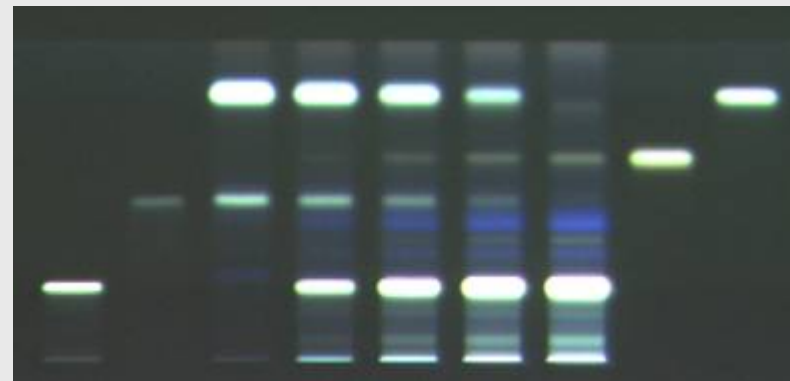


Standardized HPTLC Methods

For identification of botanicals and detection of adulteration

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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

Standardized Methodology

Is there a need for standardization?

Lab A



Lab B

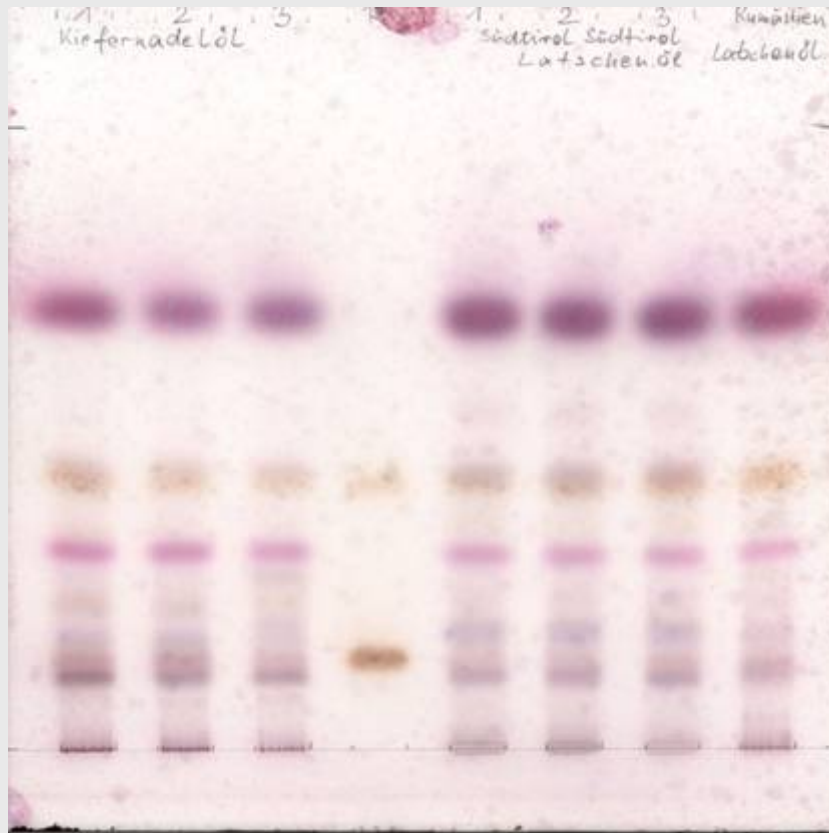


Identification test for Peppermint and Spearmint oil, Ph.Eur.5

5

TLC or HPTLC?

TLC



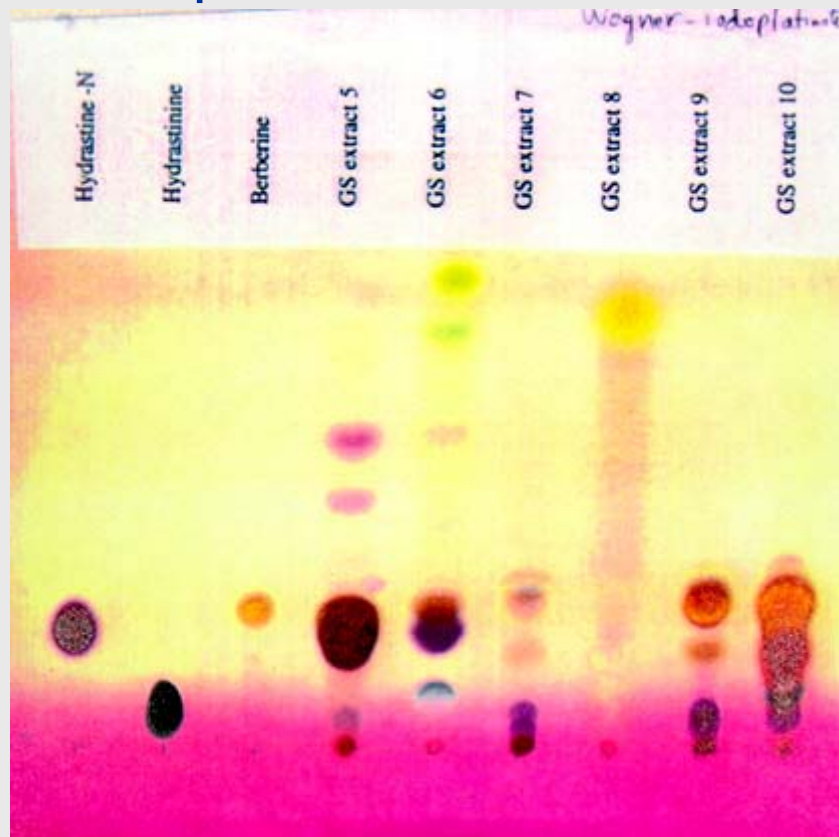
HPTLC



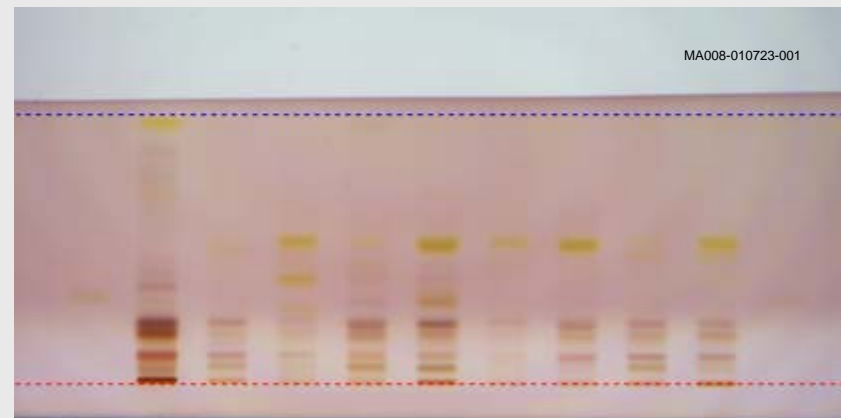
6

Spot or band application?

Spot

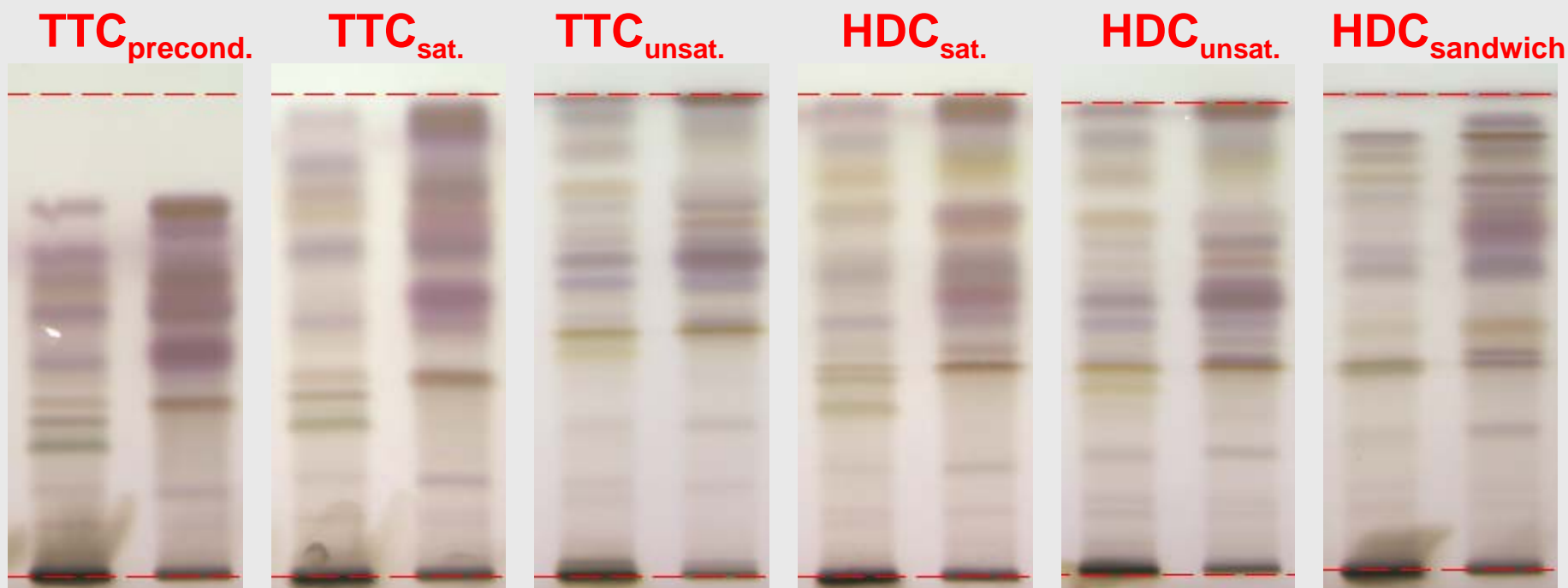


Band



Effects of chamber configuration/saturation

TTC = Twin Trough Chamber HDC= Horizontal Developing Chamber

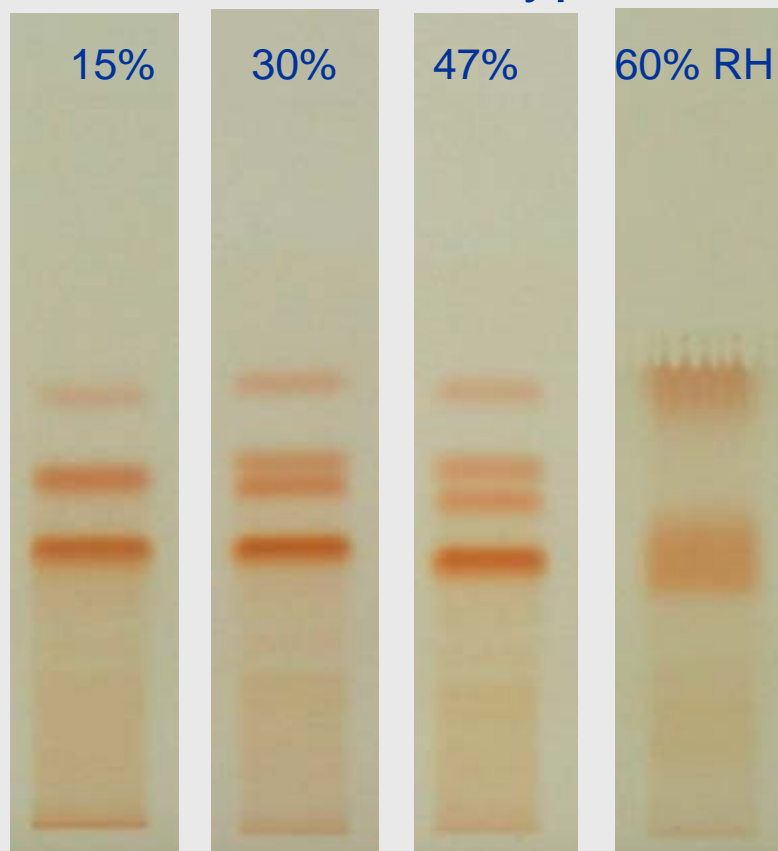


HPTLC silica gel 60 F₂₅₄, toluene - ethyl acetate - acetic acid (70 : 33 : 3)

