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Analytical and Stability Studies of Ginger Preparations

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ABSTRACT

Ginger is one of the most frequently used ingredients in Chinese folk medicine and cooking. Ginger rhizome is used in treating/relieving appetite loss and motion sickness in western world. 6-Gingerol has been used as a marker substance of ginger. An HPLC analytical procedure of 6-gingerol was described. The correlation coefficients were greater than 0.999. The described HPLC procedure in this report has been validated and can be used to check 6-gingerol stability. It is also suitable for the preformulation study of ginger. The stabilities of ginger solution at different pHs were studied. The 6-gingerol contents in different preparations were evaluated.

Key words: Ginger; 6-Gingerol; HPLC; Stability study.

INTRODUCTION

Ginger is the dried rhizome of *Zingiber officinale* Roscoe (Zingiberaceae). Zingiber is from the Arabic Zindschebil, meaning root of Indian Zindschi. Ginger is used to mediate the effects of other ingredients, to stimulate the appetite, and to calm the stomach in traditional Chinese medicine. In European herbal traditions, ginger has been studied for its antibacterial, antifungal, pain relieving, antiulcer, antitumor, and other properties. Six clinical studies have looked at ginger's potential to reduce motion sickness(1). It is primarily used to stop nausea and relieve stomach upset. The rhizome has long been valued since ancient times antiquity for its flavor and pungency. It has been verified in clinical trials that ginger rhizome could relieve manage appetite loss, motion sickness and prevent vomiting. It also tends to boost the pumping action of heart(2). Asian medicine employs it as a treatment for asthma, shortness of breath, water retention, earache, diarrhea, nausea, and vomiting; and homeopathic practitioners recommend it for sexual disorders as well(3).

Ginger rhizome contains two classes of constituents: (i) the essential oils which give the aroma, (ii) the main pungent principles: gingerols. Ginger contains 1-2% volatile oil, 5-8% resinous matter, starch and mucilage. The oil of ginger is a mixture of over 24 constituents, consisting of monoterpenes (phellandrene, carnphene, cineolc, citral, and borneol) and sesquiterpenes (zingiberine and hisabolene) etc.(4). Some chemical structures of ginger pungent principles are depicted in Fig. 1. The total content of gingerol in fresh ginger rhizomes is higher than that in solar dried ones, because gingerols become dehydrated and produced shogaols(5, 6).

6-Gingerol, the major pungent principle isolated from ginger oleoresin, has been found to possess substantial antioxidative activity as determined by inhibition of phospholipid peroxidation induced by the $FeCl_3$-ascorbate system(7). 6-Gingerol inhibits phorbol ester-induced inflammation, epidermal ornithine decarboxylase activity and skin tumor promotion in ICR mice(8). It also shows inhibitory effect on xanthine oxidase(9) which is responsible for the generation of reactive oxygen species, such as superoxide anion. Guh(10) reported concentration-dependent inhibition by 6-gingerol of arachidonic acid-induced platelet aggregation and formation of thromboxane B2 and prostaglandin D2. The ethanol extract of ginger reduced carrageenan-induced paw edema(11). Gingerol, shogaol and other structurally related substances in ginger inhibit prostaglandin and leukotriene biosynthesis through suppression of 5-lipoxygenase or prostaglandin synthetase(12,13).

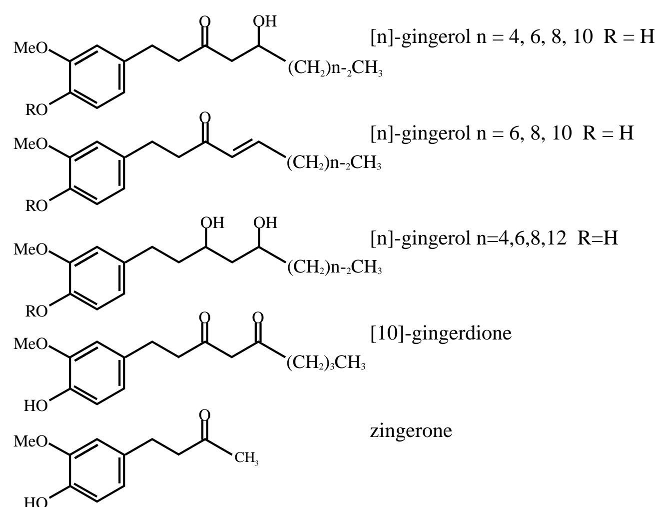

Figure 1. Ginger pungent principles discussed in this paper.

