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Reversed Phase High Pressure Liquid Chromatography Technique for Determination of Carbocisteine from Pharmaceutical Formulation

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for determination of carbocisteine from pharmaceutical formulation. The separation of drug was achieved on (Amino Propyl Silane) APS-2 hypersil (250 X 4.6 mm) 5µ column. The mobile phase consisted a mixture of buffer and methanol (65:35 v/v). The buffer was a mixture of 0.02 M potassium dihydrogen phosphate monobasic and 0.01 M 1-hexane sulphonic acid sodium salt anhydrous. The detection was carried out at wavelength 210 nm. Buffer containing 0.02 M potassium dihydrogen phosphate monobasic and 0.01 M 1-hexane sulphonic acid sodium salt anhydrous used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze carbocisteine from pharmaceutical formulation.

Keywords: Carbocisteine, Reversed phase HPLC, Potassium dihydrogen phosphate monobasic, 1-Hexane sulphonic acid sodium salt anhydrous

INTRODUCTION

In this communication the present work proposes a new HPLC method for assay of carbocisteine from pharmaceutical formulation. Its chemical name is (2R)-2-amino-3-[(carboxymethyl) sulphanyl] propanoic acid. carbocisteine is a mucolytic drug, which breaks down mucus in the body so that it can be more easily cleared from the body. In chronic obstructive pulmonary disease (COPD) symptoms involves the oversecretion of mucus, mucolytics have great potential for treatment of this disease. Additional characteristics of COPD include airflow limitation,

oxidative stress, and airway inflammation. Carbocisteine is official in British Pharmacopoeia [1] and European Pharmacopoeia [2]. In literature survey ion pair HPLC [3] method was reported. A new, simple, rapid and reliable HPLC method is developed for the determination of carbocisteine. This method can be used for the routine analysis. In the proposed method optimization and validation of this method are reported.

EXPERIMENTAL SECTION

Chemical and reagents

Reference standard of carbocisteine was obtained from reputed firm with certificate of analysis. Potassium dihydrogen phosphate monobasic, 1-hexane sulphonic acid sodium salt anhydrous were used of analytical grade and the HPLC grade water was used from Merck. Standard and sample solutions were prepared in diluent (i.e. buffer containing 0.02 M potassium dihydrogen phosphate monobasic and 0.01 M 1-hexane sulphonic acid sodium salt anhydrous.)

Instrumentation

The HPLC system, Water Alliance (2695) HPLC system equipped with separation module and DAD detector (2996), was used. The chromatogram was recorded and peak quantified by mean of PC based Empower software.
A SHIMADZU analytical balance was used.
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Preparation of Standard preparation

Standard solution

About 50 mg standard carbocisteine was weighted accurately and transferred in 50 ml volumetric flask. About 20 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The working standard solution was prepared by diluting 5 ml of 1000 µg /ml solution to 50 ml with diluent to get concentration 100 µg /ml.

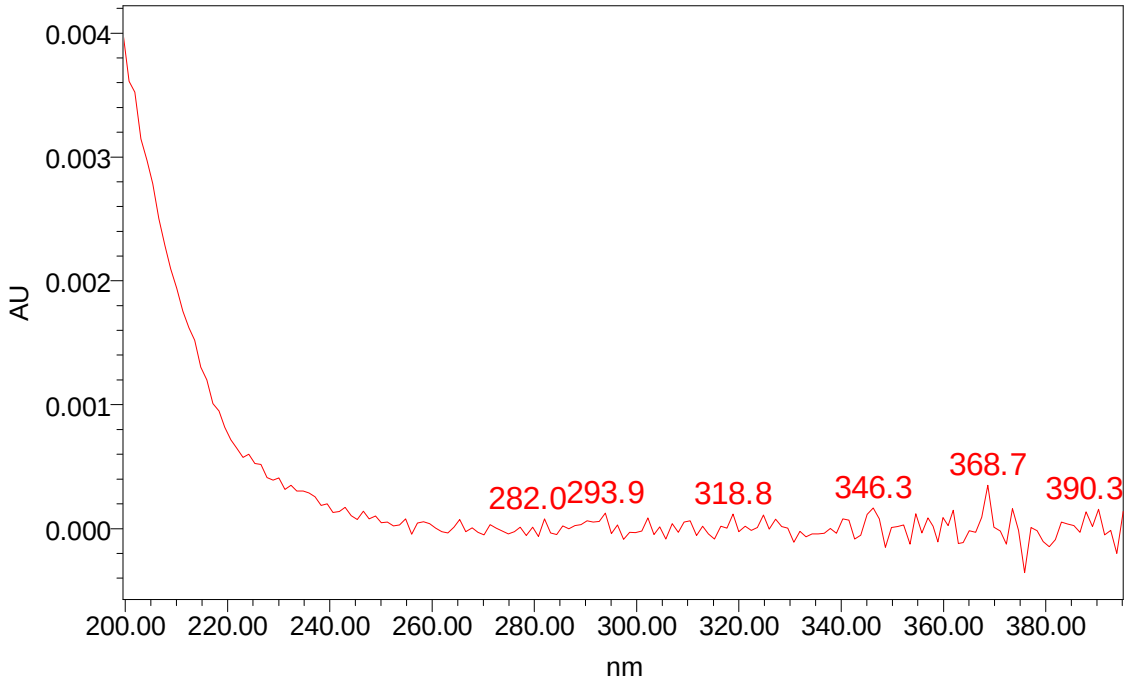
Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 50 mg of carbocisteine sample was weighted accurately and transferred in 50 ml volumetric flask. About 20 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The sample solution was prepared by diluting 5 ml of 1000 µg/ml solution to 50 ml with diluent to get concentration 100 µg /ml.

