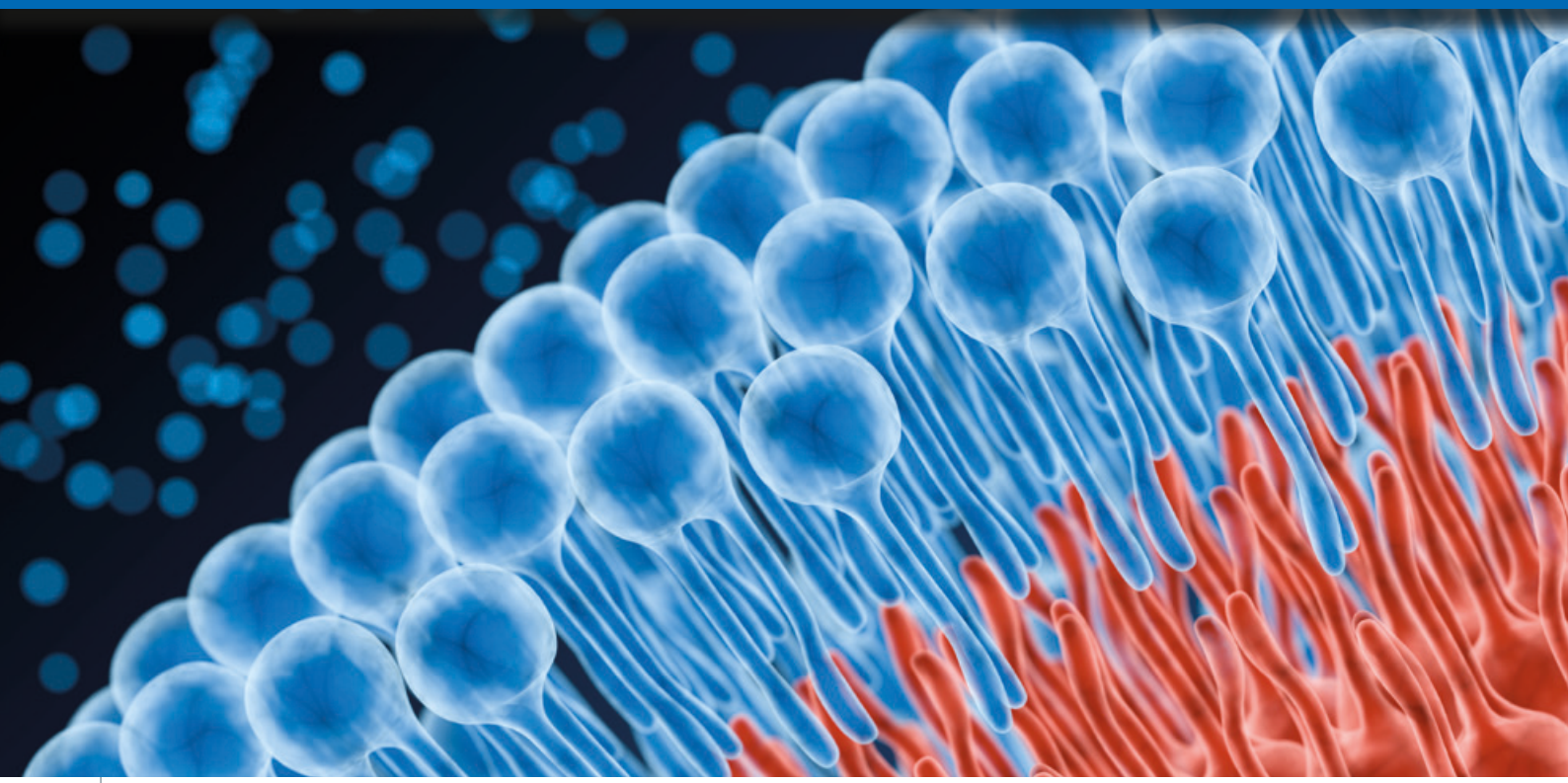

CCSPredict: Using a Machine Learning Approach for Higher Confidence in Lipid Identification

Trapped ion mobility (TIMS) mass spectrometers offer new options for higher confidence in annotations of target lipids.

Introduction

With the additional TIMS separation dimension compounds co-eluting from LC columns can be separated. This can result in cleaner MS/MS spectra – crucial for any ID in lipidomics or other small molecule workflows. Moreover, TIMS enables the determination of collisional cross sections (CCS) of ions. These values are specific properties for any ion species under given conditions (type of gas, pressure, temperature). Therefore, acquired CCS values provide increased confidence in compound identification if compared to values from literature or to predicted values generated using recently developed machine learning (ML) algorithms.