Left: *Schisandra chinensis*, right: *Schisandra sphenanthera*

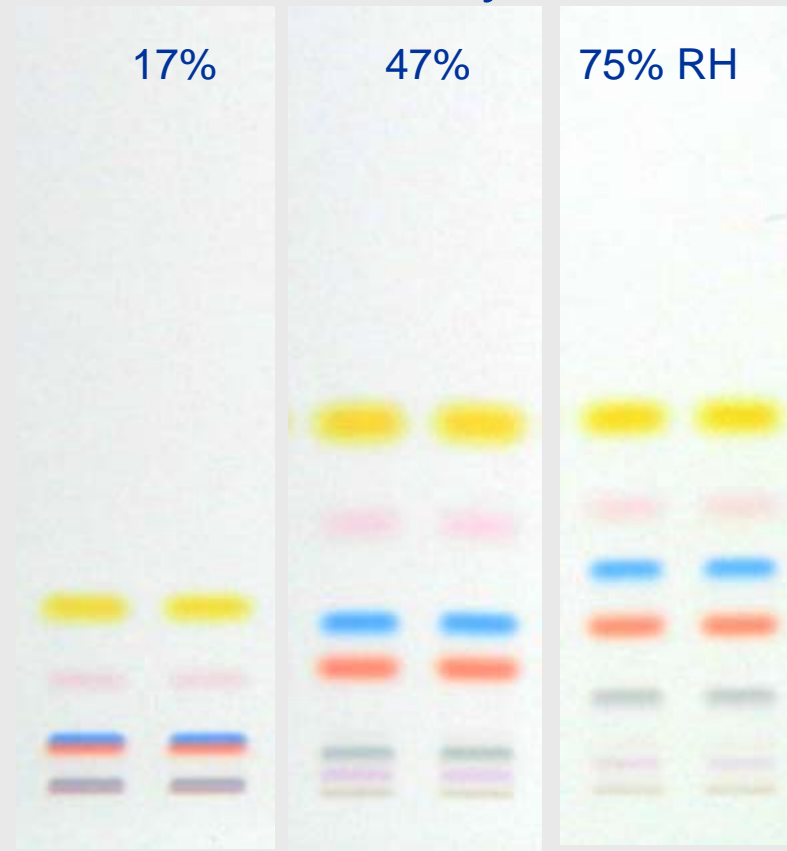
Effects of rel. humidity /activity

Green Tea: Polyphenols



Toluene, Acetone, Formic Acid (4.5:4.5:1)

Test Dye



Toluene

Spraying or dipping?

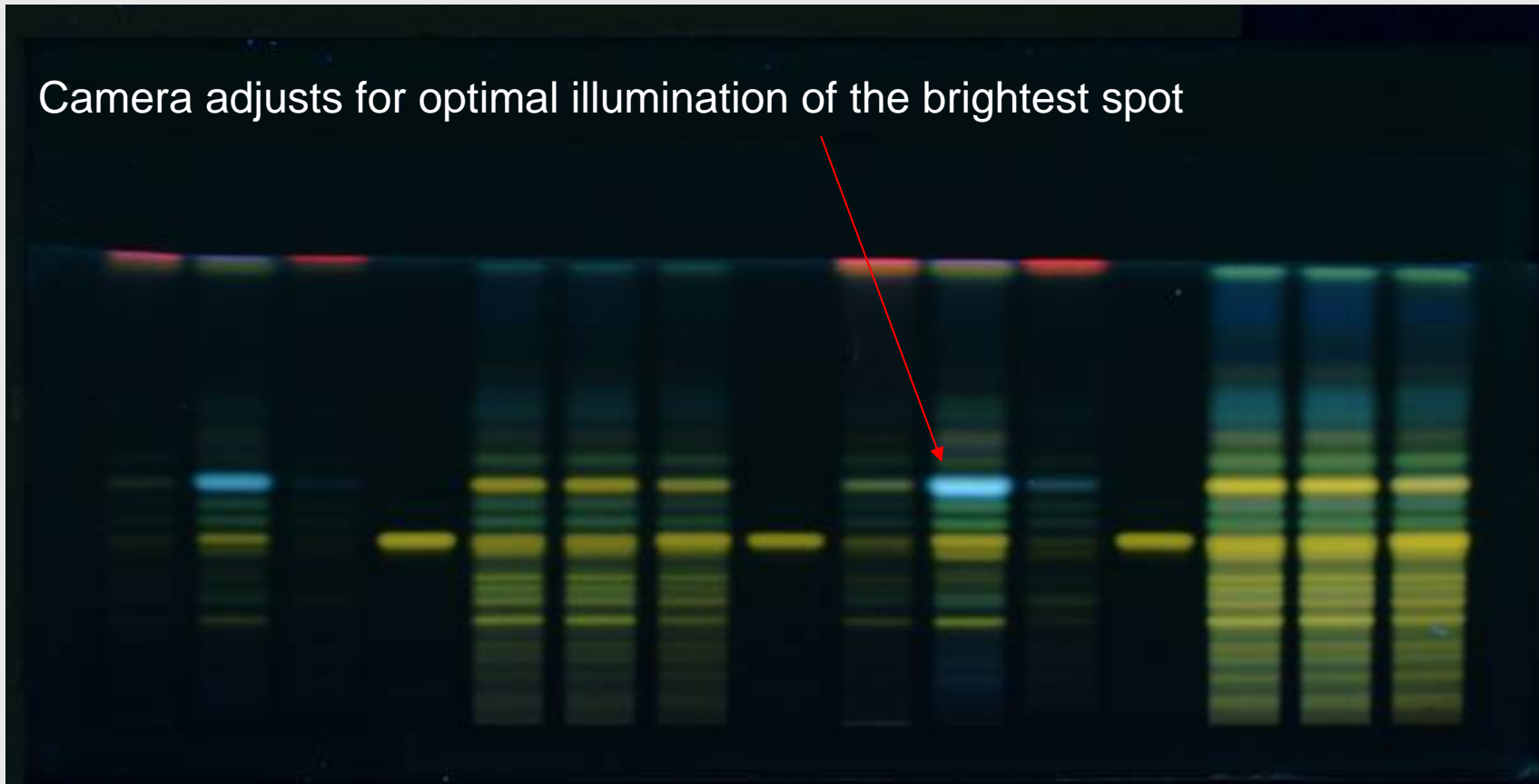


Dichloroquinone
chloroimide reagent
and ammonia vapor



Automatic image optimization

Camera adjusts for optimal illumination of the brightest spot



Our approach: 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, 8 mm (lower edge), 15 mm (left right edge)
- ▶ **Detailed description of development**
 - 6 cm, 20 min saturation (with filter paper)
- ▶ **Derivatization**
 - Dipping whenever possible
- ▶ **Densitometry**
 - MWL scan
 - Scan at the max. WL
- ▶ **Digital documentation**
 - UV 254 nm / 366 nm / white light (prior to and after derivatization)

Successful standardization – *Echinacea*

May 06, 2005 – CSI Laboratory

Image published
September 11, 2001

Echinacea Reprod.
Q154-050630-10A



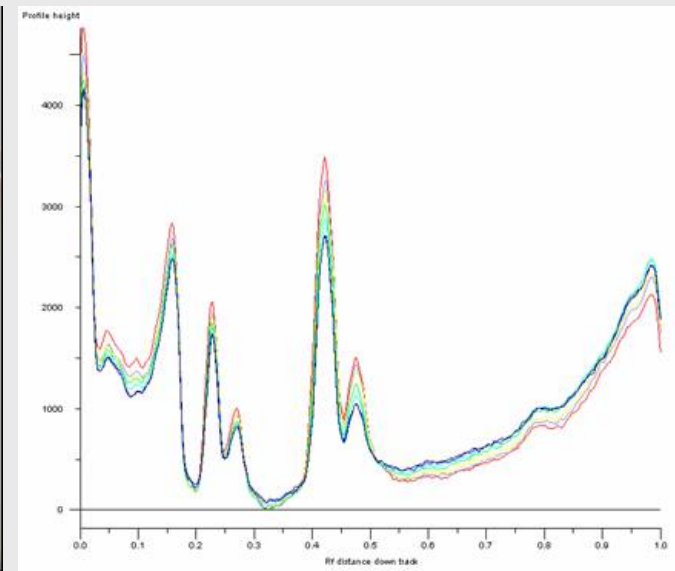
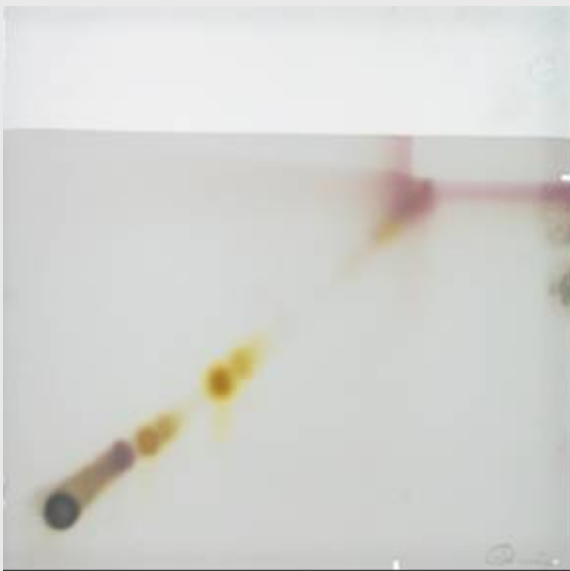
Validated Methods

Validation of qualitative methods



Stability: Licorice

- ▶ During chromatography
- ▶ In solution and on plate (3 h each)
- ▶ Result 30 min

