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ORIGINAL ARTICLE **OPEN ACCESS**

Simultaneous Estimation Quantitation of Guggulsterone E & Z from Pharmaceutical Solid Dosage form by Using High Performance Liquid Chromatography

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ABSTRACT
Guggul is the oleo-gum-resin which is obtained from deep incisions at the basal part of stem bark of Commiphora wightii belonging to Burseraceae family. It is very popular ancient Ayurvedic medicine used to cure various diseases. In Ayurveda guggul is always purified prior to use in different formulations. This process is known as sodhana. Guggulsterone E and Z are the prime constituents of Commiphora wightii. This formulation contains extract of various crude drugs which is used in the following disease condition i.e Osteoarthritis, Rheumatoid arthritis, Gouty arthritis, Lumbago, Sciatica & Spondylosis. Guggulsterone (GS) (4,17 (20)-pregnadiene-3,16-dione) is a plant polyphenol (steroid).HPLC is preferred over other analytical method due to its several advantages such as reproducible, fast, simple method and many samples as well as standards can be tested simultaneously. It is cost-effective method. In this we used methanol acetonitrile and Orthophosphoric acid HPLC Grade solvents. Run time is 35 minutes. Column used is Inertsil – ODS-4 Column, 5µ (4.6 X 250 mm). We used the tablet dosage form for the estimation and quantification of active components. Below method is validated as per ICH guidelines and results are acceptable and in the range. This method can be also used in the industry for the estimation of the Guggulsterone E &Z from the various dosage forms.
Key words: *Guggulsterone E &Z, HPLC, estimation, quantification, Validation.*

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INTRODUCTION

For the past few decades, compounds obtained from natural sources have been gaining importance because of their vast chemical diversity and activity many of them are being used as a medicine. Herbal-based medicines have traditional value in the curing of various diseases, and now it has become more popular due to its scientifically known pharmacological activity with fewer side effects [1]. Hence from last few years research activities regarding natural resources were increased. The WHO has taken a keen interest in the quality control of herbal medicine and recommends the use of High Performance Liquid Chromatography (HPLC) for the characterization and quality assurance of herbal medicines [2].

Commiphora genus has approximately 165 species. All species are very slow growing with small thorny, sturdy, highly branched small balsamiferous trees with a short trunk and thin papery bark [3]. *Commiphora wightii* (Arn.) Bhandari is one of the species which is widely cultivated in rocky tracts of the dry and semi-dry region of India, Pakistan, Bangladesh, In India, it is found in dry, rocky and sandy tracts of Rajasthan, Gujarat, Maharashtra, and Karnataka [4]. It is commonly known as "Guggul" or "Indian Bdellium" due to the presence of aromatic steroidal kenotic compounds like guggulsterone E&Z and its related guggulsterone I-IV. According to the Ayurvedic history, guggul is considered as a God gift. According to Vedas guggul is described as "Agni Sthana" and used for dhupa. Guggul was used externally as well as internally during the period of Charaka (1000 B.C.) and Sushruta (600 B.C). Vagbhata (1700 A.D.) has described the use of guggul as a drug of choice for medoroga and vatavikaras [5].

In case of dry form when it is prepared in Tablet form the amount of Guggulsterone E & Z both isomers must have to find. The amount of guggulsterone E&Z depends upon the exposer of light, temperature, packaging, duration of storage, climatic conditions under which the plants are grown and the harvesting method. The hypolipidemic activity was found to be related guggulsterone E&Z (cis- and trans-4, 17(20)-

pregnadiene-3, 16-dione) which are present in a ketonic fraction [6]. Pharmacological studies suggested that the pure guggulsterone isomers have marked hypolipidemic activity.
The main objective of the study was to develop a suitable HPLC method for simultaneous estimation of Guggulsterones [7].

MATERIAL AND METHODS

The designed method was validated as per following criteria's,

Validation:

The method was validated as per the guidelines of the International Conference on Harmonization (ICH) for the parameters like linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) [8-10].

Linearity:

The linearity of an analytical procedure is its ability to obtain test results within a given range, which are directly proportional to the concentration of the analyte in the sample. The linearity study was done by serially diluting standard stock solutions (1 mg/ml) to a given concentration range as given above. Calibration plots were constructed for all three compounds, after triplicate analysis of each calibration solution, by plotting peak area against concentration (µg/ml) of the corresponding standard solution [11-14].

