International Journal of Parma Research and Technology

Developing and Validating the Quantification of Zanamivir in Bulk Formulations using UV-Visible Spectrophotometry

Patil M.S.[1*], Desai A.A.[2]
MET Institute of pharmacy Adagaon, Nashik 422003
Ashokrao Mane College of Pharmacy, Peth Vadgaon 416112
manasvipatil2016@gmail.com

Abstract

Zanamivir has been identified in drug compounds using a straightforward, precise, accurate, affordable, quick, and sensitive UV/visible spectrophotometric approach. According to ICH criteria, the devised approach was validated. UV/Vis spectrophotometry was used to analyse drugs, and the results were confirmed for linearity, precision, precision and specificity, limits of detection, and limits of quantitation. The solvent employed was 0.1N NaOH, and the wavelength that the drug's maximum absorbance fell within was 360 nm. With y=0.099x-0.0021 and a regression coefficient of R^2=0.998, the linear response over the Zanamivir concentration range of 10 to 80 g/ml was displayed. 94.88 to 106.24% of measurements were accurate. It was discovered that intraday and intraday accuracy were within limits. The limit of detection (LOD) and limit of quantification (LOQ) of the method were established, and they were 6.65 g/ml and 26.70 g/ml, respectively. Assay results showed that the drug content was 104.6 percent. The interpretation of the UV spectrum proved the medication. The suggested method can now be utilised for regular analysis of Zanamivir in pharmaceutical dosage forms because it has been validated.

Keywords: Zanamivir, Validation, Limits

Introduction

According to reports, zanamivir exhibits antiviral activity against influenza viruses. Influenza viruses continue to have a significant negative impact on health and the global economy every year. Particularly, the A (H1N1) and H5N1 influenza viruses cause an excessive number of fatalities and raise severe concerns about pandemics over the world. Hemagglutinin and neuraminidase, two surface glycoproteins carried by influenza viruses, are necessary for the creation of new virions. By identifying the cellular sialic acid receptor, hemagglutinin attaches to the cell surface. Neuraminidase then cleaves the terminal silica acid residues and releases progeny virus from the infected cell's surface.[2]

Oseltamivir (Tamiflu®) and zanamivir are two neuraminidase inhibitors that the World Health Organization (WHO) suggests using to treat A (H1N1) and H5N1 flu. According to reports, Zanamivir is active against H1N1 and H5N1 influenza viruses that are oseltamivir-resistant. Due to its low oral bioavailability (2%), Zanamivir cannot be taken orally like oseltamivir can. According to a study, just 10 to 20 percent of Zanamivir administered by inhalation was absorbed by the body, resulting in a low serum concentration. As a result, Zanamivir has

 https://doi.org/10.5281/zenodo.7476063

only been made available on the market as a dry powder for inhalation.[3]

There haven't been any studies done or publications published regarding the mechanism of Zanamivir absorption through the human gastrointestinal tract or a convincing justification of the causes of low bioavailability. Understanding the process of absorption would be a significant step in creating a formulation that could increase Zanamivir oral bioavailability.[1]

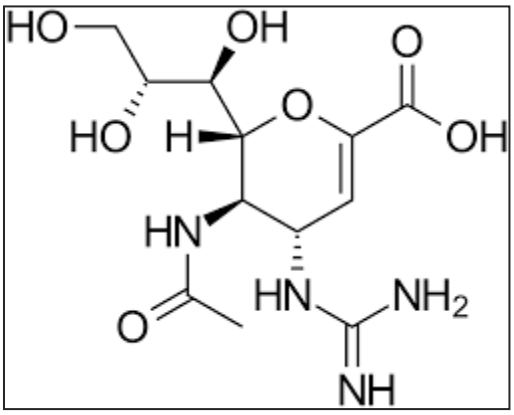

Figure No.1 Structure of Zanamivir

Material and Method

Chemicals and Reagents

All the chemicals used were of analytical grade. All the solutions were freshly prepared in 0.1N NaOH and Phosphate Buffer pH 6.8. Authentic of Zanamivir were obtained as gift samples from Emcure Laboratories Limited, Pune

Instruments: Sonicator, Weighing balance, UV-visible spectrophotometer.

Marketed Formulation: Relenza is an oral inhalation powder manufactured by GlaxoSmithKline.

Methods

Construction of Standard Curve of Zanamivir in 0.1N NaOH

Preparation of Stock Solution

Stock solution 100µg/ml of Zanamivir was prepared in 0.1N NaOH solution. This solution was approximately diluted with 0.1N NaOH to obtain a concentration of 10µg/ml. The resultant solution was scanned in the range of 200- 400 nm using UV double beam spectrophotometer (Lab India UV-2000)

Selection of Detection Wavelength

Drug solution was scanned over the range of 200- 400 nm. The wavelength of Zanamivir was determined to be 360 nm.

