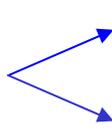


# Interest of HPTLC for hydrocarbon-related analysis and possibilities of detection by induced-fluorescence

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# Fluorescence only occurs in certain molecules

Detection of molecules by fluorescence  Native fluorescence  
Use of a derivatizing agent

Can all the non-fluorescent molecules be determined by fluorescence?

**SATURATED HYDROCARBONS**  
(Alkanes, isoalkanes and cycloalkanes)



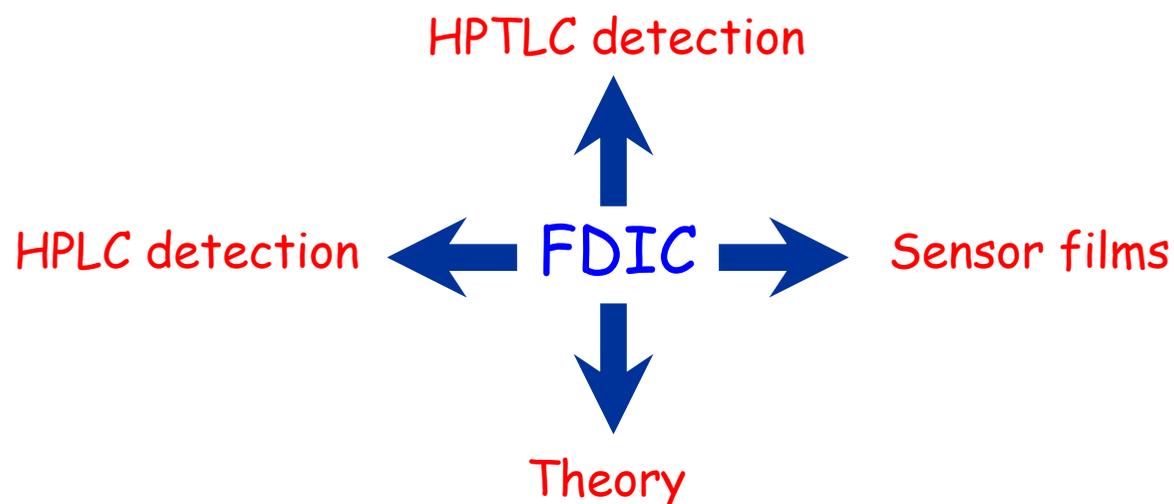
They have neither UV nor fluorescence spectra under analytical working conditions

They are in high concentration in petroleum and its derived products

They have traditionally been considered as inert molecules

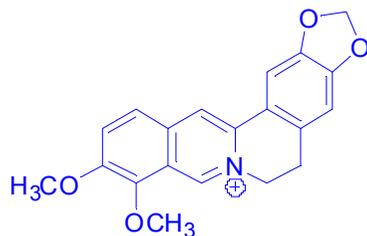
# What is Fluorescence Detection by Intensity Changes (FDIC)?

A wide number of compounds, including *non-fluorescent ones*, induce changes in the fluorescence spectra (either increase or quenching) of certain probes that exclusively affect emission intensity

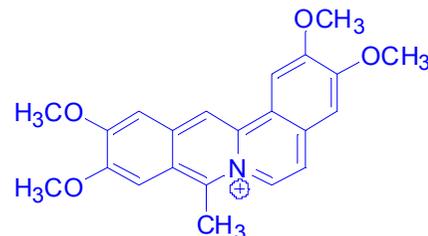


J.Chromatogr.A 2007, 1146, 251  
Anal.Chem. 2006, 78, 3699  
Anal.Chem. 2000, 72, 1759  
Org.Lett. 2000, 2, 2311

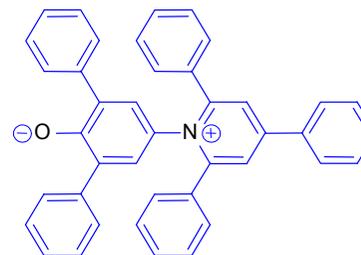
Probes:



Berberine cation



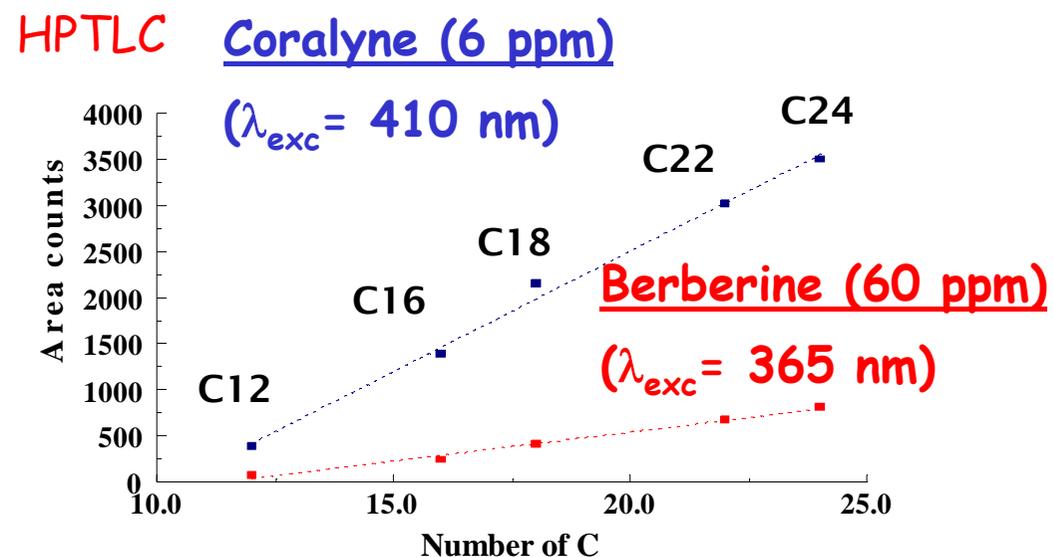
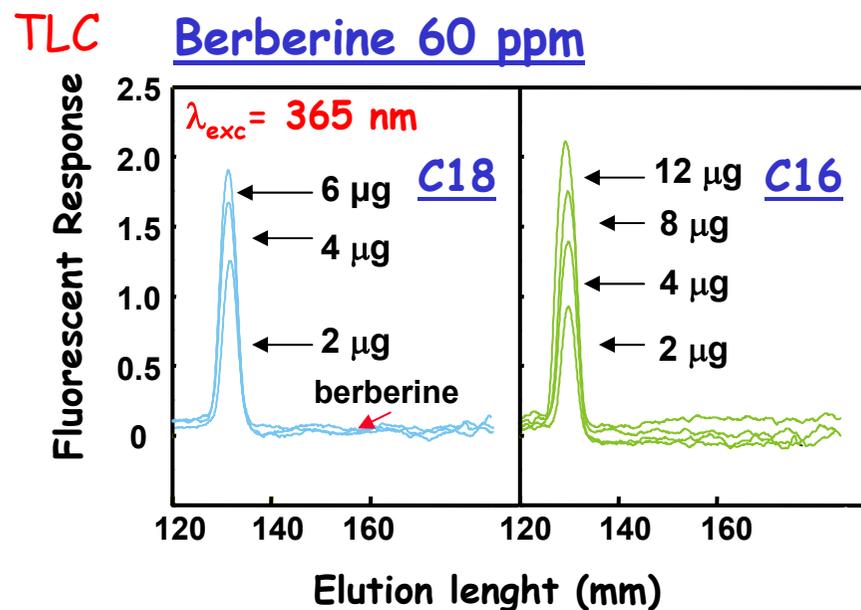
Coralyne cation



Reichardt's Dye

# FDIC produced by saturated hydrocarbons (alkanes) in HPTLC

Increase of fluorescent emission



\* Fluorescent response depends on alkane mass and chain length, and probe concentration

