Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 240–245

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Effective separation and analysis of *E*- and *Z*-guggulsterones in *Commiphora mukul* resin, guggulipid and their pharmaceutical product by high performance thin-layer chromatography-densitometric method

Syed Ghulam Musharraf*, Naveed Iqbal, Umair Gulzar, Azhar Ali, Muhammad Iqbal Choudhary, Atta-ur-Rahman

H.E.J. Research Institute of Chemistry, International Centers for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi 75270, Pakistan

ARTICLE INFO

Article history:
Received 8 February 2011
Received in revised form 11 April 2011
Accepted 15 May 2011
Available online 20 May 2011

Keywords:
E- and *Z*-guggulsterones
Commiphora mukul Engl.
HPTLC densitometric analysis
Guggul resin
Guggulipid

ABSTRACT

A high performance thin-layer chromatographic (HPTLC) method for the simultaneous determination of the hypolipidemic agents, *E*- and *Z*-isomers of guggulsterone in *Commiphora mukul* resin, guggulipid (ethyl acetate extract of resin), and its pharmaceutical formulation, was developed. The developed system was efficient enough to separate both isomers from their conger, 17,20-dihydroguggulsterone (**3**). HPTLC glass plates, pre-coated with silica gel 60F-254, were used as a stationary phase. The mobile phase consisted of toluene:acetone (9.3:0.7, v/v) which gave well resolved spots for *E*- and *Z*-guggulsterones (R_f: 0.52 ± 0.01, and 0.67 ± 0.01, respectively) following double development of chromatoplate with the same mobile phase under unsaturated conditions. The analyte stability towards the developed chromatographic procedure was also investigated by two-dimensional (2D) HPTLC analysis. 17,20-Dihydroguggulsterone (**3**) was identified by the electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI–QTOF–MS/MS) analysis.

1. Introduction

Commiphora mukul Engl. is a small thorny plant, four to six feet tall, mostly growing in different areas of India, Arabian Peninsula, Afghanistan and in North Africa [1]. In Pakistan, it is widely distributed in Baluchistan and some parts of Sindh. It is one of the most trusted herbs which is traditionally used as a purifying agent, a tonic that helps rejuvenate our cells/tissues, and plays an important role in balancing the nervous system, and solving reproductive system related complaints [2].

C. mukul produces a dense oily resin known as Guggulu or Guggul, widely used in traditional medicine for the treatment of various disorders, either by itself or fortified with other herbs to promote specific actions. It is also prescribed to treat a variety of disorders such as obesity, rheumatoid arthritis, arteriosclerosis, inflammation and cardiovascular diseases [3]. Recently, it has been found to exert cytotoxicity against human breast cancer and multiple myeloma [4]. It also inhibits tumor growth in xenografts murine model of colorectal cancer [5]. Pharmacological studies showed that guggulipid (ethyl acetate extract of "guggul") lowers blood lipids and possesses clinically proven hypolipidemic activity [6,7]. Moreover, guggul also helps to reduce high cholesterol level, by lowering low density lipoproteins (LDL-protein), and elevating the beneficial high density lipoproteins (HDL-protein) [8].

Several processed herbal supplements contain guggul for weight loss and fat reduction. Two ketosteroids are mainly responsible for most of the biological activities associated with guggul, particularly the hpypolipidemic effect, which have been identified as 4,17(20)-(*trans*)-pregnadiene-3,16-dione (*E*-guggulsterone **1**), and 4,17(20)-(*cis*)-pregnadiene-3,16-dione (*Z*-guggulsterone, **2**).

In Asia, particularly in the Indo-Pak sub-continent, plants have been used as sources of indigenous drugs for thousands of years. However, at the global level the traditional herbal medicines could not achieve significance in medical and scientific communities due to the lack of quantitative standardization and consistency. The quantitative analysis of medical plants by using TLC and HPTLC methods is increasingly popular as manufacturers, researchers, and drug regulatory authorities are recognizing their versatility and cost effectiveness. It has been demonstrated that these techniques can analyze even a mixture of 15 plants in a single analytical run [9].

