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Note d'application

Simplifying Qualitative/Quantitative Analysis in Discovery DMPK using UPLC and Xevo TQ MS

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Abstract

In this application note, we demonstrate the power of using UPLC with the Xevo TQ MS to perform both quantitative and qualitative analysis, providing high-quality MS, MRM, and MS/MS data for candidate drug molecules in discovery. Rapid data collection ensures that both MS and MS/MS peaks are well characterized and that high-quality spectra are acquired. This maximizes the return on investment for this modern tandem quadrupole instrument.

Introduction

The determination of the drug metabolism and pharmacokinetics of a candidate drug molecule early in drug discovery has revolutionized drug development. The ability to drop compounds with poor exposure or clearance in animal models has dramatically reduced the number of compounds falling out of the development program in clinical trials due to poor exposure or non-linear pharmacokinetics; thus saving millions of dollars in research expenditure. The further evaluation of compound metabolisms early in discovery has allowed the medicinal chemist to remove metabolic soft-spots or allows compounds forming active metabolites to be removed from the project.

Mass spectrometry, particularly LC-MS/MS, has facilitated this scientific revolution. The major instrument platform used for this task is the tandem quadrupole mass spectrometer. This is due to the high sensitivity and selectivity it possesses when operated in Multiple Reaction Monitoring (MRM) mode. The ability to accurately quantify compounds at very low levels allows the compounds' pharmacokinetics (PK) parameters to be established at low doses to small rodents, e.g., mice. This reduces compound requirements, allowing compounds to be evaluated very early in the discovery process and thus reducing costs.

While being the instrument of choice for quantification, tandem quadrupole MS sensitivity is limited in full scan mode due to poor duty cycle. Thus scientists must either trade-off performance or invest in a second instrument, such as a Q-Tof for qualitative work.

The Xevo TQ MS is a tandem quadrupole mass spectrometer equipped with a novel collision cell that is continuously filled with collision gas, allowing for the simultaneous collection of MRM and full scan MS or MS/MS data,1 facilitating the simultaneous collection of quantitative and qualitative data.

The collision cell incorporates the Waters T-Wave design rather than quadrupole rods. The T-Wave (Traveling Wave) is constructed from a series of charged, ring-stacked electrodes with the ion path through the collision cell controlled by a combination of DC and RF signals. T-Wave collision cell technology minimizes source-todetector ion transit times for high-speed data acquisition.

To complement this further, Xevo TQ MS/MS full scan sensitivity is significantly improved over standard quadrupole instruments with its incorporation of ScanWave technology.[2] By accumulating ions in the collision cell and synchronizing their ejection with the scanning of the final, resolving quadrupole, the overall full scan MS and MS/MS sensitivity is improved by a factor of 10 to 15X over standard instruments.

In this application note, we demonstrate use of the Xevo TQ MS for the quantitative and qualitative evaluation of the common beta blocker propranolol (Figure 1).

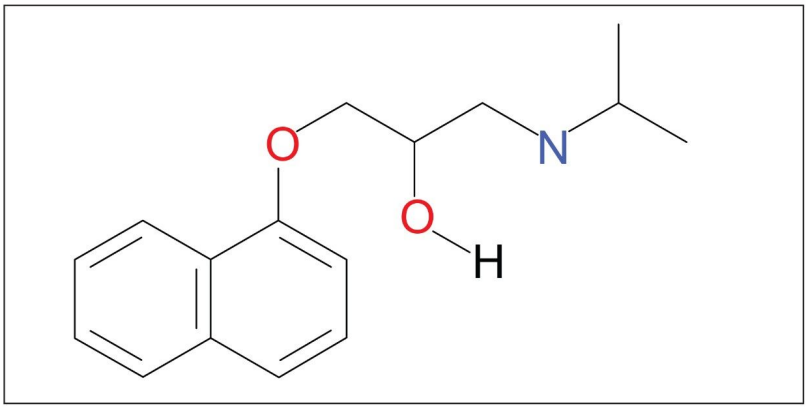

Figure 1. Propranolol.

Experimental

LC-MS Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18}

	Column 2.1 x 50 mm, 1.7 µm
Column temp.:	45 °C
Flow rate:	600 µL/min
Mobile phase A:	0.1% Formic acid
Mobile phase B:	Methanol
Gradient:	5 to 95% B/2 min
MS system:	Waters Xevo TQ MS
MS/MS transitions:	260 => 183
Dwell time:	10 and 100 ms
Ionization:	Positive ion ESI
Capillary voltage:	1.0 KV
Collision energy:	25 eV
Cone voltage:	20 V

Results and Discussion

Quantitative LC-MS/MS

During a drug discovery program, numerous compounds per week will require method development and sample analysis. Thus it is neither practical nor necessary to fully develop robust LC-MS methodology; simple generic gradient and protein precipitation is preferred. In this example, we adopt a similar approach for the analysis of propranolol. Propranolol is a non-selective, beta-adrenergic receptor- blocking agent for

treatment of hypertension. Propranolol rat plasma standards (50 µL), over a concentration range of 10 to 10,000 pg/mL, were precipitated with 100 µL of cold acetonitrile in a Sirocco 96-well extraction plate. The sample was extracted under vacuum, evaporated to dryness, and reconstituted in 100 µL of water.

