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STATISTICAL EVALUATION OF THE N-BROMOSUCCINIMIDE AND IODINE AS TITRIMETRIC METHODS FOR ESTIMATION OF VITAMIN C IN ORANGE JUICE AND PHARMACEUTICAL PREPARATIONS

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ABSTRACT

N-bromosuccinimide (NBS) and Iodine solution have been used separately for oxidation of vitamin C in orange juice and pharmaceutical preparations. The quantitative results obtained by the two methods have been statistically evaluated for evidence of systematic difference, if any, between the two methods. The t-test has shown that titration with dilute solution (0.0022 M) of NBS has produced better results as compared to Iodine method. On the contrary a concentrated iodine solution (0.0828 N) gave good results.

INTRODUCTION

Vitamin C, known as ascorbic acid, is water soluble and its most distinctive chemical property is the reducing action, or reversible oxidation to dehydro compound, $C_6H_6O_6$. Dehydroascorbic is a neutral lactone. The amount of ascorbic acid has successfully been determined by reversed phase HPLC using different columns and mobile phases in commercial non-alcoholic beverages (Nakama and Yameda, 1997), tomatoes (Abusshita *et al.,* 1997), fruits of some Turkish Rosa species (Coskun *et al.,* 1997), fresh strawberry (Wang *et al.,* 1999), selected fruits and vegetable products (Czerwiecki and Wilczynska, 1999). As a base line separation method this technique has quite efficiently been used for the separation of L-ascorbic from its epimer, D-ascorbic acid (Aboul – Enein *et al.,* 1999). Simultaneous determination of water soluble vitamins including ascorbic acid in foods (Moser *et al.,* 1999, Russell, 2000) and with diode array and UV-visible detection has been reported (Paulo *et al.,* 1999, Li, 2001). High performance capillary electrophoresis (Xiao and Li, 1997) and capillary zone electrophoresis (Choi and Jo, 1997, Fukushi *et al.,* 1997) have been found equally suitable for vitamin C determination. Micellar electrokinetic capillary chromatography also served the purpose of determination (Gomis *et al.,* 1999). Fluorimetric, spectrophotometric and FIA differential photometric methods have found wide application in the determination of vitamin C in formulations (Zhao *et al.,* 1996, Arya and Mahajan, 1997), vegetables (Chen *et al.,* 1995), pharmaceutical preparations (Ensafi and Mohsenei, 1998 and Yang, 1999), foods and other biological samples (Shiraishi and Deki, 1998). Some novel spectrophotometric methods have been successfully employed for the determination of vitamin C in simple solutions, pharmaceutical preparations and biological samples (Ding, 1999, Huang *et al.,* 1999, Ma, 1999, Kawakani *et al.,* 1997, Arya and Mahajan, 1997). Similarly this vitamin has also been determined volumetrically in presence of uric acid (Strochkova *et al.,* 1997). Recently the analytical techniques such as sequential injection analysis (Sultan and Desai, 1998), flow injection analysis (Grudpan *et al.,* 1999), differential pulse polarography (Aparicio *et al.,*

1995), chemiluminescence (Qin *et al.,* 1997 and Chen, 1997) and Voltametric (Ivanovskaya and Karpov, 1997) methods have been used for the estimation of the vitamin.

The previous work reveals that the determination of vitamin C with any accuracy requires modern and elaborate equipments which may or may not be easy for every laboratory to afford. It is for this reason that the simplest classical analytical tools have instead been sought to help achieve the same results as obtained by using the sophisticated instruments. The purpose of the present study centered around the determination of vitamin C in orange juices and pharmaceutical solid dosage formulations e.g. tablets in smaller quantities by volumetric method of analysis. For this two reagents N-bromosuccinimide (NBS) and Iodine have been selected and used separately for titration of vitamin C. The results have been compared on the basis of statistical treatment of the data to establish their validity and their individual superiority over one another.

EXPERIMENTAL

Materials:

All the reagents and chemicals used were of analytical grade.

Standard Solutions:

1. Ascorbic acid solution

50 mg of pure ascorbic acid, weighed accurately, was transferred into a 250 ml. volumetric flask, dissolved in distilled water and diluted (or made up) to the mark with distilled water. Shaken properly and prepared fresh and protected from light. This solution gave a concentration of 0.2 mg vitamin C per ml.

