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Original Article

DETERMINATION OF 10-GINGEROL IN INDIAN GINGER BY VALIDATED HPTLC METHOD OF SAMPLES COLLECTED ACROSS SUBCONTINENT OF INDIA

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ABSTRACT

Objective: A simple, sensitive, precise, and accurate stability indicating HPTLC (high-performance thin-layer chromatography) method for analysis of 10-gingerol in ginger has been developed and validated as per ICH guidelines.

Methods: The separation was achieved on TLC (thin layer chromatography) aluminum plates pre-coated with silica gel $60F_{254}$ using n-hexane: ethyl acetate 55:45 (%, v/v) as a mobile phase. Densitometric analysis was performed at 569 nm.

Results: This system was found to have a compact spot of 10-gingerol at R_F value of 0.57±0.03. For the proposed procedure, linearity (r^2 = 0.998±0.02), limit of detection (18ng/spot), limit of quantification (42 ng/spot), recovery (ranging from 98.35%–100.68%), were found to be satisfactory.

Conclusion: Statistical analysis reveals that the content of 10-gingerol in different geographical region varied significantly. The highest and lowest concentration of 10-gingerol in ginger was found to be present in a sample of Patna, Lucknow and Surat respectively which inferred that the variety of ginger found in Patna, Lucknow are much superior to other regions of India.

Keywords: Ginger, 10-gingerol, HPTLC, Validation

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INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) Fam. Zingiberaceae is a valued medicinal crop, and it has been used as a spice for over 2000 y [1]. This plant is cultivated in many tropical and subtropical countries including China, India, Nigeria, Australia, Jamaica, and Haiti. Among which, China and India are the world's leading producers of ginger [2]. Different cultivars of ginger are growing in different areas in India which were named after the localities from where they are cultivated or collected. Globally gingerols are gaining importance as a budding source of new drug(s) to combat a variety of ailments viz. antitumor, antioxidant, anti-inflammatory, antiapoptotic, cytotoxic, anti-proliferative and anti-platelet activities [3-6].

Ginger contains a number of different pungent and biologically active compounds mainly 6-gingerol, 10-gingerol, 8 gingerols, 6-shogaol, zingerone and paradol [7]. The carbon chain length has also played a significant role in making 10-gingerol (fig. 1) as the most potent antioxidant and anti-inflammatory properties among all the gingerols [8]. This component has also been reported to possess antimicrobial and antifungal properties as well as several pharmaceutical properties [9].

Because of the widespread use of ginger as a spice, dietary supplements, tea, cream, household remedy, as well as an ingredient of various herbal formulations, it is important to know the variety of ginger which contributes the highest yield. Several analytical techniques have been reported for the analysis of gingerols in ginger like high-performance liquid chromatography (HPLC) methods [10, 11], GC/MS (Gas-chromatographic–mass-spectrometric) methods [12]. UPLC-MS/MS (Ultra high-performance liquid chromatography and mass spectroscopy) method has also been developed for the analysis of gingerols [13].

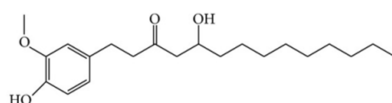

Fig. 1: Chemical structure of 10-gingerol

The literature showed that researcher only evaluated the content of gingerols in different formulations of ginger, but very few or no study has ever been reported to develop a method for quantification of 10-gingerol in different cultivars of ginger collected across subcontinent of India. Keeping in view of their biological importance and its diversity in nature, a sensitive, rapid HPTLC method was developed and validated for the estimation of 10-gingerol in different accessions of ginger. Therefore the objective of this investigation was, to develop a simple, economical, selective, precise, and sensitive HPTLC technique for analysis of 10-gingerol in its methanolic extract of different cultivars of Indian gingers.

MATERIALS AND METHODS

Chemicals, standards, and samples

Samples of *Z. officinale* were collected from different geographical regions of India (table 5). The plant specimens (GP1, GL2, GDD3, GE4, GB5, GN6, GT7, GDL8, GC9, GB10, GG11 and GS12) were identified by taxonomist Professor M. P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. The analytical standard of 10-gingerol (purity>98%) was obtained from Ms. chroma dex, anna.

