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Ratio spectra derivative and mean centering as green analytical techniques for simultaneous determination of ascorbic acid and folic acid in pharmaceutical formulations, for-ferro tablets

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Abstract

Background and objective: The reliability and efficacy of multivitamin pharmaceutical products containing ascorbic acid and folic acid vary due to differences in manufacturing processes and ingredient selection. This research paper presents the development and validation of derivative spectrophotometric and mean-centering methods for the simultaneous determination of ascorbic acid and folic acid both in their pure form and in pharmaceutical formulations. Furthermore, an assessment of the environmental impact of the proposed methods is conducted utilizing the analytical procedure index as a measure of its "greenness" profile.

Methods: Analytical procedures that are simple, accurate, and environmentally friendly were devised to concurrently determine the levels of ascorbic acid and folic acid. These methods include the ratio derivative and mean-centering spectrophotometric techniques, ensuring precise measurements of both substances**.**

Results: The ratio derivative and mean-centering spectrophotometric techniques for the determination of ascorbic acid and folic acid showed a measurable amplitude and substantial linearity. The ratio spectra derivative, at the amplitude 247.2 nm (1DD 247.2) and 270 nm (1DD 270) ascorbic acid showed linearity from (0.5-15.0 µg/mL) and (0.5-15.0µg/mL) with a detection limit of (0.17 µg/mL) and (0.298 µg/mL), respectively, while folic acid displayed observable amplitude at 314.4 nm (1DD 314.4) with linearity (2.0-15.0µg/mL) and a detection limit of (0.37 µg/mL). The mean-centering method for ascorbic acid and folic acid illustrated assessable peak-to-baseline (356.8 nm) and (346 nm) with the linearity of (0.5-12.0µg/mL) and (2.0-15.0µg/mL), and a detection limit of (0.15 µg/mL) and (0.49 µg/mL), respectively.

Conclusion: The proposed methods were effectively utilized for the simultaneous quantification of both ascorbic acid and folic acid in laboratory-prepared synthetic mixtures as well as pharmaceutical formulations with reasonable levels of precision, accuracy, and recovery.

Keywords: Ascorbic acid; Folic acid; Ratio derivative spectrophotometry; Mean centering; Greenness measurement tool.

Introduction

Vitamins are vital for the healthy growth and development of living organisms. Ascorbic acid (AA), depicted in Figure 1(a), is a natural water-soluble vitamin which is known as vitamin C. AA has several essential functions, such as being a potent reducing and antioxidant agent,[1,2] and a cofactor for various enzymatic processes.[3] Additionally, it plays a crucial role in collagen creation in different organs and tissues like skin, bones, and capillaries, and helps prevent bacterial infections.[4-6] Folic acid (FA), illustrated in Figure1(b), is the synthetic form of folate, a vitamin B9 natural form, which is

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naturally found in several foods. FA is essential for the production of red blood cells, growth, and prevention of anemia.[7] It also helps create and maintain DNA and other genetic materials.[8] The human body cannot produce or store AA and FA, so they must be obtained through diet.[9]

Various techniques have been reported for the simultaneous and individual quantification of AA and FA in pharmaceutical formulations, employing diverse methods such as HPLC,[10,11] electrochemical sensor,[12,13] and Raman spectroscopy with capillary zone electrophoresis.[14,15] Recently, the multi-component analysis found excellent results in analytical chemistry to simultaneously analyze two or more components in a sample. It also offers time and cost savings, as well as being more efficient than analyzing each component separately, for this purpose ratio spectra derivative and mean centering methods were developed and used in this study.

In the ratio derivative spectra method, the spectrum of a mixture is divided separately by the standardized spectrum of each analyte present in the mixture. This division produces a ratio that is then subjected to derivative analysis, resulting in a spectrum that is not influenced by the concentration of the analyte used as a divisor.[16] By removing the effect of the divisor, the ratio spectra derivative method can enhance the selectivity and sensitivity of spectral analysis for multi-component mixtures, allowing to improve quantification and identification of the components.[17]

However, mean centeringof ratio spectra in spectrophotometric methods involves subtracting the mean value from each data point in the spectral-ratio data.[18,19] This process aids in removing systematic variations, enhancing comparability, highlighting significant changes, and improving the analysis and interpretation of ratio spectra data.[20] The goal of this study is to create new, and extremely straightforward techniques for separating overlapping spectra in a mixture. Two different spectrophotometric methods for the simultaneous determination of FA and AA in the pharmaceutical sample were utilized. The methods are easy to use, exact, and neither complicated hardware nor software is required.

