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Development And Validation Of Novel Rp-Hplc Method For Quantification Of Anti Retroviral Drug [Covid-19] Remdesivir In Its Bulk And Its Pharmaceutical Dosage Form

M. Ashok babu[1*], K. Suneetha[2]

[1*] Department of Pharmaceutical Analysis, SSJ College of Pharmacy, Hyderabad, Telangana.

[2] Department of Pharmaceutical Chemistry, SSJ College of Pharmacy, Hyderabad, Telangana.

Abstract:
The Present work was to develop a simple, fast, accurate, precise, reproducible, Reverse Phase High Performance Liquid Chromatographic Method for estimation of Remdesivir in pure drug form. Chromatographic separation was done using Inertsil ODS column having dimension of (150*4.6, 5mm), with mobile phase consisting of 0.1% Formic acid in water: Methanol (30:70), flow rate was adjusted to 1.0ml/min and detection wavelength at 254nm. The retention time of Remdesivir was found to be 3.257. The proposed method has been validated for accuracy, precision, linearity, robustness and range were within the acceptance limit according to ICH guidelines. Linearity for Remedisivir was found in range of 50-150 mcg, mean recovery for Remedisivir was found to be 99.77%. The method was found to be robust even by change in the mobile phase and in less flow condition. The developed method can be successfully employed for the routine analysis of Remedisivir in API and Pharmaceutical dosage forms.

Index Terms: Remdesivir, RP-HPLC, Method development, Validation.

INTRODUCTION

Elite Liquid Chromatography (HPLC)

Superior Liquid Chromatography (HPLC) is the most generally utilized scientific method. The chromatography cycle can be characterized as partition strategies including mass-move among fixed and portable stages. The rule associated with high pressing factor fluid chromatography is adsorption. The detachment of the combination of segments depends on their overall affinities towards the fixed stages. The segment has a greater fondness towards the fixed stage voyages increasingly slow later. The segment has less liking towards the fixed stages voyages quicker and eluted first. Since no two segments have similar partiality towards the fixed stages so the segments were separated.

APPROVAL PARAMETERS (ICH)

Regular approval studies incorporate framework appropriateness:

I. Accuracy
II. Precision
III. Specificity
IV. Linearity
V. Detection limit
VI. Quantitation limit
VII. Range
VIII. Robustness

