Simultaneous Estimation of Ascorbic Acid and Calcium Pantothenate in Multivitamin and Multimineral Tablets by Reverse-Phase HPLC

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ABSTRACT

The present study describes A RP-HPLC method for simultaneous estimation of Ascorbic acid (AA) & Calcium pantothenate (CP) in a pharmaceutical Multivitamin & Multimineral unit dosage form. The separation was achieved on a reverse phase C_{18} column (5µm; 250x4.6 mm i.d.) with an isocratic mobile phase elution order at a flow rate of 1.0 mL/min. The mobile phase was buffer [0.1 %v/v solution of triethylamine in milli-Q water with pH 3.00 ± 0.05 (adjusted with ortho-phosphoric acid)] and methanol in the ratio of 80:20. Detection was performed with UV detector at 210nm. The method was validated with respect to linearity, precision, accuracy, and specificity according to ICH guidelines. The responses were linear in concentration range of 20-60 µg/mL for AA and 2.5-7.5 µg/mL for CP. The values of slope and correlation coefficient were found to be 15202 & 1.000 for AA; and 7583 & 1.000 for CP respectively. The %RSD value for repeatability and intermediate precision studies were 2.9 & 0.8 for AA; and 3.3 & 2.8 for CP respectively. The %recovery of the vitamins ranged between 98.0 to 99.7 for AA and 98.1 to 98.7 for CP.

Key words: HPLC, Vitamins, Simultaneous quantification, Validation.

INTRODUCTION

The purpose of this study was to develop and validate an HPLC method for the quantitation of Ascorbic acid (vitamin C) and Calcium pantothenate in pharmaceutical Multivitamin & Multimineral tablet preparations containing various active & excipients. Vitamins are non energy producing organic compounds, essential for normal human metabolism that must be supplied in small quantities in the diet. The importance of vitamins as drugs or nutritive supplements is primarily in the prevention and treatment of deficiency diseases [1].

Ascorbic acid (water-soluble vitamin), is (R)-5-[(S)-1,2-dihydroxyethyl]-3,4-dihydroxy-5(H)-furan-2-one [2]. Vitamin C occurs in the form of L -ascorbic acid (AA) and L-dehydroascorbic acid (DHAA) [3]. Vitamin C (ascorbate, AA) is a water-soluble organic compound and involved

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in many of the biological processes. It is also involved in maintaining the reduced state of metal co-factors, like, monooxygenase (Cu^+) and dioxygenase (Fe^{2+}). The other role of AA is to reduce hydrogen peroxide (H_2O_2), which preserves cells against reactive oxygen species (antioxidant effect) [4].

Calcium pantothenate is the calcium salt of (*R*)-3-(2,4-dihydroxy-3,3-dimethylbutyramido) propionic acid [2]. It is a component of coenzyme A which is essential in the metabolism of proteins, carbohydrates and fats. CP is also used as a supplement to treat, e.g. acne, osteoarthritis, rheumatoid arthritis, and to support immune system [5].

By observing the methodology and chromatographic conditions for the estimation of vitamins in literature, it was found that the mobile phases in existing methods were very much complicated with gradient elution system, ion pairing reagents were required in the mobile phase and at various wavelengths with complicated extraction procedures of sample preparation, diluent was other than mobile phase and costly detectors were used for detection like mass spectrometers and PDA [6-12]. And there was no research paper published specifically for the simultaneous estimation of Ascorbic acid & Calcium pantothenate in a pharmaceutical multivitamin and multimineral tablet dosage form.

The proposed method for simultaneous estimation of AA & CP was developed with simple chromatographic conditions with isocratic elution system using UV detector at one wavelength. The mobile phase is simple without any ion pairing reagent and was used as diluent also. The solvent system and procedure for sample preparation (vortexing for dissolution) were designed in a manner so that they don't make the Ascorbic acid unstable in solution form.

Ascorbic acid   *Calcium Pantothenate*
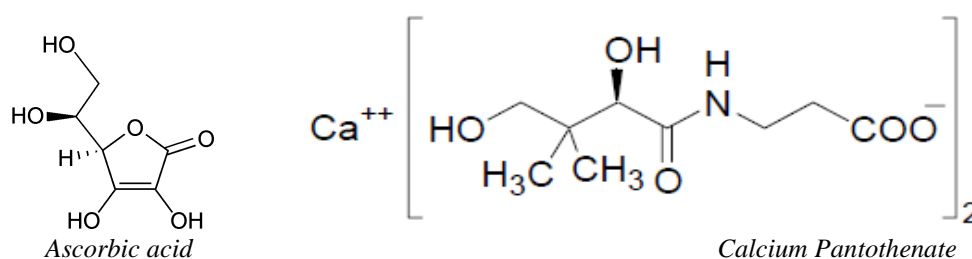
Figure 1 Chemical structure of Ascorbic acid and Calcium pantothenate

MATERIALS AND METHODS

Instrumentation
Quantitation of these two vitamins was achieved using UV detector with Dionex U3000 and Agilent1200 series high performance liquid chromatography (HPLC) systems consisting of a quaternary pump with a vacuum degasser, a thermostated column compartment & cooling autosampler.

