TLC vs LC-MS
in organic synthesis laboratories

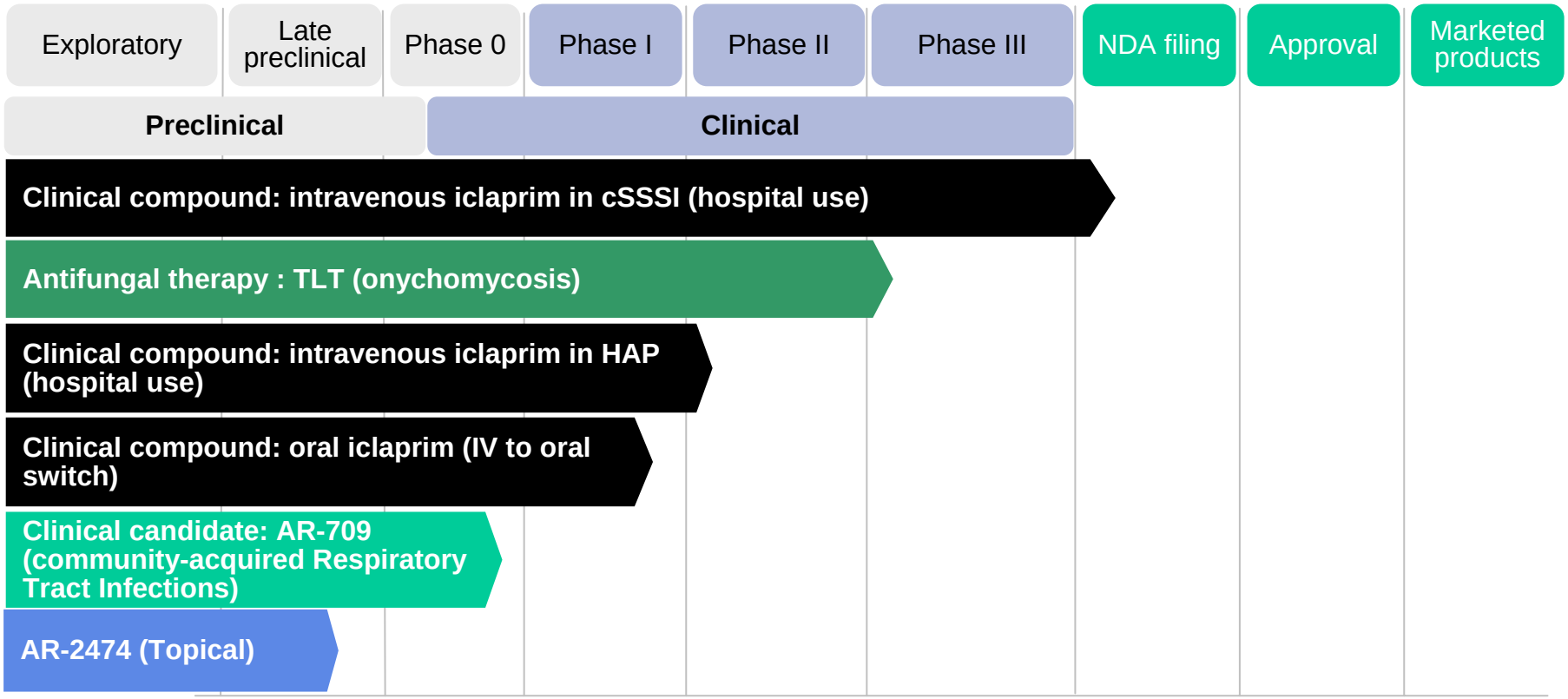
About the company…

- **Location:**
- **In Reinach- Switzerland (Near France and Germany border)-64 employees**
- **In Cambridge-USA -9 employees**

- **<u>The team in Reinach:</u>**
- **Research:**
  - **24 biologists**
  - **19 chemists**
- **Clinical development and administration**
- **Average age :37- 70% employee less than 41 years old.**
- **15 nationalities**

Arpida's pipeline

- **Arpida is a biopharmaceutical company that focuses on the discovery and development of novel drugs for the treatment of microbial infections.**



For more information: www.arpida.com

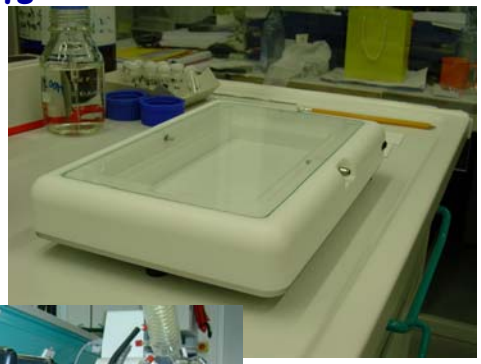
The Lab

Organic Chemistry Labs

Material

LC-MS:
10mM Formic Acid- ACN mobile phase
RP-columns Mass range 100-1000 ESI+/-
Wavelength range :190-800nm
Auto sampler

