ISSN : 0974-7419 Volume 13 Issue 1

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 13(1) 2013 [17-22]

Novel visible spectrophotometric method development for the determination of oseltamivir phosphate in capsules using NQS as a chromogenic reagent

B.Kalyana Ramu*[2], M.Syam Bab[1], U.Viplava Prasad[1]

[1]Department of Organic Chemistry& Analysis of Foods Drugs &water Laboratories, School of Chemistry, Andhra University, Visakhapatnam-530003 Andhra Pradesh (INDIA)

[2]Department of Chemistry, Maharajah's College (Aided& Autonomous), Vizianagaram-535002 Andhra Pradesh (INDIA)

E-mail : kalyanaramu23566@gmail.com; msyambab@gmail.com

ABSTRACT

A direct, simple and sensitive visible spectrophotometric method is described for the assay of oseltamivir phosphate in pure and capsule forms. The method is based on the formation of yellowish brown colored species by the drug with Folin reagent and exhibits absorption maxima at 453 nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (16-48) µg/ml. The proposed method is applied to commercial available capsules and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of the Oseltamivir phosphate in the presence of other ingredients that are usually present in dosage forms. The method offers the advantages of rapidity, simplicity and sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents

KEYWORDS

Assay;
Folin reagent;
Nucleophillic substitution;
Statistical analysis.

INTRODUCTION

Oseltamivir phosphate (OP) (Figure 1) is the best known orally active newest addition to the group of H_1N_1 and H_5N_1 neuraminidase inhibitor and an antiviral drug that slows the spread of influenza (flu) viruses (type A and B) between cells in the body by stopping the new virus from chemically cutting ties with its host cell. The drug is considered the best treatment for the bird flu disease. OP 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 (OC). OP is a white crystalline powder solid with the chemical name (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylicacid,ethylester, phosphate (1:1) and Its chemical formula is $C_{16}H_{28}N_2O_4.H_3PO_4$ representing molecular weight of 410.4.

In literature, OP can be identified by thin layer chromatography, specific optical rotation, infrared spectrophotometry and tests characteristic for ortho phosphates[1], Determination, by International Pharma-

Full Paper

copeia[2], can be done by high-performance liquid chromatography[1-2] or by titration with perchloric acid[1]. Other analytical methods such as UV spectroscopy[3-5], visible spectrophotometric[6-9], colorimetric and LC[10], spectrofluorimetric[11], HPLC with UV detection[12-19] and mass spectrometry[20-23], Micellar electrokinetic chromatography[24], capillary electrophoresis[25] voltammetry[26] and potentiometry[27] have been reported for the determination of OP in biological fluids and formulations. The main purpose of the present study was to establish relatively simple, sensitive, validated and inexpensive extraction free visible spectrophotometric method for the determination of OP in pure form and in pharmaceutical preparations, since most of the previous methods involve critical reaction conditions or tedious sample preparations and less specificity. So the authors have made some attempts in this direction and succeeded in developing the method based on the reaction between the drug and folin reagent (NQS)[28] under specified experimental conditions. The proposed method for OP determination has many advantages over other analytical methods due to its rapidity, normal cost and environmental safety. Unlike HPLC, HPTLC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. The method can be extended for the routine quality control analysis of pharmaceutical products containing OP.

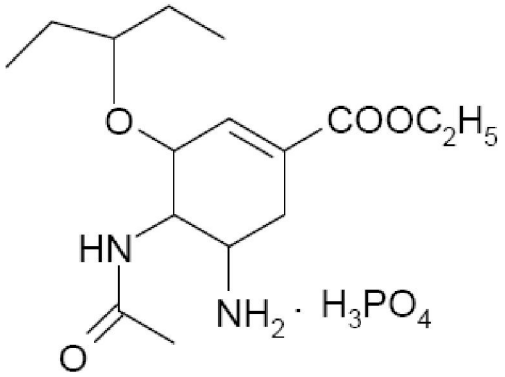

Figure 1 : Chemical structure of OP

MATERIALS & METHODS (EXPERIMENTAL)

Apparatus and chemicals

A Shimadzu UV-Visible spectrophotometer 1601 with10mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-361 was used for pH measurements. All the chemicals used were of analytical grade.

