IMPROVEMENTS IN SENSITIVITY FOR BIOANALYSIS OF WARFARIN USING ACQUITY™ PREMIER UPLC SYSTEM AND XEVO™ TQ ABSOLUTE TANDEM QUADRUPOLE MASS SPECTROMETER

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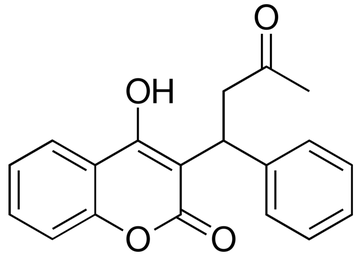
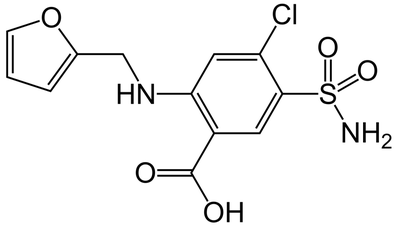
INTRODUCTION

Bioanalysis plays a critical role in drug discovery and drug development stages by providing quantitative information about the concentrations of drug, its metabolites, and related biomarkers in the body. This data enables timely decision making around the absorption, distribution, metabolism, excretion and toxicology (ADMET) profile of the drug and its progression through the various steps of the drug discovery and development pipeline. Discovery bioanalysis laboratories routinely develop LC-MS methods & analyze PK samples for hundreds of compounds per week[1]. As such, there is a drive to simplify as many steps of the sample extraction, LC and MS methods as possible. However, the practical need to have a generic method has to be balanced with the need for achieving the appropriate sensitivity for a given molecule or panel of molecules[2]. In such a scenario, having instrumentation platforms that work well for all analyte types significantly increases the chances of success. Some of the most challenging analytes that make their way through these laboratories are those which tend to have metal chelating properties due to the presence of uncharged amines, phosphates and deprotonated carboxylic acids or analytes that have a propensity to lose a proton and take up a negative charge in the source of the mass spectrometer. The lack of appropriate analytical platforms makes analysis of these types of molecules challenging. In this application note, we have used Warfarin and Furosemide as representative molecules within a typical discovery bioanalytical laboratory.

Warfarin and Furosemide extracted from human and rat plasma were quantified using ACQUITY™ Premier UPLC™ system and Xevo™ TQ Absolute mass spectrometer at LLOQ's of 25 pg/mL and 100 pg/mL respectively. %CV for all points on the calibration curve and QC's were below 13% for both analytes in both matrices



Structure of Warfarin



Structure of Furosemide

METHODS

Sample Preparation

Warfarin & Furosemide stock solutions at 100 ng/mL were used to spike rat and human plasma to create a calibration curve from 0.025 – 100 ng/mL. A separate stock solution (100 ng/mL) was used to generate QC samples at LLOQ, LQC, MQC and HQC. 100 µL of all samples were extracted in triplicate using 1:3 protein precipitation with acetonitrile. Samples were centrifuged and the supernatant was transferred to a LC-MS vial for analysis.

LC System:	ACQUIT Premier UPLC system
Detection:	Xevo TQ Absolute Mass Spectrometer
Vials:	Waters™ Total Recovery LC MS vials
Column(s):	MaxPeak ™ UPLC HSS T3 2.1x50mm column
Column Temp.:	60° C
Sample Temp.:	5° C
Injection Volume:	1 µL
Flow Rate:	600 µL/min
Mobile Phase A:	0.01% Formic acid in 100 % Water
Mobile Phase B:	0.01% Formic acid in 100% Acetonitrile
Gradient:	5-95% over 1.5 minutes

MS System:	Xevo TQ Absolute
Ionization Mode:	ESI Negative
Capillary Voltage:	0.5 kV

Analyte	Precursor (m/z)	Product (m/z)	CV (V)	CE (kV)
Warfarin	307.01	249.98	24	24
Warfarin	307.01	160.89	24	20
Furosemide	328.8	77.80	20	30

RESULTS & DISCUSSION

The ACQUITY Premier System features novel MaxPeak High Performance Surfaces (HPS) technology, which effectively reduces non-specific adsorption losses due to metal interactions. In a discovery bioanalysis laboratory setting, which sees a variety of analytes across the entire drugable physico-chemical space, technologies like these can significantly reduce the method development and sample analysis times; by removing the need for complicated sample preparation steps like derivatization and esoteric chromatographic columns, buffers and mobile phases. The Xevo TQ Absolute is a new high performance tandem quadrupole mass spectrometer with a compact design, enhanced negative ion detection and removable source shield to reduce source contamination from sample matrix and mobile phase buffer salts. These attributes are ideal for a routine, robust and sensitive detector which provides class leading sensitivity in both positive and negative modes and minimal cleaning down time for a laboratory running hundreds of samples per week.

Using the sample extraction and LC-MS method described above, we were able to achieve a LLOQ of 25 pg/mL for Warfarin from human (Figure 1A) and rat plasma (Figure 1B). These LLOQs were determined based on a signal to noise of 5 compared to the same retention time in the blank extracted sample. Similarly, the LLOQ for Furosemide was determined to be 100 pg/mL in both human (Figure 2A) and rat plasma (Figure 2B).

