# HPTLC for the identification and quality control of medicinal plants

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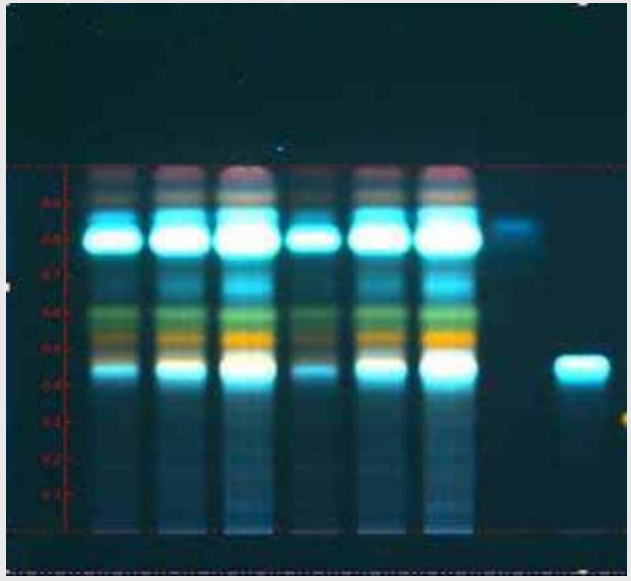

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# What is HPTLC?

**High Performance** Thin-Layer Chromatography

**Key elements**

- Instruments for all steps
  - Application
  - Development
  - Documentation
  - Densitometry
- Standardized methodology
- Validated methods

Full cGMP compliance

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# HPTLC – key advantages

- Fairly simple
- Inexpensive
- Rapid
- Extremely flexible

- Visual

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# Standardized methodology: SOP for HPTLC

- Plate material & labeling
  - pre-coated HPTLC plates, 20x10 / 10x10 cm
  - Project number_year/month/day_plate number
- Parameters for sample application
  - 8 mm bands, spray-on
- Detailed description of development
  - 6 cm, chamber saturation, humidity control
- Derivatization
  - Dipping whenever possible
- Densitometry
  - MWL scan
  - Scan at the max. WL
- Digital documentation
  - UV 254 nm / 366 nm / white light

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# Requirements for image comparison

- Fixed size (resolution / zoom)
- Fixed intensity (aperture, exposure, sensitivity, gain)
- Flat-field corrected images
- Fixed white balance
- Fixed color space
- Secure raw data
- „Certified“ images
- **Absolutely no „photoshop“**
- Color management for monitor

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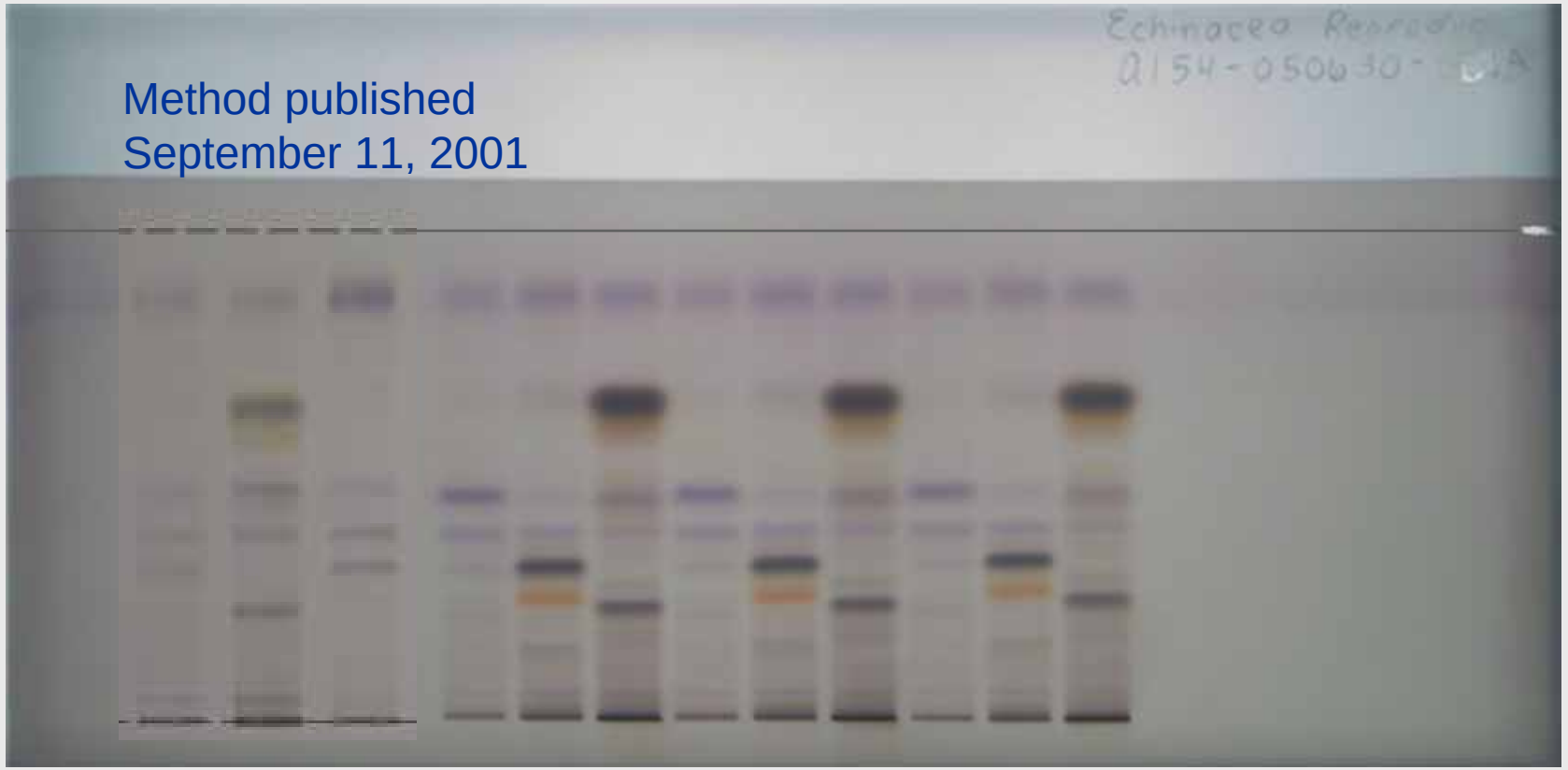
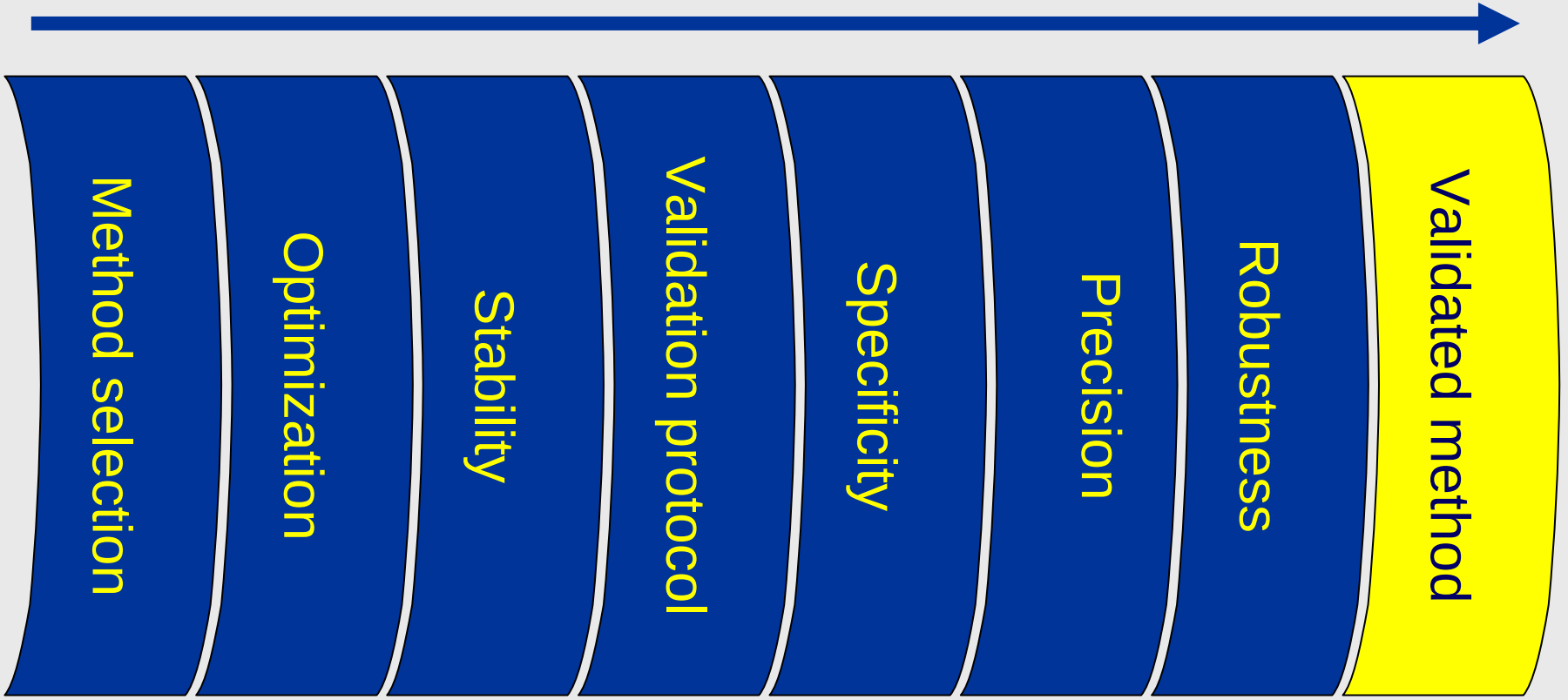
# Successful standardization – *Echinacea*

