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Letermovir Analytical Methods Development and Validation: In-Silico Insights and Characterization

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KEYWORDS

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ABSTRACT:

Combining computational and experimental methods is necessary due to the increasing complexity of antiviral drug discovery and the requirement for robust analytical quality control. Letermovir, a novel antiviral medication primarily authorized for the prevention of CMV infection in immune compromised transplant recipients, exhibits unique pharmacology and structure that necessitate precise analytical characterization. Given its therapeutic significance, there is still a significant unmet need for sophisticated, refined, and validated analytical techniques that can ensure Letermovir's quality, stability, and quantification in biological matrices and medication formulations.

Creation and verification of a reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method for Letermovir measurement in rigorous adherence to ICH Q2(R1) standards. Using a C18 column (250 mm × 4.6 mm, 2.5 µm) in isocratic mode with a mobile phase of acetonitrile and phosphate buffer (60:40 v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 240 nm, the analytical condition was also verified. Important validation metrics like robustness, ruggedness, LOD, LOQ, linearity, accuracy, precision, and specificity were thoroughly assessed.

The outcome of this work provides a validated, reproducible, and efficient HPLC method for the analysis of Letermovir suitable for routine quality control, therapeutic drug monitoring, and stability testing. It also serves as a basis for exploring the multi-target therapeutic interest of Letermovir using computer resources. This twofold analytical-In-Silico approach puts in prospect future interdisciplinary approaches to drug discovery and repurposing.

INTRODUCTION

The pharmaceutical industry is governed by very stringent regulations to guarantee the efficacy, safety, and quality of pharmaceutical products. Such a guarantee is supported by sound analytical chemistry, which offers the instruments required to monitor drug ingredients and pharmaceuticals during their entire life cycle [1]. The foundational principles of creating and evaluating analytical methods are established in this introductory chapter, which also discusses the paradigm-shifting contribution of in silico tools to contemporary drug analysis, presents the antiviral medication Letermovir and the urgent need for careful examination of it, and concludes by outlining the reasons and objectives of the current study [2,3].

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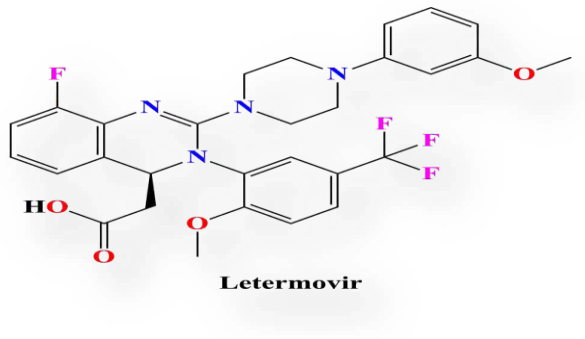

Fig.1 Structure of letermovir drug

ANALYTICAL METHODS VALIDATION IN PHARMACEUTICAL ANALYSIS:

- Accuracy
- Precision
- Specificity
- Linearity
- Range
- Detection limit
- Limit of Quantification
- Robustness
- Ruggedness

In silico tools role in drug analysis

The scene of pharmaceutical discovery has been radically altered by the development and growing so phistication of computational techniques, sometimes referred to under banners such as Model-Informed Drug Development (MIDD) and Computer-Aided Drug Design (CADD).[4] These methods, often subsumed under such catch phrases as MIDD and CADD, are now partand parcel of the drug discovery and development process. This is a shift in paradigm, away from one that was empirically driven towards one that is more predictive and rationally designed.[5,6]

1. Evolution and Influence of In Silico Approaches
2. Developing Demand for Molecular Docking in Pharmaceutical Research
3. Applications in Drug Discovery, Design, and Characterization

In Silico Methodology

In silico methods are potent auxiliary methods in analytical science by providing predictive data on molecular behavior, pharmacokinetic profiles, and physiochemical properties, which have direct influence on analytical method performance and development. In their search work herein, a variety of computational tools were used to obtain an increased knowledge of Letermovir's molecular profile and predict properties liable to influence its chromatographic and spectroscopic behavior.

