Imperial College London

The Association for Mass Spectrometry: Applications to the Clinical Lab

GC-MS determination of candidate target biomarkers for early detection of oesophageal squamous cell cancer.

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Introduction

- Branched chain amino acid metabolism is deregulated in numerous diseases.
- Aim: To develop a quantitative method for key metabolite groups in this pathway in clinical and molecular studies with the use of gas-chromatography mass spectrometry (GC-MS).

Methods

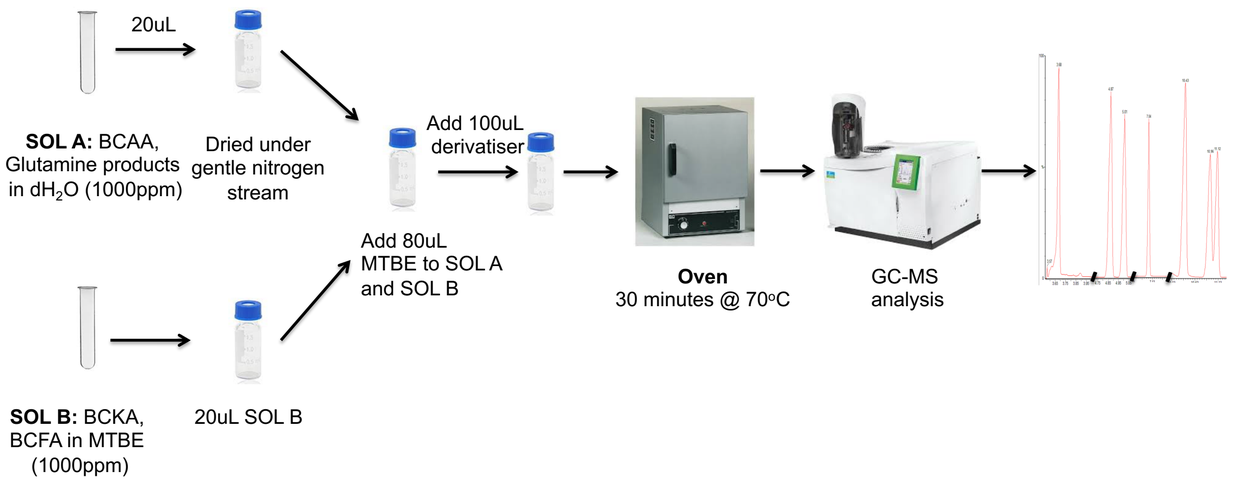

Figure 1: Method of sample processing and analysis

- Optimisation of derivatisation conditions, GC-MS oven and column parameters and compound extraction.
- Individual and compound stock solutions of branched chain compounds (BCCs) and glutamine products were prepared in methyl-tert-butyl ether (MTBE) and deionised water (for optimisation of compound extraction).
- Target compounds were: branched chain amino acids (**BCAA**) (leucine, isoleucine and valine), branched chain ketoacids (**BCKA**) (3-methyl-2-oxovaleric acid (3M2OV), 4-methyl-2-oxovaleric acid (4M2OV), 3-methyl-2-oxobutanoic acid (3M2OB)), branched chain fatty acids (**BCFA**) (isovaleric acid (IVA), 2-methyl-butyric acid (2-MB) and isobutanoic acid (IBA)), **Glutamine products**, glutamine (GLU), glutamic acid (GA), alpha ketoglutarate (a-KG) were purchased from Sigma-Aldrich.

Results

Optimisation of derivatisation:

- Two derivatising agents; *N-tert-* Butyldimethylsilyl-*N*-methyltrifluoro acetamide (MTBSTFA) and N-Methyl-N-trimethylsilyl- trifluoroacetamide (MSTFA) were selected.

