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HPTLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF 6-GINGEROL IN ORAL THIN FILM

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ABSTRACT

A HPTLC method was established for assay of 6-Gingerol and determination from herbal oral thin film (OTF).The chromatographic condition were: Toluene: Ethyl acetate: methanol: Formic Acid (5: 4:1:1v/v/v/v) as mobile phase; wavelength of the detector was 520nm; drying temperature after development was 60°C. The calibration curve was linear in the range of 100 to 600mcg/ml. With correlation coefficient of 0.999; the average recovery was found to be 100.24%, 100.45% and 100.49% at the concentration levels of 80, 100 and 120% respectively. Detection and quantification limit was fond to 80 and 120ng. The method was found to be simple, reliable, accurate and precise in accordance with ICH guidelines.

KEYWORDS: **6-Gingerol, Ginger, OTF, method development, validation, densitometry, Quantification and chromatography.**

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INTRODUCTION

Ginger is one of the more commonly used herbal supplements. Although often consumed for culinary purposes. It is taken by many patients to treat a variety of conditions. Ginger has shown to be effective for pregnancy induced and postoperative nausea and vomiting. Ginger rhizome of zingiber officinale, has been used for antiemetic effect. Several components of ginger such as 6-gingerol, 6-shagol and galanolactone have been shown to have anti-5HT activity.Literature survey reveals that the method for the determination of 6-gingerol from ginger extracts using HPTLC. But there is no method which describes the determination of gingerols from mouth dissolving thin film.The proposed HPTLC method is sensitive and accurate for the determination of 6-gingerol from mouth dissolving film.

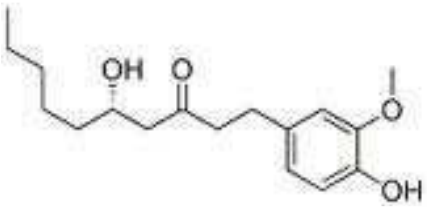

Figure 1
Chemical structure of 6-Gingerol

EXPERIMENTAL

Reagent and Chemicals

The solvents (Toluene, Methanol, Ethyl acetate, Formic acid) were of analytical laboratory grade. The standard of 6-Gingerol was purchased from Natural Remedies Bangalore.

Chromatographic Condition

A CAMAG HPTLC instrument having software version of 1.4.3 was used for the method development and validation. Toluene: Ethyl acetate: methanol: Formic Acid (5: 4:1:1v/v/v/v) as mobile phase. The sample was spotted in the form of bands with a Camag 100µl sample syringe (Hamilton, Bondouz, Switzerland) syringe on Precoated silica gel aluminium plate $60F_{254}$ (20X10) with 250µm thickness;(Merck") using a Camag Linomat 5 sample applicator (Switzerland).The linear ascending development was carried out in 20cmX 10cm twin trough chamber using Toluene: Ethyl acetate: methanol: Formic Acid (5: 4:1:1v/v/v/v) as mobile phase. TLC plates were dried on Camag TLC plate heater III. Densitometric scanning was performed on Camag TLC scanner 3 in the reflectance-absorbance mode at 520nm; after derivatization with vanillin-Sulphuric reagent and dried at 110 °C for 5 minutes.

Preparation of the standard solution

Transfer an accurately weighed 5.0mg of standard 6-gingerol in 10ml of volumetric flask, dissolved by shaking and made the volume up to the mark with methanol. Take 1.0ml of the above stock solution and dilute to get the final concentration of 100.0mcg/ml.

Preparation of sample solution

Take the average weight of the ginger OTF and transfer an accurately weighed 500.0mg of OTF with 30.0ml methanol in 125.0ml round bottom flask and allow refluxing the content for about 30 minute cool and filter. Again reflux the remaining residue with 20.0ml of methanol, cool

and filter. Combine the filtrate and evaporate it on water bath till complete dryness. Dissolve the residue in 5.0ml of methanol.

Validation of the HPTLC method

In order to confirm method suitability during routine quality control use, the following validation characteristics were performed as per ICH guidelines.

Linearity

Linearity was determined in the range 100 to 600ng/spot of working concentration of standard. The peak area responses were plotted against the corresponding concentrations and r2 values were calculated.

Precision

System Precision

Six replicate injections of a mixed standard solution at the concentration of 100ng/µl were spotted by using Linomat V semi automatic sample applicator. The percentage relative standard deviations (% RSD) in each case were calculated.

Method precision

Six replicate samples of the marketed formulation were analyzed as per the method. The mean percentage of drug content and % RSD were calculated in each case.

Intermediate precision or inter-day precision

The intermediate or inter-day precision of the method was determined by six replicate analysis of 6-Gingerol from the sample, as per the proposed method by different analyst on the same day and on different days. The average drug content and the %RSD were calculated in each case.

Accuracy (% recovery)

Recovery studies were performed by the standard addition method at three levels i.e. 80%, 100%, 120%. Known amount of standard was added to preanalyzed sample and they were subjected to the proposed HPTLC method. Result of recovery study is shown in table I.

Robustness (System Suitability)

The robustness study was done by making small changes in the optimized method parameters and results as indicated in table II.

