European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Development and ICH Q2 (R2)-Compliant Validation of Stability-Indicating Analytical Methods for Cytomegalovirus Antiviral: A Review

Article History:

Name of Author:
Deverakonda Ashlesha[1*] and Narayana Swamy Harikrishnan[2]

Affiliation: *[1]Research Scholar, Department of Pharmacy, Dr. M.G.R. Educational and Research Institute, Velappanchavadi, Chennai – 600077, Tamil Nadu, India*
[2]Faculty of Pharmacy, Department of Pharmacy, Dr. M.G.R. Educational and Research Institute, Velappanchavadi, Chennai – 600077, Tamil Nadu, India

Corresponding Author:

Deverakonda Ashlesha[1*] and Narayana Swamy Harikrishnan[2]

Received: 22-12-2025
Revised: 14-01-2025
Accepted: 30-01-2026
Published: 02-02-2026

Abstract: Cytomegalovirus (CMV) disease remains a major threat in hematopoietic stem cell and solid-organ transplant recipients and in congenital infection, where prophylaxis and pre-emptive therapy require robust drug measurement. This review develops a mechanism-based roadmap for stability-indicating analytical methods (SIAMs) for the six approved anti-CMV agents ganciclovir, valganciclovir, foscarnet, cidofovir, letermovir, and maribavir inking molecular properties and degradation pathways to platform choice and to ICH Q2(R2) validation evidence, with development concepts aligned to ICH Q14. Highly polar nucleoside/nucleotide analogues (ganciclovir, valganciclovir, cidofovir) often show poor retention on conventional C18 and benefit from HILIC, ion-pair, or mixed-mode separations, with tight control of prodrug hydrolysis (valganciclovir to ganciclovir). Foscarnet, an inorganic poly-anion with minimal UV absorbance, is most defensibly quantified by ion chromatography with suppressed conductivity or by MS in negative mode, whereas the more lipophilic agents letermovir and maribavir are readily handled by fast RP-UPLC-DAD and LC–MS/MS. Forced degradation consistent with ICH Q1A/Q1B is positioned as the core specificity experiment, supported by peak-purity assessment, mass-balance tracking, and when needed high-resolution MS-based degradant assignment. We summarize Q2(R2) requirements (specificity, linearity, accuracy, precision, LOQ/LOD, robustness, and system suitability) and bioanalytical controls aligned with ICH M10. Furthermore, the application of Quality by Design (QbD) principles is highlighted as a means to enhance method robustness and regulatory acceptance. Overall, the review emphasizes the need for tailored analytical approaches to address drug-specific challenges, ensuring accuracy, selectivity, and sensitivity across pharmaceutical and clinical applications, thereby supporting the development of safe and effective CMV antiviral therapy.

Keywords: Cytomegalovirus, Antiviral agents, RP-HPLC, LC–MS/MS, Stability-indicating methods, Quality by Design (QbD).

INTRODUCTION

Cytomegalovirus (CMV) infections remain a major cause of morbidity and mortality in immunocompromised hosts, including hematopoietic stem cell transplant (HSCT) and solid organ transplant recipients, and in congenital infection. Current clinical management relies on **six** approved antivirals ganciclovir, valganciclovir, foscarnet, cidofovir, letermovir, and maribavir whose diverse polarity, ionization behavior, and intrinsic stability govern analytical performance across quality control (QC), stability, and bioanalysis. HCMV establishes lifelong latency with periodic reactivation; in transplant recipients, CMV disease is linked to rejection risk and reduced survival, necessitating prophylaxis or pre-emptive strategies supported by reliable drug measurement (1). Each exhibits distinct chemical structures and physicochemical properties, influencing their chromatographic retention, UV absorbance, and ionization efficiency in mass spectrometry. Human cytomegalovirus (HCMV) is a β-herpesvirus that establishes lifelong latency with the potential for reactivation (2). In solid-organ and hematopoietic stem-cell transplant recipients, CMV

disease increases rejection risk and mortality, necessitating antiviral prophylaxis and pre-emptive therapy. The CMV antiviral armamentarium comprises: (i) nucleoside analogue ganciclovir and its oral prodrug valganciclovir, (ii) non-nucleoside pyrophosphate analogue foscarnet, (iii) nucleotide analogue cidofovir, and (iv) newer agents letermovir (terminase complex inhibitor) and maribavir (UL97 kinase inhibitor). Each molecule presents distinct analytical challenges due to polarity, ionizability, and susceptibility to degradation (e.g., hydrolysis, oxidation, and photolysis) (3).

METHODOLOGY

A structured narrative review was conducted to summarize stability-indicating analytical method (SIAM) development and ICH Q2(R2) aligned validation for approved CMV antivirals (ganciclovir, valganciclovir, foscarnet, cidofovir, letermovir, maribavir). PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar were searched using drug names combined with stability-indicating, forced degradation, HPLC/UPLC, HILIC, ion chromatography, LC–MS/MS, Q2(R2), and Q14. Eligible studies reported assay/impurity/stability or bioanalytical methods with extractable conditions and/or validation data. Data extracted included platform, chromatographic parameters, sample preparation, stress design, specificity evidence, and validation outcomes. Findings were synthesized by drug properties and intended use.

This review is organized around a mechanism-based analytical framework: (i) drug physicochemical properties dictate retention and peak shape (RP-HPLC vs HILIC vs ion chromatography), (ii) degradation pathways dictate the stress design and degradant profile required to prove specificity, and (iii) intended use (assay/impurities/stability vs PK/TDM) dictates validation scope and performance requirements (4). Using this framework, we synthesize reported methods and translate them into ICH Q2 (R2)-compliant validation expectations for stability-indicating procedures, complemented by the science- and risk-based development concepts in ICH Q14 (5).

Drug Profiles and Analytical Challenges

Ganciclovir (GCV)

A polar acyclic guanine nucleoside analogue with poor lipophilicity (logP ≈ –0.7), GCV demonstrates limited retention on conventional C18 columns unless mobile phases with high aqueous content or polar-embedded stationary phases are used (6). Its purine ring absorbs UV strongly at ~254 nm, enabling UV-based assays. In biological matrices, LC–MS/MS is preferred for sensitivity.

Valganciclovir (VGCV)

The L-valyl ester prodrug of GCV improves oral bioavailability but is prone to in vitro hydrolysis, necessitating cold storage and rapid analysis (7). Chromatographic conditions are similar to GCV, with slightly higher organic content improving peak shape.