LC conditions
A C_{18} Inertsil ODS-3V (250x4.6mm; 5µm particle size) reverse-phase column was used. The mobile phase consists of buffer [0.1 %v/v solution of triethylamine in milli-Q water with pH 3.00 ± 0.05 (adjusted with ortho-phosphoric acid,)] & methanol in the ratio of 80:20 with isocratic elution system. The flow rate used was 1.0 mL/min, and injection volume was 25 µL for all the analyses. The run time was 15 min for standard and 35 min for sample.

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UV condition
The UV wavelength used for determining the vitamins was 210nm.

Drug and chemicals
A single lot of multivitamin & multimineral tablets representative of a major commercial product was obtained and utilized as the test material for this method development. The label claim (80 mg for AA with 30 % overages and 10 mg with 15 % overages for CP per tablet) of the vitamins in the product was obtained from the Batch processing records. Methanol, milli-Q water and ortho phosphoric acid (HPLC grade) & triehylamine (AR grade) were used in this study. Methanol was obtained from Labchem (Mumbai), milli-Q water was obtained from the instrument Millipore (Mumbai), ortho phosphoric acid was obtained from Merck (Mumbai) & triethylamine was from Molychem (Mumbai).

Sample preparation
A composite of 20 tablets were crushed with pestle & mortar to make fine powder. Weighed accurately about 1.2950 gm (Equivalent to one tablet) of powered sample & transferred into a 200 mL volumetric flask. About 150 mL of diluent was added and vortex it for 6 min with intermittent swirling. Then it was diluted upto the mark with diluent. Further 5 mL of this solution diluted to 50 mL with diluent. Filtered through syringe filter (0.45 µm) and analyzed immediately; as Ascorbic acid is highly prone to oxidation in solution form.

Preparation of standard solutions
Standard stock solution of AA: Weighed accurately about 20 mg and transferred into a 50 mL volumetric flask. About 30 mL of diluent was added and vortex it for 6 min, then diluted upto the mark with diluent (Stock solution A).

Standard stock solution of CP: Weighed accurately about 25 mg and transferred into a 50 mL volumetric flask. About 30 mL of diluent was added and vortex it for 6 min, then diluted upto the mark with diluent. Further 5 mL of this solution diluted to 50 mL with diluent (Stock solution B).

Standard mixture solution: Pipetted out 5 mL of stock solution A and stock solution B in a 50 mL volumetric flask and diluted upto the mark with diluent. Calibration curves were generated from serial dilution of the standard stock solutions.

