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Analytical Method Development and Validation for the Determination of Acebrophylline by Rp-Uhplc in Solid Dosage Forms

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ABSTRACT:
Acebrophylline is a bronchodilator, mucolytic agent with anti inflammatory action. Acebrophylline primarily used to prevent and treat asthma and COPD (Chronic Obstructive Pulmonary Disease). In case of obstructive airway illness, the medication takes a multi prolonged strategy. The molecules contains ambroxol, which aids different steps in the creation of pulmonary surfactants, as well as theophylline- 7 acetic acid, whose carrier function elevates blood levels of ambroxol, causing surfactant production to increase quickly. A rapid, simple, accurate RP-UHPLC method has been developed for the determination of acebrophylline in pharmaceutical dosage form. The wavelength of acebrophylline was found to be 274nm. An enable C18 (250 x 4.6 mm, 5µm) column and mobile phase consisting of a acetronitrile and buffer (400:600) and method was developed. This method was validated as per ICH guidelines.

KEYWORDS: Quantitative analysis, Chromatography, HPLC, RP-UHPLC. Specificity, Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantification, Robustness, System suitability, Acebrophylline.

I. INTRODUCTION:

[1]Analytical chemistry is a branch of chemistry and it is of two types - qualitative and quantitative. Qualitative analysis deals with the identification of components or analyte of mixture or sample and the quantitative analysis deals with the determination of quantity of components of substance or samples or mixture.

[2]Method of Quantitative Analysis

There are various methods forquantitativeanalysis of pharmaceuticals. They are classified into the following types:

- Chemical Methods.
- Volumetric or Titrimetric.
- Instrumental methods.

INSTRUMENTAL METHODS

These methods are based on the relation between the content and corresponding physical or physiochemical properties of the system being analyzed.

Types of instrumental methods

- Spectral methods.
- Chromatographic methods.
- Electro analytical methods.
- Biological and microbiological methods.
- Radioactive methods.

Chromatographic methods

- Paper chromatography.
- Column chromatography.
- Thin Layer Chromatography (TLC).
- Gas chromatography.
- High Performance Liquid Chromatography (HPLC).
- High Performance Thin Layer Chromatography (HPTLC).

[3]CHROMATOGRAPHY:

The chromatography was discovered by Russian Chemist and botanist MichealTswett(1872-1919). The term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) was first used to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

[4]**Definition**:Chromatography is the separation of a mixture in to individual components using a stationary phase and a mobile phase.

Classification of Chromatography

According to nature of stationary and mobile phase

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Examples: Solid- Liquid chromatography, Liquid-Liquid chromatography, Gas-Solid chromatography, Gas -Liquid chromatography

According to principle of separation

Adsorption chromatography

Examples: Gas Solid chromatography, thin layer chromatography (TLC), Column chromatography, High performance liquid chromatography (HPLC), Affinity phase chromatography, Hydrophobic Interaction chromatography (HIC).

Partition chromatography

Examples: Gas liquid chromatography,Paper partition chromatography,Column partition chromatography.

Based on modes of chromatography

Examples: Normal phase chromatography, Reverse phase chromatography.

Other types of chromatography

Examples: Size exclusion chromatography (SEC), Gel permeation chromatography, Gel chromatography, Gel Filtration, Gel permeation chromatography, Ion exchange chromatography, chiral chromatography.

[5]ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY(UHPLC)

UHPLC is a specialised chromatographic method that runs faster, resolves better and uses less solvent than the HPLC. This encompasses LC separations using columns containing particles smaller than the 2.5-5 µm sizes typically used in HPLC. The benefit of using columns containing smaller particles (typically sub 2µm) is greater efficiency per unit time.

[6]Differences between HPLC and UHPLC

Parameters	HPLC	UHPLC
Particle size	3-5µm	2µm or less
Column dimensions	Internal diameter of 4.6 mm and length of 250 mm	Internal diameter of 2.1 mm or less and length of 100 mm
Flow rates	1-2 ml/min	0.2-0.7 ml/min
Back pressure	400-600 bar	1500 bar

[7]ANALYTICAL METHOD VALIDATION

Method validation can be defined as per ICH"Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

ICH Method validation parameter

For chromatogrpahic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B).

