Simultaneous Acquisition of Qualitative and Quantitative MS data coupled with UPLC® as a Tool for Bioanalytical Method Development

Paul D. Rainville, Joanne Mather, and Robert S. Plumb
Waters Corporation 34 Maple Street Milford, MA 01757

OVERVIEW

The process of developing LC/MS methodology for the purpose of quantitative assays for medicines and their associated metabolites is a daunting challenge. Human and animal biofluids such as urine, plasma and bile vary in components and complexity. Additionally, endogenous analytes generated by a subject's age, gender and medication regimen can further interfere, contributing to the complexity in quantitative bioassay development and validation.

Even under the best chromatographic performance co-elution can occur. Implementing an approach whereby qualitative MS scan data obtained from the matrix is simultaneously acquired with quantitative MRM MS data can aid in the monitoring of potential interfering compounds, ensuring assay robustness and reproducibility.

The fact that scan data is obtained simultaneously with the MRM data means data mining can be performed at a later time if questions arise as to the presence of analytes not targeted in the original analysis. This leads to significant time and money saving because repeat analysis is less likely to be required. It also helps maximize information where sample amount may be limited such as the case as with analysis of pediatric samples.

Here we present the utilization of simultaneous acquisition of MS scan and MRM data coupled with UltraPerformance LC® as a method development tool for quantitative bioanalytical applications.

Dual Scan MRM Mode Mass Spectrometry for the Monitoring of Matrix Effects during Drug Quantification

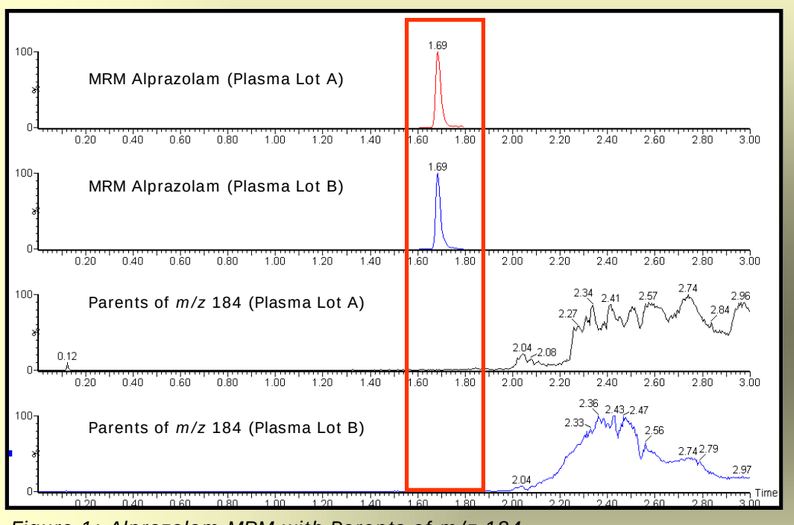

Figure 1: Alprazolam MRM with Parents of m/z 184

Alprazolam quantitation experiment (upper two traces) performed in two different plasma lots. In the bottom two traces we monitored the two different lots of matrix for phospholipids containing the choline headgroup. Here we see the differences in choline containing phospholipids present in the plasma matrix. The data indicates that there are no detected interfering matrix ions under the analyte undergoing quantitation.

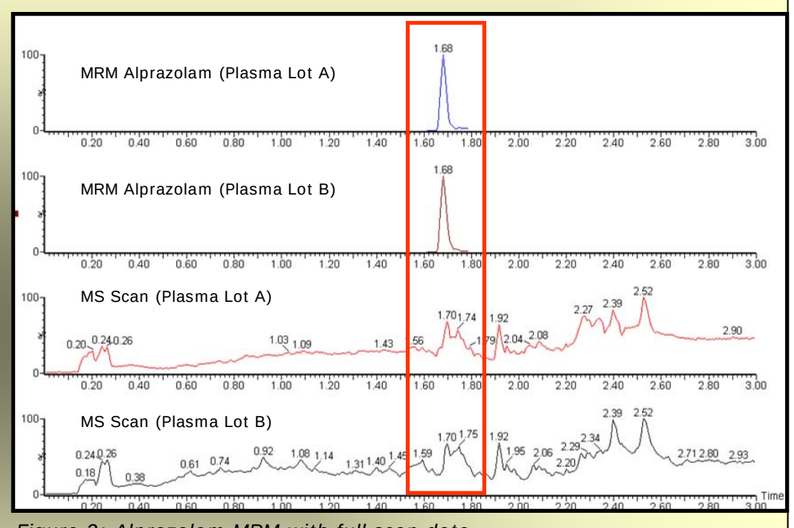

Figure 2: Alprazolam MRM with full scan data

However, Dual Scan MRM Mode displayed in the lower two traces of Figure 2 illustrates that there are other analytes in the matrix that can potentially interfere with the assay.
These matrix interferences could possibly cause ion suppression or enhancement, causing erroneous results during quantitative analysis. With this extra information the bioanalytical scientist can adjust the chromatography in order to circumvent these interferences.

