Qualitative and Quantitative Characterization of Two Licorice Root Species (*Glycyrrhiza glabra* L. and *Glycyrrhiza uralensis* Fisch.) by HPTLC, Validated by HPLC and DNA Sequencing

Débora Arruda Frommenwiler*, Valeria Maire-Widmer, Roy Upton, Judith Nichols, Günther Heubl, and **Eike Reich**

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Licorice
***Glycyrrhiza glabra* L.**
***Glycyrrhiza uralensis* Fisch. ex DC.**
Quantification

Summary

The herbal drug licorice root may be derived from the plant species *Glycyrrhiza glabra* L., *Glycyrrhiza uralensis* Fisch, and/or *Glycyrrhiza inflata* Bat. which are morphologically, chemically, pharmacologically, and toxicologically similar. However, if an ingredient of a dietary supplement is identified as a certain species and labeled as such on the product, appropriate analytical methodologies are required to assure the authenticity. Using high-performance thin-layer chromatography (HPTLC), we were able to distinguish clearly between *G. glabra* and *G. uralensis,* the most commonly used species, which allowed us to check the corresponding label claims of twenty-six dietary supplements. Two samples of *G. inflata* Bat., which were available for the study, were not distinguished from *G. glabra* by this method. Our investigation revealed that five of the twenty-eight samples made a wrong label claim. The HPTLC results were confirmed by deoxyribonucleic acid (DNA) barcoding. For the quantitative analysis of the marker 18β-glycyrrhizic acid in licorice root, we modified our HPTLC method for base-line separation of the peaks which guaranteed accurate results. Moreover, the new method is also capable to identify and distinguish both species of licorice. The quantitative HPTLC results were in accordance with the data obtained by high-performance liquid chromatography (HPLC) following the United States Pharmacopeia (USP) method on licorice root. In addition, we used two DNA candidate barcodes (internal transcribed spacer [ITS] and *psbA–trnH* intergenic spacer) for species identification.

1 Introduction

Current good manufacturing practice (cGMP) for herbal medicines internationally and dietary supplements in the United States requires proper identification of the botanical ingredients used. Internationally, ingredients used in traditional medicines must conform to standards outlined in national or international pharmacopoeias. In the United States, there is no such requirement. Rather, the United States Food and Drug Administration's (FDA) cGMP for botanical dietary supplements requires that dietary supplements be made in a manner that ensures the quality of the product, which means "the dietary supplement consistently meets the established specifications for identity, purity, strength and composition and limits on contaminants...". The emphasis of FDA has been to ensure that manufacturers perform appropriate tests to establish the identity of all the ingredients [1, 2].

Based on a review of FDA warning letters and actions, meeting these basic GMP requirements is challenging for many companies. According to *Long* [3], approximately 19% of the dietary supplement companies subject to FDA inspection in 2015 failed to set adequate specifications. An additional 16% failed to verify the identity of a dietary ingredient through an adequate test.

Oftentimes, the identity of botanical materials in trade is not clear, both when common names and Latin binomial names are used. For example, many herbal products contain licorice root (*Glycyrrhiza* species [spp.]) as an ingredient. There are three primary species of licorice root that are accepted in international pharmacopoeias (see **Table 1**). These species are often not differentiated in trade or labeling and can be misidentified. In addition, there are *G. glabra*/*G. uralensis* hybrids. While the species are morphologically, chemically, pharmacologically, and toxicologically similar, if an ingredient is identified as a certain species, appropriate analytical methodologies are required to assure authenticity. Even when Latin binomial names are used, they are often applied based on presumptions of what is recorded in the literature, not based on an identification by a botanical authority.

In recent years, molecular methodologies (*e.g.,* deoxyribonucleic acid [DNA] barcoding) have received significant attention, often with a belief of their superiority over other analytical techniques [4]. However, unlike many other techniques, DNA barcoding can never be used as a single analytical technique for medicinal plant or dietary supplement ingredient assessment as DNA barcoding cannot discern plant parts, nor can it provide

D.A. Frommenwiler, V. Maire-Widmer, and E. Reich, CAMAG Laboratory, Sonnenmattstrasse 11, CH-4132 Muttenz, Switzerland; R. Upton, American Herbal Pharmacopoeia, PO 66809, Scotts Valley, CA, USA; J. Nichols, CAMAG Scientific Inc., 515 Cornelius Harnett Dr, Wilmington, NC 28401, USA; and G. Heubl, Systematic Botany and Mycology, Department Biology I, Ludwig-Maximilians-University Munich (LMU) and GeoBio-Center (LMU), Munich, Germany.
E-mail: debora.frommenwiler@camag.com

Table 1

Accepted names and quality standards of "licorice root" in compendial references.

