Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 1, 2013

Research Article

VALIDATION OF REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS DETERMINATION OF 6-, 8-, AND 10-SHOGAOL FROM GINGER PREPARATIONS

AJINKYA R. NIKAM, LOHIDASAN SATHIYANARAYANAN, KAKASAHEB R. MAHADIK*

Centre for Advanced Research in Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune-411 038, Maharashtra, India. Email: krmahadik@rediffmail.com

Received: 08 Jan 2013, Revised and Accepted: 01 Feb 2013

ABSTRACT

Objectives: To develop simple, rapid, and accurate high-performance liquid chromatographic (RP-HPLC) method for quantitative analysis of 6-, 8-, 10-shogaol from ginger preparations.

Methods: The separation of 6-, 8-, 10-shogaol was achieved using BDS Hypersil C18 analytical column with the mobile phase composed of acetonitrile and (0.05%) ortho-phospohoric acid (85:15, v/v) at the flow-rate of 1.0 mL min^{-1} and UV detector was set at 227 nm.

Results: The retention time for 6-, 8-, 10-shogaol was found to be 4.442 min, 5.468 min and 7.258 min respectively. Linear calibration curves were obtained over concentration ranges of 0.50-10.00 µg mL^{-1} for 6-, 8-, 10-shogaol with determination coefficients more than 0.9997 for each analyte. The relative standard deviation for intra- and interday precision was found to be below 2 % for each analyte. The corresponding intra- and interday accuracy (relative error) was found to be less than 0.020 % and 0.040 % respectively.

Conclusion: In present study simple, accurate, precise and rapid HPLC method was developed for simultaneous analysis of 6-, 8-, 10-shogaol and validated in accordance with ICH guidelines. The developed method was found to be suitable for standardization of herbal extracts and polyherbal formulations for the content of 6-, 8-, 10-shogaol

Keywords: 6-shogaol; 8-shogaol; 10-shogaol; RP-HPLC; Validation.

INTRODUCTION

Rhizome of Ginger is a rich source of homologous series of phenolic ketones, accounts for pungency of Ginger [1]. 6-, 8-, and 10-shogaol [6-, 8-, 10-SGL] (Fig. 1) are one of the important bioactive phenolic ketones present in Ginger and reported to be more pungent than gingerols [1]. 6-, 8-, 10-SGL are known to occur naturally and during thermal processing (drying/heating) or long-term storage of gingerols by the elimination of the OH group at C-5 with the formation of a double bond between C-4 and C-5 [2]. Shogaols are known to exhibit antipyretic, antimicrobial, anti-inflammatory, cardiotonic, analgesic and antitussive activities along with anticancer activities [2-9]. Recently it has been proved that shogaols are having more potent anticancer properties than gingerols [10].

