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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE QUICK ESTIMATION OF GINGEROL FROM *ZINGIBER OFFICINALE* RHIZOME EXTRACT

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ABSTRACT
Ginger (*Zingiber officinale* Roscoe Family- Zingiberaceae), have been used in Chinese and Indian folk medicine for centuries. There are no reported UV-visible methods for quick estimation of this extract, which is necessary in the development of suitable formulations for this drug. Hence, a simple UV spectroscopic method was developed for direct estimation of this extract. Ginger rhizome extract obtained from simple maceration process. Calibration curve of rhizome extract was prepared in methanol on three consecutive days at λmax 281.40 nm. The absorbance values (mean of three determinations) with their standard deviations at different concentration in the range of 20-100 µg/ml was determined. Extract was found to obey Beer-Lambert's law in the concentration range of 20-100 µg/ml with regression coefficient (r2) values 0.9995. The regression equations were calculated as y = 0.0097x + 0.0132 for methanol. The developed calibration curve was validated for intra-day and inter-day variations as per ICH Q2A guideline and was found to be a stable method.

KEYWORDS: *Zingiber officinale,* Ginger extract, Maceration process, spectroscopic method, gingerol, Validation.

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe), widely used in foods as a spice around the world, and is a common condiment for various foods and beverages. It has been used as an important ingredient in Chinese, Ayurvedic and Tibb-Unani systems of medicine for centuries. Ginger has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds, catarrh, nervous diseases, gingivitis, toothache, asthma, stroke, constipation, and diabetes [1,2].

Ginger contains a number of pungent constituents and active ingredients. Steam distillation of powdered ginger produces ginger oil, which contains a high proportion of sesquiterpene hydrocarbons, predominantly zingiberene [3]. The major pungent compounds in ginger, from studies of the lipophilic rhizome extracts, have yielded potentially active gingerols, which can be converted to shogaols, zingerone, and paradol [4]. 6-gingerol (structure shown in Figure 1) is the most abundant constituent of fresh ginger but it decreases during postharvest storage and processing, especially thermal processing (He *et al*., 1998; Zhang *et al*., 1994) [5-6]. The compound 6-gingerol appears to be responsible for its characteristic taste. The compounds 6-gingerol and 6-shogaol have been shown to have a number of pharmacological activities, including antipyretic, analgesic, antitussive, and hypotensive effects [7].

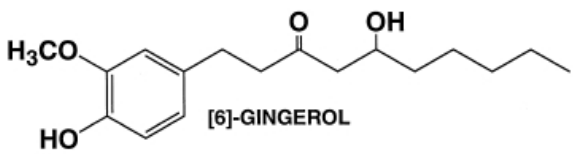

Figure-1. Chemical structure of [6]-gingerol (5-hydroxy-1-(4′hydroxy-3′-methoxyphenyl)-3-decanone)

There are no reported UV-visible methods for quick estimation of this extract, which is necessary in the development of suitable formulations for this drug. Hence, a simple UV spectroscopic method was developed for direct estimation of this extract. The calibration curve was developed using methanol. The assay validation of calibration curve was carried out as per USP guidelines in category I and as per ICH Q2A guidelines. In validation procedure, calibration curve prepared in methanol, was run in triplicate for 3 days to determine intra and inter day variations [8].

MATERIALS & METHODS

Materials

The ginger rhizome was purchased from the local market of Pune, MH, India. All the other chemicals and reagents used in this study were of AR grade and were purchased from Ranbaxy Fine Chemicals, New Delhi.

Methods

Collection and Authentication of Ginger rhizome

The Ginger rhizomes were purchased from the local market of Pune, MH, India and authenticated from Department of Botany, Hon. Balasaheb Jadhav College, Ale. The voucher specimens are preserved. (Herbarium Acc. No. 23826). The collected material was cleaned and dried under shade (at ambient temperature), and then in oven at 20-40^0 C. The dried rhizomes were weighed (1 kg) and stored in desiccator.

Extraction of plant material

Dry ginger was crushed to a coarse powder and extracted with 95% ethanol by simple maceration process. Solvent was evaporated by distillation to obtain thick pasty mass. The thick pasty mass was suspended in water. The Ginger resin precipitates in water which was removed by filtration and the residue obtained was dried under vacuum. [9]

Development of calibration curve

Selection of media

The selection of media was done on the basis of drug solubility. Methanol was selected for preparation of calibration curve. [10]

Scanning for λmax

One hundred milligrams of crude extract was dissolved in little volume of methanol and finally diluted to 100 mL in volumetric flask to get a concentration of 1000 µg/mL. This was treated as stock solution. Various aliquots of stock solution were diluted further to get different concentrations. Resultant solutions were scanned for λmax in the range of 200-400 nm using UV-spectrophotometer.

