HPTLC Association

HPTLC for describing and controlling the quality of poly-herbal formulations

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HPTLC in a nut shell

According to USP chapter <203>:

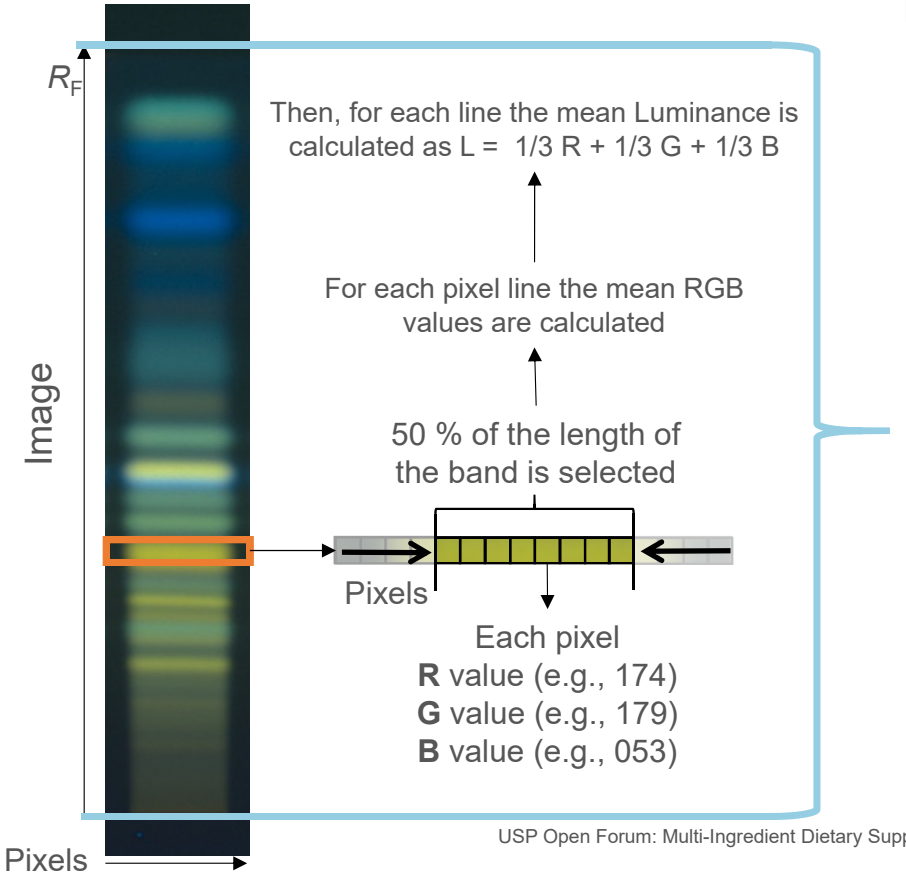
- 20x10 cm HPTLC glass plate Si 60 F_{254}
- Application: 15 tracks, 8 mm bands, 8 mm from lower edge, first track at 20 mm
- Conditioning to 33% relative humidity
- Development: 70 mm from lower edge, 20 min saturation (filter paper), 5 mm solvent level

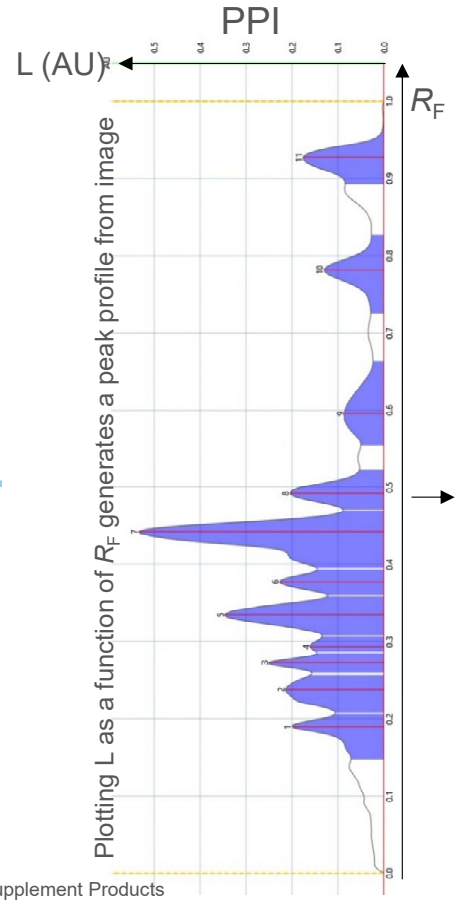
Reproducible data, every day, everywhere …

Now let's take **one step beyond** chapter <203>

Comprehensive HPTLC fingerprinting

- As discussed by USP Joint Subcommittee on Modern Analytical Methods
- Images also carry quantitative information:





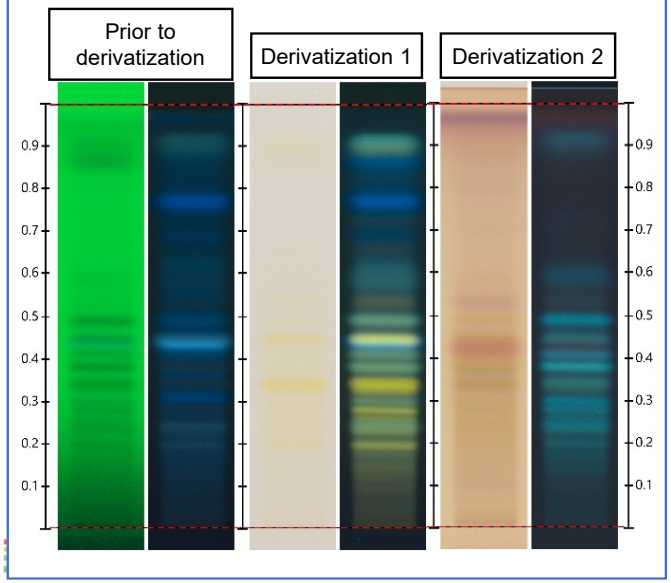
Peak	R_F	Height (AU)	Area (AU)
1	0.190	0.19973	0.007004
2	0.238	0.214429	0.009101
3	0.273	0.250716	0.005022
4	0.293	0.160755	0.003115
5	0.335	0.346191	0.012301
6	0.378	0.226948	0.006157
7	0.443	0.534302	0.022513
8	0.492	0.20429	0.0069
9	0.595	0.086192	0.006577
10	0.781	0.129996	0.006369
11	0.928	0.175959	0.007206

