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UHPLC-MS/MS: A SELECTIVE BIOANALYTICAL TECHNIQUE TO DETECT AND QUANTIFY REPURPOSED COVID-19 PROTOCOL DRUGS IN HUMAN PLASMA

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The SARS-CoV-2 caused COVID-19 disease first appeared during late 2019 and announced in March, 2020 as a global pandemic by the World Health Organization. COVID-19 was deemed to be no longer a public health emergency of worldwide concern in May 2023. Still, the virus is a potentially dangerous health risk and is still spreading throughout communities. In the current study a sensitive and rapid developed method was validated for the quantitative estimation of the drugs namely (apixaban, moxifloxacin-HCl, remdesivir and paracetamol) used in the management of COVID-19 in spiked human plasma simultaneously. The extraction solvent was diethyl ether: dichloromethane (70:30) used to extract the cited drugs and azithromycin (IS) from human plasma (liquid-liquid extraction). ultra-high performance liquid chromatography with tandem mass detection was employed for separation and quantification. In an Agilent Poroshell 120 EC-C_{18} (4.6 x 50 mm, 2.7 µm) column, a mobile phase consisting of methanol: water with 0.1% formic acid (93:7 v/v) was pumped in isocratic elution at a flow rate of 0.3 mL/min. with a total run time of 3 minutes. Multiple reaction monitoring mode (MRM) transitions were measured at the positive ionization mode for the four drugs and IS. The new approach was bio-analytically validated in accordance with the European Medicines Agency (EMA) requirements. The validated method is precise, selective, accurate and can be used in many applications including bioequivalence studies, therapeutic drug monitoring and routine drug analysis.

Keywords: *Remdesivir; apixaban; paracetamol; moxifloxacin; COVID-19*

INTRODUCTION

The SARS-CoV-2 caused COVID-19 disease first appeared during late 2019 and spread rapidly. The World Health Organization (WHO) announced on March 11, 2020, that it was a global pandemic. COVID-19 was deemed to be no longer a public health emergency of worldwide concern in May 2023. Still, the virus is a potentially dangerous health risk and is still spreading throughout communities[1]. As per the latest WHO situation reports; September 2024; over 776 million confirmed cases and over 7 million deaths have been reported globally. There are 27,227 new hospitalizations and 859 new ICU admissions were reported from 45 and 32 countries, respectively; with an overall marginal 3% decrease in hospitalization and 36% increase in new ICU admissions[2].

Upon searching literature, multiple therapeutic approaches were introduced in disease management including; **antiviral drugs** as remdesivir, and molnupiravir[3,4]. The main role of these drugs is blocking of the viral genome replication either by stopping the virus from entering host cells or by suppressing one or more of its replication processes[5]. Despite the conflicting information earlier, it was declared that using remdesivir in the beginning

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of illness resulted in lower risk of hospitalization and death and that it is the only treatment available for children under 12 with severe or critical case[6]. **Anti-inflammatory drugs** involving corticosteroids and other monoclonal antibodies as tocilizumab; which appeared to decrease mortality rate in cases suffering from acute respiratory distress syndrome[7]. **Analgesics and antipyretics** are used as symptomatic treatment in cases from mild to severe infections, the first line used drug is paracetamol[8]. The **antibiotics** empirical use is still confusing in COVID-19 disease, however the incidence of several co-infections and secondary bacterial pneumonia may necessitate their use[11,12]. Fluoroquinolones are from the highly suggested classes of antibiotics since they provide both anti-inflammatory and antibacterial properties in addition to having an immune-modulatory effect[11]. It appeared that using **antithrombotic agents** is important in cases exposed to the risk of COVID-19 associated coagulopathy[12]. The use of direct oral anticoagulants with different doses can be helpful for prophylaxis of high-risk patients as well as treatment for cases with high d-dimer level[13,14].

Determination of four medications that are indicative of the main classes utilized in COVID-19 management procedures in human plasma is the goal of the current investigation.[15]

Apixaban (APIX) (**Fig. 1a**), one of the main oral anticoagulants, it directly inhibits factor Xa which catalyses the conversion of prothrombin to thrombin**. Moxifloxacin.HCl (MOX.HCl)** (**Fig. 1b**), a member of the fluoroquinolones antibiotics; is used in COVID-19 as an additive therapy in many cases. The results of two in silico molecular docking studies that identified moxifloxacin and other class members as potential SARS-CoV-2 inhibitors based on their predicted binding affinities to the SARS-CoV-2 primary protease provide justification for utilizing a member of this class. According to this concept and due to their pharmacokinetic properties and safety profiles, Moxifloxacin and levofloxacin have been proposed for treatment of pneumonia caused by COVID-19[16,17]. **Remdesivir (REM)** (**Fig. 1c**), a monophosphoramidate prodrug of the adenine nucleoside analogue is the first FDA-approved drug in COVID-19 treatment[15,18]. Remdesivir's active form inhibits the RNA-dependent RNA polymerase (RdRp) of coronaviruses, including SARS-CoV-2, as it functions a nucleoside analogue. Although it has little effect on host RNA or DNA polymerases, the RdRp incorporates it into the developing RNA product, enabling the insertion of three additional nucleotides before RNA synthesis stalls.[19]. **Paracetamol (PAR)** (**Fig. 1d**) is the first line analgesic and antipyretic drug as it is very effective in the management of fever and body ache[15] Based on our literature search, it was found that **REM** was estimated in biological fluids such as plasma, bronchoalveolar aspirate (BAS) and Cerebrospinal fluid[20-22]. **PAR** also was determined in several biological fluids like; human plasma, serum and urine[23-25]. **APIX** was determined in human plasma[26,27] as well as **MOX.HCl**[28-30]. Furthermore; one method determined **APIX** using **MOX.HCl** as IS in rat plasma[31]. As far as we can tell, no technique for simultaneously determining the selected drugs in human plasma has been published. Considering COVID-19 as an outbreak pandemic managed with several regimens it could be useful to develop a single and rapid UHPLC-MS/MS method for the simultaneous determination of the four listed drugs and azithromycin **(IS, Fig. 1e)** in plasma while individual methods exist for these compounds, their combined determination is crucial that allows their efficient quantification for therapeutic drug monitoring in COVID-19 treatment protocols. Moreover, it would be an important method for dose adjustment based on patient needs in order to guarantee effectiveness and avoid toxicity. Furthermore, the clinical application of this method to COVID-19 treatment monitoring provides a valuable tool for optimizing patient care.

