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Research Article

Estimation of Gingerol and Curcumin in Novel herbal tablet by HPTLC

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Gingerol and Curcumin in bulk and tablet dosage form. The Ginger and Curcuminoid extracts samples were applied on TLC aluminium plate precoated with Silica gel 60 F254 and developed using the mobile phase N-hexane: Ethyl acetate for ginger and Toluene: Acetic acid for curcumin extract respectively. The bands were scanned at λ=540 nm and λ=418 nm for ginger and curcumin respectively using Camag TLC scanner 4. The detection and quantification was carried out densitometrically using an UV detector. The Rf value was found to be 0.46 for curcumin and 0.25 for gingerol. The Correlation of determination (R2) was 0.999 for ginger and for curcumin 0.992. The analysis of the in-house tablet showed that Gingerol was found to be 3% and curcumin is 65 %. This developed method can be used to analyse marketed formulation in bulk and tablets.

1. INTRODUCTION

In the past, the collection, identification, and preparation of Ayurvedic medicines were done by the Acharyas themselves; so drugs made by them were efficacious, authentic and genuine. In the present age the suppliers make the collection and there is a need to authenticate the same. There are so many drugs which lose their effectiveness with the passage of time. This lowers the quality of the drug and makes them less efficacious. Therefore when incorporated into the dosage form, there is a need to develop the method for analysis for the extracts (1). Curcumin is chemically, (1E, 6E)-1, 7-bis (4-hydroxy-3- methoxy phenyl) -1, 6-heptadiene-3,5-dione. It is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bis-

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desmethoxycurcumin. The curcuminoids are natural phenols and are responsible for the yellow color of turmeric. Curcumin has a long history of use for maintaining a healthy inflammatory response, via its effects on cyclooxygenase, prostaglandin and leukotriene metabolism. Ginger is native to Asia where it has been used as a cooking spice for at least 4000 year (2). The present work involves development of a HPTLC densitometric method for analysis of multicomponent herbal tablets containing Curcumin extract and Ginger extract as a drug candidate.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Ginger and curcuminoid extract were obtained as a gift sample from Herbal Creation, Bangalore, India. The solvents Methanol, Hexane, Ethyl acetate, Toluene, Acetic acid used were of analytical grade, procured from Vishal chem India. All the other chemicals used were also of analytical grade.

2.2. Instrumentation and conditions

TLC aluminium plates precoated with Silica gel 60 F_{254} (Merck); (20cm×10cm) were used. Densitometry was carried out with a CAMAG TLC Scanner 4, fitted with Server LABSERVER, version 3.1 software. Samples were applied to the HPTLC plates using the spray-on technique on CAMAG LINOMAT V under nitrogen gas and developed in a CAMAG twin trough chambers.

2.3. Standard preparation

A standard stock solution of ginger and curcumin extracts was prepared by dissolving 10 mg of both the extracts in 10 ml of methanol to get concentration of 1000 µg/ml. This solution was further diluted to get 100 µg/ml solution of ginger and curcumin as working standard.

2.4. Preparation of Sample solution

Tablets were prepared (in house) containing 490 mg of ginger extract and 90 mg of curcuminoid extract. Ten tablets were weighed, average weight determined. Appropriate quantity of powder equivalent to 10 mg of extract was weighed accurately and transferred to a 10 ml volumetric flask and dissolved in methanol and shaken vigorously for 5 minutes. The solution was then sonicated for 20 minutes, and volume was made up to 10 ml and filtered through the Whatman filter paper. Necessary dilutions of filtrate were made with methanol to get final concentration of 1000 µg/ml.

2.10. Chromatographic conditions

The analysis was performed on the Camag HPTLC system (Switzerland equipped with a Linomat-5 applicator,100 µl sample syringe (Hamilton, Switzerland) and Camag TLC scanner-4. Pre-coated silica gel 60 F_{254} TLC (E-Merck, Germany) plates (20x10 cm) were used as stationary phase. TLC plates were pre-washed with methanol and activated at 110ºC for 10 min prior to application. The standard samples of ginger and curcumin were spotted on pre-coated TLC plates in the form of bands of length 4 mm using 100 µl syringe with a Linomat-5 applicator. The chromatographic development was carried out using suitable mobile phase (N-hexane: Ethyl acetate::6:4 for ginger and Toluene: Acetic acid::4:1 for curcumin extract respectively.) with chamber saturation time of 20 minutes and the migration distance of 80 mm. Densitometric scanning was performed using Camag TLC scanner-4 , operated by lab server software (Version 3.1.2, Camag).