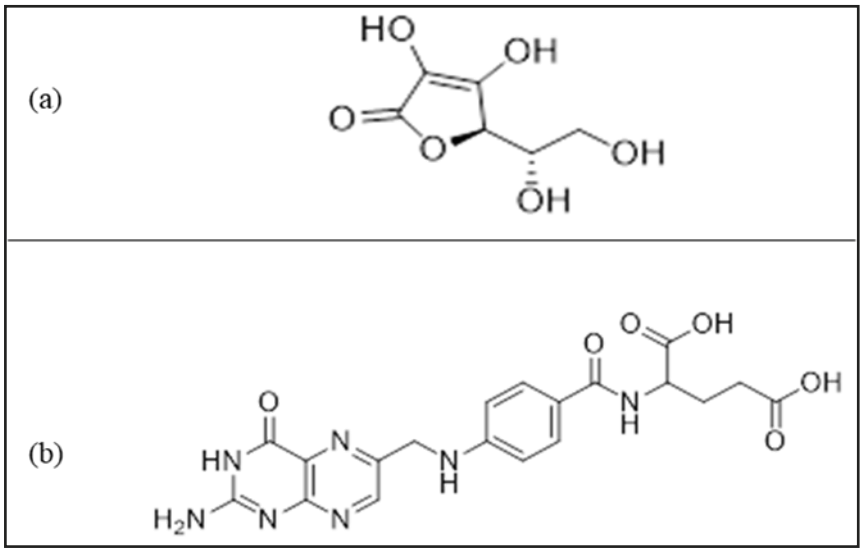

Figure 1 Chemical structure of ascorbic acid (a) and folic acid (b)

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Methods

Apparatus: To obtain the absorption spectra of AA, FA, and their mixtures, a Shimadzu UV-1800 UV-VIS double beam spectrophotometer with a 10-mm path length matches quartz cells and a computer was utilized.

Software: SHIMADZU UV-Probe data system program (Version 2.42) equipped with a Lenovo laptop was used for recording first-order derivative spectra for AA, FA, and their mixture solutions. Origin software (OriginLab) was used for the statistical treatment of the data and the construction of calibration graphs. ChemDraw software was used for drawing chemical structures.

The environmental implications of the examined methods were evaluated using the Green Analytical Procedure Index (GAPI), employing a graphical representation comprising five pentagrams. Each pentagram was assigned a specific color including green, yellow, and red. These colors are representing low, medium, and high impacts, respectively. This visual representation allowed for an assessment of the potential environmental concerns associated with the analyzed methods.[21]

Chemicals and Reagents

For the solvation process, a 0.05 mol/L potassium phosphate monobasic sodium hydroxide buffer solution with a pH of 7 was employed. To prepare the stock solutions, 0.02 g of AA (Scharlau 99%) and 0.022 g of FA (Ph. Eur. Reference standard 90.7%) were individually dissolved in phosphate buffer (pH=7) using 200 mL dark volumetric flasks individually. The resulting solutions with a concentration of 100 µg/mL were subsequently diluted with buffer solution to prepare fresh working solutions required for the measurements.

Proposed Procedure for Simultaneous Determination of AA and FA

To prepare a series of the mixture solutions with varying concentrations of AA and FA, 100 mL volumetric flasks were utilized. The concentration of FA was kept constant at 2 µg/mL while different amounts of AA were added to achieve a range of concentrations from 0.5 to 15 µg/mL. The solutions were then placed in a cell and scanned using a UV/Vis spectrophotometer over a range of 200-500 nm with a data interval of 0.2 nm, medium speed, and a slit width of 1 nm, using phosphate buffer (pH=7) as a reagent blank. The absorption spectral data were collected, and then the first ratio spectra derivatives were obtained. Similarly, a new set of absorption spectral data was obtained for a series containing a constant concentration of AA (2 µg/mL) mixed with varying concentrations of FA (0.5-15 µg/mL) using the same procedure. The obtained data were then processed to obtain the first derivative ratio spectra.

