Automated MALDI MRMS and NMR for biomarker based determination of diabetes during pregnancy

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Franklin E. Leach III[1], Christopher J Thompson[2], Jeremy J Wolff[2], Jacquelyn Welko[1], Anushka Chelliah[3], Maureen Keller-Wood[3], Gary Kruppa[2], Arthur S Edison[1]

[1]University of Georgia, Athens, GA; [2]Bruker Daltonics Inc., Billerica, MA; [3]University of Florida, Gainesville, FL

Introduction

Mass spectrometry has increasingly been applied in the clinical setting due to the high and specific information content provided to researchers that enables a positive effect on patient outcomes. A different approach that eliminates the majority of sample preparation is MALDI-MS. Beyond mixing with a suitable ionization matrix, small amounts of sample (~1 uL) can be analyzed with no prior preparation or purification after clinical collection and in a high throughput fashion via MALDI automation

Sample Prep

Serum and urine were collected for clinical patients and stored at -80C. After thawing at 4C, samples were mixed 1:1 with DHB matrix and 1 µL spotted onto a 384 sample AnchorChip target. No additional preparatory steps were required before mass spectrometry analysis.

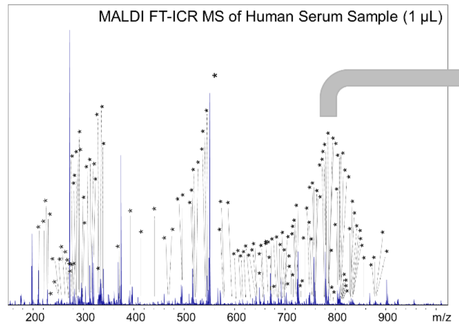

Fig. 1, left) 129 molecular compositions assigned for lipid species (*). Detected as either [M+H]+ or [M+Na]+ with Mass Error < 250 ppb. Molecular composition at *m/z* 203.05261 detected as [M+Na]+ and assigned as $C_6H_{12}O_6Na$ (49 ppb).

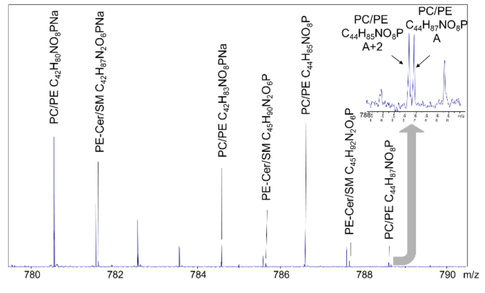

Fig. 1, right) Zoom of *m/z* 780-790 to indicate importance of resolving power to detect and assign lipid series that overlap due to variations in saturation (mass increases of 2H) resolving power ~325,000 required to make the split indicated in the inset.

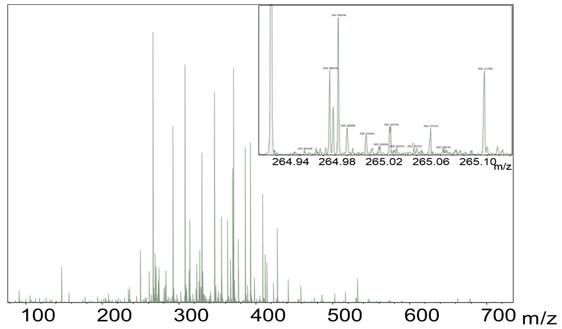

Figure. 2) MALDI MRMS analysis of 355 clinical urine samples with no sample prep beyond the addition of DHB matrix and required just under 6 hours of instrument time.

MS Method

- Urine and serum samples were analyzed on a Bruker solariX XR at either 9.4 T or 12 T using MALDI. For urine samples, a 4 M transient with a low mass of *m/z* 75 was acquired.
- 12 scans were summed, leading to an average of ~40 seconds to analyze each spot.
- In selected cases (sp. serum), 2ω and AMP were employed to increase RP.

Data Analysis

- Data Analysis was performed in DA 5.0 and Metaboscape 3.0 for multivariate analysis of large sample sets.

Results

- For example in serum, we have been able to identify molecular compositions that correspond to over 100 lipid species with a mass error less than 250 ppb shown in Figure 1 (left).
- Due to the ionization mechanism during MALDI, most analytes are observed as singly charged species.
- Seen in Figure 1 (right), a mass resolving power of approximately 325,000 is required to resolve the A+2 peak of a preceding phosphocholine/ phosphoethanlolamine (PC/PE) lipid species from the A or monoisotopic peak of the following PC/PE with one less double bond.
- For urine, the MALDI automation approach has resulted in the ability to directly measure the chemical complexity of over 300 clinical urine samples plus internal/external controls and blanks (480 total spots) in less than 6 hours.
- A typical spectrum is shown in Figure 2 and demonstrates the molecular complexity of this biofluid. Shown in the Figure 2 inset is a 0.10 Th wide excerpt of the spectrum illustrating the need for the increased mass resolving power.
- Detailed analysis of this large sample set was performed within Metaboscape 3.0, using the T-Rex-2D algorithm. Annotation was done with SF and matching to the urine HMDB (http://www.hmdb.ca/).
- A key feature of the analysis is the identification of patients with elevated urine glucose levels shown in Figure 3.

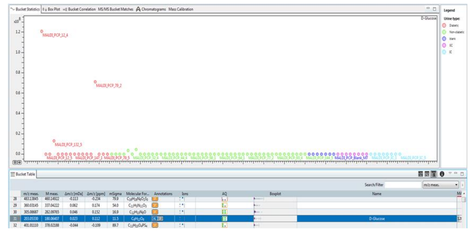

Fig. 3) The bucket at *m/z* 203.05261 was detected as $[M+Na]^+$ and assigned as D-Glucose (49 ppb). Bucket statistics for this metabolite shows that high intensities of D-Glucose can only be detected in samples of diabetic patients.

Conclusions

- Automated MALDI MRMS provides the opportunity to obtain complementary information that support NMR findings on large clinical sample sets with minimal sample prep.
- MetaboScape 3.0 enabled processing of MALDI-MRMS data facilitating this higher throughput profiling workflow.
- Future work will focus on further refining the approach by incorporating isotopic fine structure and MS/MS to increase confidence in the assignment of composition and structure and to correlate MS and NMR results.

Metabolomics