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A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF LETERMOVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The proposed work was accurate and precise stability indicating RP-HPLC method has been developed and validation of Letermovir, in tablet dosage form. The separation was achieved on a Kromasil C_{18} (4.6×250mm, 5µ) column using a mixture of Methanol: water (60: 40% v/v) as the mobile phase at a flow rate of 1.0 mL/min and detected 247 nm. The retention time of Letermovir 3.2 minutes. The linear responses in the concentration range of 10-60 µg/mL of Letermovir. The method precision for the determination of assay was less than 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Letermovir; RP-HPLC; Validation; Tablet dosage forms; PDA Detection; ICH Validation.

INTRODUCTION

The **Letermovir (LMV)** (Fig.1) is chemically 2-[(4S)-8-fluoro-3-[2-methoxy-5-(trifluoromethyl)phenyl]-2-[4-(3-methoxyphenyl)piperazin-1-yl]-3,4-dihydroquinazolin-4-yl]acetic acid.

REVYMIS contains letermovir, an inhibitor of the CMV DNA terminase complex, and is administered orally or by intravenous infusion.

PREVYMIS is available as 240 mg and 480 mg tablets. PREVYMIS tablets contain either 240 mg or 480 mg of letermovir and the following inactive ingredients: colloidal silicon dioxide, croscarmellose sodium, magnesium stearate, microcrystalline cellulose, povidone 25, and film-coated with a coating material containing the following inactive ingredients: hypromellose 2910, iron oxide red (only for 480 mg tablets), iron oxide yellow, lactose monohydrate, titanium dioxide, and triacetin, Carnauba wax is added as a polishing agent.

PREVYMIS is also available as 240 mg and 480 mg injection for intravenous infusion. This injection is a clear, preservative-free sterile solution in single-dose vials of either 240 mg or 480 mg per vial. Each 1 mL of solution contains 20 mg letermovir, hydroxypropyl betadex (150 mg), sodium chloride (3.1 mg), sodium hydroxide (1.2 mg), and Water for Injection, USP. The amount of sodium hydroxide may be adjusted to achieve a pH of approximately 7.5.

Letermovir has a molecular formula of $C_{29}H_{28}F_4N_4O_4$ and a molecular weight of 572.55. The chemical name for letermovir is (4*S*)-2-{8-Fluoro-2-[4-(3-methoxyphenyl)piperazin-1-yl]-3-[2-methoxy-5(trifluoromethyl)phenyl]-3,4-dihydroquinazolin-4-yl}acetic acid. Letermovir is very slightly soluble in water.

The chemical structure of letermovir is.

Letermovir recieved approval from the FDA on November 8th, 2017 for use in prophylaxis of cytomegalovirus (CMV) infection in allogeneic hematopoietic stem cell transplant patients.[6] It represents the first entry into a new class of CMV anti-infectives, DNA terminase complex inhibitors.[3] Letermovir has recieved both priority and orphan drug status from the FDA. It is currently marketed under the brand name Prevymis.[6]

Mechanism of action

CMV relies on a DNA terminase complex consisting of multiple subunits (pUL51, pUL56, and pUL89) for processing of viral DNA. Viral DNA is produced in a single repeating strand which is then cut by the DNA terminase complex into individual viral genomes which can then be packaged into mature viral particles.[2] Letemovir inhibits the activity of this complex to prevent production of mature viral genomes and the production of viable viral particles. The exact nature of Letemovir's binding to this complex is not currently known. Initially, the observation of resistance-causing mutations in pUL56 suggested this subunit was the location

of Letemovir binding.[3] However, resistance mutations have now been observed in pUL51, pUL56, and pUL89.[4] It is possible that changes in amino acid sequence in one subunit could result in conformational changes to interacting subunits affecting Letemovir binding or that Letemovir interacts with multiple subunits of the complex but evidence towards either of these distinctions has not yet been seen. pUL89 is known to contain the endonuclease activity of the complex but because all members of the complex are necessary for targeting as well as protection from proteosomal degradation, it is difficult to discern if Letemovir inhibits pUL89's activity directly.

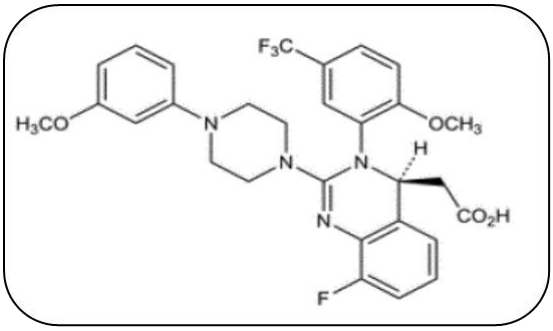

Fig.1. Structure of Letermovir.

