



Research Article

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Validation of Spectrophotometric Method for Determination of Oseltamivir in Pharmaceutical Formulation Using 7-Chloro-4-Nitrobenzo-2-Oxa-1, 3-Diazole

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Abstract

A sensitive, precise and simple spectrophotometric method has been proposed and validated for the determination of *oseltamivir* (OSL) in pharmaceutical formulations. The proposed method is based on the reaction between the OSL and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-CL) at alkaline medium (pH 10) to form deep brown adduct, exhibiting maximum absorption (λ_{max}) at 464 nm. Under optimized reaction condition, the method was linear in the concentration range 2-10 $\mu\text{g mL}^{-1}$. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.84 $\mu\text{g mL}^{-1}$, 2.66 $\mu\text{g mL}^{-1}$, respectively. The method was applied successfully to the determination of OSL in pharmaceutical formulation. A proposal of the reaction pathway has been postulated. The method is useful for routine analysis of OSL in quality control laboratories.

Keywords

Oseltamivir, Spectrophotometric, Pharmaceutical formulation, Method validation, NBD-CL

Abbreviations

OSL: Oseltamivir phosphate; OC: Oseltamivir carboxylate; LOD: Limit of detection; LOQ: Limit of quantification; SD: Standard deviation

Introduction

Oseltamivir phosphate (OSL) is the first orally available inhibitor of influenza virus neuraminidase, an enzyme involved in the release of new virus particles from infected cells. The structure of OSL indicates that it possesses a hydrophobic moiety. OSL hydrophobic group is responsible for its poor oral absorption; thus, the phosphate salt has been developed that allows oral administration of this drug. OSL phosphate is a pro drug that is rapidly and extensively metabolized via hepatic esterases to oseltamivir carboxylate (OC), the active form, a potent and selective inhibitor of influenza virus neuraminidase [1-4]. It was developed by Gilead Sciences and is currently marketed by Hoffmann-La Roche (Roche) under the trade name Tamiflu, that is the leading drug against avian flu. The manufacturer has permitted arrangements for a number of companies to produce Tamiflu under License to meet fast-growing world demand for this drug [5]. Tamiflu is already in short supply throughout the world as countries gather stockpiles of the drug among fears of a possible avian flu pandemic [6,7].

Among the various method available for the determination of drugs, spectrophotometry and spectrofluorimetry continue to be very popular, because of their simplicity, spec-

ificity and low cost. This study presents a new spectrophotometric method for the assay of oseltamivir.

A number analytical procedures have been reported for the analysis of oseltamivir in a bulk form, pharmaceutical form or biological fluids. These include high performance liquid chromatography [8-13], HPTLC [14] spectrophotometric methods [15-17], spectrofluorimetry [18] and capillary electrophoresis [19]. However, some of these methods are complicated and time consuming, involve the use of large volume organic solvents and specific reagent and requirement of expensive instruments.

7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-CL) has

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been proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group [20-43]. The applications of NBD-CL for determination of pharmaceutical bearing amine group have been reviewed by Elbashir, et al. [44,45]. The use of NBD-CL for spectrophotometric determination of OSL has not been reported yet. Therefore, in this work a sensitive and simple spectrophotometric method for determination of OSL in pharmaceutical formulations has been developed.

Experimental

Apparatus

A Shimadzu 1800UV 1800 spectrophotometer, with 1 cm quartz cells was used to record the spectrophotometric data. Mi 150 pH/Temperature Bench meter was used to adjust pH of the buffered solutions.

Reagent and solution

Oseltamivir (OSL) with purity 98.5% was kindly provided by Hoffman-La Roche. (Foster city, California, USA). Tamiflu 75 mg per capsules was obtained from local pharmacy. 4-Chloro-7-nitrobenzofurazan (NBD-CL) with purity 98% was obtained from Sigma-Aldrich (Chemie GmbH (Steinheim, Germany)). NBD-CL solution was freshly prepared in acetone at 0.7% (w/v) concentration. Buffer solution was prepared by adding 12.5 mL of 0.2 mol L⁻¹ NaOH to 5 mL 0.2 mol L⁻¹ dihydrogen sodium phosphate. All chemicals and reagent were of analytical-reagent grade. A stock solution of OSL was prepared in distilled water and diluted further with the water to obtain standard solution of 10 µg mL⁻¹.

