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Development and Validation of Analytical method for Estimation of Antiviral Drug in Solid Dosage Form by HPLC

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Abstract: *The present work aimed to develop a new simple, accurate and reproducible RP- HPLC method for the analysis of Zanamivir. The method was validated according to ICH (Q2R1) guidelines. The chromatographic conditions were effectively monitored for the elution of analyte utilizing Thermo Hypersil Gold C18 (250mm×4.6mm),5µm; water: methanol (80:20 % v/v) as a solvent system with a 1.0 mL/min of flow at detection wavelength of 233 nm. The retention of analyte was achieved at 3.290 minutes. The tablet sample was assayed with 99.44 %±0.16 purity. The system suitability parameters such as theoretical plates and retention time were found to be 7582 and 3.292, respectively. The linearity of the method achieved at the concentration range of 50-150 µg/mL with a correlation coefficient (R2) of 0.9818. The accuracy study showed 99.83%±0.28 of recovery of analyte. Precision in terms of repeatability was found within the limit (% RSD-1.21), while intermediate precision was shown % RSD of 0.23. In addition, the method found robust at a deliberate change of flow rate and solvent composition. Therefore, the results confirmed the suitability of the method for quantifying Zanamivir in their formulations.*

Keywords: Zanamivir, Method development and Validation

I. INTRODUCTION

Antiviral drugs are a class of medicines particularly used for the treatment of viral infections. Drugs that combat viral infections are called antiviral drugs.Viruses are among the major pathogenic agents that cause number of serious diseases in humans, animals and plants. Viruses cause many diseases in humans, from self resolving diseases to acute fatal diseases.1

The present work deals with the development and validation of an RP-HPLC method for the estimation of Zanamivir (ZAN) in API and tablet dosage forms.2,3 Different formulations of ZAN are being commercially available in single and in combination with other drugs (Figure 1). ZAN is an antiviral agent that works as a neuraminidase inhibitor indicated for the remedy of simple acute infection due to influenza A and B virus in adults and pediatric patients. ZAN also proven it significant inhibition of the human sialidases NEU3 and NEU2.4

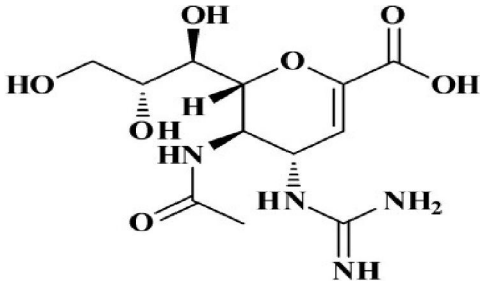

Fig 1: Chemical structure of ZAN

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II. MATERIAL AND METHODS

2.1. Materials

The solvents and chemicals employed such as water, methanol, acetonitrile, orthophosphoric acid and buffers (HPLC grade) were purchased. API of ZAN was procured from Arrow chem Mumbai. The pharmaceutical formulations were purchased from the local market.

2.2. Instrumentation

The analysis of the samples in the study were performed on Waters 600, 996 PDA Detector, Empower pro software and Thermo Hypersil Gold C18 (250mm×4.6mm), 5µm column.

2.3. Method development

2.3.1. Optimization of the method

The analyte was eluted on the Thermo Hypersil Gold C18 (250mm×4.6mm), 5µm with 1.0 ml/min flow of methanol: water (20:80 %v/v) with PDA detection. The injection volume was maintained as 20 µl in all the experiments. The ambient temperature was maintained at the column and analyte sampler throughout the elution. Run time was maintained as 8.0 min as the analyte was eluted at 3.290 minutes. single and in combination with other drugs (Figure 1). ZAN is an antiviral agent that works as a neuraminidase inhibitor indicated for the remedy of simple acute infection due to influenza A and B virus in adults and pediatric patients

2.3.2. Preparation of sample solution

Tablet powder equivalent to 5 mg of ZAN was taken in a 10 mL volumetric flask containing 2 mL of diluent and the volume was adjusted and sonicated. Pipette out 10 mL of the above stock solution into another volumetric flask (100 mL capacity) and the volume was adjusted to get a final concentration of 100 µg/mL of ZAN.

2.3.3. Preparation of standard solution

5 mg of ZAN standard was transferred into a 10 mL volumetric flask containing 2 mL of diluent, further dilutions were made to get a final concentration of 100 µg/mL of ZAN as per sample preparation.

