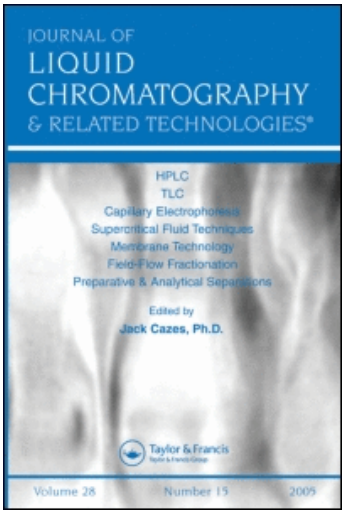

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Simultaneous Densitometric Determination of 6-Gingerol and 6-Shogaol in some Commercial Gingers (*Zingiber Officinale* Roscoe)

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Simultaneous Densitometric Determination of 6-Gingerol and 6-Shogaol in some Commercial Gingers (*Zingiber Officinale* Roscoe)

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Abstract: A simple and rapid densitometric method has been developed for determination of 6-gingerol and 6-shogaol in some commercial gingers. After extracting the samples four times with methanol, the solutions were spotted on pre-coated silica gel tlc plates, which were eluted with a mixture of n-hexane-ethyl ether (4:6, v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at $\lambda = 577$ nm after sprayed with anisaldehyde-h2so4 reagent. The tlc-densitometric method is cheap, selective, precise and accurate and can be used for routine analysis of gingers in herbal drugs industry quality control laboratories.

Keywords: Ginger, Densitometry, 6-Gingerol, 6-Shogaol, TLC, *Zingiber officinale*

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INTRODUCTION

Ginger is the rhizome of *Zingiber officinale* Roscoe (Family Zingeberacea). Its Indonesian's local name is "*Jahe*". According to Materia Medika Indonesia II,[1] ginger in Indonesia has three different varieties, i.e., "*Jahe putih besar*" or "*Jahe gajah*", "*Jahe putih kecil*", or "*Jahe emprit*" and "Jahe merah (see Fig. 1). The *gajah* variety has bigger rhizomes compared to the two other varieties, while the *merah* variety had a slightly red color when the rhizome was cut. The rhizome's size of the *emprit* and *merah* varieties was almost identical. As an herbal drug, ginger is usually used for carminative, stimulant, anti-inflammatory, and anti-emetic.[2,3] The main marker constituent of ginger is the non-volatile pungent principle 6-gingerol.[4] The corresponding anhydro compound of gingerols are shogaols.[2,4] The official monograph of ginger is available in the Indonesian *Materia Medika*,[1] British Pharmacopoeia,[5] Japanese Pharmacopoeia,[6] and the Pharmacopoeia of the People's Republic of China.[7]

Many HPLC methods for analysis of gingerols and shogaols in ginger have been reported,[8–13] while Yoshikawa et al.[14] reported the same determination using HPLC and GLC. TLC qualitative analysis of gingerols and shogaols was reported by Mukherjee,[4] Conell & Sutherland,[15] and Chen et al.[16] Recently Rai et al.[17] published HPTLC determination of 6-gingerol in ginger. To the best of our knowledge, no publication reported the simultaneous determination of 6-gingerol and 6-shogaol by using TLC densitometry.

The objective of the present work is to develop a cheap, rapid, and simple validated TLC densitometry method for simultaneous determination of 6-gingerol and 6-shogaol in ginger samples.

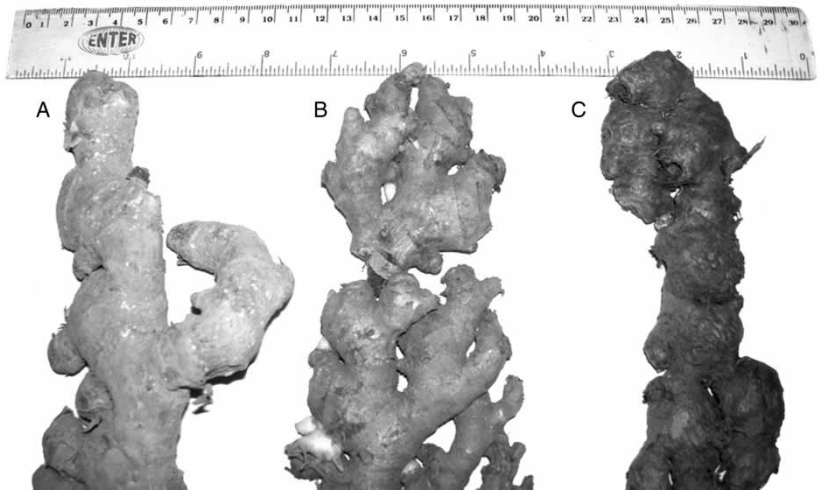

Figure 1. Fresh ginger variety "*Gajah*" (A), "*Emprit*" (B) and "*Merah*" (M).

