Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 33–41
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

HPTLC method for guggulsterone I. Quantitative determination of E- and Z-guggulsterone in herbal extract and pharmaceutical dosage form

Himani Agrawal, Neeraj Kaul, A.R. Paradkar, K.R. Mahadik*

Department of Quality Assurance Techniques, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune 411038, Maharashtra, India

Received 25 November 2003; received in revised form 20 April 2004; accepted 20 April 2004

Available online 20 June 2004

Abstract

A sensitive, selective, precise and robust high-performance thin-layer chromatographic method of analysis of E and Z stereoisomers of guggulsterone (the hypolipidemic agent in the gum-resin exudates of *Commiphora mukul*) both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene–acetone (9:1, v/v). Densitometric analysis of guggulsterone was carried out in the absorbance mode at 250 nm. This system was found to give compact spots for E- and Z-guggulsterone (R_f value of 0.38 ± 0.02 and 0.46 ± 0.02, respectively) following double development of chromatoplates with the same mobile phase. The linear regression analysis data for the calibration plots for E- and Z-guggulsterone showed good linear relationship with $r^2 = 0.9977 \pm 0.054$ and 0.9975 ± 0.068, respectively, in the concentration range of 100–6000 ng/spot. The mean value of slope and intercept were 0.11 ± 0.006 and 0.11 ± 0.005, 14.26 ± 0.56 and 10.92 ± 0.76, respectively, for E- and Z-guggulsterone. The method was validated for precision, robustness and recovery. The limit of detection and quantitation were 12, 10 and 24, 20 ng/spot, respectively, for E- and Z-guggulsterone. Statistical analysis proves that the method is repeatable and selective for the estimation of the said drug. Since the proposed mobile phase effectively resolves the E- and Z-isomers of guggulsterone, this HPTLC method can be applied for identification and quantitation of these isomers in herbal extracts and pharmaceutical dosage form.

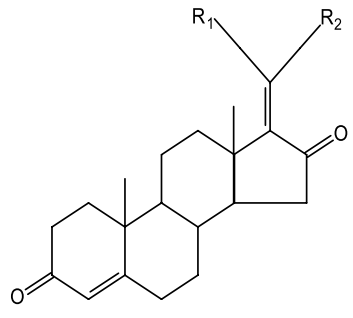
Keywords: Guggulsterone; E and Z stereo isomers; *Commiphora mukul*; HPTLC

1. Introduction

Commiphora mukul [1] is a small thorny plant indigenous to the Indian sub-continent and parts of near east [2–4]. It produces a dense, oily resin identified in Sanskrit as guggulu [5–7], but in western texts' it is generally referred to by the somewhat shortened appellation of guggul [2,3]. The traditional uses of guggul resin extract are well documented in the Ayurveda, the ancient system of traditional Indian medicine [8], where it is prescribed to treat a variety of ailments including disorders such as obesity, rheumatoid arthritis [9–12] and arteriosclerosis. It has been found to exert antioxidative and triiodothyronine enhancing activity [13]. In recent years, studies designed to elicit the scientific basis of uses have demonstrated that guggul-resin extract possesses clinically verifiable hypolipidemic activity [14–19]. Dev and co-workers carried out in depth studies on the chemistry of resins of *Commiphora mukul* [20–26]. It was found to contain diterpenoids, lipids like long chain aliphatic tetrols and various steroidal components. The steroidal constituents were isolated in various fractions during extraction. Neutral ethyl acetate soluble fraction which has shown maximum hypolipidemic activity was separated into ketonic and non-ketonic fractions. The ketonic fraction is reported to contain E- and Z-guggulsterone (43%, w/w). It also contained about 7% (w/w) components having polarity less than guggulsterone, e.g., Z-guggulsterol and 50% (w/w) of more polar constituents, e.g., guggulsterol I and III. On the

* Corresponding author. Tel.: +91 20 25436898; fax: +91 20 25439383.
E-mail address: krmahadik@rediffmail.com (K.R. Mahadik).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.04.014



	R_1, R_2	Compound	Absolute Configuration
1a	R_1 = H, R_2 = CH_3	Guggulsterone	Z –
1b	R_1 = CH_3, R_2 = H	Guggulsterone	E –

Fig. 1. Chemical structure of guggulsterone (1); Z-isomer (1a); and E-isomer (1b).

basis of characterization and elucidation of various steroidal compounds, the biogenetic pattern was proposed to be:

Cholesterol → guggulsterol II → guggulsterol I → Z-guggulsterone

Studies of patients receiving this therapy and experiments with rodent models have demonstrated that gugulipid (organic extract of this gum resin) effectively lowers serum low-density lipoprotein and triglyceride levels [27–29]. Recent findings provide significant and novel insight into the molecular targets for guggulipid and mechanism of the lipid lowering effect. Urizar [30] demonstrated that guggulsterone is an effective antagonist of farnesoid X receptor (FXR), a nuclear hormone receptor essential for the normal control of bile acid biosynthesis and transport. Wu [31] showed that the hypolipidemic natural product guggulsterone acts as an antagonist of the bile acid receptor.

The hypolipidaemic activity of guggul-resin extracts can be traced to the two closely related steroidal ketones (Fig. 1) {4,17(20)-pregnadiene-3,16-dione}, E-guggulsterone and Z-guggulsterone. Two different arrangements of CH_3 at C_{20} in three-dimensional space and the hindered rotation about the carbon–carbon double bond at C_{17} and C_{20} classifies the guggulsterone into Z-{4,17(20)-*cis*-pregnadiene-3,16-dione, 1a}and E-{4,17(20)-*trans*-pregnadiene-3,16-dione, 1b}.

