UV Specteroscopic Method Development and Validation for Estimation of Acebrophylline in Bulk and Sustained-Release Tablet Dosage Form

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Abstract: **A simple, senstitive and accurate uv spectroscopic method has been developed for the determination of acebrophylline in bulk and sustained-release tablet dosage form as per ICH guidelines. The acebrophylline is freely soluble in Ethanol. The λ max of acebrophylline was found to be 264nm. The drug obeyed the Beer's law and showed good correlation of co-efficient (R^2 = 0.9988 for bulk and R^2 = 0.9986 for sustained-release tablet dosage form) and absorption which reflect in linearity. The obtained percentage recovery value indicated the accuracy, specificity of the method. The percentage RSD was found to be 0.7038, 0.7653,0.6337,0.6585 and 0.3613, 0.4057, 0.8625 ,0.6318 the obtained values are below 2.0 for intraday and interday precision indicated that method is highly precised. The detection quantization limit of LOD is 1.028675 & 1.792253 and LOQ is 3.117198 & 5.43107 calculated respectively and final molar absorptivity was found to be 0.03812 & 0.01922. The results obtained with in the accepted criteria for the respective parameters.**

Key Words: **Acebrophylline, UV-Spectrophotometer, Validation, Accuracy, Precision, Linearity, Sustained-release tablet.**

I.INTRODUCTION

Acebrophylline is also known as ambroxol thiophyllinacetate. It is an anti-inflammatory and Bronchodilator drug used in the treatment of asthma and COPD. Acebrophylline is an adduct formed by chemical interaction between Ambroxol and Acephylline giving a distinct compound. Acebrophylline is a compound produced by salifying of Ambroxol with theophylline-7-acetic acid. Acebrophylline was initially approved by FDA in the year 2006, January 4th.

II.DRUG PROFILE

Summary: Ambroxol acefyllinate is a bronchodilator indicated in the symptomatic treatment of bronchopulmonary disorders associated with bronchospasm.

Class of Drug: A Xanthine Derivative

Structure:

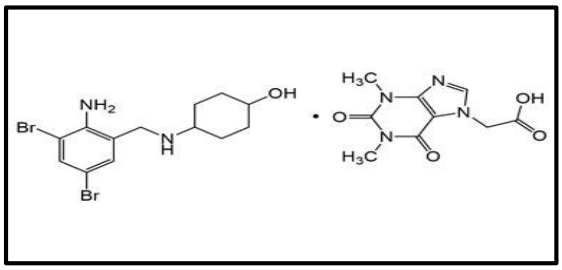

Figure 1: Chemical Structure of Acebrophylline

Chemical Formula: $C_{22}H_{28}Br_2N_6O_5$

IUPAC Name: trans-4-[[(2-Amino-3,5-dibromophenyl)methyl]amino]-cyclohexanol Mono(1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-purine-7-acetate)

Mechanism of action:

Acebrophylline acts as an anti-cholinergic receptor. Its main act is the M3 receptor-blocking action. M3 receptor is a Gi type of G-protein coupled receptor. When Acebrophylline binds to the Gi-type receptor, GDP is converted to GTP, and the alpha, beta, and gamma subunits get detached from the receptors. Followed by alpha-GTP binding to the adenylyl cyclase. It also decreases the formation of cyclic AMP. The release of the Ca2+ ion this case causes the bronchial smooth muscle to constrict. In this process, Acebrophylline binds to the Adrenaline Cyclase process and blocks the cyclic AMP cycle. It also blocks the release of Ca2+ ion, and in the same situation, K+ ion gets released in the bronchial smooth muscle which causes the broncho dilatory action. This effect releases the mucus outside.

Acebrophylline also blocks the leukotriene receptor, hence blocking allergies. It mainly blocks LTD4 and

LTC4 receptors and stops the production of allergic substances. Acebrophylline binds to the phospholipid layer and blocks the production of arachidonic acid. Also, it blocks the Phospholipase A2 which is used to convert phospholipid to Arachidonic acid.

Side Effects of Acebrophylline:
Diarrhoea, Nausea, Vomiting, Dizziness, Heartburn, Stomach discomfort, Skin rash, Drowsiness.

