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Research Article

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A validated UV spectrophotometric determination of an antiviral drug zanamvir from tablet formulations

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ABSTRACT

Simple, sensitive and specific spectrophotometric method was developed and validated for quantification of zanamvir in tablet dosage form. Drug showed the absorption maxima in water at 260nm and was linear for a range of 2-10,g/ml with a correlation coefficient of 0.9999. The validation of the above method was done by carrying out precision and accuracy studies. The Limit of detection and Limit of Quantification for zanamvir was found to be 0.82mcg/ml and 2.76mcg/ml. The percentage recovery was found to be 99.3% and showed good repeatability with relative standard deviation less than 2. So, the proposed method can be applied for the routine analysis of zanamvir from formulations

Key Words: Spectrophotometry, zanamvir, water, Beer's law. validation

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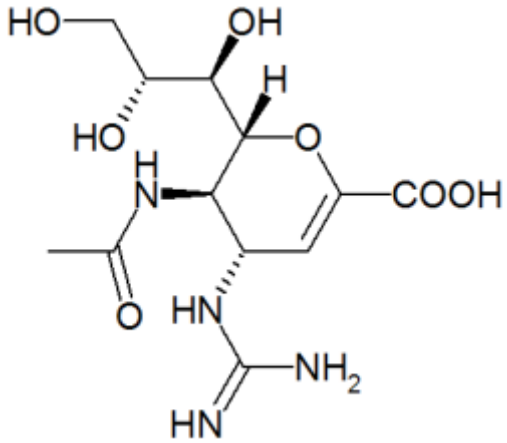
INTRODUCTION

Zanamivir is a neuraminidase inhibitor used in the treatment and prophylaxis of influenza caused by influenza A virus and influenza B virus.The chemical name of zanamvir is (2R,3R,4S)-4-guanidino-3-(prop-1-en-2-ylamino)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxylic acid [1]. Zanamivir was the first neuraminidase inhibitor commercially developed.

According to the Centers for Disease Control and Prevention (CDC), no flu, seasonal or pandemic, has shown any signs of resistance to zanamivir.[2] Zanamivir was the first of the neuraminidase inhibitors. The discovery was initially funded by the Australian biotechnology company Biota and was part of Biota's ongoing program to develop antiviral agents through rational drug design. Its strategy relied on the availability of the structure of influenza neuraminidase, by X-ray crystallography. It was also known, as far back as 1974, that 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (DANA), a sialic acid analogue, was an inhibitor of neuraminidase.[3] Computational chemistry techniques were used to probe the active site of the enzyme, in an attempt to design derivatives of DANA that would bind tightly to the amino acid residues of the catalytic site, and so would be potent and specific inhibitors of the enzyme. The GRID software by Molecular Discovery was used to determine energetically favourable interactions between various functional groups and residues in the catalytic site canyon. This showed there was a negatively charged zone in the neuraminidase active site that aligned with the C_4 hydroxyl group of DANA. This hydroxyl was therefore replaced with a positively charged amino group; the 4-amino DANA was 100 times better as an inhibitor than DANA, owing to the formation of a salt bridge with a conserved glutamic acid (119) in the active site. It was also noticed that Glu 119 was at the bottom of a conserved pocket in the active site just big enough to accommodate a more basic functional positively charged group, such as a guanidino group, which was also larger than the amino group.[4] Zanamivir, a transition-state analogue inhibitor of neuraminidase, was the result.[5] According to the CDC, Tamiflu, zanamivir's main competitor, is not as effective at treating the Influenza viruses as zanamivir, especially in H1N1 Seasonal Flu. In fact, tests showed that 99.6% of the tested strains of seasonal H1N1 flu and 0.5% of 2009 pandemic flu were resistant to Tamiflu while there have been absolutely zero flu samples

