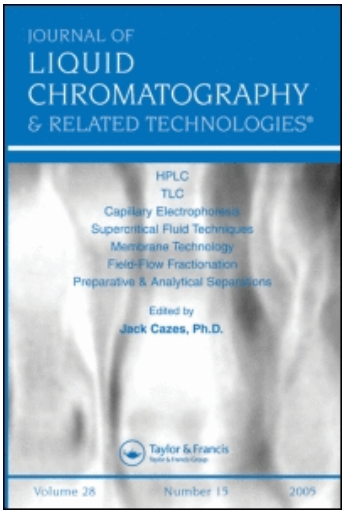

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Simultaneous Densitometric Determination of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol in Some Commercial Gingers

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To cite this Article Melianita, Fenny , Witha, Josephine , Arifin, Saiful , Kartinasari, Wiwin Farina and Indrayanto, Gunawan(2009) 'Simultaneous Densitometric Determination of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol in Some Commercial Gingers', Journal of Liquid Chromatography & Related Technologies, 32: 4, 567 — 577
To link to this Article: DOI: 10.1080/10826070802671598
URL: http://dx.doi.org/10.1080/10826070802671598

Journal of Liquid Chromatography & Related Technologies®, 32: 567–577, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802671598

Simultaneous Densitometric Determination of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol in Some Commercial Gingers

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Abstract: A simple and rapid densitometric method has been developed for determination of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in some commercial gingers. After extracting the samples four times with methanol, the solutions were spotted on precoated Lichrosphere Si 60F254 HPTLC plates, which were eluted with a mixture of toluene–ethyl acetate (3:1, v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at $\lambda = 577$ nm after being sprayed with anisaldehyde-H_2SO_4 reagent. The HPTLC-densitometric method is cheap, selective, precise, and accurate and can be used for routine analysis of gingers in the herbal drugs industry quality control laboratories.

Keywords: Densitometry, Ginger, 6-Gingerol, 8-Gingerol, 10-Gingerol, 6-Shogaol, HPTLC, *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. The main pungent compounds in fresh ginger are homologous phenolic ketones known as gingerols, the major is 6-gingerol, whereas 8- and 10-gingerol are found in smaller quantities. The corresponding anhydro compound of gingerols are shogaols.[2] The official monograph of ginger is available in the Indonesian *Materia Medika*,[1] British Pharmacopoeia,[3] Japanese Pharmacopoeia,[4] and the Pharmacopoeia of the People's Republic of China.[5]

Many HPLC methods for analysis gingerols and shogaols in ginger have been reported,[6–10] while Yoshikawa et al.[11] reported the same determination using HPLC and GLC. TLC qualitative analysis of gingerols and shogaols was reported by Mukherjee,[4] and Conell & Sutherland.[12] Rai et al.[13] published HPTLC determination of 6-gingerol in ginger. Melianita et al.[14] reported simultaneous determination of 6-gingerol and 6-shogaol in some commercial ginger by TLC. Lee et al.[15] reported the simultaneous determination of 6-, 8-, 10-gingerol and 6-shogaol using the combination of HPLC with PDA and ESI- MS/MS. Camag[16] described a validated method for identification of gingers, as marker 6-, 8-, 10-gingerols and 6-shogaol were used. To the best of our knowledge, no publication reported the simultaneous determination of 6-, 8-, 10-gingerol and 6-shogaol in ginger by densitometry.

The objective of the present work is to develop a cheap, rapid, and simple validated densitometry method for simultaneous determination of 6-, 8-, 10-gingerol and 6-shogaol in ginger samples.

EXPERIMENTAL

Materials and Reagents

Fresh gingers (3 varieties) were purchased at some local herbal drugs markets at Surabaya and Sidoarjo, East Java, Indonesia in November 2007. The three varieties of ginger, "*gajah*" (G), "*emprit*" (E), and "*merah*" (M) could be easily differentiated by their morphologic characteristics.[14] All ginger samples were washed with water, and then 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, 8 gingerol, 10-gingerol, and 6-shogaol were purchased from Chromadex (Santa Ana, Ca, USA). The substances were

used as received for preparing standard solutions. Methanol, toluene, ethyl acetate, 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, 8-gingerol, 10-gingerol, and 6-shogaol (10.0 mg each) in 25.0 mL methanol. Various standard solutions were prepared from the stock solution by dilution with methanol. Of these solutions, 6 μL (6-, 8-, and 10-gingerol), and 10 μL (6-shogaol) were spotted onto the HPTLC plate, respectively. The standard solutions were stable at least for 24 hours at room temperature (Mean ± SD%, n = 3, at 24 ± 2°C, room humidity 50 ± 10%): 99.80 ± 0.42 (6-gingerol); 99.33 ± 0.39 (8-gingerol); 103.05 ± 0.87 (10-gingerol); 99.13 ± 0.22 (6-shogaol).