2.4. Assay

Accurately weighed tablet material (equivalent to 5 mg of ZAN) was transferred into a 10 mL of the volumetric flask containing 2 mL of diluent and the volume was adjusted with diluent. Further dilutions were continued to get a final concentration of 100 µg/mL of ZAN. The resulted solution was injected into the HPLC system in triplicate. The % purity of ZAN was determined with the help of the peak area of sample and standard.

2.5. Method validation

The method developed for quantification of ZAN was validated as per ICH guidelines.

2.5.1. System suitability

System suitability parameters were evaluated by injecting the standard solution as per the optimized protocols. The standard solution was prepared by taking 5 mg of ZAN into a 10 mL volumetric flask containing 2 mL of diluent and sonicated. Further requisite dilutions were continued as per the optimized procedure. The key parameters like plate count and tailing factor, etc., were checked to assess the suitability of the system.

2.5.2. Specificity

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

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2.5.3. Linearity and range

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for ZAN found to be as 50-150 ug/ml with correlation coefficient (R2) 0.982.

2.5.4. Accuracy

The accuracy of the method was determined by standard addition at 80, 100, and 120 % w/v level to the test sample. The sample solutions were prepared by spiking 4 mg (80 % w/v), 5 mg (100 % w/v) and 6 mg (120 % w/v) of standard.The dilutions were made as per the procedure given under method optimization. The amount (% recovery) of standard recovered was determined by considering the peak area of triplicate injections. The mean % recovery was correlated with the standards of ZAN.

2.5.5. Precision Repeatability

It was illustrated in terms of assay repeatability of the test sample. The sample solution prepared from homogenous lots and injected for three determinations. The % RSD was determined for all replicate injections.

Intermediate precisions

The intermediate precision (ruggedness) of the method was determined by performing on different days by different analysts. The analysis was carried out by following repeatability protocols. The % RSD for the peak area of three replicate injections was calculated to check compliance with acceptance criteria.

2.5.6. Robustness

The solvent flow rate was monitored as 0.9 mL/min and 1.1 mL/min to assess the compliance of robustness of the optimized method. Also, the organic composition of the mobile phase was varied as + 10% (80:20). Standard solution (100 µg/mL), Change in wavelength maxima by ±2 units, was injected in triplicate with these changes and correlated with actual retention time and peak area. The % RSD of responses at these changes was assessed.

III. RESULTS AND DISCUSSION

3.1. Method development

3.1.1. Optimization of the method

The present work was intended to develop a new approach for the estimation of ZAN with the help of RP-HPLC technique. To optimize the proposed method, several trials were performed using methanol and water as a solvent system in different combinations in appropriate ratios. As per the chromatographic results, the methanol and water in the ratio of 20:80 % v/v proved to be an effective solvent system for elution of analyte. The absorption wavelength was found at 233 nm on PDA detection. The optimum flow rate was adjusted as 1.0 mL/min with an injection volume of 20 µl. The efficiency in elution was achieved at ambient temperature at the column with an auto sampling. As the analyte peak was eluted at 3.290 minutes, the run time was set as 8.0 minutes (Figure 2). The peak parameters were optimum for chromatogram in terms of asymmetric factor, plate count, tailing factor and height, etc.

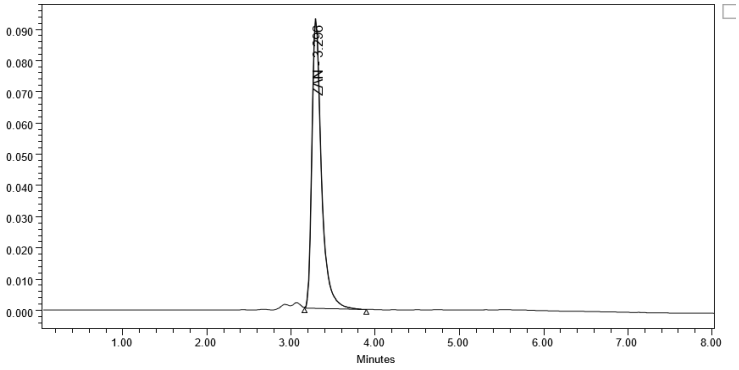

Fig 2: Chromatogram at Optimized conditions

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3.2. Assay

The ZAN in tablet formulations was assayed with the help of optimized chromatographic conditions. Triplicate injections were made to get the percentage (%) purity of analyte in the formulations. As per the proposed method, the % purity of ZAN was found to be 99.44% ± 0.42.

