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# A REVIEW ON ANALYTICAL METHODS USED FOR DETERMINATION OF ACEBROPHYLLINE IN BULK AND DIFFERENT DOSAGE FORM

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**ABSTRACT**
Acebrophylline is a Anti-inflammatory – mucosecretory and bronchodilator medicine used in the treatment of chronic or acute bronchitis, chronic obstructive pulmonary disease (COPD) and asthma. It reduces the frequency of episodes of bronchial obstruction and reduces the need for beta2-agonists, and improves indexes of ventilatory function. Different analytical method developed to get determine chemical and physical properties of chemical substance, element or mixture. In mid-1990s acebrophylline was introduced to the Indian market with successful trials and regulatory approval and the first analytical method were published in scientific literature. This survey shows which method and which solvent are more efficient to determine the acebrophylline.

**KEYWORD:** Acebrophylline, Anti-inflammatory, Mucosecretory, Bronchodilator, Analytical Method, COPD, Asthma.

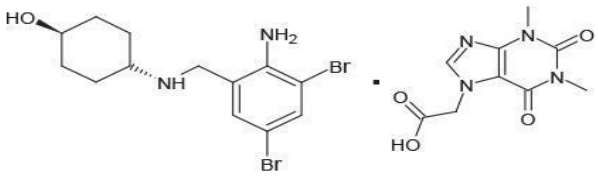
## INTRODUCTION[1-2]

Acebrophylline is an anti-inflammatory and airway mucus regulator. It contains ambroxol and theophylline-7-acetic acid, the former facilitates the biosynthesis of pulmonary surfactant while later raises blood levels of ambroxol, by stimulating surfactant production. Chemically acebrophylline is (1, 3- dimethyl-2, 6- dioxo-1, 2, 3, 6- tetrahydro-7H-purine-7yl) acetic acid-4[((2-amino-3, 5-dibromophenyl) methyl) amino] cyclohexanol. It is a salt obtained by reaction of equimolar amounts of theophylline-7-acetic acid and ambroxol.

Theophylline-7-acetate has a bronchodilator effect due to inhibition of the intracellular phosphodiesterases, followed by an increase of adenosine monophosphate cyclic levels, which promote the relaxation of bronchial muscles. Ambroxol modifies the mucous gel phase of secretions by decreasing the viscosity and increasing the serous gel phase. It increases the mucociliary clearance by stimulating cilia motility. Acebrophylline inhibits phospholipase A, and phosphatidylcholine leading to lesser production of the powerful pro-inflammatory substances like leukotrienes and tumour necrosis factor. By inhibiting the synthesis and release of these inflammatory mediators, acebrophylline reduces inflammation, a key factor in airway obstruction, especially in chronic forms.

**Drug Profile[1][3-10]**

| SR. NO. | NAME | ACEBROPHYLLINE |
|---|---|---|
| 1. | IUPAC | 4-[(2-amino-3,5 dibromophenyl)methylamino]cyclohexan-1-ol;2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetic acid. |
| 2. | Class | Anti-inflammatory |
| 3. | Category | Mucolytic and Bronchodilators |
| 4. | Generic Name | Ambroxol acefyllinate |

