ORIGINAL ARTICLE

Simultaneous estimation of Guggulsterone E & Z and Tinosporaside in Jivitprada vati by HPTLC method

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Jivitprada vati is an Ayurvedic formulation, which is consists of Guggul, Shilajit and Galodhan Guggulsterone E & Z and Tinosporaside are active constituents that are used for rheumatoid arthritis and erectile dysfunction. The purpose of this study was to develop an HPTLC method of quantitative estimation of marker compounds, Guggulsterone E & Z and Tinosporaside in laboratory prepared authentic formulation and compare with three different marketed formulation. The marker compounds were isolated from plant material and authenticated by comparing its UV spectrum, IR spectrum and GC-MS fragmentation pattern with standard marker and its literature studied. The four formulations were subjected to methanol extractions by Soxhlet apparatus. Guggulsterone E & Z and Tinosporaside were quantified in the above extracts by HPTLC method. The detection and quantification was performed at a wavelength of 230 nm. The laboratory formulation was found to contain 2.18% of Guggulsterone E, 1.898% of Guggulsterone Z and 0.954% of Tinosporaside while in case of marketed formulation MF-1, MF-2 and MF-3 was found to contain, respectively, 1.06%, 0.527%, 0.318% of Guggulsterone E and 0.914%, 0.487%, 0.24% of Guggulsterone Z in the methanolic extracts of formulations, whereas in MF-1 and MF-2 was found very less amount of Tinosporaside (respectively, 0.347% & 0.14%) except in MF-3 which was devoid of Tinosporaside. The method was found to be linear, precise and accurate for quantitative estimation of E & Z Guggulsterone and Tinosporaside in different formulation.

Key words: Guggulsterone E and Z, HPTLC, Jivitprada vati, laboratory formulation, Tinosporaside

INTRODUCTION

The recognized Indian Systems of Medicine are *Ayurveda, Siddha* and *Unani,* which use herbs and minerals in the formulations. Indian traditional systems of medicine in *Ayurveda, Siddha* and *Unani* backed by a strong manufacturing base are appropriately positioned to provide holistic health care not just within the country but internationally too1[1] Jivitprada vati consists of Guggul (*Commiphora wightii*),[2] Shilajit (Asphaltum)[3] and Galodhan (*Tinospora cordifolia*).[4] Guggulsterone E & Z[5,6] and Tinosporaside,[7,8] are active constituents of guggul and galodhan, respectively. Nowadays, Ayurvedic or Siddha formulations must require standardization.

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MATERIALS AND METHODS

Equipments

HPTLC system equipped with a sample applicator Camag Linomat 5, Camag TLC scanner 3, Silica gel coated on aluminium sheets, Twin through glass chamber, Deuterium lamp (Switzerland).

Materials

Toluene, ethyl acetate, formic acid and methanol were obtained from ACS Laboratory, Gujarat, India. Guggulsterone E & Z and Tinosporaside were isolated from *C. wightii and T. cordifolia. C. wightii, T. Cordifolia* and Shilajit were procured from Mahavir Enterprise and Hakim Chichi, Gujarat, India. These were identified and authenticated on the basis of their various morphological as well as microscopical characters.

The market formulation of "Jivitprada Vati (Tablet)" of different manufacturers (Aasfa, Surat, Shree Bhuvaneshwari Aushadashram, Gondal and Shree Mahanarayan Ayurvedic Pharmacy, Ahmadabad) was procured from the local market. These market formulations were named as MF-1 (Aasfa), MF-2 (Bhuvaneshwari), MF-3 (Mahanarayan) and fourth

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formulation was prepared in the laboratory and marked as LF (Lab or In-House formulation).

HPTLC Method for Estimation of Guggulsterone E & Z and Tinosporaside

Preparation of mobile phase

A mixture of 6 ml toluene, 2 ml ethyl acetate, 1 ml formic acid and 0.5 ml methanol previously filtered through filter paper in a flask was used as a mobile phase.