TLC Chamber:
Cyclohexane-EtOAc or Dichloromethane-MeOH are the eluents often used
TLC: MN 0.25mm SiO2 60 with fluorescent indicator
UV lamp: 254nm or 365nm
Staining reagents

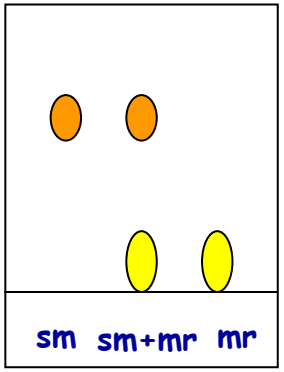


Different use of TLC

- To follow a reaction.
- Before doing a Flash Chromatography to choose the eluent.
  After a Flash Chromatography purification, to find our product.
- If degradation may occur on LC-MS or SiO_2 conditions. (2D TLC)
- If we have a doubt in our LC-MS spectra. (unknown peaks)
- To identify a functional group transformation (e.g. amine, carbonyl…) with a staining reagent.
  Derivatization method for TLC or LC-MS.

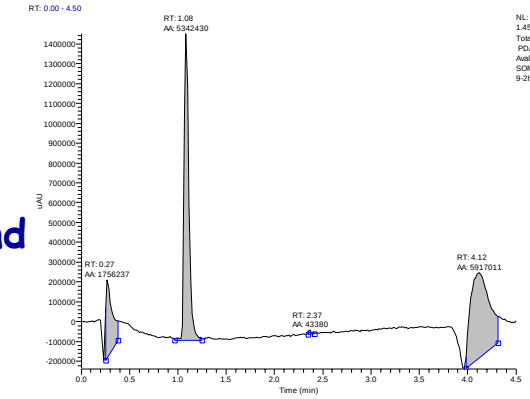
Following reactions

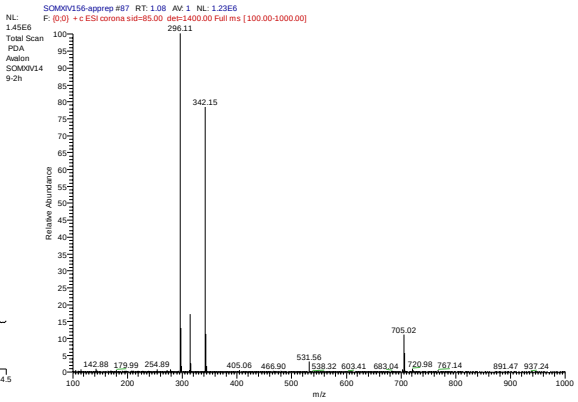
- TLC: Spotting starting material and reaction mixture
- UV: 254 or 365nm
- Rapid method (parallel)
- Information of the progress of the reaction



- LC-MS: injection of the reactional mixture
- PDA and MS combined
- Variable time (sequential)
- Information on MW (qualitative) and possible fragmentation (structure)

