https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27 (3)(September 2024); 3185-3193
Research Article

Development and Validation of RP-HPLC Method for Quantification of Quercetin in *Solanum nigrum* Extract

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ABSTRACT:
Solanum nigrum is thought to contain and demonstrate a wide range of biological activities, including anticancer, antioxidant, antiulcerogenic, and antipyretic properties. Several analytical techniques have been documented for the quantification of Quercetin. Quercetin, a flavanol, found in a variety of plants. A recent discovery in analytical chemistry offers a practical, affordable, and responsible way to build different analytical techniques. Development of RP-HPLC method using for the flavonoid (Quercetin) in the hydroalcoholic extract of *Solanum nigrum* Linn. Establishment of chromatographic method involved employee RP-HPLC method to ensure robust, precise and accurate quantification of quercetin. Acetonitrile: High-quality water in an isocratic elution mode, at a flow rate of 1 mL/min at a temperature of 37 ± 2^0C. The approach was proven to be specific for analysis of quercetin which was accurate (>90%) and precise (%RSD <2%). A calibration curve was reputable over the quercetin concentration range, with a correlation value of 0.9992. The LOD was 22.45µg and LOQ value was found to be 68.05µg. The RSD of the proposed approach was determined by assaying six replicate quercetin injections intra-day and inter-day. The average quercetin concentration of *Solanum nigrum* Linn. was determined to be 6.317 mg/kg. The method was new prepared and validated with the parameters according to guidelines.

Keywords: RP-HPLC, Quercetin, *Solanum nigrum, plant extract.*

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Received: 12/08/2024 Acceptance: 02/09/2024

DOI: https://doi.org/10.53555/AJBR.v27i3.8463

INTRODCUTION:

Nowadays, much scientific research in modern biomedicine focuses on Traditional System of Medicine (TCM), which attempts to preserve the dynamic balance of the entire body in order to promote harmony between humans and nature(1). India contains two mega-biodiversity hotspots: the Western Ghats and the Northeast, as well as the northern Himalayan area(2). These biodiversity zones provide a significant supply of herbal items for several Ayurvedic-based Indian firms. The Solanaceae family is extensively dispersed throughout the planet. It consists of around 84 genera and 3000 species(3). *Solanum nigrum* Linn. a member of the Solanaceae family, is a plant that grows abundantly in the wild of South Asia and has been utilized in Ayurveda for centuries(2). The plant *Solanum nigrum* Linn. as shown in Figure 1 is also known as black nightshade used in various health conditions as traditional medicine. It has been used to treat liver disorders, chronic

skin ailments such as psoriasis and ringworm, painful periods, inflammatory conditions, eye diseases and fever. It is also supposed to have medicinal properties which helps to cure hydrophobia. In traditional Ayurvedic medicine, black nightshade is considered to be a natural remedy for a range of health problems(4,5) and cancers treatment(1). The plant has been studied for different phytochemicals(6-8). Various portions of this plant are thought to contain and demonstrate a wide range of biological activities, including anticancer, antioxidant, antibacterial, anti-ulcerogenic, diuretic, and antipyretic properties(2,9). The plant is a key ingredient in a variety of herbal liver-support preparations. *Solanum nigrum* contains active components Quercetin is a plant-based flavonoid (flavonol) used as a dietary supplement(10–12). Flavonoids are natural compounds found throughout the plant world that are well recognized for their antioxidant, antiviral, and anticancer activities(13–15). They are found in nearly every section of the plant. Secondary plant metabolites have recently received increased interest due to their potential to prevent chronic illnesses such as cardiovascular disease. As a result, the separation, identification, and quantification of phytochemicals in food, as well as the assessment of their potential health benefits, has received attention(16–19).

Figure 1: A whole plant of *Solanum nigrum* Linn. showing plant leaves (A) and fruits (B)

Several procedures, including TLC, HPTLC and GC, have been developed to determine the value of quercetin and other phytochemicals in solanaceae family(20–22). In this work, a simple and readily accessible reversed-phase HPLC technique with no complicated requirements was devised and validated to measure quercetin content in compliance with International Conference on Harmonisation recommendations. The analytical approach was devised and validated for the measurement of quercetin in plant extract(10,23–25).

MATERIALS AND METHODS:

Materials

Solanum nigrum Linn. Extract was obtained by AMSAR Pvt. Ltd. (INDORE, INDIA). Sigma- Aldrich chemicals provided the quercetin for purchase. Merck Life Science supplied the HPLC grade acetonitrile (ACN) and methanol. Loba Chemie (Pvt. Ltd) supplied HPLC-grade glacial acetic acid. Milli-Q water having HPLC grade quality was filtered using a Milli-Q filtration system (Millipore) to meet the necessary requirements for analysis. Analytical grade chemicals, reagents and solvents were utilized throughout the analytical technique validation process, specifically HPLC-grade materials.

