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Spectrophotometric Determination Of Oseltamivir Phosphate In Bulk Drug And In Pharmaceutical Formulation.

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ABSTRACT

In the present investigation three simple and sensitive spectrophotometric methods 1,2 and 3 have been developed for the quantitative determination of oseltamivir phosphate in bulk as well as pharmaceutical formulation. Method-1 is a UV spectrophtometric method in which oseltamivir phosphate was dissolved in double distilled water and exhibited absorption maxima at 216.2 nm. Beer's law obeyed in the concentration range of 5-40 µg/ml. Method-2 is based on oxidation followed by coupling reaction. In this method oseltamivir phosphate formed a green coloured chromogen when treated with MBTH in the presence of ferric chloride. The chromogen exhibit absorption maxima at 666nm and obeyed Beer's Law in concentration range 10-50 µg/ml. Method-3 is based on the principle of oxidation followed by complex formation method. Oseltamivir phosphate react with ferric chloride and potassium ferricyanide to produce a dark green colour chromogen which exhibit absorption maxima at 760nm, and Beer's Law range was found between 2-10 µg/ml. The results of the methods have been validated statistically and by recovery studies. The proposed methods are simple, sensitive, economical and accurate for quantitative determination of oseltamivir phosphate in bulk drug and pharmaceutical formulation.

Keywords: Oseltamivir phosphate (OSP), MBTH, Potassium ferricyanide, Chromogen, Spectrophotometry.

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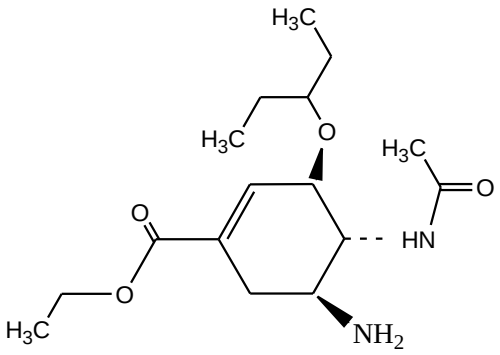
INTRODUCTION

Oseltamivir [1,2] is chemically (3R, 4R, 5S)-4-(Acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethylester. It has an antiviral activity. Its active metabolite selectively blocks the viral surface enzyme neuraminidase thereby preventing the release of virus particles from infected cells. It is active against influenza A and B virus and is the drug of choice for treatment of swine flu. It comes under the category of drugs called neuraminidase inhibitors [3]. It works by stopping the spread of flu virus in the body.



Chemical structure of Oseltamivir

The literature survey reveals that there are few reported analytical methods like HPLC [4-6], micellar electro kinetic chromatographic method [7], HPLC-Mass spectrometric [8,9] assay in plasma and urine, rapid capillary electrophoresis method, liquid chromatography-tendam mass spectrometric method. Oseltamivir phosphate (OSP) is the drug of choice for avian influenza caused by H_1N_1 virus. Due to rapid spread of pandemic influenza (swine flu) there may be chances of counterfeit products [10]. The aim of the present work is to develop simple, accurate, highly precise, economical, spectrophotometric methods for the quantitative estimation of OSP in bulk drug as well as formulation so that routine analysis and easy detection of counterfeit drugs may be possible.

MATERIAL AND METHODS

All the spectral measurements made on systronics model 119digital spectrophotometer with 10mm matched glass cells. Ferric chloride, MBTH, Potassium ferricyanide, Methanol were of analytical grade reagents procured from Ranbaxy, whereas pure sample of OSP was kindly gifted by Cipla Pvt. Ltd, Goa.

Reagents used

- Aqueous ferric chloride (1%)
- Aqueous MBTH (0.5%)
- Aqueous Potassium ferricyanide (0.1%).

- Double distilled water
- Methanol

Standard and samples preparation:

Aqueous stock solution: For UV Spectrophotometric method double distilled water was used for the preparation of stock solution. 100mg OSP was accurately weighed and dissolved in 60ml of distilled water in 100ml volumetric flask and volume was made up to the mark with double distilled water. From this solution working standard solution with final concentration of100 µg/ml was made.

Methanolic solution of OSP: For colorimetric determination stock solution of OSP was made by accurately weighing 100mg of OSP and dissolved in methanol in 100ml volumetric flask, volume made upto the mark with methanol (1^{st} stock). From this 1^{st} stock solution 2^{nd} stock was prepared with final concentration of 100µg/ml.

