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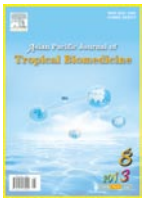

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Densitometric HPTLC analysis of 8-gingerol in *Zingiber officinale* extract and ginger-containing dietary supplements, teas and commercial creams

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable research work in which author have quantified the 8-gingerol in *Z. officinale* extract and some herbal formulation. Statically data proved that proposed method can be used in wide range for detection and quantification of 8-gingerol in herbal formulations effectively.
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ABSTRACT

Objective: To develop and validate a simple, accurate HPTLC method for the analysis of 8-gingerol and to determine the quantity of 8-gingerol in *Zingiber officinale* extract and ginger-containing dietary supplements, teas and commercial creams.

Methods: The analysis was performed on 10×20 cm aluminium-backed plates coated with 0.2 mm layers of silica gel 60 F_{254} (E-Merck, Germany) with *n*-hexane: ethyl acetate 60: 40 (v/v) as mobile phase. Camag TLC Scanner III was used for the UV densitometric scanning at 569.

Results: This system was found to give a compact spot of 8-gingerol at retention factor (*Rf*) value of (0.39±0.04) and linearity was found in the ranges 50-500 ng/spot (r^2=0.9987). Limit of detection (12.76 ng/spot), limit of quantification (26.32 ng/spot), accuracy (less than 2 %) and recovery (ranging from 98.22-99.20) were found satisfactory.

Conclusions: The HPTLC method developed for quantification of 8-gingerol was found to be simple, accurate, reproducible, sensitive and is applicable to the analysis of 8-gingerol in *Zingiber officinale* extract and ginger-containing dietary supplements, teas and commercial creams.

KEYWORDS

8-gingerol, HPTLC, ICH guidelines, *Zingiber officinale*

1. Introduction

Ginger [*Zingiber officinale* Roscoe (*Z. officinale*)] is widely used as a dietary supplement as well as a spice and flavoring agent in foods and beverages around the world. For centuries, it has been an important ingredient in Chinese, Ayurvedic, and Tibb-Unani systems of medicine and widely used in the treatment of unrelated ailments like arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, fever, infectious diseases and helminthiasis[1-2]. Ginger contains a number of different pungent and biologically active compounds which are mainly 6-gingerol, 8-gingerol, zingerone and paradol[3]. 8-Gingerol, as shown in Figure 1, is one of the principal pungent components of ginger[4]. Previous research have proved that 8-gingerol had various pharmacological functions, such as anti-platelet aggregation activities[5,6], spasmolytic activity[7], modulation of macrophage functions[8], inhibiting LPS-induced PGE 2 production and LPS-induced COX-2 expression[9], 5-HT3 receptor blocking activity and immunosuppressive activity[10,11]. 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 essential to standardize ginger formulations. Many analytical methods have been reported for the analysis of 8-gingerol in its extract, commercial formulations and biological fluids[12-14]. Most of these methods are high performance liquid chromatography (HPLC) and used for analysis of 8-gingerol either in biological fluids or in its extract. Only one high performance thin layer

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chromatography (HPTLC) densitometric method is available for the analysis of 8-gingerol in commercial ginger[12]. No HPTLC methods have been reported for analysis of 8-gingerol in teas and dietary supplements. Therefore the objective of this investigation was, to develop a simple, economical, selective, precise, and sensitive HPTLC technique for analysis of 8-gingerol in its methanolic extract, dietary supplements, teas and commercial creams. The proposed method was validated using International Conference on Harmonization Guidelines (ICH Guideline 1996)[15].

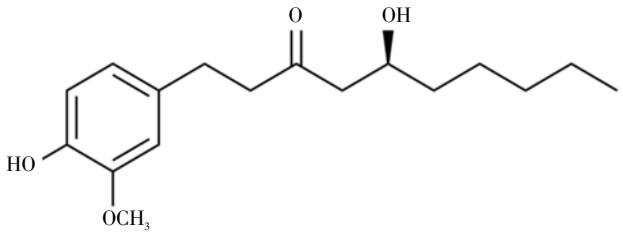

Figure 1. Chemical structure of 8-gingerol.

2. Materials and methods

2.1. Chemicals and reagents

All the solvents used were of chromatographic grade and other chemicals were of analytical reagent grade. Standard 8-gingerol was obtained from Natural Remedies (Bangalore, India). Ginger rhizomes and ginger containing dietary supplements, teas and creams were obtained randomLy from local market of Riyadh, Kingdom of Saudi Arabia.

2.2. Sample preparation

Accurately weighed 5 g of the dried whole rhizome of *Z. officinale* and refluxed with methanol (100 mL) for 1 h in water bath and filtered through Whatmann filter paper (No. 41). 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 rotary vacuum evaporator and the resulting solution was used as test solution.

