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Next-level Sensitivity for the Quantification of Warfarin and Furosemide in Human Plasma

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Introduction

This technical note demonstrates the sensitive quantification of small molecule pharmaceutical compounds extracted from human plasma using minimal sample preparation and negative ion mode-based analysis. Lower limits of quantification (LLOQs) of 3.13 pg/mL and 25 pg/mL were achieved for Warfarin and Furosemide, respectively. Quantitative performance of the assay highlighted outstanding precision, accuracy, and linearity. Enhanced assay sensitivity was achieved through the application of a high-end triple quadrupole mass spectrometer to meet the demands of routine bioanalysis in complex matrices.

Demand for improved sensitivity in bioanalytical assays continues to increase as drug discovery and development programs focus on more efficacious, lower dosage compounds and as throughput demands drive the simplification of sample extraction and LC and MS methods. In many cases, the use of a more sensitive mass spectrometer is the easiest way to meet these needs. Having a system that offers technological improvements that deliver sensitivity gains across the mass range and in both polarities offers the bioanalytical scientist maximum flexibility to address the challenges outlined above. Here, Warfarin and Furosemide were extracted from human plasma and quantified using a negative ion mode approach on the SCIEX 7500 system (**Figure 1**). The improved front-end technology of the system enabled greater ion generation, capture and transmission to improve sensitivity for routine bioanalysis.

Sample Preparation

Warfarin and Furosemide were spiked into 100 µL aliquots of human plasma at concentrations ranging from 3.13 to 100,000 pg/mL. Samples were extracted using protein precipitation with 300 µL Acetonitrile. The samples were then vortexed and centrifuged at 10,000 rpm for 25 minutes. The supernatant was collected for analysis.

LC Conditions

Column: Kinetex™ 1.7 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4475-AN
Mobile Phase: A: 0.01 % Formic Acid in Water
B: 0.01 % Formic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	15
0.25	15
3	50
3.1	95
4	95
4.1	15
5	15

Flow Rate: 0.6 mL/min
Injection Volume: 1 µL
Temperature: Ambient
LC System: SCIEX® ExionLC™
Detection: MS/MS
Detector: SCIEX Triple Quad™ 7500

MS/MS Conditions

Polarity: Negative
Source Temperature: 650 °C
GS1: 60 psi
GS2: 70 psi
CUR: 40 psi
CAD: 10
IS: 1700 V

MS/MS Transitions and Parameters

Analyte	Q1 (m/z)	Q3 (m/z)	EP (V)	CE (V)	CXP (V)
Warfarin	307.1	250.1	-10	-31	-9
Furosemide	329	77.9	-10	-40	-9

Revision: 0
PHEN-RUO-00185

Results and Discussion

Calibration curves were acquired across the concentration range of 3.13 to 100,000 pg/mL. Each concentration was analyzed in triplicate to assess method reproducibility. Strong linearity was observed across the concentration ranges analyzed, as demonstrated in **Figures 2** and **3** for Warfarin and Furosemide, respectively. **Table 1** summarizes the quantification results, including accuracy and precision. Excellent %CVs were achieved across all concentration levels with no interference in the blank human plasma samples for Warfarin and Furosemide. Using a generic sample preparation technique for the analysis of 100 µL of human plasma and a total run time of 5 minutes, the method provided LLOQs of 3.13 pg/mL and 25 pg/mL for Warfarin and Furosemide, respectively (**Figures 4** and **5**).

Figure 1. Extracted Ion Chromatograms (XICs) Representing the Matrix Blank and LLOQs of Warfarin and Furosemide Extracted from Human Plasma.