III.MATERIALS & METHOD

Chemicals & Reagents:
The raw material of Acebrophylline (99.76% W/W) was Obtained as a gift sample, which was used as reference material throughout the experiment without any prior treatment. Acebrophylline tablets were purchased from local pharmacy. Ethanol used was of analytical grade. Double distilled water was utilized throughout the process of analysis.

Instrument:
UV-Visible spectrophotometer ElicoSL196 with matched quartz cells corresponding to 10mm path length. Electronic Precision Balance (CTG302) ws used for weighing the material and Ultrasonic Bath Sonicator was used for dissolving.

IV.METHOD DEVELOPMENT

Preparation of Stock Solution:
The standard stock solution of Acebrophylline was prepared by accurately weighing 100 mg of the drug and it was kept in a 100ml volumetric flask. Half the volume of analytical grade ethanol was added. The solution was sonicated for 15 mins and then the volume was made up to the mark with ethanol. The resultant solution was suitably diluting with analytical grade Methanol to get the working standard solutions.

Preparation of Working Standard Solution:
1 ml of standard stock solution was transferred to a volumetric flask (100 ml) and the volume was made up to the mark with analytical grade ethanol so to obtain a concentration of 100 µg/ml. The solution was further diluted to get a final concentration of 10 µg/ml. The solution was used for determination of λ max.

Determination of λ max:

The standard stock solution of Acebrophylline was diluted suitably to get a concentration of 10 µg/ml. The solution was scanned with in the ran200nm-400nm.

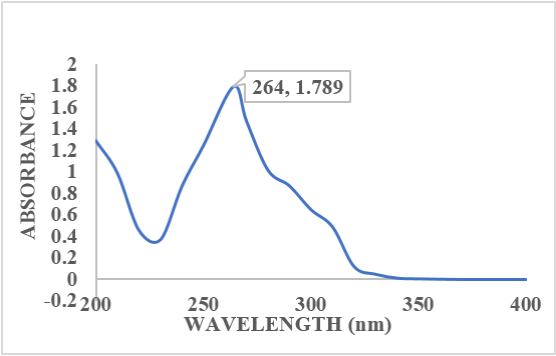

Figure 2: UV Spectrum of Acebrophylline

V.EXPERIMENTAL PROCEDURE OF ACEBROPHYLLINE

100mg standard Acebrophylline tablet powder was weighed and diluted to 100ml with analytical grade ethanol. From the above prepared standard stock solution, 1 ml of the solution was diluted to 100 ml using analytical grade ethanol. Again, from the above prepared solution, 1ml of solution was diluted to 100ml using analytical grade ethanol to get a concentration of 100µg/ml. From the above solution 1 ml, 1.5 ml, 2 ml, 2.5 ml and 3 ml of solutions were pipetted out into 5 different 100 ml volumetric flasks and the volume was made up to 100 ml using Analytical grade ethanol to get the final concentrations of 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml respectively and measured the absorbance of all solutions at 264nm.

VI.METHOD VALIDATION

The proposed method was validated according to ICH guidelines for Linearity, Accuracy, Precision, LOD, LOQ and Molar Absorptivity.

LINEARITY:
The linearity of the proposed method was studied in the concentration range 10-30 µg/ml at 264 nm. A calibration curve was plotted using concentration (on x- axis) against absorbance at 264 nm (on y – axis) from the graph linearity regression co-efficient y-intercept was calculated.

