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Chromatographic Techniques in the Quantification and Analysis of Antiviral Drugs: A Review

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Abstract :

Viruses are the primary pathogenic agents responsible for severe illnesses in humans and other organisms. Antiviral drugs as a therapeutic class have attracted significant attention from the global public. It is essential to establish accurate and precise analytical techniques for detecting antiviral drugs in different matrices. Chromatographic methods are commonly employed for quantification purposes due to their ability to simultaneously determine antiviral compounds, including Acyclovir, Amantadine, Emtricitabine, and Oseltamivir. Significant focus has been directed toward the specific elements of chromatographic assays essential for ensuring the selectivity, sensitivity, accuracy, and precision of the various methods. This review discusses analytical methods during the period from 2013 to 2025, including a variety of chromatographic techniques. HPLC with UV detection was found the favored method for many researchers, with methods developed using LC-UV.

Keywords: Antiviral drugs, Chromatographic analysis, HPLC, TLC, GC-MS, LC-MS/MS, UHPLC, RP-HPLC, Viruses .

التقنيات الكروماتوغرافية في تقدير وتحليل الأدوية المضادة للفيروسات: مراجعة

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مستخلص:

تعد الفيروسات العامل الممرض الرئيسي المسؤول عن الأمراض الشديدة لدى الإنسان والكائنات الأخرى. وقد جذبت الأدوية المضادة للفيروسات كفئة علاجية اهتمامًا كبيرًا من الجمهور العالمي. من الضروري تطوير تقنيات تحليلية دقيقة وموثوقة للكشف عن الأدوية المضادة للفيروسات في مختلف المجالات. تُستخدم الطرق الكروماتوغرافية بشكل شائع لأغراض التقدير الكمي نظرًا لقدرتها على تحديد المركبات المضادة للفيروسات بشكل دقيق، بما في ذلك (أسيكلوفير، أمانتادين، إيمترسيتابين، وأوسيلتاميفير). وقد تم توجيه تركيز كبير نحو العناصر المحددة للاختبارات الكروماتوغرافية الضرورية لضمان الانتقائية والحساسية والدقة والموثوقية لمختلف الطرق. تناقش هذه المراجعة الطرق التحليلية خلال الفترة من 2013 إلى 2025، بما في ذلك مجموعة متنوعة من التقنيات الكروماتوغرافية. وقد تبين أن طريقة HPLC مع الكشف بالأشعة فوق البنفسجية كانت الطريقة المفضلة للعديد من الباحثين.

الكلمات المفتاحية: الأدوية المضادة للفيروسات، التحليل الكروماتوغرافي , HPLC ,TLC, GC-MS, LC-MS/ MS UHPLC, RP-HPLC، الفيروسات .

Introduction

Infectious diseases have been familiar to human civilization since ancient times[1]. Infectious diseases are caused by various microorganisms, including bacteria, viruses, and fungi[2]. Viral pandemics remain a grave concern for humankind. Each year, established viruses like HIV-1 and the hepatitis B virus spread to millions of individuals worldwide[3]. Human viruses can be categorized into three main groups: DNA viruses, RNA viruses, and retroviruses[4]. Since the first antiviral drug appeared in 1963, numerous antiviral medications have been created for clinical use, providing treatment to millions of people globally[4]. However, Antiviral drugs play a crucial role in controlling influenza. Influenza leads to annual epidemics of respiratory viral infections, causing significant morbidity and mortality[5].Therefore, Favipiravir, ribavirin, and oseltamivir are known for their broad-spectrum antiviral efficacy and have been clinically examined for their effectiveness against COVID-19 [6].

