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Review Article

Analytical Techniques in Pharmaceutical Analysis

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ABSTRACT

Pharmaceutical analysis is essential for ensuring the safety, effectiveness, and quality of pharmaceutical products. As pharmaceutical research and development continue to evolve, the need for advanced analytical techniques has significantly increased. This abstract outlines the primary advanced analytical methods utilized in pharmaceutical analysis, emphasizing their importance in overcoming the challenges associated with contemporary drug formulations. These advanced techniques include a variety of methodologies that facilitate the accurate quantification and characterization of active pharmaceutical ingredients (APIs), excipients, impurities, and degradation products. High-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS) have been fundamental to pharmaceutical analysis for many years. The incorporation of these sophisticated analytical techniques into pharmaceutical research and quality control has resulted in enhanced accuracy, efficiency, and adherence to regulatory requirements.

INTRODUCTION

The advancement of analytical instruments has enabled the direct detection of intriguing analytes. Nonetheless, detecting and quantifying low-abundance analytes remains a significant challenge due to limitations in instrumental detection and the complexities of sample matrices. Biological samples, in particular, present considerable matrix interference. As a result, it is essential to isolate, separate, and purify raw

samples before analyzing trace biological targets. While electrophoresis is cost-effective and straightforward, it often requires a considerable amount of time and exhibits poor repeatability. Ultrafiltration offers high separation efficiency but does not yield dry powder of target analytes, and its membranes may adsorb biological macromolecules. Among various techniques, solid-phase extraction (SPE) stands out as a highly effective method due to its excellent selectivity

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and recovery rates. Traditional SPE methods, which involve packing adsorbents into columns, have been successfully utilized; however, they are not ideal for samples containing suspended solids or fouling components. The batch separation technique, where adsorbents are directly incubated with samples, addresses these issues. Recent innovations have introduced new materials, such as nanomaterials and mesoporous materials, in this approach. However, using these materials as affinity adsorbents for enriching biological target analytes often leads to nonreversible adsorption and necessitates high-speed centrifugation, which can result in sample loss and the co-precipitation of unwanted interferences, despite their effectiveness in removing salts and other contaminants. Consequently, the use of these advanced materials as adsorbents is significantly limited. Therefore, there is an urgent need for a rapid, convenient, gentle, and efficient sample preparation method for biological analysis. Magnetic materials have proven to be quite effective when it comes to magnetic separation techniques. This method operates on a batch scale and relies on specially designed magnetic materials. These materials are particularly good at attracting biological macromolecules because they have a large surface area, are biocompatible, can be easily modified, and are simple to handle. In a typical magnetic separation process, these magnetic materials, which are attracted to the target compounds, are mixed with a sample. During a set incubation time, the target compounds attach to the magnetic particles. Afterward, an external magnetic field is used to pull the entire magnetic complex away from the sample. Once contaminants are washed away, the target compounds can be released for further use. Compared to traditional separation methods in biosciences, magnetic separation techniques offer several benefits. The separation of enantiomers is becoming increasingly important in industries like

pharmaceuticals and food, as well as in various bioanalytical fields such as environmental and clinical analysis. Super- or subcritical fluid chromatography (SFC) is well-regarded for its ability to separate enantiomers. Its unique approach, which differs from common techniques like reversed phase liquid chromatography (RPLC) or hydrophilic interaction liquid chromatography (HILIC), along with the development of more advanced instruments, makes SFC a preferred choice for analyzing enantiopurity. Enantiomer separations are not only important for analysis but also for preparative-scale processes in the pharmaceutical industry, and their significance is growing in various bioanalytical fields. For developing methods, it's essential to have general screening and optimization strategies that allow for quick chiral separations using modern supercritical fluid chromatography. Bioanalysis refers to the process of determining drugs and related substances in biological samples like whole blood, plasma, serum, tissues, and cells. This term emerged in the 1970s, linked to techniques aimed at studying how drugs behave in the body. To boost productivity and meet the rising demand for environmentally friendly chemistry in analytical methods, the liquid-phase microextraction (LPME) technique was introduced in the mid-to-late 1990s. This method is a refined version of liquid-liquid extraction (LLE) that uses tiny amounts of solvents for extraction. LPME addresses several issues commonly faced with solid-phase microextraction (SPME), such as syringe bending, fiber coating material leaching, and fiber fragility. Microextraction techniques have been well-received, leading to various adaptations like singledrop microextraction (SDME), continuous-flow microextraction (CFME), and hollow-fiber liquid-phase microextraction (HF-LPME). More information about these techniques can be found in other sources. The drugs available on the market