Comprehensive HPTLC fingerprinting

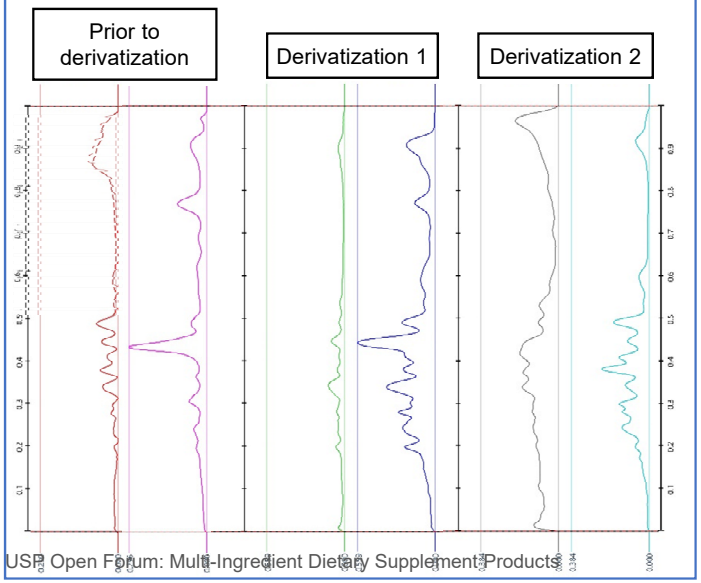
- HPTLC fingerprints, which are used for identification, contain information beyond identity…

One sample

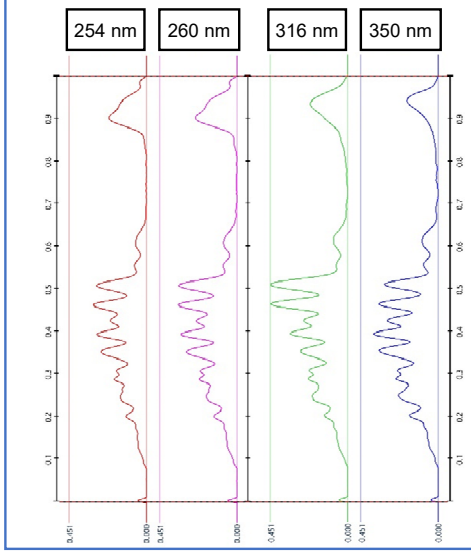
HPTLC images



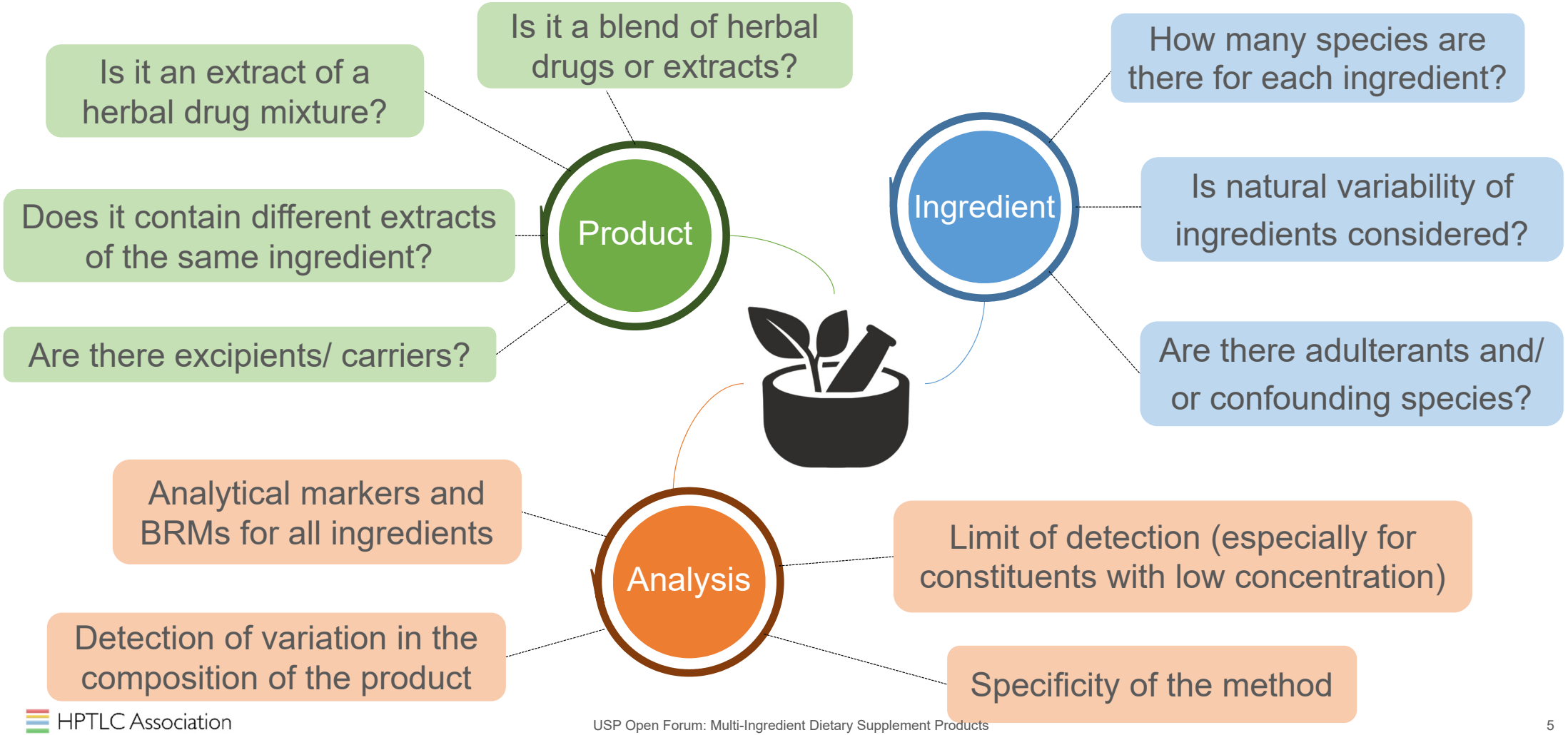
Peak Profiles from Image (PPI)



