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A metabolomics approach using Simultaneous Quantitation and Discovery (SQUAD) on high resolution accurate mass full MS[1] level

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ABSTRACT

Purpose
Develop an approach which enhances productivity by making use of a single injection for both unknown identification of potentially biological significant features as well as accurate quantitation of already known compounds combined in one high-resolution accurate mass (HRAM) based assay.

Methods
LC-MS quantitation of isotopically labeled internal standards spiked in NIST SRM 1950 plasma reference standard was performed on a Thermo Scientific™ Orbitrap Exploris™ MX mass detector using Thermo Scientific™ Hypersil GOLD™ HPLC as pre-separation technique. All data were acquired in full-scan MS[1] via alternating ESI(+) and ESI(-) polarity modes. The non-targeted identification of unknown plasma compounds was done using the very same data set.

Results
Quantitative results showed excellent sensitivity and a great dynamic range for the selected compounds in positive as well as in negative polarity mode. Data processing utilizing mzCloud™ spectral database resulted in a high rate of detected unique features with a rate of 20% coming along with a putative identification through full MS[1] level.

INTRODUCTION

There are different analytical strategies for metabolomics purposes which are split into two main sections: untargeted and targeted approaches. Untargeted setups are used for hypothesis-generating purposes which need identification of features of interest as a starting point. Targeted approaches are used for validation and translational purposes since they do offer accurate quantification but usually include few prioritized metabolites only and require known compounds.

The presented “Simultaneous Quantitation and Discovery” (SQUAD) workflow shows a combination of identification, accurate quantitation of preselected metabolites and the possibility of retrospective data mining while making use of the high-resolution accurate mass abilities of Orbitrap based systems. Therefore, the approach enhances productivity while getting the most insights out of each single injection.

MATERIALS AND METHODS

Metabolite Reference Standard NIST SRM 1950 plasma sample and isotope-labeled amino acids and organic acids were purchased from Sigma and CIL, respectively. Plasma was spiked with a dilution series (1 nM – 2.5 mM) of the labeled compounds quantified against the corresponding endogenous compounds in NIST SRM 1950 plasma. Extraction was performed with an excess of methanol. Reversed phase chromatography was applied as the technique of choice for pre-separation of the metabolites.

Figure 1 Sample Preparation

Utilizing authentic standards, isotope-labeled amino acids and isotope-labeled organic acids for confident identification and absolute quantitation. QC samples were incorporated to ensure high-quality data.

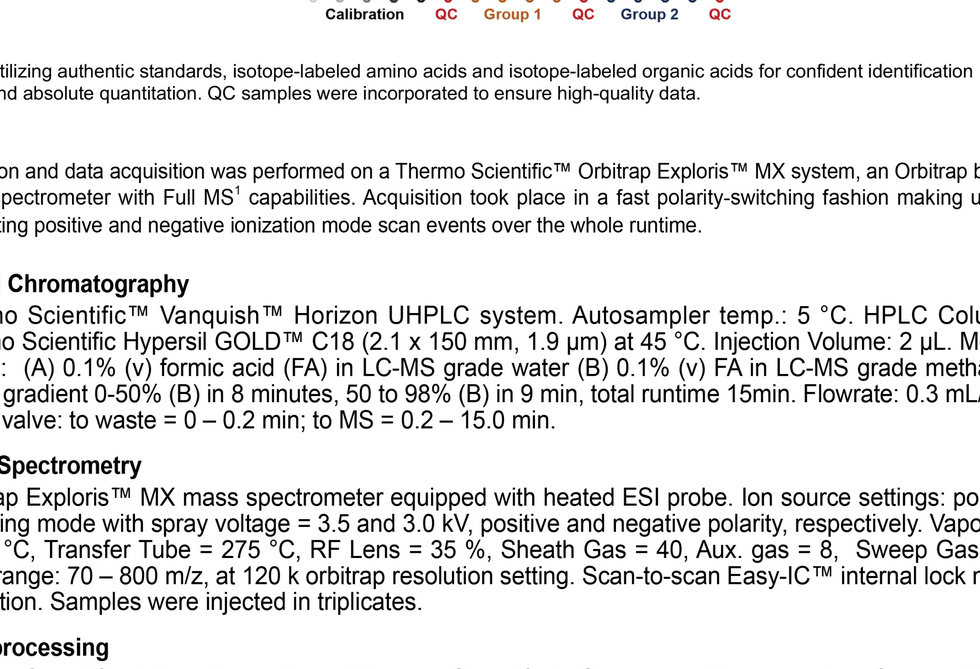
Detection and data acquisition was performed on a Thermo Scientific™ Orbitrap Exploris™ MX system, an Orbitrap based mass spectrometer with Full MS[1] capabilities. Acquisition took place in a fast polarity-switching fashion making use of alternating positive and negative ionization mode scan events over the whole runtime.

