



**DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC
METHOD FOR L-CYSTEINE IN BULK FORM BY FIRST DERIVATIVE
SPECTROPHOTOMETRIC METHOD**

CHAUHAN NPK^{1*}, SURTI N², AND PATEL MP³

1, 2: Gujarat Technological University, Ahmedabad

3: L. M. College of Pharmacy, Ahmedabad

***Corresponding Author: Nirav Pravin Kumar Chauhan: E Mail: niravpharma07@gmail.com**

Received 10th May 2022; Revised 18th June 2022; Accepted 29th Aug. 2022; Available online 1st April 2023

<https://doi.org/10.31032/IJBPAS/2023/12.4.7029>

ABSTRACT

For estimating L-cysteine from bulk and pharmaceutical formulations, a simple, rapid, accurate, and cost-powerful first-order UV-by-product spectrophotometric technique has been developed. The maximum absorbance of L-cysteine in phosphate buffer (pH 8.0) is determined to be 232nm. The first-order derivative of the equal spectrum discovered maxima at 232 nm and minima at 204 nm. With a correlation coefficient of 0.9998, the L-cysteine follows linearity inside the concentration range of 100-500µg/ml. When the proposed method was applied to find out the concentration of L-cysteine in bulk, the percent amount estimated at 99.83% was found to be in good agreement with the label claim. Recovery experiments at three different levels, 80 percent, 100 percent, and 120 percent, were used to test the method's accuracy. The percent recovery was discovered to be between 99.87 to 100.03 percent. The method's accuracy and reproducibility are indicated by the low percent R.S.D. values. The method's precision was examined in terms of intra-day, inter-day, and repeatability. The method is precise if the percent R.S.D. value is comparatively less than 2. The robustness was investigated with the help of two analysts. For routine analysis of L-cysteine in bulk, the above method was a quick and cost-effective quality-control tool.

Keywords: L-cysteine, first-order derivative, quantitative determination, phosphate buffer (pH 8.0)

INTRODUCTION

L-cysteine is a white, water-soluble solid that is chemically (2R)-2-amino-3-sulfanylpropanoic acid (**Figure 1**), according to IP (Indian Pharmacopoeia, 2007) [1]. In humans, cysteine is a sulfur-containing non-essential amino acid [2, 3]. Protein synthesis, detoxification, and a variety of metabolic functions all require cysteine [4-6]. Cysteine is found in beta-keratin, the main protein in nails, skin, and hair, and plays a role in collagen production, skin elasticity, and texture. Cysteine is a component of the antioxidant glutathione and plays a role in the metabolism of important biomolecules such as coenzyme A, heparin, and biotin [7, 8]. It is also required in the manufacture of the amino acid taurine. (NCI04) [9, 10]. L-cysteine is a form of cysteine with an L-configuration that is optically active [11]. It functions as a flour treatment agent, a human metabolite, and an inhibitor of the enzyme EC 4.3.1.3 (histidine ammonia-lyase) [12, 13]. It is a proteinogenic amino acid, a cysteine, and an L-alpha-amino acid from the serine family [14, 15]. It is an L-cysteine conjugate base. It's an L-cysteine conjugate acid (1-). It's a D-enantiomer. It's a tautomer of the zwitterion L-cysteine [16].

A review of the literature revealed a UV-spectrophotometric method for the

determination of L-cysteine in the bulk material [17, 18].

Two simple, inexpensive, and quick spectrophotometric methods for quantifying L-cysteine in bulk material were developed in this study. The developed methods were tested for precision and accuracy [19, 20].

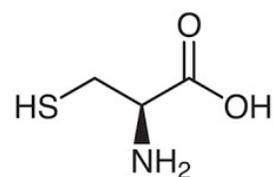


Figure 1: L-cysteine

MATERIALS AND METHODS

Instruments

Micropipette of different volumes (Microlit, India), UV-Visible double beam spectrophotometer (UV-1800, Shimadzu, Japan) with 1cm matched quartz cells, pH meter and Digital balance (Denver Instrument, Germany).

Materials

Disodium Hydrogen Phosphate, L-Cysteine was purchased from SRL Laboratories. Sodium Chloride, Potassium dihydrogen Phosphate.

