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Analytical method development and validation for genistein using UV-Vis spectrophotometry

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Abstract
A simple, precise & economically affordable UV-Visible Spectrophotometric method for estimation of Genistein according to the ICH Q2 (R1) guideline. The Genistein was scanned over UV-Visible range for its maximum absorption wavelength. The calibration curve of concentration vs. absorbance was plotted and linearity and range were calculated. The various analytical method validation parameters like accuracy, precision, Robustness were calculated using QC standards. The maximum wavelength of Genistein was found to be 259 nm. The correlation coefficient over concentration range of 0.5-6 µg/mL was found to be 0.999. The intra-day & inter-day precision shows the percentage relative standard deviations in range of 0.2742 to 0.8047 & 0.3377 and 0.8727 respectively. LOD & LOQ was found to be 0.0927 & 0.2810 µg/mL respectively. The Content of Genistein in soyabean seeds was found to be 3.25 mg/g±0.037. Developed method was found to be robust. The method was developed using economical percentage of organic solvent in aqueous media.

Keywords: Economically effective method, UV-visible spectrophotometry, genistein

Introduction
Genistein is an isoflavonoid chemically known as 5, 7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one found in soyabeans, soy products, and a few other legumes (Including peanuts & Chickpeas), which comes from the secondary metabolites of the plant [1, 2, 15]. Genistein is a phytoestrogen and has many more functions in plant physiology, such as aiding in UV protection, pigmentation, antimicrobial protection, & regulation of nitrogen-fixing symbiosis [3, 11, 13]. Genistein is potentially beneficial in treating diseases such as menopause & osteoporosis as well as prevention of cardiovascular disease [4, 5, 12]. The accurate estimation of genistein in plant extract is essential for standardization of nutraceuticals, quality control & Research applications [6, 7, 14]. UV-visible spectrophotometry remains the most popular method of quantitative analysis, as it is easy, highly sensitive, and inexpensive to apply in routine quality control [8, 9, 17]. Considering the therapeutic importance of the Genistein, there is a need of simple yet precise, economic & robust analytical methodology for the same. It was intended that development of UV-Visible Spectrophotometric method for the determination of Genistein in bulk & formulation as well as in plant extract by using co-solvent system consisting economic percentage of organic solvent will be worth.

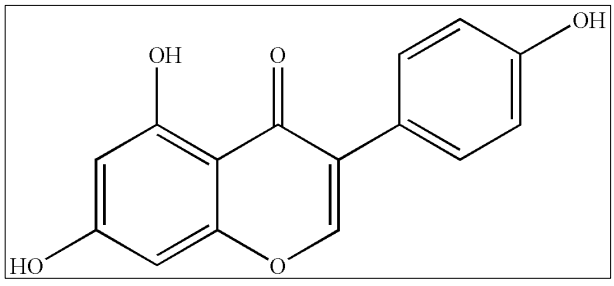

Fig 1: Chemical Structure of Genistein

Materials & Methods
Instrumentation
A pre-calibrated double-beam UV-Visible Spectrophotometer (Model UV-530, Jasco) with spectra manager software was used to develop the method. Quartz Cuvettes with a 3 cm length and a 1 cm optical path were used for the spectral analysis.

The Analytical Balance (Essae Vibra HT) equipped with an internal calibration system, was used for the weighing Purpose. For completely solubilization an ultrasonic bath (PCI Analytics, India; 6.5 L capacity) was used.

Material

Genistein was purchased from TCI Chemicals (India) Pvt. Ltd., Chennai. Dimethyl sulfoxide (DMSO) & Methanol were purchased from Merck. HPLC-grade (Type 1) water was obtained from water purification system (Lablink Xtrapure). For the study, all the chemicals used were of at least analytical grade.

Preparation of Stock Solution

Accurately weighed 10 mg of Genistein was transferred in a Volumetric flask, Mother stock was prepared with primary solvent Dimethyl Sulfoxide (DMSO) to completely dissolve Genistein. Further, it is diluted to 100 µg/mL (Stock I) with co-solvent Methanol: Water in a 50:50 (v/v) ratio and then 10 µg/mL (Stock II).

