

Conference Paper

Comparison of Vitamin C Analysis Using High-Performance Liquid Chromatography Versus Potentiometric Titration

Engrid Juni Astuti*, Agustin Rafikayanti, Diah Indri Cahyani, Diriyati Barin Putri

Department of Pharmacy, University of Muhammadiyah Malang

ORCID

Engrid Juni Astuti: <https://orcid.org/0000-0001-8703-7006>

Abstract.

Vitamin C is a water-soluble vitamin that is important for human health as it helps to maintain the body's resistance to various diseases. It functions as an antioxidant, which is a substance that gives electrons to free radicals and helps to stabilize free radicals, which protects cells from damage. Because vitamin C is easily oxidized, an analytical method is needed which measures vitamin C levels accurately and quickly. Analytical methods must be validated before being implemented to ensure the validity of the data generated. In this study, the validation of two vitamin C measurement methods were compared: high-performance liquid chromatography (HPLC) and potentiometric titration. The results of the HPLC method showed that the linearity, accuracy, and precision met the validation requirements of the official methods of analysis (AOAC). The potentiometric titration method validation results showed that the accuracy met the AOAC requirements while the precision did not meet the AOAC requirements.

Keywords: validation, HPLC, potentiometric titration, vitamin C

Corresponding Author: Engrid Juni Astuti; email: engridjuni81@umm.ac.id

Published 15 September 2022

Publishing services provided by Knowledge E

© Engrid Juni Astuti et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the ICMEDH Conference Committee.

1. INTRODUCTION

Vitamins and minerals are nutrients or substances that play an important role in the body and are determinants of health in the human body. The deficiency of vitamins and minerals can be a problem for human health, causing various diseases in the body [1].

One of the most important vitamins for the body is vitamin C. Vitamin C is a water-soluble vitamin, essential for human health, provides antioxidant protection for plasma lipids, and is required for immune functions including (leukocyte, phagocytosis, and chemotaxis), suppression of viral replication, and production interferon [2]. Vitamin C is proven to be able to maintain the body's resistance from various diseases (flu, heart disease, cancer and can increase the production of nitric oxide from the endothelium, increase vasodilation, lower blood pressure, prevent apoptosis of smooth muscle cells in blood vessels and help keep plaque more stable) [3]. Vitamin C also functions as an

OPEN ACCESS

antioxidant, giving electrons to free radicals and helps stabilize free radicals, thereby protecting cells from damage [4].

Vitamin C deficiency can be caused by low dietary intake and conditions in which the metabolism of ascorbic acid exceeds the rate of its endogenous biosynthesis, thereby increasing the turnover of the vitamin in the body. Such conditions include smoking, environmental/physical disorders, chronic disease, and diabetes. In children exposed to cigarette smoke, the concentration of ascorbic acid in plasma shows a decrease in ascorbic acid concentration [5]. In general, vitamin C has no side effects, but it allows side effects (unwanted symptoms) in certain doses. Side effects that may occur if consumed in high amounts can cause diarrhea [6]. For ten years, high doses of vitamin C can stimulate the formation of oxalate and increase the absorption of oxalate consumption which may lead to kidney stones [7].

Vitamin C is a compound that is a strong reducing agent which is very easy to reversibly oxidize to form L-ascorbic acid and L-dehydroascorbic acid, which has acted as vitamin C. L-dehydroascorbic acid is chemically very labile and can undergo further changes to become L-diketogulonic acid which no longer has the activity of vitamin C. Vitamin C is very soluble in water and is most easily oxidized rapidly in the presence of heat, light, alkali, enzymes, oxidizing agents, as well as by copper and iron catalysts [8].

Due to the nature of vitamin C, which is easily oxidized, an analytical method is needed that can measure vitamin C levels accurately and quickly. An analytical method before being used must be validated first to guarantee the validity of the data generated. Method validation is necessary for several reasons: method validation is an important element of quality control. Validation helps assure that measurements will be reliable. In some fields, method validation is a regulatory requirement [9]. Validation of analytical methods is assessing certain parameters based on laboratory experiments to prove that these parameters meet the requirements for their use. The purpose of validation of the analytical method is to prove that all methods or testing procedures are used consistently or continuously [10]. Meanwhile, according to EUROCHEM, validation is confirmation through examination and provision of objective evidence that certain requirements for certain intended uses are met [11].

