

Original Article

UV SPECTROPHOTOMETRIC ANALYSIS AND VALIDATION OF OSELTAMIVIRPHOSPHATE IN PURE AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: A new, simple, economical, precise, sensitive, linear, accurate, rapid UV spectrophotometric method has been developed for the estimation of Oseltamivir Phosphate in pure form and pharmaceutical formulation.

Methods: This UV method was developed using Methanol as a solvent. In the present method, the wavelength selected for analysis was 218 nm. UV-Visible double beam spectrophotometer (Systronic 2201) was used to carry out spectral analysis. The ICH guidelines were used to validate the method.

Results: The method was validated for linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was found in the range of 10-50µg/ml. Accuracy was performed by using a recovery study. The amount of drug recovered was found to be in the range of 99.01-100.1%. The % RSD value was found to be less than 2.

Conclusion: The developed UV spectrophotometric method was found to be simple, economic, sensitive, easy, accurate, linear, specific and highly sensitive and can be used for routine estimation of Oseltamivir Phosphate.

Keywords: Oseltamivir Phosphate, Methanol, UV-Visible spectrophotometric method, Method validation

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INTRODUCTION

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. By using UV-Visible spectrophotometry, the amount of ultraviolet or visible radiation absorbed by an analyte in a solution is determined [1].

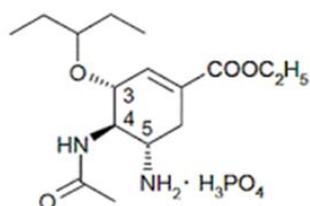


Fig. 1: Chemical structure of oseltamivir phosphate [2]

Oseltamivir Phosphate is also known as Tamiflu. Its molecular formula is C₁₆H₂₈N₂O₄. IUPAC name of Oseltamivir Phosphate is ethyl (3R, 4R, 5S)-5-amino-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (fig. 1). Oseltamivir is administered orally, it is an antiviral drug for the management of influenza A and B infections in children >1 y and adults of all ages [3]. Oseltamivir phosphate is an ethyl ester prodrug requiring ester hydrolysis for conversion to the active form, oseltamivir carboxylate. Oseltamivir carboxylate is an inhibitor of influenza virus neuraminidase affecting the release of viral particles [4]. The concentrations of oseltamivir carboxylate required for inhibition of influenza virus in cell culture were highly variable depending on the assay method used and the virus tested. Standard dose of oseltamivir in adults is 75 mg, while children have unit doses that are selected on the basis of body weight. Oral capsule (35, 40 and 75 mg) and suspension formulations are now readily available [5]. Antiviral drugs are a class of medication used specifically for treating viral infections rather than bacterial ones. Most of the antiviral drugs are available

for treating various types of viral diseases such as, HIV, influenza A and B, herpes viruses, hepatitis B and C viruses [6].

MATERIALS AND METHODS

Instruments

UV/Visible double beam spectrophotometer Systronic 2201. Standard cuvettes having 10 mm of path length are used for analysis. Ultra Sonicator (micro clean-103) was used to sonicate the formulation sample. Drug sample was weighed by using an electronic analytical balance (Shimadzu AY220).

Chemicals and reagents

Active pharmaceutical ingredient of Oseltamivir Phosphate is gifted as a sample from Zydus Cadila Healthcare Ltd, the pharmaceutical company, Vadodara, Gujarat. Marketed formulation of Oseltamivir Phosphate was procured from a local pharmacy.

Experimental work

Method development

Preparation of standard stock solution of oseltamivir phosphate

Accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask and dissolved in methanol, this was considered as a stock solution. From stock solution 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, were taken and was make up the volume to 10 ml with methanol to get respective concentrations of (10,20,30,40 and 50) µg/ml. Prepared samples were analyzed by using an ultraviolet double beam spectrophotometer at λ_{max} 218 nm.

Assay of oseltamivir phosphate capsules

Weigh 20 Capsule of Oseltamivir Phosphate equivalent to 10 mg of Oseltamivir Phosphate was weighed, transferred into 10 ml volumetric flask and dissolved in methanol. This solution was sonicated for 10 min and the final volume was made up to the mark with water. 1 ml of solution was transferred into 10 ml volumetric

flask and diluted up to 10 ml with methanol. The absorbance of this solution was measured at 218 nm.

RESULTS AND DISCUSSION

Method validation

The method was validated for several parameters like Linearity, Accuracy, Precision, Robustness, Limit of Detection (LOD), Limit of

Quantification (LOQ) and Specificity of Oseltamivir Phosphate capsule [7-10].

Linearity and range

The linear relation between absorbance and concentration of drug was evaluated using three replicates over concentration range in 10-50 µg/ml by making the replicates (table 1 and fig. 2).

Table 1: Results of linearity

| S. No. | Concentration(µg/ml) | Absorbance |
|--------|----------------------|------------|
| 1 | 10 | 0.04 |
| 2 | 20 | 0.093 |
| 3 | 30 | 0.142 |
| 4 | 40 | 0.192 |
| 5 | 50 | 0.252 |

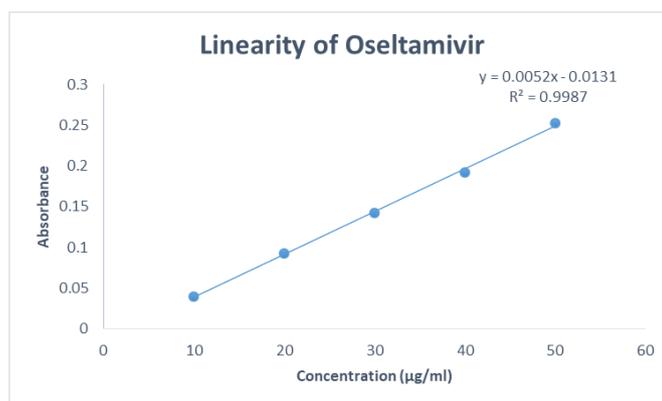


Fig. 2: Calibration curve for oseltamivir phosphate

The wavelength for linearity was scanned at 218 nm. By taking five different concentrations for linearity the regression coefficient was found to be 0.9987 i.e. in the limit of standard. Hence the linearity parameter was found to be validated.

