Review on biological activity and determination of E&Z-Guggulsterones concentration by HPLC & HPTLC Methods

Pragnesh N Dave¹*, Lakha V Chopda²

1. Department of Chemistry, Krantiguru shyamji Krishna Verma Kachchh University, Bhuj, Gujarat, India.
   [E-mail: pragneshdave@gmail.com; Tel: +91-2832-235022]
2. Chemical Engineering Department, Government Engineering College, Bhuj, Gujarat, India.

The resinous exudates of guggul known as gum-resin have important therapeutic properties reported in ancient Indian Ayurveda system. The guggul gum-resin used for the treatment of different diseases such as obesity, liver disorder, ulcers, inflammatory, cancer. An ethyl acetate extract of gum-resin known as 'guggulipid' contains hypolipidemic agents two ketosteroids E&Z-guggulsterones. Guggulsterones exhibited different biological activities such as anticholesterol, anti-diabetic, anti-inflammatory, anticancer and hepatoprotective. HPLC and HPTLC method effectively separate both isomers and method validated to determine the concentration of guggulsterones. Result showed that that ratio of guggulsterones vary with different geographical area and different climate condition. and also it was also different from claimed in the marketed products. In this review covers literature on HPLC and HPTLC method of separation and estimation of guggulsterones.

**Keyword:** Kutch, Guggul, E&Z guggulsterones, Biological activities, HPLC, HPTLC.

**Abbreviations:** COX, cyclooxygenase; GCSF, granulocytecolony–stimulating factor; GFAP, glial fibrillary acidic protein; HCC, hepatocellular carcinoma cells; HMEEC, human middle earepithelial cells; HNSCC, Head and neck squamous cell carcinoma; HPLC, high–performance liquid chromatography; HPTLC, high–performance thin layer chromatography; HUVEC, human umbilical vein endothelial cells; IAP, inhibitor of apoptosis; IL, interleukin; iNOS, inducible nitric oxide synthase; IRF, interferon-regulatory factor; IκBα, inhibitory subunit of NF-κB; JNK, c-Jun NH2-terminal kinase; LC-MS, liquid chromatography–mass spectroscopy; LPS, lipopolysaccharide; MAPK, mitogen-activatedprotein kinase; MMP, matrix metalloproteinase; PGE, prostaglandin E; PKC, Protein kinase C; RANKL, Receptor Activator of NF-kB ligand; NF-kB, Nuclear factor-kB; NO, nitric oxide; Rt, retention factor; ROS, reactive oxygen species; Rf, retention time; STAT, Signal transducer and activator of transcription; TLR, Toll-like receptors; TPA, 2-O-tetradecanoylphorbol-13-acetate; TNF, tumor necrosis factor; SREBP, Suppressing sterol regulatory element-binding protein; VEGF, vascularendothelial growth factor.

1. Introduction

The oleo gum-resin, exudates of the Commiphora wightii (Syn.Commiphora mukul) or guggul known as ‘myrth’ is used traditional medicine of ancient India Ayurveda system for treatment of different diseases such as obesity, inflammatory, coronary artery, gynecological, tumors, liver disorder etc. In Indian it is known by various name such as - guggul in Hindi, gukkulu and maishakshi in Tamil, guggulu in Sanskrit and Indian bdellium in English. The guggul plant is small shrub, near about three to six feet tall with spinescent branches, pale-gray bark and reddish-brown resinous, known as guggul gum-resin having important therapeutic property. It is mostly growing in tropical and sub-tropical climate and widely distributed in the tropical and sub tropical areas especially northeastern Africa, southern Arabia, India and Pakistan. In India, it is mostly found at arid regions of Rajasthan and Gujarat. In Gujarat, more than 70% of guggul plants are widely distributed in Kutch region (a
district of Gujarat state of India. Due to its slow growing nature, unscientific tapping technique of guggul gum-resin, poor seed set and poor seed germination it becomes endangered medicinal plant in Rajasthan and Gujarat where it founds in abundant \[1\]. An Ethyl acetate extract of guggul known as guggulipid, a drug developed by CDRI Lucknow contains two important bioactive compounds E&Z-guggulsterones as shown in Fig I.

