Quantification of 6-gingerol in Zingiber officinale extract, ginger-containing dietary supplements, teas and commercial creams by validated HPTLC densitometry

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Summary
A sensitive and accurate high-performance thin layer chromatography (HPTLC) method has been developed to determine the quantity of 6-gingerol in the methanolic extract of Zingiber officinale containing dietary supplements, teas and commercial creams. 6-gingerol was separated on aluminum-backed silica gel 60 F 254 plates with n-hexane:ethyl acetate 40:60 (%, v/v) as mobile phase. A compact band was obtained for 6-gingerol at Rf value of 0.33±0.04. The calibration plot was linear in the range of 50-1000 ng/spot of 6-gingerol and the correlation coefficient of 0.995 was indicative of good linear dependence of peak area on concentration. The developed HPTLC densitometric method was found cheap, selective, precise and accurate, and can be used for routine analysis of gingers in the quality control laboratories of herbal drug industries.

Key Words: 6-gingerol, HPTLC, ICH guidelines, Zingiber officinale.

INTRODUCTION
Ginger (Zingiber officinale Roscoe), widely used in foods as a spice around the world, has been used as an important ingredient in Chinese, Ayurvedic and Tibb-Unani systems of medicine for centuries. In the Tibb and Ayurvedic systems of medicine, preventive and ameliorative effects of ginger have been reported in the treatment of catarrh, rheumatism, nervous
diseases, gingivitis, toothache, asthma, stroke, constipation, and diabetes (1). It is a household remedy for dyspepsia, flatulence, colic and diarrhea (2). Ginger contains a number of different pungent and active ingredients. The major pungent compounds found in ginger are the gingerols (2-3). 6-gingerol (Fig.1) [5-hydroxy-1-(4′-hydroxy-3′-methoxyphenyl)-3-decanone] has been shown to have an antipyretic, antitussive, hypotensive (4), cardiotonic (5), antiplatelet (6), antiangiogenic (7), anti-inflammatory and analgesic (8), cytotoxic, apoptotic (9), antitumor (10), anticancer (11), antioxidant (12), antihepatotoxic (13), antifungal (14), vanilloid receptor agonistic (15), cholagogic (16) and antiemetic (17) activities. Because of the widespread uses of ginger as a spice, dietary supplements, tea, cream, household remedy, as well as an ingredient of various natural health products, it is essential to standardize ginger formulations. Many analytical methods have been reported for the analysis of 6-gingerol in its extract, commercial formulations and biological fluids (18-30). Most of these methods are high performance liquid chromatography (HPLC) and used for analysis of 6-gingerol either in biological fluids or in its extract. Only three high performance thin layer chromatography (HPTLC) densitometric methods are available for analysis of 6-gingerol in the extract of Z. officinale and its commercial or Ayurvedic formulations (23, 25-26). No HPTLC methods have been reported for analysis of 6-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 6-gingerol in its methanolic extract, dietary supplements, teas and commercial creams. The proposed method was validated using International Conference on Harmonization (ICH) guidelines (31).

EXPERIMENTAL

Materials
All the solvents used were of chromatographic grade and the other chemicals were of analytical reagent (AR) grade. Standard 6-gingerol was obtained from Natural remedies (Bangalore, India). Ginger root containing dietary supplements, teas and commercial creams were obtained randomly from local market of Riyadh, Saudi Arabia given in Table 4. (the authors should give the commercial name of the products)

Sample preparation
About 5 g of the dried roots of Z. officinale weighed and extracted with methanol (100 mL) for 1 h in water bath and filtered through Whatmann filter paper (No. 41) (20-25 μm, Azmiran). The marc left out was refluxed again with 50 mL 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.

Extraction procedure from teas, dietary supplements and commercial creams
The 6-gingerol composition was determined in one ginger root dietary supplement, two ginger root teas and two ginger commercial creams. For the analysis of ginger root dietary supplements, 10 capsules containing ginger powder were opened, transferred in a beaker, and mixed to insure that a homogenous sample was obtained. The ginger teas and creams were also transferred in a beaker. About 5 g each of ginger root dietary supplement, teas and creams were weighed and transferred to separating funnel. It was then extracted thrice with 70 mL each of methanol. 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.

