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Research Article

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Spectrophotometric estimation of Carbocisteine in bulk and pharmaceutical dosage form by second order derivative method

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ABSTRACT

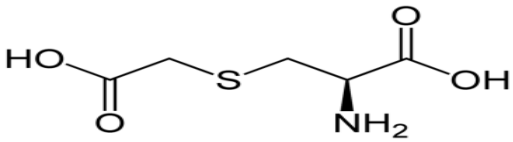
A simple and precise UV- spectrophotometric, second order derivative method have been developed and validated for the estimation of carbocisteine in bulk and its tablet formulation. The standard and sample solutions of carbocisteine were prepared in 0.1 N HCl. Carbocisteine was estimated at 208 nm for the second order derivative UV-spectrophotometric method. Beer's law was obeyed in the concentration range of 10 to 140 µg / ml with coefficient of correlation value 0.9988. The method was tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation, which was 5.146 % above method. The proposed method was successfully applied for the determination of carbocisteine in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Carbocisteine, second order derivative spectroscopy.

INTRODUCTION

In this communication the present work proposes UV spectrophotometric second order derivative 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 [7, 8] methods were reported. This method can be

used for the routine analysis and research organization. In the proposed work optimization and validation of these methods are reported.

EXPERIMENTAL SECTION

Instrument and reagents

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software. Reference standard of carbocisteine was obtained from reputed firm with certificate analysis.

Preparation of standard drug solution

100 mg standard carbocisteine was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of 0.1 N HCl for 15 minutes. The volume was made up to the mark with 0.1 N HCl to give a stock solution of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with 0.1N HCl to give a working standard solution of concentration 100 µg/ml.

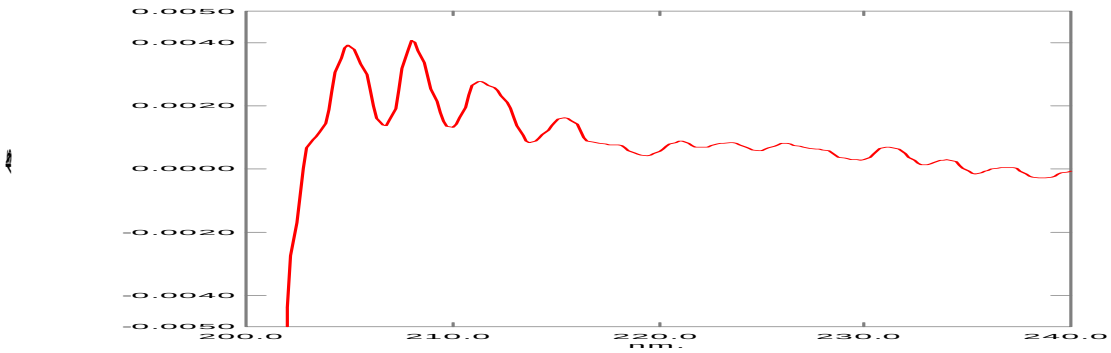
Estimation from tablets

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of carbocisteine was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1N HCl was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1N HCl to give concentration as 100 µg /ml. Such solution was used for analysis.

Method: Second order derivative method

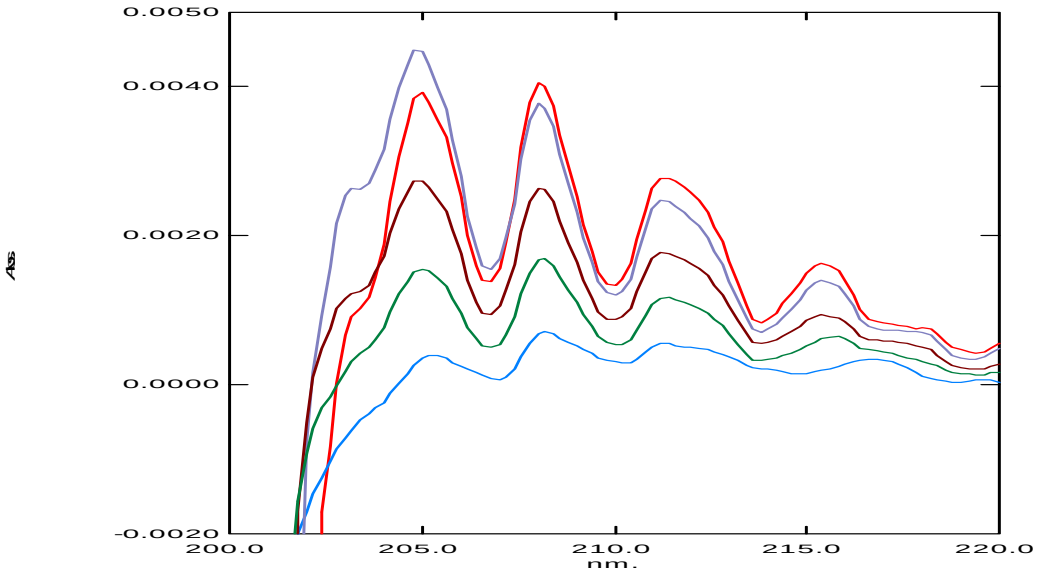
For the selection of analytical wavelength, 100 µg/ml solution of carbocisteine was scanned in the spectrum mode from 400 nm to 190 nm by using 0.1 N HCl as blank. The second order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 208 nm (Fig. 1).