EXPERIMENTAL

Materials and Reagents

Fresh gingers (13 samples) were purchased in two local herbal drugs markets at Surabaya (Code P and K), local herbal drug market at Sidoarjo (code L), and Ponorogo (code KO) in November 2006. All herbal drugs markets were located at East Java, Indonesia. The three varieties of ginger, "*gajah*" (G), "*emprit*" (E), and "*merah*" (M) could be easily differentiated by their morphologic characteristics. All ginger samples were washed with water, oven dried (50°C), cut into small pieces, and then powdered. The confirmation of the identity of all ginger was performed by spot tests according to the official method.[1]

Standards 6-gingerol and 6-shogaol were purchased from Chromadex (Santa Ana, Ca, USA). The substances were used as received for preparing standard solutions. Methanol, *n*-hexane, ethyl ether, (JT. Baker, Philipsburg, NJ, USA), sulphuric acid, anisaldehyde, acetic acid glacial (E. Merck, Darmstadt, Germany) were analytical grade reagents; the solvents and reagents were used without further purification.

Stock standard solutions were prepared by dissolving accurately weighed 6-gingerol and 6-shogaol (10.0 mg) in 25.0 mL methanol. Various standard solutions were prepared from the stock solution by dilution with methanol. For basic linearity study, the solutions were prepared containing 7.00, 10.0, 14.0, 18.0, 21.0, 24.0, 25.0, and 28.0 $\mu g\ mL^{-1}$ (for 6-gingerol), 5.00, 8.00, 10.0, 15.0, 17.0, 18.0, and 20.0 $\mu g\ mL^{-1}$ (for 6-shogaol). Of these solutions, 10 μL was spotted onto the TLC plate. The standard solutions were stable at least for 24 hours at room temperature (data for 6-shogaol: 102. $\pm$ 2.66%, n = 3, at 24 $\pm$ 2°C, room humidity 50 $\pm$ 10%).

Sample Extraction

About 1000 mg (accurate weight) of powered ginger was ultrasonicated (30 min) with 20 mL of methanol, mixed with a vortex mixer (5 min) and than filtrated. The residue was re-ultrasonicated (30 min) with 7 mL of methanol, mixed with a vortex mixer (5 min), and filtered. The re-extraction was repeated three times. All the filtrates were transferred in a 50.0 mL volumetric flask and diluted to volume by methanol. Of these solutions, 2.0 μL (for analysis 6-gingerol) and 5.0 μL (for analysis 6-shogaol), were spotted onto the TLC plate together with the standards. Details of the optimization of the extraction methods were presented Figure 4.

Chromatography

Chromatography was performed on precoated silica gel F254 aluminum back sheets (E. Merck. # 1.05554, all the precoated plates were cut into 10 $\times$ 20 cm

before used). The plates were used as obtained from the manufacturer without any pretreatment; a Nanomat III (Camag, Muttenz, Switzerland) equipped with a dispenser magazine containing 2.0 or 5.0 μL glass capillaries (Camag) was used for sample application (as spot with diameter *ca*. 2 mm). The mobile phase used in this experiment was *n*-hexane: -ethyl ether (4:6 v/v).[4] The distance from the lower edge was 10 mm; distance from the side was 15 mm, and track distance was 10 mm. Ascending development was performed in a Camag twin through chamber (for 20 × 10 cm plates) after at least 60 min of saturation; the mobile phase migration distance in all experiments was 8.0 cm. (development time *ca*. 15 min at 24 ± 2°C). The plate was air dried, sprayed with anisaldehyde-H_2SO_4 reagents (105°C for 5 min), and than scanned in the TLC scanner.

Densitometric scanning was performed with a Camag TLC-Scanner II. The purity and identity of the analyte spots were determined by scanning the absorbance – reflectance mode from 400 to 800 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at its λ maximum (ca. 577 nm; See Figure 2). The densitometric scanning parameters were: bandwidth 10 nm, slit width 4, slit length 6, and scanning speed 4 mm s^{-1}. Calculations for identity, purity checks ($r_{S,M}$ and $r_{M,E}$ where S = start, M = center, E = end spectrum), *sdv* (relative standard deviation) of the linear/calibration curve, and quantification of the analyte spots were performed by CATS version 3.17 (1995) software (Camag). Routine quantitative evaluations were performed *via* peak areas with linear regression, using 4–5 points' external calibration on each plate (80 to 120% of the targeted value). Each extract of the aliquot samples was spotted at least in duplicate.

