INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)

www.ijcrcps.com

DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 8, Issue 5 - 2021

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2021.08.05.004

Effect of Solvents on Spectrophotometric Analysis of Carbocisteine by Forming Complex with Ninhydrin

Abdul Aziz Ramadan[1*], Hasna Mandil[2], Ali Marei[3]

Department of Chemistry, Faculty of Science, University of Aleppo, Syria.

*[1]E-mail: dramadan@scs-net.org or dramadan1946@gmail.com;

[2] E-mail: promandil955@gmail.com

[3]E-mail: alooosh.7.8.93@gmail.com

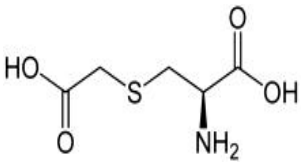
Abstract

Effect of solvents (methanol, ethanol, isopropanol and acetonitrile) on direct spectrophotometric analysis of carbocisteine in bulk and pharmaceutical formulations using complex formed with ninhydrin (NN) was studied. The method is based on reaction of ninhydrin with primary amino groups to form the purple dye. Various parameters (pH, heating time, temperature, concentration NN, etc.) affecting the carbocisteine determination were examined. The absorption spectra of this complex occur at wavelengths from 568 to 572 nm (pH 9.6, heating time 15 min, temperature 90^{o}C). The absorbance's were proportional to the concentration of carbocisteine at the range 1.00 to 100.00 µM (0.1792 to 17.919 µg.mL^{-1}) for above solvents in present 2.5x10^{-2} M of NN (which has been dissolved with used solvents). The method was validated for linearity, precision and accuracy, repeatability and robustness. The method was successfully applied for determination of carbocisteine in pure and pharmaceutical formulations samples with relative standard deviations did not exceed 3.2% for the concentrations of carbocisteine (0.1792 µg.mL^{-1}) in methanol as example.

Keywords: Solvents, Carbocisteine, Spectrophotometric method, Ninhydrin.

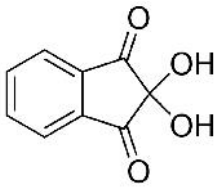
Introduction

Carbocisteine (S-carboxymethyl-L-cysteine) is mucolytic drug used for treatment of disorders of the respiratory tract associated with excessive mucus see scheme 1. Carbocisteine is an odorless white crystalline powder that has a molecular formula of $C_5H_9NO_4S$ and a molecular weight of 179.19 g. It is very slightly soluble in water and practically insoluble in ethanol 95%. It dissolves in dilute hydrochloric acid or in sodium hydroxide [1, 2].



Scheme 1: Chemical structure of carbocisteine

Ninhydrin (2,2-dihydroxyindane-1,3-dione) is a chemical used to detect ammonia or primary and secondary amines. When reacting with these free amines, a deep blue or purple color known as Rahman's purple is produced. Ninhydrin is most commonly used to detect fingerprints, as the terminal amines of lysine residues in peptides and proteins sloughed off in fingerprints react with ninhydrin. It is a white solid that has a molecular formula of $C_9H_6O_4$ and a molecular weight of 178.14 g. It is soluble in ethanol and acetone at room temperature. Ninhydrin can be considered as the hydrate of indane-1,2,3-trione [1-3], see scheme 2.



Scheme 2: Chemical structure ninhydrin (NN).

Previous research that identified carbocisteine in plasma and bulk drugs which includes fluorimetric method [4,5], in its pure form and in pharmaceutical preparations was identified according to different methods including spectrophotometry [5-12], reversed phase high pressure liquid chromatography (RP-HPLC) [13,14] and high-performance thin-layer chromatography (HPTLC) [15].

In the present work, effect of solvents (methanol, ethanol, isopropanol and acetonitrile) on direct spectrophotometric determination of carbocisteine in pure form and pharmaceutical formulations using ninhydrin (NN) was developed. The method is based on reaction of ninhydrin with primary amino groups to form the purple dye.

Materials and Methods

Instruments and apparatus

Spectrophotometric measurements were made in $T90^+$ UV-VIS with 1.0 cm quartz cells. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 10 to 5000 µL (model Piptman P, GILSON). SARTORIUS TE64 (0.01 mg) electronic balance was used for weighing.

Reagents

Carbocisteine (99.5%) was supplied by Grand Pharma (China) Co., Ltd. (Wuhan Grand Hoyo Co., Ltd.). Ninhydrin reagent is 99% of analytical grade from Surechem Products Ltd (England). All solvents and reagents were analytical grade chemicals from Merck.

A stock standard solution of carbocisteine ($1x10^{-3}$ mol.L^{-1})

Dissolving 18.01 mg of carbocisteine with 20 mL of 0.10 M NaOH after that adjusted value pH 9.6 with 0.10 M HCL. Then the volume was transferred to a 100mL volumetric flask and diluting to mark by distilled water ($1x10^{-3}$ mol.L^{-1}).

Stock standard solution of ninhydrin $5x10^{-2}$ mol.L^{-1}

We weigh 224.92 mg of ninhydrin (99%) and dissolve it with 10 mL of used solvent (methanol, ethanol, isopropanol and acetonitrile). Then the volume was transferred to a 25 mL volumetric flask and diluting to mark by the same solvent ($5x10^{-2}$ mol.L^{-1}).

Working solutions and general procedure

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of carbocisteine: 1.00, 2.00, 5.00, 10.00, 20.00, 40.00, 60.00, 80.00 and 100.0 µmol.L^{-1} (0.1792, 0.3584, 0.8960, 1.7919, 3.5838, 7.1676, 10.7514, 14.3352 and 17.919 µg.mL^{-1}) by dilution of the volumes: 5 , 10, 25, 50, 100, 200, 300, 400 and 500 µL from stock standard solutions of carbocisteine ($1x10^{-3}$ mol.L^{-1}) into 5 mL volumetric flask, then added 2.5 mL from stock standard solution of ninhydrin ($5x10^{-2}$ mol.L^{-1}), 0.4 mL of 0.10 M NaOH (adjusted pH 9.6 with 0.10 M HCl), diluted to 5 mL with distilled water and the flask was heated on a water bath at 90°C for 15 min. After the flask had been cooled to room temperature, the solution was made up to the mark with studding solvent. The absorbance of the solution was measured against a reagent blank at used λ_{max}.

