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Analysis and comparison of Radix Glycyrrhizae (licorice) from Europe and China by capillary-zone electrophoresis (CZE)

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Abstract

A simple capillary-zone electrophoresis (CZE) method for the analysis of plant specimens, *Glycyrrhiza glabra* L., *G. uralensis* FISCH. and *G. inflata* BAT. (Leguminosae) as well as commercial licorices from Europe and China was developed. Contents of glycyrrhizin (GL), glycyrrhetic acid (GA), glabridin (GLAB), liquiritin (LQ) and licochalcone A (LC_A) in ethanolic extracts were investigated. Optimum separation was achieved with sodium tetraborate buffer (pH 9.22; 70 mM); voltage, 25 kV. Recovery rate for GL was found to be $101.90 \pm 2.54\%$. Adequate correlation was observed between GL contents measured by CZE and HPLC ($r = 0.977$). Advantages over conventional HPLC analysis of *Glycyrrhiza* species are short analysis time (<15 min), simple running buffer preparation and the none-use of organic solvents.

Using the present CZE method, it was demonstrated that (1) *G. glabra* was distinguished from *G. uralensis* especially by phenolic compounds GLAB (*G. glabra*: $0.19 \pm 0.11\%$; $n = 53$) and LQ (*G. uralensis*, $1.34 \pm 0.34\%$, $n = 10$); (2) on average, GL contents were higher in Chinese commercial licorices; (3) relatively high LC_A contents were especially detected in a Chinese commercial licorice (origin estimated as *G. inflata*); (4) *Glycyrrhiza* species were also distinguished by applying PCA on the basis of CZE peak area data of GL, GLAB, GA, LQ and LC_A; and (5) liquiritin apioside was found in all samples.

Keywords: Capillary-zone electrophoresis (CZE); Radix Glycyrrhizae; Licorice; Glycyrrhizin; Glabridin; Principal component analysis

1. Introduction

Radix Glycyrrhizae, licorice, the underground material derived from species of the genus *Glycyrrhiza* L. (Leguminosae), is an extensively used herbal drug in Western and Eastern medicine, applied for diseases of the stomach, liver, catarrh of the respiratory organs and skin disorders [1,2]. A number of bioactive compounds in licorice have been described, e.g. glycyrrhizin (GL) and its aglycon glycyrrhetic acid (GA), liquiritin (LQ), liquiritin apioside (LA), isoliquiritin (IL) and glabridin (GLAB) [3–7].

The ordinary botanical sources of Radix Glycyrrhizae are *G. glabra* L., which is geographically distributed from Southern Europe to Western China, and *G. uralensis* FISCH., found from Central Asia to Eastern China [8–10]. As a further species, *G. inflata* BAT. is also mentioned in the Chinese Pharmacopoeia (2000). Many species-specific phenolics have been described such as glycycoumarin for *G. uralensis*, or the isoflavan GLAB for *G. glabra* [4]. Individual species show highly varying chemical constituents [4]. Several pharmacopoeias require definite species, such as *G. glabra* L. by the European Pharmacopoeia [20]. For these reasons, it is important to distinguish between *Glycyrrhiza* species.

Capillary electrophoresis has proven to be an efficient technique for the analysis of natural products and for fingerprinting [11,12]. Determination of GL and GA in traditional Chinese medicinal preparations by capillary electrophoresis was reported [13] as well as determination of flavonoids in Radix Glycyrrhizae by capillary-zone

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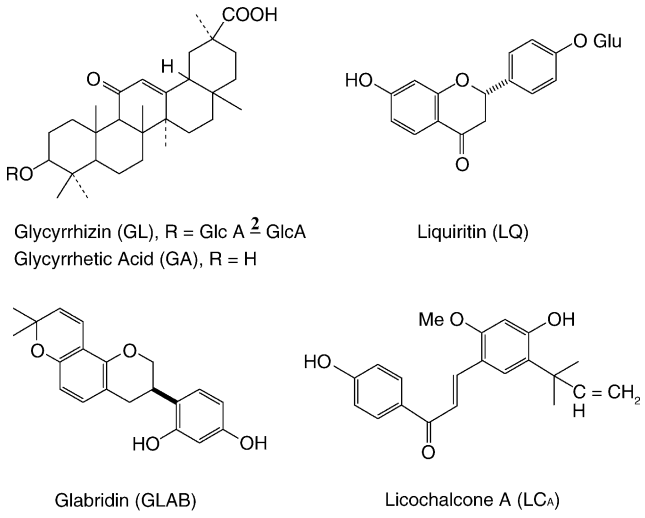

