Method Development and Validation of Gallic Acid in Liquid Dosage Form by Using RP-HPLC Method

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Abstract

A simple, rapid, precise, sensitive, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of gallic acid in the pharmaceutical dosage form. Chromatographic separation of gallic acid was achieved on Waters Alliance-e 2695, by using Waters X-Terra RP-18 (150 × 4.6 mm, 3.5 μ) column and the mobile phase containing *0.1%* formic acid and ACN in the ratio of 70:30% v/v. The flow rate was 1.0 mL/min; detection was carried out by absorption at 275 nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for gallic acid was NLT 2000 and should not be more than *2* respectively. Percentage relative standard deviation of peak areas of all measurements is always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate, and robust method for quantitative analysis of gallic acid.

51.1 Introduction

Reverse-phase chromatography or RP-HPLC is a commonly used HPLC technique for the analysis of a wide range of compounds. In this technique, the stationary phase used in nonpolar while the mobile phase is aqueous or moderately polar in nature. Hydrocarbons are used as the stationary phase and water or acetonitrile is used as the mobile phase in this kind of high-performance liquid chromatography (RP-HPLC). Solutes are eluted in decreasing polarity order in RP-HPLC. For RP-HPLC, stationary phases for the silanol group are produced by treating the surface with an organo chloro silane. Non polar hydrocarbon chains in the stationary phase interact with sample molecules to provide a retention mechanism in RP-HPLC.

Gallic acid was determined by *Carl Wilhelm Scheele* in 1786. It is a naturally occurring low-molecular-weight tri phenolic compound. Its chemical name is 3,4,5-trihydroxy benzoic acid. It is also known as trihydroxy benzoic acid. It exists both independently and as a component of tannins (specifically, gallotannin). Bark, wood, leaves, fruits, roots, and seeds are only some of the plant parts that contain gallic acid or its deriv-

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atives. Mechanism of action: Gallic acid has been shown to be selectively hazardous for cancer cells while having no effect on normal cells, inducing the death of cancer cells via mitochondria-mediated mechanisms. Gallic acid has been credited with a number of health benefits, including antioxidant, anti-inflammatory, and anticancer actions. It has a specific beneficial role in the apoptosis of cancer cells and brain health.
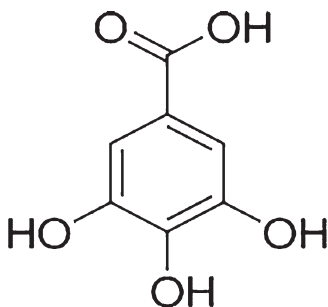
Molecular structure of gallic acid

51.1.1 Experimental Work

Materials, Reagents, and Chemicals
The pure form of gallic acid, HPLC grade water, acetonitrile, triethyl amine, and formic acid (Rankem), and pipettes, beakers, burettes, and measuring cylinder which are manufactured by Borosil.

Equipment
HPLC (Alliance) was manufactured by Waters e 2695 – Empower software 2.0 versions, UV–vis spectrophotometer by UV-1700, and ultrasonicator (UCA 701) by Unichrome and PH meter by Eutech.

51.1.2 General Preparations

Preparation of Standard Stock Solution
Accurately weigh and transfer 100 mg of gallic acid working standard into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely, and make volume up to the

mark with the same solvent (stock solution). Further pipette 5 mL of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent (100 ppm of gallic acid).

Preparation of Buffer (0.1% Formic Acid) 1 mL of the formic acid is dissolved in 1 L of HPLC water and filtered through 0.45 μ membrane filter paper.

Preparation of Mobile Phase Mobile phase was prepared by mixing 0.1% formic acid and ACN taken in the ratio 70:30. It was filtered through a 0.45 μ membrane filter to remove the impurities, which may interfere in the final chromatogram.

Chromatographic Condition
Multiple trails were run to determine the optimal chromatographic settings for this approach use suitable high-performance liquid chromatographic equipped with PDA detector.

Column:	Waters X-Terra RP-18 (150 × 4.6 mm, 3.5 μ)
Movable phase:	Acetonitrile and 0.1% formic acid (30:70)
Wavelength:	275 nm
Flow rate:	1 mL/min
Injection volume:	10 μL
Run time:	5 min

Preparation of Diluent Mobile phase was used as a diluent.

Preparation of Standard Solution
Accurately weigh and transfer 100 mg of gallic acid working standard into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely, and make volume up to the mark with the same solvent (stock solution).

Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute up to the mark with diluent (100 ppm of gallic acid).

Procedure
Inject 10 μL of the standard, sample into the chromatographic system and measure the areas for gallic acid peak and calculate the %assay by using the formulae.

Determination of Working Wavelength (λmax)
The isosbestic wavelength was utilized to make an estimate of the drug's potency. At the isosbestic point, the molar absorptivity of all interconvertible compounds is equal to one another.

That is why we relied on this wavelength for our precise medication estimations.

The wavelength of maximum absorption of the solution of the drug in a mixture of acetonitrile and 0.1% formic acid (30:70) was scanned using PDA detector within the wavelength region of 200–400 nm against acetonitrile and 0.1% formic acid (30:70) as blank. The absorption curve shows an isosbestic point at 275 nm. Thus 275 nm was selected as the detector wavelength for the HPLC chromatographic method.
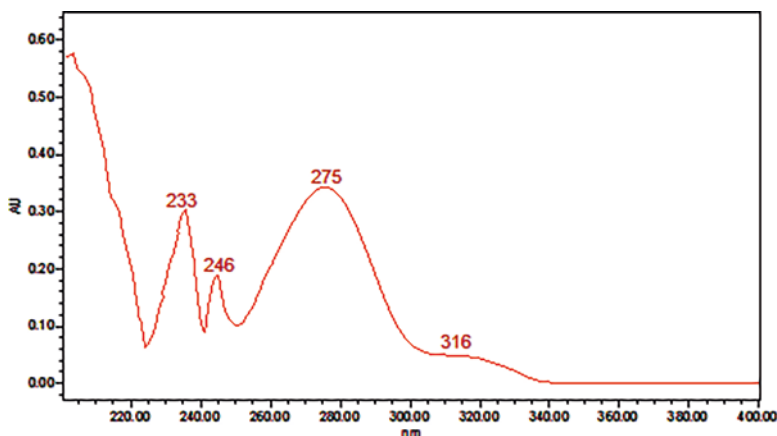
PDA – Spectrum of gallic acid

51.1.3 Syrup Formulation

Preparation of Simple Syrup
Weigh 66.35 g of sucrose. Add sucrose in hot purified water under continuous stirring until it dissolved. Kept aside for cooling.

As per IP 666.7 g of sucrose in 1000 ml of water

Preparation of Gallic Acid Syrup
Add gallic acid, preservative (sodium benzoate), diluents, and sweetener (glycerine) in the above simple syrup. Stir the solution for 20 min. After cooling, filter the final syrup through filter paper.

System Suitability
The tailing factor for the peak due to gallic acid in standard solution should not be more than 2.0.

Theoretical plates for the gallic acid peak in standard solution should not be less than 2000.

Formula for Assay

$$\%\text{Assay} = \frac{AT^*}{AS}\frac{WS^*}{DS}\frac{DT^*}{WT}\frac{\text{Average weight}^*}{\text{Label claim}}\frac{P^*}{100}100$$

where:

AT = average area counts of test (sample) preparation
AS = average area counts of standard preparation
WS = weight of working standard taken in mg
DS = dilution of working standard in mL
DT = dilution of test (sample) in mL
WT = weight of test (sample) taken in mg
P = percentage purity of working standard
LC = Label claim mg/mL

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on the X-axis concentration and on the Y-axis peak area) and calculate the correlation coefficient.

Range

The range of an analytical technique is the concentration range across which its precision, accuracy, and linearity have been shown.

Inclusion Criteria

Correlation coefficient should be not less than 0.999.

51.1.4 Preparation Accuracy Sample Solutions

For the Preparation of 50% Solution (with Respect to Target Assay Concentration)

Accurately weigh and transfer 50 mg of gallic acid standard into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely, and make volume up to the mark with the same solvent (stock solution).

Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute up to the mark with diluent (50 ppm of gallic acid).

For the Preparation of 100% Solution (with Respect to Target Assay Concentration)

Accurately weigh and transfer 100 mg of gallic acid standard into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely, and make volume up to the mark with the same solvent (stock solution).

Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute up to the mark with diluent.(100 ppm of gallic acid).

For the Preparation of 150% Solution (with Respect to Target Assay Concentration)

Accurately weigh and transfer 150 mg of gallic acid standard into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it com-

pletely, and make volume up to the mark with the same solvent (stock solution).

Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute up to the mark with diluent (150 ppm of gallic acid).

Procedure

Inject the standard solution, accuracy – 50%, accuracy – 100%, and accuracy – 150% solutions.

Inclusion Criteria

The % recovery for each level should be between 98.0% and 102.0%.

Precision

Precision is the degree of repeatability of an analytical method under normal operating conditions. Precision is of three types:

1. System precision
2. Method precision
3. Intermediate precision (a. Intraday precision, b. Interday precision)

System precision is checked by using standard chemical substances to ensure that the analytical system is working properly.