seasonal or pandemic that show any resistance to zanamivir.[1] [6] [7] Recently, the reported oseltamivir-resistance H5N1 virus neuraminidase still retaining susceptibility to zanamivir indicates that the structure of zanamivir has some advantages over oseltamivir in binding to the active pocket of H5N1 neuraminidase.[6] [7] [8] A case study from 2009 demonstrated the effectiveness of an intravenous preparation of zanamivir in the treatment of severe H1N1 pneumonitis. In this case, previous treatment with oseltamivir had failed, however there was a marked response to treatment with an extemporaneously prepared intravenous formulation of zanamivir and the patient subsequently recovered completely and was discharged from hospital soon afterwards[9] In August 2006, Germany announced that it would buy 1.7 million doses of zanamivir, as part of its preparation strategy against bird flu. "Germany's purchase shows that countries are starting to take a balanced view of influenza preparedness," says Simon Tucker, head of research at Melbourne-based Biota, where zanamivir was originally developed. [10] Zanamivir works by binding to the active site of the neuraminidase protein, rendering the influenza virus unable to escape its host cell and infect others[10] The bioavailability of zanamivir is 2%. After inhalation, zanamivir is concentrated in the lungs and oropharynx, where up to 15% of the dose is absorbed and excreted in urine.[11] Dosing is limited to the inhaled route. This restricts its usage, as treating asthmatics could induce bronchospasms.[12] The U.S. Food and Drug Administration (FDA) has issued a Public Health Advisory warning that it has received some reports of respiratory problems following inhalation of zanamivir by patients with underlying asthma or chronic obstructive pulmonary disease. The zanamivir package insert contains precautionary information regarding risk of bronchospasm in patients with respiratory disease.[13] GlaxoSmithKline (GSK) and FDA notified healthcare professionals of a report of the death of a patient with influenza who received Relenza (zanamivir) Inhalation Powder which was solubilized and administered by mechanical ventilation. [14] In April 2009 many cases of swine flu (H1N1 type virus) were reported in US and Mexico. Zanamivir is one of only two drugs that are prescribed to treat it. A study published in June 2009 emphasized the urgent need for augmentation of oseltamivir (Tamiflu) stockpiles, with additional antiviral drugs including zanamivir, based on an evaluation of the performance of these drugs in the scenario that the 2009 H1N1 swine flu neuraminidase (NA) were to acquire the Tamiflu-resistance (His274Tyr) mutation which is currently widespread in 99.6% of all tested seasonal H1N1 strains.[14] In January 2011, GSK announced it was commencing Phase III trials for Intravenous zanamivir in a study which will span 20 countries in the Northern and Southern hemispheres. [15] The present work reports the development and validation of a UV spectrophotometric method for the estimation of zanamvir in bulk and in pharmaceutical formulations. The method was validated by parameters such as correlation coefficient, intercept and slope.

FIG : 1 THE CHEMICAL STRUCTURE OF ZANAMVIR



(2R,3R,4S)-4-guanidino-3-(prop-1-en-2-ylamino)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxylic acid.

EXPERIMENTAL SECTION

CHEMICALS

zanamvir was obtained from LUPIN INDIA Ltd., Hyd, was used as such without further purification. Different brands of tablets of given drug were supplied from local pharmacy.

EXPERIMENTAL

Shimadzu UV-VIS (1700 series) double beam spectrophotometer equipped with 10mm matched quartz cells.

RECOMMENDED PROCEDURE AND CALIBRATION CURVE

zanamvir (10 mg) was accurately weighed and dissolved in 100 mL of water to form a stock solution (100 µg/mL). 10 mL of the above solution was diluted to 100 mL with water in a 100 mL volumetric flask to give a concentration of 10 µg/mL and this was then scanned in UV range. This showed an absorption maximum at 260 nm . Aliquots (2,4,6,8 and 10) mL of working standard solution (10 µg/mL) corresponding to2-10 µg were taken in a series of 10 mLvolumetric flask and volume made up with water. The absorbance measurements of these solutions were carried out against water as blank at 260 nm. A calibration curve of zanamvir was plotted. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