Reference	Accepted species and standards
European Pharmacopoeia (Ph. Eur.) [6]	Dried root and stolon of either *Glycyrrhiza glabra* L. *Glycyrrhiza inflata* Bat. *Glycyrrhiza uralensis* Fisch. ex DC. or any combination of these three species Min. 4% of 18β-glycyrrhizic acid
United States Pharmacopeia (USP) [7]	Dried root, rhizome, and stolon of either *Glycyrrhiza glabra* L. *Glycyrrhiza uralensis* Fish. ex DC. Min. 2.5% of 18β-glycyrrhizic acid
Chinese Pharmacopoeia (ChP) [8]	Dried root and rhizome of either *Glycyrrhiza glabra* L. *Glycyrrhiza inflata* Bat. *Glycyrrhiza uralensis* Fisch. ex DC. Min. 2.0% of 18β-glycyrrhizic acid Min. 0.5% of liquiritin
Korean Pharmacopoeia (KP) [9]	Dried root and rhizome of either *Glycyrrhiza glabra* L. *Glycyrrhiza inflata* Bat. *Glycyrrhiza uralensis* Fisch. ex DC. Min. 2.5% of 18β-glycyrrhizic acid Min. 1.0% of liquiritin
Japanese Pharmacopoeia (JP) [10]	Roots and stolon of either *Glycyrrhiza glabra* L. *Glycyrrhiza uralensis* Fish. ex DC.

any indication of quality. Thus, DNA barcoding must always be coupled with other analytical (*e.g.,* botanical, macroscopic, microscopic, chemical) techniques to ensure both identity and compliance with GMP requirements [5].

Based on the cross-confirmation of the identity of numerous *Glycyrrhiza* root samples both chemically and genetically, we present a high-performance thin-layer chromatography (HPTLC) method that can be used to differentiate the roots of *Glycyrrhiza glabra* and *Glycyrrhiza uralensis* with certainty. Based on a very limited number of samples, *Glycyrrhiza inflata* cannot be distinguished by HPTLC but clearly by DNA barcoding.

A second method is proposed for the quantification of 18β-glycyrrhizic acid as a quality marker for licorice root. Together, these HPTLC methods can be applied to ensure compliance with the identity, strength, and composition requirements of cGMP and to ensure that non-conforming substitutions or adulterants are absent.

2 Experimental

2.1 Materials

A total of 28 samples of raw materials (whole, chopped, or powdered) of licorice root, labeled either as *G. glabra* (19), *G. uralensis* (7), or *G. inflata* (2), were provided by the American Herbal Pharmacopoeia (AHP, Scotts Valley, CA, USA), Phyto-Technologies Inc. (Woodbine, IA, USA), Western Botanicals (Spanish Fork, UT, USA), Botanicert (Grasse, France), and Tradall (Geneva, Switzerland), or acquired in local shops in India. 18β-Glycyrrhizic acid was provided by the US Pharmacopeial Convention (USP, Rockville, MD, USA). Only one sample of *G. uralensis* (S26, from AHP) was a vouchered, botanically confirmed sample and also previously identified by DNA sequencing.

Silica gel 60 F_{254} HPTLC plates (20 × 10 cm) were manufactured by Merck (Darmstadt, Germany). Solvents of high-performance liquid chromatography (HPLC) grade were purchased from Acros (Gent, Belgium), Roth (Karlsruhe, Germany), Sigma-Aldrich (Buchs, Switzerland), and Alcosuisse (Bern, Switzerland). For DNA sequencing, all primers were synthesized by MWG Biotech (Ebersberg, Germany). For DNA isolation and amplification, NucleoSpin Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), *taq* polymerase (Hybaid, AGS, Heidelberg, Germany), Phusion polymerase (Biozym Scientific GmbH, Hessisch Oldendorf, Germany), NucleoSpin Extract II Kit (Macherey-Nagel), and BigDye-Terminator v3.1 kit (Thermo Fisher Scientific, Waltham, MA, USA) were used.

2.2 Instruments

HPTLC equipment from CAMAG (Muttenz, Switzerland) was used, including: Automatic TLC Sampler ATS 4, Automatic Developing Chamber ADC 2 with humidity control, Scanner 4, Immersion Device 3, Plate Heater, and TLC Visualizer. Other equipment included a mill (IKA, Staufen, Germany), an ultrasonic bath (Sono Swiss, Ramsen, Switzerland), a centrifuge (Hettich, Tuttlingen, Germany), and miscellaneous glassware.