Previously, a few HPLC methods for the quantitation of guggulsterones have been reported [10–17]. An HPTLC densitometric method was also reported by Mahadik et al. [18], for the quantitation of *E*- and *Z*-guggulsterones in herbal extract and in pharmaceutical dosage forms. However, the reported methods were unable to separate *Z*-guggulsterone from its conger,

* Corresponding author. Tel.: +92 21 34824930/99261701; fax: +92 21 34819018-9/99261713-4.
E-mail address: musharraf1977@yahoo.com (S.G. Musharraf).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.05.021

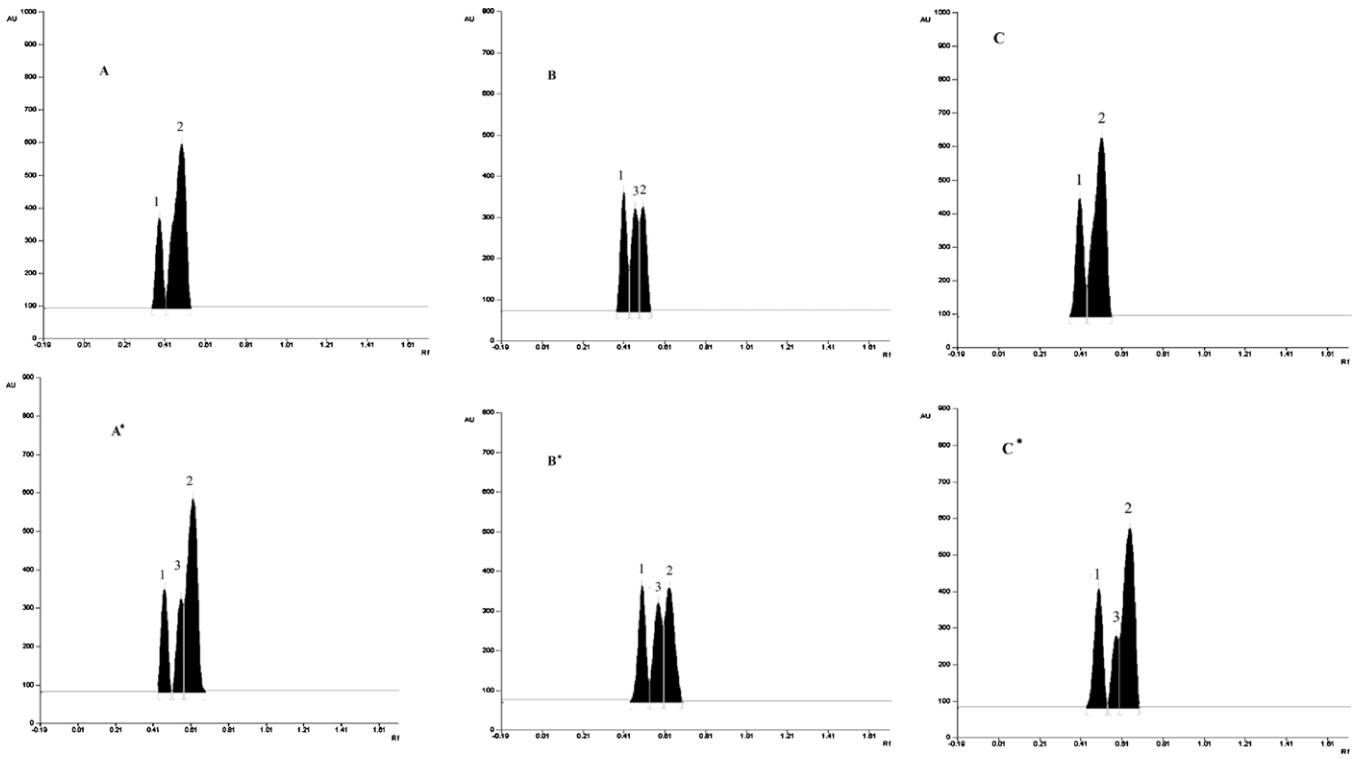

Fig. 1. Chromatogram of guggul resin, guggulipid and pharmaceutical product. Chromatogram of guggul resin after single run (A), and double run (A*). Chromatogram of guggulipid after single run (B), and double run (B*). Chromatogram of pharmaceutical product after single run (C), and double run (C*). Peak 1: *E*-guggulsterone (**1**), peak 2: *Z*-guggulsterone (**2**), peak 3: 17,20-dihydroguggulsterone (**3**).

17,20-dihydroguggulsterone (**3**), which is not a hypolipidemic agent. There was an inherent 13–30% positive error in the reported method [18] for the estimation of total *E*- and *Z*-guggulsterones content.

This paper describes an accurate and specific HPTLC-densitometric method capable of separating the hypolipidemic agents, *E*- and *Z*-isomers of guggulsterone from 17,20-dihydroguggulsterone (**3**) and provides a semi-quantitative analysis of both active ingredients. Both isomers were simultaneously analyzed in various forms of *C. mukul* resin, guggulipid, and their pharmaceutical products.

