Metabolomics Strategies Using GC-MS/MS Technology

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Introduction

The physico-chemical diversity of biological small molecules makes a metabolomic analysis very difficult. Therefore, different analytical strategies are necessary and are ideally combined. Analyses focusing on a group of metabolites or watching as many metabolites as possible at specified environmental or developmental stages is called metabolite profiling. On the other hand, hand metabolites target analyses are restricted to specific metabolites of interest, which can be selectively monitored and quantified. There is a discrepancy of detectable peaks in a typical metabolomics sample and the number of assignable chemical identifications due to limited availability of non-complete reference libraries and technical problems such as ultracomplex coelution of compounds and non-optimal mass spectral deconvolution in gas chromatograph-mass spectrometry. Therefore, we propose an integrative approach combining advantages of targeted analysis using multiple reaction monitoring (MRM) and full scan measurements to enhance the selectivity of the analysis, increase the number of identifiable and selectively quantifiable chemical structures and allow for absolute quantification strategies.

We investigated one of the key model systems for energy genomics, *Papulus sp.*, and its interaction with growth promoting endophytes. Although we have the full genome sequence, we cannot predict dynamic metabolic phenotypes. Here, metabolomics represent a key-technology to reveal the genotype – phenotype interaction [1].

**Figures and Tables**

**Figure 1.** The 2-tier strategy of combining full scan MS analysis of metabolites and targeted analysis. Full scan MS-analysis provides a completely unbiased identification of metabolites and metabolite dynamics. MRM measurements provide the targeted quantitation using the identical instrument platform.

**Table 1:** Selection of MRM-transitions for quantitation of metabolites. Using more than one transition confirms the identification of a peak and gives the possibility to select a matrix-dependent quantifier-transition. For a higher amount of SRMs it is crucial to use scheduled MRM methods in order to ensure sufficient data points for peak integration.

**Workflow Phase I: Discovery**

The discovery phase provides the identification of as many metabolites as possible by GC/MS Full Scan analysis. This phase is dominated by deconvolution of the chromatograms to extract the full and representative mass spectra for subsequent compound identification. The deconvolution step is greatly facilitated by using the AMDIS program and the NIST mass spectral library search program. Unique compound mass spectra are extracted by the analysis of all the transient ion signals allocating ion masses and relative intensities for eluting compound. Identification of metabolites is based on characteristic EI fragmentation patterns as well as on retention times (RT). The mass spectrum identification is facilitated by searching large data bases with NIST, Wiley or dedicated collections of mass spectra like GMD, Fiehn-Library, and in house metabolite databases (MOSYS database of TMS-derivatized metabolites and MRM transitions). LCQUAD (Thermo Scientific) is used for relative quantitation by peak integration of specified quantification ions. In Figure 3 an analysis of poplar leaves is shown either grown with growth-promoting endophytes or no endophytes. The chromatograms are extracted by the analysis of all the transient ion signals allocating ion masses and relative intensities for eluting compound.

**Workflow Phase II: Targeted Quantitation**

For the targeted workflow phase II suitable MS/MS transitions for each compound are used. The selected precursor ions get fragmented to structure specific product ions in the collision cell of the triple quadrupole MS. The integration of the product ion peaks provides the selective quantitation of all target metabolites. Figure 4 shows the chromatograms of IAA and glucose compounds at the level of 50 pmol injected amount. Both compounds although coeluting can be integrated independently from each other due to the different SRM transitions used.

**Conclusions**

GC-MS/MS with the TSQ Quantum provides both metabolic workflow phases on one instrument platform. This allows the application of both methods directly to the same sample providing highest convenience of sample handling and highest confidence with respect to technical variability:

**Phase I: Discovery phase analysis**

- Fast full scan analysis with deconvolution
- Access to the largest mass spectral know-how bases
- Reference library building

**Phase II: Quantitation**

- Very accurate quantitation
- High selectivity, high sensitivity by MS/MS mode
- High dynamic range for interesting metabolite markers
- Complex mixtures analysis using the MRM mode: Coeluting compounds get separated and individually quantified by compound-specific MRM

The TSQ Quantum XLS is the optimal instrument for use in metabolomics profiling to perform the metabolite screening as well as for the targeted metabolite quantitation in complex samples for the analysis of hundreds of target metabolites in one GC/MS/MS-MRM run, and high sample throughput work.

**References**


**Keywords:** metabolomics, GC-MS/MS, endophytes, metabolite discovery, coeluting compounds, targeted metabolite quantitation.