Institution: University of Birmingham		
Unit of Assessment: UoA 9, Physics		
Title of case study: Translation of Chemical Ionisation Mass Spectrometric Research to Health Care and Commercial Analytical Devices		
Period when the underpinning research was undertaken: 2003–2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Prof. Chris A. Mayhew	Head of Molecular Physics	01/10/1989–present
Dr Margaret O'Hara	Daphne Jackson Fellow	01/11/2012–30/04/2015
Dr Raquel Fernández del Río	EU Early Stage Researcher	01/03/2013–29/02/2016
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Dr David Olivenza Leon	EU Early Stage Researcher	01/07/2016–31/12/2019
Period when the claimed impact occurred: 2017–present		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Our impact in the healthcare, security, and environmental sectors is the result of breakthroughs in medical diagnosis and monitoring, and analytical technology development. We facilitated **new strategic directions and investment** at international companies. Using non-invasive and real-time breath sampling, those companies are commercialising previously impossible tests for precise monitoring of propofol (an intravenous anaesthetic) during surgery, and for detecting and monitoring liver disease. We **enabled the development of new medical diagnostic and monitoring products**, making their commercial adoption possible. Finally, we are driving the further **development and improvement of PTR-MS technology** in key international companies **for improved chemical specificity** of benefit to many areas of analytical chemistry for which real-time analysis of trace chemical compounds is required.

2. Underpinning research

Our research focuses on: (i) enabling Proton Transfer Reaction Mass Spectrometry (PTR-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) as analytical discovery tools in the health sector for determining breath volatile biomarkers of use in the clinical environment; and (ii) the technological development of PTR-MS for improved chemical selectivity, which has widespread applications for commercialisation, including specifically in the security and environmental sectors.

A. Biomarker Discovery Research – The Health Sector and Clinical Programmes

1. *Investigating breath volatile analysis for detecting liver disease and determining liver function*

Unlike an invasive liver biopsy, which samples only a small fraction of the liver and hence is prone to sampling errors, we have established that the concentrations of specific volatile trace levels in exhaled breath act as biomarkers that provide a measure of the health of the whole liver. Our research activities between 2010 and 2016 have led to the reality of non-invasive clinical tests for detecting liver disease, for monitoring liver disease drug treatments, and for determining liver function. These tests can be used to screen at-risk populations and to test the effectiveness of recently approved Food and Drugs Administration (USA) drugs for treating early-stage liver disease.

KF1: Unequivocal discovery of volatile breath biomarkers associated with liver disease

Comparing breath profiles of healthy and sick people, which is standard practice in the breath research community, is insufficient to provide complete confidence in a biomarker or set of biomarkers for use in the diagnosis and/or monitoring of disease. In our clinical studies, we took a different approach. By uniquely monitoring patients with **liver disease before and after liver transplant surgery**, we have **unequivocally shown that three volatiles in exhaled breath act as unique biomarkers for liver disease**, with one of them, limonene, which is an exogenous compound, being the most diagnostically useful [1].

KF2: Longitudinal studies of patients following liver transplant demonstrated the use of a pharmacokinetic non-invasive breath volatile test to determine liver function.

Following liver transplant, a number of patients were followed longitudinally. Control, pre-transplant, and post-transplant breath volatile concentrations were compared, with limonene showing unique washout characteristics for post-transplant patients. This demonstrated for the first time that graft liver function can be non-invasively assessed and supports the hypothesis that limonene accumulates in body fat as a consequence of a diseased liver failing to metabolise dietary limonene [2].

2. *First Investigations for real-time monitoring of the intravenous anaesthetic propofol in breath during surgery*

Propofol is the most commonly used intravenous anaesthetic for surgical procedures. However, it is not possible to directly measure blood concentrations in real-time and thereby assess how much propofol is enough to achieve adequate anaesthesia. Anaesthetists simply estimate a dose according to the patient’s body mass index, with the risk that they might inject too much or too little.

