Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 1, 2012

Research Article

# METHOD DEVELOPMENT AND VALIDATION OF OSELTAMIVIR PHOSPHATE IN BULK DRUG BY UV SPECTROSCOPY

**SANDEEP SAHU[1*], SHRUTI ACHARYA[2], AMRITA CHOURASIA[2], ANUJ ASATI[1]**

[1.] Vedica College of Pharmacy, Bhopal (M.P.), India, [2.]R.K.D.F. College of Pharmacy Bhopal (M.P.), India. Email: sandeepsahu17@gmail.com

*Received: 23 Sep 2011, Revised and Accepted: 3 Nov 2011*

**ABSTRACT**

The present research work was focused on the development of a simple, sensitive, rapid, accurate, precise and economical UV Spectrophotometric method for the estimation of Oseltamivir Phosphate in bulk and pharmaceutical dosage form which is based on the measurement of absorption maxima at 217 nm. Developed methods obeyed the Beer's law in the concentration range of 16 to 24µg/ml having line equation y = 0.015x + 0.251 with correlation coefficient of 0.999. Percentage recovery of the drug for the proposed method was from (98.76% to 98.98%), indicating no interference of the excipients. The developed method was validated with respect to linearity, precision, accuracy (recovery), limit of detection (LOD) and limit of quantitation (LOQ).

**Keywords:** Oseltamivir; Phosphate (OP); Deionised water; Absorbance maxima.

## INTRODUCTION

Oseltamivir Phosphate (OP) is an ester prodrug which is the first orally available inhibitor of influenza virus neuraminidase, an enzyme involved in the release of new virus particles from infected cells. It is used in the treatment and prophylaxis of both influenza A and influenza B [1]. The structure of Oseltamivir shows that it 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. OP is rapidly and extensively metabolized via hepatic esterases to Oseltamivir Carboxylate (OC), the active form, a potent and selective inhibitor of influenza virus neuraminidase[1,2,3]. Oseltamivir phosphate is (3R,4R,5S)-4- Acetylamino-5-amino-3-(1-ethylprop oxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate (1:1)[2]. To date there are no published methods for determination of Oseltamivir Phosphate in both bulk. For the determination of Oseltamivir there are several methods based on different techniques such as colorimetric[3], spectrofluorimetric[4], liquid chromatography with UV detection[6,7,8,9,10,11] and mass spectrophotometric detection[12,13,14,15] capillary electrophoresis [16] and micellar electrokinetic chromatography[17].

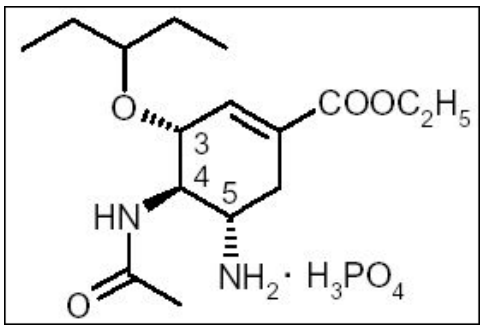


**Fig. 1: chemical structure of Oseltamivir Phosphate**

## MATERIALS AND METHODS

### Instrumentation

Double beam UV Visible Spectrophotometers, Shimadzu, Kyoto, Japan, Model UV1800 with 1cm matched quartz cells, Electronic Balance and pH Meter Sartorius, Mumbai.

### Materials and reagents

Pharmaceutical grade of OP from, Hetero Drugs Limited, Sodium hydroxide AR grade from Merck Mumbai, Hydrochloric acid AR grade RFCL New Delhi, Hydrogen peroxide AR grade Merck Mumbai, Methanol analytical grade from RFCL New Delhi, Water Milli-Q, Millipore, Mumbai, Oseltamivir Phosphate (Tamiflu) capsule was used.

### Standard solutions and calibration

The standard stock solution was prepared by weighing an accurate amount of 80 mg of OP and dissolved in 100 ml of distilled water. To get a solution of 800 µg/ml concentration, the primary stock was further diluted with distilled water. The calibration curve was prepared in the concentration range of 16 to 24 µg/ml. The absorbance was measured at 217 nm against blank solution using double beam ultraviolet spectrophotometer.

