31ème Réunion du Club de CCM
12-13 Avril 2017 à Avignon

Pierre Bernard-Savary, +33 676 29 32 81
clubccm@hptlc.com

3ème Atelier Plantes

Précédents évènements sur ce sujet, toujours en ligne sur www.clubdeccm.com:

Le 1er atelier plantes (&cos) a eu lieu le 22 Octobre 2008, près de Lyon (Les Echets). Ce fut le tout premier atelier.

Le 2ème a eu lieu le 30 Novembre 2011 près de Lyon (Ste Foy les Lyon).

3ème Atelier Plantes

D’autres évènements ont depuis traité des plantes : une après-midi (5 Juin 2013) à Forum Labo; les journées du 1er et 2 Avril 2015 (FL et YR);

A noter dans le cadre du Symposium de 2011 à Bâle le panel discussion du 7 Juillet avec Prof. Zheng-Tao WANG, Dr. Clemens ERDELMEIER, Dr. Eike REICH, Dr. Gudrun ABEL, Dr. Werner KNÖSS, Dr. Troy SMILLIE, Dr. Bernd RENGER, groupe animé par le Prof. Matthias HAMBURGER

En ligne sur www.hptlc.com


INTERNATIONAL SYMPOSIUM FOR HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY
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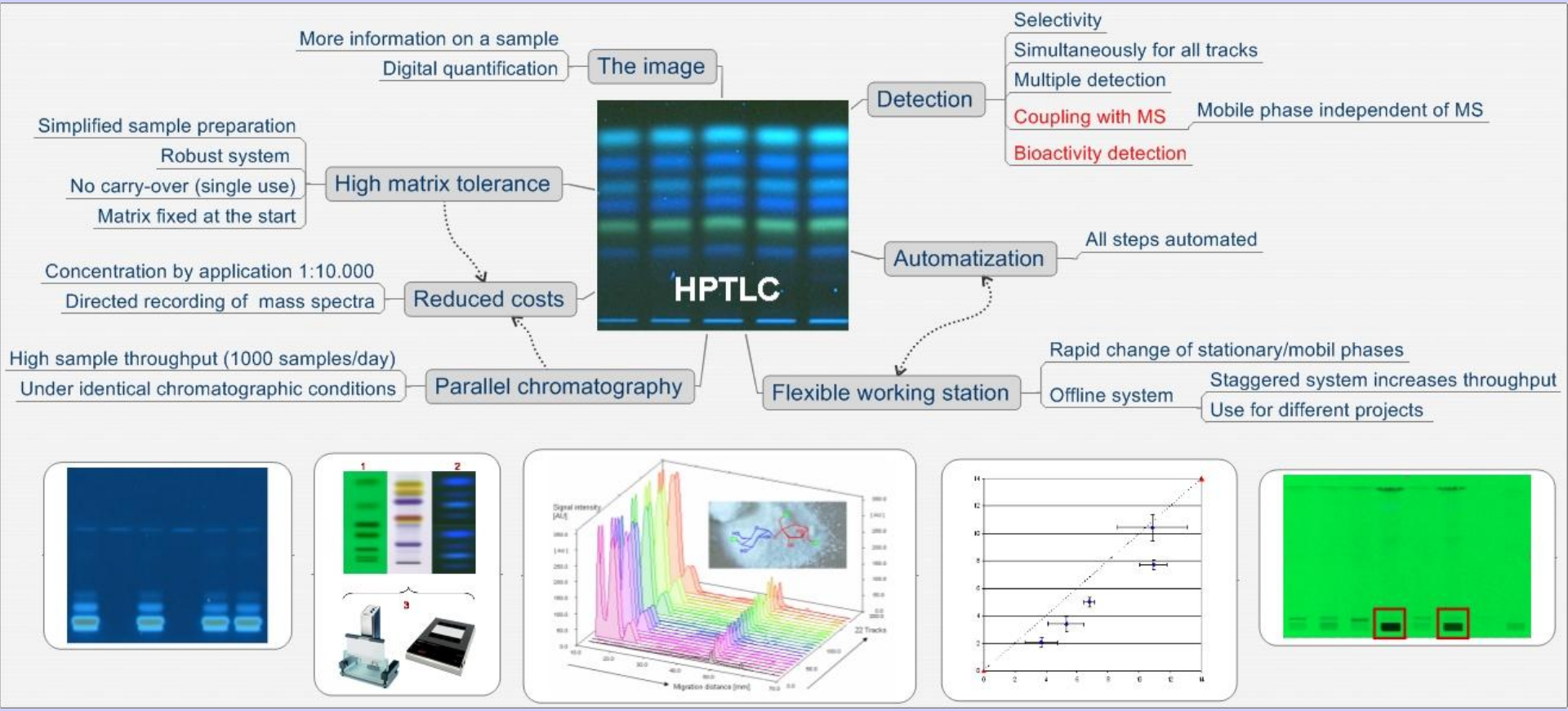
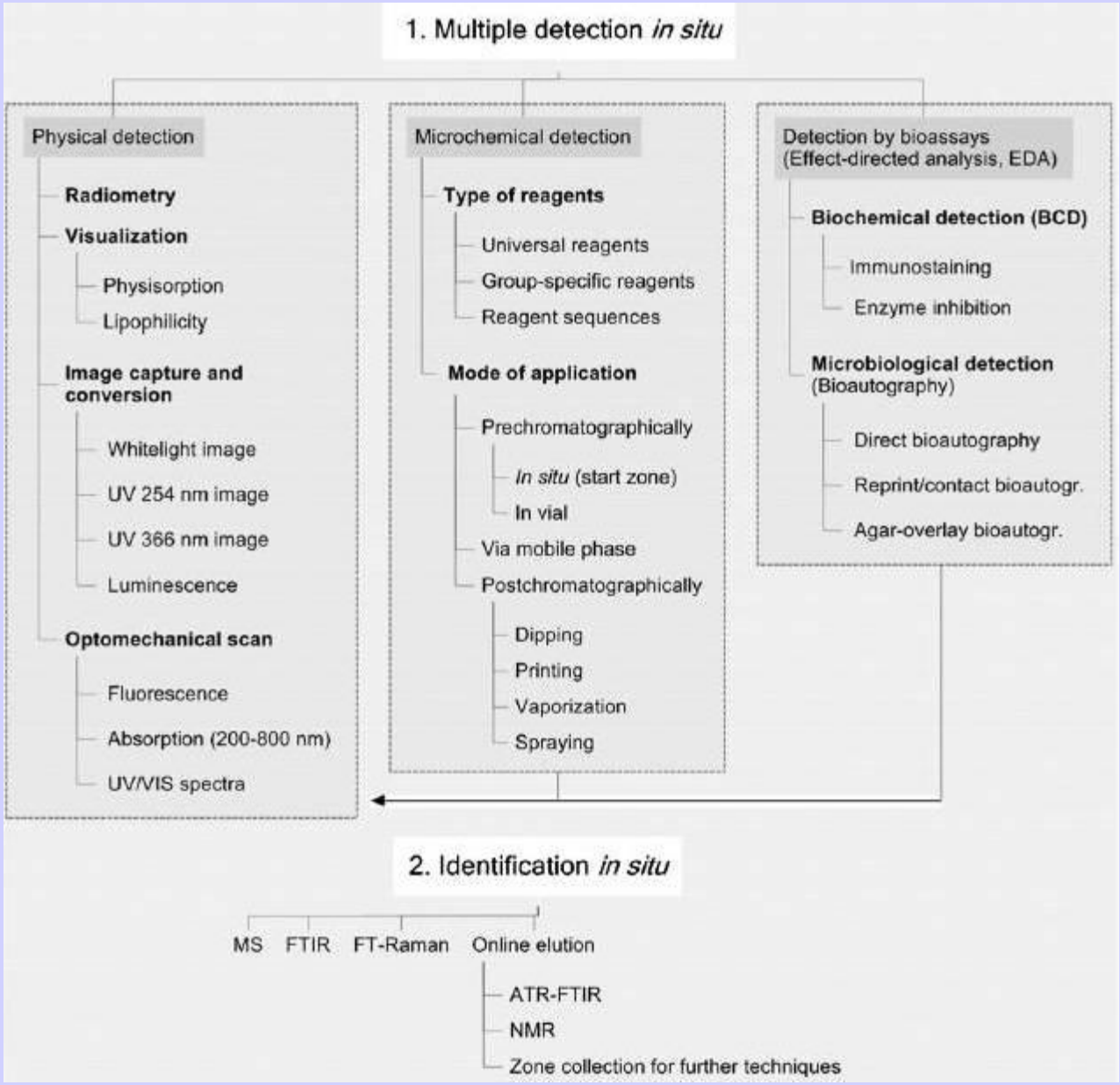
JULY 4-8 th 2017

Rappels généraux



une méthode pleine de ressources !!! … on le sait

G. Morlock, Analytica 2008



G. Morlock, Journal of Chromatography A, 1217 (2010) 6600–6609

Journal of Chromatography A, 1217 (2010) 6600–6609

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Hyphenations in planar chromatography

Gertrud Morlock*, Wolfgang Schwack

University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany

- HPTLC-UV/Vis/FLD-MS [13,14],
- HPTLC-UV/Vis/FLD-bioactivity-HRMS [15],
- HPTLC-UV-FTIR [16,17],
- HPTLC-UV/Vis/FLD-FTIR ATR [18],
- TLC-Vis-SERS [12].

ARTICLE INFO

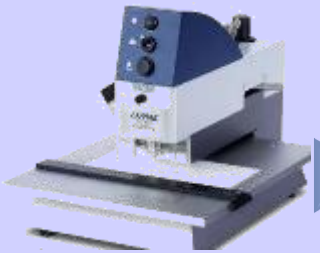
Article history:
Available online 20 May 2010