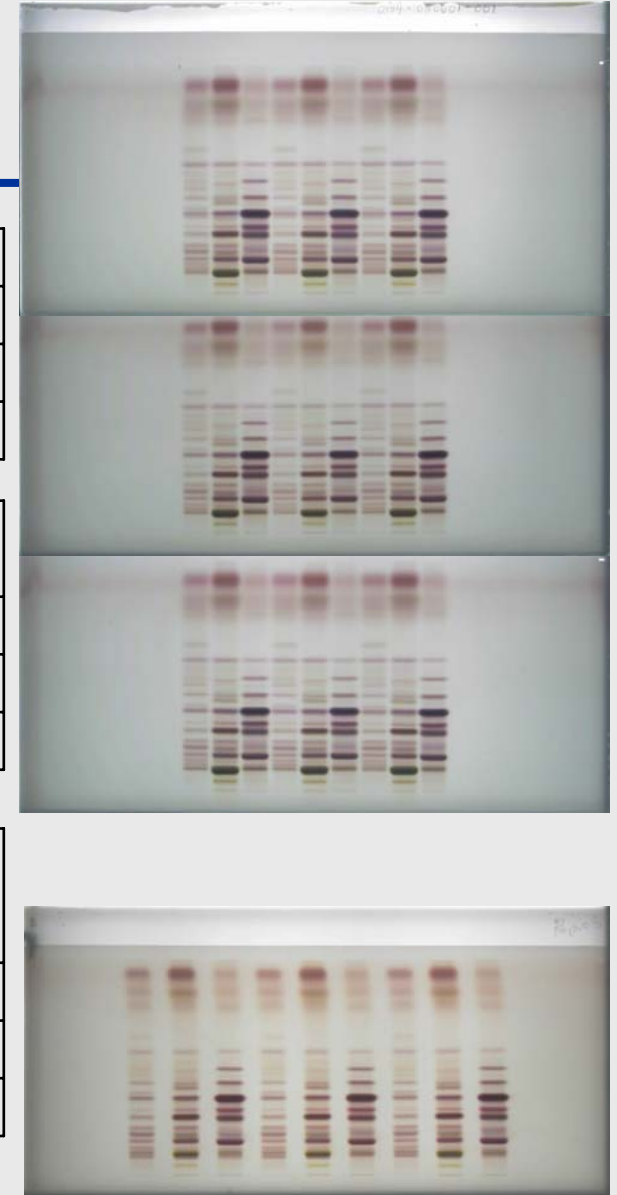


Precision: Rf of Ginsenosides

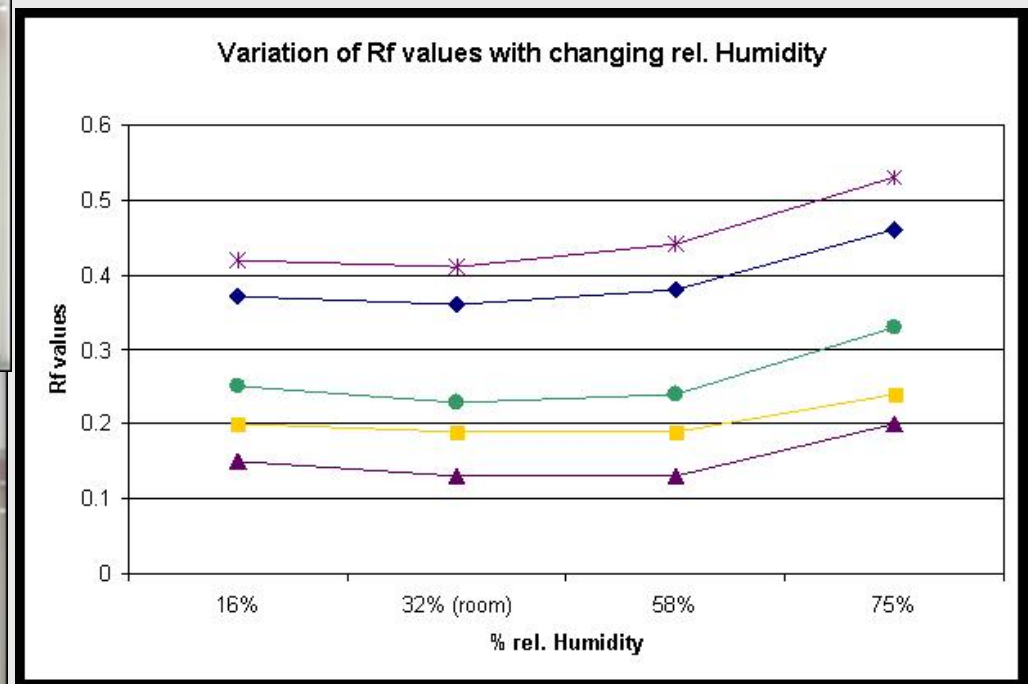
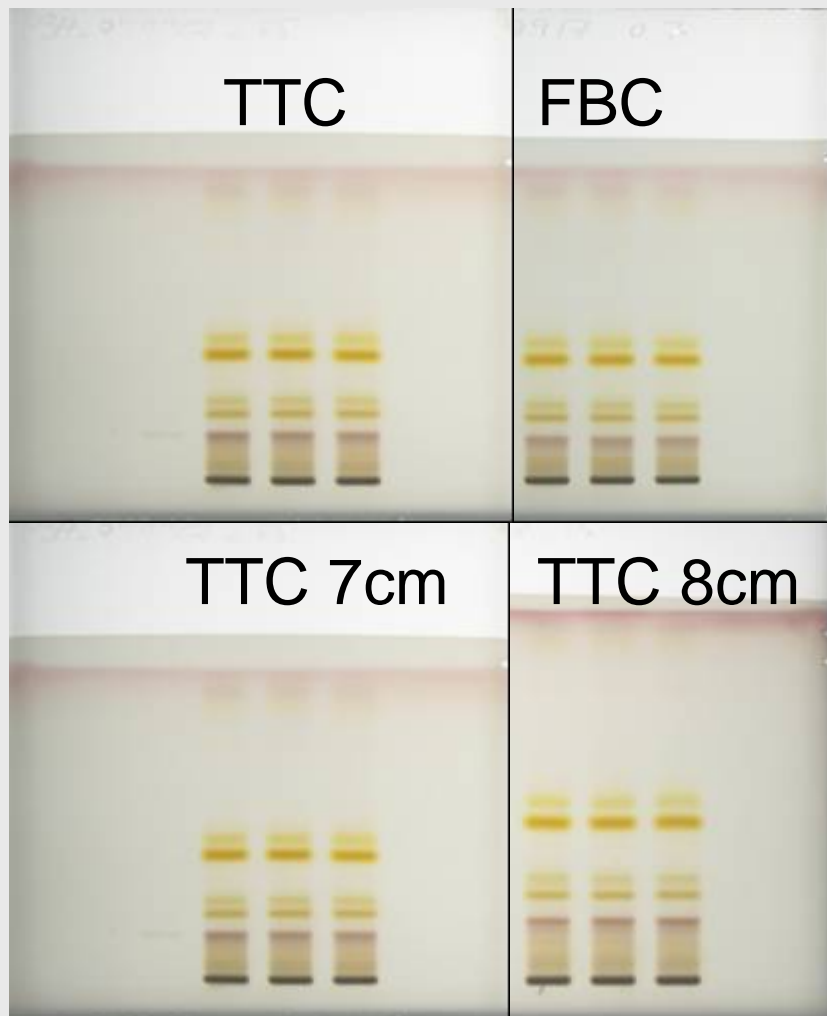
| Same day | A154-050531-01 | A154-050531-02 | A154-050531-03 | ΔRf |
|----------|----------------|----------------|----------------|-------------|
| Rg1 | 0.35 | 0.36 | 0.36 | 0.01 |
| Re | 0.26 | 0.26 | 0.27 | 0.01 |
| Rb1 | 0.15 | 0.16 | 0.16 | 0.01 |

| 3 days | A154-050531-01 (Comparison) | A154-050601-01 | A154-050606-01 | ΔRf |
|--------|--------------------------------|----------------|----------------|-------------|
| Rg1 | 0.35 | 0.35 | 0.35 | 0.0 |
| Re | 0.26 | 0.26 | 0.26 | 0.0 |
| Rb1 | 0.15 | 0.15 | 0.16 | 0.01 |

| Two Labs | Plate P47_05072 6_03 | Plate P47_05072 6_04 | Plate P47_05072 6_05 | ΔRf | A154-050531-01 (Comparison) | ΔRf |
|----------|----------------------------|----------------------------|----------------------------|-------------|--------------------------------|-------------|
| Rg1 | 0.38 | 0.38 | 0.36 | 0.02 | 0.35 | 0.03 |
| Re | 0.28 | 0.29 | 0.27 | 0.02 | 0.26 | 0.03 |
| Rb1 | 0.16 | 0.18 | 0.16 | 0.02 | 0.15 | 0.03 |



Robustness: Licorice



Validated methods for identification (of single herbs)

- ▶ Licorice
- ▶ Feverfew
- ▶ Milk thistle
- ▶ Kava kava
- ▶ Ginger
- ▶ Green tea
- ▶ Ginseng
- ▶ Eleuthero
- ▶ Echinacea
- ▶ Black Cohosh

In the process

- ▶ St. Johns Wort
- ▶ Saw Palmetto
- ▶ Hoodia
- ▶ Skullcap
- ▶ Star Anise
- ▶ Other Cimigifuga species

Identification of single species

Identification of Black Cohosh (validated, AHP)



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

Variability?



1: Chlorogenic acid; 2: Caffeic acid; 3: Isoferulic acid; 4, 16: Actein

5: *Actaea racemosa* BRM;

6: *Actaea racemosa* ;

7: *Actaea* (wild);

8: *Actaea* (cultivated);

9: *Actaea* (cultivated)*;

10: *Actaea* (cultivated);

11: *Actaea* (wild);

12: *Actaea* rhizoma conc.;

13: *Actaea racemosa* extract;

14: *Actaea racemosa* powder;

15: *Actaea racemosa* powder;

Other species



- 1: Chlorogenic acid, actein, caffeic acid, and isoferulic acid (increasing Rf values);
 2: *Actaea racemosa* BRM; 3-6: *Actaea podocarpa*; 7-8: *Actaea pachypoda*;
 9: *Actaea pachypoda* (leaves); 10: *Actaea rubra*; 11: *Actaea foetida*
 12-13: *Actaea dahurica*; 14: *Actaea heracleifolia*; 15-16: *Actaea* sp unknown
 17: *Caulophyllum thalictroides* 18-19: Guang Shengma (no *Actaea*).

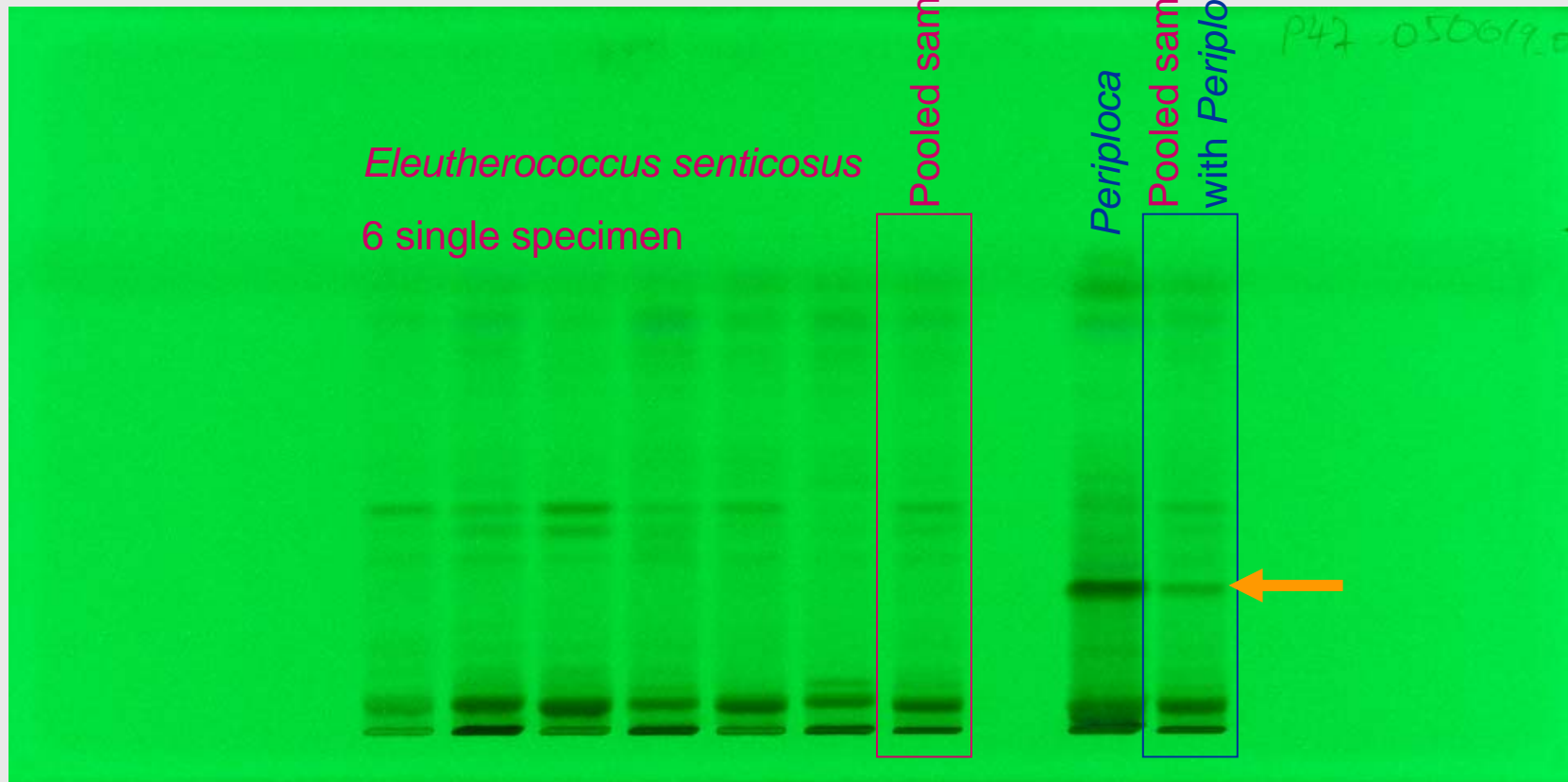
Identification of mixtures

Detection of adulterated mixtures

- ▶ Great challenge
- ▶ (Not just) identification of markers
- ▶ Representative „pooled samples“
- ▶ Representative fingerprint for adulterant
- ▶ Improved specificity
- ▶ Detection limits