Dual Scan MRM Mode Mass Spectrometry for the Detection of Metabolites During Drug Quantification

Levels of Ibuprofen in urine were measured by MRM mass spectrometry with full scan data. From the full scan data the Ibuprofen metabolites were detected. Figure 3 shows the chemical structure of Ibuprofen and some of the major in vivo metabolites

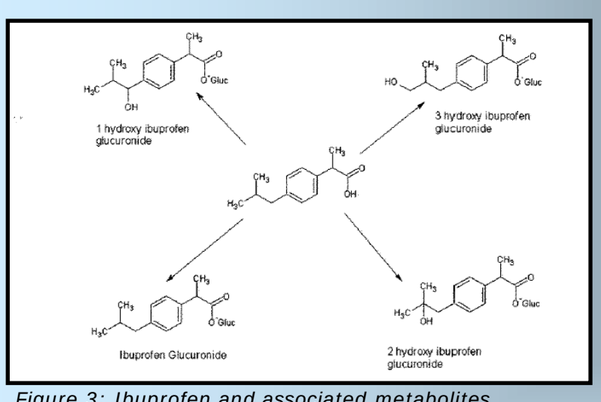

Figure 3: Ibuprofen and associated metabolites

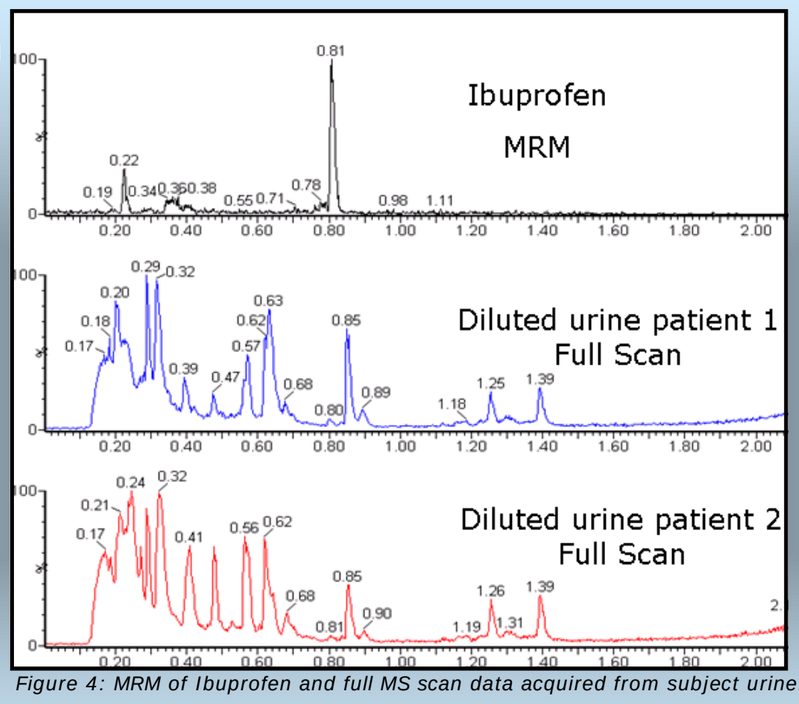

Figure 4: MRM of Ibuprofen and full MS scan data acquired from subject urine.

In this experiment, urine collected from two subjects was analyzed eight hours after dosing with 400 mg of Ibuprofen. Here we see the MRM transition data and the associated full scan data acquired during a single injection (Figure 4). The acquired full scan data was then mined for potential metabolites from the administration of Ibuprofen (Figures 5 & 6).

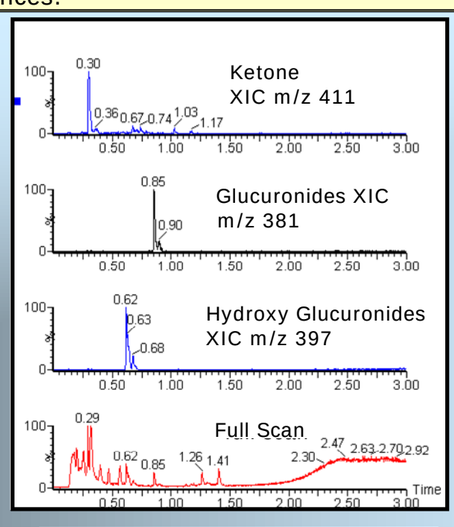

Figure 5: XIC of Ibuprofen metabolites and full scan data.

