Potential applicability of modern bioautography (BioArena) in the study of plant ingredients

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Direct bioautography

search of compounds having cell proliferation inhibiting/promoting effect

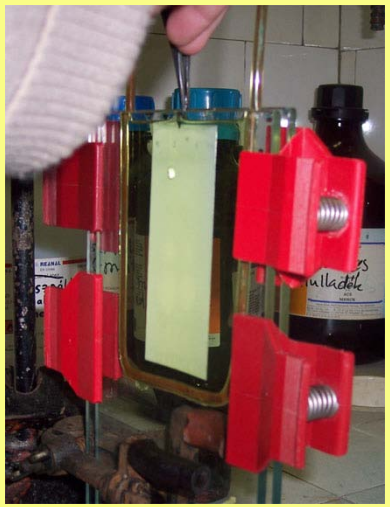
separation in thin layer
(e.g. TLC, HPTLC, OPLC)

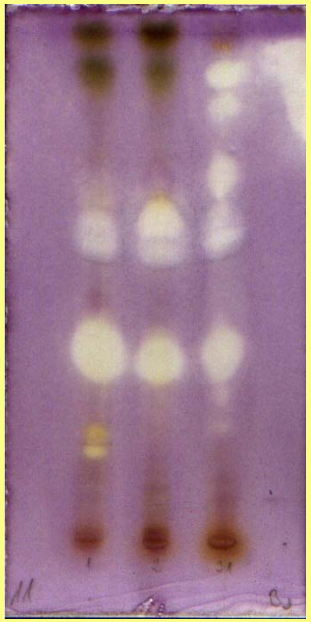
→ „inoculation" incubation → detection

The separation:
Condition of the ads. layer
Appropriate mobile phase
Remove of mobile phase
Advantages of OPLC

The developed adsorbent layer is dipped into the cell suspension or sprayed with it (antibacterial, antifungal etc. effects)

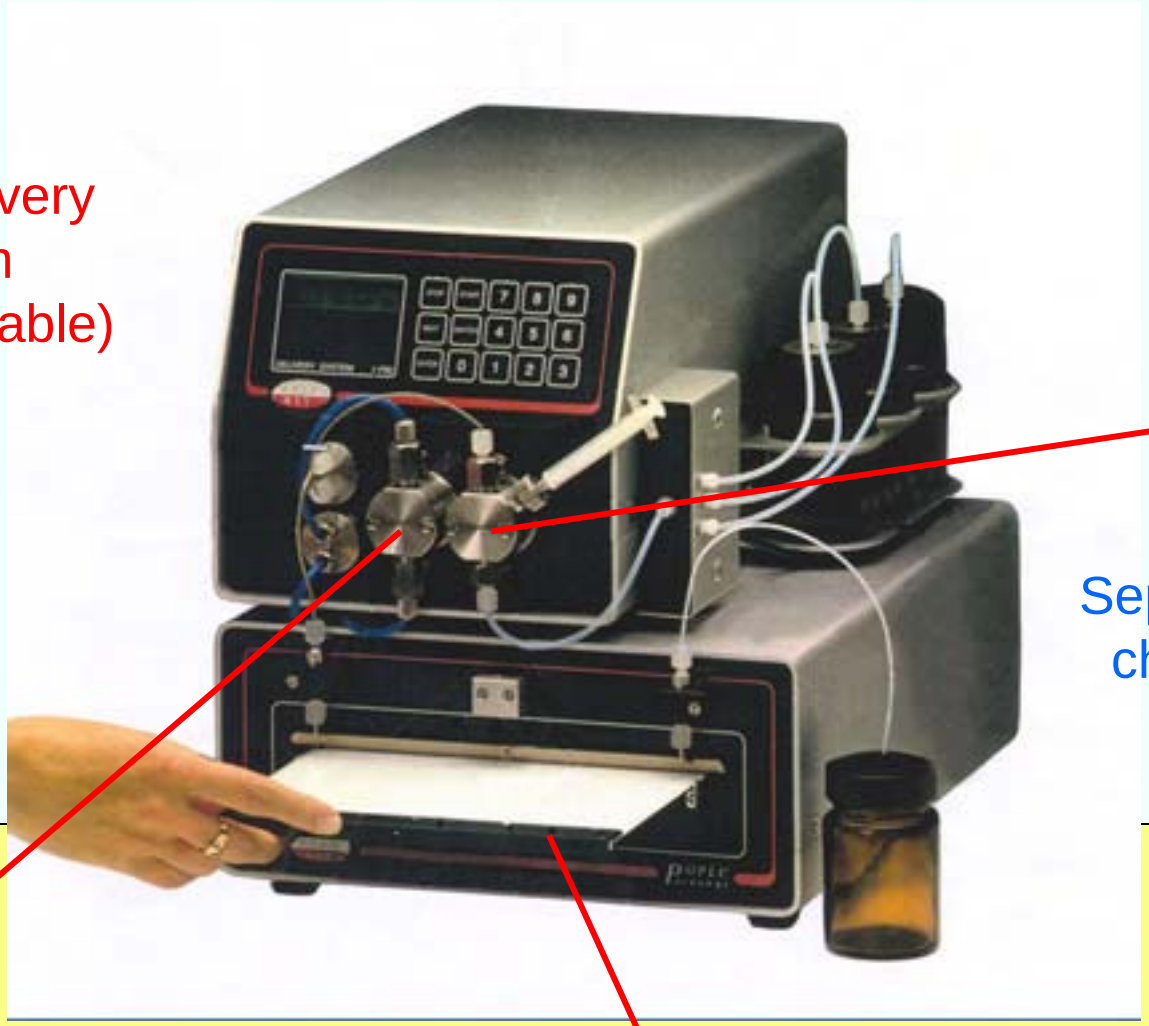
Generally by the use of vital dye (e.g. MTT) clear zones mean antibacterial effect





OPLC 50

Automatized OPLC System



Liquid delivery System (programmable)

Eluent pump

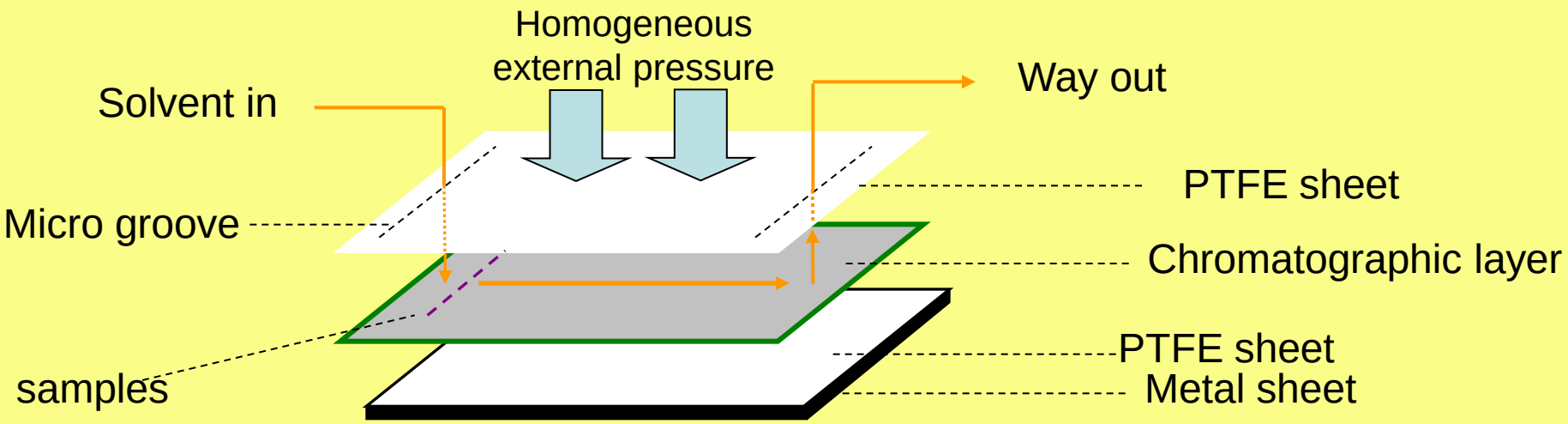
Separation chamber

Hydraulic pump

cassette

Overpressured Layer Chromatography (OPLC)