Preparation of standard solution

Stock solution of isolated E and Z – Guggulsterones and Tinosporaside (1 mg/ml) was prepared by dissolving 10 mg of reference compound E and Z – Guggulsterones and Tinosporaside in 10 ml of methanol individually. From this stock solution 100 µl/ml was prepared by transferring 1 ml stock solution to 10 ml volumetric flask and adjusted the volume with methanol.

Chromatographic conditions

Stationary phase

Pre-coated silica gel G60 – F_{254} aluminium sheet, (E. Merck, Germany) (100 × 50 mm, thickness layer 0.2 mm, pre-washed with methanol and dried at room temperature)

Mobile Phase	Toluene:Ethylacetate:Formic acid : Methanol (6 : 2 : 1 : 0.5)
Chamber saturation time	30 min
Distance run	80 millimetre
Temperature	27°C
Wavelength	254 nm

HPTLC analysis (calibration curve)

Semi-automatic spotter was used containing a syringe having capacity of 50 µl. Approximately 10 µl standard solution was filled in syringe and under nitrogen stream it was applied in form of bands of desired concentration range (200–1000 ng/spot) of standard solution on pre-coated plates. For E- Guggulsterone, Z-Guggulsterones and Tinosporaside individual plates were prepared. Plates were developed using Toluene : Ethyl acetate : Formic acid : Methanol (6:2:1:0.5) at 27°C (±2°C) and dried in air. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 230 nm using Camag TLC scanner 3. Plots of peak area vs. concentration for isolated E- Guggulsterone, Z-Guggulsterone and Tinosporaside are shown in [Figures 1–3].

Preparation of Jivitprada Vati

Firstly galodhan was treated with the Guduchi kwath in slightly warm condition and mixed properly by stirring well continuously. Then add the previously accurately weighed and prepared Suddh guggul and Suddh shilajit in this galodhan and Guduchi kwath semisolid mixture and properly mix by stirring continuously. And at that same time add the mixture mentioned in Table 1 and prepare mould Vati with hand. They are then dried under shade at sun temperature, packed in air tight container and labelled as Lab formulation Jivitprada vati [Table 1].[9]

