



# 32<sup>ème</sup> Réunion du Club de CCM 18-19 Octobre 2017

Retour sur les meilleures contributions  
de Berlin 4-8 Juillet 2017

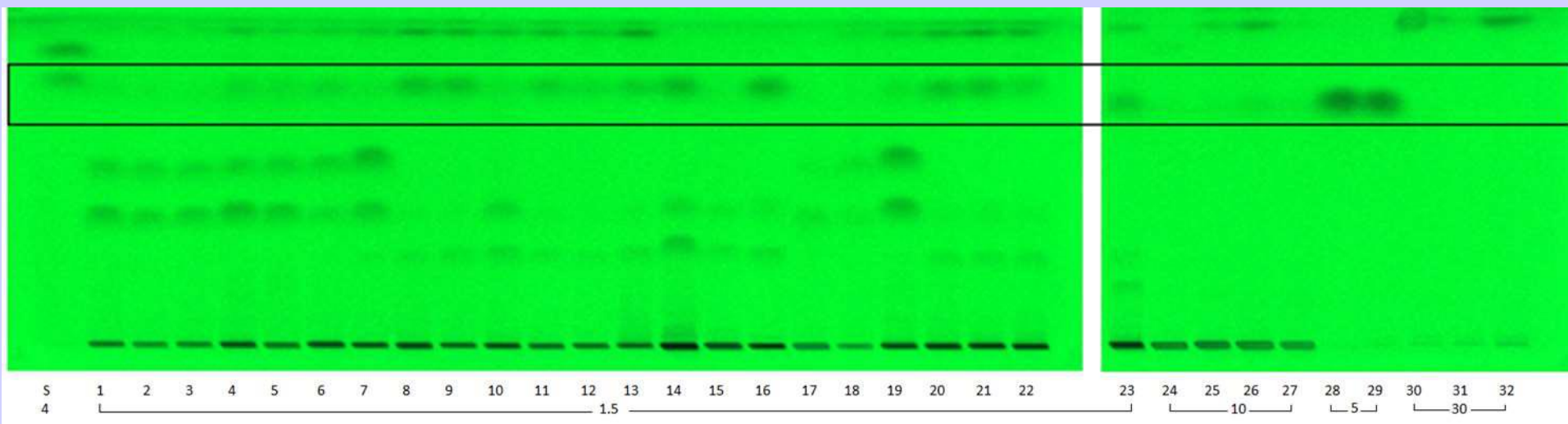
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# Contributions posters

P-13 Busso, Tomeba, Sighicelli, Morlock : évaluation de **vanilles** collectée au Sihra à Lyon lors du précédent Club CCM  
Quantification vanilline ( 1  $\mu$ g - 89 mg/g )



*HPTLC chromatograms at UV 254 nm of vanilla fruits (tracks 1-12), oleoresin (13), alcoholic extract (14-17), inner part (18), powders (19-23), aroma (24), vanilla sugars (25-27) in comparison to vanillin sugars (28-29), ice cream powder (30), puddings (31-32) and standard mixture of vanillin at hRF 72 and ethylvanillin (S)*

*B, D and E: 0.5  $\mu$ L/band; C : 2 (no. 1-23), 1.5 (no. 15), 4 (no. 28-29) and 20  $\mu$ L/band (no. 30-32)*



# Contributions posters

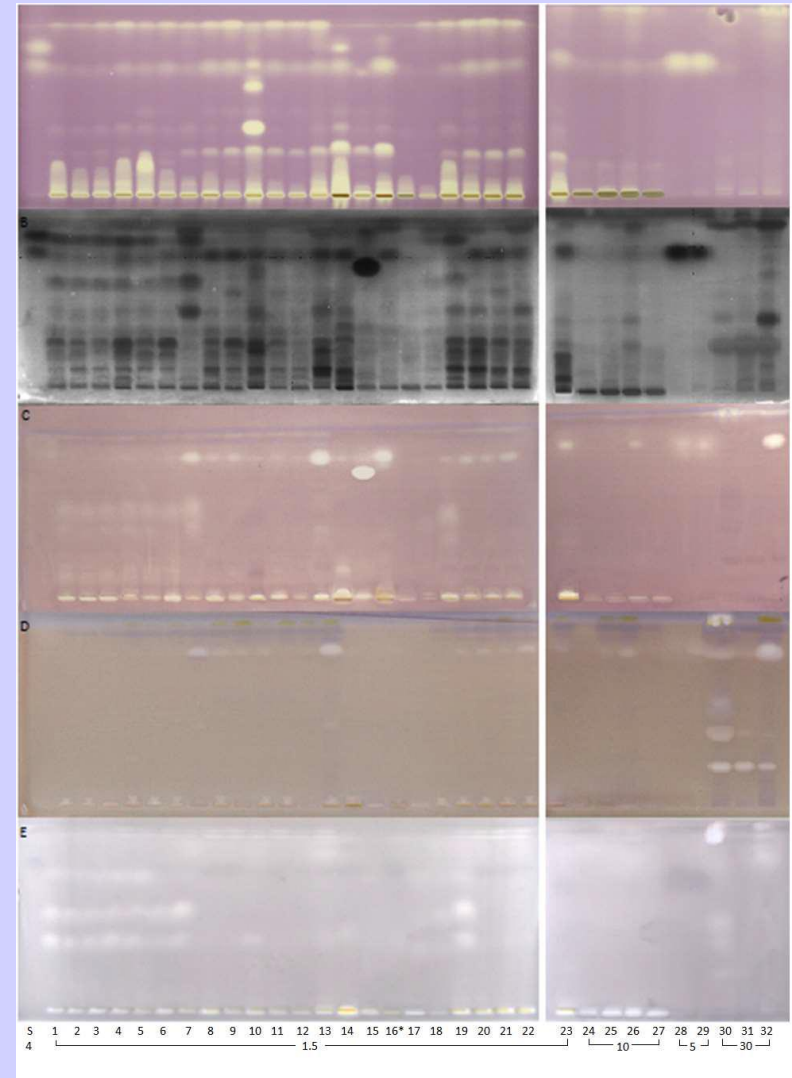


P-13 Busso, Tomeba,  
Sighicelli, Morlock : ... Bio-profiling:  
DPPH, A.V.Fischeri, B.Subtilis, AChE,  
Tyrosinase (de haut en bas)

*HPTLC chromatograms at UV 254 nm of vanilla fruits (tracks 1-12), oleoresin (13), alcoholic extract (14-17), inner part (18), powders (19-23), aroma (24), vanilla sugars (25-27) in comparison to vanillin sugars (28-29), ice cream powder (30), puddings (31-32) and standard mixture of vanillin at hRF 72 and ethylvanillin (S)*

*B, D and E: 0.5  $\mu$ L/band; C : 2 (no. 1-23), 1.5 (no. 15), 4 (no. 28-29) and 20  $\mu$ L/band (no. 30-32)*

... puis ESI-HRMS

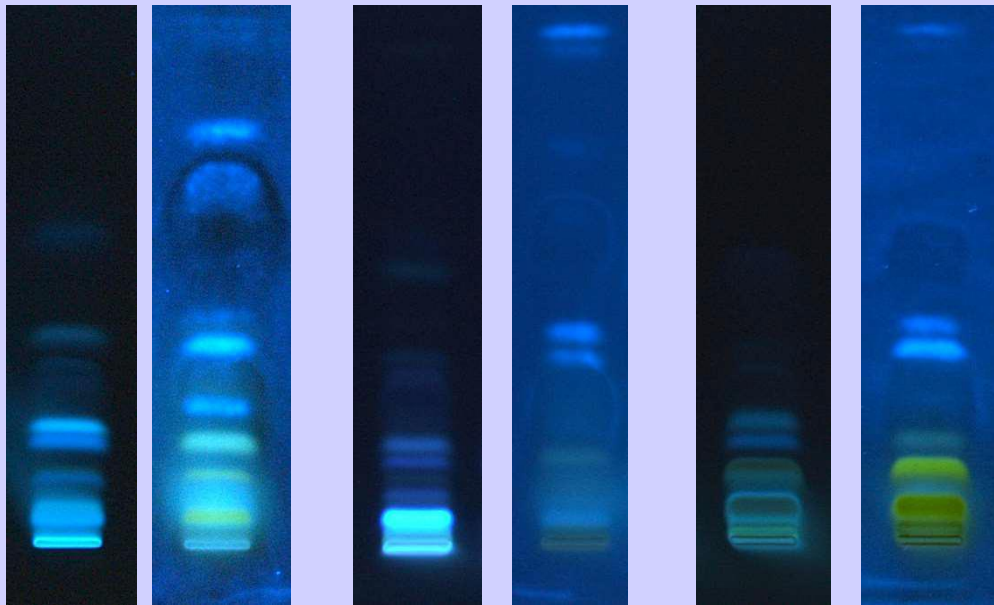




# Contributions posters

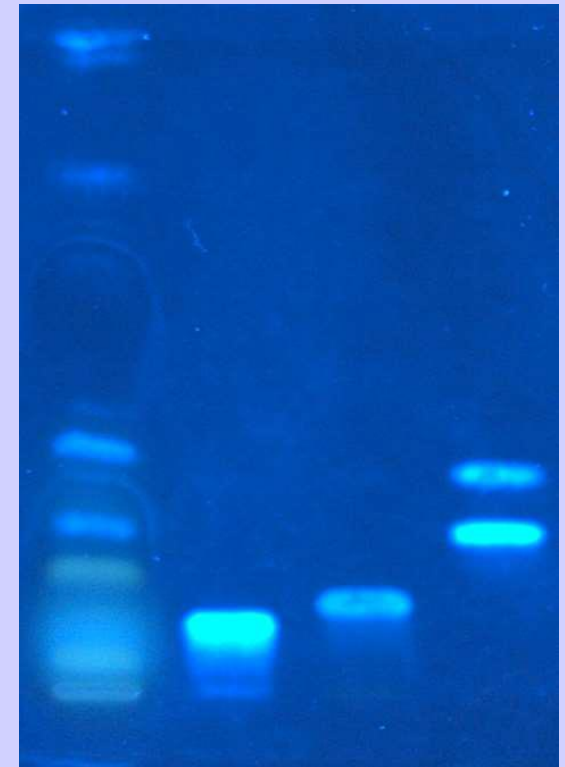


P 22-Klingelhofer, Morlock : YES-test dans les vins blancs, rosés, et rouge.



*de gauche à droite vin blanc, rosé et rouge sous 366nm et YES-test*

... puis comparaisons avec des phyto eostrogènes connus



*Comparison of white wine (track 1) with hRF values of known phytoestrogens, i. e. on track 2: genistein 3: naringenin 4: fenhexamid (haut) and fludioxonil (bas)*

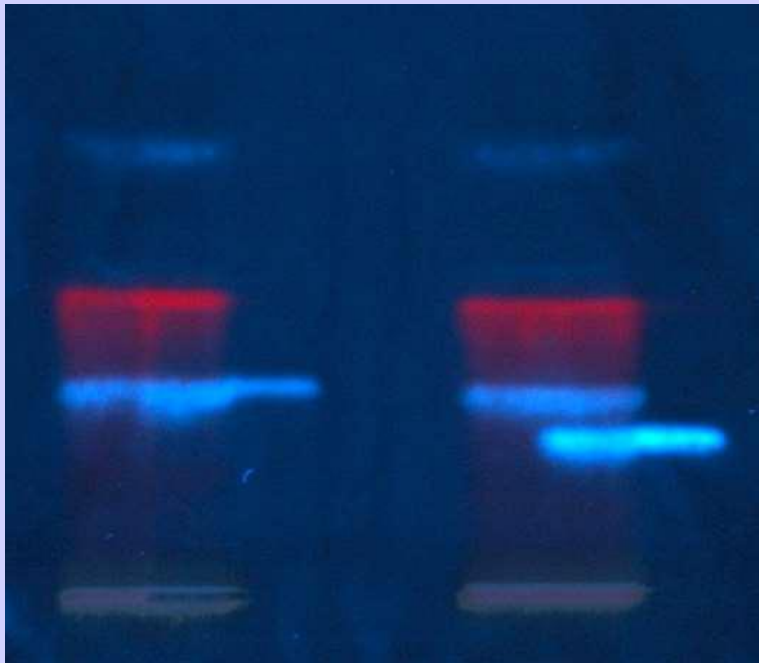




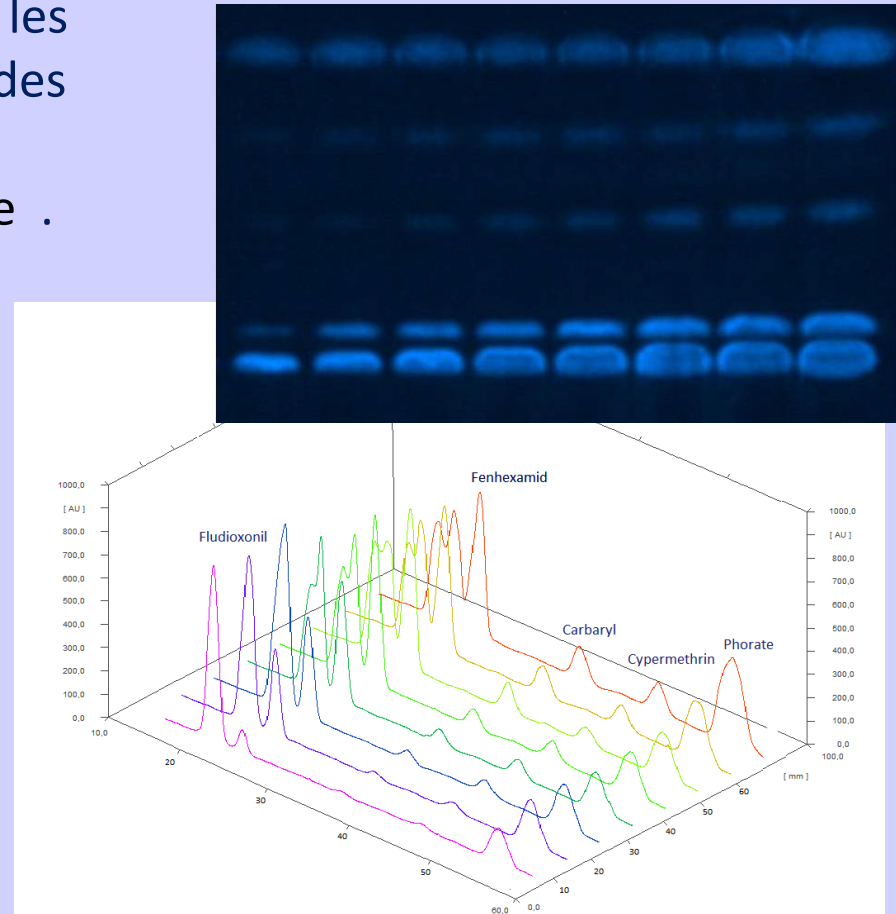
# Contributions posters



P 23-Klingelhofer, Morlock : YES-test sur les PPPs ( pdt protection des plantes) dans des échantillons et mise en évidence du fenhexamid dans du raisin blanc de table .



