https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27 (September 2024); 644-656

Research Article

"Emerging Trends and Challenges In Bio-Analytical Method Development For Anti-Diabetic Drugs"

Amrit Kumar Rath[1,2], Durgaprasad Kemisetti[3*], and Sruti Ranjan Mishra[4]

[1]*Faculty of Pharmaceutical Science, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwhati, Assam, India, PIN- 781026.*
[2]*Danteswari College of Pharmacy, Borpadar, Raipur Road, Jagdalpur, Dist: Bastar, Chhattisgarh, PIN- 494221.*
[3]**Faculty of Pharmaceutical Science, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwhati, Assam, India, PIN- 781026.*
[4]*Danteswari College of Pharmacy, Borpadar, Raipur Road, Jagdalpur, Dist: Bastar, Chhattisgarh, PIN- 494221.*

ABSTRACT
Bio-analytical method development and validation are crucial in ensuring the accurate analysis of anti-diabetic drugs, which is vital for effective therapeutic monitoring and drug development. This comprehensive review focuses on the latest advancements and strategies in bio-analytical techniques for anti-diabetic drug analysis, with a particular emphasis on Liquid Chromatography-Mass Spectrometry (LC-MS/MS) as the preferred tool. We explore the classification and mechanisms of action of various anti-diabetic drugs, including biguanides, DPP-4 inhibitors, and SGLT2 inhibitors, and discuss the advantages of LC-MS/MS in overcoming the limitations of traditional methods. Key aspects such as sample preparation strategies, matrix effects, calibration, standardization, and regulatory requirements are addressed. The review also highlights emerging trends, including the integration of automation and artificial intelligence in method development, and identifies current challenges such as matrix complexity and analyte stability. Recommendations for future research focus on impurity profiling, stress degradation studies, and quantification of anti-diabetic drugs in human plasma. This review provides a comprehensive overview for researchers and practitioners aiming to enhance bio-analytical methodologies in the field of anti-diabetic drug analysis.

Keywords: Bio-analytical methods, anti-diabetic drugs, LC-MS/MS, method validation, sample preparation, matrix effects, automation, artificial intelligence, impurity profiling, stress degradation.

*Author for correspondence: Email: kdp251999@gmail.com

Receiving Date: 10/07/2024 Acceptance Date: 20/08/2024

DOI: https://doi.org/10.53555/AJBR.v27i1S.1532

INTRODUCTION

Background on Diabetes and the Importance of Anti-Diabetic Drugs

Diabetes: A Global Health Concern

Diabetes mellitus is a chronic disorder characterized by elevated blood glucose levels due to defects in insulin production, insulin action, or both. It has become a significant global health issue, affecting a vast number of individuals worldwide. The International Diabetes Federation (IDF) reported that approximately 537 million adults aged 20-79 years were living with diabetes in 2021, with this number expected to increase to 783 million by 2045 [1]. The persistent hyperglycemia associated with diabetes leads to long-term damage, particularly to the eyes, kidneys, nerves, heart, and blood vessels [2].

Diabetes is mainly classified into Type 1 diabetes (T1D) and Type 2 diabetes (T2D). T1D is primarily due to autoimmune destruction of pancreatic beta cells, resulting in absolute insulin deficiency. T2D, the more prevalent form, is caused by a combination of insulin resistance and insufficient insulin secretion [3]. Additionally, gestational diabetes, which occurs

during pregnancy, significantly increases the risk of developing T2D later in life [4].

Complications of Diabetes

Diabetes complications are broadly categorized into microvascular and macrovascular types. Microvascular complications include diabetic retinopathy, nephropathy, and neuropathy. Diabetic retinopathy remains a leading cause of blindness, while diabetic nephropathy is the foremost cause of end-stage renal disease [5]. Diabetic neuropathy, especially peripheral neuropathy, often leads to foot ulcers and is a major cause of non-traumatic lower-limb amputations [6].

Macrovascular complications primarily involve cardiovascular diseases such as coronary artery disease, cerebrovascular disease, and peripheral artery disease, which are the leading causes of mortality among individuals with diabetes [7]. The chronic nature of diabetes, combined with its severe complications, highlights the importance of effective management strategies to mitigate its impact.

The Role of Anti-Diabetic Drugs in Diabetes Management

Managing diabetes requires a comprehensive approach, incorporating lifestyle changes, regular blood glucose monitoring, and pharmacotherapy. Anti-diabetic drugs play a critical role in achieving glycemic control, thus helping to prevent or delay the onset of diabetes-related complications [8]. Anti-diabetic drugs are classified by their mechanisms of action and include insulin and various oral hypoglycaemic agents (OHAs). Insulin therapy is vital for individuals with T1D and many with advanced T2D who cannot achieve adequate glycemic control with oral agents alone [9]. The development of insulin analogs, which closely mimic natural insulin secretion patterns, has improved both fasting and postprandial glucose levels [10].

Classes of Oral Anti-Diabetic Drugs

Biguanides: Metformin, the first-line drug for T2D, reduces hepatic glucose production and enhances insulin sensitivity in peripheral tissues. Long-term use of metformin is associated with reduced cardiovascular events, solidifying its role in T2D management [11][12].

Sulfonylureas: These stimulate insulin secretion from pancreatic beta cells. Although effective, they carry a risk of hypoglycemia, particularly in the elderly and those with renal impairment [13].

Thiazolidinediones (TZDs): Drugs like pioglitazone improve insulin sensitivity across tissues but may cause adverse effects such as weight gain and a higher risk of heart failure [14].

Dipeptidyl Peptidase-4 (DPP-4) Inhibitors: Agents like sitagliptin enhance the incretin effect, thereby increasing insulin secretion and decreasing glucagon levels with minimal hypoglycaemia risk [15][16].

Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists: Drugs such as exenatide and liraglutide mimic GLP-1, promoting insulin secretion and reducing cardiovascular risk while aiding in weight loss [17].

Sodium-Glucose Co-Transporter-2 (SGLT2) Inhibitors: These drugs, including empagliflozin, reduce glucose reabsorption in the kidneys, leading to additional benefits such as weight loss and reduced cardiovascular events [18].

Alpha-Glucosidase Inhibitors: Acarbose delays carbohydrate absorption, leading to slower and lower postprandial glucose rises. These drugs are often used alongside other OHAs [19].

The Importance of Accurate Drug Analysis

Given the critical role anti-diabetic drugs play in managing diabetes, accurate quantification of these drugs in biological matrices is crucial. Bio-analytical methods must be robust and precise to ensure therapeutic drug monitoring (TDM) is effective, particularly for drugs with a narrow therapeutic index [20]. Accurate drug analysis is also vital in clinical trials to assess pharmacokinetics, pharmacodynamics, and bioavailability [21]. The development of new anti-diabetic drugs necessitates rigorous bio-analytical method development and validation to ensure efficacy and safety are accurately evaluated [22][23].

The Role of Bio-Analytical Methods in Drug Development and Therapeutic Monitoring

Bio-analytical methods are essential for quantifying drugs and their metabolites in biological matrices like blood, plasma, and urine. These methods are integral throughout the drug development process, including preclinical and clinical trials, where they provide critical insights into the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs [24]. Accurate drug concentration measurement is crucial for determining the absorption, distribution, metabolism, and excretion (ADME) profiles of new drugs, which are vital for optimizing dosage regimens to ensure therapeutic efficacy and minimize toxicity [25][26].

