

TLC Atlas for Authentication of Chinese Crude Drugs

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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

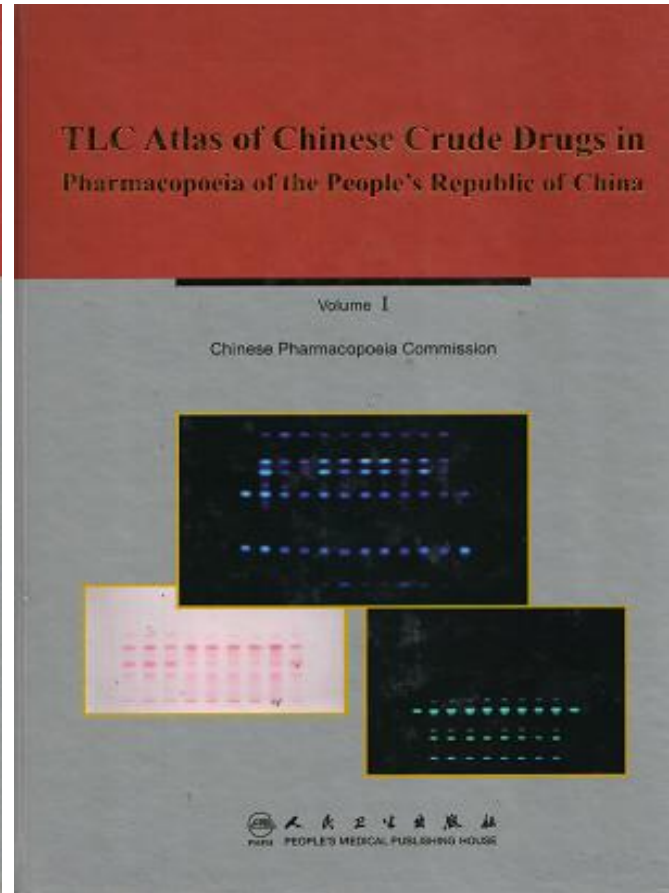
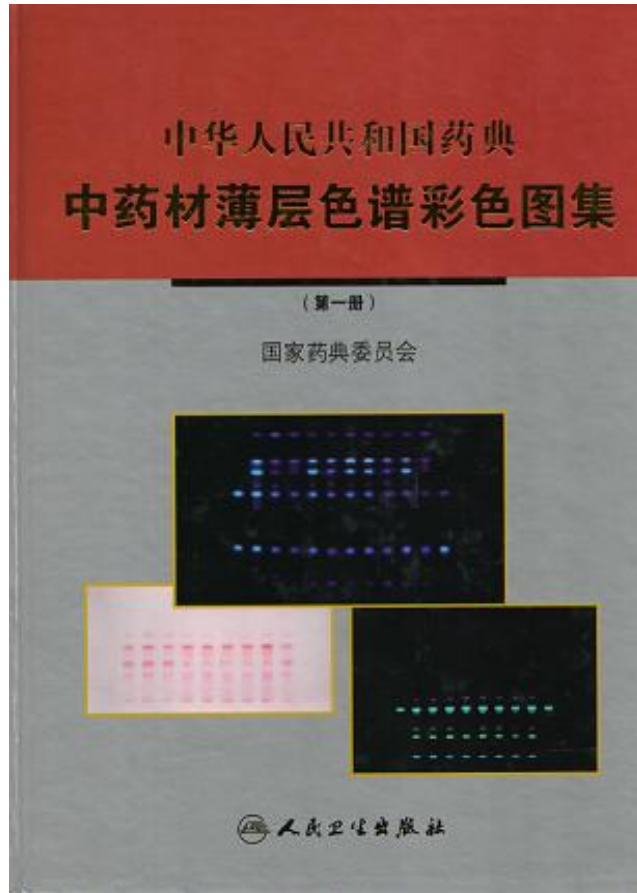
- Identification (347 monographs of crude drugs, \approx 60%)
- limit test
- Assay (13 monographs)
- Pre-coated TLC plates recommended
- Sample application: Manual, semi-automatic, automatic
- TLC images documented by electronic files



Advancements in analysis of Chinese Crude drugs in *ChP* 2010

	Identification					Limit test				Assay				
Method	microscopy	TLC	HPLC	GC	PC	General	Heavy metals	Toxic components	others	HPLC	TLCS	UV	GC	others
<i>ChP</i> 2005	339	347	5		179	726	17	17	71	175	13	20	7	30
<i>ChP</i> 2010 New admissions	374	526	3	2	26	1127	12	14	31	412	3	17	9	34
<i>ChP</i> 2010 In total	713	873	8	2	205	1853	29	31	102	587	16	37	16	64

2. TLC Atlas of Chinese Crude Drugs in Pharmacopoeia of the People's Republic of China (2005)



TLC Atlas of Chinese Crude Drugs in ChP 2005

- 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.

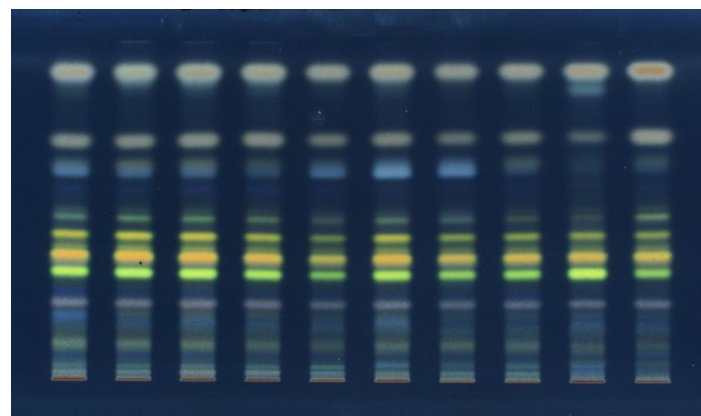
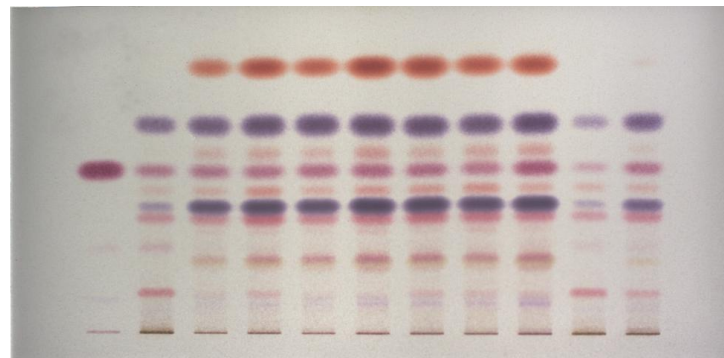
TLC Atlas of Chinese Crude Drugs in ChP 2005

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

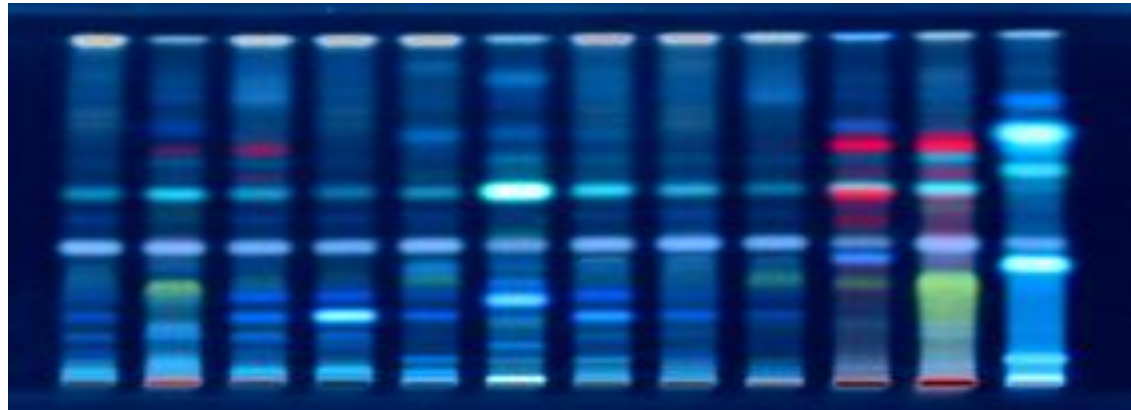
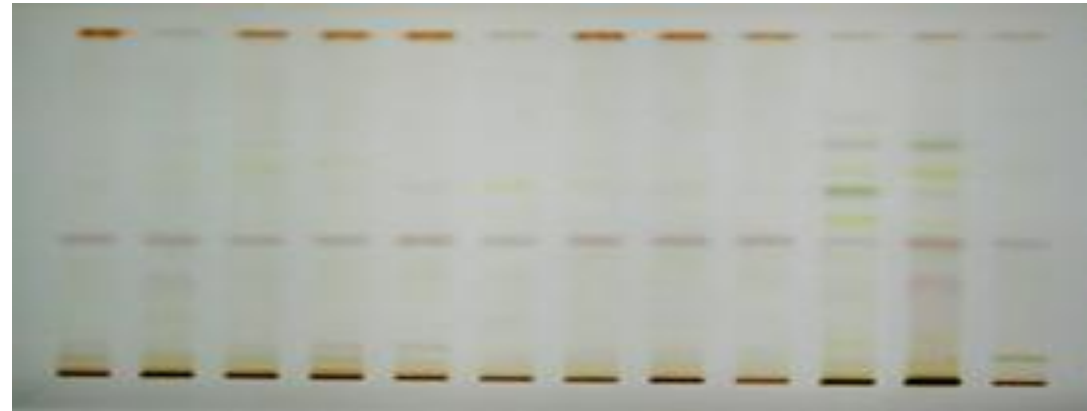
- 31 monographs
- Involved in the editing
- In charge of English version



Condition optimization and methodology evaluation for the TLC atlas

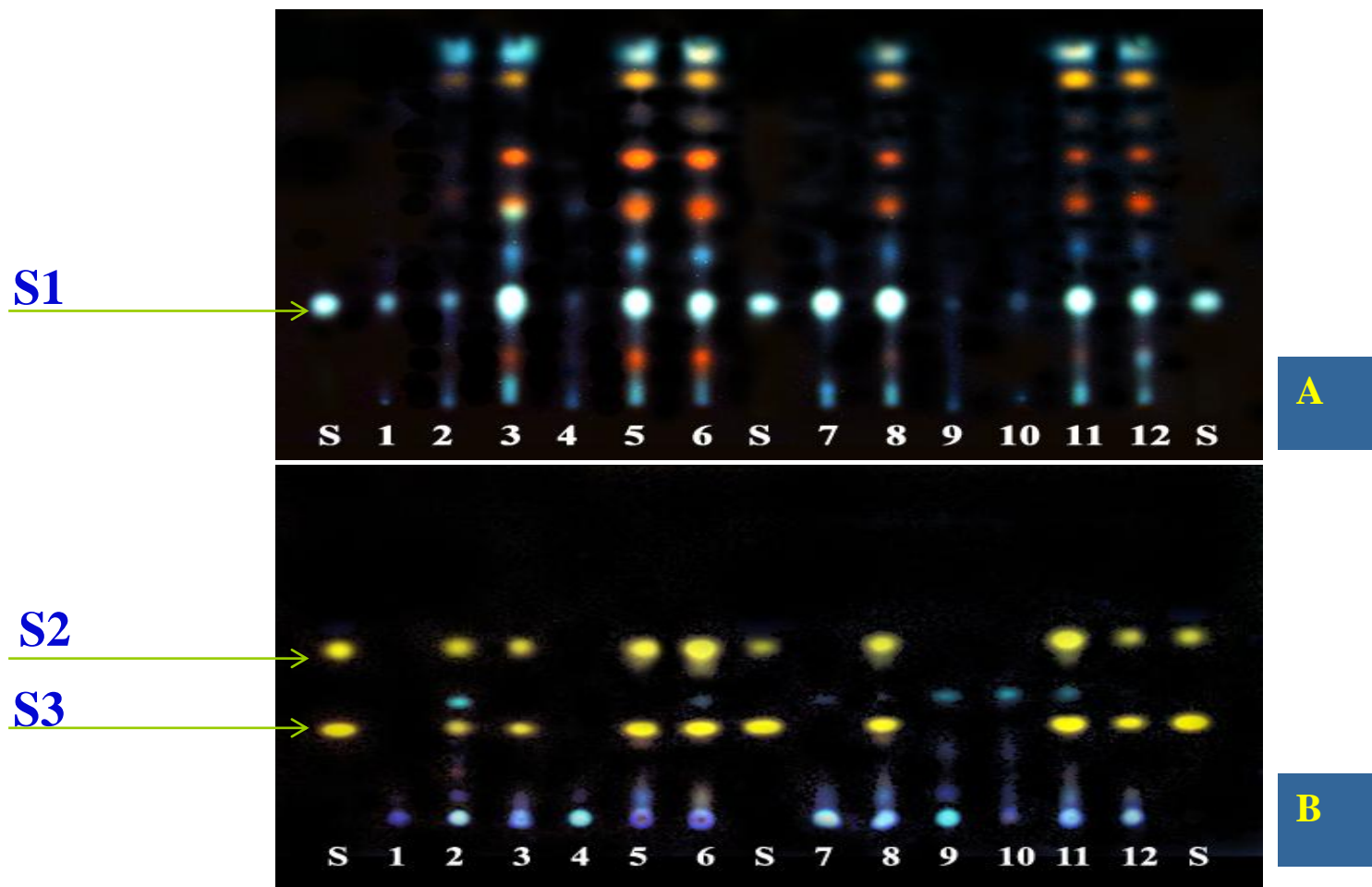
- **Representative samples for test**
- **Selection of coating materials and plates**
- **Preparation of the test solution**
- **Spotting techniques**
- **Temperature and humidity control**
- **Roles of solvent vapour**
- **Mobile phase**
- **Others**

