SHIMADZU
Excellence in Science

PO-CON1888E

A metabolomics study into influenza virus infection by HRAM Q-TOF analysis

ASMS 2019

Emily Armitage[1]; Jonathan Swann[2]; Mick Bailey[3]; Ian D Wilson[2]; Neil J Loftus[1]
[1]Shimadzu Corporation, Manchester, UK;
[2]Imperial College London, Department of Surgery and Cancer, United Kingdom;
[3]School of Veterinary Sciences, University of Bristol, Bristol, United Kingdom

A metabolomics study into influenza virus infection by HRAM Q-TOF analysis

Overview

- Influenza A viruses (IAVs) infect a variety of hosts, including humans, swine, and various avian species, however, the pathogenesis and transmission of influenza viruses in humans remains unclear.
- Untargeted metabolomics using a HRAM Q-TOF analysis with MS and DIA-MS/MS data acquired with a cycle time of less than 1 second has been applied to compare serum metabolic profiles from swine over a 14 day time-course to study the progression of influenza infection.

Introduction

Animal models help to understand mechanisms of virulence and to develop more efficacious vaccines and forms of prevention or treatment. Influenza virus infection in humans has a number of similarities with that in swine as the clinical manifestation and pathogenesis are similar. In this untargeted metabolomics study, HRAM Q-TOF analysis was used to measure the effect of influenza virus infection on host-microbial metabolism in swine and whether this differs between the early, innate response, the later adaptive response and the repair phase. Blood samples taken pre-infection and over 13 days post infection (dpi) were compared.

Materials and Methods

An untargeted metabolomics approach was applied to analyze serum extracts from swine at different stages of infection with influenza A. Pigs were acclimatized for 7 days prior to infection and pre-infection blood samples were taken from 8 pigs. Infection was via intranasal administration of H1N1pdm09 or MDCK supernatant using a mucosal atomization device. Blood samples were taken from 4 pigs at 1, 2, 3, 4, 5, 6, 7 and 9 dpi and from 8 pigs at 11 and 13 dpi. Infection was confirmed by identification of viral shedding by daily nasal swabbing and by lung pathology identified postmortem. Metabolic extracts of sera were analyzed using a HRAM Q-TOF (LCMS-9030 Shimadzu Corporation) in untargeted mode. Samples were analyzed in random order and a pooled Quality Control (QC) sample was injected several times at the start and systematically throughout the batch. A representative sample from each group was used for component detection using the Find algorithm within Insight Explore software (Shimadzu Corporation) and the generated compound table was applied to process data from all samples.

