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Review Article

ANTIVIRAL DRUGS FOR INFLUENZA VIRUSES

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ABSTRACT

Antiviral drugs have significant action against influenza viruses A and B. Virus spread deadly disease in which many people die, and the country economy greatly suffer. Presently, most of the people need to get vaccination, which is depending on the dose limit in humans. It reacts directly or sometimes indirectly in the form of metabolites. However, it is mandatory to know how much drug is absorbed or metabolites concentration after administered. Therefore, pharmacokinetics data is very crucial for all drugs. Our review discusses the mechanism of drugs action and their activity and also describes how antiviral drugs and their metabolites are determined using highly sensitive instruments such as high-performance liquid chromatography (HPLC), ultra-pressure liquid chromatography (UPLC), and mass spectrometry (MS). Therefore, the present review gives brief information about antiviral drugs, their activity, biotransformation and analytical methods for quantification and this information will be helpful for any future studies done by experts in this field and will be beneficial for research scientists and influenza experts of all over the globe.

Keywords: Antiviral drugs, Influenza virus, Oseltamivir, Laninamivir, Zanamivir, Peramivir, Metabolite, HPLC, UPLC, LCMS

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INTRODUCTION

Respiratory system is commonly infected with influenza viruses [1] and mainly orthomyxoviridae family (A, B, C). Worldwide, 2.5–5 hundred thousand people were reported death and 3-5 million diagnosed with acute illness [2] annually. Viruses classified with respect to activity of glycoproteins neuraminidase (N/NA), hemagglutinin (H/HA) and M2 ion channel. Influenza A and B are seasonal influenza with different subtypes such as H1–H16 (16 hemagglutinin) and N1–N9 (9 neuraminidase). Well-known viruses H1N1 and H3N2 are generally subtyped of seasonal virus (Influenza A). The avian influenza virus H5N1 [3–5] have great interest among the people but swine influenza (novel H1N1) is completely different from seasonal H1N1 influenza virus [6]. In the 20th century, 20–50 million people from different countries were killed with Spanish flu, 1–2 million from Asian flu, and 7 hundred thousand from Hong Kong flu [7, 8]. Virus infection was initiated by hemagglutinin after binding with a receptor called Sialic acid, 1 (fig. 1). Then other cells infected by new viruses with the help of neuraminidase [9, 10]. Life threat influenza can only be overcome with annual immunization. However, the limitation related to efficacy, timeline and design of the vaccine production increase the need for antiviral drugs. The approved antiviral drugs M2 ion channel and NA inhibitors are the basic option for therapeutic treatment with prevention. This classification based on resistance behavior, tolerance study, pharmacokinetics and drugs mechanism of action [11–17].

Neuraminidase inhibitors

Proteins (H and N) are available on the walls of influenza virus surfaces. New viral molecule was released by host cell due to enzymatic function of neuraminidase, will help to catalyzed glycoside hydrolysis. As a result, α-ketosidically connected sialic acid with glycolipid, glycoprotein, and hemagglutinin divided increased infectious molecule. However, the nascent virus is still securing with host cell due to an interaction between HA/HA receptor. The viral molecule exhibit HA/HA receptors on the surface, which enables new molecules stick together. The viral particles emerged from the clump and host cell when neuraminidase receptor removed sialic acid. In the respiratory, glycosylated components are digested by neuraminidase, contribute viral infection. Therefore, design-based modern drug act on the site of neuraminidase inhibitors, depend on its clinical efficacy, mechanism, genetic role are the key feature for success [18–20]. The neuraminidase inhibitors prompt action and excellent mortality is the main reason for its demand [21, 22]. The drugs and their pharmaceutical formulations worked for the treatment of deadly disease are *Oseltamivir* 2, *Laninamivir* 3, *Zanamivir* 4, *Peramivir* 5 [23–25] (fig. 1), established on sialic acid transformation [26–28]. Influenza experts from different countries set up a neuraminidase inhibitors susceptibility network (NISN) for the outcome of clinical result and monitoring development process. This committee is participating to monitor liability studies for encouraging the people to know the exact method of evaluation and investigation process to control influenza virus.

