TLC Atlas for Authentication of Chinese Crude Drugs

Zhengtao Wang

Key Laboratory of Standardization of Chinese, Ministry of Education
Institute of Chinese Materia Medica, SHUTCM
Shanghai R&D Centre for Standardization of Chinese Medicines
OUTLINE

- Historic use of TLC in ChP
- TLC Atlas of TCMs in ChP
- Application of TLC/HPTLC for authentication of TCM herbs
- The Ongoing work
1. Historic use of TLC for authentication of Chinese herbs in Chinese Pharmacopoeia

*ChP 1985, Volume 1*

- TLC was firstly introduced in *ChP* for authentication of crude drugs and formulated TCM preparations
  - The deficiency of the chemical reference standard (CRS) limited the application of TLC
  - Lab-coated TLC plates, poor reproducibility
  - Non-standard developing procedure
Historic use of TLC in *ChP*

*ChP 1990, Volume 1*

- TLC was widely used in the identification and quantitative determination of traditional Chinese medicines.
- TLC identification was applied in 160 monographs (20.5%)
- Reference Drug was introduced in ChP to resolve the problem of CRS deficiency. The entire image of Traditional Chinese Medicine was reflected.
TLC in *ChP 2005*

- Identification (347 monographs of crude drugs, \(\approx 60\%\))
- Limit test
- Assay (13 monographs)
- Pre-coated TLC plates recommended
- Sample application: Manual, semi-automatic, automatic
- TLC images documented by electronic files
### Advancements in analysis of Chinese Crude drugs in *ChP* 2010

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<th>Method</th>
<th>Identification</th>
<th>Limit test</th>
<th>Assay</th>
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<td>TLC</td>
<td>HPLC</td>
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<td><em>ChP 2005</em></td>
<td>339</td>
<td>347</td>
<td>5</td>
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<td><em>ChP 2010</em></td>
<td>374</td>
<td>526</td>
<td>3</td>
</tr>
<tr>
<td><em>ChP 2010</em></td>
<td>713</td>
<td>873</td>
<td>8</td>
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*ChP* 2010 New admissions

*ChP* 2010 In total
The atlas serves as one of the reference book series of the ChP, providing the reference spectra for the TLC identification of Chinese crude drugs.

The atlas has Chinese version and English version including 229 monographs.

The TLC experiments followed the methods of ChP 2005, but some methods were modified or optimized. The contents revised have been collected in the supplement of ChP 2005 Edition, as well as the latest ChP 2010 Edition.

The work started from Dec. 2004, and finished in 2007, contributed by 12 laboratories.
12 Laboratories contributed to this atlas:

- National Institute for the Control of Pharmaceutical & Biological Products,
- Shanghai Institute for Food and Drug Control,
- Zhejiang Institute for Food and Drug Control,
- Beijing Institute for Drug Control,
- Hebei Institute for Drug Control,
- Heilongjiang Institute for Drug Control,
- Guangdong Institute for Drug Control,
- Hubei Institute for Drug Control,
- Jiangsu Institute for Drug Control,
- Cromap Institute of Herbal Research,
- Northwest University,
- Shanghai R&D Center for Standardization of Chinese Medicines (SCSCM)
TLC Atlas conducted in our laboratories

- 31 monographs
- Involved in the editing
- In charge of English version
Condition optimization and methodology evaluation for the TLC atlas

- Representative samples for test
- Selection of coating materials and plates
- Preparation of the test solution
- Spotting techniques
- Temperature and humidity control
- Roles of solvent vapour
- Mobile phase
- Others
Representative samples covering different species and locations should be collected, normally more than 10 samples required, including adulterants.

