African Journal of Basic & Applied Sciences 3 (6): 323-328, 2011
ISSN 2079-2034

Dissolution Assessment and HPLC Method Development and Validation of Oseltamivir Phosphate in Pharmaceutical Formulation

[1]M.C. Sharma and [2]S. Sharma

[1]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India
[2]Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya,
Bhind (M.P) 477001, India

Abstract: A simple and precise RP-HPLC method was developed and validated for the determination of Oseltamivir Phosphate in pharmaceutical dosage forms. Chromatography was carried out using Princeton Luna C_{18} (5 mm, 25 cm 4.6 mm i.d.) Phenomenex, USA, at ambient temperature. Mobile phase consists of Sodium Acetate buffer, acetonitrile in the ratio (55:45) as the mobile phase at a flow rate 1.5 ml/min. The analyte was monitored using UV detector at 231 nm. The Retention time of the drug was 6.4min for Oseltamivir Phosphate. The proposed method was found to have linearity in the concentration range of 5-35 µg/ml with correlation coefficient of r2=0.9995. The developed method has been statistically validated and found simple and accurate. Ambroxol was used as an internal standard. The mean recoveries obtained for Oseltamivir Phosphate were in the range 100.09-103.11%. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Oseltamivir Phosphate in bulk and dosage forms.

Key words: Oseltamivir Phosphate % RP-HPLC % Acetonitrile % Validation

INTRODUCTION

Oseltamivir Phosphate is phosphoric acid salt of ethyl (3*R*, 4R, 5S)-4-(acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate, ethyl ester, phosphate (1:1) [1] (Figure-1) $C_{16}H_{28}N_2O_4$, H_3PO_4 and Mol. Wt. 410.4. Oseltamivir phosphate (OP) is an antiviral drug that is used in the treatment and prophylaxis of both influenza A and influenza B. Influenza A and B are responsible for nearly all influenza-associated clinical illnesses. There are various methods for determination of Oseltamivir Phosphate in tamiflu like liquid chromatographic [3], high performance liquid chromatography with UV detection [4, 5] capillary electrophoresis [6], Spectrofluorimetric Method [7], colorimetric [8], RPHPLC [9-10], HPTLC [11]. Author of the article and his research team has developed a HPLC method development in different pharmaceutical dosage forms [12-23]. In the present study But till this date no simultaneous method has been published anywhere for the simultaneous estimation of drug. So the aim of our study is to develop simple, fast, accurate and specific HPLC with UV detection method for simultaneous estimation of Oseltamivir phosphate in bulk and dosage formulations. The proposed RP-HPLC method was simple, precise, sensitive and accurate method for the determination of Oseltamivir phosphate its pharmaceutical dosage forms.

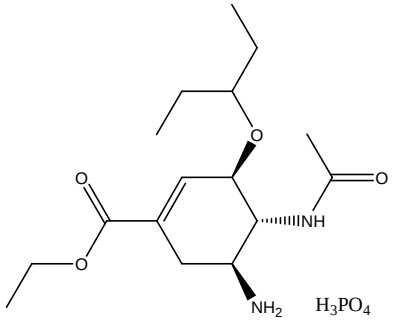

Figure: Molecular structure of Oseltamivir phosphate

MATERIALS AND METHODS

Selection of Mobile Phase: Various Mobile Phases were tried in different ratios for selection of Mobile Phase. The drug, Oseltamivir phosphate was injected with different mobile phases at different ratios with different flow rates

Corresponding Author: M.C. Sharma, School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India.

till a sharp peak, without any interference peaks containing spectrum was obtained. The different mobile phases were containing either one or the combinations of two or three of following solvents. Acetonitrile (HPLC grade). Ambroxol was used as an internal standard.