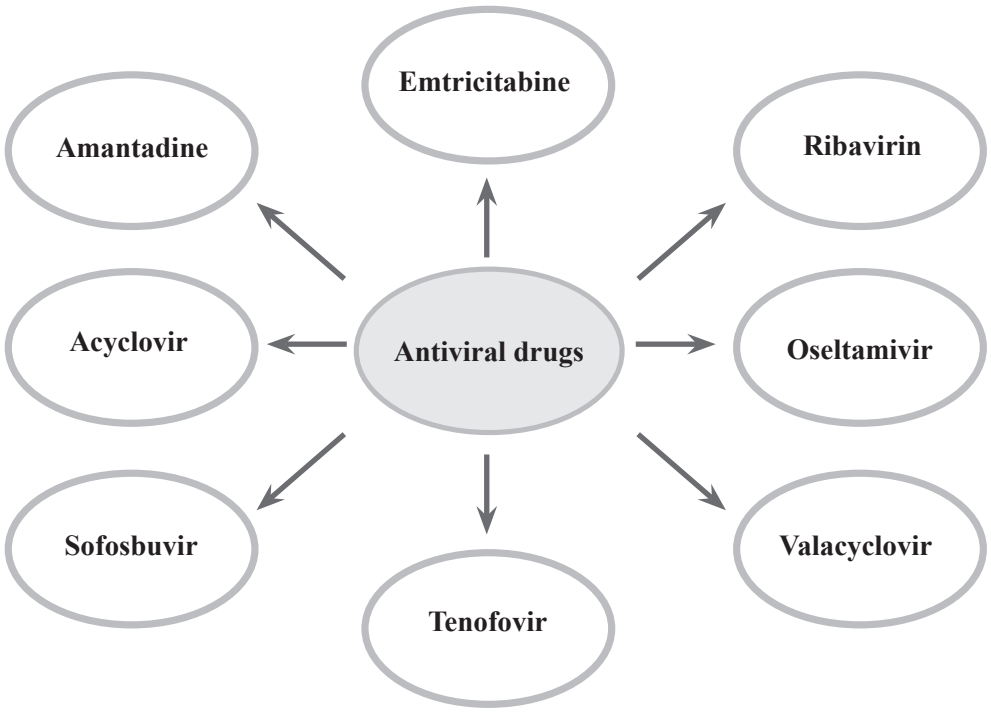

Figure 1: The types of Antiviral drugs reviewed

The appearance of various viral diseases and the currently available drug market requires new medicines to treat these diseases caused by different viruses[7]. However, despite the availability of antiviral drugs that have been discovered using different techniques, the available antiviral drugs have some problems and only neutralize some viral infections. It is absolutely important to offer new viable solutions to viruses with a high mutation rate, i.e., fast replication and easily changing strains[8]. Currently used antiviral drugs face some problems, such as viral mutation, development of resistance, changes in viral genome characteristics, toxic side effects, and reduction in daily doses[9]. The evolutionary potential of RNA viruses could lead to the emergence of viral strains capable of escaping the selective pressure of antiviral agents[10]. Mutations in regions of the viral life cycle that correspond with the mechanism of action of the drug can lead to conservation[11]. This aptly represents one of the main problems associated with long-term antiretroviral therapies. Many methods can reduce the risk of developing resistant viruses, including maintaining high plasma levels, adhering to a strict and unplanned set of drug administration, and combining different drugs that inhibit various stages in the viral life cycle[12]. Therefore, the aim of this review is to evaluate chromatographic methods that are used for the qualitative and quantitative determination of antiviral drugs in pharmaceutical formulations and biological matrices.

3. Role of Chromatography in Antiviral Drug Evaluation

A fundamental perspective of the host-virus interaction and the pharmacokinetic/pharmacodynamic parameters are essential for the development and evaluation of a novel antiviral agent. Recently, with the advancement of liquid chromatography, various analysis modes were developed based on this described system. The goal of this review to elucidate the importance of chromatography, particularly liquid chromatography, to evaluate the antiviral drug in pharmaceutical formulation and biological matrices. As a result of these efforts, a considerable amount of data characterizing in vitro antiviral

activities has been accumulated, resulting in the identification and design of emerging antiviral molecules. The in vivo pharmacokinetic and pharmacodynamic behavior of these drug types, including distribution and metabolism, requires further investigation to support efficacy assessment. High-resolution separations are, therefore, required to distinguish unknown metabolites derived from the drug versus the virus-host metabolism. Indeed, chromatography, particularly LC, plays a critical role in the degradant control and the analysis of antiviral agents in biological fluids, ensuring the success of in vivo and in vitro studies.

