Zagazig Journal of Pharmaceutical Sciences

Journal homepage: https://zjps.journals.ekb.eg/

Print ISSN: 1110-5089 Online ISSN: 2356-9786

Mini Review on different instrumental approaches applied to some selected drugs for COVID-19 treatment

Eman A. Madbouli[*1], Sobhy M. El-adl[1], Abdalla A. El-Shanawany[1]

[1] Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

ARTICLE INFO

Article History:
Received: 4 April 2023
Accepted: 26 April 2023
Published online: 3 July 2023

Key words:
Covid-19; Remdesivir; Lomefloxacin; Dexamethasone; Analytical methods.

ABSTRACT

The quickest methods for battling the new coronavirus pandemic at the beginning of the COVID-19 outbreak were therapeutic treatments based on approved drugs. Although there are now many vaccines available, it will take time for the global vaccination program to take effect. Therefore, in addition to vaccination, repurposing currently available antiviral, antibiotic, and other types of medications has been suggested as a complementary medical strategy for COVID-19 infections. Analytical methods for assessing drug concentrations in biological fluids and pharmaceutical products are necessary due to the drugs' extensive clinical potential as well as their potential side effects. The following article was introduced by presenting a mini review of the different methods used for the quantitative determination of these drugs in order to encourage and facilitate collecting literature review for other researchers who will have studies related to the presented compounds. The review discussed techniques used for the determination of each drug quantitatively involving spectrophotometric (UV and visible), spectrofluorimetric, electrochemical and chromatographic (TLC and HPLC) methods either in a biological sample or in the pharmaceutical dosage forms. All methods included in the review are validated and constructed according to ICH guidelines.

1. Introduction

The COVID-19 pandemic, the worst public health emergency in the previous 100 years, struck the world in 2020. Every continent, except for Antarctica, has been hit by the coronavirus disease-2019 (COVID-19) pandemic in December 2019. COVID-19 is a contagious illness linked to SARS-CoV-2, a new coronavirus that causes the severe acute respiratory syndrome. Even though the world has survived many pandemics in the past, this one is a unique global health challenge that has changed how we live and is having a devastating socioeconomic impact on people worldwide [1]

SARS-primary CoV-2's peculiarity is its hazy symptoms, commonly mistaken for the flu and common cold [2]. The fact that COVID-19 is unpredictable makes it even more concerning. It is especially scary because it can have fatal outcomes, such as pneumonia and acute respiratory distress syndrome, or cause non-to-mild respiratory tract symptoms in the majority of patients. Thankfully, most individuals who contract the virus can recover with only supportive treatment. This unpredictability of the virus is one more reason why it is important to take the necessary precautions to prevent its spread.

DOI: 10.21608/zjps.2023.203537.1045
*Corresponding author & copyright holder: Eman A. Madbouli Email address: eamadbouli@pharmacy.zu.edu.eg

Patients with chronic respiratory diseases, cardiovascular diseases, cancers, and immune deficiencies are susceptible to serious pathological complications and death [3]. The serious consequences led to a worldwide search for a virus-specific treatment that would be effective. The level of public mistrust toward such vaccinations is significant, despite the approval and introduction of numerous vaccine types recently. These studies showed that existing therapies could be effective against SARS-COV-2, offering hope for a quicker path to treatment. Furthermore, the results indicated that it is possible to repurpose existing drugs for the treatment of SARS-COV-2 [4].

The first recognized therapy for severe coronavirus disease is remdesivir (I) in 2019. It is a novel nucleoside analog with broad antiviral activity against RNA viruses, such as respiratory pathogens and the Ebolavirus [5]. It is an analog of adenosine and a monophosphoramidate prodrug. Remdesivir is broken down into its active form, which blocks viral RNA polymerase and avoids being checked by viral exonuclease, resulting in a reductionin the production of viral RNA. Remdesivir inhibits the nascent viral RNA of the Ebola virus through a delayed chain termination mechanism [6]. As a result of the immediate need for treatment, various experimental agents that already existed have been tried. As a result, to help control viral replication and the patient's general health, COVID- 19 patients take multiple therapy drugs from various categories [7].

Since the start of the COVID-19 pandemic, scientists have concentrated on finding new uses for current antibiotics, antivirals, and anti-inflammatory medications. Being chemically derived from quinoline, the prodrome of chloroquine, fluoroquinolones are synthetic, broad- spectrum antimicrobial agents. Curiously, fluoroquinolones have been shown to have antiviral effects on the vaccinia virus, papovavirus, CMV, VZV, HSV-1, HSV-2, HCV, and HIV [8]. Fluoroquinolones such as lomefloxacin (II) were suggested to be used as adjuncts in treating patients who have COVID-19 considering their potential antiviral activity against SARS-CoV-2, along with their immunomodulatory properties, favorable pharmacokinetics, and excellent safety profile [8]. According to coronavirus disease 2019 (COVID-19) treatment guidelines, dexamethasone can be used in the treatment protocol. Corticosteroids' ability to treat pneumonia caused by COVID- 19 and other coronaviruses was demonstrated in recent studies thatshowed this to be true both in vitro and in vivo. Dexamethasone (III) at low doses could decrease mortality in patients with severe COVID-19 disease, but it did not affect the mortality rate in patients witha milder form of the disease [9].

There is an urgent need for straightforward and reliable bioanalytical methods for drugs quantification in the human plasma matrix in order to advance clinical research and high-throughput monitoring. Here, we provide an overview of methods of instrumental analysis for drugs used in covid-19 treatment especially remdesivir, lomefloxacin and dexamethasone.

