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Research Article

APPLICATION OF POTASSIUM PERMANGANATE TO THE SPECTROPHOTOMETRIC DETERMINATION OF OSELTAMIVIR PHOSPHATE IN BULK AND CAPSULES

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ABSTRACT

A new simple, sensitive, accurate, precise, selective and robust spectrophotometric method for the determination of oseltamivir phosphate in pure and in capsule dosage form is described. The method is based on the oxidation of the drug with alkaline potassium permanganate at room temperature. The absorbance of the green colored manganate ions produced is measured at 635 nm. All the experimental variables affecting the development of the manganate ions were investigated and the conditions were optimized. The calibration graph is linear in the range of 2-20 µg/ml with an excellent regression coefficient (R^2 = 0.9984). The apparent molar absorptivity and Sandell's sensitivity values are 3.980 × 10^4 L/mole/cm and 0.0103 µg/cm^2, respectively. The Limit of detection was 0.185 µg/ml and the limit of quantification was 0.564 µg/ml. The method has been successfully applied to the determination of drug in capsule dosage forms. Good recoveries were obtained. No interferences were observed from the common excipients in the capsule dosage forms. Results of the proposed method compare very favorably with those given by the reported UV spectrophotometric method. The accuracy and validity of the method were established by recovery studies via standard addition technique.

Key words: Potassium permanganate, Spectrophotometric analysis, Validation, Capsule dosage form.

INTRODUCTION

Oseltamivir Phosphate[1-6](OSP), chemically known as Ethyl (3*R*,4*R*,5*S*)-4-(acetylamino) -5-amino -3- (1-ethylpropoxy)cyclohex-1-ene-1-carboxylate dihydrogen phosphate (Fig. 1), is an oral neuraminidase inhibitor anti-viral drug approved for the treatment of Type A and B influenza in patients one year and older who have been symptomatic for no more than 2 days. OSP is a white to off-white powder freely soluble in water and in methanol. OSP requires conversion to the corresponding carboxylate, by esterases located predominantly in the liver, to be physiologically active. OSP acts as a transition-state analogue inhibitor of influenza neuraminidase and inhibits the neuraminidase protein on the viral surface and interferes with virus particle aggregation and release.

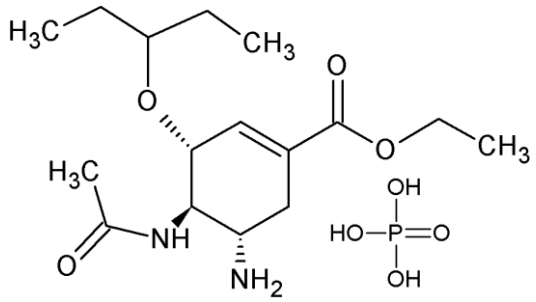

Fig. 1: Structure of oseltamivir phosphate

Different analytical techniques have been reported in the literature for the assay of OSP in bulk, pharmaceutical preparations and biological fluids. They include: Spectrofluorimetry[7], HPLC[8-12], LC-MS[13-16], Micellar electrokinetic chromatography[17], capillary electrophoresis[18], voltametry[19] and potentiometry[20]. The International Pharmacopoeia recommended HPLC and potentiometric methods for the assay of OSP[21].

Spectrophotometry is a potent analytical technique used for the determination of drugs, because of its high sensitivity and selectivity, low cost and wide availability in most of the quality control laboratories. Only few spectrophotometric methods have been reported for the determination of OSP. These methods include direct UV measurements[22], oxidative coupling with MBTH[23], formation of complex with Fe(III) chloride and potassium ferricyanide[23], formation of ion-pair associates with bromocresol green[24], bromocresol purple[24], congo red[25] and bromochlorophenol blue[25]. The reported spectrophotometric methods are insensitive, requires expensive reagent, control of pH and requires extraction step. The objective of this investigation was to develop a simple, sensitive and cost-effective viable procedure that could be used to analyze OSP in bulk drug and pharmaceutical dosage forms. The proposed method is based on the oxidation of OSP by potassium permanganate in alkaline medium resulting in formation of bluish green colored manganate ion, which exhibits maximum absorbance at 635 nm. The developed method was validated according to ICH guidelines[26].

MATERIALS AND METHODS

Instrumentation

The absorbance measurement was made on an Elico UV and Visible recording spectrophotometer (SL 159 model) with matched 1 cm quartz cells.

Reagents and materials

All chemicals used were of analytical reagent grade and all solutions were freshly prepared using double distilled water. Aqueous solutions of 0.0015 M Potassium permanganate (Merck, Mumbai, India) and 0.6 M sodium hydroxide (Merck, Mumbai, India) were prepared in double distilled water.