Preparation of calibration curve

Aliquots of the stock solution of ginger extract (1000 µg/mL) were pipetted out into a series of 10 mL volumetric flasks and diluted with methanol to get a final concentration of 20-100µg/mL. The absorbance of the resultant solutions was measured at 281.40 nm. Freshly prepared solutions were made for the calibration curve on three consecutive days.

Validation of calibration curve

Assay validation of calibration curve was carried out as per USP guidelines in category I and as per ICH Q2A guidelines. In validation procedure, calibration curve prepared in methanol was run in triplicate for 3 days to determine intra and inter day variations. [8]

RESULTS AND DISCUSSION

The ginger extract was soluble in ethanol, methanol, chloroform and Di-methyl-sulphoxide (DMSO) and was insoluble in acetone, toluene, and water. (Table1). The λmax of drug in methanol was determined using UV-Spectrophotometer (SHIMADZU-1800). The λmax was determined by scanning 100 µg/mL solution of drug in the methanol in the range of 200-400 nm. The λmax was found to be 281.40 nm and the absorbance was found to be 0.980.

Calibration curve of drug were prepared in methanol on three consecutive days at λmax 281.40 nm. The absorbance values (mean of three determinations) with their standard deviations at different concentration in the range of 20-100 µg/mL are given in Table 2. The calibration curve is given in Figure 2. The extract was found to obey Beer-Lambert's law in the concentration range of 20- 100 µg/mL with regression coefficient (r2) values 0.9995. The regression equations were calculated as $y = 0.0097x + 0.0132$ for methanol.

Accuracy can also be associated with the term bias. A biased estimate is systematically either higher or lower than the true value. Thus, for accuracy, recovery studies were carried out and the percentage recovery was found to be in the range of 100.43-100.88, which was within the recommended tolerance of 80–115% [11]. The results are shown in Table 3.

The precision of an analytical method or a test procedure is referred to as the degree of closeness of the result obtained by the analytical method or the test procedure to the true value. For evaluation of the precision, the RSD was determined and the range of %RSD was 0.41-3.54, 0.125-1.60 and 0.09-1.39 for first, second and third days, respectively, in the intraday study and was found to be in the range of 0.41-3.54 in the inter day assay. The results are given in Tables 4 to 7. It is suggested that the analytical method may be considered validated in terms of precision if the precision around the mean value does not exceed 15% RSD [12].

Linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. Linearity and range data for calibration curves prepared in methanol.

Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under a stated experimental condition and the limit Quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. These two parameters are required for assay validation as per ICH Q2A guidelines. Limit of detection and limit of Quantitation of calibration curve were calculated which was based on the standard deviation of y intercept of regression line (SD) and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD = 3.3 (SD/S) and LOQ =10 (SD/S) [13]. LOD and LOQ of calibration curve of drug prepared in methanol. The results are given in Table 8.

CONCLUSION

From the above studies, it can be concluded that the developed method of estimation of ginger extract using UV spectro-photometric technique can be used for direct and rapid measurement of the extract. This technique can be used for estimation of ginger extract in different formulations and can be highly helpful in formulation development, particularly in the dissolution studies.

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Table 1: Solubility profile of the ginger extract

Solvent	Solubility behaviour
Water	Insoluble
Tolune	Insoluble
Acetone	Insoluble
DMSO	Soluble
Ethanol	Soluble
Methanol	Soluble
Chloroform	Soluble
Benzene	Soluble
Ether	Sparingly soluble

Table 2: Calibration curve data of Ginger extract

Concentration (µg/mL)	Absorbance
20	0.200
40	0.412
60	0.591
80	0.790
100	0.980

Table 3: Recovery results of drug for determination of accuracy

Labelled amount(µg)	Amount added (µg)	Amount recovered(µg)	Percentage Recovery
25	25	50.44	100.88
50	25	75.32	100.43
75	25	100.49	100.49

Table 4: Results of intraday precision studies for day one

Conc. (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.210 0.200 0.201	0.205	3.54
40	0.408 0.415 0.411	0.412	0.87
60	0.594 0.581 0.598	0.591	1.50
80	0.787 0.789 0.794	0.790	0.46
100	0.980 0.976 0.984	0.980	0.41