Methods

Lipid Standards # 330708 Differential Ion Mobility System Suitability Lipidomix® Kit (from Avanti Polar Lipids, Inc.) were dissolved in Methanol:Water (9:1). Milk samples were extracted using a modified Bligh & Dyer [1] method. LC separation was performed using a Bruker Elute UHPLC system (20 min gradient program).

Keywords:
timsTOF Pro, timsTOF, Lipid Identification, Lipidomics, CCS values, CCS value prediction, machine learning

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Bruker Daltonik GmbH, Bremen, Germany

MS data were acquired on Bruker timsTOF and timsTOF PRO instruments with and without TIMS separation in ESI positive and negative autoMS/MS modes. CCS recalibration was performed using TuneMix as calibrant (322 m/z, 152.8 Å²; 622 m/z, 201.6 Å²; 922 m/z, 241.8 Å²; 1221 m/z, 279.9 Å²). The resulting data sets were processed in the novel MetaboScape 4.0 software (Bruker Daltonics) using the Time aligned Region complete eXtraction (T-ReX™) algorithm. T-ReX 3D was applied to LC-MS/MS data and T-ReX 4D to 4-dimensional LC-TIMS-MS/MS data. Statistical analysis, molecular formula annotation, MS/MS spectral library queries using LipidBlast [2, 3, 4] and prediction of CCS values were conducted in the same integrated client/server software solution. Lipid prediction is based on a support vector regression based machine learning approach described by Zhou et al. [5].

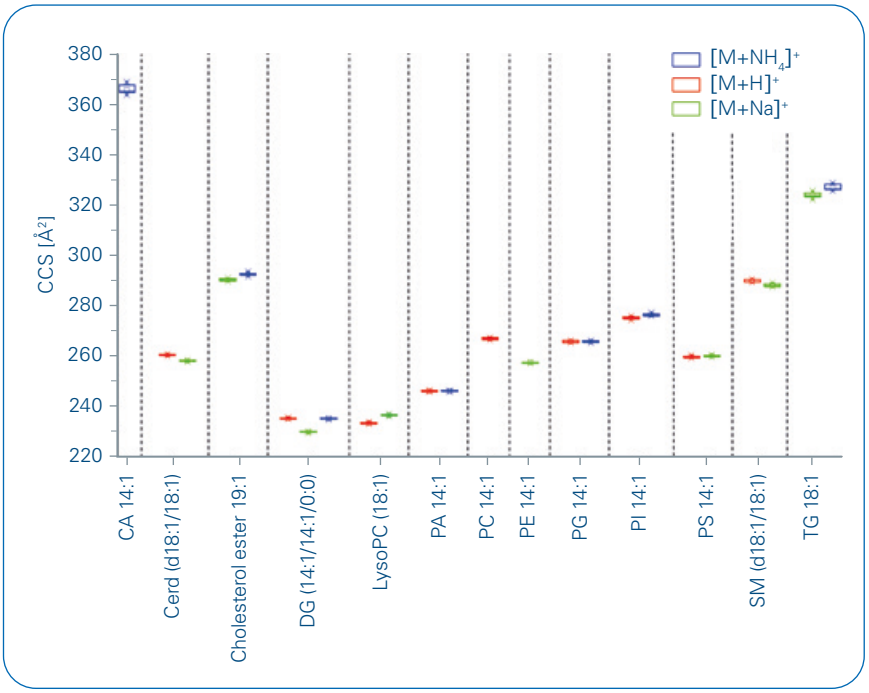

Figure 1: Highly reproducible CCS values generated by timsTOF Pro. Box plots of CCS values from 24 ion species (corresponding to 13 lipids standards from Avanti # 330708) measured in 10 consecutive timsTOF Pro runs.

timsTOF systems generate highly reproducible lipid CCS values

- 10 consecutive measurements of 24 lipids on a timsTOF Pro showed highly reproducible CCS values (Figure 1). The average RSD for all species was 0.17%. Minimum and maximum RSD observed were 0.07% and 0.49%, respectively.
- Measurements for the same lipid standard mix on a timsTOF instrument (n=3 runs) demonstrated a high instrument to instrument reproducibility of CCS values (Figure 2).

