Research Article

การพัฒนาการตรวจวิเคราะห์สารสำคัญห้าชนิดของขิงพร้อมกัน ด้วยวิธีโครมาโทกราฟีชนิดของเหลวสมรรถนะสูง

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บทคัดย่อ

การพัฒนาการตรวจวิเคราะห์สารสำคัญห้าชนิดของขิงพร้อมกันด้วยวิธีโครมาโทกราฟีชนิดของเหลวสมรรถนะสูง

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ขิง (*Zingiber officinale* Roscoe) อยู่ในวงศ์ ซิงจิเบอราซี ซึ่งส่วนเหง้านิยมใช้เป็นอาหารและยา เนื่องจากมีฤทธิ์ทางเภสัชวิทยาหลายประการ สารสำคัญของเหง้าขิงมีหลายกลุ่มโดยที่สารกลุ่มจิงเจอรอลและโชกาออลเป็นกลุ่มที่มีการศึกษามากที่สุดและเป็นสารบ่งชี้ทางเคมีของขิง **วัตถุประสงค์:** การศึกษานี้มีเป้าหมายเพื่อพัฒนาวิธีตรวจวิเคราะห์ที่ง่ายและสามารถตรวจวัดสารสำคัญ 5 ชนิดของขิงพร้อมๆ กันด้วยเทคนิคโครมาโทกราฟีชนิดของเหลวสมรรถนะสูง (เอชพีแอลซี) **วิธีการวิจัย:** ระบบเอชพีแอลซีประกอบด้วยคอลัมน์ซี 18 เครื่องวัดชนิดโฟโตไดโอดแอรเรย์ และ วัฏภาคเคลื่อนที่ชนิดเกรเดียน ได้พัฒนาขึ้น จากนั้นได้ทำการตรวจสอบความเหมาะสมของระบบและนำมาใช้ในการวิเคราะห์หาปริมาณสารสำคัญทั้ง 5 ชนิดในสารสกัดขิงที่เตรียมขึ้น **ผลการวิจัย:** ระบบเอชพีแอลซีที่พัฒนาขึ้นมีความเชื่อถือได้ เนื่องจากมีค่าความเที่ยงและความแม่นสูง ในระยะเวลา 25 นาทีภายหลังการฉีดตัวอย่าง พบว่าสามารถแยกพีคของสารสำคัญได้ชัดเจน โดยมีค่าเวลาที่คงอยู่ 7.3, 11.0, 12.0, 15.0 และ 20.3 นาทีสำหรับ 6-จิงเจอรอล, 8-จิงเจอรอล, 6-โชกาออล, 10-จิงเจอรอล และ 10-โชกาออล ตามลำดับ ค่าสัมประสิทธิ์การตัดสินใจของสารเหล่านี้เข้าใกล้ 1 (0.9997-0.9999) ให้ค่าขีดจำกัดการตรวจหา (แอลโอดี) ที่ 0.08-0.52 ไมโครกรัมต่อมิลลิลิตร และค่าขีดจำกัดการวัดเชิงปริมาณ (แอลโอคิว) ที่ 0.26-1.73 ไมโครกรัมต่อมิลลิลิตร มีความเที่ยง (Precision) ที่ดีด้วยค่าร้อยละสัดส่วนเบี่ยงเบนมาตรฐานที่ต่ำ ทั้งการวิเคราะห์ภายในวัน (0.04-0.77%) และการวิเคราะห์ระหว่างวัน (0.49-0.96%) มีความแม่นยำสูง (Accuracy) โดยมีค่าร้อยละของการได้กลับคืนอยู่ระหว่าง 99.97±0.99-102.07±1.54% วิธีทดสอบนี้สามารถตรวจสอบและแยกสารสำคัญทั้ง 5 ชนิดได้ในสารสกัดขิง พบว่าสารสกัดด้วย 95%เอทานอล มีสาร 6-จิงเจอรอลสูงสุด รองลงมาคือ 6-โชกาออล และปริมาณสารสำคัญอีก 3 ชนิดแตกต่างกันตามแหล่งของวัตถุดิบ สำหรับสารสกัดด้วย 50% เอทานอล มีรูปแบบปริมาณสารสำคัญคล้ายคลึงกัน แต่มีปริมาณต่ำกว่าของสารสกัดด้วย 95% เอทานอล แต่ไม่พบสารสำคัญทั้ง 5 ชนิดในสารสกัดด้วยน้ำเดือดทั้งการต้มเป็นเวลา 15 นาที และการต้มเคี่ยวเป็นเวลานานขึ้น **สรุปผลการวิจัย:** วิธีเอชพีแอลซีที่พัฒนานี้ง่ายและสะดวกสำหรับการตรวจสอบสารสำคัญทั้ง 5 ชนิดพร้อมๆ กันในตัวอย่างขิงและจะเป็นระบบวิเคราะห์ที่มีประโยชน์ในการควบคุมคุณภาพและพัฒนาผลิตภัณฑ์ที่มีขิงเป็นส่วนประกอบ

