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Simultaneous analysis of glycyrrhizic acid and preservatives in licorice aqueous extract by HPLC/PDA detection

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A high performance liquid chromatographic (HPLC) analytical method for the assay of glycyrrhizic acid (GLY), sodium benzoate (SB), methyl 4-hydroxybenzoate (MP), and propyl 4-hydroxybenzoate (PP) in licorice aqueous extract was developed. The method employed Lichro CART® Purospher STAR, C18, 5 µm (250 × 4.6 mm) analytical column with a simple mobile phase of potassium acetate buffer: acetonitrile (68.8: 31.2) v:v ratio. The detection was achieved by PDA detector at a wavelength of 254 nm. The developed method was considered linear within the specified range at concentrations of 80%, 90%, 100%, 110% and 120%. The method was found to be accurate, repeatable and precise. Upon storage in 0.1N HCl for 24 h, 0.1N NaOH for 24 h, and 3% H_2O_2 with exposure to UV for 24 h and heating in water bath at 90 ºC for 24 h, methyl paraben (MP) was stable upon changing conditions. The other tested components exhibited different stability properties upon changing conditions.

Keywords: glycyrrhizic acid (GLY), sodium benzoate (SB), methyl 4-hydroxybenzoate (MP), propyl 4-hydroxybenzoate (PP), validation, Arrhenius plots.

INTRODUCTION

Liquorice or licorice [1, 2] is the root of *Glycyrrhiza glabra* from which a somewhat sweet flavor can be extracted. Licorice extract is produced by boiling licorice root and subsequently evaporating most of the water, and is traded both in solid and syrup forms [3, 4, 5]. Its active component is glycyrrhizin known as glycyrrhizic acid (GLY) (Figure 1), a sweetener from 30 to 50 times sweeter than sucrose, which also has pharmaceutical effects [6].

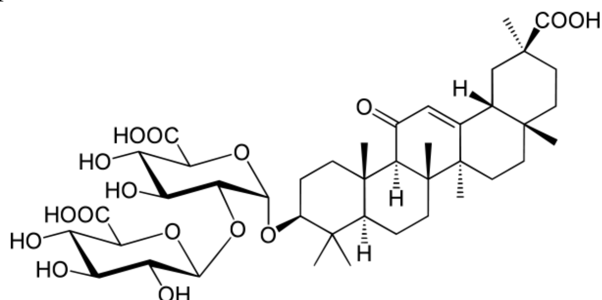

Fig. 1. The chemical structure of glycyrrhizic acid (GLY), the major active component in licorice.

Several studies have investigated the main components in licorice root extract for anti-inflammatory activity [7-10]. The effects of the aqueous extract of *Glycyrrhiza glabra* on the depression in mice using forced swim test (FST) and tail suspension test (TST) have been investigated [11]. Ethanolic extracts of the dried roots of *Glycyrrhiza glabra* were analyzed for their phytochemical constituents. The analysis showed the presence of various phytochemicals like alkaloids, cardiac glycosides, flavonoids, HCN, indoles, juglones, phenols, saponins, steroids, tannins and terpenoids [12].

An ionic liquid based single-drop microextraction procedure followed by HPLC has been developed and validated for the determination of the components of licorice tablets [13].

Several research studies have been conducted and reported in the literature on the analysis of licorice and its extract. For example, Qiao Xue and coworkers investigated the regulatory effects of licorice on bile acid metabonome in rats using liquid chromatography coupled with tandem mass spectrometry [14]. Also, Farag and coworkers utilized multi-targeted metabolic profiling and fingerprinting techniques to study the *Glycyrrhiza* species components [15]. Moreover, licorice and its extract were quantitatively determined by two-dimensional liquid chromatography [16], inductively coupled plasma atomic emission spectrometry [17], HPLC-ESI-MS/MS [18], high-performance thin-layer chromatography (HPTLC) [19,20], HPLC-UV detector with external standard

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[21], graphite furnace atomic absorption spectrometry [22], liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry [23,24], RP-HPLC/DAD method [23-25], UPLC-MS-MS [26], gas chromatography [27], first derivative spectrophotometric and Vierordt's method [28], multi-stage mass spectrometric [29] and HPLC-UV-MS [30].