EXPERIMENTAL

Method- 1

Aliquots of oseltamivir phosphate ranging from 0.5-4ml (1ml =100µg) were transferred into series of 10ml volumetric flasks and volume was made upto the mark with distilled water. The absorbance was measured at 216.2nm against solvent blank. The amount of oseltamivir in sample was computed from calibration curve.

Method-2

Aliquots of working standard solution ranging from 1 to 5mL (100µg/mL) were transferred into series of 10ml volumetric flasks, to each flask 1.5ml of MBTH (0.5%) and 1ml of ferric chloride (1%) were added. The solutions were allowed to stand for 15mins at room temperature for completion of reaction. Final volume was made upto the mark with methanol. The absorbance of green coloured chromogen was measured at 666nm against reagent blank. The content of the sample was computed from calibration curve.

Method-3

In 10ml volumetric flask different aliquots of working standard solution ranging from 0.2-1.0ml (1ml=100µg) were transferred to provide final concentration of 2-10µg/ml. To each flask 0.5ml of ferric chloride (1%) and 1ml of potassium ferricyanide (0.1%)were added. The aliquots were allowed to stand for about 20 minute for completion of reaction. The final volume was made upto the mark with methanol and the absorbance of green coloured chromogen was measured at 760nm against reagent blank. The calibration curve was

constructed by plotting the absorbance versus concentration of drug. The content of the sample was computed either from calibration curve or regression equation.

Color Stability Study

Color stability for method 2

The color was developed using 30μg/ml drug concentration, 1.5 ml of aqueous MBTH (0.5%)& 1 ml of aqueous ferric chloride (1%)were added and kept aside for 15minutes. The absorbance of the green colored species was measured at 666 nm against reagent blank. The colour was found to be stable for 2 hrs (table 8).

Color stability for method 3

The color was developed using 6μg/ml drug concentration, 0.5ml of aqueous ferric chloride (1.0%) and 1 ml of aqueous potassium ferricyanide (0.1%) were added and kept aside for 20 minutes. The absorbance of the green colored species was measured at 760nm against reagent blank. The color was found to be stable for more than 2 hrs. (table 9)

RESULTS AND DISCUSSION

Sensitivity

Molar absorptivity and Sandell's sensitivity for the proposed spectrophotometric methods were found in the range of $5.355X10^3$ to $2.613X10^4$ Lit $mole^{-1}cm^{-1}$ and 0.009 to 0.038 μg/ml respectively, which shows the high sensitivity of developed methods (table 1)

Linearity

Regression equation for the developed methods were calculated using the data obtained and correlation coefficient was found in the range of 0.9998 to 0.9999 this shows a good linear relationship between concentration of drug and absorbance.(table1)

Precision

For precision demonstration %RSD was calculated it was found in the range of 0.258 to 0.487 which is within the prescribed limits (<2%) this depicts that the developed method are highly prcise (table1).

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Assay

Assays were carried out by selecting capsules (C_1 and C_2) of two different brand (Hetero Drugs and Cipla) percentage recovery of OSP from the formulations were found between the range of 99.33 to 99.93 the results obtained reveals the suitability of the proposed methods for routine analysis of OSP in pharmaceutical formulations. The detailed information regarding the assay are given in tables 2-4

Accuracy

The accuracy of the developed methods was determined by recovery studies. Known quantity of drug was added to pre-analyzed formulation and the amount added was recovered by the proposed method. In present investigation recovery was done at three different percentage levels (80,100 and 120 %) of labelled claim. Percentage recovery by the proposed methods is found within the range 99.82 to 99.95 this represents the accuracy of the proposed methods. The results are shown in tables 5-7

Color Stability for the developed methods

The information provided in tables 8 and 9 are regarding the color stability of the developed chromogen. The green colored chromogen formed by the reaction of OSP with MBTH is found to be stable for about 2 hrs. OSP reacts quantitatively with potassium ferricyanide to form a green colored chromogen which is stable for more than 2 hrs.
Figures from 1-6 shows the absorption spectrum and calibration curves of OSP for the developed methods.

