International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 5 Sept - Oct 2025, pp: 303-311 www.ijprajournal.com ISSN: 2456-4494

Bioanalytical Approaches for the Quantitative Determination of SGLT2 Inhibitors in Biological Matrices: A Comprehensive Review

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Date of Submission: 05-09-2025 Date of Acceptance: 15-09-2025

ABSTRACT
Sodium-glucose cotransporter-2 (SGLT2) inhibitors are a significant class of antidiabetic medications that also provide documented cardiovascular and kidney protection. It is essential to quantify these drugs precisely in biological matrices such as plasma, serum, and tissues. The quantification aids pharmacokinetic studies, assists bioequivalence establishment, and facilitates therapeutic monitoring. This review outlines the key bioanalytical methods established for their quantitation. These techniques include liquid chromatography-tandem mass spectrometry (LC-MS/MS), which is the most suitable because of its high selectivity, sensitivity, and capability to detect the substance at very low concentrations. High-performance liquid chromatography (HPLC), often coupled with ultraviolet detection, is also a common and inexpensive option for regular analysis. Ultra-performance liquid chromatography (UPLC) has become popular due to its rapid run times and enhanced separation efficiency, thus being a suitable option for high-throughput analysis. The review also considers usual sample preparation techniques, including protein precipitation, liquid-liquid extraction, and solid-phase extraction, and how they impact recovery, selectivity, and matrix effects. It addresses validation criteria such as accuracy, precision, linearity, detection limits, quantitation limits, and analyte stability, according to international guidelines. Although LC-MS/MS represents the norm in practice today, UPLC methods are gaining increasing recognition for their speed and analytical potential.

KEYWORDS: SGLT2 inhibitors; bioanalytical methods; LC–MS/MS; UPLC; capillary electrophoresis; sample preparation; method validation; pharmacokinetics; biological matrices.

I. INTRODUCTION

Sodium-glucose cotransporter 2 (SGLT2) inhibitors have become an important tool in managing type 2 diabetes mellitus (T2DM). These medications work by blocking the SGLT2 protein in the kidneys' proximal tubules, which helps the body remove excess glucose through urine. This not only lowers blood sugar levels but can also lead to modest weight loss and a reduction in blood pressure, all without a high risk of hypoglycemia. Beyond these effects, clinical studies have shown that SGLT2 inhibitors can provide additional protection for the heart and kidneys, making them particularly beneficial for patients with heart failure or chronic kidney disease. As more patients use SGLT2 inhibitors, it becomes increasingly important to measure these drugs accurately in biological samples such as plasma, serum, urine, and tissues. Reliable measurements are essential for understanding how the drugs behave in the body, for bioequivalence studies, and for evaluating potential drug interactions.[1] Guidelines like ICH M10 help ensure that bioanalytical methods are properly validated, so that both preclinical and clinical studies produce dependable results.[2] Several analytical techniques are used to detect and quantify SGLT2 inhibitors. High-performance liquid chromatography (HPLC) with UV detection remains a reliable choice for routine testing, while liquid chromatography-tandem mass spectrometry (LC-MS/MS) is preferred for pharmacokinetic studies because it can detect very low concentrations from small sample volumes. Ultra-performance liquid chromatography (UPLC) offers faster analysis and improved resolution, further enhancing efficiency. Sample preparation methods including protein precipitation, liquid-liquid extraction, and solid-phase extraction are critical for minimizing interference and ensuring accurate results. Despite these advances, challenges persist. Ion suppression can affect mass spectrometry

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 5 Sept - Oct 2025, pp: 303-311 www.ijprajournal.com ISSN: 2456-4494

readings, analytes may degrade over time, and other drugs or naturally occurring compounds can interfere with measurements. Researchers are also exploring greener, faster methods that use less solvent and reduce analysis time, without sacrificing accuracy or reliability. This review aims to provide a clear overview of the strategies used to analyze SGLT2 inhibitors in biological samples. It covers traditional and modern techniques such as fluorescence spectroscopy, HPLC, UPLC, and LC-MS/MS along with sample preparation approaches and validation requirements. By highlighting the strengths and limitations of different methods, this article serves as a practical guide for researchers, laboratory analysts, and regulatory professionals working in this area.