白头翁 Pulsatillae Radix and its adulterants
Chemical diversity due to different producing and storing conditions

TLC of Fleeceflower Root

A. Showing the stilbens, and B. Enthroquinones

S1: 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glc; S2, physcion; S3, emodin
Selection of TLC plate

**Kansui Radix 甘遂**

In the *TLC Atlas (2005)*, at least five TLC plates were compared, the results were showed in the Chinese version. In the English version, only the optimized one was given.
The selection of TLC plate

TRICHOSANTHIS RADIX天花粉

DC-Fertigplatten SIL G-25UV\textsubscript{254} MN, Germany

HPSG, Yantai chemical industry research institute, China

MN plate showed better result than HPSG plate
Gentianae Radix 龙胆
SG F254 showed better resolution than MN plate.
The selection of TLC plate

NELUMBINIS PLUMULA 蓮子心

SG, Yantai chemical industry research institute, China

DC-Fertigplatten SIL G-25UV_{254} MN, Germany

Lab-coated plate (0.5% CMC-Na, 0.3 mm)

For some alkaloids, the lab-coated plate showed the best resolution
Preparation of test samples

EUPOLYPHAGA SEU STELEOPHAGA

— ChP 2005: 1g powder, ultrasonicate in 25 mL of methanol for 30 minutes.

— TLC Atlas: 1g powder, ultrasonicate in 25 mL of 6% NaOH in methanol for 30 minutes, centrifuge and collect the supernatant, evaporate to dryness. Dissolve the residue in 10 ml of water, partitioned with three 20-mL EtOAc, and use the EtOAc extract.
The semi-automatic or automatic band-wise spray sampling mode is adopted in this atlas to ensure best chromatogram quality.

NELUMBINIS PLUMULA 莲子心
MERCK TLC Silica gel 60 F_{254}

Apply in spots is better than in bands

ECLIPTAE HERBA 墨旱莲
HPSG, Yantai chemical industry research institute, China

Band-wise spray sampling mode provides more ideal separation.
Temperature and humidity

- The temperature and relative humidity marked in the monograph of the *TLC atlas, 2005* was the recording of the actual situation when testing, and is provided for reference only.

T: 22°C  RH: 62%

**Vladimiriae Radix 川木香**
Temperature and humidity

To get satisfied separation, temperature and humidity were optimized on the system suitability verification.

Different RH

<table>
<thead>
<tr>
<th>RH</th>
<th>42%</th>
<th>58%</th>
<th>65%</th>
<th>72%</th>
<th>88%</th>
</tr>
</thead>
</table>

Different Temperature

<table>
<thead>
<tr>
<th>Temperature</th>
<th>20°C</th>
<th>4°C</th>
</tr>
</thead>
</table>

ALPINIAE KATSUMADAI SEMEN 草豆蔻
Roles of solvent vapour

Chamber saturation

We rarely need to develop under a “saturated” state. instead, the vapour of the mobile phase is allowed to “equilibrate” within the chamber for a certain period of time, this is referred as “pre-equilibrate” in this atlas.

no pre-equilibrated  pre-equilibrated
Roles of solvent vapour

Coptidis Rhizoma

The amounts of ammonia used in pre-equilibrating in the TLC analysis of Coptidis Rhizoma, effect the migration of berberine (*).
Optimization of mobile phase

A. ChP 2005: Hexane-EtOAc (9: 1)
B. TLC Atlas: Hexane-Acetone (9: 1)

ECLIPTAE HERBA 墨旱莲
Mobile phase

In the *TLC atlas*, benzene was replaced by other solvents to avoid its toxicity.