Instrumentation and Chemicals Reagents: LC system (Shimadzu LC 10AT VP HPLC) used consist of pump with universal loop injector (Rheodyne 7725) of injection capacity 20 ml. Detector consists of photodiode array detector SPD-10 AVP, Shimadzu; the reversed phase column used was Luna C_{18} (5 mm, 25 cm ´4.6 mm i.d.) Phenomenex, USA, at ambient temperature. Electro lab auto sampler dissolution apparatus were used for comparative dissolution study. Mobile phase consists of Sodium Acetate buffer, acetonitrile in the ratio (55:45). Buffers were prepared by dissolving 6.0 g of sodium acetate dissolve it in 500ml of HPLC grade water. Adjust the pH to 4.5, filter through 0.45µm nylon membrane filter and degas. The mobile phase was pumped from the solvent reservoir to the column at a flow rate 1.5 ml/min. The column was maintained at 36°C and the volume of each injection was 10µL. Prior to injection of the solutions, column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluents were monitored at 231 nm.

Standard Preparation: 100 µg/ml of Oseltamivir phosphate was prepared in acetonitrile. This solution was further diluted with acetonitrile to get a solution of concentration 1 µg/ml.

Sample Preparation: Twenty tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder, dose equivalent to 75 mg was transferred to a 50 ml volumetric flask, dissolved in working mobile phase and then the solution was made up to the mark with mobile phase and filtered through 0.45 µ membrane filters. 5 ml of this solution was pipette into 10ml volumetric flask and diluted with the mobile phase to get concentration of 500 µg/ml.

Buffer Preparation: Dissolve 2 gm of sodium dihydrogen orthophosphate in to 1000 mL of Milli Q water and adjust pH 3.0 with orthophosphoric acid. Filtered it through 0.45 µ HVLP nylon filter.

Dissolution Studies: Standard stock solutions were prepared in Diluent and dilute it further for second dilution with dissolution media and then dilute 5.0 mL of this to 10.0mL with buffer solution pH 2.9 to make final concentration Oseltamivir phosphate 50 µg and domperidone 3 µg respectively. The description of the dissolution profiles was calculated by using model-independent method [24-25]. In this study, as model-independent approaches, two fit factors were applied to the dissolution data that compare the dissolution profiles of a pair of drug product. These fit factors directly compare the difference between the percent drug dissolved per unit time for a test and reference product. The fit factors are f_1 (difference factor) and f_2.

Preparation of Calibration Graph: To prepare the calibration curve for Oseltamivir phosphate, 2, 6, 10, 14, 18 and 22 ml of the stock solution of Oseltamivir phosphate (75 µg/ml) were transferred to a series of six, 50 ml volumetric flasks. The volume in each flask was adjusted to 50 ml with mobile phase and mixed the contents to obtain a final concentration in the range of about 2 to 22 µg/ml. Regression equation and coefficient of correlation for Oseltamivir phosphate were found to be (y = 633812x-143221, r = 0.9995).

Assay of Formulation: Twenty tablets of the formulation were weighed and the average weight of one tablet was calculated. All twenty tablets were crushed and grounded to a fine powder and powder equivalent to 5mg of was weighed and transferred to 50 ml coloured volumetric flask. It was dissolved in mobile phase and filtered through membrane filter (0.45µ). This solution was suitably diluted and used for analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20µl fixed sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak area were recorded.

Method of Validation: Once the HPLC method development was over, the method was validated in terms of parameters like specificity, precision, accuracy, linearity and range, LOD, LOQ, ruggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values were calculated. The proposed HPLC method was validated as per ICH guidelines.

Accuracy: To ensure the reliability and accuracy of the method recovery studies were carried out by standard

addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by proposed method and the mean % recovery were found to be 101.21 Oseltamivir phosphates.

Stability Studies: The forced degradation studies were carried out at 70° C using 2 ml of 0.1N NaOH, 0.1N HCl, 3% H_2O_2. Volumes were made up to the mark with methanol, further aliquots were diluted with mobile phase and sample solutions were injected separately and chromatograms under stress conditions were recorded. The results showed slight difference in the percent label claim as compared with normal condition. In all the stress condition was found to be more sensitive to hydrolysis and oxidation.

Precision and Intermediate Precision: Intraday and Inter day shows the % Label claim values within limits (% R.S.D. not more than 2). The method was found to be précised. The ruggedness studies were carried out using different analyst variation.

Linearity and Range: Accurately weighed quantities of tablet content equivalent to about 80, 100 and 120% of label claim of Oseltamivir phosphate were taken and dilutions were made as described under marketed formulation. The chromatograms of the resulting solutions were recorded. The plot of AUC Vs Percent label claim was found to be linear with correlation coefficient of 0.9992 Oseltamivir phosphates.