GCV and VGCV: Highly polar, multiple hydrogen-bond donors/acceptors; weak UV chromophores (π–π* transitions ≈ 250–260 nm). Retention on conventional C18 is limited without ion-pairing, HILIC, or mixed-mode phases.

Foscarnet (FOS)

An inorganic pyrophosphate analogue with negligible UV absorbance, FOS is poorly retained in RP-HPLC. Ion chromatography with conductivity detection or LC–MS/MS in negative ion mode is preferred (8). An inorganic poly-anionic phosphonoformate; lacks UV chromophore; best measured by ion chromatography with conductivity detection or derivatization for UV/fluorescence; ICP-MS can quantify phosphorus as a surrogate.

Cidofovir

Highly polar phosphonate nucleotide analogue; similar retention issues to GCV. Ion-pair RP-HPLC or HILIC are effective; LC–MS/MS is favored bioanalytically. An acyclic cytidine nucleotide with a charged phosphonate group, cidofovir requires ion-pair chromatography or HILIC for adequate retention. UV absorbance is modest (~260–270 nm). MS detection in negative mode offers higher sensitivity (9).

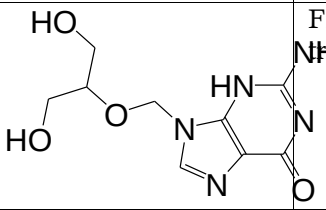
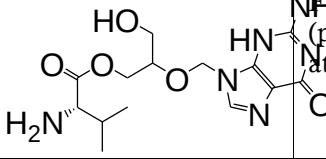
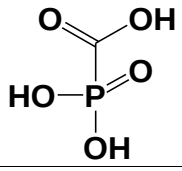
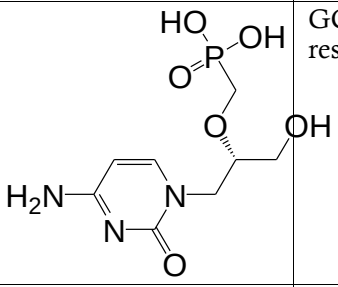
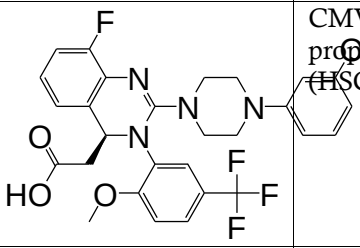
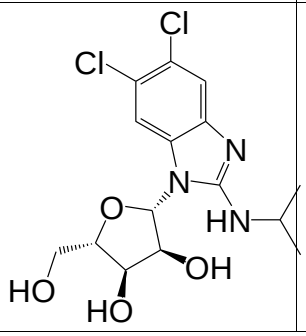
Letermovir (LTV)

More lipophilic; strong UV absorption around 240–260 nm; readily retained on RP phases; MS-friendly mobile phases enable high-sensitivity LC–MS/MS (10).

Maribavir (MBV): Moderately lipophilic benzimidazole derivative; good UV chromophore; RP-HPLC amenable; LC–MS/MS widely adopted (11).

A novel CMV terminase inhibitor with lipophilic properties and strong UV absorbance in the mid-UV range, LTV is MS-friendly (ESI^+) and easily retained on RP columns (10, 11). Table 1 provides a consolidated overview of approved anti-CMV agents, contrasting their primary clinical indications with mechanism-linked resistance pathways (UL97, UL54, UL56) and the toxicity profiles that most often determine drug selection and switching in transplant practice

Table 1: General overview of anti-cytomegalovirus (CMV) drugs: clinical use, resistance mechanisms, and toxicity

Drug	Structure	Primary Use	Resistance Mechanism	Toxicity
GCV		First-line CMV treatment	UL97 kinase mutation	Myelosuppression
VGCV		First-line (prophylaxis/treatment)	UL97 mutation	Myelosuppression
FOS		GCV-resistant CMV	UL54 mutation	Nephrotoxicity, electrolyte imbalance
Cidofovir		GCV/FOS-resistant CMV	UL54 mutation	Severe nephrotoxicity
LTV		CMV prophylaxis (HSCT)	UL56 mutation	GI upset, mild LFT elevation
MBV		The treatment of cytomegalovirus (CMV) infection in post-transplant patients	CMV UL97 gene	Dysgeusia

Analytical Techniques

For CMV antivirals, polarity and ionization dominate retention and detection performance, while the intended context (QC/stability vs PK/TDM) determines sensitivity, selectivity, and validation breadth.

Bulk Drug Analysis

Bulk assays are typically performed using RP-HPLC with PDA detection for GCV, VGCV , and LTV. Mobile phases often employ phosphate or formate buffers (pH 3–4) with acetonitrile gradients. For cidofovir, ion-pair agents (e.g., tetrabutylammonium) are incorporated. FOS analysis often relies on conductivity or MS detection due to weak UV absorption (12-14).

Dosage Form Analysis
Tablets, capsules, and injections are analyzed using gradient elution to resolve active ingredients from excipients. For VGCV, attention is required to prevent hydrolysis to GCV during sample prep. LTV's lipophilicity allows shorter run times (3–5 min) with higher organic content (12-14).

Bioanalytical Pharmacokinetic Applications
LC–MS/MS is preferred for PK studies, providing ng/mL sensitivity. GCV, VGCV, and LTV are quantified in positive ion mode, while cidofovir and FOS are measured in negative mode. Protein precipitation (PPT) is adequate for most, though SPE improves cleanliness for long runs (15-18).
UV–Visible Spectrophotometry

UV–Vis spectrophotometry remains suitable for single-component assay in uncomplicated matrices when specificity risks are low. However, UV methods are rarely stability-indicating without prior separation because degradants and excipients may share absorbance bands; therefore, UV-only methods are best positioned as screening or supportive assays rather than SIAMs (A stability-indicating analytical method). Useful for **assay of bulk drug and simple formulations** of GCV, VGCV, LTV, and MBV, where excipient interference is minimal. Methods use λ_max ~250–260 nm (drug-dependent), aqueous buffers (pH 2–7), and Beer's law verified over ≈5–50 µg/mL. Derivative spectrophotometry or chemometrics can resolve overlapping spectra in fixed-dose combinations. UV lacks specificity for complex matrices and is generally not stability-indicating without prior separation (15-18).

