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Derivative Spectrophotometric Methods for Simultaneous Determination of Quercetin and Gentisic acid in *Capparis spinosa* L.

Dlgash Hamad Maruf [1], ***Rizgar Hassan Mohammad**** [2]

[1]Department of Chemistry, College of Science, University of Soran, Erbil, Iraq.
[2]Department General Sciences, College of Education, University of Soran, Erbil, Iraq.
*Corresponding Author.

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Abstract

Capparis spinosa L. is one of the medicinal plants used in traditional medicine which contains numerous phytochemicals including polyphenolic compounds. Quercetin and gentisic acid are two important phenolic compounds found in plants which display many medicinal properties such as anti-inflammatory, antimicrobial, antioxidant and anticancer. Determination of both compounds together in a binary mixture is not achieved yet with spectrophotometric methods. In this study, two simple, rapid and accurate derivative spectrophotometric methods were developed and used for simultaneous quantification of quercetin and gentisic acid in binary mixtures of *Capparis spinosa* L. methanolic leaves extract. The first technique relies on the zero-crossing approach (first and fourth order derivatives), while the second approach is based on using ratio spectra and first-order derivative spectrophotometry. The calibration curves of the two derivative spectrophotometric techniques are linear in the concentration ranges of 2.0-30 µg/mL and 4.0-80 µg/mL for quercetin and gentisic acid, respectively, whereas the recovery percentages ranged from 94.06% - 105.98% (quercetin) and 94.29% - 113.37% (gentisic acid). The developed methods were effectively used for the quantitative determination of both phenolic compounds in *Capparis spinosa* L. leaves.

Keywords: *Capparis spinosa L*, Derivative spectrophotometry, Gentisic acid, Quercetin, Ratio spectra derivative method, Zero-crossing method.

Introduction

Medicinal plants still play vital roles in the daily lives of people living in developing countries [1]. Phytochemicals including carotenoids, flavonoids, and other phenolic compounds are abundant in wild edible plants [2]. One of the valuable medicinal plants is *Capparis spinosa* L. which is used as a traditional medicine in many countries [3, 4]. Several common names were used to describe *Capparis spinosa* L. such as Kabar, Shafallah (Arabic); Mar gir, Mara gira (Kurdish); Caper (English) [5]. It is a member of the *Capparidaceae* family, a genus of Capparis [2] and species of *Capparis spinosa* L. [6]. Caper is a prickly shrub which is 0.3-1m tall and has roots grown up to 6 to 10 m [2], widely grown in rocky areas, deserts, and arid places [7]. Numerous bioactive substances from several chemical classes, including phenolic acids, flavonoids, alkaloids, fatty acids, aldehydes, and esters, were found in capers extracts according to

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previous researches [8-12]. Furthermore, *C. spinosa* L. has shown anti-inflammatory, antimicrobial, antioxidant, and anticancer properties [2, 13].

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy 4H-chromen-4-one) with a molecular formula $C_{15}H_{10}O_7$ and chemical structure shown in Fig. 1 [14], is a polyphenolic flavonoid compound which is abundant in plants [15] and displays a wide range of medicinal properties such as anti-inflammatory, antiviral, antimicrobial, anti-bacterial [16], antioxidative, anticancer and neuroprotective [17, 18]. Recently discovered to have anti-COVID-19 properties [16]. Despite the availability of numerous analytical techniques for the quantification of quercetin, the most popular methods are high-performance liquid chromatography (HPLC) [19-21] and UV-Vis spectrophotometry [22-24]. Capillary electrophoresis with diode array detection (CE-DAD) [25] and dispersive liquid-liquid microextraction based on solidification of the floating organic droplets (DLLME-SFOD) are also used in a few cases [26].

Gentisic acid (2,5-dihydroxybenzoic acid) with molecular formula $C_6H_3(OH)_2COOH$ and chemical structure displays in Fig. 2 [27], is a diphenolic chemical compound and a benzoic acid derivative which is a member of the phenolic acids . It has a wide range of biological properties, including anti-inflammatory, anti-rheumatic, antioxidant, and antibacterial activities [28]. Most techniques that have been reported previously to determine the amount of gentisic acid present in various samples are HPLC with UV detection [29], liquid chromatography combined with mass spectrometry [30, 31], HPLC coupled with tandem mass spectrometry [32] and capillary electrophoresis [25, 33].

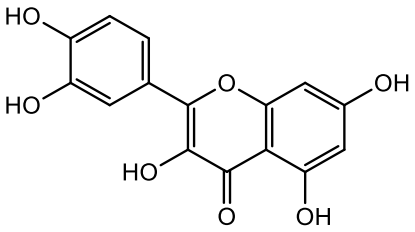

Figure 1. Chemical structure of quercetin.

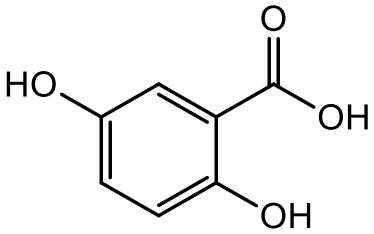

Figure 2. Chemical structure of Gentisic acid.

