



*Utilisation of Thin Layer Chromatography .
Applications in a preparative chromatography
laboratory.*



1. Introduction

2. Tools and methodology

3. Examples

4. Conclusion



Dr Jacques Servier

17 500 people

- The number one independent pharmaceutical laboratory in France, present in more than 140 countries.
- Prescription drugs
- More than 3 boxes out of 4 sold outside of France

2500 people

- A quarter of the worldwide consolidated turnover devoted to Research & Development
- Main research areas : Metabolic diseases, Cardiology, Central nervous system, Cancerology, Rhumatology.

SERVIER is involved in every stage of drug production from research to manufacturing

R & D and Chemical production

Bolbec and Baclair

Physico-chemistry and drug delivery studies

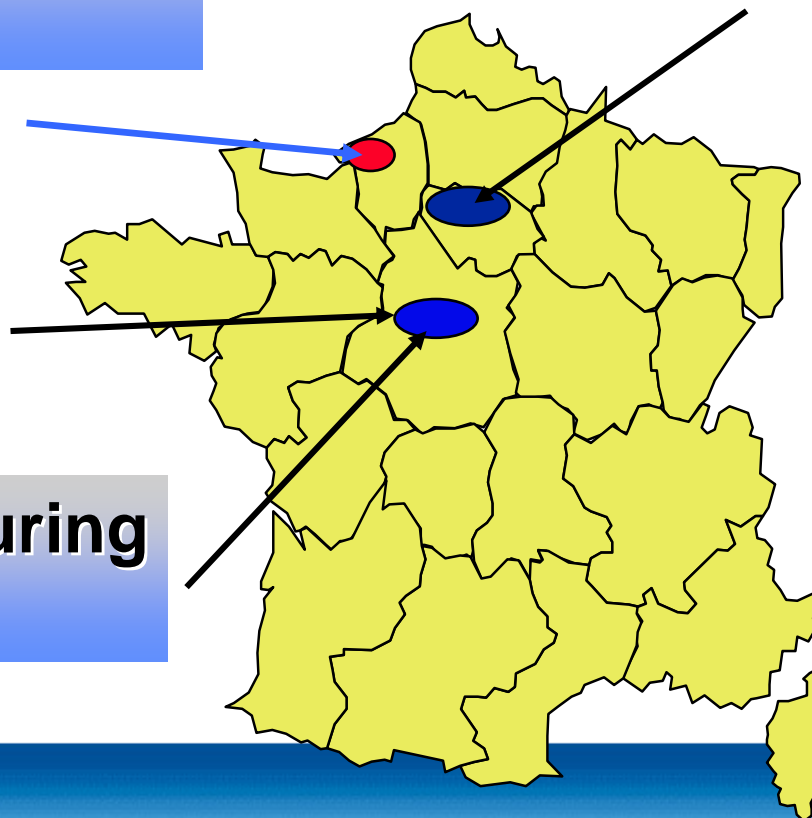
Orléans

Pharmaceutical manufacturing and toxicology center

Gidy

Drug discovery

Suresnes and Croissy



2 sites :

**1992, 2000 & 2004,
Baclair**

1960, Bolbec



**Production of
24 SERVIER API**
(active pharmaceutical ingredients)



TASKS

**Chemical Research &
Development :**

- New ways of synthesis
- Scale up
- Production of clinical batches



Main Active pharmaceutical Ingredients

Products	Active ingredients	Indications
ARCALION	Sulbutiamine	Tonic
ARTEX	Tertatolol	Hypertension
COVERSYL	Périndopril	Hypertension
DAFLON	Flavonoïdes	Vascular disorders
DIAMICRON	Gliclazide	Diabetes
DUXIL	Raubasine+Almitrine	Mental impairment in the elderly
FLUDEX	Indapamide	Hypertension
GLUCIDORAL	Carbutamide	Diabetes
HYPERIUM	Rilménidine	Hypertension
LOCABIOTAL	Fusafungine	Respiratory tract infection & inflammation
MEDIATOR	Benfluorex	Hyperlipidaemias
MUPHORAN	Fotémustine	Anti-Cancer
PNEUMOREL	Fenspiride	Asthma
PRETERAX	Périndopril+Indapamide	Hypertension
PROCORALAN	Ivabradine	Ischaemic disorders
PROTELOS	Ranélate de strontium	Ostéoporosis
STABLON	Tianeptine	Dépression
TRIVASTAL	Piribédil	Mental function imperment in the elderly
VASTAREL	Trimétazidine	Ischaemic disorders
VECTARION	Almitrine	Obstructive airway disease

1. Flash chromatography

Principle:

- Optimization of the separation of the compounds from a mixture with TLC and transfer on a column.

2. Preparative HPLC

Principle:

- Optimization of the separation of the compounds from a mixture with HPLC with an preparative available stationary phase and transfer on the column

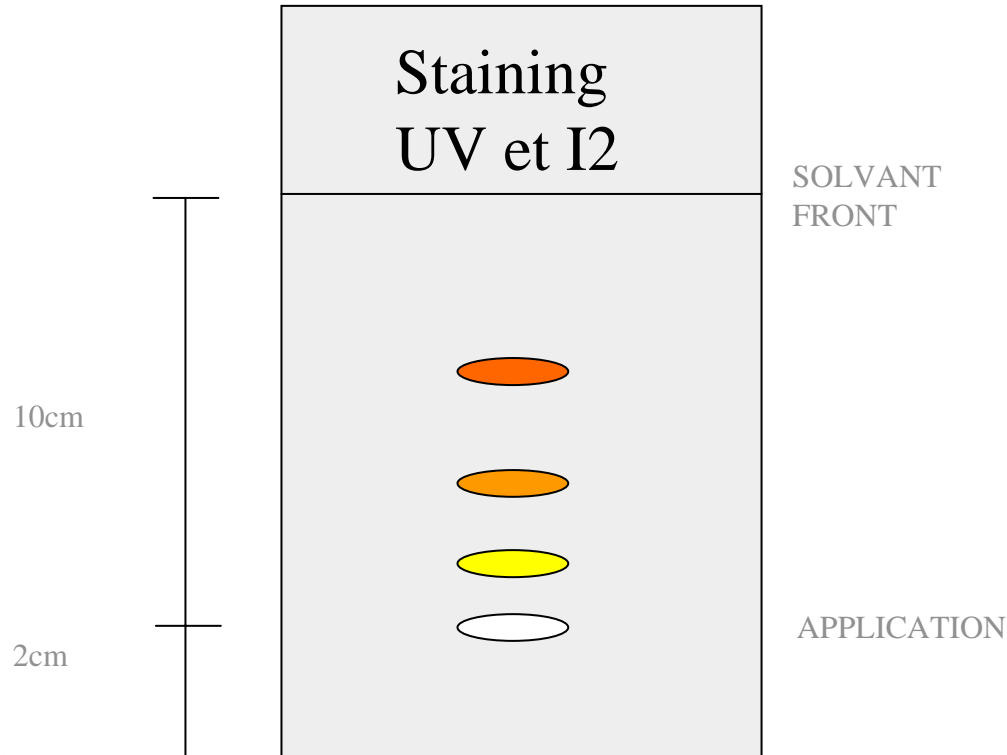
Tools



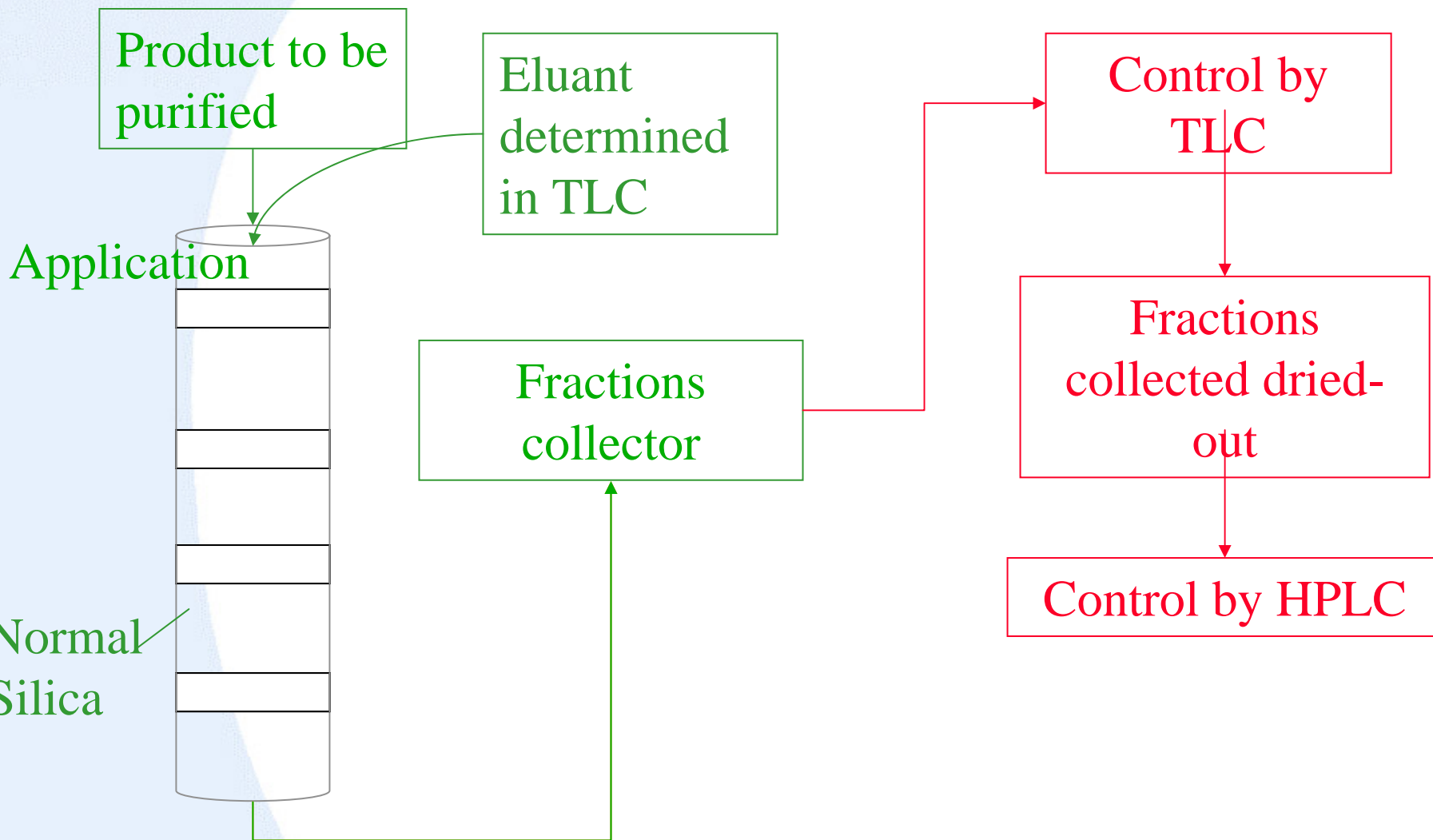


Finding a TLC solvent system before column transfer

- The choice of operating conditions for purification goes first through a good TLC :
Determination of operating conditions (choice of the solvent and of stationary phase : usually Silica gel), adapted Rfs, solvents (solubility).



