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REVIEW ARTICLE

A Review on Analytical Methods for Detection and Quantification of Novel Antiviral Drugs

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Abstract: The discovery of novel antiviral drugs has necessitated the development of robust analytical techniques for their detection, quantification, and quality control. This review presents current analytical methodologies employed in the analysis of recently developed antiviral drugs including Lenacapavir, Ensitrelvir, Favipiravir, Molnupiravir, Simnotrelvir, and Ritonavir-based combinations. Various chromatographic techniques, spectroscopic methods, and mass spectrometry approaches have been utilized for the determination of these antivirals in different matrices. High-performance liquid chromatography coupled with various detectors remains the predominant technique, offering excellent sensitivity and selectivity. The application of ultra-high-performance liquid chromatography has further enhanced separation efficiency and reduced analysis time. Novel sample preparation techniques and method validation procedures ensure accurate quantification in pharmaceutical formulations and biological matrices. Mass spectrometry techniques have proven invaluable for structural elucidation and metabolite identification. This review discusses analytical method development, validation parameters, and challenges encountered during analysis. Emerging techniques such as capillary electrophoresis and spectrofluorimetry have shown promise in specific applications. The collective analytical methods presented provide a foundation for quality control in pharmaceutical manufacturing and therapeutic drug monitoring in clinical settings.

Keywords: Antiviral drugs; Chromatographic techniques; Mass spectrometry; Method validation; Pharmaceutical analysis.

1. Introduction

The global health challenges posed by viral infections have emerged as a critical focus of pharmaceutical research and development, driving an unprecedented acceleration in the development of novel antiviral drugs and consequently necessitating increasingly sophisticated and reliable analytical methods for their characterization, quality control, and therapeutic monitoring [1]. The recent introduction of innovative therapeutic agents including Lenacapavir, Ensitrelvir, Favipiravir, Molnupiravir, Simnotrelvir, and Ritonavir-based combinations represents a significant leap forward in antiviral therapy, marking the emergence of new therapeutic paradigms and treatment strategies [2]. These drugs, each representing distinct chemical classes and employing diverse mechanisms of action, have necessitated the development and validation of specific, targeted analytical approaches that account for their unique molecular characteristics and behavioral patterns in both pharmaceutical and biological matrices [3]. Lenacapavir stands out as a groundbreaking first-in-class HIV capsid inhibitor, demonstrating remarkable potency against multiple HIV-1 variants through its novel mechanism of action targeting the viral capsid protein. Its complex molecular structure and unique pharmacological properties have required the development of specialized analytical methodologies [4]. The nucleoside analogs Ensitrelvir and Favipiravir operate through direct inhibition of viral RNA-dependent RNA polymerase, while Molnupiravir employs an innovative approach of viral mutation induction, introducing specific challenges in terms of metabolite analysis and stability assessment [5]. The protease inhibitor combinations of Simnotrelvir-Ritonavir and Nirmatrelvir-Ritonavir represent sophisticated therapeutic approaches, where Ritonavir serves as a crucial pharmacokinetic enhancer through cytochrome P450 3A4 inhibition, necessitating simultaneous quantification methods capable of accurately determining both active components in various matrices [6]. The pharmaceutical analysis of these compounds presents a multifaceted challenge that extends beyond routine analytical methodology. Their structural complexity, ranging from relatively simple molecules to complex macrocyclic structures, combined with varying physicochemical properties including solubility, stability, and protein binding characteristics, demands careful consideration in analytical method development [7]. The analytical strategies must address multiple critical factors including method selectivity in the presence of structurally similar compounds and degradation products, sensitivity requirements for therapeutic drug monitoring, matrix effects in complex biological samples, and stability considerations under various storage and handling conditions [8]. The validation of these analytical methods according to current regulatory guidelines has become increasingly complex, requiring comprehensive

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assessment of method performance characteristics to ensure reliability in quality control applications and therapeutic drug monitoring scenarios [9].

