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Review Article

A Review on Bioanalytical Method Development and Validation of Pioglitazone by RP-HPLC

Sandra Chandran*, Geetha P Nair

College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, Kerala, India 695011

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ABSTRACT

Bioanalytical method development is a process of designing and optimizing an analytical method for quantitative estimation of drug of interest in biological matrices like plasma, serum, urine, saliva etc. Pioglitazone is a thiazolidinedione antidiabetic drug, a selective agonist of Peroxisome Proliferator Activated Receptor gamma (PPAR-γ) used in the treatment of Type 2 diabetes mellitus. The determination of its concentration in biological samples is essential for pharmacokinetic studies, therapeutic drug monitoring, drug interaction assessment, bioequivalence evaluation and regulatory acceptance. This is because of its extensive therapeutic use and the growing interest in its pharmacokinetics and in achieving consistent therapeutic exposure in patients in the clinical setting. This review updates the recent advances and strategies in bioanalytical techniques for the analysis of pioglitazone with special emphasis on reverse phase high-performance liquid chromatography (RP-HPLC) as the method of choice. This review also highlights the rationale behind IS selection, including the utilization of stable isotope-labelled (SIL) compounds, structurally related drugs, which enhance the reliability and robustness of the method. Furthermore, this review addresses the significance of optimizing sample preparation techniques, such as protein precipitation (PP), liquid–liquid extraction (LLE), and solid-phase extraction (SPE), to improve analyte recovery and validate methods for reliability. This review also emphasizes emerging trends, including the integration of automation and artificial intelligence in method development, and identifies current challenges, such as matrix complexity and analyte stability. By addressing existing challenges and proposing best practices, this review contributes to the advancement of bioanalytical research in diabetes management, aiding the optimization of pharmacokinetic studies and therapeutic monitoring of Pioglitazone. Future research should focus on impurity profiling, stress degradation studies, and quantification of pioglitazone in human plasma. This review offers a comparative overview for researchers and practitioners aiming to improve bio analytical methodologies in the field of anti-diabetic drug analysis.

***Corresponding Author:** Sandra Chandran
Address: *College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, Kerala, India 695011*
Email : sandrachandran86@gmail.com

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INTRODUCTION

Diabetes mellitus, a chronic disease characterized by increased blood glucose levels, results from a deficiency in insulin production, insulin activity or both. It is a serious global health problem that affects millions of people worldwide. The International Diabetes Federation (IDF) estimates that there were 537 million adults aged 20–79 years with diabetes in 2021 and this is projected to increase to 783 million by 2045[1]. Long-term harm, particularly to the eyes, kidneys, nerves, heart, and blood vessels, is caused by persistently high blood sugar levels in diabetes. Type 1 diabetes (T1D) and type 2 diabetes (T2D) are the two main types of diabetes. The primary cause of T1D is the autoimmune death of pancreatic beta cells, which leads in a complete lack of insulin. Type 2 diabetes, the more common form, occurs due to a combination of inadequate insulin production and insulin resistance[2]. Microvascular problems such as diabetic retinopathy, nephropathy and neuropathy[20]. Macrovascular problems such as cardiovascular problems like coronary artery disease, cerebrovascular disease and peripheral artery disease[3].

Diabetes management requires a comprehensive approach, including medications, regular blood glucose monitoring, and lifestyle modifications. Antidiabetic medications are important for maintaining glycemic control and can prevent or delay complications of diabetes. These medications, which include insulin and several oral hypoglycemic agents (OHAs), are categorized based on how they work. OHAs are categorized as Biguanides (e.g., Metformin), Sulfonylureas (Glimepiride), Thiazolidinediones (Pioglitazone), Dipeptidyl Peptidase-4 (DPP-4) Inhibitors (Sitagliptin), Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists (Exenatide), Sodium-Glucose Co-Transporter-2 (SGLT2) Inhibitors (Empagliflozin), and Alpha-Glucosidase Inhibitors (Acarbose)[10,22,23].

Pioglitazone hydrochloride, a thiazolidinedione class antidiabetic drug used in the management of T2D acts as a selective agonist of peroxisome proliferator activated receptor-gamma (PPAR-γ), thereby enhancing insulin sensitivity in adipose, skeletal muscle and hepatic tissues[38]. Owing to its widespread use, extensive clinical interest exists in understanding its pharmacokinetics and ensuring consistent therapeutic exposure in patients. Its concentration is the major measured variable in plasma for pharmacokinetic studies, therapeutic drug monitoring, drug interaction studies and bioequivalence testing.