Arrangement of standard arrangement: Preparation of Standard Stock Solution of L-cysteine (10000µg/mL)

A standard stock arrangement containing 1000 µg/mL of L-Cysteine was ready by

Dissolving precisely weighed 100 mg amount of L-Cysteine in and weakening to volume with phosphate buffer pH8 in a 10 mL volumetric jar. After that Pipette out 0.1, 0.2, 0.3, 0.4 and 0.5 mL of Stock arrangement in 10 mL Volumetric jar to make 100,200,300,400 and 500 µg/mL Solution. A UV Spectrophotometer with a wavelength range of 200-400 nm was used to examine all of the aliquots listed.

Spectrophotometric Conditions

Mode: Spectrum, Scan speed: Fast, Wavelength range: 200-400 nm, Absorbance scale: 0.00A - 4.00A, Initial measure correction: Phosphate buffer (pH 8.0).

Derivative Condition

The standard drug solution was diluted so as to get the final concentration in the range of 100-500 µg/ml and scanned in the first order derivative spectra. The first order derivative spectra at showed a maxima and minima at 204 and 232 nm respectively. The amplitude of absorbance was measured at 204 nm (peak maxima) and at 232 nm (peak minima) and Take Aptitude Difference was plotted against concentration to give calibration curve, and regression equation was calculated. The amplitude was linear in the concentration range of 100-500µg/ml [21].

Accuracy (% Recovery)

To concentrate on the accuracy of the proposed techniques, and to check the interference from excipients utilized in the measurement structures, recovery tests were completed by the standard addition method. Measure the Absorbance of the standard solution, Accuracy 80%, Accuracy 100 percent and Accuracy 120% arrangements. Calculate the amount found and amount added for L-Cysteine, compute the singular recovery and mean recovery values [21]. The outcomes are displayed in **Table 2**.

Precision

Repeatability (Intra-day Precision):

Repeatability (Intra-day precision) was determined by analyzing L-cysteine standard solutions in the range 100,300 and 500µg/ml for first order derivative spectrophotometric in triplicate. Calculate % RSD for L-cysteine.

Inter day precision:

Inter-day precision was determined by analyzing L-cysteine standard solutions in the range 100,300 and 500µg/mL for first-order derivative spectrophotometric intriplicate on the different days. Calculate % RSD for L-cysteine.

Limit of Detection and Limit of

Quantification: The limit of discovery (LOD) and the limit of quantification (LOQ) were determined utilizing the standard

deviation of y-intercept of calibration curve (N) and slope (S) of the calibration curve.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ is the SD of the response S is the slope of the calibration curve.

Table 1: Linearity data for L-cysteine

Parameter	Result
Absorption maxima and minima (nm)	204 232
Beer's-Lamberts range ($\mu\text{g/ml}$)	100-500
Regression equation $y=mx+c$	$Y= 0.0003x+0.0262$
Slope(m)	0.0003
Intercept(c)	0.0262
Correlation coefficient (r ²)	0.9998

Table 2: Results of Intra-day precision and Inter-day precision

Concentration ($\mu\text{g/mL}$)	Intraday precision		Interday precision	
	Aptitude difference \pm S.D (n=3)	%RSD	Aptitude difference \pm S.D (n=3)	%RSD
100	0.050 \pm 0.00058	1.14	0.050 \pm 0.00057	1.15
300	0.101 \pm 0.000577	0.57	0.101 \pm 0.00100	0.99
500	0.150 \pm 0.00058	0.38	0.151 \pm 0.00100	0.66

Table 3: Recovery data for L-Cysteine

Amount of sample (mg/mL)	Amount of Standard added (mg/mL)	Amount found Mean* \pm S.D (mg/mL)	%Recovery	%RSD
1.2	0.96	2.16 \pm 0.282	99.90	0.28
1.2	1.2	2.4 \pm 0.440	100.03	0.44
1.2	1.44	2.64 \pm 0.300	99.87	0.30

Table 4: LOD and LOQ for L-Cysteine

L-Cysteine ($\mu\text{g/mL}$)	
LOD	LOQ
13.35	40.46

Table 5: Analysis L-Cysteine in Bulk

Drug	Amount of L-Cysteine (mg)		%Assay (n=6)	% RSD
	In Bulk	Estimated		
L-Cysteine	2.5	2.49	99.83	0.15

