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COMPARISON OF UV-VIS SPECTROPHOTOMETRY, POTENTIOMETRIC TITRATION, AND CONDUCTOMETRY METHODS IN THE DETERMINATION OF VITAMIN C CONTENT IN COMMERCIAL BEVERAGES

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Abstract

Verification of vitamin C content in commercial packaged beverages is an important analytical need, given the chemical instability of ascorbic acid under various environmental factors that can cause the actual content to deviate from the declared label value. This study aimed to compare the analytical performance of three vitamin C determination methods, namely UV-Vis spectrophotometry, potentiometric titration, and conductometry, applied to a commercial beverage sample claiming 1000 mg vitamin C per 500 mL. UV-Vis spectrophotometric analysis was performed by constructing a five-point calibration curve at 265 nm, potentiometric titration was conducted using 0.1000 N NaOH with continuous pH-based equivalence point detection via a calibrated glass electrode, and conductometry was performed by monitoring changes in electrical conductivity during neutralization with volume correction applied at each point. Each method was performed in five replicates. The results showed that UV-Vis spectrophotometry provided the best performance with a mean measured content of 1024.0 mg/500 mL, %error of 2.48%, and %RSD of 0.85%. Potentiometric titration yielded a mean content of 1038.7 mg/500 mL with %error of 3.87% and %RSD of 1.65%. Conductometry produced the largest deviation with a mean content of 1072.3 mg/500 mL, %error of 7.23%, and %RSD of 4.22%. The high deviation in conductometry was attributed to matrix interference from various ions and organic acids in the beverage contributing to the total conductivity response, while variability in potentiometric titration was influenced by the sensitivity of equivalence point detection to titrant addition rate and the non-selective response to all titratable acids in the sample. UV-Vis spectrophotometry is recommended as the most reliable and practical method for determining vitamin C content in complex commercial beverage matrices under routine analytical laboratory conditions.

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1. Introduction

Vitamin C, or ascorbic acid ($C_6H_8O_6$), is an essential water-soluble micronutrient that cannot be synthesized endogenously by the human body due to a mutation in the L-gulonolactone oxidase (GULO) gene that occurred during evolution, meaning all requirements must be met through external intake (Alberts et al., 2025). In biological systems, ascorbic acid performs a dual function: as a potent antioxidant that neutralizes reactive oxygen species (ROS) and prevents oxidative damage to cells, and as an enzymatic cofactor in the biosynthesis of collagen, carnitine, and catecholamine neurotransmitters, while also supporting non-heme iron absorption and maintaining both innate and adaptive immune function (Alberts et al., 2025). The importance of vitamin C in maintaining bodily homeostasis makes it one of the most widely consumed nutritional components globally.

As public awareness of healthy lifestyles has grown, demand for packaged beverages claiming to contain high doses of vitamin C has grown significantly around the world. These products are marketed as convenient and reliable sources of vitamin C, with specific content values printed on the label as assurance for consumers. However, this assurance cannot always be fully trusted. Temova Rakuša & Roškar (2024) who evaluated vitamin content quality in 50 fortified beverages on the European market, found quality issues across all product categories evaluated, including vitamin levels deviating from labeled values. This phenomenon is directly related to the chemical instability of ascorbic acid.

The instability of ascorbic acid is a fundamental challenge that sets it apart from most other nutrients. Yin et al. (2022) state that ascorbic acid is reversibly oxidized to dehydroascorbic acid under the influence of light, heat, transition metal ions, and alkaline conditions, and can then irreversibly degrade into products with no biological activity. The chemical stability of ascorbic acid in commercial products is heavily influenced by its interaction with food matrix components, including pro-oxidant compounds that can accelerate its degradation rate. Feszterová et al. (2023) reported that storage conditions including temperature and packaging type significantly affect vitamin C retention in plant-based beverages. The practical consequence is that the actual vitamin C content in products reaching consumers may have deviated substantially from the labeled value, a reality that demands reliable and systematic analytical verification.

