CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1214546

Available online at: http://www.iajps.com Research Article

RP-HPLC PDA METHOD FOR ESTIMATION OF ZANAMIVIR IN API AND PHARMACEUTICAL FORMULATION

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Abstract:

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Zanamivir in its pure form as well as in tablet dosage form. Chromatography was carried out on Sunfire C18 (4.6×150mm, 5µ) column using a mixture of Methanol and water (95:5 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 285nm. The retention time of the Zanamivir was 2.6 ±0.02min. The method produce linear responses in the concentration range of 40-200µg/ml of Zanamivir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Zanamivir, RP-HPLC, validation.*

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Please cite this article in press Devikasubramaniyan et al., ***RP-HPLC PDA Method for Estimation of Zanamivir in API and Pharmaceutical Formulation ,*** *Indo Am. J. P. Sci, 2018; 05(03).*

INTRODUCTION:

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity [8,21].

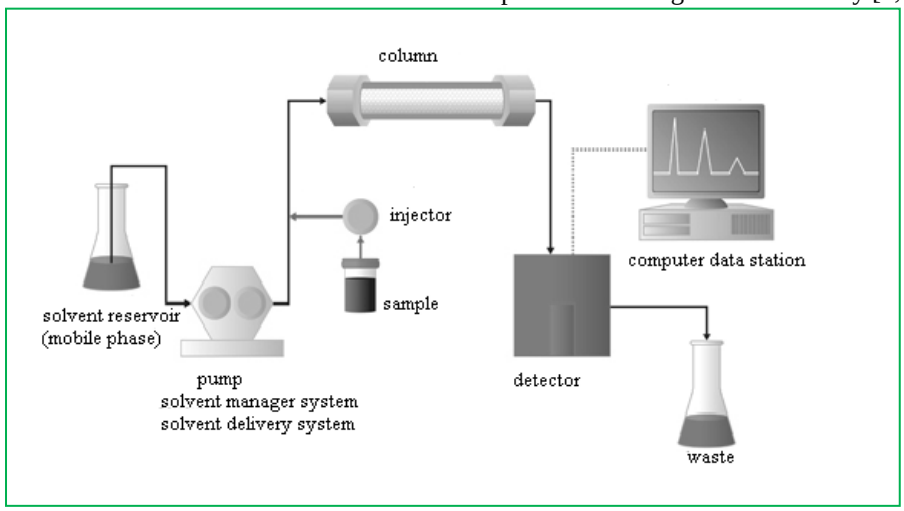

Fig.1.1: Components of HPLC instrument block diagram. [22]

Mobile phase preparation

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 µm pore size filters and be degassed before use.

Degassing of solvents

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

1. By warming the solvents
2. By stirring vigorously with a magnetic stirrer
3. By subjecting to vaccum filtration
4. By ultra sonication (using ultrasonicator)

Table 1: Physical properties of common HPLC solvents [8]

Solvent	MW	BP	RI (25°C)	UVCutoff (nm)	Density g/mL(25°C)	Viscosity (25°C)	Dielectric Constant
Acetonitrile	41.0	82	1.342	190	0.787	0.358	38.8
Dioxane	88.1	101	1.420	215	1.034	1.26	2.21
Ethanol	46.1	78	1.359	205	0.789	1.19	24.5
Ethylacetate	88.1	77	1.372	256	0.901	0.450	6.02
Methanol	32.0	65	1.326	205	0.792	0.584	32.7
CH_2Cl_2	84.9	40	1.424	233	1.326	0.44	8.93
Isopropanol	60.1	82	1.375	205	0.785	2.39	19.9
n-propanol	60.1	97	1.383	205	0.804	2.20	20.3
THF	72.1	66	1.404	210	0.889	0.51	7.58
Water	18.0	100	1.333	170	0.998	1.00	78.5

Pumping systems

The pumping system is one of the most important features of an HPLC system. There is a high resistance to solvent flow due to the narrow columns packed with small particles and high pressures are therefore required to achieve satisfactory flow rate.

The main requirements of pumping systems are:

1. Generation of pressures up to 6000 psi.
2. Pulse free output
3. Flow rates ranging from 0.01 to 10 mL/min
4. Flow control and flow reproducibility of ± 0.5%
5. Corrosion resistant components (seals of Teflon and stainless steel)
6. Should be easy to dismantle and repair.

There are three basic types of pumps in common use.

1. Reciprocating pumps.
2. Displacement pumps or syringe pumps.
3. Pneumatic pumps or constant pressure pumps [8].

Sample introduction system

Injection ports are of two basic types,

1. The sample is injected directly into the column.
2. The sample is deposited before the column inlet and then swept by a valving action into the column by the mobile phase.

