Untargeted 4D-metabolomics in animal tissues' authenticity assessment, exploiting RP- & HILIC-HRMS analytical platforms incorporated with Trapped Ion Mobility Mass Spectrometry

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Introduction

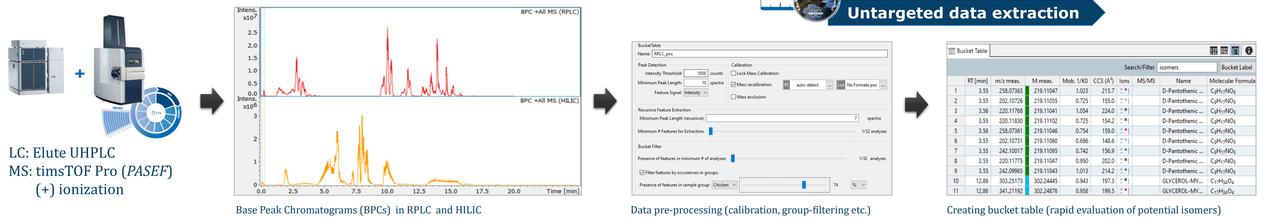
Metabolomics is being used to comprehensively understand a variety of food materials for improvement and assessment of food quality. Farm animal skeletal muscles are one of the major targets of metabolomics, aiming at the characterization of meat and the exploration of biomarkers associated with animal genetic background and sensory scores, or even feeding process treatments. In the present study, a novel 4D metabolomic approach utilizing both Reverse-Phase (RP) and Hydrophilic Interaction Liquid Chromatography (HILIC) analytical platforms is implemented to fully exploit animal muscle tissue metabolomic profiling. Trapped Ion Mobility Spectrometry (TIMS) is introduced as an additional dimension of analysis in High Resolution Mass Spectrometry (HRMS) workflows, providing a wealth of analytical information specifically in the case of animal muscle tissues.

Methods

Sample treatment was carried out utilizing a solid–liquid extraction step, including additional ultrasonic-assisted extraction. Precipitation of lipids/ proteins was performed by subjecting the extracts at −23 °C for 12 h. Further cleanup was implemented to ensure fat removal from the matrix. Samples were subjected to freeze drying before sample preparation. Analysis was performed using ultrahigh-performance liquid chromatography (UHPLC) coupled to a hybrid trapped ion mobility-quadrupole time-of-flight system (TIMS-QTOF) equipped with parallel accumulation-serial fragmentation (PASEF) (Bruker Daltonics, Bremen, Germany). DataAnalysis 5.2 software was used for initial evaluation and MetaboScape® 4.0 software and time aligned Region complete eXtraction (T-ReX) algorithm were utilized for non-targeted data treatment. Data were further evaluated through multivariate statistical analysis, including partial least squares discriminant analysis (PLS-DA).

Results

A) LC-TIMS-MS/MS for untargeted 4D-metabolomics

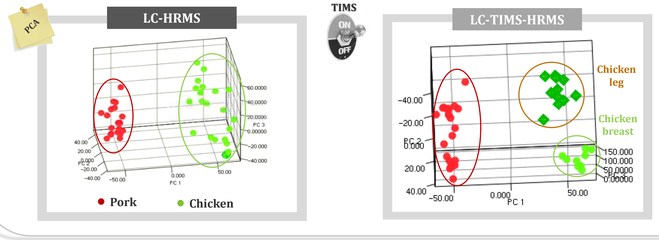


Base Peak Chromatograms (BPCs) in RPLC and HILIC

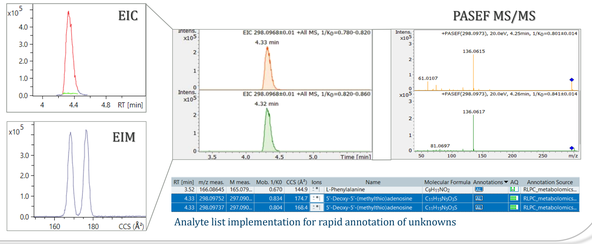
Data pre-processing (calibration, group-filtering etc.)