Sample Extraction

Sample extraction was performed according to our previous publication.[14] About 1000.0 mg (accurate weighed) 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 reultrasonicated (30 min) with 7 mL of methanol, mixed with a vortex mixer (5 min), and filtered. The reextraction was repeated three times. All the filtrates were transferred in a 50.0 mL volumetric flask and dilute to volume by methanol. Aliquots of these solutions were spotted onto the HPTLC plate together with the standards. The quantitative analysis of gingerols and shogaol was performed on the different HPTLC plates.

Chromatography

Chromatography was performed on precoated HPTLC Lichrospere Si 60 F 254 aluminum backed sheets (E. Merck. #1.05554). All the precoated plates were cut into 10 × 20 cm before being 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 toluene–ethyl acetate (3:1 v/v).[16] 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. (one development time *ca.* 30 min at

$24 \pm 2°C$). The plate was developed twice (for determination of 6-shogaol), and four times (for determination of gingerols). 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 III. 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). 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 winCATS version 1.4.2 (Camag 2006). 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 of extract 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[17,18] with modification. Accuracy, precision, and robustness evaluation were performed by using the commercial sample E1. The accuracy study was performed by the standard addition method. An aliquot of standard solutions of gingerols and 6-shogaol in methanol was added to the ginger sample, after being evaporated under nitrogen, the sample was mixed, homogenized, and than extracted, as described in sample extraction. Design and analysis of the effect of the robustness evaluation were performed and calculated by using Unscramble 9.6^{TM} (2006) software from CAMO (Bangalore, India).

RESULTS AND DISCUSSION

The method described by Camag,[16] which was used for qualitative identification of ginger should be modified for quantitative purposes. For quantitative analysis, the developing times should be done more than one time; twice for analyzing shogaol, and 4 times for analyzing 6-, 8-, and 10-gingerols. After the HPTLC-plate was eluted and sprayed with the reagent, the samples' densitogram (at 577 nm) showed all the analyte spots of gingerols and shogaols could be well separated (see Figures 1 and 2). All

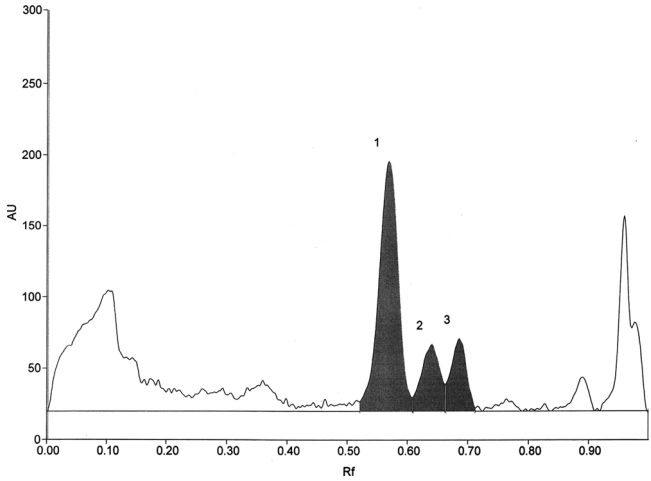

Figure 1. A typical densitograms of extract ginger measured at 577 nm. HPTLC conditions: stationary phase was precoated HPTLC Lichrospere Si 60 F 254aluminum back sheets (E. Merck. # 1.05554); mobile phase: toluene-ethyl acetate (3:1 v/v), 4 times development. Detection by Anisealdehyde-Sulphuric acid. Peak identities: 6-gingerol (1), 8-gingerol (2), 10-gingerol (3).

