



QUALITY ASSESSMENT AND QBD APPROACHED HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF GALLIC ACID AND ROSMARINIC ACID

Mahantesh Kunchanur^a, Vinodh Kumar Mannur^{a*}, Sneha Patil^b

Article History: Received: 17/03/2023

Revised: 25/04/2023

Accepted: 17/05/2023

ABSTRACT

The current work intends to investigate the quality of selected herbs and create a QbD assisted RP-HPLC method for the simultaneous estimation of gallic acid and rosmarinic acid. According to physicochemical, phytochemical, and toxic contaminant analysis, the quality of crude *Terminalia chebula* and *Ocimum sanctum* samples was assessed. The ATP and CQA's were determined, and then the method was optimized using a 2² factorial design. The tailing factor GA (R1) and theoretical plates GA (R2) were chosen as dependent variables, meanwhile the flow rate (X1) and mobile phase ratio (X2) were chosen as independent variables. The mobile phase, Methanol and Water in a 55:45 ratio, with a flow rate was selected as 1 mL/min and a detection wavelength of 298 nm, were found to be the ideal chromatographic conditions. Extrapolated from the results is that the QbD approach was employed to assure the development of a more accurate method that could generate constant, reliable, and improved data throughout the process whilst saving time.

Key Words: QbD, RP-HPLC, ATP, QTPP, CQA, Gallic acid, Rosmarinic acid

a & a*. Department of Quality Assurance, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India-590010.

b. Department of Pharmacognosy, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India-590010

DOI: 10.48047/ecb/2023.12.5.082

*Corresponding author

Prof. Vinodh Kumar Mannur, Professor
drvsmannur@gmail.com

Dept. of Pharmaceutical quality assurance,
KLE College of Pharmacy, Belagavi,
KLE Academy of Higher Education and Research,
Nehru Nagar, Belagavi-590010 Karnataka, India.
Office Tel.: 0831-2470362 Fax: 0831-2470640,
Mobile Phone: +91 9164500550

INTRODUCTION

Medicinal plants have a substantial influence on health owing to the phytoconstituents they contain. Alkaloids, glycosides, tannins, flavonoids, and phenolic compounds are among the most significant of these elements. Phenolic acids are a broad class of compounds that include hydroxybenzoic and hydroxycinnamic acids. Plant phenolic acids include ferulic acid, ellagic acid, synergic acid, caffeic acid, gallic acid, and rosmarinic acid, among others. They are also employed as natural antioxidant substitutes in the culinary, cosmetic, and pharmaceutical sectors. Gallic acid and Rosmarinic acid are two significant phenolic acids found in numerous plants, fruits, tea, coffee, and wine. It is generated by several species as free acids, esters, catechin derivatives, and hydrolysable tannins. Methylated gallic acids, such as syringic

acid, or galloyl conjugates of catechin derivatives, such as flavan-3-ols, or polygalloyl esters of glucose, quinic acid, or glycerol, can also be synthesised.^[1,2]

Gallic acid is categorized as a phenol. Its chemical formula is 3, 4, 5-trihydroxybenzoic acid. Its structure includes phenolic groups, which provide as a source of readily available hydrogen atoms, allowing radicals to be delocalized across the phenolic structure. Researchers are fascinated in the pharmacological activity of these compounds as radical scavengers. Gallic acid may be a viable starting molecule for novel medication development due to these properties.^[3]

Scarpati and Oriente isolated rosmarinic acid from *R. officinalis* in 1958. For the first time, Ellis and Towers described a RA biosynthetic route 12 years later. RA, a phenylpropanoid (i.e. C₁₈H₁₆O₈, molecular mass: 360.3 g/mol), is the caffeic acid ester of 3, 4-dihydroxyphenyllactic acid. A composition of two aromatic amino acids, phenylalanine and tyrosine the RA formed and they are joined by an ester linkage. Caffeic acid and 3, 4-dihydroxyphenyllactic acid is produced by two different pathways of the shikimic acid biosynthetic pathway. The caffeic acid component is generated primarily from phenylalanine, while the 3, 4-dihydroxyphenyllactic acid component is created exclusively from tyrosine.^[4]

