

HPTLC

Qualitative applications

Valeria Widmer
CAMAG Laboratory

Fields of interest

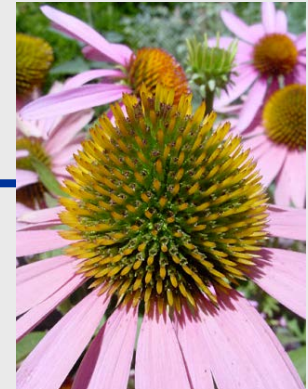
- ▶ Food
 - Sweeteners
 - Colors
 - Lipids
 - Spices
- ▶ Botanicals
 - 10 ID methods
 - Adulteration
 - Stability tests
- ▶ Environmental
 - PAH
 - Bio-Detection
- ▶ Cosmetics
 - Skin lipids

Identification - Echinacea species

E. purpurea root



Root powder



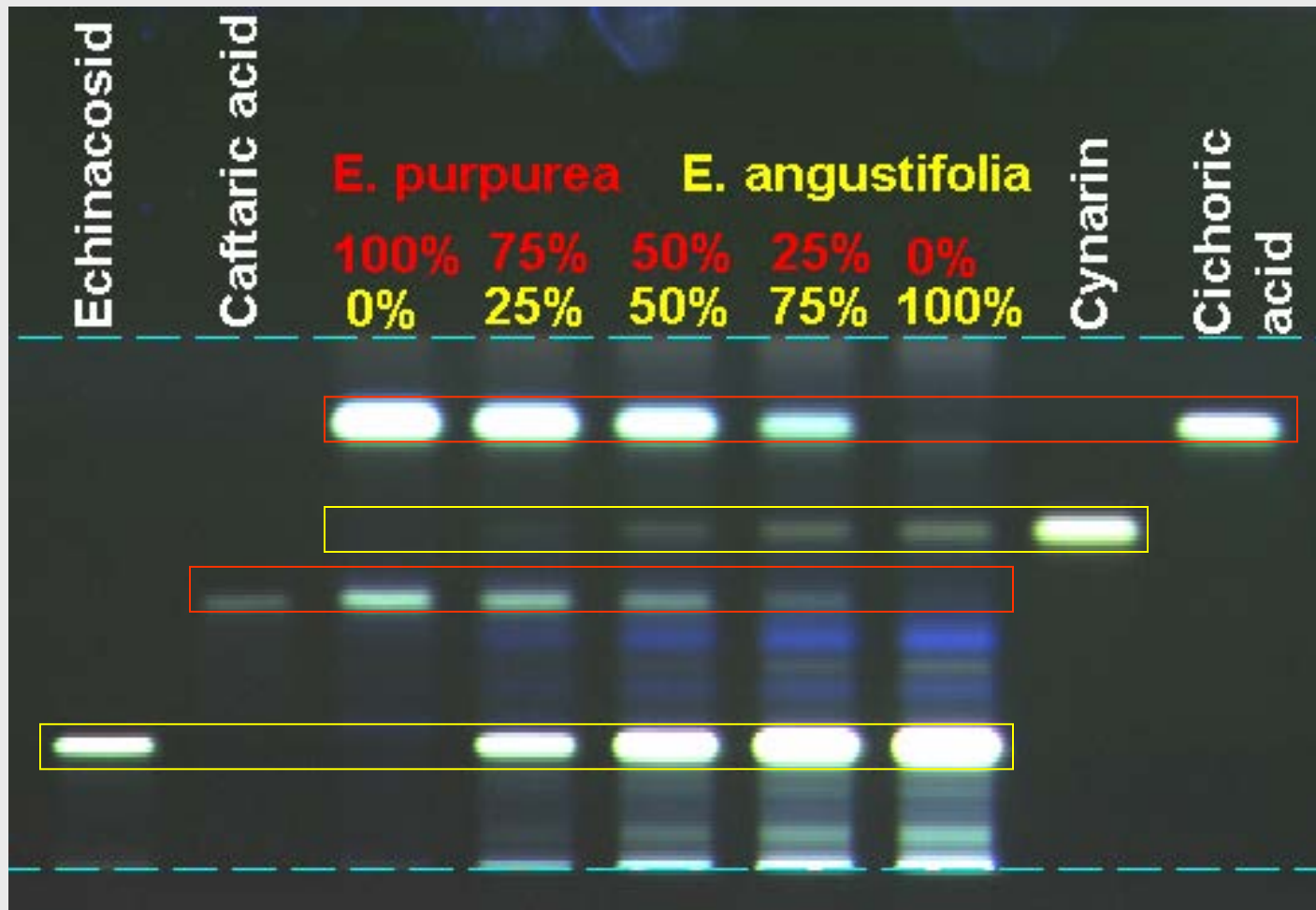
Botanical drugs



E. angustifolia root

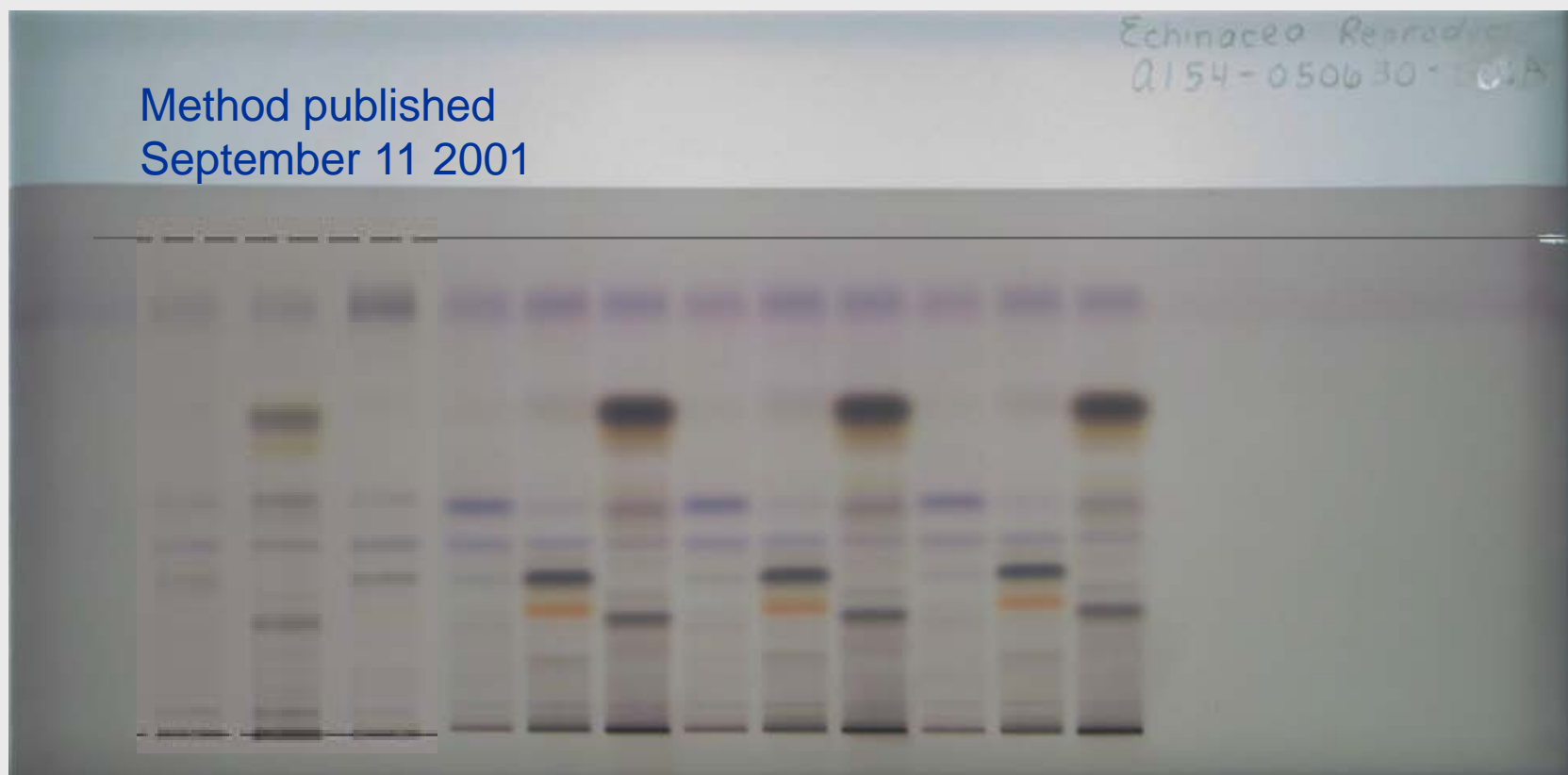


Echinacea - Identification of raw material



Successful standardization – *Echinacea*

May 06, 2005 – CSI Laboratory



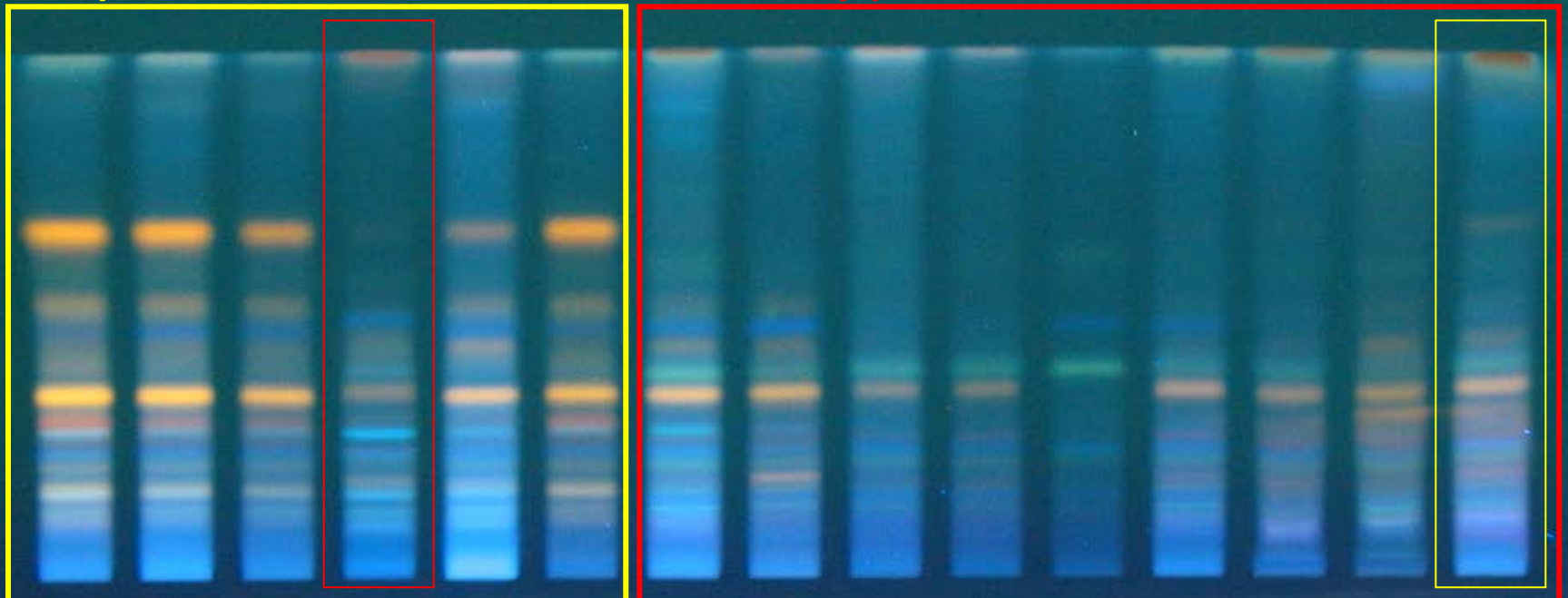
Differentiation of Red Pepper and Paprika