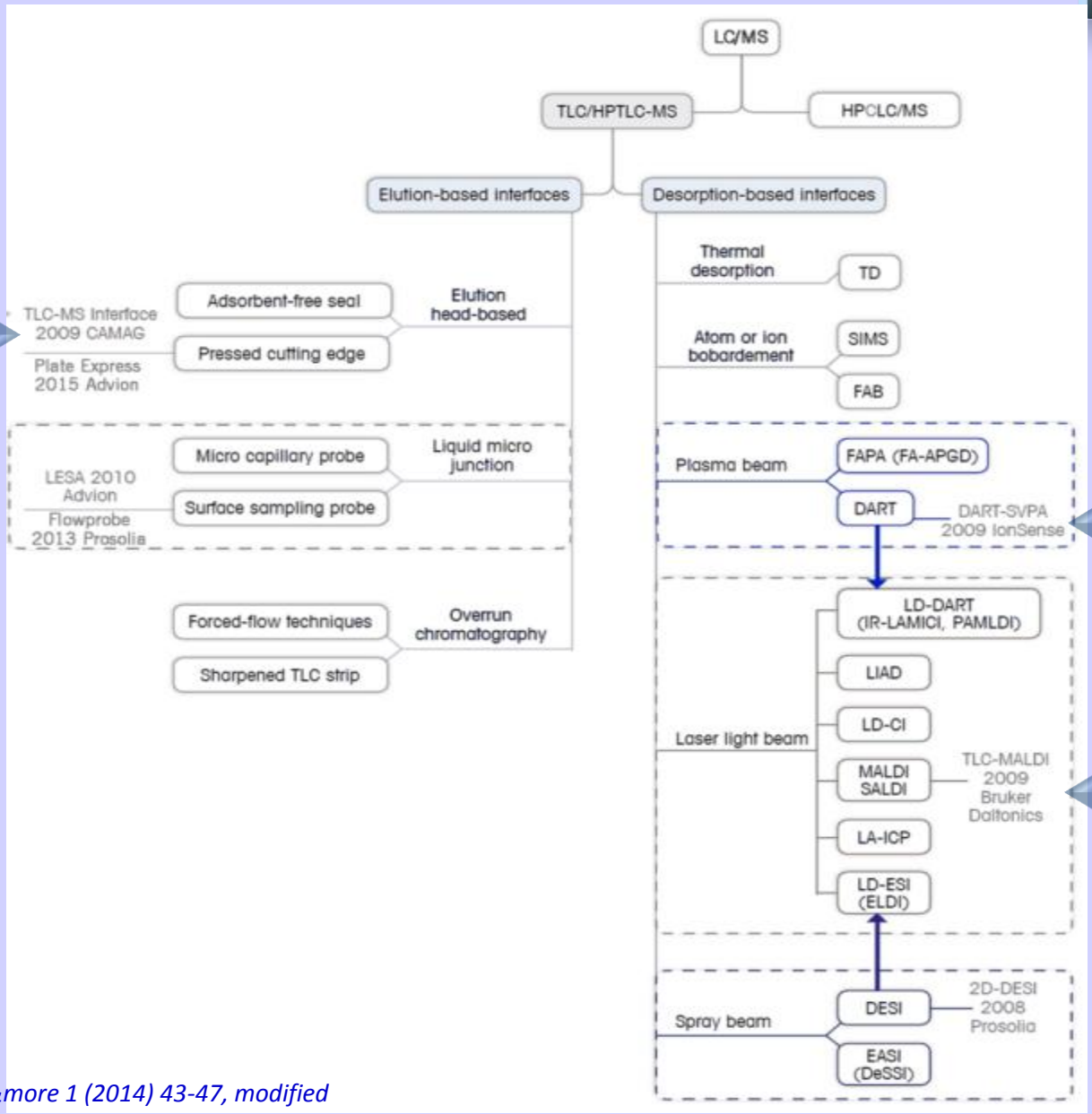
Keywords:
Mass spectrometry
High-performance thin-layer chromatography
Effect-directed analysis
Bioassays
Cost-effective analysis
High-throughput system

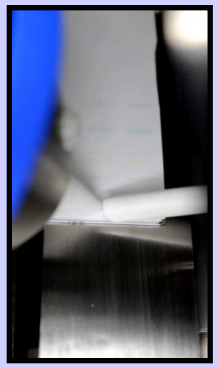
ABSTRACT

This review is focused on planar chromatography and especially on its most important subcategory high-performance thin-layer chromatography (HPTLC). The image-giving format of the open, planar stationary phase and the post-chromatographic evaporation of the mobile phase ease the performance of various kinds of hyphenations and even super-hyphenations. Examples in the field of natural product search, food and lipid analysis are demonstrated, which point out the hyphenation with effect-directed analysis (EDA) and mass spectrometry and illustrate the efficiency gain. Depending on the task at hand, hyphenations can readily be selected as required to reach the relevant information about the sample, and at the same time, information is obtained for many samples in parallel. The flexibility and the unrivalled features through the planar format valuably assist separation scientists.

*"with U/HPLC, after separation, samples go to the waste; with **HPTLC**, after separation, **samples remain on the dried plate**" (G.Morlock, HPTLC'11)*



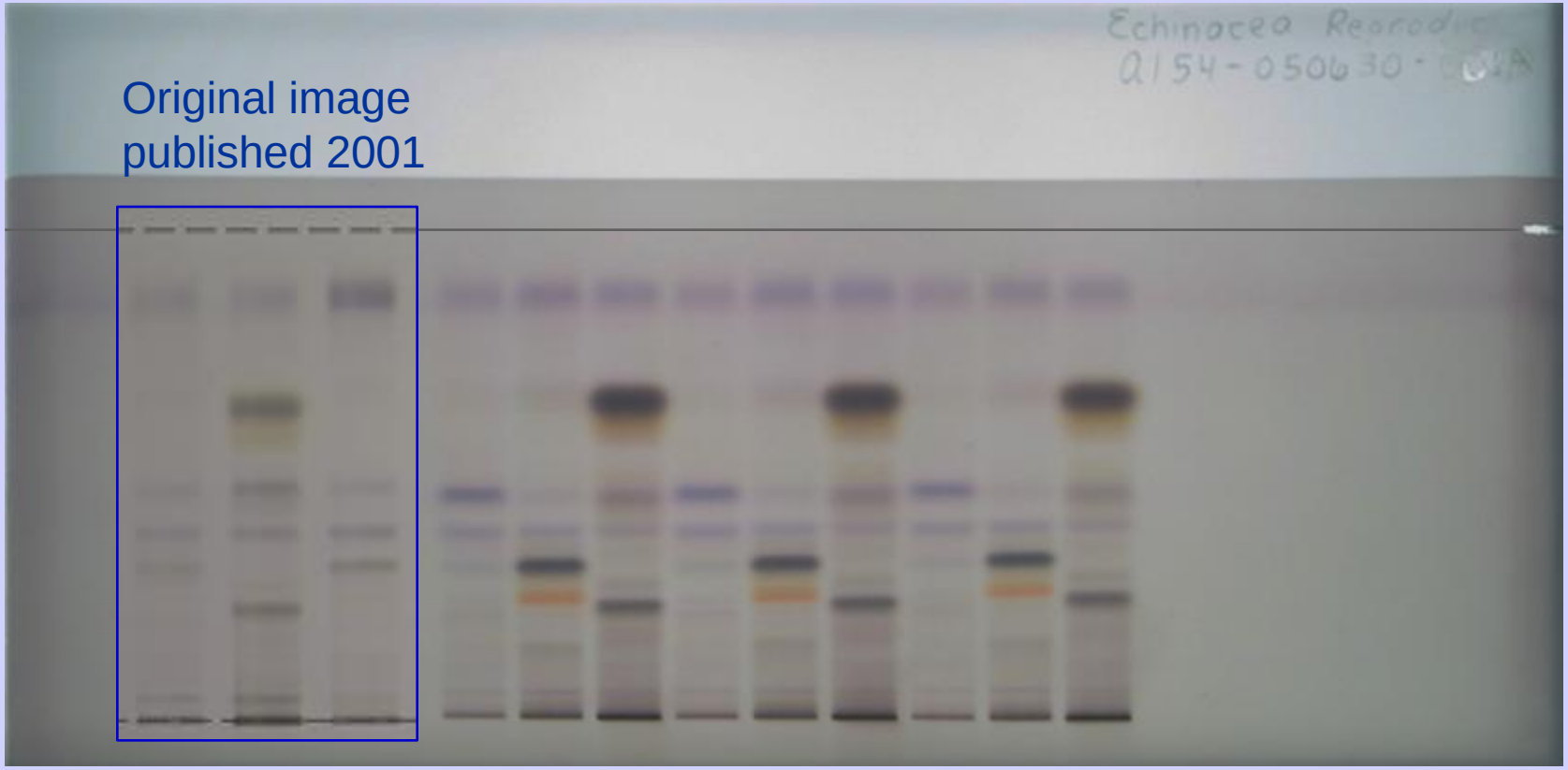




G.E. Morlock, q&more 1 (2014) 43-47, modified

Fingerprint et standardisation

Echinacea June 30, 2005 – CSI Laboratory

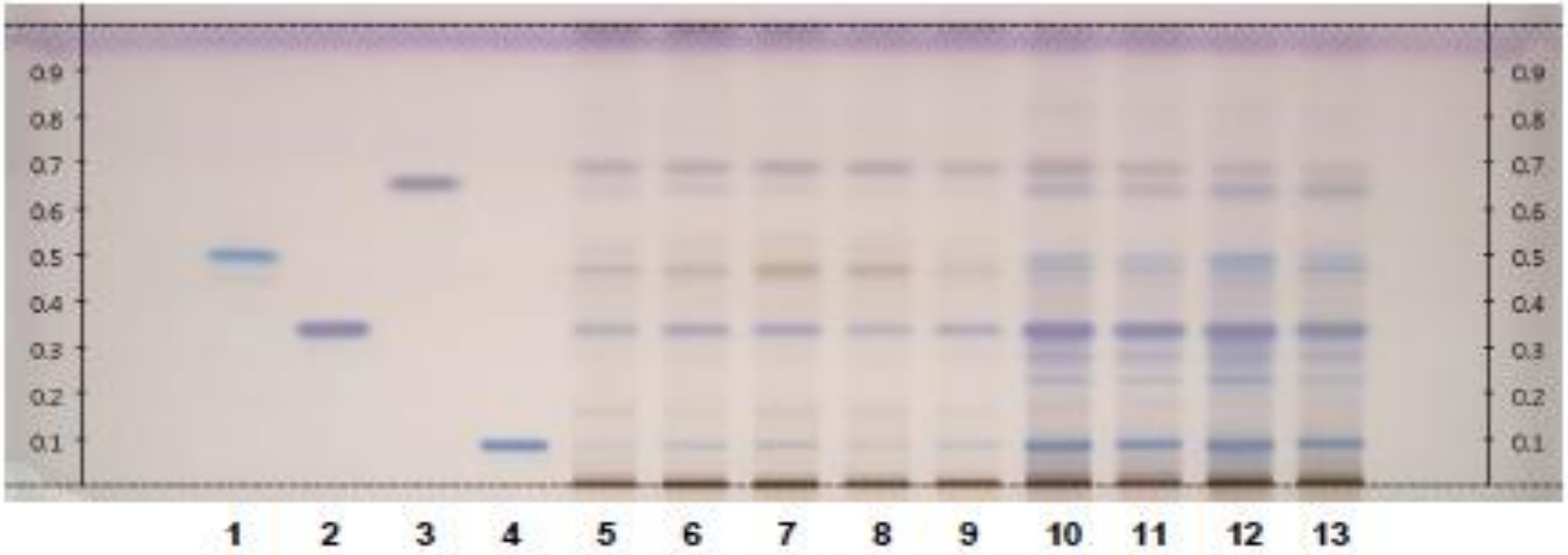


by courtesy Eike Reich CAMAG Laboratory

Guggul
Guggulsterone E and Z
alpha-Boswellic acid
beta-Boswellic acid
3-Acetyl-11-keto-beta-Boswellic acid
Acetyl-11-keto-beta-Boswellic acid
Boswellia
Anethole
Carvone
Bornyl acetate
Linalool
Myrrh

Lagerstroemia speciosa Leaf — Identification

Thin-Layer Chromatography



Typical HPTLC Chromatograms

These chromatograms are supplied for information only

Track assignment: 1) virgatic acid (0.2 mg/mL); 2) USP Corosolic Acid RS (0.2 mg/mL); 3) oleanolic acid (0.2 mg/mL); 4) asiatic acid (0.2 mg/mL); 5-9) *Lagerstroemia speciosa* Leaf, commercial samples;10) USP *Lagerstroemia speciosa* Leaf Powdered Extract (20 mg/mL);11-13) *Lagerstroemia speciosa* Leaf powdered extract, commercial samples (20 mg/mL)

