Research Article

Quantitative determination of N-Acetyl cysteine by RP-HPLC method in bulk and parenteral injection

Sreenivasa Charan Archakam[1*], Sridhar Chenchugari[2], Chandrasekhar Kothapalli Banoth[3]

[1]*Research Scholar, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Anantapuramu -515002, Andhra Pradesh, India.*

[2]*Department of Pharmaceutical Analysis, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati – 517503, Andhra Pradesh, India.*

[3]*Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapuramu -515002, Andhra Pradesh, India.*

Received: 12 July 2018 Revised: 15 August 2018 Accepted: 20 August 2018

Abstract

Objective: The aim of the present work was to develop a simple and specific RP-HPLC method for the estimation of N-acetyl cysteine (NAC) in bulk and parenteral dosage form. **Materials and methods:** Separation was carried out on a C18 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) with an isocratic mobile phase constituting of potassium dihydrogen phosphate with pH 3.0: Acetonitrile (95:5 v/v). The flow rate was kept at 1.0 mL/min with a total run time of 10 minutes. A UV-detector was employed for the detection of NAC at a wavelength of 213 nm. The developed method was validated as per ICH guidelines for various validation parameters. **Results and Discussion:** NAC showed a retention time of 4.205 min. A calibration curve was constructed in the range of 10-50 µg/ml with a correlation coefficient $R^2 = 0.9999$. Specificity was demonstrated by the absence of interference peaks of the excipients in the parenteral dosage form. The accuracy was demonstrated as mean % recovery and it was found to be 101.6 %. The assay of the commercial parenteral injection was found to be 101.69%. The method was also evaluated for robustness and ruggedness and the results obtained were satisfactory. **Conclusion:** It is concluded that the developed RP-HPLC method was specific, precise, accurate, sensitive and robust for the estimation of NAC in bulk and parenteral dosage forms.

Keywords: N-Acetylcysteine, RP-HPLC, validation, quantitative determination, parenteral

Introduction

N-Acetyl cysteine (NAC) is a precursor of L-cysteine that leads to glutathione elevation biosynthesis. Glutathione is critically important for detoxifying an array of toxic substances, including xenobiotics (chemicals foreign to biologic systems), peroxide compounds, and other free radical–generating molecules (Dickinson et al., 2003). It thereby exerts a profound protective effect on cell. NAC is also a mucolytic agent that mellows tenacious mucous discharges. It is widely used as the specific antidote for acetaminophen overdose, in prevention of chronic obstructive pulmonary disease exacerbation (Dekhuijzen, 2004), in prevention of contrast-induced kidney damage during imaging procedures, attenuation of illness from the influenza virus when started before infection, treatment of pulmonary fibrosis, and treatment of infertility in patients with clomiphene-resistant polycystic ovary syndrome. Preliminary studies suggest that N-acetyl cysteine may also have a role as a cancer chemo preventive, an adjunct in the eradication of Helicobacter pylori, and prophylaxis of gentamicin-induced hearing loss in patients on renal dialysis (Milea et al., 2009). The chemical structure of NAC was shown in figure 1. Literature review on the analytical methods for the estimation of NAC revealed that very few methods like UV-Visible spectroscopic methods (Khushboo et al., 2015), Ion pair Chromatography (IPC) method (Mathew et al., 2017) and High performance liquid

Address for Corresponding Author:
Sreenivasa Charan Archakam,
Department of Pharmaceutical Sciences,
Jawaharlal Nehru Technological University Anantapur (JNTUA), Anantapuramu-515002, Andhra Pradesh, India.
E-mail: charan4ma@gmail.com Mob: +91 94908 44432

DOI: https://doi.org/10.31024/ajpp.2018.4.5.24

chromatography (HPLC) methods (Vander Heyden, et al., 2004; Sana et al., 2012) for dosage forms of NAC like effervescent tablets, cough syrup and plasma were reported. Since there is need for analysis of N-Acetylcysteine in API and parenteral injection, the aim of the present work was to develop a reverse phase high performance liquid chromatographic method for the estimation of NAC.