3.1 Quantitative Analysis of Antiviral Drugs

The development of simple, specific, and sensitive methodology for the determination of antiviral agents in pharmaceuticals and biological fluids is essential for research in the pharmacological field. Currently, different analytical methods are involved in the analysis of antiviral drugs. Among the various techniques, gas chromatography, high-performance liquid chromatography, capillary HPLC, ion chromatography, thin-layer chromatography, and capillary electrophoresis are used for the quantitative determination of antiviral drugs. The gas chromatography system is applied to the determination of some drugs. However, gas chromatography and high-performance liquid chromatography combined with ultraviolet-visible, fluorescence, or mass spectroscopy detectors are most commonly employed for the analysis of antiviral agents. Methods for the determination of these drugs in pharmaceutical preparations and biological fluids are reviewed in this section.

The interfaces available for a combination of gas chromatography with mass spectroscopy are the hyphenated systems that exist today for the analytical approach of the relevant analytes[13] . However, half of all UV-absorbent compounds are sensitive and may degrade under the high temperatures of the gas chromatography, acutely or just a little as they pass over the red-hot coil of the gas chromatography injector, if samples are “jumped” onto the column or are retained too long for a complete separation before they arrive . Due to its interaction and re-

tention capabilities, much larger gas chromatography columns are used, mainly those that are totally non-polar or those that are non-polar with high inner-coating thicknesses for efficient but short separations, which give fast analysis without excessive resolution. The shortest columns, depending on use, are approximately 20 m in length, while the longest are about 60 m; of course, there are also intermediate-length columns[14].

3.2. Qualitative Analysis of Antiviral Drugs

The qualitative analysis of antiviral compounds is based on their mass spectral identification. The mass spectral information of reference standards is usually characterized by using liquid chromatography-mass spectrometry, together with tandem mass spectrometry and high-resolution mass spectrometry, introducing both protonated molecules for quantification and major fragment ions for their identification. Therefore, other methods were organized to evaluate antiviral agents, using mainly an absorbance in the UV range, specifically from 254 to 360 nm, depending on the antiviral compound[15].

Applications of Chromatography in Antiviral Drug Evaluation

Thin-layer chromatography (TLC)

TLC is widely utilized for the analysis of various drug materials and pharmaceutical preparations due to its notable benefits, including minimal sample preparation, a broad selection of mobile phases, flexibility in sample differentiation, high capacity for sample loading, and cost-effectiveness[16, 17]. TLC is a valuable method for screening unidentified substances within bulk drugs [18].TLC is classified as a form of liquid chromatography that utilizes a liquid mobile phase and a thin layer of material as the stationary phase positioned on a flat plate [19]. Nine TLC solvent systems were utilized within a particular schema to detect 300 target drugs[20]. In 2002, Sia and his research group evaluated acyclovir using TLC in pharmaceutical formulations[21]. In 2017, Salama *et al* used TLC with densitometric analysis to determine Ledipasvir and Sofosbuvir in the Tablet Dosage Form[22]. In 2022, Noureldeen et al , estimated remdesivir and favipira-

vir in human plasma and pharmaceutical formulations using TLC [23].

