# Comparison between a high resolution Exactive and a triple quadrupole MS for quantitative analyses of drugs

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## Abstract

We have compared the analysis performed with a LC coupled to a triple quadrupole MS or with a stand alone Orbitrap-MS (*Exactive*) in the quantitative determination of 8 Anti-Fungal Agents (AFA), 4 Immuno-Supressive Agents (ISA) and 5 Protein Kinase Inhibitors (PKI) in human plasma samples. Detection specificity, accuracy, precision etc. were evaluated in Cs, QCs and patients' samples. It indicates that extracted ion chromatograms (XIC) from high-resolution (HR) full-scan recorded in an Exactive™ benchtop LC-MS are fully capable of reliable drug quantification. Taken into account that HR full-scan acquisition shows advantages over ion transition acquisition, our results could change the way of performing routine or research and qualitative or quantitative analyses in various labs in the future.

## Introduction

Today, most quantitative analyses are performed by LC coupled to triple quadrupole-MS (TQ-MS). However, recently introduced high-resolution MS (HR-MS) have challenged TQ-MS for absolute quantification. Indeed, TQ-MS technology has some disadvantages such as extensive time and expertise required to set up ion transitions. Also, the SRM analysis is limited to molecules selected for analysis. These disadvantages could be overcome by HR-MS since the quantitative determination could be done from accurate m/z extracted chromatograms from full scan acquisition (HR-XIC). Eight anti-fungal agents (AFA: anidulafungin, caspofungin, fluconazole, itraconazole, posaconazole and voriconazole), 4 immuno-suppressive Agents (ISA: ciclosporine, everolimus, sirolimus and tacrolimus) and 5 protein kinase inhibitors (PKI: dasatinib, imatinib, nilotinib, sorafenib and sunitinib) were quantitatively determined in human plasma samples by LC coupled to TQ-MS. Then, the exact same samples were injected onto a similar LC set-up but coupled to Exactive mass spectrometer power by Orbitrap™ technology. See **Table 1**.

**Table 1.** Quantitated drugs and mode of detection: XIC of the monoisotopic m/z ([M+H]⁺) with a 10ppm window (HR-MS) or ion transitions (TQ-MS).
(■), all ISA were detected as (NH4)+ adduct; (*), z = 2

| | Drug | *monoisotopic m/z* | ion transition (*m/z* @ CE [eV]) |
|---|---|---|---|
| AFA | Anidulafungin | 1,140.5136 | 1,140.5 → 343.0 + 388.0 @ 31 |
| | Caspofungin (*) | 547.3288 | 547.3 → 86.0 @ 40 |
| | Fluconazole | 307.1113 | 307.1 → 238.1 @ 25 |
| | Hydroxy-itraconazole | 721.2415 | 721.2 → 392.3 + 406.3 @ 35 + 33 |
| | Itraconazole | 705.2466 | 705.2 → 392.2 + 432.2 @ 31 |
| | Posaconazole | 701.3370 | 701.3 → 127.0 + 683.3 @ 58 + 28 |
| | Voriconazole | 350.1223 | 350.1 → 281.1 @ 16 |
| | Voriconazole N-Oxide | 366.1172 | 366.1 → 224.1 @ 13 |
| ISA (■) | Cyclosporine A | 1219.8752 | 1220.0 → 1202.9 @ 18 |
| | Everolimus | 975.6152 | 975.6 → 908.5 @ 15 |
| | Sirolimus | 931.5890 | 931.6 → 864.5 @ 18 |
| | Tacrolimus | 821.5158 | 821.5 → 768.3 @ 20 |
| PKI | Dasatinib | 488.1630 | 488.2 → 401.1 @ 38 |
| | Imatinib | 494.2663 | 494.3 → 394.1 @ 36 |
| | Nilotinib | 530.1911 | 530.2 → 289.1 @ 38 |
| | Sorafenib | 465.0936 | 465.1 → 252.1 @ 47 |
| | Sunitinib (*) | 399.2191 | 399.2 → 283.1 @ 34 |

## Materials and Methods

### Sample Quantification

Internal standard methodology following FDA guidelines. The dynamic range of calibration curves were 500 for AFA and PKI and <50 for ISA analyses.

### Sample Preparation

**AFA analyses**: 200µL of plasma. Prot. Precipit. with 2 vol. of 0.4M ZnSO :MeOH (2/8-v/v). Direct injections of supernatants.

**ISA analyses**: 100µL of Plasma. Prot. Precipit. with 3 vol. of MeCN. Direct injections of supernatants 3x diluted.

**PKI analyses**: 100µL of Plasma. Prot. Precipit. with 7 vol. of MeCN-MeOH (6/1-v/v). Direct injections of supernatants 3x diluted.

### LC Conditions

**AFA analyses**: Analytical column: 2.1x30mm Acquity® UPLC C18 1.7µm (Waters). Mobile phase : A) 10 mM NH4 formate + 0.1% formic acid (FA) and B) MeCN + 0.1% FA; gradient: 2 to 95% of B); total run time = 7min; flow rate = 300µL/min; inj. vol. = 10µL.

