Is Titration as Accurate as HPLC for Determination of Vitamin C in Supplements?
—Titration versus HPLC for Vitamin C Analysis

Lucile Tiemi Abe-Matsumoto¹, Geni Rodrigues Sampaio², Deborah Helena Markowicz Bastos²

¹Physical, Chemical and Sensory Department, Food Center, Adolfo Lutz Institute, São Paulo, Brazil  
²Nutrition Department, School of Public Health, University of São Paulo, São Paulo, Brazil  
Email: *lucile.matsumoto@ial.sp.gov.br

Abstract

Herein, the iodometric titration and HPLC-RP methods were compared for the determination of vitamin C in vitamin and mineral supplements. The methods were validated in terms of linearity, limits of detection (LOD), limits of quantification (LOQ), precision, and recovery by using vitamin standards and a reference material SRM 3280 (multivitamin/multimineral tablets) obtained from the National Institute of Standards and Technology (NIST). A total of 22 samples of vitamin supplements, randomly acquired in local markets of Sao Paulo (Brazil) were evaluated for content of vitamin C by these two methodologies. The precision expressed as RSD was lower than 5% for both methods. LOD was 3.6 µg/mL for HPLC and 1.0 mg for titration, while LOQ were 12.0 µg/mL and 3.0 mg for HPLC and titration, respectively. Percent recoveries (%) of spiked samples ranged from 98.7 to 100.5 for HPLC and from 98 to 104 for titration. The contents of vitamin C in SRM 3280 (Certified value = 42.2 ± 3.7 mg/g) were 40.2 and 42.1 mg/g when determined by HPLC and titration respectively. Statistically, there was no difference in the analysed vitamin C content for half of the samples, irrespective of the method applied. HPLC was more sensitive, but the titrimetric method was faster and consumed less reagent. Although both methods were accurate in determining the vitamin C content in SRM 3280, the matrix constituents of some vitamin supplements may have interfered with the analysis.

Keywords

Vitamin C, Ascorbic Acid, Dietary Supplements, HPLC, Titration
1. Introduction

Vitamin C is known as ascorbic acid in its reduced form and as dehydroascorbic acid in its oxidized form. The conversion to the oxidized form is reversible and occurs in the presence of metal ions, heat, light, or mildly alkaline conditions; however, vitamin C activity decreases. Ascorbic acid can donate and receive electrons, and it supports specific enzyme reactions and plays an essential role as an antioxidant in the body. It is an important water-soluble vitamin that is not stored by the body [1]. Some studies report, that vitamin C can protect lipid peroxidation, especially that of HDL cholesterol, inducing a cardioprotective and atherogenesis inhibitory effect [2].

The vitamin C recommended daily allowance (RDA) for a healthy adult is 45 mg, although some studies suggest, that the intake of 80 to 120 mg vitamin C may reduce the risk of non-infectious chronic diseases. It was reported that smokers need more than 140 mg per day [3] [4]. The initial clinical manifestations of vitamin C deficiency are fatigue, loss of appetite, drowsiness, pallor, lack of energy in the limbs and joints, irritability, slow healing of minor injuries, and minor bleeding. The severe deficiency of ascorbic acid leads to scurvy, a disease characterized by general weakness, anaemia, gingivitis and cutaneous haemorrhages [5].

The main source of dietary vitamin C is fruits and vegetables; however, due to changes in the dietary patterns of the population, supplements did become important sources of vitamins, and therefore, consumption of vitamin and mineral nutraceutical products is common worldwide [6]. The media widely advertise the benefits of antioxidant vitamins (e.g. vitamins C and E), targeting different socioeconomic levels of the global population [7] [8]. The accuracy of the supplement’s label information with regards to the vitamin content is essential to prevent consumers from suffering adverse health consequences and economic losses.

