

Use of the Thermo Scientific™ Vanquish Horizon UHPLC and Orbitrap Exploris™ 120 in Discovery DMPK



Iain Beattie, Lucy Burton, Victoria Ford, Rina Mistry and Laie Abelló
Charnwood Discovery, Charnwood Campus, 9 Summerpool Road, Loughborough, LE11 5RD

Introduction

Discovery Drug Metabolism and Pharmacokinetics (DMPK) involves screening large numbers of compounds in a variety of assays with a Liquid Chromatography/Mass Spectrometer (LC/MS) endpoint, either for quantifying a compound or monitoring its disappearance. The triple quadrupole has, until recently, been the standard instrument of choice, however, there has been a gradual shift towards using the latest generation of high-resolution MS (HRMS) instruments. These instruments offer similar performance in terms of sensitivity and dynamic range, with the high resolution and accurate mass capability providing the selectivity. These instruments use full scan analysis negating the need to establish an MS/MS method for every compound, saving a significant amount of time. A further advantage of full scan analysis is it allows the quantification of compounds that don't fragment easily, form significant adducts (e.g. sodium), multiply charged species or undergo significant in-source fragmentation and these types of challenging compounds are being encountered more frequently with the emergence of PROTACs and peptides. Another major advantage of acquiring full scan data for quantitative work is that it can, for example, detect metabolites in metabolic stability assays or identify potential issues that would adversely affect the data (e.g. matrix interference), allowing a solution to be developed. The Thermo Scientific™ Orbitrap Exploris™ 120 with the Thermo Scientific™ Vanquish UHPLC system is being used to routinely provide quantitative data from *in vitro* and *in vivo* DMPK assays and proving to be a very capable, robust, and sensitive system.

Methods

All quantitative analyses were carried out on a 1.4 minute method with a Waters 50 x 2.1mm BEH C18 1.7 µm UPLC column and either a methanol or acetonitrile gradient with 0.1% formic acid or 0.025% ammonium acetate with 0.1% formic acid. Full scan MS data was acquired using a resolution setting of 30000.

Instrument: Thermo Scientific™ Orbitrap Exploris™ 120 Mass Spectrometer and Thermo Scientific™ Vanquish Horizon UHPLC.

System Reproducibility

Fluctuations in MS response impact data quality and may be observed due to variable injection volumes and/or source reproducibility. Ideally, a stable label internal standard of the analyte would be used to account for variability, but these are rarely available at the discovery stage. Although a close analogue or a compound known to ionize well may be used to mitigate for system variability, reproducibility was shown to be excellent without the need for an internal standard.

- Over 100 consecutive injections of compound 1, compound 2 and compound 3 were made.
- Peak areas plotted in Figure 1 with CV of <4%.

