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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF OSELTAMIVIR IN CAPSULE DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Oseltamivir in capsule dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point which is done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Oseltamivir in capsule dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, very slightly soluble in water and Insoluble in Ethanol. So, the analytical grade methanol is used as a diluent in method. The melting point of Oseltamivir was found to be 195-196˚C (uncorrected). It showed absorption maxima 218 nm in analytical grade methanol. On the basis of absorption spectrum, the working concentration was set on 100 µg/ml (PPM). The linearity was observed between 60-140 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 98.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.77% and for Ruggedness is 0.10%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Oseltamivir in capsule dosage form. The method could be considered for the determination of Oseltamivir in quality control laboratories.

KEYWORDS: Oseltamivir, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

The structure of Oseltamivir shows that it possesses a hydrophobic moiety. 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.[1] Oseltamivir Phosphates also is known as Tamiflu. Its molecular formula is $C_{16}H_{28}N_2O_4$. IUPAC name of Oseltamivir Phosphate is ethyl (3R, 4R, 5S)-5-amino-4-acetamido-3-(pentan-3-yloxy) cyclohex-1-ene-1-carboxylate. Oseltamivir is administered orally; it is an antiviral drug for the management of influenza A and B infections in children>1 y and adults of all ages.[2] OP is rapidly and extensively metabolized via hepatic esterase's to Oseltamivir Carboxylate (OC), the active form, a potent and selective inhibitor of influenza virus neuraminidase.[3] The concentrations of oseltamivir carboxylate required for inhibition of influenza virus in cell culture were highly variable depending on the assay method used and the virus tested. Standard dose of oseltamivir in adults is 75 mg, while children have unit doses that are selected on the basis of body weight. Oral capsule (35, 40 and 75 mg) and suspension formulations are now readily available.[4]

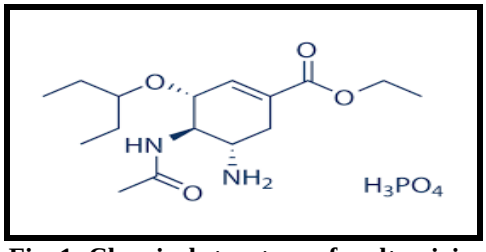

Fig. 1: Chemical structure of oseltamivir.

Literature survey revealed that a few Spectrophotometric,[5-6] HPLC,[7-8] LC-MS,[9-10]

Colorimetric,[11] methods were reported earlier for the determination of Oseltamivir in bulk and pharmaceutical dosage forms. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity, and low cost. Some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Oseltamivir alone in capsule dosage form.

MATERIALS AND METHODS

- **Instruments:**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair
Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **Materials:**

Oseltamivir was obtained as a gift sample. Oseltamivir capsules were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method development:

A. Determination of ƛ max (15 PPM)[12,13,14]

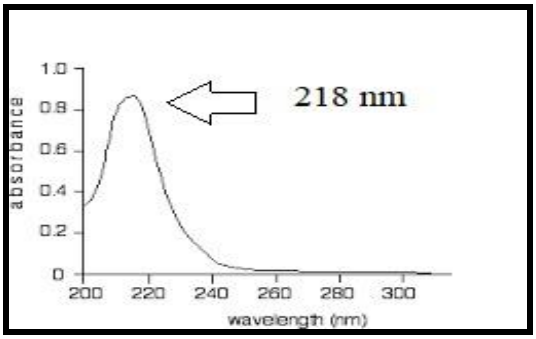

Fig. 2: Calibration curve.

50 mg weighed amount of Oseltamivir was dissolved into 50 ml of volumetric flask with analytical grade methanol. Pipette out 1 ml and added in 10 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200 - 400 nm and absorption maximum was determined.

B. Preparation of working concentration

Preparation of standard stock solution:

Standard stock was prepared by dissolving 50 mg of Oseltamivir in 50 ml of analytical grade methanol to get concentration of 1000 µg/ml (PPM).

