ISOLATION AND VALIDATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR QUANTITATION OF [6]-SHOGAOL IN GINGER (ZINGIBER OFFICINALE) ROOT EXTRACT

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TÓM TẮT

TÁCH CHIẾT VÀ THẨM ĐỊNH QUY TRÌNH ĐỊNH LƯỢNG [6]-SHOGAOL TRONG GỪNG (ZINGIBER OFFICINALE) BẰNG PHƯƠNG PHÁP SẮC KÍ LỎNG HIỆU NĂNG CAO (HPLC)

Gừng (Zingiber officinale) và các thành phần của chúng từ lâu đã có tác dụng chữa bệnh độc đáo như chống ung thư, chống oxy hóa, ngăn chặn gốc tự do, kháng khuẩn, kháng viêm… Trong số các hợp chất của gừng, [6]-shogaol là hợp chất nổi bật, có khả năng ngăn ngừa ung thư đầy hứa hẹn. Mục đích của bài báo này là nghiên cứu quá trình chiết xuất, định lượng và thẩm định quy trình định lượng hợp chất [6]-shogaol bằng phương pháp sắc ký lỏng hiệu năng cao (HPLC) với đầu dò dãy điốt quang (PDA). Củ gừng khô Zingiber officinale sau khi được chiết xuất bằng phương pháp ngâm dầm và qua các quy trình sắc kí cột (CC) thu được hợp chất [6]-shogaol (10.5 mg). Kết quả thẩm định quy trình định lượng cho thấy quy trình có độ đặc hiệu cao, đạt độ tuyến tính, đạt các chỉ tiêu về độ lặp lại và độ chính xác. Phương pháp này cho thấy độ tin cậy cao và có thể triển khai ứng dụng trong thực tế khi nghiên cứu các chế phẩm từ củ gừng.

Keywords: *[6]-shogaol, (E)-1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-on, Zingiber officinale, phenolic, analytical methods, HPLC, photodiode-array detector (PDA).*

1. INTRODUCTION

Ginger (*Zingiber officinale*) belongs to family Zingiberaceae [1], containing volatile ingredients (zingiberene), non-volatile ingredients (oleoresin) and some phenolic compounds [2]. Ginger essential oil contains hydrocarbon monoterpenoids, sesquiterpenes. The main ingredients of the spicy group are gingerol, zingeron, shogaol and zingerol [3-6] ... Ginger contains 1.0% to 3.0% volatile oils

and a number of pungent compounds [7]. Ginger is one of the medicinal species commonly used to treat diseases such as rheumatism, sore throat, abdominal pain, indigestion, vomiting, hypertension, fever, infectious diseases... [8], with prominent cytotoxic activity on cancer cells, especially breast cancer [9], ovarian cancer [10], pancreatic cancer [11]. After heat treatment, gingerols can be transformed into corresponding shogaols [12]. Among phenolic compounds of ginger, [6]-shogaol is the potential research direction in the development of a new generation of anticancer drugs.

Analysis the extraction of plant are important processes for the development, modernization, and quality control of herbal. Several analytical methods including HPLC have been employed for the estimation of [6]-shogaol in ginger with different methods [13-17]. Due to the attractive pharmacological properties of [6]-shogaol, it was evaluated as potential cancer agent propitious. The PDA detector provides HPLC-absorbance techniques by identifying the compounds both by retention time and spectral behavior. In this paper, HPLC method using reverse phase column C18 was also undertaken. The method we used for the quantification of [6]-shogaol in this study is simple, the PDA detector is used commonly, the column temperature is low, the solvent system is easy to use and the method has high accuracy. This study set the stage for phenolic could be reliably used for the standardization in herbal remedies in the future. Therefore, we conducted the study "Isolation and validation of [6]-shogaol in ginger root extract (*Zingiber officinale*) by high performance liquid chromatography (HPLC)" by extracting and isolating the [6]-shogaol compound, developing and quantitating a method for the quantification of isolated compound. The research results contribute to the standardization of raw materials containing [6]-shogaol in ginger root for the quality management of natural product in the market.