Determination of wavelength of maximum absorbance (λ_{max})

The λ_{max} of Genistein was determined using a UV-Visible Spectrophotometer. The UV Spectrophotometer was set in spectrum measurement mode and first calibrated by setting it to auto-zero with Cosolvent as blank. A working Stock II solution (10 µg/mL) of Genistein was scanned over the wavelength of 200 to 800 nm. The Scanning was repeated three times using freshly prepared Stock II Solutions.

Preparation of calibration curve

Mother Stock was adequately diluted with cosolvent to achieve a solution of strength 100 µg/mL (Stock-I). Stock-I was diluted with cosolvent to achieve a solution of strength 10 µg/mL (Stock-II) so as to achieve seven calibration standards *viz*, CAL-STD-1(0.5 µg/mL), CAL-STD-2(1µg/mL), CAL-STD-3 (2 µg/mL), CAL-STD-4 (3 µg/mL), CAL-STD-5 (4 µg/mL), CAL-STD-6 (5 µg/mL), CAL-STD-7 (6 µg/mL) respectively. The spectrophotometer was set in fixed Wavelength measurement mode, and the absorbance of each CAL-STD was measured at the pre-measured absorbance maxima of 259 nm. The procedure was repeated three times to ensure the reproducibility of the results. The results were expressed in terms of mean ± SD.

Method Validation

The proposed UV method of Genistein was validated using ICH Q2 (R1) guidelines [10]. The stated method was assessed for its linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ).

Linearity and Range

The linearity of the proposed UV-Spectrophotometric method for Genistein was evaluated using pre-established seven calibration standards, as mentioned above. By using calibration curves of absorbance vs. concentration the linear least-squares regression analysis was carried out. The linearity of the method was confirmed based on the correlation coefficient (r^2) value. The range of the UV method was stated to be in between upper and lower concentration limits with satisfactory linearity.

Accuracy: The accuracy of the UV-Spectrophotometric method for Genistein was evaluated using three quality control standards (QC-STD), such as QC-STD-1, QC-STD-2, and QC-STD-3 comprising nominal concentration of 0.6 µg/mL, 2.5 µg/mL, and 5.5 µg/mL respectively were prepared in triplicate and used for proposed study. The Said QC-STD were analysed for its Genistein content using proposed UV spectrophotometric method three different time intervals in a day. The intra-day and inter-day accuracy of the proposed method was established in terms of% difference obtained by using following formula,

$$\%\ Difference = \frac{mean\ measured\ concentration - nominal\ concentration}{Nominal\ concentration} \times 100$$

Precision

The QC-STD Proposed (0.6 µg/mL, 2.5 µg/mL, and 5.5 µg/mL) were employed to determine precision of the proposed method in terms of% Relative Standard Deviation (RSD). The intra-day and inter-day precision was established by analysing the QC-STDs at three different time intervals in a day & the same process was repeated on three consecutive days. The precision of the method was established in terms of% RSD, which was calculated by using following formula,

$$\%\ RSD = \frac{standard\ Deviation(SD)}{Mean} \times 100$$

Robustness

The robustness of the proposed UV-Spectrophotometric method for Genistein was assessed by introducing a deliberate change of two different cosolvent mixtures (Methanol: Water in ratio of 48:52 v/v and 52:48 v/v) to check whether such variation affected results. The three QC-STDs were analysed with different solvent ratios & absorbance values were used to calculate the mean standard deviation (SD), and percent relative standard deviation (%RSD). The robustness of the method was confirmed by deliberate minor variations in analytical procedure, with all calculated% RSD values remaining within the acceptable limit of ≤ 2%.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed UV method was estimated by following formula.