For HPLC, an UltiMate 3000 system from Dionex (Sunnyvale, CA, USA), consisting of a quaternary pump, an auto-sampler, a column oven and an ultraviolet–visible (UV–vis) absorbance detector (diode array detector [DAD]) was used. A 150 × 4.6 mm Kinetex C-18 (5 µm, 100 Å) analytical column from Phenomenex (Torrance, CA, USA) was used. For the DNA sequencing, an ABI 3730 sequencer (Thermo Fisher Scientific) was used.

2.3 DNA Extraction, DNA Amplification, and Marker Sequencing

Total genomic DNA was isolated from approximately 50 mg of dried root material from each sample. DNA extractions were performed using the NucleoSpin Plant Kit following the protocol given by the company, with the addition of a washing step with phenol and chloroform, to remove secondary compounds. For the amplification of the internal transcribed spacer (ITS) region, the forward primer aITS1 (5′-AGA AGT CCA CTG AAC CTT ATC-3′) and the reverse primer aITS4 (5′-CGC TTC TCC AGA CTA CAA TTC-3′) [11] were used. For the plastidal *psbA–trnH* region, the forward primer (*trnH* forward) [12] 5′-ACG GGA ATT GAA CCC GCG CA-3′ and the reverse primer (*Gly-trnHR1*) 5′-CAT ATG ACT TCA CAA TGT AAA ATC-3′ were applied to amplify the intergenic spacer region [13, 14].

Polymerase chain reaction (PCR) amplifications were performed using either the *taq* polymerase or the Phusion polymerase. The PCR reaction mixture contained: 10× PCR buffer, 5 µL;

dNTP mix (2.5 mM), 4 µL; forward primer (100 pmol/µL), 0.1 µL; reverse primer (100 pmol/µL), 0.1 µL; *taq* polymerase (1:10), 1 µL; DMSO, 2.5 µL; BSA 0, 5 µL; template DNA, 1 µL; H_20 bidest 40, 25 µL. The general PCR parameters were as follows: 94°C, 120 s; 40 cycles at 94°C, 30 s; 53°C, 30 s; 72°C, 75 s; and 72°C, 10 min. The PCR products were purified using the NucleoSpin Extract II Kit, following the manufacturer's protocol. Cycle sequencing was performed using the BigDye-Terminator v3.1 kit, and the products were run and analyzed on an ABI 3730 sequencer. Bidirectional sequence reads were obtained for all PCR products. For species identification, both a similarity analysis and a phylogenetic approach were used to compare the amplified barcode sequences to reference sequences in the NCBI GenBank (www.ncbi.nlm.nih.gov/genbank) and Barcode of Life Data System (BOLD). Similarity searches were conducted by using nucleotide Basic Local Alignment Search Tool (BLASTn). The sequences with a high similarity and maximum query coverage from NCBI were used to assign the identity of the *Glycyrrhiza* species.

2.4 Sample and Standard Preparation for Chromatographic Analysis

2.4.1 Sample Preparation

An amount of 500 mg of the powdered plant material was mixed with 10 mL (for the qualitative HPTLC analysis) or 100 mL (for the quantitative HPTLC and HPLC analysis) of 70% ethanol and extracted by sonication at room temperature (23°C) for 10 min. Following centrifugation for 5 min, the supernatant was used as the test solution.

2.4.2 Standard Preparation for Quantitative HPTLC–HPLC Analysis

A stock solution of 18β-glycyrrhizic acid was prepared in a concentration of 0.5 mg mL^{-1} in 70% ethanol. The working solutions were prepared from the stock solutions by dilution to the following concentrations: 0.10 mg mL^{-1}, 0.125 mg mL^{-1}, 0.15 mg mL^{-1}, 0.20 mg mL^{-1}, and 0.25 mg mL^{-1}.

2.5 HPTLC Conditions

The general standard operating procedure (SOP) for HPTLC, as previously published [15] was followed.

Samples and standards were applied onto plates as 8-mm bands, 11.4 mm apart, 8 mm from the lower edge, and 20 mm from the left plate edge. The application volume was 2 µL for samples and standards.

The plates were developed to a distance of 70 mm by means of the ADC 2 lined with filter paper for chamber saturation. For identification, the developing solvent consisted of ethyl acetate, formic acid, glacial acetic acid, water (15:1:1:2, *v/v*). For the HPTLC quantitative assay, the developing solvent consisted of dichloromethane, methanol, water, formic acid (12:7.5:1.5:0.1, *v/v*). Before development, the plates were conditioned to 33% relative humidity using a saturated solution of $MgCl_2$. HPTLC plates were documented and evaluated prior to derivatization under UV light at 254 nm.

