An innovative variable-energy electron ionisation technology applied to GC-TOF-MS metabolomics applications

Warwick Dunn (1), James Allwood (1), Jeremy Tomlinson (2), Simon Stevens (3), Leonhard Pollack (4), Laura McGregor (3), Nick Bukowski (3) and Steve Smith (3)

(1) School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK; (2) School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, B15 2TT, UK; (3) Markes International Ltd, Gwaun Elai Medi-Science Campus, Llantrisant, CF72 8XL, UK; (4) Markes InternationalGmbH, Schleussnerstrasse 42, Frankfurt, Germany

INTRODUCTION

- Technological advances in GC-TOF-MS instrumentation at the turn of the 21st century was a significant driver for the increased popularity of non-targeted metabolomics in biological studies
- Early applications were focused on the study of plant biochemistry; more recently the study of mammalian systems to understand molecular disease pathophysiology or identify disease biomarkers have been observed
- Current hurdles to overcome in non-targeted metabolomics studies include the acquisition of reproducible data in small and large-scale studies and the chemical identification of biologically important metabolites in these studies
- Electron impact ionisation in current instrumentation operates at 70 eV; this approach provides a high sensitivity with significant molecular ion fragmentation
- Operating at lower electron energies would provide increased sensitivity for molecular ion detection and has the potential to reduce the complexity of fragmentation mass spectra
- Here, we assess the applicability of applying low (14 eV) and traditional (70 eV) electron ionisation in a non-targeted metabolomic study of the interaction of insulin and cortisol on global human metabolism

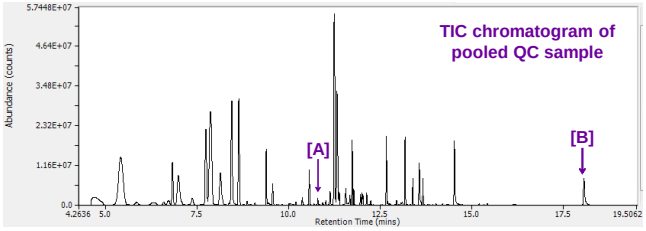
METHODOLOGY

- Plasma samples were collected from 10 healthy adults before and after a 4 hour insulin infusion (20 mU/m^2/min) at two time points corresponding to either normal or high blood cortisol concentrations
- Plasma samples were extracted in a 3-fold excess of methanol followed by centrifugation and drying [1]
- Dried extracts were chemically derivatised applying a two-stage process of methoximation followed by trimethylsilylation [1]
- Samples were analysed in a random order applying an Agilent 7890A GC coupled to a Markes International electron ionisation BenchTOF-Select™ mass spectrometer (right). All samples were analysed twice, separately applying 14 eV and 70 eV electron ionisation energies, in a Select-eV ion source

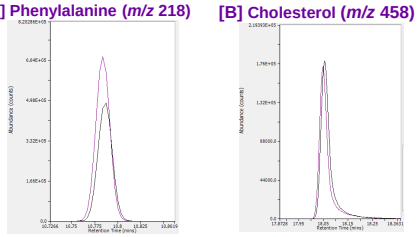
- A pooled QC sample was intermittently analysed to assess data quality [1]
- All data were processed applying Markes International TargetView 4.0 software

SENSITIVITY

- Typically, application of low electron energies is associated with a significant reduction in sensitivity
- The Select-eV source is designed to provide comparable sensitivity at 14 eV and 70 eV
- The data acquired across a range of different metabolites (including amino acids, organic and fatty acids, amines and carbohydrates) showed a similar sensitivity for data acquired at 14 eV and 70 eV
- Two examples of this comparable sensitivity are shown below for phenylalanine and cholesterol.



[A] Phenylalanine (*m/z* 218) [B] Cholesterol (*m/z* 458)



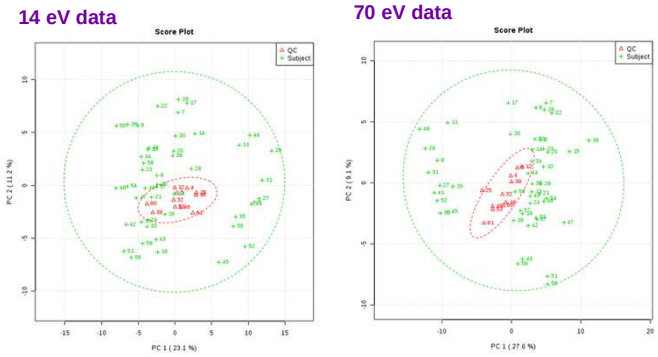
Overlaid EIC chromatograms for 70 eV (black) and 14 eV (purple) for [A] phenylalanine and [B] cholesterol.

