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Spectrophotometric Method for Determination of Oseltamivir in Capsule Dosage Form

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Abstract | **Original Research Article**

Objectives: Study was aimed to establish and vindicate a simple, accurate and precise spectrophotometric method for the quantification of oseltamivir in API and capsule dosage form. ***Materials and Methods:*** The green coloured chromogen complex absorbance which was formed by the oxidative coupling with loss of two electrons and a proton of oseltamivir with MBTH in presence of $FeCl_3$ was measured at 640nm. The amount of oseltamivir labelled in the marketed formulation (Fluvir) was determined without any interference owed with excipients. ***Results:*** A correlation coefficient of 0.999 was observed within the concentration range of 10-110µg/mL. The method was aided by various validation parameters such as LOD, LOQ and percentage relative standard deviation values (3.75 µg/mL, 9.86 µg/mL and 0.999 respectively). The percentage assay in capsule dosage form was found to be 97.6, which in conformance with ICH guidelines. ***Conclusion:*** Results were found to be within the permissible limits. Present method was verified statistically in consonance with ICH Q2R (1) guidelines. Based on above remarks, developed method may be successfully employed in regular analysis of oseltamivir in various pharmaceutical dosage forms.

Keywords: Oseltamivir, MBTH, $FeCl_3$, spectrophotometric method, oxidative coupling reaction.

1. INTRODUCTION

Oseltamivir chemically known as ethyl (3R,4R,5S)-4-acetamido-5-amino-3(1- ethyl propoxy)-1-cyclohexene-1-carboxylate phosphate belongs to a novel class of antiviral medications known as neuraminidase inhibitors, which block influenza A and B viruses.

Literature survey revealed that most of the claimed procedures[1-18] were seems to be not simple for regular analysis and require expensive or advanced instruments. Hence, we aimed to develop simple, precise and cost effective method that can be readily used for regular analysis.

2. MATERIAL AND METHODS

All chemicals used were of analytical grade. Oseltamivir (OST) was procured from Dr. Reddy's Laboratories Ltd as a gift sample, Hyderabad, India. Fluvir(75mg) formulation was obtained from a retail pharmacy. Visible spectrum was recorded using a Shimadzu UV-1800, the FTIR using IR Affinity 1, Shimadzu, Japan.

2.1. Method optimisation

The method for estimation of oseltamivir was optimized as follows:

Oseltamivir was treated with different reagents such as NQS (1,2-Naphthoquinone-4- sulfonate) reagent, salicylaldehyde, vanillin, Bratton-Marshall(N-(1-Naphthyl) ethylene diamine dihydrochloride) reagent where no absorption spectra were observed. Absorbance was notified with MBTH reagent; hence it was further used in different concentrations for optimization along with the drug. A composition of reagent with 2mL 2%MBTH and 4mL 1.5% ferric chloride was selected for the method development as it has produced spectra in compliance with the ICH guidelines.

2.1.1. Preparation of solutions

Standard stock solution (1000µg/mL) was prepared by dissolving 10mg of oseltamivir in 10mL of methanol. It was further diluted with water to prepare the working standards. Solutions of MBTH (2%), ferric chloride (1.5%) and hydrochloric acid (0.1N) were prepared with distilled water.

2.1.2. Analytical Procedure

Standard solution of oseltamivir (100µg/mL) was taken in a 10mL volumetric flask to which 2mL MBTH (2%) reagent and 4mL ferric chloride (1.5%) solutions were added. Make the volume up to the mark with water. Then the solution was allowed to stand for 10min and the absorbance was recorded at 640 nm with corresponding reagent blank.

2.1.3. Chemistry of the reaction

The proposed mechanism for the reaction of oseltamivir with MBTH along with ferric chloride involves oxidative coupling which results in the formation of oseltamivir-MBTH- $FeCl_3$ complex. MBTH generates electrophilic intermediate in presence of $FeCl_3$ by losing one proton and two electrons which is an active coupling species. It undergoes electrophilic substitution reaction with oseltamivir to generate a green coloured complex showing absorbance at 640 nm.