Derivative spectrophotometry, as a novel spectrophotometric technique, was recently developed for the simultaneous measurement of drugs in binary mixtures without prior separation stages [34]. A normal or zero order spectrum can be converted to its first, second, or higher derivative spectrum with the derivative spectroscopic method [35]. A technique was established by Salinas et al in 1990 for resolving overlapped binary mixture spectra. The ratio spectra derivative for a binary mixture is the basis for this developed method, which could be produced by dividing the mixture's absorption spectrum to the spectrum of one of the standard compounds [36]. To the best of our knowledge, no derivative spectrophotometric methods have been found to date regarding the simultaneous determination of quercetin and gentisic acid in *Capparis spinosa* plant. Consequently, this study aimed to develop derivative spectrophotometric methods for resolving a binary mixture of quercetin and gentisic acid for the simultaneous determination of both compounds in a sample of *Capparis spinosa* leaves.

Experimental

Laboratory Apparatus

All the spectral measurements were performed using a Cecil UV-visible double beam spectrophotometer (model Super Aquarius CE 9500, England) with variables 0.5, 1, 2, and 4nm bandwidth and a quartz cuvette with a path length of 1cm. The double-beam spectrophotometer was coupled to a computer to record zero order spectra and collect the absorption spectral data of quercetin and gentisic acid in their mixture solutions. UV Probe software (Version 2.42) was used to convert zero-order spectral data to the

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first and fourth orders derivative spectra (^{1}D and ^{4}D) for each quercetin and gentisic acid alone in a solution and together in binary mixture solutions. All computations were performed with Microsoft Excel.

Chemicals and Reagents

Methanol was of HPLC grade and was purchased from Scharlau Company. Standard gentisic acid was acquired from Glentham Life Sciences Company and standard quercetin was purchased from Sigma-Aldrich company.

Plant Material

The leaves of Caper plant were collected from Rawanduz in Kurdistan Region-Iraq, in May 2022. The plant was then identified and authenticated by a taxonomist at faculty of Education, University of Soran. The collected plant leaves were washed and dried in shade at room temperature 25-30 °C.

Plant Extraction

The extraction procedure was carried out according to Abdel-Sattar et al. [37] with slight modifications. The shade-dried ground plant material (20 g) was refluxed with 100 mL methanol for 2 hours on a magnetic stirrer at 80 ºC. The extract was filtrated through filter paper (CITOTEST, 15 cm), and the filtrate was then concentrated by heating at 40 ºC.

Preparation of the Sample Solution

The concentrated extract was diluted with 70% methanol in a 250 mL-volumetric flask. The spectrum of the diluted extract was complicated due to its dark green color. For this reason, 1 mL of the extract was then diluted again with 70% methanol to 25 mL in a volumetric flask. This solution showed a clear and good spectrum, and was suitable to be utilized for the applications with the proposed methods.

Preparation of Standard Stock Solution

Stock solutions (100 µg/mL) of standard quercetin and gentisic acid were prepared by dissolving 0.01 g of each compound in 100 mL of 70% methanol in volumetric flasks. The stock solutions were stored in a refrigerator at 5ºC for up to three months. All working diluted standard solutions were prepared daily by diluting these stock solutions with %70 methanol.

Calibration Graphs:

Zero-Crossing Method

Two series of different concentrations of working solutions were prepared in 10 mL volumetric flasks by diluting the standard stock solutions with 70% methanol. The prepared standard solutions' spectral data were documented on a computer after being scanned from 200 to 800 nm. The first sequence contained quercetin concentrations ranging from 0.5-30 µg/mL with a fixed 20 µg/mL concentration of gentisic acid, whereas the second sequence contained a definite amount (20 µg/mL) of quercetin with various quantities of gentisic acid 0.5-80 µg/mL. The absorption spectra of the samples were recorded from 200 nm to 800 nm where methanol (70% v/v) was employed as a reagent blank. UV Probe software was used to obtain the first and fourth derivative spectra of quercetin and gentisic acid in binary mixtures between 200 - 800 nm from converting the corresponding zero-order spectra of the compounds. The derivative spectra were affected by delta lambda (Δλ). A high signal-to-noise ratio was achieved with increasing Δλ values. Thus, different values of Δλ were tested and the appropriate value was selected. Under specific optimized instrumental parameters such as wavelength range, scaling factor and Δλ, the first and fourth derivative (^{1}D and ^{4}D) spectra were obtained; and consequently, the working wavelengths of both compounds were selected at zero-crossing points.

Ratio Spectra Derivative Method

The stored absorption spectra of different quercetin concentrations in the binary mixture were divided by a (50 µg/mL) standard absorption spectrum of gentisic acid (a divisor), and thus, the ratio spectra were attained. Different values of Δλ were tested, it was found that Δλ=12 gives the suitable signal-to-noise ratio. From the ratio spectra, the first derivative spectra, traced with an interval of Δλ=12 nm in 70% methanol, were calculated. The amplitudes of peak-to-baseline at 252.25 nm ($^1DD_{252.25}$), 277 nm ($^1DD_{277}$), 370.20 nm ($^1DD_{370.20}$) and 398.14 nm ($^1DD_{398.14}$) were chosen to quantify quercetin in the binary mixture.

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Baghdad Science Journal

Similarly, in order to achieve the ratio spectra of gentisic acid, the absorption spectra of different concentrations of gentisic acid in the binary mixture were divided by the absorption spectrum of standard quercetin (12 µg/mL), which has been opted as a divisor. The first derivative spectra of gentisic acid, traced with an interval of Δλ=12 nm in methanol (70% v/v), were then computed from the ratio spectra. The peak to baseline amplitudes at 245.72 nm ($^1DD_{245.72}$), 307.50 nm ($^1DD_{307.50}$) and 343.38 nm ($^1DD_{343.38}$) were selected for quantification of gentisic acid in the binary mixture.