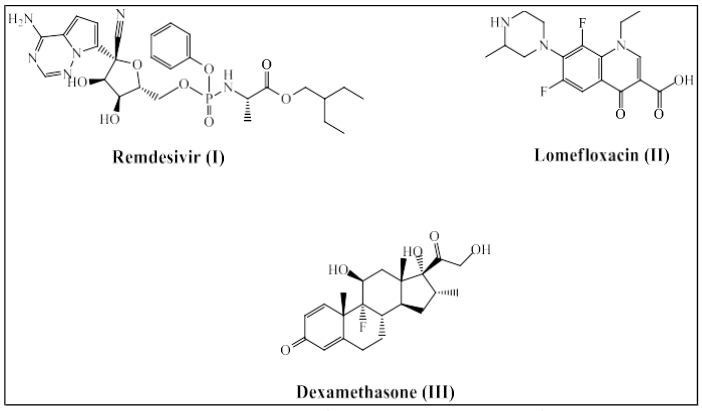

Figure 1: *Chemical structure of remdesivir (I), lomefloxacin (II) and dexamethasone (III).*

2. Methods of analysis of Selected drugs in this study Materials

2.1 Reported methods of remdesivir

2.1.1 Spectrophotometric methods

UV spectrophotometric methods have been reported for determination of remdesivir, including the following.

Formation of complex with acid dye bromophenol blue to produce a yellow ion-pair complex which can be measured at 418 nm. The best acid dye was chosen for this method by using computational and theoretical studies [10].

The synthesis of a novel charge transfer complex (CTC) between chloranilic acid (CLA), an electron acceptor, and REM, an electron donor, is described in this study for the first time. Different spectroscopic and thermal gravimetric methods were used to characterize the CTC. Through the development of a new broad absorption band with a maximum absorption peak (max) at 530 nm, UV-visible spectroscopy was demonstrated the formation of the CTC in methanol [11].

2.1.2 Spectrofluorimetric methods

Fluorescent spectroscopy has also garnered a lot of interest. Its benefits include environmental sustainability and analytical performance. Pharmaceutical quality control procedures must be delicate, quick, and economical to offer high throughput at a fair price [12]. Spectrofluorimetric methods have been reported for the determination of remdesivir,including the following:

One study was based on measurements of fluorescence (pH = 4) between 244 and 405 nm. Calibration was completed over the 1.0 - 65.0 ng/mL range to improve sensitivity at detection and quantitation limits of 0.287 and 0.871 ng/mL, respectively. Other variables affecting this technique were also studied. [13]

The fluorescence intensity for remdesivir was recorded at λ emission (410 nm) after λ excitation at 241 nm [14].

2.1.3 Electrochemical technique

In this study, an anodic process utilizing a composite of Squaraine Dye and Ag2O2 has been assessed. The electro-analytical process has a branched mechanism, which suggests relatively dynamic behavior. However, the associated mathematical model analysis, conducted using the theories of linear stability and bifurcation, supports the composite electro-analytical efficiency as an electrode modifier [15].

2.1.4 Chromatographic methods

2.1.4.1. HPLC chromatographic methods

Table 1: *HPLC methods reported for the determination of remdesivir.*

Matrix	Column	Mobile phase	Detector	Ref.
Pharmaceutical formulations	An agilent Extend C18	acetonitrile:KH_2PO_4 solution (50:50, by volume)	UV at 247 nm	(Bulduk and Akbel 2021) [16]
Plasma with Abacavir (internalstandard)	Chromosil C18	Methanol:Acetonitrile: 0.1% OPA (30:65:5 v/v)	UV at 272 nm	(Kishore, Prasad etal. 2021) [17]
Spiked humanplasma in the presence of frequently co-administered medications	Reversed phase agilentC18	Gradient elution using (a) acetonitrile (b) water acidified at pH 4with orthophosphoric acid.	Diode array detector at 240 nm Fluorescence λ_{ex} = 245 nmλ_{em} = 390 nm	(Moneim, Kamal et al. 2021) [7]
Rat plasma	Inertsil ODS column	Isocratic elution (a) Buffer of triethyl amine (b) Acetonitrile (50:50 v/v)	Triple quadrupole mass detector	(Rao, Adimulapu et al. 2022) [18]
Sublingual tabletdosage form	C18 column	Acetonitrile:Ammoniumacetate buffer (pH 4.0) (40:60 % v/v)	Photo Diode Array Detector.	(Padhye, Sonawane et al.2022) [19]
Rat plasma with Hydroxychloroquine -Favipiravir -Oseltamivir	C18 column	(a) Water (b) acetonitrile, (c) 0.1 % (v/v) formic acid	Triple QuadrupoleMS	(El Azab 2022) [20]
Plasma with -Favipiravir -Dexamethasone	Reversed phase BEH C18 column	Acetonitrile:methanol:water acidified at (pH 4) with orthophosphoric acid (35:15:50, v/v)	Diode array detector	(Emam, Abdelaleem et al. 2022) [21]

In human plasma with its active metabolite (GS441524)	Reversed phase agilentC18	Acetonitrile:Dimethyl Sulfoxide (50:50 v/v)	Triple QuadrupoleMS	(Skaggs, Zimmerman et al. 2022) [22]
In human serum with chloroquine, Hydroxychloroquine, lopinavir, Ritonavir, favipiravir, azithromycin	Reversed phase C18	(a) 0.1% formic acid (b) and acetonitrile	Triple QuadrupoleMS	(Habler, Brügel et al. 2021) [23]
In human plasmawith its active metabolite (GS-44152)	Reversed phase agilentC18	(a) formic acid (b) acetonitrile	Tandem quadrupole MS	(Kumar, Keerthana et al. 2022) [24]
In human plasma	Reversed phase C18	(a) acetonitrile (b) 0.1% formic acid	Quadrupole tandem mass spectrometer	(Alvarez, Moine et al. 2020) [25]

2.2 Reported methods of Lomefloxacin

2.2.1 Spectrophotometric methods

UV spectrophotometric methods have been reported for determination of lomefloxacin hydrochloride, including the following:

Formation of complex with praseodymium at pH 6.5–8.5 which can be measured using the second derivative spectra at 357 nm [26] (Wang, Ren et al. 2000). Measurement of the absorbance of the drug aqueous solution using distilled water at 280 nm [27].

First derivative for lomefloxacin determination in presence of its acid degradation product with zero crossing point at 295.2 nm [28].