Procedure

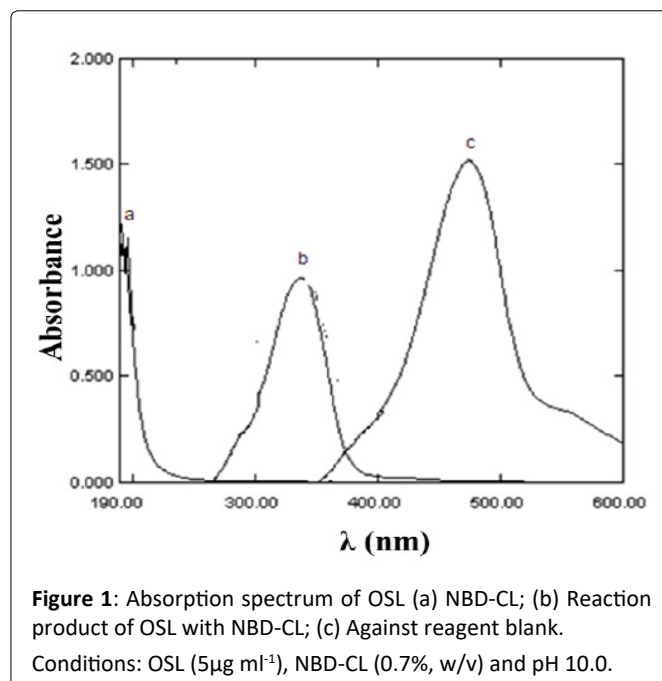
Calibration curve: An aliquot of 1.0 mL from standard solution was added to 2.0 mL buffer solution in 10 mL volumetric flask, 0.3 mL of 0.7% NBD-CL was added to the later and the volume was brought to 10 mL with water and mixed. The absorbance of the derivative was measured after heating in water bath for 25 min at 464 nm against a blank prepared similarly.

Determination of OSL in dosage Forms: For preparation of sample solution, ten capsules were weighed and powdered then a quantity of powder equivalent to 100 mg of OSL was transferred into 100 mL conical flask, about 70 mL of water was added, and the mixture was shaken for 10 min and the volume was made up with water to give a concentration of 1000 µg/mL, and then the solution was filtered.

Results and Discussion

Absorption spectra

The absorption spectrum of OSL was scanned in range from 185 nm to 600 nm against water (Figure 1), it was found that OSL exhibits a maximum absorption peak (λ_{max}) at 190 nm. Because of highly blue shifted λ_{max} of OSL, its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, deri-



