ABSTRACT
A simple, specific and accurate reverse phase high performance liquid chromatographic method was
developed for the estimation of acebrophylline in capsule dosage form. A phenomenex Gemini C18 5µm
column having 250x4.6mm i.d. in isocratic mode with mobile phase containing diammonium phosphate
buffer (pH 4):methanol (60:40) was used. The flow rate was 1mL/minute and effluents were monitored at
273nm. The retention time was found to be 4.54minutes. The linearity was in the range of 80-120 µg/mL.
The proposed method was validated and successfully applied for the estimation of acebrophylline in capsule
dosage form.

Keywords: Acebrophylline(ACE); RP-HPLC.

INTRODUCTION
Acebrophylline is chemically, 4-[(2-amino-3,5-
dibromophenyl)methylamino]cyclohexan-1-ol, compd
with 2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetic acid and
is used in the treatment of chronic obstructive pulmonary disease and bronchial asthma1-4. No method
of estimation for acebrophylline in bulk and formulation
has been reported so far. The objective of the present
work was to develop an accurate, specific and
reproducible method for the estimation of acebro-
phylline in pharmaceutical oral dosage forms.

RESULTS AND DISCUSSION
The mobile phase consisting of diamonium phosphate buffer (pH 4):methanol (60:40) was used. The flow rate was 1mL/minute and effluents were monitored at
273nm. The retention time was found to be 4.54minutes. The linearity was in the range of 80-120 µg/mL.
The proposed method was validated and successfully applied for the estimation of acebrophylline in capsule
dosage form.

EXPERIMENTAL
Instruments
Shimadzu prominence LC 20AT, UV-Visible detector
SPD-20A, Phenomenex column Gemini 5µ C18 -
spherical size 250X4.60mm.

Reagents
Methanol HPLC grade, Diammonium phosphate buffer
HPLC grade.

Preparation of standard solution
The standard solution was prepared by dissolving
adequate quantity of drug, accurately weighed and
transferred into 100mL volumetric flask with the mobile
phase. The stock solution was further diluted with
mobile phase in the concentration range of 80-120µg/

mL.

Preparation of sample solution
Twenty capsules were weighed accurately and then
emptied. The empty shells were reweighed and the
powder was mixed uniformly. The powder equivalent
to 100mg of ACE was taken in 100mL volumetric flask

and dissolved in mobile phase and sonicated for 10
minutes. The solution was made up to the mark with
the mobile phase. The resulting solution was filtered
through a nylon 0.45µm membrane filter. The first few
mL of the filtrate was discarded. Aliquot from stock
solution was further diluted with mobile phase to get
samples in the concentration range of 80-120µg/mL.

ASSAY PROCEDURE
Pharmaceutical formulation of ACE was successfully
analyzed by applying 20µL of filtered final solution to
HPLC system to obtain the chromatogram. The content
of the drug was calculated by comparing the peak area
of sample and standard5-6. The method was validated
by establishing linearity, accuracy and precision of
sample application.

RESULTS AND DISCUSSION
The mobile phase consisting of diamonium phosphate buffer (pH 4):methanol (60:40) with flow rate
of 1ml/min was found to give R 4.54minutes. Statistical
parameters such as regression characteristic, slope,
intercept, correlation co-efficient, % RSD and standard
error obtained from different concentrations were
calculated and the results are summarized in Table 1.
The chromatographic parameters were also validated
by system suitability studies(Table 2). To study the
accuracy and reproducibility of the proposed method,
recovery experiments were carried out by adding a
known amount of drug to pre analysed sample and the
percentage recovery was calculated. The results are
furnished in Table 3. The results indicate that there is
no interference from other ingredients present in the
formulation. Thus, the proposed method is cost
effective, faster, and can be used for routine analysis.
Acebrophylline RP-HPLC Method

Table 1. Statistical Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>273</td>
</tr>
<tr>
<td>Linear Range (µg/ml)</td>
<td>80-120</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>10.079</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.2508</td>
</tr>
<tr>
<td>Regression equation (y = mx + c)</td>
<td>10.079x + 0.2508</td>
</tr>
<tr>
<td>Correlation co-efficient (r)</td>
<td>0.9999</td>
</tr>
<tr>
<td>Standard Error (SE)</td>
<td>0.2430</td>
</tr>
</tbody>
</table>

Table 2. System Suitability Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (A&lt;sub&gt;1&lt;/sub&gt;,A&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>1.5</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>4.441</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.501</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.3301</td>
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</table>

Table 3. Assay and recovery of acebrophylline in dosage forms

<table>
<thead>
<tr>
<th>FORMULATION</th>
<th>LABEL CLAIM (µg)</th>
<th>AMOUNT OF DRUG (µg)</th>
<th>AMOUNT OF DRUG (µg)</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPSULE</td>
<td>101</td>
<td>103.19</td>
<td>107</td>
<td>49.99</td>
</tr>
</tbody>
</table>

* Each average of three determinations

REFERENCES