Review Article

Journal of Pharmaceutical and Medicinal Chemistry
Volume 6 Number 2, July–December 2020
DOI: http://dx.doi.org/10.21088/jpmc.2395.6615.6220.7

Recent Methods of Analysis of Ascorbic Acid: A Mini Review

Asif Husain[1], Shazia Parveen[2], Mohammad Umar[3], Harshil Prakash Jethani[4], Abubakar[5], Shivangi Kaushal[6]

[1]Professor, [2-6]UG Scholar, Department of Pharmaceutical Chemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India.

How to cite this article:
Asif Husain, Shazia Parveen, Mohammad Umar et al./Recent Methods of Analysis of Ascorbic Acid: A Mini Review. J Pharmaceut Med Chem. 2020;6(2):69–75.

Abstract

Ascorbic acid, also known as vitamin C, is an essential water soluble vitamin. Body needs vitamin C in order to form and maintain bones, blood vessels and skin, and its deficiency causes scurvy. Ascorbic acid has been widely used in chemical, pharmaceutical, cosmetic and food industry for the preparation of various preparations. Analysis of ascorbic acid is an important and inseparable part of industries involved in any form of ascorbic acid preparation. This review highlights recent methods for ascorbic acid analysis like spectrophotometric method, high pressure liquid chromatography (HPLC), etc. Former methods for ascorbic acid analysis are also presented such as Titrimetric, spectrophotometric as well as HPLC method along with different detection methods.

Keywords: Vitamin C; Assay; HPLC; Spectrophotometry.

Introduction

Ascorbic acid (vitamin C), a water soluble vitamin, is found in various foods including a variety of fruits and vegetables. Its deficiency causes disease scurvy.[1] Weakening of collagen fibers which leads to poor wound healing and impaired immunity happens due to scurvy.[2] Vitamin C is important for proper functioning of immune system.[3] Deficiency of ascorbic acid occurs due to poor dietary habits, life style changes, smoking, alcohol, drug abuse, various diseases, and pollutants.[4,5] Excess of vitamin C is also dangerous as it will lead to gastric irritation and its metabolite oxalic acid will cause renal problems.

Ascorbic acid is highly effective antioxidant due to its tendency to donate electron thereby protecting important biomolecules (such as protein, lipid, nucleic acid and carbohydrate) from damage by oxidants.[6] Vitamin C is a cofactor for the lysyl and prolyl hydroxylases required for stabilization of tertiary structure of collagen. Cofactor for two hydroxylases which is involved in carnitine biosynthesis and also a cofactor for hydroxylase enzymes which is involved in synthesis of catecholamine hormone.[7,8]

Ascorbic acid (Fig 1) is a hexanoic sugar acid having two dissociable protons therefore it occurs as an ascorbate anion.[9] Ascorbate is synthesized from glucose in plants and most of animals whereas in amphibians and reptiles synthesis of ascorbate takes place in kidney and in mammals' liver is the organ where an enzyme L-gulonolactone oxidase helps conversion of glucose into ascorbic acid.[10,11]

L-Gulonolactone oxidase is deficient in humans, some primates, and guinea pigs due to genetic mutation, making them incapable to synthesize ascorbic acid so there is requirement to take it from diet.[12] Rich sources of vitamin C (Fig 2) are fresh vegetables and fruits mainly in black currant, citrus fruit, leafy vegetables, tomatoes, green and red peppers.[13]

Corresponding Author: Asif Husain, Professor, Department of Pharmaceutical Chemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India.

Email: drasifhusain@yahoo.com

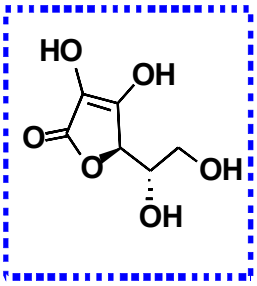

Fig. 1: Structure of Ascorbic acid.