Several methods have been used for determination of vitamin C in foods, including spectrophotometry, electrophoresis, titration, and high performance liquid chromatography (HPLC) [9] [10] [11]. The oxidation-reduction titration method using indophenol dye as indicator was established as the official method for vitamin C determination by the Association of Official Analytical Chemists (AOAC) for many years. In this method, ascorbic acid is oxidized to dehydroascorbic acid and the indophenol dye is reduced to a colorless compound, indicating the end point of the reaction [12]. The iodometric titration for vitamin C determination was the official method for Public Health Laboratories in Brazil. The endpoint of this titration is determined by the first excess of iodine in the solution, that reacts with the starch indicator, forming a complex with an intense dark blue-violet color [13]. This method is single, fast, and reliable, however, just as for the AOAC method, the end point of the titration cannot be easily detected when the food sample has intense color. The alternative to exclude color interference was to transfer the traditional iodometric titration to automatic potenti-
ometric titrator, as described in this work. Thus, the end point of titration is indicated by platinum ring electrode, and no color interference occurs. In the last decades, HPLC and UHPLC methods were developed in substitution to titration method, showing high accuracy, but these equipment cost is high [14].

New regulations on dietary supplements were published by the Brazilian Health Regulatory Agency (ANVISA) in July 2018, with a 5-year period for industries to comply with the new rules. The new legislation changed, among the other requirements, the labelling rules, and the minimum and maximum vitamin limits allowed in supplements. This means that some products considered to be medicines and that were required to be registered with the Ministry of Health will be exempted from registration as they will be considered as dietary supplements. With these changes, fiscailisation of supplements will be increasingly needed, as more products will be exempt from registration with the Ministry of Health, facilitating their commercialization [15]. To evaluate the content of vitamin C in supplements, a fast, simple, and low-cost analytical method is essential. Therefore, the objective of this work was to compare two methods, automatic potentiometric titration and HPLC, for vitamin C determination in vitamin supplements.

2. Materials and Methods

2.1. Samples

A total of 22 vitamin supplements from different brands, available in tablet form, sugar-coated tablets, hard and soft gelatine capsules, and solutions, were acquired in trade from Sao Paulo, Brazil. Their vitamin C content was evaluated using HPLC and titrimetric methods. Acquisition of supplements was made randomly according to market availability in Sao Paulo, Brazil. In order to obtain a representative sample of marketed products, the following criteria were used to select the samples: different matrices and compositions, national and imported samples, supplements from multinational and from small national companies. Most of the samples (73%) were of national origin (Table 1).

2.2. Standards and Reagents

The L-ascorbic acid standard was purchased from Sigma-Aldrich (St Louis, USA); the standard reference material SRM 3280—Multivitamin/Multielement tablet was obtained from the National Institute of Standards and Technology (NIST); analytical grade reagents potassium iodide (KI) and potassium iodate (KIO₃) were purchased from Synth (Sao Paulo, Brazil); sulfuric acid (H₂SO₄) and ortho-phosphoric acid (H₃PO₄) were from Merck (Darmstadt, Germany); sodium phosphate monobasic (NaH₂PO₄) was from Calbiochem (San Diego, EUA), and HPLC grade methanol from Carlo Erba (Milan, Italy).

A 0.05 M sodium phosphate buffer solution (pH 3.0) was prepared weekly by dissolving 6.9 g of monobasic sodium phosphate in 1 L of Milli-Q water and the pH adjusted to 3.0 ± 0.1 with addition of 85% orthophosphoric acid. The solution
Table 1. Sample forms, origin, and quantity of vitamin/mineral contents of supplements evaluated.

| Sample | Supplement Form     | Origin   | Composition Vitamin/Mineral |
|--------|---------------------|----------|----------------------------|
| 1      | Tablet              | Canada   | 13/10                      |
| 2      | Tablet              | Argentina| 13/10                      |
| 3      | Tablet              | USA      | 13/11                      |
| 4      | Tablet              | Brazil   | 13/10                      |
| 5      | Tablet              | Brazil   | 10/2                       |
| 6      | Tablet              | USA      | 13/10                      |
| 7      | Hard capsule        | Brazil   | 11/9                       |
| 8      | Tablet              | Brazil   | 1/0                        |
| 9      | Tablet              | Brazil   | 3/3                        |
| 10     | Tablet              | Canada   | 13/10                      |
| 11     | Tablet              | France   | 8/4                        |
| 12     | Sugar coated tablet | Brazil   | 9/3                        |
| 13     | Sugar coated tablet | Brazil   | 9/3                        |
| 14     | Hard capsule        | Brazil   | 12/10                      |
| 15     | Hard capsule        | Brazil   | 13/7                       |
| 16     | Hard capsule        | Brazil   | 3/4                        |
| 17     | Soft Gelatine capsule| Brazil   | 13/10                     |
| 18     | Soft Gelatine capsule| Brazil   | 8/2                       |
| 19     | Soft Gelatine capsule| Brazil   | 13/10                     |
| 20     | Soft Gelatine capsule| Brazil   | 10/2                      |
| 21     | Solution            | Brazil   | 4/2                       |
| 22     | Solution            | Brazil   | 11/1                      |

