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Original Article

HPTLC METHOD DEVELOPMENT AND VALIDATION FOR DENSITOMETRIC ANALYSIS OF CARBOCISTEINE IN DRUG FORMULATION

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ABSTRACT

Objective: Development of a simple, selective, precise, high-performance thin layer chromatographic method for the analysis of carbocisteine, mucolytic drug in pharmaceutical dosage form.

Methods: The proposed HPTLC method employed TLC aluminum plates precoated with silica gel 60_{F-254} as the stationary phase. The solvent system consisted of butanol: water: ethanol: acetic acid (5:1.5:2:1.5 v/v/v/v). This system was found to give compact spots for carbocisteine with Rf value of 0.34.

Results: The reported method is linear over the range of 50-800 ng/spot with a coefficient of correlation 0.9992. The precision study revealed that the percentage relative standard deviation was within the acceptable limit. The developed method was validated according to ICH Guidelines.

Conclusion: An economical, accurate, sensitive and precise HPTLC method was developed and fully validated for quality control analysis of carbocisteine in tablets. The developed method was validated with respect to specificity, limit of detection, limit of quantification, precision, linearity, accuracy and robustness. Validation results were found to be satisfactory. The proposed method can be used for routine analysis of carbocisteine in quality control laboratories.

Keywords: Carbocisteine, HPTLC method development, method validation, Mucolytic

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INTRODUCTION

Carbocisteine is a mucolytic agent, chemically is (*R*)-2-Amino-3-(carboxymethyl sulfanyl) propanoic acid with molecular formula $C_5H_9NO_4S$ and molecular weight of 179.2 9 [fig. 1] used in respiratory disorders associated with a productive cough that reduces the viscosity of sputum and so can be used to help relieve the symptoms of the chronic obstructive pulmonary disorder (COPD) and bronchiectasis. In addition to its much regulatory activity, carbocisteine exhibits free-radical scavenging and anti-inflammatory properties. These characteristics have stimulated interest in the potential that this and other mucoactive drugs may offer for modification of the disease processes present in COPD [1].

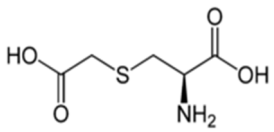

Fig. 1: Chemical structure of carbocisteine

Literature survey reveals that few methods have been reported for analysis of carbocisteine in plasma and bulk drugs which includes fluorimetric method [2], spectrophotometric method [3] and high-performance liquid chromatography (HPLC) [4-12].

There was no HPTLC method for estimation of carbocisteine has been reported so far. Hence an attempt has been made to develop a new precise and accurate HPTLC method for estimation of carbocisteine in the formulation as per ICH guidelines [13]. The advantage of HPTLC is that several samples can be run simultaneously by using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

MATERIALS AND METHODS

Chemicals and reagents

Carbocisteine pure compound was purchased from swapnaroop drugs and chemicals, Aurangabad. Carbocisteine capsules, mucodyne was purchased from a local pharmacy. All the chemicals used were of analytical grade.

HPTLC instrumentation and chromatographic conditions

The Samples were spotted in the form of bands of width 5 mm with a camag microlitre syringe on precoated silica gel aluminum plate 60F-254 (20 cm x 10 cm) with 250 µm thickness; Merck, Germany) using a Camag Linomat IV applicator (Switzerland). A constant applications rate 5 µl was employed and space between two bands was 5 mm. The slit dimension was kept at 4 mm x 0.5 mm and 20 mm scanning speed was employed.

The Mobile phase consisted of n-butanol: water: ethanol: acetic acid (5:1.5:2:1.5 v/v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min. with filter paper at room temperature. The length of the chromatogram run was 9 cm. subsequent to the development. TLC plates were dried in air with the help of an air-dryer. Ninhydrin was used as the derivatizing reagent to improve chromatographic retention. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 366 nm and operated by CATS software (V 4.06, Camag). The Source of radiation utilized was deuterium lamp.