There are few HPLC methods for quantitation of guggulsterones in biological fluids [33–39]. Singh et al. [34] reported HPLC method for quantitation of stereoisomers of guggulsterone in serum. Mesrob et al. [37] developed gradient HPLC method using PDA detector for fingerprinting and quantitative determination of E- and Z-guggulsterones in resin and its products. The quantitative analysis of several OTC products in USA by these authors has shown to contain significantly low content of guggulsterones where as one product did not contain any guggul resin extracts. Authors have attributed it mainly to the lack of quantitative analysis or application of incorrect methods of analysis. Quantitative determination by UV spectrophotometric analysis at 327 nm [32] is non-specific and may lead to erroneous results because not only guggulsterones but all non polar components of the resin show absorbance at this wavelength.

The guggul resin may exhibit significant variations in the component content depending on climatic conditions under which product is grown and harvested. Similarly there is need to differentiate between the resin obtained from different related species.

No article related to the HPTLC determination of E- and Z-guggulsterone in pharmaceutical dosage forms has been reported in literature or in pharmacopoeias. Now-a-days HPTLC is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimum sample clean-up. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. The aim of the present work is to develop an accurate, specific, repeatable and robust HPTLC method for the determination of E- and Z-guggulsterone. The proposed method was validated as per ICH guidelines [40,41] and its updated international convention [42].

2. Experimental

2.1. Materials

Standard guggulsterone mixture (containing E- and Z-isomers, purity 99.17%, w/w), standard E-isomer (purity 99.34%, w/w) and standard Z-isomer of guggulsterone (purity 99.07%, w/w) were kindly provided as a gift sample by Laila Impex, Vijaywada, India and were used without further purification. Guggulu extract and guggulu capsules were procured from the local market. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2. HPTLC instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm with 250 μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat IV (Switzerland). The plates were prewashed by methanol and activated at 60 °C for 5 min prior to chromatography. A constant application rate of 0.1 μl/s was employed and space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and 10 mm/s scanning speed was employed. The monochromatic bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase consisted of toluene–acetone (9:1, v/v) and 15 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Mut-

tenz, Switzerland) saturated with the mobile phase and the chromatoplate development was carried out for two times with the same mobile phase to get good resolution of E- and Z-isomers of guggulsterone. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C ± 2) at relative humidity of 60% ± 5. The length of chromatogram run was 8 cm. Subsequent to the scanning, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 250 nm and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Video densitometry of the chromatoplate was carried out with the help of CAMAG Reprostar 3 with cabinet cover and mounted digital camera. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

2.3. Calibration curves of standard E- and Z-isomers of guggulsterone

The stock solutions of standard E- and Z-isomers of guggulsterone (100 μg/ml) were prepared in methanol. One milliliter of standard E- and Z-stock solution was quantitatively transferred into a 10 ml volumetric flask and made to volume with methanol. Standard solutions were prepared by dilution of the diluted stock solution with methanol to give solutions containing standard E- and Z-isomers in concentration range of 0.1–6.0 μg/ml. One microliter from each standard solution was spotted on the TLC plate to obtain final concentration range of 100–6000 ng/spot. Each concentration was spotted six times on the TLC plate.

2.4. Method validation

2.4.1. Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 100% of the label claim of E- and Z-guggulsterone was accurately weighed and assayed. System repeatability was determined by six replicate applications and six times measurement of a sample solution at the analytical concentration of 500 ng/spot of each of E- and Z-guggulsterone. The repeatability of sample application and measurement of peak area for active compound were expressed in terms of relative standard deviation (R.S.D.%) and standard error (S.E.) and found to be less than 2%. Method repeatability was obtained from R.S.D. value by repeating the assay six times in same day for intra-day precision. Intermediate precision was assessed by the assay of two, six sample sets on different days (inter-day precision). The intra- and inter-day variation for determination of E- and Z-guggulsterone was carried out at three different concentration levels 300, 500 and 900 ng/spot.

2.4.2. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like toluene–acetone (9.5:0.5, v/v), toluene–acetone (8.5:1.5, v/v) were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5%. The plates were prewashed by methanol and activated at 60 °C ± 5 for 2, 5, 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 min. Robustness of the method was done at three different concentration levels 300, 500 and 900 ng/spot.

2.4.3. Limit of detection and limit of quantitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times following the same method as explained in Section 2.2. The signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting known concentrations of E- and Z-isomers of guggulsterone until the average responses were approximately three or ten times the standard deviation of the responses for six replicate determinations.

2.4.4. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spots for E- and Z-guggulsterone in sample were confirmed by comparing the R_f and spectra of the spot with that of standard. The peak purity of E- and Z-guggulsterone were assessed by comparing the spectra at three different levels, i.e., peak start, peak apex and peak end positions of the spot.

2.4.5. Recovery studies

The pre-analyzed samples were spiked with extra 50, 100 and 150% of the standard guggulsterone containing E- and Z-isomers and the mixtures were analyzed by the proposed method. The experiment was conducted six times. This was done to check the recovery of the drug at different levels in the formulations.

2.5. Estimation of E- and Z-isomers of guggulsterone in herbal extract

To determine the content of E- and Z-isomers of guggulsterone in extract, 500 mg was transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 15 min and supernatant was analyzed for drug content. Five microlitres of the filtered solution (25,000 ng/spot) was applied on the TLC plate followed by double development and scanning as described in Section 2.2.