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

Where,
SD = Standard deviation of Y-Intercept
S = Slope of the calibration curve

Estimation of Genistein in Soyabean Seeds Extract

Soyabean seeds were dried and finely grounded using a mixer grinder (Bajaj Electricals Ltd., Mumbai, India). The grinded powder was passed through 40 mesh sieves for uniform powder size. The 50 g of sieved powder was extracted using Methanol by Ultrasound assisted extraction in Ultrasonic bath for the period of 60 min. After extraction mixture was filtered using Whatman No. 1 filter paper, and filtrate was concentrated to one fourth of actual volume of extract using rotary vacuum evaporator (Heidolph Laborota 4001). Said extract was acid hydrolysed using 2N hydrochloric acid at temperature between 60-70 ºC for one hour with continuous

agitation. After completion of hydrolysis the extract was cooled and neutralized using 2 N Sodium carbonate. The neutralized material was transferred to a separating funnel & it was partitioned using ethyl acetate 1:1(v/v) and the upper layer of the mixture was collected, and partitioning process was repeated thrice to ensure complete recovery of genistein. The ethyl acetate fractions were evaporated at 40 ºC to dryness to obtain residue enriched with Genistein. Accurately weighed 3.3g of extract was transferred into the 10 ml capacity volumetric flask and dissolved using 10 mL of DMSO. One ml of said solution was diluted to 100 ml with co-solvent system to obtain a working solution, from which appropriate volumes further diluted to prepare solutions of concentration 2 µg/mL, 3 µg/mL, 4 µg/mL and analyzed for the Genistein content using proposed UV Method. The Genistein content of soyabean seeds (mg/g) was expressed in terms of mean ± SD.

Results and Discussion

Determination of the wavelength of maximum absorbance (λ_{max})

To achieve accurate quantitative estimation of Genistein by UV-Visible Spectrophotometry, it is essential to identify the wavelength of maximum absorbance λ_{max} of as it directly influences assay sensitivity & improves reliability of results. A solution containing Genistein (10 µg/mL) was repeatedly scanned between 200 to 800 nm in the spectrum measurement mode. The obtained spectrum was analyzed using the instrument's software, the maximum absorbance was found to be 259 nm, which was further selected for all analytical measurements.

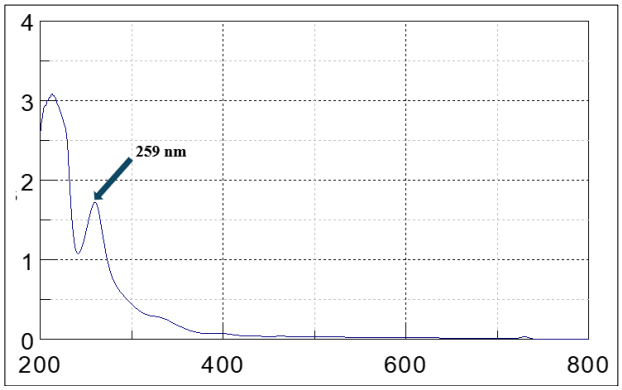

Fig 2: UV-Visible Spectrum of Genistein

Preparation of a calibration curve

For quantification of unknown samples by UV-Spectrophotometric method an equation expressing the correlation between concentration and response is required. The Equation method is more accepted and used worldwide as compared to graphical method. For quantitative estimation of Genistein, a calibration curve was developed using seven calibration standard solutions, CAL-STD-1 (0.5 µg/mL), CAL-STD-2 (1µg/mL), CAL-STD-3 (2 µg/mL), CAL-STD-4 (3 µg/mL), CAL-STD-5 (4 µg/mL), CAL-STD-6 (5 µg/mL), CAL-STD-7 (6 µg/mL) respectively. Each standard was measured three times at 259 nm in fixed wavelength mode. The average absorbance values along with their standard deviations were calculated and calibration curve was plotted.

Table 1: Calibration standard data for Genistein

Concentration (µg/mL)	Absorbance (Mean ± S.D)
0.5	0.0957±0.0037
1	0.1846±0.0104
2	0.3336±0.0047
3	0.4866±0.0035
4	0.6368± 0.0056
5	0.8039±0.0032
6	0.9363±0.0089

Method validation

Once an analytical method has been developed, it must be validated for reliability and suitability for the intended application. Method Validation ensures the quality, reproducibility, and reliability of the results. In an academic and industrial setting, this type of validation often employs the internationally accepted ICH Q2 (R1) guideline for validation of analytical methods. There are several specific parameters that are assessed and each parameter has a specific acceptance criterion. A method is considered reliable and scientifically acceptable when the parameters all are within the acceptance criteria. Throughout the validation process, the following parameters were used in the validation of the developed UV method for Genistein.