# HPTLC-FDIC application to lipids

## Separation and detection of lipid mixtures

### NEUTRAL LIPIDS (HPTLC)

**Coralyne (6 ppm).**

**Preimpregnation**

**Petroleum ether (80)-  
diethylether (20)-acetic acid  
(1), 20 min**

$\lambda_{exc} = 410 \text{ nm}; \lambda_{em} > 450 \text{ nm}$

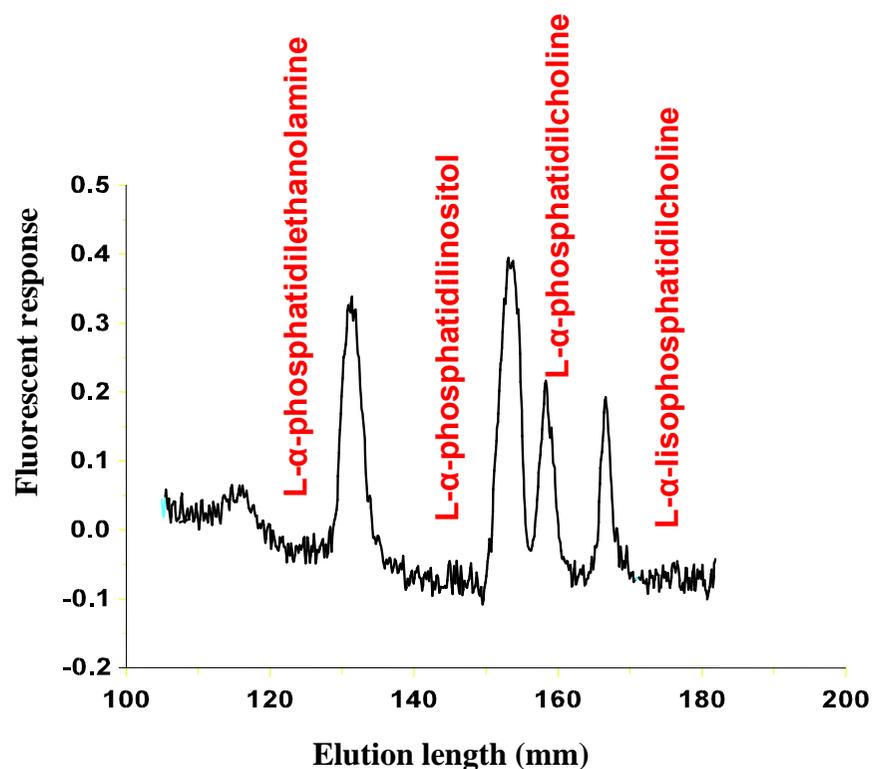
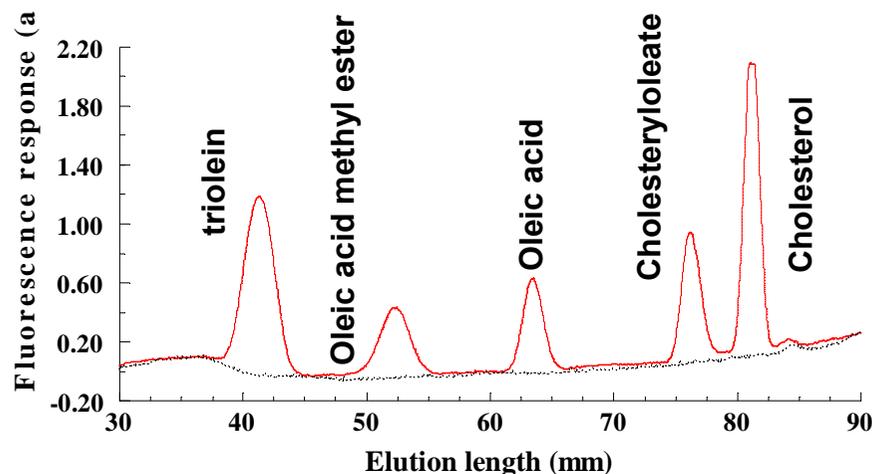
### PHOSPHOLIPIDS (in TLC plates)

**Berberine (10 ppm).**

**Postimpregnation**

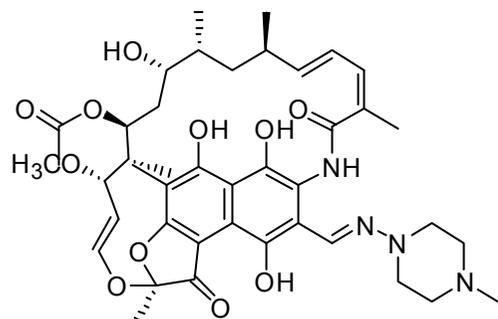
**Chloroform (65)-methanol (25)-  
water(4), 15 min**

$\lambda_{exc} = 365 \text{ nm}; \lambda_{em} > 450 \text{ nm}$



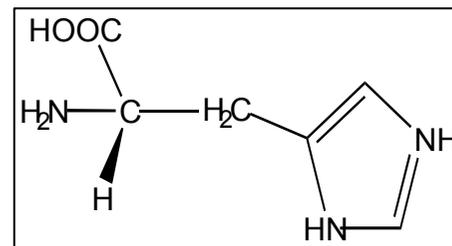
# Other compounds give quenching of emission

## A polar antibiotic

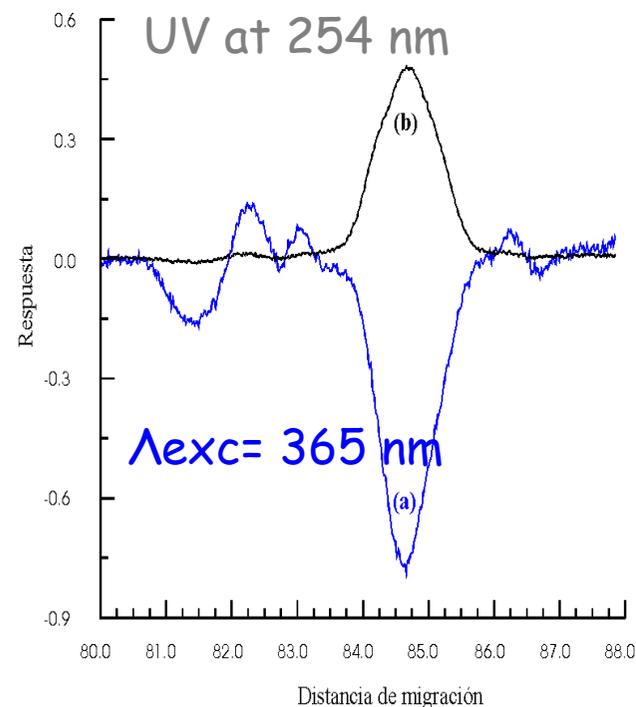
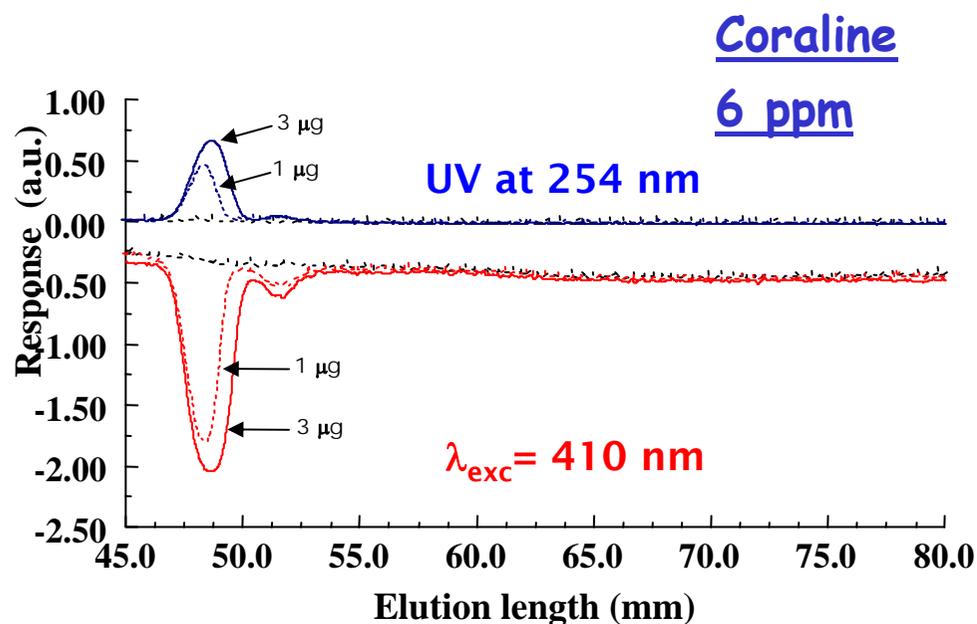