was placed in an ultrasonic bath for 10 min and filtered through 0.45 μm membranes prior to use.

2.3. Chromatographic Determination of Vitamin C

The determination of vitamin C was conducted according to Abe-Matsumoto [16]. Standard: The vitamin C standard was dissolved in 0.05 M sodium phosphate buffer and the calibration curve was constructed at five levels in the concentration range 29.8 to 476.9 μg∙mL⁻¹. About 350 mg of the grounded sample was weighed into a 50 mL screw capped polypropylene tube. 35 mL of 0.05 M so-
Dium phosphate buffer (pH 3.0) was added to the tubes, which were vortexed for 20 s, first filtered through a quantitative filter paper and then through a 0.22 µm membranes (Millipore) into amber vials. Chromatographic analyses were performed immediately after the preparation. Chromatographic conditions: Samples were analysed with high performance liquid chromatography using a LC-20AT with an autosampler SIL-20AC, controller CBM-20A, column oven CTO-20A, coupled with diode array detector PDA-20A, supplied by Shimadzu. The separation of compounds was performed using a Shim-pack C18 VP-ODS-2 reversed phase column (150 mm, 4.6 mm, 5 µm particles) from Shimadzu, operating at 35°C. The mobile phase consisted of methanol (A) and 0.05 M sodium phosphate buffer (B). A gradient mode was applied at a flow rate of 0.8 mL·min⁻¹, starting at 98% A, rising to 40% A at 9 min, returning to the baseline at 15 min, and maintaining this ratio up to 25 min to reach mobile phase equilibrium. The injection volume was 10 μL and the temperatures in the automatic injector and column oven were maintained at 15°C and 26°C, respectively. Vitamin C was detected at 254 nm. Results were expressed in milligrams (mg) of vitamin per serving of supplement. A serving portion refers to the daily intake recommended by the supplement manufacturer.

2.4. Potentiometric Determination of Vitamin C

The potentiometric determination of vitamin C was performed on the same day as the HPLC analysis to avoid potential interferences in the sample comparison due to degradation. The iodometric titration for vitamin C quantification was conducted with an automatic potentiometric titrator Titrando 905 (Metrohm Pensalab, Herisau, Switzerland). The platinum ring electrode was controlled by Tiamo® Software [14]. Between 200 and 600 mg of powdered sample was weighed into a 150 mL glass beaker. For oily matrices, amounts of samples between 150 and 500 mg were weighed in 50-mL polypropylene tubes to which 5 mL of 5% metaphosphoric acid and 5 mL of hexane were added and were vortexed for 1 min. An aliquot of the aqueous phase was pipetted into a 150-mL beaker for titration. For liquid samples, volumes between 2 and 5 mL were pipetted into a 150-mL beaker. A volume of 10 mL of 20% H₂SO₄ solution, 90 mL of distilled water and 1 mL of 10% KI solution were added to the beaker. After 30 s of homogenization with a magnetic stirrer, the sample was titrated with a 0.002 M KIO₃ solution in an automatic titrator. The results were expressed in mg of vitamin C per serving.

2.5. Validation of the Methods

The validation of the methods was carried out according to the International Conference on Harmonisation (ICH) guide, evaluating the following parameters: linearity, intraday (repeatability) and interday (intermediate precision) precision, accuracy, limits of detection (LOD) and limits of quantification (LOQ). The linearity was verified by the correlation coefficient (R²) of the calibration curve.
with samples prepared in triplicate and 5 concentration levels. The intermediate precision was determined as the relative standard deviation (% RSD) with 6 replicates in SRM 3280 reference material on two different days by the same analyst. Vitamin C analyses were performed in triplicate in three concentration with standard-spiked samples, analysed on two different days. The repeatability was evaluated by the % RSD recovery test. Accuracy was assessed with recovery standards spiked in the powder, oily and liquid matrix at three different concentration levels [17]. In addition, the standard reference material SRM 3280 was analysed in 6 replicates, and Z-scores were calculated. For determining the LOD a small concentration of analytes were spiked in blank samples, and the concentration equivalent to a peak area about three times larger than the noise was established as the LOD. By using the formula LOD = LOQ/3.3, the LOQ was established.

