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Stability Indicating Method Development and Validation for the Simultaneous Estimation of N-Acetylcysteine and Acebrophylline in Tablet Dosage form by RP-HPLC

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Abstract:- A quick, accurate, precise Stability indicating method for Simultaneous estimation of N-Acetylcysteine and Acebrophylline in Tablet dosage form was developed and validated. Chromatogram was run on Waters C18 (150 x 4.6 mm, 2 µm) Column. Mobile phase containing OPA Buffer and Methanol taken in a ratio of 10:90 % v/v was pumped at a flow rate of 1.5 mL/min through column. Cushion utilized in this strategy was OPA. Column temperature was kept at 35°C. Injection volume was 5 µL and Run time was 5 minutes. Sample was scanned at 285 nm. RT's of N-Acetylcysteine and Acebrophylline were found to be 2.646 min and 2.117 min respectively. The developed method was validated and was found to be Accurate, Precise and Linear over the Concentration Range of 25 % to 175 % of Test concentration. Retention time and Runtime are less and the method was properly validated so this method can be utilized for routine analysis and stability studies of Assay of N-Acetylcysteine and Acebrophylline in Tablet dosage form in industries.

Keywords:- *N-Acetylcysteine, Acebrophylline, Stability Indicating, Simultaneous Estimation, Tablet Dosage form, RP-HPLC.*

I. INTRODUCTION

N-Acetylcysteine is a mucolytic drug prescribed for respiratory conditions characterized by excessive mucus production. It helps to thin and break up mucus (phlegm), making it easier to expel through coughing. Additionally, it serves as an antidote in cases of paracetamol overdose by replenishing the body's levels of glutathione, a substance that helps detoxify harmful compounds.

Acebrophylline is a medication that serves as both a mucolytic and a bronchodilator. It functions by relaxing the airway muscles, thinning and loosening mucus, which facilitates easier breathing. This medication is utilized in managing respiratory conditions like asthma, characterized by the narrowing of airways that makes breathing challenging, and chronic obstructive pulmonary diseases (COPD), a group of disorders that result in obstructed airflow and breathing difficulties.

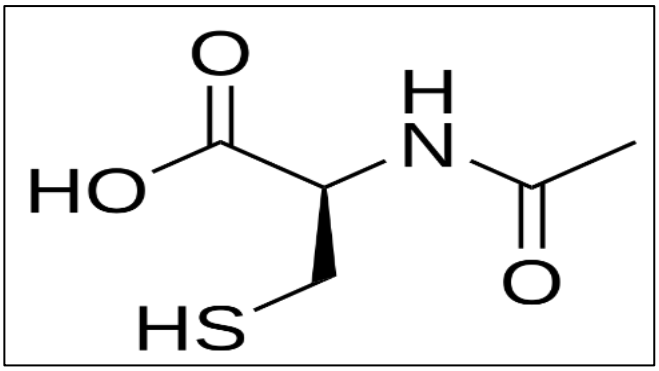

Fig 1 Structure of N-Acetylcysteine

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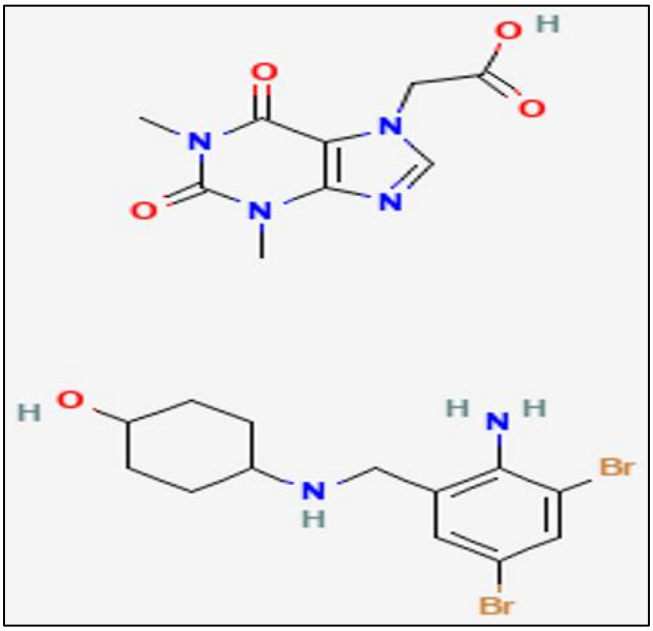

Fig 2 Structure of Acebrophylline

II. MATERIALS AND METHODS

➢ *Chemicals and Reagents:*

N-Acetylcysteine and Acebrophylline API, N-Acetylcysteine and Acebrophylline combination tablets (Pulmoclear tablets), Milli-Q Water, Methanol, Ortho phosphoric acid, All the reagents are from Merk.