Creating bucket table (rapid evaluation of potential isomers)

B) Animal Origin Discrimination



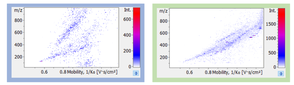
Isomers contribution to discrimination



Analyte list implementation for rapid annotation of unknowns

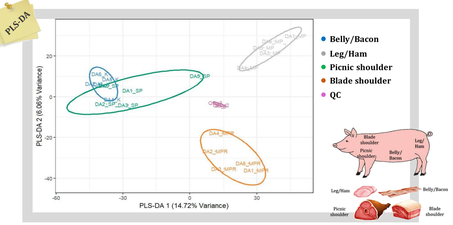
C) Animal Muscle Tissue Discrimination (pork case study)

Venn diagram of features detected in RP and HILIC

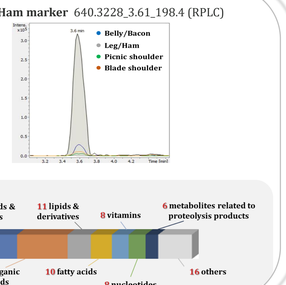


m/z-mobility heatmap in RP and HILIC (QC sample)

RP & HILIC



VIP markers



Samples of different animal muscles have been collected and analyzed in Liquid Chromatography (LC) -TIMS-HRMS utilizing both Reverse-Phase (RP) Hydrophilic Interaction Liquid Chromatography (HILIC). The combination of HILIC and RPLC coupled to High Resolution Mass Spectrometry (HRMS) expanded the number of detected analytes and provided a clear and comprehensive metabolite coverage.
Through the untargeted approach implemented, a significant number of features has been extracted, allowing the full evaluation of animal muscle tissues global profile. Data have been projected into a four-dimensional data space bounded by accurate mass, retention time, intensity and ion mobility. Compounds of different chemical classes have been successfully annotated using in-house developed databases, while potential isomers have been revealed, differentiated by their mobilities, (CCS values).
Furthermore, the combination of both RP and HILIC -TIMS-HRMS analytical platforms combined with the downstream multivariate analyses have revealed important biomarkers, of different polarity and/or hydrophobicity. Data were primarily evaluated through principal component analysis (PCA) in order to investigate potential clustering of animal muscle tissues, with the supervised chemometric technique of partial least squares discriminant analysis (PLS-DA) to follow.

Summary

A novel 4D-metabolomics approach was developed based on RP- & HILIC-HRMS workflows utilizing TIMS capabilities for animal tissues authenticity assessment. The combination of both analytical platforms, RP and HILIC, increased the depth-of-coverage, with a significant number of features being extracted from both platforms. The workflow proposed, introducing TIMS 4D analysis in the metabolomics approach, enabled the discrimination of animal origin (pork and chicken), as well regarding the animal muscle tissue in the case study of pork. Finally, compounds of different chemical compounds were identified based on our databases and notified as important authenticity markers.

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Conclusion

- The combination of RP and HILIC analytical platforms with TIMS provided a clear and comprehensive metabolite coverage in AMT authenticity study.
- The integration of TIMS in the workflow – 4D metabolomics enabled the discrimination of animal origin in the cases of pork and chicken.
- In TIMS-ON sufficient discrimination was achieved for chicken AMT (leg – breast), not enabled in TIMS-OFF.
- Different isomers were detected and co-estimated for discrimination purposes
- Adequate discrimination was accomplished in pork animal tissue authenticity challenge.
- 123 features were successfully identified with our analyte list, from 7 different chemical compounds.
- RP was proved as a capable platform to discriminate animal origin (pork – chicken)
- HILIC implementation was essential in the discrimination of AMT (with more 40 compounds being identified in the category of amino acids and derivatives).

4D-Metabolomics