![Structures of E&Z-guggulsterones](image)

**Fig I:** Structure of E&Z-guggulsterons (cis- and trans-4, 17(20)-pregnadiene-3, 16-dione)

E & Z-guggulsterones showed the hypoglycemic activity through farnesoid X receptor (FXR) antagonist which decrease the cholesterol level in the liver \[2\]. Many guggul extract or guggul capsules are available in the market - in India and outside India for treating of various diseases. Aim of this review is to provide comprehensive literature survey on separation technique such as HPLC and HPTLC of two bioactive compounds in biological fluids, natural product, marketed guggul gum extract and marketed products.

### 2. Biological activity of Guggulsterones

Wide variety of application of Guggul gum-resin such as to make lacquers, varnishes, and ointments, as a fixative in Perfumes, and also in medicine. The commiphora plant species producing gum-resin known as guggulipid. Gugulipid, a herbal extract alone and mix with other herbal extracts sold in the market for treatment of hyperlipidemia which contain two most potent bioactive ketosteroids E & Z-guggulsterones (Fig. I). Guggulsterones exhibit various activity such as hypolipidemic activity, anti-inflammatory, anticancer activity and antidiabetic activity.

#### A. Hypolipidemic activity

Guggulipid, an extract isolated from the guggul which reduced the low density cholesterol, triglyceride level and total serum cholesterol and increase high-density lipoprotein (HDL) \[3\]. First time G.V.Satyavati et al studied of guggul gum-resin of C.mukul decrease the cholesterol level in the rabbits \[4\]. Guggulsterone isolated from the C.mukul inhibited low density lipoprotein (LDL) oxidation \[5\]. Guggulipid (50 mg twice in a day for 24 weeks) decreased total cholesterol by 11.7%, LDL by 12.5%, triglyceride level by 12%, and the total cholesterol/high-density lipoprotein (HDL) ratio by 11.1% \[6\]. Gugulipid, an ethyl acetate extract isolated from C.wightii showed lipid lowering property against streptozotocin-induced memory deficits in mice \[7\]. E- and Z-guggulsterones of guggul gum are responsible for the hypolipidemic activity \[8, 9, 10\] and they act as the FXR antagonist \[2\] a nuclear hormone receptor, a bile acid receptor (BAR) antagonist \[11\] a member of the intracellular receptor superfamily and also inhibit human gene cholesterol 7 a-hydroxylase (CYP7A1) gene via activation of pregnane X receptor (PXR) \[12\].

#### B. Antidiabetic activity

Ethanol extract of C.mukul gum-resin exhibit hyperglycemic and antioxidant effect in streptozotocin induced diabetic rats \[13\]. Guggulipid containing E & Z –guggulsterons inhibit NF-κB activation also protect pancreatic β cells from cytokines which may be responsible for damage of β cell \[14\]. Guggulsterons (E & Z-guggulsterones) isolated from gum-resin of C.mukul decreases blood glucose, plasma and insulin, and increases the glycogen content in high fat diet induced diabetic rats as well as they showed the activity against G6Pase, an important antidiabetic target \[15\]. Ethanol extract of gum-resin of C.mukul increase the lipid peroxidation (LPO), Protein oxidation (PO) and enzyme activity in the diabetic rats \[16\].

#### C. Anti-inflammatory activity

Activation of NF-κB pathway leads to inflammation. It activates by LPS and cytokines. The guggul gum- resin of C.mukul and C.wightii show the potent anti-inflammatory activity. Ethylacetate extract of gum inhibit the MAPK through down regulation of TNF-α, IL-1β.
and IL-2 [17]. The methanol and EtOAc fraction of C. wightii inhibit the NO formation in LPS-activated murine macrophages with IC\textsubscript{50} values of 16.4 and 12.8 mg/ml, respectively while E&Z-Guggulsterones [18] (IC\textsubscript{50} values 3.3 & 1.1 μM respectively) found more potent compare to curcumin (IC\textsubscript{50} value 12.3 μM). The anti-inflammatory mechanism of MeOH extract by down regulation of iNOS and COX-2 gene expression [19]. Guggulipid prevented the production of NO, ROS generation and down regulation expressions of COX-2, GFAP and TNF-α [20] in rat astrocytoma cell line. E&Z-Guggulsterones inhibit the activation of NF-kB pathway [21, 14]. E-guggulsterone inhibits the COX-1 and COX-2 67% and 54% [22]. E&Z-Guggulsterones also decreases the level of inflammatory mediator such as MMP-2, NO and PGE2, and prevents the expression of inflammation related protein in eye tissues [23]. Guggulsterones [24] inhibit the TNF-α and COX-2 and IκB-α degradation and also inhibit activation of NF-kB in LPS induced in HMEEC. E-guggulsterone protects mice against the development of sign and symptoms of colon inflammation while Z-guggulsterone didn’t show any activity and also suppress the interleukin-2 and -4 and interferon-g as well as T cell proliferation [25]. Guggulsterones prevented IL-1β inflammatory mediator in the fibroblast-like synoviocytes through mechanism of inhibition of NF-kB [26]. Z-guggulsterone suppresses the activation of transcription factor IRF3 of TLR3 and TLR4 [27].