➢ *Instruments:*

- Balance-Sartorius
- p^H meter -Metrohm
- Ultra sonicator-Leela sonic
- Waters HPLC with PDA detector and Auto sampler with Empower 2 Software.

➢ *Chromatographic Conditions:*

Column: Waters C18 (150 x 4.6 mm, 2 µm), Wavelength: 285 nm, Isocratic Flow: 10:90 % v/v (OPA Buffer and Methanol), Flow rate: 1.5 mL/min, Injection volume: 5 µL, Column temperature: 35°C, Sample temperature: 20°C, Run time: 5.0 minutes.

➢ *Preparation of Solutions:*

- *Diluent:*

Based on the solubility and dissolution ability of the drugs, diluent was chosen. Methanol and Water were taken in a ratio of 55:45 % v/v.

- *Preparation of Standard Stock Solution of N-Acetylcysteine:*

Precisely weighed and transferred 200 mg of N-Acetylcysteine API into 250 mL volumetric flask. Added 25 mL of diluent and sonicated to dissolve. Volume was made up to mark by adding diluent.

- *Preparation of Standard Stock Solution of Acebrophylline:*

Precisely weighed and transferred 80 mg of Acebrophylline API into 200 mL volumetric flask. Added 20 mL of diluent and sonicated to dissolve. Volume was made up to mark by adding diluent.

- *Preparation of Standard Solution:*

Pipetted out 3 mL of N-Acetylcysteine standard stock solution and 1 mL of Acebrophylline standard stock solution into 50 mL volumetric flask and volume was made up with diluent. (Concentration of N-Acetylcysteine and Acebrophylline were 48 µg/mL and 8 µg/mL respectively).

- *Preparation of Sample Stock Solution:*

10 tablets were weighed and the average weight of 1 tablet was calculated, the tablets were crushed and the weight identical to 1 tablet was transferred into a 250 mL Volumetric flask, 30 mL of diluent was added and sonicated for 15 min. Volume was made up to mark with diluent.

- *Preparation of Sample Solution:*

2 mL of sample stock solution was transferred into 100 mL volumetric flask and volume was made up with diluent. (Concentration of N-Acetylcysteine and Acebrophylline were 48 µg/mL and 8 µg/mL respectively).

- *Preparation of Buffer Solutions:*

✓ *0.5 % OPA Buffer:*

5 mL of ortho phosphoric acid was added to 1 liter of Milli Q water.

- *Preparation of Mobile Phase:*

0.5 % OPA Buffer and Methanol were taken in ratio of 10:90 % v/v and mixed well.

➢ *Method Validation*

- *System Suitability Parameters:*

Five replicates of standard solution were injected into HPLC and analyzed to ensure that the system was fit to use.

➢ *Specificity:*

- *Blank Preparation:*

Diluent was prepared and injected into HPLC

- *Placebo Preparation:*

Weighed and transferred placebo equivalent to weight of placebo in 1 tablet into a 250 mL Volumetric flask, 30 mL of diluent was added and sonicated for 15 min. Volume was made up to mark with diluent. 2 mL of this solution was pipetted into 100 mL volumetric flask and volume was made up with diluent.

- *Impurities Preparation:*

Solutions of individual impurities of N-Acetylcysteine and Acebrophylline were prepared at 1 % level of test concentration and injected into HPLC.

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➢ *Accuracy:*

- *Preparation of 25 % Concentration Sample:*

Weighed, transferred 150 mg N-Acetylcysteine API, 25 mg of Acebrophylline API and Placebo of weight equivalent to weight of placebo in 1 tablet into a 250 mL Volumetric flask, 30 mL of diluent was added and sonicated for 15 min. Volume was made up to mark with diluent. 2 mL of this solution was transferred into 100 mL volumetric flask and volume was made up with diluent.