Results and Discussion

As a result of the entire overlapping of the normal UV absorption spectra of quercetin and gentisic acid in the wavelength range of 200 – 800 nm, Fig. 3, it is hard to quantify the amount of both phenolics simultaneously in their mixture by employing conventional spectrophotometric methods. However, utilizing derivative spectrophotometry techniques is one of the unique and satisfactorily methods to resolve the overlapping spectra and reduce the interference effects [38].

Zero-Crossing Method

The normal UV absorption spectrum of quercetin is entirely overlapped with the spectrum of gentisic acid. Fig. 3 shows the zero-order absorption spectra of quercetin, gentisic acid and their mixture where 70% methanol is used as a reagent blank. The first derivative absorption spectra of quercetin, gentisic acid and their mixture are depicted in Fig. 4. To determine the amount of quercetin in the presence of gentisic acid, data were measured at gentisic acid zero-crossing point of 271.57 nm and 396.5 nm at which gentisic acid has no UV absorption, Figs. 4 and 6. In the same manner, data for quantification of gentisic acid in the mixture solution were recorded at 306 nm where quercetin reaches the zero-crossing point, Figs. 4 and 8.

Additionally, these two phenolic compounds were also determined simultaneously in their mixture solution with the aid of the developed fourth derivative technique. As can be seen in Figs. 5, 7, and 9, a few zero-crossing points for both phenolics, quercetin at 255.70 nm, 263.80 nm, 272.50 nm and 280.40 nm, and gentisic acid at 249 nm, are indicated in this method to quantify their amounts in the binary mixture. Table 1 demonstrates the outcomes of the calibration graph's statistical analysis related to the first and fourth derivative spectrophotometric methods for simultaneous determination of quercetin and gentisic acid in the binary mixture.

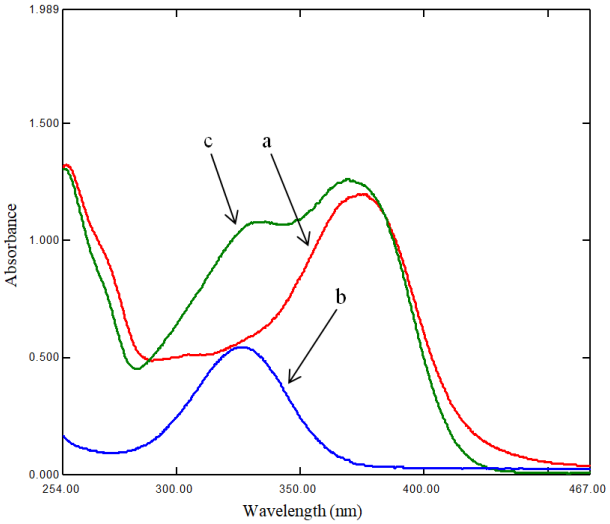

Figure 3. Normal spectra (Zero-order) of a: quercetin (20 µg/mL), b: gentisic acid (20 µg/mL) and c: their mixture in 70% methanol.

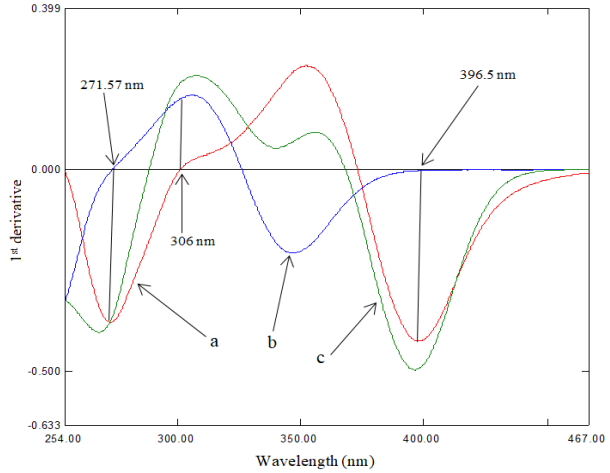

Figure 4. First order derivative spectra of a: quercetin (20 µg/mL), b: gentisic acid (20 µg/mL), and c: their mixture in 70% methanol, Δλ = 24 nm.

2024, 21(5): 1536-1548
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Baghdad Science Journal

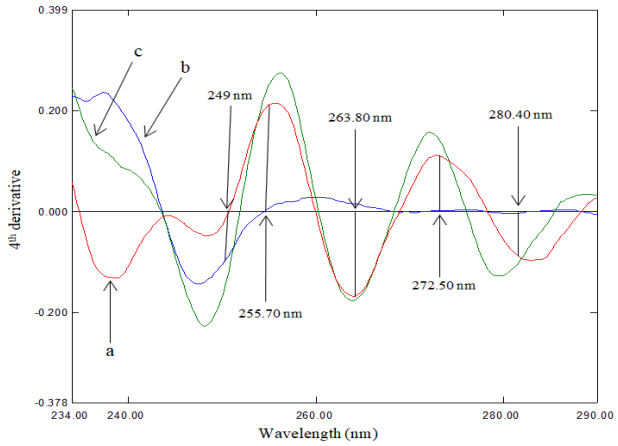

Figure 5. Fourth order derivative spectra of a: quercetin (20 µg/mL), b: gentisic acid (20 µg/mL), and c: their mixture in 70% methanol, Δλ = 12 nm.

