A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF OSELTAMIVIR PHOSPAHATE IN BULK AND CAPSULES

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S U M M A R Y

In this study, a simple, rapid and accurate spectrophotometric method has been developed for the assay of oseltamivir phosphate (OSP). The proposed method based on the charge-transfer reactions of oseltamivir, as n-electron donor, with 7,7,8,8-tetracyanoquinodimethane (TCNQ), as π-acceptors to give highly colored complexes. The experimental conditions such as reagent concentration, reaction solvent and time have been carefully optimized to achieve the highest sensitivity. Beer's law is obeyed over the concentration range of 3–30 μg/mL with correlation coefficient 0.9999. The method was applied successfully to the determination of this drug in capsules. The mean recovery for the commercial capsules was 100.03%. The suggested method could be used for the determination of OSP in pure and capsules being sensitive, simple and selective.

Ö Z E T

Bu çalışmada, oseltamivir fosfatın tayini için basit, hızlı ve doğru bir spektrofotometrik bir yöntem geliştirildi. Yöntem bir n-elektron verici olan oseltamivirin bir π-alıcısı olan 7,7,8,8-tetrasiyanokinodimetan (TCNQ) ile oldukça renkli bir kompleks oluşumuna dayanmaktadır. Analizi en yüksek hassasiyette gerçekleştirmek için belirteç miktarı, reaksiyon çözeltisi, reaksiyon zamanı gibi deneysel koşullar dikkatlice incelendi. Kalibrasyon eğrisinin 3–30 μg/mL derişim

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aralığında doğrusal olduğu görüldü (R= 0,9999). Geliştirilen yöntem ilacın kapsullerdeki tayinine başarılı ile uygulandı. Ticari kapsüllerde ortalama geri kazınım % 100.03 olarak bulundu. Önerilen yöntemin, ilaçlarda rutin oseltamivir fosfat analizinde kalite kontrol amacıyla kullanılabilecek kolay, hassas ve seçiciliği yüksek bir yöntem olduğu belirlendi.

Keywords: Spectrophotometric; oseltamivir; Charge-transfer; Drug analysis.

I N T R O D U C T I O N

Drug quality control is a branch of analytical chemistry that has a wide impact on the public health, so the development of a reliable quick and accurate method for the active ingredient determination is important.

Oseltamivir phosphate (OSP) is an antiviral drug used in the treatment and prevention of both influenza virus A and influenza virus B (Fig. 1). OSP is an ethyl ester pro-drug that is rapidly and extensively metabolized by esterases in the gastrointestinal tract and liver to its active form, oseltamivir carboxylate (OSC) (1-2).

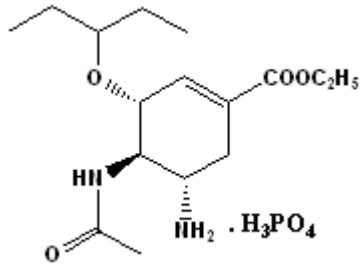

Figure 1. Oseltamivir phosphate

Several methods have been reported for the determination of OSP in pharmaceutical dosage forms and biological fluids including HPLC (3-5), liquid chromatography/mass spectrometry (LC/MS) (6), LC-tandem MS (7), a micellar electrokinetic chromatography (8) and colorimetric (5). The reported colorimetric assay for estimation of OSP in capsules [5] is not sensitive enough with the initial determined concentration of analyte (1.50 mg/mL).

Although some of the previously published methods are fairly specific, they tend to be expensive, and time consuming (6-7). This paper describes a simple, direct, sensitive and precise spectrophotometric method for the determination of OSP based on charge complexation reaction with TCNQ (Fig. 2).

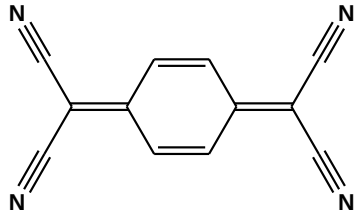

Figure 2. 7,7,8,8-tetracyanoquinodimethane

E X P E R I M E N T A L

Apparatus

Spectrophotometric measurements were carried out using a Shimadzu UV-160 A spectrophotometer with 1-cm glass cells.