May 06, 2005 – CSI Laboratory



# Validation of qualitative methods



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# Botanical ID vs. chemical profile (HPTLC)

| Botanical ID | HPTLC |
| --- | --- |
| Highly trained personnel | Basic analytical skills |
| Intact plant (parts) only | Universally applicable<br>▪ Intact plant (parts)<br>▪ Powder<br>▪ Extract |
| Single specimen | Also „pooled“ samples |
| Name | Fingerprint |
| Positive identity | Qualitative identity |
| No change with time | Can track changes |

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# The concept of similarity

- Similarity with respect to the sequence of zones in a fingerprint. It includes:
  - Number
  - Position
  - Color
  - Intensity
- Visual comparison
  - Images
  - Analog chromatograms

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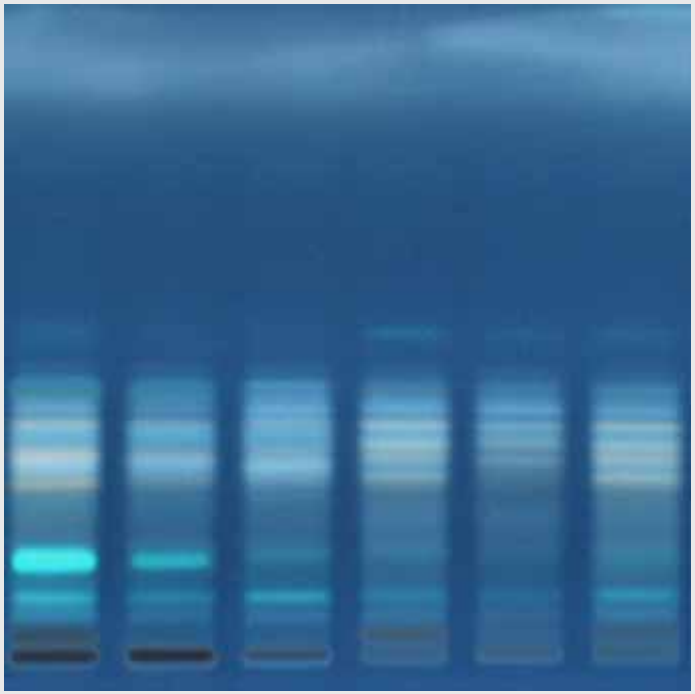
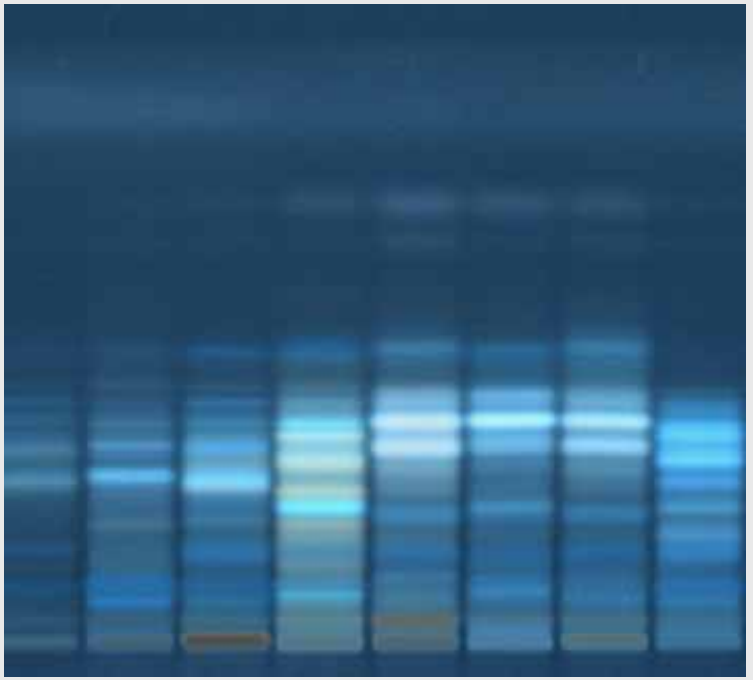
# The concept of similarity (black cohosh)



SIMILAR



DIFFERENT

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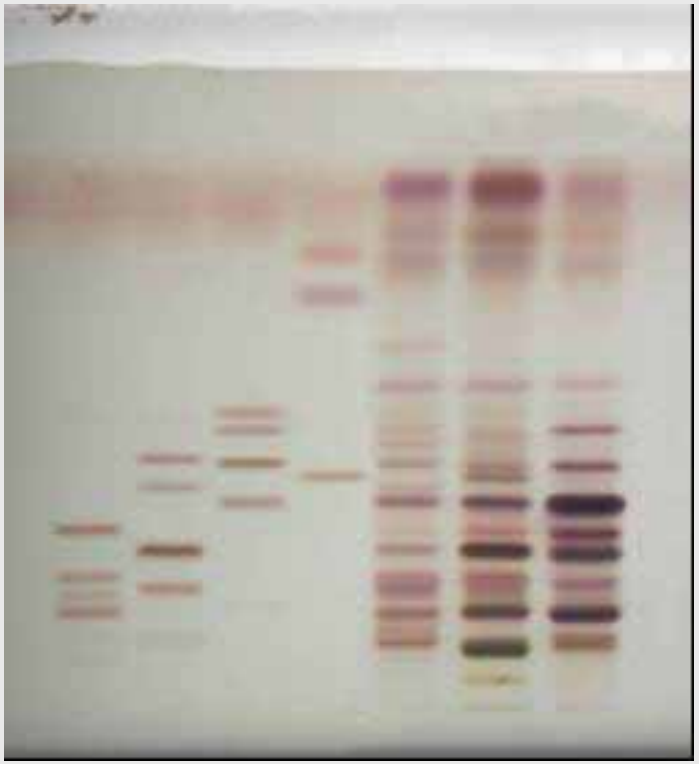
# Identification of Ginseng spp. (validated, AHP)





1 Ginsenoside Rb1, Rb2, Rc, Rd; 2 Rb3, Re, Rf, Rg3; 3 Rg1, Rg2, Rh1, Rh2;
4 Pseudoginsenoside F11, panaxatriol, panaxadiol
5 *Panax ginseng*; 6 *Panax quinquefolium*; 7 *Panax notoginseng* (syn. *P. pseudoginseng*)