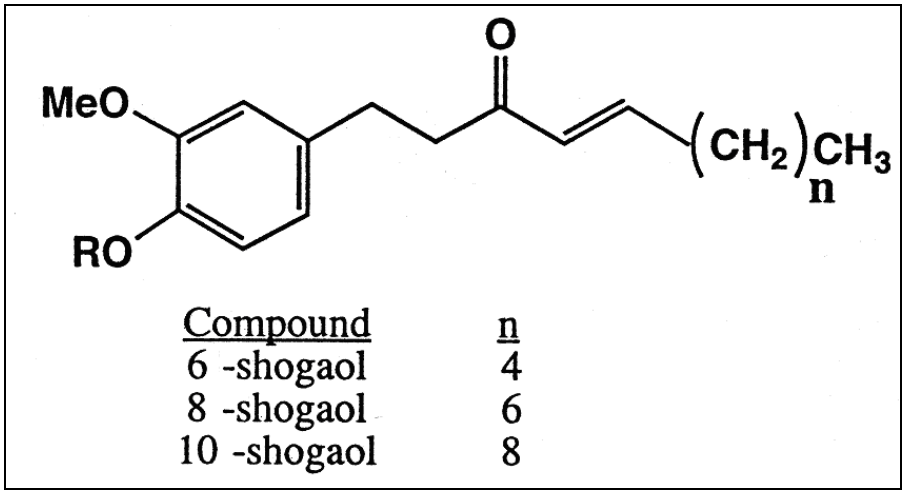


Fig. 1: Structure of 6-, 8-, 10-shogaols

Commonly shogaols are prescribed in the form of Ginger extract [GE] or Ginger oleoresin [GO]. The composition of GE or GO is very complex as both contain series of phytoconstituents along with shogaols [1, 2]. Therefore it is very difficult to identify 6-, 8-, 10-SGL alone from GE or GO. In addition, the quantities of shogaols in GE or GO may get alter with variation in species of Ginger, storage time, environmental conditions, harvest period, and processing methods. The extraction methods used to prepare GE or GO also affect the quantities of shogaols in GE or GO. Therefore, standardization of GE or GO for quantities of shogaols is of great importance in medicinal applications.

There are very few attempts made to develop reverse phase high performance liquid chromatographic (RP-HPLC) method for identification of 6-, 8-, 10-SGL [11-16]. Recently Balladin et al. [11] and He et al [12] have successfully separated 6-, 8-, 10-SGL by RP-

HPLC but they required more time (approximately 30 min) for adequate separation of 6-, 8-, 10-SGL. Kizhakkayil et al [13] and Gong et al [15] has also identified 6-, 8-, 10-SGL by Gas chromatography- mass spectroscopy (GC-MS) and RP-HPLC. However it has been reported that on exposure to heat, gingerols gets converted into corresponding shogaols [14, 15] hence it is not advisable to use GC-MS system for analysis of GE as it contains variety of phytoconstituents along with gingerols and shogaols. On repetition of RP-HPLC method reported by Kizhakkayil et al. [13] 6-, 8-, 10-SGL eluted very slowly at 40 min.

Hence the aim of present work was to develop validated high performance liquid chromatographic method for the fast and simultaneous determination of 6-, 8-, 10-SGL in GO, GE as well in commercial formulations of Ginger.

MATERIALS AND METHODS

Chemicals

GO and GE was purchased from Nisarg Biotech Satara, Maharashtra, India. The reference standard of 6-SGL (>96% purity w/w) was procured from Natural Remedies Pvt. Ltd. Bangalore, Karnataka, India. 8-SGL (>98% purity w/w) and 10-SGL (>98% purity w/w) were purchased from Wuxi App Tec (Tianjin) Co. Ltd., Tianjin, China. Herbal drug formulation used in this study, Honitus (B. No: BD0416, Dabur India Ltd. India) was purchased from the local market. All other reagents and chemicals used in this study were of analytical grade and procured from Merck Specialties Private Limited, Mumbai, India.

HPLC conditions

The HPLC system (Jasco corporation, Tokyo, Japan) consisted of a Pump (Jasco PU- 2080 Plus) with auto injector sampler programmed at 20 µL capacity per injection and UV/VIS detector (Jasco, UV 2075) was used for the analysis. Liquid chromatographic separations were performed on a BDS Hypersil C18 analytical column dimensions (mm) 250 × 4.6, Particle size (µ) 5 (Thermo Scientific, Waltham, USA). The separation was carried out with the mobile phase consisting of acetonitrile and (0.05%) ortho-phospohoric acid (85:15, v/v) at a flow-rate of 1.0 mL min^{-1}. and chromatograms were monitored at 227 nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system.

Preparation of standard solution

The stock solutions of 6-, 8-, 10-SGL (1 mg mL^{-1}) were prepared in methanol individually. The stock solutions were quantitatively transferred into a 10 mL volumetric flask to give solution of appropriate concentration range of 6-SGL, 8-SGL and 10-SGL (0.50-10 µg mL^{-1} each) and made to volume with methanol. Working standard solutions were prepared by dilution of the stock solution.

Calibration Curve

The working standard solutions 6-, 8-, 10-SGL were injected in triplicate, into the HPLC, and the peak area responses were obtained. Individually calibration curve was constructed for 6-, 8-, 10-SGL by plotting the peak areas versus the concentrations of each analyte respectively. The linear calibration curves were obtained separately by least squares linear-regression analysis for 6-, 8-, 10-SGL and linearity was established by residual analysis.