Flavonoids

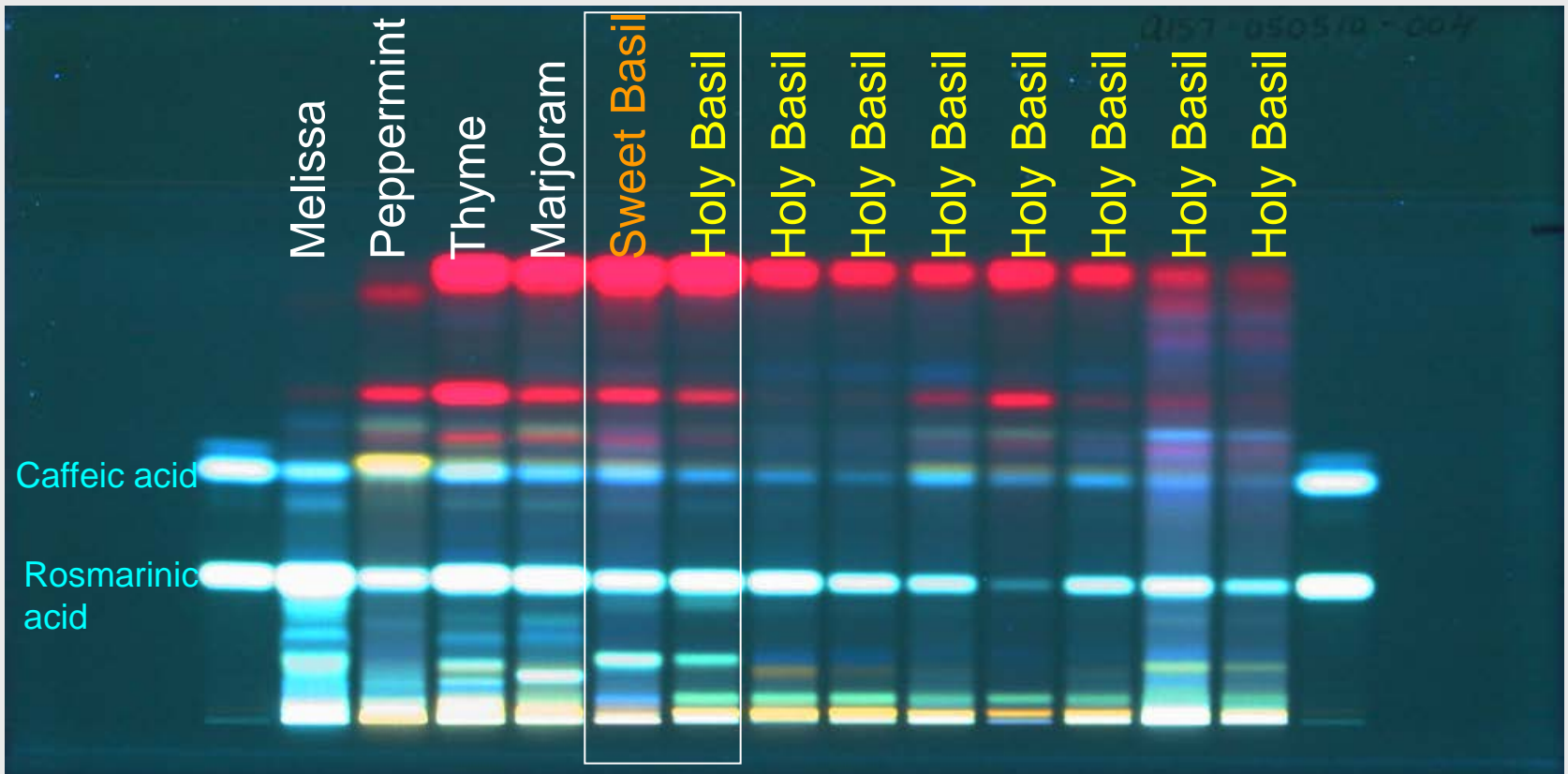


Paprika

Red Pepper

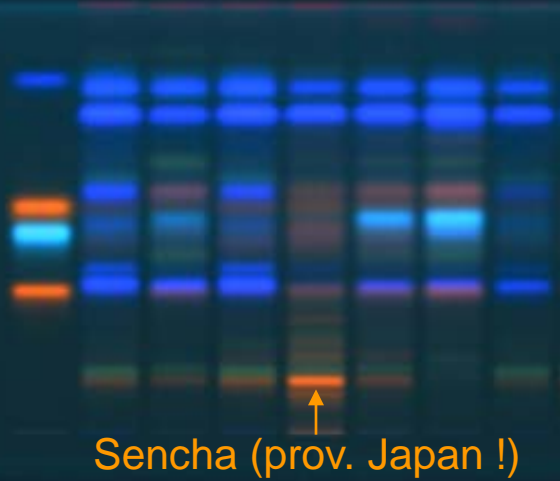


Meaningful fingerprint? No!

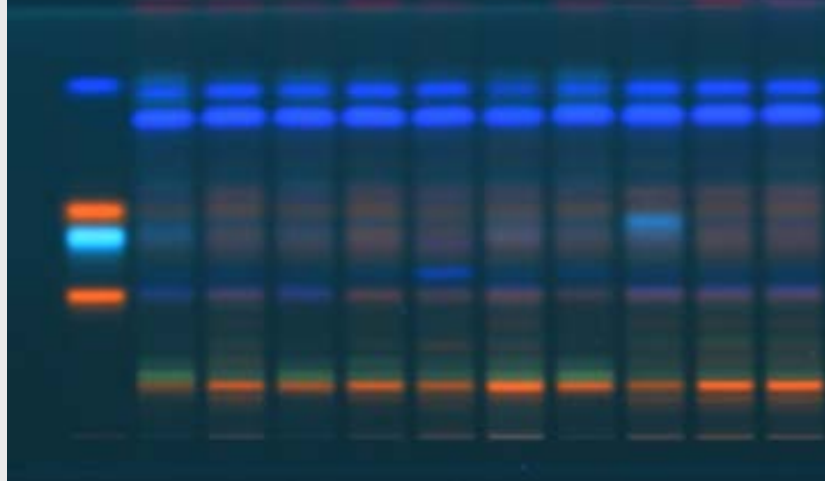


Green tea???

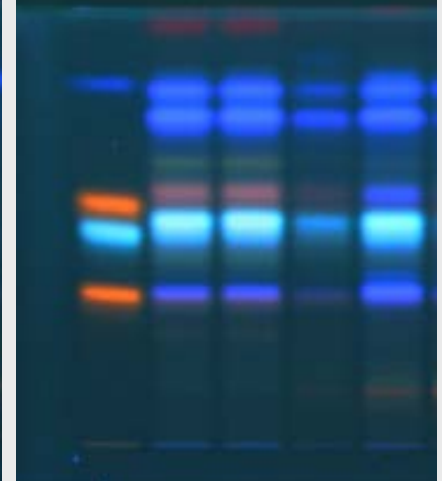
Green tea from
China



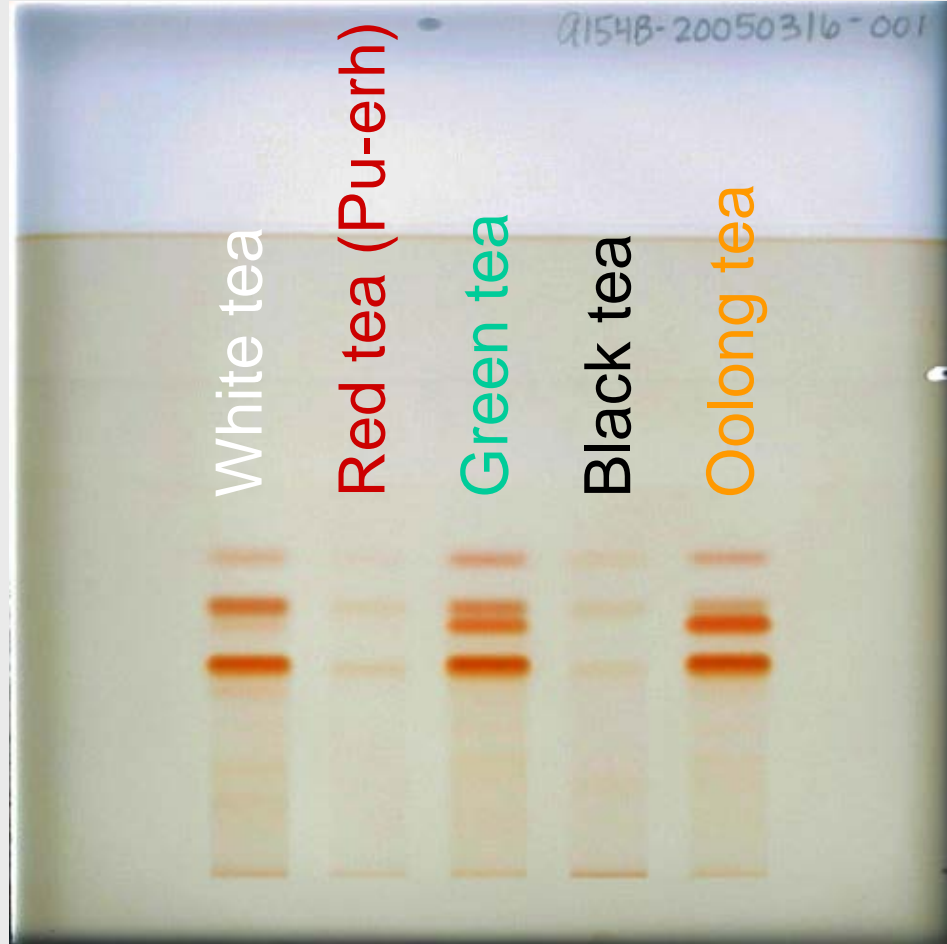
Green tea from Japan



Green tea
from India



Green tea???

