

# Value the Unique Merit of HPTLC Image Analysis and Extending its Performance by Digitalization for Herbal Medicines Quality Control

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## Abstract

It is well known that High-Performance Thin Layer-Chromatography (HPTLC), the off-line planar chromatographic technique, has been employed in the pharmacopoeias of many countries for identification by virtue of its low-cost, less dependent on expensive equipment, flexible mobile phase composition and easy post-derivatization. In particular, the unique merit of HPTLC is able to provide the picture-like images of multiple samples in parallel on the same plate for instant viewing. In addition to the two dimension parameters (migration distance ( $R_f$  value), integration peak value), the attractive color image, as the third dimension parameter, enhances the potency of HPTLC identification. The bioactivity of herbal medicines released from its compound bioactive ingredients in a holistic manner. Therefore the detected total chemical composition in the image should be reasonably more meaningful to assessing the inherent quality than only any selected single marker constituent of the given herbal drug. A vivid colorful picture-like HPTLC image could be easily recognized but not described properly by words. Hence extending the plate spot/band capacity on the plate for involving the bioactive components as much as possible. Combining digitizing the HPTLC image, the acquired infographic (HPTLC image, the digital scanning profile and the integrated parameters) can be used for establishing the fingerprint common pattern of the given species. It can be coupled with chemometrics analysis for more effectively quantifiable assessing the inherit quality of the herbal drugs. In this paper we reported methodically some Chinese herbal drugs analysis via various levels for demonstration of the practical application in QC.

**Keywords:** Unique merit of HPTLC; Herbal drug image analysis; Digital fingerprinting; Meaningful quality control

## Introduction

High-Performance Thin-Layer Chromatography (HPTLC) had its glorious past in the second half of last century [1-10]. As identification of herbal medicines, HPTLC is adopted widely in the monographs of the pharmacopoeias in various countries. This open system of planar chromatographic technique provides picture-like chromatographic images of herbal medicines for identification analysis. Taking the advantage of its low-cost, less dependent on expensive equipment, flexible mobile phase composition, easy post-derivatization and the intact images of the multiple herbal samples in parallel on the same plate for being instant comparative viewing. Therefore it is still keeping active and alive in herbal medicines quality control nowadays [11-15]. In addition to the two dimension parameters of migration distance (retention time for HPLC,  $R_f$  value for HPTLC) and integration peaks area/height, the various visible or fluorescence images is uniquely the third dimension parameter for validation of the chromatograms. The specific chemical composition of the given species is expressed by the colorful image and often recognized impressively at the first sight, but is hard described by words properly [16]. Particularly herbal's bioactive ingredients are always a complex composition, opposite to the single chemical medicine, the herbal medicines play the role mainly of adjusting the unbalance of human body's functionality based on the holistic activities of multiple bioactive substances in the herbals [17]. It would be meaningful that assessing the inherent quality of the herbals through the spectrum of the total detectable ingredients by means of HPLC, HPTLC and GC etc. Amongst them, HPTLC image analysis is the most inexpensive, and being able to compare the multiple samples on the same plate for parallel quick discrimination.

Many papers have successively been published on the perspectives of TLC/HPTLC and its widely applications [13,14,18-30]. However,

ignoring the standardized operation procedure and lack of fundamental knowledge on this open system chromatographic technique is not rare in general herbal medicine analysis laboratories. So the inferior quality of HPTLC image of herbal medicines is still quite common which either reduces the TLC/HPTLC's due performance or diminishes the operator's interest, to say nothing about exploring the potential merit of HPTLC. From pragmatic view, in addition to strengthening the SOP of TLC/HPTLC, how to exploring its potential capability to enhance the performance in routine quality herbal medicines control is now still significant.

Exploring the potential merit of the image analysis would be the most concern for strengthening the power of HPTLC image analysis. There are some directions need to be solved. (1) How to extend the spot/band capacity on the HPTLC silica gel plate so as to disclose the composition detail as possible as can be done; (2) how to recognize and reckon the HPTLC image to meet the special requirement of the herbals identification and (3) how to use chemometric algorithm to clarify logically the HPTLC images during bulk samples tested [31]. Through the selected Chinese herbal drugs the practical fruition of HPTLC image analysis are demonstrated as the following.

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## Experimental

### Samples

Bupleuri radix (*Chai Hu*), Forsythiae fructus (*Lian-Qiao*), Rehmanniae radix (*Di-Huang*), Salviae miltiorrhizae radix (*Dan-Shen*).

### Instrument and reagent

Sample applicator (Automatic or semi-automatic), Twin-rough developing chamber, Reagent sprayer or HPTLC Reagent merging device, TLC visualizer (all from CAMAG, Muttenz, Switzerland); Pre-coated Silica gel 60 HPTLC plate (Merck, Germany); Dichloromethane, Chloroform, toluene, hexane, ethyl acetate, methanol, formic acid; p-Dimethylamino-benzaldehyde (DMAB), all are analytical grade.

### Sample solution preparation

Generally take appropriate amount powdered herbal drugs, ultrasonicate twice with methanol/water for 30 min. Evaporated the filtrate or supernatant liquid to dryness, dissolve the residue with moderate solvent (e.g., methanol) and make it as sample solution (Table 1).

### Chromatographic procedure

Unless otherwise stated, the total operation procedure followed the appendix of Chinese Pharmacopoeia Volume I and the operation guidance in the first chapter of The TLC Atlas of Chinese crude drugs in Chinese Pharmacopoeia [16]. The environment conditions in the lab is controlled, the temperature kept around at 20-25°C, relative humidity < 50%.