Flash Column



- *TLC optimization:*

The composition of the mobile phase is correct if the separation of the compounds is sufficient and if the R_f of the requested compound is in between 0,1 et 0,4.

- *Transfer on column:*

Pouring the Silica in the chosen eluant

Packing under pressure (0.5 à 1 bar).

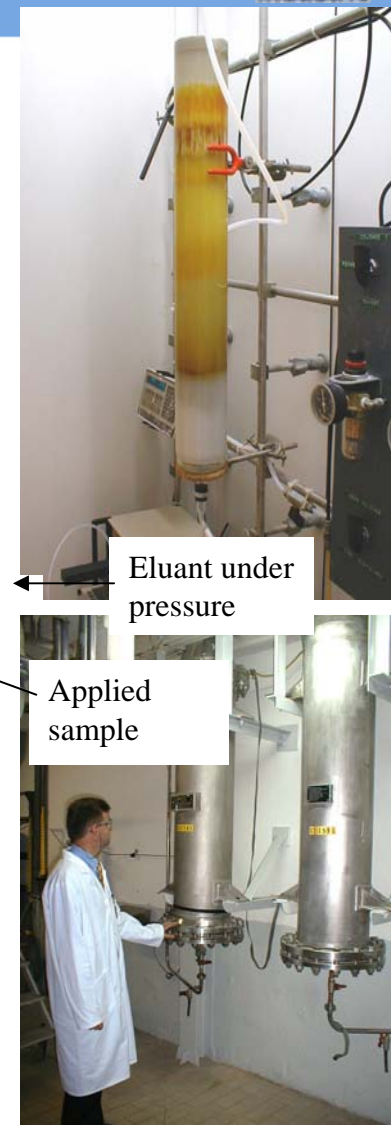
Add sand in order to avoid the dilution phenomenon during injection and disturbances of the phase layer.

Injection in the less polar solvent (or sometimes SO_2)

Elution under low pressure (0.5 à 1 bar).

Fraction collection at the column exit, purification follow-up with TLC or HPTLC, fractions put together and dried-out isolation of the compounds.

Sand from
Fontainebleau



Some hints :

Use « classical » solvent systems (Cyclohexane, Dichloromethane, Toluene, Ethyl acetate, Acetonitrile, MTBE, Ethanol, Methanol) by avoiding when possible acidic or basic additives, as the simplest systems are always the best

Another small trick is not to work with salts (Chlorhydrates...), it is necessary in this case to come back to the acid or base before injection to get good results.

The mass ratios compound(g) / silica (g) are about 1/50 à 1/100 and are dependant of the separation and injected compounds..

6 - METHODE D'ANALYSE UTILISEE POUR LA VALIDATION

Phase stationnaire

KROMASIL 100-5C18

Colonne : longueur 25 cm, diamètre interne de 4,6 mm.

Remarque :

Prévoir une boucle de préchauffage de la phase éluante dans le four (60 cm).

Calfeutrer la sortie de la colonne et l'entrée du détecteur avec de la laine de verre afin d'éviter une dérive de la ligne de base.

Température de la colonne : 70°C

Phase mobile

A = Phase aqueuse : eau + 1 % HClO₄ (à 1 litre d'eau ajouter 10 ml d'acide perchlorique à 70 %).

B = acétonitrile

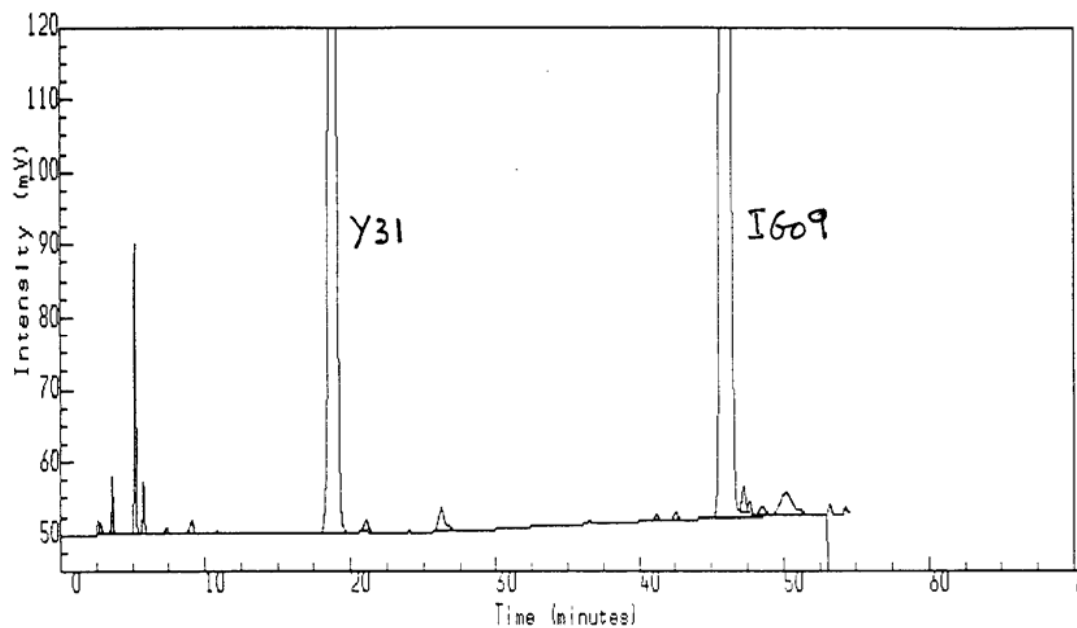
Gradient

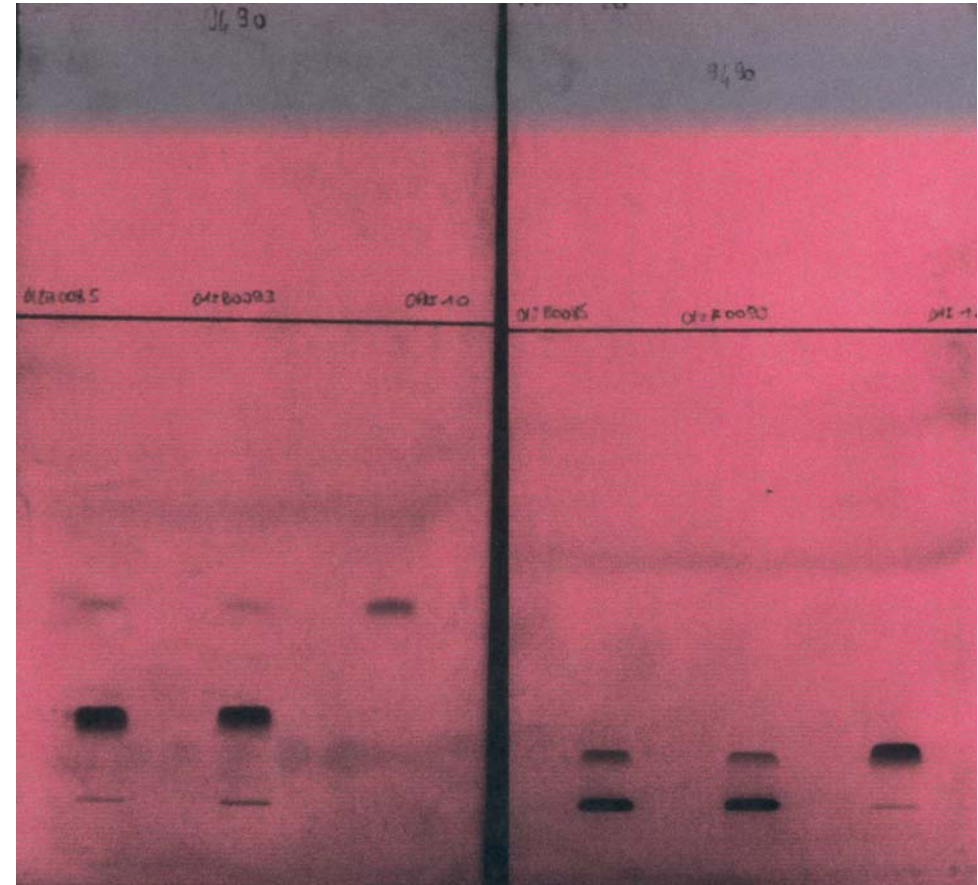
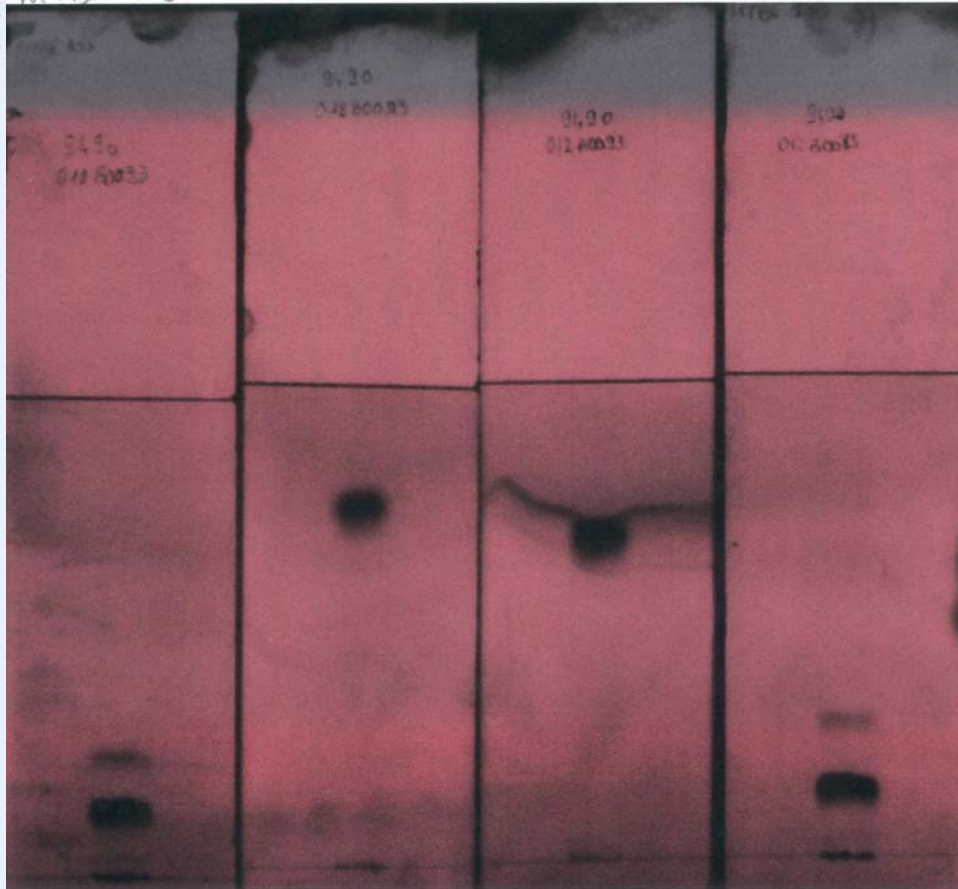
Temps (min)	Phase A %	Phase B %	Débit (ml/min)
0	60	40	0,8
20	60	40	0,8
60	20	80	0,8
70	20	80	0,8
72	60	40	0,8
80	60	40	0,8