Preparation of stock and standard solutions

A stock solution of carbocisteine (1 mg/ml) was prepared by accurately weighing approximately 10 mg of carbocisteine into 10 ml volumetric flask and volume was made up to the mark with 0.1N HCL. The standard solutions were prepared by dilution of the stock solution with 0.1N HCL to reach a concentration range 50-800 ng/µl. Each concentration was spotted six times on the TLC plate. The plate was developed on previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Preparation of sample solution

For analysis of marketed formulation, twenty capsules were accurately weighed and the average weight was determined. Powder equivalent to 100 mg of carbocisteine was weighed, transferred to 100 ml of the

volumetric flask containing 0.1N HCL, sonicated for 15 min and diluted up to the mark with 0.1N HCL. The resulting solution was filtered through whatman filter paper and used for further analysis.

Validation

Validation of method was carried as per the International Conference on Harmonization [(ICH) Q2 (Rl)] guidelines for selectivity, robustness, linearity, range, limit of detection (LOD) and limit of quantitation (LOQ), accuracy, and precision [13].

Linearity and range

Stock solutions were applied on the HPTLC plate in the range of 50-800 ng/spot of carbocisteine to evaluate linearity. Peak area versus concentration was plotted and linear regression analysis and the correlation coefficient, slope, intercept for the calibration was estimated.

LOD and LOQ

Limit of detection and limit of quantitation was estimated using formula 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot.

Precision studies

Precision was studied by intra and inter-day precision. Carbocisteine sample was prepared and analyzed six times on the same day in order to trace any variations in the results. For interday variation study, the above-mentioned drug samples were analyzed on three successive days.

Accuracy studies

The accuracy of the method was determined by estimating recoveries of carbocisteine by the standard addition method. The sample was spiked with 80, 100 and 120 % of standard solutions.

Robustness studies

In the robustness evaluation, small, deliberate changes in the analytical parameters of the proposed method were done and its effect on the peak areas of the drugs was studied.

Assay

The proposed method was applied to the determination of carbocisteine in mucodyne capsules. To determine the content of carbocisteine in capsules (label claim: 375 mg carbocisteine) 20 capsules were opened and the contents were weighed and mixed. An aliquot of powder equivalent to the weight of 1 mg was accurately weighed and transferred to 100 ml volumetric flask and was dissolved in 0.1N HCL and made up to the volume of 0.1N HCL. The volumetric flask was sonicated for 30 min to affect complete dissolution. The solutions were filtered through a 0.45 µm nylon filter. The above stock solution was further diluted to obtain sample solution at three different concentration levels of 200, 400 and 600 ng/µl respectively was applied six times to the HPTLC plate. The plate was developed in the previously described chromatographic conditions.

RESULTS AND DISCUSSION

HPTLC method development and optimization

The different solvent system was tried to optimize the method. Finally, a mobile phase consisting of butanol: water: ethanol: acetic acid (5:1.5:2:1.5 v/v/v/v) was optimized. To improve the resolution and to enhance the selectivity of carbocisteine, derivatization method has been developed for optimal and reproducible assay performances. Ninhydrin was used as the derivatizing reagent, and good resolution with R_f value of 0.34 for carbocisteine was obtained after derivatization when densitometric scanning was performed at 366 nm. The spot appeared more compact and peak shape more symmetrical. The significantly improved chromatographic retention, and the highly selective and sensitive detection afforded by the HPTLC monitoring have made the method most useful for identification of carbocisteine in pharmaceutical dosage form. The typical chromatogram of carbocisteine was shown in [fig. 2].

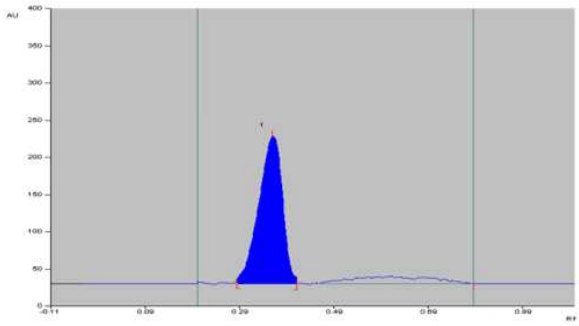

Fig. 2: Chromatogram obtained from standard solution of carbocisteine 200 ng/spot

Validation of method

The method was validated with respect to parameters including linearity, limit detection (LOD), limit of quantitation (LOQ), recovery, precision, accuracy, robustness and selectivity.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 50-800 ng per spot for carbocisteine [fig. 3]. Linear regression for carbocisteine was found to be $r^2 = 0.9992 \pm 0.034$ [table 1].