คำสำคัญ: การตรวจวิเคราะห์พร้อมกัน, เอชพีแอลซี, จิงเจอรอล, โชกาออล, สารสกัดขิง

Development of simultaneous determination of five active compounds of ginger by high performance liquid chromatographic method

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Abstract

Development of simultaneous determination of five active compounds of ginger by high performance liquid chromatographic method

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Ginger (*Zingiber officinale* Roscoe) belonging to Zingiberaceae family that its rhizome has been well known to be both food and medicine with several pharmacological activities. Among many types of constituents in ginger rhizome, gingerols and shogaols are the most studied and used as the plant chemical markers. **Objective:** This study aimed to develop the simple and simultaneous determination of five compounds by high performance liquid chromatographic (HPLC) method. **Methodology:** The HPLC system composing of C 18 column, photodiode array detector and gradient mobile phase was developed. Then the method was validated and applied for quantitation of these compounds in the prepared ginger extracts. **Results:** The developed HPLC method was reliable with high accuracy and precision. Within 25 min run, the clear and separated peaks at retention times of 7.3, 11.0, 12.0, 15.0 and 20.3 min were obtained for 6-gingerol, 8-gingerol, 6-shogaol, 10-gingerol and 10-shogaol, respectively. The coefficient of determination (r^2) of these compounds were very close to 1 (0.9997-0.9999) with low values of the limit of detection (LOD) at 0.08-0.52 µg/ml and the limit of quantitation (LOQ) at 0.26-1.73 µg/ml. The method precision was good with low percent RSD for both intra-day (0.04-0.77) and inter-day (0.40-0.96) assays and high accuracy with 99.97±0.90-102.07±1.54% recovery. The method can simultaneously determine and separate these compounds in the ginger extracts. 95% ethanol extract of ginger showed highest amount of 6-gingerol, followed with 6-shogaol and various amount of the other three compounds according to their raw material sources. The similar content pattern of these compounds was found in 50% ethanol extract of ginger, but with lower amount. Whereas these five compounds were not detected in both 15 min boiling in water extract and long-time simmer in boiling water extract of ginger. **Conclusion:** The developed HPLC method is simple and easy for simultaneous determination of these five bioactive compounds in ginger samples and would be benefit for the assay system in the quality control and the development of ginger containing products.

