http://www.e-journals.in
DOI:10.7598/cst2015.996

Chemical Science Transactions
2015, **4(2),** 478-482

RESEARCH ARTICLE

Development of Spectrophotometric Method for the Assay of Aminocaproic Acid in Dosage Forms Using Ascorbic Acid

MOHAMED ABEID ADAM, SHAZA WAGIEALLA SHANTIER*,
SARA AHMED ALFANGARI and ELRASHEED AHMED GADKARIEM

Department of Pharmaceutical Chemistry,
Faculty of Pharmacy, University of Khartoum, Sudan
sshantier@yahoo.com

Received 27 November 2014 / Accepted 16 December 2014

Abstract: A simple and selective colorimetric method was developed for the quantification of aminocaproic acid (ACA) in bulk and pharmaceutical formulations. The method was based on the coupling of ACA with ascorbic acid to give a colored product having analytically useful maxima at 390 and 530 nm. The factors; which are mainly concentration of ascorbic acid and heating time; affecting the color development were optimized and incorporated in the procedure. Regression analysis of Beer's plot showed good correlation (r=0.999) in a concentration range of 4-20 µg/mL. The molar absorptivity of the reaction product was 2492.6 L mole cm^{-1}. The limit of detection was 0.86 µg/mL and 0.29 µg/mL at 390 nm and 530 nm respectively. The average recovery for the commercial preparation (Aminocaproic acid injections, 250 mg/ 20 mL) was 108.23±1.45% (n=3) which reflected no interference by the injection excipients. Based on the molar ratio, the reaction stoichiometry was found to be 1:1.

Keywords: Colorimetric method, Aminocaproic acid, Ascorbic acid

Introduction

Aminocaproic acid (Figure 1) has many pharmaceutical uses. It is used for treatment of fibrinolysis and blood loss in patients undergoing primary, isolated coronary artery bypass surgery[1], intracranial hemorrhage[2] and cirrhosis and hyperfibrinolysis[3]. The official method of analysis is nonaquous acid base titration[4].

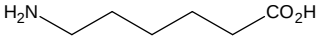


Figure 1. Aminocaproic acid structure

Many liquid chromatographic methods were reported for analysis of ACA in plasma[5] and urine[6]. Ascorbic acid has been frequently utilized as an analytical reagent in pharmaceutical analysis. It was used for determination of cefprozil[7], tobramycin[8], penicillins and cephalosporins having α-aminoacyl functions[9,10]. Based on these reports, simple, sensitive and selective assay method was developed for the determination of ACA in bulk and injection forms using ascorbic acid.

Experimental

Aminocaproic acid RS was obtained from Sigma Aldrich China. Aminocaproic acid injection (250 mg/ 20 mL), LOT No. 6489, American Reagent Inc. Ny. USA.

Reagent

All reagents used were analytical grade and were used without any further purification *L*-Ascorbi acid LR, S.d finechem limited, India, Dimethyl sulfoxide (DMSO), LOBA chemic Ltd, India. Dimethylformamide (DMF), Techno Pharmachem., India.

Instrument

Ultraviolet / Visible spectrophotometer, Perkin Elmer, England. Balance, Kern ALS 120-4, Germany.

Preparation of aminocaproic acid standard solution

0.1 g of Standard aminocaproic acid was accurately weighed and dissolved in 10 mL distilled water. The volume was then completed to 50 mL using DMSO (2000 µg/mL, solution A).

Preparation of aminocaproic acid sample solution

0.4 mL of the injection was transferred into 50 mL volumetric flask. 10 mL of distilled water was added before volume completion with DMSO (100 µg/mL, solution B).

Reagent Blank

1 mL of ascorbic acid 0.1%w/v added to 100 µL of water and the volume completed to 10 mL with dimethyl sulfoxide.

Procedures

Calibration curve

Aliquot volumes from solution A (20-100 µL) were transferred into five stoppered glass tubes, then 80, 60, 40, 20 and 0 µL of distilled water was added to produce 100 µL (maximum aqueous volume). 2 mL of 0.1%w/v freshly prepared ascorbic acid solution in DMSO was added to each tube. The volumes were then completed to 10 mL with DMSO before heating in a boiling water bath for 30 minutes. The absorbances of the resultant solutions were measured at 390 and 530 nm against the reagent blank. The graphs were then constructed by plotting the absorbance values versus drug concentration at each wave length.