Sample preparation

Weighed the accurate amount of 5 g of the dried whole rhizome of *Z. officinale* and then refluxed it for 1 h with 100 ml methanol on a water bath and filtered through Whatman filter paper (No. 42). The marc left out was refluxed again for three times with 50 ml of methanol for 1 h and filtered. The filtrates were combined and concentrated to 25 ml in a rotary vacuum evaporator, and the resulting solution was used as a test solution [14].

TLC instrumentation and conditions

The HPTLC (CAMAG, Switzerland) system, made up of a Linomat IV sample applicator fitted with a 190microliter syringe, a CAMAG twin-trough plate development chamber, CAMAG TLC Scanner 3, and Wincats integration software was used. Aluminum backed plates coated with 0.2 mm layers of silica gel 60 F254 (E. Merck, Germany),

prewashed with methanol, were used. A constant application rate 150 nL/s was employed. The composition of TLC was optimized using different solvents of varying polarity. A good resolution was achieved using n-hexane: ethyl acetate 55:45 (% v/v) a mobile phase. The plate was visualized with the use of anisaldehyde sulphuric acid reagent. The plate was immersed in the reagent for 1 sec then heated at 105 °C for 10 min and then scanned at 569 nm, using a Camag TLC scanner in absorbance mode and the deuterium lamp. The slit dimensions were 4.00×0.45 mm and the scanning speed was 20 mm/s.

Preparation of standard solution

Accurately weighed 1 mg of 10 gingerols and dissolved in 1 ml of methanol. 1 ml of this solution was diluted with methanol to 20 ml, which gives 50 µg/ml equivalent of a standard stock solution of 10-gingerol. Dilution was made in the different range from the stock and was spotted on TLC plate in six (n=6) to obtain final concentration 50-500 ng/spot and the concentration was plotted against the area.

Method validation

The developed method is validated as per the ICH guidelines [15]. Method validation is carried out to confirm that the analytical method employed for this specific analysis is suitable for its intended use. Results from method validation can be used to check its quality, reliability, and consistency. The method was validated by determining linearity, precision, accuracy, limits of detection (LOD), limits of quantification (LOQ), and recovery.

Precision and accuracy

The intra-day precision and accuracy of the assays were evaluated by performing replicate analyses (n = 6) of QC samples (100, 200, 300 and 400 ng/spot). The inter-day precision and accuracy of the assay were determined by repeating the intra-day assay on three different days. Precision was expressed as the percentage coefficient variation (CV, %) of measured concentrations for each calibration level, whereas accuracy was expressed as percent recovery.

Sensitivity and linearity

In order to estimate detection (LOD) and quantification (LOQ) limits, we spotted blank methanol (n = 6) following the same method as explained under the section of chromatographic conditions and the standard deviation (σ) of the magnitude of analytical response was determined. The LOD was expressed as (LOD = 3.3σ/slope of 10-gingerol calibration curve), whereas LOQ was expressed as (LOQ = 10σ/slope of 10-gingerol calibration curve).

RESULTS AND DISCUSSION

Selection and optimization of mobile phase

For the selection of optimum mobile phase at first hexane: ethyl acetate of varying ratios were investigated. The mobile phase hexane: ethyl acetate (55:45 v/v) gave a sharp and well-defined peak at R_F value of 0.57±0.03 for 10 gingerols (fig. 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature. For determination of the linearity curves of area vs concentration, different amounts of a stock solution of 10-gingerol was applied on the HPTLC plate and analyzed. HPTLC peak of Lucknow sample was shown in fig. 3.

Calibration curve

Calibration was linear in the concentration range 50–500 ng. The linear regression equation was Y=11.62x+1640.38, while the correlation coefficients (r2) was 0.9987±0.02, with high reproducibility and accuracy (table 1).