- 65 identified metabolites were detected applying both 14 eV and 70 eV and two metabolites were only detected applying one of two electron energies (a) mannose (detected in 14 eV data only) and (b) erythritol (detected in 70 eV data only).

METHOD REPRODUCIBILITY

- A single pooled QC sample was analysed intermittently during the batch of 61 samples.
- The reproducibility in a single analytical batch of the metabolomics data is equivalent for both electron energies applied, as shown below.
- PCA analysis (below) shows that the distribution of QC samples (the same sample analysed multiple times; red) is significantly less compared to biological samples (different subject samples; green). This indicates high reproducibility.

14 eV data 70 eV data

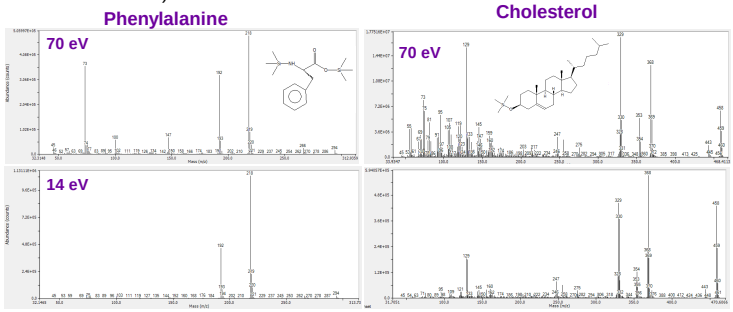


- In univariate quality assurance of these data we typically assess the relative standard deviation for QC sample responses for each metabolite – a RSD < 30% is deemed appropriate to show good quality data [1]
- In these data, 83 and 81 unique metabolite peaks were observed to provide a RSD < 30% for QC samples for the 14 eV and 70 eV data, respectively

ELECTRON IONISATION MASS SPECTRA

- The mass spectra acquired at 70 eV and 14 eV for two metabolites (phenylalanine and cholesterol) are shown below

Phenylalanine Cholesterol



- The 14 eV mass spectra show a reduction in relative intensity of the typically observed and non-specific TMS-derived fragment ions at *m/z* 73 and 147 while maintaining the metabolite-specific fragment ions applied for identification of the metabolite
- This reduces the complexity of most mass spectra, simplifying the data, while preserving the characteristic fragment ions essential for confident identification
- The loss of *m/z* 73 and 147 ions increases the similarity score when matching experimental data to an in-house mass spectral library [2] constructed on a different instrument at 70 eV.
- For example, the mass spectral similarity scores for phenylalanine at 70 eV and 14 eV are 908 and 803, respectively.
- This experimental advantage has the potential to improve metabolite identification in these studies; metabolite identification is a current bottleneck in non-targeted metabolomics studies

CONCLUSIONS

- In this study the sensitivity of data acquired at 14 eV and 70 eV were comparable
- The reproducibility of data acquired for multiple injections of a single QC sample at 14 eV and 70 eV was also comparable
- The electron ionisation mass spectra acquired at 14 eV are observed to have lower intensity *m/z* 73 and 147 ions characteristic of TMS fragments while maintaining the sensitivity for metabolic-specific characteristic ions applied for metabolite identification. Mass spectral searches applying an in-house library acquired on a different instrument at 70eV provided higher similarity scores for the 14 eV data compared to the 70 eV data.
- Operating at 14 eV provides a greater opportunity to perform robust metabolite identification and 70 eV data libraries can be applied in these non-targeted metabolomic studies
- No robust biological differences were observed in this small study

REFERENCES: [1] Dunn et al. Nature Protocols, 2011, 6, 1060-1083; [2] Brown et al. The Analyst, 2009, 134, 1322-1332

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