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Achieving comprehensive lipid profiling with a CCS, retention time and MS/MS library

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INTRODUCTION

In recent years, the benefits of using ion mobility, fragmentation, and retention time information for the identification of lipids in lipidomic workflows has been well established. However, researchers face several challenges in making the most of the currently available technologies. One major challenge is the variety of ion mobility technologies from the various vendors Since CCS databases are dependent on the technology used, there is a growing need to reconcile the various CCS measurements to make comparisons[1]. The Nature protocol published in 2017 [2], containing recommend instrument setting is a bid to improve reproducibility of results and the ability to compare between laboratories and technologies. Ideally, experimentally derived CCS values should be used to generate databases but due to a lack of authentic standards several in silico or predicted CCS models have been adopted and are in use to date. However due to the size and lack or curation of these theoretical database, searches tend to suffer from a high false positive identification rate.

Key Challenges

- Harmonisation of protocols and procedures
- Lack of authentic standards
- False positive identifications (need to curate libraries for biologically relevant search hits)

We have developed a lipid CCS database to tackle some of these challenges. Our approach has been to use a recently published predicted CCS tool [3] to aid with providing accurate CCS values. As with all predictive models, experimental validation was required to establish the accuracy of this model. The experimental validation set consisted of over 100 authentic standards, both as individual lipids and pre-mix standard solutions.

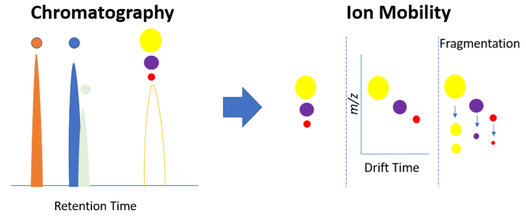
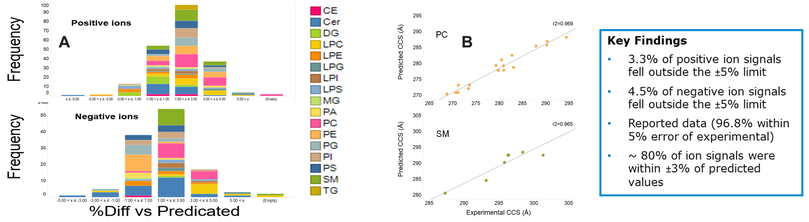


***Figure 1**. IMS-MS can be used with traditional lipidomic approaches, such as chromatography (e.g., LC) to aid identification. Orthogonal separation of lipid ions by ion mobility before MS detection is determined by their charge, size and shape. Drift-time information can be converted to CCS, a measure of the shape of molecules. [2]*

METHODS

- **Predicted CCS TWCCSN$_2$ model by Broeckling et. al model validated using over 100 authentic commercially available lipid standards (individual and premixed)**
- **High throughput Reversed-Phase Lipid profiling method for large samples sets gradient and MS conditions (12 min run time) with Nature Protocol Triwave settings [1]**
- **ACQUITY Premier UPLC I-Class and ACQUITY Premier UPLC CSH C18 (2.1 x 100 mm, 1.7 µm**
- **SYNAPT XS for HDMSe**
- **Measurements taken at 3 concentration levels**
- **Triplicate injections in both positive and negative ESI mode**
- **Data processing by UNIFI software and Progenesis QI informatics**

VALIDATION OF CCS ON DEMAND TOOL



Key Findings

- 3.3% of positive ion signals fell outside the ±5% limit
- 4.5% of negative ion signals fell outside the ±5% limit
- Reported data (96.8% within 5% error of experimental)
- ~ 80% of ion signals were within ±3% of predicted values

***Figure 2**. (**A**) Frequency graphs show that only 3.3% of experimentally derived positive ion CCS values had greater than +/- 5% difference when compared to predicate values. In negative mode that value was slightly higher at 4.5%, possible due to a lower number of ions. These values are consistent with the reported values of 96.8 % being with an error of less than 5%. (**B**) The trellis plots of predicted vs experimental values for selected examples show R^2 values between 96.4 and 96.9%*

INCORPORATING BIOLOGICAL RELEVANCE INTO THE DATABASE

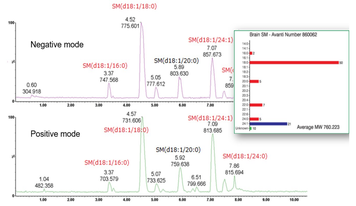


***Figure 3**. Various class-based extracts were analysed to improve the biological relevance of entries. Example chromatograms of a brain sphingomyelin extract confirms the fatty acyl chain distribution described in the certificate of analysis (insert). These extracts can be used to confirm the sum composition of lipids as well as retention times using these LC conditions.*

