International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.1, pp 180-186, Jan-Mar 2011

Estimation of Zanamivir Drug present in Tablets using RP-HPLC Method

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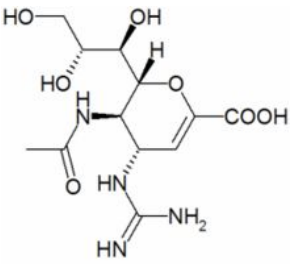
Abstract: A reverse phase isocratic high performance liquid chromatographic method was developed for the estimation of Zanamivir (ZAN) drug in tablet formulation. The separation was achieved by symmetry C8 (4.6x150 mm, 3.5 micron, make: Xterra) and Acetonitrile: potassium Dihydrogen orthophosphate buffer pH 4.0 (60:40 v/v) as mobile phase, at a flow rate of 0.5 ml/min. Detection was carried out at 230 nm. Retention time of ZAN was found to be 3.5 min+or-0.5 min. The method has been validated for linearity, accuracy, precision. Linearity of Zanamivir was in the range 20-100 mcg/ml. The mean recovery obtained for was 100.7. Developed method was found to be accurate, precise, selective and rapid for estimation of Zanamivir drug in tablets.
Key words: Zanamivir, Method development and Validation.

Introduction:

The chemical name of Zanamivir is 5-(acetylamino)-4-[(aminoiminomethyl)-amino]-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-en onic acid. It has the following structural formula[1,2] :



Zanamivir is a potent and highly selective inhibitor of the influenza A and B virus neuraminidase[3].

Mechanism of action has on viral neuraminidase catalyzes cleavages of terminal sialic acid residues attaches to glycoprotein and glycolipids, a process necessary for release of virus from host cell surfaces[4,5].ZAN poor solubility in water and shows good solubility in Acetonitrile[6,7]. Liquid chromatographic–tandem mass spectrometric method for the determination of the neuraminidase inhibitor Zanamivir (GG167) in human serum[8], Zanamivir in rat and monkey plasma by positive ion hydrophilic interaction chromatography (HILIC)/tandem mass spectrometry[9] were developed .Colorimetric method[11],HPLC methodologies available for structurally, chemistry similar oseltamavir phosphate[10] were available. Till the date there is no HPLC method available for analysis of Zanamivir

Material and Methods :

Apparatus: High Performance Liquid Chromatographic system (Waters) equipped with two LC 20AT liquid

pumps and Auto Sampler and DAD or UV detector, Rheodyne Injector (2E 7725, 20 µl loop), and Spinchrome software, Glass Van Hypodermic injecting syringe, Symmetry C8 (4.6 x 150mm, 3.5 µm, Make: Xterra) or equivalent. PH meter (polmon), analytical balance (sartorius) and Millie Q with (0.45.micron) filters for HPLC grade water

Material: water HPLC grade, Acetonitrile purchased from Merck chemicals, Potassium Dihydrogen ortho phosphate, Zanamivir Working standard, Zanamivir 5 mg Tablets from the pharmacy shops in Hyderabad.

Chromatographic condition:

Equipment : waters HPLC Auto Sampler and DAD or UV detector.

Column : Symmetry C8 (4.6 x 150mm, 3.5 µm)

Flow rate : 0.5 mL per min

Wavelength : 230 nm

Injection volume: 20 µl

Column oven : Ambient

Run time : 5 min

Preparation of Phosphate buffer: Weigh 7.0 grams of Potassium Dihydrogen ortho Phosphate into a 1000ml beaker, dissolveand diluted to 1000ml with mille Q water. Adjust the pH to 4.0 with Ortho Phosphoric acid.

Preparation of mobile phase: Mix a mixture of above buffer 400mL (40%) and 600 mL of Acetonitrile HPLC (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation: Accurately weigh and transfer 20mg of ZAN Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Sample Solution Preparation: Weigh 5 ZAN Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 20 mg of Zanamivir into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter

Calculation

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of Zanamivir obtained with test preparation, AS = Peak Area of Zanamivir obtained with standard preparation, WS = Weight of working standard taken in mgWT = Weight of sample taken in mg, DS = Dilution of Standard solution, DT = Dilution of sample solution, P = Percentage purity of working standard

Results and discussion;

Developed analytical method was a simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method for estimation of ZAN. Different mobile phases were tried and the proposed

chromatographic conditions was found to be appropriate for the quantitative determination.