Keywords: simultaneous determination, HPLC, gingerols, shogaols, ginger extract

Introduction

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is a well-known plant that has dual functions as food and medicine. Its rhizome has folkloric uses in Traditional medicine to confer health-promiting effects. Several pharmacological activities of ginger were documented including anti-motion sickness (Afzal *et al*., 2001; Ali *et al*., 2008; Stewart *et al.,* 1991), anti-inflammation (Grzanna *et al*., 2005), anti-microbial (Jagetua *et al*., 2003; Ficker *et al*., 2003) and against chikungunya virus (Kausshik *et al*., 2020). Ginger rhizome is also widely used as a flavoring agent or a spice to improve the taste and flavor of food (Aruoma *et al*., 1993; Bartley and Jacobs, 2000). Clinical studies suggested the beneficial effects of ginger in cancer patients (Uthaipaisanwong *et al*., 2020; Konmun *et al*., 2017) and antioxidant activity of ginger extract can act as a supplement in cancer patients receiving chemotherapy (Danwilai *et al*., 2017). Currently, many ginger containing products are commercial available as functional food, cosmetics and medicine. The reported constituents of ginger are categorized to be phenolics, flavonoids and essential oils (Nurhadi *et al*., 2020). Major phenolics found in ginger are gingerol, shogaol, zingeron and paradol (Li *et al*., 2016; Mao *et al*., 2019). The flavonoids contents of ginger are quercetin, rutin, catechin and epicatechin (Ghasemzadeh *et al*., 2010). Several components of essential oil give specific ginger flavor, such as zingeberene, α-curcumene, farnesene, bisabolene, sesquiphellandrene, a-pinene, cineole borneol, geraniol, geranial, and neral (Lawrence, 1996). Gingerols are major bioactive compounds that give pungent or spicy flavor, there are 4-gingerol, 6-gingerol, 8-gingerol 10-gingerol and 12-gingerol, wherein 6-gingerol is the most found and pungent (Hiserodt *et al*., 1998). Due to the presence of β-hydroxyl ketone group in gingerol structure, they became thermally labile and convert to shogaols at high temperature (Bhattarai et al., 2001; Zhang *et al*., 1994). The amounts of these compounds are varied according to many factors such as location of plantation, age and post-harvesting condition of the raw materials. Among various constituents of ginger, 6-gingerol and 6-shogaol were the most studied and used to access its quality.

The analytical methods for active compounds in ginger included several types of chromatographic technics such as gas chromatography–mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), HPLC (high performance liquid chromatography) and thin layer chromatography (TLC). However, HPLC is the most commonly used according to its simple, rapid and practical method for quality analysis of active compounds in the herbal product development. In general, 6-gingerol and 6-shogaol were the most used as standard markers of ginger studies (Tinello and Lante, 2020; Yamprasert *et al*., 2020; Kajsongkram *et al*., 2015). With the typical characteristics of plant, several compounds can be the active ingredients that confer both or either efficacy and toxicity. The simultaneous determination of herbal ingredients is an advantage method to facilitate the process of product development and the quality control of production process. Our previous reports had shown the success for using HPLC methods in the simultaneous analysis of many compounds of plant extracts such as methoxyflavones in *Kaempferia parviflora* (Mekjaraskul *et al*., 2013), diarylheptanoids in *Curcuma comosa* (Su *et al*., 2012) and phenolics in *Emblica officinale* (Chaiittianan and Sripanidkulchai, 2013). Using a system of one mobile phase and short analysis time is an ideal HPLC method for routine, practical and economical applications. This study aimed to develop the HPLC system that can simultaneously determine five bioactive compounds found in ginger extracts. The method validation was performed, then the system was used to quantitate these compounds in four different preparations of the ginger extracts. The first two extracts were prepared by maceration in 95 and 50 % ethanol; the others were extracted with hot water for two different times, i.e., 15 min and longer simmer boiling.

Materials and methods

1. Chemicals and plant material

Standard 6-gingerol, 8-gingerol, 6-shogaol, 10-gingerol and 10-shogaol were purchased from Shunbo Bioengineering, Yunan (China). The purity of all standards was above 98%. Ethanol (95%) was a product from the

Excise Department, Bangkok (Thailand). Acetonitrile and absolute methanol were HPLC grade from RCI Labscan, Bangkok (Thailand). Glacial acetic acid was purchased from Sigma-Aldrich, St. Louis, Missouri (USA). Three sources of raw materials of ginger rhizomes were obtained from the local market in Krabi, Mae Hongsorn and Petchaboon provinces.