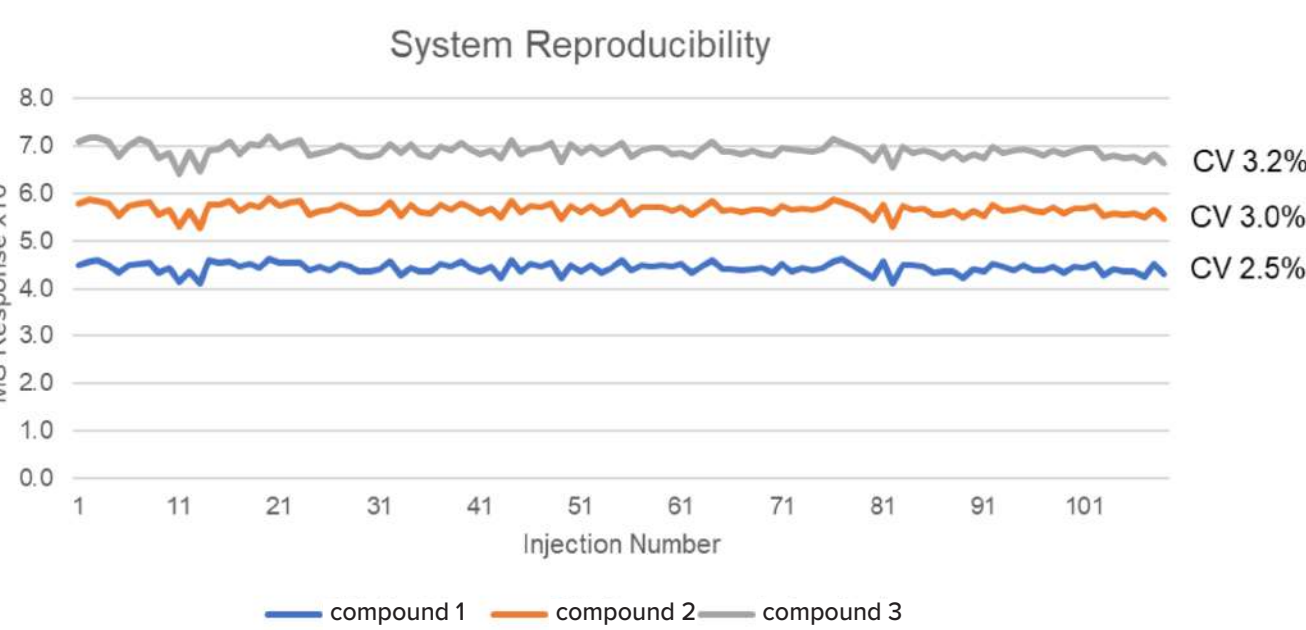


Figure 1: MS response obtained for >100 injections of compound 1, compound 2 and compound 3.

Injection Volume

For routine analyses, injection volumes are typically 0.5–2 µL, however, there are instances where lowering the injection volume can simplify sample preparation by removing the need for dilutions. The lowest injection volume available on the Vanquish Horizon system is 0.01 µL (10 nL). Injection reproducibility of compound 1 was tested at a range of volumes from 0.01–0.1 µL and good reproducibility was observed at all volumes, improving slightly as volume increased. The results are shown in Figure 2.

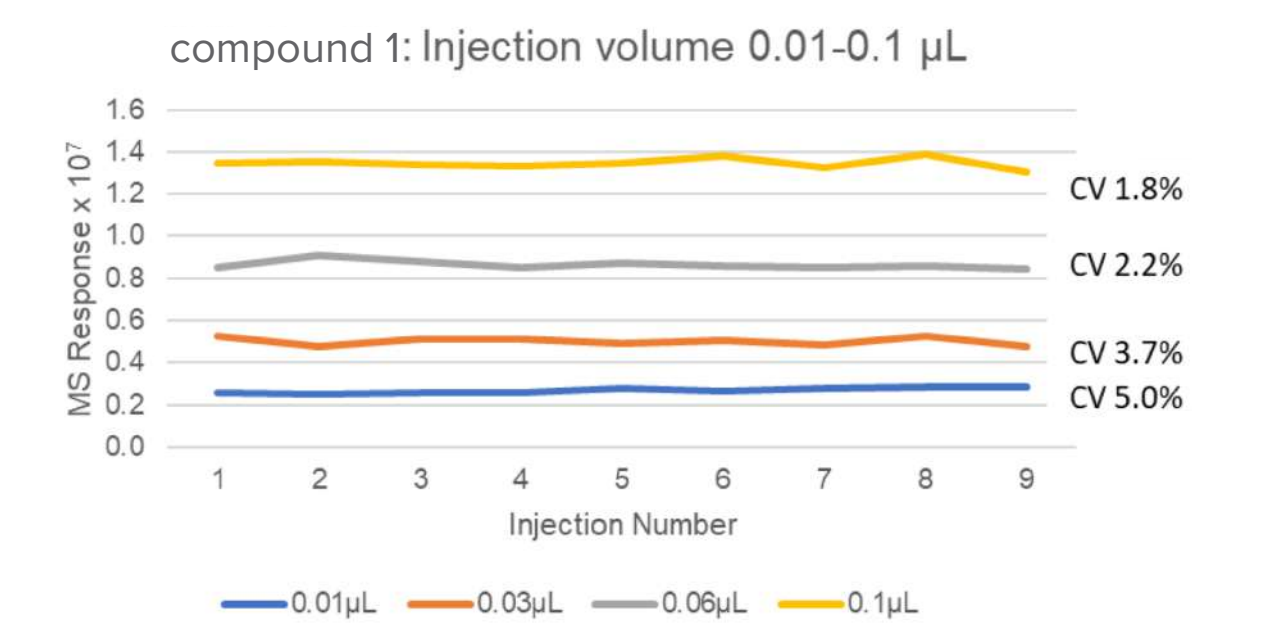


Figure 2: MS Response for compound 1 for 9 injections from 0.01–0.1 µL.

