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Spectrophotometric Estimation of Oseltamivir in Pharmaceutical Formulations

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Three simple, accurate, rapid and sensitive methods (**A**, **B** and **C**) have been developed for the estimation of oseltamivir in its pharmaceutical dosage form. The method **A** is based on the formation of orange red coloured chromogen, due to reaction of oseltamivir with *p*-dimethyl amino cinnamaldehyde (PDAC) reagent in methanol, which exhibits λ_{max} at 530 nm. Method **B** is based on the reaction of oseltamivir with 4-aminophenazone (4-AP) in the presence of sodium periodate to form a intense violet coloured chromogen, which shows maximum absorbance at 545 nm. The method **C** is based on the formation of blood red coloured chromogen with ferric chloride and 1,10-phenanthroline which shows absorption maximum at 512 nm. The absorbance-concentration plot is linear over the range of 1-7 mcg/mL for method **A**, 5-50 mcg/mL for method **B** and 1-15 mcg/mL for method **C**. Results of analysis for all the methods were validated statistically and by recovery studies. The proposed methods are economical and sensitive for the estimation of oseltamivir in bulk drug and in its formulations.

Key Words: UV-Visible spectrophotometry, Oseltamivir, Methanol, *p*-Dimethylaminocinnalmaldehyde, 1,10-Phenanthroline, 4-Aminophenazone, Ferric chloride, Sodium periodate.

INTRODUCTION

Oseltamivir[1] is a acyclic phosphonate nucleotide analogue, chemically oseltamivir is an ethyl ester prodrug which requires ester hydrolysis to be converted to the active form, oseltamivir carboxylate, [3R,4R,5S]-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate. It is official in Indian pharmacopoeia. It's molecular weight is 312.40 g/mol with molecular formula $C_{16}H_{28}N_2O_4$. Oseltamivir was the first orally active neuraminidase inhibitor commercially developed. It is a prodrug, which is hydrolyzed hepatically to the active metabolite, the free carboxylate of oseltamivir. Oseltamivir is used in the treatment and prophylaxis of both influenzavirus A and influenzavirus B[2]. Survey of literature

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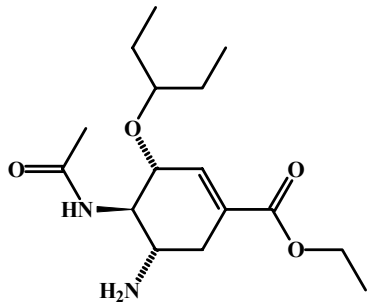
reveals that the drug is determined by using HPLC[3-6] in biological fluids and in pharmaceutical dosage forms. However, only two methods[4] has been done to estimate the oseltamivir in pharmaceutical dosage forms by extractive spectrophotometric methods. The present investigation aims to develop simple, sensitive, accurate, rapid and cost effective spectrophotometric methods **A**, **B** and **C** for the estimation of oseltamivir in its tablet formulations.



Structure of oseltamivir

EXPERIMENTAL

Elico ultraviolet-visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.

All the chemicals used were of analytical reagent grade. (1) 1,10-phenanthroline (0.2 M) AR grade: 780 mg 1,10-phenanthroline in 25 mL of in distilled water. (2) Orthophosphoric acid (0.2 M) AR grade. (3) Ferric chloride hexahydrate (0.03 M) AR grade: 405 mg of ferric chloride hexahydrate is dissolved in 50 mL of distilled water. (4) 4-Aminophenazone (4-AP) AR grade - (0.5 % w/v): 500 mg of 4-AP is dissolved in 100 mL of distilled water. (5) Sodium periodate-(0.5 % w/v) AR grade: 500 mg of sodium periodate in 100 mL of distilled water. (6) *p*-Dimethylamino cinnamaldehyde-(PDAC) AR grade (3 % w/v): 3 g of PDAC is dissolved in methanol. (7) Methanol AR grade. (8) Sulfuric acid (0.1N) AR grade.

Procedure: Standard stock solution was prepared by dissolving 10 mg of oseltamivir in distilled water. The volume was made upto 10 mL with distilled water to get a concentration of 1000 mcg/mL. This was further diluted to get the working standard solution of 20 mcg/mL for method **A** and 100 mcg/mL for method **B** and **C**.

Assay procedure

Method A: Aliquots of standard drug solution of oseltamivir 0.5-3.5 mL (20 mcg/mL) were taken and transferred into series of 10 mL graduated test tubes. To each test tube 2 mL of methanolic *p*-dimethylamino cinnamaldehyde (3 % w/v) (PDAC) and 0.5 mL of H_2SO_4 (0.1 N) were added. After thoroughly shaking, the test tubes were set aside for 10 min, for the completion of the reaction. The volumes

in each test tube were adjusted to 10 mL with methanol. The absorbances of the solutions were measured at 530 nm against reagent blank and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured and the amount of oseltamivir was determined by referring to the calibration curve.