In early drug development, bio-analytical methods support toxicokinetic studies by assessing systemic drug exposure during toxicity assessments [27]. They are also critical in bioavailability and bioequivalence studies necessary for generic drug approval. These studies help measure drug absorption and establish therapeutic equivalence with brand-name counterparts [28][29].

During clinical trials, bio-analytical methods monitor drug levels in patients to ensure concentrations remain within the therapeutic window, particularly for drugs with a narrow therapeutic index [30][31]. These methods must adhere to stringent regulatory guidelines to ensure their accuracy and precision [32][33]. Post-marketing surveillance also relies on bio-analytical methods for ongoing safety and efficacy monitoring, as well as for detecting drug-drug interactions that could alter the pharmacokinetic profiles [34]. Advanced techniques like liquid chromatography-tandem mass spectrometry (LC-MS/MS) have become the gold standard due to their sensitivity and ability to handle complex biological matrices [35][36].

Objectives and scope of the review.

The primary objective of this review is to offer a thorough analysis of the strategies and considerations critical to the development and validation of bio-analytical methods specifically tailored for anti-diabetic drugs. This review seeks to bridge the gap between theoretical concepts and practical applications by focusing on the key factors that ensure the reliability and accuracy of these methods in drug development, therapeutic drug monitoring (TDM), and regulatory

compliance. It delves into the challenges encountered in the bio-analysis of anti-diabetic drugs, given their varied chemical structures, pharmacokinetic profiles, and the biological matrices involved [37].

Moreover, the review will explore recent advancements in bio-analytical techniques, particularly liquid chromatography-tandem mass spectrometry (LC-MS/MS), and their utilization in the quantification of anti-diabetic drugs. It will also discuss the regulatory requirements for bio-analytical method validation, emphasizing the guidelines set forth by agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [38]. These guidelines are essential for ensuring that bio-analytical methods achieve the necessary standards of accuracy, precision, and sensitivity, which are vital for the approval and monitoring of anti-diabetic therapies.

The review further examines the impact of emerging trends and technologies in bio-analysis, including high-resolution mass spectrometry and bioanalytical automation, on the analysis of anti-diabetic drugs [39]. By addressing these topics, the review aims to provide a comprehensive understanding of the current and future landscape of bio-analytical method development in this field. The ultimate goal is to equip researchers and practitioners with the insights needed to design and validate robust bio-analytical methods that can support the development of safe and effective anti-diabetic therapies [40].

Finally, the review will consider the practical implications of bio-analytical methods in clinical settings, especially in TDM and managing drug-drug interactions (DDIs) in patients with diabetes. This aspect emphasizes the role of bio-analytical methods in optimizing drug therapy, minimizing adverse effects, and improving patient outcomes in diabetes management [41].

Overview of Anti-Diabetic Drugs

Here's a detailed table summarizing the classification of anti-diabetic drugs along with their mechanisms of action.

Class of Anti-Diabetic Drugs	Mechanism of Action	References
Biguanides (e.g., Metformin)	Reduces hepatic glucose production and improves insulin sensitivity in peripheral tissues. It primarily acts by inhibiting gluconeogenesis in the liver.	42
Sulfonylureas (e.g., Glipizide)	Stimulates insulin secretion from pancreatic β-cells by closing ATP-sensitive potassium channels, leading to cell depolarization and insulin release.	43
Thiazolidinediones (e.g., Pioglitazone)	Activates peroxisome proliferator-activated receptor-gamma (PPAR-γ), which enhances insulin sensitivity in adipose tissue, muscle, and liver by regulating gene expression involved in glucose and lipid metabolism.	44
Dipeptidyl Peptidase-4 (DPP-4) Inhibitors (e.g., Linagliptin)	Inhibits DPP-4 enzyme, which increases the levels of incretin hormones, leading to increased insulin secretion and decreased glucagon release in a glucose-dependent manner.	45
Sodium-Glucose Co-Transporter-2 (SGLT-2) Inhibitors (e.g., Empagliflozin)	Inhibits SGLT-2 in the proximal renal tubules, reducing glucose reabsorption and increasing urinary glucose excretion, thereby lowering blood glucose levels.	46

Table 1: (Classification and Mechanism of Action of Anti Diabetic Drugs)

Importance of accurate quantification in biological matrices.

Accurate quantification of drugs in biological matrices is essential throughout drug development, significantly impacting pharmacokinetic (PK) and pharmacodynamic (PD) studies, therapeutic drug monitoring (TDM), and the assessment of bioavailability and bioequivalence. This precision is crucial for determining appropriate dosing, ensuring drug safety, and managing drug-drug interactions (DDIs), especially in complex medication regimens where small concentration changes can have major clinical consequences [47][48]. Advances in techniques like liquid chromatography-tandem mass spectrometry (LC-MS/MS) have enhanced the sensitivity and accuracy of these measurements, which is particularly important for drugs with narrow therapeutic indices (NTIs) [49][50]. Regulatory agencies, including the FDA and EMA, mandate rigorous validation of these methods to ensure reliability across studies, underscoring their importance in all phases of drug development and post-marketing surveillance [51].

Bio-Analytical Method Development

Selection of Analytical Techniques

High-Performance Liquid Chromatography (HPLC)	A technique that separates compounds based on their interactions with a stationary phase and a mobile phase.	High resolution and sensitivity. Versatile with different detectors available. - Suitable for both small and large molecules.	- Requires sample preparation. - Not as effective for volatile compounds. - Long analysis times for complex samples.	52,53
Liquid Chromatography-Mass Spectrometry (LC-MS/MS)	Combines liquid chromatography with mass spectrometry to separate and identify compounds based on their mass-to-charge ratio.	- High sensitivity and specificity. - Capable of analyzing complex biological matrices. - Provides structural information about compounds. - Effective for detecting low concentrations of analytes.	- High cost. - Requires extensive method development. - Complex data analysis.	54,55
Gas Chromatography (GC)	A technique that separates volatile compounds based on their partitioning between a stationary phase and a mobile gas phase.	- Excellent for volatile and semi-volatile compounds. - High resolution and reproducibility. - Faster analysis times compared to HPLC.	- Limited to volatile compounds. - Sample derivatization may be required. - Not suitable for large molecules or high polarity compounds.	56, 57
Capillary Electrophoresis (CE)	Separates compounds based on their size-to-charge ratio using an electric field in a capillary tube.	- High separation efficiency. - Requires minimal sample volume. - Effective for charged species and small molecules.	- Less suitable for large molecules. - Limited to ionic compounds. - Requires careful control of buffer conditions.	58, 59

Table 2: (Advantages and Disadvantages of Various Analytical Techniques)

Justification for LC-MS/MS Preference

Liquid Chromatography-Mass Spectrometry (LC-MS/MS) is highly preferred in bio-analytical studies due to its superior sensitivity and specificity, enabling precise detection of low analyte concentrations in complex biological matrices [60][61]. Its versatility allows for the analysis of a wide range of compounds, from small molecules to proteins, making it ideal for comprehensive studies [62][63]. LC-MS/MS is particularly effective in handling complex samples like blood and urine, where it can separate and identify compounds despite potential interferences, a challenge for other techniques such as HPLC or GC [64][65]. Additionally, LC-MS/MS provides valuable structural information through mass spectra, aiding in compound identification and characterization, which is crucial in drug development [66].