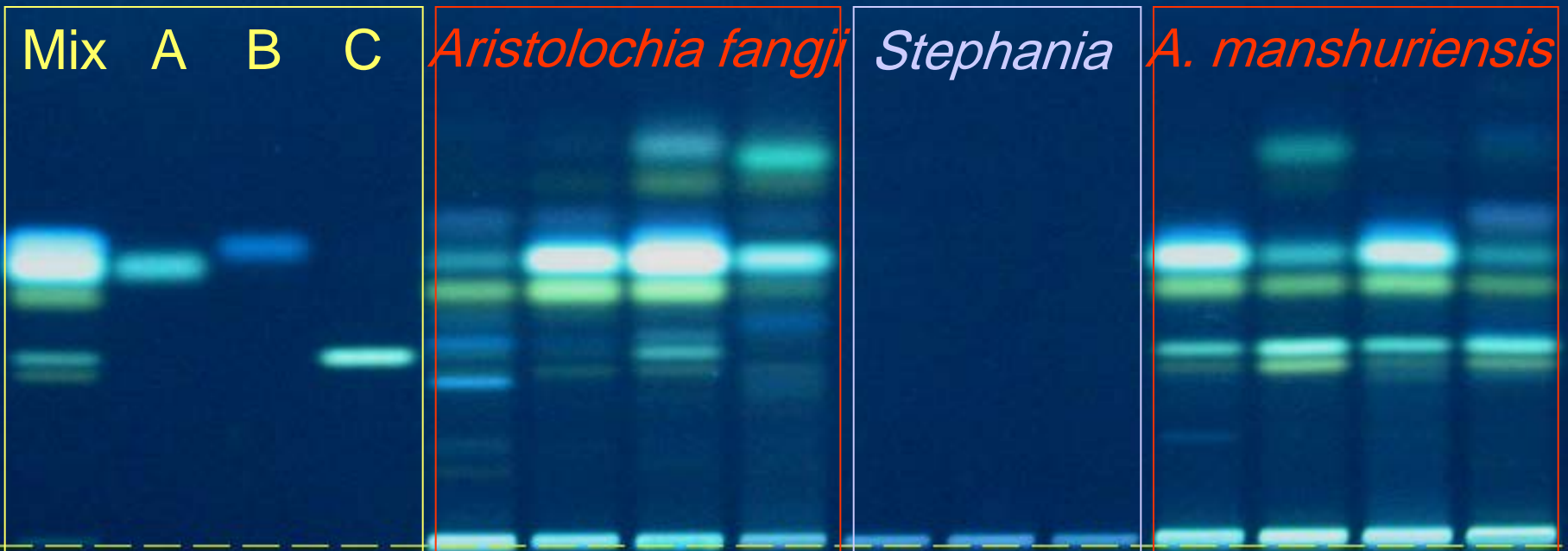


Chinese plants associated with Aristolochic acids

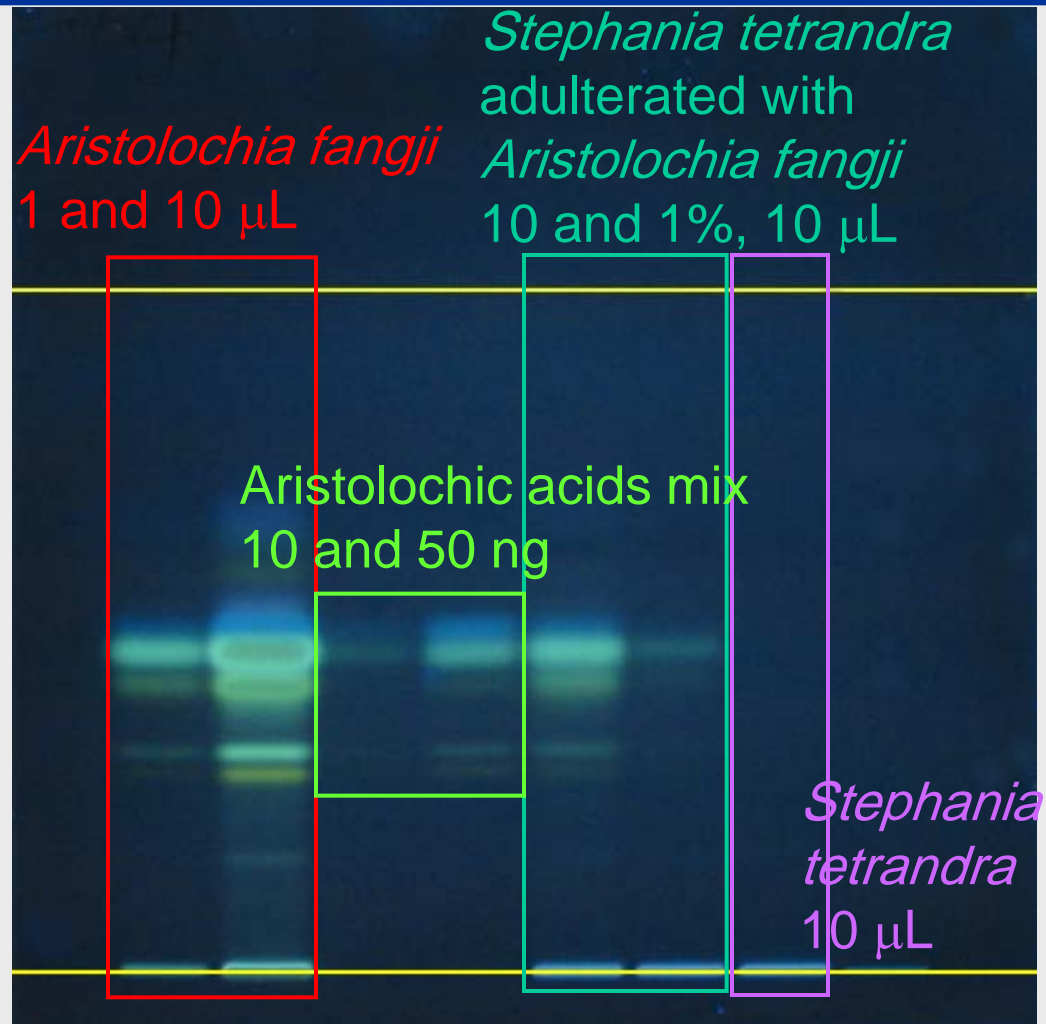
- ▶ Guang fangji, Fangji, Han fangji, Fen fangji, Mu fangji
- ▶ Guan mutong, Mutong, Bei mutong, Chuan mutong, Xiao mutong
- ▶ Qing muxiang, Muxiang, Guang muxiang, Chuan muxiang
- ▶ Xixin, Liao xixin, Bei xixin, Hua xixin
- ▶ *Aristolochia fangji*, *Stephania tetrandra*, *Cocculus sp.*
- ▶ *Aristolochia manshuriensis*, *Akebia trifoliata*, *Clematis armandi*
- ▶ *Aristolochia debilis*, *Saussurea costus* (*Aucklandia lappa*), *Vladimiria soulei*
- ▶ *Asarum heterotropoides*, *Asarum sieboldii*

12

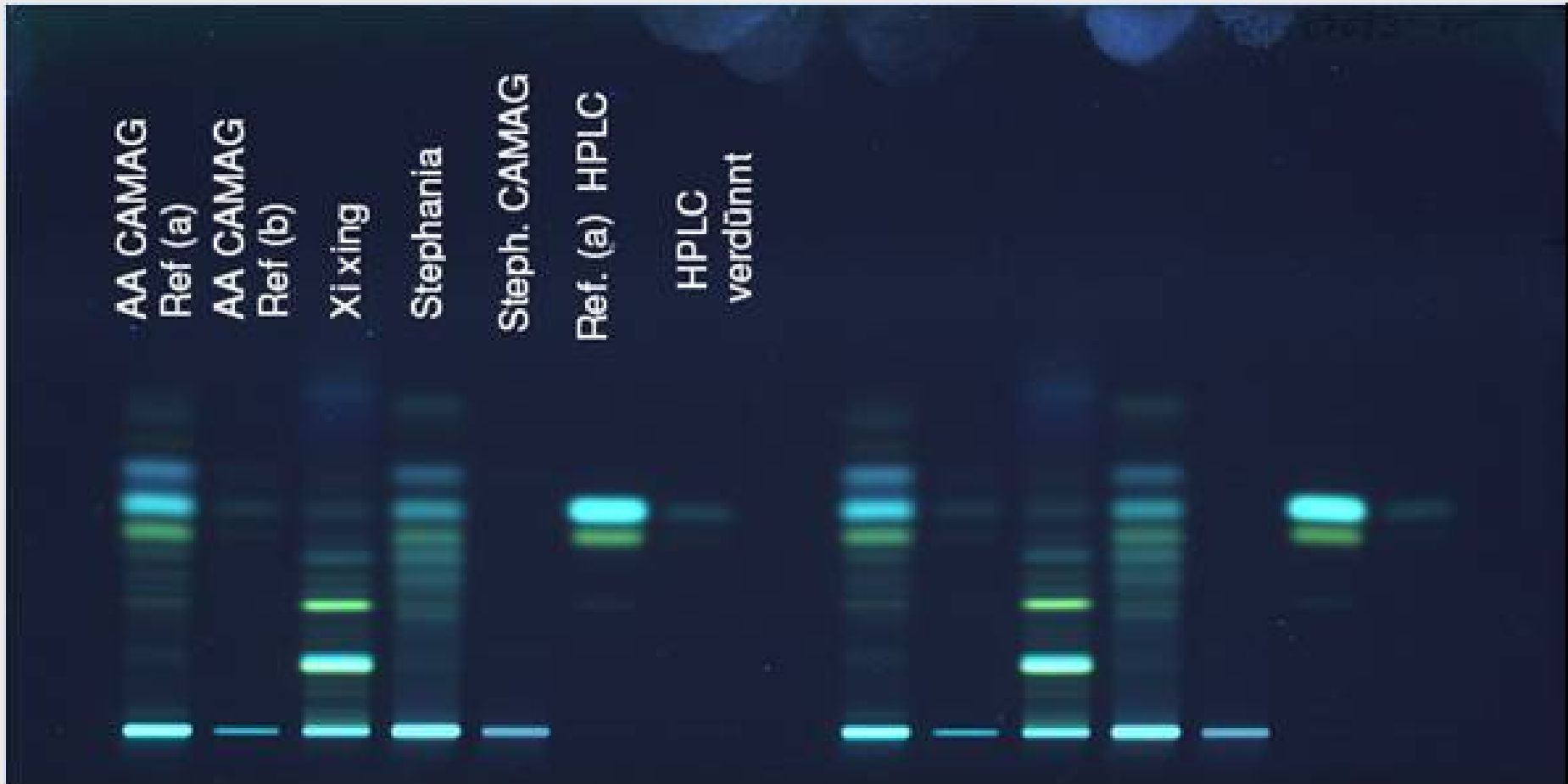
Aristolochic acids as markers of toxic plants in TCM