Literature survey showed that there was no published data related to the routine or/and stability assay of glycyrrhizic acid in the presence of a mixture of preservatives like sodium benzoate, methyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate in licorice aqueous extract by any type of analytical methods. Thus, the main purpose of this study was to develop a novel HPLC analytical method for the assay of these four components in licorice aqueous extract. The study includes the validation of the HPLC method according to FDA and ICH guidelines. The described method can be applied to similar patterns for the identification of low concentrations of extract components. For example, comparable methods were used for identification of anthocyanin derivatives in grape skin extracts [31], identification of polyphenol mixtures and plant extracts [32], identification of phenolic compounds in lemon verbena extracts [33], determination of domic acid in shellfish [34] and many other extraction products in the field.

EXPERIMENTAL

Chemicals and Materials

Licorice dry aqueous extract EP (*Glycyrrhiza glabra L.*), was purchased from Instituto Farmochimico Fitoterpico (EPO), Milano, Italy. 18-beta-glycyrrhetinic acid 98%, glycyrrhizic acid monoammonium salt trihydrate 98%, and acetonitrile HPLC grade were purchased from Acros Organics, USA. Glycyrrhizic acid 100% USP Reference STD was purchased from USP Convention, Rockville, USA. Methyl paraben EP and propyl paraben EP were purchased from KIMIA International Ltd., India. Sodium benzoate EP powder was purchased from VELSICOL, Estonia. Methanol LC grade, was purchased from MERCK, Germany. 1-Octanesulfonic acid sodium salt HPLC grade, potassium acetate reagent grade ACS, and acetic acid glacial analytical grade ACS, were purchased from Sharlau, Spain. Distilled deionized water was prepared in house by STILMAS Pharmastill MS 15045 ultra pure water system (Vaponics, USA). Certification of licorice dry aqueous extract was achieved by the HPLC analytical method specified in United States pharmacopoeia USP-27 using glycyrrhizic acid USP as a reference standard. The preservatives (methyl paraben, propyl paraben, and sodium benzoate) were certified according to European Pharmacopoeia-7th by the titrimetric analytical methods described there. The determined purity of standards is reported in table1.

Table 1. Certification of the used working standards

Working standard name	Specifications	Assay (w/w)
Licorice dry aqueous extract (LAE)	≥ 3.0 %	4.0 %
Sodium benzoate (SB)	99.0 -100.5 %	99.3 %
Methyl 4-hydroxy benzoate (MP)	99.0 -100.5 %	100.0 %
Propyl 4-hydroxy benzoate (PP)	99.0 -100.5 %	99.6 %

Instruments

The HPLC system consists of a MERCK-HITACHI gradient pump model L-7100 connected to MERCK “On-Line” Degasser L-7614, MERCK-HITACHI Autosampler L-7200, MERCK Column Oven L-7360, MERCK-HITACHI Diode Array Detector L7455, and MERCK-HITACHI Interface D7000. The HPLC system was connected to DELL PC with hp-DeskJet 845c Printer. The HPLC system was controlled by MERCK-HITACHI D-7000 HPLC system manager (HSM) software. A Purospher ® STAR, C18, (250 × 4.6 mm, 5µm) analytical column with C18 guard column was used after several optimization trials. The following helping tools were used to complete the work: Sartorius research analytical balance type R200 D from Germany; pH-meter type Orion Research Expandable ion analyzer EA 940; ultrasonic bath type FRITSCH-laborette 17.002, Germany; water bath type GFL 1003, Germany; magnetic stirrer type Gallenkamp, England; vacuum oven type Gallenkamp A-20011, England; incubator type VISMARA S.R.L F1 480 RS, Italy; oven type Heraeus T 6120, Germany; humidity oven type Heraeus VTRK 150, Germany; and centrifuge type JOUAN MR18-12, France.

Mobile phase preparation

Potassium acetate buffer solution was prepared by dissolving 0.724 g of potassium acetate in 1000 mL of deionized water using a volumetric flask. The pH was adjusted to 3.60 by glacial acetic acid (≈ 4.0 mL) with gentle mixing. Acetonitrile (454 mL) was accurately measured by using a 500-mL measuring cylinder, and mixed well with the potassium acetate buffer solution in a suitable container. The mixture was filtered through a 0.45 µm Nylon membrane filter under vacuum and degassed in a ultrasonic water bath.

