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Development and validation of HPLC method for 6-Gingerol and 6-Shogaol in ginger capsules for the treatment of chemotherapy-induced nausea and vomiting

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Keywords: Ginger; 6-gingerol; HPLC; 6-shogaol; chemotherapy-induced nausea and vomiting

Objectives: The objective of this research is to develop and validate the analytical method of 6-gingerol (6G) and 6-shogaol(6S) in ginger capsules.

Methods: The chromatographic separation was achieved by using C18 column, 150 x 4.6mm i.d., 5µ Luna, mobile phase containing acetonitrile and water (gradient elution). The flow rate was 1.0 ml/min and the absorbance was monitored at 282 nm. The retention time of 6G and 6S was found to be 22.712 and 35.879 min. The proposed method was validated in terms of the analytical parameters such as specificity, accuracy, precision, linearity, range, limit of detection (LOD), limit of quantification (LOQ) and determined based on the International Conference on Harmonization (ICH) guidelines.

Results: The linearity range of 6G and 6S was obtained over 20-60 and 3-7 µg/ml, respectively. Good linearity was observed over the above-mentioned range with linear regression equation Y= 12001x- 29861 for 6G and Y = 29550x-41406 for 6S (x is concentration of analytes in µg/ml and Y is peak area). The values of correlation coefficient were found to be 0.9993 for 6G and 0.9994 for 6S.The limit of detection (LOD) and limit of quantification (LOQ) for 6G were 1.4730 and 4.9099 µg/ml and for 6S were 0.1846 and 0.6153 µg/ml, respectively. The recovery range for 6G and 6S were found to be 93.85 to 104.25 % and 93.77 to 108.47 % for all three spiked levels. The RSD values from repeated extractions for 6G and 6S were 1.24 and 0.33%, respectively.

Conclusion: The validation of developed method on precision, accuracy, specificity, linearity and range were also performed with well-accepted results.

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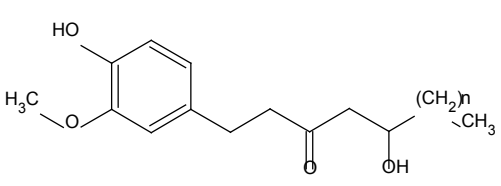
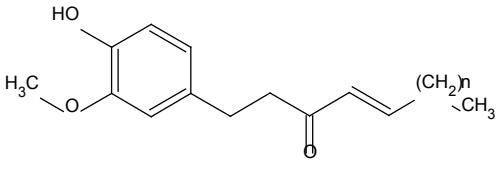
Introduction

"Ginger capsules" developed from ginger extract is a herbal nutraceutical for the treatment of Chemotherapy-induced Nausea and Vomiting; CINV. This product has passed efficacy and safety evaluation in animal testing.

Ginger rhizome (*Zingiber officinale* Roscoe) is an herb of the *Zingiberaceae* family that has been extensively used in the traditional medicine.[1] Ginger was first cultivated in Asia, and has been used as a medicinal herb for at least 2,000 years.[2] Gingerols are the most abundant compounds in fresh roots. There are several chain-lengths of gingerols and the most abundant compound is 6-gingerol. However, the dehydrated form of gingerols; shogaols are only found in small amounts of the fresh root. The shogaols are mainly found in the dried and thermally treated roots. The most abundant form of shogaols is 6-shogaol.[3,4,5] In addition, the major active components, 6-gingerol (6G) and 6-shogaol (6S) are chosen as marker substances which have been shown to have a number of pharmacological activities including: carminative, antiemetic, antinauseant, and anti-inflammatory.[6,7] The objective of this research is to develop and validate the analytical method of these markers (6G, 6S) for quality control of this product. The chemical structures of 6G (a) and 6S (b) are shown in Fig. 1.



(a)



(b)

Figure.1 Chemical Structures of (a) 6-Gingerol (b) 6-Shogaol (n=4)

Methods

A. Reagents and Samples

6G and 6S were purchased from Sigma-Aldrich, Germany. Acetonitrile and methanol were HPLC grade from Merck, Germany. All the water used in this study was Ultrapure, obtained from a Milli-Q RO system (Milipore Corporation, France). The ginger capsules were developed in our research from ginger extract.

B. Preparation of Sample Solution

The granules 40 mg was weighted and extracted with 4 ml methanol by sonicator for 15 minutes. The solution was filtered through a Whatman No.1. Then the filtrate was transferred to 10 ml volumetric flasks and the volume of each was adjusted to 10 ml with methanol. After filtering through a 0.2 µm syringe filter, the final sample was injected directly.

C. Preparation of Standard Solution

Standard stock solutions of 6G and 6S were prepared by dissolving 10 mg of 6G and 6S up to 10 ml of methanol, to get stock solution containing 1000 µg/ml of 6G and 6S.

The stock solutions were diluted to create the five-point standard curves of 6G and 6S using concentration at 20-60 µg/ml and 3-7µg/ml, respectively.

