AUTOMATIC CCS AND MS/MS LIBRARY CREATION AND APPLICATION FOR LARGE SCALE METABOLIC AND LIPIDOMIC PROFILING

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INTRODUCTION

Metabolomics and Lipidomics involve identification and quantification of chemical fingerprint of cellular processes within a biological system. Precise identification is a major challenge since polar metabolites and lipids are chemically and structurally diverse and span a wide mass range. Chromatographic separation does not typically resolve all components, and often lacks retention time reproducibility. The addition of ion mobility, a gas phase separation of ion, increases peak capacity, selectivity, and can potentially resolve isomeric/isobaric. Benefits of collision cross-section (CCS) library obtained from mobility drift times together with accurate tandem mass spectrometry data (MS/MS) of human metabolites/lipids, will be shown to be an additional source of reference to aid metabolite and lipid identification.

EXPERIMENTAL

Library standards

The library was created from compounds purchased from Sigma-Aldrich, IROA Technologies and Avanti Lipids. Steroid samples were supplied by Swansea University. The standards from IROA-MSMLS (Mass Spectrometry Metabolite Library of Standards) are a collection of high quality small biochemical molecules that span a broad range of primary metabolism and compound classification. The variety of compound classes investigated and sources are depicted in in Figure 1.

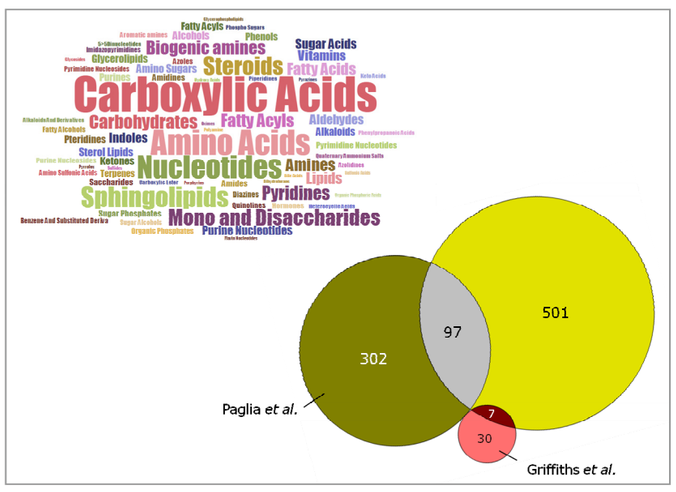


Figure 1. Library class and source composition, including CCS values and MS/MS data generated during previous studies.

IMMS

MS:	Synapt G2-Si and Vion IMS Q-ToF
Mode:	ESI (+VE/-VE) and MALDI (+VE/-VE)
Calibrant:	MajorMix (Power fit)
+VE mode:	*m/z* 152 - 1013 (130 - 306 Å2)
-VE mode:	*m/z* 150 - 1082 (131 - 322 Å2)

ESI and MALDI

For CCS and MS/MS library generation, each IROA sample was diluted to 10ng/µL and loop injected into a solvent flow (50µL/min) consisting of 50% aq. ACN containing 0.1% FA (+ve) / 0.05% NH3 (-ve) using an Acquity I-Class UPLC System. Samples for MALDI analysis were prepared to 0.5mg/ml (0.1%TFA) and 1µL spotted on target. Matrix - re-crystalised DHB 10mg/ml in 7:3 MeOH:0.2%TFA(aq) 1µL spotted on target. Samples and matrix mixed on target.

Experimental and informatics workflows

ESI-IMMS and ESI-MS/MS was used to measure CCS and fragmentation data respectively of the standards using the Synapt G2-S*i*. The data was acquired using MassLynx software which was further processed using UNIFI for generation of the individual libraries. The libraries were further used for inclusion within Progenesis QI, as shown in the workflow diagram, Figure 2, for validation of metabolomic type data acquired upon the Synapt G2-S*i* and Vion.

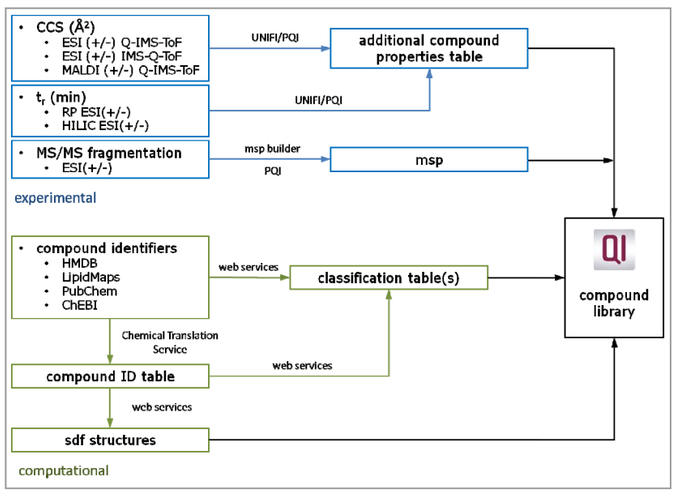


Figure 2. Experimental and computational workflow for the CCS and MS/MS library creation.

