Development and Validation of UV Spectroscopic Method for Estimation of Acebrophylline In Tablet Dosage Form

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ABSTRACT

To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Acebrophylline in tablet dosage form. The drug is freely soluble in analytical grade Ethanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade Ethanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of acebrophylline in tablet dosage form and also validated as per ICH guidelines. The drug is freely soluble in analytical grade Ethanol, slightly soluble in methanol and water. So, the analytical grade Ethanol is used as a diluent in method. The melting point of acebrophylline was found to be 213-214°C (uncorrected). It showed absorption maxima 251 nm in analytical grade Ethanol. On the basis of absorption spectrum the working concentration was set on 10µg/ml (PPM). The linearity was observed between 2-18 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.95%. A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Acebrophylline in tablet dosage form. The method could be considered for the determination of Acebrophylline in quality control laboratories.

Keywords: Acebrophylline, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Acebrophylline is chemically 4-[(2-amino-3,5 dibromophenyl)methylamino]cyclohexan-1-ol;2-(1,3- dimethyl-2,6-dioxopurin-7-yl)acetic acid [1, 2]. It is used as Bronchodilator in treatment of asthma. It is a novel drug with bronchodilating, anti-inflammatory and mucuregulating effect due to inhibition of phospholipase A, and phosphatidylcholine [2, 3]. Acebrophylline is the salt obtained by reaction of equimolar amounts of theophylline-7-acetic acid, a xanthine derivative with specific bronchodilator activity and ambroxol, a mucolytic and expectorant with molecular formula $C_{22}H_{28}Br_2N_6O_5$ and molecular weight 616.302 g/mol as shown in Figure 1 [4, 5, 6].

It is phosphorylated intracellular 1to it's active 5'-thiophosphate metabolite, acebrophylline thiophosphate (L-TP) [6, 7].
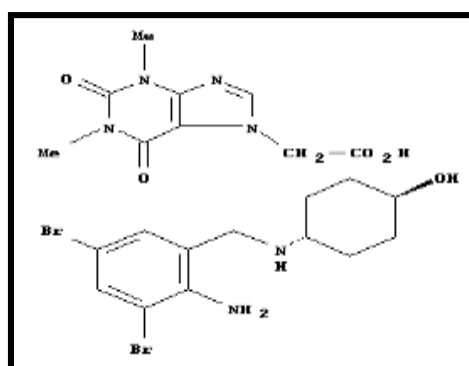

Figure 1: Chemical Structure of Acebrophylline

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 [8, 9, 10].

From literature review it's found that one method was reported on simultaneous estimation of acebrophylline and with combined dosage form [5]. Also on Q-Absorbance ratio estimation of acebrophylline and with combined dosage form [6]. Lot of work done on acebrophylline with other drug in tablet form on RP-HPLC [7], also in capsule form on RP-HPLC [8, 9, 10]. Also the method was reported on LCMS [11] and Simple TLC [12] for acebrophylline and also in combination with other drugs. But very few methods were reported on estimation of acebrophylline in tablet dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Acebrophylline in tablet dosage forms. The aim of the study was to develop a simple, precise, linear, economic and accurate UV method for determination of Acebrophylline in tablet dosage forms.

MATERIALS AND METHOD

Instruments :

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair

Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

Materials:

The pure drug Acebrophylline was obtained as a gift sample from Maceloids Pharma Pvt. Ltd. Andheri. Acebrophylline tablets were procured from local pharmacy. Ethanol used was of analytical grade. Freshly prepared solutions were employed for experiment.

Method development:

Determination of ⅄ max (10 PPM)

100 mg weighed amount of acebrophylline was dissolved into 100 ml of volumetric flask with analytical grade Ethanol. Pipette out 1 ml and added in 100 ml of volumetric flask dissolved and diluted up to the mark with analytical grade Ethanol. This solution was subjected to scanning between 200-380 nm and absorption maximum was determined [15, 16].

Figure 2: Calibration Curve

Preparation of Working concentration

Preparation of Standard stock solution:

Standard stock was prepared by dissolving 100 mg of acebrophylline in 100 ml of analytical grade Ethanol to get concentration of 1000 µg/ml (PPM).

Preparation of Standard solution:

Pipette out 1 ml from standard stock solution and diluted up to 100 ml with analytical grade Ethanol to get concentration of 10 µg/ml (PPM).