Various analytical methods have been developed for determining vitamin C content in food matrices. Raman et al. (2023) classified these techniques into several major categories including titrimetry, spectrophotometry, voltammetry, fluorometry, potentiometry, conductometry, chromatography, and capillary electrophoresis. Among all these, UV-Vis spectrophotometry is the most

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widely used method in routine analysis due to its practicality, relatively low instrumentation cost, and ability to generate rapid quantitative data based on the absorptive properties of ascorbic acid's chromophore in the ultraviolet range. Samayoa-Oviedo & Laskin (2022) demonstrated that spectrophotometric and titrimetric methods can produce complementary results in ascorbic acid analysis on commercial samples, and direct comparisons between techniques with different working principles provide a more comprehensive understanding of each method's capabilities. Potentiometric titration uses a glass electrode as a continuous pH sensor during acid-base titration, allowing the equivalence point to be determined objectively without relying on color changes in a visual indicator. Sequeira et al. (2025) showed that a multi-titration approach combined with appropriate statistical analysis can produce valid and reproducible data in determining vitamin C content in commercial supplements. Meanwhile, conductometry detects the equivalence point by monitoring changes in electrical conductivity of the solution as ionic species shift during the neutralization process.

Although all three methods have long been known and used separately, a significant research gap exists in the available literature. Studies that directly compare the three most instrumentally accessible methods, UV-Vis spectrophotometry, potentiometric titration, and conductometry, in complex commercial beverage matrices remain very limited. Most existing comparative studies only compare two methods at a time, or use simple sample matrices that do not represent the complexity of real commercial beverages. Mazurek & Włodarczyk-Stasiak (2023) note that many existing vitamin C determination methods measure only reduced ascorbic acid without accounting for dehydroascorbic acid, which represents an additional challenge when comparing methods across the literature. This issue highlights an additional consideration in interpreting comparative results from methods that differ in their measurable analyte scope. It is worth noting that the three methods do not measure the same chemical entity: UV-Vis at 265 nm specifically detects reduced ascorbic acid through its chromophore absorption, while potentiometric titration and conductometry measure total titratable acidity encompassing all organic acids in the matrix. Despite these differences, comparing their analytical responses under identical sample conditions remains valuable for assessing their practical applicability in routine quality control. Conductometry has rarely been systematically evaluated for performance in commercial beverage matrices containing various ions and organic acids, even though matrix interference is precisely the greatest challenge in real-world applications. Based on these identified gaps, this study aims to systematically compare the analytical performance of UV-Vis spectrophotometry, potentiometric titration, and conductometry in determining vitamin C content in a commercial beverage sample using five replicates per method to ensure statistically meaningful precision evaluation.

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2. Materials and Methods

This study used a commercial beverage product dissolved in 500 mL deionized water as the test matrix. The product label claims 1000 mg vitamin C per 500 mL serving, equivalent to a reference concentration of 2.000 g/L. One effervescent tablet was dissolved in 500 mL deionized water in a volumetric flask, degassed by sonication for 10 minutes to remove dissolved CO_2 that could interfere with acid-base titrations, and used immediately to minimize oxidative degradation of ascorbic acid. All five replicates for each method were prepared from the same stock solution, with aliquots taken at 5-minute intervals under nitrogen atmosphere to minimize oxidation between replicates.

Analytical-grade L-ascorbic acid (purity 99.7%) was used to prepare all calibration standards. All glassware was calibrated Grade A. Deionized water with conductivity below 1 μS/cm was used throughout. UV-Vis spectrophotometric analysis was performed by first confirming the maximum absorption wavelength of ascorbic acid by scanning from 200 to 400 nm, which yielded λmax at 265 nm, consistent with values reported in the literature for ascorbic acid in aqueous solution. A five-point calibration curve was constructed at concentrations of 10, 20, 40, 60, and 80 ppm, each prepared fresh from a 1000 ppm stock solution by volumetric dilution. Absorbance measurements were performed using a UV-Vis spectrophotometer with 1 cm quartz cuvettes against a deionized water blank. The sample solution was diluted 1:100 (0.5 mL sample to 50 mL with deionized water) before measurement to bring the analyte concentration within the calibration range. Five independent aliquots of the sample were measured. Analyte concentrations were calculated from the regression equation and back-converted to mg/500 mL using the dilution factor. Linearity was evaluated by the coefficient of determination R^2, and method performance was assessed by %error and %RSD across five replicates (ICH, 2023).