The sample was analysed using MRM LC-MS/MS. The optimal transition selected was determined to be *m/z* 260 => 183 in positive ion mode. The MRM parameters were determined automatically using IntelliStart Software for the Xevo TQ MS. IntelliStart selects the optimal MRM parameters, ionization mode, polarity, and lens voltages for the compound during the infusion of the analyte into the source – with the whole process taking just a few minutes.

The data displayed in Figure 2 shows MS/MS spectra of the propranolol peak *m/z* 260. Here we can see that the MS/MS analysis of the 260 ion gives rise to 116, 183, 157, and 165, of which the 183 is the most sensitive and specific peak as a 116 ion was observed in the blank plasma.

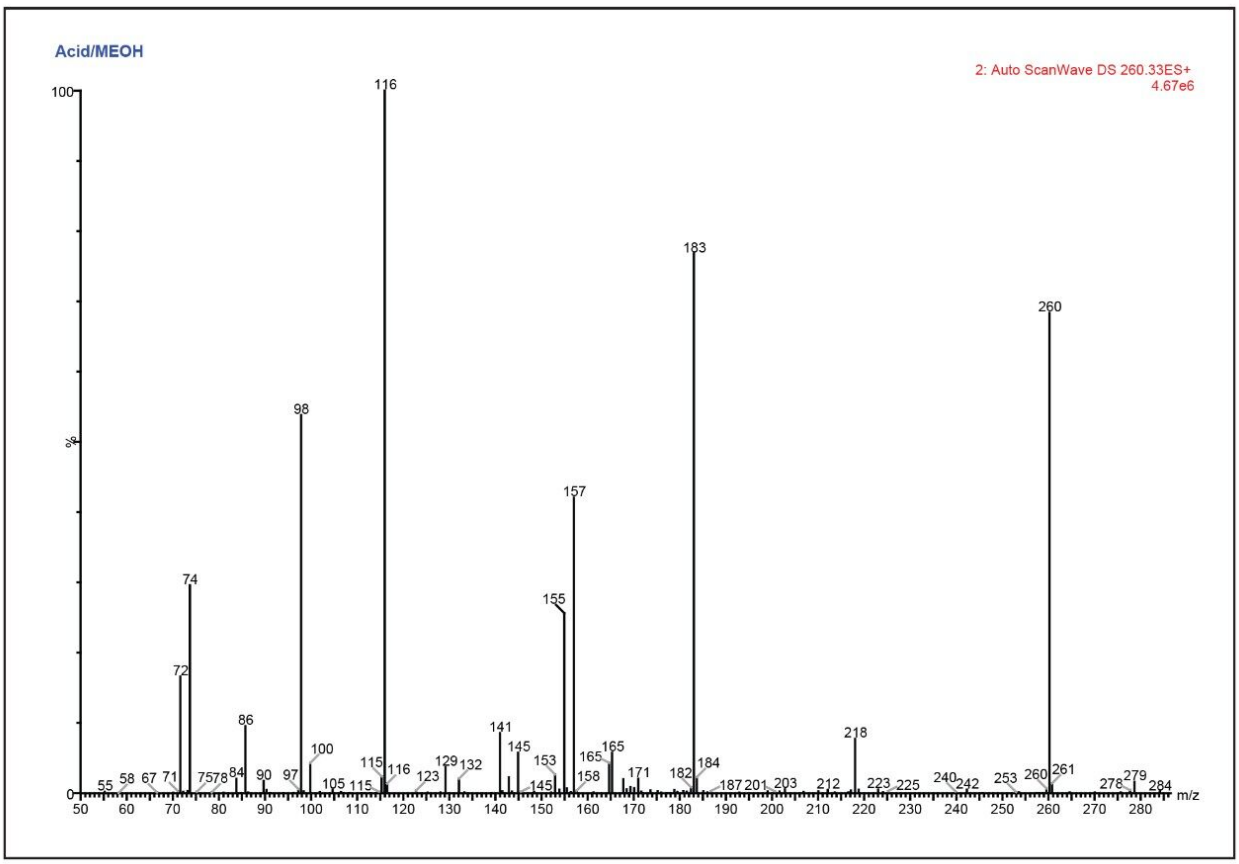

Figure 2. ScanWave product ion MS/MS of the propranolol precursor.

Following analyte reconstitution with water, 10 µL was injected onto a 2.1 x 50 mm ACQUITY UPLC Column

packed with 1.7-µm C_{18} BEH material. The column was eluted with a 5 to 95% gradient of formic acid (aq)/methanol over 2 minutes at 600 µL/min. Typical chromatograms obtained for a 10-pg/mL propranolol plasma standard and blank are shown in Figure 3.