Standard solution preparation

Accurately weighed amounts of methyl paraben (MP) and propyl paraben (EP) standardized materials equivalent to 192.4 mg and 28.8 mg, respectively, were dissolved in a 200-mL volumetric flask with the mobile phase. The solution was mixed well and 5 mL were transferred to a 100-mL volumetric flask; an amount of licorice aqueous extract (LAE) dry powder equivalent to 18 mg GLY and accurately weighed amount of sodium benzoate equivalent to 72.1 mg were added and dissolved with the mobile phase. The solution was mixed well by sonication for about 15 min. The obtained solution was centrifuged and the resulted supernatant was filtered through a membrane syringe filter having a porosity of 0.45 µm.

Test sample solution preparation

A 3 mL pipette was rinsed carefully with the mobile phase. The test sample bottle was shaken gently and 3.0 mL of licorice aqueous extract was transferred to a 100 mL volumetric flask. The solution was sonicated for about 5 min, then mixed well and filtered through a membrane syringe filter having a porosity of 0.45 µm.

Methodology of the validation characteristics

Accuracy

The accuracy of the analytical method was assessed by application of the analytical procedure to nine synthetic samples at three concentration levels, namely 80%, 100%, and 120%, three samples for each concentration. The synthetic samples were prepared by adding known quantities of the analytes to a placebo sample. Accuracy was reported as the percent recovery of the assay of known added amounts of the analytes in the synthetic samples.

Precision

Repeatability

Repeatability of the preparation of the standard solution

The repeatability of the preparation of the standard solution was assessed by application of the analytical procedure to nine determinations at three concentration levels, namely 80%, 100%, and 120%, three samples for each concentration. The repeatability of the preparation of the standard solution was reported as relative standard deviation for injections of each prepared standard solution, and for replicates of the preparation of standard solution at each concentration level: 80%, 100%, and 120%.

Repeatability of the preparation of test sample solution

The repeatability of the preparation of the test sample solution was assessed using 6 determinations at 100% of the concentration of the test sample solution; 6 sample solutions from the same container were prepared and injected into the chromatograph. The repeatability of the preparation of the test sample solution was reported as relative standard deviation for injections of each prepared test solution, and for replicates of the preparation of the test solution.

Intermediate precision

The variations that have been studied include days, analysts, and equipment. The same test sample was analyzed in different days by different analysts and equipments. The results obtained from the first and the second assay test were reported and compared.

Linearity and Range

The linearity was evaluated over the range from 80% to 120% of the concentration of the test solution. It was demonstrated by dilution of a standard stock solution. Standard stock solution was prepared in duplicate, then serial dilutions were performed for each one to obtain standard solutions having the following concentrations: 80%, 90%, 100%, 110%, and 120% of the concentration of the test solution.

Selectivity

The selectivity of the analytical method was demonstrated by evaluation of chromatographic peak purity and resolution for the analytes after storing their solutions under relevant severe conditions: light, heat, acid/base hydrolysis and oxidation. Practically, this was performed by preparing the tested samples for each analyte individually using the following media and conditions; 0.1 N NaOH, 0.1 N HCl, 3% H_2O_2, UV-radiation, and heat (90ºC).

Accelerated stability study

Container closure system

Accelerated stability testing was conducted on licorice aqueous extract packed in 25 ml-amber glass bottle containers with plastic screw caped closures.

Storage Conditions and testing frequency

The licorice aqueous extract bottles were stored in several storage conditions for 12 weeks, and were frequently tested, as shown in the following table:

Table 2: Stability study storage conditions and frequency of testing

Storage conditions	Frequency of testing (weeks)
RT/DL	1,2,3,4,5,8, and 12
25ºC ± 2ºC / 60% RH ± 5% RH	1,2,3,4,5, and 8
40ºC ± 2ºC / 75% RH ± 5% RH	1,2,3, and 4
50 ºC ± 2ºC	1,2,3,4,5,8, and 12
70 ºC ± 2ºC	1,2,3,4,5,8, and 12