In this peak area, % of drug of six determinations is measured and % RSD should be calculated.

In method precision, a homogenous sample of a single batch should be analyzed six times. This indicates whether a method is giving constant results for a single batch. In this, analyze the sample six times and calculate the % RSD.

The precision of the instrument was checked by repeatedly injecting ($n = 6$) solutions of 100 ppm of gallic acid.

Acceptance Criteria

The % RSD for the absorbance of six replicate injection results should not be more than 2%.

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.

A. The flow rate was varied from 0.9 mL/min to 1.1 m.

A standard solution of 100 ppm of gallic acid was prepared and analyzed using the varied flow rates along with the method flow rate.

On the evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence, it indicates that the method is robust even with a change in the flow rate ± 10%.

B. The variation of the Organic Phase ratio.

A standard solution of 100 ppm of gallic acid was prepared and analyzed using the varied in mobile phase ratio.

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

The limit of detection (LOD) and limit of quantification (LOQ) of the drug carry were calculated using the following equation as per international conference harmonization (ICH) guidelines.

$$LOD = 3.3X\sigma / S$$

$$LOQ = 10X\sigma / S$$

LOD for gallic acid was found to be 0.3 μg/mL and LOQ for gallic acid was found to be 1 μg/mL.

51.1.5 Degradation Studies

Preparation of Stock

Accurately weigh and transfer 100 mg of Gallic acid working standard into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely, and make volume up to the mark with the same solvent (stock solution).

Acid Degradation

Pipette 5 mL of the aforementioned solution was added to a 50 mL vacuum flask, followed by 3 mL of 1 N HCl. The vacuum flask was then maintained at 60 °C for 6 h before being neutralized with 1 N NaOH and diluted to 50 mL with

diluent. Filter the solution using 0.22-micron syringe filters and transfer it to bottles.

Alkali Degradation

Pipette 5 mL of the above solution into a 50 mL volumetric flask and add 3 mL of 1 N NaOH was added. Then, the volumetric flask was kept at 60 °C for 6 h and then neutralized with 1 N HCl and make up to 50 mL with diluent. Filter the solution with 0.22 microns syringe filters and place it in vials.

Thermal-Induced Degradation

Gallic acid sample was taken in Petri dish and kept in a hot air oven at 105 °C for 24 h. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Peroxide Degradation

Pipette 5 mL above stock solution was added to a 50 mL vacuum flask, 1 mL of 3% w/v hydrogen peroxide was added to the flask and the volume was built up to the mark using diluent. The vacuum flask was then maintained at 60 °C for 6 h. After that, the vacuum flask was left at room temperature for 15 min. Filter the solution using 0.45-micron syringe filters and transfer it to bottles.

Reduction Degradation

Pipette 5 mL of above-stock solution was added to a 50 mL vacuum flask, 1 mL of 10% sodium bisulfate was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60 °C for 6 h. After that, the vacuum flask was left at room temperature for 15 min. Filter the solution using 0.45-micron syringe filters and transfer it to bottles.

Photolytic Degradation

Gallic acid sample was placed in sunlight for 24 h. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Hydrolysis Degradation

Pipette 5 mL of above-stock solution was added to a 50 mL vacuum flask, 1 mL of HPLC grade water

was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60 °C for 6 h. After that, the vacuum flask was left at room temperature for 15 min. Filter the solution using 0.45-micron syringe filters and transfer it to bottles.
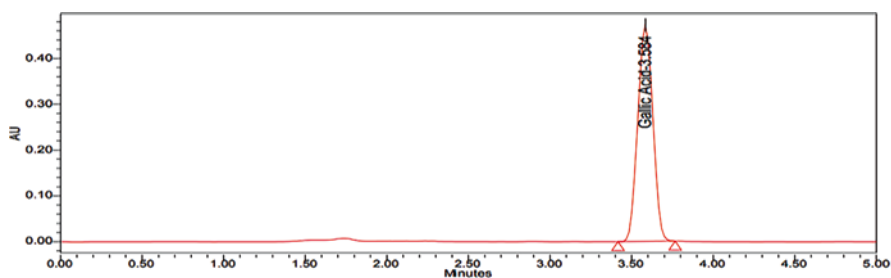
51.2   Results and Discussion

Optimization of Chromatographic Conditions (Fig. 51.1 and Table 51.1)

Specificity (Figs. 51.2, 51.3 and 51.4)

51.3   Analytical Method Validation (HPLC)

The method's linearity, accuracy, precision, and specificity were all confirmed to be satisfactory. The method was validated in accordance with ICH standards.

Linearity

Chromatogram of linearity
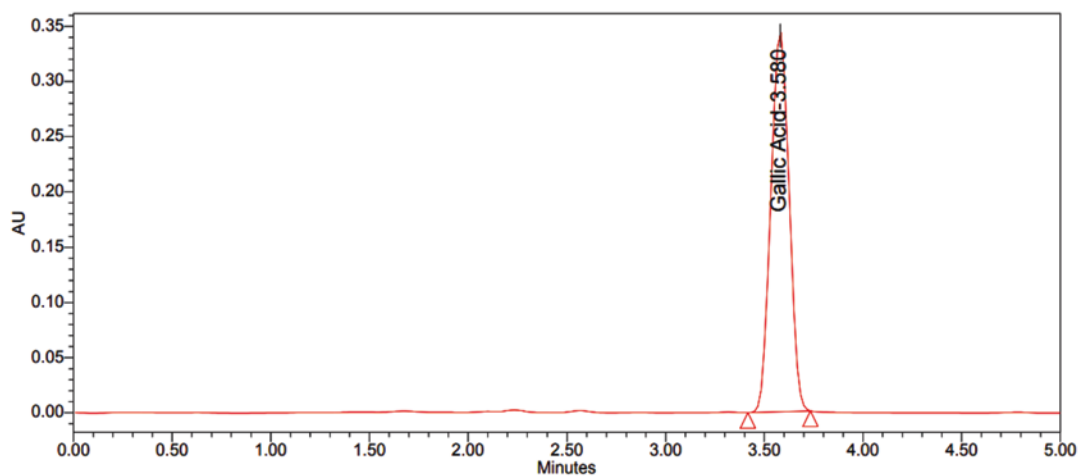
Fig. 51.1   Chromatogram of Trial-6