Bioanalytical method development is the process of developing and optimizing an analytical technique for the accurate and reproducible measurement of a drug and/or its metabolites in biological matrices such as plasma, serum, urine, or tissue. Bioanalytical methods are a series of techniques for collection, processing, storage and analysis of biological matrices containing chemical compounds[4]. Bioanalytical method validation (BMV) is done to prove that a quantitative analytical procedure is appropriate and reliable for biochemical use. Systematic validation experiments with satisfactory and repeatable results ensure the quality and reliability of the method. Validation of a bioanalytical assay also includes the stability of analytes in biological samples collected in clinical studies, and the stability of critical assay reagents such as analyte stock solutions.

Bioanalytical method validation is the process required to demonstrate that an analytical method is suitable for the quantitation of analytes in biological matrices such as blood, plasma, serum or urine and that the method is accurate, reliable and consistent. Validation is a comprehensive

laboratory procedure for establishing whether the performance characteristics of a method are appropriate for the planned analytical purpose.

The quantitative determination of medications and their metabolites in biological fluids, which supports the evaluation and interpretation of bioavailability, bioequivalency, pharmacokinetic, and toxicokinetic data, depends on the validation of bioanalytical methods. Completing regulatory reporting obligations is another reason why these investigations are crucial[28].

The quality of the data obtained is directly related to the reliability of bioanalytical investigations. It is therefore of utmost importance to establish and communicate clear validation criteria for analytical procedures in the pharmaceutical industry. RP-HPLC and LC-MS/MS are both widely used for plasma drug bioanalysis, each with their own set of advantages. Different chemicals are often evaluated using RP-HPLC with UV, PDA or fluorescence detection. These chromatographic procedures have advantages such as low detection limits, simple sample preparation requirements, possibility to obtain structural information and adaptation to analytes of different polarity[29]. Bioanalytical method validation is a series of steps to ensure an analytical method is appropriate for the quantitative determination of analytes. The main validation criteria were accuracy, selectivity, precision, linearity, recovery, robustness, stability and range. The primary objective of the validation was to demonstrate the analytical process was fit for the intended purpose. The ICH Q2 (R1) guideline is one of the most extensively used international standards for validating analytical methods in the medical and pharmaceutical sciences[39]. Regulatory bodies like the FDA and EMA have also released more thorough bioanalytical-specific guidelines under the titles "Guidance for Industry: Bioanalytical Method Validation" and "Guidelines on Bioanalytical Method Validation"[40,41]. Parameters including matrix effects, carryover, and dilution integrity have been highlighted in recent advancements in validation procedures.

Another crucial component of bioanalytical research is the evaluation of analyte stability under various experimental settings. The preparation of investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs), and related supplements incorporating clinical pharmacology, bioavailability, bioequivalency, and pharmacokinetic studies is aided by these regulatory recommendations.

Selection and Optimisation of Internal Standard

Internal standardization compensates for differences in matrix effects, extraction efficiency, and instrument responsiveness by adding a structurally comparable molecule at a predetermined concentration to every sample[24,25]. To avoid detection issues in bioanalytical testing, ISs must be kept away from untreated samples. For easy identification and detector compatibility, the chosen ISs should have a steady and distinctive structure. To evaluate the quality of separation, they should ideally elute after the analyte[5]. To guarantee comparable extraction efficiency, retention time, stability, and detection responses, the IS and analyte should share structural similarities. Additionally, by decreasing the need for reanalysis and correcting both systematic and random fluctuations, ISs aid in the reduction of measurement errors. They also assist in maintaining peak shape, identifying analytical difficulties, and guaranteeing precise retention time measurements[26,27].

Pioglitazone is often measured using high-performance liquid chromatography (HPLC) because of its accuracy, reliability, and compatibility with various biological matrices[21]. Despite these advantages, aberrations including matrix interference, inconsistent sample preparation, and detector variability can impact HPLC experiments. In order to solve these problems, internal standards (ISs) are essential. Stable isotope-labelled ISs or structurally related compounds serve as surrogates that closely mimic the retention, extraction, and analyte detection properties. Early IS introduction during protein precipitation, liquid-liquid extraction, deproteinization, or solid phase extraction improves recovery and lowers variability. Complex treatments are further stabilized and signal suppression is reduced by advanced processes, such as automated ASTED dialysis combined with SPE. By correcting response variations and increasing signal-to-noise ratios, ISs also enhance the performance of detection systems, such as UV, PDA, fluorescence, and MS. In complicated matrices like plasma, urine, and tissue, where endogenous chemicals may obstruct drug quantification, their significance is especially clear. Linearity and repeatability are strengthened by careful IS selection, which guarantees constant retention and peak area ratios.