RESULTS AND DISCUSSION

Wavelength selection

The chemical composition of the sample can provide valuable clues for the best choice of initial conditions for an HPLC separation. In this work, empirical procedures were emphasized in combination with techniques for minimizing the number of required experimental runs. However, theoretical considerations and the chemical composition of the sample were not ignored. Since the analytes have good UV-absorbance and exist in sufficient concentrations, the PDA detector was chosen as the detector. In photodiode array detection glycyrrhizic acid, sodium benzoate, propyl paraben and methyl paraben have λmax of 251 nm, 240 nm, 255 nm and 255 nm, respectively. Depending on the obtained UV-spectra the wavelength was adjusted at 254 nm.

Selection of HPLC conditions

The USP-27 assay method for licorice was tested at the beginning (figure 2A). The peak at 6.39 min consists of two components: sodium benzoate and methyl paraben. The method was considered unsuitable because the separation required further improving. After that the attention was focused on the strength and pH value of the solvent. Increasing of the concentration of acetonitrile in the eluent led to a decrease in the analytes retention and hence the capacity factor (k') decreased. There was no significant effect by changing the pH of the mobile phase in the investigated experiment for this issue. The results exhibited poor resolution of the analytes. The effect of the column length and the flow rate was tested. The increase in column length did not sufficiently improve the separation of sodium benzoate and methyl paraben peaks. The method was still not selective for sodium benzoate and methyl paraben analytes under the specified conditions of the experiments (Figure 2B). The mobile phase flow rate was increased to 1.0 ml per minute in order to reduce the run time. As the column was increased in length the analytes separated better and were longer retained due to the increase in column plate number (N), so that both k and α values of the analytes increased and the R value improved. Although the increase in the flow rate reduced the run time, it also increased the column pack pressure which was considered unfavorable because it exceeded the specified pressure limit (2000 psi).

Further separation trials were performed by using organic solvents other than acetonitrile, e.g., THF and methanol, in order to improve the selectivity of the chromatographic system. The results are still

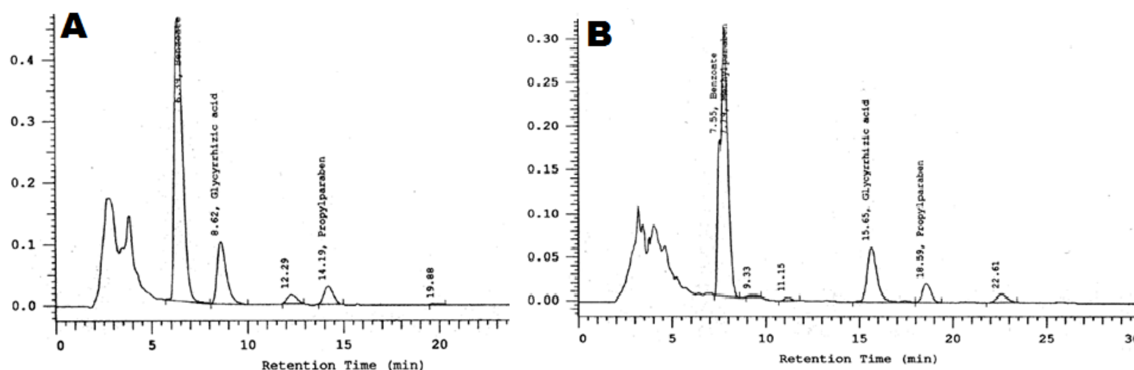

Fig. 2. A) Representative chromatogram of the sample (LAE) using the USP method. B) Representative chromatogram of the sample solution using a 25cm × 4.6mm 5µm C_{18} analytical column (modified USP-method).