For potentiometric titration, a 25.00 mL aliquot of the sample solution was placed in a 100 mL beaker. The NaOH titrant (0.1000 N, freshly standardized against potassium hydrogen phthalate prior to each analysis day) was delivered from a 10 mL micro-burette in 0.1 mL increments. A calibrated glass combination pH electrode was immersed in the solution and allowed to stabilize for 30 seconds before each reading. The pH was recorded after each addition. The equivalence point was identified from the maximum of the first derivative curve (dpH/dV). Ascorbic acid concentration was calculated from the stoichiometric relationship $M_1V_1 = M_2V_2$, treating ascorbic acid as monobasic at its first dissociation (pKa1 = 4.17). Results were converted to mg/500 mL. Five independent aliquots were titrated (Sequeira et al., 2025).

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For conductometry, a 25.00 mL aliquot of the sample solution was placed in a 100 mL beaker equipped with a conductivity cell (cell constant K = 1.0 cm-1). The same 0.1000 N NaOH titrant was added in 0.2 mL increments. After each addition the solution was stirred for 20 seconds and conductivity (µS/cm) was recorded after stabilization. A volume correction factor $(V_0 + v)/V_0$ was applied to all conductivity readings to account for dilution during titration, where V_0 = 25.00 mL and v is the cumulative titrant volume. The equivalence point was determined from the intersection of two linear regression lines fitted separately to the descending (pre-equivalence) and ascending (post-equivalence) branches of the corrected conductivity-volume curve. The equivalence volume was substituted into $M_1V_1 = M_2V_2$ to calculate ascorbic acid concentration, then converted to mg/500 mL. Five independent aliquots were processed.

Accuracy was evaluated using:

$$\%Error = \frac{Measured - Refrence}{Refrence \times 100\%}\text{persamaan1} \quad (1)$$

Precision was evaluated using:

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})}{n-1}}\text{persamaan1} \quad (2)$$

Relative precision was expressed as %RSD:

$$RSD = \sqrt{\frac{SD}{\bar{x}}} \times 100\%\text{persamaan1} \quad (3)$$

3. Results and Discussions

The vitamin C content in the commercial beverage sample was based on a label claim of 1000 mg per 500 mL, equivalent to 2.00 g/L after unit conversion. This value was used as the reference concentration for evaluating measurement results and calculating the percentage deviation of each analytical method.