Measuring the absorbance at the 287 nm using different media such as water, 0.1N HCl 0.1N NaOH and chloride buffer that used as solvent [29].

Measuring the absorbance at the 281 nm using urea solution (8M) as solubilizing agent [30].

Determination of the drug in presence of gemifloxacin mesylate and photodegradation products using two wavelengths 327 and 278 nm [31].

Measurement of the absorbance of the drug aqueous solution using distilled water at λmax= 287.1 nm [32].

2.2.2 Spectrofluorimetric and chemiluminescence methods

One method depends on complex formation between the drug and terbium ion (Tb3+) to enhance the fluorescence intensity with λ ex =320 nm and λ em =545 nm [33]. Chemiluminescence method depends on that theredox reaction between cerium (Ce4+) and Na2SO3can be greatly enhanced by the complex of terbiumion (Tb3+) and the drug with with four emissionpeaks at 490, 545, 585 and 620 nm [34].

Another method depends on native fluorescence of the drug in 0.1 N H2SO4 with λ ex =290 nm and λ em =450 nm [35]. One method depends on the quenching effect after binding of the drug to bovine lactoferrin in a dilute aqueous solution with λ ex =295 nm and emission range from 300 – 550 nm [36].

Flow injection chemiluminescence method depends on reaction of the drug with either cerium and sodium sulphite in acid medium or cerium in acid condition sensitized by rhodamine 6G or luminol-KIO4-calcein in alkali medium with emission range from 350 – 550 nm [37].

Another method was based on charge transfer complex formation between lomefloxacin hydrochloride and bromanil with λ ex =275 nm and λ em =459 nm [38].

Another process relied on the complex formation of the drug with an aluminum ion to produce a high-fluorescent end product. The amount of sodium dodecyl sulfate added increased the amount of fluorescence that was observed at 429 nm after being excited at 332 nm [39]. The developed method is dependent on the formation of a metal-chelation compound using LMX as a ligand and zinc (II) in an acetate buffer (pH 5.5). The types of metal, their concentrations, pH values, buffer types, and solvents used to dilute them were all

optimized. The best reaction conditions were determined after careful investigation to be 0.2 mM zinc, 2.0 ml acetate buffer (pH 5.5), and water as the diluting solvent. When LMX was excited at 284 nm and then excited at 450 nm, a significant increase in fluorescence intensity was achieved [40].

Also, for the purpose of lomefloxacin detection, a nanocomposite fluorescent probe based on fluorescence quenching was created. The created probe combined the excellent selectivity of molecularly imprinted polymer, the high adsorption affinity of graphene oxide, and the high sensitivity of quantum dots. For monitoring lomefloxacin, the probe demonstrated good sensitivity, high specificity, and rapidity. Lomefloxacin reduced fluorescence emission linearly from 0.10 to 50.0 $\mu g\,L^{-1}$, and the probe showed a low limit of detection of 0.07 $\mu g\,L^{-1}$ [41].

Lomefloxacin hydrochloride was found in human urine, and a quick, accurate spectrofluorometric method was developed to detect it. The technique is based on the determination of lomefloxacn's native fluorescence in $2 \times 10{-}4$ mol·L^{-1} at emission = 451 nm following excitation at 323 nm [42].

2.2.3 Electrochemical methods:

This is adsorptive voltametric method depends on using Hg electrode and supporting electrolyte containing britton-robinson buffer (pH 8.8) - 0.02 M KCl. The reduction peak of lomefloxacin hydrochloride showed a potential of -1.40 V (vs.Ag/AgCl), [43].

Another method is based on the polarographic catalytic current produced by lomefloxacin hydrochloride in a phosphate buffer (0.125 M) at pH= 6.6 and 2-iodoacetamide solution (2.5 × 10-4 M). The second-order derivative peak current of the catalytic wave of the drug is proportional to its concentration [44].

A differential pulse adsorptive stripping voltametric method using acetate buffer solution (0.04 M) at pH= 4 and accumulation potential of -0.30 V (vs.Ag/AgCl) and accumulation time was 2 minutes [45].

Using a poly-melamine layer modified glassy carbon electrode (p-(melamine)/GCE), a sensitive electrochemical method for the determination of lomefloxacin has been developed. Horizontal Attenuated Total Reflectance-Infrared Spectroscopy (HATR-IR), Field Emission Scanning Electron Microscopy (FE-SEM), and Electrochemical Impedance Spectroscopy (EIS) were used to characterize the surface morphology of the modified sensor. Square wave voltammetry and cyclic voltammetry were used to measure the electrochemical reactions. The electrode that had been modified with polymer demonstrated excellent electrocatalytic activity in the electrochemical oxidation of lomefloxacin, with a clearly defined voltammetric peak at about 980 mV [46].

This study developed a green direct potentiometric method to measure the antibacterial Lomefloxacin hydrochloride in urine using electrodes that were made in-house. The method uses non-hazardous chemicals and doesn't require sample preparation. The sensor was created using a membrane made of poly vinyl chloride, potassium tetrakis (4-chlorophenyl) borate as a cation exchanger, 2-Nitrophenyloctylether as a plasticizer, and 2-hydroxypropyl--cyclodextrin as a specific molecular recognition component. According to IUPAC recommendations, the proposed sensor was validated, and it displays a linear dynamic range from $1 \times 10{-}5$ to $1 \times 10{-}2$ mol.L^{-1}, with a Nernstian slope of 58.914 mV/decade [47].

For the first time, a novel optical sensor for lomefloxacin was based on the plasma resonance characteristics of silver nanoparticles (AgNPs). The change in color and absorption spectra of the AgNPs suspension caused by the hydrogen bonds and electrostatic force between lomefloxacin and AgNPs provided a theoretical foundation for the optical detection of lomefloxacin. Additionally, we increased the sensitivity of the AgNPs-lomefloxacin detection system by adding cystine, which allowed it to reach the critical point of discoloration. Furthermore, it was investigated how the AgNPs-lomefloxacin detection system was affected by variables like temperature, reaction time, and pH 12.

