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Quantitative analysis method for zingiber officinale water extract using high-performance liquid chromatography

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Abstract. Quantitative analysis of the Zingiber Officinale sample using subcritical water extraction (SWE) was developed employing High-Performance Liquid Chromatography (HPLC) to bolster the advancement of this innovative green extraction process. This research focuses on three principal ginger bioactive compounds: 6-gingerol, 6-shagoal, and 10-gingerol. Various stages were undertaken to establish the quantitative analysis method, encompassing the optimisation of HPLC operating conditions and the formulation of standard calibration curves, yielding individual compound equations. A robust correlation within the calibration curve was achieved, exhibiting an r^2 value of 0.9814 and an RSD of 5.00%. A simultaneous, swift, and dependable method was established with an injection time of 20 minutes and an 8-minute delay between injections, in contrast to the previous HPLC analysis requiring a 45-minute injection time for detecting and quantifying all components. Notably, no post-treatment was applied after the SWE process. This advancement allows for potential future online measurement of Zingiber Officinale bioactive compounds extracted using subcritical water extraction through this technology.

Keywords: 10-Gingerol; 6-Gingerol; 6-Shagoal; HPLC; zingiber officinale

1. Introduction

Since the 1980s, the introduction of subcritical water extraction (SWE) as a green extraction process has attracted the interest of numerous researchers exploring its applications. Most SWE applications are based on plant extraction, contributing up to 58.4% (Cheng *et al.* 2021). These applications included the extraction of Orostachys japonicus (Ko *et al.* 2020), Phyllanthus Niruri (Markom *et al.* 2010), cumin (Eikani *et al.* 2007), olive leaves (Ghoreishi *et al.* 2009), coriander seeds (Saim *et al.* 2008), and Thymbra Spicata (Mustapa *et al.* 2002).

Previous successful SWE implementations have spurred the quest for effective and economical

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methods for swift analysis of SWE samples, which comprise water-based extracts. Water-based extracts present various challenges for direct analysis, including significant vapor expansion volume, poor wettability, solubility issues in numerous stationary phases, detector problems, and chemical damage to the stationary phase (Kuhn 2002). Processes for water removal, such as liquid-liquid extraction using hexane (Eikani *et al.* 2007) and vacuum evaporation, necessitate extra energy and time, introduce harmful chemicals, and may lead to compound degradation. This highlights the criticality of selecting suitable analysis equipment. Furthermore, some extracts are thermally unstable, making Gas Chromatography (GC) unreliable (He *et al.* 1998). Hence, this study introduces a rapid analysis process for water-based extracts without pre-treatment. As a trial, the analysis of bioactive compounds in water extracts from Zingiber Officinale is chosen (Md Sarip *et al.* 2014).

Some researchers have proposed analytical methods for determining ginger bioactive compounds, notably 6-gingerol, using High Performance Liquid Chromatography (HPLC) (Schwertner and Rios 2007, Smith 1982, Wohlmuth *et al.* 2005). However, these methods require pre-treatment processes that elongate analysis time and inflate costs. Some researchers suggest some enrichment procedure for medicinal product extraction using molecularly imprinted polymers (MIPs), where the improvement improve the detection capabilities and improve the cartridges shelf life (Zakia *et al.* 2020). Other reliable methods for ginger compound analysis include High Performance Thin Layer Chromatography (HPTLC), yet its scope is confined to qualitative measurements (Foudah *et al.* 2020). Therefore, this paper aims to establish a quantitative analytical method for Zingiber Officinale extract using SWE via High Performance Liquid Chromatography (HPLC) without pre-treatment. Given that Zingiber Officinale contains three primary bioactive compounds; 6-Gingerol, 6-Shogaol, and 10-Gingerol. The study also seeks to conduct simultaneous analysis of all components in a single run to trim both analysis time and costs.

2. Materials and methods

2.1 Extraction process

In this study, SWE was carried out using the Accelerated Solvent Extraction system, ASE 200 (Dionex, USA). The experimental procedure using ASE 200 is detailed below: 1) 3±0.01 g sample was weighted and install into the extraction cell. 2) The operating condition was load into the system. 3) The extraction process takes place at 30 minutes. 4) The ginger extract was collected from the extraction vial. The SWE process was conducted at various temperatures, maintaining a constant pressure of 3.5 MPa, extraction time of 30 minutes, and a solvent-to-sample ratio of 28:3. The experiments were conducted in triplicate.

