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Determination of bioactive marker glycyrrhizin in *Glycyrrhiza glabra* root and commercial formulation by validated HPTLC–densitometric method

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PEER REVIEW

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Comments

This is a valuable research work in which authors have developed HPTLC validated method for the determination of glycyrrhizin in crude drug as well as formulation. Statically data proved that proposed method can be used in quality control against adulterant. Since, the given HPTLC method is simple, precise and specific, hence it can be employed further for the study of degradation kinetics of glycyrrhizin and determination of glycyrrhizin in plasma and other biological fluids.

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ABSTRACT

Objective: To develop a simple sensitive high performance thin layer chromatography (HPTLC)–densitometric method for quantification of glycyrrhizin in the *Glycyrrhiza glabra* root methanol extract and licorice root capsule methanol extract.

Methods: Chromatography was performed on glass–backed silica gel 60 F254 HPTLC plates with the green solvents ethyl acetate: glacial acetic acid: methanol: water in proportion of 6:2:1:0.5, v/v/v/v as mobile phase. Scanning and quantification of developed plate was done densitometrically at 254 nm. The method was validated for detection and quantification limits, precision, recovery and robustness according to International Conference on Harmonization guidelines.

Results: The system gave compact spot for glycyrrhizin ($R_f=0.280\pm0.001$). The regression curve of standard was found to be $Y=4.213x+22.078$. The limit of detection (15.7 ng per band), limit of quantification (47.1 ng per band), recovery (99.4%–99.8%) and precision ($\leq 1.62\%$ and $\leq 1.84\%$; intraday and interday) were satisfactory for glycyrrhizin. Linearity range for glycyrrhizin was 20–200 ng ($r^2=0.996$). The content of glycyrrhizin was estimated as 5.9% and 11.2% w/w in glycyrrhizin in the *Glycyrrhiza glabra* root methanol extract and licorice root capsule methanol extract, respectively.

Conclusions: This estimation technique is very much useful for the estimation of glycyrrhizin present in various formulations as well as for quality control of crude drugs containing glycyrrhizin.

KEYWORDS

Licorice root capsule, *Glycyrrhiza* root, Validation, HPTLC, Glycyrrhizin**1. Introduction**

In recent years, high performance thin layer chromatography (HPTLC) has become a conventional analytical approach for the standardization of herbal drugs due to its benefit of less expensive and high efficiency[1,2]. HPTLC is mainly applied for the identification, qualitative and quantitative estimation

of herbal and animal extracts, fermentation mixtures, drugs and excipients and formulated herbal products[3].

In HPTLC, many samples may be run simultaneously with less expense of mobile phase which leads to time and cost saving per sample analysis. These are the main advantages of HPTLC over other conventional analytical methods[4–6].

Glycyrrhiza glabra (Licorice)–Leguminosae (G.

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glabra) was used traditionally as an herbal medicine for the treatment of hepatic disorders, infections and inflammatory diseases[7]. Glycyrrhizin is a triterpenoid saponin isolated from the roots of *G. glabra*[8]. The pharmacological activity of glycyrrhizin has been widely explored and it is found to inhibit tumor growth and have antioxidant activity[9,10]. Licorice root capsule (LRC) is a premium herbal dietary supplement. The major constituent of LRC is glycyrrhizin and it is widely used medicine in China. Indication of LRC is in digestion problem, relieving stomach cramps and immune booster. Literature survey revealed that some analytical methods such as high performance liquid chromatography (HPLC), HPTLC and gas chromatography mass spectrometry (GCMS) have been reported for the quantification of glycyrrhizin in its plant extracts, commercial formulations or biological fluids[11–17], but a complete validated analytical method has not been reported yet for quantification of glycyrrhizin in the crude *Glycyrrhiza* root and LRC collected from the market of Kingdom of Saudi Arabia. Hence, this experiment was designed to develop and validate a cost effective HPTLC method for the estimation of the bioactive marker glycyrrhizin in *G. glabra* root methanol extracts (GGRMe) and LRC methanol extracts (LRCMe) marketed in Saudi Arabia.

2. Materials and methods

2.1. Materials

The commercial formulation “LRCs” and *G. glabra* root (crude drug) were collected from local market of Riyadh, Saudi Arabia.