Challenging Compounds

Compounds where the expected [M+H]⁺/[M-H]⁻ species is not the most abundant ion, forms adducts, multiply charged species or large numbers of fragments, do not lend themselves to analysis by MS/MS. For both examples shown in Figure 3 (a peptide) and Figure 4 (compound 4) improved sensitivity is obtained by utilizing an alternative to the [M+H]⁺/[M-H]⁻ ion in full scan analysis.

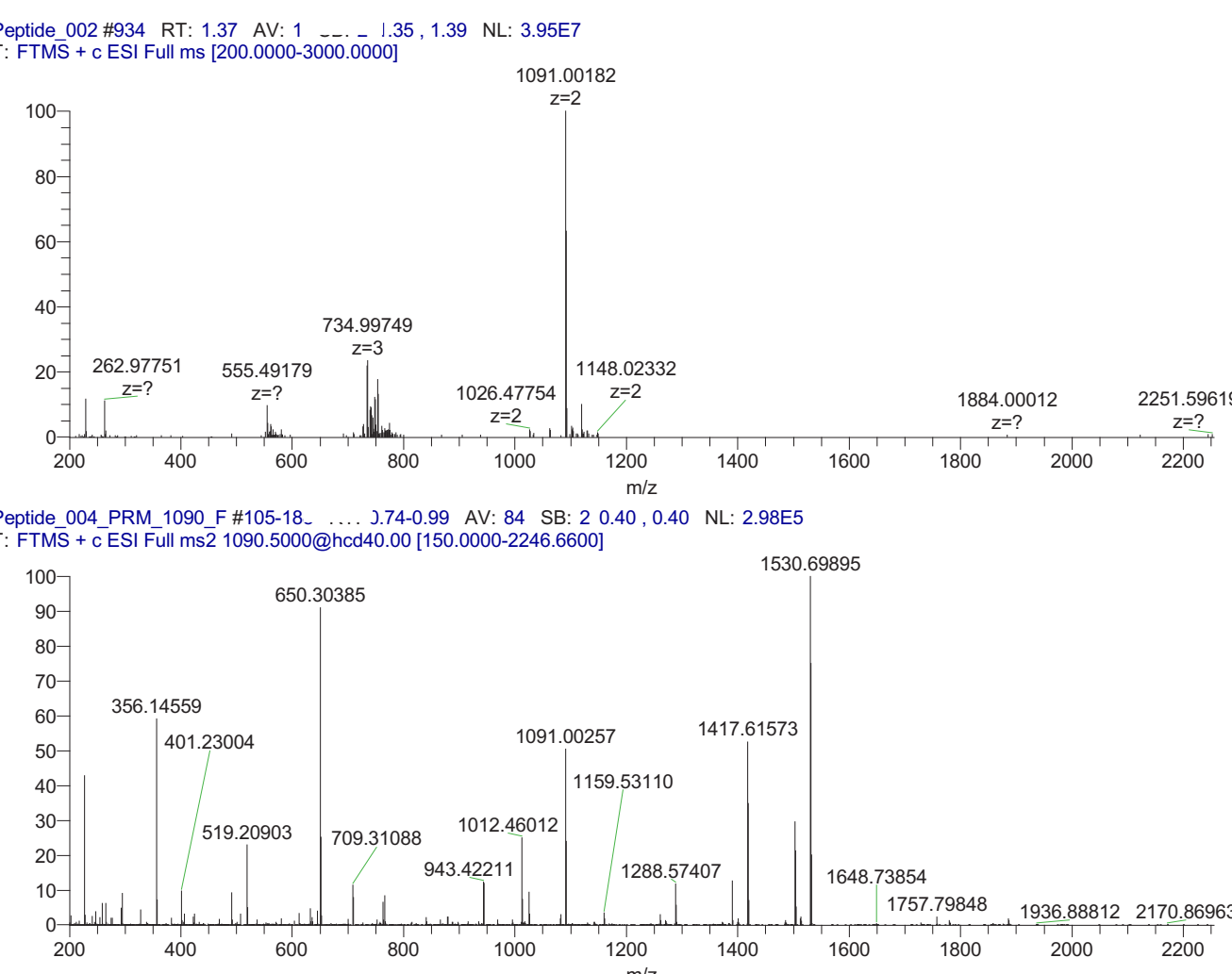


Figure 3: Peptides generally produce multiply charged species and MS/MS gives large number of fragments of similar intensity impacting sensitivity.