Methods

Latin plant name	Plant part	Date of publication
Achillea millefolium	flower	2012-04-19
Aesculus hippocastanum (Horse Chestnut)	seed	2012-12-20
Agrimonia eupatoria	flowering tops	2012-04-19
Alchemilla vulgare	herb	2012-04-19
Aloysia citriodora	leaf	2012-04-19
Alpinia officinarum	rhizome	2012-04-19
Alpinia oxyphylla	fruit	2012-04-19
Althaea officinalis (Marshmallow)	leaf	2013-02-23
Althaea officinalis (Marshmallow)	root	2013-02-23
Amomum krervanh, Amomum compactum	fruit	2012-04-19
Amomum villosum, Amomum longiligulare	fruit	2012-04-19
Angelica archangelica	root	2012-04-19
Angelica dahurica	root	2012-04-19
Angelica pubescens	root	2012-04-19
Angelica sinensis	root	2012-04-19
Arctostaphylos uva ursi	leaf	2012-04-19
Arnebia euchroma or Arnebia guttata	root	2012-04-19
Arnica montana	flower	2012-04-19
Artemisia annua	leaf	2012-04-19
Aspalathus linearis (Rooibos)	tea	2013-01-30
Astragalus membranaceus	root	2012-04-19
Avena sativa	herb	2012-04-19
Bacopa monnieri	herb	2012-04-19
Betula pendula and/or Betula pubescens	leaf	2012-04-19
Bupleurum chinense	root	2012-04-19
Calendula officinalis	flower	2012-04-19
Camellia sinensis	leaf	2012-04-19

Sweet wormwood leaf, qing hao (*Artemisia annua*)

1. Scope
This method identifies dried (and fresh) Sweet wormwood leaf (*Artemisia annua* L.) by HPTLC fingerprint and detects the adulterant Chinese wormwood leaf (*Artemisia apiacea* Hance).

2. Source of method
CAMAG

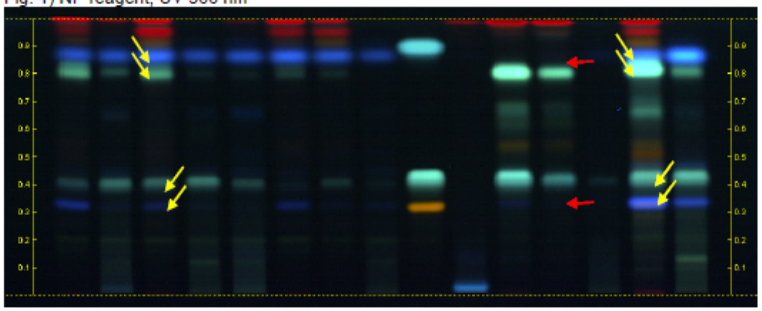
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of chlorogenic acid in 1 mL of methanol. Dissolve 1 mg of caffeic acid in 1 mL of methanol. Optional: Dissolve 1 mg of rutin in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 5 µL of test solutions
Mobile phase:	Ethyl acetate, water, acetic acid, formic acid 100:26:11:11 (v/v/v/v)
Development:	- Saturated chamber - Developing distance 70 mm from lower edge - Relative humidity 33%
Derivatization reagent:	NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate Use: Heat plate for 3 min at 100°C, then dip (time 0, speed 5)
Documentation:	1.) NP reagent, UV 366 nm

HPTLC Association

4. Results
Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Sweet wormwood fresh leaf 1 (extracted with water)	9	5 µL	Rutin, chlorogenic acid, caffeic acid (with increasing Rf)
2	5 µL	Sweet wormwood fresh leaf 1 (extracted with ethanol)	10	5 µL	Chinese wormwood fresh leaf (extracted with water)
3	5 µL	Sweet wormwood fresh leaf 1 (extracted with methanol)	11	5 µL	Chinese wormwood fresh leaf (extracted with ethanol)
4	5 µL	Sweet wormwood fresh leaf 1 (extracted with hot water)	12	5 µL	Chinese wormwood fresh leaf (extracted with methanol)
5	5 µL	Sweet wormwood fresh leaf 2 (extracted with water)	13	5 µL	Chinese wormwood fresh leaf (extracted with hot water)
6	5 µL	Sweet wormwood fresh leaf 2 (extracted with ethanol)	**14**	**5 µL**	**Sweet wormwood dried leaf 3 (extracted with methanol)**
7	5 µL	Sweet wormwood fresh leaf 2 (extracted with methanol)	15	5 µL	Sweet wormwood dried leaf 3 (extracted with hot water)
8	5 µL	Sweet wormwood fresh leaf 2 (extracted with hot water)			

Sweet wormwood fresh leaf 1: source Switzerland; Sweet wormwood fresh leaf 2: source Korea

System suitability test
Chlorogenic acid: green fluorescent zone at Rf ~ 0.40.
Caffeic acid: green fluorescent zone at Rf ~ 0.90.

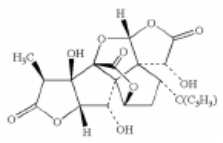
Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a green fluorescent zone at Rf ~ 0.40 corresponding to reference chlorogenic acid and below it a blue zone at Rf ~ 0.32 (yellow arrows). In the upper part of the chromatogram there is an intense green zone at Rf ~ 0.81 and a blue one just above it at Rf ~ 0.86. Below the solvent front there are two red zones. Similar but fainter zones are seen in the fresh sample (track 3).

Test for adulteration
The blue zones at Rf ~ 0.32 and Rf ~ 0.86 are missing (red arrows, Chinese wormwood leaf).

International Association for the Advancement of High Performance Thin Layer Chromatography
A non-profit organization dedicated to the promotion of HPTLC in plant analysis and other analytical fields • www.hptlc-association.org

Determination of ginkgolides A, B, and C and bilobalide in *Ginkgo biloba* dry extract by HPTLC

A-92.1



Key words:

HPTLC, densitometry, ginkgolide A, ginkgolide B, ginkgolide C, bilobalide, *Ginkgo biloba* extract

Scope:

This method is suitable for the quantification of ginkgolide A, ginkgolide B, ginkgolide C, and bilobalide in *Ginkgo biloba* dry extract.

Required or recommended CAMAG devices:

Automatic TLC Sampler 4 or Linomat 5, Automatic Developing Chamber ADC2 or Twin Trough Chamber 20 x 10 cm, TLC Scanner and winCATS software

Sample:

0.1 g of dry extract is sonicated with 10 mL of methanol for 10 min and filtered. The supernatant is used as test solution.

Standards:

A standard solution containing approx. 5 mg of bilobalide, 1 mg of ginkgolide A, 1 mg of ginkgolide B, and 1 mg of ginkgolide C in 20 µL of methanol.

Plate impregnation with sodium acetate solution:

8 g of sodium acetate are dissolved in 200 mL of ethanol, water 3:2. HPTLC plates are immersed into the solution for 2 seconds and allowed to dry at room temperature in the hood for 5 min. The plates are then heated at 90 °C for 30 min in an oven.

Derivatization reagent:

Acetic anhydride is directly used for spraying.

APPLICATION NOTES

CAMAG LABORATORY

Chromatography:

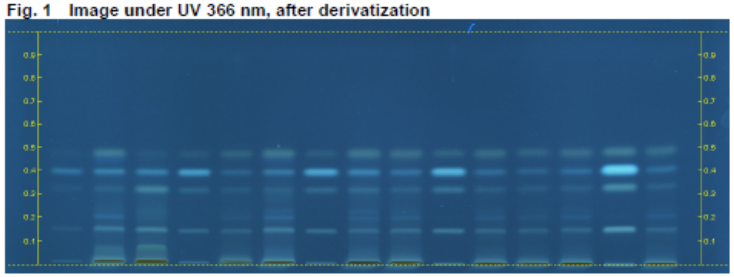
Stationary phase:	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck), impregnated with sodium acetate (see above).
Sample application:	5-15 µL each of test solution and 2, 5, 7, 10, and 25 µL of standard are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.
Developing solvent:	Toluene, ethyl acetate, acetone, methanol (20:10:10:1.2)
Development:	ADC2 or 20 x 10 cm Twin Trough Chamber, saturated for 20 min.
Developing distance:	70 mm from lower edge of plate.
Plate drying:	5 min in a stream of cold air.
Derivatization:	The plate is sprayed evenly with acetic anhydride and heated on the plate heater at 180 °C for 10 min.
Detection:	Examination under UV 366 nm.

Densitometry:

With CAMAG TLC Scanner and winCATS software in absorption mode at 300 nm (after derivatization) using a D2 lamp; evaluation via peak area, polynomial regression.

Results:

Fig. 1 Image under UV 366 nm, after derivatization

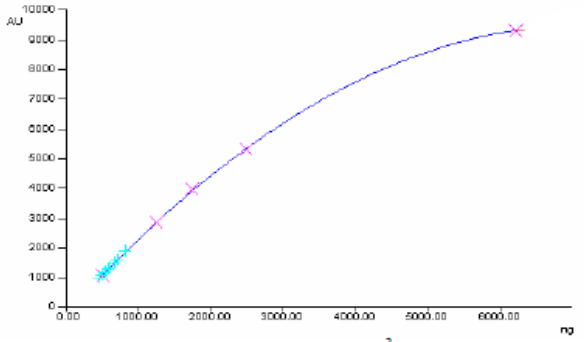


Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Standard mix (bilobalide, ginkgolides A, B, and C (with decreasing Rf value)	9	15 µL	Ginkgo dry extract #6
2	5 µL	Ginkgo dry extract #1	10	10 µL	Standard mix
3	5 µL	Ginkgo dry extract #2	11	10 µL	Ginkgo dry extract #7
4	5 µL	Standard mix	12	15 µL	Ginkgo dry extract #3
5	15 µL	Ginkgo dry extract #3	13	10 µL	Ginkgo dry extract #7
6	5 µL	Ginkgo dry extract #4	14	25 µL	Standard mix
7	7 µL	Standard mix	15	10 µL	Ginkgo dry extract #7
8	5 µL	Ginkgo dry extract #5			

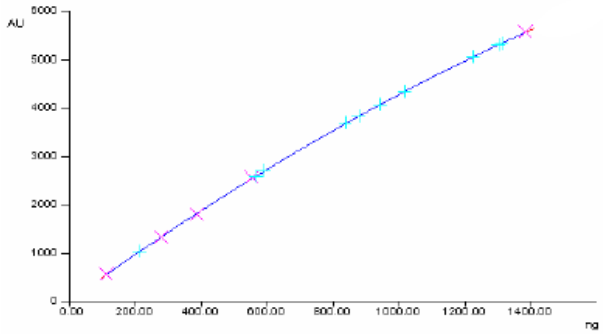
NOTE: The presented results are to be regarded as examples only!
Please contact CAMAG for more application notes and products for analysis of herbals!