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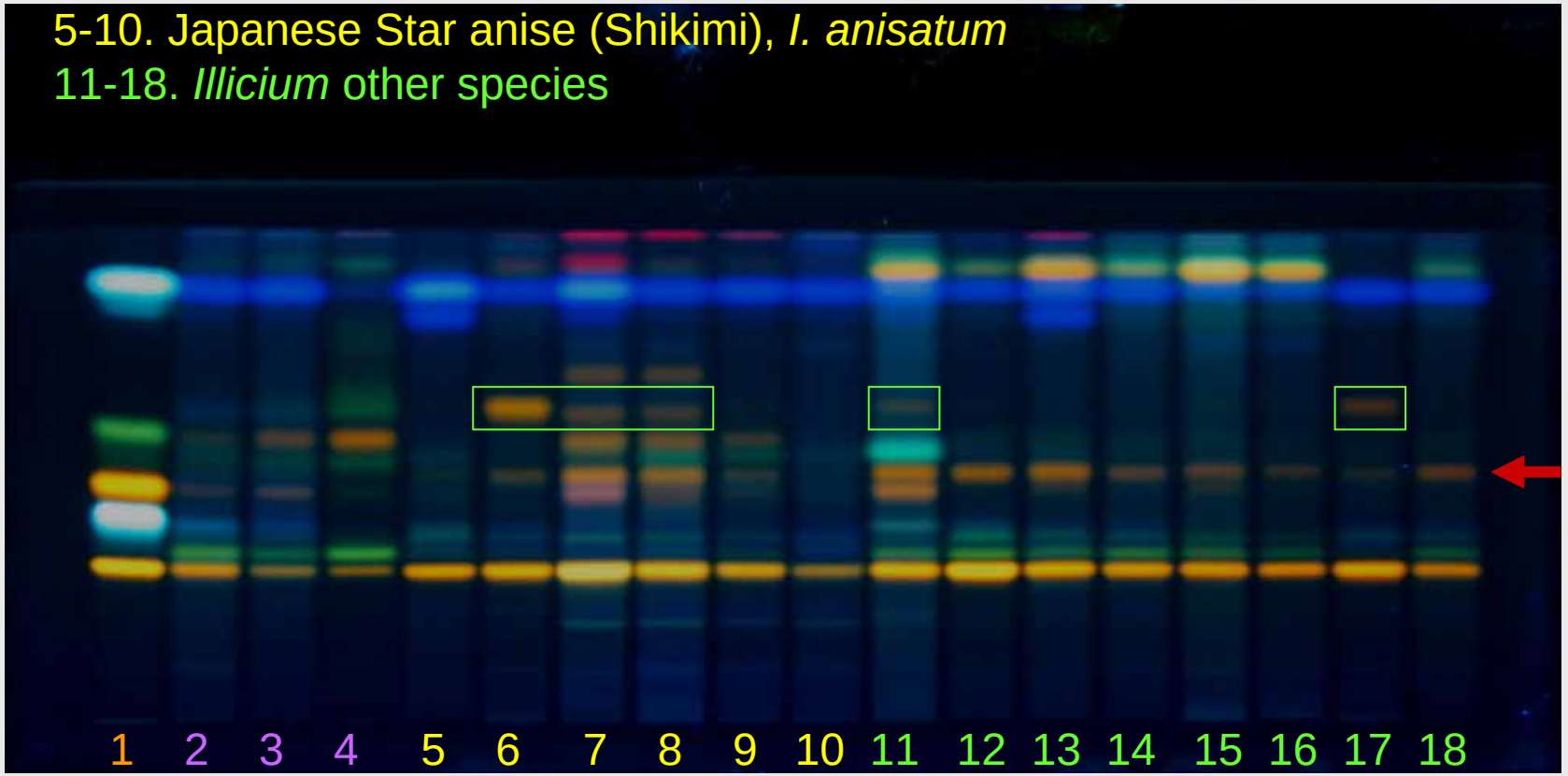
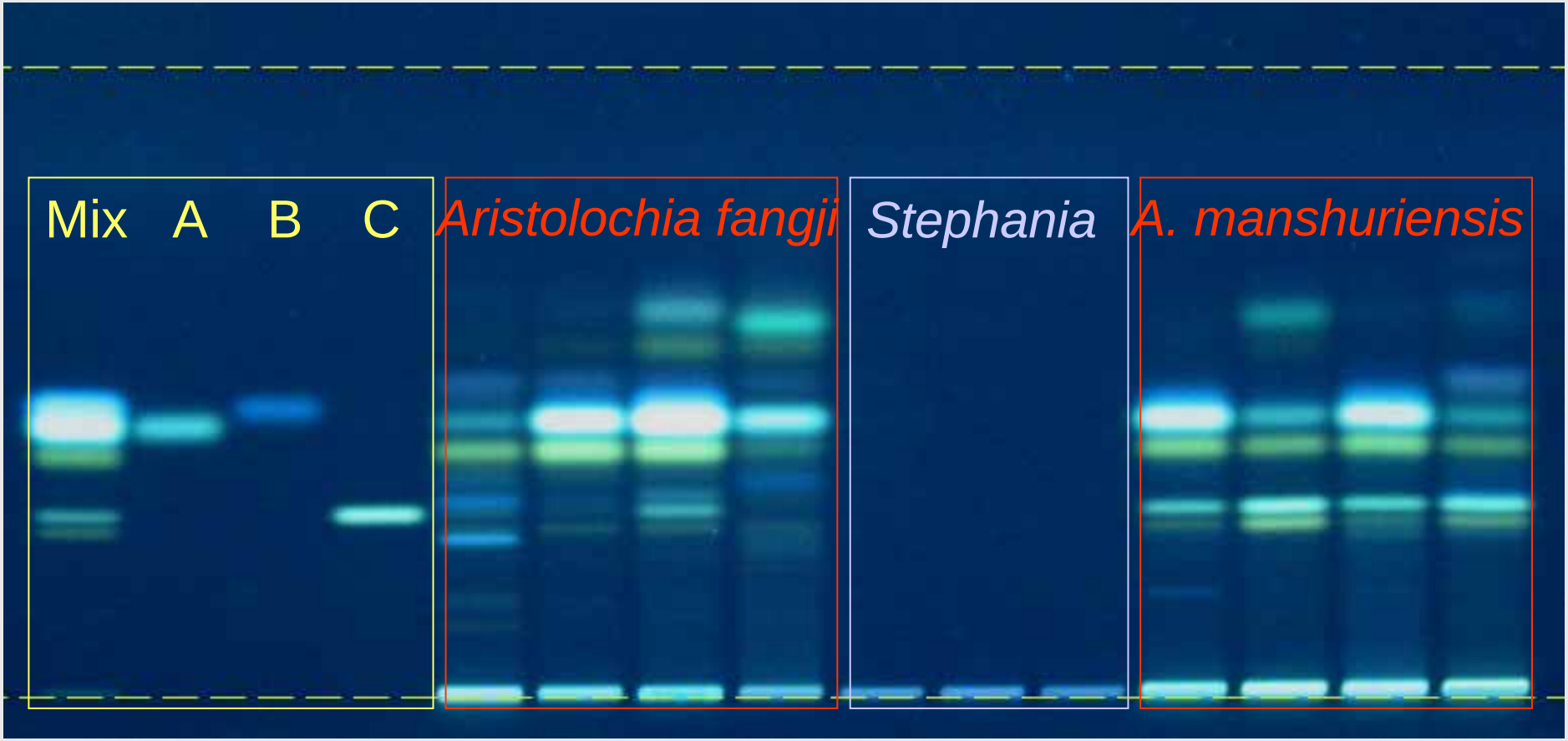
# Identification of Star anise

1. Rutin, chlorogenic acid, hyperoside, astragaline, caffeic acid
2-4. Chinese Star anise, *I. verum*
5-10. Japanese Star anise (Shikimi), *I. anisatum*
11-18. *Illicium* other species