Rifampicine

## An aminoacid



Histidine



HPTLC layer, methanol,  
5 min;  $\lambda_{em} > 450 \text{ nm}$

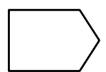
Application point

# A theoretical model to explain changes in emission

Electrostatic interactions are responsible of emission increases (MODEL)

Two effects, through polarizability  $\alpha$

$$\phi = \frac{k_r}{k_r + k_{nr}}$$



1)  $\epsilon_r, n, \alpha$

2) The higher the interaction, the higher the protection (the higher  $\alpha$ )

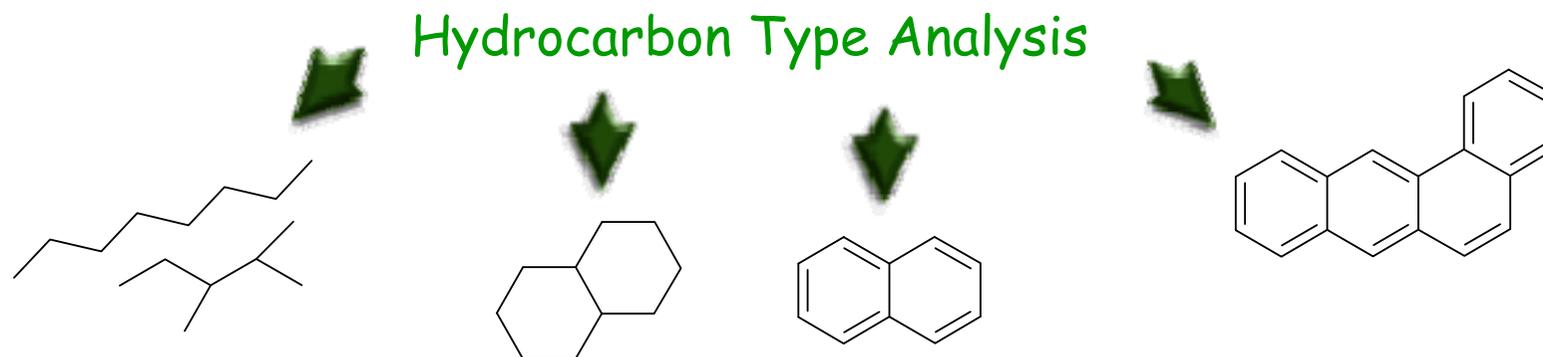


Negative peaks can be attributed to quenching produced by net specific interactions between polar compounds and probe

MODEL: non-specific, dipolar interactions between probe and the corresponding analyte contribute to the efficiency of the fluorescence emission, creating a microenvironment that isolates the fluorescent probe and prevents non-fluorescent decay mechanisms.

FDIC response depends on the balance non-specific (electrostatic) and specific interactions between probe-analyte, as well as the dielectric permittivity ( $\epsilon_r$ ) of the medium. It only involves non-covalent interactions

# Problems in hydrocarbon analysis: heavy boiling complex mixtures



**DETERMINATION:** column techniques are not satisfactory

*TLC / HPTLC*

- ▶ TLC is adequate to the analysis of complex mixtures
  - \* Analysis of the whole sample
  - \* Uneluted compounds are also detected
  - \* Scanning of the same sample under different conditions
- ▶ Almost all types of petroleum products can be analyzed using a single technique
- ▶ Direct and accurate determination of saturated compounds using FDIC

# Experimental systems used in our research

## Conventional TLC system:

TLC plates (5-25  $\mu\text{m}$  particle size)  
Autospotter for sample application  
Conventional vertical tank for elution



## HPTLC system:

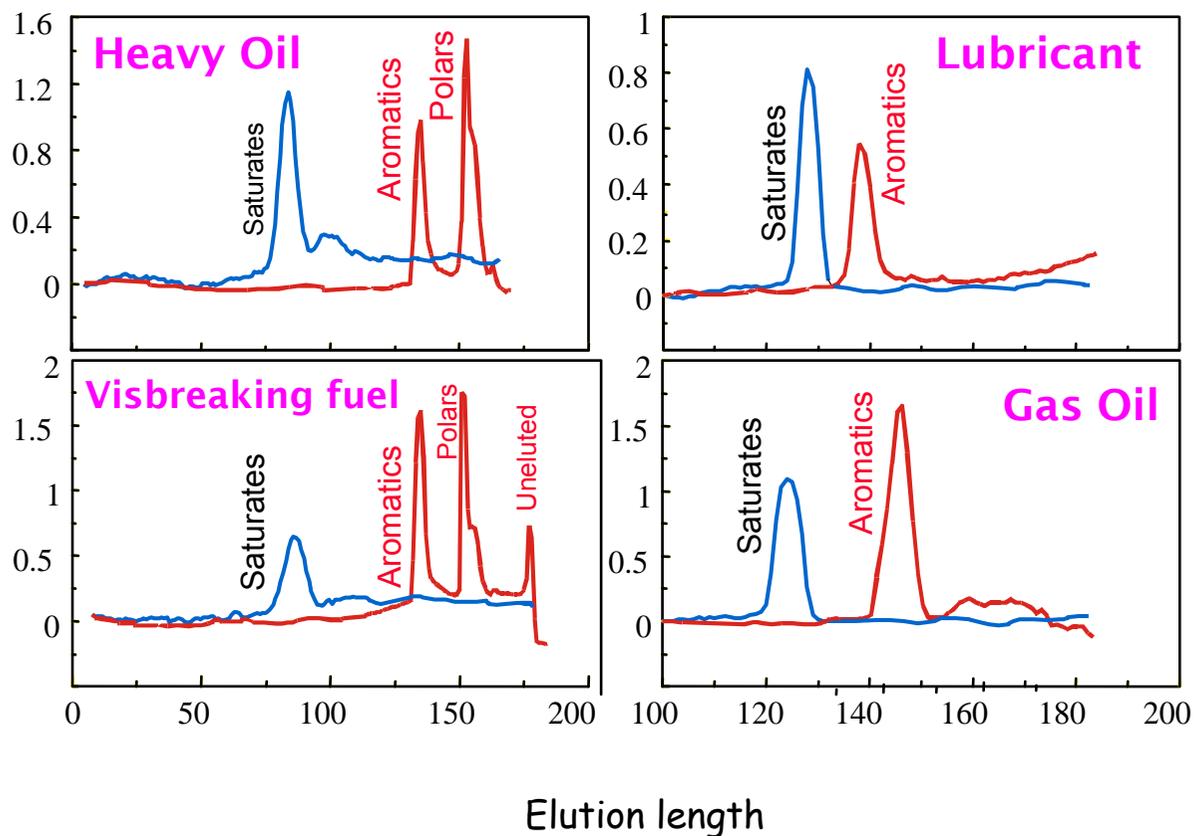
HPTLC plates (3-10  $\mu\text{m}$  particle size)

