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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ANALYTICAL METHOD FOR ESTIMATION OF ACEBROPHYLLINE AND ERDOSTEINE IN SYNTHETIC MIXTURE

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## ABSTRACT

A simple, precise, and accurate stability indicating RP-HPLC method was developed for the estimation of Acebrophylline and Erdosteine in synthetic mixture. The quantification was carried out using Zorbax C18 column (250 mm x 4.6 mm x 5 µm) and mobile phase comprised of 0.1 % Formic acid: Phosphate Buffer pH 4.8: Methanol in proportion of 60:15:25% v/v. The Flow rate was adjusted to 1.0 mL/min and the effluent was monitored at 220 nm using PDA detector. The retention time of Acebrophylline and Erdosteine was found to be 19.807 min 8.907 min respectively. The methods were validated in terms of specificity, linearity, precision, and accuracy, limit of detection, limit of quantification and robustness. The percentage assay was found to be 100.206 % for Acebrophylline and 100.154 % for Erdosteine in RP-HPLC method. Acebrophylline and Erdosteine were subjected to stress condition like Acid, Base, Oxidative, Thermal & Photo condition. Acceptable range of degradation was observed in Acebrophylline and Erdosteine in all the condition. The degradation products were well resolved from the pure drug with significant differences in their retention time. Thus, the proposed method enables rapid quantification and simultaneous estimation of

the Acebrophylline and Erdosteine drug without any interference of excipients. So, the developed and validated method can be used for routine analysis of Acebrophylline and Erdosteine in synthetic mixture.

**KEYWORDS:** Acebrophylline, Erdosteine, RP-HPLC, Stability indicating, Validation.

## INTRODUCTION

Obstructive airway diseases such as chronic obstructive pulmonary disease (COPD) and chronic bronchitis are common respiratory conditions characterized by airflow limitation and excessive mucus production, leading to symptoms like cough, breathlessness, and recurrent infections. COPD, a progressive disorder strongly associated with smoking, air pollution, and occupational exposures, involves chronic bronchitis and emphysema as its main forms, with pathological changes including inflammation, mucus gland hypertrophy, and destruction of alveolar walls.[1,2]

### Acebrophylline

Acebrophylline is a xanthine derivative with bronchodilator and mucolytic properties. It relaxes bronchial smooth muscles to improve airflow, reduces airway inflammation, and decreases mucus viscosity, making it effective in COPD, chronic bronchitis, and asthma.[3]

### Erdosteine

Erdosteine is a mucolytic and antioxidant agent that breaks disulfide bonds in mucus, improving expectoration. It also exhibits anti-inflammatory and anti-adhesive effects, reducing bacterial colonization and the frequency of COPD exacerbations.[4]

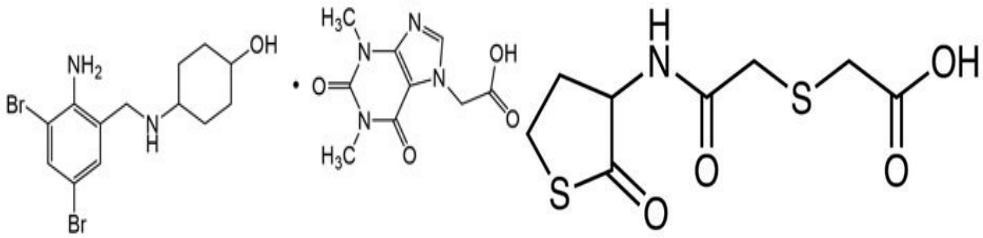



**Fig. 1: Structure of Acebrophylline and Erdosteine.**

The literature review reveals that few analytical methods were reported like RP-HPLC methods[5], Spectrophotometric method[6], HPTLC[7], UHPLC[8] and UPLC[9] in single or in Combination with other drug. But no method is reported for stability study& RP-HPLC. Hence present study aimed to develop a new Stability Indicating analytical method for

estimation of Acebrophylline & Erdosteine in synthetic mixture suitable for routine quality control analysis.

## MATERIALS AND METHODS

### Chemicals and Reagents

**Table 1: List of chemicals and reagents.**

| Material | Company | Grade |
|---|---|---|
| Water | Merck | AR Grade |
| Methanol | Merck | AR Grade |
| Acetonitrile | Merck | AR Grade |
| Formic acid | Finar | AR Grade |
| Glacial acetic acid | Finar | AR Grade |

### Preparation of Stock solution

**Preparation of Stock Solution of Acebrophylline and Erdosteine (1000 ppm)**

Accurately weighed 100 mg of drug and transferred into 100 ml volumetric flask. To this, 20 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with methanol and used as a stock solution.