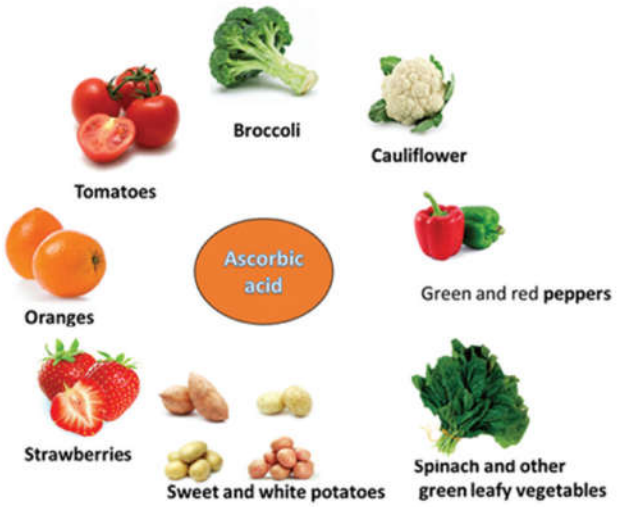

Fig. 2: Rich sources of ascorbic acid.

In pharmaceutical, chemical, cosmetic and food industry ascorbic acid is used due to its bioactivity and antioxidant property. For measuring the amount of ascorbic acid in a sample there is a need to find an accurate, reliable, rapid and easy method. For measuring the amount of ascorbic acid in sample, there is still a need to find an accurate, reliable, rapid and easy to implement method. Due to instability of ascorbic acid in aqueous solution, there has been some difficulty in quantifying ascorbic acid. Heat, light, alkali, oxygen, and contact with traces of copper and iron will easily reacts with ascorbic acid, as it is highly sensitive to these elements.[14]

In the field of biochemistry and commercial food, determination of vitamin C has become an important subject because of the role of vitamin C in maintaining proper health.[15] One of the most important properties of vitamin C is that it is an antioxidant. It scavenges nitroxide, hydroxide, superoxide, hydrogen peroxide and can reduce vitamin E.[16] It is a powerful antioxidant as it can also neutralize harmful free radicals.[17] The physical properties of ascorbic acid are presented in Table 1.[18]

Table 1: Physical properties of ascorbic acid.

Chemical name	L-ascorbic acid
Empirical formula	$C_6H_8O_6$
Molecular weight	176.1
Melting point	190°C
Appearance	White to slightly yellow is hcrystalline powder
Odour	Odourless
Taste	Strong acidic taste
Solubility	Freely soluble in water, sparingly soluble in alcohol, insoluble in ether and benzene

Methods of Analysis

Various methods for the determination of vitamin C in a natural samples, biological fluids and pharmaceutical formulations have been reported:

1. UV spectrophotometric method
2. Voltammetric method
3. Fluorometric method
4. Potentiometric method
5. High performance liquid chromatography (HPLC)
6. Gas chromatography (GC)
7. Titrimetric method

UV spectrophotometric method

Determination of ascorbic acid by UV spectrophotometer is mostly used because of simplicity, and also vitamin C absorbs UV rays easily. Earlier UV method for analysis of ascorbic acid was developed which was limited for analysis of plasma samples only and its procedures were complicated. But later on a reliable UV method was developed for analysis of ascorbic acid sample in presence of other vitamins which gives reproducible results. This method is simple and faster as compared to other old methods.[19,20].

In 2005, Zeng W. et al. developed a new UV method for the analysis of ascorbic acid with methanol as solvent. Otherstudies which are performed in this research are the effect of copper (II) concentrations on the oxidation of ascorbic acid in aqueous solution and the regularities of its oxidation in methanol, USP phosphate buffer and de-ionized water was studied. The result shows

that ascorbic acid has been found to dissolve in methanol (solubility: 81.0 mg/ml) at room temperature. This method is simple, rapid, accurate and reliable thereby used for routine determination of ascorbic acid in its granular and tablet form. With this method, the ascorbic acid bulk material from manufacturer was found to be assayed with 89.34% purity.[21]