2. RESEARCH MATERIALS AND METHODS

2.1. Reagents, chemical and plant materials

Standard [6]-shogaol (≥ 90%) was supplied by Sigma-Aldrich (Germany), lot number BCBZ1777. Acetonitrile (MeCN), methanol (MeOH), phosphoric acid (H_3PO_4), distilled water used for HPLC were supplied by Fisher Scientific Korea Ltd (Korea). *n*-hexane, ethyl acetate (EtOAc), MeOH, ethanol (EtOH) for analytical grade were provided by Chemsol, Vietnam.

Thin layer chromatography (TLC) was performed on silica gel 60 F254 (Merck, Germany), column chromatography (CC) was performed on silica gel (40-63 mesh, Merck, Germany).

Zingiber officinale (code ZO111221) was collected in Lamdong province, Vietnam on December 2021. Specimen was identified by Dr. Nguyen Ngoc Tuan - Institute of Food and Biotechnology, Industrial University of Ho Chi Minh City. Fresh gingers (5 kg) were dried at 60˚C to yield dried ginger (1 kg). Voucher specimens were stored in Department of Pharmaceutical Biochemistry, Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam.

2.2. Methods

2.2.1 Extraction and isolation

Dried materials of *Zingiber officinale* (1 kg) were extracted in EtOH 90% in 60˚C in 3 hours under condition of ultrasound wave. The ratio of material and solvent was 1:5. Evaporation of the solvents via distillation gave a crude extract (170 g). Crude extract was carried out on CC with the increasing polarity solvents *n*-hexane, EtOAc, MeOH, respectively to obtain *n*-hexane fraction (24 g), EtOAc fraction (102 g) and MeOH fraction (20 g), corresponding. The EtOAc extract (102 g) was further subjected on a silica gel CC with gradient mixture of *n*-hexane - EtOAc (50:1 – 0:1, v/v) elution to yield 7 fractions. Continuously, fraction 3 (6 g) was chosen to do CC in *n*-hexane - EtOAc (10:1) to obtain light

yellow oil (10.5 mg). This compound was clarified structure and identified by comparing with spectrum data of ^{1}H-NMR, ^{13}C-NMR and references.

2.2.2. The qualitative and quantitative process of [6]-shogaol

Chromatography conditions: VDSpher PUR 100 C18 reversed phase column (25 cm × 4.6 mm, 5 µm); mobile phase solvent: MeCN (A): 0.1% H_3PO_4 (B). The gradient elution was as follows: 0 min 10% B, 3.5 min 18% B, 4.5 min 35% B, 6 min 40% B, 10 min 20% B; flow rate 1.0 mL/min; time 10 minutes; injection volume 20 µL; column temperature 25°C, detector UV 280 nm. All samples were filtered through a 0.22 µm membrane prior to inject into the chromatography system.

Preparation of standard solution: Dissolve 10 mg of [6]-shogaol standard in 10 mL MeOH to obtain a stock solution of 1000 ppm. From the stock solution, prepare a range of standards with actual concentrations of 2, 4, 5, 6, 8, 10 ppm. Sample was filtered through a 0.22 µm membrane.

Preparation of sample solution: Dissolve 2 mg of sample in a 100 mL MeOH using volumetric flask. Sample was filtered through a 0.22 µm membrane.

The quantitative process validation method [6]-shogaol:

- *The system compatibility*

Conduct chromatography 6 times of the standard sample at a concentration of 8.0 ppm. Investigate the parameters: Peak area, number of theoretical disk, and the tailing factor.

- *The specificity*

Prepare the blank sample, the test sample ppm, and the standard sample 10 ppm. Three samples were chromatographically performed under the same chromatographic conditions.

- *The repeatability*

Prepare a 10 ppm test sample. Quantify the sample 6 times with chromatographic conditions. The results of the evaluation are based on the relative standard deviation (%RSD) of the concentration.