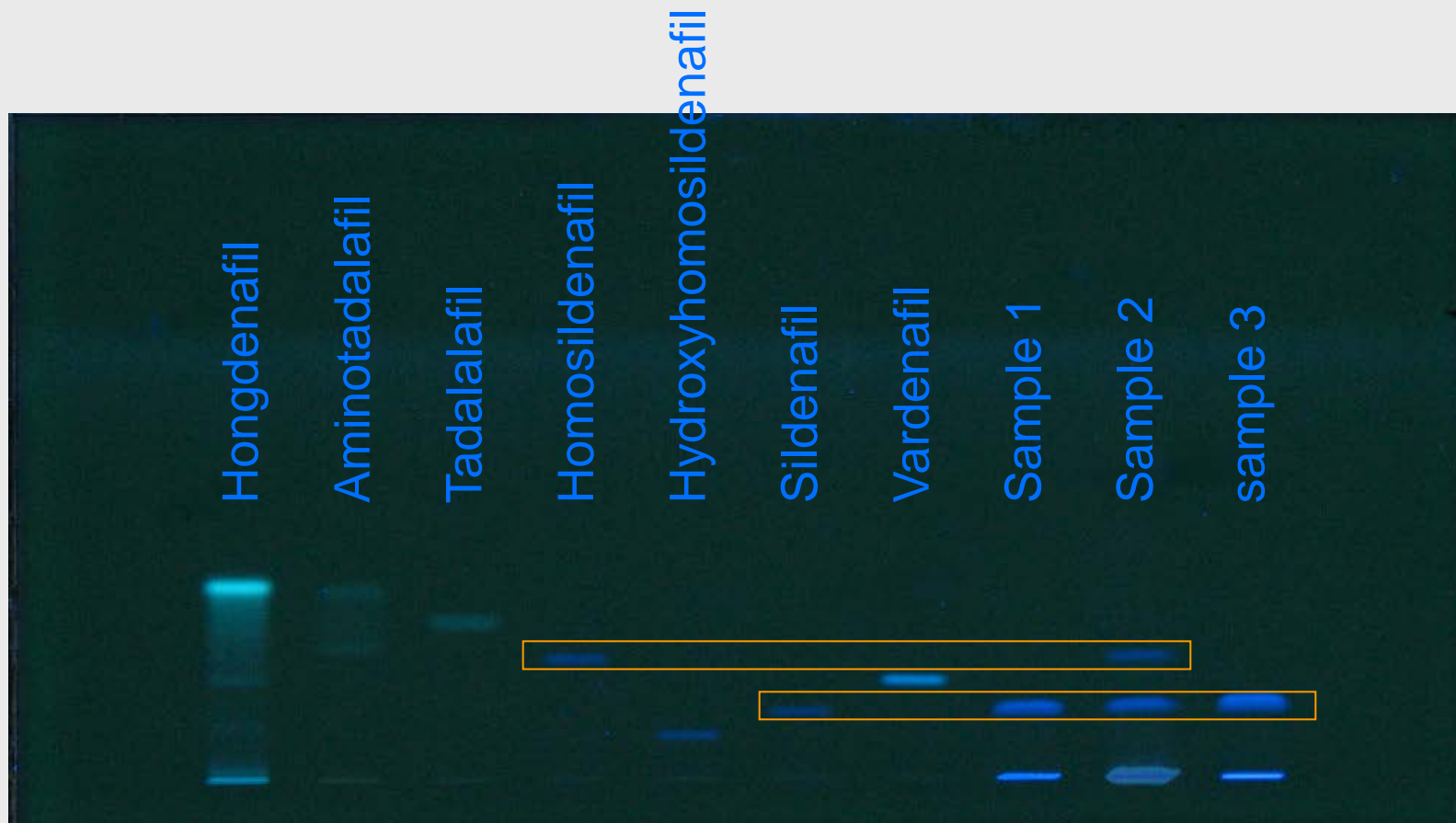
Detection of mixtures



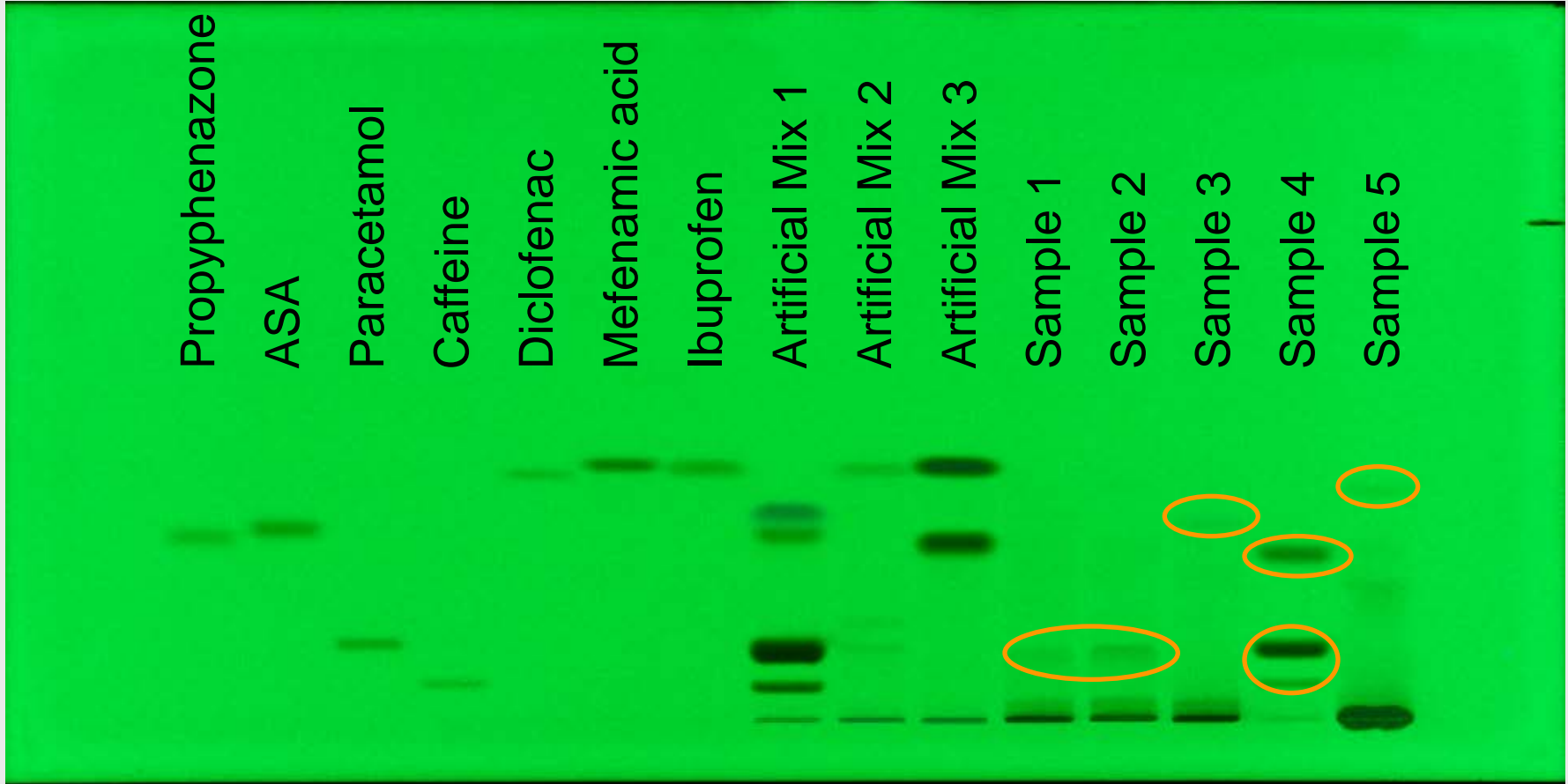
Now to become official test for Ph.Eur.