Preparation of Laboratory Mixtures

The laboratory mixtures of AA and FA were prepared by taking various ratios of previously prepared stock solutions, followed by adjusting the mixtures with phosphate buffer (pH=7) up to the mark in 100 mL volumetric flasks. The final concentrations of the constituents in the solutions were then analyzed using their respective regression equations.

Real Sample Preparation

The study involved the analysis of *For-Ferro* tablets as a real sample to determine the concentrations of AA and FA. The tablets were obtained from a local pharmacy in Sulaymaniyah city, which is produced by a pharmaceutical company, Advanced Biomedical (ABM), in Italy. To prepare the sample, ten tablets were crushed into a fine powder, and an amount of (504 mg) equivalent to one tablet was dissolved in phosphate buffer (pH=7) in a 100 mL volumetric flask.

The resulting suspension was filtered using a 0.45 µm Whatman syringe filter to obtain a colorless solution. A 1 mL portion of the solution was then diluted with phosphate buffer (pH=7) in a 100 mL dark volumetric flask. The concentrations of AA and FA were determined using first derivative ratio spectra and mean centering methods

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techniques as recommended.

Results

Normal Absorption Spectra of AA and FA

The normal spectra of AA, FA, and their mixture were measured. The highest absorption peaks were observed at 265.6 nm for AA and 281 nm for FA, both at a concentration of 2 µg/mL. However, there was a certain level of overlap in the normal spectrum of the AA and FA mixture (Figure 2), making it challenging to differentiate them based on the zero-order absorption spectrum.

First Derivative of Ratio Spectra(1DD)

The ratio spectra derivative method was utilized to determine the concentration of components in the mixture. The normal spectrum of the mixture was divided by a standard absorption spectrum of one component to obtain a ratio spectrum. Then, the first derivative of these ratio spectra was calculated which resolved the overlapping bands in the zero-order spectra, and it was then used to determine the concentration of each component in the mixture using plotted calibration curves. Two separate sets of ratio spectra derivatives were obtained for AA and FA solutions at different concentrations. The stored absorption spectrum of each concentration was divided by a standard absorption spectrum of FA (2 µg/mL), which was opted as a divisor (Figure 3).

Similarly, for FA, the absorption spectrum of each concentration was divided by the absorption spectrum of standard AA (10 µg/mL), which was chosen as a divisor (Figure 4). The selected wavelengths for determining the concentrations were 270 nm and 247.2 nm for AA and 314.4 nm for FA.