2.2. Method validation

Present method was vindicated for linearity, accuracy, precision, LOD and LOQ according to the ICH guidelines.

2.2.1. Linearity

Standard stock solution containing 1000µg/mL of oseltamivir were prepared by dissolving 10mg of drug in 1mL of methanol and then volume was made up to 10 mL using methanol. Aliquots of various concentrations (10-110µg/mL) were prepared by diluting relevant volumes of standard stock solution with 2mL MBTH (2%) reagent and 4mL ferric chloride (1.5%) solution. Water was added to made up final volumes up to the mark with and allowed to stand for 10min.Then the absorbance of green coloured chromogen was measured at 640 nm with corresponding reagent blank.

2.2.2. Accuracy

Standard addition method by calculating recoveries of oseltamivir. was applied to determine the accuracy of the method. Capsule contents (Fluvir) equivalent to 50 µg/mL of oseltamivir was transferred into three different 10mL volumetric flasks and to it 80, 100 and 120 % of standard drugs were spiked and volume was made up with MBTH, ferric chloride and water. Then solutions were allowed to stand for 10min.These solutions were further diluted with the same to get the final concentration of solutions within the linearity range. The amount of oseltamivir was estimated by measuring response at 640 nm with corresponding blank.

2.2.3. Precision

The intra-day and interday precision of the spectrophotometric method was performed by estimating the responses to the three different concentrations of osaltamavir (30,50,70 µg/mL) and the responses were recorded three times on the same day and different days. Both intra-day and inter-day precision results were reported as relative standard deviation (%RSD).

2.2.4. LOD and LOQ

The LOD and LOQ of osaltamavir were calculated by the signal-to-noise ratio (S/N, M i.e., 3.3 for LOD and 10 for LOQ) using the following equation as per ICH guidelines.

The Limit of Detection (LOD):

$$\mathbf{LOD = 3.3 \times \sigma / S}$$

Where, σ = standard deviation of the response

S = slope of the calibration curve of the analyte

The Limit of Quantification (LOQ):

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where, σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

2.3. Assay of oseltamivir marketed formulation

An amount of capsule powder (75 mg) containing 10mg of oseltamivir was added to a 10mL volumetric flask. Sonicate the flask by adding about 6 mL of methanol for 15 min and then remaining 4mL of methanol was added. Then solution was filtered through whatman filter paper (No: 41). To a 10 mL volumetric flask containing 2mL MBTH (2%) reagent and 4mL of ferric chloride (1.5%) solutions, 0.5 mL of the filtrate was transferred and final volume was made up to 10mL with water. Then the solution was allowed to stand for 10min and the coloured chromogen was measured at 640 nm with the corresponding blank. The amount of oseltamivir was determined by Beer-Lambert's plot.

3. RESULTS AND DISCUSSION

3.1. Linearity

Concentration ranges of about 10-110µg/mL of Oseltamivir was checked for the linearity of the calibration curve (absorbance vs concentration). The regression line relating the calibration curve and standard concentration of drug were linear in the taken range by regression analysis were obtained y=0.0088x+0.0164. The calibration curve was represented in Figure 3. The correlation coefficient was found to be 0.999. Mean± standard deviation (SD), intercept, slope and correlation coefficient of standard curve (n=6) were calculated as shown in table 1 that the developed method has adequate sensitivity to the concentration of the drug in the sample.

