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Simultaneous determination of gingerols and shogaol using capillary liquid chromatography and its application in discrimination of three ginger varieties from Indonesia

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ABSTRACT

A new method using reversed phase capillary liquid chromatography was developed for simultaneous determination of four bioactive compounds found in ginger (*Zingiber officinale*) namely, 6-, 8-, 10-gingerol, and 6-shogaol. The separation of these four compounds was performed using C30 as the stationary phase and 60% acetonitrile as the mobile phase in isocratic elution mode with a flow rate of 5 μL/min. All four compounds were separated within 25 min with good resolution. As the evaluation of method validation, a linear regression of the four compounds was obtained within the tested range with correlation coefficients ≥ 0.9995. The limits of detection and quantitation were between 0.034–0.039 μg/mL and 0.112–0.129 μg/mL, respectively. Intra- and inter-day precision expressed as relative standard deviations (RSD) were less than 3.1%, and the accuracy based on recovery test was ranging from 97% to 105%. Stability of the analytes within 1 day was found in the range between 1.34% and 2.93% (RSD). In addition, based on the amount of these four compounds combining with the discriminant analysis, a reliable and accurate method was developed for discrimination of three ginger varieties found in Indonesia. The results indicated that the developed method could be used as quality control for ginger raw material and its related products.

1. Introduction

Ginger, botanically known as *Zingiber officinale* belongs to the family of Zingiberaceae. It has been used worldwide as a spice and flavoring agent in foods and beverages as well as dietary supplement and herbal medicines [1,2]. Ginger is extensively used in traditional Chinese medicines, Ayurveda (traditional Indian medicines) and western herbal medicines [2,3] for treating dyspepsia, colic, nausea, vomiting, diarrhea, motion sickness, arthritis, rheumatic disorders and muscular discomfort [3–7]. Recently, ginger has been increasingly used because this rhizome has broad spectrum of biological activities and low toxicity [5]. Many countries have been including ginger in their national pharmacopeias such as China, India, Japan, Indonesia, the United Kingdom, Germany, the United States, etc.

According to the Food and Agriculture Organization (FAO) of the United Nations, Indonesia ranked 6th in the world for the production of ginger in 2010 [8] and its rhizomes have been cultivated extensively in Java Island. *Jahe* is the Indonesian name for ginger and traditionally used as an ingredient in a Javanese traditional spice drink called *wedang jahe* and it is also widely used in *jamu* (traditional Indonesian medicines). There are three varieties of ginger growth in Indonesia, namely *jahe emprit* (*Z. officinale* var *amarum*, ZOA), *jahe gajah* (*Z. Officinale* var *officinarum*, ZOO) and *jahe merah* (*Z. officinale* var *rubrum*, ZOR). ZOA and ZOR are commonly used for dietary supplement or herbal medicines, while the ZOO is usually used for cooking spices or flavor for food and beverages. These three varieties of ginger showed different level in its pungency taste due to the different amount of some homologous phenolic ketones, namely 6-, 8-, 10-gingerol and 6-shogaol that have been identified as the principal pungent compounds in ginger [9]. Differences in the amount of these pungent compounds could be due to species variation, environmental factors of growth (e.g. geographical origin, climate, soil type, and nutrient availability), harvesting, and postharvest processes (e.g. washing, drying, crushing, pulverizing, storing, and extraction for making the final products).

These four compounds especially 6-gingerol are considered to be important ingredients associated for various biological activities of ginger such as antiemetic [10], anticancer [11], antipyretic [12], antioxidant and antiinflammatory [13]. Also they have been utilized as a marker compound for quality evaluation of ginger

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based dietary supplement and herbal medicines. Differences in the amount of these bioactive compounds also may affect the resulted of biological activities from each ginger. So, in order to ensure successful use of gingers as dietary supplement or herbal medicines, accurate quantitation of the bioactive compounds mentioned above and discrimination of the three varieties of ginger to prevent an adulteration have become a crucial task.

