International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

Determination of Oseltamivir Phosphate in Capsules by Visible Spectrophotometry

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Received on: 21-10-2016; Revised on: 18-01-2017; Accepted on: 24-02-2017

ABSTRACT

A simple, sensitive, accurate and precise spectrophotometric method was described for the determination of Oseltamivir Phosphate in bulk dosage forms. The method is based on the formation of ion-pair of the drug with anionic dye such as bromophenol blue (BPB) which is extracted into chloroform having absorption maxima at 420 nm Regression analysis of the Beer's plots showed good correlation in the concentration ranges 10-100 μg/ml. The proposed method was successfully applied to the dosage forms containing the Oseltamivir Phosphate. No interference from common excipients was observed.

Keywords: Oseltamivir, anionic dye, Beer's law, Molar absorptivity, Regression analysis.

INTRODUCTION

Oseltamivir marketed under the trade name **Tamiflu**, is an antiviral drug, which may slow the spread of influenza (flu) virus between cells in the body by stopping the virus from chemically cutting ties with its host cell.[1] The drug is taken orally in capsules or as a suspension. It is used to treat influenza A virus and influenza B virus. Oseltamivir is a prodrug, a (relatively) inactive chemical, which is converted into its active form by metabolic process after it is taken into the body. It was the first orally active neuraminidase inhibitor commercially developed. Oseltamivir Phosphate is (3R,4R, 5S) -4-Acetylamino -5- amino-3- (1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate (1:1).There are several methods used, based on different techniques such as UV spectroscopy4, 5, spectrofluorimetric6, liquid chromatography with UV detection7-13 and mass spectrometry13-17,Micellar electrokinetic chromatography18 and capillary electrophoresis19 for the determination of Oseltamivir and Oseltamivir Phosphate. U.V method was developed by Ashish Thatte for the estimation of Oseltamivir in bulk and dosage forms. Colorimetric method was developed using BCG for the estimation of Oseltamivir phosphate. Another colorimetric method was developed using $KMnO_4$ and NaOH for the determination of Oseltamivir Phosphate. MBTH in presence of ferric chloride was used for the estimation of Oseltamivir phosphate.

Our aim is to develop and evaluate a colorimetric technique to measure the concentration of Oseltamivir Phosphate in pure as well as capsule dosage form. Simple and affordable colorimetric assays provide a practical means to rapidly monitor drug quality in resource-poor areas. Because Oseltamivir Phosphate (Figure 1) possesses amine group, the protonated form may act as cationic site for anionic dye such as Bromophenol blue to produce coloured ion-pairing complexes.

METHODS

Apparatus: Absorption measurements were performed using a systronics (Ahemadabad, India) model Visiscan-167 digital spectrophotometer with 1-cm matched quartz cells. Samples were weighed by Shimazdu electronic weighing balance (Tokyo, Japan) BL 220 H model. Kemi KWB 220 model water bath (Ernakulam, India) was used to control the temperature for color development.

Reagents: All chemicals used were of analytical reagent grade. All the solutions were prepared daily with distilled water. 0.1% Bromo phenol blue (BPB) was prepared by dissolving 100 mg of bromophenol blue (Sdfine-Chem limited, Mumbai) in 100 ml of distilled water. 0.862 ml of 11.6 M HCl (Fisher Scientific, Mumbai) was diluted to 100 ml with distilled water to get 0.1M HCl. Extraction of the ion-pair complex was carried out with chloroform (Fisher Scientific, Mumbai).

Preparation of stock standard solution: The oseltamivir stock solution (1 mg mL^{-1}) was prepared by dissolving 100 mg of the drug in 10 mL of water and then diluted to 100 mL with water. The stock solution was diluted with water to get working concentration of 500 µg mL^{-1} oseltamavir.

