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THE STABILITY STUDY OF GINGER EXHAUSTIVE EXTRACTION USING HPLC

ABDEL HADI AL JAFARI[*] AND AHMAD KHALAF ALKHAWALDEH
Department of Pharmaceutical Science; Faculty of Pharmacy, Jerash University, Jordan [AHAJ, AKA].
[[*]For Correspondence: E-mail: abdelhadi.aljafari@jpu.edu.jo]

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ABSTRACT

Ginger contains biologically active components that may contribute significantly to the therapeutic applications of ginger and some ginger-derived products. 6-Gingerol has long been used as a ginger marker. HPLC was used to perform quantitative measurements of 6-Gingerol and its principal degradation product. In addition, the pH factor to be one of the most important factors in the stability condition of ginger liquid extractions. The rate of degradation was shown to be pH dependent, with the maximum stability at pH 4. At 100°C and pH 1, the reversible breakdown of 6-gingerol was quite quick, reaching equilibrium in less than 2 hours. The coefficient of correlation were larger than 0.994. The HPLC approach described in this article has been validated by researchers and can be used to verify the stability of 6-gingerol. It is also suitable for ginger reformulation research. The stability of ginger solution at various pH (4-7) was investigated. The content of 6-gingerol in various formulations was assessed. Ginger stability: According to the regulations, the extract must be stable after 180 days at 40 degrees Celsius, with not more than 10% of the active component or active markers degraded. Therefore, the experimental work has been put on hold while the researchers wait for the samples to go through the process of repose at 40 degrees Celsius for a period of time, after which they will be analyzed; if the results are promising, the researchers will continue to confirm the final time of 180 days; if not, the researchers will try another theory.

Keywords: Stability study; *Zingiber officinale;* 6-Gingerol; 6-shogaol; ginger extract; roscoe ginger; HPLC.

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is a monocotyledonous plant belonging to the family zingiberaceae and is one of the world's best-known spices, cultivated in several countries and has been used since antiquity for its health benefits [1]. Ginger (*Zingiber officinale*), Roscoe belonging to the Family Zingiberaceae, is a perennial herb with thick tuberous rhizomes. The erect leafy aerial stem grows up to approximately 1 meter in height and has purple flowers. Its roots

are used as a spice in cooking zingiberene as the main component [2,3]. Ginger contains up to 3% of essential oil that causes the fragrance of the spice. The pungent taste of ginger is due to non-volatile phenylpropanoid – derived compounds, gingerols and shogaols [4,5]. The shogaols are formed from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process, and it is less pungent and has a sweet aroma [6]. Ginger is a minor chemical irritant, and has a sialagogue action, stimulating the production of saliva. Mature ginger roots are fibrous and nearly dry. Powdered dry ginger roots (ginger powder) are typically used to add spiciness to ginger bread and other recipes [7]. Although the digestion stimulating effect of this spice became known a long time ago, the stimulating effect on peptic juices, such as gastric juice, bile, pancreatic and intestinal juices, was discovered later [8]. Ginger may also decrease joint pain from arthritis, may have blood thinning and cholesterol lowering properties which may be useful for the treatment of heart and lungs diseases [9]. Ginger has been found effective by multiple studies for treating nausea caused by seasickness, morning sickness and chemotherapy. Ginger has also been reported to be effective for the treatment of inflammation, rheumatism, cold, heat cramps, and diabetes [10]. However, the stability of ginger preparations has not been extensively documented or examined [11]. Due to the loss of a hydroxyl group from gingerol during dehydration to shogaol, 6-shogaol is significantly more lipophilic than 6-gingerol in terms of lipophilic characteristics. The lipophilicity differential between these two substances would result in considerable changes in bioavailability and pharmacokinetic characteristics in vivo [12]. Moreover Gingerols have hydroxy keto functional group in their structure, which makes them thermally labile and allows for easy dehydration at high temperatures and under acidic environments. At 120°C, a kinetic study of [6]-gingerol dehydration has been carried out with a pH range of 2.4 to 7.2 [13]. The dehydration of gingerol has also been found to be pH and temperature dependent, and to follow first order kinetics in these tests. Thus the mechanism of gingerol degradation was investigated, which included a retro-aldol reaction that produced zingerone and aliphatic aldehydes, as well as dehydration to shogaol [14]. For the first time, gingerol was discovered to undergo reversible dehydration, resulting in the formation of shogaol in equilibrium with gingerol. To determine also the degradation kinetic profile for gingerol in aqueous solutions, experiments were conducted at various temperatures (S7 to 100°C) and pHs (1, 4, and 7) [15]. The goal and objective of this study was to look at the 6-gingerol content and stability of solutions made from various sections of ginger at various pH.