Preparation of standard (Quercetin) Solution

To prepare a stock solution (standard solution) containing 1000µg/mL, accurately weighed 10 mg of quercetin in 10 mL previously cleaned and dried volumetric flask. Dissolved the content properly in the mobile phase and dilute to volume using the same solvent combination.

Preparation of drug (extract) solution:

Transferred 10 mg dried extract in to a volumetric flask having capacity 10 mL containing 5mL methanol. Sonicated the solution for 30 minutes and adjusted the volume with methanol. Sonicated the solution again for 5 min. and filtered through a 0.5 µm filter.

Selection of Wavelength

The UV-vis spectrum was obtained by diluting stock solutions with concentration 1000 µg/mL in methanol as primary stock solution and created a secondary stock solution of concentration 10 µg/mL. To determine the wavelength maximum absorption (λmax), aliquot was placed in quartz cell cuvettes and scanned with a UV-vis spectrophotometer.

Method development for determination of Quercetin in the plant extract

Chromatographic condition

Quercetin was analyzed on a Phenomenex C18 column (5µ, 250 × 4.6 mm) using an elution mode with Acetonitrile (ACN), methanol, and HPLC grade water (pH 2.6, corrected with 2% w/w glacial acetic acid). Mobile phase in different proportions and compositions was prepared, filtered through 0.45-micron membrane filter, and sonicated every time for 20 minutes. HPLC system was initiated as per standard operating procedure and stabilized in a given mobile phase and column. 20 µl injection volume were injected in the column at temperature 35 ± 2ºC with flow rate 1 mL/minute and adjusted in subsequent run for development of method.

Chromatographic study of samples

The sample solutions of 20 µl was injected for each scanning and the HPLC peak parameters including retention time, peak area, area under curve (AUC), theoretical plates etc. was measured. The samples of Quercetin standard were injected to get clear retention peak by adjusting mobile phase and flow rate. The drug samples of extract were also injected in the system using optimized mobile phase proportions. Further slight modification in the mobile phase was tried to get optimized mobile phase for separation of quercetin in the extract. The optimized peak as shown in figure 4 and figure 5 for the phytochemical and extract were recorded. Similar steps were adopted for analysis of different concentrations viz. 100, 200, 300, 400, and 500 µg/mL solutions of the Quercetin in optimized mobile phase were carried out.

Method Validation

According to International Council for Harmonisation (ICH) for quercetin detection and quantification by RP-HPLC method in the plant extract was validated. ICH is technical requirements for pharmaceutical and human use. For linearity, accuracy, specificity, robustness and precision this approach was verified. The system suitability research followed United States Pharmacopoeia (USP) criteria. The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. Method validation is a practical process designed and experimentally carried out to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range of analysis. Method validation ensures the validity of a measurement before it is carried out and is essential part of quality assurance procedures. Typical validation characteristics which should be considered are listed below Specificity, Accuracy, Precision, Linearity, Limit of Detection, Limit of Quantitation, Robustness etc.

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the other component that may be expected to be present in the sample matrix. Distinguish an analyte from known impurities, synthetic precursor, metabolite or degradation product and other inactive. These should not be any peak at the R.T. of main peak. Specificity was determined for the quercetin in extract based on interference, % RSD of retention time, % RSD of area, and theoretical plates. The compliance with the limit were checked as per guidelines.

Accuracy

The accuracy of an analytical method is determined by applying the method to analyzed sample or placebo sample to which known amount of analyte have been added. The accuracy is calculated from the test result as the percentage of analyte recovered by the assay. The relative standard deviation should not be more than 2.0%. The average accuracy result for the drug should be precise and well within the acceptable limit of 98% to 102% at three different levels such as 80%, 100%, 120%.

Preparation of stock solution: To determine accuracy the standard stock solution was prepared by accurately weigh and transfer 0.01gm (10 mg) of Quercetin standard separately in to 10 ml volumetric flask and solution of concentration 1000ppm was produced which was further diluted as below:

1. For 80% concentration: For above the stock solution 1 ml from extract solution, 0.8 ml standard solution in was taken in 10 ml volumetric flask and volume was made up of standard mixture with methanol 10 ml.
2. For 100% concentration: For above the stock solution take 1 ml from extract solution,1 ml standard solution was taken in 10 ml volumetric flask and volume was made up of standard mixture with methanol 10 ml.
3. For 120% concentration: For above the stock solution take 1 ml from extract solution, 1.2 ml standard was taken in 10 ml volumetric flask and volume was made up 0f standard mixture with methanol 10 ml.
4. Sample solution: 10 mg sample was taken in 50 ml volumetric flask containing methanol and shaken vigorously, sonicated 30 min and made-up volume up to the mark with methanol. Adequate of the above solution was pipette and transferred in to a series of clean and dry 10 ml volumetric flask and diluent was added to the get final concentration of Quercetin. (i.e. 80%, 100%, 120% concentration as per above standard solution)

Precision

Precision of an analytical method is the degree of agreement among individual test result when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Determination:

1) Repeatability standard solution of Quercetin was prepared and chromatogram was recorded. Area was measure of the same concentration solution three times and %RSD was calculated.
2) Intraday precision standard mixture solution containing Quercetin was analyzed three times on the same day and %RSD was calculated.