2. N-Bromosuccinimide (0.0022 M)

NBS was prepared by dissolving 0.356 g (accurately weighed) into distilled water and making the volume to one liter in a flask using distilled water. It is better to prepare freshly every day before using.

3. 4% Potassium iodide

4 g of potassium iodide (KI) was weighed, dissolved in distilled water and made up to 100 ml with distilled water in a measuring flask

4. Acetic acid solution

A roughly 10% v/v acetic acid was prepared by dissolving 10 ml of it in water and making up the volume to mark with water in a 100 ml. volumetric flask

5. Iodine solution (N/20)

0.6345 g of iodine was dissolved in potassium iodide solution (0.825 g / 10 ml) and then made up to volume with water in a 100 ml flask.

6. Sodium thiosulphate (N/20) solution

3.1025 g of sodium thiosulphate was dissolved in water and volume made up to 250 ml with water. This was diluted with water to obtain sodium thiosulphate N/50 solution.

7. Extraction of vitamin C from Orange

Orange juice was obtained with the help of juicer machine. To this was then added sufficient filter aid to form a thin paste. This was then filtered by passing through the Buchner funnel into a clean dry flask and covered with dark brown or black paper to avoid light

8. Extraction of vitamin C from Tablets

Tablet (with label claim of vitamin C) was weighed and crushed to powder, water added and stirred for a few minutes and filtered. Washed with water and collected the washings along with filtrate. Volume was made up to 250 ml. with water in a 250 ml. volumetric flask

9. Potato starch

Starch indicator was prepared by suspending 0.5 g/100 ml of it into hot distilled water, boiled to make a clean solution and the volume made up to 100 ml with water.

Procedure:

Standardization of NBS

An aliquot of pure vitamin C solution containing a known amount of the vitamin per ml. of the solution was taken in a titrating flask. The flask contained 5 ml of 4% KI solution, 2 ml of 10% acetic acid solution and 1 ml of starch indicator. Diluted then with 10 ml of distilled water. Titrated with NBS solution from the burette until the blue starch - iodine complex end point signal was reached. The molarity (or normality) or the equivalence of vitamin C per ml. of NBS was calculated as per the model calculation.

Standardization of iodine solution

5 ml of the iodine solution was taken into a 250 ml Erlenmeyer iodine flask. Added 15 ml of diluted sulphuric acid and 1 ml of starch indicator. Titrated with standard solution of sodium thiosulphate till the colourless end point was observed.

General Procedure for Determination of Vitamin C in a sample

Method 1: (NBS method) 5 ml or 25 ml aliquots of the sample solution was taken into a 250 ml titrating flask and added 5 ml of 4% KI, 2m1 of 10% acetic acid solution followed by 1 ml of starch indicator, diluted with 10 ml of water. Titrated with the NBS solution until the blue starch - iodine complex end point signal was observed.

Method 2: (Iodine method) 5 ml of the sample solution containing vitamin C was taken into a titrating flask, containing 15 ml. of dilute sulfuric acid and 1 ml. of starch indicator. This was titrated with standardized iodine solution till blue colour, end point is reached.

Model calculation

1. Standardization of N-bromosuccinimide (NBS) solution

$$\text{mg of vitamin C per ml of NBS} = \frac{\text{Volume of known standard vitamin C for titration} \times \text{Strength (mg/ml) of the known standard vitamin C solution}}{\text{Burette reading (ml) of NBS}}$$

Amount of vitamin C in a sample is calculated on the basis of strength of NBS determined.

2. Amount of vitamin C, Iodine method

$$\text{Amount of vitamin C (mg/ml)} = \frac{\text{Burette reading of iodine solution} \times 88 \times \text{Normality of iodine solution}}{\text{Volume (ml) of the sample for titration}}$$

3. Statistical test for analytical methods

The test is based on the comparison of the mean of samples obtained separately by two methods. The null hypothesis is that there is no systematic difference between the two methods. The appropriate test statistics to calculate the effect is as below:

$$\mu = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{S_1^2 / n_1 + S_2^2 / n_2}}$$

Where μ is the population mean; $\overline{X}_1$ and $\overline{X}_2$ are the means of the sample result of the two methods; S_1 and S_2 are the standard deviations of the separate results; n_1 and n_2 are the number of observations.