Preparation of standard solution
The 6-gingerol (purity 95%), 10 mg, was weighed and dissolved in 10 mL of methanol; further 1 mL of this solution was diluted to 10 mL, which gives 95 μg/mL equivalent of standard 6-gingerol (purity 95%). This solution was used as standard stock solution of 6-gingerol (95 μg/mL), which was further diluted to get different concentrations for calibration curve.

Figure 1. Chemical structure of 6-gingerol
Figure 1A. HPTLC chromatogram of 6-gingerol standard.

Figure 1B. HPTLC chromatogram of methanol extract of Z. officinale
Figure 1C. HPTLC chromatogram of dietary supplements (GNC).

Figure 1D. HPTLC chromatogram of tea (TGA1).
Figure 1E. HPTLC chromatogram of tea (TGA2).

Figure 1F. HPTLC chromatogram of Cream A.
Chromatography
HPTLC was performed on 20×10 cm aluminum-backed plates coated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Germany). Samples were applied to the plates as 4 mm bands by use of a Camag (Switzerland) Linomat IV sample applicator fitted with a microlitre syringe. Linear ascending development of the plates to a distance of 80 mm was performed with \( n \)-hexane:ethyl acetate 60:40 (%, v/v) as mobile phase in a twin-trough glass chamber previously saturated with mobile phase vapor for 10 min at 25°C. After development, the plates were scanned using a Camag TLC scanner in absorbance mode, using the deuterium lamp. The slit dimensions were 4×0.1 mm and the scanning speed was 20 mm s\(^{-1}\). A variety of mobile phases were tried for analysis of 6-gingerol in ginger root containing dietary supplements, teas and commercial creams. These included ethyl acetate:ether 70:30 (%, v/v), chloroform:methanol 85:15 (%, v/v), ethyl acetate:methanol 90:10 (%, v/v), and chloroform:methanol:water 77:15:8 (%, v/v). For calibration, different amounts of 6-gingerol (50-1000 ng per/spot) were applied to the plate, in triplicate, by application of different volumes (1-10 \( \mu \)l) of standard stock solution. After development of the plates, peak height, peak area, and concentration data were treated by linear regression analysis.

Method Validation
The linearity of the method for 6-gingerol was checked between 50 and 1000 ng/spot and concentration was plotted against peak area. Accuracy, as recovery, was determined by the standard addition method. Pre-analyzed samples of 6-gingerol (200 ng/spot) were spiked with extra 6-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 6-gingerol at four different amounts (200, 300, 400 and 500 ng/spot) in triplicate. Limits of detection (LOD) and quantification (LOQ) were
determined by the standard deviation (SD) method (32) from the slope (S) of the calibration plot and the SD of a blank sample, by use of the equations LOD = 3.3 × SD/S and LOQ = 10 × SD/S.

Quantification of 6-gingerol in methanolic extract, dietary supplement, teas and commercial creams

The test samples were spotted, and chromatograms were obtained under the same conditions as for analysis of standard 6-gingerol. The area of the peak corresponding to the Rf value of 6-gingerol standard was recorded and the amount presented was calculated from the regression equation obtained from the calibration plot.

RESULTS AND DISCUSSION

Method development

The mobile phase composition was optimized to establish a suitable and accurate densitometric HPTLC method for analysis of 6-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 0.33 (Fig. 1). The optimum chamber-saturation time was 10 min. UV spectra measured for the bands showed maximum absorbance at approximately 533 nm; this was, therefore, chosen as the wavelength for UV densitometry (Fig. 1).

Calibration curve

The calibration plot of peak area against amount of 6-gingerol was linear in the range 50–1000 ng/spot (Fig. 2). Linear regression data for the plot confirmed the good linear relationship (Table 1). The correlation coefficient (R2) was 0.995, which was highly significant (P<0.05). The linear regression equation was Y=7.9988X+857.17, where Y is response and X is amount of 6-gingerol.

Method validation

The accuracy of the method, as recovery, was 98.16–98.84%, with RSD values in the range 1.46–1.67. These results indicated the method was accurate (Table 2). Results from determination of repeatability and intermediate precision, expressed as RSD (%) are shown in Table 3. RSD was in the range 0.69–1.14 for repeatability and 0.72–1.26 for intermediate precision. These low values indicate that the method is precise. LOD and LOQ of the proposed method were found to be 18.89 and 56.67 ng/spot respectively, which indicated that the proposed method can be used in wide range for detection and quantification of 6-gingerol effectively.