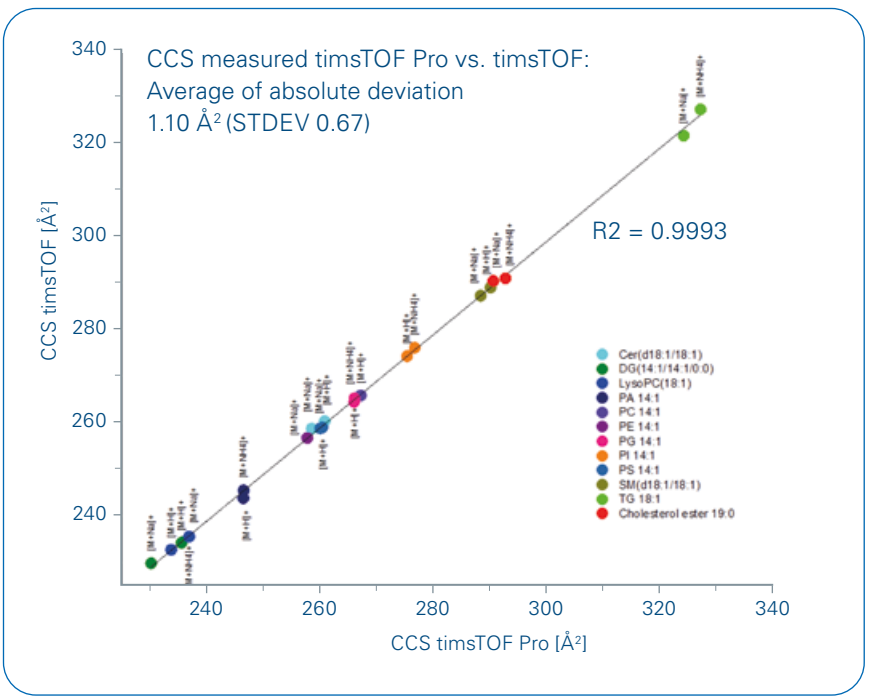

Figure 2: CCS values measured on timsTOF vs. CCS values measured on timsTOF Pro. CCS values from 23 ion species (corresponding to 12 lipids standards from Avanti # 330708) measured on a timsTOF [average of n=3] are plotted against the corresponding CCS values measured on timsTOF Pro [average of n=10].

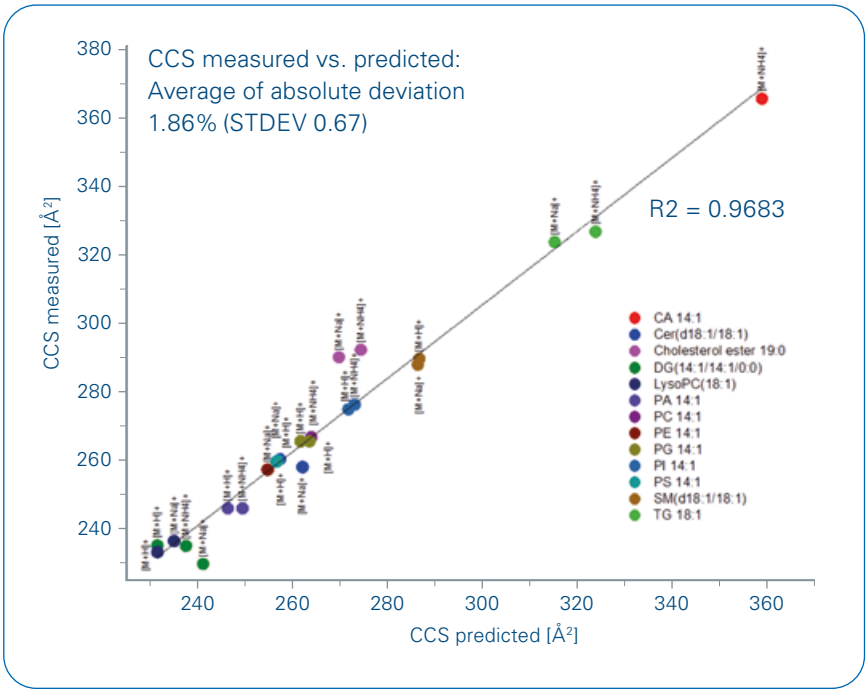

Figure 3: CCS values measured vs. CCS values predicted in MetaboScape 4.0. Average CCS values [n=10] from 24 ion species measured on timsTOF Pro are plotted vs. the CCS values predicted using a machine learning algorithm implemented in MetaboScape 4.0. The lipid class of cholesterol esters (pink) is not covered by the CCS prediction model.