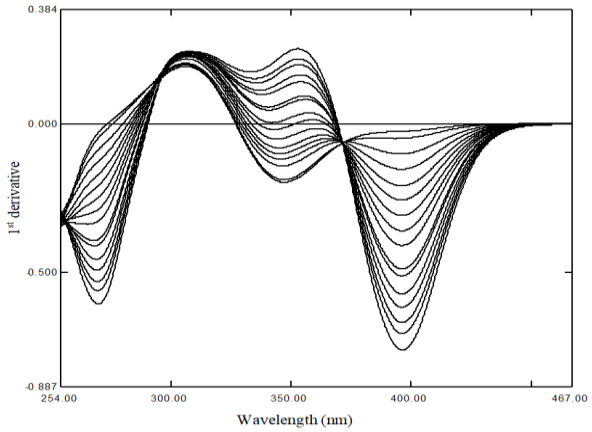

Figure 6. Derivative spectra (1st order) of a mixture containing 0.5-30 µg/mL quercetin and 20 µg/mL gentisic acid.

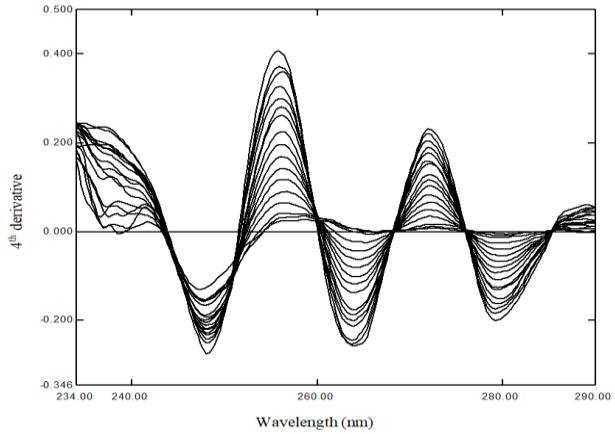

Figure 7. Derivative spectra (4th order) of a mixture containing 0.5-30 µg/mL quercetin and 20 µg/mL gentisic acid.

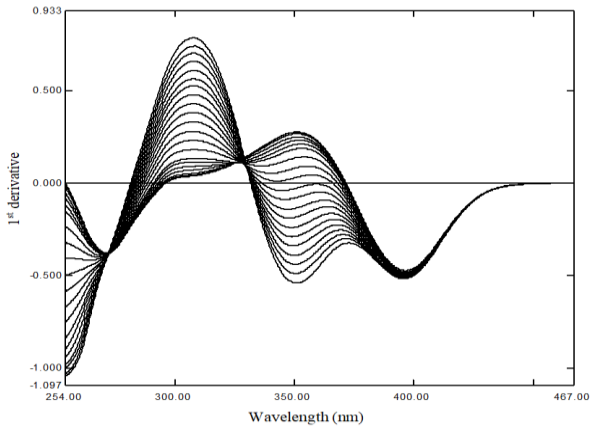

Figure 8. Derivative spectra (1st order) of a mixture containing 0.5-80 µg/mL gentisic acid and 20 µg/mL quercetin.

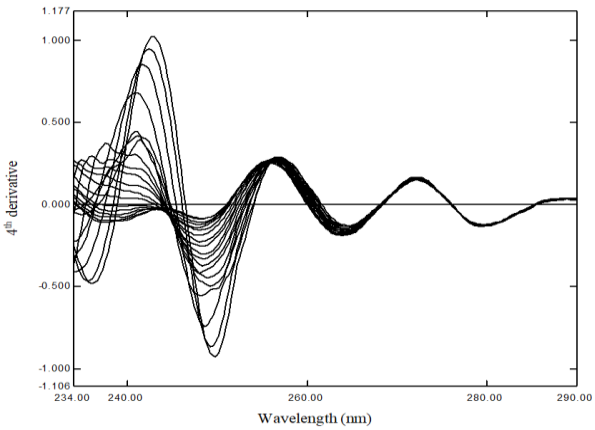

Figure 9. Derivative spectra (4th order) of a mixture containing 0.5-30 µg/mL gentisic acid and 20 µg/mL quercetin.

Ratio Spectra Derivative Method

In order to acquire the ratio spectra in the wavelength range of 200 – 800 nm, presented in Fig. 10a, the absorption spectra of quercetin at different concentrations 0.5-30 µg/mL in the binary mixture were recorded, and then the spectra were divided by the standard spectrum of gentisic acid (50 µg/mL) in 70% methanol. Afterward, the obtained ratio spectra were used to establish the first derivative (Δλ = 12 nm) as shown in Fig. 10b. The amplitudes at 252.25 nm ($^1DD_{252.25}$), 277 nm ($^1DD_{277}$), 370.20 nm ($^1DD_{370.20}$) and 398.14 nm ($^1DD_{398.14}$) were then used for the determination of quercetin concentration in the binary mixture.