- Specificity
- Linearity
- Accuracy
- Precision
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)
- Robustness
- System suitability.

[8]Specificity/Selectivity:

Specificity is ability to detect analytecomponents that are expected to be present in the sample. The terms selectivity and specificity are often used interchangeably. According to ICH the term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other.

Specificity is the ability of a method to discriminate between the analytes of interest and other components that are present in the sample. This provides the analyst with a degree of certainty that the response observed is due to the single analyte of interest. The degree of specificity testing varies depending on the method type and the stage of validation. Specificity should be evaluated continually through the drug development process.

Accuracy:

The Accuracy of analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and value found.Accuracy may be inferred once precision, linearity and specificity have been established. Accuracy for the area percent method should be established from 50% of the ICH reporting limit to the nominal concentration of drug substance in the sample solution. According to the ICH, accuracy should be determined using a minimum of nine

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determinations over a minimum of three concentration levels covering the range.

Precision:

ICH defines the precision of an analytical procedure as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiplesampling of the same homogeneous sample under the prescribed conditions.Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Linearity:

Linearity of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

- **Concentration range**

The concentration range used for linearity should be large enough to encompass the desired range of the method. A minimum of five concentration ranges should be investigated and a plot of the detector response vs. the sample concentration should be generated. It is important that the concentration ranges selected for the linearity study are relatively equally spaced throughout the range of the method (e.g., 25%, 50%, 75%, 100%, 125% and 150%), and not clustered, as this will provide a skewed estimation of linearity.

Acceptance criteria:

Solutions of known concentrations are used to determine the linearity. A plot of peak area versus concentration (in percent related substance) is used to demonstrate the linearity. Authentic samples of related substances with known purity are used to prepare these solutions. In most cases, for the linearity of a drug product, spiking the related substance authentic sample into excipients is not necessary, as the matrix effect should be investigated in method accuracy. Visual inspection is the most sensitive method for detecting nonlinearity. Therefore, the plot has to be linear by visual inspection. In addition, according to ICH guidelines, the following results should be reported: slope, correlation coefficient, y-intercept, and residual sum of squares. Under most circumstances, regression coefficient (r^2) is 0.999. Intercept and slope should be indicated.

Acceptance criteria

Test	Level	Range	Acceptance criteria
Assay	5	50% to 150%	R> 0.999,
Dissolution	5-8	10% to 150%	R > 0.99,
Impurity	5	LOQ to 2%	R > 0.98

Limit of detection:

Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Several approaches for determining the LOD are possible, depending on whether the procedure is a non-instrumental or instrumental.

Based on visual evaluation

- Based on signal-to-noise
- Based on the standard deviation of the response and the slope

The LOD may be expressed as:

$$\mathbf{LOD = 3.3\ \sigma / S}$$

Where,

σ = Standard deviation of Intercepts of calibration curves

S = Mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification:

Limit of quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Several

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approaches for determining the LOQ are possible depending on whether the procedure is a non-instrumental or instrumental.

- Based on visual evaluation
- Based on signal-to-noise Approach
- Based on the standard deviation of the response and the slope

The LOQ may be expressed as

$$LOQ = 10\,\sigma / S$$

Where, σ = Standard deviation of Intercepts of calibration curves
S = Mean of slopes of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Robustness:
The robustness of an analytical procedure is defined as a measure of its capacity toobtain comparable and acceptable results when perturbed by small but deliberate variations in specified experimental conditions. Robustness provides an indication of the test method's suitability and reliability during normal use.

System Suitability:
According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. The purpose of the system suitability test is to ensure that the complete testing system is suitable for the intended application.In general consistency of system performance, and chromatographic suitability. (Tailing factor, column efficiency and resolution of the critical pair, detector sensitivity) are the main components of system suitability.

