International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 2, 2023

Original Article

A NOVEL RP-HPLC GRADIENT ELUTION TECHNIQUE FOR BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATING GALLIC ACID IN WISTAR RAT PLASMA

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Received: 06 Jan 2023, Revised and Accepted: 02 Feb 2023

ABSTRACT

Objective: Present study aimed to develop and validate a novel, unique, simple, quick, cost-effective, sensitive, specific, accurate, precise, rugged, and robust bioanalytical method for the quantification of gallic acid in rat plasma by reverse phase high-performance liquid chromatography (RP-HPLC) using gradient elution technique.

Methods: The stationary phase was a Zorbax SB C18 5 µ (4.6*150) mm column, with the mobile phase being water with 0.1 percent formic acid (A): acetonitrile (ACN) with 0.08 percent formic acid (B). Gradient chromatographic method was used throughout this experiment from the point of view of the estimation of gallic acid from herbal formulations when present along with other phytoconstituents. So at the gradient method, all the present phytoconstituents has cleared off from the column and no any strongly adsorption of phytoconstituents occurred. The experiment was carried out at a flow rate of 1.0 ml/min at 30 °C utilising PDA detectors at 271 nm. The proposed method was validated for different parameters.

Results: The approach was found to be linear in the concentration range of 0.5-100 µg/ml, with a r^2 of 0.9998. There was not observed any interference of co-eluting peaks of endogenous compounds from the biological matrix at the same retention time (R_t) of gallic acid. The RSD (%) of intra and interday precision was found to be within acceptable limit. The overall % mean recovery was found to be 99.97%. LOD and LOQ were found to be 0.1 and 0.5 µg/ml, respectively. In terms of fluctuation in essential parameters and operating settings, the devised bioanalytical approach was shown to be rugged and resilient. Short-term, long-term, autosampler, bench-top, and freeze-thaw stability experiments revealed that gallic acid is stable.

Conclusion: The developed method described in this report was found to be well within an acceptable range. Hence, in the future, this method can be used successfully for the estimation of gallic acid alone or in combination with another analyte or marker present in bulk or an extract containing various phytoconstituents in pharmacokinetic, bioequivalence, and therapeutic drug monitoring studies in clinical laboratories.

Keywords: Gallic acid, Rat plasma, RP-HPLC, Gradient elution, Bioanalytical method, Validation

DOI: https://dx.doi.org/10.22159/ijap.2023v15i2.47278. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Gallic acid is a phenolic acidic plant metabolite that can be found all over the world. Gallic acid is a benzoic acid that has been hydroxylated three times. In the chemical formula of gallic acid (C6H2 (OH)3 CO2 H), hydroxy groups are found at positions 3, 4, and 5. Gallic acid crystals have a molar mass of 170.12 g/mol and are white, yellowish-white, or pale fawn in colour. It is soluble in alcohol, ether, glycerol, and acetone; benzene, chloroform, and petroleum ether are insoluble [1]. Gallic acid is used in a variety of industries, including pharmaceuticals, cosmetics, food, printing, and manufacturing [2]. Gallic acid is a preservative that keeps fats and oils from going rancid and rotting in a variety of foods such as sauces, confectionary, beverages, and baked goods [3]. Gallic acid also stopped melanogenesis, allowing cells to lose pigment and protect themselves from UV-B and ionising radiation. For this reason, gallic acid is employed as a major gradient in a variety of cosmetics [4, 5]. Anti-allergy, antioxidant, antibacterial and anti-inflammatory are only a few of the medical uses for gallic acid [6-9]. Gallic acid has been shown to protect neurons in a variety of cellular and animal models, both *in vitro* and *in vivo*. Gallic acid reduced neuronal death and improved learning and passive avoidance in memory through modulating antioxidants [10].

Only a few UV Spectrophotometric and HPLC [11-15] methods for detecting gallic acid alone or in combination with other drugs in tablet, extract, and other herbal formulation forms have been published, according to a literature study. The creation of a bioanalytical method for quantifying gallic acid in rat plasma has yet to be published. As a result, a simple, accurate, and sensitive bioanalytical RP-HPLC approach is required.

The goal of this research was to develop and test a new bioanalytical HPLC method for measuring gallic acid in rat plasma that was simple, cost-effective, accurate, precise, sensitive, rugged, and long-lasting.

MATERIALS AND METHODS

Reagents and chemicals

Standard gallic acid was provided by Bangalore-based Sigma-Aldrich. Merk in Mumbai, India provided HPLC-grade formic acid, methanol, and acetonitrile (ACN). The remaining chemicals were all of analytical quality.

Preparation of plasma

The Institutional Animal Ethical Committee sanctioned the experimental protocol at Crystal Biological Solutions, pune (Approval No. CRY/2122/070). The selected animals were housed in groups in stainless steel grill-top polypropylene cages with access to feeding stations. In the cages where the animals were being kept, there was a cycle of light and dark that lasted for 12 h, and the temperature was maintained at 22.3 °C with 55.5% relative humidity. A Wistar rat's blood was drawn and deposited in a centrifuge tube containing a 5% EDTA solution. The blood sample was vortex agitated for one minute before centrifugation at 4 °C at 10,000 rpm for ten minutes. The clear supernatant was separated and stored at-80 °C until it was required.

Preparation of a gallic acid standard stock solution

Weighing 100 mg of pure gallic acid and pouring it into a volumetric flask with a 50 ml capacity yielded a standard gallic acid stock solution. The mixture was then sonicated for 5 min with 25 ml methanol added. To bring the volume to 50 ml, methanol was utilised. The prepared solution was filtered using Whatman No. 41

filter paper [16]. The typical stock solution was 2000 µg/ml in concentration.