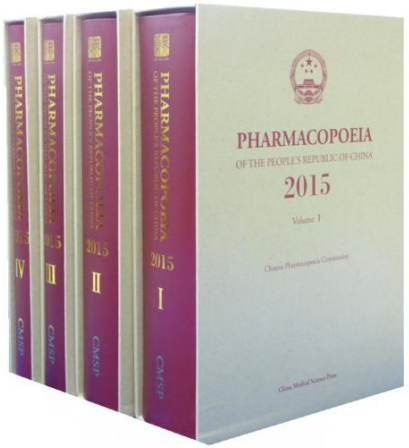
Peak Profiles from Scanning Densitometry (PPSD)

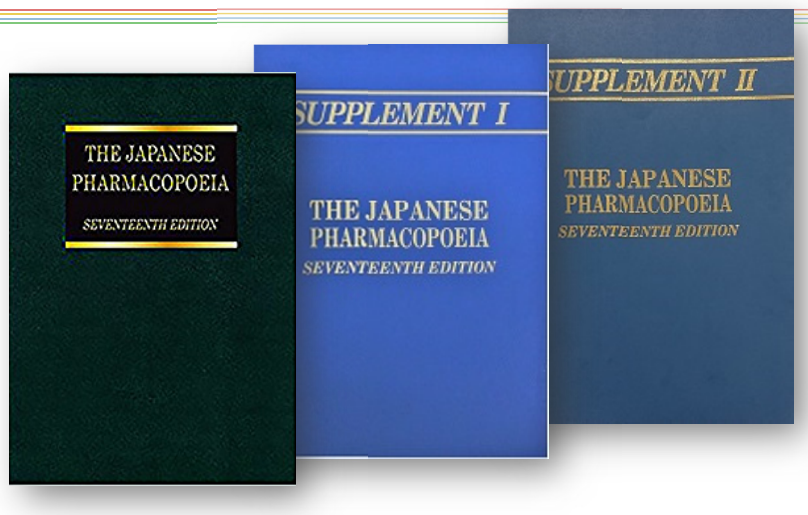


Analytical challenges of poly-herbal formulations



Poly-herbal formulations: the state of the art

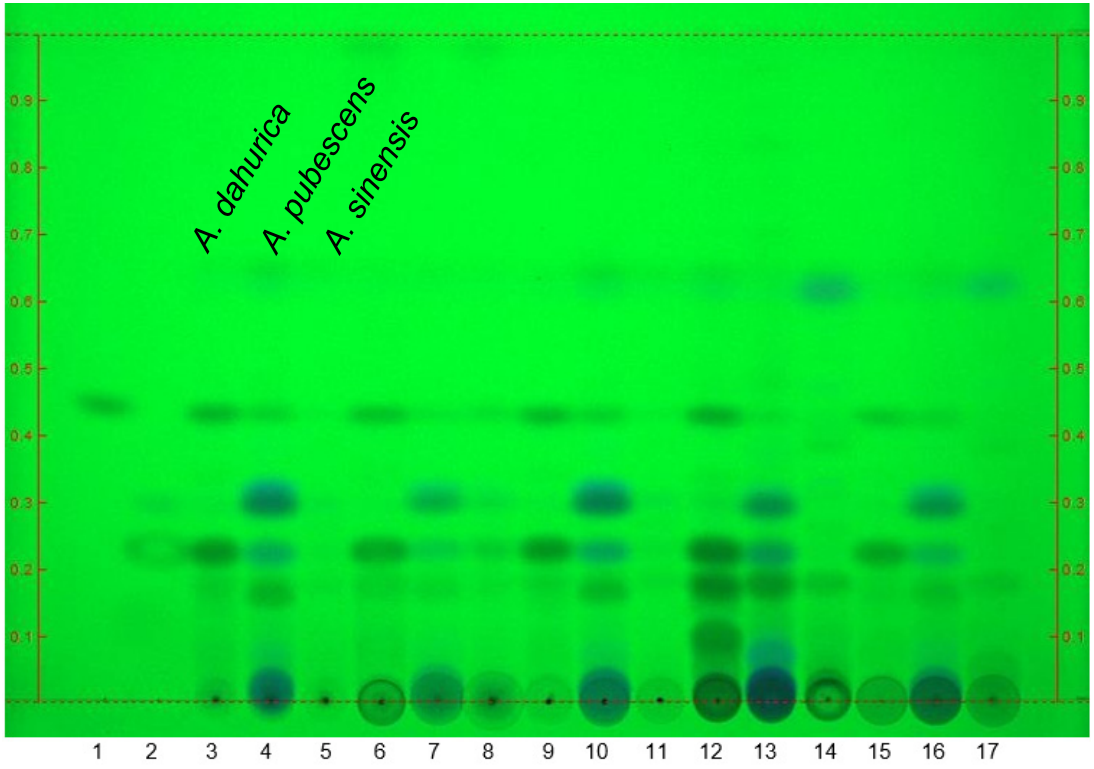


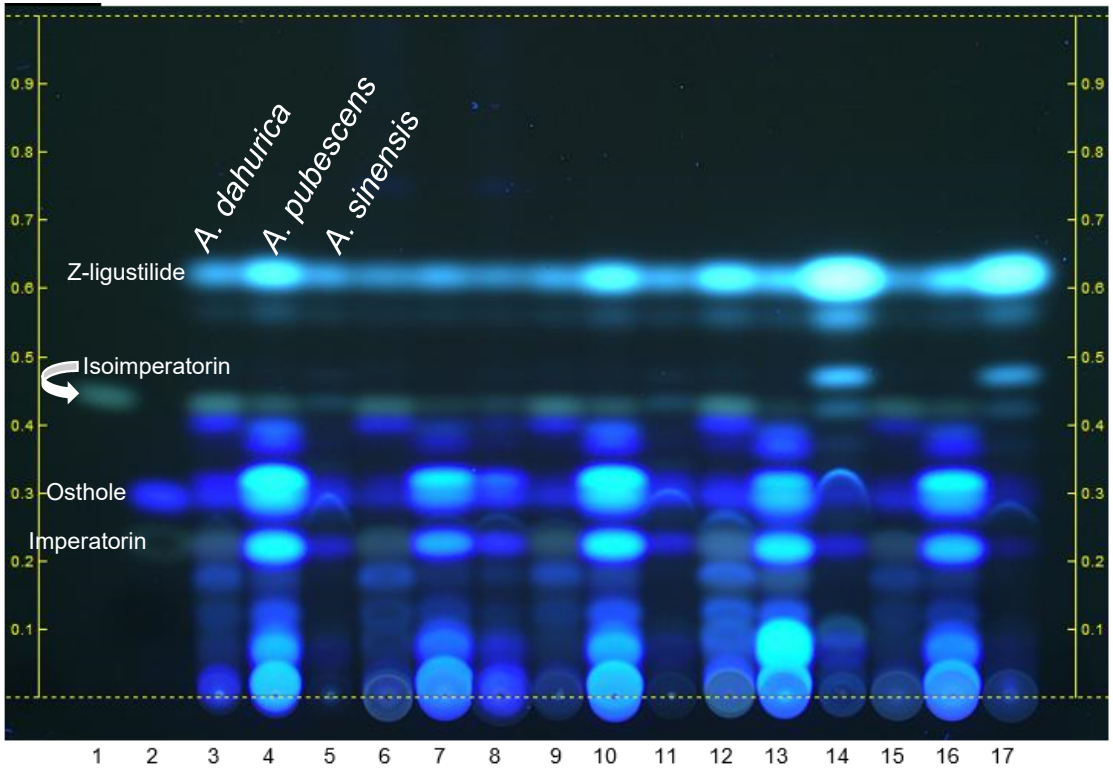


- ChP and KP → monographs for TCM and Kampo poly-herbal formulations
- Most monographs include in their identification section a TLC ID method for each ingredient