RESULTS AND DISCUSSION

Vitamin C in neat solution as well as in the real sample (orange juice) has been quantitatively determined. The Pharmaceutical preparations, e.g. tablets containing vitamin C have been analyzed for the vitamin C content. The determination of vitamin C in neat solution is presented in Table 1. N-bromosuccinimide (NBS) solutions of strengths (0.0011 M and 0.0022 M) have been used for titration. 5 ml of vitamin C solution equivalent to 0.2 mg/ml of the vitamin is titrated using 0.0022 M NBS. The percentage error (not shown in the table) varied from 1.45% to 2.3%, whereas with NBS (0.0011 M) the percentage error was not much different. Iodine method did not gave better results. Iodine (0.0828 N) was used as titrant as this concentration of iodine gave more reliable results as compared to lower concentration of iodine.

In Table 2 results of vitamin C in orange juice have been presented. Both the analytical methods were used to determine the vitamin content in the orange juice. The results obtained again showed that the two methods gave different results. The amount of the vitamin varied as the method was changed. NBS showed that the juice contained vitamin C around 21% whereas iodine method estimated the quantity to about 17.5%. This determined percentage may not be the same in the juice obtained from different grades and qualities of the ripened oranges. It was for the reason that the oranges of the same lot were analyzed for vitamin content by the two methods.

In Table 3 results of vitamin C in tablet extract are presented. The pharmaceutical preparations such as the tablets or other solid dosage forms need to be crushed to powder form for complete extraction of the vitamin by distilled water, as the vitamin is soluble in water. However, a complete extraction into water followed by filtration and making up to a suitable volume all these demand careful and analytical approach to help reduce the chances of introducing error. NBS method once again gave results which were very close to the amount calculated theoretically according to the label claim. The iodine method, however, was also found to give reasonably acceptable results. This time the concentration of iodine was little lower (0.06008 N) which indicated that the results could be improved if a suitable concentration of the titrant is chosen.

Statistical Test:

The statistical data related to results obtained by NBS and iodine methods have been presented in Table 4. Analytical methods of two different types, NBS and iodine based have been used to determine vitamin C in the same sample. From the experimental results obtained by these methods it was found that the determined quantities of vitamin C in orange juice obtained by NBS method have a standard deviation (s.d) of 0.0042722 and those obtained by iodine method have a standard deviation (s.d) of 0.371121. When the juice was tested 5 times by NBS method for the vitamin content the mean value (X_1) was found to be 0.3128. When the same was tested 5 times by

iodine method the mean value was 0.3031.

$$\text{Population mean} = \mu = \frac{0.3128 - 0.3031}{\sqrt{\frac{(0.0042722)^2}{5} + \frac{(0.0371121)^2}{5}}}$$

$$= \frac{0.0097}{\sqrt{0.00000365 + 0.000275461}}$$

$$= \frac{0.0097}{0.016706633}$$

$$= 0.58$$

As / μ / < 1.96, the null hypothesis would not be rejected if a 5% significance level were chosen. 95% confidence limits for μ (NBS) - μ (Iodine) are:

$$(0.3128 - 0.3031) \pm 1.96\sqrt{\frac{(0.00042722)^2}{5} + \frac{(0.0371121)^2}{5}}$$

= 0.0097 ± 1.96 x 0.016706633
= 0.0097 ± 0.032745 = 0.042445 and – 0.023045

Table 1
Estimation of Vitamin C in Neat Solution (NBS and Iodine Method)

S. No.	Vitamin C (0.2 mg/ml) ml		Burette reading (ml)		Known Quantity vitamin C (mg/ml)	Found Quantity Vitamin C (mg/ml)	
	NBS Method	Iodine Method	NBS Method	Iodine Method		NBS Method	Iodine Method
1	5.0	25.0	2.7	0.6	0.2	0.2089	0.1748
2	5.0	25.0	2.7	0.6	0.2	0.2089	0.1748
3	5.0	25.0	2.7	0.6	0.2	0.2089	0.1748
4	5.0	25.0	2.7	0.6	0.2	0.2089	0.1748
5	5.0	25.0	2.6	0.6	0.2	0.2010	0.1748

Table 2
Estimation of Vitamin C in Orange Juice (NBS and Iodine Method)

