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Validation of Carbocisteine by Reversed Phase High Performance Liquid Chromatography Method from Active Pharmaceutical Dosage Form

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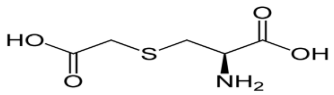
Abstract : A high performance liquid chromatography method is described for determination of carbocisteine from active pharmaceutical ingredients. The separation of drug was achieved on BDS hypersil C18 (250 x 4.6 mm i.d.) with 5 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of water and acetonitrile (95:5 % v/v). The detection was carried out at wavelength 215 nm. The mixture of water and acetonitrile (95:5% v/v) was 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 active pharmaceutical ingredients.
Keywords : Carbocisteine, Acetonitrile, HPLC.

Introduction

In this communication the present work proposes reverse phase high liquid chromatographic method for assay of carbocisteine from bulk drug and pharmaceutical formulation. Its chemical name is (2R)-2-amino-3-[(carboxy-methyl) 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 involve the over secretion of mucus, mucolytic have great potential for treatment of this disease. Additional characteristics of COPD include airflow limitation oxidative, stress and airway inflammation. The structure of carbocisteine is shown in Fig.1.

Chemical structure of carbocisteine



Carbocisteine is official in British Pharmacopoeia[1] and European Pharmacopoeia[2]. In literature survey HPLC[3-4], UPLC [5]and Ion-Chromatography [6,] Spectrophotometric [6, 7]methods were reported for validation ofdrug. In literature survey HPLC[8] Spectrophotometric [9, 10] methods were reported for validation of combined dosage form. This method can be used for the routine analysis and research organization. In the proposed work optimization and validation of these methods are reported.

Materials and methods

Chemical and reagents

Reference standard of carbocisteine was obtained from reputed firm with certificate of analysis. Tri-ethylamine, acetonitrile and acetic acid were used of analytical grade and the HPLC grade water was used from

Millipore. Standard and sample solutions were prepared in diluent [mixture of water and acetonitrile (95:5 % v/v)].

Instrumentation

The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software.

A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation

Standard solution

A 10 mg of standard carbocisteine was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent[mixture of water: acetonitrile (95:5% v/v)] 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 1 ml of 1000 µg /ml solution to 10 ml with diluent to get concentration 100 µg /ml.

Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powdered tablet equivalent to 10 mg of carbocisteine sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent [mixture of water and acetonitrile (95:5 % v/v)]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 1 ml of 1000 µg/ml solution to 10 ml with diluent to get concentration 100 µg /ml.

Chromatographic condition

Chromatographic separation was performed at ambient temperature on a reverse phase BDS Hypersil C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of water and acetonitrile (95:5 % v/v). The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 215 nm. (Fig.1) The injection volume of the standard and sample solution was set at 20 µl.

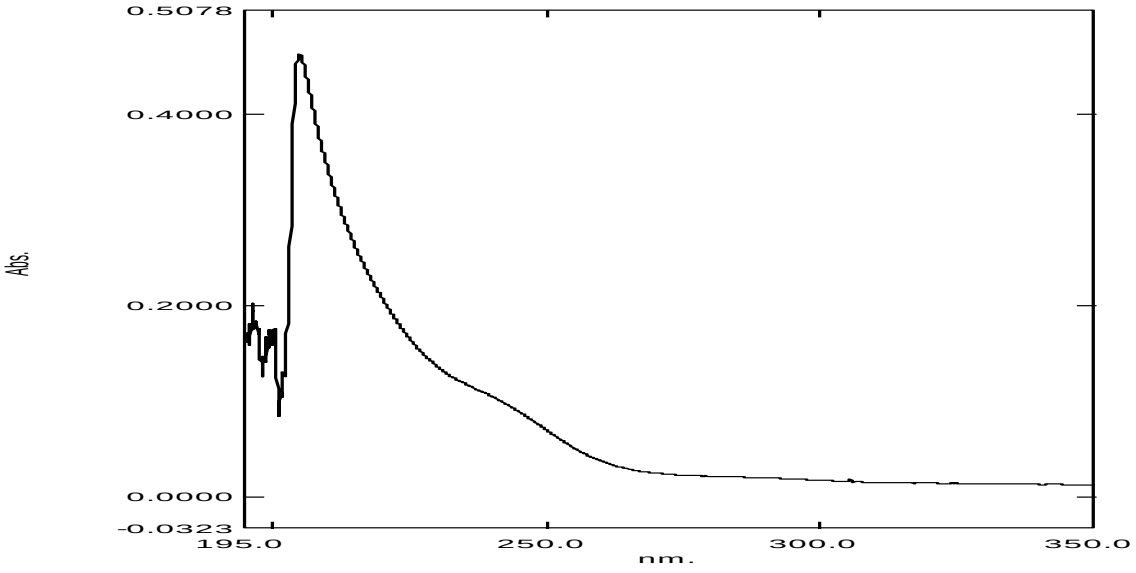


Figure 1: UV spectra of carbocisteine

Method validation

System suitability

System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, and area 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	Symmetry
1.8	1521330	**100.000**	2673	0.83

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard carbocisteinewas injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