Validation

The method was validated for linearity, detection limit (DL), quantitation limit (QL), accuracy, and precision, according to the published methods[18,19] with modification. Accuracy study was performed by the standard addition method. An aliquot of standard solutions of 6-gingerol and 6-shogaol in methanol was added to the ginger sample (KOE 01), after evaporation under nitrogen, the sample was mixed and than extracted as described in sample extraction.

RESULTS AND DISCUSSION

After the TLC plate was eluted and sprayed with the reagent, the densitogram at 577 nm of all ginger samples (Figure 3) showed three main spots of 6-gingerol (R_f ca. 0.25), 6-shogaol (Rf ca. 0.44), and unknown peak (Rf. ca 0.74). This TLC system demonstrated that all analyte spots (6-gingerol and 6-shogaol) of ginger samples furnished *in situ* VIS spectra, are identical

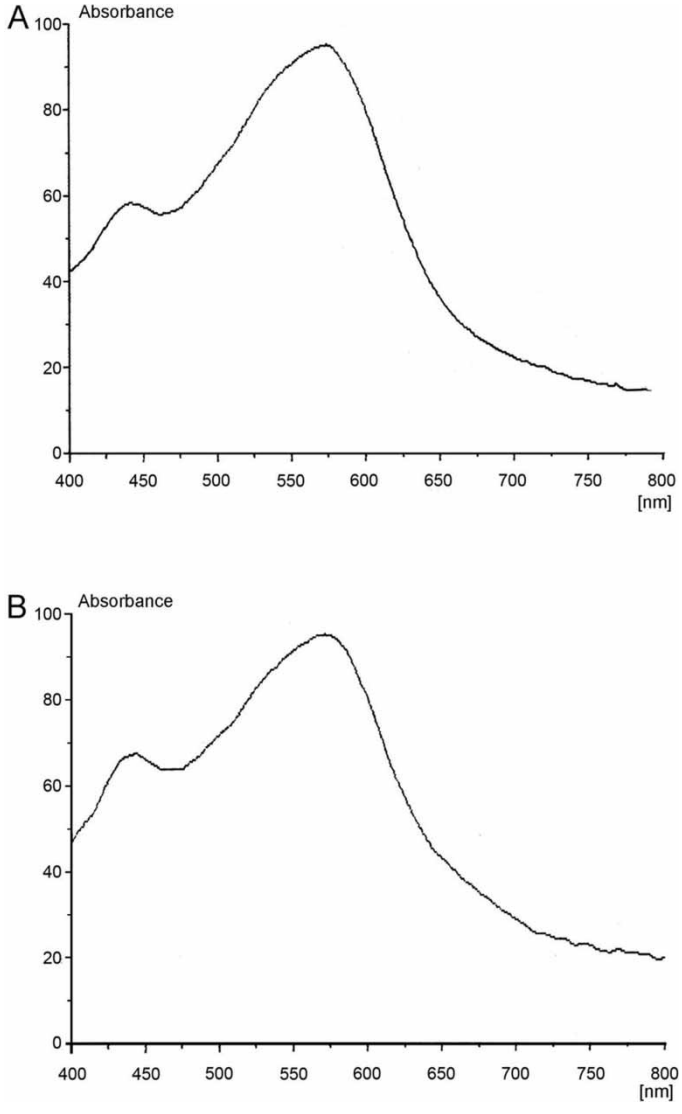

Figure 2. *In situ* absorbance-reflectance VIS -spectra of 6-gingerol (A) and 6-shogaol (B) spots (from 400 to 800 nm; maximum absorption wavelengths at ca. 577 nm). TLC conditions: stationary phase was precoated TLC plate silica gel 60 F_{254} (E. Merck); mobile phase: *n*-hexane-ethyl ether (4:6, v/v).

with those of standards ($r \geq 0.9999$). Purity check of the analyte spots using CATS software also showed that all analyte spots of the extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were ≥ 0.9999, demonstrating that the proposed TLC method is highly selective.