Sample application: appropriate amount of the sample solution and herbal reference substance (HRS) / chemical reference substance (CRS) / extractive reference substance (ERS) apply band-wise on the HPTLC silica gel plate. Band width: 8 mm, interval between bands: 4 mm; the sample-loaded plate is placed in a vacuum desiccator over P<sub>2</sub>O<sub>5</sub> (anhydrous) till development for keeping the silica gel's activity.

### Development

Ascending develops to 8 cm. T: 20-28°C, RH: 40% - 50%. (The developing chamber should be previous equilibration by the vapor of the mobile phase (solvent system) for 15 - 20 min, (put appropriate amount of solvent system in the one of the twin trough of the chamber beforehand).

### Visualization

Dry the developed plate with hair dryer to remove the remnant

solvent and visualize by means of spraying or dipping the corresponding reagents. Documentation is followed by using the appropriate facility such as TLC visualizer (CAMAG) or Digital camera (visual color image under white light).

### Digital scanning

The profile of the HPTLC image can be digital scanned with corresponding software (the associated software installed in the TLC visualizer or other commercial independent software (e.g., Origin Pro) coupled with MS Excel or self-developed software for acquiring the image profile as well as the integrated of peaks area/height value.

The brief testing conditions of the species respectively listed in Table 1.

### Documentation processing of post-chromatographic experiment

The herbal chromatographic image on the HPTLC plate can be instantly observed for identification. The general ensuing practice is to take photograph of the image via TLC visualizer or similar device for archiving. On the other hand, the HPTLC image can be scanned by means of TLC scanner to produce a curve profile with corresponding integration data (peak height/peak area) for quantitation. Alternatively the picture-like image photo, the permanent record which equals as viewing the HPTLC image on the plate, can do the same thing more rapid, low cost and convenient via software without any hardware. For ensuring the quality of the image photo, in addition to standardizing operation procedure of the experiment, the optical parameters for shot work should be carefully adjusted and recorded to ensure the photo as the original image as possible. Since the total chromatographic process goes under an open environment, some unexpected contamination possibly occurred when development and visualization, some requisite post-correction of the photo should be made appropriately through software. No matter how the image photo was processed, the resultant must closely resemble that of the direct real sight of the image.

## Results

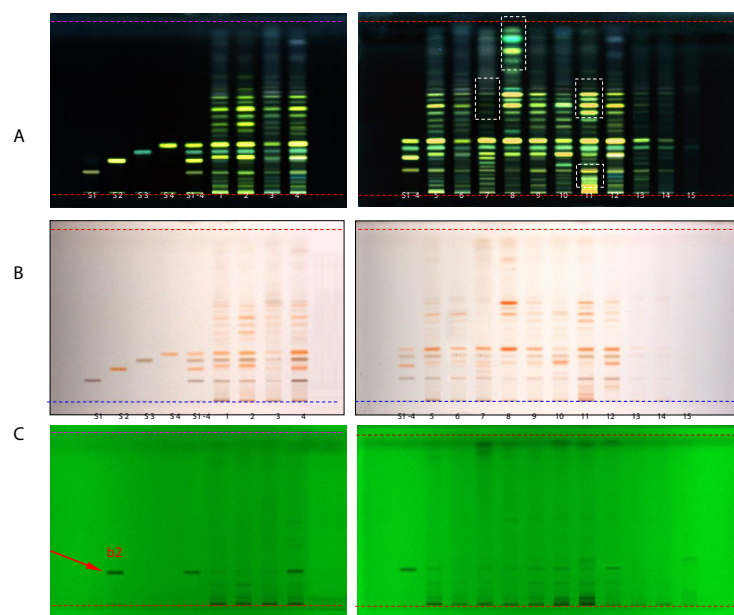
### Bupleuri radix (*Chai-Hu*)

#### Visual differentiation of various species of *Bupleurum* [31,32]:

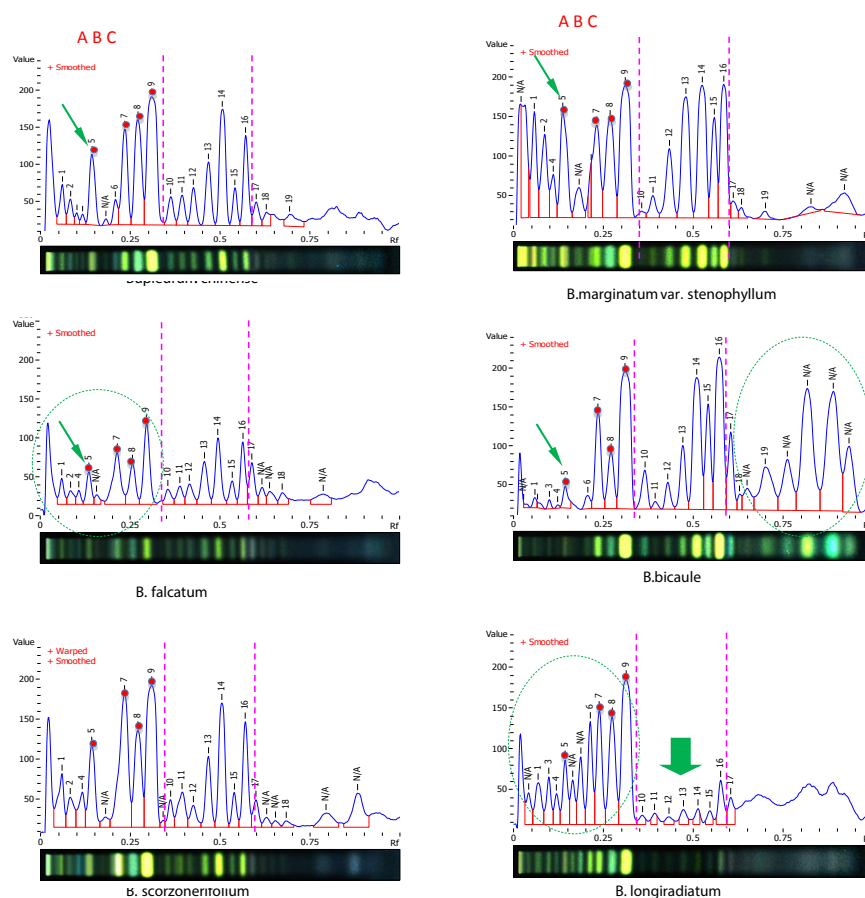
The HPTLC fluorescence images of the roots of *Bupleurum chinense* DC (*Bei-Chai-Hu*), *B. scorzonifolium* Willd (*Nan-Chai-Hu*), *B. falcatum* (San-Dao-Chai-Hu), *B. tenue* Huch. Ham. ex D. Don. (*Xiao-Chai-Hu*); *B. marginatum* Wall. ex DC. *var. stenophyllum* (Wolff) Shan et Y. Li (*Xi-Zang-Chai-Hu*), *B. marginatum* Wall. ex DC. (*Zhu-Ye-Chai-*