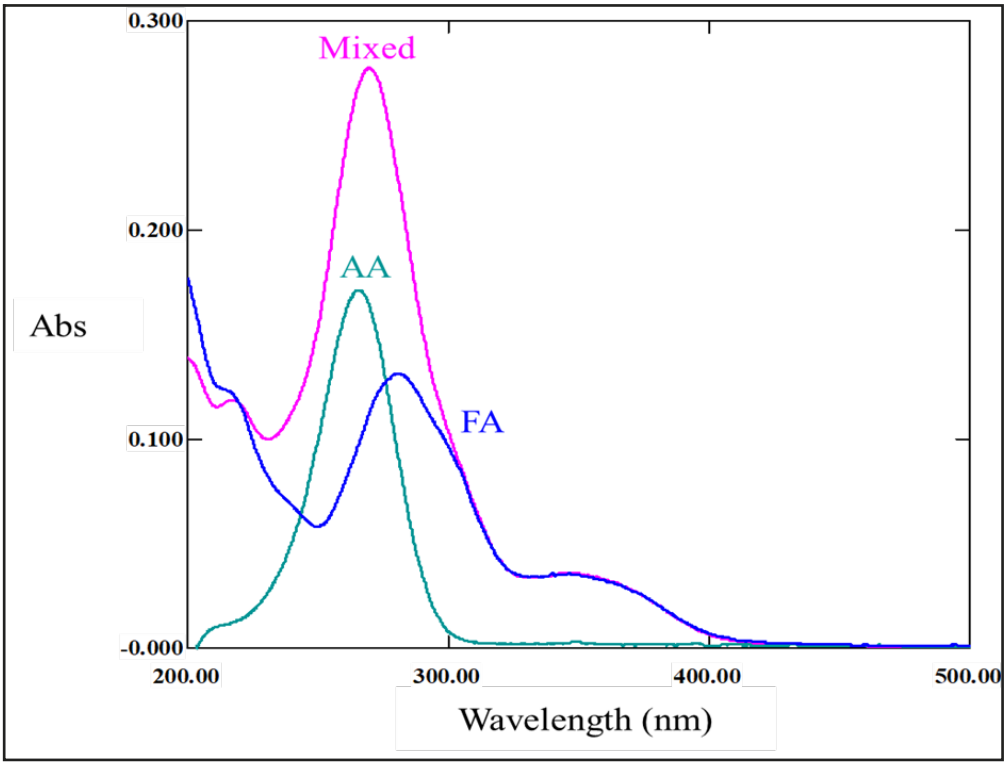

Figure 2 Zero-order absorption spectra of AA (2 µg/mL), FA (2 µg/mL), and their mixture

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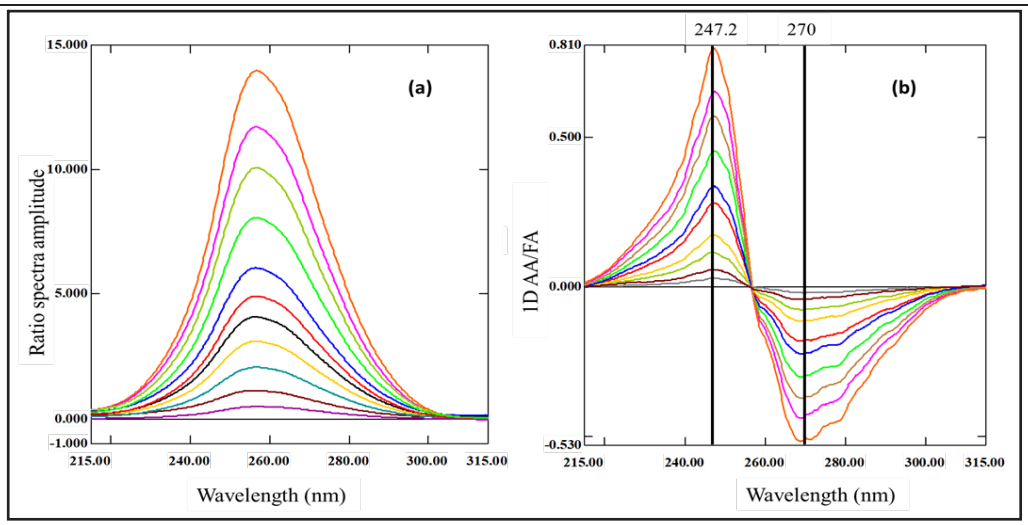

Figure 3 Graphical representation of (a) ratio spectra of AA (0.5-15.0µg/ml) using 2.0 µg/ml of FA as a divisor, (b) first derivative of the ratio spectra obtained