Détection: UV 215 nm

Durée du chromatogramme: 70 min

Quantité injectée : 10 µl des solutions témoins et essais





Flash Chromatography :

Conditions:

Glass column diameter 165 mm long 600mm

Normal Silica 15-40 μ m masse :6 Kg

Ethyle Acetate 100 %

Packing pressure :1bar

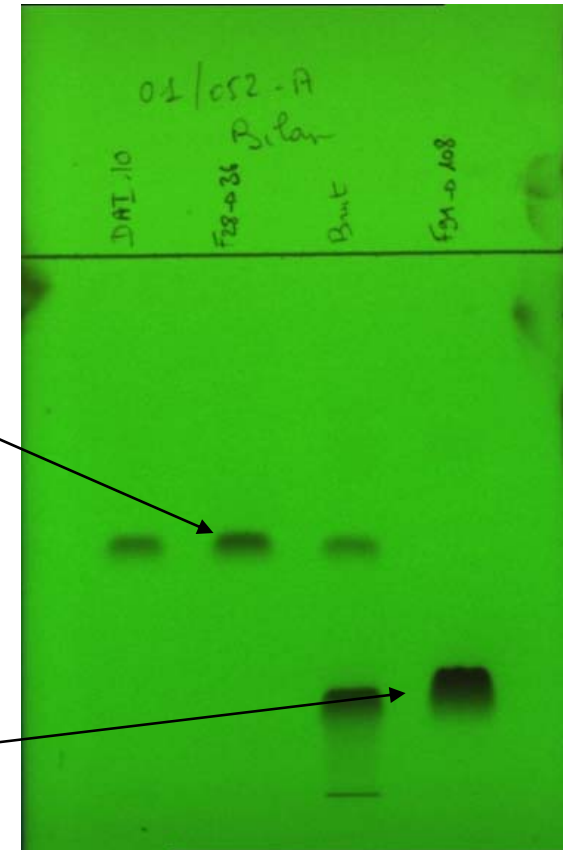
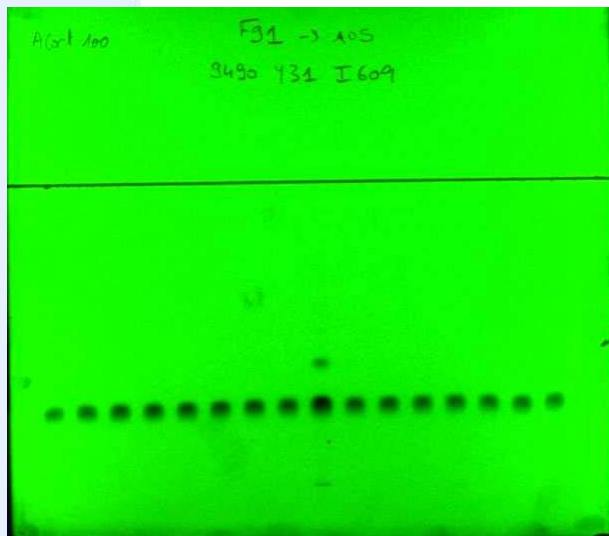
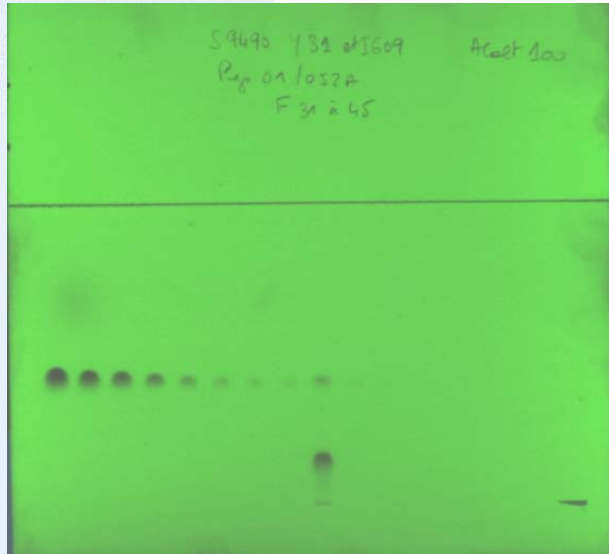
70 g of raw in ethyle acetate solution 1/5(P/V).

Collection: glass flasks

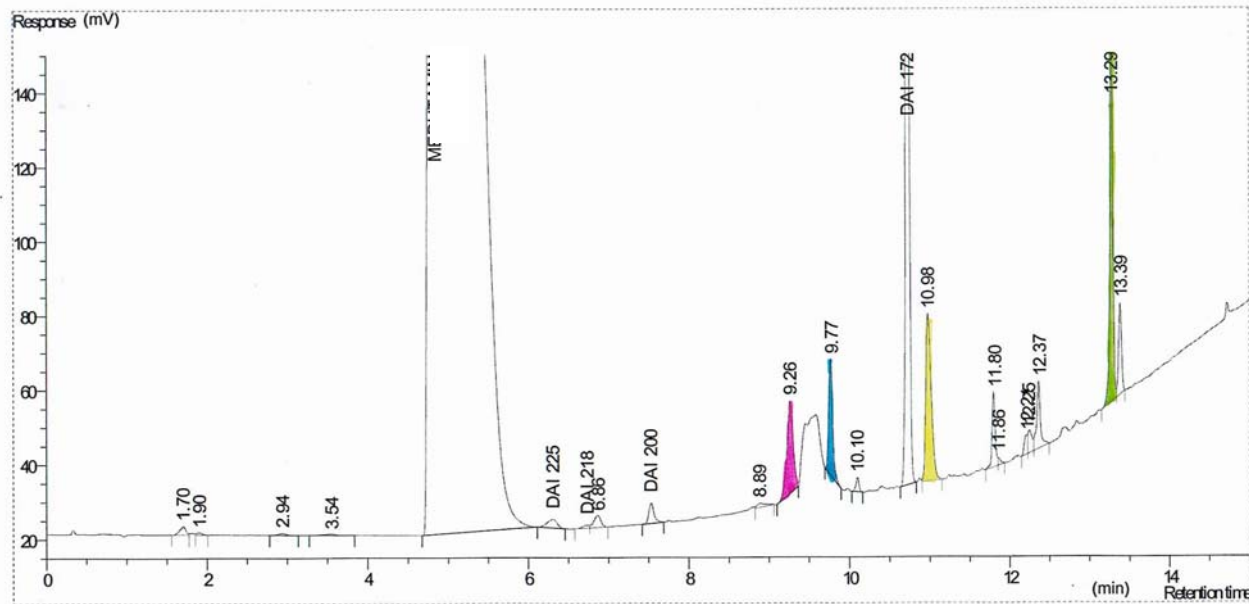
Detection : TLC Silica plate 60F254

Eluant : ethyl acetate 100%

Purification follow-up and final check



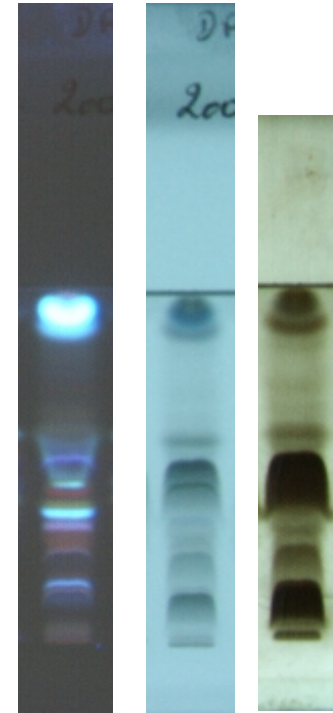
HPLC profile : negative LC/MS identification



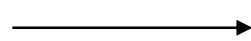
Date d'acquisition : 14/01/2005 10:20:22

Workbook modifié le 24/01/2005 à 15:54:38 par DFC_BL

HPTLC profile



HPTLC study



Flash Chromatography :

Conditions:

Glass column diameter 65 mm long 600mm

normal silica 15-40 μ m mass :6 Kg

Dichloromethan then add Methanol 95-5 et 90-10

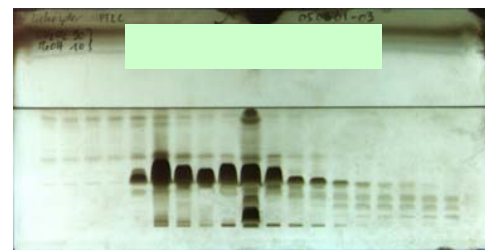
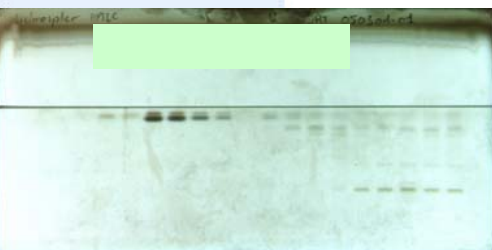
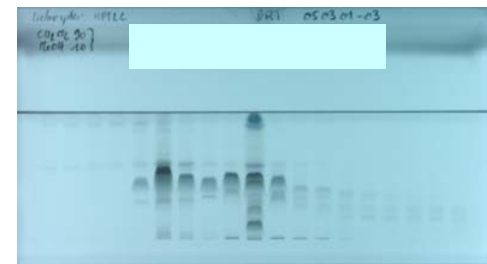
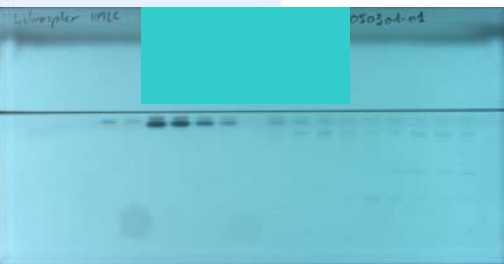
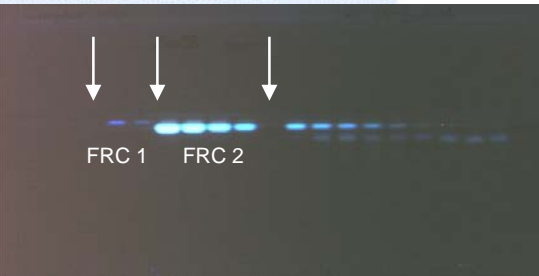
Packing pressure :1bar