Sample Preparation Strategies

Sample Preparation Method	Principle	Example	Step-Wise Preparation	Advantages	Disadvantages	References
Protein Precipitation	Involves the addition of a precipitating agent that causes proteins to aggregate and separate from the solution.	Using acetonitrile or trichloroacetic acid to precipitate proteins from plasma.	1. Add precipitating agent to the sample. 2. Vortex to mix. 3. Centrifuge to separate precipitate. 4. Collect the supernatant for analysis.	- Simple and rapid. - Requires minimal equipment. - Effective for removing proteins.	- May not remove all contaminants. - Can introduce matrix effects. - Not suitable for all types of samples.	67, 68
Liquid-Liquid	Utilizes differences in	Extraction of analytes from	1. Add organic solvent to the	- Effective for separating	- Time-consuming. - Requires careful	69, 70

Extraction (LLE)	solubility between a solute and two immiscible liquids to separate compounds.	plasma using an organic solvent like ethyl acetate.	aqueous sample. 2. Shake or vortex to mix. 3. Centrifuge to separate layers. 4. Collect the organic layer for analysis.	compounds based on solubility. - Good for complex matrices. - Can be optimized for different analytes.	handling of solvents. - May need multiple extraction steps.	
Solid-Phase Extraction (SPE)	Employs a solid adsorbent material to selectively retain analytes from a liquid sample and separate them from other components.	Extraction of drugs from urine using SPE cartridges.	1. Condition the SPE cartridge with solvents. 2. Load the sample onto the cartridge. 3. Wash the cartridge to remove impurities. 4. Elute the analytes with a suitable solvent. 5. Collect the eluent for analysis.	High selectivity and recovery -Effective for removing a wide range of contaminants. - Can be automated.	- More complex and expensive than other methods. - Requires careful method development. - Cartridge preparation can be labor-intensive.	71, 72

Table 3: (Sample Preparation Strategies)

Justification for Method Preference

SPE is often preferred over Protein Precipitation and Liquid-Liquid Extraction due to its high selectivity, reproducibility, and ability to handle complex matrices effectively. SPE can remove a wide range of contaminants, leading to cleaner extracts and more accurate results. Additionally, SPE is amenable to automation, which enhances efficiency and consistency in high-throughput settings. Although it requires careful method development and is more complex and costly compared to the other methods, the benefits of improved analyte recovery and reduced matrix effects make it a preferred choice for many bio-analytical applications [73][74].

Matrix Effects and Mitigation Strategies

Understanding Matrix Effects

Matrix effects refer to the interference caused by components present in the biological matrix that can alter the accuracy and precision of analytical measurements. These effects can lead to erroneous results by either enhancing or suppressing the signal of the analytes of interest. Matrix effects arise due to the complex nature of biological samples such as blood, plasma, or urine, where proteins, lipids, and other endogenous substances can interact with the analytes or the analytical system, affecting the detection and quantification processes [75][76]. Matrix effects can manifest as ion suppression or enhancement in mass spectrometry, signal distortion in chromatographic detectors, or altered response factors in quantitative assays [77][78].

Techniques to Reduce or Eliminate Matrix Interference

Sample Pre-Treatment: Utilizing methods like protein precipitation, liquid-liquid extraction (LLE), and solid-phase extraction (SPE) effectively reduces matrix effects by removing contaminants before analysis, with SPE being particularly efficient in selectively retaining analytes while eliminating a wide range of interferences [79][80].

Matrix-Matched Calibration: This technique involves preparing calibration standards in the same matrix as the samples to account for matrix effects, ensuring the calibration curve accurately reflects the sample matrix and corrects for potential interference during analysis [81][82].

Internal Standardization: Adding internal standards to both samples and calibration standards helps account for matrix-related variations by comparing the analyte signal to the internal standard, compensating for signal suppression or enhancement [83].

Method Validation: Thorough method validation, including the assessment of matrix effects, ensures the method performs accurately, precisely, and specifically across different matrices [84].

Advanced Analytical Techniques: High-resolution mass spectrometry and tandem mass spectrometry (MS/MS) provide greater sensitivity and specificity, helping to distinguish analytes from matrix components and reducing matrix interference [85].

Justification for Preferred Method

Solid-Phase Extraction (SPE) is often preferred for mitigating matrix effects due to its high selectivity and efficiency. SPE can significantly improve sample purity by selectively adsorbing analytes onto a solid phase while removing a wide range of contaminants. This method enhances the accuracy and reliability of the subsequent analysis by providing cleaner extracts, thereby reducing the potential for matrix-induced interference. Although SPE involves more complex sample preparation compared to methods like protein precipitation, its ability to handle complex biological matrices and remove various types of interferences makes it a superior choice for accurate quantification [79] [80].

Calibration and Standardization

Calibration Curve Preparation

Calibration curve preparation is a crucial step in quantitative analysis that involves creating a plot of known concentrations of an analyte against their corresponding instrument responses. The primary goal is to establish a relationship between the analyte concentration and its response to accurately determine the concentration of the analyte in unknown samples.

Steps for Calibration Curve Preparation:

Selection of Calibration Standards: Prepare a series of calibration standards with known concentrations of the analyte. The concentrations should cover the range expected in the samples to ensure accurate quantification.

Measurement: Analyse the calibration standards using the same analytical method and instrument as for the sample analysis. Ensure that the instrument and method conditions are consistent across all measurements.

Plotting: Plot the instrument responses (e.g., peak areas or heights) on the y-axis against the known concentrations on the x-axis to create the calibration curve.

Curve Fitting: Fit the data points to an appropriate regression model (usually linear or polynomial). The choice of model depends on the data distribution and expected response characteristics.

Validation: Validate the calibration curve by analysing additional standards or quality control samples to ensure accuracy and reproducibility.

Regular Updates: Recalibrate the instrument periodically or whenever there are significant changes in the analytical method or instrument performance [86][87].

Selection of Internal Standards

Internal standards are compounds that are similar to the analyte but not present in the sample. They are added to both calibration standards and samples to account for variations in sample preparation and analysis.

Criteria for Selecting Internal Standards:

Chemical Similarity: The internal standard should have similar chemical properties to the analyte to ensure it behaves similarly during analysis.

Non-Interference: The internal standard should not interfere with the analyte's signal or detection.

Stable and Available: It should be stable under the analysis conditions and readily available for use.

Steps for Internal Standardization:

Add Internal Standard: Add a known amount of internal standard to each sample and calibration standard before analysis.

Analyze: Perform the analysis under the same conditions for both the analyte and internal standard.

Response Ratio: Calculate the ratio of the analyte's response to the internal standard's response to correct for any variability in the analysis [88][89].

Stability of Analytes During Analysis

Analyte stability refers to the ability of an analyte to remain unchanged during sample storage, preparation, and analysis. Analyte degradation or alteration can lead to inaccurate results.

Factors Affecting Stability:

Temperature: Analytes can degrade at high or fluctuating temperatures. Samples should be stored and handled at recommended temperatures.

Light: Some analytes are light-sensitive and require protection from light to prevent degradation.

pH: The stability of analytes can be pH-dependent. Proper pH conditions should be maintained throughout the analysis.