3) Interday precision mixed solution containing Quercetin was analyzed three times on different day and %RSD was calculated. The relative standard deviation should not be more than 2% for test result.

Linearity

Linearity of an analytical method is its ability to elicit test result that is directly or by a well-defined mathematical transformation proportional in the concentration of analyte in sample within a given range. The linearity of analytical method is determined by mathematical treatment of test results obtained by analysis of sample with analyte concentration across the claimed range and area is plotted graphically as a function of analyte concentration of percentage curve fitting are calculated.

Preparation of stock solution for linearity

Standard stock solution of Quercetin prepared at different concentration level i.e., 100, 200, 300, 400 and 500ppm was used for this purpose. The peak area of chromatograms was plotted against the concentration of Quercetin to obtain the calibration curve. The concentration of standard where subjected to regression analysis to calculated calibration equation and correlation coefficient. For determination of linearity and calibration curve, quercetin diluted solutions were injected 3 times each and which was prepared by using standard solution of quercetin. A concentration vs peak area response curve for quercetin was recorded with a peak area of each concentration.

Robustness

It is measure of the method to remain unaffected by small but deliberate variation in method parameter and provides an indication of its reliability under normal usage. It measures the technique's capacity to stay unaffected by modest but decisive dissimilarities in method parameters and offers an indicator of its dependability under normal conditions(26,27).

Preparation of stock solution for robustness

Approximately 10 mg of Quercetin was accurately weighed transferred to separate 10 ml volumetric flask, dissolved mobile phase and diluted to furnish stock solution containing 1000 µgm/ml of Quercetin. 1 ml of above solution was transferred in 10 ml volumetric flask and the volume was made with dilutes. The concentration of Quercetin attained 10 µgm/ml.

Determination: The robustness of an analytical technique was assessed by analyzing aliquots from homogeneous batches using different physical characteristics that may change but remain within the parameters of the assay. For example, changes in physical parameters such as the pH of the mobile phase and its ratio. The standard preparation, placebo preparation, and sample preparation were all done in triplicate. The sample, standard and placebo were injected under various condition of chromatographs, as indicated below: Changes in flow rate (±0.1 ml/min) – changes in wavelength (±2nm).(29)

Limit of detection (LOD) and Limit of quantification (LOQ)

The method's detection was estimated by injecting quercetin standard solution into an HPLC column. The limit of detection (LOD) was given in µg/mL. This value was derived straight from the calibration graph. LOQ states as least concentration in the standard curve and that might be measured with acceptable accuracy and precision. It is stated as LOQ = 10 × SD/S, where S is the slope of the calibration graph, and SD is the standard deviation. (30)

System Suitability test

System suitability is a pharmaceutical requirement and is used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done Acceptance criteria

- RSD should not be more than 2.0% for five replicate injections of standard
- USP tailing factor is not more than 2.0
- The column efficiency is determined as a no. of theoretical plates should be more than 4500.

RESULTS AND DISCUSSION:

Selection of Wavelength

The λmax of standard was determined and found out the isobestic wavelength with the highest absorption. The Figure 2 and 3 shows UV spectra of Quercetin and *Solanum nigrum* extract. Characteristics of the phytochemical and its extracts are shown in the figures. The pattern of absorption in extract is not distinct due to number of compounds in the sample. The wavelength was selected based on literature and verified with sample absorbance.

