Determination of (E) & (Z)-Guggulsterones in Guggul Formulations by High Performance Liquid Chromatography†

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A reversed phase high performance liquid chromatography was developed to determine (E) and (Z)-Guggulsterones quantitatively in different guggul formulations. HPLC analysis was performed on a C18 column using 0.1% (v/v) phosphoric acid in water and acetonitrile (45 : 55) as mobile phase with detection at 241 nm. Seven guggul formulations available in the market were analyzed and the quantities of guggulsterones have been found to vary between 0.0022 and 1.2851 mg/capsule. Further, the claimed quantity of guggul on the label did not corroborate with the content of guggulsterones.

Key Words: Guggul formulations, (E) and (Z)-Guggulsterones, HPLC estimation.

INTRODUCTION

Guggul is the oleo-gum-resin exudate from the tree *Commiphora mukul* (Hook ex Stocks) Engl. *Syn. Balsamodendron mukul* (Hook ex Stocks). It is an opaque and reddish brown gum and carries a faintly balsamic odour[1, 2]. Traditionally, it was used as dentifrice for ulcer of mouth and an ingredient in incense and perfume by the Jews and the Egyptians for embalming and fumigations[3]. The fumes of burning guggul were recommended in the treatment of hay fever, acute and chronic nasal catarrh, chronic laryngitis, chronic bronchitis and phthisis[2]. *Shushruta Samhita*, the ancient Indian Medical Treatise[4], recorded that guggul is effective in the treatment of rheumatoid arthiritis, obesity and allied disorders, useful in bronchial diseases and reduces phlegm. Guggul resin was also reported to be useful in the treatment of osteo-arthritis, frozen shoulders, sciatica, atherosclerosis, hemiplegia, infective hepatitis and dental infections[2]. A randomized placebo controlled clinical study revealed that guggulipids reduce triglycerides level of the participants[5].

Guggul contains a complex mixture of various chemical compounds such as aliphatic esters, carbohydrates, lignins, lipids, diterpenes, steroids, etc.[2] However, two compounds, (E)-guggulsterone [4,17(20)-(*cis*)pregnadiene-3,16-dione, 1] and (Z)-guggulsterone [4,17(20)-(*trans*)pregnadiene-3,16-dione, 2], (Fig. 1), have been credited with medicinal activity of the guggul[6]. Guggulsterones have been found to be Farnesoid X receptor antagonist[7, 8] and an inhibitor of NF-kβ and 1k/βα kinase activation[9].

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Various methods have been reported in literature for the estimation of guggulsterones, including UV spectrophotometry[10, 11], colorimetry[10], thin layer chromatography[11, 12], a reversed phase high performance liquid chromatography[12, 13] and high performance thin layer chromatographic method[14].

In view of the importance of guggulsterones and large number of brands on guggul formulations available, we have estimated (E) and (Z)-guggulsterones by HPLC in the guggul formulations available in the market and the results are reported in this paper.
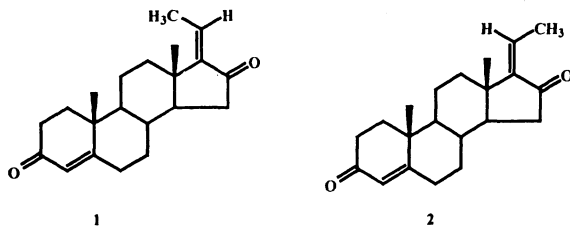

Fig. 1.   Structures of (E) and (Z)-guggulsterones

EXPERIMENTAL

(E) and (Z)-guggulsterone standards were obtained from Sterloids, Inc., USA; seven different formulations containing guggul were procured from the local market, and all solvents and chemicals used were of AR or HPLC grade.

The HPLC (Schimadzu) system used for estimation of guggulsterones was equipped with Alltima C18, 5μ (250 × 4.6 mm) column, LC-10AT pumps, SCL-10A system controller, SIL-10A auto injector, SPD-M10 AVP photodiode array detector set at wavelength of 241 nm for detection and class-M10A software were used. A Millipore Swinnex type filter (pore size = 0.45 μm) was used for filtration. Agilent 1100 series LC/MSD were used for obtaining mass spectral data.

Mobile phase preparation: 0.1% (v/v) phosphoric acid in water and acetonitrile (45 : 55) were mixed and filtered through 0.45 μm membrane filter.