II. ANALYTICAL TECHNIQUES

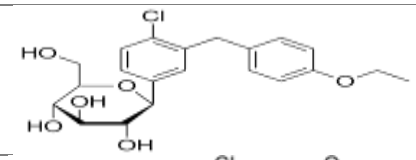
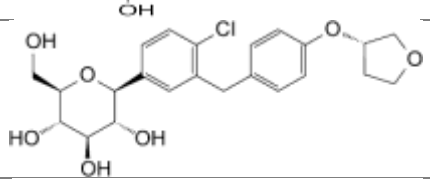
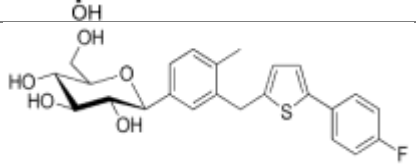
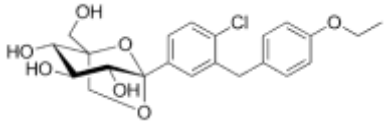
2.1 Spectrophotometric methods

2.11 fluorescence spectroscopy

Fluorescence spectroscopy is an analytical approach known for its high sensitivity, selectivity, and cost-effectiveness. It works by detecting the light emitted by molecules once they are excited at a particular wavelength. Because it can achieve detection limits down to the nanogram-per-milliliter range, this technique is especially useful for quantifying drugs present in very low concentrations within biological samples.

Several spectrofluorimetric methods have been reported for SGLT2 inhibitors in biological matrices. Omar et al. used NBD-Cl derivatization for dapagliflozin in plasma, improving sensitivity over native fluorescence but with a higher LOD and longer analysis time.[3] Rushdy et al. enhanced fluorescence of dapagliflozin and empagliflozin through β-cyclodextrin inclusion, achieving very low LODs of 1.38 ng/mL for dapagliflozin and 1.05 ng/mL for empagliflozin without derivatization.[4] For empagliflozin, Omar et al. developed a benzofurazan-based method in plasma with excitation at 455 nm and emission at 521 nm, while Ayoub et al. relied on native fluorescence in rat plasma with excitation at 226.5 nm and emission at 299.4 nm to provide a simple, low-cost assay.[5],[6] Ahmed et al. reported a green, native-fluorescence method for ertugliflozin in urine, achieving an LOD of 17.27 ng/mL and accuracy between 99.19 and 101.90 percent.[7] Elmasry et al. devised a dual assay for empagliflozin and linagliptin in plasma, combining native fluorescence for empagliflozin with NBD-Cl derivatization for linagliptin, achieving highselectivity and precision.[8]

Table 1: Marketed SGLT2 inhibitors: Chemical structure, key physicochemical properties, and dates of first approval

Drug	Chemical structure	Chemical Formula	Molecular Weight (g/mol)	LogP	Date of First Approval
Dapagliflozin		$C_{21}H_{25}ClO_6$	408.87	2.11	2014
Empagliflozin		$C_{23}H_{27}ClO_7$	450.91	1.7	2014
Canagliflozin		$C_{24}H_{25}FO_5S$	444.52	3.6	2013
Ertugliflozin		$C_{22}H_{25}ClO_7$	436.93	2.2	2017

