

DEVELOPMENT AND VALIDATION OF OSELTAMIVIR PHOSPHATE IN FLUVIR® BY UV-SPECTROPHOTOMETER

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Abstract: A simple and rapid UV-spectrophotometer estimation method for the evaluation Osetamivir (Fluvir®) is used in the treatment and prophylaxis of both influenza A and influenza B has been developed and assessed. The proposed methods were successfully applied for the estimation of Osetamivir in commercial pharmaceutical preparation with UV detection at 208.5 nm. A Shimadzu 1700 U.V visible spectrophotometer with 1cm matched quartz cells, and methanol solvent were employed in the method. Developed methods obeyed the Beer's law in the concentration range of 4-24 µg/ml. methods were validated statistically. The SD of that was found 0.01125 and RSD 0.0210. Percentage recovery of the drug for the proposed method ranged from 99.32-99.97% indicating no interference of the tablet excipients. The results of the tablet analysis were validated with respect to accuracy (recovery), linearity, limit of detection and limit of quantitation were found to be satisfactory.

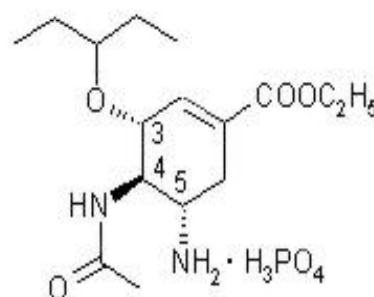
Key words: Osetamivir phosphate, UV spectrophotometry, Absorbance maxima.

Introduction

Osetamivir (Fluvir®) is an ester prodrug, which is rapidly and extensively hydrolysed in vivo to its active metabolite and extensively hydrolysed in vivo to its active metabolite influenza virus neuraminidase¹. To date there are no published methods for determination of osetamivir in Fluvir® capsules and only one published method for the determination of osetamivir in plasma using solid-phase extraction, LC-MS². There are various method for determination of Osetamivir in tamiflu like liquid chromatographic³, high performance liquid chromatography with UV detection^{5,8,15}, capillary electrophoresis⁶, Spectrofluorimetric Method⁷, colorimetric¹³, RP-HPLC¹⁴, spectrofluorimetric method^{16,17}. Osetamivir is (3R, 4R, 5S)-4-Acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate (1:1)⁴. Figure 1: Osetamivir is used

for the treatment of uncomplicated influenza infection in adults who have had symptoms for no longer than two days.⁹

Figure 1: Chemical structure of Osetamivir phosphate



Experimental

2.1. Instrumentation

A double-beam Shimadzu UV-Visible spectrophotometer, model UV-1700 with 1-cm quartz cells attached with printer of ESPON LQ 1150 II.

2.2. Materials and reagents

Pharmaceutical grade of oseltamivir phosphate from Hetero Drugs Limited Company were methanol used analytical grade from loba.chem. Capsules from Hetero Drugs Limited.

2.3. Standard solutions and calibration

Stock standard solutions of oseltamivir phosphate were prepared by accurately weigh and dissolving 10 mg in 100 ml methanol to get concentration of 100 µg/ml.

The standard solutions were prepared by dilution of the stock standard solutions with methanol (for spectrophotometric methods) to reach the concentration range of 4 µg/ml to 24 µg/ml respectively. And calibration curve was taken at 208.5nm.

2.4. Analysis of the capsule formulation:¹⁰

Twenty capsules were weighed and finely powdered weigh. A portion of the powder equivalent to about 10 mg of oseltamivir phosphate was weighed accurately, dissolved and diluted to 100 ml with methanol. The sample solution was filtered. Further dilution was carried out with methanol. The general procedures for described under calibration were followed and the concentrations of oseltamivir phosphate were calculated at 208.5nm.

3. VALIDATION:^{11, 12}

The methods were validated with respect to accuracy linearity, limit of detection (LOD) and limit of quantification (LOQ).

3.1. Accuracy (recovery test):

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for oseltamivir phosphate, by all the methods, was found in the range of 99.32-99.97(Table no.3)

3.2. Linearity:

The linearity of measurement was evaluated by analyzing different concentration of the standard

solution of oseltamivir phosphate . Beer-Lambert's concentration range was found to be 4-24 µg/ml.

