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Development of RP-HPLC method for the estimation of Oseltamivir in pharmaceutical dosage form

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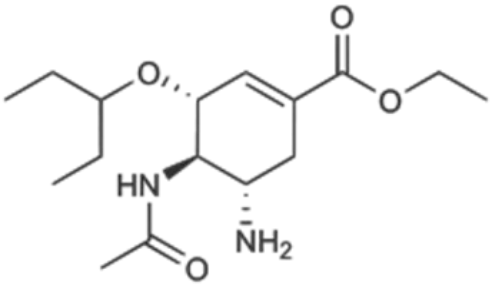
ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Oseltamivir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Luna C18 (4.6 x 250mm, 5µm) column using a mixture of Methanol and Water (75:25% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 223nm. The retention time of the Oseltamivir was 2.7 ±0.02 minutes. The method produce linear responses in the concentration range of 20-100ppm of Oseltamivir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

1. INTRODUCTION

Oseltamivir is an antiviral drug, a neuraminidase inhibitor used in the treatment and prophylaxis of both influenza A and influenza B. Oseltamivir is a prodrug (usually administered as phosphate), it is hydrolysed hepatically to the active metabolite, the free carboxylate of oseltamivir (GS4071). Like zanamivir, oseltamivir acts as a transition-state analogue inhibitor of influenza neuraminidase. It is Soluble in water (>500 mg/ml), methanol, DMSO (2 mg/ml at 25° C), and ethanol (<1 mg/ml at 25° C).



Oseltamivir

2. MATERIALS AND METHODS

HPLC used in this experiment was Waters make with Alliance 2695 separation module, Software used was Empower 2, and detector used was 996 PDA detector.

HPLC method development:

Preparation of standard solution: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.6ml of the above Oseltamivir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Validation

Preparation of mobile phase: Accurately measured 250ml (25%) of HPLC Water and 750ml (75%) of

HPLC Methanol in to a 1000ml of volumetric flask and degassed in a digital ultrasonicator for 10 minutes.

Diluent Preparation: The Mobile phase was used as the diluent.

System suitability: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of the above Oseltamivir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of the above Oseltamivir stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution: Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Oseltamivir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.6ml of Oseltamivir above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of drug solutions for linearity: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20µg/ml of Oseltamivir): Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (40µg/ml of Oseltamivir): Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (60µg/ml of Oseltamivir): Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (80µg/ml of Oseltamivir): Take 0.8ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (100µg/ml of Oseltamivir): Take 1.0ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

Preparation of Oseltamivir product solution for precision: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of the above Oseltamivir stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above Oseltamivir stock solution into a

10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.6ml of the above Oseltamivir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.9ml of the above Oseltamivir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Oseltamivir and calculate the individual recovery and mean recovery values.

Robustness: The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution: Accurately weigh and transfer 10 mg of Oseltamivir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of the above Oseltamivir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow conditions: The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

3. RESULTS AND DISCUSSION

Optimized Chromatogram (Standard):

Column	: Symmetry C18 (4.6×250mm)5µ
Column temperature	: 35ºC
Wavelength	: 223nm
Mobile phase ratio	:Methanol:Water (75:25%)V/V
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 7min

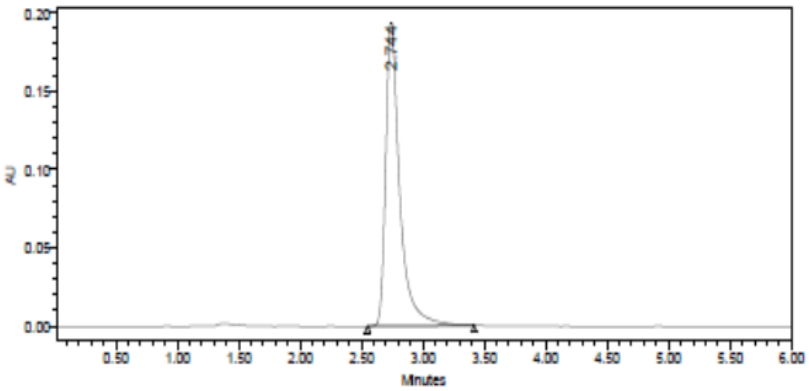


Figure.1.Optimized Chromatogram (Standard)

Table.1.Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Oseltamivir	2.744	1536490	193619	1.2	8864

Table.2.Optimized Chromatogram (Sample)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Oseltamivir	2.742	1536351	195397	1.14	7462

Optimized Chromatogram (Sample):

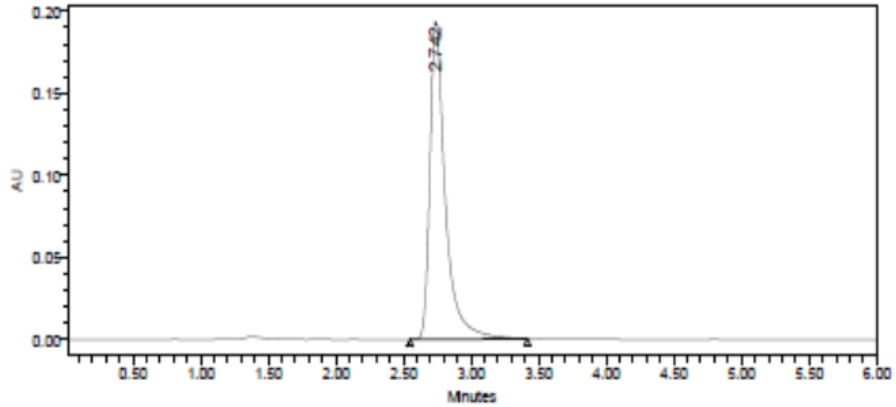


Figure.2.Optimized Chromatogram (Sample)

Acceptance criteria: Theoretical plates must be not less than 2000. Tailing factor must be not less than 0.9 and not more than 2. It was found from above data that all the system suitability parameters for developed method were within the limit.

Table.3.Results of system suitability for Oseltamivir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Oseltamivir	2.744	1536490	193619	7836	1.1
2	Oseltamivir	2.742	1536351	195397	8826	1.14
3	Oseltamivir	2.745	1539021	194759	5928	1.14
4	Oseltamivir	2.740	1539344	196639	7758	1.22
5	Oseltamivir	2.740	1540984	196731	9573	1.1
Mean			1538438			
SD			1777.251			
% RSD			0.115523			

Acceptance criteria: %RSD of five different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is suitable. It was found from above data that all the system suitability parameters for developed method were within the limit.

Table.4.Results of repeatability for Oseltamivir

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Oseltamivir	2.744	1537286	193619	8846	1.18
2	Oseltamivir	2.742	1535366	195397	7927	1.3
3	Oseltamivir	2.745	1536325	194759	7588	1.22
4	Oseltamivir	2.740	1530184	196639	6817	1.12
5	Oseltamivir	2.740	1547547	196731	9033	1.1
Mean			1537342			
SD			5662.526			
%RSD			0.368332			

Acceptance criteria: %RSD for sample should be NMT 2. The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 223nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Phenomenex Luna C_{18} because it was giving good peak. 35ºC temperature was found to be

suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Water: Methanol (25:75% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Water: Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyze gave peak around 2.7 and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 20-100ppm of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

4. CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Oseltamivir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Oseltamivir was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water. Water: Methanol (25:75% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Oseltamivir in bulk drug and in Pharmaceutical dosage forms.

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