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



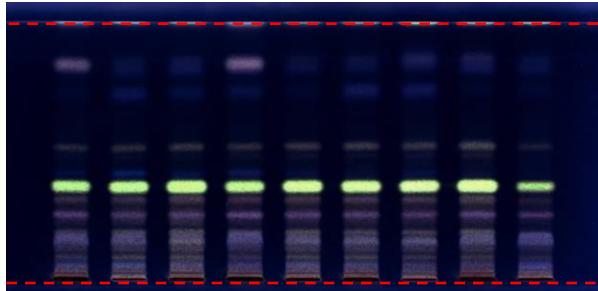
TLC of Fleeceflower Root

A. Showing the stilbens, and B. Entroquinones

S1: 2,3,5,4'-tetrahydrostilbene-2-O- β -D-glc; S2, physcion; S3, emodin

Selection of TLC plate

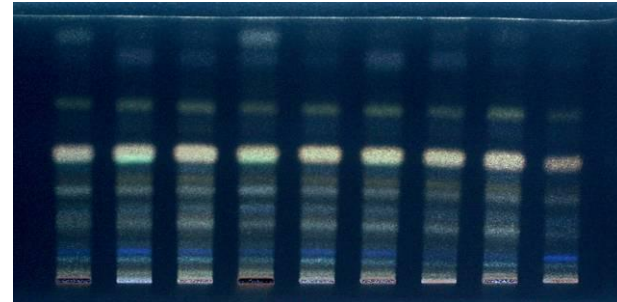
Kansui Radix 甘遂



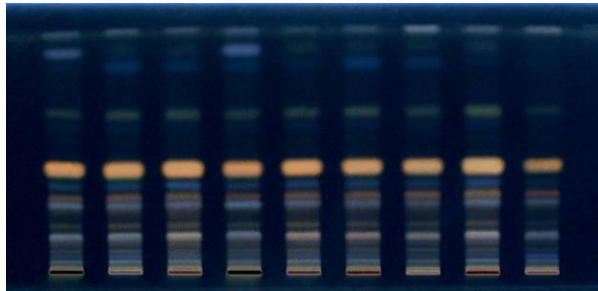
TLC pre-coated plate SIL G-25, MN, Batch: 412351

Solvent front

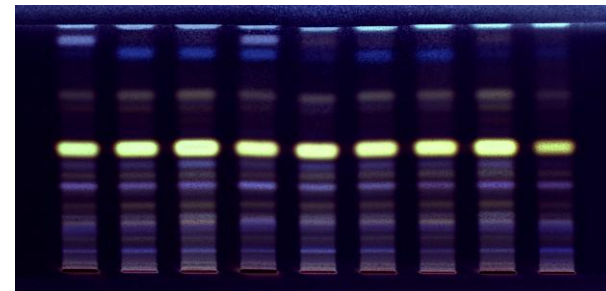
Application position



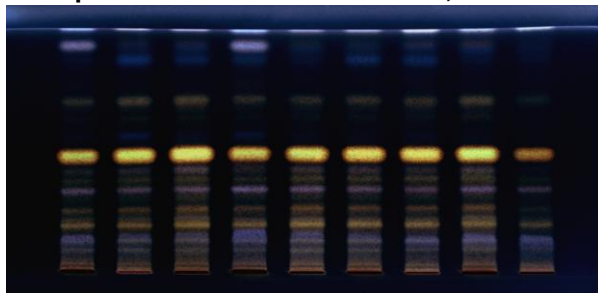
SG, Yantai chemical industry research institute, China



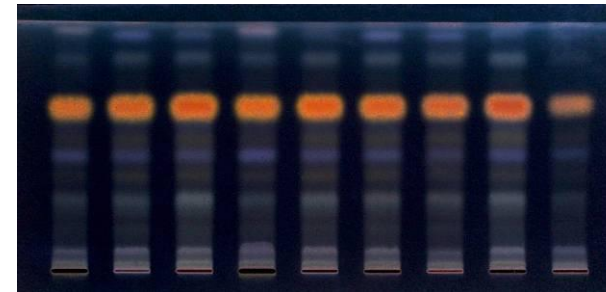
HPTLC-plate Nano-DURASIL-20, MN, Batch: 502033



HPSG, Yantai chemical industry research institute, China



TLC Silica gel 60, MERCK, Batch: OB483888

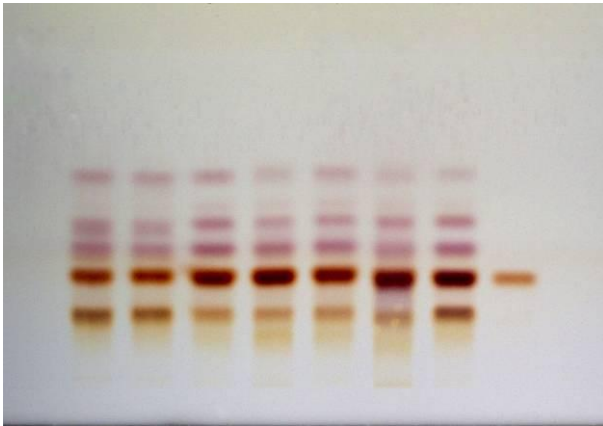


Self-coated plate (0.5% CMC-Na, 0.3 mm)

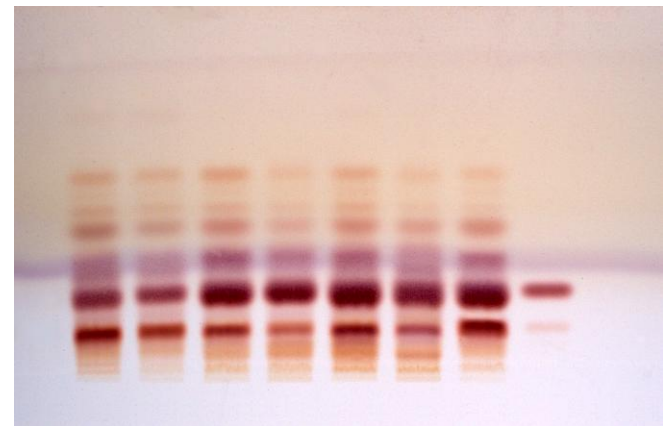
In the *TLC Atlas* (2005), at least five TLC plates were compared, the results were showed in the Chinese version. In the English version, only the optimized one was given.

The selection of TLC plate

TRICHOSANTHIS RADIX 天花粉



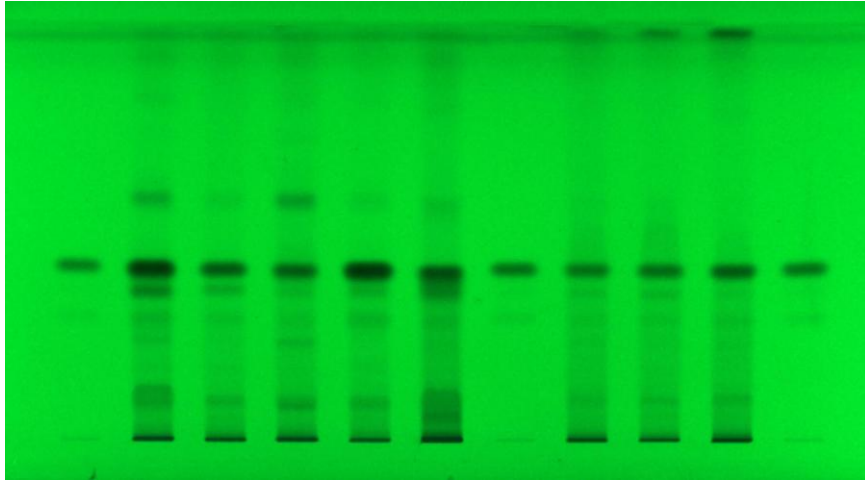
DC-Fertigplatten SIL G-25UV₂₅₄ MN, Germany



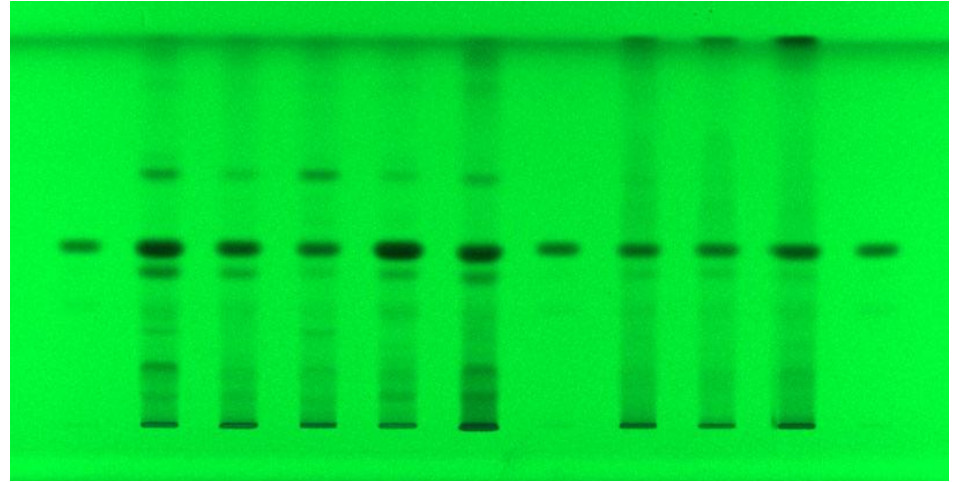
HPSG, Yantai chemical industry research
institute, China

MN plate showed better result than HPSG plate

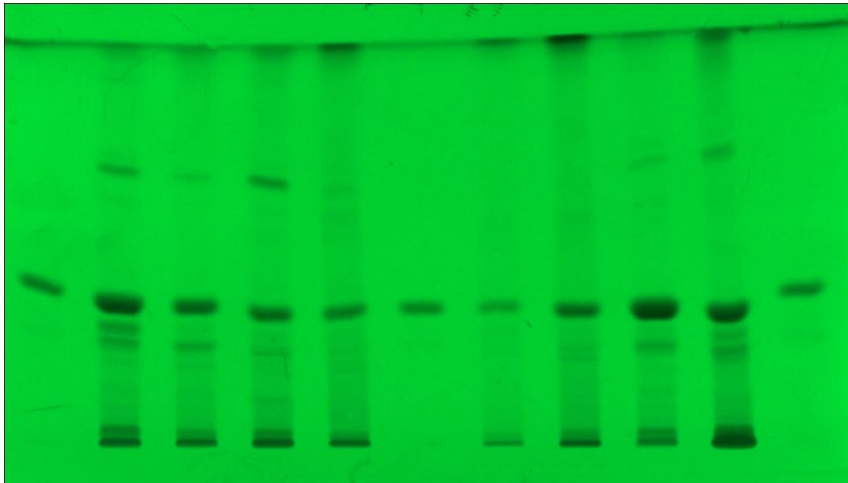
Gentianae Radix 龙胆



MN



Merck –best resolution

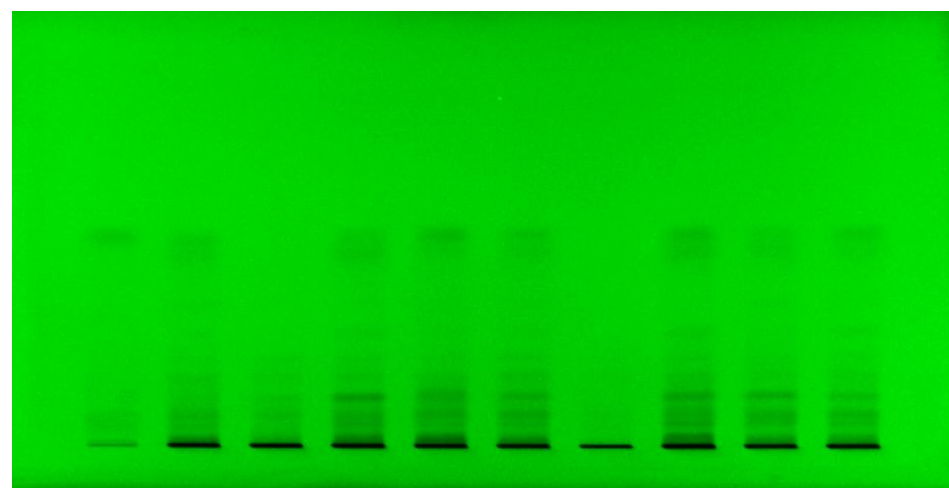
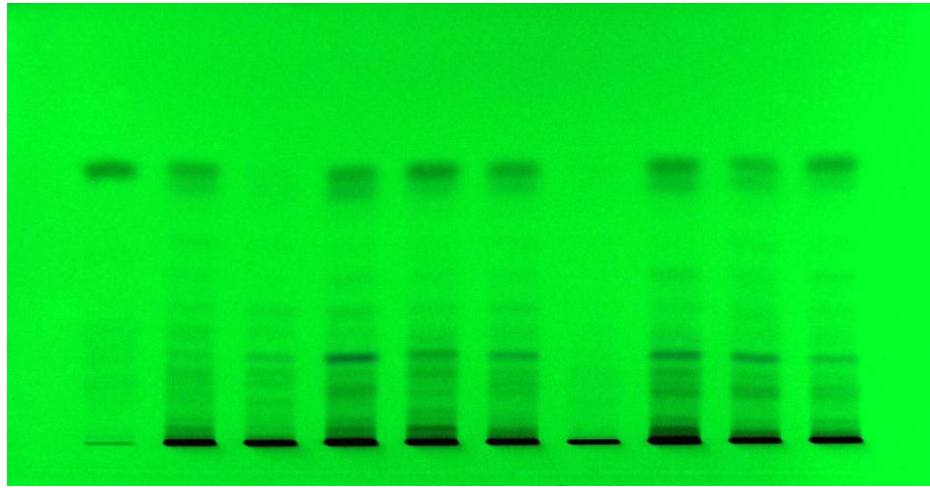