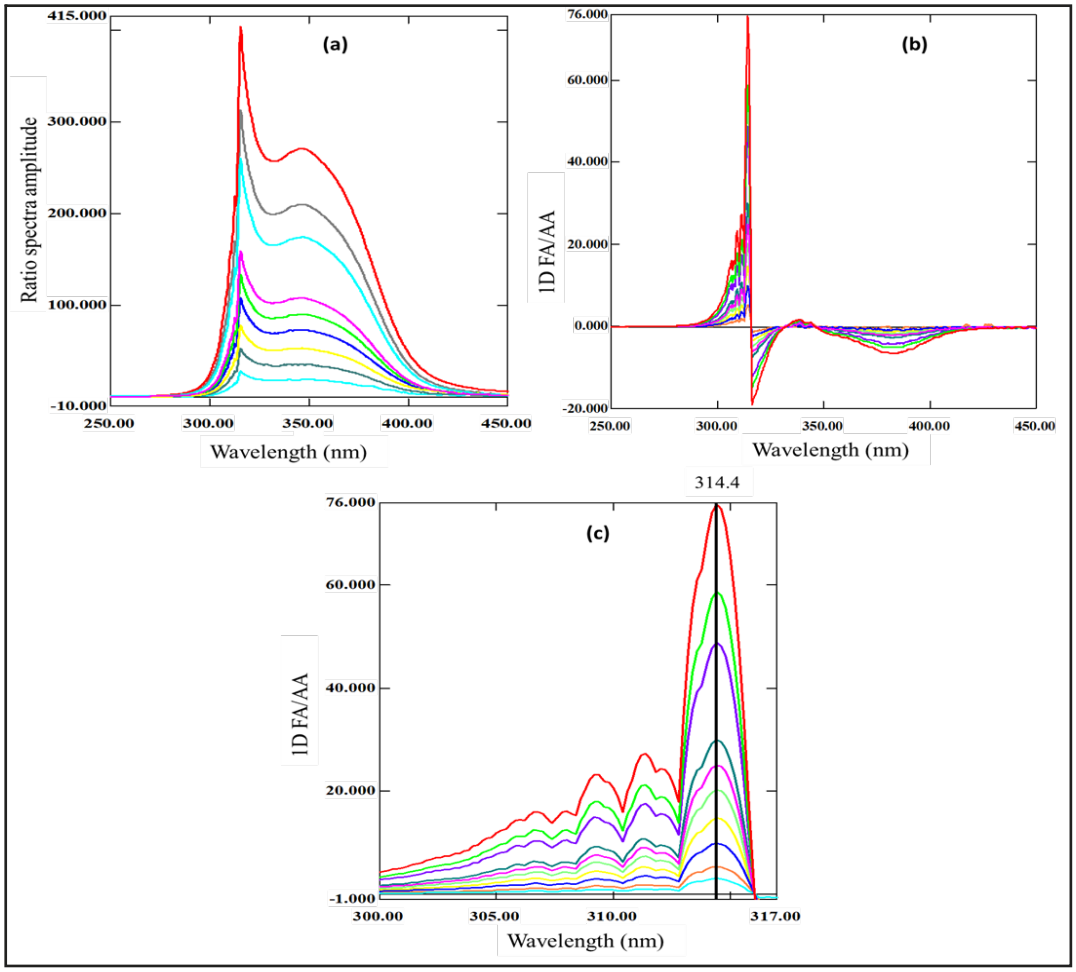

Figure 4 Graphical representation of (a) ratio spectra of FA (0.515.0-µg/mL) using 10.0µg/mL of AA as a divisor, (b) first derivative of the ratio spectra obtained, and (c) the dependent wavelength to clarify and prices the obtained ratio spectra derivative

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Mean Centering of Ratio Spectra

The mean centering technique was applied as a preprocessing step to the ratio spectra. Each series of AA and FA spectra was divided by their respective divisors, and the mean value for each wavelength or data point in the ratio spectra was calculated by averaging the ratio values across the spectrum. The calculated mean value was then subtracted from each individual data point in the ratio spectrum, effectively centering the spectrum around zero.

The selected wavelengths for determining the concentrations were 346 nm for AA and 256.8 nm for FA. The obtained mean-centering spectrum is illustrated in Figure 5.

Method Validation

The methods were validated according to ICH guidelines.[22]

Linearity and Range

The linearity of the methods for the quantification of AA and FA was evaluated across a range of concentrations. The developed methods, ratio derivative spectra and mean centering methods, showed high correlation coefficients for AA and FA. The concentration ranges for accurate quantification were determined to be 0.5-15 µg/mL for AA and 2-15 µg/mL to FA using the ratio derivative spectra method, and 0.5-12 µg/mL for AA and 2-15 µg/mL for FA using the mean centering method. The limit of detection (LOD) and limit of quantification (LOQ) values were also calculated for each method. Table 1 represents the common parameters obtained for AA and FA by both methods.

