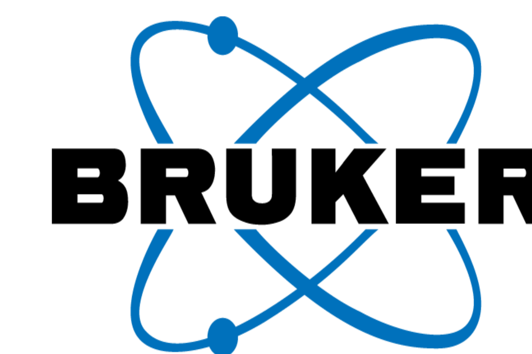
Metabolite identification by assignment of collisional-cross section and isotopic fine structure



TP 397

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Introduction

Metabolic phenotyping (phenomics) requires a comprehensive analysis of the metabolite composition of biological systems. A human phenotype is governed by the complex interplay between an individual's genetic makeup, lifestyle and environment; including diet and exercise, exposures and microbial interactions.

To understand these interactions and dissect the biological mechanisms underpinning the phenome, the analysis of complex biofluids such as urine and plasma, which possess a biochemical fingerprint of these mechanisms is necessary. Whilst commercial metabolite standards are ideal for metabolite ID assignment, the chemical diversity of the human metabonome limits this approach. Complementary instrumentation, such as QTOF, MRMS, ion mobility MS and NMR, can meet this challenge by providing comprehensive structural characterisation of unidentified metabolites and confidence in metabolite identification.

Methods

Urine was prepared using a standardised dilution approach. LC-MS platforms, including LC-QToOFMS/MS data acquired for both positive (ESI+) and negative electrospray ionization (ESI-) using the timsTOF Pro, employing trapped-ion-mobility-time-of-flight mass spectrometry and a PASEF® acquisition mode, for the measurement of structural collisional cross-sectional detail. These complementary data were used to identify metabolites of interest and ultimately characterise the small molecule composition of the biofluid. Replicate samples were then analyzed by flow injection analysis by magnetic resonance mass spectrometry (FIA-Solarix 7T MRMS), and complemented by analysis using liquid chromatography (LC), to obtain isotopic fine structure (IFS) for the observed analytes. Urine analyzed by mass spectrometry, where chromatographically separated used either a reversed-phase (C18) or HILIC column.

