SCOTTISH METABOLOMICS NETWORK

Scottish Metabolomics Network Newsletter

11th Aug 2020

IN THIS ISSUE:

Twitter updates: #ScotMetNet

Office

The offices of chair and secretary of the network require to be filled. Nominations are invited with a decision to be reached imminently. Send your nominations via the form at https://forms.gle/qkKzEeXX41y68kvTA

Communications

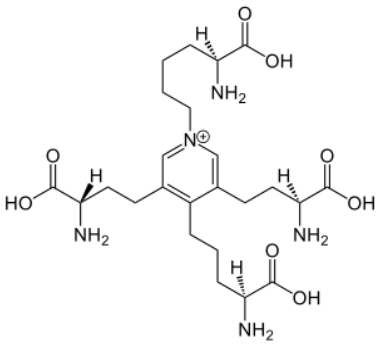
A lot has happened since the last Newsletter, not least because the last newsletter was a whole year ago. Apologies for this.

We have new mailing lists. The board can be reached at SMN-BOARD@JISCMAIL.AC.UK, and an announcements list has been set up: SCOTMETNET-ALL@JISCMAIL.AC.UK. Send your email to LISTSERV@JISCMAIL.AC.UK, with the command "sub scotmetnet-all" in the email. The list is moderated, and your default delivery will be a weekly digest.

Finally, there is SCOTMETNET-EDI@JISCMAIL.AC.UK … more on this later.

A metabolite biomarker from elastin predicts long-term cardiovascular and all-cause mortality in bronchiectasis

Jeffrey Huang, University of Dundee



In collaboration with a clinical team led by Professor Chalmers, Dr Huang 's lab at University of Dundee have demonstrated that a blood metabolite – desmosine, which is a degradation product from the crosslink moiety of elastin, predicts long-term all-cause and cardiovascular mortality in a large cohort of patients with bronchiectasis.

Bronchiectasis is a long-term respiratory condition where the airways of the lungs become abnormally widened as a result of a vicious cycle of transmural infection and inflammation. Around 210,000 people in the UK were living with this condition. Globally it affects 3 million people. Symptoms of bronchiectasis include chronic productive cough, wheeze, and dyspnoea. Patients with bronchiectasis have an increased risk of mortality compared to the general population and a high proportion of this mortality is related to cardiovascular disease. Currently no markers studied to date can identify patients at high cardiovascular risk.

The study investigated 433 patients with computed tomography–confirmed bronchiectasis enrolled in the TAYBRIDGE bronchiectasis registry and patients were followed for ~5 years. The team found that circulating desmosine levels are predictors for all-cause and cardiovascular mortality even after correcting the disease severity score, which is a composite score from key clinical features. Importantly, the authors found that the biomarker provides complementary information to the clinical based disease severity score currently used in clinics.