For derivatization, sulfuric acid reagent was prepared by mixing 20 mL of sulfuric acid with 180 mL of ice-cooled methanol. The plate was immersed into the reagent for 1 s and then heated at 100°C for 10 min. The derivatized plates were documented and evaluated under UV 366 nm and white light.

For the HPTLC quantitative measurements, the developed plate (prior to derivatization) was scanned at 254 nm in absorbance mode at 20 mm s^{-1}, data resolution of 100 µm $step^{-1}$, and slit size of 5 × 0.2 mm.

2.6 HPLC Conditions

The HPLC chromatographic parameters were taken from the USP39/NF34 monograph on powdered licorice: the isocratic mobile phase, consisting of acetonitrile and 0.66% acetic acid in water (3:2), was filtered through a 0.45-µm membrane filter under vacuum. The flow rate was 0.6 mL min^{-1}, and the injection volume was 20 µL. The chromatograms were recorded at UV 254 nm.

3 Results and Discussion

In our previously published article [16] on a general approach for the validation of HPTLC identification methods, we proposed a validated HPTLC method for the identification of licorice root. Only a limited number of samples had been available at that time, and small differences in the fingerprints of *G. glabra* and *G. uralensis* did not correlate with the name on the label. This result seemed to be in line with the acceptance of both species in the context of most pharmacopoeias.

In a further investigation, we tested 28 additional samples of licorice root (and a larger number of samples labeled as *G. uralensis*) with this method and found that introducing additional detection modes, such as evaluation of the chromatographic plate under UV 366 nm after derivatization, can lead to a much better differentiation between the individual species **(Figure 1C).** Under UV 254 nm prior to derivatization **(Figure 1A),** the sample labeled as *G. uralensis* (Gu) shows a quenching zone at R_F 0.43 (red arrow), which is absent in *G. glabra* (Gg). Under white light after derivatization **(Figure 1B),** two yellow zones are seen between R_Fs 0.4 and 0.5 in the sample of *G. uralensis* (Gu; black arrows), while the same zones are faint and diffuse in *G. glabra* (Gg). Under UV 366 nm after derivatization (Figure 1C), the sample of *G. uralensis* (Gu) shows two blue zones between R_Fs 0.25 and 0.35 (blue bracket) as well as one green and one blue zone between R_Fs 0.4 and 0.5 (green bracket), while the sample of *G. glabra* (Gg) shows no intense zone at those positions. In **Figure 1D,** where the image under UV 366 nm after derivatization is converted into profile, the intensity of the peaks between R_Fs 0.3 and 0.6 in Gu is higher than these in Gg (brackets).

Of the 28 analyzed samples, 12 were labeled as *G. glabra* (samples S1–S12; tracks 1–12; **Figure 2**). The fingerprints of those samples were consistent and different from those of seven other samples labeled as *G. uralensis* (samples S20–S26; tracks 20–26; Figure 2) which were also consistent. Seven samples were labeled as *G. glabra* (samples S13–S19; tracks 13–19; Figure 2), but their fingerprints were matching those of *G. uralensis*. The two samples labeled as *G. inflata* cannot be distinguished from *G. glabra* by means of HPTLC analysis.