This section focuses on HPLC methods for the analysis of pioglitazone and emphasizes how ISs enhance assay performance. The selection criteria for ISs, their impact on chromatographic separation, and the most effective ways to integrate ISs across detection modes are all covered. It also outlines how optimized IS strategies improve the sensitivity, accuracy, and stability of calibration curve.

The most suitable internal standard for RP-HPLC bioanalysis of pioglitazone was rosiglitazone. It is frequently used because it is in the same thiazolidinedione class and thus has similar extraction, polarity, and chromatographic properties. The similarity in structure largely eliminates the variability due to the preparation of the sample and the instrumental analysis. Nonetheless, a number of structurally unrelated substances have also been used because they offer reliable recovery, suitable retention behavior, and sufficient UV absorbance while remaining well separated from the analyte peak. Availability, chromatographic compatibility, and the lack of endogenous plasma component interference are the criteria used to choose them. The HPLC methods, including sample preparation methods, chromatographic conditions, LLOQ, and IS applications across biological matrices, are compiled in Table:1.

Table:1 Internal standards reported for RP-HPLC based bioanalysis of Pioglitazone Hydrochloride

IS	Chromatographic conditions	Detection wavelength (nm)	Sample preparation	Rt of pioglitazone (min)	Rt of IS (min)	LLOQ (ng)	Ref
Glimepiride	0.1% OPA: ACN (60:40)	220	PPE with ACN	3.553	2.208	80	11
Ethyl paraben	ACN:140Mm K2HPO4 (40:60)	269	LLE with diethyl ether	4.2	6.2	25	17
Piroxicam	ACN: 0.1M Ammonium acetate (41:59)	269	LLE with Ethyl acetate	7.418	9.944	55	16
Rosiglitazone	MeOH: ACN: Mixed phosphate	269	SPE	4.1	8.2	50	12

	buffer (pH 2.6,10mM) (40:12:48)						
Rosiglitazone	MeOH:30mM Ammonium acetate (60:40)	269	PPE with Ethyl acetate	7.07	6.1	50	18
Lansoprazole	Mixture of 0.01M Na2HPO4 and NaH2PO4: MeOH: ACN (55:30:15)	228	LLE with tert butyl methyl ether	4.6	6.1	50	19

METHOD DEVELOPMENT

The establishment of an analytical method requires the development of a process that allows the identification and quantification of the target molecule in a sample matrix. Since a compound can be analysed by a number of techniques, the technique best suited to a particular application has to be selected according to a number of parameters such as chemical properties of the analyte, range of concentration, nature of the sample matrix, cost and speed of analysis, qualitative or quantitative measurement, required precision, and equipment available. The analytical chain is the process of developing a technique. It involves sampling, sample preparation, separation, detection and interpretation of the result.

Usually reversed phase C18 columns (e.g. Hypersil ODS, Inertsil C18, Apollo C18) or C8 columns (eg: Nova-Pak C8) and mobile phases combining phosphate or acetate buffers with MeOH or ACN or water pH of which was adjusted using glacial acetic acid, perchloric acid based on the pKa of the drug of choice[37]. Flow rates were between 1.0 and 1.5 ml min−1. Detection depends on the required sensitivity. UV and PDA detectors are commonly used (220-269nm).

HPLC-UV techniques combined with protein precipitation or liquid-liquid extraction are dependable and useful methods that provide adequate sensitivity, precision and extraction recovery for high dose levels and regular treatment monitoring. For more difficult bioanalytical applications, especially those involving low analyte concentration or complex biological matrices, solid phase extraction in conjunction with automated HPLC systems or mass spectrometric detection offers improved analytical performance. Despite these benefits, HPLC is not widely used in tissue level studies and advanced metabolite characterization. There is also a growing need for more integrated imaging based approaches with chromatographic procedures and standardized, high-throughput analytical workflows. Increasing the use of HPLC in antidiabetic medication bioanalysis would necessitate developing multianalyte analytical platforms, optimising matrix specific extraction techniques etc., Optimizing matrix-specific extraction techniques, creating multi-analyte analytical platforms, and integrating pediatric-focused approaches are all necessary to increase the importance of HPLC in antidiabetic medication bioanalysis. Continued advancements in sample preparation strategies and internal standard design are essential for preserving the clinical and regulatory significance of HPLC in the rapidly evolving field of diabetes pharmacotherapy.

Sample collection

Blood, plasma, urine, and serum are common biological samples utilized for analyte assays.