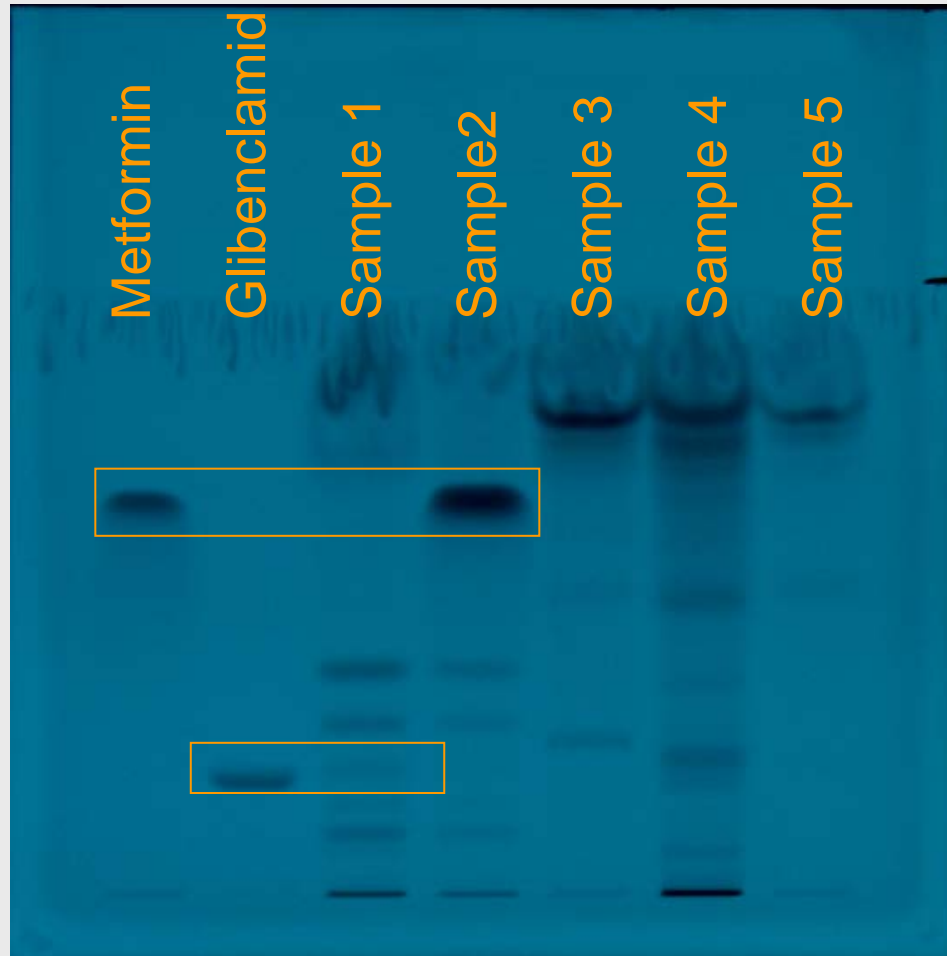
Synthetic drugs in TCM: Erectile Dysfunction



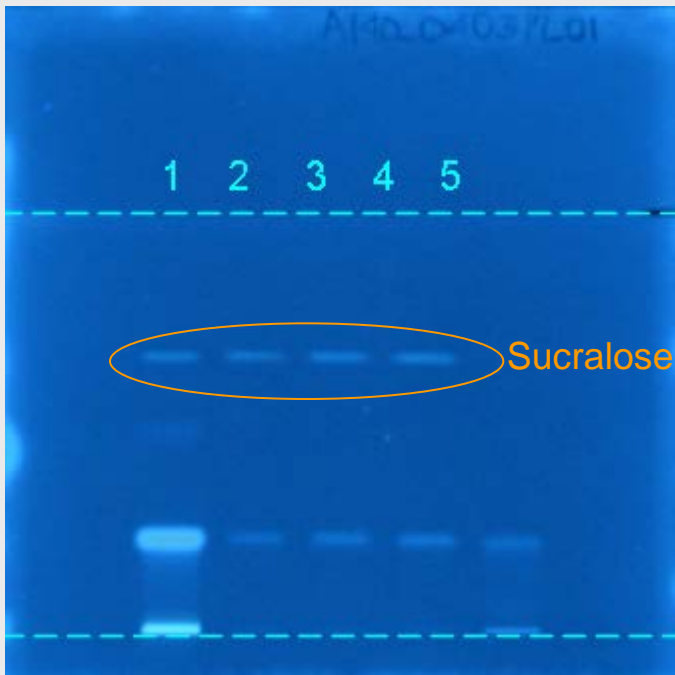
Synthetic drugs in TCM: Pain killers



Synthetic drugs in TCM: Antidiabetics



Sucralose in beverages



Analytical challenge

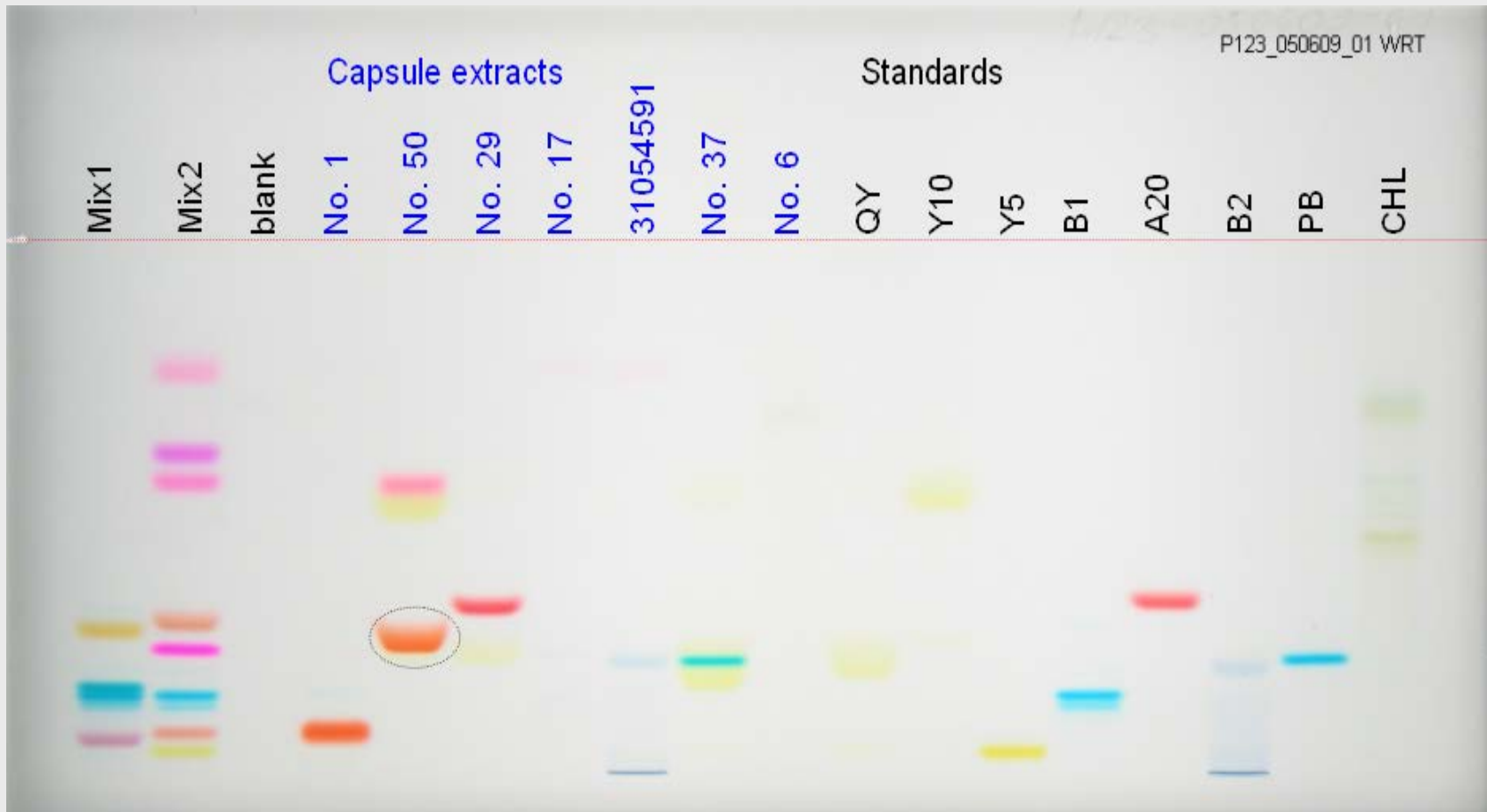
- ▶ Sucralose shows no UV absorbance. Detection for HPLC has to be performed by derivatization, refractive index, amperometric etc.
→ HPTLC is the method of choice: the amino phase allows direct derivatization by heating

- 1: Neat beverage (sucralose is in the calibration range)
2 - 4: Fructose ($R_f=0.3$) and sucralose ($R_f=0.7$)
5: Diluted beverage (fructose is in the calibration range)

