



'Analysis of flavonoids in plant extracts using TLC-MALDI-TOFMS: influence of MALDI parameters.'

David Da Silva


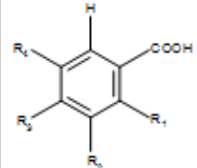
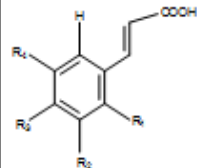
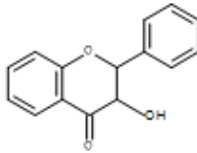
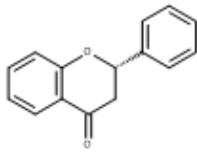
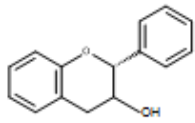
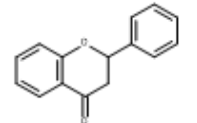
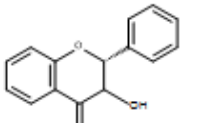
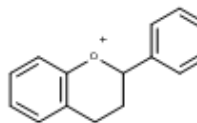
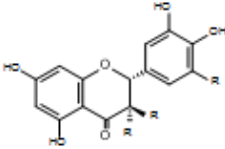
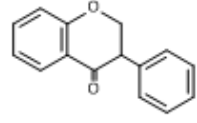
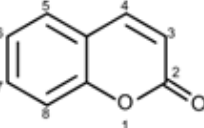
Extraction, Bioactive molecules analysis



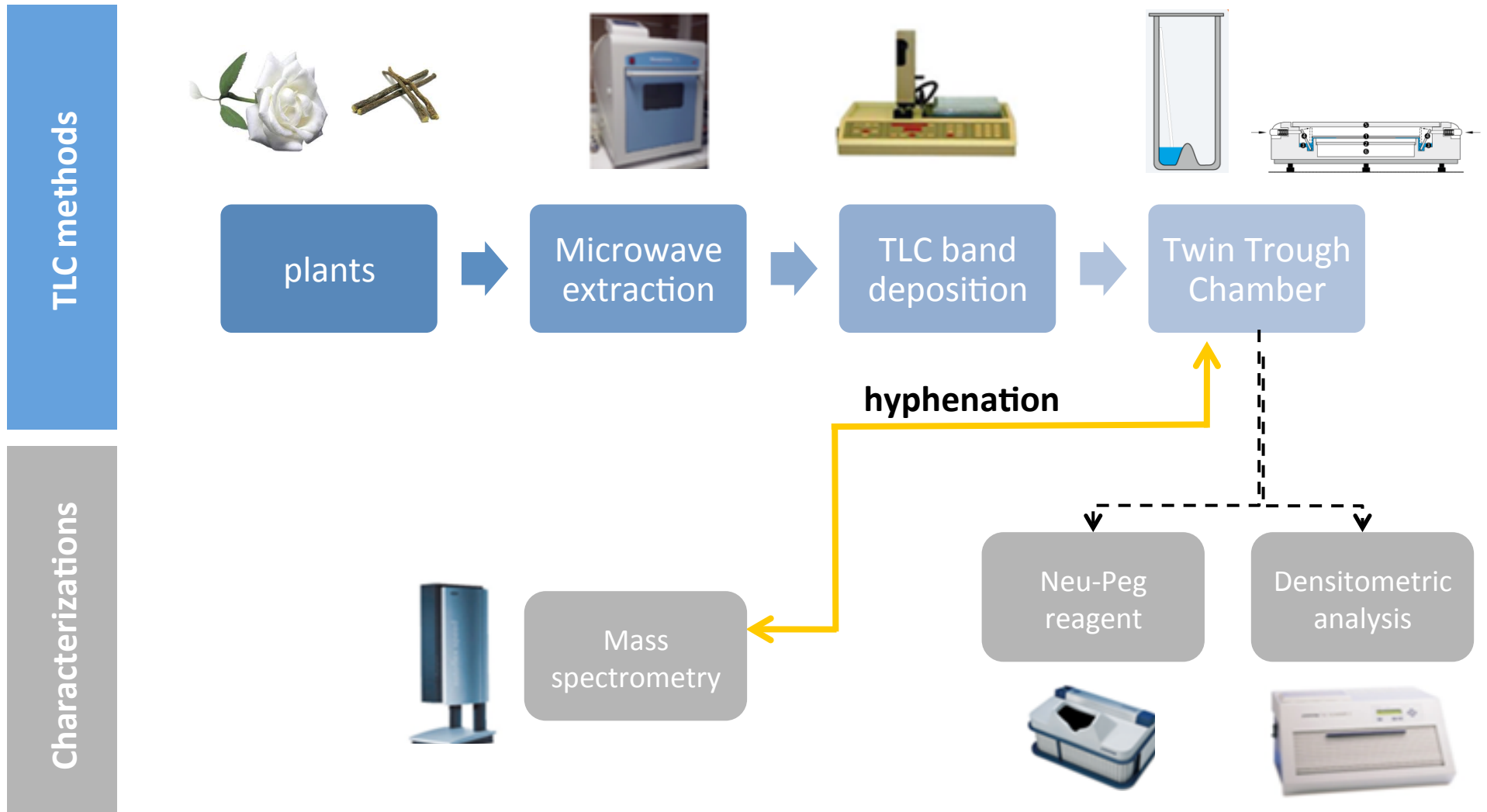
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- ☐ Plants constitute a rich source of bioactive substances