The task of maintaining the quality of medicinal herbs is crucial; factors including geographical and environmental variations in growing conditions, physical constants, adulterations, and foreign components may have an impact on the herbal drug quality and consistency from batch to batch. Nevertheless, it is crucial to evaluate the quality of herbal crude drugs, particularly in terms of parameters like pharmacognostic and phytochemical, so as to demonstrate the efficacy and safety of herbal medicines. Various analytical methods have hitherto been published for the determination of

total and individual gallic acid and rosmarinic acid in diverse matrices, notably those utilising spectrophotometric analysis for gallic acid and rosmarinic acid determination.^[5,6]

Effective component separation is better to prevent mutual interferences and achieve accurate quantification of GA and RA in various samples. As a result of their ability to separate substances, chromatographic techniques are thought to be the best methods for quantifying gallic acid and rosmarinic acid. For the separation of complex substances, HPLC methods are frequently used due to the method's simplicity and sensitivity. The excellent efficiency and quick separation of the HPLC process are well recognised.^[7,8]

There are a number of HPLC techniques documented for the separation of gallic acid and rosmarinic acid. The previously described methods, in contrast side, were centered on the time-consuming principle of altering OFAT (One Factor at a Time). But at another end, as a novel idea for developing and analysing quality pharmaceutical products, the Quality by Design method is picking up steam. When used to develop an analytical technique, this methodology is known as AQbD. The ICH Q8 and Q9 recommendations, which depend heavily on the principle of variation of specific parts and defining the risk that may result in inadequate method robustness, support the QbD approach as a systemic and risk-based strategy. Generation of Design space is the core concept of AQbD, It suggests that changing the method's parameters won't have a substantial impact on the outcomes because the experimental zone has been established. While a result, this strategy adds stability to the technique as it is being developed. The FDA has been notably proactive in advocating the QbD concept, which demands integrating efficiency Rather of attempting to assess dependability after the development stage is complete, incorporate it into the process and the product in a systematic, scientific, and risk-based approach during the development stage. The previously reported HPLC-UV methods for gallic acid and rosmarinic acid have significant drawbacks, including long separation periods, low resolution, and/or complex solvent combinations with gradient elution. The current study intends to create a QbD aided simple and quick isocratic RP-HPLC technique for the simultaneous estimation of GA and RA for quality control in the form powdered raw material.

MATERIALS AND METHODS

Chemicals and reagents

The raw materials of plants *Terminalia chebula* and *Ocimum sanctum* were purchased from BMK Ayurveda Pharmacy, Shahapur, Belagavi and the plants specimen were confirmed and verified by CRF, BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi. After being gathered, the raw materials were shade dried, crushed into a coarse powder, and then kept in airtight containers.

Quality assessment of *Terminalia chebula* and *Ocimum Sanctum*^[6,11]

By implementing the quality control parameters stated in the raw samples, the quality of *Terminalia chebula* and *Ocimum sanctum* was evaluated.

The analysis of phytochemicals and physicochemical parameters following WHO criteria, total ash value, water-

soluble ash value, acid insoluble ash value, extractive value, and moisture content examples of such parameters, were carried out.

Procurement of Marker compounds

Himalaya Wellness, Bengaluru, provided the standard marker compounds of a few chosen phytoconstituents as free samples, which were then stored in the proper containers.

Preparation of standard solution^[12,13]

The standard solutions were tried using different solvents but methanol and water was selected as optimized mobile phase for GA and RA phytoconstituents hence the different concentration solution were prepared using selected solvents & filtered using a 0.22mm membrane filter prior to being injected into the chromatographic column.

To prepare the sample, separately weighed accurately 1gm of powdered *Terminalia* and *Ocimum* samples were thoroughly refluxed for 1hr with 50mL of methanol: water. The solution was filtered & the residue was refluxed for 1 hour with 50mL of methanol and again filtered. The filtrate was homogenized and diluted to 100mL with methanol: water for further analysis.

Chromatographic instrumentation and conditions^[14,15]

The chromatographic analysis was performed out using an Agilent 1220 Infinity II LC system (Agilent technologies, Germany), which was outfitted with a system controller, online degasser, solvent supply module, low-pressure gradient pump, and manual sample injector (injection volume ranging from 5 to 20 µL), & UV-Vis detector. The Agilent OpenLab software was used to operate the instruments and process the data. For chromatographic separation, C-18 column (5 mm, 4.6mm 250 mm, ZORBAX) was utilised. The mobile phase was thought up of different ratios of Methanol & Water. The flow rate of the samples was set to 1 mL/min, and the wavelength was fixed to 298 nm. A 20 µL sample was put into the column for each analysis.

