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STABILITY INDICATING UPLC METHOD OPTIMISATION AND VALIDATION OF ACETYLCYSTEINE IN SYRUP DOSAGE FORM

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ABSTRACT:
A selective, precise, accurate and stability indicating UPLC method is validated for estimation of Acetylcysteine in syrup dosage form. The method employed, with Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column in gradient mode, with mobile phase of Methanol and Acetonitrile in the ratio of 40:60 %v/v. The flow rate was 1 ml/min and effluent was monitored at 215nm. Retention time was found to be 3.90±0.15 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100µg/ml respectively. The LOD and LOQ values for were found to be 2.10(µg/ml) and 6.3643(µg/ml) respectively. No chromatographic interference from syrup's excipients and degradants were found. The proposed method was successfully used for estimation of Acetylcysteine in syrup dosage form.

Keywords: Acetylcysteine, UPLC, Validation, stability indicating method.

1. INTRODUCTION

Acetylcysteine, a mucolytic agent used in the treatment of bronchitis or pulmonary diseases. It depolymerises mucopolysaccharides, reduces the viscosity of pulmonary secretions, chemically it is, (2R)-2-acetylamino-3-Sulfanylpropanoic acid [1-4] (Fig. 1). The analytical data are a prerequisite for correct interpretation of any dosage form. The objective of UPLC method development and validation of Acetylcysteine in syrup dosage form procedure is to provide information about its stability in stress conditions.[6-9] The validation of a specific method must be demonstrated through laboratory experiments by routinely analysing samples.[10-13]

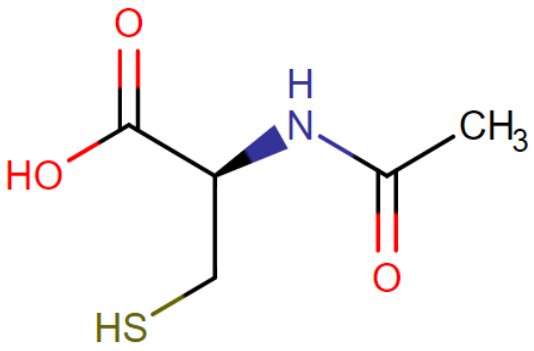


Fig.1: Molecular Structure of Acetylcysteine, (2R)-2-acetylamino-3-Sulfanylpropanoic acid.

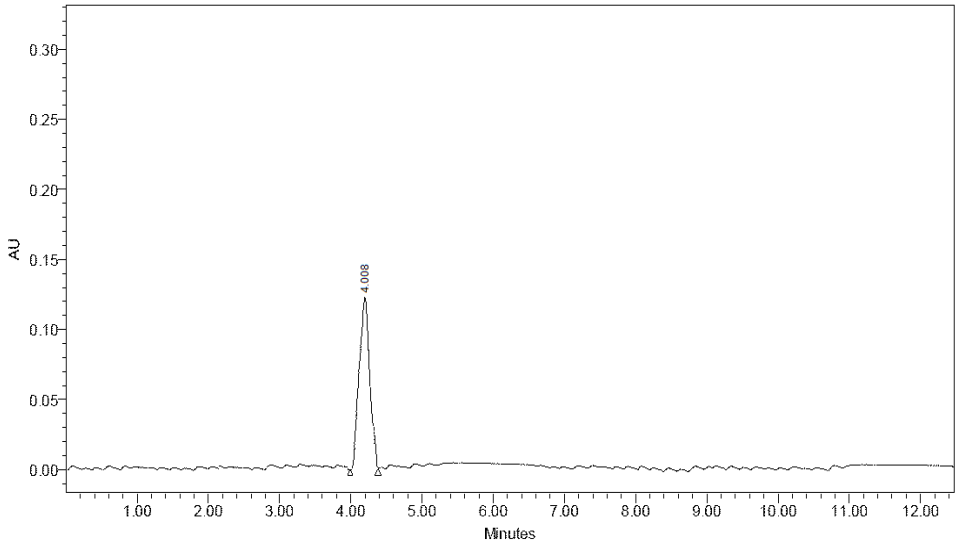


Fig. 2: Standard Chromatogram of Acetylcysteine, using mobile phase of Methanol and Acetonitrile in the ratio of 40:60 %v/v.

2. EXPERIMENTAL

Materials

Acetylcysteine (98.00 % purity) used as analytical standard was procured from Active Pharma Labs (Hyderabad). UPLC grade methanol, Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were analytical grade. The syrup - dosage (Sandoz Canada Incorporated 200 mg) Each mL of the 20% solution contains Acetylcysteine 200 mg. All chemicals used were of pharmaceutical or special analytical grade.

Instrumentation

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis. The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerex sonicator was used for enhancing dissolution of the compounds.

Chromatographic Conditions:

Table 1: Chromatographic Conditions of the validating method.