	Hydrobenzoic acid	Hydrocinnamic acid	Flavonols	Flavanones
				
	Flavan-3-ols	Flavones	Flavanonols	Anthocyanidins
				
	Tanin	Isoflavones	Coumarin	
				

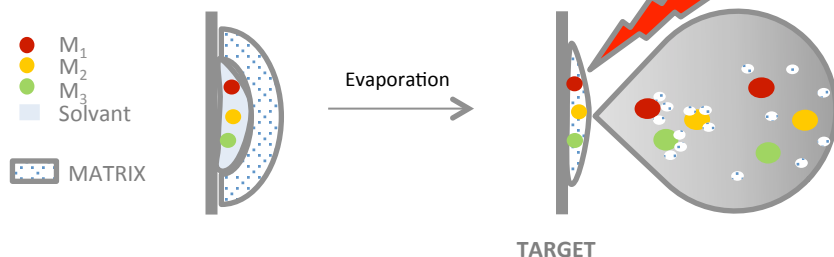
- **The search for bioactive molecules from nature:** play an important role in fashioning new cosmetic and medicinal agents.
- **Important to develop a fast and reliable characterization method of polyphenols**



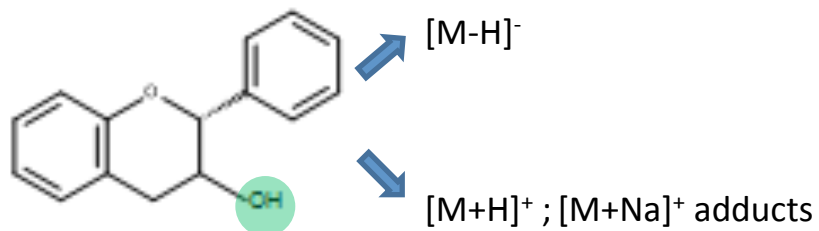
➤ TLC-MALDI-TOF-MS coupling: Performance?

MALDI Source

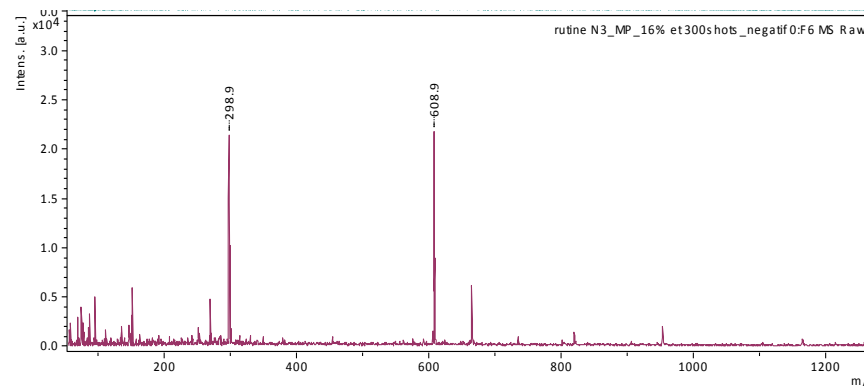
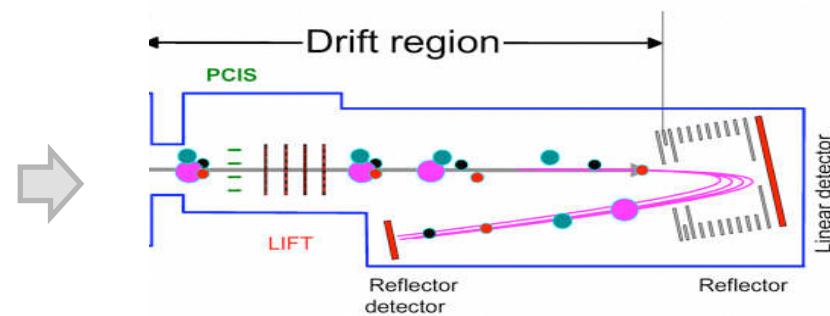
Nitrogen Laser at 337 nm