# Aristolochic acids as markers of toxic plants in TCM



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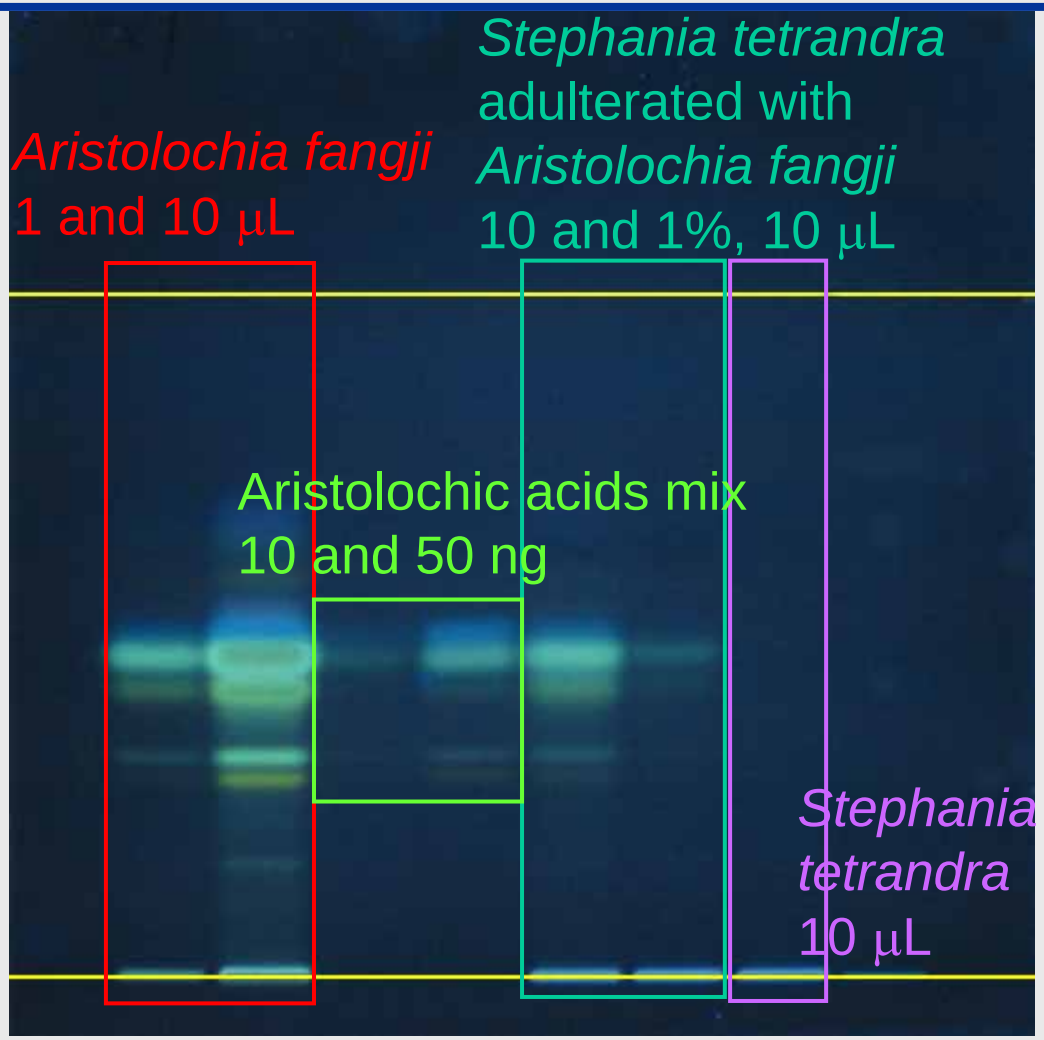
# Detection of mixtures



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# Identification of Black Cohosh (validated, AHP)


1 Chlorogenic acid; 2 Caffeic acid; 3 Isoferulic acid; 4 Actein; 5 C. *racemosa* BRM

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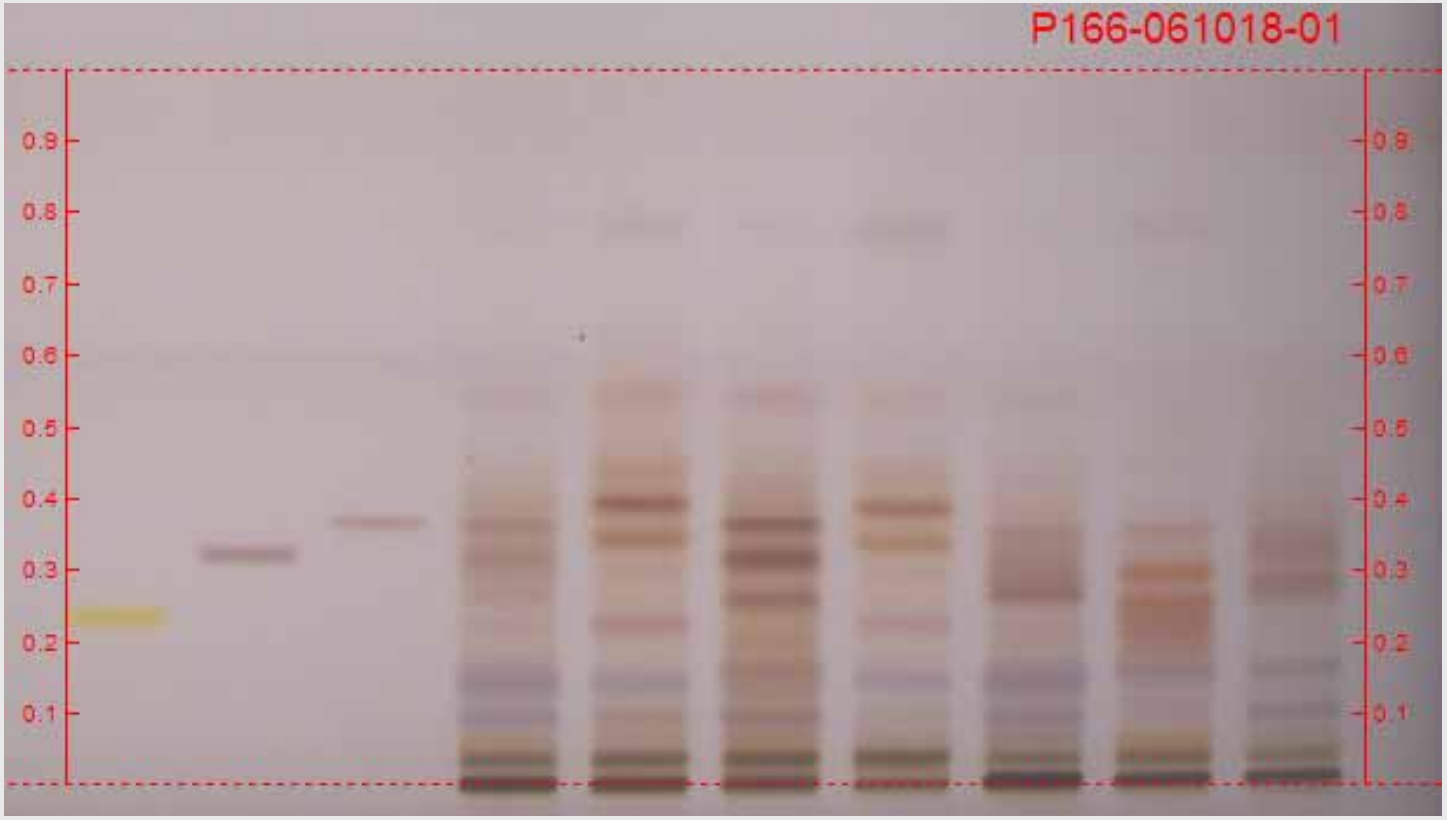
# Other *Cimicifuga* species




**1: cimifugin; 2: 27-deoxyactein; 3: actein; 4: Cimicifuga racemosa; 5: C. heracleifolia; k 6: C. foetida; 7: C. dahurica; 8: C. rubra; 9: C. americana; 10: C. pachypoda**

