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Application of microemulsion thin layer chromatography for the fingerprinting of licorice (*Glycyrrhiza* spp.)

Shufen Cui [a,b], Boqiang Fu [a], Frank Sen-Chun Lee [c], Xiaoru Wang [a,c,*]

[a] *The Key Laboratory of Analytical Science of MOE, The College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, China*
[b] *Department of Biological Applied Engineering, Shenzhen Polytechnic, Shenzhen, China*
[c] *Qingdao Key Lab of Analytical Technology Development and Standardization of Chinese Medicines, First Institute Oceanography of SOA, Qingdao, China*

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Abstract

Microemulsion thin layer chromatography (ME-TLC) has been developed for the fingerprinting of aqueous extract of licorice (*Glycyrrhiza* spp.). The separation conditions and operational processes of the method have been optimized, and its chromatographic characteristics compared with conventional TLC. The ME-TLC system is easier to operate, and with higher resolution and better reproducibility than the conventional TLC. The separation mechanism and retention behavior of ME-TLC are found to differ significantly from conventional TLC. The technique has been applied to the analysis of different licorice species including *G. uralensis*, *G. glabra* and *G. inflata*; and to monitor the dynamic accumulation of active ingredients in licorice plant harvested at different times during its growing cycle in a Good Agriculture Practice (GAP) research farm. Results show that without post-chromatographic derivatization, the ME-TLC fingerprinting images of different species appear as clear, well resolved bands and with strong intensities to reveal distinctively different compositional features of the samples. The technique has also been applied successfully to monitor the dynamic accumulation of active components in licorice plant as a function of growing time in an experimental licorice farm. The study demonstrates the potential of ME-TLC technique as a rapid fingerprinting tool for the authentication and quality assessment of licorice as well as other herbs.
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Keywords: Microemulsion thin layer chromatography; Licorice; Characteristic; Fingerprint

1. Introduction

For routine compositional analysis and quality screening of medicinal plants, thin layer chromatography (TLC) is often the method of choice when many samples have to be compared, when flexibility is of importance, and when rapid qualitative and semi-quantitative data are needed at low cost per sample. In the last few years, there has been a tremendous increase in regulatory activities in the herbal industry, and the demand for analytical methods that can help to ensure safety and quality has been growing in an accelerated pace [1–3]. As a result, a wide variety of TLC methods have been developed and successfully applied to the study of herbal drugs or implemented into pharmacopoeias all over the world.

Flexibility is one of the inherent advantages of TLC method, where a series of operational parameters such as sample application, plate development, derivatization, documentation, etc. can be optimized individually and independently. On the other hand, unless these parameters are carefully controlled and the analytical protocols well standardized, results in TLC analysis are often difficult to reproduce [4,5]. Although the reproducibility of TLC techniques has been improved significantly in recent years through the application of high-performance techniques (HPTLC) [6–8], there remains the need to develop better and standardized TLC method in real world applications [4,5,9,10]. In the past, inadequate reproducibility and relatively low resolution have been the two major factors hinder the widespread use of TLC in the analysis or quality control of herbal materials

* Corresponding author. Tel.: +86 532 8893253; fax: +86 532 8893253.
E-mail address: mt2elp@fio.org.cn (X. Wang).

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on a routine basis. In general, specific sample pretreatment (clean-up step) procedures, and the type and saturation of the developing chamber are crucial in achieving satisfactory TLC fingerprinting results [5]. These operational processes are often tedious and difficult to control. Thus, the purpose of this work is to develop a new TLC method (microemulsion TLC, ME-TLC) aiming towards the circumvention of these deficiencies. As will be described in the following text, the ME-TLC technique developed in our study demonstrates several attractive features including high peak capacity, unique separation selectivity and enhanced ultraviolet and fluorescent detection capabilities. In addition, the technique is simple and easy to operate, and thus can be readily adopted for the routine screening or quality control of herbal materials.

