Minimal-prep microsampling assay for empagliflozin quantitation using LC-MS/MS

Lakshmanan Deenadayalan[1], Sashank Pillai[1], Eshani Galermo[2], and Rahul Baghla[2]

[1]SCIEX, India and [2]SCIEX, USA

This technical note demonstrates a sensitive method for the quantitation of empagliflozin in human blood using the SCIEX 7500+ system. A lower limit of quantitation (LLOQ) of 1 ng/mL (0.12 pg on column) was achieved with a 10µL dry blood spot (DBS) from an LC-MRM analysis of 6-minute runtime (Figure 1).

Empagliflozin is a C-glycosyl compound used to manage type 2 diabetes mellitus.[1] Its therapeutic effect is mediated through selective inhibition of the sodium–glucose co-transporter 2 (SGLT2) in the proximal renal tubules, thereby reducing renal glucose reabsorption and promoting glucosuria.[2] In addition to improving glycemic control, empagliflozin has been associated with reductions in body weight and blood pressure. Ongoing research is evaluating its potential therapeutic applications in other conditions, including pediatric cardiomyopathy.[3]

To support such investigations, highly sensitive bioanalytical methods requiring minimal sample volume are essential for quantifying empagliflozin at low concentrations and for adequately characterizing its pharmacokinetic profile, thereby ensuring a robust assessment of safety and efficacy.

Key benefits of microsampling assays for empagliflozin quantitation using the SCIEX 7500+ system

- **Quantitation of empagliflozin:** Achieve 1 ng/mL LLOQ for the quantitation of empagliflozin in human blood with an on-column amount of 0.12 pg.
- **Enable true microsampling efficiency:** Perform sensitive quantitation with only 10 µL DBS using a clean, minimal extraction workflow.
- **Robust analytical performance:** Achieve accurate quantitative performance with %CV <8 at all concentration levels across a linear dynamic range (LDR) of 4 orders of magnitude.
- **Streamlined data management:** Simplify data acquisition and processing using SCIEX OS software, a 21 CFR Part 11-compliant platform.

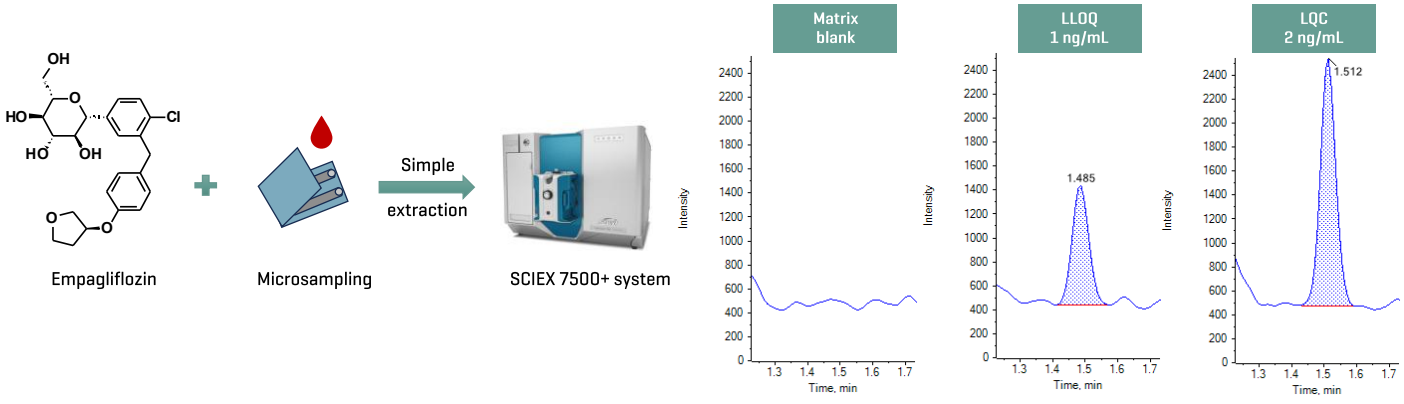

Figure 1. Simple microsampling workflow for quantitation of empagliflozin in human blood using the SCIEX 7500+ system. Representative extracted ion chromatograms (XICs) for empagliflozin in extracted human blood at matrix blank, 1 ng/mL (LLOQ), and 2 ng/mL (low quality control, LQC) are displayed. No interference was observed in the matrix blank at the retention time of empagliflozin.