Pharmaceutical grade OSP was kindly gifted by a local pharmaceutical industry. A stock standard solution containing 1 mg/ml of OSP was prepared in double distilled water. Working standard solution equivalent to 100 µg/ml of OSP was obtained by appropriate dilution of stock solution with the same solvent.

Fluvir (Hetero Drugs Ltd., Hyderabad, India) capsules containing 75mg of OSP were purchased from local commercial sources.

General assay procedure

Aliquots of 0.2-2.0 ml of OSP (100 µg/ml) were pipetted out into a series of 10 ml volumetric flasks. To each flask 2.0 ml of 0.6 M NaOH followed by 2.0 ml of 0.0015 M $KMnO_4$ were added and diluted to 10 ml with double distilled water at 25±1°C. The contents of each flask were mixed well and kept aside for 15 min at room temperature. The absorbance was measured after complete color formation at 635 nm against the reagent blank. The amount of the drug present in the sample solution was computed from the corresponding calibration curve.

Procedure for analysis of capsules

The content of ten capsules each containing 75 mg of OSP was weighed. An accurately weighed quantity equivalent to 100 mg of OSP was transferred into a 100 ml calibrated flask and dissolved in 60 ml water. The solution was shaken thoroughly for about 15–20 min, diluted to the mark with water, mixed well, and filtered using a quantitative filter paper. The suitable aliquot of the filtrate was diluted to get the working concentration of 100 µg/ml OSP for analysis by the proposed method.

Procedure for analysis of placebo

A placebo blank was prepared by mixing 30 mg starch, 20 mg lactose, 20 mg acacia, 20 mg calcium gluconate, 50 mg talc and 30 mg magnesium stearate to form a homogenous mixture. From the above prepared placebo blank, 20 mg was accurately weighed and its solution was prepared as described under "Procedure for analysis of capsules" and then subjected to analysis by the proposed method.

Procedure for analysis of synthetic mixture

A synthetic mixture was prepared by adding 50 mg of pure OSP to 100 ml of the above mentioned placebo blank and the mixture was homogenized. The synthetic mixture solution was prepared by following the "Procedure for analysis of capsules", and a suitable aliquot was subjected for investigation following the general assay procedure for the determination of OSP.

RESULTS AND DISCUSSION

Method development

Potassium permanganate is a strong oxidant with an intense violet color of λ_{max} 530 nm. The oxidation of organic compounds with potassium permanganate was found to be pH-dependent. During the course of the reaction, the valency of manganese changes and the intermediate ions have been recommended to be participating oxidants. The species that are considered as potential oxidants depend on the nature of the substrate and the pH of the medium. In strong acidic medium, potassium permanganate produces the colorless Mn^{2+}, for a net transfer of 5 electrons[27]. In neutral or basic media, colorless manganese dioxide (MnO_2) is formed with corresponding net transfer of 3 electrons[28]. In strongly alkaline solution, green manganate ion (MnO_4^{2-}) is produced[29-31]. The behavior of permanganate was the basis for its uses in development of spectrophotometric method for the determination of drugs in bulk, pharmaceutical formulations and biological samples[32-34].

The results obtained in the proposed method were due to the formation of green colored manganate ions (MnO_4^{2-}), which resulted as a result of reduction of $KMnO_4$ by OSP in alkaline medium. The green colored solution formed shows maximum absorption at 635 nm against the reagent blank. Under the experimental conditions reagent blank showed a negligible absorbance at 635 nm against the distilled water (Fig. 2).

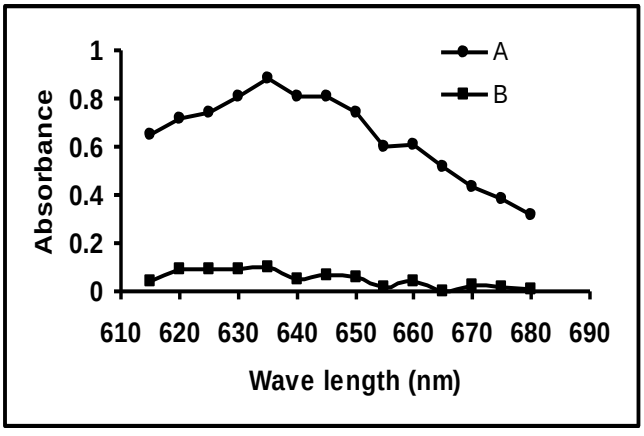

Fig. 2: (A). Absorption spectra of 10 µg/ml OSP in the presence of 0.0015 M $KMnO_4$ and 0.6 M NaOH (λ_{max}=635 nm) (B). Absorption spectra of 0.0015 M $KMnO_4$ and 0.6 M NaOH

Optimization of experimental conditions

The optimization of experimental conditions is accomplished by sequentially optimizing one variable at a time while keeping all other variables constant. In this work, the influence of concentration of reagents ($KMnO_4$ & NaOH) and time required for maximum and stable color development were studied to obtain the optimum conditions.