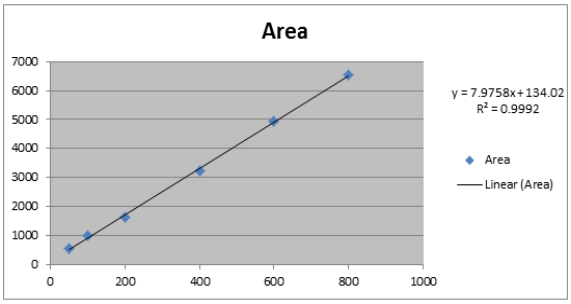

Fig. 3: Calibration curve for carbocisteine by HPTLC

LOD and LOQ

The LOD and LOQ were determined based on signal-to-noise ratios and were based on analytical responses of 3 and 10 times the background noise, respectively. The LOD and LOQ were found to be 30.191 ng/spot and 91.697 ng/spot respectively for carbocisteine.

Table 1: Statistical evaluation of calibration data

Parameter	HPTLC
Linearity Range ng/spot	50-800
r^2±%RSD	0.9992±0.034
Slope±%RSD	7.9758±1.12
Intercept±%RSD	134.02±0.86
LOD (ng spot/spot)	30.191
LOQ (ng spot/spot)	91.697

LOD = Limit of detection, LOQ = Limit of quantitation, RSD = Relative standard deviation.

Precision

Set of three different concentrations in three replicates of 200, 400 and 600 (ng/µl) were prepared. All the solutions were analyzed on the same day in order to record intra-day variations in the results. Inter-day precision study involves analysis of three different concentrations of the standard solutions in linearity range on three consecutive days**.** Precision data is summarized in table 2.

Table 2: Intra and inter-day precision results of carbocisteine

Concentration (ng/spot)	Intra-day(n=6) %R.S.D*	Inter-day(n=6) %R.S.D*
200	100.8±0.54	99.67±0.83
400	99.28±0.84	101.5±0.56
600	99.14±0.96	98.86±1.26

n = Number of determinations, *Average of six determinants.

Recovery

The proposed method was used for estimation of carbocisteine from the pharmaceutical dosage form after spiking with an additional drug. The afforded recovery was found to be 98.84-101.06 % for carbocisteine from the marketed formulation [table 3].

Robustness of the method

To evaluate HPTLC method robustness, a few parameters were deliberately varied. Results indicate that the selected factors remained unaffected by small variations of these parameters. The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2 %. The low values of % RSD indicated the robustness of the method [table 4].

Selectivity/specificity

The peak purity of carbocisteine was assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot, i.e., r (start, middle) = 0.9991 and r (middle, end) = 0.9995. A good correlation (r = 0.9993) was also obtained between standard and sample spectra of carbocisteine.

Table 3: Recovery studies (n=6) results of carbocisteine

Concentration (ng/spot)	Amount of drug added (ng/spot)	% Recovery±S. D*
200	160	98.84±0.84
200	400	99.29±0.16
200	440	101.06±0.28
mean±SD		99.73±0.42

*Average of six determinants, n = Number of determinations

Table 4: Robustness evaluation of carbocisteine for HPTLC method

Parameter	SD of peak area	%RSD*
Mobile phase composition (±0.1 ml)	0.82	0.32
Amount of mobile phase (±5 %)	1.03	1.12
Temperature (±2 %)	1.06	0.96
Relative humidity (±5 %)	1.75	1.36
Chamber Saturation (±5 min)	0.83	0.72
Time from spotting to chromatography (±20 min)	0.62	0.24
Time from chromatography to scanning (±5 min)	0.81	0.52

*Average of three determinants, SD-standard deviation, and RSD-Relative standard deviation

Assay

The drug content was found to be 100.07 %±1.57 for carbocisteine. It may, therefore, be inferred that degradation of carbocisteine had not occurred in the marketed formulations that were analyzed by the proposed method, and the low % RSD value indicated the suitability of this method for routine analysis of carbocisteine in pharmaceutical dosage form.

