Original Article

3^2 Factorial Design for Optimization of HPLC-UV Method for Quantification of Gallic acid in Lohasava and Pippalyasava

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ABSTRACT

Background: A new, simple, accurate and precise High Performance Liquid Chromatographic method (HPLC-UV) has been developed and validated in accordance with ICH guidelines for the determination of gallic acid in Lohasava and Pippalyasava. The developed method was optimised using 3^2 full factorial design by evaluating the effect of two independent variables i.e. mobile phase composition and flow rate on the various chromatographic responses such as retention time, area, number of theoretical plates and tailing factor. **Materials and Methods:** Chromatographic separation was achieved using Sun Chrome C_{18} column (250 mmX4.6 mm, 5μm) as stationary phase. The optimised mobile phase consisted of 0.05% o-phosphoric acid: Acetonitrile (93:7,v/v) and flow rate was set at 1.5 ml/min with detection at 270 nm. **Results:** The retention time of gallic acid was found to be 3.71 min. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 0.0487 μg/ml and 0.1478 μg/ml; respectively. The proposed method was found to be linear in the concentration range of 0.15-6 μg/ml for gallic acid with correlation coefficient of 0.9955. The proposed method was found to be accurate with 99.43%-100.81% recovery. The % RSD for intraday and interday precision was found to be less than 2.0 indicating that the proposed method was precise. The method was found to be robust with respect to % RSD and % w/w of gallic acid. The amount of gallic acid in Lohasava and Pippalyasava was found to be 3.00% and 0.98%; respectively. **Conclusion:** Factorial design assisted HPLC method was developed and validated for quantitative determination of gallic acid in two commercial formulations of asava. This method can be used for routine quality control of asava using gallic acid as marker compound.

Key words: Gallic acid, Factorial design, Method validation, HPLC-UV, Quality by Design.

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INTRODUCTION

Herbal formulations have achieved massive popularity as medicinal products, nutraceuticals and cosmetics in developing countries.[1] Polyherbal formulations are medicines containing a mixture of different herbal powders or extracts making it difficult to standardize.[2] Asava and Arishta are unique dosage form discovered by Ayurveda having long shelf life.[3] Lohasava is a self-fermented polyherbal formulation used in the treatment of anemia, jaundice and digestive disorders. Pippalyasava is used as a carminative, stomachic and in the treatment of dysentery, cough and loss of appetite. Lohasava mainly contains *Emblica officinalis, Terminalia belerica* and *Terminalia chebula* while Pippalyasava contains *Emblica officinalis* and other medicinal plants. Gallic acid is one of the constituents found in these medicinal plants. Chemically gallic acid is 3, 4, 5-trihydroxybenzoic acid (Figure 1). Gallic acid and its derivatives are effective antioxidants showing potential health effects. Recent research shows that gallic acid and its derivatives also possess hepatoprotec-

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tive and neuroprotective effects. It is also believed to possess free radical scavenging properties.[4,5]

Analytical Quality by Design (AQbD) is an organized approach to method development that begins with pre-specified objectives and highlights method understanding and control, based on Design of Experiment (DoE) and Quality Risk Management (QRM).[6,7] Applying AQbD helps in reduction of variations that may alter method performance. Quality is built into the method as the complete method is developed in a design space making it more robust and reducing chances of revalidation.[8-10] Figure 2 shows diagrammatic representation for approach to AQbD.

Several HPLC[11-16] and HPTLC[17-20] methods has been reported for determination of gallic acid. As per the latest literature survey, no HPLC-UV method has been optimized using DoE for quantification of gallic acid in Lohasava and Pippalyasava. Thus, the aim of present study was to develop and optimize HPLC-UV method using 3^2 factorial design followed by validation for quantification of gallic acid in Lohasava and Pippalyasava.

