Development of rapid procedure for the profiling of metabolites in dry blood spots

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Introduction and objectives

The comprehensive analysis of small molecules (metabolomics) in biofluids provides a snapshot of metabolic phenotype (metabotype) that acts as functional representation of health (Holmes et al., 2008). Investigation of metabotype variation is increasingly being applied to diagnose disease, identify risk factors and monitor treatment outcomes etc. (Sussulini, 2017).

A prominent clinical application of metabolomics is the investigation of inborn errors of metabolism (IEM), where early identification and treatment of neonates & infants is critical to prevent severe disease (Kuhara et al., 1999).

Typically, screening for IEMs in neonates and infants has been conducted via gas chromatography – mass spectrometry (GC-MS) of urine samples to identify multiple errors in amino acid, fatty acid and carbohydrate metabolism (Kennedy et al., 2016; Kuhara et al., 1999; Matsumoto and Kuhara, 1996).

Furthermore, metabolomics is widely applied to blood samples and favorably provides a comprehensive snapshot of circulating analytes and able to detect a wider range of IEMs. However, usual procedures for screening IEMs in blood require relatively large sample volumes (> 100 µL; e.g. (Bonte et al., 2019; Ford et al., 2020)) and thus are unsuitable for neonatal screening where limited sample is available.

In recent years, GC-MS based metabolite profiling methods suitable for small volume samples (< 10 µL) and/or dry blood spots (DBS) have been reported (e.g. Arakawa et al., 2021; Kong et al., 2011), with DBS analysis shown to provide comparable results to plasma (Kong et al., 2011). Yet, these procedures are relatively time-consuming and require multiple steps of analyte extraction, concentration and derivatization prior to detection.

AIM: Develop a rapid procedure to profile primary metabolites in dry blood spots suitable for automation.

Methods

An initial test to assess feasibility to develop a rapid DBS metabolite profiling method has been conducted.

DBS 'amino acid' card from the Center for Disease Control (CDC), & Prevention, USA; were selected. Holes (3 mm) were punched in card & added to glass vial with insert. Samples were directly extracted-derivatized with methoxyamine hydrochloride (MeOx; 20 ug/mL in pyridine) and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA). Derivatized extracts were analysed via gas chromatography coupled with high-resolution mass spectrometry, operating in full-scan wtth electron ionization (70 ev), as outlined in Figure 1.

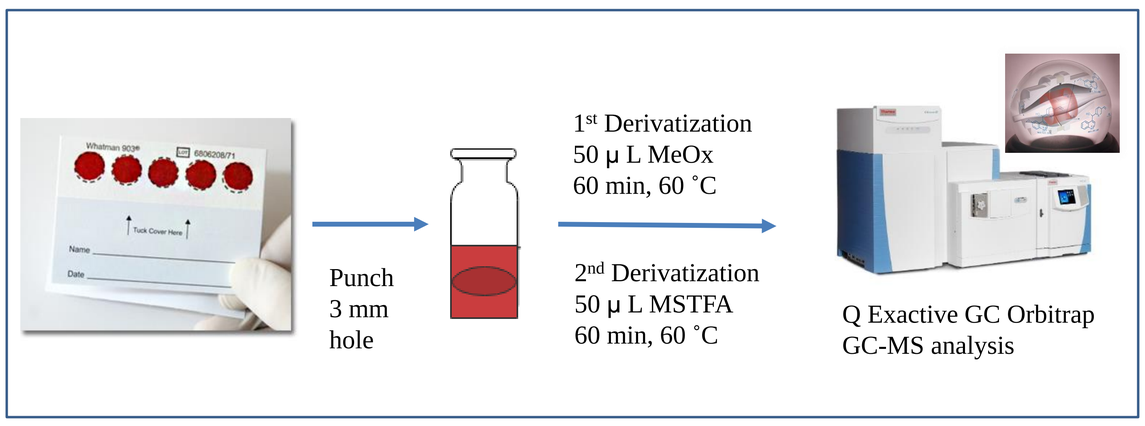

Figure 1. Outline of rapid procedure for dry blood spot metabolomics profiling via GC-HRMS.

Preliminary Results

The initial test demonstrated that direct extraction-derivatization of DBS cards is possible. Resultant chromatograms showed peaks of primary metabolites, distinguishing compounds belonging to three main classes, namely amino acids (e.g. leucine, phenylalanine), saccharides (e.g. glucose), and steroids (e.g. cholesterol), shown in Figure 2.