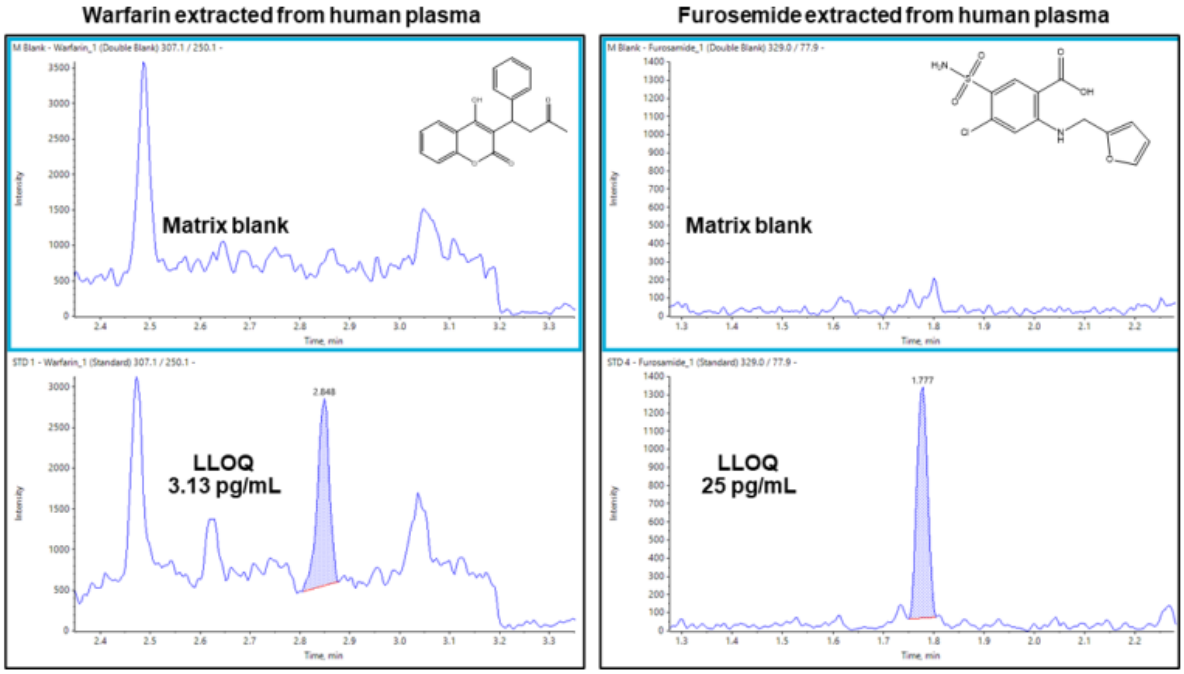


Figure 2. The Calibration Curve for Quantification of Warfarin in Human Plasma.

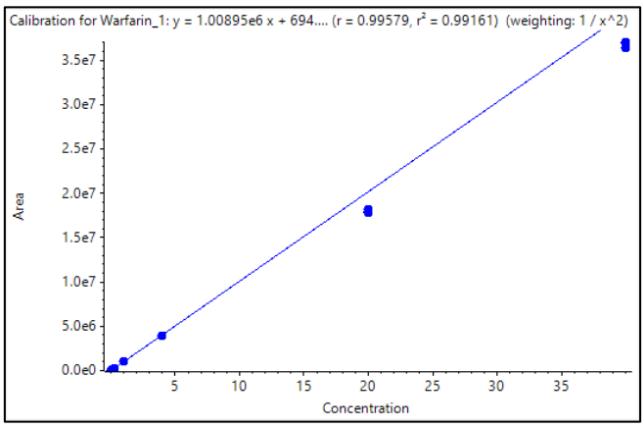


Figure 3. The Calibration Curve for the Quantification of Furosemide from Human Plasma.

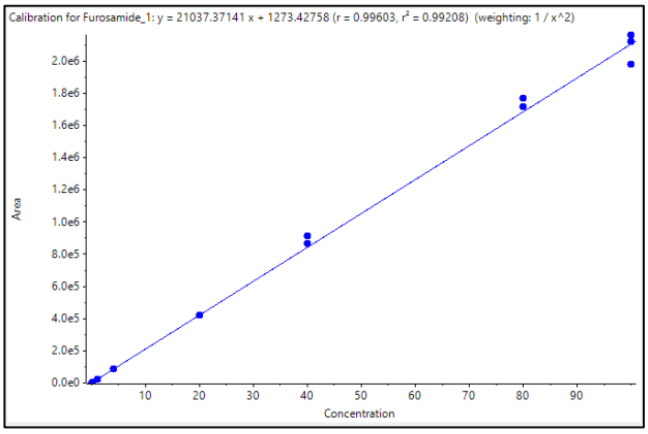


Table 1. Quantification Summary for Warfarin and Furosemide.

	Warfarin		Furosemide	
Concentration (fmol/µL)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
3.13	100	9.2	N/A	N/A
6.25	94.5	4.1	N/A	N/A
12.5	103	1.4	N/A	N/A
25.0	106	2.6	104	15.3
62.5	115	1.7	91.6	10.2
250	103	0.8	88.5	1.6
1000	100	1.5	102	1.3
4000	97.1	1.4	104	1.2
20,000	89.1	1.3	99.4	0.1
40,000	91.2	0.9	107	3.1
80,000	N/A	N/A	104	1.8
100,000	N/A	N/A	99.2	4.5

Figure 4. XICs Demostrating Warfarin Extraction from Human Plasma. a) Matrix Blank, and Warfarin present at b) 3.13 pg/mL, c) 6.25 pg/mL, d) 12.5 pg/mL, and e) 25 pg/mL.

