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Research Article

STABILITY-INDICATING RP-HPLC METHOD DEVELOPMENT FOR THE ESTIMATION OF GINGEROL

Rudra Pratap Singh [1], H.V. Gangadharappa [1]*, Ramakant M. Narke [2], Pooja V. Jadhav [3]

[1]Department of Pharmaceutics, JSS College of Pharmacy, Jagadguru Sri Shivarathreeswara University, Mysuru, India

[2]Department of Pharmaceutics, Sinhgad College of Pharmacy, Savitribai Phule University, Vadgaon (BK) Pune, India

[3]Department of Pharmaceutics, Rasiklal M Dhariwal Institute of Pharmaceutical Education & Research, Pune, India

*Corresponding Author Email: hvgangadharappa@jssuni.edu.in

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ABSTRACT

Gingerol is an analgesic, hypoglycemic, hepatoprotective, immune-stimulant, anti-inflammatory agents. In traditional medicine, gingerol has been proven plenty of pharmacological properties such as bactericidal, microbicidal, potent antioxidant, antifertility, tuberculostatic and anticancer. The key objective of this work was to develop, validate and estimate gingerol by RP-HPLC method. According to the ICH guidelines, a stability-indicating RP-HPLC method was developed and validated. In this method, reverse phase enable Cosmosil C_{18} column (250 × 4.6 mm, 5µm) was used in isocratic mode. The mobile phase consisted of acetonitrile: methanol: water (52: 8: 40 v/v) delivered at a flow rate of 1.0 mL min^{-1}. The elute injection volume was 20 µL that was analyzed by a UV detector at 280 nm. Linearity range of the concentration of gingerol was 20 µg mL^{-1}-140 µg mL^{-1} and the correlation coefficient R^2 value was found to be 0.997 ± 0.11. Recovery was found to be in the range of 98.16 -100.07 %. The limit of detection (LOD) of gingerol was found to be 2.23 µg mL^{-1} and limit of quantitation (LOQ) was 5.02 µg mL^{-1}. Gingerol was subjected to stress conditions including acidic, alkaline, neutral, oxidation, and dry heat degradation. Gingerol was more sensitive to alkaline, dry heat and oxidative degradation and it is stable at acidic conditions. The method was validated according to ICH guidelines.

Keywords: Stability-indicating, Reverse phase, HPLC method, Validation, Gingerol.

INTRODUCTION

Gingerol [5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone] is a yellowish to buff, spicy and pungent crystalline powder. It is an important phyto-constituent of ginger plant (*Zingiber officinale*, Family: Zingiberaceae). It has molecular formula $C_{17}H_{26}O_4$ and molecular weight 294.38 g/mol[1-3]. The chemical structure of gingerol is shown in Figure 1. Gingerol is insoluble in water, soluble in ethanol, methanol and other organic solvents[4-5]. Gingerol is used as an an analgesic, hypoglycemic, hepatoprotective, immune-stimulant, anti-inflammatory agents. It also has antibacterial, antimicrobial, antiparasitic, antioxidant, antifertility, tuberculostatic and anticancer properties[3-5].

The exact mechanism of action of gingerol in diseases management is unknown till now but it plays a major role to prevent diseases. Some examples of mechanism of actions of gingerol are describing that confirms it's a potent and useful agent. Gingerol prevents the damage of macromolecules, caused by the free radicals/oxidative stress and shows antioxidant activity. It also inhibits COX and inhibition of nuclear factor κB and confirms the anti-inflammatory processes. Gingerol also acts as antitumor via modulation of genetic pathways such as activation tumour suppressor gene, modulation of apoptosis and inhibition of VEGF. It also shows antimicrobial and other biological activities[3,5-6].

MATERIALS AND METHODS

Chemicals

Gingerol was procured from Sigma Aldrich chemicals Pvt. Ltd, Bangalore, India. HPLC grade acetonitrile and other solvents (Mfg by: Merck Ltd., Mumbai) were used.