Depending on the test sensitivity and study needs, blood is often drawn from human volunteers via venipuncture using a hypodermic syringe in amounts of roughly 5–7 ml. Tubes containing anticoagulants, like heparin or EDTA, are filled with the blood. After that, plasma was separated using centrifugation at 4000 rpm for 15 minutes, yielding between 30 and 50 percent of the initial sample volume[6].

Sample preparation

The analytical procedure includes an important step, sample preparation. This includes the techniques used for the isolation and concentration of analytes from complex biological matrices such as blood, serum, and tissues. Common interfering chemicals present in biological samples may complicate the detection and quantification of the target analytes. Hence, suitable sample preparation methods are needed to remove contaminants, extract analytes, and enhance detectability to achieve reliable and consistent results[7,8]. The demand for efficient, environmentally friendly sample preparation techniques is rising. The extraction methods using solvents such as protein precipitation extraction (PPE), solid-liquid extraction (SLE) and liquid-liquid extraction (LLE) were very advantageous for the bioanalytical applications of pioglitazone.. These techniques have reduced the cost of drug development and increased interest in pharmaceutical production[9]. Automation can lead to higher sample throughput, which improves accuracy and generates less hazardous waste. The main goal of sample preparation before analysis is to concentrate and purify the analyte[30]. This reduces the likelihood of chemicals interfering with the analysis, the chromatographic column or the detector. The fastest and cheapest method for pioglitazone analysis is protein precipitation, but in terms of sample purification and analytical performance, liquid-liquid extraction and solid phase extraction are better. Due to the high protein binding (>99%) of pioglitazone, simple protein precipitation using acetonitrile (ACN) or methanol (MeOH) resulted in a strong matrix effect as compared to liquid-liquid extraction (LLE) and solid-phase extraction (SPE). However, due to these problems protein precipitation extraction (PPE) still remains the method of choice for routine analysis and for pioglitazone samples at high concentrations. It is low cost, easy to operate and requires minimum sample preparation. Due to its lipophilic nature, liquid-liquid extraction is also often used to efficiently isolate pioglitazone from the aqueous plasma phase into organic solvents. This method yields better chromatograms because it separates pioglitazone and several polar components of the matrix remain in the aqueous phase. Another consequence is the decrease of limits of detection (LOD) and quantification (LOQ), crucial for pharmacokinetic and bioequivalence evaluations. If lower levels of quantification and regulatory investigations are needed then SPE is the method of choice. SPE is a method that efficiently separates medicines from plasma components using a cartridge. Table :2 lists the various sample preparation strategies employed for the bioanalysis of pioglitazone using RP-HPLC.

Table no:2 Sample reparation strategies employed for bioanalytical estimation of Pioglitazone Hydrochloride – A Comparative overview

Sample preparation method	Principle	Common solvents/ Reagents used	Merits	Demerits	Recovery	Matrix cleanliness	Sensitivity	Suitability for pioglitazone
Protein precipitation	Involves the addition of a precipitating	Acetonitrile Methanol	Simple, Rapid, Low cost,	Low cleanliness,	Moderate to high	Moderate	Moderate to high	Most commonly used for

	agent that causes proteins to aggregate and separate from the solution.	Acidified Methanol Perchloric acid Trichloro-acetic acid	Minimal sample handling	occurance of matrix interference				HPLC and LC-MS/MS Due to its simplicity and high throughput
Liquid-Liquid Extraction	Partitioning of drug between aqueous plasma and immiscible organic solvent	Ethyl acetate, Methyl tert-butyl ether, Dichloro-methane, Hexane mixtures	Cleaner extracts, better sensitivity, Reduced matrix effects	Time consuming, solvent intensive, Emulsion formation	High	Low	High	Suitable for sensitive quantitative analysis and pharmaco-kinetic studies
Solid phase Extraction	Selective adsorption of analyte on sorbent followed by elution of	C18 Catridge, HLB Catridge, Methanol, Water, Buffer	More cleaner extract than LLE, automation friendly	High cost	Very high	Very low	Very high	Preferred for highly sensitive and regulatory bio-analytical studies

BIOANALYTICAL METHOD VALIDATION (BMV)

The validation of a bioanalytical procedure aims to demonstrate the performance characteristics and reliability of the method. This helps establish confidence in the results. As Shah et al have mentioned, all bioanalytical methods must be validated if the results are to be used to support the registration of a new drug or the reformulation of an existing drug. Remember that the first validation is just the beginning. When a method is in use, it is necessary to monitor it to verify

that it is functioning as originally validated[8,31]. Validation is the demonstration of the suitability and robustness of the performance characteristics of a method for the intended analytical applications, by means of documentation supported by specific laboratory experiments.

