Simultaneous Determination of Gallic Acid and Ascorbic Acid Using First Derivative Zero-Crossing Spectrophotometric Technique

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A B S T R A C T:
A new spectrophotometric method was used for simultaneous determination of gallic acid and ascorbic acid depending on the first derivative zero-crossing technique. The first derivative spectra of these compounds permitted simultaneous determination of gallic acid and ascorbic acid over the concentration ranges of (1.0 – 30.0) μg/mL and (3.0 – 35.0) μg/mL with detection limits of 0.559 and 0.991 for gallic acid and ascorbic acid, respectively, by techniques of measuring the amplitude of peak-to-base line and zero-crossing at certain wavelengths. The proposed method showed reasonable precision and accuracy and have been applied to determine gallic acid and ascorbic acid in samples of Hibiscus sabdariffa L.

KEY WORDS: Hibiscus sabdariffa L, Gallic acid, Ascorbic acid, Derivative spectrophotometry.
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INTRODUCTION:
Roselle, Hibiscus sabdariffa L., is one of the most famous and folk medicinal plants. Many chemical compounds have been found in Rosella, such as minerals, vitamins and some bioactive components like phytosterols, organic acids, and some polyphenols which they have antioxidant properties. Due to that has a potential benefits on health including cardiovascular health promotion, preventing hypertension, diuretic, treatment of liver disorder, growth inhibition of microorganisms, digestive, sedative, and antioxidant activity (Sáyago-Ayerdí et al., 2007, Shruthi et al., 2016).

Calycs of Roselle are commonly used as a natural colorant to food and also to make herbal tea. The seeds of Roselle are a good natural source of proteins, oil and fibers (Mokhtari et al., 2018). Roselle has also received great attention in the field of industries and used in pharmaceutical and cosmetic industries, as well as a natural food colorant (Shruthi et al., 2016).

Gallic acid (GA) (3,4,5-trihydroxy benzoic acid), (Figure 1a), is a natural polyphenolic antioxidant compound that has been extracted from plant (Tashkhourian et al., 2013). It has some pharmacological activities that have been mentioned in the literature such as, anticancer, anti-HIV, anti-inflammatory, antiulcerogenic, antimicrobial, and antifungal (Fernandes and Salgado, 2016). In addition to medicinal aspects,
GA has many applications can be applied in other areas, such as it is use as a standard for estimation the total phenolic content in various samples by the Folin-Ciocalteau assay (Abbasi et al., 2011), as a chelating agent in the skin and leather industry (Fernandes and Salgado, 2016).

![Chemical structure of (a) gallic acid (b) ascorbic acid.](image)

Ascorbic acid (AA) (vitamin C), (Figure 1b), is a water-soluble, unstable antioxidant, easily oxidized acid and can be destroyed by oxygen, alkali and high temperature (Iqbal et al., 2004). It is mainly found in vegetables and fruits and is an essential nutrient needed for maintaining the human health (Sapei and Hwa, 2014). It has been used as a stabilizer for vitamin B complex in pharmaceutical industry and it is commonly added as an anti-oxidant to foods, juices and beverages. Due to its health benefits, it is used for the treatment of some diseases such as scurvy, anemia, haemorrhagic disorders etc. It is considered essential for the development and regeneration of muscles, bones, teeth and skin (Revanasiddappa and Veena, 2008, Vishnikin et al., 2010).

Derivative spectrophotometry (DS) is one of the advanced modern spectrophotometric techniques, which is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts and is now a reasonably prized standard feature of modern micro-computerized UV spectrophotometry. The derivatization of zero-order spectrum can lead to separate of overlapped signals, elimination of background caused by presence of other compounds in a sample. These properties can allow quantification of one or few analytes without initial separation or purification. This technique becomes very useful and additional tool which helps to resolve various analytical problems (Ojeda and Rojas, 2004, Patel et al., 2010, Vivek et al., 2010). DS techniques have been applied for simultaneous analysis in different fields, such as; environmental analysis, clinical and biological samples, pharmaceutical analysis, food analysis, inorganic analysis, organic compound analysis, cosmetic analysis, and recently botanical extract analysis. The detail scientific literature survey was performed in three different papers by (Ojeda and Rojas, 2004, Rojas and Ojeda, 2009, Ojeda and Rojas, 2013).

