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Applikationsbericht

Application of Exact Mass MS in Bioanalysis Discovery Quantitation using Xevo QTof MS and UPLC

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Abstract

In this application note, we present the use of a high-sensitivity quadrupole time-of-flight mass spectrometer coupled with UPLC separation technology for the quantitative analysis of a model candidate pharmaceutical in biological fluid at the levels of sensitivity required for drug discovery DMPK.

Introduction

The quantification of candidate pharmaceuticals and/or their metabolites in biological fluids plays a key role in drug discovery. The information generated is used to determine key pharmacokinetics parameters such as clearance, half-life, T_{max}, and bioavailability. During discovery ADME studies the metabolic fate of the molecule is determined as well as its pharmacokinetics. Currently this involves using two analytical instruments: one to provide the quantitative information and one for the qualitative analysis.

Quantitative information is normally derived from a tandem quadrupole instrument due to sensitivity; qualitative data is gathered either from an ion trap or quadrupole time-of-flight MS (QTof) instrument. This need for multiple instruments and analytical runs results in reduced productivity and increased instrument capital costs.

QTof technology is well-recognized as the platform of choice for exact mass MS/MS structural elucidation.[1-3] However, its use in quantitative DMPK studies has yet to be fully exploited. In this application note, we present the use of a high-sensitivity QTof mass spectrometer coupled with UltraPerformance LC (UPLC) separation technology for the quantitative analysis of a model candidate pharmaceutical at the levels of sensitivity required for drug discovery.

Figure 1. Xevo QTof MS with ACQUITY UPLC.

Experimental

A calibration line for a model drug candidate molecule was prepared in blank rat plasma at the concentration level of 50 pg/mL to 50 ng/mL. The samples were prepared by the protein precipitation of 50 µL of plasma with cold acetonitrile (2:1). The supernatant was evaporated to dryness and reconstituted in 50 µL of water for injection. An aliquot of the sample was injected onto the LC-MS system for analysis.

LC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 100 mm, 1.7 µm
Column temp.:	40 °C

Flow rate:	600 µL/min
Mobile phase A:	Aqueous formic acid (0.1%)
Mobile phase B:	Methanol
Gradient:	5 to 95% B/10 min

MS Conditions

MS system:	Xevo QTof MS Mass Spectrometer
Ionization mode:	ESI positive
Acquisition range:	100 to 800 *m/z*

Results and Discussion

The popularity of tandem quadrupole mass spectrometers for use in quantitative analysis stems from the specificity and sensitivity derived from the multiple reaction monitoring (MRM) process. These instruments still provide the most sensitive mode of analysis, especially with the complex matrices encountered in bioanalysis.

Accurate mass instrumentation generates full spectrum MS and MS/MS data that provides information regarding analytes in the sample. These accurate mass instruments, although less sensitive than tandem quadrupole instruments, can provide a similar level of specificity by using accurate mass chromatograms with small mass window ranges. The data displayed in Figure 2 shows the effect of changing the mass error range from 1 Da to 30 mDa for propranolol with a M+H mass 260.1651. Here we can see that using a narrower mass range of 50 mDa simplifies the chromatogram and reduces the detected chemical noise.