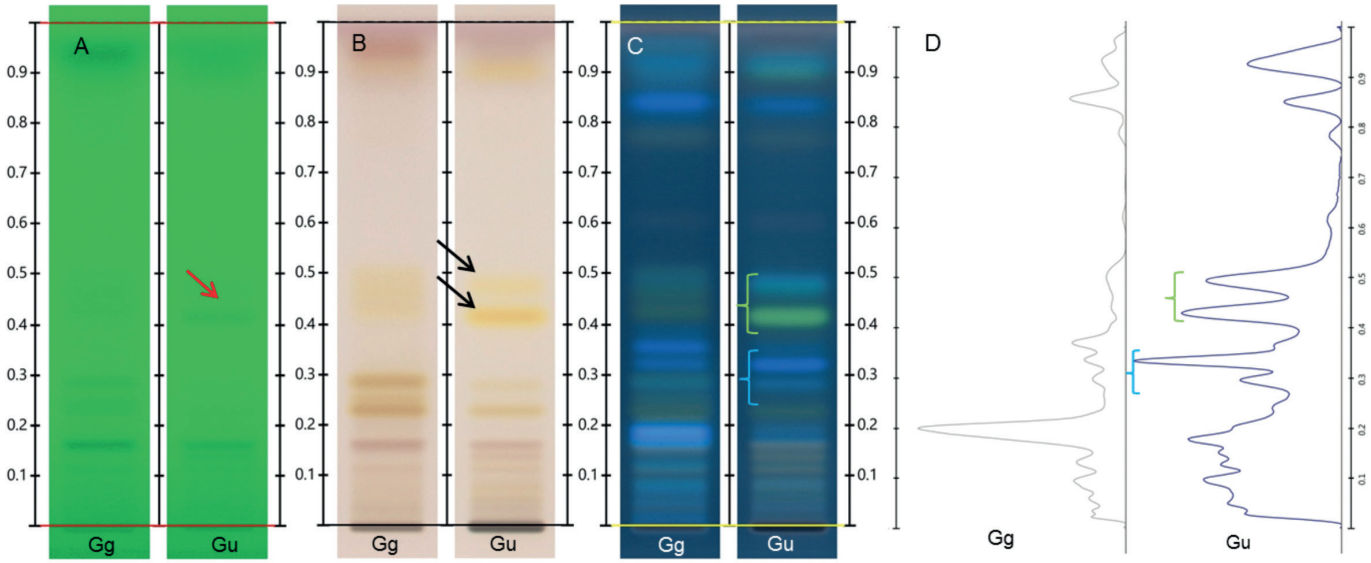

Figure 1

HPTLC chromatograms of typical samples of *Glycyrrhiza glabra* (left; Gg) and *Glycyrrhiza uralensis* (right; Gu) under UV 254 nm (A), and after derivatization with sulfuric acid reagent under white light (B) and UV 366 nm displayed as image (C) or profile (D).