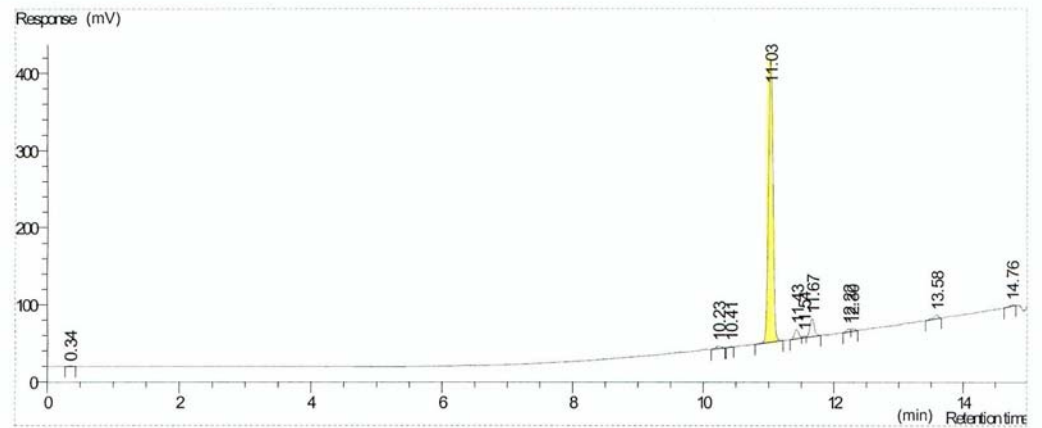
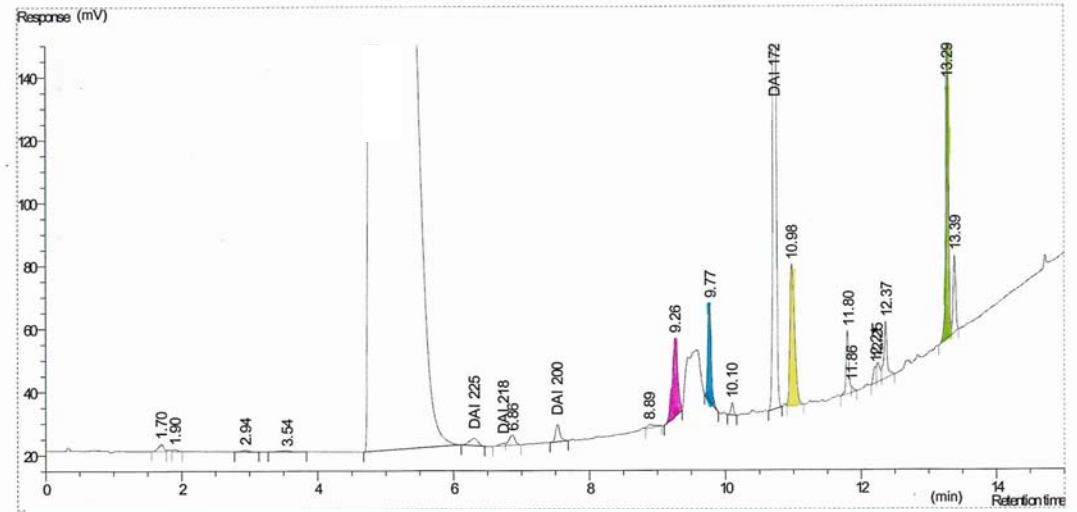
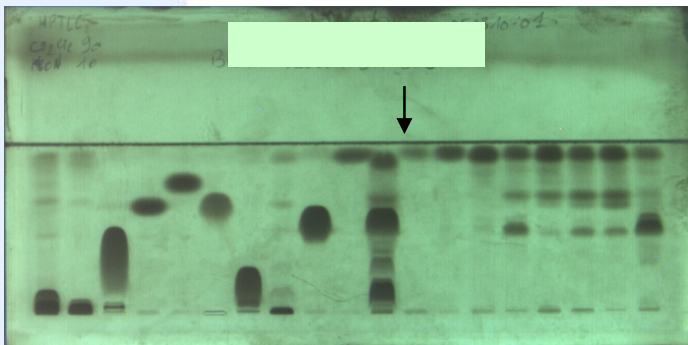
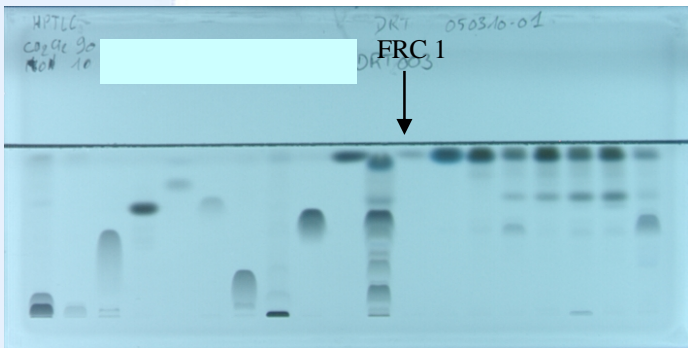
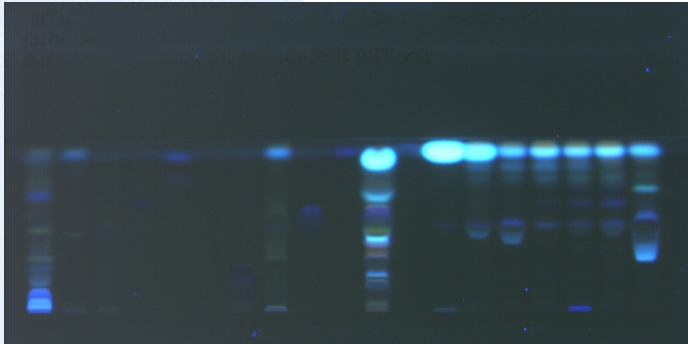
3 g of raw in dichloromethan

Collection: glass flasks

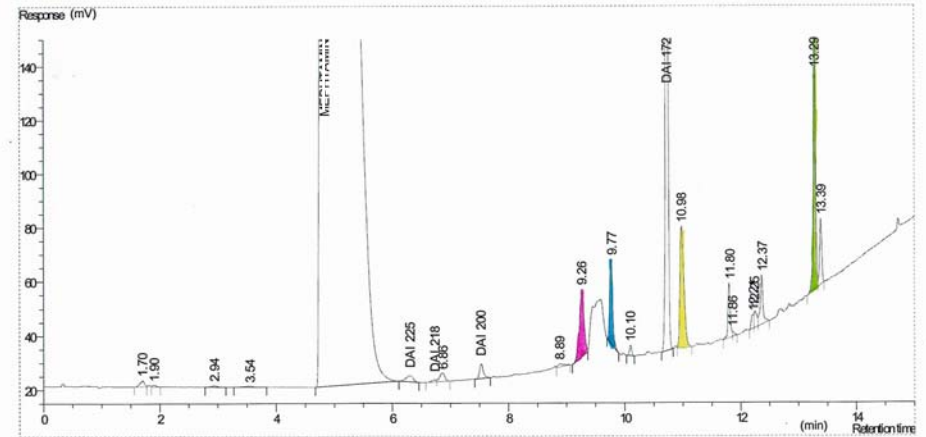
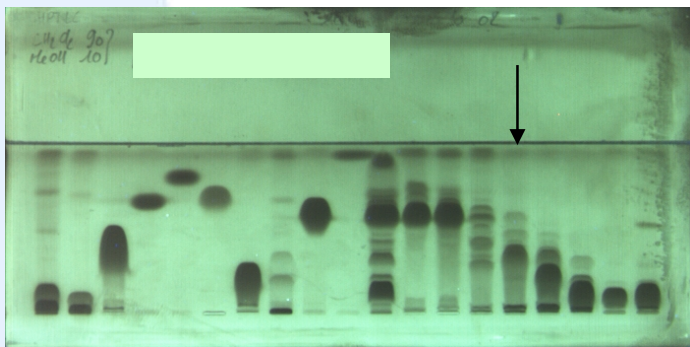
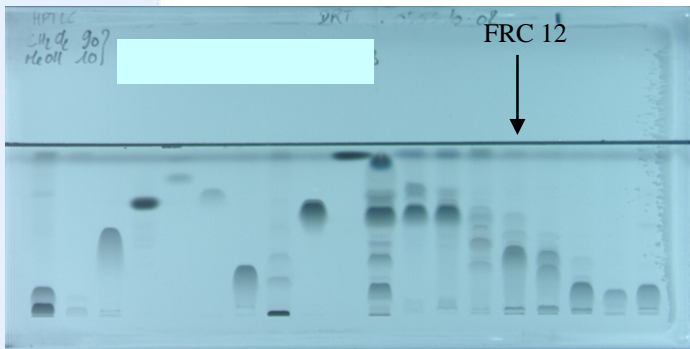
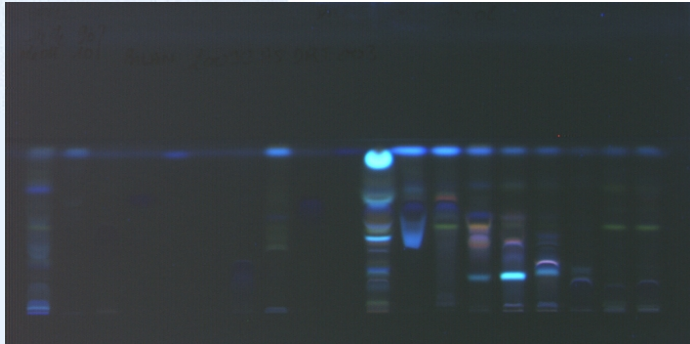
Detection : HPTLC silica plate 60F254

Eluant : Dichloromethan – Methanol 90-10





Information sur les pics



Date d'acquisition : 14/01/2005 10:20:22

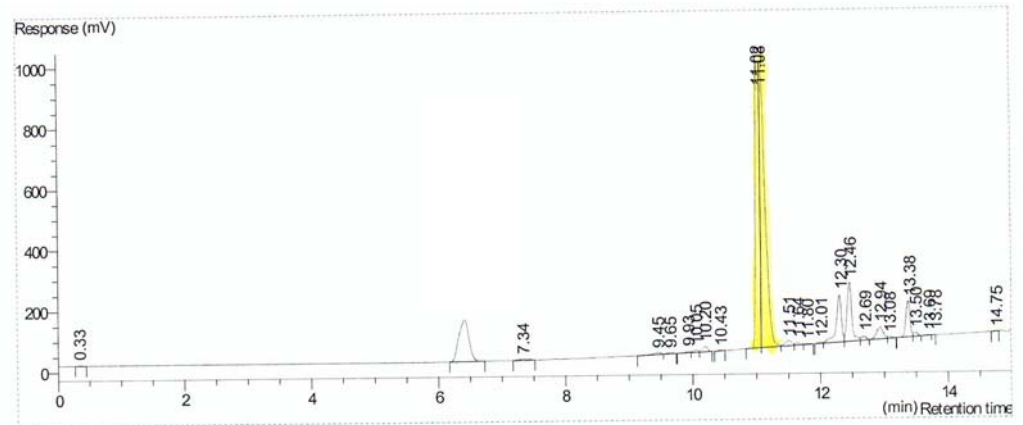
Workbook modifié le 24/01/2005 à 15:54:38 par DFC_BL