Table 1: Linearity studies of Acebrophylline in Bulk form

Concentration (ug/ml)	Absorbance
10	0.158
15	0.324
20	0.54
25	0.724
30	0.911

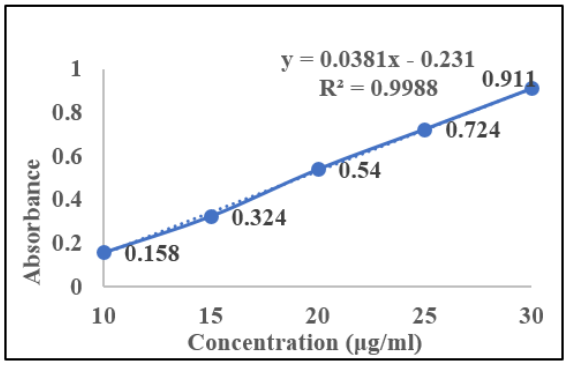

Figure 3: Calibration graph of Acebrophylline in Bulk form

Table 2: Linearity studies of Acebrophylline in Sustained-release tablet dosage form

Concentration (µg/ml)	Absorbance
10	0.114
15	0.198
20	0.286
25	0.389
30	0.499

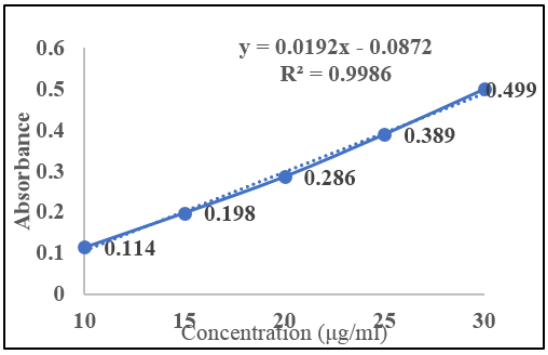

Figure 4: Calibration graph of Acebrophylline in Sustained-release Tablet dosage form

ACCURACY:

The accuracy of the method was demonstrated by recovery experiment performed at three different levels, i.e, 75%, 100% and 125%, 3 different solutions of same concentration i.e, 11.25µg/ml,15 µg/ml and 18.75 µg/ml were prepared and analysed on a day and the absorbance was noted. According to ICH guidelines the % RSD value should not exceeded upto 2%.

The formula for calculating the %RSD is %RSD = SD/Mean x 100

Table 3: Accuracy data for Acebrophylline in Bulk form

S. no	Concentration	Sample	Absorbance at 264 nm	Statistical analysis
1	75%	15	0.411	Mean=0.409331 S.D=0.001528 %RSD=0.373176
		15	0.409	
		15	0.408	
2	100%	20	0.537	Mean=0.536666 S.D=0.000577 %RSD=0.107581
		20	0.536	
		20	0.537	
3	125%	25	0.633	Mean=0.633999 S.D=0.001 %RSD=0.157729
		25	0.634	
		25	0.635	

Table 4: Accuracy data for Acebrophylline in Sustained-release tablet dosage form

S. no	Concentration	Sample	Absorbance at 264 nm	Statistical analysis
1	75%	15	0.403	Mean=0.401999 S.D=0.001 %RSD=0.248757
		15	0.402	
		15	0.401	
2	100%	20	0.541	Mean=0.542332 S.D=0.001528 %RSD=0.281659
		20	0.542	
		20	0.544	
3	125%	25	0.613	Mean=0.613666 S.D=0.001155 %RSD=0.188164
		25	0.615	
		25	0.613	

PRECISION:

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Two different concentrations of acebrophylline in the linear range (20 and 25 µg/ml) were analyzed in six independent series in the same day (intra-day precision) and in six consecutive days (inter-day precision) and results were given in Table 5,6,7&8.

Table 5: Intraday precision data for Acebrophylline in Bulk form

S.no	Sample (µg/ml)	Absorbance at 264nm	Statistical analysis
1	15	0.533	Mean=0.536656 S.D=0.003777 %RSD=0.703826
2	15	0.532	
3	15	0.536	
4	15	0.538	
5	15	0.539	
6	15	0.542	
7	20	0.632	Mean=0.634997 S.D=0.002098 %RSD=0.765317
8	20	0.633	
9	20	0.635	
10	20	0.636	
11	20	0.637	
12	20	0.637	

Table 6: Inter-day precision data for Acebrophylline in Bulk form

S.no	Sample (µg/ml)	Absorbance at 264nm	Statistical analysis
1	15	0.534	Mean=0.537164 S.D=0.001941 %RSD=0.361303
2	15	0.536	
3	15	0.537	
4	15	0.538	
5	15	0.539	
6	15	0.539	
7	20	0.635	Mean=0.63999 S.D=0.003899 %RSD=0.405755
8	20	0.637	
9	20	0.639	
10	20	0.641	
11	20	0.642	
12	20	0.646	