The analysis was repeated six times. The possibility of interference from other components of extract in the analysis was studied.

2.6. Analysis of the marketed formulation

To determine the content of E- and Z-isomers of guggulsterone in capsules (label claim: 250 mg guggulu extract/capsule), the contents of twenty capsules were weighed, their mean weight determined and they were finely powdered. The weight of powder equivalent to capsule content was transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 15 min and supernatant was analyzed for drug content. Ten microlitres of the filtered solution (25,000 ng/spot) was applied on the TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated six times. The possibility of interference from other components in the analysis was studied.

3. Results and discussion

3.1. Development of the optimum mobile phase

The TLC procedure was optimized with a view to develop a stability indicating assay method. Both the standard guggulsterone mixture (containing E- and Z-isomers) and the degraded products were spotted on the TLC plates and run in different solvent systems. Initially toluene-methanol-acetone in varying ratios was tried. It has been observed that incorporation of different volumes of methanol to the varying ratios of toluene: acetone gave poor resolution with diffused spot characteristics. Removal of methanol resulted in improved resolution. Finally, the mobile phase consisting of toluene–acetone (9.0:1.0, v/v) gave sharp and symmetrical peaks and improved spots characteristics of E- and Z-isomers were obtained. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature. The first spot at R_f 0.38 was identified as E-guggulsterone and second spot at R_f 0.46 was identified as Z-guggulsterone with the help of the chromatograms of their individual standard isomers (Figs. 2 and 3). To improve the resolution of the spots the plate was run in the same mobile phase twice (Fig. 4).

3.2. Calibration curves of standard E- and Z-isomers of guggulsterone

Calibration graph was found to be linear that is adherence of the system to Kubelka Munk theory which relies on the idea that light is travelling in all directions simultaneously within the precoated TLC plate. This is approximated as a flux of light travelling upwards and a flux travelling downwards at any depth in the plate. When this flux passes through

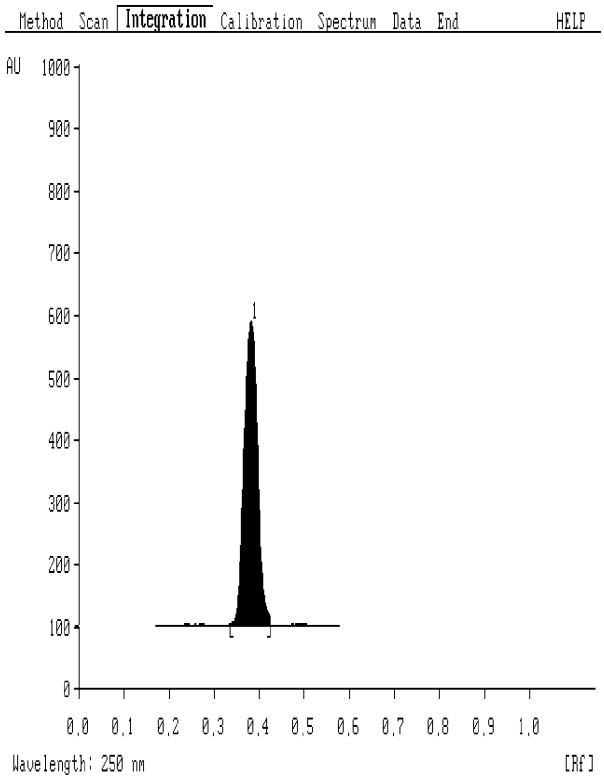


Fig. 2. Chromatogram of standard E-guggulsterone (1000 ng/spot); peak 1 (R_f: 0.38 ± 0.02), mobile phase; toluene–acetone (9.0:1.0, v/v).

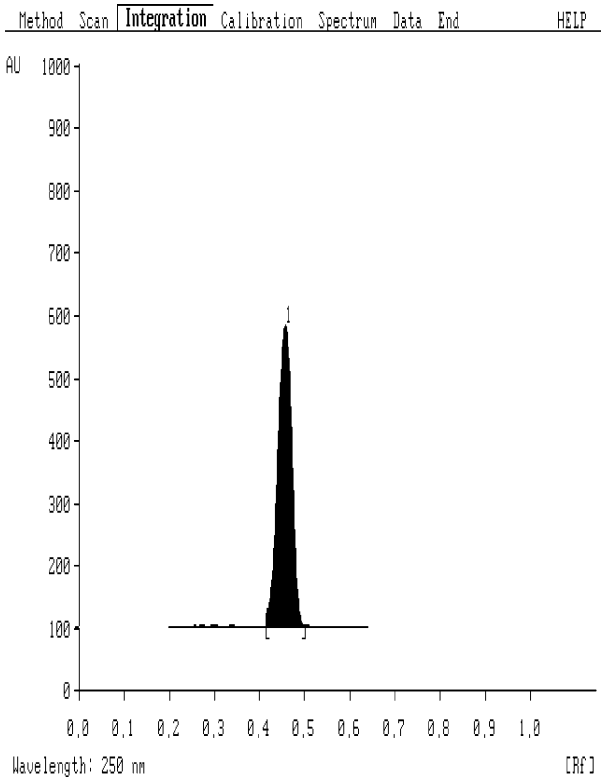


Fig. 3. Chromatogram of standard Z-guggulsterone (1000 ng/spot); peak 1 (R_f: 0.46 ± 0.02), mobile phase; toluene–acetone (9.0:1.0, v/v).