To our knowledge, there are no derivative spectrophotometric methods concerning the simultaneous determination of GA and AA in *Hibiscus sabdariffa* L. samples. The aim of this study is to develop a simple, rapid and efficient first derivative zero-crossing spectrophotometric method for the simultaneous determination of GA and AA in *Hibiscus sabdariffa* L. samples.

2. MATERIALS AND METHODS

2.1. Apparatus

A Perkin Elmer Precisely Lambda 25 double-beam scanning UV/Vis spectrophotometer equipped with 10-mm path length quartz cell and computer with Pentium IV and 512 MB RAM was used for recording zero order spectra and collecting the data of the absorption spectra for each one of GA, AA and their mixture solutions.

2.2. Software

SHIMADZU UVProbe data system program (Version 1.1) equipped with dual core processor laptop having 3.0 GB RAM (Windows 7 operating system) was used for recording the different orders (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup>) derivative spectra for each one of GA, AA and their mixture solutions.

2.3. Reagents

GA solution (250 μg/mL) (Yaccoo): 0.025 g of the compound was dissolved and diluted to 100 mL with D.W. Working solutions were freshly prepared by subsequent dilutions.
AA solution (250 μg/mL) (Scharlau): 0.025 g of the compound was dissolved and diluted to 100 mL with D.W in a dark volumetric flask and placed in a cold place. Only fresh solutions were used for the measurements. Working solutions were freshly prepared by subsequent dilutions.

2.4. Recommended procedure for simultaneous determination of GA and AA

The content of a series of 25 mL volumetric flasks, containing 125.0 μg of GA and different amounts (75.0 – 875.0) μg of AA, were diluted with D.W. The solutions were scanned (0.5 nm data intervals, 240 nm/min scan speed, and 1.0 nm slit width) against D.W. as a reagent blank in the range of 200 – 300 nm on the double-beam scanning UV/Vis spectrophotometer equipped with the sample cell and the data of the absorption spectra were collected. Then 1st derivative spectra have been recorded.

Different mixture solutions of GA and AA in a series of 25 mL volumetric flasks were prepared in a way that the concentration of AA kept constant (125.0 μg) with different concentrations (25.0 – 750.0) μg of GA, and a portion of the solution was placed in the cell and scanned (0.5 nm data intervals, 240 nm/min scan speed, and 1.0 nm slit width) against D.W. as a reagent blank in the range of 200 – 300 nm on the double-beam scanning UV/Vis spectrophotometer. The data of the absorption spectra were collected and 1st derivative spectra have been taken.

2.5. Sample preparation

The Hibiscus sabdariffa L. samples (cultivated with different nutrition system) were collected in Erbil city, and dried under shadow. The Hibiscus sabdariffa L. calyces were ground using a household blender. Ethanol:water (70:30%, v/v) solution were used for extraction of poly-phenolic compounds from the calyces of Hibiscus sabdariffa L., as follows: Hibiscus sabdariffa L. powder, 2.5 g was placed in a beaker and 25 mL of the extracting agent was added. The beaker was protected from the light with aluminum foil, and the mixture was stirred for 2.0 h. The supernatant was filtered through Whatman filter paper No. 42 and placed in a 25 mL volumetric flask then the volume was adjusted to the mark with D.W. (González et al., 2012). The solutions were evaporated on a heater before analyzing the samples in order to remove the ethanol, then the dried compound re-dissolved in D.W. and phenolic compounds of GA and AA were immediately analyzed with the aid of standard addition method by the recommended procedures for the determination of GA and AA with derivative spectrophotometric techniques.

3. RESULTS AND DISCUSSION

3.1. Absorption spectra

The normal UV absorption spectra of GA, AA and their mixture were recorded against D.W. as a reagent blank. (Figure 2) shows absorption spectrum of 5.0 μg/mL of GA solution with two maxima at 213 nm and 263 nm, curve (A), absorption spectrum of 5.0 μg/mL of AA solution with maximum absorption at 265 nm, curve (B), absorption spectrum of the mixture of 5.0 μg/mL of each one of GA and AA, curve (C). It is obvious that there is a strong overlap of the spectrum of GA and AA, therefore, their determination basing upon zero order absorption spectra measurements, when present in the same solution is very difficult due to strong overlapping and interfering between their spectra.