Linomat for sample application  
Horizontal developing chamber



# Conventional TLC for hydrocarbon analysis

# Hydrocarbon-Type Analysis of fossil fuel-derived products



## ➤ Equipment variables

- Development system
- Application system

## ➤ Analyte load and structure

## ➤ Chromatographic variables

- Probe nature
- Probe concentration
- Application volume
- Scanning beam size
- Excitation and emission
- Development length

## Two measurements:

- Saturates developed on a berberine-impregnated layer and detected by fluorescence;  $\lambda_{exc}=365\text{ nm}$ ;  $\lambda_{em}>450\text{ nm}$
- The other peaks on nonimpregnated layer and detected by UV at 254 nm

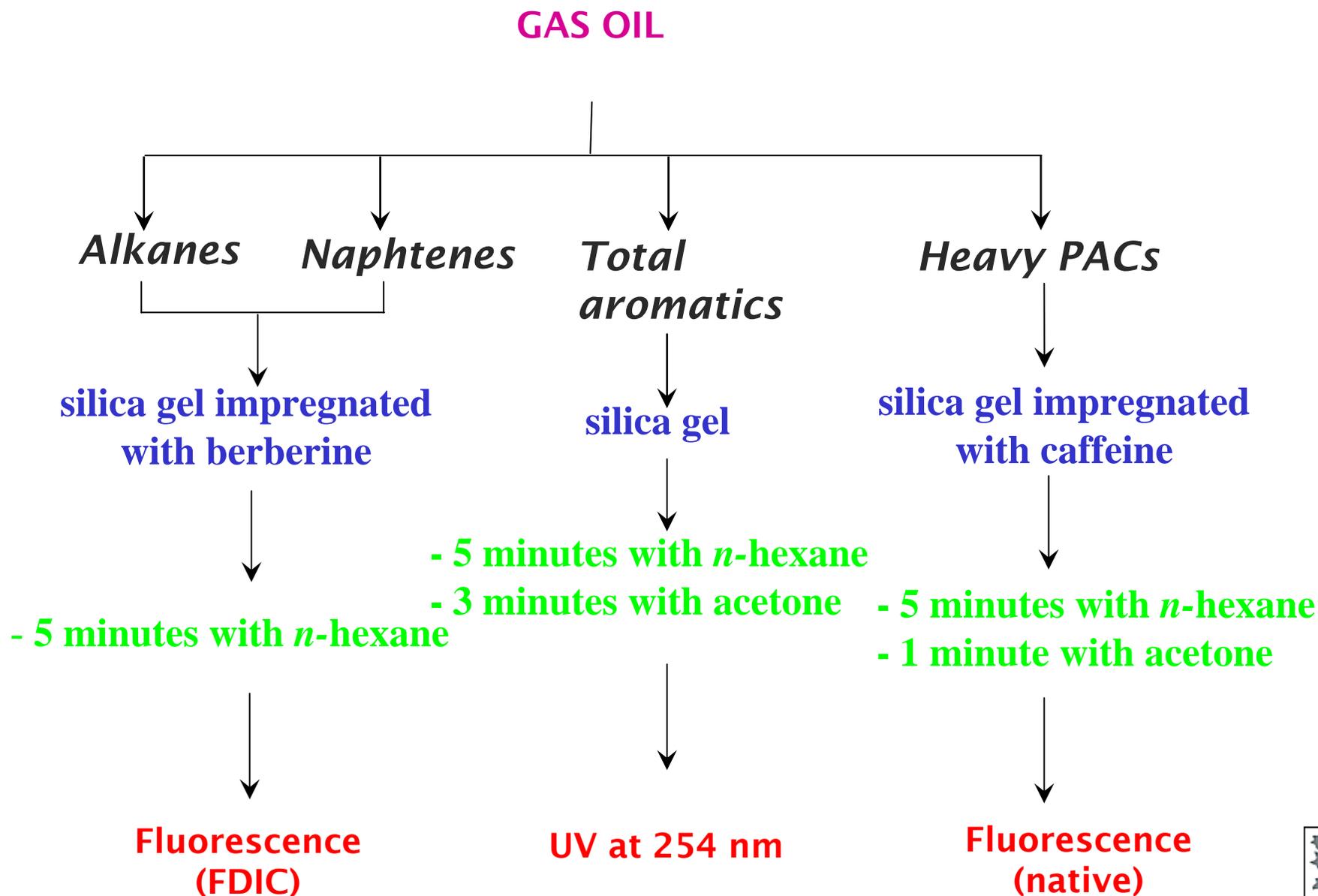
# Results using conventional TLC

Sample	SATURATES					
	wt %	ref.	% RSD	Ref.tech.	int. ( $\mu\text{g}$ )	$r^2$
Heavy oil	35,2	36,1	6,85	(TLC-FID)	3-16	0,999
Visbreaking fuel	12,3	12,1	1,85	(TLC-FID)	4-20	0,996
Lubricant	56,2	56,8	2,21	(ASTM D2007)	4-16	0,993
Gasoil	69,8	70,8		(HPLC-RI)	5-20	0,99

Percentages of saturates are in agreement with those obtained using the reference techniques

# HPTLC for hydrocarbon analysis

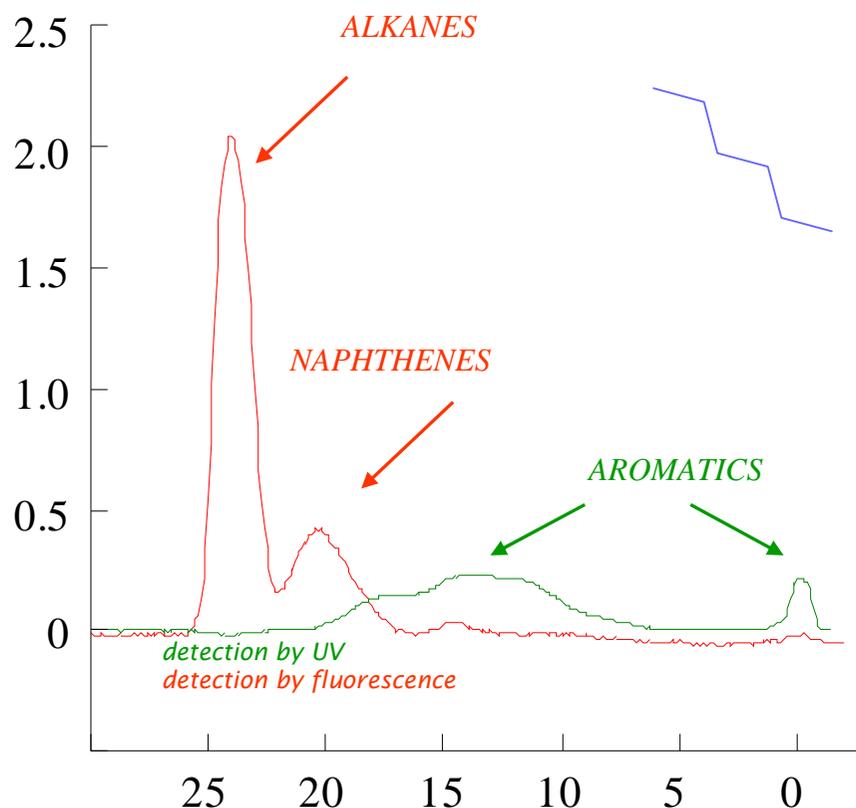
# Hydrocarbon-type analysis of a heavy gasoil



**scanning densitometry detection**

# 1. Alkanes by FDIC

berberine



- development: 5 minutes with *n*-hexane

Calibration Range : 0.05 to 1.5  $\mu\text{g}$   
 $y=1163x+744$   
 $r^2 = 0.9862$   
LOD = 0.05  $\mu\text{g}$   
LOQ = 0.15  $\mu\text{g}$