Preparation of gallic acid working standard solutions

Pipette 0.0625, 0.125, 0.25, 0.625, 1.25, 3.125, 6.25, and 12.5 ml of the standard stock solution (2000 µg/ml) into a separate series of 25 ml capacity volumetric flasks and dilute up to the mark with methanol to produce concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 µg/ml [17].

Sample preparation for linearity and quality control

Linearity and quality control (QC) samples were made by mixing 0.9 ml blank plasma with 0.1 ml working standing solutions. 0.1 ml of the working standard solution for each concentration was transferred to separate 2 ml eppendorf tubes, and 0.9 ml of blank plasma was spiked in each working standard solution in eppendorf tubes. The extracting reagent, 1 ml of methanol, was then poured to each eppendorf tube and agitated for 1 min with a vortex shaker. Each solution was maintained in a centrifuge machine and centrifuged for 15 min at 4 °C at 10000 rpm (Remi, Mumbai). Each solution's clear supernatant (1 ml) was separated and transferred to new eppendorf tubes. Before being injected into the HPLC equipment in a consecutive manner, the clear supernatant was filtered using 0.42 membrane filter paper. At concentrations of 0.5, 1, 2, 5, 10, 25, 50, and 100 µg/ml, the final linearity and quality control samples were made [18, 19].

Optimised chromatographic conditions

The quantity of gallic acid in plasma was measured using HPLC (Model: Waters 2695 alliance). The bioanalytical technique development experiment used a Zorbax SB C18 5µ (4.6*150) mm column. A Millipore 0.45 µ filter was used to filter the prepared mobile phase. The column temperature was preserved at 30 °C. The mobile phase flow rate was fixed at 1.0 ml/min. A PDA type detector was used at 271 nm. A gradient chromatographic method was used throughout the experiment. The mobile phase consisted of water containing 0.1 percent formic acid (A) and acetonitrile (ACN) containing 0.08 percent formic acid (B). The flow rate was kept constant while the mobile phase's components were altered.

Validation

Linearity

The method's linearity refers to its capacity to produce test findings that are proportionate to the analyte concentration in samples. A set of eight linearity and quality control samples was prepared by mixing 0.9 ml of blank rat plasma with varying amounts of working standard solutions. The solutions were agitated for 1 min using a vortex shaker before centrifugation. The clear supernatant solution was collected and put into eppendorf tubes. Gallic acid concentrations in the linearity and quality control samples ranged from 0.5 µg/ml to 100 µg/ml. The curve of linearity was produced by graphing peak area vs concentration in three replicates for each concentration sample. The regression equation developed was utilised to calculate the analyte concentration in each concentration sample. If all of the linearity solutions had less than 15 % RSD, the linearity curve was confirmed to be legitimate. A correlation coefficient of greater than 0.96 is preferable, as is a gallic acid LLOQ response that is at least 3 times that of blank plasma [20, 21].

Specificity or selectivity

It refers to a method's capacity to reliably quantify analyte concentrations in the presence of all other interfering sample components. If specificity isn't guaranteed, the precision, accuracy, and linearity of the procedure are all jeopardised. To create and validate an effective approach, the first step is to ensure its specificity. The recommended approach can be considered specific if there is no interference between co-eluting endogenous component peaks and analyte peaks. The chromatogram of blank Wistar rat plasma without gallic acid was analysed in three duplicates to determine the specificity and selectivity of the described method. Further plasma samples were spiked with gallic acid and evaluated in the chromatogram of rat plasma for the interference of co-eluting peaks. The retention period of a chromatographic peak of gallic acid was investigated. Changing the settings of the HPLC method was used to investigate the method's specificity. (Different gradient slopes) and looked for interference in the chromatogram from endogenous substances co-eluting peaks [22].

Accuracy and precision

When a process is repeated on a homogeneous sample, precision refers to the degree of agreement among individual test outcomes. Five replicates were tested for intraday (on the same day at different times) and interday (two consecutive days) precision and accuracy at four different QC levels: 100 µg/ml (HQC), 50 µg/ml (MQC), 10 µg/ml (LQC), and 0.5 µg/ml (LLOQ). A regression equation was used to compute the amount of plasma retrieved. Precision was measured as a percentage of the RSD, while accuracy was measured as a percentage of the recovery [23].

Recovery study

A recovery study was conducted to ensure the proposed method's reliability and appropriateness. It refers to how closely the measured value resembles the true value. The efficacy of gallic acid extraction in rat plasma samples as well as the effect of matrix was investigated in recovery research. A recovery study was conducted at three different QC concentrations: 100 µg/ml (HQC), 50 µg/ml (MQC), and 10 µg/ml (LQC). First, gallic acid was spiked in blank plasma at three QC concentration levels, and the concentration was assessed using the established HPLC bioanalytical method's chromatogram. Without the biomatrix, the same three standard concentration solutions were created. The ratio of gallic acid concentration with and without biomatrix was used to determine the extraction efficiency of gallic acid. According to FDA guidelines, drug recovery does not have to be perfect, but it should be consistent, accurate, and repeatable [24, 25].

Limit of detection

It comprises determining the analyte concentration in a sample with the least amount of analyte, however, it is rarely quantified. The LOD is related to the system's signal-to-noise ratio. To identify the smallest amount of analyte, the signal to noise ratio (S/N) of the analyte should be 3:1. The LOD was determined by injecting 0.1 µg/ml gallic acid three times into a plasma sample and comparing the chromatograms to blank plasma [26].

Limit of quantification

A sample's lowest analyte concentration must be calculated precisely and properly. The peak area of a blank plasma sample (three triplicates) was compared to spiked gallic acid in plasma at the LLOQ level (three triplicates) [27].

Ruggedness

It refers to the reproducibility of outcomes when the method is used in real-world situations. Three followings conditions were examined. The developed method was assessed for two different operators in the same lab, two different columns of the same type and manufacturer and changing sources of reagent and solvent. Gallic acid was spiked in rat plasma at one quality control concentration level only (HQC-100 µg/ml) for ruggedness research [26-28].