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm)
Mobile Phase	Methanol : acetonitrile 40:60 %v/v
Flow rate	1mL/min
Run time	12 Min.
Column Temperature	Maintained at 25°C
Injection volume	20 µL
Detection wavelength	215 nm
Diluent	Mobile Phase

Preparation of Standard Stock Solution

Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.0 mL min-1 shows good resolution. The PDA detector response of Acetylcysteine was studied and the best wavelength was found to be 215 nm showing highest sensitivity.

The mixture of two solutions methanol : acetonitrile 40:60 %v/v. The buffer used is Potassium dihydrogen phosphate transferred to 100 ml of water and sonicated well. The pH of the solution was adjusted to 3 with orthophosphoric acid solution. Gradient programming was employed to mobile phase at 1.0 mL/min flow rate was found to be an appropriate mobile phase for separation of Acetylcysteine. The column was maintained at 25°C temperature.

Preparation of internal standard solution

Weighed accurately about 10 mg of D-Phenylalanine into a clean and dry 100 mL volumetric flask, dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Acetylcysteine standard solution

Transfer accurately about 10 mg of Acetylcysteine into 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of standard stock solution of

working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. Linearity was determined in the range of 20-100 µg mL-1.

Stability Indicating Studies

Stability Indicating studies like acid hydrolysis, basic hydrolysis, dry heat degradation, wet heat degradation and oxidative degradation were carried out.

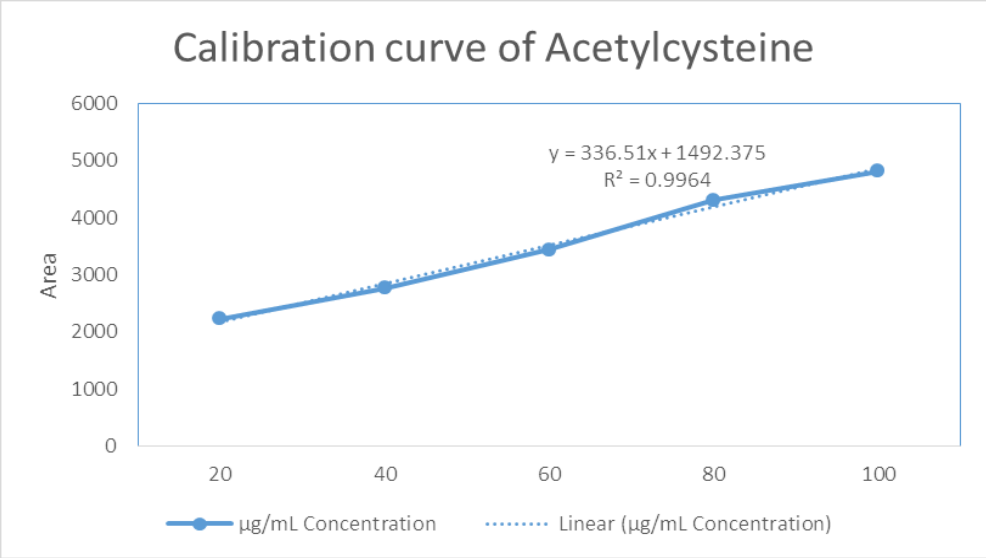
3. RESULTS AND DISCUSSIONS

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

Linearity and Range

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample. The calibration curve showed good linearity in the range of 20-100 µg/mL, for Acetylcysteine with correlation coefficient of 0.9964. A typical calibration curve has the regression equation of y = 336.51x + 1492.375 for Acetylcysteine. Results are given in Table 2.



Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Acetylcysteine were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of Acetylcysteine was found to be 2.10(µg/ml) and the LOQ of Acetylcysteine was found to be 6.3643(µg/ml). Results are given in Table 2.

Table 1: Chromatographic Conditions of the validating method.

PARAMETER	ACETYLCYSTEINE
Linearity	20 – 100 µg/ml
Intercept (c)	1492.375
Slope (m)	336.51
Correlation coefficient	0.9964
LOD	2.10 (µg/ml)
LOQ	6.3643 (µg/ml)

Precision

The Precision of the method was studied in terms of intraday and interday precision of sample injections (20 µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.11%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.09%. Low % RSD values indicate that the method is precise. The results are given in table 3.

Accuracy

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favor of (% RSD < 2%) the developed UPLC method for the analysis of Acetylcysteine. The results are given in table 5.