LOD and LOQ:

LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantified. LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantified. LOD and LOQ were experimentally verified by diluting known concentrations of E and Z-guggulsterone until the average response was approximately 3 to 10 times the standard deviation (SD) of response (peak area) for the three replicate determinations [15-19].

Precision and accuracy:

Precision is the closeness of values between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. Precision was determined as the intraday and interday variation of results from the analysis of five different standard solutions. Intraday precision was determined by triplicate analysis of each solution on a single day. Interday precision was determined by triplicate analysis of the solution on two successive days. The relative SD (RSD) of retention time and peak area of all three analytes were calculated as measures of precision and repeatability [20].
The accuracy of an analytical procedure is the closeness between the conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by application of the standard addition method [21].

Standard Testing Procedure

Requisites:

Methanol (HPLC grade)
Acetonitrile (HPLC grade)
Orthophosphoric acid (HPLC Grade)
Water (HPLC grade)
Diluent: Methanol(HPLC grade)

Chromatographic condition:

Column : Inertsil – ODS-4 Column, 5µ (4.6 X 250 mm)
Flow rate : 1 mL/min.
Column temp : 30°C ± 5°C
Sample temp : 10 °C ± 5°C
Injection volume: 10 µL
Detector : UV at 244 nm
Run time : 35 minute
Retention time : About 17and 21 minute for Guggulsterone E&Z respectively.

Mobile phase composition

Solvent A – 0.1 % Orthophosphoric acid.
Solvent B – Acetonitril

Table- 1 Details of Gradient program

Time (minute)	Flow (mL/minute)	% solvent A	% solvent B
0	1.0	50	50
20	1.0	40	60
21	1.0	10	90
30	1.0	10	90
31	1.0	50	50
35	1.0	50	50

Preparation of the standard solution Guggulsterone E :
4.0 mg GuggulsteroneE was weighed and transferred into 25 mL volumetric flask. 20 mL of diluent was added and sonicated in ultrasonic water bath for 15 minutes. The solution was cooled and volume was made with diluent.
Preparation of the standard solution Guggulsterone Z :
4.0 mg Guggulsterone Z was weighed and transferred into 25 mL volumetric flask. 20 mL2 of diluent was added and sonicated in ultrasonic water bath for 15 minutes. The solution was cooled and volume was made with diluents.
Preparation of the Mixture of standard solution of Guggulsteron E & Z:
4 mL resulting solution of Guggulsterone E and 12 mL of Guggulsterone Z we read into 20 ml volumetric flask. Volume was made with diluents. 3 ml resulting solution was diluted up to 5 mL with diluent. Resulting solution was used as mixed standard solution.

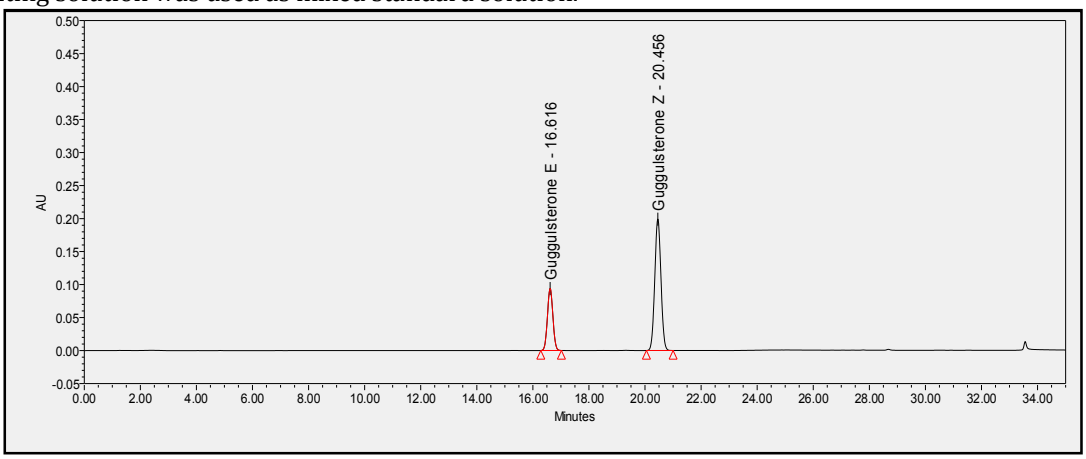


Figure 1 HPLC chromatograms of Standard solution (Guggulsterone E & Z)