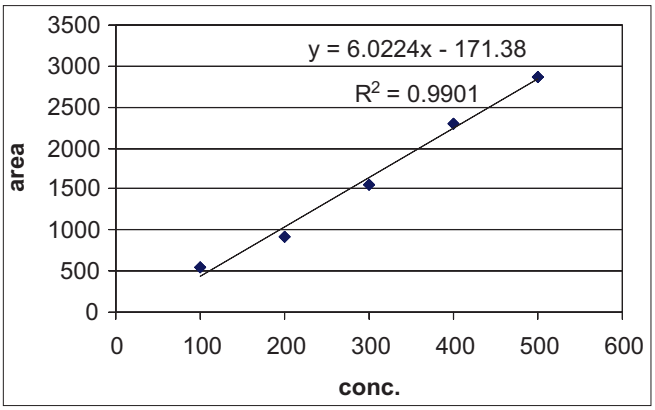

Figure 1: Calibration curve for isolated compound- E-Guggulsterone

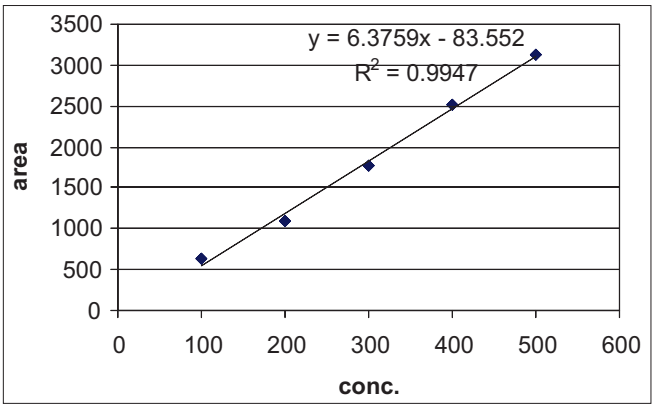

Figure 2: Calibration curve for isolated compound- Z-Guggulsterone

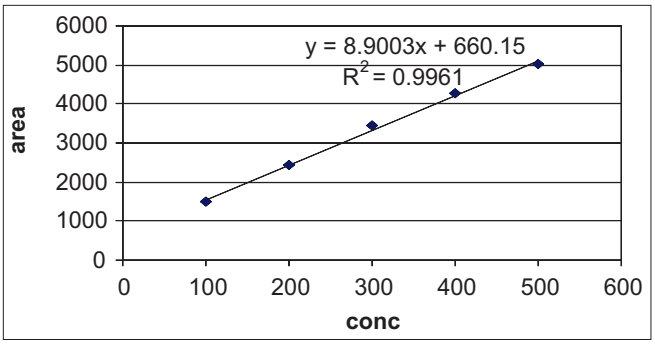

Figure 3: Calibration curve for isolated compound- Tinosporaside

Preparation of extracts

Weigh accurately 1 g of each powdered formulation and extract with methanol for four times using 25 ml methanol each time (4×25), combine the extract and concentrate to 25 ml. One millilitre of concentrated solution was taken in 100 ml volumetric flask and diluted up to 100 ml (sample solution). Sample solution was used for the quantification.

Method specifications

Injection	:	Camag Linomat 5, Switzerland
Scanner	:	Camag TLC scanner 3, Switzerland
Stationary phase	:	Silica gel coated on aluminium sheets
Development chamber	:	Twin through glass chamber
Detector	:	Deuterium lamp

Recovery study

Recovery was carried out by estimating the Guggulsterones E and Z and Tinosporaside content in the pre-analysed MF-1, MF-2, MF-3 and LF spiked with E and Z-Guggulsterones and Tinosporaside at different concentration levels.

RESULTS

Ayurvedic physician uses many traditional formulations for the treatment of arthritis. Non-medicinal person also claim to treat the disorder with the so-called plants drugs. Preparation given by such practitioners is often kept confidential, but some preparations are also available in market, whose composition is well known and not yet standardized properly. We attempted to standardize some of the formulation, containing *C. mukul, T. cordifolia* and Shilajit by determining the percentage of marker compound of drug in different formulation by HPTLC method.

Table 1: Ingredient of formulation

Ingredient	Parts
Suddh guggul	50
Galodhan	50
Suddh Shilajit	50
Ghee	5

Validation of HPTLC Method

Linearity

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 a sample within a given range. The range of the analyte method is the interval between the upper limit and lower limit of the analyte in the analytical method.

The linear range is 100–500 ng/spot for E-Guggulsterone, Z-Guggulsterone and Tinosporaside with correlation coefficient of marker compound by HPTLC was, respectively, 0.9901, 0.9947 and 0.9961.

Accuracy: (% recovery)

The accuracy of analytical method closer to the test results were obtained by the method of the value. The accuracy may often be expressed as % recovered by the assay by adding known amount of analyte. The recoveries of marker compound are shown in Tables 2–4.

Specificity

The specificity of an analytical method is its ability to measure accurately and specifically the analyte in the presence of component that may be expected to be present in the formulation. Amount of added maker compound was estimated without interference of other component present in the formulation.

Limit of detection

It is the concentration of analyte in the sample that can be detected but not necessarily quantified under the stated condition. The limit of detection was found for E-Guggulsterone, Z-Guggulsterone and Tinosporaside.