- *Preparation of 100 % Concentration Sample:*

Weighed, transferred 600 mg N-Acetylcysteine API, 100 mg of Acebrophylline API and Placebo of weight equivalent to weight of placebo in 1 tablet into a 250 mL Volumetric flask, 30 mL of diluent was added and sonicated for 15 min. Volume was made up to mark with diluent. 2 mL of this solution was transferred into 100 mL volumetric flask and volume was made up with diluent.

- *Preparation of 175 % Concentration Sample:*

Weighed, transferred 1050 mg N-Acetylcysteine API, 175 mg of Acebrophylline API and Placebo of weight equivalent to weight of placebo in 1 tablet into a 250 mL Volumetric flask, 30 mL of diluent was added and sonicated for 15 min. Volume was made up to mark with diluent. 2 mL of this solution was transferred into 100 mL volumetric flask and volume was made up with diluent.

➢ *Linearity:*

- *Preparation of Standard Solution of 25 % Concentration Level:*

Prepared and analyzed standard solution of concentration of 12 µg/mL of N-Acetylcysteine and 2 µg/mL of Acebrophylline.

- *Preparation of Standard Solution of 50 % Concentration Level:*

Prepared and analyzed standard solution of concentration of 24 µg/mL of N-Acetylcysteine and 4 µg/mL of Acebrophylline.

- *Preparation of Standard Solution of 75 % Concentration Level:*

Prepared and analyzed standard solution of concentration of 36 µg/mL of N-Acetylcysteine and 6 µg/mL of Acebrophylline.

- *Preparation of Standard Solution of 100 % Concentration Level:*

Prepared and analyzed standard solution of concentration of 48 µg/mL of N-Acetylcysteine and 8 µg/mL of Acebrophylline.

- *Preparation of Standard Solution of 125 % Concentration Level:*

Prepared and analyzed standard solution of concentration of 60 µg/mL of N-Acetylcysteine and 10 µg/mL of Acebrophylline.

- *Preparation of Standard Solution of 150 % Concentration Level:*

Prepared and analyzed standard solution of concentration of 72 µg/mL of N-Acetylcysteine and 12 µg/mL of Acebrophylline.

- *Preparation of Standard Solution of 175 % Concentration Level:*

Prepared and analyzed standard solution of concentration of 84 µg/mL of N-Acetylcysteine and 14 µg/mL of Acebrophylline.

- *Precision:*

Six individual Sample solutions were prepared and injected into HPLC and analyzed.

- *Robustness:*

Effect of Variation of Flow Rate (±0.2 mL/min), Column oven temperature (±2°C) and Mobile phase composition (±5 %) on assay was analyzed.

- *Forced Degradation Studies:*

Drug product and placebo were exposed to Acid, Base, Peroxide, Thermal, Humidity, Photolytic degradation and the net degradation of drugs were studied.

III. RESULTS AND DISCUSSION

➢ *System Suitability:*

The system suitability parameters of five replicate standard injections of N- Acetylcysteine and Acebrophylline were found to be within the acceptance limits. (Refer Table 1)

➢ *Specificity:*

None of the Blank, Placebo, Impurity peaks interfered with the retention times of N- Acetylcysteine and Acebrophylline peaks. (Refer Table 2)

➢ *Accuracy:*

Accuracy was checked at 3 concentration intervals and the % of Recovery, Mean % of Recovery, % RSD results at each level were found satisfactory. (Refer Table 3, 4)

➢ *Linearity:*

The Square of correlation coefficient and % Y-intercept at 100 % response were found to be 1.000 and 0.01 for N-Acetylcysteine, 0.999 and 0.03 for Acebrophylline respectively. (Refer Table 5, 6)

➢ *Precision:*

The percentage of assay of N-Acetylcysteine and Acebrophylline for each individual sample and mean of six samples should be between 90.0 % w/w to 110.0 % w/w of labeled quantity of N-Acetylcysteine and Acebrophylline. The percent RSD for percent assay of six sample preparations was found to be 0.3 for both N-Acetylcysteine and Acebrophylline. (Refer Table 7)

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➢ *Robustness:*

It was found that the developed method remained unaffected due to deliberate variations in Flow Rate, Column oven temperature and Mobile phase composition. Hence developed method was Robust. (Refer Table 8)