Mass spectra of product

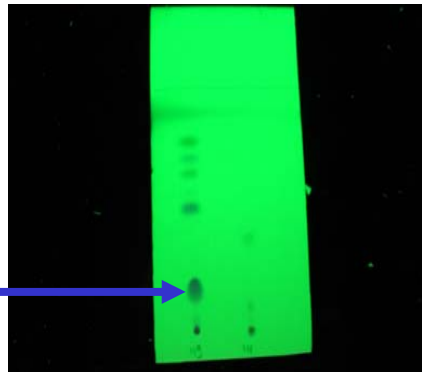




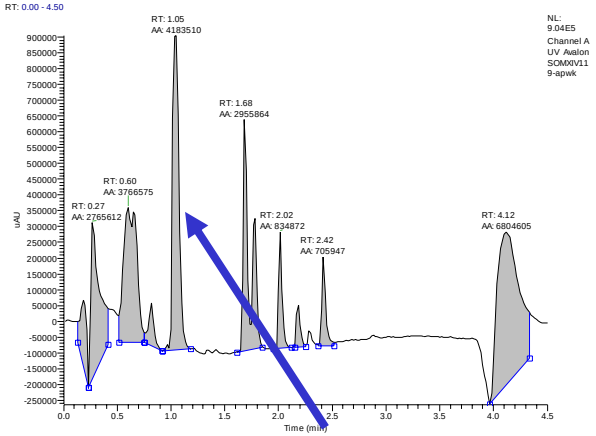
Flash Chromatography 1

- Mixture to purify
- Wanted product : RT 1.03
- TLC Cyclo-EtOAc : 1-1

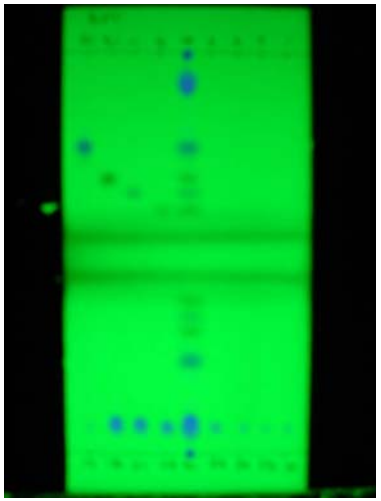
Wanted product



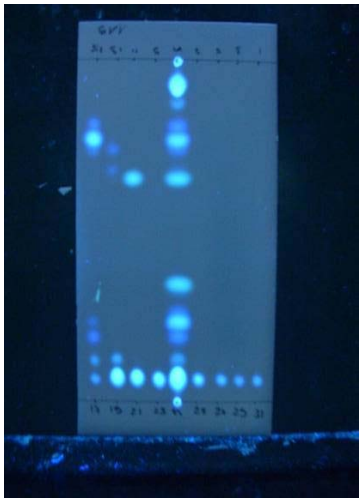
254nm



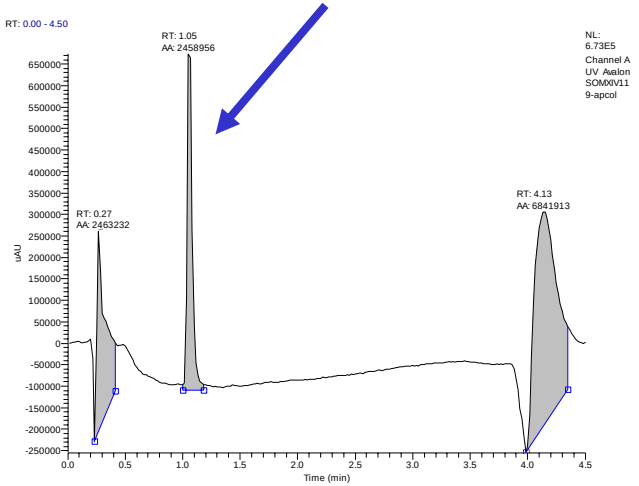
Wanted product



254nm



365nm



Flash Chromatography

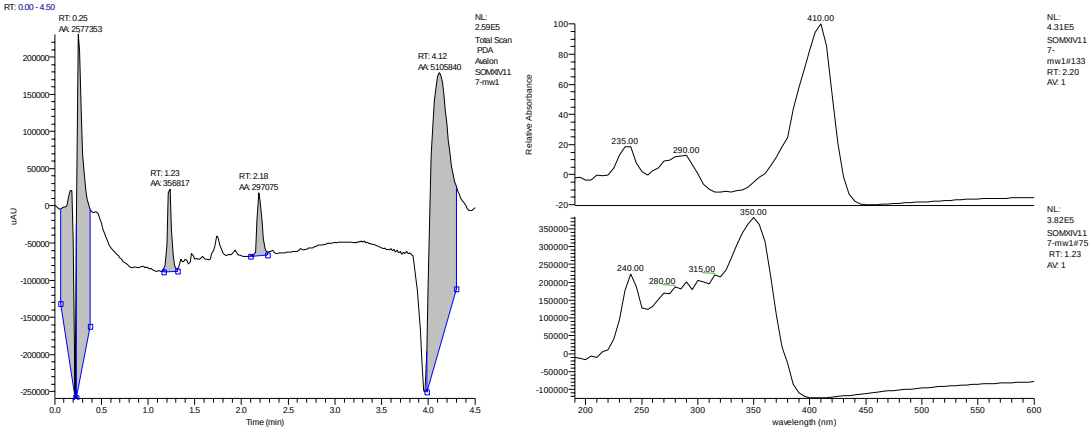
- **2 Automatic systems:**

- **<u>Optix 10</u>: 10 columns in parallel with same size but different gradient. (4g-12g-40g SiO2-AlO2)**

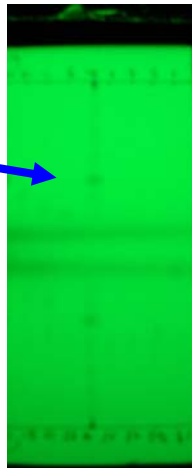
- **<u>Combiflash- companion:</u>**
  1 column 4g to 330g

Flash Chromatography 2

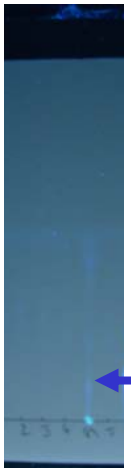
- 2 Products to separate:
- RT 2.18= product
- RT 1.23 M+H=224 ? To remove
- Cyclo-EtOAc:1-1



Product RT 2.18?