Instrument

The current research work was performed on a Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with reverse phase C_{18} column (250 mm x internal diameter 4.6 mm x particle size 5µm). Sample injection was done via a 20 µL loop. UV- Visible detector (Shimadzu SPD-20A) was used for detection purpose and output signal was monitored and integrated by LC-solution software.

Preparation of Mobile Phase and Stock Solution

Altered ratios of mobile phase compositions were tried to optimize the RP-HPLC parameters but to get a acceptable separation and good peak symmetry for gingerol, a mobile phase composition of acetonitrile, methanol and water were used in the ratio of 52: 8: 40 (v/v). Before to use the mobile phase, it was filtered under vacuum through 0.22 µm nylon membrane filter and degassed by using sonicator.

The standard stock solutions of gingerol was prepared by dissolving weighed 10 mg in 10 mL of methanol in 10 mL volumetric flask with shaking and then volume was made up to the mark of 10 mL with the methanol to get standard stock

solution of 1000 µg mL^{-1} respectively. The stock solution were degassed by using sonicator and filtered through a 0.22 µm nylon membrane filter. From this stock solution different aliquots were prepared.

Preparation of Standard Calibration curves of Gingerol

A reverse phase 4.6 × 250 mm Cosmosil C_{18} HPLC column with 5 µm (particles) packing was used as a chromatographic column for the estimation of gingerol. The column oven temperature and the HPLC system were maintained at 25 ± 2ºC. The mobile phase Acetonitrile: methanol: water (52: 8: 40 v/v) was delivered at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 µL. Elute was analyzed by a UV detector.

The standard calibration curve was prepared from the stock solutions (1000 µg mL^{-1}). The different aliquots were pipetted into a series of 10 mL volumetric flasks and the volume was made up to the mark with methanol to obtain a set of solutions of gingerol having concentration range 20-140 µg mL^{-1} each. 20 µL solutions were injected into HPLC system and chromatograms were recoded. The peak areas versus concentrations were plotted for calibration curve.

Validation of the Proposed Method

The proposed methods such as linearity, precision, repeatability and reproducibility, accuracy, limit of detection (LOD) and limit of quantification (LOQ) was validated according to the International Conference on Harmonization (ICH) guidelines[7-15].

Linearity

To determine the linearity of gingerol, a stock solution of gingerol (1000 µg mL^{-1}) was prepared in mobile phase and the linear response was observed over a range of 20-140 µg mL^{-1} by HPLC and the calibration curve was plotted between peak areas versus concentrations (Figure 2).

Precision

Method precision of experiment was performed by preparing the standard solution of gingerol (80 µg mL^{-1}) from the stock solution of gingerol (1000 µg mL^{-1}) for six times without changing the parameters of the proposed method. The results were reported in terms of percent relative standard deviation. The intra-day and inter-day precision of the proposed method was determined and analyzed at three different concentrations (20 µg, 80 µg, and 140 µg) on 3 times on the same day and on 3 different days over a period of 1 wee

Accuracy

The accuracy of the proposed method was determined by calculating the recovery of gingerol by the standard addition method. Known amounts of standard solutions of gingerol was added at 80 %, 100 % and 120 % w/w level to pre analyzed sample solutions of gingerol.

Detection limit and quantification limit

The limit of detection (LOD) and the limit of quantification (LOQ) of the gingerol were derived as per ICH guidelines using following equation:

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

Where σ = Standard deviation of the y-intercept and S = Mean slope of the calibration curve.

Force Degradation Studies

Forced degradation or stress test of gingerol was performed under acidic, alkaline, oxidative and dry heat conditions. Weighted amount of gingerol (10 mg) was transferred in 10 ml volumetric flask and dissolved in methanol to get 1000 µg mL^{-1} concentration which was used for forced degradation[7-15].