2.2.4 Chromatographic methods:

2.2.4.1 Thin layer chromatographic methods:

The first method depends on densitometric evaluation of thin layer chromatograms of lomefloxacin hydrochloride with its acid degradation product at

288 nm using ammonium chloride solution (0.3 M): n-propanol: conc. ammonia (1:8:1, by volume) as a mobile phase [28].

Another method is based on densitometric evaluation of thin layer chromatograms of lomefloxacin hydrochloride and ciprofloxacin hydrochloride in the presence of their acid induced degradation products at 288 nm using ammonia buffer and methanol (20:80, v/v) as a mobile phase [48]. Also, there is another method that relies on stability indicating densitometric evaluation of thin layer chromatograms of lomefloxacin hydrochloride in the presence of its degradation products at 288 nm using chloroform: conc. ammonia:methanol (10:3:7, by volume) as a mobile phase [49].

The last method is depending on densitometric evaluation of thin layer chromatograms in bulk drug and tablet dosage form at 288 nm using 2-propanol: conc. ammonia:water (86:6:8, by volume) as a mobile phase [50].

2.2.4.2 High & ultraperformance liquid chromatographic methods:

Table 2: *HPLC methods reported for the determination of lomefloxacin.*

Matrix	Column	Mobile phase	Detector	Ref.
In plasma and urinewith norfloxacin (internal standard)	BondaPak C18	(a) Acetate buffer at pH = 4.8 (b) Acetonitrile (80:23, by volume)	Fluorescenceλ_{ex} = 280 nmλ_{em} = 430 nm	(Shibl, Tawfik et al. 1991) [51]
In plasma with enoxacin (internal standard)	Anion exchange Vydac	acetonitrile: Phosphate buffer at pH =7 (10:90, by volume)	UV at 280 nm	(Carlucci, Cilli et al. 1993) [52]
In plasma with fenbufen and felbinacin	Anion exchange Supelcosil LC-SAX	Acetonitrile Phosphate buffer at pH= 7	UV at 280 nm	(Carlucci, Mazzeo et al. 1996) [53]
In plasma with sarafloxacin (internal standard)	Novapak C18	Acetonitrile: Phosphate buffer at = pH 3 (20:80, v/v)	Fluorescenceλ_{ex} = 338 nmλ_{em} = 425 nm	(Garcia, Solans et al. 2001) [54]
In seminal plasma with ofloxacin (internal standard)	Spherisorb S5ODS1-C18	Acetonitrile: Phosphate buffer at = pH 7 (20:80, v/v)	Fluorescenceλ_{ex} = 280 nmλ_{em} = 440 nm	(Kumar and Goyal 2017) [55]
In wastewater with: - Pipemidic acid - Norfloxacin - Ciprofloxacin - Enrofloxacin - Ofloxacin - Sarafloxacin - Difloxacin - Tosufloxacin	YMC ODS-AQ S-3C18	Gradient elution using: (A) Water (B) Acetonitrile	Tandem mass spectrometry	(Nakata, Kannan et al. 2005) [56]
In plasma with ofloxacin	Hibar Lichrospher100 - C8	0.5 % triethyl amine at pH= 2.5 with phosphoric acid: acetonitrile (85:15, v/v)	UV at 280 nm	(Zendelovska and Stafilov 2005) [57]
In raw material and tablet with excipients	Phenomenex C18	1% Acetic acid:methanol: acetonitrile (70:15:15, by volume)	UV at 280 nm	(Tozo and Salgado 2006) [58]
In pharmaceutical preparations with: - Gatifloxacin - Levofloxacin - Pefloxacin	LiChrospher 100-C18	0.3% of triethylamine at pH = 3.3 with phosphoric acid with acetonitrile:water (20:80, v/v)	UV from 279-295 nm	(Santoro, Kassab et al. 2006) [59]

In pharmaceutical preparations with degradation product	Inertsil C18	Water: acetonitrile: triethyl amine (80:20:0.6, by volume) at pH= 3 with orthophosphoricacid	UV at 328 nm	(Gupta, Yadav et al. 2014) [46]
In marine productsand animal tissues	Symmetry C18	Gradient elution using: (a) Acetonitrile (b) 0.1% formic acid at pH =2.5	Fluorescenceλ_{ex} = 280 nmλ_{em} = 450 nm	(Chang, Wang et al. 2008) [60]
In pharmaceutical preparations with excipients	Inertsil ODS-C18	Acetonitrile: 0.025 M phosphoric acid (20:80, byvolume)	UV at 287 nm.	(Amran, Hossain et al. 2011) [29]
In pharmaceutical preparations with enrofloxacin and ofloxacin	Neucleosil C18	Acetonitrile:Phosphate buffer at pH = 2.4 (20:80, v/v)	UV at 294 nm	(Amin, Dessouki et al. 2011) [61]
In pharmaceutical preparations with enrofloxacin and ofloxacin	µBondapak C18	0.31% ammonium acetate at pH= 2.2 and 0.65% sodium perchlorate and with orthophosphoric acid: acetonitrile (81:19, by volume)	UV at 294 nm	(Amin, Dessouki et al. 2011) [61]
A honey sample. With ofloxacin, ciprofloxacin, enrofloxacin, lomefloxacin, and difloxacin.	C18 column	Gradient elution using: (a) methanol (b) acetonitrile (c) 10 mmol/L NaH2PO4·2H2O at PH=3	fluorescence λem = 480 nm	(Tian, Ren et al. 2022) [62]
Oxidation of lomefloxacin and balofloxacin	Kinetex 5u XB-C18 100A column	isocratic elution using acetonitrile and 0.05 M phosphate buffer at pH = 3.20 adjusted with o-phosphoric acid (13:87 v/v for lomefloxacin; 20:80 v/v for balofloxacin).	a photodiode array detector	(Żuromska-Witek, Żmudzki et al. 2020) [63]
Poultry eggs with enrofloxacin, ciprofloxacin, ofloxacin, pefloxacin, norfloxacin, and sarafloxacin	C18 column	Gradient elution using: (a) 0.1% (V/V) formic acid (b) acetonitrile	Tandem mass spectrometry	(Huang, Fan et al. 2019) [64]
Eye drops used in cataract surgery	Phenomenex Luna® C18 column	methanol:water:formic acid (70:29:1, by volume)	Tandem mass spectrometry	(Nassar, Attia et al. 2020) [65]

2.3 Reported methods of dexamethasone

2.3.1 Spectrophotometric methods:

Dexamethasone sodium phosphate is directly determined at pH = 6 using a double-beam spectrophotometer at 242.5 nm [66] and using water:ethanol (1:2 v/v) as background electrolyte at 240 nm [67]. Oxidation of dexamethasone by iron (III) followed by complexation of iron (II) with potassium hexacynoferrate (III) to form bluish green complex with absorbance at wavelength=780 nm is another method [68].