can come in various dosage forms, and their formulations can be classified based on how they are administered.

Analytical techniques

Titrimetric techniques

The roots of titrimetric analysis can be traced back to the mid-18th century. In 1835, Gay-Lussac introduced the volumetric technique, which ultimately gave rise to the term "titration." Although this analytical approach has a rich history, it has undergone significant evolution and modernization. This evolution includes the incorporation of non-aqueous titration methods, an expansion of its use to very weak acids and bases, and improvements in precision through potentiometric endpoint detection. Furthermore, with the advent of functional group analysis techniques, titrimetric methods have demonstrated their utility in measuring reaction kinetics, thereby facilitating the determination of reaction rates. These methods offer several advantages, including time and labor efficiency, high precision, and the elimination of the need for reference standards. Historically, titrimetric methods have been utilized to quantify substances such as captopril, albendazole, and gabapentin in commercial pharmaceutical formulations, while sparfloxacin was analyzed using non-aqueous titration. In addition to drug quantification, titrimetry has also been employed in the past to evaluate degradation products of pharmaceutical compounds.

Chromatographic techniques

1] Thin layer chromatography

Thin-layer chromatography (TLC), despite being an established technique, remains crucial in the field of pharmaceutical analysis. This method involves applying a solid adsorbent in a thin layer on a solid support, which is usually made of glass, plastic, or aluminum. The effectiveness of TLC as a chromatographic separation technique is influenced by several factors. Primarily, the adsorbent must possess a high selectivity for the

target substances to ensure notable differences in their elution rates. TLC is extensively utilized for the analysis of a wide variety of organic and inorganic compounds, owing to its numerous advantages. These benefits include minimal sample preparation, the ability to select from different mobile phases, versatility in sample differentiation, a high capacity for sample loading, and cost efficiency. In the pharmaceutical sector, TLC is an effective method for screening unknown substances in bulk drugs, providing a high degree of confidence that all potential drug components are adequately separated. The specificity of TLC has also been effectively applied in quantitative analysis through spot elution followed by spectrophotometric measurement. This technique has been employed to analyze various compounds, including steroids, pioglitazone, and nescapine (Ashour et al., 2009). During the initial phases of drug development, when knowledge about impurities and degradation products in both the drug substance and drug product is scarce, TLC is particularly valuable.

2] High-Performance Thin-Layer Chromatography (HPTLC)

High-performance thin-layer chromatography (HPTLC) has become an important method for drug analysis thanks to its advancements. This technique allows for quick separation and can be used to analyze a variety of sample types. It has many benefits, such as being easy to use and having shorter analysis times, which makes it ideal for working with complex or unrefined samples.

3] High-performance liquid chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is a sophisticated technique that helps separate complicated mixtures of molecules, making it easier to identify each individual part. This method was first used for testing bulk drug materials back in 1980 and has since become widely accepted in the United States