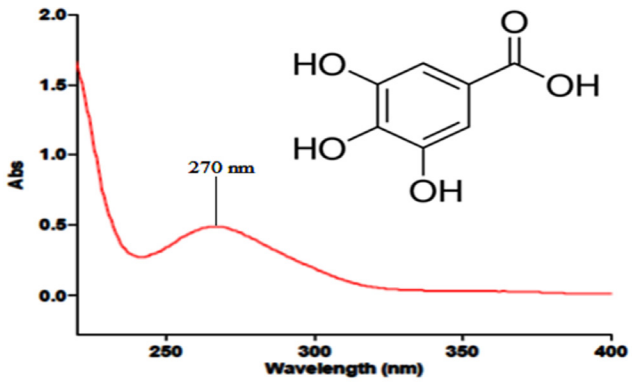

Figure 1: UV spectrum and structure of gallic acid.

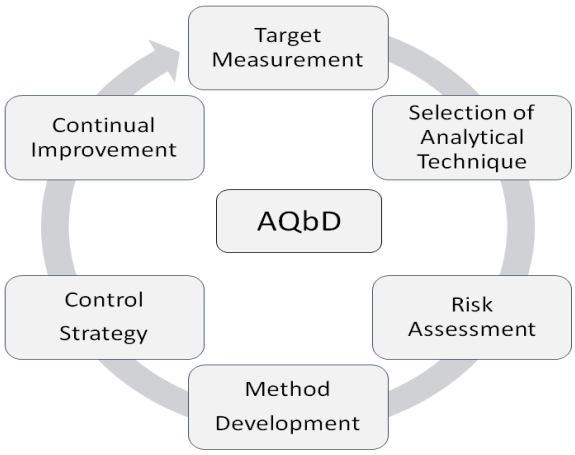

Figure 2: Schematic diagram of QbD approach to analytical methods.

MATERIALS AND METHODS

Chemicals and reagents

Reference standard of gallic acid (Purity 98% w/w) was purchased from Sigma-Aldrich Pvt. Ltd., Mumbai, India. Commercially available brands of Lohasava and Pippalyasava were procured from local market of Silvassa, India. HPLC grade acetonitrile and water were purchased from Fisher Scientific India Pvt. Ltd., Mumbai, India. HPLC grade o-phosphoric acid was purchased from Finar Chemicals Ltd., Ahmedabad, India.

Instruments

Cyber lab LC 100 HPLC system equipped with binary LC *P*-100 pump, high pressure gradient mixer (1500 µL) and a UV detector was employed for performing analysis. Data acquisition and processing were done using WS- Workstation software. UV spectrophotometer (Carry 60, Agilent Technologies) was used to check absorbance maximam of gallic acid.

Selection of detection wavelength

A standard solution containing 10 µg/ml of gallic acid was prepared using methanol. This solution was scanned in the wavelength range of 200-400 nm against methanol blank.

Method optimization

Various mobile phases like methanol: water (40:60, v/v), acetonitrile: water (50:50, v/v), methanol: 0.1 % o-phosphoric acid (50:50, v/v), methanol: 0.1 % o-phosphoric acid (90:10, v/v), methanol: 0.1 % trifluroacetic acid (75:25, v/v), methanol: 0.1 % trifluroacetic acid (90:10, v/v), methanol: 0.05% o-phosphoric acid (70:30, v/v), methanol and 0.05 % o-phosphoric acid in gradient mode as: 0 min (90:10, v/v), 0-7 min (80:20, v/v), 7-10 min (10:70, v/v) etc. at a flow rate of 1 ml/min were tried but none of them produced desired results. Various chromatographic responses like retention time, area, number of theoretical plates and tailing factor were evaluated and finally the mobile phase containing 0.05% o-phosphoric acid: acetonitrile (90:10, v/v) at a flow rate of 1 ml/min was selected to perform further optimization by factorial design.

Software aided method optimization

For optimization of chromatographic conditions, 3^2 factorial design was applied. A 3^2 full factorial design

includes three levels and two independent variables and thus it requires only 9 runs for optimization. The three selected levels were low (-1), medium (0) and high (+1) whereas the two independent variables selected were A (mobile phase ratio) and B (flow rate). The chromatographic responses recorded in the trial were retention time (Y1), area (Y2), number of theoretical plates (Y3) and tailing factor (Y4). A 3^2 full factorial design was suitable for generating response surface and creating different models with Design Expert® (Version 10.0, Trial version). The selected levels and factors along with range of dependent and independent variables for gallic acid are specified in the Table 1. The significance of the model was examined by applying Analysis of Variance (ANOVA) to the responses obtained. For visualization of effects of the independent variable and their interactions on the responses, 3D response surfaces plots and Pertubation plots were obtained. Regression equations were generated for each response showing its relationship with optimization design.