Robustness

The potential to stay unaffected by tiny but deliberate variations in technique parameters is measured by robustness. Important parameters in the method were changed systematically and measured their effect on the peak retention time and concentration through peak area. The parameters such as organic mobile phase composition of±5 %, temperature of±10 % and flow rate of±10 % were varied systematically and their chromatogram compared to the normal chromatographic method conditions. Gallic acid was spiked in rat plasma at two quality control concentration levels (HQC-100 µg/ml and LQC-10 µg/ml) to test robustness [29].

Stability study of gallic acid in rat plasma

The short-term stability of LQC and HQC samples was investigated by storing them at room temperature (25 °C) for 6 h prior to

analysis. The LQC and HQC samples were evaluated for long-term stability after 10 d of storage at room temperature (25 °C). The auto sampler's stability was evaluated by storing LQC and HQC samples for 24 h at 5 °C in the autosampler tray. The stability of LQC and HQC on the benchtop was tested by keeping samples at room temperature. These samples' LQC and HQC concentrations were compared to LQC and HQC concentrations made from scratch. LQC and HQC samples were taken out of the deep freezer at regular intervals, thawed at ambient temperature, and stored outside for 1 h as part of the freeze-thaw experiment. The samples were frozen for threefreeze–thaw cycles before being assessed at-30 °C. At LQC and HQC levels, gallic acid plasma stability was assessed and compared to the typical gallic acid content [28-29].

Statistical analysis

GraphPad Prism 8.0.1 was used to validate the results during statistical analysis.

RESULTS AND DISCUSSION

Linearity

Fig. 1, 2, 3, 4, 5 and 6 show rat plasma chromatograms, LQC, MQC, HQC, linearity overlay spectra, and a calibration curve for gallic acid in rat plasma, respectively. Tables 2 and 3 illustrate the linearity results in further depth. The gallic acid linearity curve was found to be linear using HPLC equipment for the concentration range of 0.5–100 µg/ml. 0.9998 was found to be the linearity correlation coefficient. In plasma, the linearity equation was found to be Y = 67057.54 X–39807. Gallic acid in plasma calibration curves demonstrated full linearity and a high correlation value. With the use of the calibration equation, each linearity and quality control sample was quantified, and the formed standard deviation was discovered to be less than 15%. The percent RSD was determined to be less than 7%, and the accuracy was found to be between 96 and 112 percent.

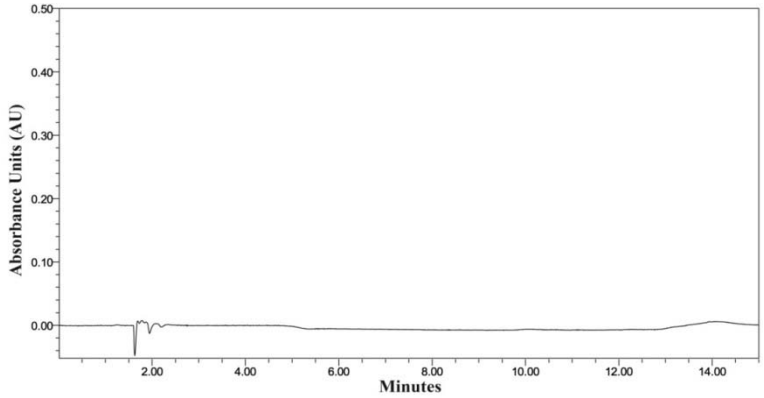

Fig. 1: Chromatogram of extracted blank rat plasma (Rt = 3.42 min)

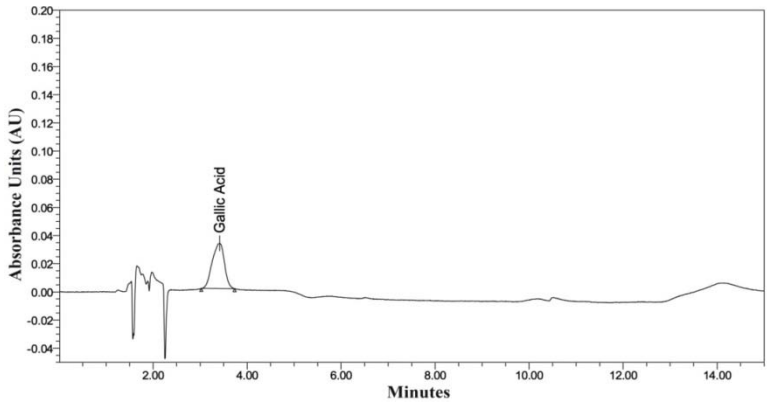

Fig. 2: Chromatogram of standard gallic acid at LQC level (10 µg/ml) spiked in rat plasma. (Rt = 3.42 min)

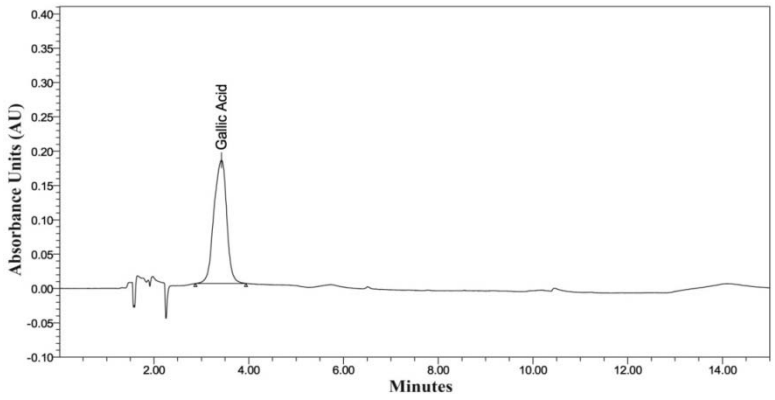

Fig. 3: Chromatogram of standard gallic acid at MQC level (50 µg/ml) spiked in rat plasma. (Rt = 3.42 min)