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Baghdad Science Journal

Likewise, the ratio spectra of gentisic acid were obtained when gentisic acid absorption spectra were at different concentrations (0.5 to 80 µg/mL) in the binary mixture divided by the standard spectrum of quercetin (12 µg/mL) in 70% methanol. Then, the ratio spectra (Δλ = 12 nm) were used to obtain the 1st derivative of gentisic acid as illustrated in Fig. 11b. The quantity of gentisic acid was then determined in the binary mixture using the peak to baseline amplitude at 245.72 nm ($^1DD_{245.72}$), 307.50 nm ($^1DD_{307.50}$) and 343.38 nm ($^1DD_{343.38}$). The optimization of concentrations of the divisor is one of the most important factors that should be performed, and therefore different divisor concentrations have been studied. It was found that using the standard solution of gentisic acid (50 µg/mL) give the higher signal-to-noise ratio, thus, it was selected as a divisor for measuring quercetin in the binary mixture. Also, quercetin standard solution (12 µg/mL) provided the best signal-to-noise ratio, and it was suitable to use as the divisor for the quantification of gentisic acid in the binary mixture. Moreover, it was noticed that delta lambda (Δλ) value had a great impact on the ratio spectra 1st order derivative. The level of noise declined noticeably as the Δλ values increased.

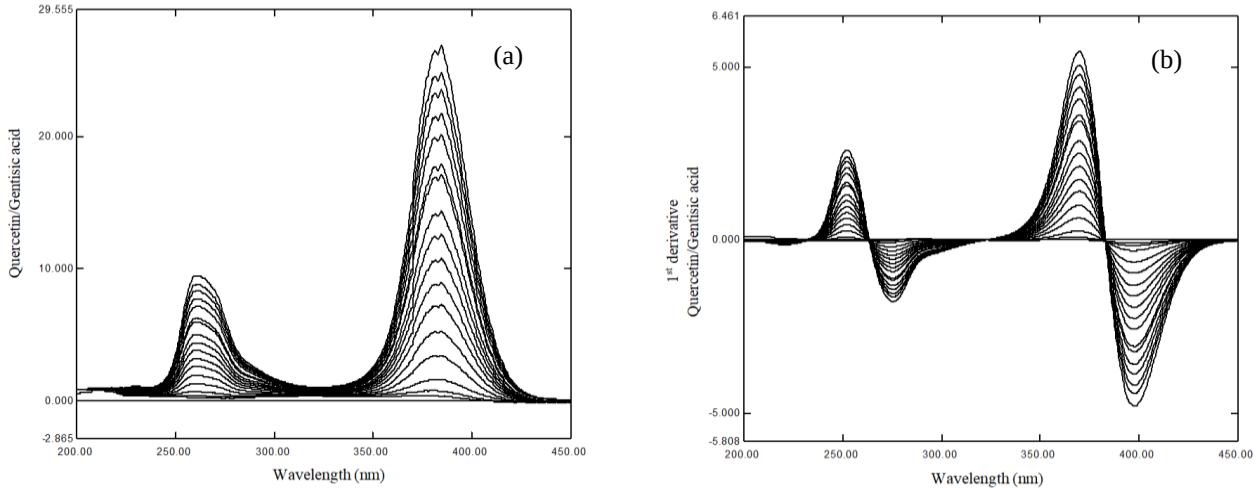

Figure 10. a: Ratio spectra and b: ratio spectra 1st order derivative (Δλ = 12 nm) of quercetin (0.5-30 µg/mL) [the divisor was gentisic acid (50 µg/mL) in 70% methanol].

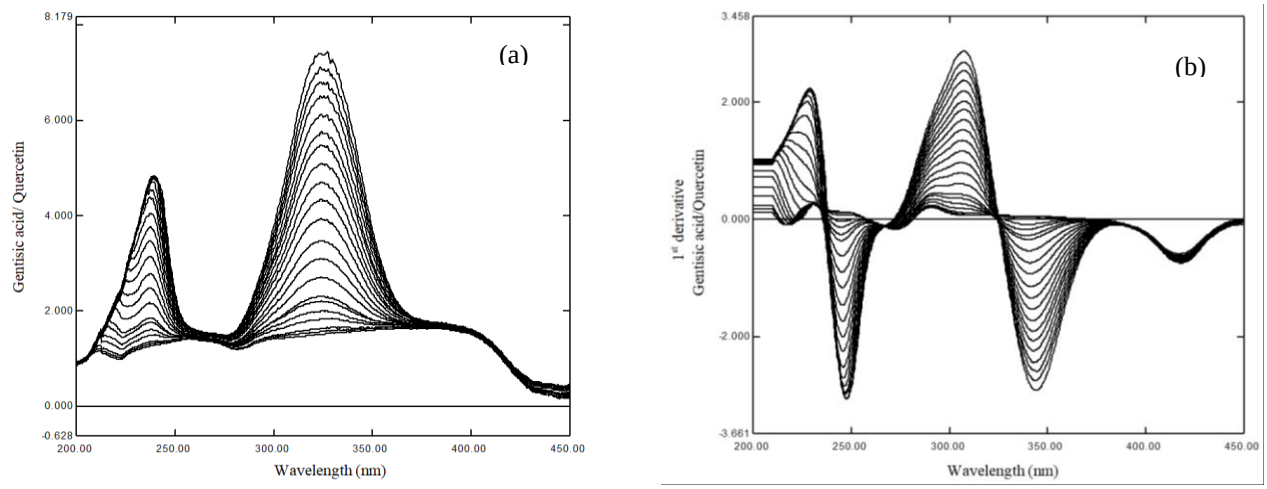

Figure 11. a: Ratio spectra and b: ratio spectra 1st order derivative (Δλ = 12 nm) of gentisic acid (0.5-80 µg/mL) [the divisor was quercetin (12 µg/mL) in 70% methanol].