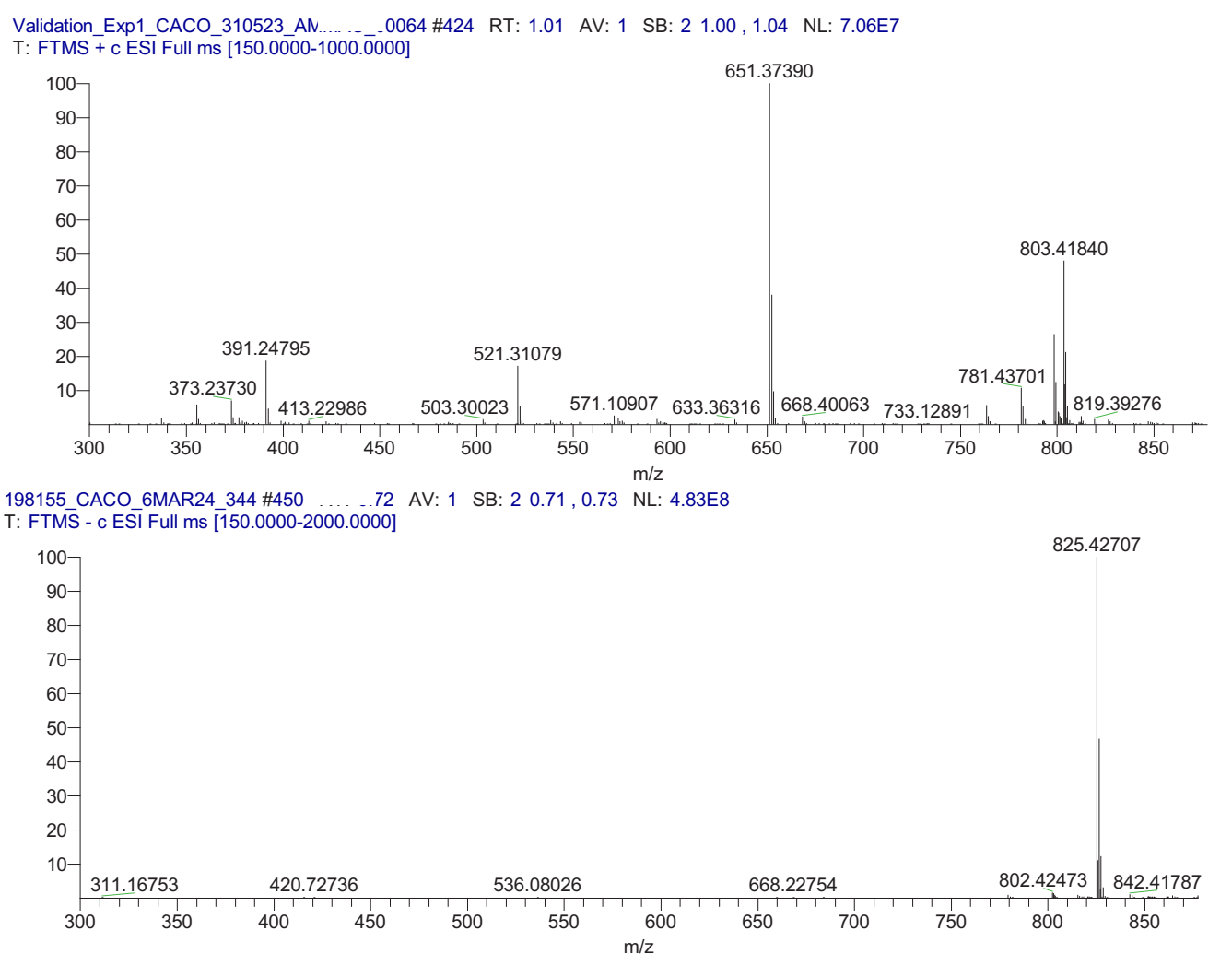


Figure 4: Compound 4 in positive mode (top). Base peak is an in-source fragment, MS response for [M+H]⁺ 10% of base peak. In negative mode (bottom) with a formic acid method. Formate adduct is the only observed molecular ion with much improved sensitivity.

Carryover

With the wide calibration ranges sometimes required, issues with carryover might be anticipated. Figure 5 shows extracted ion chromatograms (5 ppm window) for a 50000 ng/mL blood standard from a bioanalytical run, followed by injections of solvent and blood blank.