The pursuit of green analytical chemistry (GAC) is currently one of the main objectives of analytical laboratories. Every greenness assessment method is based on the twelve fundamental rules of GAC[32-34]. GAC's primary goal is to strike a compromise between lowering the environmental risks associated with analytical techniques and preserving the high Caliber of their output. Toxic solvents and/or reagents, energy-efficient devices, massive volumes of toxic waste, or threats to the environment and public health are examples of environmental hazards[35,36]. Several

assessment tools have been developed for the evaluation of influences of analytical processes on the environment. Analytical Greenness metric (AGREE)[37] along with greenness tool for sample preparation (AGREEprep)[38] were used to evaluate how environmentally friendly the sample preparation and analytical process were.

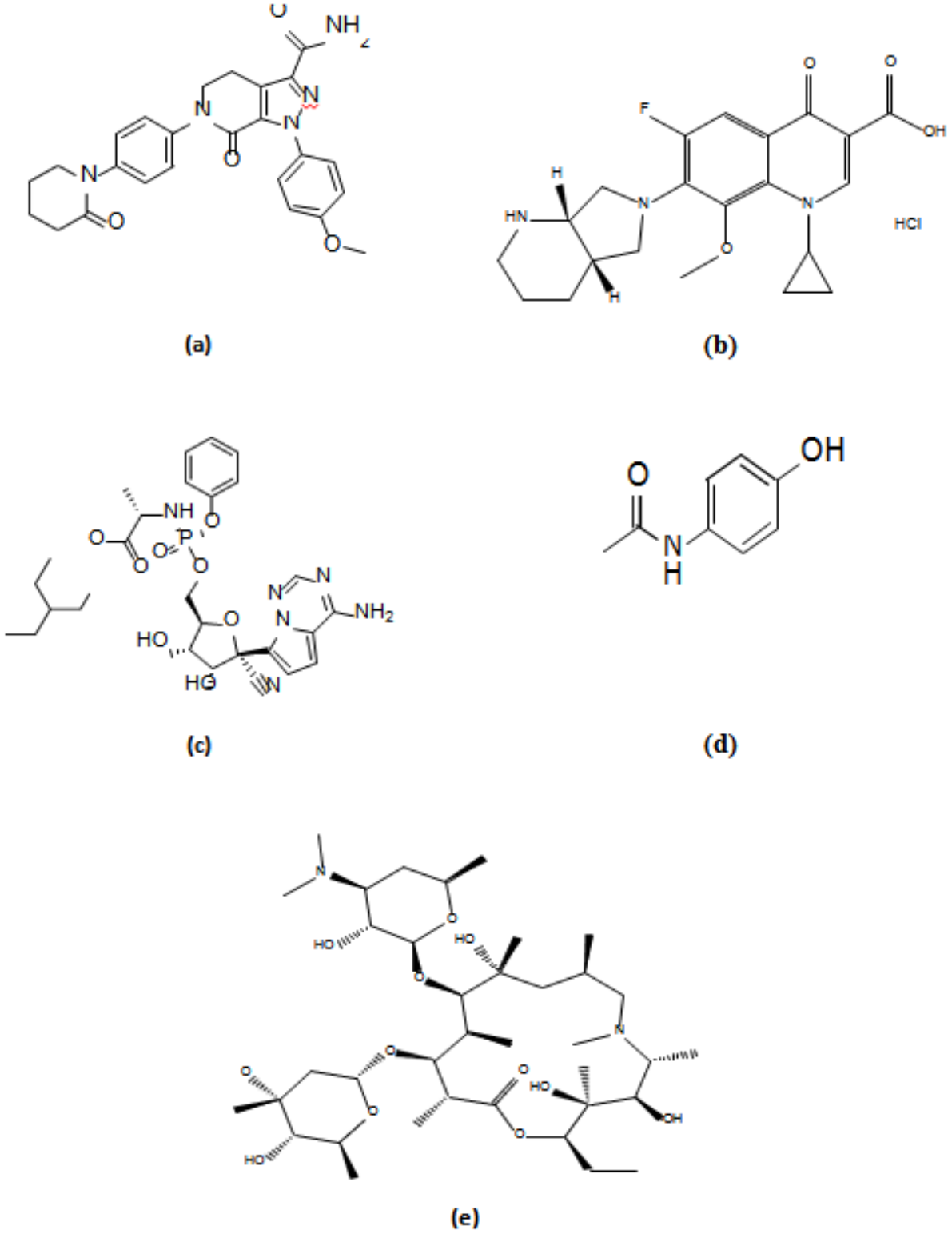


Fig. 1: Chemical structures of APIX (a), MOX.HCl (b), REM (c), PAR (d), and azithromycin (IS) (e).

Experimental

Instrumentation

Waters 3100 UPLC MS/MS (USA) consisting of Acquity ultra performance LC binary solvent manager; auto-sampler Acquity ultra performance LC Sample manager and a triple quadruple detector (Acquity ultra performance LC) were used throughout the experiment. Data acquisition was performed on (Mass lynx V4.1) analysis software. The column utilized for separation was Agilent Poroshell 120 EC-C_{18} (4.6 x 50 mm, 2.7 µm). Stuart vortex mixer (England) and Eppendorf AG 22331 evaporator (HAMBURG, 5301) were used to carry out the procedure. Human lab power sonic 405 and OHAUS frontier 5706 centrifuge were used during the current study.

MATERIALS AND METHODS

Egyptian Drug Authority (EDA), Cairo, Egypt provided the **APIX, MOX.HCl, REM, PAR and azithromycin (IS)** standards which are approved to contain 99.77 %, 99.80 %, 99.39 %, 99.84 % and 99.89 %, respectively. Methanol and acetonitrile (supplied by Merck) are of HPLC grade. The Holding Company for Biological Products and Vaccines (VACSERA, Egypt) provided the human blank plasma, which was then kept in storage at -70°C. The solvents used such as formic acid, diethyl ether and dichloromethane were supplied from Sigma-Aldrich, Germany. Double-distilled water was obtained after filtration through 0.45 µm nylon membrane filters.

Stock and working solutions

By precisely weighing each of the four mentioned drugs, four standard stock solutions of the chosen medications and IS (1 mg/mL) were produced using methanol as solvent. Further dilution was done to reach a final concentration of (200 µg/mL) for each drug. Each flask was sonicated before completing the volume with methanol.