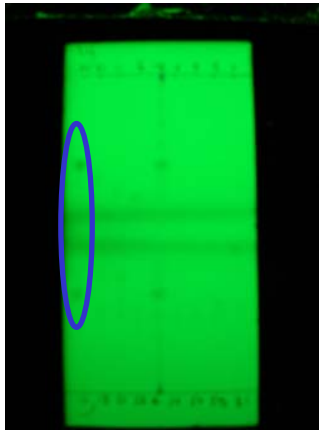
254nm



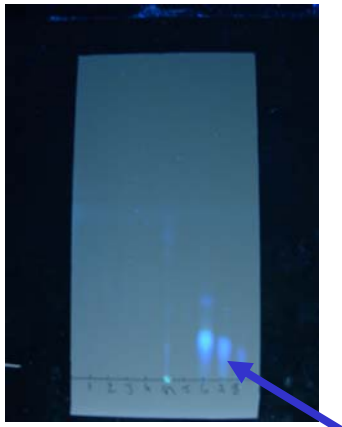
365nm

Product RT 1.23?
UV max :360nm

Results Flash Chromatography 2



254nm



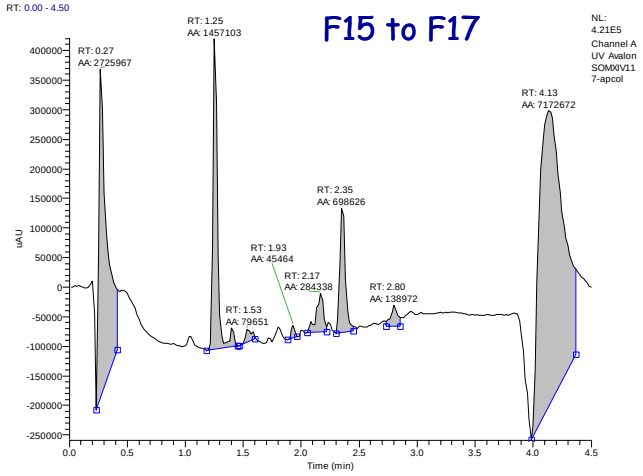
365nm

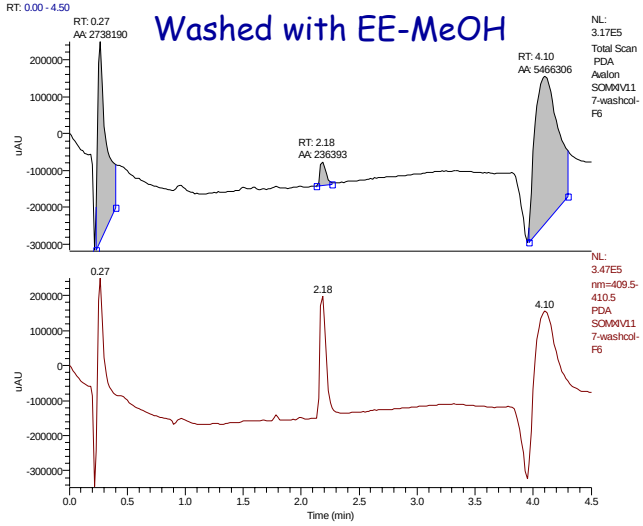
Fractions 15 to 17 mixed coming At around 30% EtOAc.

No product in the evaporated fractions.

Until 100% EtOAc, no product coming out- add 10% MeOH in eluent.

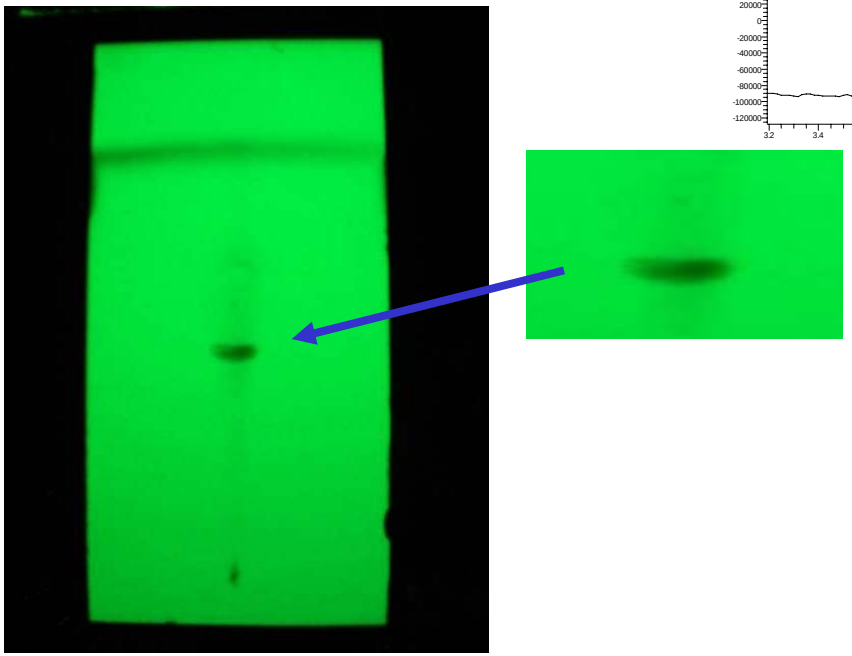
The TLC and LC-MS are not always correlating but complementary product