Chromatographic conditions

The column used for chromatographic separation was Sun Chrome C_{18} column (250 mm X 4.6 mm, 5 µm) and the software aided optimized mobile phase was 0.05 % o-phosphoric acid: acetonitrile (93:7, v/v) with the flow rate of 1.5ml/min. The injection volume was 20 µL. The detection wavelength was set at 270nm. The mobile phase was filtered before use through a 0.45 µ membrane filter (Sartorius Stedium Biotech, Germany) and sonicated for 10 min.

Preparation of mobile phase

To prepare 0.05 % o-phosphoric acid, 0.5 ml of o-phosphoric acid was accurately transferred to 1000 ml volumetric flask and volume was adjusted upto the mark with HPLC grade water. This solution was filtered through a 0.45 µ membrane filter. Required volume of the mobile phase was prepared by mixing 0.05 % o-phosphoric acid and acetonitrile (93:7, v/v). Then the mixture was sonicated for 10 min to ensure proper mixing and then filtered through a 0.45 µ membrane filter.

Preparation of standard stock solution

To prepare 100 µg/ml standard stock solution of gallic acid, accurately weighed 10 mg of gallic acid was transferred to 100 ml volumetric flask and 90 ml of mobile phase was added. The solution was sonicated for 10 min and volume was adjusted upto the mark with mobile phase.

Preparation of test solution

Each asava was successively extracted with pet ether, chloroform, ethyl acetate and methanol. The obtained extracts were dried separately. Ethyl acetate extract was found to contain gallic acid in preliminary TLC studies. Therefore, ethyl acetate extract was used for preparation of test solution.

Accurately weighed dried ethyl acetate extracts, (0.0709 g for Lohasava and 0.1047 g for Pippalyasava) were dissolved separately in 20 ml of mobile phase. These solutions were sonicated for 10 min and then filtered through a 0.45 µ membrane filter to get clear solutions. Accurately measured 0.1 ml of above solutions was transferred to 10 ml separate volumetric flasks and volumes were adjusted upto the mark with mobile phase.

Method validation

The developed and optimized analytical method was validated in accordance with ICH guidelines for various parameters such as system suitability[21] specificity, LOD, LOQ, linearity and range, accuracy, precision and robustness.[22]

Specificity

Specificity was evaluated by injecting blank, standard solution of gallic acid and test solutions of Lohasava and Pippalyasava. Any interference from the blank and sample constituents with the peak of interest was checked.

System suitability

System suitability was evaluated by applying six injections of working standards containing 3 µg/ml of gallic acid and observing parameters such as repeatability of peak area, number of theoretical plates and tailing factor.

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

To determine LOD and LOQ standard solutions of gallic acid were prepared in the range of 0.1-0.5 µg/ml. The solutions were injected in triplicate and area were recorded. Calibration curve was plotted in detection/quantitation range and LOD and LOQ of gallic acid were calculated by standard deviation method using following formula:

$$LOD = 3.3\ \sigma/S \text{ and } LOQ = 10\ \sigma/S,$$

where σ is the standard deviation of intercepts and S is the slope of the calibration curve.

Linearity and range

The linearity of proposed method was evaluated by injecting standard solutions of seven different concentrations in the range of 0.15–6 µg/ml of gallic acid in triplicate. A graph of mean area vs. concentration was plotted and regression coefficient (R^2) was calculated.

The linearity equation was obtained using GraphPad Prism software.