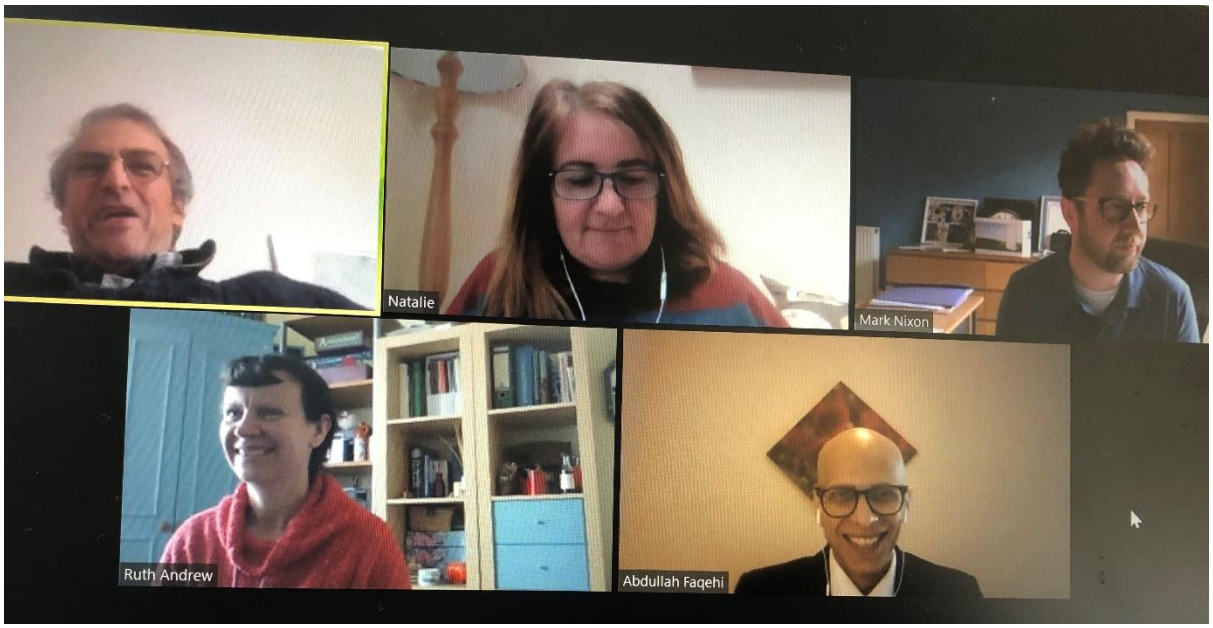
This study also provides further evidence that elastin degradation is a plausible link between airway inflammation and cardiovascular risk in bronchiectasis. Similar results were found in chronic obstructive pulmonary disease in 2016 by Huang's laboratory. These results support their hypothesis that circulating desmosine, which represents systemic elastic degradation and vascular aging, is a predictor of future mortality and particularly cardiovascular mortality.

The authors are currently validating the results in a larger European study. When validated, this marker would become the first biomarker for assessing the cardiovascular risk in bronchiectasis.

This paper is published in American Journal of Respiratory Medicine and Critical Care and is available online (https://www.atsjournals.org/doi/abs/10.1164/rccm.202002-0434LE).

Using Mass Spectrometry to profile sex steroids in metabolic diseases

Natalie Homer, QMRI Mass Spec Core



Dave Watson, Dr Mark Nixon, Ruth Andrew and Natalie Homer, Abdullah Mossa M Faqehi.

Abdullah Mossa M Faqehi has passed his viva Fri 10th July. He was supervised by Ruth Andrew and I in CVS and the Mass Spec Core, examined by Dave Watson and Mark Nixon. 'Using Mass Spectrometry to profile sex steroids in metabolic diseases'.

Congratulations!

Scottish Metabolomics Network Symposium 2019

Thanks to Dave and Nik for organising the event. (link to programme https://static1.squarespace.com/static/57df9bff46c3c466ad42bb3c/t/5dc46280c6a13f49b7b47657/1573151363070/SMN+2019+Final+Programme.pdf)

WEDNESDAY WORKSHOP

Gavin Blackburn opened the workshop with a great presentation about quality control and its importance to data processing, including the different types of replicates and their impact on statistical power, groups and controls, comprehensive metadata and advice to balance factors against biological questions, and always seek advice in this multidisciplinary area.

Nik Rattray then introduced metabolomics and free online-software MetaboAnalyst, also touching on formality, missing values, scale, networks and teased us with the possibility of Deep Learning as the future of data analysis. Nik paints a picture with words, and the whole event featured regular breaks in presentation for other information, allowing anyone falling behind for technical reasons to catch up, and modelling good multimedia learning.

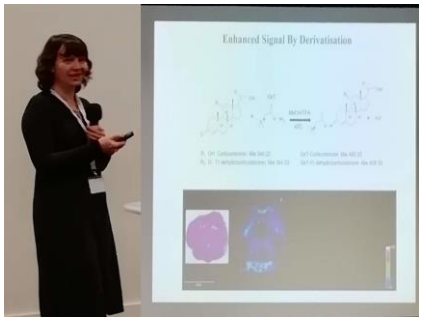
Imputation, KNN, BPCA, PPCA, normalisation, PQN, log-transform, and clustering were all mentioned. And following the questions, Gav admitted he'd "never seen a biological dataset that wasn't log-normally distributed".