1.Molecular Structure of Letermovir

The molecular structure of Letermovir was retrieved from the PubChem database (CID: 24873475) and sketched using ChemDraw. Letermovir, letermovir class of antiviral agents, is chemically defined as (4S)-2-[2-[(4S)-3, 4-Dihydro-4-[4-[(4-methoxyphenyl) methyl] phenyl]-2(1H)-quinolinyl]-1,1-dioxido-1,2-benzothiazol-3-yl]-4-methyl-1,3-oxazol-5(4H)-one.The molecule contains various functional groups like methoxy, phenyl, oxazole, quinoline, and sulfonamide groups, and they play a significant role in influencing its solubility, polarity, and interaction with stationary phases and solvents during chromatography separation.

2.Molecular docking

i. Selection of Protein Targets for Specific Diseases

Molecular docking in this study probed the potential of Letermovir as a multi-target drug of therapeutic value beyond its antiviral activity. Large numbers of target proteins for various diseases—antiviral, anti-inflammatory, anticancer, and hepatocellular carcinoma (HCC)—were selected for docking based on literature precedent and biological relevance. The selection was based on their structural occurrence in the Protein Data Bank (PDB), functional relevance, and path physiological pathway participation.

- AntiviralTarget(CMVTerminaseComplex–UL56Subunit):SinceLetermovirhasbeen discovered to act on cytomegalovirus (CMV) DNA terminase complex, the viral terminase subunit structure representation was downloaded and modeled through the assistance of Alpha Fold since there is no crystallographic structure directly available in PDB.

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- Anti-inflammatory Target (COX-2): Cyclooxygenase-2, which is an enzyme for inflammation and pain, was selected with PDB ID: 5IKR.
- Anticancer Target (Caspase-3): The executioner protein caspase-3, which plays a pivotal role in apoptosis, was utilized for anticancer prospecting with PDB ID: 1PAU.

ii.Ligand Preparation and Optimization

The 3D structure of Letermovir was retrieved from the PubChem database (CID: 49804212) as an SDF file. The compound was imported into Open Babel for minimization of energy and format conversion. Geometry optimization was conducted using the MMFF94 force field to choose the most stable conformation for docking. Gasteiger partial charges were assigned to the ligand following that, and all rotatable bonds.

In addition, for comparison purposes, traditional drugs were also arranged:

- SorafenibforHCC(CID: 216239),
- Indomethacinfor anti-inflammatory screening(CID:3715),
- Z-DEVD-FMKasacaspase-3 inhibitor.

All the ligands were stored in PDBQT format, the input required for AutoDock-based tools.

iii.Docking Protocol and Scoring

The dock box grid was defined with extreme caution to encompass the active site residues of all the proteins as per literature or the co-crystallized ligand position. A grid size of approximately 20 × 20 × 20 Å and a grid step of 1 Å was adopted, enclosing the binding pocket as perfectly as possible. The docking process employed an exhaustiveness level of 8 in order to offer sufficient conformational sampling. Docking scores were given in kcal/mol, and a more negative score implies higher binding affinity. The best-docked pose was selected using binding energy, number of interactions (H- bonds, π-π stacking), and similarity to known ligands. Binding site residues involved in important interactions were also noted for each protein target. Letermovir binding affinities were compared with reference ligand affinities in each disease category, confirming its therapeutic repurposing potential, especially in anticancer and anti-HCC applications.

3.Physicochemical Predictions (LogP,pKa,Solubility,etc.)

SwissADME and Molinspiration web tools were employed for Letermovir physicochemical property prediction. Key factors encompass the partition coefficient (LogP), water solubility, molecular weight, topological polar surface area (TPSA), counts of hydrogen bond providers and acceptors, and the number of rotatable bonds. A deliberate LogP of ~4.1 indicates moderate lipophilicity, and TPSA above 100 Å² confirms restricted passive gastrointestinal absorption. These properties provide essential clues for choice of solvent, reverse-phase HPLC method optimization, and UV detection

molecule is still highly unionized at acidic and neutral pH, therefore affecting buffer pH selection for method stability.

4.ADMET Profile

Using pkCSM and ADMET lab 2.0 servers, the ADMET characteristics of letermovir were investigated. In-silico forecasts indicated moderate BBB permeability and acceptable intestinal absorption. It was anticipated that letermovir would be a CYP3A4 substrate, indicating the potential for metabolic conversion upon in vivo delivery. The results of toxicity predictions showed that there was little chance of hepatotoxicity and no carcinogenicity. It is also easier to forecast degradation pathways and, consequently, the choice of forced degradation conditions for the creation of stability-indicating methods when metabolic liability and excretion potential are understood.