Quantification of 6-gingerol in methanolic extracts, dietary supplements, teas and commercial creams

6-gingerol peaks from methanolic extract, dietary supplement, teas and commercial creams were identified by their Rf values comparing with those of the standard obtained by chromatography under

<table>
<thead>
<tr>
<th>Excess drug added to analyte (%)</th>
<th>Theoretical content (ng)</th>
<th>Conc. found (ng) ±SD</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>300</td>
<td>296.24±4.95</td>
<td>98.74</td>
<td>1.67</td>
</tr>
<tr>
<td>50</td>
<td>450</td>
<td>441.72±6.45</td>
<td>98.16</td>
<td>1.46</td>
</tr>
<tr>
<td>100</td>
<td>600</td>
<td>588.98±9.84</td>
<td>98.16</td>
<td>1.67</td>
</tr>
<tr>
<td>150</td>
<td>750</td>
<td>741.32±11.41</td>
<td>98.84</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Table 1. Linear regression data for the calibration curve of 6-gingerol (n=3)

<table>
<thead>
<tr>
<th>Linearity range (ng/spot)</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>Slope±SD</th>
<th>Intercept±SD</th>
<th>Standard error of slope</th>
<th>Standard error of intercept</th>
<th>95% confidence interval of slope</th>
<th>95% confidence interval of intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-1000</td>
<td>Y=7.9988X+857.17</td>
<td>0.995</td>
<td>7.9988±0.94</td>
<td>857.17±10.86</td>
<td>0.542</td>
<td>6.270</td>
<td>5.666-10.331</td>
<td>830.19-884.14</td>
</tr>
</tbody>
</table>

Table 2. Accuracy of the proposed method (n=3)
the same conditions. The values were found identical (Fig. 3-8). The 6-gingerol content of *Z. officinale* extracts, dietary supplements, teas and commercial creams were quantified by the using of the linear regression equation and concentrations are given in Table 4.

**CONCLUSIONS**

This densitometric HPTLC technique is quite simple, accurate, precise and sensitive. The method was established taking to consideration requirements of high precision and economy. The method is suitable for densitometric analysis of 6-gingerol in the methanolic extracts and commercial formulations of *Z. officinale*. The method can be used for routine analysis of 6-gingerol in crude drugs and prepared formulations without interference. Its use for standardization and quality control of raw materials, and commercial herbal products containing *Z. officinale* as an ingredient can be explored.

**ACKNOWLEDGEMENT**

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**Table 3.** Precision of the proposed method

<table>
<thead>
<tr>
<th>Conc. (ng/spot)</th>
<th>Repeatability (Intraday precision)</th>
<th>Intermediate precision (Interday)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean area ±SD (n=3)</td>
<td>Standard error</td>
</tr>
<tr>
<td>300</td>
<td>3521.43±24.53</td>
<td>14.16</td>
</tr>
<tr>
<td>450</td>
<td>4014.67±35.87</td>
<td>20.71</td>
</tr>
<tr>
<td>600</td>
<td>5514.45±61.06</td>
<td>35.25</td>
</tr>
<tr>
<td>750</td>
<td>6887.89±78.92</td>
<td>45.56</td>
</tr>
</tbody>
</table>

**Table 4.** 6-gingerol in ginger-containing dietary supplement, teas, and creams

<table>
<thead>
<tr>
<th>Samples</th>
<th>Contents (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic Extract</td>
<td>0.98</td>
</tr>
<tr>
<td>GNC (GNC Nature’s Fingerprint Ginger root)</td>
<td>0.12</td>
</tr>
<tr>
<td>Tea (TGA1) (Green tea ginger)</td>
<td>0.15</td>
</tr>
<tr>
<td>Tea (TGA2) (Green tea with ginger)</td>
<td>0.08</td>
</tr>
<tr>
<td>Cream A (Ginger cream for slimming)</td>
<td>0.018</td>
</tr>
<tr>
<td>Cream B (Ginger cream for slimming)</td>
<td>0.013</td>
</tr>
</tbody>
</table>


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