2.3. Extraction procedure from teas, dietary supplements and commercial creams

The composition was determined in one ginger root dietary supplement, two ginger rhizome teas and two ginger commercial creams. For the analysis of ginger rhizomes dietary supplement, 10 capsules containing ginger powder were opened, transferred in a beaker and mixed to insure that a homogenous sample was obtained. About 5 g each of ginger rhizome dietary supplement, teas and creams were weighed and transferred to separating funnel and extracted three times with 70 mL each of methanol. The filtrates were combined and concentrated using a rotary vacuum evaporator to a final volume of 10 mL and used as test solution in the HPTLC analysis. Similarly, about 5 g of two ginger teas and two creams were separately weighed and followed same procedures for extraction as above to obtained a final volume of 10 mL for each sample for use as test solution in the HPTLC analysis.

2.4. Preparation of standard solution

The 8-gingerol, 10 mg, was weighed and dissolved in 10 mL of methanol; further 1 mL of this solution was diluted with methanol to 20 mL, which gives 50 μg/mL equivalent of standard stock solution of 8-gingerol. Different volumes of stock solution, 1, 2, 4, 6, 8, 10 μL were spotted in duplicate on TLC plate to obtain concentrations of 50, 100, 200, 300, 400 and 500 ng per spot of 8-gingerol. The data of peak area versus drug concentration were treated by linear least-square regression.

2.5. HPTLC instrumentation and conditions

HPTLC densitometric analysis was performed on 10×20 cm aluminium-backed plates coated with 0.2 mm layers of silica gel 60 F_{254} (E-Merck, Germany). Samples were applied to the TLC plates as 6 mm bands using a Camag Automatic TLC sampler 4 (ATS4) sample applicator (Switzerland) fitted with a Camag microlitre syringe. A constant application rate of 150 nL/s was used. Linear ascending development of the plates to a distance of 80 mm was performed with hexane: ethyl acetate 6:4 (%, v/v) as mobile phase in a Camag Automatic Developing Chamber 2 (ADC2) previously saturated with mobile phase vapour for 30 min at 22 °C.

2.6. Derivatization and densitometrical scanning

The plates was visualized with the use of anisaldehyde-sulphuric acid reagent. The plates were immersed in reagent for 1 seconds then heated at 105 °C for 10 min. The plate was scanned at 569, 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.

2.7. Method validation

The linearity of the method for 8-gingerol was checked between 50 and 500 ng/spot and concentration was plotted against peak area.

Accuracy, as recovery, was determined by the standard addition method. Pre-analyzed samples of 8-gingerol (200 ng/spot) were spiked with extra 8-gingerol standard (0, 50, 100, and 150%) and the mixtures were reanalyzed.

Percentage recovery and relative standard deviation (RSD, %) were calculated for each concentration level.

Precision was assessed by determination of repeatability and intermediate precision. Repeatability of sample was determined as intra-day variation, whereas intermediate

precision was determined by assessment of inter-day variation for analysis of 8-gingerol at four different amounts (100, 200, 300 and 400 ng/spot) in six riplicate.

Robustness of the proposed TLC densitometric method was determined to evaluate the influence of small deliberate changes in the chromatographic conditions during determination of 8-gingerol. Robustness was determined by changing the polarity of the mobile phase.

Limit of detection (LOD) and limit of quantification (LOQ) were determined by standard deviation (SD) method. They were determined from the slope of the calibration (S) curve and SD of the blank sample using following equations:

LOD = 3.3×SD/S

LOQ = 10×SD/S

2.8. Quantification of 8-gingerol in methanolic extract, dietary supplement, teas and commercial creams

The test samples were injected and chromatograms were obtained under the same conditions as for analysis of standard 8-gingerol. The area of the peak corresponding to the *Rf* value of 8-gingerol standard was recorded and the amount present was calculated from the regression equation obtained from the calibration plot.

3. Results

3.1. Method development

The mobile phase composition was optimized to establish a suitable and accurate densitometric HPTLC method for analysis of 8-gingerol. The mobile phase *n*-hexane: ethyl acetate 60:40 (%, v/v) resulted in a sharp, symmetrical, and well resolved peak at *Rf* value of 0.39 (Figure 2). UV spectra measured for the bands showed maximum absorbance at approximately 569 nm. The HPTLC densitograms of standard 8-gingerol. methanolic extract of *Z. officinale* and other 5 *Z. officinale* related products are shown in Figures 3-9.