Fig. 1. Reference compounds.

electrophoresis [14]. Active components GL and GA of *G. uralensis* were also characterized by micellar electrokinetic chromatography (MEKC) [15]. Hitherto, comparative studies on the compounds of *Glycyrrhiza* species have been using HPLC [10,16–19].

In this study, a capillary-zone electrophoresis (CZE) method was developed for the analysis of Radices Glycyrrhizae from Europe and China. Compared with former capillary electrophoresis analyses [13–15], the present method allows to distinguish between the ordinary botanical sources of licorice by their contents of the saponin GL and its aglycon GA, the flavonoids GLAB, LQ and licochalcone A (LC_A) (Fig. 1). The new method may also be considered as complementary to conventional HPLC analysis, for which environmentally harmful organic solvents are necessary [20,21].

Furthermore, a CZE fingerprint profile consisting of 17 characteristic peaks was developed for *G. glabra* and compared with *G. uralensis*.

2. Experimental

2.1. Material

Sixty six samples of plant specimens and commercial licorices, as divided into six groups:

(a) *G. glabra* L., 32 specimens collected during May and June 2002 in five European countries: Nos. 1–8: Southern Spain, Nos. 9–16: Southern France, Nos. 17–25: Southern Italy, No. 27: cultivated in the botanical garden of the Josephinum, Vienna, Austria, Nos. 28–33: Crete, Greece.
(b) *G. echinata* L. ($n = 1$), cultivated in the botanical garden of the Josephinum, Vienna, Austria (No. 26), collected in June 2002.
(c) Commercial Radices Glycyrrhizae ($n = 21$), all obtained during May and June 2002 from pharmacies of European and neighbouring countries. Listed below with "product name," company name, place obtained, production place and date (in parenthesis), if available: No. 34: "Regaliz raiz", Hierbas del Monte, Sevilla, Spain, Nos. 35–36: "Regaliz raiz", Aldyplant, Cordoba, Spain (Catalonia, Spain), No. 37: "Regaliz", Sattva muysano, Sevilla, Spain (Aragon, Spain), No. 38: "Reglisse baton," Phytofrance, Montpellier, France (Western France), No. 39: "Reglisse racine", Phytofrance, Montpellier, France (Western France), No. 40: "Reglisse", Gignac, France, No. 41: "Reglisse", Montpellier, France, No. 42: "Reglisse", Montpellier, France (Syria), No. 43: "Reglisse", Uzes, France (Italy), No. 44: "Reglisse", Montpellier, France, No. 45: "Radice di Liquirizia", Farvisan, Ostia, Italy (Crotone, Italy), No. 46: "Radice di Liquirizia", Carloni, Torvaianica, Italy, No. 47: "Liquirizia", Formia, Italy (Sardegna, Italy), No. 48: "Radice di Liquirizia", Napoli, Italy (Crotone, Italy), No. 49: "Oronero", Sirea, Pontesagnano, Italy (Crotone, Italy), No. 50: "Liquirizia", Margherita di Savoia, Italy, No. 51: "Shirinbayan", obtained in Teheran, Iran, (Iran), No. 52 (uncut) and 53 (cut): "Radix Liquiritiae", Klenk, Vienna, Austria (Turkey), No. 54: "Meyan", Gaziantep, Turkey (Turkey).
(d) *G. uralensis* Fisch. ($n = 3$), cultivated in the Eastern area of Nei-menggu (Inner Mongolia), China, No. 55 [4 years cult. (2001)], No. 56 [5 years cult. (2002)], No. 57 [4 years cult. (2001)]. They were the same samples as in our previous report [22].
(e) Commercial Radices Glycyrrhizae (*Gancao* in Chinese, *Kanzo* in Japanese) from China, all obtained from Tochimoto-tenkaido Co. Ltd., Osaka, Japan. They were the same samples as previously reported [22,23]. Product name, market obtained, production place and date (in parenthesis) are indicated: No. 58: "Daitou-Gancao", Hebei (Gansu, 2000), No. 59: "Kawasari (peeled)-Kanzo" (A-grade), Osaka (Ningxia, 2001), No. 60 & 64: "Seihoku-Kanzo" (2nd grade) Osaka (NW China, 1999), Nos. 61 & 63: "Dongbei-Gancao", Hebei (NE China, 2000), No. 62: "Daitou-Gancao" (Gansu, 2000). For sample No. 65 "Xinjiang-Gancao", Hebei (Xinjiang, 2000), origin was estimated as *G. inflata* Bat. by comparing its HPLC profile [16].
(f) *G. inflata* Bat. ($n = 1$), collected in September 1999 in Jiuguan/Gansu/China (No. 66).