Fig. 1. Second order derivative spectrum of carbocisteine (100 µg/ml) showing absorbance at 208 nm



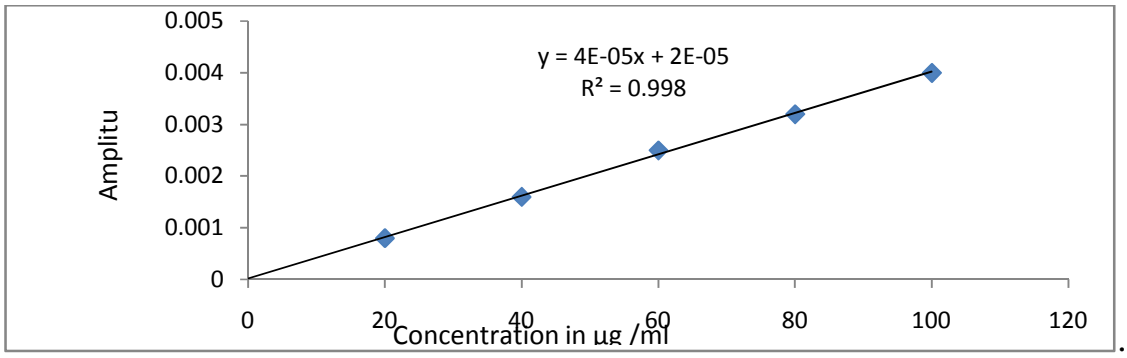
Into series of 10 ml graduated flask, varying amount of sample solutions of carbocisteine were pipette out and volume was adjusted with 0.1N HCl. Solutions were scanned between 400 nm to 190 nm in spectrum mode. The second order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 208 nm using 0.1N HCl as blank. The overlay spectra were given in fig. 2.

Fig.2. Overlay spectra of carbocisteine in the concentration of 20-100 µg /ml



The calibration curve was prepared in the concentration range of 20 to 140 µg/ml (Fig. 3).

Fig. 3. Calibration curve for carbocisteine at 208 nm by second order derivative spectroscopy



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameters	Values
Detection Wavelength (nm)	208
Beer Law Limits (µg/ml)	10-140
Correlation coefficient(r^2)	0.9988
Regression equation (y=b+ac)	
Slope (a)	0.00004
Intercept (b)	0.00002

VALIDATION

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table 2.

Table 2: Results of recovery of carbocisteine

Amount of sample added in µg /ml	Amount of standard added in µg /ml	Total amount recovered	Percentage Recovery (%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
20	**0**	20.71429	103.5714	1.88982	9.12328
20	**20**	40.71429	101.7857	1.889822	4.64166
20	**40**	60.500	100.8333	2.43242	4.02052
20	**60**	80.35714	100.4464	2.24933	2.799177
			Mean	2.115351	5.146164

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in seven replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 3.

Table 3: Precision- method precision

Experiment no.	Weight of carbocisteine taken in mg	Contents of carbocisteine in mg.
1	20	20
2	20	20
3	20	17.5
4	20	22.5
5	20	20
6	20	20
7	20	22.5
	Standard deviation	1.889822
	% R.S.D.	9.12328

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of carbocisteine was transferred to 100 ml of volumetric flask. A 30 ml of 0.1N HCl was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1N HCl to give concentration as 100 µg /ml. Such solution was used for analysis.

Solution was scanned between 400 nm to 190 nm in spectrum mode. The second order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 208 nm by using 0.1N HCl as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 208 nm for second order derivative. Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of carbocisteine was estimated by comparison with standard at 208 nm for second order derivative, table 4.

Table 4: Summary of validation parameter for intra-day and inter-day

Sr. No.	Parameters	Values
1	Intra-day precision (N=3)amount found ± % R.S.D.	101.7857 % 1.889822
2	Inter-day precision (N=3)amount found ± % R.S.D.	99.884% 2.249339
3	Ruggedness Analyst to analyst(n=3) % R.S.D.	100.012% 0.6186

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

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 5.

Table 5: Values of results of LOD and LOQ

Parameters	Values
Limit of Detection (μg/ml)	**0.001396**
Limit of Quantification (μg/ml)	**0.004233**

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of carbocisteine sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of carbocisteine was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The second order derivative UV-spectroscopic method is useful for routine analysis of carbocisteine in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 10 to 140 μg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of carbocisteine in pharmaceutical formulation.

Acknowledgement

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