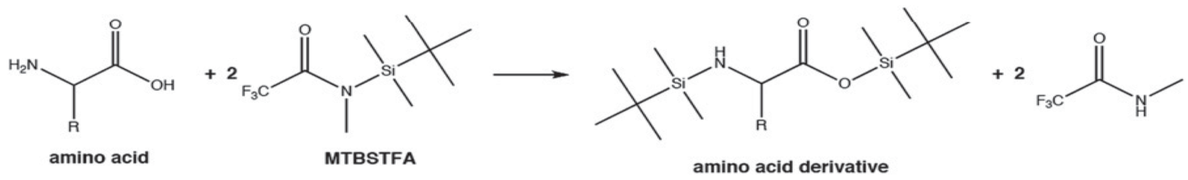

Figure 2: Equation demonstrating silylation of amino acid with MTBSTFA

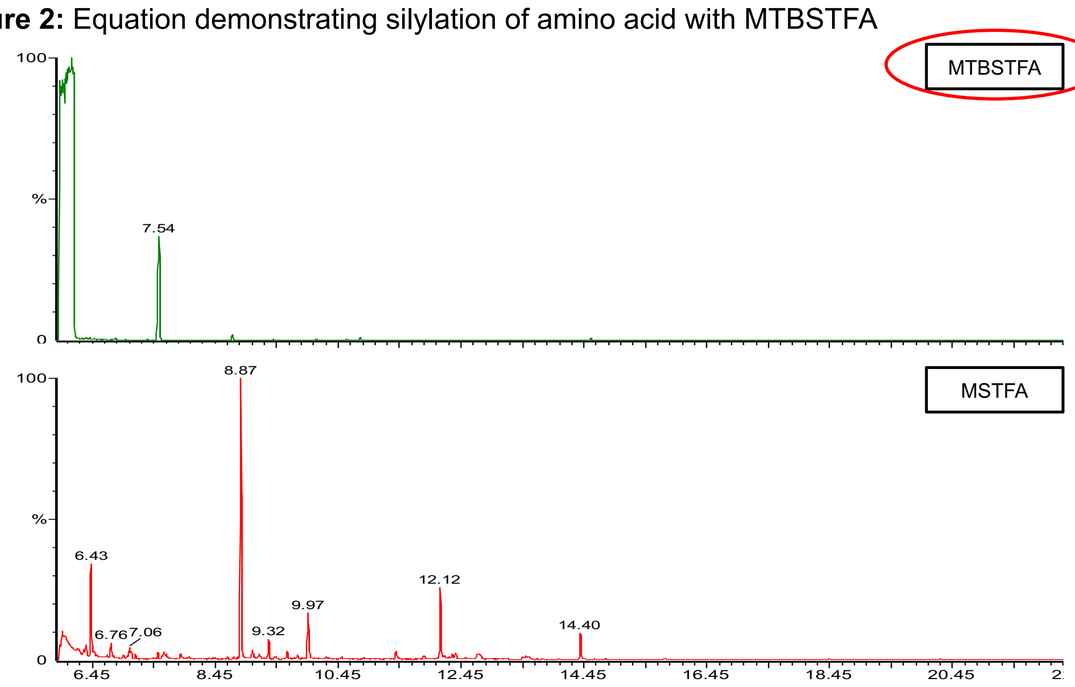

Figure 3: Chromatogram demonstrating MTBSTFA vs MSTFA derivatisation on GC-MS analysis. MTBSTFA has MTBSTFA derivatisation yieleded better identification of isomeric peaks and cleaner baseline.