2. Preparation of the ginger extracts

Four methods of extraction were conducted. For ethanolic extraction, the dried powder of ginger rhizome was separately macerated in 95% and 50% at a ratio of 1:5 for three days. After filtration through the 5 layer-gauze and a Whatman filter paper number 1, the residual part was re-macerated and the filtrates were combined. For samples using ethanol as extraction solvent, the ethanol was removed using the rotary evaporator. Then the water in all samples were eliminated by using freeze dryer for 24 h. For aqueous extraction, the dried powder of ginger rhizome in the gauze packs were separately prepared by two different conditions. The first set was boiled in a covered water pot for 15 min, the liquid part was filtered through a Whatman filter paper number 1 and freeze dried for 24 h. The second set was similarly boiled but simmered with longer time until the water level was reduced to be one-third of the original volume. After filtering through a Whatman filter paper number 1, the filtrate was freeze dried for 24 h. These four obtained extracts were named as 95% alcoholic ginger extract (95%GAE), 50% alcoholic ginger extract (50%GAE), 15-min boiling ginger extract (15GBE) and long-term boiling ginger extract (LTGBE), respectively. The extracts were weighted and the %yields were determined. All extract samples were kept at -20°C until used.

3. HPLC analytical method

3.1 Preparation of the standard mixture

To prepare the stock solutions (2 mg/ml), each 2 mg of five standard compounds (6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 10-shogaol were separately dissolved in 1 ml of absolute methanol, then filtered through a 0.22 µm syringe filter membrane. Each 200 µl of these stock solutions were mixed together to obtain a 400 µg/ml concentration of standard mixture. This standard mixture was further serial diluted with methanol to obtain five working standard mixtures at concentrations of 0.5, 1, 5, 10, 20 and 40 µg/ml.

3.2 Sample preparation

The ginger extract sample was dissolved, diluted in methanol and filtered through a 0.22 µm syringe filter before analysis with HPLC method.

3.3 HPLC condition

HPLC (Agilent 1260 Infinity II) system with ZORBAX Eclipse plus C18 column (150x4.6 mm, 3.5 µm), photodiode array detector at 280 nm, gradient mobile phase (0.5% acetic acid in water and 0.5% acetic acid in acetonitrile as shown in Table 1) and 20 µl injected volume at a flow rate of 1 ml/min was used. The column temperature was maintained at 30 °C.

Table 1: Ratio of gradient mobile phase for HPLC method

Time (min)	0.5 % acetic acid in water	0.5% acetic acid in acetonitrile
0	60	40
15	20	80
18	20	80
20	5	95
25	5	95
28	60	40

3.4 Method validation

In this study, the validation of analytical method was conducted and the following parameters including linearity, sensitivity (The limit of detection (LOD) and the limit of quantification (LOQ)), precision, accuracy and specificity were determined according to ICH (The international Conference on Harmonization) guidelines Q2A and Q2B. In this study, the specificity of HPLC method was demonstrated by running the sample of ginger extracts. The resolution between the peaks nearby the main gingerols and shogaols that could be found in ginger extracts was determined by analysis of chormatograms of standard solution and sample solution. The resolution was calculated by the formula: resolution= (Rt(2)-Rt(1))/(0.5*(width of peak 1+ width of peak2)). For linearity test, the working standard mixtures at six concentrations (0.5, 1, 5, 10, 20 and 40 µg/ml) were performed. The area under the peaks and concentrations were plotted and the correlation of determination (R^2) was determined. LOD and LOQ were calculated based on the standard deviation of the response which can be integrated by STEYX function in excel program to calculate standard error of the predicted y-value for each x in the regression and the slope (S) method using the formulae: LOD= ((STYEX)/S)×3.3, LOQ= ((STYEX)/S)×10. The precision was obtained from the intra-day assay of six repeated measurements and inter-day assay of three successive days of three concentrations of the working standard mixtures (5, 20 and 40 µg/ml). The %relative standard deviation (percent RSD) was calculated as 100xSD/mean. Finally, the accuracy was determined as %recovery of spiked samples using at least three concentrations with seven repeated measurements using the equation %recovery = 100 (C2-C1)/C3, whereas C1= concentration of un-spiked sample, C2= concentration of spiked sample and C3=concentration of added standard mixture.