Fig. 2 Calibration functions measured at 300 nm after derivatization (polynomial regression via area)

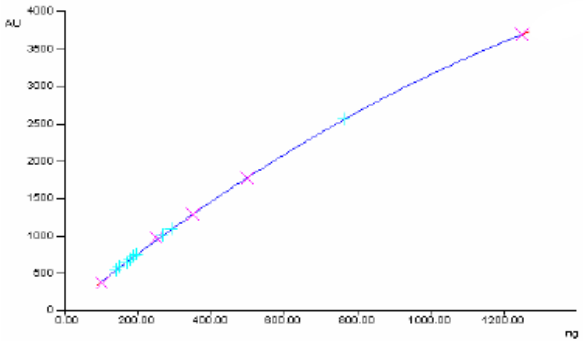
Ginkgolide A $Y = -231.6 + 2.702x - 0.0001878x^2$ r = 0.99996 sdv = 0.86%



Bilobalide $Y = 15.06 + 4.925x - 0.0006592x^2$ r = 0.99995 sdv = 1.11%



Ginkgolide B $Y = -1.006 + 3.969x - 0.0008115x^2$ r = 0.99988 sdv = 1.73%



APPLICATION NOTES

Ginkgolide C $Y = -30.2 + 2.889x - 0.0004281x^2$ r = 0.99994 sdv = 1.23%

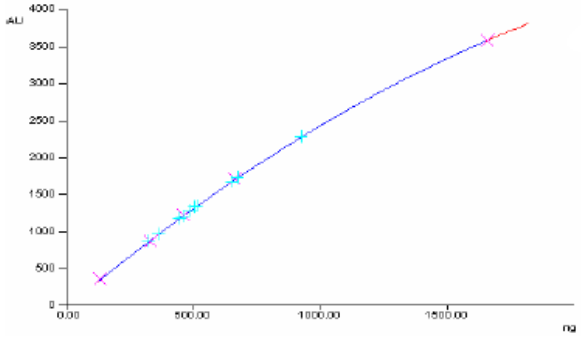
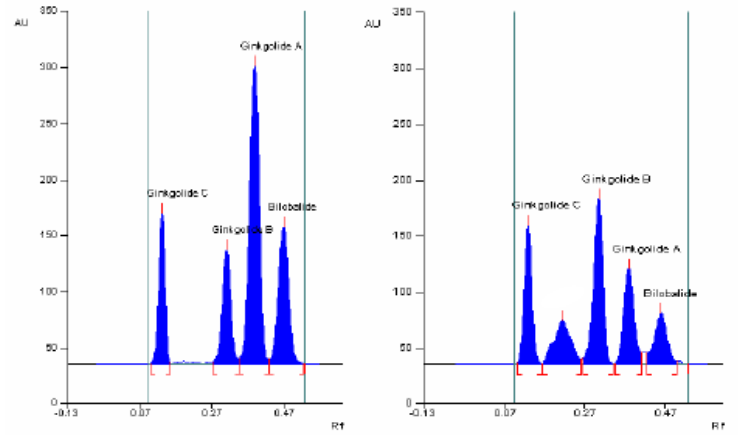


Fig. 3 Densitograms of standards (left) and a Ginkgo dry extract sample (right)



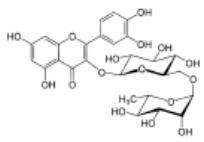
Literature

Based on the HPTLC method for identification of ginkgolides in Ginkgo, American Herbal Pharmacopoeia, 2003

NOTE: The presented results are to be regarded as examples only!
Please contact CAMAG for more application notes and products for analysis of herbals!

Determination of the flavonoid rutin in *Ginkgo biloba* dry extract by HPTLC

A-93.1



Key words:

HPTLC, densitometry, flavonoids, rutin *Ginkgo biloba* extract

Scope:

This method is suitable for the quantification of rutin in *Ginkgo biloba* dry extract. For additional visual evaluation of the HPTLC fingerprint the plate can be derivatized with natural products reagent.

Required or recommended CAMAG devices:

Automatic TLC Sampler 4 or Linomat 5, Automatic Developing Chamber ADC2 or Twin Trough Chamber 20 x 10 cm, TLC Scanner and winCATS software, Visualizer

Sample:

0.1 g of dry extract is sonicated with 10 mL of methanol for 10 min and filtered. The supernatant is used as test solution.

Standards:

A standard solution containing 0.1 mg/mL rutin in methanol.

Derivatization reagent (optional):

Natural Products reagent (NP reagent): 1 g of diphenylborinic acid aminoethylester is dissolved in 200 mL of ethyl acetate.

Macrogol reagent: 10 g of polyethylene glycol 400 (macrogol) are dissolved in 200 mL of dichloromethane.

APPLICATION NOTES

CAMAG LABORATORY

Chromatography:

Stationary phase:	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck).
Sample application:	2-5 µL each of test solution and 2, 4, 6, and 8 µL of standard are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.
Developing solvent:	Ethyl acetate, glacial acetic acid, formic acid, water (100:11:11:27)
Development:	ADC2 or 20 x 10 cm Twin Trough Chamber, saturated for 20 min.
Developing distance:	70 mm from lower edge of plate.
Plate drying:	5 min in a stream of cold air.
Derivatization (optional):	the plate is heated at 100 °C for 3 min, dipped while still hot in NP reagent, dried in a stream of cold air and dipped in Macrogol reagent.
Detection:	a) UV 254 nm b) UV 366 nm c) (optional) UV 366 nm, derivatized with NP/Macrogol reagent

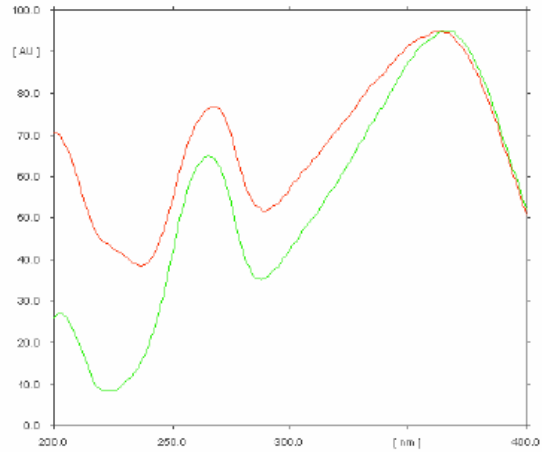
Densitometry:

With CAMAG TLC Scanner and winCATS software in absorption mode at 360 nm (prior to optional derivatization) using a D2 lamp; evaluation via peak height, polynomial regression.

Results:

Fig. 1 UV spectra of rutin standard (green) and corresponding zone in sample (red)

Based on the UV spectrum of rutin the UV_{max} of 360 nm was selected as measurement wavelength for quantification.



NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products for analysis of herbals!

Fig. 2 Linear calibration function of rutin in samples measured at 360 nm

$Y = -15.65 + 0.3664x - 8.598e-005x^2$ r = 0.99975 sdv = 1.96%

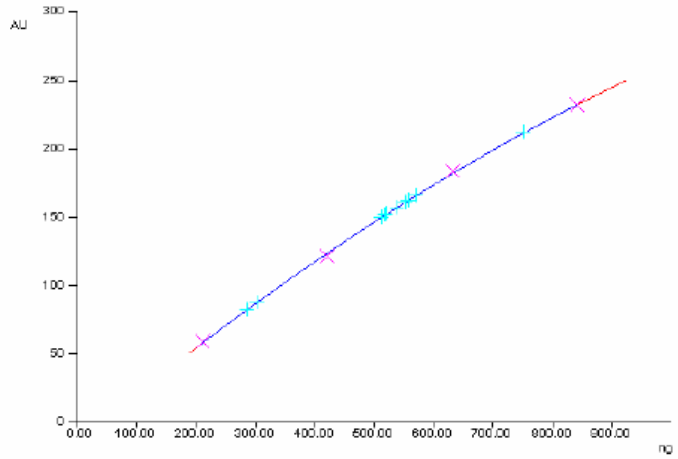
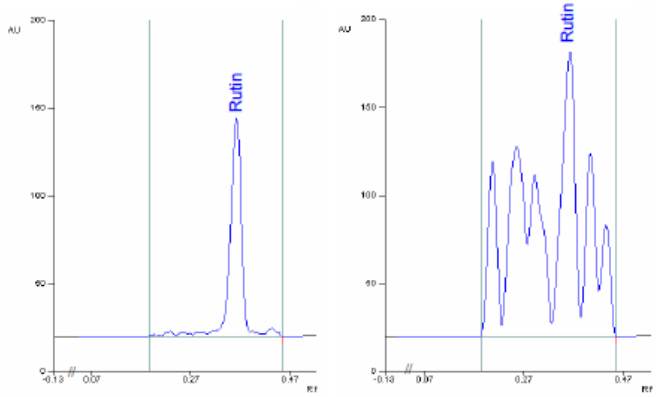
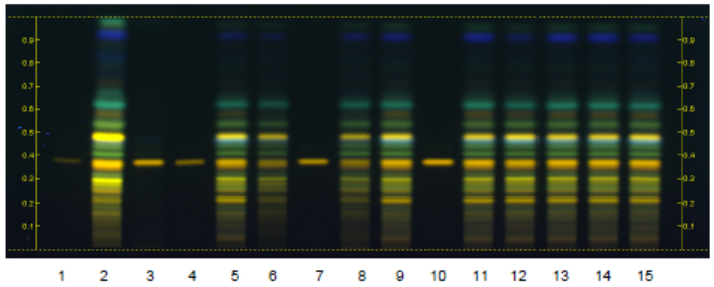


Fig. 3 Densitograms of standard (left) and a Ginkgo dry extract sample (right)



APPLICATION NOTES
CAMAG LABORATORY

Fig. 4 Image under UV 366 nm, after derivatization



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Rutin	9	4 µL	Ginkgo dry extract #6
2	2 µL	Ginkgo dry extract #1	10	8 µL	Rutin
3	2 µL	Ginkgo dry extract #2	11	4 µL	Ginkgo dry extract #7
4	4 µL	Rutin	12	7 µL	Ginkgo dry extract #3
5	7 µL	Ginkgo dry extract #3	13	4 µL	Ginkgo dry extract #7
6	5 µL	Ginkgo dry extract #4	14	4 µL	Ginkgo dry extract #7
7	6 µL	Rutin	15	4 µL	Ginkgo dry extract #6
8	6 µL	Ginkgo dry extract #5			

Literature

Based on the HPTLC method for identification of flavonoids in Ginkgo, American Herbal Pharmacopoeia, 2003.