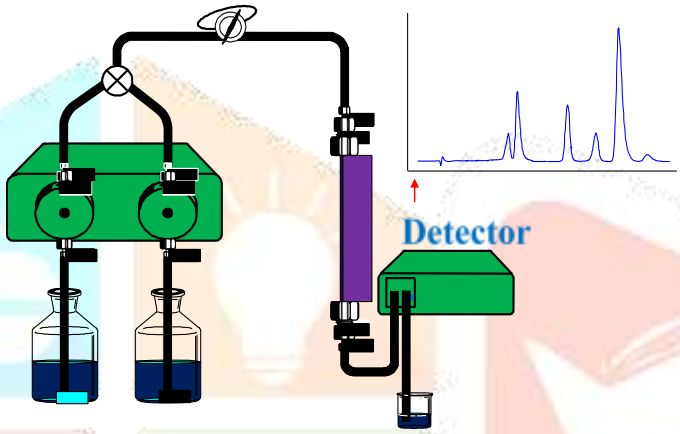

Figure 1: Instrumentation of HPLC

LITERATURE REVIEW

Warren, Travis K., et al,

In the pharmacokinetic examination, the plasma test from three uninfected male rhesus monkeys after organization were treated with a combination of methanol and acetonitrile for protein precipitation. In the wake of adding an inner norm, the example was reconstituted in a combination containing 1% acetonitrile and 99% water with 0.01% formic corrosive. A Synergi™ Hydro-RP 4 µm 75 × 2.0 mm (Part No.: 00C-4375-B0) segment was utilized at a stream pace of 0.26 mL/min. The slope was from 99% water with 0.2% formic corrosive and 1% acetonitrile, to 95% acetonitrile, 5% water with 0.2% formic corrosive over 4.5 minutes. This investigation was referenced in "Wide range antiviral GS-5734 represses both pestilence and zoonotic coronavirus"(2017). Even though GS-5734 (Remdesivir) was just utilized in clinical investigations of Ebola before the episode of COVID-19, research has discovered that it can restrain the replication of SARS-CoV and MERS-CoV in an assortment of in-vitro frameworks. In a mouse model for the pathogenesis of SARS-CoV, through the prophylactic and early remedial organization of GS-5734, it fundamentally diminished lung viral burden, decreased clinical signs, and worked on respiratory capacity. This information demonstrates that Remdesivir is probably going to have a decent inhibitory impact on COVID-19

Veena D. Singh Pt. Ravishankar Shukla, J. Daharwa;

A basic, delicate, savvy and vigorous RP-HPLC strategy for the concurrent assessment of the Lamivudine (LAM) and Remdesivir (RAL) in research facility arranged twofold combination was created, streamlined and approved. Detachment was accomplished on Phenomenex C18 section (150 X 4.6 mm id, 5µ molecule size) and portable stage was made out of 75% methanol: 15% Acetonitrile: 10 % (0.05mM) phosphate cradle (at pH 3.0), with stream rate 1.2 ml/min at 254nm. The created technique was enhanced by utilizing Box Behnken Design (BBD)accordingly surface strategy (RSM). The autonomous factors like the centralization of methanol, pH in versatile stage and stream rate were chosen for the advancement and Retention time (Rt)

were utilized as reactions for the two medications. Derringer's attractiveness work was utilized to simultaneously streamline the chose reactions. The LOD and LOQ were discovered to be 1.04 and 3.18 µg/mL for LAM and 0.36 and 1.08µg/mL of RAL. The rate recuperations were discovered to be under 2% for LAM and RAL. The maintenance season of LAM and RAL was 3.13±0.07 and 7.27±0.01 minutes separately.

T.Sudha, T.Raghupathi,et.al;

A High-Performance Liquid Chromatographic (strategy A) and Ultra Violet spectrophotometric (technique B) strategy were created and approved for quantitative assurance of Remdesivir potassium. The distinctive scientific exhibition boundaries like linearity, exactness, precision, cutoff of discovery (LOD), the breaking point of Quantification (LOQ) were resolved by International Conference on Harmonization (ICH) Q2B rules. Turn around stage High-Performance Liquid Chromatography (strategy A) and Ultra Violet (technique B) were created for the assurance of Remdesivir potassium in mass and drug measurement structures. The chromatographic division was accomplished on Symmetry C18 (4.6 x 150mm, 5 µm XTerra) section utilizing a combination of phosphate support (pH 3.0): Methanol (45:55% v/v) as the portable stage at a stream rate 0.6 mL min-1. The Ultra Violet spectrophotometric assurance was performed at 219 nm. The Linearity of the alignment bends for the analyte in the ideal fixation range is acceptable (r2 = 0.999) by both High-Performance Liquid Chromatography (strategy A) and Ultra Violet (technique B) spectroscopic technique. The two strategies (A&B) were exact and exact with recuperations in the scope of 98-100 % and rate relative standard deviation (RSD under 2%). The proposed techniques were profoundly touchy, exact, and exact and thus was effectively applied for the solid measurement of Active Pharmaceutical Ingredient content in the business definition.

DRUG PROFILE

REMDESIVIR

Structure:

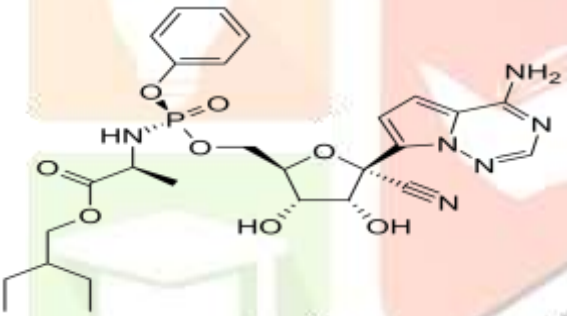

Figure 2: Structure of Remdesivir

IUPAC NAME:

2-ethylbutyl(2S)-2-{[(S)-{[(2R,3S,4R,5R)-5-{4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl}-5 cyano-3,4-dihydroxyoxolan-2-yl]methoxy}(phenoxy)phosphoryl]amino}propanoate

Molecular formula: $C_{27}H_{35}N_6O_8P$

Molecular Weight: 602.585

Protein binding: 83%

Half-Life: 9hours

Solubility (25°C) Invitro: Insoluble in Water, Soluble in Ethanol 16mg/mL (26.55mM) and DMSO 100mg/mL (165.95 Mm.

Mechanism of Action:

As an adenosine nucleoside triphosphate analog (GS-443902), the active metabolite of remdesivir interferes with the action of viral RNA-dependent RNA polymerase and evades proofreading by viral exoribonuclease (ExoN), causing a decrease in viral RNA production. In some viruses such as the respiratory syncytial virus it causes the RNA-dependent RNA polymerases to pause, but its predominant effect (as in Ebola) is to induce an irreversible chain termination. Unlike with many other chain terminators, this is not mediated by preventing addition of the immediately subsequent nucleotide, but is instead delayed, occurring after five additional bases have been added to the growing RNA chain.[33] For the RNA-Dependent RNA Polymerase of MERS-CoV, SARS-CoV-1, and SARS-CoV-2 arrest of RNA synthesis occurs after incorporation of three additional nucleotides.[34][30] Hence, remdesivir is classified as a direct-acting antiviral agent that works as a delayed chain terminator

Materials and Method

Pharmaceutical grade Remdesivir were supplied as gift sample by Hetero Pharmaceuticals Ltd, Hyderabad, Acetonitrile and HPLC grade water from Merck, Formic acid analytical grade from the Finar limited and Methanol HPLC grade from the Merck. All solvent used in this work are HPLC grade. RP-HPLC Agilent, Software, Empower 2, 2695Analytical column used for the separation of analytes Inertsil ODS 3v 150*4.6, 5mm

Methods

Selection of wavelength Standard solution of Remdesivir is prepared at the concentration of 10µg/ml scanned by UV spectrophotometer at the range of 200-400 nm. UV spectrum of Remdesivir shown below.

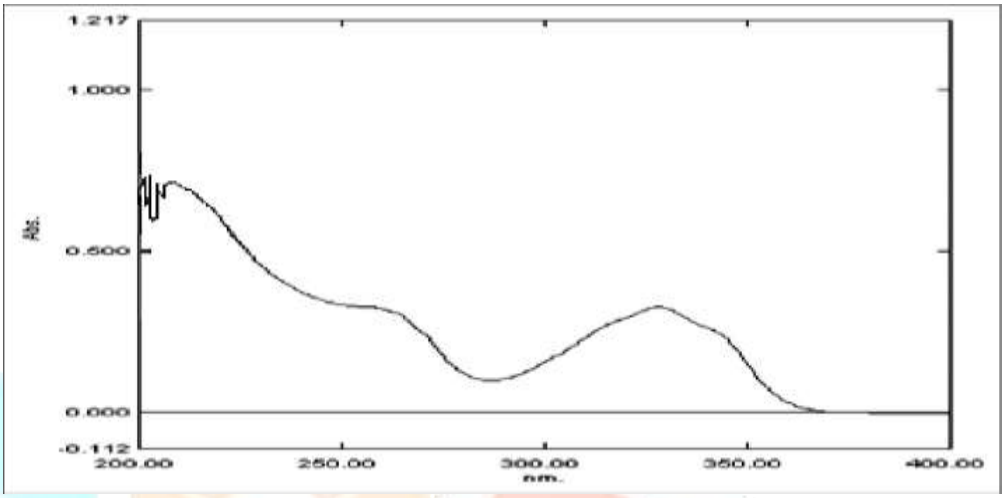

Figure 3: A typical Chromatogram

Standard stock arrangement: A 50 mg of unadulterated Remdesivir were gauged and moved to 50 ml of the volumetric carafe and disintegrated in Diluent. The carafe was shaken and volume was made up to check, with Diluent to give an essential stock arrangement containing 1000µg/ml. From the above arrangement, 2ml of arrangement is pipette out into a 10 ml volumetric flagon and volume was made up to stamp with Diluent to give an answer containing 200µg/ml of Remdesivir.

