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Development & Validation of RP-HPLC Method for the Determination of Oseltamivir Phosphate in Bulk Drug & in Dosage

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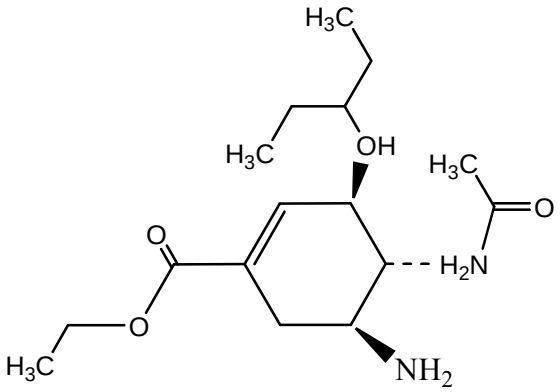
Abstract— The present study describes the development of a new, simple, reproducible RP-HPLC method for the quantitative determination of Oseltamivir phosphate (OSP). The proposed method utilizes purosphere column 250mm x 4.6mm x 5.0µm at 30°C with isocratic run using Ammonium acetate buffer 6.9 P^H and Acetonitrile (60:40 v/v) at a flow rate of 1ml/min. and UV detection at 220 nm. The method was statistically validated for linearity, ruggedness, robustness, precision, and accuracy. The calibration curve was obtained in the concentration range of 1.5-12µg/ml with correlation coefficient 0.999. The robustness of the method has been studied by slightly varying the chromatographic conditions. The ruggedness of the method was determined by carrying out the experiments on two different instruments like Shimadzu-Prominence and Agillent-1100. It was observed that there were no marked changes in the chromatograms, this shows the developed method is rugged and robust. The precision of the method was determined by inter-day and intraday variation studies having RSD values less than 1% showing high precision of the method. The tailing factor was found to be 1.34. Due to its simplicity, accuracy, high precision the proposed method may be used to determine Oseltamivir phosphate in bulk drug as well in the pharmaceutical formulations.

Keywords : Oseltamivir phosphate (OSP), RP-HPLC

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. The literature survey reveals that there are some reported analytical methods like colourimetric method[4,] micellar electrokinetic chromatographic method[5],and one HPLC-Mass spectrometric[6, 7] assay in plasma and urine. 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[8].

The aim of the present work is to develop a simple, accurate, highly precise, economical, and rapid RP-HPLC method 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.



Chemical structure of Oseltamivir

MATERIALS AND METHODS

Materials and Chemical : Acetonitrile is of HPLC grade was procured from Merck Specialties Pvt. Ltd. Mumbai, Ammonium acetate from S.D Fine chemicals Ltd, Mumbai. Pure sample of OSP was provided by Cipla Pvt.Ltd, Goa as gift sample.

Equipment :

Shimadzu prominence Isocratic HPLC system with LC 20AT pump, SPD-20A Detector, Spinchrome CFR software.

Chromatographic conditions:

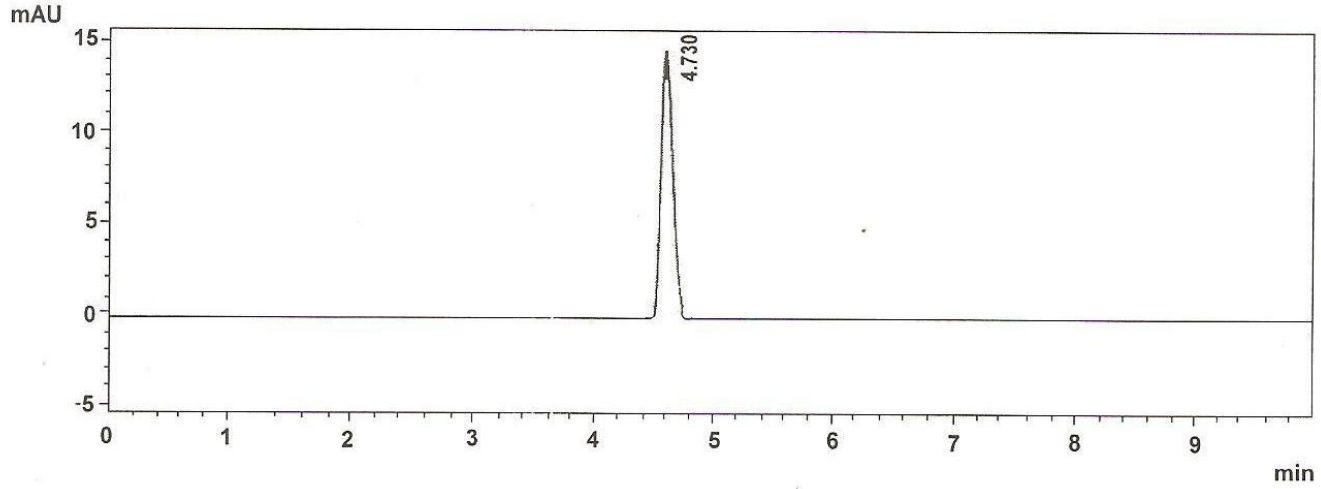
Mobile Phase	: Ammonium Acetate buffer pH6.9 and acetonitrile (60:40% v/v)
Column	: C_{18} Column (Purosphere column) 250mm X 4.6mm with 5µm particle size
Flow rate	: 1.0 ml/min
Column Temp	: 30°C
Program	: Isocratic
Wavelength	: 220 nm
Run time	: 10 mins
Sample Volume	: 10µl