2.2. Apparatus and reagents

Glycyrrhizin (standard) was obtained from Sigma Aldrich. Ethyl acetate, glacial acetic acid and MeOH were used as solvents. Solvents and reagents were of analytical grade and were purchased from WINLAB and BDH (UK). Glass-backed silica gel 60F254 HPTLC plates from E. Merck (Darmstadt, Germany) (10 cm×20 cm) were used. Methanol solution of standard (0.2 mg/mL) and *G. glabra* root and LRC extracts were applied bandwise to HPTLC plates by automatic TLC sampler (Linomat IV) (Camag, Muttenz, Switzerland) and then it was developed in automatic development chamber (ADC 2) (Camag, Muttenz, Switzerland). TLC plates were then derivatized and were documented by Camag TLC Reprostar 3 and scanned by CATS 4 (Camag, Muttenz, Switzerland).

2.3. TLC instrumentation and conditions

Chromatographic analysis was done on 10 cm×20 cm HPTLC plates[18]. Samples were applied as bands 6 mm wide and 8 mm apart by Linomat IV sample applicator. The application rate of sample on plate was 160 nL/s. The plates were developed in previously saturated 20 cm×10 cm twin-trough glass chamber [at room temperature (25± 2) °C and relative humidity (60±5)%], using green solvents ethyl acetate: glacial acetic acid: MeOH: H₂O (4:3:1:0.5, v/v/v/v) as mobile phase. The plates were dried at room temperature and then heated to identify compact bands. Quantitative analysis was performed at wavelength 254 nm in reflectance mode with CATS 4 operated by WinCATS software (Version 1.2.0).

2.4. Extraction conditions

2.4.1. Preparation of GGRMe

Root powder of *G. glabra* weighing 50 g was extracted with methanol for 1 h. The extract was filtered, concentrated and finally vacuum dried. The percentage yield of GGRMe was found to be 7.5% w/w.

2.4.2. Preparation of LRCMe

Three LRCs weighing 29.0 g (weight of one capsule is 1.45 g) were extracted with methanol for 1 h. The extract was filtered, concentrated and finally vacuum dried. The percentage yield of LRCMe was found to be 5.4% w/w.

The procedure followed for sample preparation and preparation of marker compound solution was optimized for high quality fingerprinting. Marker compound was found to be soluble in methanol hence methanol was used for the extraction of samples.

2.5. Preparation of standard

Stock solution of standard (glycyrrhizin) (10 mg/mL) was prepared in methanol. It was then diluted in the concentration of 20 ng/μL by appropriate dilution of standard solution. For calibration, glycyrrhizin standard solution (1–10 μL) was applied to a HPTLC plate to furnish amounts in the range 20–200 ng per band.

2.6. Method validation

Method validation was carried out as per International Conference on Harmonization guidelines for linearity range, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ) and recovery.

Precision (inter and intraday) and accuracy of the assay were evaluated by performing replicate analyses ($n=6$)

of glycyrrhizin at low, medium and high quality control levels of 150, 300 and 600 ng/band, respectively. Interday precision and accuracy were determined by repeating the intraday assay on three different days. Precision was expressed as percent of coefficient variation (%CV) of measured concentrations for each calibration level whereas accuracy was expressed as percentage recovery.

Robustness was studied in triplicate at 300 ng/band by making small changes to mobile phase composition, mobile phase volume, and duration of mobile phase saturation and activation of TLC plates. The results were examined in terms of % relative standard deviation [RSD (%)] and SE of peak areas. Mobile phases prepared from ethyl acetate: glacial acetic acid: MeOH: H₂O (4:3:1:0.5, v/v/v/v) in different proportions (4:2:2:0.5, v/v/v/v; 4:2:1:1, v/v/v/v; 4:3:2:1, v/v/v/v) were used for chromatography. Mobile phase volume and duration of saturation investigated were (20±2) mL (18, 20, and 22 mL) and (20±10) min (10, 20, and 30 min), respectively. The plates were activated at (60±5) °C for 2, 5, and 7 min before chromatography.

The LOD and the LOQ were calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approaching the LOD according to the formulas: $LOD=3.3\times SD/S$ and $LOQ=10\times SD/S$. The standard deviation of the response was determined based on the standard deviation of y-intercepts of regression lines.