Table 1 The statistical parameters for the determination of AA and FA

Parameters	Ratio spectra derivative method			Mean centering method	
Compounds	AA		FA	AA	FA
Wavelength (nm)	247.2	270	314.4	356.8	346
Slope	8.42	8.26	8.24	8.40	4.32
Correlation coefficient	0.9998	0.9997	0.9996	0.9994	0.9991
Intercept	0.43	0.74	0.93	0.37	0.62
Range (µg/mL)	0.5-12.0	0.5-12.0	2-15.0	0.5-12.0	2.0-15.0
LOQ (µg/mL)	0.56	0.89	1.1	0.44	1.57
LOD (µg/mL)	0.17	0.298	0.37	0.15	0.49

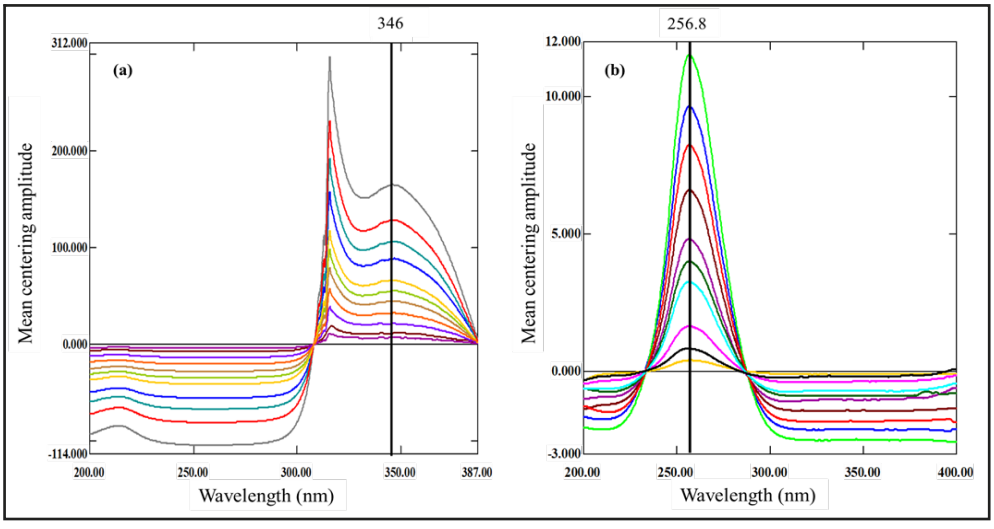

Figure 5 Graphical illustration of (a) mean centering of ratio spectra of AA series (0.5-15.0 µg/mL) and (b) mean centering of ratio spectra of FA series(0.5-15.0 µg/mL)

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Accuracy and Precision

The precision and accuracy of the proposed methods were assessed. Precision was determined by calculating the relative standard deviation percentage (%RSD) at three different concentrations with three replicated measurements for each concentration. Accuracy was evaluated by calculating the average error percentage (%E) based on the results of 9 determinations of three distinct concentrations, each with three replicated measurements. The results showed low %RSD values, (0.48% to 1.76%) for repeatability and (0.86% to 1.83%) for intermediate precision, indicating good precision of both methods. The accuracy, represented by %E values, of the developed methods for the determination of AA and FA concentrations is demonstrated in Table 2, and it is ranged from 99.37% to 101.2% for the ratio spectra derivative method and 99.49% to 99.69% for the mean centering method.

Selectivity

The selectivity of the methods was assessed by analyzing the spectra of commonly used excipients in tablets and investigating potential interferences. It was found that talc, silicon dioxide, magnesium stearate, and croscarmellose sodium did not dissolveonly required filtration, while lactose and citric acid did not interfere with the spectral analysis. A concentration of 2 µg/mL of cyanocobalamin showed interference with the spectra of AA and FA, but required dilution of the sample mitigated this interference.

Application of the Methods

The proposed method was applied to determine the concentration of AA and FA in laboratory-prepared mixtures and commercial *For-Ferro* tablets. No interference due to excipients was detected in the spectra produced. For this purpose, the recovery test using the standard addition method was carried out to confirm the validity of the methods. The recovery values ranged from 97.60% to 104.20% for the ratio derivative spectra method and from 98.67% to 103.4% for the mean centering method, further validating the selectivity of the methods is shown in Table 3 and Table 4.