In 2006, Khan M. R. et al. described a UV-spectrophotometric method to determine vitamin C content in various fruits and vegetables. The method involves the oxidation of ascorbic acid to dehydroascorbic acid by bromine water in presence of acetic acid. After coupling with 2,4-dinitrophenyl hydrazine the solution is then treated with 85% H_2SO_4 so that red color complex is formed. The absorbance is measured at 521nm spectrophotometrically. The results of this method exhibited the amount of vitamin c in the range of 12-118mg/100g in fruits, and 22- 135mg /100g in vegetables.[22]

In 2020, Panchem Y. P. et al. develop a spectrophotometric method to determine ascorbic acid in bulk powder form by using UV-1900 model. Methanol: water (50:50v/v) was taken as solvent. Ascorbic acid showed maximum absorbance wavelength at 258nm. In order to validate the method as per ICH guidelines various parameter were considered such as linearity, selectivity, specificity, precision, robustness, etc. Ascorbic acid analysis showed that this method was acceptable method.[23]

Desai A. P. et al in 2019 determined the content of ascorbic acid in different fruits by UV spectroscopy. In this method, 2,4-DNPH (2,4-dinitrophenyl hydrazine) acted as dye. Bromine water was used to oxidize ascorbic acid into dehydroascorbic acid in presence of acetic acid. After 3 hrs 85% H_2SO_4 was added which gave red color complex. The solution was then measured spectrophotometrically to determine ascorbic acid content.[24]

Voltammetric method

Determination of the contents of ascorbic acid in fifty tropical fruit samples has been reported by titrimetric method using N-bromosuccinimide along with cyclic Voltammeter using glassy carbon as working electrode. Ag/AgCl as reference and platinum as auxiliary electrode in 0.1M phosphate buffer, pH 2.0 containing 1Mm disodium EDTA were used. 200Mv to 1000Mv was the range in which measurement were done, with a scan rate of 50 Mv/s. Result showed that some fruits such as green pepper, long pepper, red pepper, lime, pawpaw, orange showed high level of ascorbic acid content.[25,26]

Nielsen S. S. et al. in 2017 determined the content of vitamin C in juices by using the reagent 2,6-dichloroindophenol. The indicator dye was reduced to a colorless solution by ascorbic acid, the unreduced dye was rose-pink in color. Using a standard ascorbic acid solution, the titer of dye could be determined. By series of steps, the amount of ascorbic acid was determined in an accurate way.[27]

Ogunlesi. M. et al. in 2010 measured vitamin C content in thirty-eight samples of green leafy vegetable and food by two methods.1st method was by cyclic voltammetry using glassy carbon, Ag/AgCl and platinum electrode system in 0.1M phosphate buffer, pH 2.0 containing 1Mm disodium EDTA in a potential range of 200Mv-1000MV with a scan rate of 50mv/s. 2nd method: Titration of aqueous mixture of sample using N-bromosccinimde. Red pepper, leaves of white camwood, black pepper, curry plant, pumpkin were the samples found to be rich in vitamin C.[28]

Fluorometric method

Fluorometric method is simple, rapid, cost-effective reliable and sensitive to analyze even the minute 2x10-3 to 0.1mg ascorbic acid/mL. This method has the capacity to analyze 50 samples/hr.[29]

Ascorbic acid is oxidized to dehydroascorbic acid in presence of activated charcoal. To produce a fluorescent compound, dehydroascorbic acid reacts with o-phenylenediamine (OPDA). The intensity of fluorescence produced indicates the concentration of ascorbic acid in sample solution. Use of charcoal will oxidize ascorbic acid slowly, so many other oxidants are used such as iodine, ferricyanide, chloramines-T, methylene blue, N-bromosuccinimide for smooth oxidation.[26,30,31]