...et analyse quantitative



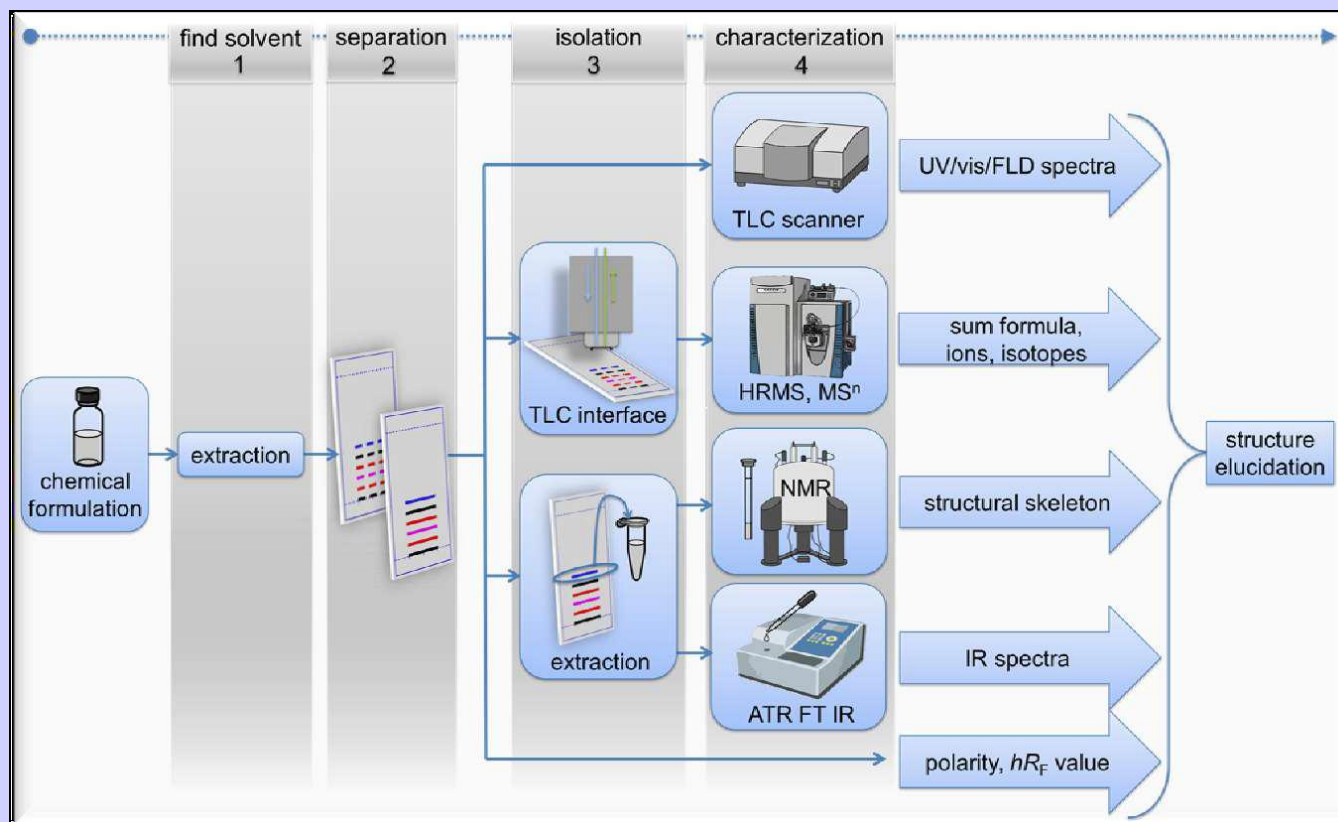
5 estrogen-effective PPPs: ( YES-test + 366 nm)  
fludioxonil (8-250 ng/ band) fenhexamid (25-500 ng/ band)  
carbaryl (8-60 µg/band) cypermethrin (6-400 µg/band)  
phorate (5-100 ng/band)





# Contributions posters

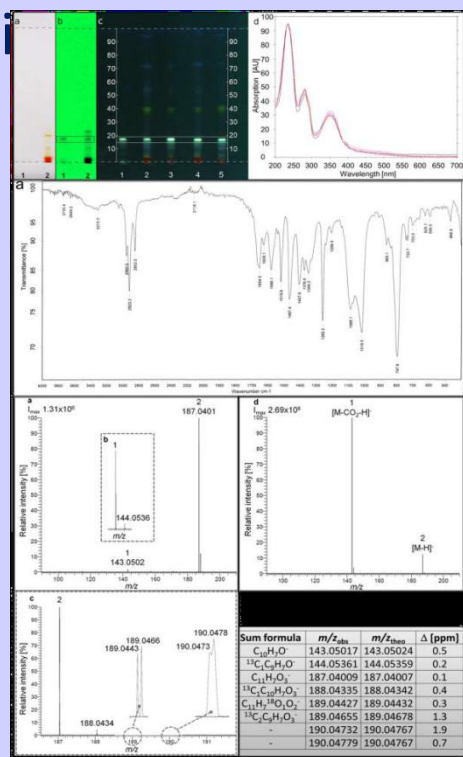
P 65-Yüce, Morlock : couplages HRMS et CCM Prep-RMN pour l'élucidation de mélanges inconnus, avec deux plaques seulement.



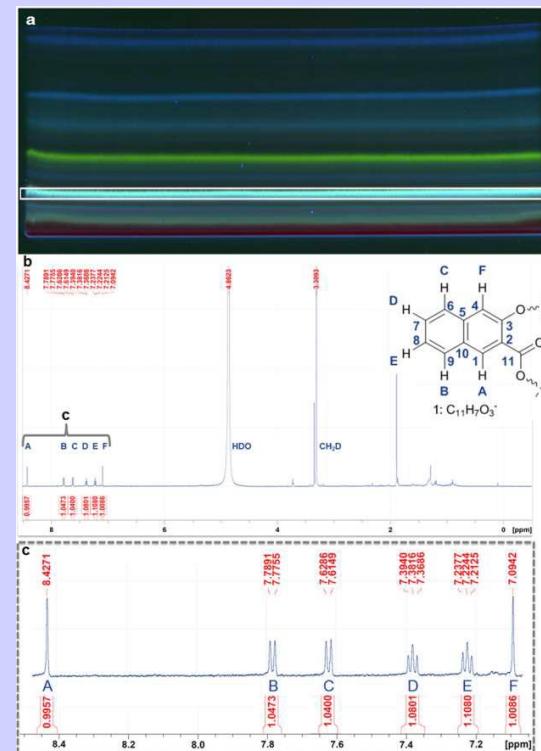
# Contributions posters



P 65-Yüce, Morlock : couplages HRMS et CCM  
Prep-RMN pour l'élucidation de mélanges



HPTLC-vis/UV/FLD (a-c),  
absorption spectra, (d), PLC-IR  
(e) and HPTLC-HRMS/MS<sup>2</sup> (f-i)

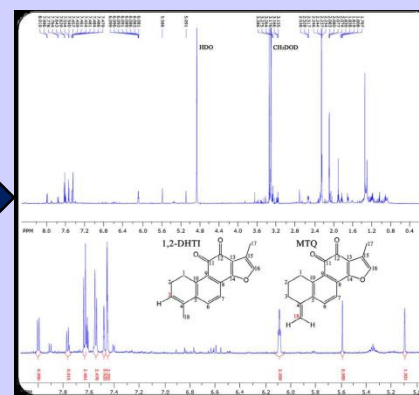
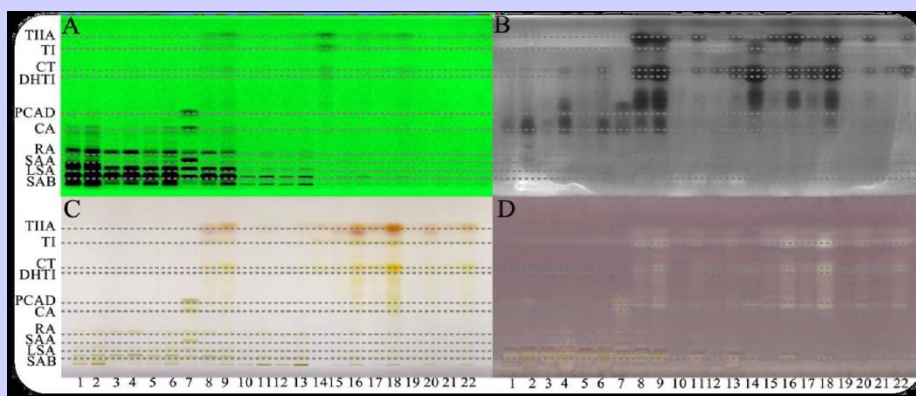


Preparative chromatogram at UV 366 nm of PR 57:1,  
batch 1472 (a, marked band of interest); respective PLC-  
1H NMR spectrum (b) and downfield area with clear  
signals for the aromatic protons (c, enlarged)



# Contributions posters

P 68-Azadniya, Morlock : étude des constituants intéressants d'un extrait de plante ( *Salvia miltiorrhiza* ) par HPTLC-EDA-HRMS et CCMPrep-RMN.



different extraction solvents: methanol (tracks 10-13), ethyl acetate extract of acidified water fraction (1, 2), same plus salt and acetone (3-6), ethanol (19-22) and ethanol – dichloromethane 4:1 (8, 9, 15-18) along with standard mixture (7, 14) at UV 254 nm (A), after *A. fischeri* bioassay, under white light illumination (C) and after *B. subtilis* bioassay (D)

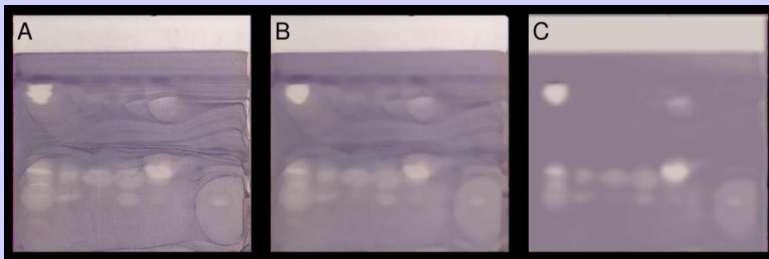
1H-NMR spectrum of unknown compound “x” led to the combination of 1,2-DHTI and MTQ



# Contributions posters TECHNOLOGY



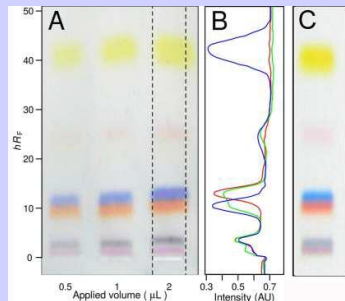
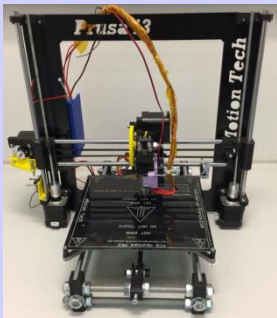
P2 P3 P4 -Fichou, Morlock : ANN, imprimantes 3D, et rTLC



Comparison des algorithmes: A original, B filtre médian, C ANN

Morlock, G.E., Ristivojevic, P., Chernetsova, E.S.  
*Journal of Chromatography A* 2014, 1328, 104–112.

...des possibilités très encourageantes



L'imprimante 3D permet de presque tout faire, phases, dépôt d'échantillon, et migration de solvant...

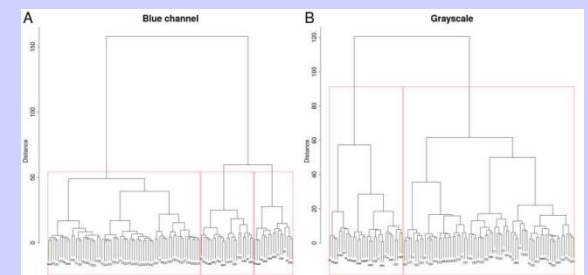
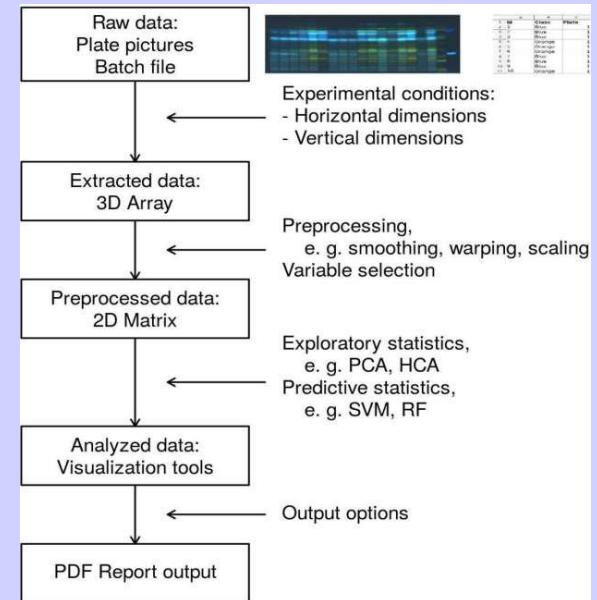


Schéma du traitement rTLC (en haut) et du résultat représenté en dendrogrammes (en bas)





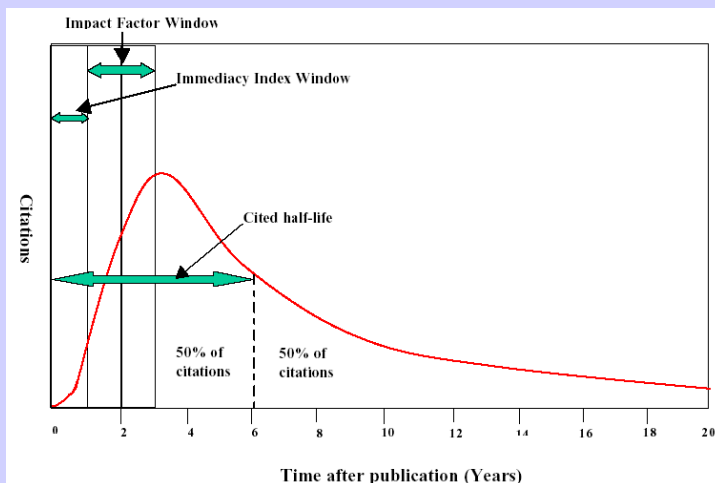
# Contributions orales



O5-Verpoorte: Publishing a world class scientific paper

IMPACT FACTOR : Exemple du Journal of Ethnopharmacology

IF 2015 = 3.055 => > 2millions de téléchargements/an, c'est-à-dire > 5000/Jour !