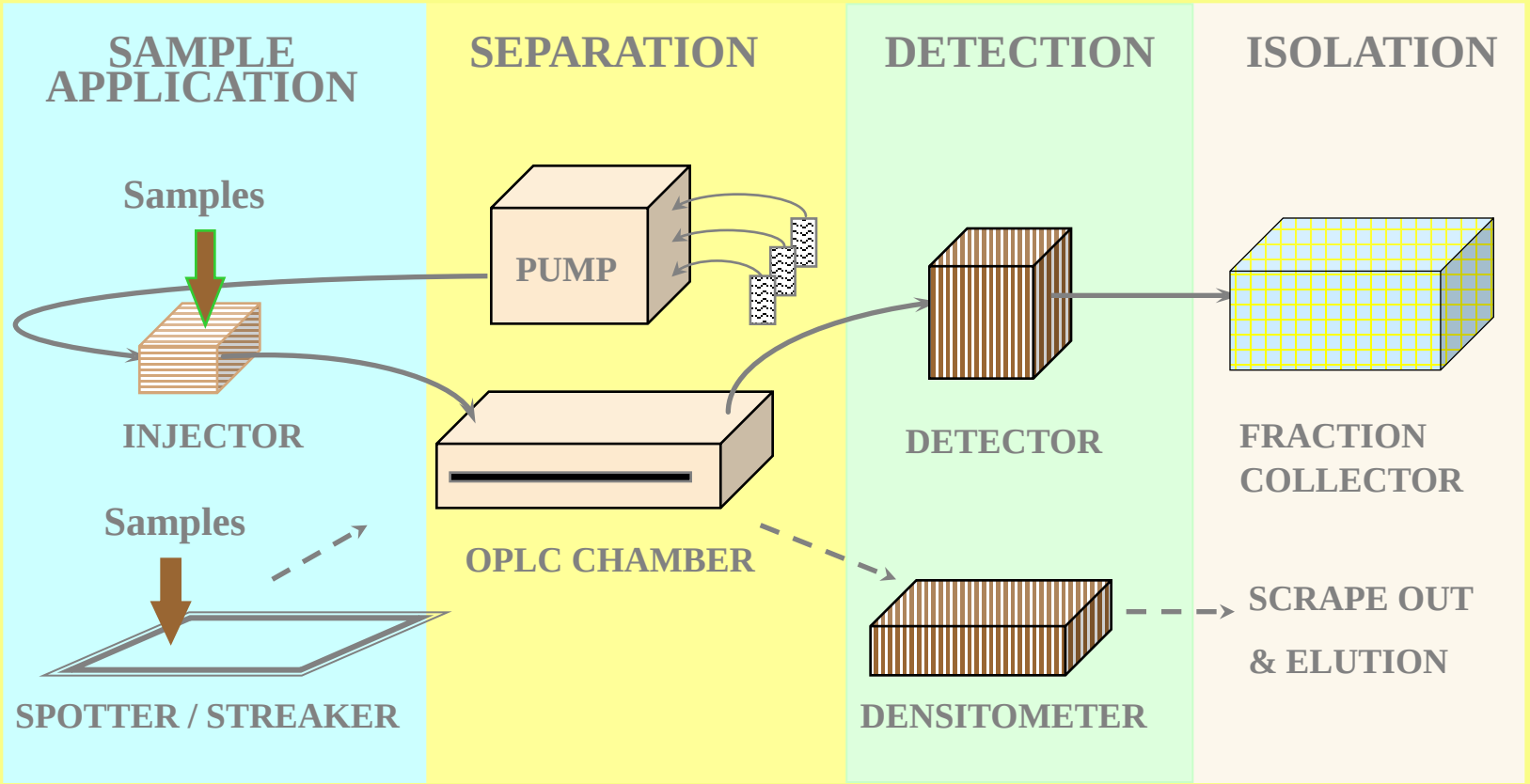
The layer is sealed around and the layer surface is covered by a flexible membrane under pressure; an eluent pump is used to deliver the mobile phase to layer.



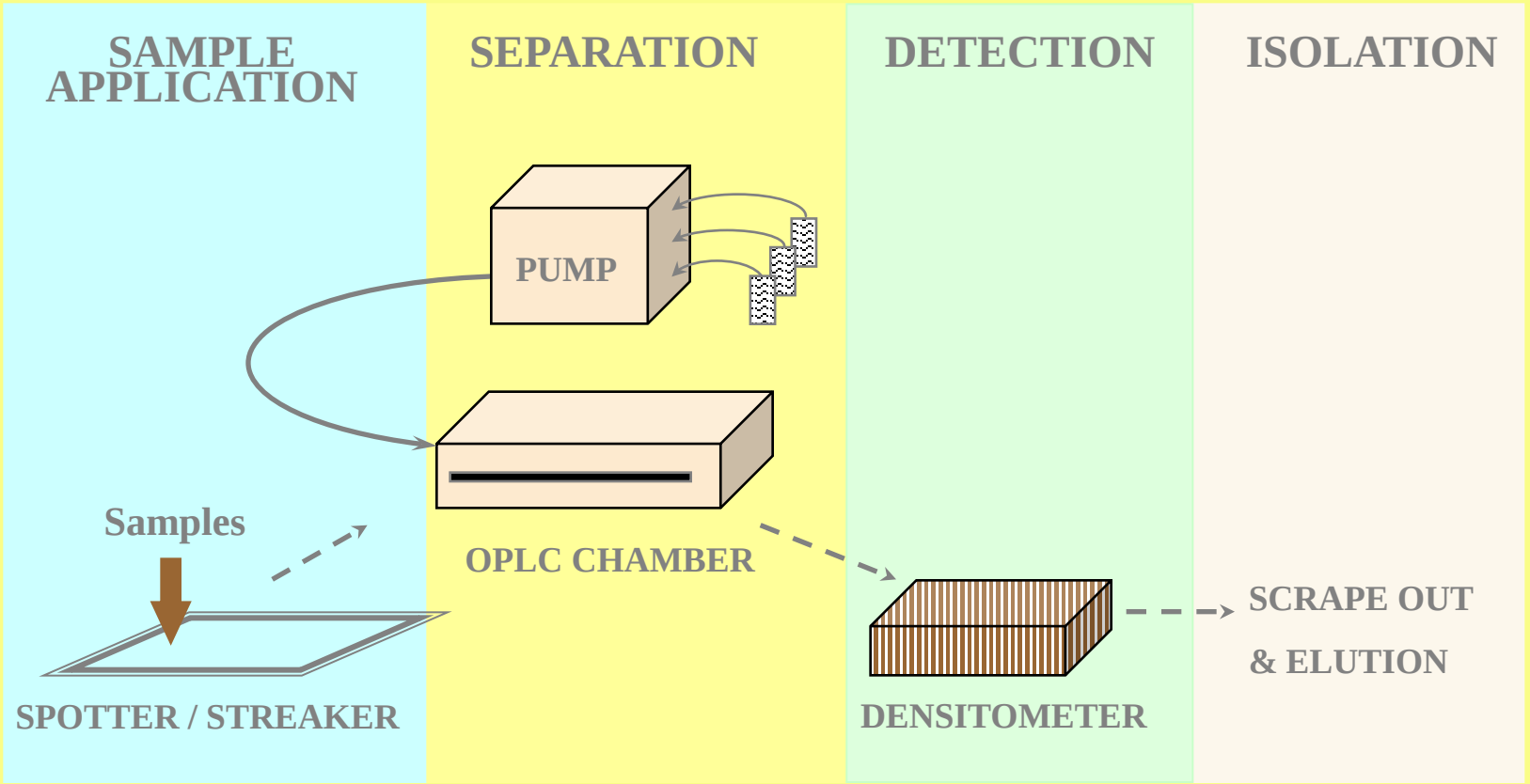
Advantages of OPLC

- Forced flow leads to a faster separation less time & solvent
- Flexibility (see later)
- Eluent flow rate is adjustable and A, B, and C solvent systems can be chosen for isocratic or step-wise gradient runs
- Longer separation distances increase zone capacity compared to classical TLC
- Constant flow rate results in almost constant theoretical plate height on the whole layer

SCHEME of OPLC PROCESSES (FLEXIBILITY of OPLC)