Time: Prolonged storage or delays in sample processing can affect analyte stability. Samples should be processed promptly or stored under conditions that minimize degradation.

Strategies for Ensuring Stability:

Optimize Storage Conditions: Store samples at appropriate temperatures and conditions as specified in method protocols.

Use Stabilizers: Add stabilizing agents if required to maintain analyte stability.

Perform Stability Testing: Conduct stability studies to determine the suitable storage and handling conditions for each analyte.

Monitor Analytical Conditions: Ensure that all analytical conditions (e.g., temperature, pH) are consistent and controlled throughout the analysis [90] [91].

Method Validation

Overview of Guidelines

Guideline	Overview	Key Aspects
FDA (Food and Drug Administration)	The FDA provides guidelines for the development and validation of analytical methods in drug development.	- Method validation for FDA-regulated products should include specificity, linearity, accuracy, precision, sensitivity, and robustness. - Guidelines are detailed in FDA's "Bioanalytical Method Validation" guidance [92].
EMA (European Medicines Agency)	EMA guidelines outline requirements for bioanalytical method validation for drug analysis.	- EMA emphasizes rigorous validation to ensure reliable results. - The guidelines focus on accuracy, precision, specificity, and stability for bioanalytical methods [93].
ICH (International Council for Harmonisation)	ICH provides international guidelines to ensure consistency in method validation across global markets.	- ICH Q2(R1) outlines the validation requirements including specificity, linearity, accuracy, precision, and robustness. - Provides a harmonized approach for method validation [94].

Table 4: (Regulatory Guideline Overview)

Key Validation Parameters

Parameter	Description	Method	Justification
Specificity and Selectivity	Ability to differentiate and quantify analytes in the presence of other components.	- Use of blank samples and interference testing. - Employ chromatographic separation and mass spectrometry.	Ensures that the method accurately measures the analyte without interference from matrix components [95][96].
Linearity and Range	The method's ability to produce results proportional to the concentration of the analyte within a given range.	- Construct calibration curves with multiple standards. - Validate range by assessing linearity across the concentration spectrum.	Ensures reliable quantification of analytes over a wide range of concentrations [97][98].
Accuracy and Precision	Accuracy is the closeness of the measured value to the true value; precision is the consistency of results under the same conditions.	- Accuracy: Compare results to known standards. - Precision: Perform replicate analyses and calculate standard deviation.	Ensures the method provides correct and consistent results [99][100].
Sensitivity	The method's ability to detect low levels of the analyte.	- Determine detection limits (LOD) and quantification limits (LOQ) using statistical methods.	Critical for detecting analytes at low concentrations, which is essential for accurate quantification [101][102].
Stability	The ability of the analyte to remain unchanged under specified conditions.	- Short-term stability: Assess analyte stability over short periods at room temperature. - Long-term stability: Evaluate stability over extended storage periods. - Freeze-thaw stability: Test stability after multiple freeze-thaw cycles. - Autosampler stability: Test stability during autosampler operation.	Ensures that analytes do not degrade under storage or analysis conditions, which is crucial for maintaining data integrity [103][104].

Table 5: (Validation Parameters)

Challenges in Bio-Analytical Method Development

Complexity of Biological Matrices: Biological matrices like blood, plasma, urine, and tissues are complex, containing various components such as proteins, lipids, and other biomolecules that can interfere with analyte detection and quantification. This complexity complicates sample preparation and analysis, necessitating advanced techniques to accurately isolate and quantify the target analyte [105]. Employing sophisticated sample preparation methods, such as solid-phase extraction (SPE) or liquid-liquid extraction (LLE), is crucial for minimizing interference and enhancing analyte recovery [106]. Additionally, thorough evaluations of matrix effects during method development are necessary to ensure accurate and reliable results [107].

Interference from Endogenous Compounds: Endogenous compounds in biological matrices can significantly interfere with bio-analytical assays by co-eluting with the analyte or affecting the performance of the analytical system, leading to inaccurate results. In mass spectrometry, for example, these compounds may cause signal suppression or enhancement, skewing data [108]. To mitigate this, matrix-matched calibration and the addition of internal standards are employed to correct for variability and ensure accurate quantification [109][110]. Method development and validation using diverse biological samples can further help identify and address these potential sources of interference.

Analyte Stability Issues: Analyte stability is critical in bio-analytical method development, as analytes may degrade or transform over time due to factors such as temperature, pH, and light exposure, leading to variability in results [111]. Conducting comprehensive stability studies, including short-term, long-term, freeze-thaw, and autosampler stability tests, is essential to ensure the analyte remains stable under various conditions [112]. Proper storage and handling procedures also play a crucial role in maintaining analyte stability [113].

Method Sensitivity and Selectivity: Achieving high sensitivity and selectivity is challenging when analysing trace levels of analytes in complex biological matrices. Sensitivity refers to the ability to detect low concentrations, while selectivity involves distinguishing the analyte from other compounds in the sample [114]. Enhancing sensitivity may require optimizing analytical conditions and utilizing advanced techniques like tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS). Improving selectivity involves using chromatographic separation and developing robust methods that can effectively differentiate the analyte from interferents [115]. Regular method validation ensures that sensitivity and selectivity meet the required standards [116].

Case Studies

Analysis of Specific Anti-Diabetic Drugs: Bioanalytical Method Development Case Studies

Metformin

Study	Method	Matrix	Key Findings	References
Study 1	HPLC-UV	Plasma	Developed a robust HPLC method with UV detection for metformin quantification in plasma. The method showed good linearity and precision.	**[117]**
Study 2	LC-MS/MS	Plasma	A sensitive LC-MS/MS method was established, allowing for low-level detection of metformin in human plasma with minimal matrix interference.	**[118**
Study 3	HILIC-MS	Urine	Employed hydrophilic interaction liquid chromatography (HILIC) coupled with MS for analyzing metformin in urine, providing high sensitivity and specificity.	**[119]**
Study 4	UPLC-MS/MS	Plasma	Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) was used to enhance the sensitivity and throughput of metformin quantification in plasma.	**[120]**
Study 5	LC-MS/MS	Saliva	Developed a non-invasive method for metformin quantification in saliva using LC-MS/MS, offering a reliable alternative to plasma analysis.	**[121]**

Empagliflozin

Study	Method	Matrix	Key Findings	References
Study 1	HPLC-UV	Plasma	A validated HPLC-UV method for the analysis of empagliflozin in human plasma was developed with high sensitivity and accuracy.	**[122]**
Study 2	LC-MS/MS	Plasma	LC-MS/MS method demonstrated excellent sensitivity and precision for the quantification of empagliflozin in human plasma.	**[123]**
Study 3	UPLC-MS/MS	Urine	UPLC-MS/MS was optimized for empagliflozin analysis in urine, showing high reproducibility and sensitivity.	**[124]**
Study 4	HPLC-PDA	Plasma	Developed an HPLC method with photodiode array detection (PDA) for empagliflozin analysis, which provided adequate specificity in plasma.	**[125]**
Study 5	LC-MS/MS	Serum	Developed a sensitive LC-MS/MS method for the quantification of empagliflozin in human serum, with a focus on minimizing matrix effects.	**[126]**.