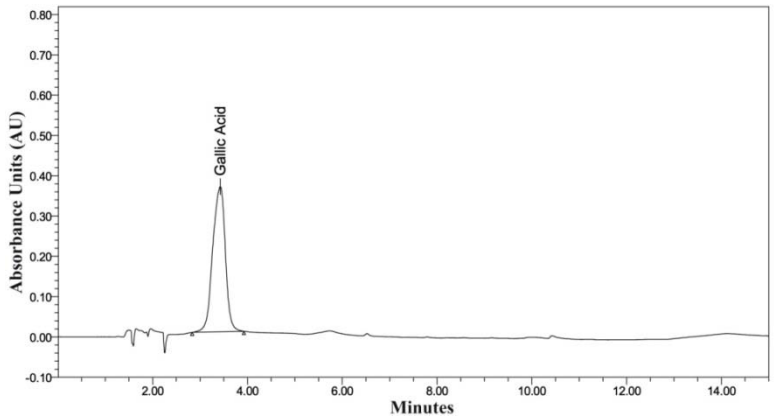

Fig. 4: Chromatogram of standard gallic acid at HQC level (100 µg/ml) spiked in rat plasma (Rt = 3.42 min)

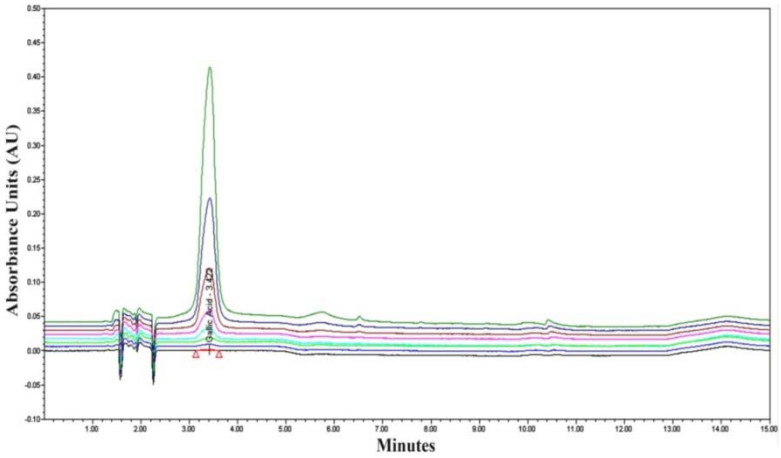

Fig. 5: Gallic acid spectra in rat plasma with linearity overlay

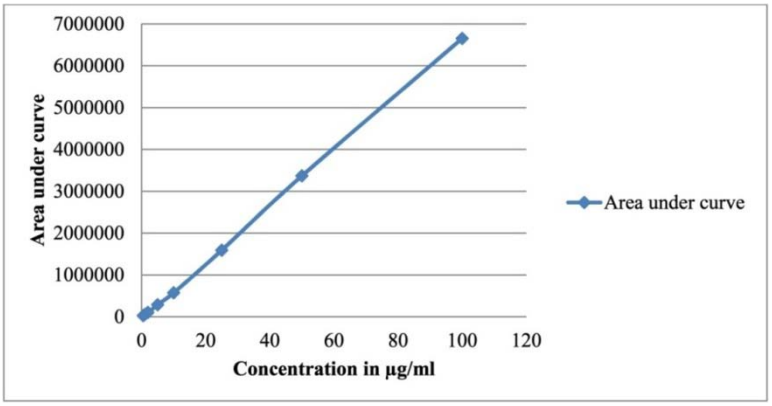

Fig. 6: Gallic acid linearity curve in rat plasma

Table 1: Optimised chromatographic conditions

Parameters	Optimized parameters
Chromatograph	Waters 2695 alliance
Chromatographic system	Gradient chromatographic method
Column	Zorbax SB C18 5µ (4.6*150)mm
Flow rate	1.0 ml/min
Temperature	30 °C
Type of detector	W2996 PDA
Detection wavelength	271.0 nm
Injection volume	30.00 µl
Mobile phase	Water with 0.1% formic acid: acetonitrile (ACN) with 0.08 % formic acid.
Run time	15 min

Table 2: Various constants for calibration curve of gallic acid in rat plasma

Parameter	Value
Beer's Low Limit	0.5–100 µg/ml
Correlation coefficient*	0.9998
Intercept*	-39807
Slop*	67057.54
Regression Equation	Y = 67057.54 X-–39807
Retention time	3.42 min

*Average of three determinations

Table 3: Gallic acid linearity in rat plasma

Spiked plasma concentration (µg/ml)	Peak area	Measured concentration (n=3) (µg/ml) (mean±SD	RSD (%)	Accuracy (%)
0.5	23860	0.48±0.03	6.41	96
1	53061	1.12±0.02	1.79	112
2	104908	2.15±0.05	2.33	107.5
5	286136	5.13±0.17	3.42	102.6
10	573726	9.96±0.33	3.34	99.6
25	1592046	24.92±0.29	1.18	99.68
50	3368523	48.13±0.31	0.63	96.26
100	6654917	98.33±3.51	3.57	98.33

(Number of an experiment, n= 3)

Specificity or selectivity

The specificity and selectivity of the new bioanalytical RP-HPLC technology were tested by comparing the chromatograms of blank rat plasma and gallic acid spiked plasma samples. Fig. 1, 2, 3, and 4 showed blank plasma and gallic acid-treated plasma at three QC levels. After comparing the peaks, it was discovered that there were no distracting peaks at the gallic acid retention time (Rt= 3.42 min). The specificity of gallic acid was investigated by adjusting the gradient slop of the mobile phase composition in the HPLC method, and no interference of co-eluting peaks of endogenous chemicals from the biological matrix was seen during the retention period of gallic acid. It can be deduced from the aforementioned observation and chromatogram that there is no interference of the co-eluting peak from rat plasma with gallic acid. As a result, the approach devised proved specific for detecting and analysing gallic acid in plasma [30].