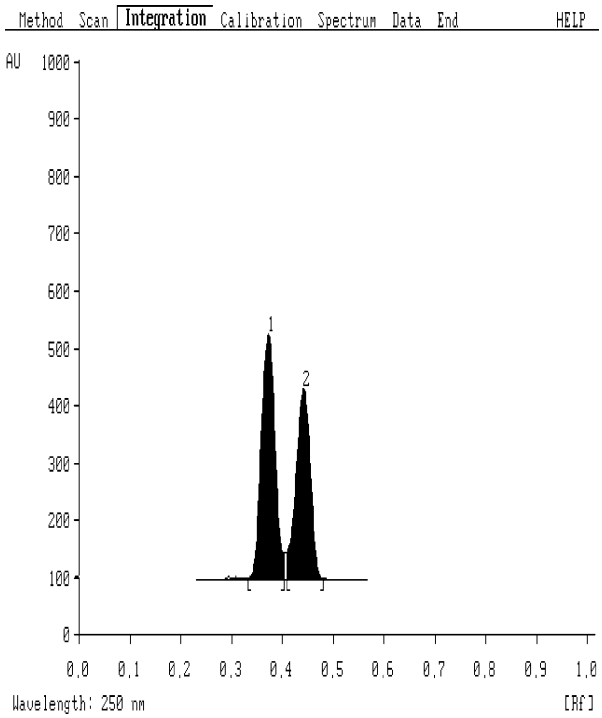


Fig. 4. Chromatogram of standard guggulsterone mixture (1000 ng/spot); peak 1 is of E-guggulsterone (R_f: 0.38 ± 0.02), peak 2 is of Z-guggulsterone (R_f: 0.46 ± 0.02); mobile phase; toluene–acetone (9.0:1.0, v/v).

a thin layer of material, some of it passes through, some of it is scattered backwards and some of it is absorbed. Linearity was found over the concentration range 100–6000 ng/spot for E- and Z-isomers with r^2 ± S.D. = 0.9977 ± 0.054 and 0.9975 ± 0.068, respectively. Linearity was evaluated by determining six standard working solutions containing 0.1–0.6 and 1.0–6.0 μg/ml of E- and Z-guggulsterone, respectively, in triplicate. Peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression data as shown in Table 1 showed a good linear relationship over the low concentration range of 100–600 ng/spot as well as over high concentration range of 1000–6000 ng/spot. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient and the S.D. for intercept value was less than 2%. No significant difference was observed in the slopes of standard curves (ANOVA; $P < 0.05$).

3.3. Validation of the method

3.3.1. Precision

The repeatability of sample application and measurement of peak area were expressed in terms of R.S.D.% and found to be 1.08, 0.98 and 0.52, 0.46 for E- and Z-guggulsterone, respectively. The results depicted in Table 2 showed that no significant intra- and inter-day variation was observed in the analysis of guggulsterone at three different concentration levels 300, 500 and 900 ng/spot. The R.S.D.% for intra- and inter-day analysis was found to be <2% in all the cases.

3.3.2. Robustness of the method

The standard deviation of peak areas was calculated for each parameter and R.S.D.% was found to be less than 2%. The low values of R.S.D.% as shown in Table 3 indicated robustness of the method.

3.3.3. LOD and LOQ

The LOD with signal/noise ratio of 3:1 was found to be 12 and 10 ng/spot for E- and Z-isomers, respectively. The LOQ with signal/noise ratio of 10:1 was found to be 24 and 20 ng/spot for E- and Z-isomers, respectively.

3.3.4. Specificity

The peak purity of individual E- and Z-isomer was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., r (start, middle) = 0.9996 and r (middle, end) = 0.9994 and the overlain spectra is shown in Fig. 5. The peak purity of E- and Z-guggulsterone was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spots, i.e., r (start, middle) = 0.9986 and r (middle, end) = 0.9984. Good correlation ($r = 0.9998$) was also obtained between standard and sample overlain spectra of guggulsterone (Fig. 6).

3.3.5. Recovery studies

The proposed method when used for extraction and subsequent estimation of E- and Z-isomers of guggulsterone from pharmaceutical dosage form after spiking with 50, 100 and

Table 1
Linear regression data for the calibration curves ($n = 6$)

Linearity range (ng/spot)	r ± S.D.	Slope ± S.D.	Confidence limit of slope[a]	Intercept ± S.D.	Confidence of intercept[a]
(a) E-isomer of guggulsterone					
100–600	0.998 ± 0.0004	0.11 ± 0.006	0.105–0.115	14.26 ± 0.56	13.81–14.71
1000–6000	0.996 ± 0.002	0.69 ± 0.008	0.683–0.696	18.61 ± 2.32	16.75–20.47
(b) Z-isomer of guggulsterone					
100–600	0.997 ± 0.0018	0.11 ± 0.005	0.106–0.114	10.92 ± 0.76	10.31–11.53
1000–6000	0.996 ± 0.005	0.70 ± 0.009	0.693–0.707	15.87 ± 2.56	13.82–17.92

[a] 95% confidence limit.