Method Validation

The developed analytical method was validated as per ICH guidelines [17] for precision, accuracy, robustness, and stability. The selectivity of the method was estimated by monitoring chromatograms of blank and spiked 6-, 8-, 10-SGL (Fig. 2) samples for probable interference of endogenous substances. Sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantitation (LOQ). Precision of the method was ensured by analyzing six replicates of 6-, 8-, 10-SGL at three different concentrations (0.5, 2.5, and 10, µg mL^{-1}). The results were expressed in relative standard deviation (% RSD). Intra- and interday precision values were determined to confirm reproducibility of method. Precision and accuracy of method was determined from the results of analysis of six determinations of 6-, 8-, 10-SGL at three different concentrations (0.5, 2.5, and 10, µg mL^{-1}). The precision and accuracy of the method was expressed in terms of % RSD and relative error respectively. Robustness of the method was analyzed by evaluating effect of variation in mobile phase composition and flow rate on the peak areas and retention time of 6-, 8-, 10-SGL at three different concentrations each (0.5, 2.5, and 10, µg mL^{-1}). The stability of standard solutions was evaluated by monitoring the peak area and retention time after 0, 8, 16, 24, 32, 40 and 48 h of storage.

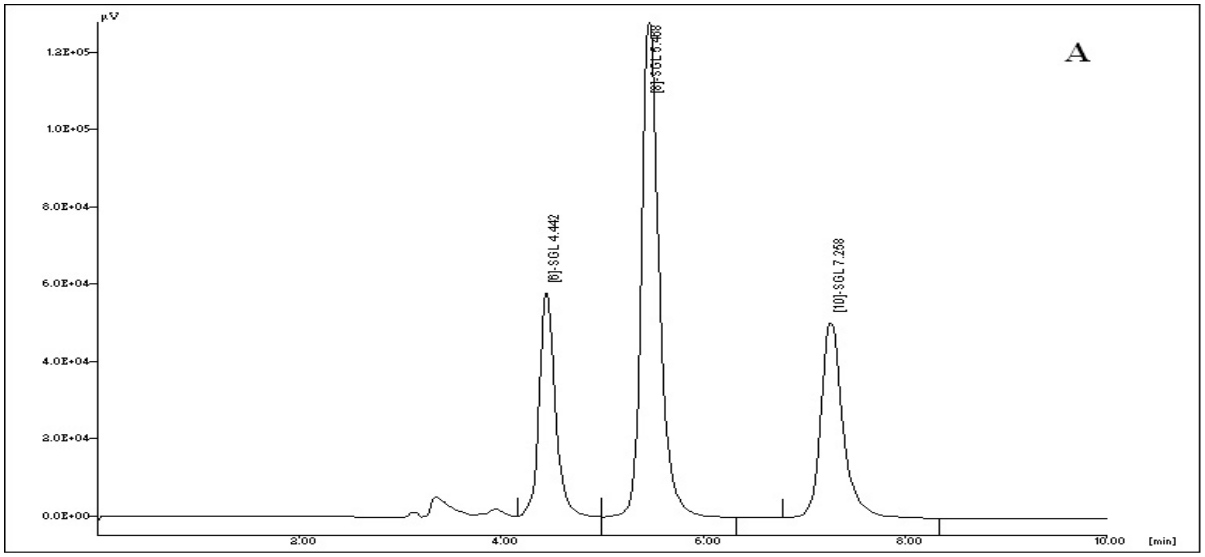
Preparation of sample solutions

GO and GE

Accurately weighed GO and GE (25 mg) were dissolved in methanol (10 mL) separately in 25 mL volumetric flask. Volume of both extracts was made with methanol up to 25 mL and filtered through 0.45 µ filter. Further both solutions were subjected to HPLC analysis for simultaneous estimation of 6-, 8-, 10-SGL.

Honitus Syrup

Accurately weighed a portion of syrup equivalent to 25 mg of Ginger (5 mL contains 25 mg of Ginger) was taken in 25 mL volumetric flask. 10 mL methanol was added and sonicated for 5-10 min to dissolve and made up volume with methanol. Final solution with concentration of 1 mg mL^{-1} of Ginger was filtered through 0.45 µ filter and subjected to HPLC analysis.