Several analytical methods have been developed for qualitative or quantitative analysis of gingerols and shogaol, and in most cases, liquid chromatography (LC) techniques such as high performance liquid chromatography (HPLC) coupled with an ultraviolet detector [1,9,14,15], a mass spectrometer [16] and an electrochemical detector [17] with octyl (C8) or octadecyl (C18) as the stationary phase packed into conventional size column (4.6 mm i.d.) are employed. Nowadays, miniaturization of a chromatographic system such as microcolumn LC (μLC) is one of the present trends in the field of separation sciences. μLC or also known as capillary LC (CLC) was firstly introduced by Horvarth et al. [18], who used a stainless steel packed column with an 0.5–1.0 mm i.d.. Thereafter, Takeuchi and Ishii also started using μLC with a slurry packed microcolumn in their works [19]. The main features of CLC compared with the conventional-sized LC are the use of columns with smaller i.d. and lower eluent flow rate. With these features, CLC offers several analytical advantages such as improved mass sensitivity, reduced consumption of packing materials, mobile phase, and the sample amounts [20]. In recent years, CLC has shown significant progress in the instrumentation and applications. There are a number of reported works using CLC in the quantitative analysis of phenolic compounds in apple juices [21], cellular flavins [22] and fat-soluble vitamins and β-carotene [23].

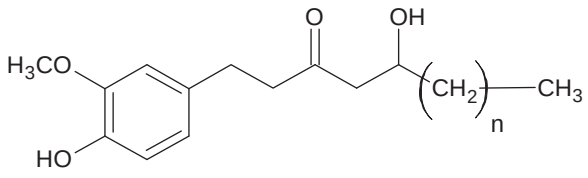
Hydrophobic stationary phases such as C8, C18 and triacontyl (C30) are widely used in reversed phase LC due to their good separation efficiency for a wide range of analytes especially organic molecules. In an early study, C8 and C18 have been used for the separation of gingerols and shogaol. It was found that the elution time for a single chromatographic run with complete separation of these compounds were 40 min using C8 under isocratic elution (65% methanol) [1] and 35 min using C18 with gradient elution as follows: 40% acetonitrile at 0–10 min; 40–90% acetonitrile at 10–40 min; 100% acetonitrile at 40.5–45 min; and 40% acetonitrile at 45.5–50 min. [9]. C30 phases are more hydrophobic than C8 and C18 and are commonly used for the separation of long chain isomeric analytes such as carotenoids [24], (polycyclic aromatic hydrocarbon) PAHs [25], tocopherols [26] etc. As we can see from the structure of gingerols and shogaol (Fig. 1), these compound have a phenyl group and a long chain structure, C30 is therefore expected to be more suitable for the separation of gingerols and shogaol, and perhaps could reduce the total analysis time by optimizing the experimental conditions.

To date, to the best of our knowledge, there was no reported work regarding quantitative analysis of gingerols and shogaol using CLC with C30 as the stationary phase. So, based on what we described above we developed for the first time a quantitative analysis method of gingerols and shogaol by CLC. In addition, we also developed a method for discrimination of three ginger varieties growth in Indonesia based on the amount of gingerols and shogaol present combined with discriminant analysis. The proposed method could be successfully applied for quality control of gingers raw material and its related products.

2. Material and methods

2.1. Chemicals and reagents

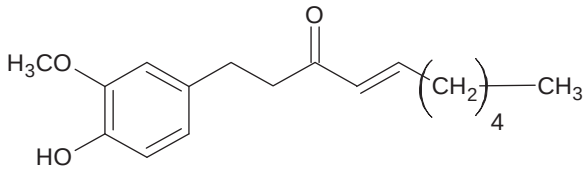
6-gingerol and 6-shogaol were obtained from Wako Pure Chemical Industries (Osaka, Japan), while 8-gingerol and 10-gingerol



n = 4; 6-gingerol

n =6; 8-gingerol

n = 8; 10-gingerol



6-shogaol

Fig. 1. Chemical structures of 6-, 8-, 10-gingerol and 6-shogaol.

