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應用手冊

# Quantification of Warfarin and Furosemide in Human and Rat Plasma for Discovery Bioanalysis Using ACQUITY™ Premier UPLC™ System and Xevo™ TQ Absolute Mass Spectrometer

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## Abstract

Accurate and precise quantification of pharmaceutical compounds is critical to the drug discovery and development process. UHPLC coupled with tandem quadrupole mass spectrometry is the gold standard in such analysis. As the importance quantifying drug candidates and biomarkers at lower concentrations increases, so does the need for more sensitive instrument platforms. In this application note, we describe a generic method for the extraction and quantification of two representative model compounds, Warfarin and Furosemide from human and rat plasma.

Warfarin is an anticoagulant used in the treatment and prevention of thromboembolism. Careful and continuous monitoring of warfarin is essential to ensure that the drug concentration stays within its narrow therapeutic window. Furosemide is a diuretic used to treat edema in patients with heart failure, liver disease or a kidney

disorder.

Using the ACQUITY™ Premier UPLC System and Xevo™ TQ Absolute Mass Spectrometer operated in negative ion ESI mode, we were able to achieve linear calibration curves from 0.025–100 ng/mL for Warfarin and 0.1–100 ng/mL for Furosemide from both rat and human plasma. %CV for all points on the calibration curve and QC's were below 13% for both analytes in both matrices.

## Benefits

- Accurate, precise, and sensitive quantification
- Warfarin and Furosemide
- ACQUITY Premier UPLC System
- Xevo TQ Absolute Mass Spectrometer

## 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 and 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.

## Experimental

Warfarin and 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 Conditions

| | |
|---|---|
| LC system: | ACQUITY™ Premier UPLC System |
| Detection: | Xevo™ TQ Absolute Mass Spectrometer |
| Vials: | Waters Total Recovery LCMS vials |
| Column(s): | MaxPeak™ UPLC HSS T3 2.1 x 50 mm 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 Conditions

| | |
|---|---|
| MS system: | Xevo™ TQ Absolute |
| Ionization mode: | ESI- |
| Capillary voltage: | 0.5 kV |

## MRM Transitions

| Analyte | Precursor (*m/z*) | Product (*m/z*) | Cone voltage (V) | Collision energy (kV) |
|---|---|---|---|---|
| Warfarin | 307.01 | 249.98 | 24 | 24 |
| Warfarin | 307.01 | 160.89 | 24 | 20 |
| Furosemide | 328.8 | 77.80 | 20 | 30 |

# Results and 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 Tandem Quadrupole Mass Spectrometer 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 rat and human plasma (Figure 1). 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 (Figure 2) in both human and rat plasma.