Preparation of (E) and (Z)-guggulsterone standard solutions: About 2.5 mg of each standard (purity: (E)-guggulsterones 99.0%, (Z)-guggulsterones 99.0%) was accurately weighed and dissolved in 25 mL of acetonitrile.

Sample preparation of guggul formulations: Twenty capsules of each formulation were weighed accurately and the average weight of each capsule was obtained. Hard gelatin shells were removed, weighed and average net content of the capsule was determined. The contents of the capsules were ground in a mortar to make homogeneous powder. About 500 mg of the above guggul powder was carefully weighed, added 25 mL of acetonitrile and dissolved by sonication. The solution was filtered on a Whatmann No. 41 filter paper and the filtrate filtered further through 0.45 μm filters before injecting into HPLC.

Chromatographic conditions: The elution was carried out with isocratic system using 0.1% (v/v) phosphoric acid and acetonitrile (45 : 55) as mobile phase with a flow rate of 1 mL/min at ambient temperature, detection at 241 nm run time about 40 min. The (E) and (Z)-guggulsterones were quantified using class M10A software.

The linearity of the method was evaluated by analyzing series of (E) and (Z)-guggulsterone standard solutions. 20 μL of each of the six standard solutions

were injected in to the HPLC. The elutions were carried out as described above. Standard calibration curve obtained by plotting the concentration of (E) and (Z)-guggulsterone standards *vs.* peak area (average of three runs). The calibration range was chosen to reflect normal guggulsterones range in guggul formulations.

Determination of (E) and (Z)-guggulsterones in guggul formulations: The sample volume was 20 μL. (E) and (Z)-guggulsterones were calculated on the basis of linear calibration functions. The content of (E) and (Z)-guggulsterones was expressed as milligrams per each capsule.

RESULTS AND DISCUSSION

The present method was developed to determine the concentrations of (E) and (Z)-guggulsterones in commercial guggul formulations. Fig. 2 represents the typical chromatogram of (E) and (Z)-guggulsterones at 241 nm. HPLC analysis of guggulsterones showed two peaks at retention times 18.0 ± 0.45 min for (E)-guggulsterone and 24.0 ± 0.50 min for (Z)-guggulsterone. Calibration graphs were prepared to determine the (E) and (Z)-guggulsterones of different guggul formula-
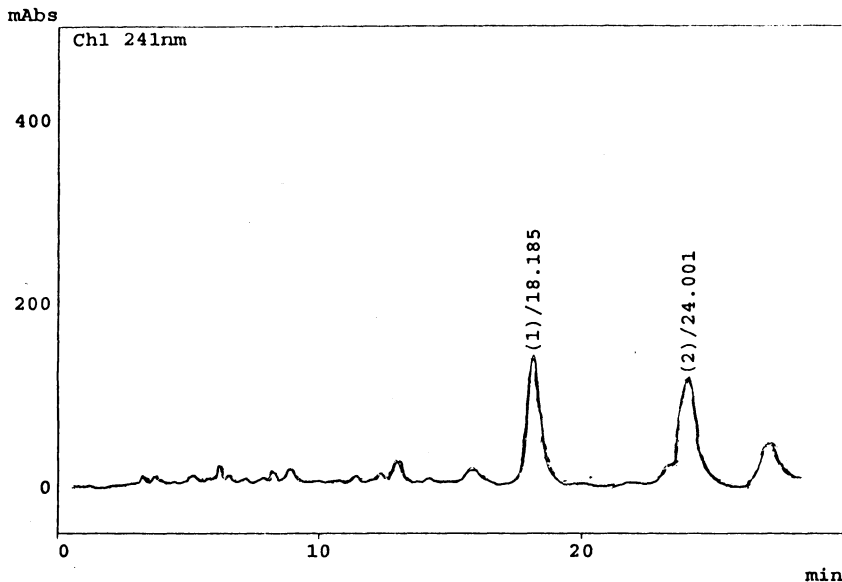