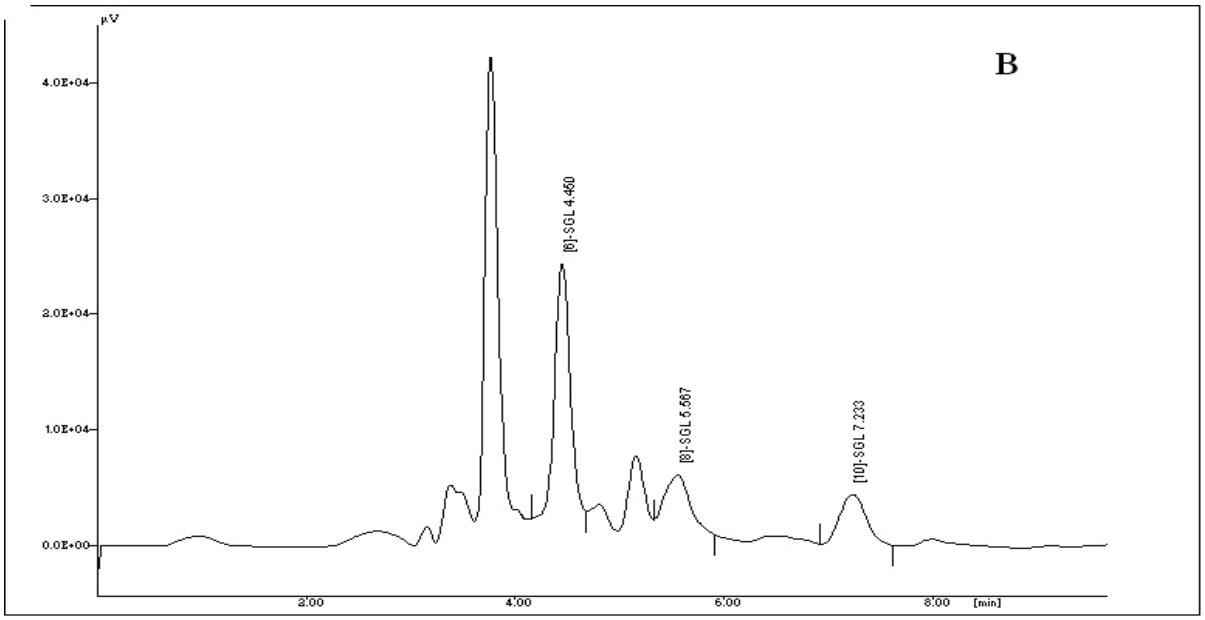


Fig. 2: Chromatogram of (A) standard solutions of the 6-, 8-, 10-shogaols, (B) Ginger Oleoresin

RESULT AND DISCUSSION

Chromatography

Recently we have reported the separation and identification of 6-SGL in rat plasma and various tissues [16]. With the reported method, acetonitrile: (0.1%) orthophosphoric acid (70:30) [16], 6-, 8-, 10-SGL was successfully separated. However 8- and 10-SGL were eluted, in 20 min, with poor baseline resolution accompanied by peak tailing (tailing factor peak >1.2). Change in mobile phase composition to acetonitrile: (0.05%) orthophosphoric acid (85:15), improves baseline resolution and reduces the peak tailing (tailing factor peak <1.2) along with elution time (10 min). The acceptable results were obtained at 1 mL min^{-1} flow rate and 227 nm wavelength (Fig. 3). Retention time for 6-, 8-, 10-shogaol was found to be 4.442 min, 5.468 min and 7.258 min respectively (Fig. 2).

Calibration Curve

Linearity of 6-, 8-, 10-SGL was evaluated by determining six working standard solutions containing 0.50–10.00 μg mL^{-1} of each analyte in triplicate. The calibration equation and correlation coefficients were calculated by least square linear regression analysis of peak area and concentrations. The linear regression data shows good linear relationship over selected concentration range (Table 1) and linearity was ascertained by residual analysis (Fig. 4).