2. Materials and methods

2.1. Materials

Standard *E*- and *Z*-isomers of guggulsterone (primary grade) were purchased from ChromaDex Inc. (USA). *C. mukul* (guggul) resins samples were obtained from various parts of Baluchistan (sample codes G1–G4) and Sindh (sample code G5, G6 and G7), provided by Medics Laboratories, Karachi (Pakistan). A sample of guggulipid (G8) was also gifted by the said company. Karshina's guggul tablets (sample code G9, a product of Planet Ayurveda, India) was imported from India. HPLC grade methanol and acetonitrile were purchased from the Fisher Chemicals Ltd. (UK). Water was purified using a Millipore Milli-Q plus system (Bedford, USA).

2.2. Instrumentation and chromatographic conditions

A CAMAG system, equipped with automatic TLC sampler (LINOMAT 5), TLC scanner 3 and integrated software of WinCats (version 1.2.3) was used for the analysis. Precoated silica gel glass Plates 60F-2 54 (batch number: HX808025, 20 cm × 10 cm, E. Merck, Darmstadt, Germany) were used for the application of samples which were spotted in the form of bands of width 6 mm with a CAMAG 100 μl syringe using a CAMAG Linomat 5 automatic sampler. A constant application rate of 0.2 μl s^{-1} was employed, and the space between two bands was 8 mm. Slit dimension was set to 4.00 mm × 0.3 mm with a scanning speed of 20 mm s^{-1} where the data resolution was kept at 100 μm $step^{-1}$. Monochromator band width was set 20 nm and lowest slope base line correction was carried out where each track was scanned thrice. Plate development was carried out in twin trough chamber (CAMAG, Muntenz, Switzerland) with 15 ml mobile phase (toluene–acetone, 9.0:0.7 v/v) under unsaturated condition. Two runs were carried out with the same mobile phase to get good separation of *E*- and *Z*-isomers of guggulsterone at the room temperature of 25 ± 3 °C and the relative humidity of 42 ± 6%. The length of the chromatogram was taken 9 cm. Prior to scanning, the plates were dried using air dryer for 10 min. Video densitometry was carried out with CAMAG Reprostar 3 and scanning was performed on CAMAG TLC Scanner III at 251 nm which is operated in reflection absorbance mode by Win Cat software. Deuterium lamp with range between 190 nm and 400 nm was used as source. Evaluation of the amount of the sample was obtained using linear regression. Vanillin was used as staining reagent, and prepared by dissolving 5 g of vanillin in 250 ml of ethanol and acidified with 2 ml of c.c. sulfuric acid. The plate was immersed into the vanillin solution with the help of CAMAG immersion device for 3 s. The stained plate was heated on CAMAG hot plate at a temperature of 150 °C for 30 s. Picture of derivatized HPTLC plate was obtained using CAMAG Reprostar 3 with cabinet cover, and mounted digital camera on white R mode.

HPLC Analysis was performed on Agilent 1200 Series, Rapid Resolution Liquid Chromatography (RRLC) system. Data acquisition and integration was controlled by Agilent Technologies ChemStation software. An Agilent Zorbax XDB-C18 column (50 mm × 4.6 mm I.D., 1.8 μm) was used. The mobile phase was a