Table 51.1 Optimized chromatographic conditions

Parameters	Observation
Instrument used	Waters HPLC with autosampler and UV detector
Injection volume	10 μL
Movable phase	Acetonitrile and 0.1% formic acid (30:70)
Column	Waters X-Terra RP-18 (150 × 4.6 mm, 3.5 μ)
Wave length	275 nm
Flow rate	1 mL/min
Runtime	5 min
Temperature	Ambient(25 °C)
Mode of separation	Isocratic mode
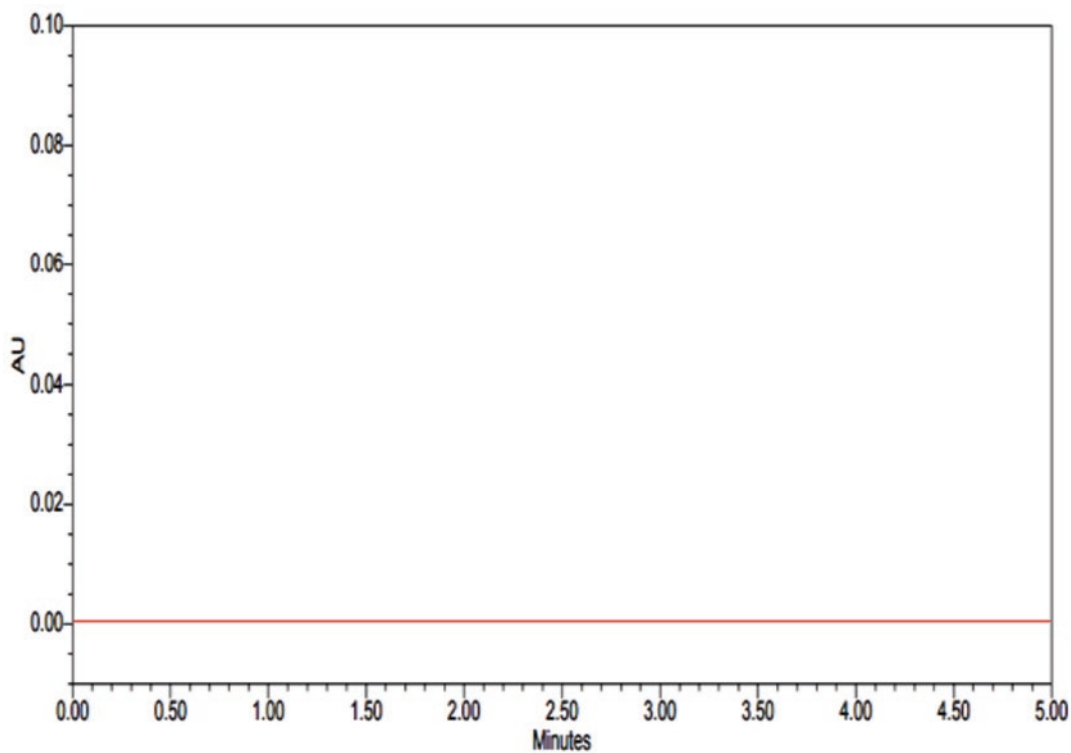
Fig. 51.2 Chromatogram of blank
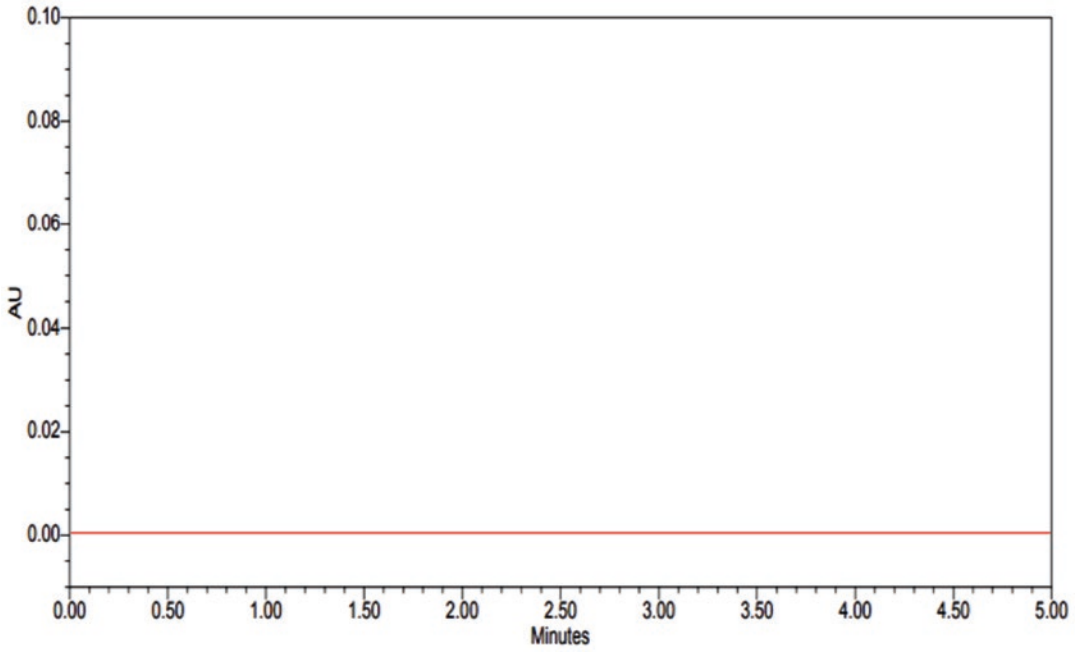
Fig. 51.3 Chromatogram of placebo
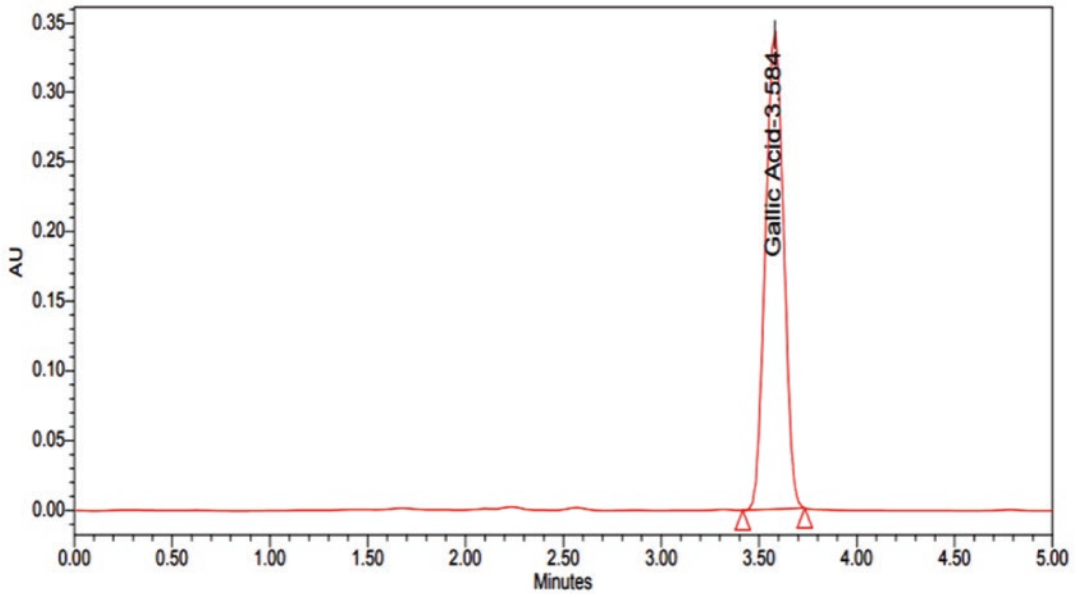
Fig. 51.4 Chromatogram of standard

Accuracy (Table 51.2, Figs. 51.6, 51.7 and 51.8)

Precision (Tables 51.3, 51.4 and Fig. 51.9)

Acceptance Criteria The % RSD for the area of six standard injections results should not be more than 2% (Table 51.5 and Fig. 51.10).

Acceptance Criteria The % RSD for the area of six standard injection results should not be more than 2%.

Robustness (Table 51.6, Figs. 51.11, 51.12, 51.13 and 51.14)

Results of Linearity for Gallic Acid (Fig. 51.5)

LOD and LOQ (Table 51.7, Figs. 51.15 and 51.16)

Degradation Studies (Figs. 51.17, 51.18, 51.19, 51.20, 51.21, 51.22, 51.23, 51.24 and Table 51.8)

51.4   Conclusion

The devised HPLC technique for estimating the target drug is easy to use, quick to implement, highly reliable, and cheap. Both the mobile phase and the solvents are easily accessible, inexpensive, dependable, sensitive, and quick to prepare. The sample recoveries revealed noninterference of formulation excipients in the estimate, and they may be utilized in labs for the regular analysis of chosen medications, all of which were in excellent agreement with their individual label claims. Since the HPLC method's system validation parameters have demonstrated good, accurate, and repeatable findings (without any interference of excipients), it can be inferred that the quick and easy procedures presented will be most beneficial for analysis. This study found that the stability indicating test technique by RP-HPLC was straightforward, reproducible, sensitive, and specific, with no cross-contamination from placebo or degradation products. So, they are suitable for regular gallic acid testing.

	Gallic acid	
S. no	Conc. (µg/mL)	Peak area
1	25.00	512,492
2	50.00	1,033,316
3	75.00	1,474,077
4	100.00	2,016,525
5	125.00	2,533,019
6	150.00	3,023,594
Regression equation	$y = 20135.78x + 3105.61$	
Slope	20135.78	
Intercept	3105.61	
R²	0.9998	
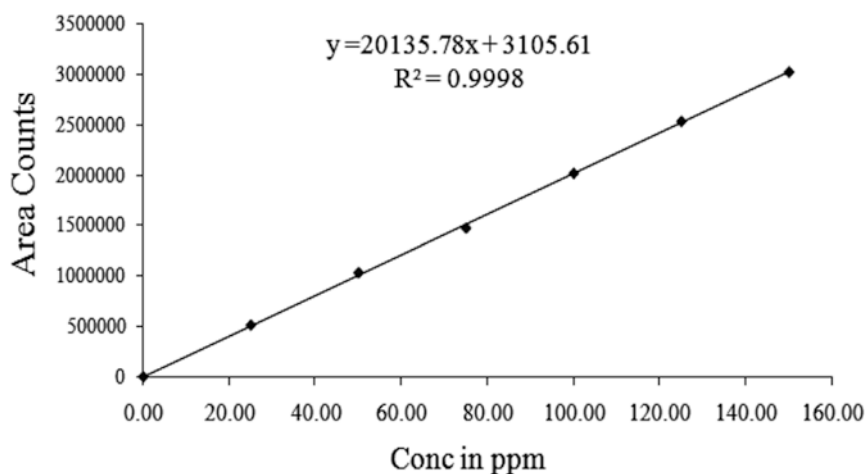
Fig. 51.5 Calibration curve for gallic acid at 275 nm