Introduction

Empagliflozin received FDA approval in June 2023 for improving glycemic control in pediatric patients aged ⩾10 years, based on evidence from Phase 3 clinical trials[4]. The approved oral tablet dosages are 10 mg and 25 mg, administered without regard to meals. Pharmacokinetic assessments in this population demonstrated a mean C_{max} of approximately 148.8003 ng/mL and a T_{max} ranging from 0.7 to 2.0 hours.[5] Ongoing investigations aim to evaluate the use of empagliflozin for the treatment of cardiomyopathy in children aged 6–18 years.[6]

However, routine therapeutic drug monitoring in this context presents logistical challenges, as current assays require rapid plasma isolation following blood collection, immediate freezing, and shipment on dry ice to specialized or research laboratories. These requirements may limit feasibility for both clinicians and patients. Adoption of DBS-based assays could offer a substantially simplified alternative, as DBS samples are minimally invasive, easier to collect (including potential self-collection), and more stable for storage and transport.[3]

Empagliflozin analysis in DBS with minimal volume requires a sensitive method with minimal sample preparation to quantify at low concentrations and assess its efficacy and safety in humans.

Methods

Standard preparation: Empagliflozin was procured from Medchem Express, and 1 mg of stock was accurately weighed and dissolved in methanol to obtain a 1 mg/mL concentration. The spiking solution was prepared using 75:25 (v/v) water: acetonitrile as the diluent.

Sample preparation: Empagliflozin was spiked into human blood at concentrations from 1 to 10,000 ng/mL. 10 µL of blood was spotted onto the Capitainer® B10 sampling cards and allowed to dry for 2 min before extraction. The DBS was transferred to a vial to which 200 µL of acetonitrile was added and vortexed for 5 min. The sample was centrifuged at 1,204 rcf for 5 min. 100 µL of supernatant was taken and diluted with 300 µL of water, vortexed, and transferred to an autosampler vial for analysis.

Chromatography: Analytical separation was performed on the ExionLC system (SCIEX) using a Phenomenex Kinetex C18 column (50 x 2.1 mm, 2.6µm) at a flow rate of 0.35 mL/min. Mobile A was 0.2% formic acid in water, and mobile phase B was 0.2% formic acid in acetonitrile. The column temperature was set to 40°C and the autosampler temperature to 15°C. The gradient condition was summarized in Table 1. A 10 µL sample was used for the LC-MS/MS analysis.

Table 1. LC gradient for empagliflozin analysis.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	75	25
3.5	10	90
4.5	10	90
4.6	75	25
6.0	75	25

Mass spectrometry: MS data were acquired using the SCIEX 7500+ system. The optimized source and gas parameters used for the analysis are listed in Table 2, and the MRM parameters are included in Table 3.

Table 2. Source and gas parameters.

Parameter	Value
Polarity	Positive
Ionization mode	ESI
Ion source gas 1	70 psi
Ion source gas 2	70 psi
Curtain gas	40 psi
Source temperature	400°C
Spray voltage	2300 V
CAD gas	5

Table 3. MRM parameters used for quantitation on SCIEX 7500+ system.

ID	Precursor ion (m/z)	Fragment ion (m/z)	CE (V)	CXP (V)	QoD (V)
Empa-01*	451.16	355.01	15	12	0
Empa-02	451.16	71.01	45	12	0

Note: *Transition used for quantitation.

Data processing: Data collection and analysis were performed using SCIEX OS software, version 4.0. Peaks were integrated

using the MQ4 algorithm, and a weighting of $1/x^2$ was used for empagliflozin quantitation.

Quantitative performance on the SCIEX 7500+ system

A triplicate injection was performed across concentrations ranging from 1 ng/mL to 10,000 ng/mL (Figure 1). An LDR of 4 orders of magnitude was achieved. A weighting factor of $1/x^2$ was used, with a coefficient of determination (r^2) >0.995, indicating excellent linearity across a wide calibration range (Figure 2).