Pharmacopoeia (USP) and, to a lesser extent, in the European Pharmacopoeia (Ph. Eur.). HPLC is known for its high specificity and precision, but to achieve such accuracy, thorough system suitability tests are necessary, which can be quite expensive. It is the most commonly used chromatographic method found in research. When it comes to effective liquid chromatography, selecting the right detection method is essential. The UV detector is a popular choice in HPLC because it can track multiple wavelengths at once, ensuring that all components that absorb UV light are detected. Photodiode arrays (PDA) are utilized for detecting multiple wavelengths at the same time, which helps in analyzing spectra and checking the purity of peaks. Refractive index detectors work best for substances that either have little or no UV absorption, while electrochemical detectors are effective for materials that can be oxidized or reduced. Fluorescence detectors are very sensitive and are commonly used in the analysis of pharmaceuticals. Reversed-phase HPLC with UV detection is often favored because it is reliable, quick, consistent, and sensitive. Many medications in pharmaceutical products and biological fluids have been examined using HPLC. Nonetheless, HPLC does have some drawbacks, such as the high costs of columns and solvents, along with issues related to long-term reproducibility due to proprietary column packing. Liquid chromatography coupled with mass spectrometry (LC-MS) has become increasingly popular and is now a preferred method for quality control and assurance in the pharmaceutical sector. HPLC-MS is extensively used for drug testing and analyzing impurities and degradation products in pharmaceuticals. This technique is versatile and powerful, playing a crucial role in pharmaceutical analysis by addressing important questions in the field. However, it's essential to keep its limitations in mind and consider advanced techniques like HPLC-MS for better performance.

4] Gas chromatography

Gas chromatography is a powerful method for separating and identifying volatile organic compounds, allowing for accurate measurement of complex mixtures, even when the concentrations are very low. In the field of pharmaceutical analysis, this technique is essential, although it does have some drawbacks when it comes to high-molecular-weight or unstable substances. One of the main issues is that many drug compounds have low volatility, which means they often need to be chemically modified to be analyzed effectively. Gas chromatography is used in testing drugs like isotretinoin and cocaine, as well as in checking for leftover solvents in betamethasone valerate. It's also important for identifying impurities in pharmaceuticals, especially those related to the manufacturing process. Furthermore, gas chromatography helps in analyzing residual solvents, which are considered impurities according to international guidelines, using different types of detectors. Overall, gas chromatography is an important tool in pharmaceutical analysis, providing precise measurements of volatile compounds in complicated mixtures, with significant uses in drug testing and impurity detection.

Spectroscopic Techniques

1] UV-VIS Spectrophotometry

A significant category of techniques that hold a key position in pharmacopoeias is spectrophotometric methods, which rely on natural UV absorption and chemical reactions. Spectrophotometry involves measuring how much light a material reflects or transmits at different wavelengths. These methods are beneficial because they save time and require less effort. Additionally, they offer great precision. Recently, the use of UV-Vis spectrophotometry for analyzing pharmaceutical dosage forms has grown quickly. Colorimetric methods typically focus on several key aspects.



- Complex-formation reaction.
- Oxidation-reduction process.
- A catalytic effect

Colorimetric methods play a significant role in the analysis of bulk materials. For instance, the blue tetrazolium assay is employed to assess corticosteroid drug formulations. Additionally,

this method is utilized for the quantification of cardiac glycosides, as outlined in the European Pharmacopoeia. Various spectrophotometric techniques for measuring active pharmaceutical ingredients in bulk drugs and formulations have been documented, with specific details provided in Table 3.

Table 3 Quantitative analysis of drugs in pharmaceutical formulations by UV-visible spectrophotometric procedures

Reagent used	Name of drug	λ_{max}	Reference
m-Cresol	Acetaminophen	640	Qureshi et al. (1992)
p-Chloranilic acid	Quetiapine fumarate	520	Vinay and Revenasiddappa (2012)
	Milrinone	519	
2,3-Dichloro 5,6-dicyano 1,4- benzoquinone	Duloxetine	477	Toker and onal (2012) Rahman and Hoda (2003)
	Amlodipine besylate	580	
7, 7, 7, 8-Tetracyanoquinodimethane	Lisinopril	743	Rahman et al. (2005b)
	Alendronate sodium	840	Raza and Haq (2011)
Folin ciocalteu phenol	Oxcarbazepine, Ampicillin, amoxicillin, and carbenicillin	760 , 750, 770, 750	Gandhimathi and Ravi (2008) Ahmad et al. (2004)