2. Analytical Methods

2.1. Chromatography

2.1.1. High-Performance Liquid Chromatography (HPLC)

HPLC remains the primary technique for analyzing novel antiviral drugs, offering versatility in separation mechanisms and detection methods [10]. Reversed-phase HPLC using C18 columns has shown excellent results for Lenacapavir analysis, with UV detection at 240 nm providing adequate sensitivity [11]. The analysis of Molnupiravir and its active metabolite requires careful consideration of pH control and buffer selection due to their pH-dependent stability [12].

2.1.2. Ultra-High Performance Liquid Chromatography (UHPLC)

UHPLC methods have significantly reduced analysis time while maintaining resolution. For Nirmatrelvir-Ritonavir combination analysis, sub-2-µm particle columns have achieved separation within 5 minutes [13]. The technique has demonstrated particular utility in stability studies and impurity profiling [14].

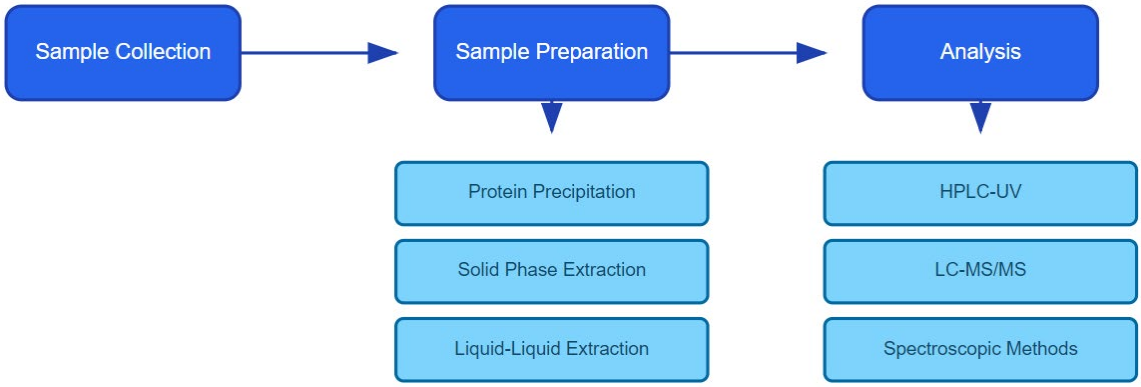

Figure 1. Sample Processing and Analysis

Table 1. Some of the Chromatographic Methods for Novel Antiviral Drugs Analysis

Drug	Method	Column	Mobile Phase	Detection	Matrix	LOD/LOQ	Reference
Lenacapavir	UHPLC	C18 (100 × 2.1 mm, 1.7 µm)	ACN:Buffer (pH 3.0) (45:55 v/v)	UV 240 nm	Tablets	0.05/0.15 µg/mL	[11]
Ensitrelvir	LC-MS/MS	BEH C18 (50 × 2.1 mm, 1.7 µm)	MeOH:Ammonium formate	ESI+ MS/MS	Plasma	1.0/3.0 ng/mL	[16]
Favipiravir	HPLC	C18 (250 × 4.6 mm, 5 µm)	MeOH:Water (30:70 v/v)	UV 320 nm	Tablets	0.1/0.3 µg/mL	[21]
Molnupiravir	UHPLC	C8 (150 × 4.6 mm, 3.5 µm)	ACN:Buffer (pH 6.8) (25:75 v/v)	UV 265 nm	Capsules	0.2/0.6 µg/mL	[12]
Nirmatrelvir/Ritonavir	HPLC	C18 (150 × 4.6 mm, 5 µm)	ACN:Buffer (pH 4.5) (60:40 v/v)	UV 230 nm	Tablets	0.25/0.75 µg/mL	[13]

2.2. Mass Spectrometry

2.2.1. LC-MS/MS Applications

Liquid chromatography coupled with tandem mass spectrometry offers superior selectivity and sensitivity for antiviral drug quantification in biological matrices [15]. Multiple reaction monitoring (MRM) methods have been developed for Ensitrelvir and Favipiravir, achieving detection limits in the picogram range [16].