Precision and accuracy

Tables 4 and 5 present the results of intraday and interday precision and accuracy tests of gallic acid in rat plasma using the suggested bioanalytical method at four QC levels. The intraday percent RSD was determined to be less than 4 %, with accuracy ranging from 95 % to 98.7 %. The difference in percent RSD between days was less than 6 %, and the accuracy ranged from 92 percent to 98.7 %. The results, which were within the acceptable range for intraday and interday precision, revealed that the proposed bioanalytical approach is accurate, precise, reproducible, and dependable [31].

Table 4: Gallic acid in rat plasma: intraday precision and accuracy data

Spiked plasma concentration (µg/ml)	Measured concentration (µg/ml) (n=5) (mean±SD)	RSD (%)	Accuracy (%)
Morning			
0.5 (LLOQ)	0.49±0.005	0.998	96
10 (LQC)	9.87±0.14	1.428	98.7
50 (MQC)	48.44±1.40	2.889	96.88
100 (HQC)	97.06±1.32	1.356	97.06
Afternoon			
0.5 (LLOQ)	0.48±0.01	2.035	96
10 (LQC)	9.54±0.12	1.254	95.4
50 (MQC)	47.50±1.78	3.742	95
100 (HQC)	97.04±2.19	2.251	97.04

All values are mean±SD values (Number of experiment, n= 5)

Table 5: Interday accuracy and precision data of gallic acid in rat plasma

Spiked plasma concentration (µg/ml)	Measured concentration (µg/ml) (n=5) (mean±SD)	RSD (%)	Accuracy (%)
First Day			
0.5 (LLOQ)	0.49±0.005	0.998	96
10 (LQC)	9.87±0.14	1.428	98.7
50 (MQC)	48.44±1.40	2.889	96.88
100 (HQC)	97.06±1.32	1.356	97.06
Second Day			
0.5 (LLOQ)	0.46±0.01	2.286	92
10 (LQC)	9.47±0.11	1.196	94.7
50 (MQC)	46.41±2.37	5.113	92.82
100 (HQC)	95.33±1.74	1.827	95.33

(Number of experiment, n= 5)

Recovery study

Gallic acid's peak areas with biomatrix (plasma) and without biomatrix were compared to compute gallic acid recovery (solvent). Table 6 shows the findings of the recovery study. Gallic acid recovery was tested in three replicates at three distinct quality control concentrations: 100 µg/ml (HQC), 50 µg/ml (MQC), and 10 µg/ml (LQC), with results of 98.19 %, 97.90 %, and 103.81 %, respectively. The overall gallic acid recovery rate was found to be 99.97 %. 3.33 % was found to be the total percent RSD. The presence of endogenous compounds in biomatrix can obstruct drug analysis in biological samples in general. The collected findings were analysed, and it was discovered that the matrix peak had no effect on the gallic acid present in the plasma sample [30-31].

Table 6: Recovery study data of gallic acid with and without biomatrix

Replicate number	LQC (Area)	LQC (Area)	MQC (Area)	MQC (Area)	HQC (Area)	HQC (Area)
	Unextracted	Extracted	Unextracted	Extracted	Unextracted	Extracted
	552317	573726	3440220	3368523	6654917	6534805
	552619	573913	3440602	3368230	6653115	6533209
	552832	573345	3440919	3368915	6654208	6534407
Mean	552589	573661	3440580	3368556	6654080	6534140
SD	258.8	289.5	350	343.7	907.8	830.7
% RSD	0.04683	0.05046	0.01017	0.01020	0.01364	0.01271
% Mean recovery	103.81		97.90		98.19	
Overall % Mean recovery	99.97					
Overall SD	3.332					
Overall % RSD	3.333%					

(Number of experiment, n= 3)

Limit of detection

0.1 µg/ml was found to be the LOD. The new bioanalytical HPLC approach is sensitive enough to detect the presence of gallic acid in rat plasma at low concentrations.

Limit of quantification

The Limit of quantification was calculated with a signal-to-noise ratio (S/N) of 10:1. The LOQ of the new bioanalytical approach was found to be 0.5 µg/ml. As a result, we were able to identify even low quantities of gallic acid in rat plasma using our method.

Ruggedness

Table 7 displays the findings of the gallic acid ruggedness study data in rat plasma. At the HQC concentration level, ruggedness was tested to examine how varied operating conditions affected retention time and concentration through peak area. At various operating circumstances, the chromatograms of spiking standard gallic acid at the HQC concentration level in rat plasma were investigated. There was no discernible difference in retention time or measured concentrations. The percent RSD was discovered to be less than 2 %. As a result, the devised method can withstand chromatographic operating conditions such as changes in operators, columns, and reagent and chemical sources [32].

Robustness

Table 8 displays the robustness of gallic acid in rat plasma. It was carried out by adjusting the parameters and determining the effect on retention duration and concentration by measuring the area. The retention duration was found to be somewhat shorter when the flow rate and temperature were increased by 10 %. When the organic mobile phase was held at+5 %, there was no discernible difference in retention time. With a flow rate of-10 %, the retention time was extended slightly from 3.42 to 3.97 min. When the temperature and organic mobile phase were held at-10 % and-5 %, there was no discernible difference in retention time. At all parameter variations, the percent RSD was found to be less than 5 %. When compared to conventional chromatographic technique parameters, the measured concentrations from acquired peak areas at+10 % of flow rate and temperature were slightly lower. When chromatographic settings were adjusted up to a particular percentage level, no substantial influence on retention and concentration was detected. As a result, it can be concluded that the established bioanalytical approach is reliable in terms of the aforementioned key criteria. As a result, it can be employed in ordinary laboratory settings [33].