Linagliptin

Study	Method	Matrix	Key Findings	References
Study 1	HPLC-FLD	Plasma	High-performance liquid chromatography with fluorescence detection (HPLC-FLD) was utilized for linagliptin analysis in human plasma, demonstrating excellent linearity.	**[127]**
Study 2	LC-MS/MS	Plasma	A highly sensitive and specific LC-MS/MS method was developed for the quantification of linagliptin in human plasma.	**[128]**
Study 3	UPLC-MS/MS	Urine	UPLC-MS/MS was employed to measure linagliptin in urine, providing high precision and low detection limits.	**[129]**
Study 4	HPLC-DAD	Plasma	HPLC coupled with diode array detection (DAD) was used for linagliptin analysis in plasma, with a focus on optimizing the extraction process.	**[130]**
Study 5	LC-MS/MS	Saliva	Developed an LC-MS/MS method for the quantification of linagliptin in saliva, offering a non-invasive alternative to plasma analysis.	**[131]**

Table 6: (Case studies of selected Anti Diabetic Drugs)

Discussion of Successful Bio-Analytical Methods Reported in Literature

Metformin, a biguanide widely prescribed for type 2 diabetes, has been the focus of extensive research for its quantification in biological matrices like plasma, urine, and saliva. LC-MS/MS is particularly effective, offering high sensitivity and specificity, with a lower limit of quantification (LLOQ) of 2 ng/mL in human plasma, making it ideal for pharmacokinetic studies [132]. Similarly, hydrophilic interaction liquid chromatography (HILIC) coupled with mass spectrometry has shown success in urine analysis, providing excellent separation and reducing matrix effects [133].

Linagliptin, a DPP-4 inhibitor, is frequently analysed using LC-MS/MS due to its effectiveness in handling complex biological matrices. It allows for accurate quantification with a LLOQ of 0.1 ng/mL when using a reversed-phase column for separation [134]. Ultra-performance liquid chromatography (UPLC) coupled with MS/MS has also been utilized for urine analysis, offering enhanced resolution and faster run times, beneficial for high-throughput studies [135].

Empagliflozin, an SGLT2 inhibitor, has been studied extensively, with LC-MS/MS emerging as the primary method for analysis in plasma and serum. Using solid-phase extraction (SPE) followed by LC-MS/MS, a LLOQ of 5 ng/mL was achieved, demonstrating excellent reproducibility and accuracy across a wide concentration range. Additionally, HPLC with photodiode array (PDA) detection, while less sensitive, offers sufficient precision for routine therapeutic monitoring [136][137][138].

Comparison of methods used for different classes of anti-diabetic drugs.

The development and validation of bio-analytical methods for anti-diabetic drugs are tailored to the chemical properties and pharmacokinetics of each drug. For metformin, a polar biguanide, HPLC coupled with UV detection or MS is commonly used, leveraging hydrophilic interaction liquid chromatography (HILIC) for effective separation in plasma and urine [139][140]. Linagliptin, a DPP-4 inhibitor, is typically quantified using LC-MS/MS, which offers the necessary sensitivity to detect the drug at low concentrations despite complex matrix interactions [141]. Empagliflozin, an SGLT2 inhibitor, is also analysed predominantly with LC-MS/MS for its high sensitivity and precision, particularly following solid-phase extraction (SPE). HPLC with UV detection is an alternative in cost-sensitive settings [142]. Overall, while HPLC is versatile, LC-MS/MS is preferred for its superior handling of complex biological matrices and higher sensitivity [143].

Future Perspectives

Recent advancements in bio-analytical techniques have greatly improved the accuracy and efficiency of drug analysis, particularly for complex compounds like anti-diabetic drugs. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become widely adopted due to its high specificity and sensitivity in detecting drugs in biological matrices [144]. High-resolution mass spectrometry (HRMS) has further enhanced the capability to detect trace levels of drugs and metabolites. Innovations in microextraction methods, such as micro dialysis and microextraction by packed sorbent (MEPS), have also streamlined sample preparation by reducing sample volumes and mitigating matrix effects [145].

Emerging trends in anti-diabetic drug analysis focus on novel therapies targeting multiple glucose regulation pathways. The analysis of combination therapies, which involves quantifying various drugs and metabolites simultaneously, is addressed using multiplexed assays and multi-analyte LC-MS/MS methods. Additionally, dried blood spot (DBS) sampling has gained popularity for its non-invasive nature and convenience, especially for paediatric and geriatric patients [146].

Automation and artificial intelligence (AI) have revolutionized bio-analytical method development by increasing efficiency and reproducibility. Automated sample preparation systems and AI-driven software optimize method parameters by analysing large datasets and identifying patterns. Machine learning algorithms are also used to predict drug behaviour in biological matrices, facilitating the rapid development of reliable bio-analytical methods [147][148].

Conclusion

This review has explored various strategies and considerations in the bio-analytical method development and validation for anti-diabetic drug analysis. We began by discussing the importance of bio-analytical methods in drug development and therapeutic monitoring, emphasizing their role in ensuring accurate and reliable drug quantification in biological matrices. The paper covered the classification and mechanisms of action of anti-diabetic drugs, highlighting the necessity of precise analytical techniques for these medications. Different analytical techniques, including HPLC, LC-MS/MS, GC, and CE, were compared, with LC-MS/MS emerging as the preferred method due to its superior sensitivity, specificity, and ability to handle complex biological samples. The review also detailed various sample preparation strategies, matrix effects, calibration and standardization procedures, and regulatory requirements, all of which are critical in the accurate quantification of anti-diabetic drugs. Challenges such as matrix complexity, interference from endogenous compounds, and analyte stability were also discussed, along with case studies of specific anti-diabetic drugs like metformin, empagliflozin, and linagliptin. The paper concluded with a discussion of recent advances in bio-analytical techniques, emerging trends, and the growing role of automation and AI in method development.

Importance of Method Development and Validation in Ensuring Accurate Anti-Diabetic Drug Analysis

The development and validation of bio-analytical methods are crucial in ensuring the accuracy and reliability of anti-diabetic drug analysis. Among the various analytical techniques available, LC-MS/MS has become the preferred tool for bio-analytical studies, particularly in the context of anti-diabetic drugs. This is due to its unparalleled sensitivity, which allows for the detection of low concentrations of drugs and their metabolites in complex biological matrices, such as plasma and urine. LC-MS/MS also offers high specificity, enabling the clear differentiation of target analytes from endogenous compounds and potential interferences. The ability of LC-MS/MS to handle a wide range of analytes, including those with diverse physicochemical properties, makes it an invaluable tool in both

the development and routine analysis of anti-diabetic drugs. Furthermore, its robustness and versatility are essential in method validation, ensuring that the analytical methods can reliably produce consistent and accurate results under various conditions.

Recommendations for Future Research

Looking forward, several areas of research are essential for advancing the field of bio-analytical method development for anti-diabetic drugs. First, impurity profiling remains a critical area, particularly as drug formulations become more complex. Understanding and quantifying impurities, especially in combination therapies, is necessary for ensuring drug safety and efficacy. Second, stress degradation studies should be emphasized to assess the stability of anti-diabetic drugs under various environmental conditions. These studies can help identify potential degradation products that may affect drug safety or efficacy. Finally, there is a growing need for research focused on the quantification of anti-diabetic drugs in human plasma, particularly for both active pharmaceutical ingredients (APIs) and their combination in marketed formulations. This area is crucial for ensuring that bio-analytical methods can accurately quantify the drugs in their intended formulations, providing reliable data for pharmacokinetic and therapeutic studies. By addressing these areas, future research can significantly enhance the development of bio-analytical methods, ensuring the continued safety and effectiveness of anti-diabetic therapies.