2.6. Statistical Analysis

The vitamin C content in vitamin supplements was expressed as mean ± standard deviation. The one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test was used to differentiate between the means of the vitamin C content (p < 0.05) determined with the HPLC and titration method. All statistical analyses were conducted using Microsoft Office Excel (2010) and Action Software [18]. The level of statistical significance was set at 5% for all analyses.

3. Results and Discussion

3.1. Validation

The linearity of the detector response was checked by linear regression. The coefficient of determination (R²) was higher than 0.99 in the tested range, for both methods (Table 2). Recovery (%) ranged from 98.7% to 100.5 and 98 to 104% for the HPLC and titration method, respectively. These results were considered as satisfactory. The values obtained for the reference material SRM 3280 are also within the expected range, being Z-score values smaller than 2 (Table 2).

Intraday and interday precision, expressed as the relative standard deviation (% RSD), were estimated at three different concentrations for each analyte. Samples were spiked with standard solutions and analysed independently on two different days. The corresponding results are summarized in Table 2 and show the good precision, with RSD values lower than 5% for both methods. The LOD was 3.6 µg∙mL⁻¹ and 1.0 mg, and the LOQ was 12.0 µg∙mL⁻¹ and 3.0 mg for the HPLC and titration methods, respectively.

3.2. Vitamin C Content in the Vitamin Supplements

According to the Brazilian legislation RDC No. 360/2003, the declared label value has a tolerance of 20%, thus the nutrient content determined is not allowed to be less than 80% or more than 120% of the declared value [3].
Table 2. Validation parameters for HPLC and titration methods.

|                        | HPLC                | Titration           |
|------------------------|---------------------|---------------------|
| **Range**              | 29.8 - 476.9 µg/mL  | 3.0 - 40.0 mg       |
| **Linearity (R²)**     | 0.99901             | 0.99999             |
| **Accuracy**           |                     |                     |
| Recovery (%)           | 98.7 ± 3.4 - 100.5 ± 4.2 | 98 ± 1 - 104 ± 2   |
| SRM (mg/g) (RV)        | 42.2 ± 3.7          | 42.2 ± 3.7          |
| SRM (mg/g) (AV)*       | 40.2 ± 0.3          | 42.1 ± 0.5          |
| Z-Score                | 0.4629              | 0.1353              |
| **Precision (%RSD)**   |                     |                     |
| Intraday               | 0.5 - 4.2           | 0.4 - 3.3           |
| Interday               | 0.6 - 4.5           | 0.6 - 3.9           |
| LOD*                   | 3.6 µg/mL           | 1.0 mg              |
| (0.36 mg/g in the sample) | (1.7 mg/g in the sample) |
| LOQ*                   | 12.0 µg/mL          | 3.0 mg              |
| (1.2 mg/g in the sample) | (5.0 mg/g in the sample) |

SRM: Standard reference material; RV: Real value; AV: Analysed value; RSD: Relative standard deviation; LOQ: Limit of quantification; LOD: Limit of detection; *Mean ± Standard deviation (n = 6).

Vitamin C levels analysed with the HPLC and titration method in supplement samples are shown in Table 3. The vitamin C value stated on the nutritional information for the supplement label on tablets, capsules and sugar-coated tablets was 45 mg/serving, while the liquid samples presented a value of 30 mg/serving.