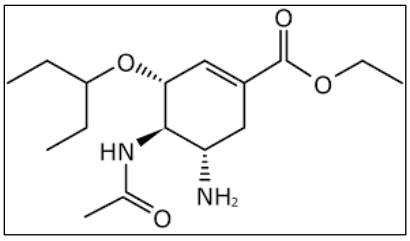

Figure 1: Chemical structure for Oseltamivir

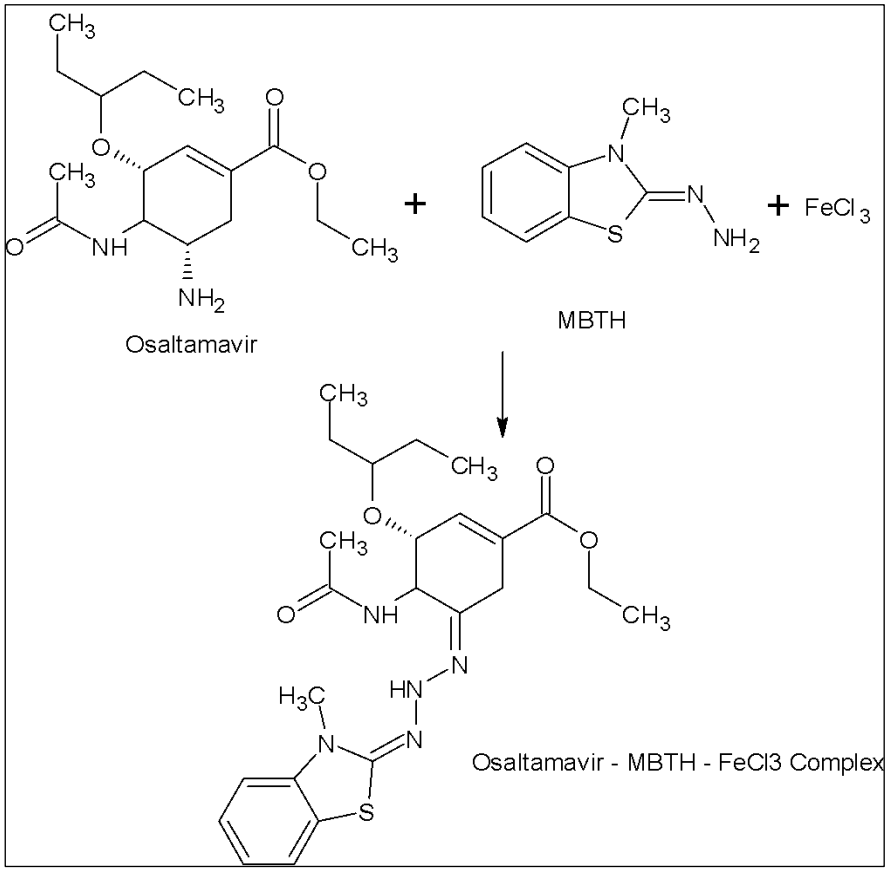

Figure 2: Chemical reaction of Oseltamivir with MBTH and $FeCl_3$

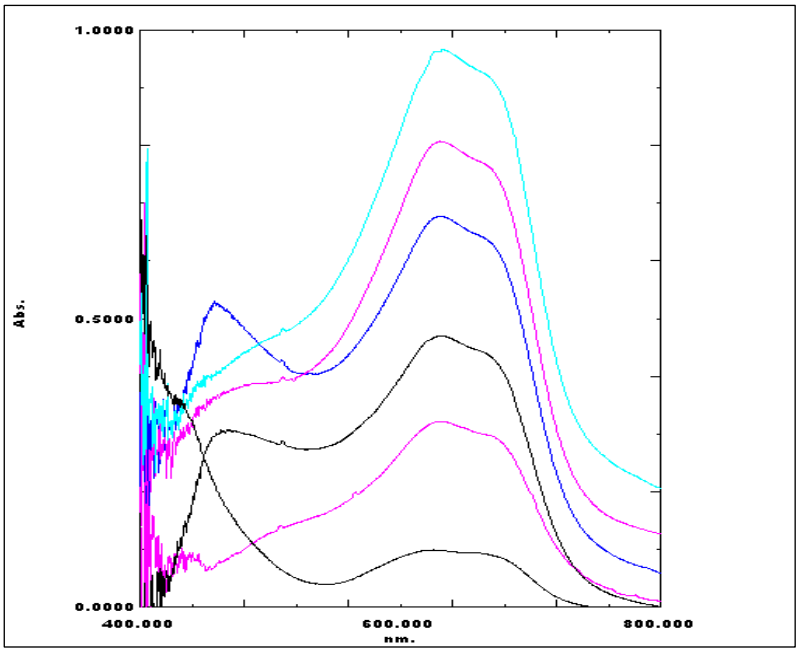

Figure 3: Absorption spectra of Oseltamivir (10-110µg/mL)

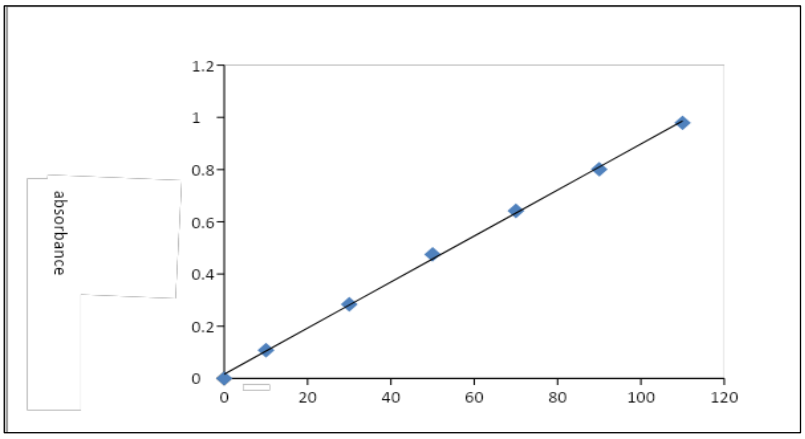

Figure 4: Calibration curve of Oseltamivir (10-110µg/mL)

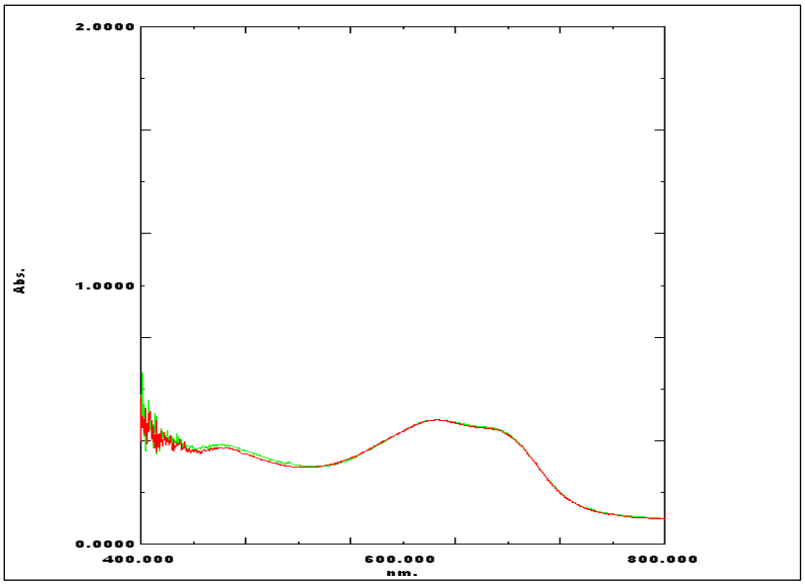

Figure 5: Selectivity graph

Table 1: Calibration curve data of Oseltamivir at λ_{max} 640nm

S. No	Concentration(µg/mL)	Absorbance (AM±SD) (n=6)
1	0	0
2	10	0.108±0.0011
3	30	0.284±0.002
4	50	0.475±0.003
5	70	0.642±0.017
6	90	0.801±0.032
7	110	0.979±0.012

AM: Arithmetic Mean**, SD:** Standard Deviation

3.2. Precision

The intra-day and interday precision of the spectrophotometric method was performed by estimating the responses to the three different concentrations of Oseltamavir (30,50,70 µg/mL) and the responses were recorded three times on the same day and different days. Both intra-day and inter-day precision results were reported as relative standard deviation (%RSD). The results recorded from precision studies were shown in table 2. The %RSD values were less than 2.0, confirming that developed method was accurate.