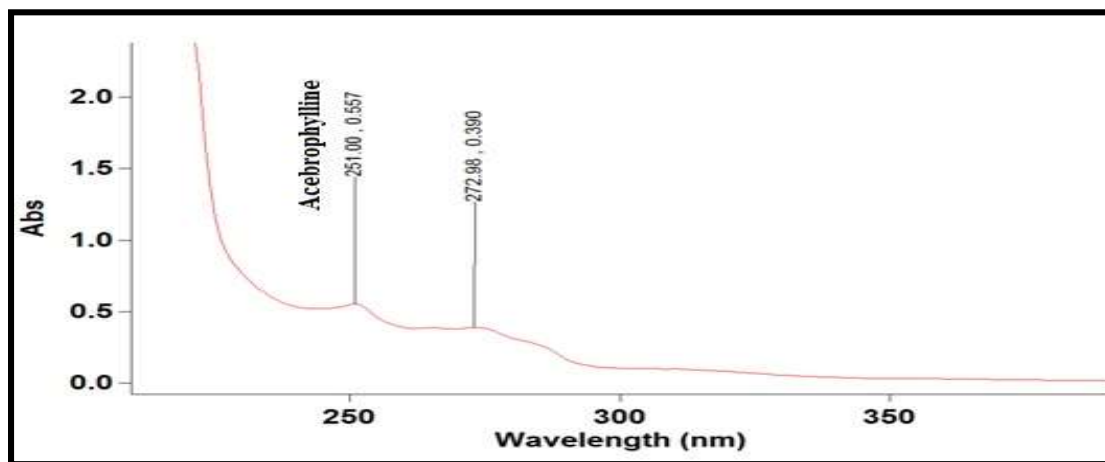**Preparation of Working concentration**

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Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade Ethanol. Wipe it with tissue paper properly then placed inside the chamber. Press Auto zero and note down the reading.

Standard Solution:

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution:

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Procedure for sample preparations

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 100 mg of acebrophylline was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml analytical grade Ethanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade Ethanol. Filtered the solution through whatmann filter paper. Pipette out 1 ml of the above solution and diluted up to 100 ml with analytical grade Ethanol. The absorbance was measured at 251 nm [18, 19, 20, 21]. The absorbance was recorded:

Table 1: Absorbance of Dosage Form

Alkem Pharmaceutical Limited (ABROFYL) (100 mg)		
Sr. no.	Sample	Absorbance
1	Blank	0.0001
2	Standard	0.5880
3	Sample	0.5840

Table 2: Dosage Form Specifications

Type	Company	M.D.	E.D.	Batch No.	Average weight (g)	Assay (%)
1	Alkem Pharma LTD (100mg) (ABROFYL)	06/2018	07/2021	TPF 07844	0.2541	99.8

Method of validation

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996 [14, 19, 20, 21].

Linearity:

The linearity of the proposed assay was studied in the concentration range 2 - 18 PPM at 251nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies

Sr. no.	Sample Concentration	Absorbance
1	2 PPM	0.1250
2	6 PPM	0.3445
3	10 PPM	0.5764
4	14 PPM	0.8241
5	18 PPM	1.0254
Correlation coefficient		**0.999**

Accuracy:

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade Ethanol and measuring the absorbance at 251nm.

Table 4: Accuracy Studies

Accuracy (%)	Qty weighed (mg)	Qty found (mg)	Recovery (98-102%)
80	0.8	0.79	98.75
100	1	1.01	101.00
120	1.2	1.19	99.17

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5784
2	Sample Solution 2	0.5668
3	Sample Solution 3	0.5735

4	Sample Solution 4	0.5787
5	Sample Solution 5	0.5687
MEAN		0.5731
SD		0.0054
% RSD		0.9498

Ruggedness:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5759	0.5784	99.38	0.2628
		0.5810			
2	Analyst 2	0.5840	0.5807	99.75	
		0.5774			

RESULTS AND DISCUSSION

Solubility of Acebrophylline

Solubility test was passed as per criteria.

Table 7: Results for solubility studies

Sr. no.	Title	Result
1	Analytical grade Ethanol	Soluble
2	Methanol	Slightly Soluble
3	Water	Slightly Soluble

Melting point of Acebrophylline

The Melting Point of Acebrophylline was found to be 213-214°C (uncorrected).

