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New RP-HPLC Method Development and Validation for Simultaneous Estimation and Forced Degradation Studies of Gallicacid and Curcumin in Solid Dosage Form

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Abstract

Objective: A New method was established for simultaneous estimation of Gallic acid and curcumin by RP-HPLC method.

Methods: Chromatographic separations were carried using Inspire (4.6 × 150 mm, 5 m) column with a mobile phase composition of 0.1% OPA buffer and Acetonitrile (30:70) have been delivered at a flow rate of 1 ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 with PDA detector 2996 at wavelength 260 nm.

Results: The retention time for Gallic acid and curcumin were 2.119 and 2.730 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 500-2500 µg/ml for Gallic acid and 5-25 µg/ml for curcumin respectively. For accuracy the total recovery was found to be 100.58% and 100.54% for Gallic acid and curcumin. LOD and LOQ for gallic acid 3.05 and 10.07. LOD and LOQ for Curcumin 2.28 and 9.98.

Conclusion: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Gallic acid and curcumin in pharmaceutical dosage form.

Keywords: Gallic acid and curcumin • RP-HPLC • Simultaneous estimation

Introduction

Gallic acid: is a phenolic acid, also known as 3,4,5-trihydroxybenzoic acid, obtained from fruit of Embelicaofficinalis belonging to family Euphorbiaceae. Gallic acid is having different pharmacological activities like anti-inflammatory, antimicrobial, antifungal, antidiabetic, anticancer, antioxidant, antiviral etc (Figure 1).

Curcumin: is a natural flavonoid obtained from the rhizome of Curcuma longa belonging to family Zingiberaceae. Curcumin is chemically 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) having various activities like antibacterial, antiprotozoal, antiviral, hypoglycemic, anticoagulant, antioxidant, antitumor, anticarcinogenic, coloring agent, flavoring agent [1].

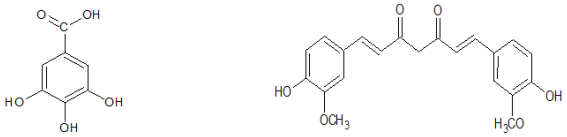

Figure 1. Structure of Gallic acid and Structure of Curcumin.

Many methods have been described in the literature for the determination of Curcumin and Gallic acid individually andin

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combination with other drugs. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. There are different dosage forms like tablet, capsule, syrup and granules having these both drugs. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of Curcumin and Gallic acid in pharmaceutical dosage forms. The present RP-HPLC method was validated as per ICH guidelines [2].

Materials and Methods

Chemicals and reagents: Curcumin and Gallic acid were obtained as a gift sample from Pharma train lab, Hyderabad. KH2PO4 was analytical grade supplied by finer chemical LTD, Mumbai, Orthophosphoric acid (MERCK), Acetonitrile (Molychem, HPLC grade) and Water for HPLC (LICHROSOLV) (MERCK), Methanol for HPLC (LICHROSOLV (MERCK).

Equipment and chromatographic conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 260 nm with column Inspire (4.6 × 150 mm, 5 m), dimensions at ambient temperature. The optimized mobile phase consists of 30% buffer 70% Acetonitrile. Buffer is 1 ml of orthophosphoric acid in 1000 ml water Flow rate was maintained at 1 ml/min and run time for 5 min (Figure 2).

Preparation of solutions: Pipette out 1 ml of Ortho Phosphoric Acid was taken in a 1000 ml volumetric flask, dissolved and diluted to 1000 ml with HPLC water and the volume was adjusted to pH 3.0 with NaOH.

Preparation of mobile phase: 300 ml (30%) of accurately measured buffer solution was mixed with 700 ml of Acetonitrile (70%) and degasified by sonicator. Finally, the solution was filtered through 0.45 µ filter.

The diluents: The Mobile phase was used as the diluent.

Preparation of standard stock solution: Accurately weigh 1000 mg of Gallic acid and 10 mg of Curcumin working standards and transfer into a 100 ml volumetric flask containing 60 mL of Diluent and sonicate to dissolve it completely. Make up the final volume to 100 mL with diluent (Stock solution). Pipette out 1.5 ml of the stock solution into a 10 ml volumetric flask and make up to the mark with diluent [3].

Preparation of Sample stock solution: Accurately weigh 1000 mg of Gallic acid and 10 mg Curcumin equivalent tablet powder and transfer into a 100 mL volumetric flask containing 60 mL of diluent and sonicate it for 30 mins to dissolve the drugs completely. Make up the final volume to 100 mL with diluent (Stock solution). After filtering the solution pipette out 1.5 ml of the stock solution into a 10 ml volumetric flask and make up to the mark with diluent [4].

Results and Discussion

Procedure: 10 L of standard and sample solutions were injected into the LC-system and measure the peak areas for Gallic acid and Curcumin.

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 5 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Inspire (4.6 × 150 mm, 5 m), the mobile phase of composition 0.1% OPA buffer and acetonitrile in the (30:70) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in (Table 1).