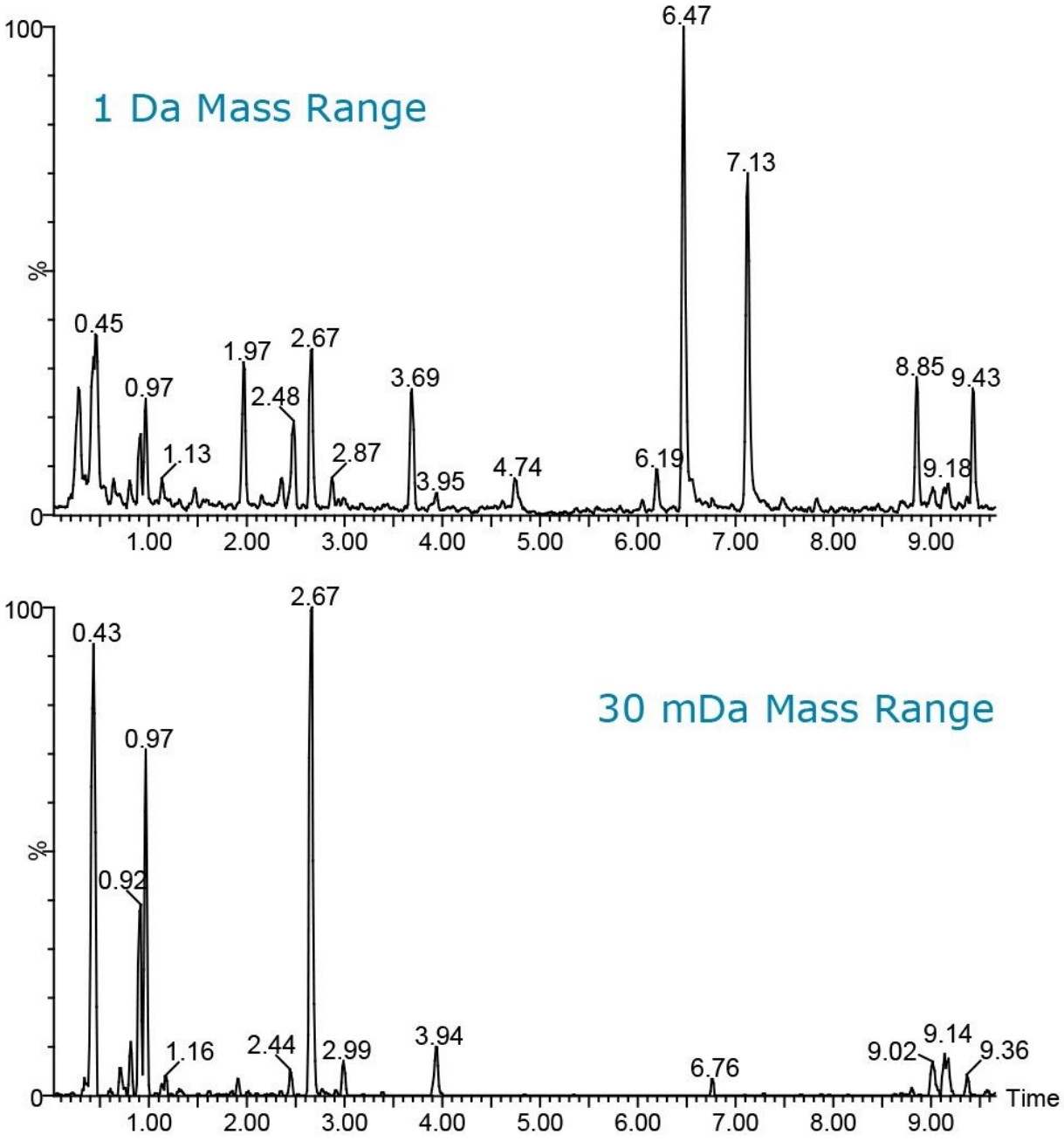


Figure 2. Exact mass chromatogram of propranolol protein-precipitated plasma sample with 1 Da and 50 mDa mass windows.

The reduction in chemical noise produced by using a smaller mass window significantly increases the signal-to-noise (S/N) ratio from 3:1 to 13:1, as shown in Figure 3. In this expanded figure we can see that when using the 30 mDa window, the propranolol peak at 3.94 min is now the biggest peak in the chromatogram

and well-defined above the noise compared to the 1 Da window.