HPLC/UPLC with UV/DAD Detection
RP-HPLC/UPLC with UV/DAD is the primary platform for assay and impurity/stability testing when analytes are adequately retained and separated. DAD adds two regulatory-useful outputs peak purity assessment and spectral comparison of parent vs degradants supporting specificity claims in SIAM (A stability-indicating analytical method) packages. For lipophilic drugs (e.g., letermovir, maribavir), RP-UPLC enables short cycles with robust retention and resolution.RP-HPLC **on C_{18}/C_8 remains the workhorse for assay, impurities, and dissolution. For very polar analytes (GCV, cidofovir),** ion-pair RP **(e.g., 2–10 mM heptafluorobutyric acid) or** HILIC (amide, zwitterionic) phases are effective.**UPLC** shortens run times and improves resolution (sub-2 µm columns). DAD enables peak purity and degradation tracking (15-18).

LC–MS/MS
LC–MS/MS is the reference approach for PK/TDM because it provides high selectivity in complex matrices and sensitivity at ng/mL levels. For stability-indicating claims, MS (and especially HRMS) strengthens degradant tracking by enabling ion-based peak assignment and structural proposals. In bioanalysis, matrix effect testing, carryover control, and internal standard strategy are central to method validity and are increasingly harmonized through modern guidance. Gold standard for **bioanalytical PK/TDM** due to sensitivity/selectivity in plasma, serum, and intracellular matrices. Typical workflows use protein precipitation or SPE, reversed-phase separation with volatile buffers (0.1% formic acid or ammonium formate), and multiple-reaction monitoring (MRM). Matrix-effect assessment and stable-isotope internal standards are critical (19-24).
Ion Chromatography (IC)

IC is most defensible for foscarnet because it lacks a useful UV chromophore and behaves as an inorganic poly-anion. Suppressed conductivity is direct and robust for ionic analytes, and IC can be paired with MS where needed for confirmation. IC is most defensible for foscarnet because it lacks a useful UV chromophore and behaves as an inorganic poly-anion. Suppressed conductivity is direct and robust for ionic analytes, and IC can be paired with MS where needed for confirmation. Best suited for FOS quantification with suppressed conductivity detection.Also useful for residual inorganic counterions and buffer species in formulations.
Capillary Electrophoresis (CE)

CE (CZE or MEKC) offers rapid, low-solvent separations for highly polar antivirals (GCV, cidofovir) and their impurities. CE–MS hyphenation improves specificity but requires careful interface optimization.
HPTLC

Applied to identity/assay screens and stability trend analyses; densitometric quantification at λ max. Less sensitive than HPLC but economical for routine QC.

Method Development and Optimization

Chromatographic mode selection:

Use RP-C18 for LTV/MBV; consider gradient (10–90% acetonitrile) with 0.1% formic acid. For GCV /cidofovir, start with HILIC (acetonitrile ≥70%, 10–20 mM ammonium formate, pH 3–4) or RP with ion-pair modifier; evaluate peak shape and robustness. For FOS, select IC with carbonate/bicarbonate eluent and suppressed conductivity (25-28).

pH and buffer:
Maintain MS compatibility (ammonium formate/acetate, ≤10–20 mM). For UV-only impurity methods, phosphate buffers (10–50 mM) yield robust retention but are not MS-friendly.

Detection:
DAD for specificity; MS/MS MRM transitions tuned for each analyte (e.g., LTV $[M+H]^+$ → prominent product ion; GCV may favor $[M+H]^+$ with low cone voltage).

Sample preparation:

- **Solid oral dosage:** simple dissolution and filtration; for impurity methods, dilute-and-shoot or SPE cleanup.
- **IV solutions:** direct dilution; protect light-sensitive analytes.
- **Plasma/serum:** protein precipitation (acetonitrile or methanol), or mixed-mode SPE for polar analytes to reduce matrix effects.

Intracellular triphosphates (GCV -TP): cell lysis with cold methanol, ion-pair or HILIC LC–MS/MS; alternatively, enzymatic dephosphorylation to parent nucleoside, then quantification (29-32).

System suitability:
Set plate count, tailing factor (≤2.0), resolution (≥2.0) between critical pairs, %RSD of replicate injections (≤2.0% assay; ≤5.0% impurities).

Stability-Indicating and Forced-Degradation Studies
Stability-indicating analytical methods are designed to separate the analyte from its potential degradation products and to demonstrate peak purity, thereby ensuring specificity and reliability of the analytical results. Forced degradation studies are performed in accordance with ICH Q1A (R2) guidelines to evaluate the chemical stability of the drug under various stress conditions (33). Hydrolytic degradation is assessed under acidic and basic environments using 0.1–1 N hydrochloric acid or sodium hydroxide at temperatures ranging from 60 to 80 °C for 2 to 24 hours, with the solutions being neutralized prior to analysis. Oxidative degradation is typically conducted with 1–3% hydrogen peroxide either at ambient temperature or at 60 °C to determine the compound's susceptibility to oxidative stress. Thermal degradation studies involve exposing the sample to dry heat and humid conditions between 60 and 80 °C to evaluate its thermal stability. Photolytic degradation is carried out in compliance with ICH Q1B, subjecting the samples to visible light exposure of 1.2 million lux hours and ultraviolet radiation of 200 watt hours per square meter, both with and without protective packaging, to assess photostability. Reductive degradation studies, when applicable, include the use of reducing agents such as sodium metabisulfite to examine the behavior of reducible moieties. Throughout all studies, the mass balance comprising the assay, degradation products, and any volatile losses is carefully monitored, and degradation kinetics are qualitatively reported. For specific compounds such as fosfomycin (FOS), evaluation focuses on pH-dependent precipitation and counterion exchange rather than classical organic degradation pathways. In the case of nucleoside and nucleotide analogues, attention is directed toward monitoring de-esterification reactions (e.g., VGCV to GCV conversion), depurination, and phosphonate hydrolysis, as seen with cidofovir.

Validation (ICH Q2 (R2))
Specificity: chromatographic separation with peak purity/DAD spectra and, where relevant, MS detection of degradants (33).

Linearity: at least 5 levels; typical ranges: assay 50–150%; impurities LOQ–150%; bioanalytical LLOQ to ≥10× therapeutic C_max.

Accuracy/Recovery: 98–102% for assay; impurity recovery within 80–120% depending on level; bioanalytical mean bias within ±15% (±20% at LLOQ).

Precision: repeatability %RSD ≤2% (assay); intermediate precision assessed across days/analysts.

LOD/LOQ: signal-to-noise or SD/slope approaches; for impurities, LOQ ≤ reporting threshold.

Robustness: deliberate small changes in pH (±0.2), organic ratio (±2%), column temperature (±5 °C), flow (±0.1 mL/min).

System suitability established and trended.