Carryover in the solvent blank is <0.1% and the blood blank is clean. Standard practice is to run a solvent blank and several matrix blanks after a high standard or QC sample to ensure no carryover issues.

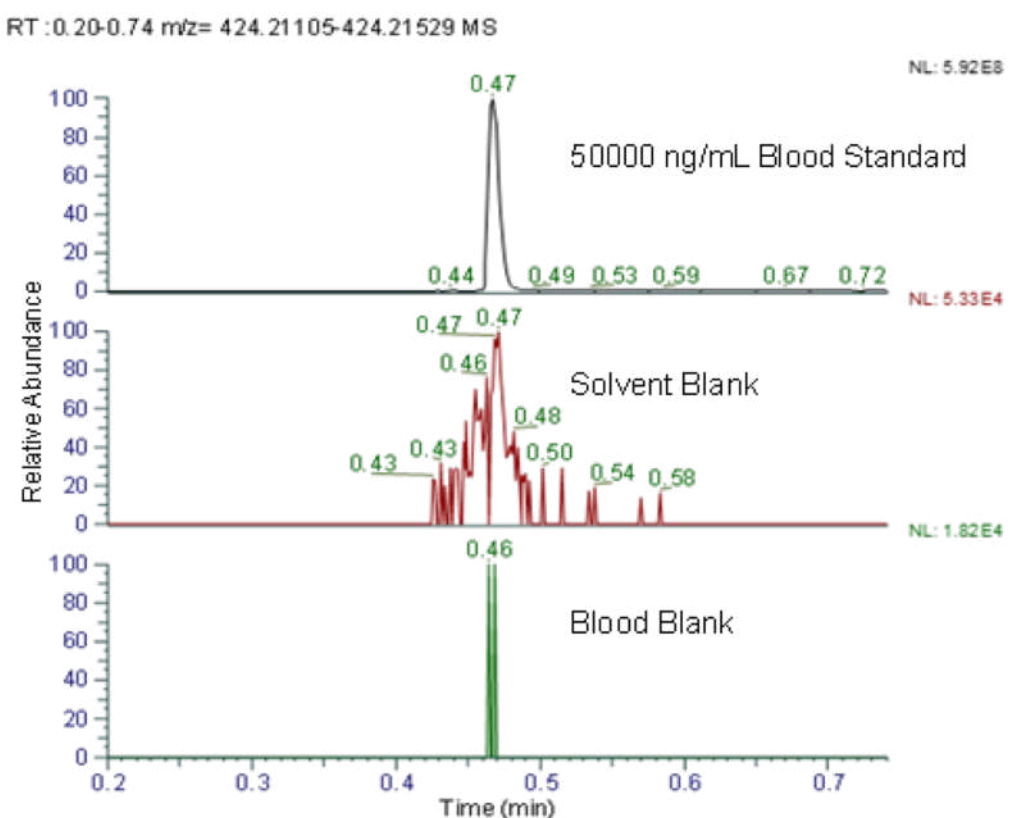


Figure 5: Extracted ion chromatograms for 50000 ng/mL blood standard and subsequent blanks.

Example DMPK Assay – In Vitro, Metabolic Stability

A key assay in Discovery DMPK is metabolic stability which often involves screening large numbers of compounds. On a triple quadrupole instrument, time is required to develop individual SRM methods and typically only [M+H]⁺/[M-H]⁻ targeted. Using the Exploris™, full scan data is acquired allowing challenging compounds to be quantified and major metabolites identified.