2] Near Infrared Spectroscopy

Near infrared spectroscopy (NIRS) is an efficient and non-invasive method that enables the analysis of multiple components within nearly any matrix. In recent years, NIR spectroscopy has become increasingly valued in the pharmaceutical sector for its applications in raw material assessment, product quality assurance, and process oversight. The rising interest in NIR spectroscopy among pharmaceutical professionals can be attributed to its significant advantages compared to other analytical methods, including straightforward sample preparation that requires no pretreatment, the ability to utilize fiber optic probes for sample measurement at various locations, and the capability to derive both chemical and physical parameters from a single spectrum. Major pharmacopoeias have largely recognized NIR methodologies, with the European Pharmacopoeia addressing this in chapter 2.2.40 and the United States Pharmacopoeia covering it in chapter 1119,

both highlighting the appropriateness of NIR instrumentation for pharmaceutical testing.

3] Nuclear Magnetic Resonance Spectroscopy

Since the initial report in 1996 detailing the application of NMR spectroscopy for drug molecule screening, the domain of NMR-based screening has advanced rapidly. In recent years, numerous cutting-edge methodologies have emerged, gaining extensive use in both pharmaceutical and academic research settings. Recently, NMR has been utilized for quantitative analysis to assess drug impurities, characterize the composition of drug products, and quantify drugs in pharmaceutical formulations and biological fluids. A significant number of reviews discussing the role of NMR in pharmaceuticals have been published.

4] Ion Mobility-Mass Spectrometry

Ion mobility-mass spectrometry (IM-MS) has attracted significant interest from researchers due to its distinctive capability to differentiate ions based on their size, shape, or charge, while also

being integrated with a high mass accuracy time-of-flight mass analyzer. Various types of ion mobility techniques have been employed for the analysis of glycopeptides. Among these, travelling wave ion mobility-mass spectrometry (TWIM-MS) stands out as the most widely used commercially available IM-MS instrument. It has been effectively utilized to comprehensively analyze the glycosylation heterogeneity profile of an IgG1 monoclonal antibody, enabling the clear identification of glycopeptides corresponding to each glycoform. Research by Li et al. demonstrated that TWIMS could effectively distinguish peptides from glycopeptides, resulting in distinct trend lines that facilitate the prediction of glycosylation status in other peptides. They also observed that ion mobility minimized chemical noise, enhancing the detection of less abundant ions. TWIMS-MS has been employed to differentiate epimeric glycopeptides derived from Muc 2 [Fig. 7A]. Two isomeric glycopeptides, which share the same peptide backbone but vary in the attachment of either α -GlcNAc or α -Gal-NAC,

were partially resolved using TWIMS-MS. Notably, multiple overlapping conformers were detected for each glycopeptide. The authors validated the identity of each glycopeptide through CID-IMS-MS [Fig. 7B], where the diagnostic oxonium ions were separated using TWIMS-MS, underscoring its utility and significance. High-field asymmetric wave ion mobility spectrometry (FAIMS) has been effectively employed to swiftly distinguish coeluting isomeric O-linked glycopeptides that vary solely by their glycosylation sites [Fig. 7C]. The separation of these coeluting peptides was achieved by modifying the applied correction voltage. The identification of the glycosylation sites was validated using electron transfer dissociation (ETD) [Fig. 7D and E], where the distinct product ions allowed for clear differentiation of the two glycosylation sites. The integration of FAIMS for the separation of isobaric peptides alongside ETD for glycosylation site confirmation has proven to be highly beneficial in the analysis of intricate glycopeptide mixtures.