Gallic acid stability in rat plasma

Table 9 shows the results of stability analysis of gallic acid in rat plasma throughout short, long, auto sampler, bench top, and freeze-thaw periods. The percent RSD ranged from 0.61 % (long-term stability) to 1.98 % (Bench top stability). In all types of stability studies, the percent RSD was determined to be less than 2 % of the real value. Even after three freeze-thaw cycles (4 h at-30 °C), a 24-h storage period at 5 °C in the auto sample tray, and a 10-day storage period at 25 °C, no substantial degradation of gallic acid was found. According to the findings of stability research, gallic acid in rat plasma was found to be stable under varied storage conditions [34-38].

Table 7: Ruggedness study data of gallic acid in rat plasma

Chromatographic operating condition	Spiked plasma concentration (µg/ml)	Retention time (min) (n=3) (mean±SD	RSD (%)	Measured concentration (µg/ml)(n=3)(mean±SD)	RSD (%)	Accuracy (%)
Operator-1	100	3.432±0.01	0.349	97.41±1.097	1.127	97.41
Operator-2	100	3.390±0.02	0.590	97.01±1.629	1.680	97.01
Column-1	100	3.410±0.02	0.525	99.49±0.974	0.979	99.49
Column-2	100	3.359±0.03	0.895	99.08±1.89	1.904	99.08
Source of reagent and chemicals (Merk, Mumbai)	100	3.440±0.03	0.769	97.84±1.53	1.563	97.84
Source of reagent and chemicals (Qualigens Fine Chemicals, Mumbai)	100	3.417±0.05	1.473	97.38±0.80	0.822	97.38

Number of experiment, n= 3

Table 8: Robustness study data of gallic acid in rat plasma

Chromatographic parameter	Spiked plasma concentration (µg/ml)	Retention time (min) (n=3) (mean±SD	RSD (%)	Measured concentration (µg/ml) (n=3)(mean±SD)	RSD (%)	Accuracy (%)
Flow rate (-10 %)	10	3.96±0.18	4.425	10.31±0.04	0.392	103.1
	100	3.97±0.11	2.806	102.1±0.29	0.281	102.1
Flow rate (+10 %)	10	3.17±0.08	2.477	8.93±0.25	2.817	89.3
	100	3.17±0.04	1.110	95.16±0.58	0.612	95.16
Temperature (-10 %)	10	3.49±0.04	1.007	9.70±0.17	1.797	97
	100	3.48±0.03	0.923	97.23±1.45	1.494	97.23
Temperature (+10 %)	10	3.24±0.04	1.170	9.45±0.08	0.847	94.5
	100	3.26±0.05	1.542	93.39±0.82	0.874	93.39
Organic mobile phase (-5 %)	10	3.47±0.03	0.927	9.88±0.03	0.268	98.8
	100	3.46±0.04	1.016	98.73±0.35	0.356	98.73
Organic mobile phase (+5 %)	10	3.40±0.01	0.294	9.56±0.08	0.856	95.6
	100	3.41±0.03	0.738	96.87±0.60	0.622	96.87

Number of experiment, n= 3

Table 9: Stability study of gallic acid in rat plasma

Type of stability	Spiked plasma concentration (µg/ml)	Measured concentration (µg/ml) (n=3) (mean±SD)	RSD (%)	Accuracy (%)
Short term stability	10	10.01±0.08	0.75	100.1
(6 h at 25 °C)	100	95.33±1.53	1.60	95.33
Long term stability	10	9.37±0.15	1.63	93.7
(10 d at 25 °C)	100	95±0.58	0.61	95
Auto sampler stability	10	9.3±0.1	1.08	93
(24 h at 5 °C)	100	94.4±0.96	1.02	94.4
Bench top stability	10	9.58±0.19	1.98	95.8
(Old solution)	100	94.6±0.99	1.05	94.6
Bench top stability	10	9.75±0.1	1.03	97.5
(Fresh solution)	100	96.6±0.66	0.68	96.6
Freeze-thaw stability	10	9.58±0.16	1.68	95.8
(cycle 3, 4 h at-30 °C)	100	95.22±0.86	0.90	95.22

Number of experiment, n= 3

CONCLUSION

This work attempted to design and test a bioanalytical RP-HPLC method for measuring gallic acid in rat plasma. The new bioanalytical RP-HPLC method was shown to be buffer-free, straightforward, precise, accurate, sensitive, rugged, durable, and highly repeatable. The proposed approach was validated for different parameters, and the results were deemed to be acceptable. The new developed bioanalytical method is particularly suitable and applicable for the estimation of gallic acid in pharmacokinetics, bioequivalence, and therapeutic drug monitoring studies due to its smaller plasma volume, the lower limit of quantification level and detection, accuracy (SD and percent RSD found within acceptable range), cost-effectiveness, and simple preparation method. This gradient elution chromatographic method can be successfully used to determine gallic acid from complex pharmacokinetic samples containing various interfering proteins as well as extracts containing various phytoconstituents with a retention time of fewer than 20 min and without the interference of late eluted peaks.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the Principal of Bharti Vidyapeeth College of Pharmacy in Kolhapur and Satara College of Pharmacy in Satara for allowing them to conduct this research. ChromeIn, Pune Chromatographic Technology and Services is also thanked for providing HPLC analytical capabilities to the authors.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