Preparation of the test solution:
20 tablets of Aricare formulation were weighed and powdered. 650 mg of powdered tablet formulation was taken into 25 mL volumetric flask. 20 mL of diluent was added and sonicated in ultrasonic water bath for 30 minutes. The resulting solution was cooled and volume was made with diluent. The content of volumetric flask was filtered through Whatman No. 41 filter paper and 0.45 µ syringe filter. Resulting solution was used as test solution.

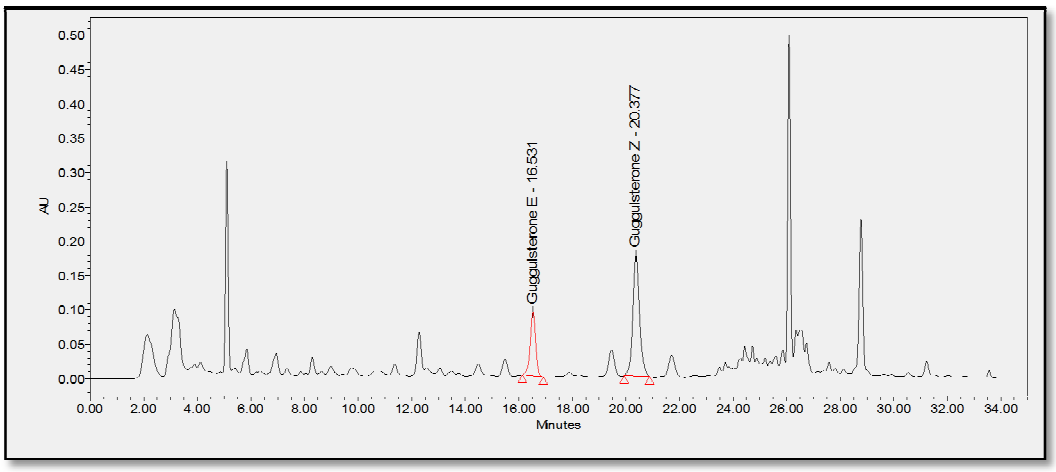


Figure 2 HPLC chromatograms of Test solution

Preparation of the Guggul Placebo solution:
Equivalent amount of Guggul placebo was taken into 25 mL volumetric flask. 20 mL of diluent was added and sonicated in ultrasonic water bath for 30 minutes. The resulting solution was cooled and volume was made with diluents. The content of volumetric flask was filtered through Whatman No. 41 filter paper and 0.45 µ syringe filter. Resulting solution was used as Guggul Placebo-solution.