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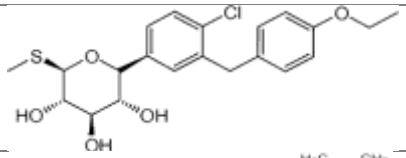
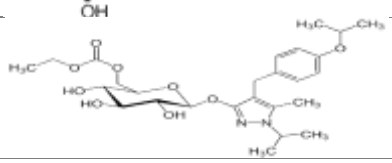
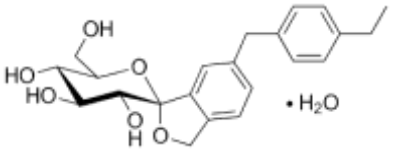
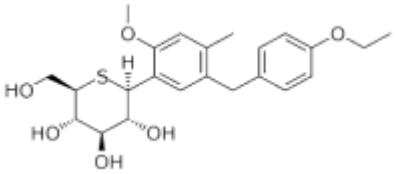
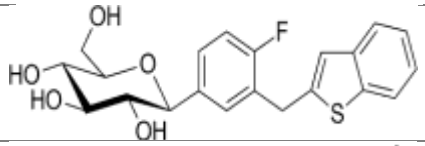
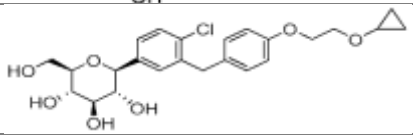
Sotagliflozin		$C_{21}H_{25}ClO_5S$	471.52	3.7	2023
Remogliflozin etabonate		$C_{26}H_{38}N_2O_9$	522.6	3.5	2019
Tofogliflozin		$C_{22}H_{26}O_6$	386.4	1.5	2014
Luseogliflozin		$C_{23}H_{30}O_6S$	434.5	2.9	2014
Ipragliflozin		$C_{21}H_{21}FO_5S$	404.5	2.5	2014
Bexagliflozin		$C_{24}H_{29}ClO_7$	464.9	2.4	2023

Table 2: Summary of spectrofluorimetric methods used for the determination of SGLT2 inhibitors and itscombinations

SGLT2 Inhibitor	Combination (if any)	Biological Matrix	λ_{ex} / λ_{em} (nm)	Linear Range (ng/mL)	LOD (ng/mL)	Reference
Dapagliflozin	None	Human plasma	453 / 522	50–1000	14.24	3
Dapagliflozin	Empagliflozin	Biological fluids	230 / 301	5.0–250.0, 10.0–300.0	1.38, 1.05	4
Empagliflozin	None	Human plasma	455 / 521	50-1000	15.55	5
Empagliflozin	None	Rat plasma	226.5 / 299.4	500–5000	Not specified	6
Ertugliflozin	None	Human urine	270 / 334	50–1000	17.27	7
Empagliflozin	Linagliptin	Human plasma	234 / 305, 469 / 538	40–1200, 3-700	12.06, 0.884	8

2.2 Chromatographic methods

Chromatographic methods play a key role in the bioanalysis of SGLT2 inhibitors. They offer the precision and sensitivity needed to measure these compounds in various biological samples. The most commonly used techniques are high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 5 Sept - Oct 2025, pp: 303-311 www.ijprajournal.com ISSN: 2456-4494

liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.2.1 High Performance Liquid Chromatography

A range of non-MS chromatographic methods have been explored for SGLT2 inhibitors in biological samples. For dapagliflozin, Dighe and Kumbhare and later Abhay et al. developed HPLC–UV assays that were reliable but lacked the sensitivity needed for very low plasma levels.[9],[10] Gopal et al. expanded this to a dapagliflozin–saxagliptin combination with consistent results, though still less sensitive than LC–MS/MS.[11] To improve speed and detection, Mabrouk and colleagues introduced a rapid UPLC–DAD method for empagliflozin and later combined it with DLLME to achieve trace-level detection of multiple gliflozins.[12],[13] More recently, Han et al. demonstrated a practical HPLC–fluorescence method for ertugliflozin in rat plasma applied to drug–drug interaction studies, while Waditake et al. validated an RP-HPLC–UV assay for remogliflozin etabonate that met ICH standards for stability and pharmacokinetic use.[14],[15] Together, these examples show that HPLC and UPLC methods with UV, DAD, or fluorescence detection can serve as accessible and dependable alternatives where mass spectrometry is not available, even if they remain less sensitive for trace concentration.