EXPERIMENTAL

Materials and Methods

The active principles were extricated from ginger by maceration at 60°C with and without steering, with different concentrations of water and glycerin (70:30, 50:50 and 30:70). To which was added different concentrations of sodium benzoate ($NaC_7H_5O_2$) under the E number E211 (0.2% & 2%), ascorbic acid ($C_6H_8O_6$) E300 (0.05% - 0.2%), citric acid ($C_6H_8O_7$) E330 (0.05% - 0.6%), and Butylated hydroxytoluene ($C_{15}H_{24}O$) E321 (0.1% 0.4%) for 4 hours. The determination of total content of gingerols and shogaols was done by reversed-phase HPLC using Capsacin ($C_{18}H_{27}NO_3$) as external reference, the Methanol, Acetonitrile, Phosphoric acid and the purified water was HPLC grade.

Solutions and sample preparation (Ginger Stability Study)

Degradation samples: Using the first extraction as an initial one, by separating 100.0 g samples to add different concentrations or mixtures of sodium benzoate (0.2% and 2%), ascorbic acid (0.05%, 0.1% and 0.2%). Citric acid (0.05%, 0.1%, 0.2 %, 0.3%, 0.4% and 0.6%) and butylated hydroxytoluene (0.1%, 0.2% 0.3% and 0.4%), and mixtures (0.2% sodium benzoate, 0.4% citric acid and 0.4% butylated hydroxytoluene), (0.2% sodium benzoate, 0.2% citric acid and 0.2% butylated hydroxytoluene), (0.2% sodium benzoate, 0.1% citric acid and 0.1% butylated hydroxytoluene). Half of the samples was left at room temperature and the other half at 40°C inside oven with continued control of temperature and humidity. As described in a former study, the pH

is a determined factor that was one of the reasons citric acid was added which plays a duple role in the samples, achieving pH near to 4, and as anti-oxidant agent, the normal pH for ginger hydroglycerin extract is 6.2 - 6.4 without adding any of the agents.

HPLC Assay

The HPLC analysis was carried out on a Dionex UltiMate 3000 UHPLC HPLC System from Thermo Scientific (Pump LPG-3400 SD, Autosampler: WPS-3000 TSL, Detector: DAD-3000 RS, Colmen compartment: TCC-3000 SD). The separation was carried out on a Thermo hypersil reversed phase column with BDS and C18 (250 X 4.6 mm, 5wm). With a flow rate of 1mL/min, the mobile phase consists of gradient solution A (Acetonitrile 100 percent) and solution B (Aqueous solution of phosphoric acid at 0.1 percent V/V). The column was kept at 30 degrees Celsius and the measurements was taken at 280 nm. For all samples, including the reference ones, each injection lasted 55 minutes. HPLC was used to determine the quantitative amounts of gingerol and shogaol.

RESULTS AND DISCUSSION

At all temperatures, the degradation of 6-gingerol followed reversible kinetics under the conditions described in this study. According to preliminary findings, 6-gingerol is relatively stable in the pH range of 1 to 7 at 50°C. At temperatures above 60°C, however, the molecule decomposes significantly. In a 24-hour incubation period at 60°C, more than half of the gingerol was destroyed. At high temperatures and in acidic conditions, 6-gingerol degraded favorably. As demonstrated in Table 1, at 80 day in 0.1 M HCl, 6-gingerol degraded in a time-dependent manner to generate shogaol.

