Next-level sensitivity for the quantification of warfarin and furosemide in human plasma

Achieve increased sensitivity for quantification in complex matrices using the SCIEX 7500 system

Ian Moore and Rahul Baghla
SCIEX, Canada

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.[1]

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.

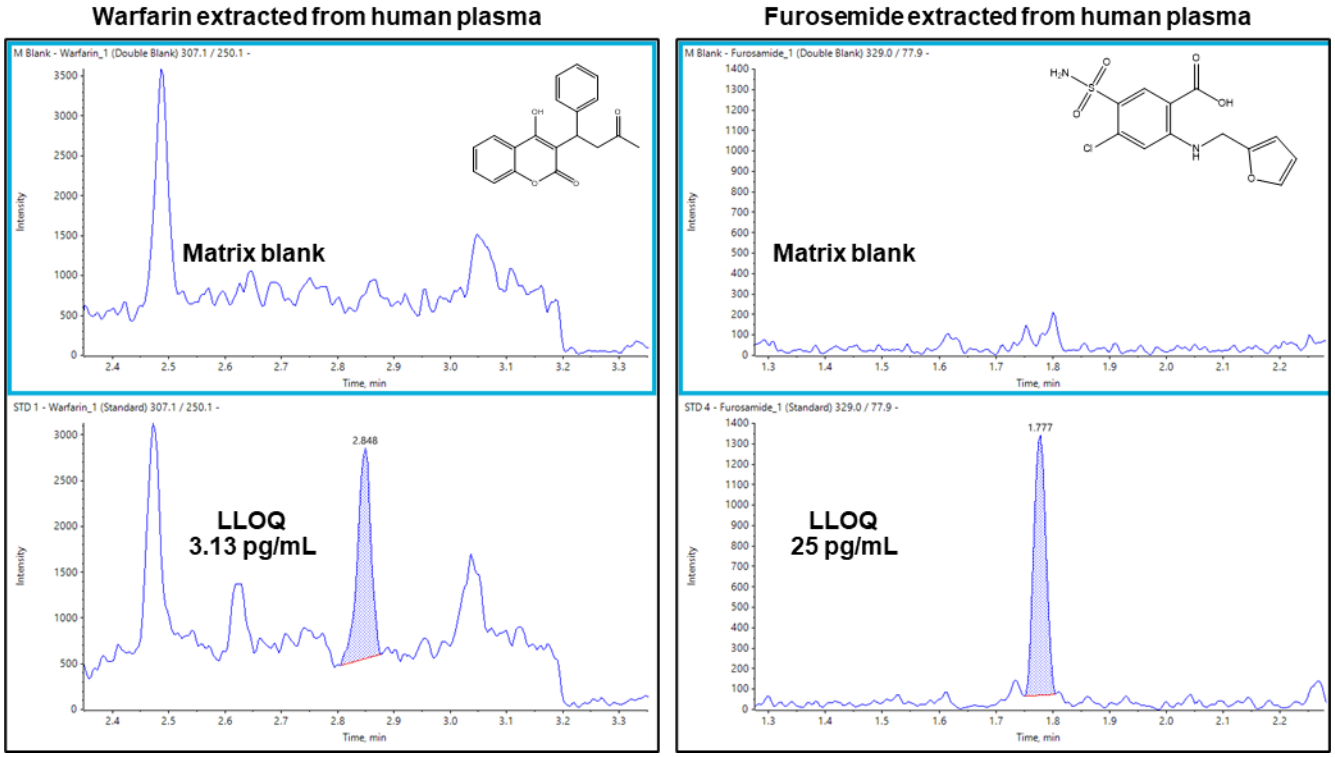

Figure 1. Extracted ion chromatograms (XICs) representing the matrix blank and LLOQs of warfarin and furosemide extracted from human plasma. An LLOQ of 3.13 pg/mL and 25 pg/mL was achieved for warfarin and furosemide, respectively. No interferences were observed in the matrix blanks of warfarin and furosemide.