Sample preparation

Commercial formulations (as capsule) were used for the analysis of carbocisteine. The pharmaceutical formulations subjected to the analytical procedure were:

(1) ***Mucolar*** capsule, ELSAAD PHARMACEUTICAL, Aleppo - SYRIA, each capsule contains 375 mg of carbocisteine (Mfg. 10/2019, Exp. 10/2023).

(2) ***Mucoxil*** capsule, Al-SHAHBA PHARMACEUTICAL, Aleppo - SYRIA, each capsule contains 375 mg of carbocisteine (Mfg. 9/2017, Exp. 9/2021).

(3) ***Carbocisteie*** capsule, Al-YOUSEF PHARMACEUTICAL, Damascus - SYRIA, each capsule contains 375 mg of carbocisteine (Mfg. 10/2017, Exp. 10/2021).

Stock solutions of pharmaceutical formulations

Contents of 20 capsules of each studied pharmaceutical formulations were weighted accurately and mixed well. An amount of the powder equivalent to the weight of tenth content of capsule of carbocisteine was solved in 40mL of 0.10 M NaOH using ultrasonic for 15 min. After that adjusted pH 9.6 with 0.10 M HCL. Then the volume was transferred to a 100 mL volumetric flask and diluting to mark by distilled water (37.5 mg/100 mL or $2.093x10^{-3}$ M).

Working solutions of pharmaceuticals

These solutions were prepared daily by diluting 100 µL (0.100 mL) from stock solutions of pharmaceutical formulations into 5 mL volumetric flask, then added 2.5 mL from suitable stock standard solution of ninhydrin ($5x10^{-2}$ $mol.L^{-1}$), 0.4 mL of 0.10 M NaOH (adjusted pH 9.6 with 0.10 M HCl) and diluted to 5 mL with distilled water and the flask was heated on a water bath at 90°C for 15 min. After the flask had been cooled to room temperature, the solution was made up to the mark with studding solvent. The absorbance of the solution was measured against a reagent blank at used λ_{max}.

Results and Discussion

Spectrophotometric results

UV-Vis spectra of carbocisteine(CS), ninhydrin(NN) and the formed complex [CS]:[NN] solutions in solvents (acetonitrile, isopropanol, ethanol and methanol with water 1:1) was obtained. CS and NN solutions do not absorb in the range 450-800 nm. [CS]:[NN] complex solutions have maximum absorption at λ_{max} 568-572 nm (ε for the complex was 8827, 11387, 13180 and 13425 $L.mol^{-1}.cm^{-1}$ in mentioned solvents, respectively). See Figure 2 as example in methanol.

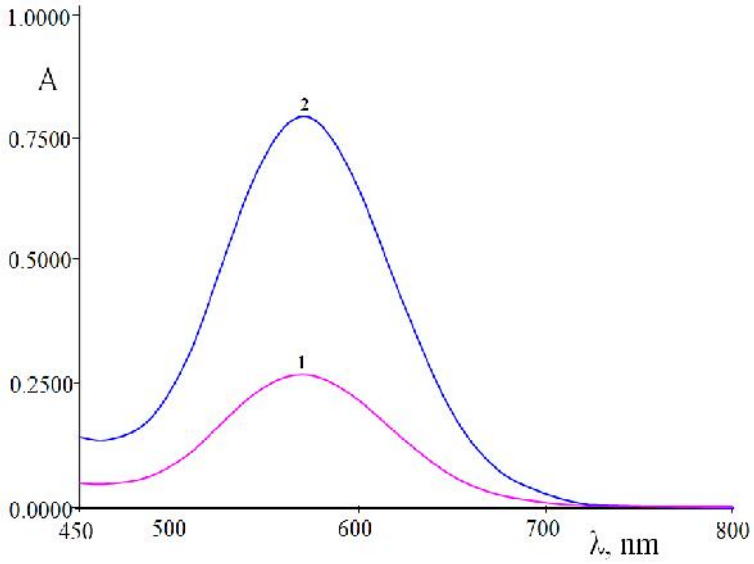


Fig.1. Spectra in methanol-water (1:1) of: 1- complex 20 µmol.L^{-1} of CS with $2.5x10^{-2}$ mol.L^{-1} of NN); 2- complex 60 µmol.L^{-1} of CS with $2.5x10^{-2}$ mol.$L^{-1}$of NN); Blank is $2.5x10^{-2}$ mol.$L^{-1}$of NN; (ℓ =1 cm. t=90°C heating time 15 min, pH 9.6).

The effect of pH

The influence of pH solutions on spectrum of complex [CS]:[NN] in methanol-water (1:1) from pH (3.0 to12) using 0.4 mL of 0.10 M NaOH (pH adjustment by 0.10 M HCl) on absorbance (A) and λ_{max} were studied. The absorbance value increases with increasing pH value of 3.0 to 5.0, then becomes semi-fixed between pH 5.0 to 8.5, after that increases until pH 9.3, then becomes semi-fixed again between pH 9.3 to 10.5 and finally decreases. While λ_{max} semi-fixed at pH (3.0 to 12), see Figure 1. The optimum pH was found to be 9.6.

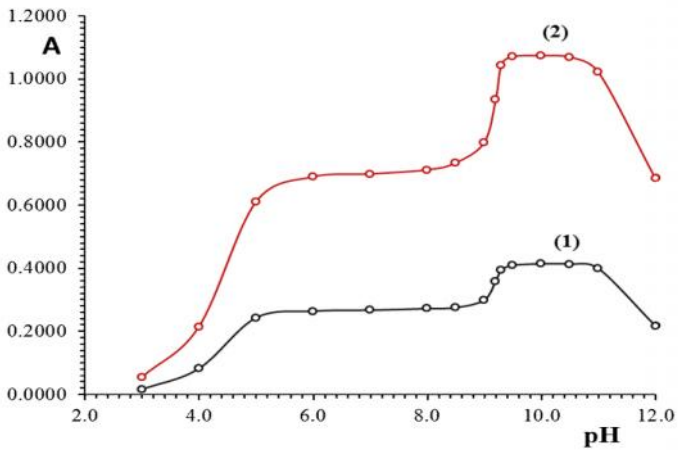


Fig.2: The effect of pH solutions on spectrum of complex [CS]:[NN] in methanol-water (1:1): 1- complex 30 µmol.L^{-1} of CS with 2.5x10^{-2} mol.L^{-1}of NN ; 2- complex 80 µmol.L^{-1} of CS with 2.5x10^{-2} mol.L^{-1} of NN (ℓ =1 cm. t= 90°C heating time 15 min)

The effect of solvent

The effect of ratio solvents –water on absorbance of formed complex [CS]:[NN]. The study revealed that the color development remained stable in 50% (V/V) methanol - water, see Figure 3 as example.