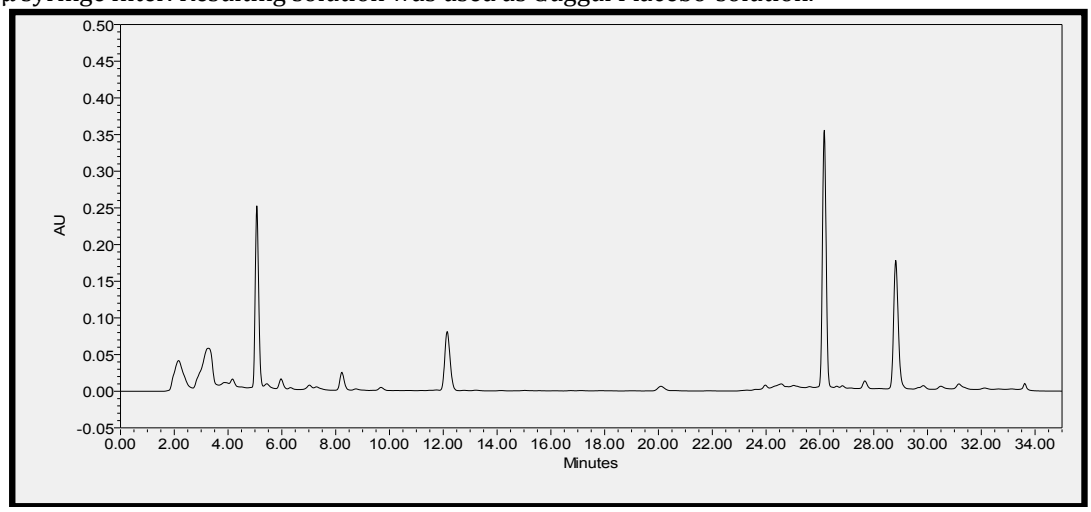


Figure 3 HPLC chromatograms of Guggul Placebo solution

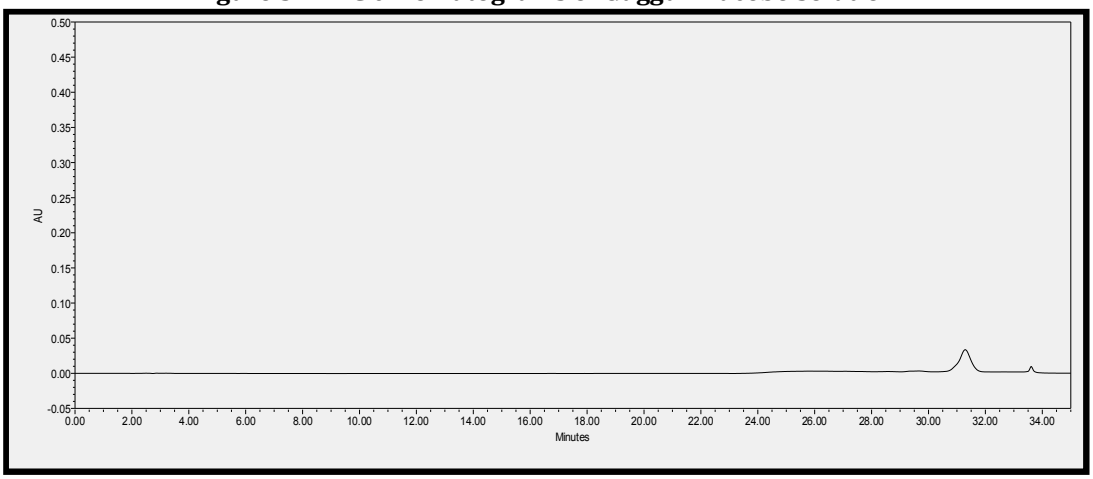


Figure 4 HPLC chromatograms of Blank solution

Validation for Assay of Guggulsterone E&Z from Aricare Tablet
Requirements

- Equipment's and materials
  1. Analytical balance
  2. Mobile phase filtration unite
  3. HPLC SYSTEM – 1
- Waters e2695 Separation module
- Waters 2996 Photo Diode Array detector
- Waters Empower 3 chromatography software
  4. HPLC SYSTEM – 2
- Waters e2695 Separation module
- Waters 2489 UV/Visible detector
- Waters Empower 3 Chromatography software

Table – 2 Standard and Test used for validation studies:

Sr. No	Name of Standard/Test	Potency (%)
1	Guggulsterone-E	99.5
2	Guggulsterone-Z	97.5
3	Aricare Tablet	NA
4	Guggul Placebo	NA

RESULTS AND DISCUSSION

Specificity

The specificity of the method for Assay of guggulsterone E and Z from Aricare tablet was demonstrated by injection of following solutions into the HPLC system.

- Blank as a Diluent
- Standard Solution
- Test Solution
- Guggul Placebo Solution

Table 3: Specificity of Guggulsterone E &Z

Sr. No.	Sample name	Analyte name	Specificity
1.	Aricare tablet	Guggulsterone E &Z	Specific
2.	Standard	Guggulsterone E &Z	Specific
3.	Blank	No Peak	-
4.	Guggul Placebo	No Peak	-

By comparing the chromatogram of the Blank, Placebo, standard solution and test solution the following evaluations were made.

- No peaks were co-eluted with both the analyte peaks from blank and Placebo solutions.
- No interfering peaks were observed from blank and Placebo at the retention time of Guggulsterone E&Z.
- No purity flag was observed for Guggulsterone E&Z from test solution and standard solution.

The method is considered to be specific as per the above mentioned observations.