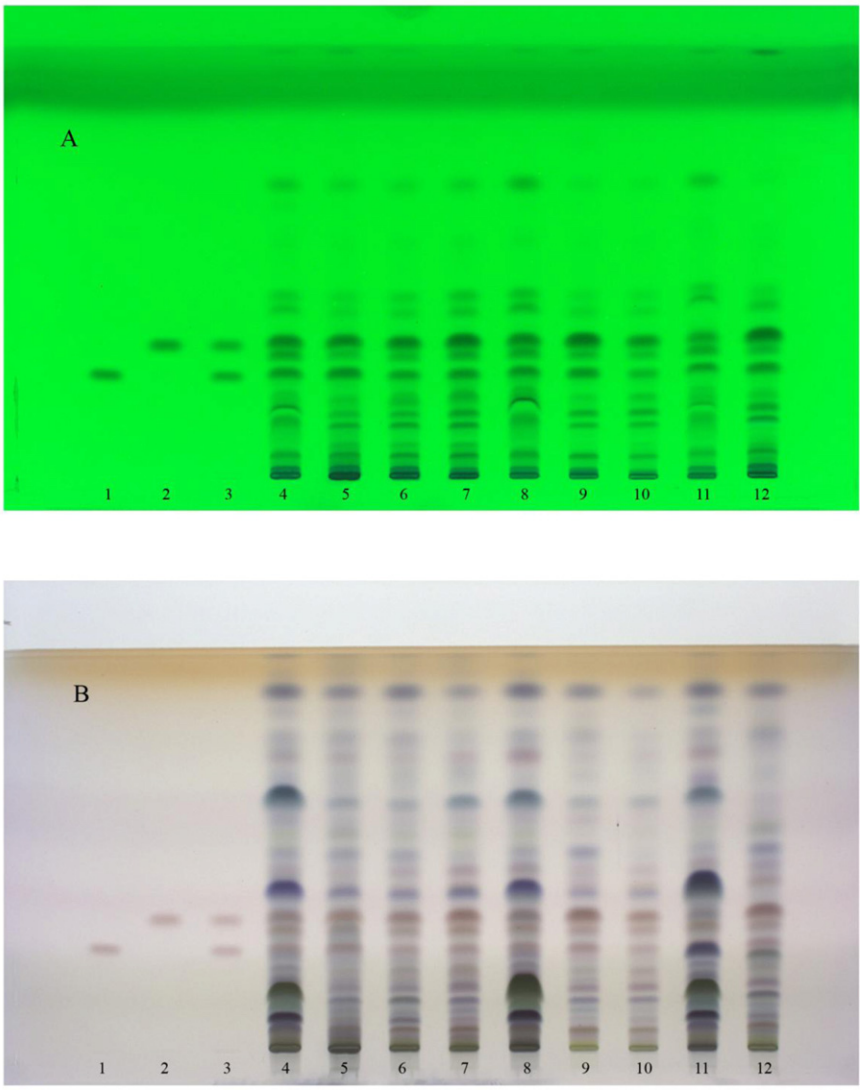

Fig. 2. HPTLC plate after double development. (A) Densitometric picture at 251 nm. (B) Densitometric picture of vanillin stained plate on white R mode. Spot 1: *E*-guggulsterone, spot 2: *Z*-guggulsterone, spot 3: *E*- and *Z*-guggulsterones, spot 4: G-1, spot 5: G-2, spot 6: G-3, spot 7: G-4, spot 8: G-5, spot 9: G-6, spot 10: G-7, spot 11: guggulipid, spot 12: marketed formulation.

binary gradient system prepared from water (eluent A), and acetonitrile (eluent B), properly filtered and degassed for 15 min in ultrasonic bath before the use. The gradient program was 20–25% B from 0 to 2 min, 25–40% B from 2 to 5 min, 40–50% B from 5 to 30 min, 5–95% B from 30 to 32 min and 95–20% B from 30 to 40 min. The flow rate was 1.0 ml min^{-1} throughout the analysis. The injection volume was 6 μl. The detection wavelength of DAD was 251 nm.

For mass spectrometric analysis, compounds were dissolved in MeOH and 0.1% HCOOH in water (9:1, v/v, 1 μg ml^{-1}), and analyzed by electrospray ionization (ESI) and collision-induced dissociation (CID) in the positive ion mode on ESI–QTOF–MS/MS instrument (QSTAR XL mass spectrometer Applied Biosystem/MDS Sciex, Darmstadt, Germany) at room temperature. High-purity nitrogen gas was used as the curtain gas and collision gas obtained from Peak Scientific Nitrogen Generator. The ESI interface conditions were as follows: ion spray capillary voltage of 5000 V, curtain gas flow rate 20 l min^{-1}, nebulizer gas flow rate 5 l min^{-1}, DP1 60 V, DP2 15 V, focusing potential of 265 V and The collision energy was swept from 15 to 25 eV for MS/MS analysis. Calibration was performed by using internal calibration process. Samples were introduced into the mass spectrometer by direct infusion using a Harvard syringe pump (Holliston, MA, USA) at a flow rate of 5 μl min^{-1}.

For the purification of compound **3**, 500 mg of guggulipid was dissolved in 3 ml of ethyl acetate, 500 μl was spotted on the HPTLC glass plates (20 cm × 10 cm) with the band width of 160 mm, and run under the developed system. A total of six plates were run. Bands between the *E*- and *Z*-guggulsterones were scratched from the HPTLC plate, the scratched silica was extracted by 100 ml ethyl acetate for 30 min at room temperature. After filtration and concentration, the afforded residue was further subjected to column chromatography with petroleum ether, and ethyl acetate gradient which yielded **3** (around 0.1 mg, with petroleum ether/ethyl acetate 82:18).