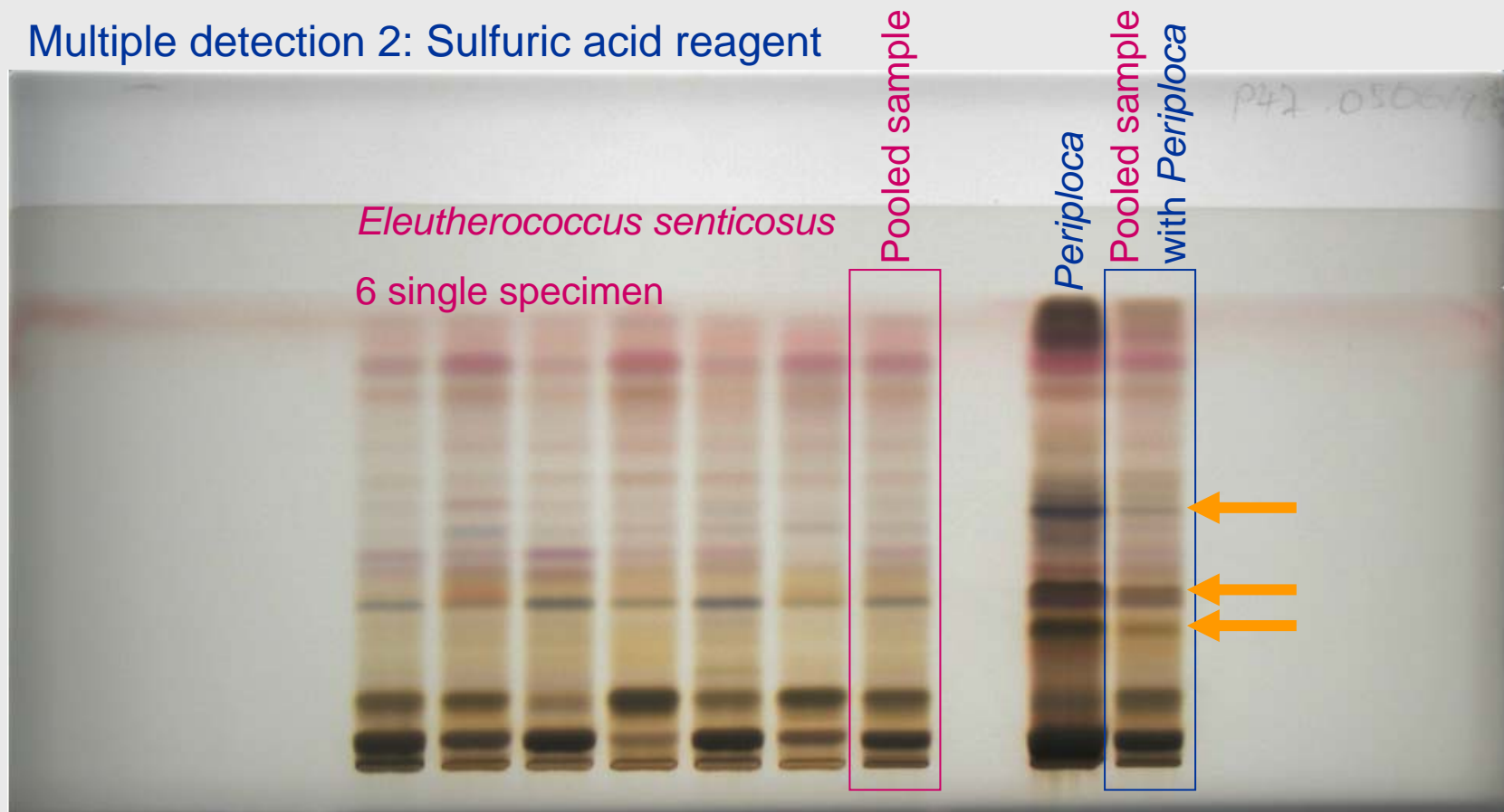
Single specimen vs. pooled sample

Multiple detection 1: UV 254 nm

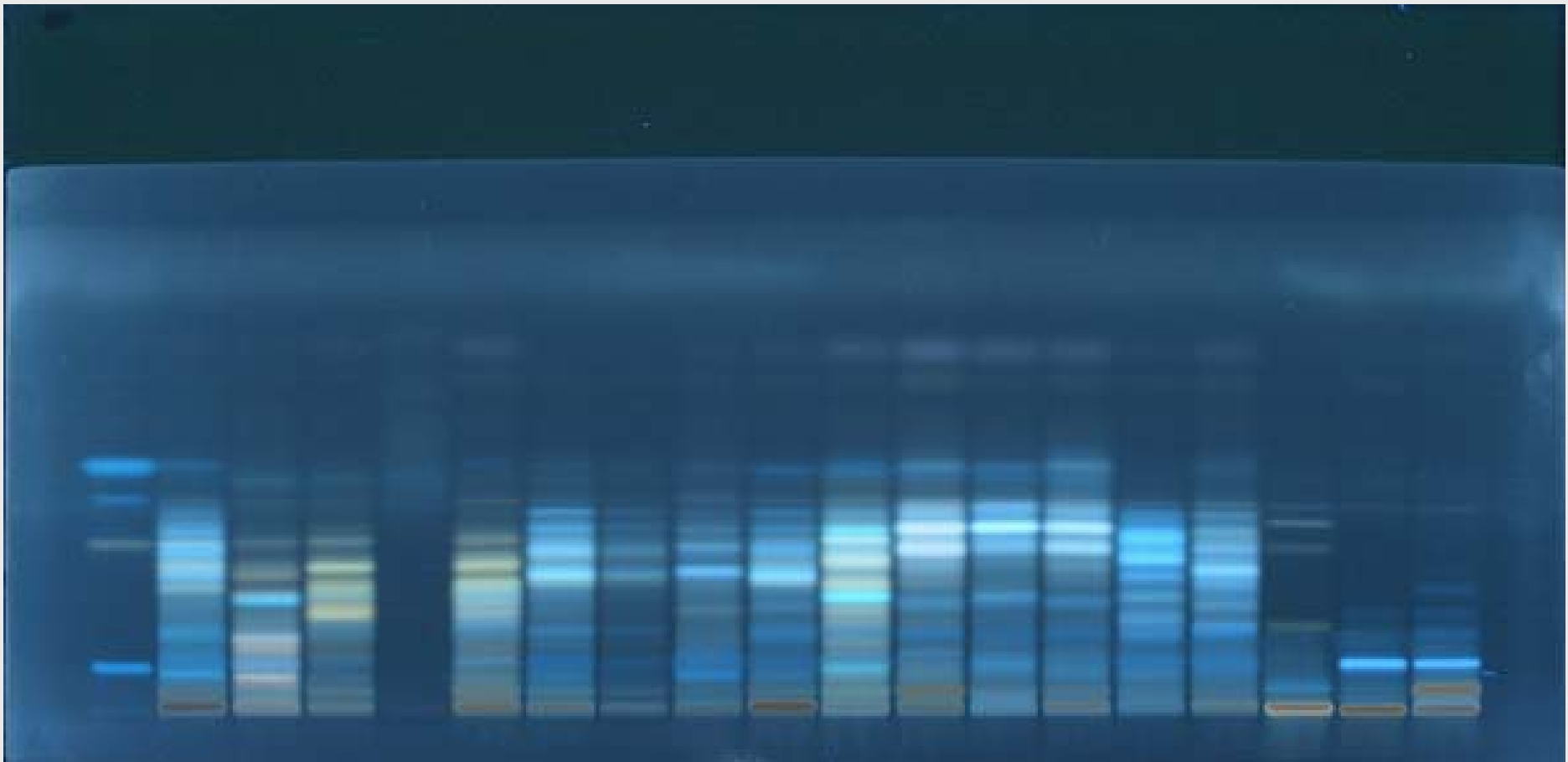


Single specimen vs. pooled sample

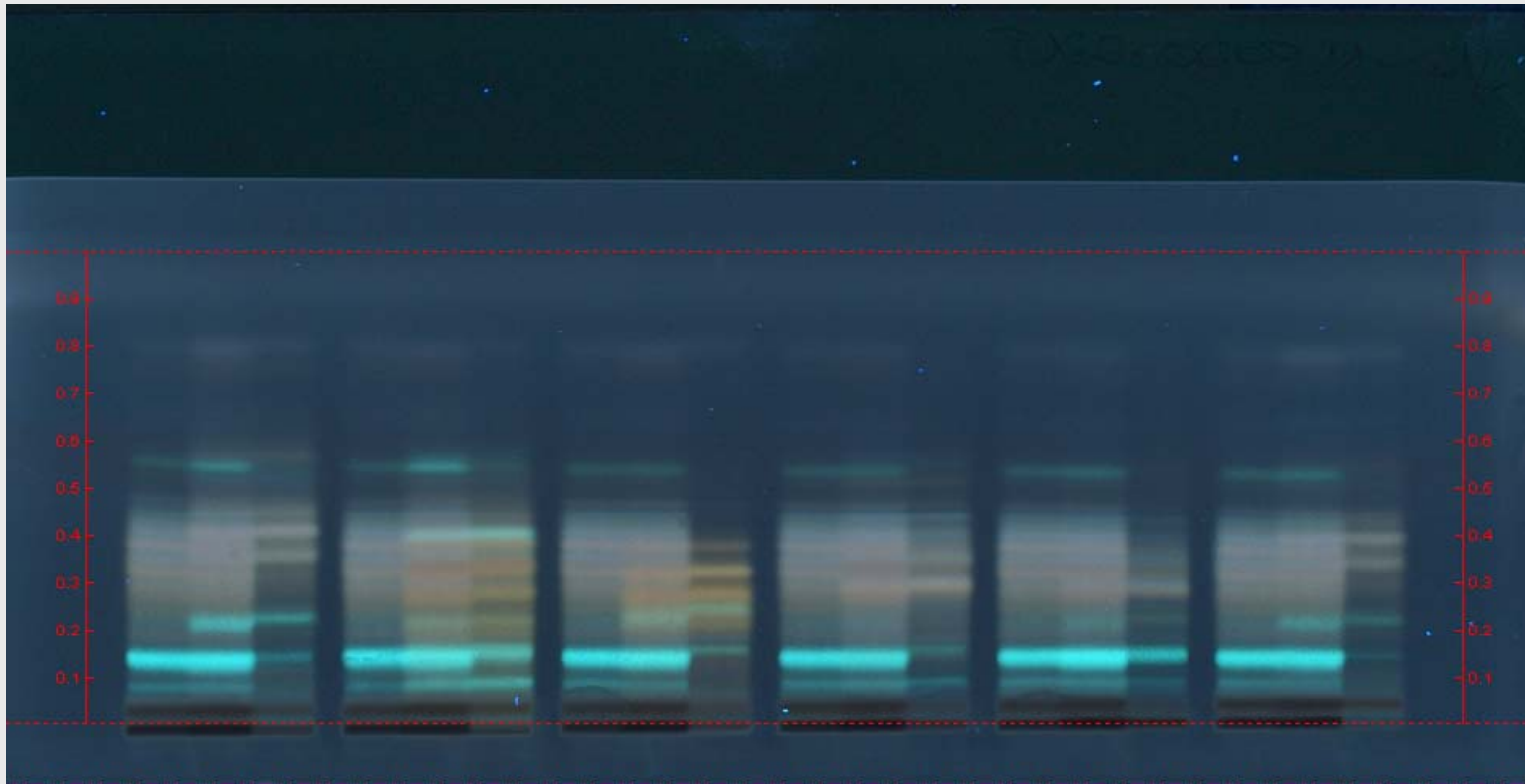
Multiple detection 2: Sulfuric acid reagent



Again: Black Cohosh and adulterants

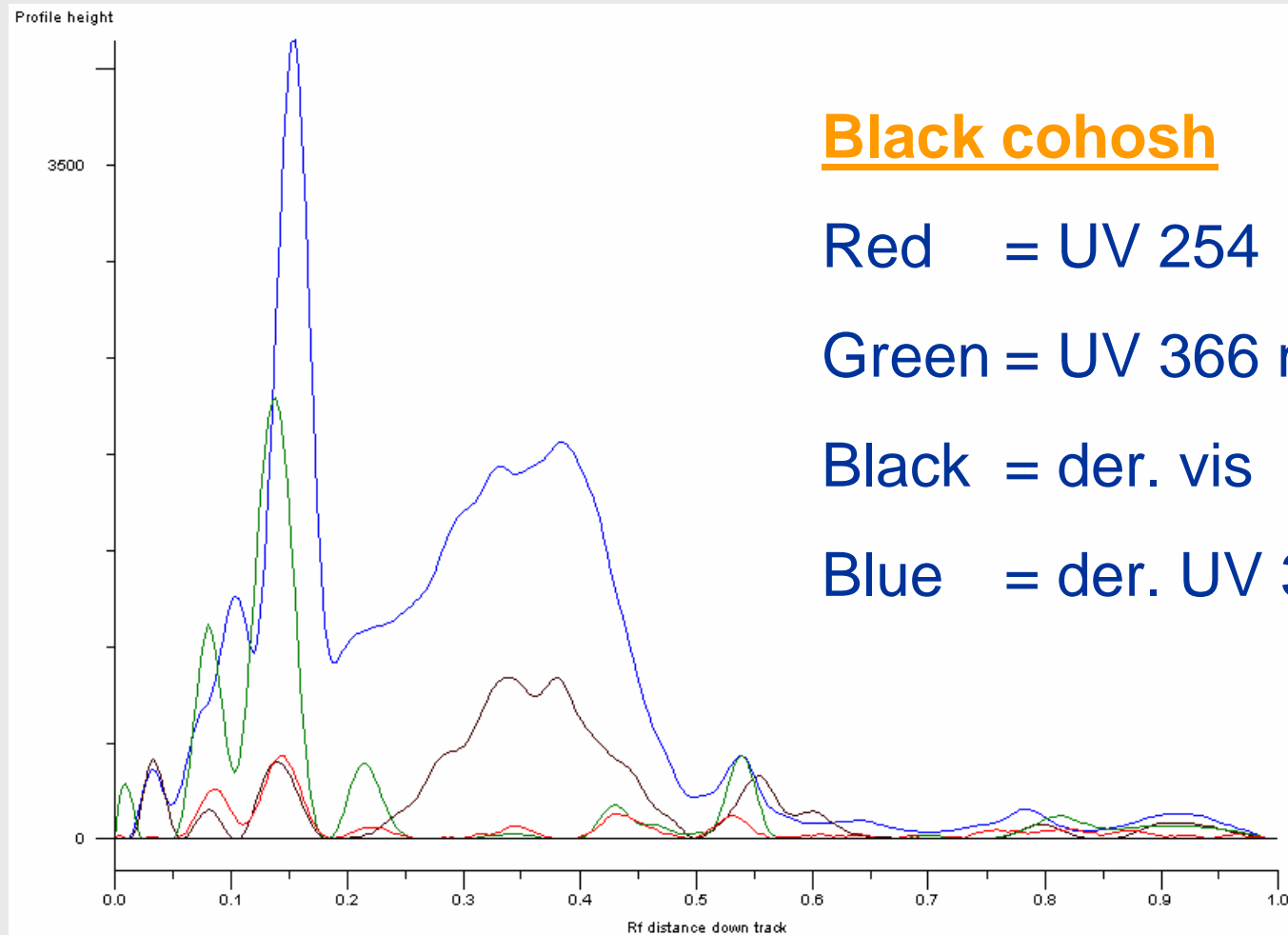


Adulteration of Black Cohosh



Actaea herae. *foet.* *podo.* *pach.* *rubr.* *dahu.*

Profile comparison (multiple detection)