D. Instrumentation and Chromatographic Conditions

The analytical method of two markers was performed on a Waters Alliance e2695 LC system connected with a Waters model 2996 photodiode-array detector. Data collection and processing were carried out using an Empower workstation. The optimum HPLC system was comprised of a C18 reverse phase column (Luna C18, 150x4.6 mm i.d., 5 µm particle size). The gradient was eluted with acetonitrile and water at a flow rate of 1.0 ml/min and PDA detection at 282 nm. The mobile phase consisted water and acetonitrile. All solutions were degassed and filtered through a 0.20 µm pore size filter (Millipore, USA).

E. Method Validation

The analytical method was validated on specificity, precision, accuracy, linearity, range, and limits of detection and quantification.

F. Statistical Calculations

Standard regression curve analysis was performed by using Micro-soft Office Excel 2007 software (Microsoft, USA), without forcing through zero. Means and standard deviations were calculated by using SPSS software version 9.5 (SPSS, Cary, NC, USA).

Results and Discussions

A. Specificity of the Developed Method

The specificity of this method was determined by analysis of the blank, placebo, standard and sample solution chromatograms (Figures. 2-5). Good separation between the peaks of 6G and 6S was achieved, with the retention times, 22.712 min for 6G and 35.879 min for 6S by comparing chromatograms of blank, placebo, standard and sample, there was no interference observed from the peaks of the blank and placebo. It showed that the method is high specificity.

B. Linearity and Range of the Developed Method

For linearity studied, five solutions in the range of 20-60 µg/ml for 6G and 3-7 µg/ml for 6S were analyzed. Each concentration was made and analyzed in triplicate. The peak areas obtained from each concentration of the analytes were used to build a linear regression equation as well as determined the value of correlation coefficient (Table 1). Good linearity was observed over the above - mentioned range with linear regression equation Y = 12001x - 29861 for 6G and Y = 29550x - 41406 for 6S (x is concentration of analytes in µg/ml and Y is peak area). The values of correlation coefficient were 0.9993 for 6G and 0.9994 for 6S.

C. Accuracy of the Developed Method

This study was performed by adding known amounts of 6G and 6S to the placebo samples. Three level of solutions were made and having concentrations at 30, 40, 50 µg/ml for 6G and 4, 5, 6 µg/mL for 6S. The recovery ranges for 6G and 6S were 93.85 to 104.25 % and 93.77 to 108.47 % respectively (limit 80 to 110%). The relative standard deviation ranged from 1.26 to 1.66 % for 6G and from 0.35 to 1.92 % for 6S.

D. Precision of the Developed Method

Repeatability was studied by calculating the relative standard deviation (RSD) from six determinations of the 100% concentration of sample. The studied was performed on the same day and under same experimental conditions. The concentrations of 6G and 6S determinations in the sample solution with the relative standard deviation were calculated (Table 3). The RSD values obtained for 6G and 6S were 1.2439 and 0.3364%, respectively (limit not less than 3.7%). The result showed that the developed method was precise.

E. Sensitivity of the Developed Method

LOD were calculated by using the following equations. LOD = 3.3 x SD/S and LOQ = 10 x SD/S, where SD = the standard deviation of the response, S = Slope of the calibration curve. The LOD values were 1.4730 and 0.1846 µg/ml and the LOQ values were 4.9099 and 0.6153 µg/ml for the simultaneous estimation of 6G and 6S, respectively. Method validation following ICH guidelines indicated that the developed method had high sensitivity.