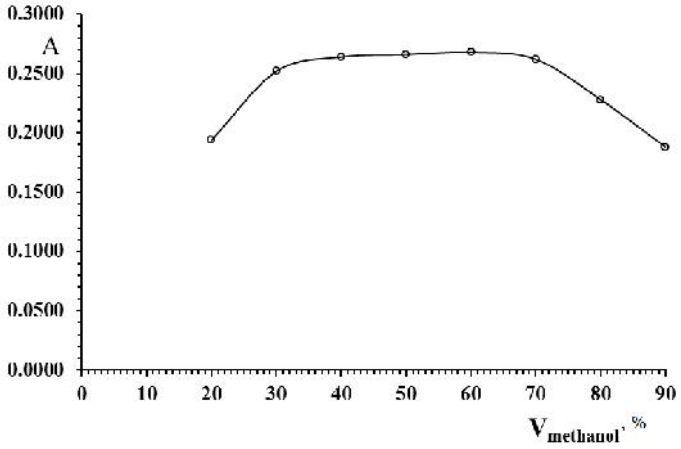


Fig.3: The effect of ratio methanol – water on absorbance of formed complex [CS]:[NN] (C_{NN} 2.5x10^{-2} M, C_{CS} 20 µM, Blank is methanol – water, ℓ =1 cm).

Effect of temperature and heating time

The effect of temperature and heating time on the complex [CS]:[NN] formation were studied with in the temperature range 15-95^{o}C and the heating time range 5-20 min. The reaction mixture was heated on a water bath at 90±2 °C. A colored product was obtained and the color intensity reached its maximum value after 15 min of heating. After reaching the ambient temperature, the reaction mixture was transferred to a 5 mL volumetric flask and diluted to the mark with used solvents. Hence, the absorbance was measured after 15 min of heating. The results are shown in Figures 4 & 5.

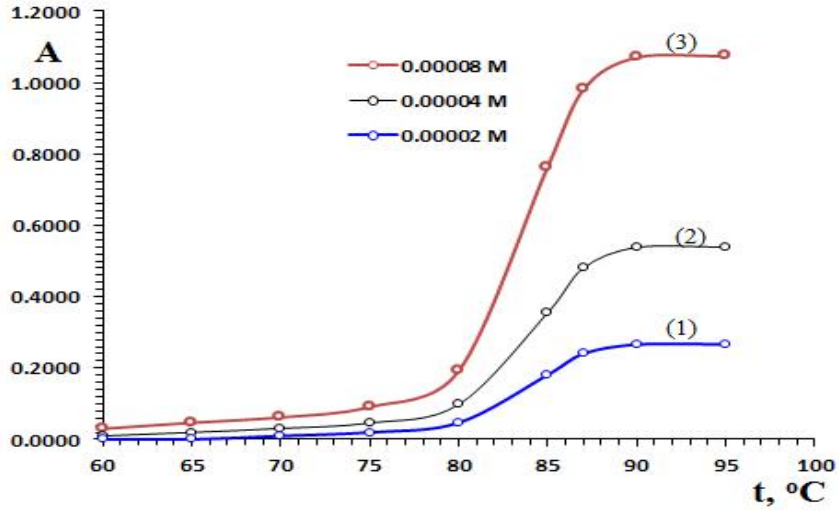


Fig.4: Effect of temperature on color development for the formation complex [CS]:[NN], C_{CS}: 1- 20 µM, 2- 40 µM, 3- 80 µM**.**

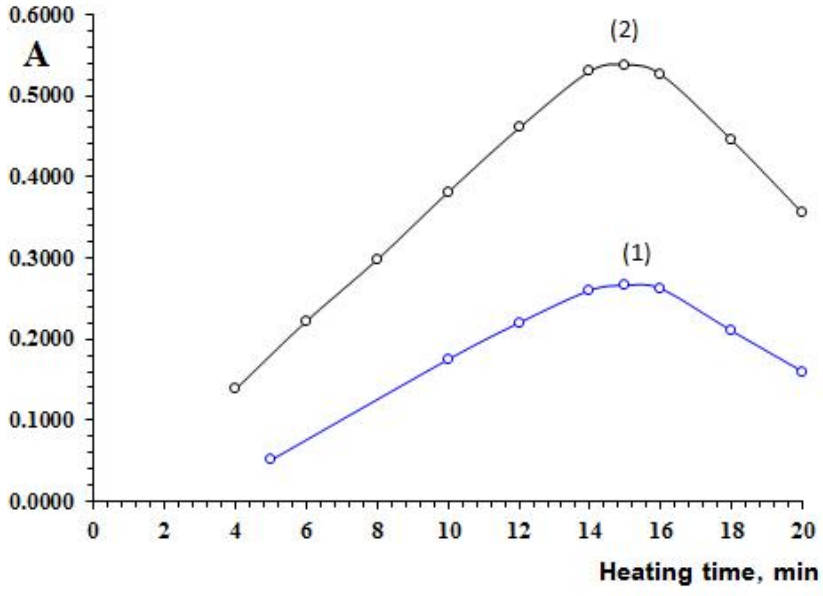


Fig.5: Effect of heating time on color development for the complex [CS]:[NN] formation, where C_{CS}: 1- 20 µM, 2- 40 µM.

Effect of ninhydrin concentration

To 0.3 mL of 0.001 M carbocisteine solution, 0.4 mL of 0.10 M NaOH (adjusted pH 9.6 with 0.10 M HCl), different volumes (0.5 - 4.8 mL) of 0.05 M ninhydrin were added, diluted to 5 mL with methanol-water (1:1). The reaction mixtures were heated for 15 min on a water bath at 90 ± 2 °C. The colored product was diluted to 5 mL with methanol after heated and the absorbance was measured against a reagent blank at λ_{max}. The results showed that the highest absorbance was obtained with 2.5 mL of 0.05 M ninhydrin solution that remained unaffected with higher amounts (Figure 6).

