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RESEARCH ARTICLE

High Performance thin layer Chromatographic Analysis of 10-Gingerol in *Zingiber officinale* Extract and Ginger-Containing Dietary Supplements, Teas and Commercial Creams

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High Performance thin layer Chromatographic Analysis of 10-Gingerol in Zingiber officinale Extract and Ginger-Containing Dietary Supplements, Teas and Commercial Creams

Summary

Ginger rhizome powder is widely used as a food spice and an herbal medicine around the world. A sensitive and accurate High Performance thin layer chromatographic (HPTLC) method has been developed to determine the quantity of 10-gingerol in Zingiber officinale extract and ginger-containing dietary supplements, teas and commercial creams on aluminum-backed silica gel 60 F_{254} plates with n-hexane:ethyl acetate 50:50 (v/v) as mobile phase. The retention factor (R_f) of 10-gingerol was found to be 0.42 ± 0.02. The calibration curve shows good linear relationship with correlation coefficient of 0.9985 in the concentration range of 200–1200 ng/spot. The proposed HPTLC method was found simple, cost effective, selective, precise and accurate, and can be used for quality control as well as routine qualitative and quantitative analysis of ginger extracts as well as ginger containing herbal formulations.

Key Words: *10-gingerol, HPTLC, ICH guidelines, Zingiber officinale.*

Zingiber officinale esktesi ve zencefil içeren diet destekleri, çaylar ve kremlerde yüksek performanslı ince tabaka kromatografisi ile 10-gingerol analizi

Özet

Zencefil rizom tozu baharat ve bir bitkisel ilaç olarak dünyada yaygın olarak kullanılmaktadır. Bu çalışmada Zingiber officinale ekstrelerinde, zencefil içeren diyet destekleri, çaylar ve kremlerde 10-gingerol miktarının tanımlanması için hassas ve kesin bir yüksek performanslı ince tabaka kromatografisi (HPTLC) metodu silika jel 60 F254 kaplı alüminyum plaklar ve n-hegzan:etilasetat 50:50 (v/v) solvan sistemi mobil faz olarak kullanılmak suretiyle geliştirilmiştir. 10-gingerolün tutunma faktörü (Rf) 0.42 ± 0.02 dir. Kalibrasyon eğrisi 200-1200 ng/spot konsantrasyon aralığında 0.9985 olan korelasyon katsayısı ile iyi bir korelasyon göstermiştir. Önerilen HPTLC metodu basit, uygun fiyatlı, seçici, etkin ve kesin bir metot olduğu, zencefil ekstrelerinin ve zencefil içeren bitkisel formülasyonların kalite kontrollerinde ve kalitatif ve kantitatif analizlerinde kullanılabileceği bulunmuştur.

Anahtar kelimeler: *10-gingerol, HPTLC, ICH kılavuzları, Zingiber officinale*

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INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is widely used as a food spice and a herbal medicine 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 is a household remedy for dyspepsia, flatulence, colic, and diarrhoea (2). Ginger contains a number of different pungent and biologically active compounds mainly 6-gingerol, 10-gingerol, 8-gingerol, 6-shogaol, zingerone and paradol (3). 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 (4). This component has also been reported to possess antimicrobial and antifungal properties as well as several pharmaceutical properties (5). The 10-gingerol compound showed the synergistic effect with aminoglycoside against vancomycin-resistant enterococci (VRE) (6). In the case of 10-gingerol, its effect on human promyelocytic leukemia (HL-60) cells is better than that of [6]-gingerols (7) and the activity of sarcoplasmic reticulum of Ca^{2+}-ATPase could be stimulated by 10-gingerol (8). 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 10-gingerol in Ginger extract, commercial formulations and biological fluids (9-12). Most of these methods are based on high performance liquid chromatography (HPLC) and used for analysis of 10-gingerol either in biological fluids or in its extract. No HPTLC methods have been reported for analysis of 10-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 the analysis of 10-gingerol in Ginger extract, dietary supplements, teas and commercial creams. The proposed method was validated using International Conference on Harmonization (ICH) guidelines (13).

MATERIALS AND METHODS

Chemicals

All chemicals and solvents used were of analytical reagent (AR) grade. Pure 10-gingerol was obtained from Natural Remedies (Bangalore, India). Ginger root containing dietary supplements, teas and creams were obtained randomly from the local market of Riyadh, Saudi Arabia.

Sample preparation

Accurately weighed 5 g of the dried *Z. officinale* were 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 with methanol for 1 h (2x50 mL) and filtered. The filtrates were combined and concentrated using rotary vacuum evaporator and the resulting residue was dissolved in methanol and the volume was accurately adjusted to 25 mL.