Microemulsions are macroscopically homogeneous, optically fully transparent fluids having more than one liquid phase. The first description of a transparent mixture of water, a hydrocarbon and a suitable hydrophilic solvent dated back to 1943 [11], and it was not until 1959 [12] that such mixtures were named specifically by the term "microemulsions". The high solubilizing ability of these emulsions [13] has been extensively utilized in the industry [14,15]. In separation sciences, microemulsions were first used in high-performance liquid chromatography (HPLC) [16]. In 1991 [17], microemulsion as a running buffer was used successfully in capillary electrophoresis (CE). Since then the total number of papers published in the area of microemulsion electrokinetic chromatography (MEEKC) has now exceeded 100. Contrary to this, microemulsion has not been applied to TLC until year 2000 [18], and so far only very few papers have been published on the subject [19–21]. In this study, the unique chromatographic characteristics of ME-TLC have been investigated in detail. Based on the findings, a simple and easily controlling ME-TLC technique has been developed. With its improved resolution and reproducibility, the developed technique further enhances the inherent advantages of conventional TLC in operational simplicity, low cost, high throughput and analytical speed.

Licorice is one of the most popular and widely consumed herbs in the world. Worldwide, it is used primarily as flavoring and sweetening additives in food products [22–24]. In the Orient, it is used extensively in medicinal formulations, and is the second most prescribed herb in China following Ginseng [25]. Licorice is the root of *Glycyrrhiza* spp. The best studied bioactive constituent found in the root of licorice is glycyrrhizin [26,27], among other components including various sugars (to 14%), starches (30%), flavonoids, sterols, amino acids, gums and essential oil [22]. Three plant species of licorice including *G. uralensis*, *G. inflata* and *G. glabra* are embodied by China Pharmacopoeia as a drug. The most popular species of licorice used in traditional Chinese herbal medicines is *G. uralensis*, which is mainly distributed in Inner Mongolia, Ganshu and Shingkiang provinces.

The developed ME-TLC technique has been used to analyze and compare the compositions of different plant species of licorice including *G. uralensis* Fisch., *G. inflata* Bat. and *G. glabra* L., and the same species of *G. uralensis* Fisch. but harvested after different growing times.

The results demonstrate that ME-TLC has the potential to be further developed as a rapid and effective fingerprinting and screening technique for the authentication and quality assessment of licorice as well as other herbs.

2. Experimental

2.1. Apparatus

Grant XB14 ultrasonic cleaners (Grant Instruments, Cambridge, UK) were used for all extractions. Sample solutions were applied onto the plates with a Linomat V semi-automated sample applier (Camag, Muttenz, Switzerland), controlled by WinCATS software. Plates were developed in the twin trough chamber (Xinyi, Shanghai, China). A TLC Scanner III with WinCATS software (Camag, Muttenz, Switzerland) was used for scanning the TLC plates. A ReproStar 3 with VideoStore 2 documentation software (Camag, Muttenz, Switzerland) was used for the imaging and archiving the TLC chromatograms. The polyamide layer sheets were purchased from TZSHSL (Taizhou, China).

2.2. Herbal materials and chemicals

All the herbs were received as gifts from Elion Bio-Pharmaceutical Company (Inner Mongolia, China). Among them, the samples of *G. uralensis* for monitoring the dynamic accumulation of active ingredients were collected in the semi-wild licorice field of Kong-Mei GAP Farm (GAP stands for Good Agriculture Practice, see reference [28]), Liang-Wai region, Inner Mongolia and the plant samples were collected during a 1-year growing cycle from July 2002 to June 2003, with the exception of the winter freeze-up period (November 2002 to February 2003). Marker compounds including glycyrrhizin (75%), 18β-glycyrrhetinic acid (98%), liquiritin (96.4%), licuraside (97%), licochalcone A (99.1%) and inflacoumarin A (99%) were separated and purified by the procedure as described in previous papers from our laboratory [29,30]. The chemical structures of the studied marker compounds are given in Fig. 1. All other reagents were of analytical reagent grade. Water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) water purification system.

2.3. Preparation of standard solutions

Individual standard solutions containing, respectively, 2.0 mg/ml of glycyrrhizin, 2.2 mg/ml of 18β-glycyrrhetinic acid, 2.0 mg/ml of liquiritin, 1.0 mg/ml of licuraside, 1.0 mg/ml of licochalcone A and 1.0 mg/ml of inflacoumarin A were all prepared in methanol solutions for general analysis.