Bioanalytical methods additionally require selectivity, carryover, matrix effect, reinjection reproducibility, stability (bench-top, freeze–thaw, autosampler), dilution integrity, and alignment with regional bioanalytical guidance. Table 2 shows that regulatory evolution affecting stability-indicating analytical procedures (SIAM) and bioanalysis.

Table 2: Regulatory evolution affecting stability-indicating analytical procedures (SIAM) and bioanalysis

Regulatory item	Focus	What changed / emphasized	Practical impact on SIAM design and validation
ICH Q1A (stability principles) + Q1B (photostability)	Stress study design	Use of stress conditions to understand degradation behavior and establish stability-indicating power	Forces deliberate degradant generation; requires mass-balance awareness, peak tracking, and rationale for stress severity
ICH Q2(R2)	Validation for release & stability procedures	Updated terminology and fit-for-purpose validation expectations for modern analytical procedures used in release/stability	Stronger specificity demonstrations; clearer linkage of reportable levels to LOQ; broader acceptance of data types when scientifically justified
ICH Q14	Analytical procedure development	Science- and risk-based development; MODR/design space concepts; complements Q2(R2)	Encourages systematic CMP identification, DoE, and control strategies that reduce post-approval friction
FDA implementation notes for Q2(R2)/Q14	Regulatory adoption	Formal posting of Q2(R2)/Q14 as guidances replacing prior drafts	Reinforces expectation that submissions justify development knowledge and lifecycle control strategy
USP <1220>	Lifecycle management	Three-stage lifecycle (design, qualification, continued verification)	Pushes ongoing monitoring and trending of system suitability/precision; supports controlled method changes
ICH M10 (bioanalytical validation)	Bioanalysis (PK/TDM)	Harmonized expectations for chromatographic bioanalysis and study sample analysis	Strengthens matrix effect, ISR, stability, and carryover controls critical for CMV TDM methods

RESULTS

GCV / VGCV

Quality control assays and impurity profiling are typically performed using hydrophilic interaction liquid chromatography (HILIC) coupled with diode array detection (DAD) or by employing reversed-phase ion-pair HPLC methods. A representative chromatographic condition involves an amide-based HILIC column (100 × 2.1 mm, 1.7 µm) with a mobile phase comprising acetonitrile and 10 mM ammonium formate buffer (pH 3.5) in a ratio of 85:15, followed by a linear gradient to 70:30 over six minutes. Detection is carried out at 254 nm with a total run time of approximately 10 minutes. The stability-indicating method ensures adequate separation of the ester prodrug from the parent drug and its hydrolysis products, effectively monitoring degradation under acidic, basic, and oxidative conditions, such as the conversion of valganciclovir (VGCV) to ganciclovir (GCV). For bioanalytical applications, quantification is achieved using LC–MS/MS with solid-phase extraction (SPE), employing multiple reaction monitoring (MRM) in the positive ionization mode. The method typically achieves

a lower limit of quantification (LLOQ) in the range of 0.01–0.05 µg/mL in plasma, suitable for therapeutic drug monitoring (TDM). Intracellular concentrations of the active metabolite, ganciclovir triphosphate (GCV-TP), can be determined using HILIC-MS/MS, often involving ion-pairing chromatography or enzymatic dephosphorylation workflows to facilitate accurate quantitation (28–30, 34, 35-40).

FOS

Quality control assays for such compounds are often performed using ion chromatography (IC) with suppressed conductivity detection, employing carbonate or bicarbonate-based eluents to ensure precise quantification of ionic species. As an orthogonal or confirmatory technique, inductively coupled plasma mass spectrometry (ICP-MS) targeting the phosphorus atom can be used to verify elemental composition or detect trace impurities. Stability studies focus on evaluating the influence of pH variations, ionic strength, and complexation with divalent cations such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), as these factors can significantly affect compound solubility and integrity. For bioanalytical purposes, ion chromatography can be applied directly to serum or urine matrices with minimal sample preparation, while advanced workflows may employ liquid chromatography–mass spectrometry (LC–MS) following derivatization for enhanced sensitivity and selectivity. In impurity profiling, reversed-phase ion-pair HPLC using mobile phases containing low concentrations of ion-pairing agents such as 5 mM heptafluorobutyric acid (HFBA) or HILIC-DAD methods are commonly utilized to assess phosphonate-related degradants and other polar impurities. For quantitative bioanalysis, LC–MS/MS in negative electrospray ionization (ESI) mode often provides superior response characteristics for acidic or phosphorylated analytes, achieving lower limits of quantification (LLOQ) in the low nanogram-per-milliliter range when combined with solid-phase extraction (SPE) enrichment (31, 34, 38, 41).

LTV

Quality control assays and impurity profiling are typically performed using reversed-phase ultra-performance liquid chromatography (RP-UPLC) with formic acid–based buffer systems, providing efficient peak resolution and reproducibility. Detection is generally achieved using a diode array detector (DAD) at approximately 254 nm, and the method allows for a total runtime of less than five minutes, ensuring rapid analysis suitable for routine quality control. For bioanalytical applications, quantification is conducted using LC–MS/MS in the positive ionization mode, following protein precipitation for sample cleanup. The method demonstrates a wide linear dynamic range, making it highly suitable for pharmacokinetic (PK) studies. Stability assessments indicate moderate sensitivity to oxidative and photolytic conditions, while the analyte remains chemically stable under neutral aqueous environments, confirming the robustness of the developed analytical procedure (25–28, 34, 42, 43).

MBV

Quality control assays and impurity profiling are generally performed using reversed-phase high-performance or ultra-performance liquid chromatography (RP-HPLC/UPLC) with a mobile phase consisting of acetonitrile and water containing 0.1% formic acid. Detection is typically carried out using a diode array detector (DAD) within the 254–280 nm wavelength range to ensure optimal sensitivity and selectivity for aromatic or conjugated analytes. For bioanalytical quantification, LC–MS/MS methods are employed utilizing deuterated internal standards to improve accuracy and compensate for matrix effects. The method further incorporates rigorous evaluation of metabolite interference to ensure specificity and reliability in complex biological matrices such as plasma or serum. Table 3 shows that the representative analytical methods for CMV antivirals (34).