The effect of NN concentration on formation complex [CS]:[NN] was investigated. It was observed that the absorbance of the formed complex increased coinciding with increasing the ratio of [CS]:[NN] until the ratio (1:250), then it becomes almost fixed.

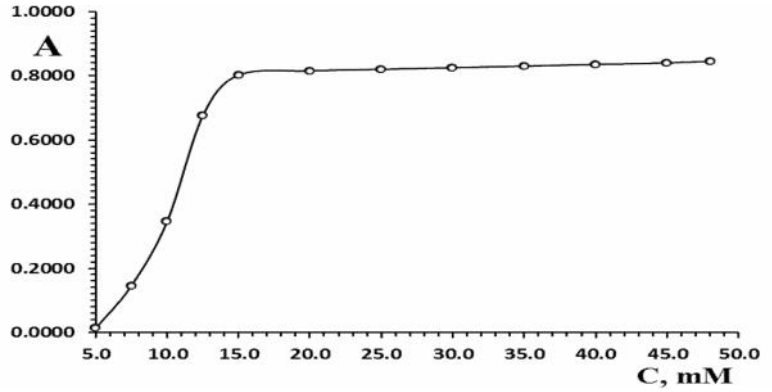
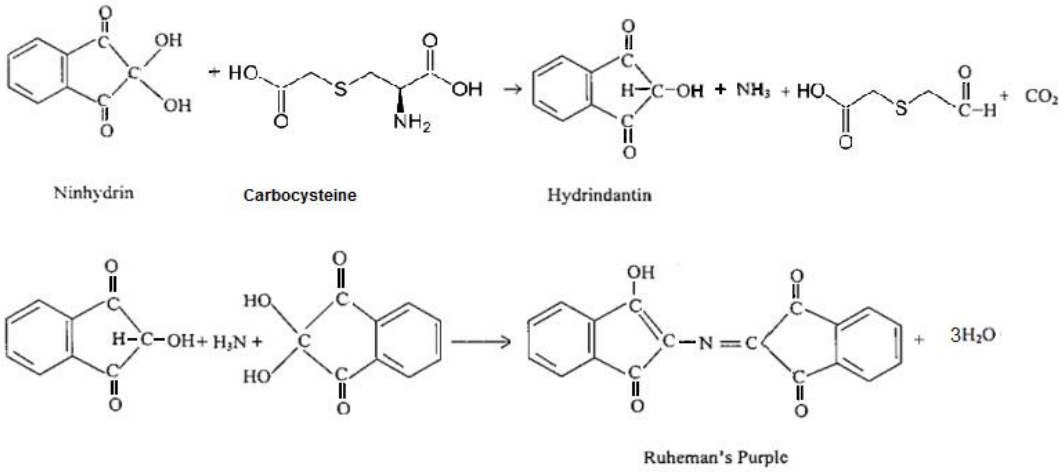


Fig.6: Effect of reagent concentration (ninhydrin) on color development for the formation complex [CS]:[NN], (Carbocisteine 60 μM).

Mechanism of reaction

Carbocisteine (CS) reacts with ninhydrin reagent in 0.10 M NaOH at pH 9.6 (pH adjustment by 0.10 M HCl) medium via oxidative deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the colored reaction product Ruhemenn's purple [16] at λ_{max} (scheme 1). To optimize the reaction conditions, a number of parameters such as heating time, reagent concentration, temperature, pH, stability of color and solvent have investigated. Varying one variable and observing its effect on the absorbance of the colored product established the optimum reaction conditions.



Scheme 3: The possible reaction mechanism of [CS]:[NN] complex formation.

Calibration curve

The calibration curve of carbocisteine in pure form through complexation with ninhydrin showed excellent linearity over concentration range of 1.00 – 100.00 µmol.L^{-1} (0.1792-17.919 µg.mL^{-1}) for the solvents (methanol, ethanol, isopropanol and acetonitrile with water ratio 1:1) in presence of 2.5×10^{-2} mol.L^{-1} of ninhydrin with good correlation coefficient in studied solvents. Regression equation at λ_{max} was as the follows:
y_1=0.0749x+0.0007 (R^2= 0.9999),
y_2= 0.0733x+0.0002 (R^2= 0.9997),
y_3= 0.0635x+0.0025 (R^2= 0.9996) and
y_4= 0.0491x+0.0002 (R^2= 0.9994),
in methanol, ethanol, isopropanol and acetonitrile, respectively. Figures 7-10 showed the spectra of [CS]:[NN] complex in presence of 2.5×10^{-2} M of NN. The spectra characteristics of the method such as the molar absorptivity (ε), Beer's law, regression equation at λ_{max} (y=a.x+b); where y=absorbance, a=slope, x=concentration of CS by µg.mL^{-1}, b=intercept, the correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) and the optimum conditions for spectrophotometric determination of CS through formation complex using NN in mentioned solvents is summarized in Table 1.