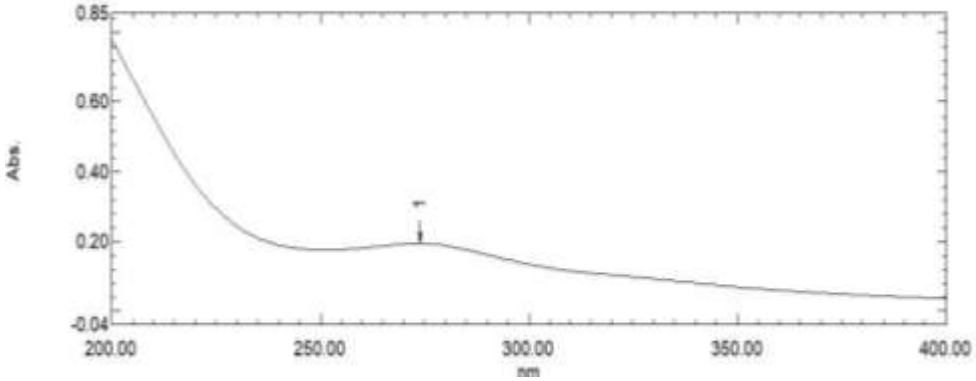

Figure 2: UV Spectrum of *Solanum Nigrum* Linn. Extract

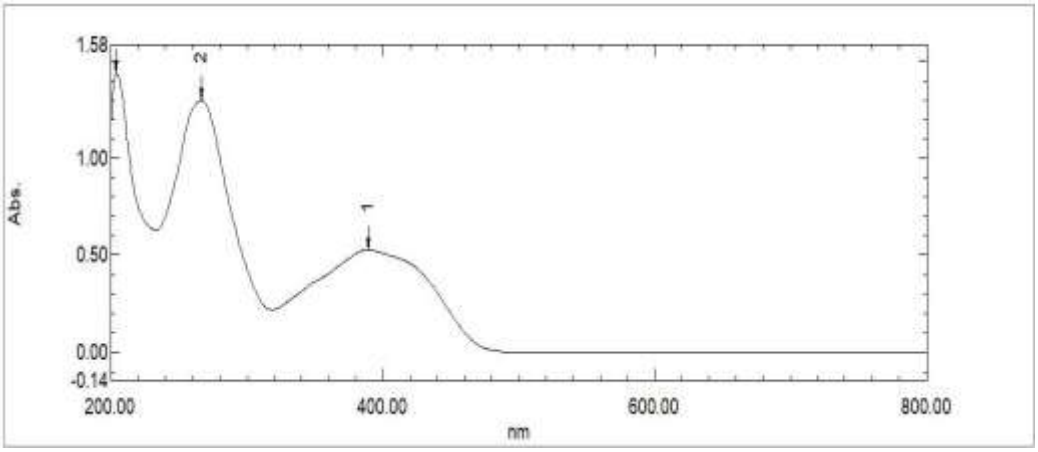

Figure 3: UV Spectrum of Quercetin

Method development for determination of Quercetin in the plant extract

The development of an RP-HPLC technique for the measurement of quercetin was studied in the given system. The quercetin was eluted through the column on retention time 4 minutes (Figure 4). System appropriateness parameters were also recorded. The chromatogram of developed system and the AUC of these samples were recorded as shown in figure 4 and figure 5.

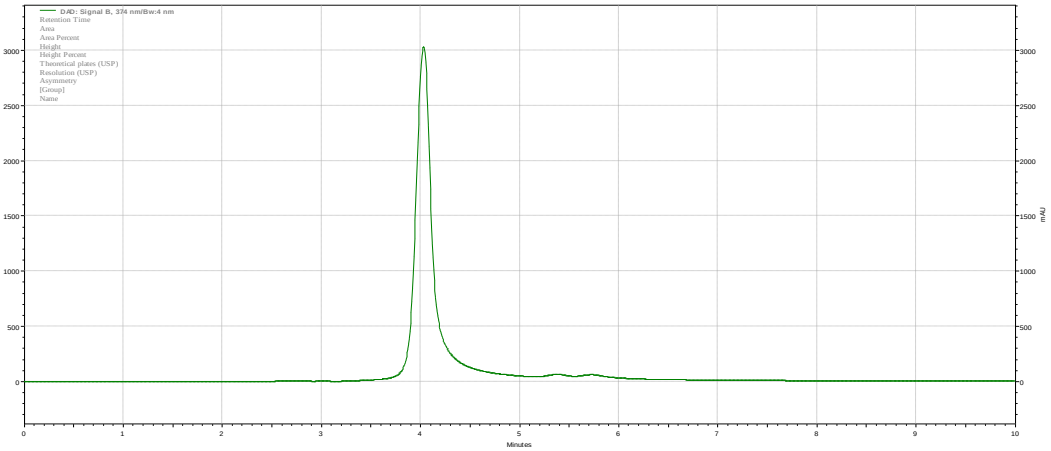

Figure 4: Chromatogram of Quercetin (retention time 4.00 min.)