Remarque : DALC1553-06 DA_BAL16 DA_LC34 CHROM-1

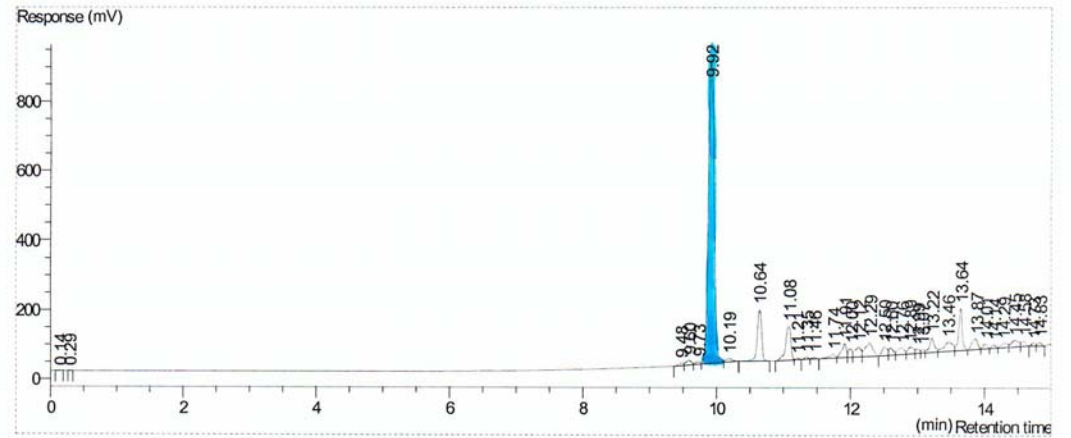
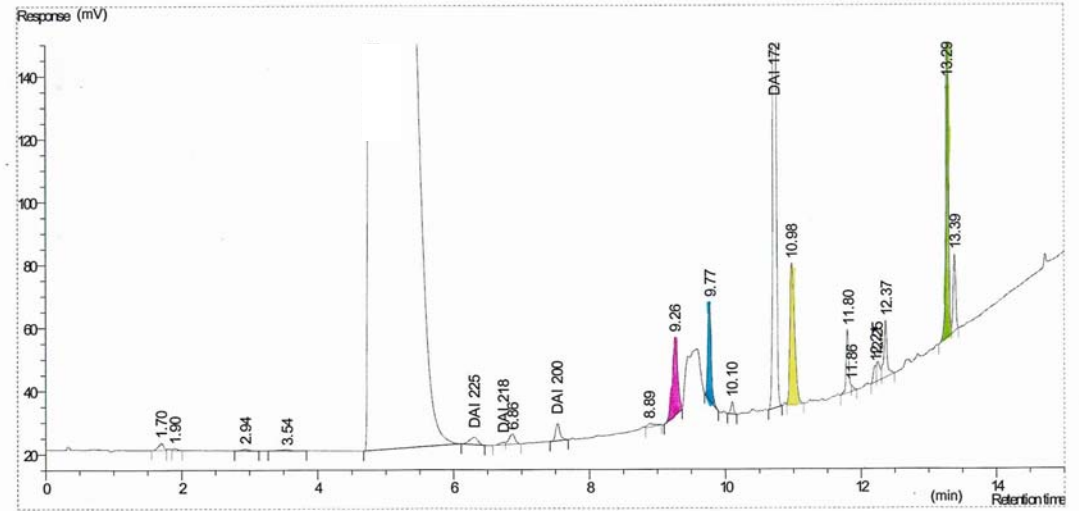
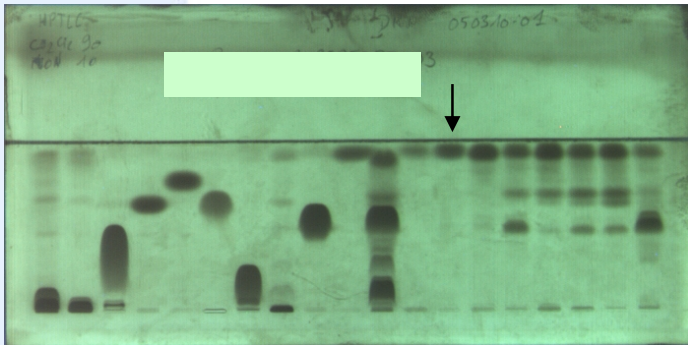
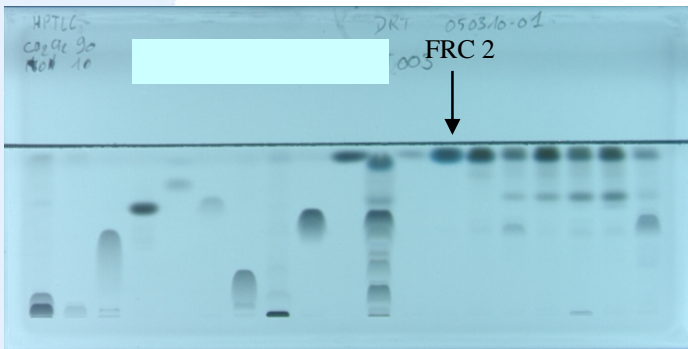
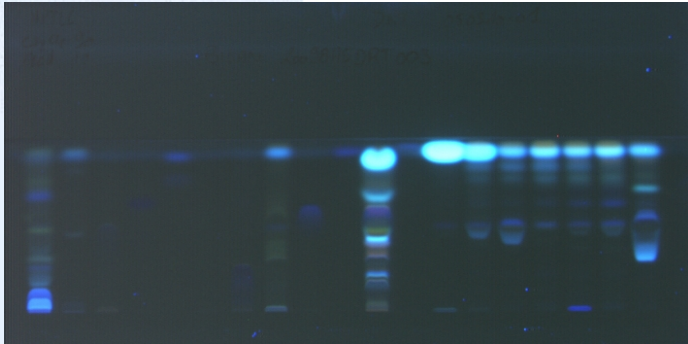
Nom de l'analyste : D.FORGET

Imprimé le 10-mars-2005 à 11:02:4 par DRT_BL

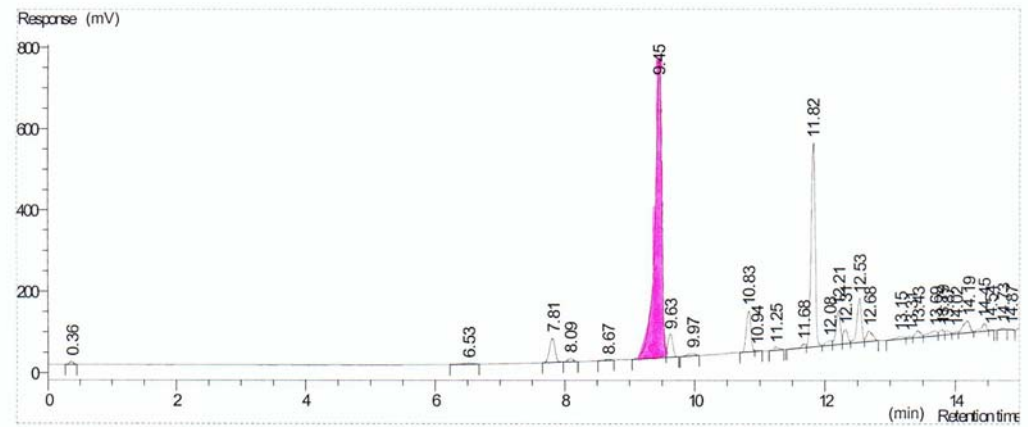
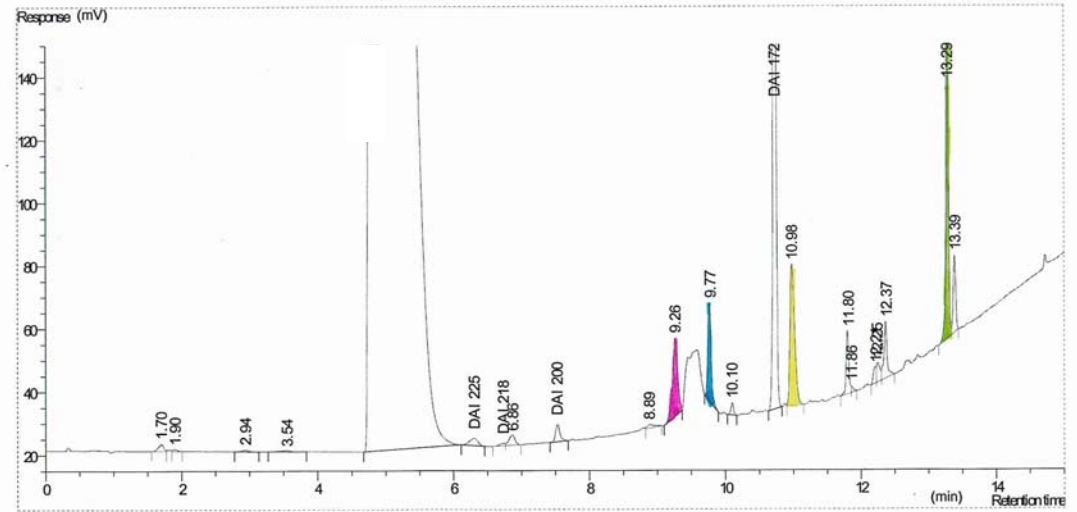
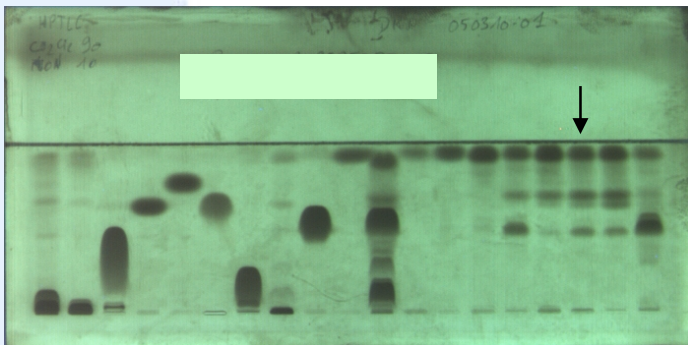
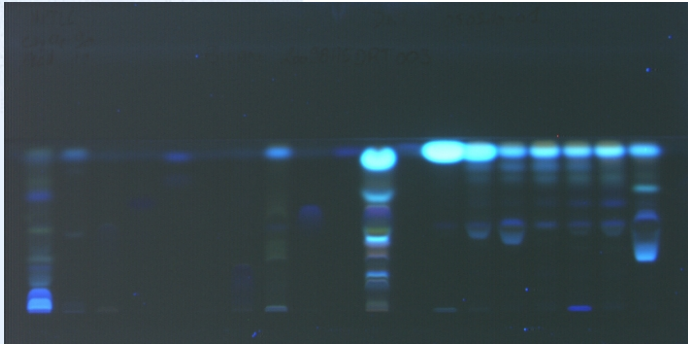
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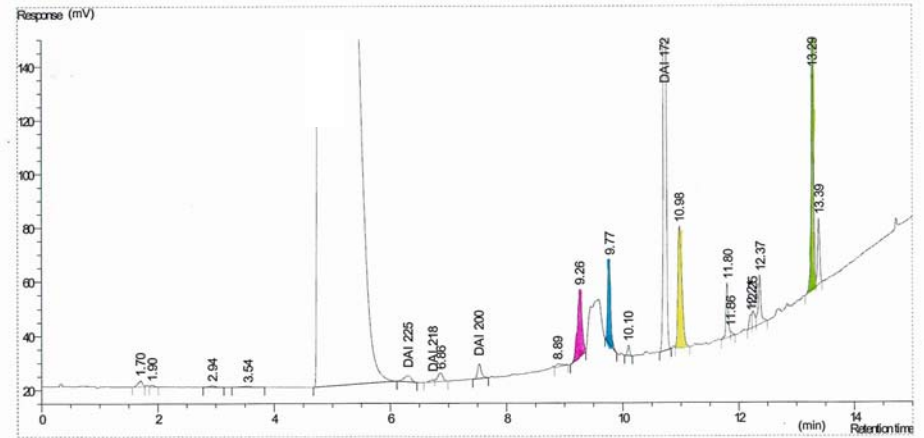
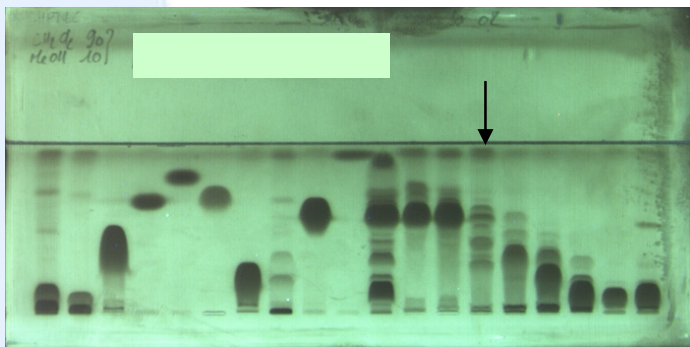
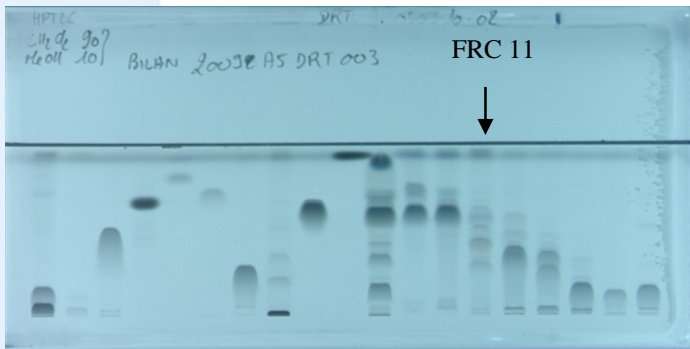
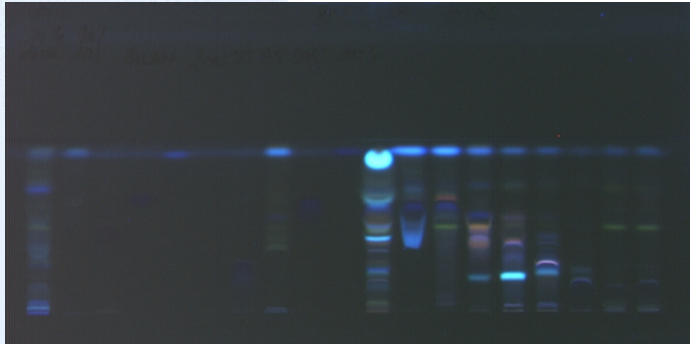
Information sur les pics