Serial dilutions of the sample stock solution B were treated as under calibration curve. Alternatively, direct sample/standard comparison was done using 60 µL of the sample solution treated as in the general procedure.

Effect of different solvents on the absorption spectra

The calibration curve was repeated using different solvents (dimethylsulfoxide (D.E. 46.7) and dimethylformamide (D.E. 36.7) for ascorbic acid dissolution and volume completion. The absorbance spectra were then recorded.

Effect of heating time on the reaction of ascorbic acid with ACA

The effect of heating time on the reaction of ascorbic acid with ACA was tested at different times (10, 20, 30 and 40 minutes) using 200 µg/mL of ACA standard solution. The corresponding absorbance was measured at 390 nm and 530 nm.

Effect of different concentrations of ascorbic acid on the color intensity

2 mL of freshly prepared ascorbic acid solutions (0.05%, 0.1%, 0.15% and 0.2% w/v) were reacted with 20 µg/mL of ACA as in the general procedure.

Added recovery

50 µL of each solution A and B were transferred to separate stoppered glass tubes. Another 50 µL of solution B was mixed with 50 µL of solution A in a third tube. The above solutions were then treated as under calibration curve. The percentage recovery was calculated using the following equation

$$\frac{Amix - Asam}{Astd} x100$$

Where A_{mix} = absorbance of the mixture solution, A_{sam} = absorbance of the sample solution A_{std} = absorbance of the standard solution

Results and Discussion

One of the targets of pharmaceutical analysis is to develop simple, accurate and precise method for the assay of drugs in bulk and pharmaceutical formulation. The advantages of spectrophotometric methods are their suitability and availability of the instruments. Some drug molecules lack a useful UV-VIS absorption that can make their assay easy. Such molecules require reaction with a certain chromogen to develop a chromophore suitable for their spectrophotometric analysis.

Ascorbic acid has been frequently used as analytical reagent in analytical chemistry. It's reported to have a selective reaction with ammonia, primary aliphatic amines of type $R\text{-}CH_2\text{-}NH_2$(11) ($\lambda_{max}$ 390 and 532 nm), amino acids with α-aminoacyl function λ_{max} about 400 nm. On the bases of these reports a simple method was developed for the determination of the amino acid analogue ACA in bulk and formulations. Most of the reported methods for analysis of ACA are time consuming, lacking selectivity require sophisticated equipment or not simple for routine analysis[12]. ACA exhibits weak UV-absorption, therefore, a suitable chromogen is needed to react with ACA to obtain a more UV absorbing chromophore for a sensitive determination of ACA. Ascorbic acid is a chromogen known to react with amino acid containing compounds. It was found to react with ACA producing a purple colored product absorbing at 530 nm and 390 nm. The different experimental factors affecting the color development, intensity and stability were studied. These factors include solvent, the reagent concentration, the reaction time and temperature. The suitable concentration of ascorbic acid was found to be 2 mL of 0.1%w/v which gave the highest intensity and stability. The obtained results for the effect of the different solvents are illustrated in Table 1

Table 1. Effect of different solvents on color formation, intensity and stability (ACA 12 µg/mL)

Solvent	Dielectric constant	Absorbance	λ_{max}	Stability	Color
DMF	36.7	0.079	530	unstable	yellow
DMSO	46.7	0.251	530	Stable	purple

The effect of heating time on product formation, color intensity and stability is shown in Table 2. The optimal heating time was found to be 30 minutes (r= 0.9997) and the color was stable for at least 24 hours.

Table 2. Estimation of ACA-ascorbic acid complex reaction boiling time and stability at 530 nm

Conc. µg/mL.	20 min	30 min	40 min
4	0.059	0.08	0.078
8	0.149	0.162	0.15
12	0.22	0.241	0.232
16	0.259	0.329	0.322
Correlation coefficient	r= 0.9866	r= 0.9997	r = 0.9997

The sequence of addition of the reagent as described under calibration was found to be essential for good reproducibility and the optimized conditions were utilized to construct the calibration curve using authentic ACA.