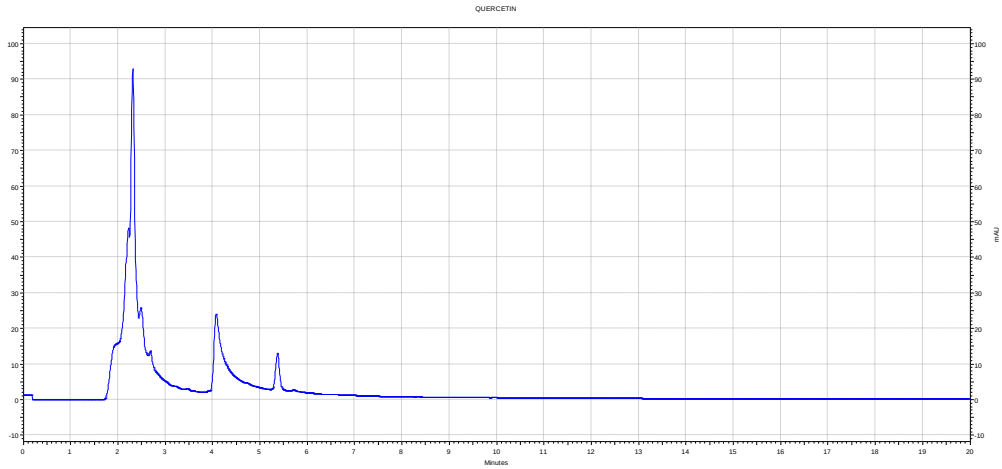

Figure 5: Chromatogram of hydroalcoholic extract of *Solanum nigrum* linn

Method Validation

Specificity

The peaks obtained for quercetin were found to have good resolution, and spacing . The results indicate that the chromatographic technique picked is suitable for additional quercetin validation and analysis. Figure 6 and table 1 reflects results of specificity.

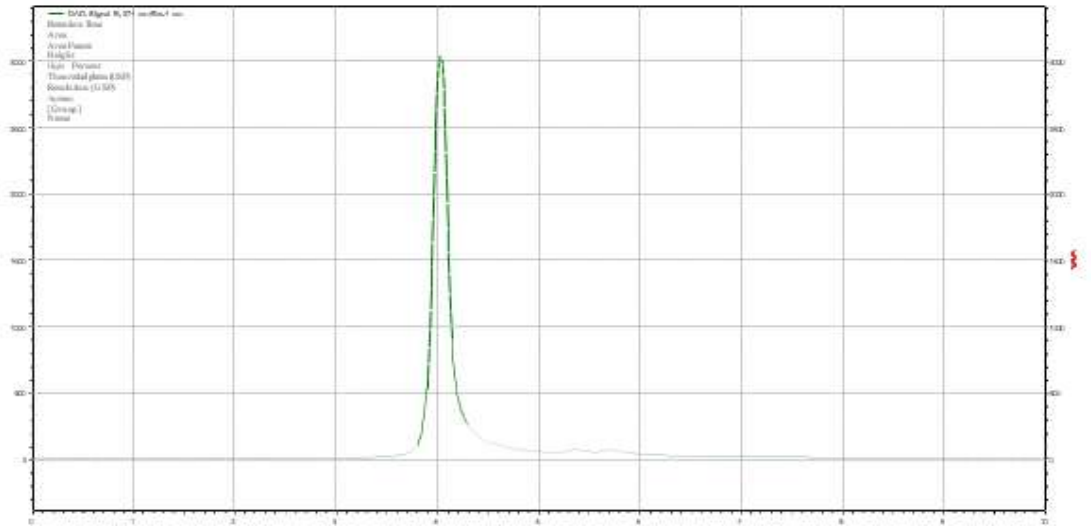

Figure 6: Specificity chromatogram of Standard Drug

Table 1: Specificity data of Quercetin standard

Sr. No.	Parameter	Result	Limit
1.	At the retention time of Quercetin standard there is no interference	Complies	Complies
2.	% RSD of retention time	1.8	NMT 2.0%
3.	% RSD of area	1.2	NMT 2.0%
4.	Theoretical plates	5493	NLT 2000

Table 2: Method Precision for Quercetin as standard

No. of Injection	Retention time (minutes)	Area	Theoretical plates (USP)
1	3.9	38250924	5583
2	3.91	38246901	5498
3	3.96	38126532	5563
4	3.99	39423215	5321
5	4.1	38472191	5463
6	4	38357189	5532
Average ± SD	3.97 ± 0.07	38479492	5493
% RSD	1.8	1.2	1.7

Note: SD is standard deviation of all readings

Table 3: Percent drug content of the Quercetin

Sr. no.	Wt. of Sample (gm)	Sample dilution (ml)	Wt. of standard (gm)	Sample Area	% Content (%w/w)
1	0.1000	10	0.100	55617646	6.32
2	0.1021	10		54523482	6.20
3	0.0995	10		56246589	6.38
4	0.1020	10		55321621	6.29
5	0.1051	10		57462431	6.51
6	0.1005	10		58234698	6.59

Average percent drug content was found to be 6.38±0.14 (% ±SD), with Relative Standard Deviation 2.2

Linearity

A concentration vs peak area response curve for quercetin was recorded with a peak area of each concentration. The standard calibration curve was found to be linear for quercetin at the concentrations of 100 µg/mL to 500 µg/mL. The determination coefficient (R^2) was obtained 0.9992 for quercetin with the help of linear regression analysis. The calibration curve equation for quercetin's mean peak and concentration (µg/mL) was y = 9586x - 5E+06 (Fig.7).