RESULTS AND DISCUSSION

Several systematic trials were performed to optimize the Chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Oseltamivir phosphate in pharmaceutical dosage forms. This method utilizes the active analogue principle that lies at the spectroscopic method [12-23]. The present method contains mobile phase sodium acetate and acetonitrile in the ratio (55:27 v/v) which was found to be the most suitable as the chromatographic peak obtained with good shape and symmetry. Hence this method was finalized for the estimation of Oseltamivir phosphate at retention time of 12.0 min. The accuracy of the method was studied by recovery studies. Where the known standard drug was added to the assay sample. The amount present was calculated and the assay amount was reduced from it, which gives the amount recovered. This recovery study was conducted in three stages, 80,100 and 120% of the assay amount. The stability of Oseltamivir phosphate in solution was determined. The samples were checked for three days of storage and the data were compared with freshly prepared sample. The solution kept in tightly closed amber coloured flask in dark was found to be stable and the RSD values of assay were well below 2% against freshly prepared sample. The linearity graphs for the proposed assay methods were obtained over the concentration range of 5-35 µg/ml. Method of least squares analysis was carried out for getting the slope, intercept and correlation coefficient values and the results were presented. Robustness of the proposed methods was evaluated by making small changes in flow rate, buffer concentration, pH of the buffer solution, organic modifier concentration and temperature. The results were found to be not affected by these small alterations. The Mean % recovery was found to be 99.96% Oseltamivir phosphate tablet. The limit for mean recovery is 99-101%. Thus the method was found to be accurate. Injection repeatability was assessed using six determinations at 100% of the test concentration (10 µg/ml). For intra-day studies three concentrations were injected in triplicate in a day and for interday studies three concentrations were injected in triplicate for three days. % RSD of repeatability, interday and intraday precision found to be 0.307 and 0.276 for Oseltamivir phosphate proves that method is precise in nature. The specification of dissolution method is set by considering the solubility, permeability, dissolution and pharmacokinetics of the drug substance. A model-independent method was used for the comparison of *in vitro* dissolution profiles. In this study f_1 (difference factor) and f_2 (similarity factor) was calculated. The use of these factors was also recommended for dissolution profile comparisons in the FDA's guides for industry. This method dissimilarity factor (f1) was found to be 3.42 and similarity factors (f2) were found to be 66.18 for Oseltamivir phosphate respectively. The contribution of another important

Table 1: Result of assay of tablet formulation

Drug	Label claim (mg/tab) (n=5)	Amount found (mg)	% of drug content	S.D.	% COV	S.E.
OP	75	75.04	100.02	0.165	0.377	0.108

OP-Oseltamivir phosphate S.D.: Standard deviation, COV: Coefficient of variance, S.E.: Standard error

Table 2: Result from system-suitability study

Property (n=5)	OP
Rt	6.73
T_f	1.25
k'	4.08
N	6592
Rs	2.05

Rt: Retention time, T_f: Tailing factor, k': Capacity factor, N: Theoretical plates number Rs: Resolution

Table 3: Result of recovery study

		Amount added at			
Drug	Amount taken (µg mL^{-1})	%	µg mL^{-1}	% recovery	%COV
OP	75	80	16	100.10±0.08	0.876
		100	20	99.96±0.27	0.217
		120	24	100.6±0.14	0.337

OP-Oseltamivir phosphate S.D.: Standard deviation, COV: Coefficient of variance.

Table 4: Result of intra day and inter day precision, LOD and LOQ study

		Inter day precision % COV				
Drug	Intraday precision (n=3) % COV	Day 1[a]	Day 2[a]	Day 3[a]	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
OP	2.0987	1.987	1.324	0.307	1.38	2.05

[a] Mean of five determination, COV: Coefficient of variance, LOD: Limit of detection, LOQ: Limit of quantitation

Table 5: Dissolution parameters and HPLC Condition Oseltamivir phosphate

Dissolution parameters:	
Medium	Phosphate buffer pH 4.5, 0.1 N HCL buffer pH-1.2 and Water.
Volume	900 mL
Apparatus	Paddle
RPM	90
Temperature	37 ± 0.5°C
Time	90minutes