| | | |
|---|---|---|
| 5. | CAS No. | 96989-76-3 |
| 6. | Synonyms | Acebrophylline<br>Ambromucil<br>Ambroxol theophyllinacetate<br>Broncomnes<br>Surfolase |
| 7. | Molecular Formula | C22H28Br2N6O5 |
| 8. | Structure |  <br>Acebrophylline |
| 9. | Molecular Weight | 616.302 g/mol |
| 10. | Appearance | White crystalline powder |
| 11. | Physical State | Powder or Tablet Form |
| 12. | Solubility | Freely soluble in analytical grade Ethanol.<br>Slightly soluble in methanol and water. |
| 13. | pKa | 15.26 (Acidic)<br>9.01(Basic) |
| 14. | Melting Point | 213-214˚ |
| 15. | Mechanism of Action | ●**Bronchodilation:** Acebrophylline acts as an anticholinergic agent by binding to M3 receptors, which are Gi-type G-protein coupled receptors in bronchial smooth muscle. This binding inhibits the formation of cyclic AMP (cAMP) and blocks the release of calcium ions (Ca2+), leading to muscle relaxation and airway dilation, which ultimately helps in mucus expulsion.<br>●**Mucoregulation:** The ambroxol component of acebrophylline regulates mucus production at the glandular level. This "regulation" of mucus production towards a less viscous form aids in its clearance from the airways.<br>●**Mucosecretory Activity:** Acebrophylline exhibits greater mucosecretory activity compared to ambroxol alone. This enhanced activity was demonstrated in animal studies where acebrophylline increased the volume of bronchial secretions and the amount of phenol red (a dye used to measure mucus secretion) secreted by the bronchial mucosa.<br>●**Mucokinetic Activity:** Acebrophylline enhances mucociliary clearance by increasing the transport of particles in the trachea and boosting the ciliary beat frequency of the respiratory mucosa. This action helps to effectively remove mucus from the airways.<br>●**Anti-inflammatory Action:** Acebrophylline demonstrates anti-inflammatory effects by inhibiting the production of inflammatory mediators. The drug reduces the synthesis and release of leukotrienes and tumor necrosis factor, which contribute to inflammation and airway obstruction<br>●**Pulmonary Surfactant Synthesis:** Acebrophylline stimulates the synthesis and release of pulmonary surfactant. This action is attributed to the combined effects of ambroxol and theophylline-7-acetic acid. Ambroxol enhances the activity of phosphocholine-cytidyl-transferase, while theophylline-7-acetic acid affects choline-kinase, both of which are involved in surfactant synthesis. |
| 16. | Half-life | 4 to 9 Hours |
| 17. | Clinical Use | Most often, this medication is used to Treat COPD and bronchial asthma. It could improve airflow while reducing Swelling and mucus production. |
| 18. | Route of administration | It is typically ingested orally. |

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|---|---|---|
| 19. | Side Effects | It is often well accepted, and any adverse effects, such as gastrointestinal issues, headaches, dizziness, and skin rashes, are typically moderate and temporary. |
| 20. | Metabolism and Monitoring | acebrophylline is processed in the liver, regular blood level monitoring is not necessary. |

**Marketed Formulation of Acebrophylline**




**Research on Acebrophylline**[13-14]

A new bronchodilator with mucosecretory action and anti-inflammatory properties in the respiratory tract, Acebrophylline is used to treat asthma, bronchospasm, and COPD. Oral bronchodilators are frequently recommended. On a clinical level, Acebrophylline is therapeutically effective in patients suffering from acute or chronic bronchitis, chronic obstructive pulmonary disease (COPD) and asthma. It functions by lowering the frequency of bronchial obstruction, relaxing muscles and widening the airways of the lungs. Acebrophylline is available in the form of a capsule, tablet and syrup. It is generally taken 100 mg twice a day for adults and 50mg/5ml of acebrophylline syrup is used for child dosage. For below 2 years of age 2.5 ml twice a day, between the age of 2 to 5 years 2.5 ml three times a day and above 5 years of age 5 ml twicw a day or as directed. This Dosage is sufficient to treat asthma, and COPD. The drug should be taken orally with food to avoid gastric discomfort.

**Introduction of Analytical Method**[15-18]

Analytical chemistry is the analysis of separation, quantification and chemical additives identification of herbal and synthetic materials constituted with one or more compounds or factors.

Analytical chemistry is separated into two predominant classes, a qualitative evaluation that is to say the identification with regard to the chemical additives exists in the sample, whereas quantitative evaluation estimates the amount of positive detail or compound within the substance, i.e. the sample.