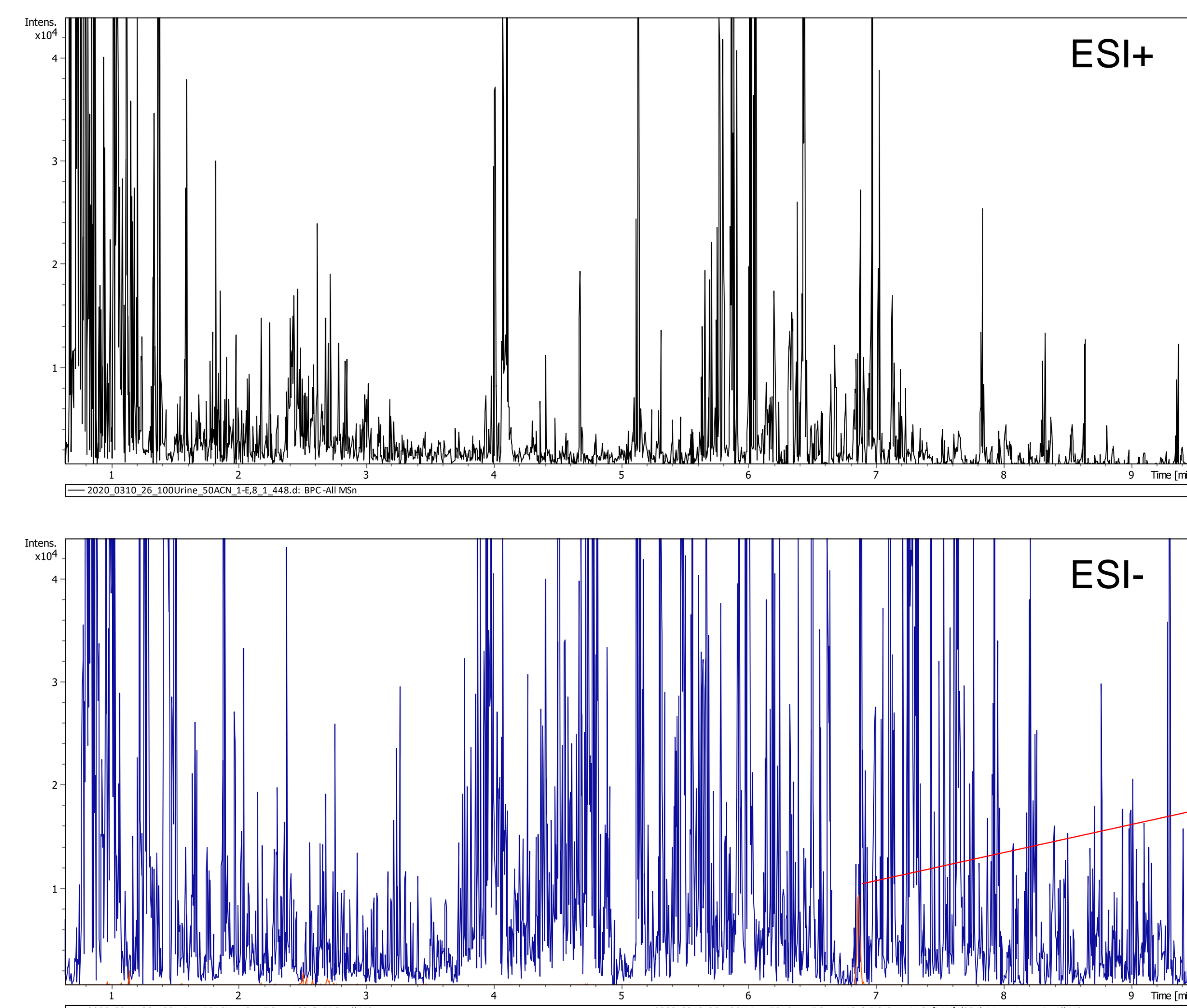