Reagents and solutions

Oseltamivir phosphate was kindly supplied by Roche Pharmaceutical (Istanbul, Turkey) and its pharmaceutical preparation (Tamiflu®) containing 75 mg of oseltamivir base (98.5 mg oseltamivir phosphate) per capsule was obtained from local drugstore. All chemicals and reagents were of analytical reagent grade.

Stock solutions of OSP was prepared by dissolving 10.0 mg in 5.0 mL of acetone and the volume was diluted to the mark in a 10 mL calibrated flask with same solvent to obtain stock solutions of 1.0 mg/mL of drug and diluted further with acetone to obtain standard solutions of 0.10 mg/mL.

7,7,8,8-Tetracyanoquinodimethane (TCNQ, Fluka Neu-Ulm, Germany) was freshly prepared as 0.392 mg/mL solutions in acetone.

General procedure and calibration curve

Into 5-mL calibrated flasks 0.15-1.5 mL aliquots of OSP standard solution were transferred and 1.5 mL of TCNQ solution were added, mixed well and then completed to mark with acetone. The reaction mixture was left for 15 min at room temperature and the absorbance was measured at 845 nm, against a reagent blank prepared in the same manner. The calibration curve was prepared by plotting absorbance vs. concentration of OSP.

Procedure for assay of capsules

The contents of the entire ten capsules were weighed and their mean mass was determined. An accurately weight of the powder equivalent to 75 mg oseltamivir base was transferred into a 100 mL calibrated flask with about 50 mL of acetone. The

solution was sonicated for 2 hour at room temperature and then completed to volume with same solvent and filtered.

Aliquots of filtrate were diluted further with acetone (concentration of final solution was 0.1 mg/mL) then proceed as described under general procedure and calibration curve. The nominal contents of the capsules were calculated using the corresponding regression equation.

Stoichiometry of the formed charge complex

The reaction stoichiometry between drug and reagent was determined by Job's continuous variation method [9-10]. For this, the drug solution with identical molar concentrations of reagent were mixed in varying volume ratios (5:1-1:5) in which the total volume of the mixtures were kept at 5 ml, and the procedures were completed as described in Section general procedure and calibration curve. The absorbance of each solution was plotted against the mole fraction drug, [drug]/ [drug] + [reagent].

RESULTS AND DISCUSSION

Molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge-transfer complexes which absorb radiation in the visible region (11). These charge complex reactions were of particular interest in the analysis of many pharmaceutical compounds (12-13). OSP, being an n-electron donor, reacts with π-acceptors giving charge transfer complexes of the n–π type which dissociate to give the colored free radical anions of the acceptors according to the following equation:

$$\underset{\text{donor}}{D} + \underset{\text{acceptor}}{A} \rightleftharpoons \underset{\text{n-}\pi\text{ complex}}{(D\text{-}A)} \overset{\text{polar solvent}}{\rightleftharpoons} \underset{\text{radical anions}}{A^{\bullet+} + D^{\bullet+}}$$

Interaction of OSP with TCNQ gives a-green chromogen which exhibits strong absorption maxima at 845, 825, 764 and 745 nm; the wavelength 845 nm is selected as it gives higher molar absorptivity with reproducible results (Fig. 3). To establish the optimum reaction conditions for OSP-TCNQ complex formation, several experimental parameters were investigated. The reaction of drug with TCNQ was investigated at room temperature, and also the heating effect was studied in the range of 50-80 ^{o}C. The results obtained indicated that, complete color development was attained with TCNQ within 15 min at room temperature. The absorbance of complex remains stable for at least 3 hour, thus permitting quantitative determination of OSP to be carried out with good reproducibility and indicating no side chemical reactions takes place. Among the solvents used acetonitrile, methanol, chloroform, dichloromethane and acetone, reaction

proceeded quantitatively in acetone. The amount of reagents on the intensity of the color development was examined by measuring the absorbance of the solutions containing a fixed concentration of OSP and varied amounts of the reagent. It was found that 6-fold molar excess of TCNQ was sufficient for the maximum absorbances (Fig. 4).