QbD based method development^[16,17]

Defining of analytical target profile (ATP) and critical quality attributes (CQA)

The utilization of the QbD approach for analytical method development implies the generation of ATP. In this section, the basic qualities that are considered to be indicators of approach performance must be identified. To achieve reliable outcomes, CQA must be recognized from the specified ATP that will be beneficial in assessing the compensating performance of the developed technique.

Optimization of method using design of experiments (DoE)

The DoE was used to evaluate the influence of chosen process factors and their responses on the HPLC procedure. To discover the optimal chromatographic procedure, a basic 2² complete factorial design with two variables and two levels was used, resulting in four experimental runs. Design-Expert software version 13.0 was employed to generate the tests (Stat-Ease Inc., Minneapolis, MN, USA). To identify the critical values required to achieve the desired response of the various independent variables the DoE software was used.

Establishment of method operable design region (MODR)

To determine its suitability, the MODR was created based on the regression models and an evaluation of the chance of failure. Furthermore, the optimal mobile phase was predicted

Validation ^[10, 18, 19]

Technique validation was carried out in compliance with ICH Q2 (R1) guidelines to validate the consistency of the optimised method. The modified technique's system applicability, linearity, LOD, LOQ, precision, and accuracy were all verified.

System Suitability ^[10, 20]

The suitability of the system was determined by injecting six replicate shots of standards (20 µg/ml), accompanied by measurement of the % RSD for the CQAs.

Linearity ^[10, 21]

Both gallic acid and rosmarinic acid standard was evaluated for linearity. Five various concentrations ranging from 5 to 30 µg/mL of gallic acid and rosmarinic acid were injected into the HPLC system to produce a linear dynamic range for each. Using least squares regression, the slope, y-intercept, and correlation coefficient (r^2) were calculated to determine linearity.

LOD and LOQ ^[10]

The sensitivity of developed method was assessed by computing LOD and LOQ, which are impacted by the SD and slope of the calibration curve. The following formulae were used to calculate LOD and LOQ.

$$LOD = 3.3 \frac{\sigma}{S}$$

using the overlay plot displaying MODR. All of the specifications indicated in the ATP are met within the design region at a given risk level. The finest run was chosen based on the criteria of the designated CQAs

Where S is the mean slope of calibration curves and σ is the standard deviation of the y-intercept.

Precision ^[10]

The optimised method's precision was evaluated in terms of intra-day and inter-day precision. The intra-day precision was achieved by assessing repeatability on the same day at different time intervals and the Inter-day precision was assessed over three consecutive days for different analyte concentrations, such as low, moderate, and high concentrations.

Accuracy ^[10, 21]

The recovery studies were used to prove the method's accuracy. Known quantities of prepared samples were spiked in triplicate injections at 50, 100, and 150% concentration levels. The mean % recovery was calculated for each standard.

RESULT AND DISCUSSION**Quality assessment of Terminalia chebula and Ocimum sanctum:**

The evaluation of the quality of herbal crude drugs is essential for determining the safety of their usage. All of the physicochemical parameters were found to be within the specified standard limits. The data is given in Table no. 1. *Terminalia chebula* and *Ocimum sanctum* preliminary phytochemical screening Secondary metabolites discovered comprise phenols, alkaloids, flavonoids, tannins, triterpenes, steroids, saponins, and proteins.

Table no. 1 Quality assessment of Terminalia chebula and Ocimum sanctum

Parameters	<i>Terminalia chebula</i>	<i>Ocimum sanctum</i>
Moisture content	2.58±0.289	0.75±0.215
Aqueous soluble extractive value	8.63±1.06	4.1±0.282
Alcohol soluble extractive value	13.2±0.616	8.7±0.163
Acid insoluble ash value	10.41±1.55	8.66±1.312
Total ash value	2.75±0.735	0.76±0.205
Water-soluble ash value	1.6±0.294	3.36±0.277

Chromatographic conditions

In the beginning, chromatographic tests were carried out by carefully reviewing the prior scientific reports on the chromatographic separation of gallic acid and rosmarinic acid that were available. The majority of previously published method i.e. for the use of acetonitrile, methanol, and other organic solvents. In order to simultaneous estimation of gallic acid and rosmarinic acid, we attempted to develop a perfect method using a systemic ratio of methanol and water as an aqueous phase with the best flow rate and wavelength.