Working solutions were prepared in two individual sets. The first set was utilized to prepare the calibrators while the quality control (QC) samples were produced from the other set. **APIX** working solutions for calibrators (10, 50, 100, 500, 1000, 5000 and 10000 ng/mL), working solutions of **MOX.HCl, REM and PAR** for calibrators (100, 500, 1000. 5000, 10000, 50000 and 100000 ng/mL) and 1000 ng/mL IS working solution were produced by diluting the appropriate stock solutions with methanol. Up until they were used, all solutions were stored in a refrigerator (2–8°C).

Calibrators and quality control samples preparation

To prepare calibration samples, 50 µL of each of the working solutions of **APIX**, **MOX.HCl, REM and PAR** added to aliquot (450 µL) of blank human plasma separately followed by 50 µL from the IS working solution. The final obtained concentrations in human plasma were (1, 5, 10, 50, 100, 500 and 1000 ng/mL) of **APIX**, (10, 50, 100. 500, 1000, 5000 and 10000 ng/mL) of **MOX.HCl, REM and PAR** and (100 ng/mL) for the IS. Likewise, three concentration levels of QC samples were prepared in plasma: low, medium and high QC samples, which are (3 QCL, 75 QCM and 800 QCH ng/mL) for **APIX** and (30, 750 and 7500 ng/mL) for each of **MOX.HCl, REM and PAR**. In addition; one blank and one zero (with IS alone) samples were created along with each calibration. Mixing of each sample takes place for 1 minute.

Extraction procedure

5 mL of the extraction solvent (70% diethyl ether (DEE): 30% dichloromethane (DCM)) was added to each plasma sample spiked with the (drug mixture and IS). The samples were then vortexed for 30 seconds, centrifuged at 3000 rpm, and allowed to sit at room temperature for 10 minutes. The upper organic layer only was transferred into a clean Wassermann tube, then evaporated using an evaporator at 45 °C. For reconstitution, the dry extract was dissolved in 200 µL of the mobile phase and then moved to a glass vial for UHPLC-MS/MS analysis.

Mass and chromatographic conditions

The Agilent Poroshell 120 EC-C18 (4.6 x 50 mm, 2.7 µm) column was used for chromatographic separation. Isocratic elution was applied, and a mobile phase consisting of (methanol: water with 0.1% formic acid (93: 7, *v/v*)) was used. Following filtration, the mobile phase was pushed through the column at a flow rate of 0.3 mL/min. Injection volume was 10

µL, and the analysis was done at 45°C. Applying these conditions resulted in a 3 min total run time and retention times of approximately 1.2, 1.76, 1.52, 1.83, and 1.74 min for **IS, APIX, MOX.HCl, REM, and PAR,** respectively; presented in **Fig. (2)**. **Table (1)** shows MRM that were recorded in positive ion mode. Peak area ratio (peak area of cited drug/ peak area of IS) calculations used to determine the cited drugs in human plasma.

Bio-analytical method validation

The validation was performed in accordance with EMA recommendations[39]. The mass spectrometric and chromatographic parameters listed in section 2.6 were used to accomplish the validation.

Selectivity

The assessment of selectivity involved identifying the four medicines along with the IS mentioned without interfering from the endogenous components of the matrix. To test the selectivity of the suggested method, six drug-free human plasma batches from different sources were chosen randomly, extracted and then analysed.

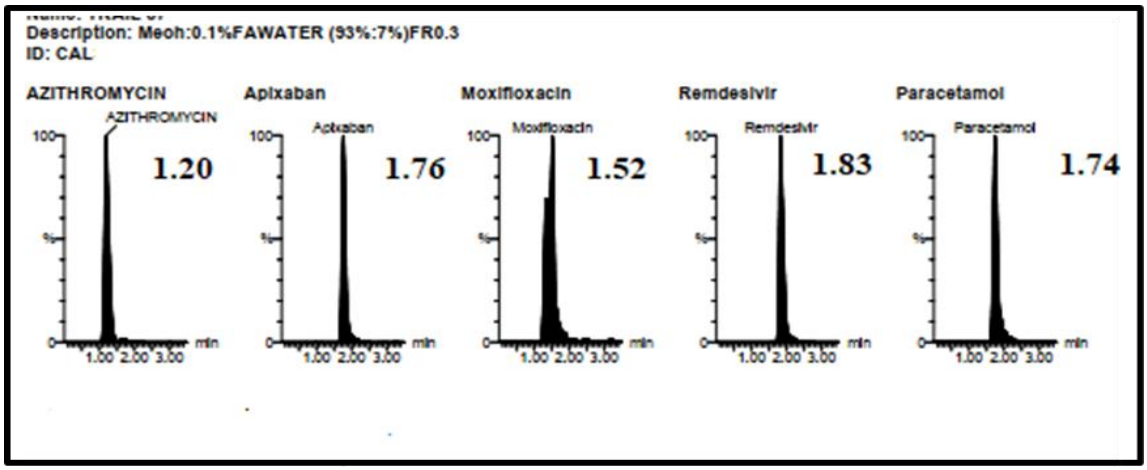


Fig. 2: MRM chromatograms of a 10 µl injection of medium quality control sample of APIX, MOX.HCl, REM, PAR and azithromycin (IS) in spiked human plasma.

Table 1: MS/MS conditions for multiple reaction monitoring (MRM) of apixaban, moxifloxacin.HCl, remdesivir, paracetamol and azithromycin (IS) in spiked human plasma

Analyte	**Ionization mode**	**CE (V)**	**FV (V)**	**Precursor ion peak**	**Product ion peak**	**Dwell time (sec)**
Apixaban	positive	25	35	460.17	443.09	0.029
Moxifloxacin.HCl	positive	25	40	402.14	384.12	0.029
Remdesivir	positive	25	35	603.19	402.06	0.029
Paracetamol	positive	25	35	151.89	109.90	0.029
Azithromycin	positive	30	45	749.47	591.38	0.029

CE: collision energy; FV: fragmentor voltage

Lower limit of quantitation and linearity

For each of the listed drugs, seven concentration levels were used to assess linearity. For **APIX**; (1, 5, 10, 50, 100, 500, and 1000 ng/mL), for **MOX.HCl, REM** and **PAR** (10, 50, 100, 500, 1000, 5000, and 10000 ng/mL) in human plasma. A zero sample (sample with IS only), drug-free sample (sample tested without IS), and seven non-zero samples were prepared to plot the six calibration curves using weighted linear regression analysis.