2.4. Preparation of samples

The powdered dried roots (0.5 g) of licorice were mixed with 40 ml of methanol. The mixture was soaked for 4 h at room temperature, and then placed in an ultrasonic bath for 30 min for licorice extraction. The extract was then concentrated to 1 ml

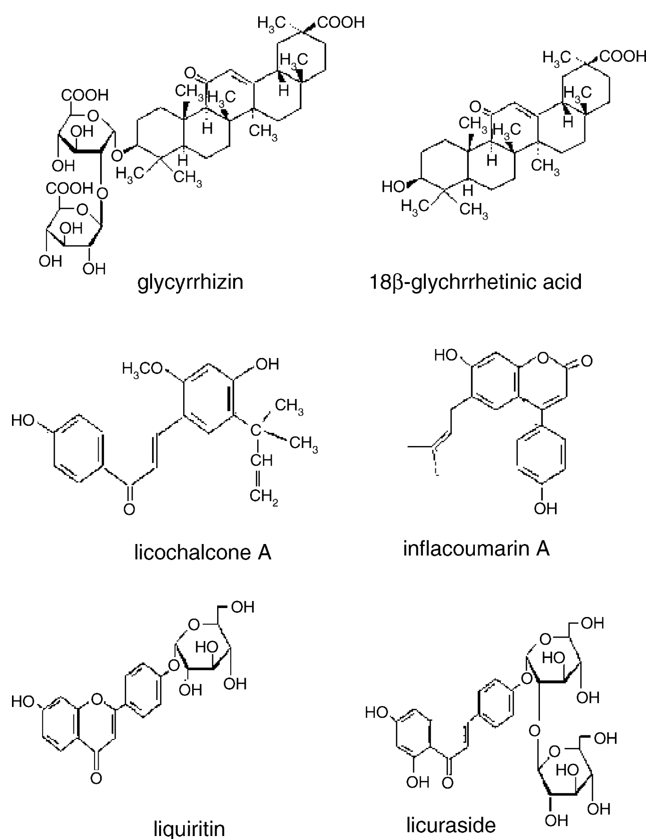

Fig. 1. Chemical structure of markers in licorice.

and filtered through a 0.45 μm filter. The filtrates obtained were used for sample application.

2.5. Preparation of microemulsion and conventional mobile phases in TLC separation

The effect of microemulsion composition on separation efficiency has been evaluated. Details of the optimization process will be published in a separate paper (in preparation). The optimum solvent system used as microemulsions in this study was prepared by mixing 2.8 ml of *n*-heptane, 19.0 ml of 1-butanol, 75 ml water and 7.7 g of sodium dodecylsulfate (SDS) in a beaker by swirling for 1–2 min. Afterwards, 14.6 ml formic acid was added to the beaker and the solution was then sonicated for 30 min to produce a transparent microemulsion solution. The emulsion was found to be stable for at least 4 months at ambient temperature.

The mobile phase for the conventional TLC consisted of a mixture of ethyl acetate–formic acid–acetic acid (17:1:1, v/v/v). The solvent system must be used within 1 day.

2.6. TLC procedures

About 1.5 μl of each sample and reference solution was spotted on a polyamide thin layer plate with the help of a semi-automatic sample applier. The plates were developed in the chosen developing system by the ascending technique. The solvent ascent was fixed at the height of 9.5 cm for identification and chromatographic characteristics evaluation, and 15 cm for monitoring tests, respectively. After development, the plates were withdrawn from the chambers and dried at room temperature. The developed plates were scanned by both 254 nm UV absorption and fluorescence detections to obtain thin layer chromatography scanning (TLCS) fingerprint profiles. The photographic TLC images were also acquired using the ReproStar 3.

2.7. Validation of ME-TLCS fingerprinting assay

In accordance with the guidelines of SFDA of China [31], the developed TLC fingerprinting method has been validated based on its performance in the three parameters of precision, reproducibility and stability, as described below.