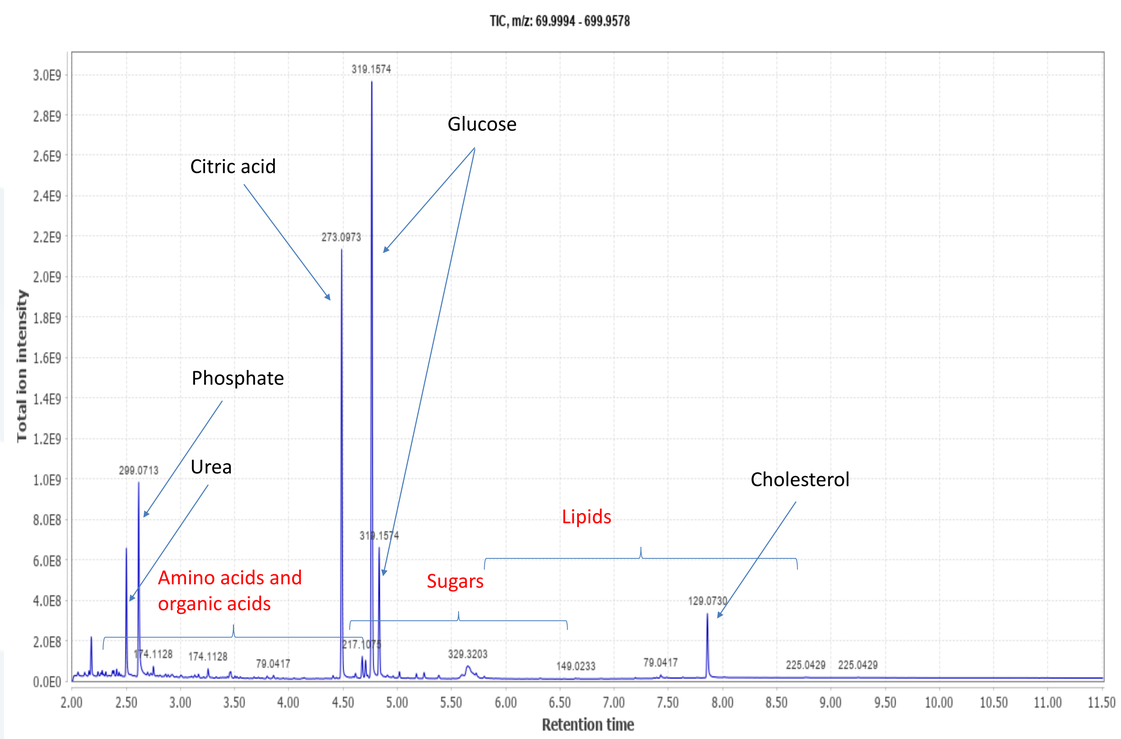

Figure 2. Example base peak chromatogram following preliminary DBS metabolite profiling assessment.

Future work

Future development will focus on optimizing direct extraction-derivatization to enhance analyte coverage, increase sample throughput and enable online automation.

In particular, various derivatization reagents will be compared (Figure 3):

- the most commonly applied two-step method of methoxyamine hydrochloride (MeOX) followed by N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) (Moldoveanu and David, 2019)
- the replacement of MSTFA with trimethylsily cyanide (TMSCN), which has been reported to enhance detection sensitivity and reduce silylation times (Khakimov et al., 2013)
- the use of N,O-Bis(trimethylsilyl)carbamate (BSMOC), which has been introduced as a single-step derivatization agent (Morvai-Vitányi et al., 1993) but rarely applied in practice

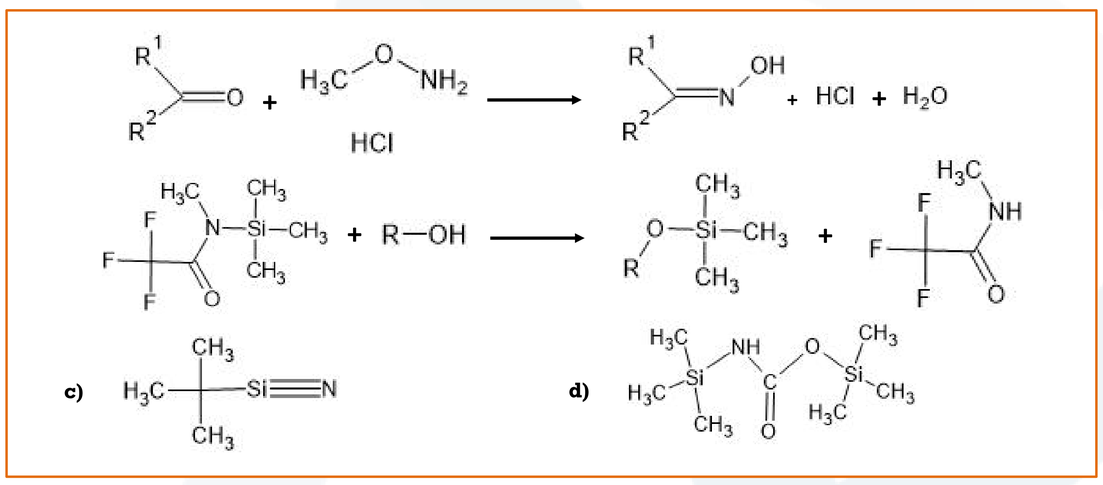

Figure 3. Derivatization reactions and reagents a) oximation reaction b) silylation reaction c) TMCSN d) BSMOC

Acknowledgments

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References

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