Table 3: Summary of different conditions for HPLC method opted to analyze SGLT2 inhibitors from various biological fluids

SGLT2 Inhibitor	Biological Matrix	Column	Mobile Phase	Linear Range	IS	Sample Prep	RT	Reference
Dapagliflozin	Human plasma	Kromasil C18 (250 × 4.6 mm)	ACN : 0.1% OPA (50:50 v/v), 1.0 mL/min	1.5–60 µg/mL	Azilsartan medoxomil	Protein precipitation	4.6 min	9
Dapagliflozin	Human plasma	Hemsil C18	Methanol : Water (80:20), 1.0 mL/min	10–50 µg/mL	Ornidazole	Liquid–liquid extraction	5.2 min	10
Dapagliflozin+ saxagliptin	Human plasma	Eclipse XDB C18 column	Water: ACN(50:50 v/v), 1.0 mL/min	0.01 to 0.50 µg/mL and 0.05 to 2.00µg/mL	linagliptin	Protein precipitation	0.49,1.07 min	11
Empagliflozin	Human plasma	BEH C18	TFA: ACN(60:40) 0.5ml/min	50-700ng	Dapagliflozin	Protein precipitation	0.511min	12
Empagliflozin+ Dapagliflozin+ Caagliflozin	Human plasma	C18 column	ACN: 0.1%TFA (40:60) 1ml/in	2-2500 ng/mL, 3.5–2500 ng/mL 1.1–2500 ng/mL	Azilsartan	Dispersive LLE	-	13
Ertugliflozin	Rat plasma	Kinetex C18 column	ACN: Phosphate buffer	4-2000ng	-	Protein precipitation	11.2min	14
Remogliflozin etabonate	Human plasma	THERMO C18	Methanol-0.1% acetic acid(80:20)	5-13 µg	-	Protein precipitation	4.46min	15

2.2.2 Hyphenated techniques

Linking HPLC with single or tandem mass spectrometry greatly enhances its sensitivity and reliability, allowing accurate measurement of active ingredients in biological samples, even when multiple components are present.[16]

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 5 Sept - Oct 2025, pp: 303-311 www.ijprajournal.com ISSN: 2456-4494

Table 4: Summary of hyphenated methods used for the determination of SGLT2 inhibitors and itscombinations

SGLT2 Inhibitor	Combination	Biological Matrix	Column	Mobile Phase	Flow Rate	Reference
Dapagliflozin	-	Rat plasma (normal & ZDF)	C18	ACN–water + 0.1% FA	0.5 mL/min	17
Dapagliflozin	D3OG (metabolite)	Human plasma	C18	ACN–water + 0.1% FA	0.4 mL/min	18
Dapagliflozin	Saxagliptin + Metformin	Human plasma	C18	ACN–buffer	1.0 mL/min	19
Canagliflozin, Dapagliflozin, Empagliflozin	-	Human plasma & urine	UPLC HSS T3	MeOH–water + 0.1% FA	0.5 mL/min	20
Empagliflozin	-	Human plasma	Synergi C18	ACN–5mM ammonium acetate + FA	0.3 mL/min	21
Empagliflozin	Metformin	Human plasma	BEH C18	ACN–0.1% FA	0.3 mL/min	22
Empagliflozin	Linagliptin	Human plasma	C18	ACN–buffer	0.4 mL/min	23
Canagliflozin	-	Rabbit plasma	C18	ACN–0.1% FA	0.8 mL/min	24
Canagliflozin	-	Human plasma	C18	ACN–buffer	0.5 mL/min	25
Canagliflozin	Metformin	Human plasma	C18	ACN–0.1% FA	0.6 mL/min	26
Ertugliflozin	Sitagliptin	Rat plasma	C18	ACN–10mM ammonium acetate	0.3 mL/min	27
Ertugliflozin	Metformin	Rat plasma	C18	ACN–0.1% FA	0.4 mL/min	28
Remogliflozin	-	Rat plasma	UHPLC C18	ACN–0.1% FA	0.3 mL/min	29
Luseogliflozin	-	Rat plasma	C18	ACN–water + 0.1% FA	0.4 mL/min	31
Ipragliflozin	-	Rat plasma	C18	ACN–0.1% FA	0.4 mL/min	32