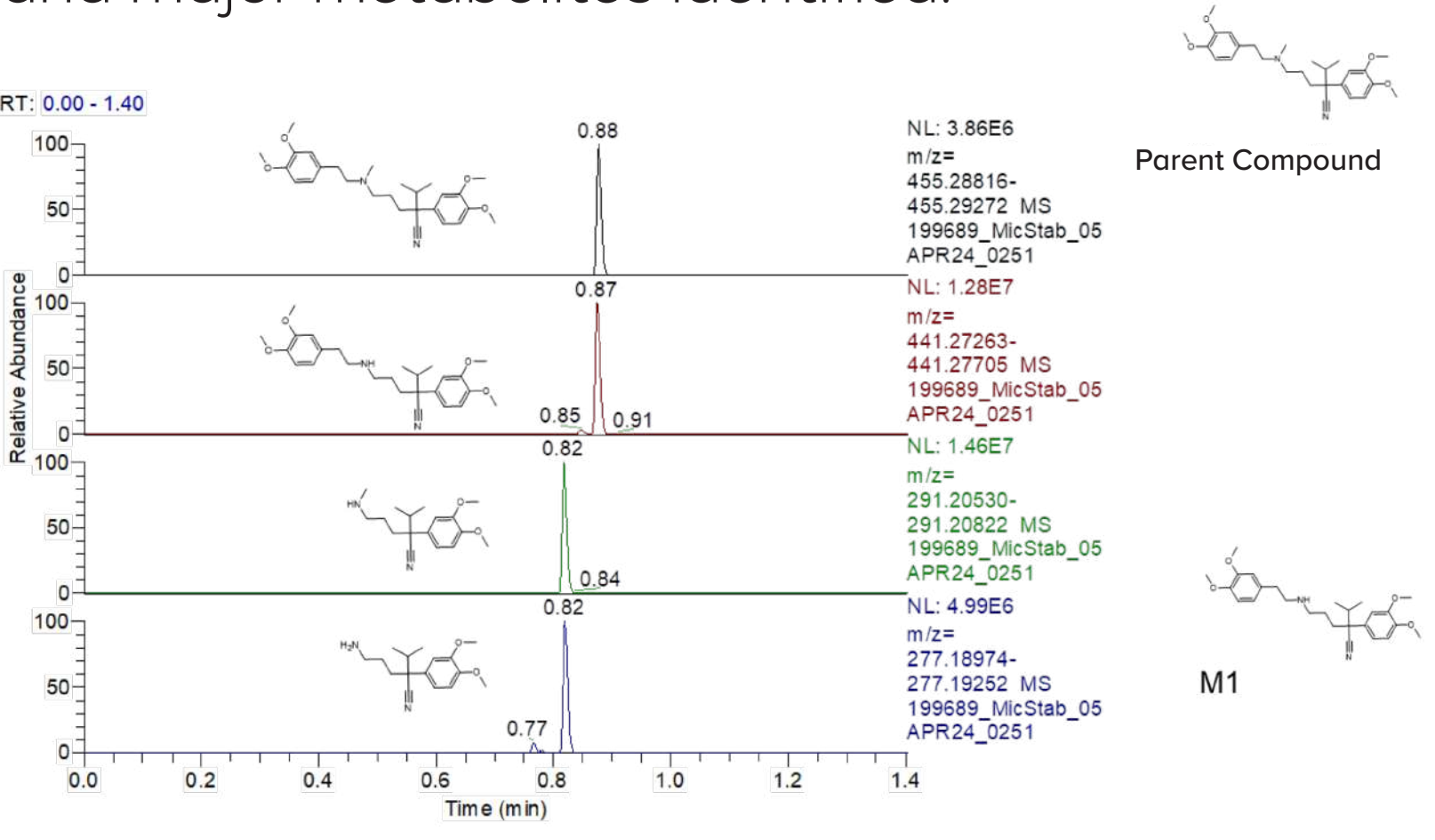


Figure 6: Extracted ion chromatograms for parent compound and major metabolites from a 1 µM, 10-minute intrinsic clearance sample. Mass error <1 ppm (not shown).

