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Research Article

A STABILITY INDICATING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC (UPLC) METHOD FOR THE DETERMINATION OF ASSAY OF CARBOCISTEINE IN VARIOUS FORMULATION PRODUCTS

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ABSTRACT

A simple, rapid, precise and accurate isocratic reversed-phase stability-indicating HPLC method was developed and validated for the determination of carbocisteine (CS) a mucolytic and expectorant drug with an amino acid structure was developed and validated in commercial tablets, capsules and syrups. The method has shown adequate separation for CST from its associated main impurities and the degradation products. Separation was achieved on a Nucleosil C18, 3µm, 100 mm × 4.6 mm. column using a mobile phase consisting of 0.01 M ammonium dihydrogen ortho phosphate buffer (pH 2.6) at a flow rate of 1ml/min and UV detection at 220 nm. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress conditions. Photoisomerization product carbocisteine lactum and sulfoxidation[1] product carbocisteine Sulfoxide were determined and separated well demonstrating the utility of the method for studies of forced degradation. The linearity of the proposed method was investigated in the range of 0.1–0.3 µg/ml (r^2 = 0.9997). The limit of detection was 0.007µg/ml and the limit of quantitation was 0.021µg/ml. Degradation products produced as a result of stress studies did not interfere with the detection of CST assay can thus be considered stability-indicating.

Keywords: Chromatography, UPLC-UV, Stability Indicating, Carbocisteine

INTRODUCTION

Carbocisteine is chemically described as (2R)-2-amino-3-[(carboxymethyl) sulfanyl] propanoic acid[2] is a mucolytic expectorant. Carbocisteine is a mucolytic that reduces the viscosity of sputum and so can be used to help relieve the symptoms of chronic obstructive pulmonary disorder(COPD) and bronchiectasis by allowing the sufferer to bring up sputum more easily. Carbocisteine should not be used with antitussives (cough suppressants) or medicines that dry up bronchial secretions. Carbocisteine is official in European pharmacopoeia and the estimation is by potentiometry. The literature survey reveals that there is no stability indicating method available for the estimation of carbocisteine formulations. A few attempts have been made as titration, potentiometric and Ion chromatographic determination. A succesful attempt was made to estimate the carbocisteine. Therefore it is worthwhile to develop and validate an accurate and rapid RP-UPLC method for estimation of carbocisteine from various formulations. The chemical structure of carbocisteine is as shown in the figure1.

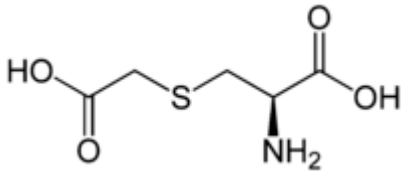

Fig. 1: Chemical Structure of Carbocisteine

MATERIAL AND METHODS

Instrumentation

For UPLC method development, waters aquity UPLC system equipped with a binary pump, an auto sampler, a thermostatted column heater/cooler and diode array detector(DAD) was used (waters, USA) Diode array data analysis was performed using waters empower2 software.

MATERIALS

Carbocisteine (99.0% purity, fig.1) used as a standard was purchased from LGC Promochem. The acetic acid, hydrochloric acid was purchased from Merck chemicals India. 5M NaOH, 5M HCl, H_3PO_4 and 30% H_2O_2 used were purchased from Merck Chemicals with analytical reagent grade. Sample preparation was made by adding 4mL of 1N hydrochloric acid with water. Ammonium dihydrogen phosphate buffer was made by adding 1.15g to 1lit of water and adjusting the pH 2.5 ± 0.05 with CH_3COOH.An in house formulation (capsule, tablet and syrup) and placebo were used for the validation of method for specificity, accuracy, precision and linearity. Each capsule contains a declared amount of 375mg of Carbocisteine. In addition, two commercially available solid dosage forms containing Carbocisteine analysed using this method.