Table 51.2 Accuracy results of gallic acid by RP-HPLC method

% Concentration (at specification level)	Area	Amount of API added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1,007,441	50	49.94	99.9	100.8
100%	2,035,921	100	100.93	100.9	
150%	3,072,308	150	152.31	101.5	
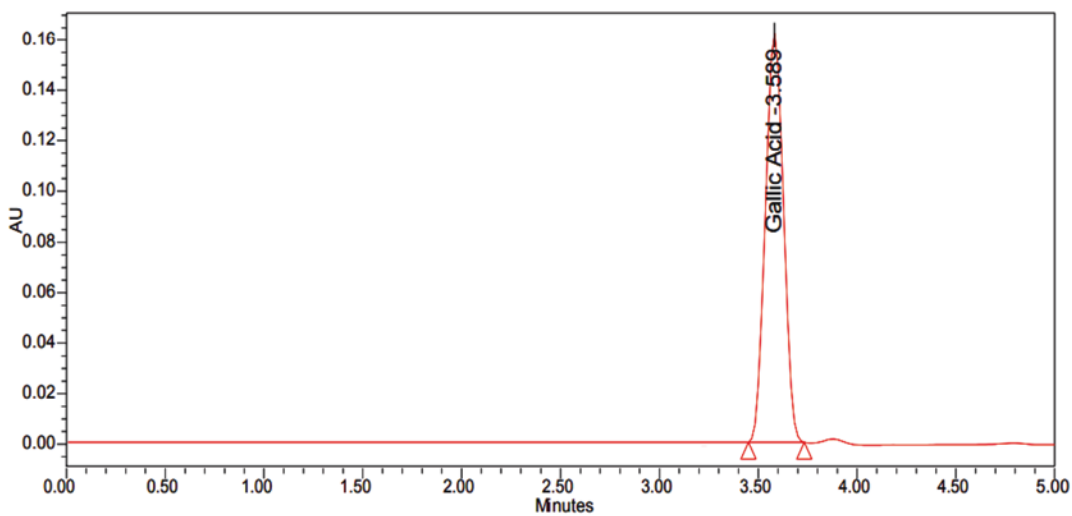
Fig. 51.6 Chromatogram for accuracy 50%
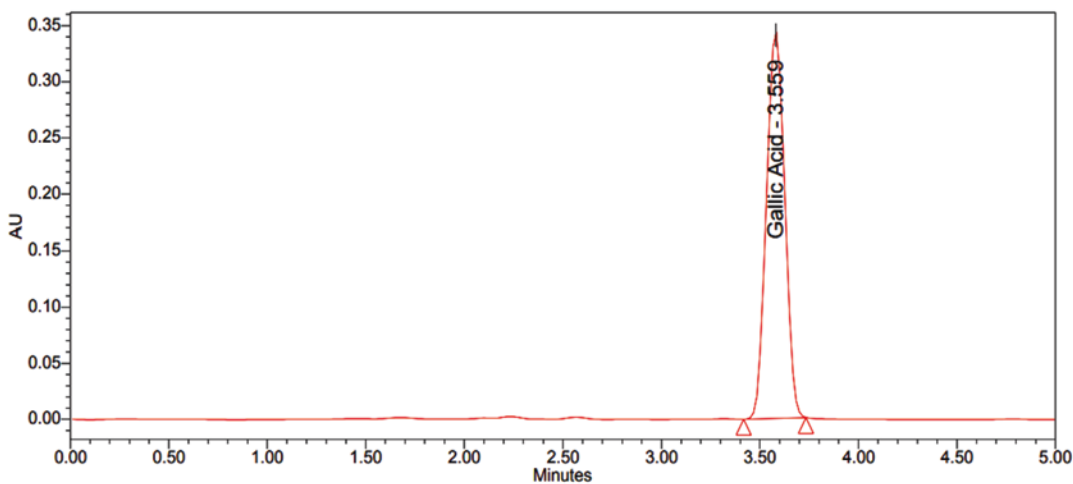

Fig. 51.7 Chromatogram for accuracy 100%
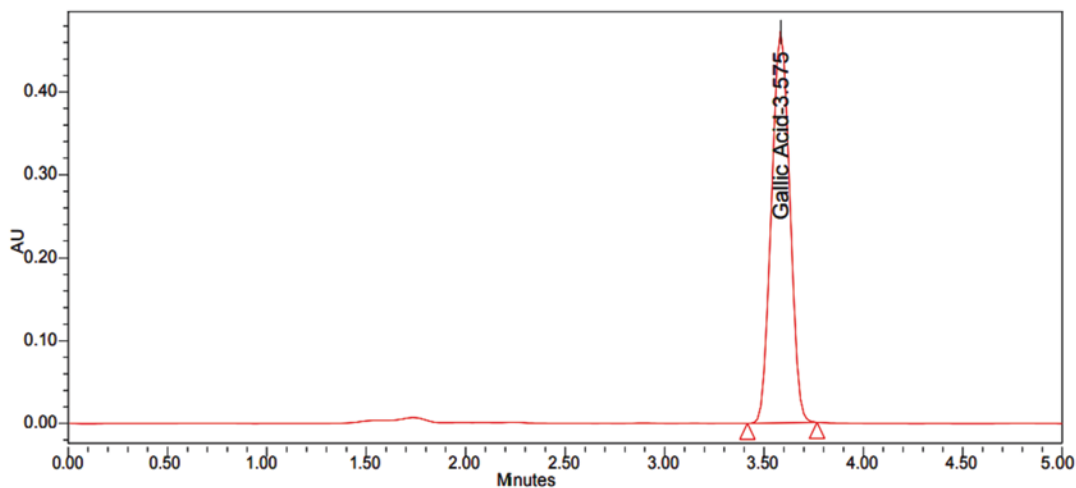
Fig. 51.8 Chromatogram for accuracy 150%

Table 51.3 Standard results for gallic acid by RP-HPLC method

Injection	Area for gallic acid
Injection-1	2,018,100
Injection-2	2,012,025
Injection-3	2,012,025
Injection-4	2,016,525
Injection-5	2,018,100
Injection-6	2,026,125
Average	2,017,150
Standard deviation	5201.49
%RSD	0.26

Table 51.4 Method precision for gallic acid by RP-HPLC method

Injection	Area for gallic acid
Method precision-1	2,005,679
Method precision-2	2,022,432
Method precision-3	2,013,387
Method precision-4	2,007,485
Method precision-5	2,022,066
Method precision-6	2,041,679
Average	2,018,788
Standard deviation	13242.329
%RSD	0.66
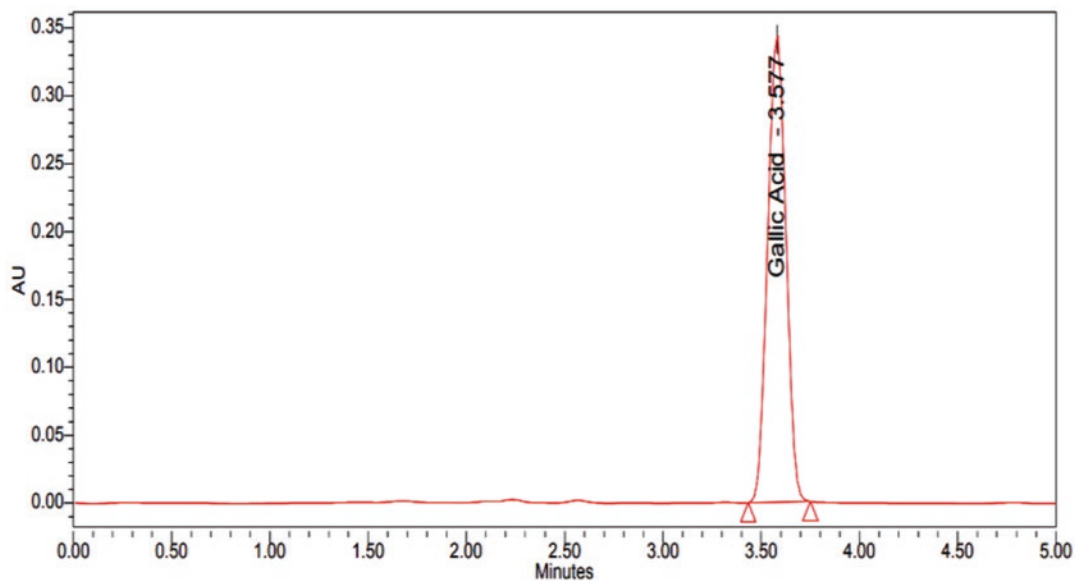
Fig. 51.9 Chromatogram of method precision

**Table 51.5** Intermediate precision for gallic acid by RP-HPLC method

Injection	Area for gallic acid	
	Day-1	Day-2
Intermediate precision-1	2,034,786	2,002,841
Intermediate precision-2	2,002,367	2,030,823
Intermediate precision-3	2,021,542	2,029,252
Intermediate precision-4	2,018,143	2,016,210
Intermediate precision-5	2,026,357	2,026,629
Intermediate precision-6	2,012,687	2,010,798
Average	2,019,314	2,019,426
Standard deviation	11193.210	11297.543
%RSD	0.55	0.56
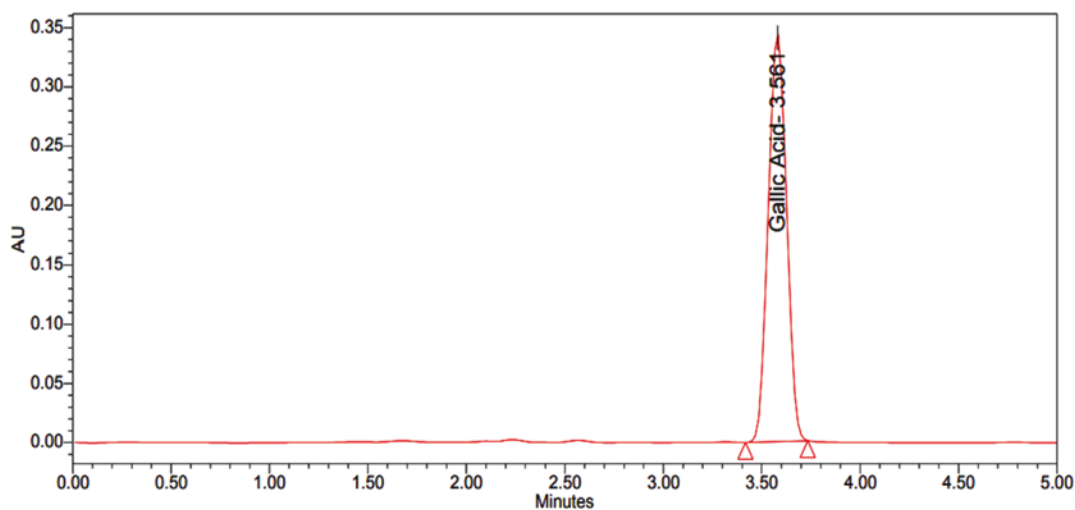
Fig. 51.10 Chromatogram of intermediate precision