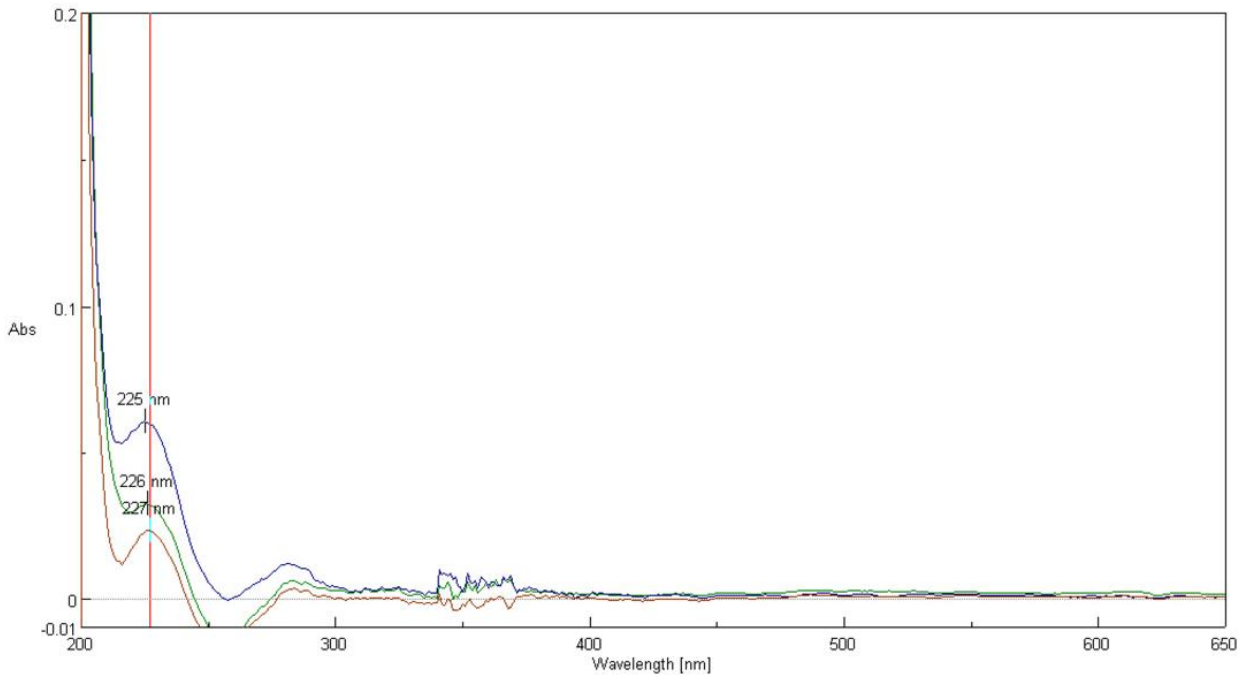


Fig. 3: Overlay of UV spectrum of 6-, 8-, 10-shogaols.

Table 1: Statistical parameters for calibration curve of 6-, 8-,10-SGL

Parameter	6-SGL	8-SGL	10-SGL
Linearity Range (µg mL^{-1})	0.50-10	0.50-10	0.50-10
Correlation Coefficient (r^2)	0.9999	0.9998	0.9999
Slope±SD	15620±81.87	37330±206.6	25690±99.17
Confidence limit of Slope[a]	15410 to 15830	36800 to 37860	25430 to 25940
Intercept±SD	-334.1±425.8	-4984±1074	-5330±515.7
Confidence limit of Intercept[a]	-1429 to 760.5	-7746 to -2222	-6656 to -4004
Sy.x	746.9	1885	904.8

[a]95 % confidence limit.

Sy.x-Standard deviation of residuals from line.