HSGF

RUBIAE RADIX ET RHIZOMA 茜草

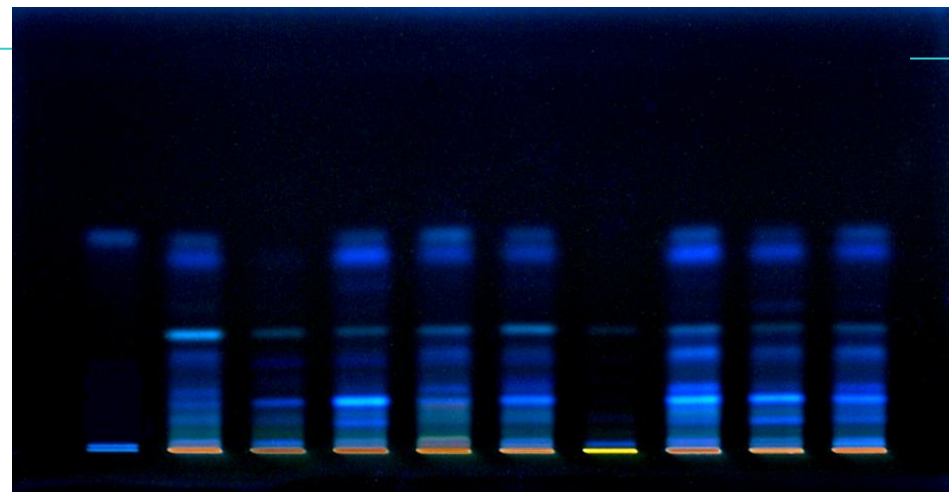
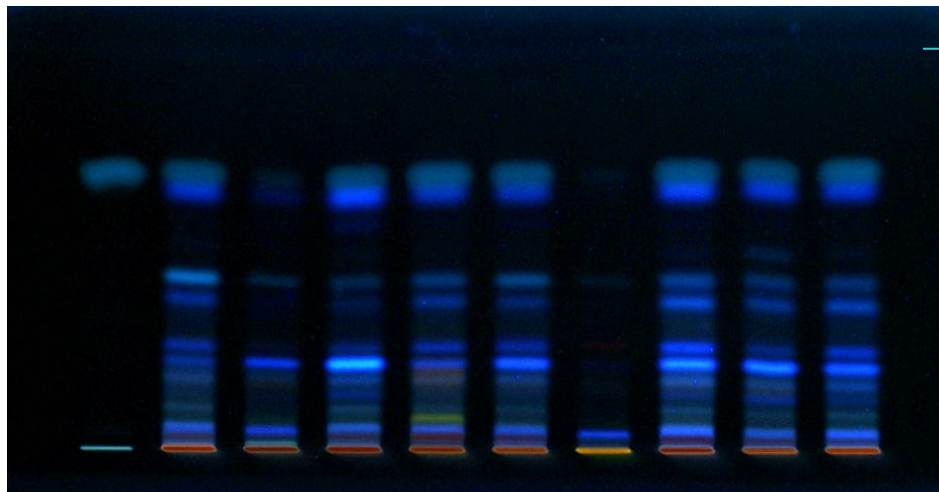
T: 12°C RH:26%

T: 14°C RH: 30%



T: 12°C RH: 26%

T: 14°C RH: 30%

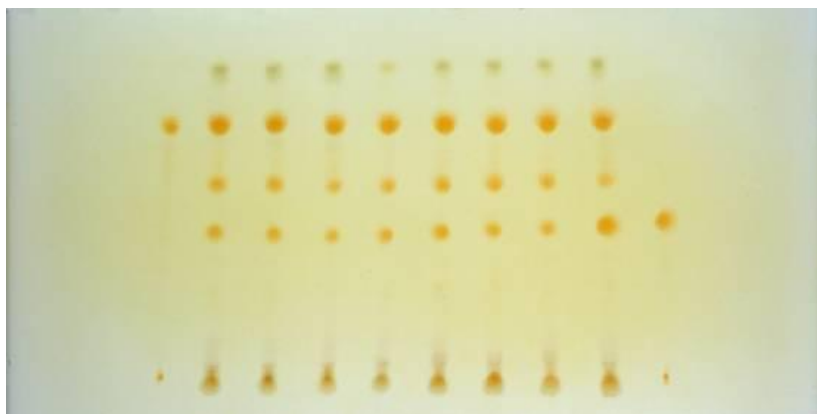


SG F₂₅₄ Showed better resolution than MN plate

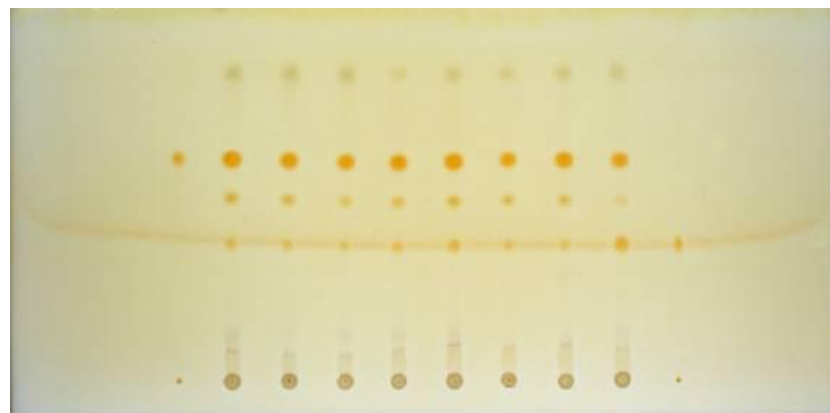
MNF254

The selection of TLC plate

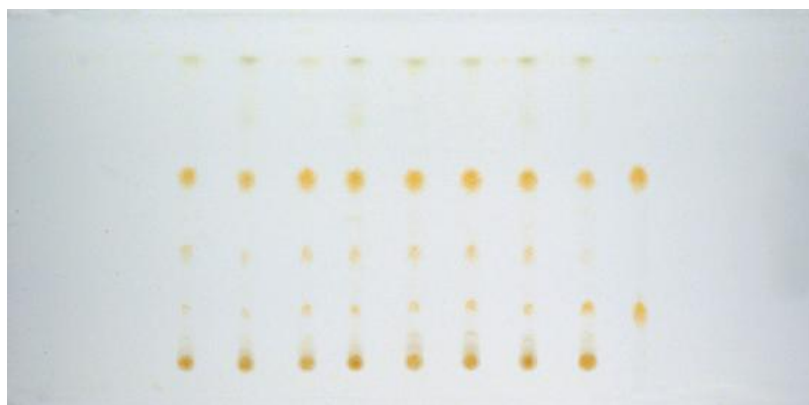
NELUMBINIS PLUMULA 蓮子心



SG, Yantai chemical industry research institute, China



DC-Fertigplatten SIL G-25UV₂₅₄ MN, Germany



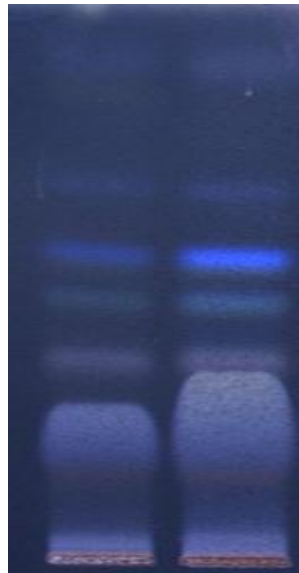
Lab-coated plate (0.5% CMC-Na, 0.3 mm)

For some alkaloids, the lab-coated plate showed the best resolution

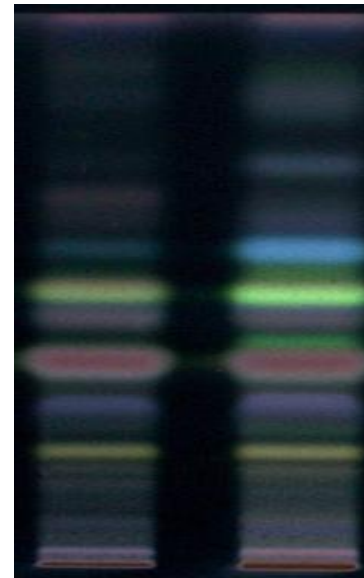
Preparation of test samples

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.



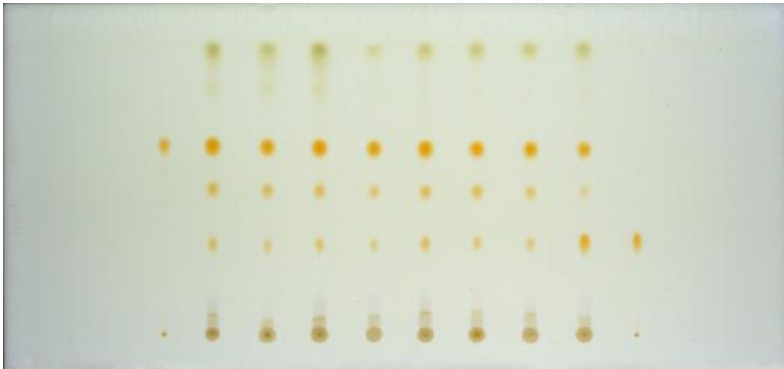
ChP 2005



TLC Atlas

Spotting techniques

The semi-automatic or automatic band-wise spray sampling mode is adopted in this atlas to ensure best chromatogram quality.

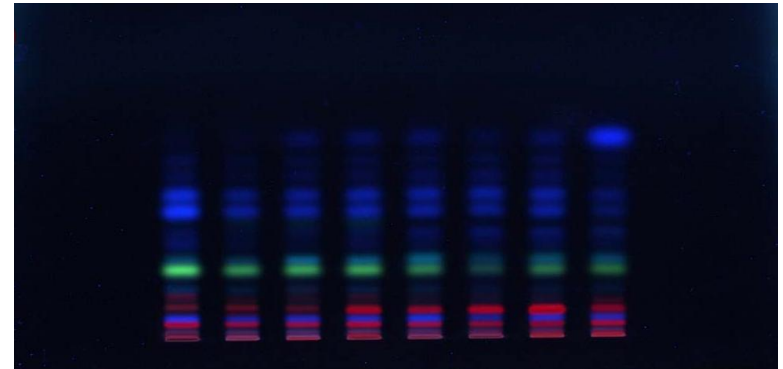


NELUMBINIS PLUMULA 蓮子心

MERCK TLC Silica gel 60 F₂₅₄



Apply in spots is better than in bands



ECLIPTAE HERBA 墨旱莲

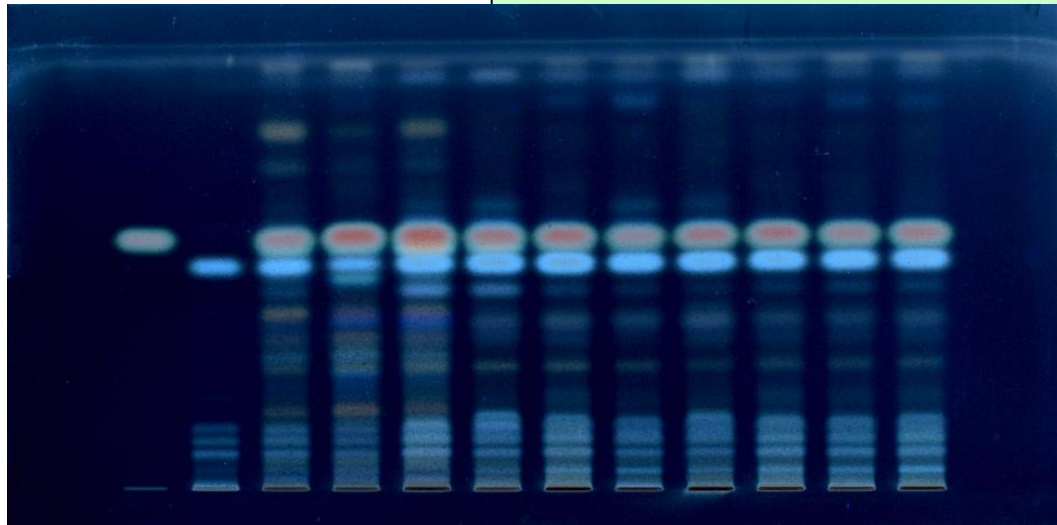
HPSG, Yantai chemical industry research institute, China

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%

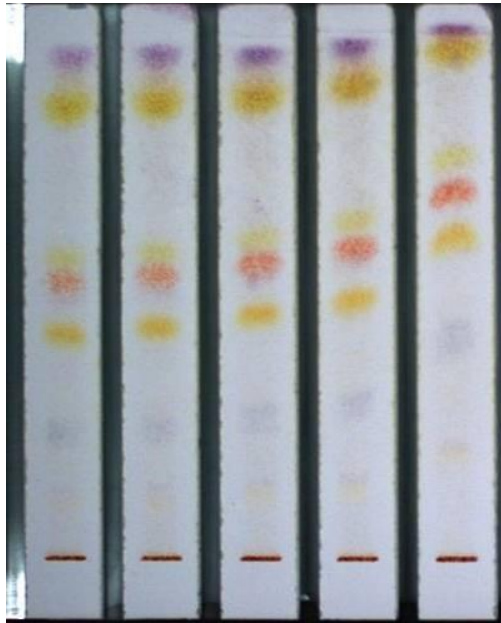


Vladimiriae Radix 川木香

Temperature and humidity

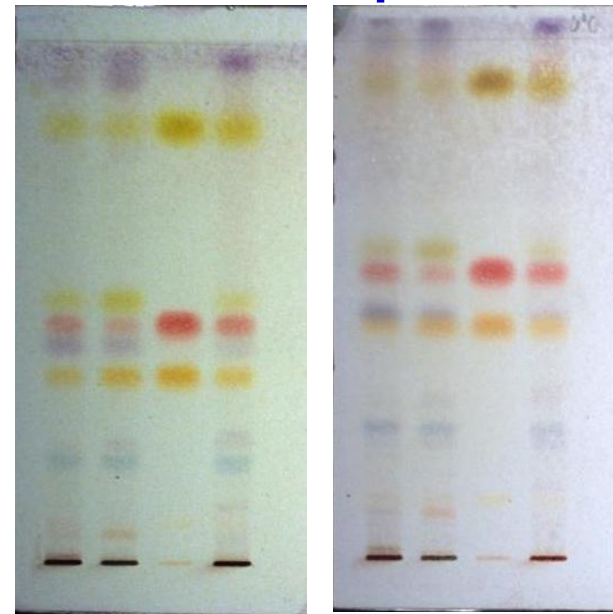
To get satisfied separation, temperature and humidity were optimized on the system suitability verification.

