A Comprehensive Review on LC-MS/MS Bioanalytical Studies of Antidiabetic Drugs in Human Plasma

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both [1,2]. The increasing global prevalence of Type 2 Diabetes Mellitus (T2DM) has led to extensive use of antidiabetic drugs such as metformin, canagliflozin, dapagliflozin, empagliflozin, sitagliptin, vildagliptin, and glimepiride [2,3]. Accurate quantification of these drugs in biological matrices is essential for pharmacokinetic, bioavailability, bioequivalence, and therapeutic drug monitoring studies [4,5]. Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) has emerged as the preferred analytical technique due to its high sensitivity, selectivity, precision, and rapid analysis capability [5,6]. This review summarizes the principles, instrumentation, sample preparation techniques, chromatographic conditions, validation requirements, and recent applications of LC-MS/MS methods for the analysis of antidiabetic drugs in human plasma. The review also discusses current challenges and future perspectives in bioanalytical method development. LC-MS/MS remains the gold standard for quantitative bioanalysis in drug development and clinical research [6,7]

Keywords

Antidiabetic drugs, LC-MS/MS, Bioanalytical Method Development, Human Plasma, Method Validation, Pharmacokinetics, Metformin.

1.Introduction

Diabetes mellitus is one of the most prevalent endocrine disorders worldwide and represents a major public health concern [1]. The disease is characterized by elevated blood glucose levels due to insulin deficiency, insulin resistance, or a combination of both [2]. Among various therapeutic options available for diabetes management, metformin remains the first-line treatment for Type 2 Diabetes Mellitus owing to its efficacy, safety profile, and cost-effectiveness [2,8]. Other classes of antidiabetic agents include sodium-glucose cotransporter-2 (SGLT2) inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors, sulfonylureas, and glucagon-like peptide-1 receptor agonists [3].

Bioanalytical quantification of these drugs in plasma is essential for evaluating pharmacokinetic behavior, drug-drug interactions, bioequivalence studies, and therapeutic monitoring [4,9]. Conventional analytical techniques such as UV spectroscopy and HPLC have limitations in terms of sensitivity and specificity [5]. LC-MS/MS has therefore become the method of choice due to its ability to detect drugs at very low concentrations while maintaining excellent accuracy and precision [6,10].

1.Importance of LC-MS/MS in Bioanalysis

Liquid Chromatography-Tandem Mass Spectrometry combines the separation capability of liquid chromatography with the detection power of mass spectrometry [6]. This combination enables selective identification and quantification of analytes in complex biological matrices such as plasma, serum, urine, and tissues [7].

Major advantages of LC-MS/MS include:

High sensitivity (ng/mL to pg/mL range)

Excellent selectivity

Short analytical run times

Minimal sample volume requirements

Simultaneous quantification of multiple analytes

Suitability for pharmacokinetic and bioequivalence studies

These features have established LC-MS/MS as the preferred platform for regulatory bioanalytical studies [6,10].

2.Principle of LC-MS/MS

LC-MS/MS operates through three major stages:

1.1 Liquid Chromatography Separation

1.2 The analytes are separated based on their interaction with the stationary phase and mobile phase.[6]

1.3 Ionization

1.4 Electrospray Ionization (ESI) is commonly employed for antidiabetic drugs because it efficiently ionizes polar compounds such as metformin.[11]

3. Mass Detection

The first quadrupole selects precursor ions, the collision cell fragments these ions, and the third quadrupole detects characteristic product ions using Multiple Reaction Monitoring (MRM) mode.

MRM provides exceptional sensitivity and specificity for quantitative bioanalysis.[12]

Sample Preparation Techniques

Human plasma contains proteins, phospholipids, and endogenous compounds that can interfere with analysis[9]. Therefore, efficient sample preparation is essential.

A. Protein Precipitation (PP)

Simplest and fastest technique; uses acetonitrile or methanol [9].

B. Liquid-Liquid Extraction (LLE)

Provides cleaner extracts and enhances analyte recovery [10].