KF3: Trace levels of propofol detected in exhaled breath during surgery using PTR-MS

In 2002–2003, our clinical study involving the University Hospital Birmingham demonstrated that propofol is present as a biomarker in exhaled breath in trace concentrations during surgery and hence can be monitored in an analogous manner to that for volatile anaesthetic agents to indirectly determine blood concentrations in real-time, which was not previously possible.

B. Technological Development – Improving the Chemical Specificity of PTR-MS

In collaboration with industrial partners, our PTR-MS research between 2009 and 2020 pioneered and demonstrated novel methods for improved chemical identification of trace compounds in complex chemical environments, without the need for pre-separation (GC) techniques [3–5]. Instead of requiring expensive changes to the design of the PTR-MS equipment to improve analytical performance, we showed that chemical specificity can be enhanced by modifying the operational conditions within the drift (reaction) tube to drive specific ion-molecule reaction pathways for the production of explicit product ions for given trace chemical compounds.

KF4: Selective Electric Field Switching – a new analytical technique

Switching the voltage across the drift (reaction) tube provokes different ion-molecule reaction pathways to produce changes in product ions for a given compound in order to distinguish isobaric and isomeric compounds [3, 4]. We call this new field of analytical science ‘selective electric field ion chemistry’. In collaboration with Kore Technology Ltd, UK, we developed rapid switching technologies (~10 Hz) so that compounds present for only short periods of time, e.g. explosives or drugs, can be quickly interrogated to provide high confidence in detection. This considerably reduces false positives, which often occur in less selective analytical systems commonly used in security areas, such as airports. We have demonstrated that improvements in selectivity are further enhanced by using a radio frequency ion-funnel [5]. We showed that the combined rapid switching of RF and DC modes of operation within the drift tube provides optimal and rapid chemical selectivity.

3. References to the research

1. Raquel Fernández del Río *et al*. "Volatile Biomarkers in Breath Associated with Liver Cirrhosis - Comparisons of Pre- and Post-liver Transplant Breath Samples", *EBioMedicine* 2 (2015), 1243−1250 (with the University Hospital Birmingham NHS Trust). DOI: 10.1016/j.ebiom.2015.07.027.
2. George Harrison *et al*. "Real-Time Breath Monitoring of Propofol and its Volatile Metabolites during Surgery using a Novel Mass Spectrometric Technique: a feasibility study", *British Journal of Anaesthesia* 97 (2003), 797−800 (with the University Hospital Birmingham NHS Trust). DOI: 10.1093/bja/aeg271.
3. Philipp Sulzer *et al*. "Proton Transfer Reaction Mass Spectrometry and the unambiguous real-time detection of 2,4,6 TNT", *Analytical Chemistry* 84 (2012), 4161−4166 (with IONICON Analytik GmbH). DOI: 10.1021/ac3004456.
4. Ramón González-Méndez *et al*. "Use of Rapid Reduced Electric Field Switching to Enhance Compound Specificity for Proton Transfer Reaction-Mass Spectrometry", *Analytical Chemistry* 90 (2018), 5664−5670 (with Kore Technology and Dstl). DOI: 10.1021/acs.analchem.7b05211.
5. Ramón González-Méndez *et al*. "Enhancement of compound selectivity using a radio frequency ion-funnel proton transfer reaction mass spectrometer: improved specificity for explosive compounds", *Analytical Chemistry* 88 (2016), 10624−10630 (with Kore Technology and Dstl). DOI: 10.1021/acs.analchem.6b02982.

4. Details of the impact

We have had international impact in the healthcare, security, and environmental sectors, influencing technological developments in companies (IONICON Analytical GmbH (Austria) and Kore Technology Ltd (UK)) and the adoption of breath biomarkers in the health sector (B. Braun Melsungen AG (Germany, propofol) and Owlstone Medical Ltd (UK, limonene as a liver disease biomarker)). Specifically, we have stimulated: 1) **strategic change and investment** to employ breath volatiles as non-invasive biomarkers; 2) **innovation and the development of new technologies**; and 3) the subsequent **commercial adoption of these new technologies** for environmental and security monitoring.