Ruggedness

The ruggedness study was done by the two analysts by using the proposed method by the same instrument on the same day. The % RSD for each analyst was found to be in the acceptable range.

RESULTS AND DISCUSSION

In a linearity study, the graphical data provethat the 6-Gingerol demonstrated linearity in the range of 100ng to 600ng with the r^2 values 0.999.In system precision study, the % RSD for 6-gingerol was found to be 0.96. The % RSD observed on the replicate indicates the precision of the system.In method precision the mean % drug content was found to be 100.96% respectively. The % RSD for 6-Gingerol is 1.2%. The results indicate that the method is validated for method precision. No interference from other components or excipients was found during determination.In intermediate or inter-day precision study, the mean % drug content for 6-gingerol was found to be 99.86%.The % RSD were found to be 1.09%. There is no significant difference by the same analyst with same instrument on same day or different day. Therefore the intermediate or inter- day precision of the method can be considered to be acceptable. Inaccuracy or recovery studies, the results are shown in table 1. The overall % recovery and % RSD in marketed ginger OTF indicated that there is no significant difference in percentage of recovery. The accuracy of the method considered acceptable as it was well within 98 to 102%.In robustness or system suitability study, there was no significant impact on the % RSD. The results of robustness study also indicated that the method is robust and is

unaffected by small variations in the chromatographic condition.In ruggedness study, the %RSD for analyst-I was 0.188 and for analyst-II was 0.35%. It is also a type of intermediate precision study. The study was found to be satisfactory.

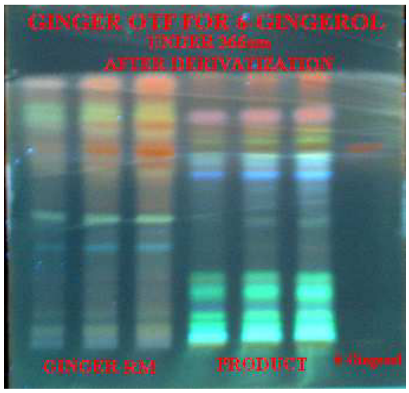

Figure 1
Chromatogram under 366 nm after derivatization showing the separated spots of 6-Gingerol standard corresponding to that of ginger raw material & product (Ginger OTF)

Figure 2
Chromatogram under day light after derivatization showing the separated spots of 6-Gingerol standard corresponding to that of ginger raw material & product (Ginger OTF)

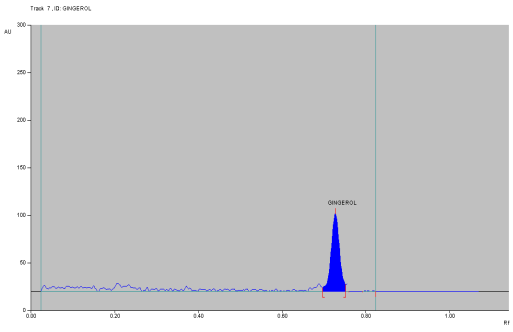

Figure 3
Densitogram of Standard 6-Gingerol (Rf = 0.73)

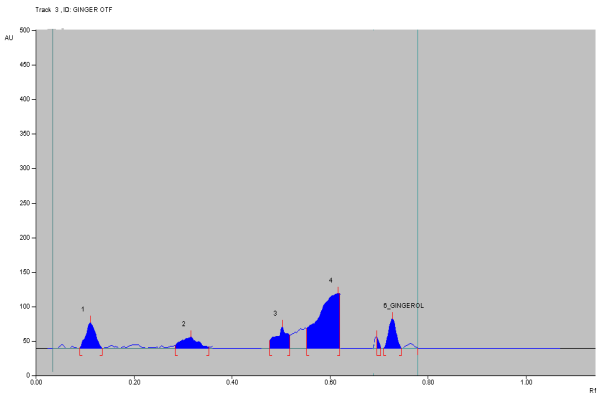

Figure 4
Densitogram of Standard 6-Gingerol in product (Ginger OTF) (Rf = 0.74)

Table I
Results of recovery studies

Name of the active Ingredients	Recovery levels	% recovery	Mean % recovery	SD	% RSD
6-Gingerol	80	99.19	100.24	1.01	1.01
	80	100.31			
	80	101.21			
	100	100.08	100.45	0.84	0.83
	100	99.86			
	100	101.41			
	120	99.90	100.49	0.75	0.75
	120	101.34			
	120	100.23			

Table II
Robustness Studies

Parameter	Initial condition	Change in condition	Effect found
Mobile phase composition	Toluene: Ethyl acetate: methanol: Formic Acid (5: 4:1:1v/v/v/v)	Toluene: Ethyl acetate: methanol: Formic Acid (5: 4:1.2:0.8v/v/v/v)	No change in Rf value
Development distance	8 cm	6cm	No Change in R_f value
Saturation time	20 min.	30 min.	No change
Extraction time	20 min.	30 min.	No change

CONCLUSION

It can be concluded that the newly developed HPTLC method was found to be simple, rapid, cost-effective, linear, accurate, precise and robust over the specified range; and selective for the estimation of 6-Gingerol without any interference from other components or additives. This method can be employed conveniently, reliably and successfully for the estimation of 6-gingerol for routine quality control and stability studies in pharmaceutical dosage formulation.

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