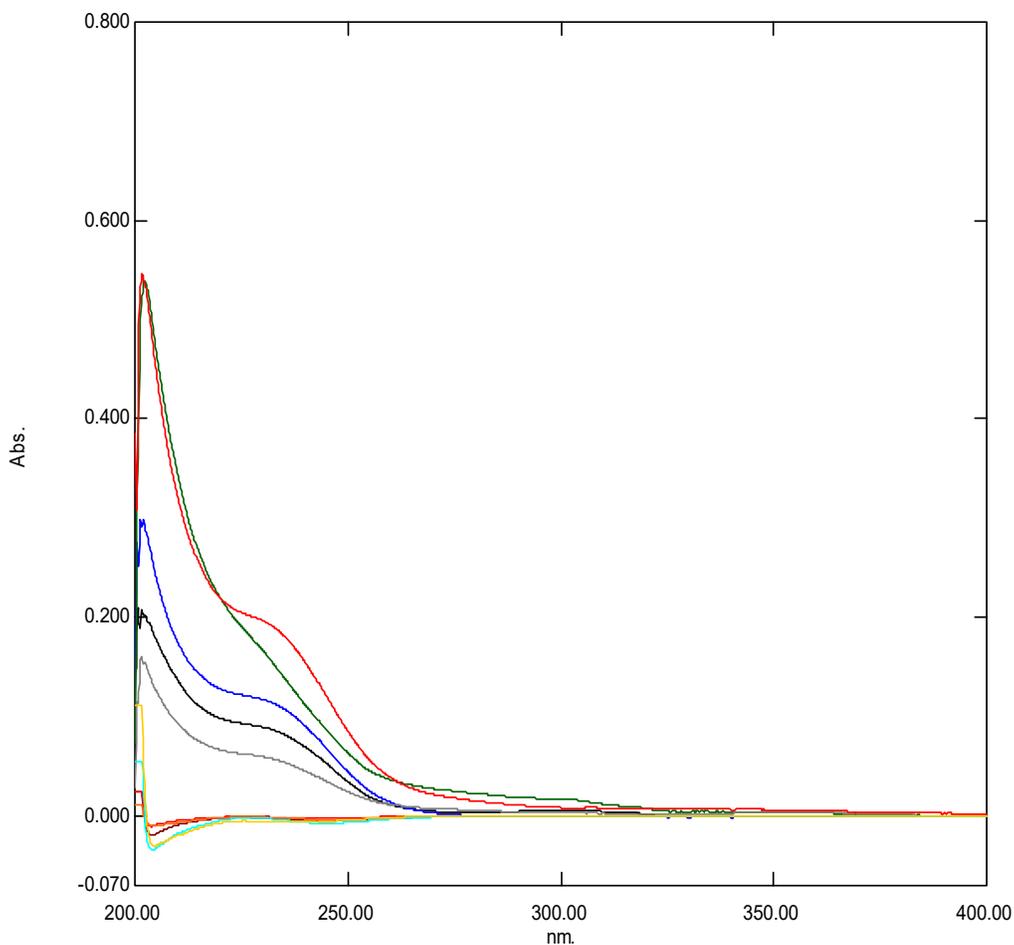


Figure 2: Linearity for first order spectra of L- Cysteine

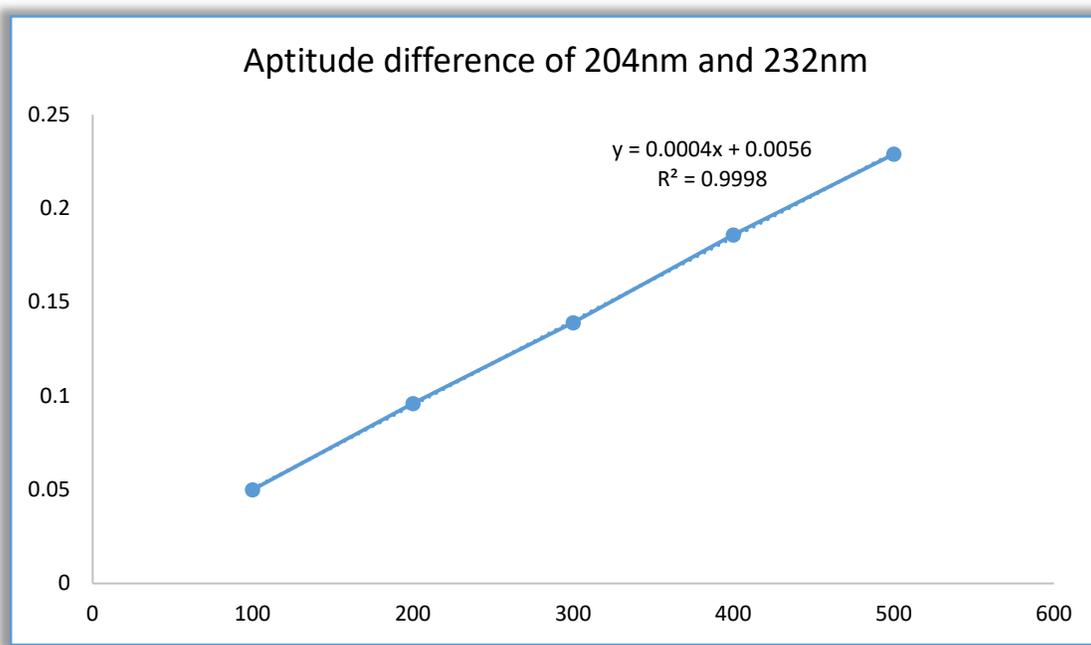


Figure 3: Calibration curve of L-cysteine

RESULTS AND DISCUSSION

The good outcomes were obtained with phosphate buffer (pH 8.0) for better reproducibility and repeatability, as L-cysteine is dissolvable in phosphate buffer. The derivative graphical method was utilized to estimate L-cysteine. The overlaid first-derivative spectrum L-cysteine was found to be appropriate for the determination of L-cysteine spectra which showed a maxima and minima in phosphate buffer. The spectrum of L-Cysteine has zero order absorbance at 232nm, where L-cysteine gives a significant derivative response, maxima at 232 nm and minima at 204 nm, which is near to the zero order absorbance.

Method Validation

Linearity

The Beer's was obeyed. A direct relationship was obtained between absorbance and concentrations of L-Cysteine in the range of 100-500 $\mu\text{g/mL}$, separately. The linearity of the calibration curve was approved by the value of correlation coefficients of the regression (r^2).

Precision

The % RSD for repeatability (intra-day) accuracy was viewed as in the range of 0.38-1.14%. While inter-day accuracy was viewed as in the range of 0.66-1.15% for L-cysteine, which demonstrates that the strategy is exact.

Accuracy (% Recovery)

The precision study was done by the standard addition method. The percent recovery was tracked down in the range of 99.87 to 100.03% for L-cysteine, which demonstrates the precision of the strategy.

Limit of recognition and Limit of Quantification:

The Limit of recognition (LOD) was viewed as 13.35 $\mu\text{g/mL}$ and the Limit of quantification (LOQ) was viewed as 40.46 $\mu\text{g/mL}$ for L-cysteine.

CONCLUSION:

For the determination of L-Cysteine in bulk, the method proposed in the above study was found to be simple, specific, economical, precise, and fast. Because it is both cost-effective and precise, the developed method may be preferred as an alternative for routine bulk L-Cysteine analysis.

Acknowledgment:

The authors would like to thank the authority of Gujarat Technological University

Financial support and sponsorship: The authors would like to thank the National fellowship for Persons with Disability for their kind support

Conflict of interest: The authors announce no conflict of interest.