Readiness of test arrangement: A comparable Lyophilized Powder of 50 mg of Remdesivir test were gauged and moved to 50 ml of the volumetric cup and disintegrated in Diluent. The jar was shaken and volume was made up to stamp with Diluent to give an essential stock arrangement containing 1000µg/ml. From the above arrangement, 2ml of arrangement is pipette out into a 10 ml volumetric flagon and volume was made up to check with Diluents to give an answer containing 200µg/ml of Remdesivir

Preparation of mobile phase.

A mixture of 0.1% Formic acid in water: Methanol (30:70) (HPLC grade). The mobile phase was sonicated for 10min to remove gases**.**

Selection of mobile phase for method Optimization and experimental condition:

Several trial has been taken for the proper optimization of RP HPLC method by changing different mobile phase with different ratio. And finally the mobile phase for Optimized condition was selected and given follows. And the Optimized parameters was for Remdesivir

Mobile Phase **:** 0.1% Formic acid in water: Methanol (30:70)
Column **:** Inertsil ODS 3v 1504.6, 5mm
Flow Rate **:** 1.0ml/min
Temperature **:** Ambient
Volume **:** 10ul
Detector **:** 254nm
Diluent **:** Water: Acetonitrile (40:60 v/v)

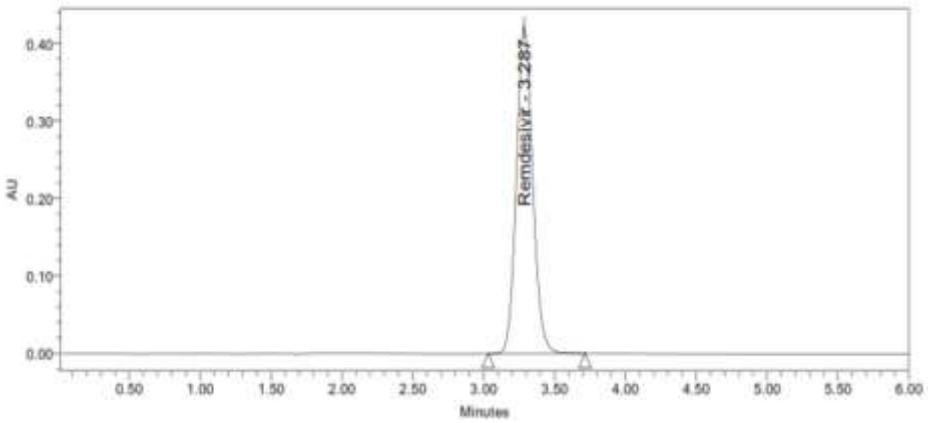

Figure 4: Chromatogram of Optimized Method - Remdesivir

S No.	Name	Retention Time	Area	USP Tailing	USP Plate Count
1	Remdesivir	3.287	3334116	1.10	4072

System Suitability:

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, retention time and asymmetric factor were evaluated

Parameter	Remdesivir	Acceptance criteria
Retention time	3.283	±10
Theoretical plates	3941	> 3500
Tailing factor	1.10	< 2.00
% RSD	0.28	< 2.00

Table 1: System suitability Results

Results:

From a singular volumetric cup of working standard plan six implantations were taken and the got locales were referred to beforehand. % RSD got as 0.28% independently for remedesivir. As the limitation of Exactness was under "2" the system precision was passed in this method.

