Automated MALDI Magnetic Resonance Mass Spectrometry (MRMS) for biomarker identification in large clinical sample sets

The selection of a MALDI MS approach in conjunction with the high mass accuracy and resolving power of the MRMS platform has enabled increased sample throughput and the direct interrogation of complex clinical biofluids such as serum and urine without the need for any advanced sample preparation or purification after collection

In conjunction with advanced automated acquisition strategies, data has been acquired in a fraction of the time previously required to facilitate high quality data with the high sample throughput required for large clinical sample sets.

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. The foundation of this application is the high sensitivity, mass resolving power, and mass accuracy in combination with the multiplex detection advantage of MRMS when compared to other analytical approaches. A major drawback though is the additional sample preparation required for biofluids prior to LC-MS or direction infusion ESI-MS. An alternative to this approach that eliminates the majority of sample preparation is MALDI-MS. Beyond mixing with a suitable ionization matrix, small amounts of sample (~ 1 µL) can be analyzed with no prior preparation or purification after clinical collection and in a high throughput fashion via MALDI

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automation. A single spectrum can typically be collected per sample spot in less than a minute resulting in the ability to measure an entire 384 spot plate in several hours. Post-acquisition, MetaboScape 3.0 enables the analysis of complete sample sets for compound identification based on isotopic fine structure (ISF) and library searches in addition to multivariate statistical analysis.

Sample Preparation

Serum and urine were collected for clinical patients and stored at -80°C. After thawing at 4°C, 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.

Mass spectrometry analysis

Urine and serum samples were analyzed on a Bruker MRMS system at either 9.4 T or 12 T using MALDI automation for increased throughput. For urine samples, a 4 M transient (1 s) with a low detection m/z of 75 was acquired. 12 scans were summed for each sample leading to an average of ~ 40 seconds to analyze each spot (including instrument overhead time). In selected cases (sp. serum), detection at twice the cyclotron frequency (2ω) and absorption mode processing were employed to increase the mass resolving power.

Data Analysis

Analysis was performed in Bruker DataAnalysis 5.0 for individual spectra and MetaboScape 3.0 for multivariate analysis of large sample sets.

Results

To date NMR data has been collected for over 300 clinical samples. Preliminary metabolic profiles have been established to determine relative differences in lipid, amino acid, and glucose levels. In our preliminary MS data, samples from three patient sub-groups have been examined in positive ion mode by MALDI-MRMS. Each acquisition required less than a minute and resulted in complex spectra. 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 (A). Due to the ionization mechanism during MALDI, most analytes are observed as singly charged species. Although MALDI reduces the spectral density of peaks when compared to ESI, these samples still benefit from the high resolving power due to the structural similarity of lipids. This intrinsic quality poses a limitation on the number of unique assignments when a mass spectrometer with insufficient mass resolving power is employed due to the overlapping nature of isotopic envelopes in lipids where a difference of 2H can arise due to bond saturation. Seen in Figure 1 (B), 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. Without the mass resolution provided by MRMS, the presence of two species could not be confirmed with a high level of certainty.

In addition to serum, urine from the same sample pool have been analyzed by automated MALDI-MRMS. Due to the increased salt content, additional sample preparation and/or chromatographic methods are required

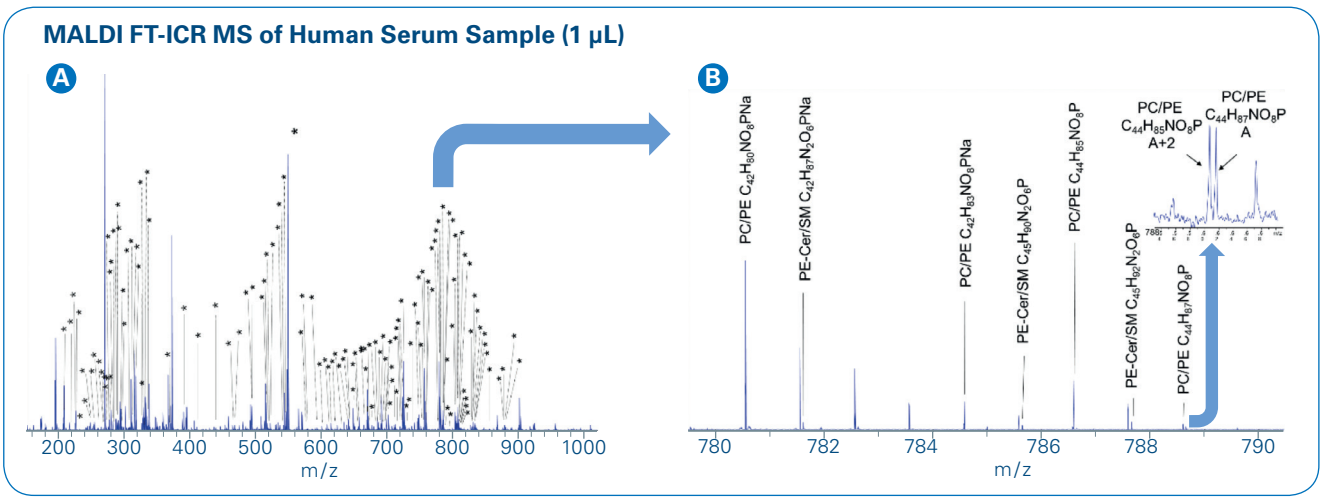

Figure 1: A 129 molecular compositions assigned for lipid species (*). Detected as either $[M+H]^+$ or $[M+Na]^+$ with mass error < 250 ppb. Molecular composition at m/z 203.05261 detected as $[M+Na]^+$ and assigned as $C_6H_{12}O_6Na$ (49 ppb). B Zoom of m/z 780-790 to indicate importance of resolving 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.