Linearity and Range

The linearity of the said method was assessed using seven calibration standards of Genistein within concentration range of 0.5-6 µg/mL, Regression analysis of calibration data demonstrated a strong linear relation between absorbance & concentration. The mean absorbance data for the corresponding calibration standards are shown in Table 1. All three calibration curves showed very linear behaviour (r^2>0.999). The least variability in slopes and intercepts, indicates the linear response is appropriate for the method as shown in the plots of figure 3 (A-C). Based on the assessment of linearity, the UV Spectrophotometric method developed was found to be linear.

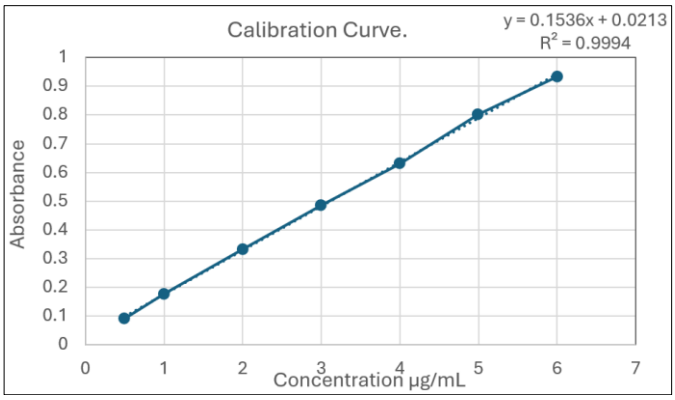

Fig 3 (A): Calibration curve of Genistein (Replicate 1)

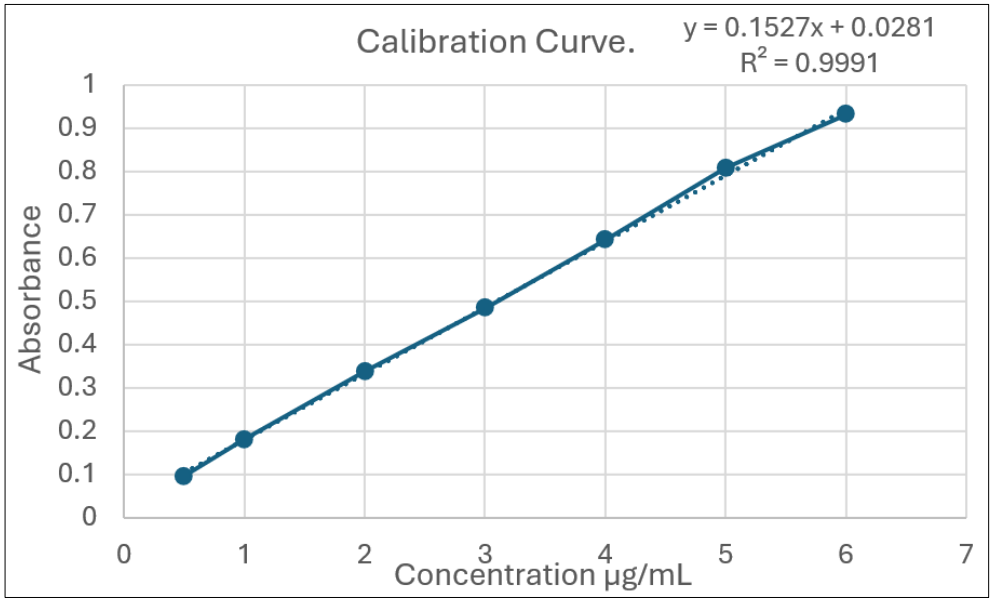

Fig 3 (B): Calibration curve of Genistein (Replicate 2)