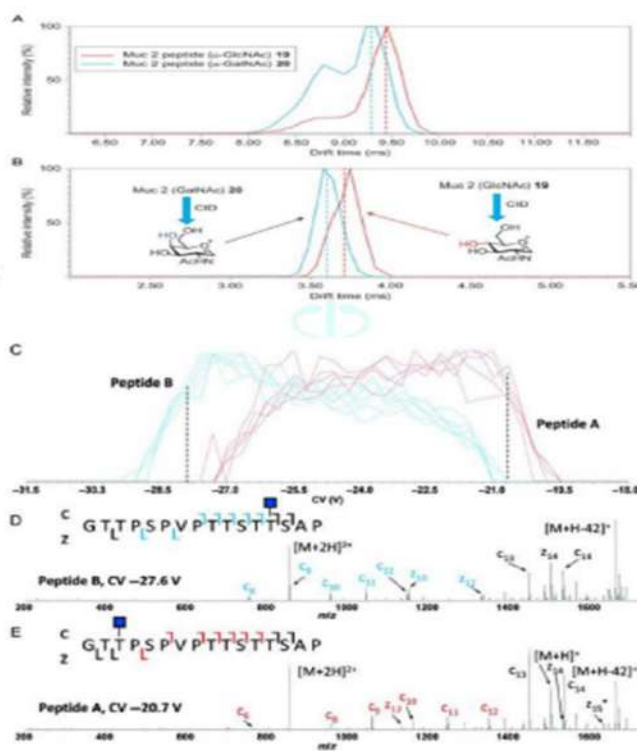


Fig. 7 Separation and analysis of glycopeptides by ion mobility-mass spectrometry.

A traveling wave ion mobility mass spectrometry (TWIMS) analysis revealed several conformers of the isobaric Muc2 glycopeptides (PTTTPITTTTTVTPPTPTGTQT with GalNAc 19 and GlcNAc 20). While TWIMS could not differentiate between the two intact glycopeptides, subsequent collision-induced dissociation (CID) allowed TWIMS-MS to identify the characteristic oxonium ions associated with each Mucin glycopeptide. High-field asymmetric wave ion mobility mass spectrometry (FAIMS-MS) successfully separated two isobaric O-linked glycopeptides that varied solely in the attachment site of the glycan. The identity of the glycopeptides was confirmed through electron transfer dissociation (ETD) MS2, with diagnostic c and z ions highlighted in blue and red, respectively.

Electrochemical methods:

In the past few years, there has been a notable rise in the application of electrochemical methods for the analysis of drugs and pharmaceuticals. This growing interest is largely due to improvements in technology and a deeper comprehension of these techniques. Different electrochemical approaches have been utilized in pharmaceutical analysis. For example, researchers created a modified glassy carbon paste electrode using Amberlite XAD-2 and titanium dioxide nanoparticles to analyze imipramine, trimipramine, and desipramine. These medications were examined through various techniques, including cyclic voltammetry, chronocoulometry, electrochemical impedance spectroscopy, and adsorptive stripping differential pulse voltammetry (Sanghavi and Srivastava, 2013).

Capsaicin-modified carbon nanotube electrodes were utilized to analyze benzocaine and lidocaine in another study. The interaction between capsaicin and benzocaine caused a reduction in the voltammetric signal, which allowed for the quantification of benzocaine. Additionally, a

glassy carbon paste electrode was created using a copper (II) complex and silver nanoparticles to measure dopamine, levodopa, epinephrine, and norepinephrine. Different electrochemical methods were used to investigate how these drugs behave. Techniques like cyclic, differential pulse, and square-wave voltammetry were applied to examine the electrochemical properties of cloquinol with a glassy carbon electrode.

Additionally, techniques like adsorptive stripping differential pulse voltammetry and capillary electrophoresis with amperometric detection have been created to identify different pharmaceutical substances, such as venlafaxine, desvenlafaxine, acetaminophen, aspirin, caffeine, levodopa, bensevazide, and bismuth. In conclusion, electrochemical methods are widely used in the analysis of pharmaceuticals, allowing for the accurate and sensitive measurement of various drugs and compounds.

Kinetic method of analysis

Kinetic analysis methods have been developing since the 1950s and are now seeing a revival. This renewed focus is due to improvements in several areas, such as basic principles, automated tools, insights into chemical processes, data analysis methods, and practical applications. In analytical chemistry, kinetic methods measure how the concentration of a reactant changes over time, usually by observing signal variations in the analyte after it is mixed with the sample and reagents, either by hand or through automated systems. Kinetic techniques provide multiple benefits compared to traditional methods that rely on equilibrium.