Gas chromatography (GC)

It is a technique that employs gases to separate and analyze compounds capable of vaporization without decomposition. To analyze a sample using GC, it is dissolved in a solvent prior to injection into the system[24]. GC is a highly effective method for separating and detecting volatile organic compounds[16]. GC is considered more environmentally friendly than HPLC as it reduces environmental pollution and conserves organic solvents[25]. GC plays a significant role in the analysis of pharmaceutical products[26]. GC–Mass Spectrometry (GC-MS) is an analytical technique that combines gas-liquid chromatography with mass spectrometry detection to identify various substances[27].GC-MS is a sensitive, precise, repeatable, quantitative, and robust instrument, ideally suited for the examination of complex mixtures[28]. Jain et al. employed GC-MS for the determination of favipiravir in biological and forensic samples[29]. Liquid chromatography plays an important role in the qualitative and quantitative estimation of antiviral drugs in pharmaceutical formulations and biological matrices. Chromatographic techniques are commonly applied in the evaluation of antiviral drugs, as outlined in the following sections.

High-performance liquid chromatography (HPLC)

HPLC is an advanced liquid chromatography method employed to separate the complex mixture of molecules present in chemical and biological systems, enhancing the understanding of the distinct roles played by individual molecules[16]. HPLC is performed using a chromatographic column where a solid or liquid sample is dissolved in an appropriate solvent[24]. The retention time of analytes is determined by the mobile phase properties such as pKa and the pH[30]. In recent years, HPLC has become the preferred technique for analyzing a wide spectrum of compounds[31]. The detection methods most commonly employed include ultraviolet, fluorescence, and MS[32]. This method is also suitable for a wide range of compounds, including those with varying polarity, molecular mass, volatility, and thermal sensitivity[33].

HPLC has the capability to simultaneously detect numerous analytes in pharmaceutical preparations. While HPLC is widely used for pharmaceutical analysis, LC–MS/MS offers higher sensitivity and selectivity for trace-level analysis in complex biological matrices[34].

A general analysis of some antiviral drugs evaluated by the HPLC method

Acyclovir (ACV)

ACV (Fig 2) is a synthetic purine nucleotide analog recognized for its antiviral activity[35]. ACV is one of the most commonly used antiviral drugs globally[36]. It represents the dawn of a new era in antiviral therapy, distinguished by its exceptional selectivity and minimal cytotoxicity[37]. ACV is the primary choice for treating herpes virus infections, largely attributed to the development of new delivery methods that significantly enhance its bioavailability[38]. In addition to its numerous applications in human medicine, it may also be utilized as a veterinary treatment for viral infections like Canine parvovirus[39]. It exhibits low solubility in water and has a short duration within the human body[40]. In 2018, a sensitive and accurate analytical technique was developed and validated for determining acyclovir levels in human plasma[41]. Malik et al, used isocratic RP-HPLC to determine of acyclovir in rabbit plasma[42]. Table 1 summarizes the methods employed for detecting acyclovir in pharmaceutical preparations and in the plasma of humans, rabbits, and rats, utilizing chromatographic techniques.