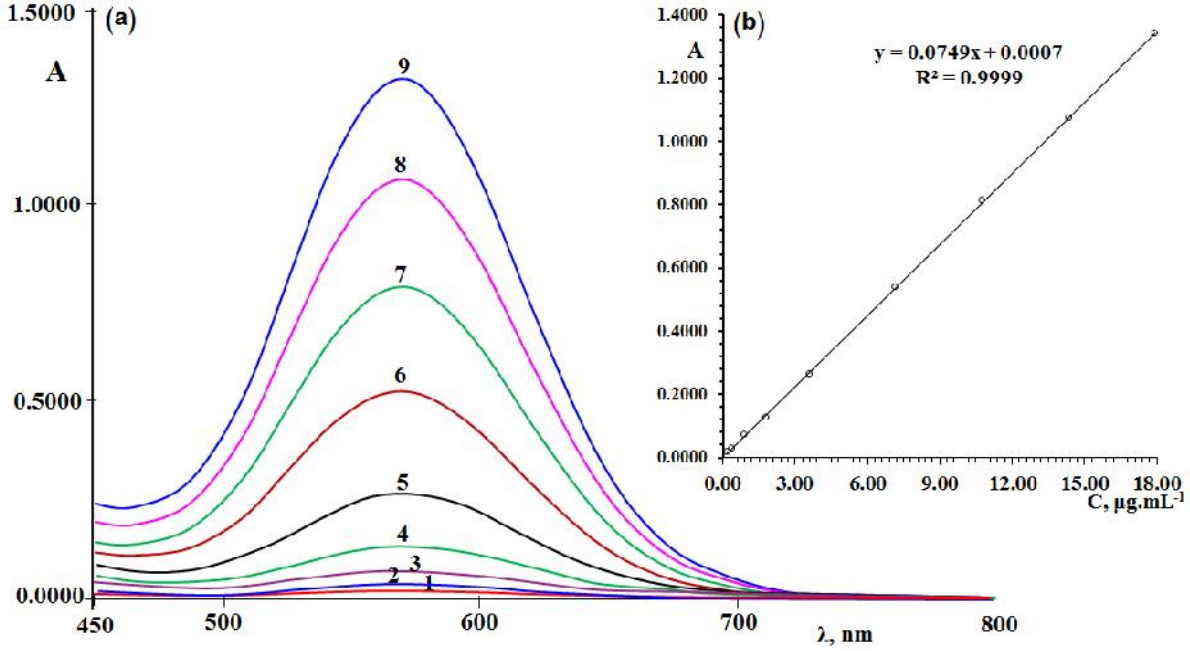


Fig.7: (a) Spectra and calibration curve of [CS]:[NN] complex in presence of 2.5×10^{-2} M of NN; where concentration of CS as the follows: 1- 0.1792, 2- 0.3584, 3- 0.8960, 4- 1.7919, 5- 3.5838, 6- 7.1676, 7- 10.7514, 8- 14.3352, 9- 17.9190 µg.mL^{-1} (ℓ = 1.0 cm , blank is 2.5×10^{-2} M of NN in methanol).
(b) Calibration curve of CS in pure form through complexation with NN in presence of 2.5×10^{-2} mol.L^{-1} of NN in methanol.

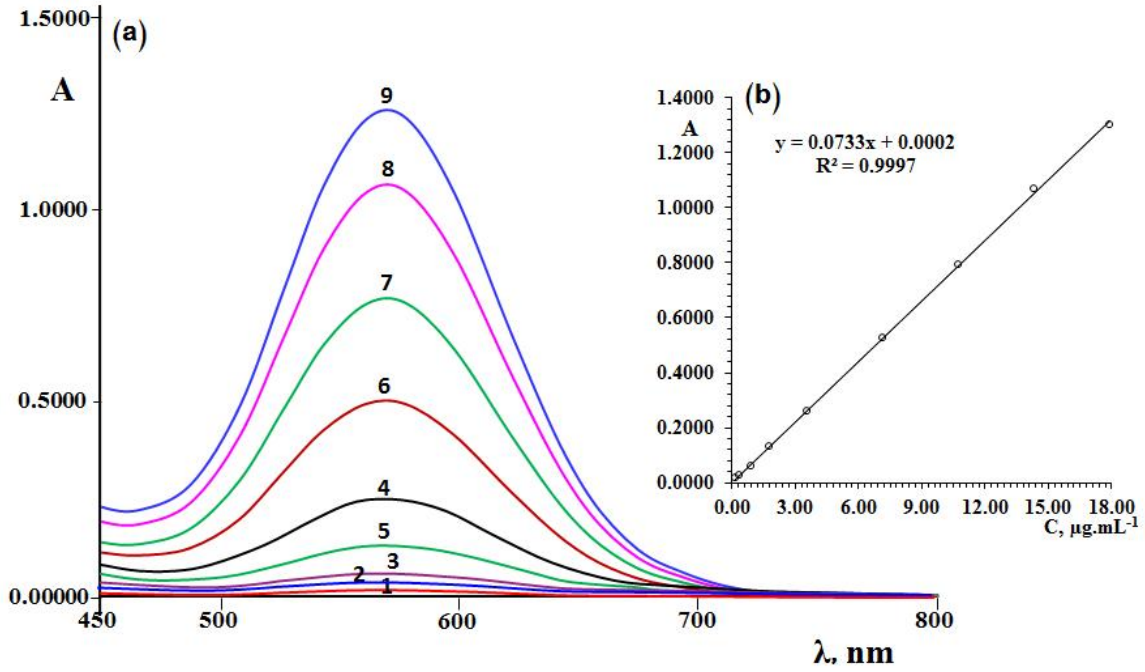


Fig. 8: (a) Spectra and calibration curve of [CS]:[NN] complex in presence of 2.5×10^{-2} M of NN; where concentration of CS as the follows: 1- 0.1792, 2- 0.3584, 3- 0.8960, 4- 1.7919, 5- 3.5838, 6- 7.1676, 7- 10.7514, 8- 14.3352, 9- 17.9190 µg.mL^{-1} (ℓ = 1.0 cm , blank is 2.5×10^{-2} M of NN in ethanol).
(b) Calibration curve of CS in pure form through complexation with NN in presence of 2.5×10^{-2} mol.L^{-1} of NN in ethanol.