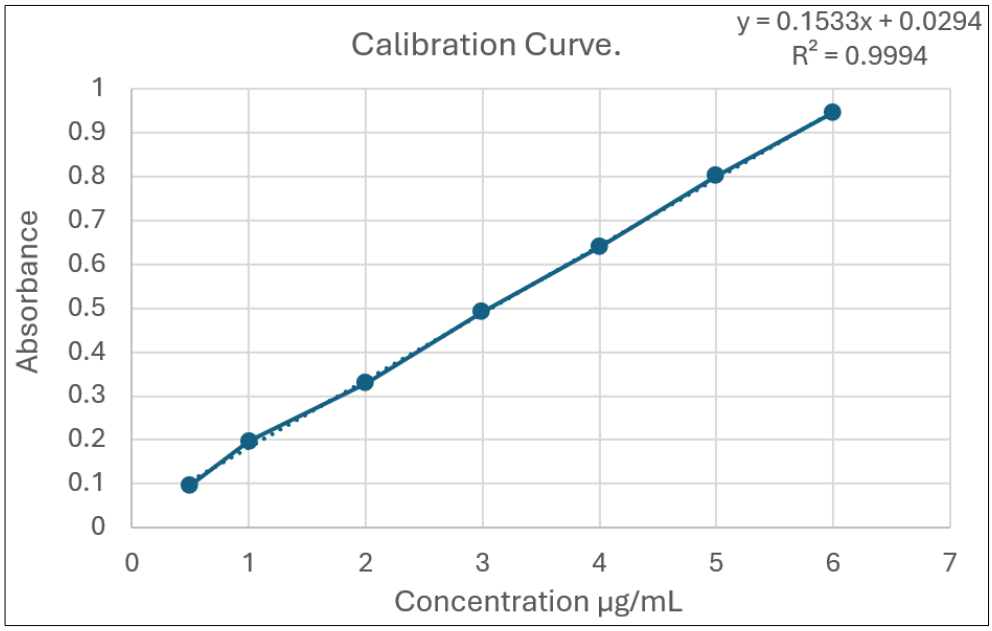

Fig 3 (C): Calibration curve of Genistein (Replicate 3)

Accuracy

Accuracy describes how close the measured value is to the actual or reference value. For an analytical method to be reliable, accuracy should remain consistent across the whole calibration range. The Intra-day and Inter-day% difference results are presented in Tables 2 & 3 respectively. The Intra-day% difference varied from + 0.3714 to + 1.4734, while the inter-day values ranged from + 0.5940 to + 1.1990. The results indicated both intra-day and inter-day percent difference values were within acceptable limits. Based on obtained results, it was envisaged proposed analytical method of Genistein offers accurate and dependable measurements across the tested concentration range.

Table 2: Intra-day accuracy data of the UV method for Genistein

Concentration Level	Nominal Concentration (µg/mL)	Mean Measured Concentration (µg/mL)	% Difference
LQC	0.6	0.6073	+ 1.2236
	0.6	0.6088	+ 1.4734
	0.6	0.6081	+ 1.3518
MQC	2.5	2.5092	+ 0.3714
	2.5	2.5269	+ 1.0780
	2.5	2.5123	+ 0.4950
HQC	5.5	5.5435	+ 0.7913
	5.5	5.5606	+ 1.1020
	5.5	5.5519	+ 0.9441

Table 3: Inter-day accuracy data of the UV method for Genistein

Concentration Level	Nominal Concentration (µg/mL)	Mean Measured Concentration (µg/mL)	% Difference
LQC	0.6	0.6067	1.1315
	0.6	0.6056	0.9470
	0.6	0.6040	0.6736
MQC	2.5	2.5170	0.6832
	2.5	2.5159	0.6367
	2.5	2.5148	0.5940
HQC	5.5	5.5417	0.7588
	5.5	5.5659	1.1990
	5.5	5.5495	0.9006

Precision: Precision refers to the degree of scatter among repeated measurements, showing how consistent the results are. An analytical method is expected to provide reproducible outcomes. The developed UV methods Intra-day and inter-day precision was determined at three different levels *Viz*. 0.6 µg/mL, 2.5 µg/mL, 6.5 µg/mL. The% RSD values of intra-day & inter-day precision were represented in table 4 and 5. Intra-day precision in terms of%RSD was found to be in the range 0.2742 to 0.8047, whereas inter-day precision of proposed method was found to be in between 0.3377 and 0.8727. The relatively low% RSD values confirms that the method offers good precision for the quantification of Genistein.