Fig. 2.   Typical chromatogram of Guggulsterones E(1) and Z(2) in formulation

tions. Calibration curves were derived from three independent injections of six concentrations of (E) and (Z)-guggulsterone standards *vs.* the peak areas (Fig. 3). Linearity was found in the concentration range between 0.1 and 5 μg of (E) and (Z)-guggulsterones with high reproducibility and accuracy. The regression analysis of the experimental data shows that the linear relationship with correlation coefficient of (E) and (Z)-guggulsterones are 0.9998 and 0.9989. Accuracy of the method was determined by spiking known amount of (E) and (Z)-guggulsterones to the pre-analyzed sample. Percentage recovery was calculated by comparing areas obtained. The results are summarized in Table-1. Using this method the (E) and (Z)-guggulsterones in seven commercial guggul formulations were estimated (Table-2). The individual peaks in all the samples were analyzed with no interfer-
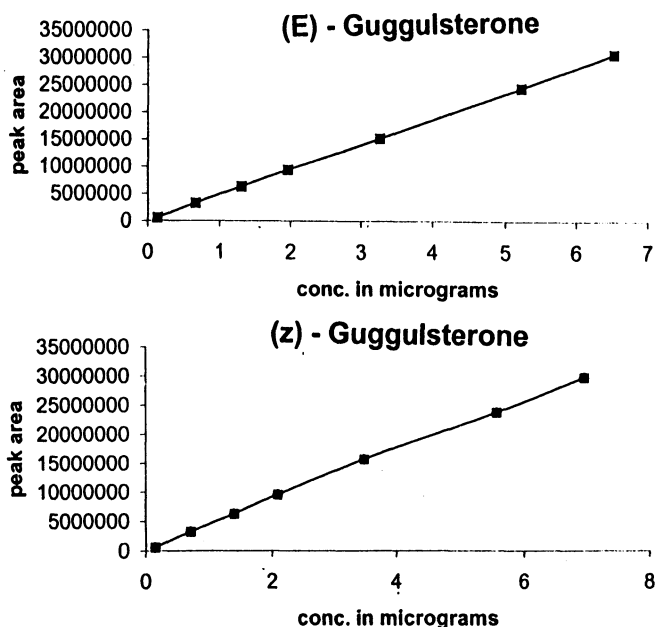
Fig. 3. Linear relationship between peak area response and concentration of (E) and (Z) guggulsterones

ence from other compounds. The efficiency of the column is 16,630 and 18,450 theoretical plates for (E) and (Z)-guggulsterones, respectively and the tailing factor is < 1.1 for analytes (E) and (Z)-guggulsterones. The identity of the each peak was confirmed by determination of retention times and by spiking with standard and also by mass spectrometry.

The quantitative studies of various guggul formulations show that the content of (E) and (Z)-guggulsterones in these materials varies widely. This is because the materials used in these formulations may be obtained from different sources. The guggul resin may exhibit significant variations in the (E) and (Z) isomer content depending on the climatic conditions under which the product is grown and harvested[14].

TABLE-1
RECOVERY STUDY

Component	Amount of guggulsterone added (mg)	Amount of guggulsterone recovered (mg)	Recovery* (%)
	1.00	0.9985	99.85 ± 0.093
(E)-Guggulsterone (1)	2.00	1.9984	99.92 ± 0.100
	3.00	2.9958	99.86 ± 0.095
	1.00	0.9981	99.81 ± 0.105
(Z)-Guggulsterone (2)	2.00	1.9976	99.88 ± 0.120
	3.00	2.9899	99.66 ± 0.112

*Average of three determinations

TABLE-2

CONTENT OF (E) & (Z)-GUGGULSTERONES IN COMMERCIAL GUGGUL
FORMULATIONS

S.No.	Name of the capsule	Label claim of guggul per capsule (mg)	Guggulsterones (mg)*		Total (E) and (Z) guggulsterones per capsule (mg)
			(E)-Isomer	(Z)-Isomer	
1.	Antharth	150.0	1.2056	0.0795	1.2851 ± 0.010
2.	Rumadap	200.0	0.1876	0.2909	0.4785 ± 0.011
3.	Rumalaya	200.0	0.0114	0.0238	0.0352 ± 0.009 ·
4.	Pain off	250.0	0.0575	0.0222	0.0797 ± 0.005
5.	R-caps	100.0	0.0063	0.0044	0.0107 ± 0.002
6.	Stretch	100.0	0.0561	0.1227	0.1788 ± 0.011
7.	C-caps	40.0	0.0022	Nil	0.0022 ± 0.001

*Average of six determinations.

The present method is simple, highly reproducible and could be used for the quantitative determination of (E) and (Z)-guggulsterones in guggul raw materials, standardized extract and guggul formulations.

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