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Simulteneous Equation Method for the Estimation of Gallic Acid and Apigenin by Uv-visible Spectrophotometry

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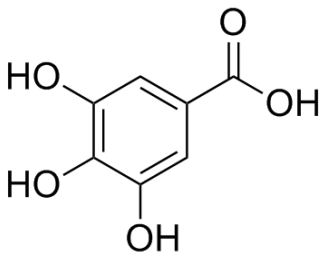
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Abstract

A simple, rapid, accurate, economic and precise UV/VIS method has been developed and validated. Choices of a common solvent were essential so various solvent ranges including methanol, ethanol, Acetonitrile and phosphate buffer and various concentrations ranges of various buffers were analyzed. Hence methanol was selected as a solvent for the proposed method. Gallic Acid and Apigenin showed maximum absorbance at 217 and 268 nm respectively. Both drugs obey Beer Lambert's law in the concentration range of 0.5-3 µg mL-1 for Gallic Acid and Apigenin respectively. The LOD and LOQ were found to be 0.00379 µg mL-1and 0.011µg mL-1for Gallic Acid, respectively. For, Apigenin the LOD and LOQ values were found to be 0.00801 µg mL-1 and 0.024µg mL-1 respectively. The method was quantitatively evaluated in terms of linearity, precision, precision, LOD, LOQ and recovery. The method is simple, convenient and suitable for the analysis of Gallic Acid and Apigenin in bulk drug.

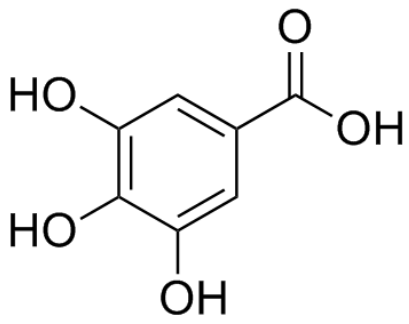
Introduction

Gallic acid is a trihydroxybenzoic acid in which the hydroxy groups are at positions 3, 4, and 5. It has a role as an astringent, a cyclooxygenase 2 inhibitor, a plant metabolite, an antioxidant, an antineoplastic agent, a human xenobiotic metabolite, an EC 1.13.11.33 (arachidonate 15-lipoxygenase) inhibitor, an apoptosis inducer and a geroprotector. It is a conjugate acid of a gallate.[2,4]



Gallic acid

Apigenin is a trihydroxyflavone that is flavone substituted by hydroxy groups at positions 4', 5 and 7. It induces autophagy in leukaemia cells. It has a role as a metabolite and an antineoplastic agent. It is a conjugate acid of an apigenin-7-olate.[1,3]



Apigenin

Literature survey revealed that several analytical methods have been described for analysis of Gallic Acid and Apigenin as single component or in combinations with other drugs (formulations and in biological samples). In biological fluid as single drug candidate, however very few methods have been reported for simultaneous estimation of Gallic Acid and Apigenin.

In combination with other drugs from biological samples. Literature survey revealed reports on analytical methods such as UV-VIS, HPLC, LC-MS, LC-MS-MS and HPTLC for the determination of Gallic Acid and Apigenin; moreover reported methods were not much cost-effective in terms of solvent consumption, so the present study was undertaken. The exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals includes these drugs in combination for the simultaneous determination of Gallic Acid and Apigenin by UV/Vis spectroscopy. So it was felt essential to develop a procedure which will serve a reliable, accurate.

Materials And Methods

Instrument: UV Spectrophotometer: Shimadzu 2700 (UV probe 2.35)

Chemicals and reagents: Methanol (Analytical Grade)

Preparation of Standard Stock Solutions: 50 mg Gallic Acid and Apigeninwere weighed separately. Transferred in to 50ml volumetric flask & dissolved in methanol. Volume was made up to mark with methanol. Make 1000 µg mL-1 of Gallic Acid and1000 µg mL-1 of Apigenin.

Preparation of Working Standard Solution: Take 1 ml from 1000 µg mL-1 and dilute up to 100 µg mL-1 to get 100 µg mL-1and makes the concentration range of 0.5, 1, 1.5, 2, 2.5 and 3 ml Gallic Acid and Apigenin. The absorbances were measured at 217 and 268 nm respectively.