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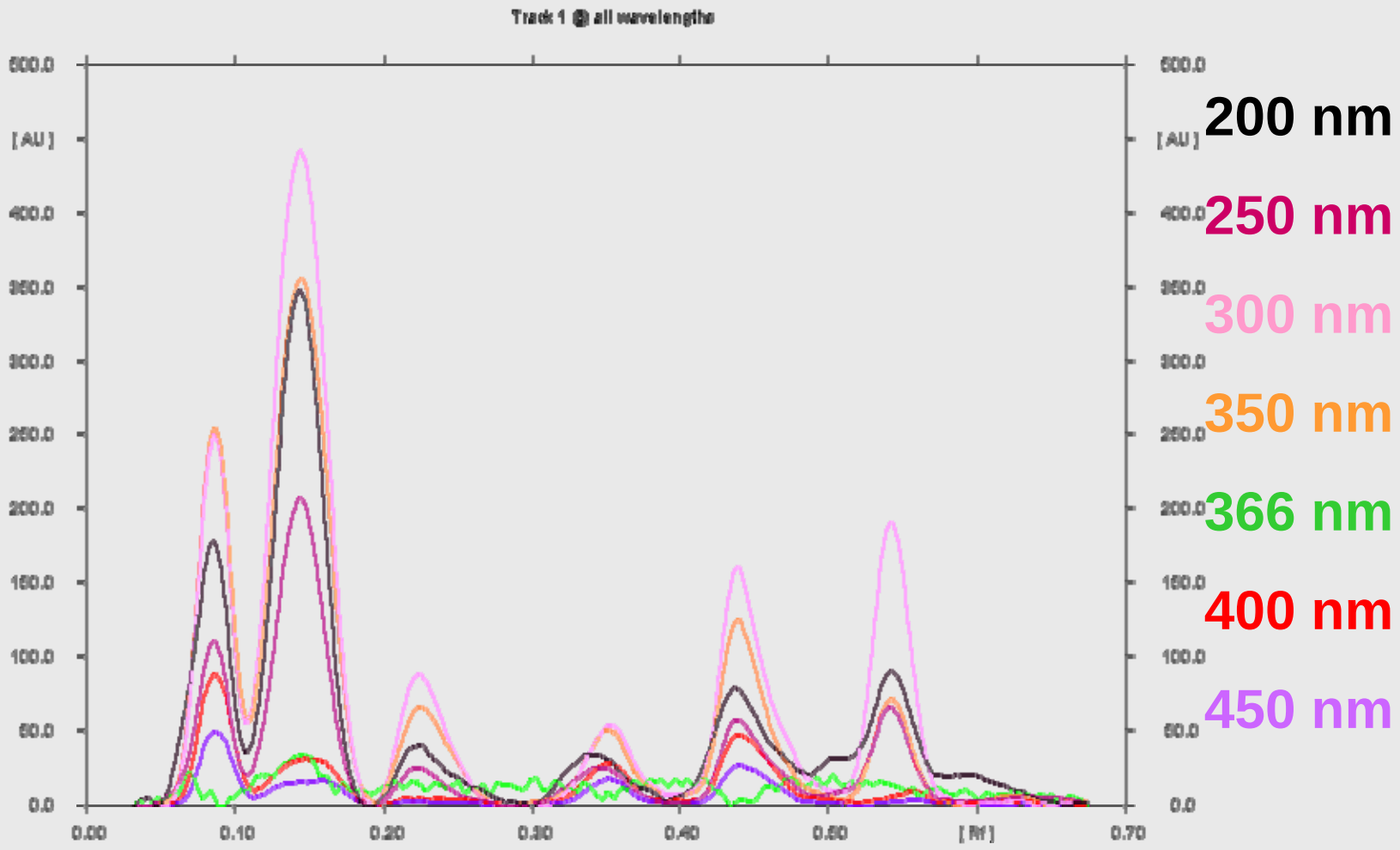
# Black Cohosh - MWL



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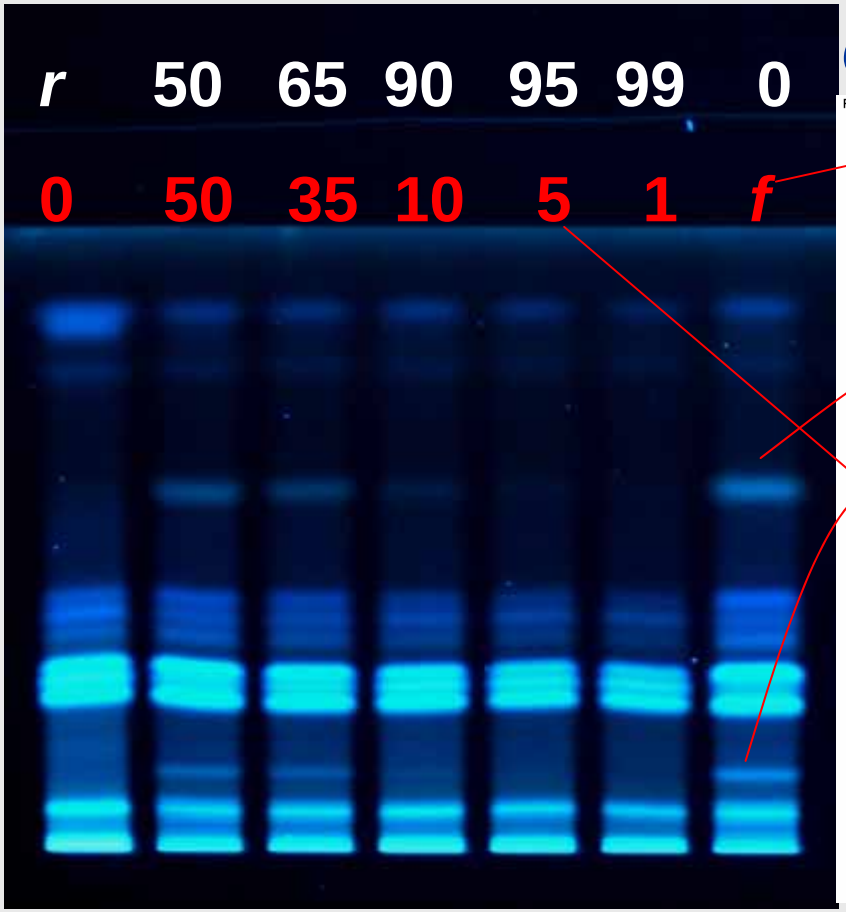
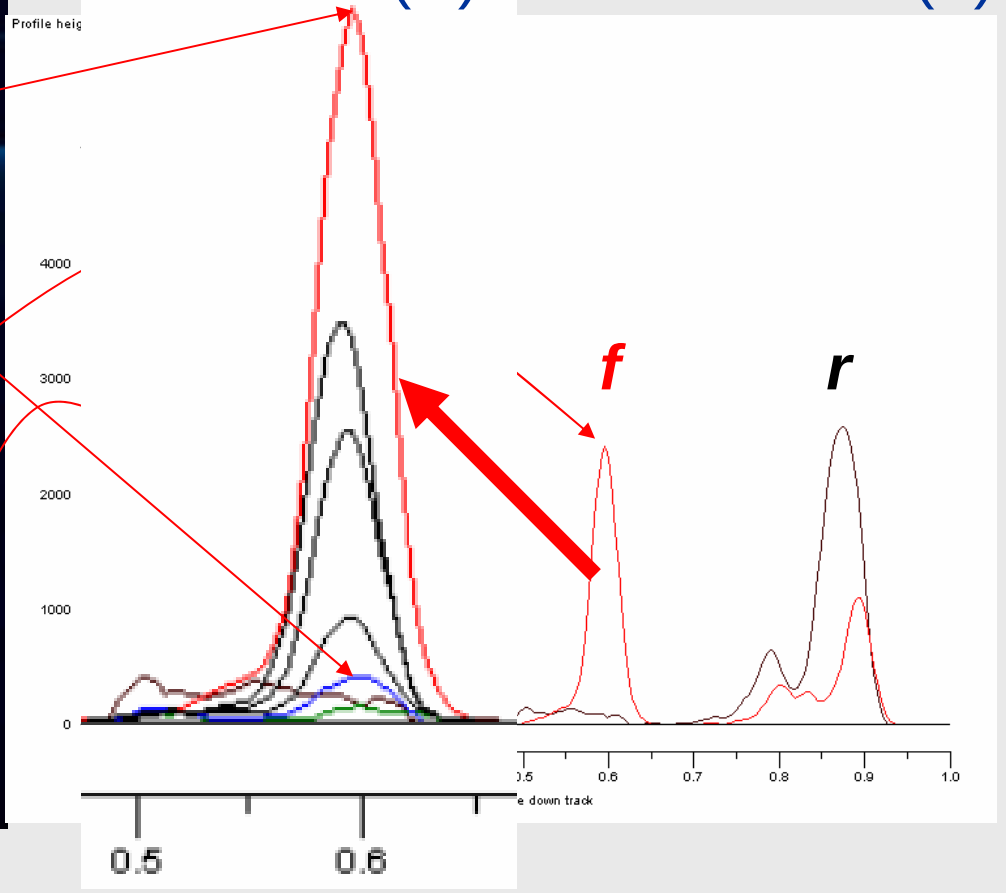
# Semi-quantitative information about adulteration