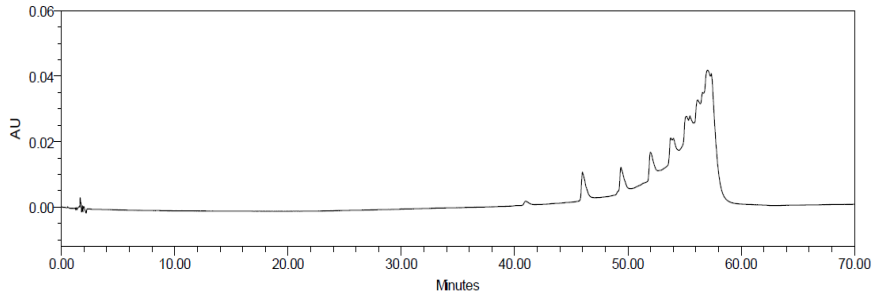


Figure.2 HPLC Chromatogram of Blank Solutions

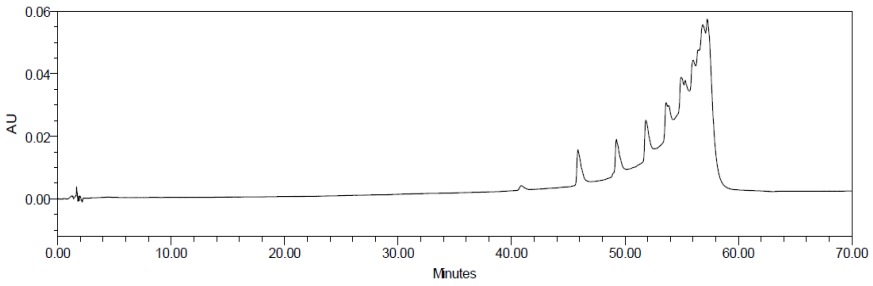


Figure.3 HPLC Chromatogram of Placebo Solutions

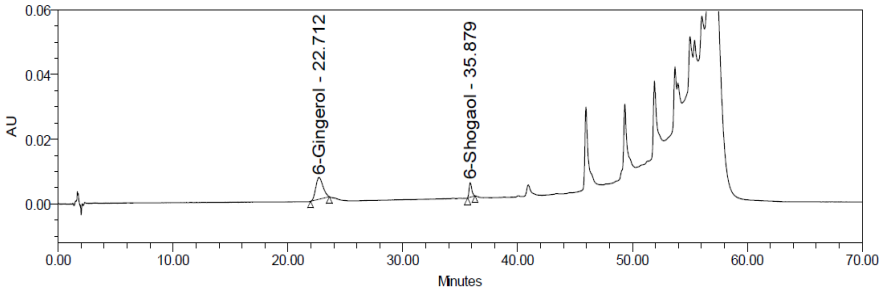


Figure.4 HPLC Chromatogram of 6G and 6S Standard Solutions

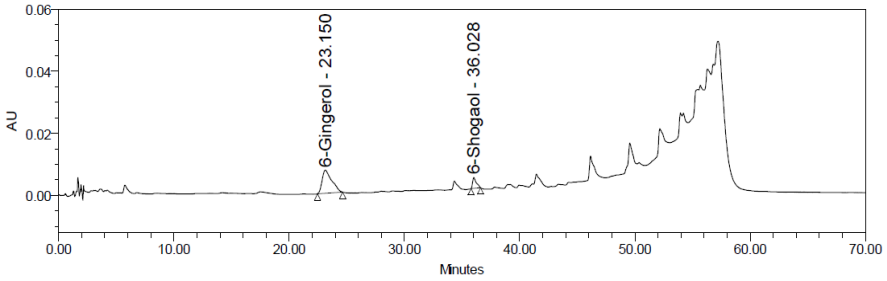


Figure.5 HPLC Chromatogram of Sample Solutions

Table 1. Linearity and Range for 6G and 6S by HPLC

Sample number	6G		6S	
	Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
1	20	207,846	3	47,393
2	30	327,824	4	77,709
3	40	459,303	5	105,372
4	50	568,256	6	134,498
5	60	687,684	7	167,998

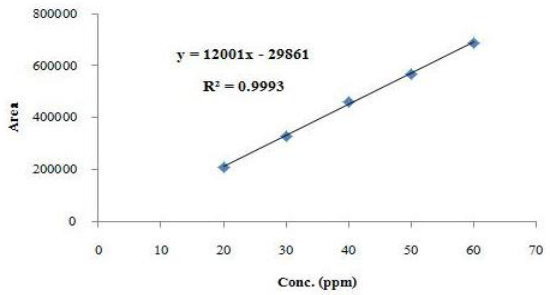


Figure.6 Calibration Curve of 6G by HPLC

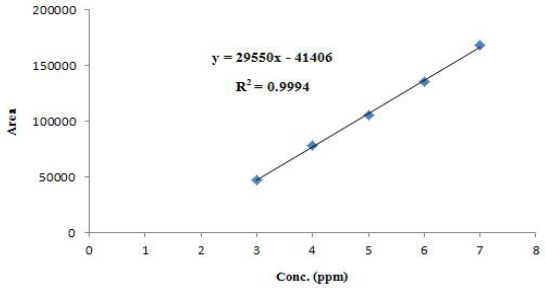


Figure.7 Calibration Curve of 6S by HPLC

Table 2. Accuracy data of 6G and 6S by HPLC

Compounds	Amount (µg/mL)	% Recovery	% RSD
6 G	30	101.96	1.66
	40	104.25	1.26
	50	93.85	1.54
6 S	4	93.77	1.61
	5	102.28	1.92
	6	108.47	0.35

Table 3.Precision Studies of 6G and 6S by HPLC

N	% W/W	
	6G	6S
1	1.0192	0.1254
2	1.0042	0.1246
3	0.9943	0.1246
4	0.9810	0.1248
5	1.0010	0.1253
6	0.9985	0.1244
% RSD	1.24	0.33

Conclusion

Using this method, 6G and 6S could be determined simultaneously, and the validity of the method was also verified. The proposed analytical method for simultaneous estimation of 6G and 6S in the ginger capsules is accurate, precise, linear, robust, reproducible and within the range.

Acknowledgements

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