Based on the Indonesian Pharmacopoeia 5th edition, the determination of vitamin C levels in pharmaceutical preparations uses iodometric titration or acid-base titration wherein the accuracy of iodometric and acid-base titrations is determined by the analyst in seeing the color change of the titration results so that the validity of the data is very less. According to Spínola, the iodometric titration method is less specific in determining the concentration of a compound. This titration method is only applied for

initial analysis in the laboratory, while using UHPLC-PDA is quite sensitive selective in the analysis process [12]. In this study, two vitamin C measurement methods will be validated using High-Performance Liquid Chromatography (HPLC) and potentiometric titration. HPLC was chosen because it is currently a popular method of pharmaceutical analysis. In addition, because of its ability to analyze quickly, it can be used for single or mixed samples in one procedure [13]. Still, this method has the disadvantage of expensive eluent and column prices. Potentiometric titration is instrumental in obtaining the reduction in the potential of redox-active centers in proteins. Also, it provides important information for understanding the role of these centers in electron and energy transfer processes [14].

Potentiometric titration has the advantage of analyzing vitamin C levels directly without the need for comparison so that the cost of analysis is cheaper than HPLC. The disadvantage of using this method is the relatively long analysis time and the possibility of a reaction between the filler and the titrant. Potentiometric titration data can be more reliable than ordinary titration data by using chemical indicators such as color change and precipitate formation. The basics of this system will be used to analyze the potentiometric titration system, in which this system does not use indicators but replaces another quantity, namely chemical potential [15]. In this study, the sample used was a vitamin drink circulating in the market in a solution.

2. RESEARCH METHODS

2.1. Materials and Equipment

The materials needed are Merck Vitamin C for analysis, samples (prepared vitamin drinks containing vitamin C), Merck KIO_3 kGaA 64271 for analysis 0.1 N, Merck KI kGaA 64271 for analysis 1%, Merck kGaA HCl 64271 for analysis 2%, aquades, aqua free of CO_2 . Aqua pro Injection, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ p.a Merck, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ p.a Merck, Phosphoric Acid p.a Merck, NaCl 0.5 N p.a Merck, Methanol pro-HPLC Merck.

The tools used are electrodes, pH meter (Schott Lab 850), Magnetic stirrer, Burette, Statip, and Burette clamps, Analytical balance (Mettler Toledo AL20), refrigerator, HPLC (Shimadzu LC-6A, UV detector, Luna 5u column). C18(2) 100A (4.6 X 250 mm), Wholesale-500mL suction flask, Filtration Buchner Funnel, Ultrasonic stirrer, Microliter spritzen, Syringe, Disposable syringe filter, Whatman eluent and sample filter 0.45 μm , Whatman filter paper, and glassware.

2.2. HPLC Method

To obtain optimum analysis results, the flow rate at HPLC instrument set to 1.00 mL/min. The composition of the mobile phase used in Phosphate Buffer pH 3: Methanol (80: 20) [8]. The wavelength used is 242nm. The linearity test made a working standard solution from the mother standard solution with a concentration of 10; 20; 30; 40; 50; 60; 75; 80; 90, and 100 ppm, filtered and injected into HPLC. The sample solution in a 1.0 ml pipette was put in a 50.0 ml volumetric flask, added with distilled water up to the marked line, and shaken until homogeneous, filtered with a membrane filter, and ready to be injected into the HPLC system. 80%, 100%, and 120% concentration were made for the accuracy test and then dissolved with aqua pro injection up to the marked line. After being shaken until homogeneous, filter the solution using a disposable syringe filter and inject it into the HPLC. For the precision test, vitamin C level of 100% was replicated 6 times, filtered, and injected into HPLC.