System suitability parameters and recommendations

Parameter	Recommendation
Capacity Factor (k')	The peak should be well-resolved from other peaks and the void volume, generally k' 1 to 20
Repeatability	RSD $\leq$ 1% for N $\geq$ 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution (R_s)	R_s of > 2 between the peak of interest and the closest eluting potential interfering (impurity, excipient, degradation product, internal standard, etc.)
Tailing Factor (T)	T of >0.5 and $\leq$ 2
Theoretical Plates (N)	N < 2000

II. MATERIALS AND METHODS

Materials:
Acebrophylline drug,HPLC, Buffer, Acetonitrile. All the above chemicals and solvents are from Rankem.

Instruments:

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- 1220 infinity LC (Agilent technologies pvt ltd)
- **Serial no:DEG00308**

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◆ **SOFTWARE:CHEMSTATION**

Methods:

Chromatographic condition:

1. Column: Inersil ODS_3V 250 mm x 4.6 mm ,5 microns
2. Wave length:274nm
3. Flow rate:1ml/min
4. Injection volume:5 µl
5. Mobilephase: Takeacetonitrile:buffer (1.3 gm of ammonium acetate in 1000 ml water and adjust pH to 3.0 with OPA- Orthophosphoric acid) at 400:600 ratio and filter through 0.45µm membrane filter and degas.

Diluent: Mobile phase

Preparation of Standard solution: Weigh accurately and transfer about 20 mg of Acebrophylline working standard into 100 ml volumetric standard flask. Add 50ml of diluent, sonicate for dissolve and makeup with the volume with diluent and mix.Further take 5 ml of this solution in 50ml volumetric flask and make up to volume withdiluent.Filter it through 0.45 micron syringe filter.

Preparation of Sample solution: Weigh about Average weight of sample into 100 ml volumetric flask add 150 ml of diluent and keep the flask in sonicator for 30 minutes intermitent vigorous shaking and cool to room temperature and make up the volume up to mark with diluent and mix.Further take 5 ml of this solution in 100ml volumetric flask and make up to volume with diluent.Filter it through 0.45 micron syringe filter.

Validation:

System suitability parameters:

The system suitability parameters was determined by preparing standard solution of Acebrophylline succinate were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.The % RSD for the area of six standard injections results should not be more than 2%.

Specificity:

Checking of the interference in the optimized method. We should not find any interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Accuracy:

Preparation of Standard stock solution: Weigh accurately and transferred about 20 mg of Acebrophylline standard into 100 ml volumetric standard
flask. Dissolved and makeup to the volume with mobile phase. Dilute 5 ml of this solution to 50 ml with mobile phase.

For preparation of 80% solution : Weigh accurately and transfer powdered sample equivalent to about 160 mg of Acebrophylline into 100 ml volumetric standard flask. Dissolve and makeup to the volume with mobile phase. Dilute1 ml of this solution to 100ml with mobile phase.

For preparation of 100% solution: Weigh accurately and transfer powdered sample equivalent to about 200 mg of Acebrophylline into 100 ml volumetric standard flask. Dissolve and makeup to the volume with mobile phase. Diluted 1 ml of this solution to 100 ml with mobile phase.

For preparation of 120% solution: Weigh accurately and transfer powdered sample equivalent to about 240 mg of Acebrophylline into 100 ml volumetric standard flask. Dissolve and makeup to the volume with mobile phase. Diluted 1 ml of this solution to 100 ml with mobile phase.

Procedure: The standard solution, Accuracy -80%, Accuracy -100% and Accuracy -120% solutions were injected.The amount found and amount added for Acebrophylline mean recovery values were calculated and the results were summarized.

III. RESULTS AND DISCUSSION

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Trial chromatogram:

Chromatographic conditions:

Mobile phase : Acetonitrile:Buffer (400:600)
Flow rate: 1 ml/min
Column : INERTSIL ODS Plus C18; 4.6mm X 450 mm;5µ
Detector wave length : 274nm
Injection volume: 5 µL
Run time: 3 min
Diluent : Mobile phase
Results: Peak not eluted properly. So, further trial is carried out.