RESULTS AND DISCUSSION

Chromatographic separation
Figure 2 & 3 shows typical chromatograms of reference standard and sample. Under the chromatographic conditions described, AA & CP had retention times of 3.0 and 7.9 min, respectively. It can be seen from Figure 3 that good separation and detectability of the multivitamin & multimineral tablets were obtained with baseline resolved peaks and chromatograms with no interference from other active constituents and excipients. The resolution between Ascorbic acid peak and its adjacent peak was found to be 2.8 which were within the limit (not less than 2.0). Developed method was validated according to ICH guidelines [13].
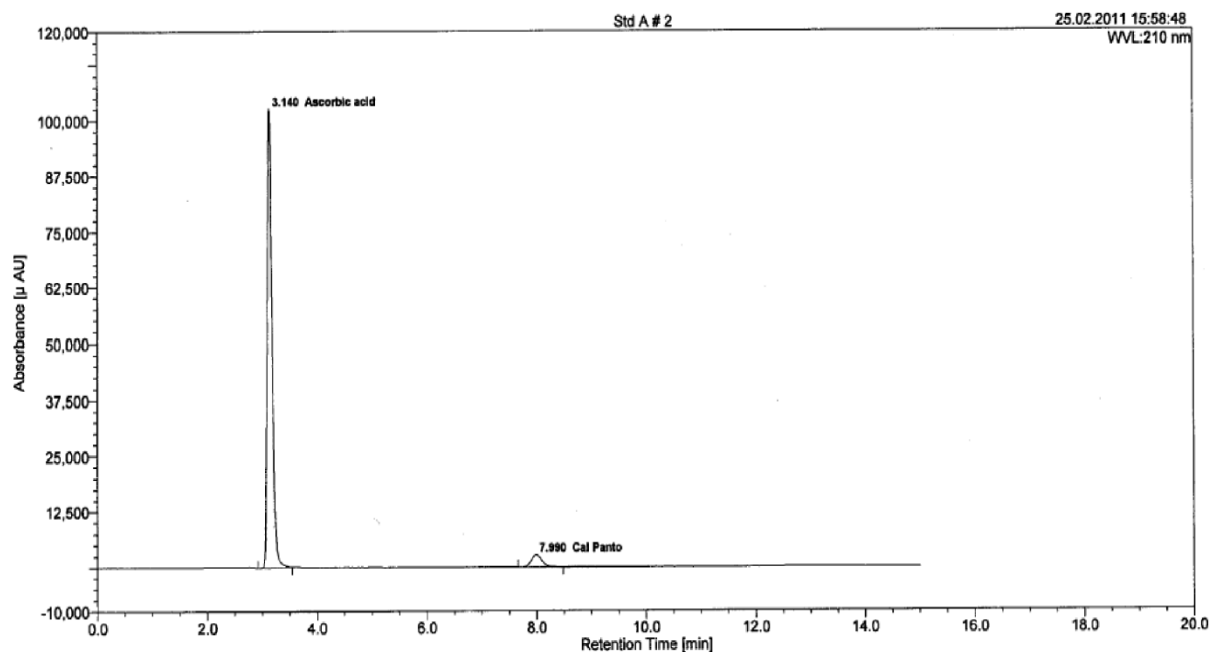
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Figure- 2 Chromatogram of standard mixture of AA & CP at 210 nm and 0.1% triethylamine (pH 3.0 with OPA): methanol as mobile phase.
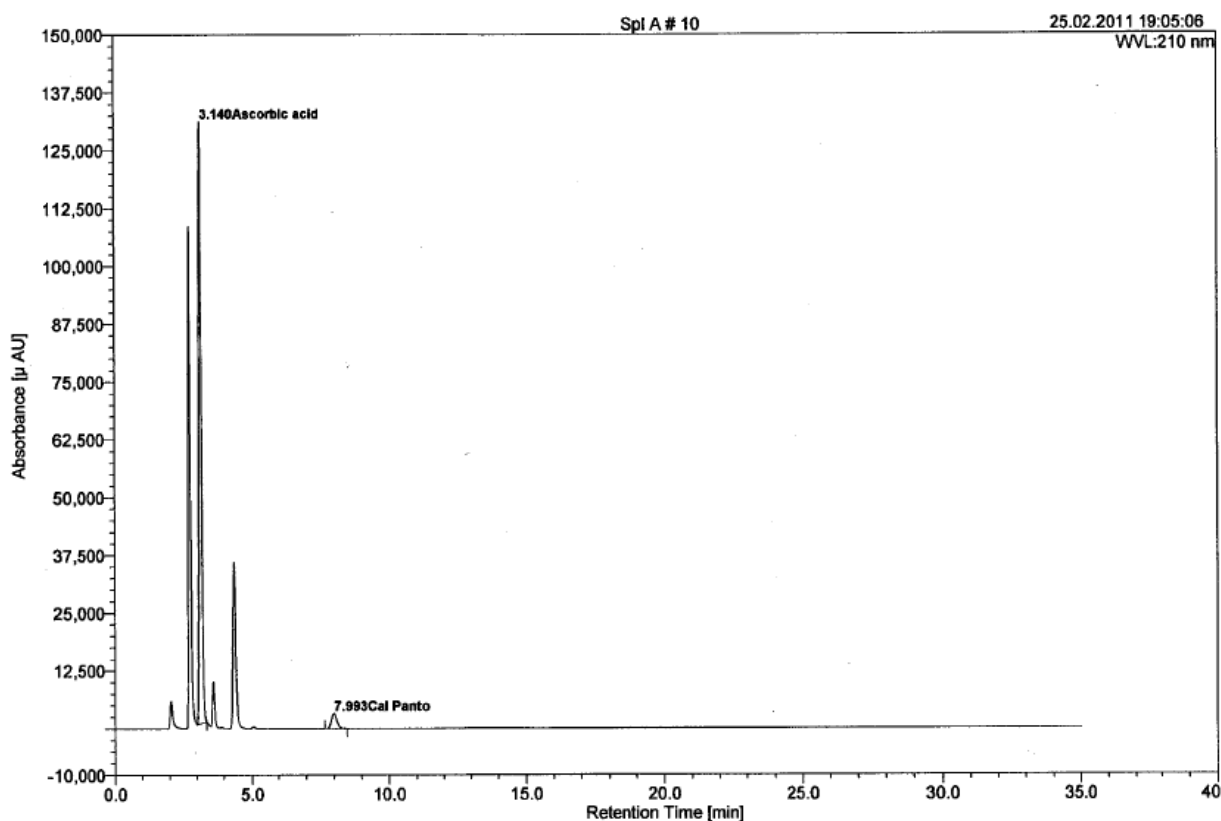