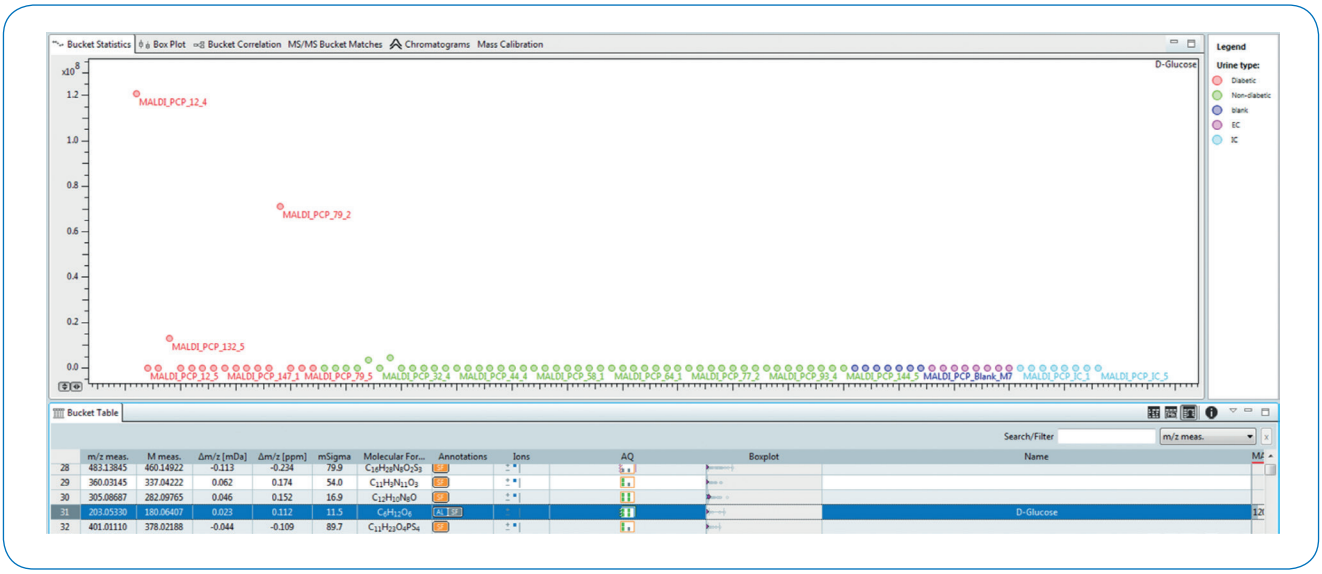

Figure 2: The bucket at m/z 203.05261 was detected as $[M+Na]^+$ and assigned as D-Glucose (49 ppb). Bucket statistics for this metabolite shows that high intensities of D-Glucose can only be detected in samples of diabetic patients.

prior to MS analysis. MALDI is more tolerant of the salt and presents a straightforward path for sample ionization. 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. For comparison, an LC-MS approach would have required approximately 120 hours based on 15 minute gradients alone and not including equilibration or washes. A typical spectrum is shown in Figure 2 and demonstrates the molecular complexity of this biofluid. Shown in the Figure 3 inset is a 0.10 Th wide excerpt of the spectrum illustrating the need for the increased mass resolving power afforded by MRMS in this experimental approach.

Detailed analysis of this large sample set was performed within MetaboScape 3.0. Automatic assignment of elemental compositions and analyte identifications was performed for detected features using accurate mass and isotopic pattern information using a novel algorithm for mass recalibration, feature extraction, and de-isotoping. The generated Bucket Table was automatically annotated by assigning analyte names based on a list of known urine metabolites obtained from the Human Metabalome Database (http://www.hmdb.ca/). Additional databases can also be imported for further assignments. A key feature of the analysis is the identification of patients with elevated urine glucose levels shown in Figure 3.

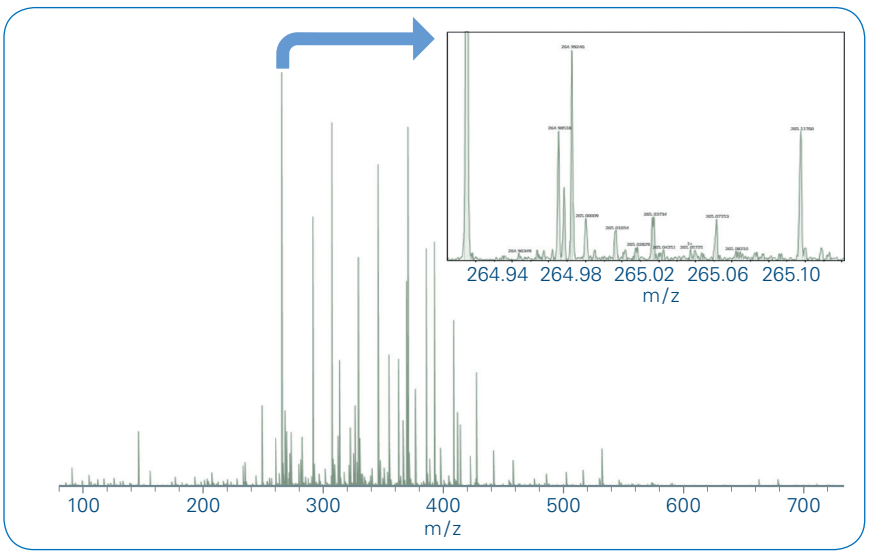

Figure 3: MALDI MRMS analysis of 355 clinical urine samples with no sample prep beyond the addition of DHB matrix and just under 6 hours of instrument time required.

Conclusion

- 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.

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