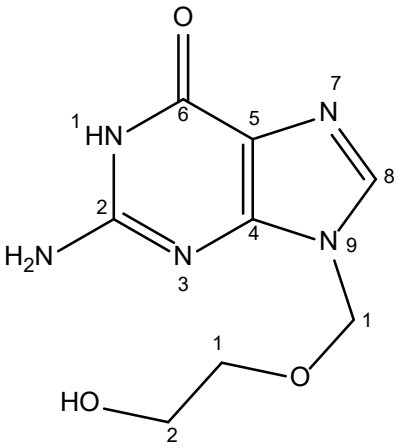

Figure 2:
Chemical structure of ACV

Table 1: Review of literature on the analysis of ACV using HPLC techniques

Technique	Column/ mobile phase/ Detection	Method Validation	Application	t_R min	Ref.
HPLC-UV	The Ace C_{18} (250 × 4.6 mm i.d.; 5 µm) MP: 0.02 mol/L CH_3COOH / MeOH (95:5) UV λ: 254 nm	35–700 µg/ml DL= 0.16 µg/ml QL= 0.048 µg/ml $R^2 > 0.99$ %Rec= 98.18 - 99.64 %RSD < 5	vitreous humor	7.427	[43]
HPLC–UV	Thermo C_{18} (250 × 4.6 mm i.d., 5µm) MP: 5 mM $NH_4C_2H_3O_2$ (pH 4.0) /ACN (40 : 60% v/v) UV λ : 290 nm	25-150 ng/ml DL= 8 ng/ml QL= 24 ng/ml %Rec= 91.0 %RSD= 4.85	Human Plasma	4.12	[44]
UPLC–PDA	C18 (5 µm,4.6 mm x 250 mm) MP: MeOH / 0.0125 M KH_2PO_4 (55:45, v/v) UV λ : 254 nm	1-64 µg/ml DL= 0.01 µg/ml LQL= 0.04 µg/ml R^2= 0.9996	Intestinal Perfusion Studies	2.8	[45]
HPLC-UV	C8 (250x4.6 mm, 5 µm) MP: 0.1 % (V/V) TEA in H_2O (pH 2.5) UV λ : 255 nm	0.1-2.0 µg/ml QL= 0.1 µg/ml R^2= 0.9985	Human plasma	3.7	[46]
HPLC-PAD	Phenomenex®C18 (4.6 × 150 mm, 5 µm) MP: ACN/0.1% H_3PO_4/ MeOH (50:40:10) UV λ: 254 nm	0.5-30 µg/ml DL= 83.62 ng/ml QL= 109.52 ng/ml R^2= 0.9998 %RSD= 1.2	polymeric microparti-cles	1.94	[47]
HPLC-PDA	Thermo Hypersil (250 mm × 4.6 mm, 5 µm) MP: MeOH / 0.03 M KH_2PO_4 adjusted with 0.1% H_3PO_4 (30:70 v/v) UV λ: 210 nm	1–100 µg/ml DL= 0.16 µg/ml QL= 0.48 µg/ml R^2=0.9999	human plasma	3.85	[41]
HPLC-UV	symmetric®C18 column (4.6 x75 mm,3.5 µm MP: H_2O /ACN (95:5 v/v) UV λ: 207 nm	2–20 µg/ml R^2 = 0.9989	tablet, cream dosage forms	0.77	[48]

Amantadine (AMD)

AMD (Fig 3) was originally created in the early 1960s and approved for its anti-influenza A2 properties in 1966[49, 50]. It is employed as a treatment for Parkinson's disease due to its observed involvement in the release of dopamine from nerve terminals[51]. AMD blocks the E channel of SARS-CoV-2, thereby preventing the virus from discharging its genetic content into the cell[52]. AMD exhibits potential anti-cancer activity by modulating cyclin D1, cyclin E, and CDK2 to inhibit cell growth, while also regulating Bax and Bcl-2 proteins to induce apoptosis[53]. Zhao et al. established a rapid analytical approach for quantifying amantadine in diverse animal-derived samples, including chicken, duck, chicken liver, and egg, through the application LC-MS/MS[54]. In 2015, AMD was assessed in Microdialysis samples obtained from rat plasma using HPLC coupled with fluorescence detection[55]. Wang and colleagues using LC-MS/MS to determine AMD in honey sample[56]. Table 2 summarizes the methods employed for detecting Amantadine in pharmaceutical preparations and in chicken muscle, rat plasma, animal food, and biological fluids utilizing chromatographic techniques.