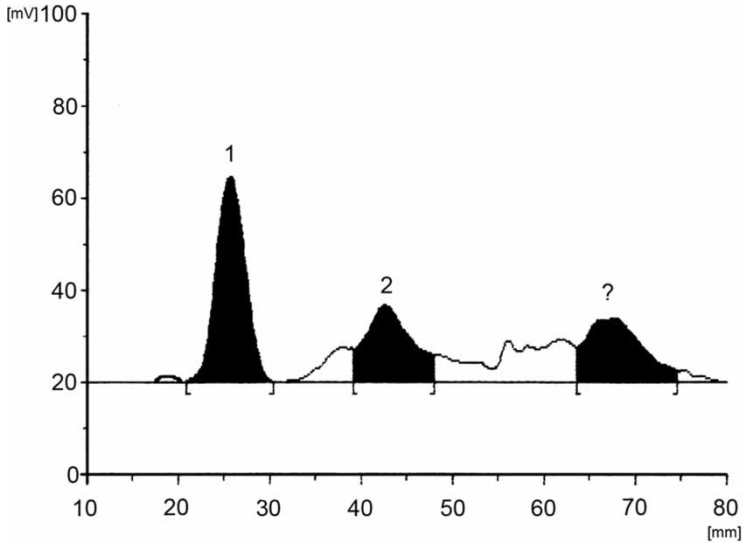

Figure 3. A typical densitograms of extract ginger measured at 577 nm. Peak identities: 6-gingerol (1), 6-shogaol (2) and unknown (3). TLC conditions: see Figure 2.

The peak area was observed to be linearity dependent of the amount of 6-gingerol within the range of 70 to 280 ng spot^{-1}, with linear regression line $Y = -76.6 + 8.18X$ (the relative process standard deviation value V_{XO}[18] was 2.44%; $n = 8$; *sdv* = 2.6; $r = 0.9984$). The calculated value of test parameter X_p (for $p = 0.05$) and r were satisfactory (23.9 ng spot^{-1} and ≥ 0.99, respectively).[18–20] For 6-shogaol, the linear range was 50 to 200 ng spot^{-1} ($Y = -76.8 + 6.64X$; Vxo = 2.99%, $n = 7$; *sdv* = 3.5; $r = 0.9978$). The ANOVA regression test for linearity testing of the regression line showed significant calculated F-value ($p < 0.0001$; $F_{calculated}$ was 1883.9 for 6-gingerol; and 1163.9 for 6-shogaol). The linearity of the basic calibration curve was also proven by the Mandel's fitting test.[18] The plots of the residuals against the quantities of the analyte confirmed the linearity of the basic calibration graph (data not shown). The residuals were distributed at random around the regression lines; neither trend nor unidirectional tendency was found. The basic linear calibration curve showed variance homogeneity over the whole range. The calculated test values PW[18] was 0.49 (6-gingerol) and 1.01 (6-shogaol), The PW values were less than the F_{table}-value (6.03 for $f_1 = 8$, $f_2 = 8$; $p = 0.01$).

All the linear regression calibration curve parameters which were used in this present work showed satisfactory results (data not shown). All values of the correlation coefficient r in this present work are >0.99; and the values of other parameters such as, Xp (less than the lower limit in the calibration range), *sdv* (<5), Vxo (<5%), and p (<0.05) for ANOVA linear test also showed satisfactory results.[18–20]