3.3. Method validation

3.3.1. System suitability

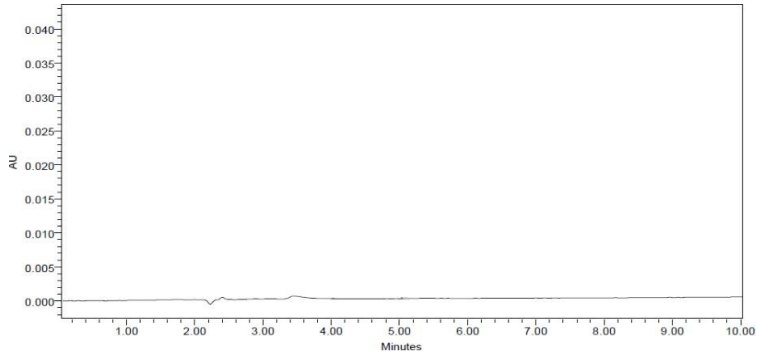
The peak parameters results met the system suitability requirements. The retention time was detected at 3.290 minutes. The number of theoretical plates were noticed as 7582

Sr. No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	ZAN	ZAN	ZAN	ZAN
1	103048	3.302	1.28	7526
2	105415	3.284	1.35	7588
3	107850	3.303	1.19	7645
4	103450	3.289	1.15	7600
5	104314	3.284	1.12	7550
mean	104815	3.2924	1.21	7582
S.D	1924.04	0.0094	0.09	56.07
%R.S.D	1.83	0.28	7.81	0.70

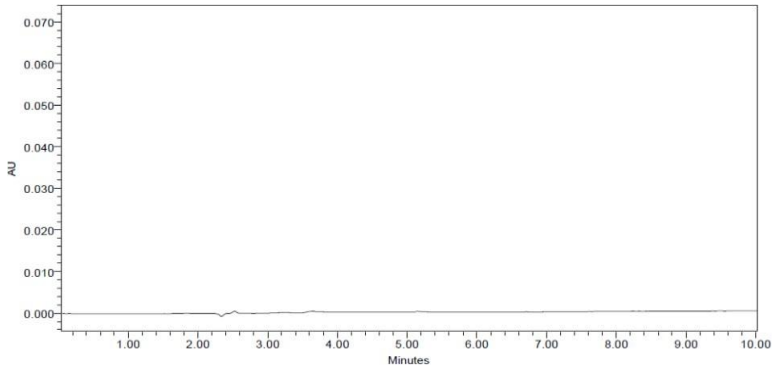
3.3.2. Specificity

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

Sample matrix



Placebo Preparation



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3.3.3. Linearity and range

The calibration curve was plotted with the peak area of ZAN obtained on elution versus corresponding concentrations to check the complying linearity (Figure 3). The linearity was attained over a concentration range of 50-150µg/ml. The calibration plot at these concentrations expressed the correlation coefficient (R2) of 0.9818 with linear regression equation of y = 16597x + 7136.2

Sr. No.	% Level	ZAN	
		Conc. (µg/ml)	Mean peak area
1	50	2.5	44090
2	80	4	77211
3	100	5	95534
4	120	6	104231
5	150	7.5	129546

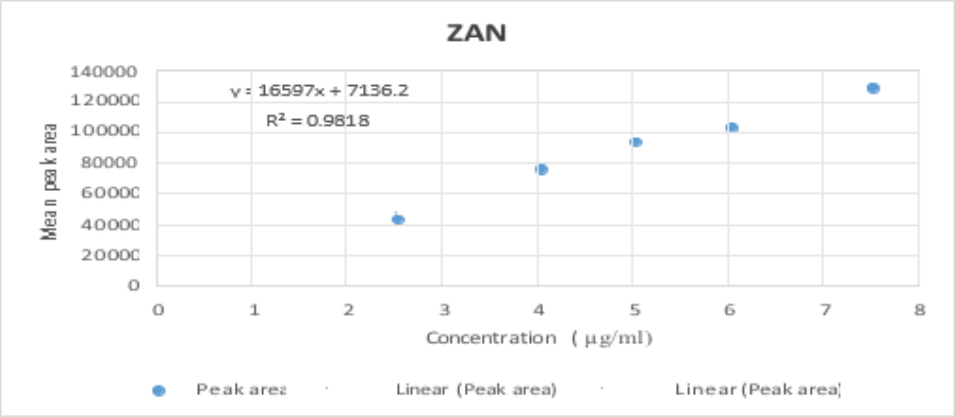

Fig 3. Plot of linearity and range study for ZAN

3.3.4. Accuracy

The accuracy samples were prepared by spiking the standard into the pre-analyzed formulation sample at different concentrations (80%,100% and 120%) and injected each in triplicate. The resultant mix was injected and recovery of standard spiked was calculated.