Intraday and Interday accuracy & Precision

Three analytical runs were conducted on six replicates of the LLOQ and the three QC levels on the same day as well as three days later in human plasma. The method accuracy was expressed as % recovery, and the method precision was assessed as coefficient of variation (CV)%.

Carry over

In order to verify carryover; It was checked at the beginning of method validation at the retention times of the upper limit of quantification (ULOQ) of the mentioned drugs and IS in a blank sample.

Dilution integrity

Accuracy and precision of the method shouldn't be affected by dilution of samples. To illustrate how dilution affects the suggested method, two dilution factors (two-fold and four-fold dilutions) were employed. Each analyte was spiked into the human plasma matrix at a concentration higher than the ULOQ; the concentration used for the 2-fold and 4- fold dilutions were 15000 ng/mL for **MOX.HCl, REM, PAR** and 1500 ng/mL for **APIX** in plasma, followed by diluting this sample with blank human plasma matrix.

Matrix effect

To evaluate the matrix effect, the drug's matrix factor (MF), IS, and IS normalized MF were computed. Six blank plasma samples that had been spiked with the aforementioned medications were used to calculate MF. The mean peak areas of the drug's QCL and QCH samples were divided by the mean peak areas of the respective standard solutions of equivalent concentration using the extraction procedure previously indicated. The normalized matrix factor was calculated by dividing the MF of the analyte by the IS.

Stability

By comparing the results of samples that were freshly prepared and analysed immediately with samples that were analysed after being stored under various conditions, QCL and QCH samples were used to assess stability in the plasma samples.

QC samples were kept at ambient temperature for six hours, then; their concentrations were compared to those of fresh samples in order to ascertain the samples short-term stability. While fresh samples with identical concentrations were compared to QCL and QCH samples that had been stored in a deep freezer at (–70 ± 5°C) for 30 days to determine long-term stability.

Three aliquots of each low and high QC sample were extracted, processed, and then stored in the auto-sampler for a full day in order to assess the auto-sampler stability of human plasma samples. Samples were examined and compared with nominal concentrations after a 24-hour period. Moreover, spiked QC samples were allowed to thaw naturally before being frozen once again for three rounds at (–70 ± 5°C) to test freeze-thaw stability.

Three aliquots of each QCL & QCH samples were extracted, processed, and then stored at the auto sampler for a full day in order to assess the auto-sampler stability. Samples were examined and compared with nominal amounts after a 24-hour period. In addition, spiked QC samples encountered three cycles of freeze-thaw stability at a temperature of –70 ± 5°C. After each cycle, the samples were allowed to thaw naturally and then frozen once more.

RESULTS AND DISCUSSION

The suggested method intended to develop a time-saving, precise and accurate analytical technique employing a fast and simple extraction protocol from human plasma along with liquid chromatography with MS detection to measure **APIX, MOX.HCl, REM and PAR** concurrently.

Based on the prescribed doses of each mentioned medication for the treatment of COVID-19, the linearity range was selected to

cover the maximum concentration (C-max) values of those medications[20,40-42]. As a result, therapeutic drug monitoring could benefit greatly from the developed approach.

Method development

Extraction protocol

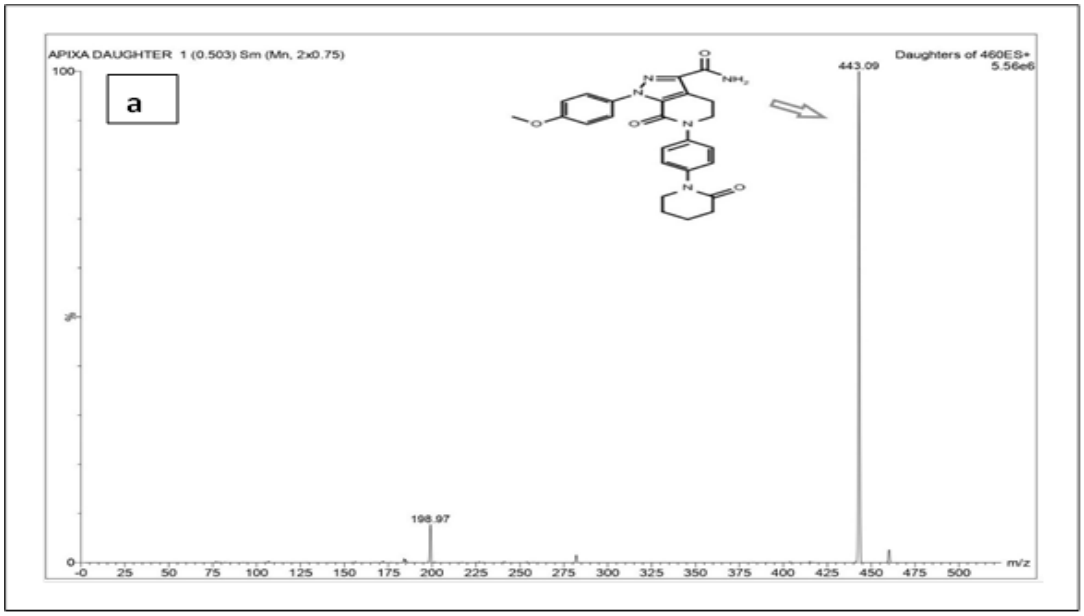
Liquid-liquid extraction was selected as the optimal extraction technique for sample preparation because it yields the highest response without interfering with the biological matrix. Protein precipitation using various acetonitrile to methanol ratios was attempted in the preliminary study, however the results showed low recoveries. On the other hand, the plasma sample was effectively de-proteinated using liquid-liquid extraction. After trying several solvents, including diethyl ether and dichloromethane, 5 mL of the extraction solvent (DEE: DCM) at a ratio of (70:30) produced good results.