NOTE: The presented results are to be regarded as examples only!
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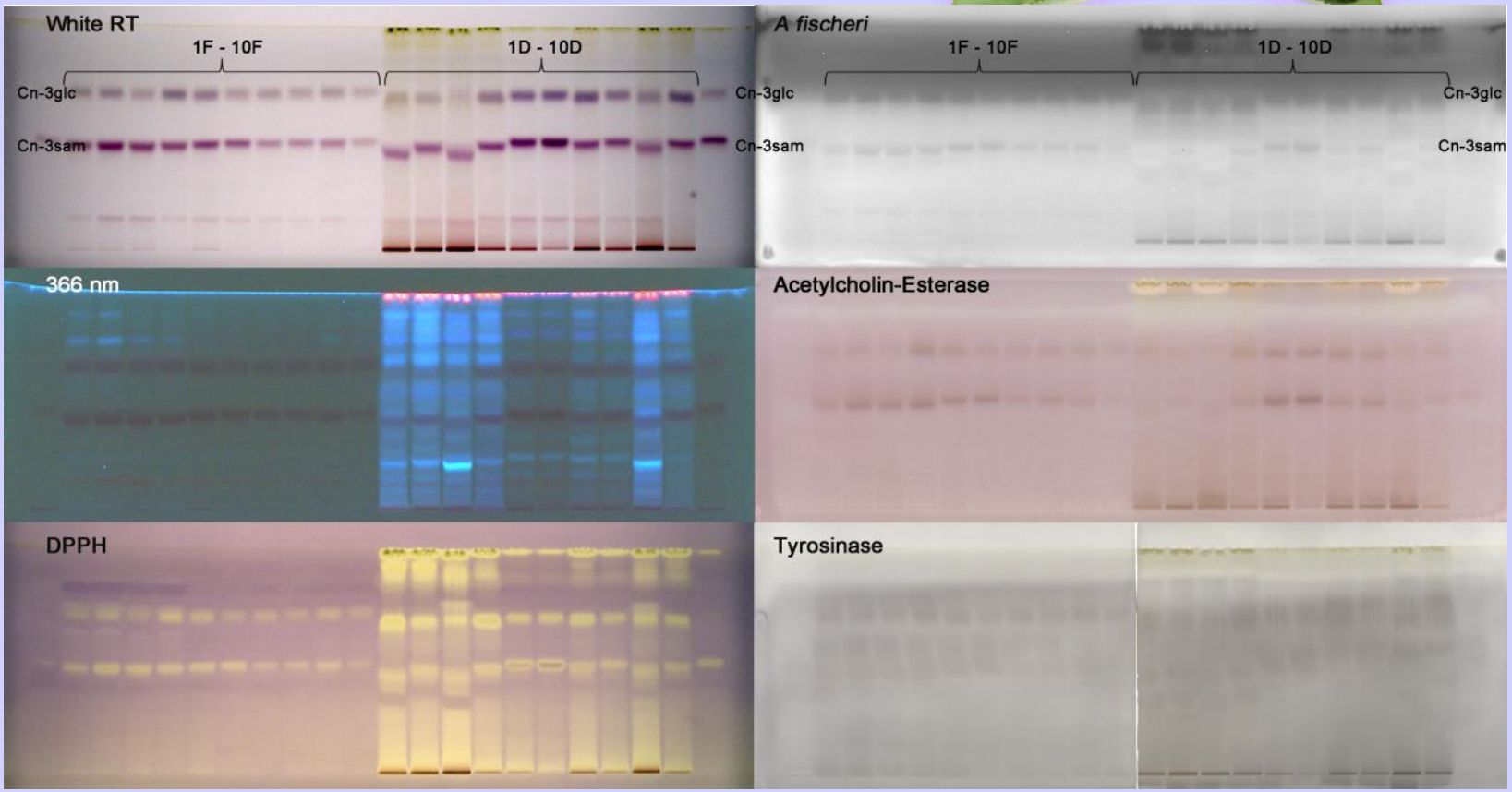
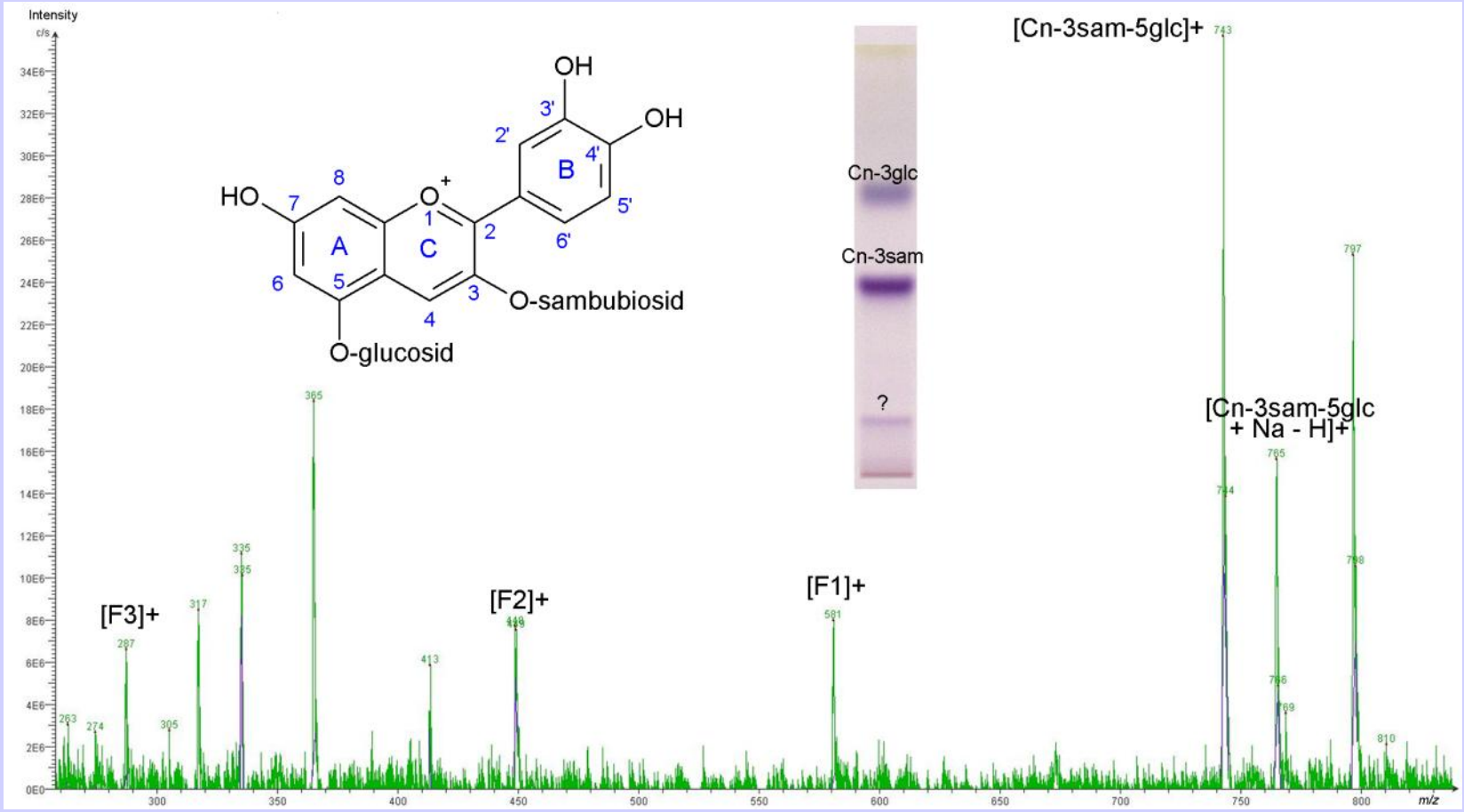
La détection non ciblée

Comparaison fruits frais et fruit séchés de sureau *(Sambucus nigra L.)*



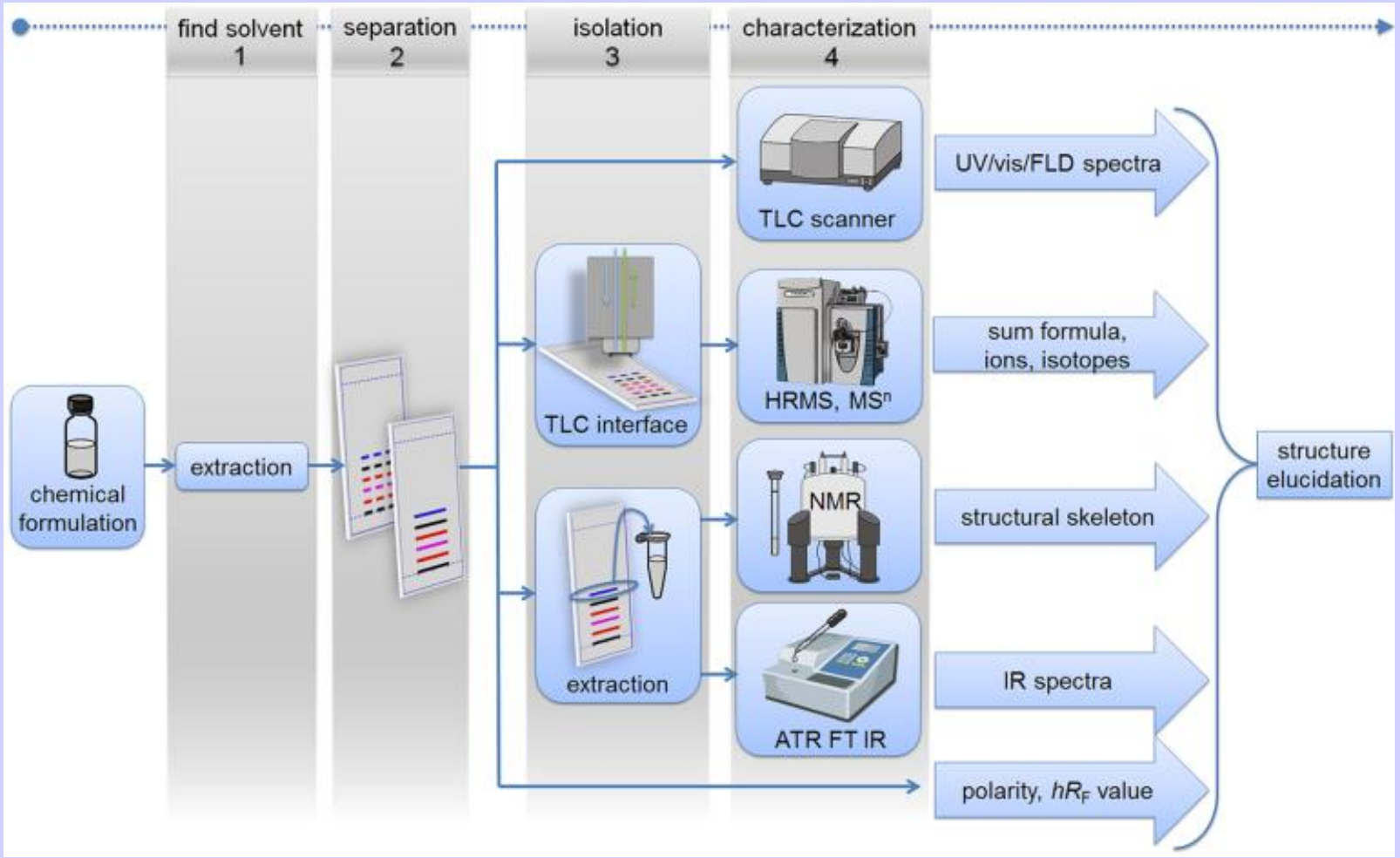
et la masse pour en savoir plus

(toujours par interface d'élution)