| Chinese herbal drug                                              | Sample solution                                                                                                                                                                                                                                                                                              | Developing solvent system                                                                                                                                                                                               | Visualization                                                                                                                                                                                                                                                                            |
|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Bupleuri radix ( <i>Chai Hu</i> ) [34,35]                        | ethanol extract (containing trace amount pyridine) – C18 cartridge clean-up – 80% eluate – methanol solution                                                                                                                                                                                                 | dichloromethane-acetyl acetate- methanol-water (30:40:15:3)                                                                                                                                                             | Dimethylamino-benzaldehyde (DMAB) (2%) in sulfuric acid alcohol solution (10%), heat at 105°C for 1 min, observe fluorescence image                                                                                                                                                      |
| Forsythiae fructus ( <i>Lian Qiao</i> ) Unpublished data         | ultrasonicate with acetone – methanol solution                                                                                                                                                                                                                                                               | (1) for higher polarity fraction:: chloroform – ethyl acetate – methanol – formic acid (30 : 5 : 10 : 1)<br>(2) for lower polarity fraction: Toluene – ethyl acetate – 36% acetic acid (90 : 25 : 2)                    | 10% sulphuric acid in ethanol, heated at 105°C until the bands color are distinct enough, examine the visible color images under day light and the fluorescence images under UV light at 366 nm.                                                                                         |
| Rehmanniae radix ( <i>Di-Huang</i> ) [37]                        | ultrasonicate at 40°C - centrifuge for 10 minutes (4000 r/min), use the supernatant as sample solution.                                                                                                                                                                                                      | dichloromethane-ethyl acetate-methanol-water-glacial acetic acid (8:4:7:1:1)                                                                                                                                            | 2% anisaldehyde in 5% sulphuric acid ethanol solution, heat at 105°C until the color of the bands are clearly visible.                                                                                                                                                                   |
| Salviae miltiorrhizae radix ( <i>Dan Shen</i> ) Unpublished data | <ul style="list-style-type: none"> <li>Lipophilic fraction: ultrasonicate with ethyl acetate extract – ethyl acetate solution;</li> <li>Hydrophilic fraction: ultrasonic extract at 60°C with water, the filtrate added small amount 10% HCl, extracted by ethyl acetate – ethyl acetate solution</li> </ul> | <ul style="list-style-type: none"> <li>Lipophilic fraction: n-Hexane - ethyl acetate - formic acid (30:10:0.5);</li> <li>Hydrophilic fraction: toluene- ethyl acetate – methanol – formic acid (5:4:0.5 : 2)</li> </ul> | <ul style="list-style-type: none"> <li>Lipophilic fraction: visible color image under day light; fluorescence-quenching image under UV 254 nm light</li> <li>Hydrophilic fraction: 5%vanillin/conc. H<sub>2</sub>SO<sub>4</sub> reagent; visible color image under day light;</li> </ul> |

**Table 1:** HPTLC experiment conditions of the species exemplified in this paper.



**Figure 1:** HPTLC image of Bupleuri radix (Chai-Hu)\*. (A) HPTLC fluorescence image (365nm); (B) HPTLC visible color image; (C) HPTLC fluorescence-quenching image (254 nm), (saikosaponin b2 can be detected under UV 254nm as a fluorescence-quenching band). (S1): saikosaponin f; (S2) saikosaponin b2; (S3) saikosaponin a; (S4) saikosaponin d; (1)(2)(5): Bupleurum chinese, (3) (6):B. scorzoneriifolium, (4) B. falcatum, (7) B. longiradiatum, (8) B.marginatum, (9) B. poly clonum, (10) B. wenchuanese, (11) B. marginatum var. stenophyllum, (12) B. falcatum, (13) B. yinchowense, (14) B. smithii var. parvifolium, (15) B. tenue.



**Figure 2:** HPTLC fluorescence images and corresponding digital scanning profiles of 6 species of Bupleuri radix. (A): main saikosaponins region; (B): inter-species identifier region; (C) low-polarity ingredients region (cf. Figure 1).