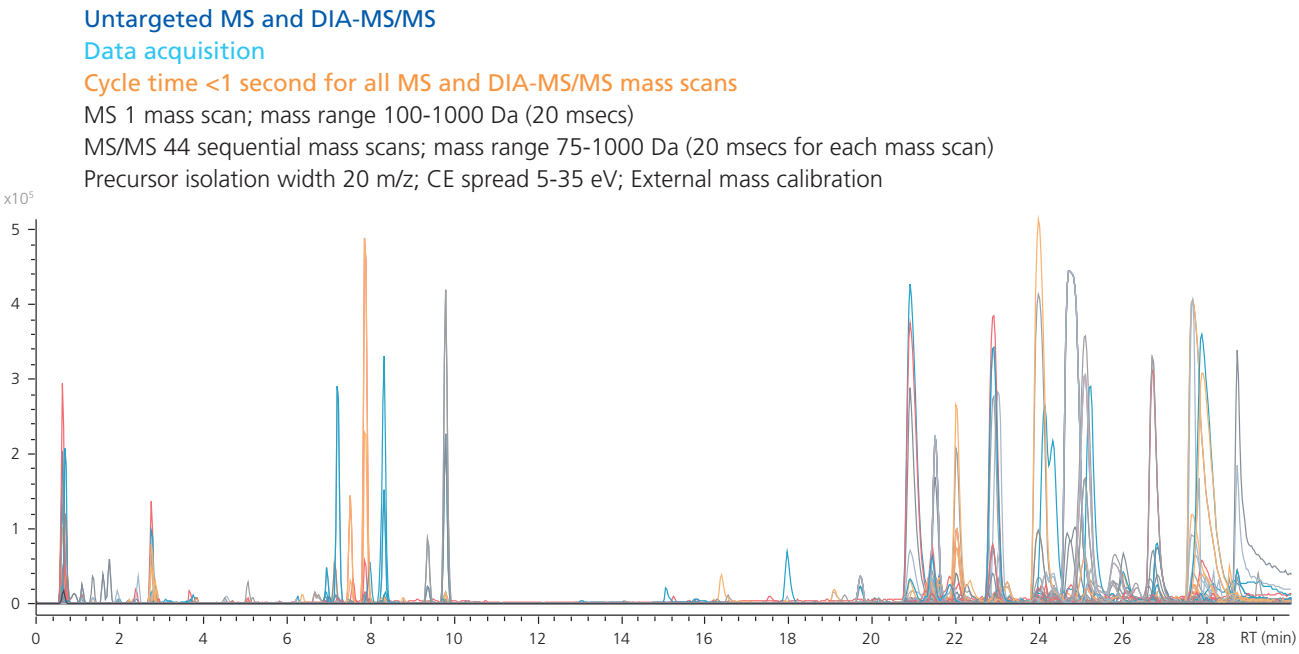


Figure 1. HRAM mass chromatograms of 716 components detected in one sample (5ppm extraction window).

Results

Component detection in all samples

A robust workflow has been devised for the processing of HRAM Q-TOF metabolomics data and applied to study the effect of influenza infection. Figure 2 shows the process of component detection in all samples to generate peak area