- The average number of citations in a year to articles in that journal published in the preceding two years
- Is used in an attempt to describe the quality of a journal, but a high impact factor means not necessarily a better journal

# Contributions orales



## O5-Verpoorte: Publishing a world class scientific paper



=



Big data > GB

- Easy to produce large data sets
- Omics are based on storing data
- Systems biology generates a combination of very different type of data

- How to deal with this
  - Save raw data or processed data?
  - Does author or publisher store the data?
  - Free access to all data?
  - Sufficient capacity?
  - Who takes responsibility?
  - Who pays the costs, user or producer?

# Contributions orales



O5-Verpoorte: Publishing a world class scientific paper

Be honest, be modest, be realistic

“Absence of evidence is not  
evidence of absence”

*Carl Sagan, 1977*

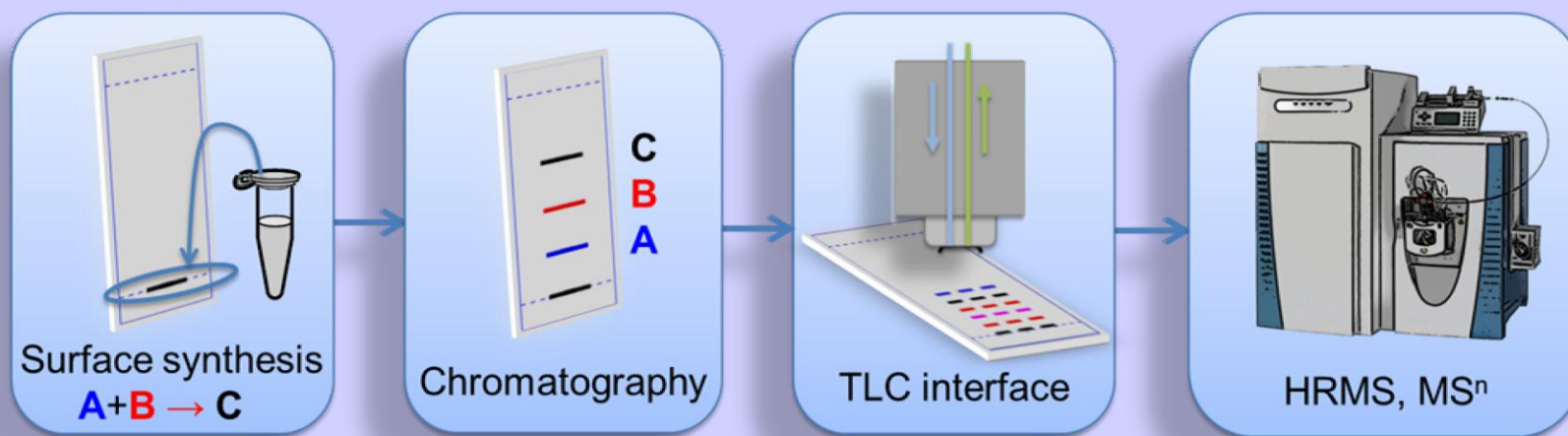




# Contributions orales

O7-Yüce, Morlock :

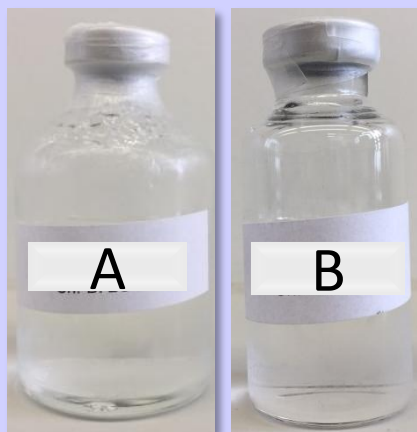
**Targeted combinatorial on-plate synthesis  
as new tool for structure elucidation**



Synthesis, purification, detection on the same layer



## Impurity analysis (< 1%)



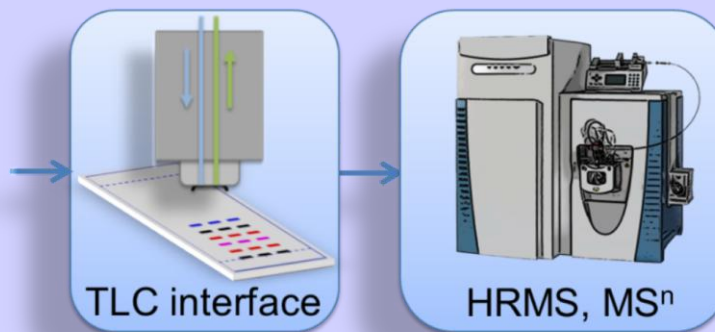
Example: Anti-cancer drug

- Establish an efficient quality control
- Guarantee a constantly high formulation quality
- Ensure the highest possible consumer protection
- Prevent impurities → knowledge about origin of unknowns
- Fast structure elucidation of impurities and contaminants, if no standard available

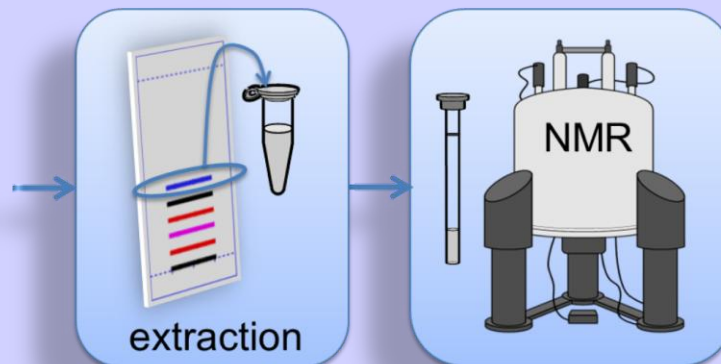
# Toolbox → Collect information



- HPTLC-HRMS
- High resolution mass spectrometry
  - 280 000 resolution
  - < 2 ppm accuracy



- PLC/HPTLC-NMR
- NMR
  - Needs high substance amount



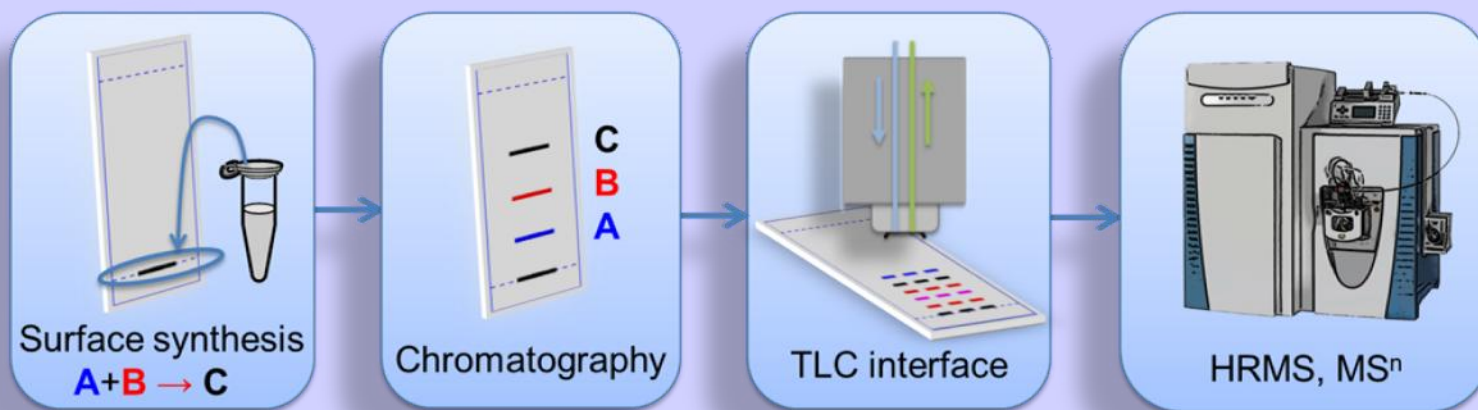




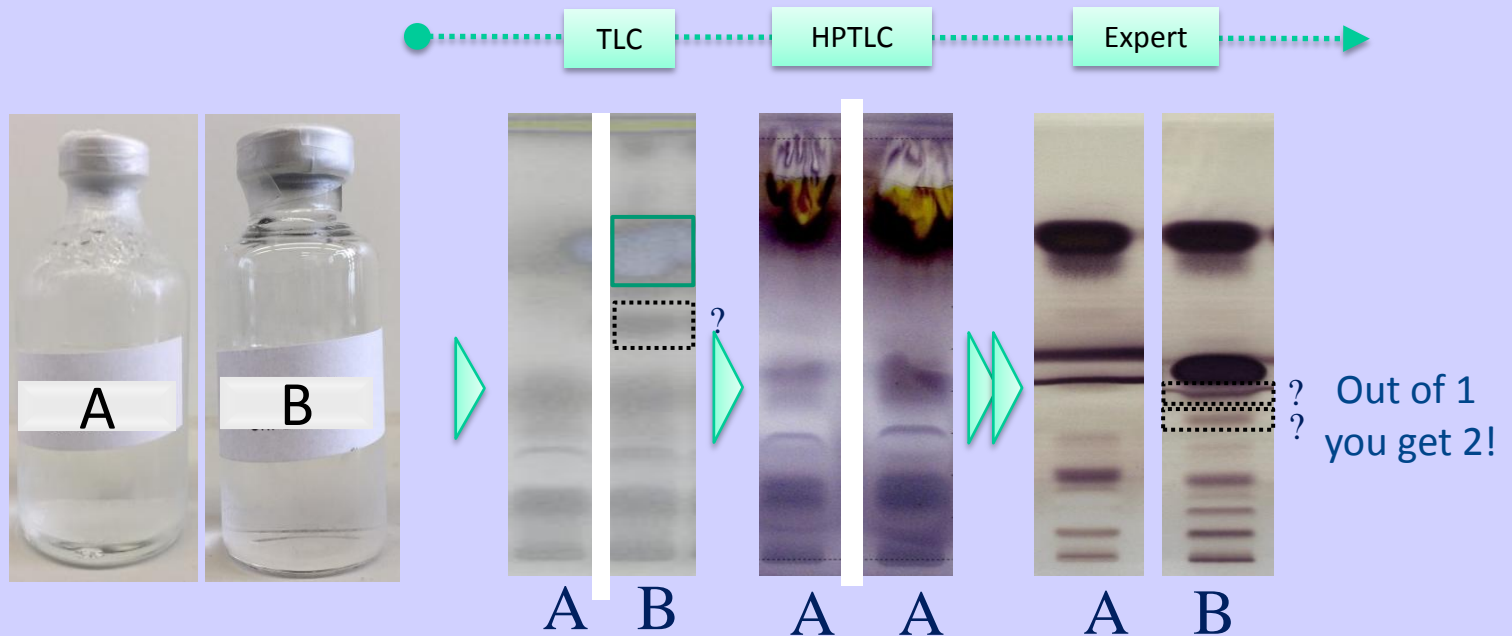
# New insight via on-plate synthesis

## Organic chemistry

- On-plate synthesis by combination of ingredients
- Understanding the synthesis way
- Determine the source of impurities ✕
- New structural information



# Anti-cancer drug – Method transfer



## Scope

- Pharmaceutical product
- TLC separation
- Not optimized derivatization
- Several impurities to identify and comply with drug agencies (FDA, EMA)

## Objectives

- Method transfer to HPTLC
- Sum formulae of the impurities
- Structures as boni

# Anti-cancer drug: Why on-plate synthesis?



NMR is difficult

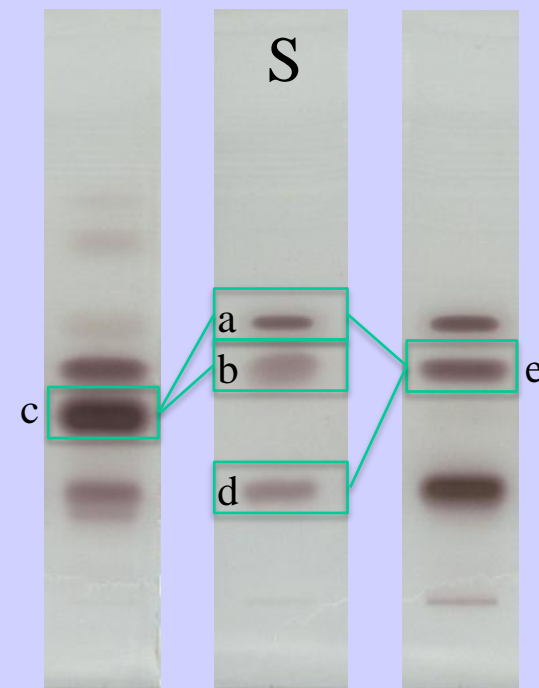
- Binder presence
- Transfer to preparative plate

Need for another way to collect information

- No standards available

On-plate synthesis

- Minimum solvent for sample application
- Reaction, purification and detection via online HRMS elution on the same layer
- Synthesis results after minutes by combining the ingredients
- 22 synthesis in parallel on one 20 x 10 cm HPTLC plate