The % Recovery was then calculated by using formula

% Recovery = A-B x 100/ C

Where-

A = Total amount of drug estimated.

B = Amount of drug found on pre analyzed basis.

C = Amount of pure drug added.

Calculate the amount recovered, % recovery, average recovery, % RSD of triplicate sample preparation, overall recovery and overall % RSD. Record the observation into the following table.

	ZAN		
	Levels		
	80%	**100%**	**120%**
Amt added (µg/ml)	4	5	6
	4	5	6
	4	5	6
Amt taken (µg/ml)	4	5	6

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	4	5	6
	4	5	6
Amt recovered (µg/ml)	3.99	4.97	6
	3.97	5	6
	4.0	5	5.97
% Recovery	99.78	99.50	100.00
	99.38	100.00	100.00
	100.00	100.00	99.59
Mean % recovery	99.72	99.83	99.86
% RSD	0.31	0.28	0.23

3.3.5. Precision

It was performed in terms of repeatability assay and intermediate precision.

Repeatability

Assay repeatability was analysed by a single analyst with a varied number of samples. The standard solution was injected three times and measured the retention time. The % RSD for the area of replicate injections was within specified limits.

Sr.no.	ZAN	
	Assay (mg)	Assay % of ZAN
1	4.975	99.5
2	4.975	99.5
3	4.925	98.5
Average	4.9	99.16
SD	0.02	0.57
% RSD	0.58	0.58

Intermediate precision

The intermediate precision was carried out by five replicate injections of ZAN standard solution. Each standard solution was injected into the chromatographic system and peak area of each injection was considered for % RSD calculations. The %RSD of intermediate precision was observed within specified limits (% RSD- 0.23).

Sr.No.	ZAN	
	Assay (mg)	Assay % of ZAN
1	4.95	99.00
2	4.95	99.00
3	4.97	99.50
Average	4.95	99.17
SD	0.01	0.28
% RSD	0.23	0.28

3.3.6. Robustness

The robustness was determined by varying the flow rate as 0.9 mL/min and 1.1 mL/min and the mobile phase ratio. The method was found robust even with a slight change in flow rate and mobile phase + 10% (80:20), Change in wavelength maxima by ±2 units,The results were depicted in Table.

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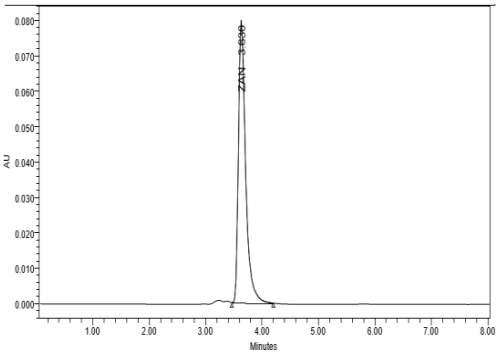
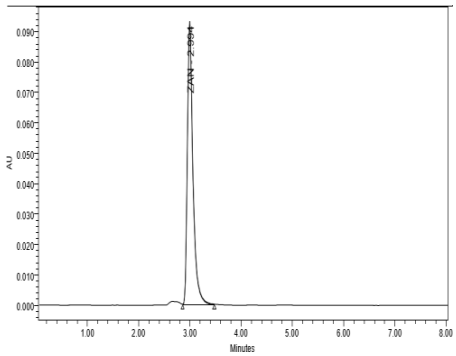