### Preparation of mobile phase

0.1 % formic acid prepared by dissolving 0.1 ml formic acid into 100 mL HPLC grade water. Diluted 100ml of 0.5M potassium dihydrogen phosphate to 800ml with water, pH of the solution was adjusted to 4.8 with diluted sodium hydroxide.

### Forced Degradation Study

**1) Acid Degradation**

Stock solution 1 ml + 1 mL 0.1 N HCl in 10 ml volumetric flask. Kept for 40 min on table top at room temperature. Neutralized with 1 mL 0.1 N NaOH. Volume made with diluent and injected into HPLC.

**2) Base Degradation**

Standard stock solution 1 ml + 1 mL 0.1 N NaOH in 10 ml volumetric flask. Kept for 30 min on table top at room temperature. Neutralized with 1 mL 0.1 N HCl. Volume made with diluent and injected into HPLC.

**3) Oxidation Degradation**

Standard stock solution of 1 ml + 1 mL 3% $H_2O_2$ in 10 ml volumetric flask. Kept for 15 min at RT. Volume made with diluent and injected into HPLC.

**4) Photolytic Degradation**

Sample and standard solutions were kept in sunlight for 90min. Volume was made with diluent. Then injected into HPLC.

**5) Thermal Degradation**

Thermal degradation was performed by keeping API and samples in hot air oven at 80 °C 10 min.

## METHOD VALIDATION[10]

**1) Linearity and Range (n=3)**

The linearity for Acebrophylline and Erdosteine were assessed by analysis of combined standard solution in range of 500-1500µg/ml. Correlation co-efficient for calibration curve Acebrophylline and Erdosteine was found to be 0.9999 and 0.9999 respetively.

**2) Precision**

Precision was evaluated at three levels: intermediate precision (intraday precision), reproducibility (interday precision), and repeatability. The solution containing 1000.0 µg/ml of Acebrophylline and Erdosteine was injected six times for repeatability study. Intermediate precision study was performed by injecting 500, 1000, 1500 µg/ml of Acebrophylline and Erdosteine solutions three times for each aliquot. The %RSD for precision was calculated.

**3) Limit of Detection and Limit of Quantitation**

The LOD and LOQ were separately determined from calibration curve. Calibration curvewas repeated for three times and the standard deviation (SD) of the intercept was calculated. Then LOD and LOQ were calculated using following equation:

$LOD = 3.3 * \sigma/S$ and $LOQ = 10 * \sigma/S$

Where, $\sigma$ = Standard deviation of Y-intercepts, S = Mean slope of calibration curve.

**4) Accuracy**

The accuracy of the method was assessed using the standard addition technique, where a known amount of the working standard was spiked at three concentration levels: 80%, 100%,

and 120%. Each solution was injected in triplicate and the recovery was calculated by measuring peak areas.

### 5) Robustness

The robustness of the analytical procedure was evaluated to assess its ability to remain unaffected by minor but deliberate variations in method parameters, ensuring its reliability during routine use. Robustness testing was conducted (n=3) by altering key parameters, including:

Flow rate of the mobile phase (± 0.2 ml/min)

pH (± 2)

Mobile phase composition (±0.2%).

## RESULT AND DISCUSSION

### Optimized Chromatographic Conditions

Agilent HPLC system was used for method development, degradation studies and validation. Data acquisition was performed on HPLC. The separations were achieved on select Agilent Zorbax C18 (250 × 4.6 mm, 5µm). The column was maintained at room temperature and the eluent was monitored at 220 nm using detector. The mobile phase of 0.1 % Formic acid: Phosphate Buffer pH 4.8: Methanol 60:15:25% v/v mixture at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20µl.