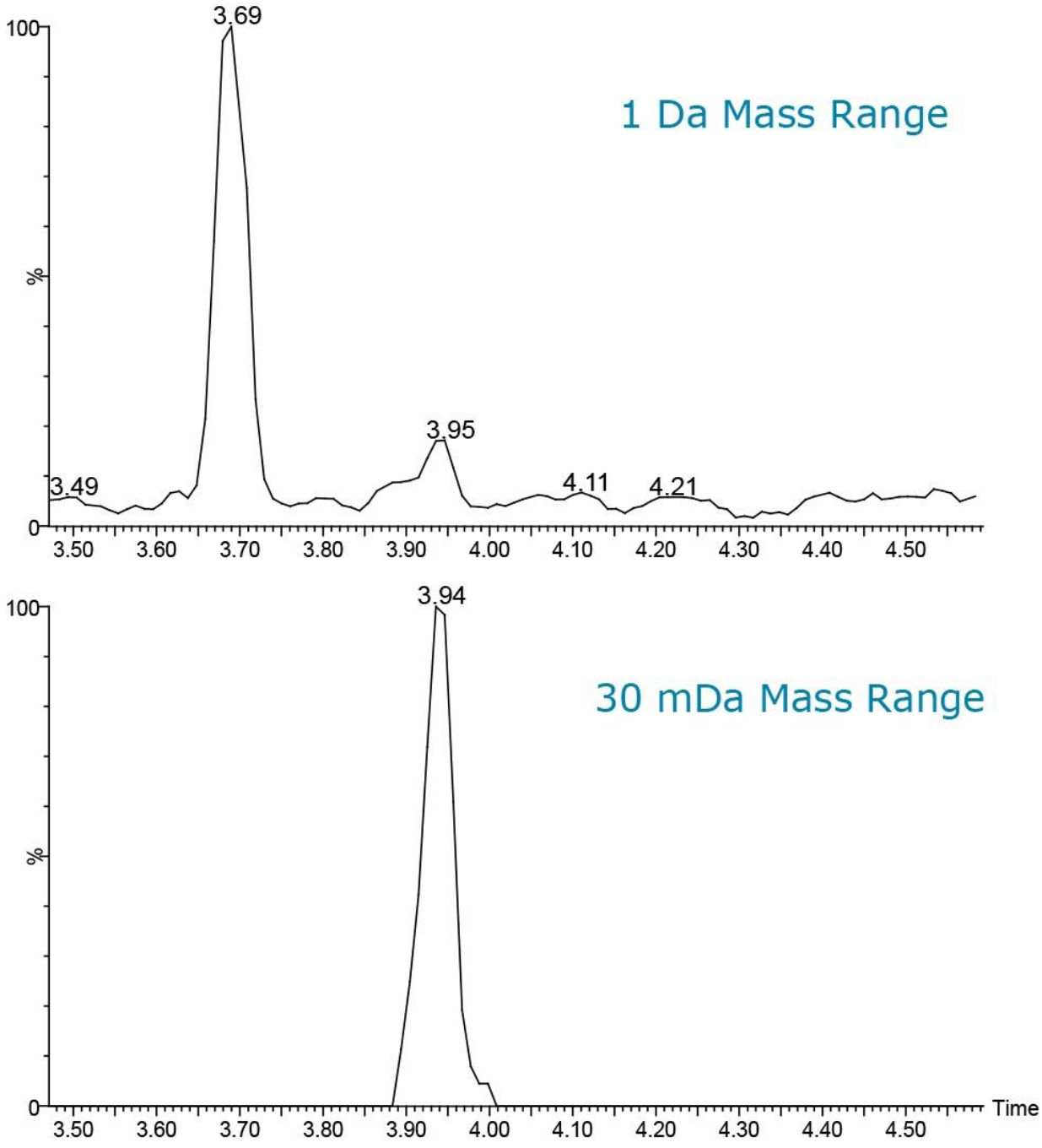


Figure 3. Expanded chromatogram window focused on propranolol peak at 3.9 min.

Linear dynamic range

The assay displayed sensitivity down to 50 pg/mL, which is more than adequate for use in discovery projects for quantification. The Xevo QTof MS provided linearity in excess of three orders of magnitude, as shown in Figure 4. The narrow peak widths produced by the ACQUITY UPLC System of 4 to 6 sec at the base required a data collection rate of 100 to 50 mSec per data point in order to correctly define the peaks for quantification.