Recovery was studied by applying the method to drug samples to which known amounts of marker corresponding to 50%, 100%, and 150% of the glycyrrhizin had been added. Each level was analyzed in triplicates. This was to check the recovery of glycyrrhizin at different levels in the extracts. Recovery of the markers at different levels in the samples was determined.

3. Results

3.1. Method development

Chromatogram was developed for glycyrrhizin under chamber saturation conditions using green solvents ethyl acetate: glacial acetic acid: MeOH: H₂O (6:2:2:1, v/v/v/v) as mobile phase or solvent system (Figure 1). The optimized saturation time was found to be 20 min. The densitometric analysis was performed at 254 nm in the reflectance mode. Compact, sharp, symmetrical and high resolution bands of glycyrrhizin were obtained at R_f 0.280±0.001 (Figure 1 and 2). The same mobile phase was also employed for the separation of GGRMe and LRCMe (Figures 3 and 4, respectively). According to literature survey, there is no HPTLC densitometric method reported

to quantify glycyrrhizin in GGRMe and LRCMe collected from Kingdom of Saudi Arabia. Therefore, attempts were made to develop and validate a cost effective, simple hyphenated HPTLC technique to quantify glycyrrhizin in crude drug and herbal formulation. Glycyrrhizin was well resolved at R_f 0.280±0.001 from GGRMe and LRCMe sample in the solvent system as is the case with standard. The developed method was found to be quite selective with good baseline resolution. The identity of the bands of compounds in the sample extracts were confirmed by overlaying their absorption spectra with those of the standards at 254 nm (Figure 5).

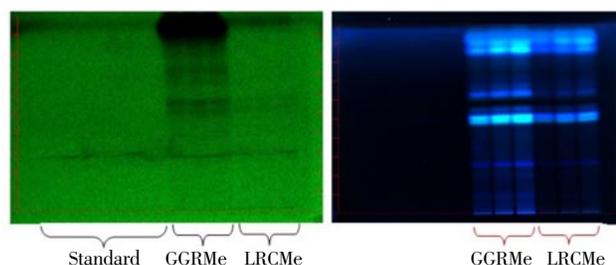


Figure 1. Picture of developed HPTLC plate at 254 nm and 366 nm.

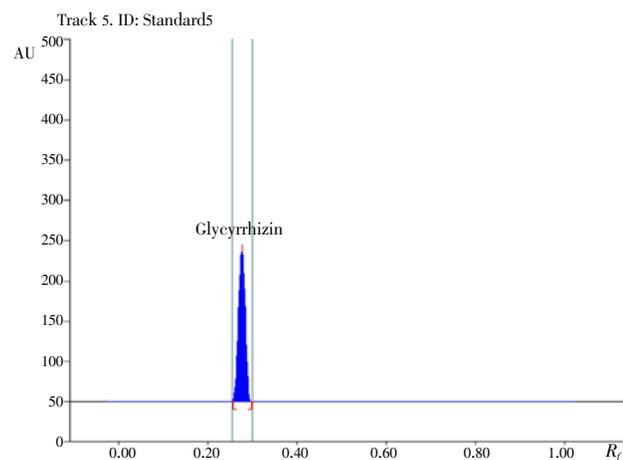


Figure 2. HPTLC chromatogram of standard glycyrrhizin scanned at 254 nm. Mobile phase–Ethyl acetate: glacial acetic acid: methanol: water (4:3:1:0.5, v/v/v/v).

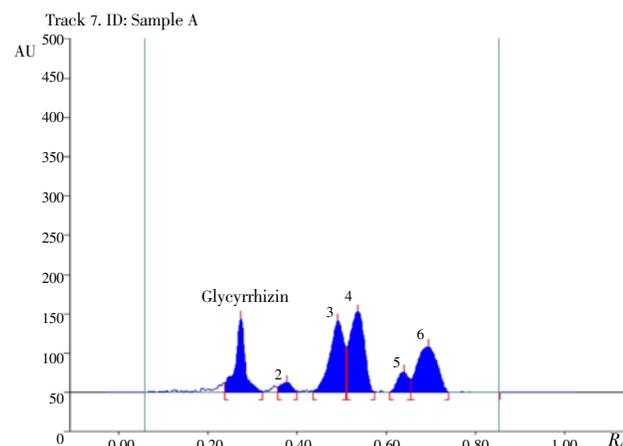


Figure 3. HPTLC chromatogram of GGRMe scanned at 254 nm. Mobile phase–Ethyl acetate: glacial acetic acid: methanol: water (4:3:1:0.5, v/v/v/v).