Table 2
Intra- and inter-day precision of HPTLC method ($n = 6$)

Amount (ng/spot)	Intra-day precision			Inter-day precision		
	S.D. of areas	R.S.D.%	S.E.	S.D. of areas	R.S.D.%	S.E.
(a) E-isomer of guggulsterone						
300	1.05	0.98	0.43	2.15	1.61	0.88
500	0.86	0.59	0.35	1.23	1.01	0.50
900	1.28	1.03	0.52	1.48	1.22	0.60
(b) Z-isomer of guggulsterone						
300	1.65	1.30	0.67	2.45	1.87	1.0
500	0.91	0.87	0.37	1.58	1.25	0.65
900	1.21	0.94	0.49	1.65	1.38	0.67

Table 3
Robustness testing ($n = 6$)

Parameter	S.D.[a] of peak area		R.S.D.%[a]	
	E	Z	E	Z
Mobile phase composition	1.58	1.72	1.12	1.34
Amount of mobile phase	1.26	1.42	1.02	1.28
Temperature	1.94	1.56	0.85	1.30
Relative humidity	1.52	1.65	1.33	1.43
Plate pretreatment	0.90	1.08	0.61	0.86
Time from spotting to chromatography	0.77	0.98	0.45	0.38
Time from chromatography to scanning	0.89	0.50	0.37	0.24

[a] Average of three concentrations 300, 500 and 900 ng/spot.

150% of additional standard guggulsterone afforded recovery of 100.16 and 100.69%, respectively, as listed in Table 4.

The data of validation parameters are listed in Table 5.

3.4. Estimation of E- and Z-isomers of guggulsterone in herbal extract

Two spots at R_f 0.38 and 0.46 (Fig. 7) were observed in the chromatogram of the drug isolated from extract along with other components. There was no interference in analysis of isomers from the other components present in the extract. These components appear in the chromatogram at significantly different R_f values. The total guggulsterone content was found to be 1.22% (w/w) of extract with a R.S.D.% of 1.91 out of which E and Z contributes 24.25 and 75.75%

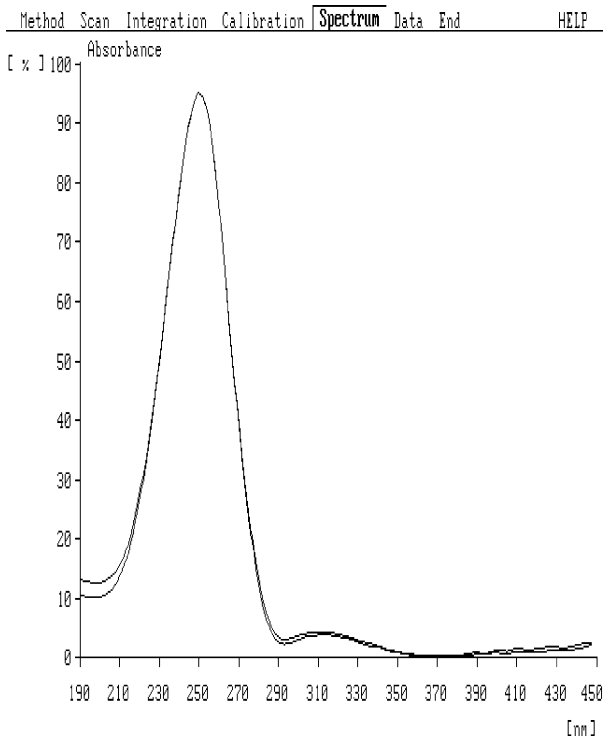


Fig. 5. Overlay spectra of standard E- and Z-guggulsterone in absorption mode in the UV range, taken on the CAMAG TLC scanner 3.

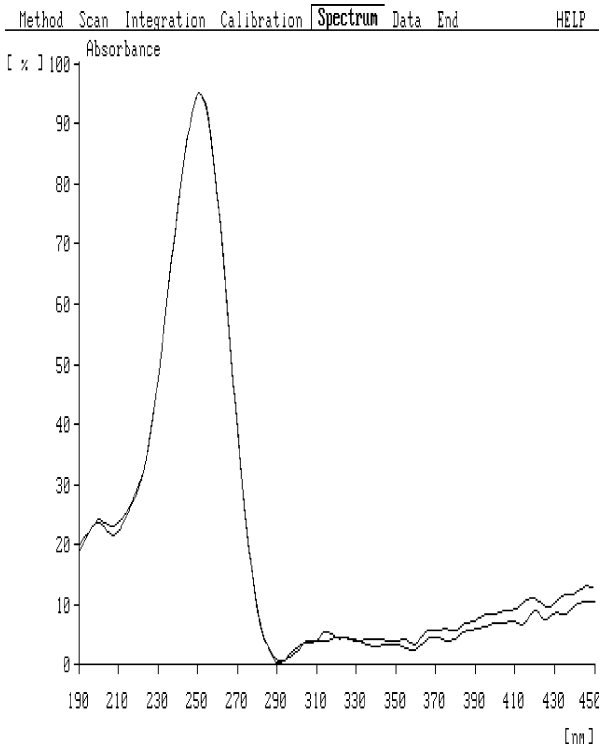


Fig. 6. Overlay spectra of standard and sample guggulsterone in absorption mode in the UV range, taken on the CAMAG TLC scanner 3.

Table 4
Recovery studies ($n = 6$)

Excess drug added to the analyte (%)	Theoretical content (ng/spot)	Recovery (%)	R.S.D.%	S.E.
(a) E-isomer of guggulsterone in capsule				
0	250	98.98	1.62	0.89
50	375	101.58	1.55	0.75
100	500	99.56	1.48	0.62
150	625	100.52	2.71	1.45
(b) Z-isomer of guggulsterone in capsule				
0	250	101.23	2.53	1.30
50	375	98.56	1.96	0.96
100	500	101.85	2.33	1.19
150	625	101.11	2.57	1.32

(w/w), respectively, of total guggulsterone content in extract (Table 6a). Student's *t*-test and the *F*-ratio at 95% confidence level were applied for statistical evaluation of the results.