Effect of 0.0015 M potassium permanganate

The influence of the volume of 0.0015 M $KMnO_4$ was observed during the formation of green colored manganate ions (MnO_4^{2-}). To study this, an aliquot of OSP containing 10 µg/ml was pipetted followed by varying volumes (0.5-4.0 ml) of 0.0015 M $KMnO_4$ and 2.0 ml of 0.6 M NaOH. It is evident from Fig. 3 that the maximum absorbance was attained with 2.0 ml of 0.0015 M $KMnO_4$; above this volume the absorbance remained constant. Therefore, 2.0 ml of 0.0015 M $KMnO_4$ was used in all further measurements.

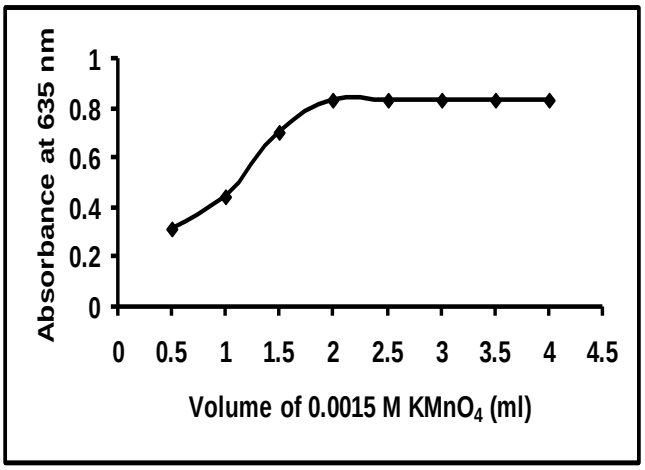

Fig. 3: Effect of the concentration of $KMnO_4$ on the formation of manganate ions (10 µg/ml OSP)

Effect of 0.6 M Sodium hydroxide

To investigate the effect of volume of 0.6 M Sodium hydroxide for green colored manganate ions (MnO_4^{2-}) development, different volumes (0.5-4.0 ml) of 0.6 M Sodium hydroxide were mixed with 1 ml of OSP (10 µg) and 2.0 ml of 0.0015 M $KMnO_4$. The results are presented in Fig. 4, which reveals that the addition of 2.0 ml of 0.6 M NaOH gave the highest absorbance, which remained constant up to 4.0 ml. Therefore, 2.0 ml of the 0.6 M NaOH was taken for the determination of the OSP throughout the experiment.

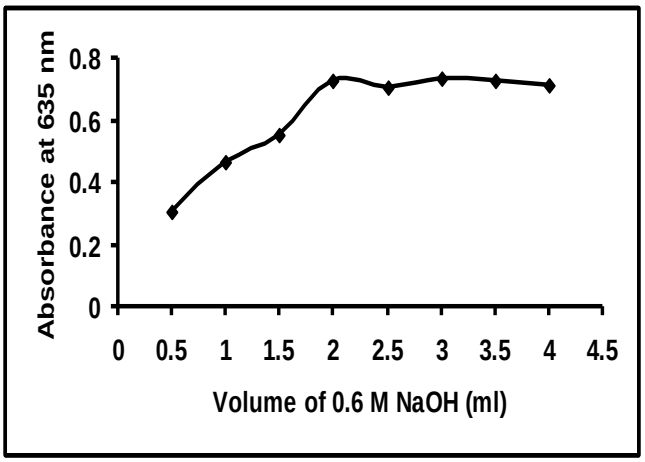

Fig. 4: Effect of the concentration of $KMnO_4$ on the formation of manganate ions (10 µg/ml OSP).

Effect of reaction time

To study the effect of reaction time for maximum color development, 1 ml of OSP (10 µg) was mixed with 2.0 ml of 0.0015 M $KMnO_4$ and 2.0 ml of 0.6 M NaOH. The contents of the mixture were kept at room temperature for varied time. The maximum intensity of color was obtained at 15 min and remained constant up to 14 hours (Fig. 5). Therefore, the optimum reaction time was fixed at 15 minutes throughout the experiment.