All samples are stored at the Institute of Natural Medicine, Toyama Medical and Pharmaceutical University.

2.2. Extract preparation

According to Japanese Pharmacopoeia (JP XIV) [21]. Briefly, 0.125 g pulverized sample was extracted with 25 ml of 50% aqueous ethanol. Internal standard (cin-

namic acid) concentration within the sample was chosen as 0.005 mg/ml.

2.3. Chemicals

Glycyrrhizin (GL), glycyrrhetic acid (GA), liquiritin (LQ), glabridin (GLAB) and cinnamic acid (IS) as well as sodium dihydrogen phosphate and sodium dodecyl sulfate (SDS) were purchased from Wako Pure Chemicals Co., Ltd., Osaka, Japan, licochalcone A (LC_A), synthetic, from Merck Biosciences, Inc., La Jolla, USA, and sodium tetraborate from Nacalai Tesque, Kyoto, Japan. All other reagents were of analytical and/or HPLC grade.

2.4. Instrumentation

All CZE experiments were performed on a Beckman P/ACE system 5510 (Beckman Instruments, Fullerton, CA, USA) using a fused silica capillary (57 cm × 50 μm i.d., Beckman Instruments, Fullerton, CA, USA) with the detector window set at 50 cm; other conditions: injection mode pressure, 0.034 atm for 5 s; applied voltage 25 kV (constant voltage, positive to negative polarity); capillary cooling at 20 °C; detector wavelength set at 254 nm.

2.5. Rinse steps and buffer preparation

Capillary was conditioned prior to first use with 1 M HCl (5 min), pure water (3 min), 0.1 M NaOH (10 min), pure water (3 min) and with running buffer (10 min). Before every run the capillary was rinsed with pure water (2 min) and running buffer (3 min). After every run capillary was rinsed with pure water (2 min) and 0.1 M NaOH (3 min). Borate buffer was prepared daily and (if necessary) brought to desired pH 9.22 with 0.2 M NaOH. All solutions were passed through 0.45 μm filter before CZE analyses.

2.6. Calibration

GL, GLAB, GA, LQ and LC_A were separately calibrated in a range sufficient to the contents reported for the analyzed *Glycyrrhiza* species [4] and calibration equations were calculated. Five concentrations (0.02, 0.04, 0.1, 0.2, 0.4 mg/ml for GL, LQ; 0.002, 0.004, 0.01, 0.02, 0.04 mg/ml for GLAB, GA; 0.01, 0.03, 0.05, 0.1, 0.2 mg/ml for LC_A) were prepared and each concentration was measured six times. For limits of quantitation (LOQ) and detection (LOD) the amount of noise was estimated by analyzing a blank sample (50% aqueous ethanol) three times. Amount of noise was measured for each compound in the corresponding migration range.