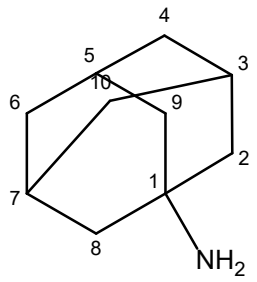

Figure 3: Chemical structure of AMD

Table 2: Review of literature on the analysis of AMD using HPLC techniques

Technique	Column/ mobile phase/ Detection	Method Validation	Application	t_R min	Ref.
UH-PLC-LTQ Orbitrap MS	Waters ACQUITY UPLC HSS T3 column (150 mm × 2.1 mm, 1.8 µm) MP: 0.1% HCOOH / ACN (gradient) Mass analyzer	1–100 µg/kg QL= 1.02 µg/kg QL=3.40 µg/kg R^2= 0.99 %Rec= 87.5 -102.4 %RSD = 3.9 - 6.3	chicken muscle	3.2	[57]
HPLC–MS/MS	XDB-C18 (2.1 × 150 mm, 3.5 mm) MP: A (0.1% volume ratio of HCOOH in H_2O) and eluent B (MeOH) gradient Mass analyzer	2-200 µg/L DL= 0.5 µg/kg QL= 1.0 µg/kg R^2= 0.9938 %Rec= 89.9- 105 %RSD ≤ 20	Animal-derived food	2.7	[54]
HPLC- FL	Hypersil C18 (150 × 4.6 mm, 5 um, MP: 5% ACN in H_2O (A) and ACN (B) (gradient) Fluorescence detector	25-500 ng/ml DL= 6.8 ng/ml QL= 20.5 ng/ml R^2= 0.9989	Rat Plasma	4.2	[55]
UPLC–MS/MS	SB-Aq (3 mm × 100 mm, 1.8µm) MP: ACN / 0.1% HCOOH in H_2O (gradient) Mass analyzer	0.1–10µg/L DL=0.05 µg/kg QL= 0.1 µg/kg R^2= 0.9995 %Rec= 98.3–102.4 %RSD =1.7–10.3%	chicken muscle	5.83	[58]
HPLC-MS/MS	Kinetex® XB-C18 (2.1 × 100 mm, 2.6 µm) MP: 0.1% HCOOH in 10 mmol/L NH_4HCOO/ACN Mass analyzer	0.5–20 µg/kg QL= 1.0 µg/kg R^2= 0.9996 %RSD= 0.11	chicken tissues, and eggs	4.14	[59]
HPLC-MS/MS	Synergi™ Hydro C18 (150× 4.6 mm, 4 mm) MP: ACN / 10 mM NH_4HCOO (80:20, v/v) Mass analyzer	0.50–500 ng/ml DL= 0.18 ng/ml QL=0.50 ng/ml R2 ≥ 0.9969 %Rec= 98.47-105.72%.	Human Plasma	1.80	[60]

Emtricitabine (EMC)

EMC (Fig 4) is a potent antiviral with a synthetic fluoro derivative of thiacytidine[61]. EMC is classified as a nucleos(t)ide analogue, is acknowledged as an essential medication by WHO. This medication has been employed globally for numerous years as a once-daily fixed-dose combination for the treatment of HIV and HBV, as well as for HIV infection prevention through pre-exposure prophylaxis[62]. Pavani and Susithra use the HPLC-PDA method to estimate EMC in pharmaceutical preparation [63]. In the same year, Kasu1 and Satyanarayana used the RP-HPLC Method to determine EMC in pharmaceutical formulations[64] .Table 3 summarizes the methods employed for detecting emtricitabine in pharmaceutical preparations utilizing chromatographic techniques.

