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(Review)

Mini analytical review of different techniques applied for oseltamivir phosphate estimation in various matrices

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ABSTRACT

Every year, the influenza virus infection causes significant morbidity and mortality due to its high contagiousness and ease of spread. The influenza virus family consists of four subtypes: A, B, C, and D. Both types of influenza viruses A and B cause outbreaks of disease in humans almost every winter throughout the world (known as flu season). So, their spreading must be controlled by using antiviral drugs such as oseltamivir phosphate (OSP). This drug is sold under the trade name "Tamiflu®" for the treatment of influenza infection. In this comprehensive review, multiple analytical techniques for quantifying OSP were used—including capillary electrophoresis, spectrophotometric analysis (UV, colorimetric and spectrofluorometric), electrochemical methods, HPLC, and LC-MS/MS—to quantify OSP in fixed-dose pharmaceutical dosage forms and other matrices such as human and animal plasma, saliva and urine. The data presented in this review paper can be used to successfully conduct additional analytical research for estimating OSP.

Keywords: Analytical approaches, Antiviral drug, Influenza infection, Oseltamivir phosphate

1. Introduction

Approximately 500,000 people die each year from influenza infections, which affect up to 25% of people worldwide (1). There are three ways that influenza viruses can spread: through aerosols, droplets, and direct contact. According to changes in temperature and humidity, small aerosol particles produced by coughing and sneezing can linger in the air for a number of hours or minutes (2). Although the role of aerosols in the spread of disease is still debatable, polymerase chain reactions have allowed for the identification of the influenza genome in aerosols (3). Thus, it is reasonable to believe that aerosols from infected individuals could be inhaled and end up in the upper or lower respiratory tract, which would spread the disease. Human-infecting influenza viruses can be divided into three major groups: A, B, and C (4). A serious type A influenza infection can result in widespread disease epidemics (3). Type A infection symptoms are frequently mistaken for those of other illnesses. While type A influenza can be life-threatening in severe cases, it can also subside on its own in some milder cases with fewer symptoms. Common infections like type A influenza can result in significant flu outbreaks. In addition to sneezing, a runny or stuffy nose, a sore throat, and fever, it can also result in body aches, headaches, chills, and fatigue (5). The most prevalent type of influenza, type A is known to cause pandemics and can pass from animals to people. Although influenza B shares many of the same signs and symptoms as influenza A, it is also very contagious and, in more serious cases, can be dangerous to health (2). However, this disease can only be transmitted from person to person. Year-round transmission of type B influenza can result in seasonal outbreaks. The influenza C virus is a less well-known strain that typically causes cold-like symptoms and sporadically lower respiratory infections, especially in young children under 2 years old. Although the virus has been found in pigs, dogs, and cattle in addition to humans, it is primarily a human pathogen. Rare swine-human transmission has also been reported (3). The two main influenza strains that commonly infect people and cause seasonal flu epidemics are influenza A and B (6). Although influenza A mutates more quickly than influenza B, both viruses are continuously evolving and giving rise to new strains from one flu season to the next.

By treating the patient's infection and assisting in preventing the serious illnesses that the flu can cause, such as bacterial pneumonia, antiviral medications are crucial tools in the fight against influenza. Antiviral medications, when used promptly, can lessen the severity of the flu's most severe symptoms and shorten the illness's duration by an average of one day (3). Oseltamivir phosphate (OSP) is an antiviral drug that decreases the influenza virus's ability to spread between body cells by preventing the virus from chemically severing ties with its host cell (7) . OSP is an antiviral

neuraminidase inhibitor that inhibits the influenza virus's neuraminidase in a powerful and selective manner (5). When hydrolyzed *in vivo* to the active form (oseltamivir carboxylate) a prodrug that is inactive, exerts pharmacologic activity. The infection of new host cells is prevented by this activated form's interference as a result of the release of influenza virus offspring from infected host cells. OSP can shorten the duration of symptoms by 0.5 to 3 days and decrease the length of shedding and viral titer (8). As it is administered via oral inhalation, it is marketed under the trade name "Tamiflu®" which administered for managing influenza infection (3)(9). When taken within 36 hours of the onset of symptoms, OSP can lessen the intensity and duration of influenza A or B-related symptoms. Recent media attention has focused on the antiviral medication OSP "Tamiflu®", which can be administered as the initial line of defense against the H5N1 and H1N1 influenza viruses. (3). Its chemical name is (3R,4R,5S) ethyl-4-acetamido-5-amino-3-(1-ethylpropoxy) phosphate carboxylate of cyclohexene, **(Figure.1)** (10). OSP is a white or nearly white powder with a molecular weight of 410.4 g/mol. It is practically insoluble in methylene chloride but freely soluble in water and methanol (10).

This review article aimed to describe several analytical methods for determining OSP in pharmaceutical dosage form, plasma, saliva, and urine. These methods comprised spectrophotometric, colorimetric, spectrofluorimetric, electrochemical, chromatographic, and capillary electrophoresis approaches.

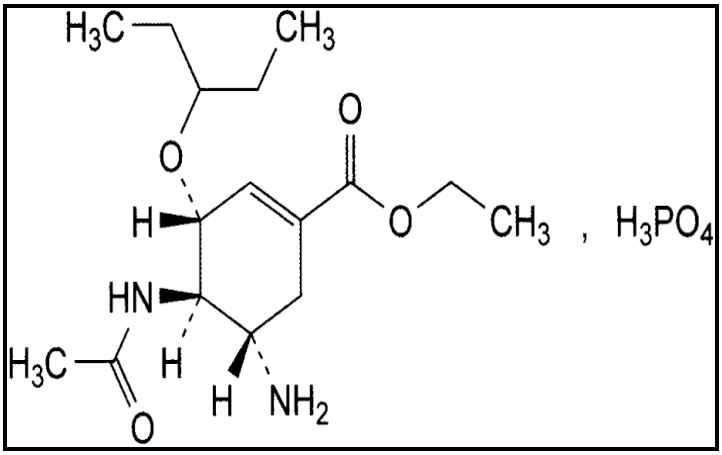

Figure.1: Chemical structure of Oseltamivir phosphate

2. Analytical techniques

2.1. Official and published analytical techniques for oseltamivir phosphate analysis

2.1.1. Official method

According to the British Pharmacopoeia (10), a reversed-phase HPLC method using octylsilyl silica gel (0.25 m x 4.6 mm, 5 m) as the stationary phase and acetonitrile, methanol, and potassium dihydrogen orthophosphate (20:40:40% w/v) as the mobile phase with a flow rate of 1.2 mL/min and UV detection was at 207.0 nm.

2.1.2. Published analytical methods

a. Spectrophotometric methods

UV spectrophotometric approaches

OSP can be detected directly in pharmaceutical preparation using methanol as a solvent at wavelengths of 208.5 nm with concentration range (4.00 -24.00 μg/mL) (11), 217.0 nm with concentration range (10.00 -70.00 μg/mL) (12) and 215.0 nm with concentration range (10.00 -60.00 μg/mL) (13).

Colorimetric methods

Colorimetric technique was employed for estimate of OSP in capsules form and its fluctuates with the reaction between OSP and 3 different chromogens: with p-dimethyl amino cinnamaldehyde to produce an orange-red colored chromogen at 530.0 nm, with 4-aminophenazone to generate an intense violet colored chromogen at 545.0 nm, and with ferric chloride and 1,10-phenanthroline to develop a blood red colored chromogen at 512.0 nm (14).

For the purpose of determining OSP in pharmaceutical dosage form, a different colorimetric method was reported that depends on the oxidation of OSP by alkaline potassium permanganate at room temperature. The resulting green manganite ions absorb at a wavelength of 635.0 nm (15).

Advanced colorometric methods (M1 and M2) for the assay of OSP in pharmaceutical preparation through the olefenic double bond using fast green FCF to estimate the amount of

unreacted potassium permanganate. Method M1 is based on the reaction of potassium permanganate with the olefenic double bond in OSP. In Method M2, the olefenic double bond in OSP is treated with a Lemieux reagent (a mixture of KMnO4 and NaIO4), and the aldehyde produced by 3-methyl-2-benzothiazolinone hydrazone (MBTH) is estimated. Maximum absorption for the color created by the M1 and M2 methods is at 620.0 nm and 654.0 nm, respectively (16) .

Anionic dyes like Congo red and bromochlorophenol blue create colored ion-pairing complexes when they react with OSP to create a colored product that can be detected at 507.0 nm for the red complex and 589.0 nm for the blue complex at a wavelength of ethyl acetate (17).

Colormetric technique for determination of OSP in pills through reaction between the OSP and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole at alkaline medium (pH 10) to form deep brown adduct, exhibiting maximum absorption (λ max) at 464.0 nm (18).

Spectrofluorimetric method

A technique for measuring OSP in pill form uses the interaction of OSP with fluorescamine in a borate buffer solution of pH 8.50 to produce highly fluorescent derivatives that are detected at 483.0 nm using an excitation wavelength of 381.0 nm (19).

A Fourier Transform Infrared Spectrophotometric method

Spectrophotometric technique for measuring OSP in pharmaceutical dosage form using a deuterated trigylcine sulfate DTGS detector. Conventional fused KBr disk spectra were recorded between 4000 and 400 cm-1, by averaging 64 scans for each spectrum (20).

b. Electrochemical determination

There have also been report of cyclic voltammetry for OSP determination in its dosage form using polycrystalline gold and glassy carbon electrodes as well as using gold electrode as a working electrode (21).

Another method for determining OSP in its dosage form was reported to use differential pulse voltammetry and a working electrode of eight microelectrodes (22).

There have also been reports of OSP determination in its capsule form using adsorption-stripping voltammetry with a thin-film mercury electrode as a working electrode (23).

A potentiometric approach using plasticized PVC membranes doped with ion-pair complexes based on drug-phosphomolybdate and drug-tetraphenylborate as electroactive materials was reported for the determination of OSP in its pharmaceutical preparation (24).

Ion Selective Electrode method for determination of OSP in its dosage form, poly vinyl chloride membrane sensors were used (25).

c. Chromatographic methods

Capillary electrophoresis

For the purpose of determining OSP in its dosage form, an electrophoretic buffer consisting of 50 mM sodium phosphate, pH 6.3, and applying a potential of 15 kV at 25 °C with UV detection at 226.0 nm was chosen (26).

High-performance liquid chromatography

As shown in **Table 1**, several liquid chromatographic techniques have been published for the detection of OSP in various metrics.

Table 1. HPLC methods for the determination of OSP in different metrics

Used Column	Mobile phase	Detection	Application	Reference
An Agilent Extend C18 (4.6 mm X 3250 mm, 5.0 µm)	20 mM potassium dihydrogen phosphate solution and acetonitrile (60:40, v/v) with flow rate of 1.2 mL/min	UV at 215.0 nm	Pharmaceutical dosage form.	(13)
X-Terra, RP C18, 4.6-× 150mm, 5.0 µm	Acetonitrile and 0.05 M bicarbonate buffer, pH 10 (30:70, v/v) with flow rate 1 mL/min	UV at 220.0 nm	Pharmaceutical dosage form.	(17)
Kromasil C18, 5m m X 250mm, 4.6 mm	Acetonitrile and 0.2% triethylamine buffer (50:50, v/v), apparent pH adjusted to 3_0.1 with 10% phosphoric acid with flow rate 1mL/min	UV at 215.0 nm	Pharmaceutical dosage form.	(27)

C18 250mm X 4.6mm, 5µm	Ammonium Acetate buffer pH 6.9 and acetonitrile (60:40, v/v) with flow rate 1 mL/min	UV at 220.0 nm	Pharmaceutical dosage form.	(28)
a Hypersil Gold column (150mm×4.6 mm,5µm)	Methanol and phosphate buffer (pH 2.5; 0.1 M) (50:50, v/v) with flow rate of 1.0 mL/min	UV at 220.0 nm	Pharmaceutical dosage form.	(29)
Column Chromolith C18 (100 mm,4.6 mm,5µm)	Format buffer (100 mM, pH 4) and acetonitrile; (80:20, v/v) with flow rate 3 mL/min	PDA at 220.0 nm	Pharmaceutical dosage form.	(30)
Inertsil® ODS-2 column (250 mm , 4.6 mm, 5 µm)	50 mM potassium phosphate buffer: MeOH (55: 45, v/v) with flow rate 1.0 mL/min	UV at 215.0 nm	Pharmaceutical dosage form	(31)
a Zorbax CN column (150mm×4.6 mm; 5 µm)	Methanol and 0.04M formic acid pH 3.0 (50:50, v/v) with flow rate 0.2 ml/ min	UV at 226.0 nm	Pharmaceutical dosage form	(32)
Shimpack (150mm×4.6mm. , 5µm) which was protected by a Shim-pack G-ODS guard column (10mm×4.0mm., 5µm)	0.05M phosphate buffer containing triethylamine (1 mL/L; pH 3.0) and acetonitrile (70:30, v/v) with flow rate of 1.6 mL/min	UV at 215.0 nm	Human plasma	(33)
Xterra_ MS C18 (2.1 X 150 mm, 3.5 µm)	Acetonitrile–20 mmol/l potassium dihydrogen phosphate buffer (pH 3.0) (8:92, v/v) with flow rate 0.2 ml/ Min	UV at 250.0 nm	Human plasma	(34)
Hypersil BDS cyano 5 µm column (250 mm X 4.6 mm)	50 mM ammonium acetate: acetonitrile with gradient system composed of 50 mM ammonium acetate: acetonitrile (95:5, v/v) for 4 min, followed by 50 mM	UV at 230.0 nm	Human plasma	(35)

	ammonium acetate: acetonitrile (70:30, v/v)) for 7 min, and finally 50 mM ammonium acetate: acetonitrile (95:5, v/v) for 4 min			

High-performance liquid chromatography tandem mass

Development of HPLC- MS/MS method for detection of OSP and its active metabolite in human plasma using mobile phase consist of acetonitrile/water (30:70, v/v) containing 0.1 % formic acid and a YMC-Triart C18 (100 × 2.0 mm, 5 µm) as stationary phase (36).

Establishing an HPLC- MS/MS technique to measure OSP in human and animal plasma and urine using mobile phase consist of MeOH–80 m*M* aqueous formic acid, pH 3 (50:50, v/v) with flow rate 0.5 mL/min and a Nova-Pak CN HP cartridge (53100 mm, 4 mm, Waters) as stationary phase (37).

Innovation of the HPLC-MS/MS approach for assessment of OSP in human plasma, saliva and urine using mobile phase consist of acetonitrile–ammonium acetate buffer (pH 3.5; 10 mM) (90: 10, v/v) at a flow rate of 500 µL/min and a ZIC-HILIC column (50 mm×2.1 mm) as stationary phase (38).

Validation parameters of some literature methods were mentioned below in **Table 2.**

Table 2: Validation parameters of some literature methods

Analytical methods	**Validation parameters**				**References**
	Linearity Range	**Correlation Coefficient**	**LOD**	**LOQ**	
Spectrophotometric method	4.00-24.00 µg/mL	0.9990	0.342 µg/mL	1.036 µg/mL	(11)

Electrochemical method	1.00 × 10-5 – 1.00 × 10-2 M	0.9987	9.31 × 10-6 M	2.793 × 10-5 M	(24)
Chromatographic method	0.075-0.75 µg/mL	0.999	0.0162 µg/mL	0.324 µg/mL	(28)

3.Conclusion

The current study summarizes the various testing techniques for identifying the presence of OSP in various matrices, including pharmaceutical formulations, serum, plasma, urine and saliva samples, that have been reported in the literature. OSP were measured using analytical methods such as spectroscopy, chromatography, and electrochemical methods in bulk pharmaceutical dosage form. The compilation of review's main objective is to compile as much data on the OSP analytical techniques as possible and carefully analyze it. The study's findings show that there aren't many analytical methods based on UV-Vis spectrophotometry and HPLC, and there aren't many papers that use hyphenated methods either. Additionally, based on the data provided for OSP analysis, the method most frequently used to measure drugs in pharmaceutical matrices and other biological matrices is HPLC with UV detection because it yields precise results with little effort. HPLC-MS/MS techniques, which offered exceptional selectivity, sensitivity, and a selection of methods, were also used to study OSP and its metabolites in biological samples.

Conflict of Interest

The Author declares no conflict of interest.

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