2.7.1. Precision assay

Precision mainly evaluates the measurement precision of the equipment, expressed as the relative standard deviation (R.S.D.) of peak area ratios (or peak heights) and relative Rf values. The values are obtained by multiple measurements of a sample solution on the same equipment. The analysis of each sample was repeated at least five times.

2.7.2. Reproducibility assay

Reproducibility is expressed as R.S.D. of the ratio of peak areas (or peak heights) and the relative Rf value. Using at least five samples of the same batch, they are prepared and analyzed by the method under constant conditions.

2.7.3. Stability

Stability mainly evaluates the stability of the sample solution. Expressed as R.S.D. of peak area ratios (or peak heights), and relative Rf values, the determination is accomplished by multiple measurements of the same sample solution at different times.

3. Results and discussion

3.1. Characteristics of microemulsion TLC process

An effective chromatographic fingerprinting method must be accompanied by proper sample pretreatment and extraction procedure, optimized operational conditions and standardized experimental protocols (including materials and facilities). In microemulsion TLC, sample preparation is benefited by the high solubilizing ability of the microemulsion system. In ME-TLC, the samples are extracted by pure methanol, and the extract requires no further clean-up process before chromatography. Whereas in conventional TLC, the extract has to be further cleaned up by rather complicated pre-treatment process before satisfactory TLC chromatogram can be obtained [27].

TLC differs from all other chromatographic techniques in that a vapor phase is present in addition to stationary and mobile phase [32]. The vapor phase can significantly influence the result of separation. The vapor environment inside the developing chamber, e.g. the extent of saturation, chamber pre-conditioning time, the shape and configuration of the TLC set-up, etc. can all

affect the reproducibility of the separation. In conventional TLC, these parameters are very difficult to control precisely because of the use of mixed organic solvent systems. In microemulsion TLC, the main component of the developing solvent is water and the vapor phase is relatively constant. The vapor environment thus plays only a minor role on the separation effect, and the run to run reproducibility is substantially improved. This is demonstrated by our result which shows the absence of effects caused by variations in the chamber conditioning processes. In addition, humidity (30–90%) and temperature (17–28%) show almost no impact on separation. Our results are consistent with those concluded by Lin in micellar TLC with similar observations [33].

3.2. Signal enhancement in microemulsion TLC

When a chemical species is positioned in a restricted space provided by cyclodextrins, micelles, vesicles and other microenvironment donors, its UV–vis absorption, fluorescence, chemiluminescence, phosphorescence, circular dichroism or nuclear magnetic resonance spectra often change from those obtained in true solutions [34]. In the present study, the fluorescence images and intensities of the separated licorice components in microemulsion TLC and conventional TLC were compared, and substantial signal enhancement was observed in microemulsion TLC.

Fig. 2 compares the fluorescence images of licorice TLC chromatograms in microemulsion and conventional TLC. Compared to conventional TLC, the image of the separated licorice components in microemulsion TLC is clearer and sharper. The separated spots are more concentrated and with less tailing, thus resulting in better sensitivity and lower detection limits.

The fluorescence fingerprinting profiles of three different licorice species including *G. uralensis*, *G. inflata* and *G. glabra* by conventional and microemulsion TLC are compared in Fig. 3. Such comparison reveals the overall signal enhancement characteristics of the microemulsion TLC technique in semi-