Lipid CCS prediction in MetaboScape for increased confidence in lipid identification

- CCS values for known standards were measured on the timsTOF Pro instrument (Figure 1). These were very similar to values predicted by a ML algorithm in MetaboScape 4.0 (Figure 3). Note that apart from LysoPC 18:1, SM 18:1, Cer 18:1, TG 18:1 the measured lipid species were not used for generating the lipid prediction model.
- CCS values match especially well for those lipid classes which were used for training the lipid prediction model (Figure 3).
- Figure 4 highlights the workflow for high confidence lipid identification using MetaboScape 4.0 based on a real-world example.

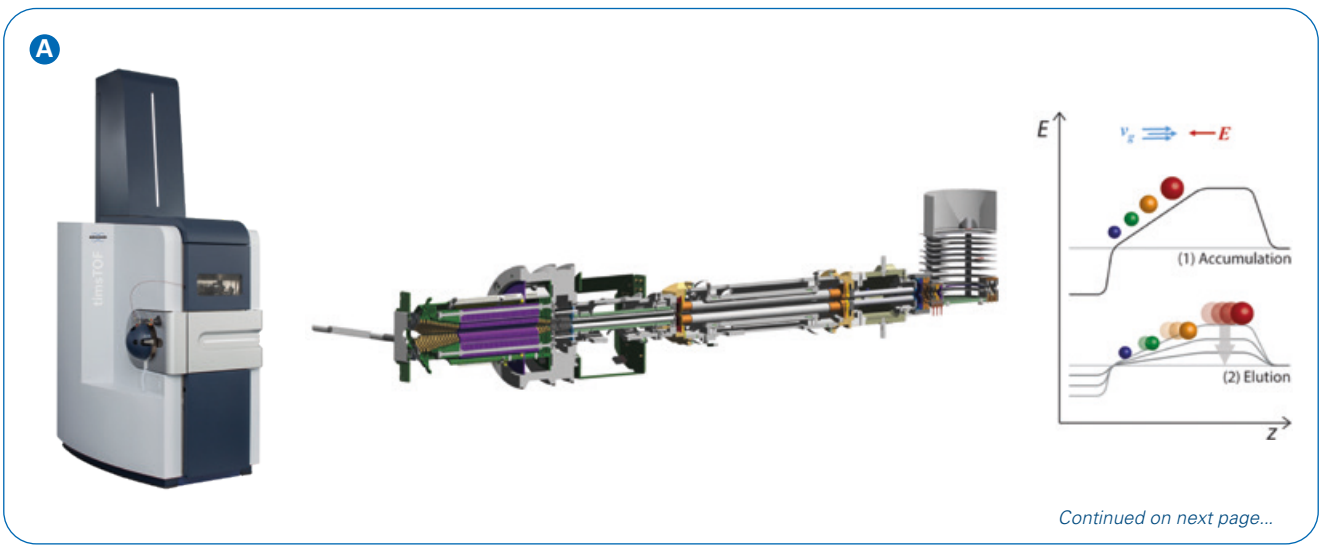

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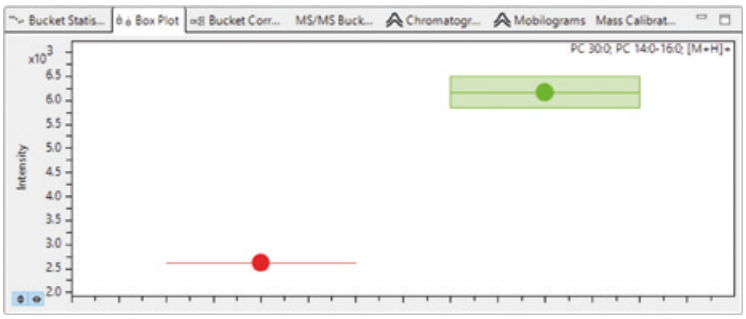
Figure 4: High confidence lipid identification workflow: A real-world example using MetaboScape 4.0. (A) Lipid extracts from two milk samples were analyzed by LC-MS/MS and LC-TIMS-MS/MS using a timsTOF. Shown are the instrument schematic and TIMS operation from ion accumulation to serial elution, where E represents electrical field and v_g the gas flow. (B) Box plot of a lipid showing different abundances in two milk lipid extracts detected in ESI positive mode. (C) Assignment of molecular formula based on accurate mass and true isotopic pattern using SmartFormula™ – $C_{38}H_{76}NO_8P$. (D) MS/MS query for ESI positive mode against LipidBlast library returns PC 30:0 as likely candidate. Characteristic head group fragment (184.07 m/z) substantiated assignment as glycerophosphocholine (PC). (E) SmartFormula3D™ algorithm: Fatty acid chains C14:0 and C16:0 could be assigned based on automatic formulae assignment for characteristic fragment ions in negative mode. (F) Very low deviation (0.8 %) between measured vs. predicted CCS values (279.7 vs. 277.5) provided further orthogonal confidence for the identification of the target lipid as: PC 14:0_16:0.