2.3. Preparation of standard and sample solutions

The stock solution of *E*- and Z-guggulsterones were prepared by accurately weighing 5 mg of *E*- and Z- isomers each, quantitatively transferred into a 10 ml volumetric flask and make up to volume with methanol. The working standard solution (50 ng μl^{-1}) was prepared by dilution of the stock solution with methanol. Guggul resin samples were powdered in liquid nitrogen with pistol

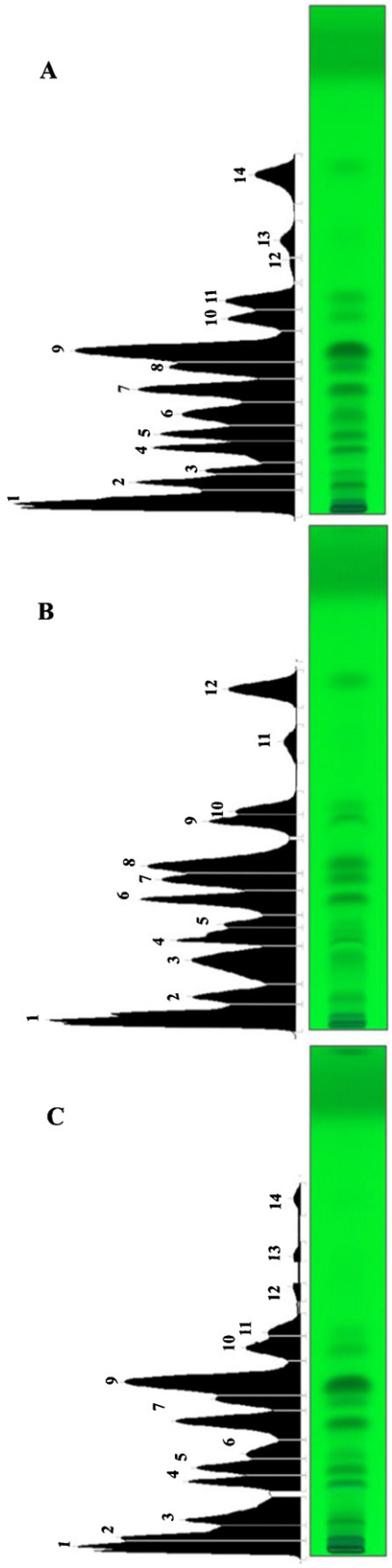

Fig. 3. UV chromatogram and HPTLC picture of (A) guggul resin (B) guggulipid (C) marketed formulation.

and mortar. 100 mg of powdered resins were accurately weighed and transferred into 10 ml volumetric flasks. Similarly, 250 mg of guggulipid extract was accurately weighed and placed in 50 ml volumetric flask. To determine the content of *E*- and *Z*-isomers of guggulsterone in the tablet, the contents of twenty capsules were weighed and finely powdered. The weight of powder equivalent to the tablet content was transferred into a 10 ml volumetric flask. Each sample was extracted with methanol solution under ultrasonic conditions for 15 min. The solution was filtered with 0.45 μm polytetrafluoroethylene (PTFE) fluoropore syringe driven filter unit (Millipore, Bedford, USA). The samples were preserved at 4 °C prior to HPTLC analysis. The samples were spotted from 10 to 20 μl.

2.4. Two-dimensional HPTLC analysis

Two-dimensional (2D) HPTLC analysis was performed by spotting 20 μl guggul sample at the lower right corner on the HPTLC plat (10 cm × 10 cm) with the band width of 0 mm which resulted the spotting in spherical shape. One-dimensional analysis was performed by placing the HPTLC plate under the developed solvent system. For 2D analysis, the developed plate was dried for 5 min, turned 90° to the right, and developed a second time in the same solvent system. After development, the plate was dried again and the picture was taken at 254 nm by CAMAG video densitometer.

3. Results and discussion

3.1. Development of the optimum mobile phase

A standard mixture of *E*- and *Z*-guggulsterones, guggul resin, guggulipid, and marketed formulation samples were spotted on HPTLC plates for the development of optimum mobile phases. Different combinations of toluene, methanol, acetone, ethyl acetate, and chloroform (toluene–acetonitrile, toluene–methanol, toluene–ethyl acetate, and toluene–chloroform) were tried as mobile phases with different ratios. Most of the combinations were able to differentiate *E*- and *Z*-guggulsterones in the standard but not in the sample due to the merging of 17,20-dihydroguggulsterone (**3**), particularly with *Z*-isomer of guggulsterone. A reported solvent system [18], toluene–acetone (9.0:1.0 v/v), under saturated conditions was also tried but it was unable to separate compound **3** from *Z*-guggulsterone (**2**), even after double development. Finally, toluene–acetone (9.3:0.7 v/v), under unsaturated conditions yielded the optimum separation with sharp, symmetrical and differentiable peaks of *E*-guggulsterone (**1**), *Z*-guggulsterone (**2**) and 17,20-dihydroguggulsterone (**3**). This separation was further optimized by two runs. The first spot at R_f 0.52 ± 0.01 (*E*-guggulsterone), and the second spot at R_f 0.67 ± 0.01 (*Z*-guggulsterone) were identified in the mixture of standard (see Supplementary material, Fig. 1). 17,20-Dihydroguggulsterone was appeared with R_f 0.60 ± 0.01, between *E*- and *Z*-isomers of guggulsterone which showed difference of 0.08 and 0.07 in R_f values from *E*- and *Z*-guggulsterone, respectively. Separation of *E*- and *Z*-guggulsterones from component **3** was also achieved in guggul resin, guggulipid, and marketed formulation samples after two runs (Fig. 1). In contrast to guggul resin and pharmaceutical products, guggulipid showed good separation of compounds **1**, **2**, and **3** even after single development of HPTLC plates while double development led to a better separation (Fig. 1B).