For half of the samples there was no statistical difference in the vitamin C content analysed with both methods. For the other half, there were differences of up to 20% observed in the vitamin C content. When comparing the analysed with the declared value on the label, four samples (2, 17, 19, and 20) presented divergent results, considering the 20% tolerance allowed by the legislation (Table 3). Taking legislation into account, samples 13 and 22 showed lower vitamin C concentrations than stated on the label, irrespective of the applied analysis method. On the other hand, samples 2 and 17 presented contents above the declared value only with the titrimetric method, while samples 19 and 20 presented values above the declared value only with the chromatographic method (Figure 1). A higher quantity of micronutrients is permitted by law to ensure the labelling stated by the expiry date. Thus, there would be no major problems in determining vitamin contents above the stated value, but there is a need to improve the extraction of compounds to limit the differences between results.

3.3. Comparison of methods

HPLC requires a vitamin extraction step, while in the titration method the sample is crushed, dissolves and analysed directly without the need for extraction,
making the analysis faster. The titration analysis takes about 3 min for a run, while the required HPLC run time is 25 min. Considering that the analysis is always performed in triplicate, HPLC takes at least 90 min per sample, while the titration method needs only 10 minutes to analyse a sample. With regards to the sensitivity of both methods, HPLC has a detection and quantification limit about 4 times lower than that of titration. In multivitamin supplements, vitamin C has the highest concentration compared to other vitamins, and therefore, is not usually analysed at low concentrations, i.e. close to the limit of quantification. Therefore, the LOD and LOQ of the methods are not critical for choosing the best method.

Table 3. Vitamin C content analysed by HPLC and titration.

| Sample | Declared (mg/per serving) | Analysed (mg/per serving) | Conformity with legislation |
|--------|---------------------------|---------------------------|----------------------------|
|        |                           | HPLC                     | Titr  | HPLC | Titr  |                          |
| 1      | 45  | 37.8 ± 0.7^a | 45.7 ± 0.4^b | c    | c    |                   |
| 2      | 45  | 49.2 ± 0.8^a | 55.9 ± 0.7^b | c    | above |                   |
| 3      | 45  | 35.9 ± 1.7^a | 37.5 ± 1.0^a | c    | c    |                   |
| 4      | 45  | 36.8 ± 0.5^a | 39.5 ± 1.7^b | c    | c    |                   |
| 5      | 45  | 55.4 ± 0.9^a | 50.5 ± 0.8^b | c    | c    |                   |
| 6      | 45  | 51.2 ± 1.6^a | 50.6 ± 2.5^a | c    | c    |                   |
| 7      | 45  | 39.9 ± 0.7^a | 45.0 ± 0.7^b | c    | c    |                   |
| 8      | 45  | 44.2 ± 0.8^a | 48.4 ± 0.4^b | c    | c    |                   |
| 9      | 45  | 39.1 ± 0.0^a | 45.7 ± 1.1^a | c    | c    |                   |
| 10     | 45  | 40.2 ± 1.7^a | 49.0 ± 1.6^b | c    | c    |                   |
| 11     | 45  | 45.7 ± 4.3^a | 52.7 ± 0.8^b | c    | c    |                   |
| 12     | 45  | 39.0 ± 0.3^a | 39.5 ± 1.4^a | c    | c    |                   |
| 13     | 45  | 33.7 ± 0.3^a | 32.9 ± 0.5^a | lower | lower |                   |
| 14     | 45  | 36.0 ± 1.8^a | 49.8 ± 0.7^a | c    | c    |                   |
| 15     | 45  | 42.4 ± 1.8^a | 47.5 ± 1.2^b | c    | c    |                   |
| 16     | 45  | 39.5 ± 0.4^a | 37.2 ± 3.62^a | c    | c    |                   |
| 17     | 45  | 45.9 ± 0.95^a | 56.9 ± 1.85^b | c    | above |                   |
| 18     | 45  | 43.2 ± 1.36^a | 43.3 ± 1.17^a | c    | c    |                   |
| 19     | 45  | 57.0 ± 2.16^a | 46.9 ± 0.18^b | above | c    |                   |
| 20     | 45  | 55.4 ± 0.91^a | 45.0 ± 0.75^b | above | c    |                   |
| 21     | 30  | 34.5 ± 0.78^a | 31.9 ± 1.16^a | c    | c    |                   |
| 22     | 30  | 20.5 ± 0.08^a | 21.6 ± 0.01^a | lower | lower |                   |

Different superscript letters in the same line indicate statistical difference (p < 0.05), according to Tukey’s Test. C: in compliance with declared value; lower: vitamin C content lower than declared; above: vitamin C content above that declared.
One of the advantages of analysing vitamin C with chromatographic methods is that if the supplement contains other water-soluble vitamins such as B1, B6, niacin and pantothenic acid, they can be analysed simultaneously.