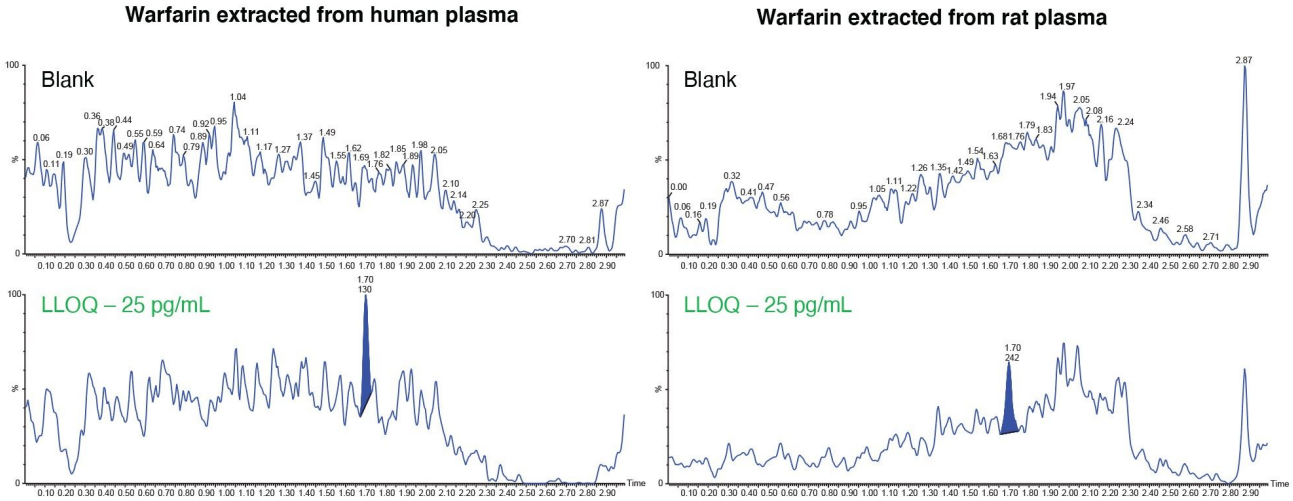


*Figure 1. LLOQ for Warfarin extracted from human and rat plasma.*

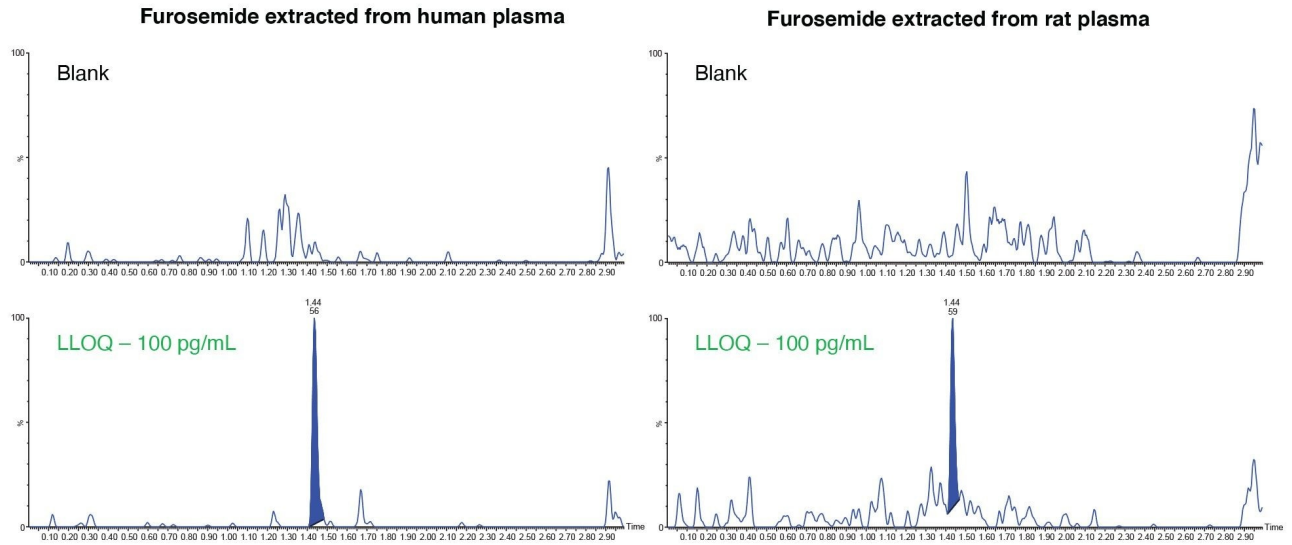


*Figure 2. LLOQ for Furosemide extracted from human and rat plasma.*

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

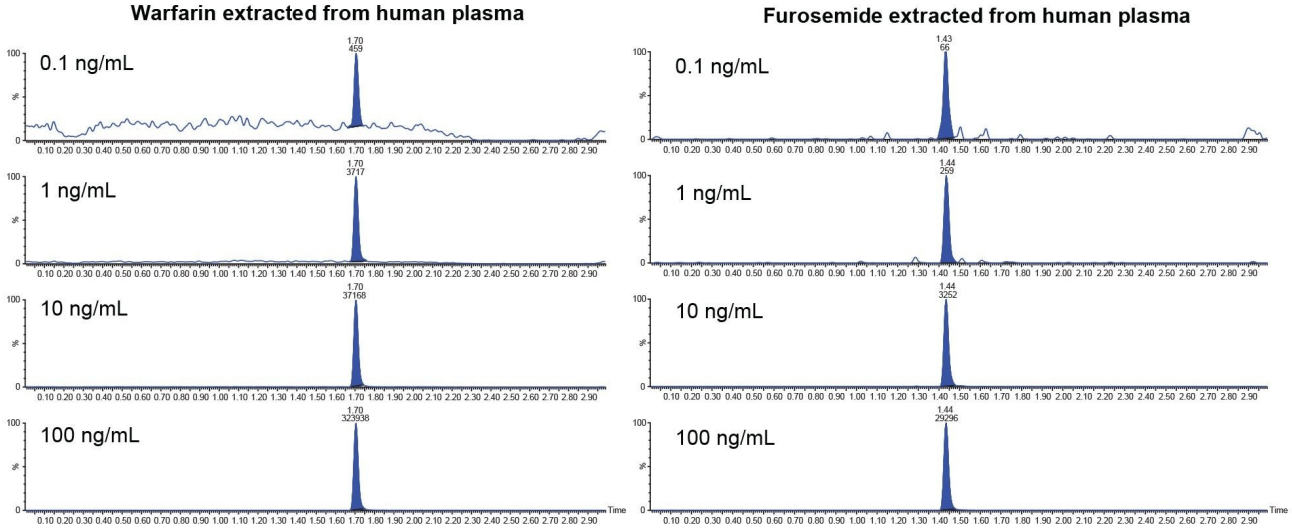


*Figure 3. Representative chromatograms for Warfarin and Furosemide extracted from human plasma at different points across the calibration curve.*

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 1).

| 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 |

*Table 1. Inter-day Accuracy and Precision for Warfarin and Furosemide extracted from human plasma (3 days, triplicates on each day).*

## Conclusion

Warfarin and Furosemide extracted from human and rat plasma were quantified using ACQUITY Premier UPLC System and Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer. 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 and Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer can become the ideal platform to use within discovery bioanalytical laboratories as it provides excellent robustness and sensitivity without compromise.

## References


1. Bateman KP, Cohen L, Emary B, Pucci V., Standardized Workflows for Increasing Efficiency and Productivity in Discovery Stage Bioanalysis, ***Bioanalysis***. 2013 Jul;5(14):1783–94.
2. Meng M, Wang L, Voelker T, Reuschel S, Van Horne K, Bennett P., A Systematic Approach for Developing a Robust LC-MS/MS Method for Bioanalysis, ***Bioanalysis***. 2013 Jan;5(1):91–115. doi: 10.4155/bio.12.295.


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