➢ *Forced Degradation:*

None of the degradant peaks interfered with the retention times of N-Acetylcysteine and Acebrophylline peaks. Peak purity of N-Acetylcysteine and Acebrophylline peaks has passed in all the stressed samples. Percentage of Net degradation was within the limits. (Refer Table 9, 10)

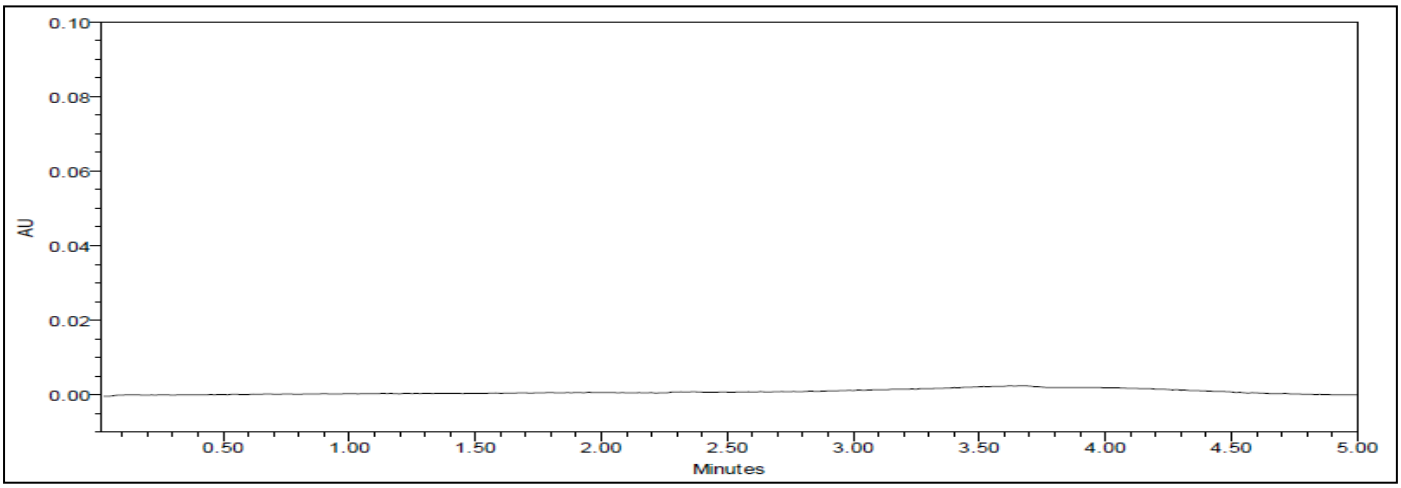

Fig 3 Blank Chromatogram

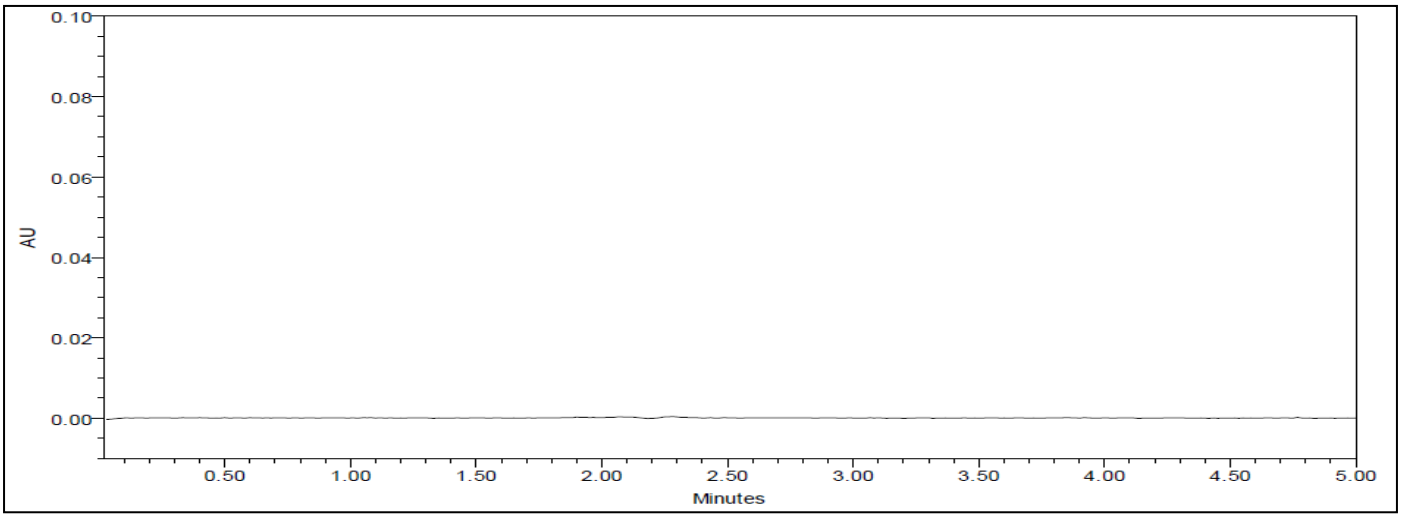