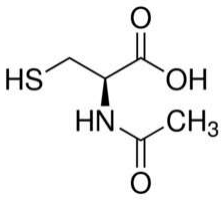

Figure 1. Chemical structure of N-Acetyl cysteine

Materials and methods

Instruments, reagents and chemicals

Shimadzu Prominence LC system equipped with LC- 20AT pump, SPD- 20A UV-Vis Detector, LC solutions data handling system, Rheodyne injection valve and Phenomenex Luna C-18 column (4.5 x 150 mm, 5µ) were used for the chromatographic separations. Sodium Hydroxide, Ortho phosphoric acid, Triethylamine, Potassium dihydrogen phosphate, HPLC grade Acetonitrile, HPLC grade Methanol and HPLC grade water were purchased from Merck Pvt Ltd (Mumbai, India). N-Acetyl cysteine was procured from Aurobindo Pharma Ltd. (Hyderabad, India). Commercial parenteral injection of NAC was purchased from local market.

Preparation of solutions

Preparation of Standard stock solutions

25mg of N-Acetylcysteine weighed and dissolved in required amount of water in a 25ml volumetric flask. The flask was shaken and volume was make up to the mark with water to give a solution containing 1000µg/ml (stock solution). From this stock solution various dilutions containing 10,20,30,40,50 µg/ml solutions of NAC were prepared.

Preparation of Test solution

1ml of parental injection of NAC was taken which consists of 200 mg/ml (200000µg/ml) of NAC. This solution was diluted to 10ml to give the concentration of 20000µg/ml (stock solution). Finally, a solution containing 20µg/ml of NAC was prepared and then filtered through 0.45 micron filter membrane. This solution was used as assay sample.

Chromatographic conditions

A series of trial runs were executed using various mobile phase compositions and chromatographic conditions. After reviewing the results of the trials, a mobile phase consisting of Phosphate buffer pH 3.0 and Acetonitrile (95.5 v/v) was used for the separation of NAC. Separation was carried out on a C18 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) with an isocratic mobile phase constituting of potassium dihydrogen phosphate. The flow rate was kept at 1.0 mL/min with a total run time of 10 minutes. A UV-detector was employed for the detection of NAC at a wavelength of 213 nm. All the solution were injected (20µl) in to the chromatographic system and then analyzed for the results.

Results and Discussion

The selected chromatographic conditions yielded good separation parameters and the retention time of NAC was observed at 4.205 min. The optimized chromatogram and chromatographic parameters were shown in figure 2 and table 1 respectively. The developed method was validated as per ICH guidelines. A calibration curve was constructed in the range of 10-50 µg/ml. The linearity was assessed from the calibration plot between concentration and peak area and it showed a correlation coefficient of R^2 = 0.9999 as