System precision

System precision was evaluated from six replicate injections of standard as per proposed method. The average and relative standard deviation was calculated from the six determinations and tabulated in table 4.

Table 4: System Precision for Guggulsterone E&Z

Inject. No	Peak Area of Guggulsterone E	Peak Area of Guggulsterone Z
1	1152790	3000239
2	1155410	3001274
3	1153689	3015615
4	1140385	3006999
5	1156820	2967298
6	1159394	3013398
Mean	1153081.3	3000803.8
%R.S.D	0.58	0.58

Acceptance Criteria:

The relative standard deviation should be within the following limits

% RSD for Area of Guggulsterone E&Z < 2.0%,

The % RSD observed within acceptable limit indicates the precision of the system.

Method precision

The six test solutions were prepared separately. Each was analyzed as per proposed procedure. The % assay for test solution with % RSD is tabulated in Table 6. The mean and %RSD were calculated and tabulated in table 5

Table 5: Method Precision for Guggulsterone E&Z

Sample No.	% Assay of Guggulsterone E	% Assay of Guggulsterone Z
1	0.625	1.938
2	0.621	1.943
3	0.631	1.950
4	0.644	1.990
5	0.639	1.979
6	0.638	1.979
Mean	0.633	1.963
% RSD	1.37	1.13

Acceptance Criteria:
The relative standard deviation should be within the following limits
% RSD for % Assay of Guggulsterone E&Z < 2.0%
The % RSD was observed within the limit indicates that the method has an acceptable level of precision.

Intermediate precision
The intermediate precision was determined by comparison of two independent Analysis on different days. The data of the 1st day was taken from the analysis of "Method precision"

Table 6: Intermediate Precision for Guggulsterone E&Z

Name of Analyte	Sr. No.	Assay (% w/w, Analysis-1)	Assay (% w/w, Analysis-2)
Guggulsterone E	1	0.625	0.652
	2	0.621	0.631
	3	0.631	0.623
	4	0.644	0.651
	5	0.639	0.648
	6	0.638	0.644
	Average	0.633	0.641
	% RSD	1.37	1.89
	Overall % RSD	1.72	
Guggulsterone Z	1	1.938	1.893
	2	1.943	1.902
	3	1.950	1.918
	4	1.990	1.884
	5	1.979	1.886
	6	1.979	1.905
	Average	1.963	1.898
	% RSD	1.13	0.67
	Overall % RSD	1.98	

Acceptance Criteria:
The relative standard deviation from day-1 and day-2 analysis should be within the following limits.
The overall % RSD for % of Guggulsterone E&Z from day-1 and day-2 should be < 2.0 %
% RSD of assay of Guggulsterone E&Z from 6 determinations ware found to be within acceptance criteria for day 1 & day2.
Hence the method of assay for Guggulsterone E & Z from Aricare Tablet is rugged.

Linearity
The linearity of peak area response for Guggulsterone E & Z was determined from 50 % to 150 % level of working concentration for Guggulsterone E & Z. The stock solutions of Guggulsterone E & Z were diluted to seven different known concentrations.
Graphs of concentration (as x-value) versus area (as y-value) were plotted. The correlation coefficient is tabulated in Table 7

Table 7: Linearity of Guggulsterone E & Z

% Level	Conc. of Guggulsterone E (ppm)	Average Peak area of Guggulsterone E	Conc. of Guggulsterone Z (ppm)	Average Peak area of Guggulsterone Z
50	10.08	587594	32.40	1536126
65	13.44	777203	43.20	2075742
80	16.80	975697	54.00	2583291
100	20.16	1150894	64.80	3014198
110	23.52	1373196	75.60	3577181
130	26.88	1589750	86.40	4093473
150	33.60	1948623	108.00	5856339
r^2	**0.9993**		**0.9995**	
Slope of Regression line	58511		46519	