3.2. First derivative mode

Derivative spectra can be used to enhance differences among spectra and to resolve overlapping bands in qualitative and quantitative analysis (Robinson et al., 2014). Thus, to select derivative spectra, different orders (1st, 2nd, 3rd and 4th) have been recorded for each one of GA and AA. The study revealed that 1st order of the derivative spectra of GA and AA, as shown in the (Figure 3) were simple and gave best results for simultaneous determinations of GA and AA basing upon zero-crossing technique. Zero-
crossing technique can be used if the measurement of the amplitude of derivative peak of an analyte is performed at those wavelengths at which spectra of other components undergo zeroing. The measured amplitude is proportional only to concentration of assayed compounds. This approach of quantitative determination allows simultaneous determination of a few analytes in a sample (Patel et al., 2010).

As shown in the (Figure 3), 1st derivative spectrum of GA solution undergoes zero absorption at 238.4 nm, while AA solution has absorption at this point. From this point of view zero-cross technique was used for determination of AA at the presence of GA in the solution. Also at 222.11 nm, GA solution has negative valleys, while AA solution has positive valleys; therefore, basing upon amplitude measurements of these negative peak-to-baselines, GA solution was determined in the presence of AA in the solution. Thus, simultaneous determination of GA and AA can be performed with 1st derivative spectrophotometric method.

In the present work, graphically studying techniques (peak-to-baseline, height measuring and zero-crossing) were used to deal with derivative spectra to carry out the simultaneous measurements. All these techniques in the 1st derivative mode show good proportionality to the GA and AA concentrations in their mixtures.

Figures (4 and 5) show sets of 1st order spectra of mixtures containing different amounts of each one of GA and AA in the presence of (5.0 μg/mL) of the other compound, respectively.

The results in the Figure 4 indicated that when the concentration of GA kept constant, the concentration of AA is directly proportional with the height at 238.4 nm (zero crossing point of GA), and the results in the Figure 5 showed that when the concentration of AA kept constant, the concentration of GA in linear proportion with peak-to-baseline amplitude at 222.11 nm (negative peaks).

3.3. Calibration graph and statistical data

The analytical characteristics and most statistical data of the calibration curves of the proposed method for simultaneous spectrophotometric determination of GA and AA using 1st derivative zero-crossing techniques are given in (Table 1).
3.4. Precision and accuracy

Under the optimum conditions, precision and accuracy of the proposed 1st derivative spectrophotometric method for simultaneous determination of GA and AA basing upon zero-cross technique were checked. Depending upon the values of the relative standard deviation (RSD %) and the relative error (Error %) for five replicate measurements of three different concentrations of GA and AA. The results are summarized in (Table 2).

Table (2): Precision and accuracy of the proposed 1st derivative spectrophotometric method for simultaneous determination of GA and AA using zero-crossing technique

| Compound | Methods of Analysis | Concentration (µg/mL) | RSD % | Error % |
|----------|---------------------|-----------------------|-------|---------|
| GA       | Peak-to-baseline at 222.11 nm | 1.0 - 30.0 | 0.559 | y=0.7661x+1.8067 0.9991 |
| AA       | Height at 238.4 nm (zero-crossing point of GA) | 1.0 | 3.64 | 4.73 |
|          |                     | 1.5 | 4.12 | 3.26 |
|          |                     | 30.0 | 3.99 | 4.33 |
|          |                     | 30.0 | 3.99 | 4.33 |
|          |                     | 30.0 | 3.99 | 4.33 |

3.5. Interferences study

The effects of different compounds, 1000 µg/mL of (scopoletine, rutine, quercetine and catechine HCl), 500 µg/mL of (chlorogenic acid, ferulic acid and citric acid) and 100 µg/mL of (caffeine, gibberlic acid, and m-coumaric acid) on the determination of 5.0 µg/mL of GA and AA with the proposed simultaneous 1st derivative zero-crossing technique were studied. Results showed that the compounds under study did not interfere in the proposed methods.

3.6. Application of the method

The proposed 1st derivative zero-crossing technique for simultaneous determination of GA and AA were successfully applied with the aid of standard addition method for simultaneous quantification of GA and AA in the samples of *Hibiscus sabdariffa* L., collected in Erbil city and cultivated with different nutrition system. The results of application and recovery study are summarized in (Table 3).

Table (3): Determination and recovery of GA and AA in *Hibiscus sabdariffa* L. samples with the proposed simultaneous method

| Samples of *Hibiscus sabdariffa* L. | Amount found (mg/g) | Recovery % |
|-----------------------------------|---------------------|------------|
| GA                                | 1                   | 5.0593     | 97.06     | 95.41     |
| AA                                | 2                   | 4.9871     | 93.99     | 94.81     |

4. CONCLUSIONS

The 1st derivative zero-crossing spectrophotometric techniques have been described for simultaneous quantification of GA and AA in *Hibiscus sabdariffa* L. samples. The proposed method is simple, rapid, non-destructive, economy and environment friendly (it does not require expensive solvents and reagents, do not use toxic and ozone depleting organic solvents and polluting reagents).