2.5. Selection of mobile phase

Mobile phases were selected as per the USP pharmacopeia of gingerols and curcumin(3).The mobile phase of N-hexane: Ethyl acetate (6:4) showed good separation for ginger. The mobile phase of Toulene: Acetic acid (4:1) showed good separation for curcumin extract.

2.6. Application of standard solutions

TLC aluminium plates precoated with Silica gel 60 F_{254} (20x10) were employed for the spotting of

standard solutions. Standard solution of Curcuminoid extract was applied to give concentration of 200, 300, 400, 500, 600 ng/spot. The standard solution of ginger extract was applied to give concentration of 200,400,600,800,1000 ng/spot on a separate plate.

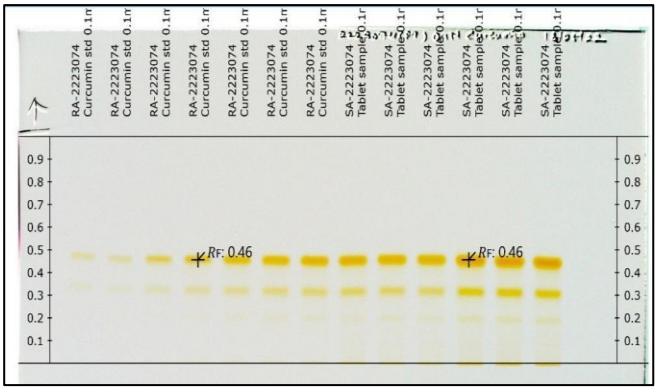

Fig no 1. Application of Standard curcumin and tablet sample on silica gel G 60 F_{254} under white light

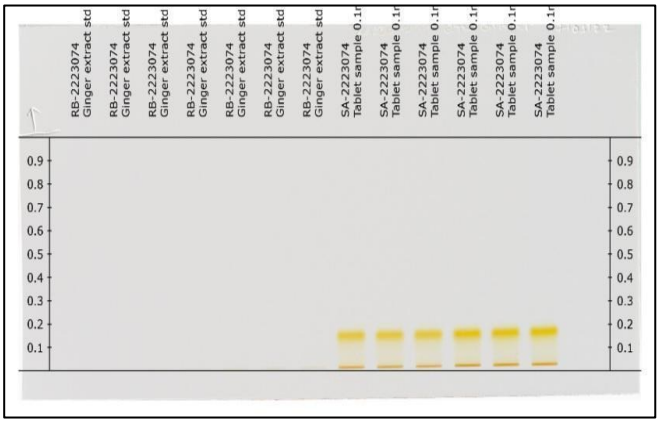

Fig no 2. Application of Standard ginger solution and tablet sample on silica gel G 60 F_{254} under white light

2.7. Application of sample solution

10 µg/ml solution of sample containing both ginger and curcumin extract were applied on TLC aluminium plates precoated with Silica gel 60 F_{254} along with standard ginger and curuminoid bands.

2.8. Development of spot

Twin Trough chamber containing 10 ml of suitable (N-hexane: Ethyl acetate 6:4 for ginger and Toluene: Acetic acid 8:2 for curcumin extract respectively) mobile phases system was used for developing the spotted plates and saturated for 15 minutes. The plates were dried after development and viewed under UV lamp to evaluate the spot obtained at 540 nm after derivatization (Anisaldehyde Sulphuric Acid Reagent) for ginger and 418 nm for curcumin. The spots were uniform and there was no tailing.

2.9. Selection of wavelength for Detection

The working standard of Ginger and curcuminoids in methanol was scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 600 nm. The wavelengths 540 nm and 418 nm were selected for detection of obtained spectrum.

Linearity and Range

The linearity was determined by using working standard solutions between 100-700 ng/spot. The spots were scanned at 282 nm. Calibration curve was constructed by plotting peak area versus concentration. Simple linear regression was performed [Table 1]. Regression equation and correlation coefficient were recorded. The regression equations for ginger was $y=3.045\times10^{-8}$ $x+4.949\times10^{-3}$ and for Curcuminoids $y=1.221\times10^{-18}$ $x^2+1.841\times10^{-10}$ -5.58×10^{-5} where, y is response and x the concentration of drug. The correlation coefficients were 0.999 for ginger and for curcumin 0.992 [Fig.3, 4]. The regression equations for ginger and curcumin were used to calculated the content in tablets.

