Analysis of Volatile Biomarkers in Exhaled Breath and other Bodily Fluids and Waste by SIFT-MS

Tuesday 18th and Wednesday 19th October 2016

Sponsored by Anatune Ltd, Cambridge, UK and Syft Technologies Ltd, New Zealand

Programme

Tuesday 18th October

12.00 Arrive and register at Down Hall

12.30 Lunch

13.30 **Introduction**
Ray Perkins, Anatune Ltd, and David Smith, Trans Spectra Ltd

13.45 **The SIFT-MS Analytical Method for Quantitative Analyses of Volatile Metabolites in Exhaled Breath: state of the art report**
Patrik Spanel, J.Heyrovsky Institute, Prague, Czech Republic

The basic physics, flow dynamics and ion chemistry kinetics that underpin the SIFT-MS analytical method will be described briefly. Then suitable gas sampling methods and reagent and analyte ion count rate data processing will be discussed that can provide accurate quantification in real time of trace compounds/volatile metabolites in exhaled breath and the headspace of aqueous liquids. The rapid acquisition rate of direct breath analyses will also be shown. The limit of detection (LOD) and limit of quantification (LOQ) achievable by SIFT-MS instruments will be also discussed. Whilst recognizing that real time analysis is not always possible and sample collection is often required, the pitfalls involved in this are alluded to. The virtues of the parallel exploitation of SIFT-MS and SPME/ATD/GC-MS in combination will be exemplified. Analysis and interpretation of breath analysis data, however acquired, needs to be treated circumspectly. In particular, the inappropriate use of statistics such as multiple comparison, PCA analysis and receiver operating characteristic (ROC) curves to treat imperfect mass spectrometry/mobility spectra should be avoided, since it can result in unjustifiable conclusions.

14.30 **Dual-Polarity SIFT-MS and its Potential Biomedical Applications**

Daniel Milligan, Barry Prince, Murray McEwan, Syft Technologies Ltd and University and University of Canterbury, Christchurch, New Zealand

Direct mass spectrometric techniques eliminate chromatography, enabling dynamic processes such as breath analysis to be monitored in real time. Further benefits of direct analysis include simplifying analysis through avoiding derivatisation, drying, pre-concentration, and other pre-treatment of samples – all of which prevent real-time analysis and can distort analysis. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a direct-analysis technique originated by Spanel and Smith (Spanel & Smith, 1996). It enables direct, real-time monitoring of analyte concentrations in breath or headspace based on known ion-molecule reaction chemistry. Traditional SIFT-MS has utilised three positively charged reagent ions that are generated in a microwave ion source operating on moist air (H_3O^+, NO^+, O_2^+). However, not all analytes can be monitored with these reagent ions. In this paper, we report an enhancement of the SIFT-MS ion source that enables generation of five negatively charged reagent ions: O^-, OH^-, O_2^-, NO_2^- and NO_3^-. These new reagent ions make accessible analytes not previously available to traditional SIFT-MS and increase selectivity for existing analytes. We also propose some biomedical applications that the additional reagent ions may enhance. Reference: P. Spanel and D. Smith (1996). Med. Biol. Eng. Comput. 34, 409.

15.15 **Tea/Coffee Break**

15.45 **High-Throughput Automation of SIFT-MS**

Mark Perkins, Anatune Ltd, Cambridge, UK

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) allows for the direct, real-time monitoring of volatile compounds. Unlike traditional chromatography based techniques, there is no requirement to separate compounds prior to analysis. This leads to a number of benefits. Not only can challenging compounds, such as formaldehyde, ammonia and small sulphur compounds, be analysed without derivatisation or pre-concentration – these analyses can be completed in seconds. By coupling a GERSTEL MPS autosampler to the SIFT-MS, analyses can be entirely automated, fully realising the potential of these fast measurements. A range of automated headspace techniques will be demonstrated, including headspace method optimisation and multiple headspace extraction (MHE) methods. By utilising GERSTEL's Maestro software PrepAhead function, techniques that are cumbersome with chromatography can be turned into practical, economic methodologies, in some cases realising a ten-fold increase in throughput.