Najwa Fatin and Azrina [19] did analyses on the vitamin C content of citrus fruits and observed significant differences between the HPLC-PDA and indophenol titration method. They indicated that at certain conditions the oxidation-reduction titration may overestimate the vitamin C content of the fruits, because the titration end point was difficult to detect, specifically when intense coloured fruits were used. In addition, reducing substances in the fruit samples can react with the indophenol dye and cause overestimation of the vitamin C content in fruit samples. In this work, the sample colour was not a problem because the end point of the titration was indicated by the platinum ring electrode.

With fruits, there may be differences in the determination of vitamin C, because fruits contain vitamin C in the form of ascorbic acid and dehydroascorbic acid. If the method does not quantify dehydroascorbic acid, the content of vitamin C may be underestimated [20]. Odriozola-Serrano et al. [21] found significant amounts of dehydroascorbic acid in strawberry, tomato, and apple, showing that quantification of this compound is important when fruits and vegetables are analysed. In supplements, the amount of dehydroascorbic acid is insignificant, as there is no mention of this compound in the NIST certified reference material SRM 3280.

4. Conclusion

Both HPLC and titration methods accurately analysed vitamin C in SRM 3280, and no statistically significant difference was observed for the results. However,
in the commercial samples, the matrix constituents of some vitamin supplements seem to interfere with the analysis, causing differences in the HPLC and titrimetric results. In samples containing other water-soluble vitamins in addition to vitamin C, such as multivitamin supplements, the HPLC method is recommended, because there is the possibility of simultaneous analysis of other water-soluble vitamins. In samples containing only vitamin C, potentiometric titration may be more advantageous. While HPLC was more sensitive, the titrimetric method was faster and consumed less reagent. The main advantage of the titration method is its simplicity, requiring only a single equipment and inexpensive chemicals.

**Funding**

This work was supported by the São Paulo Research Foundation (FAPESP) [grant numbers 2013/23006-4 and 2019/17839-0].

**Conflicts of Interest**

The authors declare that they have no conflict of interest.

**References**

[1] Moser, M.A. and Chun, O.K. (2016) Vitamin C and Heart Health: A Review Based on Findings from Epidemiologic Studies. *International Journal of Molecular Science, 17*, 1328. [https://doi.org/10.3390/ijms17081328](https://doi.org/10.3390/ijms17081328)

[2] Al-Khudairy, L., Flowers, N., Wheelhouse, R., Ghannam, O., Hartley, L., Stranges, S. and Rees, K. (2017) Vitamin C Supplementation for the Primary Prevention of Cardiovascular Disease. *Cochrane Database of Systematic Review, No. 3, Article No. CD011114.* [https://doi.org/10.1002/14651858.CD011114.pub2](https://doi.org/10.1002/14651858.CD011114.pub2)

[3] Brasil (2003) Resolução RDC nº 360, de 23 de dezembro de (2003) Aprova o Regulamento Técnico sobre Rotulagem Nutricional de Alimentos Embalados, tornando obrigatória a rotulagem nutricional. Ministério da Saúde, Brasília, DF.

[4] Raatz, S.K., Jahns, L., Johnson, L.K., Scheett, A., Carriquiry, A., Lemieux, A., Nakajima, M. and al’Absi, M. (2017) Smokers Report Lower Intake of Key Nutrients than Nonsmokers, yet Both Fall Short of Meeting Recommended Intakes. *Nutrition Research, 45*, 30-37. [https://doi.org/10.1016/j.nutres.2017.07.010](https://doi.org/10.1016/j.nutres.2017.07.010)

[5] FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements (1998) Vitamin and Mineral Requirements in Human Nutrition. Report of a Joint FAO/WHO Expert Consultation, 2nd Edition, Bangkok, Thailand.

