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Development & Validation of RP-HPLC Method for the Determination of Oseltamivir Phosphate API

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Abstract

A simple and rapid liquid chromatographic assay for the evaluation of potentially counterfeit oseltamivir phosphate has been developed and assessed. 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 C-18 column 100mm x 4.6mm at 25◦C with isocratic run using Methanol and Acetonitrile (50:50 v/v) at a flow rate of 1ml/min. and UV detection at 227 nm. The method was statistically validated for linearity, ruggedness, robustness, precision, and accuracy. The calibration curve was obtained in the concentration range of 5-35µg/ml with correlation coefficient 0.996. The robustness of the method has been studied by slightly varying the chromatographic conditions.

Keywords: Oseltamivir phosphate (OSP); RP-HPLC.

Introduction:

Oseltamivir phosphate is an ester prodrug, which is rapidly and extensively hydrolyzed *in-vivo* to its active metabolite Oseltamivir carboxylate, a potent and selective inhibitor of influenza virus neuraminidase [1]. It is considered the leading currently available antiviral to counter a serious epidemic or pandemic outbreak of influenza [2]. Oseltamivir is chemically Ethyl (3R, 4R, 5S)-4-acetylamino-5-amino-3-pentan- 3-cyclohexene-1-carboxylate phosphate. It has an antiviral activity. The structure of oseltamivir possesses a hydrophobic moiety (Fig. 1). 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. OSP is a prodrug that is rapidly and extensively metabolized via hepatic esterases to oseltamivir carboxylate (OC), the active form, a potent and selective inhibitor of influenza virus neuraminidase [3–4].

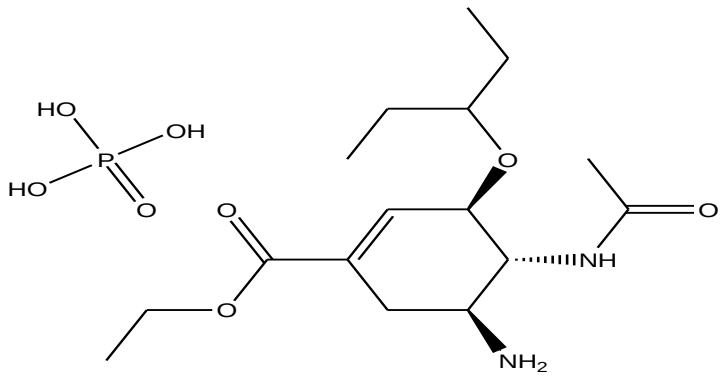


Fig1: **Chemical Structure of Oseltamivir Phosphate**

Tamiflu® will be unaffordable for many poor countries, increasing the chance of a pandemic. Generic manufacturers would fill any potential market shortages of Tamiflu® in the event of an epidemic and sell generic versions at a considerably lower price than Tamiflu® in countries where the patent is not valid. Following the potential for counterfeit copies of Tamiflu, there is a risk that medicinal products supplied may be substandard or counterfeit versions.[8]

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of OSP in Tamiflu® and generic versions. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, centrifugation, dilution; short elution time (less than 5 min) with internal standard eluted prior to OSP; short analysis time (less than 30 min); good precision (R.S.D. less than 1.5%) and high recovery (greater than 95%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH), to determination of OSP in Tamiflu® capsules and generic Tamiflu capsules has been also performed.

Summary of Reported Analytical Studies on the Drug of Oseltamivir Phosphate

S. M. Malipatil *et al*., (2011) reported RP-HPLC method for the estimation of Oseltamivir phosphate in bulk drug and in dosage form.Chromatography was carried out on purosphere column using a mixture of mobile phase i.e. Ammonium acetate buffer 6.9 pH and Acetonitrile (60:40 v/v) as the mobile phase at a flow rate of 1 mL/min with detection performed at 220 nm. The retention time was found to be (RT = 4.89 min). The detector response was linear in the concentration of 1-20 mcg/mL. The limit of detection and limit of quantification were 1.0 and 10.0 mcg/mL respectively. The percentage assay of Oseltamivir phosphate was 99.77%.