- **POSITIVE** and/or **NEGATIVE** mode



TOF-MS analyzer

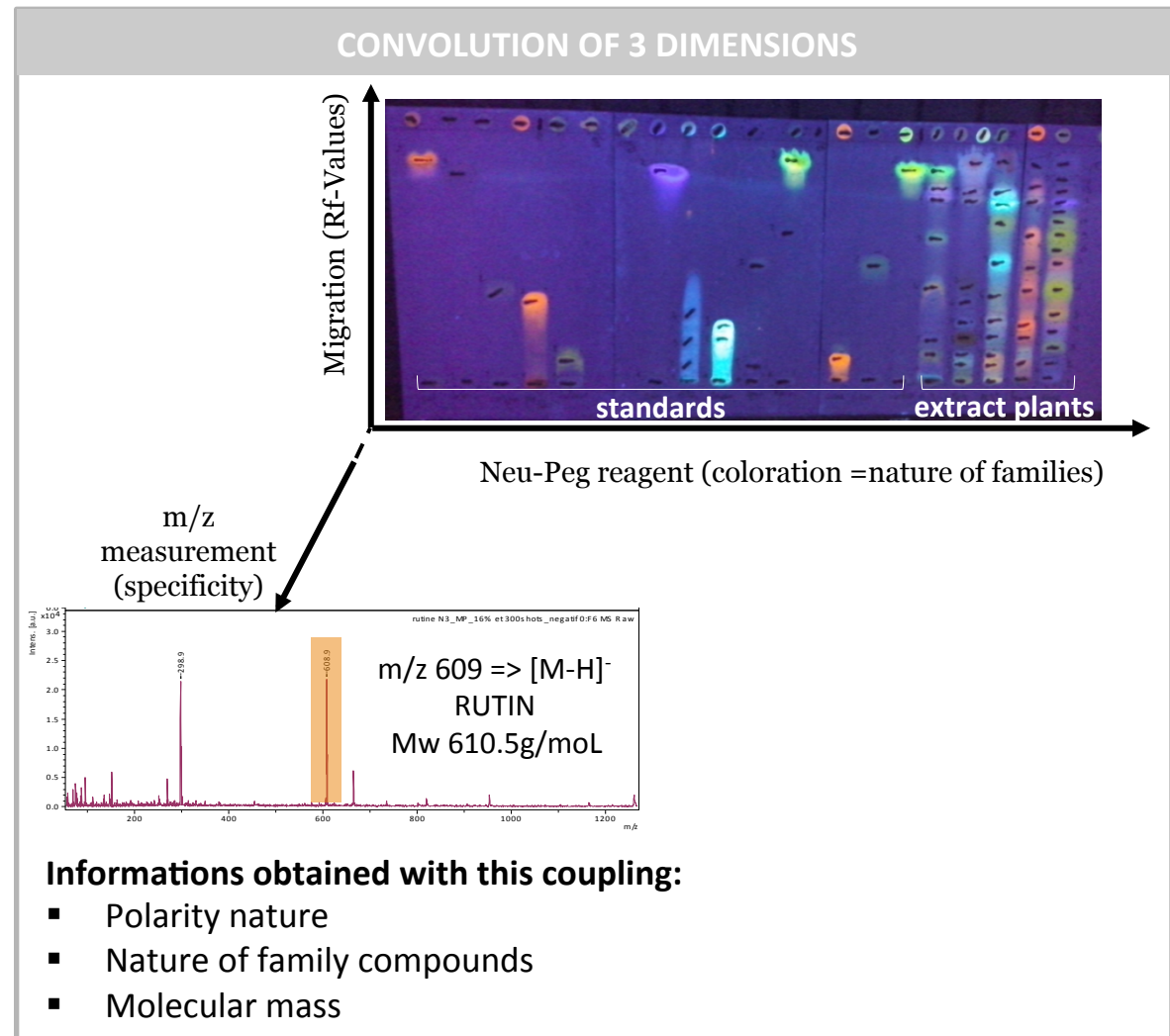


Advantages:

- lower volume of solvent, sample
- fast and superficial desorption

Limitations:

- Fixed wavelength Nitrogen laser
- Empirical process



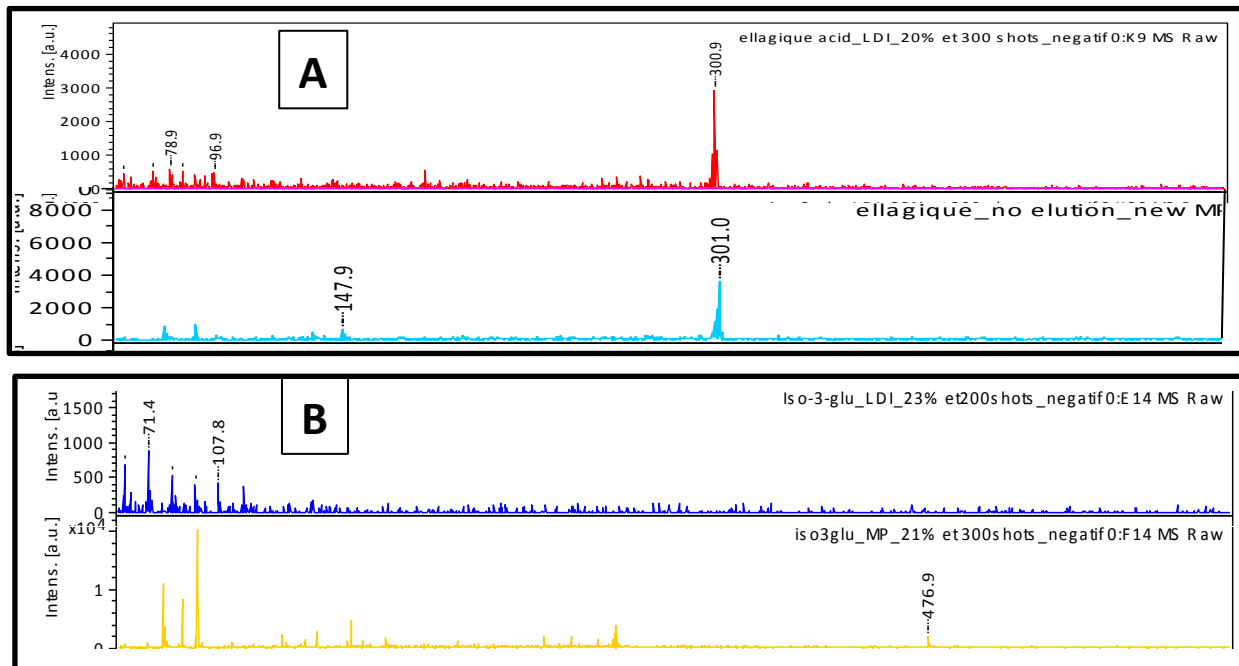
➤ **Improvement of (fast) flavonoïds characterization in complex mixture**

TLC-MALDI-MS coupling: MALDI parameters

- I. Matrix or not ?**
- II. Matrix choice ?**
- III. Deposition methods of matrix ?**