Standard Results of Remdesivir:

S No.	Sample name	RT	Area	USP plate count	USP tailing
1.	Injection 1	3.287	3361439	3961	1.11
2.	Injection 2	3.285	3355243	3934	1.11
3.	Injection 3	3.285	3354361	3923	1.10
4.	Injection 4	3.281	3344117	3907	1.10
5.	Injection 5	3.278	3338392	3979	1.10

Table 2: Standard Results of Remdesivir

Linearity:

Calibration curve was constructed by plotting concentrations of Remdesivir**.** vs. peak areas, and the regression equations were calculated. The linearity of this method was investigated by using the concentrations 50, 75, 100, 125 and 150 (µg/ml). These concentrations were prepared by diluting appropriate volume of working standard with mobile phase.

S No.	**Concentrations** (µg/ml)	**Area**
1	50	1441287
2	75	2334322
3	100	3334116
4	125	4277633
5	150	5138018
	Correlation coefficient	**0.99972**

Table 2: Linearity Results of Remdesivir

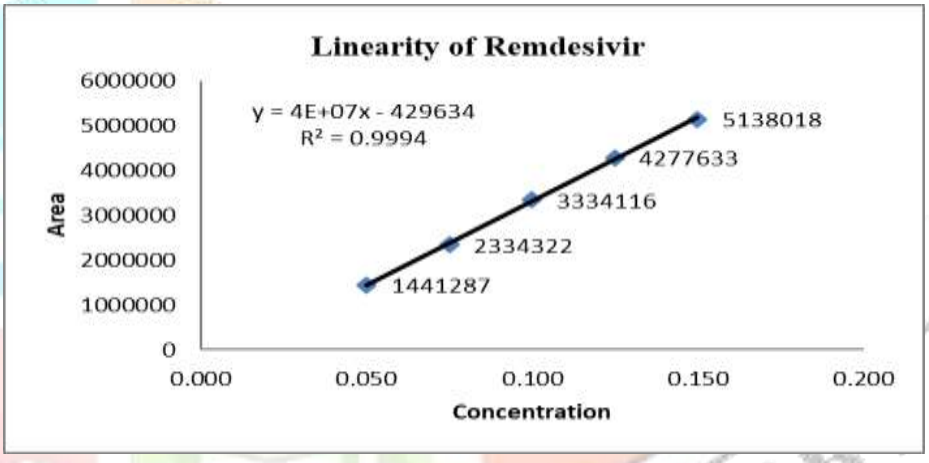

Figure 5: Linearity graph of Remdesivir

Accuracy:

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standard of the drug was added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%.

S No.	Accuracy level	injection	Remdesivir %Recovery	
1	**50%**	1	100.9	100.56
		2	101.0	
		3	100.7	
2	**100%**	1	100.6	100.76
		2	100.9	
		3	100.8	
2	**150%**	1	101.2	100.4
		2	100.1	
		3	100.9	

Table 3: Accuracy Results of Remdesivir

Results

Three levels of Accuracy tests were prepared by standard extension procedure. Three-overlap mixtures were given for every level of precision and mean %Recovery was gotten as 100.56% and 100.76, 100.4% for Remdesivir.

Precision

Intra-day precision was calculated from results obtained from six-fold replicate analysis of samples at three different concentrations of on the same day. Inter-day precision was calculated from results from the same samples analyzed on six consecutive days. Standard solution was prepared as per the test method and injected six times as per the test procedure and Relative standard deviation was calculated.

S No.	RT	Area	% Assay
Injection 1	3.276	3340306	100.7
Injection 2	3.281	3353569	100.9
Injection 3	3.282	3355671	100.8
Injection 4	3.278	3350235	101.2
Injection 5	3.277	3341561	101.6
Injection 6	3.277	3344466	101.2
		Mean	**101.1**
		Std. Dev.	**0.3327**
		%RSD	**0.33**

Table :04: Precision table of Remdesivir

Results:

Different looking at from a model stock plan was done and six working model courses of action of same obsessions were prepared, each mixture from each working model course of action was given and gotten locales were referred to in the above table. The %RSD for 0.33% independently for Remdesivir. As the limitation of Exactness was under "2" the structure precision was passed in this strategy.