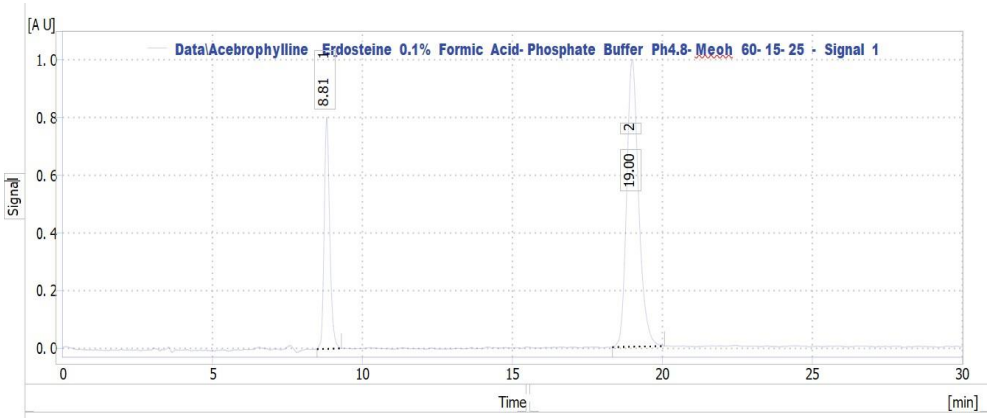
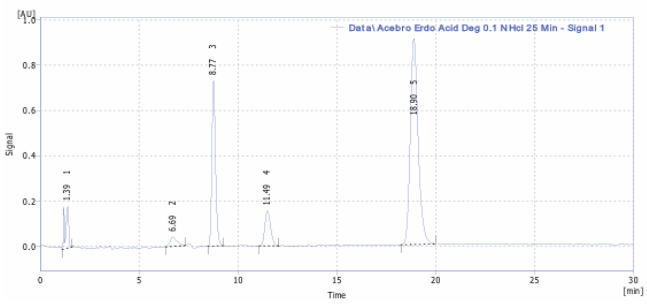



**Fig. 2 Optimized Chromatogram of Acebrophylline & Erdosteine in 0.1% Formic acid: Phosphate Buffer pH 4.8: Methanol 60:15:25% v/v.**

## Forced Degradation Study

### 1) Acid Degradation




*Result Table (Uncal - Data\Acebro Erdo Acid Deg 0.1 N Hcl 25 Min - Signal 1)*

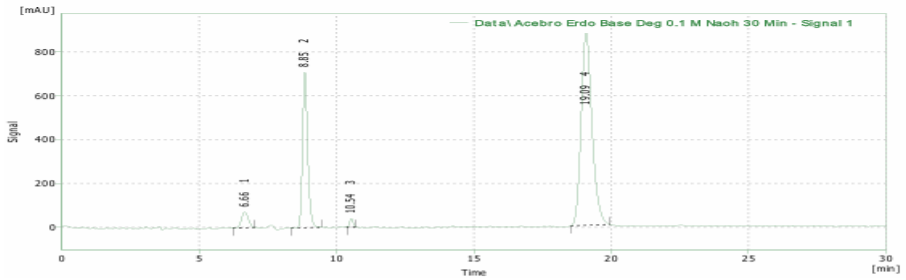
| | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] |
|---|---|---|---|---|---|
| 1 | 1.391 | 2105.759 | 184.566 | 5.2 | 9.1 |
| 2 | 6.691 | 997.412 | 41.947 | 2.5 | 2.1 |
| 3 | 8.767 | 9273.799 | 731.097 | 22.8 | 36.2 |
| 4 | 11.488 | 3135.061 | 154.862 | 7.7 | 7.7 |
| 5 | 18.904 | 25077.615 | 909.578 | 61.8 | 45.0 |
| | Total | 40589.646 | 2022.050 | 100.0 | 100.0 |

*Column Performance Table (From 50% - Data\Acebro Erdo Acid Deg 0.1 N Hcl 25 Min - Signal 1)*

| | Reten. Time [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|
| 1 | 1.391 | 0.374 | 538 | |
| 2 | 6.691 | 1.752 | 1885 | 12.414 |
| 3 | 8.767 | 1.351 | 10483 | 4.341 |
| 4 | 11.488 | 1.394 | 7031 | 6.127 |
| 5 | 18.904 | 1.346 | 11053 | 11.736 |