Table: 3, Results of Precision Studies

Acetylcysteine			
Precision Studies			
Parameter	**Peak Area**	**% RSD**	**%LC**
Intraday precision	2224.28	0.11%	99.76%
	2226.59		99.87%
	2228.98		99.98%
Inter day precision	2224.82	0.09%	99.79%
	2225.93		99.84%
	2228.76		99.97%
Instrument:1 Acquity UPLC Waters,2695H	2226.32	0.01%	99.86%
	2225.79		99.83%
	2226.39		99.86%
Instrument:2 Agilent Technologies,1290	2226.12	0.02%	99.85%
	2225.64		99.83%
	2226.33		99.86%
Average			**99.85**
Std.Dev			**0.06307**
%RSD			**0.06%**

Table: 4, Results of accuracy study.

Acetylcysteine						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	10.21	10.17	99.60	99.70%	0.0929	0.09%
100	20.40	20.35	99.75			
150	30.63	30.56	99.77			

Table: 5, Results of Robustness Studies.

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	2227.41	0.01%
	Actual	2226.95	
	Plus	2226.87	
Temperature	**Low**	2228.33	0.04%
	Actual	2227.98	
	Plus	2226.59	
Wavelength	**Low**	2228.24	0.02%
	Actual	2227.85	
	Plus	2227.53	

Results of Stability Indicating Studies

According to Singh and Bakshi, the stress testing suggests a target degradation of 20-80 % for establishing stability indicating nature of the method. UPLC study of samples obtained on stress testing of Acetylcysteine under different conditions using mixture Methanol: acetonitrile 40:60 %v/v as a mobile solvent system suggested the following degradation behaviour.

a. Acid hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 40:60 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 3.

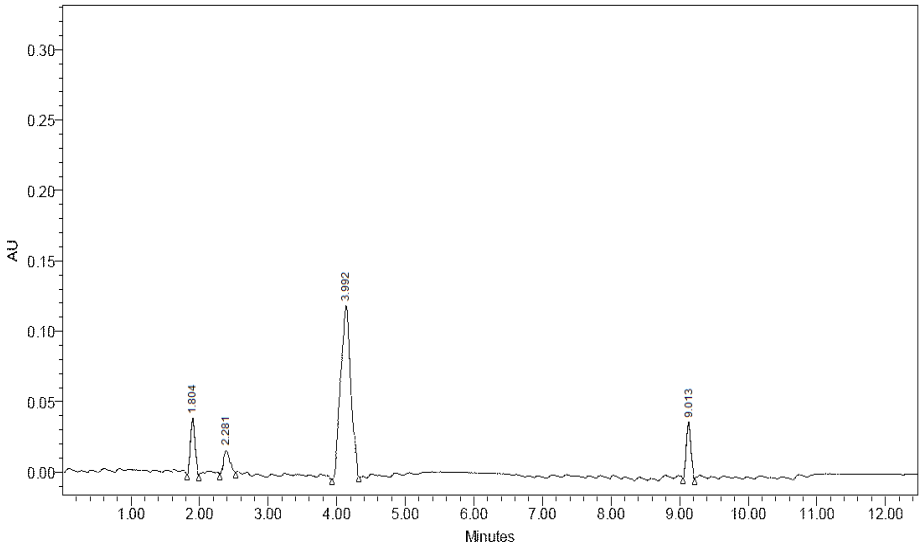


Fig. 3: Chromatogram showing the degraded products in Acidic degradation.

b. Basic hydrolysis:

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 40:60 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 4.

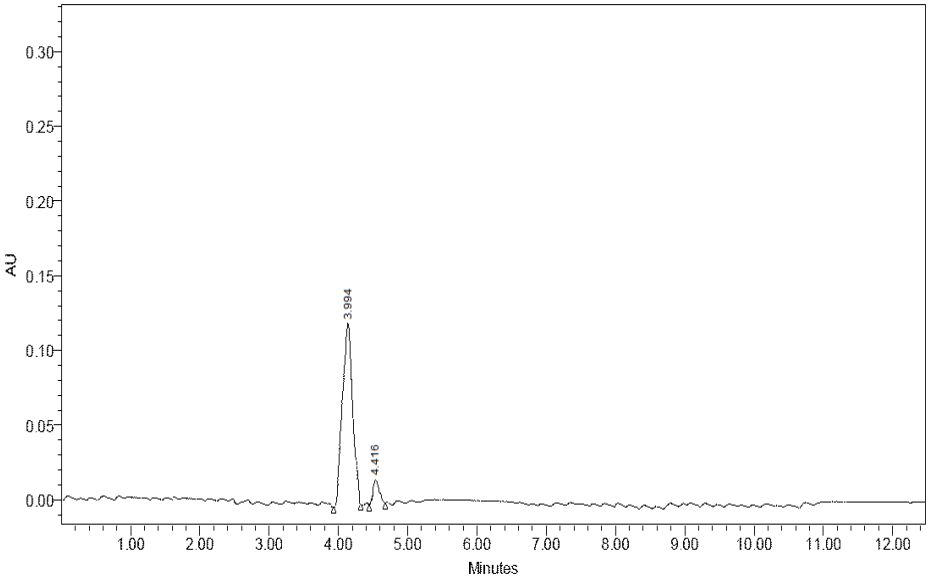


Fig. 4: Chromatogram showing the degraded products in Basic degradation.

c. Wet heat degradation

Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 40:60 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 5.