Figure 5 Linearity Graph for Guggulsterone E

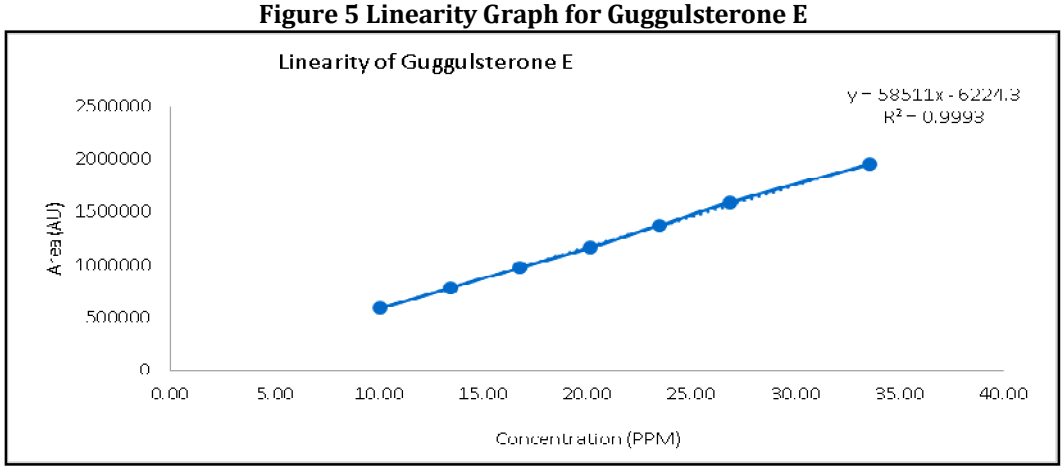
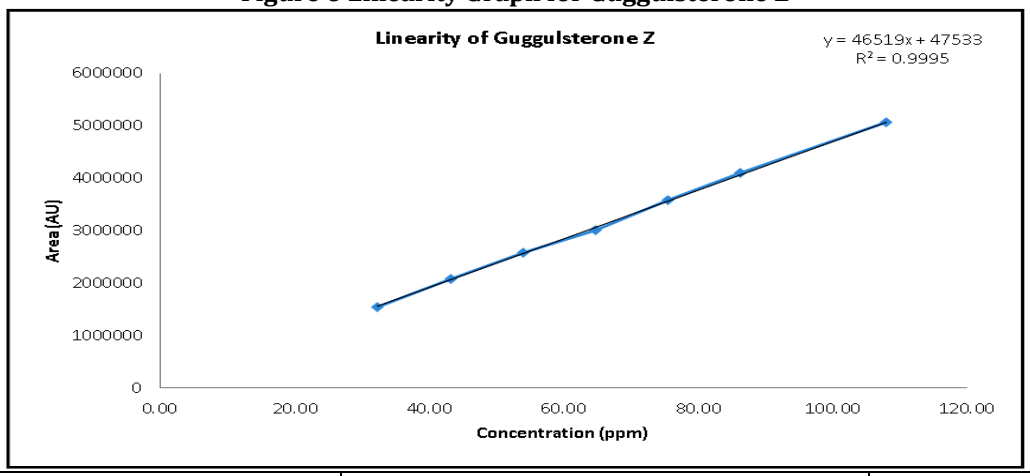


Figure 6 Linearity Graph for Guggulsterone Z



Parameter	Values		Acceptance Criteria
	Guggulsterone E	Guggulsterone Z	
Number of determinations	7	7	NA
Correlation coefficient	0.9993	0.9995	≥ 0.99
Slope of Regression line	58511	46519	NA

Method is considered to be linear as the Correlation coefficient is within acceptance criteria.

Range

The range of Guggulsterone E&Z was determined by calculating precision at 50 %, 100 % and 150 % level of the working standard concentration. The results are tabulated in Table 8

Table 8: Range of Guggulsterone E&Z

Name of Analyte	Injection No.	Peak area at 50% level	Peak area at 100% level	Peak area at 150% level
Guggulsterone E	1	588467	1156781	1948190
	2	586720	1159529	1949056
	3	587310	1159407	1957586
	Average	587499	1158572	1951611
	% RSD	0.15	0.13	0.27
Guggulsterone Z	1	1536647	3011029	5056039
	2	1535604	3015847	5078127
	3	1532419	3017366	5056639
	Average	1534890	3014747	5063602
	% RSD	0.14	0.11	0.25

Acceptance Criteria:

The relative standard deviation for each level should be within the following limits

% RSD for Area of Guggulsterone E&Z < 2.0%,

The % RSD of determinations for each level was found within acceptance criteria. This shows the range for method.