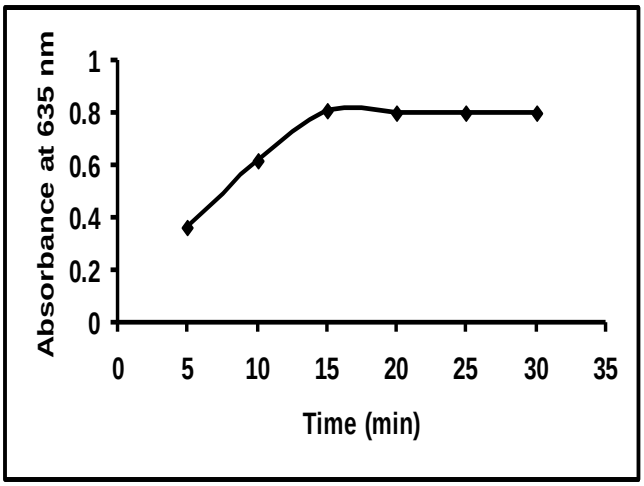

Fig. 5: Effect of time on the formation of manganate ions (10 µg/ml OSP).

Validation

The proposed method has been validated for linearity, sensitivity, precision, accuracy, recovery, selectivity and robustness.

Linearity

The linearity of the OSP was evaluated by analyzing a series of 7 concentrations (2, 4, 6, 8, 10, 15 and 20 µg/ml) of the standard solution of the OSP. The assay was performed according to the general assay procedure previously described. Under the experimental conditions, the calibration graph of the absorbance at 635 nm versus concentration of OSP was found to be linear over the range of 2-20 µg/ml for proposed method (Fig. 6). The corresponding linear regression equation and regression correlation are $y = 0.0795x + 0.0103$ and $R^2 = 0.9984$, where y is the absorbance and x is the concentration of OSP in µg/ml. The molar absorptivity and Sandell's sensitivity are 3.980×10^4 L/mole/cm and 0.0103 µg/cm^2/0.001 Absorbance units, respectively.

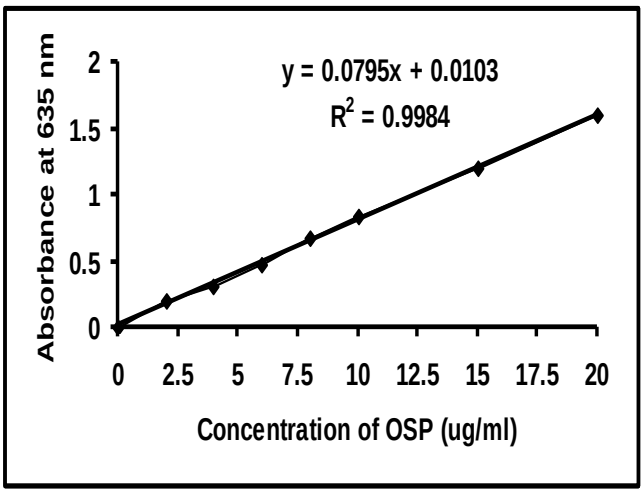

Fig. 6: Linearity of the proposed method

Sensitivity

Sensitivity of the proposed method was evaluated by calculating Limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were calculated as the ratio of 3.3 and 10 standard deviation of the blank (n=5), respectively, and the slope of the calibration line. The LOD and LOQ values were calculated to be 0.185 and 0.564 µg/ml, respectively. The values indicating high sensitivity of the proposed method.

Precision and Accuracy

The precision and accuracy of the proposed method was determined by carrying out five replicate determinations of pure OSP at three different concentration levels (5, 10, 15 µg/ml) by the proposed method. The calculated standard deviation and relative standard deviation for the proposed method showed that the precision was good (Table 1). The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage recovery. The percent recovery indicated good accuracy and an agreement between the theoretical value and the real value of concentration. The results obtained are compiled in Table 1.

Table 1: Precision and Accuracy of the proposed method.

Concentration of drug (µg/ml)		Recovery	RSD	Error
Taken	Found±SD*	(%)	(%)	(%)
5	5.02 ± 0.056	100.40	1.110	0.40
10	10.15 ± 0.023	101.50	0.226	1.50
15	15.05 ± 0.046	100.33	0.305	0.33

SD = Standard deviation
RSD = Relative standard deviation
*** = Average of five determinations**

Recovery

The accuracy and validity of the proposed method were further confirmed by performing recovery studies by standard addition method. For this purpose, a known amount of pure drug was added to pre-analyzed capsules at two different concentration levels and the nominal value of drug was determined by the proposed method. Each level was repeated five times. The mean recovery was in the range of 99.95-100.17 % with relative standard deviation in the range of 0.397-0.691 %, indicating good reproducibility of the proposed method (Table 2).

