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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF 6-GINGEROL IN HERBAL FORMULATIONS AND EXTRACTS

K. Senthil Kumar [1], B. Manasa [1], Kaliloor Rahman [1] and B. Sudhakar [2]

Sri Adichunchanagiri College of Pharmacy, B.G. Nagara- 571 448, Karnataka, India
The Himalaya drug company, Bangalore, Karnataka, India

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Correspondence to Author:

Senthil kumar K

Sri Adichunchanagiri College of Pharmacy, B.G. Nagara- 571 448, Karnataka, India

E-mail: senthilsomesh2005@gmail.com

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ABSTRACT

A simple, selective, precise and reliable HPTLC method of analysis has been developed and validated for estimation of 6-gingerol in extracts of dried rhizomes of *Zingiber officinale* and marketed formulations containing Ginger. The method employed TLC aluminium plates precoated with silica gel 60F 254 as stationary phase. The solvent system consisting of n-hexane:ether (40:60v/v). Densitometric analysis was carried out in absorbance mode at 280nm. The method was validated in terms of linearity, precision and accuracy. The LOD and LOQ were 60 and 100ng/spot respectively for 6-gingerol. The average percentage recovery of 6-gingerol was found to be 99.93%. The developed method can be successfully used for identification and quantification of 6-gingerol in extracts and formulations. The method is also suitable for rapid and simple authentication and comparison of differences among samples with identical plant resources but different geographic locations.

INTRODUCTION: Herbal medicines are increasingly used by the general population, *Zingiber officinale* Roscoe (F-Zingeberaceae) is one of the most frequently used ingredients in Ayurvedic and Chinese medicine for treatment of various infectious diseases.

Ginger has been found to posses a variety of interesting pharmacological effects, including antipyretic, cardiotonic, anti-inflammatory, anti-arthritic, in nervous diseases, asthma, gingivitis, toothache, constipation and diabetes.

Extracts of ginger have been reported to have effect as an anti-inflammatory, antioxidant, antithrombotic and anticancer agent. The constituents responsible for the pungent taste of ginger are a homologous series of phenolic ketones as 4, 6, 8, 10 and 12 gingerols and shogaols. 6-gingerol has been used as a marker substance of Ginger (**Fig. 1**). Literature survey reveals various methods for the analysis of 6-gingerol in ginger extracts including GC [4], GC-MS, HPLC [5] and TLC [6]. Very little attention has been paid for quantitative estimation of 6-gingerol by HPTLC. However, estimation of 6-gingerol in extracts and formulations using validated HPTLC methods have not been reported.

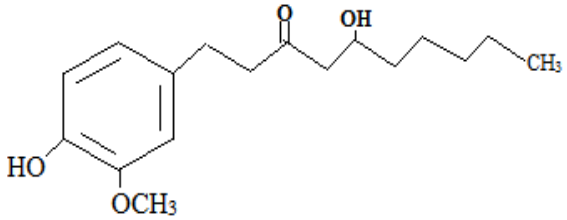

FIG. 1: STRUCTURE OF 6-GINGEROL

HPTLC is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimal sample clean-up. The major advantage of HPTLC is several samples can be run simultaneously using small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

The aim of the present work is to develop an accurate, specific, repeatable and robust HPTLC method for the standardization of extracts and formulations containing 6-gingerol. The proposed method was validated as per ICH guidelines [7-10].

MATERIALS AND METHODS:

MATERIALS: Rhizomes of *Zingiber officinale* and standard 6-gingerol were obtained from Natural remedies Bangalore, India. Samples were collected from two different geographical sources coded as G1 from Chick Magalur and G2 from Bangalore South. All chemicals and reagents used were of analytical grade. Single (Sunti capsule) and polyherbal (Myostaal forte tablet) formulations containing *Zingiber officinale* were purchased from local market.

EQUIPMENT: A Camag HPTLC system comprising of Camag linomat V automatic sample applicator, Twin trough development chamber, Hamilton syringe (100 µl), Camag TLC scanner 3, win cats software version 1.3.3, Camag reprostar 3, Camag TLC plate heater were used during the study.

SAMPLE PREPARATION: Aqueous and methanolic extracts of *Zingiber officinale* samples collected from two different geographical sources were prepared by refluxation. Solutions of aqueous (G1W, G2W) and methanolic (G1M, G2M) extracts to give a concentration of 20mg/ml and 10mg/ml respectively were prepared in methanol and the solutions were filtered through Whatman filter paper No. 1 and filtrates were used for analysis. In case of formulations, For capsules, the contents of one capsule was emptied weighed and macerated with 10ml of methanol for 24hrs, then filtered through whatman filter paper and the filtrate was used for analysis. For tablets, one tablet was weighed, powdered and macerated with 10ml of methanol for 24hrs, then filtered through whatman filter paper and the filtrate was used for analysis.

HPTLC method for estimation of Standard 6-Gingerol:

Preparation of calibration curve of Standard 6-Gingerol: 5mg of standard 6-gingerol was dissolved in 100ml of methanol to give a concentration of 50ng/µl. Calibration curve from 100-1400ng/spot was prepared and checked for linearity, reproducibility and validating the proposed method. The correlation coefficient, %RSD and linearity of results were calculated.