matrix for data analysis. The FIND algorithm was applied to detect components in a subset of samples (Step 1) which was used to build the compound table to process all samples (Step 2). Following manual review (Step 3) a total of 716 reliable components (ion signals) were considered for data analysis.

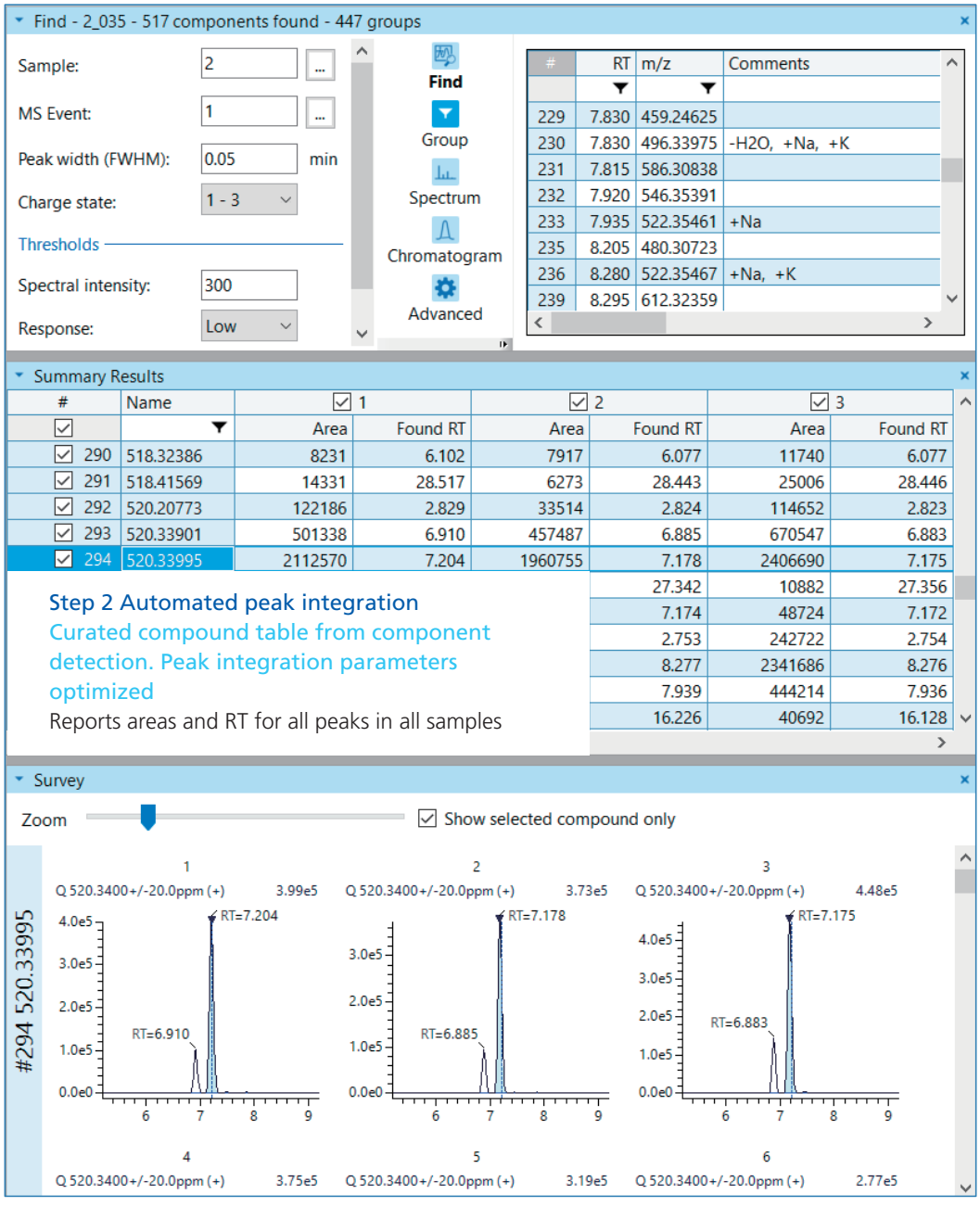
A metabolomics study into influenza virus infection by HRAM Q-TOF analysis