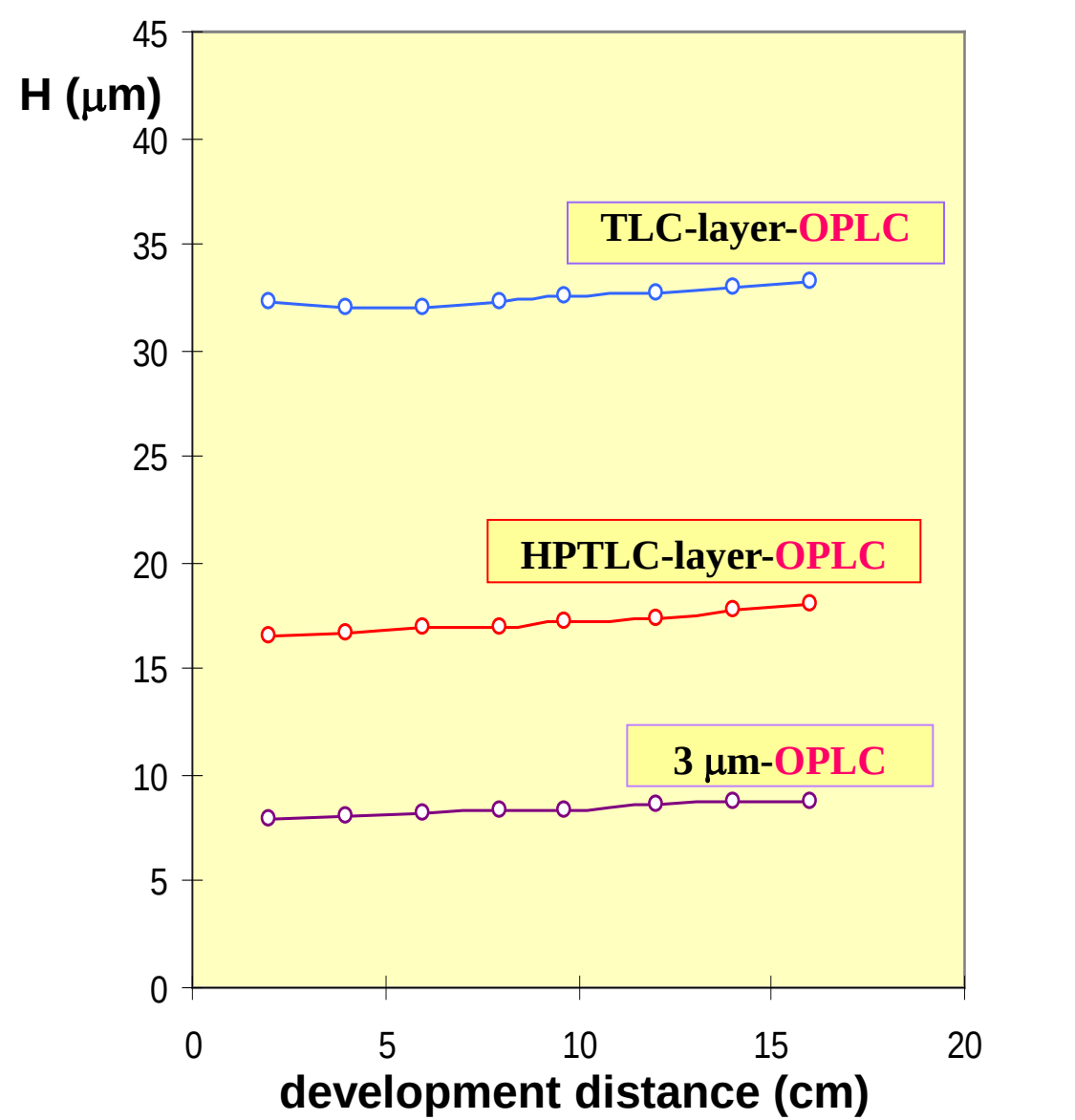


Fully off-line OPLC

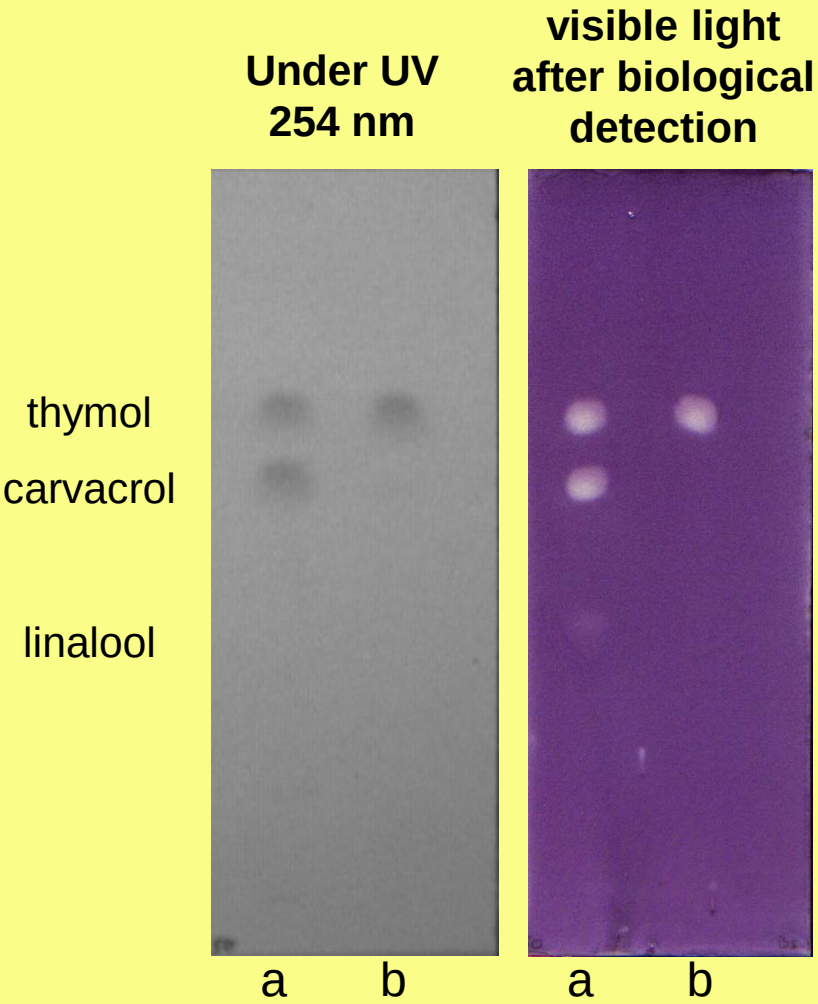


ANALYTICAL OPLC (off-line)

The correlation between average theoretical plate height (H) and the development distance in the case of fully „off-line" OPLC separation



Detection with vital dye



a - standards, 3 μg of each

b - Thyme essential oil (16 μg)

For visualisation of antibacterial effect against *Bacillus subtilis* soil bacterium the MTT dye reagent was used. Incubation time between the inoculation and the dyeing was 1h. The photo was taken 0.5 h after dyeing.

OPLC adsorbent layer: 20x20 cm, normal particle silica, F_{254} (dried 130 ^{0}C/3 h)
Eluent: chloroform
Personal OPLC 50 infusion OPLC separation, single development (1x)
External pressure: 5 MPa
Flow-rate: 300 μL/min
Rapid admission volume: 300 μL
Total volume: 4540 μL
Development time: 918 s

Bioluminescence

firefly
(*Photinus pyralis*)

Can-opener Smoothdream (*Chaenophryne longiceps*)

Bobtail Squid
(*Euprymna scolopes*)

The bioluminesence is a common phenomenon in the nature. There are many creature that can emit light.

Source: www.lifesci.ucsb.edu/~biolum/ and www.jasonpinto.wordpress.com/2009/07/

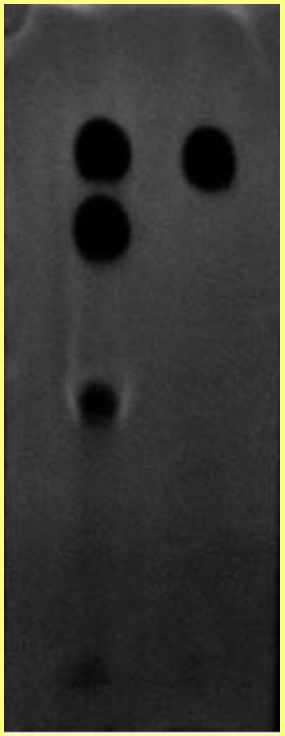
The use of luminescent bacteria

Naturally luminescent marine bacteria such as *Vibrio fischeri* or *Photobacterium phosphoreum* are applied for bioautography. Their use may be problematic because of the complex control of natural luminescence.

- Engineered bacterial cells emit light continuously dependent only on metabolic activity, thus viability
- monitored and documented by a computer-controlled camera
- Darker areas in the bioautogram indicate lack of metabolic activity.