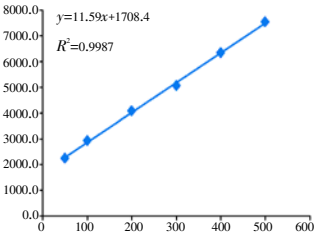

Figure 2. Linear regression curve of standard 8-gingerol.

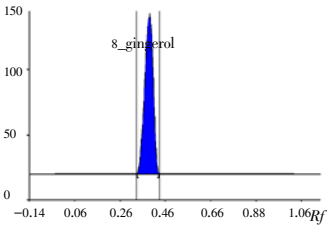

Figure 3. HPTLC densitogram of standard 8-gingerol.

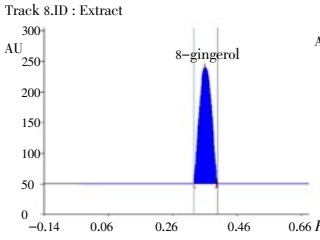

Figure 4. HPTLC densitogram of methanolic extract of *Z. officinale*.

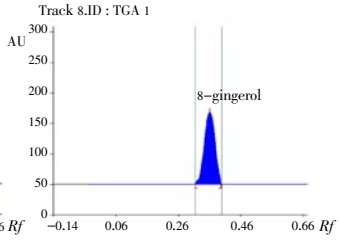

Figure 5. HPTLC densitogram of tea (TG 1).

3.2. Calibration curve

The calibration plot of peak area against amount of 8-gingerol was linear in the range 50-500 ng/spot. Linear regression data for the plot confirmed the good linear relationship (Table 1). The correlation coefficient (R^2) was 0.9987 which was highly significant ($P<0.05$). The linear regression equation was $y=11.59x+1708.4$, where y is response and x is amount of 8-gingerol (Figure 2).

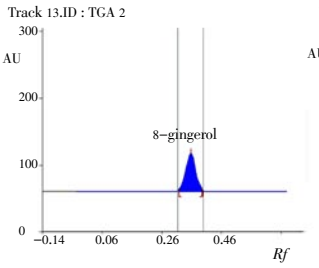

Figure 6. HPTLC densitogram of tea (TG 2).

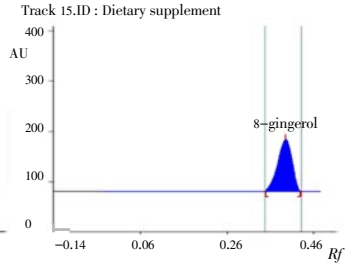

Figure 7. HPTLC densitogram of dietary supplement.

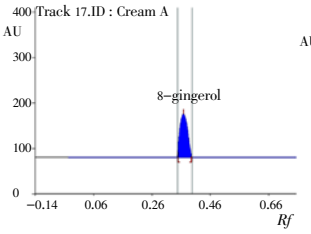

Figure 8. HPTLC densitogram of Cream A.

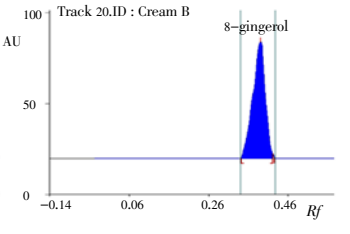

Figure 9. HPTLC densitogram of Cream B.

Table 1

Linear regression data for the calibration curve of 8-gingerol (n=6).

Linear regression data	Values
Linearity range (ng/spot)	50-500
Regression equation	y= 11.59x+1708.4
Correlation coefficient	0.9987
Slope±SD	11.59±0.48
Intercept±SD	1708.40±138.56
Standard error of slope	0.4555
Standard error of intercept	64.218
95% confidence interval of slope	12.131-13.252
95% confidence interval of intercept	1577-1883.7

3.3. Method validation

3.3.1. Precision

The accuracy of the method, as recovery, was 98.22%-99.20%, with RSD values in the range 0.70-1.41. These results indicated that the method was accurate (Table 2). Results from determination of repeatability and intermediate precision, expressed as SD (%) are shown in Table 3. RSD was in the range 0.46-1.37 for repeatability and 0.74-1.65 for intermediate precision. These low values indicate the method is precise[16].

Table 2

Accuracy of the proposed method (n=3).

Excess drug added to analyte (%)	Theoretical content (ng)	Conc. found (ng)±SD	% Recovery	% RSD
0	100	98.33±1.15	98.33	1.17
50	150	147.33±2.08	98.22	1.41
100	200	196.67±2.52	98.33	1.28
150	250	248.00±1.73	99.20	0.70

Conc.: Concentration.

Table 3

Precision of the proposed method.