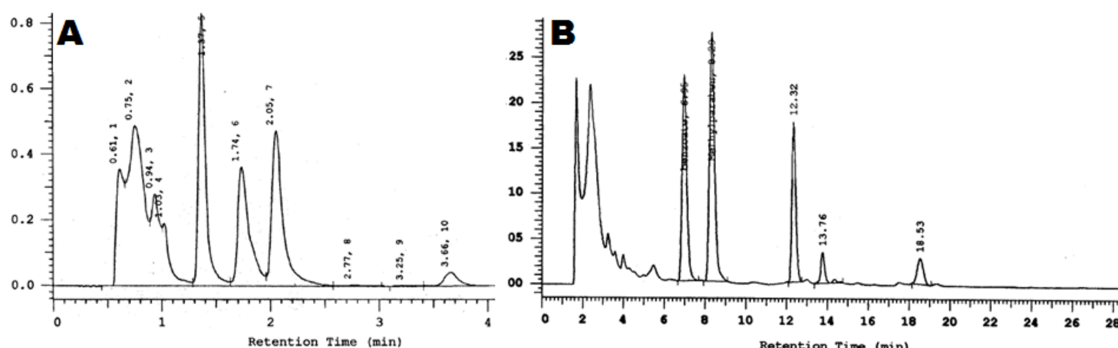

Fig. 3. A) Representative chromatogram with 12.5 cm C_8 column at FR 1.5 ml/min with 30% acetonitrile in system No.3; GLY at 1.4, SB at 1.8 min , MP at 2.0 and PP at 3.7 min. B) Representative chromatogram of gradient: (SB at 7.0 min, MP at 8.3 min, GLY at 12.3 min, and PP at 18.5 min.)

unsatisfactory. Also, packing material such as cyano and C8 columns were tested over C18 (Figure 3A). However, this type of columns did not produce the expected improvement since there was no good separation for the four analytes within the accepted run time.

Gradient elution was employed during the development trials. The most apparent problem during the development of the gradient system was the improper resolution of PP due to its low concentration which made it sensitive to the base line noise and drifts (Figure 3B).Many trials were conducted including changing the parameters mentioned above and other parameters not reported here such as ion paring and others. The results from these trials are the optimized chromatographic conditions listed in table 3. A representative chromatogram is shown in figure 4. The method provides good values for column plates number (N), capacity factor (k), selectivity α and resolution (R).

ANALYTICAL METHOD VALIDATION

Linearity study

The linearity of the analytical method was statistically evaluated by calculation of a regression line by the method of least squares. The correlation coefficient, y-intercept, and slope of the regression line are shown in table 4. Plots of the data are included in figure 5. The specified range was derived from the linearity studies and depended on the intended application of the analytical method. It was considered from 80% to 120% of the concentration of the test solution.

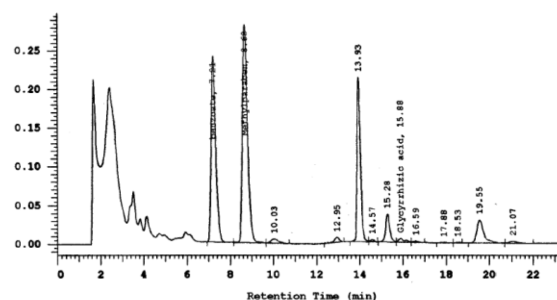

Fig. 4. Representative chromatogram under optimized chromatographic conditions: (SB at 7.2 min, MP at 8.7 min, GLY at 14.0 min, and PP at 19.6 min.)

Based on the tabulated results of the linearity study (Table 5) the developed method was considered linear within its specified range (80-120%: SB [575-865 µg/ml], GLY [144-216 µg/ml], MP [38.5-57.7 µg/ml], and PP [5.8-8.6 µg/ml] since the correlation coefficients (r) of the analytes were not less than 0.998.

Table 3: The chromatographic system of the developed method

Mobile phase	Potassium acetate buffer pH 3.60: acetonitrile (68.8: 31.2) V/ V
Column	Purospher ® STAR, C_{18}, 5µm (250 × 4.6 mm), with C_{18} guard column.
Temperature	30 °C
Detector	254 nm
Flow rate	1.3 ml/min
Injection volume	10.0 µl
Run time	22 min

Table 4: Linearity data of the analytes (average of duplicate)

Analyte Name	Area 80%	Area 90%	Area 100%	Area 110%	Area 120%
Glycyrrhizic acid (GLY)	399985	440764	491201	540321	589441
Na benzoate (SB)	547061	603990	672440	739684	817376
Methyl paraben (MP)	778294	860944	958518	1040691	1157109
Propyl paraben (PP)	97423	109388	120951	128884	141168