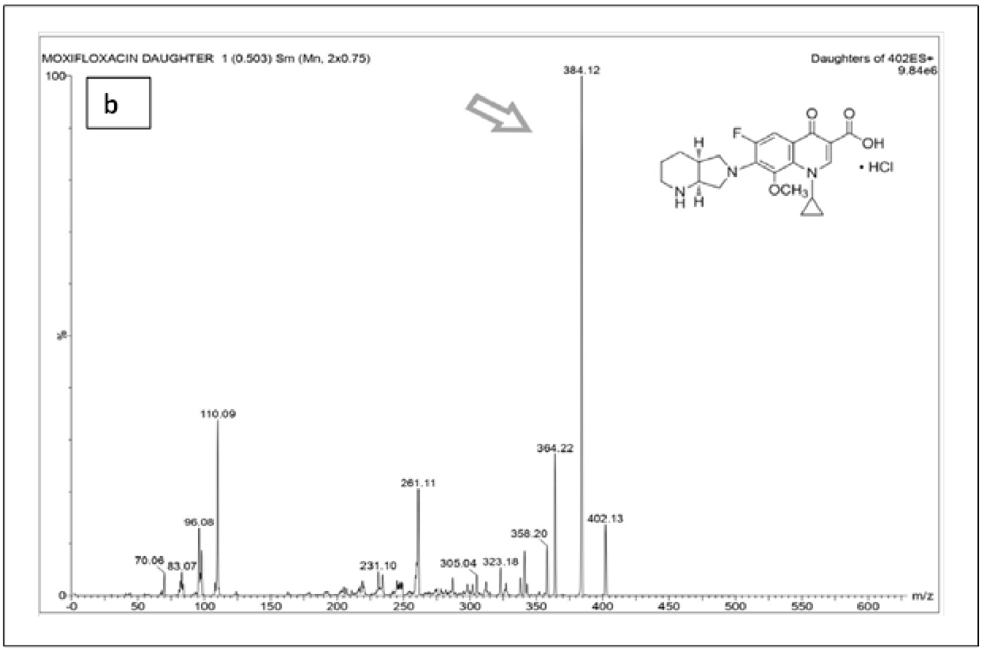
Chromatographic conditions

The primary goal of optimization was to achieve chromatographic separation in order to guarantee the accurate quantification of the mentioned drugs. Different mobile phase compositions containing methanol: acidified water in different ratios (90: 10, 95:5, 85: 15, 93:7 *v/v*) were tried. Formic acid (0.1% - 0.2%) was tried for acidification. The selected mobile phase produced an excellent peak shape and was made up of methanol: water with 0.1% formic acid (93: 7, *v/v*). In addition, short run time less than 3 min was advantageous, **Fig. (2)**. The chosen mobile phase was eluted at a flow rate of 0.3 mL/min with isocratic mode and pumped on Agilent Poroshell 120 EC-C_{18} (4.6 x 50 mm, 2.7 µm) column while keeping column at room temperature; the optimum chromatographic conditions were set for conducting the current assay.

Mass spectrometric conditions

The mass spectrometric parameters were adjusted to obtain the highest response for the drugs and the IS, and an ESI source was used to ionize the medications before they were detected by MRM mode. Positive ion mode was adopted to measure the drugs at *m/z* 460.17 > 443.09 for **APIX**, *m/z* 402.14 > 384.12 for **MOX.HCl**, *m/z* 603.19 > 402.06 for **REM** and *m/z* 151.89 > 109.9 for **PAR**, 749.47 > 591.38 for IS with a 0.029 sec dwell time for each of the four drugs and IS, **Fig. (3)**.

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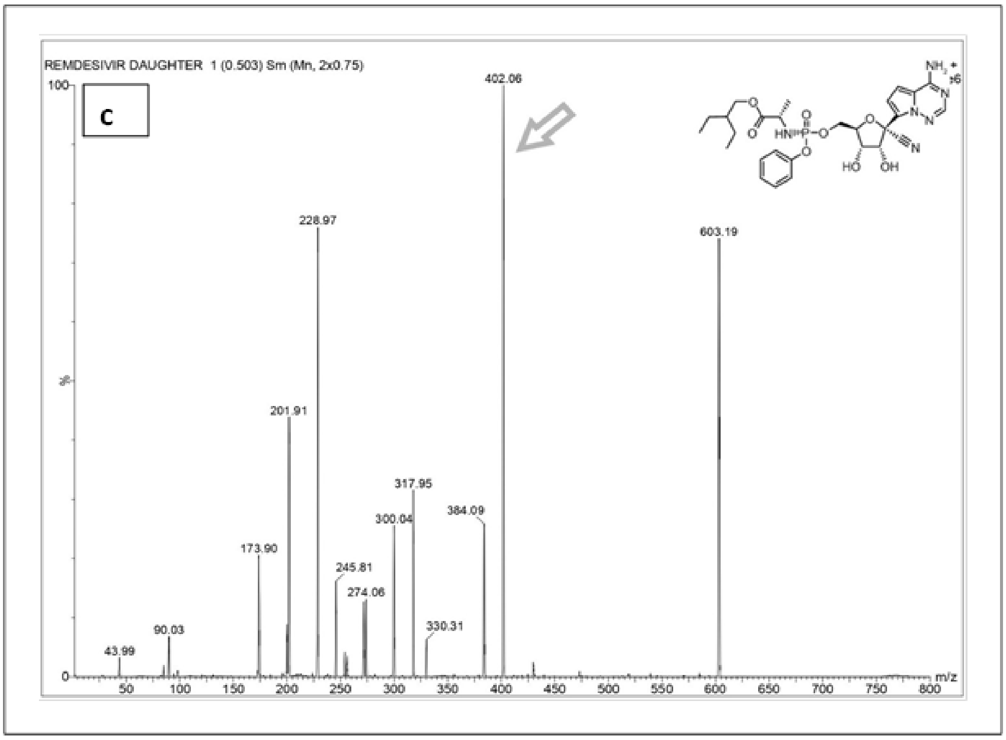
REMDESIVIR DAUGHTER 1 (0.503) Sm (Mn, 2x0.75)
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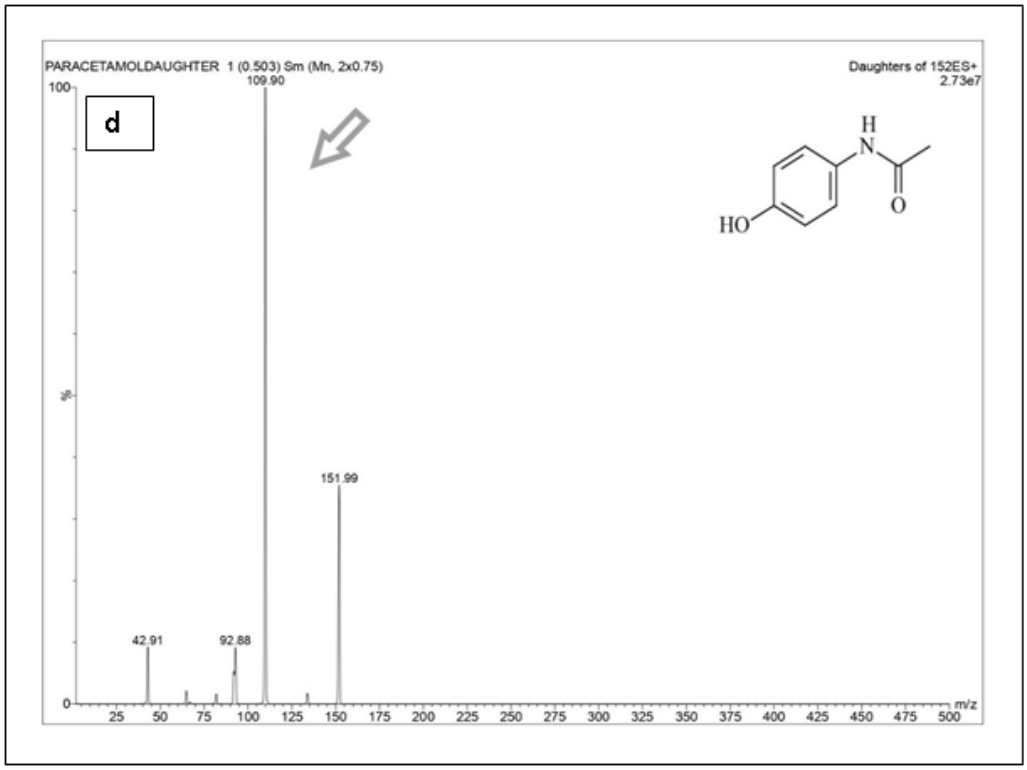