There is a time lag from the date of introduction of a medication into the market to the date of its consideration in pharmacopoeias. This occurs because of the possible vulnerabilities within the continuous and more extensive use of those drugs, reports of ongoing toxicity (bringing about their withdrawal from the market), development of affected person resistance and improvement of higher medicine with the aim of competition.

Under these situations, requirements and analytical techniques for those medicines that may not be approachable within the pharmacopoeias. It becomes necessary, so as to develop newer analytical strategies for such medications.

Analytical approach improvement and validation perform important functions in the discovery, improvement, and manufacturing of medications. The

main aim of an analytical measure is to get consistent, realistic, and correct information.

Validated analytical strategies play a significant role in achieving this goal.

Outcomes from methodology validation may be used to choose the standard, reliability, and consistency of analytical results, that is associated as an integral part of any sensible analytical practice.

**Need of Analytical Method**[15-18]

Available method can be too costly, time ingesting, power extensive. Present approach can be too much error, infection, susceptible or they may be unreliable. There won't be a suitable method for a specific analyte in the unique pattern matrix. Present approach may not offer adequate sensitivity. For regulatory necessities its miles required.

Primary drug selection standards for brand new analytical method improvement. The drug or drug mixture may not be reliable in any pharmacopoeias. A right analytical technique for the drug might not be to be had within the literature due to patent rules. Analytical techniques might not be to be had for the drug within the form of a formula due to the interference resulting from the formula excipients.

Analytical approach for the quantization of the drug in biological fluids won't be available.

Analytical techniques for a drug in aggregate with other drugs won't be available. The prevailing analytical approaches may additionally require steeply-priced reagents and solvents. It can also involve bulky extraction and separation procedures and these won't be dependable.

Review of litretures shows that very few analytical methods like Spectrometric method, HPLC method, HPTLC method, RP-HPLC method and Stability indicating HPLC method developed for the determination of the acebrophylline, individually and in combination with some other drug.

**Review of Literature**

**UV-VIS Spectrophotometry Method Development on Acebrophylline**

| Sr. No. | Title | Description | Ref. No. |
|---|---|---|---|
| 1 | Spectrometric estimation of Acebrophylline in bulk and capsule formulation. | **Solvent**- Distilled Water<br>**λmax**- 273nm(Zero order) and 266.60-287.60nm(First order)<br>**Linearity**- 10-80 µg/mL | [19] |
| 2 | Determination of Acebrophylline in bulk and pharmaceutical formulation by UV spectrophotometer. | **Solvent**- Ethanol<br>**λmax**- 274nm<br>**Linearity**- 2-20 µg/Ml | [20] |
| 3 | Development and Validation of UV Spectroscopic Method for Estimation of Acebrophylline In Tablet Dosage Form. | **Solvent**- Ethanol<br>**Λmax**- 251nm<br>**Linearity**- 2-18 µg/mL | [21] |
| 4 | Spectrometric Method for Simultaneous Estimation of Acebrophylline and Montelukast in Bulk and Marketed Formulation by UV-Spectroscopy. | **Simultaneous Equation Method:**<br>**Solvent**- Methanol<br>**λmax**- 250.14nm(ACB) and 284.79nm(MLK)<br>**Linearity**- 6-18 µg/mL | [22] |
| 5 | Simultaneous Estimation of Montelukast Sodium and Acebrophylline in Bulk and Combined Dosage Form by UV-Spectrometric Method. | **Absorption Correction Method:**<br>**Solvent**- Methanol<br>**λmax**- 313.70nm(ACB) and 345nm(MLK)<br>**Linearity**- 5-10 µg/mL(ACB) and 100-200 µg/mL (MLK) | [23] |
| 6 | Development And Validation Of Analytical Methods For Simultaneous Estimation Of Acebrophylline, Fexofenadine Hcl And Montelukast Sodium In Bulk And Pharmaceutical Formulation. | **Method A:**<br>Simultaneous Equation Method:<br>**Solvent**- Methanol<br>**λmax**- 240nm(ACB), 227nm(MLK) and 247nm(FFDH)<br>**Linearity**- 5-35 µg/mL<br>**Method B:**<br>Multicomponent analysis method:<br><br>**Method C:**<br>Derivative Spectrophotometry Method: | [24] |