- Identification is carried out by comparing the fingerprints of the product with those of the individual herbal reference drugs or analytical standards.
- Usually the assay is based on quantification of one analytical marker for each ingredient

Poly-herbal TCM formulation: Duliang Ruanjionang

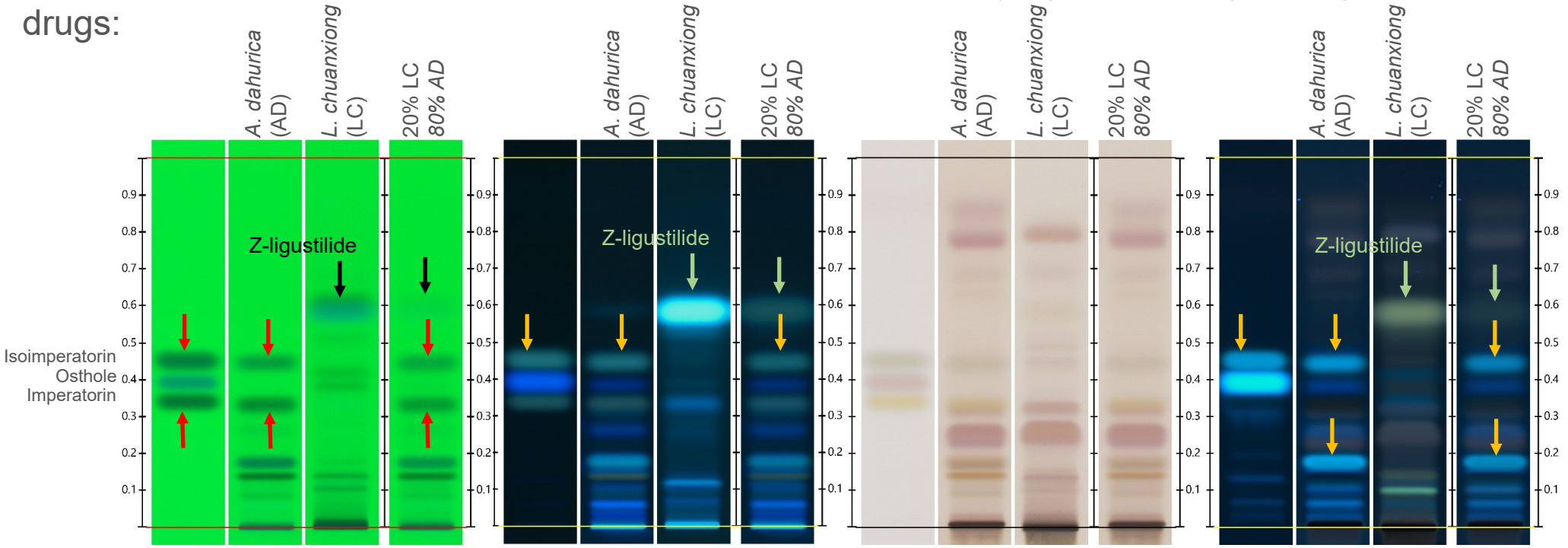
The ChP monograph for this medicine includes a single TLC method for identification of all ingredients.