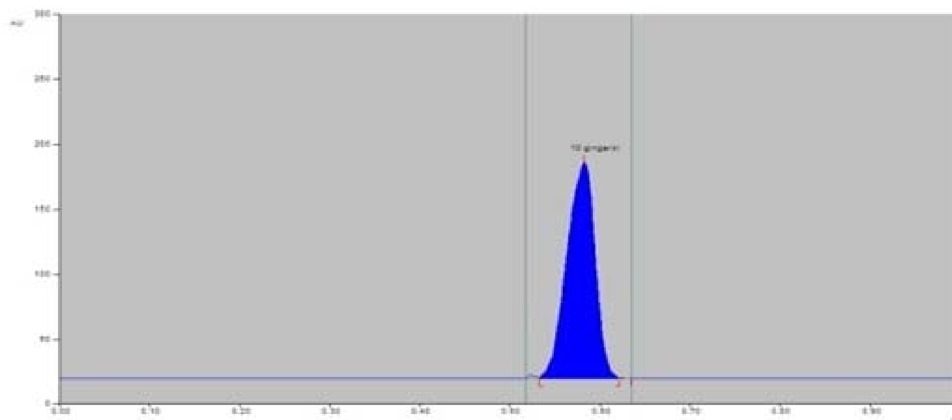

Fig. 2: HPTLC densitogram of standard 10-gingerol at R_F 0.57±0.03

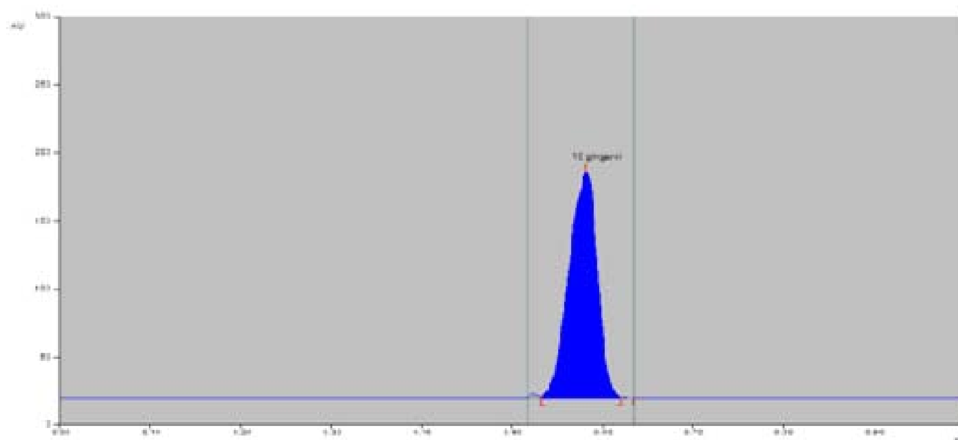

Fig. 3: HPTLC densitogram of Lucknow sample of 10-gingerol at R_F 0.57±0.06

Table 1: Linear regression data for the calibration plot (n=6)

Parameters	Observation
Linearity range (ng/spot)	50-500
Correlation coefficient (r2)	0.998±0.02
Regression equation	Y = 11.62x+1640.38
Slope±SD	11.62±0.312
Intercept b±SD	1640.38±0.997
LOD (ng/spot) (Limit of detection)	18
LOQ (ng/spot) (Limit of quantification)	48

The detection limit of 10-gingerol was determined by plotting a series of concentrations on the plate and scanning at 569 nm. The lowest amount of 10–gingerol which could be detected (LOD) was 18 ng/spot. The lowest amount of 10-gingerol which could be quantified (LOQ), was found to be 48ng/spot (table 1).

Validation of method

Recovery studies

The proposed method, when used for estimation of 10-gingerol after spiking with 50, 100 and 150% of the additional drug, afforded recovery ranging from 98.35%–100.68% for 10-gingerol was obtained as listed in table 2. The %RSD of recovery of 10–gingerol was ranged from 0.14%–0.34% (table 2).

Precision and accuracy

The intra-and inter-day precision, as the coefficient of variation (CV, %) and accuracy of the assay determined at 10-gingerol concentration of 100, 200, 300, and 400 ng/spot have been summarized in (table 3). The intra-day precision (n = 6) was ≤ 1.62%. The inter-day precision over three different days was ≤ 1.98%. The intra-day and inter-day accuracy were in the range of 98.60%–100.50% and 98.37%–99.73%, respectively. The repeatability of the method was studied by assaying six samples of 10-gingerol at same concentration under the same experimental conditions. The values were within the acceptable range, and so we concluded that the method was accurate, reliable and reproducible (table 3).

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and %RSD was found to be in the acceptable range. The % RSD (0.85-1.87) obtained after introducing small deliberate changes in the developed HPTLC method indicated the robustness of the method (table 4).

