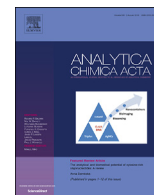
Analytica Chimica Acta 930 (2016) 13–22

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca



# Improving global feature detectabilities through scan range splitting for untargeted metabolomics by high-performance liquid chromatography-Orbitrap mass spectrometry

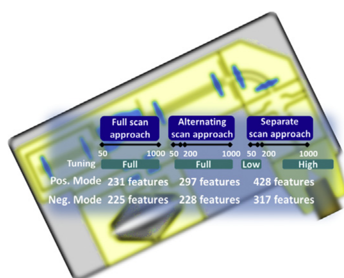
Christina Ranninger [a], Lukas E. Schmidt [a], Marc Rurik [b, c], Alice Limonciel [d], Paul Jennings [d], Oliver Kohlbacher [b, c, e, f, g], Christian G. Huber [a, *]

[a] Department of Molecular Biology, Division of Chemistry and Bioanalytics, University of Salzburg, Salzburg, Austria
[b] Center for Bioinformatics, University of Tübingen, Tübingen, Germany
[c] Department of Computer Science, University of Tübingen, Germany
[d] Department of Physiology and Medical Physics, Division of Physiology, Medical University of Innsbruck, Innsbruck, 6020, Austria
[e] Quantitative Biology Center, University of Tübingen, Germany
[f] Faculty of Medicine, University of Tübingen, Germany
[g] Biomolecular Interactions, Max Planck Institute for Developmental Biology, Tübingen, Germany

## HIGHLIGHTS

- The influence of tuning parameters on metabolite detectabilitiesin an Orbitrap mass spectrometer is shown.
- Scanning the whole mass range in two smaller segmentsimproves the number of detected metabolite features by 42–102%.
- Splitting the mass ranges leads to an improved detection of metabolites with higher *m/z* values.
- Ion abundance is significantly enhanced, allowing acquisition of MS/MS spectra of higher quality.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 16 February 2016
Received in revised form
13 May 2016
Accepted 16 May 2016
Available online 25 May 2016

*Keywords:*
Untargeted metabolomics
High-performance liquid chromatography

## ABSTRACT

Untargeted metabolomics aims at obtaining quantitative information on the highest possible number of low-molecular biomolecules present in a biological sample. Rather small changes in mass spectrometric spectrum acquisition parameters may have a significant influence on the detectabilities of metabolites in untargeted global-scale studies by means of high-performance liquid chromatography-mass spectrometry (HPLC-MS). Employing whole cell lysates of human renal proximal tubule cells, we present a systematic global-scale study of the influence of mass spectrometric scan parameters and post-acquisition data treatment on the number and intensity of metabolites detectable in whole cell lysates.

Ion transmission and ion collection efficiencies in an Orbitrap-based mass spectrometer basically depend on the *m/z* range scanned, which, ideally, requires different instrument settings for the respective

*Abbreviations:* EICC, extracted ion current chromatogram; ESI-MS, electrospray ionization mass spectrometry; GC-MS, gas chromatography-mass spectrometry; RP-HPLC, reversed-phase high-performance liquid chromatography; NMR, nuclear magnetic resonance spectroscopy; $MS^2$, tandem mass spectrometry; PBS, phosphate-buffered saline; QC, quality control; RF, radiofrequency; RP, reversed-phase; RPTEC/TERT1, TERT immortalized human renal proximal tubule epithelial cell line; RTICC, reconstructed total ion current chromatogram.

* Corresponding author. Department of Molecular Biology, Division of Chemistry and Bioanalytics, University of Salzburg, Hellbrunnerstraße 34, 5020, Salzburg, Austria.
*E-mail addresses:* christina.Ranninger@sbg.ac.at (C. Ranninger), lukas.schmidt-home@gmx.de (L.E. Schmidt), rurik@informatik.uni-tuebingen.de (M. Rurik), alice.limonciel@i-med.ac.at (A. Limonciel), paul.jennings@i-med.ac.at (P. Jennings), oliver.kohlbacher@uni-tuebingen.de (O. Kohlbacher), c.huber@sbg.ac.at (C.G. Huber).

http://dx.doi.org/10.1016/j.aca.2016.05.017
0003-2670/© 2016 Elsevier B.V. All rights reserved.

Electrospray ionization mass spectrometry
Data evaluation
Scan range optimization
Orbitrap

mass ranges investigated. Therefore, we split a full scan range of *m/z* 50–1000 relevant for metabolites into two separate segments (*m/z* 50–200 and *m/z* 200–1,000), allowing an independent tuning of the ion transmission parameters for both mass ranges. Three different implementations, involving either scanning from *m/z* 50–1000 in a single scan, or scanning from *m/z* 50–200 and from *m/z* 200–1000 in two alternating scans, or performing two separate HPLC-MS runs with *m/z* 50–200 and *m/z* 200–1000 scan ranges were critically assessed. The detected features were subjected to rigorous background filtering and quality control in order to obtain reliable metabolite features for subsequent differential quantification.