3.5. Analysis of the marketed formulation

Two spots at R_f 0.38 and 0.46 (Fig. 8) were observed in the chromatogram of the drug samples extracted from capsules. There was no interference in analysis of isomers from the other components present in the capsules. These components appear in the chromatogram at significantly different R_f values. The total guggulsterone content was found to be 0.88% (w/w) with a R.S.D.% of 1.67 out of which E and Z contributes 34.21 and 65.79% (w/w), respectively, of total guggulsterone content in capsule (Table 6b). Statistical evaluation of the results was performed with regard to accuracy and precision using Student's *t*-test and the *F*-ratio at 95% confidence level. The low R.S.D.% value indicated the suitability of this method for routine analysis of E- and Z-isomers of guggulsterone in pharmaceutical dosage form.

Table 5
Summary of validation parameters

Parameter	Data of guggulsterone	
	E-isomer	Z-isomer
Linearity range	100–6000 ng/spot	100–6000 ng/spot
Correlation coefficient	0.9977 ± 0.054	0.9975 ± 0.068
Limit of detection	12 ng/spot	10 ng/spot
Limit of quantitation	24 ng/spot	20 ng/spot
Recovery ($n = 6$)	100.16 ± 1.13	100.69 ± 1.45
Precision (R.S.D.%)		
Repeatability of application ($n = 7$)	1.08	0.52
Repeatability of measurement ($n = 7$)	0.98	0.46
Inter-day ($n = 6$)	1.28	1.50
Intra-day ($n = 6$)	0.87	1.04
Robustness	Robust	Robust
Specificity	Specific	Specific

The HPTLC method has been developed and validated. There are very few methods reported where the resolution of stereoisomers has been carried out. The HPLC technique reported by Mesorb et al. is gradient with photo diode array detection. Retention times of E- and Z-guggulsterones are 27.4 and 32.8 min, respectively, along with some slow eluting components with retention times up to 65 min. Therefore time required for each run is significantly high. The linearity was found in the range of 15–85 μg/ml for E-guggulsterone and 25–130 μg/ml for Z-guggulsterone which is higher as compared to 100–6000 ng/spot in the present method. HPTLC technique may provide a suitable alternative for

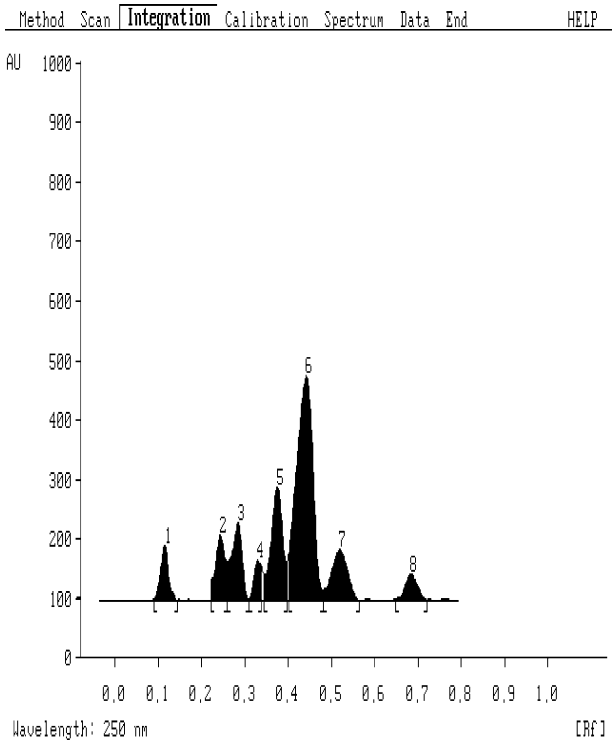


Fig. 7. Chromatogram of guggulsterone extract (25,000 ng/spot); peaks 1, 2, 3, 4, 7 and 8 belongs to other components present in the extract. Peak 5 is of E-guggulsterone (R_f: 0.38 ± 0.02), peak 6 is of Z-guggulsterone (R_f: 0.46 ± 0.02); mobile phase; toluene–acetone (9.0:1.0, v/v).

Table 6
Applicability of the HPTLC method

Guggulsterone	Label claim	Guggulsterone content (%, w/w)	R.S.D.%	S.E.	t	F	t^a	F^a
(a) Analysis of the herbal extract ($n = 6$)								
E-isomer		24.25 ± 1.63	1.46	1.06	0.71	2.64	2.44	9.27
Z-isomer		75.75 ± 1.86	1.84	1.26	0.87	2.58	2.44	9.27
Total content (E and Z)		1.22 ± 1.92	2.44	2.10	1.98	3.85	2.44	9.27
(b) Analysis of the pharmaceutical formulation ($n = 6$)								
Guggulu extract	250 mg	0.88 ± 2.42	2.54	1.58	0.83	2.35	2.44	9.27
E-isomer	–	34.21 ± 1.83	1.14	0.96	1.01	3.64	2.44	9.27
Z-isomer	–	65.79 ± 1.89	1.34	1.05	1.28	3.98	2.44	9.27

[a] Theoretical values for t and F.

the reported HPLC technique by providing fast and reliable analysis. The video densitometric image of chromatoplate has also clearly shown very low concentration of E- and Z-guggulsterone in the extract and marketed capsules as shown in Fig. 9. These results are in accordance with the findings reported by Mesorb et al. Rajpal has reported that standard extract contains 3% (w/w) guggulsterone as estimated by spectrophotometric method but as absorbance at this wavelength is erroneous, it could not be considered for analysis. Along with all other factors considered by Mesorb et al. possibility of degradation of drug during processing and storage can not be excluded. Therefore development of stability-indicating method is essential which may be used as a tool to investigate possible degradation during storage.