Robustness

The influence of slightly changed parameters of the chromatographic conditions was tested according to ICH guidelines to demonstrate sufficient robustness of the method. The tests are carried out by injecting Blank and standard solution by varying each of the parameters of chromatography mentioned above as follows.

Flow (1.0 mL/min) : 0.9 and 1.1 mL/min
Temperature (30°C) : 25°C and 35°C
Wavelength (244 nm) : 239 nm and 249 nm

Acceptance Criteria:

The RSD of peak area response due to Guggulsterone E&Z in 5 replicate injections of standard solution should be less than 2.0 % and system suitability parameters should be passed. The % RSD and system suitability parameters for results obtained with varied chromatographic conditions are within the limits. Hence, the method is robust.

Accuracy

The accuracy of method was determined from recovery studies. A known but varying amount of Guggulsterone E&Z was spiked into Guggul Placebo solution at 80%, 100% and 120% recovery levels of working standard in triplicate. The spiked test solutions were analyzed according to the proposed procedure. The percentage recoveries were calculated against respective levels and mentioned in Table 10.

Table 9: Robustness parameter for Guggulsterone E&Z

Robustness parameter		% RSD	Peak tailing	Theoretical plates	Remark
Guggulsterone E					
Wavelength (nm)	239	0.20	1.19	22568	Pass
	244	0.20	1.19	22572	Pass
	249	0.20	1.19	22575	Pass
Temperature (°C)	25	0.31	1.20	21904	Pass
	30	0.20	1.19	22572	Pass
	35	0.33	1.17	23872	Pass
Flow (mL/min)	0.9	0.23	1.19	23253	Pass
	1.0	0.20	1.19	22572	Pass
	1.1	0.39	1.19	21867	Pass
Guggulsterone Z					
Wavelength (nm)	239	0.21	1.19	26819	Pass
	244	0.20	1.19	26768	Pass
	249	0.20	1.19	26824	Pass
Temperature (°C)	25	0.30	1.20	26109	Pass
	30	0.20	1.19	26768	Pass
	35	0.32	1.17	28555	Pass
Flow (mL/min)	0.9	0.18	1.19	27575	Pass
	1.0	0.20	1.19	26768	Pass
	1.1	0.39	1.19	26133	Pass

Table 10: Recovery study for Guggulsterone E&Z

Analyte	Recovery level	% Recovery	Average % Recovery
Guggulsterone E	80% - 1	97.68	97.72
	80% - 2	97.86	
	80% - 3	97.62	
	100% - 1	101.65	100.35
	100% - 2	99.55	
	100% - 3	99.84	
	120% - 1	99.60	100.30
	120% - 2	99.43	
	120% - 3	101.86	
Guggulsterone Z	80% - 1	98.79	98.98
	80% - 2	98.83	
	80% - 3	99.30	
	100% - 1	102.67	101.04
	100% - 2	99.75	
	100% - 3	100.71	
	120% - 1	99.25	99.59
	120% - 2	98.63	
	120% - 3	100.90	

Acceptance criteria:
% Average recovery should be in the range of 95-105%.
The % Average recovery of Guggulsterone E&Z in Aricare Tablet observed within acceptance criterion of 95-105 indicates the accuracy of the method.

Stability in standard and test solution
The standard and test solutions were prepared as per the proposed method and kept at room temperature. The standard and test solutions were analyzed at initial and at different time intervals.

Acceptance criteria:
The percentage change of Guggulsterone E&Z with respect to initial in standard and Test solutions **< 5.0 %**. The percent change of Guggulsterone E&Z is within limit, Standard solution and Test solution are stable up to 30 hours at Room temperature.

CONCLUSION
The Specificity of Assay Method for quantitation of Guggulsterone E&Z from Aricare tablet was proved by chromatographic comparison. The test for linear correlation of the concentration values measured and those given for Guggulsterone E&Z was within a range of 50 to 150 % of working concentration. The exactness of the method as defined by precision and accuracy of the method was proved by recovery within a range from 80 to 120% of the working concentration complies. Standard solution and test solution are stable up to 30 hours at Room temperature. The present validation proves that the HPLC-method is suitable for the quantitation of Assay of Guggulsterone E&Z from Aricare Tablet at prescribed conditions as per the ICH Guidelines.

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