2.2 Analysis method

The simultaneous analysis of bioactive compound in ginger water extract was done using High Performance Liquid Chromatography (Water, USA) with Proto Iodide Detector (Water, USA). Column type Lichrocart 250-4, 6 Purospher Star RP-8E (5 Mym) by Merck (USA) were utilized in this study. The modified method from Schwertner and Rios (2007) used in this analysis process. The 20 minutes run time analysis process and 8 minutes next injection delay were implementation in this method. Two mobile phases used are acetonitrile (Merck, USA) and methanol (Merck, USA). The

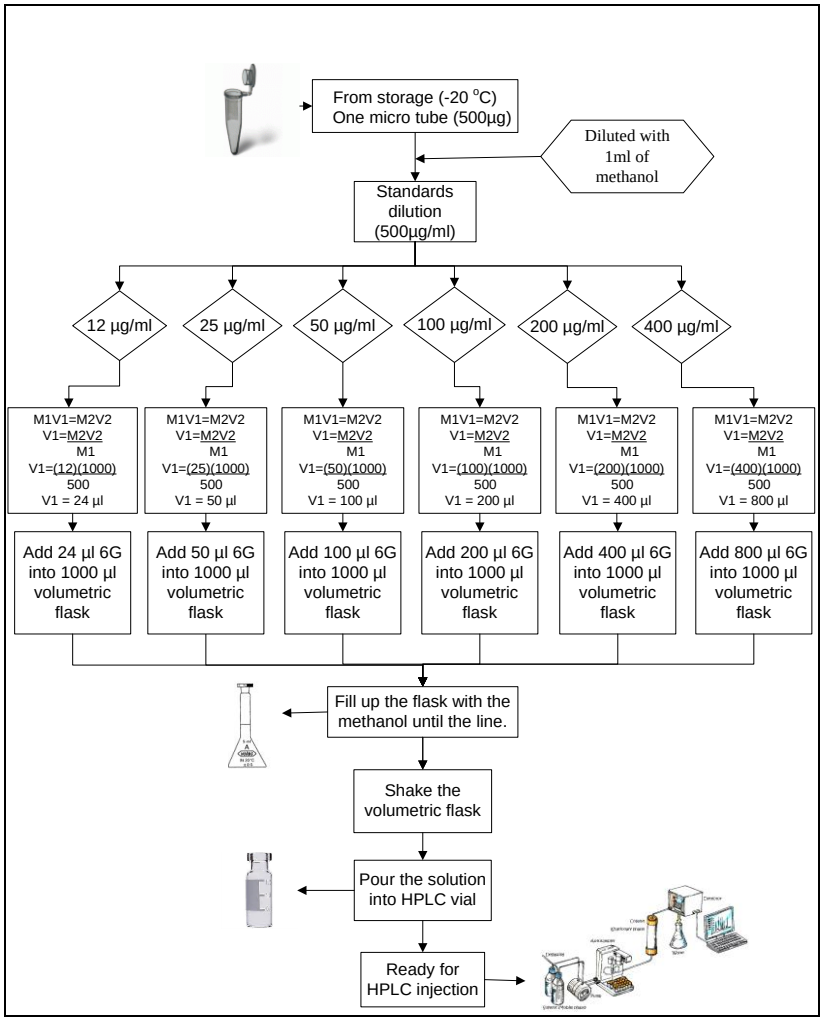


Fig. 1 Procedure for preparation and injection for ginger bioactive compound standards

percentage of mixture mobile phase increases gradually during the analysis from 20% acetonitrile to 50 % acetonitrile. During the process, two mobile phases was used which are solution A; 100% acetonitrile and solution B; 65% methanol: 35% water. During the analysis, the percentage of the solvent A to solvent B was increased gradually from 20 % to 50 % at flow rate of 1.20 ml/min. Meanwhile, the column temperature was set at 35-38 ºC. The identification for the bioactive component in water extract was conducted based on calibration curve developed from the ginger bioactive compounds standards namely 6-gingerol, 6-shagoal and 10-gingerol provided by Chromodex, UK. Procedure for the injection procedure is shown in Fig. 1. Each injection was run 5 times.