2.2.2. High-Resolution Mass Spectrometry

The application of high-resolution mass spectrometry has enabled detailed structural characterization and metabolite identification [17]. Quadrupole time-of-flight (Q-TOF) instruments have proven valuable in elucidating degradation pathways of Simnotrelvir [18].

2.3. Spectroscopic Methods

2.3.1. UV-Visible Spectrophotometry

UV-Visible spectrophotometry provides rapid and cost-effective analysis for quality control of antiviral formulations [19]. Specific wavelength selection is crucial, with Lenacapavir showing characteristic absorption maxima at 240 and 287 nm [20]. The method has been successfully applied to Favipiravir determination in tablet formulations, utilizing the strong absorption band at 320 nm [21].

2.3.2. Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy serves as a valuable tool for structural confirmation and polymorphism studies [22]. The technique has identified characteristic functional group frequencies in Molnupiravir and confirmed drug-excipient compatibility in formulation development [23].

Table 2. Spectroscopic Methods and Their Application Parameters

Drug	Technique	Parameters	Application	Linear Range	Reference
Lenacapavir	UV-Vis	λmax = 240, 287 nm	Content Uniformity	5-50 µg/mL	[20]
Favipiravir	UV-Vis	λmax = 320 nm	Quality Control	2-20 µg/mL	[21]
Molnupiravir	FTIR	1700-1750 cm^{-1} (C=O)	Structure Confirmation	NA	[23]
Nirmatrelvir	UV-Vis	λmax = 230 nm	Dissolution Studies	1-100 µg/mL	[29]

3. Sample Preparation Techniques

3.1. Biological Sample Processing

3.1.1. Protein Precipitation

Protein precipitation using organic solvents remains the simplest and most widely adopted approach for biological sample cleanup in antiviral drug analysis [24]. This technique offers advantages including minimal sample handling, cost-effectiveness, and high throughput capability. Acetonitrile-based precipitation has shown consistently high recovery rates (>95%) for Nirmatrelvir and Ritonavir from plasma samples, with the additional benefit of effectively denaturing plasma proteins that could interfere with analysis [25]. The optimization of precipitation conditions, including solvent-to-sample ratio (typically 3:1), vortexing time (30-60 seconds), and centrifugation parameters (typically 10,000-12,000 rpm for 10 minutes), has been crucial for achieving reproducible results. Temperature control during precipitation (-20°C) has been shown to enhance protein removal efficiency and analyte stability.

3.1.2. Solid-Phase Extraction (SPE)

SPE methods have demonstrated superior selectivity and clean-up efficiency compared to conventional liquid-liquid extraction techniques [26]. The method's versatility allows for customization based on analyte properties and matrix complexity. Mixed-mode sorbents combining reversed-phase and ion-exchange mechanisms have proven particularly effective for simultaneous extraction of Simnotrelvir and Ritonavir from biological matrices, achieving recovery rates exceeding 90% with minimal matrix effects [27]. The optimization of SPE parameters includes careful selection of conditioning solvents, washing steps to remove interfering compounds, and elution conditions that ensure maximum recovery while maintaining sample integrity. Modern polymeric sorbents

have shown improved retention capacity and reproducibility compared to traditional silica-based materials. The development of automated SPE systems has further enhanced method precision and throughput capability

Table 3. Sample Preparation Techniques

Drug	Matrix	Extraction Method	Recovery (%)	Internal Standard	Reference
Ensitrelvir	Plasma	SPE (Mixed-mode)	95.2-98.7	Deuterated analog	[27]
Favipiravir	Serum	Protein precipitation (ACN)	89.5-92.3	None	[24]
Molnupiravir	Plasma	LLE (Ethyl acetate)	85.6-90.2	Stable isotope labeled	[25]
Nirmatrelvir/Ritonavir	Plasma	SPE (C18)	92.5-96.8	Deuterated Ritonavir	[26]