S. No.	Pure Orange Juice (ml)	Burette reading (ml)		Found Quantity Vitamin C (mg/ml)	
		NBS (0.0022M)	Iodine (0.0828N)	NBS Method	Iodine Method
1	5.0	4.1	0.20	0.3175	0.2914
2	5.0	4.0	0.20	0.3097	0.2914
3	5.0	4.0	0.22	0.3097	0.3208
4	5.0	4.1	0.20	0.3175	0.2914
5	5.0	4.0	0.22	0.3097	0.3208

Table 3
Estimation of Vitamin C in Pharmaceutical Preparation (NBS and Iodine Method)

S. No.	Extract from Tablet (500 mg / 250 ml)	Burette reading (ml)		Known Quantity Vitamin C (mg / ml)	Found Quantity Vitamin C (mg / ml)	
		NBS (0.0022M)	Iodine (0.05008N)		NBS Method	Iodine Method
1	5.0	25.7	2.8	2.0	1.9902	2.4679
2	5.0	25.8	2.8	2.0	1.9979	2.4679
3	5.0	25.8	2.7	2.0	1.9979	2.3798
4	5.0	25.7	2.8	2.0	1.9902	2.4679
5	5.0	25.7	2.7	2.0	1.9902	2.3798

Table 4
Statistical Data for Testing the Analytical Methods

S. No.	Quantity of Vitamin C NBS Method (mg)	Quantity of Vitamin C Iodine Method (mg)	Mean		Standard Deviation	
			NBS Method	Iodine Method	NBS Method (S_1)	Iodine Method (S_2)
1	0.3175	0.2914	0.3128	0.3031	0.004272	0.037112
2	0.3097	0.2914				
3	0.3097	0.3208				
4	0.3175	0.2914				
5	0.3097	0.3208				

The fact that the 95% confidence interval, which is - 0.23045 to 0.042445, does include zero. This is linked with the acceptance of the null hypothesis (N.H) at the 5% level.

The hypothesis is thus tested that the two analytical methods have $\mu_1 = \mu_2$ and are equally good for quantitative estimation of vitamin C in a real sample.

REFERENCES

Aboul - Enein, H.Y., Al-Duraibi, I.A., Stefan, R., Radoi, C. and Avvamescu, A. (1999). Analysis of L-and D-ascorbic acid in fruits and fruit drinks. *Semin. Food Anal*. **4**(1): 31-37.

Abusshita, A.A., Hebshi, E.A., Daood, H.G. and Biacs, P.A. (1997). Determination of Antioxidant vitamin in tomatoes. *Food Chem*. **60**(2): 207-212.

Aparicio, P., Farre, P.R. and Frigola, A. (1995). Differential pulse polarographic determination of ascorbic acid in vegetables. *Bromatol* **44**(4): 257-261.

Arya, S.P. and Mahajan, M. (1997). Nitroso-R salt as a sensitive spectrophotometric reagent for the determination of vitamin C. *Anal. Lett*. **30**(14): 2541-2553.

Arya, S.P. and Mahajan, M. (1997). Colorimetric determination of ascorbic acid in pharmaceutical preparation and biological samples. *Mikrochim. Acta.* **127**(1-2): 45-49.

Chen. H., Qin, W. and Zhang, Z. (1997). Highly sensitive chemiluminescence system for determination of ascorbic acid. *Fenxi Huaxue,* **25**(9): 1079-1081.

Chen, J., Tian, A. and Di, Y. (1995). Determination of ascorbic acid in vegetables by fluorescence