Need for bioanalytical method validation

Bioanalytical method validation is conducted to demonstrate that an analytical method is reliable, accurate, precise and fit for the intended purpose for the quantification of pharmaceuticals and their metabolites in biological matrices such as plasma, serum, urine or tissues.

- To obtain dependable and interpretable results, it is vital to employ bioanalytical methods that are thoroughly characterized and fully validated.
- Bioanalytical techniques are constantly evolving and improving, representing the forefront of technological advancement.
- Each approach has unique characteristics and each is dependent on the analyte. Each molecule may require a different set of validation criteria.
- The applicability of the method also depends on the overall goal of the study. When the analyses are carried out in different sites the validation must be carried out in each site with proper documentation to assure consistency and inter laboratory dependability[32].

Various important parameters that need to be validated includes selectivity, accuracy, precision, linearity and range, limit of detection, limit of quantification, recovery, robustness and stability.

Table: 3 Validation parameters[10]

Parameter	Description	Method	Justification
Specificity and Selectivity	Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present whereas Selectivity is the capacity to distinguish and measure analytes when other elements are present[33].	Chromatographic analysis of IS-spiked plasma, blank plasma, and LLOQ sample	Verifies that the analyte or IS do not overlap with any interference peaks from the blank plasma matrix.
Linearity and Range	Shows how the nominal analyte concentration and the analytical platform's reaction to the analyte are related.	Spike blank plasma using IS and calibration standards. Determine the peak area ratio by extracting from the plasma and recording the peak areas of both.	Guarantees accurate analyte measurement across a broad range of concentrations
Accuracy and Precision	Precision is the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions, whereas accuracy is the degree of closeness of the observed value to the true value[34,35].	Four levels are usually tested: LLOQ, LQC, MQC, and HQC. Assess interday precision and accuracy over a minimum of three distinct runs.	Guarantees the technique yields accurate and reliable results
Sensitivity	The technique's capacity to identify low analyte concentrations.	Utilizing statistical techniques, ascertain the quantification limits (LOQ) and detection limits (LOD).	Vital for accurately quantifying analytes at low concentrations..
Stability	Analyte's capacity to stay unaltered under particular circumstances.	Short-term stability: Analyte stability should be evaluated for brief intervals at room temperature. Long-term stability: Assess stability of analyte in matrix during prolonged durations of storage which should equal or exceed the time period between the date of first sample collection and date of last sample analysis[36]. Freeze-thaw stability: After several freeze-thaw cycles, assess stability. Auto sampler stability: Examine stability when t the autosampler is in use.	Makes ensuring analytes don't deteriorate during storage or analysis, which is essential for preserving data integrity.

The comparison of the published RP-HPLC methods for quantification of Pioglitazone in various biological samples shows that the validated methods fulfill the regulatory standards of bioanalytical method validation. Most of the methods exhibited good linearity (correlation coefficient > 0.99) over the individual calibration ranges indicating the accurate quantification of the analyte over wide range of concentration. The accuracy was within acceptable limits of nominal concentrations and the intra-day and inter-day precision values are typically < 15 indicating good reproducibility and reliability. The main variations in the reported procedures were in the sample preparation methods and detection systems. The stability experiments consistently demonstrated the stability of Pioglitazone in different biological samples under various bench top, freeze thaw, autosampler and long term storage conditions, suggesting the suitability of the methodologies for pharmacokinetic and bioequivalence studies. Overall, most of the reported RP-HPLC methods employed for the bioanalysis of Pioglitazone showed comparable validation performance, with variations mainly arising from extraction procedures, chromatographic conditions and analytical sensitivity rather than from fundamental differences in method reliability. Table: 4 depicts the summary of validation parameters reported for RP-HPLC based bioanalysis of Pioglitazone.

Table:4 Comparative summary of validation parameters of reported RP-HPLC methods for the bioanalysis of Pioglitazone in different biological samples

Sample preparation	Biological sample	LLOQ (ng/ml)	Accuracy (% recovery)	Precision (%CV)	Calibration range (ng/ml)	Stability	Ref
PPE	Human plasma	80	99.15-101.14	0.19-2.2	80-3200	Performed stability at bench top, freeze thaw, -28ºC, -80ºC At all conditions, %CV, % mean recovery were within the limits at LQC and HQC	11
SPE	Human plasma	50	90.3-97.6	0.9-8.7	50-2000	Deviation of mean test response at LQC and HQC were within limits of appropriate controls in all stability tests of Pioglitazone in human plasma.	12
LLE	Human plasma	50	79.28-82.10	1.62-2.56	50-2000	---	13
LLE	Mice plasma/ tumor/ liver tissues	50 (plasma) 100 (tumor and liver)	99.30-105.21 (tumor) 98.95-113.82 (liver)	2.2-5.1	50-2500 (plasma) 100-500 (tumor)	----	15
LLE	Human plasma	25	77.4-83.2	2.34-6.78	25-1500	Plasma samples of Pioglitazone was found to be stable in freeze thaw test and	17