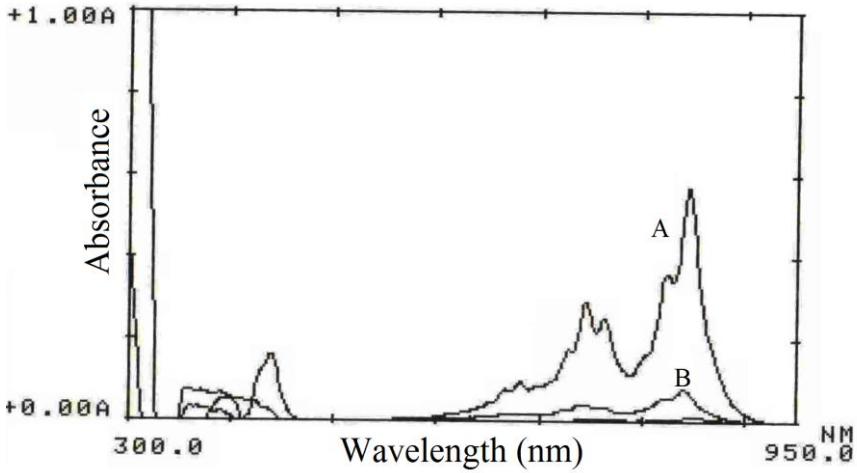

Figure 3. Absorption spectra of OSP–TCNQ complexes in (A) acetone and (B) methanol. The complex did not give absorption in chloroform (on the bottom). OSP concentration was 20.0 μg/mL.

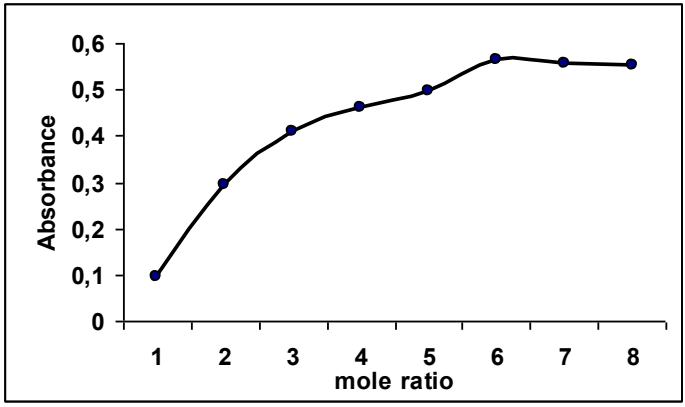

Figure 4. Effect of the reagent concentration on the absorbance of the complex reaction.

The stoichiometry of the reaction of the drug with used reagents in the proposed methods was determined by Job's method of continuous variation (9-10) and was found to be 1:1 (Fig. 5).

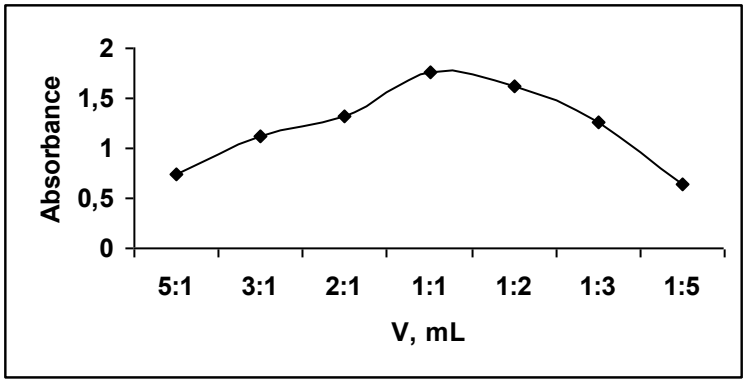

Figure 5. Job's plot for OSP–TCNQ complex (drug = reagent = 1.0×10^{-3} M).

The developed analytical method was validated as ICH guideline (14) applying a pharmaceutical preparation analysis. Under the described experimental conditions, calibration curve for proposed method was constructed. Table 1 summarizes the values for Beer's law limit, molar absortivities (ε), regression equations, correlation coefficients. For evaluation of linearity, determination of OSP was done at 6 concentration levels, and each concentration was analyzed for 4 times. Beer's law was obeyed over the concentration ranges of 3.0–30.0 µg/ mL, with correlation coefficients 0.9999. The detection limit (LOD) (14) of the method were calculated as $C = 3\sigma/s$, where C is the limit of detection, σ is the S.D. of the intercept, and s the slope of the standard curve, and found to be 0.541µg/ mL. The quantification limit (LOQ) of the method were calculated as $C = 10\sigma/s$ and found to be 1.804 µg/mL.

