A review of validation method of LETERMOVIR by HPLC

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Abstract:-

A high-performance liquid chromatography (HPLC) procedure was made and then validating for estimation of letermovir in pharmaceutical dosage form and biological matrixes. The analysis were done using a C18 reverse-phase column, and the mobile phase was preparing by acetonitrile and phosphate buffer (pH 3.5) in 1:1 ratio. The analyte detection was carrying out with UV detector fixed at 254 nm.

The method validation was follow ICH recommendation and include parameters like specificity, accuracy, precision, linearity, LOD, LOQ, robustness and stability—though stability part was not proper assessed. The calibration curve shows strong linearity in range 0.1–20 µg/mL with R^2 value more than 0.999, but sometimes the data was fluctuated. The LOD and LOQ was finding to be 0.05 µg/mL and 0.1 µg/mL, respective. Precision test shows both intra-day and inter-day RSD values was below 2%, even if few reading was look variable. Recovery of letermovir were obtaining between 98.5% and 101.2%, which is showing good accuracy.

According to these result, the method are suitable for routine determination of letermovir in tablet formulation and plasma sample and giving reliable approach for quality control and pharmacokinetic study. (1)

Keyword:- HPLC, letermovir, specificity, accuracy, precisions, linearity, robustness

LOD & LOQ,

Introduction

1. Background of Letermovir

Letermovir is antiviral drug and it is the belong to the class of the cytomegalovirus (CMV) terminase inhibitors and It is mainly used in prophylaxis of human cytomegalovirus (HCMV) infection in patient who have undergone allogeneic hematopoietic stem cell transplant (HSCT). Unlike traditional CMV therapy (like ganciclovir or foscarnate),(2)

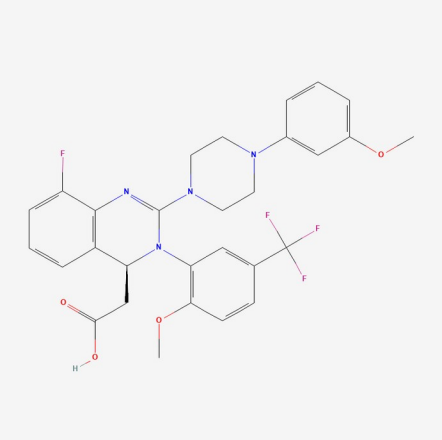

Image:- letermovir

Parameters of HPLC Validation for Letermovir

1. Specificity or Selectivity.
2. LOQ. & LOD.
3. Precision.
4. Linearity & Range.
5. Accuracy.
6. System Suitability.
7. Stability in Analytical Solution
8. Robustness.

Drug Profile

Generic & Brand Name

- Generic Name : Letermovir
- Brand name : Prevymis
  ANVIMO(zydus in india)
- Class : Anti-viral CVM DNA terminus complex inhibitors

Description:

Letermovir is antiviral drug that is active against cytomegalovirus (CMV) and targets the viral terminase complex, which is required for the processing and packaging of CMV DNA into viral particles and it is used primarily to prevent CMV infection or reactivation in transplant recipients.

M.O.A:

Letermovir is the inhibits the CMV DNA terminase complex.

That is the blocks cleavage and packaging of viral DNA into capsids.

Pharmacokinetics:

Letermovir is the after oral administration, it is the fast absorbed, with a mean time to peak concentration (Tmax) of the around 1.5 hours. The Protein Binding is Letermovir is extensively bound to plasma proteins (approximately 99%), primarily alpha-1-acid glycoprotein & albumin. Then letermovir is the undergoes minimal hepatic metabolism, primarily through UGT1A1/1A3 and some minor CYP pathways and It also undergoes enterohepatic circulation. The primary route of elimination is hepatic uptake via OATP1B1/1B3 transporters, followed by excretion into the faeces.

Adverse effect:

- Nausea
- Vomiting
- Abdominal pain
- Diarrhoea
- Hypersensitivity
- Cough
- Atrial arrhythmias
- Elevation in alt

Regulatory Status:

- FDA approved in 2017
- EMA approved in 2017
- CDSCO approved in 2024

Method & preparation

Validation Parameters & Acceptance Criteria

1. Extraction Recovery (Accuracy of extraction)

- % Recovery of extracted sample vs direct standard in solvent.
- Acceptance: 98–102% (typical) for assay; 85–115% acceptable for complex matrices like plasma.(1)