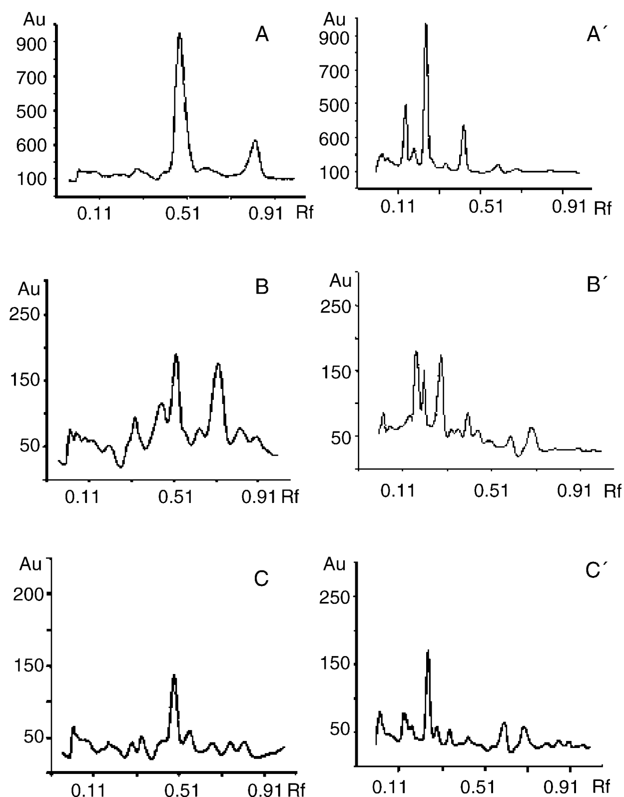

Fig. 3. Comparison of microemulsion and conventional TLC fluorescent fingerprinting profiles of licorice extracts. Scanning at 366 nm using fluorescence mode with 6.00 mm × 0.45 mm slit dimensions and 20 mm/s scanning speed. K400 was chosen as optical filter. Developing distance: 9.5 cm. A, B and C are the chromatograms of *G. uralensis*, *G. inflata* and *G. glabra*, respectively, with microemulsion mobile phase; A′, B′ and C′ are the chromatograms of *G. uralensis*, *G. inflata* and *G. glabra*, respectively, using organic solvents as the mobile phase.

quantitative terms. A comparison of the fluorescence fingerprinting profiles of *G. uralensis* between Fig. 3A (conventional TLC) and Fig. 3A′ (microemulsion TLC) shows that the qualitative features of the two chromatograms are also different. Thus, for

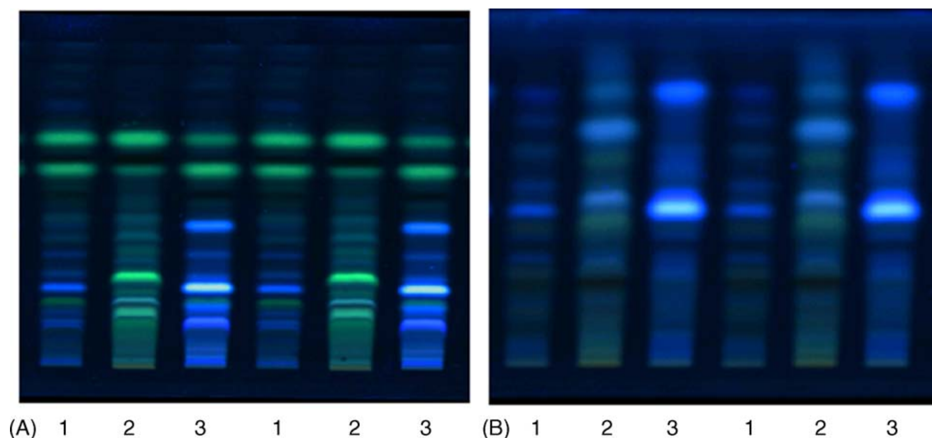

Fig. 2. Comparison of microemulsion and conventional TLC fingerprint images of licorice extract. (A) Microemulsion TLC; (B) conventioanl TLC with mobile phase of:ethyl acetate–formic acid–acetic acid (17:1:1, v/v/v) detection at 366 nm. Samples on different tracks: 1, *G. glabra*; 2, *G. inflata*; 3, *G. uralensis*. Development with microemulsion over 9.5 cm requires 135 min, separation by conventional TLC over 9.5 cm requires pre-saturation of the plate for 80 min followed by 35 min development.

instance, microemulsion TLC shows more peaks, higher detection sensitivity and better separation resolution than those of conventional TLC. Similar characteristics are observed in the TLC chromatograms of the other two licorice species, i.e. *G. inflata* (Fig. 3B and B′) and *G. glabra* (Fig. 3C and C′).