Method Validation:

The proposed RP-HPLC method was validated as per ICH guidelines.

System suitability;

Standard solution is injected five times and Flow rate was maintained at 0.5 ml/min. temperature of column kept ambient and the column effluents were monitored at 230 nm chromatograms were taken and System suitability parameters were computed. the system suitability were calculated as per ICH guidelines

Calibration Curve;

Calibration curves were prepared by taking appropriate aliquots of ZAN stock solution in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 20, 40, 60, 80 and 100 mcg/ml. These solutions (n=6) were injected and chromatogram were taken. Flow rate was maintained at 0.5 ml/min. temperature of column kept ambient and the column effluents were monitored at 230 nm. Calibration curve was constructed by plotting peak area Vs concentration and regression equation was computed. R^2 values of was found to be as 0.999.

Specificity:

The peak purity of ZAN was assessed by comparing the retention time of standard ZAN sample good correlation was obtained between the retention time of standard and sample. Placebo and blank was injected and there were no peaks. There are no interferences hence method is specific.

Chromatograms

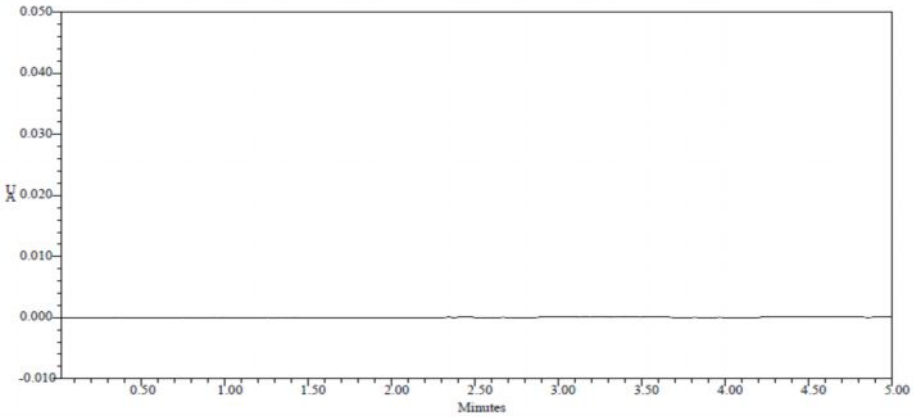


Figure 1: Chromatogram for blank

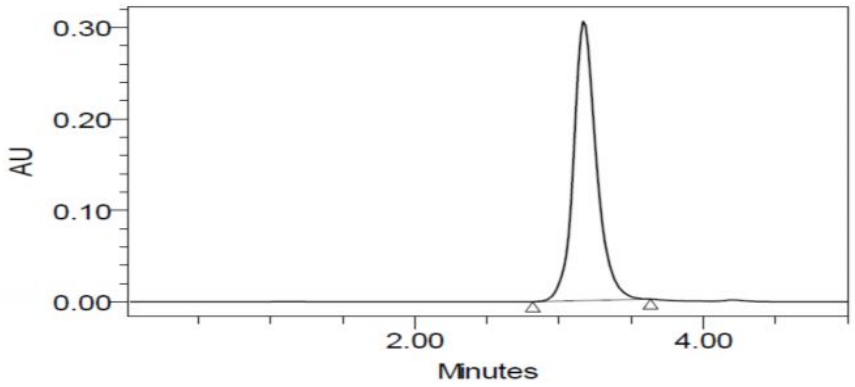


Figure 2: Chromatogram of Standard

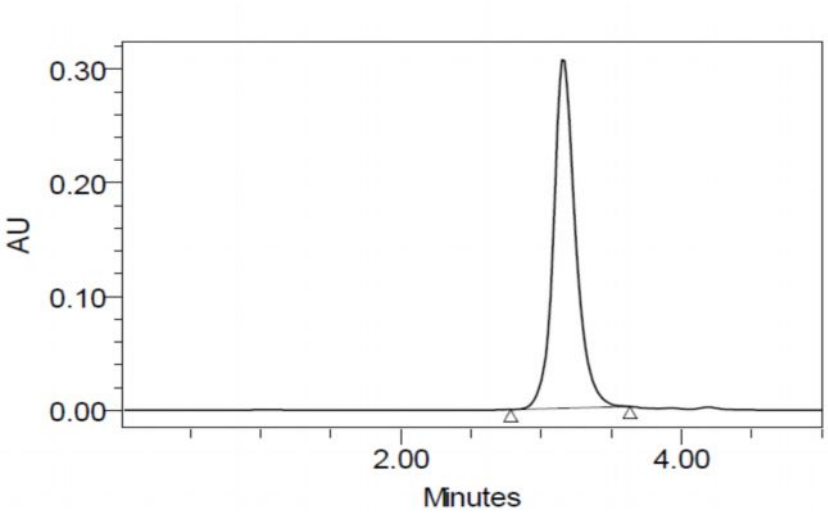


Figure 3: Chromatogram of the sample

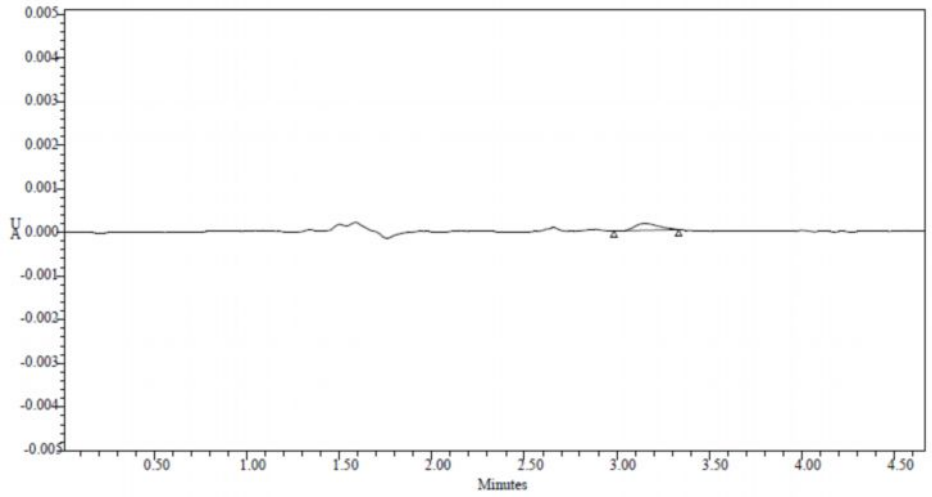


Figure 4: Chromatogram for LOD

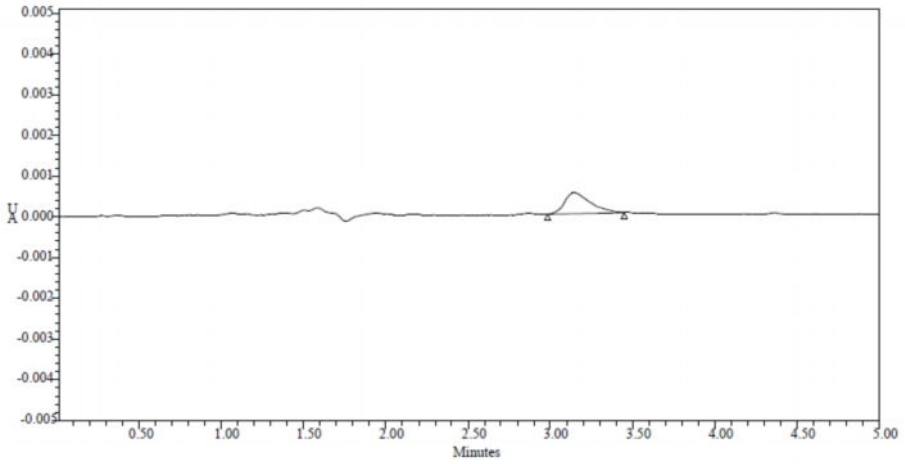


Figure 5: Chromatogram of LOQ

Linearity:

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for ZAN found to be as 20-100mcg/ml with correlation coefficient (R^2) 0.999.