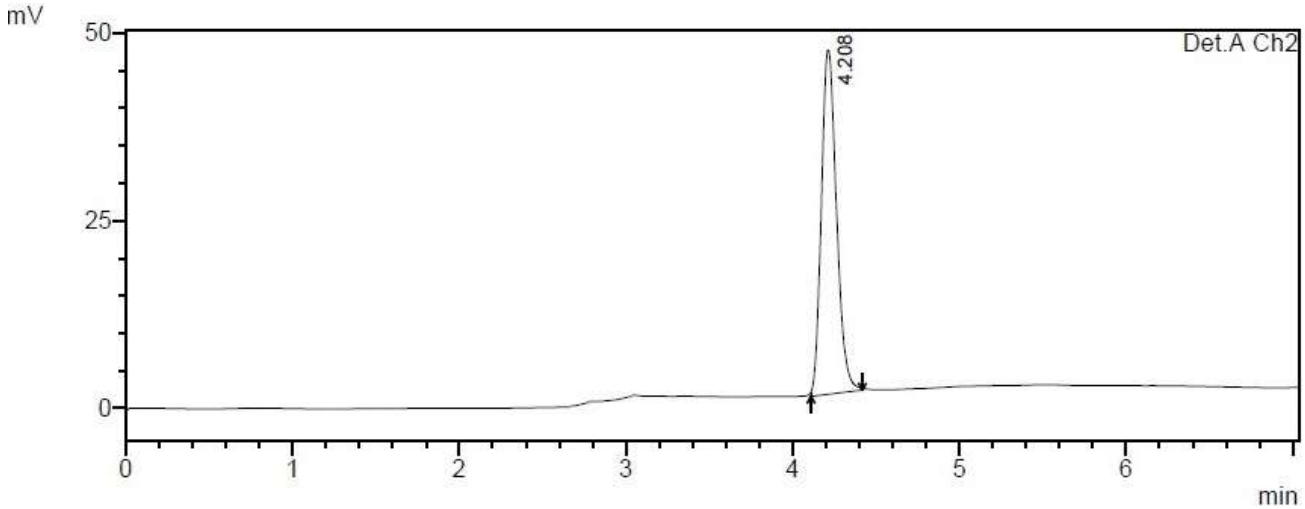

Figure 2. Optimized chromatogram of NAC

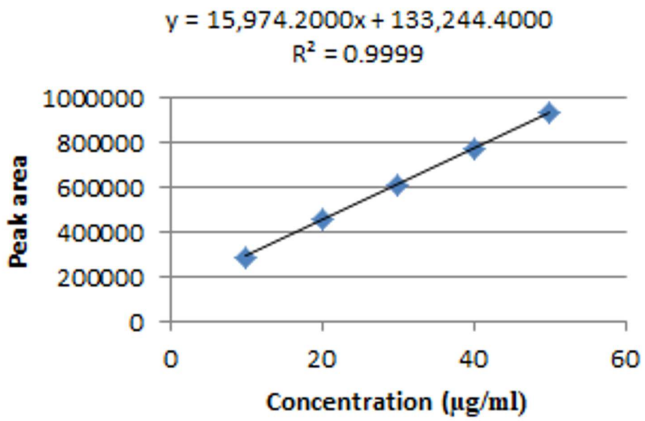

Figure 3. Calibration curve of NAC

shown in figure 3. Specificity was demonstrated by the absence of interference peaks of the excipients in the parenteral dosage form. The precision of the developed method was demonstrated by system precision and method precision. Both the parameters showed a % RSD less than 2%. The accuracy was demonstrated as mean % recovery using method of standard addition and it was found to be 101.6 % as shown in table 2. The method was also evaluated for robustness and ruggedness including various parameters like change in flow rate. Change in mobile phase composition and pH, change in detection wavelength and analyst to analyst variation. All the results obtained were satisfactory. The assay of the commercial parenteral dosage form was found to be 101.69%. The assay chromatogram was shown in figure 4.

Conclusion

The developed RP-HPLC method was specific, precise, accurate, sensitive and robust for the estimation of NAC in bulk and parenteral dosage forms. This method can be routinely employed in various quality control laboratories and in academic research activities.

Table 1. Optimized chromatographic conditions

Parameter	Optimized Conditions
Column	Phenomenex C18 5µ (250 x 4.6 mm)
Mobile phase	Phosphate buffer pH 3-Acetonitrile (95:5); Isocratic conditions
Flow rate	1.0 mL/min
Injection volume	20µL
Temperature	Ambient Temperature
Detection Wavelength	UV-Visible detection at 213nm
Run time	10 min
Retention Time for NAC	4.205 ± 0.003 min

Table 2. Accuracy and % Recovery data

% level	Sample area	Average % recovery
80%	1309201	98.6%
100%	1468265	102.8%
120%	1501358	103.4%
Mean recovery		101.6 %

*All data mean of six determinations

Acknowledgements

The authors would like to thank Management and Principal of Sri Padmavathi School of Pharmacy, Tiruchanoor for providing facilities to carry out this work.

Conflict of interest

All authors have none to declare.