Pseudomonas syringae* pv. *maculicola – chromosomally tagged with lux CDABE gene (from *Photorhabdus luminescens*); constitutive kanamycin promoter (J. Fan et al. Plant J. 2008, 53:393-9.)

Luminescent
Pseudomonas syringae pv. maculicola



a - standards, 3 μg of each
b - Thyme essential oil (16 μg)

The influence of the quality of the adsorbent layer on the biological detection

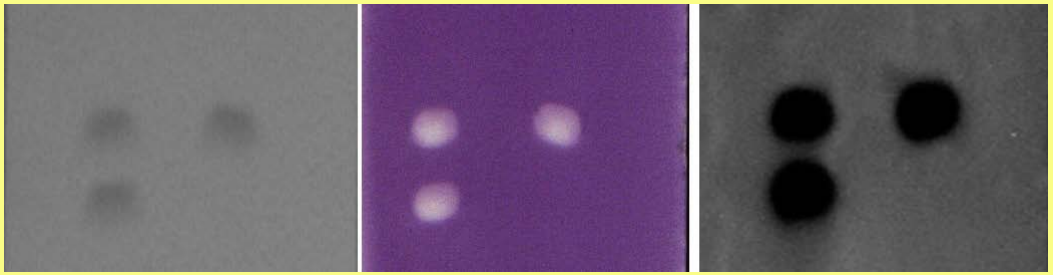
- overloaded condition – no characteristic difference in the separation efficiency
- difference in the sensitivity

(thickness, binding material, pH, trace elements)

Infusion OPLC: 20x20 cm Silica gel 60 layer, (dried 130 ^{0}C/3 h)
Eluent: chloroform
External pressure: 5 MPa

λ=254 nm *Bacillus subtilis* *Pseudomonas maculicola*

Merck TLC layer



a b

Flow-rate: 300 μL/min;
Rapid admission volume: 300 μL;
Total volume: 5540 μL;
Development time: 918 s

Merck HPTLC layer

a b

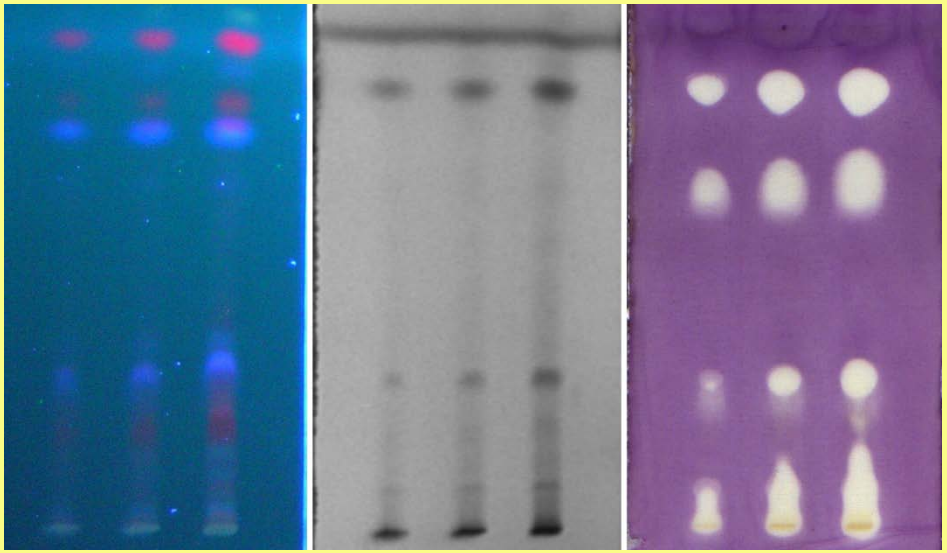
Flow-rate: 450 μL/min;
Rapid admission volume: 300 μL;
Total volume: 4250 μL;
Development time: 572 s

a - standards, 3 μg of each thymol and carvacrol

b - Thyme essential oil (16 μg)

The influence of the quality of the adsorbent layer on the biological detection

Merck TLC layer

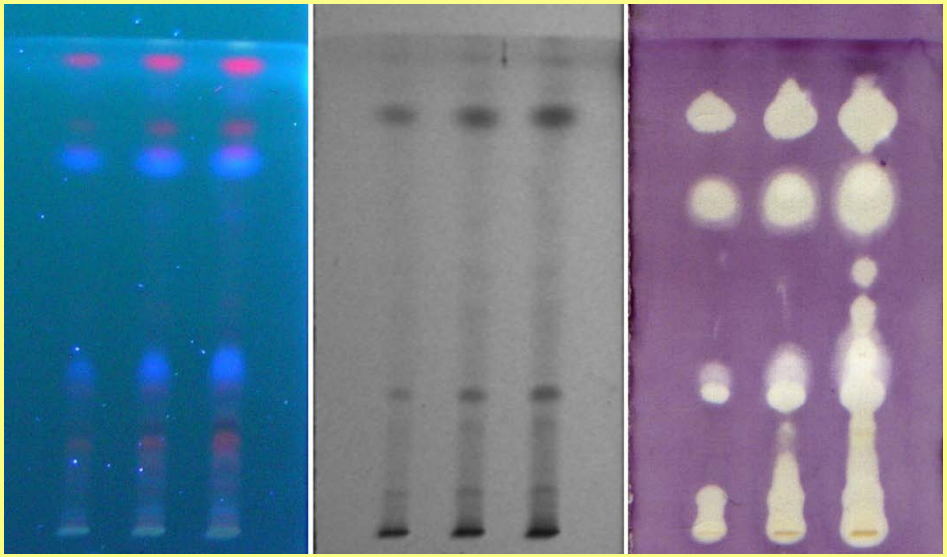


λ=365nm λ=254 nm *Bacillus subtilis*

conventional layer chromatography

mobile phase: chloroform-acetone 9:1 (v/v)