CONCLUSION

There was no HPTLC method for estimation of carbocisteine has been reported so far. Hence a new precise and accurate HPTLC method consisting butanol: water: ethanol: acetic acid (5:1.5:2:1.5 v/v/v/v) as mobile phase was optimized. Ninhydrin was used as the derivatizing reagent and good resolution with R_f value of 0.34 for carbocisteine was obtained at 366 nm. The drug shows linearity over the concentration range of 50-800 ng per spot and linear regression for carbocisteine was found to be r^2 = 0.9992±0.034. The developed method was validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are subtle, indicating a high degree of precision of the methods. The results of the recovery studies performed to show the high degree of accuracy of the proposed methods. All the parameters like precision, accuracy, specificity, ruggedness, robustness were done and found to be within the acceptance criteria. From the experimental data, it can be concluded that the developed chromatographic method is accurate, precise and selective and can be employed successfully for the estimation of carbocisteine in pharmaceutical dosage form.

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CONFLICT OF INTERESTS

Authors do not have any conflict of interest

REFERENCES

1. Hoopr C, Calvert J. The role for S-carboxymethyl cysteine (carbocisteine) in the management of the chronic obstructive pulmonary disease. Int J Chronic Obstruct Pulm Dis 2008;3:659–69.
2. Walash MI, Amina M-EB, Mohamed EM, Amina AA. Fluorimetric determination of carbocisteine and ethionamide in drug formulation. Acta Chim Slov 2004;51:283–91.
3. Walash MI, Amina M-EB, Mohamed EM, Amina AA. Spectrophotometric determination of penicillamine and carbocisteine based on the formation of metal complexes. IL FARMACO 2004;59:493–503.
4. Wahl O, Holzgrabe U. Impurity profiling of carbocisteine by HPLC-CAD, qNMR and UV/vis spectroscopy. J Pharm Biomed Anal 2014;95:1-10.
5. Nikolaos CM, Michael AK. Ion chromatographic determination of carbocisteine in pharmaceuticals based on the non-

suppressed conductimetric detection. J Chromatogr A 2004;1026:167-74.
6. Rele RV, Patil SP. Reversed phase high-pressure liquid chromatography technique for determination of carbocisteine from the pharmaceutical formulation. J Chem Pharm Res 2010;2:24-30.
7. Sarode SK, Pise ST, Dhore P, Mundhada DR, Bhaskaran S. Development of an analytical method for simultaneous estimation of amoxicillin and carbocisteine in solid dosage form by RP-HPLC. Biomedrx 2013;1:360-2.
8. Bron J. HPLC determination of carbocisteine in Human Plasma. Pharm Weekbl Sci 1986;8:134-8.
9. Argekar AP, Raj SV, Kapadia SU. Simultaneous determination of cephalexin and carbocisteine from capsules by reverse phase high-performance liquid chromatography. Anal Lett 1997;30:821-31.
10. Nadimintri A, Gunda A, Karnaker RT, Abbaraju PL, Kedarnath JA, Nagubandi S. Simultaneous estimation of amoxicillin trihydrate and carbocisteine drug present in the formulation by RP-HPLC method and its validation. J Pharm Res 2012;13:1889-95.
11. Burugu R, Venkateshwarlu G. A stability indicating ultra-performance liquid chromatography method for the determination of assay of carbocisteine in various formulation products. Int J Pharm Pharm Sci 2012;4:653-6.
12. Li Rui. Determination of carbocisteine tablets by HPLC. Chin Pharm; 2006. p. 6.
13. International Conference on Harmonization (ICH), Q2 (R1) Validation of Analytical Procedures: Text and Methodology, ICH Harmonized Triapartite Guideline; 2005.

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