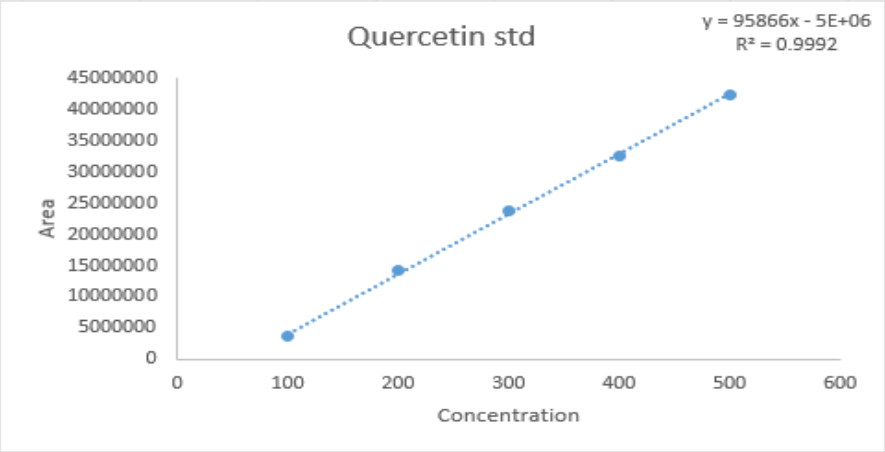

Figure 7: Linearity graph of Quercetin by RP-HPLC

Robustness

The sample, standard and placebo were injected under various condition of chromatographs, as indicated below: alterations in flow rate (±0.1 mL/min) result in wavelength alterations (±2 nm)(28).

Robustness reports indicated that the outcomes of the method used remained unchanged by trivial deliberate modifications in the mobile-phase composition, flow rate, and wavelength.

Table 4: Drug content of Quercetin in the plant extract

Sr. No.	Weight of sample (gm)	Weight of standard (gm)	Area of Standard	Area of Sample	% Content (% w/w)
1.	0.1000	0.1	30226357	55617646	6.317

Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection and limit of quantification of the quercetin in the extract is reports as shown in table 5.

Table 5: LOD and LOQ of Quercetin in the extract

Sr. No.	Parameter	Quercetin
1	LOD	22.45 µg
2	LOQ	68.05 µg

CONCLUSION:

A simple HPLC approach was devised for quantifying flavonoid (quercetin) in extracts high in quercetin from the flavanol family. This approach has the benefit of being quick to analyze and not requiring any specific instruments. Quercetin, a flavanol, is often found in a variety of plants. The method described here may be used to quantify quercetin and other flavanols from the same family in a variety of herbal extracts and formulations, and it is particularly useful for quality control testing of raw material extracts and herbal formulations including quercetin. In our laboratory, it has proven to be an efficient method for measuring quercetin for chemical standardization in extracts of *Solanum nigrum* and other flavonoid-rich plant. The technique is appropriate for assaying flavanols in a range of studies and innovative formulations due to its broad range of linearity, accuracy, and precision. Research is underway by quantifying Quercetin and other Flavonols in biological fluids to create a suitable high- performance liquid chromatography (HPLC) method for quantifying Quercetin and other Flavonols in biological fluids.

CONFLICT OF INTEREST:

The authors declare no any conflict of interest for the article

REFERENCES:

1. Wang, L., Gao, G., Bai, Y., Luo, W., Lin, C. and Jia, Q., 2011. Fingerprint quality detection of Solanum nigrum

using high-performance liquid chromatography–evaporative light scattering detection. *Pharmaceutical biology*, *49*(6), pp.595-601. https://www.tandfonline.com/doi/pdf/10.3109/13880209.2010.535171