# Anti-cancer drug – Impurity c

Reaction  $a + b \rightarrow c$

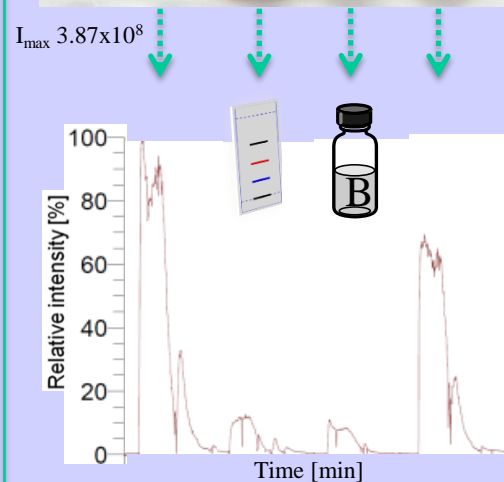
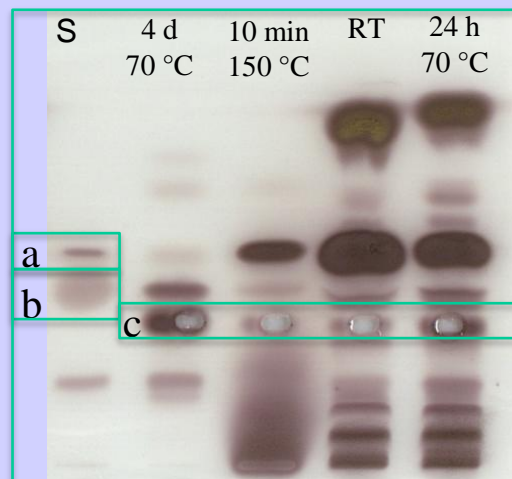
- Traditional synthesis 1 h
- On-plate synthesis 10 min

→ HPTLC-ESI<sup>+</sup>-HRMS

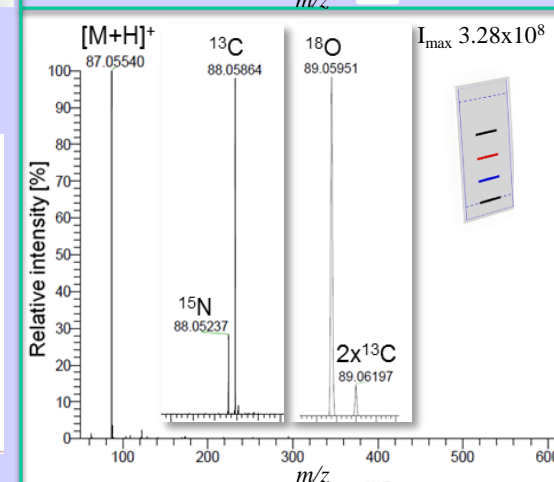
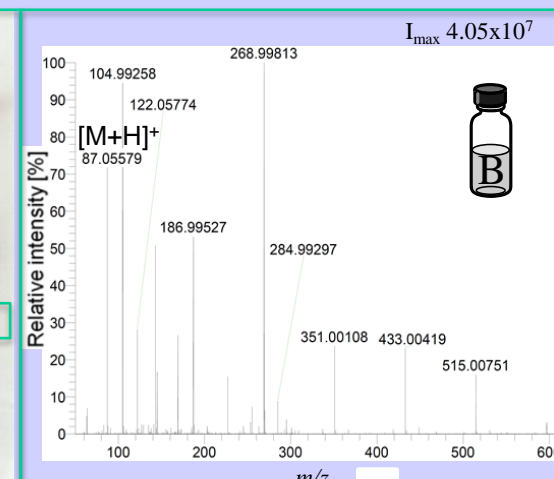
- $[M+H]^+$ :  $[C_3H_7N_2O]^+$
- $m/z$  87.05540  $\pm$  1.3 ppm



HPTLC & EIC at  $m/z$  87.06



HRMS



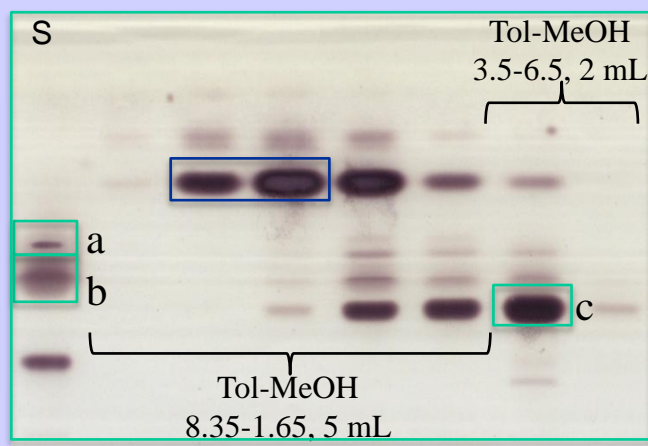
# Anti-cancer drug: SPE-NMR



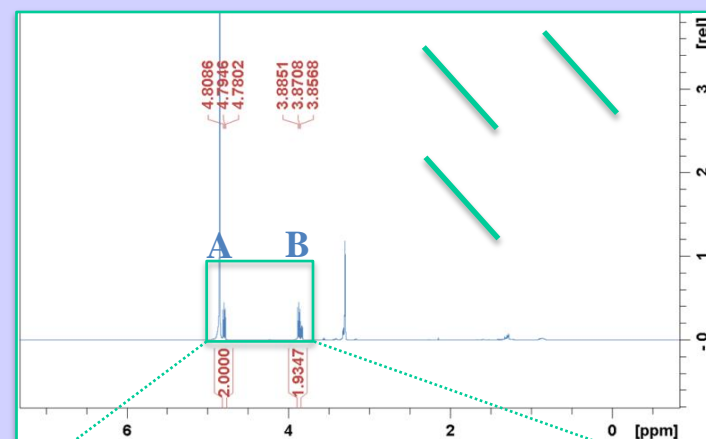
Reaction/SPE



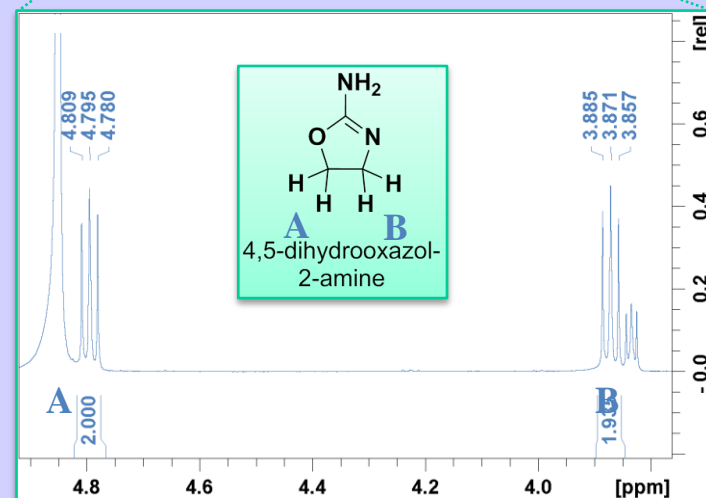
Isolation of c



NMR



- Reaction conditions: toluene, 1 h, 110 °C
- Isolation by SPE-NMR and HPTLC monitoring
- NMR-<sup>1</sup>H: 600 MHz, <sup>13</sup>C: 150 MHz; CD<sub>3</sub>OD
- <sup>1</sup>H:  $\delta = 4.79$  (t,  $J = 8.5$  Hz, 2H),  
3.87 (t,  $J = 8.5$  Hz, 2H)
- <sup>13</sup>C:  $\delta = 163.6, 70.8, 43.5$





# Anti-cancer drug – Impurity e

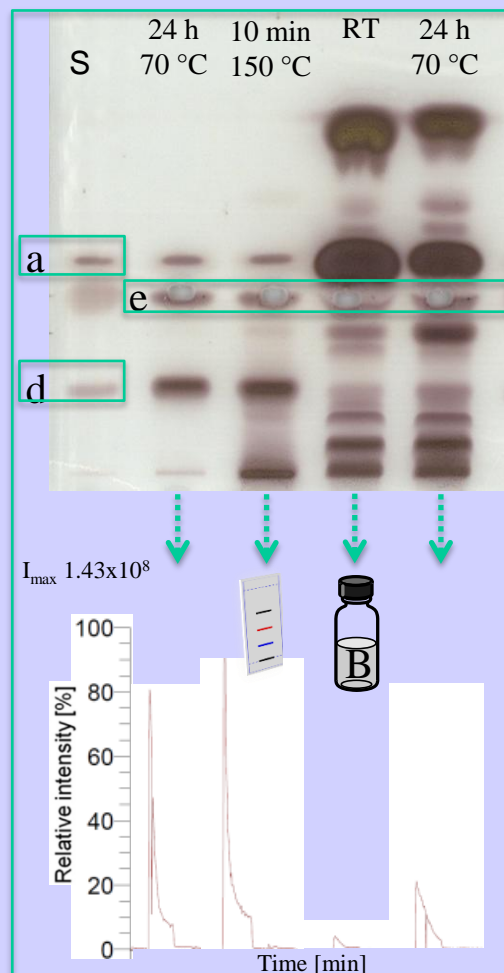
- Reaction  $a + d \rightarrow e$

→ HPTLC-ESI<sup>+</sup>-HRMS

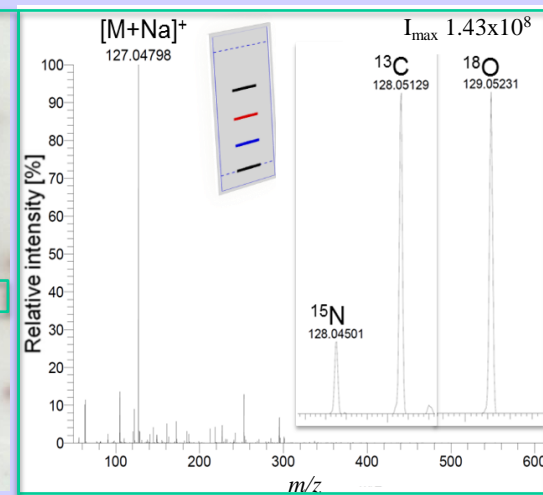
- $[M+Na]^+$ :  $[\text{NaC}_3\text{H}_8\text{N}_2\text{O}_2]^+$
- $m/z$  127.0480  $\pm$  1.4 ppm
- On-plate synthesis showed same band pattern of *e* as traditional synthesis



HPTLC & EIC of  $m/z$  127.05



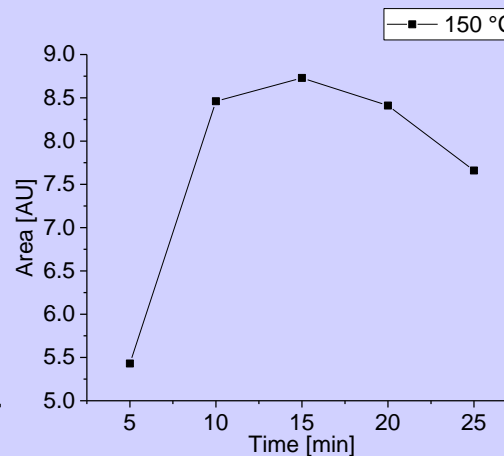
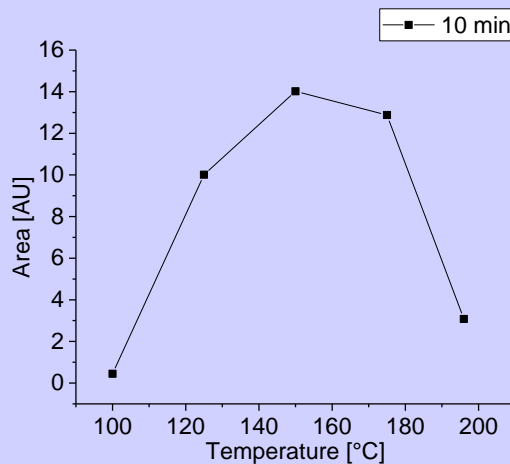
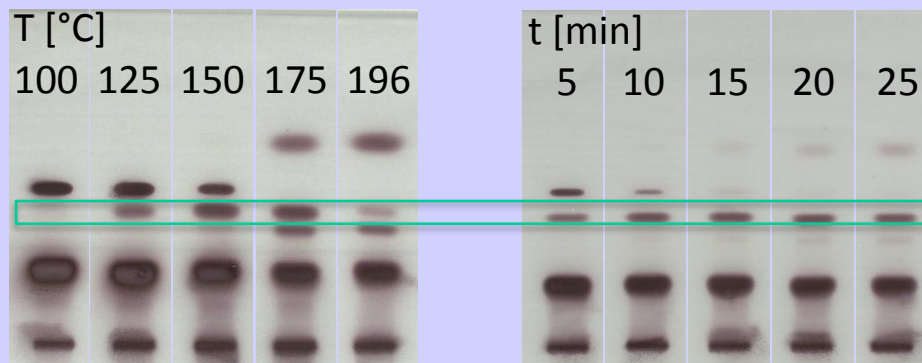
HRMS





# Anti-cancer drug – Impurity e

- Reaction control via videodensitometry
- Derivatized bands unstable → scanning not possible
- Optimization of reaction conditions



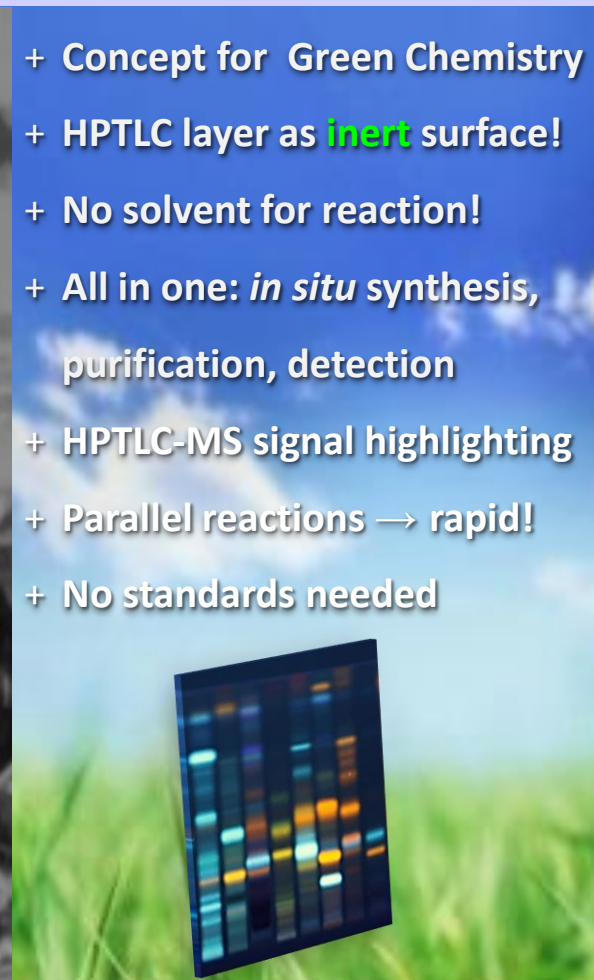
# Conclusion



## Conventional synthesis *versus* surface synthesis

- Solvent
- Time-consuming steps
- Parallel reactions difficult

- + Concept for Green Chemistry
- + HPTLC layer as **inert** surface!
- + No solvent for reaction!
- + All in one: *in situ* synthesis, purification, detection
- + HPTLC-MS signal highlighting
- + Parallel reactions → rapid!
- + No standards needed