M. Espinosa Bosch *et al.*, (2010) developed the stability of Oseltamivir Phosphate in oral aqueous solutions containing the preservative sodium benzoate was studied by a stability indicating HPLC-method. The separation was achieved on RP-18 column using a gradient of mobile phase A (aqueous solution of 50 mM ammonium acetate) and mobile phase B (60% acetonitrile/40% mobile phase A), at a flow rate 0.5 mL/min. UV detection at 220 nm, and the retention time was found to be (RT = 5.0 min).

Michael D. Green *et al*., (2008) developed and validated a simple method for the determination of Oseltamivir phosphate quality by colorimetric and liquid chromatographic methods. The mobile phase contained a combination of 30% acetonitrile and 70% 0.05 M bicarbonate buffer, pH 10, at flow rate 1 mL/min to achieve component separation. Absorbance was monitored at 254 and 220 nm. Total analysis time was found to be 4 min.

Balasubramanian Narasimhan *et al.*, (2008) reported stability indicating RP-HPLC method development and validation for Oseltamivir active pharmaceutical ingredient (API). The proposed RP-HPLC method utilized Kromasil C_{18}, 5 microm, 250 mm x 4.6 mm i.d. column (at ambient temperature), gradient run (using acetonitrile and triethylamine as mobile phase), effluent flow rate was (1.0 ml/min). UV detection at 215 nm for analysis of Oseltamivir and retention time was found to be 4.2 min.

Joseph-Charles *et al*., (2007) reported development and validation of a rapid HPLC method for the determination of oseltamivir phosphate in Tamiflu and generic versions. The proposed HPLC method utilized Zorbax CN column (150 mm x 4.6mm; 5 microm), a mobile phase consisted of methanol and 0.04 M formic acid pH 3.0 (50:50, v/v) at flow rate 1.2 (mL/min) absorbance was monitored at 226 nm and the retention time was found to be 3.40-2.25 min.

A. P. Winiarski *et al*., (2007) reported preparation and stability of extemporaneous oral liquid formulation of Oseltamivir using commercially available capsules. RP C18 column (250 x 4.6mm, 5 µm) was used. The ratio of mobile phase was 0.075 mol/L potassium phosphate monobasic solution (pH-3): methanol 89:11 (v/v). UV detection was performed at 215nm. The retention time was detected at (RT = 4.3 min).

Material and Method:

Material and Chemicals:

- Acetonitrile and Methanol of HPLC grade was procured from SIGMA –ALDRICH chemicals Pvt. Ltd. Maharashtra, India.
- Oseltamivir phosphate was provided by MATRIX PHARMACEUTICALS, Hyderabad; India as a gift sample.

Equipment:

- Waters 2487 Isocratic HPLC system with 515 LC pump, SPD-20A Detector, EMPOWER -2software.

Chromatographic conditions:

- **Mobile Phase :** Methanol and Acetonitrile (50:50% v/v)
- **Column:** C18 Column (Purosphere column) 250mm X 4.6mm with 5µm particle size.
- **Flow rate :** 1.0 ml/min
- **Column Temp :** 25^{o}C
- **Program :** Isocratic
- **Wavelength :** 227 nm
- **Run time :** 1.542 mint
- **Sample Volume :** 20µl

Procedure:

Preparation of Standard Oseltamivir Phosphate Solution

Preparation of Standard Solution: Accurately weighed 10.0 mg of pure drug Oseltamivir phosphate was taken in clean, dry 10 ml volumetric flask and dissolved in 10 ml volume of mobile phase (Methanol: Acetonitrile) resulting in 1000.0 µg/ml of drug concentration (Stock solution). Now pipette out 1 ml of this solution and diluted to 100 ml with the above solvent system, resulting in 100 µg/ml of the drug concentration. Now made different working solutions of the drug (having different concentrations) by pipette out different volume from the stock solution, and make the volume of all the working solutions upto 10 ml with above mobile phase.

Assay of the Formulation: 20 capsule of OSP each containing 75 mg of OSP were taken and the powder was mixed. An accurately weighed portion of the powder to 100mg was taken and dissolved up to 100ml with the mobile phase (Methanol: Acetonitrile) i.e. called 1st stock . 10ml of this solution is taken into 100ml volumetric flask and dissolved up to the mark with the above solvent system(2nd stock) Now 1ml of the sample solution from 2nd stock is taken in a 10 ml volumetric flask and the volume is made up to the mark with above solvent system 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.