Table 51.6 Robustness results of gallic acid by RP-HPLC

| Parameter | Gallic acid | | | | | |
	Condition	Retention time (min)	Peak area	Resolution	Tailing	Plate count
Flow rate change (mL/min)	Less flow (0.9 mL)	3.955	2,241,736		1.05	6056
	Actual (1 mL)	3.580	2,018,100		0.97	6021
	More flow (1.1 mL)	3.277	1,992,709		0.91	6012
Organic phase change	Less Org (27:73)	4.814	2,442,811		1.08	6078
	Actual (30:70)	3.584	2,012,025		0.95	6023
	More Org (33:67)	2.847	1,771,340		0.89	5984
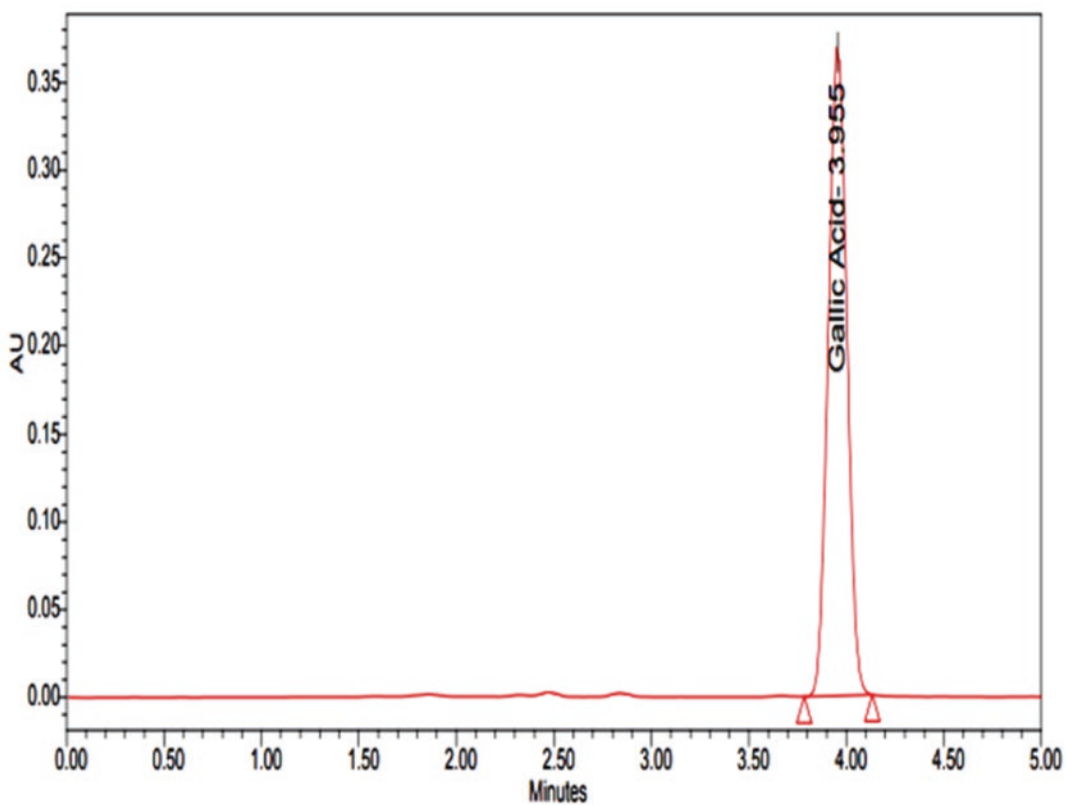
Fig. 51.11 Chromatogram for less flow rate (0.9 ml)
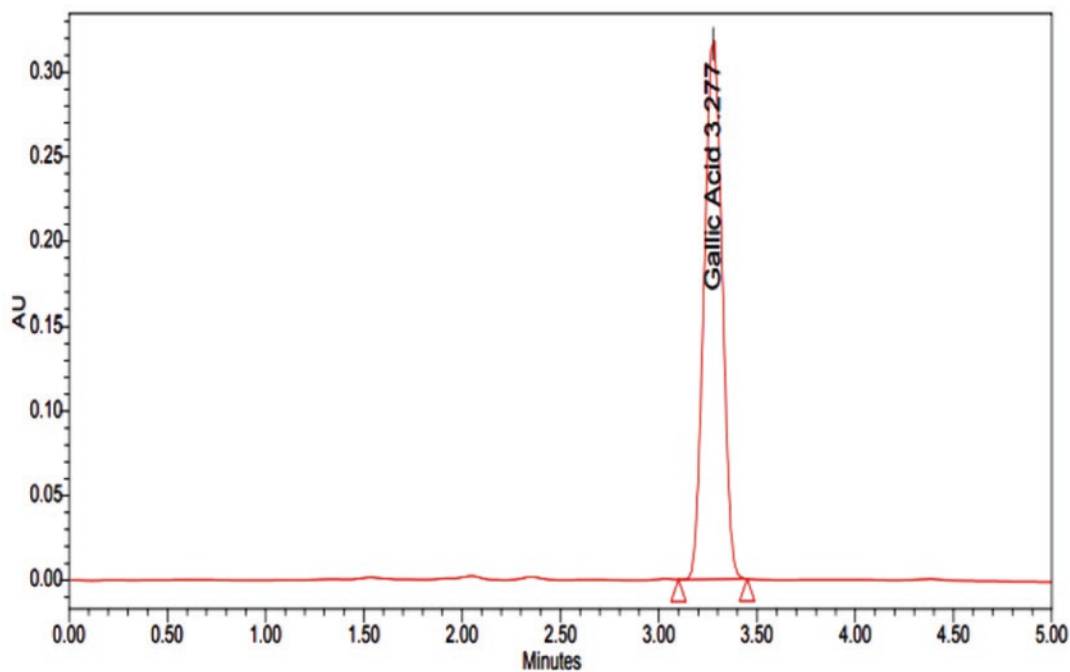
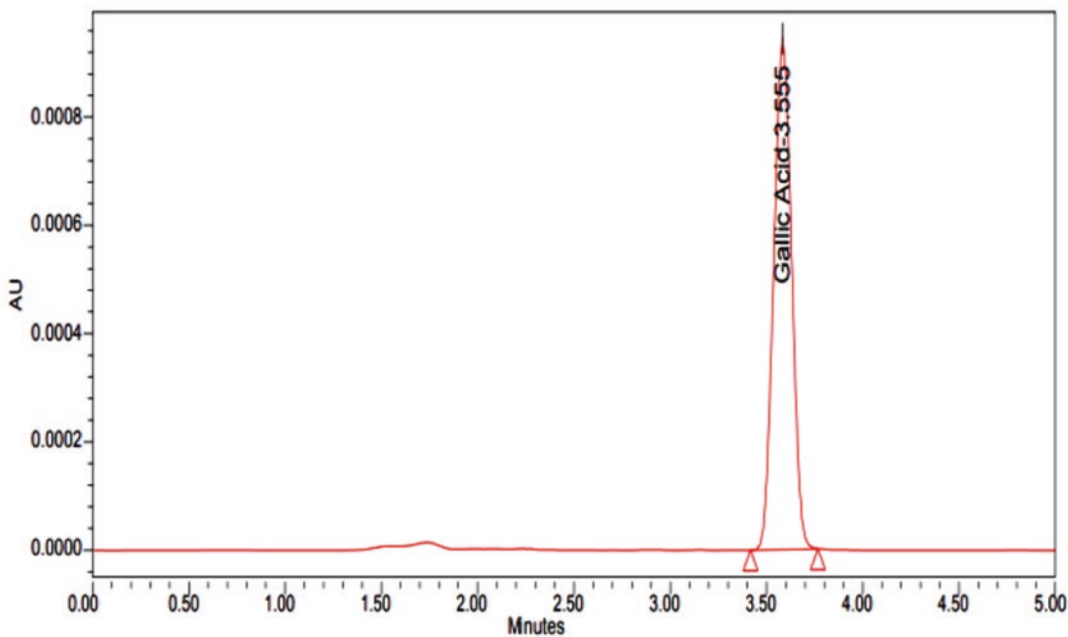**Fig. 51.12** Chromatogram for more flow rate (1.1 mL)
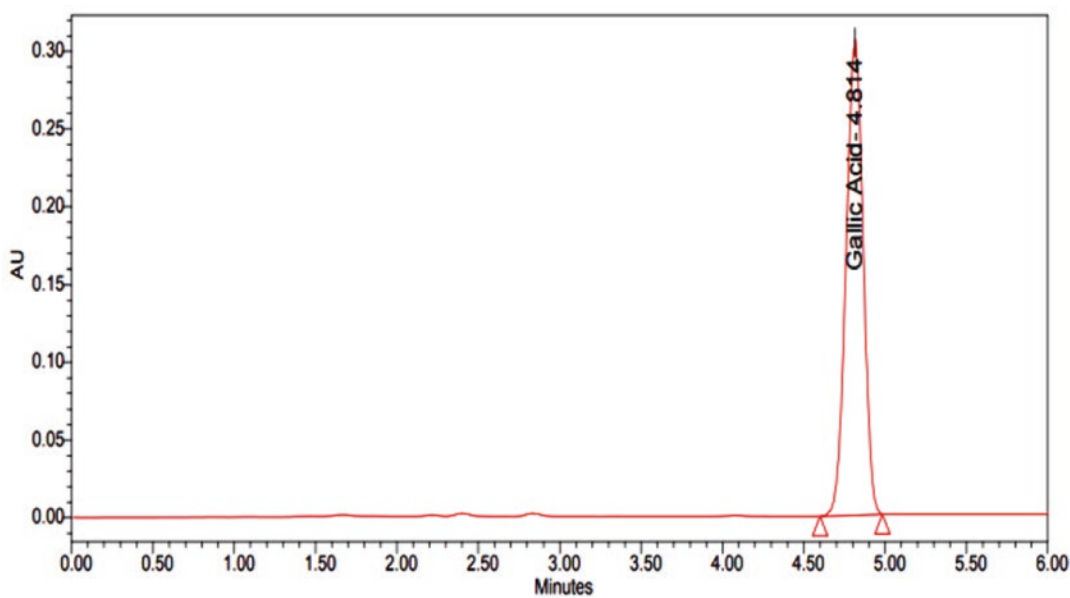
Fig. 51.13 Chromatogram for less organic phase (27:73)
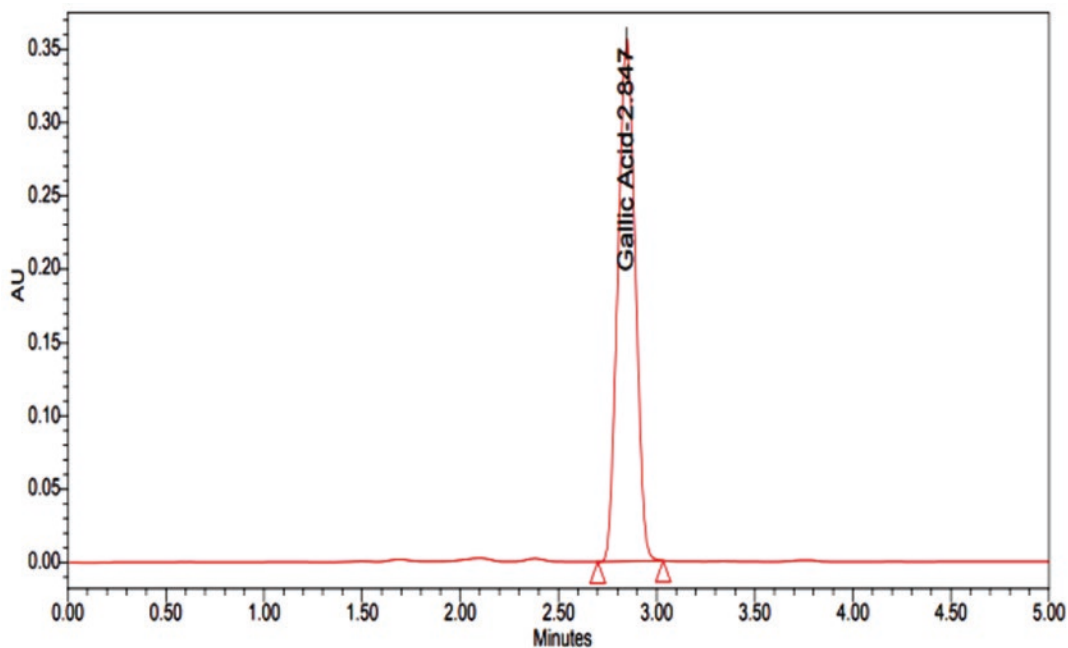
Fig. 51.14 Chromatogram for more organic phase (33:67)