Liquid Chromatographic Conditions and Mobile Phase:

The column used was a Nucleosil C_{18} (100 x 4.6 mm) 3µm column. The mobile phase was 100% of buffer pH 2.5 with H_3PO_4. The flow rate was 1.0mL/min, the wavelength was 220nm, the injection volume was 10µL,the column temperature was thermostatted at 25°C,the run time was 5min,the diluent was hydrochloric acid and water and quantitation was performed using peak area counts.

Standard Preparation

Weighed and transferred accurately about 45 mg of Carbocisteine working standard into a 50 ml clean and dry volumetric flask, added 1ml of 1M hydrochloric acid and about 30 ml of water, sonicate to dissolve and made up to volume with water and mix. Further dilute 5ml of this solution to 20ml with mobile phase.

Sample Preparation

Weighed and transferred an accurate quantity of the powder, equivalent to about 375mg of, carbocisteine in to a 200 ml clean and dry volumetric flask. Added about 4ml of 1M hydrochloric acid and about 150ml of water and sonicate for about 20 minutes and make up to volume with the water. Further diluted 3ml of this solution to 25ml with mobile phase. Filtered through 0.45 µ membrane filter.

RESULTS AND DISCUSSION

Method Development

As the substance is official in European pharmacopoeia with potentiometric[2] assay, and there are certain methods like Ion chromatographic conductimetric detection method[3], HPLC[4,5,6,7] and the experiment was started using different buffers and mobile phases. A shorter column Nucleosil C18 (100 x 4.6mm) ID, 3µm was used in order to improve the overall separation. UV detection was performed at 220nm.The flow rate was 1.0mL/min and the column was used to evaluate temperature 30°C,35°C and 40°C. Increasing the temperature shortened retention times for

carbocisteine and it's degradant, with minor selectivity changes, and room temperature was chosen because of improvements in column efficiency compared to temperature. Increasing the buffer strength increased carbocisteine retention relative to the degradant, with 0.01M ammonium acetate buffer being chosen as optimal. The UV absorbance for carbocisteine was highest at 220 nm, and was chosen. The pH 2.6 was selected depending on the pKa value of carbocisteine and ratio of acetonitrile to buffer were optimized with the set conditions at room temperature,220 nm wavelength and 1.0 ml min^{-1} flow rate. The pH (2.0, 2.2, and 3) and percent methanol (5, 10, and 15) were varied, and retention time data on carbocisteine and its degradant were entered into empower software for analysis. The optimal conditions were finalized to those listed in the Section 2. Fig. 2 shows typical HPLC chromatograms of carbocisteine standard and carbocisteine in a pharmaceutical formulation.