2.3. Potentiometric Titration Method

Weigh carefully the powder containing approximately 100 mg of ascorbic acid, put it in a 100 mL beaker. Add \pm 50 mL of carbon dioxide-free aquadest, give a magnetic rod stirrer, then add 0.5 mL of 1% KI and 2 mL of 2% HCl (1 N). Pair the reference electrode and the indicator electrode, making sure that the tip of the electrode is not touched by the stirring rod when the magnetic stirrer is running (the electrode membrane must be immersed in the solution). Turn on the potentiometer and magnetic stirrer, then titrate with 0.1 N KIO₃. The orientation titration is carried out 1 time, and the actual titration is 3 times. To test the accuracy of vitamin C titration, the comparison standard with concentration of 80%, 100%, and 120% dissolved in CO₂-free distilled water and titrated, each concentration was replicated 3 times. For precision test by titrating vitamin C comparator with 100% level 6 times.

3. RESULT AND DISCUSSION

3.1. HPLC Method

Based on the results of the optimization of the mobile phase above, the optimal mobile phase is obtained, namely methanol: phosphate buffer pH 3 (20:80) with a flow rate of 1 ml/minute and gives the best peak profile, which is symmetrical following the Gaussian profile in the direction of the flow of the mobile phase so that it is not fronting. or

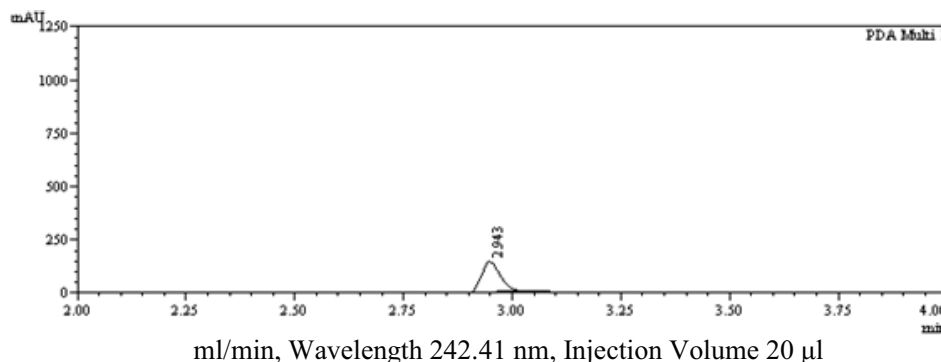


Figure 1: Methanol Mobile Phase: Phosphate Buffer pH 3 (20:80) with a Flow Rate of 1 ml/min, Wavelength 242.41 nm, Injection Volume 20 µl .

tailings [16]. The peak of vitamin C was read at a retention time (Rt) of 2.9 minutes and a wavelength of 242 nm. So that the analysis of vitamin C using the HPLC method only requires an analysis time of 4 minutes for each sample. The results of the mobile phase optimization chromatogram can be seen in Figure 1.

3.2. Linearity

The standard chromatogram data for comparing vitamin C obtained the area at each concentration which can be seen in table 1.

TABLE 1: Concentration and Standard Area of Action of Vitamin C.

Vitamin C Standard	Work	Concentration (ppm)	Area (uAU)
Work standard 1		10,7	410.425
Work standard 2		21,4	775.681
Work standard 3		42,8	1.644.154
Work standard 4		64,2	2.426.434
Work standard 5		85,6	3.273.738
Work standard 6		96,3	3.652.669

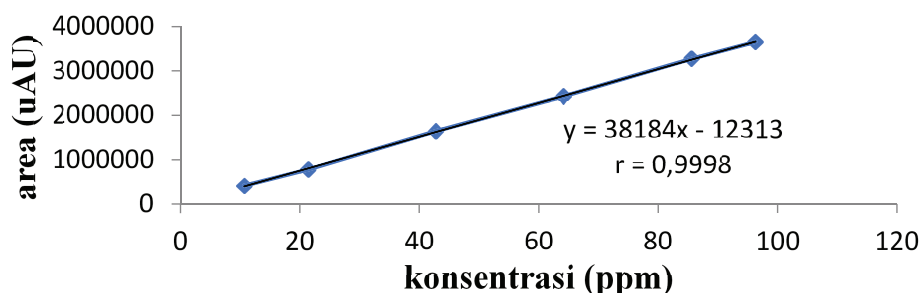


Figure 2: Vitamin C Area Regression Curve against the Comparative Standard Concentration of Vitamin C from table 1.