Table 2: Recovery study of 10-gingerol

Excess drug added to the analyte (%)	Theoretical content(ng)	%Recovery	%RSD
0	100	98.98	0.34
50	150	99.56	023
100	200	98.04	0.18
150	250	100.25	0.14

Table 3: Intra and inter day precession of HPTLC method (n=6) for 10-gingerol

Nominal concentration/ng per spot	Concentration found[a]/ng per spot	Precision[b] (CV, %)	%Accuracy[c]
Intra-day			
100	98.65	1.62	98.65
200	198.65	1.32	99.32
300	296.87	1.12	98.95
400	406.10	1.32	101.50
Inter-day			
100	99.73	1.98	99.73
200	197.56	1.04	98.78
300	295.12	1.23	98.37
400	398.34	1.98	99.58

[a]Mean of six determinations (n = 6), [b]Precision as coefficient of variation (CV, %) = standard deviation divided by concentration found x100, [c]% Accuracy = concentration found/nominal concentration x 100

Table 4: Robustness of the method (n=6) for 10-gingerol

10-gingerol	Mobile phase composition	
Amount (ng per spot)	n-hexane: ethyl acetate 50:50 (%, v/v) % RSD	n-hexane: ethyl acetate 55:45 (%, v/v) % RSD
200	1.54	0.89
400	0.85	1.87

LOD and LOQ

The calibration curve in this study was plotted between the amount of analyte versus average response (peak area) and the regression equation was obtained Y =11.62x+1640.38 over the concentration range 50-500 ng/spot with respect to the peak area with a regression coefficient of 0.998. Limit of detection (LOD) and limit of quantification (LOQ) was calculated by the method as described in validation section and was found to be 18 and 42 ng respectively, which indicates the ample sensitivity of the method. Different samples of gingerols in ginger were observed and calculated (table 5). The proposed method is simple, precise, specific, accurate, less time consuming and cost effective. Statistical analysis proved that the method is evitable for the analysis of 10-gingerol. HPTLC method

is helpful in determining the qualitative and quantitative analysis of herbal and dietary supplements, nutraceuticals, and various types of medicines. This method can also be used in quality control and in purity checks, in the detection and identification of pharmaceutical raw materials, drugs and their metabolites in biological media. HPTLC method is also a very influential tool for identification of the presence of adulterants in herbal products based on the distinguishing image produced and much useful for determining the presence and the quantification of both unintended substitutions as well as deliberate adulteration of drugs [16]. Hasan and Saleh [17] reported the different contents of 10 gingerols in the ginger extract, ginger-containing dietary supplements, teas and commercial creams but these data only showed the content of 10 gingerols in three different types of preparations. In this proposed experiment different accessions have been studied and reported that highest and lowest percentage of 10-gingerol was found to be in a sample of Lucknow, Patna (east) and Surat (west) samples respectively. The experimental result shows that the variety of *Z. officinal* of Patna and Lucknow samples are superior to other regions. This experimental result endorsed the Patna and Lucknow variety of ginger are of best quality among all varieties found in India. These results are also confirmatory to finding of Ashraf *et al.* by UPLC-MS method.

Table 5: Concentration (mg/g) of 10-gingerol in different samples of *Z. officinale* by HPTLC method

S. No	Samples	10-gingerol content(mg/g)
1	Patna (Bihar)	0.43±0.013
2	Lucknow (Uttar Pradesh)	0.43±0.021
3	Dehradun (Utrakhand)	0.35±0.011
4	Erode (Tamilnadu)	0.39±0.121
5	Bangalore (Karnataka)	0.35±0.012
6	Nashik (Maharashtra)	0.43±0.014
7	Trivandrum (Kerala)	0.39±0.105
8	Delhi (Delhi)	0.34±0.101
9	Chandigarh (Punjab)	0.28±0.011
10	Bhopal (M. P.)	0.34±0.021
11	Guwahati (Assam)	0.41±0.012
12	Surat (Gujrat)	0.21±0.101

CONCLUSION

The HPTLC method for quantitating 10-gingerol is simple, accurate, reproducible and sensitive and is applicable to the analysis of a wide variety of ginger-containing products. In this experimental results showed that highest and lowest percentage of 10-gingerol was found to be in a sample of Lucknow, Patna, and Surat samples respectively. These variations could be due to different environmental and soil conditions of different areas. The method established in this study could be used for the quality control of herbal medicine derive from ginger species. From this experiment, it is concluded that variety of *Z. officinale* of Patna and Lucknow are superior to other regions and these could be used for large-scale production. This research could provide important input for making strategies for cultivation of this plant.

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CONFLICT OF INTERESTS

Declared none

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