Table 51.7 Sensitivity parameters (LOD and LOQ) by RP-HPLC

Name of drug	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	0.3	1

Fig. 51.15 Chromatogram of LOD
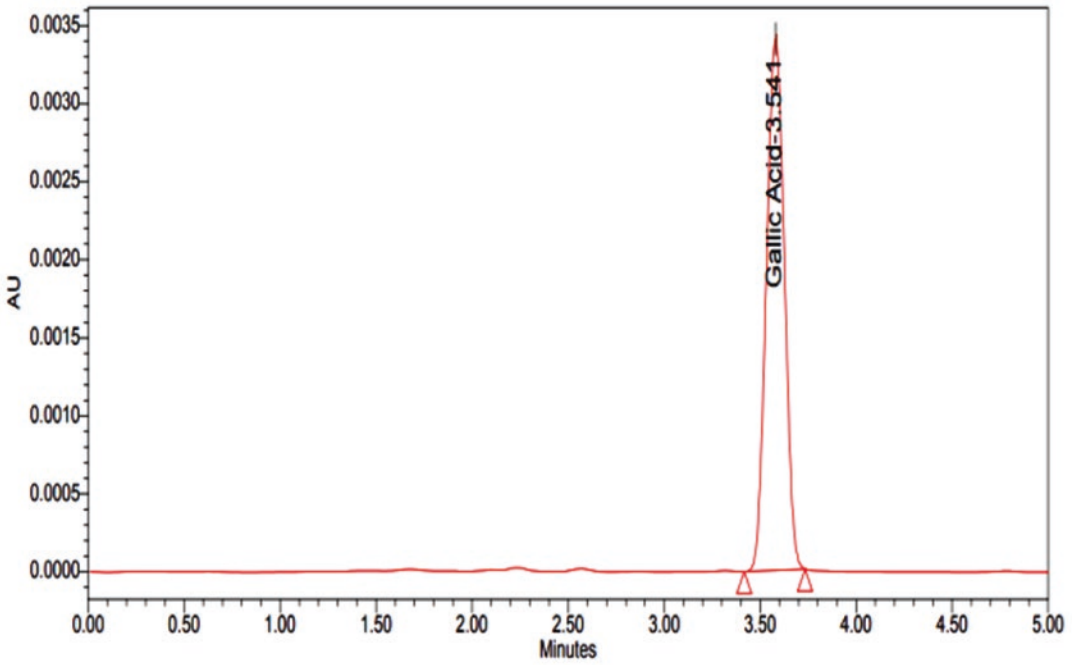
Fig. 51.16 Chromatogram of LOQ
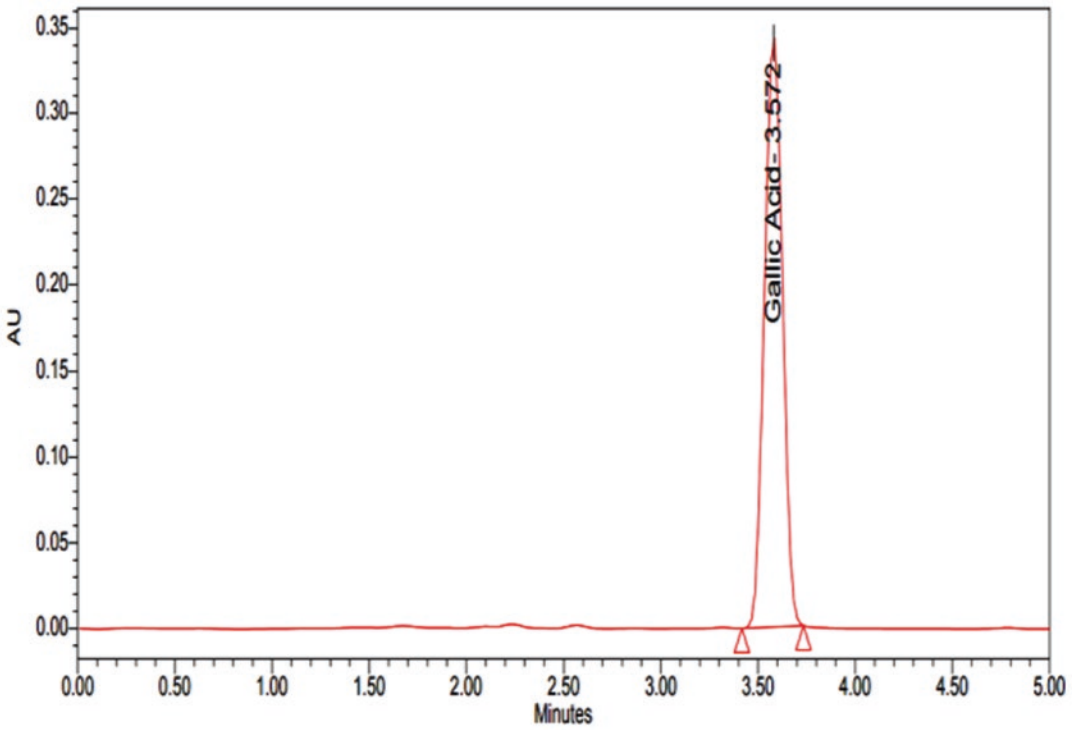
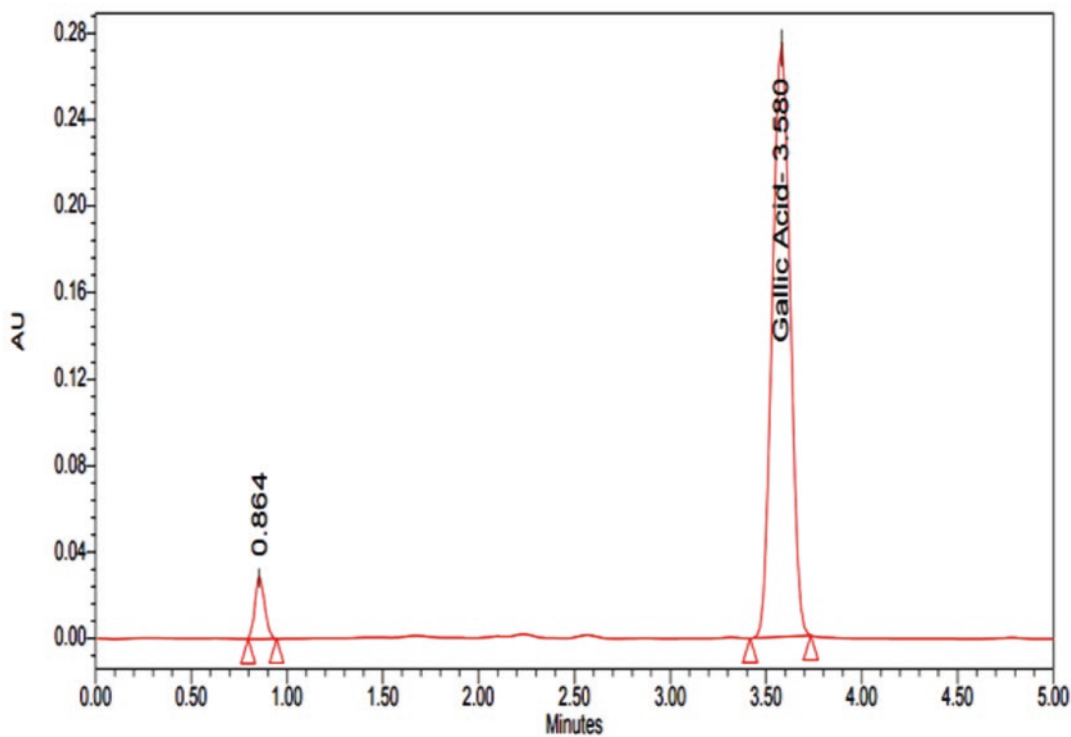**Fig. 51.17** Chromatogram of control degradation