GC-MS oven temperature optimisation:

Table 1: Established parameters for GC-MS oven temperature programme

Step	Rate (°C/min)	Temperature (°C)	Hold time (min)	Run Time (min)
Initial	0	60	0	0
Ramp 1	23.5	100	9.65	11.35
Ramp 2	40.0	300	5.00	10

GC-MS column optimisation

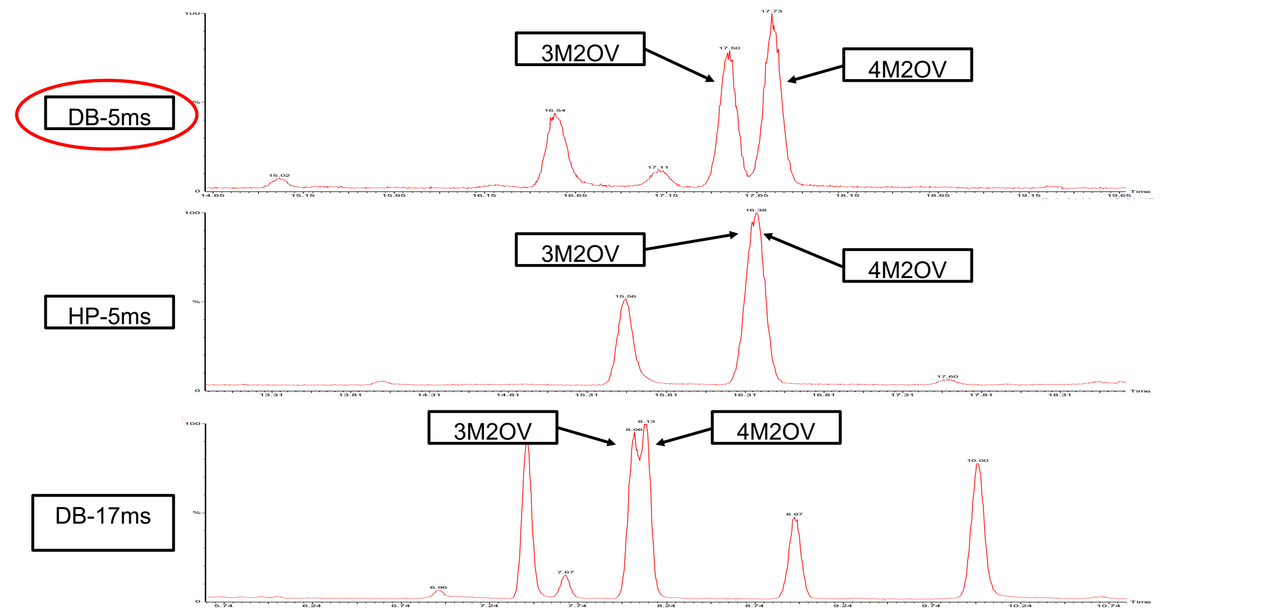

Figure 4: Chromatogram demonstrating different columns used to achieve optimal chromatographic separation of isomeric peaks.