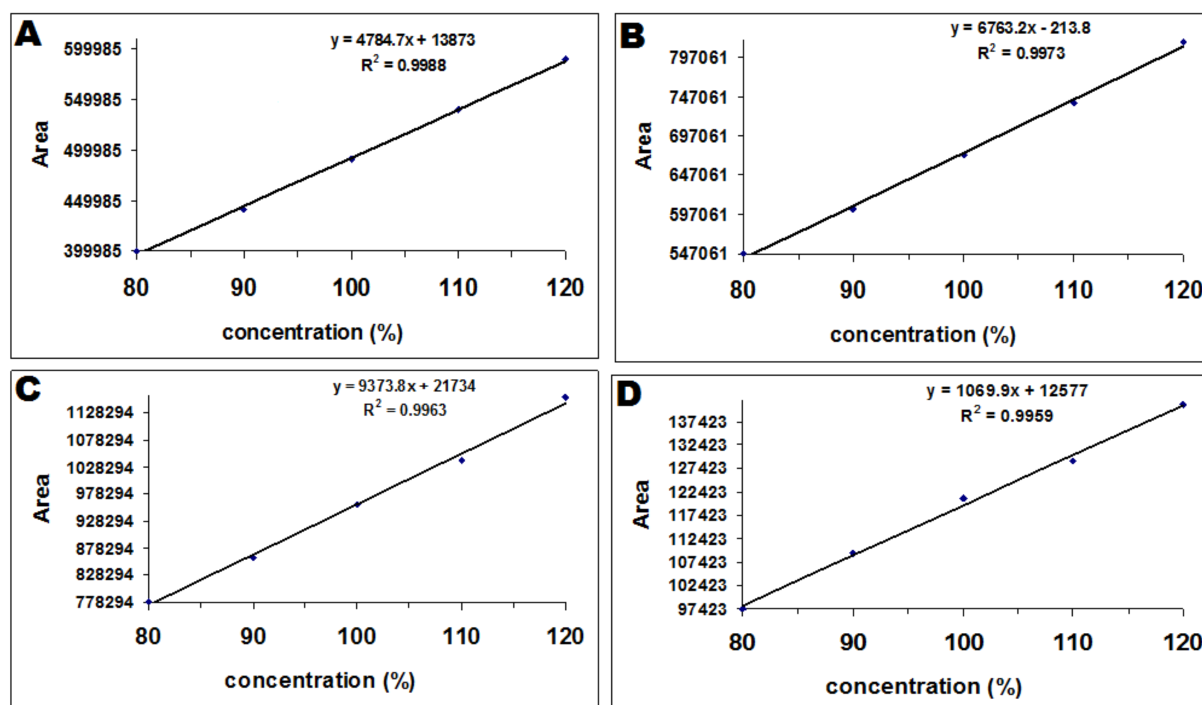

Fig. 5. Calibration curve for A: glycyrrhizic acid, B: sodium benzoate, C: Methyl paraben, D: Propyl paraben. The concentration points are 80%, 90%, 100%, 110% and 120%.

Table 5: Results of the linearity studies for the analytes

Analyte Name	Correlation coefficient (r)	Y-intercept	Slope of the regression line
Glycyrrhizic acid	0.9994	13873	4785
Na benzoate	0.9986	- 214	6763
Methyl Paraben	0.9982	21734	9374
Propyl paraben	0.9980	12577	1070

Table 6: Accuracy study results of glycyrrhizic acid

Target Concentration		Spiked Concentration (mg/mL)	Measured Concentration (mg/mL)	Percentage (%)	Average Recovery (%)	RSD (%)
	S1	0.144	0.1475	102.4		
80% (144 µg/ml)	S2	0.144	0.146	101.5	102.0	0.44
	S3	0.143	0.146	102.0		
	S1	0.179	0.180	100.8		
100% (180 µg/ml)	S2	0.179	0.182	101.5	100.7	0.85
	S3	0.179	0.179	99.8		
	S1	0.2155	0.217	100.7		
120% (216 µg/ml)	S2	0.2158	0.216	100.2	100.7	0.55
	S3	0.2155	0.218	101.3		

The RSDs for SB, MP and PP are 0.55 to 0.80; 0.53 to 0.94; and 0.94 to 1.84, respectively.