3.3. Selectivity and separation efficiency in microemulsion TLC

It is well documented that when surfactants were added into the mobile phase in a chromatographic process, the resulted micellar chromatography often displays different selectivity and separation characteristics from the conventional ones. Thus, for instance, micellar chromatography can separate simultaneously both charged and neutral compounds, and also species with much wider range of polarity [35]. Microemulsion and micelle phases are similar in physiochemical properties as they both exist as homogenous, transparent, isotropic and thermodynamically stable dispersions. However, microemulsion has higher solubilization power and lower interfacial tension. Thus, the solutes are better able to penetrate the surface of a microemulsion droplet than the more rigid surface of a micelle [36]. In microemulsion TLC, the retention of analytes is controlled by the distribution of the solute molecules among the external aqueous or oil phase, the stationary phase and the droplets of microemulsion. In the process of development, several factors such as adsorption, distribution, electrostatic interaction, steric bulkiness, etc. could all be responsible for the retention pattern of different solutes.

The retention (migration) behavior of several components of licorice in microemulsion and conventional TLCS are compared to gain understanding on the separation characteristics of microemulsion TLC. Table 1 compares the retention factor (Rf) of several marker compounds of licorice on microemulsion TLC and conventional TLC. Results show that the retention behavior for these compounds on the two systems are quite different. Thus, for instance, glycyrrhizin shows the highest Rf value in microemulsion TLC whereas its Rf value in conventional TLC is the lowest. Licuraside and liquiritin are more strongly retained in conventional TLC than they are in microemulsion TLC. As for the other three markers: inflacoumarin A, licochalcone A and 18β-glycyrrhetinic acid, they are all more strongly retained than the other three components described earlier in microemulsion TLC. This is opposed to what is observed in conventional TLC where the three species are the least retained among all components.

In conventional adsorptive TLC, the separation mechanism is based on the dynamic equilibrium between adsorption and desorption; and the mobility of individual solute is mainly controlled by the difference in adsorptivities of the analytes. For polyamide absorbent, the adsorptions of analytes on polyamide surfaces occur by the formation of hydrogen bonds between the amide or carbonyl functionalities of the polymer and the analytes molecules. The mobility of individual analyte is mainly influenced by the polarity of the analytes, i.e. the more polar components are adsorbed more strongly (lower Rf value) than the less polar components. For example, glycyrrhizin is a pentacyclic triterpene with two moieties of glucuronic acid, and glycyrrhizin can be transformed into 18β-glycyrrhetinic acid and two molecules of glucuronic acid via hydrolysis with acid or enzyme. Thus, the Rf values of glycyrrhizin and 18β-glycyrrhetinic acid in conventional TLC differ widely (0.08 and 0.92, respectively) because of their difference in polarities. Compared to those in conventional TLC, there are two developing solvent front in ME-TLC for the adsorption of surfactant on the TLC stationary phase. The first faster-moving front is mainly aqueous and the second more viscous and slower-moving front is the microemulsion phase. The analyte is distributed among the external aqueous phase, the stationary phase and the droplet of microemulsion. Thus, the Rf value of glycyrrhizin changed from the lowest in conventional TLC to the highest in ME-TLC because of the high solubility of the species in the microemulsion phase.

The separation efficiencies of microemulsion and conventional TLC have also been compared. We choose the number of real plate (N_{real}) to evaluate the separation efficiencies of ME-TLC and conventional TLC, and the equation for N_{real} [37] is:

$$N_{\text{real}} = \frac{5.54 \times Z_s^2}{(b_{0.5} - b_0)^2}$$

where N_{real} is the number of real plates, Z_s is the distance from the center of the sample application zone to the center of the sample zone, $b_{0.5}$ is the half peak width of the sample zone and b_0 is the half peak width of the sample application zone. Table 2 compares the number of real plate (N_{real}) of several marker

Table 1
Retention factor (Rf) of several marker compounds of licorice observed in microemulsion TLC and traditional TLC

Marker	Rf (M)	Rf (O)
Inflacoumarin A	0.18	0.48
Licochalcone A	0.27	0.69
18β-Glycyrrhetinic acid	0.54	0.92
Liquiritin	0.59	0.41
Licuraside	0.61	0.31
Glycyrrhizin	0.76	0.08

Notes: Rf (M) and Rf (O) are retention factors in microemulsion TLC and traditional TLC, respectively; M and O are abbreviation of microemulsion and organic agent, respectively.