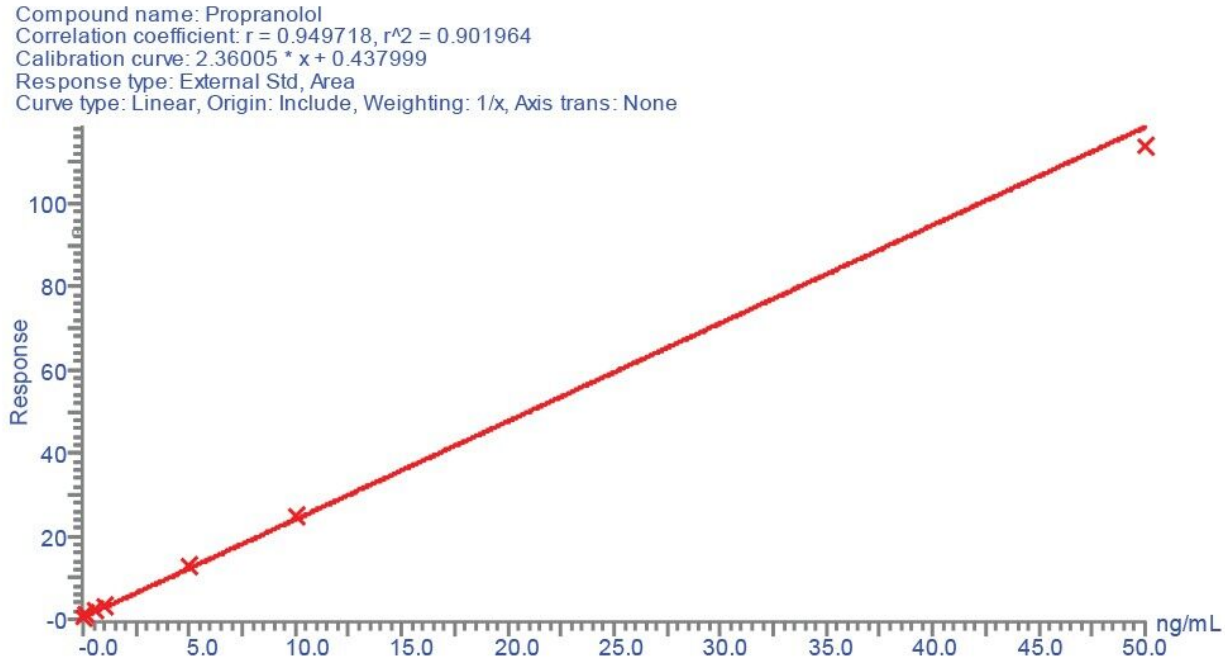
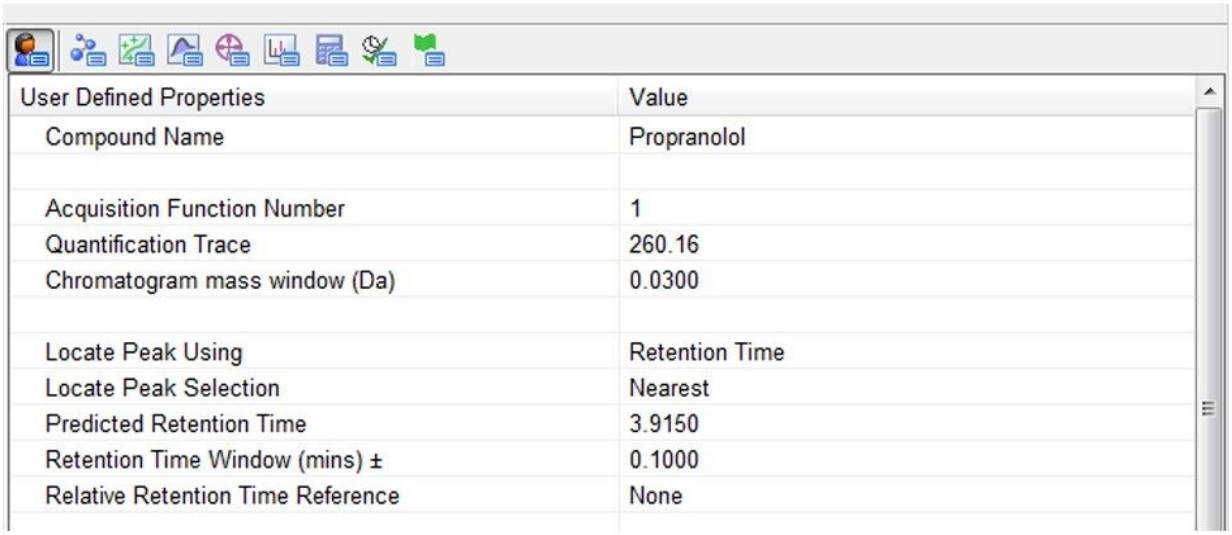