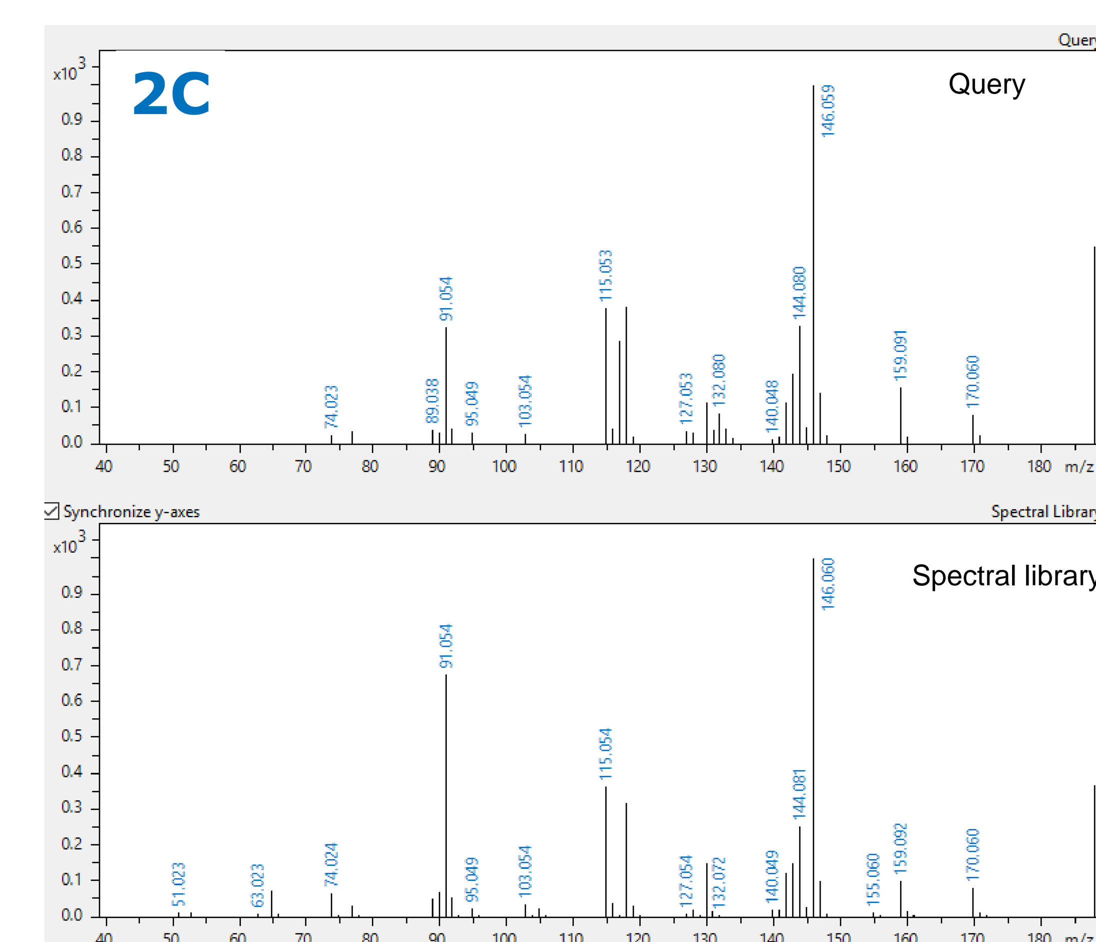


