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

Eike Reich, Débora Frommenwiler
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 ...
Comprehensive HPTLC fingerprinting

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

\[
L = \frac{1}{3} R + \frac{1}{3} G + \frac{1}{3} B
\]

Then, for each line the mean Luminance is calculated as \( L \) = \( \frac{1}{3} R + \frac{1}{3} G + \frac{1}{3} B \)

For each pixel line the mean RGB values are calculated

50% of the length of the band is selected

Each pixel
- \( R \) value (e.g., 174)
- \( G \) value (e.g., 179)
- \( B \) value (e.g., 053)

Plotting \( L \) as a function of \( R_F \) generates a peak profile from image

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

**Product**
- Is it an extract of a herbal drug mixture?
- Does it contain different extracts of the same ingredient?
- Are there excipients/ carriers?

**Ingredient**
- How many species are there for each ingredient?
- Is natural variability of ingredients considered?
- Are there adulterants and/or confounding species?

**Analysis**
- Limit of detection (especially for constituents with low concentration)
- Specificity of the method
- Detection of variation in the composition of the product
- Analytical markers and BRMs for all ingredients
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.
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

**USP Open Forum: Multi-Ingredient Dietary Supplement Products**
Polyherbal Kampo medicine formulation: Orengedokuto

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

Preparation of Orengedokuto decoctions:

<table>
<thead>
<tr>
<th></th>
<th>1)</th>
<th>2)</th>
<th>3)</th>
<th>4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coptis Rhizome</td>
<td>1.5 g</td>
<td>1.5 g</td>
<td>2 g</td>
<td>2 g</td>
</tr>
<tr>
<td>Phellodendron Bark</td>
<td>1.5 g</td>
<td>3 g</td>
<td>2 g</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Scutellaria Root</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Gardenia Fruit</td>
<td>2 g</td>
<td>3 g</td>
<td>2 g</td>
<td>2 g</td>
</tr>
</tbody>
</table>

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 H₂O, 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)
- **Detection mode not specified**

**TLC method 3 (Scutellaria root):**
- **Sample prep:** Shake 1.0 g of dry extract with 10 mL of H₂O, 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*

Different sample preparation methods, application volumes and derivatization reagents were used!
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

- Developing solvent: Dichloromethane, methanol, water (14:6:1 v/v/v)
- Derivatization: Sulfuric acid in methanol
- PPSD detection is suitable for *Centella asiatica*

Method still requires optimization and validation.

Preliminary results are shown!
HPTLC for describing and controlling the quality of poly-herbal formulations

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

Availabledrug samples with new method → set min and max limits
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.
THANK YOU!

eike.reich@hptlc-association.org