Table 2: %yields and contents of five bioactive compounds of four different ginger extracts from three sources of the raw materials

Source/Extraction method	%yield	Content (mg/g) [a]				
		6-gingerol	8-gingerol	6-shogaol	10-gingerol	10-shogaol
Krabi						
95%GAE	9.90	46.43±0.88	5.68±0.22	29.72±0.51	7.39±0.31	8.50±0.27
50%GAE	8.72	25.24±0.92	3.91±0.34	12.89±1.07	6.63±1.15	2.68±0.46
15GBE	2.02	N/D	N/D	N/D	N/D	N/D
LTGBE	7.26	N/D	N/D	N/D	N/D	N/D
Mae Hongsorn						
95%GAE	9.22	47.27±1.92	6.25±0.35	31.28±1.46	8.39±0.76	9.37±0.65
50%GAE	9.14	16.56±0.18	2.44±0.13	8.19±0.88	3.76±0.63	1.60±0.35
15GBE	2.32	N/D	N/D	N/D	N/D	N/D
LTGBE	7.50	N/D	N/D	N/D	N/D	N/D
Petchaboon						
95% GAE	11.22	56.02±8.53	4.72±1.05	19.84±3.25	4.94±2.02	0.00±0.00
50%GAE	7.12	23.57±5.54	2.44±0.65	12.28±2.38	3.59±1.02	2.05±0.42
15GBE	2.98	N/D	N/D	N/D	N/D	N/D
LTGBE	7.72	N/D	N/D	N/D	N/D	N/D

[a] Data was expressed as mean±SD (n=3)

95% GAE= 95% alcohol extract, 50% GAE= 50% alcohol extract, 15GBE= 15-min boiling water extract, LTGBE= long-term simmer in boiling water extract, N/D = not detected