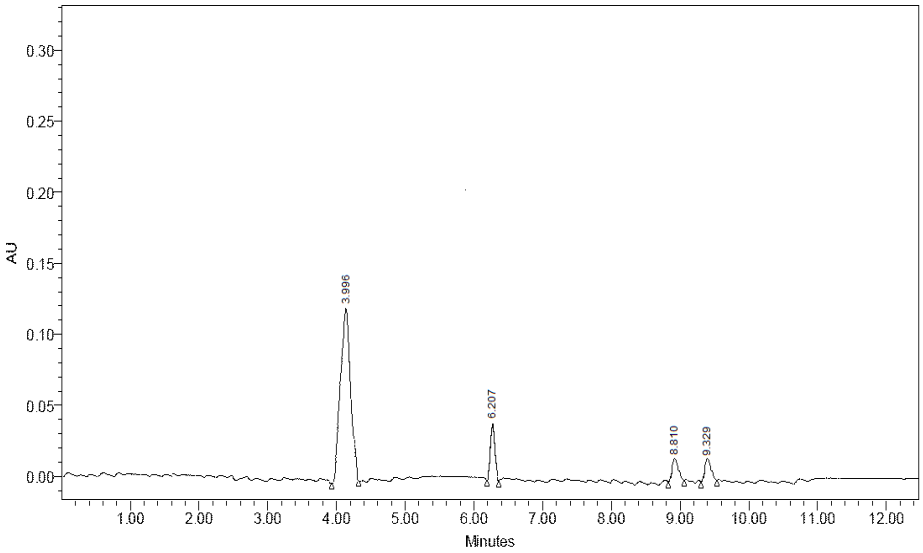


Fig. 5: Chromatogram showing the degraded products in Wet heat degradation.

d. Oxidation with (3%) H_2O_2

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Fig. 6.

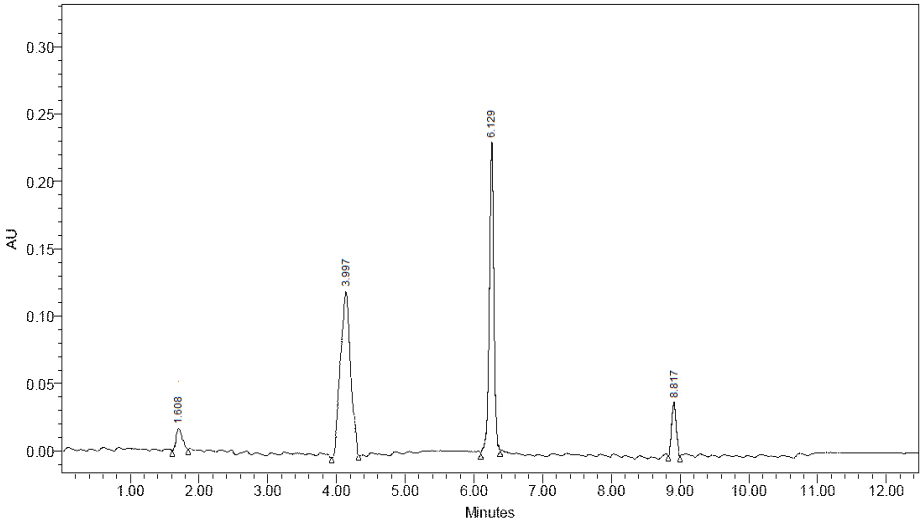


Fig. 6: Chromatogram showing the degraded products in H_2O_2.

In all degradation studies, there was a significant formation of degradation products when compared to that of a standard. This indicates that, the drug may be degraded to low molecular weight non-chromophoric compounds.

Table 6: Stability Indicating study for the developed method.

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)
Acidic	60°C	3	3 (1.804, 2.281, 9.013)
Basic	60°C	9	1 (4.416)
Oxidative	RT	48	3 (1.608, 6.129, 8.817)
Wet Heat	105°C	24	3 (6.207, 8.810, 9.329)

4. CONCLUSION

A selective and sensitive stability indicating UPLC method has been validated for the analysis of Acetylcysteine in bulk drug and syrup dosage form. Based on peak purity results, obtained from the analysis of stability indicating studying samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Acetylcysteine indicated that the developed method is specific for the estimation of Acetylcysteine in presence of degradation products. Further the proposed UPLC method has excellent precision, sensitivity and reproducibility. Even though no attempt has been made to identify the degraded products, proposed method can be used as stability indicating method for assay of Acetylcysteine in commercial formulations.

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