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Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0053 - Guggul for Guggulsterones by HPLC

Botanical Name: *Commiphora mukul; Balsamodendron mukul; Commiphora wightii*

Common Names: Bdellium tree, false myrrh, guggulu

Parts of Plant Used: Gum resin

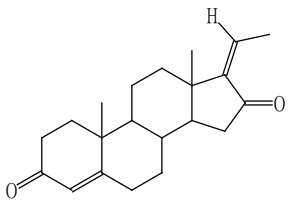
Uses: Treatment of hyperlipidemia, reduction of lipid and cholesterol levels

Modes of Action:

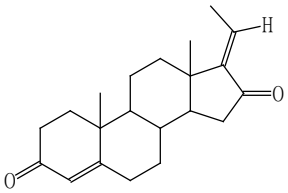
Several clinical trials have established the activity of guggul[1,2] and guggulsterones were found to be the bioactive components.[3] Guggulsterones were found to be an antagonist of the farnesoid X receptor and an antagonist of the bile acid receptor, to decrease expression of bile-acid-activated genes, and to be a farnesoid X receptor antagonist in coactivator association assays but guggulsterones act to enhance transcription of the bile salt export pump.[4–6]

Chemical Markers:

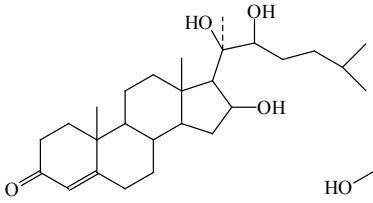
Guggul resin is a complex mixture of various types of compounds including sterols, diterpenes, triterpenes, lignans, lipids, aliphatic esters, and ferulates.[7–13] The Z-guggulsterone and E-guggulsterone were found to be the bioactive components responsible for the lipid- and cholesterol-lowering activities. Other sterols purified from the gum include guggulsterols I, II, III, IV, V, and VI. The essential oil of guggul gum resin was found to include α-pinene, myrcene, cadinene, geraniol, methylheptanone, and eugenol.[14] E- and Z-Guggulsterones are used as marker compounds for quality control of guggul gum and gum extracts.



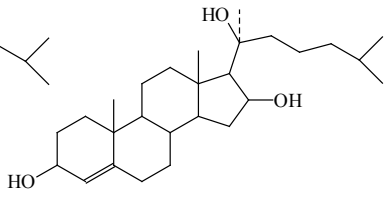
Z-Guggulsterone



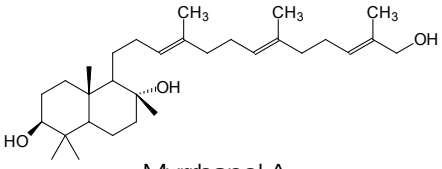
E-Guggulsterone



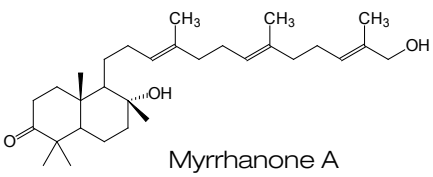
Guggulsterol I



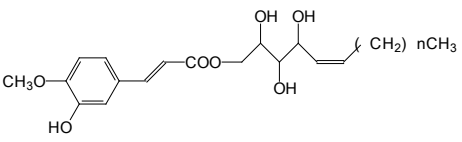
Guggulsterol II



Myrrhanol A



Myrrhanone A



Ferulates: n=16, 17, 18, 19

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Methods of Analysis

Several HPLC methods have been developed to determine E- and Z-guggulsterones; an LC–MS method also was reported that could identify the chemical components of guggul.

E- and Z-Guggulsterones can be extracted by organic solvents such as ethyl acetate and methanol. The E- and Z-guggulsterones also can be extracted from a guggul extract with 60% acetonitrile.

Method 1:

The method of Mesrob et al. was used.[15]

Sample Preparation:

Accurately weigh 25 to 30 mg of the resinous extract; dissolve in 2 mL of ethyl acetate, and then dilute to 10 mL with methanol for HPLC analysis.