Accuracy study

Based on the tabulated results of the accuracy studies, the method was found to be accurate since the average recovery values of the analytes were 100.0% ± 2.0%. Table 6 shows the results of glycyrrhizic acid; other detailed results for SB, MP and PP are not shown here for space issue.

Precision study

Repeatability

Repeatability of the preparation of the standard solution

Based on the tabulated results of the precision studies the developed method was found to be repeatable and precise since the relative standard deviations for different injections from the same test solution and for different preparations of the test solution were not more than 2.0%.

Table 7: Repeatability precision study results of Glycyrrhizic acid

Target Concentration		Measured Area	Average of Measured Area	SD	RSD (%)
	S1	402280			
80% (144 µg/ml)	S2	398429	400037	2002	0.50
	S3	399402			
	S1	491852			
100% (180 µg/ml)	S2	495208	491361	4114	0.84
	S3	487024			
	S1	589643			
120% (216 µg/ml)	S2	586198	589553	3311	0.56
	S3	592818			

Table 8: Repeatability of the preparation of the test sample solution for the assay

Analyte	Prep. No.1	Prep. No.2	Prep. No.3	Prep. No.4	Prep. No.5	Prep. No.6	Average %	RSD %
GLY	100.5	100.8	99.2	101.3	101.0	100.0	100.5	0.76
SB	99.6	100.8	98.8	99.4	100.3	100.1	99.8	0.71
MP	99.7	99.6	98.7	99.9	99.6	101.3	99.8	0.86
PP	101.2	99.6	98.1	100.5	101.2	100.5	100.2	1.18

Table 7 shows the results of glycyrrhizic acid; other detailed results for SB, MP and PP are not shown here for space issue. The RSDs for SB, MP and PP are 0.50 to 0.94; 0.50 to 0.97; and 1.36 to 1.82, respectively.

Repeatability of the preparation of the test sample solution

Based on the tabulated results of the intra-assay repeatability (Table 8) the developed method was found to be repeatable and precise since the relative standard deviations for different assay preparations from the same test solution were less than 2.0%.

Intermediate precision

As shown in Table 9, the deviations in the assay results for the analytes were less than 2.0% for two separate analyses (A & B) of the same sample bottle by different analysts and instruments at different days, which indicates a good precision of the developed analytical method.

Analysis B was performed by a different analyst using a different instrument at a different day.

Selectivity

Representative chromatograms were run for each tested sample of the analytes and the chromatographic peak purity and resolution were evaluated.

Table 9: Results of the intermediate precision study

Analyte name	Analysis A (Assay)	Analysis B (Assay)	Deviation %
GLY	99.5 %	100.0 %	0.5
SB	100.6 %	99.7 %	0.9
MP	99.3 %	99.9 %	0.6
PP	100.8 %	99.5 %	1.3

Stability

The analytes under study were put to vigorous conditions, namely, storage in 0.1N HCl for 24 h, storage in 0.1N NaOH for 24 h, heating in water bath at 90 ºC for 24 h and storage in 3% H_2O_2 with exposure to UV for 24 h. Table 10 shows the results of the stability experiments. Methyl paraben was not affected by the different conditions. It was found that the degradation products did not interfere with the analytes peaks and their assays were not affected.

Storage conditions

The pH and appearance of the stored extract were evaluated in addition to the assay of the

Table 10: The results of stability tests under different conditions.

Conditions	Storage in 0.1N HCl for 24 h	Storage in 0.1N NaOH for 24 h	Heating in water bath at 90 ºC for 24 h	Storage in 3% H_2O_2 with exposure to UV for 24 h
GLY	☑	☒	☑	☑
SB	☒	☒	☑	☒
MP	☒	☒	☒	☒
PP	☒	☑	☒	☑

☑ Degradation products appeared in the chromatogram and/or the analyte peak disappeared
☒ not affected

components of interest. The obtained results of the accelerated stability study for glycyrrhizic acid, sodium benzoate, methyl 4-hydroxybenzoate, and propyl 4-hydroxybenzoate in licorice aqueous extract during 12 weeks showed that there were significant changes in the pH values of the stored samples of LAE at high temperatures (50 ºC and 70 ºC) and in the assay results of their components of interest. GLY was the most affected analyte while PP was the least affected one.