Table 2: Results of recovery studies by standard addition method

Nominal amount (mg/Capsule)	Amount of drug added (mg)	Found$ ± SD	RSD (%)	Recovery (%)
75	30	104.95± 0.726	0.691	99.95
75	60	135.24± 0.538	0.397	100.17

$ = average of five determinations
SD = Standard deviation
RSD = Relative standard deviation

Selectivity

The selectivity of the proposed method was evaluated by analyzing the placebo blank and synthetic mixture containing OSP. The absorbance values of the placebo blank extract were almost equal to the absorbance of the reagent blank which revealed no interference from the excipients.

To study the role of excipients added to the tablets, suitable aliquot of the synthetic mixture solution was assayed by the proposed method. The mean percentage recovery was found as 99.956% (n=5) with RSD of 0.2381 % demonstrated the accuracy as well as the precision of the proposed method (Table 3). This confirms the selectivity of the proposed method.

Table 3: Results of selectivity studies of the proposed method.

Concentration of OSP (mg)		Recovery (%)
Synthetic mixture	Found	
50	49.89	99.78
50	50.12	100.24
50	49.86	99.72
50	49.93	99.86
50	50.09	100.18
X= 99.956 %		
SD= ± 0. 2380		
RSD= 0.2381 %		

X = Mean percentage recovery
SD = Standard deviation
RSD = Relative standard deviation

Robustness

The robustness of an analytical method refers to its capability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability in regular analysis. Robustness test of the proposed method was performed with deliberate small changes at volume of 0.0015 M $KMnO_4$ (± 0.2 ml), volume of 0.6 M NaOH (± 0.2 ml) and reaction time (± 2 minutes). Only one parameter was changed in the each experiment. Each deliberate small change was analyzed 5 independent series containing 20 µg/ml OSP. The results (Table 4) showed good recovery (97.80–100.45%) with low relative standard deviation (0.542–1.190 %). The results indicated that the small variations in any of the variables did not significantly affect the results. Therefore the proposed method is robust to the small changes in experimental conditions.

Table 4: Results of robustness test of the proposed method

Method Parameter	Concentration of OSP (µg/ml)		Recovery (%)	RSD (%)
	Taken	Found ± SD$		
0.0015 M $KMnO_4$ (± 0.2 ml)	20	19.56±0.156	97.80	0.782
0.6 M NaOH (± 0.2 ml)	20	19.83±0.237	99.15	1.190
Reaction time (± 2 minutes)	20	20.09±0.109	100.45	0.542

$ = average of three determinations
SD = Standard deviation
RSD = Relative standard deviation

Application of the proposed method to analysis of OSP in capsules

Oseltamivir phosphate in capsules was analyzed through the procedure as explained in "Procedure for analysis of capsules". Analysis was performed under optimum conditions. Capsule was analyzed five independent determinations. The obtained results for OSP were compared with reported UV spectrophotometric method[22]. The statistical comparison of two methods was done by applying the Student's t-test and F-test for accuracy, precision respectively. The calculated t-value and F-value at 95% confidence level did not exceed the tabulated values of 2.77 and 6.39, respectively, for eight degrees of freedom. The results showed that there was no significant difference between proposed and reference UV spectrophotometric methods (Table 5) with respect to accuracy and precision.

CONCLUSION

In this study a simple, sensitive, precise, accurate, selective and robust visible spectrophotometric method for the determination of OSP in bulk and in capsule dosage forms has been developed and validated. The developed method is cheaper and simpler than spectrofluorimetric, HPLC, LC-MS, micellar electrokinetic chromatography, capillary electrophoresis, voltametric and potentiometric methods for analysis of OSP. Therefore, the proposed method can be used easily for the routine analysis of OSP in bulk and capsule dosage forms.

Table 5: Comparison of the results obtained by proposed and reference methods for the assay of capsule containing 75 mg OSP.

Capsule	Proposed method	Reference method
	õ: 75.08	õ: 74.95
Fluvir*	SD: ± 0.567	SD: ± 0.438
(75 mg OSP)	RSD: 0.755 %	RSD: 0.584 %
	R : 100.10 %	R: 99.93 %
	t- value: 1.24	F- value: 2.58

*** = Hetero Drugs Ltd., Hyderabad, India**
õ = Mean of five determinations
SD = Standard deviation
RSD = Relative standard deviation
R = Recovery

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