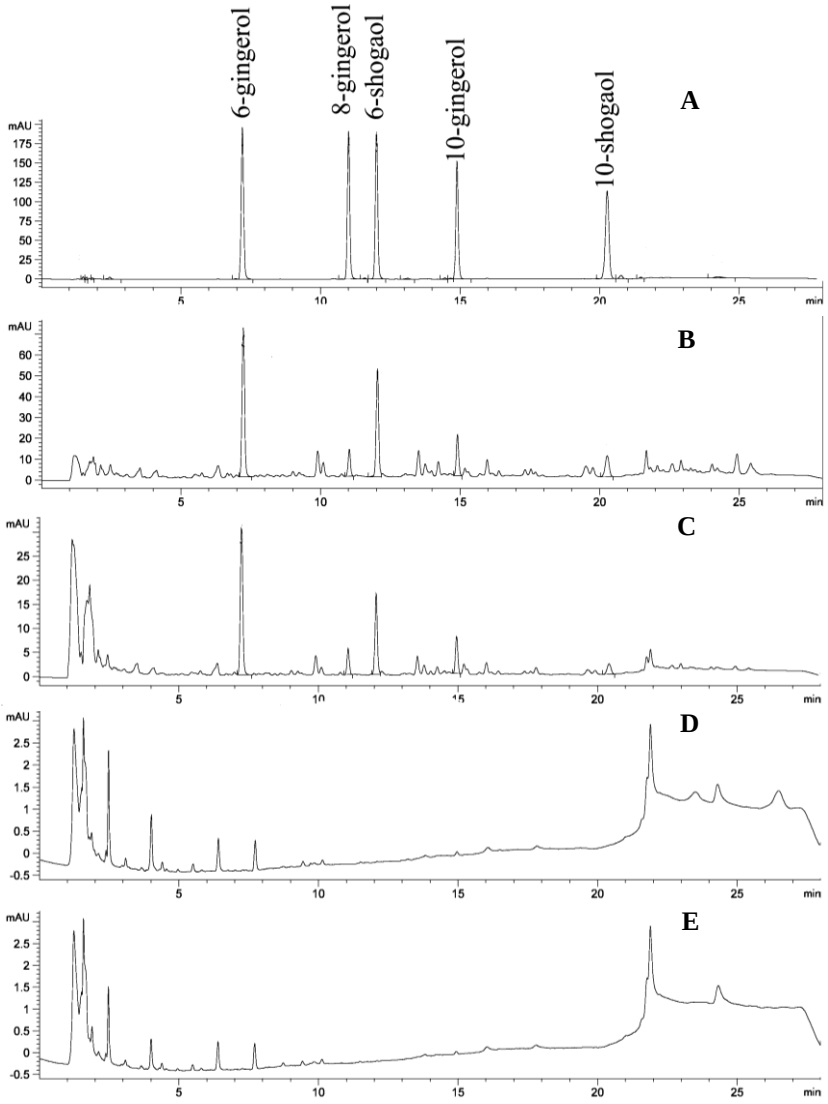

Figure 1: Representative HPLC chromatograms of standard mixture (A), 95% ethanol (B), 50% ethanol (C), 15 min boiling in water (D), and simmer for long time in boiling water (E) ginger extracts.

Results

1. Preparation of the ginger extract

From four different methods of extraction, the % yields of extracts of three sources of ginger raw materials were different (Table 2). In general, the alcohol extraction revealed higher % yield of extracts than those of the boiling in water. 95% alcohol extraction gave highest % yield, followed with 50% alcohol extraction, long-term simmer in boiling water and 15-min boiling in water. The ginger rhizome from Petchaboon province provided highest % yield of 95% alcohol extract, followed with those from Krabi and Mae Hongsorn provinces. The order of higher to lower % yield of 50% alcohol extracts of ginger rhizome sources was from Mae Hongsorn >Krabi>Petchaboon provinces. Moreover, the water extraction demonstrated that the long-term boiling gave higher % yield than those of the 15-min boiling with the similar amounts of three sources of the ginger raw materials.

2. Validation of HPLC method

The HPLC chromatograms of five standard compounds were clearly observed with the retention times of 7.3, 11.0, 12.0, 15.0 and 20.3 min for 6-gingerol, 8-gingerol, 6-shogaol, 10-gingerol and 10-shogaol, respectively (Figure 1A). The specificity of the method was evaluated by analysis of the standard and sample solution

chromatograms. For example, the resolution of these major gingerols and shogaols as calculated from the nearby peaks of 95% GAE was between 2.06-5.74. The linear equation of these standard compounds were obtained with y=11.486x+3.257, y=11.135x+2.795, y=11.235x+2.984, y=9.036x+8.046, and y=10.037x+2.395; and R^2= 0.9999, 0.9999, 0.9999, 0.9997 and 0.9999 for 6-gingerol, 8-gingerol, 6-shogaol, 10-gingerol and 10-shogaol, respectively. The LOD and LOQ values of these five compounds were shown in Table 2, in which 6-shogaol gave the lowest and 6-gingerol gave the highest LOD and LOQ values. The method precision provided the low percent RSD for both intra-day (0.04-0.77) and inter-day (0.40-0.69) assays and high accuracy 99.97±0.90-102.07±1.54% (Table 3).

3. Detection of five standard compounds in the extracts

The developed HPLC method can clearly separate these five standard compounds in the standard mixture and the extract samples (Figure 1 B-E). However, these five standard compounds were detected only in the alcohol extracts, but not in the aqueous extracts (Table 2). The highest contents of these compounds were found in the 95%GAE in which 6-gingerol was its highest content. Whereas 8-gingerol was the lowest content of the 95%GAE of the raw materials from Krabi and Mae Hongsorn. In contrast, the 95%GAE of the raw materials from Petchaboon did not have 10- shogaol. Considering the total content of these five compounds, the 95%GAE of the raw materials from Mae Hongsorn showed highest level, followed with those from Krabi and Petchaboon. These five compounds were also detected in the 50%GAE, but with lower amount than those of the 95%GAE. 6-gingerol was highest found in the 50%GAE and either 8-gingerol or 10-shogaol was lowest detected. In general, the total content of these five compounds of the 50%GAE was about one-third to half of those amount in the 95%GAE based on their sources of raw materials.

