HPTLC

For the analysis of botanical materials and medicinal plants

Eike Reich

CAMAG Laboratory
Sonnenmattstrasse 11
4132 Muttenz / Switzerland
What will be discussed?

1. What exactly is HPTLC?
2. The elements of a standardized HPTLC methodology
3. Changing TLC methods into HPTLC methods
4. Development and validation of ID Methods
5. How new developments in HPTLC may affect the analysis of plants
Thin-layer chromatography (TLC) always was and still remains an important tool for the analysis of plants.

Today there are two principal applications in this context: research and quality control. Both benefit from the advantages of the planar off-line principle and also in particular from low cost, simplicity, and flexibility.

For decades TLC is integral part of monographs for medicinal plants in all pharmacopoeias and the primary method of identification.

Growing expectation regarding performance characteristics have brought TLC methods to the limits.

Since the turn of the century pharmacopoeias recognize the technical progress in instrumentation and improvements offered by high performance plates.

Most recently HPTLC is being discussed as alternative to classical TLC.
The planar off-line principle

application

development

detection and quantitative evaluation

documentation
But what about reproducibility?

The central problem:

- When two (or more) labs do the „same“ or think that they are doing the same, the results are not necessarily equal.
- Reason is the general method description in the Pharmacopoeias (EP 2.2.27, USP <201>, <621>, PhPRC ap. VI) which define „suitable“ equipment and give ranges instead of values.
- JPXV 2.03 is still centered around self made 20x20 TLC plates!
- A table (EP) or a result description (USP, JP) can only define the most important aspects of a TLC chromatogram. That leaves room for interpretation. An atlas (PhPRC) provides clear guidance.
- Example: how can a color be described correctly?
What is blue?

And how about the color blind...
PhEur: Possible choices in methodology

- TLC layer
- Manual application
- Transparent container (Pickle jar?)
- UV-Lampe (\(\lambda\)?)
- Manual spraying / immersion

- HPTLC layer
- Automatic application
- Automatic Developing Chamber
- Scanner
- Automatic immersion / spraying
Identification of *Acanthopanax*
Identification of Peonies
Identification of Fleece flower
Goal: International standardization of HPTLC

- What
  - HPTLC definition
  - Methodology
  - Equipment

- Why
  - Reproducibility of results
  - Validity of official methods
  - Quality assurance in a globalized world
  - Quality of published research

- How
  - International collaboration
  - Top down
  - Publication
TLC or HPTLC

- Pharmacopoeias see difference primarily in the plate yet assume similar results
Comparison TLC-HPTLC of flavonoids

TLC plate 20 x 20 cm (135 mm)
HPTLC plate 20 x 10 cm (60 mm)
TLC or HPTLC?
What is TLC?

- Chromatography for the poor (cheap)
- Simple manual chromatography for everyone (students?)
- Rapid
- Flexible
- Reference and test solution side by side
- “Just” qualitative, preliminary estimation at best

- Unpredictable
- Unreliable

→ Manual technique, simple instruments, TLC plates
What is HPTLC?

**High Performance Thin-Layer Chromatography**

TLC for the 21st century
- Instrumental TLC
  - Application
  - Development
  - Documentation
  - Densitometry
- Truly „plug and play“
- Fully cGMP compliant

A new concept
- Suitable instruments
- Scientific basis
- Standardized methodology
- Validated methods
“HPTLC” on TLC plates?

- What is the point?
  - Saturation 1h vs. 20 min
  - Twice (10x) the solvent volume
  - 3x the developing time (15 vs. 6 cm)
  - Same cost per plate (20x20 vs. 10x10 cm)
  yet
  - Less resolution
  - No control of the development process
Standardization of methodology

- Plate setup and handling
- Sample application (as band)
- Chamber geometry and saturation
- Humidity control
- Developing distance
- Derivatization procedure
- Documentation (electronic images)
- Evaluation

SOP for HPTLC

- Should be the basis for all work (in participating labs)
- Applies to all methods
- All deviations need to be recorded

Our SOP is in full compliance with PhEur, USP, ChP

Available at: www.camag-laboratory.com (homepage)
The basic HPTLC setup

Applicaton  Development  Derivatization  Documentation

NOTE: standardization does not require specific equipment!
Only agreement about all parameters
Standardized basic equipment

- Clear specification of HPTLC plates (type, format, manufacturer)
- Software control for “absolute” reproducibility of all parameters
- Independence from environmental factors (humidity !)
- Eliminating human factors
- Emulation by “manual” operation still possible to a certain degree
Sources of methods