Figure 4. Propranolol calibration line from 50 pg/mL to 50 ng/mL.

The Xevo QTof MS is capable of acquiring data at acquisition speeds of 50 mSec per spectra without reduction in mass accuracy or spectral quality. This mass stability allows the use of a narrow mass window for data processing, improving the specificity and signal-to-noise obtained for the analysis. Using a data capture rate of 50 spectra/sec, a calibration line was generated over four orders of magnitude, from 50 pg/mL to 50 ng/mL.

Data were processed using TargetLynx Application Manager for MassLynx Software. TargetLynx automates sample data acquisition, processing, and reporting for quantitative results. It incorporates a range of confirmatory checks that identify samples that fall outside user-specified or regulatory thresholds. The TargetLynx method editor allows the mass window to be selected for the quantification of the analyte(s) of interest. This allows very narrow mass windows to be selected, improving the mass selectivity. The example shown in Figure 5 illustrates the selection of the mass window. Here we can see that a mass window of 30

mDa has been employed for integration. The lower the mass window that can be employed, the more specific the analysis; however, the ability to use very low mass windows relies on the mass stability of the mass spectrometer. The Xevo QTof MS is equipped with LockSpray Technology, which allows for stable, long-term operation with low mass drift.



User Defined Properties	Value
Compound Name	Propranolol
Acquisition Function Number	1
Quantification Trace	260.16
Chromatogram mass window (Da)	0.0300
Locate Peak Using	Retention Time
Locate Peak Selection	Nearest
Predicted Retention Time	3.9150
Retention Time Window (mins) ±	0.1000
Relative Retention Time Reference	None

Figure 5. TargetLynx exact mass window selection for quantification.

The Xevo QTof MS's exact mass MS and MS/MS capability also makes it ideal for de novo identification of small molecules such as drug metabolites and impurities. The Xevo QTof MS has been shown to provide excellent sensitivity and spectral quality in both MS and MS/MS modes for metabolite identification,1-3 especially when combined with the data processing power of the MetaboLynx XS Application Manager. This combination of qualitative and quantitative capability of the Xevo QTof MS makes it an ideal tool for early drug discovery metabolism studies.

Conclusion

The quantification of candidate pharmaceuticals in biological fluids plays a key role in drug discovery DMPK. The drive to maximize productivity and instrument usage in discovery means modern LC-MS systems must be able to perform bioanalysis quantification and metabolite identification.

- The use of narrow mass windows provides high selectivity
- The Xevo QTof MS provides sufficient sensitivity for use in drug discovery quantification applications
- The data collection rate is sufficiently fast to accurately quantify the narrow peaks produced by UPLC's sub-2-µm particle LC
- The ability of the Xevo QTof MS to acquire accurate mass data allows both quantitative and qualitative data to be acquired simultaneously

References

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