Polyherbal TCM formulation → Duliang Ruanjionang

- The HPTLC Association has an harmonized method for identifying and distinguishing both herbal drugs:



HPTLC method:

- DS: Toluene, ethyl acetate and acetic acid (90:10:1 v/v/v)
- Derivatization: Sulfuric acid reagent in methanol

Polyherbal Kampo medicine formulation: Orengedokuto

Most of the Kampo medicines of JP contain aqueous extracts (decocts). Preparation is described in a general section.

https://kampo.ca/herbs-formulas/formulas/orengedokuto/

Phellodendron spp. cortex

Coptidis spp. rhizome

Gardenia jasminoides fruit

Scutellariae Radix

Preparation of Orengedokuto decoctions:

	1)	2)	3)	4)
Coptis Rhizome	1.5 g	1.5 g	2 g	2 g
Phellodendron Bark	1.5 g	3 g	2 g	1.5 g
Scutellaria Root	3 g	3 g	3 g	3 g
Gardenia Fruit	2 g	3 g	2 g	2 g

Definition:

- NLT 20 mg and NMT 80 mg of berberine (Coptis and Phellodendron)
- NLT 80 mg and NMT 240 mg of baicalin (Scutellariae Radix)
- NLT 30 mg and NMT 90 mg geniposide (Gardenia Fruit)

Polyherbal Kampo medicine formulation: Orengedokuto ...

- Preparation of decoction
  - 1.5 – 3.0 g of material
  - 1 hour extraction (reflux) + ~ 30 min drying the **aqueous extract**
- Identification test (TLC):
  - 4 different TLC methods (~ 3 – 4 hours for each method)
  - 3 different sample preparations (some of them very cumbersome and the extract is not entirely dissolved)
  - 4 different analytical standards**: coptisine chloride**, **limonin**, **wogonin**, and **geniposide**

TLC method 1 (Coptis rhizome):
- **Sample prep**: Shake 0.5 g of dry extract with 10 mL of methanol, centrifuge, and use the supernatant.
- **DS**: ethyl acetate, ammonia solution and methanol (15:1:1 v/v/v)
- **Evaluation** in 365 nm before derivatization

TLC method 2 (Phellodendron bark):
- **Sample prep**: Shake 0.5 g of dry extract with 5 mL of H_2O, then add 25 mL of EtOAC. Dry the EtOAC fraction and dissolve the residue in 1 mL of methanol.
- **DS:** ethyl acetate and hexane (5:1 v/v)
- **Derivatization:** vanillin-sulfuric acid reagent
- **Detection mode not specified**

TLC method 3 (Scutellaria root):
- **Sample prep**: Shake 1.0 g of dry extract with 10 mL of H_2O, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant
- **DS:** ethyl acetate, hexane and acetic acid (10:10:1 v/v/v)
- **Derivatization:** iron (III) chloride-methanol reagent
- **Detection mode not specified**

TLC method 4 (Gardenia Fruit):
- **Sample prep**: Shake 0.5 g of dry extract with 10 mL of methanol, centrifuge, and use the supernatant.
- DS: ethyl acetate, methanol and water (20:3:2 v/v/v)
- Derivatization: 4-methoxybezaldehyde-sulfuric acid reagent
- Detection mode not specified

Ayurvedic preparation containing 4 ingredients

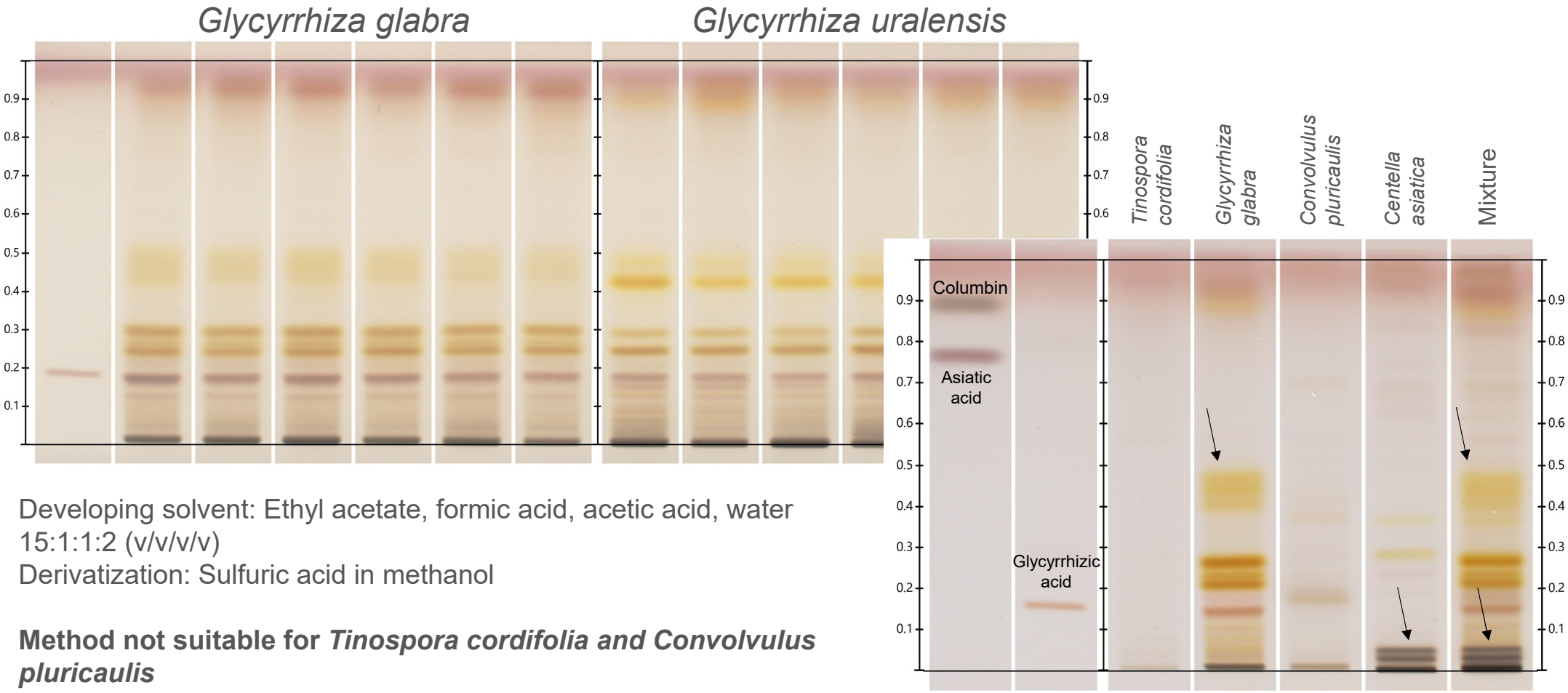
- Maharish Ayurveda have their own formulation with Ayurvedic herbs called MA 3
- It is used for brain health and memory
- It contains:

- *Tinospora cordifolia* stem
- *Glycyrrhiza glabra* root
- *Convolvulus pluricaulis* W.P.
- *Centella asiatica* W.P.

- Goal of the HPTLC method: to define “positive markers” for each herbal drug
- Method evaluation:

1. Evaluate the individual herbal drugs and the mixture thereof with the methods for each herbal drug. Source of the methods: QSIMP; USP DSC and BP.
2. Sample preparation: use a universal method → 20 mg/mL in methanol, sonication for 10‘

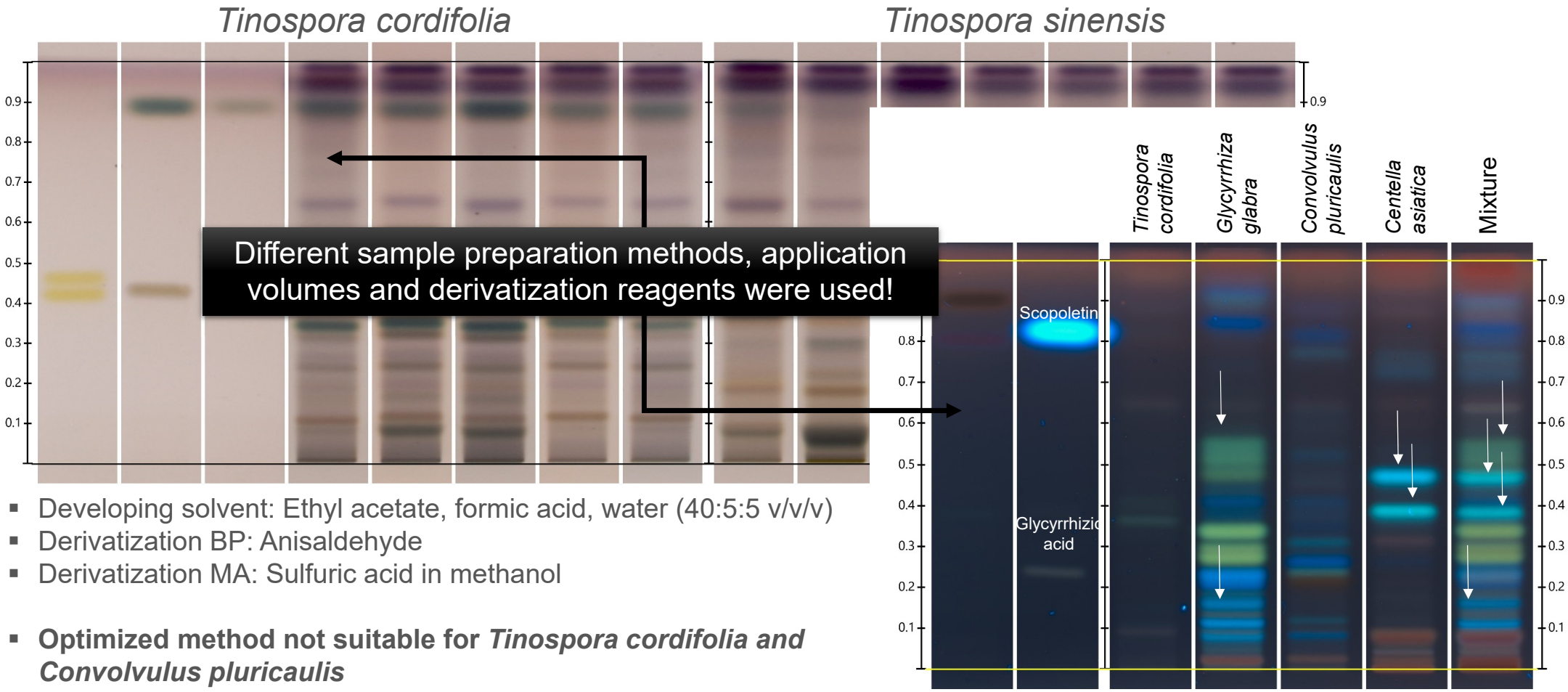
Method evaluation → *Glycyrrhiza spp* Root and Rhizome; USP DSC