Folin reagent (NQS) solution (Loba, 0.5%, $1.92x10^{-2}$M prepared by dissolving 500mg of NQS in 100 ml of distilled water), phosphate buffer of pH 8.0 (prepared by mixing 30 ml of 0.067M potassium hydrogen phosphate and 970 ml of 0.067M disodium hydrogen phosphate and pH adjusted to 8.0) were prepared.

Preparation of standard stock solution

The standard stock solution (1mg/ml) of OP was prepared by dissolving 100mg of OP in 10 ml 0.1M sodium hydroxide and the volume was brought to 100 ml with distilled water. The working standard solution of OP (400 µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent. The prepared stock solution was stored at 4p C protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

Preparation of sample solution

About 20 capsules were weighted to get the average capsule weight and pulverized. The powder equivalent to 100mg of OP was weighed, dispersed in 25ml of Isopropyl alcohol, sonicated for 15 minutes and filtered through Whatman filter paper No 41. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Determination of wavelength maximum (λ_{max})

The 3.0 ml of working standard solution of OP (400µg/ml) was taken in 25ml standard flask. To this, 1.0ml of folin reagent ($1.092x10^{-2}$M), 5.0 ml of buffer pH 8.0 and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water and sonicated for 1 min. to get a concentration of 48µg/ml. In order to investigate the wavelength maximum, the above standard stock solution was scanned in the range of 360-560nm by UV-Visible spectrophotometer. From the spectra (Figure 2), it was concluded that 453nm is the most appropriate wavelength for analyzing OP with suitable sensitivity.

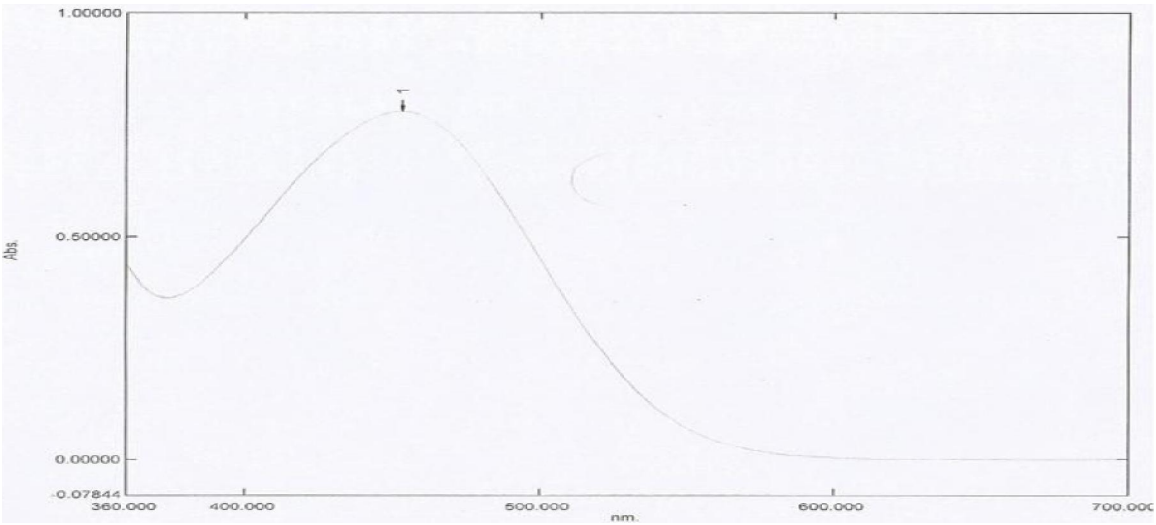

Figure 2 : Absorption spectra of OP-NQS system

Preparation of calibration curve

Aliquots of the standard OP solution [1.0-3.0ml, 400µg/ml] were placed in a series of 25ml standard flask. Then 1.0ml of folin reagent ($1.092x10^{-2}$M), 5.0 ml of buffer pH 8.0 and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water and sonicated for 1 min. The absorbance was measured at 453 nm against a reagent blank within the stability period (5minutes to 30min). The calibration graph was constructed by plotting the drug concentration versus absorbance. The amount of drug was computed from its calibration graph (Figure 3).