D. Anticancer activity
Guggulsterone suppresses RANKL and tumor cell - induced osteoclastogenesis via inhibition of the activation of NF-kB pathway [28]. Z-guggulsterone reduced the tumor incidence, lower tumor body burden and delayed tumor appearance in TPA induced SENCAR mouse skin tumorigenesis model through inhibition of phosphorylation MAPKs, activation of NF-kB pathway [29]. Z-guggulsterone inhibits the activation of NF-kB and STAT-3 in smokeless tobacco and nicotine-induced HNSCC [30]. Z-guggulsterone suppress the growth of HNSCC mediated through inhibition of signal transducer and activator of transcription-3 and also decrease the growth of tumor in a xenograft model by using treatment of Guggulipid [31]. E & Z-guggulsterones inhibited the proliferation of wide variety of human tumor cells such as leukemia, head and neck carcinoma, multiple myeloma, lung carcinoma, melanoma, breast carcinoma, and ovarian carcinoma and they also showed the activity against the drug-resistant cancer cells gleevac-resistant leukemia, dexamethasone-resistant multiple myeloma, and doxorubicin-resistant breast cancer cells and mechanism of inhibition by the proliferation of cells through down regulation of DNA synthesis, producing cell cycle arrest in S-phase as well as they induce apoptosis through the activation of JNK, suppression of Akt, and down regulation of antiapoptotic protein expression [32]. Guggulsterone significantly increases apoptosis in HT-29 cells by activating caspases-3 and -8 and it also decreases cIAP-1, cIAP-2, and Bcl-2 levels and increases the levels of truncated Bid, Fas, p-JNK, and p-c-Jun as well as inhibited tumor growth in murine colorectal cancer xenografts [33]. Z-guggulsterone inhibits the growth PC-3 and LNCaP cells by inducing apoptosis and it was mediated by Bax and Bak [34], reactive oxygen intermediate dependent activation of JNK and inhibition of androgen receptor [35]. Xio et al study Z-guggulsterone in vitro and in vivo on HUVEC cells, and migration in HUVEC and DU 145 cells, result showed that it inhibited the capillary-like tube formation through inhibition of angiogenesis by suppressing of secretion of proangiogenic growth factors (VEGF and GCSF), down-regulation of VEGF receptor 2 (VEGF-R2) protein level, and inactivation of Akt and decrease the tumor in tumor burden ,microvessel area and VEGF-R2 protein expression in male nude respectively [36]. Z-guggulsterone inhibits [37] both constitutive and IL-6 induced Signal STAT-3 in HMM cells by activation of protein tyrosine kinase phosphate JAK2 and c-Src. Z-guggulsterone prevents the migration of sodiumdeoxycholate (DC) released osteoblast-like MG 63 cells and human bone tissue and induced the apoptosis in breast cancer cells [38]. Guggulsterone sensitizes HCC trail -
induced apoptosis through the induction of CHOP-dependent death receptor 5 and ROS-dependent ER-stress. Z-Guggulsterone inhibited P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) in multidrug-resistant human cancer cell lines.

E. Miscellaneous activities
Guggulsterone prevents Liver X receptor-α (LXR-α) mediated SREBP-1c dependent hepatic steatosis through PKC dependent pathway and used for treatment of nonalcoholic fatty liver disease. Tripathi et al reported that Z-guggulsterone isolated from the resin of C. mukul increased the iodine-uptake by thyroid, and enhanced the thyroid peroxidase and protease activity in vivo.