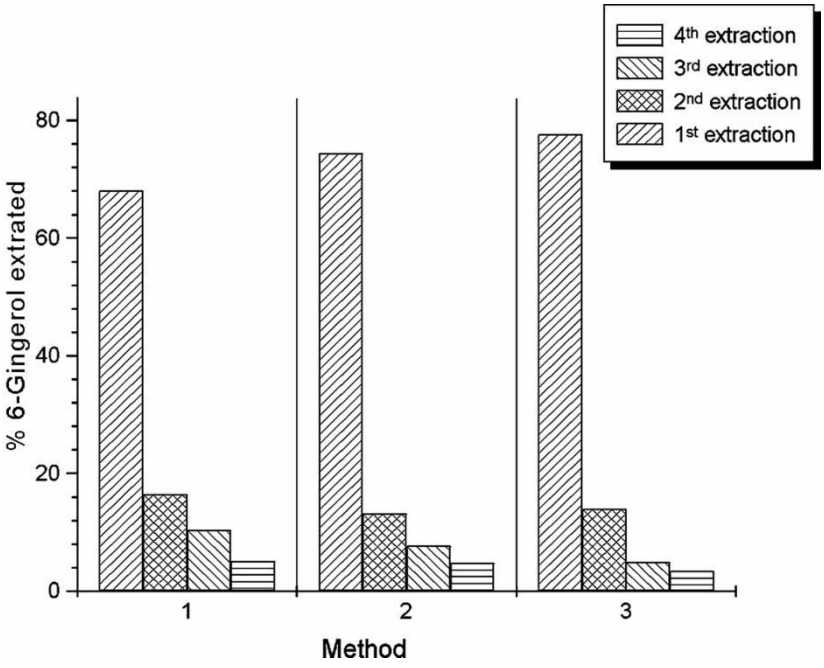

Figure 4. Optimization of extraction methods. Data presented as % 6-gingerol of each extract from total. Method 1: Ultrasonication time was 15 min for each extraction; the amount of MeOH was 15 mL (1st extraction), mixed with vortex mixer (5 min), filtered and diluted to 25 mL with MeOH before analysis. For 2nd–4th extraction, 5 mL MeOH was used and final volume before analysis was 10 mL. Method 2: Ultrasonication time was 30 min for each extraction; the amount of MeOH was 15 mL (1st extraction; final volume 25 mL) and 5 mL (2nd–4th extraction, final volume 10 mL). Method 3: Ultrasonication time was 30 min for each extraction; the amount of MeOH was 20 mL (1st extraction; final volume 25 mL) and 7 mL (2nd–4th extraction; final volume 10 mL).

DL was determined by making a linear regression of relatively lower concentration of 6-gingerol (50 to 250 ng spot^{-1}) according to the method of Funk et al.[18] The calculated equation of the regression line was $Y = -129 + 9.98\ X$ ($n = 6$; $V_{XO} = 3.85\%$; $r = 0.9970$; $sdv = 3.2$). The calculated value of test parameter X_p (for $p = 0.05$)[18] was 39 ng spot^{-1}. In this case, the value of DL = X_p.[18] According to Carr and Wahlich,[21] the value of the QL could be estimated as 3 times of the DL-value (117 ng spot^{-1}). With the same method,[18] DL and QL for 6-shogaol were 27 and 81 ng spot^{-1}, respectively ($Y = 20.7 + 8.69\ X$; $n = 7$; range: 35 to 190 ng spot^{-1}, $Vxo = 4.06\%$; $r = 0.9964$; $sdv = 4.0$).

Optimization of the extraction method of the analyte from the ginger was performed by variation of the time of extraction and the amount of the solvent

Table 1. Results of the accuracy evaluation using sample Ginger code KOE 01

Analyte	Amount found[a] (% dr.wt)	Amount added (% dr. wt.)	Theoretical value (% dr. wt.)	Found[a] (% dr. wt.)	Recovery (%)
6-Gingerol	0.815 ± 0.004	0.195	1.010	1.014 ± 0.029	100.40
6-Shogaol	0.250 ± 0.002	0.057	0.307	0.319 ± 0.002	103.91
6-Shogaol	0.250 ± 0.002	0.084	0.334	0.347 ± 0.008	103.89

[a]Mean ± SD (n = 3); dr. wt.: dry weight.

used. Figure 4 showed that after the 4th extraction of method 3 almost 97% of 6-gingerol was extracted, so, for further studies, this method was used.

Table 1 demonstrated good accuracy as revealed by the percentage of mean recovery data of the ginger sample KOE 01. The results of the determination of 6-gingerol and 6-shogaol in all ginger samples were presented in Figure 5, and the summary of their relative standard deviation (RSD) values (repeatability) were presented in Table 2. All RSD were below 5%. For bio analytical study, the accuracy and precision should not be more than $\pm 15/20\%$.[22] It seemed that the contents of 6-gingerol and 6-shogaol could not be used for differentiating the three varieties of ginger. For differentiating the three varieties of ginger, other methods of metabolite profiling using FTIR, HPLC, and GC-MS are in progress at our laboratory.