2024, 21(5): 1536-1548
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Baghdad Science Journal

Table 1. The statistical parameters attained in the quercetin and gentisic acid determination using 1st derivative, 4th derivative and ratio spectra derivative spectrophotometric methods.

Methods	Compounds	λ_{max} (nm)	Linear ranges (µg/mL)	Regression equations	r^2	LOD (µg/mL)	LOQ (µg/mL)
Zero crossing	Quercetin	$^1D_{271.57}$	2.0-30.0	y = 0.0203x - 0.0076	0.999	0.000	0.000
1st derivative		$^1D_{396.5}$	4.0-30.0	y = 0.0256x + 0.002	0.998	0.040	0.123
	Gentisic acid	$^1D_{306}$	8.0-80.0	y = 0.0095x + 0.0379	0.999	0.109	0.332
Zero crossing	Quercetin	$^4D_{255.70}$	4.0-30.0	y = 0.0131x + 0.0113	0.998	0.159	0.482
4th derivative		$^4D_{263.80}$	6.0-30.0	y = 0.0095x - 0.0103	0.995	0.407	1.235
		$^4D_{272.50}$	6.0-30.0	y = 0.0075x + 0.0049	0.997	0.278	0.842
		$^4D_{280.40}$	6.0-30.0	y = 0.0064x + 0.0017	0.998	0.532	1.612
	Gentisic acid	$^4D_{249}$	8.0-60.0	y = 0.0076x + 0.0633	0.997	0.558	1.692
RSD	Quercetin	$^1DD_{252.25}$	4.0-30.0	y = 0.0896x - 0.088	0.998	0.011	0.035
		$^1DD_{277}$	2.0-30.0	y = 0.0602x - 0.051	0.999	0.028	0.087
		$^1DD_{370.20}$	4.0-30.0	y = 0.1863x - 0.0952	0.999	0.000	0.000
		$^1DD_{398.14}$	4.0-30.0	y = 0.1606x + 0.0113	0.999	0.009	0.030
	Gentisic acid	$^1DD_{245.72}$	4.0-55.0	y = 0.0536x - 0.1678	0.999	0.031	0.096
		$^1DD_{307.50}$	8.0-80.0	y = 0.0355x + 0.0599	0.999	0.043	0.132
		$^1DD_{343.38}$	8.0-80.0	y = 0.0377x - 0.0209	0.998	0.042	0.128

RSD: ratio spectra derivative method; LOD: limits of detection; LOQ: limits of quantification.

Table 2. Accuracy and precision of the developed techniques including 1st derivative, 4th derivative and ratio-derivative spectra for quercetin and gentisic acid determination in binary mixtures.

Compounds	Techniques of analysis	Concentrations (µg/mL)	Recovery (%)	RSD (%)	Error (%)
Quercetin	Zero-crossing technique at $^1D_{271.57nm}$	2.0	95.07	2.94	2.46
		12.0	99.17	0.68	1.55
		30.0	99.44	0.53	-1.93
	Zero-crossing technique at $^1D_{396.5nm}$	4.0	97.65	1.76	1.56
		12.0	99.93	0.88	1.49
		30.0	98.95	0.37	-1.45
	Zero-crossing technique at $^4D_{255.70nm}$	4.0	100.57	2.76	3.62
		12.0	97.77	1.76	-2.22
		30.0	100.17	0.54	-1.29
	Peak-to-baseline at $^4D_{263.80nm}$	6.0	95.26	4.29	-3.30
		12.0	97.63	4.32	1.49
		30.0	99.40	0.94	0.45
	Zero-crossing technique at $^4D_{272.50nm}$	6.0	95.77	3.77	-2.40
		12.0	105.66	2.23	2.11
		30.0	98.26	1.27	1.11
	Zero-crossing technique at $^4D_{280.40nm}$	6.0	99.73	4.11	-1.30
		12.0	99.34	1.69	-2.21

2024, 21(5): 1536-1548
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Baghdad Science Journal

		30.0	98.59	0.92	0.88
	Ratio spectra derivative	4.0	97.65	0.96	-1.78
	$^1DD_{252.25nm}$	12.0	97.75	1.06	-2.19
		30.0	99.85	0.46	-0.83
	Ratio spectra derivative	2.0	93.85	3.98	-4.40
	$^1DD_{277nm}$	12.0	99.11	1.04	-1.71
		30.0	99.44	1.07	-0.73
	Ratio spectra derivative	4.0	97.58	1.01	-2.09
	$^1DD_{370.20nm}$	12.0	99.44	0.45	-0.61
		30.0	98.93	0.25	-1.06
	Ratio spectra derivative	4.0	96.93	0.79	-3.03
	$^1DD_{398.14nm}$	12.0	99.92	0.50	-0.60
		30.0	99.08	0.19	-1.04
Gentisic acid	Zero-crossing technique at	8.0	100.13	2.30	4.60
	$^1D_{306nm}$	30.0	102.49	4.04	1.08
		80.0	98.03	0.23	-2.35
	Zero-crossing technique at	8.0	91.61	1.36	-2.79
	$^4D_{249nm}$	30.0	102.06	0.38	1.00
		60.0	101.46	0.65	1.46
	Ratio spectra derivative	4.0	97.85	4.60	-1.67
	$^1DD_{245.72nm}$	30.0	101.83	0.80	2.16
		55.0	97.51	1.73	-2.46
	Ratio spectra derivative	8.0	100.38	1.16	0.95
	$^1DD_{307.50nm}$	30.0	103.20	2.51	2.33
		80.0	98.70	0.45	-1.34
	Ratio spectra derivative	8.0	94.13	1.62	-4.94
	$^1DD_{343.38nm}$	30.0	102.73	0.49	2.90
		80.0	97.50	1.09	-2.35