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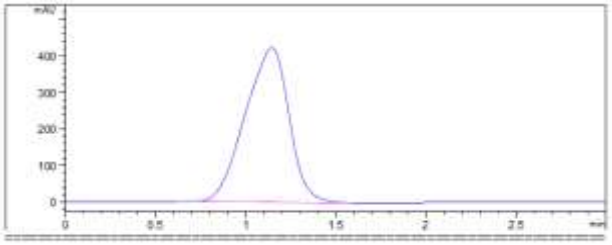
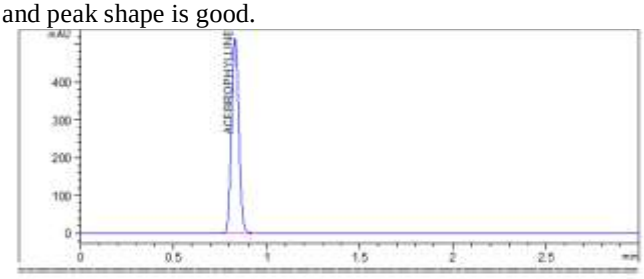


Trial chromatogram
Optimized conditions
Chromatographic conditions:
Mobile phase: Acetonitrile:Water (400:600)
Flow rate: 1 ml/min
Column : INERTSIL ODS C18; 4.6mm X 50 mm; 1.8µ
Detector wave length : 274nm
Injection volume: 3 µL
Run time: 3 min
Diluent : Mobile phase
Results : Retention time and peak shape is good.



Optimized chromatogram
Observation: Acebrophylline was eluted at 0.381 min. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.
System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.
System suitability parameter for ACEBROPHYLLINE

Sample ID	**ACEBROPHYLLINE**	
	RT	**AREA**
Injection -01	0.831	1103.5670
Injection -02	0.833	1106.4580
Injection -03	0.835	1100.8480
Injection -04	0.832	1107.7590
Injection -05	0.846	1106.7510
Average :		1105.0766
SD :		2.831
% RSD :		0.26

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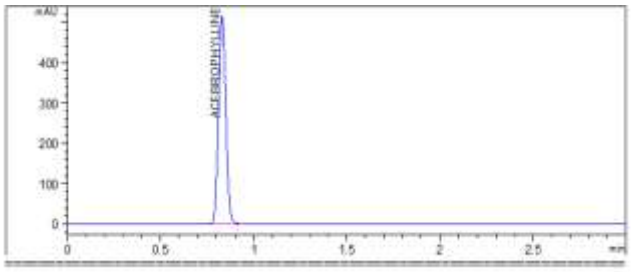


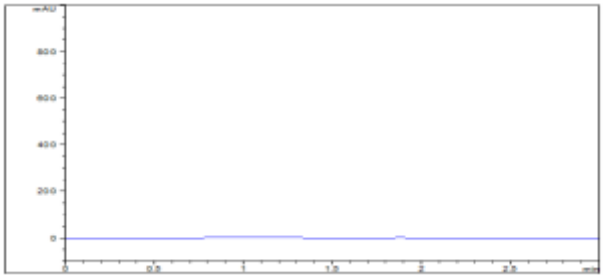
Systemsuitability Chromatogram

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

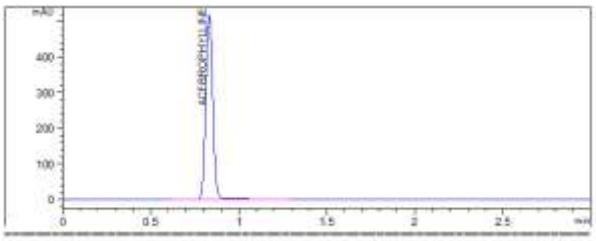
Specificity:

Specificity table for ACEBROPHYLLINE

Sample ID	ACEBROPHYLLINE	
	RT	**AREA**
BLANK	0.831	No peak observed
STANDARD	0.831	1100.8480
PLACEBO	0.831	No peak observed



Chromatogramof blank.