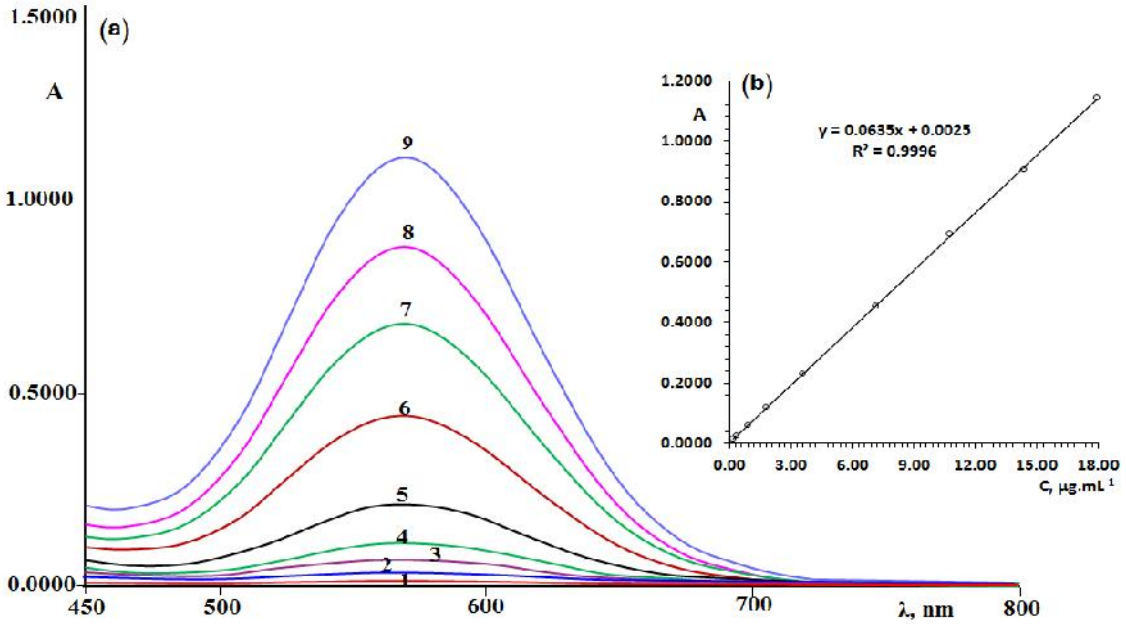


Fig. 9: (a) Spectra and calibration curve of [CS]:[NN] complex in presence of 2.5×10^{-2} M of NN; where concentration of CS as the follows: 1- 0.1792, 2- 0.3584, 3- 0.8960, 4- 1.7919, 5- 3.5838, 6- 7.1676, 7- 10.7514, 8- 14.3352, 9- 17.9190 µg.mL^{-1} (ℓ = 1.0 cm, blank is 2.5×10^{-2} M of NN in isopropanol).
(b) Calibration curve of CS in pure form through complexation with NN in presence of 2.5×10^{-2} mol.L^{-1} of NN in isopropanol.

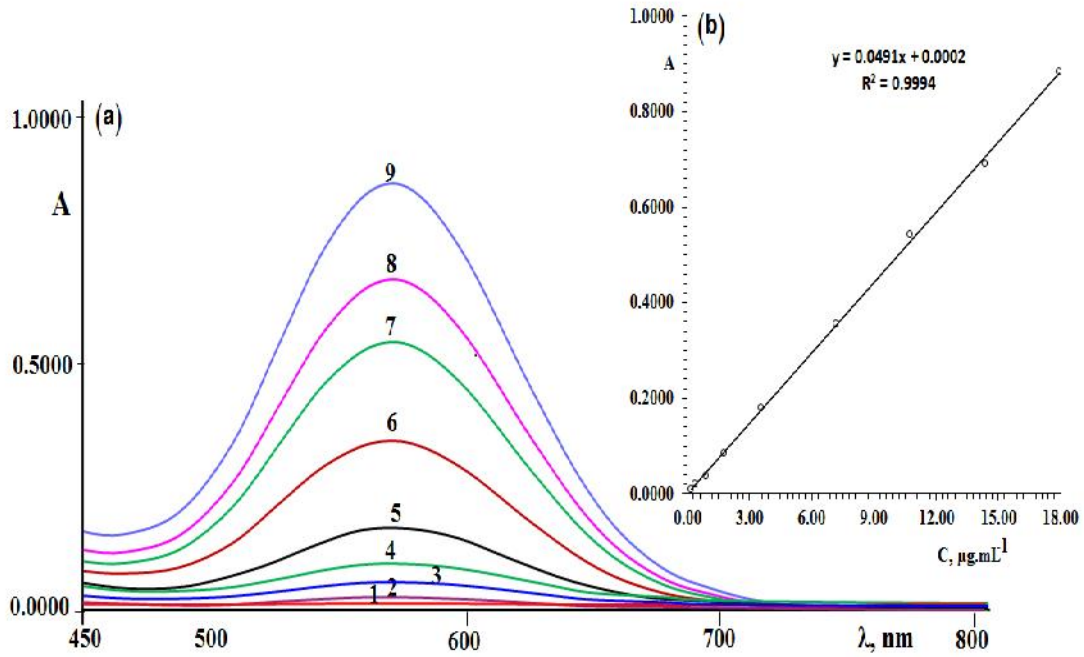


Fig. 10: (a) Spectra and calibration curve of [CS]:[NN] complex in presence of 2.5×10^{-2} M of NN; where concentration of CS as the follows: 1- 0.1792, 2- 0.3584, 3- 0.8960, 4- 1.7919, 5- 3.5838, 6- 7.1676, 7- 10.7514, 8- 14.3352, 9- 17.9190 µg.mL^{-1} (ℓ = 1.0 cm , blank is 2.5×10^{-2} M of NN in acetonitrile).
(b) Calibration curve of CS in pure form through complexation with NN in presence of 2.5×10^{-2} mol.L^{-1} of NN in acetonitrile.

Table 1. The parameters established for spectrophotometric determination of CS by formation complex with NN in studied solvent-water (1:1).

Parameters	Operating values			
	Methanol	**Ethanol**	**Isopropanol**	**Acetonitrile**
λ_{max} of CS:NN complex, nm	570	572	569	**568**
Beer's Law Limit by µmol.L^{-1}	1.00-100.00			
Beer's Law Limit by µg.mL^{-1}	0.1792–17.9190			
ε of complex (L.mol^{-1}.cm^{-1})	13425	13180	11387	8827
Y=a.x+b for complex at λ_{max}	y=0.0749x+0.0007	y=0.0733x+0.0002	y=0.0635x+0.0025	y=0.0491x+0.0002
Slope	0.0749	0.0733	0.0635	0.0491
Intercept	0.0007	0.0002	0.0025	0.0002
Correlation coefficient (R^2)	0.9999	0.9997	0.9996	0.9994
C_{NN}:C_{CS}, M	≥250			
Stability	24 hour			
Temperature of solution	90±2°C			

n=5, t=2.776.