Liquid Chromatography
Thermo Scientific™ Vanquish™ Horizon UHPLC system. Autosampler temp.: 5 °C. HPLC Column: Thermo Scientific Hypersil GOLD™ C18 (2.1 x 150 mm, 1.9 µm) at 45 °C. Injection Volume: 2 µL. Mobile Phase: (A) 0.1% (v) formic acid (FA) in LC-MS grade water (B) 0.1% (v) FA in LC-MS grade methanol. HPLC gradient 0-50% (B) in 8 minutes, 50 to 98% (B) in 9 min, total runtime 15min. Flowrate: 0.3 mL/min. Divert valve: to waste = 0 – 0.2 min; to MS = 0.2 – 15.0 min.

Mass Spectrometry
Orbitrap Exploris™ MX mass spectrometer equipped with heated ESI probe. Ion source settings: polarity switching mode with spray voltage = 3.5 and 3.0 kV, positive and negative polarity, respectively. Vaporizer = 320 °C, Transfer Tube = 275 °C, RF Lens = 35 %, Sheath Gas = 40, Aux. gas = 8, Sweep Gas = 1. Scan range: 70 – 800 m/z, at 120 k orbitrap resolution setting. Scan-to-scan Easy-IC™ internal lock mass correction. Samples were injected in triplicates.

Data processing
Thermo Scientific™ TraceFinder™ and Thermo Scientific™ Compound Discoverer™ software utilizing mzCloud™ spectral database were used for data processing, analytes quantitation, and unknown annotation.

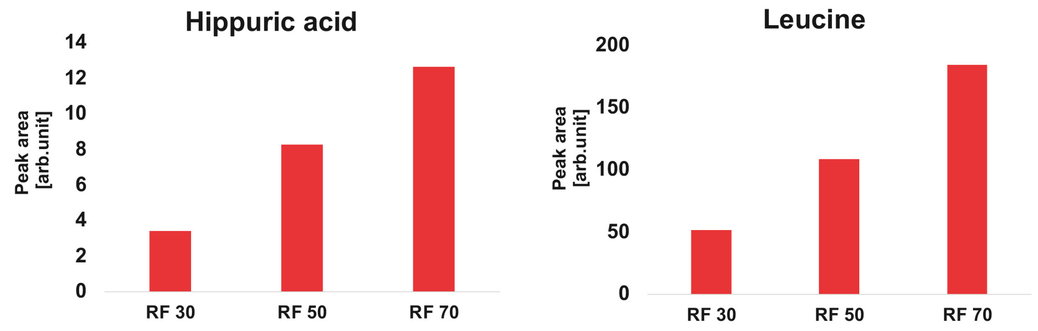
Figure 2 Instrumentation and post-processing Software



Data acquisition was performed on an Thermo Scientific™ Orbitrap Exploris MX instrument coupled to a Vanquish Horizon UHPLC system. For detection, fast polarity switching was applied ensuring a wider coverage. For data analysis, sophisticated and comprehensive software solutions were used that enable fast data processing, accurate quantification of metabolites, advanced differential analysis, confident metabolite annotation utilizing spectral libraries and databases, and biological interpretation.

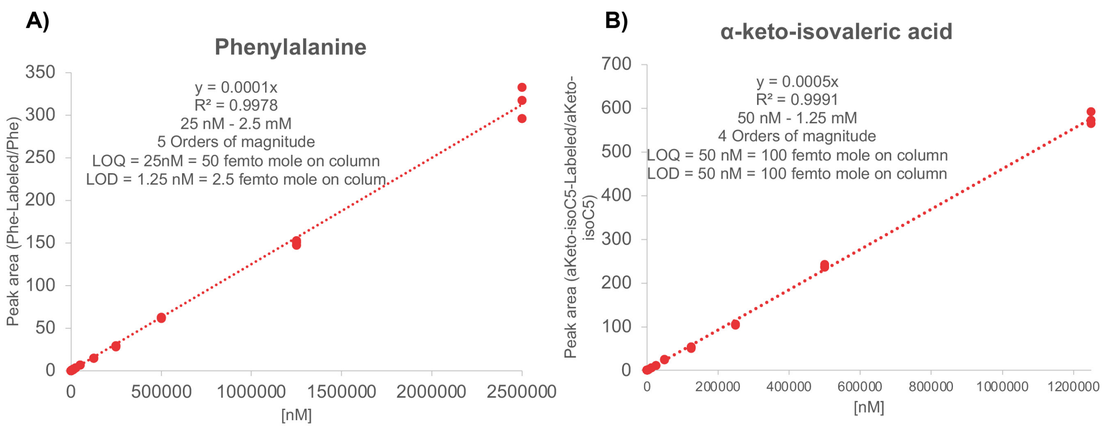
RESULTS

Figure 3 Method Optimization



Exemplarily shown method optimization for two compounds regarding the applied RF amplitude [%] on the S lens electrodes for an optimization towards best transmission over a broad variety of compounds and mass-to-charge ratios in a single full scan event.

