FlashNote

Confident metabolite annotation in the absence of reference CCS and MS/MS data

Trapped Ion Mobility Spectrometry (TIMS) enables accurate measurement of metabolite Collisional Cross Section (CCS). These CCS values are a core component of the 4D-Metabolomics™ workflow, providing confidence in compound annotation that augments the accurate mass, isotopic pattern fit, retention time, and MS/MS data routinely generated in high resolution mass spectrometry workflows.

Challenge

MS/MS spectral libraries and CCS repositories are being generated and shared at a rapid rate, including the Unified CCS Compendium [1] which houses >3800 values from traceable reference standards.

Still, many MS/MS libraries used for large-scale annotations in metabolomics and other small molecule research areas lack reference CCS values. Additionally, for many compounds, reference MS/MS spectra are not available and hence cannot serve as input for automatic annotation routines in non-targeted metabolomics applications.

Solution

The new extended Spectral Library annotation workflow in MetaboScape® addresses lacking of CCS reference values by extending MS/MS spectral libraries with predicted CCS values. These are generated on-the-fly using the machine learning-based prediction model CCSPredict Pro, resulting in highly accurate predicted CCS values. Target List annotation in MetaboScape combines this new functionality with MetFrag *in-silico* fragmentation [2, 3] routines to enhance metabolite annotation confidence in the absence of MS/MS reference data. Any Target List containing the common InChI identifier for chemical substances is readily made into a searchable CCS library by the CCSPredict Pro model. Simultaneously, MetaboScape performs automatic MS/MS matching based on the InChI encoded structures using the MetFrag algorithm. Together, these data allow users to leverage the value of CCS measurement and MS/MS fragmentation without restriction or dependence on their availability in data repositories.

MetaboScape provides CCS-enabled annotation confidence

CCS-enabled profiling workflows provide higher confidence for compound annotation even if reference CCS values and / or MS/MS spectra are not available. On-the-fly structure-based CCS prediction and *in-silico* MS/MS fragment matching can substantiate annotations before purchasing or synthesizing reference standards for final validation of compound IDs.

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CCSPredict workflow in MetaboScape turns any MS/MS spectral library with structure information into a CCS-enabled library

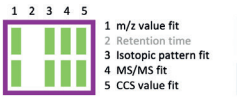
Spectral libraries without CCS references

InChI structure

Spectral library annotations enhanced with CCS information from CCSPredict Pro predictions

CCS-enabled spectral library annotation of human urine data acquired by 4D-Metabolomics

Automated Annotation Qualtiy (AQ) and AQ Details reporting:

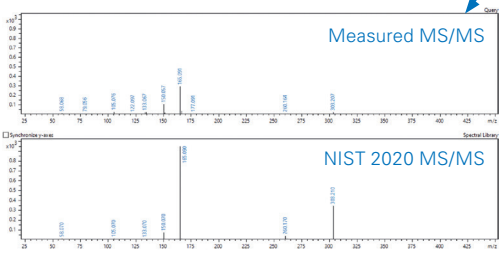


CCSPredict Pro

MS/MS library

Name	Annotations	AQ	Annotation Source	AQ Details	MS/MS score	ΔCCS [%]
1,3-Dimethyluric acid	SL		Bruker NIST 2020 MSMS ...		912.4	0.4
Hippuric acid	SL		Bruker NIST 2020 MSMS ...		982.2	-0.8
Verapamil	SL		Bruker NIST 2020 MSMS ...		987.7	-0.1
Phenylacetyl-L-glutamine	SL		Bruker NIST 2020 MSMS ...		958.5	-1.8
Paraxanthine	SL		Bruker NIST 2020 MSMS ...		983.2	-1.3
Kynurenic acid	SL		Bruker NIST 2020 MSMS ...		744.5	-1.3
Uric acid	SL		Bruker NIST 2020 MSMS ...		825.5	0.8
L-Histidine	SL		Bruker NIST 2020 MSMS ...		970.2	-0.8

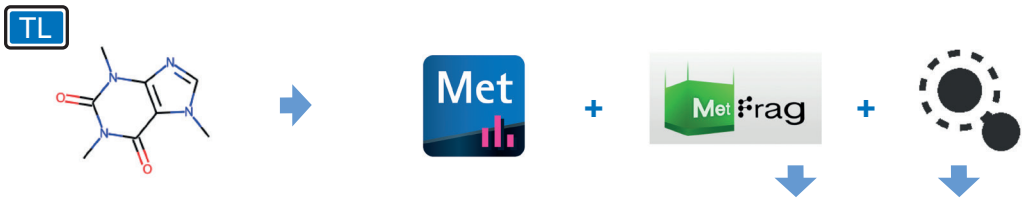
Validation of spiked-in Verapamil



Human urine samples were measured with LC-TIMS-PASEF MS/MS using a timsTOF Pro 2 MS instrument. Metabolite annotation was performed in MetaboScape using the NIST 2020 MS/MS library. Forty unique annotations fit the criteria for **maximum annotation confidence** based on mass accuracy (mass dev. <2 ppm), isotope pattern (mSigma <20), MS/MS spectra (MS/MS score > 900) and predicted CCS value (CCS deviations measured vs. prediction <2%) matching.

CCS-based annotation quality scores were automatically calculated within MetaboScape and predicted values were clearly labelled in the Annotation Quality (AQ) Details column. To validate the workflow, the annotation of spiked-in Verapamil was confirmed with both reference retention times and CCS values.