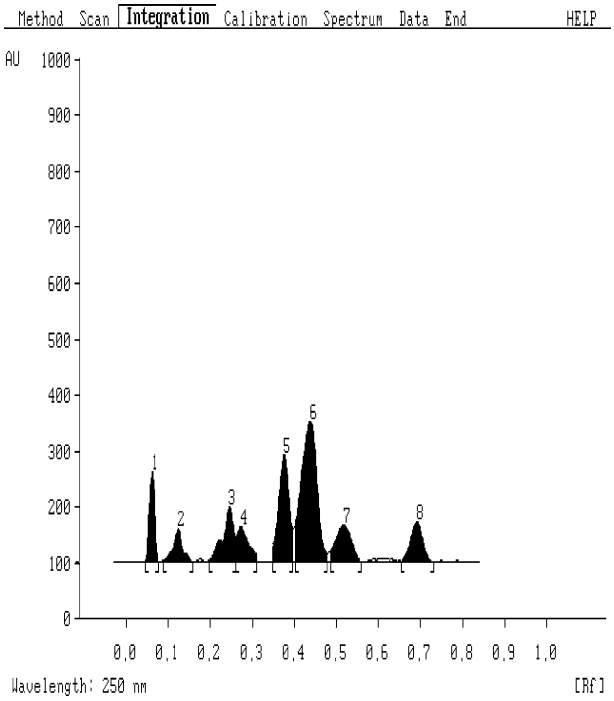


Fig. 8. Chromatogram of guggulu capsule (25,000 ng/spot); peaks 1, 2, 3, 4, 7 and 8 belongs to other components present in the capsule. Peak 5 is of E-guggulsterone (R_f: 0.38 ± 0.02), peak 6 is of Z-guggulsterone (R_f: 0.46 ± 0.02); mobile phase; toluene–acetone (9.0:1.0, v/v).

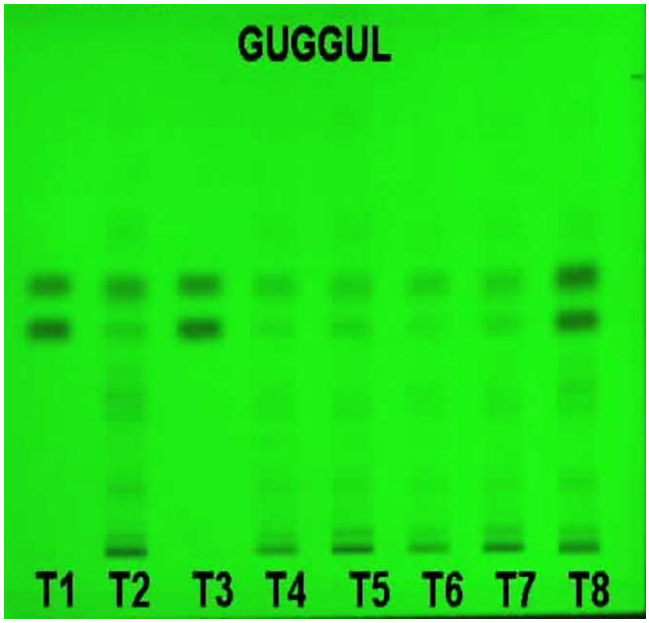


Fig. 9. Video densitometry of guggulsterone. T1, T3 are standard guggulsterone (1.0 μg/spot), T2 is extract (5.0 μg/spot), T4–T7 are marketed formulation (5.0 μg/spot), and T8 is standard added to marketed formulation (6.0 μg/spot).

4. Conclusion

The developed HPTLC technique is precise, specific, accurate and robust for determination of two isomers of guggulsterone. Statistical analysis proves that the method is repeatable and selective for the analysis of two biologically active components E- and Z-isomers of guggulsterone as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities which allows the determination of variations in the guggul resin component's content in products grown and harvested at different climatic conditions. Further the proposed method would also be useful for comparing the resinous extracts of related plant species. Interestingly, the Z-isomer of guggulsterone contributes almost 75 and 65% (w/w) of total guggulsterone in herbal extract and pharmaceutical dosage form, respectively, whereas in case of standard guggulsterone mix-

ture, the amount of Z-isomer is 45% (w/w) of total guggulsterone. It may be extended to study the degradation of guggulsterone under different stress conditions as per the recommendations of ICH guidelines.

Acknowledgements

The authors thank Laila Impex, Vijaywada, India for gift sample of standard guggulsterone. Authors are thankful to Mr. Rama Raju (Director, Laila Impex) and Dr. G.V. Subbaraju (Vice-President, Laila Impex) for providing standards of E- and Z-isomers of guggulsterone. Himani Agrawal is thankful to Council of Scientific and Industrial Research (CSIR), New Delhi for financial support of the project. Neeraj kaul and Himani Agrawal are thankful to Anchrom HPTLC Technologists, Mumbai for providing the facilities for completion of the project. KRM is thankful to University Grant Commission (UGC) for sanction of UGC major research project.