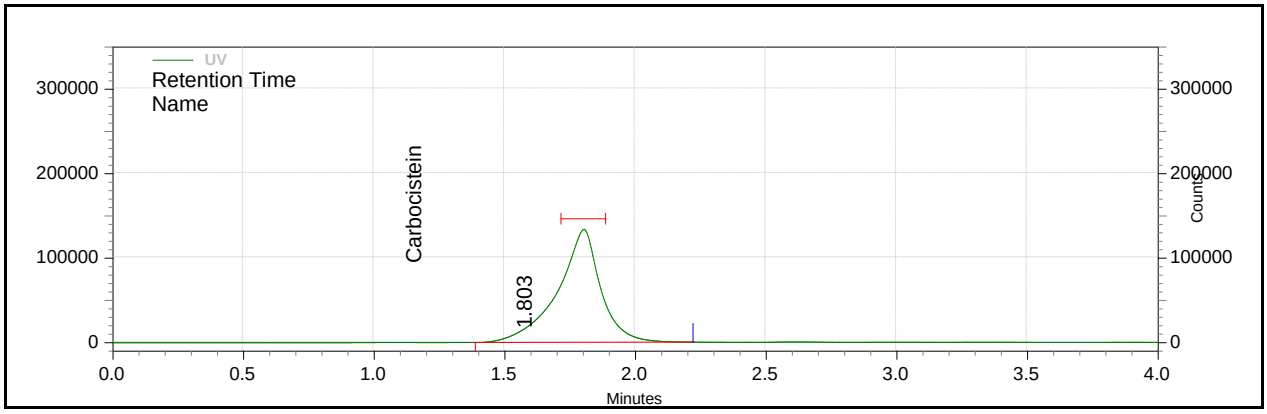


Figure 2: Typical chromatogram of carbocisteine(standard)

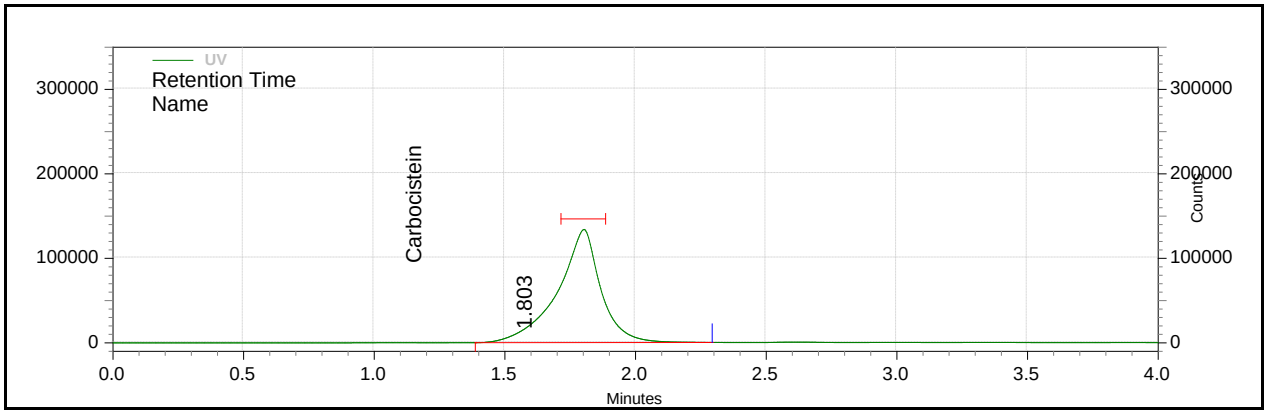


Figure 3: Typical chromatogram of carbocisteine(sample)

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

Parameters	**Values**
Correlation Coefficient (r)	0.9999
% Intercept (y)	37546
Slope (m)	11267

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 80 %, 100 % and 150 %. 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

level	**test**	**wt in mg**	**area**	**quantity added in µg /ml**	**quantity recovered in µg /ml**	**% recovery**	**mean recovery**
80%	1	10.28	1253764	16.64	17.12	102.88	**102.86**
	2	2.07	1253650	16.64	17.12	102.87	
	3	2.06	1253327	16.64	17.11	102.84	
100%	1	2.09	1522489	20.8	20.79	99.94	**99.98**
	2	2.11	1522967	20.8	20.79	99.97	
	3	2.08	1523821	20.8	20.81	100.03	
150%	1	2.07	1825807	24.96	24.93	99.88	**99.93**
	2	2.09	1826671	24.96	24.94	99.93	
	3	2.10	1827906	24.96	24.96	99.99	

Mean recovery of all level	**100.93**

Precision

The method precision was established by carrying out the analysis of. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. 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

Test	**wt of test**	**Area**	**% assay**
Solution-1	10.22	1524195	99.47
Solution-2	10.25	1524944	99.81
Solution-3	10.24	1525424	99.75
Solution-4	10.19	1523821	99.15
Solution-5	10.26	1522967	99.78
Solution-6	10.27	1522489	99.85
	Mean Assay		**99.64**
	SD		**0.271**
	RSD		**0.272**

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 ± 0.2 ml /min
Variation in mobile phase composition by ± 2 %
Variation in wavelength ± 5 nm

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

Method application

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of carbocisteinesample was weighted accurately and transferred in 10 ml volumetric flask. About 5 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 1 ml of this solution was diluted to 10 ml with diluent to give 100 µg /ml of ambroxal hydrochloride. From this solution 1.0 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 carbocisteinein the product meets the requirement.

Result and conclusion

The reproducibility, repeatability and accuracy of the proposed method were 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. Thus the proposed RP-HPLC method is used for estimation of carbocisteinefrom active pharmaceutical ingredient. It is more precise, accurate, linear, robust, simple and rapid method. 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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