2. Ahmad, R., Gupta, A., Fatima, A., Husain, I. and Srivastava, A.N., 2017. The evaluation of biological activity of methanolic extracts of Solanum nigrum and molecular docking analysis of selected phytoconstituents against Vimentin. *Journal of Intercultural Ethnopharmacology*, *6*(4), pp.391-400.
3. Sammani, A., Shammaa, E. and Chehna, F., 2013. Qualitative and quantitative steroidal alkaloids of solanum species distributed widely in Syria by TLC and HPLC. *Int. J. Pharm. Sci. Rev. Res*, *23*, pp.23-27.
4. Saleem, T.M., Chetty, C., Ramkanth, S., Alagusundaram, M., Gnanaprakash, K., Rajan, V.T. and Angalaparameswari, S., 2009. Solanum nigrum Linn.-A review. *Pharmacognosy reviews*, *3*(6), p.342.
5. Al Sinani, S.S.S. and Eltayeb, E.A., 2017. The steroidal glycoalkaloids solamargine and solasonine in Solanum plants. *South African Journal of Botany*, *112*, pp.253-269. https://www.sciencedirect.com/science/article/pii/S0254629917305185
6. Ravi, V., Saleem, T.S.M., Maiti, P.P., Gauthaman, K. and Ramamurthy, J., 2009. Phytochemical and pharmacological evaluation of Solanum nigrum Linn. *Afr J Pharm Pharmacol*, *3*(9), pp.454-457. https://academicjournals.org/article/article1380791142_Ravi%20et%20al..pdf
7. Gogoi, R., Sarma, N., Pandey, S. and Lal, M., 2021. Phytochemical constituents and pharmacological potential of Solanum khasianum CB Clarke., extracts: Special emphasis on its skin whitening, anti-diabetic, acetylcholinesterase and genotoxic activities. *Trends in Phytochemical Research*, *5*(2), pp.47-61. https://oiccpress.com/tpr/article/view/11796
8. Weissenberg, M., 2001. Isolation of solasodine and other steroidal alkaloids and sapogenins by direct hydrolysis-extraction of Solanum plants or glycosides therefrom. *Phytochemistry*, *58*(3), pp.501-508. https://www.sciencedirect.com/science/article/abs/pii/S0031942201001856
9. Nawaz, A., Jamal, A., Arif, A. and Parveen, Z., 2021. In vitro cytotoxic potential of Solanum nigrum against human cancer cell lines. *Saudi Journal of Biological Sciences*, *28*(8), pp.4786-4792. https://www.sciencedirect.com/science/article/pii/S1319562X21003715
10. D'Mello, P.M., Joshi, U.J., Shetgiri, P.P., Dasgupta, T.K. and Darji, K.K., 2011. A simple HPLC method for quantitation of quercetin in herbal extracts. *Journal of AOAC International*, *94*(1), pp.100-105. https://academic.oup.com/jaoac/article-abstract/94/1/100/5655334
11. Thongchai, W., Liawruangrath, B., Liawruangrath, S. and Saysin, S., 2011. High performance liquid chromatographic determination of solasodine in solanum species. *Asian J. Chem*, *23*, pp.2407-2410.
12. Garg, P., 2021. HPLC Estimation of Flavanoid (quercetin) of leaves and stem extracts of Ocimum sanctum and Tinospora cordifolia. *The Journal of Phytopharmacology*, *10*(4), pp.220-224. https://www.phytopharmajournal.com/Vol10_Issue4_01.pdf
13. Wach, A., Pyrzyńska, K. and Biesaga, M., 2007. Quercetin content in some food and herbal samples. *Food chemistry*, *100*(2), pp.699-704.
14. Braga, L.R., Rosa, A.A. and Dias, A.C.B., 2014. Synthesis and characterization of molecularly imprinted silica mediated by Al for solid phase extraction of quercetin in Ginkgo biloba L. *Analytical Methods*, *6*(12), pp.4029-4037. https://pubs.rsc.org/zh-tw/content/getauthorversionpdf/C4AY00471J
15. Chaudhari, V.S., Borkar, R.M., Murty, U.S. and Banerjee, S., 2020. Analytical method development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers. *Journal of Pharmaceutical and Biomedical Analysis*, *186*, p.113325. https://www.sciencedirect.com/science/article/abs/pii/S0731708520300121
16. Hadjmohammadi, M. and Sharifi, V., 2009. Investigation of optimum extraction conditions for determination of quercetin and kaempferol in coriander (Conundrum sativum L.) by using experimental design and HPLC. *Journal of Food and Drug Analysis*, *17*(4), p.7. https://www.jfda-online.com/journal/vol17/iss4/7/
17. Heimhuber, B., Galensa, R. and Herrmann, K., 1988. High-performance liquid chromatographic determination of polymethoxylated flavones in orange juice after solid-phase extraction. *Journal of Chromatography A*, *439*(2), pp.481-483. https://www.sciencedirect.com/science/article/abs/pii/S0021967301838656
18. Rowland, I., 1999. Optimal nutrition: fibre and phytochemicals. *Proceedings of the nutrition Society*, *58*(2), pp.415-419. https://www.cambridge.org/core/services/aop-cambridge-core/content/view/6E4B17CF4E2A1F4B3537C7D4665349FC/S0029665199000543a.pdf/optimal_nutrition_fibre_and_phytochemicals.pdf
19. Silva, E.L., Almeida-Lafetá, R.C., Borges, R.M. and Staerk, D., 2017. Dual high-resolution inhibition profiling and HPLC-HRMS-SPE-NMR analysis for identification of α-glucosidase and radical scavenging inhibitors in Solanum americanum Mill. *Fitoterapia*, *118*, pp.42-48. https://www.sciencedirect.com/science/article/pii/S0367326X17300242
20. Chakraborty, A., Bhattacharjee, A., Dasgupta, P., Manna, D. and Chun, W.O.H., 2016. Simple method for standardization and quantification of linoleic acid in Solanum nigrum berries by HPTLC. *J Chromatogr Sep Tech*, *7*(342), p.2. https://www.omicsonline.org/open-access/simple-method-for-standardization-and-quantification-of-linoleic-acid-insolanum-nigrum-berries-by-hptlc-2157-7064-1000342.php?aid=83193