Fig 4 Placebo Chromatogram

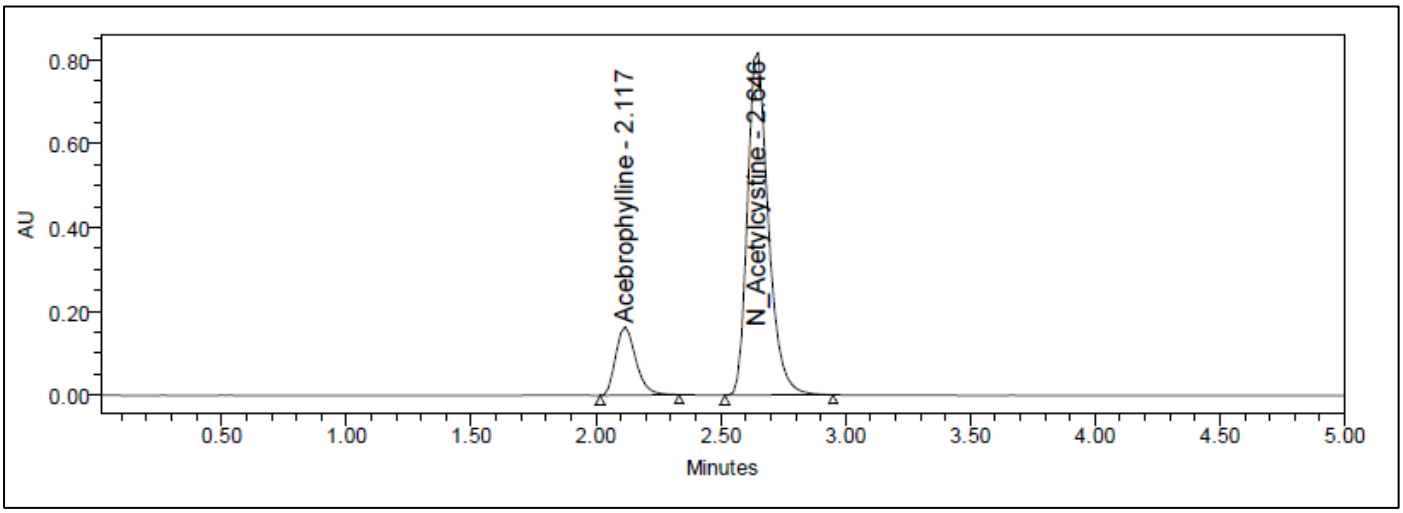

Fig 5 Standard Chromatogram

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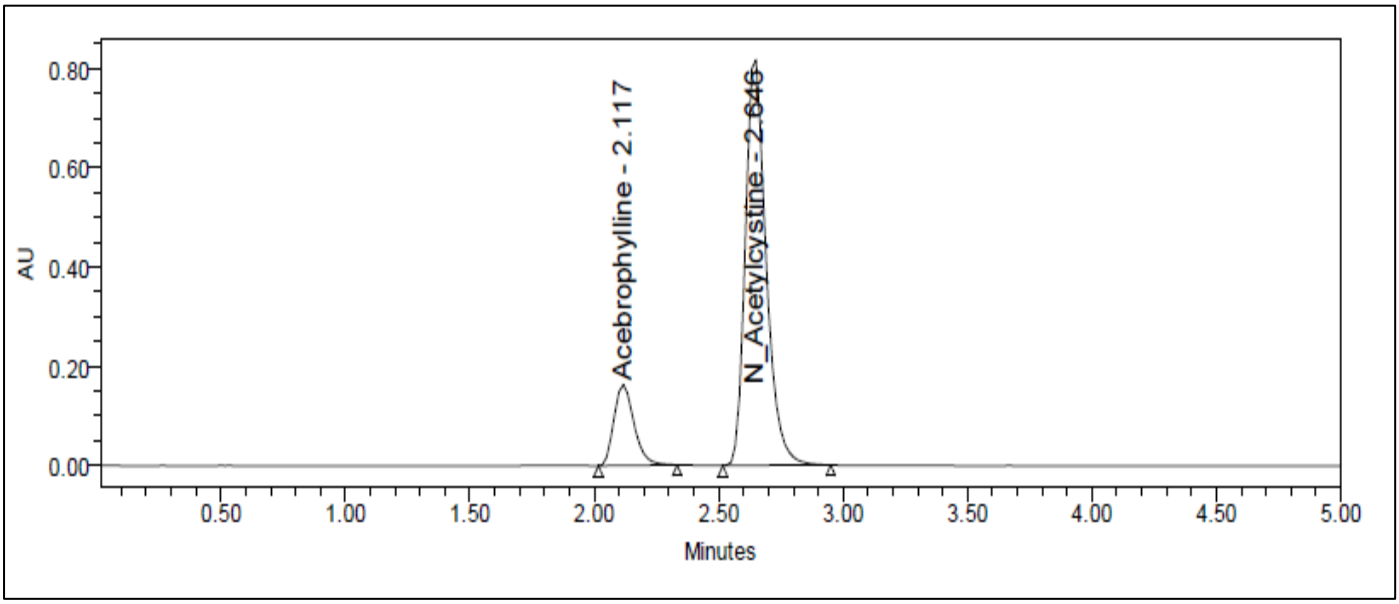

