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# Quantitative performance of a quadrupole-orbitrap-MS in targeted LC–MS determinations of small molecules

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ABSTRACT

High-resolution mass spectrometry (HRMS) has been associated with qualitative and research analysis and QQQ-MS with quantitative and routine analysis. This view is now challenged and for this reason, we have evaluated the quantitative LC–MS performance of a new high-resolution mass spectrometer (HRMS), a Q-orbitrap-MS, and compared the results obtained with a recent triple-quadrupole MS (QQQ-MS). High-resolution full-scan (HR-FS) and MS/MS acquisitions have been tested with real plasma extracts or pure standards. Limits of detection, dynamic range, mass accuracy and false positive or false negative detections have been determined or investigated with protease inhibitors, tyrosine kinase inhibitors, steroids and metanephrines. Our quantitative results show that today's available HRMS are reliable and sensitive quantitative instruments and comparable to QQQ-MS quantitative performance. Taking into account their versatility, user-friendliness and robustness, we believe that HRMS should be seen more and more as key instruments in quantitative LC–MS analyses. In this scenario, most targeted LC-HRMS analyses should be performed by HR-FS recording virtually "all" ions. In addition to absolute quantifications, HR-FS will allow the relative quantifications of hundreds of metabolites in plasma revealing individual's metabolome and exposome. This phenotyping of known metabolites should promote HRMS in clinical environment. A few other LC-HRMS analyses should be performed in single-ion-monitoring or MS/MS mode when increased sensitivity and/or detection selectivity will be necessary.



## 1. Introduction

Actual LC-high-resolution MS instruments (HRMS) have the versatile capability to perform reliable and sensitive quantitative and qualitative analyses or both (Quan/Qual) while recording high-resolution full scan (HR-FS) or MS/MS mode [1–5]. Therefore, most actual HRMS instruments can be seen as a true alternative to triple-quadrupole MS (QQQ-MS) in routine and quantitative LC–MS analyses [6]. Today's affordable HRMS are (quadrupole)-orbitrap-MS (e.g. Q-/Exactive®; Q-orbi-MS) and quadrupole-time-of-flight-MS (Q-TOF).

In contrast to ion transition monitoring (SRM), HR-FS acquisition gives a much more complete overview of the content of a sample extract. HR-FS allows robust targeted quantifications with the construct of extracted ion chromatograms (XIC) using a narrow mass-extraction-window (MEW) that is centered on the theorerical *m/z* [6]. Moreover, HRMS allows compound identification, metabolite phenotyping, data mining for biomarker discovery and retrospective data treatment which can be a true advantage for research investigations or legal claims. A global sample picture recorded by HR-FS or simultaneous HR-FS and MS/MS acquisitions (e.g. data independent acquisition, DIA) could be preferred by Authorities as it gives a certain "traceability". HRMS versatility is an undeniable asset and will be the driving force to promote the shift in favor of HRMS [1,2]. In addition, the use of the same MS technology for quantitative and qualitative analyses should result in workflow optimization and more efficient lab organization.

Whereas the HRMS capability for qualitative (Qual) analyses, is "unquestionable", there are still remaining questions for the quantitative (Quan) performance of newest HR instruments, especially in comparison to QQQ-MS performing SRM acquisitions. Already, accumulating data shows that the quantitative performance of HRMS are slightly below, similar or better than the performance of QQQ-MS [5,7,8].

Some authors have mentioned some possible hindrances [9–11] such as 1. the inutility to record large HR-FS data or 2. the very

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distinct knowledge between bioanalysts that are specifically dedicated to quantitative or to qualitative analyses, suggesting that there are possibly no advantages to use one technology for both quantitative or qualitative workflows [9]. These points have been addressed elsewhere [1,2,9–11] and these reports support the use of HRMS technology. Here, we aimed at focusing our work on evaluating the quantitative performance of a new Q-orbi-MS in a clinical environment.

Typical evaluation of quantitative performance in LC–MS analyses, is listed in the following points: #1: accuracy and precision of quantified analytes, #2: linearity of calibration curves and dynamic range, #3: sensitivity, #4: detection reliability (false positive or false negative detections as a results of mass accuracy deviation), #5: detection selectivity, #6: user-friendliness in running the analysis and in data treatment and #7: availability of standard operating procedures and Authorities' guidelines for quantification in certified labs.

All of the above-mentioned points have been already investigated to some degree. Point #1: many published data show similar precision and accuracy in the quantification of xenobiotics or endogenous metabolites in plasma using HRMS or QQQ-MS (e.g. Passing and Bablok regressions) [4,5,12–14]. Point #2: there are similar dynamic range and linearity with newest HRMS [7,12] whereas this was not the case with "old" Q-TOF MS [15]. Point #3: there are comparable limits of detection, even if it is still under debate in part because the latest hardware resulting in sensitivity improvement, are first installed on QQQ-MS [4]. Point #4: robust quantification and reliable detection have been reported for various kinds of compounds and hundreds of real samples [13] but need appropriate MEW width, mass accuracy and mass calibration [16]. Point #5: when the resolution is > 25,000 and the MEW is ≤ 10 ppm, the HR-FS acquisition is as selective as ion transitions with low resolution MS [17–20], even if in some cases MS/MS acquisition performed on HRMS appears necessary [8]. Point #6: HRMS technology is user-friendly (e.g. easier troubleshooting in HR-FS, no collision energies to tune, etc.) and allows running and processing hundreds of samples. Point #7: data are compatible with certified laboratories and authority guidance [9].