Chromatographic condition

Chromatographic separation was performed at ambient temperature on a reverse phase (Amino Propyl Silane) APS-2 hypersil (250 X 4.6 mm) 5µ column. The mobile phase consisted a mixture of buffer and methanol (65:35 v/v). The buffer was a mixture of 0.02 M potassium dihydrogen phosphate monobasic and 0.01 M 1-hexane sulphonic acid sodium salt anhydrous. The flow rate of the mobile phase was adjusted to 1.0 ml /min. The detection was carried out at wavelength 210 nm. The injection volume of the standard and sample solution was set at 10 µl. The UV spectra of carbocisteine are given in figure no.1

Figure 1: UV spectra of carbocisteine



Method validation

System suitability

System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), tailing factor, resolution (R) and relative standard deviation were determined. The results are shown in table 1 which indicates good performance of the system.

Table 1: System suitability parameters evaluated on standard solution of carbocisteine

Retention Time	Area	Area %	USP Plate Count	USP Tailing
7.03 minutes	562765	100.0	11434	1.28

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank (diluent), L-cysteine, carbocisteine were injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

Figure 2: Typical chromatogram of carbocisteine (standard)

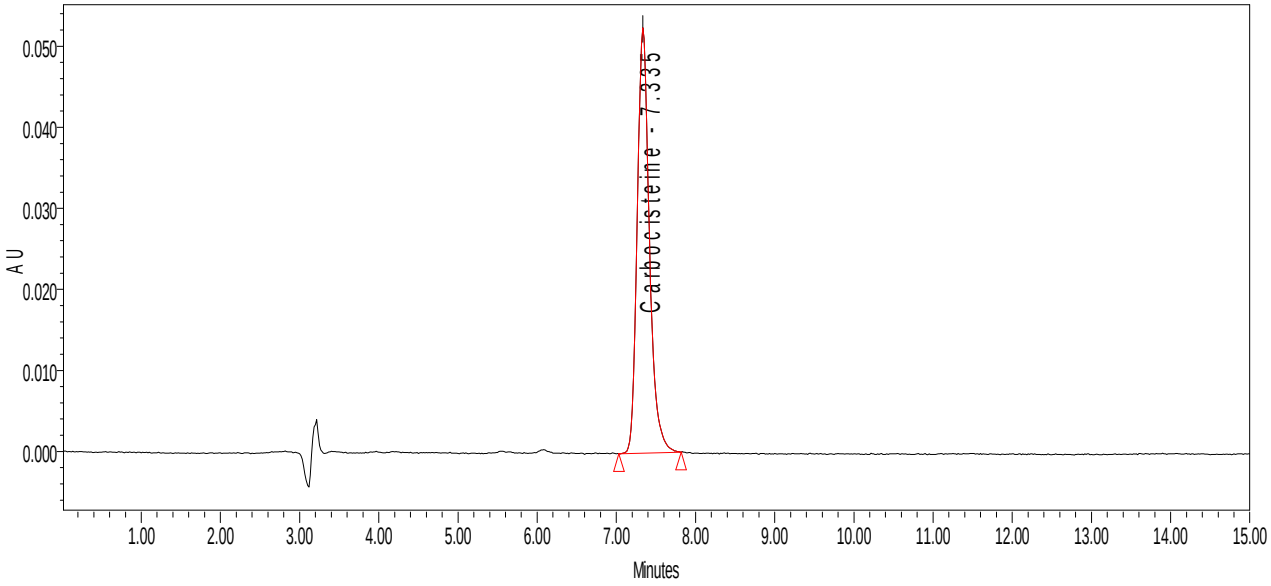
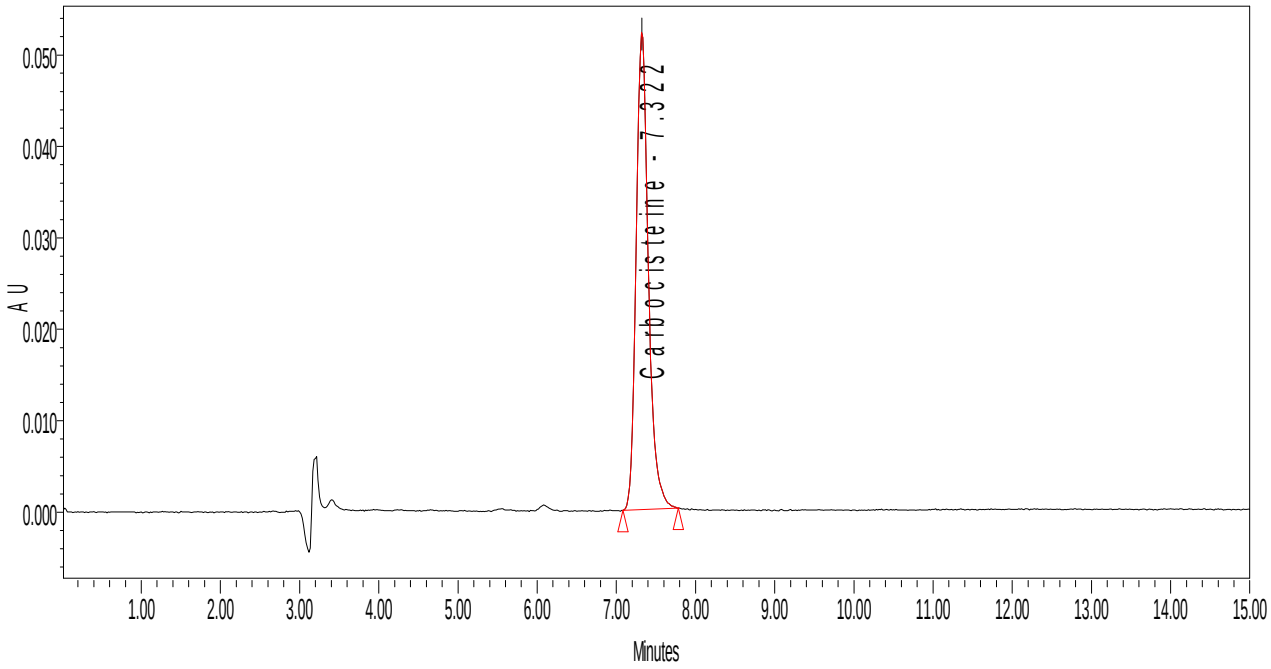


