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Short communication

Development and application of a novel UV method for the analysis of ascorbic acid

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Abstract

A UV method for the analysis of ascorbic acid with methanol as solvent to prepare a sample has been developed and applied. The effect of copper(II) concentrations on the oxidation of ascorbic acid in aqueous solution has been studied in detail, and the regularities of ascorbic acid oxidation in methanol, USP phosphate buffer (pH 2.50) and de-ionized water have been found. Upon experiments ascorbic acid has been found to dissolve in methanol, and its solubility in it has been measured to be 81.0 mg/ml at room temperature (22 °C). The ascorbic acid bulk material from a manufacturer has been assayed to be 89.34% with this method, in good agreement with the assay value (89.58%) from the titration method. The ascorbic acid granule and tablet content uniformity also has been tested using this method. This method is simple, rapid, accurate and reliable, and can be adopted for the routine determination of ascorbic acid in its granule and tablet formulations.
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Keywords: Ascorbic Acid; Assay; UV method; Methanol; Solubility

1. Introduction

Ascorbic acid (Vitamin C) has been widely used in the pharmaceutical, chemical, cosmetic and food industry by its bioactivity and antioxidant. Therefore, there is a need to find an accurate, reliable, rapid, and easy-to-implement method for measuring the amount of ascorbic acid in a sample. However, there have been some difficulties in quantifying ascorbic acid due to its instability in aqueous solution. The instability of ascorbic acid is due to its oxidation to dehydroascorbic acid, which is a reversible reaction, and subsequently to 2,3-diketo-L-gulonic acid. The later reaction is irreversible. Ascorbic acid is highly sensitive to heat, alkali, oxygen, light and contact with traces copper and iron [1]. Copper(II) and iron(III) can greatly speed up the oxidation of ascorbic acid in aqueous solution.

Various methods have been employed for the analysis of ascorbic acid in pharmaceutical formulations, fruit juices, urine, plasma etc. These include titration [2,3], HPLC [1,3–5], UV [6–8], fluorimetry [9,10], etc. The titration is a classic and time consuming method, and its experimental error changes with different operators to determine the end point of titration. The HPLC and fluorimetry methods have demonstrated good sensitivity and specificity, but their implementation requires specialized equipment and the procedures may be rather lengthy.

Tulley has invented a UV method for the analysis of ascorbic acid [6], but its procedures are complicated and it only applies to analysis of the plasma samples. A UV method for assaying ascorbic acid based on its stability studies has been reported [7], which is very useful for the ascorbic acid analysis in the presence of other Vitamins. However, this method is time dependent and needs strict carefulness to achieve reliable and reproducible results. Recently, Kwakye [8] has developed a UV method for analyzing ascorbic acid in the commercial tablets by adding thiosulphate (0.04%, w/v) to stabilize ascorbic acid in aqueous medium. The UV method is simpler and faster, but cannot be used for the analysis of ascorbic acid in the multivitamin–mineral formulations containing interfering copper [1].

The oxidation of ascorbic acid during the sample preparation has caused much attention [1,4], especially, in the presence of copper(II). An aqueous sample containing about

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0.2 ppm of copper(II) only obtained about 50% of recovery if copper(II) was not effectively chelated [1]. To date, however, there have not been any systematic researches into the effect of copper(II) concentrations on the oxidation of ascorbic acid in aqueous solution. Also, there have not been any quantitative studies on the oxidation of ascorbic acid during the sample preparation.

In the present research, on the basis of investigation into the effect of copper(II) concentrations on the oxidation of ascorbic acid and the regularities of its oxidation in methanol, USP phosphate buffer (pH 2.50) and de-ionized water (D-H_2O), and of measurement of its solubility in methanol, a novel UV method has been developed by using methanol as solvent to prepare a sample. Then, this method has been applied to the ascorbic acid bulk assay and its granule and tablet content uniformity testing.

2. Experimental

2.1. Reagents

ACS grade $CuSO_4 \cdot 5H_2O$, KH_2PO_4, and Na_2HPO_4 were purchased from Caledon Laboratories Ltd. (Georgetown, Ontario, Canada). ACS grade H_3PO_4 and HPLC grade methanol were purchased from EMD Chemicals Inc. (Gibbstown, NJ). USP grade ascorbic acid reference standard (RS) was purchased from Sigma Chemical Co. (Saint Louis, MO). The ascorbic acid bulk material from Northern Pharmaceutical Co. (Liaoning, China) was used to manufacture the ascorbic acid granules and tablets. De-ionized water (D-H_2O) from the Pilot Plant of Toronto Institute of Pharmaceutical Technology (TIPT) was utilized throughout the experiment.