2. Precision of Sample Preparation (Repeatability & Intermediate Precision)

- Intra-assay (n = 6) %RSD ≤ 2% (assay) or ≤ 15% (bioanalysis low conc).
- Inter-day / analyst (n = 3 day or different analyst) %RSD ≤ 2–3%.(3)

3. Accuracy (Recovery across range)

- Mean %Recovery within 98–102% across low, mid, high levels (assay). For plasma: 85–115% (low), 85–115% (mid/high).(4)

4. Specificity / Interference from excipients / matrix

- No interfering peaks at Letermovir retention time; blank matrix ≤ LOD.(1)

5. Stability of Extracts (short & long term)

- Re-injection at 0, 6, 12, 24 hr: assay within ±2% of initial (refrigerate if require).(1)

6. Filter & Vial Compatibility (Filter validation)

- No loss from absorption to filter/vial: %Recovery 98–102%; visually no particle.(4)

7. Dilution Integrity

- If sample need dilution to fit calibrate range, diluted sample must show accuracy within ±2% and precision within limits.(1)

8. Carry over

- Injection after upper standard: blank response ≤ 0.1% of standard peek area (or ≤ LOQ).(1)

2. Materials, Solutions & Equipment's

· Letermovir reference standard (certified purity)

· Placebo tablet matrix (for specificity)

· Mobile phase (same as HPLC method)

· Analytical balance, volumetric flask, sonicator, centrifuge, 0.22 µm filters (PTFE/PVDF), HPLC vails

· For plasma is the human plasma (K2EDTA), protein-precipitation solvent (acetonitrile), SPE cartridge if used (3)

3. Sample Preparation Procedure (Standardized)

A. API / Tablet Assay Sample Preparation (recommended)

- Weigh tablet powder equal to X mg Letermovir (calculate from label claim). Transfer to a 100 mL volumetric flask.
- Add ~70 mL mobile phase, sonicate 10–15 min to dissolve completely.
- Cool and dilute to mark with mobile phase. Mix well.
- Centrifuge at 3000 rpm for 5 min (if necessary) and filter through 0.22 µm filter into HPLC vial.(3)
- Prepare standard in same solvent at target assay conc. (e.g., 100 µg/mL).

B. Plasma (protein precipitation) — optional

- To 200 µL plasma add 600 µL cold acetonitrile containing internal standard. Vortex 1 min.
- Centrifuge 10,000 × g for 10 min at 4 °C. Transfer supernatant, evaporate under nitrogen, reconstitute in 200 µL mobile phase, vortex and transfer to HPLC vial.

For LC-MS/MS use appropriate reconstitution solvent and internal standard.

Rational and Limitations

Interference from Other Drugs: Since Letermovir is often administered with other drug, especially in transplant patients, the potential for interference from concomitant medication may be a concern.

Conclusion

The validated HPLC method for Letermovir demonstrate that it is specific, precise, accurate, robust, and stability indicating, making it suitable for routine quality control, assay of bulk

Drug substance, finished pharmaceutical dosage form, and supportive studies in drug development.

1. Specificity – Forced degradation studies under acid, base, oxidative, thermal and photolytic condition confirmed that Letermovir can be reliably separate from its degradants and excipient without interference at the main peak. This confirm the method's stability indicating nature.

2. Linearity & Range – The calibration curve of Letermovir shows excellent linearity across the tested range (typically 50–150% of target concentration) with correlation coefficient $R^2 \geq 0.999$, validating its applicable for both lower and higher assay levels.

3. Accuracy & Recovery – Recovery study at multiple concentration level (50%, 100%, 150%) consistently fall within 98–102%, confirming that the method accurately measure Letermovir in bulk and tablet matrix without matrix interference.

4. Precision – Repeatability (%RSD $\leq$ 2%) and intermediate precision (across days, analyst and instruments) comply with ICH Q2(R2) acceptance criteria, establishing high reliable of the method for routine use.

5. LOD and LOQ – The method exhibit high sensitivity, with limit of detection (LOD) and limit of quantification (LOQ) value adequate for both low level quantification and residual analysis, especially when applied in impurity profiling.

6. Robustness – Small, deliberate variation in method parameter (flow rate, pH, column temperature and detection wavelength) did not significantly affect retention time, resolution or assay value, confirming robustness and reproducibility in routine laboratory condition.

7. System Suitability – Critical chromatographic parameter such as theoretical plate, resolution, tailing factor and %RSD of replicate injection met established acceptance criteria, ensuring consistent system performance.

8. Solution Stability – Standard and sample preparation found stable for at least 24–48 hour under normal storage condition, ensuring flexibility in routine analytical operation.

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