Extraction procedure from teas, dietary supplements and commercial creams

The composition was determined in one Ginger rhizome dietary supplement, two ginger root teas and two Ginger commercial creams. For the analysis of Ginger rhizome dietary supplements, 10 capsules containing Ginger powder were opened, and their contents were mixed. Accurately weighed 5 g each of Ginger rhizome dietary, teas and creams were transferred to separating funnel. It was then extracted with methanol (3x70mL) and filtered. Filtrates were

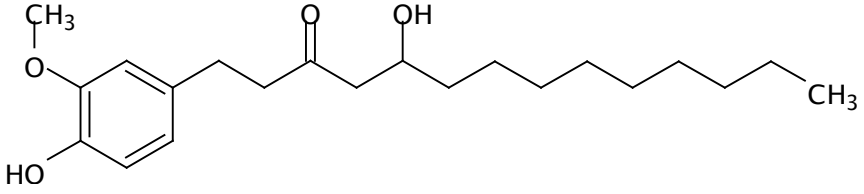

Figure 1: Chemical structure of 10-gingerol

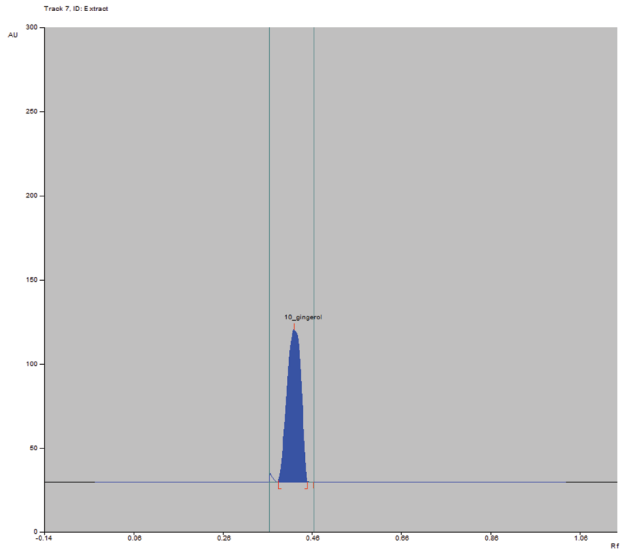

Figure 2: HPTLC densitogram of methanolic extract

combined and concentrated using rotary vacuum evaporator and final volumes were accurately adjusted to 10 mL and used as test solutions in the HPTLC analysis.

Preparation of standard solution

The 10-gingerol (purity 95%), 1 mg, was dissolved in 10 ml of methanol, which gives 100 µg/mL equivalent of standard 10-gingerol. Different volumes of stock solution, 2, 4, 6, 8, 10, 12 were spotted in duplicate on TLC plate to obtain concentrations of 200, 400, 600, 800, 1000 and 1200 ng per spot of 10-gingerol. The data of peak area versus drug concentration were treated by linear least-square regression.

Chromatography

The HPTLC (CAMAG, Switzerland) system, made up of a Linomat IV sample applicator fitted with a microlitre 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. Plates were developed to a distance of 8 cm with mobile phase n-hexane: ethyl acetate 50:50 (%, *v*/*v*).

Method Validation

The linearity of the method for 10-gingerol was checked between 200 and 1200 ng/spot and concentration was plotted against peak area. Accuracy, as recovery, was determined by the standard addition method. Pre-analyzed samples of 10-gingerol (200 ng/spot) were spiked with extra 10-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 10-gingerol at four different amounts (200, 400, 600 and 800 ng/spot) in triplicate.

Quantification of 10-gingerol in methanolic extract, dietry supplement, teas and commercial creams

The test samples were spotted and chromatograms were obtained under the same conditions as for analysis of standard 10-gingerol. The area of the peak corresponding to the R_f value of 10-gingerol standard was recorded and the amount present was calculated from the regression equation obtained from the calibration plot.

RESULTS

Method development

The mobile phase composition was optimized to establish a suitable and accurate densitometric HPTLC method for analysis of 10-gingerol. The mobile phase n-hexane: ethyl acetate 50:50 (%, *v*/*v*) resulted in a sharp, symmetrical, and well resolved peak at R_f value of 0.42 (Figure 1). UV spectra measured for the bands showed maximum absorbance at approximately 533 nm; this was, therefore, choosen as the wavelength for UV densitometry (Figure 1).

Calibration curve

The calibration plot of peak area against amount of 10-gingerol was linear in the range 200-1200 ng/spot. Linear regression data for the plot confirmed the good linear relationship (Table 1). The correlation coefficient (R_2) was 0.9985, which was highly significant ($P<0.05$). The linear regression equation was $Y = 4.89x + 1027.3$, where Y is response and X is amount of 10-gingerol.