Figure- 3 Chromatogram of sample containing ascorbic acid & calcium pantothenate at 210 nm and 0.1% triethylamine (pH 3.0 with OPA): methanol as mobile phase.

Linearity and range

Calibration curves for the standards (AA and CP) were obtained using a series of concentrations of these compounds in the range of 20 to 60 µg/mL for AA and 2.5 to 7.5 µg/mL for CP. The

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calibration curves for the two standards were linear. The regression coefficients were 1.000 as shown in (Table 1). Figures 4 & 5 show the calibration curves for these two standards. The linearity equations were y = 15202x – 9361 & y = 7583x + 307 for AA and CP respectively.

Table 1 Results for linearity of AA and CP

Name	AA	CP
Equation	y = 15202x - 9361	y = 7583x + 307
R^2	0.999	0.999
Response factor	1.7	0.6
% Y-intercept	-1.5	0.8
% RSD of area	0.5	0.4
% RSD of RT	0.0	0.0
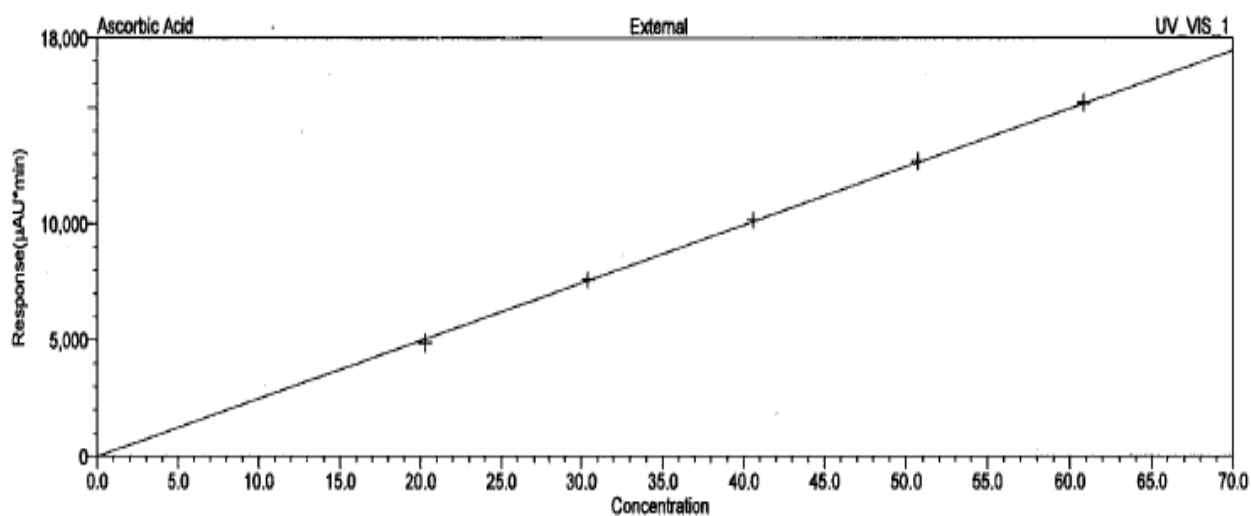
Figure- 4 Linearity plot of AA in concentration range (20-60 µg/mL)
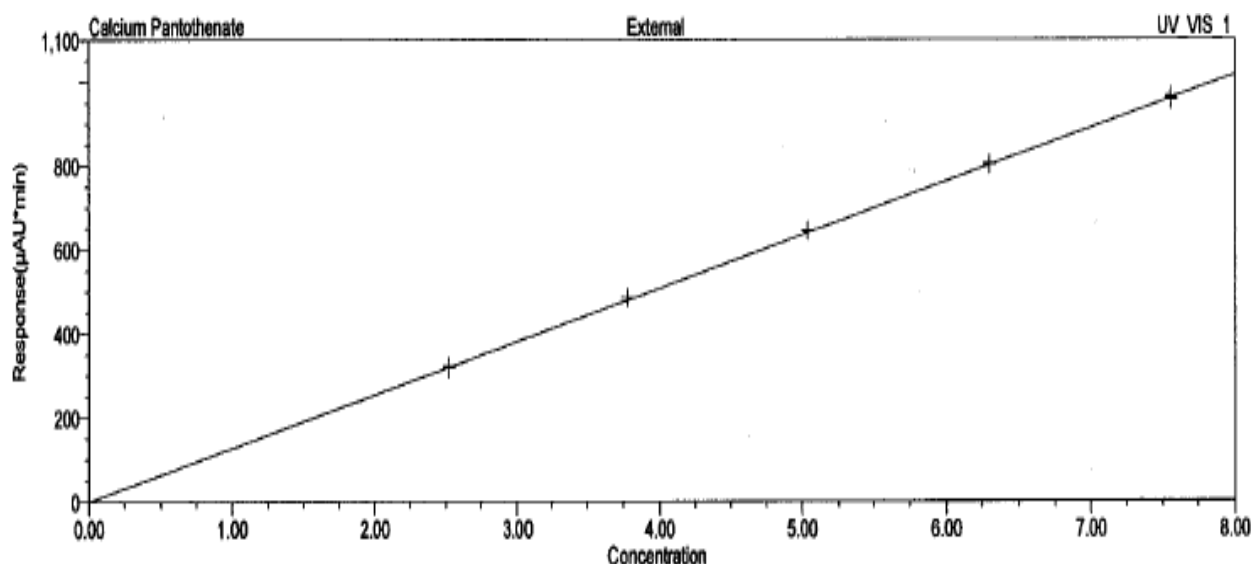
Figure- 5 Linearity plot of CP in concentration range (2.5-7.5 µg/mL)