- European Pharmacopoeia (EP)
  - New monographs feature TLC and HPTLC in parallel
- British, French, German, Swiss Pharmacopoeias
  - Offer specific monographs not found in EP
- The USP Dietary Supplement Compendium
  - TLC and state of the art HPTLC
- Chinese Pharmacopoeia
  - HPTLC atlas as a supplement of 2005 ed.
- Japanese Pharmacopoeia
  - Only simple TLC
More sources of methods

- American Herbal Pharmacopoeia (19 monographs)
- Quality Standards of Indian Medicinal Plants (8 volumes)
- Indian Herbal Pharmacopoeia (54 monographs)
- Quality Standards of Traditional Chinese Medicines (Chinese only)
- Wagner, H. and Bladt, S. „Plant Drug Analysis“
Converting existing TLC methods to HPTLC

Assumptions:

- Results on HPTLC and TLC plates are similar if
  - No changes are made to chromatographic system
  - Same equipment is used
  - Original TLC method was optimized
- Due to the higher separation power HPTLC plates
  - Usually give improved result
  - Require shorter developing distance → less time
- HPTLC with no instruments looks bad, but so does TLC
Converting existing TLC methods to HPTLC

Practical aspects (I)

- Do not change chromatographic system (chamber configuration, mobile phase, stationary phase)
- Reduce application volume (generally) to 1/5 (typically 2 μL)
- Employ standardized methodology (based on SOP):
  - Fixed (x, y) application positions, (e.g.) 8 mm bands
  - Use 60 mm developing distance
  - Fixed drying time and temperature
  - Use dipping instead of spraying if possible
  - Fixed waiting times between derivatization and evaluation
  - Obtain multiple images (if possible) of plate (e.g. UV 254, UV 366 prior to derivatization, and white light, UV 366 after derivatization
Converting existing TLC methods to HPTLC

Practical aspects (II)

- Use same samples on TLC and HPTLC
- Evaluate whether changes in the result can
  - Fall under “additional weak zones may be seen”
  - Are due to natural variability of plant
- Color description is always subjective: e.g.
  - blue, bluish
  - blue white
  - blue green, etc.
- Rf is predictable only for validated methods!
Example: USP method for chamomile

HPTLC application volume 2 μL
Developing an ID method from scratch

- Review literature for related plants
- Obtain multiple samples from different accessions
- Obtain samples of related plants and known adulterants
- Optimize sample preparation and detection
- Avoid toxic solvents
- Start with silica gel, select mobile phase

Reich, E., Schibli, A.: HPTLC for the analysis of medicinal plants, chapter 5, Thieme 2007
Envisioning the Future...
Methods for identification of plants

- SOP is the basis for an HPTLC method template (instruments)
  → software template
- A (PhEur, USP, etc.) monograph or validated methods is basis for HPTLC method document → Method for identification of Thyme
- Result table/description is replaced by a reference image
- Corresponding instrument methods are derived from software template → instrument method
- Results on plate are qualified by a System Suitability Test (SST)
- Comparison of results with reference images
Thyme leaf plates 1 - 3
33

Thyme leaf
Goal:
The CAMAG HPTLC method collection

- Identification of 150 (+) plants from East and West
- Predictable results everywhere
- Convenient comparison against reference images
- Compatible with (current and future) harmonized description of HPTLC
- Based on ATS4, ADC2, Visualizer and Software
Goal: CAMAG Laboratory Network

- Collaboration on development and validation of HPTLC methods for identification of plants
- Setting global quality standards for HPTLC
- Providing training and research opportunities
- Offering analytical services for customers
Who is part of the network?

- CAMAG Laboratory Muttenz
- CAMAG Scientific Inc. Wilmington (NC)
- Shanghai University of TCM (Prof. Wang)
- University of Applied Sciences Wädenswil (Prof. Meier)
- University of Regensburg (Prof. Heilmann)
- University of Barcelona (Prof. Canigueral)
- University Sapienza Rome (Prof. Nicoletti)
- University of Graz (Prof. Bauer)
Examples from other labs (HS Wädenswil)

**Oswego tea**

![Image of TLC plate showing bands](image)