**Fig. 3: Chromatogram of Acid Degradation on Sample.**

### 2) Base Degradation




*Result Table (Uncal - Data\Acebro Erdo Base Deg 0.1 M Naoh 30 Min - Signal 1)*

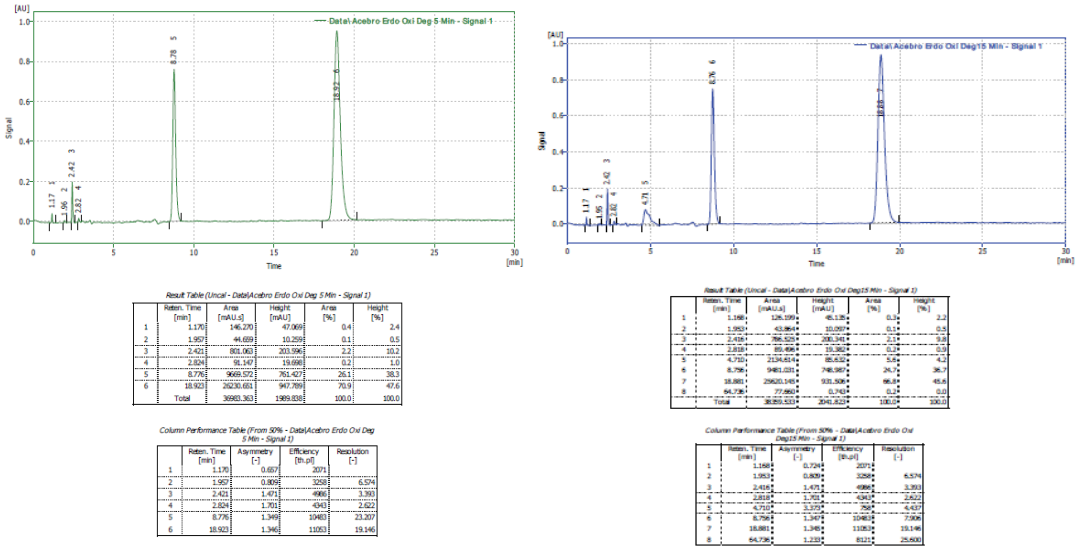
| | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] |
|---|---|---|---|---|---|
| 1 | 6.656 | 1228.652 | 71.576 | 3.5 | 4.2 |
| 2 | 8.855 | 9133.329 | 708.807 | 26.2 | 41.8 |
| 3 | 10.544 | 341.619 | 39.879 | 1.0 | 2.4 |
| 4 | 19.094 | 24100.316 | 876.269 | 69.2 | 51.7 |
| | Total | 34803.915 | 1696.532 | 100.0 | 100.0 |

*Column Performance Table (From 50% - Data\Acebro Erdo Base Deg 0.1 M Naoh 30 Min - Signal 1)*

| | Reten. Time [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|
| 1 | 6.656 | 1.254 | 3022 | |
| 2 | 8.855 | 1.350 | 10483 | 5.310 |
| 3 | 10.544 | 1.187 | 23227 | 5.441 |
| 4 | 19.094 | 1.332 | 11053 | 17.090 |

**Fig. 4: Chromatogram of Base Degradation on Sample.**

### 3) Oxidation Degradation




Result Table (Uncal - Data\Acebro Erdo Oxi Deg 5 Min - Signal 1)

| | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] |
|---|---|---|---|---|---|
| 1 | 1.170 | 146.270 | 47.069 | 0.4 | 2.4 |
| 2 | 1.957 | 44.699 | 10.259 | 0.1 | 0.5 |
| 3 | 2.421 | 801.963 | 203.996 | 2.2 | 10.2 |
| 4 | 2.824 | 91.147 | 19.698 | 0.2 | 1.0 |
| 5 | 8.776 | 9669.572 | 761.427 | 26.1 | 38.3 |
| 6 | 18.923 | 26230.681 | 947.789 | 70.9 | 47.6 |
| | Total | 36983.363 | 1989.838 | 100.0 | 100.0 |

Result Table (Uncal - Data\Acebro Erdo Oxi Deg15 Min - Signal 1)

| | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] |
|---|---|---|---|---|---|
| 1 | 1.168 | 126.199 | 45.135 | 0.3 | 2.2 |
| 2 | 1.952 | 43.864 | 10.097 | 0.1 | 0.5 |
| 3 | 2.414 | 766.526 | 200.341 | 2.1 | 9.8 |
| 4 | 2.818 | 89.498 | 19.382 | 0.2 | 0.9 |
| 5 | 4.710 | 2134.614 | 85.632 | 5.8 | 4.2 |
| 6 | 8.756 | 9481.035 | 748.982 | 25.7 | 36.7 |
| 7 | 18.881 | 25620.148 | 931.506 | 69.4 | 45.6 |
| 8 | 64.736 | 77.860 | 0.743 | 0.2 | 0.0 |
| | Total | 38359.530 | 2041.823 | 100.0 | 100.0 |

Column Performance Table (From 50% - Data\Acebro Erdo Oxi Deg 5 Min - Signal 1)

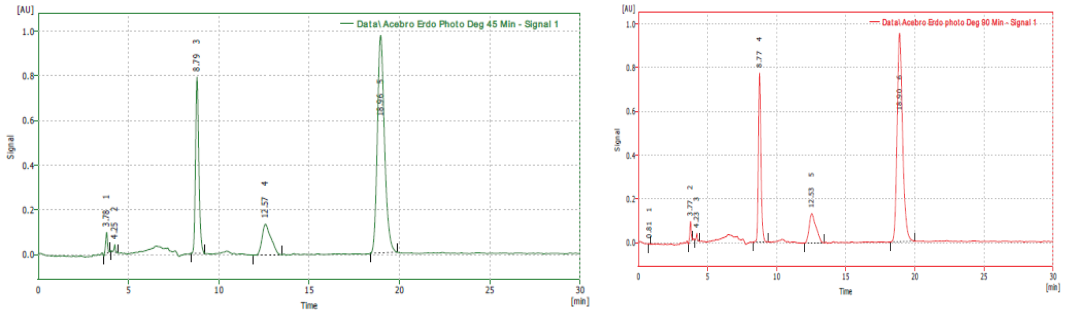
| | Reten. Time [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|
| 1 | 1.170 | 0.657 | 2071 | |
| 2 | 1.957 | 0.895 | 3258 | 6.574 |
| 3 | 2.421 | 1.471 | 4986 | 3.393 |
| 4 | 2.824 | 1.701 | 4343 | 2.622 |
| 5 | 8.776 | 1.349 | 10483 | 23.207 |
| 6 | 18.923 | 1.346 | 11053 | 19.146 |