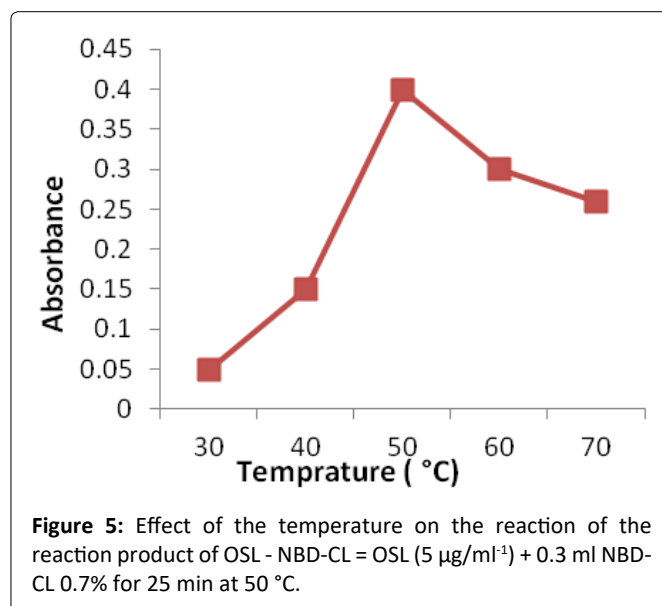
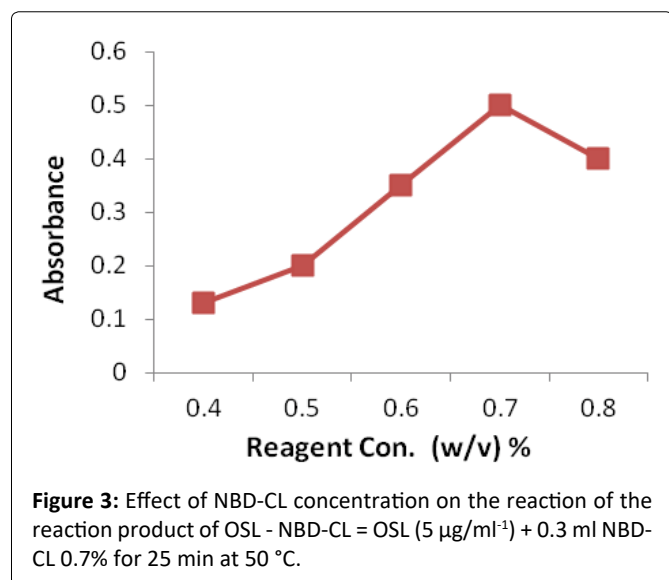
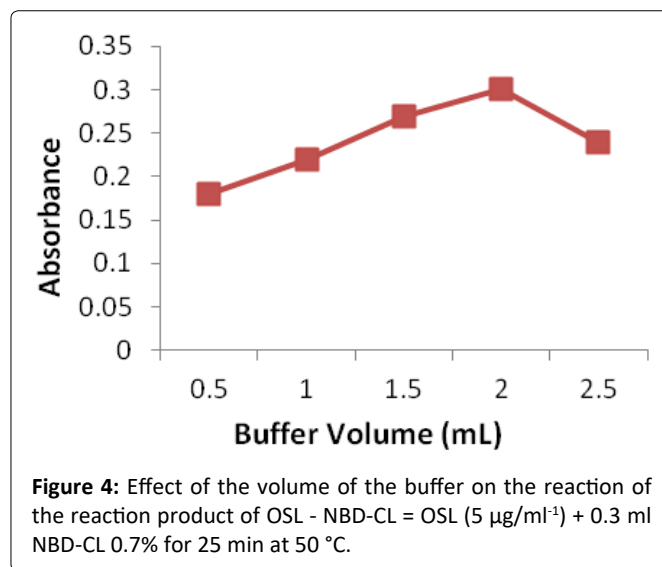
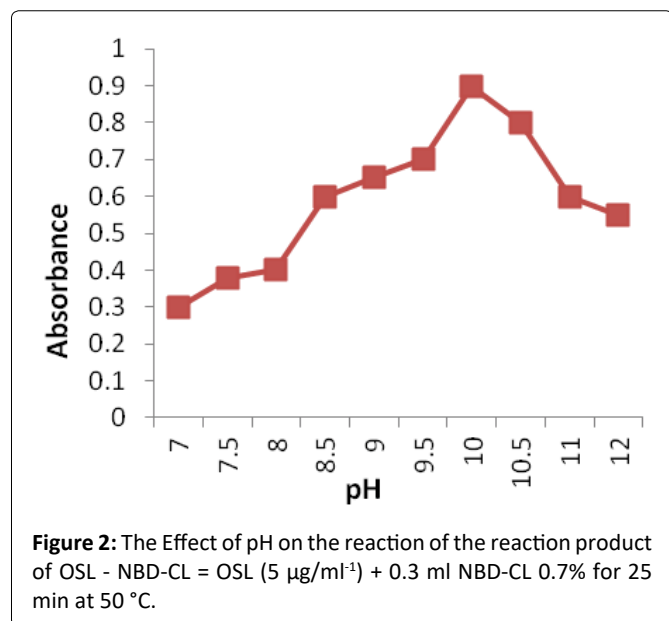
vatization of OSL red-shifted light-absorbing derivative was necessary. The reaction between OSL and NBD-CL was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is brown colored exhibiting λ_{max} at 464 nm, and the λ_{max} of NBD-CL was 342 nm. The λ_{max} of OSL-NBD-CL derivative was red-shifted, eliminating any potential interference. Therefore, the measurements were carried out at 464 nm.

Optimization of reaction conditions

The optimum conditions for the development of method were established by varying the parameters one at a time while keeping the others fixed and observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as, pH, concentration of NBD-CL temperature, and time of heating were studied. Observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as, pH concentration of NBD-CL temperature, and time of heating were studied.

The influence of pH on the absorbance of product was investigated in the range of 7.0-12.0, the absorbance of the solution increases rapidly up to pH 10 and then decrease (Figure 2). At pH 10.0, the absorbance reaches its maximum; in other words, the degree of the nucleophilic substitution reaction is also maximal. At pH > 10.0, the absorbance of solution decreases sharply. Presumably it may be that the increase of hydroxide ion holds back the nucleophilic substitution reaction between OSL and the chromogenic reagent. Consequently, the absorbance of the solution reduces. In order to keep the high sensitivity for the determination of OSL, pH 10.0 was selected for optimal experimental conditions.