The breakdown of [6]-gingerol and [6]-shogaol in Water/ Glycerin 50:50 with constant steering followed reversible kinetics involving dehydration and hydration processes, respectively. Water dehydration causes gingerol breakdown, which results in the formation of shogaol as a main product, and vice versa. pH (4-7) and temperature were both important factors in this process. Because no substantial fluctuations in gingerol content was found over 24 hours, 6-gingerol was determined to be relatively stable over a pH range of 1 to 7.

All samples were stable for two weeks, results in Table 2 shows these promising results, starting the third week degradation take over; the best results was achieved with the combination of (0.2% sodium benzoate, 0.4% citric acid and 0.4% butylated hydroxytoluene) with pH 4.05, at 40°C. When the samples was kept inside the oven for the period of 80 days, some were between 80 days and 130 days the degradation took place and samples lost 12% of the total content of gingerols and shogaols, and at 180 days it showed 20% of degradation, compared to the results at the starting point as shown in Table 3.

Table 1. Ginger first extraction with Water/ Glycerin 50:50 with constant steering

Sample	Av.mg/g	Gingerol%	shogaol%	Gingerol & shogaol%	6-Gingerol %	6-shogaol %
Ginger 1st ext.4h 10%	**0.531**	68.9	12.1	81.0	55.9	9.8
Ginger 1st ext.4h 10%	**0.508**	45.6	24.9	70.5	34.9	21.0
Ginger 1st ext.4h 10%	**0.468**	32.2	29.6	61.8	24.1	25.1
Ginger 1st ext.4h 10%	**0.419**	19.7	27.6	47.3	14.1	22.8

Table 2. Ginger first extraction with Water 100% with constant steering and without steering

Sample	Av.mg/g	Gingerol %	shogaol %	% Gingerol & shogaol	6-Gingerol %	6-shogaol %
Ginger 1st ext.4h 10%	**0.320**	63.9	11.9	75.8	53.5	8.4
Ginger 1st ext.4h 10%	**0.472**	57.3	15.1	72.4	42.5	11.3

Table 3. Ginger first extraction with (Water/ Glycerin 50:50) with constant steering and without steering

Sample	Av.mg/g	Gingerol %	Shogaol %	% Gingerol & shogaol	6-Gingerol %	6-shogaol %
Ginger 1st ext.4h 10%	**0.517**	77.3	12.8	90.2	63.1	10.3
Ginger 1st ext.4h 10%	**0.293**	72.1	10.8	82.9	62.0	9.3