The stability of ginger preparation has not been reported or studied extensively(6,14,15). In this paper, we report the 6-gingerol contents and stability of solutions of different parts of ginger and at different pHs.

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MATERALS AND METHODS

I. *Reagents and Materials*

6-Gingerol, lot No. LDQ7867, was purchased from Wako Pure Chemical Industries (Osaka, Japan). Methanol, HPLC grade, was obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade and were used without further purification. Ginger was purchased from Taiping, Taichung, and was identified as the dried rhizome of *Zingiber officinale* Roscoe by Mr. Nien-Yuan Chiou (an instructor of China Medical College).

II. *Instruments and Conditions*

A Shimadzu liquid chromatographic system (Kyoto, Japan) equipped with a Model LC-10 AS solvent delivery pump, a Rheodyne 7125 injector (loop volume 10 µL), and an SPD-6AV variable wavelength UV detector was used. A stainless-steel column (150 × 4 mm I.D.) packed with Spherisorb S5 C-18 was purchased from Fisons (Mainz-Kastel, Germany). For the mobile phase system: methanol and water were used in this study. Through varying the composition of methanol in water, an optimal ratio of these two solvents could be determined.

III. *Purity Determination of 6-Gingerol*

The purity of 6-gingerol was checked with HPLC and detected at 210 nm. A 6-gingerol aqueous solution with concentration of 124.2 µg/mL was used. Flow rate was 1 mL/min. The mobile phase system used in this study was composed of methanol : water (65:35, v/v). The whole chromatogram took 25 minutes. The ratio of peak area of 6-gingerol over that of total peaks is a purity reference of 6-gingerol.

IV. *Preparation of Calibration Curve for 6-Gingerol*

6-Gingerol aqueous solutions were prepared in concentrations of 124.2, 103.5, 82.8, 62.1, 41.4, and 20.7 µg/mL for the calibration curve. The mobile phase system used in this study was composed of methanol: water (60:40, v/v). The flow rate was 1.0 mL/min. The eluted substances were detected at 281 nm. Every sample was analyzed at least in triplicate and the results were averaged.

V. *The Stability of 6-Gingerol and Ginger Juice*

6-Gingerol aqueous solutions were prepared in concentrations of 103.5 µg/mL with 0.1N HCl and 0.1N KOH solutions and stored at room temperature. At appropriate time intervals, samples were withdrawn and stored in the freezer. When the experiment was finished, the samples were thawed and analyzed by the HPLC method. The chromatographic fingerprints of each sample were determined by a mobile phase system composed of methanol: water (60:40,v/v).

Ginger juice was obtained via pressing and diluted with equal volume of pH 4.0 acetate buffer and stored at 36˚C incubator. At t = 0, 75, and 250 min. samples were withdrawn and stored in a freezer. The kinetic profiles of peak area of No. 1-5 were determined. The concentration of the remaining 6-gingerol was determined by the described HPLC conditions. A volume of 500 mL distilled water was added to 100 g of ginger rhizome and blended. The ginger solution was filtered and then adjusted separately to obtain pHs of 2.64, 3.56, 4.36, 5.44, 6.76, 7.58, and 8.70 by adding proper amount of 1.0 N HCl or KOH solution. They were stored at room temperature. The appearance, odor, clarity, height of precipitate and color of the ginger solution on the initial day (day 0), day 1 and day 4 were observed.

All the samples were withdrawn at appropriate time intervals and stored in the freezer. When the experiment was finished, samples were thawed. The fingerprint and the concentration of 6-gingerol were analyzed by the described HPLC conditions.

VI. *Comparison among Different Ginger Segments in Chromatography*

The ginger skin was obtained from ginger rhizome with a peeler (width = 1mm). Twenty grams each of ginger skin, ginger rhizome, ginger leaf, and ginger bud were ground in a mortar then adjusted to 25 mL volume with pH 4.0 acetate buffer solution. The mobile phase was methanol: water (65:35, v/v), The ginger segments with the highest quantity of HPLC peak area of 6-gingerol was set as 100%. A bar chart of relative peak area versus ginger segments was then drawn. The peaks of chromatogram among three different segments were compared.