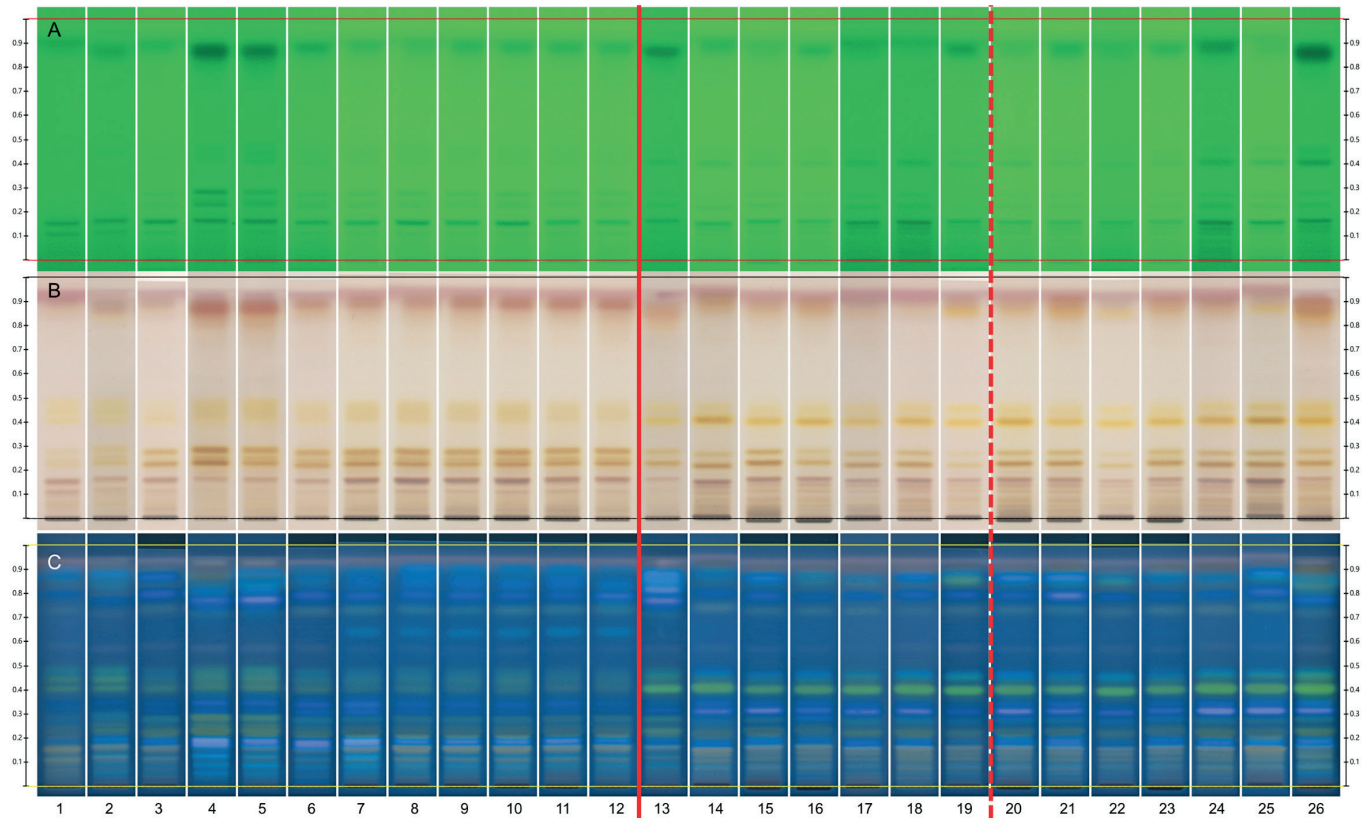

Figure 2

Comparison of fingerprints of all analyzed samples under UV 254 nm (A), after derivatization with sulfuric acid under white light (B) and UV 366 nm (C). Tracks 1–19, licorice samples labeled as *G. glabra* (samples S1–S19); tracks 20–26, licorice samples labeled as *G. uralensis* (samples S20–S26).

At this point, it is not clear whether the chemical profile of these two samples is representative for the species. *Simmler et al.* [14] successfully distinguished *G. uralensis, G. glabra,* and *G. inflata* by ultra high-performance liquid chromatography (UHPLC)–UV, based on the abundance of flavones and chalcones in each species in addition to the evaluation of species-specific metabolites (glabridin for *G. glabra,* licochalcone A for *G. inflata,* and glycycoumarin for *G. uralensis*). They also performed tests by nuclear magnetic resonance (NMR) combined with principal component analysis (PCA), as a more

holistic approach to detect UV–visible and invisible metabolites, which also distinguished the three species. However, as the chemical profile of plants varies naturally, it is difficult to compare the results of studies that are based on different and limited sets of samples. Glabridine, the positive marker for *G. glabra,* co-elutes with other zones just below the solvent front and thus is not suitable to distinguish *G. glabra* from *G. inflata* in our method.

To further investigate the identity of the questionable samples, we used comparative DNA sequencing. Twenty-one samples were analyzed: 8 of the 12 samples identified by HPTLC and labeled as *G. glabra* (samples S1–S6 and S11–S12; tracks 1–6, 11, and 12; Figure 2), 6 of the 7 samples identified by HPTLC and labeled as *G. uralensis* (samples S20–S24 and S26; tracks 20–24 and 26; Figure 2), and 6 of the 7 samples identified by HPTLC as *G. uralensis* but labeled as *G. glabra* (samples S13–S17 and S19; tracks 13–17 and 19; Figure 2) and one sample labeled as *G. inflata*.

Kondo et al. [13] and, in recent time, *Simmler et al.* [14] analyzed four DNA regions in their studies on species identification of licorice using genetic markers, the nuclear ITS region, the plastidal *rbcL* and *matK* genes, and the *psbA–trnH* intergenic spacer. Following this framework, nine genotypes (TG1–TG9 plus amphipathic dependent degradation [ADD] genotype which is typical for hybrids) were recognized as combinations of the sequence data obtained from the four DNA regions (see **Table 2**). Following this concept, *G. uralensis* is defined by four genotypes (TG6–9), *G. glabra* is characterized by two genotypes (TG2, TG3), and *G. inflata* comprises also 2 genotypes (TG4 and TG5). The sequence alignments of each DNA marker suggested that reliable identification of *Glycyrrhiza* species is even possible using only the genetic information obtained from the ITS and *trnH–psbA* intergenic region.

Our DNA barcoding analyses based on a combination of ITS and *psbA–trnH* confirmed that the sample labeled as *G. inflata* showed DNA sequences identical to the TG4 genotype (allele combination I-2/T-3) which are indicative for *G. inflata*. Eight samples labeled as *G. glabra* (tracks 1–6, 11, and 12) are characterized by the TG3 genotype (allele combination I-2/T-2) which confirms the correct labeling of this species. Five samples labeled as *G. glabra* (tracks 14–17 and 19; Figure 2) have DNA sequences identical with the *G. uralensis* barcode corresponding to the TG6 genotype (allele combination I-3/T-1) [13] (see Table 2). The HPTLC fingerprints which show also the *G. uralensis* pattern confirm the wrong labeling of these samples. Five samples labeled as *G. uralensis* (tracks 20–22, 24, and 26) show DNA sequences which correspond to the TG6 genotype (allele combination I-3/T-1) typical for *G. uralensis*. These findings are highly supported by the HPTLC fingerprint analyses (see Figure 2). One questionable sample, which shows an HPTLC fingerprint similar to that of *G. uralensis,* but is labeled as *G. glabra* (track 13; Figure 2), and one sample labeled as *G. uralensis* (track 23; Figure 2), which shows an HPTLC fingerprint in compliance with this species, exhibit a DNA sequence which is characterized by overlapping peaks at the specific sites in the ITS chromatograms. It is hypothesized that these samples may be hybrids with an ADD allele type also reported by *Kondo et al.* [13]. Within this ADD type, different nucleotides were observed at four variable sites: Y (C or T) at position 187 and yeast rough microsomes (YRM) (C or T, A or G, A or C) at position 411–413 in the ITS sequence. Therefore, it was considered that the ADD type is a combination of the I-2 (T, CAA) and I-3 (C, TGC) alleles.