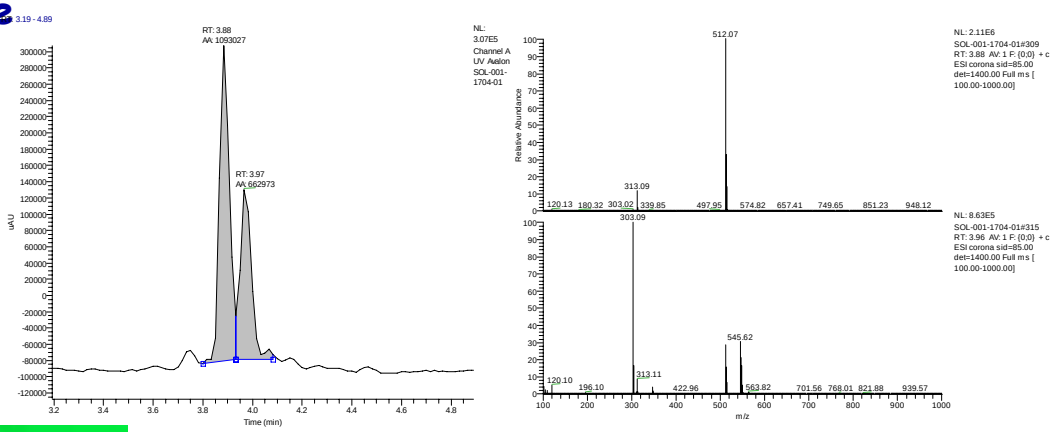


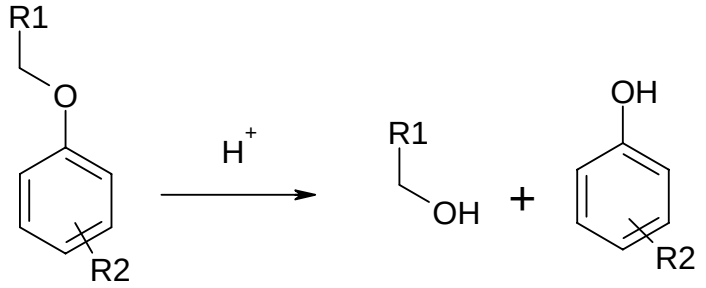


Case of degradation in Acidic Conditions

- Product not stable due to presence of acid in mobile phase
- Check the purity per TLC

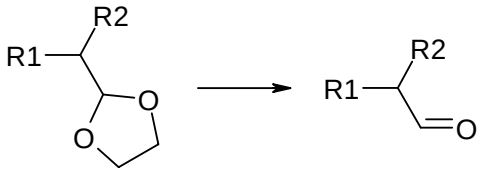


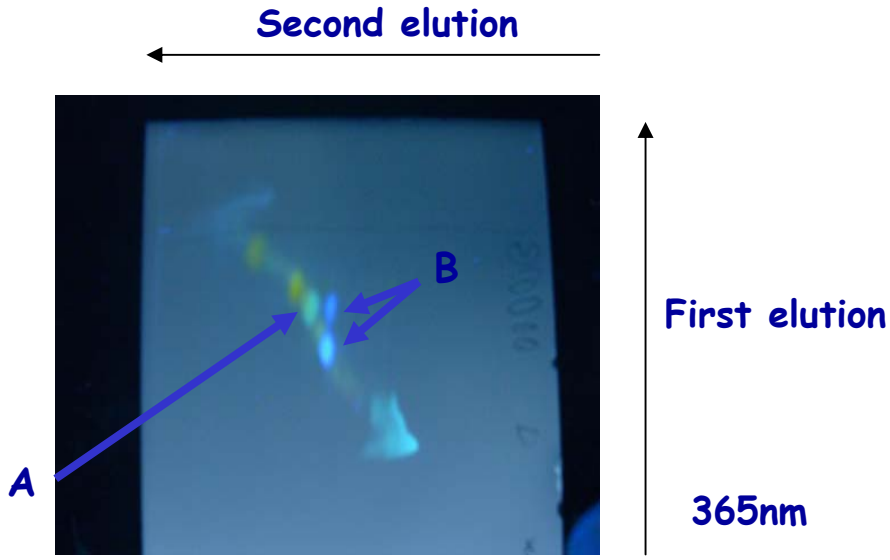




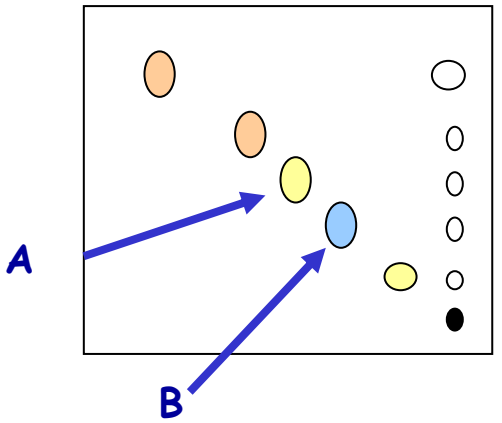
Case of non- stable product

- Not stable, transform it self by heating, under light….
- TLC 2D is a good method to confirm the degradation.
- Yellow spot A transformed in blue spot B
- In fact we observe a deprotection on the TLC:





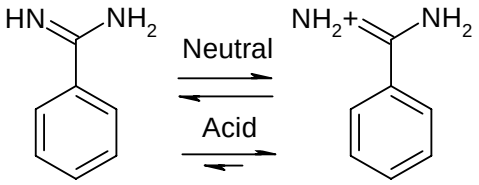
If the product was stable:



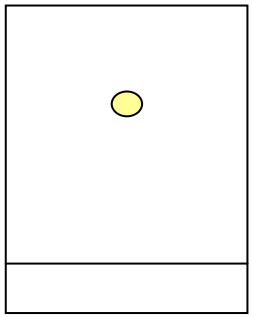
Protonation-Deprotonation on TLC-LCMS

- **Balance between the protonated and deprotonated product.**
- **Balance depending on the pH.**
- **Explanation:**

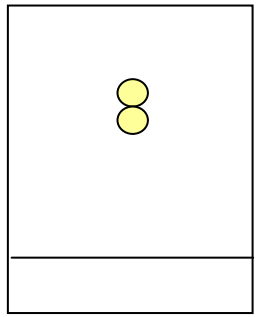
pKa=11



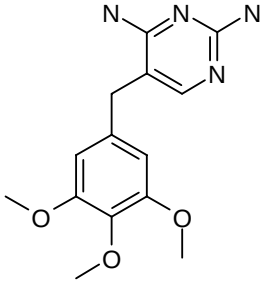
EtOAc-Butanol-H_2O-AcOH 4-1-1-1



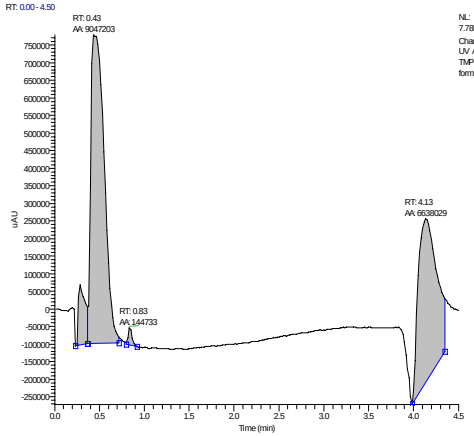
CH2Cl2-MeOH 9-1



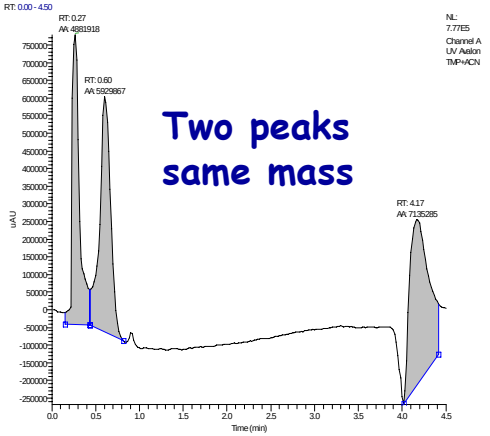
- **Similar effect with Trimethoprim:**



Sample in 100% Formic acid



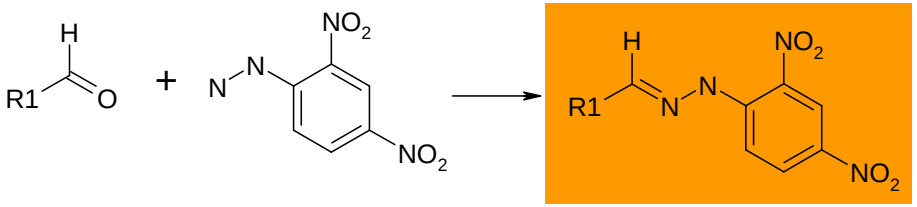
Sample in 100% ACN



Two peaks same mass

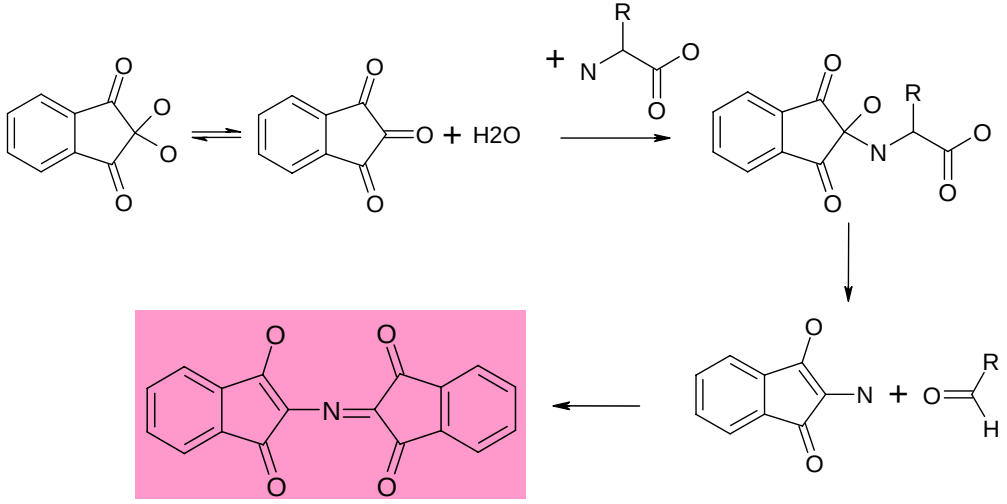