were obtained from Chromadex Inc. (Santa Ana, CA, USA). All solvents used were analytical or HPLC grade and obtained from Kanto Chemicals (Tokyo, Japan), membrane filters of Ekicrodisc 25R (0.45-μm pore size; PTFE; P/N E252) obtained from Gelman Science Japan Co. (Tokyo, Japan) were used for the filtration of the mobile phase and the real samples solution. Develosil C30-UG-5 (C30; mean particle diameter, 5 μm; Nomura Chemical, Seto, Japan) and C18 (L-column2 ODS, Chemical Evaluation and Research Institute, Tokyo, Japan) were used as the stationary phases. These stationary phases were packed into fused-silica tubes (150 mm in length × 0.32 mm i.d. × 0.46 mm o.d.; GL Sciences, Tokyo, Japan).

2.2. Plant materials

Thirty seven samples from three varieties of gingers were collected from various locations in Java Island, Indonesia (Table 1). All of the samples were identified in Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Indonesia and voucher specimens were deposited at Biopharmaca Research Center, Bogor Agricultural University, Indonesia (BMK 2012050001–BMK 2012050037). All samples were sieved, dried and pulverized prior to use.

2.3. Apparatus and chromatographic conditions

An ultrasonication device (USD-3R; As One, Osaka, Japan) was used for samples extraction. CLC system used in this work consists of an L.TEX 8301 Micro Feeder (L.TEX Corp., Tokyo, Japan) equipped with an MS-GAN 050 gas-tight syringe (0.5 mL Ito, Fuji, Japan) as a pump, a model M-435 microinjection valve (Upchurch Scientific, Oak Harbor, WA, USA) with the injection volume of 0.2 μL as an injector, a 0.32 mm i.d. × 150 mm microcolumn and a UV-2705 UV detector (JASCO, Tokyo, Japan). The data were acquired by a Chromatopac C-R4A data processor (Shimadzu, Kyoto, Japan). Acetonitrile aqueous solution was used as the mobile phase in the isocratic elution mode with a flow rate of 5 μL/min and monitored at 280 nm. Peaks of 6-, 8-, 10-gingerol and 6-shogaol were identified by comparing the retention times with the standards.

Table 1
Sources of sample and the amounts of the four bioactive compounds in gingers.