Selection of Analytical Wavelength: From standard solution of Gallic Acid (100 µg mL-1) and Apigenin(100 µg mL-1) prepare 1 µg mL-1 for Gallic Acid and Apigenin. The scanning for solution of Gallic Acid and Apigeninwere carried out in the range of 200-400 nm against using methanol as a blank. The maximum absorption (λmax) of Gallic Acid and Apigenin was found at 217 and 268 nm (Fig. 1and

Fig. 2). Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths. The two wavelengths selected should be such that at each wavelength the difference in the absorptivity between the two components should be as large as possible.

Determination: The standard solution of Gallic acid was scanned in the range of 200-400 nm and the λmax was found to be 217 nm against methanol (Fig. 1). Similarly, the standard solution of Apigenin was scanned in the range of 200-400 nm and the λmax was found to be 268 nm against methanol (Fig. 2).

The absorbances of both the drugs were recorded at 217 and 268 nm and molar absorptivity (e) for both the drugs which was calculated from the formula.

e = A/C

Where, A = absorbance, C = concentration of analyte

Simultaneous equation method: If a sample contains two absorbing drug each of which absorbs at the λmax of the other, it may be possible to determine both drugs by the technique of simultaneous equation. Two wavelengths selected for the development of the simultaneous equations are 217 and 268 nm. The absorptivity values determined for Gallic acid are 0.124 (ax1), 0.154 (ax2) and for Apigenin are 0.143 (ay1), 0.189 (ay2) at 217 and 268 nm respectively. These values are means of six estimations. The Absorptivity data are shown in Table I. The absorbance's and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of both drugs.

Cx = A2ay1 – A1ay2/ ax2ay1 – ax1ay2 ---------- equation no – 1.

Cy = A1ay2 – A2ay1/ ax2ay1 – ax1ay2 ---------- equation no – 2.

Where Cx and Cy are concentration of Gallic Acid and Apigeninrespectively in µg mL-1. A1 and A2 are the absorbance of the mixture 217 and 268 nm respectively.

Method validation: The method was developed and validated according to analytical procedure as per the ICH guidelines for validation of analytical procedures in order to determine linearity, precision, LOD, LOQ and accuracy for the analyte.[5.6.7]

Linearity: The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Gallic Acid and Apigenin. For both the method, the Beer-Lambert's concentration range was found to be 0.5, 1, 1.5, 2, 2.5 and 3 µg mL-1 for Gallic Acid and Apigeninrespectively.

The linearity of the relationship between absorbance's and concentration was determined by plotting the calibration curve for Gallic Acid and Apigenin respectively shown in Fig. 4 and Fig. 5.

Limit of Detection and Limit of Quantitation: Limit of detection (LOD) is the lowest amount of an analyte that can be detected but not necessarily as an exact value. Limit of quantification (LOQ) is the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and

accuracy 24. The LOD and LOQ were separately determined which is based on calibration The standard deviation of y intercept of regression line may be used. The given concentration was used for this and the absorbance was taken and then calculates the standard deviation and slope by following formula. Results are shown in Table III.

LOD = 3.3 X σ/s,

LOQ= 10 X σ/s

Where,

σ=Standard deviation of y intercept of regression lines,

S =Slope of calibration Curve

Precision: Repeatability measurement carried out by analyzing solution containing 1.5 µg mL-1 of Gallic Acid and Apigenin six times and %RSD was calculated. Repeatability of method was established by analyzing various replicates samples of Gallic Acid and Apigenin Precision were carried out by performing inter day variation and intraday variation. In Inter day variation the sample was analyzed on three consecutive days the interday's and intra precision was determined using10µg mL-1 concentration. In intraday variation the absorbances were measured three times in a day.

Intraday Precision: The Intra-day precision was determined for 0.5, 1 and 1.5 mg/mL standard solution of Gallic Acid and Apigenin for the three times on the same day. Percentage relative standard deviation (% RSD) should be considered.