**ISA analyses**: Column switching set-up.Trap and analytical columns: 2.1x10mm and 50mm resp. XTerra® HPLC C18 5µm (Waters). Mobile phase : A) 2mM NH4 acetate + 0.1% formic acid (FA) and B) MeOH+ 0.1% FA; gradients: 5 to 100% and 65 to 100% of B), resp.; total run time = 14 min; both flow rates = 400µL/min; inj. vol. = 50µL

**PKI analyses**: Analytical column: 2.1mmx50mm XTerra® dC18 5µm analytical column (Waters). Mobile phase: A) 20mM NH4 acetate pH2.2 (with FA)and B) MeCN; gradient: 5 to 100% of B); total run time = 20min; flow rate = 300µL/min; inj. vol. = 20µL.

### MS Detection

ESI *discovery* and *ion max* sources, positive mode. Triple Quad. MS discovery (ISA) and Ultra (AFA + PKI) from Thermo, performing ion transitions and Exactive-Orbitrap-HR-MS from Thermo performing HR full scans (a MS full scan and a HCD MS full scan set at resp., 50K and 10K resolution -*R*-). See **Table1**.

## References

Bateman KP et al., J Am Soc Mass Spectrom 2009; Décosterd LA et al. AAC 2010; Haouala A. et al., J Chrom B 2009; Kaufmann A et al., RCMS 2011; Scigelova and Makarov, Bioanalysis, 2009; Zhang NR et al., RCMS 2009

## Conclusion

The comparative analysis of three classes of drugs shows -

- HR-AM analysis to be as specific as SRM analysis
- HR-AM analysis is compatible with sample preparation methods used for SRM analysis
- HR-AM assay is as precise as SRM assay
- HR-AM analysis leads to significant time saving in method development
- HR-AM analysis is an excellent alternative for routine labs that lack high end mass spectrometry expertise.

## Results.

According to the chromatograms (**Fig. 1**)., m/z distribution (**Fig. 2**). and mass deviation (<3ppm), the Exactive-MS shows excellent detection specificity without any constraints (lock mass or frequent mass calibrations). No difference of selectivity were observed between the 2 acquisition types.

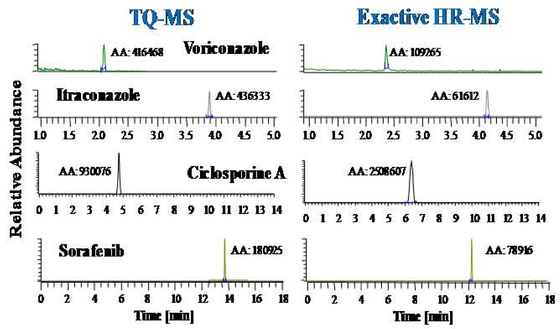


**Fig. 1.** Typical LC-TQ and LC-Exactive HR-MS chromatograms of human plasma extracts.

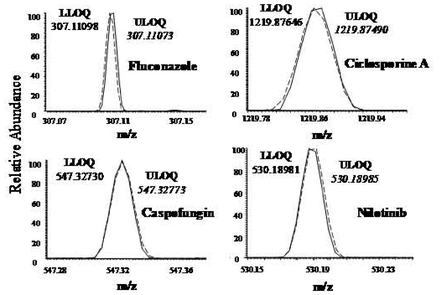


**Fig. 2.** Representative m/z distribution at LLOQ and ULOQ (straight and dashed lines, resp.)

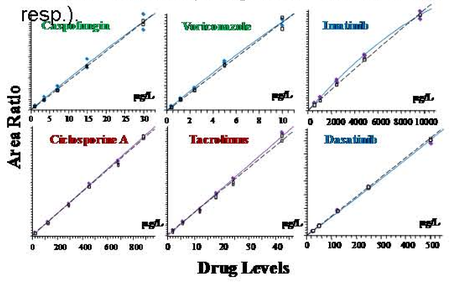


**Fig. 3.** Typical superimposed calibration curves obtained by LC-TQ (blue lines) and LC-Exactive-MS (dashed lines) from human plasma extracts.

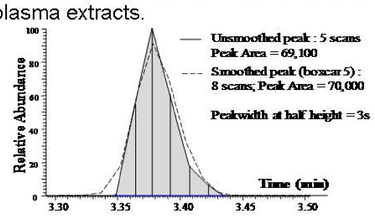


**Fig. 4.** UPLC peak of posaconazole at LLOQ levels obtained with Exactive-MS at *R*=50,000.

Considering the superimpositions of the calibration curves obtained with the TQ and HR-MS (**Fig. 3**), the CV (%) of Cs and QCs samples and the number of rejected Cs and QCs (data not shown), no differences were observed between TQ and HR-MS.

In UPLC analyses of AFA, the number of scans per peak (≥2.5 sec at half height) were in general ≥5 (**Fig. 4**). However, it did not modify precision or peak area. Moreover, 7 scans per peak could have been acquired if only one full scan would have been recorded.

93 patients' drug levels determined by TQ-MS and by HR-MS, were compared (**Fig. 5**). Only 5 determinations show an accuracy below 80% that is due to drug degradation (voriconazole-NO) or unidentified reasons.

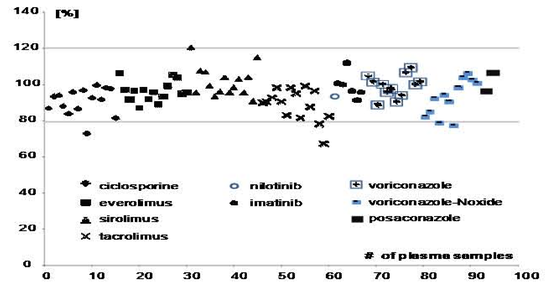


**Fig. 5.** Accuracy of 93 drug levels measured by HR-MS relatively to the levels measured by TQ-MS