2.3.2 Chromatographic techniques

2.3.2.1 Thin layer chromatographic method

Development of HPTLC method for determination of dexamethasone using hexane– propan-2-ol (90:10, by volume) as a mobile phase [69] (Huetos, Ramos et al. 1999).

2.3.2.2 High and ultraperformance liquid chromatographic methods:

Table 3: *HPLC methods reported for the determination of remdesivir.*

Matrix	Column	Mobile phase	Detector	Ref.
In microemulsions	RP-18 column	Water :methanol (35:65; by volume)	UV at 239 nm	(Urban, Mainardes et al. 2009) [70]
Pharmaceutical Formulations	C_{18} column	Ammonium acetate buffer (5mM) :methanol :acetonitrile(43:25:32, v/v)	UV at 240 nm	(Duarah, Sharma et al. 2021) [71]
Pharmaceutical formulations with ofloxacin	C 18 column	Acetonitrile : phosphate buffer at pH= 4 (50:50, v/v)	UV at 236 nm	(Sireesha and Prakash 2012) [72]
Pharmaceutical Formulations with moxifloxacin.	BDS Hypersil C8 column	20 mM phosphate buffer , 0.1% (v/v) triethylamine, atpH = 2.8) and methanol (38.5:61.5 v/v)	diode array detector at 254 nm	(Razzaq, Ashfaq et al. 2017) [73]
Pharmaceutical Formulations with granisetron.	CPS Hypersil CN column	Acetonitrile: 100 mM buffer Triethylamine at pH = 3.0 with orthophosphoric acid (25:75 by volume)	UV at 242 nm	(Heda, Kathiriya et al. 2011) [74]
Human plasma	a Sphereclone ODS2 column	10 mM phosphate buffer at pH = 7.0: acetonitrile (68:32,v/v)	UV at 240 nm	(Song, Park et al. 2004) [75]
In dried blood spot samples	a Zorbax EclipsePlus C18 colum	Water and acetonitrile with formic acid	Mass spectrometer	(Patel, Tanna et al. 2010) [76]

3. Conclusion

In this review, simple and clear highlights for the collected information about the most instrumental methods used for determination of compounds used in covid-19 treatment are reported. This review includes HPLC technique, spectroscopy, flourimetry and electrochemical methods. It was obvious that HPLC technique was the most developed method followed by spectroscopy. The presented methods were validated according to ICH guidelines in addition to some of them were assessed using various green tools.

Conflict of interest statement

Authors declare that there is no conflict of interest.

References

1. Joshi, S., J. Parkar, A. Ansari, A. Vora, D. Talwar, M. Tiwaskar, S. Patil and H. Barkate (2021). "Role of favipiravir in the treatment of COVID-19." International Journal of Infectious Diseases 102: 501-508.

2. Magro, G. (2020). "COVID-19: Review on latest available drugs and therapies against SARS-CoV-2. Coagulation and inflammation cross-talking." Virus research 286: 198070.

3. Venkatasubbaiah, M., P. D. Reddy and S. V. Satyanarayana (2020). "Literature-based review of the drugs used for the treatment of COVID-19." Current medicine research and practice 10(3): 100- 109.

4. Scavone, C., S. Brusco, M. Bertini, L. Sportiello, C. Rafaniello, A. Zoccoli, L. Berrino, G. Racagni, F. Rossi and A. Capuano (2020). "Current pharmacological treatments for COVID-19: What's next?" British journal of pharmacology 177(21): 4813-4824.

5. Malin, J. J., I. Suárez, V. Priesner, G. Fätkenheuer and J. Rybniker (2020). "Remdesivir against COVID-19 and other viral diseases." Clinical microbiology reviews 34(1): e00162-00120.

6. Warren, T. K., R. Jordan, M. K. Lo, A. S. Ray, R. L. Mackman, V. Soloveva, D. Siegel, M. Perron, R. Bannister and H. C. Hui (2016). "Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys." Nature 531(7594): 381-385.

7. Moneim, M. M. A., M. F. Kamal and M. M. Hamdy (2021). "Rapid sensitive bioscreening of remdesivir in COVID-19 medication: Selective drug determination in the presence of six co- administered therapeutics." Reviews in Analytical Chemistry 40(1): 323-333.

8. Karampela, I. and M. Dalamaga (2020). "Could respiratory fluoroquinolones, levofloxacin and moxifloxacin, prove to be beneficial as an adjunct treatment in COVID-19?" Archives of medical research 51(7): 741-742.

9. Ahmed, M. H. and A. Hassan (2020). "Dexamethasone for the treatment of coronavirus disease (COVID-19): a review." SN comprehensive clinical medicine 2: 2637-2646.

10. Abdelazim, A. H. and S. Ramzy (2022). "Spectrophotometric quantitative analysis of remdesivir using acid dye reagent selected by computational calculations." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 276: 121188.

11. Darwish, I. A., N. Y. Khalil, H. W. Darwish, N. Z. Alzoman and A. M. Al-Hossaini (2022). "Synthesis, spectroscopic and computational characterization of charge transfer complex of remdesivir with chloranilic acid: Application to development of novel 96-microwell spectrophotometric assay." Journal of Molecular Structure 1263: 133104.