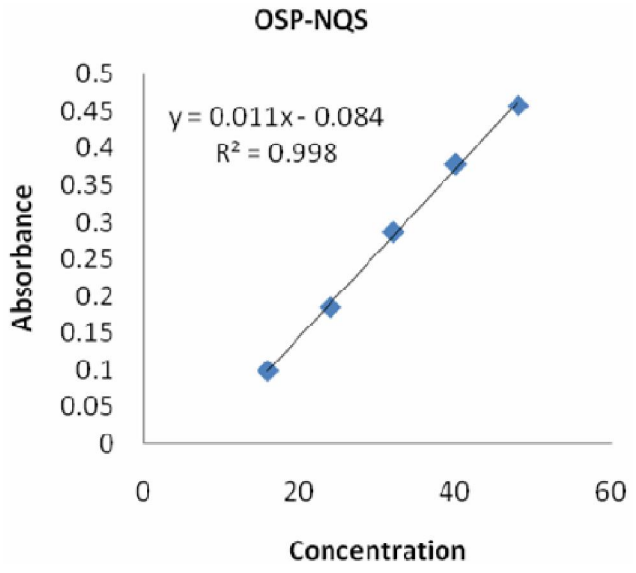

Figure 3 : Calibration graph of OP-NQS system

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, the order of addition of reagents, pH buffer solutions and solvent for final dilution of the colored species were studied. Distilled water was found to be best solvent for final dilution. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile have no additional advantage in increasing the intensity of the color. The optical characteristics such as Beer's law

TABLE 1 : Optical characteristics, precision and accuracy of proposed method.

Parameter	Values
λ_{max} (nm)	453
Beer's law limit (µg/ml)	16 - 48
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.004475524
Molar absorptivity (Litre/mole/cm)	91698.75
Regression equation (Y)*	
Intercept (a)	-0.084
Slope (b)	0.011
%RSD	1.68
% Range of errors (95% Confidence limits)	
0.05 significance level	1.76
significance level	2.76

***Y = a+ b x, where Y is the absorbance and x is the concentration of OP in µg/ml**

Full Paper

limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing 3/4th of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in TABLE 1.

Natflu capsules containing OP were successfully analyzed by the proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in TABLE 2.

Chemistry of colored species

In the present investigation, the presence of aliphatic secondary amino group of OP permits the development of visible spectrophotometric method for its determination. Yellowish brown colored species (N-alkyl amino napthaquinone) was formed by replacement of the sulphonate group of the napthaquinone sulphonic acid by a secondary amino group of drug. The formation of colored species with the reagent may be assigned through above analogy as shown in scheme (Figure 4).

TABLE 2 : Analysis of sumatriptan succinate by proposed and reference methods.

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	f		
NQS	capsule-1	30	29.61 ± 0.12	1.45	1.82	29.80±0.16	98.71 ± 0.40
	capsule-2	75	73.22 ± 1.79	2.77	3.9	74.65±0.91	97.63 ± 2.38

*** Capsule- 1 and capsule-2: Natflu capsules of NATCO PHARMA LIMITED, Hyderabad (INDIA); **Average ± Standard deviation of eight determinations, the t- and f-values refer to comparison of the proposed method with reference method. (UV). Theoretical values at 95% confidence limits t =2.57 and f = 5.05; # Recovery of 10mg added to the pre analyzed sample (average of three determinations); Reference method (reported UV method) using 0.1M NaOH (2û $_{max}$=216nm).**