Black cohosh

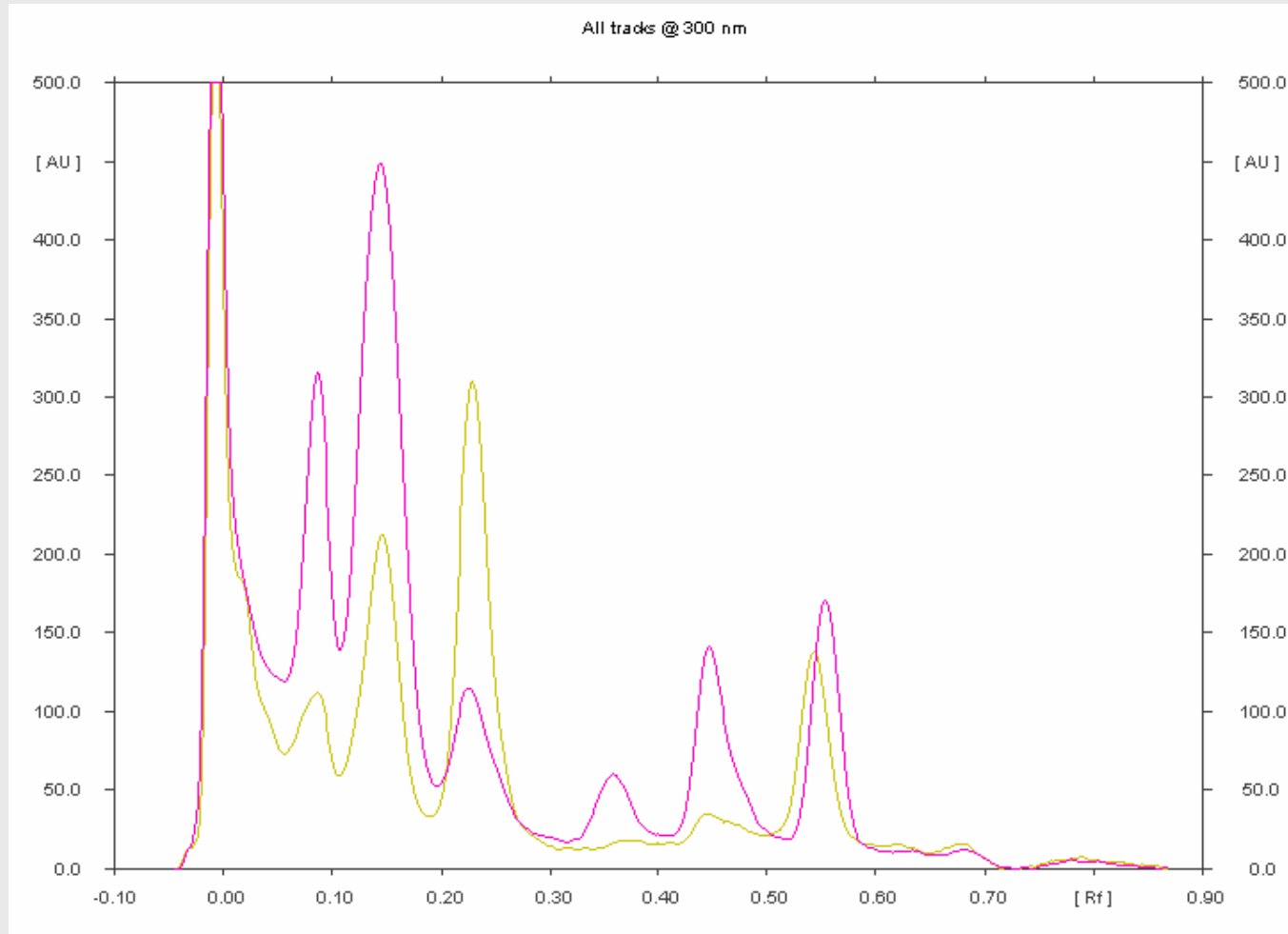
Red = UV 254

Green = UV 366 nm

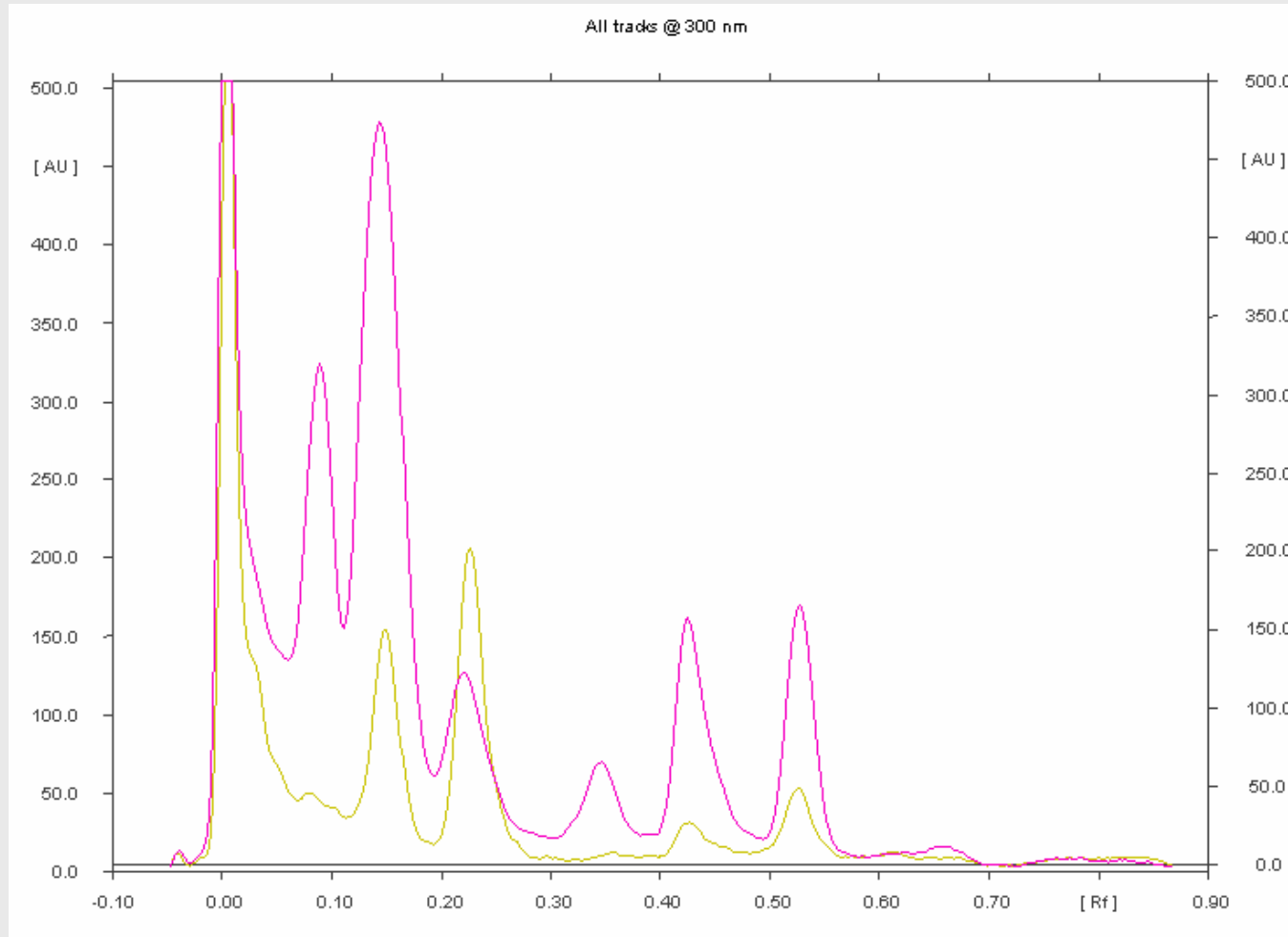
Black = der. vis

Blue = der. UV 366 nm

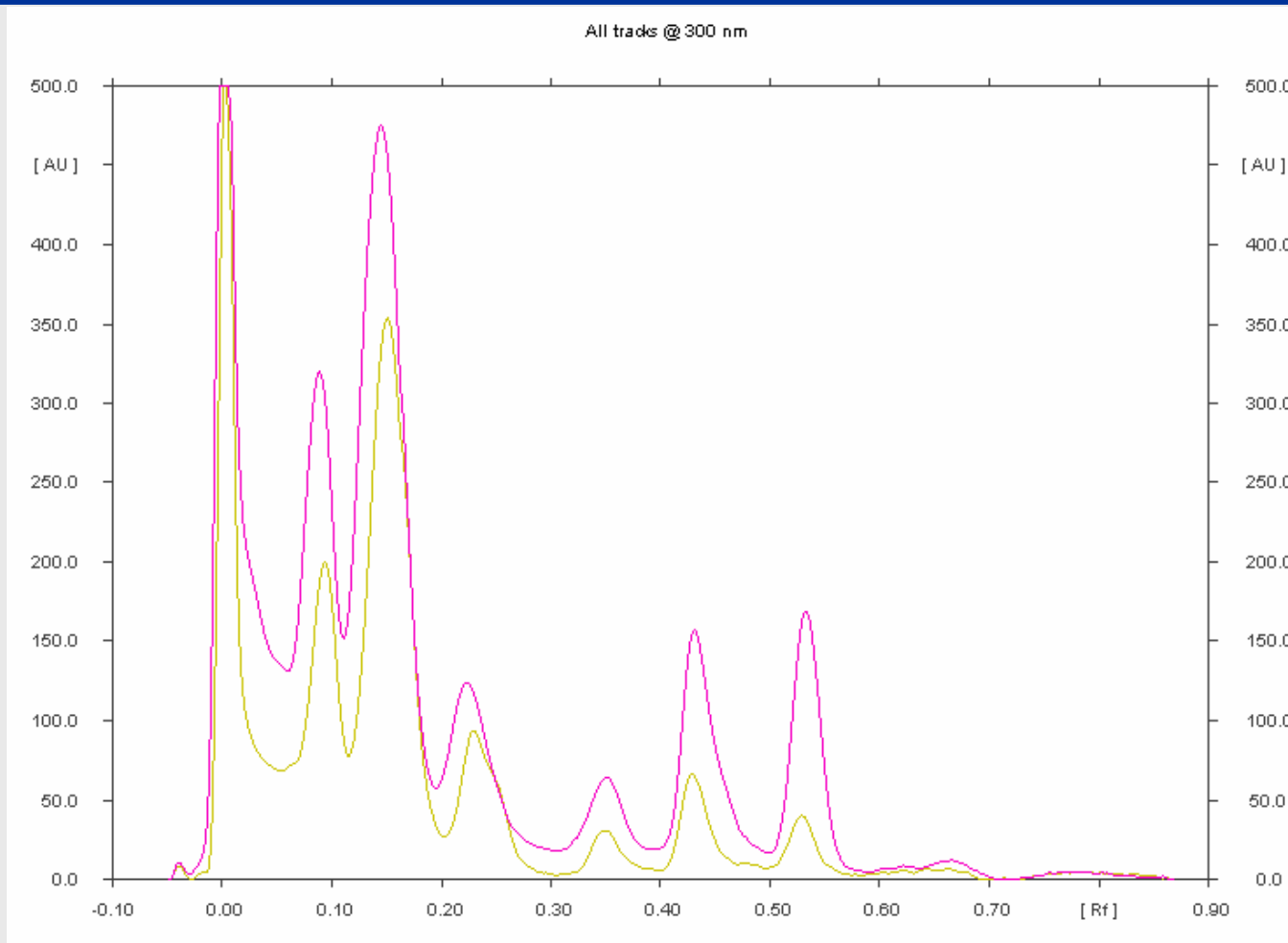
A. racemosa vs *A. heracleifolia*



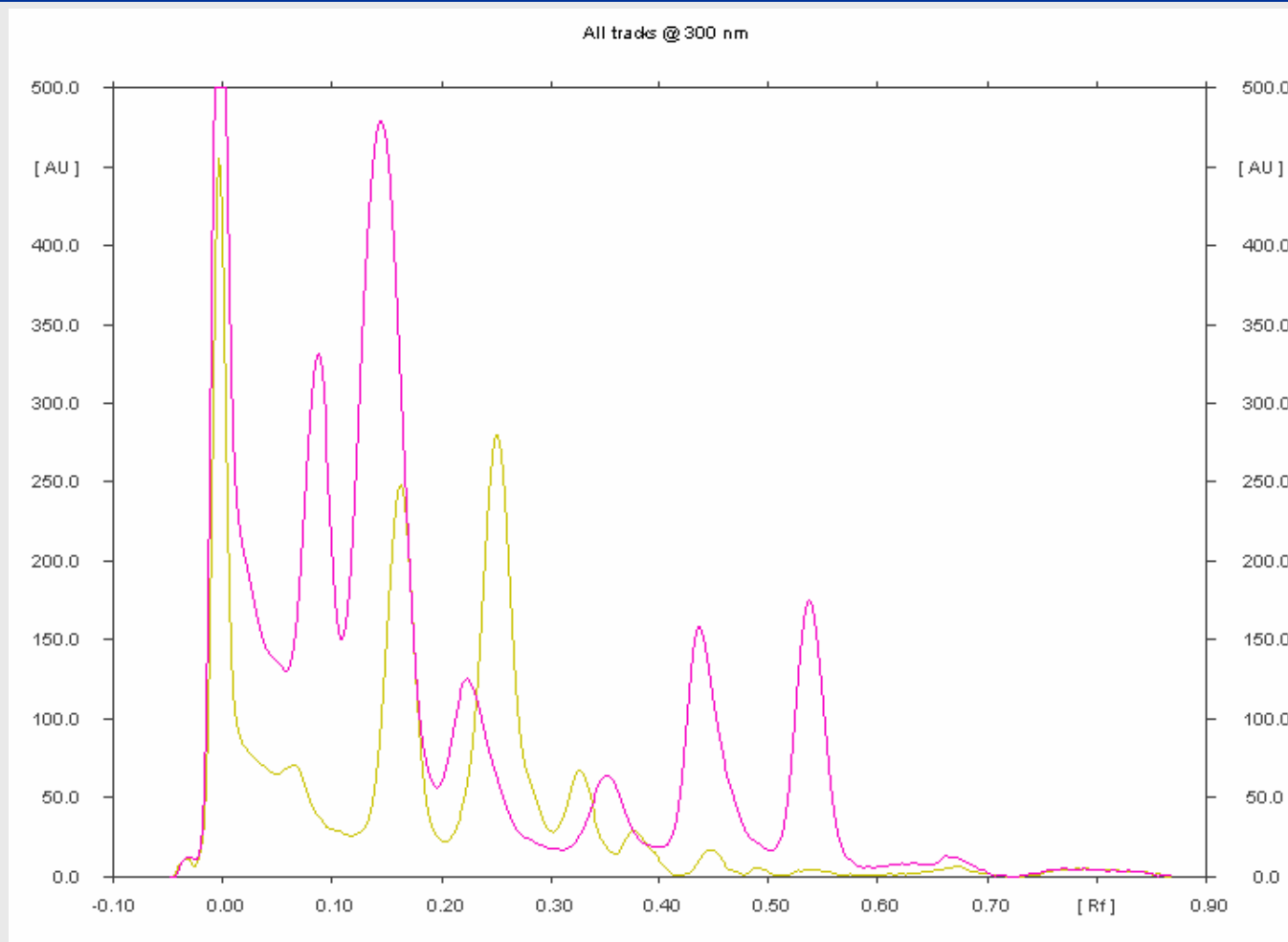