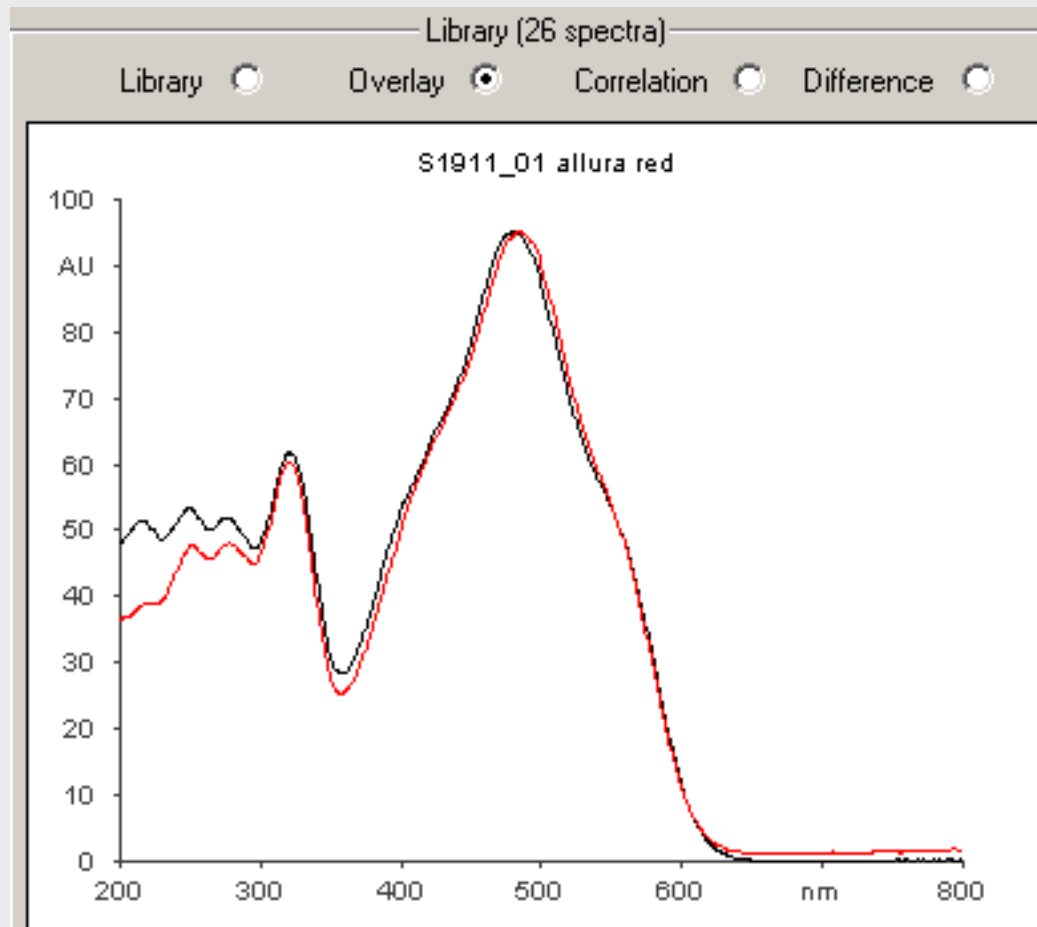
CAMAG Application Note A-83.1

Determination of sucralose and fructose in food and beverages

Analysis of food colors from capsules

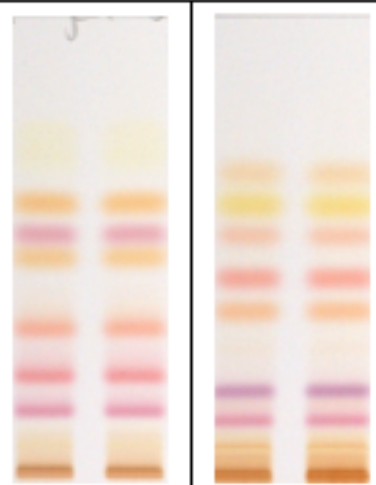


Analysis of food colors from capsules



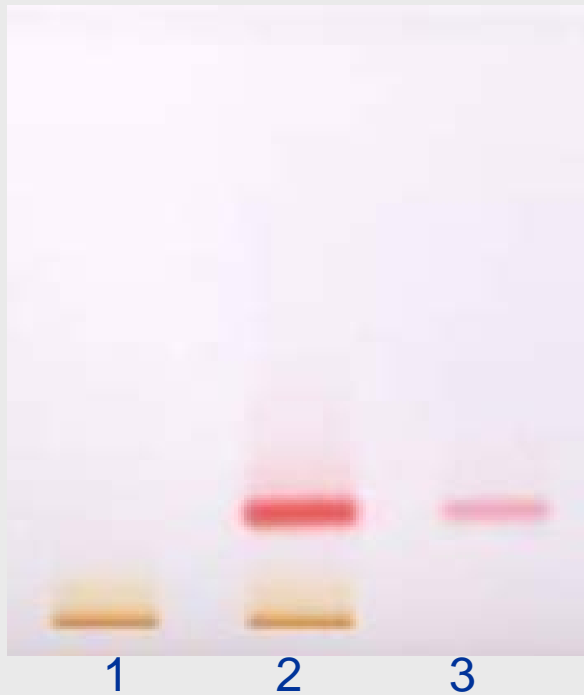
Illegal dyes in spices

► Screening of 15 illegal dyes

Mix 1	R _F	Relative R _F			Relative R _F	R _F	Mix 2
Para Red	0.60	1.22			1.38	0.66	Disp. Orange 11
Citrus Red 2	0.54	1.10			1.23	0.59	Butter Yellow
Sudan I*	0.49	1.00			1.10	0.53	Toluidine Red
Sudan II	0.33	0.67			1.00	0.48	Sudan Red G*
Sudan III	0.23	0.47			0.83	0.40	FD&C Orange 2
Sudan IV	0.16	0.33			0.44	0.21	Sudan Red 7B
-	-	-			0.31	0.15	Sudan Red B

Illegal dyes in spices

Analytical challenges



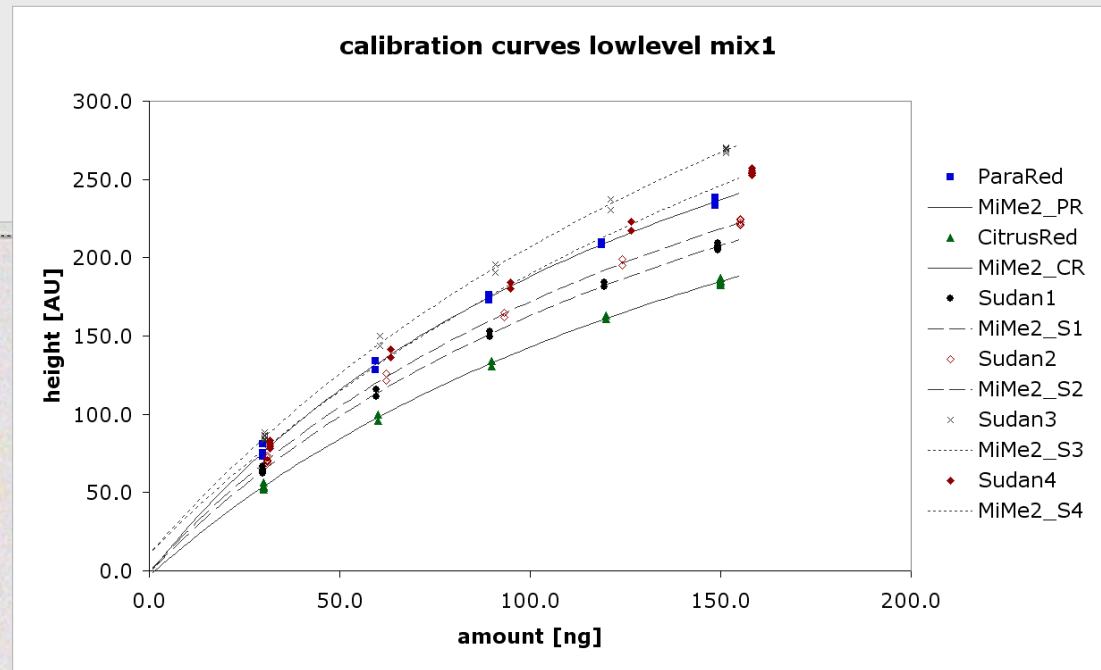
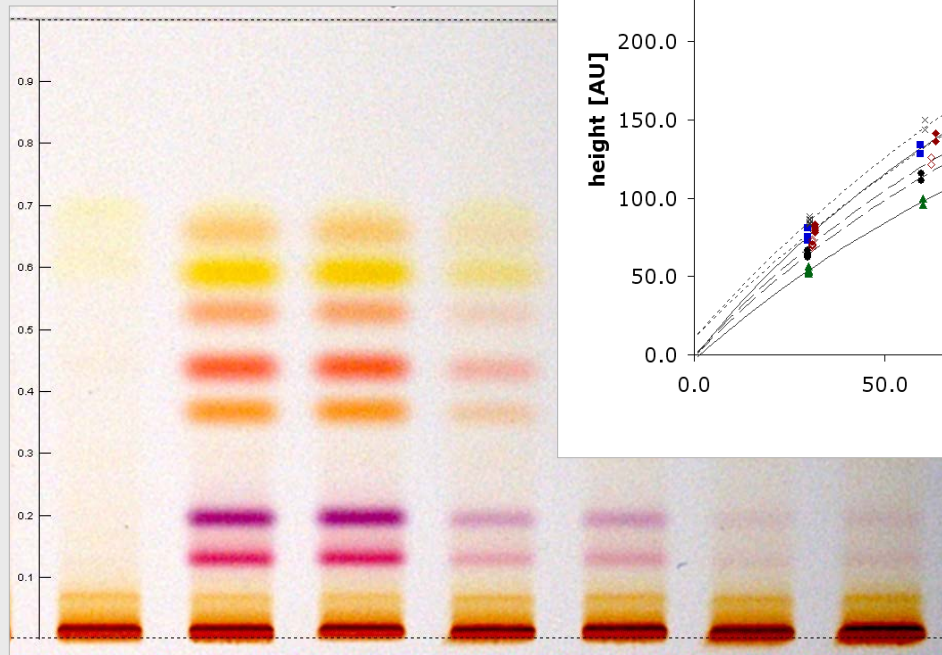
1: Paprika extract

2: Paprika spiked with Sudan III

3: Sudan III

- ▶ A broad range of illegal dyes with different chemical properties shall be detected
→ TLC is the method of choice
- ▶ Interferences by sample matrix and natural dyes (carotinoids)
→ Selective oxidation of carotinoids