The regression equation between concentration (ppm) and standard area of action of vitamin C at various concentrations from table 1 and Figure 2 is as follows:

$$y = 38183,72933 x -12312,6859$$

$$r = 0,9998$$

The calculated r-value obtained is greater than the r table ($r_{table(5\%,6)} = 0.811$), then the calculated r-value has met the requirements and can be used to calculate the concentration (ppm) of the sample [17].

3.3. Accuracy

The accuracy is expressed as the ratio between the results obtained with the actual results. Vitamin C was made in concentrations of 80%, 100%, and 120%, each replicated 3 times. Filtered using filter paper and filter holder then injected.

TABLE 2: Accuracy Results.

Replication	Area	Concentration earned (mg/kg)	Real Concentration (mg/kg)	% recovery	Average recovery	%
80%-1	1.499.573	39,59	40,40	98,01	98,02	
80%-2	1.499.952	39,60	40,40	98,02		
80%-3	1.500.114	39,61	40,40	98,04		
100%-1	2.256.913	59,41	60,60	98,07	98,12	
100%-2	2.259.116	59,49	60,60	98,16		
100%-3	2.258.390	59,47	60,60	98,13		
120%-1	3.015.927	79,31	80,80	98,16	98,16	
120%-2	3.016.144	79,31	80,80	98,16		
120%-3	3.015.981	79,31	80,80	98,16		
Average					98,10 %	

The validation requirements of the method accuracy according to the AOAC are 92-105% [18]. Then from the validation results of the accuracy method, the average data recovery for each concentration in table 2 is 98.10%. Based on the results of the retrieval, the accuracy requirements are met.

3.4. Precision

The precision test shows the degree of correspondence between the results of individual tests carried out using vitamin C levels of 100% replicated 6 times. Filtered using filter paper and filter holder then injected.

TABLE 3: Precision Test Results.

Replication	Area
1	3.534.905
2	3.522.679
3	3.527.700
4	3.528.826
5	3.520.120
6	3.534.402
Total	21.168.632
average	3.528.105,3333
Standard Deviation	5.996,2951
% RSD	0,16995 %

Precision measures the repeatability of the analytical method documented by the standard deviation (SD) and RSD values. Precision validation requirements for RSD 2% [17]. Repeatability (precision) is said to be good according to AOAC International, 2002 at a concentration of 0.1% is 3%. Validation of the precision method from table 3 obtained a standard deviation value (SD) = 5,996.2951 and RSD = 0.17 %. Based on the assessment criteria, the precision requirements are met.

3.5. Result of Concentration Sample Using HPLC Method

Calculation of vitamin C concentration in the sample is obtained by entering the value of the peak area of the sample into the standard regression of the obtained vitamin C work, namely:

$$y = 38183,72933 + (-12312,6859)$$

From the above equation, the sample level is obtained in ppm (e.g. sample level: Xppm).

$$\text{Sample (mg)} = \frac{X \text{ mg}}{1000 \text{ ml}} \times \frac{1 \text{ ml}}{100 \text{ ml}} = y$$

$$\% \text{sample level} = \frac{y}{\text{sample weighing}} \times 100\%$$

The following is the data from the calculation of the levels obtained from the analysis using HPLC.

In the results of the study, it was found that the percentage of concentration in the bottle of vitamin C used as a sample with 3 different replication bottles in table 4 obtained concentration of 100.00%. Where if we refer to the requirements in the Indonesian Pharmacopoeia edition 5 for injection preparations in the form of liquid containing ascorbic acid or vitamin C not less than 90% and not more than 110% as

TABLE 4: Data Analysis of Calculation of Vitamin C concentration in Vitamin Drinks using HPLC.

Sample	Ppm	mg/sample weight	mg/kg sample	%	% average	% concentration in bottle
1	55,0753	$5,51 \times 10^{-4}$	0,5508	$5,51 \times 10^{-5}$	$5,48 \times 10^{-5}$	100,00
2	54,7355	$5,47 \times 10^{-4}$	0,5474	$5,47 \times 10^{-5}$		
3	54,6417	$5,46 \times 10^{-4}$	0,5464	$5,46 \times 10^{-5}$		

stated on the label, so the HPLC method, when used to measure vitamin C beverage preparations non-pharmaceutical preparations can still meet these requirements.