Key features of the SCIEX 7500 system for high sensitivity bioanalysis

- **Sensitive quantification:** Achieve low-pg/mL level LLOQs for the quantification of warfarin and furosemide extracted from human plasma using minimal sample preparation and negative ion mode-based analysis
- **Excellent quantitative performance:** Achieve outstanding accuracy, precision and linearity for the analysis of small molecule pharmaceutical compounds using the SCIEX 7500 system featuring improved front-end technology
- **Streamlined data management:** Increase productivity with a user-friendly interface and integrated platform for data acquisition, processing and management for routine bioanalysis using SCIEX OS software

Methods

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.

Chromatography: Samples were analyzed using the ExionLC AC system at a flow rate of 0.6 mL/min using a Phenomenex Kinetex C18 column (2.1 x 50 mm, 1.7 µm, 100 Å) with a 5-minute gradient (Table 1). Mobile phase A was 0.01% formic acid in water and mobile phase B was 0.01% formic acid in acetonitrile. A 1 µL injection volume was used for analysis.

Table 1. Chromatographic gradient.

Time (min)	Flow (mL/min)	%B Conc
0.25	0.600	15.0
3.00	0.600	50.0
3.10	0.600	95.0
4.00	0.600	95.0
4.10	0.600	15.0
5.00	0.600	15.0

Mass spectrometry: Samples were analyzed using the SCIEX 7500 system operating in negative ion mode equipped with the OptiFlow Pro ion source. The system was controlled by SCIEX OS software. The optimized MS parameters are listed in Table 2.

Data processing: Data processing was performed with SCIEX OS software, version 3.0, using the Analytics module. A $1/x^2$ weighting was applied for the quantification of warfarin and furosemide.

Table 2. Optimized MS parameters.

ID	Q1/Q3 (*m/z*)	EP (V)	CE (V)	CXP (V)
Warfarin	*307.1/250.1*	*-10*	*-31*	*-9*
Furosemide	*329.0/77.9*	*-10*	*-40*	*-9*

Source parameters	Value	Source parameters	Value
Curtain gas	*40 psi*	*CAD gas*	*10*
Ion source gas 1	*60 psi*	*Ion spray voltage*	*1700 V*
Ion source gas 2	*70 psi*	*Source temp*	*650°C*

Quantitative performance

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 3 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

Table 3. Quantification summary for warfarin and furosemide.

	Warfarin		Furosemide	
Concentration (pg/mL)	Average accuracy (%)	CV (%)	Average 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*

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).

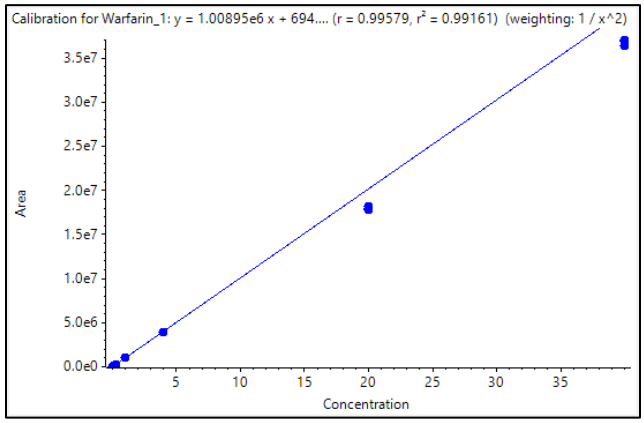

Figure 2. The calibration curve for quantification of warfarin in human plasma. The calibration range covered 3.13 to 40,000 pg/mL.

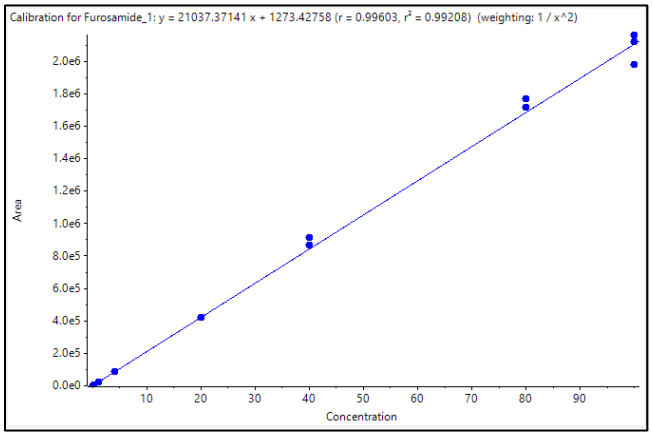

Figure 3. The calibration curve for the quantification of furosemide from human plasma. The calibration range covered 25 to 100,000 pg/mL.