						also when tested after 2 months	
PPE	Rat serum	50	96.5-98.1	0.75-4.76	0.1-10000	---	18
LLE	Human plasma	50	90-100	2.5-5.8	50-5000	Performed on processed and unprocessed samples at various conditions and also subjected to 3 freeze thaw cycles. The plasma samples of Pioglitazone were found to be stable under the given conditions.	19
PPE	Human plasma	44.2	96.29-101.59	0.27-4.51	50-2000	Stability test performed at 3 QC levels by storing the samples in different conditions including acidic condition. The results obtained were well within the limits of nominal concentration of sample in human plasma	14
LLE	Human plasma	55	78.34-80.59	1.53-2.45	55-2000	No significant degradation of Pioglitazone was observed in plasma under the studied concentration at different conditions indicating that plasma samples containing Pioglitazone can be handled under normal laboratory conditions without significant loss of compound.	16

CHALLENGES ENCOUNTERED IN BIOANALYTICAL METHOD DEVELOPMENT

For an analytical chemist, developing bioanalytical methods is a difficult endeavor since it involves not only the chemistry of the medication but also a delicate balancing act between biological complexity, technical sophistication, and precision requiring extreme discipline. In addition to being time-sensitive, matrix-dependent, and involving an effective extraction procedure, the approach must adhere to regulatory requirements.

Various challenges in the development of a bioanalytical method for Pioglitazone include;

- **Complexity of Biological matrices**

Biological samples such as plasma, serum and urine contain proteins, lipids and other endogenous components which can affect the concentration of analytes. This complexness makes the sample preparation more difficult and requires advanced techniques for the precise isolation and quantification of the target molecule. Pioglitazone is bound with plasma proteins (> 99%), thus expensive and time-consuming sample preparation techniques such as SPE and LLE techniques are required to minimize the interference and increase the analyte recovery. Also, during method development, it is important to carefully evaluate matrix effects to provide reliable results to meet regulatory standards for drug development and approval.

- **Interference from Endogenous Compounds**

Interference in bioanalytical experiments and wrong results may be caused by naturally occurring substances in biological matrices that co-elute with the analyte or affect the instrument performance. However pioglitazone is a highly protein-bound drug and this interference may be detrimental to routine analysis. Variability is corrected and accurate quantification is ensured using internal standards and matrix matched calibration. Testing a variety of biological samples during the development and validation steps of the technique assists in identifying and controlling these interferences.

- **Analyte stability issues**

Analyte stability in biological matrices is of utmost importance in the development of bioanalytical methods, as analytes can be altered or degraded due to temperature, pH or light exposure. This instability can produce different results. Extensive stability studies including short term, long term, freeze thaw and autosampler testing are required to confirm analyte stability under different conditions. Proper handling and storage procedures further ensure analyte integrity.

- **Method Sensitivity and Selectivity**

High sensitivity and strong selectivity are needed to detect trace analytes in complex matrix. Sensitivity ensures that the analyte can be detected at very low concentrations, and selectivity allows the analyte to be distinguished from other components of the matrix. The sensitivity can be improved by optimization of the analytical conditions and the use of sophisticated instruments such as tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS). Chromatographic separation and the development of robust methods for separating analytes from interferents can improve selectivity. The continuous validation is a way to make sure that these parameters are up to the required standards.

FUTURE PERSPECTIVES

Advancements in Bioanalytical Techniques

Recent innovations in bioanalytical techniques have greatly enhanced the accuracy and effectiveness of drug analysis. Liquid chromatography–tandem mass spectrometry (LC MS/MS) and ultra performance liquid chromatography–mass spectrometry (UPLC-MS) are widely used for identification of drugs in biological samples because of their excellent specificity and sensitivity. High resolution mass spectrometry (HRMS) leads to further improvement of the sensitivity for detection of drug and metabolite traces. Developments in microextraction methods, for example micro-dialysis and microextraction by packed sorbent (MEPS) have simplified sample preparation,

reducing the required amounts and restricting matrix effects.

Emerging Trends in Anti-Diabetic Drug Analysis

Current research is directed toward therapies that target multiple mechanisms of glucose control. In order to deal with combination therapy requiring simultaneous detection of multiple drugs and metabolites, multiplexed assays and multianalyte LC MS/MS techniques are applied. Also the non-invasive and practical nature of Dried Blood Spot (DBS) sampling has received attention, especially with regard to elderly and pediatric patients.