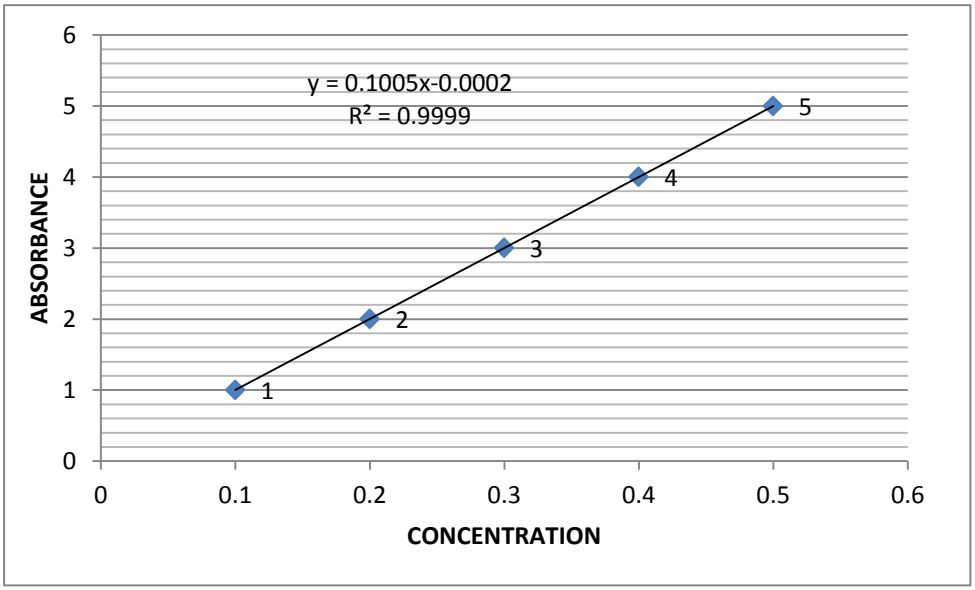


FIG : 2 Standard plot of zanamvir

Zanamvir tablets namely REBETOL (LUPIN) and VIRAZOL (LUPIN) were purchased from local pharmacy. The contents of 20 tablets were mixed and accurately weighed amount of the contents equivalent to10 mg of zanamvir was transferred into a 100 mLvolumetric flask. 70 mL of water was added and the contents of the flask were shaken for 5 min.The solution was then diluted to the mark with the water to get a stock solution of 100 µg/mLThe content of the flask was filtered through Whatman filter paper No.1 and 10 mL of the filtrate was diluted to 100 mL with water in a100 mL volumetric flask to give a concentration of 10 µg/mL Suitable volume of this solution was taken in 10 mL volumetric flask and volume was made up with water. Absorbances were read and concentrations of zanamvir determined using the calibration curve. Calculations were then made with the dilution factor to find out the concentration of the drug in tablets. The experiments were repeated five times to check its reproducibility.

RESULTS AND DISCUSSION

The proposed method for determination of zanamvir showed molar absorptivity of 0.97 x 104 L/mol.cm. Linear regression of absorbance on concentration gave the equation y = 0.1005 x- 0.0002 with a correlation coefficient (r) of 0.9998. The optical characteristics such as Beer's law limit, Sandell's sensitivity, % Range of error (0.05 and 0.01 confidence limits) were calculated and are summarized in Table 1. Statistical analysis and recovery studies of commercial formulations has been shown in Table 2 and Table 3.

Table 1 Optical characteristics of zanamvir

Parameters	**values**
λmax, nm	260
Beer's law limit, ,g/mL	2-10
Molar absorptivity, (L mole-1 cm-1)	0.97x104
Sandell's sensitivity (µg cm-2 / 0.001 absorbance unit)	0.0319
Regression equation	(Y = a+bC)
Slope (b)	0.0294
Intercept (a)	0.0014
Correlation coefficient (r)	0.9999
% Range of error (Confidence limits)	
0.05 level	0.1923
0.01 level	0.2845

Table 2 Statistical analysis of zanamvir tablets.

Brand	Label mount mg/capsule	AmountFound mg/capsule	% label claim ± FD*
REBETOL	10	9.9965	99.98+ 0.14
VIRAZOL	10	9.9997	99.99+ 0.23

**average of five determinations*

Table 3 Recovery studies of zanamvir tablets

Brand	**Amount added** (Mg)	**Amount Found** (Mg)	**% recovery ± FD***
REBETOL	5	14.98	99.73±0.24
VIRAZOL	5	14.96	99.66±0.21

CONCLUSION

In this study a simple, rapid, sensitive, accurate and precise UV spectrophotometric method for the determination of zanamvir in bulk and pharmaceutical formulation has been developed and validated. It was found that the common excipients present in the formulation did not interfere with the proposed method and can be used for the routine quality control analysis of zanamvir in bulk as well as in marketed tablets.

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REFERENCES

[1] http://en.wikipedia.org/wiki/Zanamivir
[2] "2008-2009 Influenza Season Week 32 ending August 15, **2009**". *Flu Activity & Surveillance*. Centers for Disease Control and Prevention (CDC). August 21, **2009**.
[3] Meindl P, Bodo G, Palese P, Schulman J, Tuppy H (April **1974**). *Virology* 58 (2): 457–63.
[4] Laver, Graeme (March **2007**). "Flu drugs - pathway to discovery". *Education in Chemistry*. Retrieved 2009-11-11. ISSN 0013-1350
[5] von Itzstein M, Wu WY, Kok GB, *et al.* (June **1993**). *Nature* 363 (6428): 418–23.
[6] Collins PJ, Haire LF, Lin YP, Liu J, Russell RJ, Walker PA, Skehel JJ, Martin SR, Hay AJ, Gamblin SJ. (**2008**). *Nature* 453 (7199): 1258–61.
[7] Garcia-Sosa AT, Sild S, Maran U. (**2008**). *J. Chem. Inf. Model.* 48 (10): 2074–2080.
[8] Du QS, Wang SQ, Chou KC (October **2007**). *Biochemical and Biophysical Research Communications* 362 (2): 525–31.
[9] Kidd IM et al. **2009**. *The Lancet* 374: 1036.
[10] Cyranoski D (September 2005). *Nature Medicine* 11 (9): 909.
[11] Moscona A (September **2005**). *The New England Journal of Medicine* 353 (13): 1363–73.
[12] Hayden FG (December **2001**). *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 356 (1416): 1877–84. doi:10.1098/rstb**.2001**.1007.
[13] "Safe and Appropriate Use of Influenza Drugs". *Public Health Advisories (Drugs)*. U.S. Food and Drug Administration (FDA). April 30, 2009. Retrieved **2009**-11-11.
[14] www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm186081.html
[15] Soundararajan V, Tharakaraman K, Raman R, Raguram S, Sasisekharan V, Sasisekharan R (June **2009**). *Nature Biotechnology* 27 (6): 510–3.