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Development and validation of stability-indicating spectrofluorometric method for determination of H1N1 antiviral drug (Oseltamivir phosphate) in human plasma through Hantzsch reaction.

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Abstract

Simple and sensitive spectrofluorimetric method has been described and validated for the determination of Oseltamivir phosphate (OSP) in pure form and pharmaceutical dosage forms. The method is based on the reaction of acetylacetone and formaldehyde with primary amino group of OSP through Hantzsch reaction forming a yellow fluorescent dihydropyridine derivative measured spectrofluorimetrically at 475 nm (excitation at 408 nm). At the optimum reaction conditions, the fluorescence intensity concentration plot is rectilinear over the range of 0.4-2.8 μg mL^{-1}. The lower limits of detection and quantitation were 0.08 and 0.24 μg mL^{-1}, respectively. The developed method was successfully applied for determination of OSP in its commercial capsules and suspension with average percentage recovery of 99.86 ± 1.20 and 98.36 ± 2.42, respectively (n=5) without interference from common excipients. The proposed method was utilized for in vitro determination of the cited drug in spiked human plasma, the percent mean recovery (n=3) 98.22 ± 1.27%. Furthermore, the proposed method was extended to study the stability of OSP under different stress conditions; such as hydrolysis (acidic and alkaline), oxidation (30% H_2O_2 v/v), photolysis, (As per ICH guideline Q1B option 1) and thermal degradation study. Also the developed

method was used to investigate the kinetic of the alkaline and acidic degradations of the cited drug.

Keywords: Oseltamivir phosphate**,** spiked human plasma, spectrofluorimetric, Acetylacetone, formaldehyde**,** Hantzsch reaction, degradation**.**

1. Introduction

Oseltamivir phosphate, (OSP, (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy) -1-cyclohexene-1-carboxylic acid ethyl ester phosphate), (Fig. 1) is an antiviral drug used in the treatment and prophylaxis of both Influenza virus A and B. OSP is a pro-drug that is rapidly and extensively metabolized by esterase enzyme in the gastrointestinal tract and liver to its active form, Oseltamivir carboxylate (OSC)[1-5]. Some analytical methods were reported for the analysis of OSP or its active metabolite, OSC in the biological fluids and pharmaceutical preparations. These methods include spectrophotometric[6-12], spectrofluorometric[13, 14], chromatographic[15-22], capillary electrophoretic[23], and voltammetric methods[24]. Our finding indicates that the reported spectrophotometric methods for the determination of OSP in capsule have low sensitivity[6-12]. Until now, two spectrofluorimetric methods have been published for the determination of OSP. The first method used fluorescamine[13] while the second used o-phthalaldehyde[14] as derivatizing agents. The linear ranges were 50-450 and 50-1000 ng mL^{-1} with LOD of 1.2 and 5 ng mL^{-1}, for both methods, respectively. Advantages of the proposed method (LOD and linear range were 0.081 and 0.4-2.8 μg mL^{-1}, respectively) over the previously published spectrofluorometric method include; using a cheaper reagents (acetylacetone and formaldehyde) which are widely available in most quality control laboratories. As well as, the method is rapid

compared to the second reported method which requires 45 minutes to complete the reaction.

Our target in this study is to develop a simple, rapid and sensitive spectroflourimetric method for determination of OSP in pharmaceutical dosage forms (capsules and suspension) and spiked human plasma, based on Hantzsch condensation reaction. The developed method will be extended to study the stability of OSP under different stress conditions such as, acidic, alkaline, oxidative, thermal and photolytic conditions according to ICH guidelines[25], Furthermore the kinetic of the acidic and alkaline degradations of OSP will be studied for the first time using spectrofluorimetric technique.

2. Experimental

2.1. Apparatus

A Perkin Elmer LS 45 luminescence spectrometer (United Kingdom) equipped with 150-watt xenon arc lamp and 1 cm quartz cell. Slit width for both monochromators were set at 10 nm. The spectrometer is connected to an IBM PC computer loaded with the FL WINLAB^{tm} software, Laboratory centrifuge 4000c/s (Bremsen ECCO, Germany), MLW type thermostatically controlled water bath (Memmert GmbH, Schwabach, Germany) was used for heating purposes. Digital analytical balance (AG 29, Mettler Toledo, Glattbrugg, Switzerland) and Milwaukee SM 101 pH meter Portugal.