method. *Shaanxi Shida Xuebao Ziran Kexueban*. **23**(2): 69-71.
Choi, O.K. and Jo, J.S. (1997). Determination of L-ascorbic acid in foods by capillary zone electrophoresis. *J. Chromatogr*. **781**(1-2): 435-443.
Coskun, M., Kurucu, S. and Kartal, M. (1997). HPLC analysis of ascorbic acid in the fruits of some Turkish Rosa species. *Sci. Pharm*. **65**(3): 169-173.
Czerwiecki, L. and Wilezynska, G. (1999). Determination of vitamin C in selected fruit and vegetable products. *Rocz Panstw. Zakl. Hig*. **50**(1): 77-87.
Ding. S. (1999). Study on catalytic reaction system of $KBrO_3$ – methylene green-vanadium and its application to determination of vitamin C. *Lihua Jianyan, Huaxue Fence*. **35**(8): 346-347.
Ensafi, A.A. and Mohsenei, S. (1998). Kinetic determination of ascorbic acid with spectrophotometric detection. *J. Sci. Islamic Repub. Iran*. **9**(4): 226-230.
Fukushi, K., Takeda, S. Wakida, S., Yamane, M., Highashi, K. and Hiiro, K. (1997). Determination of ascorbic acid in vegetables by capillary zone electrophoresis. *J. Chromatogr*. **772**(1-2): 313-320.
Gomis, D.B., Gonzalez, L.L. and Alvarez, D.G. (1999). Micellar electrokinetic capillary chromatography analysis of water-soluble vitamins. *Anal. Chim. Acta.* **396**(1): 56-60.
Grudpan K., Kamfoo, K. and Jakmunee, J. (1999). Flow injection spectrophotometric or conductometric determination of ascorbic acid in vitamin C tablet using permaganate or ammonia. *Talanta*. **49**(5): 1023-1026.
Huang, B., Luo, Z., Fang, Y. and Lin, J. (1999). Spectrophotometric determination of vitamin C in fruits and vegetables by Fe(II)-1,10-phenanthroline-bromopyrogallol red system. *Huaxue Shijie*. **40**(6): 319-321.
Ivanovskaya, E.A. and Karpov, R.S. (1997). Determination of ascorbic acid in biological media by stripping voltammetry. *J. Anal. Chem.* **52**(7): 698-699.
Kawakami, T., Honda, K. and Igarashi, S. (1997). Spectrophotometric determination for 10^{-8} M levels of L-ascorbic acid using the diazotization of 5,10,15,20-tetrkis-(4-aminophenyl) porphine. *Chem. Pharm. Bull.* **45**(8): 1376-1378.
Li, H.B. and Chen, F. (2001). Simultaneous determination of nine water-soluble vitamins in pharmaceutical preparations by high-performance liquid chromatography with diode array detection. *J. Sep. Sci*. **24**(4); 271-274.
Ma, W. (1999). Determination of ascorbic acid in medicine by sulfosalicylic and spectrometry. *Guangpuxue Yu Guangpu Fenxi.* **19**(3): 507-508.
Moser, M.A., Dorfner, H.H. and Kniel, B. (1999). A new method for the quantitative determination of ascorbic acid in flour by high performance liquid chromatography. *Dtsch. Lebensm. Rundsch.* **95**(5): 180-184.
Nakama, A. and Yamada, K. (1997). Determination of vitamin C in commercial non-alcoholic beverages. *Seikatsu Eisei*. **41**: 183-188.
Paulo, M.G., Cabral Margues, H.M., Moris, J.A.G. and Almeida, A.J. (1999). An isocratic LC method for the simultaneous determination of vitamins A, C, E and B-carotene. *J. Pharm. Biomed. Anal*. **21**(2): 399-406.
Qin, W., Zhang, Z.J. and Chen, H.H. (1997). Highly sensitive chemiluminescence flow sensor for ascorbic acid. *Fresenius J. Anal. Chem.* **358**(7-8): 861-863.
Russell, L.F. (2000). Quantitative determination of water-soluble vitamins. *Food Sci. Technol*. **100**: 403-476.
Shiraishi, M. and Deki, K. (1998). Quantitative determination of ascorbic acid, in Kokai Tokkyo Koho Jp, p.4.
Strochkova, E.M., Turyan, Y.I., Kuselman, I. and Shenhar, A. (1997). Simultaneous voltammetric determination of uric and ascorbic acid in urine. *Talanta*. **44**(10): 1923-1928.

Sultan, S.M. and Desai, N.I. (1998) Mechanistic study and kinetic determination of vitamin C employing the sequential injection technique. *Talanta.* **45**(6): 1061-1071.

Wang, X., Gao, S. and Yu, J. (1999). Determination of water-soluble vitamin in fresh strawberry by HPLC. *Shipin Kexue*. **20**(5): 52-53.

Xiao, L, and Li, G. (1997). Determination of ascorbic acid in Soy Sauce by high performance capillary electrophoresis. *Fenxi Ceshi Xuebao*. **16**(3): 79-82.

Yang, M. (1999). Determination of ascorbic acid in pharmaceutical preparation by indirect spectrophotometry. *Huaxue Yanjiu*. **10**(2): 54-56.

Zhao, Z., Zhang, D. and Li, S. (1996). Determination of vitamin C content in drug preparations by FIA differential photometry. *Zhongguo Shenghua Yaown Zazhi.* **17**(6): 259-261.