Table 3: Comparative overview of analytical methods for CMV antivirals: separation conditions, detection modes, retention time, and LOD/LOQ

Drug	Column	Mobile Phase & Gradient	Flow Rate (mL/min)	Injection Vol (µL)	Detection	Retention Time (min)	LOD/LOQ
GCV (28)	C18, 150×4.6 mm, 5 µm	A: 10 mM ammonium formate pH 3.5; B: ACN; 95:5→85:15 in 6 min	1.0	20	UV 254 nm / ESI+	2.1	0.05/0.15 µg/mL

VGCV (29,30)	Polar-embedded C18, 150×4.6 mm	Same as GCV; 90:10→80:20 in 6 min	1.0	20	UV 254 nm / ESI+	4.3	0.08/0.25 µg/mL
FOS (31)	Anion-exchange, 250×4.0 mm	5 mM ammonium bicarbonate, isocratic	1.0	50	Conductivity / ESI–	1.8	0.1/0.3 µg/mL
Cidofovir (32)	HILIC amide, 150×4.6 mm	70:30 ACN:10 mM ammonium formate	0.8	10	UV 265 nm / ESI–	3.0	0.06/0.20 µg/mL
LTV (25-28)	C18, 100×4.6 mm, 3 µm	40:60→20:80 in 4 min	1.0	10	UV 300 nm / ESI+	7.8	0.02/0.06 µg/mL

Bioanalytical / TDM Method (LC–MS/MS)

Plasma samples ranging from 50 to 200 µL are prepared by adding a stable isotope-labeled internal standard (IS) to account for extraction variability and ion suppression. Proteins are precipitated using three to four times the plasma volume of cold acetonitrile (ACN), followed by centrifugation to obtain a clear supernatant, which is subsequently diluted with mobile phase before injection. For highly polar analytes such as ganciclovir (GCV) or cidofovir (CDV), improved sample cleanliness and reduced matrix effects are achieved using mixed-mode weak anion or cation exchange solid-phase extraction (SPE) cartridges. Chromatographic separation is typically achieved on a C18 column (2.1 × 50 mm, 1.7–3 µm) using a mobile phase consisting of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) with a fast linear gradient over 2–4 minutes, ensuring high throughput and reproducible retention. Mass spectrometric detection is performed on an LC–MS/MS system, where source parameters are optimized for each analyte, and at least two multiple reaction monitoring (MRM) transitions per compound are monitored to confirm identity one serving as the quantifier and the other as a qualifier ion. The validated method achieves lower limits of quantification (LLOQ) suitable for clinical monitoring, such as 0.05–0.1 µg/mL for ganciclovir, ensuring accurate measurement within the typical therapeutic trough concentration range (0.5–2 µg/mL). Matrix effects, including ion suppression or enhancement, are evaluated using post-extraction addition techniques, with precision and bias maintained within ±15%, and ±20% at the LLOQ, in accordance with regulatory bioanalytical method validation guidelines. Stability assessments encompass short-term, long-term (−20 to −80 °C), freeze–thaw (≥3 cycles), autosampler (4–24 h), and processed sample stability to ensure analyte integrity throughout sample handling. For intracellular nucleotide triphosphate metabolites, quantification is performed using HILIC or ion-pair LC–MS/MS, and when direct triphosphate detection is unstable, enzymatic dephosphorylation protocols are adopted to improve robustness and reproducibility (40-45).

Impurity Profiling and Identification

The characterization of process- and degradation-related impurities is performed in compliance with ICH guidelines, establishing appropriate reporting, identification, and qualification thresholds to ensure product safety and analytical integrity. Comprehensive peak tracking is carried out using diode array detection (DAD) to assess peak purity and high-resolution mass spectrometry (MS) to determine accurate mass values for empirical formula estimation. Further MS/MS fragmentation analysis aids in the elucidation of structural proposals for degradation products and process-related impurities. Orthogonal analytical confirmation is employed where feasible, utilizing nuclear magnetic resonance (NMR) spectroscopy to confirm the structure of major degradants, while ion chromatography (IC) is applied for the detection and quantification of inorganic or counterion-related impurities, particularly relevant for FOS and other phosphonate-containing compounds. A robust control strategy is implemented through spiking studies, determination of relative response factors (RRFs), and comprehensive mass balance evaluations, ensuring the analytical method is capable of accurately detecting, quantifying, and differentiating all relevant impurities, thereby confirming its suitability as a stability-indicating method.

Quality by Design (QbD) Considerations

Define Analytical Target Profile (ATP) (e.g., quantify LTV 80–120% label claim; Rs ≥ 2.0 to nearest impurity; LOQ ≤0.05%). Employ risk assessment (Ishikawa/FMEA) to identify critical method parameters (CMPs)—pH, % organic, column temperature, buffer strength. Use DoE (fractional factorial/central composite) to map design space and establish a control strategy (in-method system suitability limits and lifecycle trending per ICH Q14/USP <1220> principles) (Table-4).

Table 4: QbD Considerations for CMV Antiviral Drugs

Drug	Analytical Target Profile (ATP)	Critical Method Parameters (CMPs)	DoE / MODR Highlights	Control Strategy	References
GCV	Assay accuracy 98–102%; %RSD ≤2.0%; Rs ≥2.0 vs degradant/VGCV; LOQ ≤0.05%; tailing ≤1.5	%ACN, buffer type/strength, pH 3–4, column chemistry (HILIC vs ion-pair RP), temperature	HILIC: 74–78% ACN; 10–15 mM ammonium formate, pH 3.3–3.7; 35–40 °C; Rs ≥2.0	Autosampler ≤10 °C; light protection; injection solvent ≥80% ACN; system suitability Rs ≥2.0	(28–30, 34, 36, 39)
VGCV	Quantify prodrug & GCV; Rs ≥2.0 VGCV/GCV; assay accuracy 98–102%; LOQ ≤0.05%; %RSD ≤2.0%	pH (ester hydrolysis), autosampler temp, %ACN, buffer strength, ion-pair concentration	HILIC: 73–77% ACN; 10–15 mM buffer, pH 3.3–3.6, 35–40 °C; RP ion-pair: HFBA 3–6 mM, 10–18% ACN	Cold/rapid prep; autosampler ≤8 °C; monitor VGCV/GCV ratio; solution stability checks	(29, 30, 34, 37, 40)
FOS	Specific assay vs inorganic ions; accuracy 98–102%; %RSD ≤2.0%; Rs ≥2.0; runtime ≤10 min	Eluent strength (NH_4HCO_3 3–7 mM), flow, suppressor performance, temperature, matrix ions	IC MODR: 4–6 mM NH_4HCO_3, 0.9–1.1 mL/min, 30–35 °C; Rs ≥2.0 vs PO_4^{3-}	Chelators for Ca^{2+}/Mg^{2+}; suppressor calibration; conductivity baseline stability; system suitability vs phosphate	(31, 34, 38, 41)
CDV	Assay accuracy 98–102%; %RSD ≤2.0%; Rs ≥2.0 vs phosphonate degradants; LOQ ≤0.05%	%ACN, buffer strength, pH 3–4, ion-pair concentration, temperature, injection solvent	HILIC MODR: 72–78% ACN; 10–15 mM buffer; pH 3.3–3.6; 35–40 °C; Rs ≥2.0	Mixed-mode SPE (bioanalysis); solvent matching; guard cartridge; allowable adjustments pH ±0.2, ACN ±2%	(32, 34, 39, 42)
LTV	Fast RP-UPLC (≤6 min); assay accuracy 98–102%; Rs ≥2.0 vs impurity; LOQ ≤0.05%; tailing ≤1.5; carryover ≤20% LLOQ	Gradient start/end, slope, %ACN, pH (~3), temperature 35–45 °C, wash solvents, injection volume	MODR: Start 32–38% ACN → 72–80% by 4–5 min; buffer 5–10 mM; 38–42 °C; Rs ≥2.0	Needle wash (2-step), bracketed calibration, peak purity (DAD/MS), carryover monitoring	(25–28, 34, 40, 43)