Table 2 Accuracy and precision of the proposed methods for simultaneous detection of AA and FA

Parameters	**Ratio spectra derivative method**		**Mean centering method**	
Compounds	**AA**	**FA**	**AA**	**FA**
Wavelength (nm)	247.2	270	314.4	356.8	346
Accuracy (%E)	0.3	1.4	2.36	0.35	1.26
Repeatability (%RSD)	1.07	1.76	0.48	0.79	0.86
Intermediate precision (%RSD)	1.83	1.21	0.86	0.98	0.88

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Table 3 Recovery of ratio spectra derivative method for determination of AA and FA

Compound	Wavelength (nm)	Sample	Amount found in sample (µg/mL)	Spiked amount (µg/mL)	Amount found in Spiked sample (µg/mL)	Recovery %
AA	247.2	Synthetic	4.20	3	7.34	103.33
		For-Ferro	7.15	3	10.11	99.44
	270	Synthetic	3.95	3	7.14	104.81
		For-Ferro	7.24	3	10.26	100.27
FA	314.4	Synthetic	3.66	3	6.52	95.62
		For-Ferro	4.99	3	7.98	99.80

Table 4 Recovery of mean centering method for AA and FA

Compound	Wavelength (nm)	Sample	Amount found in sample (µg/mL)	Spiked amount (µg/mL)	Amount found in Spiked sample (µg/mL)	Recovery %
AA	256.8	Synthetic	4.07	3	7.21	103.44
		For-Ferro	7.17	3	10.09	98.88
FA	346	Synthetic	3.78	3	6.69	97.62
		For-Ferro	5.18	3	8.22	100.77

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Greenness Assessment

The environmental sustainability of the developed methods was assessed using the GAPI (Green Analytical Procedure Index) tool. The GAPI analysis considered various factors involved in the analytical process, including sample preparation, sample handling, chemicals used, and instrumentation.[23-25] According to a color-coding system in this tool, the green, yellow, and red colors indicate minimal, medium, and high negative environmental impacts, respectively.[26]

The GAPI assessment of the proposed methods revealed a positive sustainability profile. As shown in Figure 6 (a), a significant portion of the proposed methods' chart analysis was designated as green, indicating a low environmental impact of the methods. Also, in terms of greenness, the reported HPLC[10] method for simultaneous quantification of AA and FA signifies a high environmental impact compared with the proposed methods Figure 6 (b).

