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Research Article

Development and Validation Of Spectrophotometric Methods For The Estimation of Oseltamivir Phosphate

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ABSTRACT
Oseltamivir phosphate (OP) is chemically ethyl (3R, 4R, 5S)-4-acetamino-5-amino-3-(ethylpropoxy)-1-carboxylate phosphate used to treat flu virus (influenza).The drug is commercially available as tablets for oral administration. In the present work two simple, economical, precise and accurate UV spectrophotometric methods have been developed for the estimation of oseltamivir phosphate in bulk and pharmaceutical formulation .Method A is absorption maxima method in which λmax was found to be 217.06nm. Method B is first order derivative spectroscopy where drug showed λmax at 214.35 nm. Amplitude (dA/dλ) was calculated and was plotted against concentration and regression equation was calculated. Linearity was observed in the concentration range 5-25µg/ml (r =0.999) for the two methods. The % assay for the marketed formulation for absorption maxima, first order derivative method was found to be 97.62%, 98.02% respectively. The methods were validated with respect to linearity, precision and accuracy studies. Recovery studies for absorption maxima, first order derivative was found to be 98.61%, 98.71%respectively. The methods were found to be simple, precise and accurate and can be employed for routine quality control analysis of oseltamivir phosphate in bulk as well as from its dosage form.
Key words: Oeltamivir phosphate, UVspectroscopy, Absorption maxima, First order derivative.

INTRODUCTION

Oseltamivir phosphate (OP) is chemically ethyl (3R, 4R, 5S)-4-acetamino-5-amino-3-(ethylpropoxy)-1-carboxylate phosphate. It is classified as antiviral agent, enzyme inhibitor. Oseltamivir phosphate is an oral prodrug which undergoes hydrolysis by hepatic esterases to form active oseltamivir carboxylate. Literature survey reveals that 4HPLC methods, 3biological methods, 2electrophorotic methods,1mass spectroscopic method has reported for the estimation of oseltamivir phosphate in bulk, biological fluids and pharmaceutical dosage forms. No derivative spectrophotometric method for the estimation of oseltamivir phosphate as a single dosage form. In the present work an attempt has been made to estimate the drug by spectrophotometric methods.

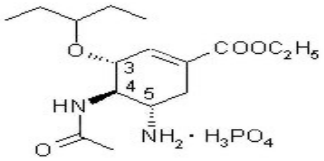

Figure 1 Structure of ethyl (3R, 4R, 5S)-4-acetamino-5-amino-3-(ethylpropoxy)-1-carboxylate phosphate

METHODS AND MATERIALS

For the present study Perkin-Elmer UV-Visible double beam spectrophotometer (lamda-25) was used with slit width fixed at 1.5nm, a pair of 1-cm matched quartz cells were used to measure the absorbance of solution. The samples were weighed on electronic analytical balance (Adventure Essac).

Reagents

Oseltamivir phosphate (OP) was obtained as a gift sample from hetero drugs ltd, hyd. The marketed formulation used for tablet analysis was Oseltamivir by hetero drugs. The label claim states that each uncoated tablet contains 45 mg of Oseltamivir phosphate.

Solvent- Distilled water spectroscopic grade

Preparation of standard stock solution:

Accurately weeighted 100mg of Oseltamivir phosphate was transferred into 100ml volumetric flask. The powder was dissolved completely in few ml of distilled water. Later, the volume of the solution was made up to 100ml with water to obtain the concentration of 1000µg/ml. Aliquots of standard stock solution were suitably diluted with distilled water to get working standard solutions of concentration of 5, 10, 15, 20 and 25µg/ml. These were scanned in the wavelength range 200-800 nm.

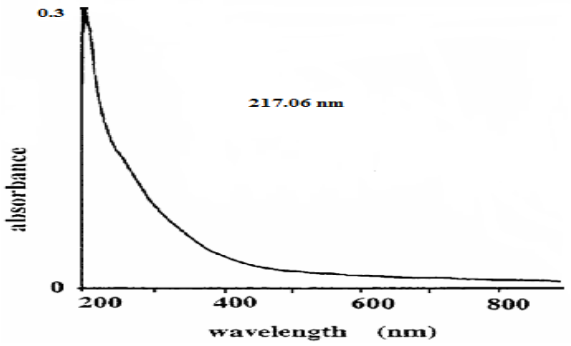

Figure 2 Spectra of Oseltamivir phosphate (concentration 10µg/ml)

Method A: Absorbance Maxima Method

Aliquots of standard stock solution of concentration 1000µg/ml were taken and suitably diluted with distilled water to get working standard solutions in the increasing concentration range. These were scanned in the range of

200-400 nm. The absorbance maximum was found to be at 217.06 nm (Fig.3). The calibration curve was plotted with concentration v/s absorbance and regression equation was calculated.

Method B: First order derivative spectroscopy

The first order derivative spectra showed λmaxima 214.35 (Fig.4).The absorbance difference at n=1 (dA/dλ) was calculated by the inbuilt software of the instrument. The derivative amplitudes were calculated by considering the maxima of the curve. Amplitude was measured for the respective concentration of standard and was plotted against concentration and regression equation was calculated. The concentration range of 5-25 µg/ml for OP was chosen for the derivative analysis.