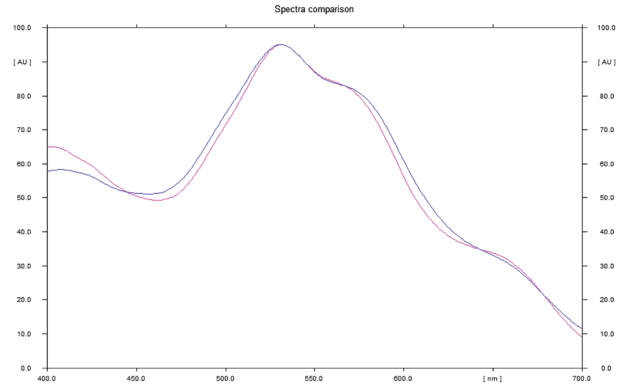

Figure 3: Overlaid UV spectra of both spots

Method validation

The accuracy of the method, as recovery, was 98.58-99.19%, with RSD values in the range 1.00-1.10. These results indicated 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 0.51-1.32 for repeatability and 0.43–0.83 for intermediate precision. These low values indicate that the method is precise.

Quantification of 10-gingerol in methanolic extract, dietary supplement, teas and commercial creams

10-gingerol peaks from methanolic extract Figure 2, dietary supplements, teas and commercial creams were identified by comparing their R_f values and overlaid UV spectra at 533 nm (Figure 3) with those obtained by chromatography of the standard under the same conditions. The 10-gingerol content of the *Z. officinale* extract, dietry supplement, teas and commercial creams were quantified by the use of the linear egression equation and concentration are given in Table 4.

Table 1: Linear regression data for the calibration curve of 10-gingerol (n=3)

Linearity range (ng/spot)	200-1200
Regression equation	Y= 4.8935X+1027.3
Correlation coefficient	0.9985
Slope ± SD	4.894 ± 0.091
Intercept ± SD	1027.3 ± 71.63
Standard error of slope	0.0531
Standard error of intercept	41.36
95% confidence interval of slope	4.781-5.006
95% confidence interval of intercept	939.63-1115.0

DISCUSSION

The HPTLC method developed here for quantitation of 10-gingerol was found to be simple, accurate, cost effective, reproducible and sensitive and is applicable to the analysis of a wide variety of Ginger-containing products. The method was established taking into consideration the requirements of high precision and economy. The validation parameters for the developed method were the specificity, calibration curve, precision (repeatability), recovery and accuracy. The mobile phase n-hexane: ethyl acetate 50:50 (%, *v/v*) resulted in a sharp, symmetrical, and well resolved peak at R_f value of 0.42. Linear regression data for the plot confirmed the good linear relationship and the resulting equation was operational in the concentration range of 200-1200 ng/spot. The method was accurate 98.58-99.19%, with RSD values in the range 1.00-1.10 after spiking the 10-gingerol with different

Table 2: Accuracy of the proposed method (n=3)

Excess drug added to analyte (%)	Theoretical content (ng)	Conc. found (ng) ±SD	% Recovery	% RSD
0	400	394.34±4.26	98.58	1.08
50	600	559.11±6.54	99.19	1.10
100	800	790.78±7.90	98.85	1.00
150	1000	988.23±10.09	98.82	1.02

Table 3: Precision of the proposed method

Conc. (ng/spot)	Repeatability (Intraday precision)			Intermediate precision (Interday)		
	Mean area ±SD (n=3)	Standard error	% RSD	Mean area ±SD (n=3)	Standard error	% RSD
200	1977.54±24.53	26.01	1.32	1994.18±16.48	25.39	0.83
400	2987.50±35.87	23.15	0.71	2981.28±16.43	23.69	0.55
600	4050.67±61.06	55.25	0.98	4068.32±32.81	55.25	0.81
800	5071.83±78.92	18.84	0.51	5066.19±21.58	18.84	0.43

concentrations of standard. The current method was applied for the analysis of 10-gingerol in the methanol extract, dietary supplements, teas and commercial creams. The 10-gingerol spots were identified by comparing their R_f values with those obtained by chromatography of the standard under the same conditions. The 10-gingerol content of the *Z. officinale* extract, dietry supplement, teas and commercial creams were quantified by the use of the linear regression equation. The proposed HPTLC method can be used for quantitative monitoring of 10-gingerol in crude drugs and prepared formulations without interference. In conclusion, HPTLC is an analytical technique that can be utilized for standardization and quality control of raw materials, and commercial herbal products of traditional medicine containing *Z. officinale* as an ingredient can be explored.

Table 4: Contents of 10-gingerol in its methanolic extract, dietary supplement, teas, and commercial creams

Samples	Contents (% w/w)
Methanolic extract	0.07
Dietary supplements	0.04
Tea (TG1)	0.03
Tea (TG)	0.03
Commercial cream A	0.03
Commercial craem B	0.02

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