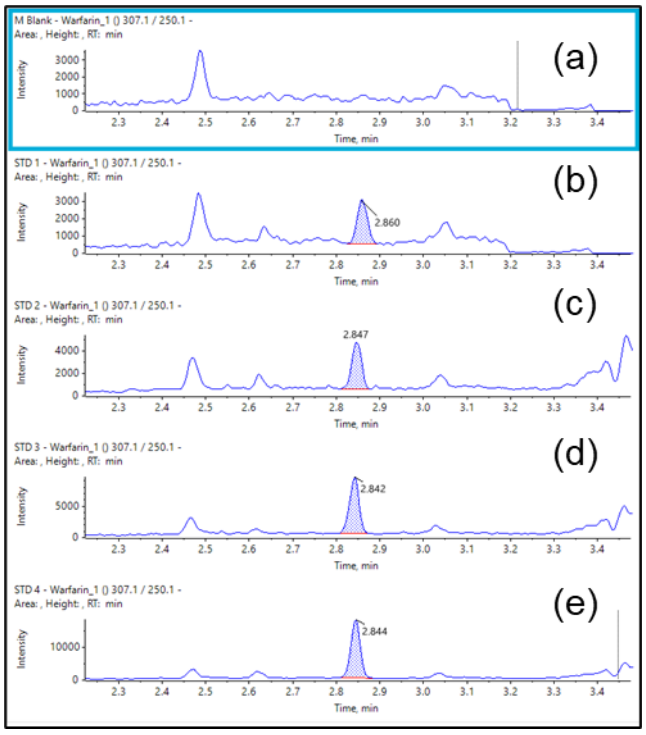

Figure 4. XICs demonstrating warfarin extraction from human plasma. XICs of the matrix blank (a) and warfarin present at 3.13 pg/mL (b), 6.25 pg/mL (c), 12.5 pg/mL (d) and 25 pg/mL (e) are shown.

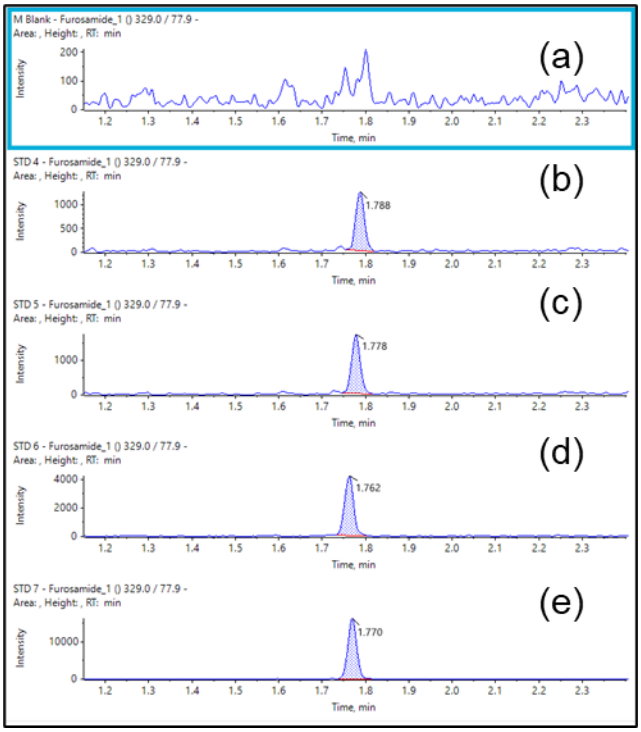

Figure 5. XICs demonstrating furosemide extraction from human plasma. XICs of the matrix blank (a) and furosemide present at 25 pg/mL (b), 62.5 pg/mL (c), 250 pg/mL (d) and 1000 pg/mL (e) are shown.

Conclusion

- 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, SCIEX 7500 system enables pharmaceutical researchers maximum flexibility to explore lower dosage, higher efficacy compounds and improve the efficiency of bioanalysis

References

1. Achieve next level sensitivity for the evolution of routine bioanalysis. SCIEX technical note, RUO-MKT-02-14859-A

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices