

Development, Validation and Application of UV-Spectrophotometric Method for the determination of Oseltamivir phosphate in Bulk and Pharmaceutical Dosage Form

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Abstract: Oseltamivir Phosphate is used in the treatment and prophylaxis of both influenza A and influenza B. The present research work discussed the development of a simple, sensitive, rapid, accurate, precise and economical UV-Spectrophotometric method for the evaluation Oseltamivir Phosphate in bulk and pharmaceutical dosage form which is based on the measurement of absorption maxima at 221.4 nm. A Shimadzu 1800 U.V visible spectrophotometer with 1cm matched quartz cells, and de-ionized water as solvent were used. Developed methods obeyed the Beer's law in the concentration range of 20-70 μ g/ml having line equation $y = 0.020x + 0.168$ with correlation coefficient of 0.999. Method was validated statistically. Percentage recovery of the drug for the proposed method ranged from (99.2280-99.5320 \pm 0.1670) indicating no interference of the capsule excipients. The developed method was validated with respect to precision, accuracy (recovery), linearity, limit of detection and limit of quantitation.

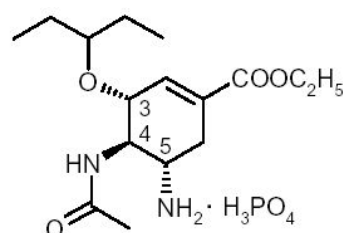
Key words: Oseltamivir Phosphate(OP), deionised water, Absorbance maxima.

Introduction

Oseltamivir Phosphate (OP) is an ester prodrug which is the first orally available inhibitor of influenza virus neuraminidase, an enzyme involved in the release of new virus particles from infected cells. It is used in the treatment and prophylaxis of both influenza A and influenza B. The structure of Oseltamivir shows that it possesses a hydrophobic moiety (Fig. 1). Oseltamivir's hydrophobic group is responsible for its poor oral absorption; thus, the phosphate salt has been developed that allows oral administration of this drug. OP is rapidly and extensively metabolized via hepatic esterases to Oseltamivir Carboxylate (OC), the active form, a potent and selective inhibitor of influenza virus neuraminidase^{[1][2][3]}. Oseltamivir phosphate is

(3R,4R,5S)-4-Acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate (1:1)

Figure 1: chemical structure of Oseltamivir Phosphate



To date there are no published methods for determination of Oseltamivir Phosphate in both bulk and formulation (Fluivir capsules). For the determination of Oseltamivir there are several methods based on different techniques such as colorimetric^[4], spectrofluorimetric^[5], liquid chromatography with UV detection^{[6][7][8][9][10][11]} and mass spectrophotometric detection^{[12][13][14][15]}, capillary electrophoresis^[16] and micellar electrokinetic chromatography^[17].

Materials and Methods:

Instruments and materials:

A Shimadzu UV-1800 UV/VIS Spectrophotometer was used with 1 cm matched quartz cell. All the chemicals used were of analytical grade. An analytically pure sample of Oseltamivir Phosphate was procured as gift sample from Cipla Pharmaceuticals Ltd. (pune, India)

Preparation of standard stock solution and calibration curve:

Standard stock solution of Oseltamivir Phosphate was prepared by dissolving 10 mg in 100 ml of deionised water to get concentration of 100 μ g/ml. The aliquots of 2 to 7 ml of standard stock solution were transferred into series of 10ml volumetric flask and made up to mark with deionised water to reach the concentration range of 20 μ g/ml to 70 μ g/ml respectively. And calibration curve was taken at 221.40 nm.

Preparation of sample solution

Twenty capsules were finely powdered and weighed. A portion of the powder equivalent to about 10 mg of Oseltamivir Phosphate was weighed accurately, dissolved and diluted to 100 ml with deionised water. The sample solution was filtered. Further dilution was carried out with deionised water. The general procedures described under standard stock solution and calibrations were followed and the concentrations of Oseltamivir Phosphate were calculated at 221.4nm.