Tim Ebbels kicked of the next part of the day with a revision of statistical tests, multiple testing corrections and some more MetaboAnalyst. We learned how to load, filter, impute, normalise, transform and scale data in MetaboAnalyst, did multivariate analysis using PCA, PLS-DA and O-PLS-DA, and importantly Tim gave us a visual, mechanical understanding of how these approaches work. He warned of the overfitting "Danger Zone" and how to use and interpret the validation tools in the online software.

SMN2019

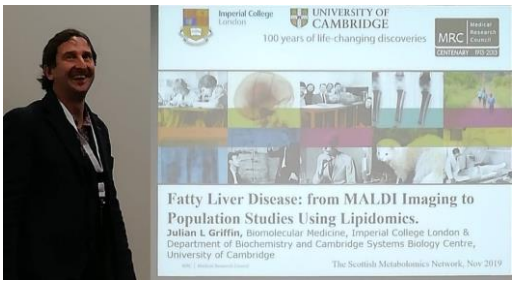
Dr Tim Ebbels finished the day with the Symposium's opening keynote, asking the question 'Computational Metabolomics: The Key to the Future?' He highlighted the importance and outlook of computational metabolomics, metabolite annotation and data integration as data complexity and availability increases.

Karl introduced Day 2. Andrew Finch and Karl Burgess are stepping down from the SMN board and we call for nominations to the positions of President and Secretary. Ruth Andrew will stay on as Treasurer.



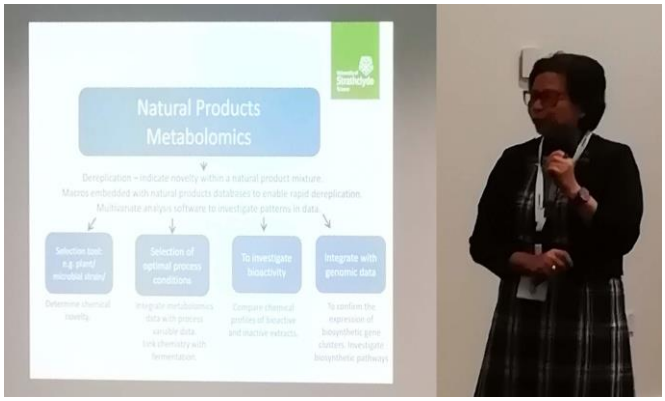
Dave, retiring this year, then chaired the first session, which kicked off with his previous PhD student Ruth Andrew's beautifully layered roundup of recent advances in her lab, including (but not limited to) Natalie Homer's multi-sterol assays, mass-spec imaging and understanding receptor action. It was a great start to a great meeting. I'm not going to detail every talk, as you can reference the program for that. Suffice to say that were all equally engaging and valuable, including the sponsor talks.

Julian Griffin closed the second day showcasing large-scale high-throughput metabolomics technologies that allow population-level dataset generation, whose appropriate analysis ultimately link de novo lipogenesis to insulin resistance in fatty liver disease.



The programme continued to deliver on schedule, with the exceptions that Rebecca Goss presented in place of Hannah Lawther (you can follow the @GossGroup on Twitter) and Sufyan Pandor represented Agilent in place of Hannah Florence.

Centrally located and with stunning views, the Technology and Innovation Centre, University of Strathclyde was a nice choice. But, I'll also remind everyone of RuAngelie Edrada Ebel, of the host institution, being thrice rudely interrupted by the fire alarm test automated announcements:



"NO ACTION IS REQUIRED"

– dealt with very professionally"

From blackcurrants to imprecision, from UHI to Imperial, from junior to senior, the talks and posters had the diversity and breadth of appeal that we have all come to anticipate and relish in our annual meeting.

Finally, Roy Goodacre took us on a journey through significant advances in large-scale clinical phenotyping, in his plenary lecture, ranging from the first large-scale serum metabolomics in the Husermet Project to the frailty biomarkers discovered in fRaill.