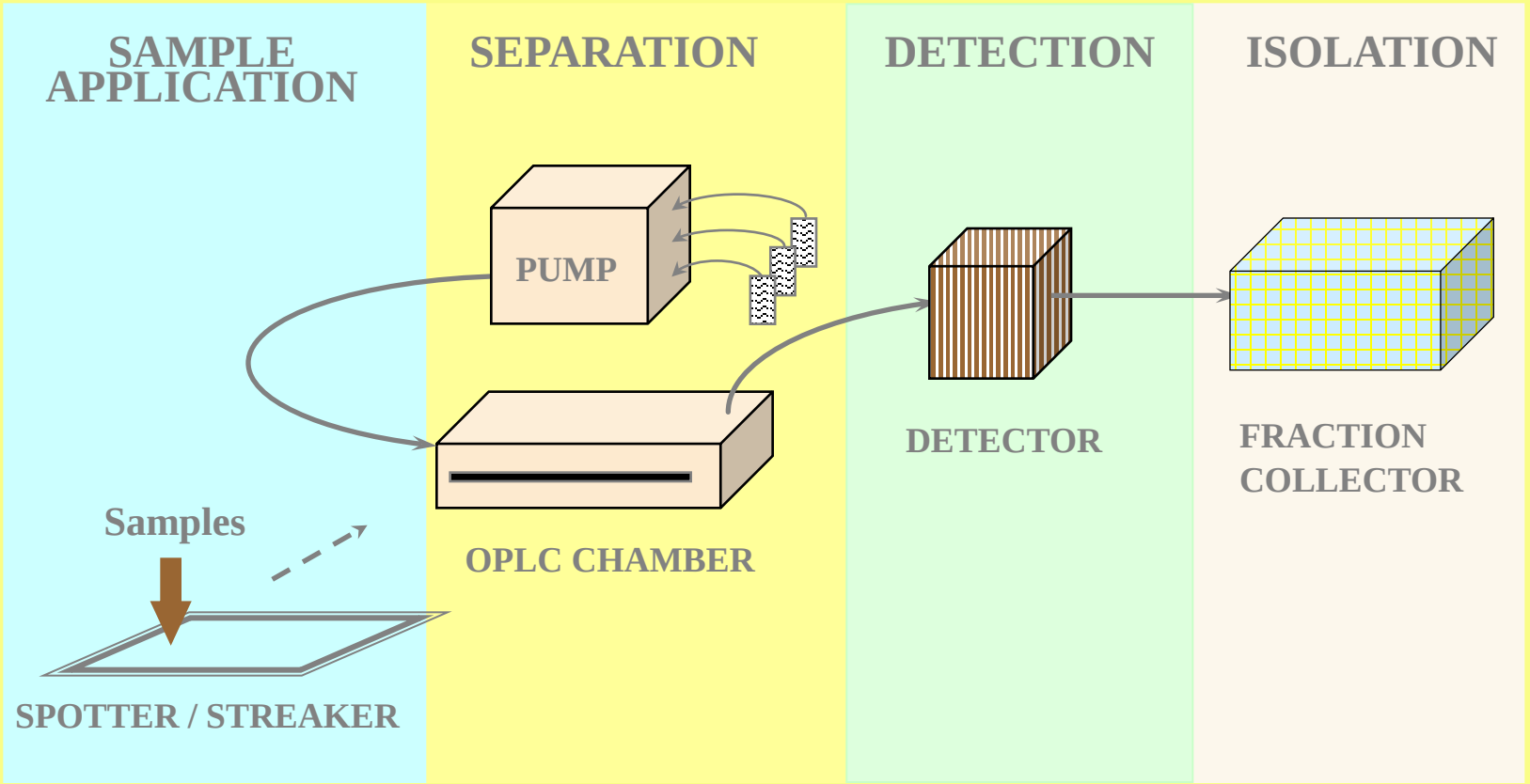
Merck HPTLC layer



3 6 9µl

plant extract

Fractionation with OPLC

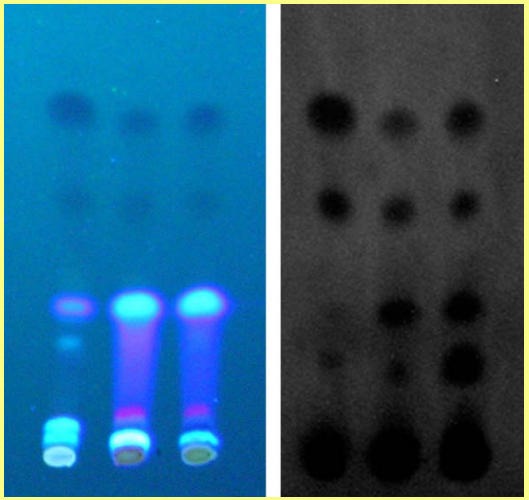


PREPARATIVE OPLC

(off-line sample application & on-line detection)