References

[1] W.R. Benn, R.M. Dodson, J. Org. Chem. 29 (1964) 1142–1154.
[2] S. Dev, Proc. Ind. Natl. Sci. Acad. 54 (1988) 12–18.
[3] M.T. Murray, The Healing Power of Herbs: The Enlightened Person's Guide to the Wonders of Medicinal Plants, second ed., Prima, 1995 (Chapter 18).
[4] R.N. Chopra, S.L. Nayar, I.C. Chopra, Full Botanical Name: *Commiphora mukul* (Hook ex. Stocks) Engl; Syn.: *Balsamodendron mukul* Hook. Ex. Stocks, in: Glossary of Indian Medicinal Plants, CSIR, New Delhi, 1956, pp. 75–82.
[5] The Wealth of India (Raw Materials), vol. II, Council of Scientific and Industrial Research, Delhi, 1950, pp. 313–315.
[6] N.K. Kapoor, S. Nityanand, Ind. J. Heart Res. 1 (1980) 22–28.
[7] V.D. Patil, V.R. Nayak, S. Dev, Tetrahedron 28 (1972) 2341–2348.
[8] A.K. Nadkarni, Indian Mater. Med. (1954) 167–170.
[9] M.L. Gujral, K. Sareen, K.K. Tangri, M.K.P. Amma, A.K. Roy, Indian J. Physiol. Pharmacol. 4 (1960) 267–271.
[10] V.L. Mehta, C.L. Malhotra, N.S. Kalrah, Indian J. Physiol. Pharmacol. 12 (1968) 91–98.
[11] G.V. Satyavati, C. Dwarakanath, S.N. Tripathi, Indian J. Med. Res. 57 (1969) 1950–1956.
[12] C.L. Malhotra, Y.K. Agarwal, V.L. Mehta, S. Prasad, Ibid 58 (1970) 394–400.
[13] S. Panda, A. Kar, Life Sci. 65 (1999) 137–141.
[14] A.G. Bajaj, S. Dev, Tetrahedron 38 (1982) 2949–2954.
[15] Y.B. Tripathi, P. Tripathi, O.P. Malhotra, S.N. Tripathi, Planta Med. (1988) 271–279.
[16] Y.B. Tripathi, O.P. Malhotra, S.N. Tripathi, Planta Med. (1984) 78–84.
[17] N.K. Kapoor, S. Nityanand, Indian J. Pharm. 7 (1975) 160–165.
[18] O.P. Asthana, P.P. Gupta, B.N. Dhavan, N.K. Kapoor, S. Nityanand, Indian J. Pharm. 13 (1981) 59–65.
[19] V.P. Arya, Drugs Future 13 (1988) 618–624.
[20] V.D. Patil, U.R. Nayak, S. Dev, Tetrahedron 28 (1972) 2341–2352.
[21] V.D. Patil, U.R. Nayak, S. Dev, Tetrahedron 29 (1972) 341–348.
[22] V.D. Patil, U.R. Nayak, S. Dev, Tetrahedron 29 (1972) 1595–1598.
[23] R.S. Prasad, S. Dev, Tetrahedron 32 (1976) 1437–1441.
[24] A.G. Bajaj, S. Dev, Tetrahedron 38 (1982) 2949–2954.
[25] R.P. Singh, H.N. Subbarao, S. Dev, Tetrahedron 37 (1981) 843–847.
[26] V. Kumar, S. Dev, Tetrahedron 43 (1987) 5933–5948.
[27] S.C. Malhotra, M.M.S. Ahuja, Indian J. Med. Res. 59 (1971) 1621–1627.
[28] S.C. Malhotra, M.M.S. Ahuja, Indian J. Pharmacol. 4 (1972) 110–116.
[29] R.B. Singh, Cardiovasc. Drugs Ther. 8 (1994) 659–664.
[30] N.L. Urizar, Science 296 (2002) 1703–1706.
[31] J. Wu, Mol. Endocrinol. 16 (2002) 1590–1597.
[32] V. Rajpal, Standardization of Botinacals, vol. 1, Eastern Publishers, New Delhi, 2002, pp. 83–91.
[33] T.H. Sane, V.R. Bhate, V.B. Malkar, U.R. Nayak, R.M. Kothurkar, Indian Drugs 28 (1990) 86–91.
[34] S.K. Singh, N. Verma, R.C. Gupta, J. Chromatogr. B 670 (1995) 173–176.
[35] S.K. Roy, R. Pal, J.P.S. Sarin, Indian J. Pharm. 51 (1989) 251–258.
[36] N. Verma, S.K. Singh, R.C. Gupta, J. Chromatogr. B 708 (1998) 243–248.
[37] B. Mesrob, C. Nesbitt, R. Misra, R.C. Pandey, J. Chromatogr. B 720 (1998) 189–196.
[38] Indian Pharmacopoeia, vol. 1, Controller of Publication, New Delhi, 1996, pp. 356–358.
[39] T. Hung, S. Strum, H. Stuppner, Sci. Pharm. 64 (1996) 471–479.
[40] ICH, Q2A validation of analytical procedure: methodology, in: Proceedings of the International Conference on Harmonization, Geneva, October 1994.
[41] ICH, Q2B validation of analytical procedure: methodology, in: Proceedings of the International Conference on Harmonization, Geneva, March 1996.
[42] ICH guidance on analytical method validation, in: Proceedings of the International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, September 2002.