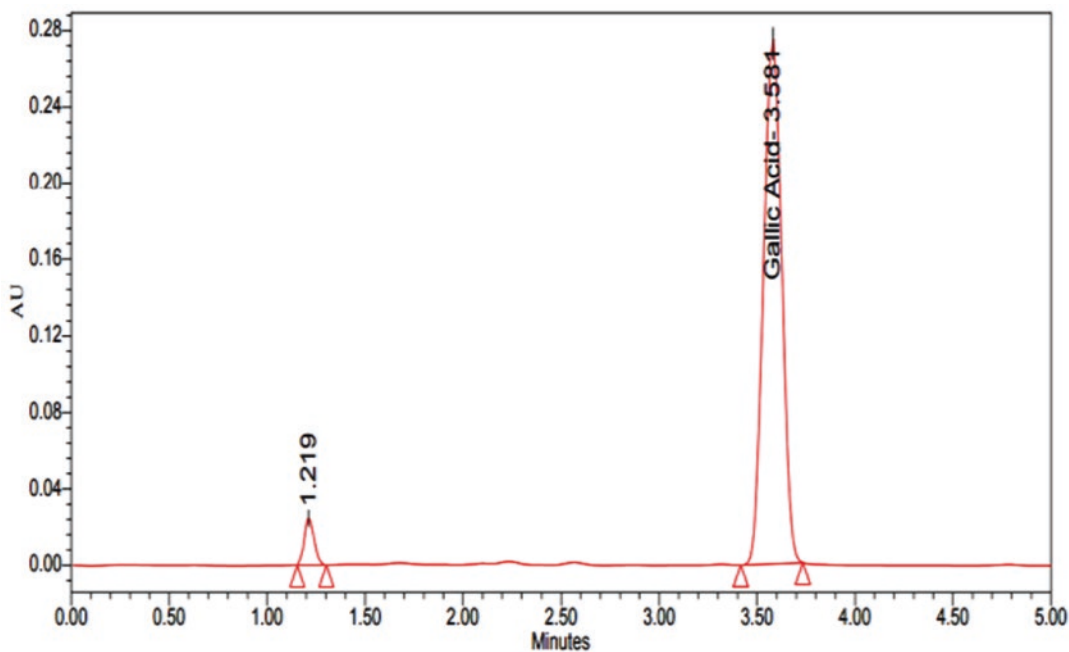**Fig. 51.18** Chromatogram of acid degradation

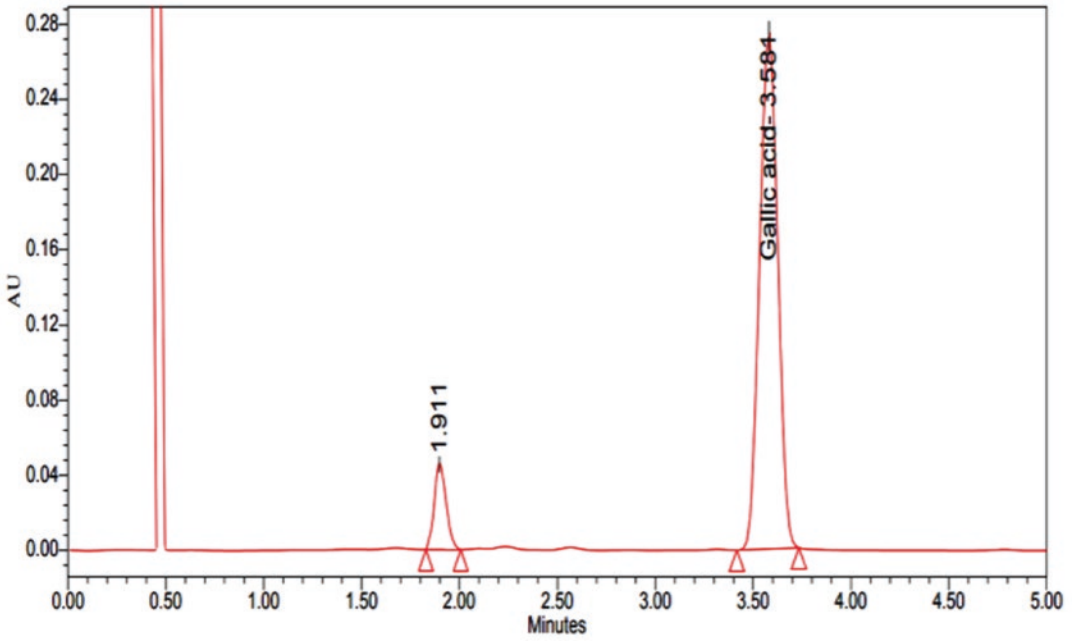**Fig. 51.19** Chromatogram of alkali degradation