Hu), *B. longiradiatum* Turcz (*Da-Ye-Chai-Hu*), *B. polyclonum* Y. Li et S. L. Pan (*Duo-Zhi-Chai-Hu*), *B. Wenchuanense* Shan et Y. Li (*Wen-Chuan-Chai-Hu*), *B. yinchowense* Shan et Y. Li (*Yin-Zhou-Chai-Hu*), *B. simithii* Wolff. var. *parvifolia* Shan et Y. Li (*Xiao-Ye-Yin-Chai-Hu*) are demonstrated as Figures 1 and 2.

The major hydrophilic bioactive ingredient in *Chai-Hu* roots is triterpenoid saponin named saikosaponin which can generate fluorescence under UV 365 nm whilst spraying the Reagent of Dimethylamino-benzoic acid (DMAB) (2% in sulfuric acid 10 % alcohol solution on the Plate). The formed HPTLC fluorescence color images of saikosaponins under UV 365 nm light illustrated the components composition of various species of *Bupleurum* (Figure 1). For easy recognition, the whole images are divided into three regions. Region (A) includes 9 peaks of the main saponins, all tested species share the common features, and except the root of *B. longiradiatum* contains more 2 peaks than the others. The digital scanning profiles of those images can be quantifiably compared the different concentration distribution among the tested species (Figure 2). Region (B) assigned as inter-species identifier. The peaks abundance in the image of *B. marginatum* var. *stenophyllum* is the highest and *B. longiradiatum* the lowest. Region (C) contains the low-polar ingredients, *B. bicauli* express specifically six stronger peaks in this region, and the species had barely some small irregular heaves in the profiles. All the features disclosed in the image and the corresponding digital scanning profiles.

### Forthysiae fructus (*Lian-Qiao*)

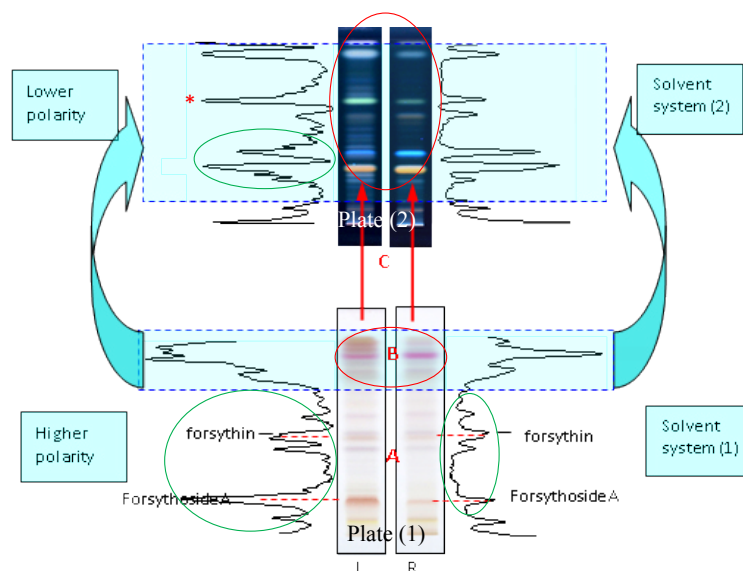
**Extended view of the detectable bioactive ingredients of *Lian-Qiao* by means of the seamless association of two fractions of HPTLC images:** Forsythoside A and Forsythin (phyllyrin) are the main bioactive components of phenylethanol and lignan derivatives in *Forsythiae fructus* (*'Lian-Qiao'*). A HPTLC with the solvent system (A). Table 1 distinguishes clearly the two components in the fingerprint (image plus scanning curve profile) of phenylethanols fraction, but the low polar bioactive compounds crowd on the front edge of the

image. As conventional practice, that part on the front edge would be neglected. But it involves bioactive ingredients too. For separation of such crowded constituents, the sample was dedicatedly developed on another plate by using solvent system (B) (Table 1). Connecting the two HPTLC images and their profiles constitutes the intact HPTLC fingerprint of *'Lian-Qiao'*. So the assessment of quality of *Lian-Qiao* is obviously far meaningful than the single image let alone the selecting one or two marker for identification. There are two grades of *Lian-Qiao*: the immature fruits (*Qing-Qiao*; *'Qing'* means immature here) and mature fruits (*Lao-Qiao*; *'Lao'* means aged, mature). The images of both polarities show that the *Qing-Qiao* contains consistent higher contents than that in the mature fruits (*Lao-Qiao*) (Figure 3). In methodology view, this example focus on how to select the boundary line to divided the two polarities fractions to ensure no overlapping of the components in the two fractions. From view point of herbal drugs application, it is obviously that content-consistent *'Qing-Qiao'* (immature fruits) is better than the *'Lao-Qiao'* (mature fruits).