Acidic degradation

For acid induced degradation, the stock solution (10 mL) of gingerol (1000 µg mL^{-1}) was treated with 1 mL of 0.1M HCl and this reaction mixture was refluxed at 70ºC for about 1 h. After 1 h, the solution was neutralized using 1 mL of 0.1M NaOH solution and then injected into HPLC system.

Alkaline degradation

Forced degradation in alkaline condition was performed by treating of stock solution (10 mL) of gingerol (1000 µg mL^{-1}) with 1 mL of 0.1M NaOH and this reaction mixture was refluxed at 70ºC for about 1 h. After 1 h, the solution was neutralized using 1 mL of 0.1M HCl solution and then injected into HPLC system.

Oxidative degradation

For oxidative degradation, the Stock solution (10 mL) of gingerol (1000 µg mL^{-1}) was transferred to separate round bottom flask, to this 10 ml of 3% hydrogen peroxide was added and this reaction mixture was kept for 1 h at 70ºC. Sample was diluted and mixed well and injected into HPLC system.

Dry heat degradation

In dry heat degradation condition, accurately weighed 10 mg of gingerol spread in a petri dish and kept in oven at 105ºC for about 8 h and then gingerol was diluted with mobile and filter through 0.45µm filter and injected into HPLC system.

RESULTS

Method development

Chromatographic condition

Numerous numbers of chromatographic conditions were examined to optimize the estimation of gingerol. Optimization of RP-HPLC chromatographic conditions are shown in Table 1.

Selection of mobile phase

Several mobile phase compositions were tried to validate RP-HPLC method and to estimate gingerol. A satisfactory separation, good peak symmetry, better reproducibility and repeatability of gingerol were obtained with a mobile phase comprising of Acetonitrile: methanol: water (52: 8: 40 v/v) at a flow rate of 1.0 mL min^{-1}. Quantification was achieved with UV Visible detector at 280 nm.

Proposed method validation

Linearity

In proposed validation methods, retention time of blank (Figure 3) and gingerol was recorded and it was found at 7.48 min as shown in Figure 4. The calibration graphs for gingerol was constructed by plotting the area versus their corresponding concentrations, good linearity was found over the range of 20-140 µg mL^{-1} for gingerol with co-efficient of correlation (R^2) =

0.997 ± 0.11. The regression characteristics and validation parameters are reported in Table 2.

Precision and Accuracy

The repeatability, intra-day and inter-day precision of gingerol are summarized in Table 3, Table 4 and Table 5, respectively. The accuracy of gingerol was determined by recovery study and the mean recoveries were found 100.39 ± 0.94 % respectively for gingerol by the standard addition method shown in Table 6.

Limit of detection and limit of quantitation

The limit of detection and limit of quantitation of gingerol was found to be 2.23 µg mL^{-1} and 5.02 µg mL^{-1} respectively. The summary of proposed validation parameters of gingerol was shown in Table 7.

Forced degradation study
Acidic degradation

For this study, 0.1 M HCl and 0.1M NaOH was used. The stock solution of gingerol was treated with 1 mL of 0.1M HCl and this reaction mixture was refluxed at 70ºC for about 1 h. After 1 h, the solution was neutralized using 1 mL of 0.1M NaOH solution and then injected into HPLC system. The chromatogram of acidic degradation study of gingerol results are shown in Figure 5.

Alkaline degradation

Forced degradation in alkaline condition was performed by treating of stock solution of gingerol with 1 mL of 0.1M NaOH and this reaction mixture was refluxed at 70ºC for about 1 h. After 1 h, the solution was neutralized using 1 mL of 0.1M HCl solution and then injected into HPLC system. The chromatogram results are shown in Figure 6.

Oxidative degradation

For oxidative degradation, the Stock solution of gingerol was transferred to separate round bottom flask, to this 10 ml of 3% hydrogen peroxide was added and this reaction mixture was kept for 1 h at 70ºC. Sample was diluted and mixed well and injected into HPLC system. The chromatogram results are shown in Figure 7.