Detection by FDIC  $\left\{ \begin{array}{l} \lambda_{\text{exc}} = 365 \text{ nm} \\ \lambda_{\text{em}} = > 450 \text{ nm} \end{array} \right.$

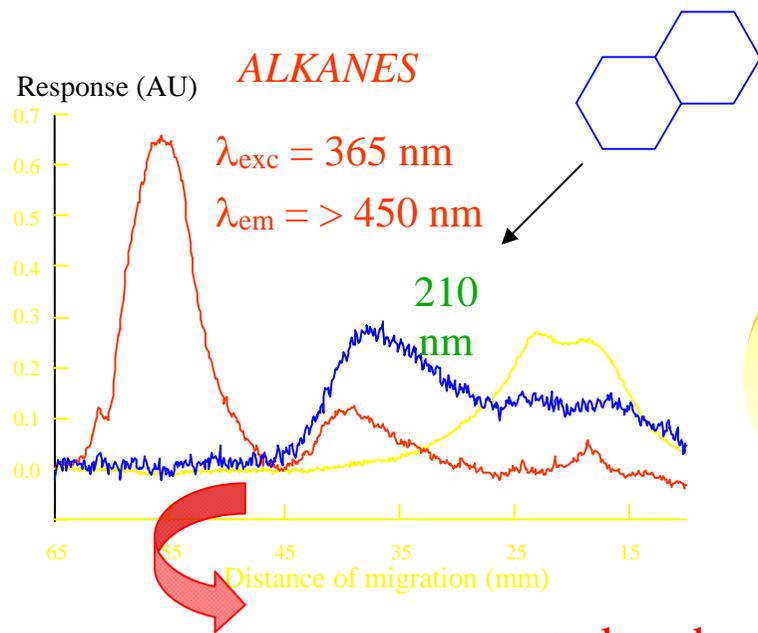
Detection by UV

Either on the same impregnated plate  
or on a non-impregnated plate

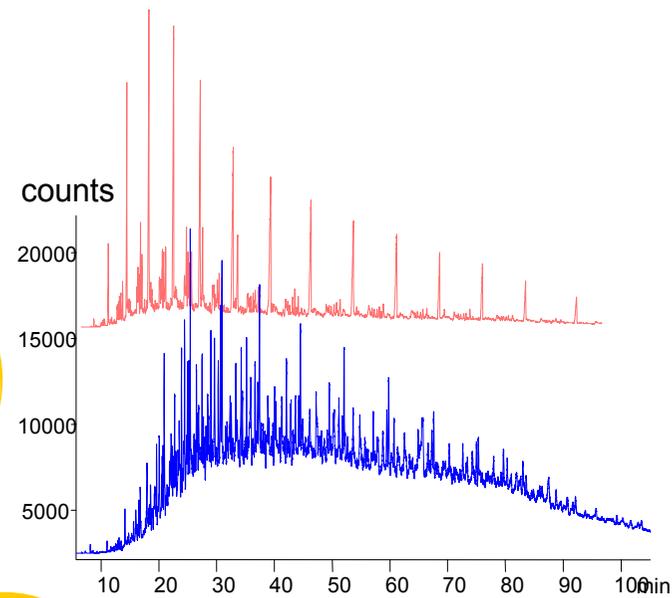
Is it possible to determine all families by FDIC?

# 2.- Naphthenes by FDIC

Calibration Range : 0.6 to 2.4  $\mu\text{g}$   
 $y=655x-89$   $R^2 = 0.9097$   
 LOD = 0.1  $\mu\text{g}$  LOQ = 0.3  $\mu\text{g}$



**GC-FID**



**IRFT**

Effect of berberine on separation?  
 Charge-transfer?

**NAPHTHENES** absorb at 210 nm

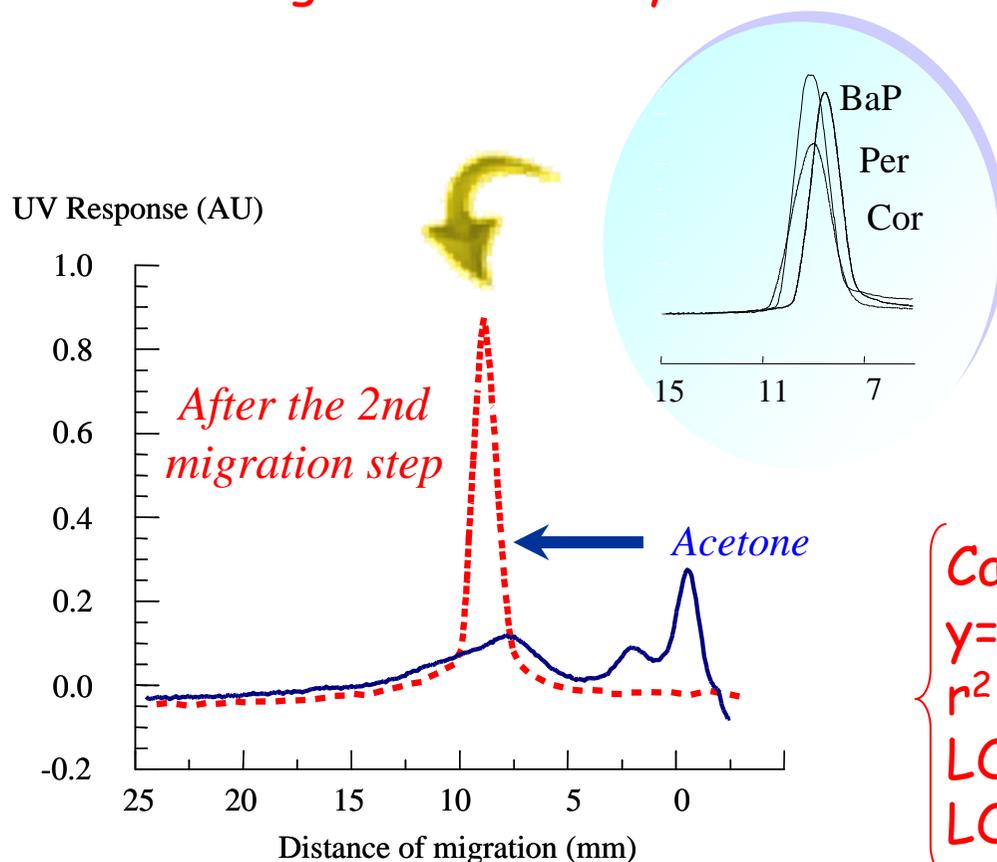
**$^1\text{H-NMR}$**

Increasing of the ratio  $\text{CH}_2/\text{CH}_3$   
 (1459  $\text{cm}^{-1}$  - 1380  $\text{cm}^{-1}$ )

Chemical shifts and coupling constant typical of cyclic  $-\text{CH}_2-$

### 3.- Total aromatics by UV

#### UV Scanning Densitometry



Calibration Range : 0.1 to 2.0  $\mu\text{g}$   
 $y=873x+89$   
 $r^2 = 0.9684$   
LOD = 0.06  $\mu\text{g}$   
LOQ = 0.18  $\mu\text{g}$