Standard Calibration of Zanamivir in 0.1N NaOH

100 mg of Zanamivir was accurately weighed and dissolved in100 ml of 0.1N NaOH to obtain a concentration of 1000 µg/ml. From the above 10 ml was withdrawn and diluted to 100 ml to obtain a concentration of 100µg/ml. From this stock solution aliquots were diluted in 10 ml volumetric flask with phosphate buffer to give concentrations in range of 10µg/ml to 80µg/ml respectively, absorbance was measured at 360 nm.

Construction of Calibration Curve

Pipette out 1,2,3,4,5,6,7, and 8 ml of working solution and transfer into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with water to get solution of concentrations to 2, 4, 6,8,10,12,14,16 µg/ml respectively.

UV Method Validation[7-9]

The ultraviolet spectrophotometric method was verified for robustness, accuracy, linearity, and precision.

Linearity

Appropriate aliquots of Tamiflu working standard solutions were taken in different 10 ml volumetric asks and diluted up to the mark with distilled water to obtain final concentrations of 10-80µg/ml. Calibration

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curves were constructed by plotting absorbance versus concentrations and regression equations were calculated for both the drugs.

Range

The Range of the analytical method was decided from the interval between the upper and lower level of the calibration curve by plotting curve.

Precision

Intraday precision was determined by analyzing the drugs at concentrations (6µg/ml) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis is carried out daily, for two consecutive days. Repeatability (intraday) of the method was determined by analyzing six samples of the same concentrations of the drug (6g/ml). The absorbance of each was measured and reported in terms of relative standard deviation to obtain the variation.

Accuracy

The accuracy of the method was determined by calculating recoveries of Zanamivir by method of standard additions at three different levels 50, 100 and 150 %. Mean percentage recovery was determined. Recovery values were calculated and shown in table 1.

Table No 1: Calibration Curve Data for Zanamivir in 0.1 N NaOH

Sr. No.	Drug Conc. (µg/ml)	Stock solution of macitentan (ml)	0.1 N NaOH (ml)	Mean absorbance ±SD (n=3)	% RSD
1	2	2	18	0.0974±0.0003	0.68
2	2	2	16	0.267±0.001	0.61
3	3	3	14	0.462±0.001	0.34
4	4	4	12	0.594±0.001	0.47
5	5	5	10	0.596±0.002	0.49
6	6	6	8	0.678±0.001	0.32
7	7	7	6	0.856±0.003	0.36
8	8	8	4	0.889±0.001	0.24

Detection limit

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 detection limit (LOD) may be expressed as. LOD= 3.3σ/S Where σ = Relative standard deviation of the response. S = the slope of the calibration curve (of the analyte).

Quantitation Limit

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation Limit (LOQ) may be expressed as: LOQ = 10σ/S Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Assay Marketed Formulation[4-6]

Weighed and transferred 10 Zanamivir tablets powder after crushing (equivalent to 10mg of Zanamivir) into 100ml volumetric flask, added about 40 ml of 0.1N NaOH and sonicated for 10 minutes with intermittent shaking, further added 60 ml 0.1N NaOH . Pipette out 1 ml of filtered solution into 10 ml volumetric flask, and made the volume up to the mark with water and mixed well. 1 ml of the above was again diluted with distilled water to obtain 10 µg/ml of Zanamivir.

Results & Discussion[11,12]

Analytical Method for Drug Concentration Measurements (UV/VIS Method)

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Selection of detection wavelength: The wavelength of Zanamivir was determined to be 360 nm as shown in figure 2.

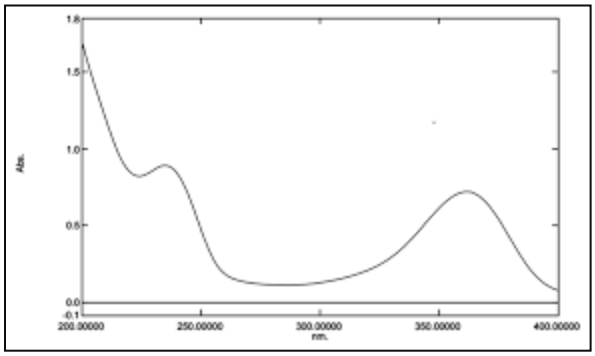

Figure No 2: λ_{max} of Zanamivir in 0.1N NaOH

Preparation of standard plot for Zanamivir: Absorbance of the resultant solution was measured at 360 nm using blank. A graph was plotted between the concentrations and their respective absorbance. The response of the drug was found linear in the entire investigational range of 10 to 80µg/ml as shown in table 2. The calibration curve showed the linear equation as, y = 0.099x - 0.0021, with a correlation coefficient, R^2 = 0.998, where y represents absorbance (optical density) and x represents the concentration (µg /ml) as shown in figure 3.