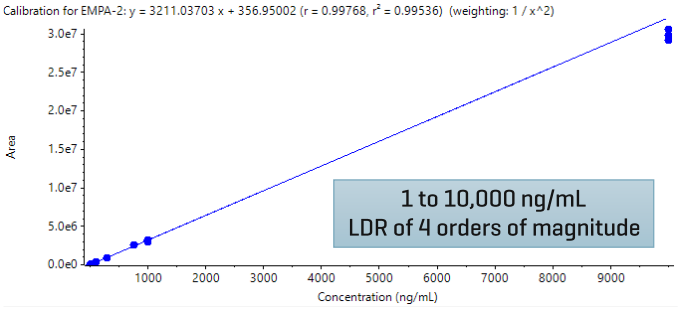

Figure 2. Calibration curve for quantitation of empagliflozin (451.1→355.1). The calibration curve was generated using a weighing factor of $1/x^2$.

Analytical performance was evaluated based on the requirement that the accuracy of the calculated mean should be between 80% and 120% at the LLOQ and between 85% and 115% at higher concentrations.

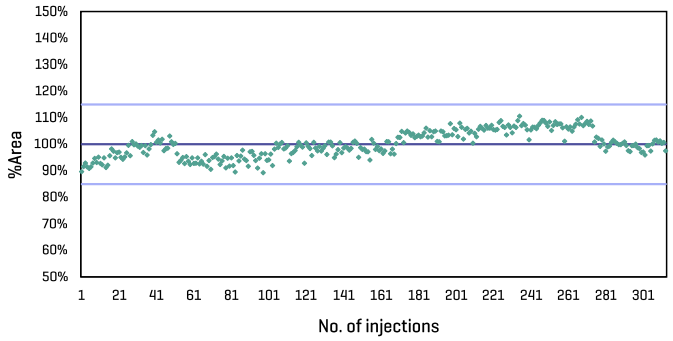

Figure 4. Stability over an extended sample run. Over 300 injections of 1000 ng/mL were performed, indicating a stable response throughout, with a mean response within ±15%.

The %CV of the calculated mean should be below 20% at the LLOQ and below 15% at all higher concentrations.[7]

The accuracy was within ±7% of the nominal concentration, and the %CV was <8 for the quantitation of empagliflozin in human blood (Figure 3). Calculated %accuracy and %CV values were within the acceptance criteria at each concentration level.

Recovery was evaluated for empagliflozin at 2 different concentrations (1 and 300 ng/mL). A triplicate injection of the blood-extracted sample was compared against the neat standard samples. An average recovery of 74.4% was achieved with average %CV <5 (Table 4).

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	EMPA-2	1.000	3 of 3	0.968	0.071	7.28	96.8
2	EMPA-2	2.000	3 of 3	2.110	0.123	5.81	105.
3	EMPA-2	5.000	3 of 3	5.096	0.346	6.79	102.
4	EMPA-2	25.000	3 of 3	25.664	1.103	4.30	103.
5	EMPA-2	50.000	3 of 3	46.850	0.266	0.568	93.7
6	EMPA-2	100.000	3 of 3	106.597	2.497	2.34	107.
7	EMPA-2	300.000	3 of 3	292.805	12.407	4.24	97.6
8	EMPA-2	750.000	3 of 3	790.195	1.782	0.225	105.
9	EMPA-2	1000.000	3 of 3	967.281	23.032	2.38	96.7
10	EMPA-2	10000.000	3 of 3	9311.782	212.297	2.28	93.1

Figure 3. Quantitative performance for empagliflozin (455.1→355.1) analysis. Reproducibility and accuracy results were determined from the calibration curve standards across 3 replicates at each concentration. Statistical results were summarized using the Analytics module in SCIEX OS software.

Table 4. Recovery was evaluated using 1 ng/mL and 300 ng/mL of empagliflozin. Each concentration was evaluated in triplicate injections.