Cheng X. et al in 2019 described the ratio metric fluorescent assay to determine the enzyme activity in ascorbic acid forming reaction. As fluorescent indicator blue-emitting carbon dots (bCDs; with excitation/emission wavelength at 380/450nm was served) via an inner filter effect their fluorescence was reduced by Fe^{3+} ions. As internal reference yellow-emitting CDs with excitation/emission wavelength at 380/550nm was served, as their fluorescence was insensitive to Fe^{3+}. When this was exposed to ascorbic acid, Fe^{3+} was reduced to Fe^{2+}, the fluorescence by bCDs was restored. Thereby, the enzymes in ascorbic acid reaction such as alpha-

glucosidase and alkaline phosphatase could be measured.[32]

In 2019 Ni P. et al. reported a fluorometric method to determine the alkaline phosphatase (ALP) activity and its inhibitors using ascorbic acid. As fluorescent probe, nitrogen and boron co-doped carbon dots (C-dots) with excitation/ emission peaks at 490/540nm. The C-dots modified by boronic acid binds to ascorbic acid generated by ALP-catalyzed hydrolysis of ascorbic acid-2-phosphatase. This method determined the ALP activity in acceptable range.[33]

Maki T. et al. in 2011 presented a simple and sensitive flow injection fluorometric method to determine ascorbic acid. A fluorescent reagent, perylenebisimide-linked nitoxide (PBILN), was used which allowed the selective determination of ascorbic acid. When solution of ascorbic acid was merged with the stream of PBILN, the ascorbic acid reacted with nitroxide moiety of PBILN to form hydroxylamine thereby the fluorescence properties of perylenebisimide moiety were recovered. A peak shaped fluorescence signal was produced which was detected by the detector. This method was applied to determine the concentration of ascorbic acid in several soft drink beverages.[34]

Wang Y. et al. in 2019 developed a dual-method to determine the ascorbic acid and ascorbic acid oxidase activity. The advantages of ratiometric fluorometry and colorimetry were combined together. In this assay, the oxidation of o-phenylediamine (OPDA) was done by permanganate ($KMnO_4$). In the presence of oxidant, a yellow substance (oxOPDA) with an absorption peak at 425 nm was produced rapidly. This oxOPDA would reduce the blue fluorescence of carbon dots peaking at 450nm, and new emission peak could be found at 565nm. The results showed that fluorometric assay for ascorbic acid had a linear range that extends from 0.6 to 40 μM and colorimetric assay from 0.2 to 70 μM.[35]

Xu W. et al. in 2019 prepared water-dispersed quantum dots (SiQDs) by using aminopropyl-trimethoxysilane as silicon source and ascorbic acid as reduction reagent. SiQDs are fluorescent with high quantum yield and high quality. Due to their low cost, good compatibility and superb electronic and mechanical property they are becoming popular. As SiQDs cannot be dispersed in water therefore fluorescent water-dispersible SiQDs are developed which have various optical-based applications. The SiQDs display fluorescence with excitation/emission peaks at 350nm/440nm.[36]

Non-electrochemical techniques to determine ascorbic acid- Titration with an oxidant solution such as dichlorophenol indophenol (DCPIP)[37], potassium iodate[38] or bromate[39] are the traditional methods used to determine ascorbic acid.

Potentiometric method

Potentiometric method gives much faster result for the assay of many active pharmaceutical ingredients. The fats, oils, resin, wax are determined potentiometrically. Potentiometry as an electroanalytical tool does not apply current or potential modulator and the apparatus used in potentiometry are simple, easy to handle and the sample can be seen as single independent variable. It gives rapid and reproducible results. Potentiometry has also been successfully applied to analyze different types of ascorbic acid samples.[40,41]

Total antioxidant capacity of ascorbic acid is utilized by Potentiometry by recording the generated potential from reaction of $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ by interaction with sample antioxidant by using platinum electrode as detector.[42,43]