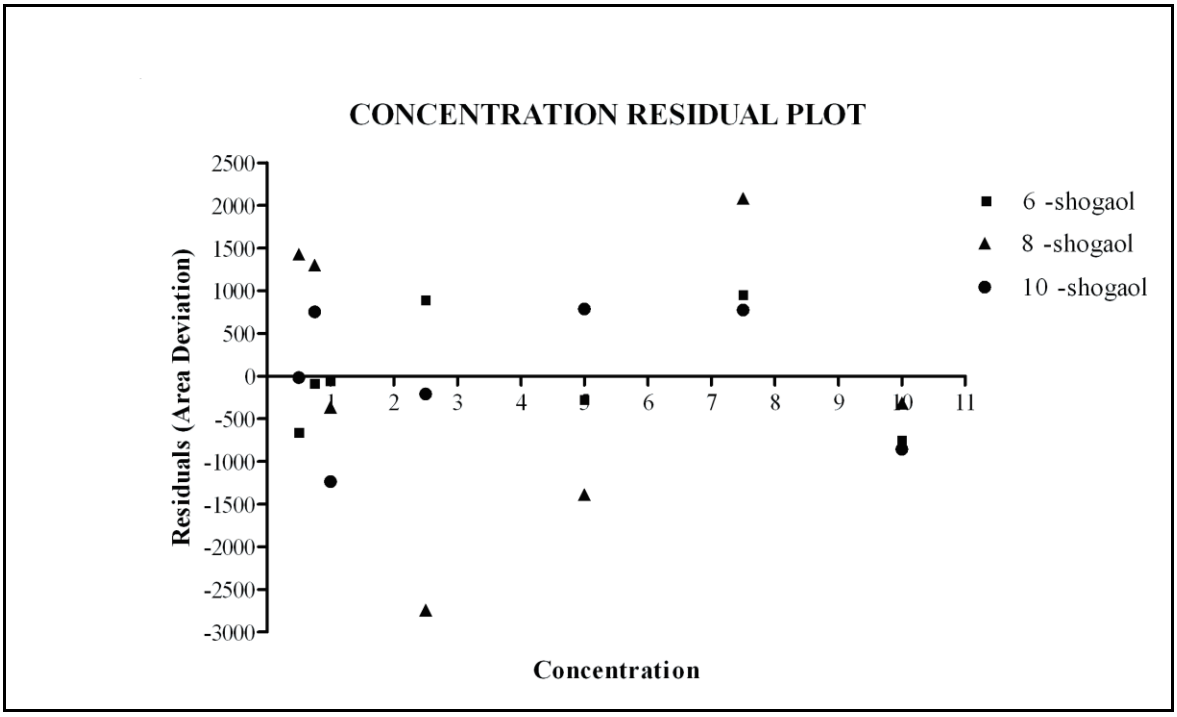


Fig. 4: Concentration versus Residual plot of 6-, 8-, 10-shogaols.

Method Validation

Selectivity and sensitivity

No interference was observed at the retention time when chromatograms of blank and spiked 6-, 8-, 10-SGL samples (n=6) were monitored (Fig. 2). LOD and LOQ were calculated by using slope and the residual standard deviation of a regression line. LOD values were 0.145 µg mL^{-1}, 0.153 µg mL^{-1}, 0.104 µg mL^{-1}, for 6-, 8-, 10-SGL respectively. The LOQ for 6-, 8-, 10-SGL was found to be 0.436 µg mL^{-1}, 0.461 µg mL^{-1}, 0.312 µg mL^{-1}, respectively.

Precision and accuracy

The intra- and interday precision of the analytical method was tested by confirming the intra- and interday variation of 6-, 8-, 10-SGL. The developed method was found to be precise since the relative standard deviation (% RSD) values for intra- and interday precision did not exceed 2 % for selected concentrations (n=6) (Table 2). The accuracy of the method was evaluated by investigating the relative error (% error) of 6-, 8-, 10-SGL at three different concentration levels (n=6) and was found to be less than 0.035 for 6-, 8-, 10-SGL respectively (Table 2).

Table 2: Intra- and interday Precision and Accuracy (n=6)

Analyte	Concentration mg mL^{-1}	Intraday			Interday		
		Mean±SD	% RSD	%Error	Mean±SD	% RSD	%Error
6-SGL	**0.5**	0.49±0.00	0.00	0.020	0.48±0.00	0.00	0.040
	2.5	2.49±0.01	0.40	0.004	2.48±0.01	0.40	0.007
	10	9.98±0.02	0.20	0.002	9.95±0.01	0.10	0.005
8-SGL	**0.5**	0.50±0.00	0.00	0.000	0.49±0.00	0.00	0.020
	2.5	2.51±0.03	1.20	-0.004	2.45±0.04	1.63	0.019
	10	10.04±0.16	1.59	-0.004	9.99±0.17	1.70	0.001
	0.5	0.50±0.00	0.00	0.000	0.49±0.00	0.00	0.020
10-SGL	**2.5**	2.49±0.02	0.80	0.004	2.49±0.01	0.40	0.004
	10	9.99±0.02	0.20	0.001	9.97±0.02	0.20	0.003