2.7. Reproducibility

For this study, three samples were chosen: No. 60 for reproducibility of GL and LQ, No. 3 for GLAB, GA and No. 65 for LC_A. Samples were analyzed under the mentioned CZE conditions for 3 days, nine times each day (3× morning, 3× noon, 3× evening).

2.8. Recovery

Recovery study was examined for GL. Three amounts of standard GL (0.3, 0.8, 1.5 mg) were added to each 10 ml of Chinese sample No. 39 and filled up to 20 ml. The measured concentration was compared with the theoretical concentration to calculate the recovery rate.

2.9. Data treatment

Integrated peak areas of the five reference compounds GL, GLAB, GA, LQ and LC_A were corrected by migration time to exclude changes in velocity. Internal standard was applied to correct differences in injected sample volume and to avoid matrix effects.

2.10. Principal component analysis (PCA)

PCA on the basis of CZE peak area data of GL, GLAB, GA, LQ, LC_A and of the characteristic fingerprint peaks of *G. glabra* were examined for 65 samples by using Statistica 6.0 software (StatSoft, Tulsa, USA).

Fingerprint of *G. glabra* L.: from the 32 samples of *G. glabra* L., 1 ml each of the 50% aqueous ethanolic extracts were mixed and this solution was analyzed by CZE. The resulting electropherogram was examined for characteristic peaks.

2.11. HPLC experiments

For HPLC the same extracts were used as for CZE. Amount of GL in samples was determined according to JP XIV. Briefly, 20 μl reference standard solution (0.025 g/l GL in 50% aqueous ethanol) were injected and measured by HPLC five times, followed by 20 μl sample extract; flow (0.7 ml/min) was adjusted, so that GL eluted after 10 min; mobile phase: acetic acid – pure H_2O – acetonitrile (1:2:2, v/v/v) (pH 2.00); all solutions were ultrasonified and samples were filtered (0.45 μm) before analyses. HPLC experiments were performed on a Shimadzu C-R6A Chromatopac system, equipped with a Jasco 880-PU pump unit and a Jasco 875-UV UV–vis detector with wavelength set at 254 nm, column HiQsil C18V (4.6 mm × 250 mm, Kya Tech Corporation, Japan).

3. Results and discussion

3.1. Development of CZE method

Beginning with micellar electrokinetic chromatography (MEKC), phosphate and borate buffers with sodium dodecyl sulfate (SDS) as surfactant were tested. These buffers