**Alpiniae Katsumadai Semen 草豆蔻**

![Image of TLC plates with bands]

**Mobile phase:**

*Chp 2005*: benzene-ethyl acetate - methanol (15:4:1)

*TLC Atlas*: toluene- ethyl acetate - formic acid (10:1:1)
**Asteris Radix et Rhizoma**

**Derivatization reagent:**

*ChP 2005*: 2,4-Dinitrophenylhydrazine TS

*TLC Atlas*: 10% sulfuric acid in ethanol

**Extract solvent:**

*ChP 2005*: petroleum ether

*TLC Atlas*: chloroform

This revised method was adopted in *ChP 2010.*
Derivatization

天麻 Gastrodiae Rhizoma

Mobile phase

**Chp 2005**: acetate-methanol-water (9:1:0.2)

**TLC Atlas, 2005**: chloroform-ethyl acetate-methanol-water (2.5:1:1:0.1)

derivatization regent

**ChP 2005**: 10% Phosphomolybdic acid in ethanol

**TLC Atlas**: 10% sulfuric acid in ethanol

TLC Silica gel 60, MERCK
3. Application of TLC/HPTLC in Identification of Traditional Chinese Medicines
TLC identification is more widely used in *ChP 2010*

- Authentication of crude drugs from different species,
- Specificity of the marker components,
- Identification using multi-marker components,
- More informative chromatograms,
- Use of new methods and new technologies
Identification of Ginseng, Notoginseng, and American Ginseng

HPTLC chromatograms for three species of Coptidis Rhizoma

The 3 species of Coptidis Rhizoma were distinguished by the same TLC identification method
TLC identification of Schisandriae chineses Fructus and Schisandriae sphenantherae Fructus

北五味子
Fructus Schisandriae Chineses

南五味子
Fructus Schisandriae Sphenantherae

Reference

g-schisandrin（五味子乙素）
Schizandrin（五味子甲素）
Schisantherin (Gomisin C)（五味子酯甲）
Schisandrin (五味子醇甲)
TLC Directed Identification of Diagnostic components of Chinese Gentian and Largeleaf Gentian Root

Chinese Gentian

Largeleaf Gentian

Substitutes from Gentiana

Chinese Gentian adulterant

roburic acid (11)
TLC identification of Isatidis Radix and Rhizoma et Radix Baphicacanthis Cusiae

1. (R,S)-goitrin is the active and specific constituent in Isatidis Radix;
2. The two herbs can be distinguished by this marker compound.
MOUTAN CORTEX  牡丹皮  ChP, 2005

CYNANCHI PANICULATI RADIX ET RHIZOMA  徐长卿 ChP, 2005

Paeonol CRS，5%FeCl$_3$ in ethanol
MOUTAN CORTEX 牡丹皮

Method in *TLC Atlas, 2005*

**Mobile phase:**

*Chp, 2005:* cyclohexane-ethyl acetate (3:1)

*TLC Atlas:* cyclohexane-ethyl acetate-glacial acetic acid (4:1:0.1)

**Derivatization reagent:**

*Chp, 2005:* 5% FeCl$_3$ in ethanol

*TLC Atlas:* 2% solution of vanillin in ethanolic sulfuric acid (10%)

This revised method was adopted in *ChP 2010*
CYNANCHI PANICULATI RADIX ET RHIZOMA 徐长卿

Method in ChP, 2005 is remained

New method in TLC Atlas

This revised method was adopted in ChP, 2010.
Radix Linderae 乌药

• Combined Spicebush Root is the dried root tuber of Lindera strychnifolia (Sims) Kosterm.

• **Action**: To smooth the flow of qi and relieve pain, and warm and tonify the kidney and urinary bladder

• **The goal of this experiment**: establish TLC identification for Radix Linderae.

• **Chemical Constituents**: volatile oil, Sesquiterpene, isoquinoline alkaloids and polyphenol
Method 1: Macerate 1 g of powder in 30 ml ethyl ether for 30 min, ultrasonicate for 10 min, filter, evaporate the filtrate to dryness, dissolve the residue in 1 ml ethyl acetate as the test solution.

Method 2: Macerate 1 g of powder for one night in 30 ml ethyl ether, filter, ...