Fig 6 Sample Chromatogram

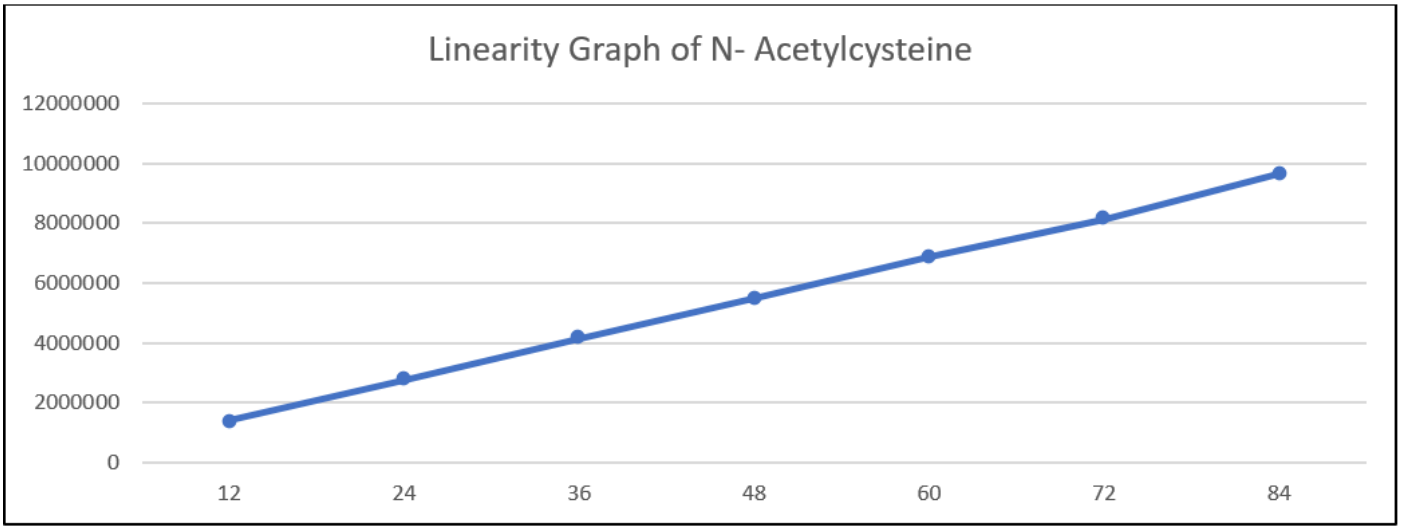

Fig 7 Linearity Graph of N-Acetylcysteine

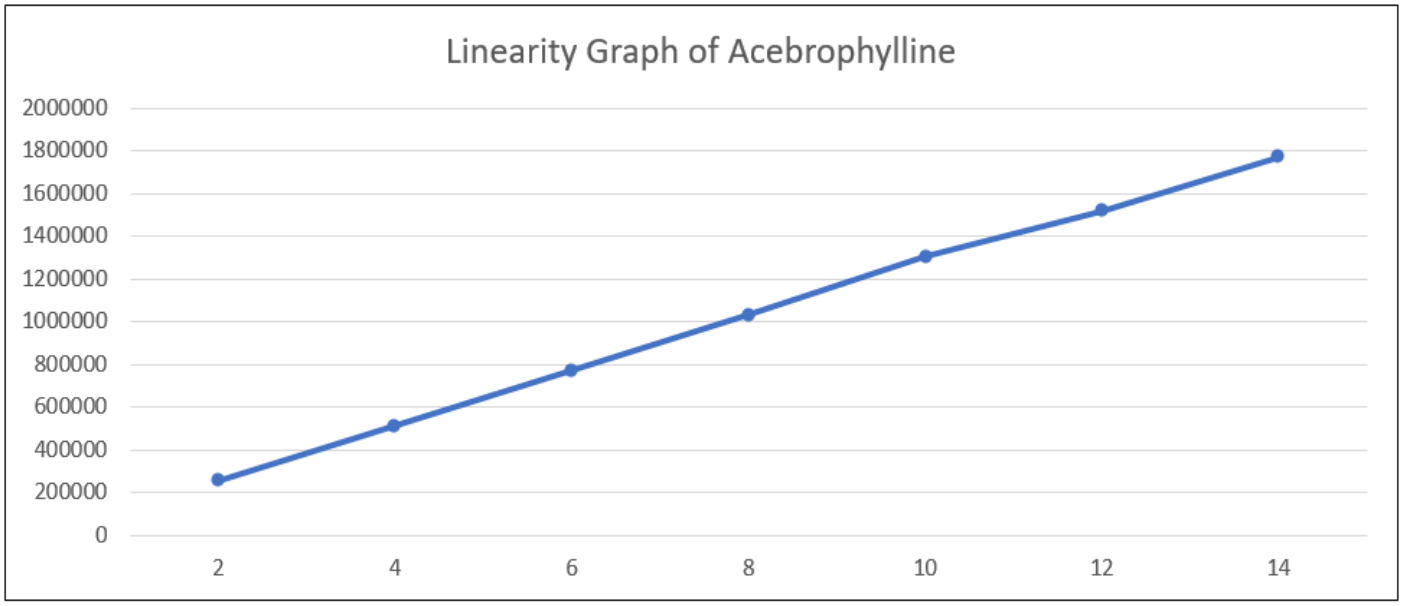

Fig 8 Linearity Graph of Acebrophylline

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Table 1 Results of System Suitability

Parameter	Observed Value		Acceptance Criteria
	N- Acetylcysteine	Acebrophylline	
%RSD (peak area)	0.7	0.6	NMT 2.0
Tailing factor	0.9	0.7	NMT 2.0
Plate count	6001	7200	NLT 2000

Table 2 Results for Specificity

Result	Acceptance Criteria
No peaks interfered with the retention times of N-Acetylcysteine and Acebrophylline peaks.	None of the Blank, Placebo, Impurity peaks should interfere with the retention times of N- Acetylcysteine and Acebrophylline peaks.