Dry heat degradation

In dry heat degradation condition, accurately weighed 10 mg of gingerol spread in a petri dish and kept in oven at 105ºC for about 8 h and then gingerol was diluted with mobile and filter through 0.45µm filter and injected into HPLC system. The chromatogram results are shown in Figure 8.
The results of forced degradation study of gingerol under different degradation conditions are summarized in Table 8.

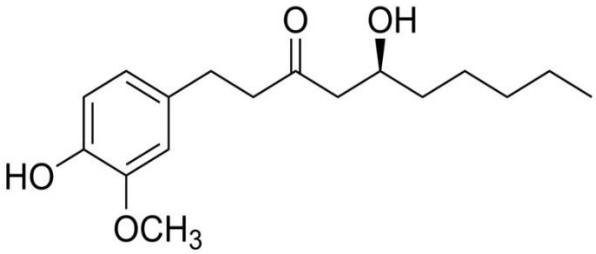

Figure 1: Chemical structure of Gingerol

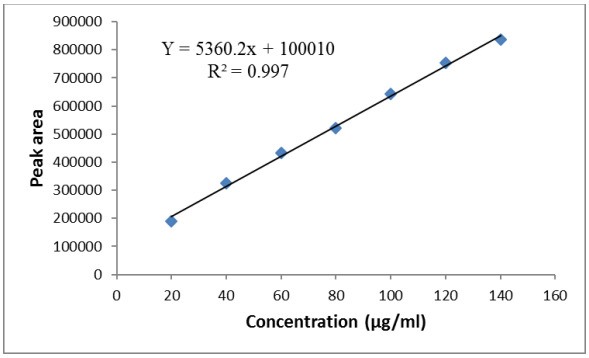

Figure 2: Standard calibration curve of Gingerol

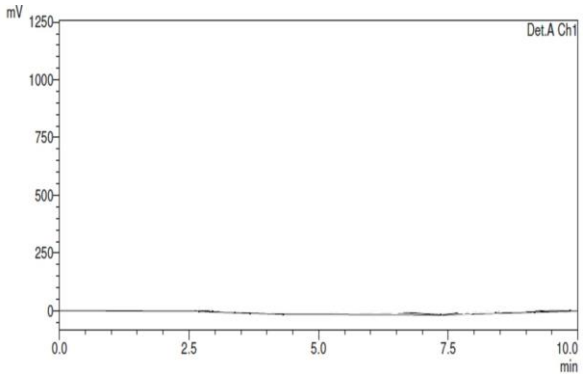

Figure 3: Chromatogram of blank sample

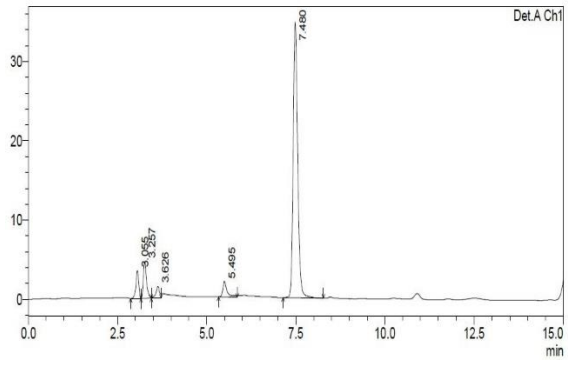

Figure 4: Chromatogram of Gingerol

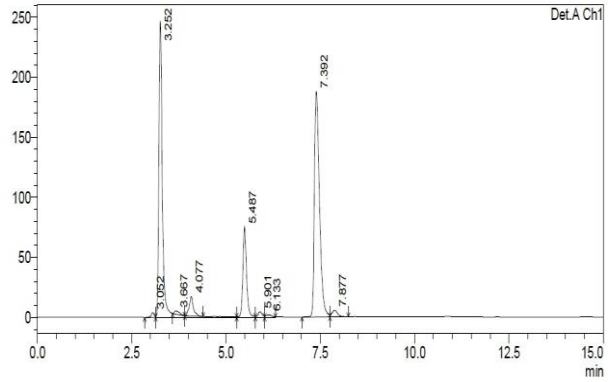

Figure 5: Chromatogram of Acid degradation of Gingerol

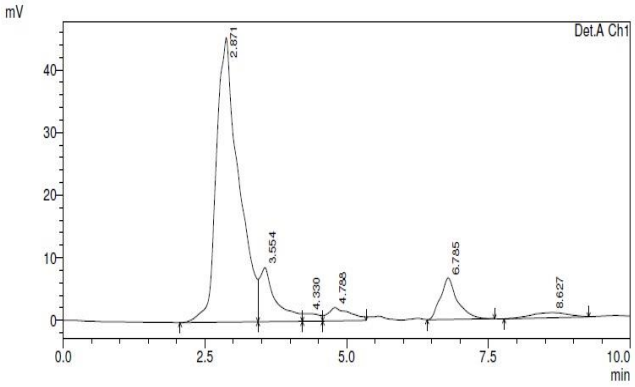

Figure 6: Chromatogram of Alkaline degradation of Gingerol

