RESEARCH

Simultaneous spectrophotometric determination of recombined sofosbuvir, ledipasvir and paracetamol together as commonly repurposed drugs for COVID-19 treatment

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Abstract

Background COVID-19 has emerged as the most serious outbreak in recent years. Certain medications such as sofosbuvir (SOF), ledipasvir (LDV) and paracetamol (PAR) were proposed as a safer and recommended substance to control symptoms of COVID-19.

Results Using built-in spectrophotometer software, zero order and derivative spectra of the studied components, two extremely clear, quick, and sensitive spectrophotometric techniques for simultaneous determinations of SOF, LDV, as well as PAR have been developed. LDV was calculated using a zero order absorption spectrum at wavelength maxima of 333 nm. SOF and PAR were evaluated simultaneously using a first derivative spectrophotometer at 247.2 nm and 260.8 nm, respectively. The calibration graphs for SOF, LDV, and PAR are linear over ranges of concentrations of 8–60 µg/mL, 4–22 µg/mL, and 2–14 µg/mL, consequently. The suggested methodologyspecificity was investigated using laboratory manufactured (different ratios) mixtures, which were then effectively used to the analysis of Mpi-viropack plus® and Panadol® pills. Valid limitations included accuracy, precision, and specificity. The methodologies were validated in accordance with some ICH standards.

Conclusions The methods proposed were simple, accurate, precise, and neither require any complex equipment nor specific software.

Keywords Sofosbuvir, Ledipasvir, Paracetamol, COVID-19, Spectrophotometry

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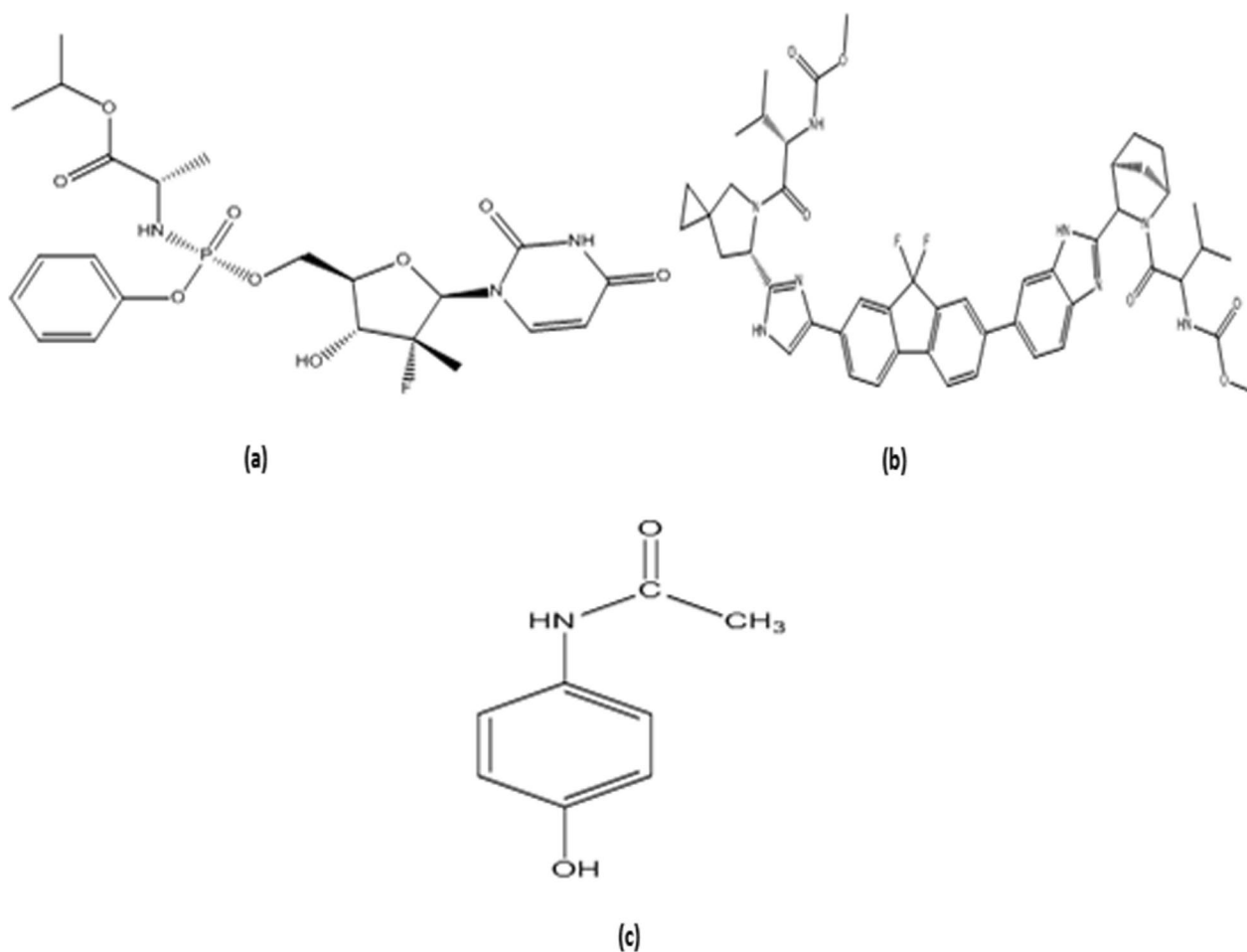
Background

