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Development and Validation of Oseltamivir Phosphate API by UV-Spectrophotometer

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Abstract: A simple and rapid UV-spectrophotometer estimation method for the evaluation of Oseltamivir Phosphate is used in the treatment and prophylaxis of both influenza A and influenza B viruses has been developed and assessed. The proposed methods were successfully applied for the estimation of Oseltamivir Phosphate in commercial pharmaceutical preparation with UV detection at 217 nm. A Shimadzu 1700 UV-visible spectrophotometer with 1cm matched quartz cells and distilled water solvent were employed in this method. Developed methods obeyed Beer's law in the concentration range of 10 to 70ìg/ml having correlation coefficient of 0.999. Method was validated statistically. Percentage recovery of the drug for the proposed method ranged from (99.85%) indicating no interference of the excipients. The developed method was validated with respect to linearity, precision, accuracy (recovery), limit of detection (LOD) and limit of quantitation (LOQ).

Key words: Oseltamivir Phosphate (OP) · UV Spectrophotometry · Absorbance Maxima

INTRODUCTION

Oseltamivir phosphate (OP) is an ester prodrug which belongs to a new class of drugs termed neuraminidase inhibitors, which are active against both influenza viruses type A and B. [1, 2]. 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. [3, 4] 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 [5, 6].

In-vitro resistance to oseltamivir occurs from mutations in either the hemagglutinin or neuraminidase genes, or both [7, 8]. However, resistance emergence due to loss of neuraminidase susceptibility has been uncommonly recognized during therapeutic use of oseltamivir and has not been documented during prophylactic use [9, 10].

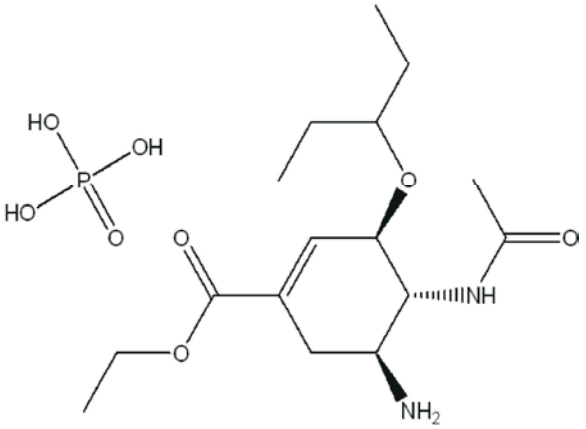

Fig. 1: Chemical structure of Oseltamivir phosphate

Oseltamivir-phosphate (O-phosphate) is a prodrug that was developed to enhance the oral bioavailability of the parent compound and antiviral molecule, oseltamivir-carboxylate (O-carboxylate) [11, 12]. Oral bioavailability of O-carboxylate is low (5%). The absolute oral bioavailability of O-carboxylate from the prodrug averages is 79% and plasma protein binding of the prodrug and

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its active metabolite is low, 42% and 3%, respectively. O-phosphate undergoes rapid, extensive de-esterification conversion by hepatic esterases to O-carboxylate. O-carboxylate is eliminated primarily by glomerular filtration and renal tubular secretion. Oseltamivir phosphate is an ethyl ester pro-drug that is rapidly and extensively metabolized by esterases in the gastrointestinal tract and liver to its active form, oseltamivir carboxylate [13, 14]. There are some analytical methods reported in the literature for the analysis of both compounds in the biological fluids and pharmaceutical preparations [15, 16].

MATERIALS AND METHODS

Instrumentation: A double-beam Shimadzu UV–Visible spectrophotometer, model UV-1700 with 1cm quartz

Cells attached with printer of ESPON LQ 1150 II.

Materials and Reagents: A Shimadzu UV-1700 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 Matrix Pharmaceuticals Ltd. (Hyderabad, India)

Preparation of Standard Stock Solution and Calibration Curve: Accurately weighed 50.0 mg of pure drug Oseltamivir phosphate was taken in clean, dry 50 ml volumetric flask and dissolved in 50 ml volume of methanol, resulting in 1000.0 µg/ml of drug concentration (Stock solution). Now pipette out 10 ml of this solution and diluted to 100 ml with distilled water, resulting in 100 µg/ml of the drug concentration. Now made different working solutions of the drug (having different concentrations) by pipette out different volume from the stock solution and make the volume of all the working solutions upto 10 ml with distilled water.

RESULTS

Linearity Plot: Linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. Linearity and range data for calibration curves prepared in distilled water.

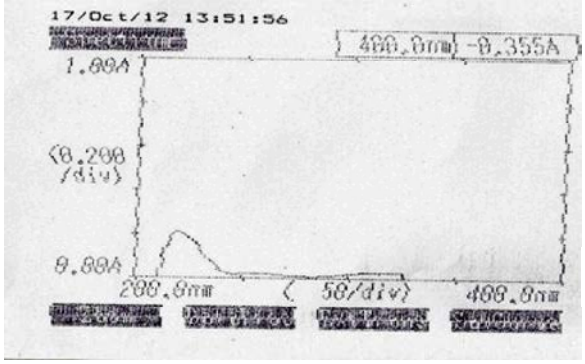

Fig. 2: Zero order spectra of oseltamivir phosphate

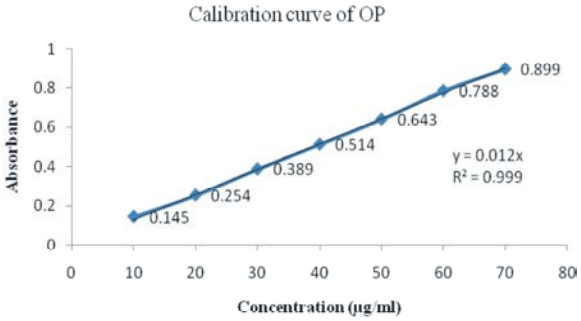

Fig. 3: Calibration curve of oseltamivir phosphate

Table 1: Optical characteristics and Other Parameters

Parameters	Results
Absorption maxima	217 nm
Beer's – Lambert' range (µg/ml)	10-70
Regression equation (y)	Y= 0.012x
Correlation coefficient	0.999
System precision (% RSD)	0.643
Method precision (%RSD)	1.0368
Limit of detection (µg/ml)	76.07 µg
Limit of quantification (µg/ml)	230.5µg

Accuracy (Recovery Test): The accuracy of an analytical procedure expresses the true value or reference value recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for OP by all the methods was found to be 99.85.

The proposed UV method for estimation of related substances for Oseltamivir Phosphate was carried out as per USP/ICH guidelines. The method was found to be specific for the sample and standard preparation. The method was also stability indicating as evident by degradation studies. The method was found to be linear in the specified range. Accuracy of the method was also established for the drug product. The method was found to be precise and robust. LOD and LOQ established by the method are sufficient enough to quantitate the trace

Table 2: Recovery study data

Level (Approx)	Std. Added (mg)	ABS	Std. Recovered (mg)	% Recovered	Mean Recovery	RSD
80%	40	0.519	40.232	100.58	099.85	0.6378
100%	50	0.641	49.69	99.38		
120%	60	0.771	59.77	99.61		

of impurities in the sample. The analytical method for the estimation of OP was developed and validated using UV-Spectroscopy technique. The proposed stability indicating assay methods have proved to be simple, specific, accurate and sensitive. The drug was found to be stable in extreme condition of the stress. Therefore the method has been successfully applied for the estimation of OP in bulk drug. From the above observation, the method showed all the simplicity, rapidity, reproducibility and economy of the proposed methods completely fulfill the objective of this research work.

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