Enhanced 4D workflows using TIMS for advancing small molecule research

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Introduction

Small molecule analyses in metabolomics, pharma and applied research often face similar challenges requiring the analysis of complex samples for detection, identification and (semi-) quantification of known and unknown analytes across a broad mass and analyte concentration range. Recent advances in high resolution LC-MS in combination with trapped ion mobility separation (TIMS) address the need for combining rapid analyses with confident identification. The ability of TIMS to separate coeluting isobaric and isomeric analytes and measure collisional cross sections (CCS) can increase confidence in compound identification. The TIMS-enabled PASEF data acquisition mode provides extensive MS/MS precursor coverage in every injection. Here were present an optimized workflow for 4D metabolomics using LC-MS and PASEF data acquisition capable of capturing analytes across a wide mass range in a single analysis, with dedicated data processing for broad profiling/screening applications.

Methods

Samples: Human urine; centrifuged and filtered (0.22µm). Methanolic human plasma (SRM 1950) extract. Custom standard mix including Restek Pharmaceutical Mix 1, Agilent Forensic Toxicology Comprehensive Mix, Imidazole, Tylosine A (both Sigma Aldrich).

LC: Elute UHPLC, Intensity Solo C18 column (Bruker).

- LC gradient according to T-ReX LC-QTOF Solution (Bruker), allows matching of retention times for >600 metabolomics relevant compounds [1].
- LC gradient according to TargetScreener 15 min gradient allows for annotation of > 2800 drugs, pesticides or mycotoxins [2].

MS: timsTOF Pro 2 (Bruker) equipped with VIP-HESI source

Acquisition: Optimized broad range PASEF and bbCID acquisitions

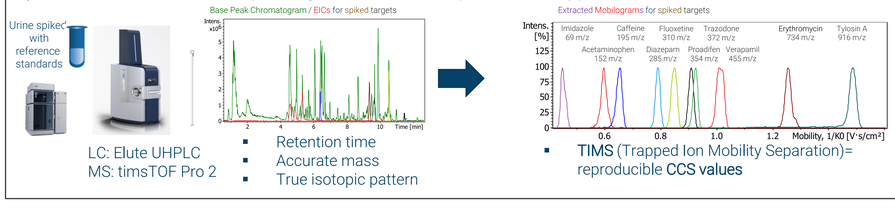
Software: TASQ and MetaboScape 2022b, preliminary version (Bruker).

Target Lists for annotation in MetaboScape:

- Target List 1: Compounds contained in Unified CCS Compendium [3] containing CCS, appended with retention times (RT) from Bruker HMDB Personal Library 2.0 and MS/MS references from Bruker HMDB Personal Library 2.0, Bruker MetaboBase Personal Library 3.0 and NIST 2020, respectively.
- Target List 2: Metabolites present in serum derived from HMDB 5.0 [4] including name, molecular formula and structure but no assigned MSMS or CCS reference values.

Results

A) LC-TIMS-MS/MS for broad profiling in metabolomics, pharma and applied research



LC: Elute UHPLC
MS: timsTOF Pro 2

- Retention time
- Accurate mass
- True isotopic pattern

- TIMS (Trapped Ion Mobility Separation)= reproducible CCS values

The novel data acquisition utilizes TIMS stepping and provides broad profiling and screening/quantitation in a single acquisitions for target samples.

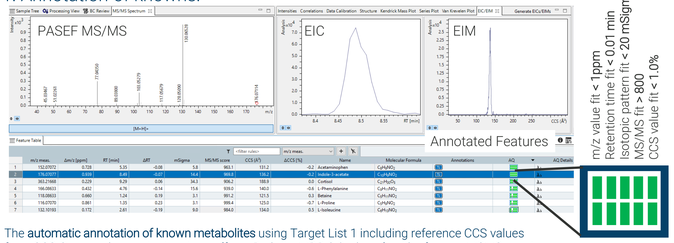
4D-Metabolomics™ data acquisition of a human urine sample spiked with a custom reference standard mix highlights wide mass and mobility transfer:
Spiked compounds detected from 69 m/z (Imidazole) to 916 m/z (Tylosin A).

This optimized transfer methods were combined with two complementary MS/MS acquisition modes. Parallel accumulation serial fragmentation (PASEF) (see B) and broad band CID (bbCID) (see C) which are routinely used for profiling and screening/quantitation workflows, respectively.