It is soluble slightly in water (pH 1.2-7.7), freely soluble in ethanol and acetone, soluble in 2-propanol, and insoluble in heptanes.[3]

Literature survey review reveals a few HPLC methods, LC method have been used. The objective of the proposed work was to develop simple, rapid, accurate and specific RP-HPLC stability indicating method.

The proposed work aim was to develop and validate a simple, rapid and reliable isocratic RP method with PDA detection for the determination of Letermovir tablet dosage form. The novelty of the proposed method included simple sample treatment with sonicator of small amount of powder sample at ambient temperature, short retention time (less than 5 min) good precision (R.S.D. below 2%).

The aim of the current work was to develop and validate a simple, fast and reliable isocratic RP.

Method with UV detection for the determination of Letermovir in bulk form. The important features and novelty of the proposed method included simple sample treatment with sonicator

of small amount of powder sample at ambient temperature, shot elution time(less than 5 min) LMV, good precision (R.S.D. less than 2%).

Conformation of the applicability of developed method validated according to the international conference on Harmonization (ICH).[6]

MATERIALS AND METHODS

Chemicals

Letermovir and other chemicals were procured from Synergy Pharmaceuticals and Merck Earn Lab Pvt. Ltd. Mumbai.[7]

Reagents

Methanol (HPLC grade), Water (HPLC grade), Potassium dihydrogen phosphate (GR grade), Orthophosphoric acid (GR grade).

Instruments and Equipments

All the chemicals High Performance Liquid Chromatography (Waters 2695 HPLC, Class) with 2487 pumps, auto injector with loop volume of 10 µl (Rheodyne), Programmable variable wavelength PDA detector.[8]

Preparation of mobile phase

Mix a mixture of Methanol 60 ml and HPLC grade Water 40 mL (60:40%) and degas in ultrasonic water bath for 5minutes.Filter through 0.45µ filter under vacuum filtration.

Preparation of standard and sample solutions

Weighed accurately and transferred in to the clean and dry 100 ml volumetric flask and made up to the mark with the diluent (1000µg/mL). Standard solutions of LMV were prepared in the range of 20 µg/mL to 100 µg/mL by diluting the stock solution with mobile phase. The eluate was monitored at 260nm. Each solution was then injected into the column and chromatogram was recorded.

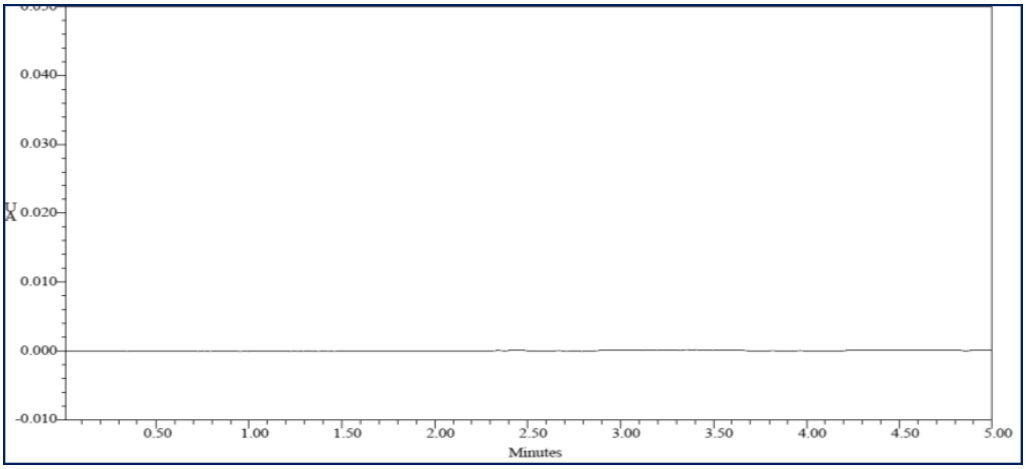

Fig 2. Chromatogram showing blank (mobile phase preparation)