Column Performance Table (From 50% - Data\Acebro Erdo Oxi Deg15 Min - Signal 1)

| | Reten. Time [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|
| 1 | 1.168 | 0.726 | 2071 | |
| 2 | 1.952 | 0.839 | 3258 | 6.574 |
| 3 | 2.414 | 1.475 | 4986 | 3.393 |
| 4 | 2.818 | 1.701 | 4343 | 2.622 |
| 5 | 4.710 | 1.375 | 758 | 4.437 |
| 6 | 8.756 | 1.347 | 10483 | 7.006 |
| 7 | 18.881 | 1.346 | 11053 | 19.146 |
| 8 | 64.736 | 1.233 | 8121 | 25.600 |

**Fig. 5: Chromatogram of Oxidative Degradation on Sample.**

### 4) Photolytic Degradation




Result Table (Uncal - Data\Acebro Erdo Photo Deg 45 Min - Signal 1)

| | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] |
|---|---|---|---|---|---|
| 1 | 3.780 | 677.614 | 94.932 | 1.6 | 4.7 |
| 2 | 4.245 | 183.919 | 33.734 | 0.4 | 1.7 |
| 3 | 8.793 | 10107.820 | 790.181 | 23.8 | 38.9 |
| 4 | 12.573 | 4719.332 | 138.359 | 11.1 | 6.8 |
| 5 | 18.961 | 26694.338 | 973.751 | 63.0 | 47.9 |
| | Total | 42383.022 | 2030.953 | 100.0 | 100.0 |

Result Table (Uncal - Data\Acebro Erdo photo Deg 90 Min - Signal 1)

| | Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] |
|---|---|---|---|---|---|
| 1 | 0.806 | 18.312 | 3.640 | 0.0 | 0.2 |
| 2 | 3.768 | 621.394 | 90.380 | 1.5 | 4.5 |
| 3 | 4.232 | 177.605 | 32.804 | 0.4 | 1.7 |
| 4 | 8.766 | 9946.373 | 772.600 | 23.9 | 38.9 |
| 5 | 12.538 | 4572.818 | 134.770 | 11.0 | 6.8 |
| 6 | 18.900 | 26262.022 | 952.532 | 63.1 | 47.9 |
| | Total | 41598.866 | 1986.976 | 100.0 | 100.0 |

Column Performance Table (From 50% - Data\Acebro Erdo Photo Deg 45 Min - Signal 1)

| | Reten. Time [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|
| 1 | 3.780 | 0.923 | 3954 | |
| 2 | 4.245 | 0.948 | 9773 | 2.262 |
| 3 | 8.793 | 1.331 | 10483 | 17.700 |
| 4 | 12.573 | 1.796 | 2734 | 5.807 |
| 5 | 18.961 | 1.338 | 11053 | 7.610 |

Column Performance Table (From 50% - Data\Acebro Erdo photo Deg 90 Min - Signal 1)

| | Reten. Time [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|
| 1 | 0.806 | 1.241 | 359 | |
| 2 | 3.768 | 0.868 | 3954 | 14.465 |
| 3 | 4.232 | 1.096 | 9773 | 2.262 |
| 4 | 8.766 | 1.330 | 10483 | 17.700 |
| 5 | 12.538 | 1.799 | 2734 | 5.807 |
| 6 | 18.900 | 1.346 | 11053 | 7.610 |