RESULTS AND DISCUSSION

The purity of 6-gingerol reference standard under the mentioned HPLC method showed a good purity of 99.52%. The calibration graph for 6-gingerol was obtained over the range of 124.2-20.7 µg/mL. The result, by linear regression analysis, showed a very good linear relationship between peak area and concentration (Fig. 2.).

The correlation coefficient was 0.9994. The repeatability and the accuracy were determined by repeating the analysis six times at the concentration of 20.7, 62.1, and 103.5 µg/mL, the C.V.'s were 2.49, 2.12, and 1.9%, respectively. The relative errors were found to be -1.5, -0.9, and -0.6%, respectively, at the concentrations mentioned above.

A stability indicating method assay should be capable of discriminating between the active ingredient from any degradation product[16]. It should be used to describe the kinetic model of a degraded active ingredient. In this study, the kinetic profiles of 6-gingerol, both in acidic and alkaline media, indicated that degradation of 6-gingerol followed the zero order reaction model. The kinetic plots were shown in Fig. 3. The slopes were -82.8 µg/mL/hr and -311.6

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Table 1. The physical properties of ginger aqueous solutions in various pH media after 4 days

pH	2.64	3.56	4.36	5.46	6.67	7.58	8.70
Appearance	Phase separated	Phase separated	Phase separated	Suspension	Suspension	Suspension	Suspension
Clarity	++++	+++	++	+			
Height of ppt.	+++	++++	++	++	+	+	+
Odor	+++	+	+			Unpleasent	Unpleasent
Color	Light yellow to pink	Light yellow to pink	Light yellow	Light yellow	Light yellow to yellow	Light yellow to yellow	Light yellow to dark yellow

Number of "+" represent the strength of that item.

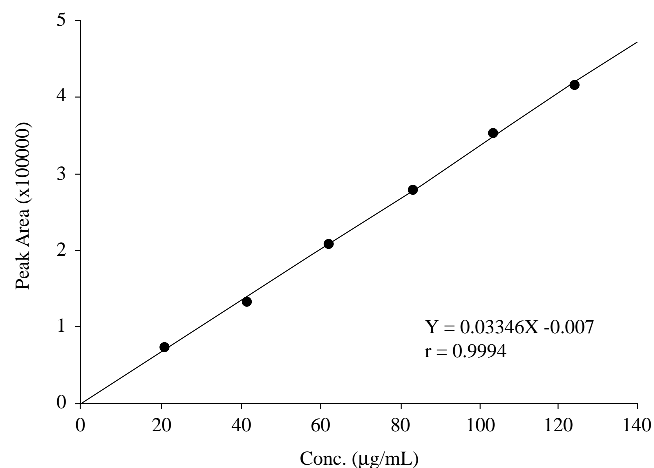

Figure 2. The calibration graph of 6-gingerol determined by HPLC assay.

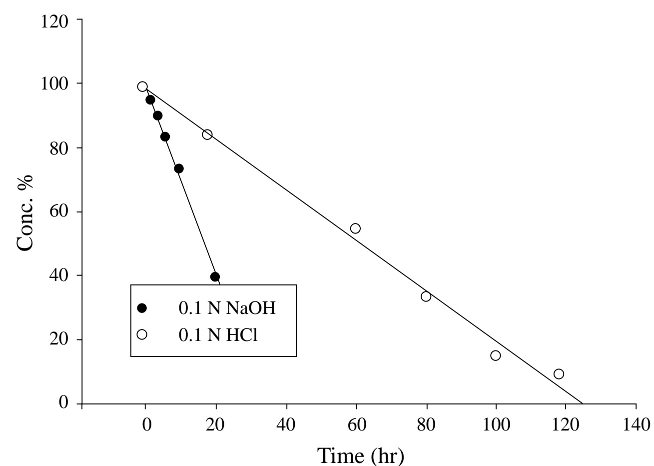

Figure 3. Apparent zero-order plots for the degradation of 103.5 μg/mL 6-gingerol at acid/alkaline medium and determined by HPLC assay.