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|---|---|---|---|
| 7 | Spectrophotometric Estimation of Acebrophylline in Bulk and Capsule Formulation. | **Solvent-** Distilled Water<br>**λmax-** 273 nm<br>**Linearity-** 10-80 µg/mL | [25] |
| 8 | Visible Spectrophotometric Estimation of Acebrophylline in Bulk and Capsule Formulation. | **Method A:** Formation of orange red colored chromgen<br>**Solvent- Distilled Water**<br>**λmax-** 522nm<br>**Linearity-** 10-100 µg/mL<br><br>**Method B**: Formation of yellow coloured chromgen<br>**Solvent-** Distilled Water<br>**λmax-** 456nm<br>**Linearity Range-** 50-300 µg/mL | [26] |
| 9 | Development and Validation of Spectroscopic Method for Simultaneous Estimation of Acebrophylline and Acetylcysteine in Capsule Dosage Form. | **Simultaneous Equation Method:**<br>**Solvent-** Distilled Water<br>**λmax-** 273(ACB) and 220(ATC)<br>**Linearity-** 1 to 6 µg/ ml (ACB) and 6 to 36µg/ml (ATC) | [27] |

**HPLC Method Development on Acebrophylline**

| SR. NO. | TITLE | DESCRIPTION | REF. NO. |
|---|---|---|---|
| 1. | Analytical method development and validation for the simultaneous estimation of montelukast and Acebrophylline by RP-HPLC method. | **Mobile Phase-** Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v<br>**Stationary Phase-** Inertsil C18 column C18 (4.6 x 150mm, 5□m)<br>**λmax-** 260 nm<br>**Flow Rate-** 0.8 ml/min<br>**Retention Time-** 3.842 min(ACB) and 2.569 min(MLK)<br>**Injected Volume-** 20 µL<br>**Concentration Range-** 1-5 µg/ml(ACB) and 100-500 µg/ml(MLK) | [28] |
| 2. | Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Qualification of Fexofenadine and Acebrophylline Drug in Bulk and Tablet Dosage Form. | **Mobile phase-** 40:60% v / v Phosphate buffer and acetonitrile<br>**Stationary Phase-** Hypersil BDS C18 (250/4.6 mm, 5 µm)column<br>Wave Length- 230nm<br>**Flow Rate-** 1 mL / min<br>**Retention Time-** 6.76 min(ACB) and 3.06 min(FFD)<br>**Injected Volume-**<br>**Concentration Range-** 23.78–71.20 µg / mL(ACB) and 12.56–37.68 µg / mL(FFD) | [29] |
| 3. | Stability Indicating HPLC Method for Simultaneous Estimation of Acebrophylline and Doxofylline in Pharmaceutical Dosage Form. | **Mobile Phase-** Acetonitrile: 10 mM n-hexane sulfonic acid buffer (80: 20, v/v)<br>**Stationary Phase-** HiQSil C18 Column (250 × 4.6 mm, 5µm)<br>**λmax-** 250 nm<br>**Flow Rate-** 1.0 ml/min<br>**Retention Time-** 2.77 min(ACB) and 9.56 min(DXF)<br>**Injected Volume-** 20 µL<br>**Concentration Range-** 1-10 µg/ml(ACB) and 4-24 µg/ml(DXF) | [30] |
| 4. | Development and Validation of Acebrophylline and its Derivatives from Marketed Preparations. | **Mobile Phase-** 25 mM Ammonium Acetate: Acetonitrile (pH 7.3) in ratio of 3:7 v/v<br>**Stationary Phase-** SGE SS Wakosil-II 5C18AR, 250 mm(length) x 4.6mm(I.D) , 5 µm (particle size)<br>**λmax-** 257nm | [9] |