**Fig. 6: Chromatogram of Photolytic Degradation Standard and Sample.**

### 5) Thermal Degradation

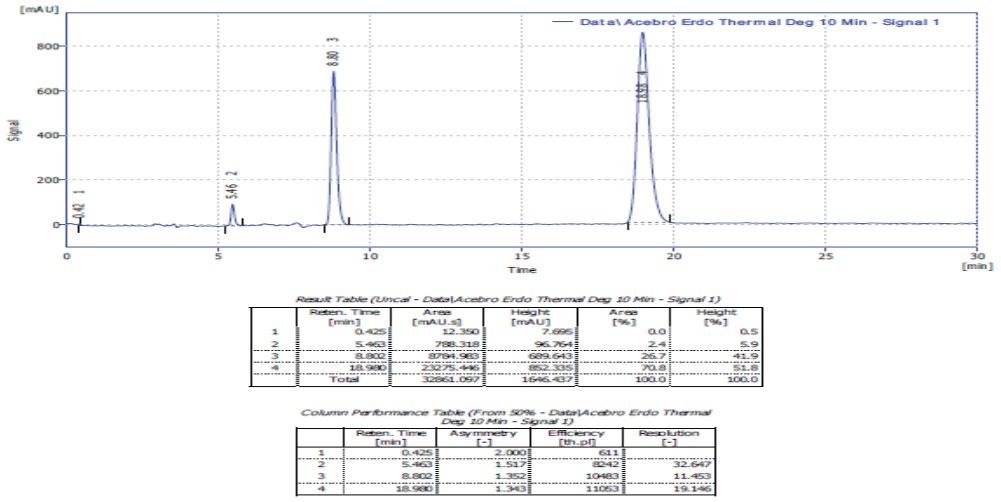



**Fig. 7: Chromatogram of Thermal Degradation Standard and Sample.**

**Table 2: Forced degradation summary of Acebrophylline and Erdosteine.**

| **Acebrophylline Standard Area** | | **27410** |
|---|---|---|
| **Degradation Condition** | **Area after degradation** | **% Degradation** |
| Acid | 22740 | 17.0 |
| Base | 24100 | 12.1 |
| Peroxide | 25620 | 7.0 |
| Thermal | 23275 | 15.1 |
| Photo | 26262 | 4.0 |
| **Erdosteine Standard Area** | | **10203** |
| Acid | 8607 | 16.0 |
| Base | 9133 | 10.0 |
| Peroxide | 8785 | 14.0 |
| Thermal | 9481 | 7.0 |
| Photo | 9946 | 3.0 |

### Validation of RP-HPLC method

#### Linearity

Linearity was assessed by preparing five solutions of Acebrophylline and Erdosteine. The method demonstrated linearity over the concentration range of 500-1500 µg/ml with a correlation coefficient ($R^2$ = 0.9999).

**Table 3: Linearity Data for Acebrophylline and Erdosteine.**

| **Acebrophylline** | |
|---|---|
| **Concentration (µg/mL)** | **Peak Area (n=5)** |
| 500 | 13760.67 |
| 750 | 20683.02 |

| | |
|---|---|
| 1000 | 27448.97 |
| 1250 | 34394.32 |
| 1500 | 41353.63 |
| **Linear Regression Equation** | Y = 27.5588x - 30.7718 |
| **Regression Coefficient ($R^2$)** | $R^2$ = 0.9999 |
| **Erdosteine** | |
| 500 | 5105.57 |
| 750 | 7664.39 |
| 1000 | 10212.72 |
| 1250 | 12737.43 |
| 1500 | 15334.78 |
| **Linear Regression Equation** | Y = 10.2125x – 1.6044 |
| **Regression Coefficient ($R^2$)** | $R^2$ = 0.9999 |