Extracted Ion Chromatograms (XIC) were generated relating to the ketone glucuronide (m/z 411) glucuronide (m/z 381) and hydroxy glucuronide metabolites (m/z 397).[1]

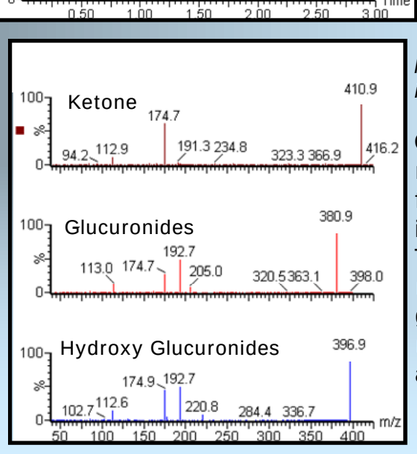

Figure 6: Product ion spectra of ibuprofen metabolites.

Confirmatory product ion MS revealed several diagnostic fragment ions indicative of ibuprofen metabolites.
These were:
m/z 193 and 175 for the glucuronide acid moieties, m/z 221 for the aglycone and m/z 113 for ibuprofen.[1]

Peak Tracking utilizing PRODUCT ION CONFIRMATION (PIC): A New Tool For Bioanalytical Method Development

In complex matrices situations can arise where closely related compounds or matrix interferences can give rise to spurious signals even in MRM mode. This can lead to ambiguity and result in the need for a second qualitative experiment to be performed. A product ion confirmatory scan provides a means of verifying that the signal from the MRM peak is from the compound of interest. With conventional instrumentation these experiments require separate analytical experiments. Many conventional tandem quadrupole MS instruments are unable to perform MRM and scan experiments simultaneously, in the timescale of an LC peak, without significantly compromising data quality. Xevo™ TQ mass spectrometer is equipped with a novel collision cell design where the collision gas is always on allowing both quantification (MRM) and characterization to be performed simultaneously on the peak as it elutes from the LC or UPLC column while maintaining good data quality.[2]

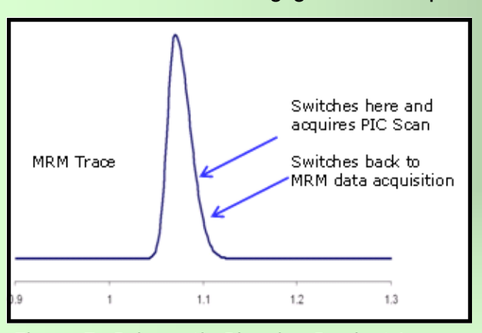

Figure 7: Schematic Showing Product Ion Confirmation Switching After the Peak Top.

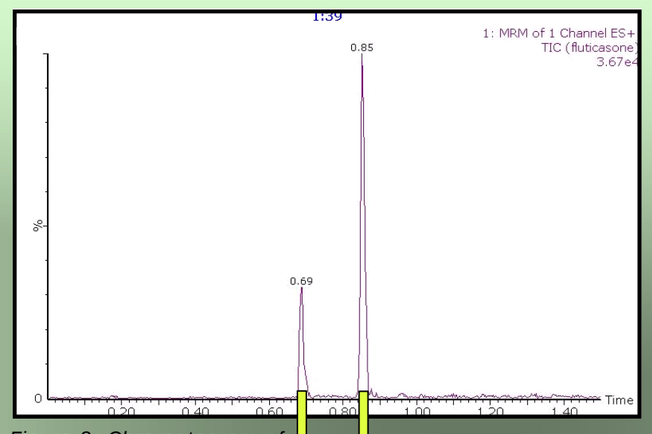

Figure 8: Chromatogram of MRM 501>293

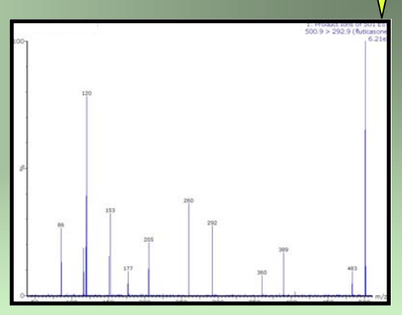

Figure 9: PIC ScanWave™ DS Spectrum of peak @ retention time 0.69 minutes.

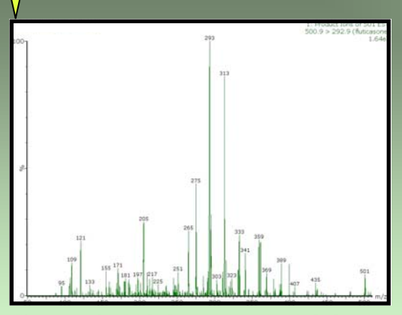

Figure 10: PIC ScanWave™ DS Spectrum of peak @ retention time 0.85 minutes.