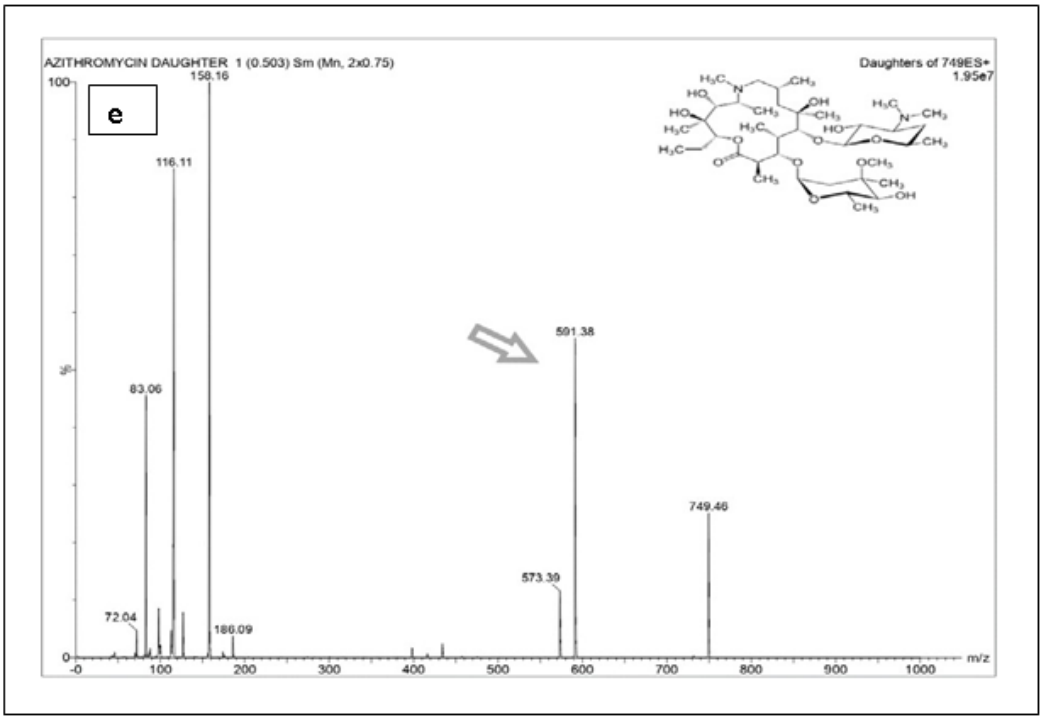


Fig. 3: Product ion scan of APIX (a), MOX.HCl (b), REM (c), PAR (d) and azithromycin (IS) (e).

Bio-analytical method validation

Selectivity

As seen in **Fig. (4)**, MRM chromatograms of extracted drug-free human plasma revealed no interfering peaks at the retention times of **APIX, MOX.HCl, REM, PAR and IS.**

Lower limit of quantification and linearity

Over the concentration range of (1–1000 ng/mL) for **APIX** and (10–10,000 ng/mL) for **MOX.HCl, REM and PAR**, the method demonstrated good linearity. The results of the back calculations are accepted, and the calibration curves are linear, **Table (2)**.

Since the analyte signal of the LLOQ sample must be at least five times that of a blank sample (according to EMA guidelines), the lowest standard in the calibration curve was considered to be the LLOQ. The results for **APIX** (1 ng/mL) and **MOX.HCl, REM, PAR** (10 ng/mL) were deemed acceptable in terms of accuracy and precision, as shown in **Table (3).**

Intraday and interday accuracy and precision

Table (3) shows that all acceptance criteria in EMA guidelines concerning accuracy and precision were achieved.

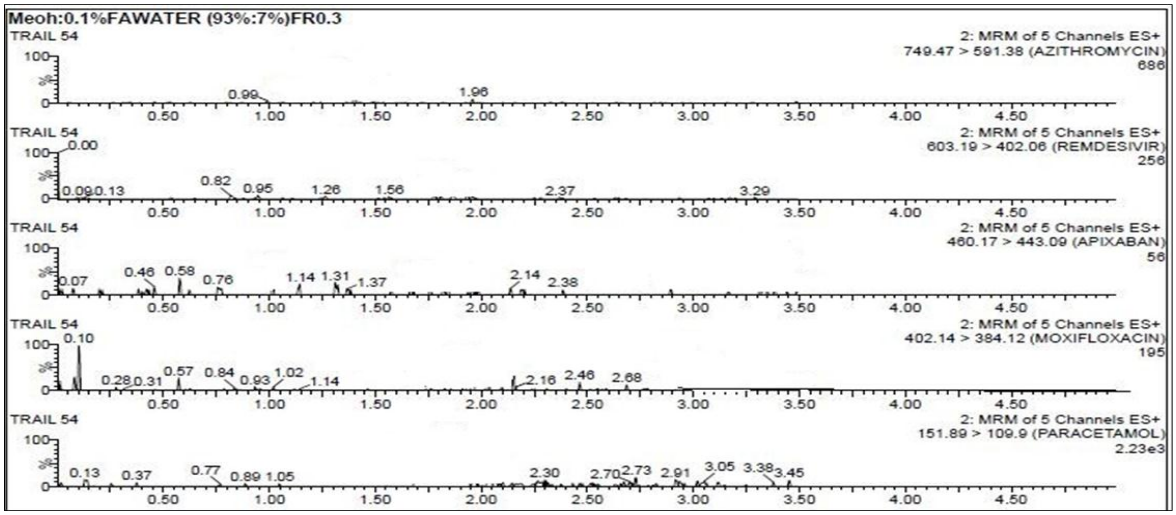


Fig. 4: MRM chromatogram of extracted drug-free human plasma samples.