1. TLC - bioautography

UV (365 nm) Psmlux

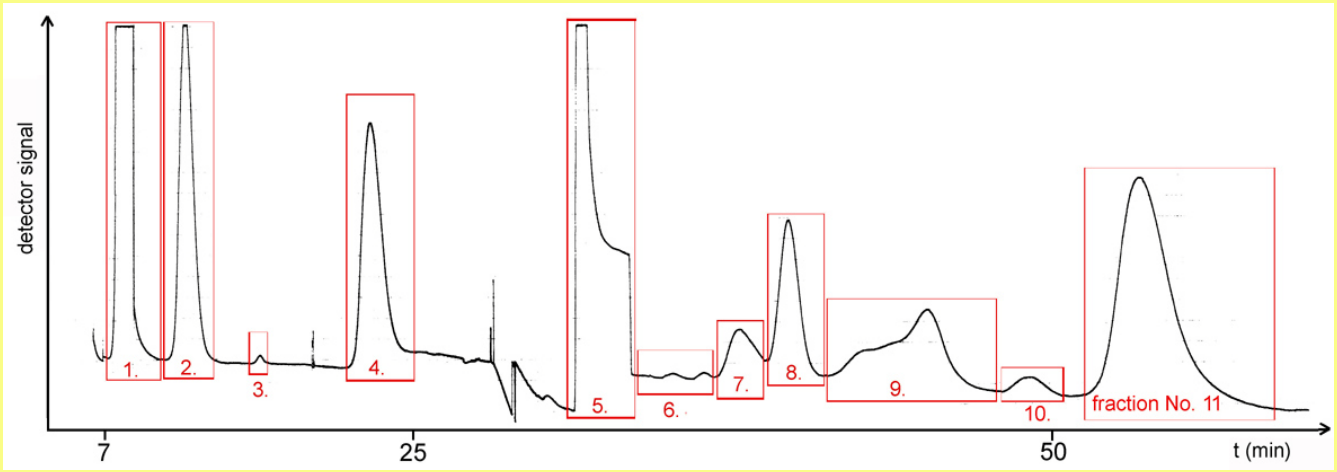


chamomile

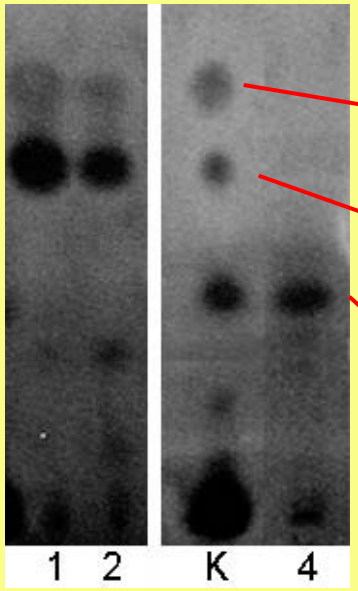
root leaf flower

2. OPLC with on-line detection

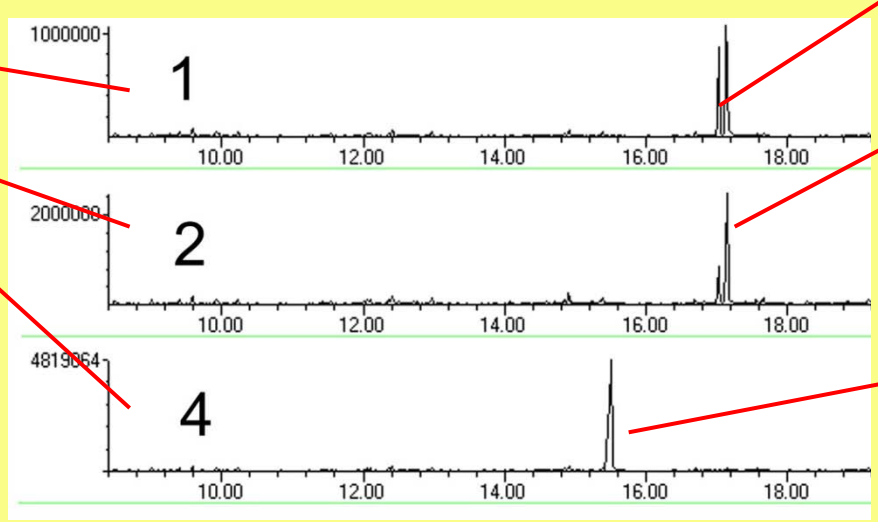
the fractionation of chamomile extract

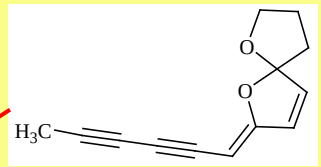


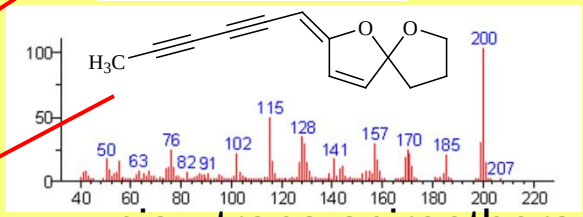
3. Checking of the fractions



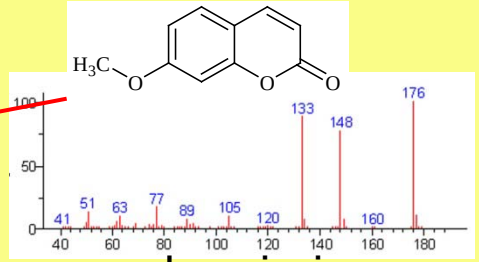
4. GC-MS analysis of the fractions



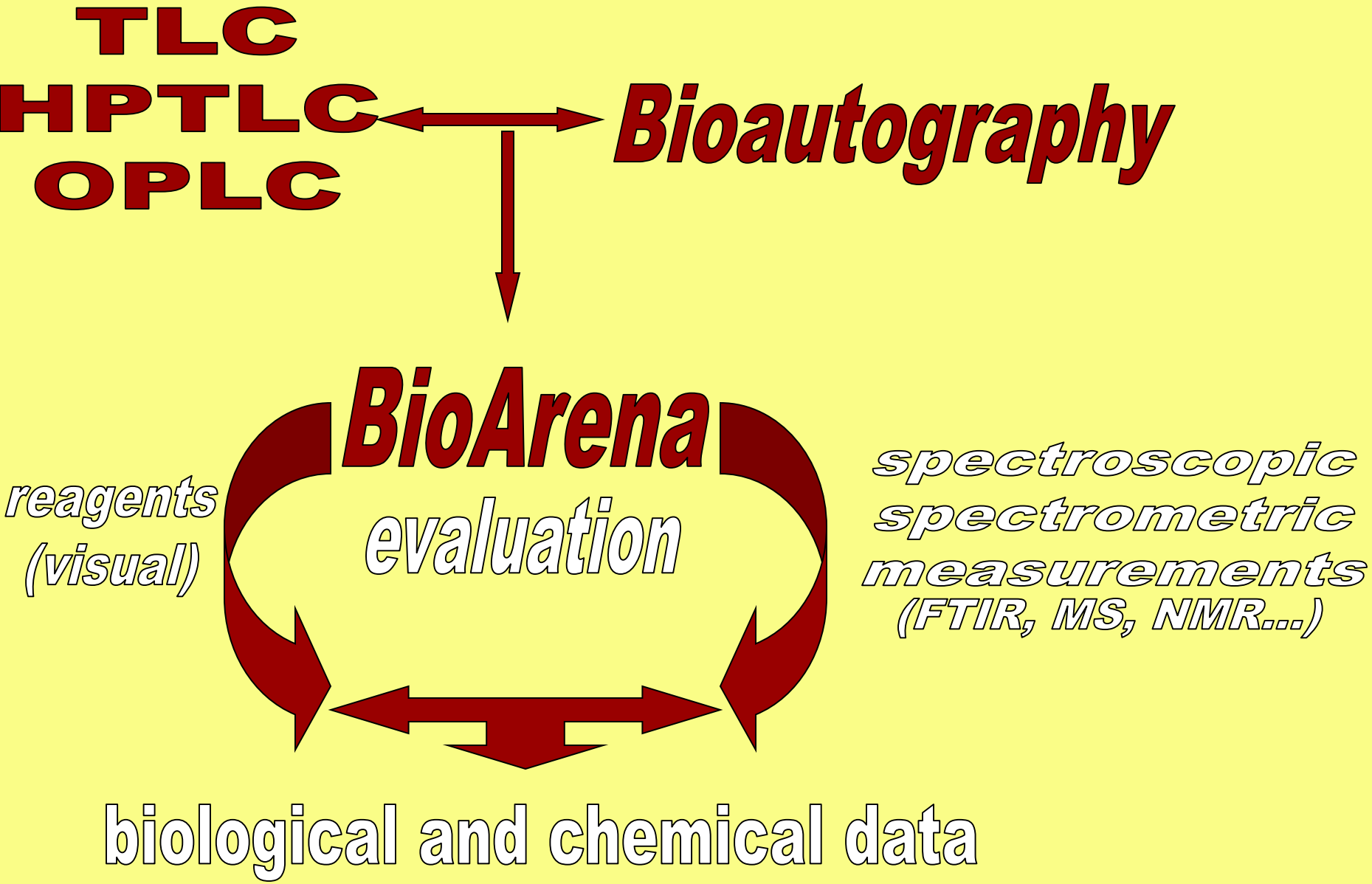




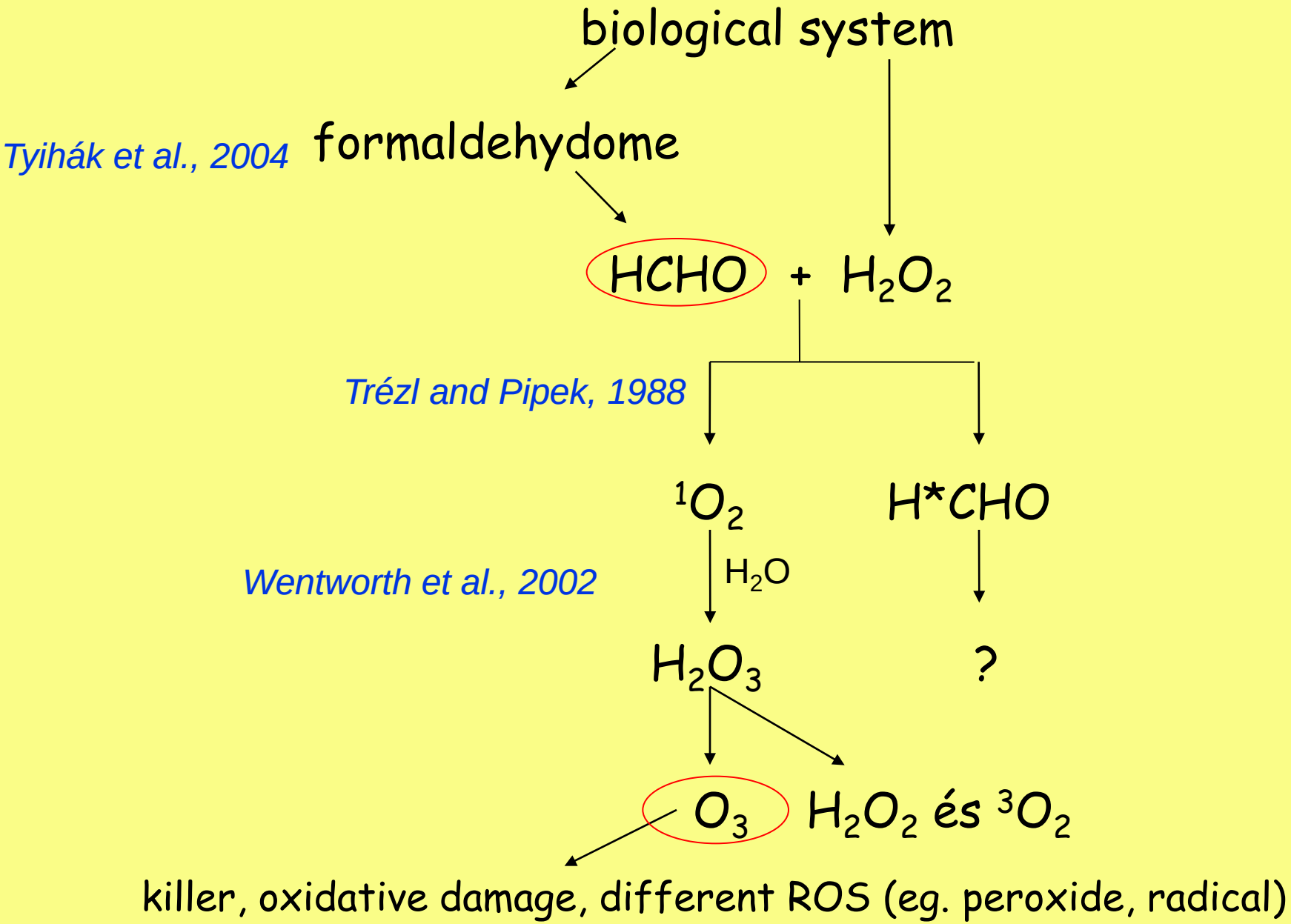
cis-, trans-spiroethers



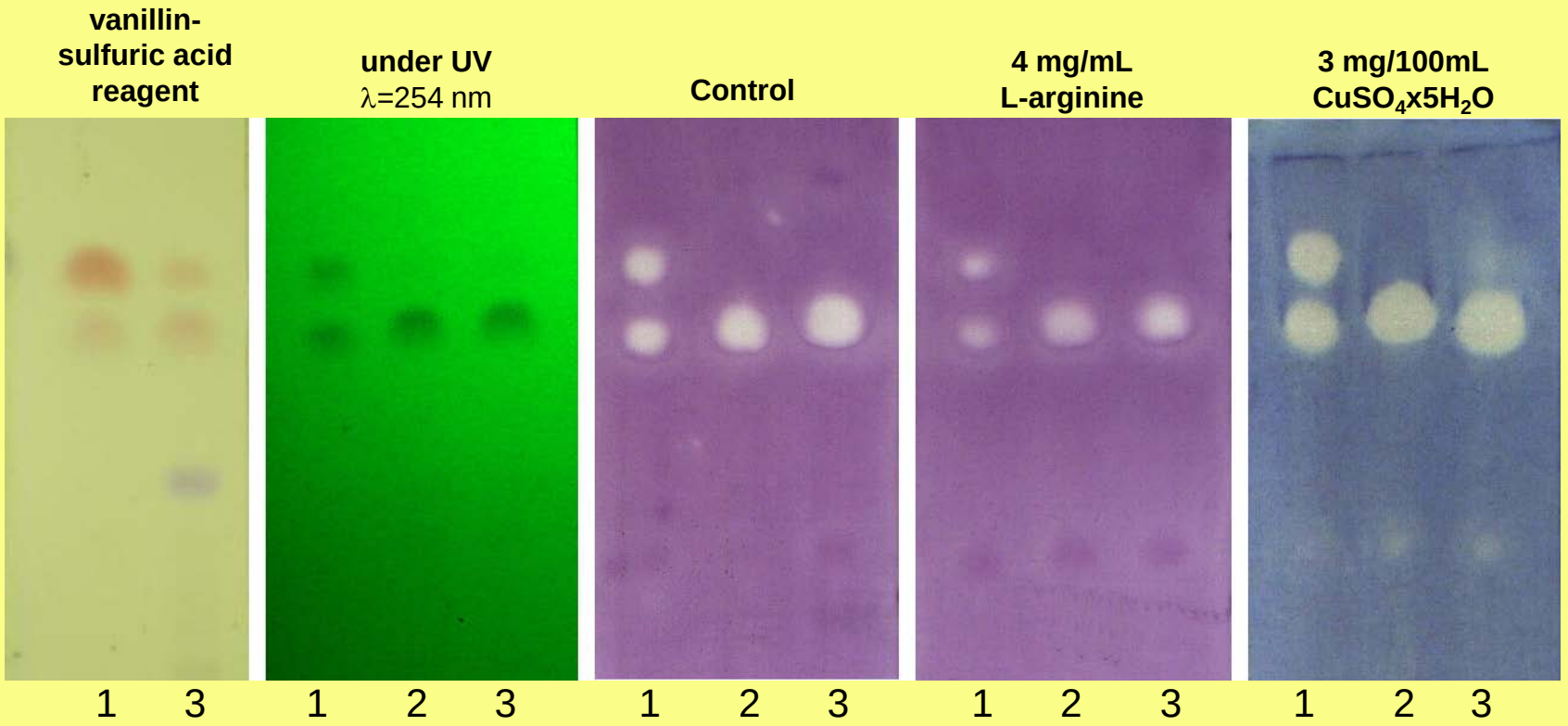
herniarin


TLC
HPTLC
OPLC
Bioautography
BioArena
evaluation
reagents
(visual)
