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ADVANCEMENTS IN HPLC METHOD DEVELOPMENT AND VALIDATION STRATEGIES FOR ANTI-VIRAL AGENTS: A COMPREHENSIVE REVIEW

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ABSTRACT

High-performance liquid chromatography (HPLC) remains the most widely adopted analytical tool for the estimation, purity assessment, and stability profiling of anti-viral agents. Significant advancements in column technologies, mobile phase optimization, detectors, and analytical quality-by-design (AQbD) approaches have enhanced the robustness and reliability of HPLC methods. Anti-viral drugs-including nucleoside analogues, protease inhibitors, integrase inhibitors, polymerase inhibitors, and fixed-dose combinations-require highly selective and stability-indicating methods due to their complex structures, degradation pathways, and combination formulations. This review highlights the fundamental principles of HPLC method development, recent advancements in stationary phases, ultra-high-performance liquid chromatography (UHPLC), AQbD-driven method optimization, and green analytical approaches. Detailed discussion on validation parameters based on ICH Q2 (R2), emerging trends, and comparative evaluation of published methods is provided. The review summarizes more than 20 years of literature to present a comprehensive understanding of how HPLC continues to evolve for the estimation of anti-viral agents.

KEYWORDS: HPLC, Anti-viral drugs, Method development, Validation, AQbD, UHPLC, Chromatography,

1. INTRODUCTION

Anti-viral agents play a foundational role in the management of viral infections such as HIV, hepatitis B (HBV), hepatitis C (HCV), influenza, herpes simplex, COVID-19, Ebola, dengue, and emerging viral diseases. With continuous discovery of new viral targets and drug classes, analytical scientists are required to establish accurate, precise, and stability-indicating methods for quantification of these drugs in bulk, formulations, and biological matrices.[1]

HPLC has long been the analytical workhorse in pharmaceutical sciences. Its versatility allows separation of a wide range of molecules with different polarities, functional groups, molecular weights, and lipophilic properties.[2] Anti-viral drugs often possess complex structures such as nucleoside analogues (e.g., zidovudine, acyclovir, Remdesivir), integrase inhibitors (dolutegravir, Raltegravir), protease inhibitors (Lopinavir, Darunavir), and neuraminidase inhibitors (oseltamivir), requiring robust method development strategies.[3]

The introduction of newer technologies including UHPLC, monolithic columns, superficially porous particles (SPPs), AQbD, gradient elution techniques, photodiode array (PDA), charged aerosol detectors (CAD), MS-compatible mobile phases, and green

chromatography has made HPLC more efficient and environmentally sustainable.[4]

Method validation is equally critical, ensuring the method consistently produces reliable results. ICH Q2(R2) guidelines emphasize parameters such as accuracy, precision, specificity, LOD, LOQ, linearity, robustness, and system suitability. For anti-viral agents that are prone to hydrolysis, photo-degradation, oxidation, and thermal decomposition, stability-indicating methods are mandatory.[5-6]

This review aims to provide a detailed overview of HPLC method development strategies, technological advancements, and validation approaches applied for the estimation of anti-viral drugs, supported by extensive literature and comparative tables.[7-9]

2. OVERVIEW OF ANTI-VIRAL DRUG CLASSES

Anti-viral agents may be classified as:

2.1 Nucleoside and Nucleotide Analogues

Examples:
Acyclovir, Zidovudine, Lamivudine, Tenofovir, Remdesivir
Features: high polarity, require reverse-phase or ion-pair chromatography.[10]

2.2 Protease Inhibitors

Lopinavir, Ritonavir, Darunavir
Hydrophobic molecules → C18 columns with gradient elution

2.3 Integrase Strand Transfer Inhibitors (INSTIs)

Raltegravir, Dolutegravir, Bictegravir
Stable under acidic conditions but degrade in alkaline medium.[11]

2.4 Polymerase and Reverse Transcriptase Inhibitors

Sofosbuvir, favipiravir, Entecavir

2.5 Neuraminidase Inhibitors

Oseltamivir, Zanamivir
Highly polar → need ion-pair or HILIC columns

2.6 COVID-19 Related Anti-Virals

Remdesivir, Molnupiravir, Favipiravir
Require stability-indicating approaches due to rapid degradation

3. HPLC METHOD DEVELOPMENT FOR ANTI-VIRAL DRUGS

3.1 Selection of Stationary Phase[12]

C18 columns: Most widely used
C8: Faster elution

Phenyl-hexyl: Good for aromatic or conjugated antiviral molecules

HILIC columns: For highly polar drugs (Tenofovir, Zanamivir)

Monolithic columns: Faster separation and higher throughput

SPP (core-shell) columns: Higher efficiency under lower backpressure

Figure (Text-based)
Column Technology Evolution for Anti-Viral HPLC Analysis
C18 → C8 → Phenyl → HILIC → Monolithic → UHPLC (1.7 µm) → SPP Columns

3.2 Mobile Phase Optimization[13]

Commonly used solvents
* Methanol
* Acetonitrile
* Water
* Buffer: phosphate, ammonium acetate, formate, trifluoroacetic acid (TFA)

pH Selection[14]
Anti-viral drugs contain:
* Primary/secondary amines
* Heterocyclic nitrogen
* Carboxyl groups

Thus pH affects:
* Retention
* Peak shape
* Ionization

Role of Buffer System
* Improves peak symmetry
* Enhances reproducibility
* Essential for MS compatibility (volatile buffers)

3.3 Mode of Elution

Isocratic: For simple formulations[15]
Gradient: For combinations like Lopinavir/ritonavir, dolutegravir/lamivudine.
Ion-pair chromatography: For highly polar molecules
HILIC mode: For hydrophilic antivirals.