Figure 1: Total ion chromatograms of human urine (**above**) acquired using RP-chromatography with ionization by ESI+ (**black**), and HILIC chromatography with ESI- (**blue**); with the target ion of interest, tryptophan, displayed by extracted ion chromatogram (**red**).

Figure 2: Comparison of accurate mass measurements using simulated isotopic pattern of predicted formulae and spectra library. **A)** High resolution spectra of tryptophan with timsTOF Pro and further characterized with isotopic fine structure by Solarix MRMS **(B)**. **C)** Primary metabolite identification achieved by MS/MS spectral library match.



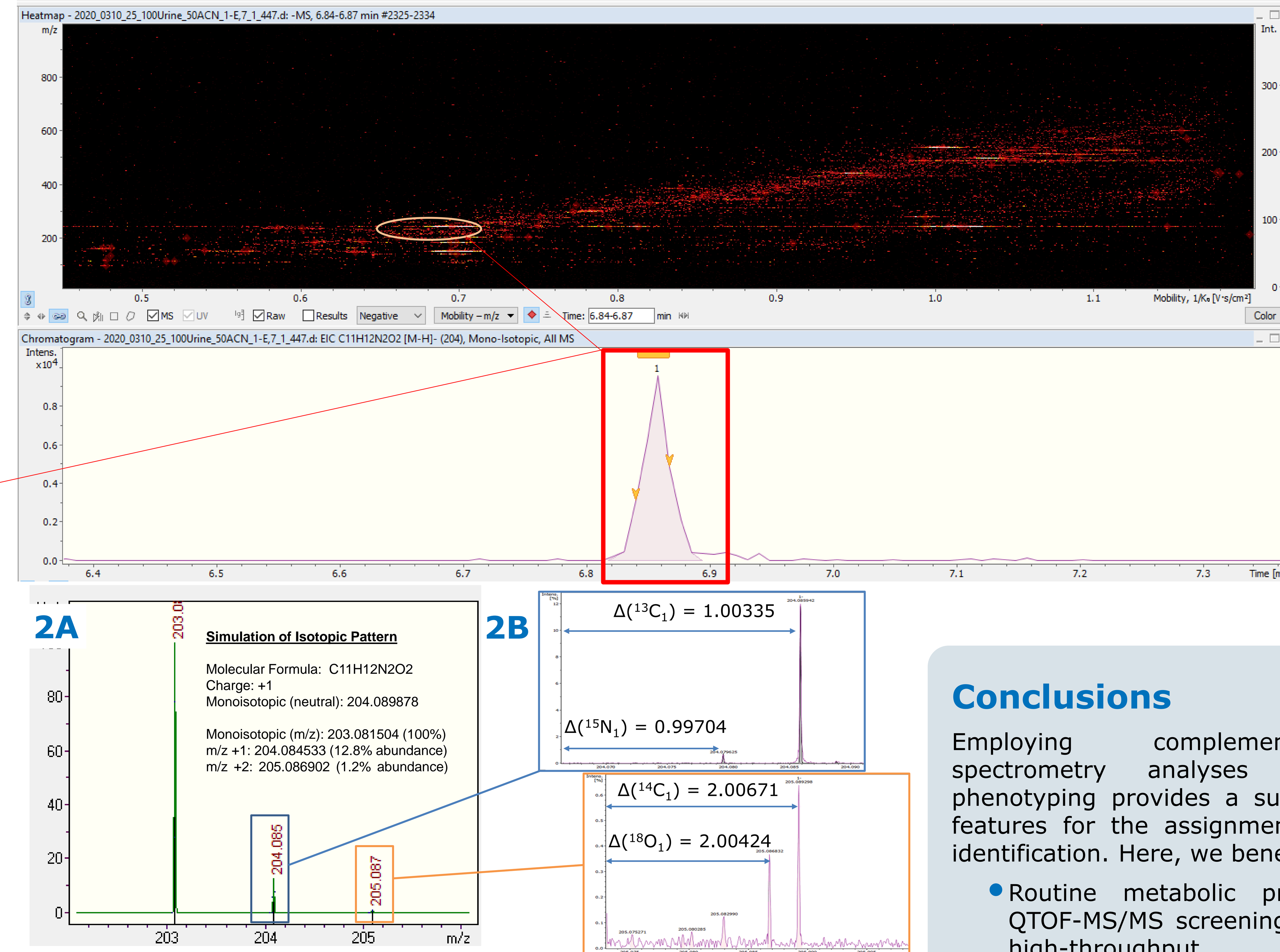


Figure 3: Complementary analysis using the 4D-Metabolomics™ pipeline (**left**) provides highly reproducible collisional cross-sectional measurements and identifies metabolite class-specific gas-phase separation of the target ion.

Results

Urine samples were analysed in the laboratory of the Australian National Phenome Centre (ANPC) using the suite of analytical platforms. Mass spectra were identified against mass spectral libraries (Bruker HMDB Metabolite Library 2.0, CCS compendium and MetaboBase® Personal Library 3.0) and metabolites identified by accurate mass precursor ion, MS/MS, collisional cross section and chemical fine structure. PASEF® data was processed in MetaboScape® and included automatic CCS value determination. Features extracted by this 4-dimensional peak picking and alignment algorithm were annotated based on precursor mass accuracy and isotopic pattern, retention time, MS/MS spectrum and CCS value. Analysis of the urinary metabolites by LC-QTOF-MS/MS showed that more than 50% of the features had MS/MS spectra acquired. Co-eluting metabolites were successfully resolved using the additional separation dimension of ion mobility, improving MS/MS spectral quality and accomplishing greater detail in characterising the biofluid beyond routine profiling by LC-QTOF-MS. TIMS increased the resolving power of the MS for co-eluting compounds, resulting in a cleaner baseline and ultimately cleaner MS/MS spectra, which is essential for accurate ID. TIMS also provided reliable CCS values toward metabolite identification. The ultra-high resolution mass accuracy and isotopic fine structure data obtained by MRMS provides integral detail toward metabolite structural elucidation and identification.

Conclusions

Employing complementary mass spectrometry analyses for metabolic phenotyping provides a suite of molecular features for the assignment of metabolite identification. Here, we benefited from:

- Routine metabolic profiling by LC-QTOF-MS/MS screening of biofluids for high-throughput metabolic phenotyping, using the Bruker Impact II series.
- The use of an additional separation dimension by ion mobility by timsTOF-MS/MS, resulting in clean MS/MS spectra, highly beneficial for accurate ID in small molecule workflows.
- The measurements of Highly reproducible CCS^{TIMS} values for confidence in metabolite ID
- The complimentary use of isotopic fine structure as a final and highly useful identification tool.

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