B) Profiling and annotation: DDA workflows with PASEF & MetaboScape

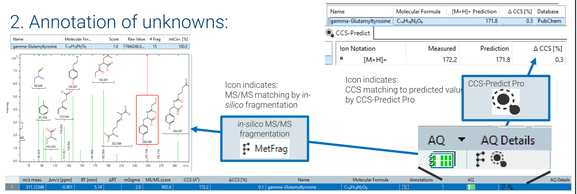
For routine profiling of complex samples the optimized transfer method was combined with parallel accumulation serial fragmentation (PASEF) data dependedent MSMS acquisition. PASEF provides fast MS/MS acquisition, increasing the depth of coverage for all small molecules. For methanolic extracts of human plasma this provided an MS/MS coverage of ~60-70% of extracted features. TIMS intrinsically generates ion mobility information which is automatically recalibrated and transformed into CCS values in MetaboScape® by the T-ReX® 4D feature extraction algorithm. CCS values in combination with MS/MS, retention time, accurate mass, and isotopic pattern fit allow for annotation of known target compounds with high confidence.

1. Annotation of knowns:



The automatic annotation of known metabolites using Target List 1 including reference CCS values from CCS Compendium, retention times (from Bruker HMDB 2.0 Library) and reference MSMS spectra, allowed for confident assignment of e.g. Indole-3-acetate. Mass error was below 1 ppm, retention time deviation below 0.1 min vs. HMDB 2.0 reference, MS/MS score >990 and CCS below 1% vs. CCS Compendium reference [3].

2. Annotation of unknowns:

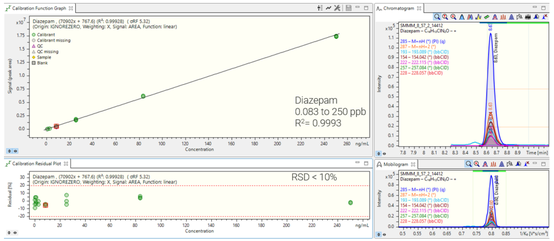


Annotating the human plasma data set with Target List 2 of compounds reported to be present in serum (derived from HMDB 5.0 [4]) enabled tentative annotation of further metabolites.
Note: This Target List could be appended with RTs, MS/MS spectra and/or CCS values from reference repositories as highlighted in B1. In case these are not readily available, MetaboScape can perform automatic CCS prediction and MS/MS matching based on InChI encoded structures. This is based on the CCS-Predict Pro model and MetFrag [5, 6], respectively.
Here it permitted tentative annotation of > 50 extracted features (not shown) with high scoring (<1ppm precursor mass, <20 mSigma, >800 MetFrag MSMS score, < 2% CCS vs. prediction).
The example highlighted shows the tentative annotation of gamma-Glutamyl-tyrosine MetFrag MS/MS score 992 (max. 1000); delta CCS vs prediction 0.3%.
Next step: Validate by comparison to reference standard.

C) Screening and quantitation: DIA workflows with bbCID and TASQ

Data independent broadband CID (bbCID) acquisition continuously cycles between low collision energy and elevated collision energy (bbCID) MS/MS. This provides seamless collection of accurate mass precursor, true isotopic pattern intensities and fragment qualifier ion data in a single analysis. In combination with the optimized method for broad transfer, TIMS-bbCID provides enhanced quantitation capabilities by mobility separating target compounds from co-eluting background noise.
Evaluating this data and considering high resolution diagnostic qualifier ion information and retention time information from the TargetScreener HR solution [2] means false positive detections are minimized, delivering maximum confidence in screening result.

A dilution series acquired from reference standards using TIMS-bbCID data was investigated using the TASQ software solution and linear dynamic ranges of quantitation were determined. The example shown for Diazepam shows >3.0 orders of magnitude linear dynamic range (0.1 to 250 ppb). The residuals plot shows deviations of <10% for all concentration levels, proofing the capability of the established method to enable quantitation across wide dynamic ranges.



Summary

We developed a CCS-enabled, single shot data acquisition for routine small molecule applications. This new workflow can be used for both targeted and untargeted samples and combines the confidence of high MS/MS coverage with CCS, resulting in a powerful workflow for broad screening, profiling, quantitation and ID capabilities for small molecule research.

References

[1] https://www.bruker.com/en/applications/academia-life-science/metabolomics/metabolomics-solution.html
[2] https://www.bruker.com/en/products-and-solutions/mass-spectrometry/ms-solutions/targetscreener.html
[3] https://doi.org/10.1039/C8SC04396E
[4] https://hmdb.ca/
[5] https://doi.org/10.1186/1471-2105-11-148
[6] https://doi.org/10.1186/s13321-016-0115-9

Note: HMDB and CCS Compendium are no Bruker products.

Conclusion

- The CCS-enabled, single shot data acquisition routines provide deeper insights for metabolomics, pharma and applied research.
- The CCS-enabled profiling workflow provides high confidence for automated compound annotation.
- The CCS-enabled screening/quantitation provides more than three orders of magnitude in linear dynamic range, separating target compounds from co-eluting background noise.
- Both workflows (profiling using PASEF MS/MS and screening/quantitation using TIMS-bbCID MS/MS) provide up to five confidence criteria for evaluation in MetaboScape and TASQ, respectively:
  - Accurate mass
  - Isotopic pattern fit
  - Retention time
  - MS/MS information
  - CCS values

4D-Metabolomics