Figure 4 Absolute quantitation results for A) phenylalanine and B) alpha-keto-isovaleric acid spiked in NIST SRM 1950 plasma reference standard.

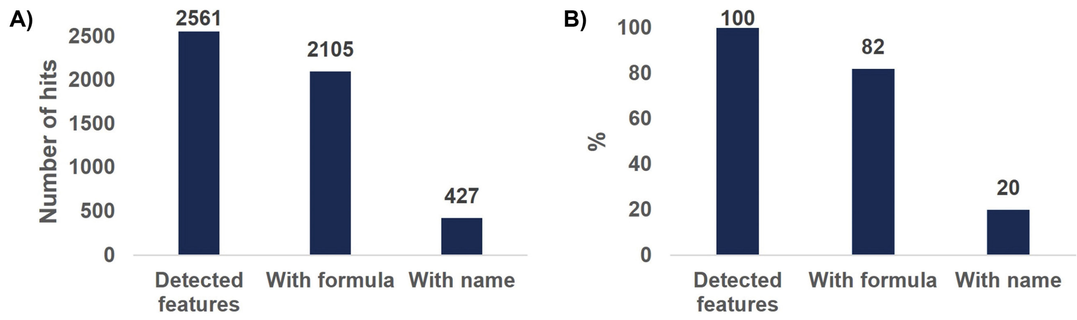


Absolute quantitation results exemplarily shown for phenylalanine (detected in positive ionization mode) and α-keto-isovaleric acid (detected in negative ionization mode). Quantitation is performed on the labeled compounds spiked in the plasma sample against their corresponding endogenous metabolites. While applying fast polarity switching for Orbitrap detection, both modes could be applied in the same run while acquiring sufficient data points for accurate and precise quantitation exhibiting excellent sensitivity in both ionization modes.

Table 1 Linear dynamic range, lower limits of quantitation (LOQ) and lower limits of detection (LOD) of the analyzed compounds using the outlined SQUAD approach, targeted portion.

Analyte	Calibration linear dynamic range	LOQ (femto mole on column)	LOD (femto mole on column)
Phenylalanine	25 nM – 2.5 mM (5 orders of magnitude)	50	2.5
isoleucine	25 nM – 2.5 mM (5 orders of magnitude)	50	10
Leucine	25 nM – 2.5 mM (5 orders of magnitude)	50	10
Tyrosine	25 nM – 2.5 mM (5 orders of magnitude)	50	10
Tryptophan	25 nM – 2.5 mM (5 orders of magnitude)	50	5
Valine	25 nM – 2.5 mM (5 orders of magnitude)	50	25
3-Hydroxybutyrate	250 nM – 2.5 mM (4 orders of magnitude)	500	500
a-keto-isovaleric acid	50 nM – 1.25 mM (4 orders of magnitude)	100	100
a-keto-isocaproic acid	50 nM – 2.5 mM (4 orders of magnitude)	100	100
Hippuric acid	12.5 nM – 0.5 mM (4 orders of magnitude)	100	100
Succinic acid	50 nM – 0.5 mM (4 orders of magnitude)	100	100
Uric acid	25 nM – 2.5 mM (5 orders of magnitude)	50	50
Creatine	25 nM – 2.5 mM (5 orders of magnitude)	50	50

Figure 5 Identification capabilities of the full MS[1] SQUAD approach



Data processing utilizing mzCloud™ spectral database resulted in a high rate of detected unique features and compounds with a rate of 20% coming along with a putative identification based on the accurate mass capabilities and the excellent isotope fidelity of the assay. The results are showing unique features only, excluding background ions, charge states and adducts. Identification capabilities are shown A) in absolute numbers and B) in percentage of overall detected unique features.

CONCLUSIONS

Overall, we developed an approach which enhances productivity by making use of a single injection for both unknown identification of potentially biological significant features as well as accurate and precise quantitation of already known compounds combined in one HRAM based, future-proof assay.

TRADEMARKS/LICENSING

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