REFERENCES

- [1] R W, L O, V S, G M. A quick, convenient, and economical method for the reliable determination of methylglyoxal in millimolar concentrations: the N-acetyl-L-cysteine assay. *Analytical and bioanalytical chemistry* [Internet]. 2012 Jul [cited 2022 Jun 6];403(9). Available from: <https://pubmed.ncbi.nlm.nih.gov/22580513/>
- [2] Basic Principles and Analytical Application of Derivative Spectrophotometry | IntechOpen [Internet]. [cited 2022 Jun 6]. Available from: <https://www.intechopen.com/chapters/37663>
- [3] Petri L, Szijj PA, Kelemen Á, Imre T, Gömörly Á, Lee MTW, et al. Cysteine specific bioconjugation with benzyl isothiocyanates. *RSC Adv.* 2020 Apr 8;10(25):14928–36.
- [4] Karpińska J. Derivative spectrophotometry—recent applications and directions of developments. *Talanta.* 2004 Nov 15;64(4):801–22.
- [5] Rojas FS, Ojeda CB, Pavon JM. Derivative ultraviolet-visible region absorption spectrophotometry and its analytical applications. *Talanta.* 1988 Oct;35(10):753–61.
- [6] N E. Derivative-differential UV spectrophotometry and compensation technique for the simultaneous determination of zidovudine and lamivudine in human serum. *Die Pharmazie* [Internet]. 2004 Feb [cited 2022 Jun 6];59(2). Available from: <https://pubmed.ncbi.nlm.nih.gov/15025177/>
- [7] El-Yazbi FA, Hammud HH, Assi SA. Derivative-ratio spectrophotometric method for the determination of ternary mixture of aspirin, paracetamol and salicylic acid. *Spectrochim Acta A Mol Biomol Spectrosc.* 2007 Oct;68(2):275–8.
- [8] Forsyth RJ, Ip DP. Determination of imipenem and cilastatin sodium in Primaxin® by first order derivative ultraviolet spectrophotometry. *Journal of Pharmaceutical and Biomedical Analysis.* 1994 Oct 1;12(10):1243–8.
- [9] Determination of mirtazapine in tablets by UV spectrophotometric and derivative spectrophotometric methods. - Abstract - Europe PMC [Internet]. [cited 2022 Jun 6]. Available from:

- <https://europepmc.org/article/med/11108534>
- [10] First-order derivative spectrophotometric estimation of gemifloxacin mesylate and ambroxol HCl in tablet dosage form | GSC Biological and Pharmaceutical Sciences [Internet]. [cited 2022 Jun 6]. Available from: <https://gsconlinepress.com/journals/index.php/gscbps/article/view/gscbps-2021-0034>
- [11] First-order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities - ScienceDirect [Internet]. [cited 2022 Jun 6]. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0731708503002048>
- [12] Tietje C, Brouder A, editors. International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use. In: Handbook of Transnational Economic Governance Regimes [Internet]. Brill | Nijhoff; 2010 [cited 2022 Jun 6]. p. 1041–53. Available from: https://brill.com/view/book/edcoll/9789004181564/Bej.9789004163300.i-1081_085.xml
- [13] Yin J, Ren W, Yang G, Duan J, Huang X, Fang R, et al. L-Cysteine metabolism and its nutritional implications. *Mol Nutr Food Res*. 2016 Jan;60(1):134–46.
- [14] De Vries N, De Flora S. N-acetyl-l-cysteine. *Journal of Cellular Biochemistry*. 1993;53(S17F):270–7.
- [15] (PDF) Optimizing derivatizing conditions using experimental design and simultaneous estimation of artemether and lumefantrine by ratio first order derivative spectrophotometric method [Internet]. [cited 2022 Jun 6]. Available from: https://www.researchgate.net/publication/308344501_Optimizing_derivating_conditions_using_experimental_design_and_simultaneous_estimation_of_artemether_and_lumefantrine_by_ratio_first_order_derivative_spectrophotometric_method
- [16] Dh B, Gl CM. Pharmacologic role of cysteine in ameliorating or exacerbating mineral toxicities. *The*

- Journal of nutrition [Internet]. 1987 Jun [cited 2022 Jun 6];117(6). Available from: <https://pubmed.ncbi.nlm.nih.gov/3298579/>
- [17] Game1 MD, Gabhane1 KB, Sakarkar2 DM. Quantitative analysis of clopidogrel bisulphate and aspirin by first derivative spectrophotometric method in tablets. Indian Journal of Pharmaceutical Sciences. 2010;72(6):825.
- [18] Quantitative Determination of Fluconazole In Syrups By First Order Derivative Spectrophotometry: Analytical Letters: Vol 35, No 7 [Internet]. [cited 2022 Jun 6]. Available from: <https://www.tandfonline.com/doi/abs/10.1081/AL-120005972>
- [19] Rodenas V, García MS, Sánchez-Pedreño C, Albero MI. Simultaneous determination of propacetamol and paracetamol by derivative spectrophotometry. Talanta. 2000 Jun 30;52(3):517–23.
- [20] Awasthi A. Novel Validated UV Spectroscopic Method for Routine Analysis of Decitabine Drug Substance. Indian Journal of Pharmaceutical Education and Research. 2020; 54(3s):s667–70.
- [21] Text and methodology, in the International Conference on Harmonization (ICH): Validation of Analytical Procedure, Q2 (R1), ICHPMA, Geneva, Switzerland, 2005.