Chromatogramof placebo

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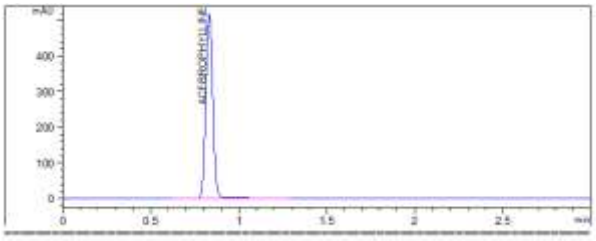

Typical Chromatogram

Discussion: Retention time of Acebrophylline was eluted at 0.381 min. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to bespecific.

Accuracy:
Accuracy for acebrophylline
Label claim : Acebrophylline 200 mg
Average weight of a tablet : 350 mg
Factor : 1
Purity of std : 99.22 %
Standard values:
1103.5670, 1106.4580, 1100.8480, 1107.7590, 1106.7510
Average : 1105.0766
Standard deviation : 2.831
% RSD : 0.26
Sample preparations:
80 % sample:
80 % sample -01 : 280.00 mg diluted to 100 ml, further 1 ml diluted to 100 ml.
80 % sample -02 : 280.00 mg diluted to 100 ml, further 1 ml diluted to 100 ml.
80 % sample -03 : 280.00 mg diluted to 100 ml, further 1 ml diluted to 100 ml.
100 % sample:
100 % sample -01 : 350.00 mg diluted to 100 ml, further 1ml diluted to 100 ml.
100 % sample -02 : 350.00 mg diluted to 100 ml, further 1ml diluted to 100 ml.
100 % sample -03 : 350.00 mg diluted to 100 ml, further 1ml diluted to 100 ml.
120 % sample:
120 % sample -01 : 420.00 mg diluted to 100 ml, further 1ml diluted to 100 ml.
120 % sample -02 : 420.00 mg diluted to 100 ml, further 1ml diluted to 100 ml.
120 % sample -03 : 420.00 mg diluted to 100 ml, further 1ml diluted to 100 ml.

Accuracy data for Acebrophylline

Sample ID	Sample wt. (mg)	Sample area	Calculated Content (in mg)	Calculated Content (in %)
80% sample 01	280.00	882.3660	200.381	100.19
80% sample 02	280.00	890.6330	202.259	101.13
80% sample 03	280.00	883.3660	200.608	100.30
100% sample 01	350.00	1100.0875	199.862	99.93
100% sample 02	350.00	1103.3660	200.455	100.23
100% sample 03	350.00	1108.6695	201.419	100.71
120% sample 04	420.00	1325.0000	200.601	100.30
120% sample 02	420.00	1326.0000	200.752	100.38
120% sample 03	420.00	1327.0000	200.904	100.45

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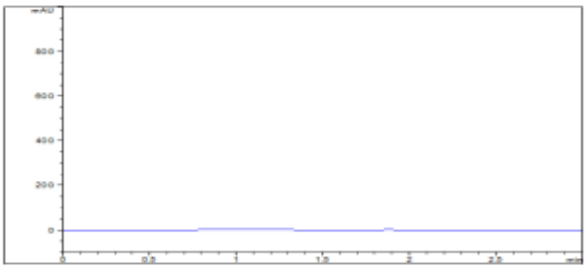
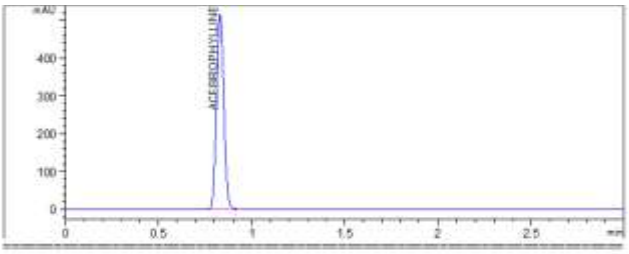
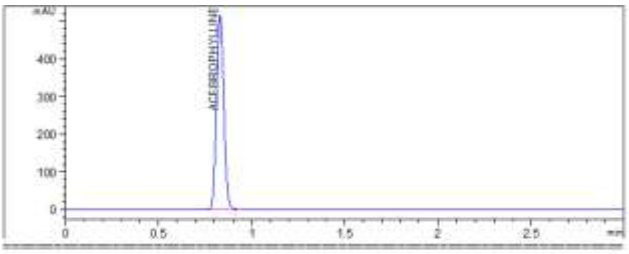
Average : 100.40
SD : 0.344
RSD : 0.34