Amini M. K. et al. in 2001 constructed a chemically modified electrode by incorporating iron (II) phthalocyanine [Fe(II)Pc] into carbon -paste matrix used as a sensitive potentiometric sensor to detect ascorbic acid. The formed electrode had catalytic property for electrooxidation of ascorbic acid. The results showed a linear response in concentration range from 10^{-6} to 10^{-2} M (0.18-1800 μg/ml) was obtained with a detection limit of 5×10^{-7} M for the potentiometric determination of ascorbic acid. This electrode showed a fast response time (<10s), good stability and had an extended lifetime.[44]

Tonelli D. et al. in 2011 developed a polymeric membrane-based ion selective electrode to determine L-ascorbic acid. By modifying the glassy carbon electrode with molecularly imprinted polypyrrole the sensor was fabricated. The performance of sensor was improved by the molecularly imprinted polymer. This imprinted polymer shows near Nernstian response to the ascorbate at concentration range between 5×10^{-6} M and 2×10^{-3} M. This potentiometric sensor could be used for some weeks and helps to determine concentration of ascorbate in food and pharmaceutical samples.[45]

High-performance liquid chromatography (HPLC)

In high-performance liquid chromatography, analytes (samples to be analyzed) are separated

by column chromatography and measured by post column detectors. HPLC methods are also developed to reduce the drawbacks associated with other method. For measuring ascorbic acid in biological samples, HPLC with UV detection is the simplest system. UV detection is possible due to vitamin optical absorbance spectrum having peak value at 265-266 nm. HPLC/UV assays are used to measure the content of ascorbic acid in lymphocytes, urine, tears, aqueous humor, plasma and in food.[46,47]

In 2011 Mitic S. S. et al. developed and validated an accurate and reproducible HPLC method for measuring the content of ascorbic acid in pharmaceutical samples. The drug and standard were eluted from Superspher RP-18 at 200°C. By carefully adding acetic acid (500 mL) to 1.5g of 1-hexanesulfonic acid sodium salt, mobile phase was prepared. The flow rate was 0.7 mL/min. To monitor the effluent, a UV detector was set at 280nm. Result showed that, each analysis of sample took 4 min, limit of quantitation was 1.95 μg/L. Recovery % ranged from 99.58 to 101.93.[48]

Li X. et al. in 2009 developed a robust and rapid high pressure liquid chromatography-electrochemical detection (HPLC-ECD) for an accurate determination of ascorbic acid and uric acid in human plasma. For accurate quantification of analytes, a stable electrochemical active internal standard (homogentisic acid) was added. The analysis was done on a reverse-phase column with HPLC and ultra-HPLC. Result showed that U-HPLC had increased sensitivity with detection limit of 0.05ng which was lower as compared to conventional HPLC. A good accuracy and precision had been shown by both analyses.[49]

Gas chromatography (GC)

GC method is rapid, reliable and highly sensitive to analyze even very minute quantities of ascorbic acid.

Silva F. O. et al in 2005 developed a new and highly sensitive method to determine the content of ascorbic acid by using gas chromatography and it was compared to HPLC. The analysis was performed by simple clean up techniques a freeze-drying condition. The total content of ascorbic acid was analyzed after reduction to dehydro-ascorbic acid with the help of dithiothretinol.[50]

Vecchi M. et al. reported a method of gas chromatography to determine vitamin C by the use of a silylating agent, N-trimethylsilylacetamide. The result showed that the reaction proceeds quantitatively and the results were accurate and reproducible.[51]

Titrimetric method

Titration methods are simple, cost-effective and also give much faster results. Narong Lenghor et al. in 2002 developed two sequential injection titration systems with spectrophotometric detection to measure ascorbic acid. The first system was based on redox reaction between ascorbic acid and permanganate in an acidic medium which lead to decrease in color intensity of permanganate.[52]

Suntornsuk L. et al. in 2002 determined the content of vitamin C in fresh and freeze-dried herbal juice of guava, lemon, sweet pepper and passion fruit by direct titration with iodine. This method showed excellent linearity over the concentration range tested (100-500%) of amount found in juice sample with good precision and recovery.[53]

Conclusion

Ascorbic acid (vitamin C) is an important water soluble vitamin, and its deficiency could lead to various health issues. Variety of natural products and market preparations are available for human intake. Analysis of ascorbic acid in different products is an important aspect of quality control. There are various methods available for the analysis of ascorbic acid. Majority of these methods are reproducible, rapid and give accurate results. However, there is still a need to find novel, accurate, reliable, rapid, cost-effective and easy to implement method(s) to determine ascorbic acid.