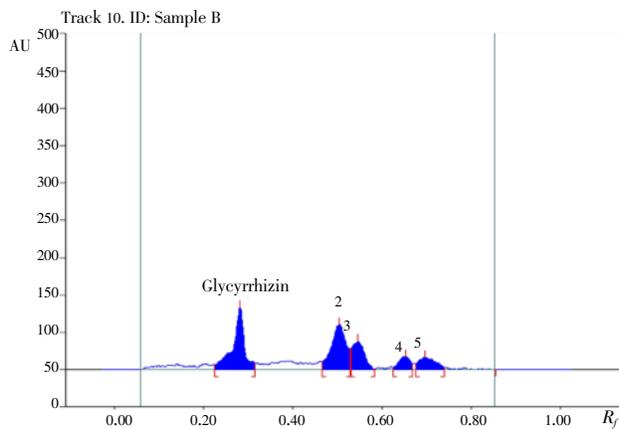


Figure 4. HPTLC chromatogram of LRCMe scanned at 254 nm.

Mobile phase–Ethyl acetate: glacial acetic acid: methanol: water (4:3:1:0.5, v/v/v/v).

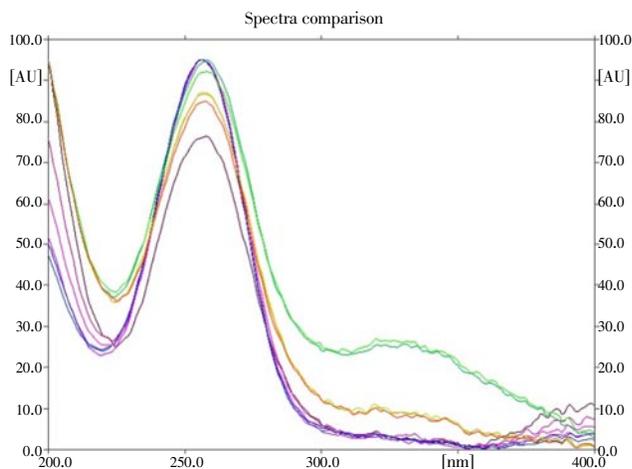


Figure 5. Spectral comparison of glycyrrhizin and different samples.

3.2. Method validation

Linearity of compound glycyrrhizin was validated by the linear regression equation and correlation coefficient. The six-point calibration curve for glycyrrhizin was found to be linear in the range of 20–200 ng. Regression equation and r^2 for the reference compound were $Y=4.213X+22.078$ and 0.996 which revealed a good linearity response for developed method (Table 1). The mean value with \pm SD of the slope was 4.213 ± 0.030 and intercept was 22.078 ± 0.050 for glycyrrhizin. No significant difference was observed in the slopes of standard plots ($P>0.05$). Table 2 presents intraday and interday precision and accuracy of the assay for glycyrrhizin at three quality control levels (150, 300 and 600 ng/band). Both intraday and interday precision was determined in terms of %CV. Intraday and interday precisions ($n=6$) for glycyrrhizin were found to be 1.46%–1.62% and 1.66%–1.84%, respectively, which demonstrated the good precision of proposed method. Intraday and interday accuracy of glycyrrhizin were observed as 98.9%–99.6% and 99.2%–99.7%, respectively. These results indicated the accuracy of the proposed method. The SD and %RSD were also calculated at each concentration level of glycyrrhizin. The low value of SD and %RSD obtained

after introducing small deliberate changes in the method indicated that the method was robust (Table 3). LOD and LOQ were determined from the slope of the lowest part of the calibration plot. LOD and LOQ for glycyrrhizin were found to be 15.7 and 47.1 ng/band respectively (Table 1). Good recoveries were obtained by the fortification of the sample at three quality control levels of glycyrrhizin. It is evident from the results that the percent recoveries for glycyrrhizin after sample processing and applying were in the range of 99.4%–99.8% as shown in Table 4.