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Gallic acid and Curcumin in their tablet dosage form. The result obtained for Gallic acid and Curcumin was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method: Linearity and Range: Stock solution was prepared by dissolving the appropriate amount of Gallic acid and Curcumin in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 500 µg/ml to 2500 µg/ml of Gallic acid and 5 µg/ml to 25 µg/ml of Curcumin. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [5]. The results are shown in (Table 3).

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Gallic acid and Curcumin and calculate the individual recovery and mean recovery values. The results are shown in (Table 4).

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in Table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in (Table 5-10).

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The Organic composition in the Mobile phase was varied from 10% to 10%. LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines [6]?

- LOD=3.3 σ/S and
- LOQ=10 σ/S, where

- σ=Standard deviation of y intercept of regression line,
- S=Slope of the calibration curve

Forced degradation studies and Acid degradation condition:

Accurately 3.0 ml of stock solution into a 1 0 ml volumetric flask and 3 ml of 0.1 N HCl was added. Then, the volumetric flask was kept at 60°C for 6 h and then neutralized with 0.1 N NaOH and makeup to 10 ml with diluent. The solution was filtered through 0.45 µ filter, and then filtrate was injected into system and percentage of degradation was calculated [7].

Alkali degradation condition: Accurately 3.0 ml of stock sample into a 10 ml volumetric flask and add 3 ml of 0.1 N NaOH was added. Then, the volumetric flask was kept at 60°C for 6 h and then neutr alized with 0.1 N HCl and makeup to 10 ml with diluent. The solution was filtered through 0.45 filters and then the filtrate was injected into the system and percentage of degradation was calculated (Figure 3-6).

Thermal-induced degradation condition: 3 ml of stock sample was taken in petri dish and kept in hot air oven at 110°C for 24 h. The samples were then placed in a desiccator till reaching the room temperature. The content in the flasks was dissolved using methanol and diluted up to the mark. Then the sample was taken and diluted with diluents and injected and percentage of degradation was calculated [8].

Photolytic degradation condition: Accurately 3.0 ml of stock sample was exposed to sunlight for about 6 h and then the sample diluted with 5 ml of mobile phase and percentage of degradation [9-10].

Oxidative degradation condition: Accurately 3.0 ml of stock sample into a 10 ml volumetric flask, 1 ml of 3.0 ml of 3% H_2O_2 was added and the volume was made up to the mark with diluents. The volumetric flask was then kept at room temperature for 15 min. The solution was filtered through 0.45 µ filter and then filtrate was injected into the chromatography system and the percentage of degradation was calculated. Force degradation results are shown in (Table 11-13)

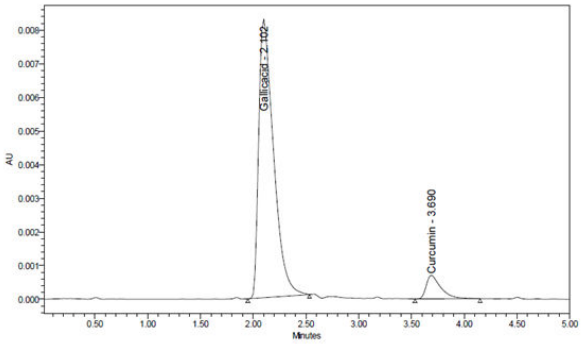

Figure 2. Standard chromatogram.

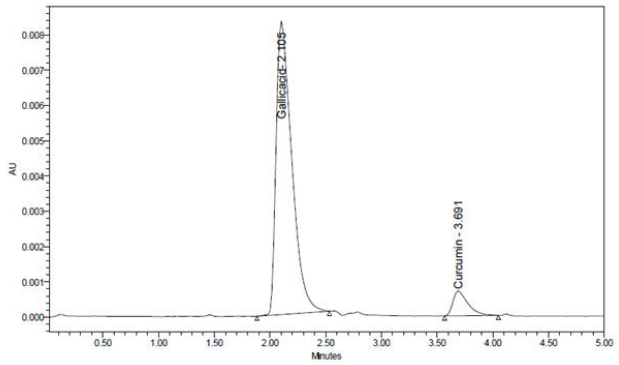

Figure 3. Sample chromatogram.

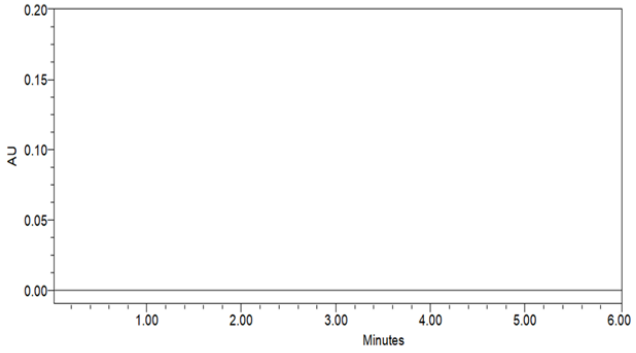

Figure 4. Blank chromatogram.