2.2. Material and chemicals

OSP was obtained as a gift from El-Nile Chem. and Pharm. Co., (Cairo, Egypt). Hydrochloric acid, hydrogen peroxide (30 % v/v), disodium hydrogen phosphate, citric acid, and sodium hydroxide were purchased from El-Nasr Chemical Co. (Cairo,

Egypt). Tamiflu® capsule (F. Hoffmann- La Roche Ltd, Basel, Switzerland) labeled to contain 75 mg of OSP per capsule. Taminile N® suspension labeled to contain 12 mg oseltamivir base (15.768 mg oseltamivir phosphate)/mL (El-Nile Chem. and Pharm. Co., (Cairo, Egypt). Acetylacetone (99.0 %, El-Nasr Chemical Co., Egypt) was freshly prepared as 1.5 % v/v solution by mixing 2.1 mL acetyl acetone with 10 mL Mcllvaine buffer (pH 2.5)[26] and diluting to 25 mL with distilled water, and then 4.5 mL of this solution was diluted to 25 mL with distilled water. Formaldehyde (36 %, El-Nasr Chemical Co.,) was prepared as 7 % v/v solution by diluting 5 mL formaldehyde to 25 mL with distilled water. O-Phthalaldehyde (OPA) and 2-mercapto-ethanol were purchased from Sigma (St. Louis, MO, USA). Human plasma samples were kindly provided by Minia University Hospital, Minia, Egypt and were kept frozen at -20°C until assay after gentle thawing. All chemicals and reagents used were of analytical grade.

2.3. Standard solution preparation

Stock solution of OSP was prepared in 100-ml volumetric flask by dissolving 20 mg of the drug using 100 mL of distilled water. Working solution (20 μg mL^{-1}) was prepared by diluting 10 mL of the stock solution with distilled water to 100 mL. The solutions are stable for at least 15 days when kept in the refrigerator.

2.4. General Analytical procedure:

Aliquot volume of the working solution equivalent to 4-28 μg mL^{-1} of OSP was transferred into 10-mL test tube. One mL of 1.5 % v/v acetylacetone and 0.7 mL of 7% v/v formaldehyde solutions were added. The mixture was heated on a boiling water bath for 25 min. The content of the test tube was cooled, transferred quantitatively to 10-mL volumetric flask and diluted to 10 mL with distilled water.

The fluorescence intensity of the final solution was measured at 475 nm after excitation at 408 nm. Blank experiment was carried out simultaneously following the same procedure omitting the drug.

2.5. Determination of the studied drug in pharmaceutical preparations

An accurate weight of the mixed content of ten capsules or measured volume of Taminile N® suspension equivalent to 20 mg OSP was transferred into a 100-mL calibrated volumetric flask. The solution was sonicated with 25 mL of distilled water for 30 min. and then completed to volume with distilled water and filtered. The first portion of the filtrate was discarded and 2 mL of this solution was diluted to 20 mL with the same solvent. Aliquot volume in the working concentration range of 4-28 μg mL^{-1} of OSP was transferred into 10 mL volumetric flasks and analyzed as described under "general analytical procedures".

2.6. Procedures for spiked human plasma:

The authors got the permission for using plasma sample of human volunteers from Minia Hospital according to institutional guidelines. In all cases, informed written consent was obtained from all participants. Five mL of drug-free human blood sample was taken from healthy volunteers into a heparinized tube, centrifuged at 4000 rpm for 30 minute. Into 10 mL stoppered calibrated tube, 1.0 mL of the drug free plasma (supernatant) was spiked with 1.0 mL of OSP containing 40-280 μg mL^{-1}. Two mL of acetonitrile was added as precipitating agent for protein and the resultant mixture was diluted to 10.0 ml with distilled water to achieve final concentration of 4-28 μg mL^{-1}, and then centrifuged at 4000 rpm for about 20 min, and then the general procedure was followed on the obtained

supernatant. A blank value was determined by treating the OSP-free blood sample in the same manner.

2.7. Procedures for stability indicating assay:

2.7.1. Base induced degradation:

Into 10-mL volumetric flasks, 1 mL of OSP solution (0.2 mg mL^{-1}) was transferred and 1 mL of 1 M NaOH was added. The solution was left at room temperature for 0.5, 1, 1.5, 2, 2.5 and 3 hours. After the specified time, the solution was neutralized to pH 7 using 1 M HCL solution and was completed to the volume with distilled water. The solution was then filtrated and 1.2 ml from the filtrate was taken and general analytical procedure was applied to detect the remaining intact drug content.

2.7.2. Acid induced degradation:

Into 10-mL test tube, 1 mL of OSP solution (0.2 mg mL^{-1}) was transferred and 1 mL of 2 M HCl was added. The solution was heated at 80ºC for 0.5, 1, 1.5, 2, 2.5 and 3 hours. After the specified time, the solution was neutralized to pH 7 using 2 M NaOH solution. The solution was transferred quantitatively into 10-mL volumetric flask and was completed to the volume with distilled water. The solution was then filtrated and 1.2 ml from the filtrate was taken and general analytical procedure was applied to detect the remaining intact drug content.