Table 2: Summary of back calculated standards of apixaban (APIX), moxifloxacin (MOX.HCl), remdesivir (REM) and paracetamol (PAR) in human plasma.

Concentration of drug in plasma (ng/mL)	Accuracy (%)			
	APIX	**MOX.HCl**	**REM**	**PAR**
1*, 10**	110.00	115.00	96.00	105.71
5*, 50**	102.00	108.50	104.00	95.43
10*, 100**	103.00	105.25	112.60	110.00
50*, 500**	92.00	111.55	102.40	110.34
100*, 1000**	108.50	110.43	113.94	101.63
500*, 5000**	100.66	103.76	103.30	97.72
1000*, 10000**	103.24	107.28	104.53	90.38
Mean weighed regression coefficient	APIX 0.9963, MOX.HCl 0.9990, REM 0.9970, PAR 0.9917			
Mean intercept	APIX 0.0023, MOX.HCl -0.0004, REM 0.0813, PAR 0.0266			
Mean slope	APIX 0.0010, MOX.HCl 0.0004, REM 0.0005, PAR 0.0007			

*Concentrations for APIX

** Concentrations for MOX.HCl, REM and PAR

Table 3: Intraday and interday results of apixaban (APIX), moxifloxacin (MOX.HCl), remdesivir (REM) and paracetamol (PAR) in human plasma.

Accuracy and precision	Concentration of drug in plasma (ng/mL)	Accuracy (%)				CV (%)			
		APIX	MOX.HCl	REM	PAR	APIX	MOX.HCl	REM	PAR
Intraday	1*, 10**	100.00	110.83	94.00	94.29	10.00	6.89	4.26	3.03
	3*, 30**	92.22	102.22	95.78	92.06	2.09	4.98	5.05	2.33
	75*, 750**	113.33	89.63	100.89	103.58	1.16	4.05	7.18	2.03
	800*, 7500**	93.94	91.16	102.20	92.43	6.59	1.43	2.01	2.31
Interday	1*, 10**	106.67	108.33	93.33	98.57	14.32	8.74	9.66	7.25
	3*, 30**	97.78	101.39	97.56	93.49	5.21	5.83	12.83	4.45
	75*, 750**	110.40	93.82	104.76	99.26	3.51	8.48	7.90	6.50
	800*, 7500**	93.58	96.11	96.37	96.88	6.04	6.18	4.48	5.40

*Concentrations for APIX
** Concentrations for MOX.HCl, REM and PAR
CV: coefficient of variation

Carry over

No discernible carryover was seen since it was less than 5 % of the IS and 20 % of LLOQ of the cited drugs.

Dilution integrity

The 2-fold dilution CV% was found to be 0.64%, 0.84%, 3.06% and 0.71 %, in addition; the accuracy results were found to be 94.61 %, 99.84 %, 103.35 % and 104.60 % for **APIX, MOX.HCl, REM and PAR**, respectively. While the 4-fold dilution CV% was found to be 2.58%, 4.73%, 0.47% and 2.78% and the accuracy results were found to be 104.63 %, 106.18 %, 111.5 % and 102.62 % for **APIX, MOX.HCl, REM** and **PAR**, respectively. All results of accuracy and precision met the acceptance criteria.

Matrix effect

As presented in **Table (4);** CV % of the (IS normalized MF) calculated from the six plasma QCL and QCH samples revealed that the plasma matrix has no significance on the extraction process and on the drugs and IS response. The resulting drug recoveries ensured that no additional treatment was required to overcome plasma protein binding and that the drugs were fully extracted from plasma samples using liquid-liquid extraction.

Stability

According to CV% and recovery% results, **Table (5)** demonstrated that all drugs were stable in human plasma under the tested stability conditions.

Table 4: Matrix effect results of apixaban (APIX), moxifloxacin (MOX.HCl), remdesivir (REM) and paracetamol (PAR) in human plasma.

Drug	Mean drug MF		CV % of drug MF		Mean IS MF		CV % of IS MF		Mean normalized MF		CV % of normalized MF	
	QCL	QCH	QCL	QCH	QCL	QCH	QCL	QCH	QCL	QCH	QCL	QCH
APIX	0.95	0.98	2.70	1.81	0.91	1.01	5.35	5.82	1.04	0.97	7.26	4.29
MOX.HCl	0.92	0.90	3.19	3.64	0.91	1.01	5.35	5.82	1.00	0.90	7.89	8.57
REM	0.92	0.92	12.87	3.99	0.93	0.97	2.98	3.71	0.99	0.94	13.76	3.87
PAR	0.97	0.92	2.87	3.56	0.93	0.97	2.98	3.71	1.04	0.94	2.21	4.92

CV: coefficient of variation.
MF: matrix factor.