### *Rehmanniae radix* (*Di-Huang*)

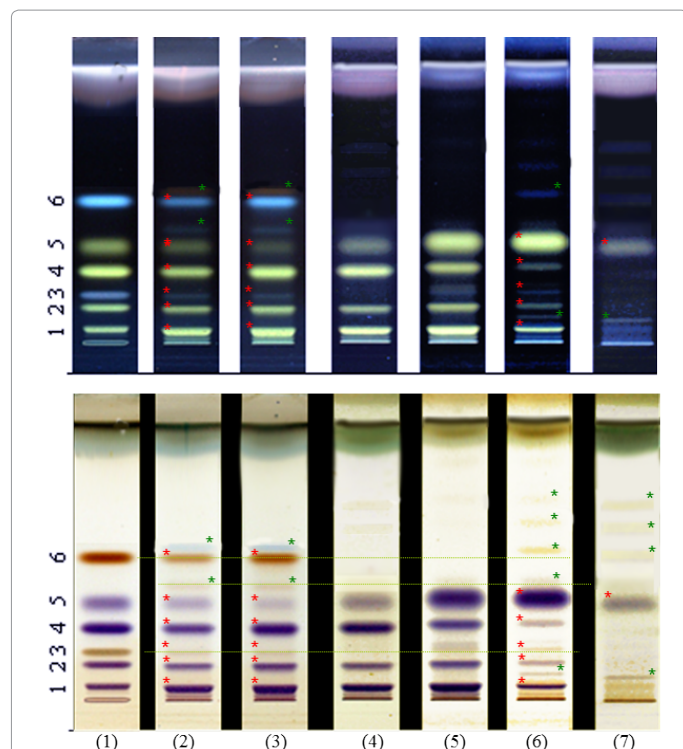
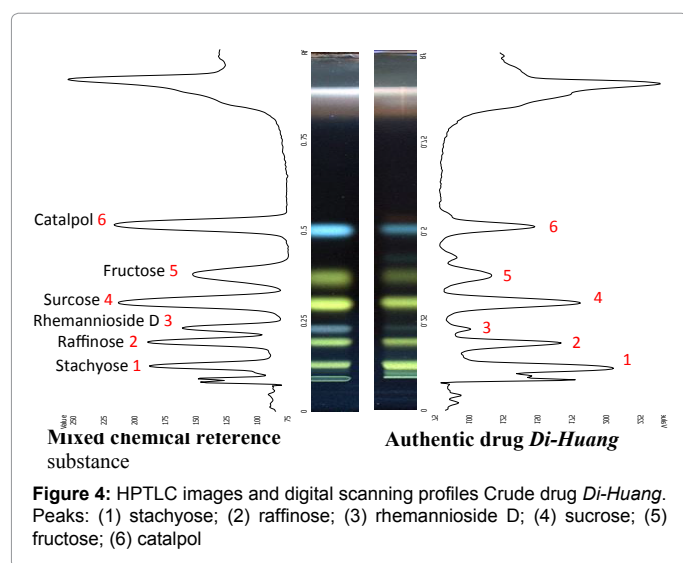
Reveal the relationship between the dynamic change of the bioactive constituents and the transformation of "drug property" between the sun-dried drug and the steaming-processed drug.

*Rehmanniae radix* (*Di-Huang*) is the tuberous roots of *Rehmannia glutinosa* Libosch, belonging to the family Scrophulariaceae. There are two grades of *Di-Huang* available in the market: Sun-dried or mediate-heating dried drug (*Sheng-Di-Huang*, *'Sheng'* means raw) and steam-heating processed drug (*Shu-Di-Huang*, *'Shu'* means processed). The two kinds of *Di-Huang* have different clinical efficacy according to the traditional Chinese medicine's experience due to their different 'medical properties'. It was described that the *'Sheng-Di-Huang'* has 'cold' property and the *Shu-Di-Huang* has a 'warm' property. The change in herbal 'property' from 'cold' to 'warm' implies that some significant alteration of the inherent secondary metabolites in *Di-Huang* roots occurs before and after the steam-heating processing



**Figure 3:** Extending separation capacity of HPTLC image and digital scanning profile of *Forsythiae fructus* with two plates associated seamlessly. (L) immature fruits (*'Qing-Qiao'*) (R) mature fruits (*'Lao-Qiao'*)  
A: visible color image; higher polarity fraction developed with solvent system (1);  
B: lower-polarity fraction, squeezed on the front of the image (A);  
C: fluorescence image of B (lower polarity fraction) developed with solvent system (2)