Robustness of the method

The effect of small and deliberate variations in the mobile phase composition and flow rate on the peak areas (n=6) and retention time (n=6) of 6-, 8-, 10-SGL was monitored. The relative standard deviation (% RSD) of peak areas and retention time for each drug was calculated and found to be less than 2 % (Table 3). The lower values of relative standard deviation (% RSD) confirm robustness of method.

Solution stability

The standard solutions were found to be stable up to 48 h as no unknown peak was observed. For evaluation of stability, standard solution was stored at ambient temperature away from light. There was no change observed in peak areas and retention time of standard solution over the period of 48 h. The %RSD of peak areas (n=3) and retention time (n=3) of standard solution at different times was found to be less than 2 %.

The data of summary of validation parameters are listed in Table 4.

Methods Comparison

Balladin et al. [11] and He et al [12] have reported separation and identification of 6-, 8-, 10-SGL from ginger preparation by RP-HPLC and LC-MS respectively. However, these methods suffer from drawbacks such as poor base line resolution and poor separation. He et al [12] used gradient elution method and elution time of both methods was more than 30 min whereas the proposed method uses simple isocratic elution with mobile phase of acetonitrile and (0.05%) ortho-phospohoric acid (85:15, v/v) that require the elution time of only 10 min. Besides, in comparison with LC–MS, LC with UV detection is chiefly characterized by its simplicity of operation, inexpensive and widespread.

Analysis of GO, GE and marketed herbal formulation

Developed method was applied for standardization GO, GE and marketed herbal formulation Honitus for content of 6-, 8-, 10-SGL. There was no interference from other phytoconstituents or excipients present in extracts or herbal formulation. The percent contents for each component identified are summarized in Table 5.

Table 3: Robustness study (n=6)

Analyte	Change in Flow Rate (±0.1 mL min^{-1})				Change in % of Acetonitrile (±1%)			
	RT		Area		RT		Area	
	SD	% RSD	SD	% RSD	SD	% RSD	SD	% RSD
6-SGL	0.07	1.59	891.68	1.18	0.07	1.55	1488.51	1.68
8-SGL	0.09	1.54	1014.19	1.08	0.09	1.56	1055.31	1.20
10-SGL	0.11	1.49	1677.55	1.05	0.11	1.56	2065.32	1.36

Table 4: Summary of Validation Parameters.

Parameters	6-SGL	8-SGL	10-SGL
Linearity range (µg mL^{-1})	0.50-10	0.50-10	0.50-10
Correlation coefficient	0.9999	0.9998	0.9999
Limit of detection (µg mL^{-1})	0.15	0.15	0.10
Limit of quantitation (µg mL^{-1})	0.44	0.46	0.31
Precision (% RSD)			
Intraday (n = 6)	0.20	0.93	0.33
Interday (n = 6)	0.17	1.11	0.20
Accuracy (% Error)			
Intraday (n = 6)	0.009	-0.003	0.002
Interday (n = 6)	0.017	0.013	0.009
Robustness	Robust	Robust	Robust
Specificity	Specific	Specific	Specific

Table 5: Determination of 6-, 8-, 10-SGL from marketed formulation, GE and GO

Analyte	Amount of Ginger (mg)	Amount found (%)		
		6-SGL	8-SGL	10-SGL
Honitus Syrup	25	0.068	0.029	0.044
GO	25	0.997	0.185	0.307
GE	25	0.897	0.158	0.273

CONCLUSION

In the present study a simple, accurate, precise and rapid HPLC method was developed and validated for simultaneous analysis of 6-, 8-, 10-SGL. The method was found to be suitable for the standardization of herbal extracts or formulations for the content of 6-, 8-, 10-SGL. The simplicity of the procedure, combined with excellent sensitivity, resolution, and short analysis time, makes this method a useful tool for identification of 6-, 8-, 10-SGL from herbal medicines.