Table 4: Intra-day precision data of the UV method for Genistein

	Morning			Afternoon			Evening		
Conc. Range (µg/mL)	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD
0.6	0.6073	0.0032	0.5343	0.6053	0.0036	0.5995	0.6039	0.0018	0.3010
2.5	2.5093	0.0202	0.8047	2.5270	0.0131	0.5171	2.5124	0.0060	0.2742
5.5	5.5435	0.0280	0.5059	5.5606	0.0427	0.7672	5.5519	0.0279	0.5021

Table 5: Inter-day accuracy data of the UV method for Genistein

	Day 1			Day 2			Day 3		
Conc. Range (µg/mL)	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD
0.6	0.6068	0.0040	0.6656	0.6057	0.0020	0.3377	0.6040	0.0030	0.4943
2.5	2.5171	0.0220	0.8727	2.5159	0.0120	0.5083	2.5140	0.0130	0.5413
5.5	5.5417	0.0270	0.4869	5.5659	0.0370	0.6642	5.5490	0.0330	0.5945

Robustness

Robustness is the method's capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness tests, examine the effect of operational parameters on the analysis results. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study.

To establish robustness of the proposed analytical method of Genistein, solvent composition was initially modified. The absorbance values obtained under the modified conditions showed no significant deviations from original measurements. All calculated% RSD values remained the acceptable limit of ≤2% as shown in table 6. The results demonstrated the proposed analytical method of Genistein is robust & remains reliable under deliberate variations.

Table 6: Robustness data of the UV method for Genistein

Concentration (ug/mL)	Methanol: Water (V/V)	Absorbance (Mean ± S.D.)	%RSD
0.6	48:52	0.0925±0.0015	1.6258
2.5	48:52	0.3868±0.0041	1.0802
5.5	48:52	0.8616±0.0035	0.4126
0.6	52:48	0.0975±0.0015	1.5560
2.5	52:48	0.3823± 0.0021	0.5635
5.5	52:48	0.8246±0.0016	0.2003

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantification (LOQ) represents the lowest concentration of an analyte that can be measured with acceptable precision & accuracy. In the present study, the proposed UV-Spectrophotometric method showed an LOD 0.0927 µg/mL& LOQ of 0.2810 µg/mL as summarized in table 7.

Table 7: LOD & LOQ data of Genistein

Parameter	Values
LOD	0.0927 µg/mL
LOQ	0.2810 µg/mL

Estimation of Genistein in Soyabean Seeds Extract

Developed UV method was successfully applied for estimation of Genistein content in soyabean seeds. The Genistein Content in soyabean seeds was expressed in terms of mean ± SD. By proposed UV method, Genistein content in soyabean seeds was found to be 3.25±0.037 mg/g feed which is in good agreement with the existing data available. The proposed UV-Visible spectrophotometric method was found to estimate the Genistein content with sufficient accuracy which proved that said method can be used for the routine analysis of Genistein in near future.

Conclusion

A simple yet precise & accurate, cost -effective method using UV-Visible Spectrophotometric method has been successfully developed & validated for quantitative estimation of Genistein. Linearity has been proved within the concentration ranges with correlation coefficients falling in the acceptance limits. Accuracy, Precision, LOD & LOQ were found to be in within acceptable limits as per ICH guidelines. Proposed method was found to be robust with no significant interference. The results confirm the methods is simple, reliable & can be adopted for regular quality control analysis as well as quantitative estimation of Genistein.