2.3 Data analysis and linear regression model

The result was analyzed using the statistical mean and comply with the WADA technical report

(WADA technical report 2010) and EURACHEM guide (EURACHEM 1998). The relative standard deviation, RSD (%) for each data can be calculated which referring as the percentage errorEURACHEM (EURACHEM 1998) . Other statistic value such as mean, μ and standard deviation, σ is also important for this measurement. μ, σ and RSD (%) was calculated using the Eqs. (1), (2) and (3), respectively.

$$\mu = \frac{1}{N}\int_{i=1}^{N} X_i \tag{1}$$

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(X_i - \mu)^2} \tag{2}$$

$$RSD = \frac{\sigma}{\mu} \times 100 \tag{3}$$

where,

μ=Mean

σ=Standard deviation

RSD=Relative standard deviation

N=No of replicate

i=No of experiment

X_i=Value at no i experiment

The relationship between HPLC response and compound concentration was conducted using linear regression with intercept -y is equal to zero. The assumption is based on the concentration are equal to zero when the HPLC response were zero. The validity of the equation was examined through the linearity of curve with the coefficient of determination, r^2>0.96 (Miller 1991) using Eq. (4).

$$r = \frac{n\sum x_i y_i - (\sum x_i)(\sum y_i)}{\sqrt{n\sum x_i^2 - (\sum x_i)^2}\sqrt{n\sum y_i^2 - (\sum y_i)^2}}, 1 \geq r \geq 0 \tag{4}$$

For the HPLC validation, limit of detection, LOD had been calculated using Eq. (5) (IUPAC 1997). LOD was the measurement of the lowest concentration that can be measured for the certain analysis method. It can be expressed as the lowest concentration, c_l which that can be measured from the lowest measurement (Eq. (5)), x_L.

$$x_l = x_{bl} + ks_{bl} \tag{5}$$

where,

x_l=Lowest measurement

x_{bl}=Mean of the blank measurement.

s_{bl}=Standard deviation of the blank measurement.

k=Numerical factor chosen according to the confidence level desired.

3. Result and discussions

Fig. 2 shows the HPLC chromogram for 6-Gingerol, 6- Shogaol and 10-Gingerol. As shown in Fig. 2, three main bioactive components in ginger were well separated at the retention time of 4.595 minute for 6-Gingerol, 6.628 minute for 6-Shogoal and 8.848 minute for 10- Gingerol. An improvement of the retention time is detected as compared to the previous HPLC analysis which