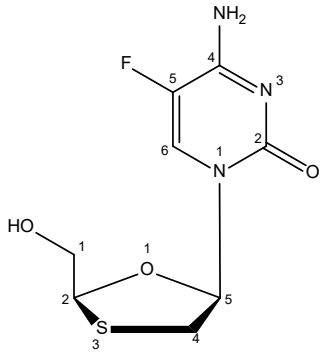

Figure 4: Chemical structure of EMC

Table 3: Review of literature on the analysis of EMC using HPLC techniques

Technique	Column/ mobile phase/ Detection	Method Validation	Application	t_R min	Ref.
HPLC-PDA	Inertsil ODS 3V (150mm × 4.6mm i.d., 5 µm) MP: 20 mmol L^{-1} of KH_2PO_4 / ACN (70: 30 v/v) UV λ: 25٦ nm	20–300 µg/ml DL=0.36 µg/ml QL=1.19 µg/ml R2= 0.9997 %Rec=99-101 RSD= 0.3	pharmac	2.0 ±0.38	[65]
HPLC-UV	Phenomenex C18 (250 mm × 4.6 mm I.D., 5 µm) MP: 40 mM $H_2PO_4^-$ buffer (pH 6.8)/MeOH /2% ACN (83: 15: 2, v/v/v) UV λ: 280 nm	0.040 - 2.0 µg/ml DL= 0.024 µg/ml QL= 0.036 µg/ml R2= 0.9982 %RSD<2	pharmac	4.39	[66]
UPLC– UV	Phenomenex Gemini C18 (150 mm × 4.6 mm i.d., 5 µm) MP: ACN/ KH_2PO_4 buffer (20 mM, pH 3.3) /TEA (58.72: 41.23 : 0.05 (v/v/v) UV λ: 270 nm	28–84 ng/ml DL= 1.90 ng/ml QL= 0.43 ng/ml R2= 0.999 %Rec= 99.50 %RSD= 0.044	pharmac	2.6	[67]
HPLC-UV	Phenomenox C18 (250mm x 4.6mm,5µm) MP: NaH_2PO_4 / MeOH (50:50v/v) UV λ: 280 nm	80-240 µg/ml DL= 0.0112 µg/ml QL= 0.0375 µg/ml R^2= 0.9990 %Rec= 99.53-100.01 %RSD<2	Synthetic Mixture	3.5	[68]
HPLC-PDA	Inertsil ODS 3V C18 (250 m×4.6 mm, 5 µm) MP: KH_2PO_4: H_2O (30:70 v/v) UV λ: 240 nm	20-240 µg/ml DL= 0.02 µg/ml QL= 0.06 µg/ml R2 > 0.999 %Rec= 108.56 RSD<2	pharmac	3.719	[69]
HPLC-PDA	A Phenomenex C18 (250 x 4.6mm, 5µm) MP: 10mM NH_4CH_3COO/ ACN (gradient) UV λ: 265nm	20-100 µg/ml DL= 0.1017 µg/ml QL= 0.3083 µg/ml R2= 0.9998 %Rec= 98 -102 %RSD<2	Pharmac, in vitro disso-lution samples	4.0	[70]

Oseltamivir (OSM)

OSM (Fig 5) is classified as a primary antiviral medication, particularly in hospital settings[71]. OSM is a neuraminidase inhibitor authorized for treating and preventing influenza A and B[72, 73]. It has the capability to inhibit the propagation of the influenza virus and minimize viral shedding in respiratory secretions within the human body[73]. Numerous clinical studies have confirmed that initiating oseltamivir treatment early leads to improved outcomes in critically ill patients afflicted with influenza[74-76]. During the current COVID-19 outbreak, OSM has been commonly employed by symptomatic COVID-19 patients[71]. Huang and his colleagues used HPLC-MS/MS to determine OSM prodrugs [77]. Babu and Sharma utilized RP-HPLC to accurately determine OSM levels in pharmaceuticals [78]. Table 4 summarizes the methods employed for detecting oseltamivir in pharmaceutical preparations and human plasma utilizing chromatographic techniques.