Procedure: Into a series of 50 mL separating funnels, aliquots of working oseltamavir [0.2-2.0 mL) solution were transferred. The volume in each separating funnel was adjusted to 2.0 mL with distilled water. Then, to each separating funnel 1 mL of 0.1 N HCl and 1.0 mL of bromophenol blue solution were transferred and mixed well. The funnels were shaken vigorously with 5 mL of chloroform for 2 min. The funnels were allowed to stand for the clear separation of the two phases. The separated chloroform phase was transferred into a 10 mL volumetric flask, made up to the mark with chloroform and mixed well. The absorbance of the chloroform phase was measured at 420 nm against reagent blank.

RESULTS AND DISCUSSION

The ion-pair complex is a special form of molecular complex resulting from two oppositely charged ions extractable into organic solvents from aqueous phase at suitable pH[2-4]. Initially, the field of physical chemistry has investigated the ion pair complex formation which is now being applied widely for the chemical as well as pharmaceutical analyses [5]. By using an anionic dye as a reagent and organic solvent as an extractant the quantification of several pharmaceutical compounds that possess basic moieties (secondary or tertiary amino group) is done by the ion-pair extractive spectrophotometry. As bromophenol blue is an anionic dye ; it involves in the formation of ion pair complex. Because of this nature, it contributes to simple and rapid spectrophotometric determination of many organic compounds of pharmaceutical significance.

The results obtained in the proposed method were based on the tendency of the drug to form chloroform extractable ion-pair complex with anionic dye, bromophenol blue, under experimental conditions. The positively charged primary nitrogen of Oseltamivir in acid medium is likely to attract the negatively charged part of the anionic dye, bromophenol blue and forms an ion-pair complex held together through electrostatic attraction. Ion-pair complex showed maximum absorbance at 420 nm. The ion-pair complex was stable for 1 hour at room temperature.

The different experimental parameters affecting the formation of colored ion-pair complex was studied and optimized to obtain the maximum color intensity. The effect of bromophenol blue concentration was studied by adding different volumes (0.5-2.5) of 0.1 % solution of BPB to a constant concentration of Oseltamivir (50 µg/mL). Maximum absorbance of ion-pair complex was found at 1.0 mL of 0.1 % solution of BPB (Fig 3). Beyond this value the absorbance decreases. Hence, 1.0 mL of the dye solution was used throughout this study.

The influence of acidity on the development of colored ion-pair complex using different volumes of 0.1 N HCl (0.5–2.5 mL) to a fixed concentration of Oseltamivir (50 µg/mL) was tested in this study. The maximum color intensity was observed with 1 mL of 0.1 N HCl (Figure 4). Therefore 1 mL of 0.1 N HCl was used throughout the experiment.

Method validation was performed by following the International Conference on Harmonization (ICH) guideline [6] for analytical method validation. Under the experimental conditions described, the Beer's law was obeyed over the concentration ranges of 10-100 µg/mL. The linear regression analysis using the method of least square was made to assess slope, intercept and regression coefficient (Table 1). High values of the regression coefficient and the small values of the intercepts of the regression equations proved the linearity of the calibration curve.

The sensitivity parameters such as molar absorptivity, Sandell's sensitivity, limit of detection and limit of quantification were calculated and are presented in Table 1. The high values of molar absorptivity and low values of Sandell's sensitivity, limit of detection,

and limit of quantification reveal the high sensitivity of the proposed method.

The accuracy and precision of the proposed method was assessed by determining the concentration of Oseltamivir at three different concentration levels (low, medium and high) within one day and on five consecutive days. The intra day and inter day assays were performed by performing six independent analyses at the 10,50 and 100 µg/mL concentration levels using BPB method. The standard deviations, relative standard deviation, and mean recoveries obtained by intraday and inter day assays for BPB method was calculated and are summarized in Table 2. The results of the assays are acceptable and can be considered to be very reasonable.