2.7.3. Hydrogen peroxide-induced degradation:

Oxidative degradation with hydrogen peroxide was carried out by transferring 1.0 mL of OSP working solution (1.0 mg mL^{-1}) into 10 mL volumetric flask then 1 mL of hydrogen peroxide (30 % v/v) was added. The solution was kept at room temperature for 2 h. The volume was completed to the volume with distilled water. The resulting solution was spotted on silica gel $60F_{254}$ plates and developed using mobile phase

consisting of chloroform: methanol: ammonia (5:1.5:0.2 v/v/v). The spots were visualized using UV lamp at 254 nm.

2.7.4. Thermal degradation:

Ten mg of OSP powder was transferred into test tube and heated at 100 ºC for 1 h using paraffin oil bath. The degraded OSP powder was dissolved with distilled water to 100 mL. Two mL of the obtained solution was diluted to 10 mL with distilled water. The solution was then filtrated and 1.2 ml from the filtrate was taken and general analytical procedure was applied to detect the remaining intact drug content.

2.7.5. Photochemical degradation product.

The photochemical stability of OSP was studied by exposing the stock solution (20 µg mL^{-1}) to direct artificial day light for two days. The remaining drug content was analyzed using 1.2 mL solution as described under "General analytical procedure".

3. Results and discussion

Hantzsch reaction has been applied for the spectrophotometric and/or spectrofluorometric determination of several pharmaceutical compounds[27-29]. In most cases, acetylacetone and formaldehyde were reacted with the aliphatic amino group of these compounds to form colored and/or florescent product. In the present study, the primary amino group of OSP was condensed with acetylacetone and formaldehyde in a buffered solution to form a yellow fluorescent product, reaction pathway is postulated as shown in fig.2. The formed product exhibited maximum fluorescence emission at 475 nm after excitation at 408 nm. Neither the drug nor the reagents possess such fluorescence prosperity (Fig.3).

3.1. Optimization of the reaction conditions

The experimental parameters affecting the development and stability of the reaction product were investigated and optimized. Each parameter was changed individually while the others were kept constant. These parameters include; pH, concentration of acetylacetone and formaldehyde, reaction time and temperature.

Effect of pH:

The effect of the solution pH on the reaction product formation was studied in the pH range of 2.0–5.5. The fluorescence intensity of the reaction product was highly dependent on pH. The highest intensity was obtained in the pH range of 2.4-2.8. Higher or lower pH solution resulted in decrease on the fluorescence intensity. Therefore the selected pH was 2.5 using Mcllvaine buffer solution (Fig.4).

Effect of the concentration of acetylacetone

Different concentrations of acetylacetone were used in performing the general analytical procedure. Increasing acetylacetone concentration produced a gradual increase in the fluorescence intensity until reaching a steady state at 1.2-1.8 % of acetylacetone. Further increase in the concentration resulted in slight decrease in the fluorescence intensity. Consequently, 1.5 % v/v acetylacetone was chosen for subsequent works (Fig.5).

Effect of formaldehyde concentration

The fluorescence intensity increased by increasing formaldehyde concentration. The intensity reached maximum value at 4.2 % v/v and remained constant till 5.6 % v/v. Further increase in formaldehyde concentration produced a gradual decrease in the fluorescence intensity. Therefore, the chosen formaldehyde concentration was 5 % v/v (Fig 5).

Effect of heating time and temperature

After mixing the reagents together with the drug solution, the mixture was heated at different temperature. The fluorescence intensity was dependent on temperature of the reaction. Maximum fluorescence intensity was obtained at 100ºC (Fig. 6). The heating time was also investigated. As shown in Fig. 6, the fluorescence intensity increased until 20 min. and remains constant for further 10 min. Consequently, heating the reaction mixture at 100 ºC for 25 min was the most suitable condition to produce maximum fluorescence intensity.

Effect of diluting solvent

In order to select the most appropriate diluting solvent, the reaction mixture was diluted using different solvents (distilled water, ethanol, acetonitrile, methanol, acetone or DMF).The results showed that distilled water was the best solvent for dilution as it achieves the highest fluorescence intensity.

3.2. Method validation

The proposed method has been validated according to ICH guidelines[25]. The investigated parameters include: linearity and range, sensitivity, precision, accuracy, recovery and robustness

Linearity and range:

Using the optimum reaction condition, a series of solutions containing 0.4, 0.6, 1, 1.4, 2, 2.4 and 2.8 μg mL^{-1} of OSP was analyzed. Calibration curve was constructed by plotting the obtained relative fluorescence intensity at 475 nm versus the corresponding OSP concentration. The relationship between the concentration and the intensity was linear in the range of 0.4-2.8 μg mL^{-1} with correlation coefficient of 0.9990. Other analytical parameters were summarized in table 1.