RSD%: percentage relative standard deviation; Error %: error percentage

Limits of Detection (LOD) and Quantification (LOQ)

The lowest concentration that may be observed accurately and precisely is referred to the limit of detection. The quantification and detection limits of the proposed techniques are computed as LOQ = 10 σ/S and LOD = 3.3 σ/S, where S represents the slope of calibration curves and σ denotes the reagent blank's standard deviation [27, 39].

Accuracy and Precision

Based on the error percentage calculation (Error %) for three different standard concentrations of each phenolic in the binary mixture with five replicated measurements, the accuracy of the proposed methods was evaluated. Likewise, precision was assessed by calculating the percentage of relative standard deviation (RSD %) at three distinct binary mixture concentrations with five replicated measurements for each concentration.

Calibration Graph and Statistical Data:

In order to simultaneously quantify quercetin and gentisic acid using two different spectrophotometric methods, all analytical parameters and calibration curve's statistical data of the suggested approaches were calculated for each compound, as illustrated in Table 1. These parameters include LOQ, LOD, relative standard deviation, the linear range of the calibration graph and correlation coefficients. In all the proposed methods, the high values of correlation coefficients ($r^2 \geq 0.9952$) and excellent linearity for the calibration curves of both phenolic compounds were observed, Table 1. Under the conditions of the described analytical methods, the lowest limit of detection (LOD) and limit of quantification (LOQ) were achieved which indicates the sensitivity of the methods. As shown in Table 2, the suggested spectrophotometric methods allowed good precision at which the relative standard deviations for both chemicals were less than or equal to 4.32% for the five replicated measurements of the three different

2024, 21(5): 1536-1548
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Baghdad Science Journal

standard concentrations. Also, the developed methods showed excellent accuracy at which satisfactory recovery percentage (93.85 - 105.66%) and relative error percentage (Error %, -4.94% to 4.6%) were obtained when the determination of both phenolics carried out in quintuplicate at three different concentrations, Table 2. All the results including relative standard deviations, recovery and error percentages are illustrated in Table 2.

Application of the Methods:

Quercetin and gentisic acid were quantified successfully using both zero crossing and ratio spectra derivative methods in *Capparis spinosa* leaf extract sample. Standard addition method was applied to find the recoveries and concentration of the phenolic compounds. At three different spiking concentrations, the recoveries percentage was calculated by spiking with 6.0, 8.0 and 10.0 µg/ml for both compounds. Table 3 provides the results of recovery percentages of quercetin and gentisic acid in the sample at three fortification levels. The average recoveries were between 94.06% and 113.37% in all situations for quercetin and gentisic acid, respectively. The derivative spectrophotometric techniques were effectively applied for the simultaneous determination of the actual amounts of quercetin and gentisic acid present in the real sample (*Capparis spinosa*), as shown in Table 4.

Table 3. Recovery percentages of quercetin and gentisic acid determination in *Capparis spinosa* sample by the proposed methods.

Concentration (µg/mL)	Quercetin			Gentisic acid		
	^{1}D	^{4}D	^{1}DD	^{1}D	^{4}D	^{1}DD
	R %	R %	R %	R %	R %	R %
6	98.71	105.98	96.0	111.41	107.58	113.37
8	94.90	102.26	94.29	108.13	105.72	110.02
10	94.06	102.63	94.70	100.89	97.32	104.03

Table 4. The residues (µg/mL) of recovery percentage of quercetin and gentisic acid found in *Capparis spinosa* using the developed spectrophotometric methods.

Compounds	Methods	Actual amount found in the sample (*Capparis spinosa*) (µg/mL)	Spiked amount (µg/mL)	Total amount found in spiked sample (µg/mL)	Recovery%
Gentisic acid	1st Derivative	2.32	10	12.43	100.89
	4th Derivative	2.31	10	11.98	97.32
	RSD method	2.67	10	13.18	104.03
Quercetin	1st Derivative	15.54	10	24.023	94.06
	4th Derivative	16.23	10	26.92	102.63
	RSD method	13.03	10	21.81	94.70

Comparison with other Spectrophotometric Methods:

In order to compare the proposed methods for simultaneous determination of gentisic acid and quercetin in *Capparis spinosa* with other previously reported spectrophotometric methods, various analytical variables obtained from these procedures could be utilized for the comparison. Fortunately, neither classical spectrophotometric and nor derivative spectrophotometric methods have been done yet for the determination of gentisic acid alone or simultaneously in binary mixtures with other phenolic compounds especially quercetin. As shown in Table 5, the UV spectrophotometric methods reported in the literature for the estimation of quercetin displayed the linearity from the concentrations of 2-12 µg/mL, while the methods developed in this study covered a wider linear range from 2-30 µg/mL. It can also be noticed from Table

2024, 21(5): 1536-1548
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Baghdad Science Journal

5 that the proposed methods are more precise and sensitive than other published spectrophotometric methods. As a result, the derivative spectrophotometric methods have high accuracy and precision, better linearity and good recovery percentages for the determination of quercetin and gentisic acid in a binary mixture.