In addition to proper identification, cGMP requires the determination of "strength", *i.e.,* by assaying the content of selected marker compounds, as one of the quality proofs for an herbal

Table 2

DNA data and total genotypes (TGs) defined by *Kondo et al.* [13] used for authentication of medicinal licorice *(Glycyrrhiza)* species based on the nuclear ITS region and the plastidal psbA–trnH intergenic spacer. The ADD genotype characteristic for some hybrids is a combination of the alleles I-2 and I-3. Specific sites in the ITS and *psbA–trnH* intergenic region contributing to the identification of the alleles I-2, I-3, T-1, T-2, and T-3 are indicated.

		DNA Marker	
Species	Genotype (TG)	ITS allele	*psbA–trnH* allele
G. glabra	TG3	I-2	T-2
G. uralensis	TG6	I-3	T-1
G. inflata	TG4	I-2	T-3

	Nucleotide sequence				
Allele type	ITS site		*psbA–trnH* site		
	187	411–413	72	125	171
I-2	T	CAA			
I-3	C	TGC			
ADD	Y	YRM			
T-1			C	A	T
T-2			C	A	G
T-3			T	A	T

product. This is to ensure that the herbal ingredient has at least the same potency as specified in the monograph, thus avoiding the use of "weaker" or "bad quality" material. The current monographs on licorice root of the European Pharmacopoeia [6], the United States Pharmacopeia [7], the Korean Pharmacopoeia [9], and the Pharmacopoeia of the Peoples Republic of China [8] include HPLC assays of markers for this purpose (Table 1), typically 18β-glycyrrhizic acid.

In practice, an additional analytical test requires more time, material, equipment, and expertise to prepare samples, analyze them, and interpret the results. As a consequence, the cost of "ensuring good quality of a product" increases dramatically. To simplify the quality-control process, we attempted to use the HPTLC identification method also for quantifying the marker 18β-glycyrrhizic acid in licorice root. However, the corresponding zone (between R_Fs 0.1 and 0.2) was not sufficiently separated from a neighboring peak (with lower R_F value) under UV 254 nm (blue profile; **Figure 3**) and the concentration of the sample solutions was out of the linear range.

Therefore, a new HPTLC method for the assay of 18β-glycyrrhizic acid was developed. For this test, five samples of *G. glabra,* five samples of *G. uralensis,* and five samples with the wrong species name on the label were utilized. The test solutions were diluted 1:10 to fit in the linear calibration range and analyzed by HPTLC using the mobile phase dichloromethane–methanol–water–formic acid (12:7.5:1.5:0.1, *v/v*). The solutions to establish a calibration curve, with five concentration levels, were applied on each plate. After development, first, an electronic image of the chromatogram was taken, and then it was evaluated by densitometry at UV 254 nm. The new method affords sufficient separation of the target compound 18β-glycyrrhizic acid from other constituents **(Figure 4).** To test the reliability of the HPTLC quantification, the same samples and

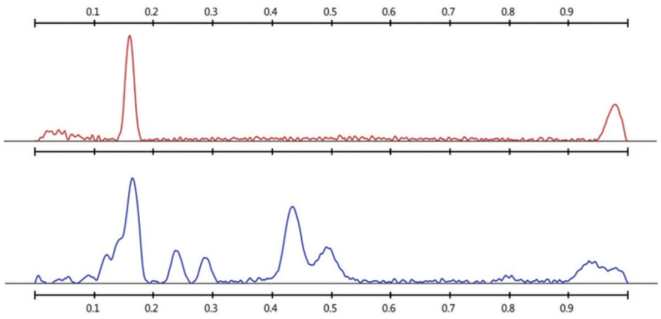

Figure 3

Image of the profiles under UV 254 nm; mobile phase ethyl acetate–formic acid–glacial acetic acid–water (15:1:1:2, *v/v*); red profile: 18β-glycyrrhizic acid; blue profile: *Glycyrrhiza glabra* root sample.