Method B: Aliquots of standard solutions of oseltamivir containing 0.5-5.0 mL (100 µg/mL) were transferred into series of 10 mL graduated test tubes, 1 mL of 4-AP (0.5 % w/v) and 1 mL of sodium periodate (0.5 % w/v) were added to each test tube. The volume was made up to 10 mL with distilled water. The absorbance of the violet coloured species was measured at 545 nm against reagent blank. The coloured species is stable for 0.5 h. The amount of oseltamivir present in the sample solution was computed from its calibration curve.

Method C: Aliquots of standard drug solution of oseltamivir containing 0.1-1.5 mL (100 mcg/mL) were taken and transferred into series of graduated test tubes. To each test tube 2 mL of ferric chloride (0.03 M) and 2 mL of 1,10-phenanthroline (0.2 M) and 0.2 mL of orthophosphoric acid were added. The test tubes were allowed to stand in water bath at 70 ºC for 20 min. The test tubes were then cooled to room temperature and the solutions were made upto 10 mL with distilled water. The absorbance of the red coloured chromogen was measured at 512 nm against reagent blank and a calibration curve was constructed. The absorbance of the sample solution was measured and the amount of oseltamivir was determined by referring to the calibration curve.

The methods were extended for the determination of oseltamivir from tablet formulations. Twenty tablets of oseltamivir (Fluvir 75 mg, Hetero Drugs) were accurately weighed and powdered. Tablet powder equivalent to 100 mg of oseltamivir was dissolved in 50 mL of distilled water, sonicated for 15 min, filtered and washed with distilled water. The filtrate and washings were combined and the final volume was made to 100 mL with distilled water for all the three methods. The solution was suitably diluted and analyzed as given under the assay procedure for bulk samples.

The analysis procedure was repeated three times with tablet formulations and the results of analysis are shown in Table-2.

Recovery studies: To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The percentage recoveries thus obtained were given in Table-2.

RESULTS AND DISCUSSION

In the present study, the method **A** involves quantitative reaction of the drug with PDAC reagent. The reaction is based on the condensation of oseltamivir with methanolic *p*-dimethylaminocinnamaldehyde, in acidic media thereby producing orange red coloured chromogen with maximum absorbance of 530 nm. Stability

study of the developed chromogen was carried out by measuring the absorbance values at time intervals 15 min for 3 h and it was found to be stable for more than 3 h at room temperature. The linearity was found to be in the concentration range of 1-7 mcg/mL.

The method **B** is based on the reaction between 4-aminophenazone (4-AP) and oseltamivir in the presence of sodium periodate results on resulting in formation of violet coloured antipyrine dye, with absorption maximum at 545 nm. The linearity was found to be in the concentration range of 5-50 mcg/mL. The coloured chromogen was stable for 0.5 h.

The method **C** is based on the reduction of ferric chloride to ferrous form by the drug, which forms complex with 1,10-phenanthroline to yield red coloured chromogen, having absorbance maximum at 512 nm. The linearity was found to be in the concentration of 1-15 mcg/mL. The coloured chromogen was stable for 3 h.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-1. The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION DATA

Parameters	Method **A**	Method **B**	Method **C**
λ_{max} (nm)	530	545	510
Beer's law limits	1-7	5-50	1-15
Molar absorptivity (L/mol cm)	5.68×10^3	0.928×10^3	1.5×10^3
Sandell's sensitivity (mcg/cm^2/0.001 absorbance unit)	0.0750	0.8045	0.2750
Regression Equation* (Y)			
Slope (m)	0.0150	0.0020	0.0060
Intercept (c)	-0.0073	0.0110	0.0265
Correlation coefficient (r)	0.9998	0.9997	0.9995
Precision (% relative standard deviation)	0.6890	0.0720	0.0812
Standard error of estimate	0.0174	0.0265	0.0255

*Y = mX + c, where X is the concentration in mcg/mL and Y is absorbance unit.

The reproducibility and precision of the methods are very good as shown by the low values of coefficient of variance (CV). The mean percentage recovery value of 99.6 % for method **A**, 100.4 % for method **B** and 99.5 % for method **C**, indicates non-interferences from the formulation excipients. All the validated parameters are summarized in Table-2.

In conclusion, the proposed methods are simple, sensitive, accurate and economical for the routine analysis of oseltamivir in bulk and in its formulations.

TABLE-2
ASSAY OF OSELTAMIVIR IN TABLET FORMULATIONS

Tablet formulation	Labelled amount (mg)	Amount obtained (mg)* by proposed method			**% Recovery by the proposed method		
		Method A	Method B	Mehtod C	Method A	Method B	Method C
1	75	74.5	74.7	74.8	99.5	99.9	99.7
2	75	74.8	75.3	75.2	99.4	101.2	99.3
3	75	75.5	75.8	75.2	99.9	100.1	99.5

*Average of three determinations, **After spiking the sample.

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