The goal of this study was to evaluate the quantitative performance of a recent Q-orbi-MS on 4 types of compounds: protease inhibitors (PI), tyrosine kinase inhibitors (TKI), metanephrines and steroids. These compounds are measured routinely in our hospital by LC–MS with QQQ-MS systems. First, plasma samples were extracted following validated methods. Exact same calibration samples of PI, TKI were injected on HRMS and QQQ-MS systems performing with the same type of LC pumps, autosamplers, exact same LC columns (same serial number) and LC conditions. External calibrations (compound peak area) were determined to establish the dynamic ranges (linear curve behavior, #2), limits of detection (LOD, #3) and analytical workflow (#6). These results were compared head-to-head with those obtained on a QQQ-MS. Secondly, additional quantitative evaluations were performed only on the Q-orbi-MS and took into account different HRMS acquisitions, mass accuracy (risk of false detections), ion source and mass resolution values.

## 2. Experimental

### 2.1. Materials, LC–MS systems and parameters

The MS instruments were a Q-Exactive Focus® HRMS or a TSQ Quantum ultra QQQ-MS (both from Thermo Fischer Scientific, USA) and were connected to a similar LC system. The chromatographic systems consisted of a UHPLC quaternary pump (Rheos, Flux. Switzerland), an autosampler (CTC Analytics AG, Switzerland) maintaining injection vials at 10 °C.

For PI and TKI, plasma samples were extracted and injected onto various C18 analytical columns (30–50) x 2.1 mm (L x i.d.), <2 μm or 3.5 μm, part. size. For PI, TKI and steroids, the mobile phases were composed of, respectively, (a) 2 mM or 20 mM ammonium acetate with 0.1 or 1% formic acid and (b) MeCN with 0.1–1% formic acid (pH 2.8) and were delivered to the heated electrospray ion source (H-ESI-II) at 0.3–0.6 mL/min using stepwise gradients (5–100% of B but with a slight gradient to 30% or 50% of B). Exact same ESI source design (H-ESI-II, Thermo, USA), position and conditions were used on the HRMS and QQQ-MS instruments. Typically, ion source spray voltage, capillary temperature, sheath and auxiliary nitrogen flow rate were set at 4 kV, 350 °C, 35 and 10 (arbitrary units), respectively. Nitrogen gas temperature was deactivated unless otherwise mentioned. Total analytical run times were between 10 and 20 min. See references for additional details [21,22].

For metanephrines the analyses were performed according to Peatson et al. [23] The analytical column consisted in an Atlantis HILIC Silica (2.1 × 30 mm, L x i.d., 3 μm, part. size; Waters Corp., USA) and mobile phases of (A) 100 mM of ammonium formate in water (pH 3.0) and (B) MeCN. The gradient was from 95% of B to 70% B and the flow rate was 0.35 mL/min [23].

High resolution MS full scans (HR-FS) were acquired on a mass range ≥200 Da (typically, from $m/z$ 200–1000) with an AGC (automatic gain control) and a resolution set at $1 \times 10^6$ ions and 70,000 (at $m/z = 200$), respectively. Parallel-reaction-monitoring (PRM) and Data-Independent-Acquisition (DIA) were also tested as MS/MS mode of acquisition. PRM are product ion scan acquisition with precursor isolation usually <10 Da following with the XIC construct with product ion(s). In our PRM acquisition, resolution, isolation width and AGC values were set at 35,000, 4 Da, and $1 \times 10^5$ ions. Collision energies were set at 30 and 35 arbitrary units for TKI and PI drugs, respectively. DIA is a product ion scan acquisition recorded in parallel to HR-FS acquisition. In DIA acquisition, isolation widths of precursor ions are usually >20 Da. In our DIA analyses, HR-FS and DIA were set at a resolution of 70,000 and 35,000, respectively. Collision energies were similar to PRM acquisitions. Isolation width and AGC were 50 Da and $5 \times 10^5$ ions, respectively.

LC-HRMS analyses were performed with an internal mass calibration using a lock-mass recalibrating the MS from 0 to 0.25 min (<void volume) in each run. The calibrator ion was a plasticizer contaminant, $C_{10}H_{15}NO_2S$, found in the mobile phase at $m/z$ 214.08963 and 212.07507 in $ESI^+$ and $ESI^-$ mode, respectively. Mass recalibration in each run is not mandatory but can reduces the mass shift by a couple of ppm. Please, refer to the *Results* for more details.

Extracted ion chromatograms (XIC) were based on a mass-extraction-window (MEW) centered on the theoretical $m/z$ ($m/z_{theor}$). MEW widths were ± 5 or 10 ppm. LC-HRMS data acquisition, peak integration, and quantification were performed using Xcalibur software (Thermo, USA).

On the QQQ-MS, ion transitions were performed at unit resolution (*full-width-half maximum* set at 0.7 Da). Ion transitions (parent-to-product ions) and collision energies of PI and TKI can be found in the previously published methods [21,22].

### 2.2. Sample biomatrices and preparations

Human plasma samples were prepared from whole blood withdrawn in collection tubes (Sarstedt® S-monovettes with EDTA anticoagulant, Germany). Exact same extracts were spiked with protease inhibitors (PI) and tyrosine kinase inhibitors (TKI) and injected on an LC-QQQ-MS and LC-HRMS for a head-to-head comparison.

For TKI (dasatinib, imatinib, nilotinib sorrafenib and sunitinib), plasma samples were prepared and analyzed according to