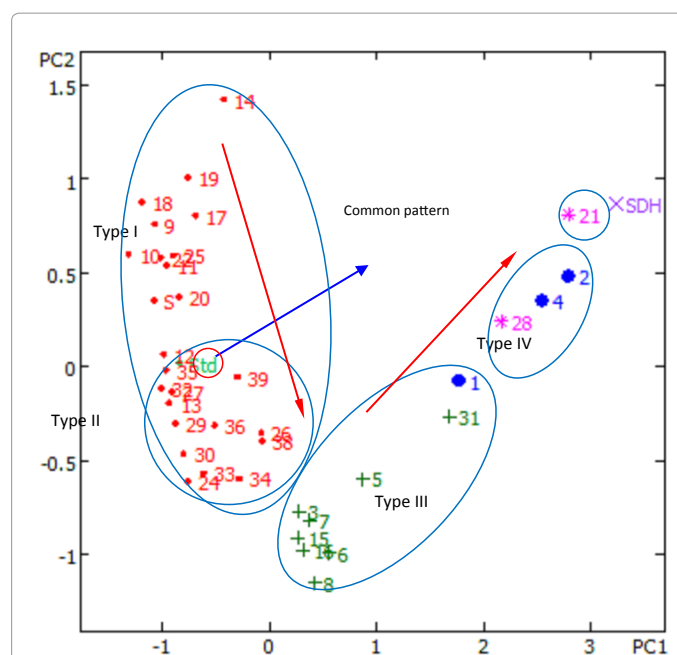


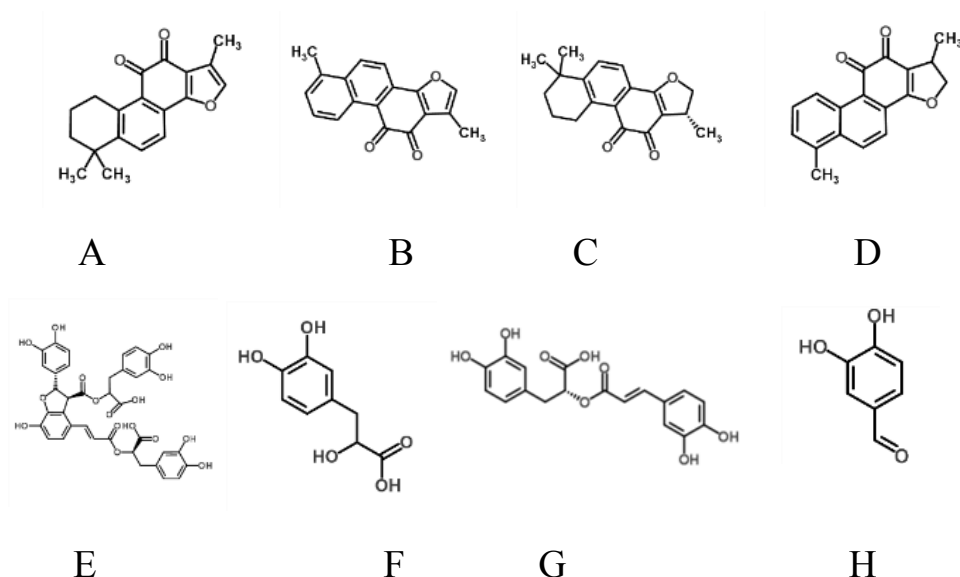
cycles. The HPTLC images of *Di-Huang* unfolded the dynamic change of the iridoid glycosides and oligosaccharides, the two major bioactive constituents, from *Sheng-Di-Huang* to *Shu-Di-Huang*. The HPTLC fingerprints showed that the iridoids represented by catalpol in *Sheng-Di-Huang* could be easily decomposed by steam-heating, even disappeared in *Shu-Di-Huang*. The oligosaccharides including stachyose (tetrasaccharide), raffinose (trisaccharide) were gradually hydrolyzed into sucrose (disaccharide) and glucose and fructose (monosaccharide) by successive steam-heating cycles. But the stachyose was hydrolyzed steadily, hence it still existed in the proper processed roots, nevertheless the others disappeared from the fingerprint (Figures 4 and 5). Referring to bioactive research as reported in the literature, it could be understood the iridoids ingredients in *Sheng-Di-Huang* expressed mainly the 'cold' property, and the stachyose is the prime component in *Shu-Di-Huang* exerts seemingly the role of the 'warm' property [33].

Observation in depth will find the traditional processing method being uncontrollable so as to be difficult ensuring the consistent composition. The Principal Component Analysis (PCA) of the samples tested showed the dynamic change trends (Figure 6). It hints that the steam-heating process of *Di-Huang* should be improved to keep the oligosaccharides pattern consistent in the final processed entities (*Shu-Di-Huang*).

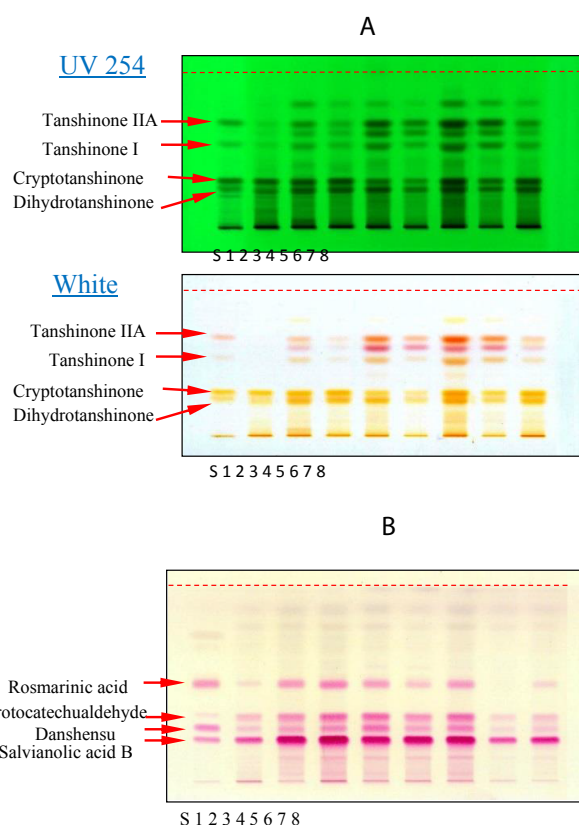
#### Salviae miltiorrhizae radix (*Dan-Shen*)

**Comprehensive quality assessment of by multi-levels HPTLC fingerprint analysis:** There are two kinds of bioactive compounds, diterpene quinones derivatives (lipophilic) and mono- and poly-phenols (hydrophilic) in the roots (Figure 7). As a cost/effective offline HPTLC technique plays the role for comprehensive control the quality in a holistic manner. The common patterns of the images both lipophilic and hydrophilic ingredients in *Dan-Shen* serve as the chemical fingerprint. Comparative observation disclosed The image pattern are very similar within the same species (*Salvia miltiorrhizae*),





**Figure 7:** Chemical structure of major bioactive components in *Dan-Shen*. (A) Danshinone IIA; (B) Danshinone I; (C) Cryptotanshinone; (D) Dihydrotanshinone; (E) Salvianolic acid B; (F) Danshensu; (G) Rosmarinic acid; (H) protocatechualdehyde.