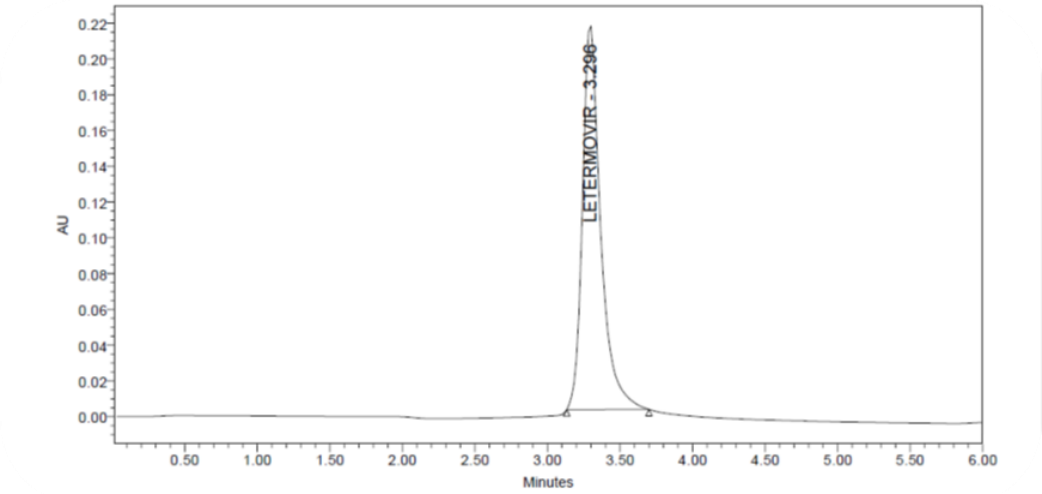

Fig. 3 Chromatogram of standard Letermovir.

Table 1: Results of system suitability for Letermovir.

S.No	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	Letermovir	3.2	2857505	7462	1.1
2	Letermovir	3.2	2868475	7462	1.1
3	Letermovir	3.2	2855847	6472	1.1
4	Letermovir	3.2	2862642	7183	1.1
5	Letermovir	3.2	2841645	7428	1.1
Mean			2835223		
Std. Dev.			7114.704		
% RSD			0.24075		

Table 2: Optimized method of parameters.

Column C_{18}	Hypersil C18 (4.6×150mm) 5µ
Mobile Phase	Methanol:Water (60:40%)
Flow Rate	1.0 mL /min.
Run Time	7 min.
Retention time	3.2 min
Column Temp.	Ambient
Volume Of Injection Loop	10µL
Detection Wave Length	247 nm
Linearity Range	10-60 µg/mL

Table 3: Calibration data of Letermovir.

Concentration µg/ml	Average Peak Area
10	983048
20	1873321
30	3655166
40	7263921
50	1406038
60	28932421
Slope (m)	40735
Intercept (C)	13723
r^2	0.9997

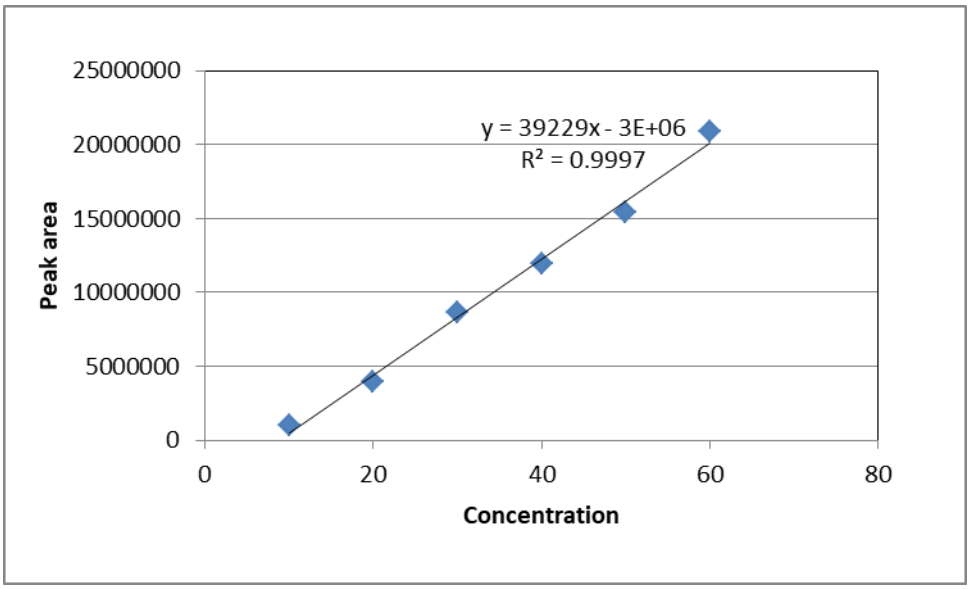

Fig .03: Calibration graph of Letermovir.