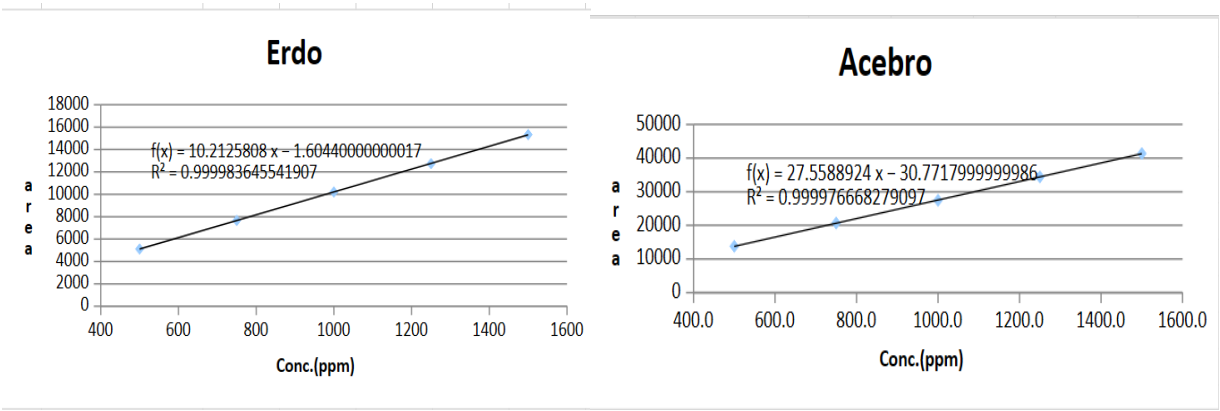



**Fig. 8: Calibration Curve of Acebrophylline and Erdosteine.**

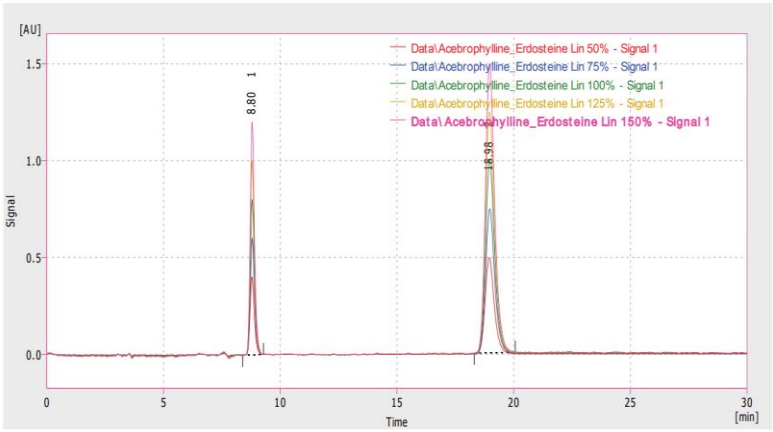



**Fig. 9: Overlay of Linearity Chromatogram of Acebrophylline and Erdosteine Precision.**

**Table 4: Repeatability Data Acebrophylline and Erdosteine.**

| Sr. No. | Acebrophylline | Erdosteine |
|---|---|---|
| | 1000.0 µg/mL | 1000.0 µg/mL |
| **1** | 27448.64 | 10212.72 |
| **2** | 27445.90 | 10210.62 |

| | | |
|---|---|---|
| **3** | 27438.52 | 10216.35 |
| **4** | 27441.05 | 10209.66 |
| **5** | 27443.14 | 10211.49 |
| **6** | 27440.01 | 10213.56 |
| **Mean** | 27442.87 | 10212.398 |
| **SD** | 3.821 | 2.388 |
| **%RSD** | 0.014 | 0.023 |

**Table 5: Intraday and Inter Day Precision Data of Acebrophylline.**

| **Precision** | **Intraday** | | **Interday** | |
|---|---|---|---|---|
| **Drug** | **Acebrophylline** | **Erdosteine** | **Acebrophylline** | **Erdosteine** |
| **Concentration (µg/ml)** | 500 | | 500 | |
| | 1000 | | 1000 | |
| | 1500 | | 1500 | |
| **Mean peak area ± SD (n = 3)** | 13758.851 ± 2.120 | 5103.407 ± 2.280 | 13764.165 ± 1.950 | 5105.932 ± 1.260 |
| | 27447.444 ± 2.472 | 10212.076 ± 1.951 | 27442.719 ± 2.820 | 10214.528 ± 2.119 |
| | 41351.455 ± 2.365 | 15333.513 ± 2.288 | 41353.207 ± 3.274 | 15335,259 ± 2.144 |
| **% RSD** | 0.015 | 0.044 | 0.014 | 0.025 |
| | 0.009 | 0.019 | 0.010 | 0.021 |
| | 0.006 | 0.015 | 0.008 | 0.014 |

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

Using slope and Y-intercept, the determined values of LOD and LOQ were evaluated. LOD and LOQ value for Acebrophylline was found to be 7.276 µg/ml and 22.047 µg/ml respectively. LOD and LOQ value for Erdosteine was found to be 6.091 µg/ml and 18.459 µg/ml respectively.

**Accuracy (n=3)**

Accuracy of the method was confirmed by recovery study at three levels (80%, 100% and 120%) of placebo addition.

**Table 6: Accuracy Data of Acebrophylline.**

| **Level of Spiking** | **Amount of Drug Present (µg/mL)** | **Amount of drug added (µg/mL)** | **Amount of Drug recovered (µg/mL)** | **% Recovery** | **Avg. %Recovery** | **% RSD** |
|---|---|---|---|---|---|---|
| **80 %** | 1000 | 800 | 800.45 | 100.06 | 100.10 | 0.13 |
| | 1000 | 800 | 801.99 | 100.25 | | |
| | 1000 | 800 | 800.01 | 100.00 | | |
| **100%** | 1000 | 1000 | 1000.67 | 100.07 | 100.02 | 0.10 |
| | 1000 | 1000 | 999.04 | 99.90 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 1000 | 1000 | 1000.96 | 100.10 | | |
| **120%** | 1000 | 1200 | 1200.77 | 100.06 | 100.07 | 0.05 |
| | 1000 | 1200 | 1200.30 | 100.03 | | |
| | 1000 | 1200 | 1201.41 | 100.12 | | |