- Flavonoïds absorption : 255-366 nm
- Laser desorption at 337 nm

Evaluation : 17 polyphenol standards analyzed in absence and presence of matrix



Samples

- volume: 1 μ L
- standards: 1000 ppm in EtOH

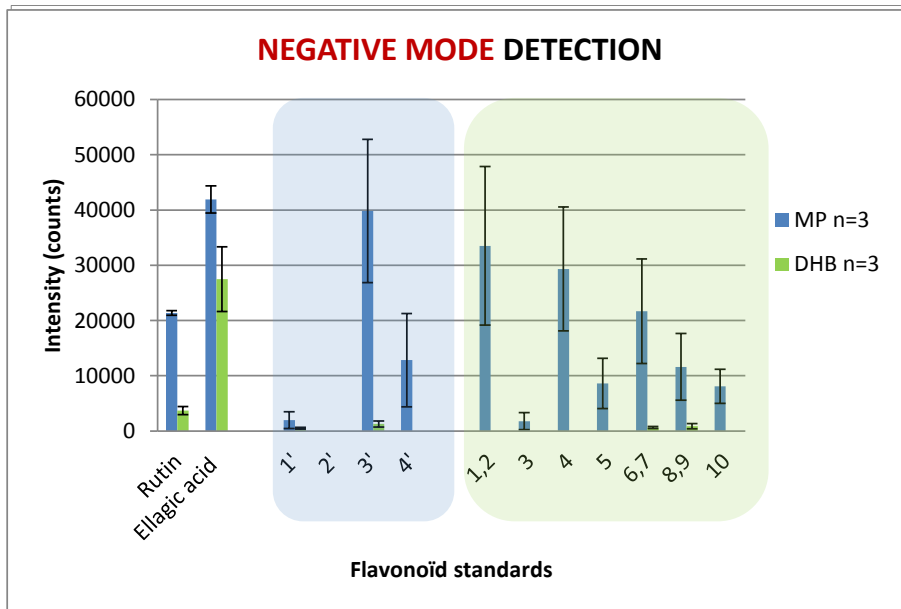
Matrice

- volume : 1 μ L
- amount : 10mg/mL in EtOH
- Dried droplet deposition
- TLC silica aluminum



Matrix	Absence	Presence
Compounds detection	30%	100%

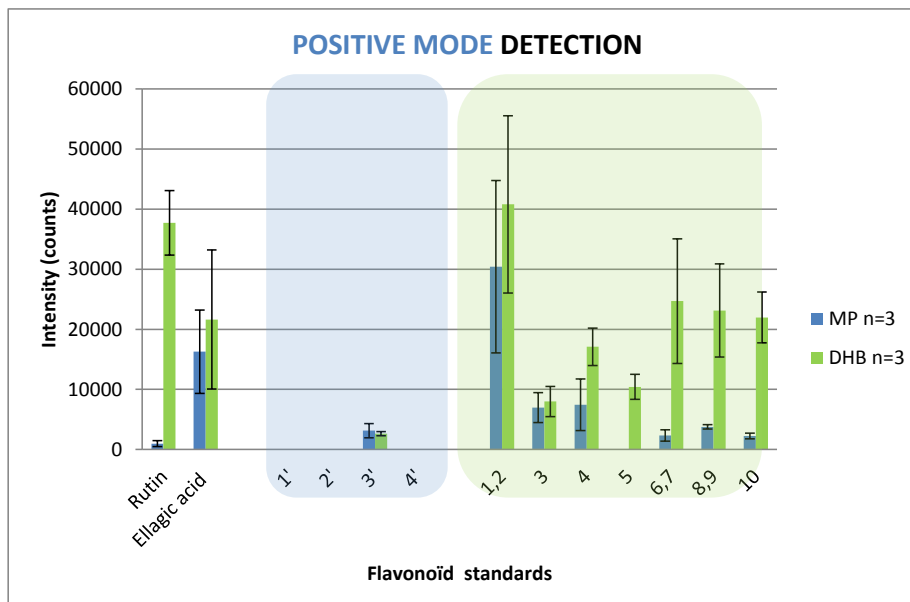
➤ **The use of a matrix is essential**



In negative mode :

- ✓ higher sensitivity with MP > DHB > 9AA
- ✓ Low adducts form
- ✓ Easy identification with MP