Procedure:

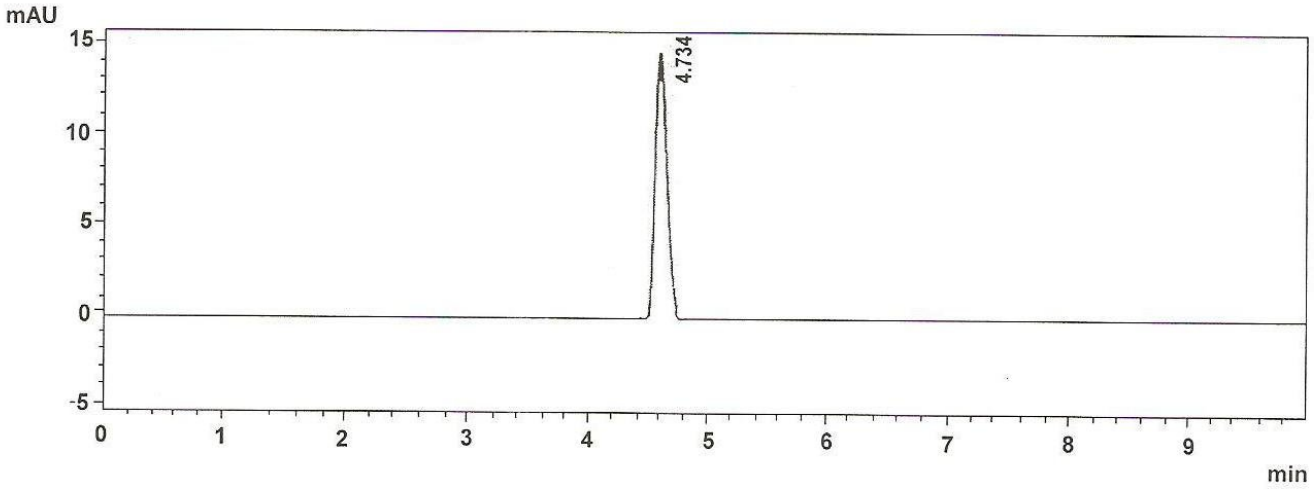
Preparation of Diluent: **7.7g** of ammonium acetate is weighed accurately and dissolved in about 750ml of water then make up the volume to 1000ml with distilled water. Add 0.1 ml of triethylamine and the solution was sonicated for 15min. Degas the buffer solution by passing through 0.45 mμ membrane.

Preparation of Standard Solution : About 50mg of OSP is weighed accurately and dissolved in 40ml pf diluent in 50ml volumetric flask, volume is made up to the mark with diluent. About 10ml of this solution is taken in 100ml volumetric flask and the volume is made up to the mark with diluent (2^{nd} stock). From this 2^{nd} stock solution subsequent dilutions were made to get concentration ranging from 1.5- 12μg/ml. 10 μl of the standard solution prepared as above at each concentration level, were injected 3times into the column at a flow rate of 1ml/min. The peak area of the drug was calculated.

Assay of the Formulation : 20 Capsules of OSP each containing 75mg of OSP were taken and the powder was mixed. An accurately weighed portion of the powder equivalent to 100mg was taken and diluted up to 100ml with the diluent. 10ml of this solution is taken into 100ml volumetric flask and diluted up to the mark with the diluent.(2^{nd} stock) Now 1ml of the sample solution from 2^{nd} stock is taken in a 10 ml volumetric flask and the volume is made up to the mark with diluent i.e.(10μg/ml) Solution. The mean of peak area of drug solution for n=6 were calculated and the drug content in the tablet was quantified by using the regression equation. The same procedure was followed for the estimation of OSP in other pharmaceutical formulations.



Typical Chromatogram of Standard Oseltamivir Phosphate



Typical Chromatogram of Sample Oseltamivir Phosphate

RESULTS

As per the ICH guidelines the method validation parameters checked were linearity, precision, LOQ, LOD, accuracy, ruggedness and robustness.

Linearity: The method was linear in the range of 1.5-12µg/ml

Precision: Precision was demonstrated by Interday and intraday variation studies. In the intraday and Interday studies the solutions were injected 6times and %RSD was calculated which was found to be less than 1%. From the data obtained the RP-HPLC method appeared precise.