Accuracy

The accuracy of the proposed method was evaluated by standard addition method. The study was carried out at three different concentration levels (50 %, 100 % and 150 %) by spiking different concentration of standard solution of gallic acid (1.5 µg/ml, 3 µg/ml and 4.5 µg/ml) into pre-quantified (1 µg/ml) test solution. Area was measured in triplicate at each level and % recovery of gallic acid was determined.

Precision

The precision studies were carried out at different time periods as inter-day and intra-day precision. Precision studies were performed at 50 %, 100 % and 150 % concentration level i.e. 1.5 µg/ml, 3 µg/ml and 4.5 µg/ml of gallic acid; respectively. Intraday precision studies were performed on the same day at different time intervals whereas interday studies were carried out on three different consecutive days. Area of gallic acid at each concentration level was measured thrice and % RSD was calculated.

Robustness

The evaluation of robustness was carried out by changing method parameters such as flow rate (1.4 ml/min and 1.6 ml/min); detection wavelength (269 nm and 271 nm) and mobile phase composition (94:06 v/v and 92:08 v/v). It was evaluated by injecting six injections of standard solution of gallic acid (3 µg/ml) and two injections of test solution of gallic acid (3 µg/ml). The value of % w/w of gallic acid, mean area, mean retention time and % RSD were calculated and the data were evaluated using one way Analysis of Variance (ANOVA).

Stability of studies of gallic acid solution

Stability of the gallic acid test solutions was tested at 24, 48 and 72 hr after preparation and storage at 4°C and 25°C separately. Stability was assessed by comparing the chromatographic parameters of the solutions after storage with the same characteristics of freshly prepared solutions.

Quantification of gallic acid in Lohasava and Pippalyasava using proposed HPLC method

The test solution of Lohasava and Pippalyasava was analysed in triplicate. Area of gallic acid was measured and the % content of gallic acid in both Lohasava and Pippalyasava was calculated using the following formula:

$$\%\ w/w \text{ of gallic acid in Lohasava} = \frac{\text{Area of test}}{\text{Area of standard}} * \frac{W}{100} * \frac{0.3}{10} * \frac{20}{W1} * \frac{10}{0.1} * P$$

$$\%\ w/w \text{ of gallic acid in Pippalyasava} = \frac{\text{Area of test}}{\text{Area of standard}} * \frac{W}{100} * \frac{0.3}{10} * \frac{20}{W2} * \frac{10}{0.1} * P$$

Where, W is the weight of standard gallic acid, W_1 and W_2 are the weights of ethyl acetate extracts of Lohasava and Pippalyasava; respectively and P is the purity of standard gallic acid.

RESULTS AND DISCUSSION

Initial method development

The absorbance maximum of gallic acid was found to be 270 nm from the spectrum obtained (Figure 1). Thus, 270 nm was selected as detection wavelength for the development of HPLC-UV method for gallic acid.

Design of Experiment (DOE)

A 3^2 full factorial design using 9 experimental runs was carried out for the optimization of proposed HPLC method. The independent and dependent variables selected for the DOE are given in Table 1. Mobile phase composition and flow rate were selected as independent variables as they were key factors to change chromatographic behaviour of gallic acid.

The results of 9 experimental runs carried out for gallic acid along with the chromatographic conditions and observed responses are specified in Table 2. The best fitted model for retention time, area and tailing factor was found to be quadratic model except for number of theoretical plates where the best fitted model

Table 1: Independent and dependent variables selected for the Design of Experiment (DoE).

Independent variable			
Factor	**Level used**		
	Low (-1)	**Medium (0)**	**High (+1)**
A= Mobile phase ratio (0.05 % OPA: acetonitrile) (v/v)	95:5	90:10	85:15
B= Flow rate (ml/min)	0.5	1.0	1.5
Dependent variable			
Chromatographic response	**Value**		
Y1= Retention time (min)	2.537 ≤ Y1 ≥ 12.017		
Y2= Area (mAU)	416485 ≤ Y2 ≥ 1213880		
Y3= No. of theoretical plates	8901.93 ≤ Y3 ≥ 19659.7		
Y4= Tailing factor	1.52 ≤ Y4 ≥ 2.87		

was found to be 2FI model (Table 3). The difference between predicted R^2 and adjusted R^2 was not more than 0.2 for all responses demonstrating that there was a reasonable agreement between them. Adequate precision greater than 4.0 indicated that there was adequate signal for all responses.