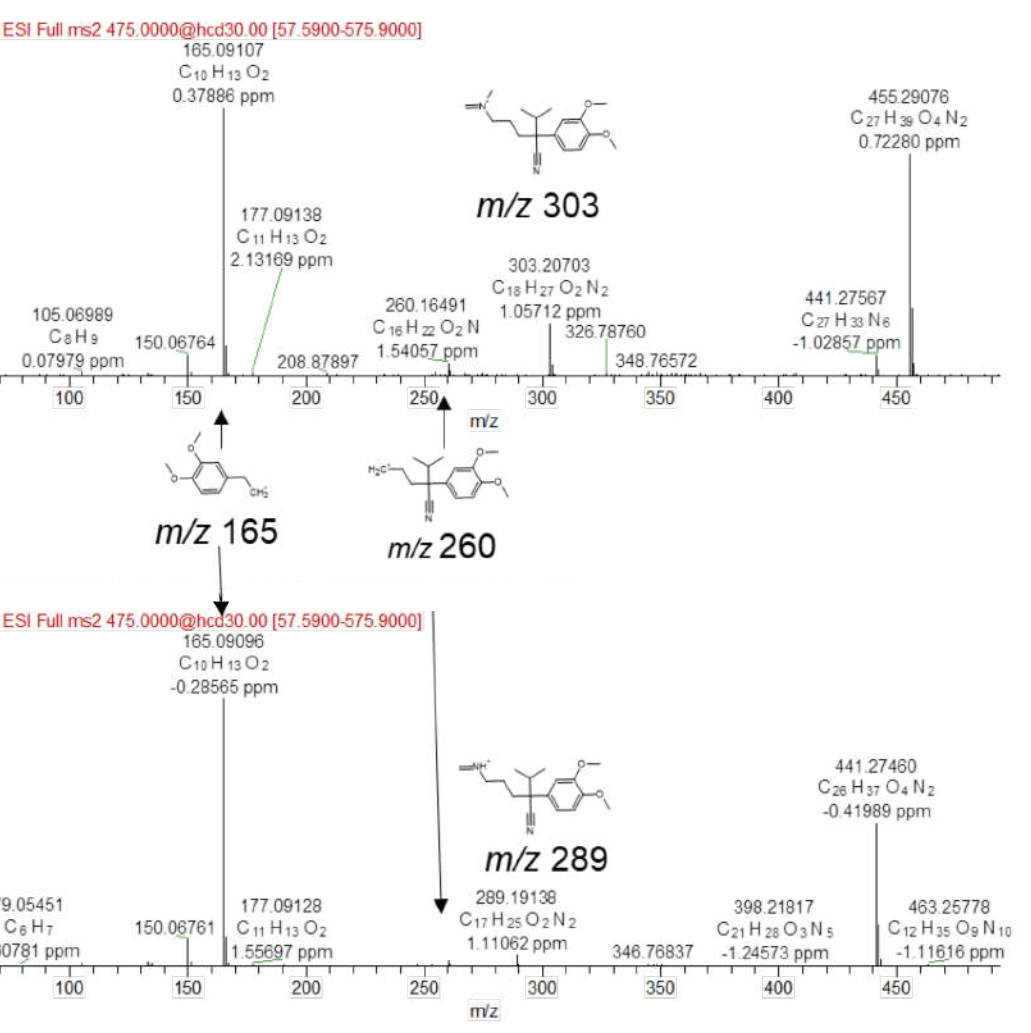
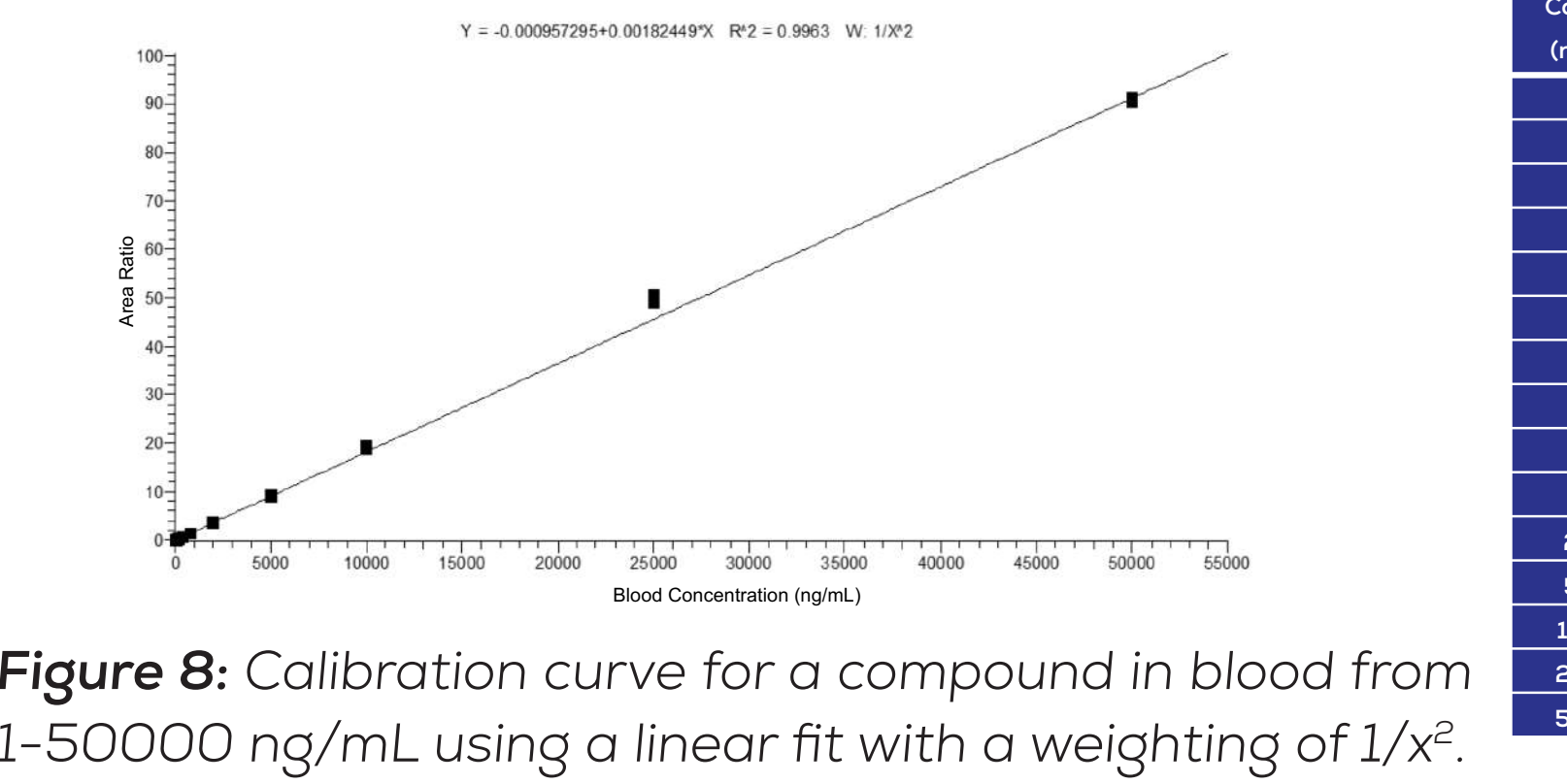