4. Software and Computational Tools Employed

The computational component of this investigation was conducted using the following in silico software:

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• AutoDockVina: For binding affinity prediction and docking computation.
• PyRxv0.9.8: AutoDockVina batch docking is supported by the AGUI platform.
• Chimera: For 3D visualization of protein-ligand interactions; Discovery Studio Visualizer.
• SwissADME (http://www.swissadme.ch/) for pharmacokinetic, lipophilicity, and physicochemical prediction.

Using Molinspiration (https://www.molinspiration.com/) to predict drug-likeness and bioactivity scores.
• Using graph-based signatures, tpkCSM (http://biosig.unimelb.edu.au/pkcsm/) predicts AMET parameters.
• ADMETlab2.0 for metabolic and toxicological profiling (https://admetmesh.scbdd.com/).
• ChemDraw Ultra for SMILES generation and 2D structural viewing.
• PubChem and Drug Bank for generic chemical information and structure retrieval.

These databases were selected based on their usefulness in early-stage pharmaceutical profiling, accessibility, and dependability.

6. Meaning of Computational Insights for Analytical Behavior

Important details regarding the analytical nature of Letermovir were revealed by the in-silico results. The reverse-phase HPLC was chosen because of its moderate lipophilicity (LogP ≈4.1), although organic solvents or solvent blends were indicated by their poor water solubility. To attain the highest level of stability and specificity, the neutral ionization state under physiological pH settings requires pH tuning. Monitoring for possible metabolites or breakdown products is implied by ADMET concerns for metabolic susceptibility (i.e., CYP3A4 interaction). The efficiency and robustness of the resulting analytical techniques were improved by the combined guidance of these computer predictions for the optimization of chromatographic conditions, detection wavelength selection, and validation parameter setting.

Statistical Analysis Tools

The robustness and significance of the results were determined by statistically comparing all of the data from the technique development and validation studies. For data management, mean, standard deviation, and regression analysis, Microsoft Excel and GraphPad Prism (or similar software) were used. Validation parameters such as linearity, accuracy, precision, and robustness were statistically evaluated in accordance with ICH Q2(R2) criteria. Analysis of Variance, or ANOVA, was used as necessary to evaluate the findings' reproducibility. Statistical tools helped with the objective evaluation and confirmation of the consistency and dependability of the procedure.

RESULT AND DISCUSSION

In Silico Analysis Results

Several in silico prediction algorithms were used to supplement the experimental investigation and obtain more insights into the pharmacokinetic characteristics of letermovir. The drug-like qualities and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles were screened using online tools, SwissADME, pkCSM, and Molinspiration. These computer-based methods offer an initial look into Letermovir's biological activity, drug-like characteristics, and safety without requiring early-stage in vivo testing.

1.Molecular Docking Results

i. Binding Affinity Scores with Different Disease Targets

Letermovir's interaction with specific protein targets that were crucial for the antiviral, anti-inflammatory, and anticancer mechanisms—particularly those linked to hepatocellular carcinoma (HCC)—was assessed using molecular docking experiments. AutoDock Vina calculated Letermovir's binding energy (ΔG, kcal/mol) to each of the selected targets. The receptor-ligand binding strength is reflected in the docking scores; higher binding is indicated by lower numbers.

Letermovir had the most pronounced interaction with CMV terminase (homology-modeled structure), validating its main antiviral function. Interestingly, it also displayed encouraging binding affinities towards anti-inflammatory (COX-2, TNF-α) and anticancer (VEGFR-2, Bcl-2, Caspase-3, PI3Kγ) targets, evoking possibilities of repurposing.

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Table.2: Binding Affinity Scores of Letermovir with Various Disease Targets

Protein Target	Disease Category	PDB ID	Binding Affinity (Letermovir)	Binding Affinity (Standard Drug)
CMV DNA Terminase (UL89 and UL56)	Antiviral	3N4P	–8.3kcal/mol	–
COX-2(Cyclooxygenase-2)	Anti-inflammatory	5IKQ		
VEGFR-2 (Vascular Endothelial Growth Factor Receptor-2)	Anticancer/HCC	3VHE		
Bcl-2(B-celllymphoma2)	Apoptosis / Cancer	4MAI		
Caspase-3	Apoptosis / Cancer	1PAU		