Different RH



42% 58% 65% 72% 88%

Different Temperature



20°C

4°C

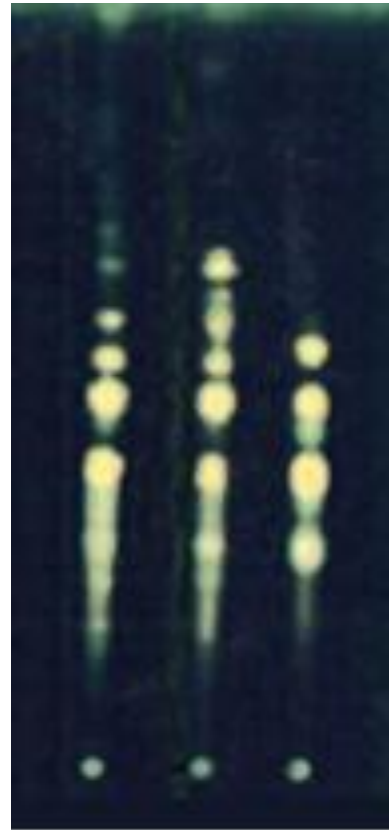
ALPINIAE KATSUMADAI SEMEN 草豆蔻

Roles of solvent vapour

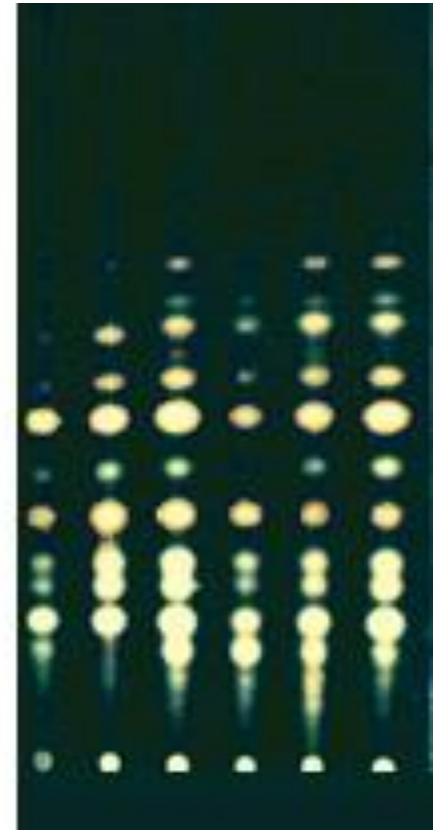
Chamber saturation

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.

Ginseng Radix



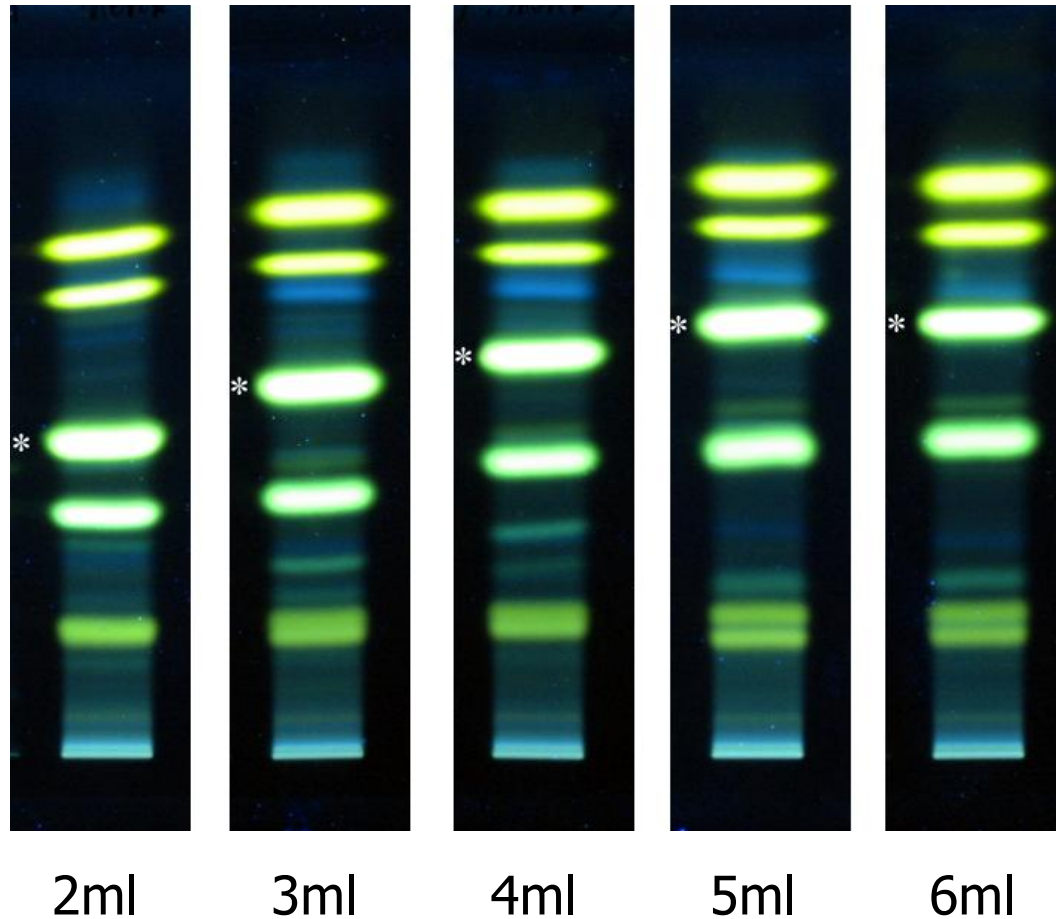
no pre-equilibrated



pre-equilibrated

Roles of solvent vapour

Coptidis Rhizoma

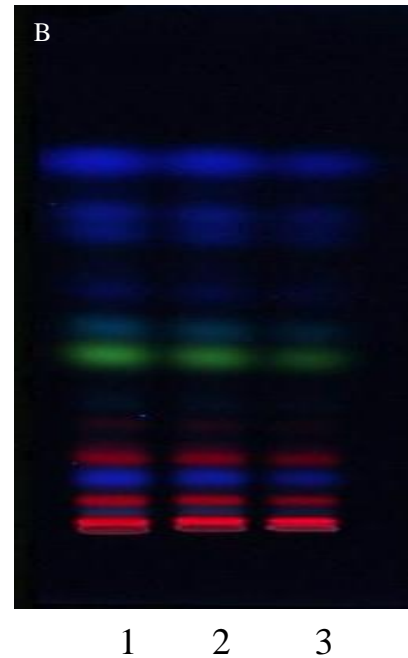
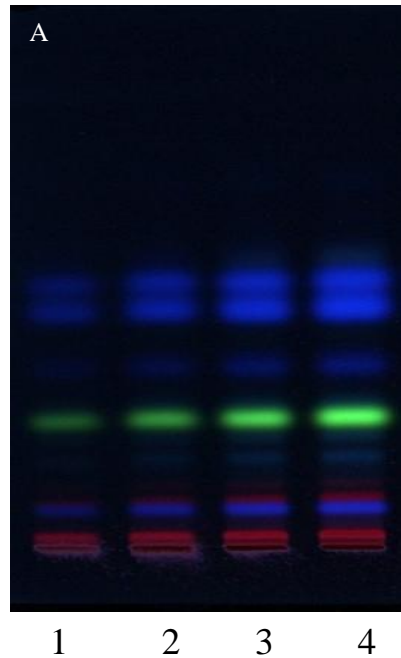


The amounts of ammonia used in pre-equilibrating in the TLC analysis of Coptidis Rhizoma, effect the migration of berberine (*)

Optimization of mobile phase

A. ChP 2005 : Hexane-EtOAc (9: 1)

B. TLC Atlas: Hexane-Acetone (9: 1)



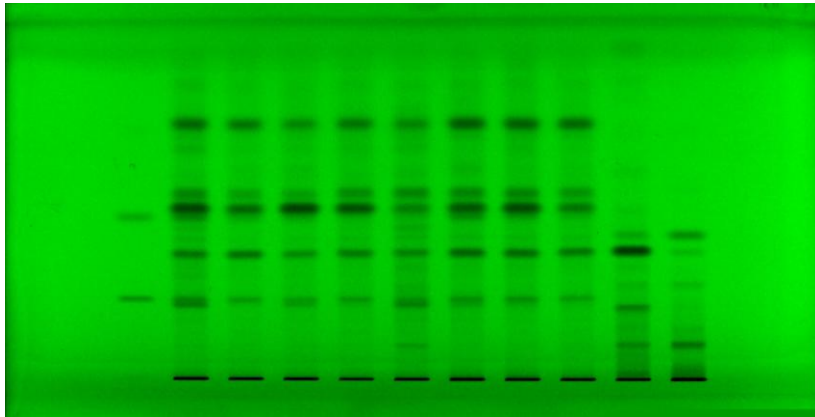
ECLIPTAE HERBA 墨旱莲

Mobile phase

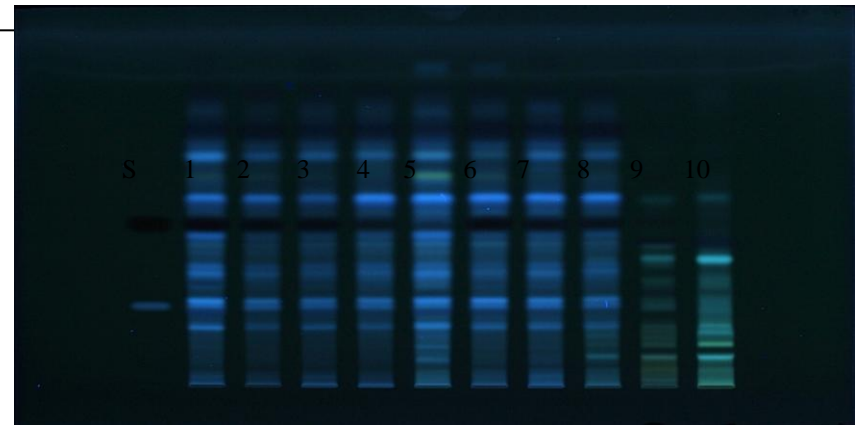
In the *TLC atlas*, benzene was replaced by other solvents to avoid its toxicity.

Alpiniae Katsumadai Semen 草豆蔻

T: 21 °C RH: 62%



T: 21 °C RH: 62%



DC-Fertigplatten SIL G-25UV₂₅₄ (MN)

S. cardamomin and alpinetin CRS (from up to down); 1~10. sample

Mobile phase:

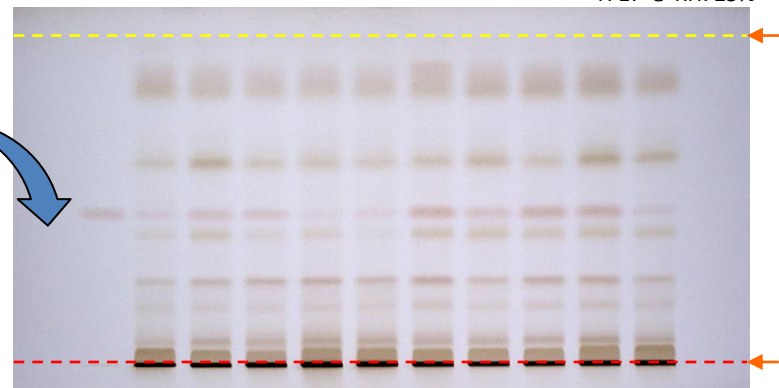
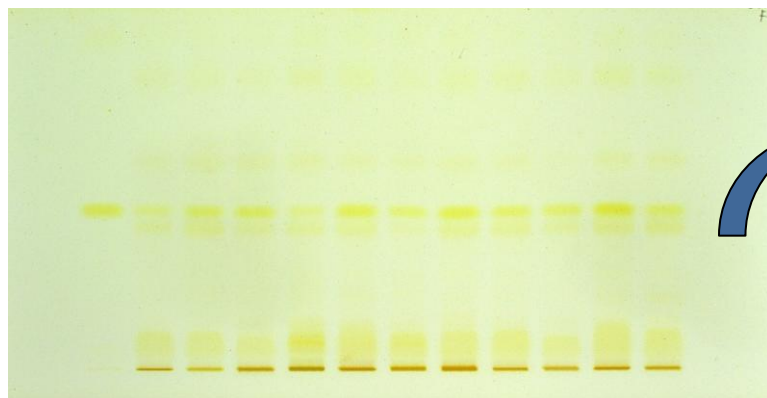
Chp 2005: benzene-ethyl acetate - methanol (15:4:1)

TLC Atlas: toluene- ethyl acetate - formic acid (10:1:1)



Derivatization

Asteris Radix et Rhizoma 紫菀



In *Chp 2005*

2,4- DINITROPHENYLHYDRAZINE TS

Derivatization reagent:

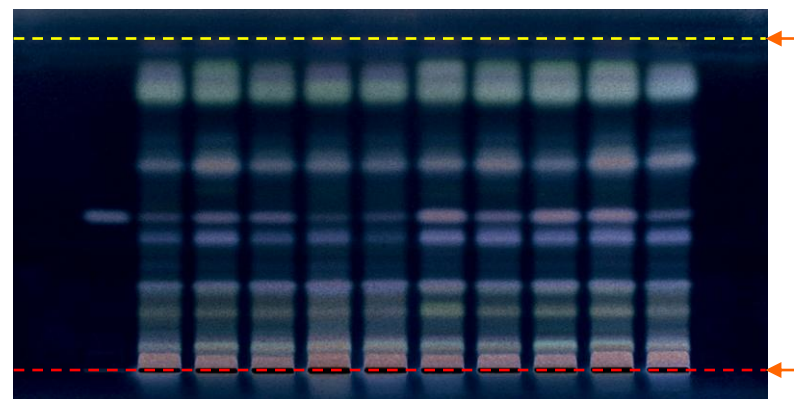
ChP 2005: 2,4-DinitrophenylhydrazineTS

TLC Atlas: 10% sulfuric acid in ethanol

Extract solvent:

ChP 2005: petroleum ether

TLC Atlas: chloroform



In *TLC Atlas*

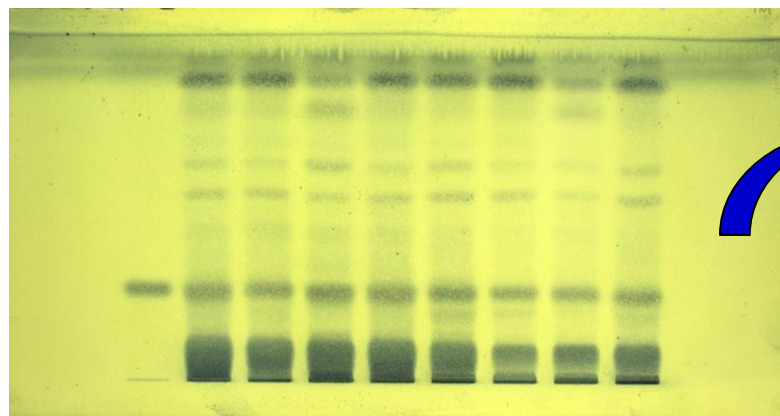
10% sulfuric acid in ethanol

This revised method was adopted in *ChP 2010*.

Derivatization

天麻 *Gastrodiae Rhizoma*

ChP 2005



(DC-Fertigplatten SIL G-25 (MN))

Mobile phase

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

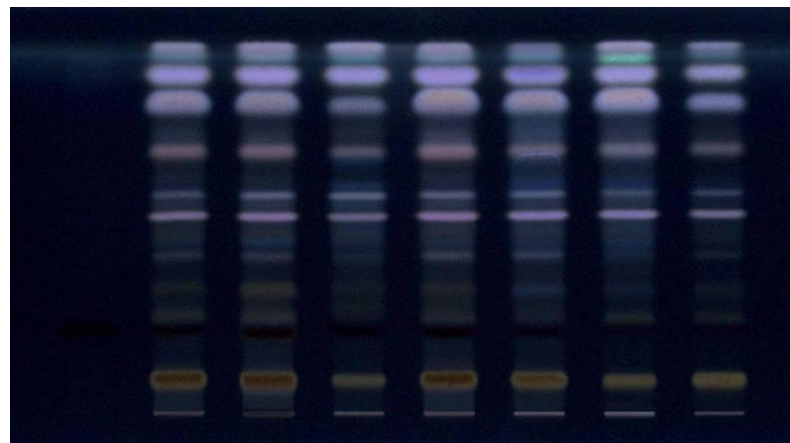
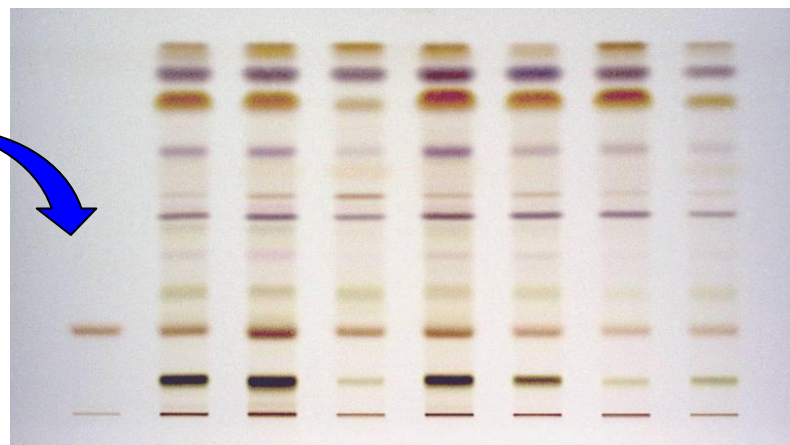
TLC Atlas,2005: chloroform-ethyl acetate-methanol-water (2.5:1:1:0.1)

derivatization reagent

ChP 2005: 10% Phosphomolybdic acid in ethanol

TLC Atlas: 10% sulfuric acid in ethanol

TLC Atlas



TLC Silica gel 60, MERCK

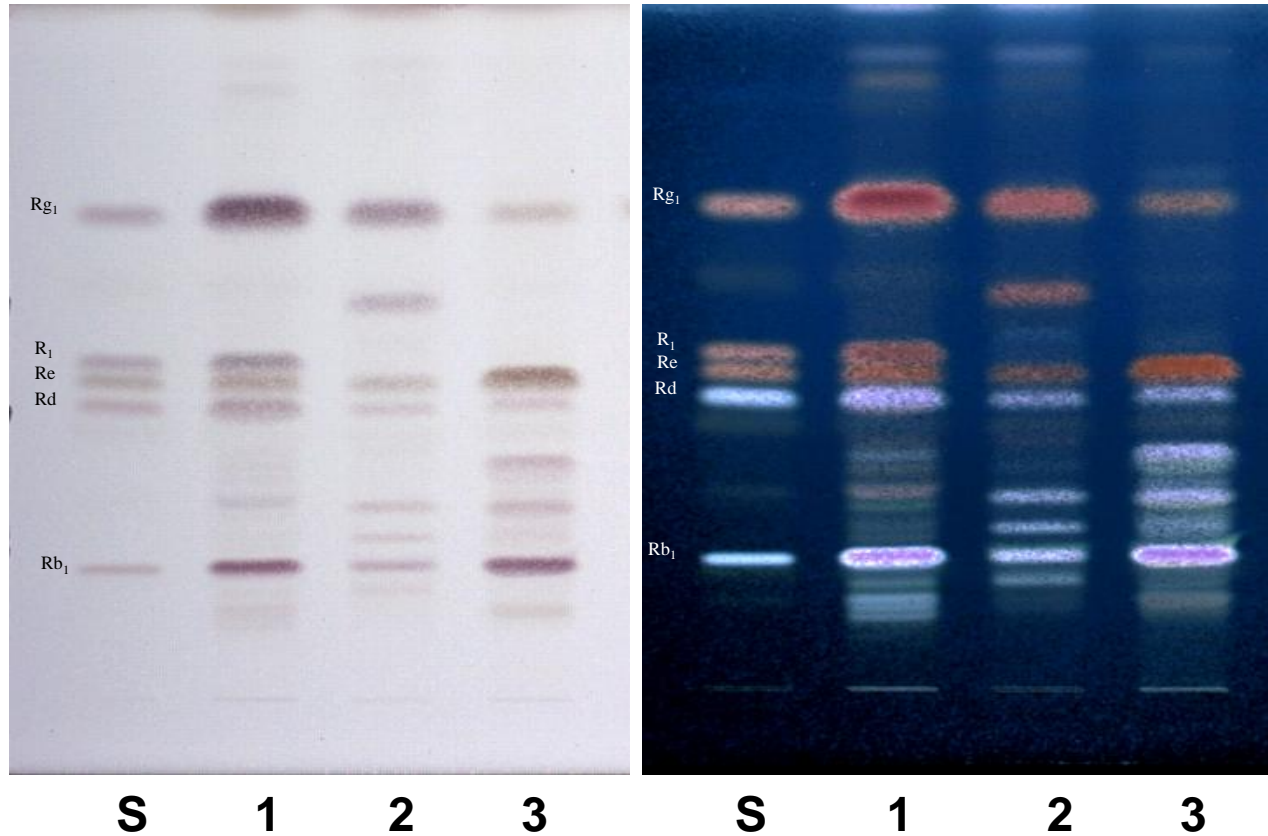
3. Application of TLC/HPTLC in Identification of Traditional Chinese Medicines

TLC identification is more widely used in

ChP 2010

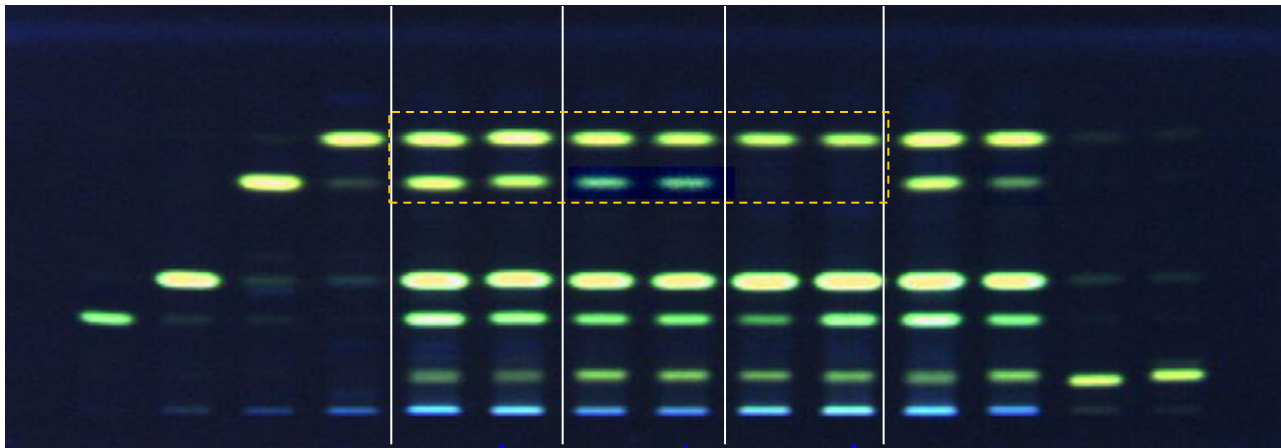
- **Authentication of crude drugs from different species,**
- **Specificity of the marker components ,**
- **Identification using multi-marker components ,**
- **More informative chromatograms ,**
- **Use of new methods and new technologies**

Identification of Ginseng, Notoginseng, and American Ginseng



S. reference standards 1. Notoginseng 2. Ginseng 3. American Ginseng

HPTLC chromatograms for three species of *Coptidis Rhizoma*



味连(Rhizome of *Coptis chinensis*)

雅连(Rhizome of *Coptis deltoidea*)

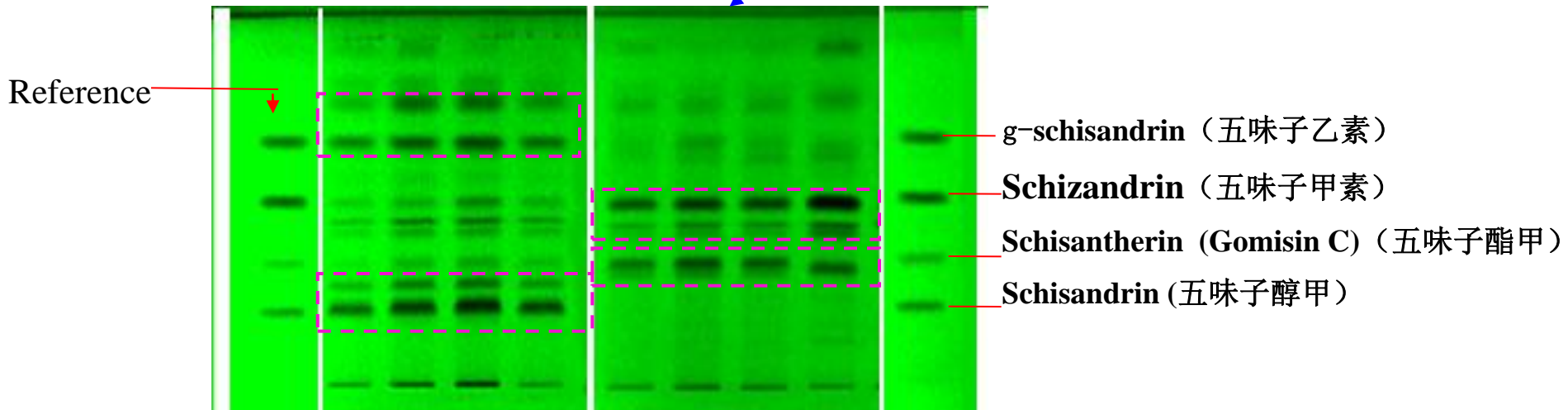
云连(Rhizome of *Coptis teeta*)

The 3 species of *Coptidis Rhizoma* were distinguished by the same TLC identification method

