APPLICATION OF EXACT MASS MS IN BIOANALYSIS DISCOVERY QUANTITATION WITH UPLC AND XEVO QTOF MS

Robert S. Plumb and Marian Twohig, Waters Corporation, Milford, MA, U.S.

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:	Waters® 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:	Waters Xevo™ QTof MS Mass Spectrometer
Ionisation mode:	ESI positive
Acquisition range:	100 to 800 m/s

RESULTS

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.

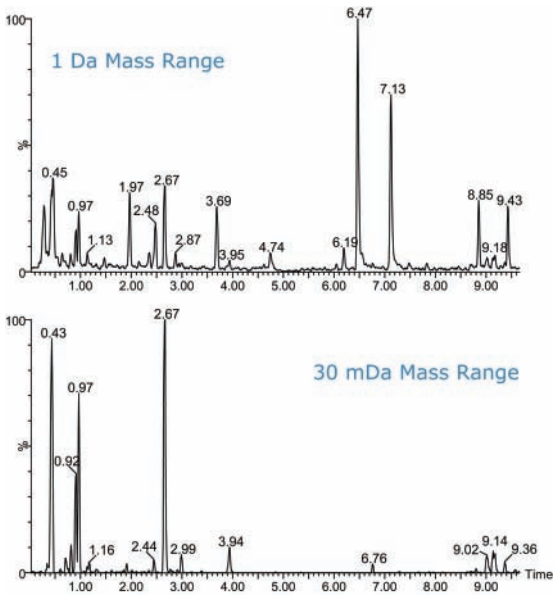


Figure 2. Exact mass chromatogram of propranolol protein-precipitated plasma sample with 1 Da and 50 mDa mass windows.

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 minuntes is now the biggest peak in the chromatogram and well-defined above the noise compared to the 1 Da window.

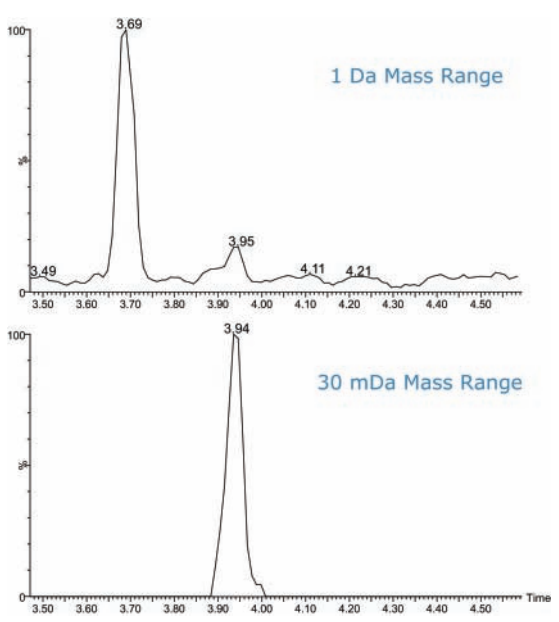


Figure 3. Expanded chromatogram window focused on propranolol peak at 3.9 minutes.

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.

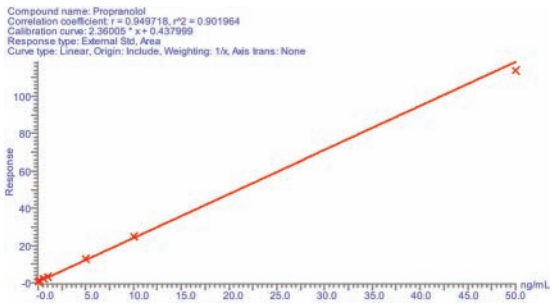


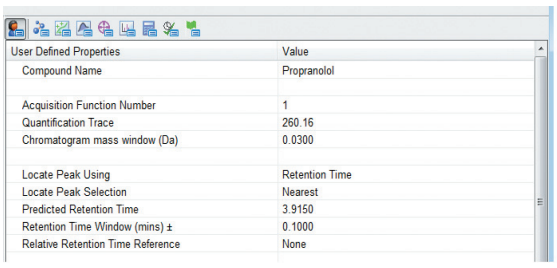
Figure 4. Propranolol calibration line from 50 pg/mL to 50 ng/mL.

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.

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.



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.

CONCLUSIONS

The quantification of candidate pharmaceuticals in biological fluids plays a key role in drug discovery DMPK. The drive to maximise 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

[1] Castro-Peres J, Yu K, Shockcor J, Shion H, Marsden-Edwards E. Fast and Sensitive in vitro Metabolism Study of Rate and Routes of Clearance for Ritonavir using UPLC coupled with the Xevo QTof MS System. Waters Application Note. 2009; 720003025en.

[2] Bateman KP, Castro-Perez J, Wrona M, Shockcor JP, Yu K, Oballa R, Nicoll-Griffith DA. MSE with Mass Defect Filtering for In Vitro and In Vivo Metabolite Identification. Rapid Commun Mass Spectrom. 2007; 21(9): 1485-96.