3. Isolation of Guggulsterons from oleo-gum resin
Gum-resin of guggul is the complex mixture of steroids, terpenoids, flavanoids, oils and minerals. Extract with EtOAc yield soluble fraction and insoluble fraction. Soluble fraction consists of 45% gum-resin while 55% insoluble fraction contains carbohydrate, there is no any therapeutic property reported. Further soluble fraction divided into acid, base and neutral fraction. Neutral fraction showed the hypoglycemic activity and it is divided into ketonic and non ketonic. The ketonic fraction contains E & Z-guggulsterones. Ethyl extract of guggul known as guggulipid, a drug developed by CDRI Lucknow mostly used for treatment of obesity. Details extraction and separation process was developed by sukh dev et al.

A. HPLC methodology for determination of concentration of E&Z-guggulsterons
To the best of our literature survey, scores of papers of guggulsterons on different biological activities and modern target reported during 1995 onwards. Before 1990, few HPLC techniques on separation of guggulsterons from biology fluids and marketed products were reported. Separation technique of guggulsterons predominant after 1990 due to their highly medicinal properties. This review summarizes HPLC and HPTLC technique of both isomers.

B. Determination of Z-guggulsterone in serum
High performance chromatography liquid chromatography assay developed and validated method for determination of the concentration of 2 in the spiked human serum. Hexane used for the extraction of 2 from serum and C18 reverse phase column for the separation with acetonitrile-H2O as mobile phase and photodiode detector. Endogenous impurities added into the column. Serum standard containing 100 ng/ml of 2 (D). Serum sample from a rat treated with a single 50 mg/kg oral dose of 2. Rf of 2 was 6.2 ± 0.2 min. Endogenous didn’t interfere, maybe they eluted before or after 2. The quantitation limit of 2 in serum was 10 mg/ml after 2-fold concentration using 0.5 ml of serum. Peak purity almost greater than 90% showed that didn’t interfere with endogenous impurities. Extraction efficiency of 2 range of concentration 10,100 and 500 ng/ml 93.21%, 91.66% and 101.02% and Coefficient variations 3.97%, 6.70% and 5.33% respectively. This method require less amount of quantity of 2 compare to previously published method. This is first method of determination of E & Z-guggulsterones in the spiked serum and 50 mg/kg dosed into the rat. C18 used as column with methanol, acetonitrile, buffer and tetrahydrofuran as a mobile phase and monitor of using photodiode array detector at 248 nm. Serum sample of rat at 24 h (2 of 24.61 ng/ml and 1 of 28.44 ng/ml) post-oral dose of 2 of 50 mg/kg). Rf of 1 & 2 are 5.5±0.2 and 7.2±0.2 respectively. Samples of each concentration run per day. Coefficients of variation less than 3 and 4% of 1 & 2 respectively within day while less than 3% in variation of coefficients in five different days of each concentration day to day analysis of 1 & 2. Recovery of E & Z-guggulsterones are greater than 90% from serum each concentration. HPLC profile of synthesized E-guggulsterone reported by Gioiello et al normal Phase HPLC method use for quantification of guggulsterone. Chiralpak IB column (250×4.6 mm I.D) made of cellulose tris (3, 5-dimethylphenylcarbamate) immobilized

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onto silica gel act as stationary phase. Mobile phase consist of hexane/chloroform/IPA-90/8/2 (v,v,v) (system–1) and [n-hexane/acetone-85/15 (v,v)] (system–2). Purity of Z & E-guggulsterones 98.8%, 98.1% (System-1) and 98.2% & 97.5% (system-2) respectively. Retention time of E & Z-guggulsterones 4.23, 2.62 min (System-1) while 2.27 and 1.53 min (system-2) respectively. Separation & Rf of E & Z-guggulsterones 1.48, 1.61 & 7.98 10.79 respectively.