Information sur les pics



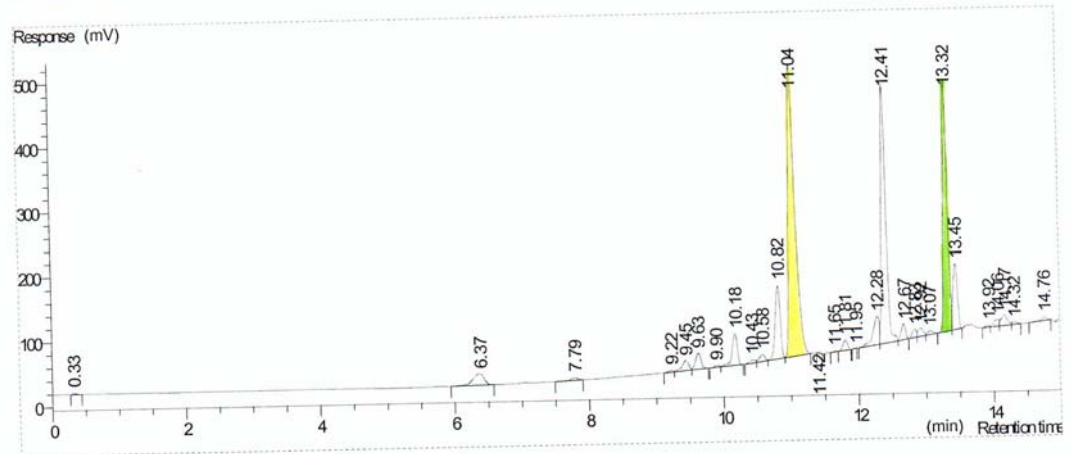
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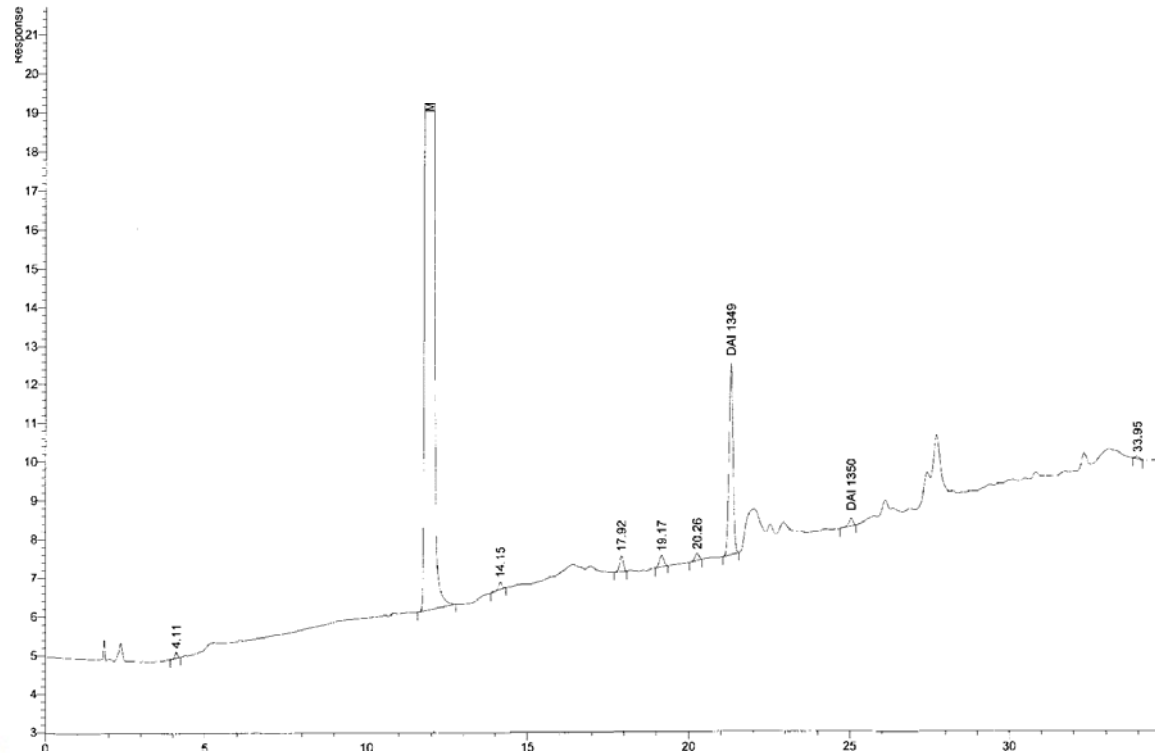
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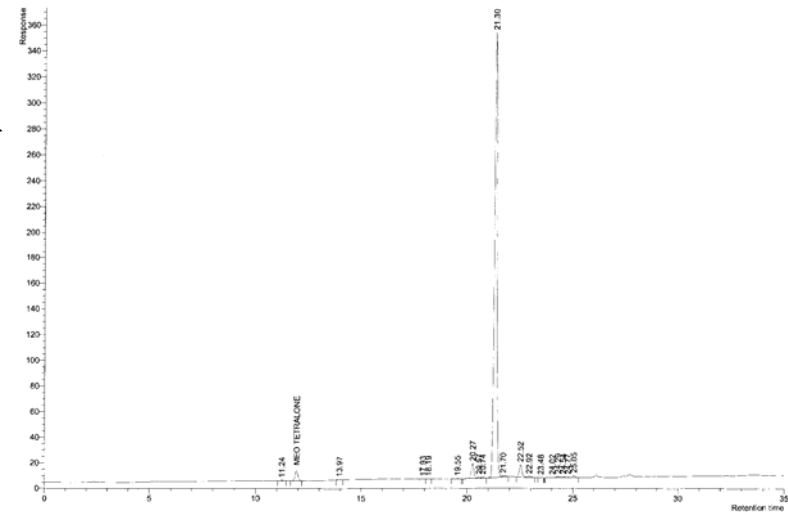
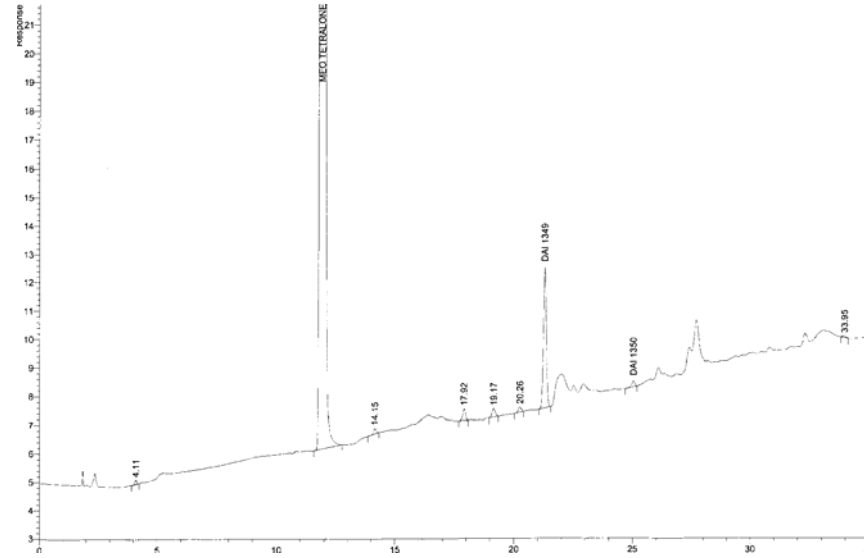
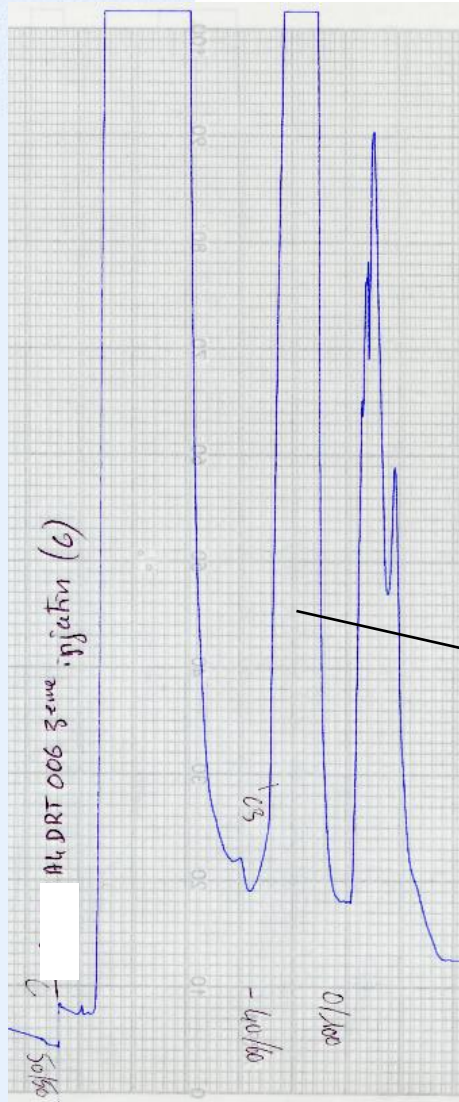
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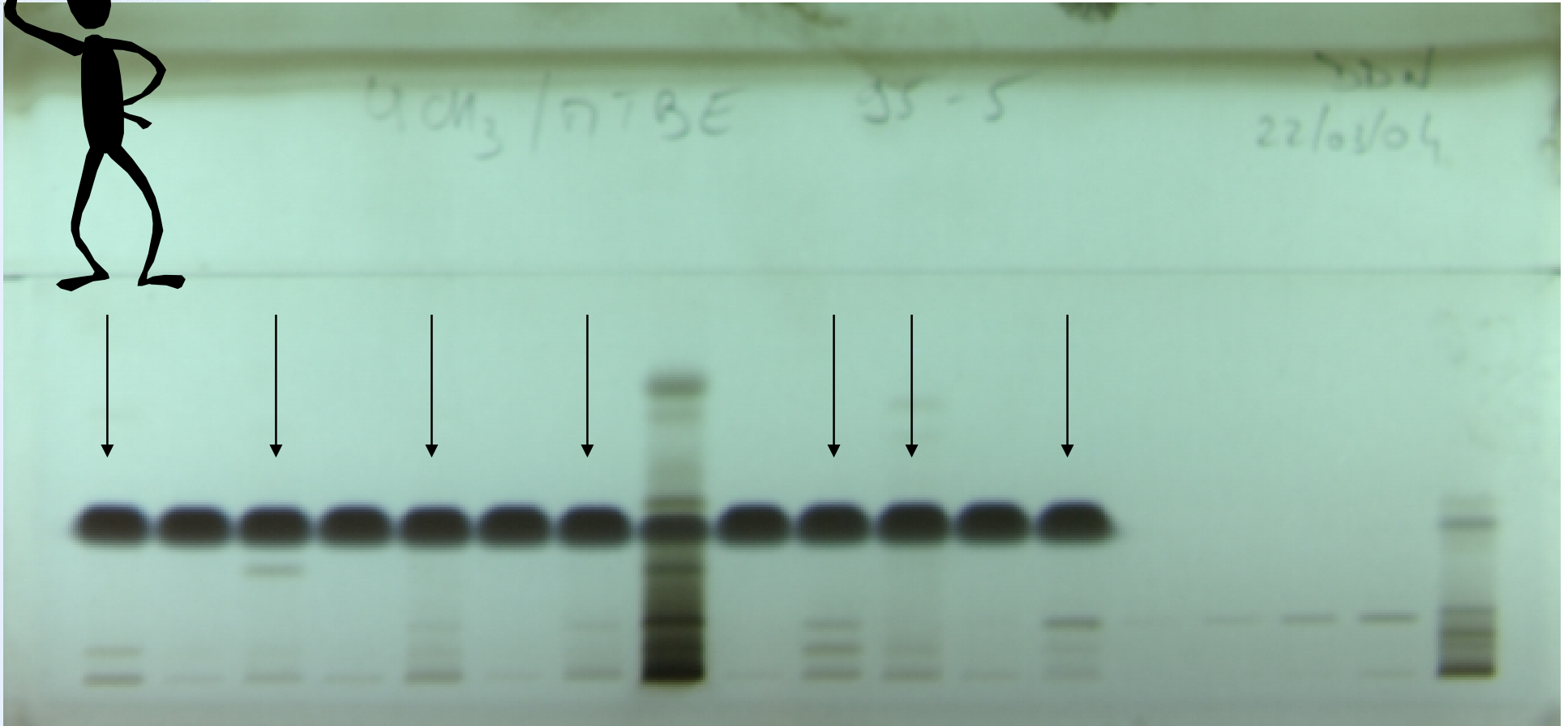