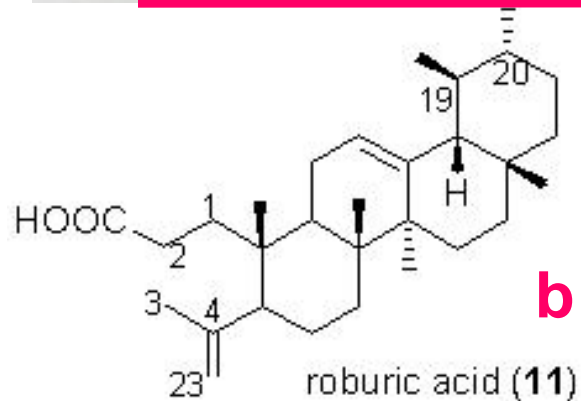
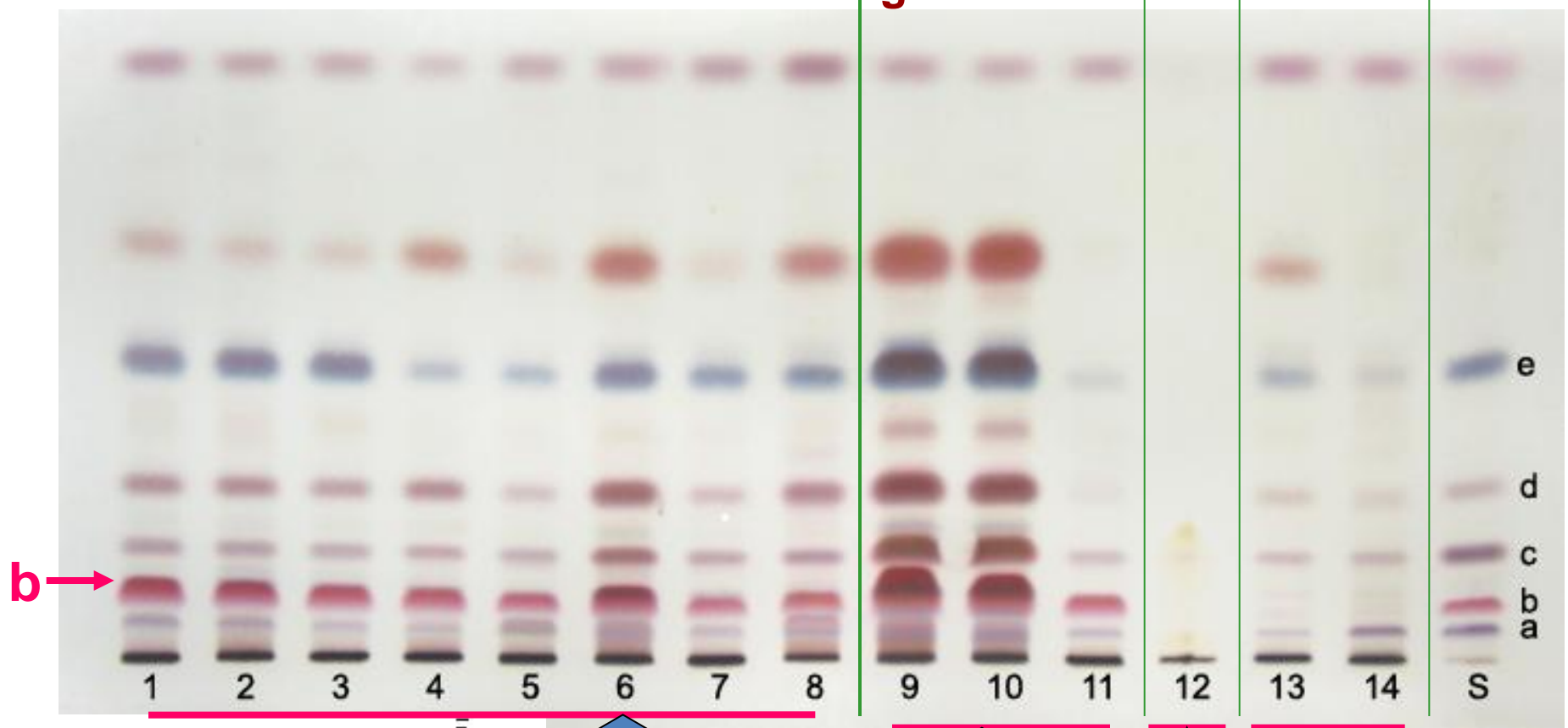
TLC identification of Schisandriae chinesis Fructus and Schisandriae sphenantherae Fructus

北五味子
Fructus Schisandriae Chinesis

南五味子
Fructus Schisandriae Sphenantherae



TLC Directed Identification of Diagnostic components of Chinese Gentian and Largeleaf Gentian Root



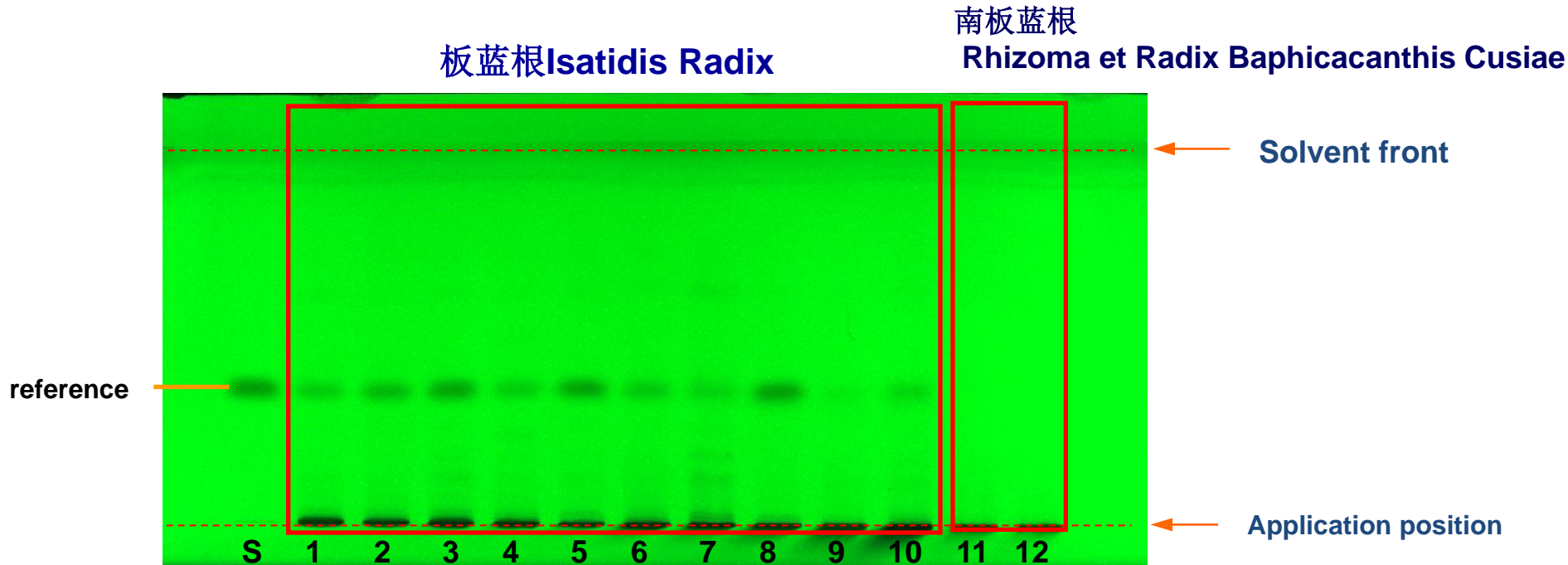
Largeleaf Gentian

Substitutes
from Gentiana

adulterant

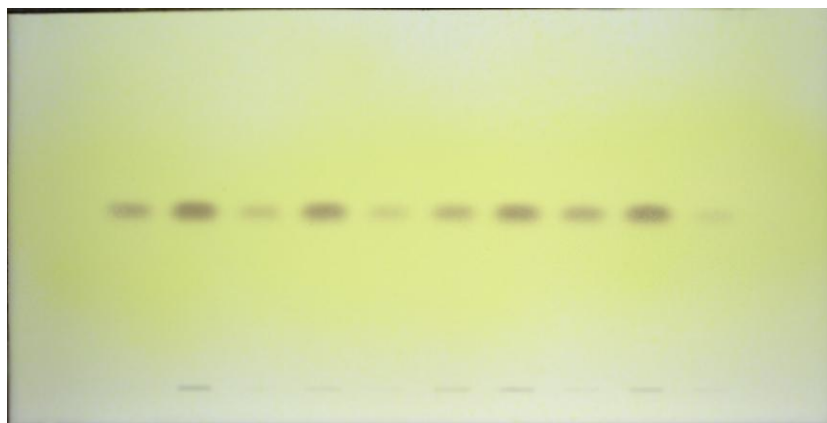
Chinese Gentian

TLC identification of Isatidis Radix and Rhizoma et Radix Baphicacanthis Cusiae

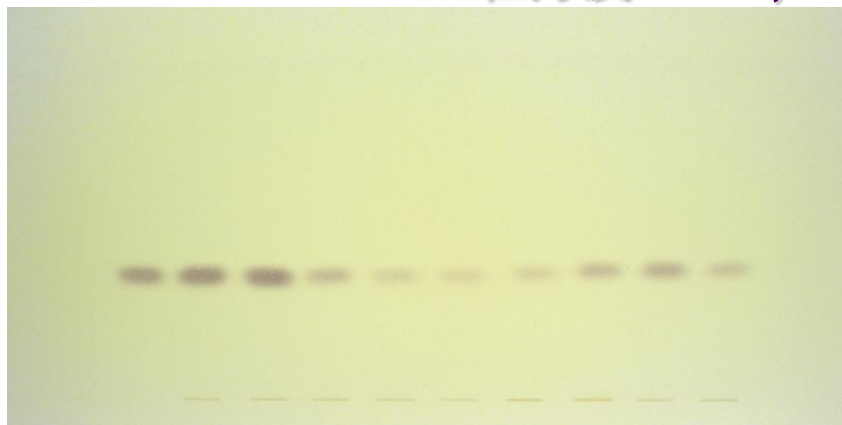


S. (R,S)-goitrin; 1-12. samples

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*

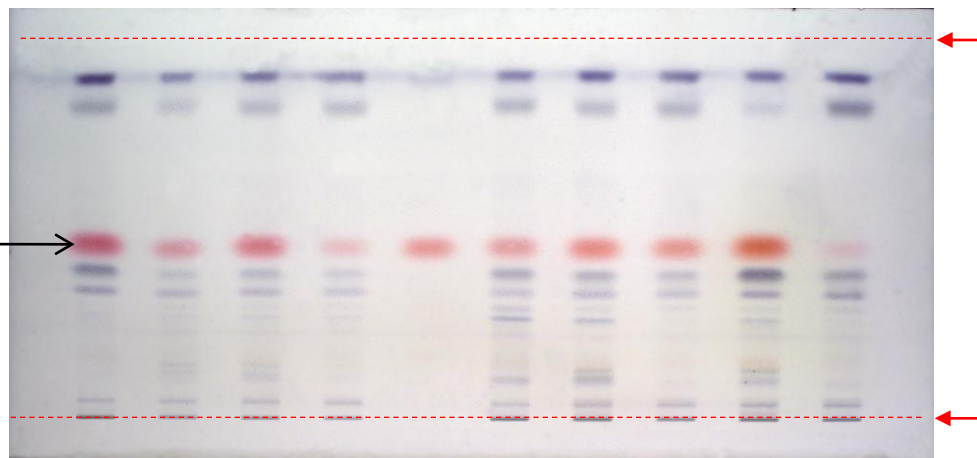


CYNANCHI PANICULATI RADIX ET RHIZOMA 徐长卿 *ChP, 2005*

Paeonol CRS, 5% FeCl₃ in ethanol

MOUTAN CORTEX 牡丹皮

T: 22°C RH: 70%



Paeonol

Method in *TLC Atlas, 2005*

Mobile phase:

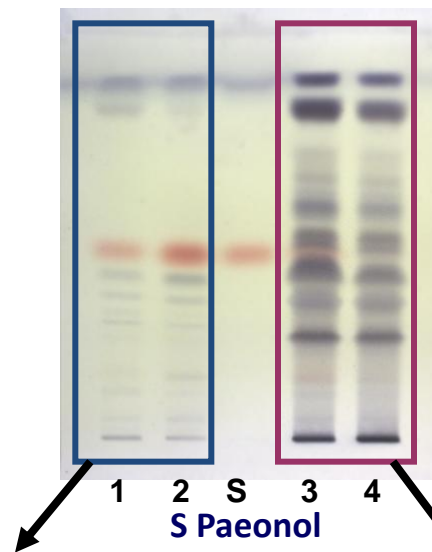
Chp,2005: cyclohexane-ethyl acetate (3:1)

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

Derivatization reagent:

Chp, 2005: 5%FeCl₃ in ethanol

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



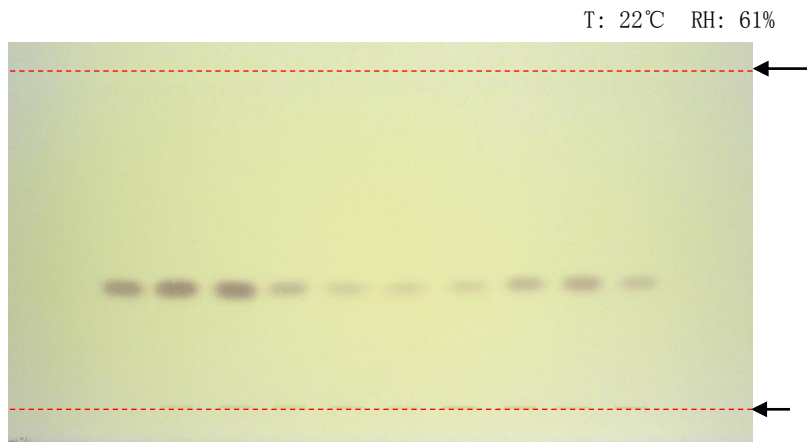
Paeonia suffruticosa

Cynanchum paniculatum

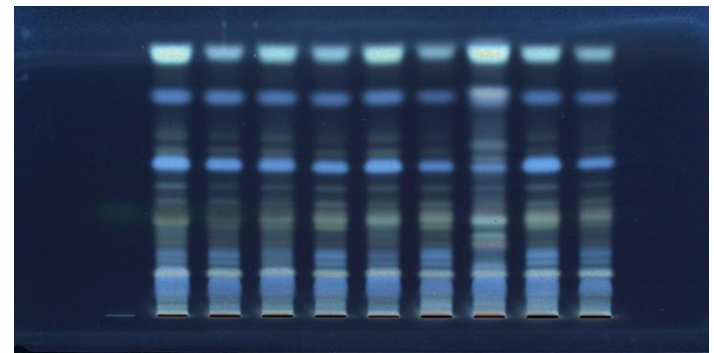
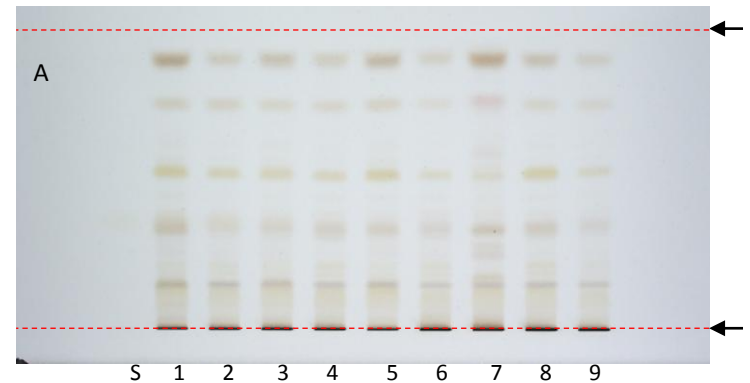
This revised method was adopted in *ChP 2010*