A. racemosa vs *A. dahurica*



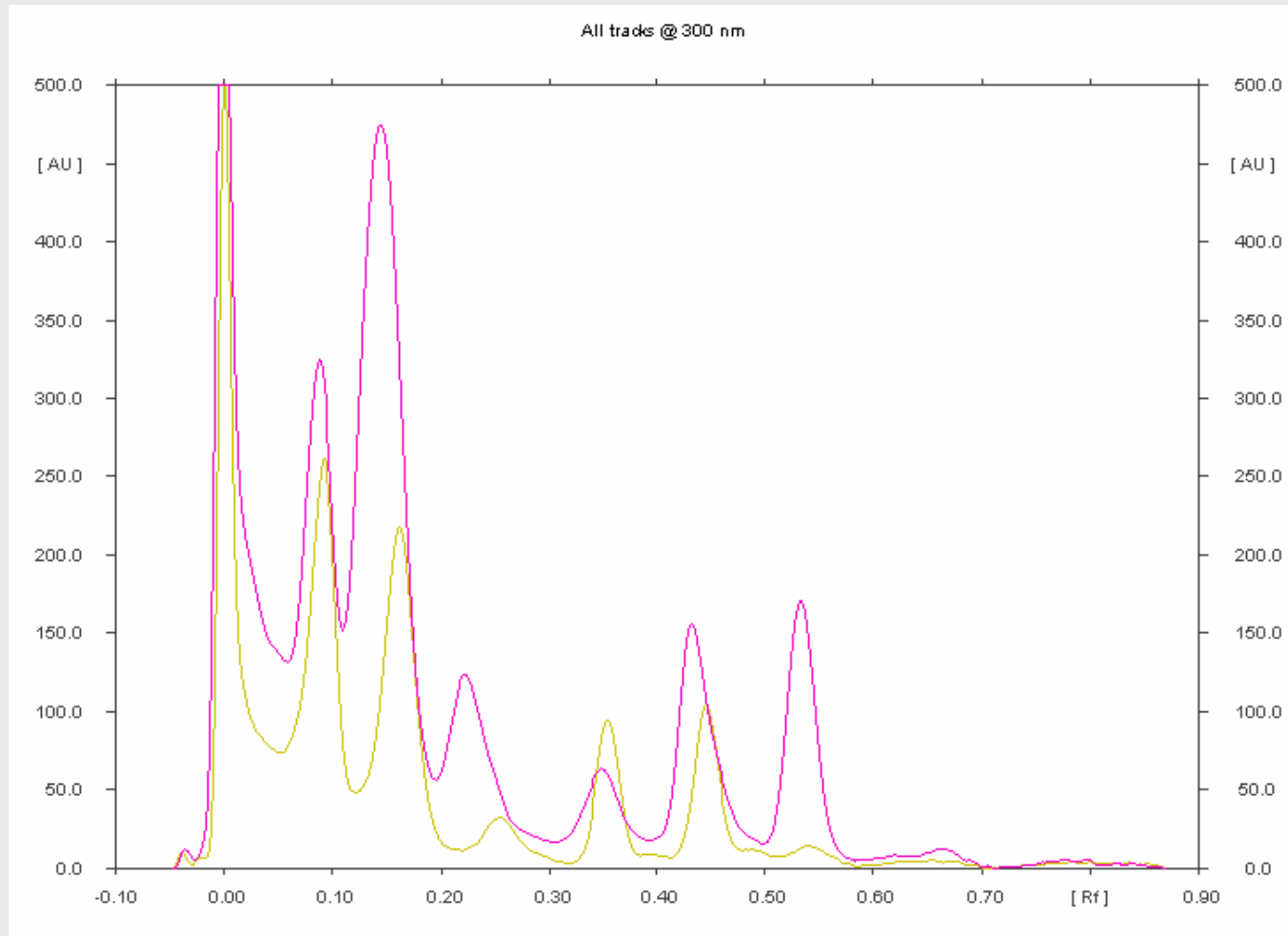
A. racemosa vs *A. rubra*



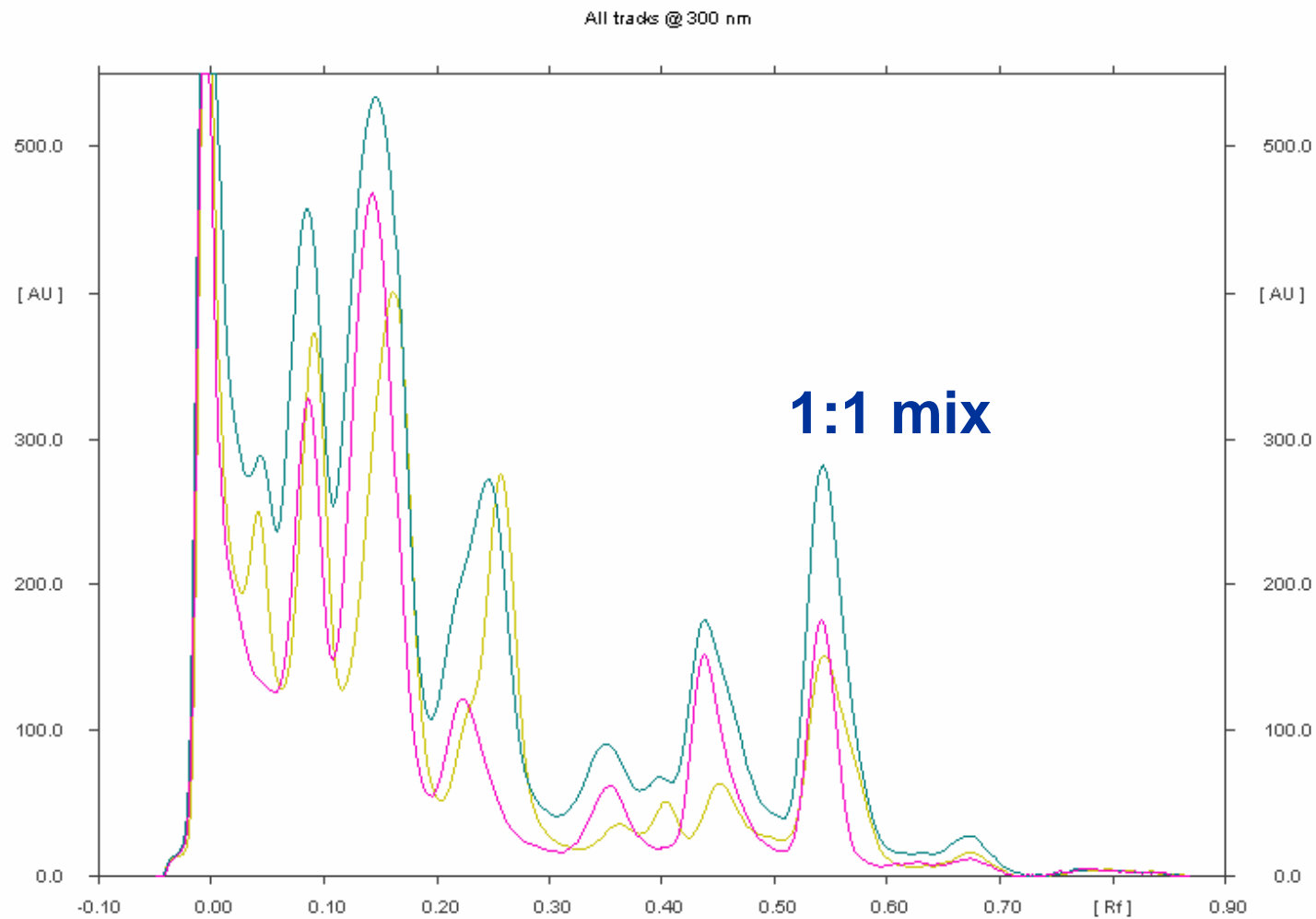
A. racemosa vs *A. podocarpa*



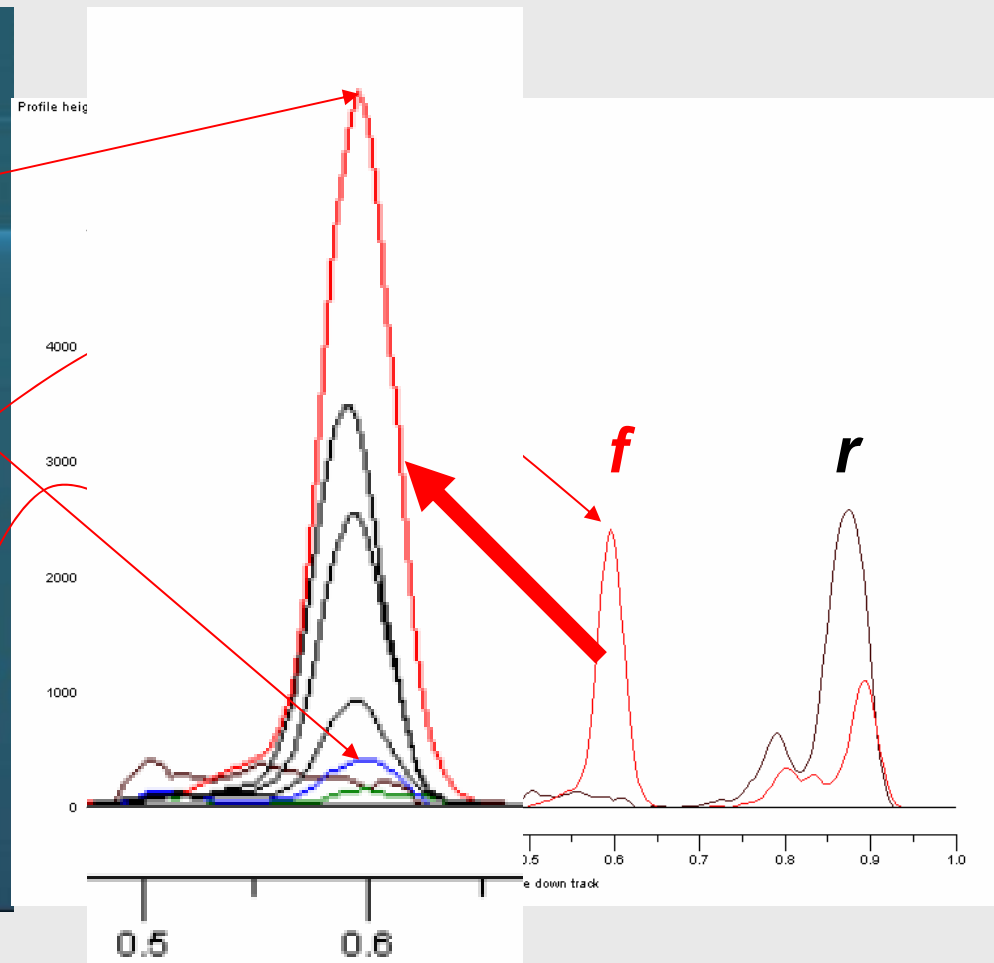
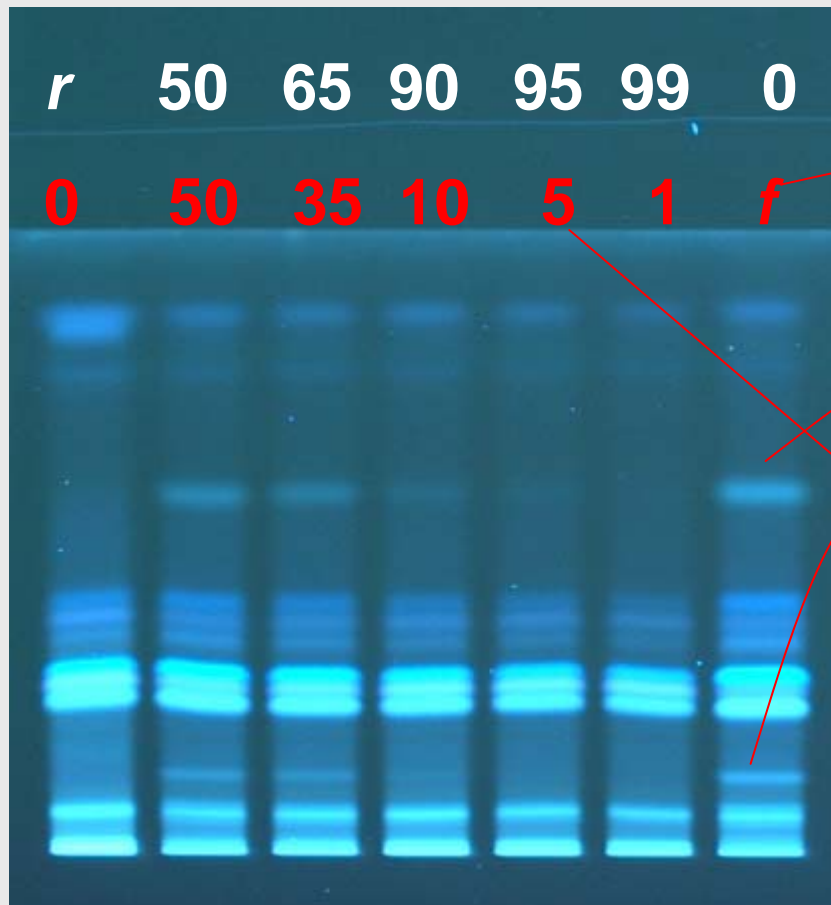