Figure 7: MS/MS spectra for parent compound (top) and M1 (bottom) the N-demethylated metabolite from the intrinsic clearance sample.

Example DMPK Assay – In Vivo Bioanalysis

Compounds with suitable *in vitro* properties can be dosed to animals to determine their pharmacokinetic profile in the whole animal. Bioanalysis of the samples is typically performed on a triple quadrupole instrument, however, the Exploris™ is proving to be capable of handling samples from these studies too, exhibiting good sensitivity and dynamic range. A standard curve in rodent blood from 1–50000 ng/mL is shown in Figure 8 with back calculated concentrations taken from the same analysis run shown in Table 1.



Cal Level (ng/mL)	STD Replicate 1 (ng/mL)	%Diff	STD Replicate 2 (ng/mL)	%Diff	STD Replicate 3 (ng/mL)	%Diff
1	11	7.6	Not Found		Not Found	
2	1.8	-9.5	2.1	3.4	2.1	6.5
4	3.9	-1.4	3.4	-14.7	3.7	-6.4
8	7.9	-1.0	7.9	-1.5	6.9	-13.3
16	15.7	-1.8	15.5	-3.1	15.6	-2.7
32	34.4	7.5	31.3	-2.2	32.4	1.2
60	85.9	7.4	80.8	0.9	80.6	0.8
16	159	-0.6	154	-3.8	151	-5.4
400	392	-1.9	379	-5.5	381	-4.7
800	814	1.7	775	-3.1	764	-4.5
2000	2074	3.7	1995	-0.2	1980	-1.0
5000	5087	1.7	5076	1.5	5209	4.2
10000	10682	6.8	10324	3.2	10507	5.1
25000	26847	7.4	27761	11.0	26894	7.6
50000	--	--	49976	0.0	49583	-0.8

Figure 8: Calibration curve for a compound in blood from 1–50000 ng/mL using a linear fit with a weighting of 1/x².

Summary and Conclusion

- The Orbitrap Exploris™ 120 is an excellent all-round instrument for both qualitative and quantitative applications.
- The system demonstrates good signal reproducibility with very low carryover.
- The system is capable of supporting *in vivo* bioanalytical studies with a wide dynamic range and good sensitivity.
- Acquiring all data in full scan mode allows quantitative analysis of more challenging compounds and can identify potential issues (e.g. matrix interference).
- Mass accuracy is routinely within 1 ppm, which is ideal for metabolite identification.