Chromatographic Conditions: Samples were spotted in the form of bands of width 6mm on precoated silica gel aluminium plate 60 F-254 (20×10cm with 0.2mm thickness, Merck Germany) using Camag linomat V. The space between the bands was 8mm. The mobile phase was n-hexane:ether (40:60v/v). Linear ascending development was carried out in twin trough saturated glass chamber. The optimized chamber saturation time was 20 minutes at room temperature. Length of chromatogram run was 75mm. Densitometric scanning was performed using Camag TLC scanner 3 in absorbance mode at 280nm. The source of radiation was deuterium and tungsten lamp keeping the slit dimension at 6×0.45mm.

Aliquots of 2, 4, 6, upto 14µl were spotted in duplicate on TLC plate to obtain a concentration of 100-1400ng per spot. Plate was dried and analysed as described earlier. Standard calibration curve was generated and evaluation was via peak areas with linear regression.

Validation of the Method: The developed method was validated in terms of linearity, limit of detection, limit of quantification, intra day and inter day precision and accuracy.

Analysis of Extracts and Marketed Formulations: 2, 4, 6, upto 14µl of standard 5µl of all extracts and formultions were applied as bands on precoated plates followed by development and scanning. The analysis was repeated in triplicate and the content of drug was calculated from the peak areas recorded.

RESULTS AND DISCUSSION: TLC procedure was optimized. The mobile phase n-hexane:ether (40:60v/v) gave sharp and well defined peak with significant Rf value 0.25±0.05 was desired for quantification of 6-gingerol in extracts and formulations.

The calibration curve in this study was plotted between amount of analyte versus peak area and the regression equation was obtained (Y=18.66+1.109X) with a regression coefficient of 0.9967. The linear regression data (**Table 1**) showed good linear relationship over a concentration range of 100-1400ng/spot for 6-gingerol. In order to determine the detection limit and quantification limit 6-gingerol concentration in the lower part of calibration curve was used. 6-gingerol solutions of 5ng/µl was prepared and applied in duplicate(7-14µl).

TABLE 1: METHOD VALIDATION PARAMETERS

Parameters	Values
Linearity range (ng)	100-1400
Correlation coefficient (r)	0.9967
Limit of detection (LOD)	60ng/spot
Limit of quantification (LOQ)	100 ng/spot

The amount of gingerol by spot versus peak area was determined. LOD was determined at the minimum concentration where the peak was detectable. And LOQ was determined by spotting 1-6µl of 50ng/µl solution in duplicate. The intra day precision was determined by application and measurement of peak areas using six replicates of same spot (300ng/spot of 6-gingerol) two times on the same day and inter day variations were determined similarly on consecutive days.

The repeatability of sample application was assessed by spotting 6µl of sample solution six times on TLC plate followed by development and recording of peak areas for six spots. The % RSD for peak values of 6-gingerol were found to be 1.04% and 1.46% for intra and inter-day precision respectively. The % RSD and results are depicted in **Table 2**, which reveal intra and inter day variations of 6-gingerol at a concentration of 300ng per spot. Recovery studies of the samples were carried out for the accuracy parameter. These studies were carried out at three levels. Sample solutions of 50µg/ml were prepared dilutions were made and recovery studies were performed.

TABLE 2: INTRA AND INTER DAY PRECISION OF HPTLC METHOD (N=6)

Amount (ng/spot)	Mean area	S D	Intra-day % RSD	Inter-day % RSD
300	1063	1.27	1.04	1.46

Percentage recovery was found to be within the limits as listed in **Table 3**. The total 6-gingerol content in the aqueous (G1W) and methanolic (G1M) extracts per 100mg of *Zingiber officinale* were found to be 15.5mg and 27.6mg respectively for samples collected from Chick Magalur in comparison to samples collected from Bangalore as shown in **Table 4.**

TABLE 3: RECOVERY STUDIES (n=6)

Excess drug added to analyte (%)	Amount of 6-gingerol found (ng)	% Recovery	Average
50	49.13±0.51	99.92	
100	99.1±1.27	100.1	99.94
150	148.7±0.91	99.79	

TABLE 4: ANALYSIS OF *ZINGIBER OFFICINALE* EXTRACTS

Sample code	Amount of 6-gingerol (ng/spot)	Amount of 6-gingerol per 100g of extract
G1W	895	15.5
G1M	1070	27.6
G2W	707	11.4
G2M	849	18.4

When two marketed formulations containing different amount of *Zingiber officinale* were analyzed, there was a wide variation in the amount of 6-gingerol with respect to their label claim as shown in **Table 5**.

TABLE 5: ANALYSIS OF MARKETED FORMULATIONS

Brand name	Label claim (mg)	Amount found (mg)	% RSD
Sunti capsule	400	0.49	0.18
Myostal forte tablet	100	2.25	0.34

Hence, HPTLC is a useful tool in standardizing formulations for marker compounds.

The low % RSD value indicate suitability of this method for routine analysis of 6-gingerol in extracts and dosage forms. Statistical analysis proves that the method is repeatable and selective for analysis of 6-gingerol in extracts and formulations. The developed HPTLC technique is simple, precise and accurate. The method can also be used to determine the purity of the drug available from different geographical sources.

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