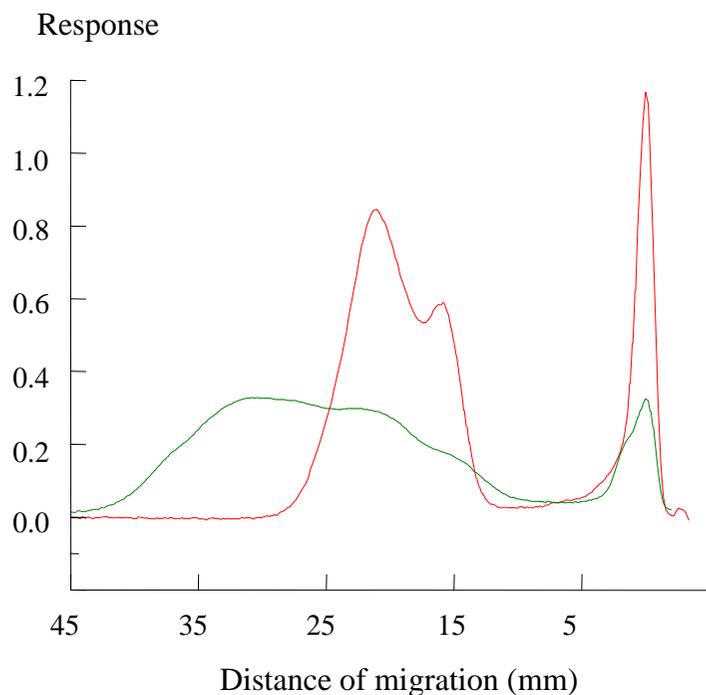
On a non-impregnated silica gel HPTLC plate because berberine is highly soluble in acetone

## HPTLC for aromatics (PACs)

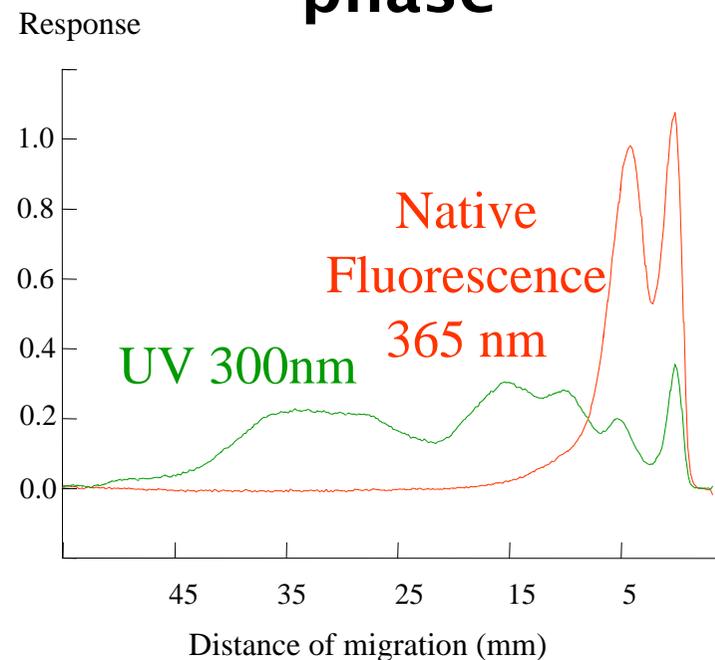
# Influence of caffeine in separation of aromatics