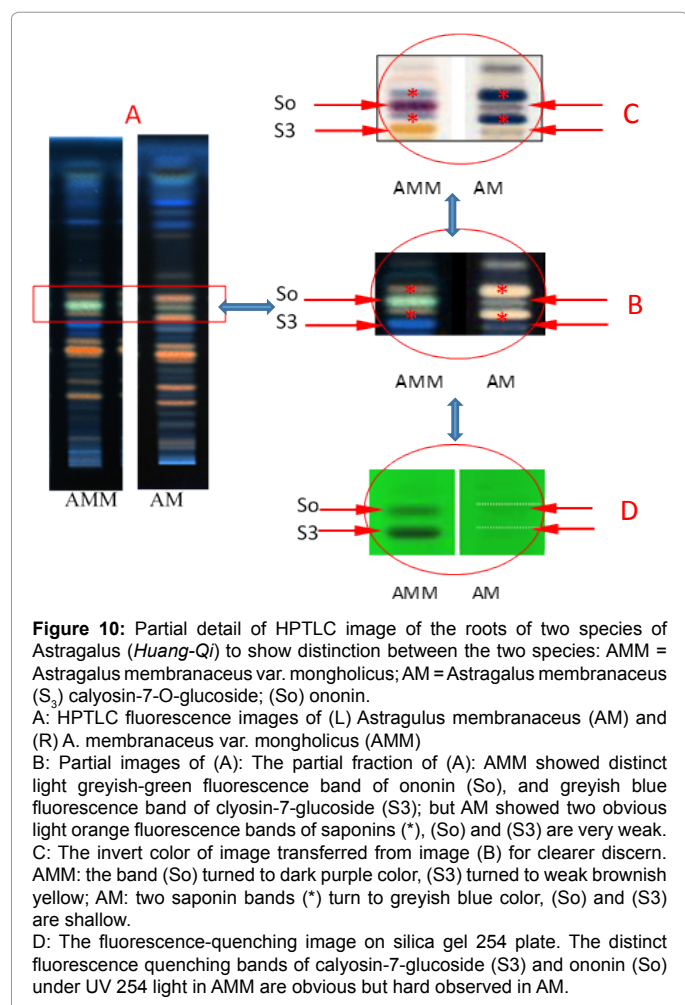
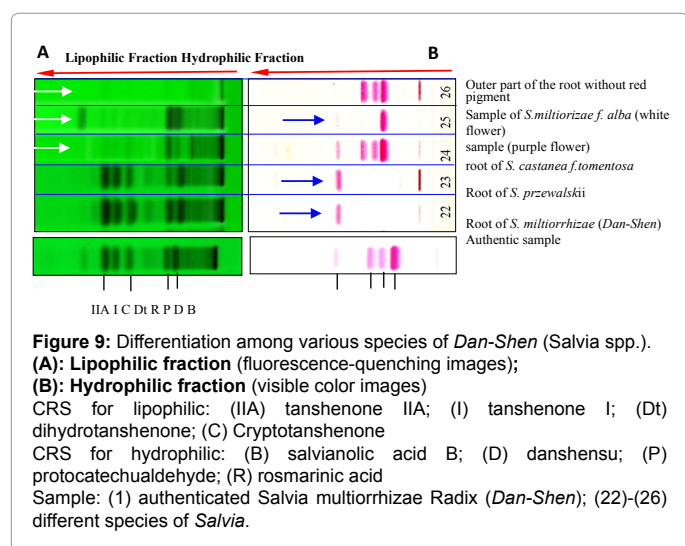
**Figure 8:** HPTLC fluorescence-quenching images and visible color images of *Dan-Shen*.

(A): Lipophilic fraction (fluorescence-quenching images and visible color images); (B): Hydrophilic fraction (visible color image); Track (S): CRS for lipophilic (upward): dihydrotanshinone, Cryptotanshinone, tanshinone I, tanshinone IIA; CRS for hydrophilic (upward): salvianolic acid B, danshensu, protocatechualdehyde, rosmarinic acid. Sample: (1)–(7) commercial samples of *Dan-Shen*; (8) authenticated *Salvia miltiorrhizae* Radix (*Dan-Shen*).

but the concentration of the ingredients differ. That showed lower concentration should be inferior commerce. The patterns among different species (*S. miltiorrhizae*, *S. miltiorrhizae* f. *alba*, *S. przewalskii*, *S. castanea* f. *tomentosa*) are diverse each other. The associated two (lipophilic and hydrophilic ingredients) images strengthen the specificity for identification (Figures 8 and 9).