3.6. Potentiometric Titration Method

After optimizing the orientation titration, the results of the addition of titrant for the actual titration are 4.00 mL and require the amount of titrant that has been used up (0.1N potassium iodate solution) of approximately 6 mL so that the total analysis time for each sample is approximately 60 minutes per sample.

3.7. Accuracy

Accuracy is expressed as the ratio between the results obtained with the actual results, namely by titrating the standard vitamin C concentration of 80%, 100%, and 120% each with 3 replications. Then, the titration is carried out.

TABLE 5: Accuracy Test Parameter Results.

Weighed Vitamin C Weight	Replication	% recovery
80 mg	1	101,15%
	2	107,75%
	3	96,67%
100 mg	1	110,84%
	2	101,94%
	3	131,89%
120 mg	1	98,17%
	2	104,10%
	3	102,63%
Average		106,13%

For the accuracy validation test from table 5, the average percent value obtained is 106.13%. Because it follows the % recovery range set by AOAC International, 2002 for

a concentration of 0.1% is 90-108%, the accuracy of using the potentiometric titration method still meets the requirements.

3.8. Precision

Precision is a measure that shows the degree of correspondence between the results of individual tests carried out by titrating vitamin C with concentration of 100%, which is carried out six times.

TABLE 6: Precision Test Parameter Results.

Replication	% concentration
1	103,45%
2	107,30%
3	103,75%
4	100,26%
5	91,45%
6	107,30%
Average	102,2517
Standard Deviation (SD)	5,9211
RSD $RSD = \frac{SD}{average} \times 100\%$	5,79%

From the calculations, for the precision test parameters from table 6, the RSD value is 5.79%. Repeatability (precision) is said to be good according to AOAC International, 2002 at a concentration of 0.1% is 3%. This shows that the precision value obtained does not meet the requirements due to several factors. Among them is the accuracy of the tools or analysts who work on them.

3.9. Result of Concentration Sample Using Potensiometric Method

After optimizing the orientation titration, then the actual titration was carried out with three replications. Titration was carried out every 5 minutes (recorded volume and potential results). After obtaining the TAT (the endpoint of the titration) is calculated by taking the value from one level above and 2 levels below it. After the calculation is obtained, it is entered into the formula:

Equivalent Volume

$$x = TAT \text{ vol} + \frac{\text{early } \Delta \text{value}}{\text{early} + \text{end } \Delta \text{value}} \times \text{decrease in each titration}$$

Example:

$$x = 5,40 \text{ mL} + \frac{4020}{4878} \times 0,20 \text{ mL} = 5,5649$$

Then look for Mgrek titrant = Mgrek Sample

$$5,5649 \times 0,1009 \text{ N} \times 0,5 \times 176,13 = 49,4484 \text{ mg}$$

Looking for vitamin C level and vitamin C levels

Vitamin C level

$$49,4484 \text{ mg} / 25 \text{ ml} = x / 500 \text{ ml}$$

$$X = 988,97 \text{ mg}$$

Vitamin C level

$$988,97 \text{ mg} / 1000 \text{ mg} \times 100 \% = 98,89 \% \text{ (b/b)}$$

TABLE 7: Data Analysis of Calculation of Vitamin C Levels in Vitamin Drinks.

Sample	% concentration	% the average concentration in the bottle
1	101,38%	101,89%
2	102,30%	
3	101,98%	

In the study results, it was found that the % concentration in the bottle of vitamin C, which were used as samples with 3 different replications in Table 7, obtained levels of 101.89%. Where if we refer to the requirements in the Indonesian Pharmacopoeia edition 5 for injection preparations in the form of liquid containing ascorbic acid or vitamin C not less than 90% and not more than 110% stated on the label, so the potentiometric titration method is used to measure vitamin C beverage preparations. non-pharmaceutical preparations can still meet these requirements.

In this study, we compared the validation of two vitamin C measurement methods using High-Performance Liquid Chromatography (HPLC) using a DAD detector and using potentiometric titration. The results of the HPLC method show linear results between grade and area, accuracy, and precision, and meet the validation requirements of the AOAC and require an analysis time of 4 minutes per sample so that it does not take a long time for sample analysis. The potentiometric titration method requires an analysis time of approximately 5 minutes per sample, and the validation results show that the accuracy meets the AOAC requirements while the precision does not meet the AOAC requirements.