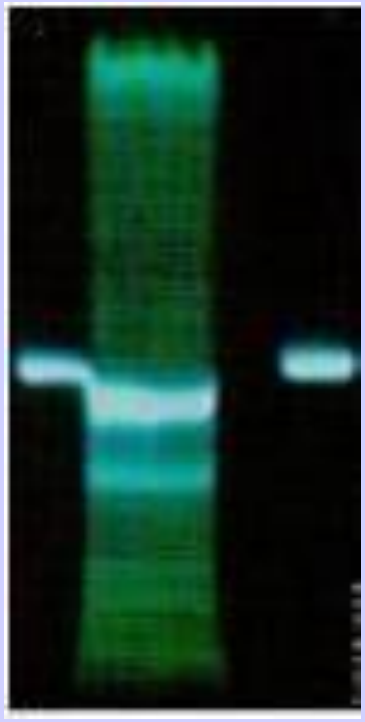
S. Krüger, M. Mirgos, G. Morlock,en préparation

Elucidation structurale en 2 plaques

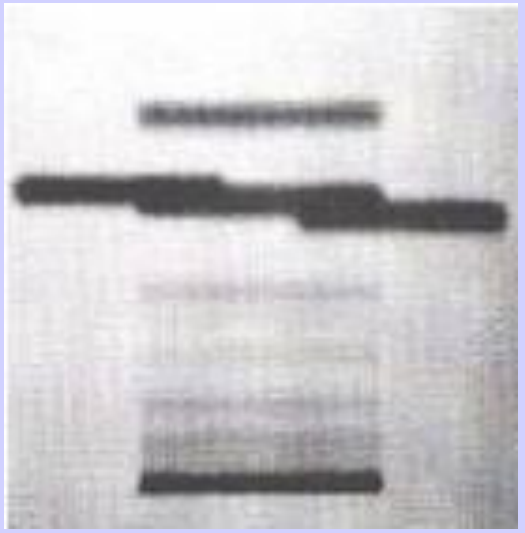


I. Yüce, G. Morlock, J Chromatogr A J Chromatogr A 1469 (2016) 120-127

2 choses pratiques: 1) superposition



Superposition partielle de dépôt simple et efficace permet de **vérifier l'identité ou pas** de molécules proches dands un échantillon complexe par rapport à un témoin qui ne migrerait pas forcément au même Rf du fait de la matrice. (nécessite deux tables de dépôt)

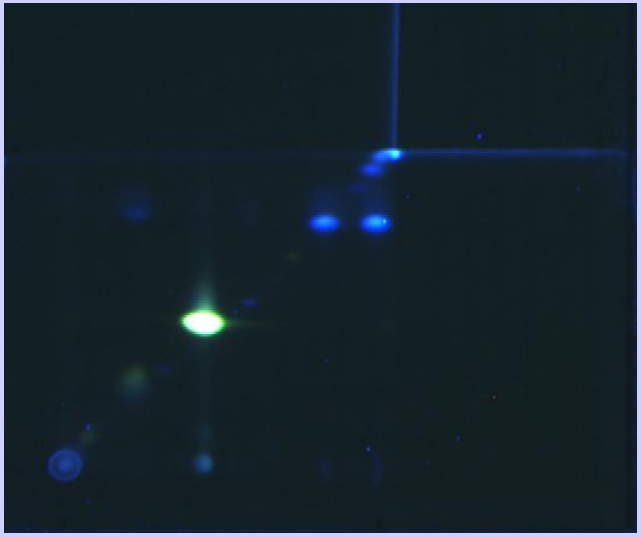


identiques

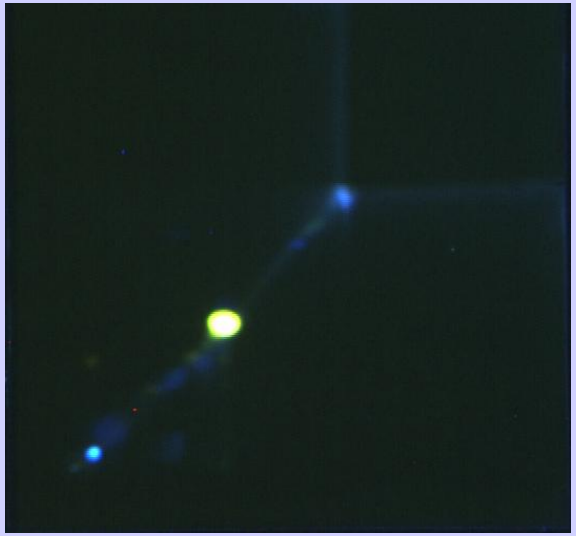
différentes

2 choses pratiques: 2) stabilité

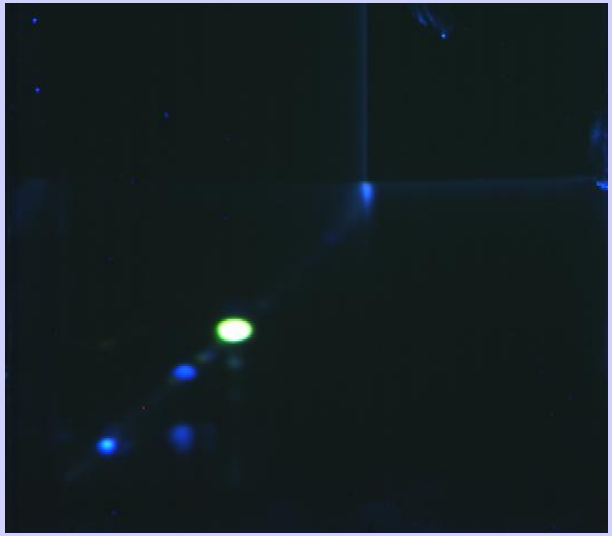
Migration bi-dimensionelle permettant de vérifier la stabilité d'un mélange sur une plaque donnée avec un solvant donné. Sinon changer le solvant, retirer le MeOH par exemple, puis changer de plaque et passer sur Diol… (ici méthode Hydrastis)



Pharmacopée Chinoise



Méthode CAMAG

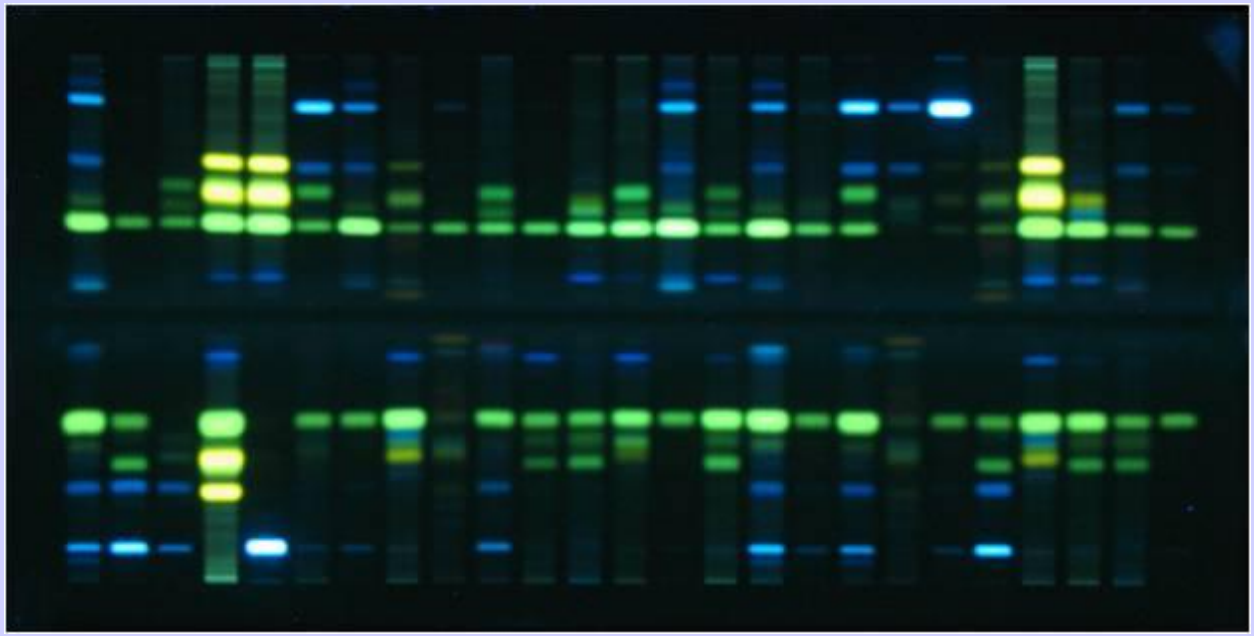


Pharmacopée US

Intérêt du haut débit ?

avec une cuve horizontale 20x10

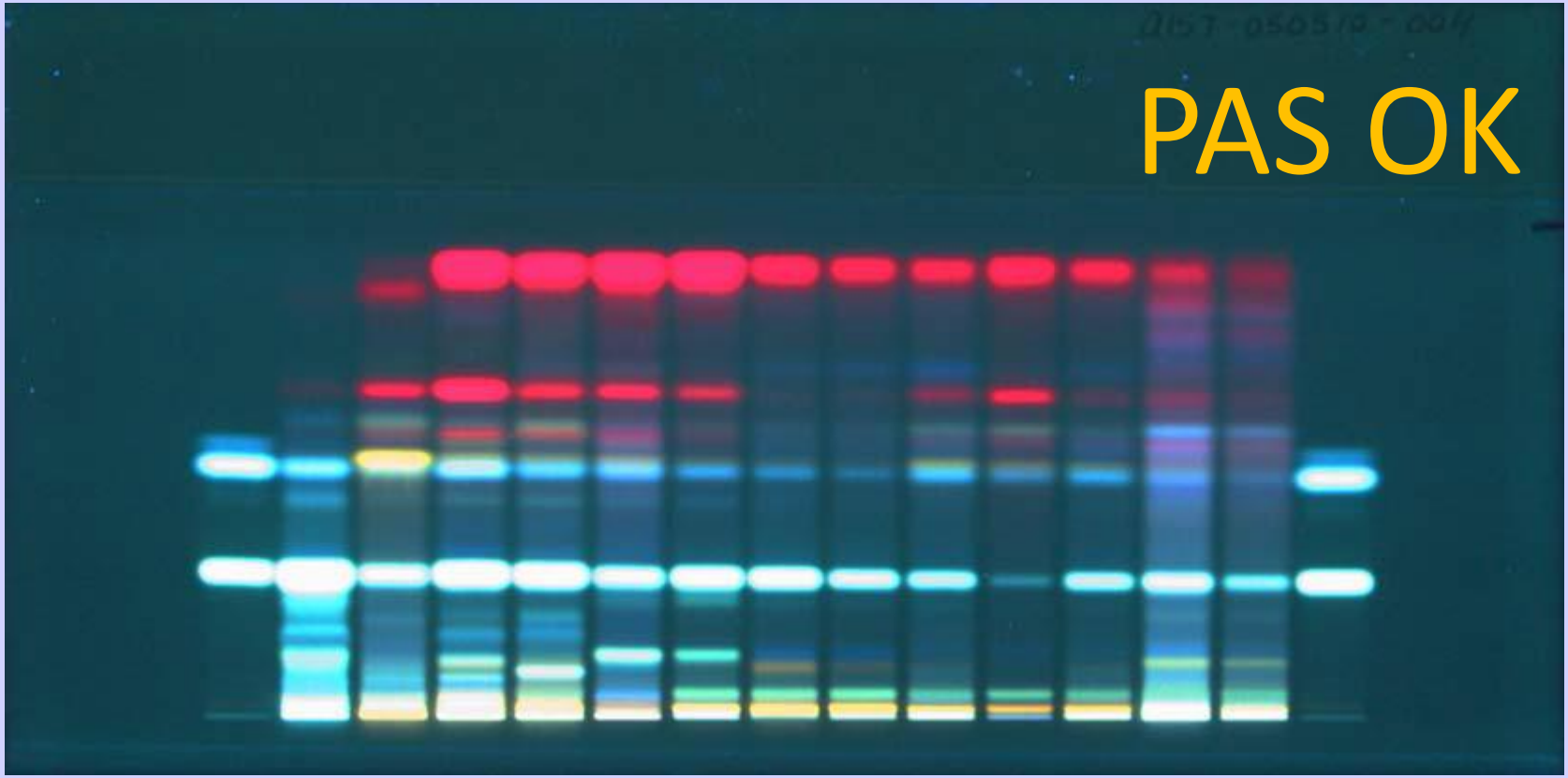
(2x plus d'échantillons; 5 mL de solvant en tout; s'arrête toute seule; optimisation de la distance de migration)