- *The linearity*

From the 1000 ppm stock standard solution, prepare standard solution samples at concentrations from 1 ppm to 10 ppm. Conduct chromatography of 6 standard samples and determine the correlation curve between the peak area and the standard concentration to prepare the regression equation.

- *Limit of detection and limit of quantification*

The LOD and LOQ can be determined based on the slope of the standard curve and the standard deviation of the measured signal, according to the following formula:

$$LOD = \frac{3{,}3 \times SD}{a}$$

$$LOQ = 3.3 \times LOD$$

With:

- SD: Standard deviation of the signal
- a: slope of the standard curve

- *The precision*

Add 0.5 mg of sample [6]-shogaol dissolved in MeOH and dilute to a 100 mL volumetric flask to obtain solution A with a theoretical concentration of 5 ppm.

Add 0.4 mg, 0.5 mg, and 0.6 mg standard samples dissolved in a 10 mL volumetric flask with MeOH, respectively. Add 1 mL of the standards with concentrations of 40 ppm, 50 ppm, and 60 ppm, and dilute in a 10 mL volumetric flask with solution A, obtain a solution with a concentration of the standard sample that varies by 80 %, 100 % and 120 % compared with the test sample.

Carry out the analysis at each concentration three times under the same chromatographic conditions. Calculate the content of the samples to which the standard was added at each concentration, % recovery (%R) at each concentration added, and the relative standard deviation (%RSD).

2.3. Statistical analysis

Statistical analysis was performed using Origin Pro 8.5 (OriginLab Inc., Northampton, USA). All experiments were carried out at least three times, and the data are represented as the mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Determining the structure of the isolated compound

^{1}H-NMR (500 MHz, CD_3OD) δ_H: 0,89 (3H, t, J = 6,8 Hz), 1,30 (4H, m), 1,45 (2H, m), 2,19 (2H, q, J = 7,2 Hz), 2,85 (4H, m), 3,87 (3H, s), 6,09 (1H, d, J = 15,6 Hz), 6,69 (1H, d, J = 8,0 Hz), 6,71 (1H, brs), 6,82 (2H, m).

^{13}C-NMR (125 MHz, CD_3OD) δ_C: 30.4 (C-1), 42.5 (C-2), 200.4 (C-3), 130.9 (C-4), 148.5 (C-5), 31.9 (C-6), 28.3 (C-7), 32.5 (C-8), 23.0 (C-9), 14.5 (C-10), 133.8 (C-1'), 111.7 (C-2'), 146.9 (C-3'), 144.4 (C-4'), 114.9 (C-5'), 121.4 (C-6'), 55.5 (3'-OCH_3).

From the ^{1}H-NMR it was observed that there are three aryl protons, two olefinic proton, methoxy groups (δ_H 3.87), and doublet for H-4 proton (δ_H 6,09). This arrangement of groups is revealed that the molecular has phenyl and alkyl group. The ^{13}C-NMR and HSQC spectrum indicate presence of unsaturated alkyl groups containing 10 carbons. Clarifying the three quaternary carbon locations in aryl group. In addition, a carbonyl group liking with alkyl group at (δ_C 200.4). In HMBC correlation, two olefinic proton at δ_H 6.09 (δ_C 130.9) and δ_H 6.11 (δ_C 148.5) correlated to each other and to the ketone carbon at (δ_C 200.4). Along with other data was summarized [18], the molecular structure was figured out. The NMR results revealed that the isolated compound is (E)-1-(4-Hydroxy-3-methoxyphenyl)-dec-4-en-3-one which common name is [6]-shogaol. Structure of this compound on the basis data presented above would be as shown in Figure 1.

