BUILDING MULTIDIMENSIONAL IN-HOUSE METABOLOMICS LIBRARIES FOR UNTARGETED METABOLOMICS WITH OPEN-SOURCE TOOLS

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IMSC 2022

INTRODUCTION

Metabolite annotation is crucial in untargeted metabolomics but remains a major challenge. The large pool of metabolites analyzed under various instrumental conditions (MS/MS with different collision energies, retention time with different columns) is underrepresented in publicly available databases.

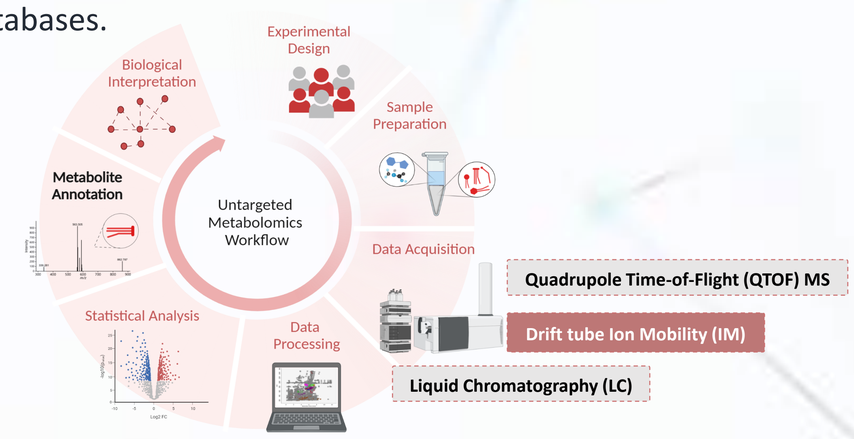


Figure 1. Untargeted metabolomics general workflow.

A gas-phase separation method, **ion mobility** (**IM**) spectrometry hyphenated to LC-HRMS, is gaining significant interest to help increase confidence in annotation by using **collision cross section** (**CCS**) information.

Goal: Build an in-house and easy to share metabolite library with retention time (RT), MS/MS spectra and CCS values using open-source tools

WORKFLOW

Data were acquired using electrospray ionization (ESI) in positive (+) and negative (-) using an ACQUITY UPLC BEH C18 column (150 × 2.1 mm, 1.7 µm). The mobile phase consisted of (A) MeCN/5 mM of NH_4COOCH_3 (30/70, v/v) and (B) IPA/MeCN/5 mM NH_4COOCH_3 (88/10/2, v/v/v). In ESI+, 0.1% (v/v) of HCOOCH3 was added to the aqueous fraction.