- Developing solvent: Ethyl acetate, formic acid, acetic acid, water 15:1:1:2 (v/v/v/v)
- Derivatization: Sulfuric acid in methanol

- **Method not suitable for *Tinospora cordifolia and Convolvulus pluricaulis***

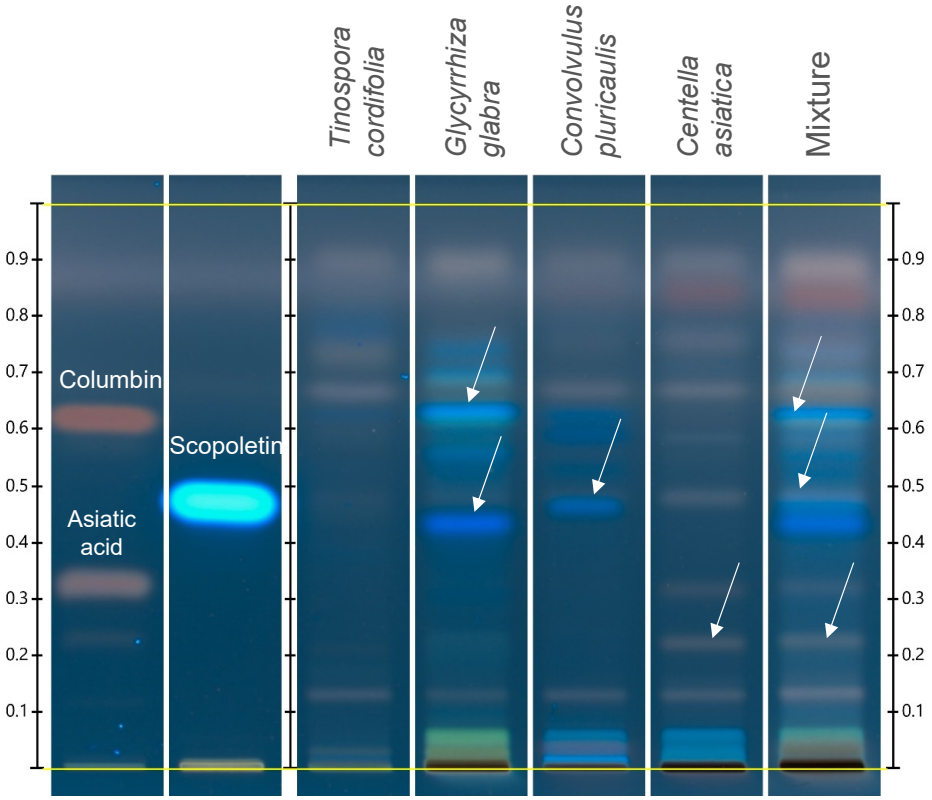
Method evaluation → *Tinospora cordifolia* stem, proposed to the BP



- Developing solvent: Ethyl acetate, formic acid, water (40:5:5 v/v/v)
- Derivatization BP: Anisaldehyde
- Derivatization MA: Sulfuric acid in methanol

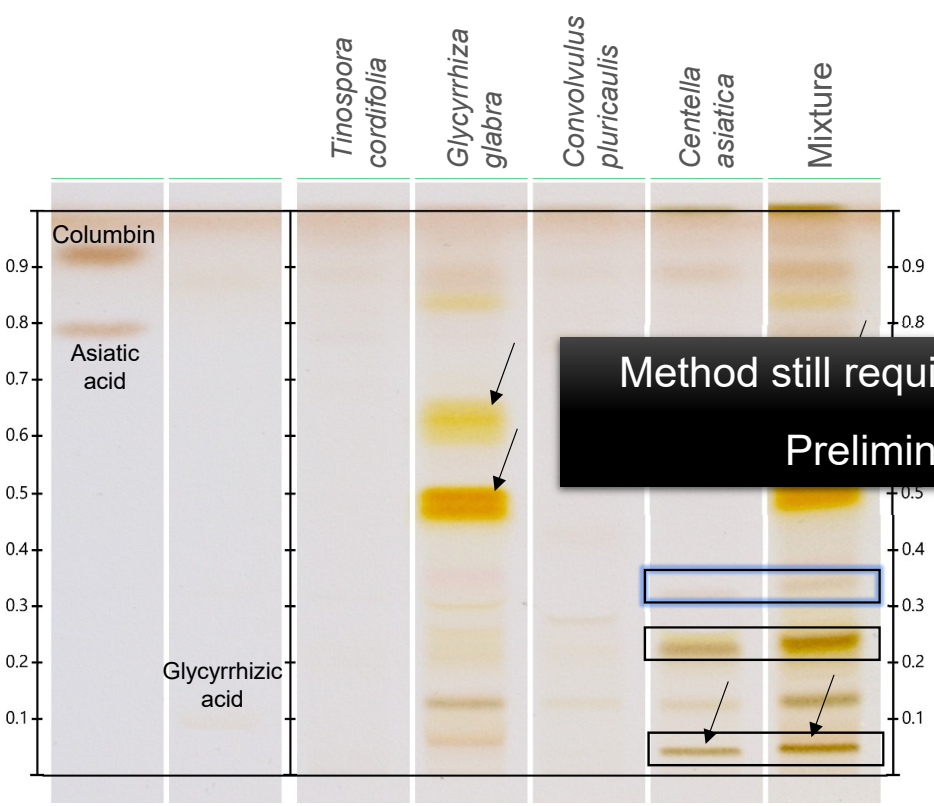
- **Optimized method not suitable for *Tinospora cordifolia and Convolvulus pluricaulis***