Table 2: Percentage recovery of E-Guggulsterone

Powdered formulation (g)	E-Guggulsterone (mg)	E-Guggulsterone added (mg)	E-Guggulsterone expected (mg)	E-Guggulsterone found (mg)	Percentage recovery	Average percentage recovery
6	0.36	0.25	0.61	0.58	95.08	96.95%
6	0.36	0.5	0.86	0.85	98.83	

Table 3: Percentage recovery of Z-Guggulsterone

Powdered formulation (g)	Z-Guggulsterone (mg)	Z-Guggulsterone added (mg)	Z-Guggulsterone expected (mg)	Z-Guggulsterone found (mg)	Percentage recovery	Average percentage recovery
6	0.40	0.25	0.65	0.64	98.46	100.33%
6	0.40	0.5	0.9	0.92	102.2	

Table 4: Percentage recovery of Tinosporaside

Powdered formulation (g)	Tinosporaside (mg)	Tinosporaside added (mg)	Tinosporaside expected (mg)	Tinosporaside found (mg)	Percentage recovery	Average percentage recovery
6	0.27	0.25	0.52	0.48	92.3	93.55%
6	0.27	0.5	0.77	0.73	94.80	

Limit of quantification

It is the lowest concentration of analyte in the sample that can be determined with acceptable decision and accuracy under the stated experimental conditions. The limit of quantification for different marker compound was 70 ng/spot, 80 ng/spot and 10 ng/spot.

Precision

It is a measure of reproducibility of data within a series of results. Results within a series, which agree closely with one another, are said to be precise. Precision data of E-Guggulsterone, Z-Guggulsterone and Tinosporaside showed that the method was found to be precise [Tables 5–7].

Repeatability

It is defined as precision under the same concentration over a short interval time. It can be affected by factor such as analyst, environment condition or instrument error. Summary of all validation parameters of E and Z-Guggulsterone and Tinosporaside are shown in Tables 8–10.

Table 5: Precision data for E-Guggulsterone using HPTLC method

Area	Concentration (ng/spot)	Mean	% RSD
1547.8	300	1552.6	0.27%
1555.4	300		
1554.8	300		

Table 6: Precision data for Z-Guggulsterone using HPTLC method

Area	Concentration (ng/spot)	Mean	% RSD
1765.3	300	1765.5	0.30%
1760.3	300		
1770.9	300		

Table 7: Precision data for Tinosporaside using HPTLC method

Area	Concentration (ng/spot)	Mean	% RSD
3443.9	300	3442.1	0.04%
3440.5	300		
3441.9	300		

Table 8: Summary of validation parameter of E-Guggulsterone

Parameters	Results
Linearity range	100–500 ng/spot
Linearity	0.9901
Accuracy (%)	96.95%
Limit of detection	20 ng/spot
Limit of quantification	70 ng/spot
Precision	0.27%

Table 9: Summary of validation parameter of Z-Guggulsterone

Parameters	Results
Linearity range	100–500 ng/spot
Linearity	0.9947
Accuracy (%)	100.3%
Limit of detection	20 ng/spot
Limit of quantification	80 ng/spot
Precision	0.30%

Table 10: Summary of validation parameter of Tinosporaside

Parameters	Results
Linearity range	100–500 ng/spot
Linearity	0.9961
Accuracy (%)	93.55%
Limit of detection	60 ng/spot
Limit of quantification	10 ng/spot
Precision	0.04%

DISCUSSION

Jivitprada Vati (tablet) was prepared according to the Nidan Chikitsha Hastamalka by Ranjitrai Desai and also by slightly modification in the method of preparation. Quality control parameters according to WHO guidelines were examined, such as extractive value, Ash value, volatile oil content, moisture content, tablet weight variation, disintegration study, friability etc. The disintegration time noted were slightly higher than recommended limit but it would not adversely affect the dose delivery to patients. Proximate analysis of formulation was carried out and higher water-soluble extractive value indicated the presence of more of polar constituents. Successive solvent extraction with different solvents like petroleum ether, chloroform, acetone, methanol and water were carried out and their extractive values were found.