*C. racemosa* (R) and *C. foetida* (F)



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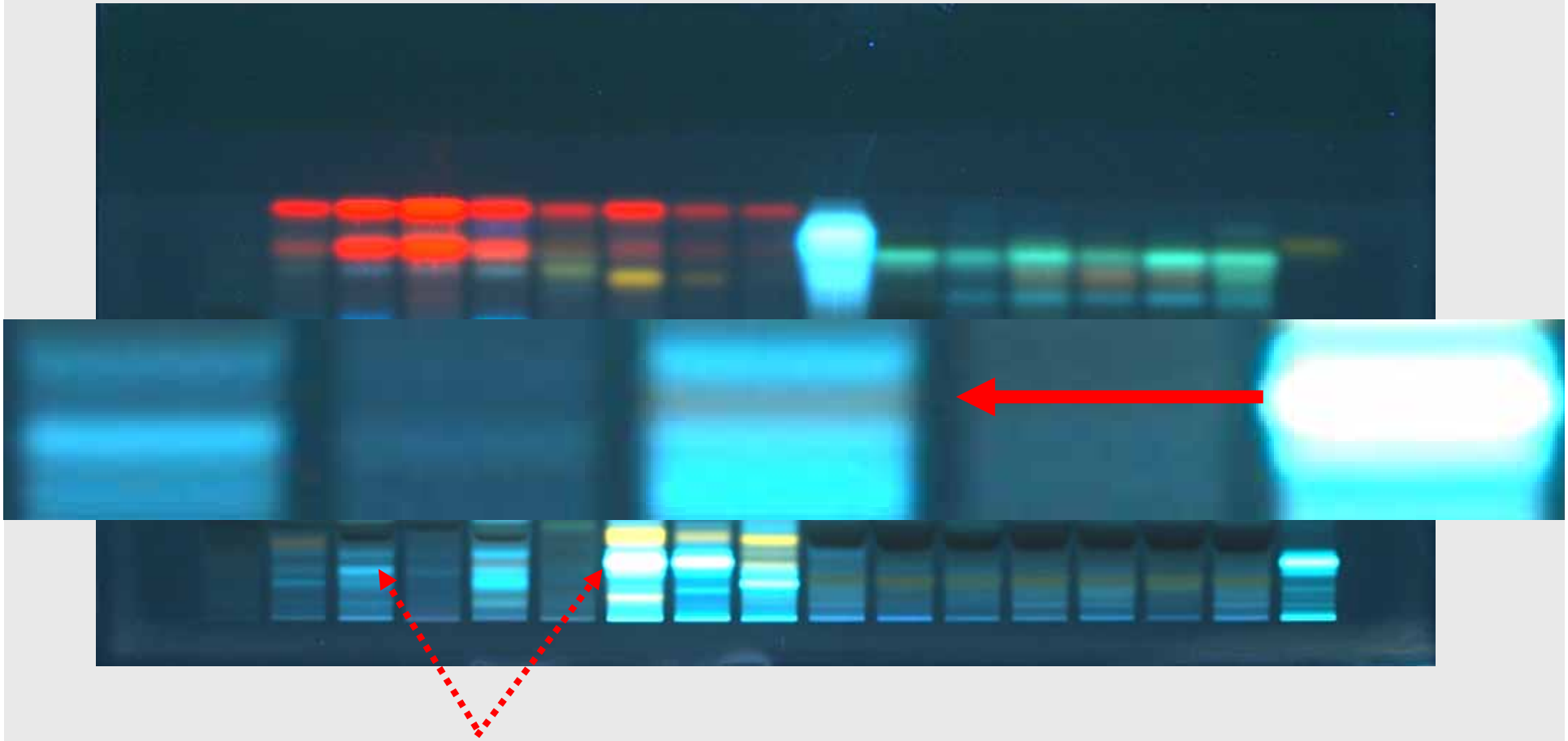
# Identification of Skullcap (AHP method)