Table 5. Comparison of the proposed methods with some other methods for determination of quercetin.

Analytical parameters	Zero crossing	RSD	Reported method [40]	Reported method [41]
Linearity range (µg/mL)	2.0 – 30.0	2.0 - 30	2 – 12.0	2 – 12.0
LOD (µg/mL)	0.532	0.028	0.150	0.817
Recovery (%)	94.06 - 105.98	94.29 - 96.0	99.00	98.27 - 100.84
RSD (%)	Below 4.32	Below 3.98	0.33	Below 2.00
Application	*Capparis spinosa*	*Capparis spinosa*	*Tagetes Erecta*	*Calendula*

RSD: ratio spectra derivative method; RSD%: percentage relative standard deviation.

Conclusion

This is the first research describing simultaneous derivative spectrophotometric methods for the quantification of quercetin and gentisic acid in the plant leaves sample, *Capparis spinosa*, collected from Iraqi Kurdistan Region – Rawanduz. In the current study, first and fourth derivative zero-crossing methods along with ratio spectra first derivative methods have been developed and used for simultaneous determination of quercetin and gentisic acid in the binary mixtures. Derivative spectrophotometry is a novel, simple and rapid technique which allows the determination of compounds in binary or ternary mixtures without the need for prior separation. The normal spectra of quercetin and gentisic acid are completely overlapped with the classical spectrophotometry, thus quantification of the two phenolic compounds is difficult with this method, whereas with the proposed derivative methods, this problem was easily tackled and consequently the overlapping spectra resolved in their mixtures. The presented statistical analysis showed that there is no obvious difference between the proposed approaches for the determination of quercetin and gentisic acid amounts in *Capparis spinosa*. Good recoveries, achieved from *Capparis spinosa*, range between 94.06% - 105.98% for quercetin and 94.29% - 113.37% for gentisic acid, which indicates that the developed approaches are simple, accurate, fast and economical. Moreover, the suitable results of relative standard deviation (RSD %) and the relative error percentage (Error %) were achieved which indicates good precision and accuracy for the suggested approaches. To sum up, the suggested derivative spectrophotometric techniques could be considered a very successful method for the quantification of quercetin and gentisic acid simultaneously in a binary mixture and *Capparis spinosa*, in comparison with other classical spectrophotometric techniques.

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Authors' Declaration

- Conflicts of Interest: None.

- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Soran.

Authors' Contribution Statement

DHM carried out the practical part of research and wrote the research manuscript. RHM planned the conception and design of the research and supervised the project with providing support and giving the critical feedback during writing the manuscript. Both authors discussed the results and contributed to the final manuscript.

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طرق قياس الطيف الضوئي المشتقة لتقدير الآنى لمادة كويرستين وحامض الجنتسيك في نبات الشفلح

دلكش حمد معروف[1]، رزكار حسن محمد[2]

[1]قسم الكيمياء، كلية العلوم، جامعة سوران، أربيل، العراق.
[2]قسم العلوم العامة، كلية التربية، جامعة سوران، أربيل، العراق.

الخلاصة

الشفلح هو أحدى النباتات الطبية الذي يستخدم في الطب التقليدي و يتضمن المواد الكيميائية النباتية العديدة مثل المركبات بوليفينولية. تعتبر كلتا المركبتين، كويرستين وحامض الجينتسيك، كمركب فينولية مهمة اللتان توجدان في النباتات ولهما الخصائص الطبية الكثيرة مثل مضاد الالتهابات، مضاد الميكروبات، مضاد الأكسدة ومضاد للسرطان. لم يتم التحقق بعد من تحديد كلا المركبين معًا في مزيج ثنائي باستخدام طرق القياس الطيفي. في هذه الدراسة، تم تطوير طريقتين طيفيتين مشتقتين بسيطتين سريعتين و دقيقتين لاستخدامهما للتقدير الكمي الآنى لمادة كويرستين وحامض الجينتسيك في المزيج الثنائية وخلاصة أوراق الشفلح. الطريقة الأولى تعتمد على استخدام طريق العبور الصفري (المشتق الأول والرابع) ، بينما الطريقة الثانية هي المشتق الأول لأطياف النسبة. الخطية لمنحنيات المعايرة لكلتا الطريقتين الطيفية المشتقة يتراوح تركيزهما بين 2.0-30 ميكروغرام / مل و 4.0-80 ميكروغرام / مل لكويرستين وحامض الجينتسيك، على التوالي، في حين تراوحت نسب الاسترداد من 94.06٪ الى 105.98٪ (كويرستين) و 94.29٪ الى 113.37٪ (حمض الجينتسيك). تم استخدام الطرق المطورة بشكل فعال في التقدير الكمي لكل من المركبات الفينولية في أوراق الشفلح..

الكلمات المفتاحية: الشفلح، المشتق القياس الطيفي، كويرستين، حامض الجينتسيك، طريقة نسبه المشتق الاطياف، طريقة معبر الصفر.