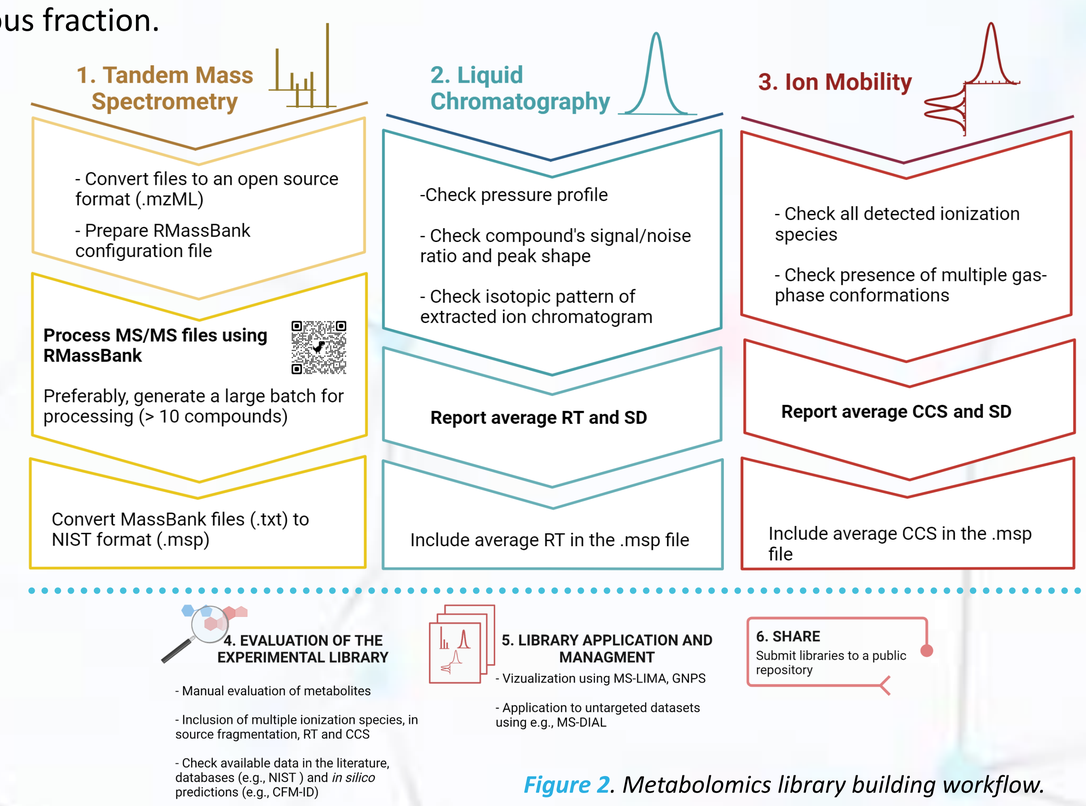


Figure 2. Metabolomics library building workflow.

RESULTS

3D-Metabolite library

Data heterogeneity

Proof of concept: 100 metabolites from nine RefMet superclasses.
539 MS/MS (1-3 collision energies, different ionization species),
2 methods (ESI+/-)^2 → 194 RT values and 177 CCS values

CCS values can increase confidence in annotation after RT and MS/MS spectral matching

High repeatability of the $^{DT}CCS_{N2}$ measurements is reflected by > 85% of $^{DT}CCS_{N2}$ showing an SD ≤ 0.1 Å² (*N*=3).

Use of the in-house library to evaluate CCS *in silico* prediction tools

Coverage

Prediction for 195 ionization species:
AllCCS → 174, CCSbase → 157 and DeepCCS → 149.

Number of CCS values with errors <3%

CCSbase showed the highest accuracy (87%) followed by AllCCS (81%) and DeepCCS (56%).

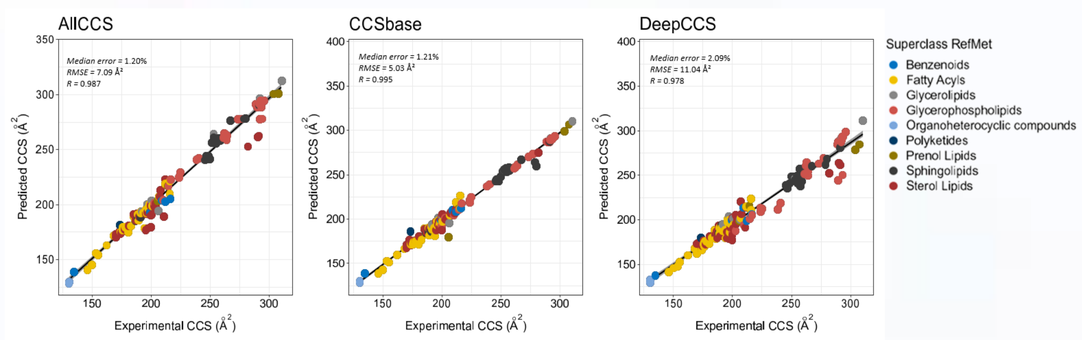


Figure 3. Correlation between experimental acquired $^{DT}CCS_{N2}$ values for reference standards and predicted CCS values.

One of the challenges of IM data…

Different gas-phase conformations can be observed for some ions. Suggestion **CCS compendium**: Report all calculated CCS.

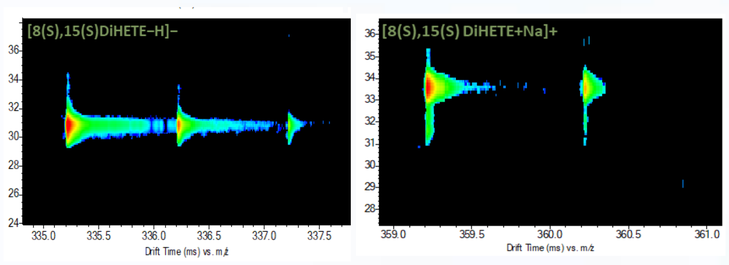


Figure 4. Ion mobility spectrum of [M-H]⁻ and [M+Na]⁺ of 8,15-dihydroxy eicosatetraenoic acid. Dihydroxy modification is observed for the sodium adduct since coordination can occur in two different sites.