Method 3: Macerate 1 g of powder for 30 min in 30 ml petroleum ether (30~60℃), ultrasonicate for 10 min, filter, …
Extracting method
1. Ethyl ether, ultrasonicate;
2. Ethyl ether, macerate for one night
3. Petroleum ether, ultrasonicate;

- Extraction efficiency is same for the three methods;
- Ultrasonicate is more simple than macerate for one night, time-saving
- Using petroleum ether is better than ethyl ether

- Left: 1% solution of vanillin in sulfuric acid
- Right: 10% sulfuric acid reagent

The final extraction method: Macerate 1 g of powder in 30 ml petroleum ether (30~60°C), ultrasonicate for 10 min, filter, evaporate the filtrate to dryness, dissolve the residue in 1 ml ethyl acetate as the test solution
Optimization of mobile phase

Petroleum ether - ethyl acetate (5:1)

n-hexane - ethyl acetate (5:1)

toluene - ethyl acetate (15:1)

chloroform - ethyl acetate (20:1)

Other three systems are all good except chloroform system
Optimization of mobile phase and visualization solvent

- Petroleum ether-ethyl acetate (5:1)
- n-hexane-ethyl acetate (5:1)
- Toluene-ethyl acetate (15:1)

10% sulfuric acid in ethanol

1% solution of vanillin in sulfuric acid

Uv 366nm – the result is same for three mobile phase
Visible light - more spots were found in the left plate (use 1% solution of vanillin in sulfuric acid as derivatization reagent)

One more zone

Toluene system is the final mobile phase to be selected.
Humidity control

using different concentration of sulfuric acid solution: 88%, 75%, 65%, 58%, 47% and 32%

Mobile phase: toluene-ethyl acetate (15: 1);
derivatization: 10% solution of sulfuric acid in ethanol, heat at 105°C to zones distinct
plate: silica gel G60-TLC (Merck);
Develop vertically for 8cm;

Better performance was obtained in lower humidity (47%~65%)
TLC condition after Optimization

Mobile phase: toluene-ethyl acetate (15: 1);
Derivatization I: 10% solution of sulfuric acid in ethanol, heat at 105°C to zones distinct
Derivatization II: 1% solution of vanillin in sulfuric acid
plate: silica gel G60-TLC (Merck);
Develop vertically for 8 cm;
S. Linderane, 1~11 samples

This method was adopted in ChP 2005.
Application of TLC-bioautographic technology in *ChP*

TLC of *Cortex Magnoliae Officinalis*

TLC bioautography Apply in the screening the antioxidant component in Chinese crude drugs

TLC of *Fructus Perillea*

DPPH显色

扫描轮廓叠加图
Semen Alpiniae katsumadai

1% vanillin in sulphiric acid

Form up to down:
alpinetin
cardamonin
Pinocembrin

(E,E)-1,7-Diphenyl-4,6-Heptadien-3-One

1 mg/ml DPPH in ethanol regent (visible light)
Radix rehmanniae

10% $\text{H}_2\text{SO}_4$

DPPH

1 mg/ml DPPH in ethanol regent (visible light)

acteoside
Other research for ChP 2010

- Monographs finished
  - Crude drugs: 29
  - Medicinal Slices: 22
  - Extraction: 1
  - Formulated preparations: 2
  - New methodologies: 2
  - New reference substances: 34
- The English Version of ChP 2010
More than 400 chemical references have been prepared and distributed to the markets in China and overseas.
4. Ongoing work

- The status of TLC atlas 2005
  Published 3-years later, neither coincided with ChP 2005, nor be in line with ChP 2010

- TLC atlas 2010?
  The same status if editing TLC atlas 2010

- ChP 2015: Carry out at the same time planed by ChP
  - I have been pointed in charge of TLC methodology
  - International collaboration, with Eike and other experts
  - Inter-lab validation
  - harmonization
Thanks and welcome to visit our institute!

1200 Cailun Road, Pudong District, 
Institute of Chinese Meteria Medica, 
Shanghai University of Traditional Chinese Medicine, 
201203, Shanghai, China