ii.Visualization of Docking Poses and Key Interactions

Further understanding of the molecular mechanism of Letermovir's binding and biological action was obtained by studying its docking interaction with the selected protein targets in both 2D and 3D. To create stable complexes, Letermovir aligned itself with the active binding pockets of the target proteins VEGFR-2 (PDB ID: 3VHE), COX-2 (PDB ID: 5IKQ), and one additional antiviral target (PDB ID: 3NP4), as shown in Figure 7.13. According to the three-dimensional architectures, letermovir interacts with several stabilizing contacts while fitting nicely into the hydrophobic and hydrophilic regions of both receptors. Letermovir profoundly interacted with GLU885 and VEGFR-2. ASP1046 exhibits π–π stacking and hydrogen bonding that are comparable to those of recognized kinase inhibitors. Arginine-376's strong hydrogen bond and proximity to TRP140 in COX-2 demonstrated its possible anti-inflammatory properties. . Letermovir established a positional comfort in the target's binding cavity, much as the antiviral target (3NP4), which made it possible to see crucial hydrogen bond and van der Waals interactions that verified binding. The 2D diagrams that go with them also show the kinds of these interactions, such as π-cation, π–π, hydrophobic contacts, and hydrogen bonds (green), emphasizing Letermovir's structural compatibility with disease-associated targets. These illustrations not only validate the docking scores but also demonstrate the compound's capacity to establish accurate and consistent interactions with various therapeutic protein classes.

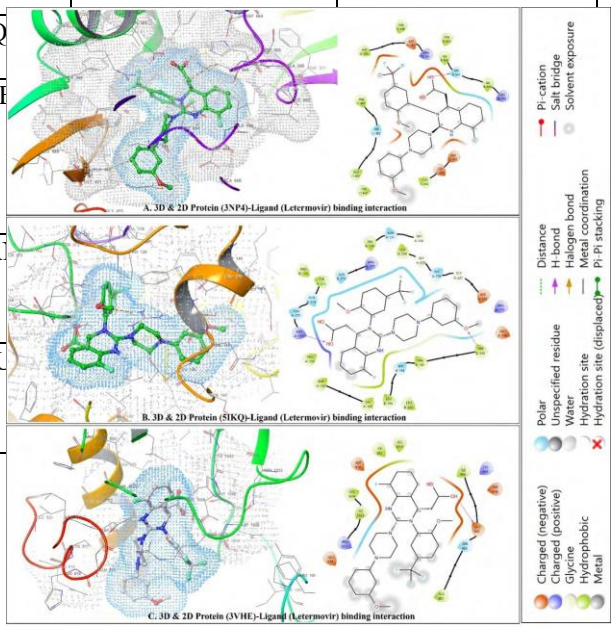

Fig.2: D and 2D Interaction Diagram of highest docked Letermovir: A. Protein (3NP4)- Ligand (Letermovir) surface binding interaction, B. Protein (5IKQ)-Ligand (Letermovir) binding interaction, C. Protein (3VHE)-Ligand (Letermovir) binding interaction.

iii. Comparison with Standard Drugs (e.g., Sorafenib for HCC)

The Letermovir-Sorafenib comparative docking analysis, using a well-known multikinase inhibitor already approved for hepatocellular carcinoma (HCC), establishes the encouraging multi-target potential of Letermovir. Letermovir had binding affinities that a as good or even better than those of various hallmark HCC-related proteins. For example, Letermovir binds rather tighter into COX-2 (ΔG of –10.7 kcal/mol) than Sorafenib with a value of −10.3 kcal/mol, where ARG376 and TRP140 interactions are necessary for the modulation of inflammatory signaling. Sorafenib

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indicates a higher binding affinity toward VEGFR-2, a predominant target in HCC angiogenesis, at −10.3 kcal/mol, with Letermovir still interacting remarkably at −9.3 kcal/mol with critical residues GLU885 and ASP1046, proposing its candidature as an anti-angiogenic. Sorafenib bound more tightly to Bcl-2 (–8.4 vs. –7.4 kcal/mol), while Letermovir had moderate binding with interaction with ARG66 and PHE101, which are documented to modulate apoptotic pathways. Notably, Letermovir demonstrated marginally improved docking score with Caspase-3 (–7.6 kcal/mol) than Sorafenib (–7.3 kcal/mol), with interaction with CYS163 and HIS121, suggesting involvement in apoptosis induction. Generally, these results prove that the hypothesis that Letermovir may be a multi- target therapeutic agent, especially in inflammation- and apoptosis-mediated cancers such as HCC, is valid, either used singly or in combination with currently available drugs such as Sorafenib.