Table 1. Analytical Parameters for the Determination of OSP by proposed method

Parameters	Proposed method
λ_{max}, nm	845
Beer's law range (µg/mL)[a]	3.0-30.0
Apparent molar absorptivity (L mol^{-1} cm^{-1})	1.08 x10^4
LOD (µg/mL)	0.541
LOQ (µg/mL)	1.804
Regression equation Y[b]	
Slope (a)	0.0313
Intercept (b)	0.0034
Correlation coefficient (r)	0.9999
Relative standard deviation, %	2.28

[a] Data obtained from 4 determination (n = 4).

[b] Y= aX + b, where Y is the absorbance of a 1 cm layer of solution, a is the slope, b is the intercept and X is the concentration of the drug in µg/mL.

The intraday and interday accuracy and precision were examined by analysis of OSP with the concentrations of 6.0, 10.0 and 25.0 µg/ml (each *n* =5) for five consecutive days. The intraday studies were performed during 1 day, and the interday studies were performed on five different days. The RSD values for intraday and interday precision were 0.03 - 0.74 % and 0.12- 0.94%, respectively. The obtained results are summarized in Table 2.

Table 2. Interday and intraday assay of oseltamivir base by the proposed method (n = 5)

Proposed method	Concentration (µg/mL)		Recovery (%)	R.S.D.(%)
	Taken	Found ± S.D.		
Interday	6.0	6.04 ± 0.06	100.66	0.94
	10.0	10.01 ± 0.04	99.20	0.37
	25.0	25.11 ± 0.03	100.44	0.12
Intraday				
	6.0	6.00 ± 0.002	100.00	0.03
	10.0	9.92 ± 0.04	99.20	0.37
	25.0	25.30 ± 0.19	101.16	0.74

To check accuracy of the proposed methods, the standard addition method was applied. A different amount of pure sample solution was added to a known amount of capsule solutions and assayed. The percent recovery of the added standard to the assay samples was calculated from: Recovery %= $[(C_t-C_u)/ C_a]$ x 100, where C_t is the total concentration of the analyte found; C_u, concentration of the analyte presents the formulation; C_a, concentration of the pure analyte added to the formulation.The results of analysis of the commercial dosage forms and the recovery study are shown in Table 3. The average percent recoveries obtained were 101.28%, indicating good accuracy of the method.

Table 3. Determination of oseltamivir base in capsules by standard addition method (n =5)

Concentration (µg/mL)			Recovery (%)	R.S.D.(%)
Taken	Added	Found ± S.D.		
10.0	5.0	15.22 ± 0.11	101.49	0.69
10.0	10.0	20.06 ± 0.13	100.40	0.59
10.0	20.0	30.59 ± 0.10	101.96	0.31

The effect of excipients frequently found in formulations, such as starch, lactose, glucose and magnesium stearate, was evaluated using the proposed method. It was found that the common excipients did not interfere with the determination.

The proposed method was applied successfully to the determination of OSP in capsules. The results given in Table 4 reveal that the recovery was 100.03% reflecting the high accuracy and precision as indicated by low value of R.S.D. (0.60%). Statistical analysis of the results obtained, compared with those of reported method (5), showed no significant difference in the accuracy and precision of the two methods.

Table 4. Statistical evaluations of the results obtained by the proposed and comparison methods for the assay of OST in capsules (each capsules contains 75 mg of oseltamivir base)

Statistical values	Proposed methods	Reference method (5)
Mean (mg)[a]	75.02	75,89
Recovery (%)[a]± S.D.	100.03 ± 0.45	101.19 ± 0.94
R.S.D. (%)[b]	0.60	1.24
Confidence limits	75.02 ± 0.37	
t-calculated [b]	2.03	
F-calculated [b]	0.23	

[a] Each values is the average of six determination
Theoretical values at 95% confidence limit; $t = 2.23$ and $F = 5.05$

The suggested method has the advantage of being simple, accurate, sensitive and suitable for routine quality control of the drug alone and in pharmaceutical formulations without fear of interference used by excipients expected to present in formulations. Rapid and stable formation of the colored complex with no need for extraction process is advantages of the developed method over the previously reported spectrophotometric method.

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