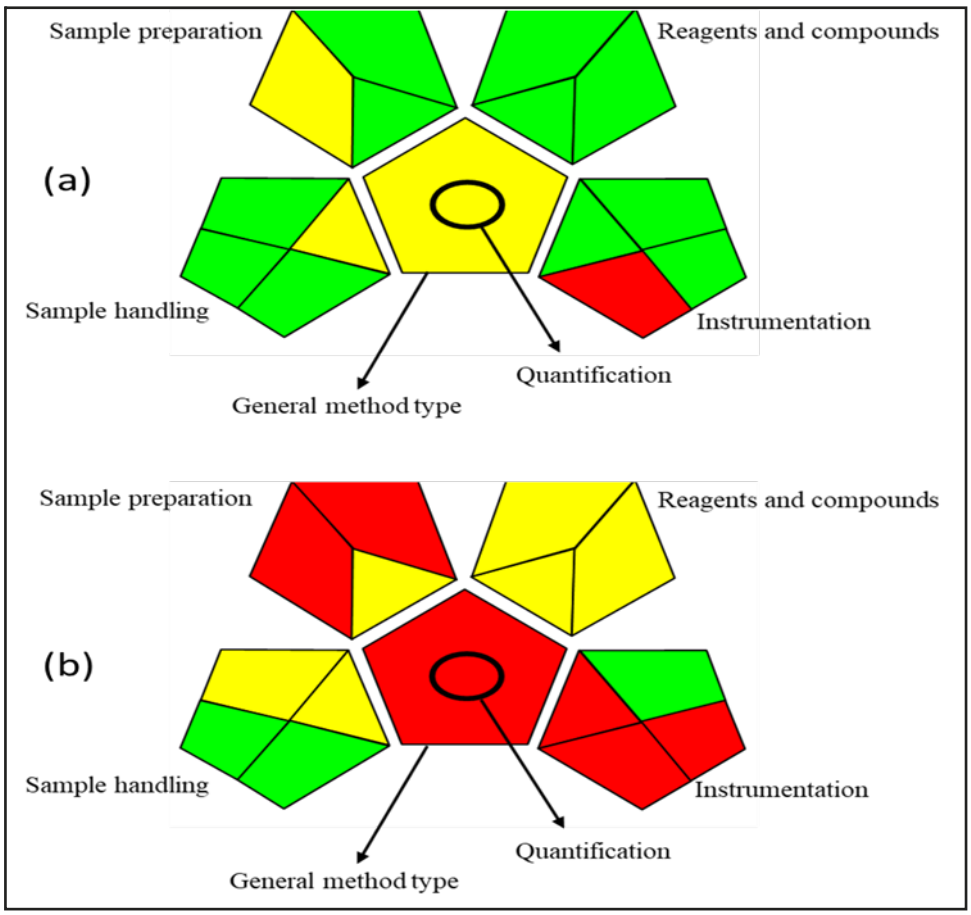

Figure 6 GAPI representation of (a) the proposed methods and (b) reported HPLC method.[10]

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Discussion

The normal spectra provided information about the absorption characteristics of AA, FA, and their mixture. However, due to the overlap in the spectra of the mixture, it was challenging to distinguish between AA and FA using only the zero-order absorption spectra.

To overcome this challenge, the ratio spectra derivative method was employed. By dividing the spectral data of AA and FA solutions by their respective divisors, the ratio spectra were obtained. The first derivative of the ratio spectra was attained which resulted in obtaining ratio derivative spectra, and as a consequence, the overlapping bands in the zero-order spectra were resolved. This allowed for the determination of component concentrations using the plotted calibration curves. Additionally, the mean centering method was applied to the ratio spectra as a preprocessing step. This technique helps to eliminate unwanted variations and enhances the detection of the spectra. By subtracting the mean value from each data point in the ratio spectra, facilitating further analysis and interpretation.

The linearity assessment demonstrated that both the ratio spectra derivative and mean centering methods have a wide linear range for the quantification of AA and FA. The high correlation coefficients indicate a strong linear relationship between the concentration and the corresponding response. This suggests that both methods are suitable for accurately determining the concentrations of AA and FA over a broad range of concentrations.

The precision results indicate good repeatability and intermediate precision of the methods. The low %RSD values for the precision parameters demonstrate the reliability and consistency of the methods in measuring AA and FA concentrations. The accuracy results, represented by %E values, indicate that the methods provide accurate measurements. These findings confirm the accuracy of the methods in quantifying AA and FA in the tested sample.

The selectivity assessment revealed the potential interferences from excipients commonly found in tablets. The need for filtration and dilution to eliminate or mitigate interferences demonstrates the selectivity of the methods in specifically detecting and quantifying AA and FA. The recovery test further confirmed the absence of significant matrix effects and interference from excipients, which validates the selectivity and application of the methods.

The greenness assessment using the GAPI tool provides valuable insights into the environmental impact of the developed methods. By considering various aspects of the analytical process, such as sample preparation, handling chemicals, and instrumentation, the GAPI analysis helps to identify areas where improvements can be made to minimize environmental impact.

The significant number of green zones in the GAPI assessment of the proposed methods indicates that they are environmentally friendly. As shown, the proposed methods have eleven green zones in the GAPI assessment, while the previously published HPLC method had only three green zones. This indicates that the proposed methods have a more environmentally friendly profile compared to the HPLC method[10] This result can be attributed to several factors. First, the use of derivative spectrophotometry techniques reduces the need for complex and resource-intensive instrumentation, such as HPLC systems, which typically have a higher environmental impact. Second, the proposed methods require lower sample volumes and use fewer chemicals, reducing waste generation and chemical consumption. Third, the methods employ more sustainable sample preparation techniques, such as filtration.

Conclusion

This research focused on the development and validation of two green

spectrophotometric methods, namely ratio spectra derivative and mean centering, for the simultaneous determination of AA and FA in their combined dosage form. The methods demonstrated good linearity, accuracy, precision, and selectivity alongside confirming the successful quantitative analysis of the studied drugs (AA and FA) in both their pure and combined pharmaceutical tablet form. In addition, the greenness assessment using the green analytical procedure index indicated a positive sustainability profile for the methods.

Competing interests

The authors declare that they have no competing interests.

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