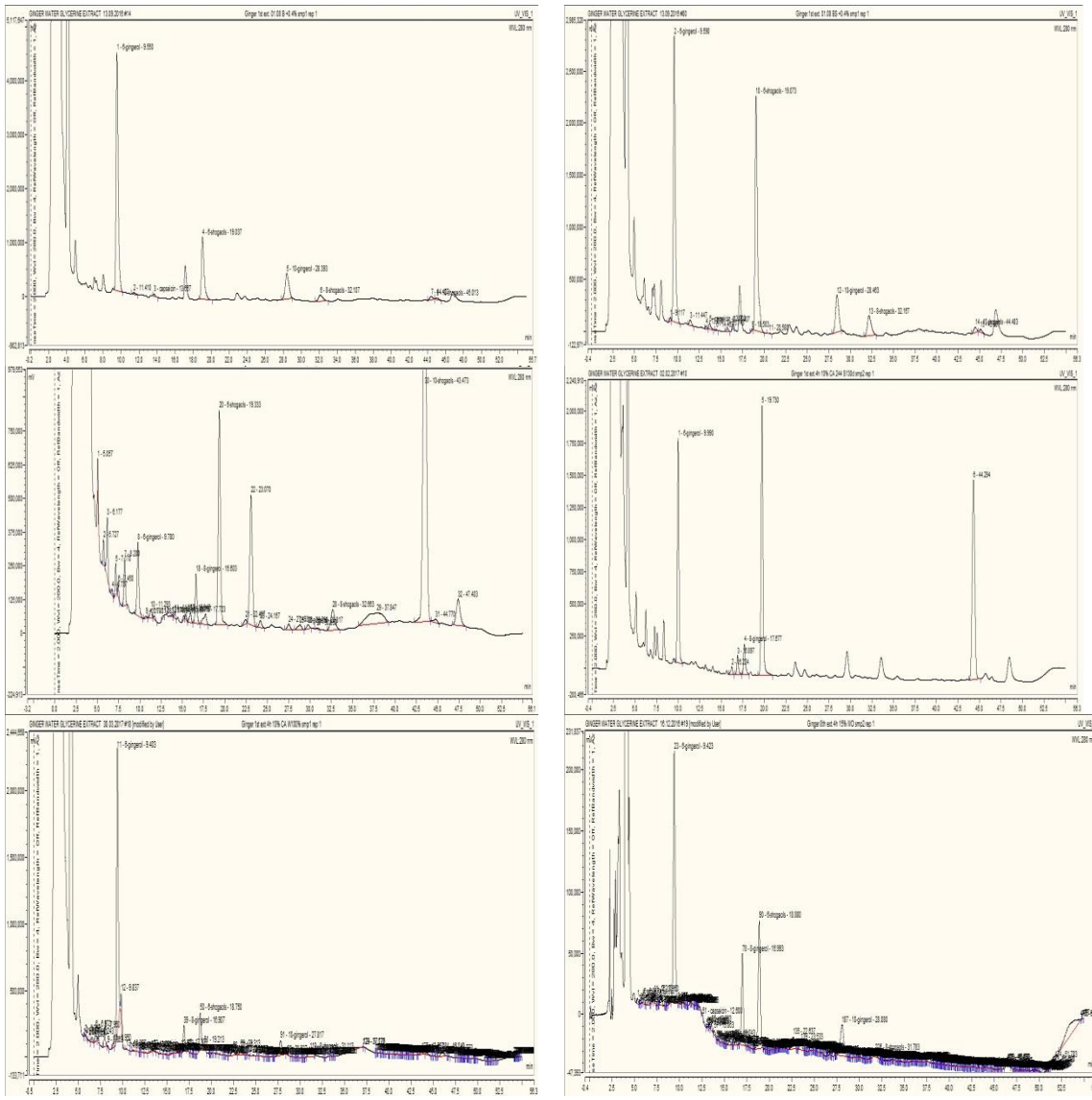

Fig. 1. Ginger first extraction with (Water/ Glycerin 50:50) with constant steering and without steering

The stability of 6- shogaol was also examined under comparable conditions. After a 24-hour incubation period, nearly half of the shogaol concentration was destroyed. The β-hydroxy group of gingerol is thought to undergo catalytic dehydration to generate shogaol in an acidic environment.

At higher temperatures, the amount of products (gingerol and shogaol) measured at equilibrium was significantly different from that at lower temperatures. The parent molecule gingerol predominated at lower temperatures, whereas the dehydration product shogaol was found in abundance at higher temperatures, such as 100°C. At a higher temperature (100°C), the reaction moved quickly and achieved equilibrium in less than 2 hours. At a lower temperature, such as 60°C, however, the reaction was slower and did not reach equilibrium even after 24 hours (results not shown). This suggests that at higher temperatures, equilibrium favors the creation of shogaol. At low pH, where dehydration of gingerol occurred to a considerably greater amount, the reversible process was likewise more favorable.

CONCLUSIONS

Ginger stability: The regulation demands are that after 180 days at 40°C the extract should be stable with acceptable degradation of not more than 10% of the active ingredients or active markers.

1. Experimental work with the percentage of water glycerin has failed to show any improvement in the stability.

2. Adding agents like Citric acid, Ascorbic acid or Butylated hydroutoluene has shown good results up to 30 days; after which, it starts degradation.

3. The mixture of all Citric acid, Ascorbic acid and Butylated hydroxytoluene had promising results up to 100 days, but also somewhere between 80 days and 130 days it start degradation, to reach 12% of degradation, latter on at 180 days it showed 20% of degradation.

4. The other theory that the research came out with, is that as the ginger total content is stable in the product Soothex, Soothex ingredients should be used while eliminate one ingredient or more each time, researchers should have the ingredient or ingredients responsible for its stability. Therefore the experimental work is now on hold, waiting for these samples to pass through the process of repose at 40°C, for some time after which should be analyzed, if the results are promising he researchers will then confirm the final time of 180 days, if not, another theory will be tried.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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