QbD assisted RP-HPLC method development

The analytical target profile is a description of the different qualities that are unquestionably representative of technique performance and consequences. The selection of an analytical target is influenced by taking the technique objective into consideration. In the AQbD approach, defining ATP in relation to quality attributes is the first step. The objective of the proposed analytical method in the current investigation was to obtain a good separation for the measurement of gallic acid and rosmarinic acid with an acceptable analysis time and a modest tailing factor. CQAs were recognised as tailing factor (Not More Than 2) and theoretical plates based on the aforementioned Analytical target profile (Not less than 3000).

The rationale for choosing the tailing factor and theoretical plates as CQAs depends on the accuracy of each marker compound's quantitation. Tailing factor and theoretical plates have been chosen in order to achieve precise peak integration because quantitation accuracy declines as tailing factor increases. To accurately quantify the two chosen marker compounds, this is also crucial.

Method optimization by DoE

Two independent variables, notably mobile phase ratio (X1) and flow rate (X2), were varied at two different levels that were coded for low and high (-1 and +1, respectively), in accordance with the applied 2² complete factorial design. For the experiments, response factors such as the tailing factor and theoretical plates were used. Table no. 2 shows the selected experimental design variables resulting from four chromatographic experiments. The analytical method was further statistically improved by analysing numerous statistical parameters offered by Design-Expert Software, Version 13. Table no. 3 summarises the statistical data for the used design. The relationship between the independent variables (Mobile phase ratio (X1) and Flow rate (X2) and responses (Tailing factor of GA (R1), theoretical plates of GA (R2), Tailing factor of RA (R3), theoretical plates of RA (R4)) was represented by mathematical expressions in the form of polynomial equation

Table no. 2 Experimental design for screening of independent variables and responses

Code	Coded levels		Actual levels		Responses			
	X ₁	X ₂	X ₁	X ₂	Tailing factor GA R ₁	Theoretical plates GA R ₂	Tailing factor RA R ₃	Theoretical plates RA R ₄
Trail 1	1	1	55:45	1.1	1.46	6263	1.48	6161
Trail 2	1	-1	55:45	1.0	1.21	6624	1.29	6641
Trail 3	-1	1	50:50	1.0	1.69	4798	1.62	4329
Trail 4	-1	1	50:50	1.1	1.47	5012	1.45	4976

X₁: Mobile phase ratio, X₂: Flow rat

Table no. 3 Summary of statistical parameters and polynomial equations.

Response		p-value	Model significance	Polynomial equation
G A	R ₁	0.0441	Significance	+1.45-0.122*X ₁ +0.1175*X ₂
	R ₂	0.0469	Significance	+5674.2+769.2*X ₁ -143.7*X ₂
R A	R ₁	0.0426	Significance	+1.46-0.075*X ₁ +0.0900*X ₂
	R ₂	0.0454	Significance	+5526.7+874.25*X ₁ -281.75*X ₂

A positive coefficient sign signifies a synergistic impact, whereas a negative term signifies an antagonistic influence on the reaction. A higher coefficient indicates that the independent variable has a significant influence on the result. The Response Surface Plots were generated in order to graphically illustrate the each factor's impact on the responses

shown in Figure 1. The response surface plots demonstrate the connection between each dependent variable (CQA) and the independent variables. The Response Surface Figure showed that blue indicates a reduced tailing factor and red indicates a greater tailing factor in the case of response, R1 (Tailing factor GA), and R3 (Tailing factor RA).

Factor Coding: Actual

tailing factor 1

Design Points:

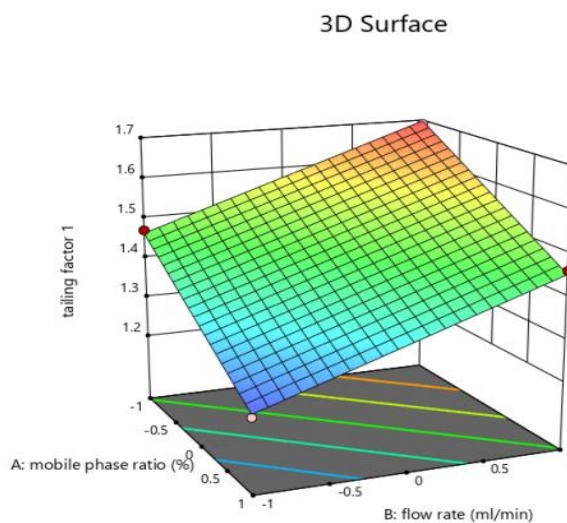
● Above Surface

○ Below Surface

1.21 1.69

X1 = A

X2 = B



(A)

Factor Coding: Actual

T plates 1

Design Points:

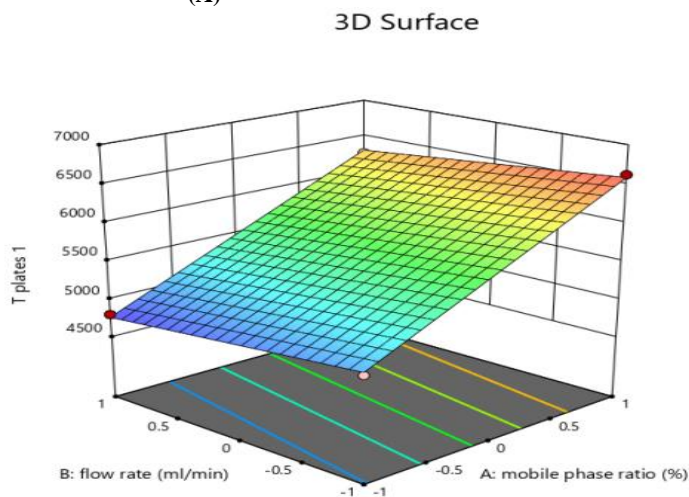
● Above Surface

○ Below Surface

4798 6624

X1 = A

X2 = B



(B)

Factor Coding: Actual

tailing factor 2

Design Points:

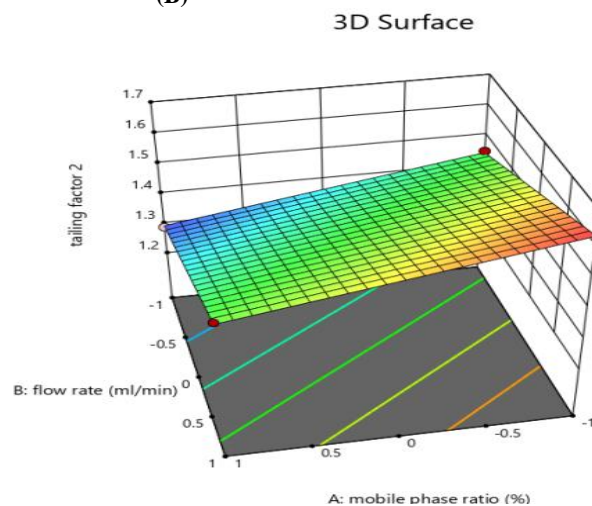
● Above Surface

○ Below Surface

1.29 1.62

X1 = A

X2 = B



(C)

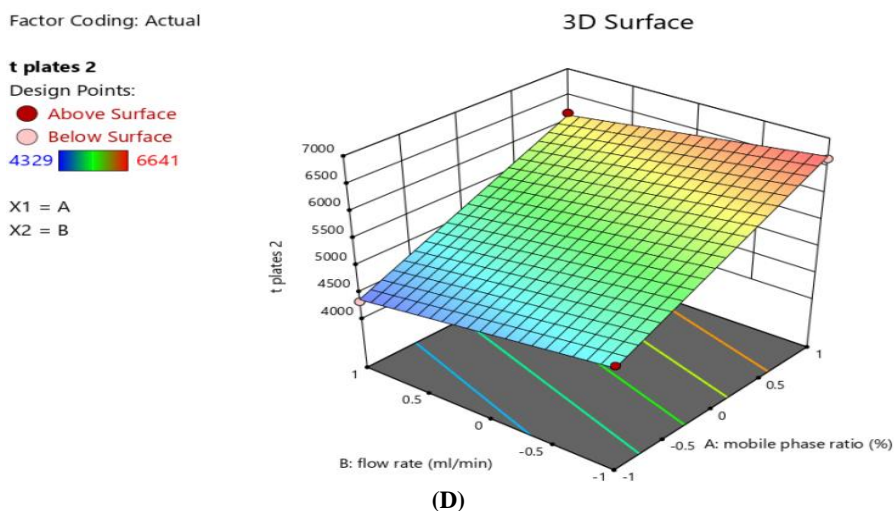


Figure 1. 3D response surface plots for dependent valuables, tailing factor of GA (A), theoretical plates of GA (B), tailing factor of RA (C), theoretical plates of R (D).