Table 1 : Optical characteristics and precision

Parameter	Method A	Method B	Method C
λmax (nm)	216.2	666	760
Beer's law limits (μg/ml)	5-40	10-50	02-10
Molar absorptivity (lit. mol^{-1} cm^{-1})	$1.03422x10^4$	$5.355x10^3$	$2.61303 x10^4$
Sandell's sensitivity (μg/ml 0.001 abs unit)	0.018	0.038	0.009
LOD (μg/ml)	0.173	0.481	0.045
LOQ (μg/ml)	0.523	1.458	0.150
Regression equation (Y*)			
Slope (b)	0.02489	0.012932	0.06173
Intercept (a)	0.008357	0.000321	-0.00182
Correlation coefficient (r)	0.99983	0.9999	0.9998
% RSD	0.2586	0.487	0.342
Range of Errors**			
Confidence limit with 0.05 level	± 0.001088	± 0.00158	± 0.000107
Confidence limit with 0.01 level	± 0.001609	± 0.00233	± 0.001585

Y* = bc + a, where C is the concentration of Oseltamivir phosphate in μg/ml and Y is the absorbance at the respective λmax **for eight measurements.

ISSN: 0975-8585

Table 2:Assay of Oseltamivir Phosphate in Pharmaceutical dosage form (capsule) by Method A

Capsules	Labelled amount (mg)	Amount obtained (mg) by Proposed Method	% recovery*
C_1	75	74.95	99.93
C_2	75	75.03	100.04

*average of 5 determinations, C_1 and C_2 are capsules from Hetero drugs and Cipla.

Table 3: Assay of Oseltamivir Phosphate in Pharmaceutical dosage form (capsule) by Method B

Capsules	Labelled amount (mg)	Amount obtained (mg)		% recovery*
		Proposed Method	Reference method (UV Method)	
C_1	75	74.65	74.95	99.53
C_2	75	74.50	75.03	99.33

*average of 5 determinations, C_1 and C_2 are capsules from Hetero drugs and Cipla

Table 4: Assay of Oseltamivir Phosphate in Pharmaceutical dosage form (capsule) by Method C

Capsules	Labelled amount (mg)	Amount obtained (mg)		% recovery*
		Proposed Method	Reference method (UV Method)	
C_1	75	74.68	74.40	99.57
C_2	75	74.80	74.71	99.73

*average of 5 determinations, C_1 and C_2 are capsules from Hetero drugs and Cipla

Table 5 :Recovery Studies of Oseltamivir Phosphate for Method 1

Level of % Recovery	Labelled Amount (mg)	Amount of Standard Drug Added (mg)	*Mean Recovery	%Recovery	± Std Deviation	%RSD
80	75	60	59.97	99.94	0.055	0.092
100	75	75	74.95	99.93	0.051	0.068
120	75	90	89.94	99.93	0.049	0.054

* Five determinations

Table 6 :Recovery Studies of Oseltamivir Phosphate for method 2

Level of % Recovery	Labelled Amount (mg)	Amount of Standard Drug Added (mg)	*Mean Recovery	%Recovery	± Std Deviation	%RSD
80	75	60	59.91	99.85	0.04	0.066
100	75	75	74.95	99.93	0.077	0.10
120	75	90	89.86	99.84	0.036	0.040

*Five determinations

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Table 7: Recovery Studies of Oseltamivir Phosphate for method 3

Level of % Recovery	Labelled Amount (mg)	Amount of Standard Drug Added (mg)	*Mean Recovery	%Recovery	± Std Deviation	%RSD
80	75	60	59.89	99.82	0.07	0.116
100	75	75	74.92	99.89	0.037	0.050
120	75	90	89.91	99.9	0.04	0.044

*Five determinations

Table 8: Color stability data for method 2

Conc µg/ml	Time (mins)											
	10	20	30	40	50	60	70	80	90	100	110	120
30	0.389	0.389	0.389	0.389	0.388	0.388	0.387	0.387	0.386	0.385	0.384	0.382

Table 9: Color stability for method 3

Conc µg/ml	Time (mins)											
	10	20	30	40	50	60	70	80	90	100	110	120
6	0.375	0.375	0.375	0.374	0.375	0.374	0.374	0.373	0.373	0.373	0.373	0.371

Fig 1:UV-spectrum of Oseltamivir Phosphate (20 µg/ml)