16.30 **SIFT-MS for Medical Diagnostics**

Claire Turner, Open University, Milton Keynes, UK

Volatile organic compounds (VOCs) are emitted by all biological systems, including humans, and a change in that system, such as illness, will result in a change in VOCs. Analysing these changes can thus be used to detect disease. SIFT-MS is an ideal technique for monitoring VOCs as it is direct, quantitative and very fast. VOCs can be detected in breath, above urine, faeces, blood and from skin. Analyses of these samples have shown promise in monitoring diabetes, detecting cancer and inflammatory bowel conditions as well as infectious disease. This talk will discuss a range of conditions and sample types and discuss the potential for VOC analysis in replacing existing screening and diagnostic tests. It will highlight issues that need to be solved and yet offer an insight into the extraordinary potential for this approach to monitoring human health.

17.15 **Poster Session**

19.30 **Pre-dinner Drinks in the bar**

20.00 **Dinner**

Wednesday 19th October

09.00 **Concentrations of Specific Volatile Metabolites in Breath as Biomarkers of Disease: concepts, achievements and challenges**

David Smith, Trans Spectra Ltd, UK

It has become common practice in trace gas/breath analysis using mass spectrometry to analyse the exhaled breath/urine headspace samples obtained from cohorts of patients with particular identified disease, e.g. cancer and respiratory infection, for volatile trace compounds and to distinguish the combinations of volatile compounds revealed from those present in the breath of healthy controls. However, it should be understood that recognition of combinations of VOCs in breath only, will not be taken seriously until they are replicated in other laboratories and clinics. Yet it is surely most desirable to properly identify and accurately quantify single metabolites as biomarkers of disease/infection that can be confidently utilized clinically as exemplified by the widely used single breath biomarkers NO (asthma) and H_2 (bowel bacterial overgrowth) and very recently by selected ion flow tube mass spectroscopy (SIFT–MS), including hydrogen cyanide (*Pseudomonas aeruginosa* airways infection), n-pentane (IBD) and acetic acid (mucus pH in CF).

Hopefully, these latter discoveries will provide encouragement to research workers to be more open-minded on this important and desirable issue of single biomarker identification.

09.45 **A Review of the Total Volatiles from the Healthy Human Body**

Norman Ratcliffe, Ben De Lacy Costello and Oliver Gould, University of the West of England, UK

A compendium of all the volatile organic compounds (VOCs) emanating from the human body (the volatilome) was, for the first time reported recently by the author and collaborators. 1840 VOCs have been assigned from breath (872), saliva (359), blood (154), milk (256), skin secretions (532) urine (279), and faeces (381) in apparently healthy individuals and this has information has been updated to include quantitative information. This lecture describes how the compounds have been grouped into tables according to their chemical class or functionality to permit easy comparison. Some clear differences are observed, for instance, a lack of esters in urine with a high number in faeces. A large number of volatiles were reported from skin which is partly due to the methodologies used, e.g. collecting excretions on glass beads and then heating to desorb VOCs. It is the authors' intention that this information will not only be a useful database of VOCs listed in the literature, but will stimulate further study of VOCs from healthy individuals. Establishing a list of volatiles emanating from healthy individuals and increased understanding of VOC metabolic pathways is an important step for differentiating between diseases using VOCs. The role of SIFT-MS versus GCMS and other technologies for aiding this work will be described.

10.30 **Tea/Coffee Break**

11.00 **SIFT-MS – Future Role in Clinical Practice**

Sheraz Markar, Dept. of Cancer and Surgery, Imperial College London, UK

SIFT-MS permits real-time online analysis that facilitates rapid potentially point-of-care medical testing. SIFT-MS may also be used in an offline fashion with the advantage of rapid diagnostics and high throughput analysis allowing large-scale clinical trials. Despite these advantages clinical application of SIFT-MS remains limited with large scale investment of time and resources required to facilitate integration into clinical practice. In this discussion we will present the current and future work being undertaken at Imperial College London. Including large-scale multi-centre clinical validation studies, cross platform validation to confirm VOC identity and most importantly thorough mechanistic investigation to enhance understanding of the origin of VOC production in cancer states.

11.45 **Open Discussion – the way forward for SIFT-MS**
led by Patrik Spanel, J. Heyrovsky Institute, Prague, Czech Republic and Daniel Milligan, Syft Technologies Ltd, Christchurch, New Zealand

12.30 **Lunch and Close**