Fixed-time and initial rate methods are often used to measure drugs in pharmaceutical products. Automated kinetic techniques typically rely on open systems, like the stopped flow system and the continuous addition of reagent (CAR) method. The CAR method has been used to analyze different drugs using photometric and fluorimetric



detection. It's possible to use catalysts to speed up analytical reactions, which can help with both reaction rate and equilibrium calculations. Recently, micellar media have become more popular in kinetic methods because they can boost reaction rates through micellar catalysis, which might lead to better sensitivity and selectivity while also cutting down on analysis time.

Multicomponent kinetic estimations, often referred to as differential rate methods, are becoming more popular in pharmaceutical research. Moreover, two new techniques, the kinetic wavelength pair method and the H-point standard addition method, have been introduced to tackle the issue of overlapping spectra in binary mixtures.

Electrophoretic methods:

Capillary electrophoresis (CE) plays an important role in the field of pharmaceutical analysis. This modern analytical method separates charged substances in a tiny capillary tube using an electric field. As the solutes move through a detector, they are identified as peaks, and the size of each peak corresponds to its concentration, making it possible to measure quantities accurately. CE isn't just useful for pharmaceuticals; it is also applied in studying biopolymers and analyzing inorganic ions. Capillary electrophoresis (CE) has a lot of benefits. It's usually more efficient and works on a quicker timescale. Plus, it only needs tiny amounts of samples, often just in the nanolitre range, and can be done in water-based conditions. These features have been really useful in many areas of pharmaceuticals. Numerous studies have pointed out how CE is used for regular drug testing.

Multiple types of capillary electrophoresis have been established and utilized in the assessment of pharmaceutical purity and drug bioanalysis. These types encompass capillary zone electrophoresis, micellar electrokinetic chromatography, isotachopheresis, capillary gel electrophoresis, isoelectric focusing, and affinity capillary

electrophoresis. Capillary electrophoresis serves as an essential analytical method in the field of pharmaceutical analysis, providing benefits such as effective separation, minimal sample requirements, and versatility across a range of pharmaceutical applications.

Flow injection and sequential injection analysis:

Laboratory automation began to take shape in the latter part of the 20th century, spearheaded by innovators such as Steward in the United States and Ruzicka and Hansen in Denmark. They introduced flow injection analysis (FIA), a groundbreaking method that revolutionized the automation of chemical processes. This technique represented a major advancement in chemical analysis automation, allowing for instrumental measurements to occur independently of physical and chemical equilibria.

Flow injection analysis (FIA) is a technique that entails the introduction of a liquid sample into a continuous and uninterrupted stream of an appropriate carrier liquid. This injection creates a separate zone of the sample that moves toward a detector, which consistently monitors variations in absorbance, electrode potential, or other physical parameters as the sample traverses the flow cell.

The FIA technique has played a crucial role in enhancing automation within pharmaceutical analysis, with its benefits extensively detailed in numerous review articles and a specialized monograph. Its applications span a diverse array of matrices, such as solid substances, pastes (including ointments and creams), liquids (encompassing emulsions, suspensions, and solutions), and a variety of active ingredients with distinct therapeutic effects. By optimizing the economical use of reagents and improving sampling frequencies, many of these applications focus on identifying active ingredients to maintain quality control in pharmaceutical products.



CONCLUSION

The primary objective of pharmaceutical drugs is to benefit humanity by alleviating potential illnesses and preventing diseases. For medications to fulfill their intended functions, they must be devoid of impurities or any other contaminants that could pose risks to human health. This review aims to emphasize the significance of various analytical instruments in the assessment of pharmaceuticals and provides a comprehensive literature overview of the instrumentation utilized in pharmaceutical analysis. Additionally, the review underscores the evolution of techniques, starting from traditional titrimetric methods and progressing to sophisticated hyphenated techniques.

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