Qualitative chemical examination of various extracts of formulation revealed the presence of different phytoconstituents like alkaloids, glycoside, steroids, essential oil, terpenes, fixed oils, saponins etc. Thin layer chromatography of formulation was carried out to confirm the presence of E and Z Guggulsterone and Tinosposide [Figures 4–6]. TLC fingerprints of formulations were carried out to confirm the presence of E and Z Guggulsterone and Tinosposide. HPTLC method was developed for quantification of E and Z Guggulsterone and Tinosposide and results show the moderate amount of E and Z Guggulsterone and Tinosposide in market formulation as compared with the laboratory formulation. The method was validated and method is found simple, accurate,

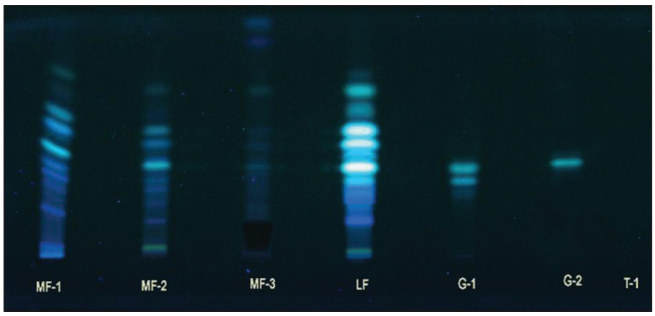

Figure 4: UV-366 photos with various formulations and compare with marker

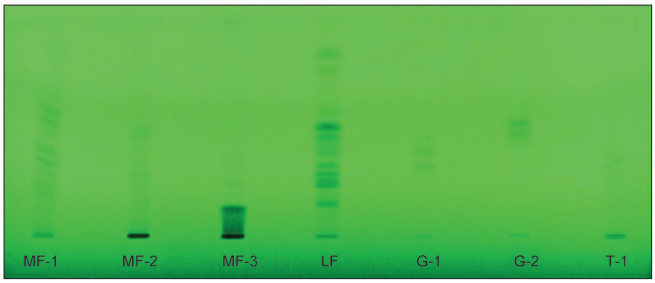

Figure 5: UV-254 photos with various formulations and compare with marker

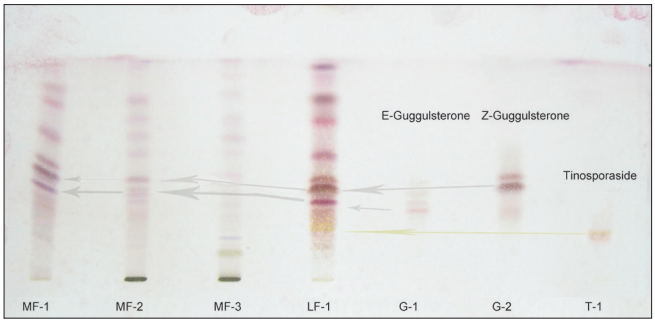

Figure 6: Derivation photos with various formulations and compare with marker (MF-1: Market formulation-1, MF-2: Market formulation-2 MF-3: Market formulation-3 LF: Lab or Inhouse formulation, G-1: E-guggulsterone, G-2: Z-guggulsterone, T-1: Tinosporaside)

precise and specific. It can be used for quantification of marker compound for market formulation.

CONCLUSION

The developed HPTLC method was found to be rapid, simple and accurate of quantitative estimation of E and Z Guggulsterone and Tinosporaside in different formulation. The recovery value of E and Z Guggulsterone and Tinosporaside shows the reliability and suitability of the method. The coefficient of variation for E and Z Guggulsterone and Tinosporaside proves the accuracy and precision of the analysis. The method was found to be useful in detecting the genuineness of the formulation and thus suitable to evaluate various formulation available in the market. The method also revealed the suitability of methanol solvent in extracting the phytomarkers present in the formulation. The proposed solvent system and the scanning wavelength are suitable to identify and estimate for E and Z Guggulsterone and Tinosporaside simultaneously.

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