Table 2
Number of real plates of several marker compounds of licorice in microemulsion TLC and conventional TLC

Marker	*N* (M)	*N* (O)
Inflacoumarin A	1086	631
Licochalcone A	1418	1341
18β-Glycyrrhetinic acid	1539	2216
Liquiritin	1931	1564
Licuraside	4659	333
Glycyrrhizin	7584	89

Notes: *N* (M) and *N* (O) are number of real plate in microemulsion TLC and conventional TLC, respectively; M and O are abbreviation of microemulsion and organic agent, respectively.

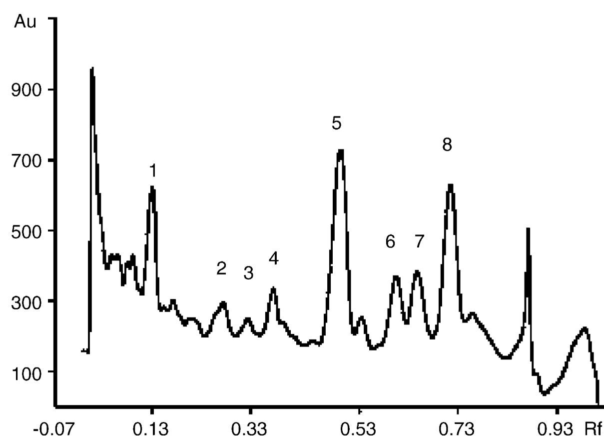

Fig. 4. Representative ME-TLC absorption scanning fingerprints of Liangwai *G. uralensis*. Scanning wavelength: 254 nm; slit dimension: 6.00 mm × 0.45 mm; scanning speed: 20 mm/s scanning speed. Peaks 1–8 are the common peaks in all samples, and peak 5 is identified as liquiritin and peak 8 is identified as glycyrrhizin.

compounds of licorice on the two TLC systems. In general, ME-TLC is superior in separation efficiency as demonstrated by the higher theoretical plates. The exceptions are 18β-glycyrrhetinic acid and glycyrrhizin but both values are less reliable because of their exceptionally high Rf values. The overall results highlight the technical merits of the microemulsion TLC technique comparing to conventional TLC or other micellar chromatography techniques in general since the latter techniques are known to suffer low separation efficiency problems [38–40]. More in-depth study along this direction is in progress in our lab.

3.4. Development of ME-TLC fingerprint of G. uralensis by absorption scanning

So far, more than 100 active components have been separated in licorice [41]. Thus, the mobile phase developing distance of TLC was extended to 15 cm in order to provide better separation of these components in ME-TLCS fingerprinting assay. The representative ME-TLC fingerprinting profiles of *G. uralensis* samples from absorption scanning can be seen in Fig. 4. There are more than 20 well resolved peaks in ME-TLC separation of *G. uralensis*, compared to only about 12 in the conventional TLC assay [42].

There are eight common peaks in the ME-TLC chromatograms of the eight samples of *G. uralensis* investigated in this work. Among these eight common peaks, peak 8 has been identified as glycyrrhizin by matching its Rf value and the UV spectra with the standards. The relative peak height and Rf values of the eight common peaks are calculated against those of glycyrrhizin, which is used as the marker species. The equations for calculating the relative peak height and relative Rf [37] are given below:

$$\text{relative height of the common peak } n = \frac{\text{peak height of the common peak } n}{\text{peak height of glycyrrhizin}}$$

$$\text{relative Rf of the common peak } n = \frac{\text{Rf of the common peak } n}{\text{Rf of glycyrrhizin}}$$

Table 3
Results from precision study of ME-TLCS fingerprinting of *G. uralensis*

No. of common peaks observed in TLC	% R.S.D. of relative peak height ($n=6$)	% R.S.D. of relative Rf ($n=6$)
1	0.83	0.68
2	1.08	0.83
3	1.27	1.02
4	2.06	1.25
5	1.45	1.17
6	2.06	1.14
7	2.15	1.23
8	0	0

3.5. Validation of ME-TLC fingerprinting assay for G. uralensis

3.5.1. Precision

The R.S.D. values for precision are summarized in Table 3. All of them were less than 3%, which met the demands of the national standard.