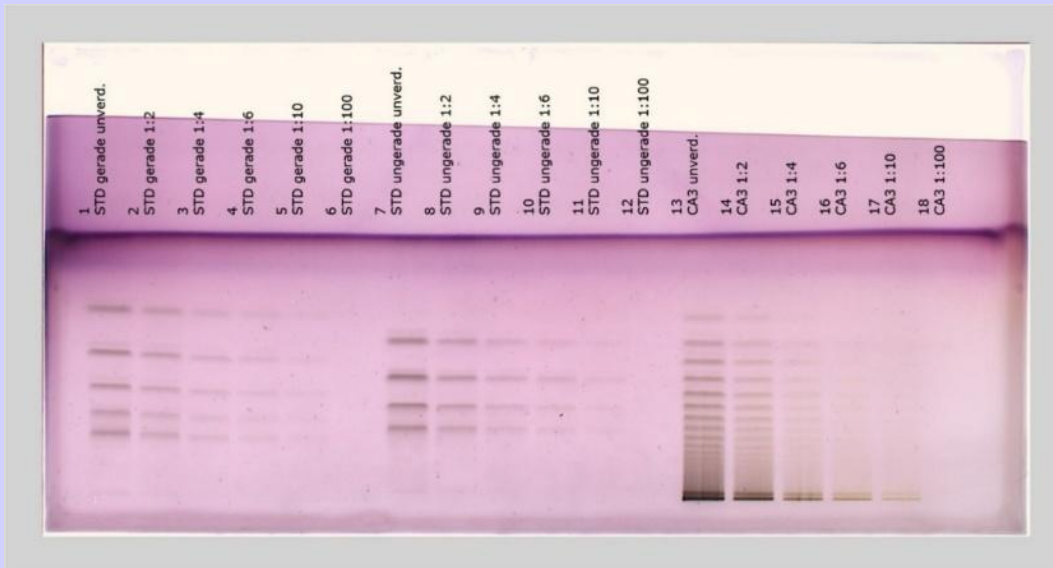




# Contributions orales



O8- Oberlerchner :  
**Bridging the Analytical Gap –  
Comprehensive Analysis of  
Cellooligosaccharides by HPTLC**



$g \cdot mol^{-1}$

390

678

966

1255

1543

1831

2120

2408

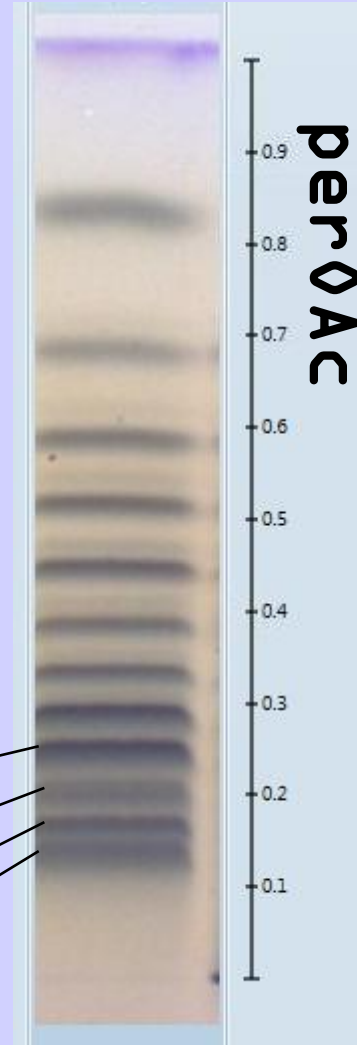
2696

2984

3273

3561

01igo ladder  
perOAC



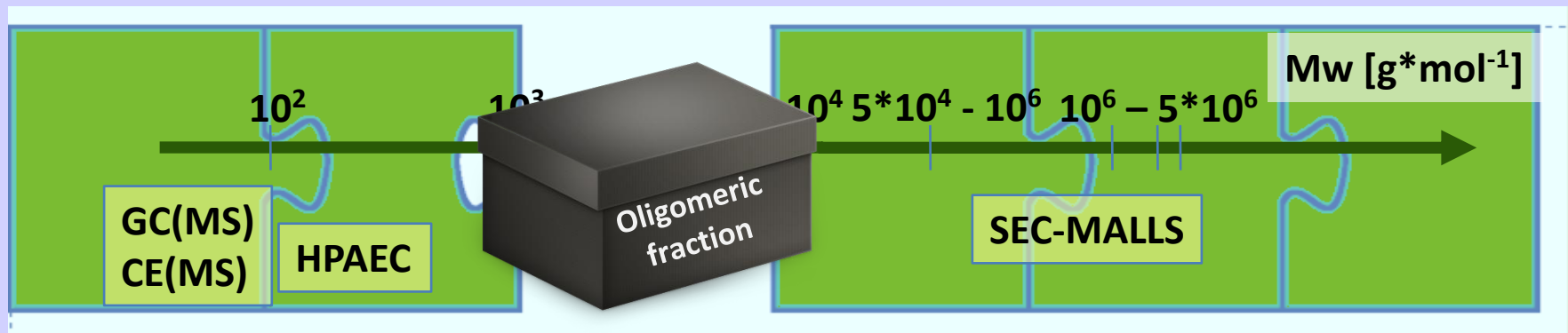
# Contributions orales



O8- Oberlerchner :

## Bridging the Analytical Gap – Comprehensive Analysis of Cellooligosaccharides by HPTLC

- Preparation of cellooligomers yielded valuable standard compounds
- Direct mass spectrometry from plates (MALDI Coupling)
- New HPTLC method for cellooligomers
- Introduction of the Oligo-Ladder







# Contributions orales

O9-Reich, Frommenwiler :

**How can HPTLC help the botanical industry to improve quality of botanical products in a pragmatic way?**

Participation in **5** case studies over the last 3 years, involving dietary supplements (DS), and medicines (Traditional Herbal Registration THR):



Ginkgo

The header for the Ginkgo case study features a green rectangular box with the name 'Ginkgo' in white text. Above the box are two circular icons: the Union Jack flag and the European Union flag.

35 samples  
Products  
(DS)



SJW

The header for the SJW case study features a green rectangular box with the name 'SJW' in white text. Above the box are three circular icons: the European Union flag, the Union Jack flag, and the United States flag.

84 samples  
Herbal drug, extracts  
and Products  
(DS and THR)



Black cohosh

The header for the Black cohosh case study features a green rectangular box with the name 'Black cohosh' in white text. Above the box is a circular icon of the United States flag.

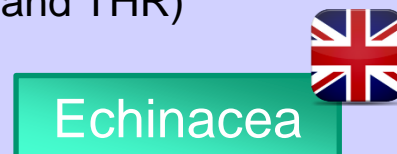
74 samples  
Ingredients and  
products (raw material  
and DS)



Milk thistle

The header for the Milk thistle case study features a green rectangular box with the name 'Milk thistle' in white text. Above the box is a circular icon of the Union Jack flag.

31 samples  
Products  
(DS and THR)



Echinacea

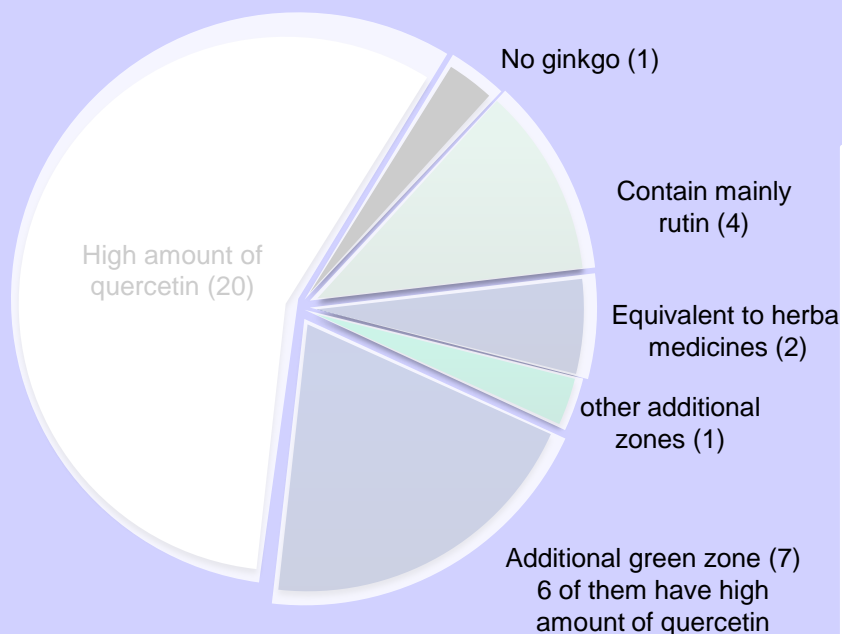
The header for the Echinacea case study features a green rectangular box with the name 'Echinacea' in white text. Above the box is a circular icon of the Union Jack flag.

23 samples  
Products  
(DS and THR)



# Ginkgo products - the findings:

Total: 35 samples



- The two most expensive products contain a large amount of quercetin / fingerprints are similar to those of other cheap products
- The products equivalent to herbal medicines cost around € 15,00
- The “No ginkgo” products cost around € 22,00

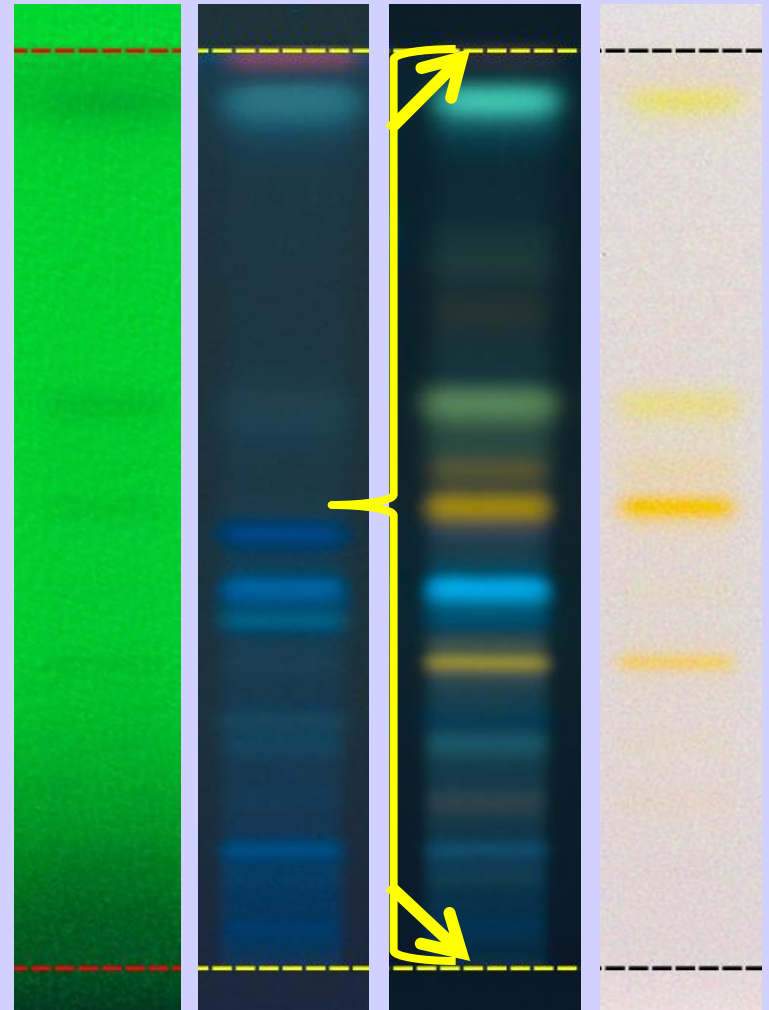


# HPTLC Fingerprint (part 1): single track



## The HPTLC fingerprint:

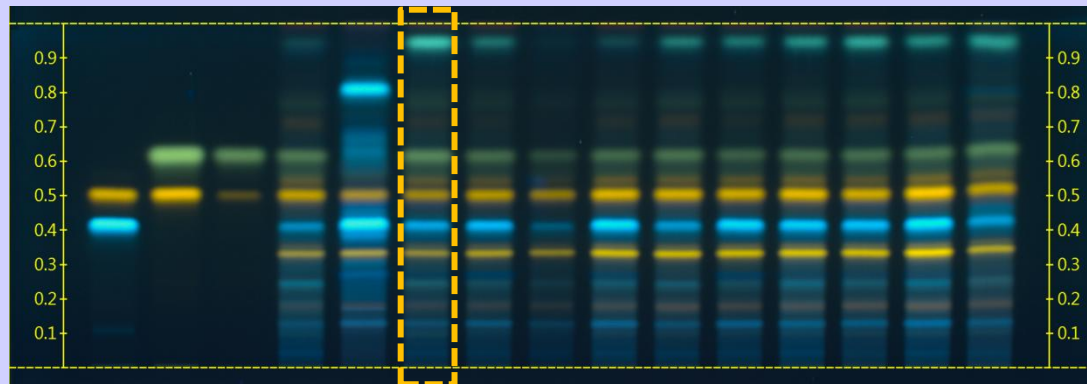
- is the (electronic) image of the visual HPTLC – chromatogram
- represents the sample (sample ID)
- includes application and front position
- consists of a sequence of (colored) zones
- may be a stack of multiple images in different detection modes



# HPTLC Fingerprint (part 2): the plate



The HPTLC fingerprint is part of the image taken of the entire HPTLC plate:



- Up to 14 fingerprints may be on the same plate
- One track (usually the first) is assigned as SST
- Clearly structured chromatograms, zones parallel and horizontal
- $R_f$  scale is aligned with application position and front

Through the image of the plate the fingerprint is linked to the plate identity:

- Chromatographic conditions are recorded
- Compliance with cGMP is assured

As long as the chromatographic conditions are the same and the SST is passed, fingerprints from different plates can be compared!