Illegal dyes in spices



HPTLC detection of the azo dye amaranth as an adulterant of Bilberry extract

CAMAG Application



Image white light

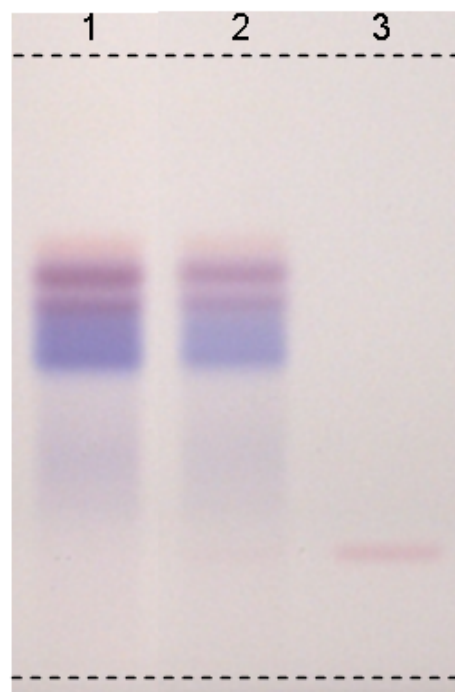
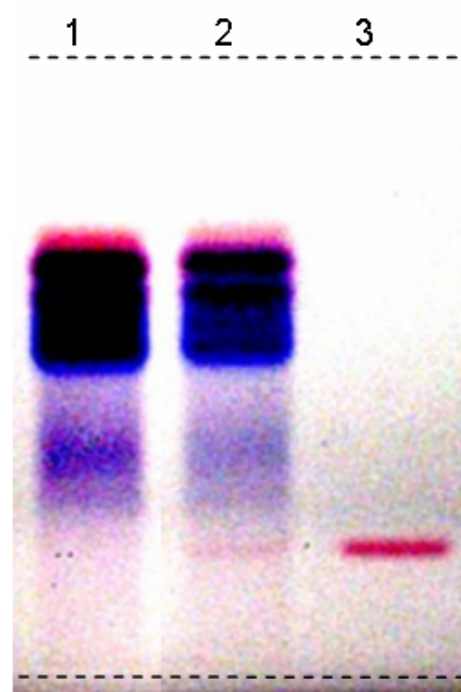
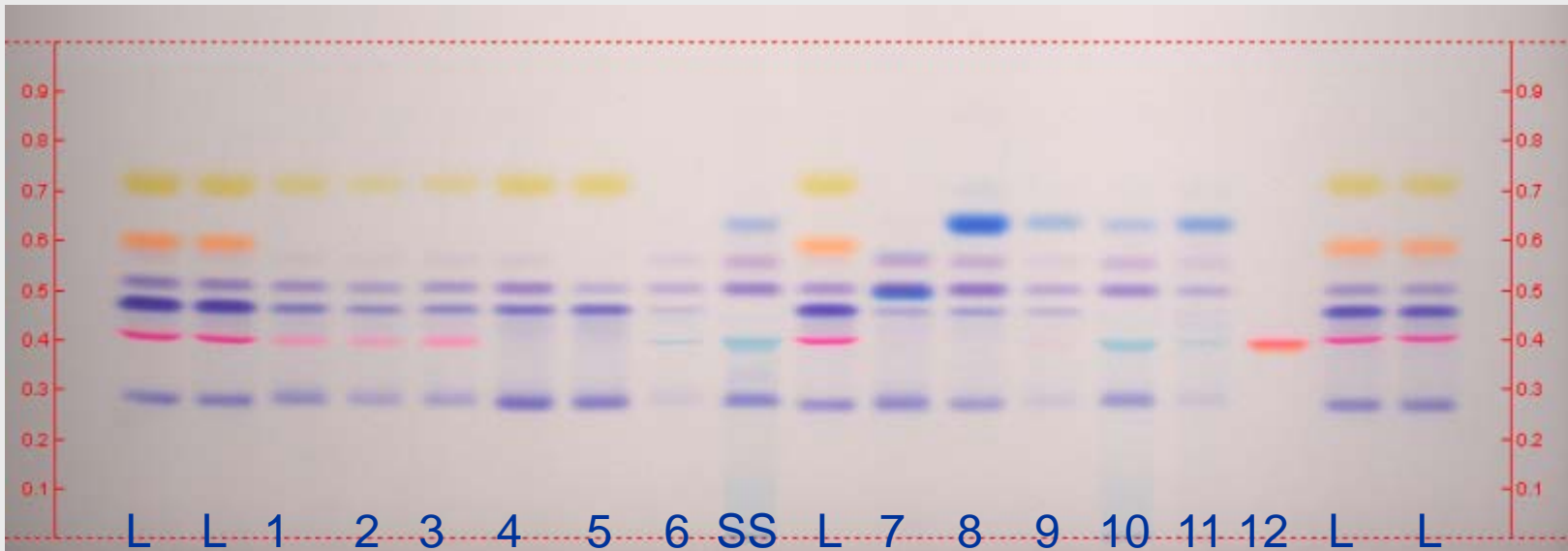


Image white light (enhanced)



- 1: Bilberry dry extract
- 2: Bilberry dry extract spiked with amaranth (spiking level 0.25 %)
- 3: Amaranth

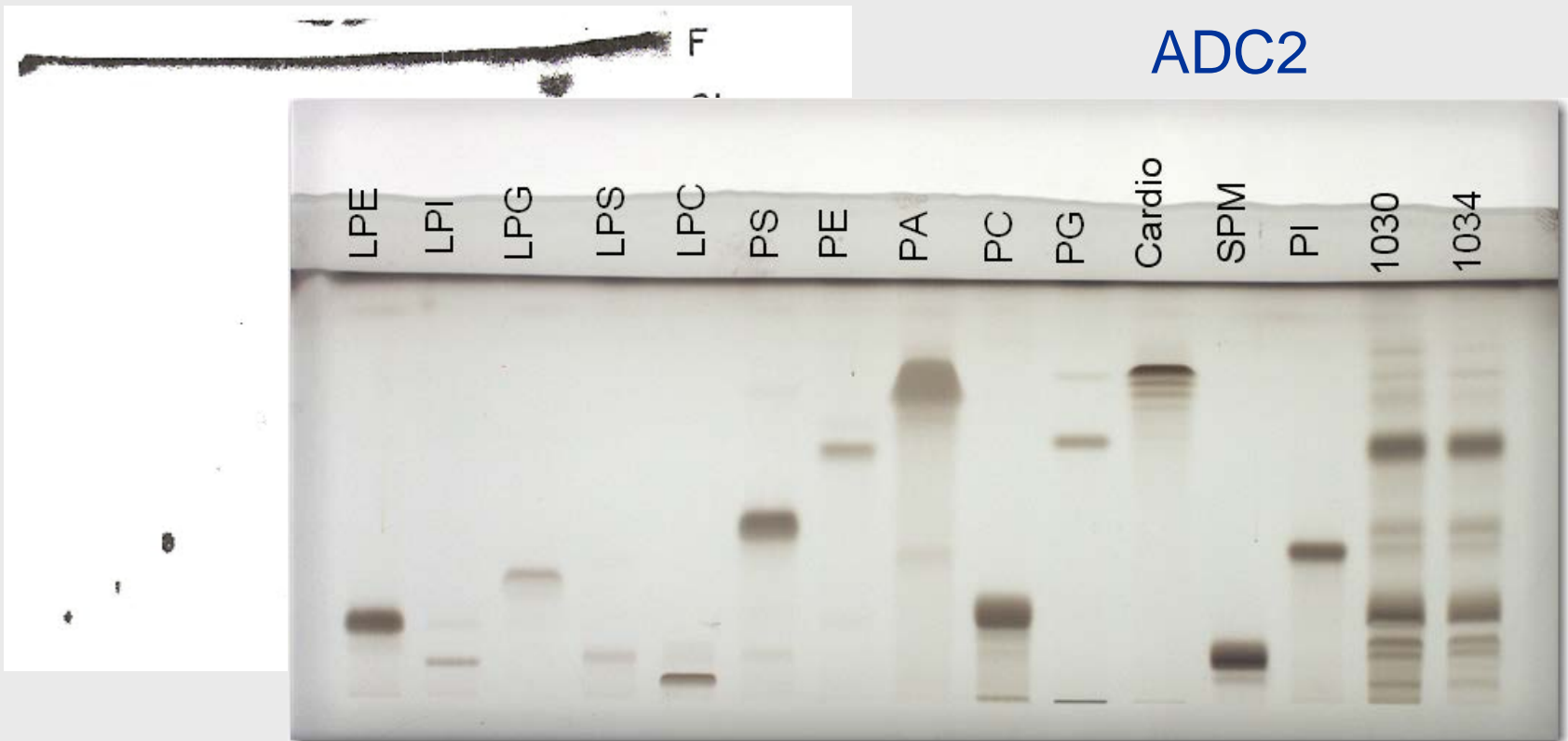
HPTLC analysis of inks



- L: Ladder (Standard mixture)
SS: System suitability test
1-12: Various samples of ball point inks

Phospholipids

Method from Literature



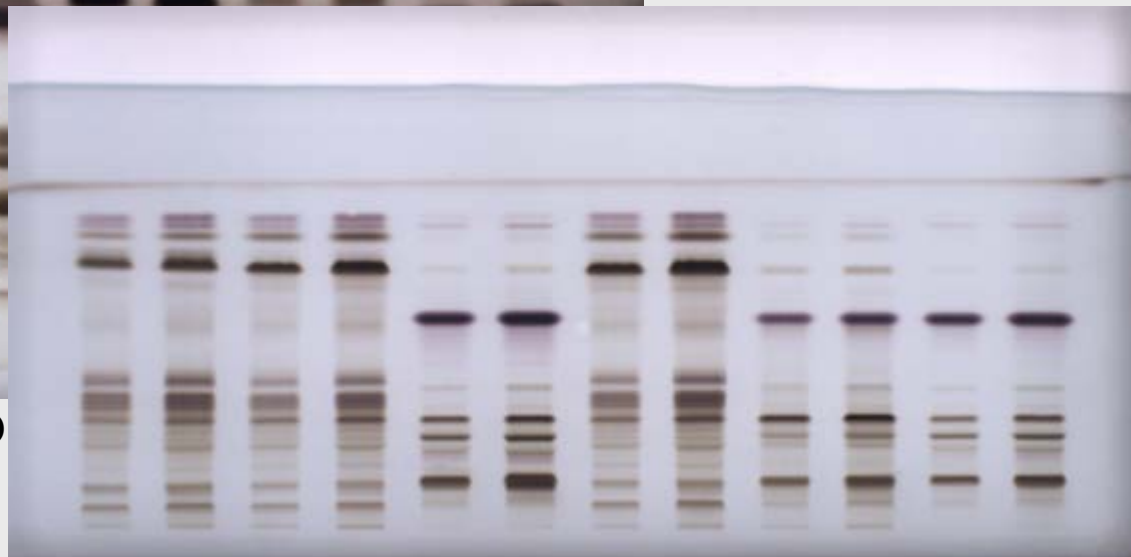
Phospholipids

ADC 2



Mobile Phase: CHCl_3 , MeO
Humidity control (47%)