RESULTS AND DISCUSSION

CCS and MS/MS library generation

The usefulness of ion mobility to aid metabolomic and lipidomic type workflows has previously been described by Paglia *et. al* [1,2]. This study extends the previous work by offering a greater number of both CCS and accurate mass MS/MS libraries for inclusion into searchable databases within Progenesis QI and UNIFI.

Figure 1 shows the total number of compounds studied. The yellow section indicated the number of new CCS values measured during this study and the intersection the number of CCS values common to this and the previous study with Paglia *et. al* [1]. These 97 common compounds provided a means of CCS validation between the two studies. 30 steroid standard CCS measurements were obtained from a previous in-house study of which 7 were common to both studies.

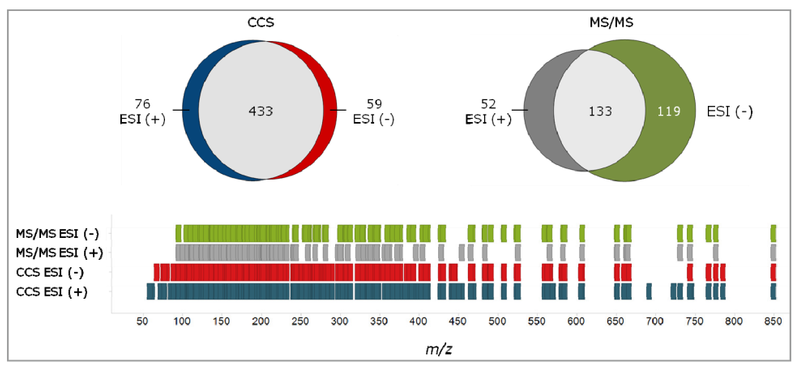
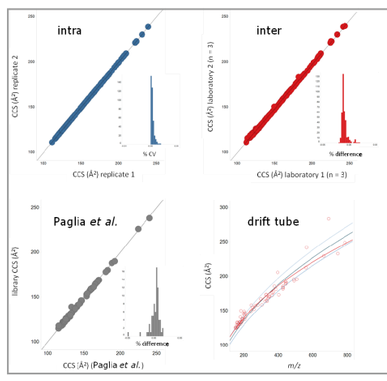


Figure 3. CCS and MS/MS coverage of the IROA compounds studied. The total number of CCS values determined equalled 568. 10% and 13% of the CCS values were unique to ESI -VE and ESI +VE respectively. Also shown is the total number of MS/MS spectra generated (304). 39% and 17% of the MS/MS data were unique to ESI -VE and ESI +VE respectively.

CCS intra- and inter-laboratory validation

The primary objective in generating CCS measurements is the reliability of intra- and inter-laboratory values. In order to make this assessment, Synapt G2-S*i instruments* located at three different sites were used. The laboratories used identical instrumental tuning conditions together with *m/z* and T-Wave ion mobility calibration procedures. CCS values were obtained through post-processing of the MassLynx data using UNIFI software.

Figure 4 shows the correlation between the intra- (top LHS) and inter-laboratory (top RHS) CCS values obtained from laboratories 1 and 2. The % coefficient of variability (CV) was <1% for both intra- and inter-laboratory measurements. The CCS difference between this study and the previous study by Paglia *et al.* [1,2] also shows excellent correlation with the %CV <1.4% between both modes of ESI ionisation (bottom LHS).