Derivatization on TLC

- **Principle of aldehyde derivatization:**



Very colored product : orange

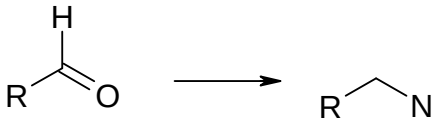
- **Principle of ninhydrine derivatization:**



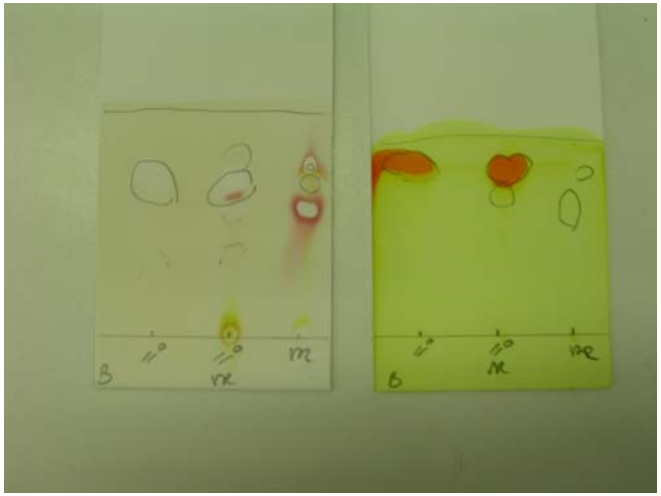
Very colored product : pink

Example where derivatization is needed

- Reaction:
- Starting material is an aldehyde.
- Product should be an amine.



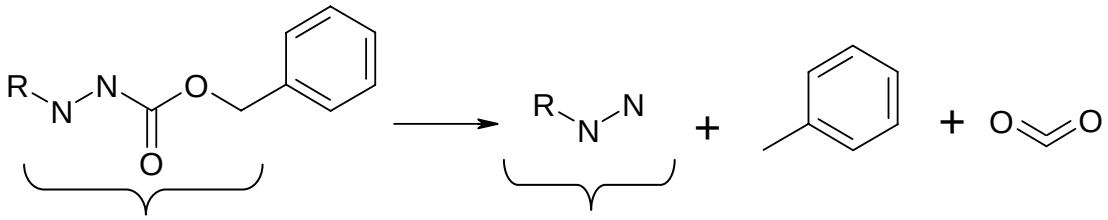
Aldehyde → **Amine** + side product



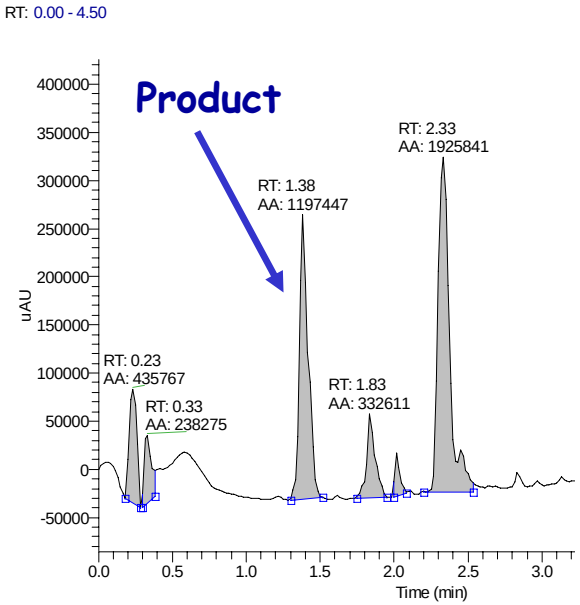
- No ionisation- no mass found in LCMS
- TLC: confirmation of product

Derivatization for LC-MS

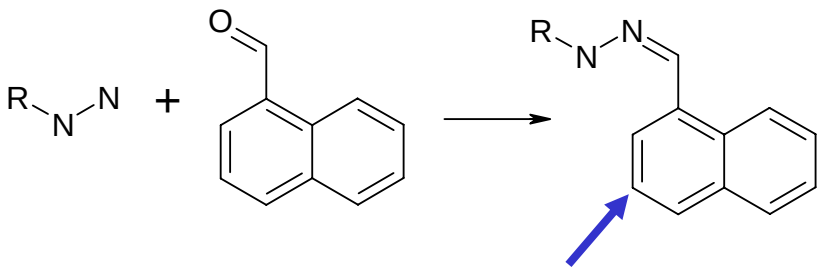
- **Reaction of deprotection: product obtained:**