REFERENCES

1. Naaz H, Srikanth P, Rudrapal M, Sarwa KK. Development and validation of UV spectrophotometric and RP-HPLC methods for the estimation of gallic acid in the herbal formulation of amalaki. Asian J Chem. 2020;32(10):2469-74. doi: 10.14233/ajchem.2020.22737.
2. Gao J, Hu J, Hu D, Yang X. A role of gallic acid in oxidative damage diseases: A comprehensive review. Nat Prod Commun. 2019 Aug;14(8). doi: 10.1177/1934578X19874174.
3. Brewer MS. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev Food Sci Food Saf. 2011 Jun 14;10(4):221-47. doi: 10.1111/j.1541-4337.2011.00156.x.
4. Kosuru RY, Roy A, Das SK, Bera S. Gallic acid and gallates in human health and disease: do mitochondria hold the key to success? Mol Nutr Food Res. 2018 Jan;62(1). doi: 10.1002/mnfr.201700699, PMID 29178387.
5. Su TR, Lin JJ, Tsai CC, Huang TK, Yang ZY, Wu MO. Inhibition of melanogenesis by gallic acid: possible involvement of the PI3K/Akt, MEK/ERK and Wnt/β-catenin signaling pathways in B16F10 cells. Int J Mol Sci. 2013 Oct 14;14(10):20443-58. doi: 10.3390/ijms141020443, PMID 24129178.
6. Sawa T, Nakao M, Akaike T, Ono K, Maeda H. Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor-promoter effect of vegetables. J Agric Food Chem. 1999 Feb;47(2):397-402. doi: 10.1021/jf980765e, PMID 10563906.
7. Shao D, Li J, Tang R, Liu L, Shi J. Inhibition of gallic acid on the growth and biofilm formation of escherichia coli and streptococcus mutans. J Food Sci. 2015 Jun;80(6): M1299-305. doi: 10.1111/1750-3841.12902, PMID 25974286.
8. Sorrentino E, Succi M, Tipaldi L, Pannella G, Maiuro L, Sturchio M. Antimicrobial activity of gallic acid against food-related pseudomonas strains and its use as biocontrol tool to improve the shelf life of fresh black truffles. Int J Food Microbiol. 2018

Feb 2;266:183-9. doi: 10.1016/j.ijfoodmicro.2017.11.026. PMID 29227905.