Accuracy

The percentage recovery results for Oseltamivir Phosphate were varied from 99.01% to 100.1% at three different concentration levels that is (30, 40, 50 µg/ml), and the results were shown in table 2. Based on the % recovery data, it was concluded that the developed method is capable for the estimation of Oseltamivir Phosphate drug substance and is adequate for routine analysis.

Precision

Method precision was determined by analyzing the test solution of six determinations, and the observed values of % RSD were shown in table 3 and 4. The % RSD for Oseltamivir Phosphate in the test

solution for six formulations was not more than 2%. The solution was analyzed in 6 replicates for intra-day precision and in two successive days for inter-day precision.

Results confirmed that the precision of the method was found to be accepted. Precision results were given in table 3 and table 4 for intra and inter-day precision respectively.

Robustness

The robustness of the proposed method was performed by preparing the standard solutions and test solutions of Oseltamivir Phosphate at 100% level were analyzed by a change in wavelength for absorbance readings. The wavelength selected was ± 2 nm to the λ_{max} , i.e., 218 and 234 nm for Oseltamivir Phosphate drug for standard and sample solutions. Robustness was carried out on two different instruments and also carried out by using two different analysts (table 5).

Table 2: Results of accuracy

| Name of drug | Recovery levels | Concentration (µg/ml) | Amount recovered | % Recovery with SD |
|--------------|-----------------|-----------------------|------------------|--------------------|
| Oseltamivir | 80 % | 30 | 30.002 | 100.02±0.80 |
| | 100 % | 40 | 40.001 | 100.01±0.26 |
| | 120% | 50 | 50.004 | 100.02±0.5 |

Table 3: Results for Intra-day precision

| S. No. | Concentration (µg/ml) | Absorbance |
|--------|-----------------------|------------|
| 1 | 30 | 0.142 |
| 2 | 30 | 0.143 |
| 3 | 30 | 0.141 |
| 4 | 30 | 0.142 |
| 5 | 30 | 0.143 |
| 6 | 30 | 0.144 |
| SD | | 0.001049 |
| %RSD | | 0.736006% |

Table 4: Results for Inter-day precision

| S. No. | Concentration ($\mu\text{g/ml}$) | Absorbance (Day1) | Absorbance (Day2) |
|--------|------------------------------------|-------------------|-------------------|
| 1 | 30 | 0.142 | 0.144 |
| 2 | 30 | 0.143 | 0.143 |
| 3 | 30 | 0.141 | 0.145 |
| 4 | 30 | 0.142 | 0.142 |
| 5 | 30 | 0.143 | 0.143 |
| 6 | 30 | 0.144 | 0.144 |
| SD | | 0.001049 | 0.001049 |
| %RSD | | 0.736006% | 0.730877% |

For Intra-day and the inter-day precision relative standard deviation is in the limit.

Table 5: Results for robustness

| | 218 nm | 234 nm |
|---------------|---------------------|---------------------|
| Wavelength | 218 nm | 234 nm |
| Concentration | 25 $\mu\text{g/ml}$ | 25 $\mu\text{g/ml}$ |
| Absorbance | 0.111 | 0.113 |
| | 0.112 | 0.112 |
| | 0.114 | 0.111 |
| | 0.112 | 0.112 |
| | 0.111 | 0.111 |
| | 0.115 | 0.114 |
| SD | 0.001643 | 0.001169 |
| % RSD | 1.460593 | 1.042239 |

By change in concentration and wavelengths i.e. 218 nm and 234 nm % RSD is less than 2% i.e. within the range. So the parameter was validated.

Table 6: Results for ruggedness

| Concentration | Analyst 1 | Analyst 2 |
|-------------------------|-----------|-----------|
| 20 ($\mu\text{g/ml}$) | 0.093 | 0.092 |
| | 0.092 | 0.091 |
| | 0.094 | 0.095 |
| | 0.091 | 0.093 |
| | 0.095 | 0.094 |
| | 0.093 | 0.092 |
| SD | 0.001414 | 0.001472 |
| %RSD | 1.52066% | 1.585594% |

By change in analyst and laboratory, there is no effect on absorbance with the same conditions (table 6). Hence, the parameter was validated.

Ruggedness

The degree of reproducibility of test results of the same sample within different laboratories and different analysts under the same condition with the same concentration.

Limit of detection (LOD)

The limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected but not necessarily to be determined quantitatively under specified experimental conditions. The limit of detection, as calculated statistically for Oseltamivir Phosphate was found to be 2.08 $\mu\text{g/ml}$. The low value of Limit of detection suggests that a very low concentration of drugs can be detected.

Limit of quantitation (LOQ)

The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be quantitatively determined within an acceptable level of accuracy and precision under the stated operational conditions of the method. The limit of quantification was found to be 6.93 $\mu\text{g/ml}$. Hence, a very low concentration of drug can be quantified satisfactorily.

CONCLUSION

The developed UV spectrophotometric method was found to be simple, economic, easy, accurate, precise, linear, specific, and highly sensitive and can be used for routine estimation of Oseltamivir Phosphate.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

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