Considerations for MS/MS spectra

RMassBank performs formula assignments for fragment ions. ➡ − Noise Signals in MS/MS; + Retain (low signal) informative fragments

Rule-based fragmentation of lipids can be used as a tool to evaluate the quality of experimental libraries containing these compounds. For the others, a literature review of common fragments can be helpful.

≠ Ionization species can have ≠ fragmentation patterns

Suggestion: Include all possible ionization species (considering MP modifiers)

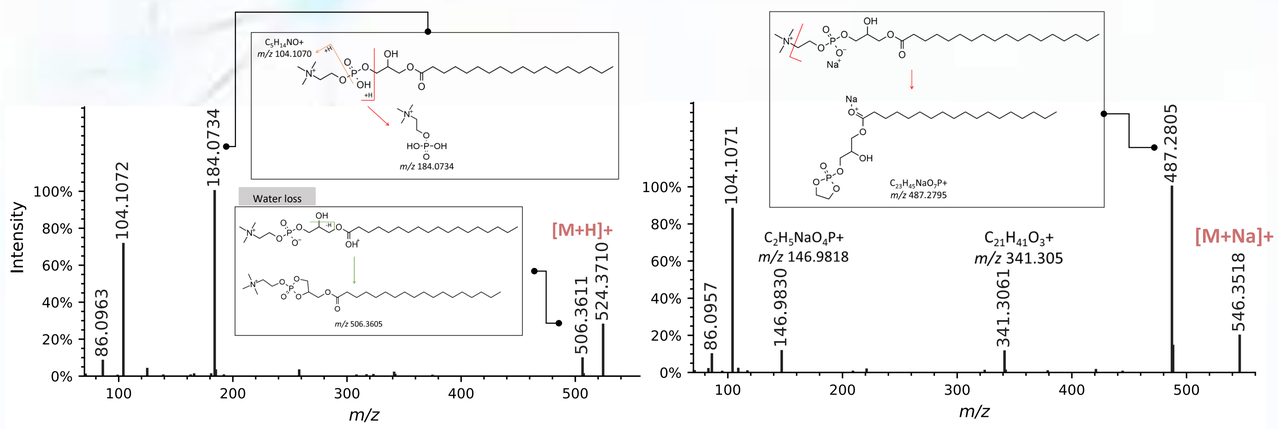
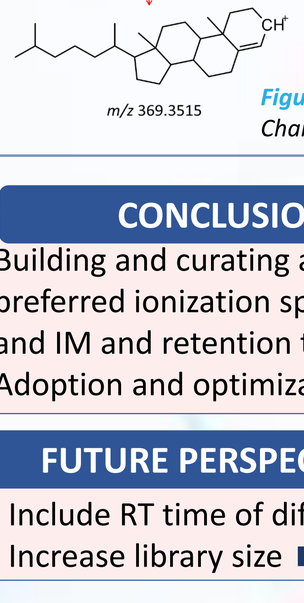


Figure 5. Example of MS/MS spectra of $[M+H]^+$ and $[M+Na]^+$ of LPC 18:0 at CID 20 eV.

Radical Ions (odd-electron ions) are present in ESI and CID-based fragment ions. They can provide important structural information for different classes, including **benzenoids, carotenoids, sterols, and fatty acids.**



In source fragmentation (ISF) is a common effect in ESI that cannot be completely avoided. MS/MS information of ISF ions can be very informative, but it should be combined with retention time information to reduce false positive annotations. ISF in suspect lists (MS only libraries)

Figure 6. Cholestene ion, ISF of cholesterol in ESI+. Characteristic fragment of cholesterol esters.

CONCLUSIONS

Building and curating a metabolite library allow to obtain in-depth knowledge of the preferred ionization species formed, in source fragmentation, characteristic fragments, and IM and retention time patterns for different metabolite classes.
Adoption and optimization of open-source workflows ➡ **FAIR** RESEARCH

FUTURE PERSPECTIVES

Include RT time of different chromatographic modes (e.g., HILIC)
Increase library size ➡ Acquire more standards + collaborations (e.g., mFAM)

REFERENCES & ACKNOWLEDGEMENTS

RMassBank

Metabolite Families (mFAM) Contact for sharing raw data and msp files

Analytical methods

NIST msp files

KMS: BOF DOCPRO 4
MvdL.: BOF – Antigoon ID 46315
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