Accuracy: The accuracy of the method was determined by adding known quantities of drug to previously analysed formulations and reanalysed by the proposed method. The accuracy of the method was supported by high recovery values obtained from developed method.

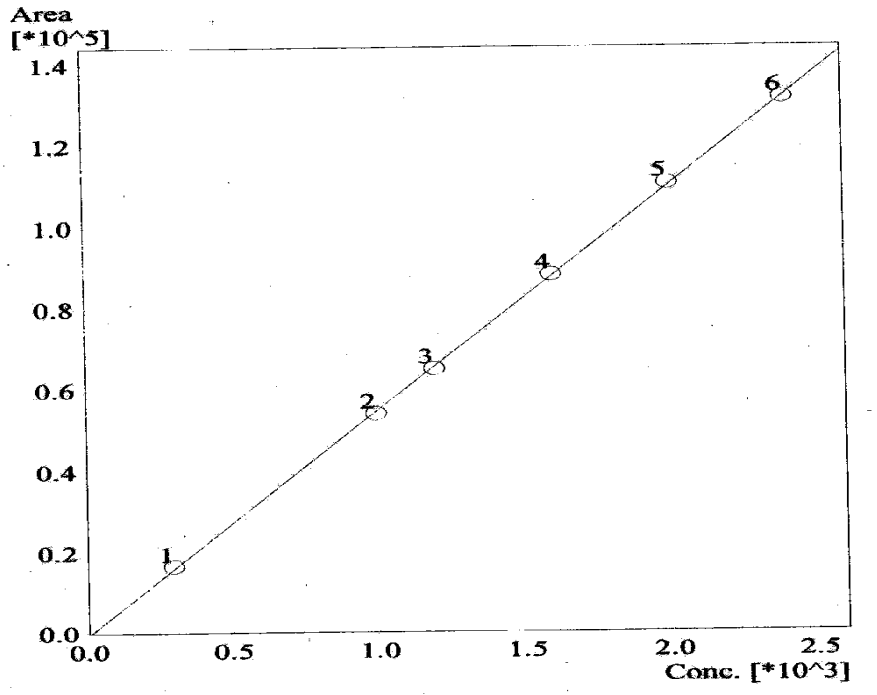
LOD and LOQ: LOD and LOQ was determined for the proposed method. Mean for the six(6) readings were taken for the same concentration and the limit of detection was found to be 0.495 µg/ml and quatitation limit was 1.5µg/ml, this shows the sensitivity of the method.

Robustness: Robustness of the method was observed by making slight deliberate changes in the chromatographic conditions like variation in the pH of mobile phase (i.e.6.8 and 7.0) and also by varying the flow rate (i.e. 0.9ml/min and 1.1ml/min). It was observed that there were no marked changes in chromatograms, which demonstrate the robustness of the method.

Ruggedness Studies: were carried out with agillent-1100 and Shimadzu Prominence , %RSD was calculated for six readings and was found to be 1.306 and 0.260 respectively.

CONCLUSION

The present developed RP-HPLC method is sensitive, precise, economical and rapid. The retention time was 4.897min. Whereas the run time was set for 10min. the method was used for the quantitative estimation of two different samples and the %age purity was found to be 99.66% and 99.78%. Hence the proposed RP-HPLC method can be conveniently adopted for the routine analysis and quality control of the drug.



I - Calibration of HPLC method for the estimation of Oseltamivir Phosphate.

Concentration of OSP (µg/ml)	Peak area	CV (%)
1) 1.5	16518	1.72

2) 5	53962	1.14
3) 6	64925	0.712
4) 8	87954	0.17
5) 10	110357	0.45
6) 12	131248	0.28

II - Inter and intraday precision for Oseltamivir Phosphate assay in Pharmaceutical dosage form.

OSP. Concentration (µg/ml)	Concentration of OSP found			
	Intraday		Interday	
	Mean (n=6)	CV %	Mean (n=6)	CV %
05µg/ml	54725	0.9993	53260	0.762
10µg/ml	110626	0.3080	110677.833	0.3161

III - Recovery studies for Oseltamivir Phosphate using proposed RP-HPLC method.

% Concentration at Specification level	Area	Amount Added	Amount found	% Recovery	Mean recovery
62.5%	53962	5µg	5.04	100.81	100.2%
100%	87787	8µg	7.98	99.75	
125%	110848	10µg	10.04	100.44	
150%	131122	12µg	11.97	99.8	

IV - Mean (S.D) amount of Oseltamivir Phosphate in tablet dosage form by proposed HPLC method.

Tablet	Labelled amount of drug (mg)	Mean amount found (n=6)	Mean % purity
T_1	75mg	74.835	99.78
T_2	75mg	74.74	99.66

T_1 = Fluvir (Hetero drugs) T_2 = Tamiflu (Cipla)

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