Accuracy:

Standard addition method was applied for capsules and suspension to examine of the accuracy of the proposed methods. Known amounts of pure drug were added to a previously analyzed sample solution. The mixtures were re-analyzed by the proposed procedures in six replicates and the percentage recovery was calculated. The calculated high percentage average recovery and low standard deviation indicate the suitable accuracy of the method (Table 2).

Precision:

The interday and intraday precisions[25] were examined by analyzing six replicates of OSP solutions at four concentration levels (0.6, 1, 1.4 and 2 μg mL^{-1}) in seven successive days. The percent relative standard deviations of the measurements were calculated and found to be less than 1.3 % and 1.7 % for intraday and interday precisions, respectively (Table 3). The low value of RSD indicates fairly the good, repeatability and reproducibility of the proposed method.

Sensitivity

Sensitivity of the proposed method was evaluated by calculating limit of detection (LOD) and limit of quantification (LOQ) using the formula; "$x = n \sigma / S$", where x is LOD or LOQ, n is a numerical value equal to 3.3 or 10 for LOD or LOQ, respectively and σ is the standard deviation of intercept and S is the slope of calibration graph[25]. The calculated LOD and LOQ values were 0.08 and 0.24 μg mL^{-1}, respectively. The low values of these parameters indicate the high sensitivity of the proposed method.

Robustness

The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variation in method parameters and provides an indication of its reliability in routine analysis. Robustness was performed by making small changes

in general analytical procedure, such as acetylacetone (± 0.2 ml), and formaldehyde (± 0.1 ml) volumes, heating time (± 5 min.) and solution pH (± 0.2 unit). The obtained results shown in table 4 indicated that small variation in any of these parameters did not significantly affect the results as the overall calculated % RSD not exceeds 2%, therefore the proposed procedure is considered a robust one.

Specificity

The Specificity of the method was investigated by observing any interference encountered from the common capsule and suspension excipients, such as starch, talc, lactose, sodium lauryl sulphate, magnesium stearate, mannitol. This study indicates that the presence of these excipients did not interfere with the proposed method as proved by the excellent recoveries obtained, as shown in Table 5.

3.3. Stability-indicating studies

OSP was exposed to different stress conditions including acid and alkali degradation, oxidative, photo- and thermal degradations. After applying the degradation procedure, the proposed method was applied for the determination of the remaining intact drug content. The degradant was identified as oseltamivir carboxylic acid, the active metabolite of OSP (fig.1) which lack native fluorescence. This degradant was water insoluble and do not react with acetylacetone/formaldehyde reagents. As a result, the acidic and alkaline degradations were accompanied by decrease in the fluorescence intensity. Therefore our proposed method is considered selective for determination of OSP in presence of its degradation product Oseltamivir carboxylic acid. We found that fluorescence intensity not affected and no difference in fluorescence intensity

between authentic and degradation solution when making degradation of OSP with other solvent than distilled water as DMSO (solvent can dissolve the degradant).

Acid induced degradation was performed using 2 M HCL at 80 ºC while alkaline degradation was carried out using 1 M NaOH at room temperature. Both degradation procedures were performed for different times. The acidic and alkaline degradations were found to be time dependent. The apparent first order degradation rates constant were calculated according to the following equation:

$K = 2.303/t \log A^{o}/A$ and $t_{1/2} = 0.693/K$

Where K is reaction rate constant, A^{o} is initial concentration of the drug, A is the remaining concentration of the cited drug, t is the time of degradation, $t_{1/2}$ is the half life time.

The calculated rates constant were 0.26 and 0.33 K h^{-1} and half time were 2.7 and 2.1 h for acidic and alkaline degradation, respectively (table 6).

Photo-degradation was carried out by exposing the drug solution to direct sunlight for two days while thermal degradation was performed by heating the drug powder for one hour at 100ºC. It was found that OSP is stable against photolytic degradation and minor degradation was observed by thermal degradation which produced about 11 % decomposition of the drug.

Oxidative degradation was carried out using hydrogen peroxide (30.0 % v/v) for 2 hr at room temperature. Monitoring the oxidative degradation by the proposed procedure was not possible because hydrogen peroxide easily oxidize formaldehyde to formic acid. Instead, the analysis was performed using thin layer chromatography. Two spots were obtained at the TLC plate. The first one ($R_f = 0.51$) corresponds to the oxidative degradation product while the second ($R_f = 0.81$) is for the intact drug. This indicates

that the degradation product is more polar than the parent drug which is in agreement with the previously reported results[13, 22].

3.4. Application to pharmaceutical preparations

The proposed method was applied for the determination of the drug in its pharmaceutical preparations. The mean recovery values were 99.86 ± 1.20 and 98.36 ± 2.42 for capsules and suspension, respectively. The results of the proposed method was statistically compared with that of the reference method[14] regarding t- and F- tests at 95 % confidence level and this is the first time to make comparison between results of the proposed method and reference one for OSP in suspension dosage form. As shown in table 7. There is no significant difference between the results of both methods, as the calculated values is less than the tabulated one. This is an indication of the good accuracy and precision of the proposed method.