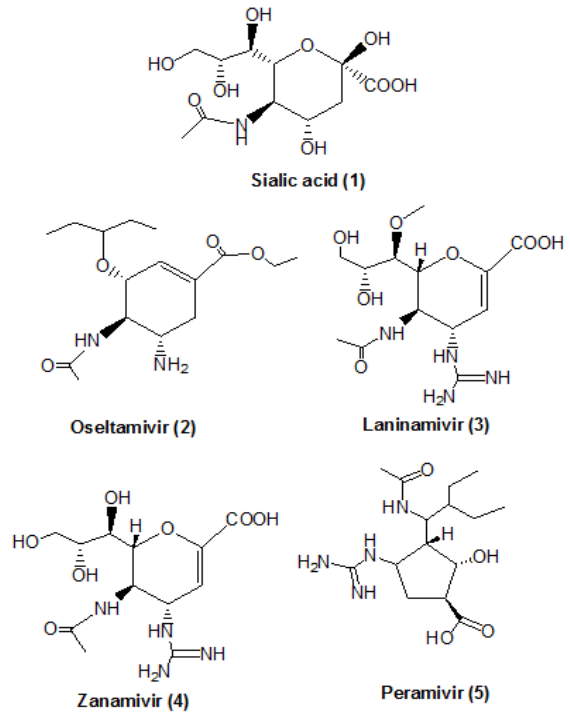

Fig. 1: Chemical structures of *Sialic acid* 1, *Oseltamivir* 2, *Laninamivir* 3, *Zanamivir* 4, and *Peramivir* 5

M2 ion channel inhibitors

Amantadine 6 and *Rimantadine* 7 (fig. 2) licensed by United States of America (USA) in the years 1966 and 1993 respectively for the treatment of influenza A as M2 ion inhibitor. Allosteric inhibition controls the movement and activity of M2 by the presence of methyl group in amantadine; consequently, it blocks the release of RNA. It was found that M2 ion is successful for influenza A but ineffective towards influenza B. Therefore, these drugs are precise only for influenza A. M2 inhibitor has extraordinary response to antibiotic resistance [29, 30], but it is very less compared to neuraminidase inhibitors [31, 32]. Through endocytosis process, host cell allows viruses to enter. At low pH, M2 channel permits the virus to viral interior due to an activity of endosome responsible for maturation and growth of molecule. The reproduction is continued in cytoplasm, viral ribonucleic acid (RNA) and genetic material released from protein matrix [33]. Therefore, it is very important to develop inhibitors functioning against the virus but in native condition.

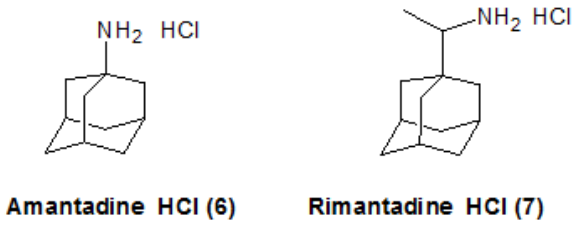

Fig. 2: Chemical structures of *Amantadine* 6 and *Rimantadine* 7

Biotransformation

Oseltamivir

Oseltamivir phosphate 8 is significant ester prodrug, applied orally for prevention and curing of influenza disease (A and B). It's brand name Tamiflu is available in quantity of 75 mg. The approved regimen for adults is 75 mg daily two times for 5 d with respect to influenza (A and B), but it is different for the prophylactic regimen. For control and prevention, daily dose is 75 mg once; for a minimum of 10 d up to 42 d. The intake dose for healthy participants follows linear pharmacokinetics in the range of 75–675 mg [34–36]. *Oseltamivir* is absorbed in the gastrointestinal tract then metabolized and converted into *Oseltamivir carboxylate* 9 (fig. 3) in the liver and later on, uniformly distributed in the body. After oral administration, therapeutic action continues for 30 min with the metabolite (80%) but the remaining 20% available 3–4 h. in plasma. The drug half-life is 1.8h. It was found that renal disease may affect active metabolite clearance and found that cytochrome P450 has only 3% interaction with plasma protein. Similar action occurs during glucuronosyl transferases [37–38].

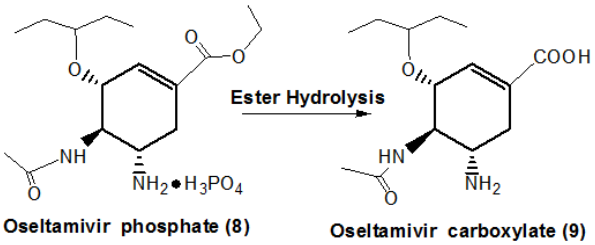

Fig. 3: Chemical structures of *Oseltamivir phosphate* 8 (prodrug) and *Oseltamivir carboxylate* 9 (active metabolite)