References

1. "Vitamin C". https://en.wikipedia.org/wiki/Vitamin_C
2. Hemilä H. Vitamin C and Infections. Nutrients. 2017, 29; 9(4): 339.
3. "Vitamin C". Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: The National Academies Press. 2000. pp. 95–185.
4. Maggini, S.; Beveridge, S.; Sorbara, J.; Senatore, G. Feeding the immune system: The role of micronutrients in restoring resistance to infections. CAB Rev. 2008, 3; 1–21.
5. Huskisson, E.; Maggini, S.; Ruf, M. The role of vitamins and minerals in energy metabolism and well-being. J. Int. Med. Res. 2007, 35; 277–289.

6. Carr, A.; Frei, B. Does vitamin C act as a pro-oxidant under physiological conditions? FASEB J. 1999, 13; 1007–1024.
7. Mandl, J.; Szarka, A.; Banhegyi, G. Vitamin C: Update on Physiology and Pharmacology. Br. J. Pharmacol. 2009, 157; 1097–1110.
8. Englard, S.; Seifter, S. The biochemical functions of ascorbic acid. Annu. Rev. Nutr. 1986, 6; 365–406.
9. Müller L.D. Improved extraction methods for avoiding the interference of copper in the LC determination of ascorbic acid in multivitamin-mineral tablets. J. Pharm. Biomed. Anal. 2001, 25(5-6); 985-994.
10. Erdurak-Kilic, C.S.; Uslu, B.; Dogan, U.; Ozgen, S.A.; Ozkan, M.; Coskun, Anodic voltammetric behavior of ascorbic acid and its selective determination in pharmaceutical dosage forms and some Rosa species of Turkey. J. Anal. Chem. 2006, 61; 1113.
11. Du, J.J.; Cullen, G. R. Buettner, Ascorbic acid: Chemistry, Biology and the treatment of cancer, Biochim. Biophys. Acta. 2012, 1826; 443.
12. Stone, I. The natural history of ascorbic acid in the evolution of mammals and primates and its significance for present-day man, J. Orthomol. Psychiatry. 1972, 1; 82.
13. Valpuesta, V.; Botella, M. A. Biosynthesis of l-ascorbic acid in plants: new pathways for an old antioxidant, Trends Plant Sci. 2004, 9; 573.
14. Padayatty, S. J.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J. H.; Chen, S. Vitamin, C as an antioxidant: evaluation of its role in disease prevention, J. Am. Coll. Nutr. 22 (2003) 18.
15. Hodgkinson A. Oxalic acid in biology and medicine. FEBS Lett. 1979, 101(2); 421.
16. Bendich, A. Vitamin C- Safety in Humans. In: Vitamin C in Health and Disease, Packer, L and J. Fuchs (Eds.); Marcel Dekker Inc New York, USA, 1997, pp: 367-379.
17. Hickey, S.; Roberts, H. J. Ascorbate: The Science of Vitamin C. Lulu press, 2004.
18. Izuagie, A. A.; Izuagie, F. O. Iodimetric determination of ascorbic acid (Vitamin C) in citrus fruits. Res. J. Agr. Biol. Sci. 2007, 3(5); 367-369.
19. Tulley, R. T.; Inventor; Louisiana State University, Mechanical College, assignee. Method for the analysis of Vitamin C. United States patent US 5,441,872. 1995.
20. Kwakye, J. K. West Afr. J. Pharm. 1997, 11; 65–75.
21. Zeng W.; Martinuzzi, F., MacGregor, A. Development and application of a novel UV method for the analysis of ascorbic acid. J. Pharm. Biomed. Anal. 2005, 36(5); 1107-1111.