Parameters	Gallic acid	Curcumin
Retention time	2.991	5.931
USP Plate count	2940	3415
USP Tailing	1.87	1.84

Table 1. System suitability parameters.

	Label Claim (mg)	% Assay
Gallic acid	1000	101.03
Curcumin	10	99.95

Table 2. Assay results for gallic acid and curcumin.

Gallic acid		Curcumin	
Concentration(µg/ml)	Area	Concentration(µg/ml)	Area
500	34517	5	2647
1000	63997	10	5201
1500	93332	15	8029
2000	123766	20	10737
2500	154482	25	13743
Correlation coefficient	0.999	Correlation coefficient	0.999

Table 3. Linearity results for gallic acid and curcumin.

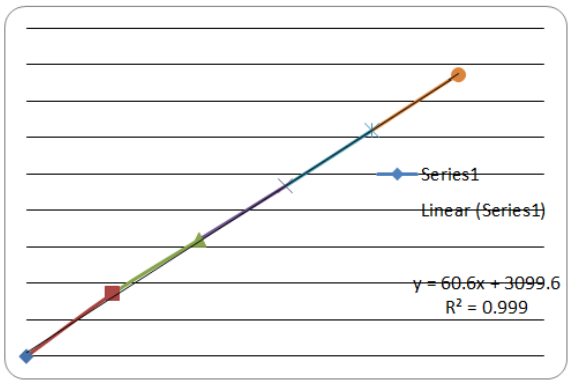

Figure 5. Linearity graph for Gallic acid.

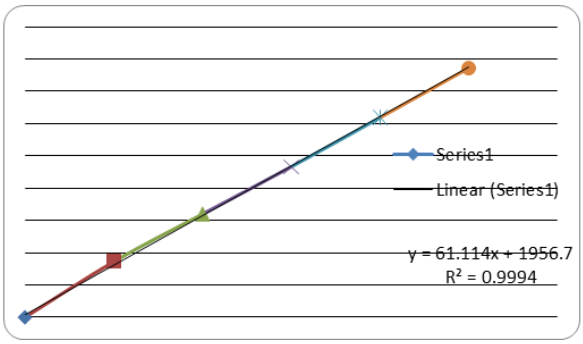

Figure 6. Linearity graph for curcumin.

%Concentration (At specification Level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
0.5	47085	500	503.48	100.7	100.58
1	94332	1000	1008.69	100.87	
1.5	140531	1500	1502.7	100.18	

Table 4. Showing accuracy results for gallic acid.

Injection	Area for Gallic acid	Area for Curcumin
Injection-1	94432	8439
Injection-2	94332	8446
Injection-3	95132	8352
Injection-4	95632	8565
Injection-5	95632	8558
Injection-6	95132	8429
Average	238049	8465
Standard Deviation	563.6	82.2
%RSD	0.6	1

Table 5. Precision results for gallic acid and curcumin.

Injection	Area for Gallic acid	Area for Curcumin
Injection-1	97532	8829
Injection-2	99523	8695
Injection-3	96832	8755
Injection-4	97332	8523
Injection-5	98332	8762
Injection-6	96332	8645
Average	97649	8702
STD Deviation	1142.7	107.6
%RSD	1.2	1.2

Table 6. Intermediate precision results for gallic acid and curcumin.

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2075.75	1.86
2	1	2978	1.82
3	1.2	2694.04	1.63

Table 7. Robustness results for gallic acid (flow rate change).

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	4078.38	1.89
2	1	3415.94	1.84
3	1.2	3196.52	1.47

Table 8. Robustness results for curcumin (flow rate change).

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2193.04	1.94
2	*Actual	2978	1.82
3	10% more	2133.74	1.68

Table 9. Robustness results for gallic acid (Mobile phase composition change).

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3869.45	1.25

2	*Actual	3415.94	1.84
3	10% more	2910	1.89

Table 10. Robustness results for curcumin (Mobile phase composition change).

Drug	LOD	LOQ
Gallic acid	3.05	10.07
Curcumin	2.98	9.98

Table 11. LOD, LOQ of gallic acid and curcumin.

Sample Name	Gallic acid		
	Area	% Degraded	Peak purity
Standard	93332		
Acid	84646	9.31	Passes
Base	88401	5.28	Passes
Peroxide	87925	5.79	Passes
Thermal	87133	6.64	Passes
Photo	88359	5.33	Passes

Table 12. Degradation results for gallic acid.

Sample Name	Curcumin		
	Area	% Degraded	Peak purity
Standard	8418		
Acid	7716	3.77	Passes
Base	7607	6.19	Passes
Peroxide	7753	5.44	Passes
Thermal	7707	3.39	Passes
Photo	7634	6.42	Passes

Table 13. Degradation results for curcumin.

Conclusion

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Gallic acid and curcumin in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Gallic acid and curcumin in pure and its pharmaceutical dosage forms.

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