Future research and practical implications:

For QC and stability-indicating methods, future work should prioritize lifecycle-ready control strategies, including predefined performance criteria, robustness-supported operating ranges, and transparent change-management logic consistent with the Q14/Q2(R2) framework.

For clinical PK/TDM, expanding validated microsampling and high-throughput LC–MS/MS

workflows can improve feasibility in transplant and pediatric settings, while maintaining rigorous control of matrix effects and stability.

DISCUSSION

consistent message across the CMV antiviral space is that *chemistry dictates chromatography*. The six approved agents span inorganic/poly-anionic structures (foscarnet), highly polar nucleos(t)ide analogues (ganciclovir, valganciclovir, cidofovir), and more lipophilic small molecules (letermovir, maribavir). That spread explains why a single "universal" stability-indicating platform is rarely optimal. Instead, the most defensible approach is a mechanism-based analytical framework where (i) polarity/ionization guides the separation mode, (ii) plausible degradation pathways guide stress design, and (iii) the intended use (release/stability vs PK/TDM) sets the validation depth and acceptance criteria (3–5). In practice, this framework prevents two common failure modes: forcing very polar analytes onto conventional RP conditions with poor retention, or claiming stability-indicating power without proving degradant resolution and peak purity.

RP-HPLC/UPLC with UV/DAD remains the mainstay for assay and stability testing of letermovir and maribavir, where retention is reliable and UV response is strong. Here, shorter UPLC cycles can be justified without sacrificing resolution, provided system suitability and robustness are explicitly demonstrated (12–14). For **ganciclovir/valganciclovir/cidofovir**, the literature shows a recurring pattern: conventional C18 methods often require *very aqueous mobile phases, ion-pairing, mixed-mode phases, or HILIC* to achieve acceptable retention and peak shape (6,9). Among these, **HILIC** is frequently the cleanest scientific choice because it improves retention for polar species while remaining compatible with MS-friendly buffers (ammonium formate/acetate). That said, HILIC introduces its own control needs equilibration time, water content in injection solvent, and sensitivity to small composition shifts making robustness studies and well-defined operating ranges essential (25–28).

Foscarnet sits apart analytically. Its negligible UV absorbance and inorganic poly-anionic character make **ion chromatography with suppressed conductivity** the most defensible routine option (8). Attempts to "adapt" foscarnet into UV workflows via derivatization or surrogate approaches can be valuable for specific contexts, yet they raise traceability and specificity questions unless thoroughly justified. For confirmatory characterization or sensitive quantification in complex matrices, LC–MS in negative mode can work, but the analytical burden shifts to ionization control, matrix effects, and ion suppression management (19–24).

Forced degradation is the centrepiece of any SIAM package because it underwrites **specificity**. The practical goal is not maximal destruction; it is *informative* degradation that produces relevant degradants, enables peak tracking, and supports mass balance. The stress palette described acid/base hydrolysis, oxidation, heat/humidity, and photolysis aligned with ICH Q1A/Q1B concepts matches the degradation liabilities expected for these chemotypes (33). Still, the quality of evidence varies across reports. Strong SI claims are typically supported by (i) clear separation of parent from degradants, (ii) DAD peak purity and spectral congruence checks, and (iii) orthogonal confirmation (MS/HRMS where feasible). When only UV trace overlays are presented without peak purity or degradant assignment, the "stability-indicating" label becomes fragile, particularly for polar nucleos(t)ides where early-eluting impurities may co-elute.

Drug-specific degradation logic also matters. **Valganciclovir** is a clear example: ester hydrolysis to ganciclovir can occur during sample handling, so cold conditions, rapid processing, and autosampler controls are not optional details; they are integral parts of method validity (7). For **cidofovir** and other phosphonate-containing molecules, stress behavior may reflect pH-driven transformations and ionic interactions rather than classic oxidative fragmentation, which changes how one interprets degradant patterns and mass balance. For **foscarnet**, solubility, counterion exchange, and complexation with divalent cations can dominate apparent "instability," so study designs must distinguish chemical degradation from precipitation or speciation changes.

When mapped to ICH Q2(R2) expectations, most reported CMV antiviral methods address the core pillars linearity, precision, accuracy, LOD/LOQ, and robustness reasonably well, especially for assay in simple dosage forms. For PK and TDM, **LC–MS/MS** dominates because it provides sensitivity and selectivity in complex matrices, and it can support clinical decision-making when trough levels matter (15–18). However, the quality of a bioanalytical method is rarely limited by the chromatogram alone. It is constrained by **matrix effects, carryover, internal standard design, and stability controls** (19–24). This is particularly relevant for polar CMV antivirals: simple protein precipitation may be adequate for throughput, yet mixed-mode SPE can markedly improve cleanliness and robustness, especially when long analytical runs and high sample counts amplify ion suppression (29–32). The review's QbD elements are not academic extras; they are practical tools to reduce post-approval friction and

unexpected failures (5).

Limitations of the evidence base As a narrative synthesis, this review is constrained by heterogeneity in published reporting. Many studies present partial validation datasets, use differing acceptance criteria, or omit critical details (sample handling temperatures for VGCV, HILIC equilibration practices, impurity spiking levels, or mass balance logic). In several cases, stability-indicating claims rely on limited degradant characterization, which weakens the transferability of conclusions across laboratories and instruments.