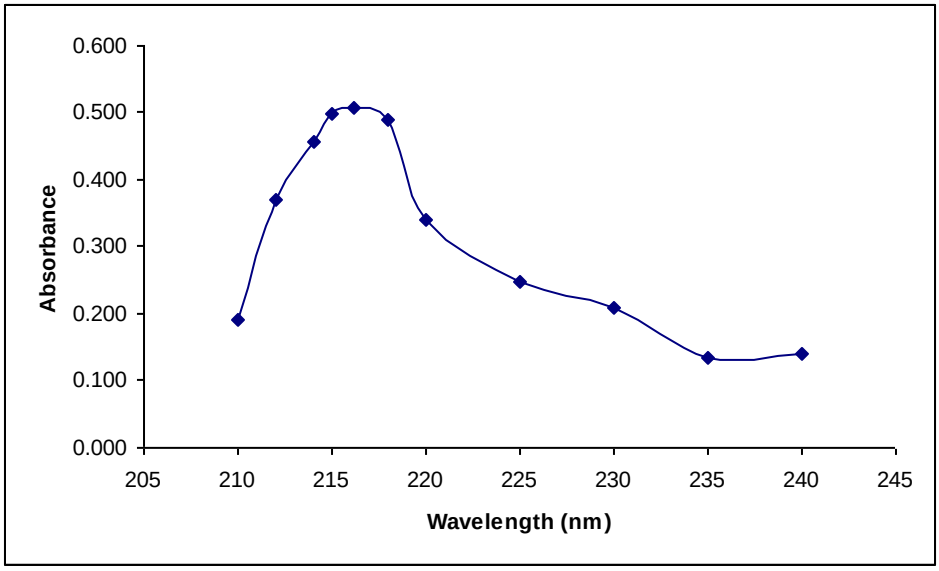

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Fig 2: Calibration curve of Oseltamivir Phosphate for UV-method