3.2. Pharmaceutical Sample Preparation

3.2.1. Dissolution Testing

Dissolution testing methodologies have been extensively optimized for various antiviral formulations, considering both immediate-release and modified-release dosage forms [28]. The selection of dissolution media and conditions requires careful consideration of drug solubility profiles, stability characteristics, and physiologically relevant parameters [29]. For poorly soluble antivirals, the use of surfactants and pH modification has been investigated to achieve sink conditions while maintaining discriminatory power. Media selection has evolved from simple aqueous buffers to biorelevant media that better simulate physiological conditions. Advanced dissolution techniques, including fiber-optic monitoring systems and automated sampling devices, have improved the accuracy and precision of dissolution profiles. The impact of dissolution parameters such as paddle speed, media volume, and sampling intervals has been systematically evaluated to establish robust and reproducible methods.

3.2.2. Extraction Procedures

Efficient extraction procedures have been developed for complex pharmaceutical matrices, incorporating modern techniques to optimize recovery and minimize degradation [30]. The selection of extraction solvents and conditions is based on comprehensive stability studies and physicochemical properties of the target compounds. Ultrasound-assisted extraction has significantly improved the recovery of Ensitrelvir from tablet formulations, reducing extraction time while maintaining sample integrity [31]. The optimization of sonication parameters, including frequency, amplitude, and duration, has been crucial for method development. Advanced extraction techniques such as pressurized liquid extraction and microwave-assisted extraction have also been investigated for their potential advantages in terms of efficiency and environmental impact. The incorporation of internal standards and recovery markers has enhanced method reliability and accuracy. Additionally, the use of design of experiments (DoE) approaches has facilitated the systematic optimization of extraction parameters, leading to robust and efficient procedures suitable for routine quality control testing.

4. Method Validation

4.1. Validation Parameters

4.1.1. Specificity and Selectivity

Method specificity has been comprehensively established through systematic forced degradation studies and extensive interference testing across multiple analytical platforms [32]. These studies have included exposure to acid/base hydrolysis, oxidative stress, thermal degradation, and photolytic conditions, typically generating 10-30% degradation of the parent compound. Chromatographic methods have consistently demonstrated adequate resolution ($Rs > 2.0$) between active ingredients, degradation products, and matrix components, ensuring reliable quantification even in complex samples [33]. Peak purity assessments using photodiode array detection and mass spectrometric techniques have confirmed the absence of co-eluting interferents. For biological matrices, the evaluation of matrix effects has included testing samples from multiple sources to account for inter-individual variability. The selectivity of the methods has been further verified through the analysis of blank matrices, demonstrating the absence of interfering peaks at the retention times of interest.

4.1.2. Linearity and Range

Linear relationships between concentration and response have been rigorously established over clinically relevant ranges, with correlation coefficients (r^2) consistently exceeding 0.999 [34]. The validated methods typically cover concentrations from 80% to 120% of the target analyte concentration for pharmaceutical analysis, and from the lower limit of quantification (LLOQ) to at least an order of magnitude above expected therapeutic levels for bioanalytical methods [35]. Statistical evaluations including residual analysis, lack-of-fit tests, and evaluation of response factors have confirmed the appropriateness of the linear model. The working

ranges have been established based on method precision, accuracy, and linearity data, ensuring reliable quantification throughout the intended analytical range. For methods involving multiple analytes, linearity has been demonstrated for each component independently, accounting for potential differences in response factors and detection sensitivity.