In this experiment the eluent from an SPE experiment was analysed. Two analytes were detected with the same MRM channel (*m/z* 501 > 293) (Figure 8).
PIC data acquired in the same analytical run as the MRM channel showed that the two analytes were separate compounds. PIC data indicated that the analyte at retention time 0.85 minutes was the peak of interest. This was confirmed by comparing the PIC DS data to the Daughter Scan of Fluticasone acquired during sample tuning.

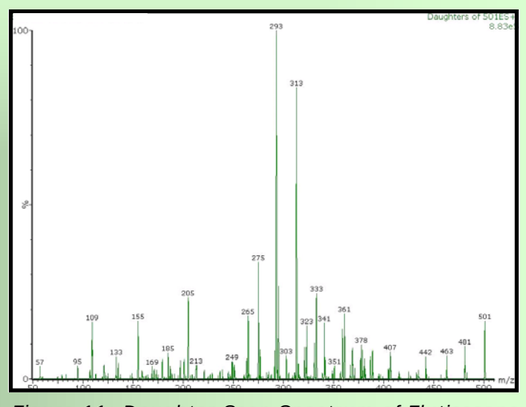

Figure 11: Daughter Scan Spectrum of Fluticasone.

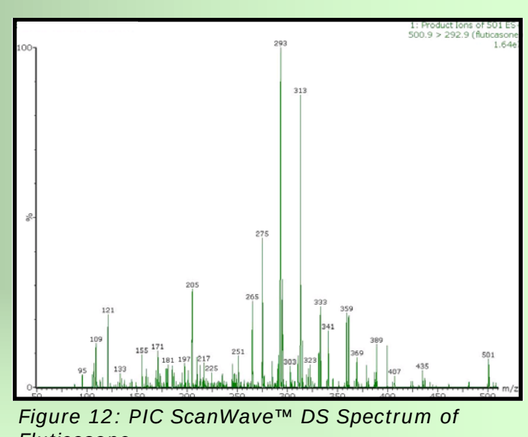

Figure 12: PIC ScanWave™ DS Spectrum of Fluticasone.

METHODS

LC System:	**Waters® ACQUITY UPLC® System**
Column:	ACQUITY UPLC® BEH C18 Column 2.1 x 50 mm, 1.7 µm
Column Temp:	40 ˚C
Flow Rate:	600 µL/min.
Gradient:	5-95% B/2 min.
MS System:	**Waters Xevo™ TQ MS**
Desolvation Temp:	550 ˚C
Desolvation Gas:	1000 L/Hr
Source Temp:	150 ˚C

Alprazolam

Mobile Phase A:	0.1% NH_4OH
Mobile Phase B:	MeOH
Ionization Mode:	ESI Positive
Capillary Voltage:	1600 V
Cone Voltage:	50 V
Collision Energies:	MRM data 26 V, Full scan data 3 V
MRM Transition:	*m/z* 309 > 281

Ibuprofen

Mobile Phase A:	0.1% NH_4OH
Mobile Phase B:	Acetonitrile
Ionization Mode:	ESI Negative
Capillary Voltage:	2000 V
Cone Voltage:	15 V
Collision Energies:	MRM data 7 V, Full scan data 3 V
MRM Transition:	*m/z* 205 > 161

Fluticasone

Mobile Phase A:	0.1% NH_4OH
Mobile Phase B:	MeOH
Ionization Mode:	ESI Positive
Capillary Voltage:	1000 V
Cone Voltage:	30 V
Collision Energies:	MRM data 17 V, Full scan data 3 V
MRM Transition:	*m/z* 501 > 293

CONCLUSION

- Xevo TQ MS™ has the ability to produce MS & MS/MS data in one analytical experiment.
- The ability to produce MRM & Scan data in one experiment ensures matrix interferences can be accurately monitored during the method development process.
- Metabolite information can be obtained from full scan data whilst simultaneously performing quantitation on a drug.
- PIC data was used to confirm analyte identity during method development without the requirement for further experiments.

REFERENCES

1. Plumb *et al.* Rapid communications in mass spectrometry 2007;**21**:4079 4085.
2. Twohig *et al*. Simultaneous Confirmation and Quantitation using Xevo™ TQ MS: Product Ion Confirmation (PIC). Waters literature code: 720002829en.
3. Rainville *et al*. A Novel Method for Monitoring Matrix Interferences Utilizing Simultaneous Acquisition of Full Scan and MRM Mass Spectrometry Waters literature code: 720002830en.
4. Rainville *et al*. Novel Dual Scan MRM Mode Mass Spectrometry for the Detection of Metabolites During Drug Quantification. Waters literature code: 720002832en.

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