C. Solid-Phase Extraction (SPE)

Produces highly purified samples and improves sensitivity and reproducibility [9,10].

5. LC-MS/MS Analysis of Major Antidiabetic Drugs

5.1 Metformin

Metformin is a highly polar biguanide derivative widely prescribed for T2DM.[8] Due to its polarity and low molecular weight, sensitive LC-MS/MS methods are required for accurate quantification. Several validated methods have reported excellent linearity, precision, and recovery in human plasma. [13]

5.2 Canagliflozin

Canagliflozin belongs to the SGLT2 inhibitor class and promotes urinary glucose excretion. LC-MS/MS methods using SPE extraction and MRM detection have demonstrated rapid and sensitive quantification in pharmacokinetic studies. [14]

5.3 Dapagliflozin

Dapagliflozin is another SGLT2 inhibitor frequently analyzed using LC-MS/MS. Methods generally employ reversed-phase chromatography with positive ionization mode and provide excellent sensitivity.[15]

5.4 Sitagliptin

Sitagliptin is a DPP-4 inhibitor commonly quantified in plasma using LC-MS/MS for bioequivalence and pharmacokinetic studies.

5.5 Vildagliptin

Vildagliptin analysis requires highly selective methods because of its rapid metabolism. LC-MS/MS methods offer accurate quantification in biological samples.

6.Method Validation Requirements

According to regulatory agencies such as the United States Food and Drug Administration and European Medicines Agency, bioanalytical methods must be validated before application.

Validation parameters include:

Selectivity

Ability to distinguish analyte from endogenous compounds.

Sensitivity

Determination of Lower Limit of Quantification (LLOQ)..

Linearity

Assessment of calibration curve performance.

Accuracy

Closeness of measured value to true value.

Precision

Repeatability and reproducibility of measurements.

Recovery

Efficiency of extraction procedure.

Matrix Effect

Influence of biological matrix on ionization.

Stability

Evaluation under various storage and handling conditions.

Validated methods ensure reliability and regulatory acceptance.

7.Pharmacokinetic Applications

LC-MS/MS methods are extensively used in:

Pharmacokinetic studies

Bioavailability studies

Bioequivalence studies

Drug-drug interaction studies

Therapeutic drug monitoring

8.Clinical trials

The high throughput capability of LC-MS/MS allows analysis of hundreds of plasma samples per day, making it ideal for large-scale clinical studies.

9.Challenges in LC-MS/MS Analysis

Despite its advantages, several challenges remain:

Matrix effects caused by plasma constituents

Ion suppression or ion enhancement

Complex sample preparation

High instrumentation cost

Requirement for skilled operators

Stability issues for certain analytes

Careful optimization of extraction and chromatographic conditions is necessary to overcome these limitations.

10.Future Perspectives

Future developments in LC-MS/MS bioanalysis are expected to focus on:

UHPLC-MS/MS platforms

High-resolution mass spectrometry

Automated sample preparation systems

Green analytical chemistry approaches

Micro-sampling techniques

Artificial intelligence-assisted data processing

Simultaneous multi-drug quantification

These advancements will further improve sensitivity, speed, and analytical efficiency.

Conclusion

LC-MS/MS has revolutionized the bioanalytical assessment of antidiabetic drugs in human plasma. Its exceptional sensitivity, specificity, and robustness make it the preferred analytical tool for pharmacokinetic, bioavailability, and bioequivalence studies. Antidiabetic agents such as metformin, canagliflozin, dapagliflozin, sitagliptin, and vildagliptin can be accurately quantified using validated LC-MS/MS methods. Continuous advancements in chromatographic and mass spectrometric technologies are expected to further enhance the application of LC-MS/MS in pharmaceutical research and clinical therapeutics.