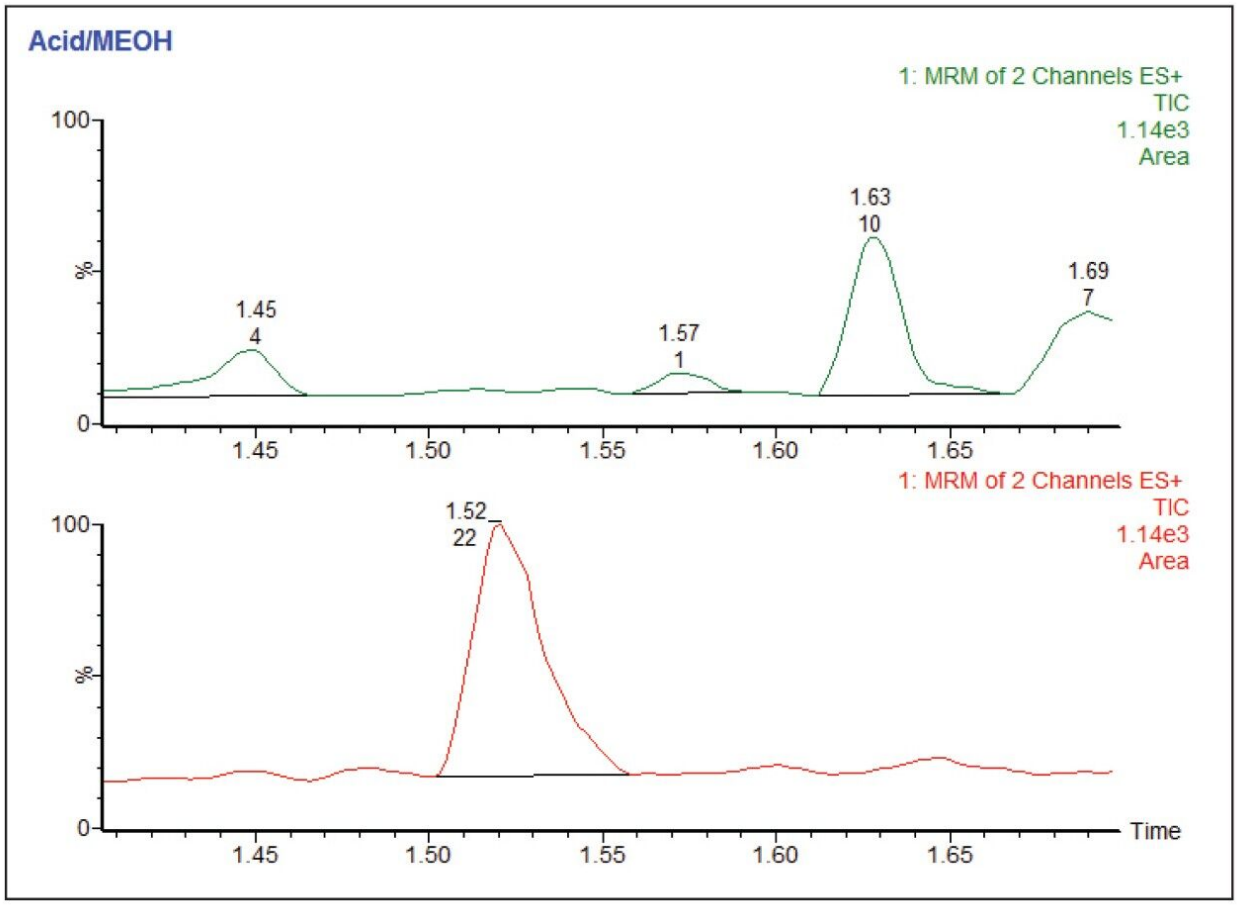

Figure 3. LC-MS/MS chromatogram of blank (top) and 10 pg/mL standard (bottom). Detection positive ion MRM, transition 260 to 183.

As we can see from the data displayed in Figure 3, the propranolol peak eluted with a retention time of 1.52 minutes. A typical calibration line obtained is shown below in Figure 4, with the lower concentration levels highlighted. The response was found to be linear using 1/x weighting, even without the use of an internal standard.