Sample	Amount ng/spot	Rf	Area
Ginger	200	0.281	0.00137
	400	0.294	0.00253
	600	0.297	0.00362
	800	0.297	0.00468
	1000	0.297	0.00531

Curcumin	200	0.477	0.01036
	300	0.485	0.01451
	400	0.482	0.01769
	500	0.479	0.02047
	600	0.482	0.02260

Table no 1. Calibration curve of gingerol and curcumin along with Rf value

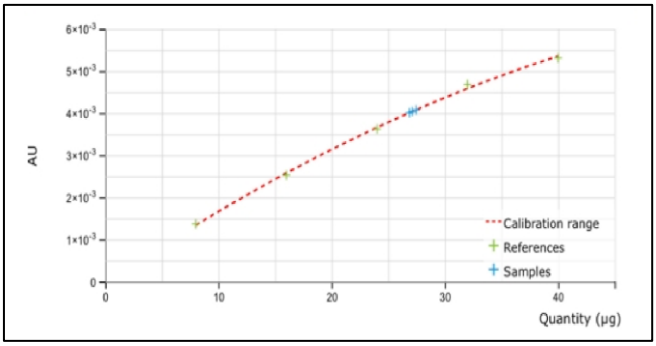

Fig no 3. Calibration curve of gingerol at 540nm after derivatization

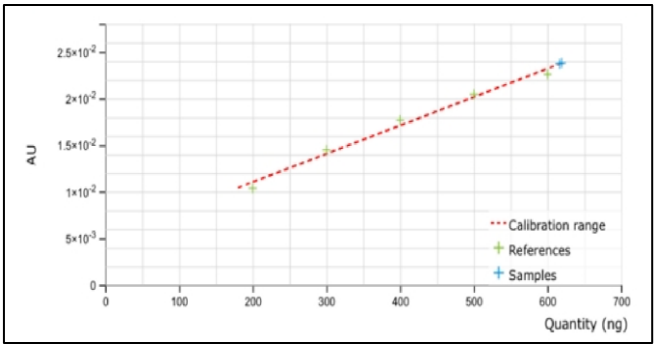

Fig no 4. Calibration curve of curcumin at 418 nm

2.11 Assay for tablet preparation

Twenty tablets were weighed, and average tablet weight was determined. The tablet 1 gram powder equivalent to a weight of 98 mg ginger and 487 mg curcumin was accurately weighed, transferred to a 100 ml of volumetric flask dissolved in methanol separately then solution was ultrasonicated for 20 min and diluted up to mark with methanol then filtered with Whatman filter paper No. 41. This filtrate was further diluted with the same solvent and subjected for HPTLC study (Table1). The plate was developed under previously described chromatographic conditions. The content of the tablets was calculated from the regression equations.

Sample	Sample solution concentration(ng/spot)	Sample solution area	% Content
Ginger	600	0.00401	102
	600	0.00408	
	600	0.00404	
Curcuminoids	200	0.02381	72
	200	0.02371	
	200	0.02380	

Table no 2.

RESULT

The calibration curve was plotted of Ginger and curcumin peak area versus Concentration. The generated regression equation was $y=3.045\times10^{-8}$ $x+4.949\times10^{-3}$ for ginger and for curcumin $y=1.221\times10^{-18}$ x2 $+1.841\times10^{-10}$ -5.58×10^{-5}. The r^2 value as 0.999 and 0.992 indicates that developed method was linear. The calibration curve was obtained in the range of 200-600 ng/spot and 200, 400, 600, 800, 1000 ng/spot for curcuminoids. The proposed method was found to be precise and satisfactory. The average gingerol and curcumin content found in the tablet preparation was 102 % of gingerol and 72 % of curcumin, which is within the specification of ginger and curcuminoids extracts according to the COA of both the extracts. Hence, it can be said that this method was accurate.

DISCUSSION

The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the gingerol and curcumin in solid dosage form (Tablet, capsule, MUPS).

CONCLUSION

The developed HPTLC method is found to be rapid, accurate, precise and economical, thus can be used for routine analysis of gingerol and curcumin in solid dosage form.

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GRAPHICAL ABSTRACT