Interday Precision: The Interday precision was determined for 0.5, 1 and 1.5 mg/mL standard solution of Gallic Acid and Apigenin for the three times on the same day. Percentage relative standard deviation (% RSD) should be considered.

Accuracy (% Recovery): The accuracy study was performed using the standard addition method. The pre-quantified 1 µg mL-1sample solution of Gallic Acid and Apigenin were spiked with an extra 80, 100, and 120% of the standard Gallic Acid and Apigenin. Absorbances were measured at 217 nm and 268 nm (λmax of Gallic Acid and Apigenin, respectively) and the concentration of both drugs can be determined. These mixtures were analyzed by the developed method. The experiment was performed in six times. The percentage recovery of the samples, % RSD and the percentage were calculated at each concentration level.

Results And Discussion

Overlain spectra of the Gallic Acid and Apigenin depicted occurrence of two peaks at 217 nm and 264 nm, shown in Fig. 3. To optimize the UV parameters, several conditions were tried to achieve a good absorbation and peak shape for Gallic Acid and Apigenin. Several solvents of different compositions

were tried to provide sufficient selectivity towards the drugs. Methanol as organic components resulted in better sensitivity.

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of Gallic Acid and Apigenin. In simultaneous equation method, wavelengths selected for analysis were 217 nm for Gallic Acid and 268 nm for Apigenin (Fig. 4). The absorbance of Gallic Acid is 0.119 to 0.999 and for Apigenin is 0.046 to 0.448, shown in table ii. Selected methods linearity was observed in the concentration range of 0.5 to 3 µg mL−1for Gallic Acid and Apigenin. in this method concentration of the individual drug was determined by solving the simultaneous equation at 217 nm and 268 nm using the respective absorptivity value. Percent recovery for Gallic Acid and Apigenin, by both the methods, was found in the range of 98 % to 102.21 %.

Linear correlation was obtained between absorbance Vs concentration of Gallic Acid and Apigenin; calibration curves were linear in the concentration range from 0.5 to 3 µg mL−1. Linearity of the calibration curves were validated by the value of correlation coefficients of the regression (r2). The correlation coefficients were 0.997 for Gallic Acid and 0.99 for Apigenin. The results of the linearity experiment are listed in Table III.

Intra-day precision of the method ranged from 0.75 to 0.67 %RSD for Gallic Acid and 0.64 to 0.31 for Apigenin (Table IV). Inter-days precision of the method was found to be 1.51 to 0.59 %RSD for Gallic Acid and 0.90 to 1.09 for Apigenin (Table V), which indicate that the developed method is precise. The low values of the RSD (%) indicate the repeatability of the proposed method. The LODs for Gallic Acid and Apigenin were 0.00379 and 0.00801 found to be µg mL-1, while the LOQs for Gallic Acid and Apigenin were 0.011 and 0.024 µg mL-1, respectively (Table III). The accuracy experiments were carried out by the standard addition method. The percentage recoveries for Gallic Acid and Apigenin were found in the range of 98-102.62% and 98-102.21% respectively. The values of the recovery (%) and %RSD are shown in Table VI and VII, which indicated the accuracy of the proposed method.

Conclusion

All the above factors lead to the conclusion that the proposed method is simple, accurate, precise, sensitive and cost effective and can be applied successfully for the estimation of Gallic Acid and Apigenin in herbal formulation.

Declarations

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Tables

Table I: Absorptivity data for Gallic Acid and Apigenin

Conc of Apigenin in ppm	Absorptivity of Apigenin		Conc of Gallic acid in ppm	Absorptivity of Gallic acid	
	ax1	ax2		ay1	ay2
0.5	0.182	0.27000	0.5	0.22	0.08
1	0.143	0.18900	1	0.124	0.154
1.5	0.125	0.15267	1.5	0.092666667	0.166666667
2	0.117	0.1375	2	0.076	0.183
2.5	0.112	0.13000	2.5	0.066	0.181
3	0.107	0.12667	3	0.06	0.183
Avg	**0.131**	**0.16764**	**Avg**	**0.10636**	**0.15788**