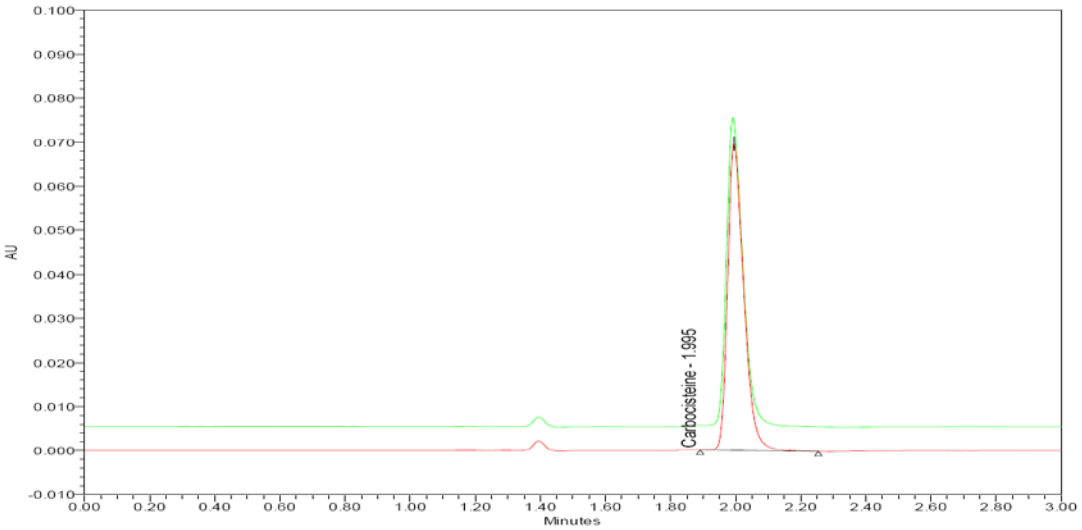

Fig. 2: Standard and sample chromatogram of carbocisteine

System Suitability

A system suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability/calibration standard and one injection of a check standard were made. Area relative standard deviation, tailing factor, and efficiency for the five suitability injections were determined. The check standard was quantified against the average of the five suitability injections. For all sample analyses, the tailing factor was ≤2.0; efficiency ≥2000, %R.S.D. ≤2.0%, and 100±2.0% check standard recovery.

Linearity

A set of six standards at the following concentrations were prepared: 0.11, 0.16, 0.23, 0.27, 0.32 and 0.34, mg mL^{-1} of carbocisteine. This set ranges from 50% to 150% of the nominal assay concentration of 0.225 mg/ml of carbocisteine. Each of the six standards was analyzed. Table 1 shows the results. The calibration curve was constructed by plotting the peak area against the concentration using linear regression analysis. It showed that the slope was 3932473 with a *y*-intercept of -14179.33 and a correlation coefficient or regression coefficient of 0.9997, indicates an excellent linearity.

Table 1: Linearity Results

% Nominal(Assay)	Concentration mg/mL	Peak Area	
50	0.11	454183	
75	0.16	640177	**Y=3932473x-14179.33**
100	0.23	907541	**r^2 = 0.9998**
120	0.27	1078368	**Slope=3932473**
140	0.32	1268507	
150	0.34	1359737	

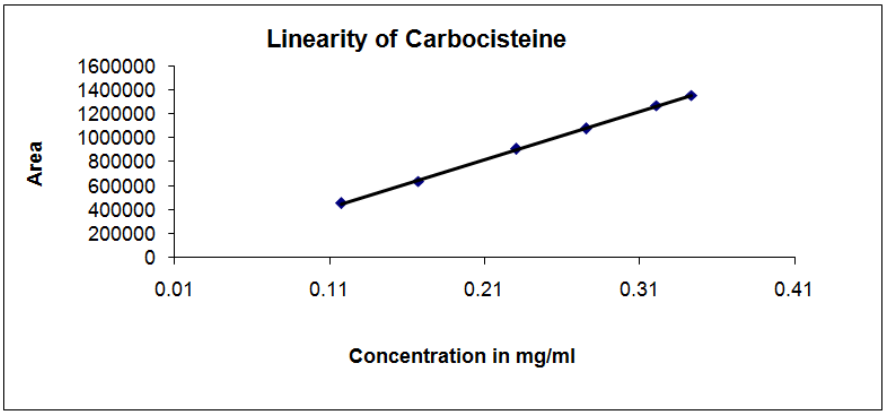

Fig. 3: Linearity graph of carbocisteine

Accuracy

The accuracy was demonstrated by preparing placebo samples that were additionally spiked approximately 80%,100% and 120% of the theoretical carbocisteine concentration level in sample. The spiked placebo samples at the 80%,100%, and 120% level were prepared in triplicate, using carbocisteine in the solid form for spiking the placebo. The results in Table 2 shows that the average recovery at each level was within 100.0±2.0% and the %R.S.D. at each level was ≤2.0%.

Specificity/Forced degradation studies

The forced degradation study was conducted by subjecting raw material, placebo, and capsules, tablets formulation samples to heat, oxidation, acid, and alkali degradation. The samples were appropriately neutralized and analyzed using the method. Single wavelength data at 220 nm was collected by following the method. Additional photodiode array data was collected for the purposes of the peak purity evaluation. Thermal degradation was induced by storing the samples at 105°C±2◦ C for a period of 24 hours. Oxidative degradation was induced by treating with 30% hydrogen peroxide for a period of 1hour. The specificity experiments showed that, in all the conditions carbocisteine degraded was less than 20%.Baseline resolution between carbocisteine and degradation product peaks was achieved. Diode array detection peak homogeneity tests showed that no peak interfered with the carbocisteine peak. Peak purity for carbocisteine was established with the help of empower2 software. The purity angle is less than purity threshold indicating the peak is pure.