REFERENCES

ABBASI, S., DANESHFAR, A., HAMDGHADAREH, S. & FARMANY, A. 2011. Quantification of sub-nanomolar levels of gallic acid by adsorptive stripping voltammetry. *Int J Electrochem Sci*, 6, 4843-4852.

FERNANDES, F. H. A. & SALGADO, H. R. N. 2016. Gallic acid: review of the methods of determination and quantification. *Critical reviews in analytical chemistry*, 46, 257-265.

GONZALEZ, C. S., BALDERAS, F. T. V., REGULES, A. O. & BELTRÁN, J. A. G. 2012. Antioxidant properties and color of Hibiscus sabdariffa extracts. *Ciencia e investigación agraria: revista latinoamericana de ciencias de la agricultura*, 39, 79-90.

IQBAL, K., KHAN, A. & KHATTAK, M. M. A. K. 2004. Biological significance of ascorbic acid (Vitamin C) in human health—a review. *Pakistan Journal of Nutrition*, 3, 5-13.

MOKHTARI, Z., ZARRINGHALAMI, S. & GANJLOO, A. 2018. Evaluation of Chemical, Nutritional and Antioxidant Characteristics of Roselle (Hibiscus sabdariffa L.) Seed. *Nutrition and Food Sciences Research*, 5, 41-46.

OJEDA, C. B. & ROJAS, F. S. 2004. Recent developments in derivative ultraviolet/visible absorption spectrophotometry. *Analytica Chimica Acta*, 518, 1-24.
OJEDA, C. B. & ROJAS, F. S. 2013. Recent applications in derivative ultraviolet/visible absorption spectrophotometry: 2009–2011: a review. Microchemical Journal, 106, 1-16.

PATEL, K. N., PATEL, J. K., RAJPUT, G. C. & RAJGOR, N. B. 2010. Derivative spectrometry method for chemical analysis: A review. Der Pharmacia Lettre, 2, 139-150.

REVANASIDDA PPA, H. & VEENA, M. 2008. Sensitive spectrophotometric methods for the determination of ascorbic acid. Journal of Chemistry, 5, 10-15.

ROBINSON, J. W., FRAME, E. S. & FRAME II, G. M. 2014. Undergraduate instrumental analysis, CRC press.

ROJAS, F. S. & OJEDA, C. B. 2009. Recent development in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008: A review. Analytica Chimica Acta, 635, 22-44.

SAPEI, L. & HWA, L. 2014. Study on the kinetics of vitamin C degradation in fresh strawberry juices. Procedia Chemistry, 9, 62-68.

SáYAGO-AYERDI, S. G., ARRANZ, S., SERRANO, J. & GOñI, I. 2007. Dietary fiber content and associated antioxidant compounds in roselle flower (Hibiscus sabdariffa L.) beverage. J. of Agricultural and Food Chemistry, 55, 7886-7890.

SHRUTHI, V., RAMACHANDRA, C., NIDONI, U., HIPEGoudAR, S., NAIK, N. & KURUBAR, A. 2016. ROSELLE (HIBISCUS SABDARIFFA L.) AS A SOURCE OF NATURAL COLOUR: A REVIEW. Plant Archives, 16, 515-522.

TASHKHOURIAN, J., ANA, S. N., HASHEMNIA, S. & HORMOZI-NEZHAD, M. 2013. Construction of a modified carbon paste electrode based on TiO 2 nanoparticles for the determination of gallic acid. Journal of Solid State Electrochemistry, 17, 157-165.

VISHNIKIN, A. B., SVINARENKO, T. Y., SKLENáŘOVá, H., SOLICH, P., BAZEL, Y. R. & ANDRUCH, V. 2010. 11-Molybdobismuthophosphate — A new reagent for the determination of ascorbic acid in batch and sequential injection systems. Talanta, 80, 1838-1845.

VIVEK, M., JIGAR, A., SHWETA, S. & SUNIL, R. 2010. First and second derivative spectrophotometric methods for determination of olanzapine in pharmaceutical formulation. International Journal of ChemTech Research, 2, 756-761.