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|---|---|---|---|
| | | **Flow Rate-** 0.7 mL/min<br>**Retention Time-** 3.05 and 8.59 min<br>**Injected Volume-** 100 µ L<br>**Concentration Range-** 2 to 14 µg/ml | |
| 5. | Devlopment and Validation of Stability Indicating RP-HPLC Method for Simultanous Estimation of Doxofylline and Acebrophylline in their Combine Dosage Form. | **Mobile phase-** Phosphate Buffer (pH 3.0): Acetonitrile (40:60)<br>**Stationary Phase-** Agilent (250 cm × 4.6 cm) 0.5µm column<br>**λmax-** 274 nm<br>**Injected Volume-** 250 µg/ml(DXF) and 100 µg/ml(ACB)<br>**Concentration Range-** 125-375 µg/ml(DXF) and 50-150 µg/mL(ACB) | [31] |
| 6. | RP-HPLC Method Development and Validation for Estimation of Acebrophylline. | **Mobile Phase-** 25M Ammonium acetate: Acetonitrile (3:7) v/v<br>**Stationary Phase-** SGE SS Wakosil-II 5C18AR( 250 mm(length) x 4.6 mm(I.D) 5 µm<br>λmax- 257nm<br>**Flow Rate-** 0.7 mL/min<br>**Retention Time-** 3.05 and 8.59 min<br>**Injected Volume-**100 µ L<br>**Concentration Range-** 0.6, 0.5, 0.4 and 0.3 µg/Ml | [4] |
| 7. | Simultaneous estimation of acebrophylline, montelukast, and levocetirizine dihydrochloride in marketed formulation by high-performance liquid chromatography method. | **Mobile Phase-** Ammonium acetate buffer of pH 3.5 (pH adjusted with glacial acetic acid) and methanol in the ratio 15:85 v/v<br>**Stationary Phase-** Macherey-Nagel C18 4.6 mm * 250 mm (5 µm)<br>**λmax-** 230 nm<br>**Flow Rate-** 0.6 ml/min<br>**Retention Time-** 5.287 min(ACB), 26.856 min(MLK) and 6.440 min(LCZ)<br>**Injected Volume-** 20 µL<br>**Concentration Range-** 6–18 µg/ml(ACB and MLK) and 3–12 µg/ml(LCZ) | [32] |
| 8. | Development and Validation of a Stability-Indicating HPLC Method for the Determination of Acarbose in Pharmaceutical Dosage Forms. | **Mobile Phase-** Acetonitrile–0.007 M phosphate buffer (pH 6.7) (750 : 250, v/v)<br>**Stationary Phase-**Lichrospher R–100–NH2<br>**λmax-** 210nm<br>**Flow Rate-** 2 mL/min<br>**Injected Volume-**10 µL<br>**Concentration Range-** 2.5–20 mg/mL | [33] |
| 9. | Validated RP-HPLC Method Development for the Simultaneous Estimation of Acetylcysteine and Acebrophylline in Capsule Formulation. | **Mobile Phase-** 0.02M Potassium dihydrogen orthophosphate (KH2PO4) buffer : acetonitrile (90:10 % v/v pH 3.2)<br>**Stationary Phase-**Hypersil BDS, C18, 100 x 4.6 mm, 5µm particle size column<br>**λmax-** 260 nm<br>**Flow Rate-** 0.9 ml / min<br>**Retention Time-** 5.505 min(ACB) and 2.365 min(ACT)<br>**Injected Volume-** 10 µL<br>**Concentration Range-** 25-150 µg/ml(ACB) and 150-900µg/ml(ACT) | [34] |
| 10. | RP-HPLC Analysis of Acebrophylline in API and Capsule Dosage Form**.** | **Mobile phase-** Acetonitrile : water (70:30%v/v)<br>**Stationary Phase-** Enable C18 (250x4.6mm) 5µ<br>**λmax-** 274nm<br>**Flow Rate-** 1mL/min | [1] |