Table 1

R_f : linear regression data for the calibration curve and sensitivity parameter for glycyrrhizin.

| Parameters | Glycyrrhizin |
|-----------------------------------|--------------------|
| R_f | 0.280 \pm 0.01 |
| Linearity range (ng/band) | 20–200 |
| Regression equation | 4.213X+22.078 |
| Correlation coefficient (r^2) | 0.996 |
| Slope \pm SD | 4.213 \pm 0.030 |
| Intercept \pm SD | 22.078 \pm 0.050 |
| Standard error of slope | 0.005 |
| Standard error of intercept | 0.007 |
| LOD | 15.7 ng/band |
| LOQ | 47.1 ng/band |

Table 2

Precision and accuracy of glycyrrhizin.

| Nominal concentration | Glycyrrhizin obtained ^{a,b} | Precision ^c | Accuracy ^d |
|-----------------------|--------------------------------------|------------------------|-----------------------|
| Intraday batch 150 | 148.4 | 1.62 | 98.9 |
| 300 | 297.8 | 1.52 | 99.2 |
| 600 | 597.6 | 1.46 | 99.6 |
| Interday batch 150 | 148.8 | 1.84 | 99.2 |
| 300 | 298.2 | 1.77 | 99.4 |
| 600 | 598.3 | 1.66 | 99.7 |

a: concentration in ng/band; b: mean from six determination ($n=6$); c: precision as coefficient of variation (CV,%)=[(standard deviation)/(concentration found)] \times 100; d: accuracy (%)=[(concentration found)/(nominal concentration)] \times 100

Table 3

Robustness of the method.

| Optimization condition | Glycyrrhizin | |
|---|--------------|-------|
| | SD | %RSD |
| Mobile phase from ethyl acetate: GAA: MeOH: H ₂ O (6:2:2:1, v/v/v) | 4.52 | 0.020 |
| v/v; 6:2:2:0.5, v/v/v/v; 6:2:1:1, v/v/v/v; 6:3:2:1, v/v/v/v/v) | | |
| Mobile phase volume (18, 20 and 22 mL) | 3.85 | 0.011 |
| Duration of saturation (10, 20 and 30 min) | 4.21 | 0.016 |
| Activation of TLC plate (2, 5 and 7 min) | 4.48 | 0.020 |

Table 4

Recovery studies of glycyrrhizin.

| Concentration added to analyte (%) | Theoretical (ng) | Added (ng) | Detected (ng) | Recovery (%) | RSD (%) |
|------------------------------------|------------------|------------|---------------|--------------|---------|
| | 300 | | | | |
| 50 | | 450 | 447.4 | 99.4 | 1.48 |
| 100 | | 600 | 597.6 | 99.6 | 1.22 |
| 150 | | 750 | 784.2 | 99.8 | 1.10 |

3.3. HPTLC analysis of glycyrrhizin in GGRMe and LRCMe

The utility of the proposed method was evaluated by applying this method for the quantification of glycyrrhizin in GGRMe and LRCMe. The content of glycyrrhizin in

the GGRMe and LRCMe were found to be 5.9% and 11.2%, respectively. It is for the first time, a simple, accurate and rapid HPTLC method has been developed for the quantification of bioactive marker glycyrrhizin in crude drug as well as commercial formulation marketed in Saudi Arabia.