3.4 Flow Rate and Temperature Optimization

* Flow rate: 0.8–1.5 mL/min
* Column temperature[8]: 30–40°C optimizes peak shape

3.5 Detection Strategies

- **UV detection (210–260 nm)** is most common
- **PDA:** Identifies impurities and degradation peaks[16]
- **FLD:** Used for nucleoside derivatives
- **MS Detection**

* High sensitivity
* Used widely for Remdesivir, dolutegravir, and Tenofovir

4. VALIDATION STRATEGIES (ICH Q2 (R2))[17]

4.1 Specificity

Ability to measure analyte in presence of:

* Excipients
* Degradants
* Impurities
* Co-Administered Antivirals

Forced degradation studies include
* Acid/alkali hydrolysis[18]
* Oxidation
* Thermal degradation
* Photolysis

4.2 Linearity and Range
Typical concentration ranges[19]
* 5–50 µg/mL
* 10–100 µg/mL
Correlation coefficient (R^2) ≥ 0.999

4.3 Accuracy
Expressed as % recovery
Acceptance criteria: 98–102%
4.4 Precision
Includes:
* Repeatability
* Intermediate precision
* %RSD ≤ 2%

4.5 LOD and LOQ
Calculated by[20]
* Signal-to-noise ratio
* Calibration curve method

4.6 Robustness
Analyzed by small variations in
* Flow rate
* Temperature
* Organic phase concentration
* pH

4.7 System Suitability Parameters[21]
* Theoretical plates
* Tailoring factor
* Resolution
* Capacity factor

5. LITERATURE REVIEW SUMMARY[22-26]

Table-1: Summary of Reported HPLC Methods for Anti-Viral Agents.

Drug	Column	Mobile Phase	Detection	LOD/LOQ	Key Findings
Zidovudine	C18	ACN: Buffer	UV 265 nm	0.5/1.5 µg/mL	Simple RP-HPLC
Lamivudine	C8	Methanol: Water	UV 280 nm	0.2/0.6 µg/mL	Stability-indicating
Tenofovir	HILIC	ACN: Ammonium Formate	PDA	0.1/0.3 µg/mL	Highly polar
Dolutegravir	C18	ACN: Formate Buffer	UV 258 nm	0.05/0.15 µg/mL	Compatible with FDCs
Remdesivir	C18	ACN:Water (0.1% FA)	PDA	0.3/0.9 µg/mL	MS compatible
Favipiravir	C8	Methanol: Buffer	UV 322 nm	0.1/0.25 µg/mL	Good peak shape

6. FIGURE (TEXT REPRESENTATION)

Chromatographic Workflow for Anti-Viral Drugs
Sample Prep → Column Selection → Mobile Phase → Optimization → Validation → Application (Bulk/Formulation)

7. DISCUSSION

HPLC continues to evolve rapidly, significantly improving analytical performance for anti-viral drugs. Increasing drug complexity, emergence of pandemic-related antivirals, and fixed-dose combinations create challenges in developing robust analytical methods. Recent advancements such as UHPLC, AQbD-driven optimization, and SPP columns have considerably reduced retention times, improved separation efficiency, and allowed multi-component analysis within short runtime.

AQbD stands out as the most significant advancement, enabling systematic development through risk assessment, design of experiments (DoE), and establishment of a method operable design region (MODR). These tools have shown proven benefits in analyzing combination antiviral products such as Lopinavir/ritonavir and dolutegravir/lamivudine/Tenofovir.

Another notable shift is the increased preference for MS-compatible mobile phases and HILIC techniques to address polarity issues associated with nucleotide analogues and hydrophilic antivirals. Stability-indicating methods derived from forced degradation studies are highly important due to sensitivity of antivirals to hydrolysis, oxidation, and temperature.

Overall, modern HPLC techniques provide high sensitivity, reduced solvent consumption, improved resolution, and longer column life compared to conventional methods.

8. CONCLUSION

Method development and validation for anti-viral agents using HPLC has seen remarkable advances due to emerging analytical technologies and regulatory demands for high-quality chromatographic methods. With the rapid introduction of new antiviral drugs, optimized HPLC methods must be selective, sensitive, and stability-indicating. AQbD approaches, UHPLC systems, monolithic and SPP columns, and green analytical chemistry principles have transformed the classical approach to method development.
Future analytical methods must focus on:
* Eco-friendly solvents
* Real-time analytical monitoring
* Automation
* MS-based high-throughput methods
* enhanced stability-indicating capabilities

HPLC will continue to be the gold standard for antiviral drug quantification due to its adaptability, precision, and robustness.

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