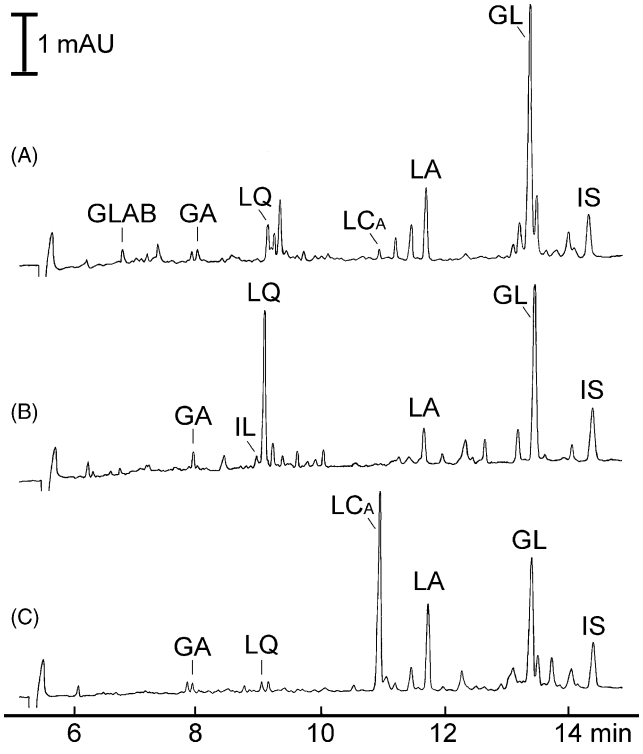

Fig. 2. Electropherograms of *G. glabra* (A), *G. uralensis* (B) and *G. inflata* (C); GLAB: glabridin, GA: glycyrrhetic acid, IL: isoliquiritin, LQ: liquiritin, LCA: licochalcone A, LA: liquiritin apioside, GL: glycyrrhizin, IS: internal standard cinnamic acid; (A) plant specimen No. 13, (B) cultivated plant specimen No. 56, (C) Chinese commercial licorice No. 65; CZE conditions: fused silica capillary [57 cm (50 cm effective length), 50 μm i.d.]; running buffer, 70 mM borate (pH 9.22); injection, pressure, 0.034 atm for 5 s; voltage, 25 kV (constant voltage, positive to negative polarity); temperature, 20 °C; detection, UV at 254 nm.

only allowed separation for GL and LQ. Organic modifiers (ethanol, methanol, acetonitrile) did not increase separation efficiency. During MEKC experiments, the species-specific GLAB in European samples as well as GA and LC_A could not be separated from other compounds. Applying capillary-zone electrophoresis (CZE) with only borate as buffer, the four selected reference compounds could be separated sufficiently by optimizing borate concentration (70 mM, pH 9.22), capillary temperature (20 °C) and voltage (25 kV, positive to negative polarity). Detector wavelength was set at 254 nm. Under optimum conditions, analysis was finished within less than 15 min (Fig. 2), whereas previous HPLC analyses of species used for Radix Glycyrrhizae took between 25 and 60 min [10,16–19]. Moreover, it was not necessary to use organic solvents as modifiers and thus an environmental-friendly analytical method was created.

3.2. Calibration

Calibration curves for the five reference compounds were linear across the examined concentration range. LOD (signal to noise ratio 3) ranged from 0.0005 to 0.0050 mg/ml, LOQ (signal to noise ratio 5) ranged from 0.0009 to 0.0064 mg/ml. Results have been summarized in Table 1.

3.3. Reproducibility

Reproducibility for the reference compounds was assessed in terms of relative standard deviation (R.S.D.) (Table 2). R.S.D. for GL was 1.95% (intraday) and 2.02% (interday). Due to its low content, maximum R.S.D. values (intraday 5.39%; interday 6.09%) were obtained for GLAB.

3.4. Recovery

Values for GL were between 96.31 and 104.32%, with an average recovery of 101.90 ± 2.54%.

3.5. CZE results

For all samples ($n = 66$), their contents (% of dry weight) of GL, GLAB, GA, LQ and LC_A were examined.

Under the described extraction and analytical conditions, European and Chinese samples were distinguished. On average, plant specimens of *G. glabra* and commercial licorices from Europe had quite comparable CZE patterns,

Table 1
Calibration data

Analyte	Average mobility (cm/V s)	Average T_m (min) (S.D.)	% R.S.D.
Glycyrrhizin	0.0001394	13.63 (0.19)	1.39
Glabridin	0.0002911	6.53 (0.11)	1.62
Glycyrrhetic acid	0.0002439	7.79 (0.02)	0.25
Liquiritin	0.0002157	8.81 (0.03)	0.35
Licochalcone A	0.0001742	10.91 (0.14)	1.31

Analyte	Concentration range (mg/ml)	Slope	Intercept	r^2	LOQ (S/N = 5) (mg/ml)	LOD (S/N = 3) (mg/ml)
Glycyrrhizin	0.02–0.4	25.023	0.0083	0.9995	0.00640	0.00498
Glabridin	0.002–0.04	34.594	0.0033	0.9963	0.00258	0.00157
Glycyrrhetic acid	0.002–0.04	44.360	0.0115	0.9994	0.00092	0.00050
Liquiritin	0.02–0.4	33.244	−0.0158	0.9994	0.00212	0.00159
Licochalcone A	0.01–0.2	70.981	−0.0890	0.9994	0.00177	0.00097

T_m: migration time; S/N: signal to noise ratio.