2.2. Oxidation of ascorbic acid in aqueous solution with different copper(II) concentrations

Weigh 195.0 mg of $CuSO_4 \cdot 5H_2O$ (equivalent to 50 mg Cu) into a 100-ml volumetric flask, dissolve in D-H_2O, add it to volume and mix to obtain 500 ppm of Cu^{2+} solution.

Transfer 0, 2, 20, and 200 μl of 500 ppm Cu^{2+} solution into 100-ml volumetric brown flasks containing 3 mg of ascorbic acid RS, respectively, add D-H_2O to volume, and mix, before testing on a Ultrospec 3000 spectrometer from Pharmcia Biotech Co. (Cambridge, England, UK) at the wavelength of 255 nm with D-H_2O as blank. Determine the absorbance of each solution at different time intervals at room temperature (22 °C) until almost all ascorbic acid of each solution is oxidized.

2.3. Oxidation of ascorbic acid in methanol, USP phosphate buffer (pH 2.50) and de-ionized water

Weigh 3.0 mg of ascorbic acid RS into two100-ml volumetric brown flasks respectively, dissolve in methanol and de-ionized water respectively, add both to volume separately, and mix. Determine the absorbances of both solutions at different time intervals until 24 h using the same spectrometer as above at the wavelengths of 245 and 255 nm with methanol and D-H_2O as blank, respectively.

Dissolve 3.90g of Na_2HPO_4 and 3.05 g of KH_2PO_4 in 500 ml of D-H_2O, and adjust with H_3PO_4 to a pH of 2.50 to obtain the USP phosphate buffer. Weigh 3.0 mg of ascorbic acid RS into a 100-ml volumetric brown flask, dissolve in the buffer, add it to volume and mix. Measure the absorbance of the solution at different time intervals until 24 h using the same spectrometer as above at the wavelength of 245 nm with the buffer as blank.

2.4. Preparation of UV calibration curve for ascorbic acid in methanol

Weigh 10.0 mg of ascorbic acid RS into a 50-ml volumetric brown flask, dissolve in methanol, add it to volume and mix to obtain 200 ppm of stock solution. Transfer 0.3125, 0.625, 1.25, 2.5, 3.75, 5.0, and 6.25 ml of the stock solution, respectively, into 25-ml volumetric flasks, add methanol to volume and mix to obtain 2.5, 5, 10, 20, 30, 40, and 50 ppm of calibrators, respectively. After preparation, immediately measure the absorbances of these calibrators using the same spectrometer as above with methanol as blank. Record the data to obtain a regression equation between the absorbances and concentrations.

2.5. Measurement of solubility of ascorbic acid in methanol

Weigh 500.0 mg of ascorbic acid RS into three 10-ml glass tubes, separately. Add 4.0 ml of methanol into each tube under stirring, seal them with parafilm, and place them on a table in the R&D lab at the TIPT to equilibrate for 24 h at room temperature (22 °C).

After 24 h, filter a saturated solution to obtain a supernatant, transfer 1.0 ml of the supernatant into a 100-ml volumetric brown flask, add methanol to volume and mix. Then, transfer 1.0 ml of the above solution into a 25-ml volumetric brown flask, add methanol to volume and mix. Measure the absorbance of the last dilution. Repeat the procedure for another two saturated solutions.

Calculate the solubility of ascorbic acid for each saturated solution in accordance with the above calibration curve.

2.6. Application of this UV method in ascorbic acid bulk assay and its granule and tablet uniformity testing

Accurately weigh 200.0 mg of ascorbic acid RS into a 200-ml volumetric flask, dissolve in methanol filled to two-third of volume, add it to volume, and mix. Filter the above solution with a 0.45-μm filter paper. Transfer 1.0 ml of the filtrate into a 50-ml volumetric flask, dilute to volume with methanol, and mix. Immediately, test the absorbance of the diluted solution, which is referred to as the standard solu-

tion, on the same spectrometer as above at the wavelength of 245 nm with methanol as blank.

Follow the same procedure as the standard solution to obtain the absorbance of the diluted solution from the ascorbic acid bulk, which is referred to as the assay solution. Calculate the assay value by the absorbance of the assay solution divided by the absorbance of the standard solution.