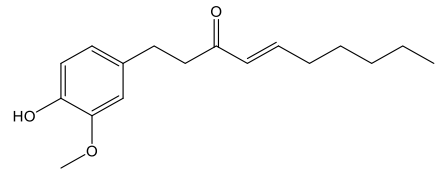

Figure 1. Structure of [6]-shogaol

3.2. Validation of the quantitative process [6]-shogaol in *Zingiber officinale* by HPLC method

3.2.1. The qualitative result of [6]-shogaol by HPLC method

Table 1. The qualitative result of [6] shogaol by HPLC method

Samples	Blank sample	Standard sample	Test sample
Retention time (t_R)	There is no peak	4.185	4.171
		4.188	4.167
Mean ± SD		4.178 ± 0.010	

3.2.2. Validation of the quantitative process of [6]-shogaol by HPLC method

- *The system compatibility*

The [6]-shogaol standard was analyzed six times with a concentration of 8.0 ppm and was recorded the chromatograms (Figure 4). The result of retention time was determined 4.194 ± 0.0059 minutes (x ± SD, RSD = 0.14%), the peak area was 1353588.4 ± 20946.1 (x ± SD, RSD = 1.547%), RSD value of retention time, peak area < 2%, tailing factor was 1.01, solvent system and flow rate did not cause peak widening. The theoretical disk values > 4000 showed that no peak overlapping at the [6]-shogaol peak position. The [6]-shogaol peak signal wasn't interfered with by other substances. Conclusion, the process achieved the system compatibility.

Table 2. The system compatibility of [6]-shogaol

Std. samples	Retention time t_R (mins)	Peak area (mAU)	The tailing factor	The theoretical disk
2	4.200	1321655.4	1.01	4044
1	4.197	1207002.9	1.00	4849
3	4.197	1353625.2	1.03	4382
4	4.192	1361703.8	0.99	5207
5	4.197	1212083.3	1.03	4345
6	4.182	1259268.4	1.00	4702
Mean	4.194	1353588.4	1.01	4588
SD	0.0059	20946.1		
RSD (%)	0.14	1.547		

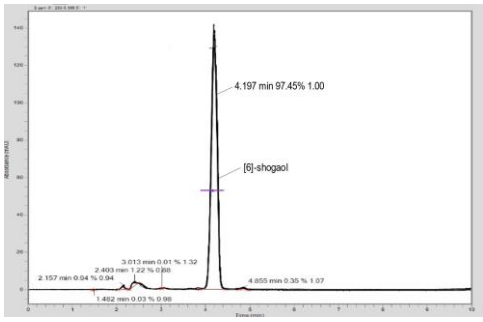

Figure 2. The system compatibility chromatogram of [6]-shogaol

- *The specificity*

The [6]-shogaol standard 10 ppm, the test sample 10 ppm and the blank sample were simultaneously analyzed under the selected chromatographic condition. Table 3 showed the specificity of the method. The results had the peak appearance of the standard sample chromatogram, the corresponding peak appears on the test sample chromatogram, no corresponding peak on the blank sample. The peak of [6]-shogaol on a well-balanced, sharp sample.

Table 3. The specificity of [6]-shogaol

Samples	Retention time (t_R)	Peak area (mAU)
Blank sample		
Standard sample	4.172	3102498.7
Test sample	4.082	1386073.6
	4.109	1539096.0
Mean	4.121	

- *The linearity*

[6]-shogaol standard series at concentrations of 2, 4, 5, 6, 8, 10 ppm were analyzed under the selected condition. In the concentration range investigated, there was a linear relationship between peak area and concentration of analyzed compound. The linear regression equation for [6]-shogaol is y = 166129.76x + 22743.83 with the correlation coefficient R^2 = 0.9997 (Figure 3 and 4).