Analysis Using UV-Vis Spectrophotometry

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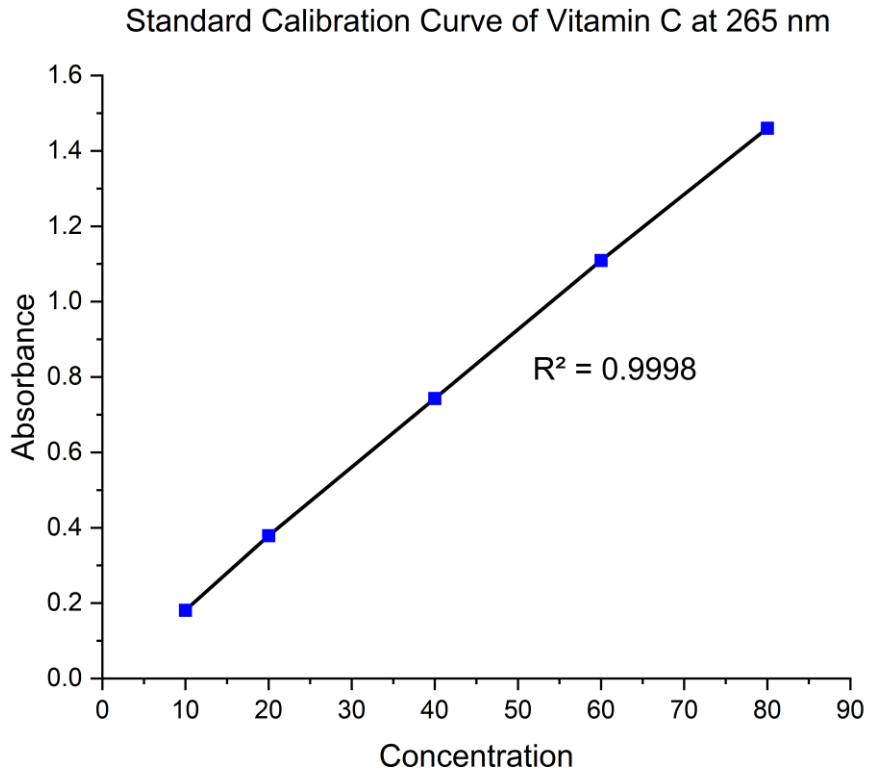

Figure 1. Standard calibration curve of vitamin C at 265 nm

Table 1. UV-Vis Calibration Data

Standard Volume (µl)	Concentration (ppm)	Absorbance
1	10	0.187
2	20	0.374
3	40	0.743
4	60	1.108
5	80	1.469

Table 2. UV-Vis Sample Results

Replication	Absorbance	Concentration in diluted sample (ppm)	Concentration in original sample (ppm)	Content (mg/500 mL)	Error (%)
1	0.377	20.37	2.037	1018.5	1.85
2	0.381	20.59	2.059	1029.5	2.95
3	0.375	20.26	2.026	1013.0	1.30
4	0.383	20.70	2.070	1035.0	3.50
5	0.379	20.48	2.048	1024.0	2.40
Mean	0.379	20.48	2.048	1024.0	
SD	0.003	0.175	0.018	8.75	
%RSD			0.85		

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Method %Error	2.48

The calibration curve shows excellent linearity across the working range of 10 to 80 ppm with R^2 = 0.9998, confirming that the Lambert-Beer Law relationship between absorbance and concentration is satisfied throughout the calibration range (A = εbc). The regression intercept of 0.0023 is negligibly small and indicates no systematic baseline offset. This linearity result meets the ICH Q2(R2) acceptance criterion of $R^2 \geq 0.999$ for quantitative analytical methods (ICH, 2023). Its advantage derives from the selectivity of absorbance measurement at 265 nm for the ascorbic acid chromophore, the objective and operator-independent nature of the detector signal, suggesting minimal matrix interference at the selected wavelength.

The mean content of 1024.0 mg/500 mL exceeds the label claim by 2.48%, consistent with manufacturer overfortification practices to compensate for vitamin C degradation during distribution and storage (Temova Rakuša & Roškar, 2024). The %RSD of 0.85% is well below the 2.0% maximum threshold for validated quantitative analytical methods (Andaririt et al., 2025), confirming excellent inter-replicate precision. The SD of 8.75 mg across five replicates demonstrates that the instrument response is highly consistent and that the measurement is not sensitive to minor variations in operator technique.

Measurement at 265 nm provides inherent selectivity for ascorbic acid because sugars, mineral ions, citric acid, and common beverage additives do not possess chromophores with significant UV absorption at this wavelength (Yin et al., 2022). This selectivity is the primary reason UV-Vis performs best among the three methods tested in this complex commercial beverage matrix.