Sample code	Sources (subdistrict, regency, province)	Amounts (mg/g ± SD), $n=5$							
		6-gingerol	RSD (%)	8-gingerol	RSD (%)	10-gingerol	RSD (%)	6-shogaol	RSD (%)
ZOA-1	Arjosari, Pacitan, East Java	11.53 ± 0.11	0.94	2.47 ± 0.06	2.24	3.07 ± 0.02	0.67	2.56 ± 0.07	2.60
ZOA-2	Pacitan, Pacitan, East Java	9.54 ± 0.16	1.70	2.19 ± 0.05	2.20	2.73 ± 0.11	4.36	2.75 ± 0.09	3.29
ZOA-3	Tegal Ombo, Pacitan, East Java	8.85 ± 0.06	0.62	2.56 ± 0.04	1.56	3.54 ± 0.02	0.62	1.89 ± 0.04	1.98
ZOA-4	Ponorogo, Ponorogo, East Java	6.48 ± 0.11	1.66	1.44 ± 0.04	2.77	2.13 ± 0.05	2.38	2.34 ± 0.07	2.94
ZOA-5	Pulung, Ponorogo, East Java	9.28 ± 0.30	3.21	1.85 ± 0.05	2.50	2.25 ± 0.09	4.10	2.31 ± 0.06	2.79
ZOA-6	Tawangmangu-1, Karanganyar, Central Java	12.91 ± 0.20	1.55	2.88 ± 0.05	1.62	3.36 ± 0.05	1.59	3.23 ± 0.06	2.03
ZOA-7	Tawangmangu-2, Karanganyar, Central Java	6.78 ± 0.24	3.40	1.52 ± 0.06	3.69	2.18 ± 0.06	2.91	1.75 ± 0.03	1.87
ZOA-8	Ngadirojo, Wonogiri, Central Java	14.6 ± 0.22	1.53	2.68 ± 0.05	1.83	2.74 ± 0.05	1.87	3.05 ± 0.09	2.94
ZOA-9	Tirtomoyo, Wonogiri, Central Java	9.00 ± 0.28	3.11	2.14 ± 0.01	0.63	2.66 ± 0.04	1.35	3.27 ± 0.09	2.60
ZOA-10	Wonogiri-1, Wonogiri, Central Java	11.29 ± 0.09	0.76	1.97 ± 0.03	1.63	2.35 ± 0.02	0.97	2.84 ± 0.03	1.07
ZOA-11	Wonogiri-2, Wonogiri, Central Java	7.29 ± 0.12	1.66	1.56 ± 0.03	1.61	2.05 ± 0.03	1.44	2.47 ± 0.05	2.17
ZOA-12	Kutoarjo, Purworejo, Central Java	9.86 ± 0.37	3.71	2.07 ± 0.05	2.32	2.58 ± 0.07	2.87	3.53 ± 0.12	3.50
ZOA-13	Ciampea, Bogor, West Java	18.83 ± 0.28	1.47	3.07 ± 0.03	1.01	2.63 ± 0.09	3.47	2.16 ± 0.03	1.27
ZOO-1	Arjosari, Pacitan, East Java	4.46 ± 0.11	2.57	0.75 ± 0.01	1.80	1.20 ± 0.03	2.16	1.31 ± 0.05	3.47
ZOO-2	Pacitan, Pacitan, East Java	7.63 ± 0.21	2.77	1.31 ± 0.03	2.31	2.13 ± 0.07	3.09	1.78 ± 0.03	1.89
ZOO-3	Tegal Ombo, Pacitan, East Java	6.84 ± 0.13	1.90	1.34 ± 0.04	3.04	2.33 ± 0.04	1.52	1.39 ± 0.04	2.73
ZOO-4	Ponorogo, Ponorogo, East Java	8.79 ± 0.20	2.28	1.34 ± 0.07	4.86	2.01 ± 0.03	1.58	1.72 ± 0.06	3.49
ZOO-5	Slahung, Ponorogo, East Java	6.49 ± 0.22	3.43	1.08 ± 0.01	1.35	1.65 ± 0.05	2.79	2.21 ± 0.06	2.60