<table>
<thead>
<tr>
<th>Track</th>
<th>Volume</th>
<th>Sample</th>
<th>Track</th>
<th>Volume</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 µL</td>
<td>Oswego tea, herb 1</td>
<td>9</td>
<td>275 µL</td>
<td>Chlorogenic acid, proanthocyanidin with increasing Rf</td>
</tr>
<tr>
<td>2</td>
<td>4 µL</td>
<td>Oswego tea, herb 1</td>
<td>10</td>
<td>4 µL</td>
<td>Oswego tea, herb 1</td>
</tr>
<tr>
<td>3</td>
<td>6 µL</td>
<td>Oswego tea, herb 1</td>
<td>11</td>
<td>4 µL</td>
<td>Oswego tea, herb 2</td>
</tr>
<tr>
<td>4</td>
<td>2 µL</td>
<td>Oswego tea, herb with flower</td>
<td>12</td>
<td>4 µL</td>
<td>Oswego tea, herb 3</td>
</tr>
<tr>
<td>5</td>
<td>4 µL</td>
<td>Oswego tea, herb with flower</td>
<td>13</td>
<td>4 µL</td>
<td>Oswego tea, herb 4</td>
</tr>
<tr>
<td>6</td>
<td>6 µL</td>
<td>Oswego tea, herb with flower</td>
<td>14</td>
<td>4 µL</td>
<td>Oswego tea, herb with flower</td>
</tr>
<tr>
<td>7</td>
<td>2 µL</td>
<td>Rutin, naringen, hesperidin, glycosides with increasing Rf</td>
<td>15</td>
<td>4 µL</td>
<td>Oswego tea, flower</td>
</tr>
<tr>
<td>8</td>
<td>2 µL</td>
<td>Naringen, naringen (with increasing Rf)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**System suitability test**

- Rutin: orange fluorescent zone at Rf ~ 0.32 (UV 366 nm).  
- Hesperidin: orange fluorescent zone at Rf ~ 0.58 (UV 366 nm).
Examples from other labs (Uni Regensburg)

Coptis rhiz.

<table>
<thead>
<tr>
<th>Track</th>
<th>Volume</th>
<th>Sample</th>
<th>Track</th>
<th>Volume</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 µL</td>
<td>Palmating and berberine</td>
<td>9</td>
<td>10 µL</td>
<td>Coptis testa rhizome</td>
</tr>
<tr>
<td>2</td>
<td>10 µL</td>
<td>Coptis</td>
<td>10</td>
<td>10 µL</td>
<td>Chinese cork tree bark</td>
</tr>
<tr>
<td>3</td>
<td>10 µL</td>
<td>Coptis rhizome # 1</td>
<td>11</td>
<td>10 µL</td>
<td>Chinese mahonia bark</td>
</tr>
<tr>
<td>4</td>
<td>10 µL</td>
<td>Coptis rhizome # 2</td>
<td>12</td>
<td>10 µL</td>
<td>Tinospora root</td>
</tr>
<tr>
<td>5</td>
<td>10 µL</td>
<td>Coptis rhizome # 3</td>
<td>13</td>
<td></td>
<td>blank</td>
</tr>
<tr>
<td>6</td>
<td>10 µL</td>
<td>Coptis rhizome # 4</td>
<td>14</td>
<td></td>
<td>blank</td>
</tr>
<tr>
<td>7</td>
<td>10 µL</td>
<td>Coptis rhizome # 5</td>
<td>15</td>
<td></td>
<td>blank</td>
</tr>
<tr>
<td>8</td>
<td>10 µL</td>
<td>Coptis deltoida rhizome</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

System suitability test:
- Palmating: fluorescent zone at Rf ~ 0.14
- Berberine: fluorescent zone at Rf ~ 0.23
- Coptis: fluorescent zone at Rf ~ 0.60
A new software concept!
### Sequence Table Definition