Table II: Linearity study for Gallic Acid and Apigenin

Sr. No.	Gallic acid		Apigenin	
	Conc. (µg/ml)	Mean Absorbance at 217 nm (A2)	Conc. (µg/ml)	Mean Absorbance at 268 nm (A1)
1	0.5	0.119	0.5	0.046
2	1	0.261	1	0.099
3	1.5	0.435	1.5	0.189
4	2	0.645	2	0.284
5	2.5	0.795	2.5	0.366
6	3	0.999	3	0.448

Table III: Linear regression analysis of calibration curves (n = 6)

Parameter	Gallic acid	Apigenin
Wavelength (nm)	217	268
Linearity	0.5-3µg/ml	0.5-3µg/ml
Linear regression equation		
Intercept (c)	**0.078**	**0.057**
Slope (m)	0.355	0.168
Correlation coefficient (R2)	0.997	0.997
LOD	0.00379	0.00801
LOQ	0.011	0.024
Intercept (c)	**0.078**	**0.057**

Table IV: Intraday Precision for Gallic Acid and Apigenin (n = 6)

Sr. No.	Conc. (µg/ml)	Absorbance (Mean) at 217 nm (n=3)	SD	% RSD	Absorbance (Mean) at 268 nm (n=3)	SD	% RSD
1	0.5	0.134	0.0010	0.75	0.090	0.001	0.64
2	1	0.185	0.0015	0.82	0.143	0.001	0.40
3	1.5	0.229	0.0015	0.67	0.187	0.001	0.31

Table V: Interday Precision for Gallic Acid and Apigenin (n = 6)

Sr. No.	Conc. (µg/ml)	Absorbance (Mean) at 217 nm (n=3)	SD	% RSD	Absorbance (Mean) at 268 nm (n=3)	SD	% RSD
1	0.5	0.038	0.0006	1.51	0.111	0.001	0.90
2	1	0.159	0.0015	0.96	0.125	0.001	0.80
3	1.5	0.260	0.0015	0.59	0.141	0.002	1.09

Table VI: Statistical Validation for recovery studies of Gallic acid (n = 6)

Sr. No.	Level of Spiking	Conc. Taken (µg/ml)	Amount of standard Gallic acid added (µg/ml)	Amount found (µg/ml)	% Recovery ± SD	RSD
1	Control	1	0	0.98	98 ± 0.0008	0.57854
2	80 %	1	0.8	1.84	102.62 ±0.0015	0.47157
3	100 %	1	1	2.02	102±0.0008	0.24531

Table VII: Statistical Validation for recovery studies of Apigenin (n = 6)

Sr. No.	Level of Spiking	Conc. Taken (µg/ml)	Amount of standard Apigenin added (µg/ml)	Amount found (µg/ml)	% Recovery ± SD	RSD
1	Control	1	0	0.98	98 ± 0.0008	0.62286
2	80 %	1	0.8	1.82	101.61 ± 0.0008	0.40953
3	100 %	1	1	2.04	102.21 ± 0.0017	0.73676