spectroscopic
spectrometric
measurements
(FTIR, MS, NMR...)
biological and chemical data

The formation of singlet oxygen and ozone



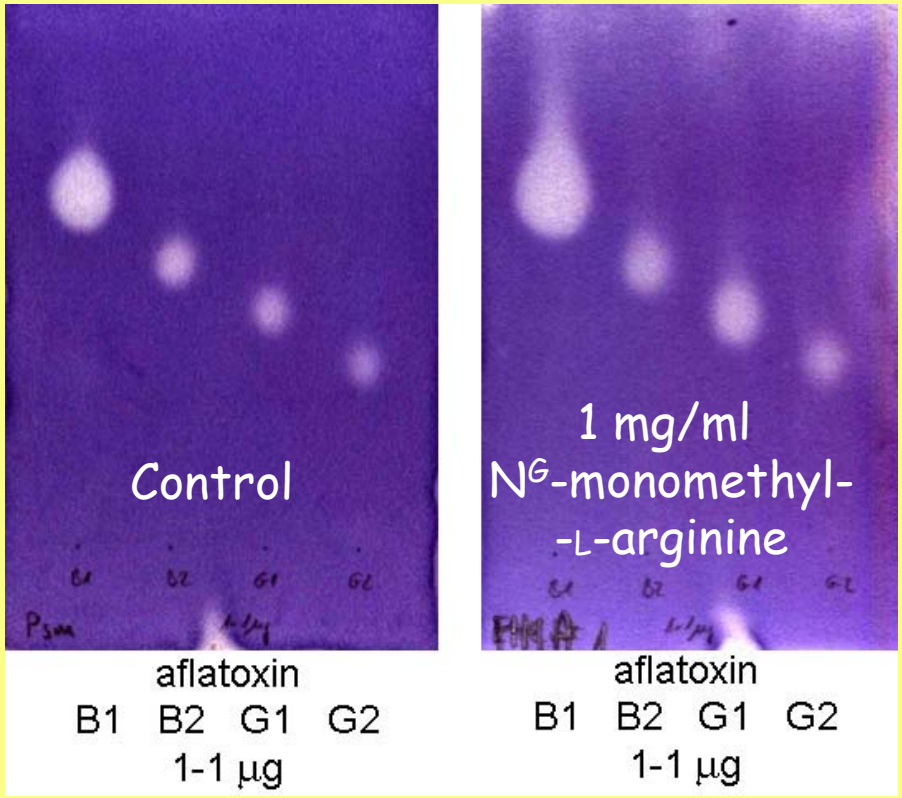
The influence of L-arginine and Cu(II) ions on the antibacterial effect of *Origanum onites* oil components against *Bacillus subtilis*

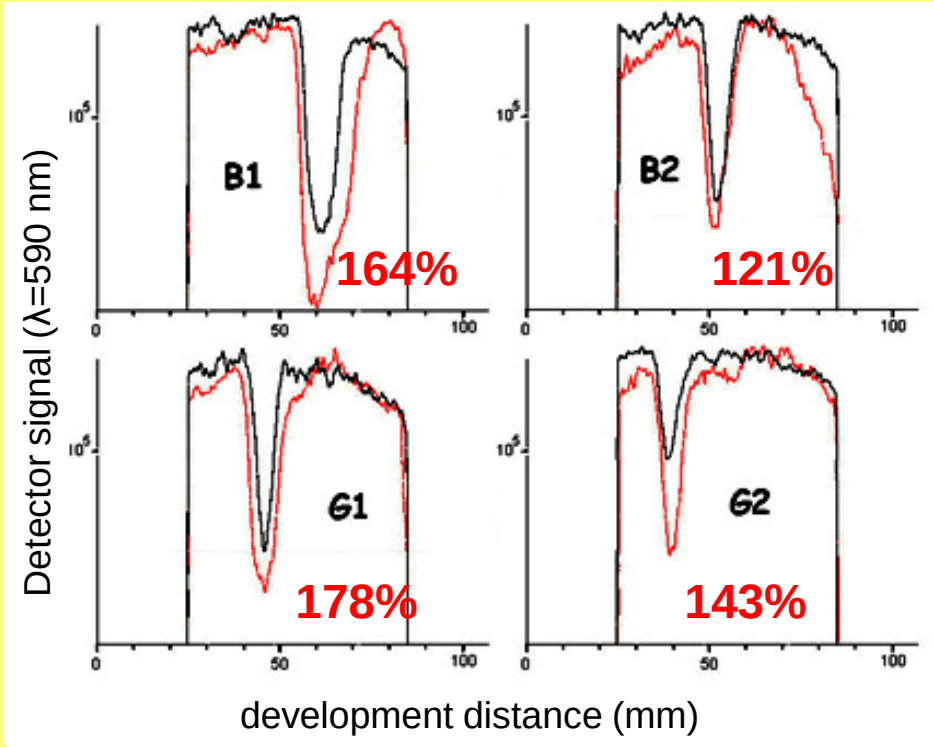


1. Standards, 10 μg of each thymol (A) and Carvacrol (B)
2. Origanum oil (FK 3A-1) (30 μg)
3. Origanum oil (FK 3A-2) (30 μg)

Infusion OPLC: 20x20 cm layer, (dried 130 ^{0}C/3 h); **Eluent:** dichloromethane; **External pressure:** 5 MPa
Flow-rate: 400 μL/min; **Rapid admission volume:** 450 μL; **Total volume:** 4847 μL; **Development time:** 738 s