CYNANCHI PANICULATI RADIX ET RHIZOMA 徐长卿

New method in TLC Atlas



Method in *ChP*, 2005 is remained



S 1 2 3 4 5 6 7 8 9

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

Radix Linderae 乌药

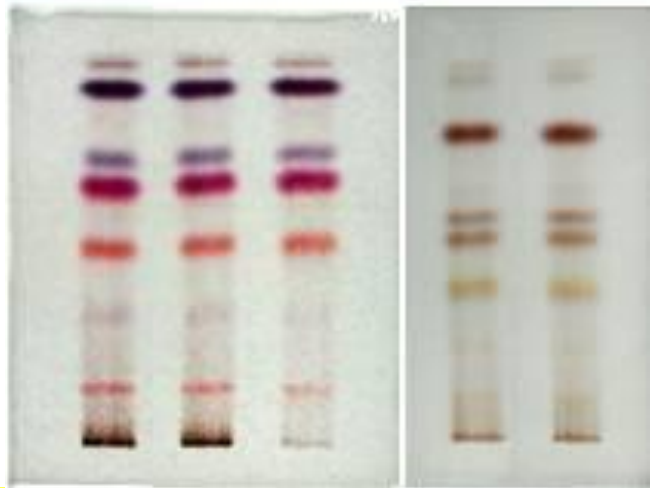
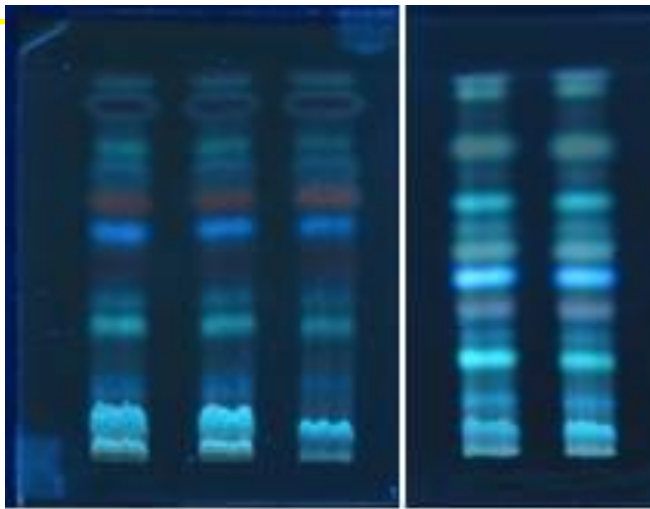
- Combined Spicebush Root is the dried root tuber of *Lindera strychnifolia* (Sims) Kosterm.
- **Action:** To smooth the flow of qi and relieve pain, and warm and tonify the kidney and urinary bladder
- **The goal of this experiment:** establish TLC identification for Radix Linderae.
- **Chemical Constituents** :volatile oil, Sesquiterpene, isoquinoline alkaloids and polyphenol

Optimization of extraction method

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 30min in 30 ml petroleum ether (30~60°C), ultrasonicate for 10 min, filter, ...



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

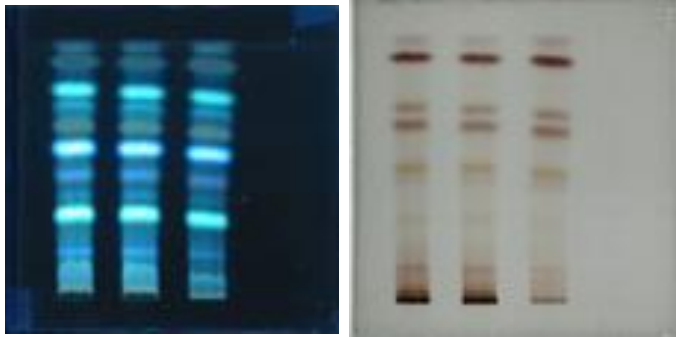
- ❑ Left: 1% solution of vanillin in sulfuric acid
- ❑ Right: 10% sulfuric acid reagent

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

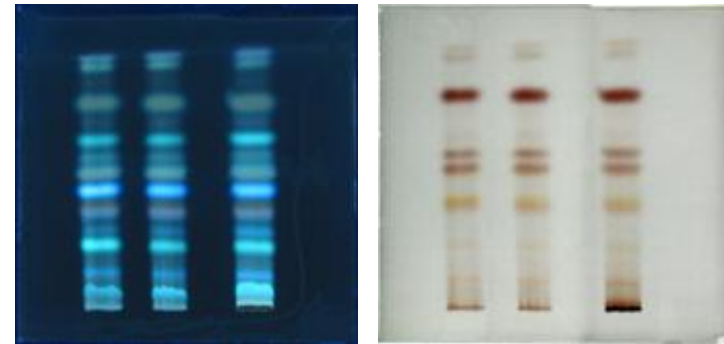
Extracting method

1. Ethyl ether, ultrasonicate;
2. Ethyl ether, macerate for one night
3. Petholeum ether, ultrasonicate;

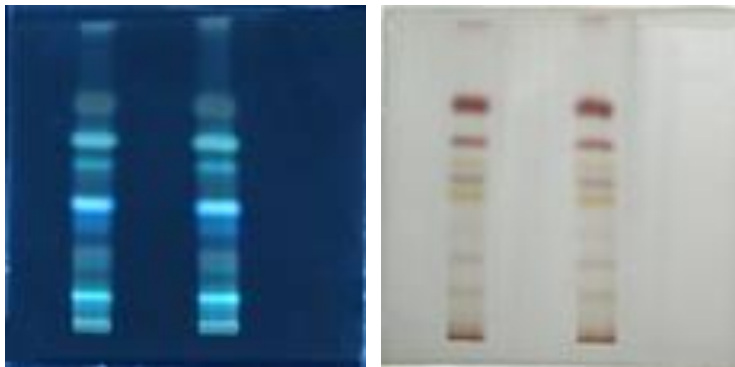
Optimization of mobile phase



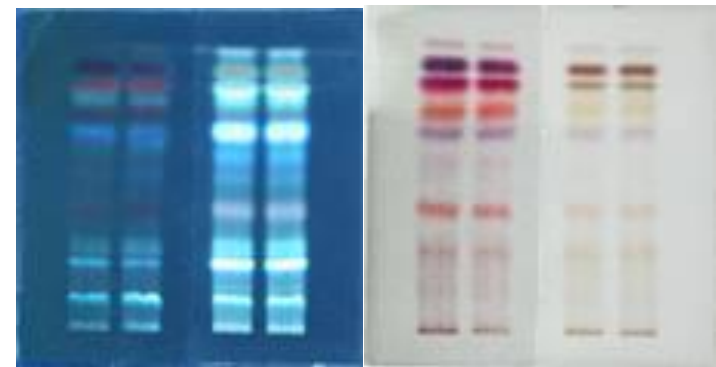
Petroleum ether-ethyl acetate(5:1)



n-hexane-ethyl acetate(5:1)



toluene-ethyl acetate(15:1)

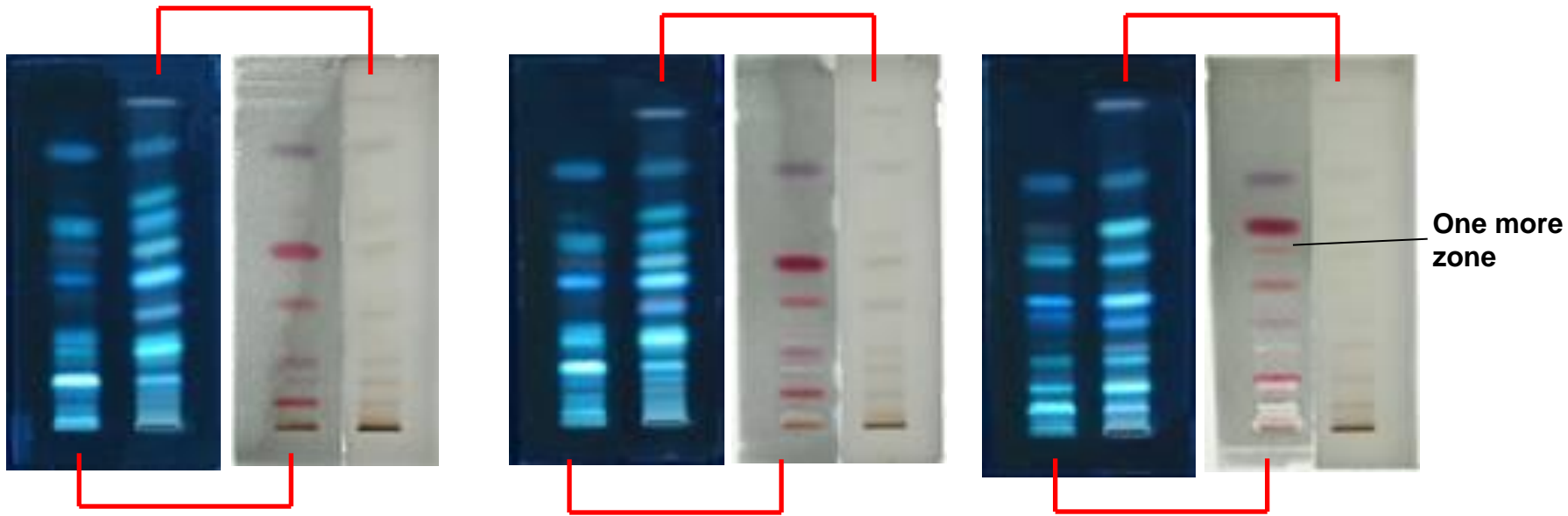


chloroform-ethyl acetate(20:1)

Other three systems are all good except chloroform system

Optimization of mobile phase and visualization solvent

10% sulfuric acid in ethanol



1% solution of vanillin in sulfuric acid

Petroleum ether-ethyl acetate(5:1)

n-hexane-ethyl acetate(5:1)

Toluene-ethyl acetate(15:1)

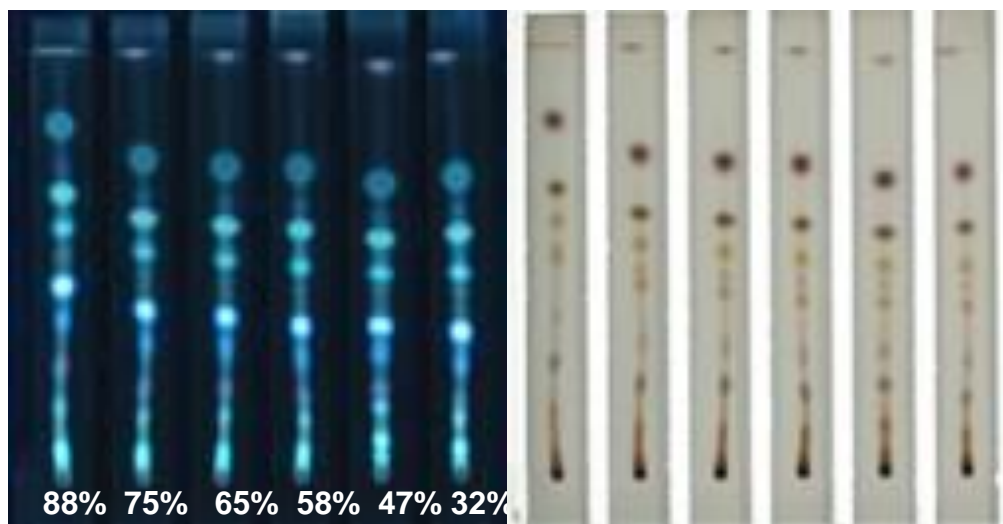
Uv 366nm – the result is same for three mobile phase

Visible light- more spots were found in the left plate (use 1% solution of vanillin in sulfuric acid as derevatization reagent)

Toluene system is the final mobile phase to be selected.