Table 5: Results of intraday precision studies for day two

Conc. (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.199 0.201 0.205	0.201	1.60
40	0.402 0.404 0.403	0.403	0.248
60	0.590 0.591 0.594	0.592	0.358
80	0.787 0.780 0.784	0.784	0.450
100	0.974 0.974 0.976	0.975	0.125

Table 6: Results of intraday precision studies for day three

Conc. (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.196 0.196 0.198	0.197	0.63
40	0.394 0.398 0.405	0.399	1.39
60	0.586 0.582 0.587	0.585	0.45
80	0.791 0.790 0.790	0.790	0.09
100	0.971 0.974 0.977	0.974	0.31

Table 7: Results of inter day precision studies of the calibration curves of ginger extract

Conc. (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.210 0.200 0.201	0.205	3.54
40	0.408 0.415 0.411	0.412	0.87
60	0.594 0.581 0.598	0.591	1.50
80	0.787 0.789 0.794	0.790	0.46
100	0.980 0.976 0.984	0.980	0.41

Table 8: Different validation parameters of the calibration

Parameters	Results
Linearity correlation coefficient	0.9995
y- intercept	0.0132
Slope	0.0097
Range	20-100 µg/ml
LOD	4.5 µg/ml
LOQ	13.6 µg/ml

Figure 1: UV Scan of ginger extract

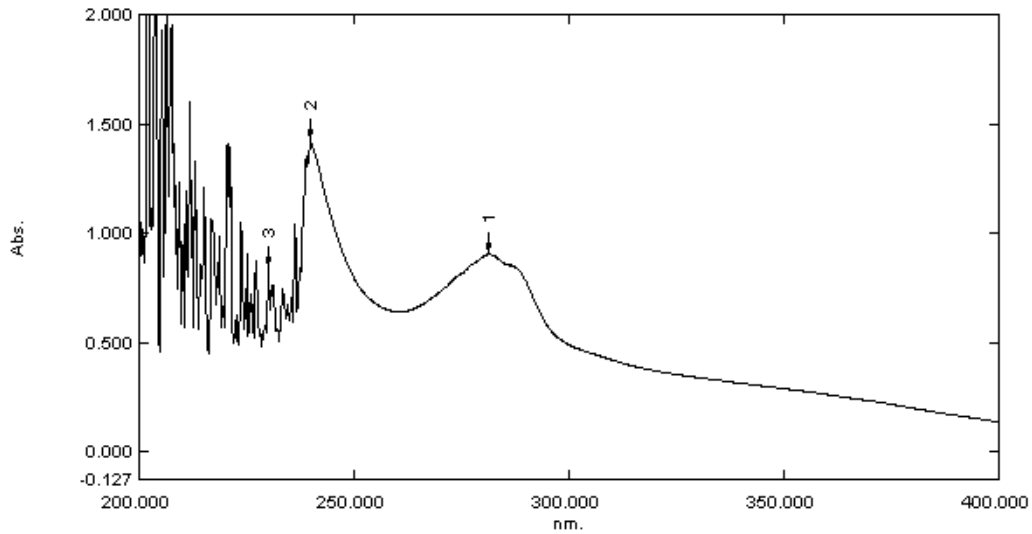
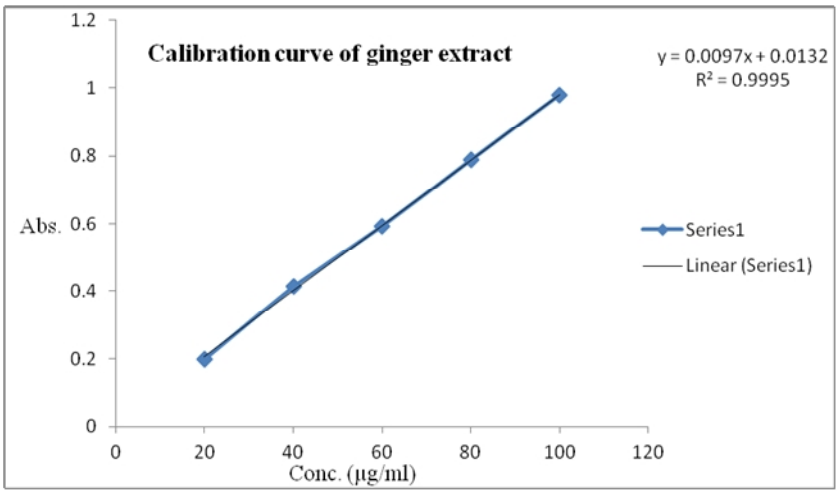


Figure 2: Calibration curve for ginger extract



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