**Table 7: Accuracy Data of Erdosteine.**

| Level of Spiking | Amount of Drug Present (µg/mL) | Amount of drug added (µg/mL) | Amount of Drug recovered (µg/mL) | % Recovery | Avg. %Recovery | % RSD |
|---|---|---|---|---|---|---|
| **80 %** | 1000 | 800 | 800.88 | 100.11 | 100.01 | 0.10 |
| | 1000 | 800 | 799.29 | 99.91 | | |
| | 1000 | 800 | 800.12 | 100.02 | | |
| **100%** | 1000 | 1000 | 1000.18 | 100.02 | 99.98 | 0.04 |
| | 1000 | 1000 | 999.37 | 99.94 | | |
| | 1000 | 1000 | 999.73 | 99.97 | | |
| **120%** | 1000 | 1200 | 1200.44 | 100.04 | 100.00 | 0.04 |
| | 1000 | 1200 | 1199.55 | 99.96 | | |
| | 1000 | 1200 | 1200.01 | 100.00 | | |

**Robustness**

**Table 8: Robustness Data of Acebrophylline.**

| Parameter | Level of change | Area | Mean | %SD | %RSD |
|---|---|---|---|---|---|
| **Flow rate (± 0.02)** | -0.02 | 26887.578 | 26835.446 | 46.450 | 0.173 |
| | | 26820.302 | | | |
| | | 26798.459 | | | |
| | +0.02 | 28414.447 | 28458.593 | 39.183 | 0.138 |
| | | 28489.247 | | | |
| | | 28472.084 | | | |
| **pH (± 2)** | -2 | 28189.478 | 28166.017 | 61.232 | 0.217 |
| | | 28096.523 | | | |
| | | 28212.049 | | | |
| | +2 | 28502.981 | 28500.211 | 35.647 | 0.125 |
| | | 28463.259 | | | |
| | | 28534.392 | | | |
| **Mobile Phase composition (± 2)** | -2 | 26389.243 | 26326.820 | 219.804 | 0.835 |
| | | 26508.661 | | | |
| | | 26082.557 | | | |
| | +2 | 29794.352 | 29704.376 | 282.301 | 0.950 |
| | | 29930.723 | | | |
| | | 29388.054 | | | |

**Table 9: Robustness Data of Erdosteine.**

| Parameter | Level of change | Area | Mean | %SD | %RSD |
|---|---|---|---|---|---|
| **Flow rate (± 0.02)** | -0.02 | 93789.147 | 93711.942 | 88.111 | 0.094 |
| | | 93615.954 | | | |
| | | 93730.724 | | | |

| | | | | | |
|---|---|---|---|---|---|
| | +0.02 | 12808.478<br>12804.889<br>12807.547 | 12806.971 | 1.862 | 0.015 |
| **pH (± 2)** | -2 | 12789.548<br>12480.236<br>12610.902 | 12626.895 | 155.275 | 1.230 |
| | +2 | 12990.118<br>12899.026<br>12942.531 | 12943.892 | 45.561 | 0.352 |
| **Mobile Phase composition (± 2)** | -2 | 13095.225<br>13467.391<br>13279.840 | 13280.819 | 186.085 | 1.401 |
| | +2 | 15522.321<br>15164.259<br>15379.804 | 15355.461 | 180.268 | 1.174 |

**Assay of Synthetic Mixture**

Applicability of the proposed method was tested by analyzing the Synthetic Mixture. Results as % Assay is shown in Table 6.18.

**Table 10: Assay of Synthetic Mixture (Acebrophylline & Erdosteine).**

| Drug | Area of Sample | %Assay | %Avg. Assay | % SD | % RSD |
|---|---|---|---|---|---|
| **Acebrophylline** | 27502.239 | 99.938 | 100.206 | 0.233 | 0.232 |
| | 27611.371 | 100.335 | | | |
| | 27614.771 | 100.347 | | | |
| **Erdosteine** | 10189.147 | 100.030 | 100.154 | 0.140 | 0.139 |
| | 10199.011 | 100.127 | | | |
| | 10217.186 | 100.305 | | | |

These results indicates that the developed method is specific, accurate, precise, simple, sensitive, robust and rapid.

## CONCLUSION

Based on the experimental results, the proposed method is accurate, novel, simple, precise, linear, sensitive, robust, and stable for the estimation of Acebrophylline and Erdosteine in synthetic mixtures.

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