The regression equations for various chromatographic responses obtained from ANOVA analysis are given in Table 4. A positive value in the equation represented the factor that favours the optimization, while a negative value indicated an inverse relationship between the independent variable and the response. It is clear from the equations that variable A (mobile phase) had a positive effect on number of theoretical plates and a negative effect on retention time, area and tailing factor. Variable B (flow rate) had a negative effect on all the chromatographic responses. Interaction of A and B had a positive effect on retention time and area and it had a negative effect on number of theoretical plates and tailing factor. The square of variable A (A^2) had a positive effect on all the chromatographic responses following quadratic model. The square of variable B (B^2) had a positive effect on retention time and area whereas it had positive effect on tailing factor.

The Sum of Squares (SS) in ANOVA indicated that the contribution of variable B (SS 52.87) was more on retention time as compared to variable A (SS 15.67), the contribution of variable B (SS 859800000000) was more on area as compared to variable A (SS 938500000), the contribution of variable B (SS 71420000) was more on number of theoretical plates as compared to variable A (SS 9762000) and the contribution of variable A (SS 1.54) was more on tailing factor as compared to variable B (SS 0.22). For each chromatographic response, all the model terms were found to be significant. The calculated F-value for the models of retention

Table 2: Observed responses for 9 experimental runs for gallic acid.

Run	Level	Factor		Response[a]			
		Mobile phase (v/v)	Flow rate (ml/min)	Retention time (min)	Area (mAU)	No. of theoretical plates	Tailing factor
1	0, 0	90:10	1	4.29	601896.40	11148.87	1.77
2	0, +1	90:10	1.5	2.97	416484.95	9695.18	1.58
3	0, -1	90:10	0.5	8.53	1179578.4	17522.46	1.87
4	+1, 0	85:15	1	3.65	620512.75	11356.71	2.73
5	+1, +1	85:15	1.5	2.53	423787.55	8901.93	2.17
6	+1, -1	85:15	0.5	7.00	1138143.7	19659.70	2.87
7	-1, 0	95:5	1	6.64	623631.45	11077.15	1.53
8	-1, +1	95:5	1.5	4.23	419970.55	9535.83	1.52
9	-1, -1	95:5	0.5	12.01	1213883.4	11652.08	1.68

a=Average of three measurements

Table 3: Summary of statistical analysis for various chromatographic responses.

Response	Model	R^2	Adjusted R^2	Predicted R^2	SD	% CV	Adequate precision
Retention time	Quadratic	0.9979	0.9965	0.9797	0.16	2.93	86.677
Area	Quadratic	0.9993	0.9989	0.9942	9577.92	1.38	123.007
Number of theoretical plates	2F1	0.8675	0.8234	0.6326	1301.64	10.91	15.541
Tailing factor	Quadratic	0.9851	0.9744	0.8488	0.069	3.60	29.898

Table 4: Regression equations for various chromatographic responses.

Response	Regression equation
Retention time	$Y1 = +4.32-1.62*A-2.97*B+0.83*AB+0.78*A^2+1.38*B^2$
Area	$Y2 =+604600-12506.89*A-378600*B+19889.16*AB+10877.24*A^2+186800*B^2$
Number of theoretical plates	$Y3 = +11934.26+1275.55*A-3450.22*B-2160.38*AB$
Tailing factor	$Y4 = +1.77+0.51*A-0.19*B-0.14*AB+0.35*A^2-0.057*B^2$

time, area, number of theoretical plates and tailing factor were found to be 676.87, 2137.72 19.65 and 92.48; respectively.