Establishment of MODR

Analytical Trial 2 (Methanol and Water Ratio 55:45) from the method operable Design incorporates various into the area of successful operating ranges and satisfies the ATP and CQA criteria for HPLC method depicted in Figure 2 The effectiveness of chromatographic conditions to produce

decreased tailing factor and peak width was a criterion for choosing the best run. Consequently, trial 2 with Methanol and Water (55:45) was selected as the most effective HPLC method and tabulated in Table no. 4 and The HPLC chromatogram for standard gallic acid and rosmarinic acid samples have been shown in Figure 3.

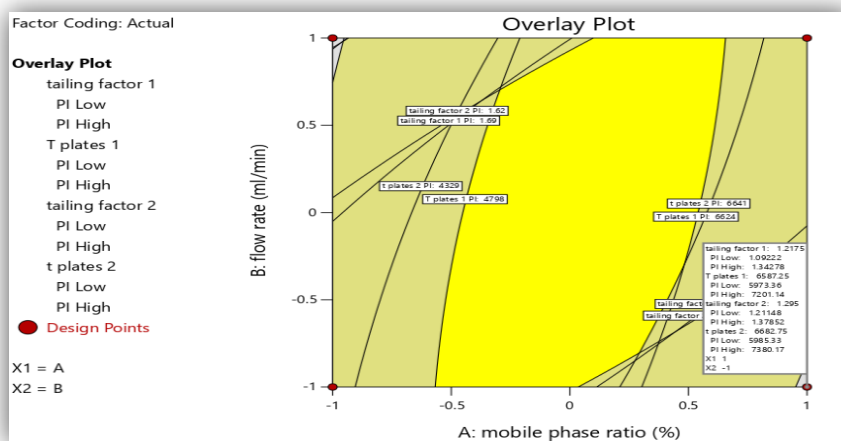


Figure 2. Overlay plot showing the design space with optimal analytical conditions.

Table no. 4. Optimized Chromatographic Conditions.

Parameters	Chromatographic conditions
Stationary phase	ZORBAX C18 (250mm 4.6mm, 5m) column
Mobile phase	Methanol: Water
Mobile phase ratio	55:45
Flow rate	1 mL/min
Detection wavelength	298nm
Injection volume	20µL
Retention time of Gallic acid	2.6 min
Retention time of Rosmarinic acid	4.3 min

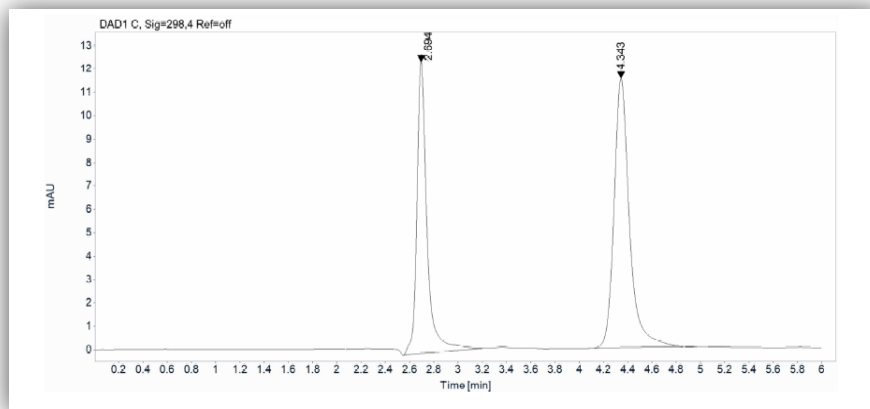


Figure 3. The HPLC chromatogram of simultaneous estimation of gallic acid and rosmarinic acid.

Method validation:

Table 5 provides a summary of the method validation data for the proposed RP-HPLC method. The % RSD of various parameters, including peak area, retention time, and tailing factor, was utilized to confirm the developed method's system suitability. The % RSD of peak area, retention time, and tailing factor were all within acceptable ranges. For the measured concentration ranges, the linear calibration curve for GA and RA was obtained. The LOD and LOQ were

determined using the data from the calibration curve's linear regression equation. The reproducibility and repeatability of an analytical method are the representations of its precision. The % RSD of intra-day and inter-day for gallic acid and rosmarinic acid reveals the developed method's high precision. The percentage recovery was calculated by analysing the produced sample at three distinct concentration levels, namely 50, 100, and 150%. As a conclusion, the observed results show that the developed method is reliable.

Table 5. Validation data of the proposed method.