22. Khan, M. R.; Rahman, M. M.; Islam, M. S.; Begum, S. A. A simple UV-spectrophotometric method for the determination of vitamin C content in various fruits and vegetables at Sylhet area in Bangladesh. J. Biol. Sci. 2006, 6(2); 388-392.
23. Pancham, Y. P.; Girish, B.; Sanjay, S. S. UV-Spectrophotometric method for quantification of ascorbic acid in bulk powder. The Pharma Innov. J. 2020, 9(5); 05-08.
24. Desai, A. P.; Desai, S. UV Spectroscopic Method for Determination of Vitamin C (Ascorbic Acid) Content in Different Fruits in South Gujarat Region. Int. J. Env. Sci. Nat. Resources. 2019, 21(2); 41-44.
25. Okiei, W.; Ogunlesi, L.; Azeez, L.; Obakachi, V.; Osunsanmi, M.; Nkenchor, G. The voltammetric and titrimetric determination of ascorbic acid levels in tropical fruit samples. Int. J. Electrochem. Sci. 2009, 4; 276-287.
26. Barakat, M. Z.; El-Wahab, M. F. A.; El-Sadr, M. M. Action of N-Bromosuccinimide on Ascorbic Acid. Anal. Chem. 1955, 27(4); 536-540.
27. Nielsen, S. S. Vitamin C Determination by Indophenol Method. Food Science Text Series, 2003, 45–50.
28. Ogunlesi, M.; Okiei, W. O.; Azeez, L.; Obakachi, V. Vitamin C contents of tropical vegetables and foods determined by voltammetric and titrimetric methods and their relevance to the medicinal uses of the plants. Int. J. Electrochem. Sci. 2010, 5; 105-115.
29. Roy, R. B.; Conetta, A.; Salpeter, J. Automated Fluorometric Method for the Determination of Total Vitamin C in Food Products. J. Assoc. Off. Anal. Chemists. 1976, 6(1); 1244-1250.
30. Deutsch, M. J.; Weeks, C. E. Microfluorometry assay for Vitamin C. J. Assoc. Off. Agric.. Chem. 1965, 48; 1248-1256.
31. Horwitz, W. Analytical Official methods of AOAC International. 17th Edn., Washington, DC: 2002.
32. Cheng, X..; Xu, J.; Wang, L.; Xu, G.; Wei, F.; Chai, Y.; Hu, Q.; Cen, Y. A redox modulated ratiometric fluorometric method based on the use of dual-color carbon dots for determination of the activity of enzymes participating in ascorbic acid-related reactions. Mikrochimica Acta. 2019, 186 (12); 818.
33. Ni, P.; Xie, J.; Chen, C.; Jiang, Y.; Lu, Y.; Hu, X. Fluorometric determination of the activity of alkaline phosphatase and its inhibitors based on ascorbic acid-induced aggregation of carbon dots. Microchimic Acta. 2019, 186; 202.
34. Maki, T.; Soh, N.; Nakano, K.; Imato T. Flow injection fluorometric determination of ascorbic acid using perylenebisimide-linked nitroxide. Talanta. 2011, 85(4);1730-1733.
35. Wang Y, Yang Y, Liu W, Ding F, Zou P, Wang X, Zhao Q, Rao H. A carbon dot-based ratiometric fluorometric and colorimetric method for determination of ascorbic acid and of the activity of ascorbic acid oxidase. Microchimica Acta. 2019, 186(4); 246.

36. Xu, W.; Yu, L.; Xu, H.; Zhang, S.; Xu, W.; Lin, Y.; Zhu, X. Water-dispersed silicon quantum dots for on-off-on fluorometric determination of chromium (VI) and ascorbic acid. Microchimica Acta. 2019, 186(10); 673.