Humidity control

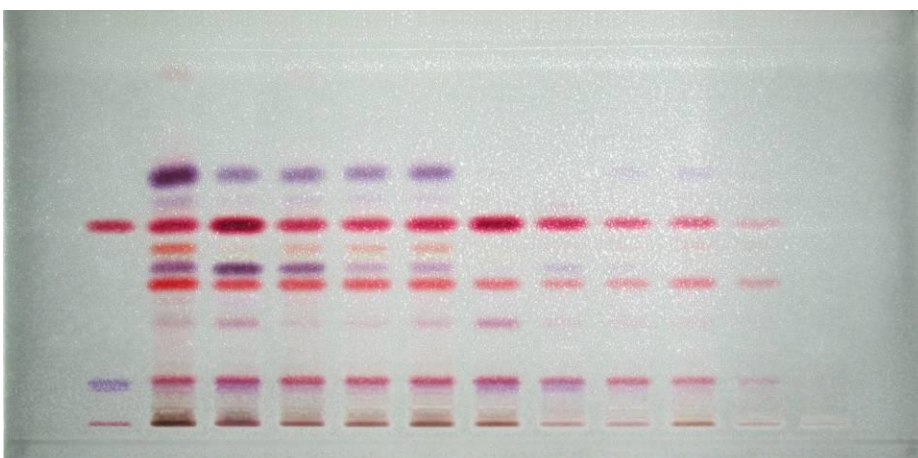
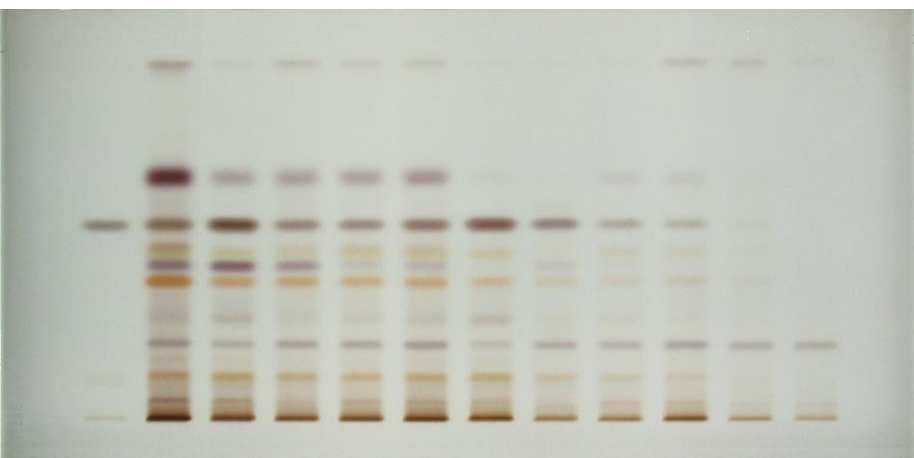
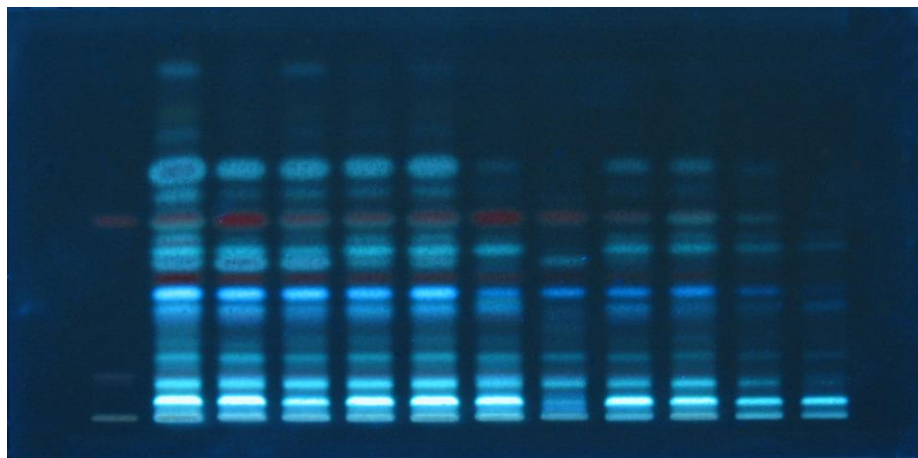
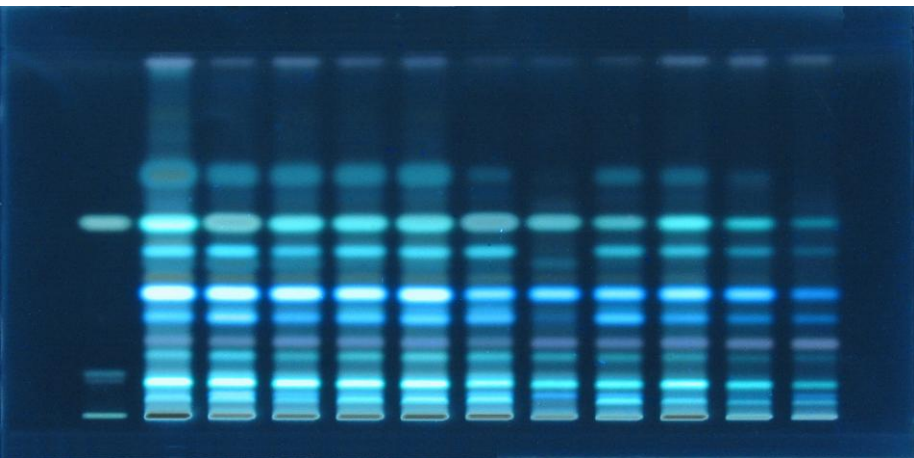
using different concentration of sulfuric acid solution: 88%, 75%, 65%, 58%, 47% and 32%



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



S 1 2 3 4 5 6 7 8 9 10 11

S 1 2 3 4 5 6 7 8 9 10 11

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

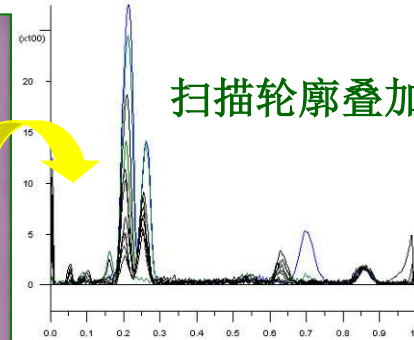
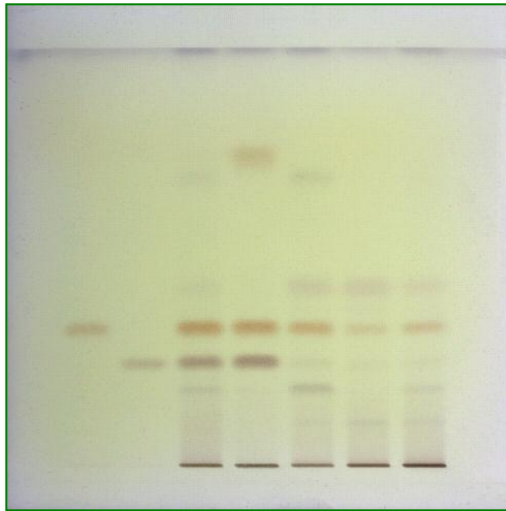
plate: silica gel G60-TLC (Merck) ;

Develop vertically for 8 cm;

S. Linderane ,1~11 samples

This method was adopted in *ChP* 2005.

Application of TLC-bioautographic technology in *ChP*

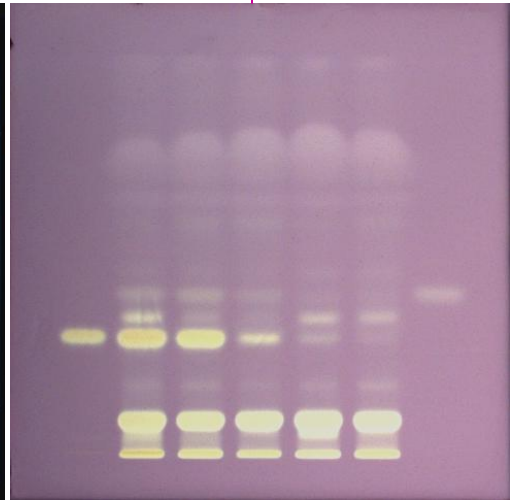
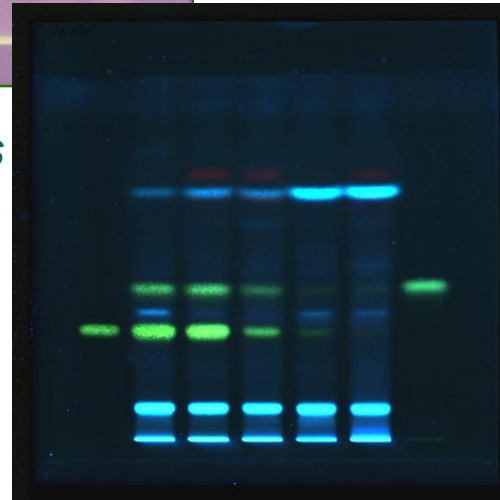


扫描轮廓叠加图

DPPH显色

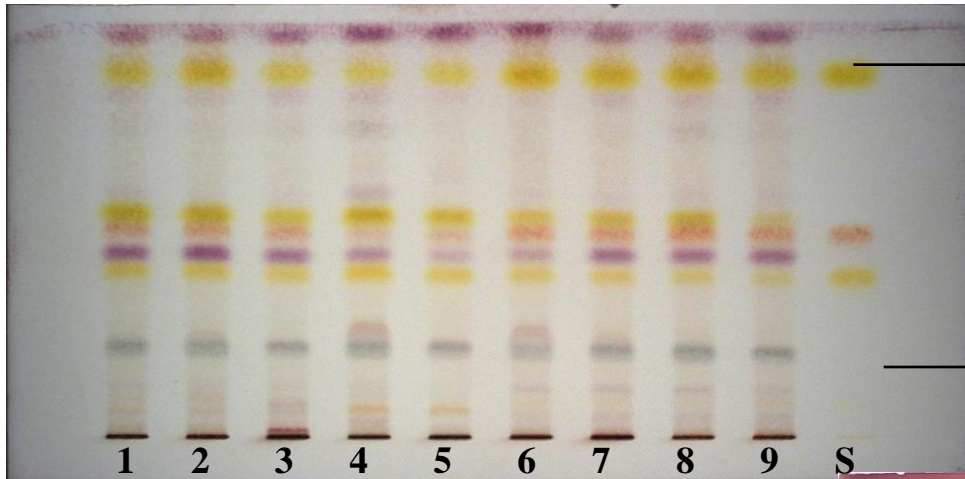
TLC of *Cortex Magnoliae Officinalis*

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



TLC of *Fructus Perillea*

Semen Alpiniae katsumadai



alpinetin

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

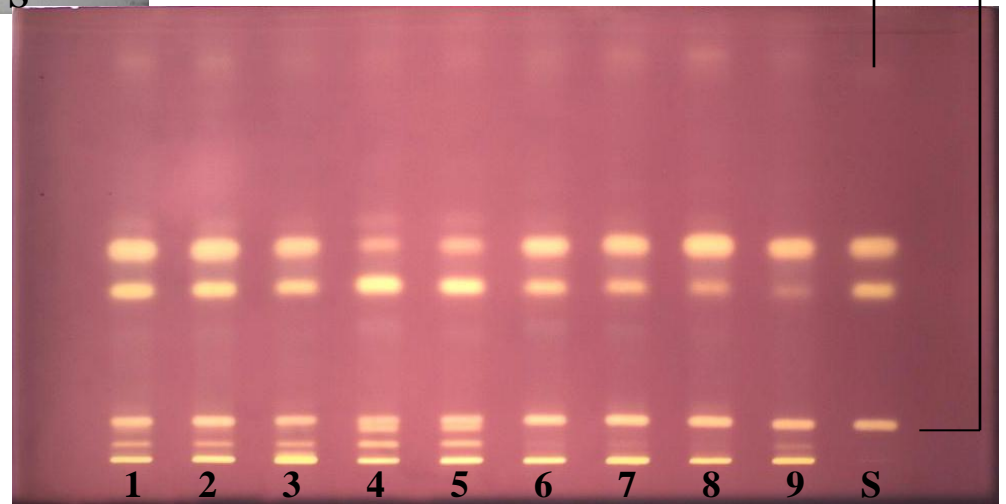
1% vanillin in sulphuric acid

From up to down:
alpinetin

cardamonin

Pinocembrin

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



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

Radix rehmanniae

ChP 2005

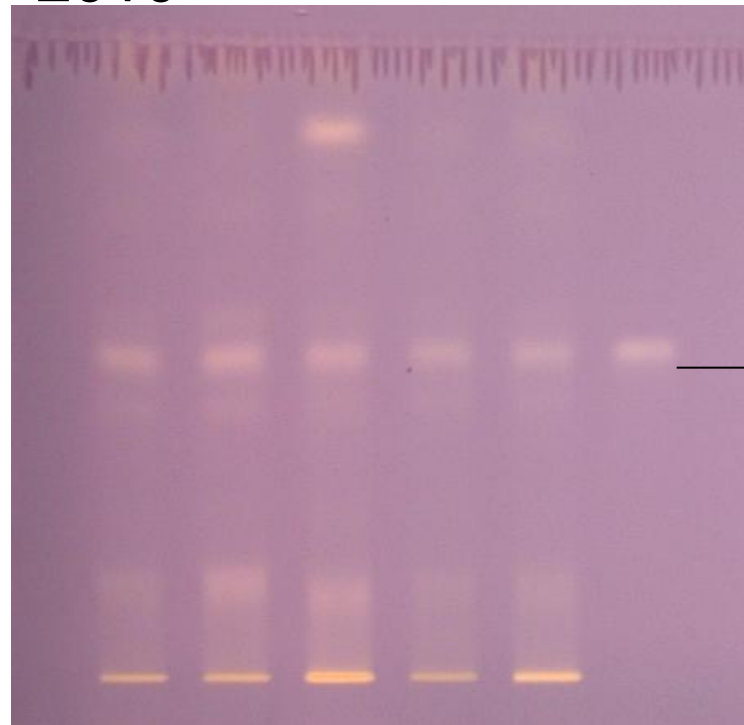


10% H₂SO₄

ChP 2010



DPPH



— acteoside

1 2 3 4 5 S

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

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**

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!

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Shanghai University of Traditional Chinese Medicine,
201203, Shanghai, China**