Separately take a sample from each top point of a triangle in a round plate filled with the ascorbic acid granules for each batch. Then, obtain the absorbance of the assay solution from each sample in terms of the same procedure as the standard solution. Calculate the ascorbic acid content in each sample according to the method of computing the above assay value, and then calculate the relative standard deviation (R.S.D.) of the three contents of ascorbic acid in the three different locations for the granule of each batch. If the R.S.D. < 2.2%, then it shows uniformity of the granule. If the R.S.D. > 2.2%, then it indicates non-uniformity of the granule.

Randomly weigh 20 tablets of the ascorbic acid from TIPT (Lot #: VTC100-07), grid them into powder in a mortar with a pestle. Calculate an average weight of the 20 tablets. Accurately weigh the average weight from the powder into a 100-ml volumetric flask, and then obtain the absorbance of the assay solution following the same procedure as the standard solution. Calculate the amount (mg) of ascorbic acid ($C_6H_8O_6$) in each tablet by the absorbances of the assay and standard solutions, the concentration of the standard solution and the dilution factor of the assay solution. Finally, compute the percentage of ascorbic acid in each tablet compared to the label claim.

Repeat the same procedures to obtain the percentage of ascorbic acid in each tablet for the lot VTC100-08 and VTC100-09.

3. Results and discussion

3.1. Effect of copper(II) concentrations on oxidation of ascorbic acid in aqueous solutions

Fig. 1 shows that the oxidized percentage of ascorbic acid in aqueous solution with 0, 0.01, 0.1, and 1.0 ppm Cu^{2+} during the sample preparations (time = 0 in Fig. 1) is 9.2, 11.2, 12.6, and 32.0%, respectively, relative to the assumption of its zero oxidation in methanol during the sample preparation. With increasing copper(II) concentrations, its rate of oxidation obviously increases, which demonstrates that copper(II) can greatly accelerate the oxidation of ascorbic acid in aqueous solution [1]. Within 10 min, 64.7% of ascorbic acid in aqueous solution with 1.0 ppm Cu^{2+} is oxidized. Within 30 min, the oxidized percentage of ascorbic acid in aqueous solution with 0, 0.01, 0.1, and 1.0 ppm Cu^{2+} is 24.7, 30.0, 43.3, and 96.8%, respectively. Within 1 h, almost all ascorbic acid (98.5%) in aqueous solution with 1.0 ppm Cu^{2+} is oxidized, while the oxidized percentage of ascorbic acid in aqueous solution with 0.1, 0.01, and 0 ppm Cu^{2+} is 63.7, 45.0, and 36.2%, respectively.

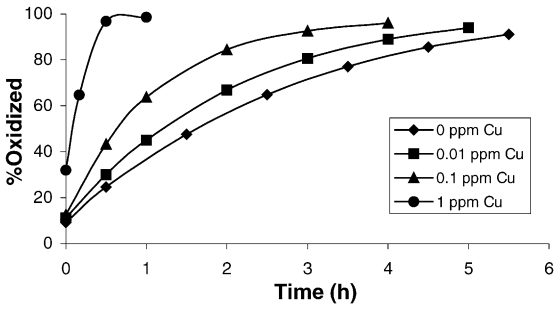

Fig. 1. Effect of Cu^{2+} concentrations on oxidation of ascorbic acid in D-H_2O at room temperature (22 °C).

Ascorbic acid in aqueous solution is slowly oxidized by O_2 in the air. Traces of common metal ions such as Fe^{3+} and Cu^{2+} can serve as catalysts to accelerate the oxidation of ascorbic acid by O_2. The mean concentration of copper(II) in drinking water was 60 μg/l (0.06 ppm) [11]. This might explain why the ascorbic acid in water was oxidized so fast.

3.2. Oxidation of ascorbic acid in methanol, USP phosphate buffer (pH 2.50) and de-ionized water

The UV absorbance spectra of ascorbic acid in D-H_2O, USP phosphate buffer (pH 2.5), and methanol shown in Fig. 2 indicate that the maximum wavelengths of ascorbic acid in D-H_2O, the buffer, and methanol are 255, 245, and 245 nm, respectively. These wavelengths are separately employed for different sample analysis in this research.

As shown in Fig. 3, ascorbic acid in methanol is the most stable at room temperature (22 °C) with only 0.7% of oxidation within 1 h based on the assumption of its zero oxidation during the sample preparation, whereas ascorbic acid in D-H_2O is the most unstable with 46.0% of oxidation within 1 h and the oxidized rate of ascorbic acid in the buffer is in between with 1.3% of oxidation within 1 h, relative to the oxidation of ascorbic acid in methanol at time zero.

Fig. 3 also shows no oxidation of ascorbic acid in the buffer and 14.9% of ascorbic acid was oxidized in D-H_2O during the sample preparation compared to its oxidation in methanol during the sample preparation.