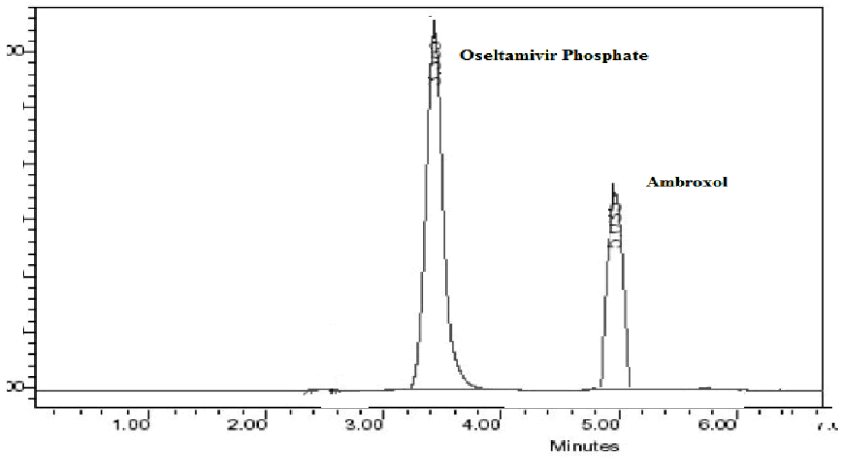

Fig. HPLC chromatograms Oseltamivir phosphate and Ambroxol (internal standard)

factor is its LOD. Dissolution testing is very important test to evaluate drug product. Ambroxol was used as an internal standard.

CONCLUSION

An RP-HPLC method has been developed for the simultaneous estimation of Oseltamivir phosphate in tablet dosage forms, using UV-detector. The developed method was validated as per ICH guidelines and specificity, linearity and range, accuracy, precision and robustness was performed. It is evident from the study that the developed method is simple, specific, precise and accurate. This newly developed method can be used for routine analysis as method for the simultaneous estimation of Oseltamivir phosphate in pharmaceutical tablet dosage forms.

ACKNOWLEDGEMENTS

The author wishes to express gratitude to Head, School of Pharmacy, Devi Ahilya Vishwavidyalaya for providing facilities to carry out the work.

REFERENCES

1. Indian pharmacopoeia., 2007, 2: 866-867.
2. http://www.medicinenet.com/oseltamivir/article.htm
3. Lindegardh, N., T.T. Hien, J. Farrar, P. Singhasivanon, N.J. White and N.P.J. Daya, 2006. A simple and rapid liquid chromatographic assay for evaluation of potentially counterfeit Tamiflu®. J. Pharm. Biomed. Anal., 42: 430-433.
4. Charles, J.J., C. Geneste, E.L. Kummer and R.H. Gheyouche, 2007. Development. Boudisb and HPLC. validation of a rapid method for the determination of oseltamivir phosphate in tamiflu® and generic versions Dubost. J. Pharm. Biomed. Anal., 44: 1008-1013.
5. Bahrami, G., M. Bahareh and K. Amir, 2008. Determination of oseltamivir carboxylic acid in human serum by solid phase extraction and high performance liquid chromatography with UV detection. J. Chromato. B., 864: 38-42.
6. Evelyne, L.K., K. Gaudin, C.J. Joseph, R. Gheyouche, H. Boudis and J.P. Dubost, 2009. Development and validation of a rapid capillary electrophoresis method for the determination of oseltamivir phosphate in Tamiflu® and generic versions. J. Pharm. Biomed. Anal., 50: 544-546.
7. Aydo™muþ, Z., 2009. Simple and Sensitive Spectrofluorimetric Method for the Determination of Oseltamivir Phosphate in Capsules through Derivatization with Fluorescamine. J. Fluoresc., 19: 673-679.
8. Green, M.D., H. Nettey and R.A. Wirtz, 2008. Determination of Oseltamivir Quality by Colorimetric and Liquid Chromatographic Methods. Emerg. Infect. Diseases., 14: 552-556.
9. Balasubramanian, N., A. Khan and S. Kona, 2008. Stability indicating RP-HPLC method development and validation for oseltamivir API. Chem. Pharm. Bull., 56(4): 413-7.
10. Nagarajan, J.S.K. and S.A. Muralidharan, 2009. Validated RP-HPLC method for estimation of Oseltamivir in pharmaceutical formulation, Der. Pharma. Lett., 1: 162-168.
11. Sharma, M.C. and S. Sharma, 2010. Development and Validation of An HPTLC Method for Determination of Oseltamivir phosphate in pharmaceutical dosage form. Indian Drugs., 47: 68-72.
12. Sharma, S., M.C. Sharma, D.V. Kohli and S.C. Chaturvedi, 2010. Isocratic Reverse Phase HPLC estimation method of Torsemide and Spironolactone in pharmaceutical combined dosage form. J. Optoel. and Biomed. Mate-Rapid Communications, 4: 234-237.
13. Sharma, S., M.C. Sharma, D.V. Kohli and S.C. Chaturvedi, 2010. Study of stressed degradation behaviour of Pioglitazone hydrochloride in bulk and pharmaceutical formulation by HPLC assay method. J. Optoel. and Biomed. Mate., 1: 17-24.
14. Sharma, S., M.C. Sharma and S.C. Chaturvedi, 2010. Development and validation of isocratic reversed-phase HPLC method for simultaneous estimation of torsemide and spironolactone in bulk and pharmaceutical combined tablet dosage form. J. Optoel. and Biomed. Mate-Rapid Communications, 4 ISS., 3: 427-430.
15. Sharma, S., M.C. Sharma and S.C. Chaturvedi, 2010. Simultaneous estimation of Rosuvastatin calcium and Ezetimibe in tablet dosage form by reverse phase high performance liquid chromatography. Optoel. and Biomed. Mate-Rapid Communications, 4: 238-241.
16. Sharma, M.C. and S. Sharma, 2011. A Facile and Isocratic RP-HPLC Method and Spectroscopy for Simultaneous estimation of Ramipril in Formulation: dissolution method. Intl. J. Chem. Tech. Res., 3(2): 992-996.