| | | | |
|---|---|---|---|
| | | **Retention Time**- 1.75 min<br>**Concentration Range**- 5 to 50µg/mL | |
| 11. | Stability Indicating HPLC Method Development for Estimation of Montelukast<br>Sodium and Acebrophylline in Combined Dosage Form. | **Mobile Phase**-Acetonitrile: Methanol (60:40 %v/v, pH 3.2 adjusted with O-phosphoric acid)<br>**Stationary Phase**- Reverse phase C18 column (Hibar Lichrospher® 100, RP-18e 5 µm, 250 mm L × 4.6 mm diameter in size)<br>**λmax**- 260nm<br>**Flow Rate**- 0.8ml/min<br>**Retention Time**-15.49 min(MLK) and 3.45 min(ACB)<br>**Injected Volume**- 20 µL<br>**Concentration Range**-5-25 µg/ml(MLK) and 100-500 µg/ml(ACB) | [35] |
| 12. | Estimation of Acebrophylline in Pharmaceutical Oral Dosage Form by RP-HPLC. | **Mobile phase**- Diammonium phosphate buffer (pH 4):methanol (60:40)<br>**Stationary Phase**- A phenomenex Gemini C18, 5µm column having 250x4.6mm<br>**Wave Length**- 273nm<br>**Flow Rate**- 1mL/min<br>**Retention Time**- 4.54min<br>**Concentration Range**- 80-120 µg/Ml | [36] |

**HP-TLC Method Development on Acebrophylline**

| SR. NO. | TITLE | DESCRIPTION | REF. NO. |
|---|---|---|---|
| 1. | Analytical Method Development and Validation for Simultaneous Estimation of Acebrophylline and Montelukast Sodium in their<br>Pharmaceutical Dosage Form. | **Mobile Phase**-<br>Chloroform : Ethyl acetate : Methanol : Triethylamine (6 : 4.5 : 2.5 : 0.8, v/v/v/v)<br>**Stationary Phase**-<br>HPTLC aluminium plates of silica gel G60 F254, (10 × 10 cm)<br>250 µm thickness<br>**Wave Length**- 272 nm<br>**Rf Value**- 0.23 (ACB) and 0.83 (MLK)<br>**Concentration Range**- 12000 – 20000 ng/spot (ACB) and 600-1000 ng/spot (MLK) | [2] |
| 2. | Development and Validation of Stability Indicating HPTLC Method for Estimation of Acebrophylline in<br>Their Dosage Form. | **Mobile Phase**- Toluene: Methanol: Acetone (8: 2: 2v/v)<br>**Stationary Phase**- Pre-coated 6 x 10 silica gel 60F254 TLC plate<br>**Wave Length**- 247nm<br>**Rf Value**- 0.49<br>**Concentration Range**- 600-2200 ng/band | [37] |

## CONCLUSION

"A Review on Analytical Methods Used for Determination of Acebrophylline in Bulk and Different Dosage Form" provides a comprehensive overview of the various analytical techniques developed for the quantification of acebrophylline, a medication used primarily for respiratory conditions such as chronic obstructive pulmonary disease (COPD) and asthma.

The review concludes that several analytical methods, including UV-VIS spectroscopy and High-Performance Liquid Chromatography (HPLC), have been successfully developed and validated for the determination of acebrophylline in various formulations. These methods demonstrate varying degrees of sensitivity, specificity, and reproducibility, making them suitable for both bulk and pharmaceutical preparations. The article highlights the importance of continuous development and validation of analytical techniques to ensure accurate and reliable measurement of acebrophylline, which is critical for its therapeutic efficacy and safety in clinical use. The findings underscore the necessity for standardized methods to facilitate regulatory approval and quality control in pharmaceutical applications.

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