CONCLUSION

CMV antivirals span extreme polarity and markedly different degradation behavior, so no single analytical platform is universally adequate. This review contributes a fit-for-purpose roadmap that links molecular properties and degradation pathways to method selection (RP-UPLC, HILIC/ion-pair, ion chromatography, LC–MS/MS) and to ICH Q2(R2) validation evidence for procedures used in release and stability testing. ICH Database A practical implication is that stability programs for polar nucleoside/nucleotide analogues must prioritize retention control (HILIC/ion-pair design), interconversion prevention, and orthogonal confirmation of degradant peaks, while lipophilic agents can often meet SIAM requirements with fast RP-UPLC-DAD supplemented by MS where degradant assignment is needed.

The main limitation across the literature is uneven SIAM documentation forced degradation severity is not always justified, mass balance is inconsistently discussed, and degradant identity confirmation is sometimes limited to peak tracking rather than structural evidence. Future work should therefore focus on: (i) standardized, reportable SIAM checklists aligned to Q2(R2), (ii) broader use of HRMS/MS libraries for degradation product elucidation, (iii) Q14-consistent reporting of MODR/control strategies to support lifecycle method changes, and (iv) harmonized intracellular metabolite workflows to strengthen clinical TDM interpretation.

Abbreviations

QbD: Quality by Design, CMV: Cytomegalovirus, HSCT: hematopoietic stem cell transplant, Human cytomegalovirus: HCMV, IC: Ion Chromatography, CE: Capillary Electrophoresis, GCV: Ganciclovir, VGCV: Valganciclovir, FOS: Foscarnet, LTV: Letermovir, MBV: Maribavir.

Acknowledgment

The authors sincerely acknowledge the Department of Pharmaceutical Sciences for providing the facilities and academic environment that supported this work. We are grateful to colleagues and mentors for their constructive suggestions and encouragement during the preparation of this manuscript. The authors also extend appreciation to the wider scientific community whose research and publications contributed to the development of this review.

Authors' Contributions

Deverakonda Ashlesha: Conceptualization, manuscript writing, data interpretation, and revision. Narayana Swamy Harikrishnan: manuscript review and editing.

Conflicts of Interest

None

Ethics approval

None

Funding

None

Declaration of Generative AI and AI Assisted Technologies in the Writing Process:
Not used any AI tools

REFERENCES

1. Boeckh M, Geballe AP. Cytomegalovirus: pathogen, paradigm, and puzzle. The Journal of clinical investigation. 2011;121(5):1673-80.
2. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, Humar A, The Transplantation Society International CMV Consensus Group. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102(6):900-31.
3. Bottino P, Pastrone L, Curtoni A, Bondi A, Sidoti F, Zanotto E, Cavallo R, Solidoro P, Costa C. Antiviral approach to cytomegalovirus infection: an overview of conventional and novel strategies. Microorganisms. 2023;11(10):2372.
4. McMahon ME, Abbott A, Babayan Y, Carhart J, Chen CW, Debie E, Fu M, Hoaglund-Hyzer C, Lennard A, Li H, Mazzeo T. Considerations for updates to ICH Q1 and Q5C stability guidelines: embracing current technology and risk assessment strategies. The AAPS journal. 2021;23(6):107.
5. Ermer J. ICH Q2 (R2): validation of analytical procedures. Method validation in pharmaceutical analysis: a guide to best practice. 2025:351-72.
6. Teshima D, Otsubo K, Yoshida T, Itoh Y, Oishi R. A simple and simultaneous determination of acyclovir and ganciclovir in human plasma by high-performance liquid

chromatography. Biomedical Chromatography. 2003;17(8):500-3.
7. Derangula VR, Talluri MR, Ponneri V, Polagani SR, Adireddy V. Development and validation of a sensitive and rugged LC-MS/MS method for evaluation of valganciclovir and its active metabolite ganciclovir in human plasma. Indian J Pharm Sci. 2019;81(4):747-56.
8. Dinh NP, Shamshir A, Hulaj G, Jonsson T. Validated modernized assay for foscarnet in pharmaceutical formulations using suppressed ion chromatography developed through a quality by design approach. Separations. 2021;8(11):209.
9. Lecomte F, Hubert C, Demarche S, De Bleye C, Dispas A, Jost M, Frankenne F, Ceccato A, Rozet E, Hubert P. Comparison of the quantitative performances and measurement uncertainty estimates obtained during method validation versus routine applications of a novel hydrophilic interaction chromatography method for the determination of cidofovir in human plasma. Journal of pharmaceutical and biomedical analysis. 2012;57:153-65.
10. Chemaly RF, Ullmann AJ, Stoelben S, Richard MP, Bornhäuser M, Groth C, Einsele H, Silverman M, Mullane KM, Brown J, Nowak H. Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. New England Journal of Medicine. 2014;370(19):1781-9.
11. Sun K, Fournier M, Sundberg AK, Song IH. Maribavir: Mechanism of action, clinical, and translational science. Clinical and Translational Science. 2024;17(1):e13696.
12. Sturkenboom M, van der Lijke H, Jongedijk E, Greijdanus B, Kok W, Uges D, Alffenaar JW. Quantification of isoniazid, pyrazinamide and ethambutol in serum using liquid chromatography-tandem mass spectrometry. Journal of Applied Bioanalysis. 2015;1(3):89-98.
13. Dogan-Topal B, Uslu B, Ozkan SA. Development and validation of an RP-HPLC method for determination of valganciclovir in human serum and tablets. Chromatographia. 2007;66(1):97-101.
14. Isberner N, Aghai F, Schirmer D, Kraus S, Grigoleit GU, Klinker H. Quantification of letermovir in human serum using high-performance liquid chromatography with diode array detection. Journal of Chromatography B. 2020; 1159:122399.
15. Franzin M, Ruoso R, Del Savio R, Addobbati R. Development and Validation of an HPLC-UV Method for the Quantification of Acyclovir and Ganciclovir in the Plasma of Pediatric Immunocompromised Patients. International Journal of Molecular Sciences. 2024;25(5):2685.
16. Badgujar VM, Jain PS. Analytical Method Development and Validation of an RP-HPLC Method for Stability-Indicating Determination of Idelalisib. Pharmaceutical Chemistry Journal. 2024;58(7):1176-81.
17. Junapudi S, Godela R, Srinivas M, Namadi S. Method development and validation for simultaneous estimation of dasatinib and lenvatinib by Using RP-HPLC in pharmaceutical dosage form. Inven Rapid: Pharm Anal Qual Assur. 2017; 4:1-5.
18. Annapurna MM, Lakshmi SK, Sirichandra M. Stability indicating liquid chromatographic method for the quantitative determination of valganciclovir in pharmaceutical dosage forms. J Drug Deliv and Ther. 2013;3(3):64.
19. Singh O, Saxena S, Mishra S, Khuroo A, Monif T. Determination of valganciclovir and ganciclovir in human plasma by liquid chromatography-tandem mass spectrometric detection. Clin Biochem. 2011;44(10-11):907-15.
20. Heinig K, Wirz T, Gajate-Perez A, Belli S. Determination of ganciclovir and its prodrug valganciclovir by hydrophilic interaction liquid chromatography–tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879 (5-6):436-42.
21. Gunda S, Earla R, Cholkar K, Mitra AK. Pharmacokinetic studies and LC–MS/MS method development of ganciclovir and dipeptide monoester prodrugs in sprague dawley rats. European journal of drug metabolism and pharmacokinetics. 2015;40(3):325-34.
22. Pigliasco F, Cafaro A, Simeoli R, Barco S, Magnasco A, et al. A UHPLC–MS/MS method for therapeutic drug monitoring of aciclovir and ganciclovir in plasma and dried plasma spots. Biomedicines. 2021;9(10):1379.
23. Şanlı S, Kaynak MS, Şanlı N, Balta EE. Investigation of the Permeability of Antiretroviral Drugs Lamivudine and Valganciclovir via Single-Pass Intestinal Perfusion (SPIP) Method. Current Pharmaceutical Analysis. 2024;20(7):585-96.
24. Guo D, Wang X, Li P, Shang Y, Zhang L, Chen Y, Kong X, Chen W. Efficacy and toxicity analysis of ganciclovir in patients with cytomegalovirus lung infection: what is new for target range of therapeutic drug monitoring. Microbiology spectrum. 2025;13(8):e00461-25.