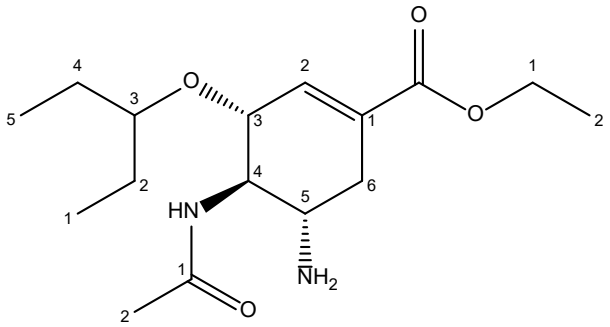

Figure 5:
Chemical structure of OSM

Table 4: Review of literature on the analysis of OSM using HPLC techniques

Technique	Column/ mobile phase/ Detection	Method Validation	Application	t_R min	Ref.
LC-MS/MS	ASynergiHydroC_{18} (150 mm × 2.0 mm,4µm) MP: 0.1% (40:60 v/v) HCOOH / H_2O (A) and 100% MeOH (B) Mass analyzer	3–300 ng/ml LQL=3 ng/ml R2= 0.9935 %Rec=88.6-103 RSD≤ 7.46	human fluoride EDTA plasma	5.0	[79]
LC-MS/MS	Symmetry C18 (100 mm × 4.6 mm, 5 mm) MP: 10 mM NH_4HCOO /ACN (30:70, v/v) Mass analyzer	0.5–200 ng/ml LQL= 0.5 ng/ml R2=0.9976	human plasma	1.56	[80]
HPLC– UV	KromasilC_{18} (250 mm x 4.6 mm, 5 µm) MP: NH_4CH_3COO buffer / ACN (60:40 v/v) UV λ: 227 nm	5-35 µg/ml DL= 5.57 µg/ml QL= 16.90 µg/ml R2= 0.996 %Rec>95 %RSD<1.5	pharmac	1.542	[81]
HPLC-UV	Phenomenex Luna C18 (4.6 x250mm, 5µm) MP: MeOH / H_2O (75:25% v/v) UV λ: 223 nm	20-100 µg/ml %Rec= 98.0-102 %RSD<2	pharmac	2.7 ±0.02	[82]
HPLC-UV	Agilent Extend C18 (4.6 mm 3 250 mm, 5.0 mm MP: 20 mM KH_2PO_4 / ACN (60:40 v/v) UV λ: 215 nm	10–60 µg/ml DL= 0.40 µg/ml QL= 1.20 µg/ml R2= 0.999	pharmac	3.010	[83]
LC-MS/MS	Dikma Inspire (4.6 mm × 250 mm, 5µm) MP: 0.1% H_3PO_4 / ACN (83:37 v/v) UV λ: 254nm	15–75 µg/ml DL= 0.74 µg/ml QL= 2.46 µg/ml R2=0.999 %Rec= 100.13	Pharmac	2.5	[78]

Abbreviation

CH_3COOH	Acetic acid
MeOH	Methanol
ACN	Acetonitrile
$NH_4C_2H_3O_2$	Ammonium acetate
KH_2PO_4	potassium dihydrogen phosphate
TEA	Triethylamine
H_3PO_4	phosphoric acid
HCOOH	Formic acid
NH_4HCOO	Ammonium formate
NaH_2PO_4	Sodium dihydrogen phosphate
PO_4^-	Phosphate ion
$C_3H_5O(COO)_3^{-3}$	Citric acid
$H_2PO_4^-$	Dihydrogen phosphate ion
t_R	Retention time
Ref	References

Conclusion

The interest in the chromatographic analysis of antiviral drugs in different samples has increased in conjunction with the development of more aggressive antiviral-oriented therapies. These therapies required more detailed analytical studies with a more demanding limit of quantification, program of quality control, and methodology validation. In this review, we compiled various, rapidly evolving trends in the application in recent years on the chromatographic methodology for antiviral drugs in all classes and common formulations. It is worth mentioning that there is no technique or methodology that can be considered the best approach for the chromatographic analysis of antivirals. Every approach has advantages and leads to complementary information that can be useful for the development of pharmacokinetic/pharmacodynamic knowledge and therapeutic support protocols. The demand for more and better detection levels leads to the development of new methodologies and the exploitation

of the combination of different chromatographic techniques and analytical strategies. The present review offered a comprehensive overview of the current state-of-the-art chromatographic approaches for the evaluation of antiviral drugs in biological and other samples which plays a critical role in the antiviral-oriented therapy. Various research papers and some reviews presented antiviral drugs as solid references in the application of the chromatographic methodology, with the availability of some quantification and program of quality control. They increased their knowledge of both the methodology and the analytical protocol while providing useful support tools for more accurate treatment.

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