Validation parameters	Gallic acid	Rosmarinic acid
System suitability		
Retention time Mean±SD and % RSD	2.68±0.0168 and 0.89%	4.27±0.127 and 1.02%
Theoretical plates Mean±SD and % RSD	6624±0.0324 and 0.68%	6641±0.0781 and 1.12%
Tailing factor Mean±SD and % RSD	1.510±0.0254 and 0.85%	1.358±0.0874 and 0.65%
Linearity		
Linearity range µg/mL	5-25	5-25
Correlation coefficient	0.9983	0.9984
LOD (µg/mL)	0.146128	0.146509
LOQ (µg/mL)	0.442813	0.443968
Precision		
Intra-day (% RSD)	1.84	1.31
Inter-day (% RSD)	1.55	1.12
Accuracy		
50% recovery	95.70182	96.44044
100% recovery	99.02062	98.86455
150% recovery	98.8373	99.3223

n=3

CONCLUSION:

The research study reveals the effective application of analytical QbD for the development of easy and reliable HPLC method for the simultaneous estimation of GA and RA.

The quality and safety of the crude sample were confirmed by the positive results of all the quality control tests used to evaluate the quality of the sample of crude herbs. Quite few more advantages of the newly developed method include reduced analytical time, successful separation in terms of well-defined peaks, and the employment of a simple mobile

phase combination. Through the use of QbD concepts and DoE tools, it was possible to determine the influencing factors that were thought to be crucial for achieving the ideal chromatographic conditions for the precise measurement of both markers. It can be stated that using the QbD approach culminated in the development of a more robust approach that can generate dependable, consistent, and high-quality data throughout the process while also reducing time. In addition, the method was validated in compliance with ICH requirements

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this research.

ACKNOWLEDGMENTS:

The authors are very thankful to Principal, KLE College of Pharmacy Belagavi and Nipani. The authors are also thankful to Himalaya wellness, Bengaluru for providing gift samples