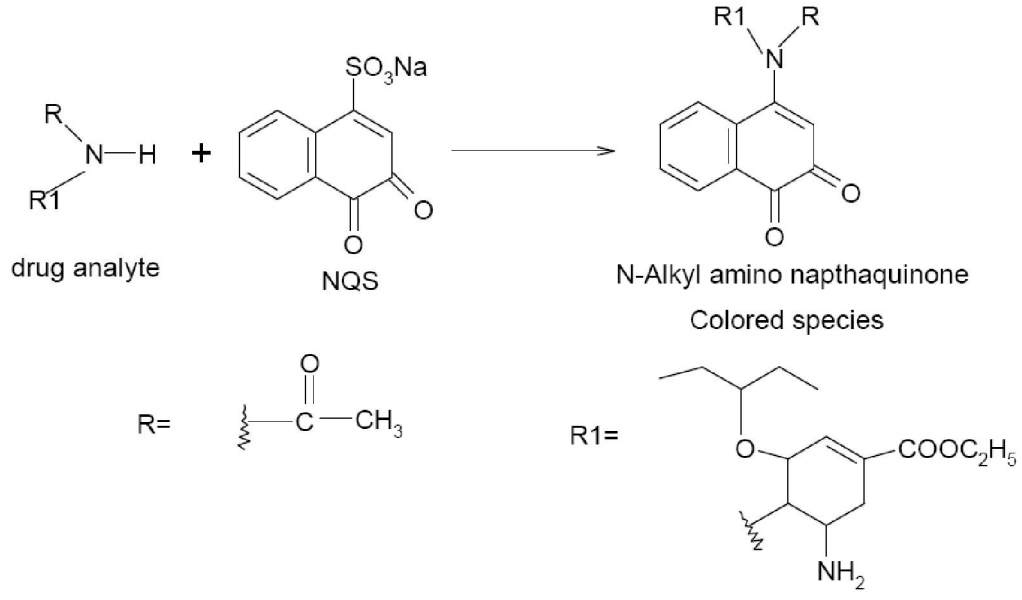

Figure 4 : Probable scheme of the reaction

CONCLUSION

The reagents utilized in the proposed method are normal cost, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed colorimetric methods possesses reasonable precision, accuracy, and are simple, sensitive and can be used as alternative methods to the reported ones for the routine determination

of OP depending on the need and situation.

ACKNOWLEDGEMENTS

The authors (MS Bab & BK Ramu) are thanks to the University Grants Commission, New Delhi for providing financial assistance under teacher fellow ship and also thanks to University authorities for providing facilities in this work.

REFERENCES

[1] AHFS Drug information; 782 **(2008)**.
[2] WHO, Oseltamivir phosphate, Final text for addition to The International Pharmacopoeia; Document QAS/06.190/FINA., December **(2008)**.
[3] A.Chourasia, S.Acharya, S.K.Sahu; Method development and validation of oselamivir phosphate in bulk drug by UV spectroscopy, International Journal of Pharmaceutical and Biological Research, **2(5)**, 132-136 **(2011)**.
[4] D.Mehar, Y.Rajesh, V.Rajakumar, B.V.V.Ravi kumar, S.Arun Kumar; Development, estimation and validation of oseltamivir phosphate in bulk and in its pharmaceutical formulations by UV spectroscopic method, International Journal of Pharma and Bio Sciences, **1(4)**, 579-586 **(2010)**.
[5] C.S.Rault, D.S.Gharge, P.N.Dhabale, I.D.Onjari, A.H.Hosmani, H.Hosmani Abhijeet; Development and validation of oseltamivir phosphate in fluvir by UV-spectrophotometer, International Journal of Phar.Tech.Research, **2(1)**, 363-366 **(2010)**.
[6] J.V.Shanmuka Kumar, S.Prasanthi, M.Guravaiah, C.H.B.Sakaram; Application of potassium permanganate to the spectrophotometric determination of oseltamivir phosphate in bulk and capsules, Asian Journal of Pharmaceutical Clinical Research, **5(2)**, 18-22 **(2012)**.
[7] Ashish Ashok Thatte, T.Pramila; Simple extractive colorimetric determination of oseltamivir phosphate by ion-pair complexation method in bulk and capsule dosage form. International Journal of Research in Pharmaceutical and Biomedical Sciences, **2(2)**, 543-547 **(2011)**.
[8] Z.Aydogmus, F.Sari; A new spectrophotometric method for the determination of oseltamivir phosphate in bulk and capsules, OZET, **40**, 47-55.
[9] K.Jahn, M.Deepthi, S.Malipatil; Spectrophotometric determination of oseltamivir phosphate in bulk drug and pharmaceutical formulations, Res.J.Pharm Biol.Chem.Sci., **1(4)**, 933 **(2010)**.
[10] D.Michael.Green, Henry Nettey, A.Wirtz Robert; Determination of oselamivir quality by colorimetric and liquid chromatographic methods, Emerging Infectious Diseases, **14(4)**, 552-556 **(2008)**.
[11] Z.Aydogmus; Simple and sensitive spectrofuorometric method for the determination of oseltamivir phosphate in capsules through derivatization with fluorescamine, Journal of Fluorescence, **19(4)**, 673-679 **(2009)**.
[12] Z.Aydogmus, S.Caglar, S.Toker; RP-HPLC methods for determination of oseltamivir phosphate in capsules and spiked plasma, Analytical Letters, **43(14)**, 2200-2209 **(2010)**.
[13] L.Lindegardh, T.T.Hein, J.Farrar, P.Shinghasivanon, N.J.White, N.P.J.Day; A simple and rapid LC assay for evaluation of potentially counterfeit Tamiflu, J.Pharm.BiomedAnal., **42**, 430-433 **(2006)**.
[14] J.Joseph-Charles, C.Geneste, E.Laborde-Kummer, R.Gheyouche, H.Boudis, J.P.Dubost; Development and validation of a rapid HPLC method for the determination of oseltamivir phosphate in Tamiflue and generic versions, J.Pharm.Biomed Anal., **44**, 1008-1013 **(2007)**.
[15] B.Narasimhan, K.Abida, K.Srinivas; Stability indicating RP-HPLC method development and validation for oseltamivir API, Chem.Pharm.Bull., **56**, 413-417 **(2008)**.
[16] C.Fuke, Y.Ihama, T.Miyazaki; Analysis of oseltamivir active metabolite, oseltamivir carboxylate, in biological materials by HPLC-UV in a case of death following ingestion of Tamiflue Legal Medicine, **10**, 83-87 **(2008)**.
[17] G.Bahrami, Mohammadi Bahareh, Amir Kiani; Determination of oseltamivir carboxylic acid in human serum by solid phase extraction and HPLC with UV detection, Journal of Chromotography B, **864**, 38-42 **(2008)**.
[18] J.S.K.Nagarajan, S.Muralidharan; A validated RP-HPLC method for estimation of oseltamivir in pharmaceutical formulation, Der Pharmacia Lettre, **1(1)**, 162-168 **(2009)**.
[19] E.J.Eisenberg, K.C.Cundy; High performance liquid chromatographic determination of GS4071, a potent inhibitor of influenza neuraminidase in plasma by precolumn fluorescence derivatization with naphthalenedialdehyde, J.Chrom.B.Biomed.Sci. Appl., **716**, 267-273 **(1998)**.
[20] H.Wiltshire, B.Wiltshire, A.Citron, T.Clarke,