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 mL of volume with high reproducibility and under high pressure (up to the 4000psi).They should also produce minimum band broadening and minimize possible flow disturbances. The most useful and widely used sampling device for modern LC is the micro sampling injector valve. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperatures. High-performance valves provide extra column band-broadening characteristics comparable or superior to that of syringe injection [8].

Columns

Typical analytical columns are 10, 15 and 25 cm in length and are fitted with extremely small diameter (3, 5 or 10 µm) particles. The internal diameter of the columns is usually 4 or 4.6 mm; this is considered the best compromise among sample capacity, mobile phase consumption, speed and resolution. Preparative columns are of larger diameter. Packing of the column tubing with the small diameter particles requires high skill and specialized equipment. For this reason, it is generally recommended that the most experienced chromatographers purchase prepacked columns, since it is difficult to match the high performance of professionally packed LC columns without a large investment in time and equipment.

The column can be classified based on the material bonded to the silica packed surface such as C_4, C_8, C_{18}, phenyl, chiral, cyanomicrobore columns (1mm to 100cm), U shaped and coiled columns are available. Guard columns are used before the analytical columns to increase the life of analytical columns by retaining non eluted components and particulate matter. The flow chart in the Table 1.3 can assist one in determining which columns to examine.

Zanamivir : (2R,3R,4S)-4-[(diaminomethylidene)amino]-3-acetamido-2-[(1R,2R)-1,2,3-trihydroxypropyl]-3,4-dihydro-2H-pyran-6-carboxylic acid. The proposed mechanism of action of zanamivir is via inhibition of influenza virus neuraminidase with the possibility of alteration of virus particle aggregation and release. By binding and inhibiting the neuraminidase protein, the drug renders the influenza virus unable to escape its host cell and infect others.

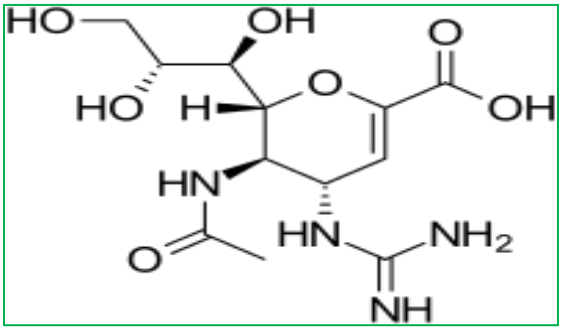

Fig. 2: chemical structure of Zanamivir

MATERIALS AND METHODS:

Accurately weigh and transfer 10 mg of Zanamivir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Instrumentation and Chromatographic conditions

The analysis was performed by using Sunfire C18 column, 4.6×250mm internal diameter with 5 micron particle size column and UV detector set at 285nm nm, in conjunction with a mobile phase of Methanol in the ratio of 100v/v (pH 5 adjusted with OPA) at a flow rate of 0.8 ml/min. The retention time of Zanamivir was found to be 2.262 minute. The 10µl of sample solution was injected into the system

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Zanamivir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1.2ml of the above Zanamivir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 50:50 v/v respectively.

Optimization of Column:

The method was performed with column like Sunfire C18 (4.6×250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow

(Optimized chromatogram):

Column : Sunfire C18 (4.6×250mm) 5µ
Column temperature : Ambient
Wavelength : 285nm
Mobile phase ratio : Methanol:Water (95:5% v/v)
Flow rate : 0.8ml/min
Injection volume : 10µl
Run time : 4minutes

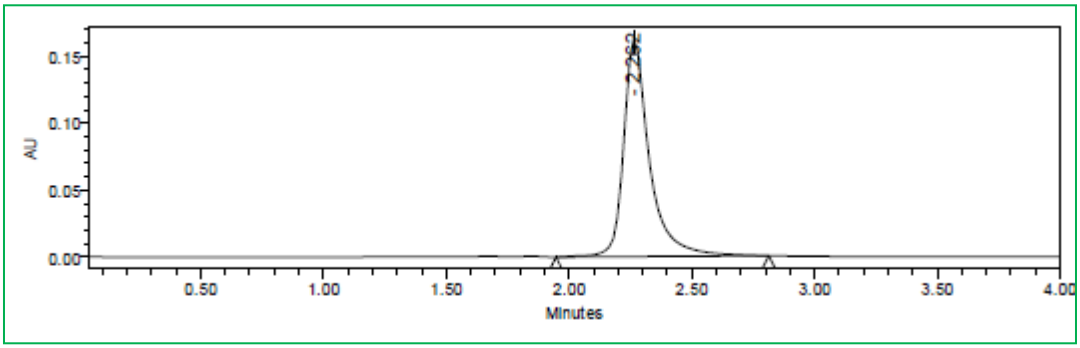

Fig. 3: Typical chromatogram of mixture of Standard solution

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 950ml (95%) of HPLC Methanol and 50ml of HPLC Water (5%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Linearity