Step 1 Find algorithm to detect components

MS1 event for any sample

Typical peak width indicated, charge state and spectral intensity defined

Generates a list of all detected peaks with adducts and neutral losses grouped



Step 2 Automated peak integration

Curated compound table from component detection. Peak integration parameters optimized

Reports areas and RT for all peaks in all samples

Step 3 Survey mode to review peaks

Manual review of peak integration and identification

Peak integration or identification can be corrected for any individual peaks which may have be missed in the automated data processing

Figure 2. Insight Explore interface for component detection in all samples.

Metabolite and lipid identification with DIA MSMS

The formula prediction algorithm in LabSolutions Insight – Explore was used to generate candidate formulae for all components. Accurate masses and predicted formulae were searched in the METLIN database. Identification was performed by comparison of experimental DIA-MS/MS spectra to MS/MS spectra available in METLIN, or manually interpreted where spectra were not available. Figure 3 shows the process using *sn-1* isoform of lysophosphatidylcholine 18:2 as an example.

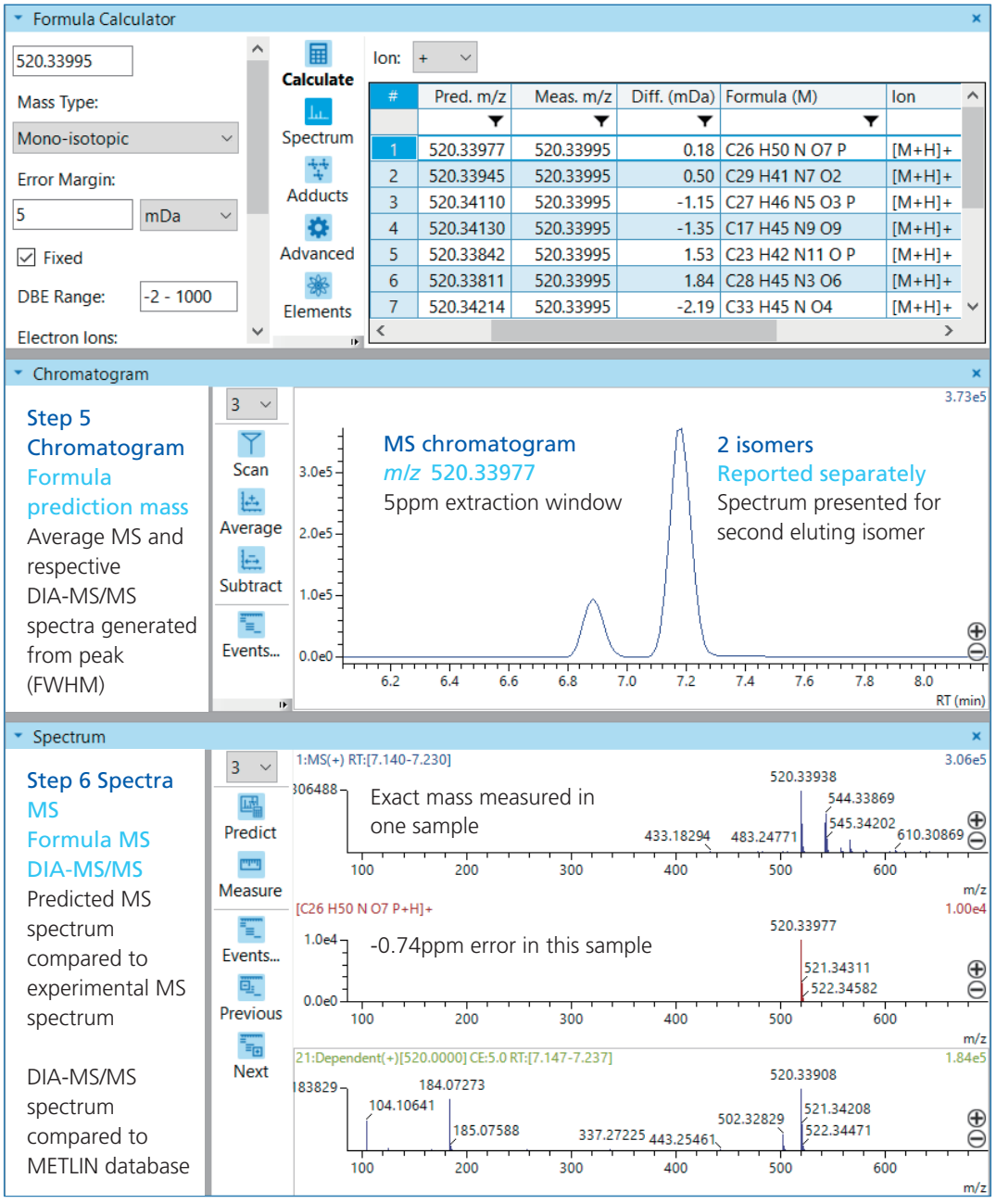


Figure 3. Insight Explore application for data review and component verification.

Isomers are separated by RT and a distinctive fragment (protonated choline at *m/z* 104), characteristic of the *sn-1* isoform known to exceed a 30 fold difference in intensity relative to the *sn-2* isoform (Han and Gross 1996). All other fragments in the spectrum corresponded to this ID: neutral water loss (*m/z* 502), loss of phosphocholine (*m/z* 337) and protonated phosphocholine (*m/z* 184).

Analysis of trends to study progression of infection

To assess the impact of influenza virus infection on swine a number of statistical and trend analysis tools were used in the comparison of metabolite profiles. The effect of influenza in swine is expected to be most pronounced at 3-5dpi, however the effect on the serum metabolic profile appeared limited despite confirmed virus shedding and lung pathology. Several metabolites and lipids were reported in a study of serum from a murine model of influenza infection including hippuric acid and SM d18:0/18:1 which were found to increase at 6dpi (Cui *et.al*. 2016). These metabolites were detected in the present study, however trends were not observed (figure 4). Sampling was limited to 4 replicate pig serum samples for most time-points.