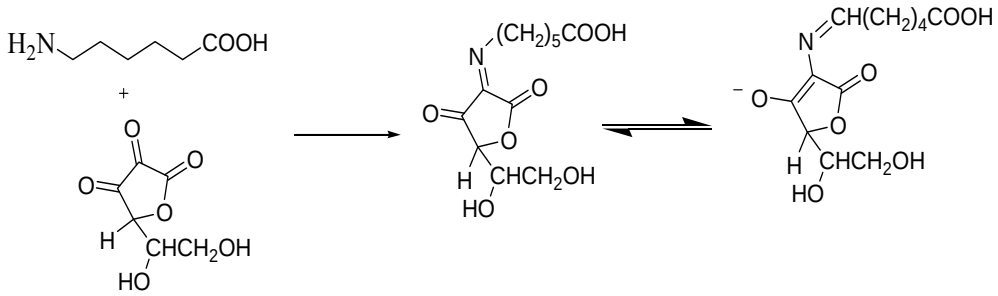
Beer's law was obeyed for ACA concentration 4-20 µg/mL. The regression analysis data for the curve at 390 nm and 530 nm were found to be $A= 4.6x10^{-3} + 0.0548x$ (r= 0.99998) and $A= 1.6x10^{-3}+0.0183x$ (r=0.99994) respectively which indicated good linearity. The detection limit of the studied drug was 0.86 µg/mL and 0.287 µg/mL at 390 nm and 530 nm respectively; which represents the minimum absorbance value that can be measured for color produced by complex formed.

The accuracy of the procedure and freedom from interference of by the injection excipients was confirmed by the obtained results for recovery testing of added amount of authentic ACA to the injection solution in ratio of1:1.The results showed good recovery for the injection(X=108.23±1.45, n=3). This together with the good reproducibility reflected the good accuracy and precision of the method.

The method was applied for drug samples using Aminocaproic acid (250 mg/ 20 mL) made by American Reagent Inc. The results were found to be 101.2% ± 1.78 (n=4).

Proposed pathway for the reaction

According to the molar ratio method, the reaction stoichiometry was found to be a 1:1 ratio reaction. Accordingly, the proposed reaction pathway between the drug and the reagent is expected to proceed as illustrated in Scheme 1.



Dehydroascorbic acid

Scheme 1. Proposed reaction pathway between ascorbic acid and ACA

Conclusion

The developed method was proved to be simple, accurate and precise. Ascorbic acid was found to be a suitable reagent for the determination of ACA in pure form and its dosage forms without interference from excipients.

References

1. Greilich P E, Jessen M E, Satyanarayana N, Whitten C W, Nuttall G A, Beckham J M, Wall M H and Butterworth J F. *Anesth Analg*., 2009, **109(1)**, 15-24; DOI:10.1213/ane.0b013e3181a40b5d
2. Liu-DeRyke X and Rhoney D, *Pharmacotherapy, J Human Pharmacol Drug Therapy,* 2008, **28(4)**, 485-495; DOI:10.1592/phco.28.4.485
3. Gunawan B and Runyon B, *Aliment Pharmacol Thera.,* 2006, **23(1),** 115-120; DOI:10.1111/j.1365-2036.2006.02730.x
4. United States Pharmacopeia Convention, USP 32-NF 27, 2009, p188.
5. Stanley L, *Biomed Chroma.,* 1990, **4**, 175-177; DOI:10.1002/bmc.1130040414
6. Phillips D, Tebbett I and Kalita S, *Chromatographia,* 1990, **30(5-6)**, 309-310; DOI:10.1007/BF02319713
7. Gadkariem E A, Mutasim M M, Ibrahim K E and El-Obeid H A, *J Biomed Sci.,* 2009, **5**, 267-274.
8. Shantier S W, Gadkariem E A and Ibrahim K E, *Int J Dru Form Res*., 2012, **2**, 260-272.
9. El-Shafie F S, GadKariem E A, Al-Rashood K A, Al-Khamees H A and El-Obeid H A, *Anal Lett*., 1996, **29(3)**, 381–393; DOI:10.1080/00032719608000405
10. El-Obeid H A, Gad-Kariem E A, Alrashood K A, Al-Khamees H A, El-Shafie F S and Bawazeer G A M, *Anal Lett.,* 1999, **32**, 2809–2823; DOI:10.1080/00032719908543008
11. Pischetsrieder M, Larisch B, Muller U and Severin T, *J Agric Chem*., 1995, **43(12)**, 3004-3006; DOI:10.1021/jf00060a002
12. Pinto P CA G, Saraiva M L M F S, Santos J L M and Lima J F L, *Talanta*, 2006, **68**, 857-862; DOI:10.1016/j.talanta.2005.06.008