ZOO-6	Tirtomoyo, Wonogiri, Central Java	3.77 ± 0.10	2.70	0.65 ± 0.03	4.27	1.13 ± 0.02	1.41	1.76 ± 0.04	2.32
ZOO-7	Wonogiri-1, Wonogiri, Central Java	10.52 ± 0.11	1.08	2.00 ± 0.05	2.30	3.01 ± 0.06	2.13	2.15 ± 0.05	2.15
ZOO-8	Wonogiri-2, Wonogiri, Central Java	7.29 ± 0.11	1.48	1.35 ± 0.03	2.32	2.10 ± 0.08	4.01	1.86 ± 0.07	3.59
ZOO-9	Ciemas, Sukabumi. West Java	11.94 ± 0.19	1.58	1.84 ± 0.04	2.20	2.85 ± 0.03	1.08	2.49 ± 0.05	2.14
ZOO-10	Nagrak, Sukabumi, West Java	12.23 ± 0.16	1.27	1.54 ± 0.02	1.18	2.29 ± 0.06	2.47	3.02 ± 0.04	1.38
ZOO-11	Ciampea, Bogor, West Java	11.76 ± 0.34	2.87	1.86 ± 0.05	2.57	2.97 ± 0.15	4.97	2.53 ± 0.06	2.41
ZOO-12	Leuwiliang, Bogor, West Java	2.98 ± 0.06	2.17	0.52 ± 0.02	3.32	0.84 ± 0.03	3.00	1.24 ± 0.03	2.76
ZOR-1	Pacitan, Pacitan, East Java	5.35 ± 0.17	3.10	1.06 ± 0.04	4.21	1.18 ± 0.02	1.96	1.56 ± 0.03	1.73
ZOR-2	Tegal Ombo, Pacitan, East Java	7.25 ± 0.10	1.40	1.41 ± 0.02	1.59	1.41 ± 0.04	3.00	1.76 ± 0.02	1.29
ZOR-3	Ponorogo, Ponorogo, East Java	6.43 ± 0.13	2.04	1.38 ± 0.04	2.59	1.37 ± 0.01	0.91	3.12 ± 0.04	1.17
ZOR-4	Slahung, Ponorogo, East Java	7.29 ± 0.33	4.59	1.74 ± 0.05	2.73	1.84 ± 0.07	3.85	2.16 ± 0.11	5.43
ZOR-5	Wonogiri-1, Wonogiri, Central Java	5.86 ± 0.20	3.35	1.39 ± 0.06	4.05	1.55 ± 0.06	3.82	1.79 ± 0.04	2.40
ZOR-6	Wonogiri-2, Wonogiri, Central Java	3.30 ± 0.09	2.87	0.89 ± 0.02	2.78	1.06 ± 0.04	4.13	1.24 ± 0.03	2.06
ZOR-7	Kutoarjo, Purworejo, Central Java	10.29 ± 0.15	1.49	1.80 ± 0.06	3.51	1.38 ± 0.06	4.02	2.55 ± 0.09	3.42
ZOR-8	Purworejo, Purworejo, Central Java	5.02 ± 0.14	2.84	1.12 ± 0.02	1.73	1.34 ± 0.05	3.59	1.71 ± 0.04	2.39
ZOR-9	Ciampea-1, Bogor, West Java	15.34 ± 0.42	2.72	2.41 ± 0.06	2.53	1.39 ± 0.03	2.11	2.20 ± 0.02	0.99
ZOR-10	Ciampea-2, Bogor, West Java	7.86 ± 0.17	2.17	1.76 ± 0.06	3.58	2.04 ± 0.06	2.72	1.80 ± 0.05	2.71
ZOR-11	Leuwiliang, Bogor, West Java	5.62 ± 0.14	2.46	0.81 ± 0.02	2.38	0.79 ± 0.01	0.78	1.45 ± 0.05	3.35
ZOR-12	Nagrak, Sukabumi, West Java	14.88 ± 0.17	1.12	2.12 ± 0.04	2.04	1.70 ± 0.06	3.43	2.12 ± 0.03	1.31