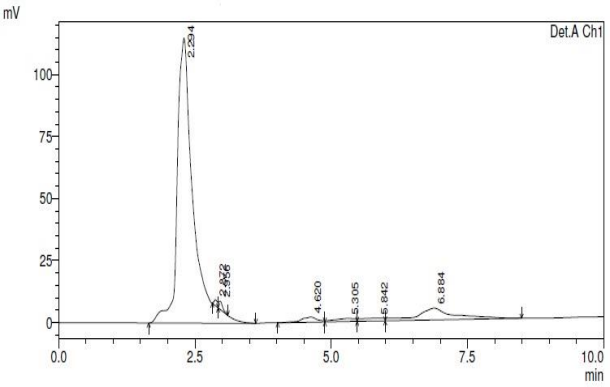

Figure 7: Chromatogram of Oxidative Degradation of Gingerol

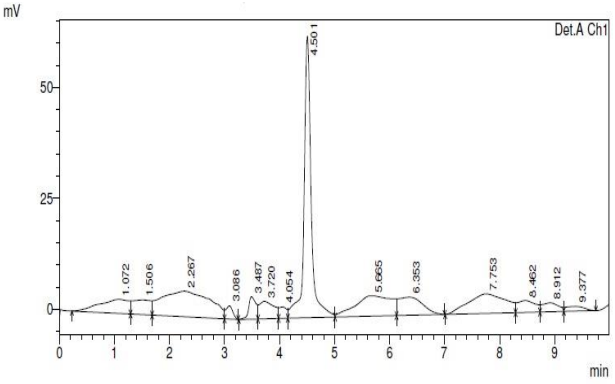

Figure 8: Chromatogram of Dry heat Degradation of Gingerol

Table 1: Optimization of RP-HPLC method

Method parameter	Optimized value
Column	C_{18} (250 mm x 4.6 mm x 5µm)
Wavelength of detection	280 nm
Mobile phase	Acetonitrile: methanol: Water (52: 8: 40 v/v)
Pump mode	Isocratic
Flow rate	1.0 mL min^{-1}
Run time	10 minutes
Volume of injection	20 µL
Temperature	25 ± 2ºC

Table 2: Linear regression data for calibration curves

Parameters	Gingerol
Linearity range µg/ml	20-140
r^2 ± SD*	0.997 ± 0.11
Slope ± SD*	5360.2 ± 2169.02
Intercept ± SD*	100010 ± 0862.15
Y= mx + c	Y = 5360.2x + 100010

Table 3: Repeatability studies

Precision	Amount (µg mL^{-1})	Area	Mean Area ± SD*	% RSD*
Repeatability (n=6)	80	520896	520327.33 ± 137.02	1.32
	80	519033		
	80	520255		
	80	520417		
	80	520691		
	80	520672		