3.5. Application to spiked human plasma

The high sensitivity of the proposed method allowed the determination of the investigated OSP in spiked human plasma. Thus, it has been tested on spiked human plasma. The concentration of the investigated OSP was computed from its responding regression equations. The obtained mean recovery values of the obtained amount were 96.91 - 99.45 ± 1.41 - 2.28 as shown in table 8. It can be seen that the proposed method is suitable for the analysis of the investigated OSP in spiked human plasma.

4. Limitation

Our proposed method could not used for determination of OSP in real human plasma due to maximum peak plasma concentration(C_{max}) is lower than limit of quantitation of our proposed method[30].

5. Conclusion

The present study described a simple, rapid, accurate, reliable and validated spectrofluorimetric method for the analysis of Oseltamivir phosphate in pure form, pharmaceutical formulations and spiked human plasma. The stability indicating assay and kinetic of the alkaline and acidic degradations were studied. The proposed method is of a great value in quality control analysis of the cited drug owing to its improved simplicity, sensitivity, low-cost, and its independence on expensive instruments, or critical analytical reagents.

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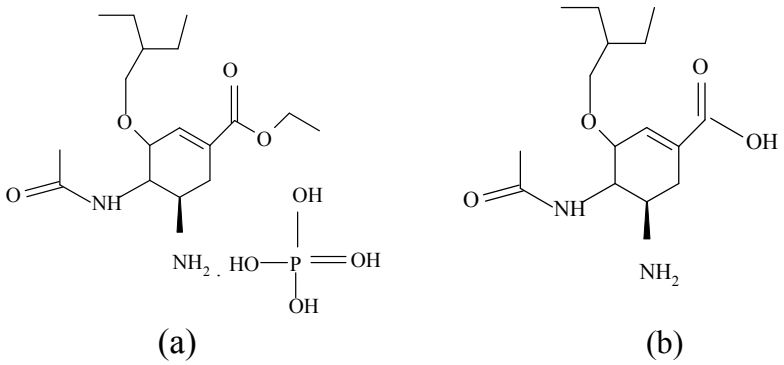

Fig.1. Chemical structure of (a) oseltamivir phosphate and (b) oseltamivir carboxylic acid.

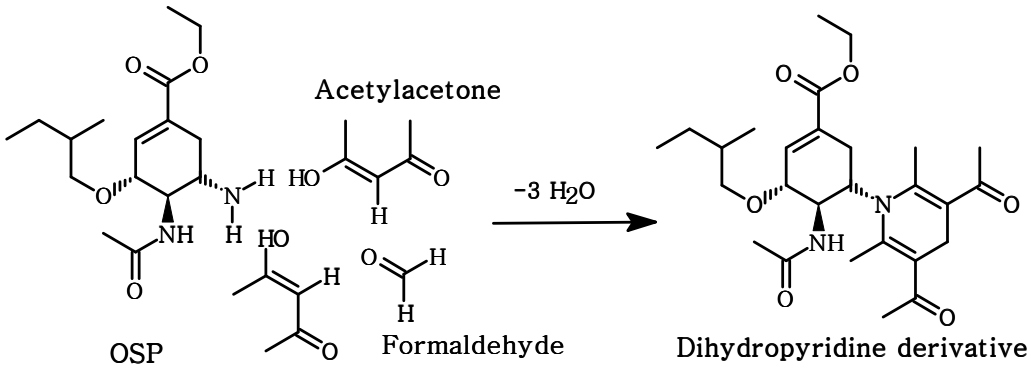

Fig.2. Suggested pathway of the reaction between the OSP and acetylacetone/formaldehyde reagent.