Method evaluation → *Convolvulus microphyllus,* QSIMP, Vol 2, p 70



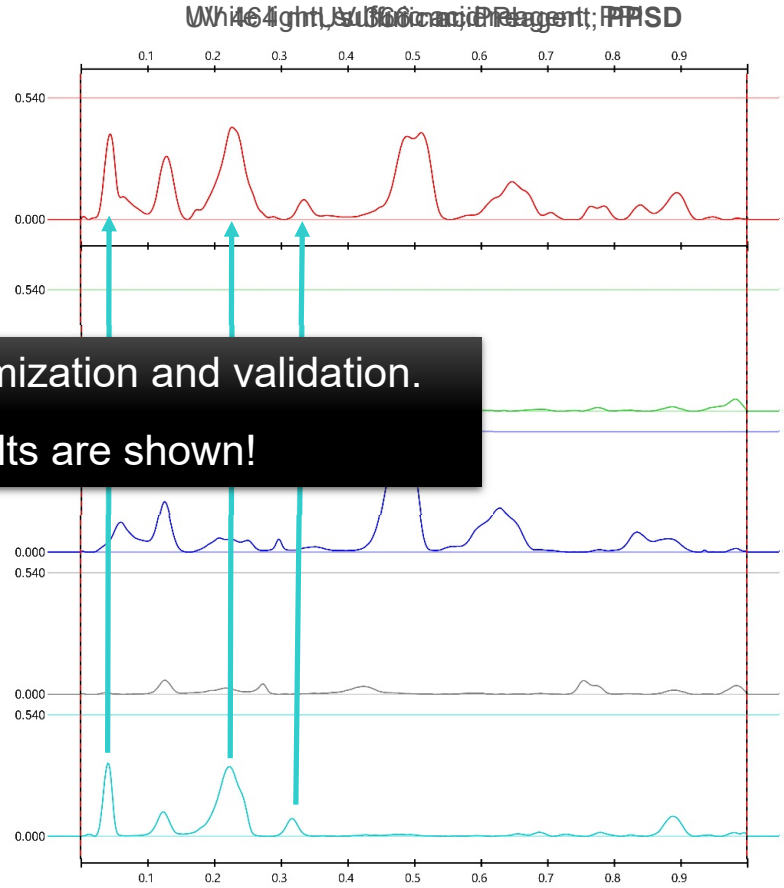
- Developing solvent: Ethyl acetate, toluene, acetic acid (5:4:1 v/v/v)
- Derivatization: Sulfuric acid in methanol
- Method not suitable for *Tinospora cordifolia*

Method evaluation → *Centella asiatica* aerial parts USP 41 - NF36



Method still requires optimization and validation.

Preliminary results are shown!



Finished product

Ingredient 1: *Tinospora cordifolia*

Ingredient 2: *Glycyrrhiza glabra*

Ingredient 3: *Convolvulus pluricaulis*

Ingredient 4: *Centella asiatica*

- Developing solvent: Dichloromethane, methanol, water (14:6:1 v/v/v)
- Derivatization: Sulfuric acid in methanol

- PPSD detection is suitable for *Centella asiatica*

HPTLC for describing and controlling the quality of poly-herbal formulations

→ Each individual ingredient with natural variability;
→ Adulterants/confounding species

ID Method (compendial)

Mixture:
→ All ingredients
→ excipients

Method development

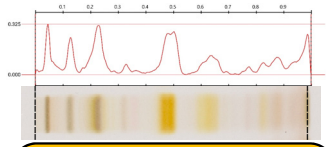
Evaluate all herbal drug samples with new method → set min and max limits

Establish acceptance criteria

Create an overall fingerprint describing the mixture and showing markers for each ingredient

Method validation

Mixture as working standard for ID

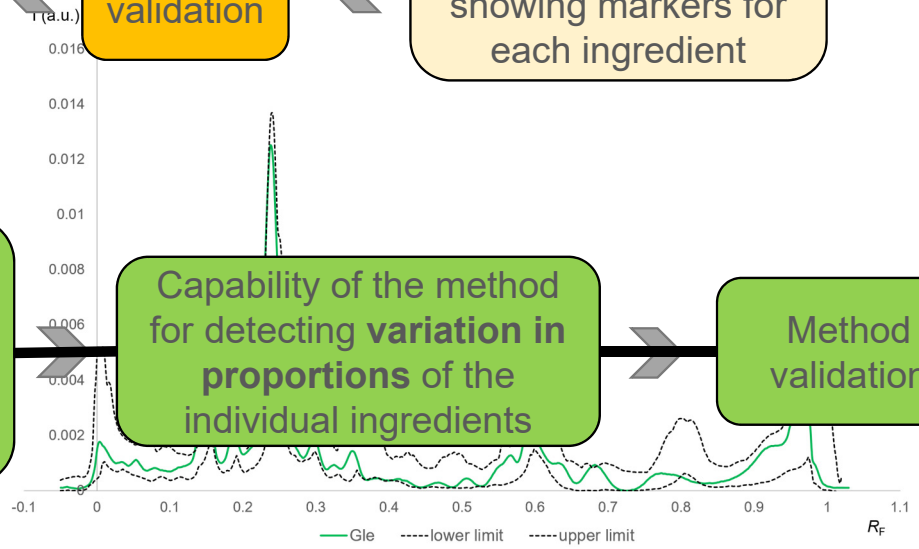


Production of the "**article**"

Identification of each batch against internal standard

Quantitative assessment: With multiple batches of finished products the acceptance criteria **(electronic standard)** for the "article" can be established.

Capability of the method for detecting **variation in proportions** of the individual ingredients



Method validation

Summary

- HPTLC <203> and comprehensive HPTLC fingerprinting are the ticket for pragmatic description and control of quality of poly-herbal formulations, allowing proper identification and determination of identity and strength of ingredients (raw material) and finished products.
- Method development and validation are straight forward and not time consuming.
- Electronic standards can be developed for ingredients and finished products.
- Qualitative and quantitative assessments are based on the same analysis.
- No special equipment required.

HPTLC Association

THANK YOU !

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