Precision:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample preparation was carried out in same manner as described in sample preparation. Percentage Relative

Standard Deviation (% RSD) was found to be less than 2% that proves method is precise.

Accuracy (Recovery studies);

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100% and 120% concentration levels. Known amounts of standard CAP was added to the pre-analyzed samples and subjected to the proposed HPLC method. Results of recovery studies are shown in table no.3

Robustness:

The Robustness of method as carried out by changing the Chromatographic conditions such as Flow rate and Temperature variations. With the change of Flow rate of 0.4 ml, 0.5 ml and 0.6ml, change of organic solvent portion of the mobile phase with of 10% less, actual, 10% more and their tailing factor, plate count obtained within the limit.

Ruggedness:

The ruggedness of method carried out by using the different HPLC system .with change of the system mean area found to be 98.5 and percentage RSD found to be 0.10 .

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD concentration obtained is 0.023mcg/ml (or) 0.04% with respect to working concentration of 0.8mg/ml. The LOQ concentration obtained is 0.078mg/ml (or) 0.13% with respect of working concentration of 0.8mg/ml.

Table 1:Linearity

Linearity levels (mcg/ml)	Peak areas
20	1404812
40	2303535
60	3382511
80	4461052
100	5551532

Figure 6: Linearity graph

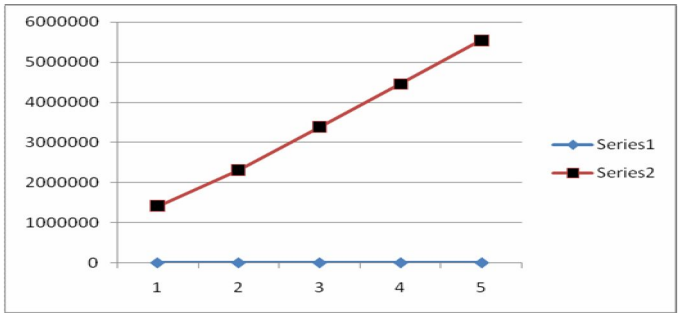


Table 2: Precision

Injection	Area
Injection-1	3313918
Injection-2	3384074
Injection-3	3362752
Injection-4	3388053
Injection-5	3343227
Average	3358405
Standard Deviation	30684.9
%RSD	0.91

Table 3: Accuracy

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1710826	10.2	10.23	100.3%	100.7%
100%	5089935	20.0	20.1	100.5%	
150%	5089935	30.0	30.4	101.3%	

Table 4: Ruggedness

Injection	Area
Injection-1	3377344
Injection-2	3381214
Injection-3	3383251
Injection-4	3385436
Injection-5	3384943
Average	3382438
Standard Deviation	3292.9
%RSD	0.10

Table 5: Robustness (flow rate variation)

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.4	2178	1.1
2	0.5	2232	1.1
3	0.6	2027	1.12

Table 6: Robustness (Mobile phase variation)

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2014	1.1
2	*Actual	2232	1.1
3	10% more	2041	1.2

Table 7: LOD

Component	Working conc. (mcg/ml)	LOD Conc. (mcg/ml)	Signal To Noise Ratio
Zanamavir	60	0.023	2.8

Table 8: LOQ

Component	Working conc. (mcg/ml)	LOD Conc. (mcg/ml)	Signal To Noise Ratio
Zanamavir	60	0.078	9.62

Conclusion:

The proposed method is simple, specific, accurate and precise and hence can be used in routine for estimation of ZAN in tablet dosage. Statistical analysis of the results has been carried out revealing high accuracy and good

precision. The percentageRSD for all parameters was found to be less than two, which indicates the validity of the method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of ZAN in tablet dosage form.

Acknowledgement

The authors are thankful to Mr. K. Chandra Shekar, Managing Director of Pharma training, Balanagar, and Hyderabad for providing necessary facilities and T.Karnaker Reddy. for his support during this work. We are also thankful to Dr. A. Sunil kumar Reddy, for his moral support and guidance.

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