Automation and Artificial Intelligence

Automation and artificial intelligence are changing the development of bioanalytical methods by improving productivity and repeatability. Automation for preparing samples makes the process easier and the use of intelligent software allows for the analysis of large data sets to optimize the parameters of the technique. The use of machine learning (ML) models to predict drug behaviour in biological matrices is on the rise, thus facilitating the development of reliable bioanalytical techniques.

CONCLUSION

This review explored various strategies and considerations in the bio-analytical method development and validation for Pioglitazone in biological sample with RP-HPLC as the preferred analytical tool. The review began by discussing the importance of bioanalytical methods in drug discovery, development and its marketing approval, highlighting their role in ensuring accurate and reliable drug quantification in biological matrices. It covered the importance of optimization of internal standards in bioanalysis, various sample preparation techniques and their role in improving the reliability and robustness of the method, matrix interference, regulatory requirements. The review concluded with the detailing of the various challenges encountered in bioanalysis, emerging trends and expanding role of automation and artificial intelligence in bioanalytical method development.

REFERENCES

1. Aerts J, Huitema AD, Schellens JH. A comparative study of LC-MS/MS and LC-UV for pharmacokinetic analysis. Journal of Pharmaceutical Biomedical Analysis. 2008 Dec 1;48(3):923-930.
2. Alaa M, Abdel-Fattah T, Khaled SM. LC-MS/MS determination of metformin in human plasma and its application to a pharmacokinetic study. Biomed Chromatography. 2019;33(9).
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014 Jan;37(Suppl 1).
4. Kirthi A, Shanmugam R, Shanti Prathyusha M, Jamal Basha D. A review on bioanalytical method development and validation by RP-HPLC. Journal of Global Trends in Pharmaceutical Sciences.2014;5(4):2265-71.
5. Jadhav S, Auti S, Sharma S. Optimizing Internal Standard Selection for Precise Bioanalytical Quantification Of Antidiabetic Drugs: A Critical Review. Chromatographia.2025 Sep;88(9):637-57.
6. European Medicines Agency Guidelines on Bioanalytical Method Validation: Committee for Medicinal Products for Human Use.
7. Cho SJ, Vinter S, Garofolo F. Crucial importance of evaluating internal standards (IS) response and troubleshooting in effective LCMS method development, validation and sample analysis. Bioanalysis. 2019 Sep 1;11(18):1645–7.
8. Goodwin JM, Kuiper HC, Brister B, Vesper HW. Impact of internal standard selection on measurement results for long chain fatty acids in blood. Journal of Mass Spectrometry and