Robustness

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 ml/min to 0.8 ml and 1.2 ml/min., temperature of column oven (±27C) and 33°C unit. The mobile phase composition also little changed

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	3.036	6237	1.23
Increased flow rate (1.2ml/min)	2.501	5165	1.18

Table 5: Robustness Results of Remdesivir

Results:

Strength conditions like Stream less (0.8ml/min), Stream not withstanding (1.2ml/min), compact stage short (55B:45A), flexible stage notwithstanding (45B:55A), temperature less (27°C) and temperature notwithstanding (33°C) was stayed aware of and tests were implanted in duplicate manner. Structure suitability limits next to no influenced and all of the were passed. % RSD was inside in the end

LOD & LOQ:

Drug	LOD	LOQ
Remdesivir	**0.27**	**0.46**

Table 6: LOD & LOQ Results of Remdesivir

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.27 & 0.46 µg/ml respectively.

Estimation of Remdesivir in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to work out the typical weight. On top of weighed tablets were finally pulverized and triturated well. A amount of powder cherish twenty five mg of medicine were transferred to twenty five cc meter flask, build and resolution was sonicated for quarter-hour, there once volume was created up to twenty five cc with same solvent. Then ten cc of the on top of resolution was diluted to a hundred cc with mobile part. The answer was filtered through a membrane filter (0.45 µm) and sonicated to remove. The answer ready was injected in 5 replicates into the HPLC system and therefore the observations were recorded. A duplicate injection of the quality resolution was conjointly injected into the HPLC system and therefore the peak areas were recorded. The info square measure shown in Table-7.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak space of drug obtained with check preparation
AS = Peak space of drug obtained with normal preparation
WS = Weight of operating normal taken in mg
WT = Weight of sample taken in mg
DS = Dilution of normal resolutio
DT = Dilution of sample resolution
P = proportion purity of operating normal

Drug Remdesivir	Labelled amount of Drug(mg)	Mean (±SD) Amount (mg) found by the proposed method	Assay % (±SD)
100mg	100 mg	99.29 (± 0.659)	99.48 (± 0.891)

Table 7: Assay Results of Remdesivir

Result:

The amount of drug in Remdesivir Vial was found to be 99.29 (± 0.659)mg/tab for Remdesivir & % assay was 99.48 %.

SUMMERY

S No.	PARAMETER	RESULT	ACCEPTANCE CRITERIA
1	**System suitability** **Theoretical plates** **Asymmetry** **Retention time** **%RSD**	 6706 1.91 5.630 0.5	 Not less than 2000 Not more than 2 Not more than 2
2	**Specificity** **a) Blank interference** **b) Placebo interference**	 Specific	 Specific
3	**Method precision(%RSD)**	0.32	Not more than 2.0%
4	**Linearity parameter** **Slope** **Intercept** **Correlation coefficient(r^2)**	50-150 mcg/ml 0.9994	 Not less than 0.999
5	**Accuracy** **(Mean % recovery)** **50%** **100%** **150%**	 100 100 100	 97 - 103%
6	**Robustness** **a) Flow rate variation** **b) Temperature variation**	All the system suitability parameters are within the limits.	Complies
7	**LOD** **LOQ**	**0.27** **0.46**	NMT 3 NMT10

Table 8: Summary – Method Validation Results of Remdesivir

CONCLUSION

We present in this article simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Remdesivir. Remdesivir was found to be soluble in water, Soluble in Acetonitrile or Methanol. Remdesivir was found to be freely soluble in methanol and ethanol. Mobile Phase: 0.1% Formic acid in water: Methanol (30:70) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Remdesivir in Pharmaceutical dosage forms. By studying various media and conditions, the HPLC method was optimized. The Sample recoveries were in excellent accordance with their respective labeled claims in all formulations and the proposed method was validated in all suitable parameters as per ICH guidelines. This method can be used in quality control testing process of Antiretroviral Drug (Covid-19) Remdesivir in pharmaceutical dosage forms

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