4. CONCLUSION

From the comparison results of the vitamin C analysis method validation using the HPLC method and potentiometry in vitamin drinks, it was found that the analysis using HPLC was better than using potentiometric titration, especially in precision data.

5. Acknowledgement

DPPM University of Muhammadiyah Malang for research funds that have been given by Number: E.2.a/1411.a/BAA-UMM/XII/2015.

References

- [1] A. Labellapansa, "Defisiensi Vitamin dan Mineral.," *Informatika*. vol. 10, p. 2016.
- [2] S.H. Mitmesser, Q. Ye, M. Evans, and M. Combs, "Determination of plasma and leukocyte vitamin C concentrations in a randomized, double-blind, placebo-controlled trial with Ester-C®.," *SpringerPlus*. vol. 5, no. 1, pp. 1–11, 2016.
- [3] M.A. Moser and O.K. Chun, "Vitamin C and heart health: a review based on findings from epidemiologic studies.," *International journal of molecular sciences*. vol. 17, no. 8, p. 1328, 2016.
- [4] Williams and Wilkins, *Ilmu Gizi Menjadi Sangat Mudah Edisi Kedua.*, 2011.
- [5] A.M. Preston, C. Rodriguez, C.E. Rivera, and H. Sahai, "Influence of environmental tobacco smoke on vitamin C status in children.," *The American journal of clinical nutrition*. vol. 77, no. 1, pp. 167–172, 2003.
- [6] J.D. Cook and M.B. Reddy, "Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet.," *The American journal of clinical nutrition*. vol. 73, no. 1, pp. 93–98, 2001.
- [7] L.K. Massey, M. Liebman, and S.A. Kynast-Gales, "Ascorbate increases human oxaluria and kidney stone risk.," *The Journal of nutrition*. vol. 135, no. 7, pp. 1673–1677, 2005.
- [8] S. Ullah, A. Hussain, J. Ali, and A. Ullah, "A simple and rapid HPLC method for analysis of vitamin-C in local packed juices of Pakistan.," *Middle East Journal of Scientific Research*. vol. 12, no. 8, pp. 1085–1091, 2012.
- [9] Ph. D Riyanto, *Validasi & Verifikasi Metode Uji: Sesuai dengan ISO/IEC 17025 Laboratorium Pengujian dan Kalibrasi.*, 2014.
- [10] Harmita, *Petunjuk pelaksanaan validasi metode dan Cara Perhitungannya.*, 2004.

- [11] S. Watanabe, “[Drug information for safe use].,” *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan*. vol. 134, no. 3, pp. 351–353, 2014.
- [12] V. Spínola, B. Mendes, J.S. Câmara, and P.C. Castilho, “Effect of time and temperature on vitamin C stability in horticultural extracts. UHPLC-PDA vs iodometric titration as analytical methods.,” *LWT-Food Science and Technology*. vol. 50, no. 2, pp. 489–495, 2013.
- [13] A. Requirements, “Alpha Lipoic Acid Tablets.,” no. c, pp. 2011–2012, 2012.
- [14] J.J. Wright, E. Salvadori, H.R. Bridges, J. Hirst, and M.M. Roessler, “Small-volume potentiometric titrations: EPR investigations of Fe-S cluster N2 in mitochondrial complex I.,” *Journal of inorganic biochemistry*. vol. 162, pp. 201–206, 2016.
- [15] Suyanta, *Potensiometri.*, 2013.
- [16] I.G. Gandjar and A. Rohman, “Kimia farmasi analisis.,” Yogyakarta: Pustaka Pelajar. vol. 224, p. 228, 2007.
- [17] H. Harmita, “Petunjuk pelaksanaan validasi metode dan Cara Perhitungannya.,” *Majalah Ilmu Kefarmasian*. vol. 1, no. 3, p. 1, 2004.
- [18] W. Horwitz, “AOAC guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals.,” Gaithersburg, MD, USA: AOAC International. pp. 12–19, 2002.