Figure 2: Zero order spectra of Oseltamivir Phosphate

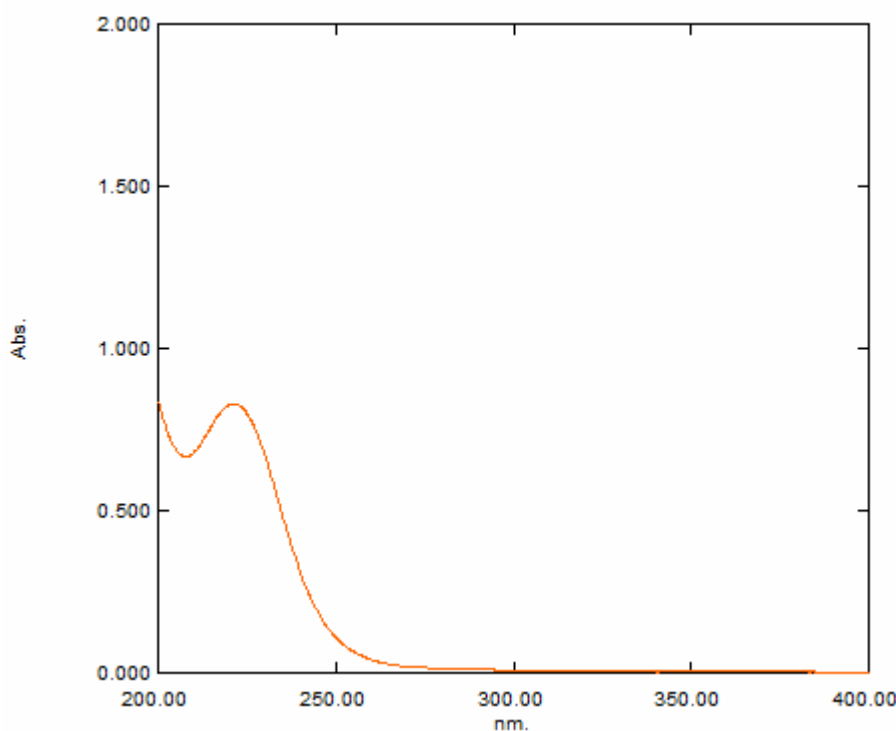


Table no. 1 Optical characteristics and Other Parameters

Sr. No.	Parameters	Observations
1	Absorption Maxima (nm)	221.4
2	Beer's range ($\mu\text{g/ml}$)	20-70
3	Molar absorptivity (L/mol.cm)	6.81445×10^3
4	Sandell's sensitivity ($\mu\text{g/cm}^2$ x 0.001 absorbance unit)	0.060241
5	Regression equation (y)* Slope (m) Intercept (c)	Y = mx+c 0.020 0.168
6	Correlation coefficient(R^2)	0.999
7	LOD ($\mu\text{g/ml}$)	0.165
8	LOQ ($\mu\text{g/ml}$)	0.500

*y= mx × c; where x is the concentration in $\mu\text{g/ml}$ and y is absorbance.

Table no.2 Result of Analysis of Oseltamivir Phosphate in marketed tablet formulation

Sr. No.	Lable claim (mg)	Amount found*(mg)	%estimated	S.d.*(±)	%R.S.D
1	75	74.68	99.5733	0.0023	0.2705

*indicates average of 6 readings.

Table no. 3 Recovery study data.

Sr. No.	Amount of drug sample (μg)	Level of recovery (%)	Amount added* (μg)	Amount found* (μg)	Recovery* (%)	S.D(±)	%R.S.D
1	50	80	40	39.6912	99.2280	0.3249	0.3274
2	50	100	50	49.7660	99.5320	0.2930	0.2943
3	50	120	60	60.7000	99.5000	0.3832	0.3851

*indicates average of 6 readings

Results and Discussion:

Validation parameters:

The method was validated with respect to precision, accuracy, linearity, limit of detection (LOD) and limit of quantification (LOQ).

Precision:

To determine the precision of the method, Oseltamivir Phosphate concentrations were analysed six times in a day (intra-day precision) and for six continuous days (inter-day precision). SD and %RSD were 0.0026, 0.4050 and 0.0030, 0.4630 respectively.

Accuracy (recovery study):

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for Oseltamivir Phosphate, was found to be as in table no. 2.

Linearity:

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Oseltamivir Phosphate. Beer-Lambert's concentration range was found to be 20-70 $\mu\text{g/ml}$.

Limit of detection (LOD) and limit of quantitation (LOQ):

The LOD and LOQ of Oseltamivir Phosphate were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ was found to be as in table no.1

Determination of active ingredients in capsule formulation:

The validated method was applied to the determination of Oseltamivir Phosphate in capsules. Twenty capsules were assayed and results are shown in table no. 3 indicating that the amount of drug in capsule sample met with requirements.

Conclusion:

The developed method was found to be simple, sensitive, accurate, precise, economic and can be used for routine quality control analysis of Oseltamivir Phosphate in bulk as well as in pharmaceutical dosage form.

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