3D surface plots (Figure 3a-3d) and Pertubation plots (Figure 4e-4h) were obtained to determine the relationship between the variables and various chromatographic responses. The surface plots indicated a negative effect of mobile phase and flow rate on retention time and area. As the proportion of acetonitrile in the mobile phase and flow rate decreased, the retention time and area increased. As per surface plots, mobile phase had positive effect of on number of theoretical plates and tailing factor. As the proportion of acetonitrile in the mobile phase was increased, the number of theoretical plates and tailing factor also increased. It also indicated negative effect of flow rate on number of theoretical plates and tailing factor. Pertubation plots are plotted by changing one variable over its range while holding other variables constant. A steep plot indicates a relatively high sensitivity of the response. As per Pertubation plots, lower concentration of acetonitrile in mobile phase and lower flow rate led to increase in the values of all responses except tailing factor. The responses area and number of theoretical plates were highly influenced by flow rate while tailing factor was highly influenced by proportion of acetonitrile in mobile phase. High proportion of acetonitrile in mobile phase was responsible for high tailing factor.

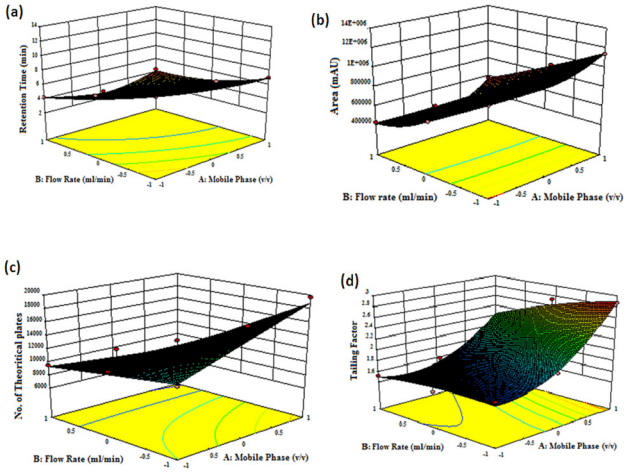

Figure 3: 3D surface plots of gallic acid for response a) retention time b) area c) theoretical plates d) tailing factor.

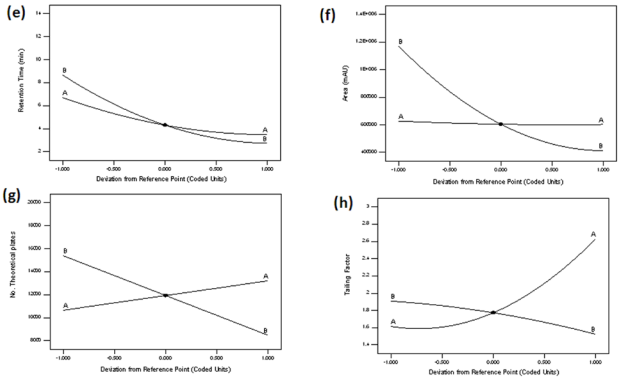

Figure 4: Pertubation plots of gallic acid for response e) retention time f) area g) theoretical plates h) tailing factor.

Method validation

Specificity

The proposed HPLC method was found to be specific as there was no interference found from mobile phase or any other components present in the sample of asavas. Chromatogram of standard gallic acid, ethyl acetate extract of Lohasava and ethyl acetate extract of Pippalyasava using the optimised mobile phase and flow rate are shown in Figure 5-7; respectively.

System suitability

The results of system suitability analysis for proposed method are shown in Table 5. The number of theoretical plates was found to be more than 8000 and the tailing factor was found to be less than 2.0. The % RSD for peak area was found to be less than 2.0%. As the results of system suitability study were within the standard specifications, the method was found to be suitable for intended purpose.

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

The LOD and LOQ of the proposed HPLC method were found to be 0.0487 µg/ml and 0.1478 µg/ml; respectively. The developed method was found to be sensitive as it could detect and quantitate microgram levels of gallic acid.