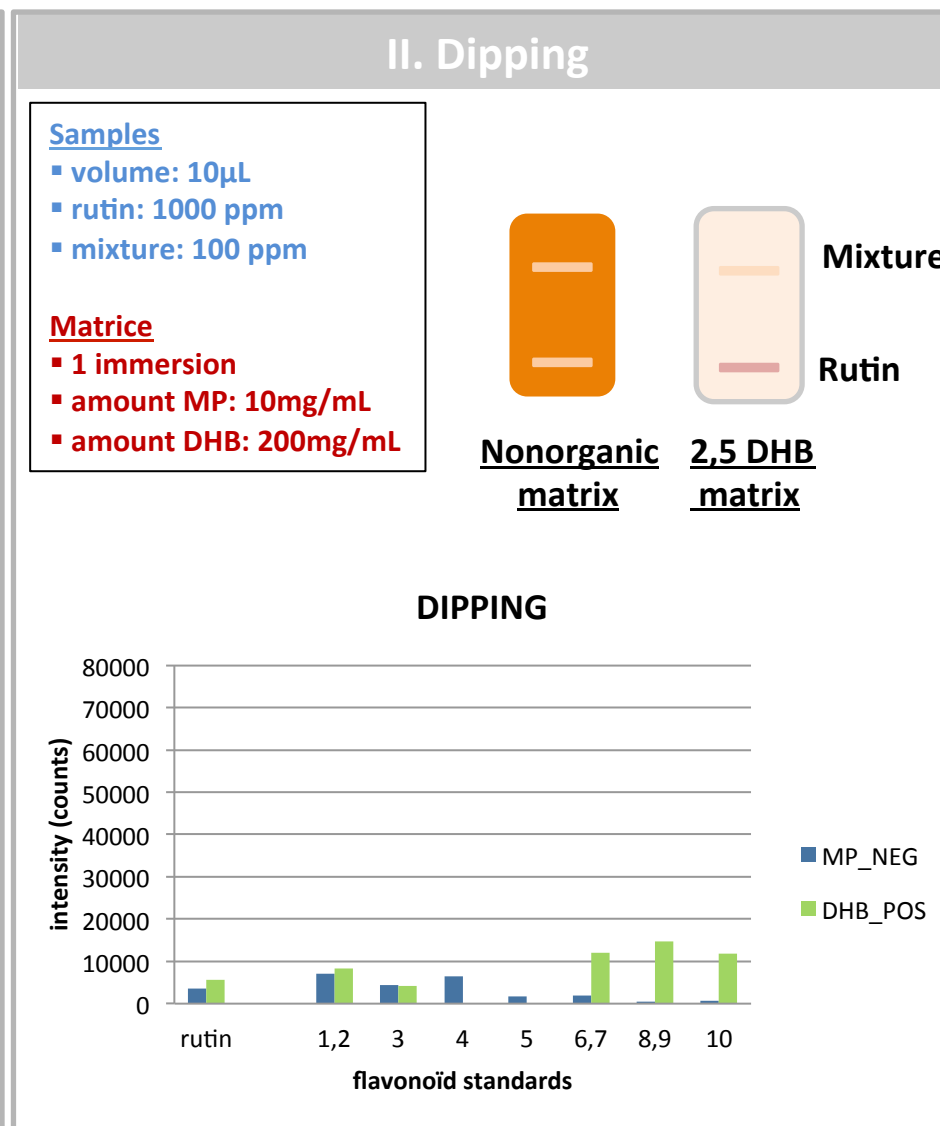
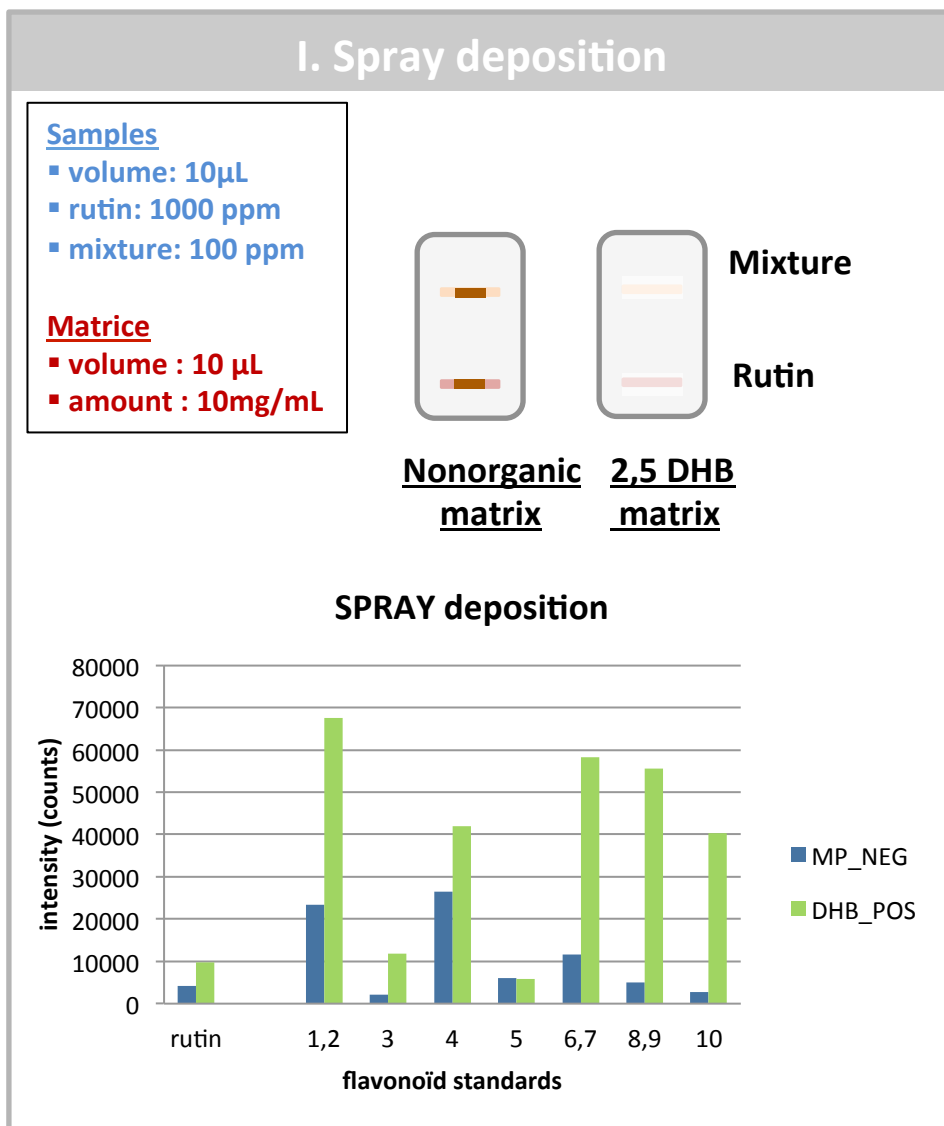
✓ **Selection of nonorganic (MP)**



In positive mode :

- ✓ higher sensitivity with DHB > MP > 9AA
- ✓ high adducts form
- ✓ Identification more complex with DHB