[3] Wrona M, Mauriala T, Bateman KP, Mortishire-Smith RJ, O'Connor D. 'All-in-one' Analysis for Metabolite Identification using Liquid Chromatography/hybrid Quadrupole Time-of-flight Mass Spectrometry with Collision Energy Switching. Rapid Commun Mass Spectrom. 2005; 19(18): 2597-602.

World's Fastest and Most Sensitive LCMS System

Shimadzu has introduced a new compact LCMS-2020 single quadrupole mass spectrometer, which features the world's fastest scanning capabilities. Utilising patent pending ultrafast (UF) technology, the LCMS-2020 has faster measurement speed and significantly higher sensitivity than any other single quadrupole analyser. This provides more accurate detection of trace impurities in pharmaceuticals, environmental pollutants and other contaminants.

The new UFscanning technology achieves mass spectrum measurement speeds of 15,000 u/sec without sacrificing sensitivity or resolution, thus obtaining the best chromatography for the fastest LC conditions. The novel UFswitching technology achieves industry-leading 15 ms polarity switching, enabling accurate data from even the fastest chromatographic peaks without any loss of peak height.

Shimadzu redesigned the ion transfer section to provide greater sensitivity than other quadrupole analysers for commonly measured substances. Users can now inject less and keep the analyser cleaner for longer. It also has improved high mass operation with sensitivity increased for masses above 1,000. In addition to better performance, the LCMS-2020 allows easier maintenance, permitting users to replace the ionisation unit and inlet capillary to the MS from the LC, without breaking the vacuum.

LCMS-2020 control and data processing are handled by an updated version of LCMSsolution software. The new software easily displays multiple sets of LC or MS data, allowing overlay and analysis of multiple data results for easy searching and comparison. The new software controls the LCMS-2020 and is fully integrated with Shimadzu's prominence series of ultrafast and nano HPLCs.

Circle no. 359

Award for Outstanding Research Publication

Waters Corporation announced that the American Society for Mass Spectrometry (ASMS) presented Professor Alison Ashcroft of the University of Leeds with the first Ron Hites Award for Outstanding Research Publication in the Journal of the American Society of Mass Spectrometry (JASMS). Professor Ashcroft's paper titled, Monitoring Copopulated Conformational States During Protein Folding Events Using Electrospray Ionisation-Ion Mobility Spectrometry-Mass Spectrometry, appeared in the December 2007 issue of JASMS and was co-authored by Waters scientists Kevin Giles and Robert Bateman along with University of Leeds researchers David Smith and Professor Sheena Radford. Professor Ashcroft's team acquired the research data for the publication on a Waters® SYNAPT High Definition MS™ (HDMS™) System.

ASMS judged the publication on the basis of its 'innovative aspects, technical quality, likely stimulation of future research, likely impact on future applications, and the quality of the presentation'.

Professor Ashcroft received the Award and $2,000 cash at the 57th ASMS Conference on Mass Spectrometry and Allied Topics.

The Award is named in honour of Professor Ronald A. Hites of Indiana University who spearheaded the creation of JASMS in 1988 while President of ASMS. JASMS is devoted to the publication of research papers covering all aspects of mass spectrometry from all fields of science including chemistry, physics, geology, environmental, biological, health and life sciences.

Shortly after purchasing her SYNAPT™ HDMS System in 2007, Professor Ashcroft had this to say about it: "It's adding a new dimension to our research. We can now quantify the amount of protein that is in its native state and the amount that is unfolded and partially folded. We can also monitor which particular conformers are consumed during the assembly process. This is providing important new insights and detail into how biomolecules work at the molecular level."

Circle no. 360

High-Resolution VOC Quantification in the Single-Digit pptv-Range

Ionicon presents the new PTR-TOFMS series, taking online mass spectrometry for trace VOC analysis another step ahead.

The new PTR-TOFMS series offers to the scientific market two very attractive variants: the IONICON PTR-TOF 8000 and the IONICON PTR-TOF 2000.

Both instruments combine the legendary features of Proton Transfer Reaction (PTR) technology that is being ultra-sensitive to VOCs (single-digit pptv-range), with the speed and resolution of time-of-flight mass spectrometry.

The flagship of this series is the IONICON PTR-TOF 8000 providing an incredible resolution of up to 8000 m/Δm thus resolving isobaric compounds, and a very low detection limit of < 10 pptv.

The IONICON PTR-TOF 2000 focuses on sensitivity and speed reaching a limit of detection below 5 pptv and very high ion count rates but nevertheless features a mass resolution of up to 2000 m/Δm.

The PTR-TOFMS series as well as the classic PTR-QMS Series (based on quadrupole MS) can be equipped with Switchable Reagent Ions (PTR+SRI-MS) where instead of the single precursor ion (H3O+) used so far in PTR-MS systems also NO+ and O_2+ can be used to chemically ionise trace constituents in gas samples. The benefits are extraordinary as not only isomeric VOC compounds can be separated and instantaneously quantified but also more substances can now be detected.

Circle no. 361