C. Quantitative determination of guggulsterones in Commiphora mukul resin and its commercial products

Two HPLC methods were used for the quality identification and quantitative estimation of guggulsterones. Method 1 was used for the qualitative identification of compounds in the guggul gum-resin in the commercial products and second method developed for quantitative estimate of E & Z-guggulsterones. In both procedure Chromatography analysis was carried out on reversed-phase C$_{18}$ column using an acetonitrile–water as mobile phase using two different instruments Perkin-Elmer Integral 4000 equipped with PDA detector and Waters Alliance (Waters, Milford, MA, USA.) also equipped with PDA detector [48]. Retention time of E & Z-guggulsterones are 27.4 min and 32.8 min of while 30.6 min for unknown compound of C.mukul resin using method 1. Method II provides better separation. LC-MS result showed the peak of Z-guggulsterone and E-guggulsterone at 313 [M+H]$^+$ . The method validated range of 25–130 µg/ml and 15–85 µg/ml of Z-guggulsterone and E-guggulsterone respectively. Recovery near about 99.5% and precision 62% S.D. where as standard curve correlation coefficients 0.992 Method 2 used for the estimation of quantity of guggulsterones of six commercial products. The ratio of guggulsterones of resin extracts and formulated products are same from the result of HPLC. Concentration of guggulsterones were significantly different of the claimed. Another HPLC methodology developed by Gottumukkala et al for determination of E & Z-guggulsterons in commercial available guggul formulation [49]. Experiment carried out on reverse phase C$_{18}$ column using 0.1% phosphoric acid in H$_2$O and acetonitrile (45:55) used as mobile phase and detection at 240 nm about 40 min.Rf of E & Z-guggulsterones were 18.0±0.45 and 24±0.5 min respectively. Linearity of calibration curve for E&Z-guggulsterones 0.1 and 5 µg range of concentration. Correlation Coefficient of E & Z-guggulsterones are 0.9998 and 0.9989 respectively. Recoveries of both isomers are nearly about 99%. The efficiency of column for both isomers 16,630 and 18,450 respectively where as tailing factor is < 1.1 Determination of concentration of both isomers in seven commercial guggul products by running six times of each samples of standard concentration. Quantity of E & Z-guggulsterons have been found in between 0.0022 to 1.2851 mg/capsule. It was found that there is variation in concentration of guggulsterones compare to claim in the label. Vineet soni [50] et al. evaluated concentration guggulsterones of different part of Rajasthan, India. There is great variations found in the amount of guggulsterones on seasonal and geographically. HPLC analysis results show that central, northen and western part of the Rajasthan produced quantity amount of guggulsterons ((1.87–2.76%) compare to southern part (0.61–1.19%). The highest concentration of both isomers were found from plants collected from rocky arid tracks of Mangliawas village, Ajmer (26.25 N, 74.51 E) whereas lowest concentration found from the plant collected from Pratapgarh (24.03 N, 74.78 E). Another observation also reported that ratio of guggulsterones also depends on annual rain fall. Higher concentration of guggulsterones (more than 1.87%) observed rain fall in between 15 to 55 cm and lowest concentration (less than 1.63%) found highest rain fall (greater than 60 cm). High concentration of guggulsterones exhibited during May to July where al lowest concentration found in the August to October. The highest and lowest guggulsterons ratio found in month of May and December respectively. Summer is the best season for collecting oleo gum-resin compare to other season. Ratio of Z-guggulsterone higher than E-guggulsterone in all samples.
D. Estimation of Guggulsterones by HPTLC

Himani Agrawal et al. developed HPTCL methodology for quantification of E & Z-guggulsterones from the herbal extract and the pharmaceutical drugs. The method developed by using stationary phase aluminum plates precoated with silica gel 60F-254 and mobile phase toluene–acetone (9:1, v/v). Rf values of E & Z-guggulsterones were 0.38±0.02 and 0.46±0.02, respectively. The linear regression analysis data for the calibration plots for E- and Z-guggulsterones \( r^2 = 0.9977\pm0.054 \) and 0.9975±0.068 respectively in the concentration range of 100–6000 ng/spot and 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. Validation parameters summarized in the Table I. Rf values found for E & Z-guggulsterones in the herbal extract and drug samples were 0.38 and 0.46 along with others compounds.

Table I: Validation parameters (Journal of Pharmaceutical and Biomedical Analysis, (2004); 36:33–41.)

| Parameter | Data on guggulsterone |  
|-----------|------------------------|---|
|           | E                      | Z  |
| Limit of quantitation | 24 ng/spot | 20 ng/spot |
| Limit of detection  | 12 ng/spot | 10 ng/spot |
| Recovery (n=6)      | 100.16±1.13 | 100.69±1.45 |
| Precision (R.S.D.%) | 1.08 | 0.52 |
| Repeatability of application (n=7) | 0.98 | 0.46 |
| Intra-day (n=6)    | 0.87 | 1.04 |
| Inter-day (n=6)    | 1.28 | 1.50 |
| Robustness         | Robust | Robust |
| Specificity        | Specific | Specific |
| Robustness         | Robust | Robust |