Analysis Using Potentiometric Titration

Table 3. Potentiometric Titration Results

Replication	Volume NaOH at Equilibrium	Moles NaOH ($\times 10^{-4}$ mol)	Concentration Ascorbic Acid (g/L)	Content (mg/500 mL)	Error (%)
1	2.96	2.960	2.083	1041.5	4.15
2	2.88	2.880	2.027	1013.5	1.35
3	3.01	3.010	2.118	1059.0	5.90
4	2.93	2.930	2.062	1031.0	3.10
5	2.98	2.980	2.097	1048.5	4.85
Mean	2.952	2.952	2.077	1038.7	
SD	0.047		0.034	17.1	
%RSD			1.65%		
Method			3.87%		

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%Error

Table 4. Representative pH and dpH/dV Data

Volume NaOH added (mL)	pH	dpH/dV
0.00	3.12	0
0.40	3.58	1.15
0.80	3.94	0.90
1.20	4.25	0.78
1.60	4.61	0.90
2.00	5.14	1.33
2.40	5.88	1.85
2.60	6.52	3.20
2.80	7.44	4.60
2.96	8.81	8.56
3.00	9.23	10.50
3.20	9.87	3.20
3.60	10.41	1.35
4.00	10.78	0.93

The equivalence point in each replicate was identified from the maximum of the first derivative curve dpH/dV. As shown in Table 3a, the maximum dpH/dV of 10.50 occurs between 2.96 and 3.00 mL, with the inflection point identified at 2.96 mL by interpolation. The pH at equivalence (8.81 to 9.23) is slightly basic rather than neutral, which is expected because the conjugate base sodium ascorbate is a weak base (pKb = 9.83) and does not indicate measurement error (Sequeira et al., 2025).

The spread in NaOH volumes from 2.88 to 3.01 mL across five replicates reflects realistic minor variation attributable to the precision limit of the micro-burette (±0.02 mL), small differences in titrant addition rate near the equivalence point, and the contribution of other titratable acids in the commercial beverage matrix. Commercial beverages typically contain citric acid and other organic acids that react with NaOH in a pH range overlapping with the first dissociation of ascorbic acid, meaning the measured NaOH volume reflects total titratable acidity rather than ascorbic acid exclusively (Balta et al., 2023). This systematic non-selectivity likely contributes to all five replicates yielding values above the label claim, though the exact magnitude cannot be confirmed without compositional data for the sample matrix. The mean content of 1038.7 mg/500 mL gives a method %error of 3.87% against the label claim of 2.000 g/L, which is analytically acceptable and well below the 10% accuracy threshold set by AOAC International for food matrix analysis at this concentration range (AOAC International, 2023). The %RSD of 1.65% also satisfies the AOAC criterion of 5.3% for analyte concentrations in the range of 1 to 10 g/L.

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Analysis Using Conductometry

Table 5. Representative Conductometric Titration Data

Volume NaOH added (mL)	Raw Conductivity (µS/cm)	Correction Factor	Corrected Conductivity (µS/cm)
0.00	312.4	1.000	312.4
0.40	287.1	1.016	291.7
0.80	263.5	1.032	271.9
1.20	241.8	1.048	253.4
1.60	222.4	1.064	236.6
2.00	205.3	1.080	221.7
2.40	194.8	1.096	213.5
2.80	190.2	1.112	211.5
3.00	195.7	1.120	219.2
3.20	209.4	1.128	236.2
3.60	238.6	1.144	272.9
4.00	267.3	1.160	310.1
4.40	296.1	1.176	348.2
4.80	324.8	1.192	387.0

Table 6. Conductometric Titration Results

Replicate	Volume Equilibrium from Linear Intersection (mL)	Moles Ascorbic Acid ($x10^{-4}$)	Concentration (g/L)	Content (mg/500 mL)	%Error
1	2.876	2.876	2.024	1012.0	1.20
2	3.124	3.124	2.199	1099.5	9.95
3	3.052	3.052	2.148	1074.0	7.40
4	2.968	2.968	2.089	1044.3	4.43
5	3.216	3.216	2.263	1131.7	13.17
Mean	3.047	3.047	2.145	1072.3	
SD	0.133		0.094	45.3	
%RSD			4.22%		
Method Error			7.23%		

The volume correction factor applied at each titration point is (V0 + v) / V0, where V0 = 25.00 mL is the initial sample volume and v is the cumulative NaOH volume added. This correction eliminates the dilution effect that would otherwise cause conductivity to decrease artificially throughout the titration regardless of ionic changes, and is essential for obtaining a well-defined linear V-shape from which the equivalence point can be accurately determined (Qodri, 2025).