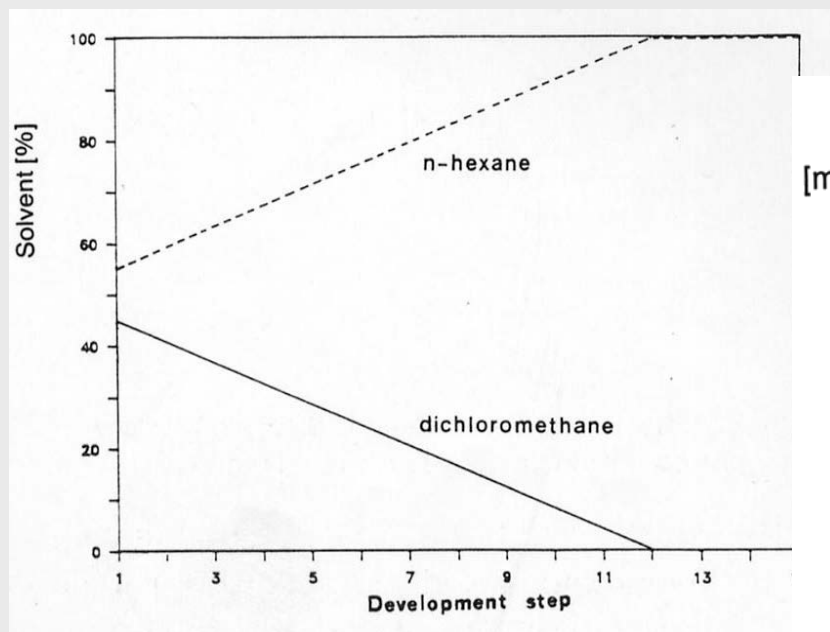
AMD 2



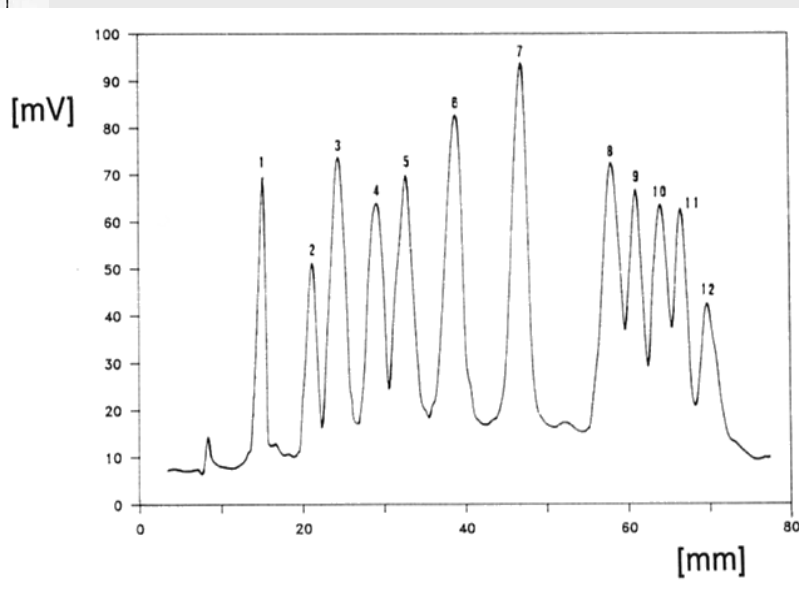
Universal gradient with 20 steps from MeOH/H₂O 98/2 to CHCl_3 to hexane, preconditioned with 1 N NH_3 25%

Organochlorine pesticides by AMD

Methoxychlor, Dieldrin, δ -HCH, Heptachlorepoxide, β -HCH, Lindane, γ -HCH, p,p'-DDT, o,p'-DDE, p,p'-DDE, Aldrin, HCB



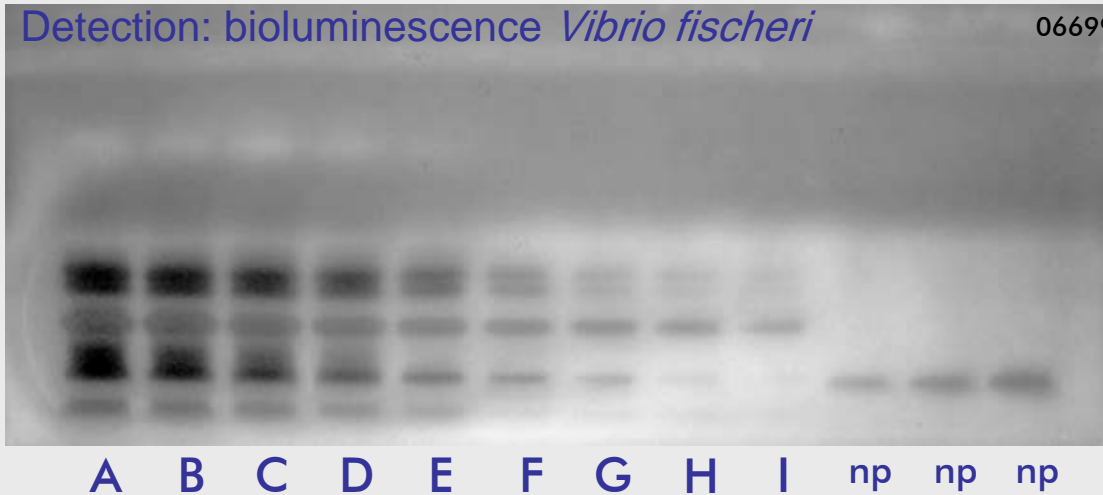
AMD gradient elution



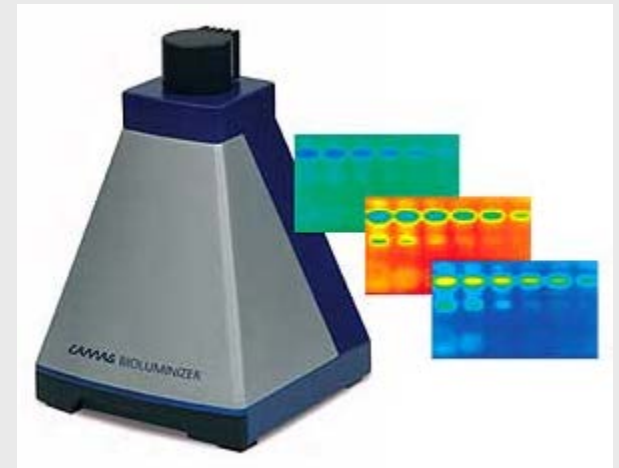
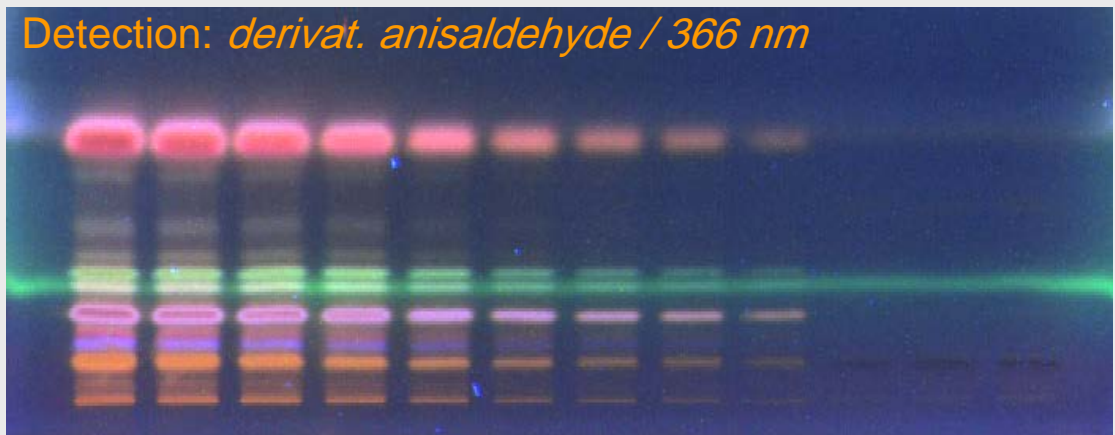
Chromatogram after gradient separation

Bioluminescence – BioLuminizer™

Detection: bioluminescence *Vibrio fischeri* 06699

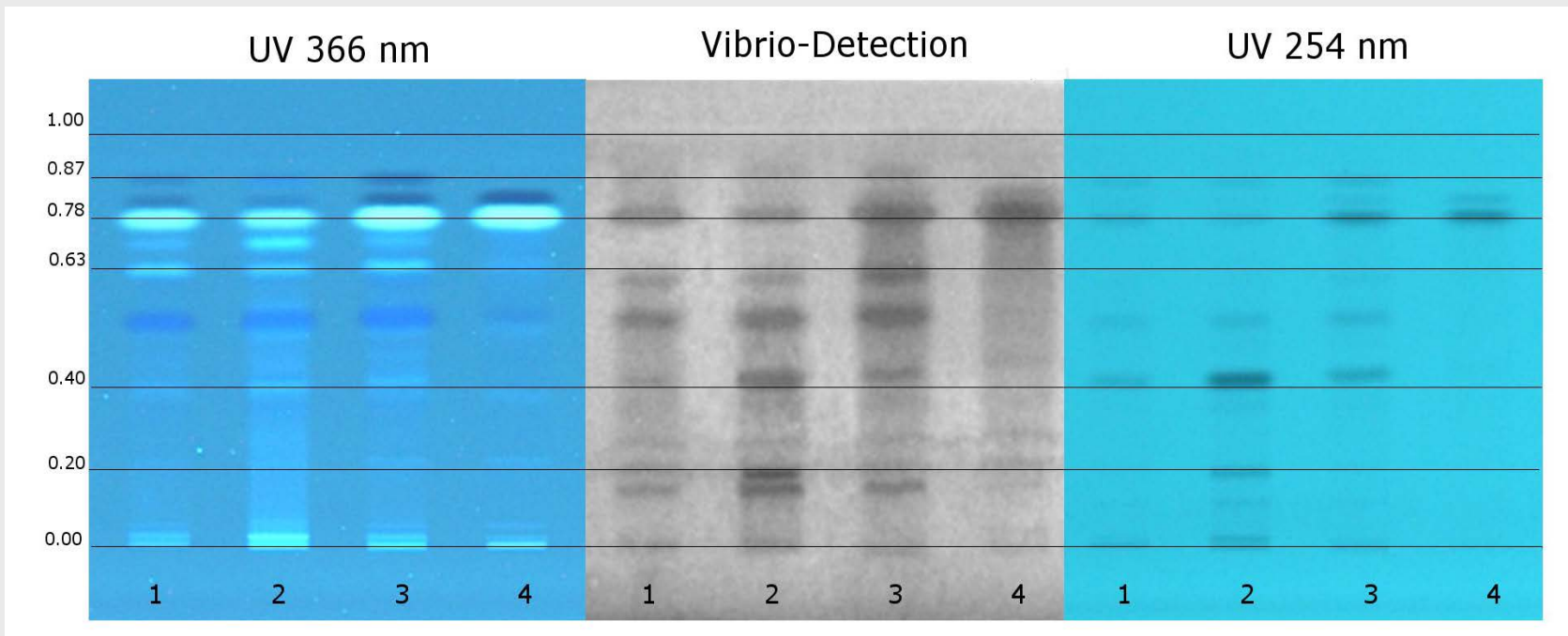


Detection: *derivat. anisaldehyde* / 366 nm



Bioluminescence – BioLuminizer™

Stability of sunscreen



- 1 Sunscreen irradiated on skin
- 2 Sunscreen irradiated in Suntest CPS+
- 3 Sunscreen irradiated on microscopic slide
- 4 Reference (Sunscreen not irradiated, on microscopic slide)

Bioluminescence – BioLuminizer™