Analytical results

Spectrophotometric determination of carbocisteine through complexation with ninhydrin in solvents (methanol, ethanol, isopropanol and acetonitrile) within optimal conditions using calibration curve was applied. The results, summarized in Table 2, showed that the determined concentrations of CS were rectilinear over the range of 1.00 to 100.00 µM (0.1792 to 17.919 µg.mL^{-1}) with relative standard deviation (RSD) not more than 3.2% for the concentrations of CS (0.1792 µg.mL^{-1}) in methanol as example. The results obtained from the developed method have been compared with the official RP-HPLC method [13]. The compatibility between them was good.

Table 2: Spectrophotometric determination of CS through formation complex with NN within optimal conditions using calibration curve in studied solvent.

X_i, µg.mL^{-1} (Taken)	**Solvents**	*** $\bar{x}$ ±SD, µg.mL^{-1} (Found)**	**$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, µg.mL^{-1}**	**RSD%**
0.1792	Methanol	0.1822±0.0058	0.1822±0.0072	3.2
	Ethanol	0.1853±0.0061	0.1923±0.0076	3.3
	Isopropanol	0.1811±0.0063	0.1811±0.0078	3.5
	Acetonitrile	0.1772±0.0073	0.1772±0.0091	**4.1**
0.3584	Methanol	0.3645±0.0109	0.3645±0.0135	**3.0**
	Ethanol	0.3386±0.0108	0.3386±0.0134	3.2
	Isopropanol	0.3684±0.0125	0.3684±0.0156	3.4
	Acetonitrile	0.3796±0.0144	0.3796±0.0179	3.8
0.8960	Methanol	0.9115±0.0273	0.9115±0.0339	3.0
	Ethanol	0.8758±0.0271	0.8758±0.0336	3.1
	Isopropanol	0.9055±0.0299	0.9055±0.0371	3.3
	Acetonitrile	0.8438±0.0312	0.8438±0.0387	3.7
1.7919	Methanol	1.7872±0.0518	1.7872±0.0643	2.9
	Ethanol	.7981±0.05571	.7981±0.06921	3.1
	Isopropanol	.8189±0.05821	.8189±0.07231	3.2
	Acetonitrile	1.7426±0.0627	1.7426±0.0778	3.6
3.5838	Methanol	3.5587±0.0996	3.5587±0.1237	2.8
	Ethanol	3.5643±0.1070	3.5643±0.1328	3.0
	Isopropanol	.5984±0.11163	.5984±0.13863	3.1
	Acetonitrile	3.6112±0.1264	3.6112±0.1569	3.5
7.1676	Methanol	7.1623±0.1934	7.1623±0.2401	2.7
	Ethanol	7.1863±0.2084	7.1863±0.2587	2.9
	Isopropanol	7.1389±0.2142	7.1389±0.2659	3.0
	Acetonitrile	7.2261±0.2457	7.2261±0.3050	3.4

10.7514	Methanol	10.8718±0.2827	10.8718±0.3510	**2.6**
	Ethanol	10.8158±0.3028	10.8158±0.3759	2.8
	Isopropanol	10.8740±0.3153	10.8740±0.3914	2.9
	Acetonitrile	11.0153±0.3415	11.0153±0.4240	3.1
14.3352	Methanol	14.3325±0.3583	14.3325±0.4448	2.5
	Ethanol	14.5218±0.3776	14.5218±0.4688	2.6
	Isopropanol	14.2598±0.3993	14.2598±0.4957	2.8
	Acetonitrile	14.1326±0.4240	14.1326±0.5264	3.0
17.9190	Methanol	17.8866±0.4293	17.8866±0.5330	2.4
	Ethanol	17.5756±0.4570	17.5756±0.5674	2.6
	Isopropanol	17.9610±0.4849	17.9610±0.6020	2.7
	Acetonitrile	17.9796±0.5394	17.9796±0.6700	3.0

n=5, t=2.776.

Table 3: Determination of carbocisteine in some Syrian pharmaceutical preparations using spectrophotometric method through formation complex with 2.5×10^{-2} M of NN within optimal conditions using calibration curve in methanol.

Dosage form	Label Claim of CS, mg/cap	*Mean ±SD CS, mg/cap	RSD%	Assay %	*Mean ±SD CS, mg/tab by RP-HPLC [13]	* Assay %, by RP-HPLC[13]
Mucolar capsule, ELSAAD PHARMACEUTICAL	375	385±10.01	2.6	102.7	383±10.25	102.1
Mucoxil capsule, Al-SHAHBA PHARMACEUTICAL	375	380±10.26	2.7	101.3	381±10.52	101.6
Carbocisteie capsule, Al-YOUSEF PHARMACEUTICAL	375	373±10.07	2.7	99.5	374±11.0	99.7

***** n=5.

Method validation

The developed method for simultaneous estimation of carbocisteine has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [17].

Linearity

Several aliquots of standard stock solution of carbocisteine were taken in different 5 mL volumetric flask and diluted up to the mark with studied solvents such that their final concentrations were 0.1792-17.919 μg.mL^{-1} of CS. Absorbance was plotted against the corresponding concentrations to obtain the calibration graph, see Figures 7-10 and Table 2. Linearity equations obtained at λ_{max} suitable for used solvent were applied, see Table 1.

Precision and Accuracy

The precision and accuracy of proposed method was checked by recovery study by addition of standard drug solution to pre-analyzed sample

solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for carbocisteine. The basic concentration level of sample solution selected for spiking of the carbocisteine standard solution was 7.1676 µg.mL^{-1}. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of carbocisteine in pure and dosage forms with percent recoveries ranged from 99.2% to 101.3%, see Table 4 (in methanol as example).

Table 4: Results of recovery studies (in methanol as example).

Level	% Recovery
80%	99.2
100%	101.3
120%	101.2

n=5

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 10.7514 µg.mL^{-1} of carbocisteine using the studied spectrophotometric method under the optimum conditions. The found amount of carbocisteine ($\bar{x}$ ±SD) 10.8716 ± 0.2718 µg.mL^{-1} (in dichloromethane as example). The percentage recovery was found to be 101.1 ± 2.5 with RSD of 0.025. These values indicate that the proposed method has high repeatability for carbocisteine analysis.

Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the LOD and LOQ. The values of LOD and LOQ for carbocisteine are 0.019 and 0.058 µg.mL^{-1} (in methanol as example).