Before we all went home, the presentations honoured:

- Dave Watson: retiring
- Andy Finch: relocating
- Elena Conti: best talk
- Gio Rodriguez-Blanco: 2nd best talk
- Ioannis Stasinopoulos: Poster prize (judged)
- Thomas Grove: Poster prize (people's choice)

Andrew, Burgess, Finch, Watson, Conti, Rattray

Andrew, Burgess, Finch, Rodriguez-Blanco, Watson, Rattray

Andrew, Burgess, Finch, Stasinopoulos, Watson, Rattray

Andrew, Burgess, Finch, Grove, Watson, Rattray

Scottish Metabolomics Network Anti-Racism Commitment

The following statement was circulated across SMN PIs on June 4th, with a majority response and unanimous approval; the final version was issued (in 4 tweets) on June 9th, 2020. The SMN Twitter profile has included the #BlackLivesMatter hashtag since 4th June…

> The Scottish #Metabolomics Network enjoys the mandated human rights of education and scientific progress. The value of what we do lies in its humanitarian and cultural contribution, so it will be no surprise that we are also the kind of people who adopt an #AntiRacist position.
>
> Like everyone, we are sickened by the killing of Mr. George Floyd and many others by an unequal system of justice. This is not just a political issue, but one of human rights. While many consider Britain a more progressive country, #racism remains a problem right here in the UK.
>
> #ScotMetNet is a diverse network, in terms of our science, the technologies we use, and the people we are. Hopefully, it is obvious that we value #DiversityInSTEM. But valuing the things that make us all unique is clearly not enough. We must all do something to #FightRacism.
>
> #ScotMetNet aims to develop a formal commitment to anti-racism, first starting a network-wide equality, diversity and inclusivity group who can further develop our strategy. Stay tuned for more news on this. We'll do our best not to be part of the problem! #blacklivesmatter

Since then, the promised EDI group has been formed and met for the first time on 8th July. We agreed to meet roughly quarterly, to develop strategies that encourage inclusivity within our network, be good example to other communities, and to fight racism at large, which is yet a problem not acknowledged by many of our peers in Scotland.

If you have something to say on this matter, would like to join the EDI group and make a difference, or need support, you can confidentially contact us on SCOTMETNET-EDI@JISCMAIL.AC.UK

Book of the quarter: *Black and British: A Forgotten History* by David Olusoga – available from many libraries, online bookshops, and as an audiobook. Read more about this book: https://www.goodreads.com/book/show/32809816-black-and-british

Scottish Metabolomics Network Papers

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Industry news

SPECTRALWORKS DART-MS DISTRIBUTION IN THE UK AND IRELAND

Mass Spectrometry software company SpectralWorks Limited has been working with IonSense in the US to develop novel data processing solutions for data acquired using their Direct Analysis in Real Time (DART) ionisation sources. The DART ionisation source is a vendor neutral add-on to existing or new MS instruments and matches SpectralWorks' own vendor neutral software approach to MS data processing. It was a logical step for SpectralWorks to become the distributor for these products in the UK and Ireland.

A short application note on the use of the DART system and AnalyzerPro® XD processing software is shown.

RAPID DART-MS ANALYSIS OF METHADONE, CODEINE AND FENTANYL IN URINE. INTRODUCTION

In the late 1990s, pharmaceutical companies reassured the medical community that patients would not become addicted to prescription opioid pain relievers, and healthcare providers began to prescribe them at greater rates. This subsequently led to widespread diversion and misuse of these medications before it became clear that these medications could indeed be highly addictive[1,2].

In 2017 the US Department of Health and Human Services (HSS) declared a public health emergency to combat *the US Opioid Crisis*[3].

2018 data shows that every day, 128 people in the United States die after overdosing on opioids[4]. Clearly, the misuse of and addiction to opioids, including prescription pain relievers, and synthetic opioids such as Methadone and Fentanyl, affects public health as well as social and economic welfare.

A rapid and selective method for Methadone, Codeine and Fentanyl was developed using Direct Analysis in Real Time – Mass Spectrometry (DART-MS) to allow high through put analysis.