Laboratory / instrument	CCS %CV (n)[‡]	
	ESI (+VE)	ESI (-VE)
intra		
laboratory 1	0.2 (349)	0.1 (433)
laboratory 2	0.1 (419)	0.1 (428)
intra (m/z > 150)		
laboratory 1	0.2 (260)	0.1 (303)
laboratory 2	0.1 (309)	0.1 (290)
inter		
laboratories 1 and 2*	0.5 (280)	0.8 (378)
laboratories 1 and 2**	0.5 (493)	0.7 (484)
inter (m/z > 150)		
laboratories 1 and 2*	0.4 (214)	0.8 (262)
laboratories 1 and 2**	0.4 (359)	0.7 (331)
inter		
Synapt G2-S*i*/Vion***	0.8 (21)	1.2 (15)
Library/Paglia *et al.*	1.4 (36)	0.7 (84)
ESI/MALDI***	1.5 (12)	--

* intersection
** combined
*** average CCS difference between G2-Si and Vion and ESI and MALDI outliers and multiple adduct types (H^+, Na^+, K^+) allowed/not removed

Figure 4. Inter– and inter-laboratory T-Wave CCS measurements together with a plot of the T-Wave CCS measurements vs. drift tube ion mobility measurements.

The measured CCS values obtained during this study (excluding lipid species) were contrasted with previously measured carbohydrate CCS values obtained using a drift tube IM_MS [3] (bottom RHS). The dark blue line is a power fit of the carbohydrate drift tube *m/z vs.* CCS data of the form y= $11.6x^{0.5}$ and the light blue lines represent ± 5% deviation from the fit. The red line represents a power fit of *m/z* v CCS values obtained during this study, representing multiple compound classes. These results supplement confidence in the T-Wave ion mobility measurements. The bar graph inset summarises the relative standard deviation (RSD) of the CCS measurements obtained from laboratory 1. For ions detected with *m/z* > 150 the intra CCS RSD vales were < 0.65%.

Class-specific ion mobility separation

Ion mobility has the potential to facilitate detection of class-specific ions in two ways. Firstly, where there are *m/z* interferences, if the species have different mobilities, then they can be temporally separated and independently detected; secondly, even if *m/z* interferences are not present, mass spectral complexity can be high and software-based removal of mobility separated background ions can simplify the resultant mass spectrum and aid detection.

For 'shotgun' omic approaches that do not employ a chromatographic step such as DESI or MALDI the usefulness of ion mobility and CCS is particularly useful. Figure 5 CCS *vs. m/z* for a number of different chemical classes. The data are fitted with a power function and show class-specific separation due to conformational differences by virtue of ion class specific-N_2 interaction differences. These power trend lines could be further used for screening/filtering/sorting of unknown metabolites into their respective classes based upon their position within the scatter plot to aid metabolic screening.

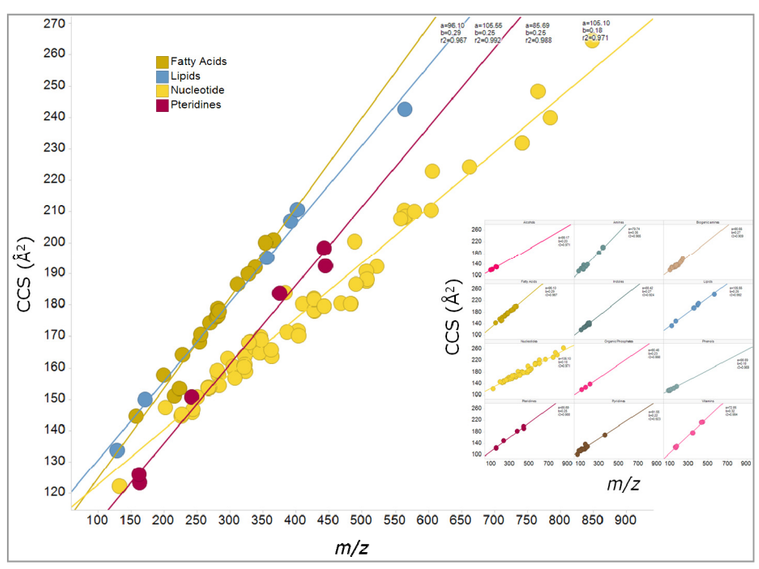


Figure 5. Ion mobility separation of some of the conformationally different molecular classes.

Application library

A mixture of standard lipids containing a range of classes (PC, PA, DG, PE, LPC, SM, PS, PG, PI, TAG) were spiked into increasing levels of bovine heart extract (BHE) matrix. The concentration of BHE was increased from 1-100:1. Figure 6 shows an example of PA lipid (*m/z* 647.4) that has a matrix interference detected at *m/z* 646.4. The second isotope of which overlaps with the monoisotopic ion of the PA. As the level of BHE is increased the mass spectral complexity is shown to be high in the 2D plot (BHE:LipidoMIX, 20:1).