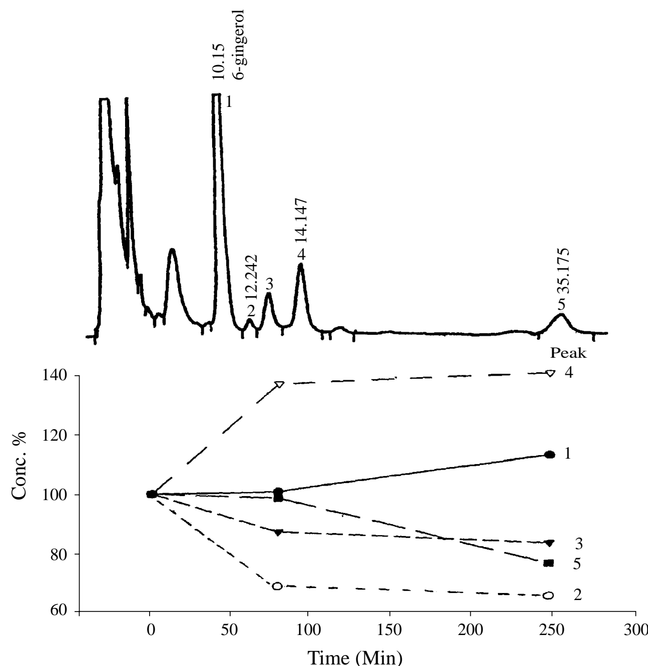

Figure 4. The chromatogram of ginger solution in pH 4.0 acetate buffer at 36˚C and the kinetic profile of peak No. 1-5.

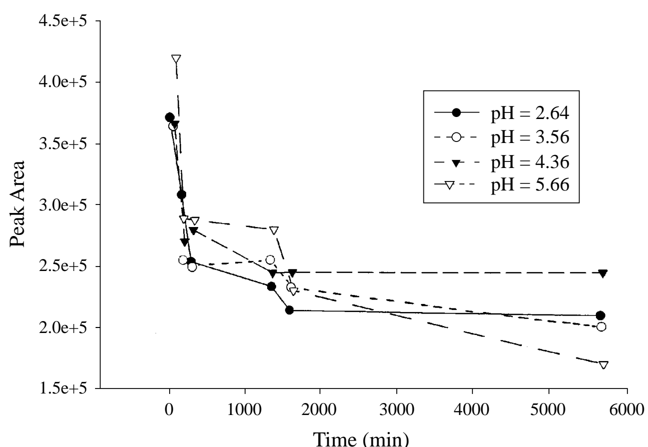

Figure 5. The kinetic profiles of 6-gingerol in pH = 2~5 ginger solutions at 25˚C.

μg/mL/hr, respectively. The correlation coefficient was 0.996 in acidic medium and 0.997 in alkaline medium, demonstrating that this HPLC method is suitable for stability study. The 6-gingerol is more stable in acid than in alkaline.

Gingerol has acidic methylene protons and tends to undergo dehydration to form shogaols[6,17]. Both pH and higher temperature affect stability of gingerols. When ginger juice in pH 4.0 acetate buffer was stored at 36˚C, the chromatogram was shown in Fig. 4. The kinetic profiles of peak No. 1-5, labeled in the chromatogram of Fig. 4, were determined.

The chromatographic peaks # 2, 3, and 5 tended to

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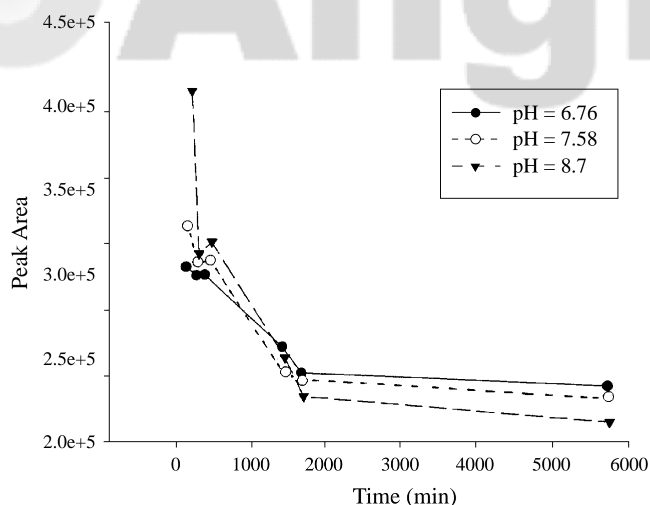

Figure 6. The kinetic profiles of 6-gingerol in pH = 6~8 ginger solutions at 25˚C.