9. Oh E, Jeon B. Synergistic anti-campylobacter jejuni activity of fluoroquinolone and macrolide antibiotics with phenolic compounds. Front Microbiol. 2015 Oct 13;6:1129. doi: 10.3389/fmicb.2015.01129, PMID 26528273.
10. Fu R, Zhang Y, Peng T, Guo Y, Chen F. Phenolic composition and effects on allergic contact dermatitis of phenolic extracts Sapium sebiferum (L.) Roxb. leaves. J Ethnopharmacol. 2015 Mar 13;162:176-80. doi: 10.1016/j.jep.2014.12.072, PMID 25576898. jep.2014.12.072.
11. Korani MS, Farbood Y, Sarkaki A, Fathi Moghaddam H, Taghi Mansouri M. Protective effects of gallic acid against the chronic cerebral hypoperfusion-induced cognitive deficit and brain oxidative damage in rats. Eur J Pharmacol. 2014 Jun 15;733:62-7. doi: 10.1016/j.ejphar.2014.03.044, PMID 24726557.
12. Zakaria F, Wan Ishak WR, Wan Ahmad WAN, Safuan S, Tengku Ismail TA. Hypoglycaemic and protective effects of Benincasa hispida aqueous extract in streptozotocin-induced diabetic rats. Sains Malays. 2022 Mar 31;51(3):783-93. doi: 10.17576/jsm-2022-5103-12.
13. Kshirsagar RR, Vaidya SA, Jain V. Development and validation of a novel RP-HPLC method for the simultaneous quantification of ascorbic acid, gallic acid, ferulic acid, piperine, and thymol in a polyherbal formulation. Indian J Nat Prod Resour. 2020. doi: 10.56042/ijnpr.v11i4.30002.
14. Fernandes FHA, Batista RSdA, de Medeiros FD, Santos FS, Medeiros ACD. Development of a rapid and simple HPLC-UV method for determination of gallic acid in schinopsis brasiliensis. Revista Brasileira de Farmacognosia. 2015;25(3):208-11. doi: 10.1016/j.bjp.2015.05.006.
15. Al-Rimawi F, Odeh I. Development and validation of an HPLC-UV method for determination of eight phenolic compounds in date palms. J AOAC Int. 2015 Sep-Oct;98(5):1335-9. doi: 10.5740/jaoacint.15-010, PMID 26525252.
16. Khatri S. Bioanalytical method development and validation for the estimation of levocetirizine in blood plasma by using Rp-hplc. J Drug Delivery Ther. 2018 Oct 1;8(5-s):288-92. doi: 10.22270/jddt.v8i5-s.1977.
17. Babu R, Rao A, Rao JV. Bioanalytical method development and validation for simultaneous estimation of paracetamol and cefixime by using RP-HPLC in rabbit plasma. Orient J Chem. 2016 Mar 25;32(1):701-7. doi: 10.13005/ojc/320178.
18. Satyavert, Gupta S, Nair AB, Attimarad M. Development and validation of bioanalytical method for the determination of hydrazinocurcumin in rat plasma and organs by HPLC-UV. J Chromatogr B Analyt Technol Biomed Life Sci. 2020;1156:122310. doi: 10.1016/j.jchromb.2020.122310. PMID 32835908.
19. Bhandari R, Kuhad A, Paliwal JK, Kuhad A. Development of a new, sensitive, and robust analytical and bio-analytical RP-HPLC method for *in vitro* and *in vivo* quantification of naringenin in polymeric nanocarriers. J Anal Sci Technol. 2019 Mar 1;10(1). doi: 10.1186/s40543-019-0169-1.
20. Reddy BKK, Sekhar KBC, Mohan CK. Bioanalytical method development and validation for the simultaneous determination of vildagliptin and telmisartan in rabbit plasma using RP-HPLC. J Pharm Res Int. 2021 Feb 13:76-86. doi: 10.9734/jpri/2021/v33i131141.
21. GA, KB, Prasad K. Development and validation of bioanalytical hplc method for simultaneous estimation of cilnidipine and nebivolol in human plasma. Int J Pharm Pharm Sci. 2017 Oct 2;9(10):253. doi: 10.22159/ijpps.2017v9i10.20237.
22. Ajitha A, Sujatha K, Abbulu K. Bioanalytical method development and validation of garenoxacin mesylate in human plasma by RP-HPLC. International Journal of Research in Pharmaceutical Sciences 2019;10(2):1314-20. doi: 10.26452/ijrps.v10i2.534.
23. Moni F, Sharmin S, Rony SR, Afroz F, Akhter S, Sohrab MdH. Bioanalytical method validation of Esomeprazole by high-performance liquid chromatography with PDA detection. ACHROM. 2021 Mar 19;33(2):120-6. doi: 10.1556/1326.2020.00769.
24. Kanala K, Hwisa TN, Chandu BR, Katakam P, Khagga M, Challa BR, Khagga B. Bioanalytical method development and validation of milnacipran in rat plasma by LC-MS/MS detection and its application to a pharmacokinetic study. J Pharm Anal. 2013 Dec;3(6):481-8. doi: 10.1016/j. jpha.2013.03.009.
25. D'cruz D, Babu A, Joshy E. Bioanalytical method development and validation of ticagrelor by RP-HPLC. Int J App Pharm. 2017 May 1;9(3):51. doi: 10.22159/ijap.2017v9i3.17452.
26. Bhowmick M, Bhowmick P, Sengodan T, Thangavel S. Development and validation of bioanalytical Rp hplc method for the estimation of metoprolol tartrate in rabbit plasma after transdermal and oral administration: application in pharmacokinetic studies. J Drug Delivery Ther. 2015 Jul 15;5(4). doi: 10.22270/jddt.v5i4.1118.
27. Bhalme VM, Jumade PP, Bawankar RD, Wanjari DS, Mundhada DR. Method development, validation and stability indicating studies for simultaneous estimation of anti-hypertensive drugs from the pharmaceutical formulation by RP-HPLC. J Drug Delivery Ther. 2020 Nov 30;10(6):120-32. doi: 10.22270/jddt.v10i6.4571.
28. Kitzman D, Cheng KJ, Fleckenstein L. HPLC assay for albendazole and metabolites in human plasma for clinical pharmacokinetic studies. J Pharm Biomed Anal. 2002 Oct;30(3):801-13. doi: 10.1016/s0731-7085(02)00382-5, PMID 12367706.
29. Gültekin Y, Ozturk N, Filazi A, Deniz A, Korkmaz C, Pezİk E. Simultaneous determination of albendazole sulfoxide and praziquantel from PLGA nanoparticles and validation of new HPLC method. JRP 2022;26(6):1608-18. doi: 10.29228/jrp.252.
30. Mujewar IN, Bhusnure OG, Jagtap SR, Gholve SB, Giram PS, Savangikar AB. A review on bioanalytical method development and various validation stages involved in method development using RP-HPLC. J Drug Delivery Ther. 2019 Aug 25;9(4-s):789-95. doi: 10.22270/jddt.v9i4-s.3422.
31. Kumar S, Debnath M, Rao J, Sankar D. A new bioanalytical method development and validation for simultaneous estimation of esomeprazole and naproxen in human plasma by using RP-HPLC. Br J Pharm Res. 2014 Jan 10;4(19):2312-27. doi: 10.9734/BJPR/2014/10918.
32. Kumar SA, Debnath M, Rao JV, Sankar DG. New validated stability-indicating Rp-HPLC method for simultaneous estimation of atorvastatin and ezetimibe in human plasma by using PDA detector. Adv Pharm Bull. 2015 Sep;5(3):385-91. doi: 10.15171/apb.2015.053, PMID 26504761.
33. Ramesh D, Habibuddin M. Development and validation of Rp-hplc method for the determination of alvimopan in rat plasma. Int J Pharm Pharm Sci. 2018 Oct 1;10(10):124. doi: 10.22159/ijpps.2018v10i10.29001.
34. Gurav P, Damle M. Bioanalytical method for estimation of teriflunomidein human plasma. Int J Pharm Pharm Sci. 2022 Sep 1:19-23. doi: 10.22159/ijpps.2022v14i9.45151.
35. Sellappan M, Devakumar D. Development and validation of rp-hplc method for the estimation of escitalopram oxalate and flupentixol dihydrochloride in combined dosage form and plasma. Int J Pharm Pharm Sci. 2021 Feb 1:61-6. doi: 10.22159/ijpps.2021v13i2.30158.
36. Kondeti HP, Sankar Dannana G. Development and validation of a stability-indicating rp-hplc method for the estimation of metformin, saxagliptin, and dapagliflozin. Asian J Pharm Clin Res. 2022 Feb 4:72-7. doi: 10.22159/ajpcr.2022.v15i3.42117.
37. AS, Prasad Panigrahy U. Stability indicating method development and validation of fimasartan by reverse-phase high-performance liquid chromatography in bulk and pharmaceutical dosage form. Asian J Pharm Clin Res. 2021 Feb 7:138-46. doi: 10.22159/ajpcr.2021.v14i2.39919.
38. Shah SK, Dey S, De S. Simultaneous determination of atorvastatin and atenolol in rabbit plasma by RP-HPLC method and its application in pharmacokinetic study. Int J Curr Pharm Sci 2022;14:72-8. doi: 10.22159/ijcpr.2022v14i2.1968.