Chromatography:

Column: Alltech Adsorbophere HS-C18, 5 µm, 150 × 4.6 mm.
Mobile phase: Solvent A = water, solvent B = acetonitrile.
Gradient:

Time (minutes)	%A	%B	Curve
0	64	36	-
30	64	36	-
50	55	45	6
56	55	45	-
66	0	100	1
67	64	36	6
76	64	36	-

Flow rate: 1.2 mL/minute
Injection volume: 20 µL
Detection wavelength: 245 nm

Validation Data:

Linearity: For Z- and E-guggulsterones, 15 to 85 and 25 to130 mcg/mL, respectively, with a correlation coefficient over 0.992.
Accuracy: The percent recoveries were from 100 to 103.9 for Z- and E-guggulsterones with two different HPLC systems.
Precision: Not specified
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: Not specified

Method 2:

The method of Nagarajan et al. was used.[16]

Sample Preparation:

Sonicate sample equivalent to 3.0 mg of E- and Z-guggulsterones with acetonitrile in a 50-mL volumetric flask for 30 minutes.

Chromatography:

Column: Waters Symmetry C18, 4 µm, 150 × 3.9 mm, with a Sentry C18 guard column, 4 µm, 20 × 3.9 mm.
Mobile phase: Solvent A = water, solvent B = acetonitrile; A:B = 54:46.
Flow rate: 1.0 mL/minute
Injection volume: 20 µL
Column temperature: 25°C
Detection wavelength: 242 nm

Validation Data:

Linearity: For both compounds, 0.01 to 0.2 mg/mL with a correlation coefficient over 0.999.
Accuracy: The percent recoveries were 98.7, 99.0, and 97.7 with 50%, 100%, and 150% spiking levels, respectively.
Precision: RSD is less than 2.3%.
Ruggedness: Not specified
Robustness: Not specified
Selectivity: Peak identification was determined against standards.
LOD/LOQ: LOD = 0.005 mg/mL, LOQ = 0.014 mg/mL.

Method 3:

The Chromadex method was used; it can be found at www.chromadex.com.

Sample Preparation:

Extract about 350 mg of guggul extract with 50 mL of 60% acetonitrile in a 100-mL volumetric flask. First shake the sample for 15 minutes and then sonicate for 15 minutes. Cool to room temperature and fill to volume with 60% acetonitrile.

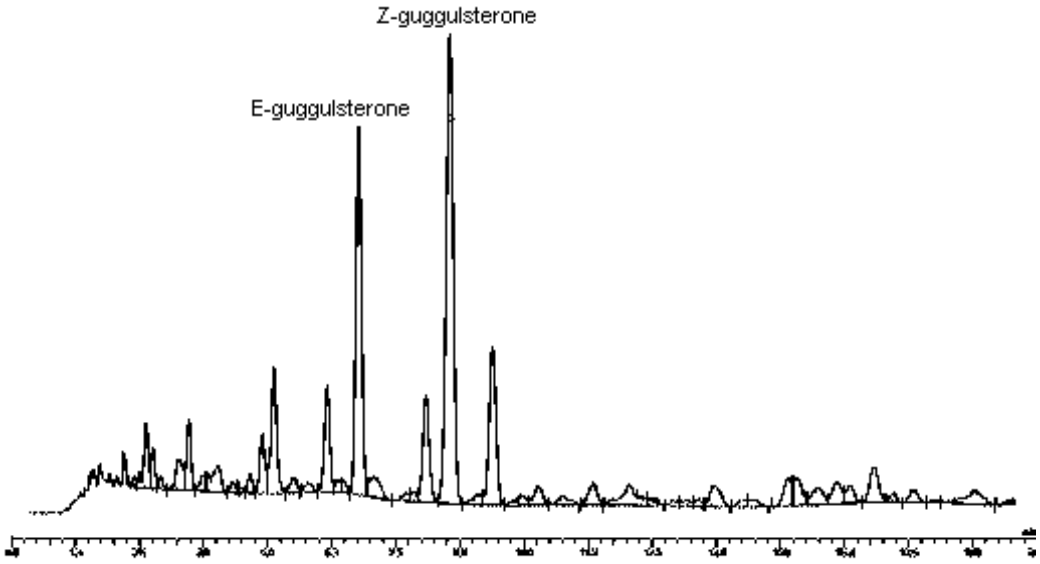
Chromatography:

Column: Phenomenex Luna C18 (2), 5 µm, 4.6 × 150 mm.
Mobile phase: Solvent A = water–acetonitrile–85% phosphoric acid (50:50:0.1), solvent B = water–acetonitrile–85% phosphoric acid (25:75:0.1).
Gradient:

Time (minutes)	%A	%B
0	100	0
20	0	100

Flow rate: 1.5 mL/minute
Injection volume: 20 µL
Detection wavelength: 241 nm
Column temperature: 25°C

Representative HPLC Chromatogram of Guggul Run by Method 3.



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