Plate impregnated with Caffeine : electron acceptor

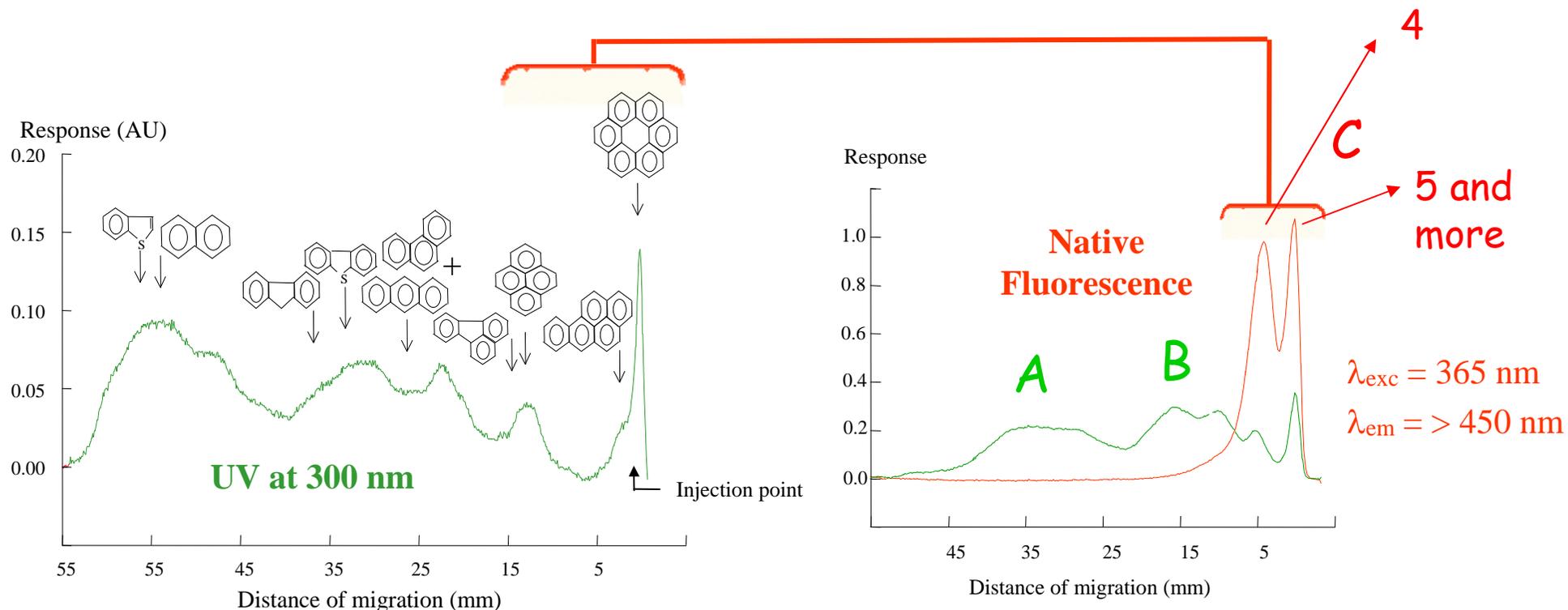
## Silica gel



## Caffeine stationary phase



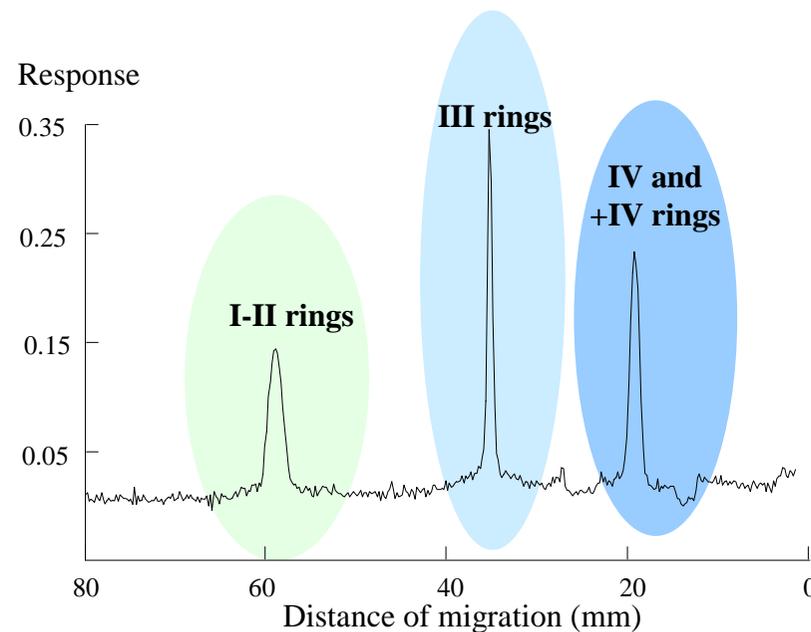
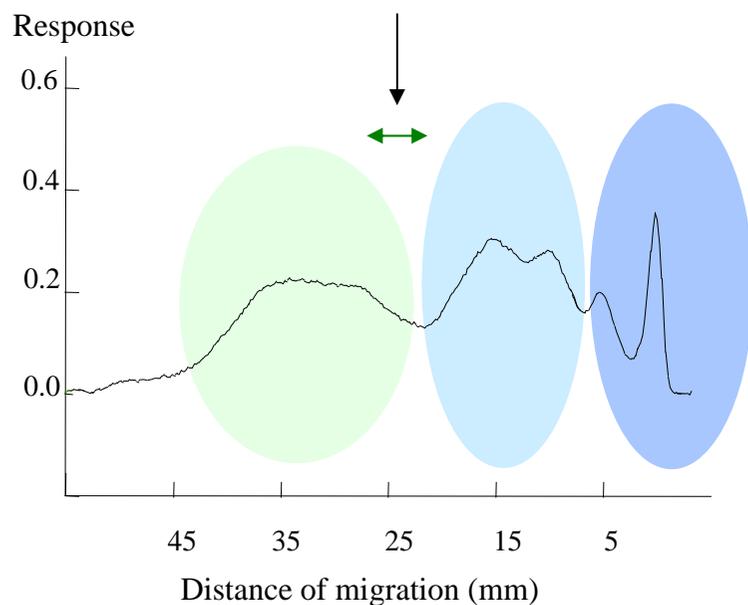
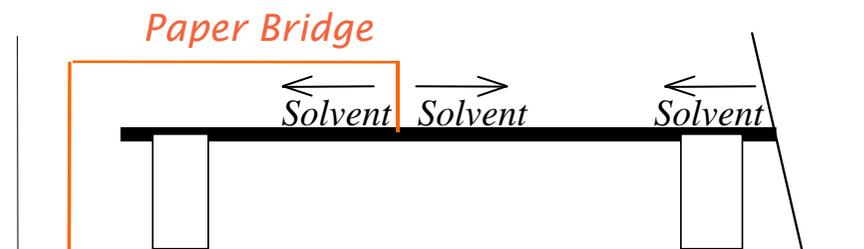
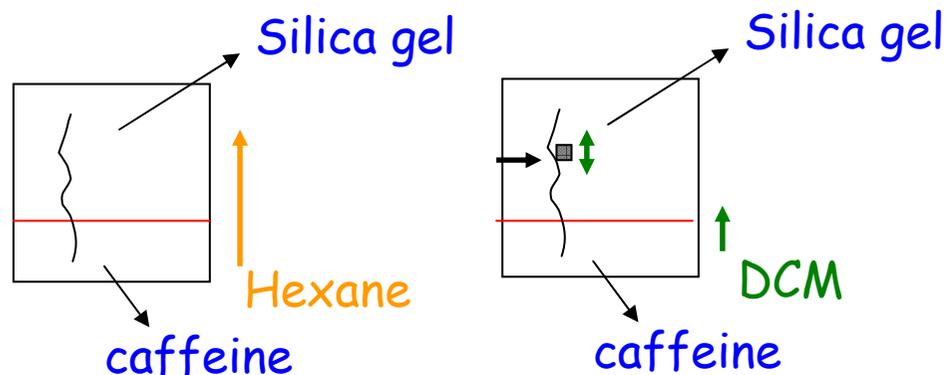
# Selective detection and quantification of HEAVY PACs



2nd migration step (EtOH or acetone) to merge PACs  $\left\{ \begin{array}{l} \text{Calibration Range : 0.1 to 2.0 } \mu\text{g} \\ y=898x+8 \text{ } R^2 = 0.9970 \\ \text{LOD} = 0.07 \mu\text{g} \text{ } \text{LOQ} = 0.25 \mu\text{g} \end{array} \right.$

- A:** 2 aromatic rings and benzothiophenes
- B:** 3 aromatic rings + fluorenic+ dibenzothiophenic
- C:** four and more aromatic rings

# Separation of aromatics according to ring number



...and AMD?

# Calibration in Hydrocarbon-Type Analysis

# Calibration: which standards?

One molecule

Its commercial standard

Complex mixtures

One molecule as representative standard ?

Detection method is chosen regarding to the structure of molecules



Response factor is generally structure dependent

Possibilities:

Internal standard

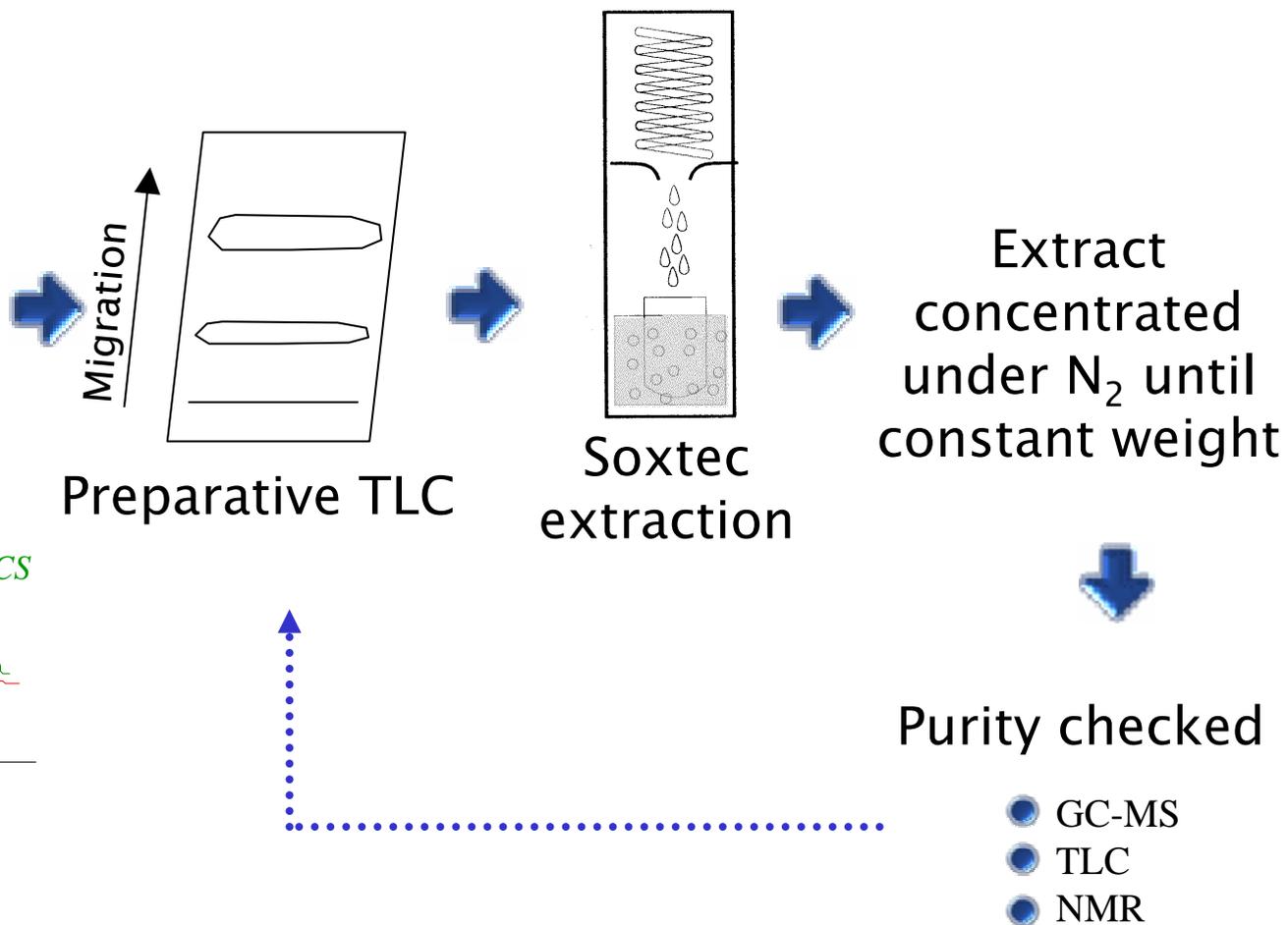
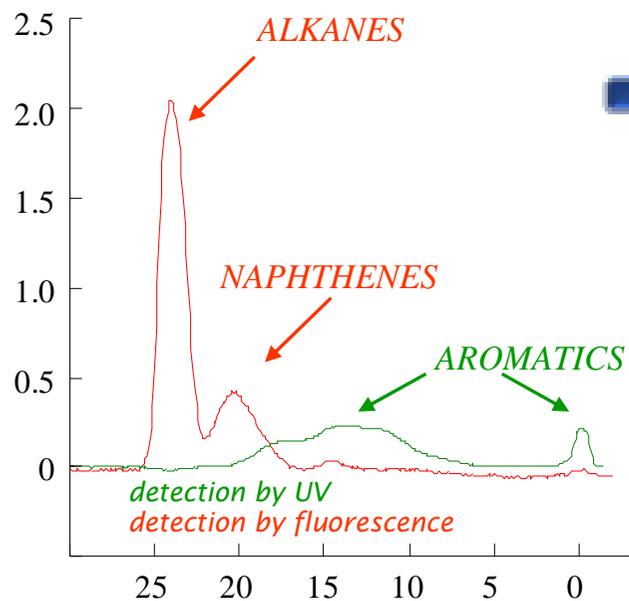
A representative alkane (SIMDIS concept)