Figure 3: Typical chromatogram of carbocisteine (sample)



Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2: Statistical evaluation of the data subjected to regression analysis

No.	Parameters	Carbocisteine
01.	Correlation Coefficient (r)	1.000
02.	% Intercept (y)	5570.220
03.	Slope (m)	5706.6000

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing know amount of drug corresponding to 80 %, 100 % and 120 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3.

Table 3: Statistical evaluation of the data subjected to accuracy of carbocisteine

Injection	Level (%)	Conc. of spiked Carbocisteine (ppm)	Area	Amount found in ppm	% Recovery
01	80 %	**80**	451339	79.48	99.4
02			454739	80.07	100.1
03			450180	79.27	99.1
01	100 %	**100**	562809	99.10	99.1
02			562296	99.01	99.0
03			563150	99.16	99.2
01	120 %	**120**	674708	118.81	99.0
02			675616	118.97	99.1
03			678340	119.45	99.5
				Mean	**99.3**
				Standard Deviation	**0.213854**
				% RSD	**0.22**

Precision

The method precision was established by carrying out the analysis of carbocisteine. The assay was carried out of the drug using analytical method in six replicates. The value of relative standard deviation lies well with the limits (0.10 %). The results of the same are tabulated in the table no. 4.

Table 4: Statistical evaluation of the data subjected to method precision of carbocisteine

Sr. No.	Sample name	Assay (%)
01	Test solution – 1	99.6
02	Test solution – 2	99.7
03	Test solution – 3	99.8
04	Test solution – 4	99.7
05	Test solution – 5	99.8
06	Test solution – 6	99.8
	Mean	**99.7**
	Standard Deviation	0.10088
	% RSD	**0.10**

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in the flow rate by $\pm$ 0.2 ml /min

Variation in wavelength by $\pm$ 5 nm

Variation in concentration of mobile phase by $\pm$ 2 %

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Stability of solution

The stability of the solution under study was established by keeping the solution at room temperature for 12 hours and 24 hours. The results indicated no significant change in assay results of the solutions. In confirmed the stability of the drug in the solvents used for the analysis.

Method application

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 50 mg of carbocisteine sample was weighted accurately and transferred in 50 ml volumetric flask. About 20 ml diluent was added and sonicated for 10 min to dissolve it. Further volume was made up to the mark with the diluent to give 1000 µg /ml. Further the 5 ml of this solution was diluted to 50 ml with diluent to give 100 µg /ml of carbocisteine. From this

solution 10 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4. It indicates the amount of carbocisteine in the product meets the requirement.

RESULT AND CONCLUSION

The reproducibility, repeatability and accuracy of the proposed method was found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. In the proposed method retention time is very less and resolution is much better compared to method suggested in literature [3]. Thus the proposed RP-HPLC method is used for estimation of carbocisteine from active pharmaceutical ingredient. It is more precise, accurate, linear, robust, simple and rapid method than reported method in literature. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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