### Analysis of the capsule formulation

10 tablets OP 5 mg were accurately weighed and average weight of the tablet was calculated. Weight equivalent to 80mg was transferred to 100ml volumetric flask and made up to volume with distilled water and sonicated for 15minutes. The resultant 1mg/ml of the solution was further diluted to get a concentration of 100mcg/ml. Accurately pipetted out 2, 2.25, 2.5, 2.75 and 3ml of the above solution into 10ml volumetric flasks and the volumes were made up using distilled water. This gave sample solution having concentration 16,18,20,22 and 24mcg/ml. The absorbance of each concentration was measured at 217 nm.

### Validation

The methods were validated with respect to Linearity, Accuracy, Precision, Robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

### Linearity

The linearity was evaluated by analyzing different concentration of the standard solution of OP Beer-Lambert's concentration range was found to be 16-24(µg/ml).

### Accuracy (Recovery test)

The accuracy of an analytical procedure expresses the true value or reference value. Recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for OP by method was found in the range of 98.76%-98.98%.

### Precession

The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From these absorbance's, Mean, Standard deviation, % RSD was calculated.

**Table 1: Linearity table of Oseltamivir in Working Standard**

| S. No. | Concentration µg/ml | Absorbance |
|---|---|---|
| 1 | 16 | 0.498 |
| 2 | 18 | 0.529 |
| 3 | 20 | 0.561 |
| 4 | 22 | 0.59 |
| 5 | 24 | 0.622 |
| | Mean | 0.56 |
| | SD | 0.0437 |
| | %RS | 0.7 |
| | $R^2$ | 0.999 |

**Table 2: Accuracy Readings**

| Recovery Level | Absorbance | | Mean Absorbance | % Recovery of 80% |
|---|---|---|---|---|
| Sample | I | 0.499 | | |
| Preparation | II | 0.497 | 0.498 | 98.76% |
| (~80%) | III | 0.498 | | |
| Sample | I | 0.567 | | |
| Preparation | II | 0.565 | 0.565 | |
| (~100%) | III | 0.564 | | |
| Sample | I | 1.056 | | |
| Preparation | II | 1.057 | 1.056 | |
| | III | 1.055 | | |

**Table 3: Precision readings**

| S. No. | Sample preparation no. | Sample-I Concentration ( µg/ml) | Absorbance | Sample-II Concentration ( µg/ml) | Absorbance |
|---|---|---|---|---|---|
| 1 | 1 | 20 | 0.567 | 22 | 0.595 |
| 2 | 2 | 20 | 0.566 | 22 | 0.589 |
| 3 | 3 | 20 | 0.567 | 22 | 0.596 |
| 4 | 4 | 20 | 0.565 | 22 | 0.598 |
| 5 | 5 | 20 | 0.566 | 22 | 0.596 |
| 6 | 6 | 20 | 0.567 | 22 | 0.595 |
| | | Mean | 0.566333 | Mean | 0.594833 |
| | | S.D | 0.00748 | S.D | 0.002794 |
| | | %RSD | 0.13 | %RSD | 0.46 |

**Limit of detection (LOD) and limit of quantitation (LOQ)**

The LOD and LOQ of OP were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines[5]. The limit of detection (LOD) for OP was 0.02 µg/ml and the limit of quantification (LOQ) for OP was 0.04 µg/ml.

**RESULTS AND DISCUSSIONS**

The proposed UV method for estimation of related substances for Oseltamivir phosphate was carried out as per USP/ICH guidelines. The method was found to be specific for the sample and standard preparation. The method was found to be linear in the specified range. Accuracy of the method was also established for the drug product. The method was found to be precise and robust. LOD and LOQ established by the method are sufficient enough to quantitate the trace of impurities in the sample. Hence, this method stands validated and may be used for routine and stability sample analysis.