3.3. Limit of detection (LOD) and limit of quantitation (LOQ):

The LOD and LOQ of oseltamivir phosphate were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines⁵.The LOD and LOQ was found to be as in table no.1

Results and Discussion

The methods discussed in the present work provide a convenient and accurate way for analysis of oseltamivir phosphate in its pharmaceutical dosage form. Absorbance maxima of oseltamivir phosphate at 208.5 nm were selected for the analysis. Linearity for detector response was observed in the concentration range of 4-24 µg/ml. Percent label claim for oseltamivir phosphate in tablet analysis, was found close to 100 %. Standard deviation for six determinations of tablet sample, was found to be less than ± 2.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for oseltamivir phosphate, was found in the range of close to 100% and values of standard deviation was satisfactorily low indicating the accuracy of all the methods(Table no.3).

Conclusions

The developed method was found to be simple, sensitive, accurate, precise, reproducible, and can be used for routine quality control analysis of oseltamivir phosphate in bulk and pharmaceutical formulation.

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Table No.1: Optical characteristics and Other Parameters

Sr. No.	Parameters	Observation
1	λ_{\max} (nm) / wavelength range (nm)	208.5
2	Beer's-Lambert's range ($\mu\text{g/ml}$)	4-24
3	Coefficient of Correlation	0.9990
4	Slope	0.0373
5	Y - Intercept	0.003867
6	Sandell's Sensitivity ($\text{mg/cm}^2/0.001$ absorbance unit) ¹²	0.026959
7	Molar absorptivity	15222.905254
8	LOD ($\mu\text{g/ml}$)	0.342
9	LOQ ($\mu\text{g/ml}$)	1.0367

Table No.2: Result of Analysis of oseltamivir phosphate in marketed tablet formulation

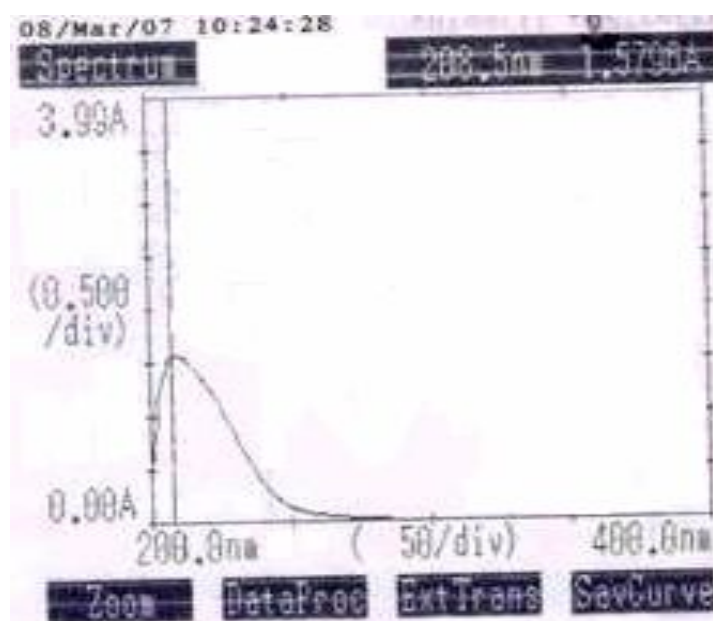
Sr.No.	Lable Claim (mg)	Amount Found* (mg)	% Estimated*	S.D.* (\pm)	R.S.D.*
1	75	75.519	101.103	0.01125	0.021069

Where, * indicates mean of six determinations.

Table No.3: Recovery study data.

Sr.No.	Lable Claim (mg)	Level of Recovery(%)	Amount Added (mg)	Amount Found*(mg)	Recovery* (%)	S.D.* (\pm)	R.S.D.*
1	75	80	60	134.96	99.97	0.3040	0.00225
2	75	100	75	149.41	99.32	0.3380	0.00226
3	75	120	90	164.73	99.83	0.2503	0.00152

Where, * indicates mean of six determinations.

Figure 2: Zero order spectra of oseltamivir phosphate

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