Table 2
Reproducibility data

Analyte	% R.S.D. – interday[a]	% R.S.D. – intraday[b]
Glycyrrhizin	2.02	1.95
Glabridin	6.09	5.39
Glycyrrhetic acid	4.05	3.56
Liquiritin	2.49	2.44
Licochalcone A	2.11	2.16

[a] Mean of 27 analyses over 3 days.
[b] Mean of daily means.

indicating botanical origin of the commercial licorices as *G. glabra* L. All Chinese samples [except Xinjiang-Gancao (No. 65)] showed similar electropherograms and reference compounds were detected in amounts as reported for *G. uralensis* [4]. European samples could mainly be distinguished from the Chinese by the presence of GLAB and only low LQ contents as well as lower GL contents on average. Apart from some European commercial licorices, GA was detected in only low amounts within the analyzed material. Also for LC_A, contents were relatively low, except Xinjiang-Gancao and *G. inflata* (No. 66).

The mean contents of all five reference compounds in European and Chinese samples have been summarized in Table 3.

3.6. Glycyrrhizin

Glycyrrhizin (GL) was the dominant compound in all samples. It is typical for underground parts of *G. glabra*, *G. uralensis*, *G. inflata* and also found in other species of this genus [4]. During a field survey of *G. glabra* in Europe (Sicily and Northern Spain) Hayashi et al. [9] detected GL contents between 0.7 and 4.4%. These data were within the range of GL contents of *G. glabra* in the present study (mean $2.39 \pm 1.05\%$, $n = 32$). Relatively low GL contents between 0.12 and 2.24% were found in Italian *G. glabra* [26].

Out of the 21 European commercial licorices, only 2 (No. 46 from Italy, No. 54 from Turkey) fulfilled the criterion of 4% minimum GL content according to the European Pharmacopoeia [20]. Compared to the three samples of *G. uralensis* from China, the seven Chinese commercial licorices had a higher GL content (mean $3.37 \pm 1.99\%$) on average and five of them fulfilled the criterion of 2.5% minimum GL content according to the Japanese Pharmacopoeia (JP XIV). Xinjiang-Gancao (origin estimated as *G. inflata*) with GL content of 2.20% is officially used in China but not mentioned as drug source for Radix Glycyrrhizae in the JP XIV. GL content of the only *G. inflata* plant specimen No. 66 was 5.81%.

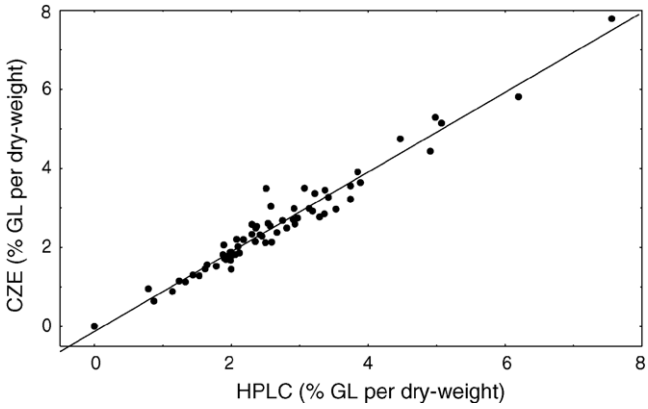

Fig. 3. Correlation of glycyrrhizin (GL) contents obtained by CZE and HPLC; calibration equation: $y = 1.009x - 0.129$; correlation coefficient $r = 0.977$.

GL contents obtained in Chinese licorices and plant specimens were comparable with those found in previous investigations [16,17]. However, Yamamoto and Tani [23], who investigated Chinese licorice over 15 years, found average GL contents for Seihoku-Kanzo ($4.36 \pm 1.45\%$) and Dongbei-Gancao ($5.16 \pm 1.00\%$), which were much higher than the present findings.

The GL contents of all samples analyzed in the course of CZE experiments were also analyzed by HPLC, applying the method described in the JP XIV. Overall, GL contents measured by CZE could be confirmed by HPLC, with a correlation coefficient of $r = 0.977$ (Fig. 3).