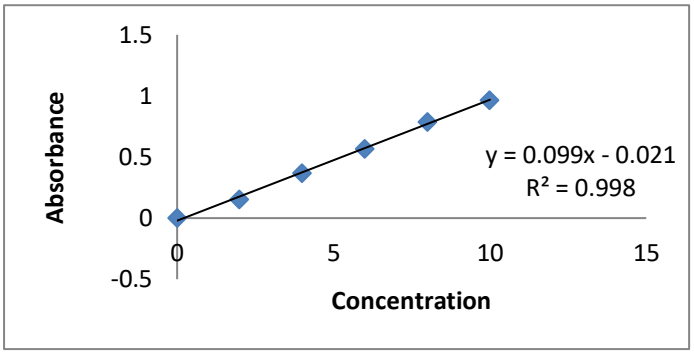

Figure No 3: Calibration Curve of Zanamivir in 0.1N NaOH

Method Validation: The developed method was validated as per ICH guidelines for the following parameters:

Linearity: The linearity for Zanamivir was found to be linear in the range of 10-70 µg/ml. The regression equation was found to be y = 0.099x - 0.0021, R^2 = 0.998.

Range: The observed range of Zanamivir in test solution was observed from 0.0981±0.0004 to 0.881± 0.001.

Accuracy: The accuracy of the analytical method for Zanamivir was determined at 50%, 100% and 150% levels of standard solution. Absorbance was measured at 360 nm and results were expressed in terms of % recoveries in table 2.

Table No 2: Results of Accuracy of Zanamivir

Sr. No.	Level of % Recovery	Amount of tablet sample (ml)	Amount of standard drug added (µg/ml)	Amount added (µg)	Amount found (µg/ml)	% Recovery

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1	0	1	0	0	0	0
2	50	1	0.1	15	16	94.88%
3	100	1	5	20	22	100%
4	150	1	10	25	28	106.24%

Precision: The same optimal circumstances were used for the intra-day and inter-day precision measurements. As shown in tables 3 and 4, respectively, the precision results (measurement of inter-day, intra-day repeatability) demonstrated good reproducibility with the relative standard deviation (% RSD) < 2.0%. This demonstrated how exact the procedure was.

Table No 3: Results of Intra-Day Precision of Zanamivir in Solvent

Sr. No.	Day Time	Concentration (µg/ml)	Absorbance	Mean Absorbance 360nm±SD (n=6)	%RSD
1	**Intraday morning precision**	6	0.821	0.824±0.001	0.16%
2			0.820		
3			0.824		
4			0.826		
5			0.827		
6			0.827		
1	**Intraday morning precision**	6	0.823	0.825±0.003	0.48%
2			0.825		
3			0.825		
4			0.829		
5			0.824		
6			0.821		
1	**Intraday morning precision**	6	0.812	0.819±0.005	0.62%
2			0.817		
3			0.819		
4			0.812		
5			0.823		
6			0.819		

Table No 4: Results of inter-day Precision of Zanamivir in Solvent

Sr. No.	Day Time	Concentration (µg/ml)	Absorbance	Mean Absorbance 360nm±SD (n=6)	%RSD
1	**Interday morning precision**	6	0.819	0.823±0.004	0.52%
2			0.821		
3			0.826		
4			0.827		
5			0.821		
6			0.822		

 https://doi.org/10.5281/zenodo.7476063

1	**Interday morning precision**	6	0.831	0.831±0.002	0.20%
2			0.832		
3			0.831		
4			0.830		
5			0.829		
6			0.832		
1	**Interday morning precision**	6	0.844	0.842±0.001	28%
2			0.845		
3			0.841		
4			0.843		
5			0.841		
6			0.841		

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ of method were determined to be 6.65 µg /ml and 26.70 µg /ml respectively. LOD and LOQ indicate that method was highly sensitive and fast.

Assay Marketed Formulation

The solution was then analyzed by developed UV-Visible spectroscopy method, and the results were indicated by % drug content. The % drug contents were found to be 104.6. As shown in table 5.[13,14]

Table No 5: Assay Results of Formulation

Drug	Brand Name	Label Claim	Conc. Prepared	Amount Found (µg /ml)	%Assay	±SD	%RSD
Zanamivir	Tamiflu	70 mg	10 µg/ml	10.4	104.6	0.598	0.587

Conclusion

According to ICH criteria, the approach was validated and determined to be straightforward, sensitive, accurate, and exact. Less than 2% RSD was found for the validation parameters. For routine examination of these medicines in pharmaceutical dose forms, the proposed approach may therefore be applied. The proposed method's accuracy was verified using accuracy studies that produced results that fell within the expected range. The proposed UV method's accuracy was verified by running intra-day and inter-day precision tests. Results exceeded acceptance criteria, demonstrating the method's high scope for detecting Zanamivir in pharmaceutical dosage forms and bulk.

Acknowledgement

The authors are thankful to the MET Institute of pharmacy Adagaon, Nashik (India) for unconditional support for the work.

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