REFERENCES

1. Yuan, H., Ma, Q., Piao, G. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*, 2016, 21(559), 1-18. DOI: [10.3390/molecules21050559](https://doi.org/10.3390/molecules21050559).
2. Shi, Q., Song, D., Li, Q., Yang, H., Qi, L., Xin, Z., Wang, Q., Song, P., Chen, J., Hao, H., Identification of Effective Combinatorial Markers for Quality Standardization of Herbal Medicines. *Journal Chromatography A*, 2014, 1345, 78–85. DOI: [10.1016/j.chroma.2014.04.015](https://doi.org/10.1016/j.chroma.2014.04.015).
3. Nayeem, N., Asdaq, S., Salem, H., Alfqy, S., Gallic Acid: A Promising Lead Molecule for Drug Development. *Journal of Applied Pharmacy*, 2016, 8, 213–6. DOI: [10.4172/1920-4159](https://doi.org/10.4172/1920-4159).
4. Sik, B., Kapcsandi, V., Szekelyhidi, R., Hanczue, E., Ajtony, Z., Recent Advances in the Analysis of Rosmarinic Acid from Herbs in the Lamiaceae Family. *Natural Product Communications*, 2019, 1, 1-10. DOI: [10.1177/1934578X19864216](https://doi.org/10.1177/1934578X19864216).
5. World Health Organization. *Quality Control Methods for Medicinal Plant Materials*; WHO: Geneva, 1998.
6. World Health Organization. *WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues*; WHO: Geneva, 2007.
7. Sylvester, B., Tefas, L., Vlase, L., TomutTa, I., Porfire, A., A Quality by Design (QbD) Approach to the Development of a Gradient High-Performance Liquid Chromatography for the Simultaneous Assay of Curcuminoids and Doxorubicin from Long-Circulating Liposomes. *J. Pharm. Biomed. Anal.* 2018, 158, 395–404. DOI: [10.1016/j.jpba.2018.06.018](https://doi.org/10.1016/j.jpba.2018.06.018).
8. Gokbulut, A., Validated RP-HPLC Method for Quantification of Phenolic Compounds in Methanol Extracts of Aerial Parts and Roots of *Thymus sipyleus* and Evaluation of Antioxidant Potential. *Tropical Journal of Pharmaceutical Research*, 2015, 14(10), 1871-7. DOI: <http://dx.doi.org/10.4314/tjpr.v14i10.19>.
9. Gaonkar, V., Mannur, V., Mastiholimath, V., Hullatti, K., Development and Evaluation of Herbal Supplement: A Quality by Design Approach. *Indian Journal of Pharmaceutical Sciences*, 2020, 82 (4), 640-9. DOI: [10.36468/pharmaceutical-sciences.690](https://doi.org/10.36468/pharmaceutical-sciences.690).
10. ICH guideline Q2 (R1): Validation of analytical procedures. In *International Conference on Harmonisation, ICH, Geneva*, 2003.
11. Wootisin, N., Kongkiatpaiboon, S., Sukprasert, S., Sathiraku, K., Development and Validation of Stability Indicating HPLC Method for Determination of Caffeic Acid, Vitexin and Rosmarinic Acid in *Thunbergialaurifolia* Leaf Extract. *Pharmacogn J*, 2020, 12(3), 611-8. DOI: [10.5530/pj.2020.12.91](https://doi.org/10.5530/pj.2020.12.91).
12. Gaonkar, V., Mannur, V., Hullatti, K., Quality assessment and Analytical Quality by Design- based RP- HPLC method development for quantification of Piperine in *Piper nigrum* L. *Future Journal of Pharmaceutical Sciences*, 2022, 8(16), 2-9. DOI: <https://doi.org/10.1186/s43094-022-00405-9>.
13. Thyagaraj, V., Koshy, R., Kachroo, M., Mayachari, A., Sawant, L., Balasubramaniam, M., A validated RP-HPLC-UV/DAD method for simultaneous quantitative determination of rosmarinic acid and eugenol in *Ocimum sanctum* L. *Pharmaceutical Methods*, 2013, 4, 1-5. DOI: <http://dx.doi.org/10.1016/j.phme.2013.08.003>.
14. Baranauskaite, J., Kubiliene, A., Marksa, M., Petrikaite, V., Vitkevilius, K., Baranauskas, A., Bernatoniene, J., The Influence of Different Oregano Species on the Antioxidant Activity Determined Using HPLC Postcolumn DPPH Method and Anticancer Activity of Carvacrol and Rosmarinic Acid. *BioMed Research International*, 2017, 1, 1-7. DOI: <https://doi.org/10.1155/2017/1681392>.
15. Mesquita, M., Leao, W., Ferreira, M., Paula, J., Espindola, L., Soares, L., Reversed- phase- liquid chromatography method for separation and quantification of gallic acid from hydroalcoholic extracts of *Qualeagrandiflora* and *Qualeaparviflora*. *Pharmacognosy Magazine*, 2015, 11(44), 316-21. DOI: [10.4103/0973-1296.166062](https://doi.org/10.4103/0973-1296.166062).
16. Palakurthi, A., Dongala, T., Katakam, N., QbD based development of HPLC method for simultaneous quantification of Telmisartan and Hydrochlorothiazide impurities in tablets dosage form. *Practical Laboratory Medicine*, 2020, 21, 1-11. DOI: <https://doi.org/10.1016/j.plabm.2020.e00169>.
17. Kim, M., Park, G., Hong, S., Jang, Y., Analytical Quality by Design Methodology Approach for Simultaneous Quantitation of Paeoniflorin and Decursin in Herbal Medicine by RP-HPLC Analysis. *Natural Product Sciences*, 2021, 27(4), 264-73. DOI: <https://doi.org/10.20307/nps.2021.27.4.264>.
18. Garg, P., HPLC Estimation of Flavanoid (quercetin) of leaves and stem extracts of *Ocimum sanctum* and

- Tinosporacordifolia. The Journal of Phytopharmacology, 2021, 10(4), 220-4.
DOI: [10.31254/phyto.2021.10401](https://doi.org/10.31254/phyto.2021.10401).
19. Urmi, K., Nawaz, S., Islam, A., Analytical quality by design approach to RP- HPLC method development and validation for simultaneous estimation of esomeprazole and naproxen in modified- release dosage form. Future Journal of Pharmaceutical Sciences, 2022, 8(8), 2-16.
DOI: <https://doi.org/10.1186/s43094-021-00396-z>.
 20. Zacharis, C., Vastardi, E., Application of analytical quality by design principles for the determination of alkyl p-toluenesulfonates impurities in Aprepitant by HPLC. Validation using total-error concept. Journal of Pharmaceutical and Biomedical Analysis. 2018, 150,152-61.
DOI: <https://doi.org/10.1016/j.jpba.2017.12.009>.
 21. Ullaha, S., Rauf, N., Hussain, A., Sheikh, A., Farooq, M., HPLC profile of phenolic acids and flavonoids of Ocimum sanctum and O. basilicum. International Journal of Plant based pharmaceuticals, 2022, 1(2), 205-9.
DOI: <https://doi.org/10.29228/ijpbp.1>