2.4. Column preparation

The packing technique employed in this work was almost the same as previously reported [19]. Fused silica tubes were used as the column with both ends of the tube were inserted into PTFE tubes (0.26 mm i.d. × 2 mm o.d.) and doubly covered with another large-bore PTFE in order to eliminate leakage due to the high pressure. Quartz wool was also inserted into one end of the fused silica tube in order to prevent the loss of packing material from the column during packing process. The packing material (C18 and C30) was dispersed in methanol by ultrasonication for a few minutes and then was fed into PTFE tube and filled the column by manually forcing methanol from a gas-tight syringe.

2.5. Standard solutions and sample preparation

Accurately weighed powdered samples (50 mg) were sonicated with methanol (5 mL) for 1 h at room temperature. The samples extracts were filtered through a 0.45 μm membrane filter before being injected into CLC. Standard stock solutions of the 6-, 8-, 10-gingerol and 6-shogaol were prepared in methanol at concentrations of 1600 μg/mL for 6-gingerol and 1000 μg/mL for 8-gingerol, 10-gingerol and 6-shogaol. An appropriate amount of each standard stock solution was mixed and diluted with methanol to obtain seven concentrations of the working standard solutions of the four analytes for constructing the calibration curves.

2.6. Method validation

Validation of the method was evaluated following the guidelines of the International Conference on Harmonization (ICH) by determining the linearity of the calibration curves, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and stability [27]. The sample used for the validation tests was ZOA-6.

2.7. Data analysis

In this work, discriminant analysis was used to build a model for discrimination of the three ginger varieties and it was performed in XLSTAT software version 2012.2.02 (Addinsoft, New York, NY, USA). We used the amounts of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol as the variables.

3. Results and discussions

3.1. Optimization of CLC condition

In order to obtain good separation of the analytes in the chromatogram, the type of stationary phase and the mobile phase composition were optimized in this study. For the detection wavelength we used 280 nm according to the previous reference [9]. We examined and compared reversed phase C18 and C30 as the stationary phase with acetonitrile–water as the mobile

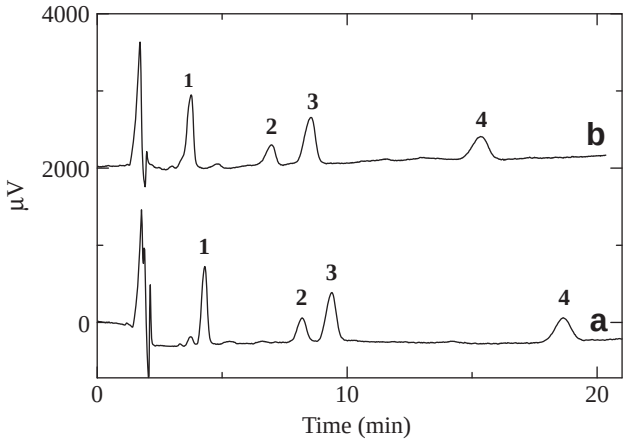


Fig. 2. CLC chromatogram of standard solution of gingerols and shogaol on the C30 and C18 stationary phases. Column: C30 (a) or C18 (b), 100 × 0.32 mm i.d. Mobile phase 60% acetonitrile. Flow-rate: 5.0 μL/min. Sample: 6-gingerol (1), 8-gingerol (2), 6-shogaol (3) and 10-gingerol (4); 10 μg/mL each except for 8-gingerol, 5 μg/mL. Sample injection volume: 0.2 μL. Wavelength of UV detection: 280 nm.

phase in different compositions (60%, 70% and 80%). The flow rate was maintained at 5 μL/min. The resolution of each analyte and the total analysis time were used as the parameters for choosing the optimized chromatographic conditions. Representative chromatograms from the standard solution of analytes are shown in Fig. 2. From the results obtained, by using C30 as stationary phase and 60% acetonitrile as mobile phase, a good separation was achieved for all analytes with a separation factor of each peak greater than 1.5 and total analysis time within 25 min. Separation of these four compounds were also achieved using C18 as the stationary phase and 60% acetonitrile in a shorter time compared to C30 with the same mobile phase composition, but the gingerol peak is not pure because there is an impurity peak overlapped with the 6-gingerol peak as we can see in Fig. 2.

3.2. Method validation

Validation of the method was evaluated in terms of linearity of calibration curves, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and stability. Calibration was performed at seven concentration levels with three replicates injection over the concentration range 5–320 μg/mL by plotting the peak areas versus the concentration of each analyte. Linearity of the calibration curves was determined by the correlation coefficient (r^2). Good linearity was obtained with a mean correlation coefficient value greater than 0.9995 for all analytes within the test range. LODs ($S/N=3$) and LOQs ($S/N=10$) were found to be between 0.034–0.039 μg/mL and 0.112–0.129 μg/mL, respectively. Detailed information for the calibration curves, LOD and LOQ are summarized in Table 2.