Zanamivir

Relenza is the trade name of *Zanamivir*, an anti-influenza inhibitor, which obtained the regulatory approval in 1999. It targets respiratory site and available as inhaled powder. The pharmaceutical formulation has high potential against people diagnosed with deadly disease influenza A (H5N1). The maximum amount of 20 mg (10 mg twice) is allowed daily as inhalation, continued for 5 consecutive days. However, for prophylaxis, time and quantity limit is 10 mg daily for 28 d for children age five or more, and same for adults [39–40]. The nasal spray set down into posterior nasal and nostril approximately 88% but 13% in lung, 78% in oropharynx deposited active site for replication of influenza virus [41]. Settle down in stomach (17%) and lung (2%) dose intake orally after fifty minutes [42]. Sputum and nasal wash sample resulted concentration about 50% inhibitory after 24 and 12 h. respectively [43] for healthy volunteers with 10 mg daily dose but 20 mg amount with infected sample quantified after 4 d [44]. The unchanged drug (90%) was found with excreted urine sample recommended without biotransformation [41]. Therefore, the drug not metabolized in the liver.

Peramivir

Peramivir is a predominant cyclopentane based neuraminidase inhibitor. The binding capacity of the inhibitor will be half (IC50) with a concentration in the range 0.09–1.39 nM and 0.6–10.8 nM respectively for influenza A and B strains. This value is for *Oseltamivir carboxylate* 0.01–2.24 nM, 6.39–24.3 nM and for *Zanamivir* 0.30–2.32 nM, 1.53–17.0 nM [45]. The drug is productive without any complication for seasonal influenza viruses [46]. The *Peramivir* IV was first launched with trade name Rapiacta in Japan (2010). The drug prescribed for acute treatment in adults is 300 mg daily but for severe cases, hospitalized patients, 600 mg daily dose is recommended. The drug eliminated through renal and not metabolized in the human liver.

Laninamivir

The function of *Laninamivir* 3 is similar to *Zanamivir*, potent neuraminidase inhibitor for influenza viruses (A and B) [47–48]. *Laninamivir octanoate* is an inactive prodrug and the commercially approved product (inavir) is available in Japan since 2010 [49]. Single dose of *Laninamivir octanoate* hydrate is limited to children with age above 10 y is 40 mg and below 10 y is 20 mg. *Laninamivir*, active metabolite of *Laninamivir octanoate* (LO) formed within 24 h due to hydrolysis in the octanoyl ester site. When LO is mixed with water in the human body, equilibrated to form 3-acyl form 10, 2-acyl form 11 (fig. 4) known as major and minor metabolites [50].

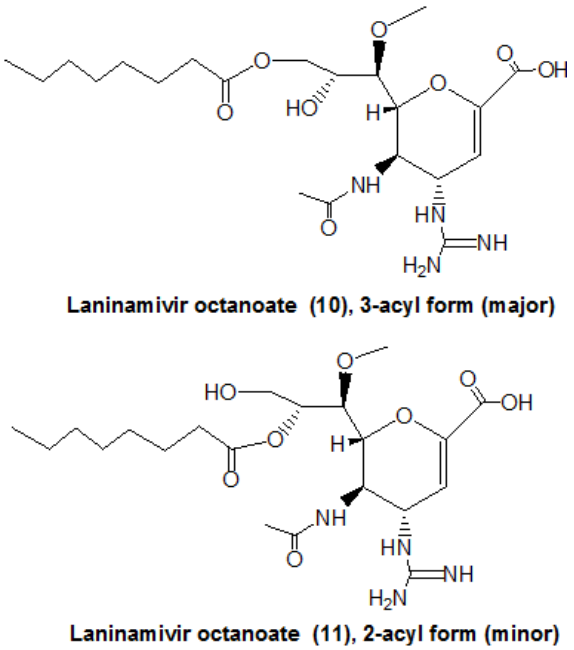

Fig. 4: Chemical structures of *Laninamivir octanoate* 3-acyl form 10 and 2-acyl form 11

Amantadine

Amantadine 6 is a very important drug as M2 proton channel inhibitor for influenza A. Symmetrel is the brand name normally used for *amantadine*. Its rate of absorption is high for young people compared to elder independents. Renal insufficiency does not appear to affect absorption, nor does the formulation used. First relative bioavailability studies of the oral administration reported that 86% recovery of the original dose (2–4 mg/kg) within 96 h from urine samples of five healthy men [51]. In case of ingestion after 72 h, 105 mg (52.5%) of the original dose was found in old man (84 y) urine [52]. Eight metabolites originated during metabolism. Besides the major metabolite produced by N-acetylation which is N–acetylamantadine 12; other metabolites were observed. These are N–methylamantadine 13, N–dimethylamantadine 14, N–methyleneamantadine 15, N–formylamantadine 16, and metabolites 17, 18 and 19 (fig. 5) [53].

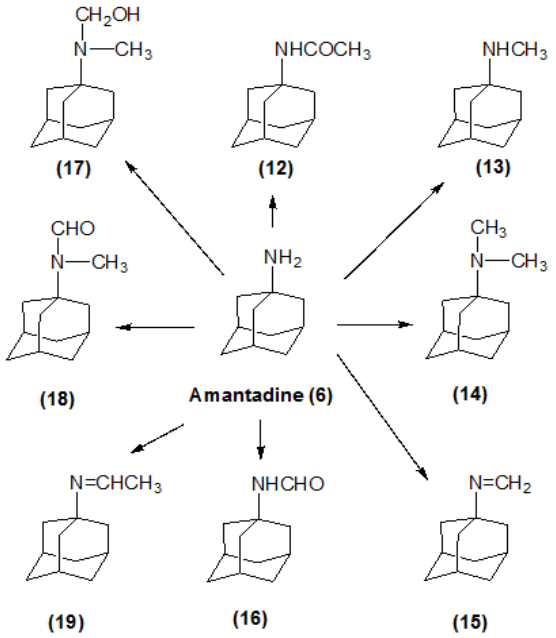

Fig. 5: Chemical structures of *amantadine* and *amantadine* metabolites

Rimantadine

Pharmaceutical formulation of *Rimantadine* is known as flumadine. Recommended daily dose for adults is 200 mg for 11–42 d, for children of 1–9 y (5 mg/kg) 150 mg is the maximum limit. Several studies concluded that 10% of the amantadine dose and 75% of the *Rimantadine* dose are metabolized in the liver [54–57]. The metabolites are determined as free *Rimantadine* 7, m–hydroxyrimantadine 20, p–hydroxyrimantadine (equatorial 21 and axial 22) (fig. 6) [58–62].

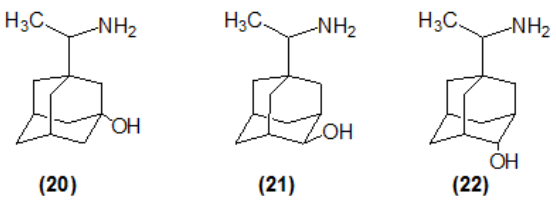

Fig. 6: Chemical structures of *m*-hydroxyrimantadine (20) and the equatorial (21) and the axial (22) epimers of *p*-hydroxyrimantadine

Analytical techniques

UV–visible spectrophotometer

UV–visible spectrophotometer is a simple and common technique for quantification of pure, pharmaceutical formulations and biological fluids. First order derivative spectrophotometry applied for Oseltamivir capsules having sophisticated additional software for pharmaceutical industry [63]. The drug (λmax=217, 208.5 nm) is stable and suitable with different stress condition in dosage form [64, 65]. Potassium permanganate as oxidizing agent in alkaline medium, no interference from excipients for capsules [66]. The pure drug with formulation is determined by monitoring reaction mechanism in buffer medium with 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole [67]. *Zanamivir* is evaluated in tablet formulation at 260 nm, validated respect to an international conference on harmonization guidelines [68, 69]. Amantadine derivative and ion pair complex was developed with 1, 2-naphtoquinone-4-sulfonate, bromocressol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and methyl orange in pharmaceutical preparation and plasma samples [70–73]. Rimantadine can also be determined spectrophotometrically using 1, 2-naphtoquinone-4-sulfonic acid sodium salt as derivatizing reagent in pharmaceutical dosage form [74].

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is an accurate instrument for pharmaceutical analysis. The inhibitor demand is increasing with time in the world because influenza viruses causes significant health alert. All determined drugs either applying ultraviolet detector or fluorescence detector. As well as used C_{18} and C_8 column with a different manufacturer. The single mobile phase is usually a combination of two or more solvents. The ratio of the mobile phase is changed with time; gradient method investigation resulted in drugs with a short time. During the analysis buffer also added for the preparation of the mobile phase. Therefore, for the quantification of neuraminidase and M2 ion channel inhibitor, numerous HPLC methods are developed based on the mobile phase composition and the stationary phase for pure bulk drug, tablet dosage form and biological fluids (table 1) [75–102].

High-performance thin layer chromatography

Currently, the high-performance thin layer chromatography (HPTLC) has high demand because it involves fewer samples clean-up, high throughput value, low-cost methodology. The fundamental advantage of HPTLC leads to compute several samples at one time. *Oseltamivir* study performed on silica gel 60F-254 coated aluminum plate consists of mobile phase (toluene: methanol: ammonia=3.5:1.5:0.2, v/v/v), detected at R_f value (0.45±0.02) for bulk and its dosage form [103]. This technique applied to evaluate Zanamivir in a pharmaceutical formulation having a correlation coefficient close to one [90, 104].

Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) is strong enough to large molecules additionally for small (organic/inorganic), separation technique controlled by electric field inside narrow bore capillaries. The method has several benefits with respect to fewer amounts of solvent, analyte and high resolution, applicable to forensic, environmental, clinical and pharmaceutical industry. It is a specific technique for the separation of the molecule along with enantiomers. The generic version of Oseltamivir was separated on fused capillary under potential (−15 kV) at room temperature. The analysis time is 1.5 min and detected at 226 nm included phosphate buffer [105]. Unmodified silica capillary, 4-methylbenzylamine in ethanol (20%) as background electrolyte, maintained+20 V for separation at 210 nm within 3 min in Rimantadine tablets [106]. Pharmaceutical products can be estimated with derivatization agent 1, 2-naphthoquinone-4-sulfonic acid by CZE in alkaline medium [107].

Table 1: Important parameters for the determination of drugs using HPLC

Drug	Mobile phase	Stationary phase	Flow rate (ml/min)	Detector (nm)	Application	Reference
Oseltamivir	Acetonitrile: Methanol = 50:30, v/v	Purosphere C_{18} (250×4.6 mm, 5µm)	1.0	227	API	[75]
	Potassium dihydrogen orthophosphate (pH 3.2): Acetonitrile: Methanol = 60:20:20, v/v/v	YMC Pro C_8 (150×4.6 mm, 5µm)	1.5	207	Capsules	[76]
	Triethylamine (0.1%): Methanol = 60: 40, pH 10.04 with Conc HCl	Water's X Bridge C_{18} (250×4.6 mm, 5µm)	0.9	226	Bulk and pharmaceutical formulations	[77]
	Phosphate buffer (0.02 M, pH 5 with 0.02 M TEA): Methanol = 50:50, v/v	Purosphere Star RP–18e (150×4.6 mm, 5 µm)	1.5	215	Bulk drug and capsules	[78]
	Mobile phase A–KH_2PO_4 buffer (0.05 m, pH 6): Acetonitrile = 90:10, v/v Mobile phase B–KH_2PO_4 buffer (0.05 m, pH 6): Acetonitrile = 10:90, v/v Gradient Programme	ODS (50×4.6 mm, 5µm)	1.5	220	Bulk drug	[79]
	Ammonium acetate buffer (pH 6.9): Acetonitrile = 60:40, v/v	Purosphere C_{18} (250×4.6 mm, 5µm)	1.0	220	Bulk drug and dosage	[80]
	Sodium acetate buffer (pH 4.5): Acetonitrile = 55:45, v/v	Phenomenex Luna C_{18} (250×5 mm, 5 mm)	1.5	231	Bulk and dosage form	[81]
	Acetonitrile: Nitric acid (10 mmol, pH 3) = 60:40, v/v	Phenomenex C_{18} (250x4.6 mm, 5 µm)	1.0	λex 470 λem 541	Capsules and spiked plasma	[82]
	Potassium dihydrogen orthophosphate (pH 3.5 with OPA): Acetonitrile = 50:50, v/v	Princeton Spher C_{18} (250x4.6 mm, 5 µm)	1.0	254	Pharmaceutical dosage form	[83]
	Bicarbonate buffer (0.05 M, pH 10): Acetonitrile = 70: 30, v/v	X-Terra RP_{18} (4.6×150 mm)	1.0	220	Pharmaceutical product	[84]
	Mobile phase A: Triethylamine (0.2%, pH 3 with OPA) buffer Mobile phase B: Acetonitrile Gradient programme	Kromasil C_{18} (250×4.6 mm, 5 µm)	1.0	215	Quality control sample	[85]
	Potassium dihydrogen orthophosphate (0.05 mmol, pH 3 with OPA): Methanol: Acetonitrile = 60:25:15, v/v/v	Oyster RP18e (250×4.6 mm, 5 µm)	1.0	207	Bulk and dosage form	[86]
	Phosphate buffer (0.05 M, pH 3) with triethylamine (1 ml/l): Acetonitrile = 70:30, v/v	Shimpack ODS (150×4.6 mm, 5 µm)	1.6	215	Human serum	[87]
	Formic acid (0.04 M, pH 3 with NaOH): Methanol = 50:50, v/v	Zorbax CN (150×4.6 mm, 5 µm)	1.2	226	Capsule	[88]
Zanamivir	Methanol: Water = 95:5, v/v	Sunfire C_{18} (4.6×250 mm, 5µm)	1.0	285	Tablet dosage form	[89]
	Phosphate buffer (0.02 M, pH 5): Methanol = 50:50, v/v	Agilent zorbax eclipse C_{18} (150× 4.6 mm, 5 µm)	1.0	230	Bulk and capsule	[90]
	Potassium dihydrogen orthophosphate (pH 4): Acetonitrile = 40:60, v/v	Xterra Symmetry C_8 (4.6×150 mm, 3.5 µm)	0.5	230	Tablets	[91]
	Ultrapure water: Acetonitrile = 98:2, v/v	BDS Hypersil Cyano (250×4.6 mm; 5 µm)	0.5	230	Bulk drug	[92]
	Acetonitrile: Water = 50:50, v/v	Supelcocil C_{18} (150×4.6 mm, 5 µm)	1.2	230	Human plasma and pharmaceutical formulations	[93]
Amantadine	Mobile phase A: Acetonitrile (5%) in water Mobile phase B: Acetonitrile Gradient programme	Hypersil C_{18} (150×4.6 mm, 5 µm)	1.0	λex 262 λem 430	Rat plasma	[94]
	Ammonium acetate buffer (0.02 M): Methanol = 12:88, v/v	Inertsil ODS-3V (250×4.6 mm, 5 µm)	1.5	226	Bulk and formulation	[95]
	Water: Acetonitrile = 40:60, v/v	Phenomenex RP C_{18} (250×4.6 mm, 5 mm)	1.0	273	Bulk and dosage form	[96]
	Acetonitrile: Sodium acetate buffer (10 mmol, pH 3.5 with acetic acid): Methanol = 20:70:10, v/v/v	Nucleosil CN (250x 4.6 mm, 5 µm)	1.5	λex 293. λem 382	Human plasma	[97]
	Mobile phase A: 0.1% trimethylamine solution (pH 3)	Diamonsil C_{18} (200x 4.6 mm, 5 µm)	1.0	210, 280	Quality control granules	[98]

	Mobile phase B: Acetonitrile Gradient programme					
	Water: Methanol = 15:85, v/v	Lichrospher C_{18} (150×6 mm, 5 µm)	1.0	256	Rat plasma	[99]
	Methanol: Acetic acid (pH 7) with water = 4:1, v/v	Kanto C_{18} (150×4.6 mm, 5 µm)	0.8	λex 342 λem 410	Rat plasma	[100]
Rimantadine	Ethylnitrile and octane sulfonic acid buffer (0.005 M, pH 6.7) = 60:40, v/v	C_{18} (250× 4.6 mm)	1.0	259	Medicinal form	[101]
	CH3OH: phosphate buffer, 25 mmol/l (pH 3) = 50:50, v/v	Monolithic RP (100×4.6 mm)	0.5	λex 340 λem 455	Human urine	[102]
	Methanol: Acetic acid (pH 7) with water = 4:1, v/v	Kanto C_{18} (150×4.6 mm, 5 µm)	0.8	λex 342 λem 410	Rat plasma	[100]

Ultra pressure liquid chromatography

Ultra pressure liquid chromatography (UPLC) is obligatory in respect to the analysis time; resolution enhanced its reliability in the pharmaceutical industry. The achieved data from developed methods are robust and accurate. The common available columns for UPLC are acquity BEH C_8, acquity BEH C_{18}, acquity BEH RP_{18} and acquity BEH phenyl. UV–Visible detector is commonly used detector for UPLC analysis. The method for *Oseltamivir* and its degraded product is established within 5 minute using mobile phase composition phosphate buffer, pH 3.5 and methanol (80: 20, v/v), detected at 207 nm [108].

Liquid chromatography-mass spectrophotometry

Liquid chromatography-mass spectrophotometry technique is involved in the physical separation of compounds by liquid chromatography (LC) combined mass spectrometry (MS) for mass analysis [109–113]. Liquid chromatography can be high pressure or ultra-pressure. The structure of molecule elucidate after confirming the chemical composition of charged particles with respect to mass-to-charge ratio. The molecule ionized by MS to produce fragments and its mass-to-charge ratio measured. This technique maintains high sensitivity compared to traditional techniques and selective to many applications. It has an excellent impact in the bioanalysis field mainly confronted to pharmacokinetics investigation of pharmaceuticals. Generally, LC installed octadecyl (C_{18}) silica column but nowadays hydrophilic interaction based liquid chromatography (HILIC) has received more attention and recommended due to its potential to retain highly polar molecule with hydrophilic nature, but it needs to apply buffer as the mobile phase to minimize analyte and stationary phase ionic interaction. The LCMS method that was reported for inhibitors is presented in table 2 [114–137].