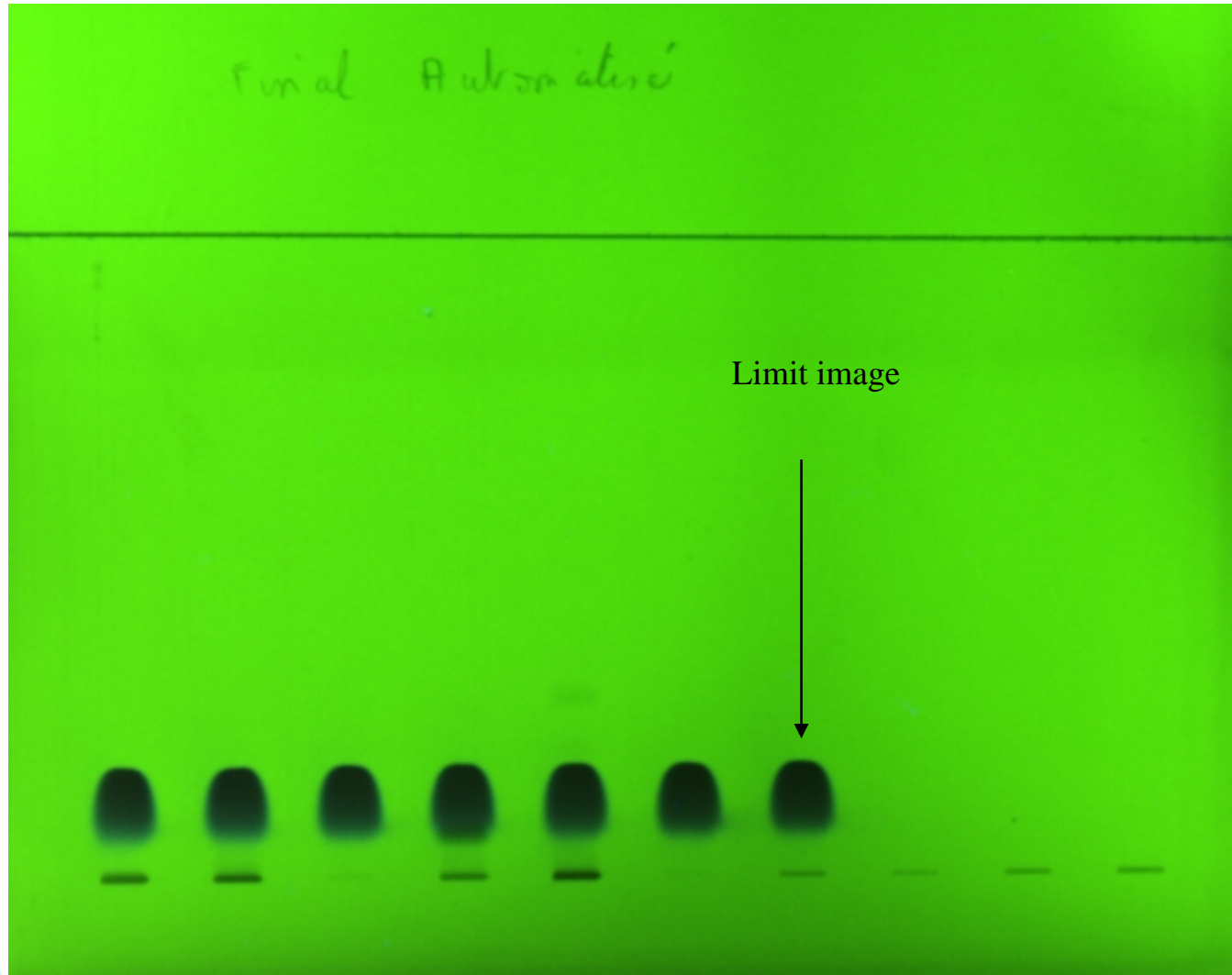
Information sur les pics

Show-up by HPLC of a « poison » substance present in the RM and blocking the aromatisation reaction .