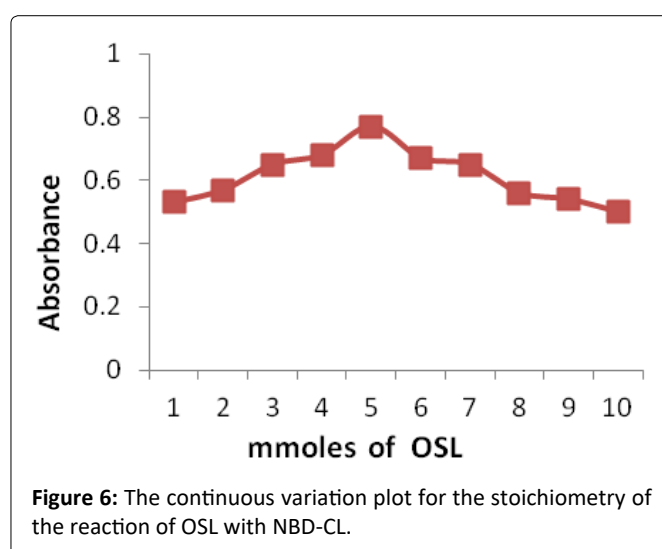
The effect of NBD-CL concentration was studied over the range 0.4-0.8% (w/v) as shown in (Figure 3). Increasing the concentration of NBD-CL results in more products up to an



amount of 0.7%, after which the absorbance of the solution reduced. Therefore, a concentration of 0.7% NBD-CL was considered optimum. Keeping pH at 10.0, the effect of amount of buffer solution on the absorbance of product was also studied. It shows that the absorbance of product enhances rapidly with the rise of amount of buffer solution, and becomes maximal when the amount of buffer solution is 2.0 mL. Therefore, the amount of 2.0 mL buffer solution was selected to ensure the highest absorbance of product, as shown in (Figure 4).

The effect of temperature on the reaction was studied by carrying out the reaction at different temperatures (30-70 °C). It was found that the reaction of OSL with NBD-CL was affected by increasing the temperature, and the reaction at 50 °C went to completion in 25 minutes, as shown in (Figure 5) and therefore, further experiments involving NBD-CL reagent were carried out at 50 °C for 25 minutes.

From the above experiments, the optimized conditions used for the assay were: pH 10.0, NBD-CL concentration 0.7% w/v, volume of the buffer 2.0 mL, reaction time 25 min and temperature 50 °C.



Furthermore, the molar ratio of NBD-CL to OSL in the reaction mixture was studied according to Job's method of continuous variation [46]. A $5.0 \times 10^{-4} \text{ mol L}^{-1}$ standard solution

of OSL and solution of NBD-CL were used. The reaction stoichiometry was found to be a good approximation 1:1 ratio (drug/reagent), confirming that one molecule of OSL reacts with one molecule of NBD-CL (Figure 6). Based on the observation molar ratio, the reaction pathway was postulated to proceed as shown in (Scheme 1).

Analytical method validation

Under the described experimental conditions, linear rela-

Table 1: Parameter for the performance of the proposed method.

Parameter	value
Measurement wavelength	464 nm
Linear range	2-10 $\mu\text{g mL}^{-1}$
Regression equation	$Y = 0.00125X + 0.289$
Slope \pm SD	0.00125 ± 0.00005
Intercept \pm SD	0.289 ± 0.000332
Correlation coefficient (r^2)	0.997
Limit of Detection (LOD)	$0.8448 \mu\text{g ml}^{-1}$
Limit of Quantification (LOQ)	$2.656 \mu\text{g ml}^{-1}$
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	4.64×10^3

ationship was found between the absorbance at λ_{max} 464 nm and the concentration of the drug. The regression equation was found to be as $Y = 0.00125X + 0.289$ ($r^2 = 0.997$, $n = 7$) (where A is the absorption, and c is the concentration of OSL in $\mu\text{g mL}^{-1}$) the limits of detection (LOD) and limits of quantitation (LOQ) where determined using the formula:

$$\text{LOD or LOQ} = K S D. a / b \quad (1)$$

Where,

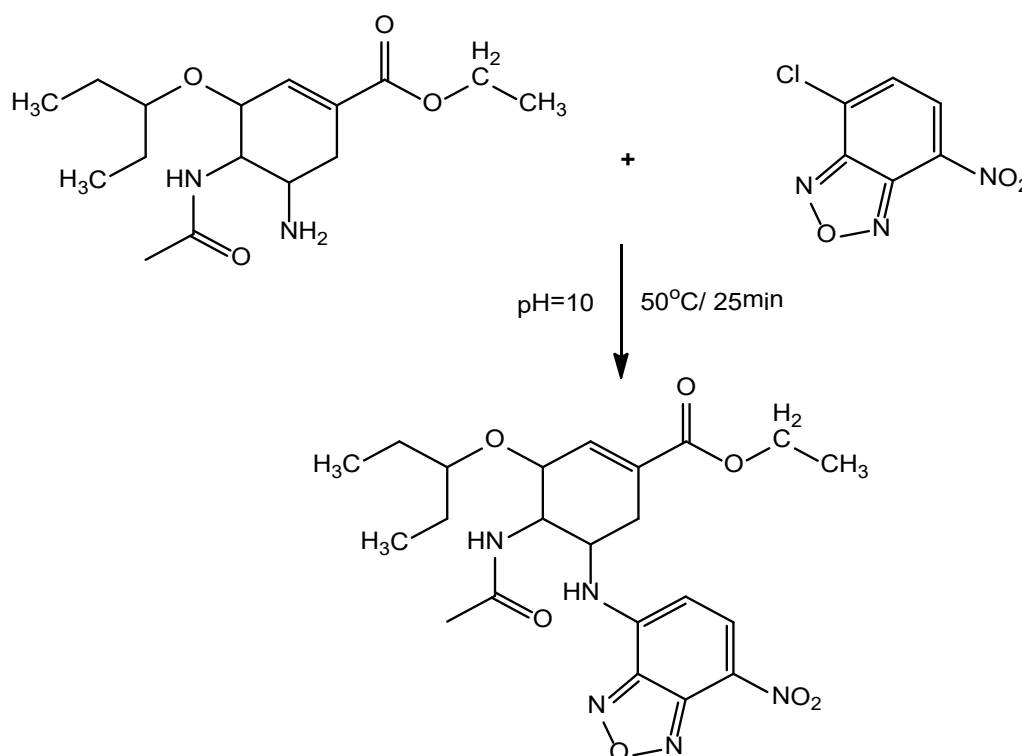
$K = 3.3$ for LOD and 10 for LOQ, S. D. a is the standard deviation of the intercept, and b is the slope, the obtained results are summarized on (Table 1).

The accuracy of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to a known concentration of the drug sample. The average percent recoveries obtained in range 99.25-100.75 (Table 2).

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that variation in the NBD-CL concentration of $0.7 \pm 0.02\%$ (w/v) and

Table 2: Recovery studies for the determination of OSL by proposed method.

Sample No	Sample content ($\mu\text{g mL}^{-1}$)	OSL amount added	Amount Found	Recovery
1	4	3.5	7.53	100.75%
2	4	7.0	10.90	97.5%
3	4	12.0	15.97	99.25%



Scheme 1: Reaction pathway of OSL with NBD-CL.

Table 3: Results of robustness for determination of OSL by the proposed method.

	Parameter	Recovery (%)
	Recommended conditions	101.36
NBD-CL concentration	NBD-CL concentration (%0.68)	99.00
	NBD-CL concentration (%0.72)	100.00
Buffer pH	Buffer pH 9.5	98.20
	Buffer pH 10.5	99.40
Temperature	Temperature (48 °C)	99.80
	Temperature (52 °C)	98.20
Reaction time	Reaction time (min) 23	102.22
	Reaction time (min) 27	96.60

optimal experimental conditions of temperature (50 ± 2 °C), time (25 ± 2 min) and pH (10.0 ± 0.5), did not significantly affect the procedures and recovery values were 96.6-102.2% and the RSD values did not exceed 0.497% (Table 3).

Conclusion

In this work, the product of the derivatization reaction between OSL and NBD-Cl has been utilized to develop a simple accurate and sensitive spectrophotometric method for OSL analysis in pharmaceutical formulation. The derivatization reactions conditions were optimized. The suggested mechanism was postulated. The method was validated with respect to linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy and robustness. The method is also cost effective and environmentally friendly; therefore, the proposed method can be used advantageously as a routine method for the determination of OSL in quality control and industry.

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