4. Discussion

According to literature survey, there is no HPTLC densitometric method reported to quantify glycyrrhizin in GGRMe and LRCMe collected from Kingdom of Saudi Arabia. Therefore, attempts were made to develop and validate a cost effective, simple HPTLC technique to quantify glycyrrhizin in crude drugs and herbal formulations. A validated HPTLC method has been developed for the simultaneous determination of glycyrrhizin in *G. glabra* root and commercial formulation LRC collected from Riyadh, Saudi Arabia. Intraday and interday accuracy and precision study revealed that the method is highly accurate and precise. The LOD and LOQ indicated that the proposed method exhibits a good sensitivity for the quantification of glycyrrhizin. The recovery of the method was 99.4%–99.8% which is very good taking into account the complexity of herbal matrix. This study clearly gave a very good analytical method for the quantitation of the bioactive compound glycyrrhizin in GGRMe and LRCMe. Statistical analysis proved that the method was evitable trace out the small variations in glycyrrhizin. The developed HPTLC method for quantification of glycyrrhizin is simple, precise, specific, sensitive and accurate. The results revealed that glycyrrhizin content in GGRMe is almost 50% of that in LRCMe. This might be due to the extraction method as it is known that the extraction is difficult from the roots compared to the capsule powdered content. Alternatively it might be due to the variation in the source of licorice. This is why this method is of critical importance since it will be a great tool to distinguish good quality licorice from the poor quality ones. This will be of special importance for the regulatory authorities to check such products for possible degradation and/or counterfeiting. Moreover, this technique is getting popularity nowadays in the identification and authentication of herbal products. This utility will make checking purity and identity an easy job for both suppliers and the regulatory authorities. The importance of this method is represented also by the fact that triterpenoid saponins are difficult to be assessed by gas liquid chromatography due to the poor volatility and by HPLC due to the lack of UV activity and the contradictory properties of this moiety being highly nonpolar in a side and highly polar in the other side a typical feature for triterpenes saponins. The analytical range (20–200 ng) covers the wide spectrum of

the expected variation in the herbal products. The LOD was 15.7 ng/band which was quite fine since no activity might be claimed for a product that has this level of glycyrrhizin.

This method will be helpful for the manufacturer for quality control and standardization of commercial herbal formulations. The present study clearly gave evidence of the bioactive quantification of glycyrrhizin in GGRMe and LRCMe.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Standardization of herbal drugs in terms of quality of raw materials, manufacturing practices and composition is important to ensure quality and optimum levels of active principles for their bio–potency. Bioactive marker glycyrrhizin (a triterpenoid saponin) has been reported to be useful in several diseases. It was an effort to develop a validated HPTLC–densitometric method for quantification of biomarker glycyrrhizin in the crude drug as well as herbal formulation of *G. glabra* collected from Riyadh, Kingdom of Saudi Arabia.

Research frontiers

The aim of this research work was to develop a simple, sensitive HPTLC–densitometric method for quantification of glycyrrhizin in GGRMe and LRCMe.

Related reports

Few methods are reported in the literature regarding the quantification of glycyrrhizin but the method use in this research work is quite different and selective from the other methods.

Innovations & breakthroughs

The developed HPTLC method for quantification of glycyrrhizin is simple, precise, specific, sensitive and accurate. The results revealed that glycyrrhizin content

in GGRMe is almost 50% of that in LRCMe. This might be due to the extraction method as it is known that the extraction is difficult from the roots compared to the capsule powdered content. Alternatively it might be due to the variation in the source of licorice. This is why this method is of critical importance since it will be a great tool to distinguish good quality licorice from the poor quality one. This will be of special importance for the regulatory authorities to check such products for possible degradation and/or counterfeiting.

Applications

The proposed HPTLC method is quite selective and reproducible. It can be proved more useful in the correct determination of the quantity and quality of glycyrrhizin in the different extracts and pharmaceutical preparations. This method will also be helpful for the manufacturer for quality control and standardization of commercial herbal formulations.

Peer review

This is a valuable research work in which author has developed HPTLC validated method for the determination of glycyrrhizin in crude drug as well as formulation. Statically data proved that proposed method can be used in quality control against adulterant. Since, the given HPTLC method is simple, precise and specific, hence it can be employed further for the study of degradation kinetics of glycyrrhizin and determination of glycyrrhizin in plasma and other biological fluids.