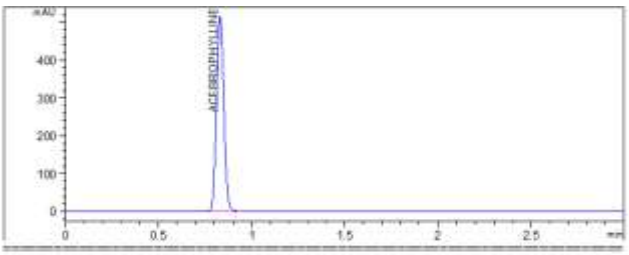
Blank chromatogram



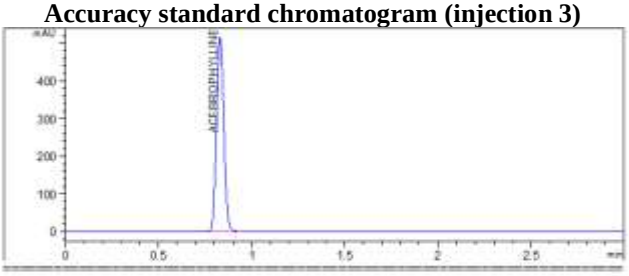
Accuracy standard chromatogram (injection 1)



Accuracystandardchromatogram (injection 2)



Accuracy standard chromatogram (injection 3)



Accuracy standard chromatogram (injection 4)

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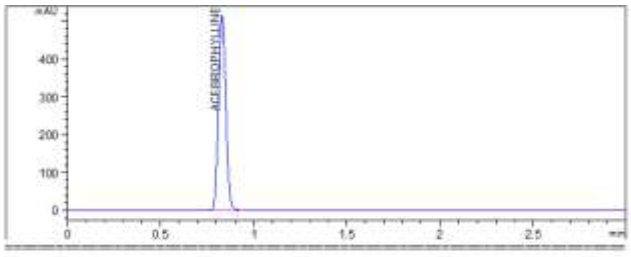
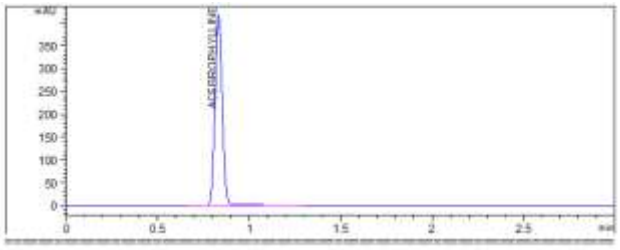
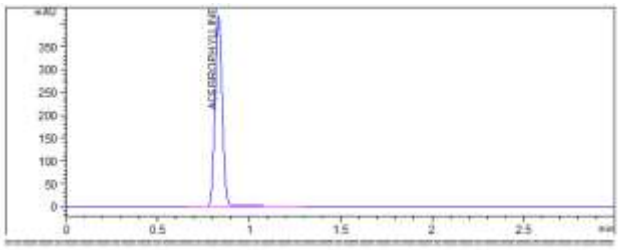


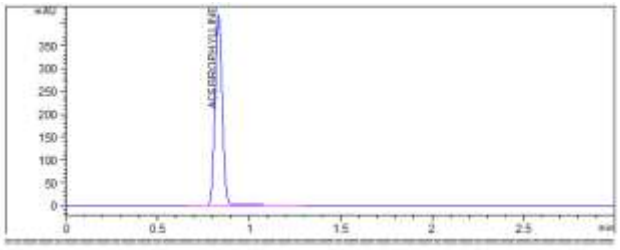
Accuracy standard chromatogram (injection 5)