3.2. Analysis of E- and Z-guggulsterones in C. mukul resin, guggulipid and marketed formulation

Two peaks of *E*- and *Z*- isomers of guggulsterone at R_f: 0.52 ± 0.01 and 0.67 ± 0.01, respectively, were observed in the chromatogram along with the peak of 17,20-dihydroguggulsterone (**3**)

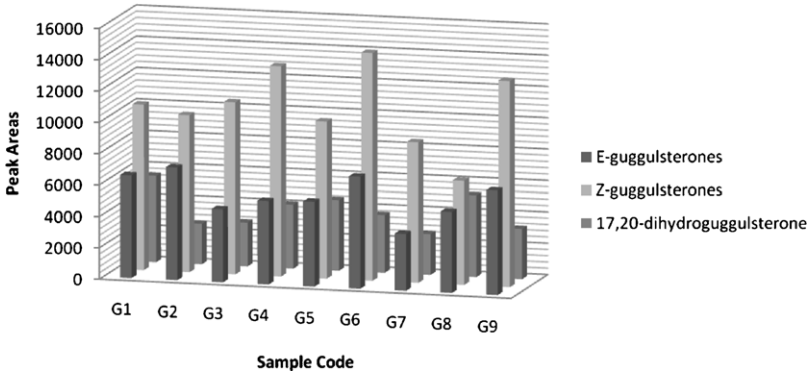

Fig. 4. Peak areas of *E*- and *Z*-guggulsterones and compound **3** in guggul resin (G1–G7) guggulipid (G-8) marketed formulation (G-9).

at R_f 0.60 ± 0.01, which was clearly separated from the *Z*-isomer of guggulsterone in all resin samples, guggulipid and marketed formulations (Fig. 2). The developed system was able to differentiate many peaks in guggul resin sample, guggulipid and marketed formulation on HPTLC plate and similar pattern was observed in UV-chromatogram (Fig. 3) while staining with vanillin showed a complex but characteristic fingerprint. Moreover, isomers **1**, and **2**, and structural analogue **3** showed characteristic and differentiable staining color with vanillin (Fig. 2B). A comparison of the peak areas of *E*- and *Z*-guggulsterones and compound **3** were plotted in Fig. 4. The maximum guggulsterone content was found in the resin samples of Sindh (sample codes G5–G7), while the minimum in the resin samples of Baluchistan (sample codes G1–G4). The variation in the samples was likely due to the different climatic conditions in which the plants grew and were harvested. Compound **3** was found to be 13–24%, 30%, and 14% in guggul resin sample, guggulipid and marketed formulation, respectively with reference to the total % peak area of *E*- and *Z*-guggulsterones.

3.3. Two-dimensional (2D)-HPTLC and HPLC analysis

The stability of the analyte on the plate, in solution, and during the chromatography was investigated by two-dimensional (2D) development of HPTLC, a reported tool for the investigation of artifacts formation during the chromatographic processing [19]. The chromatogram showed that all components detected on the line connecting the application position and the intersection of the two solvent fronts and there were no spots observed across the diagonal line, therefore no artifacts were detected and the sample is stable during the chromatographic processes. 17,20-Dihydroguggulsterone (**3**) was observed in between the *E*- and *Z*-guggulsterones (Fig. 5). Similarly, HPLC analysis of guggul resin was also conducted for the further validation of **3**. The HPLC chromatogram showed that *E*- and *Z*-guggulsterones were eluted at R_t 12.53 and 15.05, respectively, while compound **3** was eluted between the isomers, at R_t 14.99, as observed by HPTLC analysis (see Supplementary material, Fig. 2).