Table 2: LCMS method for neuraminidase and M2 ion channel inhibitors

Mobile phase	Stationary phase	Sample	Flow rate (ml/min)	Run time(min)	Application	Reference
Ammonium formate (10 mmol, pH 3): Acetonitrile=20:80, v/v	Synergi C_{18} (150×4.6 mm, 4 µm)	Amantadine	0.8	2.5	Human plasma	[114]
Mobile phase A: Ammonium formate (5 mmol, in water) Mobile phase B: Acetonitrile; Gradient programme	Eclipse Plus C_{18} (50x3 mm, 3.5 µm)	Amantadine	---	5.3	Human plasma	[115]
Mobile phase A: Ammonium formate (10 mmol/l, 0.1% formic acid) Mobile phase B: Acetonitrile; Gradient programme	Kinetex XB C_{18} (2.1× 100 mm, 2.6 µm)	Amantadine, Rimantadine	0.3	15	Chicken tissue and eggs	[116]
Mobile phase A: Formic acid (0.1%) in water Mobile phase B: Acetonitrile; Gradient programme	Eclipse Plus C_{18} (100x2.1 mm, 1.8 µm) Zorbax SB C_{18} (100 ×2.1 mm, 3.5 µm)	Amantadine, Rimantadine	0.35	5	Chicken jerky	[117]
Mobile phase A: Formic acid (0.1%) in water Mobile phase B: Formic acid (0.1%) in acetonitrile; Gradient programme	ZIC-HILIC (50×2.1 mm, 5 µm)	Zanamivir	0.4	15	Milli-Q water, sewage effluent, influent and surface water	[118]
Acetonitrile: 0.1% Formic acid = 90:10, v/v	Hydrosphere C_{18} (150x4.6 mm, 5µm)	Oseltamivir and oseltamivir carboxylic acid	0.5	5.5	Human plasma	[119]
Mobile phase A: Formic acid (0.1%) in water Mobile phase B: Methanol; Gradient programme	XDB C_{18} (2.1×150 mm, 3.5 µm)	Amantadine, Rimantadine	0.3	10	Chicken, pig, pork, duck (liver, kidney, egg)	[120]
Mobile phase A–Methanol: Water=1:99, v/v Mobile phase B–Methanol: Water=99:1, v/v Gradient programme	Eclipse Plus C_{18} (50×2.1 mm, 5µm)	Oseltamivir and oseltamivir carboxylate	---	4.5	Human plasma	[121]
Ammonium formate (10 mmol): Acetonitrile=30:70, v/v	Symmetry C_{18} (100×4.6 mm, 5 µm)	Oseltamivir and oseltamivir carboxylate	1.0	2	Human plasma	[122]
Ammonium acetate (10 mmol,	ZIC-HILIC (150x2.1	Oseltamivir and	0.3	10	Rat Plasma	[123]