17. Sharma, M.C. and S. Sharma, 2011. Isocratic RP-HPLC Method for Simultaneous Estimation of Paracetamol and Lornoxicam in combined tablet Dosage Form and its Dissolution Assessment. Intl. J. Chem. Tech. Rese., 3(2): 997-1002.
18. Sharma, M.C. and S. Sharma, 2011. Dissolution Studies and RP-HPLC Method for the Simultaneous Determination of Satranidazole and Ofloxacin in Pharmaceutical Dosage Form. Intl. J. Chem. Tech. Rese., 3(2): 1003-1007.
19. Sharma, S., M.C. Sharma, R. Sharma and A.D. Sharma, 2011. High Performance Liquid Chromatographic Assay method for the determination of Paracetamol and Caffeine in Tablet Formulation-*in vitro dissolution studies*. J. Pharma. Res., 4(5): 1559-1561.
20. Sharma, S., M.C. Sharma and A.D. Sharma, 2011. Application to Dissolution Assessments; A Facile and Rapid procedure of Atenolol and Hydrochlorothiazide in Pharmaceutical Formulation by RP-HPLC assay Method. Drug. Invention. Today, 3(6): 100-101.
21. Sharma, S., M.C. Sharma and A.D. Sharma, 2011. Facile and Rapid Simultaneous Estimation of Dexrabeprazole and Domperidone by RP-HPLC assay Method in Combined Dosage Form-In Application *Dissolution Assessment*. Drug. Invention. Today, 3(6): 98-99.
22. Sharma, S., M.C. Sharma and A.D. Sharma, 2011. Study of stressed degradation behavior of Amlodipine Besylate and development of Reverse phase HPLC assay method. J. Pharma. Res., 4(5): 1579-1581.
23. Sharma, M.C. and S. Sharma, 2011. Stability Indicating RP-HPLC Method for Determination and Validation of Repaglinide in Pharmaceutical Dosage Form. Intern. J. Chem. Tech. Res., 3(2): 210-216.
24. Morre, J.W. and H.H. Flanner, 1996. Mathematical comparison of dissolution profiles. Pharm. Techno., 6: 64.
25. Shah, V.P., Y. Tsong, P. Sathe and R.L. Williams, 1999. Dissolution profile comparison using similarity factor, f_2. Dissolution. Technol., 6: 15.