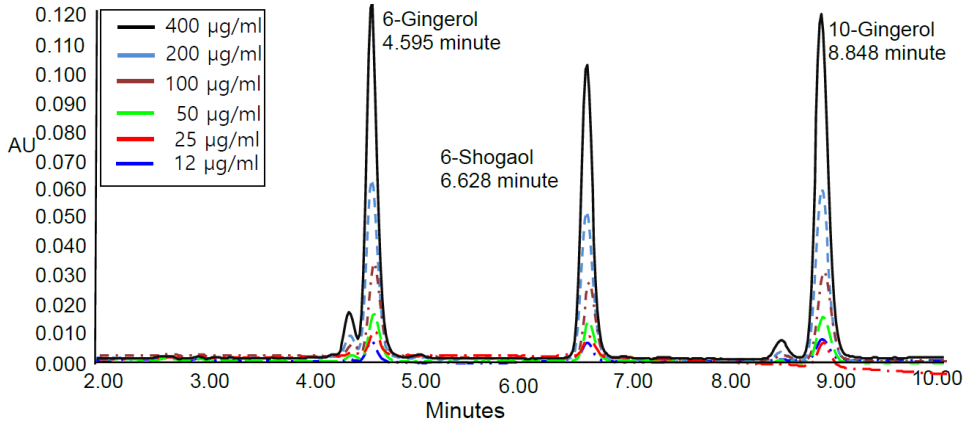


Fig. 2 The ginger bioactive standard chromatogram at different concentration

Table 1 The calibration curve analysis data

Compound	Compound concentration, µg/ml	Mean peak area, Au	Standard deviation, σ	Relative standard deviation, RSD %
6-Gingerol	12	42671.0	894.6907	2.0967
	25	99755.0	4063.0563	4.0730
	50	202310.0	6274.0752	3.1012
	100	417502.0	5755.5726	1.3786
	200	836054.0	5130.8680	0.6137
	400	1713340.2	23745.1098	1.3859
6-Shogoal	12	59495.4	1221.1070	2.0524
	25	122433.4	5173.2826	4.2254
	50	247790.6	5827.5174	2.3518
	100	497604.2	5410.2221	1.0873
	200	1033674.6	14041.3665	1.3584
	400	2327817.4	24898.3103	1.0696
10-Gingerol	12	40515.0	1105.4377	2.7285
	25	87161.8	1436.1689	1.6477
	50	182062.0	8137.4842	4.4696
	100	336722.4	2512.0988	0.7460
	200	663190.0	5995.6358	0.9041
	400	1454550.4	25122.0235	1.7271

involve up to 45 minute (Schwertner and Rios 2007). Based on standard injection, 6-gingerol, 6-shagoal and 10-gingerol was identify at 4.595 minute, 6.628 minute and 8.848 minute, respectively.

Then, the data had been analyzed statistically as tabulated in Table 1. The maximum percentage RSD for the data was 4.4696 % with the average value of 2.0565 which is acceptable under the EURACHEM guide (Magnusson and Örnemark 2014). The mean peak area for each concentration of the standard was used for the development of the standard calibration curve.

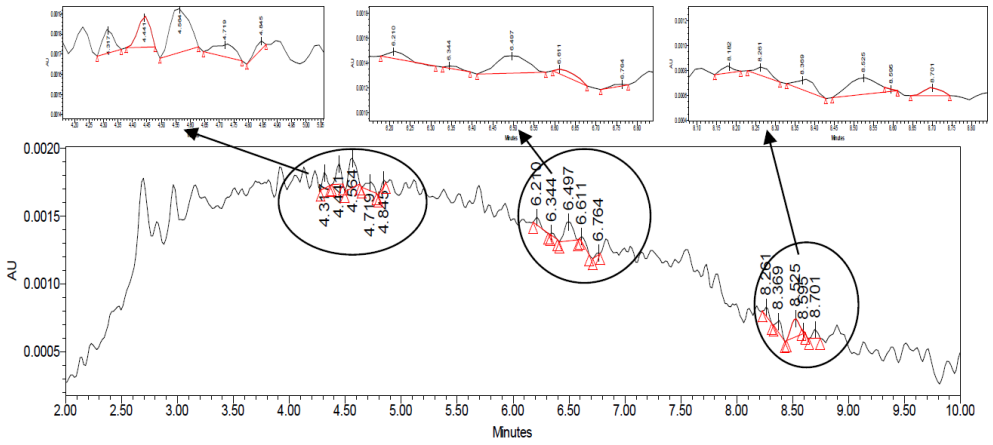


Fig. 3 The blank HPLC profile for specific compounds

Table 2 The calculation of LOD for 6-gingerol, 6-shagoal and 10-gingerol

Compound	x_{bl}, AU	s_{bl}, AU	x_l, AU	c_l, µg/ml
6-gingerol	434.60	345.78	437.60	0.10
6-shogaol	234.80	292.56	237.80	0.04
10-gingerol	259.40	269.96	262.40	0.07

The r^2 values for calibration curve of 6-gingerol, 6-shagaol and 10-gingerol were 0.9947, 0.9974 and 0.9997, respectively and considered as the good linear correlation, as suggested by Miller (1991). The correlation equations for each bioactive compound were generated as shown in Eqs. (6)-(8).

