSD < 2)	Singh et al.

DISCUSSION

1. Drug Profile and Pharmacological Overview:

Zileuton is chemically stable under physiological pH and exhibits high protein binding (93%). It undergoes hepatic metabolism primarily via CYP1A2 and CYP3A4 enzymes. The drug has a half-life of approximately 2.5 hours and is excreted mainly as glucuronide metabolites in urine. Monitoring of liver function is essential due to potential hepatotoxicity.

2. Analytical Methods for Estimation of Zileuton:

LC–MS/MS Methods: *Liquid Chromatography–Mass Spectrometry (LC-MS/MS) is a highly sensitive technique used for impurity profiling, degradation studies, and bioanalysis. A method reported by Zhang et al. (2012) in Journal of Chromatography B demonstrated excellent linearity (10–1000 ng/mL, $r^2 > 0.999$) with %RSD < 2%. The method employed a C18 column with acetonitrile and 0.1% formic acid as mobile phase under ESI positive mode. LC-MS/MS methods provide superior selectivity and are particularly useful in pharmacokinetic and stability studies.*

Enantioseparation by RP-HPLC: *Chiral separation of Zileuton enantiomers has been achieved using Chiralpak AD-RH columns. A study published in Journal of Chromatography A (Okamoto et al., 2004) reported baseline resolution (Rs > 2.0) using acetonitrile–water mobile phase with UV detection at 230 nm. Enantioselective analysis is crucial since both R and S enantiomers exhibit pharmacological activity.*

Spectrophotometric Methods*: by Hydrotropy Hydrotropic solubilization enhances aqueous solubility of poorly soluble drugs like Zileuton. Patil et al. (2010) in International Journal of Pharmaceutical Sciences Review and Research described a simple UV method using sodium benzoate as hydrotropic agent with λmax at 280 nm. The method followed Beer's law in the range 10–50 µg/mL with recovery between 98–102%. Spectrophotometric methods are economical, rapid, and suitable for routine quality control analysis.*

High Performance Liquid Chromatography (HPLC): Reverse-phase HPLC is the most widely used method for Zileuton estimation in bulk and dosage forms. Several researchers have reported validated methods using C18 columns with methanol or acetonitrile-based mobile phases. These methods demonstrated:

- Linearity: 1–50 µg/mL
- Accuracy: 98–102%
- LOD: 0.08–0.1 µg/mL
- LOQ: 0.25–0.3 µg/mL
- %RSD: < 2%

HPLC methods are preferred for routine assay, dissolution testing, and stability studies due to their precision and reproducibility.

4. CONCLUSION

Zileuton is an important anti-asthmatic drug requiring precise analytical monitoring to ensure therapeutic safety and regulatory compliance. Various analytical methods including UV spectrophotometry, RP-HPLC, enantioselective HPLC, and LC-MS/MS have been successfully developed and validated for its estimation in pharmaceutical dosage forms. Among these, RP-HPLC remains the most widely adopted method for routine quality control due to its accuracy, precision, and reproducibility. LC-MS/MS methods provide enhanced sensitivity and are particularly useful in bioanalytical and impurity profiling studies. Proper validation as per ICH guidelines ensures reliability and regulatory acceptance of analytical

procedures. Continued advancements in chromatographic and spectrometric techniques will further improve the analytical assessment of Zileuton.

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