4-dimensional annotation of Metabolomics features: CCS values as an additional source for higher confidence

ASMS 2020, ThP 383

Ulrike Schweiger-Hufnagel[1]; Matthias Szesny[1]; Aiko Barsch[1]; Melvin Gay[2]; Torben Kimhofer[3]; Joel Gummer[3]; Luke Whiley[3]; Jeremy Nicholson[3]

[1]Bruker Daltonik GmbH, Bremen, Germany;
[2]Bruker Pty. LTD., Australia, Preston, Australia;
[3]Murdoch University, Perth, Australia

Introduction

Reliable annotation of small molecules for LC-MS/MS based data requires the adept combination of many parameters. To extend our parameter portfolio we analyzed trapped ion mobility spectrometry (TIMS) data. We investigated the reproducibility of CCS values for intra- and inter-lab measurements. Furthermore, we compared the measured CCS values to those from literature. From the present results we conclude that CCS value serve as an excellent additional filter for metabolite annotation.

Methods

- Sample preparation and data acquisition in Perth (Australia) and Bremen (Germany) and following the same standardized protocol
- Samples: Urine (TRX-3178-R) NovaMT (Alberta, CA)
- LC separation: UHPLC, RP column, 15 min gradient
- MS instruments: timsTOF Pro (Bruker)
- Data acquisition:
  - ESI positive and negative
  - PASEF mode, 100ms ramp time: generating MS data and up to 60 MSMS spectra per second
- Processing: MetaboScape 2021 software, preliminary version.
- Annotation: Analytes extracted from Unified CCS compendium (1). The list was combined with retention times and MS/MS spectra from the Bruker HMDB Personal Library 2.0.

Results

PASEF data (4 technical replicates) was acquired for a urine sample prepared following an identical protocol on two Bruker timsTOF Pro instruments in Perth and Bremen. Processing was performed in MetaboScape based on the T-ReX® 4D algorithm and included automatic CCS value determination. Features extracted by this 4-dimensional peak picking and alignment algorithm were annotated based on precursor mass accuracy and isotopic pattern, retention time, MS/MS spectrum and CCS value.

Intra-lab reproducibility of measured CCS values

- Box and Whiskers plots (Figure 1) revealing high stability of CCS values for each measurement.
- Average standard deviations:

Polarity/ lab	Perth	Bremen
positive	0.27 Å²	0.37 Å²
negative	0.21 Å²	0.18 Å²

- Small absolute difference between the CCS values determined in Perth and in Bremen

Inter-lab comparison of CCS values

- Average |ΔCCS| for Bremen vs Perth (Figure 2).
  - 0.25 % for positive ionization mode
  - 0.15 % for negative ionization mode

Comparison of CCS values determined in Perth/ Bremen and Unified CCS Compendium

- The measured CCS values were similar to those from the Unified CCS Compendium (Ref 1, Figure 3).
- Average | ΔCCS|:

Polarity/ lab	Perth	Bremen
positive	0.94 %	0.93 %
negative	0.88 %	0.79 %

Figure 4: 4D-Metabolomics™ annotation shown for Paraxanthine.
- Annotation Quality symbol indicating the quality of the annotation in all dimensions.
- Feature table (bottom)
- MS/MS spectrum. The measured spectrum is very clean due to PASEF. For comparison, the Spectral library spectrum is shown.