1) Changing company strategy and investment through the development of non-invasive tests for liver disease and liver function – Owlstone Medical Ltd

Owlstone Medical's strategic change in direction was a direct result of our demonstration at an international conference [KF1 and KF2] that **breath biomarkers are ideal for use in the diagnosis and monitoring of liver disease**. Owlstone Medical is a Cambridge-based medical device company, whose stated vision is to "save 100,000 lives and $1.5B in healthcare costs" [E1]. We contributed to a **new commercial opportunity by materially influencing the development of breath tests** to detect liver disease and for determining liver function (e.g. following drug treatments and/or liver transplant [E2–E6]).

As highlighted by the company's CEO, our work **improved on Owlstone Medical's commercial viability and competitiveness**: it has "provided clear evidence of the importance of a limonene breath test and has been **a driving force behind our current investment and development**" [E2]. In a webinar, the CEO states that our research "really changed [his] view of what we can do in breath" and that "exogenous volatile compounds no longer should be considered as noise" [E3, E4]. The use of these exogenous breath biomarkers specifically for liver disease has been confirmed in a collaborative peer-reviewed clinical study with Owlstone and the University of Cambridge [E5] and further described in a webinar [E6].

KF1 and KF2 thus "inspired [Owlstone] to take the UoB research forward to **develop a breath test for limonene**" in the following clinical applications: a **national screening programme** for the detection of early-stage liver disease; a **programme for determining liver function** and monitoring after treatment; and **a clinical method to determine the efficacy of drugs** employed in treating non-alcoholic steatohepatitis and non-alcoholic fatty liver disease [E2 and E3].

2) Impacting the development of new medical diagnostic and monitoring products and commercial adoption of a new concept: Real-time monitoring of propofol during surgery

We directly enabled **innovation and entrepreneurial activity** through the **design and delivery of a new commercial device** at the German company B&S Analytik. Our discovery [KF3] that trace quantities of propofol and associated metabolites are present in the breath of patients undergoing surgery has been described as "a seminal investigation" providing "proof of concept of reality to the conjecture of pulmonary propofol elimination and its measurement" [E7]. B&S Analytik has **developed a low-cost Ion Mobility Spectrometer** (IMS) to monitor breath propofol levels during surgery based on ion-molecule processes similar to those used in our original PTR-MS study. Founded by Professor Jörg Baumbach, the company first developed the IMS which is now being marketed by B. Braun Melsungen AG under the name EDMON (Exhaled Drug Monitor) [E8], with a worldwide sales forecast of 2,000 instruments per year [E9].

Our contribution has been explicitly recognised by the founder of B&S Analytik, who stated that our work "was ultimately **the scientific door opener** showing the possibility to detect propofol using mass spectrometry technology in exhale **which finally led to the independent development of a commercial CE marked clinical instrument** based on IMS technology" [E9].

3) Technological developments improving the chemical accuracy of PTR-MS products – adopted for environmental and security real-time monitoring through collaboration with industry and Dstl

Our impact on the **development and improvement of existing PTR-MS technology** as a result of KF4 has arisen from direct collaboration with industry and the **commercial adoption of PTR-MS** [E10–E12]. PTR-MS has been adopted as a major analytical tool for the real-time detection of compounds in trace quantities (less than a ppbv) in many fields of application, ranging from atmospheric chemistry through to homeland security. The advantages of real-time analysis come with a cost in chemical selectivity. In the absence of pre-separation of compounds, as used in GC-MS, PTR-MS cannot easily distinguish between different but similar substances, leading to 'false positive' readings that are problematic in many applications. KF4 provided additional analytical capabilities for improved chemical selectivity to existing and new PTR-MS instruments, making them analytically more multidimensional whilst retaining their real-time analysis capabilities. This enabled the following developments.