Figures

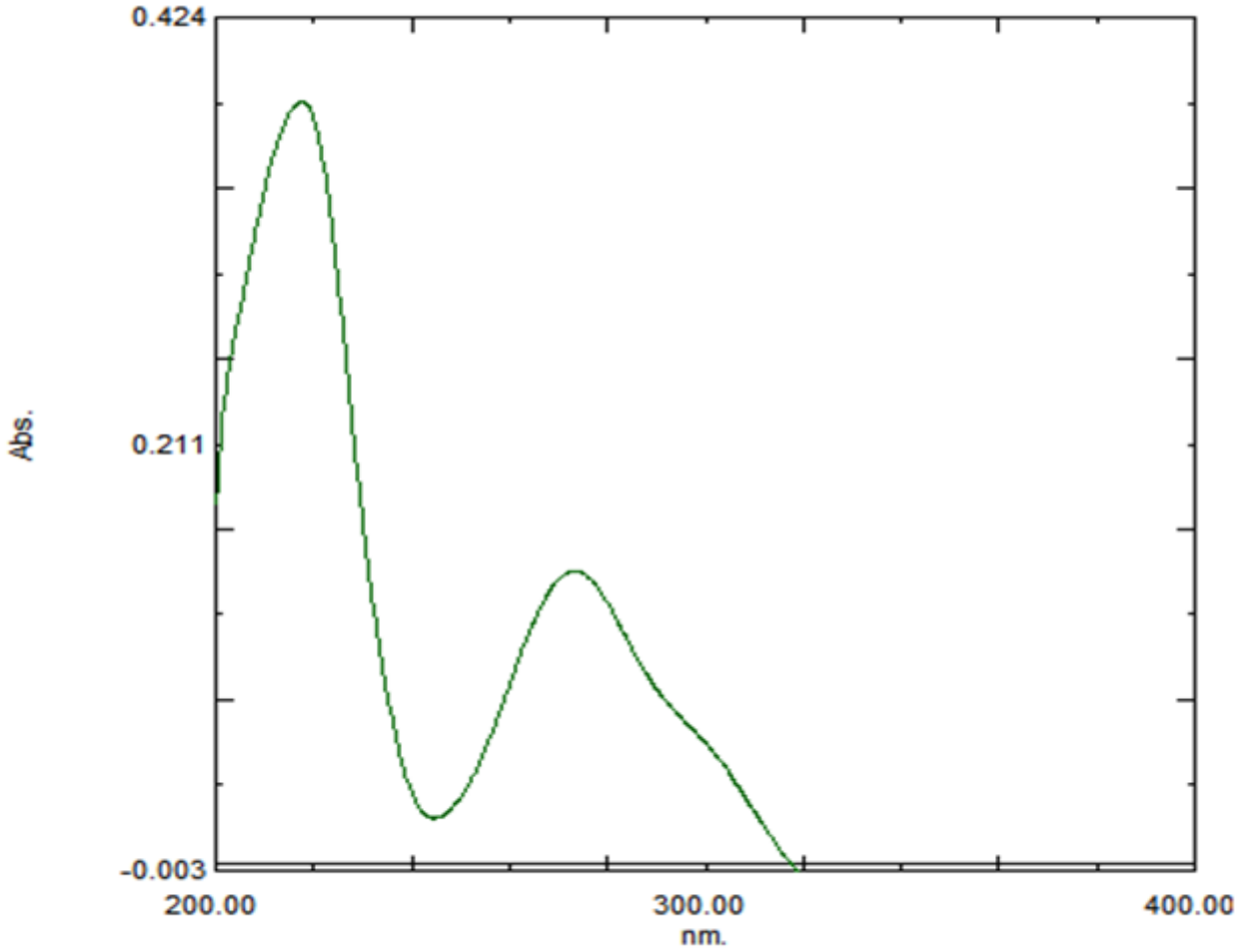


Figure 1

UV Spectrum of Gallic acid

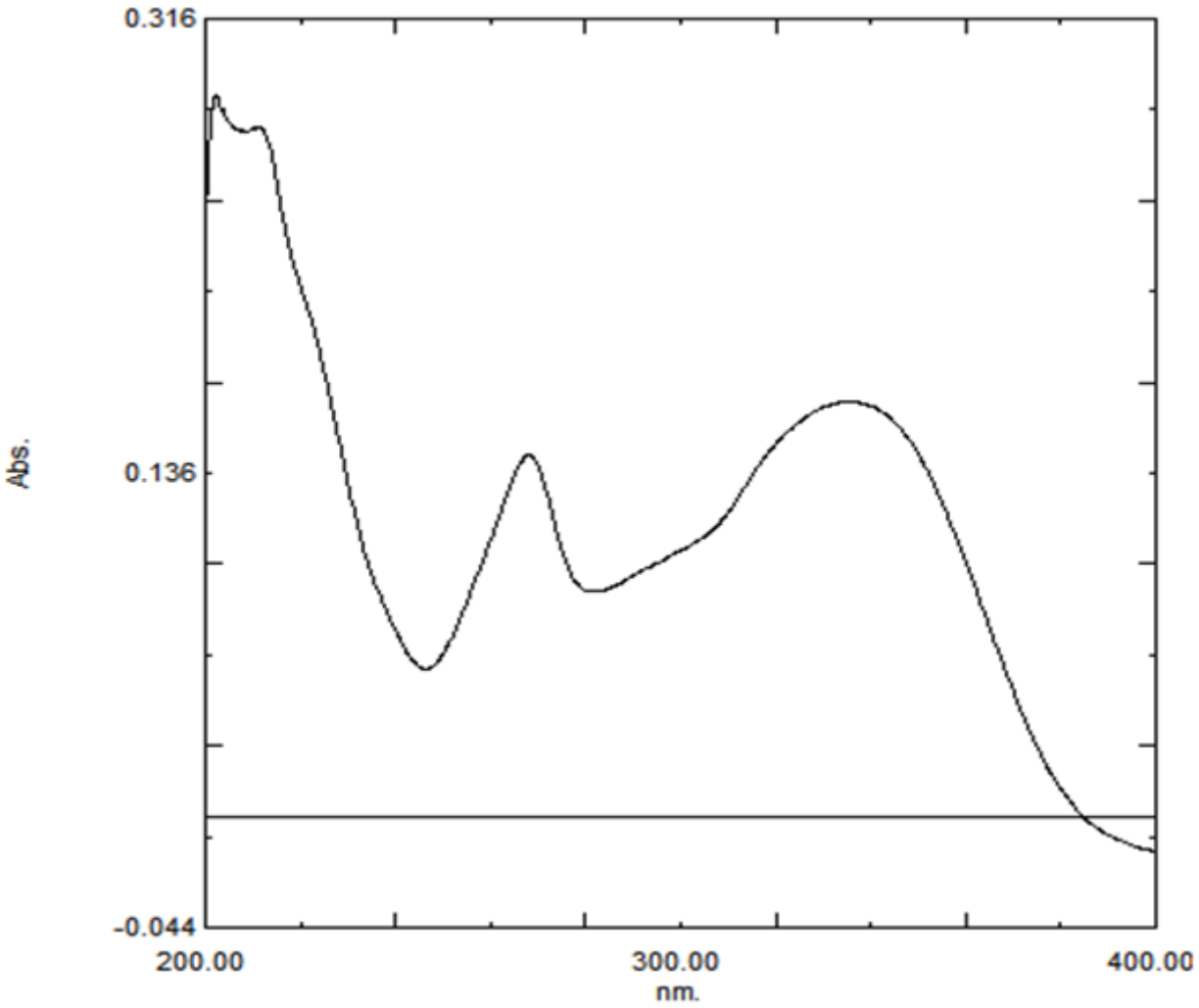


Figure 2