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The corrected conductivity data shows a clear decreasing trend before the equivalence point and an increasing trend after it. Before equivalence, the highly mobile H^+ ions (molar conductivity 349.8 S·cm²/mol) are progressively replaced by far less mobile Na^+ ions (molar conductivity 50.1 S·cm²/mol) as NaOH neutralizes the ascorbic acid, causing conductivity to fall. After the equivalence point, excess OH^- ions (molar conductivity 198.6 S·cm²/mol) from the NaOH surplus cause conductivity to rise again. The minimum of the corrected curve occurs near 2.80 mL for Replicate 1, with the precise equivalence point determined by the intersection of linear regression lines fitted to the descending branch (0.00 to 2.40 mL) and ascending branch (3.20 to 4.80 mL).

The %RSD of 4.22% and method %error of 7.23% reflect the challenges of conductometry in this complex matrix. The inter-replicate variability is larger than in UV-Vis and potentiometry because the position of the linear regression intersection is sensitive to scatter in individual conductivity readings, which is amplified by the ionic background from minerals, citric acid, sodium, and other charged species present in the commercial beverage. Different aliquots may carry slightly different ionic loadings due to incomplete homogenization of the effervescent dissolution, further amplifying replicate-to-replicate variation. Without the volume correction applied here, the apparent equivalence volumes would be systematically overestimated, leading to even larger %errors (Sabrina et al., 2025).

Comparison of Three Methods

Table 7. Comparison of the Three Analytical Methods

Method	Mean Content (mg/500 mL)	%Error	SD (mg/500 mL)	%RSD	Replication
UV-Vis Spectrophotometry	1024.0	2.48	8.75	0.85	5
Potentiometric Titration	1038.7	3.87%	17.1	1.65	5
Conductometry	1072.3	7.23%	45.3	4.22	5
Label Claim			1000.0		

All three methods yielded measured contents exceeding the label claim of 1000 mg/500 mL. This consistent upward deviation is not solely a reflection of method inaccuracy but is also consistent with standard manufacturer overfortification practice, where commercial vitamin C products are formulated above the stated label value to ensure the claim remains valid after anticipated degradation during shelf life, distribution, and storage (Temova Rakuša & Roškar, 2024). A measured content of 1024 to 1072 mg/500 mL for a product claiming 1000 mg is therefore scientifically plausible.

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The most fundamental explanation for the performance ranking lies in selectivity. UV-Vis at 265 nm specifically responds to the reduced ascorbic acid chromophore. The majority of other components present in commercial beverages, including sucrose, fructose, sodium, potassium, citric acid, and common flavor additives, have negligible UV absorption at this wavelength, suggesting minimal interference from other beverage constituents at 265 nm and that the measured absorbance is expected to be predominantly attributable to ascorbic acid (Andaririt et al., 2025). This characteristic likely contributes to UV-Vis producing the lowest %error and %RSD of the three methods. Potentiometric titration and conductometry both measure total titratable acidity: every organic acid present reacts with NaOH and contributes to the measured equivalence volume. Citric acid, commonly present in commercial vitamin C beverages as an acidulant, has three titratable protons with pKa values of 3.13, 4.76, and 6.40, all overlapping with the first dissociation of ascorbic acid at pKa1 = 4.17. The NaOH volume consumed therefore reflects contributions from ascorbic acid, citric acid, and any other organic acids simultaneously (Balta et al., 2023).