Precision of the method was examined by intra- and inter-day repeatability of six individual samples in each day within three consecutive days. The precision was expressed as relative standard deviation (RSD) and the values obtained for intra- and inter-day were found to be less than 3.1%, which showed good repeatability of the method. The accuracy of the method was evaluated by carrying out recovery test with three different concentration levels and triplicate measurements at each level. The average percentages of recovery for all analytes were found to be in the range of 97–105% with RSD values below 4%. These results demonstrated that the established method was reliable and accurate. Stability of analytes in sample solution was evaluated by analyzing the sample solution within 1 day at 0, 4, 8, 12, and 24 h at room temperature. The analytes were found to be stable in the sample solution with RSD values range between 1.34% and 2.93% for all compounds. Analytical data for precision, accuracy and stability were tabulated in Table 3.

Table 2
Calibration curves, LOD and LOQ for quantitative analysis of the four bioactive compounds.

Analyte	Regression equation[a] ($y=a+bx$)	Correlation coefficient (r^2)	LOD (μg/mL)	LOQ (μg/mL)
6-gingerol	$-1394.2+1640.9x$	0.9995	0.034	0.112
8-gingerol	$-46.1+1429x$	0.9996	0.039	0.129
6-shogaol	$-740.46+1523.3x$	0.9997	0.036	0.121
10-gingerol	$-2391.5+1555.9x$	0.9997	0.036	0.118

[a] Concentration range for all analytes: 5–320 μg/mL (triplicate measurement).

Table 3
Analytical data for precision, recovery and stability of the proposed method.

Analyte	Precision (RSD, %)		Recovery[a]		Stability[b] ($n=5$)
	Intraday ($n=6$)	Interday ($n=3$)	Average recovery (%)[a]	RSD (%) ($n=3$)	
6-gingerol	Day 1: 2.42 Day 2: 1.98 Day 3: 2.55	1.69	105	1.33	1.61
8-gingerol	Day 1: 2.21 Day 2: 1.34 Day 3: 2.79	2.49	104	2.46	1.35
6-shogaol	Day 1: 2.48 Day 2: 2.91 Day 3: 3.06	2.62	97	3.60	2.94
10-gingerol	Day 1: 2.29 Day 2: 1.40 Day 3: 2.24	2.30	100	2.37	2.24

[a] Three levels of added standard compounds (6-gingerol: 50, 125 and 200 μg; 8-gingerol: 45, 110, and 175 μg; 10-gingerol: 50, 120 and 190 μg; 6-shogaol: 45, 120, 190 μg) in the sample solution with each level measured in triplicate.

[b] For five measurements at 0, 4, 8, 12 and 24 h after the extraction of the sample.

3.3. Quantitative analysis of gingerols and shogaol in three varieties of ginger

The established CLC method was applied to the simultaneous determination of gingerols and shogaol in the 37 samples of gingers consisting of 13 ZOA samples, 12 ZOO samples and 12 ZOR samples. Each sample was analyzed with five replicate measurements to determine the mean amount of each analyte. Fig. 3 shows representative chromatograms of the three varieties of ginger and the target analytes. It can be seen that the objective components are well separated from coexisting species.

As shown in Table 1, the amounts of 6-, 8-, 10-gingerol and 6-shogaol in all samples were ranging from 2.98–18.83, 0.52–3.07, 0.79–3.54 and 1.24–3.53 μg/g, respectively. 6-gingerol was found to be the most dominant compound, while 8-gingerol was the lowest in amount in majority of the raw material samples investigated. This variation in the amount of gingerols and shogaol may be due to the variations of geographical origin, harvest time, environmental growth conditions and post-harvest processes. Due to these great differences in the amount of gingerols and shogaol in the three varieties of gingers, we could not discriminate them only based on the amount of these four bioactive compounds. Therefore, chemometric method, which is widely employed for discriminating closely related medicinal plants, was also used.