C.Serpe, D.Gray, Herron; Development of a high-performance liquid chromatography-mass spectrometric assay for the specific and sensitive quantification of Ro-64-0802, an anti-influenza drug and its prodrug, oseltamivir, in human and animal plasma and urine. J.Chrom.B Biomed.Sci.App., **745**, 373-388 **(2000)**.

[21] N.Lindegardh, W.Hanpithakpong, Y.Wattanagoon, P.Singhasivanon, N.J.White, N.P.J.Day; Development and validation of a liquid chromatographic-tandem mass spectrophotometric method for determination of oseltamivir and its metabolite oseltamivir carboxylate in plasma, saliva and urine, Journal of Chromatography B, **859**, 74-83 **(2007)**.

[22] K.Heinig, F.Bucheli; Sensitive determination of oseltamivir and oseltamivir carboxylate in plasma, urine, cerebrospinal fluid and brain by liquid chromatography-tandem mass spectrometry, Journal of Chromatography B, **876**, 129-136 **(2008)**.

[23] Q.Chang, M.S.Chow, Z.Zuo; Studies on the influence of esterase inhibitor to the pharmacokinetic profiles of oseltamivir and oseltamivir carboxylate in rats using an improved LC/MS/MS method, Biomed Chromatography, **23(8)**, 852-857 **(2009)**.

[24] F.Jabbaribar, A.Mortazavi, R.Jalali-Milani, A.Jouyban; Analysis of oseltamivir in Tamiflu capsules using Micellar electrokinetic chromatography, Chem.Pharm.Bull., **56**, 1639-1644 **(2008)**.

[25] E.Laborde-Kummer, K.Gaudin, J.Joseph-Charles, R.Gheyouche, H.Boudis, Dubost Jean-Pierre; Development and validation of a rapid capillary electrophoresis method for the determination of oseltamivir phosphate in Tamiflu and generic versions, Journal of Pharmaceutical and Biomedical Analysis, **50**, 544-546 **(2009)**.

[26] L.Milka Avramov Ivic, D.Slobodan Petrovic, Z.Dusan Mijin, M.Katica Drljevic-Duric; The qualitative determination of oseltamivir phosphate in tamiflu capsule by cyclic voltammetry, Hem.Ind., **65(1)**, 87-91 **(2011)**.

[27] M.Salem, M.H.Hamza Nashwa, A.B.Rizk Hamdy, A.Matter; Novel potentiometric method for determination of oseltamivir phosphate (Tamiflu) and its pharmaceutical applications. International Journal of Pharma. Research & Development, **2(6)**, 1-11 **(2010)**.