		Recovery		
Level (ng/mL)	Avg. area neat samples (n=3)	Avg. area in post-spiked samples (n=3)	%CV	%Recovery
1	5228±408.3	3466±184.8	5.33	68.9
300	1103821±22021.9	940565±32527.9	3.46	79.9
		Average	4.39	74.4

Column and method stability were evaluated by performing over 300 injections at 1000 ng/mL (MQC level), with the mean peak area within ±15%, as shown in Figure 4.

The data demonstrates consistent stability of the Phenomenex Kinetex C18 column and method over long batches, validating a fast, minimal-prep microsampling strategy for empagliflozin analysis.

Compliance-ready SCIEX OS software

Equivalent SCIEX OS software capabilities for regulated bioanalysis can be executed on the SCIEX 7500+ system, ensuring high fidelity when performing method transfers while retaining critical compliance features.

SCIEX OS software is a closed system and requires records and signatures to be stored electronically, in compliance with 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing workstations.

Figure 5 illustrates the features of SCIEX OS software used to monitor the audit trail, acquire and process data, and configure user access. The audit trail feature enables users to audit critical user actions and locks in data integrity.

The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards. The configuration module allows users to assign roles and access as the administrator, method developer, analyst, and reviewer.

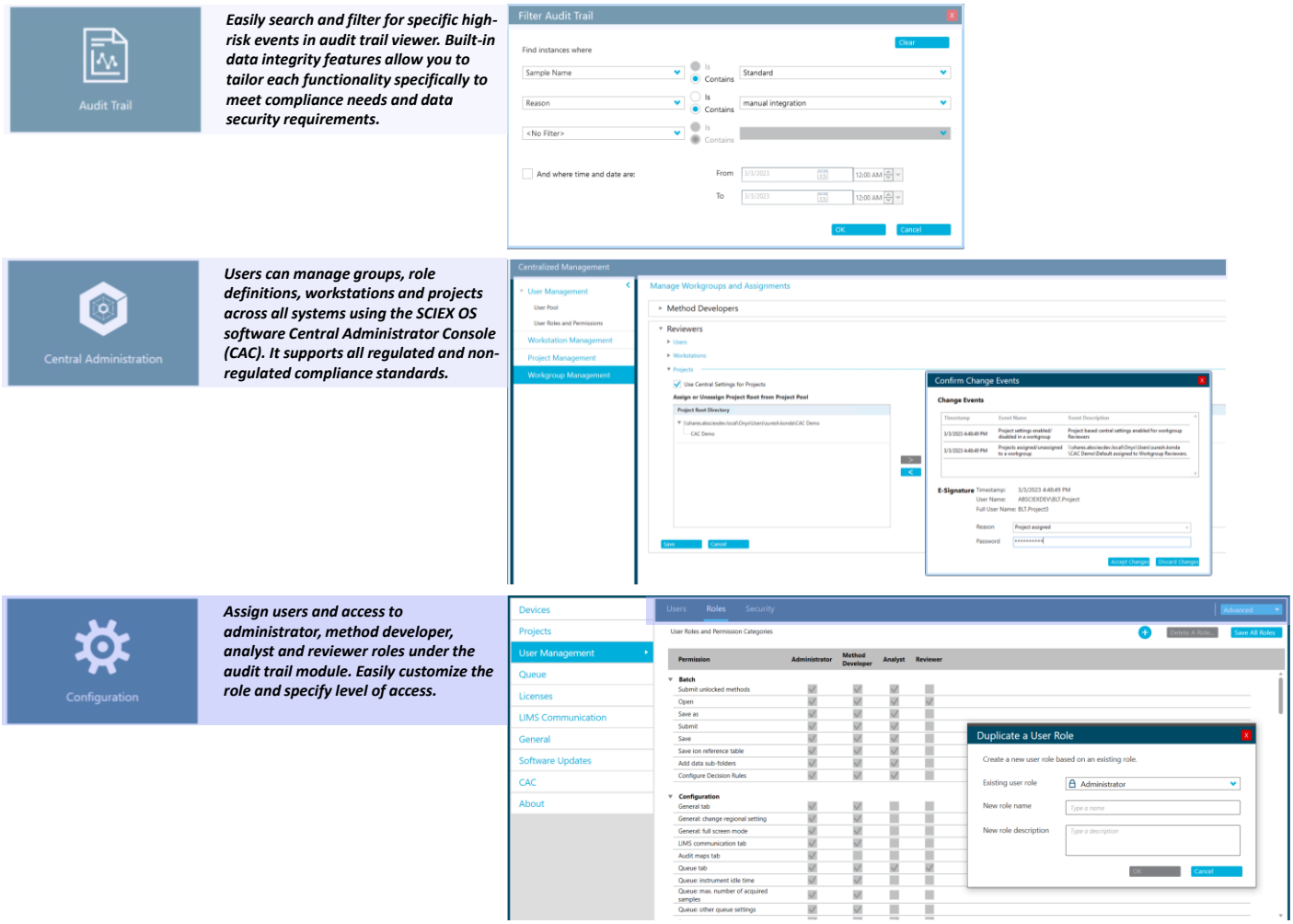

Figure 5. Features of SCIEX OS software for monitoring user access and evaluating the audit trail. The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) to manage users and groups, role definitions, workstations, and projects across all systems. The CAC feature supports both regulated and non-regulated compliance standards. The configuration module enables users to quickly set up roles and access levels for the administrator, method developer, analyst, and reviewer.

Conclusions

- **Quantitation of empagliflozin**
  - A LOQ of 1 ng/mL (on column concentration of 0.12 pg/mL) was achieved for the quantitation of empagliflozin.
  - Good quantitative performance was demonstrated, with accurate and highly reproducible results (%CV <8%) on the SCIEX 7500+ system.