pH 6): Acetonitrile=17:83, v/v	mm, 3.5 µm)	oseltamivir carboxylic acid				
Mobile phase A: Formic acid (50 mmol) in water Mobile phase B: Formic acid (50 mmol) in acetonitrile; Gradient programme	Symmetry C_{18} (3×150 mm, 5 µm) Hypercarb (3×100 mm, 5 µm)	Oseltamivir, oseltamivir carboxylate, zanamivir, amantadine, rimantadine	0.4	20	Poultry muscle	[124]
Mobile phase A: Formic acid (0.1%) Mobile phase B: Methanol; Gradient programme	Synergi C_{18} (150×2.0 mm, 4 µm)	Oseltamivir and oseltamivir carboxylate	0.25	9	Human plasma	[125]
Mobile phase A: Ammonium acetate (10 mmol, 1% acetic acid) Mobile phase B: Acetonitrile; Gradient programme	ZIC-HILIC (50x2.1 mm, 5 µm)	Zanamivir	0.5	4	Human plasma	[126]
Ammonium acetate (10 mmol, pH 3.5): Formic acid (0.1%) in acetonitrile=20:80, v/v	Chromatopack C_{18} (50×3 mm, 3.0 µm)	Oseltamivir and oseltamivir carboxylate	0.6	1	Human plasma	[127]
Mobile phase A: Ammonium acetate (10 mmol, 1% methanol) Mobile phase B: Acetonitrile; Gradient programme	ZIC-HILIC (50x2.1 mm, 3 µm)	Zanamivir	0.3	5	Rat and monkey plasma	[128]
Water: Acetonitrile = 60: 40 v/v, with 5 g/l formic acid	Phenomenex Luna C_8 (100×2 mm, 3 µm)	Amantadine	0.2	3	Human serum	[129]
Mobile phase A: Formic acid (0.1%) in water Mobile phase B: Methanol; Gradient programme	XDB C_{18} (2.1× 150 mm, 3.5 µm)	Amantadine, Rimantadine	0.3	10	Feed pigs and chicken	[130]
Mobile phase A: Formic acid (0.1% solution) Mobile phase B: Methanol; Gradient programme	Agilent SB-Aq (3× 100 mm, 1.8 µm)	Amantadine, oseltamivir, rimantadine, oseltamivir carboxylate	0.3	11	Chicken muscle tissues	[131]
Mobile phase A: Formic acid (0.1%) in water Mobile phase B: Acetonitrile; Gradient programme	BEH C_{18} (2.1× 100 mm, 1.7 µm)	Peramivir, laninamivir, oseltamivir, oseltamivir carboxylate, zanamivir	0.35	8	River waters, sewage effluent	[132]
Mobile phase A: Formic acid (0.1% solution) Mobile phase B: Acetonitrile; Gradient programme	Acquity HSS T3 (150×2.1 mm, 1.8 µm)	Zanamivir, Oseltamivir	0.7	8.5	Waste and surface water	[133]
Mobile phase A: Ammonium acetate (10 mmol), 5% acetonitrile adjusted pH 5 with acetic acid Mobile phase B: Ammonium acetate (10 mmol), pH 5 with acetic acid; Gradient programme	Acquity HILIC (100×2.1 mm, 1.7 µm)	Osetamivir phosphate, oseltamivir carboxylate	0.7	8.5	River water	[134]
Mobile phase A: Formic acid (0.1% solution) Mobile phase B: Acetonitrile; Gradient programme	Acquity HSS T3 (150×2.1 mm, 1.8 µm)	Amantadine, Rimantadine	0.25	12	Chicken muscle	[135]
Mobile phase A: Formic acid (0.1%) in water Mobile phase B: Methanol; Gradient programme	BEH C_{18} (2.1×100 mm, 1.7 µm)	Amantadine, Rimantadine	0.3	10	Chicken muscle	[136]
Mobile phase A: Formic acid (0.1%) in water Mobile phase B: Formic acid (0.1%) in methanol; Gradient programme	Ascentis C18 (100×2.1 mm, 2.7µm)	Oseltamivir, oseltamivir carboxylate	0.35	6	Human plasma	[137]

The LCMS/MS investigation revealed that low concentration of amantadine present in plasma sample, does not require any derivatization during solid phase extraction, the developed method reported successfully bioequivalence study of healthy volunteers [114]. Amantadine–d15 internal standard can be used based on protein precipitation [115]. QuEChERS extraction method applied for chicken muscle, egg, pet treat sample [116, 117]. *Zanamivir* found in effluent sample and water from river in Japan [118]. The antiviral drugs and metabolite quantified applied flow rate (0.2–1 ml/min) having run time between 1–20 minute with biological fluids and water sample [119–129]. Ultra pressure liquid chromatography combined with the mass spectrophotometer (triple quadrupole ion

trap) reported in feed sample illegal addition of *amantadine* and *rimantadine* [130]. Fourteen antiviral drugs detected within short time of 11 minute, multiple reaction monitoring ensured method specificity [131]. After winter season the inhibitors found in river water [132–134]. *Amantadine* and *rimantadine* are quantified in chicken muscle using different sorbent [135–136]. The UPLC method was first reported with whole blood sample, assay calculated using dried blood spot [137]. The minimum amount of sample required, elute from the column within 6 minute. The correlation coefficient of *Oseltamivir* and its active metabolites *Oseltamivir carboxylate* was greater than 0.99. Sample collection is very important parameter for this analysis. Therefore, the developed method validated successfully for human plasma of healthy volunteers.

CONCLUSION

Antiviral drugs are very useful versus influenza viruses and molecules that are actively participated for deadly diseases. The developed novel drugs are showing great potential for the prevention of influenza. It was evident that for controlling influenza, administered single dose drug to human and all animals including farm animals is required. All inhibitors can be applied during epidemic or seasonal conditions. Their main function, through vaccination, is to control the activity of viruses, reduce and manage pandemic viruses. All these mentioned drugs are also important for patients hospitalized due to influenza. Although the existing drugs are doing well, influenza experts are looking forward to identify other new drugs that exert antiviral activity like the approved ones and can be applicable as future antiviral agents for influenza viruses. Therefore, the present review gives brief information about antiviral drugs, their activity, biotransformation and analytical methods for quantification and this information will be helpful for any future studies done by experts in this field.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Both authors report no declaration of interest

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