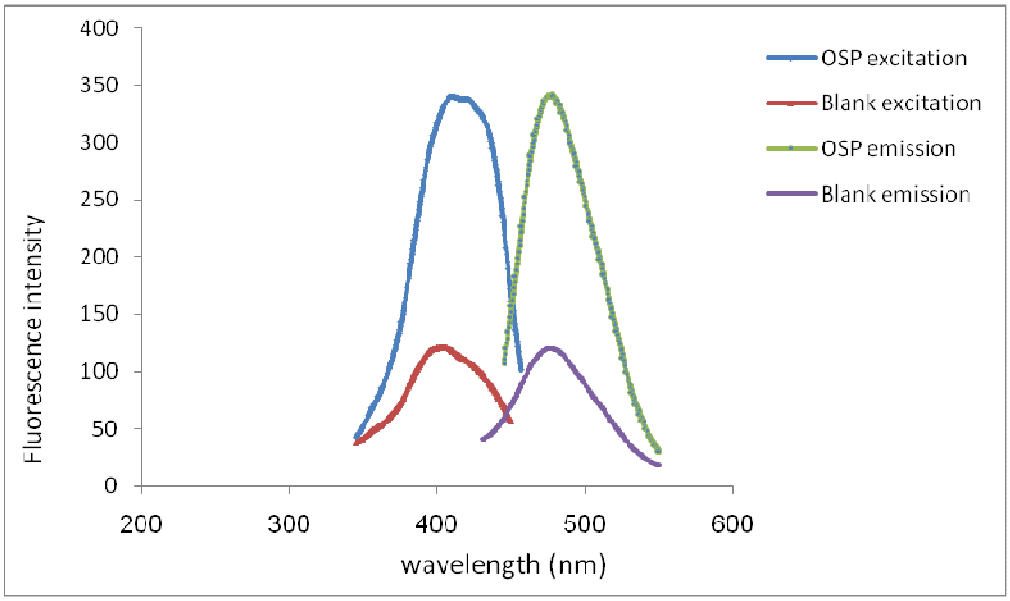

Fig. 3. Excitation and emission spectra of OSP (1.4µg/mL) after condensation with acetylacetone (1.5 % v/v) and formaldehyde (7 % v/v) at Mcllvaine buffer (pH 2.5), and after heating on boiling water bath for 25 minutes.

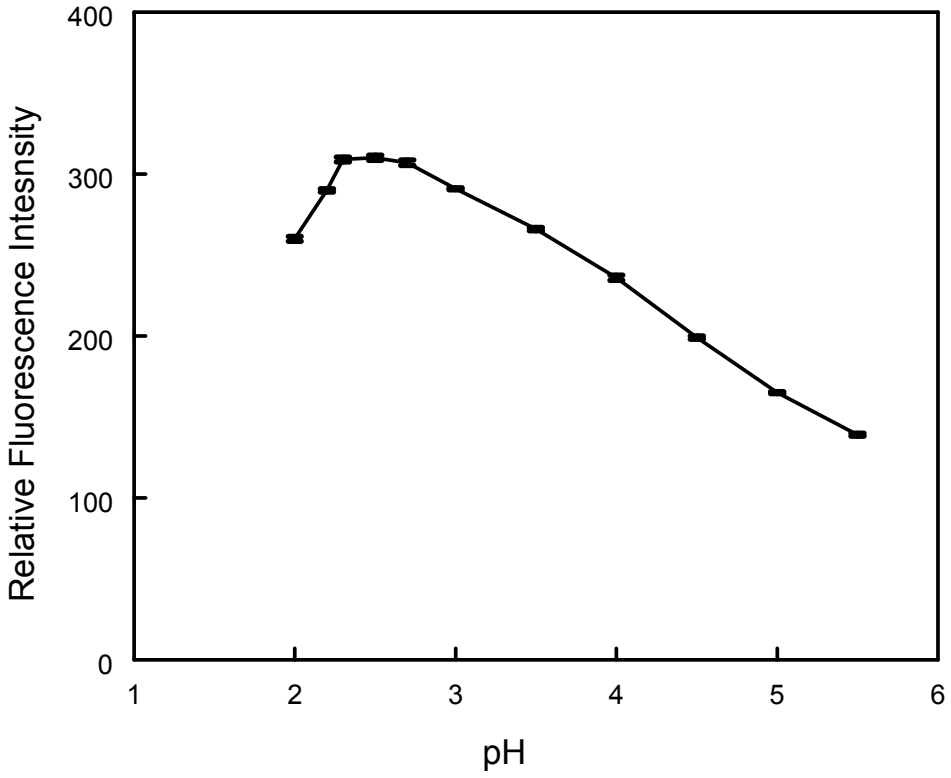

Fig. 4: Effect of the pH on the fluorescence intensity of the condensation product of OSP (1.4µg/mL) with acetylacetone (1.5 % v/v) and formaldehyde (7 % v/v), after heating in boiling water bath for 25 minutes.