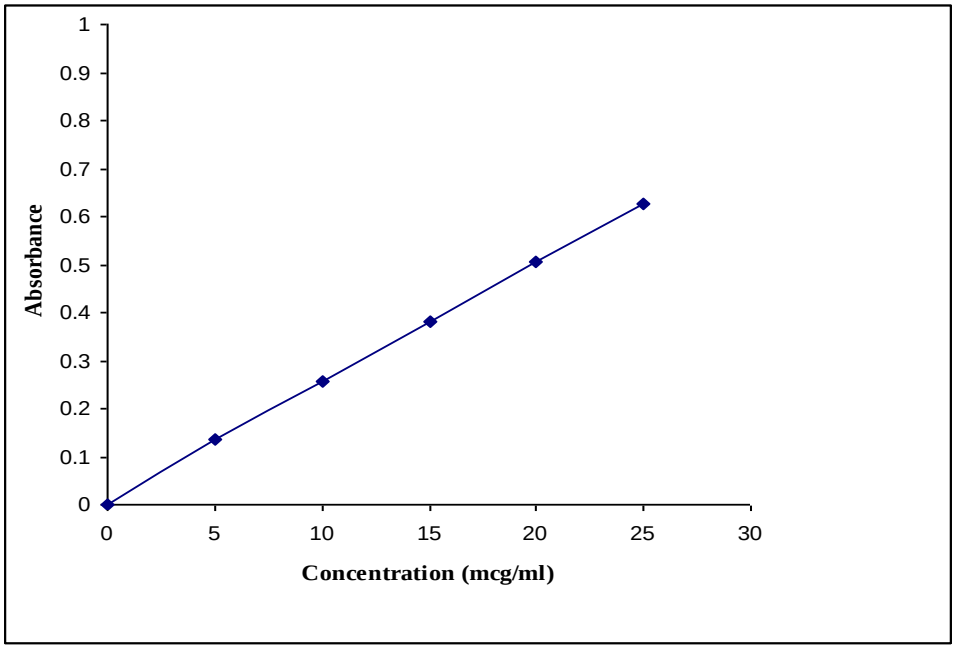

Fig 3: Absorption Spectrum of Oseltamivir Phosphate

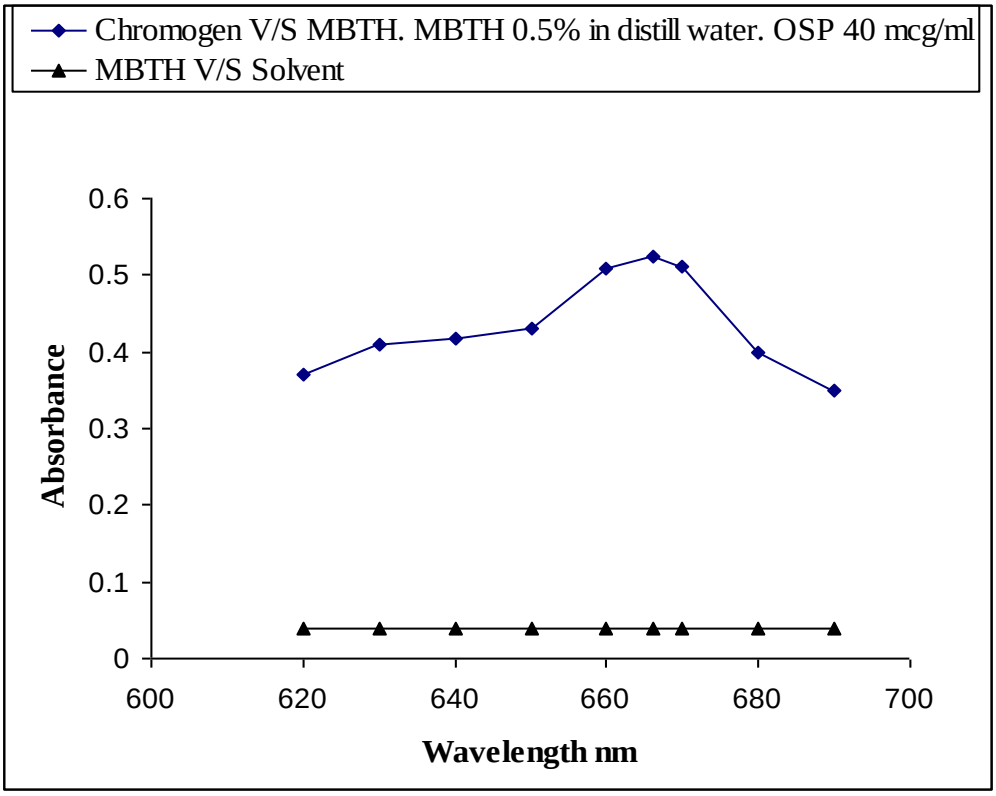

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Fig 4: Calibration curve of Oseltamivir Phosphate for method 2
Absorption Spectrum of Oseltamivir Phosphate