## Discussion

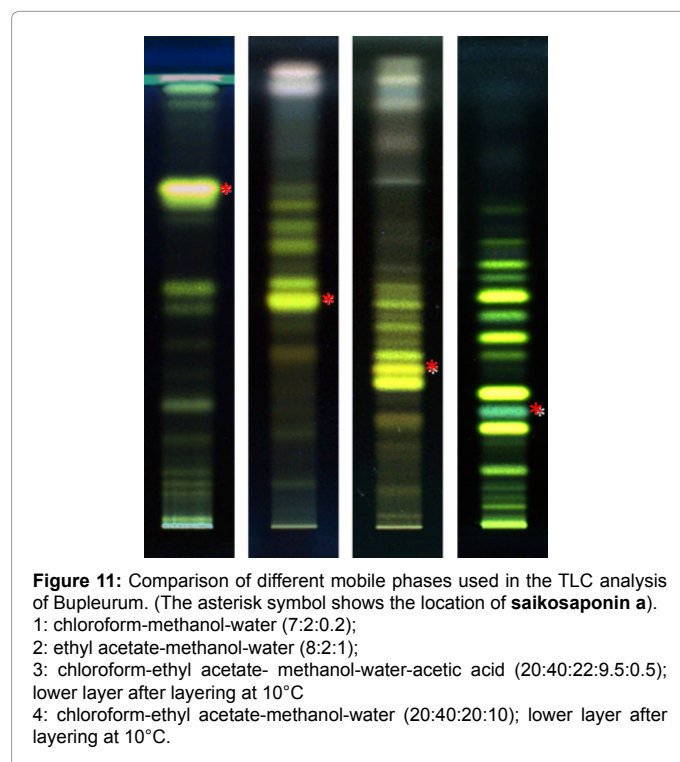
1. A colorful picture-like HPTLC image of the herbal drugs is the unique advantage compared with the column chromatography. The examples in this paper demonstrated the vivid image is visually recognized and evaluated the quality in parallel among the various samples on the same plate. Particularly the subtle but significant specific part in some kindred species such as differentiation between the roots *Astragalus membranaceus* (AM; *Mo-jia-Huang-Qi*) and *A. membranaceus* var. *mongholicus* (AMM; *Meng-Gu-Huang-Qi*) in the HPTLC images counts on the subtle difference in the middle part of the images, which is hard to be literal described (Figure 10). Hence the elaborated high quality of HPTLC image is the vital prerequisite.
2. Offline operation of HPTLC is often daunting to the inadequate-trained practitioners. But the other side of offline makes flexible selection of the solvent systems (mobile phase), thus the separation would be much more selective and specific (Figure 11). Opposite to Reverse-phase HPLC, medium-polar to non-polar compounds are separated more effectively by HPTLC, so both HPTLC and HPLC are complementary separation techniques.
3. In HPTLC, the vapors of the solvent systems in the chamber also participates the chromatographic behavior and afford unique influence on the separation. Hence the solvent vapor is the 'third phase (vapor phase)' in addition to the mobile phase (solvent system) and the stationary phase (silica gel). That is the reason why the chamber often needs to be equilibrated with the solvent mixture for a certain time before developing in most cases [2].



- The routine HPTLC can generally provide a better image of medium polarity components, but the low polarity components is often jam-packed on the front edge of the solvent on the plate by using the same solvent system. For disclosing the low polarity substances squeezed on the front edge on the first plate, the second development with suitable solvent system on

another plate should be done to achieve the purpose. Hence the boundary of the both higher and lower polarity fractions must be defined in case unexpected overlap. The two images together virtually doubled the plate length. Seamless association of the two images constitutes the 'whole view' of the detectable bioactive compounds (Fig. 3). Generally, the bigger the gap between the two polarities fractions, the more easily affirming the boundary between both, then the more clarity of the whole image is recognized.

- Multiple levels of visualization of the HPTLC image is another benefit for chromatographic identification. There are a lot of chemical reagents which react with the various metabolic compounds in the images of the herbals, various visible color, fluorescence as well as fluorescence-quenching HPTLC images can then be obtained. Two kinds of chemical reagents, the general reagents and special reagents, can fit to react with various categories of compounds [34]. For pilot screening test, multiple levels of visualized image can be produced on the same plate by means of spraying several reagents one-by-one sequentially if only the used reagents without interference each other. For example, it can be observed sequentially multiple levels of images from the same plate: (1) the compounds' original color image (e.g., some pigments), (2) fluorescence-quenching image (Silica gel 254 plate for aromatic compounds, observed under UV 254 nm), (3) the compounds original fluorescence image (UV 366nm, e.g., berberine, quinine, coumarins), (4) fumigating with ammonia vapor (some flavonoids, coumarins, anthroquinones), (5) spraying AlCl<sub>3</sub> reagent (some flavonoids, phenols), (5) then spraying selected FeCl<sub>3</sub> reagent (for mono- or poly-phenols) or Dragendorff reagent (alkaloids) or sulfuric acid ethanol/water solution (general reagent, generating fluorescence image of some of triterpenoids, steroids, saponins, some flavonoids, coumarins, saccharides observed under UV





366 nm) or vanillin or anisaldehyde/sulfuric acid (essential oils, saponins, saccharides) etc. [33]. Then the acquired successively various images can be investigated and documented sequentially on one plate. Thereby the multiple levels information (the possible compounds detected, the suitability of the mobile phase), positive or negative, in a very rapid, cost/effective way. It is most useful in screening pilot test for herbal drugs samples.

6. The obvious benefit is of easy-doing, low cost, no time bound, and suitable in general herbal laboratories, in-process quality control of the herbal industry and surveillance in the market. If using calibrated Extractive Reference Substance (ERS) replace the pure chemical standard substance as the external standard, it can be used for not only quantitative assay, but also for integrative quantifiable fingerprint analysis [35]. Furthermore, the infographic obtained from all the image and the corresponding parameters is aided by chemometric analysis makes the quality evaluation more legible (Figure 6).

## Conclusion

By comparison, HPLC is mainly reversed phase chromatography suitable for analyzing higher or medium polarity compounds. The opposite, HPTLC is mainly normal phase chromatography in an open system. It is adept at separation of lower and medium polar compounds. Particularly, facing the complicated chemical composition in the herbal drugs, there are too many unknown ingredients to be separated satisfactory with any single separation technique as routine analysis. The both chromatographic techniques are complementary. Any undervaluing the merits of HPTLC should be inadvisable.

A satisfactory and reproducible experiment relies on the standardized operation, qualified instrument and relative consistent environment condition. In addition to the TLC ADC apparatus (CAMAG, Mutenz, Switzerland) for controlling the relative humidity, A well-designed small customized lab with controllable temperature and relative humidity will be easily assembled within about 10 m<sup>2</sup> area for keeping a consistent environmental condition. Anyway, the most important is the practitioners should participate a short-term pre-job-training by the eligible tutors.

## Acknowledgement

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