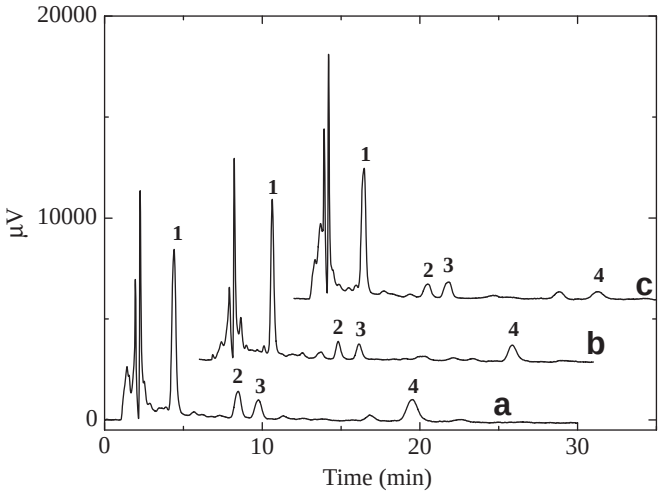


Fig. 3. Representative CLC chromatogram of real samples on the C30 stationary phase. 6-gingerol (1), 8-gingerol (2), 6-shogaol (3) and 10-gingerol (4). ZOA (a), ZOO (b) and ZOR (c). Operating conditions as in Fig. 2.

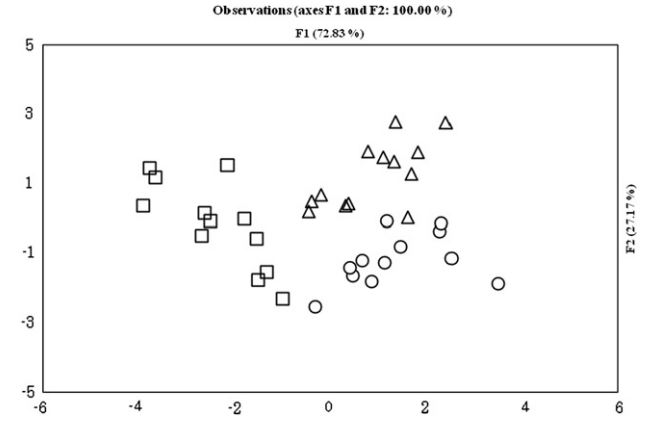


Fig. 4. Discrimination of three ginger varieties by discriminant analysis. ZOA (Δ), ZOO (□) and ZOR (○).

3.4. Discrimination of three ginger varieties

In order to develop a method for discriminating the three varieties of ginger, we employed a combination of LC analysis with a chemometric method. This combination has become one of the most frequently applied approaches for classification, authentication and discrimination of medicinal plants for recognition of geographical origin, detection of adulteration and discrimination of closely related species. Discriminant analysis is one of the multivariate statistical analyses that can be used for this purpose. Discriminant analysis will construct discriminant functions for each group by finding the linear combinations of features that give better separation in two or more groups of observations. In this study, the predictive model of the variety group is built based on the amount of gingerols and shogaol as the variables.

The result of discriminant analysis showed that 100% of each observation groups (ZOA, ZOO and ZOR) were correctly classified (as shown in Fig. 4), or in other words, the discriminant functions obtained discriminate well between each group. Cross-validation was used to evaluate the predictive ability of the discriminant model and it was found that 94% from all of the samples were correctly classified while 2 samples (ZOA-13 and ZOR-12) were misclassified. So, based on these results, the amount of gingerols and shogaol are good predictors for discrimination of the three varieties of ginger used in this study.

4. Conclusion

The CLC method was successfully developed for simultaneous quantitative analysis of 6-, 8-, 10-gingerol and 6-shogaol in raw materials of the three varieties of gingers with good accuracy and reliability. Combination between the amount of gingerols and shogaol determined by CLC with discriminant analysis could discriminate the three varieties of ginger tested. The simultaneous quantitative analysis in combination with discriminant analysis was proven to be an efficient method and practically could be applied for the quality control of ginger raw materials and its related products.

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