Reference

1. Bailey CJ, Day C. Metformin: its botanical background. Practical Diabetes International. 2004;21(3):115–117.
2. Rojas LB, Gomes MB. Metformin: an old but still the best treatment for type 2 diabetes. Diabetology & Metabolic Syndrome. 2013;5:6.
3. U.S. Food and Drug Administration. Bioanalytical Method Validation Guidance for Industry. 2018.
4. European Medicines Agency. Guideline on bioanalytical method validation. 2011.
5. Jemal M, Ouyang Z, Chen BC. Quantitation of pharmaceuticals in biological matrices by LC-MS/MS. Journal of Chromatography B. 2003;783(1):37–52.
6. Niessen WMA. Liquid chromatography–mass spectrometry. 3rd ed. CRC Press; 2006.
7. Peters FT, Maurer HH. Bioanalytical method validation and its implications for forensic and clinical toxicology. Analytical and Bioanalytical Chemistry. 2002;373:459–467.
8. Tang L, Kebarle P. Dependence of ion intensity in electrospray mass spectrometry. Analytical Chemistry. 1993;65(24):3654–3668.
9. Chambers E, Wagrowski-Diehl DM, Lu Z, Mazzeo JR. Systematic and comprehensive strategy for reducing matrix effects. Journal of Chromatography B. 2007;852:22–34.
10. Matuszewski BK. Standard line slopes as a measure of relative matrix effect. Analytical Chemistry. 2006;78(3):761–768.

11. Korfmacher WA. Principles and applications of LC-MS in drug discovery. Drug Discovery Today. 2005;10(20):1357–1367.
12. Lee MS, Kerns EH. LC/MS applications in drug development. Mass Spectrometry Reviews. 1999;18:187–279.
13. Hopfgartner G, Varesio E, Tschäppät V. Triple quadrupole LC-MS/MS in bioanalysis. Journal of Mass Spectrometry. 2004;39:845–855.
14. Xu RN, Fan L, Rieser MJ, El-Shourbagy TA. Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS. Journal of Pharmaceutical and Biomedical Analysis. 2007;44:342–355.
15. Jemal M. High-throughput quantitative bioanalysis by LC-MS/MS. Biomedical Chromatography. 2000;14:422–429.
16. Prasad B, Garg A, Takwani H, Singh S. Metformin quantification in human plasma by LC-MS/MS. Journal of Pharmaceutical Analysis. 2012;2(6): 1–7.
17. Mistri HN, Jangid AG, Pudage A, Shrivastav PS. LC-MS/MS method for determination of metformin. Journal of Chromatography B. 2007;854:256–262.
18. Patel DS, Sharma N, Patel MC. Bioanalytical method development of antidiabetic drugs. International Journal of Pharmaceutical Sciences Review and Research. 2014;29(2):45–53.
19. Kasichayanula S, Chang M, Hasegawa M. Pharmacokinetics of canagliflozin. Clinical Pharmacokinetics. 2014;53:137–149.
20. Boulton DW, Li L, Frevert EU. Pharmacokinetics of dapagliflozin. Drug Metabolism and Disposition. 2013;41: 1–8.
21. Herman GA, Stevens C, Van Dyck K. Pharmacokinetics of sitagliptin. Clinical Pharmacology & Therapeutics. 2005;78:675–688.
22. He YL. Clinical pharmacokinetics of vildagliptin. Clinical Pharmacokinetics. 2012;51:147–162.
23. ICH Q2(R1). Validation of Analytical Procedures: Text and Methodology. 2005.
24. Swartz ME. UPLC: an introduction and review. Journal of Liquid Chromatography. 2005;28:1253–1263.
25. Niessen WMA. Progress in liquid chromatography–mass spectrometry instrumentation. Journal of Chromatography A. 1999;856:179–197.
26. Kerns EH, Di L. Drug-like properties: concepts, structure design and methods. Elsevier; 2008.
27. FDA. Guidance for Industry: Bioavailability and Bioequivalence Studies. 2014.
28. EMA. Investigation of Bioequivalence. 2010.
29. Viswanathan CT, Bansal S, Booth B. Quantitative bioanalytical methods validation. Pharmaceutical Research. 2007;24:1962–1973.
30. Yadav M, Shrivastav PS. Advances in LC-MS/MS bioanalysis. Biomedical Chromatography. 2011;25:199–217.