Informatics-based removal of mobility separated background ions simplifies the resultant mass spectrum and aids in detection and identification. The PA has a CCS library entry of 257 Å^2 whilst the matrix interference of the same nominal mass had a measured CCS of 264 Å^2 . Thus, in this simple case, the use of ion mobility together with *m/z* shows no ambiguity in identification and the use of CCS to searchable databases increases identification confidence within metabolic workflows.

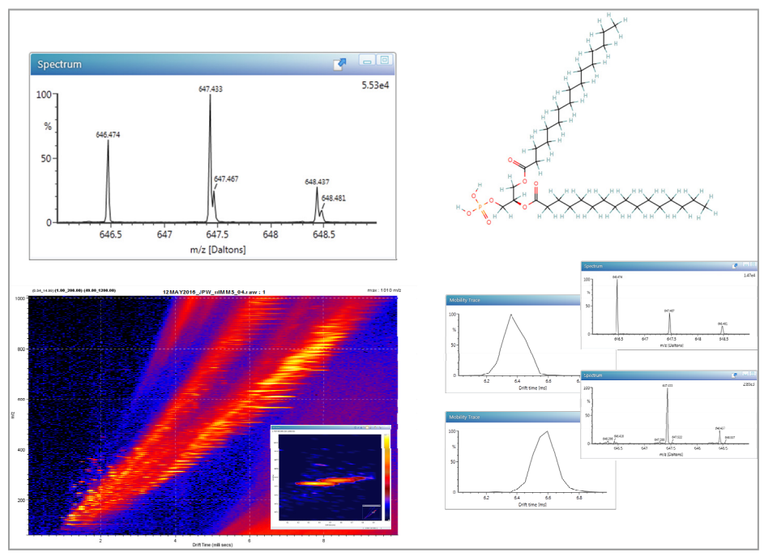


Figure 6. Drift time vs. m/z. The inset shows the drift time (ms) differences of the PA ion at m/z 647 and BHE matrix interference. Due to the mobility differences these ions can be independently detected and database searched. A compound search using the CCS library identified the peak with a drift time of 6.4 ms as the known PA of CCS 257 Å^2.

The use of the CCS library was further explored for lipid identification with additional parameters included in the database search within Progenesis QI such as accurate mass measurement and isotopic fit against an LC-ion mobility-MS acquisition of a human plasma lipid extract. The sample preparation has previously been described by Sarafian *et. al* [4].

The compound database search was conducted with and without CCS as a search parameter As can been seen in Figure 7, an increase in score/confidence is observed with CCS included in compound searches. The total number of other lipids within the extract detected with and without CCS is also shown.

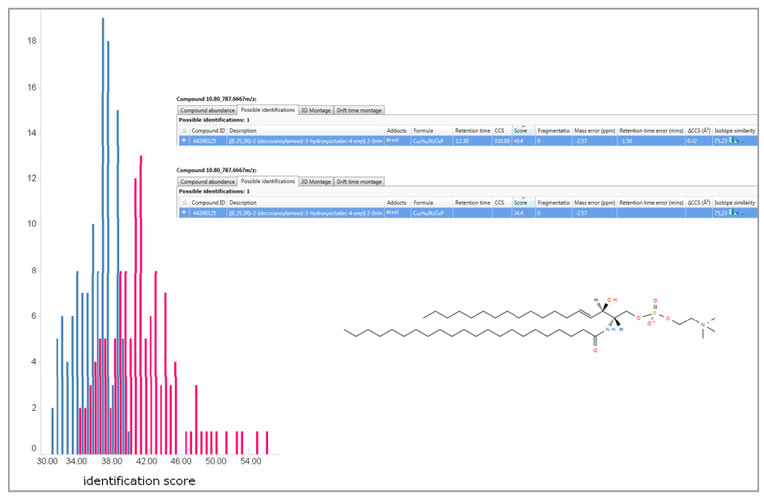


Figure 7. Progenesis QI output of a compound search with and without CCS as a search parameter. Top compound example ID with CCS; bottom without CCS for a lipid PC detected at m/z 787.6. Score distribution identification score for all IDs: Red = with CCS; blue = without CCS for compounds identified in common with both searches.

CONCLUSIONS

- **The combination and use of LC with ion mobility-MS/MS to metabolic profiling has been shown to increase the specificity of analyses and increase the metabolite identification confidence**
- **The increase in specificity using searchable CCS and MS/MS libraries together with RT, accurate mass information and isotopic fit will reduce false positive metabolite identifications**
- **CCS and MS/MS library containing databases to augment metabolite identification will be particularly attractive to Shotgun IMMS-based approaches that use direct infusion or direct ionisation techniques**

Acknowledgments and references

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