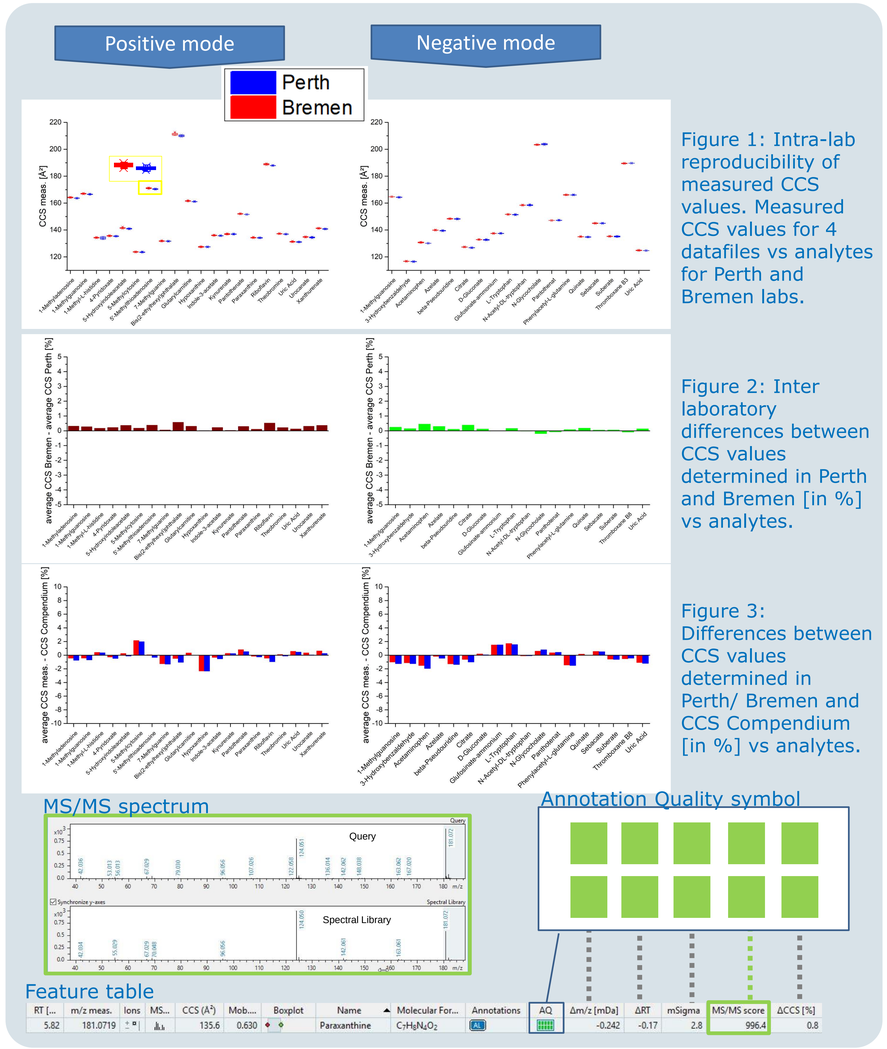

Figure 1: Intra-lab reproducibility of measured CCS values. Measured CCS values for 4 datafiles vs analytes for Perth and Bremen labs.

Figure 2: Inter laboratory differences between CCS values determined in Perth and Bremen [in %] vs analytes.

Figure 3: Differences between CCS values determined in Perth/ Bremen and CCS Compendium [in %] vs analytes.

Summary

- High intra-lab reproducibility for CCS values. Standard deviation < 0.4 Å²
- Low inter-lab differences in measured CCS values < 0.3 %
- High accordance of measured CCS values to those from Unified CCS Compendium: Average | ΔCCS [%] | < 1 %

Conclusions

The PASEF acquisition mode proved to be highly beneficial for metabolomics research in several aspects:

- Higher confidence in ID
  - Measured CCS^{TIMS} values are highly reproducible – intra and inter-lab.
  - The deviation of CCS^{TIMS} from literature CCS^{DT} values is low.
  - This allows to use CCS values as filter for 4D annotation – in addition to retention time, precursor mass and isotopic pattern and MS/MS spectrum.
- PASEF utilizes this additional separation dimension by ion mobility, resulting in cleaner MS/MS spectra. This is crucial for accurate ID in small molecule workflows.

4D-Metabolomics

References

(1) Unified CCS Compendium list
https://mcleanresearchgroup.shinyapps.io/CCS-Compendium/)