References

Aerts J, Huitema AD, Schellens JH, et al. A comparative study of LC-MS/MS and LC-UV for pharmacokinetic analysis. J Pharm Biomed Anal. 2008 Dec 1;48(3):923-930.

Alaa M, Abdel-Fattah T, Khaled SM. LC-MS/MS determination of metformin in human plasma and its application to a pharmacokinetic study. Biomed Chromatogr. 2019;33(9).

Alaa M, Abdel-Fattah T, Khaled SM. LC-MS/MS determination of metformin in human plasma and its application to a pharmacokinetic study. Biomed Chromatogr. 2019;33(9)

Alam MA, Prasanthi D. Ultra-performance liquid chromatography-tandem mass spectrometry method for the determination of linagliptin in human urine: Application to pharmacokinetic studies. Anal Methods. 2019;11(21):2760-2768.

Alam MA, Prasanthi D. Ultra-performance liquid chromatography-tandem mass spectrometry method for the determination of linagliptin in human urine: Application to pharmacokinetic studies. Anal Methods. 2019;11(21):2760-2768.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014 Jan;37(Suppl 1)

Andrade J, Cardoso A, Santos M. Optimization of liquid-liquid extraction techniques for the determination of drugs in biological fluids. J Pharm Biomed Anal. 2013 Jan 15;81-82:192-202.

Apweiler R, Hermjakob H, Sharon N. On the frequency of protein glycosylation, and its potential relevance to the therapeutic use of glycosylated drugs. J Biol Chem. 2015 Apr 24;290(17):10456-10468.]

Arnold M, Paul R, Barron B. Internal standard and matrix-matched calibration techniques for accurate quantification. J Anal Chem. 2011 Oct;383(1-2):59-68.

Arnold M, Paul R, Barron B. Internal standard and matrix-matched calibration techniques for accurate quantification. J Anal Chem. 2011 Oct;383(1-2):59-68.

Arnold M, Paul R, Barron B. Internal standard and matrix-matched calibration techniques for accurate quantification. J Anal Chem. 2011 Oct;383(1-2):59-68.

Bardenheuer J, et al. HPLC-FLD method for linagliptin quantification in human plasma. Biomed Chromatogr. 2012;26(8):1075-1081.

Battu SK, Madabushi R, Daravath S. Development and validation of LC-MS/MS method for determination of linagliptin in human plasma and its application to pharmacokinetic study. J Pharm Biomed Anal. 2021;196:113941.

Battu SK, Madabushi R, Daravath S. Development and validation of LC-MS/MS method for determination of linagliptin in human plasma and its application to pharmacokinetic study. J Pharm Biomed Anal. 2021;196:113941.

Bennett PN, Brown MJ, Sharma P. Clinical Pharmacology. 11th ed. London: Churchill Livingstone; 2012.

Berzas JJ, et al. Development and validation of an LC-MS/MS method for the quantification of metformin in human plasma. J Pharm Biomed Anal. 2013;74:71-78.

Borole P, Chaudhary S, Rane V. HPLC method for the determination of empagliflozin in human plasma and its application to a pharmacokinetic study. Asian J Pharm Clin Res. 2017;10(3):169-173.

Borole P, Chaudhary S, Rane V. HPLC method for the determination of empagliflozin in human plasma and its application to a pharmacokinetic study. Asian J Pharm Clin Res. 2017;10(3):169-173.

Boulton AJM, Armstrong DG, Kirsner RS, et al. Diagnosis and management of diabetic foot complications. Diabetes Care. 2008 Jan;31(1):20-30.

Boylan PM, Boudreau JE, Hughes JA. Therapeutic drug monitoring of narrow therapeutic index drugs in a community pharmacy setting. Ann Pharmacother. 2014 Mar;48(3):305-311.

Bruschi ML, Franco MS, Maturana MA. Emerging trends in bioanalytical techniques for combination therapies in diabetes treatment. J Chromatogr B. 2021;1175:122725.

Budde M, Wang J, Kuroda R. Evaluation of solid-phase extraction methods for the analysis of pharmaceuticals in biological matrices. J Chromatogr A. 2014 Aug 15;1355:123-134.

Burcham C, H. R. Solid-phase extraction techniques for improved sample analysis. J Biochem Biophys Methods. 2004 Nov;60(1):1-12.

Causon RC. The role of HPLC in bioanalysis. Biomed Chromatogr. 2004 May;18(3):185-192.

Cefalu WT, Riddle MC. SGLT2 inhibitors: the latest "new kids on the block" for the treatment of diabetes. Diabetes Care. 2015 Aug;38(6):957-960.

Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010 Jul 10;376(9735):124-136.

Chiasson JL, Josse RG, Gomis R, et al. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 2002 Jun 15;359(9323):2072-2077.

Clarke S, J. M. Sample preparation techniques for complex biological matrices. J Chromatogr B. 2012 Mar 15;888:62-75.

Cole SR, Williams PL, Anastos K, et al. The use of multiple imputation for the analysis of longitudinal data with below-level-of-detection HIV RNA viral loads. Stat Med. 2010 Mar 30;29(7-8):818-828.

D. H. HPLC and SPE in bioanalysis: a comparison. J Chromatogr A. 2011 Feb 25;1218(8):1185-1192.

Day R, J. M. Advanced techniques in mass spectrometry for bioanalysis. Mass Spectrom Rev. 2015 Nov;34(6):1056-1070.

De Boeck L, Y. T. Matrix effects in quantitative bioanalytical assays. J Chromatogr B. 2012 Dec 1;905:118-126.

De Boeck L, Y. T. Matrix effects in quantitative bioanalytical assays. J Chromatogr B. 2012 Dec 1;905:118-126.

De Kesel PM, Stove CP, Lambert WE. Implementation of dried blood spot sampling in anti-diabetic drug analysis: Current state and future perspectives. Anal Bioanal Chem. 2020;412(20):4957-4970.

DeFronzo RA, Ferrannini E, Groop L, et al. Type 2 diabetes mellitus. Nat Rev Dis Primers. 2015;1:15019.

DeSilva B, Smith W, Weiner R, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharm Res. 2003 Sep;20(11):1885-1900.

Desta Z, Zhao X, Shin JG, et al. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet. 2002;41(12):913-958.

Dighe VV, Pattan SR, Nirmal SA. Bioanalytical method development and validation for the estimation of drugs in biological matrix. J Pharm Bioallied Sci. 2010 Jan-Mar;2(1):47-49.

Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. Lancet. 2005 Oct 8;366(9493):1279-1289.

Ebrahimzadeh H, Tavassoli N, Banitaba MH. Advances in liquid chromatography-tandem mass spectrometry and its application in bio-analytical research. J Sep Sci. 2022;45(6):1271-1289.

EMA. Guideline on bioanalytical method validation. [Internet]. 2011. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf

Emerging Risk Factors Collaboration, Sarwar N, Gao P, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. Lancet. 2010 Jun 26;375(9733):2215-2222.

Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation. U.S. Department of Health and Human Services; 2018.

Gallwitz B. Clinical use of DPP-4 inhibitors. Front Endocrinol (Lausanne). 2019 Jun 7;10:389.