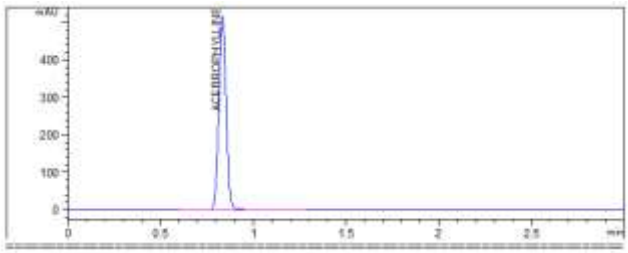
Accuracy Samplechromatogram (80% - injection 1)



AccuracySample chromatogram (80% - injection 2)



Accuracy Sample chromatogram (80% - injection 3)



AccuracySamplechromatogram (100%- injection 1)

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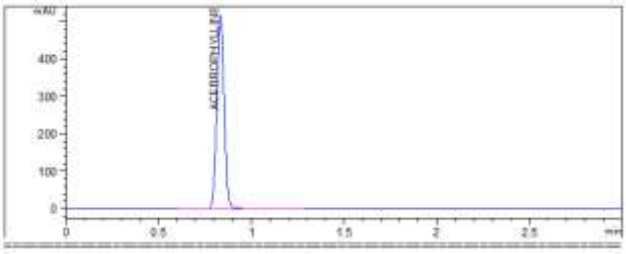
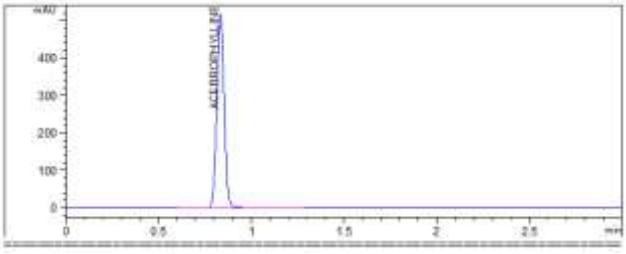
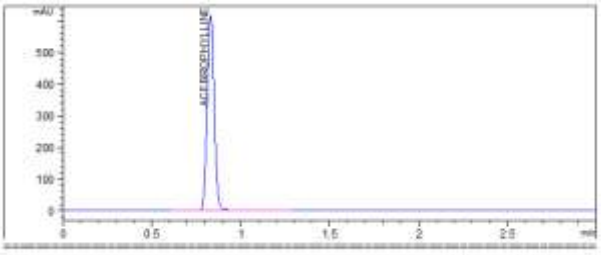


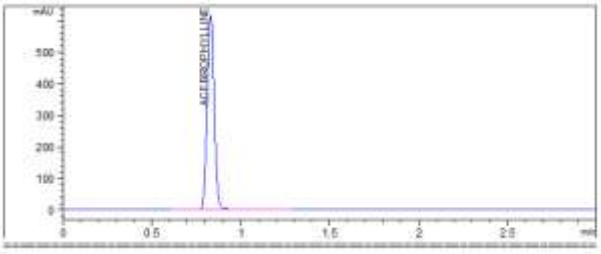
AccuracySample chromatogram (100%- injection 2)



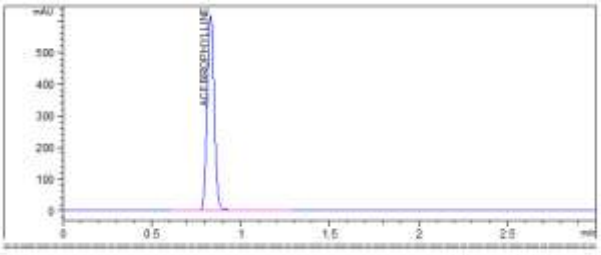
Accuracy Sample chromatogram (100%- injection 3)



AccuracySamplechromatogram (120% - injection 1)



AccuracySample chromatogram (120% - injection 2)



AccuracySample chromatogram (120% - injection 3)

Discussion:
Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean. Percentage recovery was obtained as 99.93% and 101.13% for Acebrophylline.

IV. CONCLUSIONS:

A simple, accurate, precise method was developed for the simultaneous estimation of the Acebrophylline in solid dosage form. Retention time of Acebrophylline were found 0.381 minrespectively. %RSD of the Acebrophylline found 0.26 respectively. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in industries.Percentage recovery was obtained as 99.93% and 101.13% for Acebrophylline.

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