✓ **Selection of DHB**

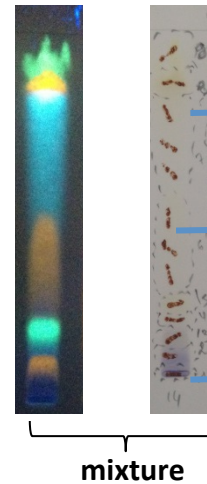
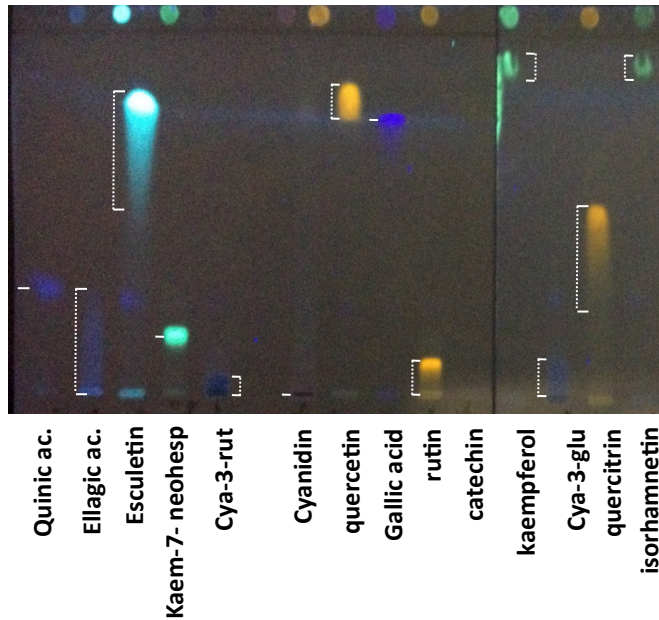


➤ **Spray deposition: Best sensitivity (higher Signal/Noise)**

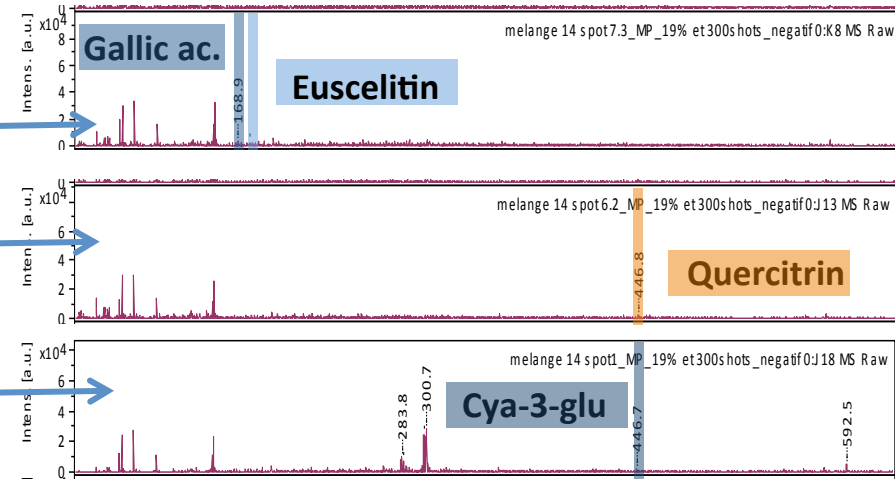
Complex mixtures studied by TLC-MALDI-MS coupling:

- ✓ negative mode
- ✓ nonorganic matrix
- ✓ spray deposition

i) Neu-Peg ii) TLC



iii) Mass spectra in negative mode

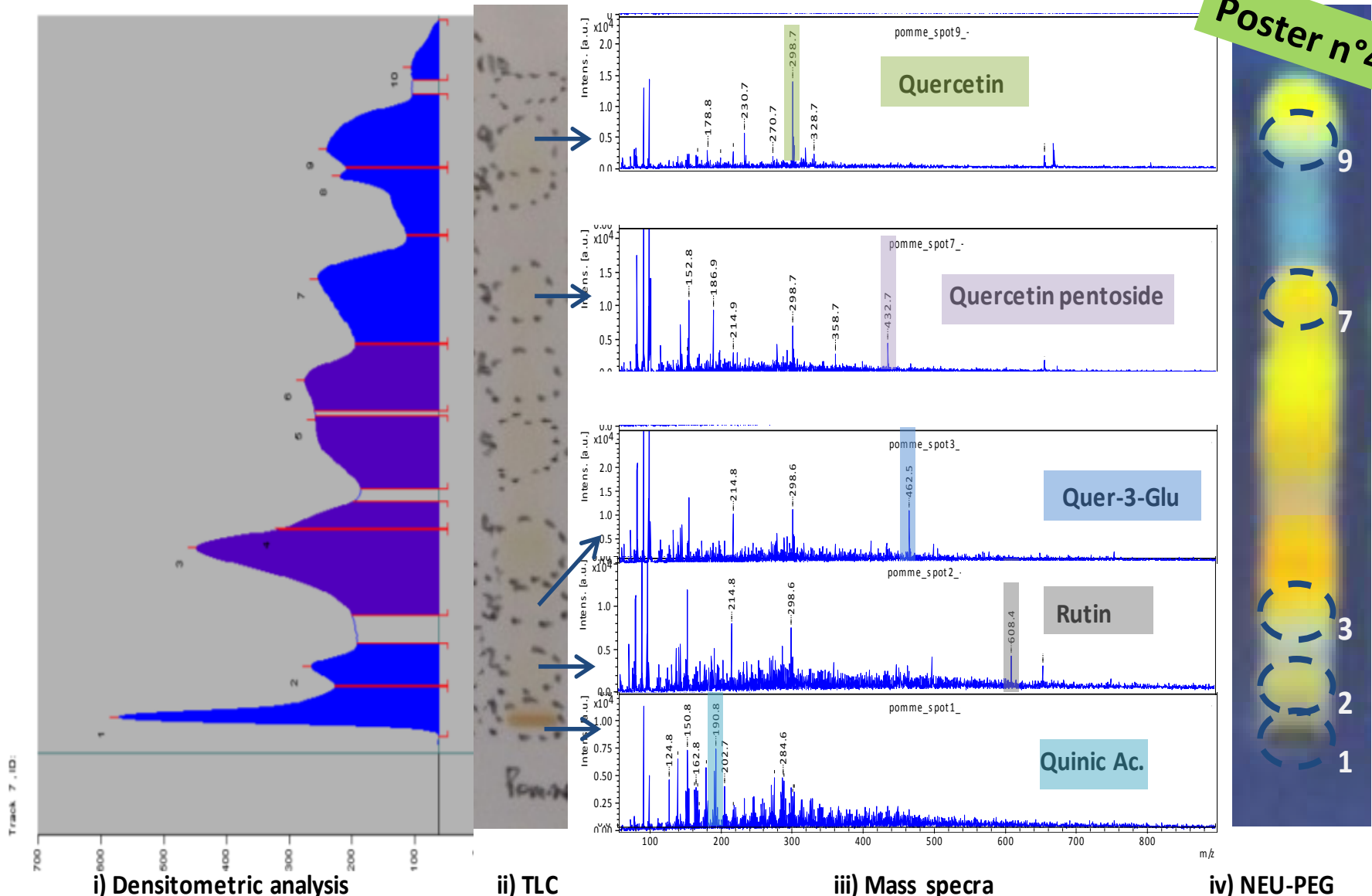


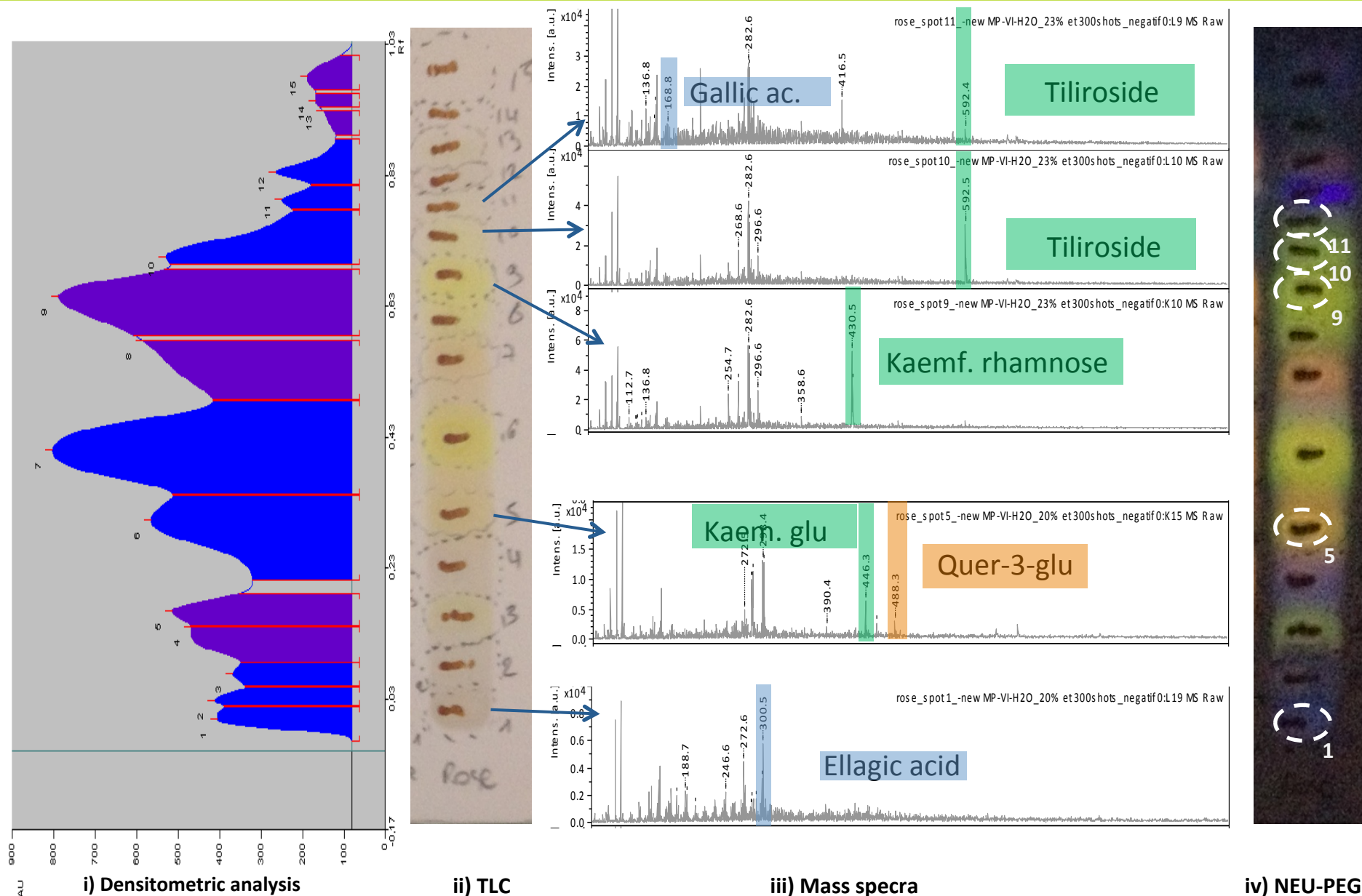
➤ **Characterization of full mixture of polyphenol standards**

Information obtained with this coupling:

- Polarity nature
- Nature of family compounds
- Molecular mass

Poster n°41

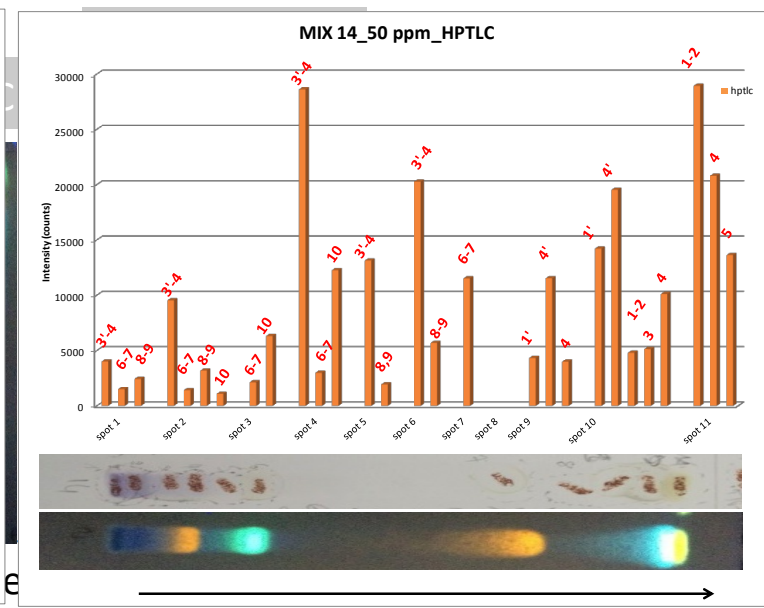
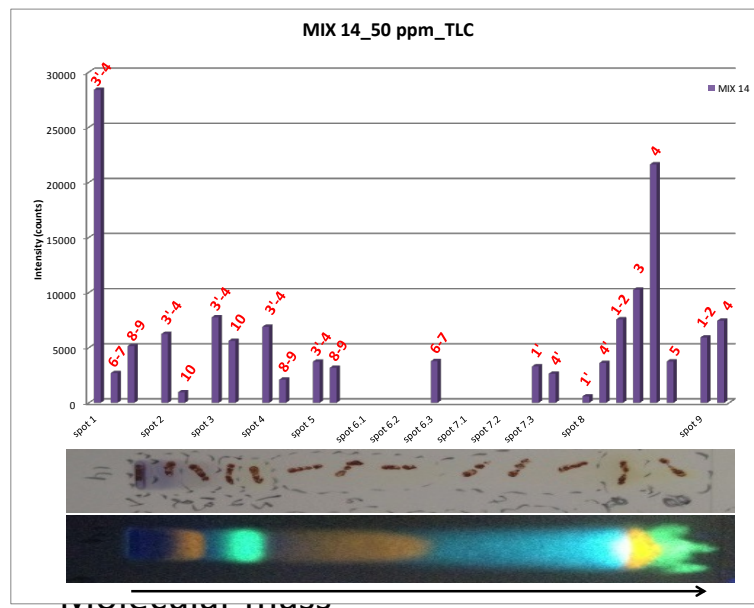




Conclusions and outlook

- TLC-MALDI-TOF-MS coupling process:
 - **Matrix or not ? YES**
 - **Matrix choice ? MP in negative mode**
 - **Deposition methods of matrix ? SPRAY DEPOSITION**

- Characterization of polyphenol compounds:
 - **Standards and mixtures: 50-1000 ppm**
 - **8 Extract plants: unknown concentration per molecules**





Acknowledgements

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Extraction, Bioactive molecules analysis

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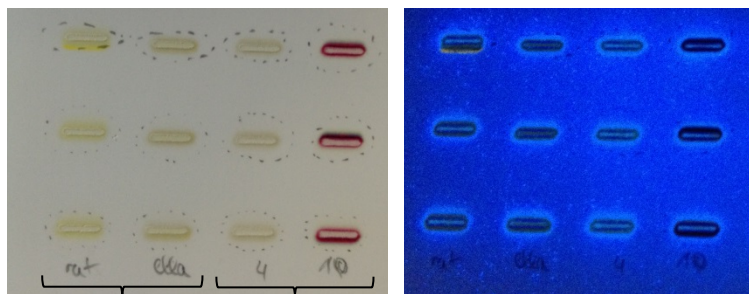
La Région Centre



Merck KGaA (Germany)

2,5 DHB matrix

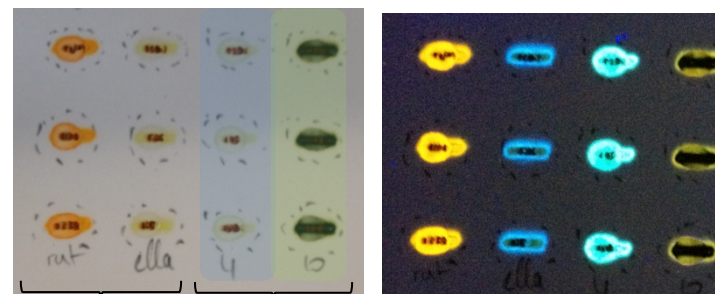
Revelation NEU-PEG



compound Mixtures

Nonorganic matrix

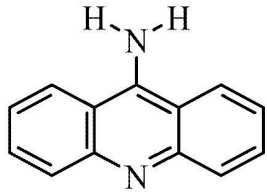
Revelation NEU-PEG



compound Mixtures

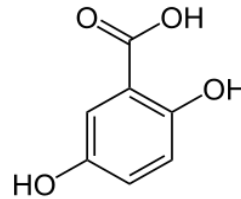
Classiquement utilisées en Maldi-Tof

En mode negatif



9 amino-acridine
(9AA)
194.23 g/mol

En mode positif



2,5-dihydroxybenzoïque
(DHB)
154.12 g/mol

En mode negatif/positif

Matrice inorganique
synthétisée par le
laboratoire (MP)