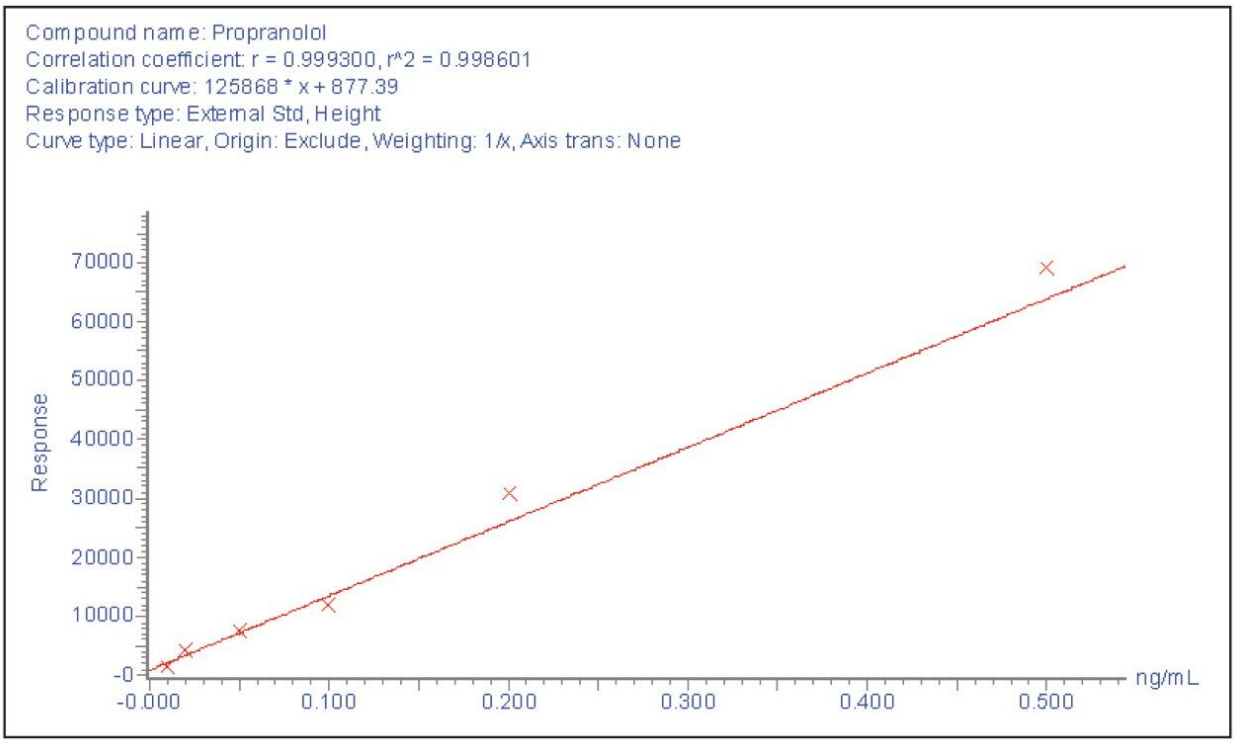

Figure 4. Typical calibration line for propranolol.

Qualitative LC-MS/MS

During the drug discovery process, it is necessary to quickly identify the route and rate of metabolism to allow confident compound selection. The use of tandem quadrupole MS for qualitative analysis has been hampered by the need to perform several analytical runs to obtain the MS and MS/MS data necessary to identify the structure of the drug metabolites. The unique collision cell design of the Xevo TQ MS allows for the simultaneous collection of MS, MS/MS, neutral loss, or precursor ion scan data, all in a single analytical run. The fast-electronics design of the mass spectrometer allows this switching to be performed with sufficient rapidity to correctly define a narrow UPLC peak that is just a few seconds wide.

Detection of metabolites can be carried out using two different approaches:

1. Unbiased collection of LC-MS data and post-acquisition processing
2. Targeted collection of LC-MS data based on the combination of known metabolic biotransformation and fragmentation pattern of the parent compound

Using the second approach a series of MRM transitions are generated based on the proposed metabolite

fragmenting to the product ions detected for the parent. When an ion is detected in one of the MRM channels, the instrument then switches to acquire MS/MS data on the base peak in the spectrum. This approach generates multiple MRM channels per metabolite, leading to potentially hundreds of MRM transitions.

This factor, combined with the switch to MS/MS modes, results in a total duty cycle of 2 to 2.5 seconds, which limits its use with modern high-resolution chromatography such as UPLC, where peak widths are of the order of 2 to 4 seconds per data point. An alternative approach is to collect full scan MS data and then switch to MS/MS data when a peak is detected in the full scan LC-MS chromatogram. This Survey Scan approach is easily set up in the Xevo TQ MS from the Experiment Editor page in MassLynx Software (Figure 5).