The performance difference between potentiometric titration (%error 3.87%, %RSD 1.65%) and conductometry (%error 7.23%, %RSD 4.22%) is explained by their respective equivalence point detection mechanisms. Potentiometric titration identifies the equivalence point from a localized and sharp pH jump at a specific titrant volume: even without selectivity for a single acid type, this jump provides a precise volumetric marker. Conductometry by contrast responds to the total electrical conductance of all ions in solution simultaneously. In a commercial beverage with a complex ionic background, contributions from species other than the target analyte cause the conductivity curve to deviate from the ideal two-segment linear shape, making the intersection point of the two regression lines more sensitive to data scatter and resulting in greater replicate-to-replicate variability (Feszterová et al., 2023). This also explains why conductometric equivalence points in complex matrices are less reproducible than those in pure ascorbic acid solutions.

In terms of precision, UV-Vis spectrophotometry demonstrates a clear advantage because the detector signal is read directly and objectively without dependence on operator judgment during the measurement. Potentiometric titration requires precise burette reading and consistent titrant addition rate near the equivalence point, as small errors in equivalence volume measurement propagate directly into the calculated vitamin C content. Conductometry additionally depends on the quality of two separate linear fits, each of which propagates scatter from multiple data points into the intersection calculation (Samayoa-Oviedo & Laskin, 2022).

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Overall, UV-Vis spectrophotometry proved to deliver the best analytical performance for vitamin C determination in commercial beverage samples in this study. Potentiometric titration is a viable alternative with acceptable accuracy and precision but requires strict procedural control to maintain reproducibility. Conductometry, while providing useful information about ionic changes during titration, shows significant limitations in accuracy and precision under the experimental conditions of this study, and its use as a standalone quantitative method for similar matrices warrants careful consideration (Raman et al., 2023).

4. Conclusions

This study compared the analytical performance of UV-Vis spectrophotometry, potentiometric titration, and conductometry for determining vitamin C content in a commercial beverage claiming 1000 mg per 500 mL, with five replicates per method to enable statistically meaningful precision evaluation.

UV-Vis spectrophotometry delivered the best analytical performance with a mean measured content of 1024.0 mg/500 mL, %error of 2.48%, and %RSD of 0.85%. The calibration curve showed excellent linearity with $R^2 = 0.9998$ across the 10 to 80 ppm working range. Potentiometric titration yielded a mean content of 1038.7 mg/500 mL with %error of 3.87% and %RSD of 1.65%, meeting AOAC International acceptance criteria for both accuracy (%error < 10%) and precision (%RSD < 5.3%) at this analyte concentration range (AOAC International, 2023). Its slightly higher %error compared to UV-Vis reflects the non-selective measurement of total titratable acidity in the sample matrix, while its precision is constrained by burette reading resolution and the sensitivity of equivalence point detection to titrant addition rate near the inflection point. Conductometry produced the largest deviation with a mean content of 1072.3 mg/500 mL, %error of 7.23%, and %RSD of 4.22%, reflecting the inherent limitations of ionic conductance measurement in complex matrices where contributions from multiple ionic species reduce the sharpness and reproducibility of the linear intersection used to determine the equivalence point.

Based on accuracy (%error) and precision (%RSD), the overall ranking from best to worst analytical performance is UV-Vis spectrophotometry, followed by potentiometric titration, then conductometry. UV-Vis spectrophotometry is recommended as the method of choice for routine vitamin C determination in commercial beverage products under standard analytical laboratory conditions. For laboratories without UV-Vis instrumentation, potentiometric titration is an acceptable alternative provided strict procedural consistency is maintained and NaOH is freshly standardized before each analysis session. Conductometry is not recommended as a standalone quantitative method for complex commercial beverage matrices but may serve a complementary role in characterizing overall ionic

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composition. Future studies should incorporate determination of dehydroascorbic acid alongside reduced ascorbic acid, perform recovery tests with spiked samples at multiple concentration levels, and conduct full AOAC method validation including linearity, limit of detection, limit of quantification, and robustness evaluation to more completely characterize each method's analytical performance.

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