ACKNOWLEDGMENT

The authors are thankful to the Council of Scientific and Industrial Research (CSIR) (Grant No.:8/281(0020)/2011-EMR-I), New Delhi, India for financial assistance to carry out the research and also thankful to the Poona College of Pharmacy, for providing necessary facilities to carry out the work efficiently.

REFERENCES

1. Bailey-Shaw YA, Williams LA, Junor GO, Green CE, Hibbert SL, Salmon CN, et al. Changes in the contents of oleoresin and pungent bioactive principles of jamaican ginger (*zingiber officinale* roscoe.) during maturation. J Agric Food Chem 2008; 56: 5564–71.
2. Ali BH, Blunden G, Tanira MO, Nemmar A Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. Food Chem Toxicol 2008; 46: 409-20.
3. Chrubasik S, Roufogalis BD, Pittler MH Zingiberis rhizoma: A comprehensive review on the ginger effect and efficacy profiles. Phytomedicine 2005; 12: 684-701.
4. Vaiyapuri M, Namasivayam N Chemopreventive efficacy of ginger, a naturally occurring anticarcinogen during the initiation, post-initiation stages of 1,2 dimethylhydrazine-induced colon cancer. Clin Chim Acta 2005; 358: 60-67.

5. Jennifer R, Sarah F, Suzanna Z, Wahl H, Griffith KA, Huang J, et al. Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. BMC Complement Altern Med 2007; 7: 44.
6. Ishiguro K, Ando T, Maeda O, Ohmiya N, Niwa Y, Kadomatsu K, et al. Ginger ingredients reduce viability of gastric cancer cells via distinct mechanisms. Biochem Biophys Res Commun 2007; 362: 218–23.
7. Iwasaki Y, Morita A, Iwasawa T, Kobata K, Sekiwa Y, Morimitsu Y, et al. A nonpungent component of steamed ginger-10-shogaol-increases adrenaline secretion via the activation of TRPV1. Nutr Neurosci 2006; 9(3/4): 169–78.
8. Chen C, Cheng K, Chang A, Lin Y, Hseu Y, Wang H 10-shogaol, an antioxidant from *zingiber officinale* for skin cell proliferation and migration enhancer. Int. J. Mol. Sci 2012; 13: 1762-77.
9. Shukla Y, Singh M Cancer preventive properties of ginger: A brief review. Food Chem Toxicol 2007; 45: 683–690.
10. Wu H, Hsieh M, Lo C, Liu CB, Sang S, Ho C, et al. 6-Shogaol is more effective than 6-gingerol and curcumin in inhibiting 12-O-tetradecanoylphorbol 13-acetate-induced tumor promotion in mice. Mol Nutr Food Res 2010; 54(9): 1296–1306.
11. Balladin DA, Headley O, Chang-Yen I, Mcgaw DR High pressure liquid chromatographic analysis of the main pungent principles of solar dried West Indian ginger (Zingiber officinale Roscoe). Renewable Energy 1998; 13: 531-36.
12. He X, Bernart MW, Lian L, Lin L High-performance liquid chromatography–electrospray mass spectrometric analysis of pungent constituents of ginger. J Chromatogr A 1998; 796: 327–34.
13. Kizhakkayil J, Sasikumar B Characterization of ginger (Zingiber officinale Rosc.) germplasm based on volatile and non-volatile components. African Journal of Biotechnology 2012; 4: 777-86.
14. Harvey D Gas chromatographic and mass spectrophotometric studies of ginger constituents. Identification of gingerdions and new hexahydrocurcumin analogous. J. Chromatography 1981; 212: 75-84.
15. Gong F, Fung YS, Liang YZ Determination of volatile components in ginger using gas chromatography-mass spectrometry with resolution improved by data processing techniques. J Agric Food Chem 2004; 52(21): 6378-83.
16. Nikam AR, Sathiyanarayanan L, Mahadik KR Mapping pharmacokinetic and tissue distribution profile of 6-shogaol from ginger oleoresin. Int J Pharm Pharm Sci 2013; 5(1): 185-189.
17. International Conference on Harmonization ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, The International Federation of Pharmaceutical Manufacturers & Associations, Geneva, Switzerland 2005.