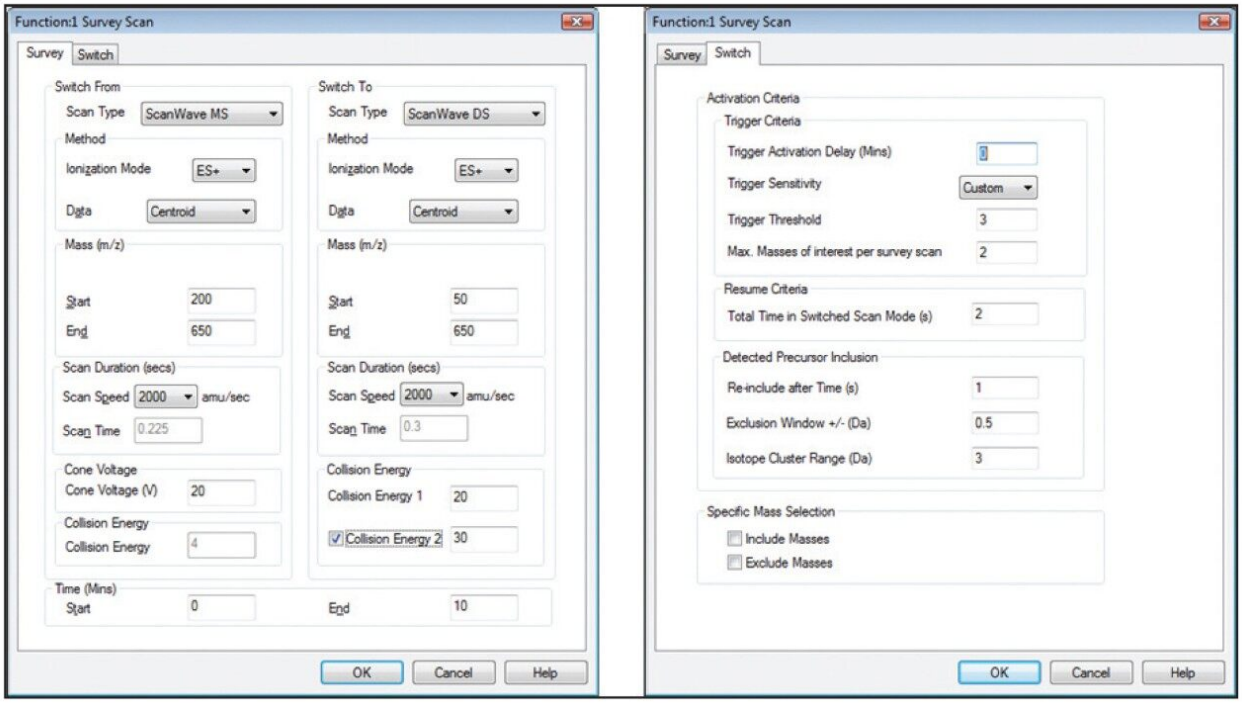

Figure 5. Survey Scan instrument parameter method definition.

The LC-MS chromatogram obtained from the full scan MS analysis of 1-µmol propranolol incubated with rat liver microsomes spiked into rat plasma, analyzed with a 10-minute gradient, is shown in Figure 6. Here we can see that there are several potential peaks in the chromatogram that require investigation to identify the metabolites. Previous work by Wilson *et al.*[3] showed that the major metabolites of propranolol are the hydroxy, glucuronide, and the hydroxy-glucuronides. These had *m/z* values of (M+H) 276, 436, and 452

respectively.