Plaque HPTLC avec 50 échantillons méthode Hydrastis

Remerciements à Daniel Handloser, CAMAG Laboratory pour le Club de CCM, 2005

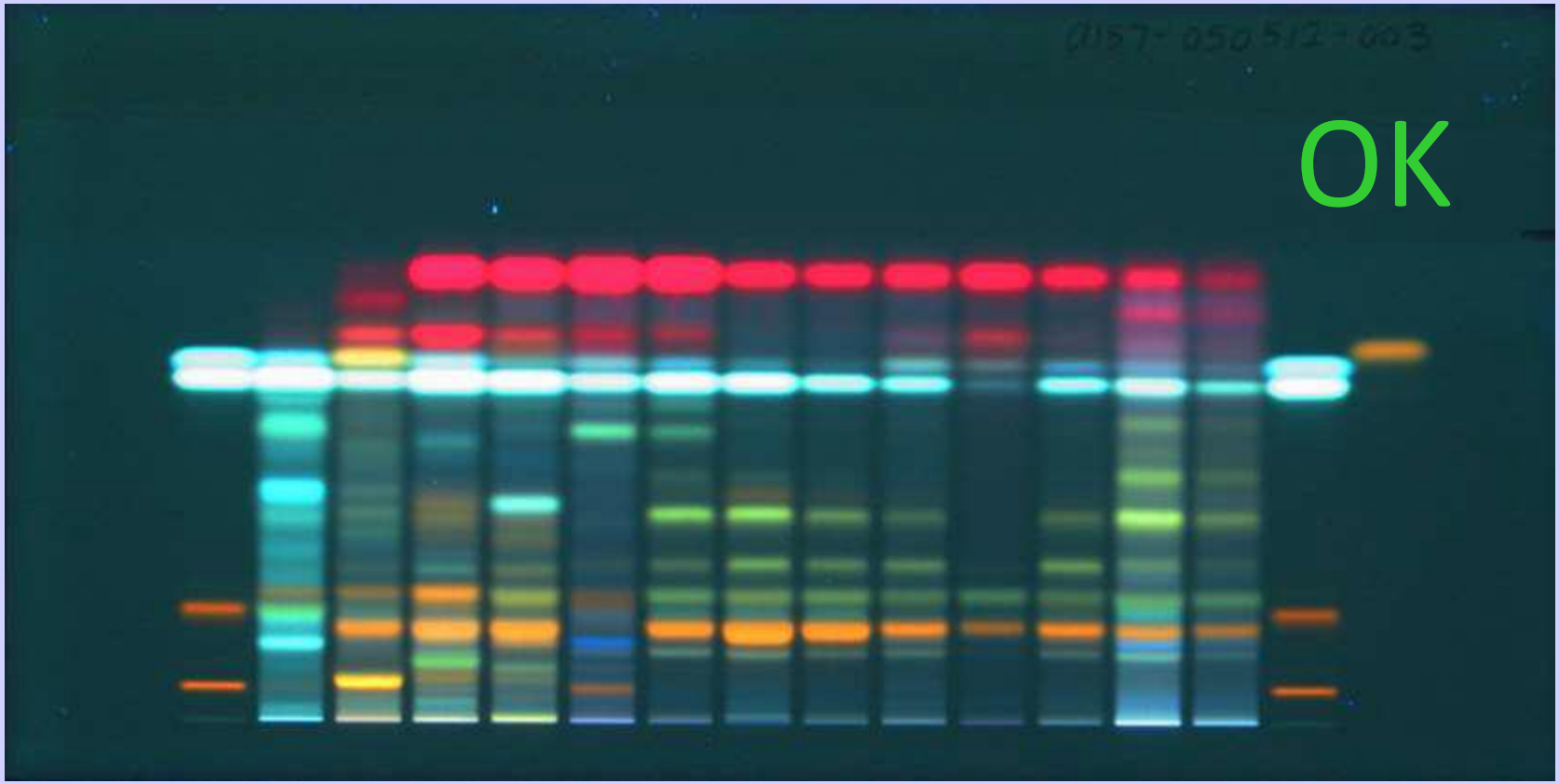
Fingerprint et mise au point



Ac. Rosmarinique et caféique; mélisse; menthe poivrée; marjolaine; basilic doux (sweet basil); grand basilic x8 (holy basil).

Eike Reich, CAMAG Laboratory pour le Club de CCM, Avril 2015

Fingerprint et mise au point



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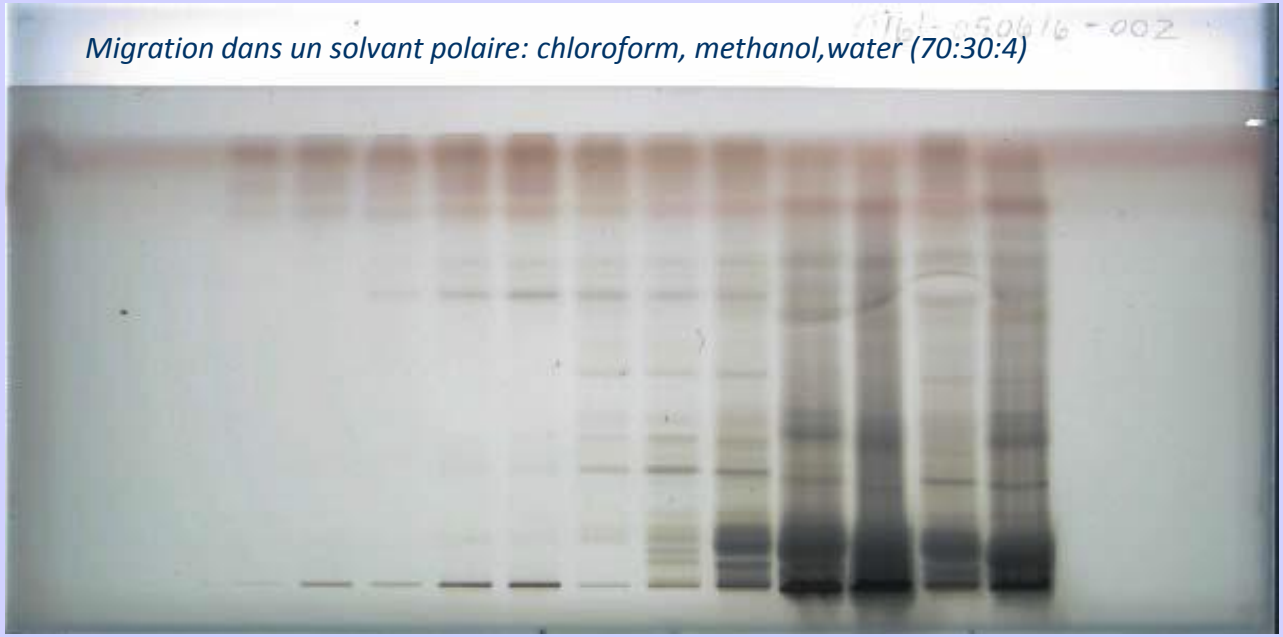
Eike Reich, CAMAG Laboratory pour le Club de CCM, Avril 2015

L'extraction est importante



Solvants d'extraction: 1: heptane; 2: toluene; 3: MTBE; 4: DCM; 5: chloroform; 6: acetone; 7: ethanol; 8: methanol; 9: ethanol-water(7:3); 10: methanol-water (8:2); 11: methanol-acetic acid (9:1); 12: methanol-ammonia 25% (8:2).

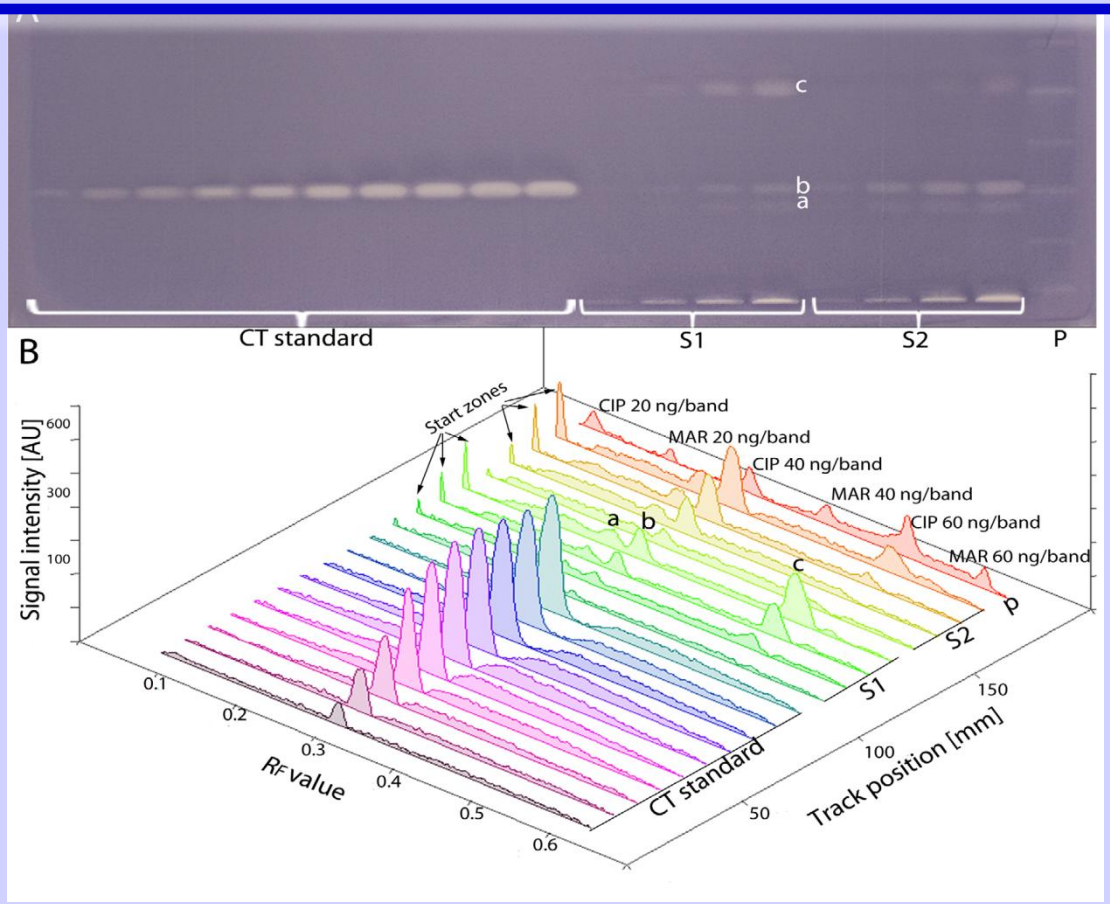
co Extraction ?