References

1. Lee SY, Yoo JI. Soybean isoflavones potentially prevent sarcopenia: a systematic review. J Ethn Food 2023;10:48. https://doi.org/10.1186/s42779-023-00210-6
2. Spagnuolo C, Russo GL, Orhan IE, Habtemariam S, Daglia M, Sureda A, *et al*. Genistein and cancer: current

status, challenges, and future directions. Advances in nutrition (Bethesda, Md.) 2015;6(4):408-419. https://doi.org/10.3945/an.114.008052
3. Kim IS. Current perspectives on the beneficial effects of soybean isoflavones and their metabolites on plants. Food Sci Biotechnol 2022;31(5):515-526. DOI: 10.1007/s10068-022-01070-7
4. Mahrous RS, Fattah BA, Rahman YA, *et al*. Green and Efficient Extraction of Soybean (*Glycine max* (L.) Merr.) Isoflavones Using Natural Deep Eutectic Solvents-Based Ultrasound-Assisted Extraction. Food Bioprocess Technol 2025;18:10419-10433. https://doi.org/10.1007/s11947-025-04031-0
5. Hong H, Landauer MR, Foriska MA, Ledney GD. Antibacterial activity of the soy isoflavone genistein. Journal of basic microbiology 2006;46(4):329-335. https://doi.org/10.1002/jobm.200510073
6. Krizova L, Dadakova K, Kasparovska J, Kasparovsky T. Isoflavones. Molecules 2019;24:1076. 10.3390/molecules24061076
7. Krenn L, Pötsch V. An efficient HPLC method for the quantification of isoflavones in soy extracts and soy dietary supplements in routine quality control. Die Pharmazie 2006;61(7):582-585.
8. César I, Braga F, Soares C, Nunan E, Pianetti G, Moreira-Campos L. Quantitation of genistein and genistin in soy dry extracts by UV-Visible spectrophotometric method. Quimica Nova - QUIM NOVA 2008;31. 10.1590/S0100-40422008000800003
9. Mazumder MAR, Hongsprabhas P. Detection of Genistein in Soy Protein Isolate and Soymilk Powder by Spectrophotometric and Chromatographic Method. Journal of Food Science and Technology Nepal 2019;11:69-73. https://doi.org/10.3126/jfstn.v11i0.16920
10. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. 2005.
11. Sharifi-Rad J, Quispe C, Imran M, Rauf A, Nadeem M, Gondal TA, *et al*. Genistein: An Integrative Overview of Its Mode of Action, Pharmacological Properties, and Health Benefits. Oxid Med Cell Longev 2021;2021:3268136.
12. Spagnuolo C, Russo GL, Orhan IE, Habtemariam S, Daglia M, Sureda A, *et al*. Genistein and Cancer: Current Status, Challenges, and Future Directions. Advances in Nutrition 2015;6(4):408-419. https://doi.org/10.3945/an.114.008052
13. Rahman Mazumder MA, Hongsprabhas P. Genistein as antioxidant and antibrowning agents in *in vivo* and *in vitro*: A review. Biomedicine & pharmacotherapy Biomedecine & pharmacotherapie 2016;82:379-392.
14. Magiera S, Baranowska I, Lautenszleger A. UHPLC-UV method for the determination of flavonoids in dietary supplements and for evaluation of their antioxidant activities. Journal of pharmaceutical and biomedical analysis 2015;102:468-475. https://doi.org/10.1016/j.jpba.2014.10.004
15. Uifălean A, Farcaş A, Ilieş M, Hegheş SC, Ionescu C, Iuga CA. Assessment of isoflavone aglycones variability in soy food supplements using a validated HPLC-UV method. Clujul medical (1957) 2015;88(3):373-380.
16. Crupi V, Ficarra R, Guardo M, Majolino D, Stancanelli R, Venuti V. UV-vis and FTIR-ATR spectroscopic techniques to study the inclusion complexes of genistein with beta-cyclodextrins. Journal of pharmaceutical and biomedical analysis 2007;44(1):110-117. https://doi.org/10.1016/j.jpba.2007.01.054