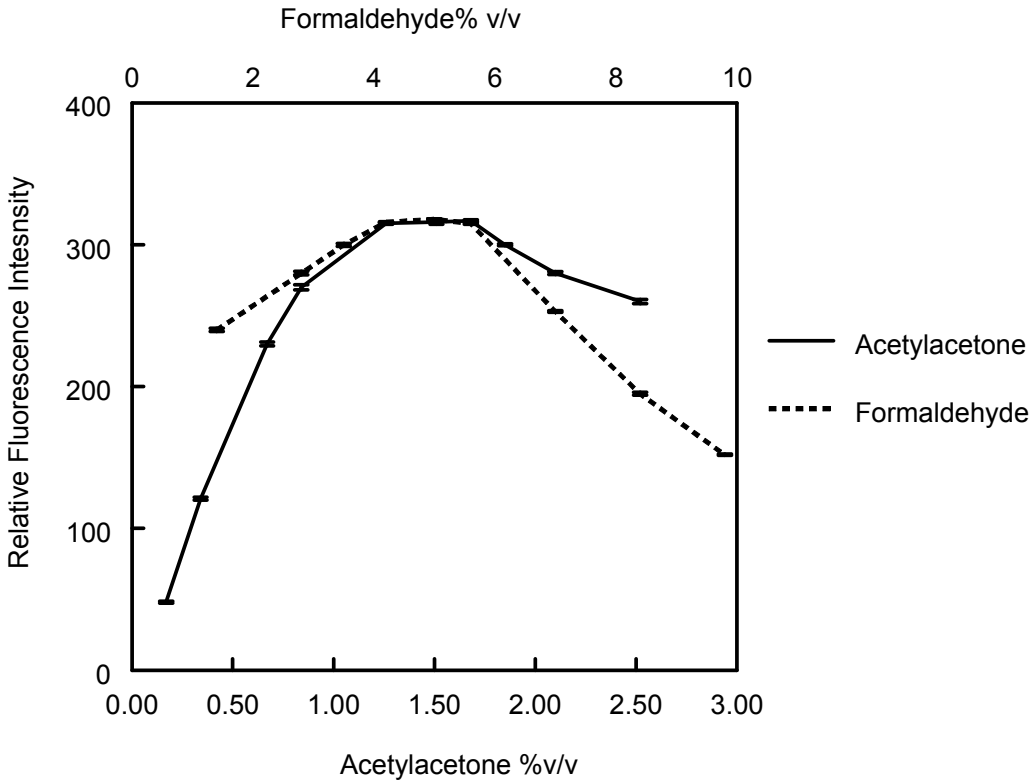

Fig. 5: Effect of the concentration of acetylacetone and formaldehyde reagents on the fluorescence intensity of the condensation product of OSP (1.4μg/mL), at Mcllvaine buffer (pH 2.5) and heating on boiling water bath for 25 minutes.

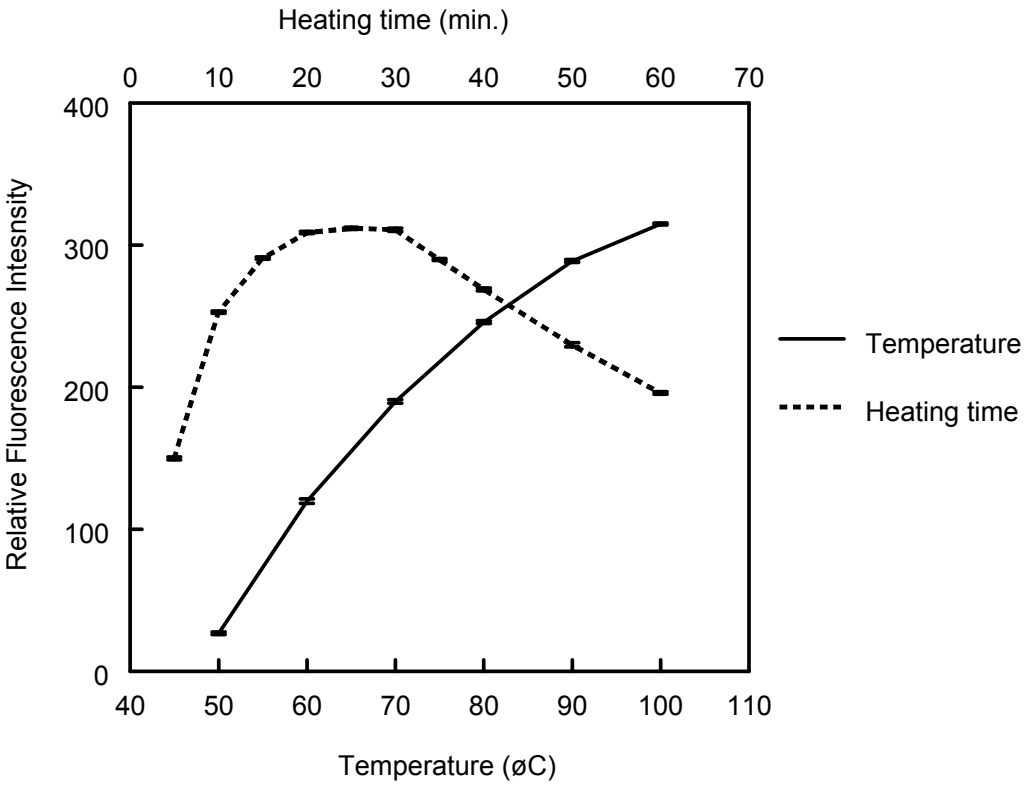

Fig. 6: Effect of the reaction temperature and heating time on the fluorescence intensity of the condensation product of OSP (1.4µg/mL) with acetylacetone (1.5 % v/v) and formaldehyde (7 % v/v), at pH 2.5 (Mcllvaine buffer).