Advances in the Clinical Lab.2024 Aug1; 33:22-30.
9. Fachi MM, Leonart LP, Degaut Pontes FL, Oliveira Vilhena RD, Cerqueria LB, Pontarolo R. Bioanalytical methods for the detection of antidiabetic drugs: a review. Bioanalysis. 2017 Dec 1; 9(24):2015–25.
10. Rath AK, Kemisetti D, Mishra SR. Emerging Trends and Challenges In Bioanalytical Method Development For Antidiabetic drugs. African Journal of Biomedical Research.2024:644-56.
11. Hephzibah K, Shanmugasundaram S. Validated Stability-indicating RP-HPLC Method for Simultaneous Estimation of Candesartan and Pioglitazone in Human Plasma. International Journal of Health Sciences.;6(S6):10226-39.
12. Sripalakit P, Neamhom P, Saraphanchotiwitthaya A. High-performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study. Journal of chromatography B. 2006 Nov 7;843(2):164-9.
13. Saha SK, Chowdhury AA, Bachar SC. Pharmacokinetics study of pioglitazone (30 mg) tablets in healthy volunteers. Dhaka University Journal of Pharmaceutical Sciences. 2014;13(2):181-6.
14. Islambulchilar Z, Valizadeh H, Zakeri-Milani P. Rapid HPLC determination of pioglitazone in human plasma by protein precipitation and its application to pharmacokinetic studies. Journal of AOAC International. 2010 May 1;93(3):876-81.
15. Morosi L, Matteo C, Meroni M, Ceruti T, Nerini IF, Bello E, Frapolli R, D'Incalci M, Zucchetti M, Davoli E. Quantitative measurement of pioglitazone in neoplastic and normal tissues by AP-MALDI mass spectrometry imaging. Talanta. 2022 Jan 15;237:122918.
16. Alim M, Nawaz R, Shahid M, Asi MR, Iqbal T, Anwar F. HPLC method for the determination of pioglitazone in human plasma and its application to pharmacokinetic study. Asian Journal of Chemistry. 2010 Jan 1;22(1):213.
17. Souri E, Jalalizadeh H, Saremi S. Development and validation of a simple and rapid HPLC method for determination of pioglitazone in human plasma and its application to a pharmacokinetic study. Journal of chromatographic science. 2008 Oct 1;46(9):809-12.
18. Ravikanth CH, Kumar AA, Kiran VU, Prashanth S, Madhu B, Reddy Y. Sensitive and rapid HPLC method for the determination of pioglitazone in rat serum. Int. J. Pharm. Sci. Drug Res. 2011;3(1):38-41.
19. Hussein RF, Hammami MM. Determination of pioglitazone level and its stability in human plasma by validated HPLC assay. World J. Pharm. Pharm. Sci.. 2015 Jul 8;4(9):11-22.
20. Types of diabetes Diabetes UK. https://www.diabetes.org.uk/diabetes-the-basics/types-of-diabetes. Accessed 3 Apr 2024.
21. Gedawy A, Al-Salami H, Dass CR (2019) Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. J Food Drug Anal 27:315–322.
22. Dowarah J, Singh VP. Anti-diabetic drugs recent approaches and advancements. Bioorganic & medicinal chemistry. 2020 Mar 1;28(5):115263.
23. Tripathi KD (2019) Pharmacological classification of drugs with doses and preparations. Jaypee Brothers Medical Publishers.
24. Siddiqui MR, Al Othman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arabian Journal of chemistry. 2017 Feb 1;10:S1409-21.
25. Panuwet P, Hunter Jr RE, D'Souza PE, Chen X, Radford SA, Cohen JR, Marder ME, Kartavenka K, Ryan PB, Barr DB. Biological matrix effects in quantitative tandem mass spectrometry-based analytical methods: advancing biomonitoring. Critical reviews in analytical chemistry. 2016 Mar 3;46(2):93-105.

26. Kauna-Czaplińska J. Current applications of gas chromatography/mass spectrometry in the study of organic acids in urine. Critical Reviews in Analytical Chemistry. 2011 Apr 29;41(2):114-23.
27. Cho SJ, Vinter S, Garofolo F. Crucial importance of evaluating internal standards (IS) response and troubleshooting in effective LCMS method development, validation and sample analysis. Bioanalysis. 2019 Sep 1;11(18):1645-7.
28. Ludwing H. Validaton of Analytical Methods. Agilnet Tech. 2010;1:65.
29. Kalakuntla RR, Kumar KS. Bioanalytical method validation: A quality assurance auditor view point. Journal of Pharmaceutical Sciences and Research. 2009 Sep 1;1(3):1.
30. Rosing H, Man WY, Doyle E, Bult A, Beijnen JH. Bioanalytical liquid chromatographic method validation. A review of current practices and procedures.
31. Shah VP. The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. The AAPS Journal. 2007 Feb 16;9(1):E43.
32. Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. Pharmaceutical methods. 2010 Oct 1;1(1):25-38.
33. Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. Clinical Cancer Research. 2008 Oct 1;14(19):5967-76.
34. Causon R. Validation of chromatographic methods in biomedical analysis viewpoint and discussion. Journal of Chromatography B: Biomedical Sciences and Applications. 1997 Feb 7;689(1):175-80.
35. Rozet E, Marini RD, Ziemons E, Boulanger B, Hubert P. Advances in validation, risk and uncertainty assessment of bioanalytical methods. Journal of pharmaceutical and biomedical analysis. 2011 Jun 25;55(4):848-58.
36. Sekar V, Jayaseelan S, Subash N, Udhaya KE, Perumal P, Venkatesh RP. Bioanalytical method development and validation of letrozole by RP-HPLC method. Int J Periodont Restorat Dent. 2009;1:1-7.
37. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. John Wiley & Sons; 2012 Dec 3.
38. Wisher D. Martindale: the complete drug reference. Journal of the Medical Library Association. 2012 Jan 1;100(1):75-7.
39. Lang JR, Bolton S. A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry—1. Experimental considerations. Journal of pharmaceutical and biomedical analysis. 1991 Jan 1;9(5):357-61.
40. Blume H, Brendel E, Brudny-Klöppel M, Grebe S, Lausecker B, Rohde G, Siethoff C. Workshop/conference report on EMA draft guideline on validation of bioanalytical methods. European Journal of Pharmaceutical Sciences. 2011 Feb 14;42(3):300-5.
41. U.S Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research : bioanalytical method validation.

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