The observed MS response for both analytes increased linearly as shown by the representative chromatograms from levels across the calibration curve (Figure 3A & 3B).

All points on the calibration and QC's were extracted on triplicate on each day. The % CVs for all points across all levels were <12%. (Table 1A & 1B)

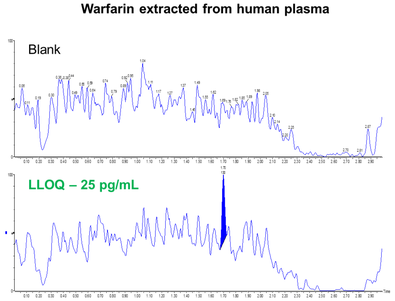


Figure 1A - Representative chromatogram for the LLOQ of Warfarin extracted from human plasma

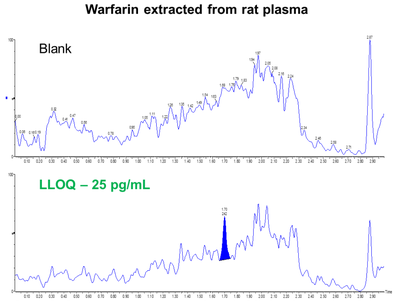


Figure 1B - Representative chromatogram for the LLOQ of Warfarin extracted from rat plasma

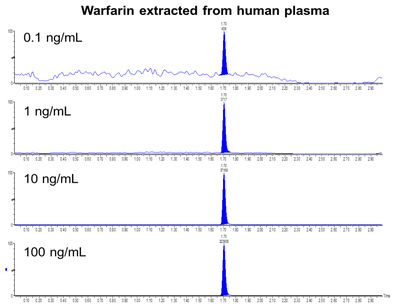


Figure 3A - Representative chromatogram for Warfarin extracted from human plasma across the calibration curve

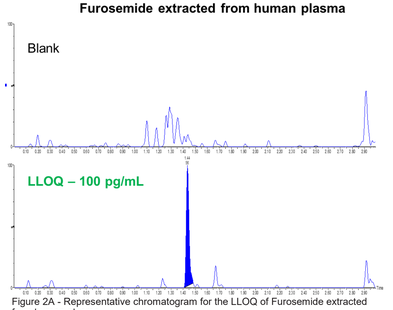


Figure 2A - Representative chromatogram for the LLOQ of Furosemide extracted from human plasma

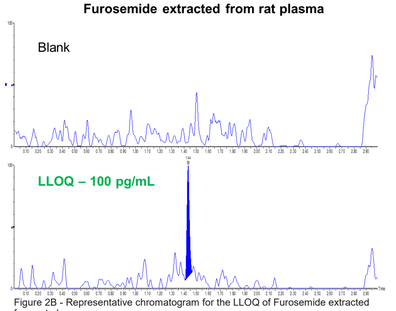


Figure 2B - Representative chromatogram for the LLOQ of Furosemide extracted from rat plasma

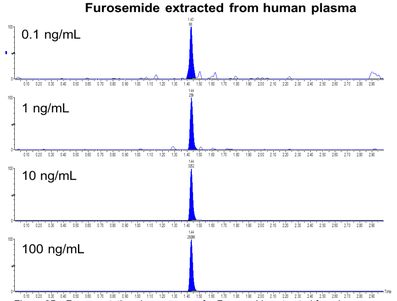


Figure 3B - Representative chromatogram for Furosemide extracted from human plasma across the calibration curve

WARFARIN IN HUMAN PLASMA					
	Expected Concentration (ng/mL)	Mean (ng/mL)	Standard Deviation	% CV	% Accuracy
LLOQ	0.075	0.074	0.002	9.26	98.44
LQC	1	1.01	0.08	8.37	100.66
MQC	10	10.71	0.46	4.30	107.05
HQC	75	77.87	3.75	4.81	103.82

FUROSEMIDE IN HUMAN PLASMA					
	Expected Concentration (ng/mL)	Mean (ng/mL)	Standard Deviation	% CV	% Accuracy
LLOQ	0.25	0.26	0.01	11.42	101.88
LQC	1	0.97	0.04	8.94	97.08
MQC	10	10.75	0.50	4.63	107.75
HQC	75	76.32	1.77	2.33	101.76

CONCLUSION

Warfarin and Furosemide extracted from human and rat plasma were quantified using ACQUITY Premier UPLC system and Xevo TQ Absolute. The observed results were as follows:

- LLOQ for Warfarin and Furosemide were 25 pg/mL and 100 pg/mL from both human and rat plasma
- % CV's for all points across the calibration curve and QC levels were <12%, well within the allowed bioanalytical acceptance criteria.

ACQUITY Premier UPLC system with MaxPeak HPS technology columns and Xevo TQ Absolute can become the ideal platform to use within discovery bioanalytical laboratories as it provides excellent robustness and sensitivity without compromise.

References

1. Standardized workflows for increasing efficiency and productivity in discovery stage bioanalysis, Bateman KP, Cohen L, Emary B, Pucci V., Bioanalysis. 2013 Jul;5 (14):1783-94
2. A systematic approach for developing a robust LC-MS/MS method for bioanalysis, Meng M, Wang L, Voelker T, Reuschel S, Van Horne K, Bennett P., Bioanalysis. 2013 Jan;5(1):91-115. doi: 10.4155/bio.12.295

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