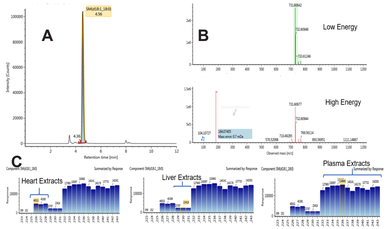


***Figure 4**. (A) Shows a chromatogram of endogenous lipid Sphingomyelin SM(d18:1/18:0) and the positive mode spectrum (B). The the response of SM(d18:1/18:0) detected in various matrices which include Heart, Liver and plasma extracts are shown below (C).*

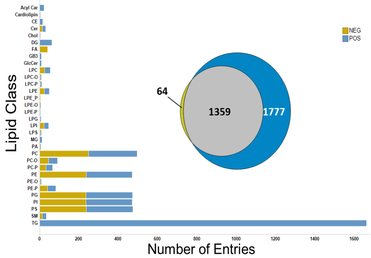
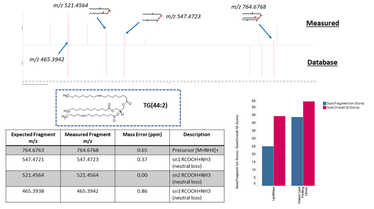


***Figure 5**. Overview of the lipid entries included within the library. A total of 3200 curated lipid species are included covering the major classes, representing both positive and negative ESI.*



Expected Fragment m/z	Measured Fragment m/z	Mass Error (ppm)	Description
764.6763	764.6768	0.65	Precursor [M+NH4]+
547.4721	547.4723	0.37	sn1-RCOOH+NH3 (neutral loss)
521.4564	521.4564	0.00	sn2-RCOOH+NH3 (neutral loss)
465.3938	465.3942	0.86	sn3-RCOOH+NH3 (neutral loss)

***Figure 6**. Representative lipid TG(44:2) identified using either the Waters lipid library or LipidMaps. The mirror plot (top) compares the experimental (measured) with the library (database). Matched fragment ions are highlighted in red. The bar chart compares the fragment ion scores (blue) is shown to be significantly higher when searched against the Waters library. Furthermore, incorporating CCS as part of the seacrh space and increasing the specificity shows the overall score generated with the Waters library to be higher.*

METABOLIC SYNDROME PILOT STUDY

***Figure 7**. Metabolic syndrome pilot study with data collected on a cyclic IMS instrument. Data from 4 healthy, 6 obese and 6 diabetic samples were processed using Progenesis QI with additional statistical analysis conducted using Metoboanalyst [4].*

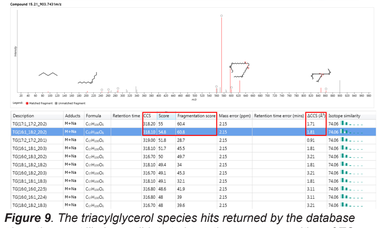


***Figure 9**. The triacylglycerol species hits returned by the database show the most likely candidate to have the sum composition of TG (54:5). The top hits based on the fragmentation score and the low CCS difference are the most likely candidates. Since these are biological samples, it is plausible that the TG with 16 and 18 carbon chain length is the most likely candidate over the odd chain fatty acyl chain TG's, however additional experiments would be required to confirm this.*

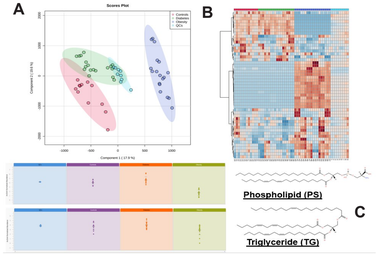


***Figure 8**. (**A**) Supervised PLS-DA models which used pareto scaling show how the various samples cohorts to be distinct with the QC samples tightly clustering, highlighting high technical reproducibility; (**B**) The heatmap is comprised of the top 50 statistically significant features; (**C**) Example lipids from the heatmap include a PL and TG species (see Figure 9 and 10).*

***Figure 10**. Using the high fragmentation score of 97.9 and the low CCS difference we can confidently assign this identification as PS (38:0). Further experiments would be required in order to differentiate between the PS(16:0/22:0) and PS(18:0/20:0).*

CONCLUSION

- **A curated lipidomic database containing over 3200 lipid species, which have previously been reported in human plasma and tissue samples.**
- **An easy to deploy library enabling increased customer productivity.**
- **Validated predicted (in silico) CCS model used to increase coverage.**
- **Assigned measured RTs to reduce false-positives identification.**
- **Comprehensive MS/MS coverage incorporated for increased confidence.**
- **Fast data processing and visualization using UNIFI and Progenesis QI informatics for maximum flexibility.**

References

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3. Broeckling, C. D. et al. Application of Predicted Collisional Cross Section to Metabolome Databases to Probabilistically Describe the Current and Future Ion Mobility Mass Spectrometry. J. Am. Soc. Mass Spectrom. (2021) doi:10.1021/jasms.0c00375.
4. Xia, J., Psychogios, N., Young, N. and Wishart, D.S. (2009) MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucl. Acids Res. 37, W652-660.