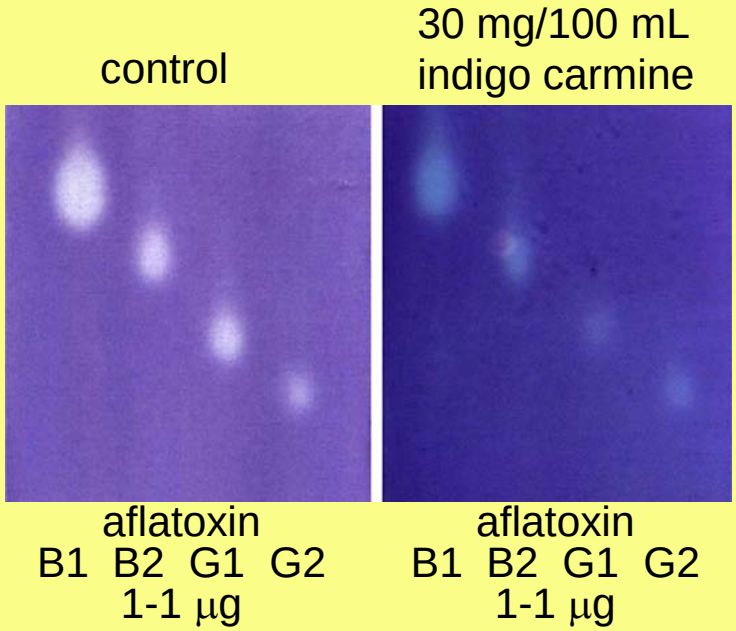
The influence of the N^G-monomethyl-L-arginine on the antibacterial effect of aflatoxins

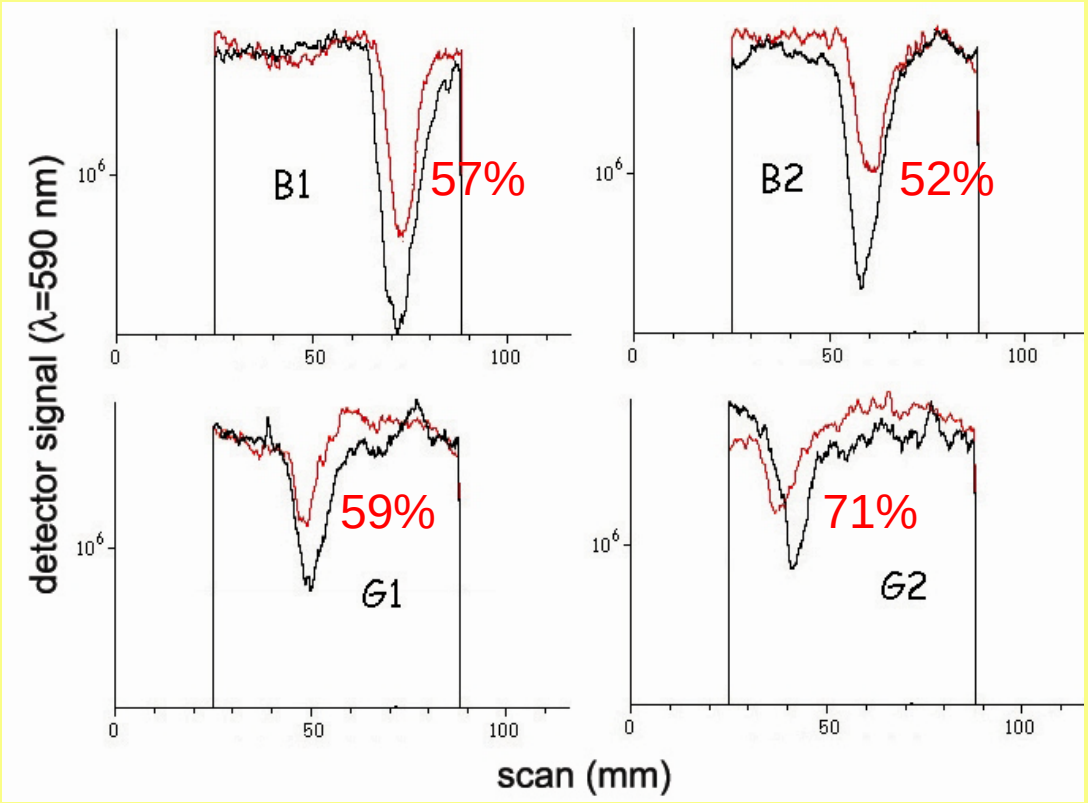




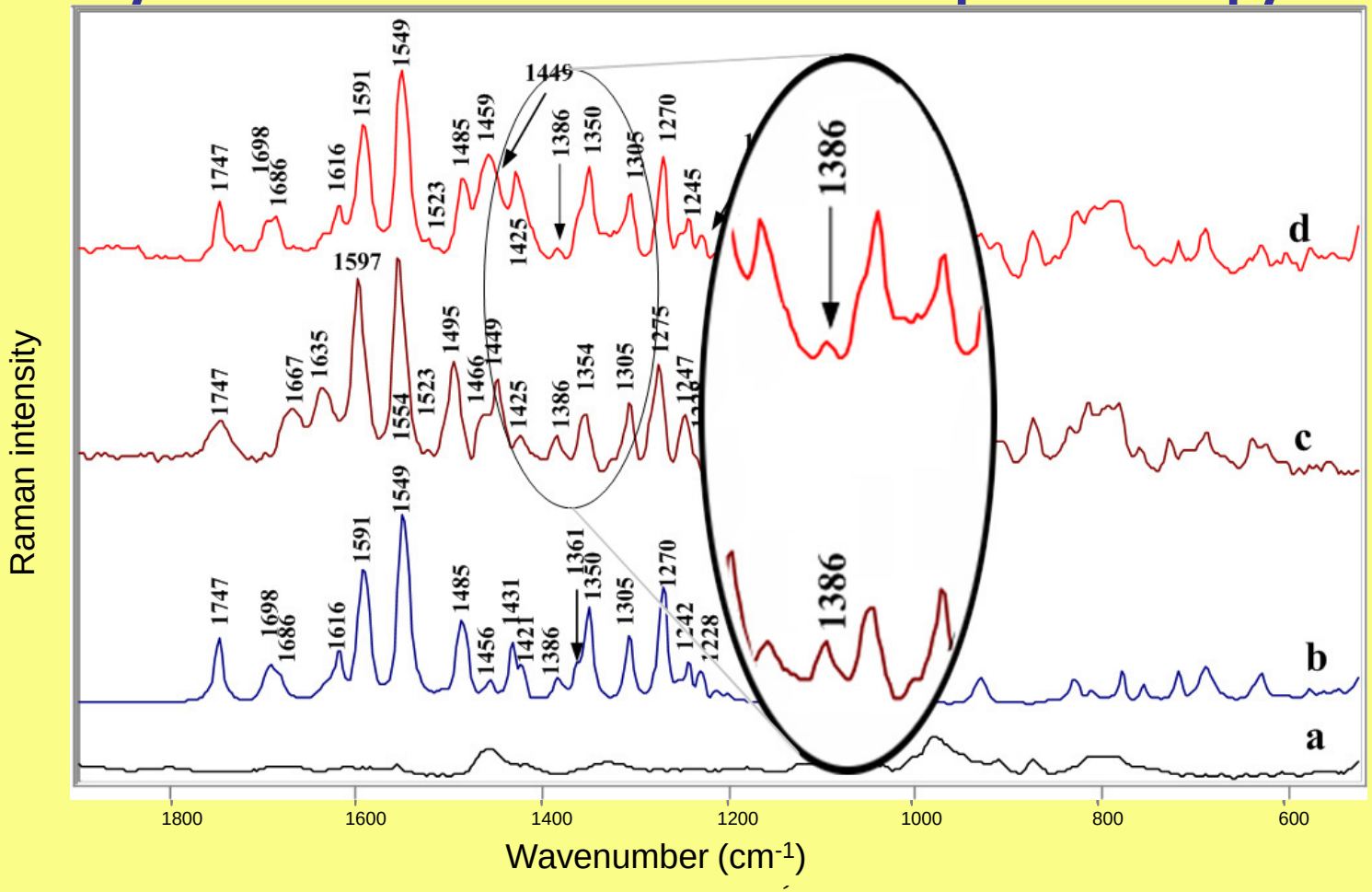
OPLC: 20x20 cm layer, (dried 130 ^{0}C/3 h); **Eluent:** chloroform-acetone 9:1 (v/v); **External pressure:** 5 MPa
Flow-rate: 400 µL/min; **Rapid admission volume:** 400 µL; **Total volume:** 4500 µL; **Development time:** 685 s

The influence of indigo carmine on the antibacterial effect of aflatoxins





In situ evaluation of the aflatoxin B1 chromatographic spot by Fourier transform Raman spectroscopy



(a) a background spot in inoculated TLC layer
(b) AFB1 standard (in powder form)
(c) the AFB1 spot in bacteria-free TLC layer
(d) the AFB1 spot in TLC layer inoculated with Psm cell suspension

Supporters:
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OTKA grant No. PD83487

Thank you for your kind attention!

Origanum onites

Thymus vulgaris