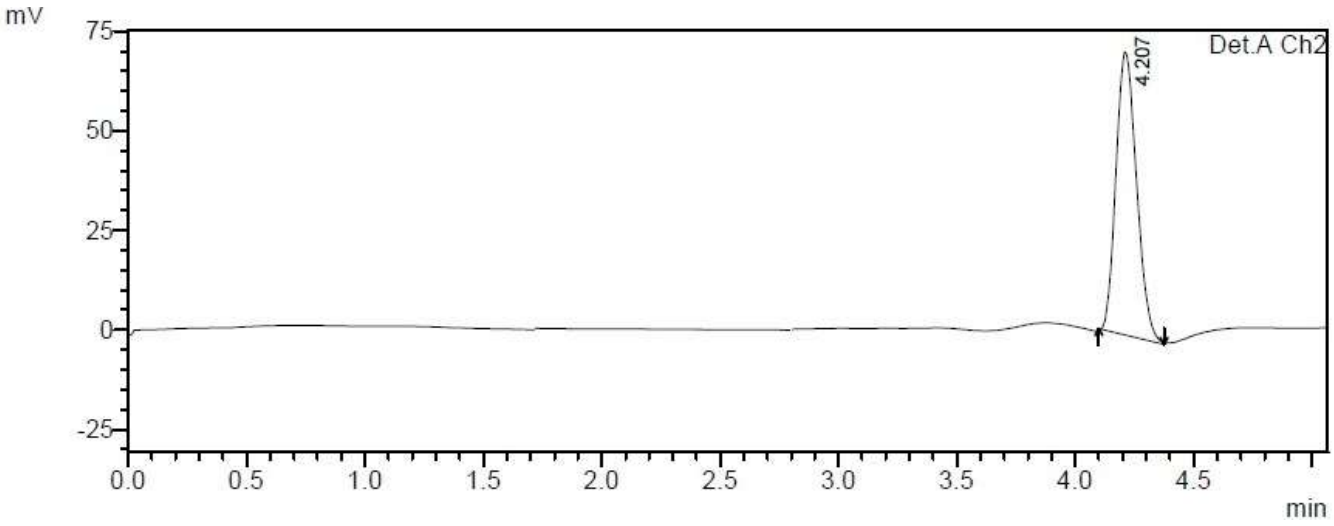

Figure 4. Assay chromatogram of NAC Parenteral Injection

References

Dekhuijzen PNR. 2004. Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. European Respiratory Journal, 23:629-636.

Dickinson DA, Moellering DR, Iles KE, Patel RP, Levonen AL, Wigley A, Darley-Usmar VM, Forman HJ. 2003. Cytoprotection against oxidative stress and the regulation of glutathione synthesis. The Journal of Biological Chemistry, 384(4):527-537.

Khushboo S, Padmavathi PP, Subramanyam EVS, Ramakrishna S. 2015. Development of new analytical methods and their validation for the determination of and N-Acetylcysteine in bulk and marketed formulations. International Journal of Pharma Sciences and Research, 6(4):773-776.

Magesh AR, Dasaratha Dhanaraju M. 2017. Simultaneous Determination of N-Acetyl Cysteine and Taurine by HPTLC Method in Active Pharmaceutical Ingredient and Pharmaceutical Dosage Form. American Journal of Analytical Chemistry, 8:742-751.

Mathew EM, Ravi A, Rameshwar N, Sudheer M, Krishnamurthy B. 2017. Development and Validation of an Analytical Method for Related Substances in N-acetyl–L-cysteine Effervescent Tablets by RP-HPLC. Indian Journal of Pharmaceutical Education and Research, 51(4):626-636.

Millea PJ. 2009. N-acetylcysteine: multiple clinical applications. American family physician, 80(3):265-9.

Sana S, Rajani A, Sumedha N, Pravin P. 2012. Development and Validation of RP-HPLC Method for the Estimation of N Acetylcysteine in Wet Cough Syrup. International Journal of Drug Development & Research, 4 (2):284-293.

Vander HY, Mangelings D, Van Brempt J, Spapen H. 2004. Development and validation of an HPLC method with post-column derivatisation for assay of N-Acetylcysteine in plasma. Acta Chromatographica, 14:149-164.