Robustness

The robustness of the method adopted is demonstrated by the constancy of the absorbance with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients, temperature (90±2°C), stability (23-25 h) and reaction time (15±1 min), see Table 5 which indicates the robustness of the proposed method. The absorbance was measured and assay was calculated for five times (in methanol as example).

Table 5: Robustness of the proposed spectrophotometric method (in methanol as example).

Experimental parameter variation	Average recovery (%)*	
	C_{CS}	
	0.3584 µg.mL^{-1}	1.7919 µg.mL^{-1}
Temperature 88°C 92°C	 99.6 100.5	 99.8 100.6
Stability 23 hour 25 hour	 99.9 100.2	 100.0 100.5
Reaction time 14.0 min 16.0 min	 99.2 100.6	 99.4 100.8

* n=5.

Selectivity

Several other components were examined under the conditions that had been optimized for carbocisteine determination. The results show that guaifenesin and oxomemazine did not interfere when it presents at same amount with carbocisteine.

Specificity

The specificity of the method was ascertained by analyzing standard CS in presence of excipients. These findings prove that the suggested methods are specific for determination of the investigated drugs without interference from the co-formulated adjuvants.

Conclusion

Effect of solvents (methanol, ethanol, isopropanol and acetonitrile with water ratio 1:1) on direct spectrophotometric determination of carbocisteine (CS) in pure form and pharmaceutical formulations using ninhydrin (NN) was studied. The method is based on reaction between CS and NN and formation complex. The peak of these complex's occurs at wavelengths from 568-572 nm. The absorbance's were proportional to the concentration of CS at range 1.00 to 100.00 µM (0.1792 to 17.919 $\mu g.mL^{-1}$) in present $2.5x10^{-2}$ M of NN. The method was validated for linearity, precision and accuracy, repeatability and robustness. The method was successfully applied for determination of CS in pure and pharmaceutical formulations samples with relative standard deviations did not exceed 3.2% for the concentrations of CS (0.1792 $\mu g.mL^{-1}$) in methanol as example.

References

1. Neil J.O., 1995 - "The Merck Index, Merck Research Laboratories". 12th Ed., Martindale, Merck.
2. Budavari S, 2011-"The Merck Index" 13th Ed. Merck & Co. Inc. 856.
3. British Pharmacopoeia, 2009- Her Majesty's Stationary Office, London. Volume I, II, and III,
4. Walash M.I., El-Brashy A.M., Metwally M.E., Abdelal A.A., 2004- Fluorimetric determination of carbocisteine and ethionamide in drug formulation, Acta Chim. Slov, 51: 283-291.
5. Ayad M.M., Abdellatef H.E., Hosny M.M., Sharaf Y.A., 2013- Application of 4-chloro-7-nitrobenzofurazan for the analysis of propafenone and diltiazem hydrochlorides using kinetic spectrophotometric and spectrofluorimetric methods, European Journal of Chemistry, 4(1): 35-43.
6. Walash M.I., El-Brashy A.M., Metwally M. E., Abdelal A.A., 2004- Spectrophotometric determination of penicillamine and carbocisteine based on formation of metal complexes, IL FARMACO, 59: 493-503.
7. Rele R.V., 2014 - Simultaneous spectrophotometric estimation of amoxicillin and carbocisteine by second order derivative spectroscopy method in combined dosage form, Int. J. Chem. Sci, 12(4):1587-1595.
8. Rele R.V., Patil S.S., 2013- Ultra-violet spectrophotometric method for validation of carbocisteine from bulk drug and pharmaceutical formulation, Int J Pharm Bio Sci, 4(2): 847 – 851.
9. Li S.Y., Guo Q.L., Yuan W., Hou Y.C., Du L.M., 2010- Spectrophotometric study of the charge transfer complexation of some amino acid derivative drugs as electron donors with 7, 7, 8, 8 - tetracyanoquinodimethane, Bull. Chem. Soc. Ethiop, 24(1): 21-30.
10. Wu F.Y., Liao W.S., Wu Y.M., Wan X.F., 2008- Spectroscopic determination of cysteine with alizarin red S and copper, Spectroscopy Letters, 41:393–398.
11. Taha E.A., Hassan N.Y., Abdel Aal F., El-Sayed Abdel Fattah L., 2008- Kinetic spectrophotometric determination of acetylcysteine and carbocisteine in bulk powder and in drug formulations, Science Asia, 34:107-113.

12. Walash M.I., Metwally M.E., El-Brashy A.M., Abdelal A.A., 2003- Kinetic spectrophotometric determination of some sulfur containing compounds in pharmaceutical preparations and human serum, Il Farmaco, 58 : 1325-1332.
13. Rele R. V, Patil S. P. 2010-Reversed Phase High Pressure Liquid Chromatography Technique for Determination of Carbocisteine from Pharmaceutical Formulation, J.Chem.Parm.Res., 2(4), 24-30.
14. Rele R.V, Rane D.G., 2017- Validation of Carbocisteine by Reversed Phase High Performance Liquid Chromatography Method from Active Pharmaceutical Dosage Form, International Journal of ChemTech Research, 10(5):583-589.
15. Chauhan K., Mujawar A., Quazi I., 2016- HPTLC method development and validation for densitometric analysis of carbocisteine in drug formulation, International Journal of Applied Pharmaceutics, 8(4):22-25.
16. Siddiqui F.A., Sher N., Naz A., Hasan N., Shah S.N., Shehzad M., 2019- Kinetic Approach to Determine 3-Amino Methyl Hexanoic Acid in Pharmaceutical Formulations, Afr. J. Chem.,72:189–194.
17. ICH: Proceedings of the International Conference on Harmonization of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonized Tripartite Guidelines), 2005.

Access this Article in Online

Website: www.ijcrcps.com

Subject: Chemistry

Quick Response Code

DOI: 10.22192/ijcrcps.2021.08.05.004

How to cite this article:
Abdul Aziz Ramadan, Hasna Mandil, Ali Marei. (2021). Effect of Solvents on Spectrophotometric Analysis of Carbocisteine by Forming Complex with Ninhydrin . Int. J. Curr. Res. Chem. Pharm. Sci. 8(5): 27-41.
DOI: http://dx.doi.org/10.22192/ijcrcps.2021.08.05.004

