P07

The feasibility of MALDI-MS for small molecules as a future fast screening alternative in toxicology. Preliminary results.

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1. Introduction

Matrix Assisted Laser Desorption Mass Spectrometry (MALDI-MS) is a fast technique (no chromatographic separation step) allowing the simultaneous analysis of multiple compounds. Well established for proteins, its use for small molecules is only just being considered. MALDI-MS seems, nevertheless, promising as the procedure is simple: add matrix to the sample and let it co-crystallize on a stainless steel target plate. A single spot analysis then takes from seconds to minutes. Accurate mass is, however, vital for identification purposes.

2. Aims

- Evaluate feasibility to analyze small (< 1000 Da), toxicologically relevant molecules using MALDI-MS.
- Investigate fundamental pre-requisites for MALDI in a toxicological setting:
  - Assess interference of MALDI matrix ions.
  - Investigate signal intensity, signal quenching and sensitivity.
  - Evaluate accurate mass measurement potential in terms of identification potential of small molecules.
  - Assess the quantitative characteristics.

3. Methods

- Different instruments: high resolution (Q-Tof) and "super-high" resolution (FTICR). Positive ion mode.
  - Micromass MALDI Q-Tof Ultima, around 10,000 FWHM resolution (V-mode). External calibration. Broad, defocused laser spot.
  - Bruker APEX III FTICR (7 Tesla magnet) with SCOUT 100 MALDI source, > 100,000 FWHM resolution (external calibration). 16x20 laser shots accumulated.
- Test mix (MeOH/CH_3CN, 50/50) with couples of substances having closely similar masses, spread out over mass range m/z 50 – 700. Three concentrations: 5, 10, and 25 µg/mL.

  Morphine (MM 285.13595) and pentazocine (MM 285.20872); methadone (MM 309.20872) and fluoxetine (MM 309.13351); methaqualone (MM 250.11007) and sulfadiazine (MM 250.05190); trazodone (MM 371.15074) and thioridazine (MM 370.15320); MDMA (MM 193.10974) and caffeine (MM 194.07983); clofentezine (MM 303.01988) and cocaine (MM 303.14651); chlorhexidine (MM 504.20265) and dipyridamole (MM 504.31671); amiodarone (MM 645.02314) and aconitine (MM 645.31437).
- Nalorphine (MM 311.15160) as IS (50 µg/mL in MeOH/CH_3CN 50/50).
- Alkaline L/L extracts (hexane/EtAc) of spiked blank urine and blood (same standard compounds, spiked to the same concentration).
- 1 µL each, sample, IS, and matrix sampled on target plate (dried droplet). Matrix either 2,5–dihydroxybenzoic acid (DHB) of α-cyano-4-hydroxycinnamic acid (CN), 20 mg/mL.

4. Results

- Signals distinguishable (except <m/z 200) from matrix and noise. Signal intensity decreases with decreasing mass.
- No obvious quenching or interference for spiked urine and blood samples.

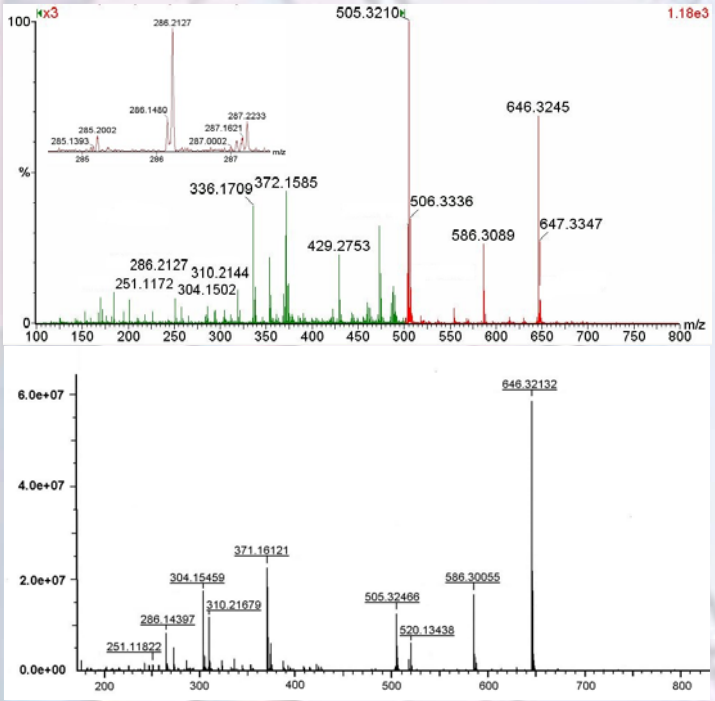

Fig. 1: Top: MALDI-MS spectrum for 5 µg/mL standard on a Q-Tof. Insert shows resolution between morphine and pentazocine. Bottom: MALDI-MS spectrum for 5 µg/mL spiked urine sample on a Bruker FTICR. Peak labels are of $[M+H]^+$ ions.

- Mean mass accuracy 0.827 ppm for FTICR and 7.7 for Q-Tof (no lock mass correction). Mass errors allow unequivocal molecular formula to be established, isomers excluded → technique limited to rapid screening.
- NO QUANTITATIVE RELATIONSHIP (SO FAR) !
  - sweet spots (non-uniform crystallization of compound(s) and IS)
  - compound/matrix molar ratio has limited dynamic range

    ⇨more investigation needed

4. Conclusion

Despite popular belief that MALDI ionization is only useful for biopolymers, we have shown that it might have a future potential for small molecules too. In a toxicological context, it could be a fast screening alternative, provided a protocol for at least semi-quantitative measurements can be established.