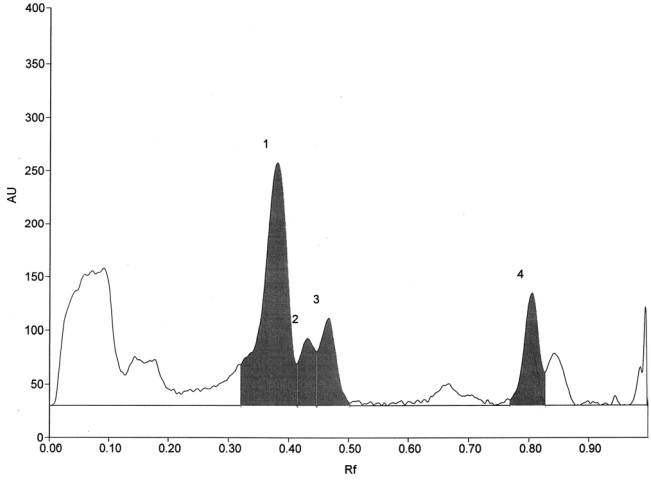

Figure 2. A typical densitograms of extract ginger measured at 577 nm. HPTLC condition: see Figure 1 (twice development). Peak identities: 6-gingerol (1), 8-gingerol (2), 10-gingerol (3), 6-shogaol (4).

Table 1. Linearity, Homogeneity, DL, and QL data of the analytes

Analyte	Linear regression curve	n	Range (ng spot^{-1})	r	*sdv*[a]	Vxo[b]	F[c]	PW[d]	DL[e]	QL[f]
6-Gingerol	$Y = -913.1 + 18.73\ X$	9	96.0–480	0.999	3.62	1.84%	4931	7.10	25.8	77.4
8-Gingerol	$Y = -315.8 + 15.20\ X$	9	39.4–196	0.998	3.90	2.86%	2030	2.02	16.4	47.2
10-Gingerol	$Y = -229.2 + 11.20\ X$	9	48.5–242	0.998	2.98	2.38%	2930	1.13	16.8	50.4
6-Shogaol	$Y = -300.1 + 34.25\ X$	7	50.0–256	0.997	3.69	3.87%	826	7.86	33.2	99.6

[a]Caculated by using winCats 1.4.2 (Camag).
[b]Relative process standard deviation of the linear curve.[17]
[c]$F_{calculated}$ of ANOVA linearity testing ($p < 0.0001$).
[d]Test value for homogeneity of the linear curve[17] ($p < 0.05$).
[e]Detection Limit (ng spot^{-1}), calculated from Xp value.[17]
[f]Quantification Limit (ng spot^{-1}), calculated as 3 times of DL value.[19]

Table 2. Results of accuracy studies for Sample E1

Analyte	Original content (% DW[ab])	Addition (%)[c]	Theoretical (%)	Found (%)[b]	Recovery (%)
6-Gingerol	0.821 (0.80)	48.7	1.22	1.27 (0.67)	104
8-Gingerol	0.139 (3.81)	70.8	0.237	0.245 (1.32)	103
10-Gingerol	0.168 (3.97)	72.1	0.301	0.300 (0.67)	99.7
6-Shogaol	0.370 (1.76)	69.2	0.638	0.652 (0.69)	102

[a]DW = dry weight.
[b]Values were expressed as Mean (% dry weight; n = 3); values in parentheses showed RSD (in %).
[c]% from original content.

analyte spots (6-, 8-, 10-gingerol and 6-shogaol) of ginger samples furnished *in situ* VIS spectra, are identical with those of standards ($\mathbf{r} \geq 0.999$). All the VIS spectra of 6-, 8-, 10-gingerol and 6-shogaol showed almost identical spectra of 6-gingerol and 6-shogaol, which were published previously.[14] A purity check of the analyte spots using winCats software also showed that all analyte spots of the extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were $\geq$0.999, demonstrating that the proposed HPTLC method is highly selective.

The peak area was observed to be linearity dependent of the amount of analytes (see Table 1). In this case the values of DL can be calculated from the value of Xp,[17] and QL can be estimated at 3 times DL.[19] The linearity of the basic calibration curve was also proved by the Mandel's fitting test.[17] 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.

Table 3. Results of precision evaluation for sample E1[a]

Analyte	1st measurement[b]	2nd measurement[b]	3rd measurement[b]
6-Gingerol	0.62	0.14	0.50
8-Gingerol	4.85	2.95	2.65
10-Gingerol	3.20	1.52	0.99
6-Shogaol	1.11	0.68	0.96

[a]Data presented as RSD (%) for n = 3.
[b]Each measurement was performed by a different analyst on the different days, and HPTLC plates within one laboratory.