The most efficient approach in terms of feature number, which forms the basis for statistical analysis, identification, and for generating biological hypotheses, was the separate analysis of two different mass ranges. This lead to an increase in the number of detectable metabolite features, especially in the higher mass range (*m/z* greater than 400), by 2.5 (negative mode) to 6-fold (positive mode) as compared to analysis involving a single scan range. The total number of features confidently detectable was 560 in positive ion mode, and 436 in negative ion mode.

© 2016 Elsevier B.V. All rights reserved.

## 1. Introduction

Metabolomic studies involve the global analysis of small molecules (below 1000 Da) representing the non-polymeric set of intracellular compounds, such as amino acids, mono- and disaccharides, nucleotides, steroids, fatty- and dicarboxylic acids, or phospholipids [1–4]. In order to obtain an unbiased and comprehensive view on a biological system, non-targeted metabolomic investigations intend to gather as many metabolite signals as possible [5,6]. Due to the enormous structural diversity, metabolites turn out to be quite challenging analytes – particularly when it comes to obtaining the broadest spectrum of analytical information possible [7–9]. Hence, it is very demanding to find experimental conditions, including sample preparation and mass spectrometric data acquisition, that are optimal for all metabolites of interest, such that an all-encompassing methodology for metabolome analysis is currently not available [10].

One approach – albeit laborious and time-consuming – to handle this diversity of analytes is to measure a sample with a toolbox of different analytical methods including high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) [11], gas chromatography-mass spectrometry (GC-MS) [12], and nuclear magnetic resonance spectroscopy (NMR) [13] to obtain comprehensive (and complementary) information about a metabolite sample [11]. Nevertheless, the use of HPLC-ESI-MS alone has been shown to be a highly powerful tool for metabolomic studies capable of detecting more than 3000 features in a biological sample [14–16] – at clearly reduced effort and complexity compared to the combination of complementary analytical techniques. Since analytes of highly divergent physical and chemical properties are detected in a single analytical setup, experimental parameters usually need to represent a viable compromise enabling the detection of a broad range of chemically diverse metabolites.

Much effort has been put into maximizing the coverage of the metabolome as well as into providing sufficient ion abundance for high-quality tandem mass spectra ($MS^2$) required for metabolite identification. A better data-dependent-$MS^2$ efficiency was demonstrated by using multiple staggered *m/z* windows for gas-phase fractionation, which featured more uniform selection of precursor ions by encompassing low, medium, and high *m/z* ranges within a single experiment [17,18]. Nevertheless, these studies utilize different *m/z* ranges in the first quadrupole of a quadrupole-linear ion trap or a quadrupole-time-of-flight instrument primarily to “fractionate” candidate precursor ions under otherwise identical conditions and do not optimize tuning parameters in the different scan segments. The better MS/MS performance is mainly attributed to the reduced number of concurrent precursor ions selected for fragmentation. In another approach, the scan range in full-scan data acquisition was set as 50–500 for the first 2 min segment and 100–1,100 for the following segment in order to detect bile acids of different molecular mass, but a possible effect on the detectability of metabolites was not further studied [19]. Other experimental approaches to enhance the detectability of structurally divergent metabolites involved the use of different extraction solvents and conditions, the implementation of various chromatographic modes, as well as the addition of ammonium fluoride to the eluent to improve ionization efficiency in ESI-MS [20,21].

In this work we systematically investigate a way to improve small molecule detection by tailoring the operating parameters to molecular size and splitting the scan range acquired by the mass spectrometer into separate low and high mass ranges. The approach is based on our experience that mass-range dependent tuning parameters have an enormous impact on the detectabilities of proteins and nucleic acids [22]. Moreover, we recently published a computational data evaluation workflow, which, after strict background subtraction and quality control (QC), enables the fully automated extraction of significant metabolite features from highly complex HPLC-MS datasets obtained for untargeted metabolome analysis [23]. This provides us with a valuable tool for the evaluation of large-scale metabolomics datasets on a real sample containing several hundreds to thousands of metabolites over a high dynamic range instead of a small subset of low-molecular standard compounds, which may not be representative for highly complex samples of biological origin. In order to demonstrate the applicability in a large-scale metabolomics study, we systematically investigate the detectability of the methanol-extractable metabolites from lysates of a human renal proximal tubule epithelial cell line (RPTEC/TERT1) under three different data acquisition conditions. As a metric for the quality of the method, we will consider - after rigorous filtering and quality control - the number and signal intensity of analyte features that are confidently extracted from a large-scale metabolomic raw dataset.

## 2. Material and methods

### 2.1. Chemicals and samples

Acetonitrile for LC-MS was purchased from VWR (VWR International GmbH, Darmstadt, Germany). High-purity water was produced using a Milli-Q Integral 3 purification system from Merck (Darmstadt, Germany). Reagent-grade formic acid, methanol, and 3-nitrotyrosine were obtained from Sigma-Aldrich (St. Louis, MO, USA). The human renal proximal tubule epithelial cell line (RPTEC/

Download English Version:

https://daneshyari.com/en/article/1162755

Download Persian Version:

https://daneshyari.com/article/1162755

Daneshyari.com