The linearity of was obtained in the concentration ranges from 40-200 µg/ml

Table 1: Linearity data of Zanamivir

Concentration Level (%)	Concentration µg/ml
60	40
80	80
100	120
120	160
140	200

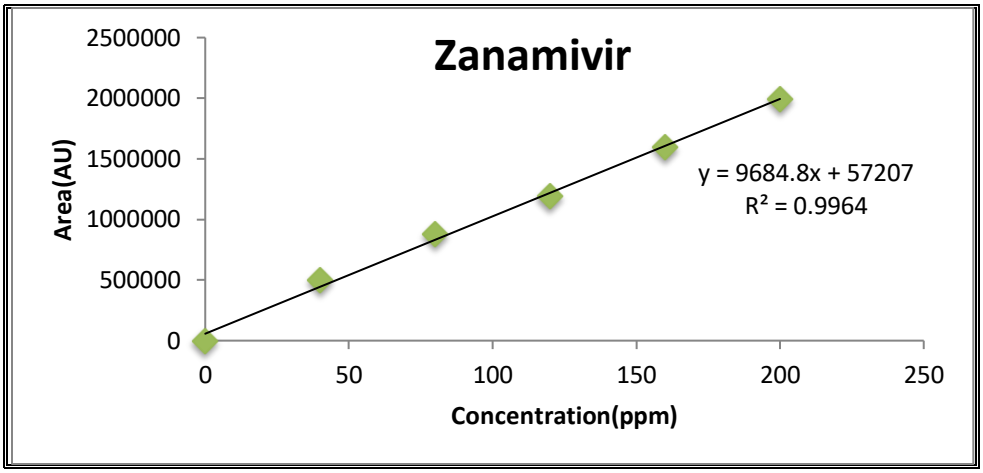

Fig.4: calibration graph of Zanamivir

LINEARITY PLOT

Linearity of detector response of assay method was found by injecting seven standard solutions with concentration ranging from 40-200 µg/mL for Zanamivir. The graph was plotted for concentration versus peak area. The results were shown in Table-1 and fig 4.

Precision

Repeatability

The precision of test method was determined by preparing six test preparations using the product blend and by mixing the active ingredient with excipients as per manufacturing formula. And the relative standard deviation of assay results was calculated. The results were shown in Table 2

Table 2: Results of repeatability for Zanamivir

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Zanamivir	2.269	1187187	159416	6622.7	1.4
2	Zanamivir	2.264	1188125	161793	8758.1	1.5
3	Zanamivir	2.267	1189202	161854	8700.8	1.4
4	Zanamivir	2.270	1191196	159246	7619.9	1.5
5	Zanamivir	2.262	1192867	162665	6652.7	1.4
Mean			1189715			
Std.dev			2308.166			
%RSD			0.19401			

Accuracy

Zanamivir tablets content were taken at various concentrations ranging from 50 % to 150 % (50 %, 75 %, 100 %, 125 %, and 150 %) to accurately quantify and to validate the accuracy. The assay was performed in triplicate. The results were shown in Table-4

Table 4: The accuracy results for Zanamivir

%Concentration (at specification Level)	Peak area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	605972	37.5	37.4	99.7	99.7
100%	1195448.66	75	74.8	99.7	
150%	1822551	112.5	112.39	99.9	

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.The LOD value for Zanamivir 10.0 µg/ml.

Quantitation limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The LOQ values for Zanamivir 30.3 µg/ml

ROBUSTNESS

The robustness was performed for the flow rate 0.8ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Zanamivir. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard samples of Zanamivir were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Table 5

Table 5: Results for Robustness of Zanamivir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.8mL/min	1294261	2.262	7452	1.1
Less Flow rate of 0.7mL/min	1294613	2.995	5927	1.2
More Flow rate of 0.9mL/min	1274532	1.925	8462	1.3
Less organic phase (about 5 % decrease in organic phase)	1273762	3.936	9265	1.2
More organic phase (about 5 % Increase in organic phase)	1205836	1.927	6647	1.1

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 285nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Sunfire C18 (4.6 x 150mm, 5µm) because it was giving good peak. 35º C temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: water was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Zanamivir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Zanamivir was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: water 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.

Table 6.Summary data for Zanamivir

Parameters	Zanamivir
Retention Time (min.)	2.262
Linearity (µg/ml)	40-200
Correlation Coefficient (r^2)	0.996
Slope	9684
Y - intercept	26877
LOD (µg/ml)	10.0
LOQ (µg/ml)	30.3
Repeatability (% RSD) n=6	0.19401
Intraday Precision (% RSD) n=6	0.161136
Interday Precision (% RSD) n=6	0.377309

ACKNOWLEDGEMENT:
The authors are thankful to Sura Pharma Labs, Dilshuknagar, Hyderabad for providing necessary facilities for this entire research work

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