NE L'OUBLIONS PAS !

(que l'échantillon soit connu, ou pire inconnu !...)

Eike Reich, CAMAG Laboratory pour le Club de CCM, Avril 2013

NEWS : Bioéquivalence quantitative

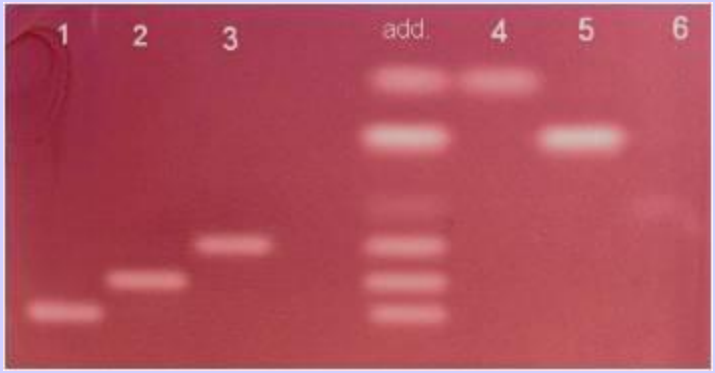


HPTLC-B. subtilis bioautograms *documented under white light illumination (A) for the bioprofiling of substances a–c in the two different S. miltiorrhiza samples (S1, S2), CT standard (20–200 ng/zone), and a positive control pattern of ciprofloxacin (CIP) and marbofloxacin (MAR) (20, 40, and 60 ng/zone each) applied on the edge track (P); respective biodensitograms recorded by inverse scanning at 546 nm (B).*

M. Jamshidi-Aidji, G. E. Morlock, poster SEP 2017, Paris

Leadership et compétences

Sur les inhibiteurs d'AchE: comparaison de quelques nanogrammes de références extraites de *Peucedanum ostruthium L.* à gauche et quelques picogrammes de témoins d'organochlorés ci-dessous



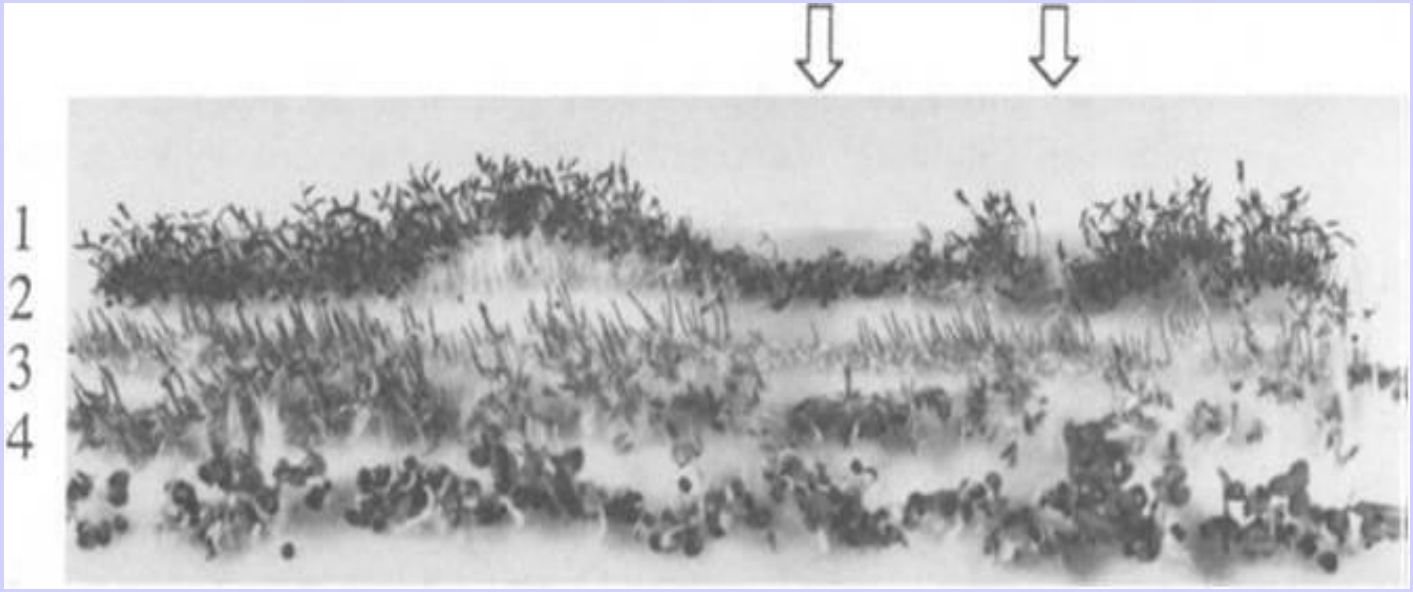
Akkad R., ***Schwack W****., Journal of Planar Chromatogr. 21 (2008) 411-415*

(avec un facteur d'environ 1000 en sensibilité)

Urbain A., Marston A., ***Hostettmann K.*** *(2005) Pharm Biol 43(8): 647-650.*

On parle de plantes…

anthraquinone and naphtoquinone derivatives proved to be potent germination inhibitors using this TLC bioassay (Meyer & coll. 2007)



Growth inhibition TLC bioautographic assay of fractions from Polygonum sachalinese (5 days). Arrows point to areas of inhibited plant activity. (1) green amaranth, (2) timothy grass, (3) crab grass, (4) Chinese cabbage.

3ème Atelier Plantes

contributions : … ?

3ème Atelier Plantes

Question : que pouvons nous, avec l'HPTLC, apporter à ce domaine ?

- Méthodologie (CAMAG /Association HPTLC / AHPA / pharmacopées) quoi d'autre ?
- Liens : Aferp sponsorisé / GA ?
-Instrumentation dédiée au top (visionCATS) mais ?
- autres : ...?

3ème Atelier Plantes

et la question du jour: comment faire en sorte de développer la quantification et la diffusion des détections biologiques ?

- Monographies (ex alcaloïdes totaux / poster Boiron)
- Publications ?
- Formations spécifiques à refaire ?
- Quoi d'autre ?

Quel est votre avis ?

Ateliers “AUTRE”

prochains ateliers :
3 short practical courses à HPTLC 2017
(HPTLC-MS API, lipides quali quanti, **plantes**)
4 Juillet après-midi à l’université TU Berlin
(nombre limité, animé par Eike Reich 100€)

Formation une semaine à IFZ Giessen (labo Gerda MORLOCK) **semaine 34**, limité à 15 personnes (une semaine 3000€/ modulaire)
mise au point et couplages HPTLC MS & EDA

Ateliers “AUTRE”

prochains ateliers :

à l’automne Sanofi Vitry
18 Octobre (à confirmer)

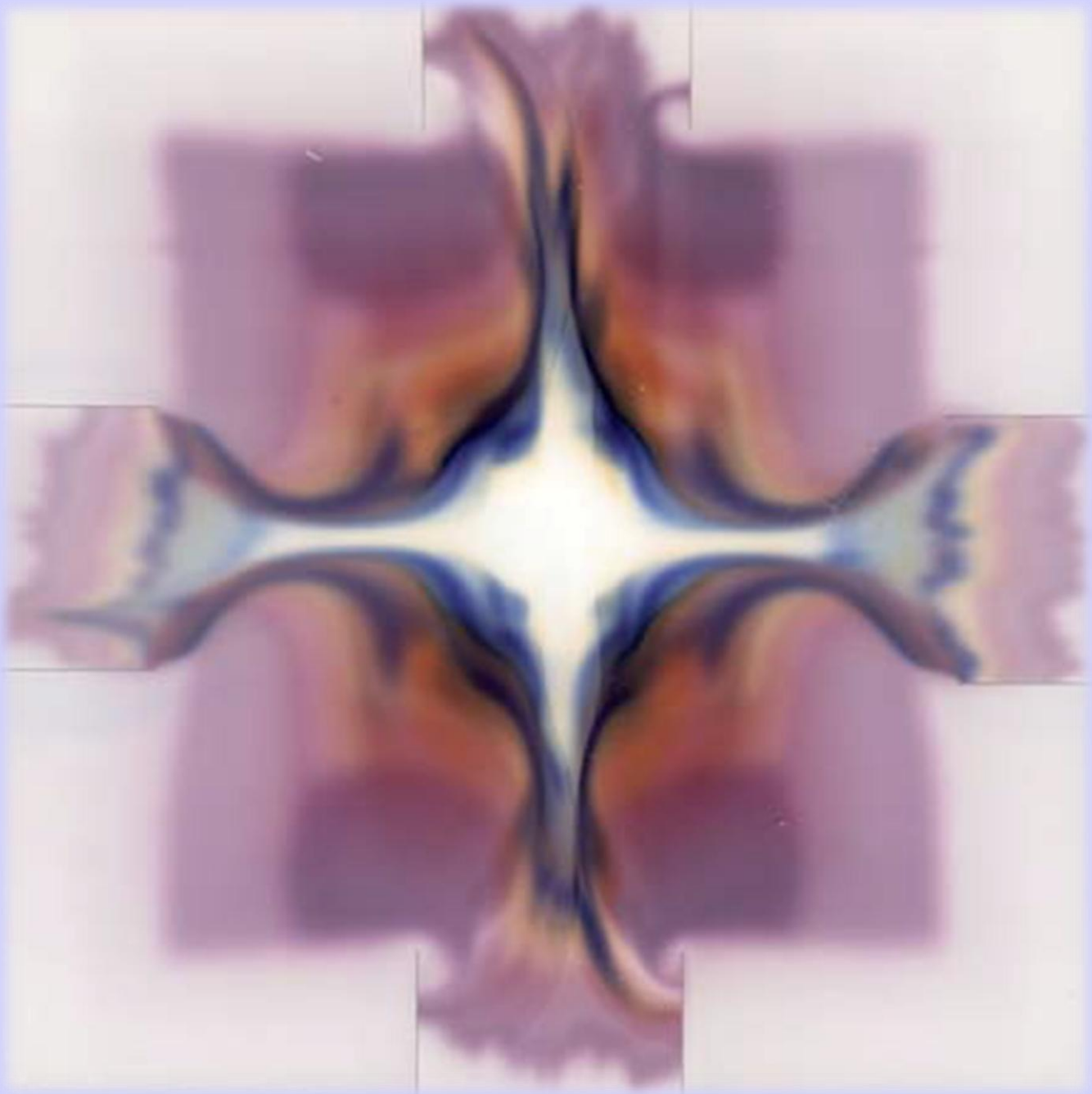
API contrôle in-process > mise au point de méthode, dosage d’impuretés, purification,...

Ateliers “AUTRE”

Et en 2018 , … ? : vos avis sont les bienvenus

-Lipides
-Couplages
-Validation
-Plantes (encore ?...)

où: Montpellier, Clermont Ferrand, Genève ?



“Chromart” by Herbert Halpaap in 1986-1987