Table 4. The calibration curve of [6]-shogaol

Samples	1	2	3	4	5	6
Std conc. (ppm)	2	4	5	6	8	10
Peak area (mAU)	357556	684520	841332	1031872	1355249	1680474
The linear regression equation: y = 166129.8x + 22743.8						
The correlation coefficient: R^2 = 0.9997						

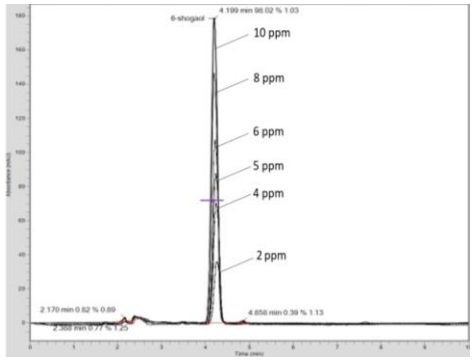

Figure 3. The linear range chromatogram of [6]-shogaol

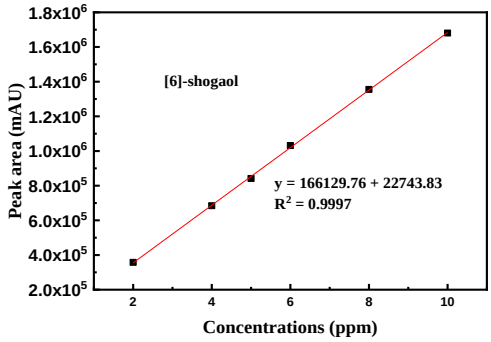

Figure 4. The calibration curve of [6]-shogaol

- *The precision*

Determining the recovery rate with the standard sample was added to the test sample at 80%, 100%, and 120% on the test sample 5 ppm, respectively. Each concentration was carried out 3 times with the same condition, the results were presented in Table 5.

In all tests, the RSD value were less than 2%, the average recovery rate reached the limit from 98% to 102%, so the method had satisfactory precision.

Table 5. The precision of [6]-shogaol

No.	% Std. addition	Peak area	Theoretical conc. (ppm)	Actual conc. (ppm)	Recovery efficiency (%)	Mean (%)	RSD %
1	80%	913287.9	4	4.10	102.0	99.33	1.89
2		879911.3		3.90	98.0		
3		882843.7		3.92	98.0		
4	100%	1045295.3	5	4.90	98.0	98.87	1.24
5		1052866.8		4.90	98.0		
6		1068383.5		5.03	100.6		
7	120%	1237863.9	6	6.05	100.8	99.72	0.89
8		1215788.8		5.92	98.7		
9		1224978.6		5.98	99.7		
Mean						99.30	1.34

▪ *The repeatability*

Conducted six separate experiments for 6 test samples with the concentrations of 10 ppm into the HPLC system at 100% concentration, the results were presented in Table 6.

Table 6. The repeatability of [6]-shogaol

Samples	Peak area (mAU)	Concentration (ppm)
1	254382.6	1.46
2	247883.2	1.42
3	256213.4	1.47
4	254131.6	1.39
5	255960.4	1.46
6	256722.7	1.45
Mean	254215.7	1.44
RSD (%)		1.99

The results of Table 6 showed that the repeatability was acceptable with RSD = 1.99% (< 2%). The test results were highly reproducible, the analytical method was less affected by systematic errors and random errors. Therefore, the process was applicable to the analysis of the test sample.

▪ *Limit of detection and limit of quantification*

$$LOD = \frac{3.3 \times 20946.1}{167761} = 0.41 \text{ (ppm)}$$

$$LOQ = \frac{10 \times 20946.1}{166130} = 1.25 \text{ (ppm)}$$

The results showed that the limit of detection and limit of quantification of [6]-shogaol were 0.41 ppm and 1.25 ppm, respectively.

3.2.3. Quantification of [6]-shogaol by HPLC method

HPLC analysis was determined that the [6]-shogaol content in crude extract EtOH was 3.6%, in fraction 3 of EtOAc extract was 15.6% and in the purified was 96.3%.

4. CONCLUSION

The result of the [6]-shogaol content in raw material sample, fraction 3 and extracted product are respectively 3.6%, 15.6% and 96.3%. The HPLC method for quantitation of [6]-shogaol in ginger root extract is suitable for the validation process in accordance with ICH guidelines. The validation results show that the method developed in this study has a wide linear range, good specificity, repeatability and recovery rate. Therefore, it is a suitable process for the quantification of [6]-shogaol in ginger-derived products.

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