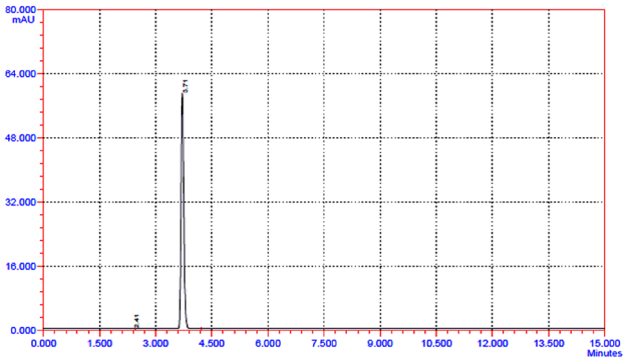

Figure 5: HPLC chromatogram of standard gallic acid.

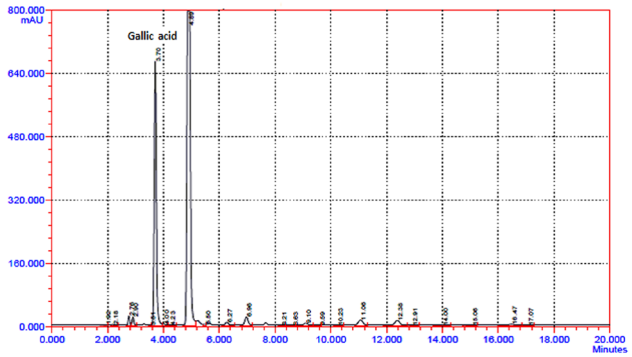

Figure 6: HPLC chromatogram of ethyl acetate extract of Lohasava.

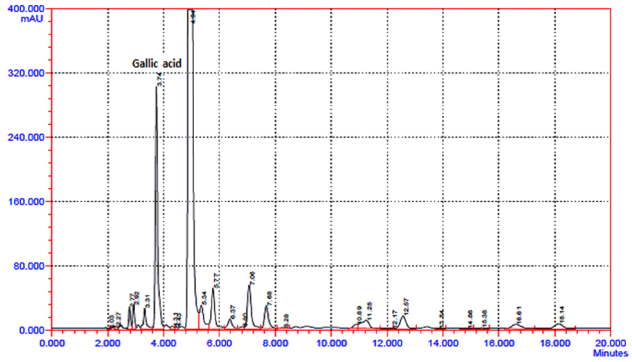

Figure 7: HPLC chromatogram of ethyl acetate extract of Pippalyasava.

Linearity and range

The proposed method showed linearity over concentration range of 0.15-6 µg/ml for gallic acid with regression equation y = 3559x + 557.7 and regression coefficient 0.9955.Statistically calculated F value for linear regression was found to be Fcal 4216 as compared to Fcri 4.3807 (DFn, DFd = 1.0, 19.0) indicating the statistical significance of method linearity. As the regression coefficient was obtained near 1.0, the method was found to be linear.

Accuracy

The percentage recovery of gallic acid was found in the range between 98.0 – 102.0 % (Table 6). As the % recovery for gallic acid was near to 100 %, the proposed method was found to be accurate.

Precision

Precision studies of the method were carried out in terms of intraday and interday precision studies. In each case, the % RSD was found to be less than 2.0 (Table 7). Thus, the proposed method was found to be precise.

Table 5: Results of system suitability parameters of method for gallic acid.

Parameter	Mean[b] ± SD	%RSD[b]
Area	9560.3 ± 172.0101	1.7992
No. of theoretical plates	8406.99 ± 146.1362	1.7382
Tailing factor	1.64 ± 0.0183	1.1176

b= triplicate measurements

Table 6: Accuracy of the proposed HPLC method for gallic acid.

% Level	Amount present (µg/ml)	Amount recovered (µg/ml)	% Recovery[c] ± SD
50	1.50	1.50	100.43 ± 1.1629
100	3.00	3.02	100.81 ± 0.8648
150	4.50	4.47	99.43 ± 0.9219

c=triplicate measurements

Table 7: Evaluation of intra-day and inter-day precision studies of method.

Concentration (µg/ml)	Intraday Precision (%RSD[d])		Interday Precision (%RSD[d])	
	Day 1	Day 1	Day 2	Day 3
1.5	0.5200	0.3033	1.1339	0.5012
3.0	0.2211	0.3676	1.6827	0.7157
4.5	0.0264	0.5157	0.2195	0.5210

d=triplicate measurements

Robustness

In robustness study, the calculated F-value for many parameters was higher than the critical F-value but the value of % RSD obtained for area, retention time and % w/w of drug was found to be less than 2.0 (Table 8).