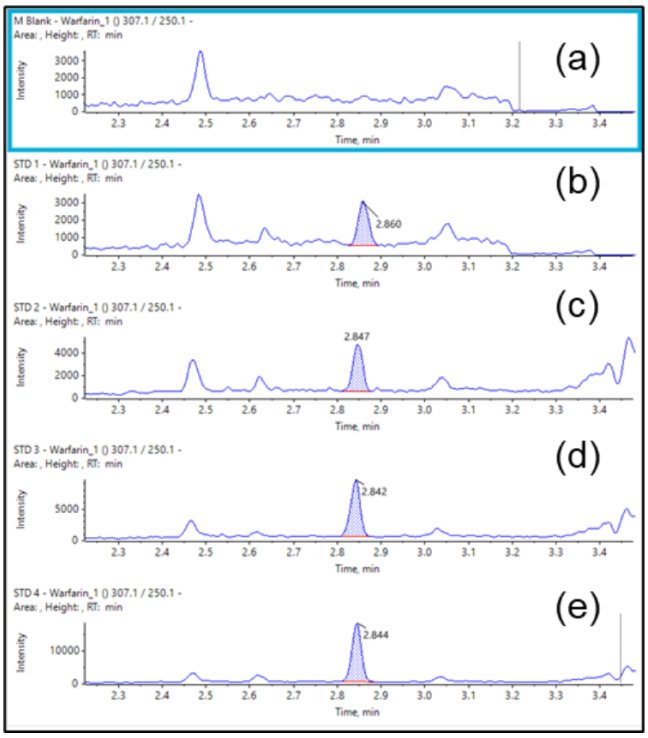
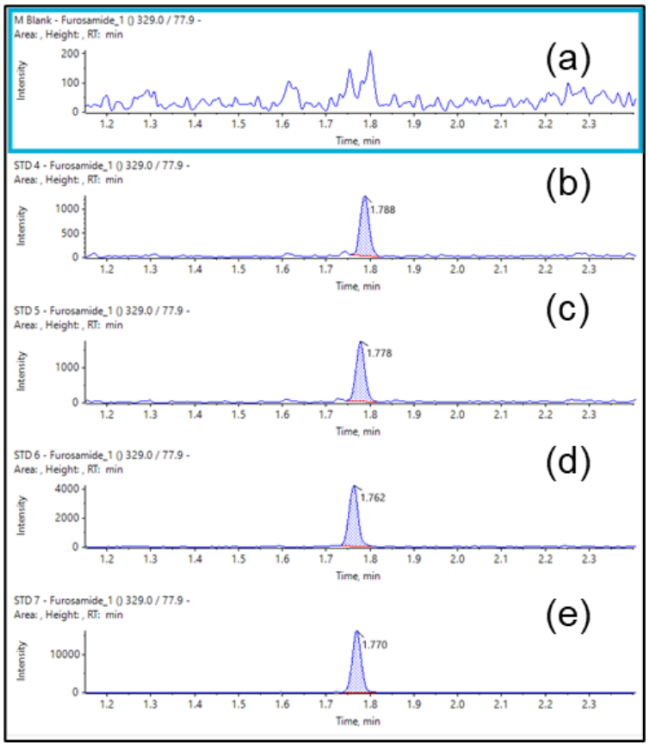


Figure 5. XICs Demostrating Furosemide Extraction from Human Plasma. a) Matrix Blank, and Furosemide present at b) 25 pg/mL, c) 62.5 pg/mL, d) 250 pg/mL, and e) 1000 pg/mL.



Conclusions

Low-pg/mL level LLOQs were reached for Warfarin and Furosemide extracted from human plasma using minimal sample preparation. A highly sensitive assay for the quantification of small molecule pharmaceutical compounds requiring negative ion mode-based analysis was demonstrated on the SCIEX® 7500 system. The method demonstrated excellent accuracy, precision, and linearity at all concentration levels. A single platform for streamlined data acquisition, processing, and management with SCIEX OS software was presented. Overall, the SCIEX 7500 system enables pharmaceutical researchers the maximum flexibility to explore lower dosage, higher efficacy compounds, and improve the efficiency of bioanalysis.

Kinetex™ Ordering Information

1.7 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	—	00B-4726-AN	00D-4726-AN	00F-4726-AN	AJ0-9298
Biphenyl	00A-4628-AN	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209
XB-C18	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN	AJ0-8782
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782
C8	00A-4499-AN	00B-4499-AN	00D-4499-AN	00F-4499-AN	AJ0-8784
HILIC	00A-4474-AN	00B-4474-AN	00D-4474-AN	—	AJ0-8786
Phenyl-Hexyl	—	00B-4500-AN	00D-4500-AN	00F-4500-AN	AJ0-8788
F5	—	00B-4722-AN	00D-4722-AN	00F-4722-AN	AJ0-9322

for 2.1 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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