Conc. (ng/spot)	Repeatability (Intraday precision)			Intermediate precision (Interday)		
	Avg conc.±SD (n=3)	Standard error	% RSD	Avg conc.±SD (n = 3)	Standard error	% RSD
100	3104.48±16.59	9.58	0.53	3067.00±50.74	29.30	1.65
200	4255.33±58.40	33.72	1.37	4221.00±54.67	31.56	1.30
300	5150.33±52.31	30.20	1.02	5235.67±40.07	23.13	0.78
400	6241.33±28.92	16.70	0.46	6233.33±46.06	26.59	0.74

Conc.: Concentration.

3.3.2. Robustness of the method

Results of robustness are shown in Table 4. Low values of % RSD (0.75-1.52) were obtained after introducing small deliberate change into the densitometric TLC procedure, which proved the robustness of the proposed HPTLC method[17].

Table 4

Robustness of the proposed HPTLC method.

Conc. (ng/spot)	Mobile phase composition (Hexane: ethyl acetate)			Results		
	Original	Used		Area±SD (n=3)	% RSD	*Rf*
300	6: 4	8:2.1	-0.1	5100±38	0.75	0.40
		8:2	0.0	5115±63	1.23	0.39
		8:1.9	+0.1	5089±77	1.52	0.38

3.3.3. Limit of detection and quantification

LOD and LOQ of the proposed method was found to be 12.76 and 26.32 ng/spot for 8-gingerol, which indicated that the proposed method can be used in wide range for detection and quantification of 8-gingerol effectively[18].

3.4. Quantification of 8-gingerol in methanolic extract, dietary supplement, teas and commercial creams

8-gingerol peaks from methanolic extract, dietary supplements, teas and commercial reams were identified by comparing their *Rf* values with those obtained by chromatography of the standard under the same conditions. The 8-gingerol content of the *Z. officinale* extract, dietry supplement, teas and commercial creams were quantified by use of the linear regression equation and results are given in Table 5.

Table 5

Contents of 8-gingerol in its methanolic extract, dietary supplement, teas and commercial creams.

Samples	Contents (% w/w)
Methanolic extract	0.11
Dietary supplement	0.07
Tea (TG1)	0.05
Tea (TG2)	0.04
Commercial cream A	0.04
Commercial cream B	0.05

4. Discussion

The HPTLC method for quantitating 8-gingerol is simple, accurate, reproducible and sensitive and is applicable to the analysis of a wide variety of ginger-containing products. The method was established taking requirements of high precision and economy into consideration. The validation parameters for the developed method were the specificity, calibration curve, precision (repeatability), recovery and accuracy. The mobile phase *n*-hexane: ethyl acetate 60: 40 (%, v/v) resulted in a sharp, symmetrical, and well resolved peak at *Rf* value of 0.39. Linear regression data for the plot confirmed the good linear relationship and the resulting equation was operational in the concentration range of 50-500 ng/spot. The method was accurate 98.22%-99.22%, with RSD values in the range 0.70-1.41 after spiking the 8-gingerol with different concentrations of standard. The current method to analyse 8-gingerol from methanolic extract, dietary supplements, teas and commercial creams were identified by comparing their *Rf* values with those obtained by chromatography of the standard under the same conditions. The 8-gingerol content of the *Z. officinale* extract, dietry supplement, teas and commercial creams were quantified by use of the linear regression equation. The proposed HPTLC method can be used for quantitative monitoring of 8-gingerol in crude drugs and prepared formulations without interference. Its use for standardization and quality control of raw materials and commercial herbal products of traditional medicine containing *Z. officinale* as an ingredient can be explored.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Since 8-gingerol is principal bioactive component of ginger rhizome, simple robust method is required for quantification of this active

constituent which has been used for quality control and standardization of this plant and its formulation as a marker compound.

Research frontiers

The present aim of this research work is to develop and validate a simple, accurate HPTLC method for the analysis of 8-gingerol in *Z. officinale* extract and ginger-containing dietary supplements, teas and commercial creams.

Related reports

No similar reports were found in the literature regarding this plant specifically, since it is being evaluated using this technique for the first time as per literature review available in our resources.

Innovations and breakthroughs

The proposed HPTLC method can be used for quantitative monitoring of 8-gingerol in crude drugs and preparing formulations without interference.

Applications

The proposed method for quantification of 8-gingerol is the first validated HPTLC method. Statistical analysis proves that the method is reproducible and selective for the analysis of 8-gingerol with added advantages of speed and minimal, low cost of reagents, satisfactory precision and accuracy. This method may be used for quality control and standardisation of *Z. officinale* extracts and marketed herbal formulations.

Peer review

This is a valuable research work in which authors have quantified the 8-gingerol in *Z. officinale* extract and some herbal formulations. Statistical data proved that the proposed method can be used in wide range for detection and quantification of 8-gingerol in herbal formulations effectively.

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