Table 1. Regression equation and validation parameters for the proposed spectrofluorimetric method.

Parameters	Proposed method
λ_{ex}(nm)	408
λ_{em}(nm)	475
Linear range (µg/mL)	0.4-2.8
Correlation coefficient (r)	0.9995
Determination coefficient (r^2)	0.9990
Intercept ± SD	9.664 ± 3.642
Slope ± SD	147.86 ± 2.099
LOD (µg/mL)	0.081
LOQ (µg/mL)	0.24

Table 2. Analysis of oseltamivir phosphate in capsules and suspension by standard addition method.

Dosage form	Taken concentration (µg/mL)	Added concentration (µg/mL)	Found concentration (µg/mL)	%Recovery [a] ± SD
Tamiflu® capsule	1	0	0.989	98.90± 0.061
	1	0.5	1.48	98.66± 0.032
	1	1	2.03	101.50± 0.015
	1	1.5	2.51	100.40± 0.123
Taminile N® suspension	1	0	0.99	99.00± 1.112
	1	0.5	1.52	101.33± 0.834
	1	1	1.99	99.50±0.086
	1	1.5	2.48	99.20± 1.162

[a]; the value is the average of six determinations.

Table 3. The Intra- and inter-day precision for the determination of oseltamivir phosphate by the proposed spectrofluorimetric method.

Amount (µg/mL)	Intra-day precision		Inter-day precision	
	% Recovery [a] ± % RSD	SE	% Recovery [a] ± % RSD	SE
0.6	98.50 ± 1.310	0.645	100.75 ± 1.695	0.853
1	99.87 ± 0.858	0.428	99.77 ± 1.533	0.765
1.4	99.09 ± 0.873	0.432	99.55 ± 0.398	0.229
2	98.57 ± 0.687	0.399	99.45 ± 0.496	0.494

[a] the value is the average of six determinations.

Table 4. Robustness for determination of oseltamivir phosphate (1 μg/mL) by the spectrofluorimetric method

Method Parameters	(%) Recovery [a]± SD	% RSD
Volume of Acetylacetone		
0.8	99.03±0.01	0.010
1	99.45±0.52	0.523
1.2	98.26±1.20	1.2221
Mean	98.91±0.603	0.610
Volume of formaldehyde		
0.6	99.98±0.128	0.128
0.7	99.37± 0.81	0.815
0.8	98.66±0.015	0.015
Mean	99.33±0.66	0.664
Time of heating(minutes)		
20	101.32±0.06	0.592
25	99.99±0.135	0.135
30	98.54±1.30	1.319
Mean	99.95±1.39	1.391
Buffer solution (pH)		
2.3	98.33±0.020	0.020
2.5	98.19±0.315	0.321
2.7	100.03±0.161	0.161
Mean	98.85±1.024	1.036

[a] the value is the average of three determinations

Table 5. Analysis of the investigated drug (1 µg/mL) in presence of some common excipients using the proposed spectrofluorimetric method.

Excipients	Amount Added (µg /mL)	(%) Recovery [a]± SD
Starch	50	102.36 ± 1.82
Mg stearate	10	98.73± 1.43
sodium lauryl sulphate	10	99.63± 0.862
Talc	5	100.46± 0.981
Lactose	10	99.01± 1.76
mannitol	10	100.73± 0.687

[a] the value is the average of three determinations

Table 6. Results of the degradation study of OSP under different stress conditions

Degradation condition	Reaction rate constant (K, hr^{-1})	Half life time ($t_{1/2}$)
Alkaline degradation	0.327	2.11
Acidic degradation	0.258	2.68

Alkaline degradation (1M NaOH, room temperature) and acidic degradation (2M HCL at 80 °C)

Table 7. Analysis of pharmaceutical dosage forms containing oseltamivir phosphate using the proposed and reference methods.

Dosage form	Labeled content	% Recovery [a] ± SD		t-value[b]	F-value[b]
		Reference method	Proposed method		
Tamiflu® capsules	75 mg	99.39 ± 2.04	99.86 ± 1.20	0.4404	2.911
Taminile N® suspension	12 mg	99.42 ± 1.34	98.36 ± 2.42	0.0431	3.023

[a] Average of five determinations.

[b] tabulated values at 95% confidence limit are t=2.306, F=6.338.

Table 8. Application of the proposed method for the determination of OSP in spiked human plasma.

	Concentration added (µg/mL)	% Recovery [a] ± SD
	0.4	98.31 ± 2.28
	0.6	99.45 ± 2.15
	1	96.91 ± 1.41
Mean±SD	98.22 ± 1.27	
% RSD	1.29	

[a] :Mean of three replicate measurements