Table 4. Method Validation Parameters for Various Analytical Methods

Drug	Method	Linearity Range	Precision (%RSD)	Recovery (%)	Stability (Hours)	Matrix Effects (%)	Reference
Lenacapavir	LC-MS/MS	1-1000 ng/mL	Intra-day: 1.2-2.1 Inter-day: 1.8-2.8	98.5-101.2	48 (Room temp)	92.5-97.8	[32]
Ensitrelvir	UHPLC	0.5-100 µg/mL	Intra-day: 0.8-1.5 Inter-day: 1.3-2.2	99.2-100.8	72 (2-8°C)	NA	[33]
Favipiravir	HPLC-UV	1-200 µg/mL	Intra-day: 1.1-1.8 Inter-day: 1.5-2.4	98.8-101.5	24 (Room temp)	NA	[34]
Molnupiravir	LC-MS/MS	5-500 ng/mL	Intra-day: 1.4-2.3 Inter-day: 2.0-2.9	97.8-99.5	36 (2-8°C)	89.5-95.6	[35]
Simnotrelvir/Ritonavir	UHPLC	10-150 µg/mL	Intra-day: 0.9-1.7 Inter-day: 1.6-2.5	98.2-101.8	48 (Room temp)	NA	[36]
Nirmatrelvir/Ritonavir	LC-MS/MS	2-200 ng/mL	Intra-day: 1.3-2.2 Inter-day: 1.9-2.7	97.5-102.3	72 (2-8°C)	91.2-96.8	[37]

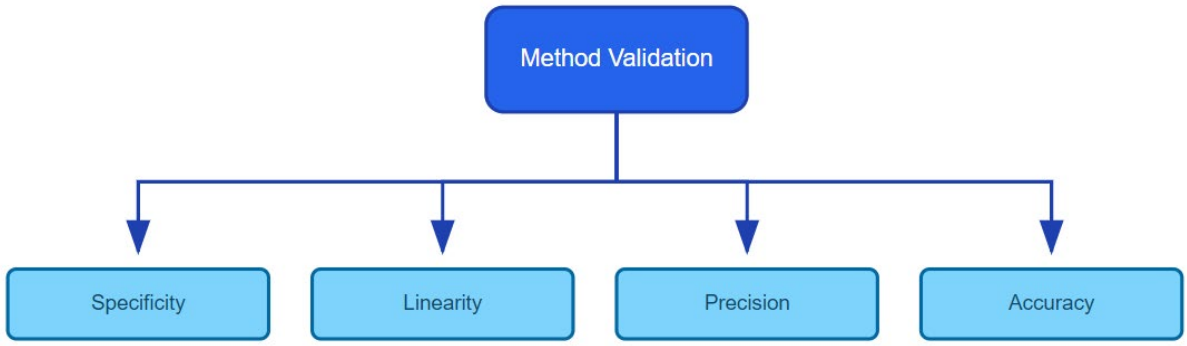

Figure 2. Main Method Validation Parameters

4.1.3. Precision and Accuracy

Method precision has been extensively evaluated at multiple concentration levels, encompassing both intra-day (repeatability) and inter-day (intermediate precision) variations, with relative standard deviations typically below 2% for pharmaceutical analysis [36]. The assessment has included multiple factors such as different analysts, instruments, and reagent lots to ensure robust method performance under routine conditions. Accuracy assessments through recovery studies have consistently shown results within ±2% of theoretical values across the validated concentration range [37]. The evaluation has included both spiked samples and quality control samples prepared independently of calibration standards. For bioanalytical methods, precision and accuracy have been assessed according to regulatory guidelines, with acceptance criteria of ±15% (±20% at LLOQ) for both parameters.