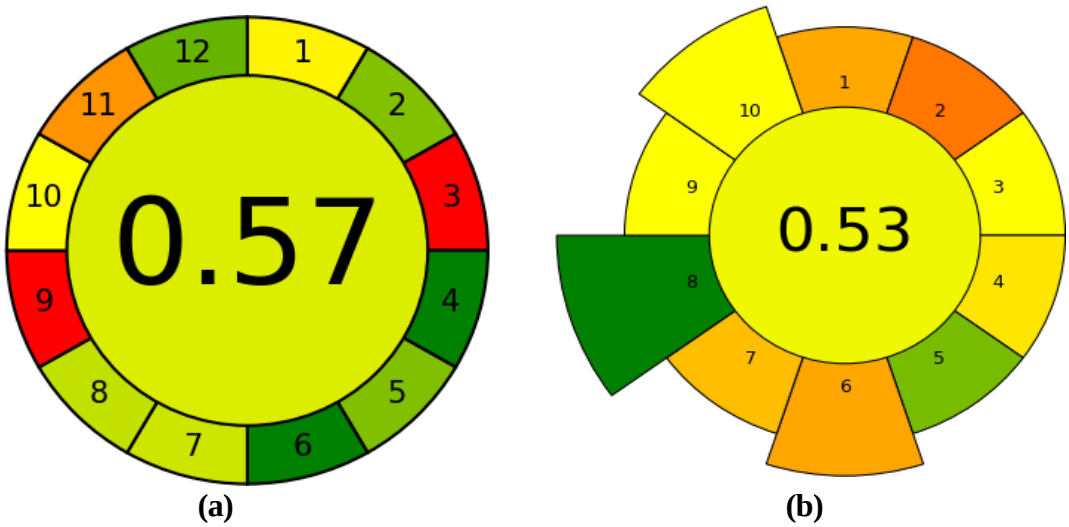
Table 5 : Summary of stability data of apixaban (APIX), moxifloxacin (MOX.HCl), remdesivir (REM) and paracetamol (PAR) in human plasma.

Stability term	Concentration of the drug (ng/mL)	Accuracy (%)				CV (%)			
		APIX	MOX. HCl	REM	PAR	APIX	MOX. HCl	REM	PAR
Short term	3*, 30**	92.22	101.39	97.56	93.49	2.09	5.83	12.83	4.45
	800*, 7500**	93.94	96.11	96.37	96.88	6.59	6.18	4.48	5.40
Long term	3*, 30**	103.33	99.44	103.33	96.35	9.68	5.45	7.10	1.24
	800*, 7500**	92.73	106.65	90.54	104.87	7.30	1.82	2.77	6.19
Auto-sampler	3*, 30**	91.11	99.50	94.44	92.86	2.11	3.30	4.14	1.54
	800*, 7500**	96.96	94.57	102.91	91.68	2.09	2.51	1.41	1.64
Freeze and thaw	3*, 30**	93.33	94.17	87.56	96.03	3.57	4.42	1.16	6.45
	800*, 7500**	88.71	106.05	96.44	91.12	1.71	1.56	2.37	3.79

*Concentrations for APIX.
** Concentrations for MOX.HCl, REM and PAR.
CV: coefficient of variation.

Greenness Assessment

The significance principles serve as the foundation for AGREE, which measures how environmentally friendly analytical processes are. The developed approach was evaluated for greenness using the AGREE tool. In addition, the AGREEprep tool was also evaluated. Recently, AGREEprep, a measuring tool that prioritizes sample preparation, was released. Important steps in the analytical process include target analyte separation and enrichment, matrix interference removal or minimization, and/or ensuring compatibility with the measurement method and sample preparation. At the same time, sample preparation is acknowledged as one of the most important phases by the GAC perspective. It was clear that AGREEprep provided a more detailed picture of the sample preparation than AGREE. A score of 0.57 and 0.53 was captured by the proposed method for AGREE and AGREEprep, respectively which proves the current method as an eco-friendly one, **Fig. (5)**.



(a) **(b)**

Fig. 5: AGREE (a) and AGREEprep (b) evaluation of the proposed method.

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Conclusion

In conclusion, the development of a selective and fast chromatographic technique that involves simple sample preparation represents a significant advancement in the ability to analyze simultaneously the four drugs **APIX, MOX.HCl, REM** and **PAR** used in the treatment of COVID-19. All of the parameters needed to ensure the method's applicability and reliability for the simultaneous detection of the four mentioned drugs in plasma are included in the bio-analytical method validation. Good recoveries and short run time not exceeding 3 minutes make it advantageous to be used for therapeutic drug monitoring.

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كروماتوجرافيا السائل ذات الاداء العالي باستخدام مقياس الكتلة الترادفي في البلازما : تقنية تحليل حيوي انتقائية لاكتشاف وقياس أدوية بروتوكول كوفيد-١٩ المعاد استخدامها في البلازما البشرية

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ظهر فيروس SARS-CoV-2 المسبب لمرض كوفيد-١٩ لأول مرة في أواخر عام ٢٠١٩ وأعلنته منظمة الصحة العالمية في مارس ٢٠٢٠ باعتباره وباءً عالميًا. في مايو ٢٠٢٣، تم اعتبار فيروس كورونا (COVID-19) بمثابة حالة طوارئ صحية عامة تثير قلقًا عالميًا. ومع ذلك، يمثل الفيروس خطرًا صحيًا خطيرًا ولا يزال ينتشر في جميع أنحاء المجتمعات. في الدراسة الحالية، تم التحقق من صحة طريقة حساسة وسريعة التطور للتقدير الكمي للأدوية وهي (ريمديسيفير، موكسيفلوكساسين-حمض الهيدروكلوريك، الباراسيتامول والأبيكسابان) المستخدمة في إدارة كوفيد-١٩ في البلازما البشرية المعززة في وقت واحد. كان مذيب الاستخلاص هو إيثر ثنائي إيثيل: ثنائي كلورو ميثان (٧٠:٣٠) المستخدم لاستخلاص الأدوية المذكورة والأزيثروميسين (IS) من البلازما البشرية (استخلاص سائل-سائل). تم استخدام تحليل كروماتوجرافي سائل فائق الأداء مع الكشف الجماعي الترادفي للفصل والقياس الكمي. في عمود كربون ١٨اجيلنت (٤.٦ × ٥٠ مم، ٢.٧ ميكرومتر)، تم ضخ طور متحرك يتكون من الميثانول: ماء يحتوي على ٠.١% من حمض الفورميك (٩٣:٧ فولت/حجم) في شطف إسقراطي بمعدل تدفق قدره ٠.٣ مل/دقيقة. مع إجمالي وقت التشغيل ٣ دقائق. تم قياس التحولات في وضع مراقبة التفاعل المتعدد (MRM) في وضع التأين الإيجابي للأدوية الأربعة وIS. تم التحقق من صحة النهج الجديد من الناحية التحليلية الحيوية وفقًا لمتطلبات وكالة الأدوية الأوروبية (EMA). الطريقة المعتمدة دقيقة وانتقائية ودقيقة ويمكن استخدامها في العديد من التطبيقات بما في ذلك دراسات التكافؤ الحيوي ومراقبة الأدوية العلاجية والتحليل الروتيني للأدوية.