Evaluation of the metabolomic tool: MALDI-MS for the analysis of small molecules

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1. Introduction

The most recent 'omics' technology applied to marker discovery is metabolomics, which can be defined as the study of the collection of small molecule (<1000 Da) metabolites in biofluids to elucidate differences in population groups due to genetic modification, disease state and environmental stress.

Spectroscopic techniques such as H-NMR have been applied in a number of studies but also complementary MS technology, especially exact-mass LC-MS, has been introduced. LC-MS does not provide complete metabolomic coverage. In particular, very polar compounds, such as simple sugars and many amino acids, elute in the void of the column. Another area of improvement is the cycle time of the LC-MS analysis and interpretation of results.

Because approaches to quantitative small molecule MALDI-MS have been reviewed recently, its inherent robustness, low sample consumption, no preceding separation and high sensitivity, we want to evaluate this technique based on MALDI as ionisation technique and accurate mass analysis, in our metabolomics study. By using this method in combination with multivariate techniques, we aim to distinguish groups based on their differences on metabolite level.

2. Aim

Exploration of the operational parameters concerning the application of MALDI-ionisation technique for small molecules on a Q-TOF mass spectrometer. Evaluation of its potential relative quantitative character.

3. Materials

Ionisation

- MALDI targetplates Waters
- Drug compounds, amino acids, carbohydrates, nucleosides, base-derivatives, nucleotides, planthormones, fatty-acids, steroids, in ng to mg/ml concentrations dissolved in a methanol-water mixture
- Matrix: 2 to 20 mg/ml of an acidic matrix (DiHydroxyBenzoic acid, CyanoCinnamicAcid) in a mixture of acetonitrile and water (2:1, v/v, 0.1% TFA) at instances with an equimolar amount of an organic base (TriButylAmine, Pyridine or Methylimidazol) added to obtain ionic liquid matrices

Mass spectrometry

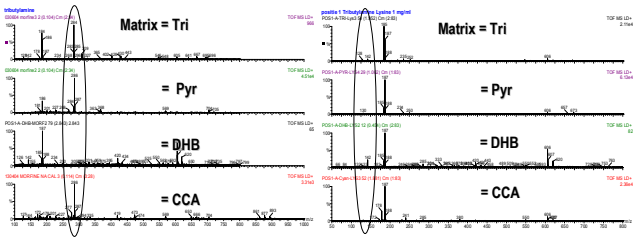
- Micromass MALDI-Q-TOF hybrid mass spectrometer
- Controlled using masslynx software version 4.0
- 337 nm nitrogen laser
- MCP detector

4. Methods

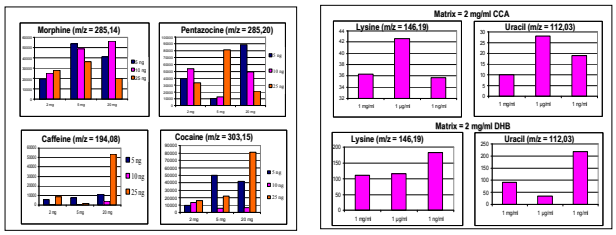
- After an optimized effective calibration of the mass spectrometer with a PEG-mixture, evaluation of different parameters such as laser firing rate, speed, collision energy, MS profile, ionisation mode, with the help of clearly measurable drug compounds. The optimized conditions remain intact for the tests of different matrices by variable matrix-compound ratio.
- Based on the preceding results, optimization of the analysis of metabolites on a MALDI-Q-TOF.
- Testing of a subset of these small molecules (acids, phosphates) in negative mode, using the acidic matrices as well as the ionic liquid matrices.
- In view of the strikingly different signals of total ion current between the drug compounds and the metabolites, examination of the relationship between TIC signal and structure (aromatic ring, base-group,...) of the analytes.
- In the light of given experiences with a MALDI-TOF (M@LDI instrument), investigation into the influence of the laser energy by changing the lens position of the nitrogen laser.

5. Results

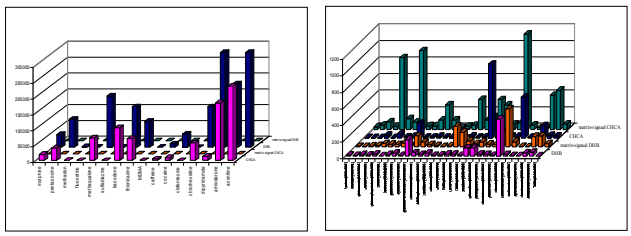
- Intensity signals of drug compounds ([M-H+] morphine = 286,14) and metabolites ([M-H+] lysine = 147,11) by using different matrices.



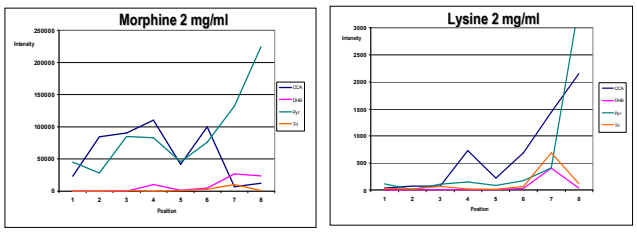
- Intensity signals for different quantities analyte at variable matrix (CCA) concentration (2, 5 and 20 mg/ml).



- Commonly observed interfering background matrix peaks from CCA and DHB.



- Each matrix, once crystallised has an optimal energy band where ions are emitted. By changing the position of the micro bench lens from source block the matrix receives a different amount of energy.



6. Conclusion

- Analysis of some small pharmaceutical drugs is possible, but no quantitative results are observed. Not even a roughly increasing response for a growing amount of analyte, nor better ionisation by increasing matrix quantity.
- Moreover a clear signal for metabolites was never really obtained, under no circumstances, with none of the matrices. Logically a quantitative relationship was absolutely out of the question.
- Based on the different experiments using various operational conditions as well as different small molecules, in an effort to correlate ionisation succes with physico-chemical characteristics, we conclude that the ionisation of small molecules using MALDI is largely based on coincidence and unsuitable for a screening analysis.
- The results, based on experiences with a M@LDI instrument gives no correlation between the amount laser energy and the bad TIC-signals of the analytes.

7. Acknowledgements

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