  - Linearity was achieved at concentrations ranging from 1 ng/mL to 10,000 ng/mL with an r^2 >0.995 for empagliflozin with an LDR of 4 orders of magnitude.
- **Minimal-prep microsampling strategy**
  - A simple sample extraction procedure was employed with a minimal sample volume of 10 µL.
  - The method and analytical column were stable with over 300 injections of the extended batch in a complex matrix.
  - An average recovery of 74.4% was achieved with an average %CV <5 for the blood-extracted samples compared against the neat samples.
- **Software compliance**
  - Retain data management and compliance-readiness (21 CFR Part 11) features using SCIEX OS software to support bioanalysis on the SCIEX 7500+ system.

References

1. CenJun Li, Chao fan, Chunchao Wang, Jianchao Chen Chengjun Wu, TieMin Sun. Synthesis, crystal structure, DFT calculation, and confirmation of absolute configuration of empagliflozin. Journal of molecular structure .2024, Apr. doi.org/10.1016/j.molstruc.2023.137331
2. Annemarie B. van der Aart-van der Beek, A. Mireille A. Wessels, Hiddo J.L. Heerspink , Daan J. Touw. Simple, fast and robust LC-MS/MS method for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin in human plasma and urine. Journal of Chromatography B. 2020, Jun. doi.org/10.1016/j.jchromb.2020.122257
3. Joseph P. Dewulf, Nathalie Chevalier, Sandrine Marie, Maria Veiga-da-Cunha. DBS are suitable for 1,5-anhydroglucitol monitoring in GSD1b and G6PC3-deficient patients taking SGLT2 inhibitors to treat neutropenia. Molecular Genetics and Metabolism. 2023. doi.org/10.1016/j.ymgme.2023.107712
4. FDA Approves New Class of Medicines to Treat Pediatric Type 2 Diabetes. U.S. Food and Drug,2023 Jun
5. Sreeraj Macha, Tobias Brand, Thomas Meinicke, Jasmin Link, Uli C. Broedl,. Pharmacokinetics and Pharmacodynamics of Twice Daily and Once Daily Regimens of Empagliflozin in Healthy Subjects. Clinical Therapeutics. 2015, Aug. 10.1016/j.clinthera.2015.06.003
6. Repurposing Empagliflozin for DMD-associated Cardiomyopathy in Children 6-18 Years of Age (REDMeD) Sebastiano Lava, Centre Hospitalier Universitaire Vaudois, 2025.
7. Bioanalytical Method Validation, May 2018

Headquarters
250 Forest Street | Marlborough,
MA 01752 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