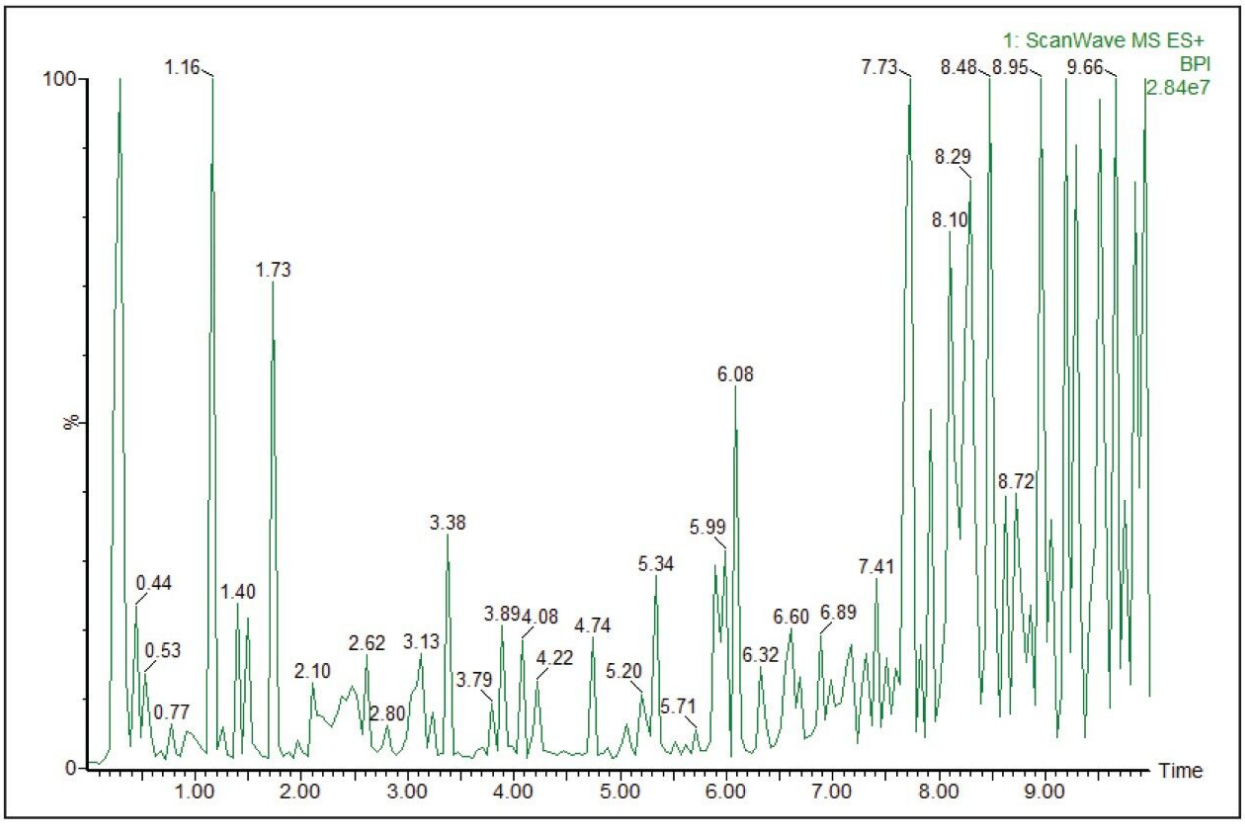

Figure 6. LC-MS chromatogram of protein-precipitated rat plasma containing propranolol metabolites.

Based on the spectra obtained in Figure 2, if the MRM-to-MS/MS approach was applied to detect the metabolite we would use the transitions X => 183, X => 157, X => 116, and X => 96 where X = 276, or 436, or 452. This is four transitions per metabolite; thus for a new compound – even with a truncated list of possible phase I and phase II metabolites – the number of MRM transitions could easily exceed 60. This would significantly affect the duty cycle and hence compromise the chances of detecting the metabolites when using narrow UPLC peaks.

Survey Scan MS/MS

The Survey Scan MS/MS chromatogram obtained from the analysis of the propranolol rat liver microsome is shown in Figure 7. Here we can see that a significant number of peaks were detected and selected for MS/MS analysis. The mass spectrometer's ability to rapidly switch between MS and MS/MS allowed the narrow UPLC peaks to be correctly defined. In this approach, MS and MS/MS data are collected in an

unbiased manner and can be mined to detect the drug metabolites.

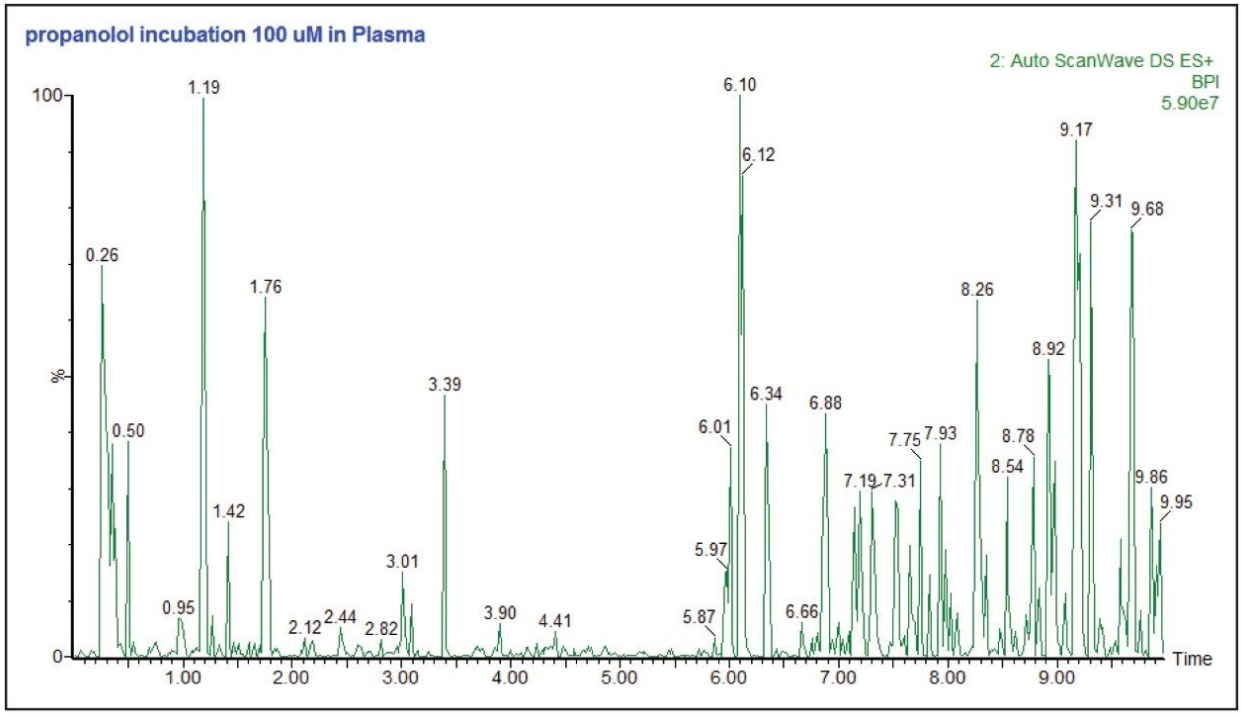

Figure 7. Survey Scan LC-MS/MS data rat liver microsomal incubation of propranolol.