12. El-Awady, M., H. Elmansi, F. Belal and R. A. Shabana (2022). "Insights on the Quantitative Concurrent Fluorescence-Based Analysis of Anti-COVID-19 Drugs Remdesivir and Favipiravir." Journal of Fluorescence 32(5): 1941-1948.

13. Elmansi, H., A. E. Ibrahim, I. E. Mikhail and F. Belal (2021). "Green and sensitive spectrofluorimetric determination of Remdesivir, an FDA approved SARS-CoV-2 candidate antiviral; application in pharmaceutical dosage forms and spiked human plasma." Analytical Methods 13(23): 2596-2602.

14. Attia, T. Z., J. M. Boushra, A. F. Abdel Hakiem, A. S. Lashien and D. A. Noureldeen (2022). "Spectrofluorimetric determination of the anti- Covid 19 agent, remdesivir, in vials and spiked human plasma." Luminescence 37(7): 1192-1199.

15. Tkach, V. V., M. Kushnir, S. C. de Oliveira, J. Ivanushko, A. V. Velyka, A. F. Molodianu, P. I. Yagodynets, Z. O. Kormosh, L. Vaz dos Reis and O. V. Luganska (2021). "Theoretical description for anti-COVID-19 drug Remdesivir electrochemical determination, assisted by squaraine Dye–Ag2O2 composite." Biointerface Research in Applied Chemistry 11(2): 9201-9208.

16. Bulduk, I. and E. Akbel (2021). "A comparative study of HPLC and UV spectrophotometric methods for remdesivir quantification in pharmaceutical formulations." Journal of Taibah University for Science 15(1): 507-513.

17. Kishore, D., K. Prasad, C. Darapureddy and R. Phani (2021). " Development and validation of a new HPLC bioanalytical internal standard method for the analysis of redmisivir in human plasma" Rasayan Journal of Chemistry: 2639-2644.

18. Rao, N. S., A. Adimulapu, B. N. Babu and G. Rambabu (2022). "Development and Validation of an HPLC-MS/MS Method for the Determination of Remdesivir in Rat Plasma." Journal of Pharmaceutical Research International 34(28B): 23-31.

19. Padhye[1], H., B. Sonawane, V. K. Munipalli, A. Paranjpe, R. M. Singh, S. Nayak[1] and V. Bhaskar[1] "Stability Indicating RP-HPLC Method for Determination of Remdesivir in Sublingual Tablet Dosage Form".

20. El Azab, N. F. (2022). "A validated UHPLC-MS/MS method for simultaneous quantification of some repurposed COVID-19 drugs in rat plasma: Application to a pharmacokinetic study." Microchemical Journal 178: 107321.

21. Emam, A. A., E. A. Abdelaleem, E. H. Abdelmomen,R. H. Abdelmoety and R. M. Abdelfatah (2022). "Rapid and ecofriendly UPLC quantification of Remdesivir, Favipiravir and Dexamethasone for accurate therapeutic drug monitoring in Covid-19 Patient's plasma." Microchemical Journal 179: 107580.

22. Skaggs, C., H. Zimmerman, N. Manicke and L. Kirkpatrick (2022). "Development and validation of a paper spray mass spectrometry method for the rapid quantitation of remdesivir and its active metabolite, GS-441524, in human plasma." Journal of Mass

Spectrometry and Advances in the Clinical lab 25: 27-35.

23. Habler, K., M. Brügel, D. Teupser, U. Liebchen, C. Scharf, U. Schönermarck, M. Vogeser and M. Paal (2021). "Simultaneous quantification of seven repurposed COVID-19 drugs remdesivir (plus metabolite GS-441524), chloroquine, hydroxychloroquine, lopinavir, ritonavir, favipiravir and azithromycin by a two-dimensional isotope dilution LC–MS/MS method in human serum." Journal of pharmaceutical and biomedical analysis 196: 113935.

24. Kumar, J. S., M. Keerthana, N. Varsha, P. Asha⁴, T. S. Kumari7, P. V. Babu and B. S. Prasad (2022). "Development and validation of liquid chromatography, mass spectrometer methods for determination of remdesivir and its metabolite GS-441524 in plasma and their applications in COVID-19-related clinical studies"

25. Alvarez, J.-C., P. Moine, I. Etting, D. Annane and I. A. Larabi (2020). "Quantification of plasma remdesivir and its metabolite GS-441524 using liquid chromatography coupled to tandem mass spectrometry. Application to a Covid-19 treated patient." Clinical Chemistry and Laboratory Medicine (CCLM) 58(9): 1461-1468.

26. Wang, N., X. Ren, Z. Si, W. Jiang, C. Liu and X. Liu (2000). "Derivative spectrophotometric determination of praseodymium in rare earth mixtures with lomefloxacin." Talanta 51(3): 595-598.

27. Gomes, G. C. and H. R. N. Salgado (2005). "Validation of UV spectrophotometric method for determination of lomefloxacin in pharmaceutical dosage form." Acta farmaceutica bonaerense 24(3): 406.

28. Salem, M. Y., N. M. El-Guindi, H. K. Mikael and L. E.-S. Abd-El-Fattah (2006). "Stability indicating methods for the determination of some fluoroquinolones in the presence of their decarboxylated degradates." Chemical and pharmaceutical bulletin 54(12): 1625-1632.

29. Amran, M. S., M. R. Hossain, M. A. Baki, F. M. Amjad, S. Sultana and M. A. Hossain (2011). "Analysis of lomefloxacin by spectrophotometry: development of simple quantitative analytical method." Bangladesh Pharm. J. 14: 31-35.

30. Jain, R., V. Jain, N. Jain, D. K. Jain and S. Jain (2012). "Eco friendly spectrophotometric method for quantitative estimation of lomefloxacin using hydrotropic approach." Journal of Applied Pharmaceutical Science(Issue): 111-114.

31. Tammam, M. H. (2014). "Photostability studies on gemifloxacin and lomefloxacin in bulk powder and dosage forms." European Journal of Chemistry 5(1): 73-80.