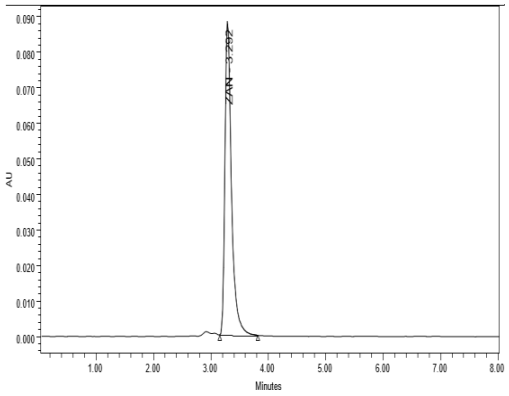
0.9 ml/min



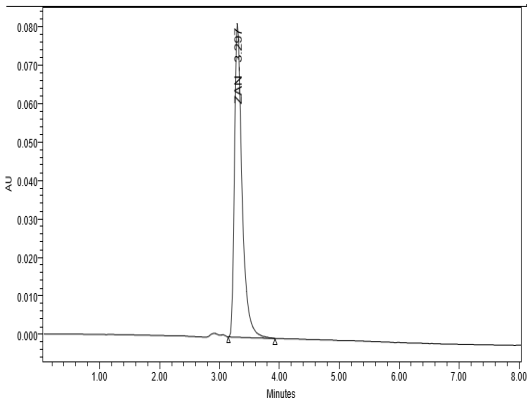
1.1 ml/min

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchang ed	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	ZAN	1.22	1.52	1.55	NMT 2.0
2	Theoretical plates	ZAN	7497.53	7438.7	7457.9	NLT 2000
3	Tailing factor	ZAN	1.48	1.61	1.56	NMT 2.0
4	Retention Time (Min)	ZAN	3.290	3.635	2.994	

Table: System suitability of change in Flow Rate



-10% MET: (BUFF: MET 82:18)



+10% MET: (BUFF: MET 78:22)

Sr. No.	System Suitability parameter		Observations			Limits
			Unchang ed	- 10%	+ 10%	
1	The % RSD of peak area response for five replicate injections	ZAN	1.17	1.55	1.46	NMT 2.0
2	Theoretical plates	ZAN	7397.53	7496	7347.6	NLT 2000
3	Tailing factor	ZAN	1.65	1.70	1.42	NMT 2.0
4	Retention Time (Min)	ZAN	3.290	3.297	3.292	

Table: System suitability of change in Organic Composition

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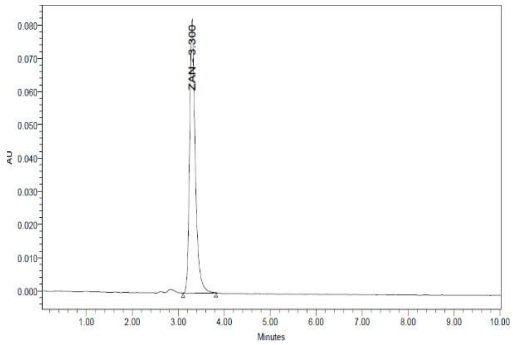
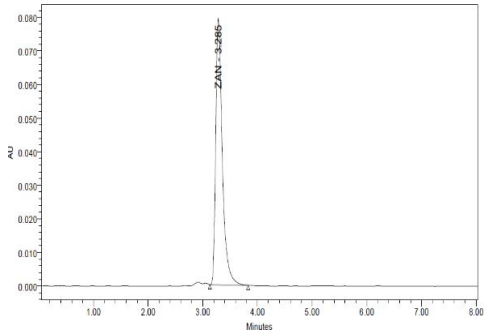


231 nm



235 nm

Sr. No.	System Suitability parameter		Observations			Limits
			Unchanged	231nm	235nm	
1	The % RSD of peak area response for five replicate injections	ZAN	1.17	1.55	1.46	NMT 2.0
2	Theoretical plates	ZAN	7397.53	7496	7347.6	NLT 2000
3	Tailing factor	ZAN	1.6	1.70	1.4	NMT 2.0
4	Retention Time (Min)	ZAN	3.290	3.300	3.285	

Table : System suitability of change in Wavelength

IV. CONCLUSION

A RP-HPLC approach was established for the estimation of ZAN and it was fulfilled to all the validation criteria as per ICH guidelines. The analyte separation was achieved with Thermo Hypersil Gold C18 (250mm×4.6mm), 5µm column by water and methanol in the ratio of 80:20
% v/v at 1.0 mL/min flow. The eluted analyte was monitored at 233 nm on PDA detector. The analyte peak was eluted at 3.290 mins. The results were within the acceptable range for accuracy, precision, linearity, and specificity. Therefore, the proposed method could be applied for the determination of analyte drug in bulk and marketed tablet formulations.

V. ACKNOWLEDGEMENTS

The authors are thankful to the Principal and Management, P. Wadhwani College of Pharmacy, Yavatmal for providing the facilities to carry out the research work.

VI. ABBREVIATIONS

RP-HPLC: Reverse Phase-High Performance Liquid Chromatography. API: Active Pharmaceutical Ingredient SD: Standard Deviation %RSD: Percent relative standard deviation. ICH: International Conference on Harmonization

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