3.7. Glabridin (GLAB)

Glabridin (GLAB) was found in all 32 samples of *G. glabra* (mean $0.19 \pm 0.09\%$) and in 18 out of 21 European commercial licorices (mean $0.21 \pm 0.12\%$). GLAB is a species-specific, minor compound of *G. glabra* [4], for *G. uralensis* only traces were reported [24]. For European *G. glabra*, collected in Sicily (Italy) and Northern Spain,

Table 3
Mean contents (% per dry weight) of glycyrrhizin (GL), glabridin (GLAB), glycyrrhetic acid (GA) liquiritin (LQ) and licochalcone A (LC_A) in all samples

Sample[a]	GL	GLAB	GA	LQ	LC_A
G. glabra, plant spec., $n = 32$	2.39 ± 1.05	0.19 ± 0.09	0.09 ± 0.08	0.26 ± 0.17	0.05 ± 0.03
Comm. licorices, Europe, $n = 21$	2.55 ± 1.09	0.17 ± 0.12	0.33 ± 0.34	0.22 ± 0.12	0.12 ± 0.04
G. uralensis, plant spec., $n = 3$	2.17 ± 0.09	n. d.	0.06 ± 0.04	1.32 ± 0.19	0.02 ± 0.00
Comm. licorices, China, $n = 7$	3.37 ± 1.99	n. d.	0.09 ± 0.05	1.35 ± 0.40	0.05 ± 0.02
Comm. Xinjiang-Gancao[b], $n = 1$	2.20	n. d.	0.07	0.16	1.11
G. inflata, plant spec., $n = 1$	5.81	n. d.	0.06	1.10	0.32

[a] European *G. echinata* was excluded from calculations due to lacking all five reference compounds.
[b] Origin estimated as *G. inflata*; spec.: specimen, comm.: commercial, n.d.: not detected.

Hayashi et al. [9] found GLAB contents between 0.07 and 0.80%. GLAB ranging from 0.15 to 0.57% has also been found in *G. glabra* from China [17].

3.8. Glycyrrhetic acid (GA)

Glycyrrhetic acid (GA) was detected as minor compound in both the Chinese ($0.09 \pm 0.04\%$, $n = 10$) and the European samples ($0.21 \pm 0.26\%$, $n = 47$), except for a few commercial samples (e.g. No. 50 from Italy, No. 54 from Turkey) from Europe, which had contents of up to 1.19%.

3.9. Liquiritin (LQ)

Liquiritin (LQ) in minor quantities was found in all European samples ($0.24 \pm 0.15\%$, $n = 53$). According to former investigations, LQ contents in Chinese *G. glabra* between 0.15 and 0.47% were detected [16,17,19,25].

The Chinese samples all had higher LQ contents ($1.34 \pm 0.34\%$, $n = 10$) than the European samples, except for No. 65 (origin *G. inflata*) with 0.16%. LQ is a major compound, in particular of *G. uralensis* (0.6–3.7%) [4]. For *G. inflata*, LQ contents between 0.05 and 0.59% were reported [16,17,19,25]. Therefore, the present finding of 1.10% LQ in No. 66 *G. inflata* is unusually high.

The highest amounts of licochalcone A (LC_A) were contained in No. 66 *G. inflata* (1.11%) and No. 65 Xinjiang-Gancao (0.32%). In all other samples, LC_A contents were markedly lower.

In the Austrian cultivated plant specimen of *Glycyrrhiza echinata* L. (No. 26) neither the five observed reference compounds nor IL and LA were detected.

3.10. Principal component analysis (PCA)

PCA was applied to 65 samples (except for *G. echinata*) in order to investigate and visualize chemical relationship to each other (Fig. 4).

By applying PCA European and Chinese samples were distinguished. European commercial licorices were closely related to *G. glabra* plant specimens. Some of the European commercial licorices (No. 50 from Italy, No. 54 from Turkey) were positioned apart from *G. glabra* due to specific patterns of GL and GA. The same is stated for Chinese commercial licorice No. 58 with a maximum GL content of 7.79%. The other commercial licorice No. 65 Xinjiang-Gancao and plant specimen No. 66 *G. inflata*, both with high LC_A contents, were distinguished from other samples. The present PCA result of Xinjiang-Gancao obtained by CZE analysis is similar to that obtained by hierarchical cluster analysis using HPLC data [27].