Method

The DART ionization source can be interfaced to a number of different mass spectrometers. A high resolution ThermoFisher Orbitrap was used for the Methadone analysis and a Waters QDa (single quadrupole, nominal mass resolution) for the Codeine and Fentanyl analysis. Deuterated standards of each of the target species were spiked into the urine specimens. The samples were then deposited on a sampling mesh substrate for analysis and the resulting data files were imported to AnalyzerPro XD for data processing.

Data Processing

1. A data file containing 96 samples was loaded to the software.

2. From the total ion chronogram (TIC) the starting and end points for splitting the data file were designated by the user. The software's automatic peak detection identified all peaks between those two points (Figure 1.) and parsed them into individual data files.

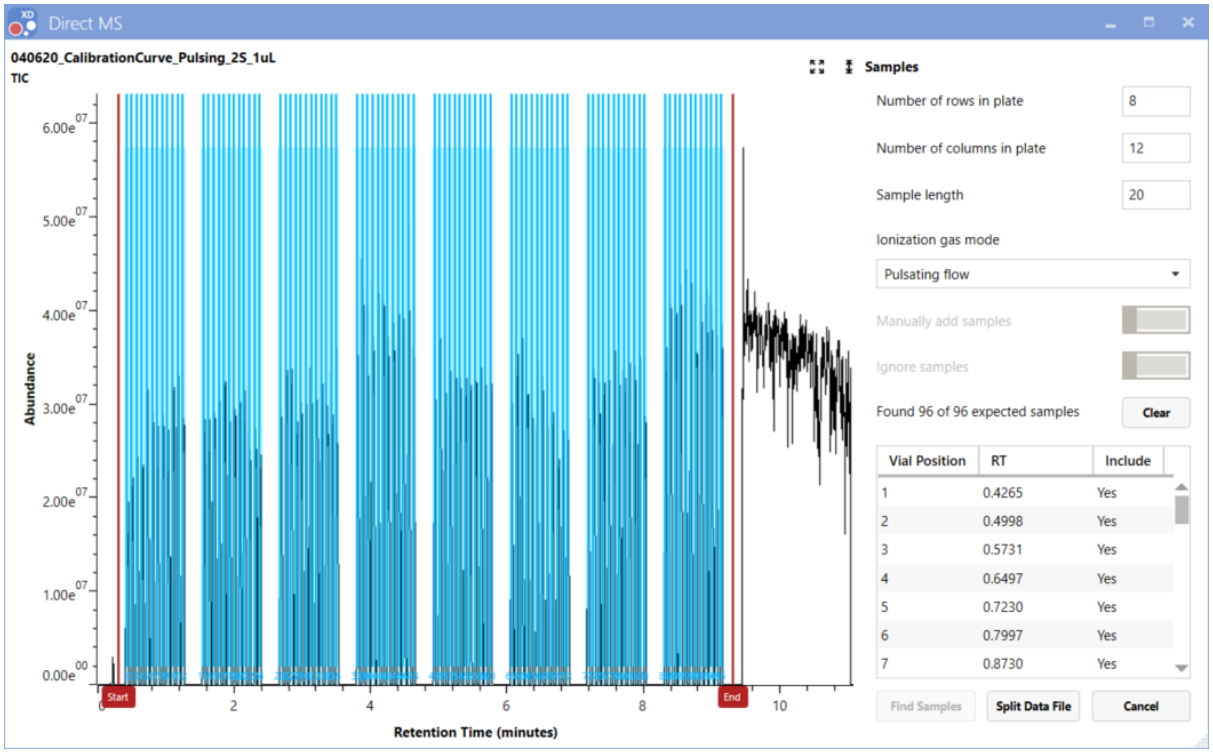

Figure 1. Automatic Peak Detection and Sample File Splitting.

3. Each individual data file was automatically integrated and the relevant averaged mass spectrum for each sample are provided.

4. Specific masses for the native and deuterated were selected and calibration curves generated using standard amounts of native and deuterated compounds.

Results

As an example, the calibration curve for Methadone using Methadone-D3 as an internal standard is shown in Figure 2.