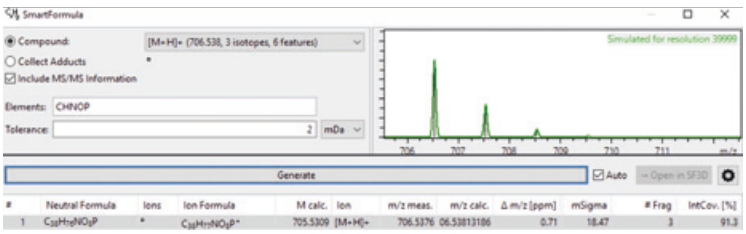
...Figure 4 continued

B



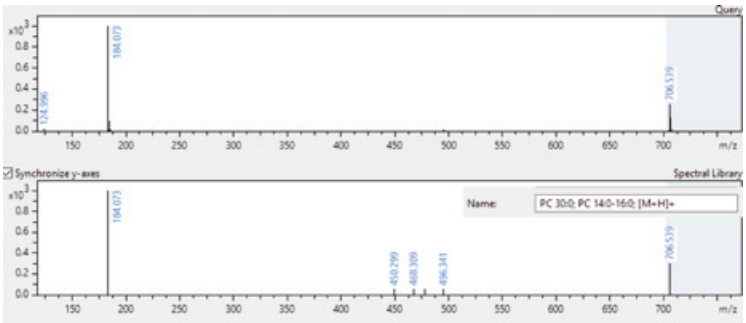
Target lipid – different abundances

C



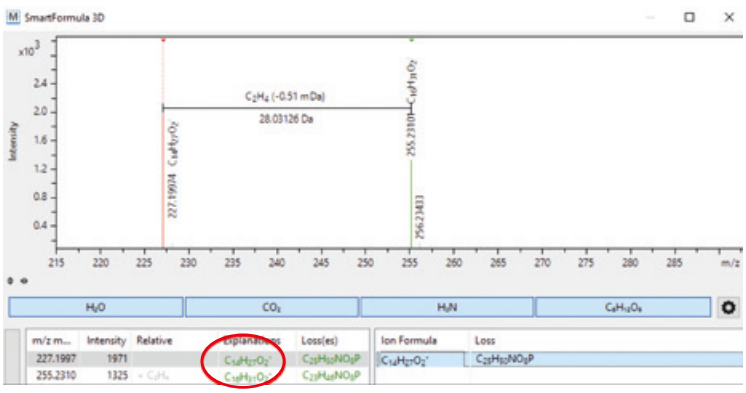
Accurate m/z & True Isotopic Pattern

D



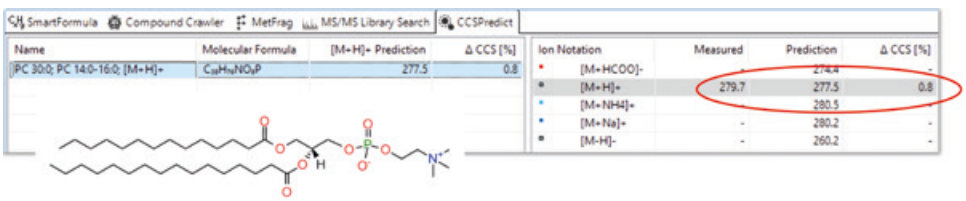
MS/MS positive

E



MS/MS negative

F



CCS Prediction

Identification Confidence

Acknowledgements

We thank Prof. Peter Hoffmann and Dr. Mark Condina (Future Industries Institute, The University of South Australia) for providing the lipid milk extracts.

Conclusions

- timsTOF Pro instruments provide highly reproducible CCS values (0.17% RSD).
- CCS values can be determinded reproducibly on timsTOF and timsTOF Pro instruments (the two instruments agree on average within 1.1 Å²).
- MetaboScape 4.0 enables prediction of lipid CCS values based on machine learning.
- CCS values increase confidence in lipid annotation as orthogonal information in addition to accurate mass, true isotopic pattern, MS/MS and retention time information.
- MetaboScape provides a fully integrated solution for confident lipid annotation.

Learn More

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www.bruker.com/metaboscape

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