A. racemosa vs *A. pachypoda*



A. racemosa vs *A. foetida*



A. racemosa – *A. foetida*

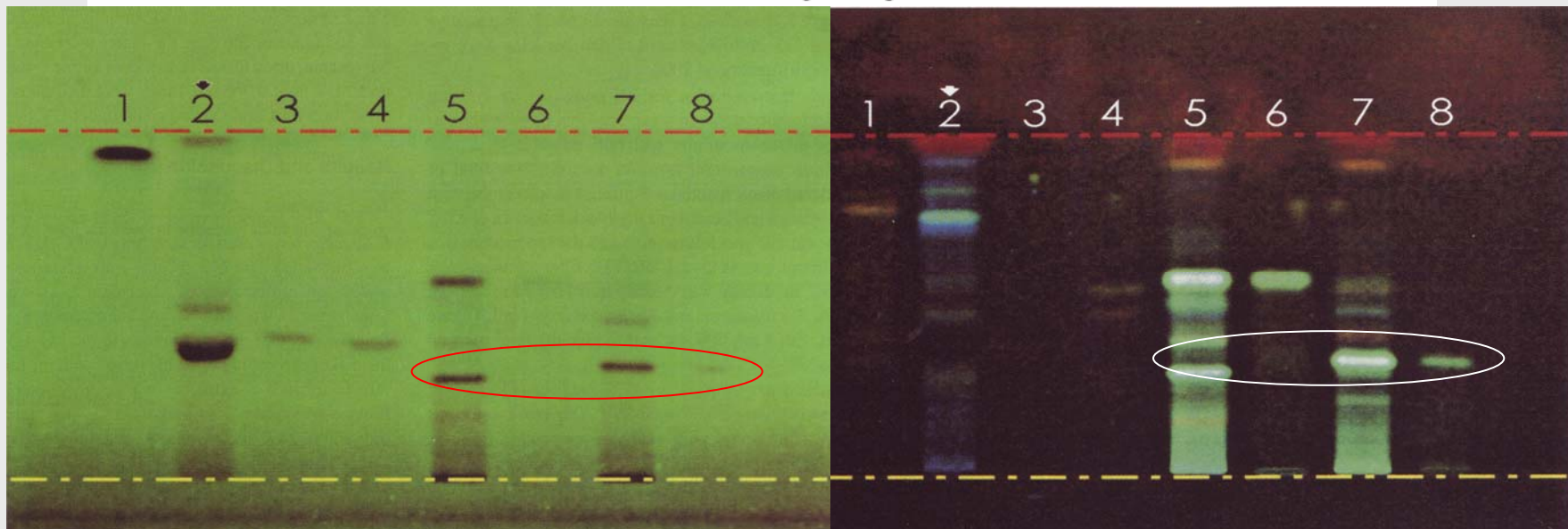


Identification of Skullcap

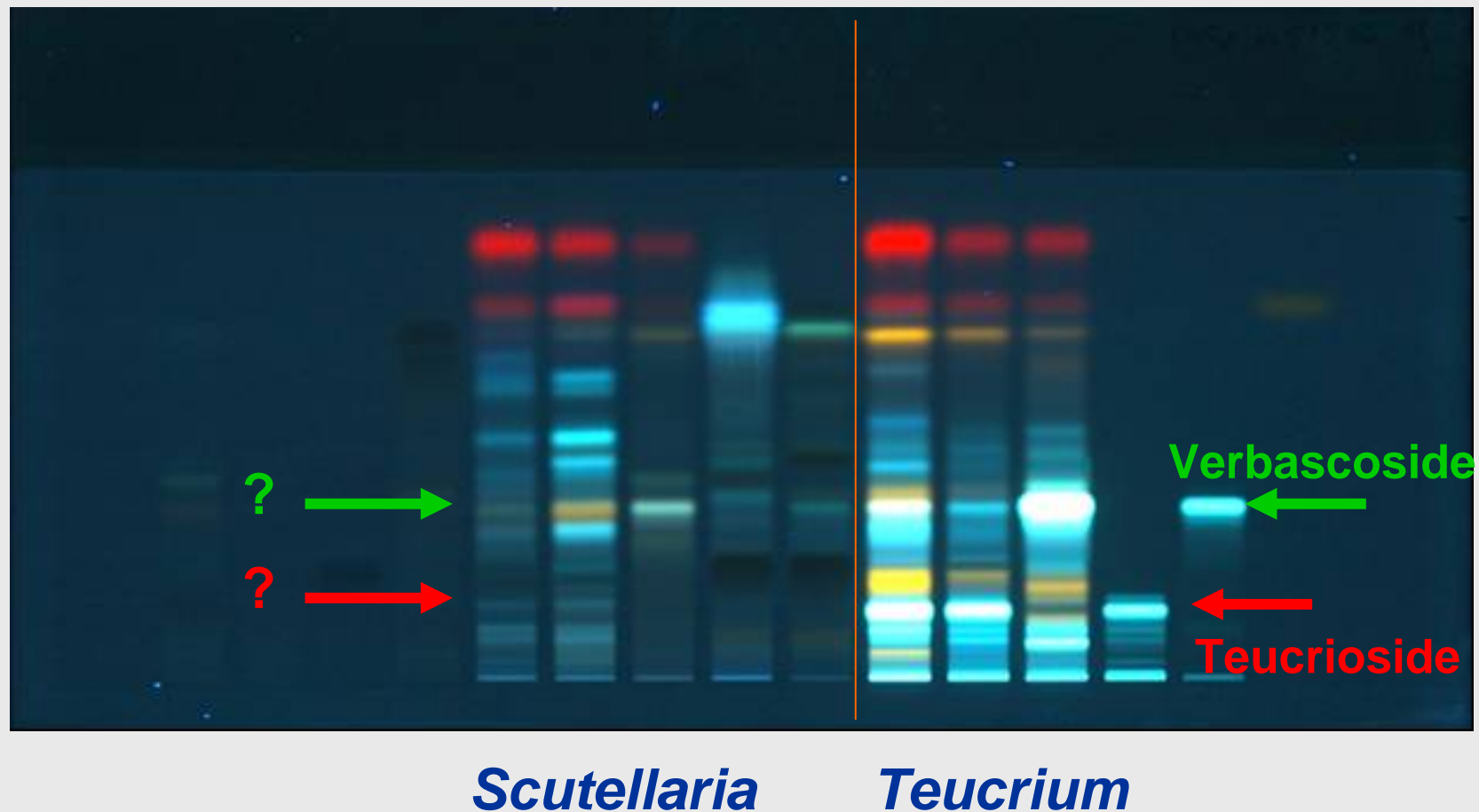
GAFNER ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 86, No. 3, 2003 453