The data of the analysis of LAE were obtained at the start point (Table 11) and after different time periods at the following conditions:

Table 11: Zero time analysis results of LAE stored samples.

Test	Results at zero time
Appearance	Dark brown liquid
pH	5.32
Assay	
Na-benzoate	101.5 %
Methyl paraben	101.0 %
Propyl paraben	99.6 %
Glycyrrhizic acid	102.3 %

The pH values for LAE samples stored during 1-12 weeks at RT/DL, 25 ºC/65%RH, 50 ºC and 70 ºC were: 5.30-5.25, 5.29-5.24, 5.25-5.13 and 5.18-4.29, respectively. The assay values for GLY samples stored during 1-12 weeks at RT/DL, 25 ºC/65%RH, 50 ºC and 70 ºC were 102.3-91.8%, 101.7-91.0%, 101.3.0-91.6%, 101.3-76.5%. The assay values for SB samples stored during 1-12 weeks at RT/DL, 25 ºC/65%RH, 50 ºC and 70 ºC were 102.4-93.0%, 102.0-91.0%, 100.2.0-93.9%, 101.6-91.7%. The assay values for methyl paraben samples stored during 1-12 weeks at RT/DL, 25 ºC/65%RH, 50 ºC and 70 ºC were 101-91.2%, 101.9-91.1%, 99.3.0-90.6%, 100.2-81.7%. The assay values for propyl paraben samples stored during 1-12 weeks at RT/DL, 25 ºC/65%RH, 50 ºC and 70 ºC were 97.6-88.2%, 101.0-NT%, 94.0.0-87.3%, 96.72-80.1%.

The stability data of LAE samples stored during 4 weeks were obtained at 40 ºC /75%RH. The results are not shown here.

CONCLUSIONS:

A high performance liquid chromatographic (HPLC) analytical method using a PDA detector at 254 nm and a Lichro CART® Purospher STAR, C18, 5µm (250 x 4.6 mm) analytical column with a simple mobile phase of potassium acetate buffer pH 3.60: acetonitrile (68.8: 31.2) v:v was successfully developed for the assay of glycyrrhizic acid, sodium benzoate, methyl 4-hydroxybenzoate, and propyl 4-hydroxybenzoate in licorice aqueous extracts.

The method was simple and reliable and did not need long extraction procedures.

The validation of the analytical method indicated its suitabilility for the intended use. The method could be applied for stability studies of the analytes in licorice aqueous extracts, thus it was considered a stability indicating method. It was selective, accurate, precise, and linear within its specified range (80-120%). The method can be applied in similar patterns for the identification of low concentrations of extract components.

The developed RP-HPLC analytical method for the routine assay of GLY and SB in alcoholic liquid extracts was validated for its intended use.

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ЕДНОВРЕМЕНЕН АНАЛИЗ НА ГЛИЦИРОВА КИСЕЛИНА И КОНСЕРВАНТИ ВЪВ ВОДНИ ЕКСТРАКТИ ОТ ЖЕНСКО БИЛЕ ЧРЕЗ HPLC/PDA

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(Резюме)

Успешно е разработен аналитичен метод за високо-ефективна течна хроматография (HPLC) за определянето на глицирова киселина (GLY), натриев бензоат (SB), метил 4-хидроксибензоат (MP), и пропил 4-хидроксибензоат (PP) във водни екстракти от женско биле. Методът използва аналитична колона Lichro CART® Purospher STAR, C18, 5µm (250 x 4.6 mm) с проста подвижна фаза от калиев ацетатен буфер:ацетонитрил в обемно съотношение (68.8: 31.2). Анализът се извършва при PDA-детектор при дължина на вълната 254 nm. Методът дава линейна зависимост в интервал от концентрации 80%, 90%, 100%, 110% and 120%. Методът е точен възпроизводим и прецизен. Метил-парабенът (MP) е стабилен при променливи условия при съхранение в 0.1N HCl за 24 часа, 0.1N NaOH за 24 часа и 3% H2O2 и облъчване с ултравиолетова светлина и нагряване на водна баня. Другите изпитани компоненти показват различна стабилност при променящи се условия.