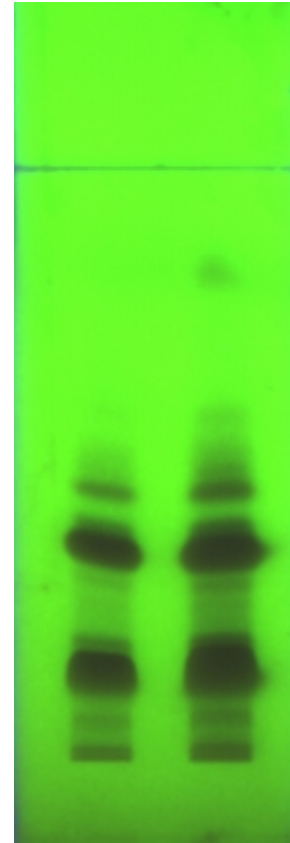




- Goal : isolation of the major impurity in order to make solubility tests in the aim of studying a re-treatment way and getting the pure main compound

- Used analytical system: TLC
Dichloromethan-Methanol-Ammonia

- Optimized TLC system : Dichloromethan-
Methanol



TLC Study

→ Flash Chromatography :

Conditions:

Glass column diameter 65 mm long 600mm

Normal Silica 15-40 μ m mass :6 Kg

Dichloromethan - Methanol 90-10

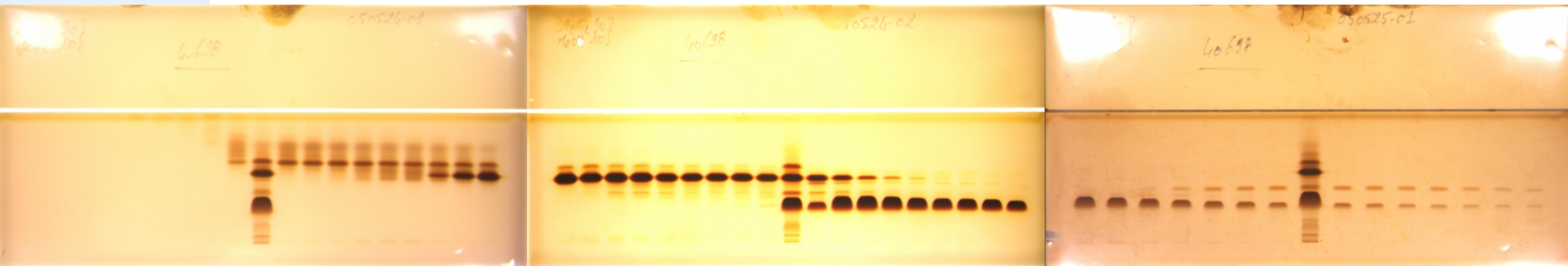
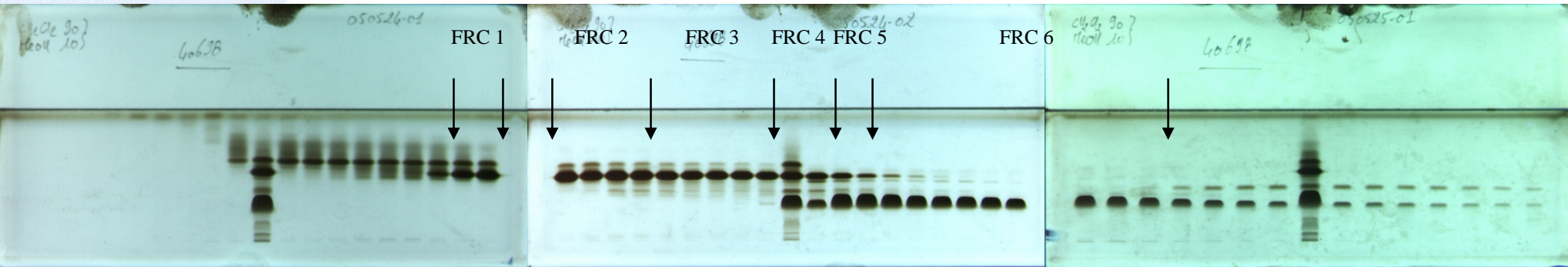
Packing pressure :1bar

100 g of raw in solution in the dichloromethan

Collection: glass flasks

Detection : HPTLC plaque silice 60F254

Eluant : Dichloromethan – Methanol 90-10



FRC 1 = 7.2 g

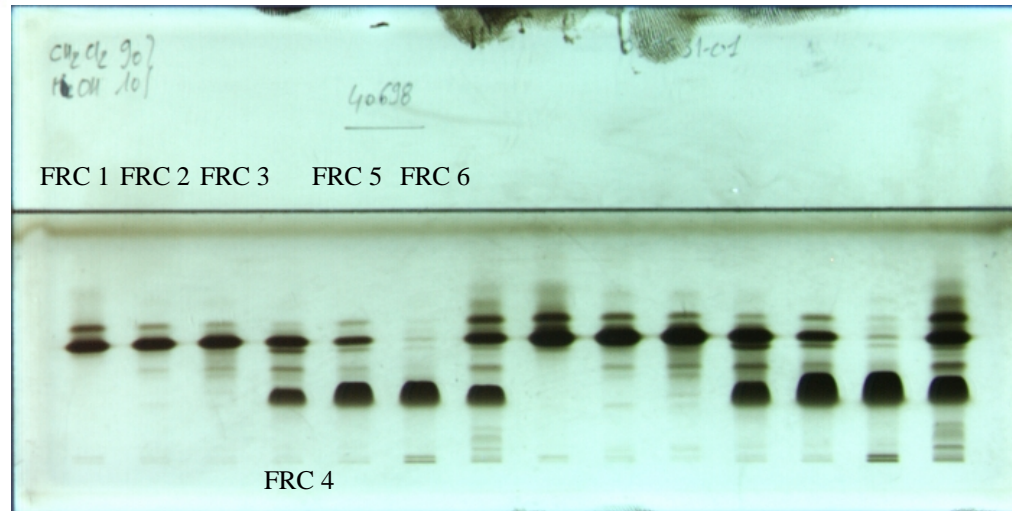
FRC 2 = 22.9 g

FRC 3 = 13.4 g

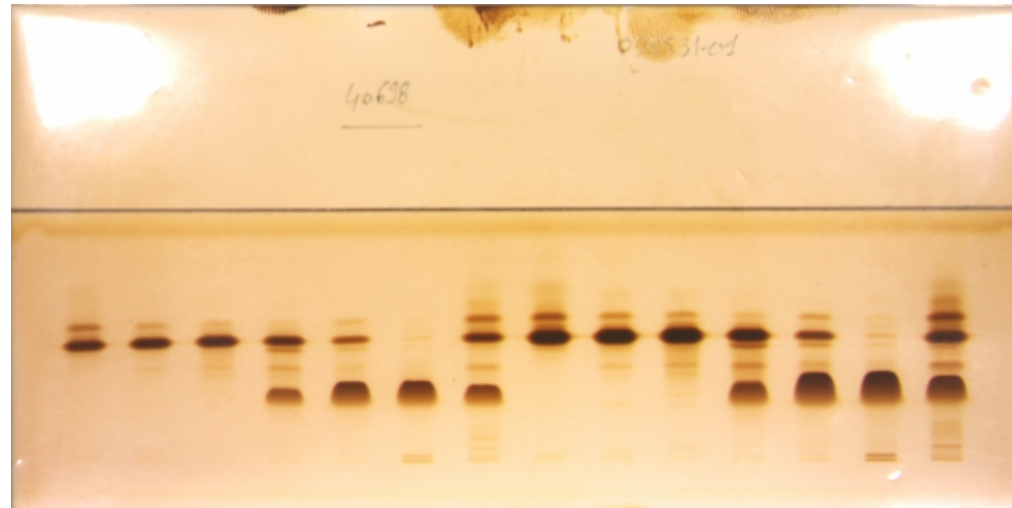
FRC 4 = 5.1 g

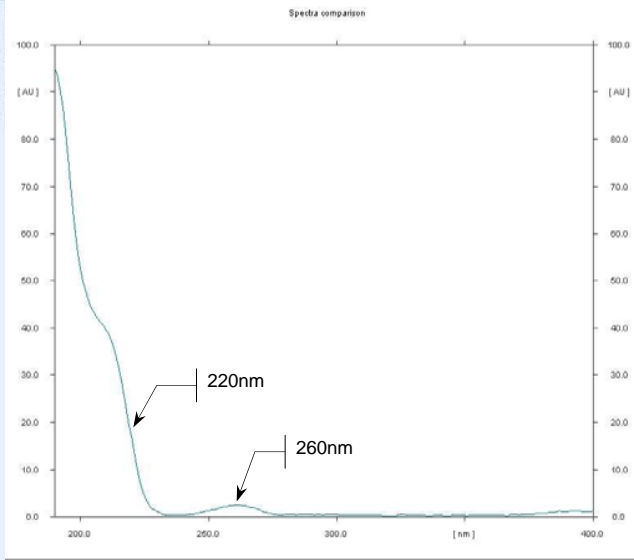
FRC 5 = 14.3 g

FRC 6 = 32.1 g

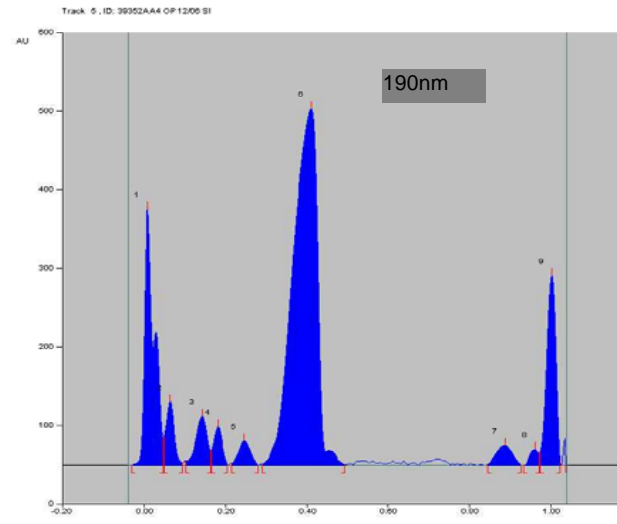


purification
efficiency: 95%

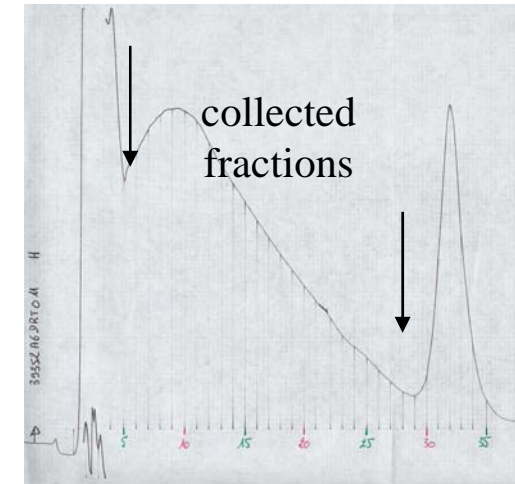




UV spectrum of the compound



Analytical HPTLC at 190nm



LC Prep at 220 nm

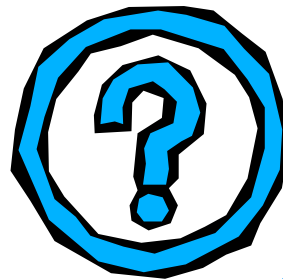
→ The HPTLC possibilities for optimization and follow-up at **190 nm** (without staining) allow a safe transfer on a new 50mm dynamic axis compression column (50 ml/mn).

→ After direct transfer on 450mm column (250L/h) : time saving = 34h/batch !

- TLC is a complementary method to HPLC.
- TLC is a method which enables visualisation of the whole application and especially the compounds remaining at Rf 0.
- Fast and simple technique, easily transferable on a column allowing the isolation of impurities for structural identification and the purification of synthesized compounds.
- L'HPTLC being a more sensitive and selective method allows this technique to be a quantitative method.

**Merci
de
votre
attention**

(Thank you for listening)



(Do you have any questions)