Table 2: Data for precision of analytical method

S.No	Concentration (µg/mL)	Intra –day precision		Inter- day precision	
		Concentration estimated (µg/mL) (AM±SD)	%RSD[a]	Concentration estimated (µg/mL) (AM±SD)	%RSD[a]
1	30	28.6±0.3744	1.29	27.3±0.542	1.9
2	50	48.6±0.866	1.74	49.5±0.964	1.45
3	70	69.2±0.1159	0.158	65.2±0.987	1.52

[a]Acceptance criteria: %RSD should not be more than 2.0.

3.3. Accuracy

The accuracy of the developed method was validated by recovery studies and was found to be significant under limits, within % recovery and %RSD. The results reported less than 2 for the drug in Table 3.

Table 3: Data of Accuracy studies of Oseltamivir

Analyte	Recovery level%	Conc of sample(µg/mL)	Conc of standard spiked (µg/mL)	Total amount(µg/mL)	Amount recovery (AM±SD) (µg/mL) (n=3)	%Recovery	%RSD[a]
Oseltamavir	80	50	40	90	38.74±0.144	98.65%	1.214
	100	50	50	100	49.12±0.615	98.32%	1.25
	120	50	60	110	64.9±0.518	108.16%	0.79

[a]Acceptance criteria: %RSD should not be more than 2.0.

3.4. Limit of Detection (LOD), Limit of Quantification (LOQ)

LOD was found to be 3.75µg/mL and LOQ was found to be 9.36 µg/mL for Oseltamivir respectively as shown in Table 4.

Table 4: Table of LOD & LOQ

Drug	Parameter	Values
Oseltamivir	Slope(s)	0.01
	Standard deviation(σ)	0.0088
	LOD(µg/mL)	3.75 µg/mL
	LOQ(µg/mL)	9.86 µg/mL

3.5. Assay of formulation:

The assay results were found to be 94.6%. The results were reported in Table 5, denoting that the assay results were consistent with the respective labelled claim and there was no interference of excipients from formulation at the λ_{max} of Oseltamivir.

Tables 5: Assay of Oseltamivir in the marketed formulation

Formulation	Label claim(mg)	Amount found (AM+SD) (mg)(n=3)	%Assay	%RSD
Oseltamivir	75	70.26±0.654	94.6	0.976

3.6. Selectivity

Based on the selectivity parameter both the Oseltamivir (API) and formulation were estimated for the interference that the sample may have and the graph obtained is shown in Figure 5.

Table 6: System suitability parameters

Parameters	Oseltamavir
Absorption Wavelength(nm)	640
Linearity range (µg/mL)	10-110
Slope(m)	0.0088
Intercept(c)	0.0164
Regression equation(y)	Y=0.0088x+0.0164
Correlation coefficient(r^2)	0.999
Accuracy(%RSD)	Less than 2.0
Precision(%RSD)	Less than 2.0
LOD(µg/mL)	3.75
LOQ(µg/mL)	9.36
Assay(%)	94.6

4. CONCLUSIONS

The results acquired in the present study demonstrated the developed spectrophotometric method was simple, selective, precise, accurate, and linear. The assay values were in good credence with their corresponding label claim suggesting no interference with excipients in capsule dosage form and the results obtained were validated.

The sensitivity of the developed method was supported by LOD and LOQ values. These advantages of the developed method were encouraging to employ this method in the regular analysis of respective drugs in their pharmaceutical dosage forms.

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