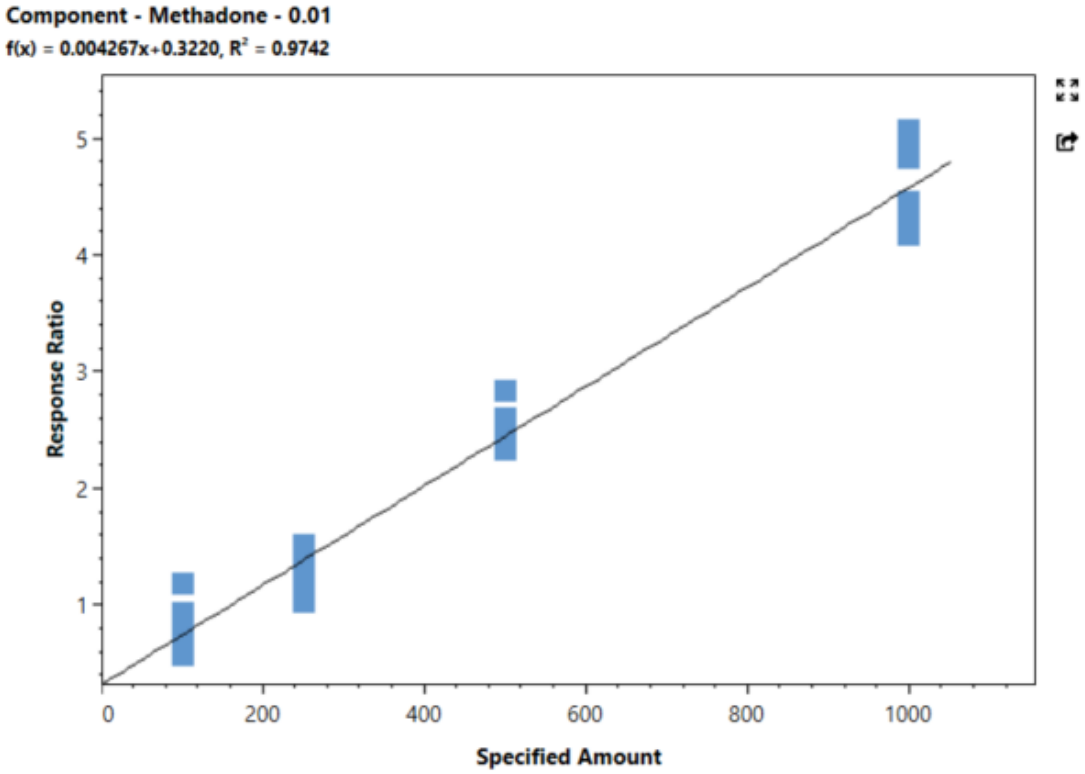

Figure 2. Methadone Calibration Curve. (100 to 1000ppb).

The calibration curve can be used to generate the calculated amounts from unknown samples as shown in Figure 3.

Calibration Component Results - Methadone - 0.01

	Vial Position	Sample Type	Response	IStd Response	Response Ratio	Calculated Amount	Units
32	C:8	Unknown	59190.6	83299.3	0.7106	88.2787	ppb
30	C:6	Unknown	53827.9	82435.2	0.6530	74.7376	ppb
29	C:5	Unknown	0.0	45436.8	0.0000	0.0000	ppb
10	A:10	Unknown	84495.5	71350.4	1.1842	199.6198	ppb

Figure 3. Calculated amounts of Methadone Samples.

Plate View

A unique representation of the data is available in the Plate View. The Plate View can display specific Quantitation Components or specific masses. Relative or absolute responses can be displayed, and the parameters set to provide a variation of colour or a simple red light / green light display of the sample plate (Figure 4).