Precision

The % RSD in repeatability was found to be 2.9 and 3.3 for AA & CP respectively. The %RSD in intermediate precision was found to be 0.8 and 2.8 for AA & CP respectively. The acceptance criterion for % RSD in repeatability and intermediate precision is 5.0 for degradable products.

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The % variation between average % assay values of repeatability & intermediate Precision was found to be 2.0 & 0.99 (Table 2) for AA & CP respectively. The acceptance criterion for % variation is 5.0 for degradable products.

Table 2 Results for % variation between repeatability and intermediate precision studies

Name	AA	CP
% Assay of repeatability	132.1	121.1
% Assay of intermediate precision	129.4	119.9
% Variation	2.0	0.99

Accuracy

The recoveries at three different concentrations (50, 100 and 150 % of label claim) were found to be within the range of 98 to 102 % as per ICH guidelines. Mean % recovery was found to be 98.9 and 98.4 for AA & CP respectively. The results indicated that the recoveries of AA & CP at three different concentrations were between 98.0 to 99.7 % and 98.1 to 98.7 % respectively. Results are given in (Table 3).

Table 3 Results for recovery studies (accuracy) for AA & CP

Level	Amount added (µg/mL)		Amount recovered (µg/mL)		% Recovery	
	AA	CP	AA	CP	AA	CP
50% level	20.2	2.58	19.8	2.54	98.0	98.4
100% level	40.2	5.2	39.8	5.1	99.0	98.1
150% level	60.3	7.8	60.1	7.7	99.7	98.7

LOD and LOQ

The LOD and LOQ of the present method were calculated based on the standard deviation of the responses of blank and slope of linearity curve. The values of LOD and LOQ were 0.05 and 0.08 for AA, and 0.13 and 0.27 for CP respectively.

The optimized conditions for simultaneous estimation of AA & CP were established by performing the trials for mobile phase selection, selection of one concentration for sample preparation & for wavelength. The mobile phase was optimized after adding peak modifier (triethylamine) to eliminate the baseline drift before AA peak. The wavelength 210 nm (λ_{max} of CP) was selected because at this wavelength the response of AA got reduced, and CP was showing good response in the chromatogram. The proposed method was found to be advantageous over the existing methods with respect to following points: a) mobile phase is very simple with isocratic elution order, and without using any ion-pairing reagent; b) UV detector was used because the estimation was done at one wavelength only (210 nm); c) method of sample preparation is simple without any extraction procedure and using the mobile phase as diluent.

CONCLUSION

This method provides good recovery (accurate) and precision and it is simple, specific and robust in both chromatographic conditions and sample preparation. Furthermore, the analytical methodology is easy to handle and suitable for routine analysis of a large number of samples. The method can be used in analytical drug control laboratory for routine quality control of pharmaceuticals containing AA & CP.

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