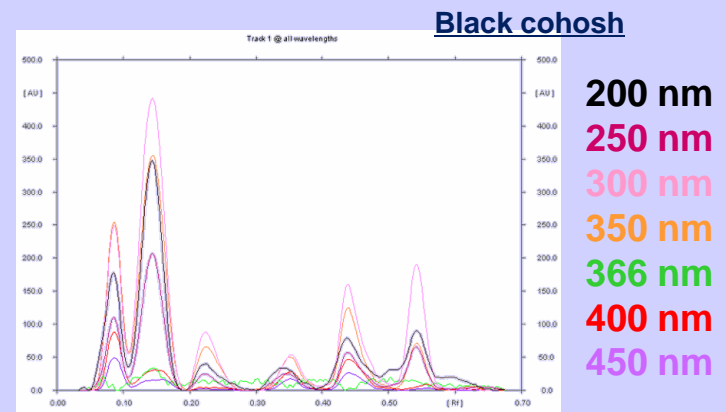


# HPTLC Fingerprint (part 3): profiles

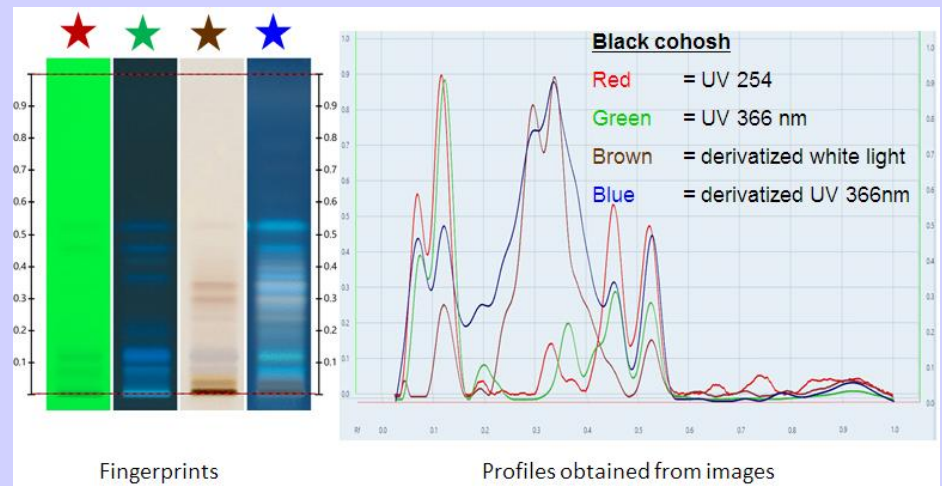


HPTLC chromatograms can also be presented as profile

- Information from scanning densitometry
  - 200 nm to 800 nm
  - Absorption and/or fluorescence measurement

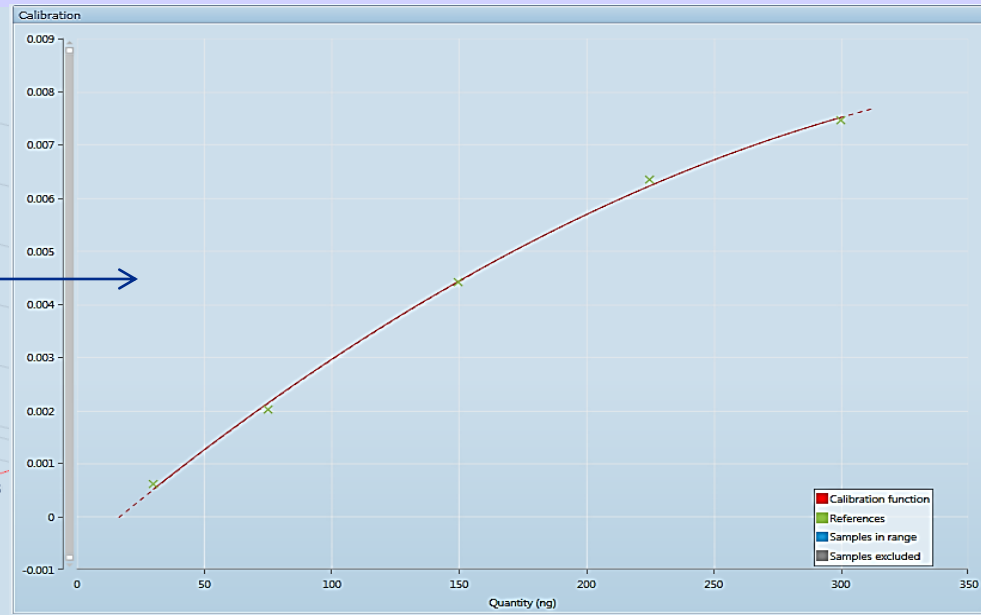
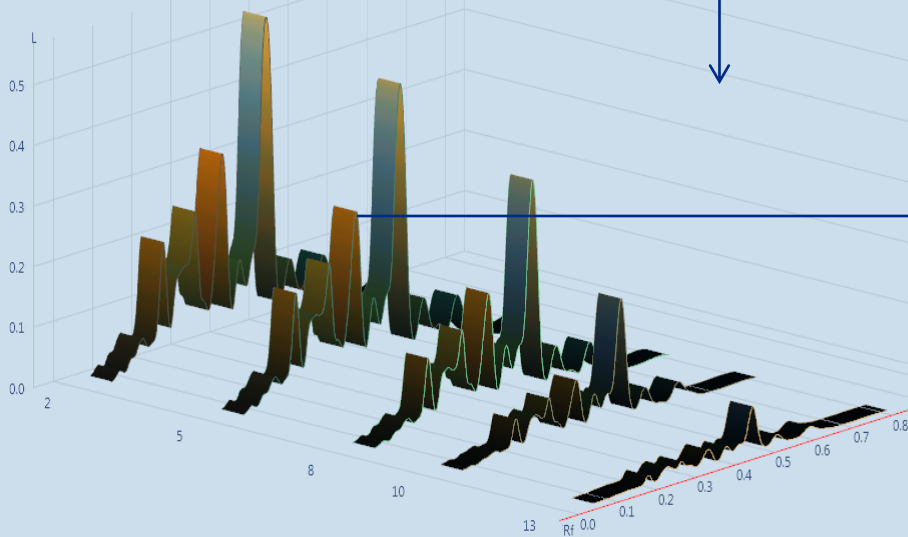
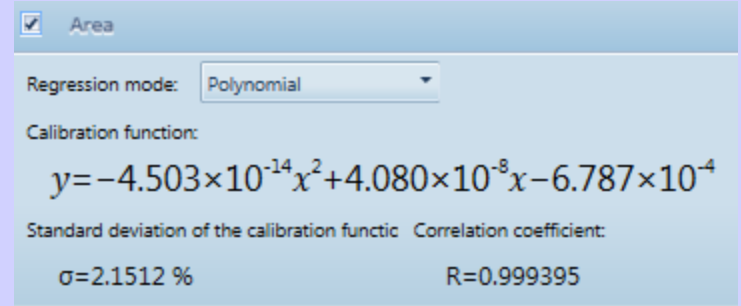
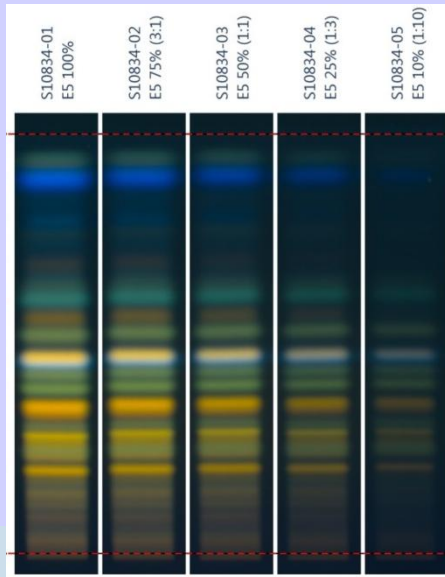


- Information from image raw data
  - UV 254 nm, UV 366 nm, (white light) prior to derivatization
  - (UV 254 nm), UV 366 nm, white light after derivatization





# HPTLC Fingerprint (part 4): Quantitative information





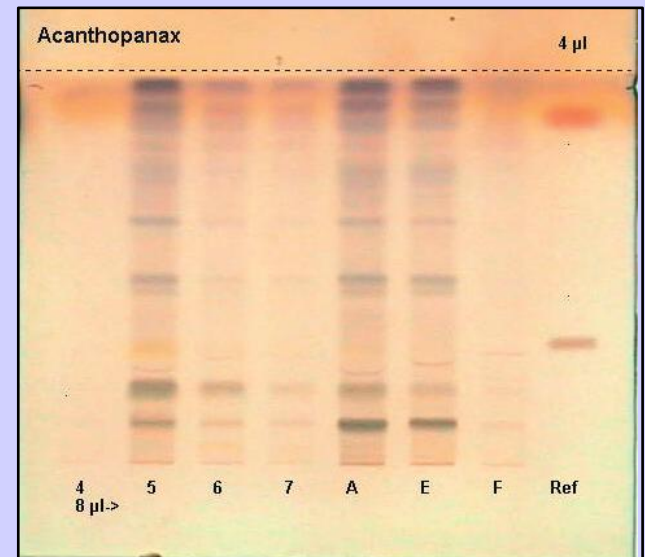
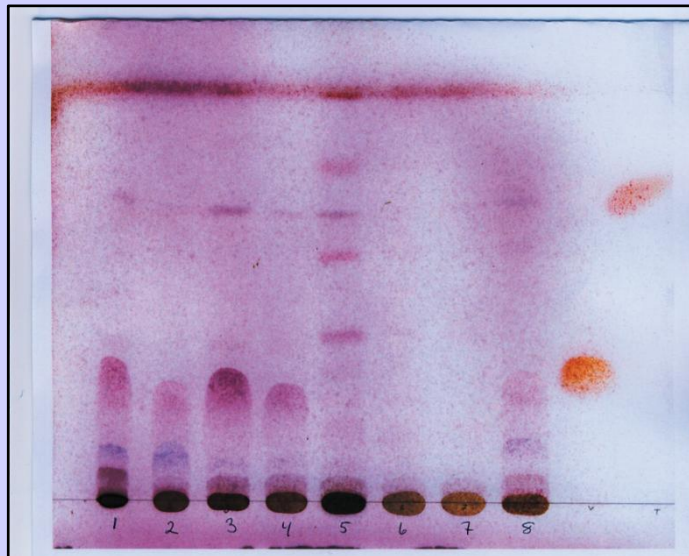


# Contributions orales


O11-Canigüeral:

## HPTLC for herbal drugs and herbal drug preparations in the European Pharmacopoeia

... ou comment lutter contre la variabilité des analyses sur plaque et améliorer la qualité et la régularité des médicaments à base de plantes.



### 2.8.25 High-performance thin-layer chromatography of herbal drugs and herbal drug preparations



a monograph, for example: *lactic reagent R* which is used to show the presence of various features, 10 per cent V/V alcoholic solution of *phloroëluçinol R* and *hydrochloric acid R*, which are used for the detection of starch in tissues. The test is performed as follows: a solution of the preparation to be examined is placed on a glass plate coated with a uniform, porous layer (average pore size 6 nm), typically 200 µm thick, of irregular particles of silica gel between 2 µm and 10 µm in size and with an average size of 5 µm, a polymeric binder and a fluorescence indicator (F<sub>254</sub>). The results are qualified using a system-specific suitability test.

01/2017:20825

#### 2.8.25. HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY OF HERBAL DRUGS AND HERBAL DRUG PREPARATIONS

High-performance thin-layer chromatography (HPTLC) is used for qualitative analysis of herbal drugs and herbal drug preparations. It is a thin-layer chromatographic technique (2.2.27) that, unless otherwise stated in an individual monograph, uses a glass plate coated with a uniform, porous layer (average pore size 6 nm), typically 200 µm thick, of irregular particles of silica gel between 2 µm and 10 µm in size and with an average size of 5 µm, a polymeric binder and a fluorescence indicator (F<sub>254</sub>). The results are qualified using a system-specific suitability test.

General Notices (1) apply to all monographs and other texts

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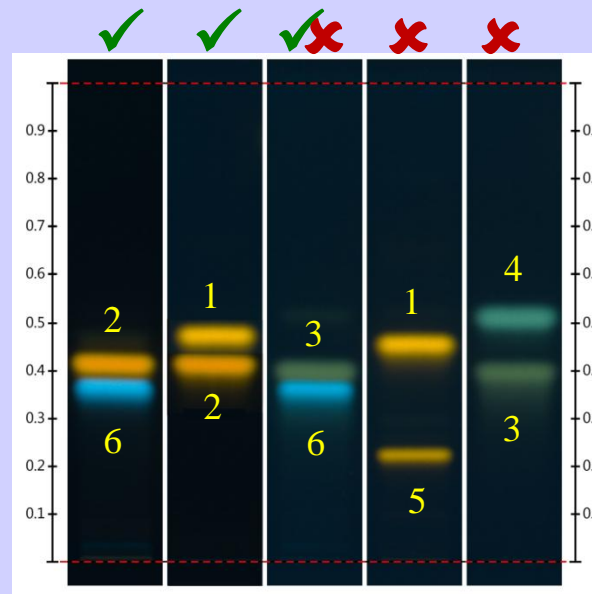
## *Ph. Eur.* improvements (chapter 2.8.25)

### 1. Improvement of reproducibility

- ✓ Introduction of HPTLC
- ✓ Standardisation of methodology
- ✓ **Introduction of a system suitability test** (qualification of the plate)

#### Flavonoids

Developing solvent:  
Ethyl acetate / formic acid / water  
(80:10:10)



1. Isoquercitrin
2. Hyperoside
3. Isovitexin
4. Apigenin
5. Rutin
6. Chlorogenic acid

# Introduction of an intensity marker



Ph. Eur. 2.8.25

**Example of intensity marker:** chlorogenic acid (CA)

## Visual intensity description

### Intense zone:

More intense than CA zone intensity (a)

### Zone with no descriptor for intensity:

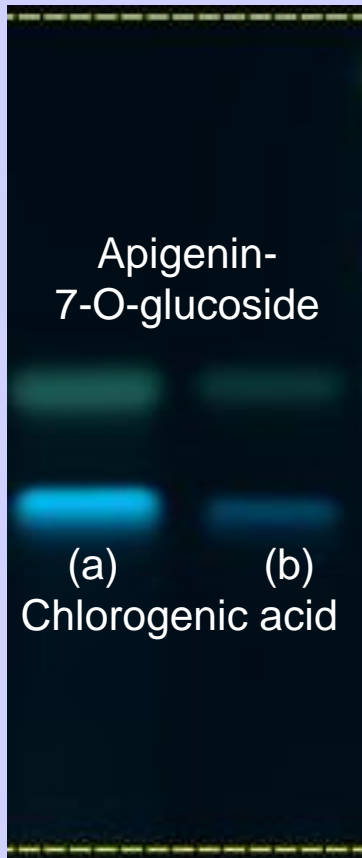
Similar in intensity to CA zone intensity (a)

### Faint zone:

Less intense than CA zone intensity (a) but equal to or more intense than CA zone intensity (b)

### Very faint zone:

Less intense than CA zone intensity (b)



R → R<sup>1/4</sup>  
4-fold dilution

**R and R<sup>1/4</sup>:** Reference solutions





# Contributions orales



O30-Shiea :

## TLC Combined with Flame Atmospheric Pressure Chemical Ionization Mass Spectrometry (FAPCI/MS) for Sample

**Analysis** *Shiea, J et al.; Rapid Commun. Mass Spectrom. 2016, 30, 890-896.*



*avant*

Summary



*après*

- The charged species generated in a flame were used to react with analytes to form analyte ions.
- No need to use high voltages and inert gas for ionization.
- Analytes on TLC plate can be directly desorbed and ionized by desorption-FAPCI/MS.
- A laser beam was used to desorb analytes on a TLC plate for FAPCI/MS analysis.
- Volatile and semi-volatile compounds could be detected by TLC/DFAPCI/MS and TLC/LD-FAPCI/MS.