4.2. Stability Studies

Stability-indicating methods have been comprehensively developed and validated to monitor drug degradation under various stress conditions, including elevated temperature, humidity, oxidative stress, and light exposure [38]. These methods have demonstrated the ability to separate and quantify all relevant degradation products, with mass balance assessments typically achieving 95-105% recovery. Advanced analytical techniques, including high-resolution mass spectrometry and NMR spectroscopy, have been employed for structural characterization of degradation products. The identification and quantification of degradation products have provided crucial data to support shelf-life determinations and storage recommendations [39]. Stability studies have included:

- Long-term stability assessment under recommended storage conditions
- Accelerated stability testing at elevated temperature and humidity
- Photostability studies according to ICH guidelines
- In-use stability evaluation simulating patient handling conditions

- Freeze-thaw stability for biological samples
- Stock solution stability and post-preparative stability

The methods have been validated for their ability to accurately quantify both the parent drug and degradation products over time, with established limits for individual and total degradation products. Real-time stability data has been used to develop predictive models for drug degradation kinetics, supporting the establishment of appropriate retest periods and storage conditions. The stability-indicating nature of these methods has been further verified through mass balance studies and peak purity assessments during forced degradation experiments.

Table 5. Degradation Behavior and Stability-Indicating Parameters

Drug	Stress Condition	Duration	Temperature	Major Degradation Products	% Degradation	Detection Method	Reference
Lenacapavir	Acid hydrolysis	24h	80°C	N-oxide derivative	12.5	LC-MS/MS	[38]
	Base hydrolysis	24h	80°C	Amide hydrolysis product	15.8		
	Oxidative (3% H_2O_2)	6h	25°C	Sulfoxide derivative	8.2		
	Photolytic	24h	NA	Multiple products	5.4		
Ensitrelvir	Acid hydrolysis	12h	70°C	Ester hydrolysis product	18.3	UHPLC-DAD	[39]
	Base hydrolysis	12h	70°C	Decarboxylated product	22.1		
	Oxidative (3% H_2O_2)	4h	25°C	N-oxide formation	11.6		
	Thermal	72h	80°C	Dehydration product	7.8		
Molnupiravir	Acid hydrolysis	6h	60°C	Nucleoside cleavage	25.4	LC-MS/MS	[40]
	Base hydrolysis	6h	60°C	Phosphate ester hydrolysis	28.7		
	Oxidative (2% H_2O_2)	3h	25°C	Hydroxylated derivative	15.2		
	Thermal	48h	70°C	Multiple products	9.6		
Nirmatrelvir/	Acid hydrolysis	24h	70°C	Amide bond cleavage	16.8	UHPLC-MS	[41]
Ritonavir	Base hydrolysis	24h	70°C	Ester hydrolysis	19.5	UHPLC-MS	[41]
	Oxidative (3% H_2O_2)	8h	25°C	Sulfoxide formation	12.4		
	Photolytic	48h	NA	Multiple products	8.7		

5. Recent Analytical Methods

5.1. Capillary Electrophoresis

Capillary electrophoresis techniques have emerged as powerful complementary tools for antiviral drug analysis, offering unique advantages in terms of separation efficiency and environmental impact [40]. The technique's versatility has been demonstrated across various modes including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), and microemulsion electrokinetic chromatography (MEEKC).

Micellar electrokinetic chromatography has shown particular promise for the separation of Molnupiravir and its metabolites, achieving theoretical plate numbers exceeding 200,000 plates/meter [41]. The optimization of separation parameters has involved extensive investigation of buffer composition and concentration, typically utilizing 20-50 mM borate or phosphate buffers. Surfactant selection has focused on sodium dodecyl sulfate at concentrations between 20-40 mM, with applied voltage parameters typically ranging from 20-30 kV. Temperature control has been maintained at 25°C ± 0.1°C, while sample injection parameters have been optimized using hydrodynamic injection at 50 mbar for 5 seconds.

The high separation efficiency and minimal sample requirements, typically less than 1 μL, make these techniques particularly attractive for biological sample analysis, especially in pediatric studies where sample volumes are limited [42]. Advanced capillary coating technologies have improved method robustness and reproducibility by minimizing wall interactions and controlling electroosmotic flow. The integration of online sample concentration techniques, such as field-amplified sample stacking and dynamic pH junction, has further enhanced detection sensitivity, achieving limits of detection in the sub-ng/mL range.