[6] Bailey, R.L., Gahche, J.J., Miller, P.E., Thomas, P.R. and Dwyer, J.T. (2013) Why US Adults Use Dietary Supplements. *JAMA Internal Medicine, 173*, 355-361. [https://doi.org/10.1001/jamainternmed.2013.2299](https://doi.org/10.1001/jamainternmed.2013.2299)

[7] Pullar, J.M., Carr, A.C. and Vissers, M.C.M. (2017) The Roles of Vitamin C in Skin Health. *Nutrients, 9*, 866. [https://doi.org/10.3390/nu9080866](https://doi.org/10.3390/nu9080866)

[8] Shaw, G., Lee-Barthel, A., Ross, M.L.R., Wang, B. and Baar, K. (2017) Vitamin C-Enriched Gelatin Supplementation before Intermittent Activity Augments Collagen Synthesis. *American Journal of Clinical Nutrition, 105*, 136-143. [https://doi.org/10.3945/ajcn.116.138594](https://doi.org/10.3945/ajcn.116.138594)

[9] Spinola, V., Llorent-Martínez, E.J. and Castilho, P.C.J. (2014) Determination of Vitamin C in Foods: Current State of Method Validation. *Journal of Chromatography*
[10] Baghizadeh, A., Karimi-Maleh, H., Khoshnama, Z., Hassankhani, A. and Abbaspour, M. (2015) A Voltammetric Sensor for Simultaneous Determination of Vitamin C and Vitamin B6 in Food Samples Using ZrO2 Nanoparticle/Ionic Liquids Carbon Paste Electrode. *Food Analytical Methods, 8*, 549-557. https://doi.org/10.1007/s12161-014-9926-3

[11] Skrovankova, S., Mlcek, J., Sochor, J., Baron, M., Kynicky, J. and Jurikova T. (2015) Determination of Ascorbic Acid by Electrochemical Techniques and Other Methods. *International Journal of Electrochemical Sciences, 10*, 2421-2431.

[12] AOAC (Association of Official Analytical Chemists) (2012) Official Methods of Analysis. 19th Edition, Association of Official Analytical Chemists, Gaithersburg.

[13] Instituto Adolfo Lutz and Ministério da Saúde (2005) Agência Nacional de Vigilância Sanitária. IAL. Métodos físico-químicos para análise de alimentos, 4th Edition, 1018 p.

[14] Klimczak, I. and Gliszczyńska-Świglo, A. (2015) Comparison of UPLC and HPLC Methods for Determination of Vitamin C. *Food Chemistry, 175*, 100-105. https://doi.org/10.1016/j.foodchem.2014.11.104

[15] Brasil (2018) Resolução RDC nº 243, de 26 de julho de 2018. Dispõe sobre os requisitos sanitários dos suplementos alimentares. Ministério da Saúde, Brasília, DF.

[16] Abe-Matsumoto, L.T., Sampaio, G. R. and Bastos, D.H.M. (2018) Do the Labels of Vitamin A, C, and E Supplements Reflect Actual Vitamin Content in Commercial Supplements. *Journal of Food Composition and Analysis, 72*, 141-149. https://doi.org/10.1016/j.jfca.2018.07.001

[17] International Conference on Harmonization (ICH) (1996) Validation of Analytical Procedures: Methodology, Q2B.

[18] Equipe Estatcamp (2014) Software Action. Estatcamp—Consultoria em estatística e qualidade. http://www.portalaction.com.br

[19] Najwa Fatin, R. and Azrina, A. (2017) Comparison of Vitamin C Content in Citrus Fruits by Titration and High-Performance Liquid Chromatography (HPLC) Methods. *International Food Research Journal, 24*, 726-733.

[20] Phillips, K.M., Council-Troche, M., McGinty, R., Rasor, A.M. and Tarrago-Trani, M.T. (2017) Stability of Vitamin C in Fruit and Vegetable Homogenates Stored at Different Temperatures. *Journal of Food Composition and Analysis, 45*, 147-162. https://doi.org/10.1016/j.jfca.2015.09.008

[21] Odriozola-Serrano, I., Hernández-Jover, T. and Martín-Beloso, O. (2007) Comparative Evaluation of UV-HPLC Methods and Reducing Agents to Determine Vitamin C in Fruits. *Food Chemistry, 105*, 1151-1158. https://doi.org/10.1016/j.foodchem.2007.02.037