21. Macêdo, S.K., Almeida, T.S., Alencar Filho, J.M.T., Lima, K.S.B., Libório, R.C., Costa, M.M., Rolim Neto, P.J., Rolim, L.A. and Nunes, X.P., 2021. Phytochemical identification and quantification of quercetin in Triplaris gardneriana wedd. leaves by HPLC-DAD with evaluation of antibacterial activity. *Natural product research*, *35*(18), pp.3083-3088.
22. Khaled, J.M., Alyahya, S.A., Kanisha, C.C., Alharbi, N.S., Kadaikunnan, S., Ramachandran, G., Alanzi, K.F., Rajivgandhi, G., Vimala, R.T.V. and Manoharan, N., 2021. Anti-biofilm activity of LC-MS based Solanum nigrum essential oils against multi drug resistant biofilm forming P. mirabilis. *Saudi Journal of Biological Sciences*, *28*(1), pp.302-309. https://www.sciencedirect.com/science/article/pii/S1319562X20304836
23. Abdullatef, O., Khalifa, Y., Morshedy, S. and Talaat, W., 2022. Green Bio Estimation of Co-Administered Non-Steroidal Anti Inflammatory Drug Diclofenac and Leflunomide in Rat Plasma by New HPLC Method. *Egyptian Journal of Chemistry*, *65*(132), pp.1307-1316. https://ejchem.journals.ekb.eg/article_271104_bda83abc9bd328ce54f7ab1b52fb300b.pdf
24. Salman, A.T., 2021. RP-HPLC estimation of ceftriaxone sodium in pharmaceuticals. *Egyptian Journal of Chemistry*, *64*(9), pp.4901-4906. https://journals.ekb.eg/article_165227_f1b94119469e248b3edd7879d56e3f48.pdf
25. El-Haddad, A.E., Gendy, A.M., Amin, M.M., Alshareef, W.A. and El Gizawy, H.A., 2022. Comparative characterization of carob pulp and seeds extracts: HPLC, antimicrobial, anti-inflammatory, and cytotoxic studies. *Egyptian Journal of Chemistry*, *65*(10), pp.279-284. https://ejchem.journals.ekb.eg/article_221194_f7b7ccf3e7f62f51e82c87f43895918c.pdf
26. Mohamed, S.H., Youssef, A.F.A., Issa, M., Abdel Salam, H. and El-Ansary, A., 2021. Validated HPLC method for quantitative analysis of gallic acid and rutin in leaves of Moringa oleifera grown in Egypt. *Egyptian Journal of Chemistry*, *64*(3), pp.1583-1591. https://journals.ekb.eg/article_123806_96dd999898d9e37b797fb082c8e50b3e.pdf
27. Saeed, N., Afridi, M.S.K., Latif, A., Fahham, H.H., Aslam, I., Mazhar, M. and Afridi, M.S.K., 2022. Development and validation of HPLC method for quantification of daclatasvir in pure and solid dosage form. *Egyptian Journal of Chemistry*, *65*(2), pp.81-91. https://ejchem.journals.ekb.eg/article_187912_2fba188d6afc582ed20ba97b8f443d2b.pdf
28. Alnedawi, Z., Hassan, A.M., Hadi, H. and Shabana, A., 2022. Development HPLC technique for determining Oxymetazoline and Isoxspurine in pharmaceutical formulations. *Egyptian Journal of Chemistry*, *65*(11), pp.779-784.
29. Ghosh, S., Mandal, S., Roy, T. and Ravikumar, B.V.V., 2021. A Novel RP–HPLC analytical method development and validation of fulvestrant injection as per ICH guidelines. *Egyptian Journal of Chemistry*, *64*(4), pp.2047-2055.
30. Girme, A., Bhoj, P., Saste, G., Pawar, S., Mirgal, A., Raut, D., Chavan, M. and Hingorani, L., 2021. Development and validation of RP-HPLC method for vicenin-2, orientin, cynaroside, betulinic acid, genistein, and major eight bioactive constituents with LC-ESI-MS/MS profiling in ocimum genus. *Journal of AOAC International*, *104*(6), pp.1634-1651. https://drive.google.com/file/d/1bQT6vJmftQOgUsG8U1I6Mglkf492L_ns/view