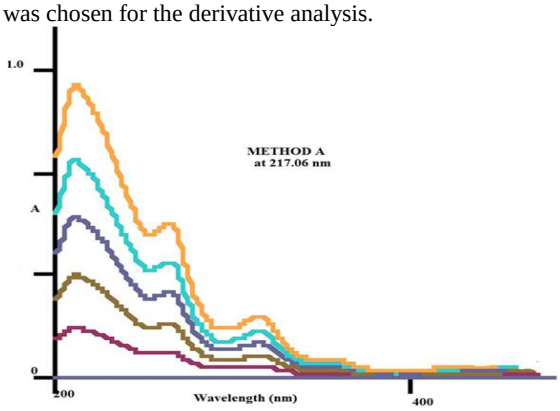

Figure 3 Absorbance maxima method for Oseltamivir phosphate (concentration 5-25µg/ml)

Analysis of Tablet Formulation

For the estimation of oseltamivir phosphate in the commercial formulations, ten tablets each containing 45 mg of OP were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent to 100 mg of OP was transferred to 100 ml volumetric flask and dissolved in sufficient quantity of water. It was sonicated for 15 mins and through Whatmann filter paper # 42. Further dilutions of the stock solution were made in distilled water to get required concentration. In method A, the concentration of OP was determined by measuring absorbances of sample solutions at 217.06 nm (Fig.3). In method B i.e first order derivative spectroscopy the concentration of OP was determined by measuring amplitude at λmaxima 214.35 nm. Results of tablet analysis are shown in Table 1. The assay procedure was repeated three times (n=3).

Table 1 Result of Marketed Formulation Analysis

Method	Label claim	Mean	%RSD	%Assay
A	45mg	43.92	1.365	97.62
B	45mg	44.11	0.395	98.02

Validation

The methods were validated according to ICH guidelines to study linearity, accuracy and precision

Linearity

The linearity was evaluated by analyzing different concentrations of the standard solutions of OP. From the standard stock solution of 1000µg/ml appropriate dilutions were made in distilled water .These were scanned in the wavelength range 200-400 nm. Beer's law was obeyed in the concentration range 5-25 µg/ml for all the two methods. The correlation coefficient was found to be 0.999 (Figures 5, 6)

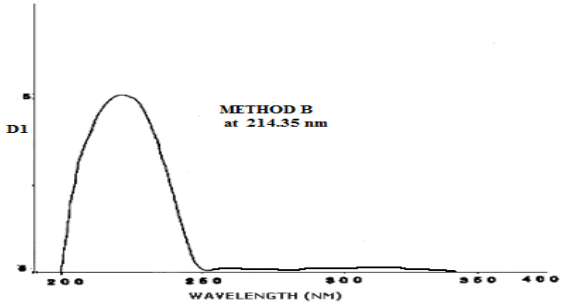

Figure 4 First Order Derivative Spectroscopy

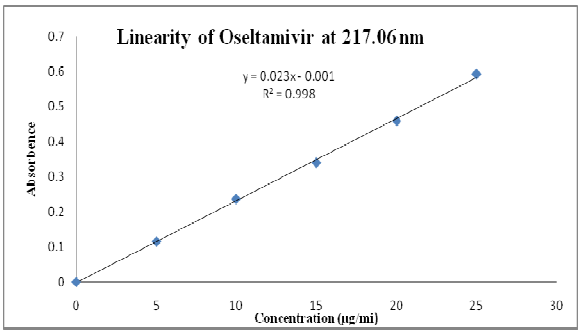

Figure 5 Calibration Curve For Oseltamivir phosphate zero order

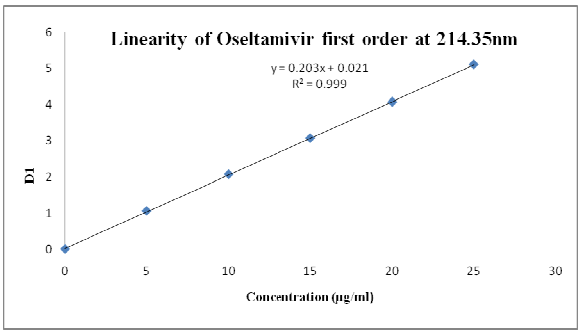

Figure 6 Calibration Curve For Oseltamivir phosphae first order

Accuracy (Recovery studies):

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (100%, 150% and 200%). Percent recovery for OP by all the two methods, was found in the range of 97%- 103% (Table 2)

Precision

The reproducibility of the proposed methods was determined by performing tablet assay at different time intervals on same day (Intraday precision) and on three different days (Inter-day precision).

RESULTS AND DISCUSSION

For method A, the absorbance maxima was found to be at 217.06nm, for method B λmaxima at 214.35nm was

selected for first order derivative spectra selected for the analysis. The % assay by the two methods was found to be in the range 99.03-100.16% for Oseltamivir phosphate. No interference was observed from the pharmaceutical excipients. The % recovery obtained for absorption maxima, first order derivative spectroscopy was found to be in the range of 97% –103%, respectively. Hence, the proposed methods were validated in terms of linearity, precision and accuracy (Table No.3). The present work provides an accurate and sensitive method for the analysis of OP in bulk and tablet formulation.

Table 2 Result Of Recovery Studies

Amount present (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)		%Recovery		%RSD	
		A	B	A	B	A	B
10	10	19.96	19.94	99.63	99.42	1.876	0.365
10	15	24.87	24.89	98.77	98.95	1.365	0.395
10	20	29.85	29.75	98.53	97.56	1.546	0.876

Table 3 Results of the two methods

Parameter	Method A	Method B
λmax	217.06	214.35
Linearity range	5-25	5-25
Correlation coefficient	0.9992	0.9999
Regression equation	Y= mX+C	Y= mX+C
Slope	-0.0036	0.2056
Intercept	0.0236	0.0259
RSD (%)	1.365	0.3955
Assay (%)	97.62	98.02
Recovery (%)	98.61	98.71
Precision (% RSD)	1.365	0.395

CONCLUSION

The two spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of oseltamivir phosphate in bulk and formulation. The proposed methods were found to be simple, economical, rapid, precise and accurate for the determination of OP in tablet dosage form. Thus, it can be easily and conveniently adopted for routine quality control analysis.

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