Contribution of first and second principal component obtained, using the peak area data of GL, GLAB, GA, LQ and LC_A, was together 60.30%, representing variation within the data.

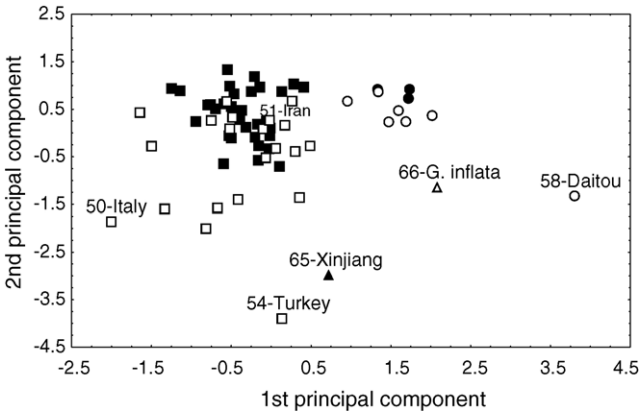

Fig. 4. European and Chinese samples; scatterplot of first and second principal component obtained by PCA on the basis of CZE peak area data of glycyrrhizin, glabridin, glycyrrhetic acid, liquiritin and licochalcone A; first principal component: eigenvalue 1.7755, contribution 35.51%; second p.c.: eigenvalue 1.2396, contribution 24.79%; (■) *G. glabra*, Europe, (□) commercial licorice, Europe, (●) *G. uralensis*, China, (○) commercial licorice, China, (▲) Xinjiang-Gancao, China, (△) *G. inflata*, China; No. 26 (*G. echinata*) was excluded from PCA.

3.11. CZE fingerprint of G. glabra

The fingerprint electropherogram of *G. glabra* was investigated and 17 peaks, including GL, GLAB, GA, LQ and LC_A, as well as LA, but not IL, were selected as characteristic, present in all 32 *G. glabra* specimens. Relative peak area variation (R.S.D.) was between 10 and 80%, revealing variable chemical composition within *G. glabra* L. from different regions in Southern Europe.

By applying PCA to all samples based on the 17 peak areas, the result was similar to the one illustrated in Fig. 4 and *G. glabra* could again be distinguished from *G. uralensis*. First and second principal component together represented 54.48% of data variation.

3.12. Comparison of glycyrrhizin contents in 50 and 70% aqueous ethanolic extracts

In the course of CZE experiments, GL contents in 50 and 70% aq. ethanolic extracts were tested. Both extracts were prepared from the same sample powder and compared under the same CZE conditions. Separate validation for GL using 70% aq. ethanol as solvent was completed. The comparison was carried out with the 32 *G. glabra* plant specimens and the 21 European commercial licorices.

Only 15 samples had 0.28% more GL in the 50% ethanolic extracts on average, whereas 39 showed higher GL contents in the 70% extracts, with an average difference of 0.39%. Therefore, it is considerable changing the extract preparation of licorice as described in the JP XIV in order to get licorice extracts yielding maximum GL content.

4. Conclusions

A CZE method for the analysis and comparison of Radix Glycyrrhizae from Europe and China was developed. By using 70 mM borate buffer, *G. glabra* and European commercial licorices were distinguished from *G. uralensis* and Chinese commercial licorices especially by phenolic compounds glabridin (only in *G. glabra*) and liquiritin (major contents in *G. uralensis*). Glycyrrhizin contents were on average higher in Chinese commercial licorices. The highest contents of licochalcone A were found in *G. inflata* and Xinjiang-Gancao, of which botanical origin was also estimated as *G. inflata*. Except for a few European commercial licorices, glycyrrhetic acid was only found in low amounts within the material analyzed. The new CZE method allows differentiation between *G. glabra* and *G. uralensis*, the two main sources for the herbal drug Radix Glycyrrhizae. Furthermore, higher glycyrrhizin contents were achieved by extract preparation with 70% aq. ethanol compared with 50% aq. ethanol according to the JP XIV.

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