Stability studies:

Stability of gallic acid in the test solution was evaluated at 4°C and 25°C for 3 days to verify whether spontaneous degradation occurred. The results were calculated as the percentage of non-degraded gallic acid at the specified time intervals. Both test samples showed less than 1.0 % degradation indicating that the samples were stable at 4°C and 25°C for 3 days.

Quantification of gallic acid in Lohasava and Pippalyasava using proposed HPLC method

The ethyl acetate extracts of both Lohasava and Pippalyasava were analysed by the proposed HPLC method. The % w/w of gallic acid in Lohasava and Pippalyasava was found to be 3.00±0.0085 and 0.98 ± 0.0016; respectively.

Table 8: Statistical evaluation of robustness for determination of gallic acid.

Parameter	Average[e] (% RSD[e])		
	Retention time	Area	% Content
Flow rate (ml/min)			
1.4	4.04 (0.4141)	10112.4 (0.9989)	101.12 (0.0359)
1.5	3.75 (0.4350)	9560.3 (1.7992)	100.06 (0.5506)
1.6	3.56 (0.6797)	8810.7 (0.9260)	98.90 (0.5047)
Fcal/Fcri	50.7517	9.1846	2.6521
Wavelength (nm)			
269	3.85 (0.1420)	10237.2 (1.1155)	99.70 (0.5978)
270	3.75 (0.4350)	9560.3 (1.7992)	100.06 (0.5506)
271	3.84 (0.1342)	9262.9 (0.6919)	99.82 (0.2775)
Fcal/Fcri	17.7061	5.3287	0.3247
Mobile phase ratio (0.05 %OPA: acetonitrile)			
94:06	4.28 (0.2447)	9260.5 (1.0377)	99.57 (0.0697)
93:07	3.75 (0.4350)	9560.3 (1.7992)	100.06 (0.5506)
92:08	3.57 (0.1443)	10706.7 (1.2489)	100.98 (0.5539)
Fcal/Fcri	336.6982	10.2613	1.3180

e=six measurements

CONCLUSION

Factorial design was employed for optimization of HPLC method for quantification of gallic acid in ethyl acetate extract of Lohasava and Pippalyasava. Factorial design had helped in identification of best chromatographic conditions for desired response. Interactions between mobile phase and flow rate were evaluated. Flow rate was major influencing variable than mobile phase in optimization. All the responses were significantly affected by flow rate except tailing factor. The method was validated for various parameters. The proposed method was found to be sensitive, accurate, precise and robust.

CONFLICT OF INTEREST

Authors declare that there are no conflict of interest.

ABBREVIATIONS

AQbD: Analytical Quality by Design; **ANOVA:** Analysis of Variance; **CV:** Coefficient of Variance; **DoE:** Design of Experiment; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **OPA:** Ortho Phosphoric Acid; **HPLC:** High Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **ICH:** International Conferences on Harmonization; **QRM:** Quality Risk Management; **SD:** Standard Deviation**; SS:** Sum of Square; **RSD:** Relative Standard Deviation; **UV:** Ultra Violet.

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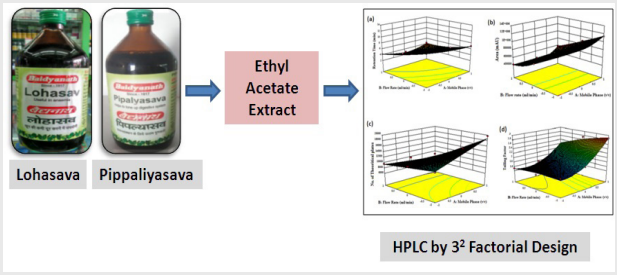
PICTORIAL ABSTRACT



SUMMARY

Factorial design based analytical method development provides better tool as compared to one factor at a time approach. Gallic acid was estimated in marketed asava using 3^2 factorial based HPLC method.

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