3.4. Identification of 17(20)-dihydroguggulsterone (3)

HPTLC Analysis of guggul samples showed that there are many UV inactive compounds, in between the UV active compounds. In such cases, the identification of unknown compounds *via* online LC–MS analysis will be ambiguous despite the excellent LC profile. Therefore, compound **3** was purified by preparative HPTLC method followed by the column chromatography. The purified compound **3** was subjected to the ESI–QTOF–MS scan (+ve mode), and showed the protonated pseudo-molecular ion peak $[M+H]^+$ at *m*/*z* 315.2309, corresponding to the formula $C_{21}H_{31}O_2$ (calc. 315.2324). Compound **3** was searched in the updated Dictionary of Natural Products (DNP, version 19.1) on the basis of their deprotonated molecular masses, and respective formula. The search was narrowed down to the plant species (*C. mukul*) and to the class of compound (guggulsterone). Two hits were observed including 16-hydroxypregna-4,17(20)-dien-3-one and 17,20-dihydroguggulsterone (pregn-4-ene-3,16-dione), which are the reduced forms of *Z*-guggulsterones and *E*- or *Z*-guggulsterones, respectively. ESI–QTOF–MS/MS analyses of *E*- and *Z*-guggulsterones were also conducted to generate the reference spectra. MS/MS spectra of compound **3** was also recorded and found to be very similar to *E*- and *Z*-isomers of guggulsterone and showed characteristics fragments at *m*/*z* 109 and 97 (see Supplementary material, Fig. 3). One of the DNP hits, 16-hydroxypregna-4,17(20)-dien-3-one possessed a hydroxyl group which shows dehydrated peak $[M+H-H_2O]^+$ under collision induced dissociation (CID) reaction, while $[M+H-H_2O]^+$ peak was absent in the MS/MS spectra of the compound **3**, even at low collision energies (10–20 eV). MS/MS spectra of 17α-methyltestosterone was also recorded, which possessed hydroxyl group on five-member ring and showed very similar fragmentation pattern as the standards, except for the appearance of a dehydration peak. Therefore, the structure of isolated compound was identified as 17,20-dihydroguggulsterone (**3**).

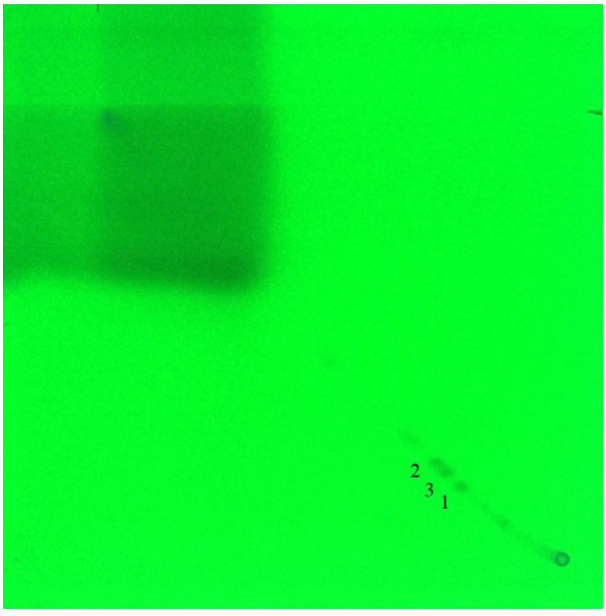

Fig. 5. 2D-HPTLC analysis of guggul resin. *E*-guggulsterone (**1**), *Z*-guggulsterone (**2**), 17,20-di hydroguggulsterone (**3**).

4. Conclusion

An improved HPTLC-densitometric method that allows separation of *E*- and *Z*-isomers of guggulsterone from 17,20-dihydroguggulsterone (**3**) and reduces the risk of 13–30% positive error in the quantitation of *E*- and *Z*-guggulsterones has been developed. Moreover, this method can be successfully applied for the simultaneous determination of the above mentioned drug components in a variety of guggul samples, that are involved in the processing and the preparation of pharmaceutical products including guggul resin (raw material), guggulipid (ethyl acetate extract of guggul resin) and the pharmaceutical samples (finished product). Hence this method can serve as a quick method for the determination of both isomers of guggulsterone and to determine the quality of the raw material by utilizing their analytical amount before harvesting. It can therefore prevent the premature and wasteful harvesting of the plant. Similarly, quick analysis of guggulipid indicates the efficiency of the extraction procedure and presence of active constituents before processing in bulk. The pharmaceutical samples analysis also validates the biomarkers components in the sample and allows standardization of the whole process starting from the raw materials to the finished products.

Acknowledgements

The authors thanks to Medics Laboratories, Karachi (Pakistan), for kindly supplying the gift of *Commiphora mukul* resins, gugulipid, and tablets samples for this study. This work was sponsored by the Pak-US Joint Academic & Research Program (grant number 1-5/ILS-US/HEC/2004).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.05.021.