Table 7: Intraday precision data for Acebrophylline in Sustained-release Tablet dosage form

S.no	Sample (µg/ml)	Absorbance at 264nm	Statistical analysis
1	15	0.531	Mean=0.534324 S.D=0.003386 %RSD=0.633744
2	15	0.532	
3	15	0.532	
4	15	0.534	
5	15	0.538	
6	15	0.539	
7	20	0.634	Mean=0.63997 S.D=0.004195 %RSD=0.658558
8	20	0.635	
9	20	0.637	
10	20	0.638	
11	20	0.639	
12	20	0.639	

Table 8: Inter-day precision data for Acebrophylline in Sustained-release Tablet dosage form

S.no	Sample (µg/ml)	Absorbance at 264nm	Statistical analysis
1	15	0.535	Mean=0.540817 S.D=0.004665 %RSD=0.862583
2	15	0.537	
3	15	0.539	
4	15	0.542	
5	15	0.545	
6	15	0.547	
7	20	0.638	Mean=0.641156 S.D=0.00407 %RSD=0.631834
8	20	0.642	
9	20	0.643	
10	20	0.645	
11	20	0.648	
12	20	0.649	

LIMIT OF DETECTION (LOD):

The limit of detection (LOD) was determined based on standard deviation of calibration curve. The standard deviation of absorbance of calibration curve and slope of calibration curve were used. According to formula LOD was calculated and found to be 1.028675&1.792253 µg/ml.

$$LOD = 3.3 \times SD/S$$

Where,

S = slope of calibration curve

SD = standard deviation of calibration curve

LOD OF ACEBROPHYLLINE IN BULK FORM:

$$LOD = 3.3 \times SD/Slope$$
$$= 3.3 \times 0.011882761/ 0.03812$$
$$= 1.028675\mu g/ml$$

LOD OF ACEBROPHYLLINE IN SUSTAINED-RELEASE TABLET DOSAGE FORM:

$$LOD = 3.3 \times SD/Slope$$
$$= 3.3 \times 0.010435516/ 0.01922$$
$$= 1.792253\mu g/ml$$

LIMIT OF QUANTIFICATION (LOQ):

The limit of Quantification (LOQ) as determined based on standard deviation and slope of calibration

curve. According to the formula LOQ was calculated and found to be 3.117198&5.43107µg/ml.

LOQ = 10 x SD/S

Where,

S = slope of calibration curve

SD = standard deviation of calibration curve

LOQ OF ACEBROPHYLLINE IN BULK FORM:

LOQ = 10 x SD/Slope

= 10 x 0.011882761/0.03812

= 3.117198 µg/ml

LOQ OF ACEBROPHYLLINE IN SUSTAINED-RELEASE TABLET DOSAGE FORM:

LOQ = 10 x SD/Slope

= 10 x 0.010435516/ 0.01922

= 5.43107 µg/ml

MOLAR ABSORPTIVITY:

Molar Absorption coefficient is a spectrophotometric unit indicating the light a substance absorbs with respect to length, usually centimeters and concentration usually moles per liter. Molar absorptivity is particularly useful in spectrometry for measuring the concentration of chemical solutions.

Molar absorptivity is calculated by using the following formula:

Molar absorptivity = Slope / Pathlength

Where,

Slope = log (y2-y1 / x2-x1)

MOLAR ABSORPTIVITY OF ACEBROPLYLLINE IN BULK FORM:

Molar absorptivity = Slope/ Pathlength

= 0.03812/1

= 0.03812 $mol^{-1}cm^{-1}$

MOLAR ABSORPTIVITY OF ACEBROPHYLLINE IN SUSTAINED- RELEASE TABLET DOSAGE FORM:

Molar absorptivity = Slope /Pathlength

= 0.01922/1

= 0.01922 $mol^{-1}cm^{-1}$

VII. RESULTS AND DISCUSSION

Acebrophylline in Bulk Form:

The method was developed and validated as per ICH guidelines. The method was validated in terms of Linearity, Precision, Accuracy, LOD, LOQ and Molar absorptivity. Detection wavelength was selected at 264 nm. Linearity in response was observed on 10-30 µg/ml. The linearity equation was found to be y = 0.0381 x – 0.231, R^2 = 0.9988. The precision results showed a % RSD = 0.703826 & 0.765317 at intraday, % RSD = 0.361303 & 0.405755 at inter-day clearly indicating that the method was precise enough for the analysis of Acebrophylline. The accuracy of the method was checked by recovery studies. The LOD = 1.028675 µg/ml and LOQ = 3.117198 µg/ml indicate sensitivity of the method. The molar absorptivity was found to be 0.03812 $mol^{-1}cm^{-1}$.

Acebrophylline in Sustained-release Tablet dosage Form:

The method was developed and validated as per ICH guidelines. The method was validated in terms of Linearity, Precision, Accuracy, LOD, LOQ and Molar absorptivity. Detection wavelength was selected at 264 nm. Linearity in response was observed on 10-30 µg/ml. The linearity equation was found to be y = 0.0192 x – 0.0872, R2 =0.9986. The precision results showed a % RSD = 0.633744 & 0.658558 at intraday, %RSD = 0.862583 & 0.631834 at inter-day clearly indicating that the method was precise enough for the analysis of Acebrophylline. The accuracy of the method was checked by recovery studies. The LOD = 1.792253 µg/ml and LOQ = 5.43107 µg/ml indicate sensitivity of the method. The molar absorptivity was found to be 0.01922 $mol^{-1}cm^{-1}$.

RESULTSTABLE OF ACEBROPHYLLINE IN BULK AND SUSTAINED-RELEASE TABLET DOSAGE FORM:

Characteristic Parameters	Acebrophylline in Bulk form			Acebrophylline in Sustained-release Tablet Dosage form		
λ Max	264 nm			264 nm		
Beer's Law limit (µg/ml)	10 – 30 (µg/ml)			10 – 30 (µg/ml)		
Linearity						
Correlation Coefficient (R^2)	R^2 = 0.9988			R^2 = 0.9986		
Regression Equation	y = 0.0381 x - 0.231			y = 0.0912 x – 0.0872		
Slope (m)	0.03812			0.01922		
Intercept (c)	-0.231			-0.0872		
Accuracy						
Concentration (µg/ml)	75%	100%	125%	75%	100%	125%
%RSD	0.3731	0.1075	0.1577	0.2487	0.2816	0.1881
Precision						
Inter-day precision						
Concentration (µg/ml)	Mean	SD	%RSD	Mean	SD	%RSD
15	0.537	0.001	0.3613	0.540	0.004	0.8625
20	0.639	0.003	0.4057	0.644	0.004	0.6318
Intraday precision						
Concentration (µg/ml)	Mean	SD	%RSD	Mean	SD	%RSD
15	0.536	0.0037	0.7038	0.534	0.0033	0.6337
20	0.634	0.0020	0.7653	0.636	0.0041	0.6585
LOD (µg/ml)	1.028675			1.792253		
LOQ (µg/ml)	3.117198			5.43107		
Molar absorptivity ($Mol^{-1}cm^{-1}$)	0.03812			0.01922		

CONCLUSION

A Validation UV spectrophotometric method has been developed for the estimation of acebrophylline in bulk and sustained-release tablet dosage form. In this proposed method the linearity was Observed in the concentration range of 10-30µg/ml with correlation coefficient R2 - 0.999 for acyclovir at 264 nm. The developed method was found to be simple, accurate, precise, specific, reproducible and linear over the concentration range studies. The proposed method can be used for routine analysis of acebrophylline. The method was validated as per ICH guideline.

ABBREVATIONS

1. nm – Nanometer
2. UV – Ultra violet
3. ICH – International Council for Harmonization
4. RSD – Relative Standard Deviation
5. SD – Standard Deviation
6. LOD – Limit of Detection
7. LOQ – Limit of Quantification

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