References

- [1] Kaul N, Agrawal H, Patil B, Kakad A, Dhaneshwar SR. Application of stability-indicating HPTLC method for quantitative determination of metadoxine in pharmaceutical dosage form. *Farmaco* 2005; **60**: 351–360.
- [2] Alqasoumi SI, Alam P, Al-Rehaily AJ, Shakeel F, Abdel-Kader MS. Stability-indicating densitometric HPTLC method for qualitative and quantitative analysis of hydroquinone in commercial whitening creams. *J Planar Chromatogr* 2011; **24**: 48–52.
- [3] Biringanine G, Chiarelli MT, Faes M, Duez P. A validation protocol for the HPTLC standardization of herbal products: application to the determination of acteoside in leaves of *Plantago palmata* Hook. f.s. *Talanta* 2006; **69**: 418–424.
- [4] Faisal MS, Naz Z, Shakeel F, Ahmed S, Kohli K, Khar RK. A new TLC densitometric method for stability assessment of modafinil. *Chem Analityczna* 2009; **54**: 77–88.
- [5] Alam P, Alqasoumi SI, Shakeel F, Abdel-Kader MS. HPTLC densitometric analysis of arbutin in bulk drug and methanolic extracts of *Arctostaphylos uva-ursi*. *Nat Prod Res* 2011; **25**: 1671–1675.
- [6] Ali B, Mujeeb M, Aeri V, Mir SR, Ahmad S, Siddique NA, et al. High-performance thin layer chromatographic quantification of bioactive psoralen and daidzein in leaves of *Ficus carica* L. *Nat Prod Res* 2011; **25**: 1666–1670.
- [7] Amirghofran Z. Herbal medicines for immunosuppression. *Iran J Allergy Asthma Immunol* 2012; **11**: 111–119.
- [8] Hasanzadeh E, Shamsa F, Ohtsuki K, Rezazadeh Sh. The anti-inflammatory and anti-viral effects of an ethnic medicine: glycyrrhizin. *J Med Plants* 2010; **9**: 1–28.
- [9] Amirghofran Z. Medicinal plants as immunosuppressive agents in traditional Iranian medicine. *Iran J Immunol* 2010; **7**: 65–73.
- [10] Sheela ML, Ramakrishna MK, Salimath BP. Angiogenic and proliferative effects of the cytokine VEGF in Ehrlich ascites tumor cells is inhibited by *Glycyrrhiza glabra*. *Int Immunopharmacol* 2006; **6**: 494–498.
- [11] Yang HJ, Ma JY, Weon JB, Lee B, Ma CJ. Qualitative and quantitative simultaneous determination of six marker compounds in Soshiho-tang by HPLC-DAD-ESI-MS. *Arch Pharm Res* 2012; **35**(10): 1785–1791.
- [12] Gupta S, Sharma R, Pandotra P, Jaglan S, Gupta AP. Chromolith method development, validation and system suitability analysis of ultra-sound assisted extraction of glycyrrhizic acid and glycyrrhetic acid from *Glycyrrhiza glabra*. *Nat Prod Commun* 2012; **7**(8): 991–994.
- [13] Seo CS, Lee JA, Jung D, Lee HY, Lee JK, Ha H, et al. Simultaneous determination of liquiritin, hesperidin, and glycyrrhizin by HPLC-photodiode array detection and the anti-inflammatory effect of Pyungwi-san. *Arch Pharm Res* 2011; **34**(2): 203–210.
- [14] Seo CS, Lee MY, Lim HS, Kim SJ, Ha H, Lee JA, et al. Determination of 5-hydroxymethyl-2-furfural, albiflorin, paeoniflorin, liquiritin, ferulic acid, nodakenin, and glycyrrhizin by HPLC-PDA, and evaluation of the cytotoxicity of Palmul-tang, a traditional Korean herbal medicine. *Arch Pharm Res* 2012; **35**(1): 101–108.
- [15] Yang HJ, Ma JY, Weon JB, Ma CJ. Simultaneous determination of eight marker compounds in the traditional herbal medicine, Sipjundaebotang by HPLC-DAD. *Arch Pharm Res* 2011; **34**(9): 1503–1508.
- [16] Gantait A, Pandit S, Nema NK, Mukjerjee PK. Quantification of glycyrrhizin in *Glycyrrhiza glabra* extract by validated HPTLC densitometry. *J AOAC Int* 2010; **93**(2): 492–495.
- [17] Okamura N, Miki H, Orii H, Masaoka Y, Yamashita S, Kobayashi H, et al. Simultaneous high-performance liquid chromatographic determination of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin in Kampo medicines. *J Pharm Biomed Anal* 1999; **19**(3–4): 603–612.
- [18] Faiyazuddin Md, Baboota S, Ali J, Ahuja A, Ahmad S, Akhtar J. Validated HPTLC method for simultaneous quantitation of bioactive citral isomers in lemongrass oil encapsulated solid lipid nanoparticle formulation. *Int J Essen Oil Ther* 2009; **3**: 142–146.