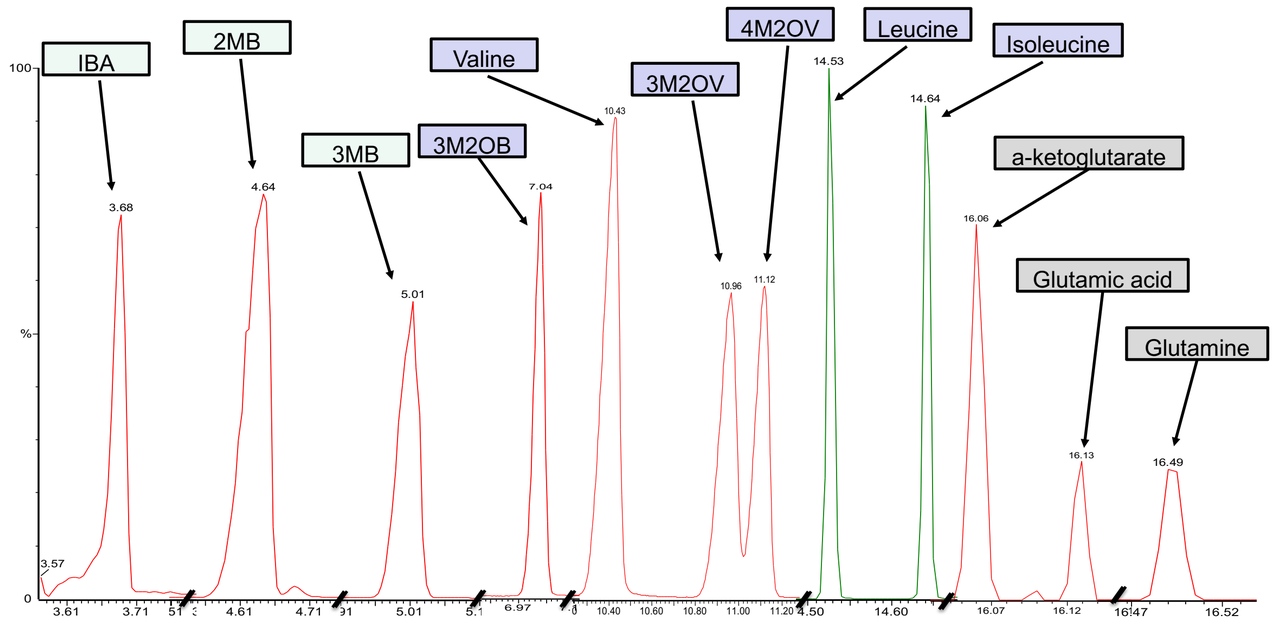

Figure 5: Chromatogram demonstrating peaks of all target compounds

Optimisation of sample processing conditions

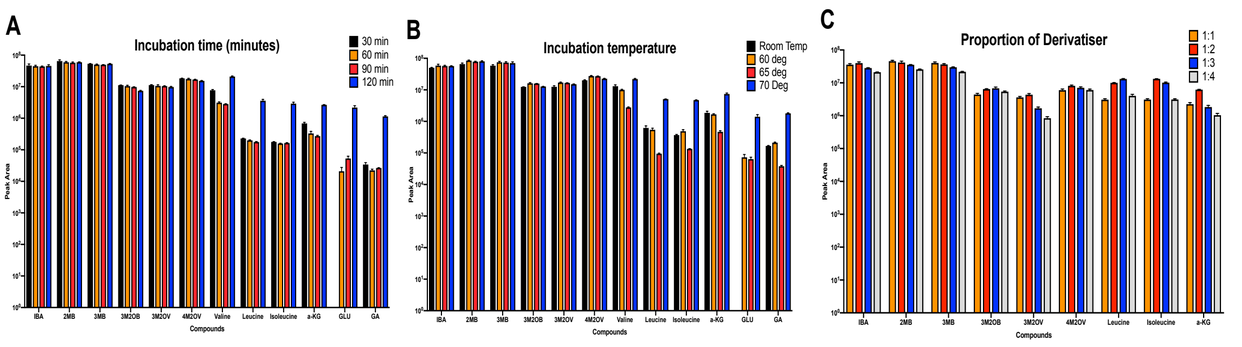

Figure 6: Optimisation of sample processing conditions. A: Incubation time B: Incubation temperature C: Proportion of derivatiser

Discussion

- All 12 target compounds can be identified with optimised GC-MS oven and column parameters.
- Saliva and cell media sample processing with the use of liquid-liquid extraction has successfully demonstrated target compound peaks low method limits of detection (low ppb).
- Further work to address recovery and accuracy in accordance to the ICH guidelines will need to be performed.

Conclusion

- This proposed method demonstrates the ability for streamlined sample preparation and analysis for candidate biomarkers with GC-MS for use in liquid phase matrices.

References

1. Moreau N., Goupry S., Antignac J., Monteau F., Le Bizec B., Champ M., et al. Simultaneous measurement of plasma concentrations and 13C-enrichment of short-chain fatty acids, lactic acid and ketone bodies by gas chromatography coupled to mass spectrometry. J Chromatogr B. 2003 Feb 5;784(2):395–403.
2. Marchesan JT, Morelli T, Moss K, Barros SP, Ward M, Jenkins W, et al. Association of Synergistetes and cyclodipeptides with periodontitis. J Dent Res. 2015;