32. Singh, S. B. and S. Singh (2014). "Validated UV-Spectrophotometric method for quantitative estimation of Lomefloxacin HCl in bulk and pharmaceutical dosages forms." World Journal of Pharmaceutical Sciences: 1520-1525.

33. Tieli, Z., Z. Huichun and J. Linpei (1999). "Photochemical fluorescence enhancement of the terbium–lomefloxacin complex and its application." Talanta 49(1): 77-82.

34. Nie, L.-H., H.-C. Zhao, X. Wang, L. Yi, Y. Lu, L.-P. Jin and H.-M. Ma (2002). "Determination of lomefloxacin by terbium sensitized chemiluminescence method." Analytical and bioanalytical chemistry 374: 1187-1190.

35. Salem, H. (2005). "Spectrofluorimetric, atomic absorption spectrometric and spectrophotometric determination of some fluoroquinolones." American Journal of Applied Sciences 2(3): 719- 729.

36. Chen, X., J.-C. Fan, Y. Wang, C.-P. Fan and Z.-C. Shang (2006). "Fluorometric study on the interaction between lomefloxacin and bovine lactoferrin." Analytical sciences 22(3): 427-430.

37. Ling–boa, Q., Z. Jie and L. Jian–jun (2007). "Determination of lomefloxacin by flow injection chemiluminescence." Anal Chem Indian J 4(1-3): 43-48.

38. Salem, H., L. Fada and W. Khater (2007). "Spectrofluorimetric determination of certain fluoroquinolones through charge transfer complex formation." American journal of pharmacology and toxicology 2(1): 18-25.

39. Derayea, S. M., Y. F. Hassan, M. A. Hammad, Y. M. Alahmadi, M. A. Omar and E. Samir (2023). "Feasible spectrofluorimetric approach for the ultrasensitive determination of lomefloxacin based on synergistic effects of micellization and metal complexation." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 292: 122399.

40. Attia, T. Z., M. A. Omar, D. A. Nour El-Deen, A. Mohamed Abbas and A. A. Mohamed (2022). "A novel spectrofluorimetric method for determination of lomefloxacin adopting on zinc (II) chelation strategy: Application in human plasma." Luminescence 37(2): 255-262.

41. Orachorn, N. and O. Bunkoed (2019). "A nanocomposite fluorescent probe of polyaniline, graphene oxide and quantum dots incorporated into highly selective polymer for lomefloxacin detection." Talanta 203: 261-268.

42. Boltia, S. A., A. T. Soudi, E. S. Elzanfaly and H. E. Zaazaa (2019). "A new plan for determining drug pharmacokinetics by establishment of urinary excretion pattern. Spectrofluorimetric application on Lomeflox® tablets." Microchemical Journal 148: 419-423.

43. CEZYG, H. (2001). "Adsorptive voltammetric behavior of lomefloxacin and its application." J Beijing Normal Univ 2: 22.

44. Song, J.-f., Y. Shao and W. Guo (2001). "Determination of lomefloxacin, an antibacterial drug, in pharmaceutical preparations based on its polarographic catalytic wave in the presence of 2-iodoacetamide." Analytical sciences 17(10): 1145-1148.

45. Vílchez, J. L., L. Araujo, A. Prieto and A. Navalón (2001). "Differential-pulse adsorptive stripping voltammetric determination of the antibacterial lomefloxacin." Journal of pharmaceutical and biomedical analysis 26(1): 23-29.

46. Gupta, P., S. K. Yadav and R. N. Goyal (2014). "A sensitive polymelamine modified sensor for the determination of lomefloxacin in biological fluids." Journal of The Electrochemical Society 162(1): H86.

47. Boltia, S. A., A. T. Soudi, E. S. Elzanfaly and H. E. Zaazaa (2019). "Development and Greenness Evaluation of a Potentiometric Method for Lomefloxacin Hydrochloride Determination in Urine and Establishment of Cumulative Excretion Pattern." Journal of The Electrochemical Society 166(2): B141.

48. Hassib, S. T., R. El-Bagary, H. M. Hashem and M. M. El-Hakim (2007). "Simultaneous determination of intact Lomefloxacin and Ciprofloxacin in the presence of their acid degradation products." Bulletin of Pharmaceutical Sciences. Assiut 30(2): 241-258.

49. Chitlange, S. S., M. Ranjane, S. B. Wankhede and D. M. Sakarkar (2009). "Stability-indicating HPTLC method for estimation of lomefloxacin hydrochloride in pharmaceutical dosage form." Int.J. PharmTech Res. 1(3): 844-851.

50. Rajasree, R., K. Radha, S. Bernard, K. Girija and A. Nair (2013). "Estimation of Lomefloxacin hydrochloride in Bulk and Tablet dosage form by HPTLC method." Hygeia: Journal for Drugs and Medicine 5(1): 141-147.

51. Shibl, A. M., A. F. Tawfik, S. El-Houfy and F. J. Al- Shammary (1991). "Determination of lomefloxacin in biological fluids by high- performance liquid chromatography and a microbiological method." Journal of clinical pharmacy and therapeutics 16(5): 353-359.

52. Carlucci, G., A. Cilli, M. Liberato and P. Mazzeo (1993). "Determination of lomefloxacin in human plasma by solid-phase extraction and high-performance liquid chromatography with UV detection." Journal of pharmaceutical and biomedical analysis 11(11-12): 1105-1108.

53. Carlucci, G., P. Mazzeo and G. Palumbo (1996). "Simultaneous determination of lomefloxacin, fenbufen and felbinac in human plasma using high performance liquid chromatography." Chromatographia 43: 261-264.

54. Garcia, M., C. Solans, A. Calvo, M. Royo, E. Hernandez, R. Rey and M. Bregante (2001). "Determination of lomefloxacin in plasma samples by HPLC with fluorescence detection. Application to pharmacokinetic studies." Chromatographia 54: 577-580.