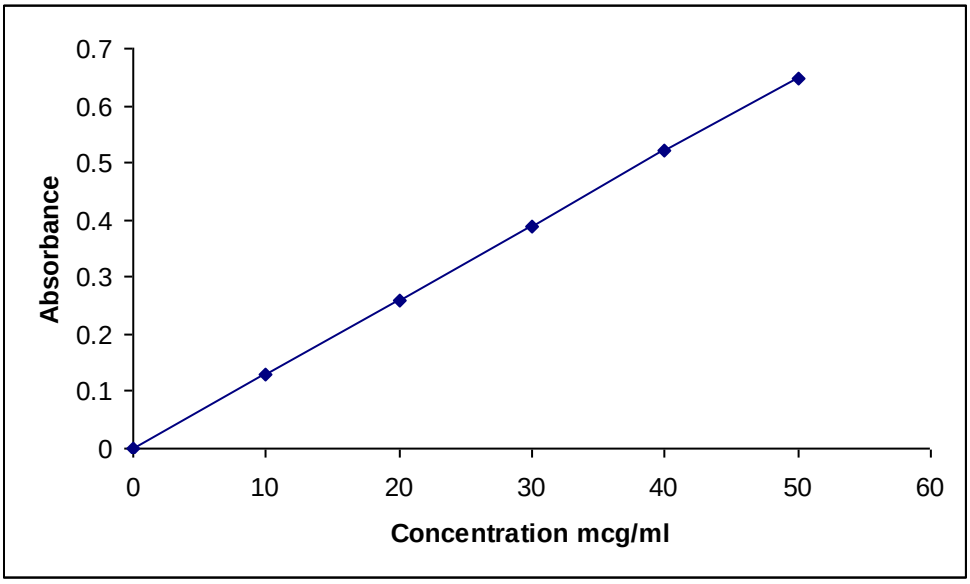


Fig 5: absorption spectrum of Oseltamivir Phosphate

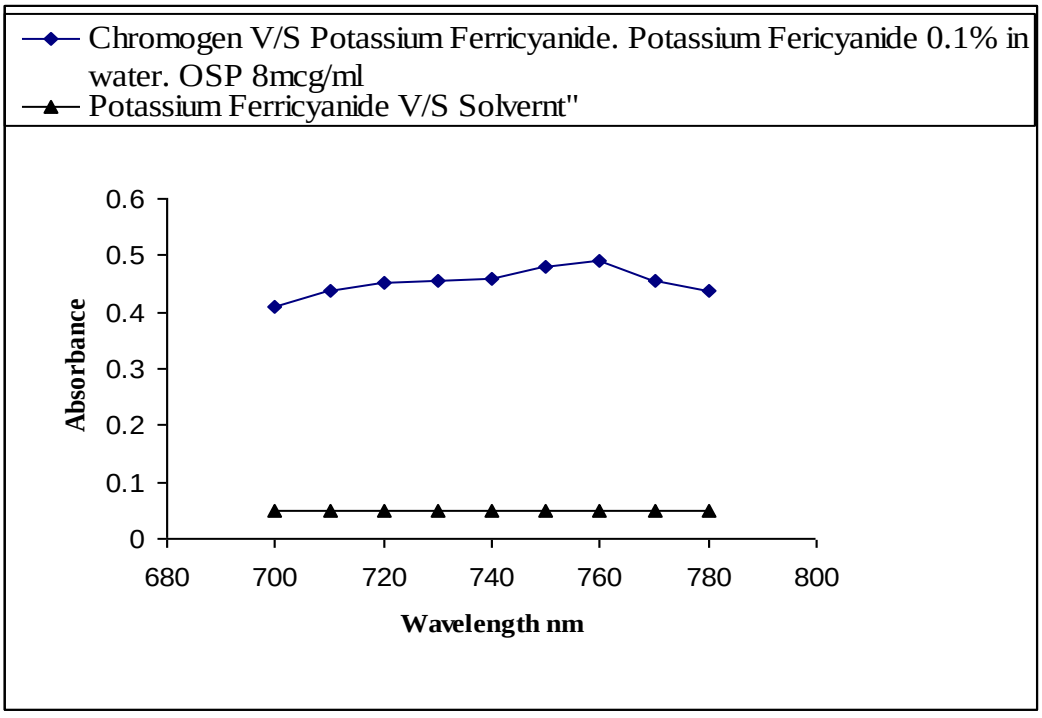
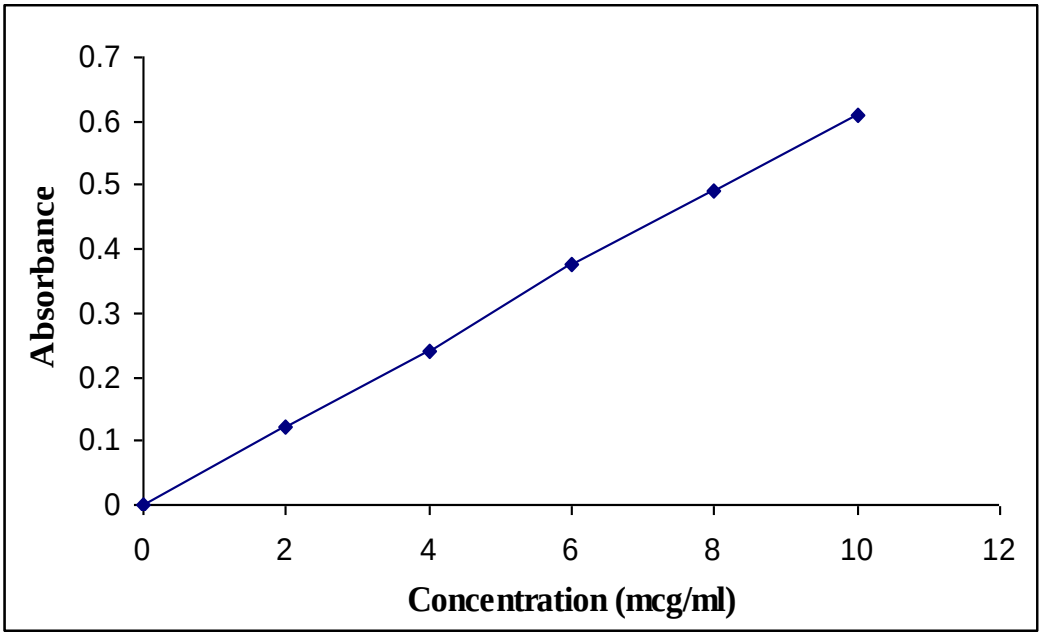


Fig 6: Calibration Curve of Oseltamivir Phosphate for Potassium Ferricyanide Method



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