The extracted ion chromatograms for the parent compound and hydroxyl, glucuronide, and hydroxy-glucuronide (*m/z* 260, 276, 436, and 452) are shown in Figure 8. The two *m/z* 436 peaks eluting after 4.5 were shown not to be drug-related by MS/MS.

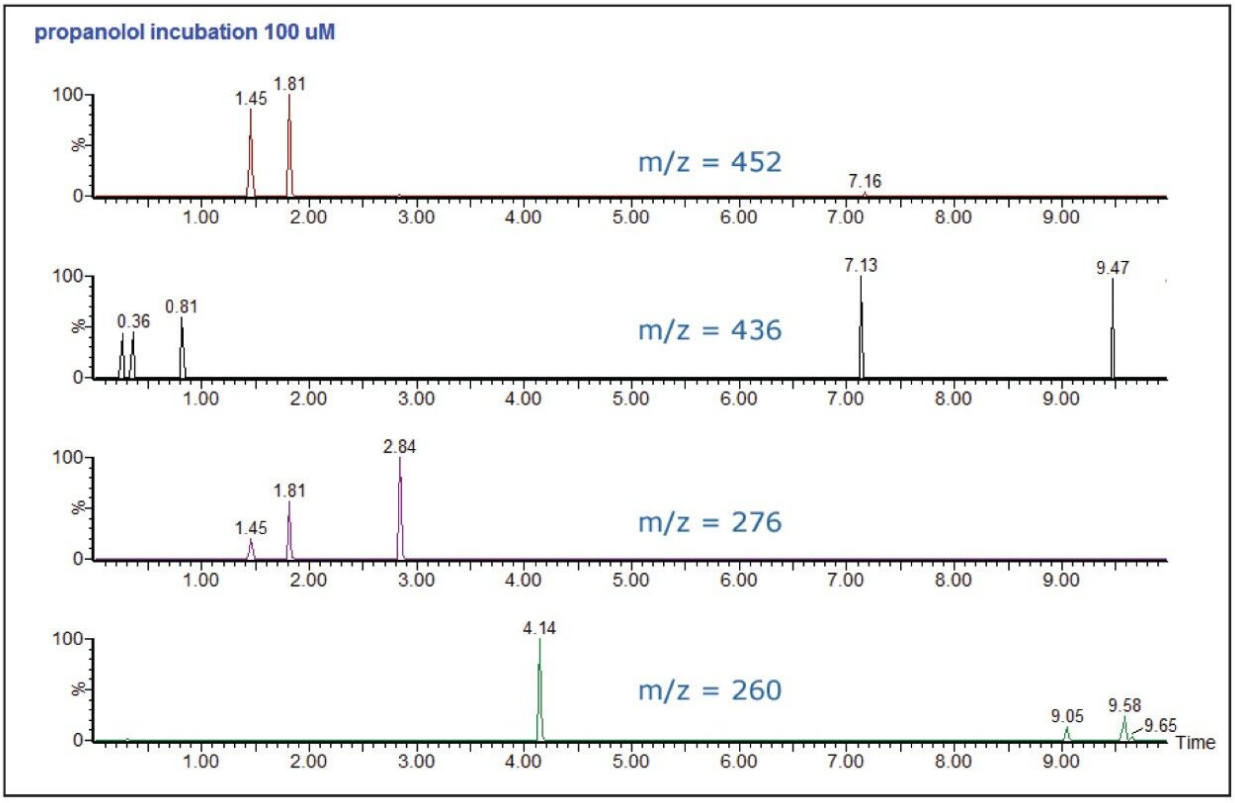

Figure 8. Extracted ion chromatograms for m/z 260, 276, 436, and 452 from the rat liver microsomal incubation of propranolol.

Here we can see that there is one peak in the *m/z* 260 channel, three peaks in the *m/z* 276 and 436 channels, and two peaks in the *m/z* 452 channel. The MS/MS spectrum of the *m/z* 260 propranolol peak is displayed in Figure 9. This peak showed the characteristic *m/z* 183 and 157 ion. The peak eluting with a retention time of 2.8 minutes corresponded to the hydroxy metabolite, but this did not show the characteristic fragment ions.