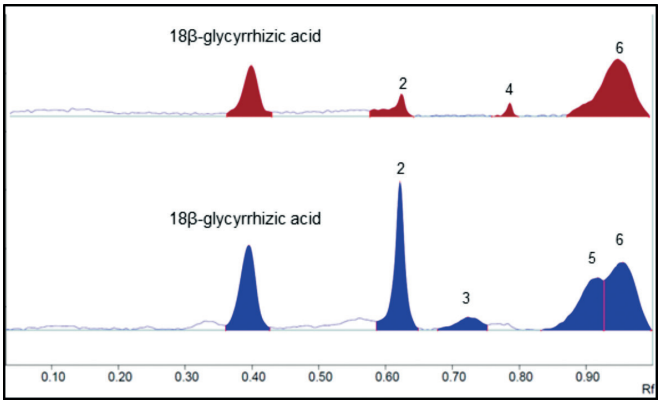

Figure 4

Densitograms of 18β-glycyrrhizic acid (0.25 mg mL^{-1}; red) and a *G. glabra* sample (blue) under UV 254 nm after development with the mobile phase dichloromethane–methanol–water–formic acid (12:7.5:1.5:0.1, *v/v*). Peaks 2, 4, and 6 are due to secondary fronts in the chromatogram.

Table 3

Calibration data obtained by HPTLC and HPLC using the same set of standards.

	HPTLC	HPLC
Regression mode	Linear	Linear
Calibration function	$y = 307.99x + 153.79$	$y = 23.219x - 3.3717$
Coefficient of variation (CV)	3.08%	1.61%
Correlation coefficient (R^2)	0.9920	0.9989

Table 4

Amount of 18β-glycyrrhizic acid found in 15 licorice samples, expressed in percentage of the powdered drug.

HPTLC data			HPLC data			HPTLC–HPLC correlation
Samples	Area	%	Samples	Area	%	
std 2%	42.32	2.00	std 2%	757.79	2.00	–
std 2.5%	54.52	2.50	std 2.5%	913.33	2.50	–
std 3%	67.03	3.00	std 3%	1081.88	3.00	–
std 4%	90.65	4.00	std 4%	1439.49	4.00	–
std 5%	111.74	5.00	std 5%	1658.26	5.00	–
S09	68.89	3.92	S09	1362.53	3.11	1.26
S10	109.66	6.20	S10	2062.40	4.87	1.27
S11	63.61	3.57	S11	1251.84	2.88	1.23
S12	90.03	5.15	S12	1740.57	4.02	1.28
S13	55.07	3.10	S13	1108.81	2.52	1.23
S16	63.54	3.50	S16	1235.31	2.88	1.21
S17	102.91	4.75	S17	1637.64	4.58	1.03
S18	34.12	2.05	S18	763.45	1.61	1.27
S20	48.47	2.99	S20	1069.25	2.23	1.34
S21	28.42	1.53	S21	595.49	1.37	1.11
S22	49.00	2.99	S22	1074.32	2.26	1.32
S23	34.01	2.07	S23	790.81	1.61	1.28
S24	18.17	1.16	S24	511.37	0.93	1.24
S25	18.17	1.69	S25	674.23	1.40	1.20
S27	76.41	4.15	S27	1432.92	3.44	1.20

standard solutions used for HPTLC were evaluated by HPLC, using the chromatographic conditions as described in the USP monograph on licorice root. More information is displayed in **Table 3.**

The content of 18β-glycyrrhizic acid determined in 15 samples by HPLC and HPTLC is presented in **Table 4.** Both sets correlate well, the HPTLC data being 1.2 times higher at an average. Using HPTLC, 5 of the 15 samples show a value below the 2.5% limit of the USP monograph (samples S18, S21, S23, S24, and S25). Using HPLC, seven samples fail (samples S18, S20, S21, S22, S23, S24 and S25). HPTLC and HPLC data for quality compliance with pharmacopoeial standards are not in agreement for two samples.

4 Conclusion

Using HPTLC, the most widely used two species of licorice root, *G. glabra* and *G. uralensis,* can be clearly distinguished. The method is suitable to check label claims concerning the presence of those species in a dietary supplement. *G. inflata* and *G. glabra* show the same fingerprint and cannot be distinguished. The DNA of the only *G. inflata* sample analyzed shows sequences characteristic of *G. inflata* and *G. glabra*. Further studies on a large sample population may change that situation. An investigation of 28 samples revealed that 7 samples made a wrong claim on the label. All of them claim to be *G. glabra,* but were identified as *G. uralensis* by HPTLC.

The ability of HPTLC to correctly establish the identity of 2 licorice species was confirmed by DNA barcoding. HPTLC is also able to quantify the content of 18β-glycyrrhizic acid in licorice samples. The HPTLC results correlate with the data obtained by HPLC following the USP method on licorice root.

Compared to other published methods, HPTLC represents a much simpler and very cost-efficient approach to the identification of licorice root.

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