Several LC–MS/MS methods have been established for dapagliflozin, covering both preclinical and clinical use. Aubry et al. were among the first to describe an assay in rat plasma, using solid-phase extraction with negative-mode electrospray ionization. Their method was linear from 5 to 2000 ng/mL and showed good precision and stability, making it reliable for pharmacokinetic studies in animals.[17] In human plasma, Gu et al. developed a highly sensitive and fully validated LC–MS/MS method that could measure dapagliflozin along with its major glucuronide metabolite, providing valuable support for metabolic and PK evaluation.[18] Work has also focused on combination therapies: El-Zaher et al. designed a plasma assay that quantified dapagliflozin or saxagliptin in the presence of metformin, while van der Aart-van Lennep et al. created a fast, selective method for simultaneously analyzing dapagliflozin, empagliflozin, and canagliflozin in plasma and urine. Their protocol offered short run times and high reproducibility,

International Journal of Pharmaceutical Research and Applications
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highlighting the practicality of multi-analyte monitoring in clinical settings.[19],[20]Jagadeesh and Kumar reported a simple plasma assay on a Synergi C18 column that delivered results in just three minutes, with excellent recovery and picogram-level sensitivity.[21] For combination therapies, Abou-Omar et al. developed a sensitive UPLC–MS/MS method for empagliflozin with metformin, while Shah et al. designed a reliable plasma method for empagliflozin with linagliptin using mixed-mode solid-phase extraction.[22],[23] Collectively, these studies show how LC–MS/MS offers unmatched speed, sensitivity, and flexibility for both single-drug and multi-drug analysis of empagliflozin. For canagliflozin,Bhatt and Rajkamal developed a validated method in rabbit plasma that worked well for pharmacokinetic studies, delivering consistent accuracy, precision, and sensitivity.[24] Extending to human applications, Deepan et al. designed and validated an assay in plasma that provided excellent linearity, reproducibility, and low detection limits, making it well suited for clinical pharmacokinetic and bioanalytical studies.[25]Mohammed also reported a method for simultaneous estimation of canagliflozin and metformin using propranolol and tadalafil as IS.[26]Qiu et al. developed a UPLC–MS/MS method for ertugliflozin with sitagliptin in rat plasma, validated over 1–1000 ng/mL and applied to PK studies.[27] Rao et al. reported an LC–MS/MS assay for ertugliflozin with metformin in rat plasma, achieving a 0.1 ng/mL LLOQ and supporting pharmacokinetic analysis. Both highlight LC–MS/MS as a sensitive, reliable tool for ertugliflozin bioanalysis in preclinical models.[28]Patel reported an UHPLC-MS/MS for the determination of remogliflozin in rat plasma.[29]Kobuchi and his coworkers reported LC-MS/MS method for quantification of tofogliflozin in rat plasma using empagliflozin as IS.[30]later, they also developed separate method for quantification of luseogliflozin and ipragliflozin in rat plasma.[31],[32]

III. CONCLUSION

Over the years, several bioanalytical methods have been developed to measure SGLT2 inhibitors in biological samples. Among them, HPLC and UPLC continue to be widely used because they are reliable, straightforward, and well-suited for routine laboratory analysis. Spectrofluorimetric methods provide a simple and cost-effective alternative, though their lower sensitivity limits their role in detailed pharmacokinetic studies. In contrast, LC–MS/MS stands out as the gold standard, offering unmatched sensitivity, accuracy, and the ability to quantify multiple drugs at once, making it especially valuable for pharmacokinetic profiling, bioequivalence studies, and drug–drug interaction assessments. Even with this progress, challenges such as matrix interferences, analyte instability, and the need for affordable high-sensitivity methods remain. Future work is likely to focus on greener techniques, faster workflows, and microsampling approaches to reduce both analysis time and sample requirements.

In conclusion, while HPLC, UPLC, and spectrofluorimetry have their place in routine analysis, LC–MS/MS remains the benchmark for comprehensive bioanalysis of SGLT2 inhibitors. Continued innovation in these methods will play a key role in advancing pharmacokinetic research and improving therapeutic monitoring in clinical practice.

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