The total guggulsterone content was found to be 1.22% (w/w) of extract with a RSD % of 1.91 out of which Z & E contribute 75.75% and 24.25 (w/w) respectively in the herbal extract while guggulsterone 0.88% (w/w) with a RSD % of 1.67 out of which Z contributes 34.21 and 65.79% (w/w), respectively in the drugs. Low ratio of E- and Z-guggulsterones found in the extract and marketed in form of capsules. HPTLC method also used for the study of stress degradation study of guggulsterones according to International Conference on Harmonization (ICH) conditions such as acid and base hydrolysis, wet and dry heat degradation, photo degradation and oxidation. The separation carried out on stationary phase TLC aluminum plates precoated with silica gel 60F-254 as a stationary phase using mobile phase made of toluene–acetone (9:1, v/v). The Rf values of E & Z-guggulsterones 0.38±0.02 and 0.46±0.02, respectively after double development of TLC in same solvent system. Rf values of degradation products are different from the Rf values of both isomers. Musharraf et al. demonstrated HPTLC methodology for the simultaneous estimation of guggulsterones from the Commiphora mukul resin, guggulipid and its pharmaceutical formulation. HPTLC glass plates, pre-coated with silica gel 60F-254, was used as a stationary phase. Using different combination of solvent system such as methanol, toluene, ethylacetate, chloroform and acetone able to resolve both guggulsterones isomers but unable to resolution sample containing guggulsterones and 17, 20-dihydroguggulsterone. Also tried with reported solvent system toluene:acetone (9.0:1.0 v/v v/v) couldn’t able to separate Z-guggulsterone from 17, 20-dihydroguggulsterone even though double development. After using solvent system toluene–acetone (9.3:0.7 v/v) gave better resolution of both isomers and 17, 20-Dihydroguggulsterone with Rf 0.52±0.01, 0.67±0.01 and 0.60±0.01 respectively. Guggul resin and guggulipid exhibited good resolution after single running and much better in second running. Similar pattern was also observed in UV chromatogram.E&Z-guggulsterones and 17, 20-dihydroguggulsterone showed characteristic and differentiable staining color with vanillin. The maximum guggulsterone content was found in the resin samples of while the minimum in the resin samples of Baluchistan (sample codes G1–G4). In 2D-HPTLC 17, 20-dihydroguggulsterone was observed in between both isomers and similar observation obtained in HPLC i.e retention time of E & Z- guggulsterones were 12.53 and 15.05 min respectively while 17, 20-
dihydroguggulsterone was 4.99 min and it was confirmed by LC–MS analysis. Quantification of E & Z-guggulsterones by using HPTLC methodology of five commercial formulations was also developed and validated[84]. Aluminum-coated silica gel 60F254S HPTLC plates act as stationary phase while mobile phase consists of petroleum ether–ethyl acetate–formicacid, 3+1+0.1 (v/v) (20 mL) and Rf values of guggulsterones E and Z were 0.25 and 0.33, respectively. This method used for the quantification of the E and Z guggulsterones of commercial products of guggul.

4. Conclusion
The Present review discusses the biological activities of guggulsterones and their separation method of HPLC & HPTLC. Provide detailed analysis of the literature of separation technique of both isomers developed after of 2000. Gum-resin of guggul contains two important bioactive ketosteroids E & Z (1 & 2). Guggulsterones 1 & 2 displayed potent biological activities such as anticholesterol, antidibetic, anticancer and anti-inflammatory activities with modern target of drug development. Concentrations of both isomers are not same in guggul gum resin. Z-gugglsterone is more potent active compare to E-guggulsterone. It is very important to determine their concentration in marketed guggul herbal extract and capsules. After 1990 separation field of both isomers had lot attained to scientific community. HPLC method useful for determination concentration of Z- guggulsterone as well as simultaneous resolution &estimated their concentration from serum. Two HPLC method developed for qualitative &quantitative analysis of E & Z-guggulsterones. These methods are highly selective, reproducible and accurate for the estimation of guggulsterones from gum-resin of c.mukul and marketed products of guggulipid. HPLC method also used for the estimation of guggulsteones of gum-resin collected from the different part of the Rajasthan state of India where guggul plant widely distributed. Result showed that ratios of both isomers depend on the different geographical area of Rajasthan and its climate conditions. Chiral stationary phase effectively separated both isomers in synthesis method of E-gugglsterone. HPTLC method developed effectively separated both isomers using different composition of solvent system and also used for quantitative analysis of both isomers in gum-resin and herbal extract as well as also determined the concentration of both isomers in degradation products in the different condition.

5. References


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