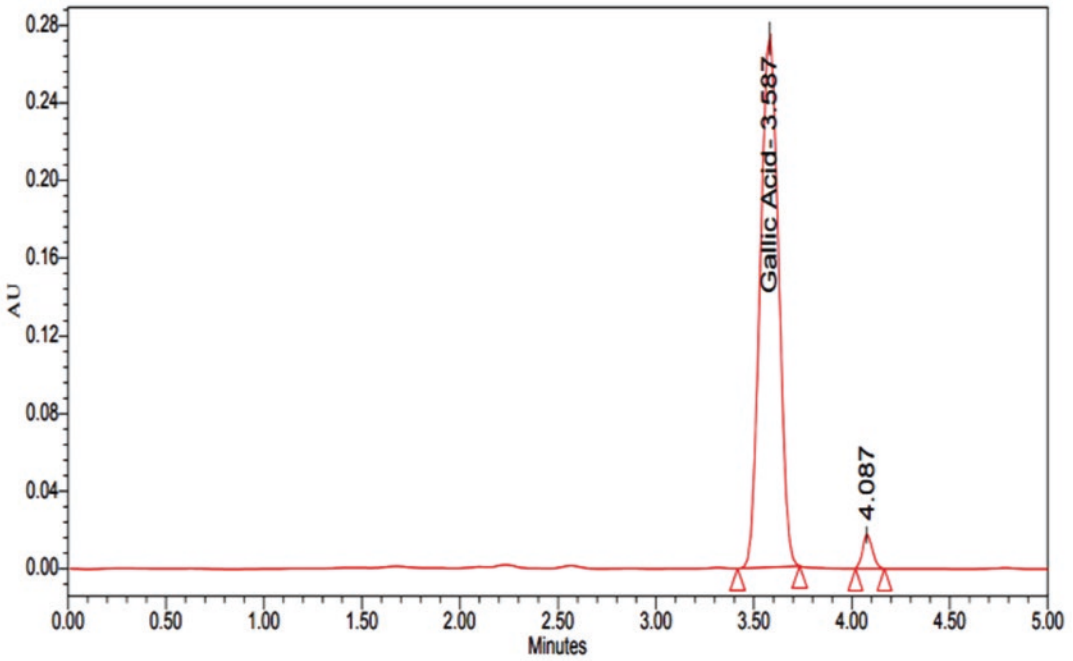**Fig. 51.20** Chromatogram of peroxide degradation

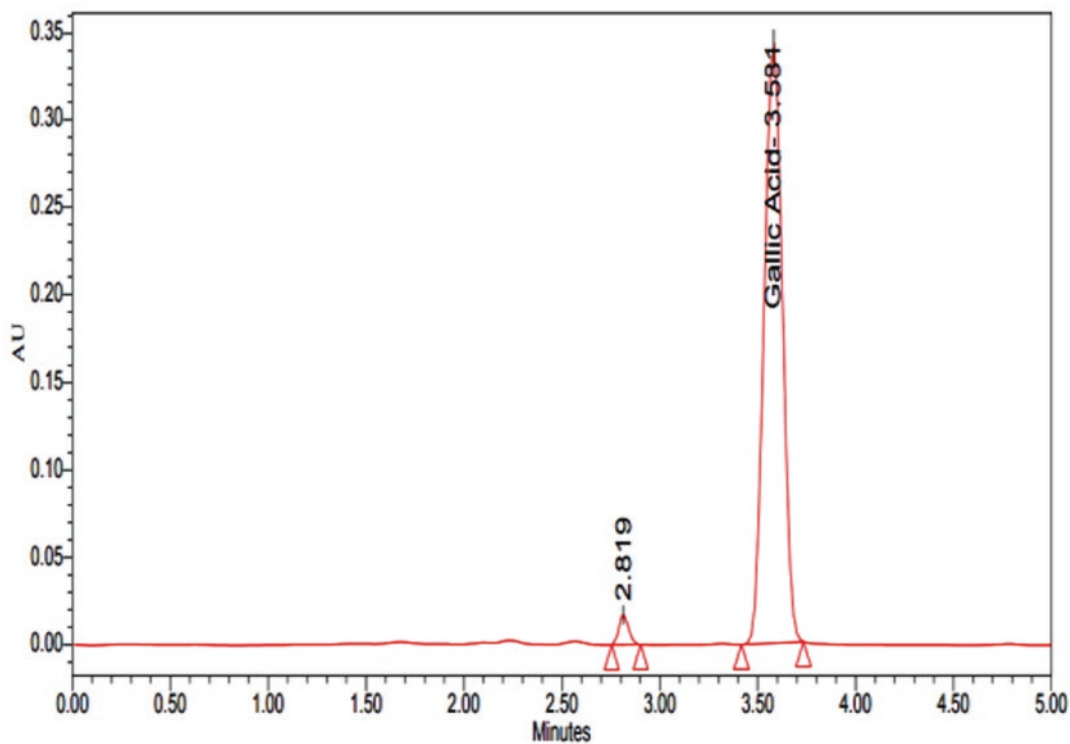**Fig. 51.21** Chromatogram of reduction degradation

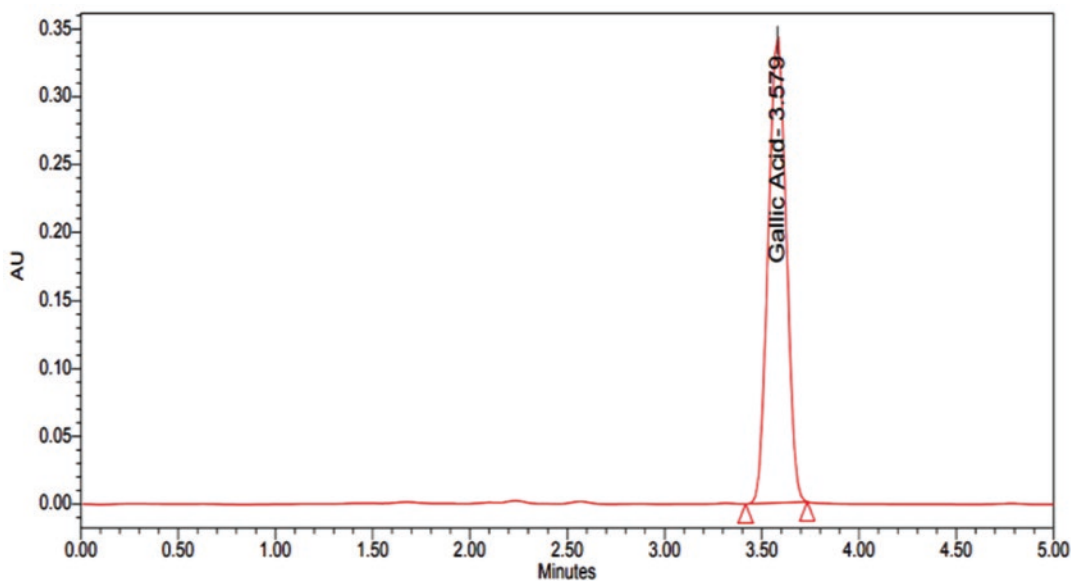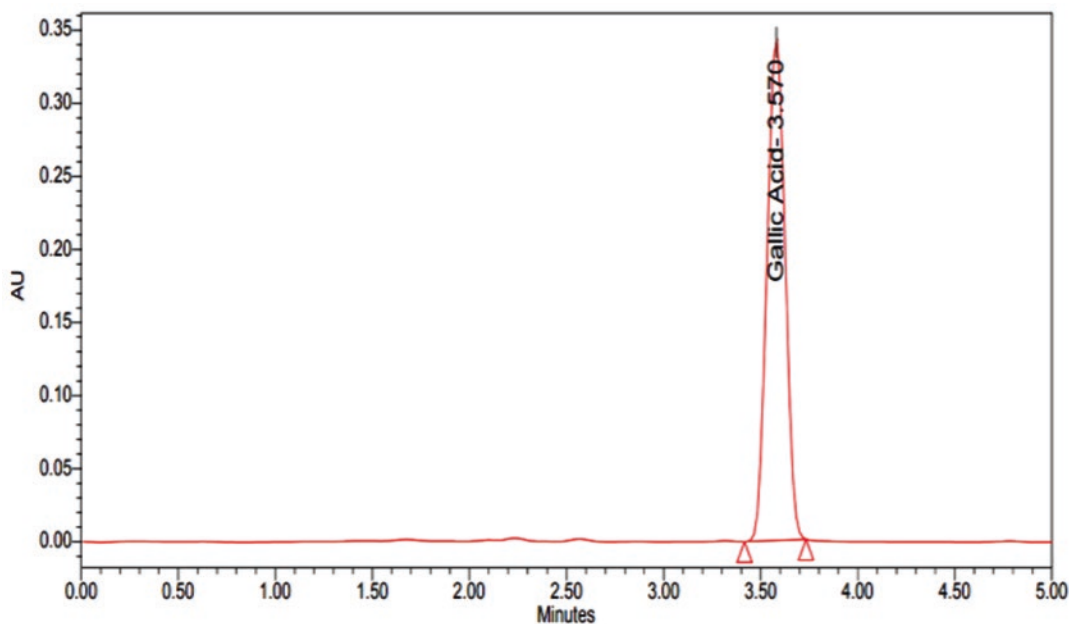**Fig. 51.22** Chromatogram of thermal degradation

Fig. 51.23 Chromatogram of hydrolysis degradation

Fig. 51.24 Chromatogram of photolytic degradation

Table 51.8 Forced degradation results for gallic acid

Results: % degradation results	Gallic acid	
	Area	% Degradation
Control	2,015,530	0
Acid	1,724,763	14.4
Alkali	1,732,274	14.0
Peroxide	1,690,715	16.1
Reduction	1,775,289	11.9
Thermal	1,802,177	10.6
Photolytic	1,991,436	1.2
Hydrolysis	2,006,401	0.4

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