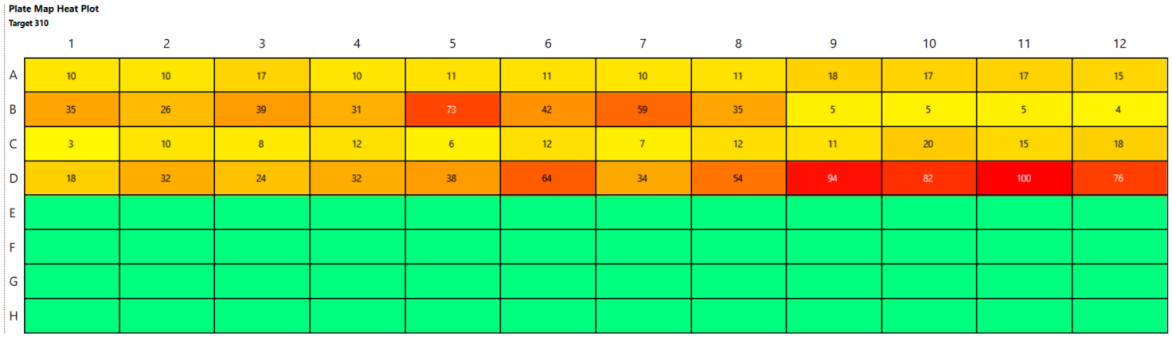

Figure 4. Plate Heat Map of Methadone Standards and Sample.

Principal Component Analysis

Statistical analysis using PCA can be used to determine the specific features that are differentiating between samples. In this case the features are the individual ions in each sample and the software allows direct interaction between the PCA plots and the underlying mass spectra (Figure 5). This allows the ions of interest to be easily identified from the Loadings plot and the specific samples to be visualised in the Scores plot.

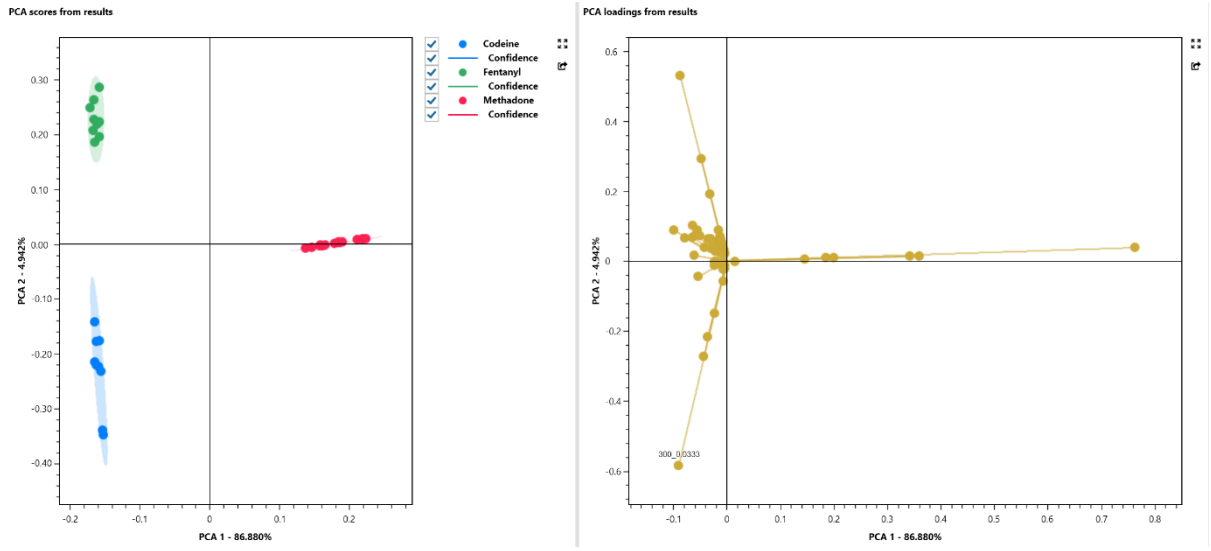

Figure 5. PCA Scores and Loadings Plots from Feature Analysis.

Conclusion

DART-MS combines the speed of the Direct Analysis in Real Time with the specificity of low or high resolution mass spectrometry. Fast qualitative and quantitative results from large sample batches can be quickly produced.

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Acknowledgements

PHOTOGRAPHS:

1. Symposium photos by Jimi Wills
2. Abdullah's viva, photo courtesy of Natalie Homer

Thanks to everyone for your contributions. Thanks to Nik for helping write up the symposium. Any corrections or last-minute updates for the web version, let me know jimi.wills@ed.ac.uk.