The **reduction of false positives** is evidenced by two users, Dstl Fort Halstead and Dstl Porton Down, who **have validated the capability of reducing false positives for explosive detection** which are common in homeland security technologies, such as IMS. This improvement to the real-time (seconds) analytical accuracy (chemical specificity) of PTR-MS, was achieved by manipulating conditions within the reaction tube [KF4], allowing retrofit and thus avoiding expensive changes to older equipment. **Two of the three global manufacturers of PTR-MS**, Kore Technology and IONICON Analytik, adopted this improvement leading to **product development and improvement**. This is confirmed by Kore's Project Manager and Senior Researcher who stated that "Chris Mayhew and his group have had an active role in affecting the content and effectiveness of different aspects of our PTR-TOF-MS instruments" [E10], have changed the company's "fundamental understanding" of ion-molecule reactions for PTR-MS, and led to the testing and developing of new hardware and software [E10]. As a result, "every commercial PTR instrument produced by Kore now has an ion funnel, [this …] has been vital in offering us analytical flexibility to our customers" [E10; KF4].

We have also contributed to Kore's **strategic direction**. The transfer of **two highly skilled people into specialist roles that draw on their research** has enabled take up by environmental agencies. For example, in 2019, Kore Technology built the first of seven compact PTR-MS instruments for use in China, with applications of the new technology in environmental monitoring in the more than thirty Chinese provinces [E10; KF4].

Finally, since 2019, IONICON Analytik GmbH (Austria) have re-developed their instruments to include rapid electric switching, making IONICON more competitive [E11; KF4]. This is confirmed by the company which states that our work led to “a step change in PTR-MS instrumentation, helping in IONICON’s commercial growth” and that it has “significantly contributed to IONICONs innovation and entrepreneurial activities” [E11]. Evidence of strong collaborative R&D programmes between us and IONICON Analytik GmbH and Kore Technology Ltd is provided in numerous peer-reviewed publications [E12].

5. Sources to corroborate the impact

(A) Health sector

E1. Owlstone Medical, Breath Biopsy Tests [accessed 2/2/2021].

E2. Testimony from the CEO of Owlstone Medical Ltd.

E3. Webinar, “Targeted breath analysis: exogenous volatile organic compounds (EVOC) as targeted metabolic probes in Breath Biopsy,” Billy Boyle (CEO of Owlstone Medical Ltd) [accessed 15/2/2021].

E4. Gaude *et al*. “Targeted breath analysis: exogenous volatile organic compounds (EVOC) as metabolic pathway-specific probes”, *Journal of Breath Research* 13 (2019), 032001. DOI:10.1088/1752-7163/ab1789.

E5. Giuseppe Ferrandino *et al*. “Breath Biopsy assessment of liver disease using an exogenous volatile organic compound - towards improved detection of liver impairment”, *Clinical and Translational Gastroenterology* 11(9) (2020), e00239. DOI: 10.14309/ctg.0000000000000239. [Clinical trial led by Owlstone Medical Ltd].

E6. Webinar, “Measuring Exhaled Limonene in Cirrhosis Patients” [accessed 16/11/2020].

E7. Dr E. D. Kharasch, “Every Breath You Take, We’ll Be Watching You”, Editorial, *Anesthesiology* 106(4) (2007), 652−654.

E8. B Braun, EDMON [accessed 16/11/2020].

E9. Testimony from the founder and former CEO of B&S Analytik GmbH (original development company) (dated 17/11/2020).

(B) PTR-MS Instrumental Development for Improved Selectivity

E10. Testimony from the Project Manager and Senior Researcher at Kore Technology Ltd (dated 4/1/2019).

E11. Testimony from the Science Manager at IONICON’s (dated 7/5/2019).

E12. List of collaborative research papers with Kore Technology Ltd. and IONICON Analytik GmbH.