<table>
<thead>
<tr>
<th>Track</th>
<th>Vial ID</th>
<th>Description</th>
<th>Volume</th>
<th>Position</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R6424</td>
<td>Rosmarinic acid</td>
<td>2.0</td>
<td>A1</td>
<td>Standard</td>
</tr>
<tr>
<td>2</td>
<td>R7637</td>
<td>Rutin</td>
<td>2.0</td>
<td>A2</td>
<td>Standard</td>
</tr>
<tr>
<td>2</td>
<td>R7638</td>
<td>Hyperosid</td>
<td>2.0</td>
<td>A3</td>
<td>Standard</td>
</tr>
<tr>
<td>2</td>
<td>S2930</td>
<td>Serpylli herba concisa</td>
<td>2.0</td>
<td>B1</td>
<td>Sample</td>
</tr>
<tr>
<td>3</td>
<td>S6522</td>
<td>Thymus serpyllum</td>
<td>2.0</td>
<td>B2</td>
<td>Sample</td>
</tr>
<tr>
<td>4</td>
<td>S8790</td>
<td>Thymus serpyllum</td>
<td>2.0</td>
<td>B3</td>
<td>Sample</td>
</tr>
<tr>
<td>5</td>
<td>S8798</td>
<td>Thymus serpyllum pulvis</td>
<td>2.0</td>
<td>B4</td>
<td>Sample</td>
</tr>
<tr>
<td>6</td>
<td>S6587</td>
<td>Thymian</td>
<td>2.0</td>
<td>B5</td>
<td>Sample</td>
</tr>
<tr>
<td>7</td>
<td>S8457</td>
<td>Thymus zygis Loefl. Ex L.</td>
<td>2.0</td>
<td>B6</td>
<td>Sample</td>
</tr>
<tr>
<td>8</td>
<td>S8460</td>
<td>Thymus hyemalis Lange</td>
<td>2.0</td>
<td>B7</td>
<td>Sample</td>
</tr>
</tbody>
</table>

Plate Layout Preview

- Left
- Center
Three channels per track
PCA (Master thesis R. Ambühl, Uni Basel)
HPTLC as research tool

- Evaluation of column fractions
- Identification by MS
- Screening for bio-activity
Application of HPTLC-MS for the identification of flavonoids in herbal extracts

Bauer R1, Meier M1, Pflerscher-Wenzel E1, Wohlgart K1, Reich E2

1 CAMAG Laboratory, Sonnenalpplatz 11, CH-5210 Muttenz, Switzerland
2 Uni Graz, Austria

Introduction

Recently, HPTLC-MS has become a powerful tool for the identification of flavonoids in herbal extracts. The technique allows for the simultaneous detection and quantification of multiple compounds, providing valuable information for the quality control of medicinal products.

Principle

The samples are extracted and analyzed on a CAMAG TLC-MS interface, which is a combination of high-performance liquid chromatography (HPLC) and mass spectrometry (MS). This allows for the separation and identification of flavonoids with high accuracy.

Summary

The method described in this study is a reliable and efficient approach for the identification of flavonoids in herbal extracts. It can be used to ensure the quality of medicinal products and to provide scientific evidence for the use of traditional supplements.

Results and Discussion

Rutin, hyperoside, vitexin, quercetin, and morin were successfully identified in the herbal extract by HPTLC-MS. The method showed high specificity and sensitivity, allowing for the detection of even trace amounts of these compounds.

Experimental

The extraction of the sample was performed using methanol as a solvent. The extraction conditions were optimized to maximize the yield of flavonoids. The samples were then analyzed using the CAMAG TLC-MS interface.

References

Identification of alkaloids in *Sophora flavescens*
Identification of alkaloids in *Sophora flavescens*
<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>np</th>
<th>np</th>
<th>np</th>
</tr>
</thead>
</table>

**detection:** bioluminescence *Vibrio fischeri*

**BioLumineX - BioLuminizer**

**detection:** derivat. anisaldehyde / 366 nm

A - I: chamomile oil  np: 4-nitrophenol
Bio-assays: DPPH

Anti-oxidative properties of essential oils

Silica gel 60 F$_{254}$
Toluene : ethyl acetate
95:5

Bio-assays: DPPH

Anti-oxidative properties of flavonoids

Silica gel 60 F$_{254}$

Ethyl acetate, acetic acid, formic acid, water
100:11:11:27

Thank you!

eike.reich@camag.com

www.camag-laboratory.com
New Application Notes
Content uniformity test of Coenzyme Q10
HPTLC assay of metronidazole
Determination of apolar lipids from human skin by HPTLC
Determination of ceramides from human skin by HPTLC

New Publications
Quality control of multicomponent herbal drugs: example from TCM

HPTLC of dyes

New CAMAG TLC-MS Interface:
For direct extraction of compounds from TLC/HPTLC layers into MS.
More details >>

CAMAG Laboratory
a center for applied HPTLC
- Broad range of services
- Dedicated to research
- Source of information

Services
Method development and validation
- Download new CAMAG Lab flyer (pdf)
- Feasibility studies
- Contract analyses
- Training

Publications
- Validated HPTLC method for the determination of illegal dyes in spices
- Separation of phospholipids by HPTLC
- Validation of HPTLC identification methods for botanicals
Download SOP for HPTLC (pdf)

Last update: 05.07.2011