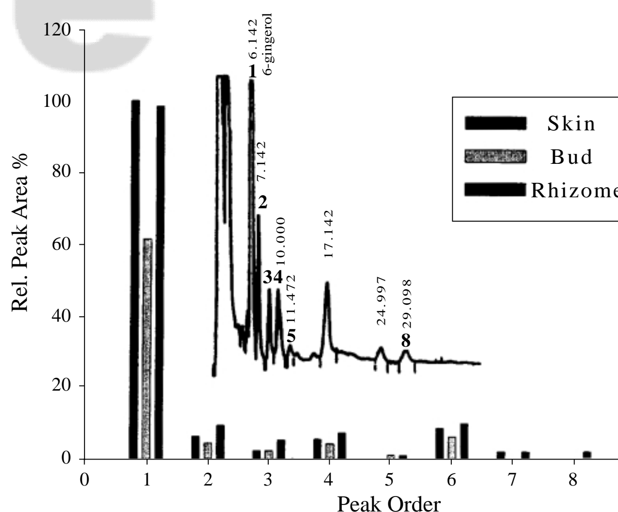

Figure 7. The chromatograms and the finger-prints of different segments of ginger.

Table 2. pH values of ginger solution at different times

pH at t=0 hr	2.64	3.56	4.36	5.46	6.76	7.58	8.7
pH at t=22.17 hr	2.75	3.78	4.58	5.13	5.29	5.25	5.35
pH at t=93.83 hr	2.70	4.49	4.76	5.09	6.45	5.39	4.77

degrade in Fig. 4. The degradation occurred rapidly within 75 mins. First, the ginger preparations were formulated with acetate buffer and phosphate buffer. However, a white precipitation formed immediately after phosphate buffer added and the odor of acetic acid was unpleasant. In the stability study of ginger solution, only 1.0 N HCl or 1.0 N KOH solution was used to adjust the pH of ginger solutions to avoid possible interaction of acetate or phosphate ion with ginger preparation. The kinetic profiles of 6-gingerol in ginger solutions over the range of pH 2.64~8.70 at 25˚C were shown in Figs. 5 and 6.

Some physical properties of ginger aqueous solutions in various pH media after 4 days were shown in Table 1. It indicated that the ginger solutions had better physical and chemical stability between pH 4.0~5.0. The pH of ginger solution at different times had been measured. Ginger solutions at day 4 had pH values mostly within 4 to 5 (Table 2).

The nature of the complex mixture of relatively unstable phenolic compounds in ginger have been outlined by Balladin[5] and Denniff[18]. Gingerols are dehydrated, under acidic condition and at higher temperature in order to produce shogaols. Both of gingerols and shogaols undergo the retro-aldol cleavages to form zingerone and corresponding aldehydes. Zingerone and aldehydes under acidic condition are condensed to form corresponding gingerols. Gingerols, shogaols, gingediols, gingerdione, and zingerone may react to form one another through the mechanism mentioned above. Their stability study in ginger must be very complicated.

The chromatograms of different segments of ginger had some differences (Fig. 7).

From the chromatogram in Fig. 7, eight peaks were chosen. Only peak #1 was identified as 6-gingerol with authentic sample. The other seven peaks were unknown. Figure 7 showed that the ginger skin had a higher amount of 6-gingerol than the bud. Peaks # 5 and #8 were not detectable in ginger skin. Peaks #7 and #8 were not detectable in ginger bud.

Ginger rhizome and skin had about 1.67 times more of 6-gingerol than ginger bud and about 39.0 times more than ginger leaf. The amount of 6-gingerol was about 0.823 mg/g of fresh weight in ginger skin and about 0.806 mg/g of fresh weight in ginger rhizome.

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薑配製物之分析與安定性研究

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摘　　要

薑是國人常用的藥物和食品。在歐美，薑的根部用於治療無食慾及暈車病。6-Gingerol是薑製劑品管的標誌物。本報告描述了6-gingerol的HPLC分析與安定性研究。其標準曲線之線性相關係數大於0.999。6-gingerol的HPLC分析方法已確認可作為安定性研究用，亦適合作為薑的預配方研究。本研究也報告薑配製物在不同酸鹼度下之安定性。

關鍵詞：薑；6-Gingerol ；HPLC ；安定性研究