DIETARY SUPPLEMENTS

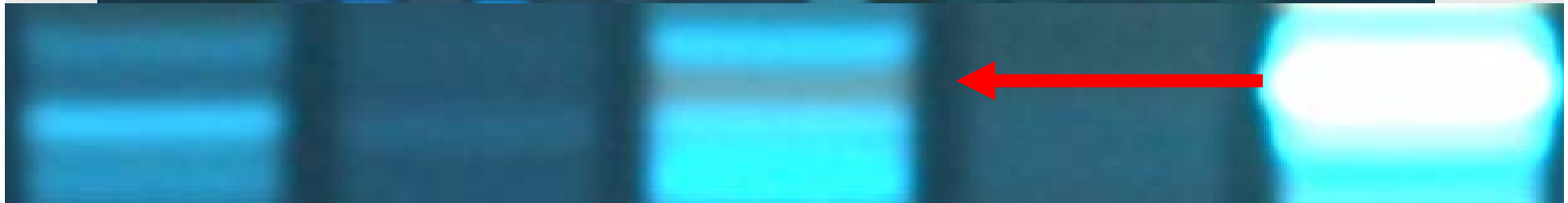
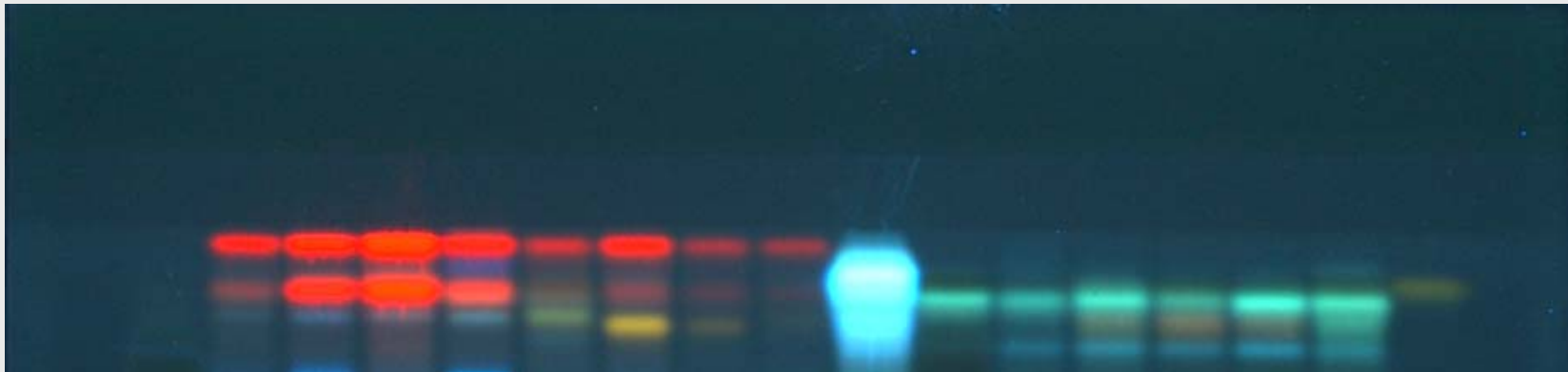
Analysis of Scutellaria lateriflora and Its Adulterants Teucrium canadense and Teucrium chamaedrys by LC-UV/MS, TLC, and



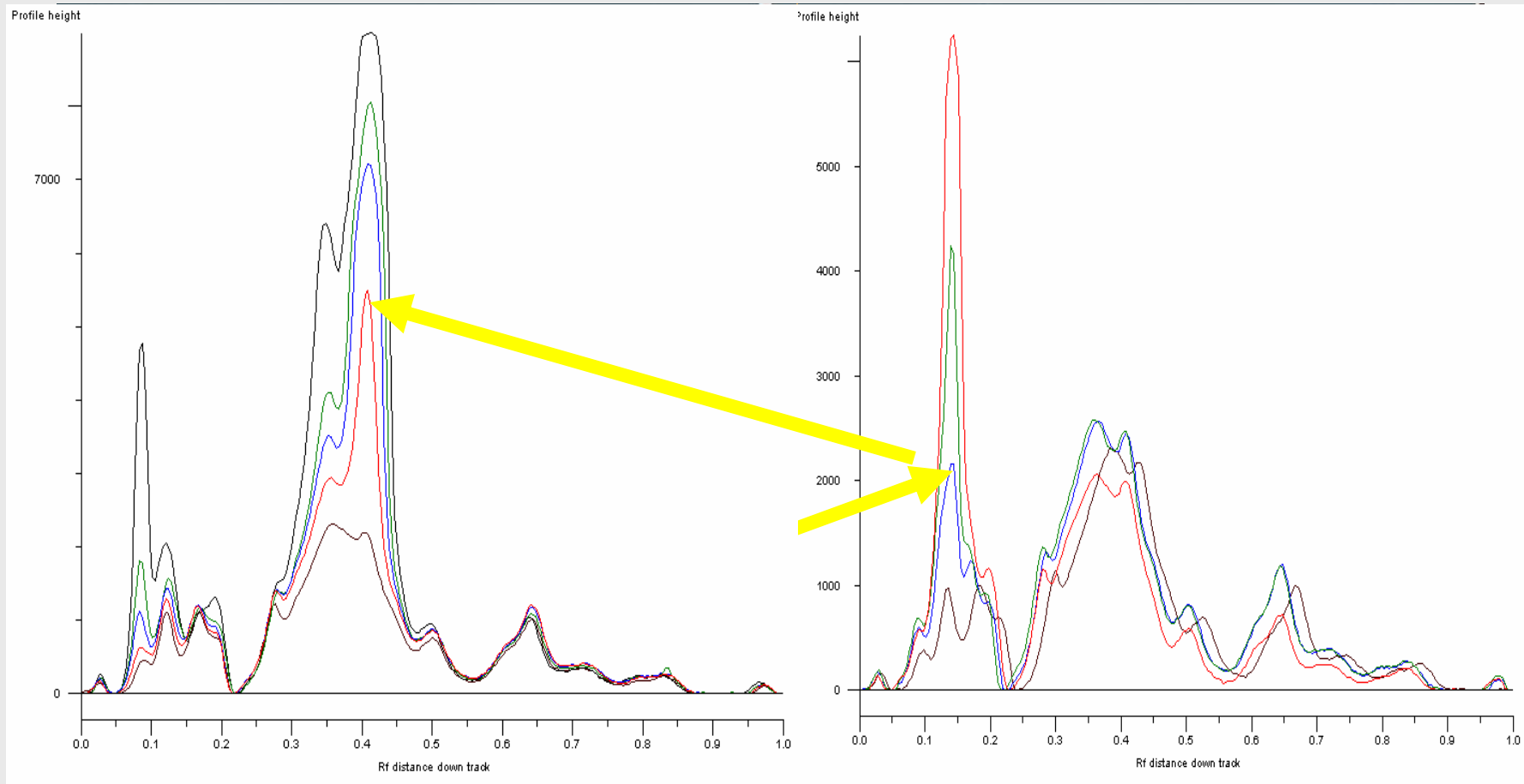
Identification of Skullcap (Gaffner et.al.)



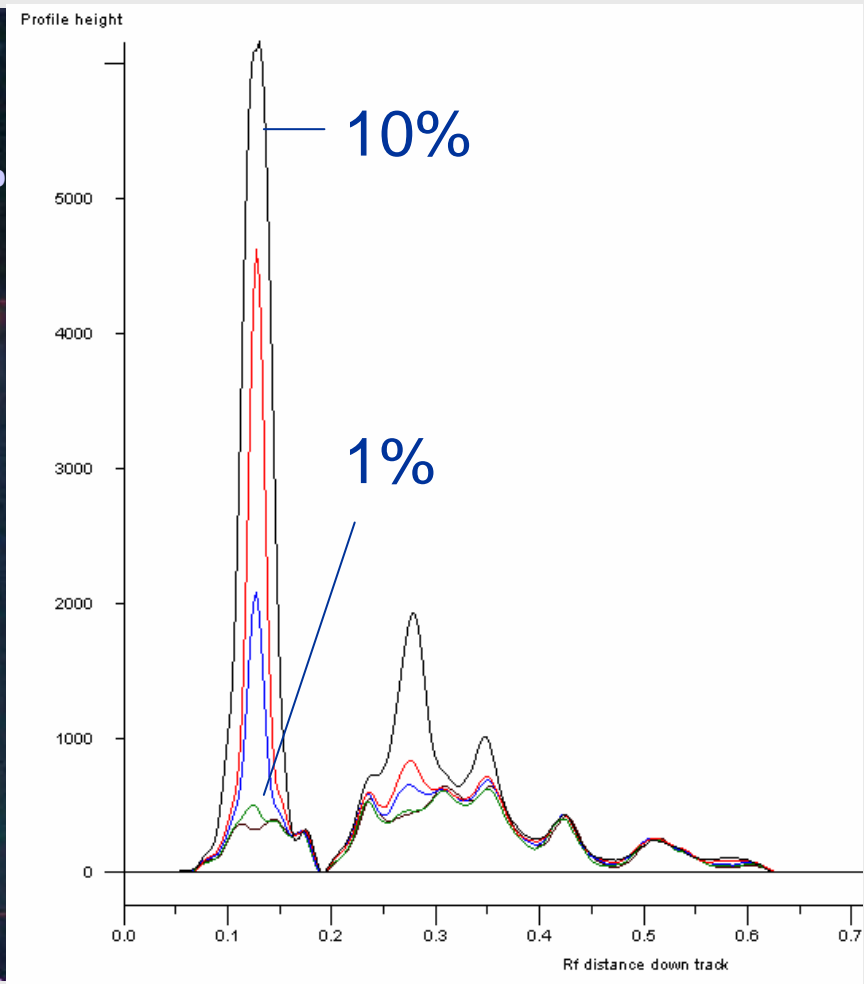
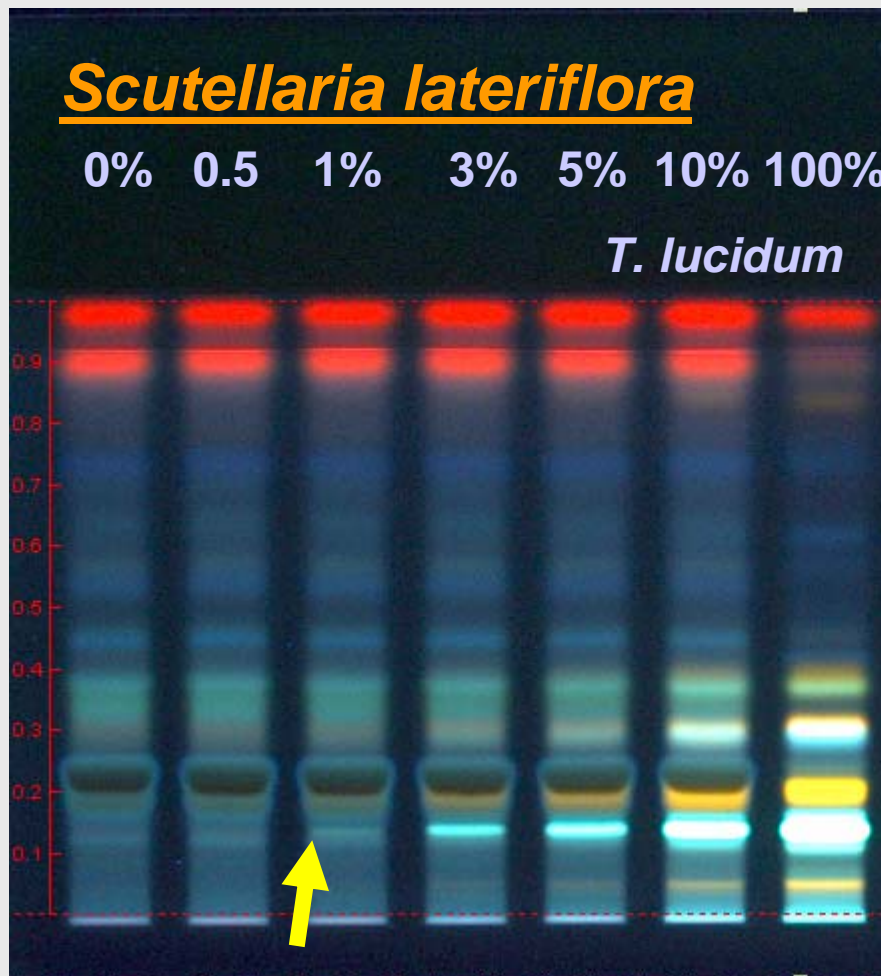
Identification of Skullcap (AHP method)



What about mixtures?



What about mixtures?



Summary

- ▶ Standardization and validation are prerequisites for reliable results.
- ▶ Validated HPTLC methods are complementary to more sophisticated techniques.
- ▶ They allow identification of pure samples and mixtures with certainty.
- ▶ HPTLC is a very flexible and inexpensive analytical tool.

Questions?

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