Table 4: Intraday precision studies

Precision	Amount (µg mL^{-1})	Area	Mean Area ± SD	% RSD*
Intra-day (n=3)	20	190163	190137.67 ± 721.01	0.382
	20	190098		
	20	190152		
	80	520896	520806.31 ± 304.73	0.751
	80	520715		
	80	520808		
	140	836055	836013 ± 195.05	0.989
	140	835983		
	140	836001		

Table 5: Inter-day precision studies

Precision	Amount (µg mL^{-1})	Area	Mean Area ± SD	% RSD*
Inter-day (n=3)	20	190163	190137.67 ± 721.01	0.382
	20	190098		
	20	190152		
	80	520896	520806.31 ± 304.73	0.751
	80	520715		
	80	520808		
	140	836055	836013 ± 195.05	0.989
	140	835983		
	140	836001		

Table 6: Results for accuracy studies

Level (n=3)	Amount Added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% recovery
80%	50	49.0857	98.1714
		49.0731	98.1462
		49.0922	98.1844
100%	75	75.0102	100.0136
		74.9096	99.8794
		75.0028	100.0037
120%	100	100.210	100.210
		100.017	100.017
		100.003	100.003

Table 7: Summary of the validation parameters

Parameters (Unit)	Gingerol
Linearity range (µg mL^{-1})	20 -140
Correlation Coefficient ± SD, n = 6	0.997 ± 0.11
Precision (%RSD)	
Inter day % RSD	0.989
Intraday % RSD	0.382
Recovery (%), n=3	100.076
Limit of detection (µg mL^{-1})	2.23
Limit of quantitation (µg mL^{-1})	5.02

Table 8: Summary of forced degradation studies of gingerol

Stress conditions	Time (h) and Temperature (ºC)	Amount of gingerol degraded (%)	Amount of gingerol recovered (%)
Acid (0.1 M HCl)	1 h at 70°C	11.74	88.26
Alkali (0.1M NaOH)	1 h at 70°C	58.30	41.70
Oxidative H_2O_2 (3%)	1 h at 70°C	62.46	37.54
Dry heat	8 h at 105°C	39.07	60.93

DISCUSSION

The present research work describes a simple and sensitive RP-HPLC method for the estimation of gingerol. Various ratio of Acetonitrile: methanol: water mobile phase were tried and found that (52: 8: 40 v/v) proportion of the mobile phase favored the separation and elution of gingerol. In RP-HPLC method, the linearity range was in between 20-140 µg mL^{-1} for gingerol with co-efficient of correlation (R^2) = 0.997 ± 0.11. The results of accuracy and precision were in good agreement with the threshold limits of validation parameters as per ICH guidelines. The intra-day and inter-day variability and accuracy results of gingerol were found in acceptable limit. The LOD and LOQ values of gingerol were found 2.23 µg mL^{-1} and 5.02 µg mL^{-1} respectively.

In this research, gingerol was applied for the stability studies and it subjected to different stress conditions and evaluated by

HPLC, where gingerol was more sensitive to alkaline (2.87 min), dry heat (4.50 min) and oxidative degradation (2.29 min). It was found to be stable under the acidic condition (7.39 min).

CONCLUSION

The developed and validated RP-HPLC method for gingerol estimation was found suitable and accurate. The Simplicity of the method, economical nature and low limit of detection and quantification makes the method superior to the other reported HPLC methods.

LIST OF ABBREVIATIONS

% - Percent
λ_{max}- Wavelength of maximum absorbance
°C- Degree celcious
µg - Micro gram
h - Hour
LOD - limit of detection
LOQ - limit of quantification
Mg - Milligram
Min - Minute
mL - Milliliter
µL - Microliter
r^2- Regression coefficient
RP-HPLC- Reverse phase-high performance liquid Chromatography
SD - Standard deviation
t_R - Retention time
UV - Ultra Violet

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