3.5.2. Reproducibility

By preparing five sample solutions and then analyzing them according to procedures described in Sections 2.4 and 2.6, the reproducibility of the method is obtained as shown in Table 4. All of the R.S.D. values for reproducibility are below 3%.

3.5.3. Stability

The samples were analyzed by TLC after different sample storage times, i.e. 0, 6, 24 and 48 h. The results show very minor differences in the values of relative height and relative Rf for all the eight common peaks, indicating satisfactory stability of the sample solution. In addition, to evaluate the stability of the developed plates, scannings are made at different times after development, i.e. 0, 6, 24 and 48 h. Good reproducibility was observed as the R.S.D.s of the relative peak height of the eight common peaks are all within 5%.

Table 4
Results from reproducibility study of ME-TLCS fingerprinting of *G. uralensis*

No. of common peaks observed in TLC	% R.S.D. of relative peak height ($n=5$)	% R.S.D. of relative Rf ($n=5$)
1	0.92	0.69
2	1.27	0.80
3	1.30	1.15
4	2.16	1.29
5	1.65	1.20
6	2.19	1.08
7	2.51	1.19
8	0	0

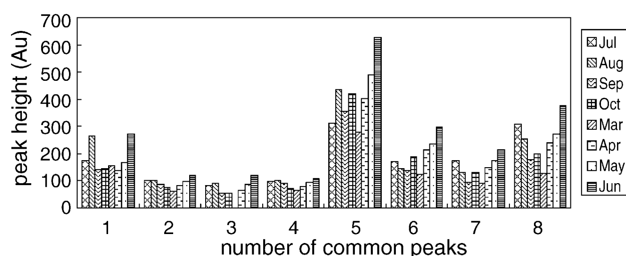

Fig. 5. Comparison of ME-TLC absorption scanning fingerprints of Liangwai *G. uralensis* samples harvested at different times during a 1-year growing period.

3.6. Monitoring the dynamic accumulation of active ingredients in G. uralensis

Botanicals could have variable chemical compositions or contents of specific components because of variations in soil types, climates as well as harvest time or growing period. Because of the large number of samples involved in this type of research, it is highly desirable to develop rapid and simple analytic methods capable of field applications for screening or quality control purposes. The ME-TLC fingerprinting technique has been applied to monitor the dynamic accumulation of active ingredients in licorice as a function of growing time, which is part of a program involving the search of best harvest time for licorice plant grown in Inner Mongolia [43].

In this study, the total quantity of active ingredients in licorice is represented by adding up the intensities of the eight common peaks (Rf values between 0.12 and 0.85) in TLC. Results for all samples analyzed are displayed on a bar chart shown in Fig. 5. The bar chart is plotted by placing the number of the common peaks in the TLC fingerprints on the *X* coordinate, and the peak heights of the respective peaks of licorice samples collected monthly on the *Y* coordinate. Change of active ingredients contents with licorice growth period suggests that June is the best harvest time whereas March and April are the worst in terms of yields of active ingredients.

4. Conclusion

The mobile phase used in ME-TLC developing system is aqueous phase, which is non-toxic, non-flammable and operationally more controllable and less expensive than the organic solvents used in conventional TLC. The high solubilization power of the mobile phase used in microemulsion TLC simplifies the sample preparation process and minimizes material losses during analysis. The mobile phase used in microemulsion TLC shows good long-term stability (at least 4 months at ambient temperature), making it ideally suited for routine fingerprinting or screening type of applications.

The separation mechanism and analyte retention of ME-TLC have been found to differ significantly from conventional TLC. Compared to conventional TLC, the ME-TLC technique shows better reproducibility, requires less involved sample pretreatment and development procedures, and offers higher detection sensitivity because of sharper band images.

The ME-TLC images of different plant species of licorice including *G. uralensis*, *G. glabra* and *G. inflata* show distinctive fingerprints. The technique is therefore ideally suited for species authentication and quality control purposes. The technique has also been applied successfully to monitor the dynamic accumulation of active components in licorice plant as a function of growing time in an experimental farm. The information is significant not only in basic research but also in practical applications for the selection of best harvest times for licorice plant.

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