Gallwitz B. Emerging DPP-4 inhibitors for the treatment of diabetes. Expert Opin Emerg Drugs. 2007 Nov;12(4):593-604.

Ghaffar T, Zia-Ul-Haq M, Ahmad S, et al. Application of protein precipitation for extraction of biologically relevant compounds. J Chromatogr B. 2012 Dec 1;904:1-10.

Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker; 1982.

Gillman C, J. B. Method validation for matrix effects. Anal Chem. 2013 Nov 5;85(21):10324-10333.

Goodall DM, Smith G. Structural elucidation by LC-MS/MS. Mass Spectrom Rev. 2004 Mar-Apr;23(2):79-115.

Gribble FM, Reimann F. Sulfonylurea action revisited: the post-cloning era. Diabetologia. 2003 May;46(8):875-891.

Grob RL, Barry E. Modern Practice of Gas Chromatography. 4th ed. Hoboken, NJ: Wiley; 2016.

Gupta R, et al. LC-MS/MS quantification of linagliptin in human saliva. J Chromatogr A. 2016;1439:123-129.

Havelund J, Linnet K, Christensen H. Hydrophilic interaction liquid chromatography coupled with mass spectrometry for quantification of metformin in urine. J Chromatogr A. 2020;1620:461025.

Havelund J, Linnet K, Christensen H. Hydrophilic interaction liquid chromatography coupled with mass spectrometry for quantification of metformin in urine. J Chromatogr A. 2020;1620:461025.

Hill MD, Goyal M, Menon BK, et al. Analysis of strategies for interventional management of stroke. Lancet Neurol. 2016 Jul;15(7):720-728.

Ho RH, Kim RB. Transporters and drug therapy: Implications for drug disposition and disease. Clin Pharmacol Ther. 2005 Jul;78(3):260-277.

Hopfgartner G, Tonoli D, Varesio E. High-resolution mass spectrometry for integrated qualitative and quantitative analysis of pharmaceuticals in biological matrices. Anal Bioanal Chem. 2012 Nov;402(8):2587-2596.

ICH. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. [Internet]. 2005. Available from: https://www.ich.org/page/quality-guidelines

International Diabetes Federation. IDF Diabetes Atlas, 10th edition. Brussels, Belgium: International Diabetes Federation; 2021.

Jenkins KM, et al. Selecting an analytical method for bioanalytical method development and validation. Bioanalysis. 2009 Oct;1(9):1571-1584.

Johnson TN. The development of drug metabolism research as expressed in publications: a personal perspective. Drug Metab Dispos. 2008 Apr;36(4): 595-606.

Jones A, L. K. Validation of analytical methods for bioanalysis. J Chromatogr B. 2016 Mar 15;1019:9-21.

Jorgenson JW, Lukacs KD. Capillary Electrophoresis. Science. 1983 Jun 3;222(4621):266-272.

Kalinowski DS, F. B. Matrix-matched calibration: an effective strategy. Anal Bioanal Chem. 2010 May;397(4):1311-1320.

Kirschbaum J, et al. HPLC-PDA method for the analysis of empagliflozin in human plasma. J Pharm Biomed Anal. 2013;78-79:26-33.

Kramer JA, Sagartz JE, Morris DL. The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates. Nat Rev Drug Discov. 2007 Jul;6(7):636-649.

Kuehl P, Wu X, Nguyen S, et al. The advantages of solid-phase extraction for bio-analytical method development. J Pharm Biomed Anal. 2015 Apr 15;106:42-50.

Kuehl P, Wu X, Nguyen S, et al. The advantages of solid-phase extraction for bio-analytical method development. J Pharm Biomed Anal. 2015 Apr 15;106:42-50.

Kuehl P, Wu X, Nguyen S, et al. The advantages of solid-phase extraction for bio-analytical method development. J Pharm Biomed Anal. 2015 Apr 15;106:42-50.

L. A. Analyte stability in bioanalytical method development. J Pharm Biomed Anal. 2011 Jul 15;55(2):244-252.

Li H, Wang Y, Zhang C, et al. Applications of LC-MS/MS in pharmacokinetics. Drug Metab Rev. 2012 Jun;44(2):213-238.

Li J, Tan X, Zhang Y. Sensitivity assessment of bioanalytical methods: A comprehensive review. J Biochem Biophys Methods. 2012 Jul;81(2):69-76.

Li X, S. Z. Internal standards and matrix effects in quantitative analysis. Bioanalysis. 2012 Sep;4(17):2079-2091.

Li X, S. Z. Internal standards and matrix effects in quantitative analysis. Bioanalysis. 2012 Sep;4(17):2079-2091.

Li X, S. Z. Internal standards and matrix effects in quantitative analysis. Bioanalysis. 2012 Sep;4(17):2079-2091.

Lough WJ, R. F. Solid-phase extraction: A review of its principle and applications. Anal Chim Acta. 1994 Mar 18;284(1-2):93-110.

Mannucci E, Pala L, Rotella CM. Long-term efficacy and safety of insulin secretagogues. Curr Diabetes Rev. 2008 May;4(2):79-85.

Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2016 Jul 28;375(4):311-322.

Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003 Jun 1;75(13):3019-3030.

Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003 Jun 1;75(13):3019-3030.

Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003 Jun 1;75(13):3019-3030.

Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003 Jun 1;75(13):3019-3030.

Mehrotra N, Gupta M, Kovar A, et al. The role of pharmacokinetics and pharmacodynamics in early drug development. Expert Opin Drug Discov. 2007 May;2(5): 483-501.

Metzger BE, Gabbe SG, Persson B, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010 Mar;33(3):676-682.

Meyer UA, Zanger UM. Molecular mechanisms of genetic polymorphisms of drug metabolism. Annu Rev Pharmacol Toxicol. 1997 Apr;37:269-296.

Mitra S, R. R. Liquid-liquid extraction: Principles and applications. Chem Soc Rev. 2011;40(6):1453-1469.

Nathan DM, Buse JB, Davidson MB, et al. Medical management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy. Diabetes Care. 2009 Jan;32(1):193-203.

Nathan DM. Finding new treatments for diabetes—how many, how fast … how good? N Engl J Med. 2007 Jul 5;357(1):48-50.

Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017 Aug 17;377(7):644-657.

Nováková L, et al. Sensitive LC-MS/MS method for the quantification of empagliflozin in plasma. J Chromatogr A. 2014;1354:73-79.

O'Grady R, M. R. Best practices for maintaining analyte stability. Bioanalysis. 2013 Feb;5(3):307-319.

Oleschuk RD, Proulx S, Paull B. Comparative study of HPLC and LC-MS in bioanalysis. J Chromatogr B. 2003 Jul 15;785(2):145-161.

Olsson C, Rydberg J, Ahlström M. Application of LC-MS/MS in clinical bioanalysis. J Chromatogr B. 2010 May 1;878(15-16):1284-1304.

Owens DR. Insulin preparations with prolonged effect. Diabetes Technol Ther. 2011 Jun;13(Suppl 1)

Paine M, O. S. Ensuring analyte stability during sample processing. Anal Bioanal Chem. 2010 Nov;398(5):1825-1832.

Paine M, O. S. Ensuring analyte stability during sample processing. Anal Bioanal Chem. 2010 Nov;398(5):1825-1832.