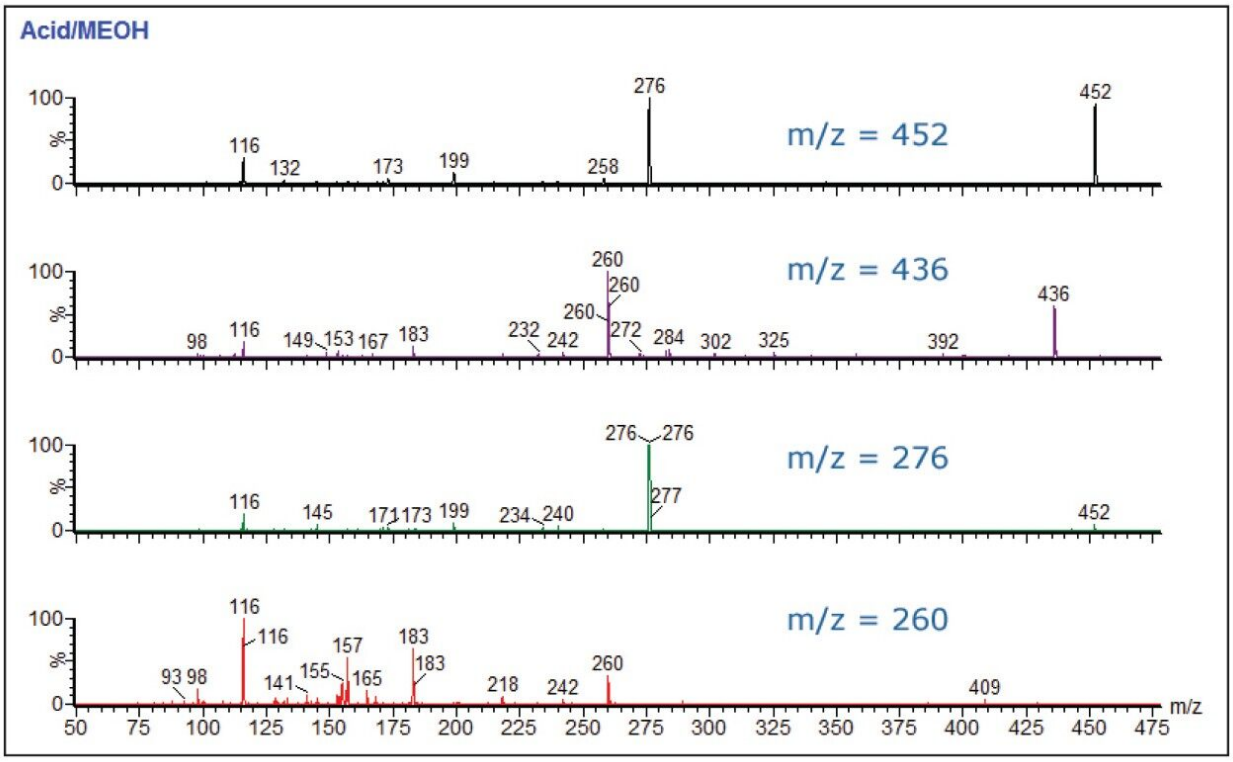

Figure 9. MS/MS spectra of peaks eluting at 1.8, 0.8, 2.8, and 4.1 minutes (top to bottom)

The MS/MS spectrum of the peak at 0.8 minutes was confirmed to be the glucuronide metabolite. The MS/MS data from the peaks eluting at 0.26 and 0.36 minutes detected in *m/z* 436 channel showed no diagnostic ions relating to the drug molecule and hence are endogenous and not drug-related. The two peaks detected in the *m/z* 452 channel showed the characteristic fragment ions related to the hydroxy-glucuronide, with the 276 and 116 ion being present, but not the *m/z* 183, 157, or 165 ions. Thus MRM data collection using these product ions would not have detected these metabolite peaks. The two peaks detected in the *m/z* 276 at retention times 1.45 and 1.85 minutes are, in fact, actually fragment ions from the hydroxy-glucuronide metabolites. The metabolites detected here were in agreement with those previously reported by Wilson ***et al.*** Despite the narrow peak widths, the fast data capture ability of the Xevo TQ MS allowed for rapid collection of MS and MS/MS data sufficient to accurately quantify the peaks of interest and obtain good quality MS/MS spectra.

Conclusion

- Modern drug discovery requires rapid quantitative and qualitative analysis of candidate drug molecules.
- To maximize return on investment, modern tandem quadrupole instruments must be able to perform both quantitative and qualitative analysis, providing high-quality MS, MRM, and MS/MS data.
- The ability of the Xevo TQ MS to rapidly switch between MS and MS/MS modes has been exploited using Survey Scan MS for the rapid detection of drug-related metabolites.
- Rapid data collection ensures that both MS and MS/MS peaks are well characterized and that high-quality spectra are acquired.
- These features combine with the high resolution of UPLC to make the Xevo TQ MS the ideal instrument for qualitative/ quantitative DMPK applications in drug discovery.

References

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3. Athersuch TJ, Sison RL, Kenyon AS, Clarkson-Jones JA, Wilson ID. Evaluation of the use of UPLC-TOF MS with simultaneous [14C]-radioflow detection for drug metabolite profiling: application to propranolol metabolites in rat urine. *J Pharm Biomed Anal.* 2008; 10, 48 (1): 151-7.

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