**REFERENCES**

1. Hill G, Cihlar T, Oo C, Ho ES, Prior K, Wiltshire H, et al. Lack of Pharmacokinetic Interaction between the Oral Anti-Influenza Prodrug Oseltamivir and Aspirin. Drug Metab Dispos 2002;30: 13–19.
2. Accinelli C, BarraCaracciolo A, Grenni P. Degradation of the antiviral drug oseltamivir-carboxylate in surface water samples. Int J Environ Anal Chem 2007;87: 579–587.
3. The International Pharmacopoeia (December 2008), 4th Edition by WHO, Document QAS/06.190/FINAL December 2008.
4. Bahrami G, Mohammadia B, Kiani A Determination of oseltamivir carboxylic acid in human serum by solid phase extraction and high performance liquid chromatography with UV detection. Journal of Chromatography B, 2008;64: 38–42.
5. Arora M, Quality Control in Pharmacy; Pharmaceutical Chemistry-Inorganic. 2nd ed. Himalaya Publication House, Mumbai. 1999, vol-1, 25-27. (8-15)
6. Backett, AH, Stenlake JB, Davidson A. Instrumental Method in the development and use of Medicines; Practical Pharmaceutical Chemistry. 4th ed. CBS Publishers and Distribution, New Delhi, 2002, vol-2, 1-8, 85-174. (8)
7. Ahuja S, Scypinski S, Crowther JB. Validation of Pharmaceutical Text Method; Handbook of Modern Pharmaceutical Analysis. Academic Press, London, 2001, 415-445.
8. Sherman, RE, Rhodes LJ. Analytical Instrumentation; Practical Guides for Measurement and Control, Instrument Society of America.1996, 647-648. (8-15)
9. Gennaro AR, Karen, BM, Medwick T. Remington ;The Science and Practice of Pharmacy, Mack Publishing Company, Pennsylvania, 19th ed., 1995,vol-1,437-490.

10. Willard HH, Merritt LL, Dean JA, Settle FA. HPLC Theory and Instrumentation; In Instrumental Methods of Analysis. 8th ed. CBS Publishers and Distributors, New Delhi. 2002, 1-12. (9-15)
11. F.G. Hayden, R.L. Atmar, M. Schilling, C. Johnson, D. Poretz, D. Paar, L. Huson, P. Ward, R.G. Mills, N. Engl. Pneumococcal and Influenza Vaccination: Current Situation and Future Prospects, J. Med. 341 (1999) 1336–1343.
12. K.G. Nicholson, F.Y. Aoki, A.D.M.E. Osterhaus, S. Trottier, O. Carewicz, C.H. Mercier, A. Rode, N. Kinnersley, P. Ward, Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial, Lancet 355 (2000) 1845–1850. (8).
13. World Health Organization, WHO Media Centre, Counterfeit medicines, Fact sheet No. 275, Revised February 2006, Available from http://www.who.int/mediacentre/factsheets /fs275/en/.
14. W. Li, P.A. Escarpe, E.J. Eisenberg, K.C. Cundy, C. Sweet, K.J. Jakeman, J. Merson,W. Lew, M.Williams, L. Zhang, C.U. Kim, N. Bischofberger, M.S. Chen, D.B. Mendel, Antimicrob. Agents Chemother: 42 (1998) 647–653.
15. H. Wiltshire, B. Wiltshire, A. Citron, T. Clarke, C. Serpe, D. Gray, W. Herron, Development of a high-performance liquid chromatographic–mass spectrometric assay for the specific and sensitive quantification of Ro 64-0802, an anti-influenza drug, and its pro-drug, oseltamivir, in human and animal plasma and urine J. Chromatogr. B: Biomed. Sci. Appl.745 (2000) 373–388. (16-20)
16. N. Lindegardh, T.T. Hien, J. Farrar, P. Singhasivanon, N.J. White, N.P.J. Day, A simple and rapid liquid chromatographic assay for evaluation of potentially counterfeit Tamiflu, J. Pharm. Biomed. Anal. 42 (2006) 430–433. (16-20)
17. International Conference on Harmonisation, ICH Guidelines Q2(R1): Validation of Analytical Procedures, Text and Methodology Ref CPMP/ICH/381/95, Available from URL: http://www.ich.org/cache/ compo/276-254-1.html.