Table 4: Precision studies of Letermovir.

S. No	Name of drug	Retention time	Peak area	Plate Count	Tailing
1.	Letermovir	3.2	2758357	7579	1.2
2.	Letermovir	3.2	2751049	7293	1.1
3.	Letermovir	3.2	2759294	8274	1.1
4.	Letermovir	3.2	2753391	7358	1.1
5.	Letermovir	3.2	2750744	9345	1.1
Mean			2754562		
Std.dev			3028.083		
%RSD			0.236334		

Degradation study

The proposed research work to determine whether the analytical methods were stable for Letermovir dosage forms are stressed on the different conditions to applied degradation studies. The ICH guidelines are expressed in ICH Q2A, Q3B, Q2B & FDA 21 CFR section of 211 all the required for development & for the validation of stability study.

The Forced degradation studies was conducted on Letermovir and the equivalent to the weight of each tablet was transfer into 100 ml flask & it was treated under the acidic, alkaline, thermal, oxidizing and photolytic conditions. When degradation was complete the solution were left to equilibrate to the room temperature and diluted with mobile phase to furnish the solutions of a concentration equivalent to a 30 µg/mL of Letermovir. The specific degradative conditions are described below mentioned Table 5.

Acid degradation study: The Acid degradation was done by sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs at 60ºC. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC. The degrading drug content was found up to 10.5% in the acidic condition.

Alkaline degradation: The Alkaline degradation was done by sample was treated with 3ml of 1N sodium hydroxide and kept the sample for 10hr. After 10hr solution was neutralized to add 3ml of 1N hydrochloric acid, made the volume up to the mark with irrelevant media and analyzed using HPLC. In alkali degradation study, it was found to be 8.05% of the degraded drug.

Oxidative degradation: The oxidative degradation was done by sample was mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the

volume upto the mark with mobile phase and analyzed using HPLC. In oxidative degradation, it was found to be 14.15% of the degraded drug.

Photolytic degradation: The photolytic degradation was done by exposing of drug content under the UV light for 15mins to 7days. There is 8.45% of the drug degradation observed in the above specific photolytic degradation condition.

Thermal degradation: The Thermal degradation is to be performing by the exposing the solid drug at the 80°C for 15mins to 60mins and at 220°C for 2-5mins. Resultant chromatogram of thermal degradation study was indicates that the drug was found to be slightly stable under thermal condition. It was only 2.64% of the drug content were degraded.

RESULTS AND DISCUSSION

The present study was developed and validated RP-HPLC using RP-C_{18} column for using entire the procedure. Atypical Chromatogram obtained by using the mobile phase (Figure No 2). The precision and Accuracy of the method was determined. The precission study was performed in two consecutive days. The method was validated for linearity, precision and accuracy parameters.[9] The calibration curve of the method was studied by injecting six concentrations of drug prepared in the mobile phase in the range 10-60 µg/mL and solutions are analyzed through the high pressure liquid chromatographic technique (Fig. 3). The peak area were plotted against concentration was subjected to linear plot and the results present in table (Table no.3). Precision of this method was studied in inter day and intraday variation.[12] The precision of intraday studies was repeated on two consecutive days. The developed method was found to be precise as the percentage of RSD values for inter-day and intra –day precision studies were found to be less than 2%.

Table 5: Percentage of degradation of Letermovir.

Drug Name		Acid	Alkali	Oxidative	Photolytic	Thermal
Letermovir	Std Area	279605				
	Sample Area	270837	2728744	277136	279461	265381
	% of Degradation	2.1%	5.05%	3.14%	8.43%	2.68%
% Average of Degradation		3.5%	4.05%	5.15%	8.45%	2.64%

CONCLUSION

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Letermovir from pure and its dosage forms. The mobile phase is simple to prepare and economical. The developed method is accurate, precise and reliable for the

analysisof Letermovir in Pharmaceutical formulations. This method was validated for linearity, accuracy and precision of Letermovir drug. The RSD values for all parameters were found to be <2, which indicates the validity of method and results obtained by this method is with fair agreement. Hence, this method can be easily and conveniently adopted for routine analysis of Letermovir in pure form and also can be used for dissolution or similar studies.

Conflict of interest statement

The authors declare that there are no conflicts of interest in this study. The authors alone are responsible for the content and writing of the paper.

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