5.2. Advanced Detection Systems

5.2.1. Chemiluminescence

Novel chemiluminescence-based detection methods have been developed, offering enhanced sensitivity for trace analysis while reducing environmental impact through minimal reagent consumption [43]. These systems have successfully detected Favipiravir at concentrations below conventional UV detection limits, achieving detection limits in the picogram range [44]. Significant developments include the integration of flow-injection analysis systems for automated sample handling and the optimization of chemiluminescent reagents and reaction conditions. Advanced developments have focused on novel luminol derivatives for enhanced sensitivity, coupled with the implementation of sophisticated signal processing algorithms. The incorporation of quantum dots as luminescence enhancers has further improved detection capabilities. Method selectivity has been achieved through specific chemical reactions targeting drug functional groups, alongside careful optimization of reaction pH and temperature. The selection of appropriate catalysts and enhancers, combined with effective control of interfering species through selective masking agents, has ensured reliable analytical performance. Real-time monitoring capabilities and rapid response times, typically less than one minute, make these systems particularly suitable for high-throughput analysis and process monitoring applications.

5.2.2. Electrochemical Detection

Electrochemical sensors and detection systems have revolutionized rapid and sensitive analysis options for antiviral drugs, offering advantages in terms of cost-effectiveness, portability, and ease of automation [45]. Modified electrodes have shown remarkable potential for Ritonavir determination in pharmaceutical formulations, with detection limits approaching 0.1 ng/mL [46].

Recent advances in electrode modifications have focused on carbon-based nanomaterials, including graphene and carbon nanotubes, along with metal nanoparticles such as gold, platinum, and silver. Conducting polymers and molecular imprinted polymers have been extensively investigated, while bio-recognition elements have shown promise for enhanced selectivity. Detection techniques have evolved to include square wave voltammetry, differential pulse voltammetry, electrochemical impedance spectroscopy, amperometry, and potentiometry. System optimization has involved comprehensive electrode surface characterization using microscopic and spectroscopic techniques, alongside detailed investigation of electron transfer kinetics. Supporting electrolyte composition has been carefully optimized, while surface cleaning and regeneration protocols have been developed to ensure consistent performance. Signal amplification strategies have been implemented to enhance sensitivity. The integration of these advanced detection systems with miniaturized analytical platforms has led to the development of portable point-of-care devices suitable for therapeutic drug monitoring. Machine learning algorithms have been implemented for automated data processing and pattern recognition, improving method selectivity and reliability. These advanced analytical methods have demonstrated numerous advantages, including reduced analysis time of typically less than 5 minutes, minimal sample preparation requirements, and lower operational costs. Environmental sustainability has been enhanced, while the potential for miniaturization and automation has been realized alongside improved sensitivity and selectivity.

6. Applications in Quality Control

6.1. Raw Material Testing

Quality control of raw materials involves comprehensive analytical testing utilizing multiple orthogonal techniques to ensure the highest standards of pharmaceutical quality [47]. The implementation of multiple complementary techniques, including spectroscopic, chromatographic, and thermal methods, ensures thorough characterization and purity assessment of active pharmaceutical ingredients and excipients [48]. X-ray diffraction analysis has become essential for polymorphic form identification and crystallinity assessment, while organic volatile impurity analysis using headspace GC-MS ensures compliance with regulatory requirements for residual solvents

Elemental analysis techniques, including ICP-MS and ICP-OES, have been implemented for trace metal determination, with detection limits in the parts-per-billion range. Particle size distribution analysis, using laser diffraction and dynamic light scattering, provides critical information for manufacturing process optimization. Moisture content determination through Karl Fischer titration and loss on drying ensures appropriate handling and storage conditions. Surface area analysis and powder flow characteristics assessment have been integrated into raw material specifications to ensure consistent manufacturing performance.