37. Nielsen, S.S. Food analysis laboratory manual, Second Edition, chap.7, Springer Science, Bussines Media, New York, Dordrecht, Heidelberg, London, 2010, p.55.

38. Deshmukh, G. S.; Bapat, M. G. Determination of ascorbic acid by potassium iodate, Z. Anal. Chem. 1955, 145; 254-256.

39. Skoog, D. A.; West, D. M.; Holler, F. J. Fundamentals of Analytical Chemistry, Saunders College Publishing, 7th Edition, Philadelphia, 1998, p 845.

40. Tessutti, L. S.; Macedo, D. V.; Kubota, L. T.; Alves, A. A. Measuring the antioxidant capacity of blood plasma using potentiometry. Anal. Biochem. 2013, 441; 109–114.

41. Abdullin, I. F.; Turova, E. N.; Ziyatdinova, G. K.; Budnikov, G. K. Potentiometric determination of ascorbic acid: Estimation of its contribution to the total antioxidant capacity of plant materials. J. Anal. Chem. 2002, 57; 353–355.

42. Shpigun, L. K.; Arharova, M. A.; Brainina, K. Z.; Ivanova, A. V. Flow injection potentiometric determination of total antioxidant activity of plant extracts. Anal. Chim. Acta 2006, 573; 419–426.

43. Brainina, K. Z.; Gerasimova, E. L.; Varzakova, D. P.; Balezin, S. L.; Portnov, I. G.; Makutina, V. A.; Tyrchaninova, E. V. Potentiometric method for evaluating the oxidant/antioxidant activity of seminal and follicular fluids and clinical significance of this parameter for human reproductive function. Open Chem. Biomed. Methods J. 2012, 5; 1–7.

44. Amini, M. K.; Shahrokhian, S.; Tangestaninejad, S.; Mirkhani, V. Iron (II) phthalocyanine-modified carbon-paste electrode for potentiometric detection of ascorbic acid. Analytical Biochemistry. 2001, 290(2); 277-282.

45. Tonelli, D.; Ballarin, B.; Guadagnini, L.; Mignani, A.; Scavetta, E. A novel potentiometric sensor for l-ascorbic acid based on molecularly imprinted polypyrrole. Electrochimica acta. 2011, 56(20); 7149-7154.

46. Lewin, S. Vitamin C: Its Molecular Biology and Medical Potential. Academic Press, New York, 1976, pp.5-39.

47. Bolanca, T.; Ukic, S.; Novak, M.; Rogosic, M. Computer assisted method development in liquid chromatography.Croatica Chemica Acta. 2014, 87(2); 111-122.

48. Mitic, S. S.; Kostic, D. A.; Naskovic-okic, D. C.; Mitic, M. N. Rapid and reliable HPLC method for the determination of vitamin C in pharmaceutical samples. Trop. J. Pharm. Res. 2011, 10(1); 105-111.

49. Li, X.; Franke, A. A. Fast HPLC–ECD analysis of ascorbic acid, dehydroascorbic acid and uric acid. J. Chromatogr. B. 2009, 877(10); 853-856.

50. Silva, F. O. Total ascorbic acid determination in fresh squeezed orange juice by gas chromatography. Food Control. 2005, 16(1); 55-58.

51. Vecchi, M, Kaiser, K. The gas chromatographic determination of ascorbic acid in the form of its trimethylsilyl ether derivative. J. Chromatogr. A. 1967, 26; 22-29.

52. Lenghor, N.; Jakmunee, J.; Vilen. M.; Sara, R.; Christian, G. D.; Grudpan, K. Sequential injection redox or acid–base titration for determination of ascorbic acid or acetic acid. Talanta. 2002, 58(6); 1139-1144.

53. Suntornsuk, L.; Gritsanapun, W.; Nilkamhank, S.; Paochom, A. Quantitation of vitamin C content in herbal juice using direct titration. J. Pharm. Biomed. Anal. 2002, 28(5); 849-855.