The accuracy and validity of the proposed method was also checked by performing recovery experiments through standard addition technique. For this purpose, a known amount of Oseltamivir was added to pre-analyzed dosage forms at three different percentage levels (50%, 100% and 150% of that in tablet) and then determined by the proposed method. The results (Table 3) showed that no interference from the common excipients was observed and establishes some degree of selectivity of the proposed method.

For the assessment of method robustness, some experimental parameters were interchanged; dye concentration (1±0.1 mL) and HCl concentration (1±0.1 mL). The analysis was performed at the deliberately varied experimental conditions by taking two different concentrations of Oseltamivir (BPB method – 10 and 100 µg/mL). The ability remained unaffected by small deliberate variations. The results, presented in Table 4, indicate acceptable robustness of the proposed method.

The proposed method was applied for the quantification of Oseltamivir in capsule dosage forms purchased from a local pharmacy store. The results, shown in Table 5, suggest that the method is suitable for the determination of oseltamivir with good accuracy and precision. The excipients in the dosage forms do not interfere in the assay procedure.

CONCLUSION

Spectrophotometric method was developed for the quantification of Oseltamivir using BPB. The developed method was validated as per ICH guidelines. It was observed that all validation parameters such as linearity, precision, accuracy, selectivity, and robustness convene the predetermined acceptance criteria. Thus, it has been concluded that the proposed method was validated for the routine analysis of the drug in pure and capsule dosage form.

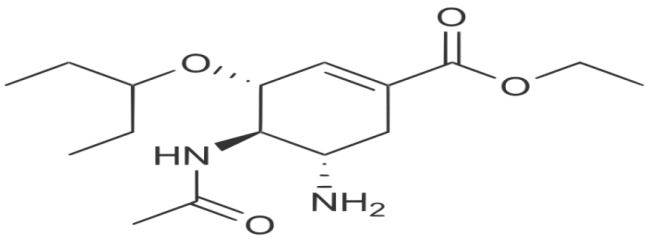

Figure 1: Structure of Oseltamivir

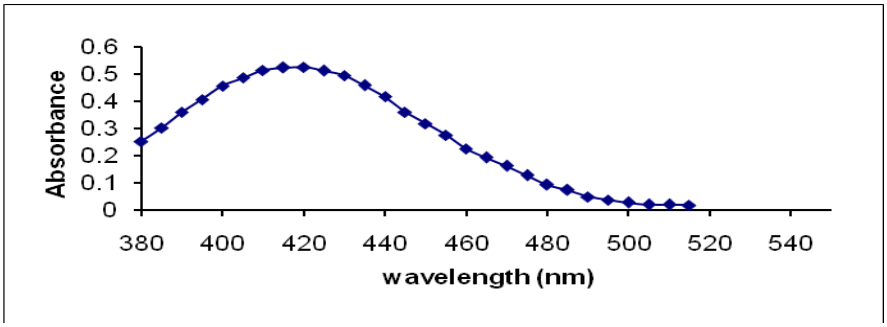

Figure 2: Absorption Spectrum of Oseltamivir-BPB ion pair complex

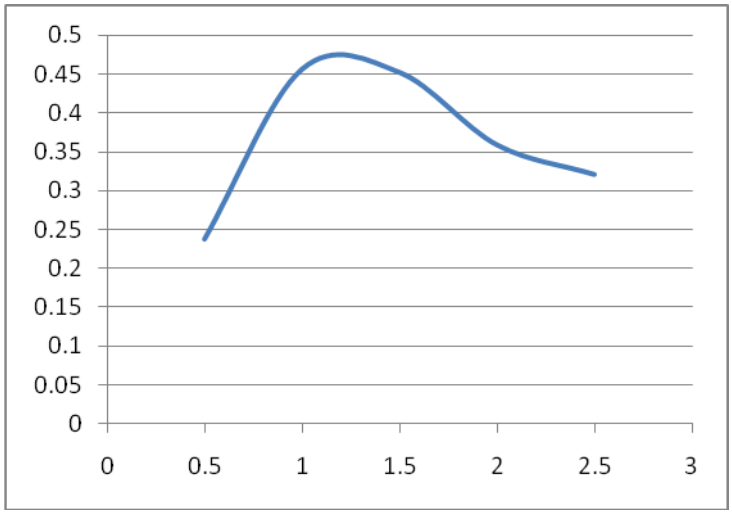

Figure 3: Effect of concentration of dye: 1. BPB method, 0.1 % BPB solution, λmax-420 nm;