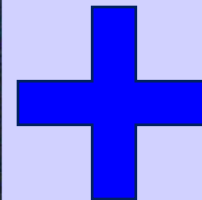
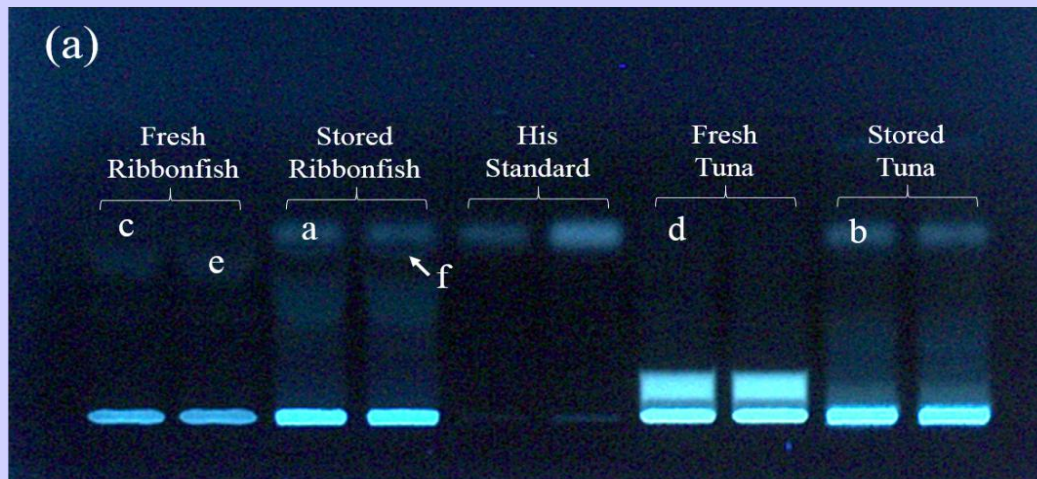


# Contributions orales



O33-Chen :

## TLC-SERS as an effective tool for Histamine screening



## Facile analysis at hand

SERS provides a cost-efficient and user-friendly tool for molecule-specific identification of TLC results. With the popularizing of portable Raman spectroscopy devices, this strategy is of a promising future in food and drug screening, particularly attractive to laboratories with limited resources.



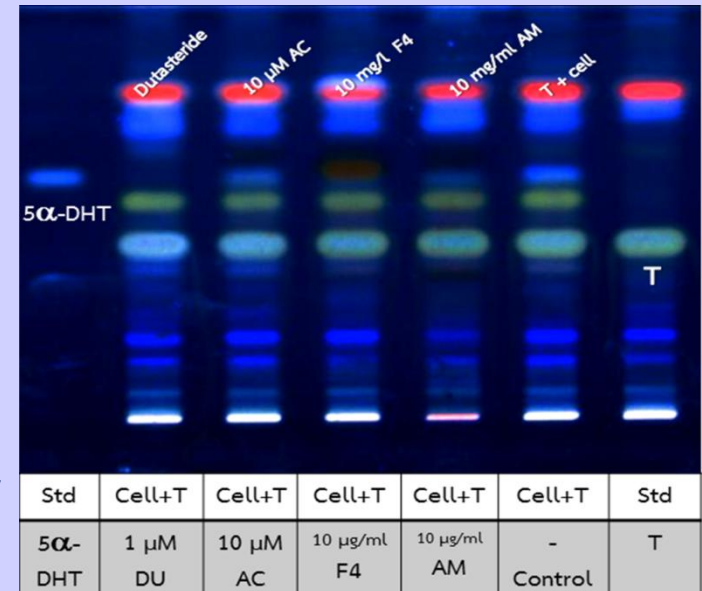
# Contributions orales



O36-Chen De-Eknamkul :

## HPTLC detection of steroid $5\alpha$ -reductase activity from a non-radioactive cell-based assay

- We have successfully developed an HPTLC method for detection of  $5\alpha$ -R activity without using radioactive substrate and sensitive enough to be used for cell-based assay
- The method allowed us to find -an active plant extract of *Avicennia marina* among many with clear  $5\alpha$ -R inhibitory activity, and its active constituent, avicequinone C, was isolated for detailed studies
- Both AM extract and AC compound could inhibit not only the conversion of T to DHT but the translocation of AR-ligand into the nucleus
- The treated human dermal papilla cells could produce two of the four key hair growth-promoting growth factors, VEGF and HGF
- More results about safety, efficacy, formulation is under studies.



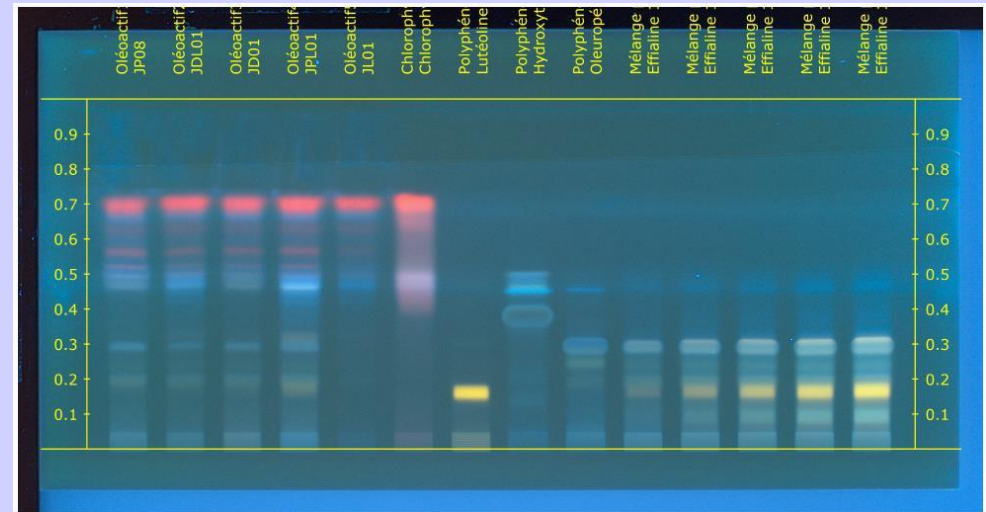
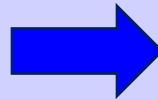
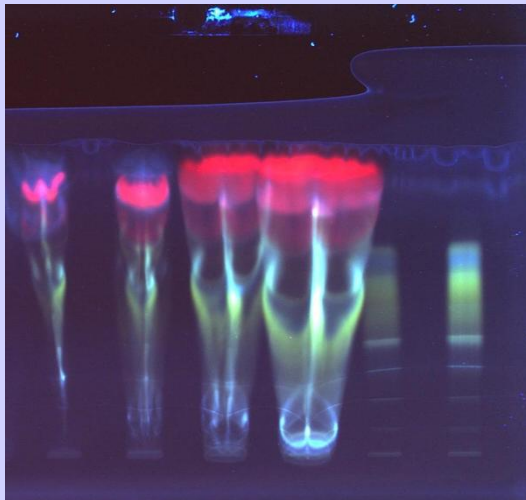


# Contributions orales



O37-Fadel :

## Antioxydants in structured vegetable oils : identification via HPTLC



5 oléoactifs®

4 témoins

Polyphénols  
de feuilles  
d'olivier

Avec de la persévérance et un minimum d'équipement  
il est possible de bien caractériser des oléoactifs®



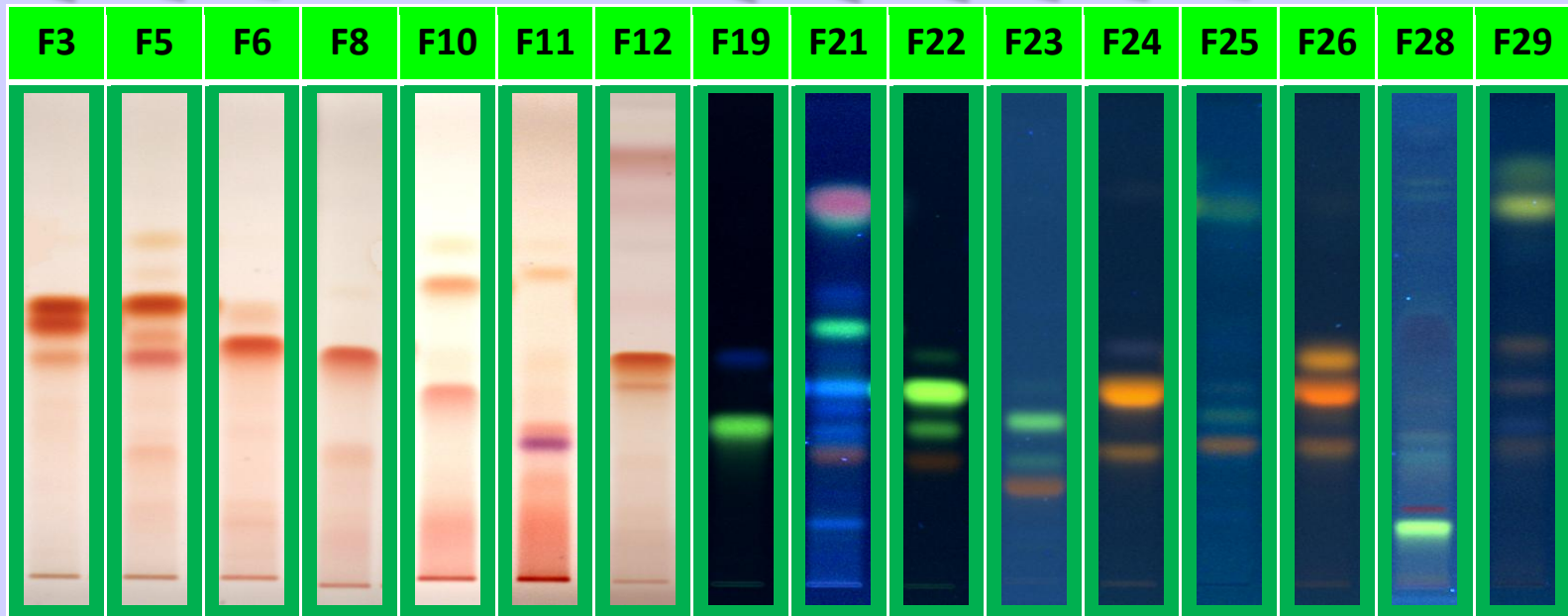
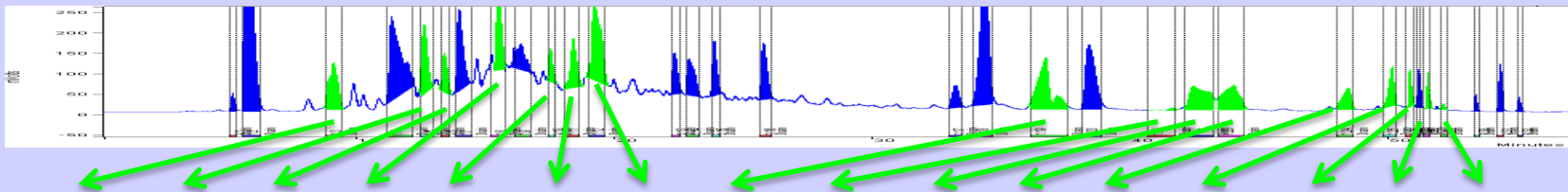




# Contributions orales

O38-Ballert :

## Multidimensional Chromatography (HPLC-HPTLC) for Identification of Antifungal Substances in Rheum Root Extracts







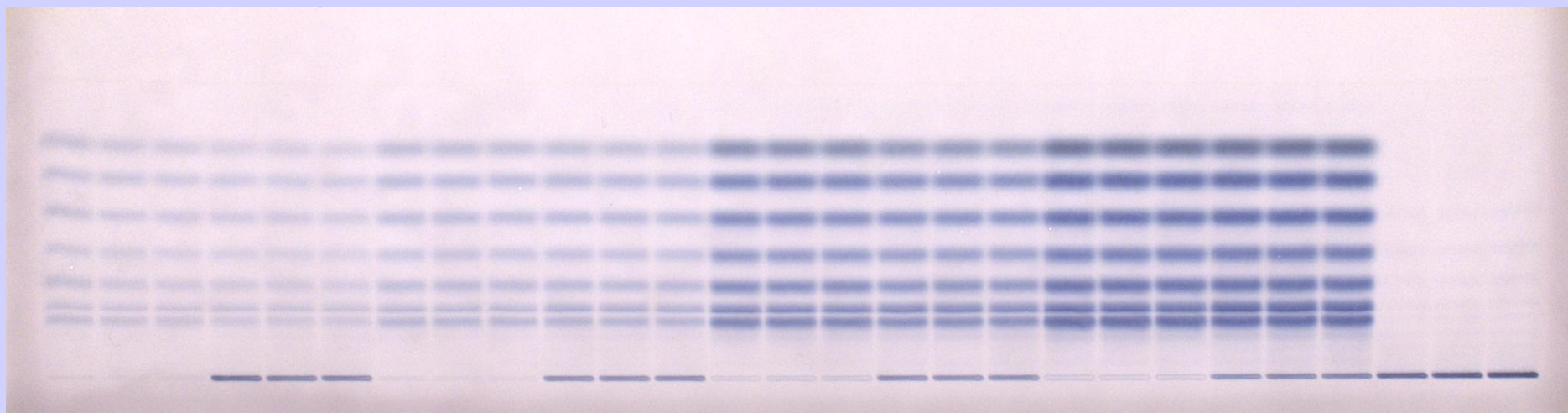
# Contributions orales

O58-Baeyens, Luquet, Roussel :