UV Spectrum of Apigenin

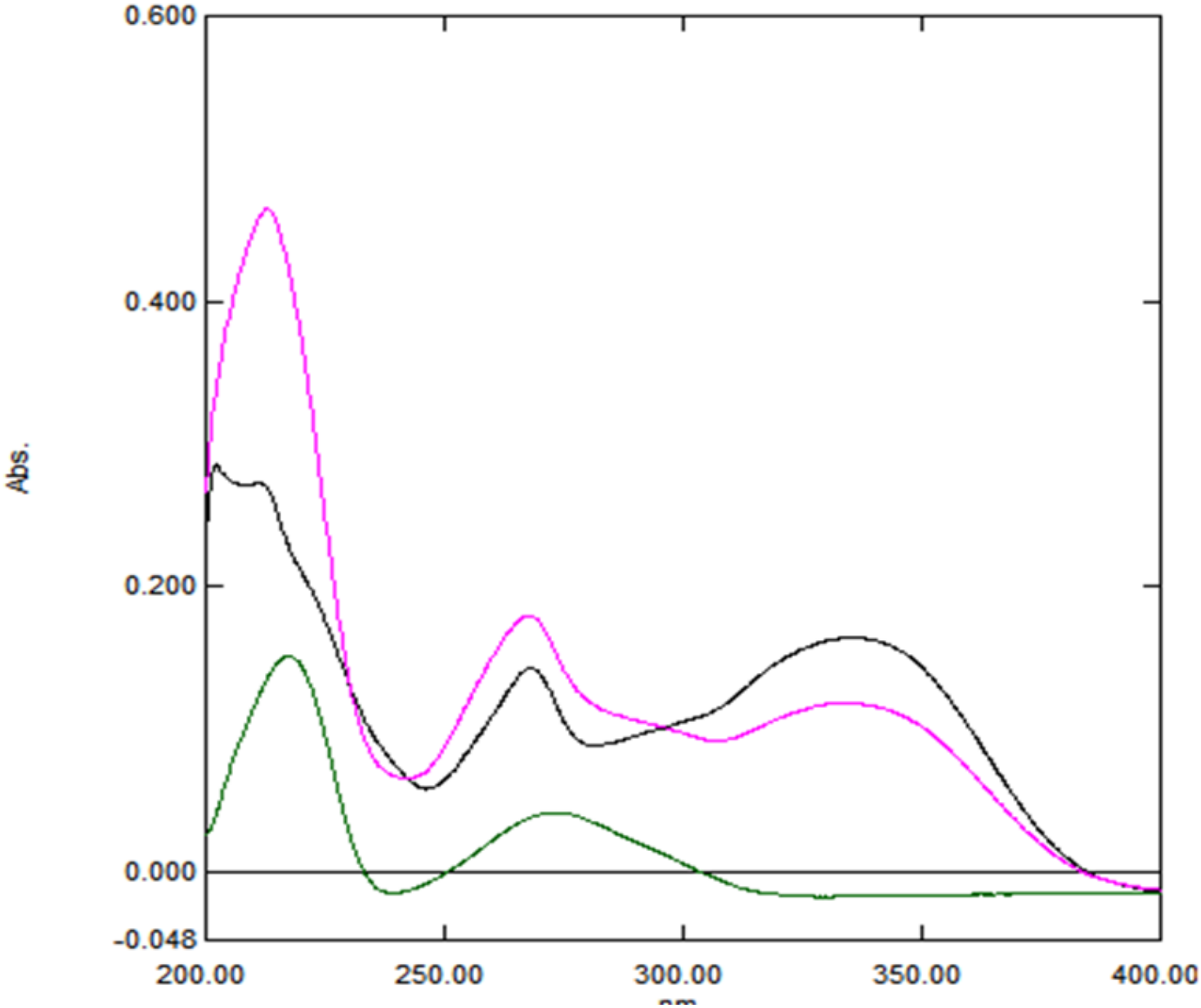


Figure 3

Overlap spectra of Gallic Acid and Apigenin

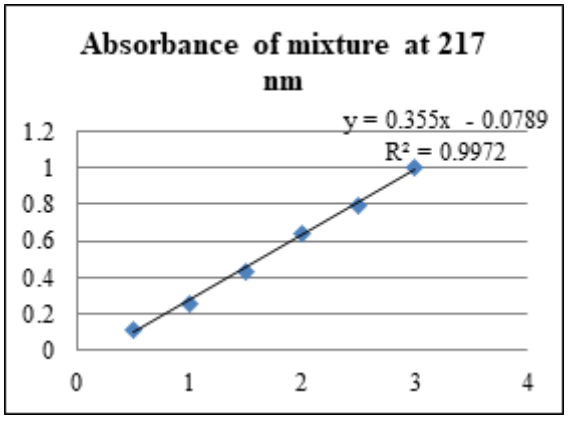


Figure 4

Calibration curve of Gallic acid at 217 nm

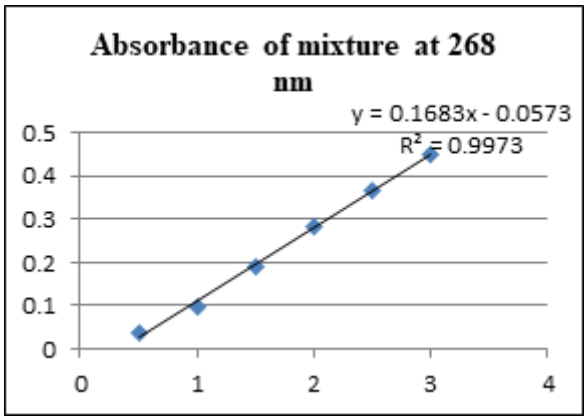


Figure 5

Calibration curve of Apigenin at 268 nm