Table 3 Results of Accuracy of N-Acetylcysteine

Accuracy of N- Acetylcysteine					
% Level	Added Amount (mg)	Found Amount (mg)	% of Recovery (Limit:98.0 to 102.0 %)	Mean % of Recovery (Limit:98.0 to 102.0 %)	% RSD (NMT 2.0)
25%	150.3	150.1	99.9	100	0.1
	150.0	150.0	100.0		
	150.2	150.3	100.1		
100%	600.5	600.5	100.0	99.9	0.1
	600.9	600.3	99.9		
	600.7	600.0	99.9		
175%	1050.7	1048.0	99.7	99.8	0.1
	1050.2	1049.1	99.9		
	1050.5	1047.0	99.7		

Table 4 Results of Accuracy of Acebrophylline

Accuracy of Acebrophylline					
% Level	Added Amount (mg)	Found Amount (mg)	% of Recovery (Limit:98.0 to 102.0 %)	Mean % of Recovery (Limit:98.0 to 102.0 %)	% RSD (NMT 2.0)
25%	25.6	25.2	98.4	98.3	1.0
	25	24.8	99.2		
	24.8	24.1	97.2		
100%	100.2	100.5	100.3	99.8	0.5
	100.5	100.1	99.6		
	100.8	100.2	99.4		
175%	175.7	175.0	99.6	99.6	0.1
	175.2	174.3	99.5		
	175.8	175.1	99.6		

Table 5 Results of Linearity of N- Acetylcysteine

Linearity of N- Acetylcysteine	
Concentration (µg/mL)	Peak Area
12.04	1379340
24.08	2761023
36.06	4154192
48.10 (100 % Level)	5473553
60.12	6871894
72.11	8138462
84.14	9654390
Square of correlation coefficient (R^2) (Limit: should not be less than 0.999)	1.000
% Y-intercept at 100% response (Limit: should be within ±2.0)	0.01

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Table 6 Results of Linearity of Acebrophylline

Linearity of Acebrophylline	
Concentration (µg/mL)	**Peak Area**
2.01	252712
4.03	511421
6.02	773636
8.03 (100 % Level)	1028729
10.10	1302899
12.13	1517632
14.15	1767999
Square of correlation coefficient (R^2) (Limit: should not be less than 0.999)	0.999
% Y-intercept at 100% response (Limit: should be within ±2.0.)	0.03

Table 7 Results of Method Precision

Sample Number	**% Assay of N- Acetylcysteine**	**% Assay of Acebrophylline**
1	100.7	99.5
2	100.8	99.7
3	101.5	99.8
4	101.1	99.8
5	100.8	100.2
6	100.9	100.1
Mean	101	100
% RSD (NMT 2.0)	0.3	0.3

Table 8 Results of Robustness

Result	**Acceptance Criteria**
System suitability parameters were within the acceptance criteria for N- Acetylcysteine and Acebrophylline for variations in Flow Rate, Column oven temperature and Mobile phase composition.	System suitability parameters should meet for variations in Flow Rate, Column oven temperature and Mobile phase composition.

Table 9 Results of Forced Degradation of N- Acetylcysteine

Forced Degradation Studies of N- Acetylcysteine			
Degradation Condition	**% Assay of N-Acetylcysteine**	**% Degradation of N-Acetylcysteine**	**Peak Purity**
Control Sample	98.5	NA	No impurity detected
Acid	93.5	5	No impurity detected
Base	94.2	4.3	No impurity detected
Peroxide	95	3.5	No impurity detected
Thermal	96.4	2.1	No impurity detected
Humidity	96.5	2.0	No impurity detected
Photilytic	96.2	2.3	No impurity detected

Table 10 Results of Forced Degradation of Acebrophylline

Forced Degradation Studies of Acebrophylline			
Degradation Condition	**% Assay of Acebrophylline**	**% Degradation of Acebrophylline**	**Peak Purity**
Control Sample	99	NA	No impurity detected
Acid	94.2	4.8	No impurity detected
Base	93.9	5.1	No impurity detected
Peroxide	93.7	5.3	No impurity detected
Thermal	97.8	1.2	No impurity detected
Humidity	97.5	1.5	No impurity detected
Photilytic	97.2	1.8	No impurity detected

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IV. CONCLUSION

A Stability indicating method was developed and validated as per ICH guidelines for Simultaneous estimation of N-Acetylcysteine and Acebrophylline in Tablet dosage form. The method was found to be Accurate, Precise and Linear in the Concentration Range of 25 % to 175 % of Test concentration. Forced degradation studies were performed and the stability of N-Acetylcysteine and Acebrophylline were determined. The method resisted the deliberate variations that were made to the method parameters, Hence the method was found be Robust and can be used in industries for routine analysis and stability studies of Assay of N-Acetylcysteine and Acebrophylline in Tablet dosage form.

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