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Extraction, Bioactive molecules analysis

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Plants: unexploited source

I. Context

Plants constitute a rich source of bioactive substances

- 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

II. Materials & Methods

III. Coupling

IV. Application

V. Conclusions
Plant extracts characterization process

I. Context
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- TLC-MALDI-TOF-MS coupling: Performance?
I. Context

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MALDI-TOF-MS principle

- **MALDI Source**
  - Nitrogen Laser at 337 nm
  - **POSITIVE and/or NEGATIVE** mode
  - $[\text{M-H}]^-$
  - $[\text{M+H}]^+$; $[\text{M+Na}]^+$ adducts

- **TOF-MS analyzer**
  - Drift region
  - Linear detector

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

Limitations:
- Fixed wavelenght Nitrogen laser
- Empirical process
General outlook of TLC-MALDI-TOF-MS coupling

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

**TLC-MALDI-TOF-MS coupling**

- Nitrogen Laser $\lambda$: 337 nm
- TLC plate
- MALDI-TOF-MS

**CONVOLUTION OF 3 DIMENSIONS**

- m/z measurement (specificity)
- m/z 609 => [M-H]$^-$
- RUTIN
  - Mw 610.5 g/mol
- Neu-Peg reagent (coloration = nature of families)

**Informations obtained with this coupling:**

- Polarity nature
- Nature of family compounds
- Molecular mass
TLC-MALDI-MS coupling: MALDI parameters

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

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

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Matrice</th>
</tr>
</thead>
<tbody>
<tr>
<td>volume: 1μL</td>
<td>volume: 1 μL</td>
</tr>
<tr>
<td>standards: 1000 ppm in EtOH</td>
<td>amount: 10mg/mL in EtOH</td>
</tr>
<tr>
<td></td>
<td>Dried droplet deposition</td>
</tr>
<tr>
<td></td>
<td>TLC silica aluminum</td>
</tr>
</tbody>
</table>

Matrix | Absence | Presence
---|---------|---------
Compounds detection | 30% | 100%

The use of a matrix is essential
II. Matrix choice?

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
III. Deposition methods of matrix?

### I. Spray deposition

<table>
<thead>
<tr>
<th>Samples</th>
<th>Volume: 10 µL</th>
<th>Rutin: 1000 ppm</th>
<th>Mixture: 100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrice</td>
<td>Volume: 10 µL</td>
<td>Rutin: 10 µL</td>
<td>Mixture: 10 µL</td>
</tr>
</tbody>
</table>

#### SPAY deposition

- **Nonorganic matrix**: 2,5 DHB matrix
- **Mixture**: Rutin

#### Graph

- Intensity (counts)
- Flavonoid standards: rutin, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10

- **MP_NEG**
- **DHB_POS**

#### Notes

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

### II. Dipping

<table>
<thead>
<tr>
<th>Samples</th>
<th>Volume: 10 µL</th>
<th>Rutin: 1000 ppm</th>
<th>Mixture: 100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrice</td>
<td>1 immersion</td>
<td>Amount MP: 10 mg/mL</td>
<td>Amount DHB: 200 mg/mL</td>
</tr>
</tbody>
</table>

#### DIPPING

- **Nonorganic matrix**: 2,5 DHB matrix
- **Mixture**: Rutin

#### Graph

- Intensity (counts)
- Flavonoid standards: rutin, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10

- **MP_NEG**
- **DHB_POS**

### III. Coupling
Complex mixtures studied by TLC-MALDI-MS coupling:

- negative mode
- nonorganic matrix
- spray deposition
polyphenol mixture standards: TLC-MS

i) Neu-Peg

ii) TLC

iii) Mass spectra in negative mode

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
Apple extract characterization: TLC-MS

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Rose extract characterization: TLC-MS

I. Context

ii) TLC

iii) Mass spectra

iv) NEU-PEG

Tiliroside

Kaemf. rhamnose

Quer-3-glu

Ellagic acid

Gallic ac.
Conclusions and outlook
Conclusions

- 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
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Extraction, Bioactive molecules analysis

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Compatibility with revelation

2,5 DHB matrix

Revelation NEU-PEG

Nonorganic matrix

Revelation NEU-PEG

compound Mixtures

compound Mixtures
II. Matrix choice ?

Classiquement utilisées en Maldi-Tof

**En mode négatif**

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

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

**En mode positif**

- Matrice inorganique synthétisée par le laboratoire (MP)

**En mode négatif/positif**

- Flavonoid compounds

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