MetaboScape's structure-based CCS prediction and *in-silico* fragmentation



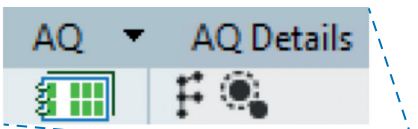
InChI structure
from Target List

Name	Annotations	AQ	AQ Details	MS/MS score	ΔCCS [%]
Proline betaine	TL SL			1000.0	-0.9

Compound annotations enhanced with CCS predictions and *in-silico* MS/MS fragmentation

Structure based annotation of human plasma data acquired by 4D-Metabolomics

Annotation Quality scores featuring
CCS prediction and *in-silico* fragmentation

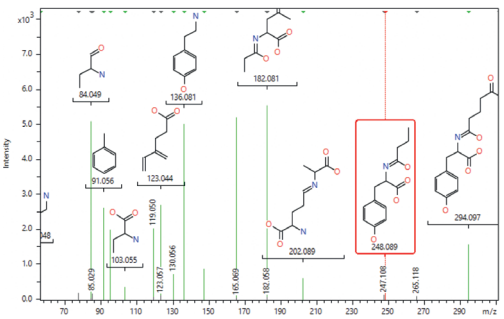


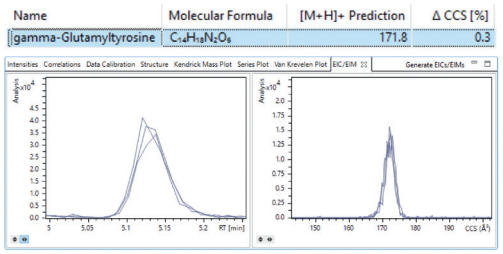
	m/z meas.	Δm/z [ppm]	RT [min]	mSigma	MS/MS score	CCS (Å²)	ΔCCS [%]	Name	Molecular Formula	Annotations	AQ	AQ Details
16	144.10187	-0.267	1.39	9.5	1000.0	127.2	-0.9	Proline betaine	$C_7H_{13}NO_2$	TL		
17	197.12846	-0.208	6.23	1.3	845.7	144.1	0.7	Fasoracetam	$C_{10}H_{16}N_2O_2$	TL		
18	181.07207	0.398	5.73	9.6	952.3	134.1	-1.2	Paraxanthine	$C_7H_8N_4O_2$	TL		
19	246.16981	-0.356	6.52	9.6	851.0	161.0	1.2	2-Methylbutyroylcarnitine	$C_{12}H_{23}NO_4$	TL		
20	282.11948	-0.335	2.09	8.6	938.8	164.7	1.8	N6-Methyladenosine	$C_{11}H_{15}N_5O_4$	TL		
21	134.05991	-0.956	6.17	16.1	966.0	124.0	0.5	Isoindolin-1-one	C_8H_7NO	TL		
22	732.55343	-0.255	16.44	15.9	978.3	280.4	-0.8	PC(16:0/16:1(9Z))	$C_{40}H_{78}NO_8P$	TL		
23	303.23165	-0.666	14.46	18.3	979.3	179.5	1.6	14(R)-Hydroxy-retro-vitamin A	$C_{20}H_{30}O_2$	TL		
24	301.21596	-0.771	12.65	5.4	849.7	180.7	1.8	9-cis-Retinoic acid	$C_{20}H_{28}O_2$	TL		
25	269.12468	0.922	1.55	18.0	909.1	160.1	0.5	Pentostatin	$C_{11}H_{16}N_4O_4$	TL		
26	311.12348	-0.901	5.14	2.0	992.6	172.2	0.1	gamma-Glutamyltyrosine	$C_{14}H_{18}N_2O_6$	TL		

A human plasma (NIST SRM 1950) 4D-Metabolomics data set was annotated with a Target List of compounds reported to be present in blood products (derived from HMDB 5.0 [4]). This enabled the **annotation** of >50 metabolites **based on structural information alone** (10 shown) with high scoring: <1ppm precursor mass, <20 mSigma, >800 MetFrag MS/MS score, <2% CCS vs. prediction. Annotation Quality scoring gives direct feedback on the quality identification results – at a glimpse!

MS/MS matching
by MetFrag *in-silico*
fragmentation

CCS matching to predicted
value by CCSPredict Pro





The example selected above shows the annotation of gamma-Glutamyltyrosine with a **MetFrag MS/MS score of 992** (max. 1000) and a **delta CCS vs. prediction 0.3%**. Visualization of MetFrag *in-silico* generated fragment structures matching measured MS/MS peaks (left) as well as CCS prediction and mobilogram plots (right) facilitate data evaluation to substantiate annotation confidence.

MetaboScape provides CCS-enabled annotation confidence

- Novel workflows using the machine learning model CCSPredict Pro unlock higher annotation confidence.
- Automated CCS prediction for MS/MS spectral library annotations provides CCS-enabled annotation of any user-created or publicly available MS/MS library.
- CCSPredict Pro combined with MetFrag *in-silico* fragmentation routines enhance metabolite annotation confidence in the absence of reference CCS and MS/MS data.

> "The Bruker timsTOF Pro's combination of high scanning speed and the ability to generate CCS values is a unique combination, which can significantly improve our capabilities in high-throughput metabolomics."

Dr. Zheng-Jiang Zhu, Principal Investigator and Director of Metabolomics Research Center of Interdisciplinary Research Center on Biology and Chemistry, Chinese Academy of Sciences

References

[1] https://doi.org/10.1039/C8SC04396E
[2] https://doi.org/10.1186/1471-2105-11-148
[3] https://doi.org/10.1186/s13321-016-0115-9
[4] https://hmdb.ca/

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