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Mass spectrometric recommendations for Quan/Qual analysis using liquid-chromatography coupled to quadrupole time-of-flight mass spectrometry

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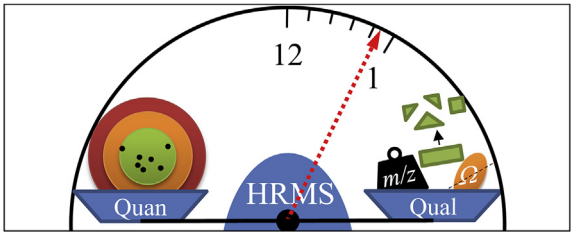
HIGHLIGHTS

- A widely applicable Quan/Qual method using high-resolution MS is proposed.
- Resolution, scan mode, scan rate and smoothing affect Quan/Qual performance.
- Recommendations are provided for future Quan/Qual method development.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: High-throughput simultaneous quantitative and qualitative (Quan/Qual) analysis is attractive to combine targeted with non-targeted analysis, e.g. in pharmacometabolomics and drug metabolism studies. This study aimed to investigate the possibilities and limitations of high-throughput Quan/Qual analysis by ultra-high performance liquid chromatography (UHPLC) coupled with high-resolution mass spectrometry (HRMS), to develop a widely applicable Quan/Qual UHPLC-HRMS method and to provide recommendations for Quan/Qual method development.
Methods: A widely applicable 4.25-min UHPLC method for small-molecules was used to investigate and optimize mass spectrometric parameters of a Synapt G2S for Quan/Qual analysis. The method was applied on a rat metabolomics study investigating the effect of the fasting state and administration of a dosing vehicle on the rat plasma metabolic profile.
Results: Highly important parameters for high-throughput Quan/Qual analysis were the scan mode and scan rate. A negative correlation was found between the amount of qualitative information that a method can provide and its quantitative performance (accuracy, precision, sensitivity, linear dynamic range). The optimal balance was obtained using the MS^E scan mode with a short scan time of 30 ms. This 4.25-min Quan/Qual analysis method enabled quantification with accuracy and precision values $\leq$ 20% at the lowest quality control (QC) level and $\leq$15% at higher QC levels for 16 out of 19 tested analytes. It provided both parent *m/z* values and fragmentation spectra for compound identification with limited loss of chromatographic resolution and it revealed biologically relevant metabolites in its application to the metabolomics study.
Conclusion: Quan/Qual method development requires balancing between the amount of qualitative data, the quality of the quantitative data and the analysis time. Recommendations are provided for MS

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resolution, scan mode, scan rate, smoothing and peak integration in Quan/Qual method development and analysis.

1. Introduction

High-resolution mass spectrometry (HRMS) has become increasingly popular over the last decade for use in quantitative bioanalysis [1]. It offers the possibility to do simultaneous quantitative and qualitative (Quan/Qual) analysis, which is attractive for studies combining targeted and non-targeted analysis, such as those investigating the absorption, distribution, metabolism and excretion (ADME) of a (radiolabeled) drug and pharmacometabolomic studies. Today, Quan/Qual analysis applying HRMS is still mostly applied in non-regulated early stage drug discovery and metabolomics. It is expected, however, that its use will expand to regulated bioanalysis [2], where currently the triple-quadrupole mass spectrometer (QqQ-MS) still runs the show. In order to compete with the contemporary short UHPLC-QqQ-MS analysis methods for large scale studies, a Quan/Qual analysis method needs to be fast and sensitive, and this represents a challenge for the high resolution mass spectrometer. Various Quan/Qual methods have already been published (e.g. Refs. [3,4]), but this work does not only provide a Quan/Qual method that is widely applicable for targeted and non-targeted analysis of small molecules, it focuses on the mass spectrometric challenges encountered when applying Quan/Qual in a high-throughput fashion and provides recommendations how to deal with these challenges. In addition, the applicability of the method is demonstrated in a metabolomics study.

Previously, we evaluated various sub-2 μm particle size ultra-high performance liquid chromatography (UHPLC) columns and mobile phases to develop a fast (4.25 min) and easy-to-use chromatographic method with an excellent chromatographic resolution [5]. In the present study, we aim to evaluate how to optimally use HRMS to achieve reliable quantification and collect qualitative information applying this chromatographic method, minimizing compromises to its chromatographic resolution.