Result And Discussion

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 5-30µg/ml.

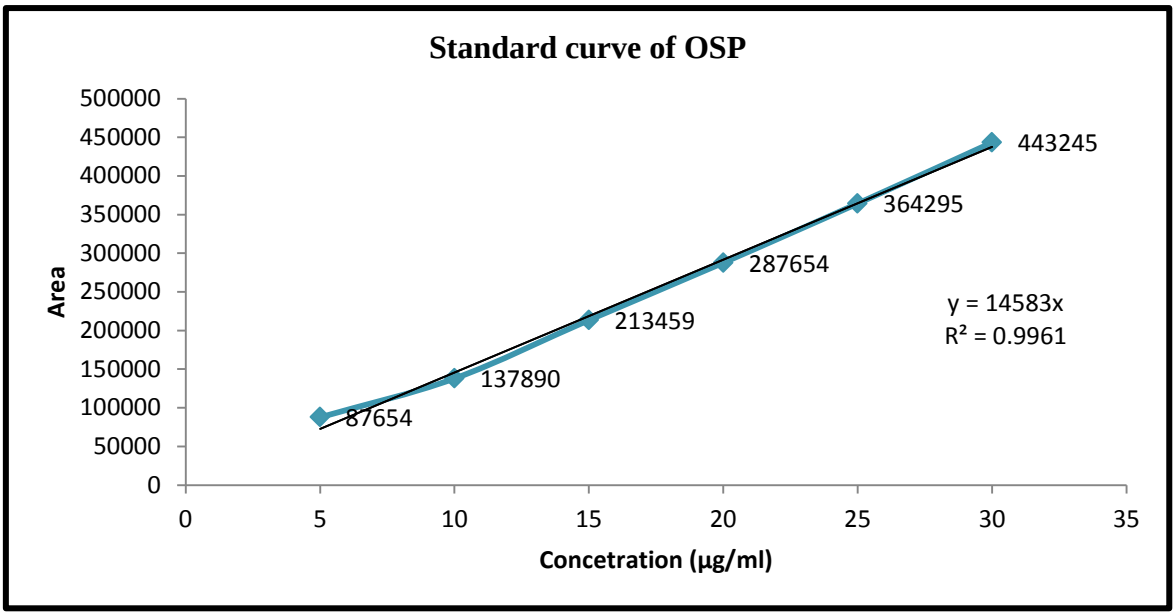
Precision: Precision was demonstrated by Interday and intraday variation studies. In the intraday and Interday studies the solutions were injected 6times and %RSD was calculated which was found to be less than 1.5%. From the data obtained the RP-HPLC method appeared precise.

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 were determined for the proposed method. Mean for the six readings were taken for the same concentration and the limit of detection was found to be 5.57µg/ml and quantitation limit was 16.90µg/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 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 Waters 2487 with the software of Empower 2 system, %RSD was calculated for six readings and was found to be 1.206 and 0.5943 respectively.



I - Standard Curve of HPLC Method for the Estimation of Oseltamivir Phosphate.

Sr. No.	Conc. (µg/mL)	Peak area
1.	5	87654
2.	10	137890
3.	15	213459
4.	20	287654
5.	25	364295
6.	30	443245

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

Level (Approx)	Standard Added (mg)	Peak Area	Standard Recovered (mg)	% Recovery	Mean Recovery	RSD
80%	40	320681	39.884	099.71	**099.92**	**0.2669**
100%	50	401334	49.915	099.83		
120%	60	483527	60.132	100.22		

Conclusion

The present developed RP-HPLC method is sensitive, precise, economical and rapid. The retention time was 1.522min. 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.

Table 1: HPLC Estimation of Oseltamivir Phosphate

Parameters	Results
Calibration Range (µg/ml)	5-30 µg/ml
LOD	5.57 µg
LOQ	16.90 µg
Regression Coefficient	0.996
Slope (Y)	14583x
System precision	1.4364
Method precision	1.2481
Retention Time	1.542 mint

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