Table 4. The content of gingerols and shogaol from some commercial samples[a]

Analyte	Commercial samples[b]								
	G1	G2	G3	E2	E3	E4	M1	M2	M3
6-gingerol	0.60 (1.34)	0.19 (1.13)	0.26 (0.97)	0.34 (0.89)	0.42 (1.03)	0.85 (1.12)	0.44 (0.66)	0.35 (0.87)	0.25 (1.44)
8-gingerol	0.14 (2.44)	0.04 (1.43)	0.04 (2.50)	0.14 (0.01)	0.07 (3.67)	0.14 (3.02)	0.09 (1.17)	0.10 (1.68)	0.06 (2.75)
10-gingerol	0.24 (1.45)	0.08 (2.49)	0.05 (4.03)	0.20 (0.50)	0.15 (1.66)	0.20 (2.84)	0.14 (0.85)	0.12 (3.06)	0.08 (1.40)
6-shogaol	0.15 (4.11)	0.08 (1.96)	0.13 (2.42)	0.15 (2.04)	0.12 (0.97)	0.36 (2.31)	0.18 (0.63)	0.15 (2.09)	0.10 (2.37)

[a]Values were expressed as Mean (% dry weight; n = 3); values in parentheses showed RSD (in %).
[b]Number after the code of the varieties (G, E, M) means samples from different markets.

Table 5. Effect of the mobile phase compositions[a] on % analyte content[b] of sample E1

No	Toluene	Ethyl acetate	6-gingerol	8-gingerol	10-gingerol	6-shogaol
1	17	5	0.809 (0.47)	0.160 (3.12)	0.178 (0.57)	0.357 (2.92)
2	19	5	0.821 (0.31)	0.158 (1.01)	0.172 (0.34)	0.369 (0.72)
3	17	7	0.812 (0.12)	0.163 (0.36)	0.176 (0.01)	0.367 (4.46)
4	19	7	0.820 (0.14)	0.159 (3.16)	0.175 (2.31)	0.360 (1.55)
5	18	6	0.816 (0.49)	0.157 (1.45)	0.178 (0.65)	0.363 (2.31)

[a]Mobile phase composition presented in v/v.
[b]Values were expressed as Mean (% dry weight; n = 3); values in parentheses showed RSD (%).

All the linear regression calibration curve parameters of those 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.[17,18,20]

Table 2 demonstrated good accuracy as revealed by the percentage of mean recovery data of the used ginger sample E1. The data of repeatability and intermediate precision of ginger sample E1 was presented in Table 3. All RSD (relative standard deviation) were below of 5%. For the bioanalytical study, the accuracy and precision should be not more than ±15/20%.[21] Table 4 showed the content (in %) of all analytes in 9 commercial Gingers; again the data showed that the RSD of the results was relatively accurate (<5%).

In order to evaluate the robustness of the proposed method, the influence of small variations on the composition of the mobile phase on the values and % content of the analytes was analyzed. The data were presented in Table 5. Analysis of effect of the data was performed by using Unscrambler 9.6™ software. A higher order interaction effect

Table 6. Analysis of effect of the robustness data (HOIE method)[a,b]

Variable (Mobile Phase)	6-gingerol	8-gingerol	10-gingerol	6-shogaol
Toluene	NS (p = 0.126)	NS (p = 0.205)	NS (p = 0.395)	NS (p = 0.836)
Ethyl acetate	NS (p = 0.704)	NS (p = 0.295)	NS (p = 0.874)	NS (p = 0.966)

[a]Calculated from data presented on Table 5.
[b]Calculation was performed by using Unscrambler 9.6 software (CAMO) NS = not significant (for p = 0,05); p = probability value.

(HOIE) method showed that the % gingerols and 6-shogaol were not significantly affected by these small variations (Table 6; $p > 0.05$).

The present work showed that the proposed densitometric method is suitable for the routine analysis of ginger samples in herbal drugs industry quality control laboratories. Our experiences showed that the (HP) TLC methods are less expensive 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), so the densitometric method is better. For developing countries in which the price of HPLC grade solvents and columns are relatively very expensive, the availability of an alternative cheap (HP)TLC method is essential.

ACKNOWLEDGMENTS

The authors are very grateful to Camag, Muttenz, Switzerland for providing method MOA 010, and Mr. Fajar Zulkarnain Lubis (Assessment, Service Unit, Faculty of Pharmacy, Airlangga University) for preparing the figures.

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Received August 20, 2008
Accepted September 15, 2008
Manuscript 6386