The slowest rate of the oxidation of ascorbic acid should result from the absence of copper(II) in the HPLC grade methanol which also has been directly demonstrated by the fact that $CuSO_4 \cdot 5H_2O$ does not dissolve in the methanol in our exploratory experiments. The fastest oxidized rate of ascorbic acid in D-H_2O might be attributed to the presence of copper(II) as discussed above [11] and demonstrated in our previous experiments. It has been shown that ascorbic acid in an oxygen-saturated solution in the presence of copper(II) is more stable at pH 3–4.5 than at pH 6–7 [1]. This might explain why the oxidized rate of ascorbic acid in the buffer (pH 2.5) is much slower than that in D-H_2O.

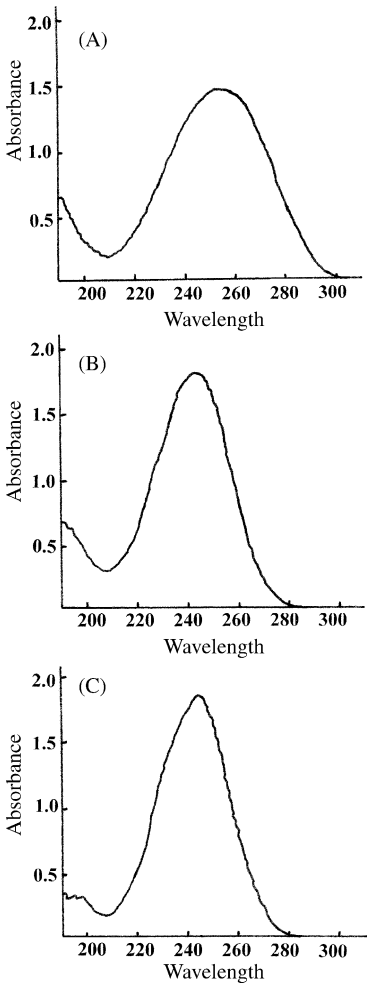

Fig. 2. UV absorbance spectra of ascorbic acid in: (A) D-H_2O (30 ppm); (B) USP phosphate buffer (pH 2.5, 30 ppm); and (C) in methanol (30 ppm).

3.3. UV Calibration curve of ascorbic acid in methanol

The calibration equation of ascorbic acid in methanol from 2.5 to 50 ppm is absorbance = 0.0559 concentration − 0.0238 with a correlation coefficient of 0.999.

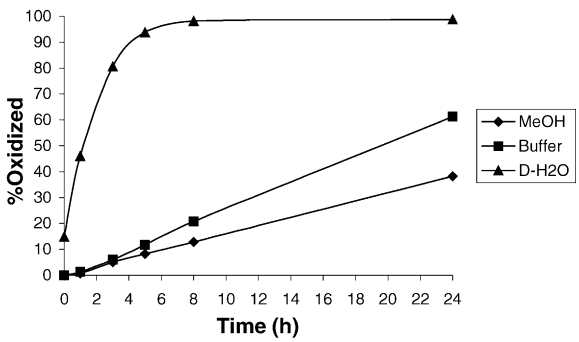

Fig. 3. Oxidation of ascorbic acid in methanol, USP phosphate buffer (pH 2.50) and D-H_2O at ambient temperature (22 °C).

3.4. Solubility of ascorbic acid in methanol

The average solubility of ascorbic acid in methanol from three measurements was measured as 81.0 mg/ml with an R.S.D. of 0.7% in this work, which is about four-times lower than its solubility in water (333.0 mg/ml) [12]. It is the first time to have found ascorbic acid to dissolve in methanol and measured its solubility in methanol [13].

The solubility of ascorbic acid in methanol is moderate and it is stable in methanol within 1 h as discussed in the previous section. Hence, using methanol as solvent to prepare the ascorbic acid sample used for the UV analysis is much better than using water. This UV method can be explored for the analysis of ascorbic acid in all solid materials containing ascorbic acid in the pharmaceutical, chemical, cosmetic, and food industry.

3.5. Ascorbic acid bulk assay and its granule and tablet uniformity testing

The mean percentage of ascorbic acid in the bulk material from three measurements using this method is 89.34% with an R.S.D. of 0.2%, in good agreement with the value (89.58%) assayed by the titration method, which indicates that this method is accurate and reliable.

Table 1 shows that the granules of three batches are all homogeneous because their R.S.Ds. are all less than 2.2%. The average content of ascorbic acid in the three batches (VTC-CL-ST01, VTC-CL-ST02, and VTC-CL-ST03) is 87.1, 86.0, and 85.3%, respectively, which will be used for the tablet's weight adjustments in terms of different ascorbic acid doses.