Results for linearity for assay method of Acebrophylline

The linearity of method was determined at concentration level ranging from 2 to 18 μg/ml (PPM).

The correlation coefficient value was found to be **(R^2) 0.999.**

Acebrophylline Linearity

$y = 0.0573x + 0.0049$
$R^2 = 0.9994$

$y = 0.0573x + 0.0049$
$R^2 = 0.9994$

Absorbance

Series1, 18, 1.0254
Series1, 14, 0.8241
Series1, 10, 0.5764
Series1, 6, 0.3445
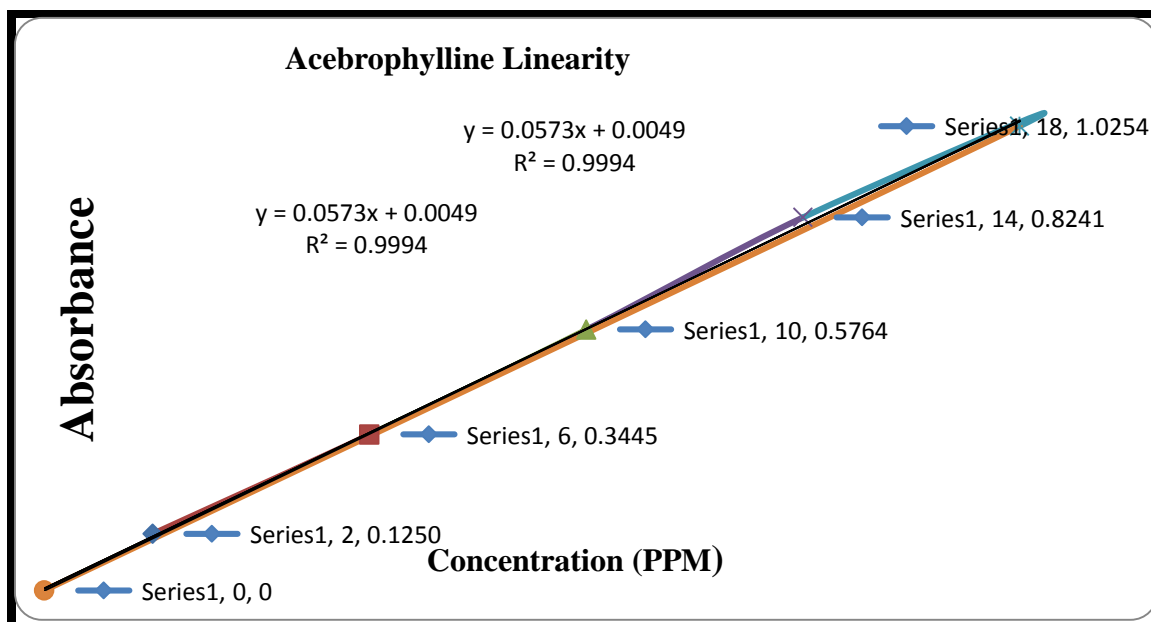Series1, 2, 0.1250
Concentration (PPM)
Series1, 0, 0

Figure 3: Acebrophylline Standard Curve

Results for accuracy for assay method of Acebrophylline

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

Results for precision for assay method of Acebrophylline

The % RSD for different sample of precision was found to be 0.9498 and it is within acceptance criteria represented in Table - 5.

Results for ruggedness for assay method of Acebrophylline

The %RSD for different sample of ruggedness was found to be 0.2628 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Acebrophylline in tablet form has been developed. From the spectrum of Acebrophylline, it was found that the maximum absorbance was 251 nm in analytical grade Ethanol. A good linear relationship was observed in the concentration range of 2-18 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of acebrophylline in solid dosage forms. Hence, the method could be considered for the determination of acebrophylline in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. HBV - Hepatitis B virus
6. DNA - Deoxyribonucleic acid
7. HIV - Human Immunodeficiency Virus
8. ICH - International Council for Harmonization
9. RSD - Relative Standard Deviation
10. SD - Standard Deviation
11. Qty - Quantity
12. C - Celsius
13. M.D. - Manufacturing Date
14. E.D. - Expiry Date

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