Various mass spectrometers of several vendors are being or could be used for Quan/Qual analysis. Examples are the TripleTOF® series from Sciex, the Synapt® and Xevo® series from Waters, the 6500 series QTOF LC-MS and 6200 series TOF LC-MS systems from Agilent Technologies, the LCMS-IT-TOF MS from Shimadzu, the compact, impact™ and maXis™ series ESI-QTOF Instant Expertise™ mass spectrometers from Bruker and the Q-exactive™ from Thermo Scientific, each with their own advantages and limitations [6]. In the present study, we used the Synapt G2S mass spectrometer (Waters). An advantage of this instrument is the variety of scan modes, of which TOF-MS, MS^E and $HDMS^E$ provide an increasing amount of qualitative information. In the TOF-MS mode, the collision energy is at a fixed low level in order to detect the parent ions. In the MS^E mode, low and high collision energy scans are alternated to provide both accurate parent ion masses and data-independent fragmentation spectra of all ions. In the $HDMS^E$ mode, ion mobility separation is applied, providing an additional dimension of separation and an additional identifier of an ion, i.e. the collisional cross section, which is related to the drift time.

To obtain our goals, first the MS resolution mode and scan rate of a Synapt G2S mass spectrometer were optimized to accommodate the high-throughput Quan/Qual analysis. Then the TOF-MS, MS^E and $HDMS^E$ scan modes were evaluated for their quantitative performance in terms of accuracy, precision, linear dynamic range and sensitivity, using a test set of 19 small-molecule drugs, selected for their diversity in molecular masses (151–749 Da), hydrophobicity (log P of 0.91–6.7) and pKa values (ranging from an acidic pKa of 3.77 to a basic pKa of 9.68). The qualitative performance of the MS^E and $HDMS^E$ mode were evaluated based on an *in-vitro* drug metabolism study.

An ideal example application of the developed Quan/Qual analysis method is to simultaneously investigate drug metabolism and changes in endogenous metabolism related to the drug administration. The problem in this use is however that *in vivo* ADME studies often lack (appropriate) control samples and make use of generic blank control samples. In this case, drug metabolites may still be identified based on radioactivity measurements (in case of radiolabeled mass balance studies) or fragmentation patterns. However, differences between endogenous metabolite levels which changed upon drug administration are more complicated to find and may include many false positives that are only related to e.g. fasting state or to a dosing vehicle. In this perspective, Fiebig et al. recently recommended the use of control samples of the same gender and intravenously dosed with the same dosing vehicle, for ADME studies in rat [7]. Here we demonstrate the applicability of the Quan/Qual method for metabolomics studies in a similar set-up, investigating differences in rat plasma metabolome caused by a dosing vehicle, the fasting state or by bench instability (i.e. short-term storage at room temperature). The results show that the developed method is not only fast and widely applicable, but also capable to provide biologically relevant information.

2. Experimental

2.1. Chemicals

Water was obtained from a Milli-Q Purification System from Millipore (MA, USA). Acetonitrile, methanol (both Ultra LC-MS grade) and isopropanol (HPLC-MS grade) were supplied by Actu-All Chemicals (Oss, the Netherlands) and dimethylsulfoxide (DMSO, ≥99.7%) and formic acid (ULC-MS grade) by Biosolve (Valkenswaard, the Netherlands). Ammonium acetate (>99%) and the drug product standards of acetaminophen, tolbutamide, 19-norethindrone, omeprazole, prednisone, buspirone hydrochloride, (+/−)-verapamil hydrochloride, nefazodone hydrochloride and loperamide hydrochloride originated from Sigma-Aldrich (St. Louis, MO, USA). Abiraterone was supplied by Cambridge Major Laboratories, Inc. (WI, USA). Cilag AG (Schaffhausen, Switzerland) provided darunavir ethanolate and midazolam was obtained from Actavis (Dublin, Ireland). Janssen Research and Development (Beerse, Belgium) supplied the drug product standards of galantamine hydrobromide, rilpivirine, risperidone, bedaquiline and simeprevir. An overview of the physicochemical properties of the compounds is provided in Supplementary Table 1. The internal standards acetaminophen-d4, tolbutamide-d9, galantamine-*O*-methyl-d3, risperidone-d4, verapamil-d6 hydrochloride and bedaquiline-d6 (mixture of diastereomers) and the metabolites 1′-hydroxy midazolam and 4-hydroxy midazolam were obtained from Toronto Research Chemicals (Toronto, Canada).

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