Method Precision

The repeatability of the method was demonstrated by preparing and analyzing six sample replicates from a homogenous composite blend of 20 tablets. The result in Table 3 shows that the average recovery was within 100.0 ±2.0% of label claim, and the %R.S.D. was below 1.0%, indicating excellent precision.

Table 2: Accuracy Results

% Level	Sample	% Recovery	Mean	% R.S.D.
80	1	99.2	98.9	0.33
	2	98.5		
	3	98.8		
100	1	99.8	99.7	0.50
	2	99.2		
	3	100.2		
120	1	99.1	98.8	0.39
	2	98.9		
	3	98.4		

Table 3: Precision Results

Sample	Carbocisteine 375mg/caps	%Assay	Carbocisteine 750mg/tabs	%Assay	Carbocisteine 250mg/5ml	%Assay
1	372.1	99.2	750.1	100.0	248.5	99.4
2	369.5	98.5	748.9	99.9	249.2	99.7
3	371.6	99.1	749.4	99.9	246.7	98.7
4	372.6	99.4	748.6	99.8	247.3	98.9
5	371.1	99.0	745.2	99.2	248.4	99.4
6	369.8	98.6	740.9	98.8	246.8	98.7
Mean	**371.1**	**99.0**	**747.2**	**99.6**	**247.8**	**99.1**
%RSD	**0.33**	**0.33**	**0.47**	**0.49**	**0.41**	**0.43**

Solution Stability

The stability of carbocisteine in standard and sample preparation was evaluated. The solutions were stored at ambient temperature and tested at intervals of 0, 2, 4, and 48 hours. The responses for the aged solutions were evaluated using a freshly prepared standard. The results in Table 4 show that sample and standard solutions retained a potency of 100.0±2.0% as compared with the fresh solution over a time of 48 hours.

Table 4: Solution stability results

Interval	Standard Solution Stability	Sample Solution Stability
Initial	99.9	99.5
1 hours	99.6	99.1
6 hours	99.7	99.6
12 hours	99.2	98.9
24 hours	98.9	99.1
48hours	98.7	98.4

Application to commercial Dosage Forms

The developed method was applied for the determination of carbocisteine content in three marketed products contains single active carbocisteine. Products are in tablets dosage form, capsules dosage form and liquid dosage form. The method was also used for analyzing the stability samples of formulation. The samples were stored at 40°C/75%RH, pulled periodically and analyzed for the active content. The chromatograms from stability samples and commercial products showed the well separation of carbocisteine peak with other degradants or components. The purity of the carbocisteine peak was checked by PDA and found to be pure, demonstrating that no interference existed inside carbocisteine and degradants or other components. The results showed the method was suitable for stability-indicating analysis and assay for commercial products.

Robustness

The influence of chromatographic parameters on the separation was investigated. The parameters examined were the amount of acetonitrile in mobile phase, flow and pH. Their effects on the resolution of different peaks were evaluated by means of an experimental design.The chromatographic parameter settings in the experimental design were showed in the Table 5 and results were tabulated in table 6.

Table 5: Robustness parameters

Parameter	Lower Value(-)	Central Value	High Value(+)
Acetonitrile	0	2	4
Flow	0.8	1.0	1.2
pH	2.4	2.6	2.8

Table 6: Robustness Results

Parameter	Variation	% Assay
Acetonitrile	**0**	**99.8**
	4	**100.1**
Flow	0.8	99.6
	1.2	98.9
pH	2.4	99.1
	2.8	99.8

CONCLUSION

A simple HPLC method using a nucleosil column was developed for the analysis of carbocisteine in solid and liquid dosage formulations. This method was also successfully used for the analysis of carbocisteine in various different marketed formulations. Since the forced degradation and stability studies of the in-house formulation showed no interference with the carbocisteine peak, the method is specific and stability-indicating. The method is also accurate, linear and precise. Hence, the method is recommended for routine quality control analysis.

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