References

[1] M.T. Murray, The healing power of herbs, in: The Enlightened Person's Guide To the Wonder of the Medicinal Plants, 2nd ed., Prima, 1995 (Chapter 18).
[2] R.B. Singh, M.A. Niaz, G. Ghosh, Hypolipidemic and antioxidant effects of *Commiphora mukul* as an adjunct to dietary therapy in patients with hypercholesterolemia, Cardiovasc. Drugs Ther. 8 (1994) 659–664.
[3] D. Ruitang, Therapeutic effects of guggul and its constituent guggulsterone: cardiovascular benefits, Cardiovasc. Drug Rev. 25 (2007) 375–390.
[4] H. Ichikawa, B.B. Aggarwal, Guggulsterone inhibits osteoclastogenesis induced by receptor activator of nuclear factor-Kβ ligand and by tumor cells by suppressing Nuclear factor-Kβ activation, Clin. Cancer Res. 12 (2006) 662–668.
[5] M.J. An, J.H. Cheon, S.W. Kim, E.S. Kim, T.I. Kim, W.H. Kim, Guggulsterone induces apoptosis in colon cancer cells and inhibits tumor growth in murine colorectal cancer xenografts, Cancer Lett. 279 (2009) 93–100.
[6] P.O. Szapary, M.L. Wolfe, L.T. Bloedon, A.J. Cuccharia, A.H. Dermarderosian, M.D. Cirigliane, D.J. Rader, Guggulipid for the treatment of hypercholesterolemia: a randomized controlled trial, JAMA 290 (2003) 765–772.
[7] S.P. Gaur, R.K. Garg, A.M. Kar, Gugulipid, a new hypolipidaemic agent, in patients of acute ischaemic stroke: effect on clinical outcome, platelet function and serum lipids, Asia Pacif. J. Pharm. 12 (1997) 65–69.
[8] J.P. Kotiyal, D.S. Singh, D.B. Bisht, Gum guggulu (*Commiphora mukul*) fraction 'A' in obesity. A double-blind clinical trial, J. Res. Ayurveda Siddha 6 (1985) 20–35.
[9] M. Paramasivam, A. Wasim, R. Poi, H. Banerjee, A. Bandyopadhyay, Occurrence of curcuminoids in *Curcuma longa*: a quality standardization by HPTLC, Bangladesh J. Pharmacol. 3 (2008) 55–58.
[10] T.H. Sane, V.R. Bhate, V.B. Malkar, U.R. Nayak, R.M. Kothurkar, Reversed-phase high-performance liquid chromatography determination of guggulsterones from pharmaceutical preparation and from blood plasma, Indian Drugs 28 (1990) 86–89.
[11] S.K. Singh, N. Verma, R.C. Gupta, Sensitive high-performance liquid chromatographic assay method for the determination of guggulsterone in serum, J. Chromatogr. B 670 (1995) 173–176.
[12] S.K. Roy, R. Pal, J.P.S. Sarin, TLC separation and quantitative determination of guggulsterones, Indian J. Pharm. 51 (1989) 251–253.
[13] N. Verma, S.K. Singh, R.C. Gupta, Simultaneous determination of the stereoisomers of guggulsterone in serum by high-performance liquid chromatography, J. Chromatogr. B 708 (1998) 243–248.
[14] B. Mesrob, C. Nesbitt, R. Misra, R.C. Pandey, High-performance liquid chromatographic method for fingerprinting and quantitative determination of *E*- and *Z*-guggulsterones in *Commiphora mukul* resin and its products, J. Chromatogr. B 720 (1998) 189–196.
[15] M. Nagrajan, T.W. Waszkuc, J. Sun, Simultaneous determination of *E*- and *Z*-guggulsterones in dietary supplements containing *Commiphora mukul* extract (guggulipid) by liquid chromatography, J. AOAC Int. 1 (2001) 24–28.
[16] A.L. Gottumukkala, T. Ramakrishna, S.K. Babu, G.V. Subbaraju, Determination of *E* and *Z*-guggulsterones in guggul formulation by high performance liquid chromatography, Asian J. Chem. 17 (2005) 1789–1793.
[17] I. Haque, M. Kumar, K. Mukhopadhyay, A rapid and simple UPLC–MS–MS based simultaneous determination of the medicinally important *E*- and *Z*-guggulsterone from oleogum-resins of naturally occurring *Commiphora wightii* plants, Chromatographia 70 (2009) 1613–1619.
[18] H. Agrawal, N. Kaul, A.R. Paradkar, K.R. Mahadik, HPTLC method for guggulsterone. I. Quantitative determination of E- and Z-guggulsterone in herbal extract and pharmaceutical dosage form, J. Pharm. Biomed. Anal. 36 (2004) 33–41.
[19] E. Reich, A. Schibli, A. DeBatt, Validation of high-performance thin-layer chromatographic methods for the identification of botanical in cGMP environment, J. AOAC Int. 91 (2008) 13–20.