The mean percentage of ascorbic acid from three measurements in its tablets for the lot VTC100-07, VTC100-08, and VTC100-09 is 102.6, 101.6, and 102.9% with an R.S.D. of 0.5, 0.8, and 0.6%, respectively. This in turn demonstrates that the analytical results of the granules using this method are accurate and reliable because the ascorbic acid tablet's weight adjustments were based on the above assay values of the ascorbic acid granules.

Table 1
Results of ascorbic acid granule content uniformity testing

Batch #	Location	%AA	%AA (mean)	%R.S.D.
VTC-CL-ST01	L1	87.3		
	L2	86.8	87.1	0.3
	L3	87.1		
VTC-CL-ST02	L1	85.8		
	L2	86.7	86.0	0.8
	L3	85.4		
VTC-CL-ST03	L1	85.1		
	L2	85.3	85.3	0.2
	L3	85.5		

%AA: percentage of ascorbic acid in the granule; R.S.D.: relative standard deviation; L1, L2, and L3: one top point of a triangle in a round plate filled with the granule of each batch.

As indicated in the previous section, in measuring the absorbance of a sample from the granules and tablets, methanol was used as blank. The effects of starch and other excipients in the ascorbic acid formulations on the absorbance of ascorbic acid have been studied prior to testing the samples, showing no effects of them on it. This is because the starch and other excipients do not dissolve in methanol.

All above results show that this UV method is simple, rapid, accurate and reliable, and its analytical results are comparable with the titration method. In comparison with the titration and HPLC methods for quantifying ascorbic acid, from the sample preparation to the absorbance testing it is much easier and faster than the HPLC and titration methods. The titration method requires the operators to take time to prepare and standardize the standard titration solution and to have more patience to do titration so as to accurately determine the end point of titration.

The HPLC method requires special equipments and the column used for analyzing ascorbic acid often needs to be regenerated and changed. In addition, the repeatability of analytical results from the HPLC method is poor due to the oxidation of ascorbic acid during the sample preparation and analytical process as discussed above. This might explain why BP and USP still utilize the traditional titration method for assaying ascorbic acid [2,3].

However, the UV method developed in this research does not require special reagents, equipments and operations, and only needs to dissolve ascorbic acid in methanol and then to measure the absorbance, which saves much time and money. The authors therefore recommend this method for the routine determination of ascorbic acid in its granule and tablet formulations.

4. Conclusions

On the basis of research into the effect of copper(II) concentrations on the oxidation of ascorbic acid and the regularities of its oxidation in methanol, USP phosphate buffer (pH 2.50) and de-ionized water, and of measurement of its solubility in methanol, a novel UV method has been successfully developed by using methanol as solvent to prepare a sample. Copper(II) can greatly accelerate the oxidation of ascorbic acid in aqueous solutions. Within 30 min at ambient temperature (22 °C), the oxidized percentage of ascorbic acid in aqueous solution with 0, 0.01, 0.1, and 1.0 ppm Cu^{2+} is 24.7, 30.0, 43.3, and 96.8%, respectively. Ascorbic acid in methanol at room temperature (22 °C) is the most stable with only 0.7% of oxidation within 1 h, while the most unstable in de-ionized water with 46.0% of oxidation at the same period, and in between in the USP phosphate buffer (pH 2.50) with 1.3% of oxidation within 1 h. Compared to the oxidation of ascorbic acid in methanol during the sample preparation, no oxidation of ascorbic acid in the buffer is found, whereas 14.9% of ascorbic acid in de-ionized water is oxidized during the sample preparation. Ascorbic acid has been experimentally found to dissolve in methanol, and its solubility in it has been measured at ambient temperature (22 °C) to be 81.0 mg/ml. The assay value of the ascorbic acid bulk from the Northern Pharmaceutical Co. using this UV method is 89.34%, in good agreement with the assay value (89.58%) from the titration method. The average content of ascorbic acid analyzed with this method for the granule batch VTC-Cl-ST01, VTC-CL-ST02, and VTC-Cl-ST03 from the Toronto Institute of Pharmaceutical Technology is 87.1, 86.0, and 85.3%, respectively, and the mean percentage of ascorbic acid in each tablet for the tablet lot VTC100-07, VTC100-08 and VTC100-09 is 102.6, 101.6, and 102.9%, separately. This UV method can be adopted for the routine determination of ascorbic acid in its granule and tablet formulations.

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