UV-detectable **Non UV-detectable**



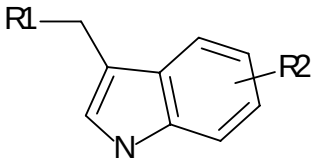
•Derivatization:



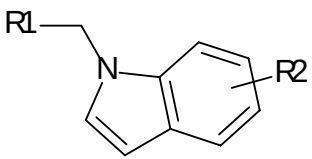
RT 1.38: product naphtaldehyde derivated
RT 2.33: naphtaldehyde excess
RT 2.37 toluene

UV detectable product

Example where TLC and LC-MS are not enough to identify the molecule

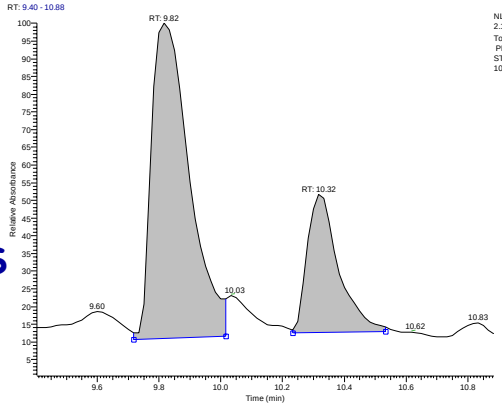


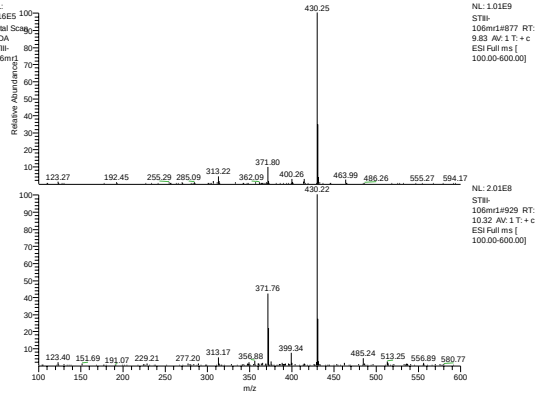
4': C-alkylated



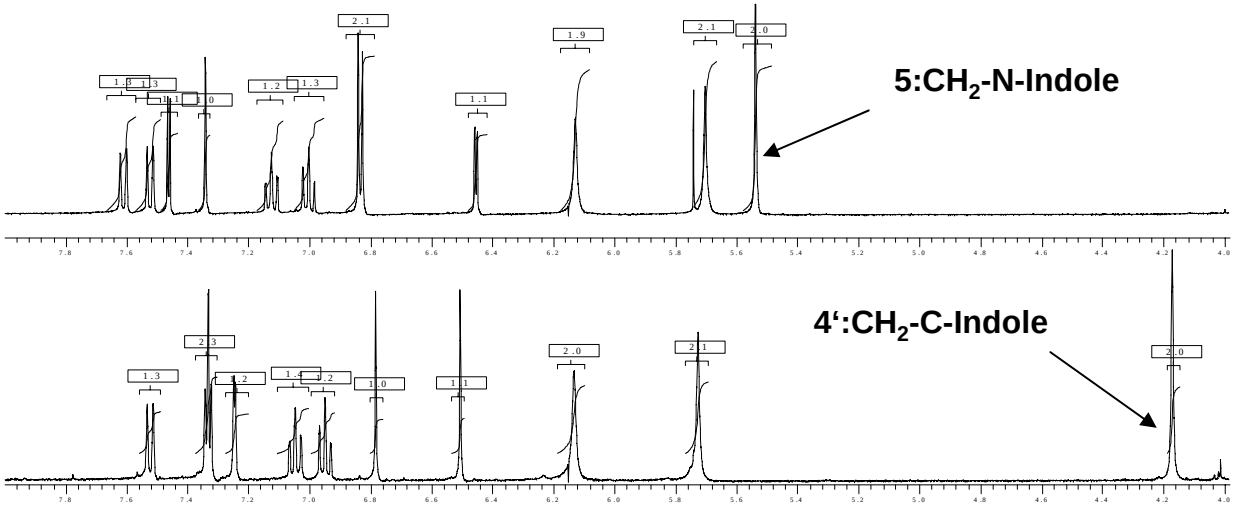
5: N-alkylated

Same profile on TLC- same mass in LC-MS





1H-NMR profile of **4'** and **5** region (8-4 ppm)



We can only differentiate both products in NMR

400mHz- ^{1}H

Conclusion

- TLC:
- Cheap and fast method.
- Parallel method.
- Possibilities to identify different functional group. (staining reagent)

- LC-MS:
- Identity molecule and structure.
- Sequential method.

- Both methods are useful, sometimes combined or not.
- But some additional analytical methods can be needed: IR (function), NMR (1D,2D, ^{1}H, ^{13}C, ^{19}F)

Acknowledgements

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