A representative fraction



Preparative step based on TLC

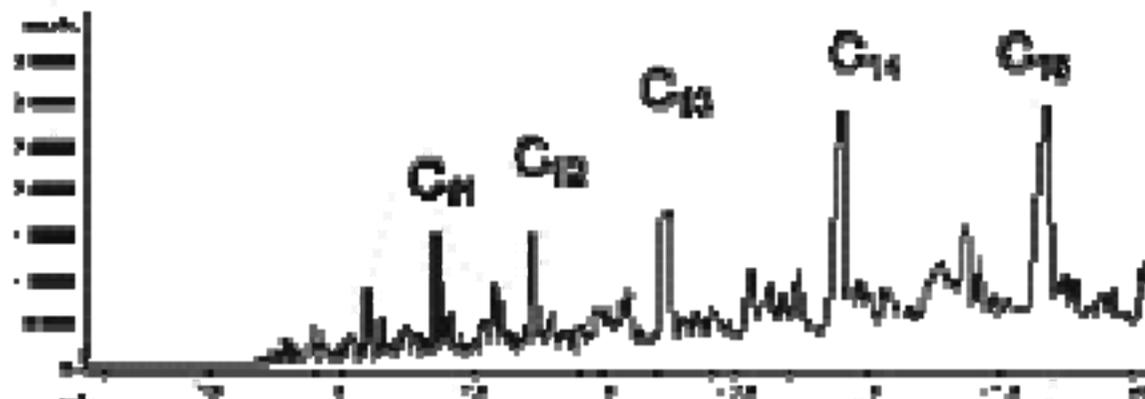
# Standard purification



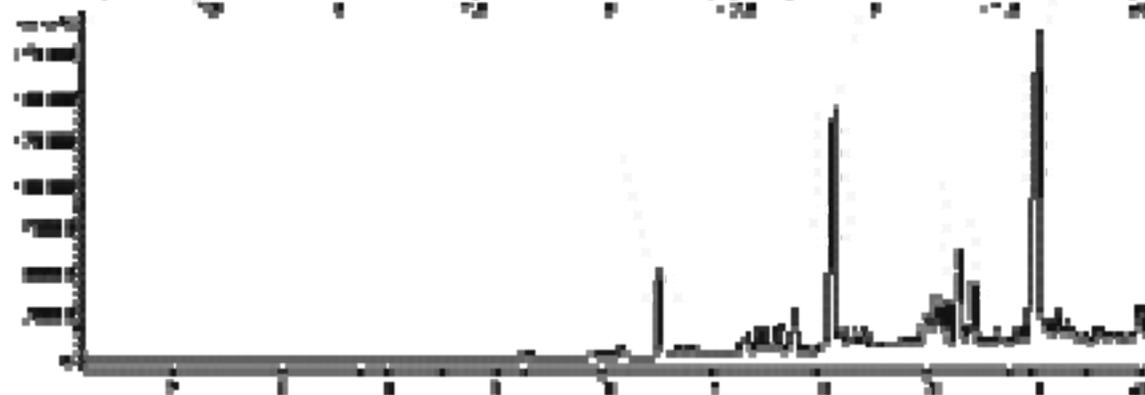
Each fraction will be a standard

# Evaluation of evaporation losses during purification

GC-FID of gas oil



GC-FID of alkanes fraction



▶ 6 % for alkanes

▶ 1 % for aromatics

# Accuracy

Standard Addition

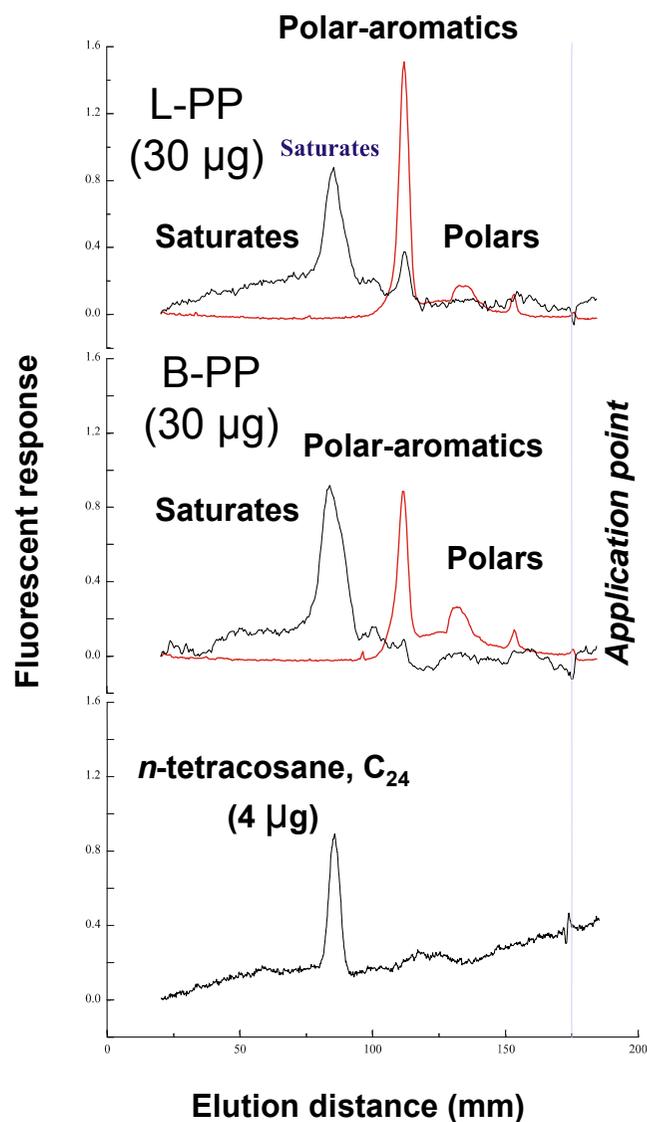
External Standard

	Percentage (m/m) determined by standard addition	Percentage (m/m) determined by external standard (RSD%)
LCO	57.7 %	57.5 % (4.3)
GO	16.1 %	17.0 % (3.9)

## Preparation of Synthetic Fuel

Theoretical value (%)	Measured value (%) (RSD%)	Recovered value (%)
16.8	15.3 (6.5)	91.1
22.2	22.7 (20.0)	102.2
32.2	29.8 (10.0)	92.5
52.3	47.1 (4.1)	90.1

# HPTLC-FDIC application to other hydrocarbon-related samples



Liquids from lignine-polypropylene copenolysis

Liquids from beech-polypropylene copenolysis

Merci de votre attention

## Research team on FDIC phenomena



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