Figure 6 shows the 2D scatter plot of 6-gingerol and 6-shogaol contents in ginger samples and its linear regression curve (n = 39, r = 0.460, $p < 0.01$),

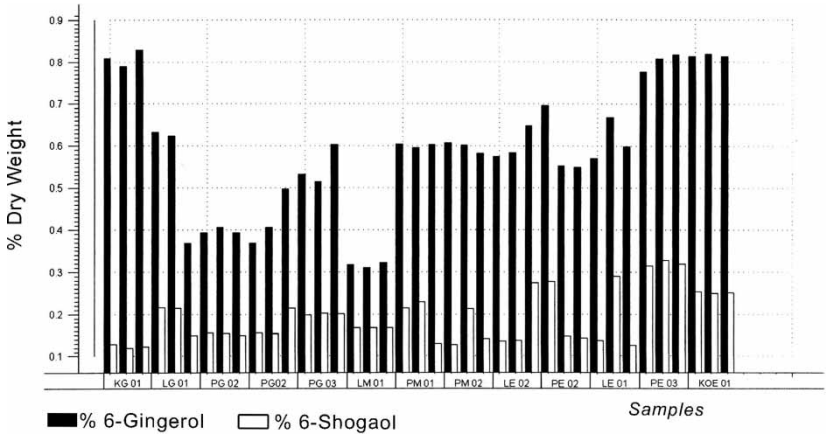

Figure 5. Results of the determination of 6-gingerol and 6-shogaol in ginger samples presented as % dry weight. Each sample was determined in three replicates, and their RSD were presented in the Table 2.

Table 2. Repeatability of the results of the determination of 6-gingerol and 6-shogaol in all ginger samples[a]

		RSD (%, n = 3)	
Variety of Ginger	Code	6-gingerol	6-shogaol
Emprit	KOE 01	0.393	0.922
	PE 02	3.481	3.177
	LE 01	1.952	3.637
	LE 02	0.958	2.151
	PE 03	2.687	2.191
Gajah	PG 03	3.450	4.029
	LG 01	1.945	3.665
	PG 01	2.106	2.065
	KG 01	2.374	4.290
	PG 02	4.960	3.024
Merah	PM 02	0.750	2.736
	LM 01	4.558	0.406
	PM 01	0.652	3.814

[a]The content of 6-gingerol and 6-shogaol (% dr. wt) in ginger samples were presented in Figure 5.

$F_{calculated}$ of ANOVA linearity testing was 9.94 ($p = 0.003$). It seemed the ratio of 6-shogaol to 6-gingerol in ginger KG 01 was smaller compared to the average of other ginger samples, whilst for ginger PE 03 the ratio was bigger. These were maybe due to the differences of the maturity of the

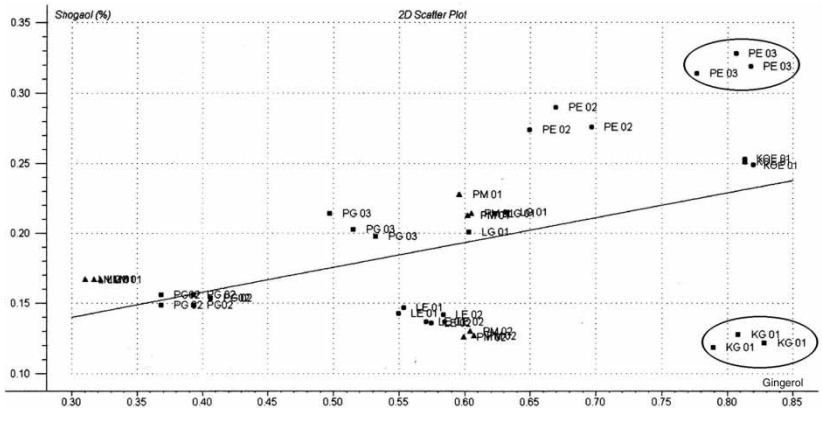

Figure 6. 2D (two dimensional) scatter plot of the content of 6-gingerol and 6-shogaol in ginger samples with its linear regression line ($Y = 0.086 + 0.178\ X$, $n = 39$; $n = 39$, $r = 0.460$, $p < 0.01$).

rhizomes and storage condition after harvesting. If the data of KG 01 and PE 03 were deleted, the r value increased significantly to 0.667 ($n = 33$, $p < 0.01$), and the $F_{calculated}$ of ANOVA linearity testing value was 24.9 ($p < 0.001$). This showed that gingerol degraded to shogaol during storage and drying process. Fresh ginger usually did not contain shogaol.[4]

The present work showed that the proposed TLC densitometric method is suitable for the routine analysis of ginger samples in herbal drugs industry quality control laboratories, especially for developing countries like Indonesia. Our experiences showed that the TLC methods are very cheap compared to the LC-MS, GC-MS, and even with HPLC equipped with DAD/UV detector. The disadvantages of using LC with fixed UV detector and GC-FID are the inability for proving the identity and purity of the analyte peak(s). For developing countries in which the price of HPLC grade solvents and columns are relatively very expensive, the availability of an alternative cheap TLC method is essential.

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