$$C_{6G} = \frac{4254.8}{Peak\ Area} \tag{6}$$

$$C_{6S} = \frac{Peak\ area}{5644.5} \tag{7}$$

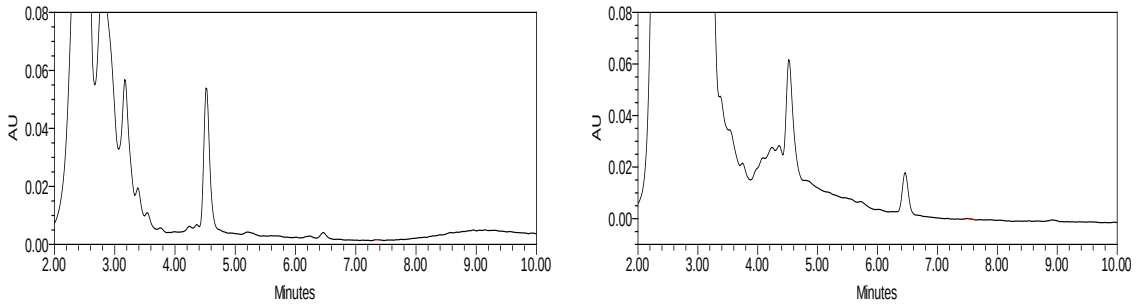
$$C_{10G} = \frac{Peak\ area}{3563.1} \tag{8}$$

3.1 Limit of detection (LOD) for the analysis

The limitation of the bioactive compound measurement was determined though the limit detection (LOD) measurement. The LOD for each compound were calculated based on the mobile phase or blank injection. Fig. 3 show the HPLC profile for the blank at the specific retention time as identify earlier for 6-gingerol, 6-shagoal and 10-gingerol.

The calculation for the LOD is shown in Table 2. The *k* value used in this study was three as suggested by Hayashi et. al (1995).

As seen in Table 2, minimum concentrations for the measurement of 6-gingerol, 6-shogaol and 10-gingerol are 0.10 µg/ml, 0.04 µg/ml and 0.07 µg/ml respectively using this method. Sample with the concentration this value is consider insignificant or non-detected.



(a) 120 ℃ (b) 160 ℃

Fig. 4 HPLC profiles for SWE extract at (a) 120 ℃ and (b) 160 ℃ constants pressure (3.5 MPa), extraction time (30 minute) and solvent to sample ratio (28:3)

3.2 Subcritical water extraction of zingiber officinale

Fig. 4 shows the HPLC profile for subcritical water extraction sample at the temperature of 120 ℃ and 160 ℃, and constants pressure (3.5 MPa), extraction time (30 minute) and solvent to sample ratio (28:3).

The concentration of 6-gingerol decreased from 84.33 µg/ml to 61.13 µg/ml as the temperature rose from 120 to 160 ℃. A different trend emerged for 6-shogaol concentration, which increased from 0 µg/ml (undetected) at 120 ℃ to 62.13 µg/ml at 160 ℃. This suggests a possible selective extraction process in Zingiber Officinale, wherein higher temperatures reduce the dielectric constant of water, potentially enhancing the extraction rate of specific components, such as 6-shogaol (Bertolini *et al.* 1982).

Furthermore, there's the likelihood of a dehydration process occurring in 6-gingerol, involving the dehydration of the β-hydroxy keto group within its structure, resulting in the formation of 6-shogaol and water. This phenomenon is supported by Ali *et al.* who indicated the dehydration of 6-gingerol into 6-shogaol at elevated temperatures (Ali *et al.* 2008). Consequently, establishing a rapid analysis method for all bioactive compounds aids in identifying these phenomena without the need for post-treatment processes. Introducing post-treatment processes might alter the concentrations of certain compounds, leading to potentially misleading conclusions. In conclusion, the crucial role of rapid analysis methods for determining bioactive components lies in eliminating potential errors arising from post-extraction treatments that could result in component degradation.

4. Conclusions

The quantitative and qualitative analysis method for the water-based ginger extract was successfully developed using HPLC.

- A more reliable and faster method was established, with an injection time of 20 minutes and an 8-minute delay between injections, compared to the previous HPLC analysis, which required a 45-minute injection time.
- Additionally, the analysis exhibited Limit of Detection (LOD) values of 0.10µg/ml for 6-gingerol, 0.04µg/ml for 6-shagaol, and 0.07µg/ml for 10-gingerol. Robust calibration curves were established for the ginger bioactive compounds—6-gingerol, 6-shagaol, and 10-gingerol—

with r^2 values of 0.9947, 0.9974, and 0.9997, respectively.
• This newly established method offers a swift and cost-effective process for analysing water-based zingiber officinale bioactive compounds.
• Moreover, the potential for future online measurement of zingiber officinale bioactive compounds extracted using subcritical water extraction exists with this technology.

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