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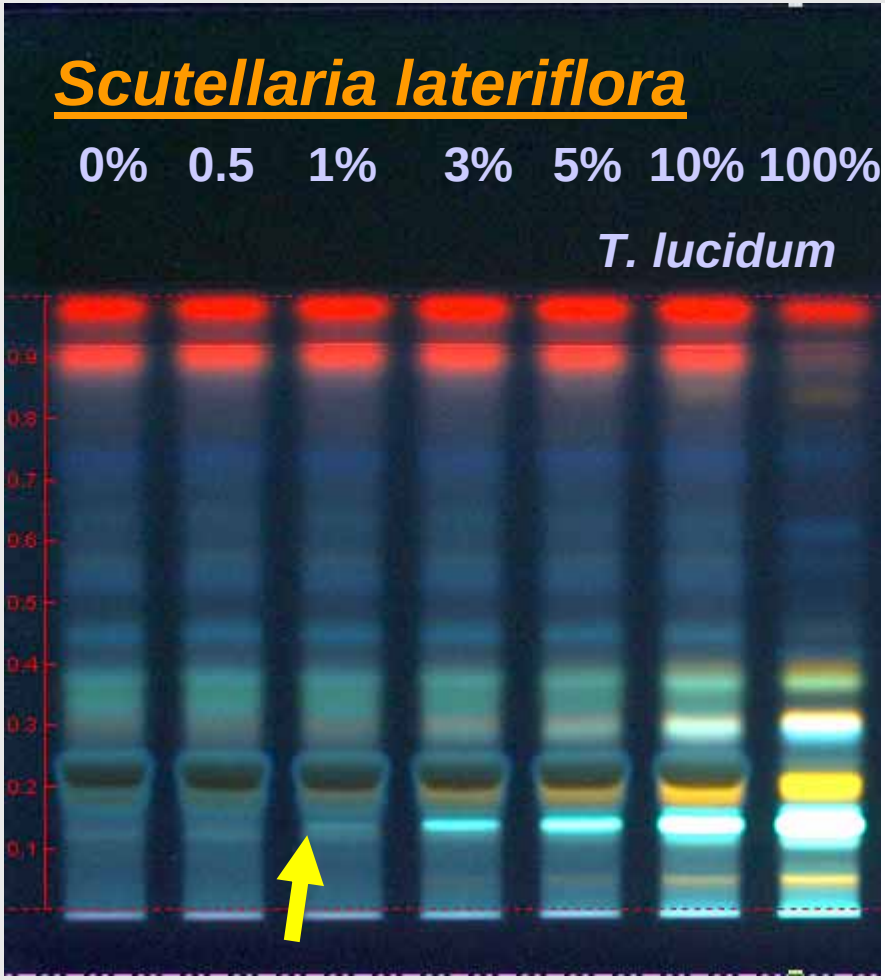
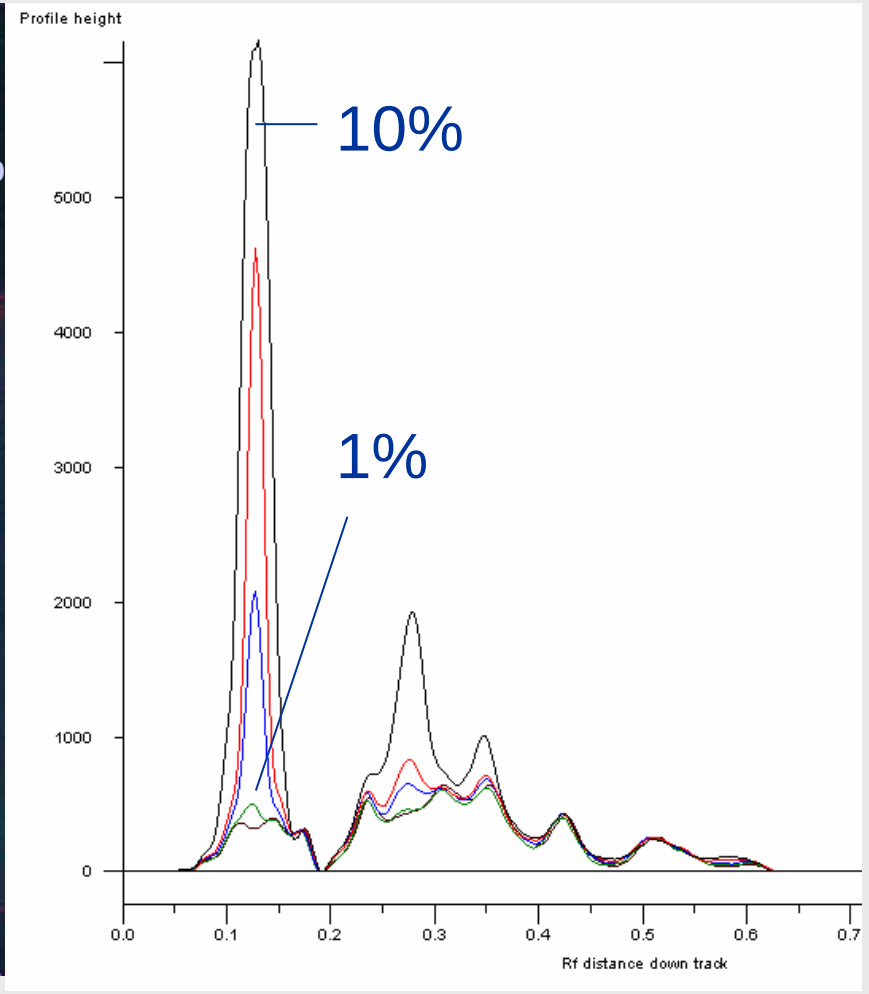
# What about mixtures?



# Your questions please?

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www.camag-laboratory.com

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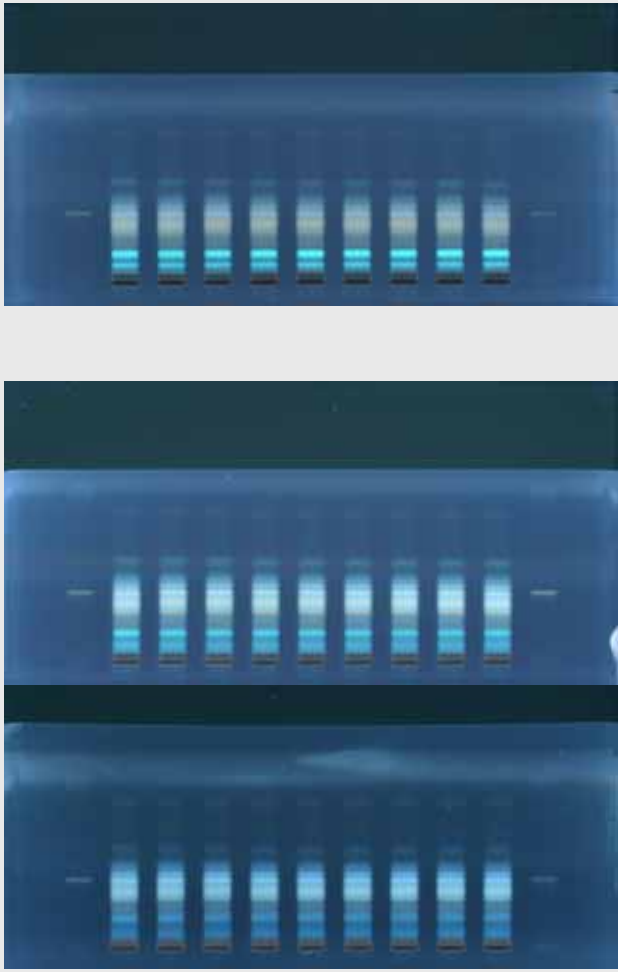
# Reproducibility (black cohosh)

| 1 day | **P119_050830_01** | P119_050830_02 | P119_050830_03 | ΔRf |
|---|---|---|---|---|
| F1 (Actein) | 0.36 | 0.35 | 0.35 | 0.01 |
| F2 | 0.30 | 0.30 | 0.30 | 0.0 |
| F3 | 0.25 | 0.25 | 0.25 | 0.0 |

| 3 days | **P119_050830_01** | P119_050831_01 | P119_050901_01 | ΔRf |
|---|---|---|---|---|
| F1 (Actein) | 0.36 | 0.34 | 0.38 | 0.04 |
| F2 | 0.30 | 0.28 | 0.33 | 0.05 |
| F3 | 0.25 | 0.24 | 0.28 | 0.04 |

| 2 labs | A160-050830-01 | A160-050830-02 | A160-050830-03 | ΔRf | **P119_050830_01** | ΔRf |
|---|---|---|---|---|---|---|
| F1 (Actein) | 0.33 | 0.33 | 0.33 | 0.0 | 0.36 | 0.03 |
| F2 | 0.28 | 0.28 | 0.28 | 0.0 | 0.30 | 0.02 |
| F 3 | 0.23 | 0.23 | 0.23 | 0.0 | 0.25 | 0.02 |