Modern HPTLC methods validation

Application of prediction intervals to dextrine profiles of enzymatic digestion of starch and baking products

## ■ Specificity



Std level  
1

Spiked  
matrix  
50 ng

Std level  
2

Spiked  
matrix  
100 ng

Std level  
3

Spiked  
matrix  
300 ng

Std level  
4

Spiked  
matrix  
500 ng

Unspiked  
matrix



# Contributions orales

O58-Baeyens, Luquet, Roussel :

Modern HPTLC methods validation

Application of prediction intervals to dextrine profiles of enzymatic digestion of starch and baking products

- Regarding HPTLC methods performance assessment:
  - Method performance may be checked with not more than 5 HPTLC plates !
  - Powerful method validation tools, using total error, exist
    - Standards and official proposals already use these tools (NF V03-110, USP <1210> (PF42(5)), ISO 21748...)
  - Accuracy profiles and uncertainty profiles are easy to obtain and both give a clear view on the real performance of analytical methods,

**Who may still say that HPTLC methods performance cannot be checked and that HPTLC is not a quantitative method ?!**



# **New approach to development of planar chromatograms (O19)**

A. Hałka-Grysińska, K. Skop, M. Gorzkowska,  
A Klimek-Turek, Tadeusz H. Dzido

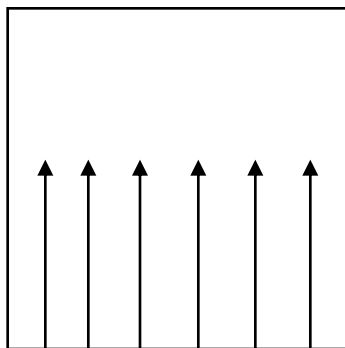
**Medical University of Lublin, Lublin, Poland**

**HPTLC 2017, Berlin**

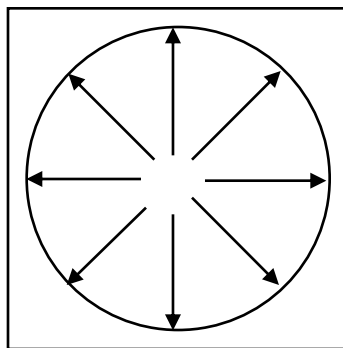
# Introduction - modes of chromatogram development

Capillary forces – conventional development

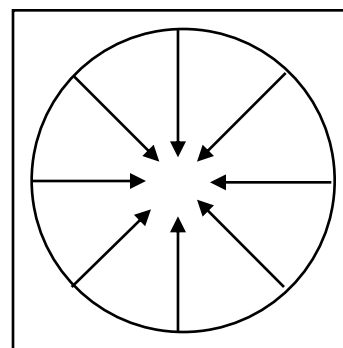
a) Linear



b) Circular



c) Anticircular



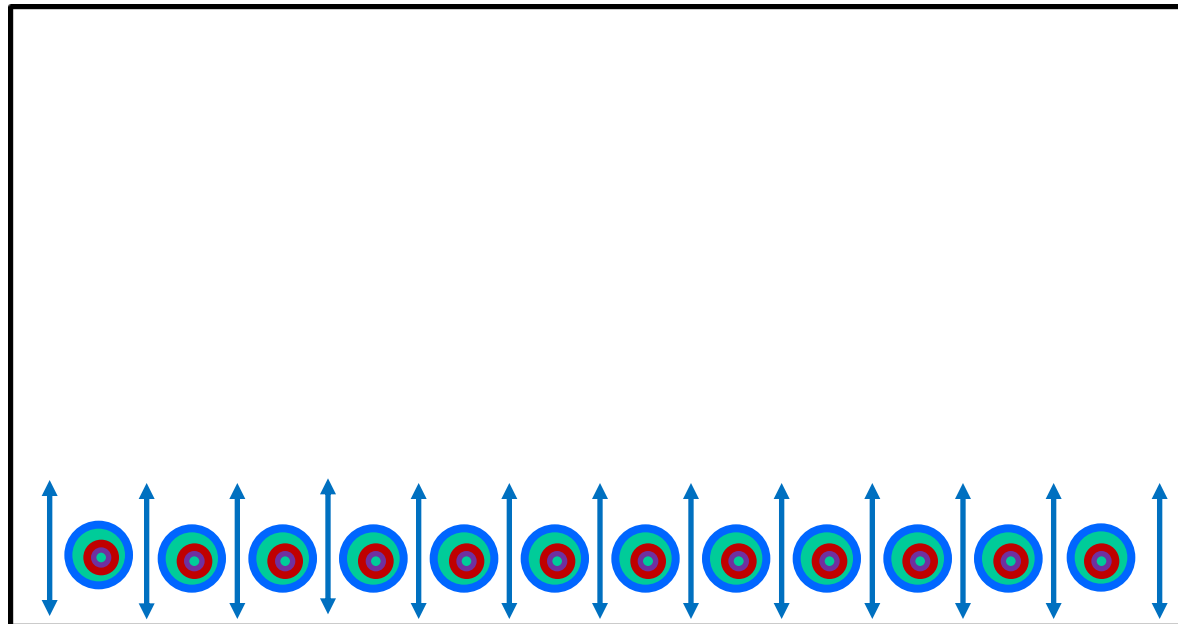
Forced flow:

hydrodynamic (pumped) flow

centrifugal force

electrokinetic phenomena

# Sample preparation to instrumental analysis







# Contributions orales



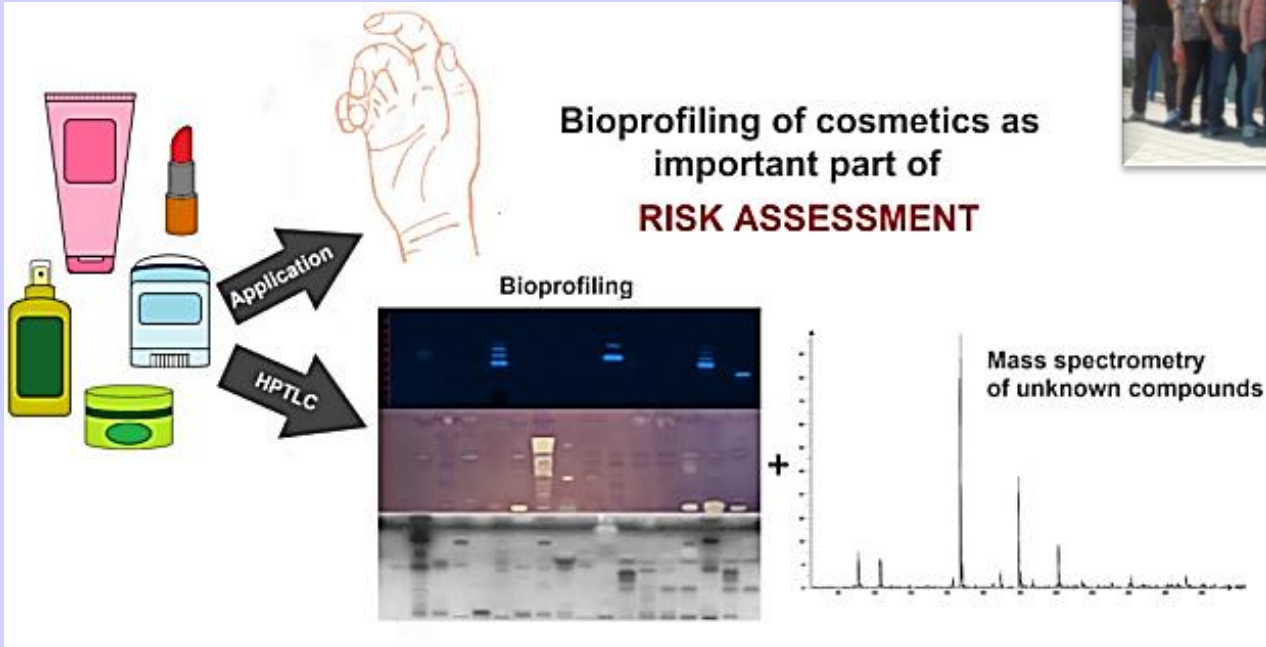
O46-Poole:

What Every Chromatographer Should Know About Solvents

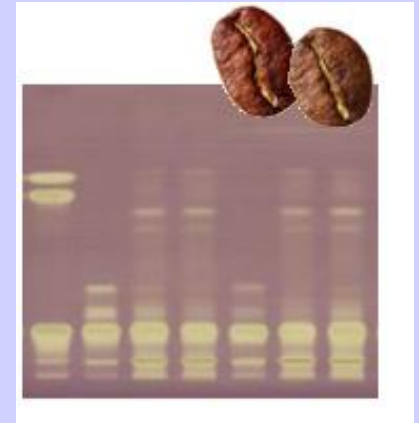
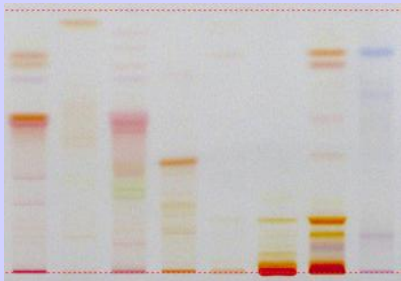
- Available in a pure form at a reasonable cost
- Reasonable shelf-life (stable)
- Low viscosity (water 1 cP, propan-2-ol 2.4 cP)
- Low surface tension (water 73 mN/m)
- Modest vapor pressure at room temperature (50<bp<125°C)
- Capability to form mixtures with other liquids
- Safe to use (Reactivity/Toxicity/Flammability)
- Compatible with the detection system
  - Column chromatography: critical for on-line detection
  - Planar chromatography: not important for volatile solvents



# We love HPTLC!



## Thank you!



O7-Stiefel, Morlock :  
Application of hyphenated HPTLC in food,  
commodity and cosmetics analysis

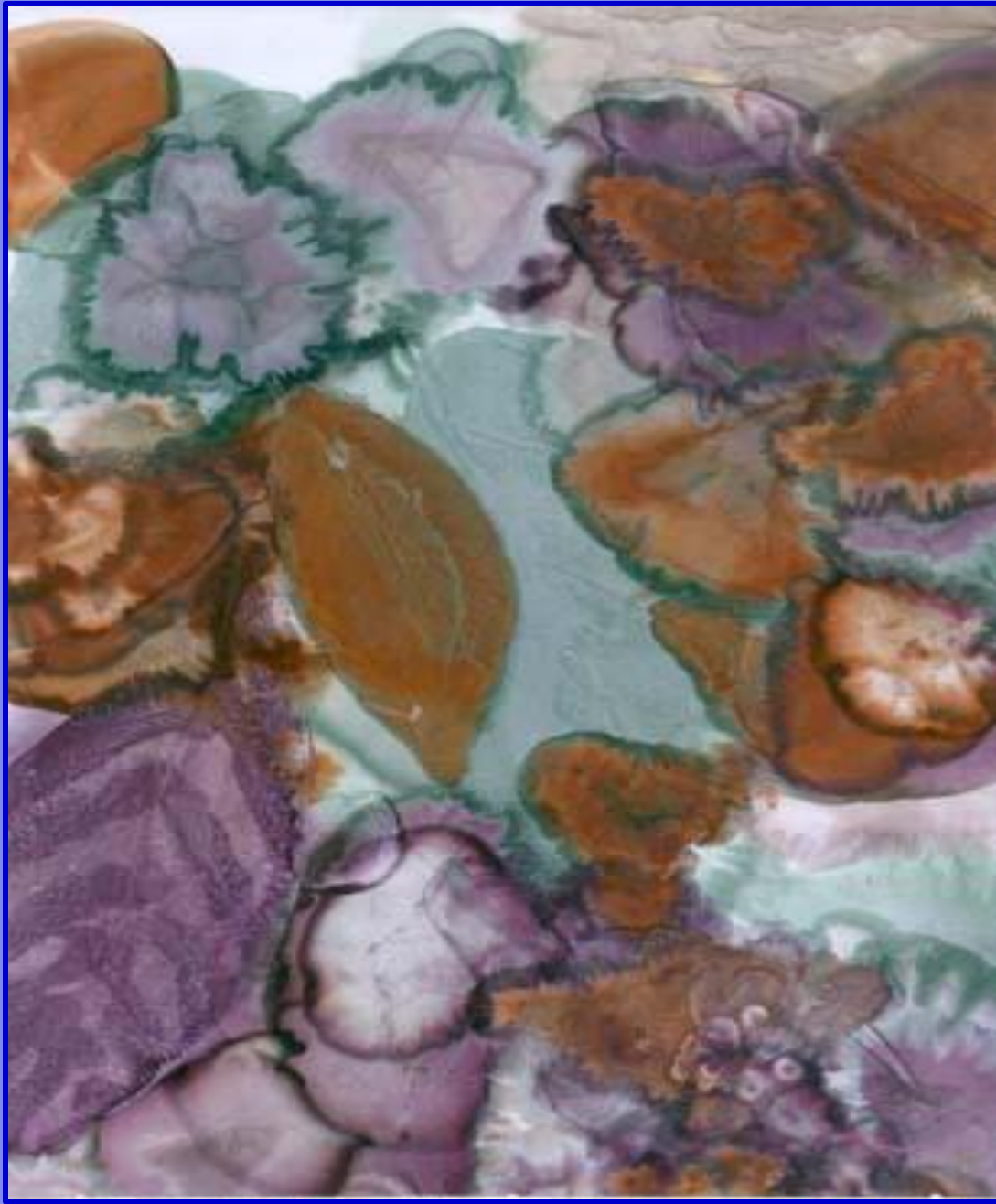
# We love HPTLC!



*explore nature through HPTLC plate window*

photo: Ebrahim Azadniya  
Prof. Morlock HPTLC Lab 2016-05-05

O7- Azadniya, Morlock (aussi P68):  
HPTLC-EDA-HRMS and **PLC-NMR**  
to reveal co-eluting isomers of bioactive zones



**JE VOUS REMERCIE ! III !II**



*"Chromart" by Herbert Halpaap in 1986-1987*