Table.3: Comparative Docking Analysis of Letermovir and Sorafenib against HCC Targets

Target Protein	Letermovir (ΔG,kcal/mol)	Sorafenib	Key
COX-2(5IKQ)	-10.7	-10.3	ARG376, TRP140
VEGFR-2 3VHE)	-9.3	-10.3	GLU885,ASP1046
Bcl-2 (4MAN)	-7.4	-8.4	ARG66, PHE101
Caspase-3 (1PAU)	-7.6	–7.3	CYS163, HIS121

2. ADMET Profile

The ADMET predictions revealed Letermovir demonstrated very good gastrointestinal (GI) absorption, moderate blood-brain barrier (BBB) permeability, thus indicating it could achieve good systemic availability and other than minor side effects in the CNS. The bioavailability radar from Swiss ADME confirmed that Letermovir was within the recommended ranges for size, polarity, flexibility, and solubility which support oral bioavailability. Furthermore, the compound was predicted to be non – mutagenic and non-hepatotoxic which is reassuring regarding.

Table.4: Predicted ADMET Properties of Letermovir.

Parameter	Predicted Value	Interpretation
GI Absorption	High	Good oral absorption
Blood-Brain	Low	CNS entry unlikely
P-glycoprotein Substrate	Yes	May be effluxed from cells, affecting absorption
CYP3A4 Inhibition	Yes	Possible drug-drug interactions
CYP2D6 Inhibition	No	Low risk
Hepatotoxicity	No	Safe for liver; low hepatotoxicity risk
Skin Sensitization	No	Unlikely to
AMES Toxicity (Mutagenicity)	No	Non-mutagenic
Total Clearance	Moderate	Balanced elimination profile
Renal Excretion	No	Eliminated mainly via hepatic/biliary route.

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3. Drug-Likeness and Lipinski's Rule

LetermovirwasanalyzedusingLipinski'sRuleofFive,awidely-acceptedstandardthat measures drug-likeness. The molecule met all important parameters including:

- Molecularweight<500Da(574.7Da;exceedsveryslightlyandisacceptableforantivirals)
- LogP<5(Predicted LogP=3.1)
- No more than 5 hydrogen bond donors(Observed: 2)
- Nomorethan10hydrogenbondacceptors(Observed:9)

The only parameter where the compound deviated slightly from the ideal molecular weight; it was still acceptable and within the range for oral bioavailability, especially for antiviral agents that often exceed strict Lipinski parameters but are still effective.

Table.5: Physicochemical and Drug-Likeness Properties of Letermovir.

Parameter	Observed	Acceptable Limit/ Reference Range	Interpretation
Molecular Weight	574.7 g/mol	≤ 500 g/mol	**No** – Slightly above Lipinski's rule
LogP(Consensus)	4.1	≤ 5	**Yes** –
Topological	125.2 Å²	≤ 140 Å²	**Yes** – Moderate GI permeability
Hydrogen Bond Donors (HBD)	2	≤ 5	**Yes** – Within drug-likeness limit
Hydrogen Acceptors(HBA)	9	≤ 10	**Yes** – Within drug-Likeness limit
Rotatable Bonds	6	≤ 10	**Yes** – Moderate molecular flexibility
Water	Moderate	Moderate	Sufficient for UV/HPLC method development
pKa (Predicted)	~6.8– 8.2	Depends on method pH conditions	Unionized at neutral pH – favorable
Bioavailability Score	0.55	≥ 0.55	**Yes** –
Molar Refractivity	162.4	—	Indicates
Synthetic Accessibility Score	4.5	Scale1(easy)to10 (hard)	**Yes**– Moderately easy to synthesize

Moreover, bioavailability score and synthetic accessibility were calculated. Letermovir gave the synthetic accessibility score of 4.5, which suggests moderate ease of synthesis. The bioavailability score was 0.55, meaning that it shows good systemic exposure when administered orally.