25. Kodad S, Sabry W, Elsayed A, Goubran H, Elemary M. Viral infections in allogeneic hematopoietic stem cell transplant recipients: literature review. OBM Transplantation. 2019;3(3):1-22.
26. Wildum S, Zimmermann H, Lischka P. In vitro drug combination studies of Letermovir (AIC246, MK-8228) with approved anti-human cytomegalovirus (HCMV) and anti-HIV compounds in inhibition of HCMV and HIV replication. Antimicrobial agents and chemotherapy. 2015;59(6):3140-8.
27. Bhupatiraju RV, Kumar BS, Peddi P, Tangeti VS. An effective HPLC method for evaluation of process related impurities of Letermovir and LC-MS/MS characterization of forced degradation compounds. J. Chem. Metrol. 2023;2(2):181-98.
28. Wang J, Ming M, Yu L, Chen G, Hao N, Meng Y, Fang L. LC–MS/MS determination of buparlisib, a phosphoinositide 3 kinase inhibitor in rat plasma: Application to a pharmacokinetic study. Biomedical Chromatography. 2020;34(6):e4816.
29. Ljungman P, De la Camara R, Milpied N, Volin L, Russell CA, Crisp A, Webster A, Valacyclovir International Bone Marrow Transplant Study Group. Randomized study of valacyclovir as prophylaxis against cytomegalovirus reactivation in recipients of allogeneic bone marrow transplants. Blood, The Journal of the American Society of Hematology. 2002;99(8):3050-6.
30. Abhigna GV, Sundararajan R. Analytical method development and validation for determination of valganciclovir by using RP-HPLC. Int. J. Pharm. Invest.. 2023;13(3):617-24.
31. Wagstaff AJ, Bryson HM. Foscarnet: a reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic use in immunocompromised patients with viral infections. Drugs. 1994;48(2):199-226.
32. Irie K, Hiramoto N, Ishikawa T, Fukushima S. Use of liquid chromatography–tandem mass spectrometry for foscarnet quantification in human serum and cerebrospinal fluid. Rapid Communications in Mass Spectrometry. 2022;36(7):e9255.
33. González-González O, Ramirez IO, Ramirez BI, O'Connell P, Ballesteros MP, Torrado JJ, Serrano DR. Drug stability: ICH versus accelerated predictive stability studies. Pharmaceutics. 2022;14(11):2324.
34. Faulds D, Brogden RN. Didanosine: a review of its antiviral activity, pharmacokinetic properties and therapeutic potential in human immunodeficiency virus infection. Drugs. 1992;44(1):94-116.
35. Momper JD, Zhang S, Randhawa PS, Shapiro R, Schonder KS, Venkataramanan R. Determination of cidofovir in human plasma after low dose drug administration using high-performance liquid chromatography–tandem mass spectrometry. Journal of pharmaceutical and biomedical analysis. 2010;53(4):1015-21.
36. Raćkowska E, Bobrowska-Korczak B, Giebułtowicz J. Development and validation of a rapid LC–MS/MS method for determination of methylated nucleosides and nucleobases in urine. Journal of Chromatography B. 2019; 1128:121775.
37. McGavin JK, Goa KL. Ganciclovir: an update of its use in the prevention of cytomegalovirus infection and disease in transplant recipients. Drugs. 2001;61(8):1153-83.
38. Faulds D, Heel RC. Ganciclovir: a review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. Drugs. 1990;39(4):597-638.
39. Boivin G, Goyette N, Gilbert C, Humar A, Covington E. Clinical impact of ganciclovir-resistant cytomegalovirus infections in solid organ transplant patients. Transplant infectious disease. 2005;7(3-4):166-70.
40. Ärlemalm A, Helldén A, Karlsson L, Carlsson B. Rapid determination of acyclovir, its main metabolite 9-carboxymethoxymethylguanine, ganciclovir, and penciclovir in human serum using LC–MS/MS. Biomedical Chromatography. 2022;36(4):e5315.
41. Madhusudhan Patlola VR, Singh S. Determination of assay and validation of stability indicating RP-HPLC method for ganciclovir in nciclovir drug substance. International Journal of Scientific & Technology Research. 2020;9(02): 4191-95.
42. Lin K, Kong X, Tao X, Zhai X, Lv L, Dong D, Yang S, Zhu Y. Research methods and new advances in drug–drug interactions mediated by renal transporters. Molecules. 2023;28(13):5252.
43. Ahmed A. Antiviral treatment of cytomegalovirus infection. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders). 2011;11(5):475-503.
44. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. Clinical microbiology reviews. 2010;23(4):689-712.
45. Limaye AP, Boeckh M. CMV in critically ill patients: pathogen or bystander? Reviews in medical virology. 2010;20(6):372-9.