Table 3: Validation of HPLC method of five active compounds from ginger extract

Standard compound	Concentration (ug/ml)	Precision (%RSD)		Accuracy[a] (%recovery)	Linear equation y=ax+b (R^2)	LOD (µg/ml)	LOQ (µg/ml)
		(intra-day)	(inter-day)				
6-gingerol	5	0.09	0.67	100.66±0.53	y = 11.486x+3.257 (R^2=0.9999)	0.52	1.73
	20	0.05	0.41	100.74±2.83			
	40	0.05	0.42	99.97±0.90			
8-gingerol	5	0.09	0.68	100.17±1.14	y = 11.135x+2.795 (R^2=0.9999)	0.10	0.35
	20	0.05	0.42	100.60±3.38			
	40	0.05	0.43	100.33±0.50			
6-shogaol	5	0.07	0.69	100.19±0.54	y = 11.235x+2.984 (R^2=0.9999)	0.08	0.26
	20	0.23	0.40	100.38±1.83			
	40	0.05	0.58	100.41±0.97			
10-gingerol	5	0.11	0.62	100.24±1.55	y = 9.0361x+8.046 (R^2=0.9997)	0.44	1.45
	20	0.77	0.45	102.07±1.54			
	40	0.04	0.58	100.38±0.53			
10-shogaol	5	0.07	0.69	100.77±0.41	y = 10.037x+2.395 (R^2=0.9999)	0.37	1.25
	20	0.04	0.43	100.23±2.06			
	40	0.05	0.42	100.38±1.05			

[a] Data was expressed as mean±SD (n=3)

Discussion and conclusion

The HPLC method was developed to simultaneously determine five major compounds of ginger in this study. Within 25 min run, the HPLC chromatogram showed clear and well-separated peaks of 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol and 10-shogaol. According to ICH guideline, specificity of the HPLC method is the capability of the method to separate the analytes which might include impurities, degradants or matrix from main component. In this study, the resolution between the peaks nearby the major gingerols and shogaols was higher than 2, indicating a high degree of peak separation (CDER guideline). Although several compounds were reported in ginger rhizome, but based on the limitation of commercial availability, only five compounds were used as standards in this study. Previous reports had shown the simultaneous determination of bioactive compounds in ginger, such as two compounds (6-gingerol and 6-shogaol) by RP-HPTLC (Foudah *et al*., 2020), four compounds (6-gingerol, 8-gingerol, 10-gingerols and 6-shogaol) by RPHPLC (Antony *et al*., 2020). With our attempt, the minor compound (10-shogaol) was included to simultaneous determination of total five compounds. The validation data of this method provided high linearity, accuracy, precision and low limit of detection/quantitation. The method was used to quantitate and differentiate the amount of these compounds in four preparations of the ginger extracts. This method is reliable as observed that these compounds were found only in the ethanolic extracts (50%GAE and 95%GAE). The method did not detect these five compounds in the aqueous extracts (15GBE and LTGBE). Based on their lipophilic properties, these five compounds could be extracted by alcohol, but not by water. The findings suggest the appropriate use of the developed HPLC method for quality control of the product development of ginger rhizome and for standardization of the ginger extract as well. Moreover, the quality of raw materials can also be differentiated in their contents by this HPLC method. As observed that there were different in these five compounds of the extracts from different sources of plantation. The advantage to determine these five compounds simultaneously was important for saving time and cost of analysis and would be practical to be used for in-house assay. Based on several compounds contain in herbs, the more chemical markers used in the analysis, the higher accuracy of content analysis of herbal extracts and products. Somehow, the sum-up contents are alternative indicators to show the chemical ingredients of herbs and they are very useful for the quality control in the product development. Moreover, the simultaneous determination of several compounds of the extracts were successfully used in the pharmacokinetic study of many herbal extracts such as the study on increasing in the bioavailability of *K. parviflora* (Mekjaraskul *et al*., 2013) and diarylheptanoids of *C. comosa* (Su *et al*., 2012) and to evaluate the topical films containing phytoestrogenic diarylheptanoids (Tantiyasawasdikul *et al*., 2018). However, to determine more compounds, the future study needed to overcome the limitation of a commercial availability of the other compounds found in ginger. In conclusion, the developed HPLC method was simple, quick and easy to determine five bioactive compounds of ginger extract and can be applied for the quality assessment of the plant raw materials as well as the product development.

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