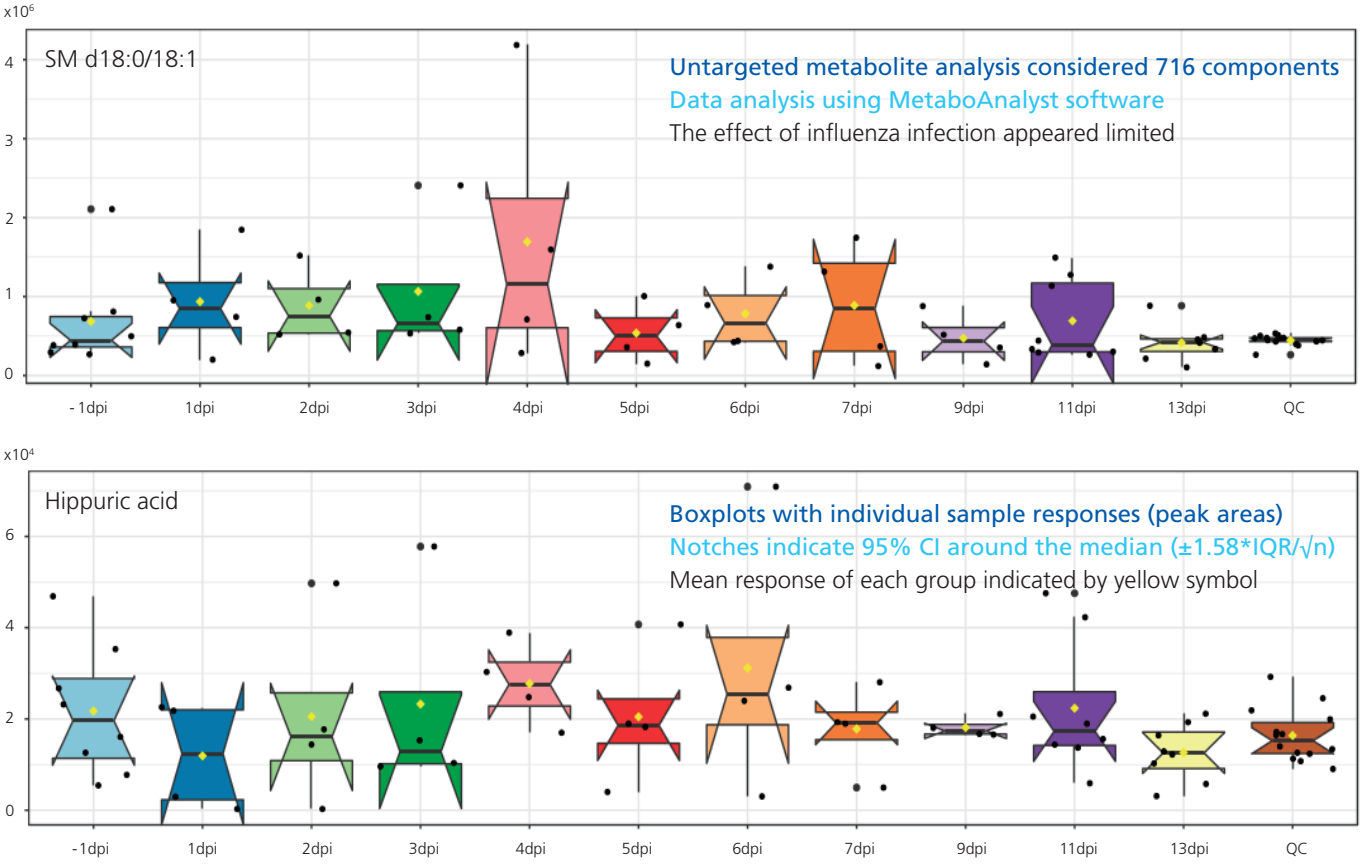


Figure 4. Boxplots of responses (peak areas) from samples at each group (days post infection) presented for SM d18:0/18:1 and hippuric acid.

A metabolomics study into influenza virus infection by HRAM Q-TOF analysis

Conclusions

- An LC-MSMS method has been developed for the untargeted metabolomics analysis of serum extracts.
- HRAM Q-TOF (LCMS-9030 Shimadzu Corporation) acquired MS and DIA-MS/MS data with a cycle time of 0.9 seconds over the MS/MS mass range of 50-1000 Da. The robust workflow for data processing and analysis is presented an appears well suited to metabolomics workflows.
- The method was applied to study the progression of influenza A virus in swine by assessing the metabolic profile of serum from samples taken before infection and up to 13 dpi.
- Around 700 metabolic features were detected across all samples and the relative concentrations between samples were compared. In this study, despite the robust workflow for sample analysis and data processing, metabolite profiles showed high variability between the groups and minimal differentiation by univariate and multivariate statistical analysis.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures. Not available in the USA, Canada and China.

First Edition: July, 2019

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
Company names, products/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation, its subsidiaries or its affiliates, whether or not they are used with trademark symbol "TM" or "®".
Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2019