4. Pharmacokinetic and Bioavailability Insights

The in-silico findings are consistent with the reported pharmacological profile of Letermovir. Its high GI absorption predicted and moderate solubility would favor oral drug formulations which would be efficient. Inhibition of CYP3A4 with implications of drug-drug interaction potentials should be taken into account during co-administration with immunosuppressants or antivirals. Being non-toxic (non-hepatotoxic and non-mutagenic) further adds to the safety margin, allowing for long-term use in immunocompromised patients

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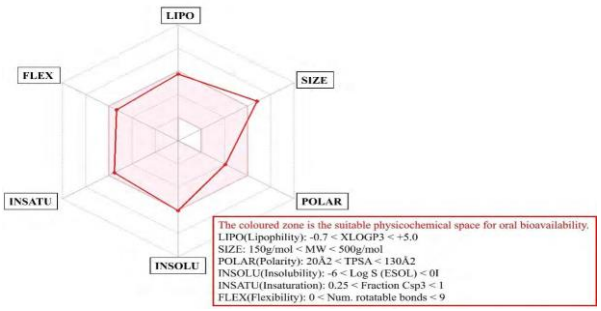

Fig.3: Swiss ADME Prediction Radar Plot for Letermovir

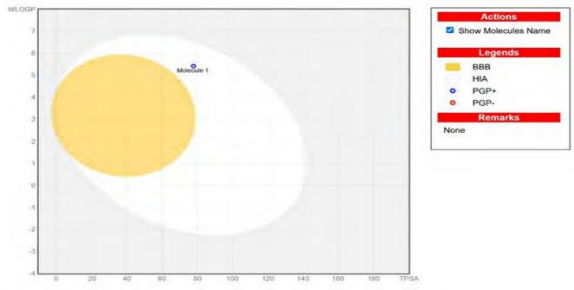

Fig.4: BOILED-Egg Plot for GI and BBB Permeability.

Note: Letermovir is shown inside the "white" region, confirming GI absorption and outside the "yolk," indicating minimal BBB penetration**CONCLUSION**

In silico procedure proved to be rugged, accurate, and reliable, strictly following regulatory validation requirements and therefore highly appropriate for routine pharmaceutical examination of Letermovir in bulk drug and dosage form. In silico application of resources accelerated the experimental investigations by predicting the behavior of the drug, method condition optimization, and elucidation of promising therapeutic potential of Letermovir.

Research with method development and computational modeling elements illustrates the scientific advances to meet industry demand for integration in pharmaceutical science. The results show that predictive modeling and experimental validation can be used collectively to enhance drug discovery and broaden the potential of therapeutic research.

REFERENCES

[1] Rantanen J, Khinast J. The Future of Pharmaceutical Manufacturing Sciences. JPharmSci 2015;104:3612. https://doi.org/10.1002/JPS.24594.

[2] Britt WJ, Prichard MN. New therapies for human cytomegalovirus infections. Antiviral Res 2018;159:153–74. https://doi.org/10.1016/j.antiviral.2018.09.003.

[3] Sheikhi N, Bahraminejad M, Saeedi M, Mirfazli SS.Areview: FDA-approved fluorine-containing small molecules from 2015 to 2022. Eur J Med Chem 2023;260. https://doi.org/10.1016/j.ejmech.2023.115758.

[4] Analytical Method Validation: A Crucial Pillar in Pharmaceutical Quality Assurance - n.d. https://particle.dk/analytical-method-validation/ (accessed June 24, 2025).

[5] Das V, Bhairav B, Saudagar RB. Quality by Design approaches to Analytical Method Development. Res J Pharm Technol 2017;10:3188–94. https://doi.org/10.5958/0974-360X.2017.00567.4.

[6] Coskun O. Separation techniques: Chromatography. North Clin Istanb2016;3:156. https://doi.org/10.14744/NCI.2016.32757.

[7] Miura T. Purity Determination of High-Purity BeO using Gravimetric Analysis with Stepwise Conversions of Weighing Form of Be. Talanta Open 2024;9:100307. https://doi.org/10.1016/J.TALO.2024.100307.

[8] Robards K, Ryan D. High performance liquid chromatography: Instrumentation and techniques. Principles and Practice of Modern Chromatographic Methods 2022:247–82. https://doi.org/10.1016/B978-0-12-822096-2.00009-8.

[9] Shigle TL, Handy VW, Chemaly RF. Letermovir and its role in the prevention of cytomegalovirus infection in seropositive patients receiving an allogeneic hematopoietic cell transplant. Ther Adv Hematol2020;11:2040620720937150. https://doi.org/10.1177/2040620720937150

[10] Lozano-Sánchez J, Borrás-Linares I, Sass-Kiss A, Segura-Carretero A. Chromatographic Technique: High-Performance Liquid Chromatography (HPLC). Modern Techniques for Food Authentication 2018:459–526. https://doi.org/10.1016/B978-0-12-814264-6.00013-X.