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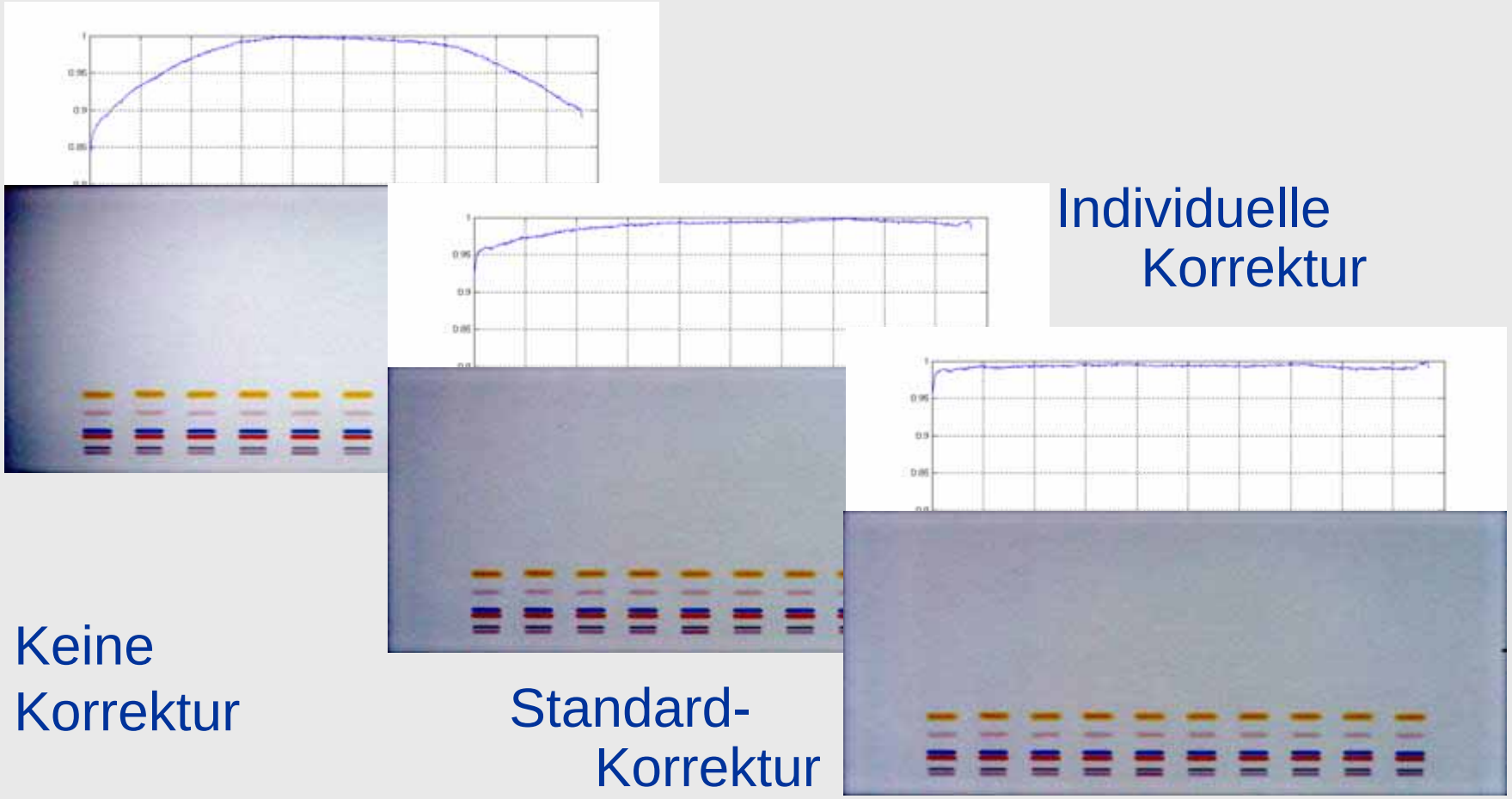
# Bildkorrektur - Auflicht



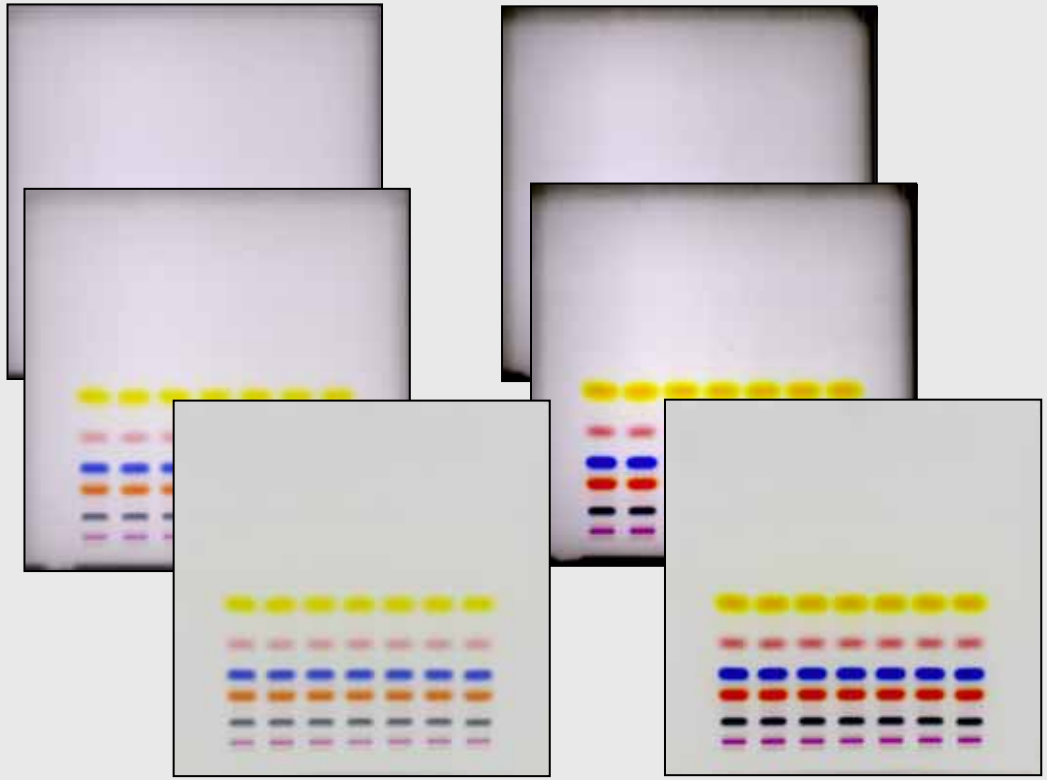
Keine Korrektur

Standard-Korrektur

Individuelle Korrektur

# Auf-und Durchlichtmessung Testfarbstoffe

- Testfarbstoffe (Reproduzierbarkeit)
- Leere Platte
- Chromatogramm
- Kompensiertes Ergebnis



→ Gute Basis für quantitative Analysen

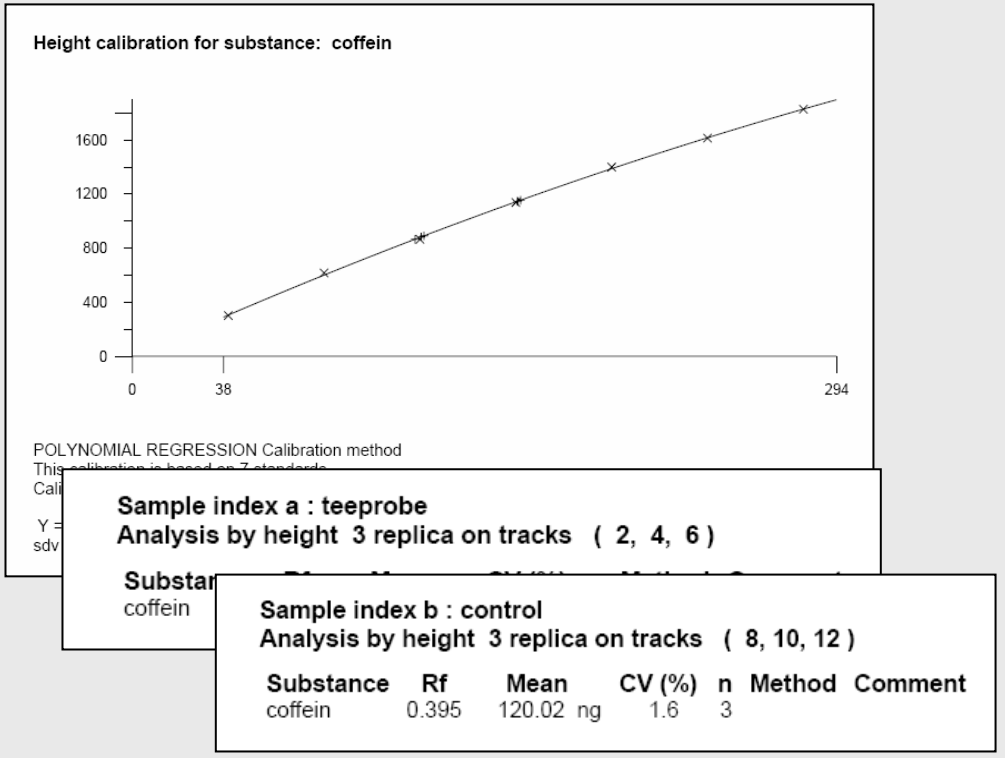
# Quantitative Analyse unter UV 254 nm

- Koffein im Tee
  (3 Kontroll + 7pt.Kal.)
- Leere Platte
- Chromatgramm
- Kompensiertes Ergebnis

→ Exzellentes Ergebnis, wenn Substanzen sichtbar sind

# Ergebnis

- CAMAG VideoScan



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# VideoScan – Software zur Bildauswertung