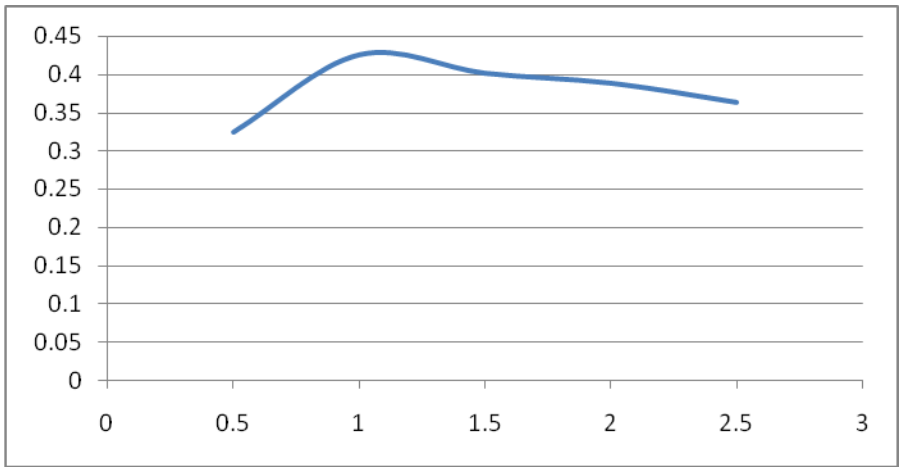

Figure 4: Effect of acidity : BPB method λmax-420 nm

Table 1: Linearity and sensitivity data

λ_{max} (nm)	420 nm
Linear range (µg ml^{-1})	10-100 µg ml^{-1}
Molar Absorbtivity (L $mole^{-1}$ cm^{-1})	3.07×10^{4}
Sandell's sensitivity (µg cm^{-2}/0.001 Absorbance unit)	0.010
Regression equation (Y= mx + c)$	0.009x+0.020
Slope (m)	0.009
Intercept (c)	0.020
Regression coefficient (R^2)	0.998
LOD (µg ml^{-1})	0.183
LOQ (µg ml^{-1})	1.11

Table 2: Precision and accuracy data

Drug taken (µg/mL)	Drug found (µg/mL) ± SD$	% RSD	% Recovery
10	9.81±0.0008	0.81%	98.1%
50	49.80±0.0008	0.18%	99.6%
100	99.78±0.0007	0.08%	99.78%
10	9.83±0.0007	0.63%	98.1%
50	49.8±0.0008	0.16%	99.6%
100	99.78±0.003	0.37%	99%

Table 3: Results of recovery experiments

Labeled claim (mg)	Pure drug added (mg)	Found ± SD$	% RSD	% Recovery
30	15	44.67±0.0015	0.37%	99.26%
30	30	59.49±0.0015	0.26%	99.15%
30	45	74.26±0.0025	0.35%	99%

Table 4: Robustness

Parameter	Drug taken (µg/mL)	Drug found (µg/mL) ± SD$	% RSD	%Recovery
BPB	10	9.9±0.0005	0.48%	99%
	100	99.89±0.0005	0.06%	99.89%
HCl	10	9.9±0.0005	0.49%	99%
	100	99.89±0.0005	0.06%	99.89%

Table 5: Evaluation of Osseltamivir in capsule dosage forms.

.Formulation	Labeled Claim (mg)	Found ± SD$$	% RSD	% Recovery
TAMIFLU	30 mg	29.16±0.050	0.17%	97.2%

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