Preparation of standard solution:

Pipette out 1 ml from standard stock solution and diluted up to 10 ml with analytical grade methanol to get concentration of 100 µg/ml (PPM).

C. Procedure for UV reading

Blank solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard solution:

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample solution:

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[12,13,14]

For analysis of commercial formulations; twenty capsules are taken weighed it and powdered. The powder equivalent to 50 mg of Oseltamivir was accurately weighed and transferred into the 50 ml of volumetric flask, added 35 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted up to 50 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 1 ml of the filtered solution and diluted up to 10 ml with analytical grade methanol. The absorbance was measured at 218 nm. The absorbance was recorded.

Table 1: Absorbance of dosage form.

Cipla Pharma Pvt. Ltd. (Oseltamivir 300 mg Capsules)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.5239
3	Sample	0.5174

Table 2: Dosage form specifications.

Type	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	Antiflu ® - 75 Cipla Pharma Pvt LTD (75 mg)	9/2022	8/2024	EDG 15478	0.1442	98.76

E. Method of validation[15,16,17,18]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity:

The linearity of the proposed assay was studied in the concentration range 60 - 140 PPM at 218 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies

Sr. no.	Sample concentration	Absorbance
1	60 PPM	0.3174
2	80 PPM	0.4259
3	100 PPM	0.5274
4	120 PPM	0.6369
5	140 PPM	0.7484
Correlation coefficient		0.9998

Accuracy:

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 10 ml of volumetric flask with analytical grade methanol and measuring the absorbance at 218 nm.

Table 4: Accuracy studies.

Spectrophotometric method			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75
100	1	0.98	98.00
120	1.2	1.19	99.17

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5215
2	Sample Solution 2	0.5305
3	Sample Solution 3	0.5214
4	Sample Solution 4	0.5215
5	Sample Solution 5	0.5251
MEAN		0.5239
SD		0.0040
% RSD		0.7652

Ruggedness:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for ruggedness studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5219	0.5223	99.68	0.0947
		0.5226			
2	Analyst 2	0.5245	0.5230	98.81	
		0.5214			

RESULTS

1. Solubility of oseltamivir

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Methanol	Freely Soluble
2	Methylene Chloride	Practically Insoluble
3	Ethanol	Insoluble

2. Melting point of oseltamivir

The melting point of Oseltamivir was found to be 195-196˚C (uncorrected).

3. Results for linearity for assay method of oseltamivir

The linearity of method was determined at concentration level ranging from 60 to 140 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9998**

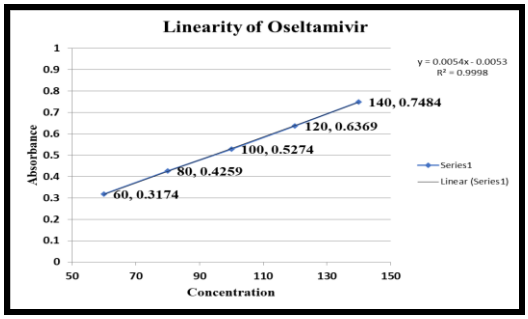

Fig. 3: Oseltamivir standard curve.

4. Results for accuracy for assay method of oseltamivir

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of oseltamivir

The % RSD for different sample of precision was found to be 0.7652 ~ 0.77 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of oseltamivir

The %RSD for different sample of ruggedness was found to be 0.0947 ~ 0.10 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Oseltamivir in capsule form has been developed. From the spectrum of Oseltamivir, it was found that the maximum absorbance was 218 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 60-140 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Oseltamivir in solid dosage forms. Hence, the method could be considered for the determination of Oseltamivir in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. MS - Mass Spectroscopy
6. LC - Liquid Chromatography
7. ICH -
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. °C - Degree Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date
14. µg/ml - Microgram per milliliter
15. Avg - Average
16. Wt - Weight
17. g - gm
18. OP - Oseltamivir-phosphate
19. OC - Oseltamivir Carboxylate

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