55. Kumar, N. and R. N. Goyal (2017). "Gold-palladium nanoparticles aided electrochemically reduced graphene oxide sensor for the simultaneous estimation of lomefloxacin and amoxicillin." Sensors and Actuators B: Chemical 243: 658-668.

56. Nakata, H., K. Kannan, P. D. Jones and J. P. Giesy (2005). "Determination of fluoroquinolone antibiotics in wastewater effluents by liquid chromatography–mass spectrometry and fluorescence detection." Chemosphere 58(6): 759-766.

57. Zendelovska, D. and T. Stafilov (2005). "Development and validation of high-performance liquid chromatographic method for determination of ofloxacin and lomefloxacin in human plasma." Journal of the Serbian Chemical Society 70(12): 1451-1460.

58. Tozo, G. C. and H. R. Salgado (2006). "Determination of lomefloxacin in tablet preparations by liquid chromatography." Journal of AOAC International 89(5): 1305-1308.

59. Santoro, M. I. R., N. M. Kassab, A. K. Singh and E. R. Kedor-Hackmam (2006). "Quantitative determination of gatifloxacin, levofloxacin, lomefloxacin and pefloxacin fluoroquinolonic antibiotics in pharmaceutical preparations by high-performance liquid chromatography." Journal of Pharmaceutical and Biomedical Analysis 40(1): 179-184.

60. Chang, C.-S., W.-H. Wang and C.-E. Tsai (2008). "Simultaneous determination of eleven quinolones antibacterial residues in marine products and animal tissues by liquid chromatography with fluorescence detection." Journal of Food and Drug Analysis 16(6): 2.

61. Amin, A. S., H. A. Dessouki and I. A. Agwa (2011). "Ion-pairing and reversed phase liquid chromatography for the determination of three different quinolones: enrofloxacin, lomefloxacin and ofloxacin." Arabian Journal of Chemistry 4(3): 249-257.

62. Tian, C., X. Ren, M. He, B. Chen and B. Hu (2022). "Core-shell magnetic porous organic polymer for magnetic solid-phase extraction of fluoroquinolone antibiotics in honey samples followed by high-performance liquid chromatography with fluorescence detection." Journal of Separation Science 45(4): 874-882.

63. Żuromska-Witek, B., P. Żmudzki, M. Szlósarczyk, A. Maślanka and U. Hubicka (2020). "Development and validation of Stability-indicating HPLC methods for the estimation of lomefloxacin and balofloxacin oxidation process under ACVA, H2O2, or KMnO4 treatment. Kinetic evaluation and identification of degradation products by mass spectrometry." Molecules 25(22): 5251.

64. Huang, K., X. Fan, Q. Cao, Z. Han, L. Zhang, H. Wang and J. Guo (2019). "Rapid determination of 7 quinolone residues in poultry eggs using QuEChERS and ultra performance liquid chromatography-tandem mass spectrometry." Food and Fermentation Industries 45(16): 261- 265.

65. Nassar, M. W., K. A. Attia, A. A. Mohamed, R. A. Said, A. El-Olemy and M. A. Hasan (2020). "Validated Liquid Chromatography–Tandem Mass Spectroscopic Method for Simultaneous Determination of Certain Drugs Used in Cataract Surgery in Rabbit Aqueous Humor." Journal of Chromatographic Science 58(9): 814-822.

66. Al-Owaidi, M. F., S. L. Alkhafaji and A. M. Mahood (2021). "Quantitative determination of dexamethasone sodium phosphate in bulk and pharmaceuticals at suitable pH values using the spectrophotometric method." Journal of Advanced Pharmaceutical Technology & Research 12(4): 378.

67. Sversut, R. A., J. C. Vieira, A. M. Rosa, A. K. Singh, M. S. do Amaral and N. M. Kassab (2015). "Improved UV Spectrophotometric Method for Precise, Efficient and Selective Determination of Dexamethasone in Pharmaceutical Dosage Forms." Orbital: The Electronic Journal of Chemistry: 5-9.

68. Singh, D. and R. Verma (2008). "Spectrophotometric determination of corticosteroids and its application in pharmaceutical formulation".

69. Huetos, O., M. Ramos, M. M. de Pozuelo, T. B. Reuvers and M. San Andrés (1999). "Determination of dexamethasone in feed by TLC and HPLC." Analyst 124(11): 1583-1587.

70. Urban, M. C. C., R. M. Mainardes and M. P. D. Gremião (2009). "Development and validation of HPLC method for analysis of dexamethasone acetate in microemulsions." Brazilian journal of pharmaceutical sciences 45: 87-92.

71. Duarah, S., M. Sharma and J. Wen (2021). "Rapid and simultaneous determination of dexamethasone and dexamethasone sodium phosphate using HPLC-UV: Application in microneedle-assisted skin permeation and deposition studies." Journal of Chromatography B 1170: 122609.

72. Sireesha, K. R. and K. Prakash (2012). "HPLC-UV method for simultaneous determination of ofloxacin and dexamethasone sodium phosphate." Int. J. Pharm. Pharm. Sci 4(1): 415-418.

73. Razzaq, S. N., M. Ashfaq, I. U. Khan, I. Mariam, S. S. Razzaq and W. Azeem (2017). "Simultaneous determination of dexamethasone and moxifloxacin in pharmaceutical formulations using stability indicating HPLC method." Arabian Journal of Chemistry 10(3): 321-328.

74. Heda, A., J. Kathiriya, D. Gadade and P. Puranik (2011). "Development and validation of RP-HPLC method for simultaneous determination of granisetron

and dexamethasone." Indian Journal of Pharmaceutical Sciences 73(6): 696.

75. Song, Y. K., J. S. Park, J. K. Kim and C. K. Kim (2004). "HPLC determination of dexamethasone in human plasma." Journal of liquid chromatography & related technologies 27(14): 2293-2306.

76. Patel, P., S. Tanna, H. Mulla, V. Kairamkonda, H. Pandya and G. Lawson (2010). "Dexamethasone quantification in dried blood spot samples using LC–MS: the potential for application to neonatal pharmacokinetic studies." Journal of Chromatography B 878(31): 3277-3282.