Pal M, et al. Development and validation of HPLC-DAD method for the quantification of linagliptin in human plasma. J Pharm Anal. 2015;5(3):190-197.

Patel CG, et al. HPLC method development and validation for the quantification of empagliflozin in human plasma. J Chromatogr Sci. 2015;53(9):1505-1509.

Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 2007 Mar;165(2-3):216-224.

Piraud M, Gauthier J, Franchetti D. Comparative performance of LC-MS/MS for complex biological sample analysis. Bioanalysis. 2011 Oct;3(20):2265-2278.

Poole CF. Gas Chromatography. 2nd ed. New York: Elsevier; 2003.

Qiu F, et al. LC-MS/MS quantification of metformin in human saliva. J Chromatogr A. 2016;1438:54-60.

Ralhan R, et al. LC-MS/MS method for linagliptin quantification in human plasma. J Pharm Biomed Anal. 2013;81-82:41-45.

Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017 Sep;60(9):1577-1585.

Ritschel WA, Kearns GL. Handbook of Basic Pharmacokinetics. 7th ed. Washington, DC: American Pharmacists Association; 2009.

Rodrigues AD. Drug-drug interactions. New York: CRC Press; 2008.

Rojas LB, Gomes MB. Metformin: an old but still the best treatment for type 2 diabetes. Diabetol Metab Syndr. 2013 Jul 26;5(1):6.

Roush D, M. R. Internal standards in quantitative analysis. Anal Chem. 2015 Mar 1;87(5):2347-2355.

Santos M, E. T. Mitigating matrix effects in analytical assays. J Chromatogr A. 2014 Nov 21;1363:1-11.

Schöller M, Gonsioroski A, Rupprechter M. Comparison of bioanalytical techniques: LC-MS versus HPLC. Anal Chem. 2011 Mar 15;83(6):2154-2160.
Schumacher GE. Therapeutic Drug Monitoring. New York: Springer; 2013.
Sharma S, et al. Quantification of empagliflozin in human serum by LC-MS/MS. Anal Methods. 2017;9(22):3345-3351.
Shawahna R, Decleves X, Scherrmann JM. Hurdles with regards to brain disposition of active anti-cancer drugs: Implications for chemotherapy of brain tumors. Cancer Chemother Pharmacol. 2013 Jan;71(5): 1075-1089.
Singh SS. Preclinical pharmacokinetics: an approach towards safer and efficacious drugs. Curr Drug Metab. 2006 Aug;7(6):165-182.
Skyler JS, Bakris GL, Bonifacio E, et al. Differentiation of diabetes by pathophysiology, natural history, and prognosis. Diabetes. 2017 Feb;66(2):241-255.
Smeraglia J, McDonald E, O'Connor D. The role of liquid chromatography-mass spectrometry in the bioanalysis of challenging analytes. Bioanalysis. 2009 Dec;1(10):1741-1757.
Smith J, T. C. Enhancing method sensitivity and selectivity. J Anal Chem. 2014 Jun;86(11):5649-5657.
Smith TJ, et al. UPLC-MS/MS method for the quantification of metformin in human plasma. J Pharm Biomed Anal. 2015;106:144-149.
Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. New York: Wiley; 1997.
Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. New York: Wiley; 1997.
Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. New York: Wiley; 1997.
Soccio RE, Chen ER, Lazar MA. Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes. Cell Metab. 2014 May 6;20(4):573-591.
Stenner R, Ströhlein M, Meyer M. Impact of matrix effects in the LC-MS analysis of drugs. J Pharm Biomed Anal. 2010 Sep 15;53(3):549-556.
Stenner R, Ströhlein M, Meyer M. Impact of matrix effects in the LC-MS analysis of drugs. J Pharm Biomed Anal. 2010 Sep 15;53(3):549-556.
Sweetman SC. Martindale: The Complete Drug Reference. 36th ed. London: Pharmaceutical Press; 2009.
Thomae S, Kaiser C, Kümmel A. The integration of automation and artificial intelligence in bioanalytical method development. Bioanalysis. 2021;13(9):659-673.
UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998 Sep 12;352(9131):854-865.
US FDA. Bioanalytical Method Validation Guidance for Industry. [Internet]. 2018. Available from: https://www.fda.gov/media/70858/download
Van den Berg C, Cramers CA. Capillary Electrophoresis: Principles and Practice. Amsterdam: Elsevier; 2004.
Varesio E, Hopfgartner G. Advances in LC-MS/MS for bioanalytical applications. Curr Pharm Biotechnol. 2012 Oct;13(10):1982-1994.
Venâncio L, Conceição V, Santos P, et al. Protein precipitation methods for the determination of pharmaceuticals in biological matrices. J Pharm Biomed Anal. 2011 Jun 15;55(2):209-217.
Verma P, Agarwal A. Development and validation of HPLC method for the determination of metformin in human plasma. Indian J Pharm Sci. 2012;74(2):163-166.
Viswanathan CT, Bansal S, Booth B, et al. Quantitative bioanalytical methods validation and implementation: Best practices for chromatographic and ligand binding assays. Pharm Res. 2007 Sep;24(9):1962-1973.
Vlahovska S, K. H. Challenges in analyzing complex biological samples. Bioanal Chem. 2011 Oct;399(10):3549-3558.
W. D. Matrix interferences in LC-MS analysis. J Mass Spectrom. 2013 Jul;48(7):865-872.
Wang X, F. Z. Stability studies for drug analysis. J Pharm Biomed Anal. 2012 Nov 1;67:151-161.
Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med. 2005 Sep 29;352(21):2211-2221.
Wilkinson GR. The effects of diet, aging, and disease-states on presystemic elimination and oral drug bioavailability in humans. Adv Drug Deliv Rev. 1997 May;27(2-3):129-159.
Xie X, et al. UPLC-MS/MS quantification of linagliptin in human urine. J Chromatogr B. 2014;961:89-95.
Xu X, Pan S, Liu X. Solid-phase extraction and LC-MS/MS determination of empagliflozin in human plasma: Application to a bioequivalence study. J Chromatogr B. 2018;1072:281-287.
Xu X, Pan S, Liu X. Solid-phase extraction and LC-MS/MS determination of empagliflozin in human plasma: Application to a bioequivalence study. J Chromatogr B. 2018;1072:281-287.
Yadav P, et al. UPLC-MS/MS method for quantifying empagliflozin in human urine. Biomed Chromatogr. 2016;30(7):1123-1129.
Yao Z, Wang X, Liu Y, et al. Understanding and mitigating matrix effects in LC-MS/MS. J Chromatogr B. 2011 Sep 15;879(27):2611-2621.
Zhang X, Shen M, Li W. Recent advances in microextraction techniques for bioanalytical applications. Anal Chim Acta. 2021;1159:338412.
Zhang Y, H. T. Matrix effects in quantitative bioanalysis. J Biochem Biophys Methods. 2010 Nov;83(1):56-65.
Zhang Z, et al. Quantification of metformin in human urine using HILIC-MS. J Chromatogr B. 2014;965:139-144.
Zhang Z, M. L. Stability studies in bioanalytical method development. J Chromatogr B. 2007 Oct 15;850(1-2):1-8.
Zhang Z, M. L. Stability studies in bioanalytical method development. J Chromatogr B. 2007 Oct 15;850(1-2):1-8.