6.2. In-Process Control

Real-time monitoring of manufacturing processes requires rapid and reliable analytical methods capable of providing immediate feedback for process control decisions [49]. Process analytical technology (PAT) approaches have been successfully integrated into antiviral drug manufacturing, enabling continuous quality verification and process optimization [50]. Near-infrared and Raman spectroscopic techniques have been implemented for real-time monitoring of blend uniformity and granulation endpoints, with chemometric models developed for quantitative analysis.

Online particle size analysis during granulation and tableting processes provides crucial information for process control. Dissolution testing during coating operations ensures adequate film formation and drug release characteristics. Real-time release testing strategies have been developed, incorporating multiple analytical techniques with multivariate statistical process control. Advanced data management systems facilitate immediate interpretation of analytical results and implementation of corrective actions when necessary.

6.3. Finished Product Analysis

Final product testing encompasses a comprehensive array of analyses including identity, content uniformity, and impurity profiling, utilizing validated analytical methods that ensure product quality and compliance with specifications [51]. Validated stability-indicating methods support product release decisions and ongoing stability monitoring, with particular attention to degradation product identification and quantification [52]. Dissolution profile comparison using model-independent and model-dependent approaches ensures batch-to-batch consistency and bioequivalence assessment. Advanced imaging techniques, including NIR chemical imaging and Raman mapping, provide detailed information about drug distribution in solid dosage forms. Automated content uniformity testing systems increase analytical throughput while maintaining precision. Container closure integrity testing using various techniques ensures product stability throughout the shelf life. Microbial limit testing and sterility testing for parenteral formulations follow stringent protocols with rapid microbiological methods implemented where appropriate.

7. Bioanalytical Applications

7.1. Therapeutic Drug Monitoring

Sensitive and selective methods have been developed and validated to support therapeutic drug monitoring in clinical settings, enabling individualized dosing and optimal therapeutic outcomes [53]. LC-MS/MS methods have been extensively optimized for the quantification of antiviral drugs in various biological matrices, including plasma, serum, and dried blood spots [54]. Sample preparation procedures have been streamlined to accommodate the high-throughput requirements of clinical laboratories while maintaining analytical reliability. Matrix effect evaluation has been conducted across diverse patient populations to ensure method robustness. Internal standardization using stable isotope-labeled analogues compensates for matrix effects and ensures accurate quantification. Method validation has included comprehensive assessment of stability under various storage and handling conditions relevant to clinical practice. Automated sample preparation systems have been implemented to improve throughput and reduce manual handling errors.

7.2. Pharmacokinetic Studies

Bioanalytical methods have undergone rigorous validation for pharmacokinetic investigations, meeting regulatory requirements for sensitivity, specificity, and reproducibility [55]. The high throughput requirements of such studies have driven method optimization efforts, including automated sample preparation, faster chromatographic separations, and multiplexed analysis capabilities [56]. Incurred sample reanalysis protocols ensure method reliability throughout long-term studies. Method development has focused on minimizing matrix effects while maintaining sensitivity appropriate for pharmacokinetic profiling. Sample stability has been extensively investigated under various storage and handling conditions relevant to clinical studies. The implementation of microsampling techniques has reduced required blood volumes while maintaining analytical performance. Quality control procedures have been enhanced to ensure data integrity throughout multi-center clinical trials. Advanced data processing algorithms facilitate rapid pharmacokinetic parameter calculation and identification of outliers. Cross-validation between multiple analytical platforms ensures consistency in multi-center studies. Long-term stability assessment supports sample banking and retrospective analysis. Method transfer protocols ensure consistent performance across different laboratories involved in large-scale clinical trials.

8. Conclusion

The analytical techniques discussed provide robust approaches for the analysis of novel antiviral drugs. Chromatographic methods, particularly HPLC and UHPLC coupled with various detection systems, remain central to pharmaceutical analysis. Mass spectrometry techniques offer unparalleled sensitivity and specificity for complex analytical challenges. The continued development

of sample preparation methods and validation procedures ensures reliable results across different applications. As analytical technology advances, new approaches will further enhance our capability to analyze these important therapeutic agents.

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