Sofosbuvir (SOF) (Fig. 1a); (S)-isopropyl-2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydro-furan-2-yl)methoxy-phenoxy-phosphoryl)amino)propanoate. SOF is a direct acting antiviral agents (DAAS) that inhibits RNA-dependent RNA polymerase (RdRp) of the hepatitis C virus (HCV). Similar to the HCV, SARS-CoV2 genome is characterized by single-strand RNA and share a similar replication mechanism requiring RdRp that catalyzes the synthesis of

Fig. 1 Chemical structures of **a** SOF, **b** LDV and **c** PAR drugs

viral RNA and thus plays a central role in the replication and transcription cycle of SARS-COV2 [1]. SOF's assessment was achieved via some recent analysis tools examples like; spectrophotometric[2], fluorometric [3], HPTLC [4] and HPLC [5] methods.

Ledipasvir (LDV) (Fig. 1b); methyl *N*-[(2S)-1-[(6S)-6-[5-[9,9-difluoro-7-[2-[(1R,3S,4S)-2-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl]-2-azabicyclo[2.2.1]heptan-3-yl]-3H-benzimidazol-5-yl]fluoren-2-yl]-1H-imidazol-2-yl]-5-azaspiro[2.4]heptan-5-yl]-3-methyl-1-oxobutan-2-yl]carbamate. LDV is an antiviral medication that targets a protein (NS5A) in the HCV. LDV reduces the quantity of virus in infected patients since this protein is involved in viral replication [6]. LDV was analyzed using some recent analysis tools examples like; spectrophotometric [7], fluorometric [8], HPTLC [9] as well as HPLC [10].

Paracetamol (PAR) (Fig. 1c); Analgesic and antipyretic N-(4-hydroxyphenyl) acetamide. Titrimetric [11], spectrophotometric [12], fluorometric [13], HPTLC

[14], and HPLC [15] procedures were used to assess PAR.

A series of viral pneumonias that occurred in Wuhan, China, in December 2019 were diagnosed as Corona virus disease (COVID-19), which was brought on by the SARS-CoV-2 corona virus. Fever, coughing, shortness of breath, and exhaustion occur frequently among patients. Over two million COVID-19 cases and 160 thousand fatalities have been reported. Pharmacological tools that have been demonstrated to be efficient in vitro or in vivo against SARS-CoV-2 and/or related viruses like SARS and MERS are among the options and resources being tested against the virus in addition to the significant global effort aimed at finding a treatment for COVID-19 [17].

The HCV and other RNA viruses have identical life cycles, and both require the same proteins and enzymes for viral replication [18]. An FDA-approved drug combination for treating HCV infection is SOF/LDV [19]. LDV suppresses NS5A, a crucial protein for RdRp function.

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SOF inhibits NS5B-RdRp, a key enzyme in the replication of the hepatitis virus [20]. Many heterocyclic containing compounds were repurposed for their wide pharmaceutical use [21]. SOF/LDV may be a possible pharmacological therapy for COVID-19 treatment based on in vitro docking experiments [6].

At present, the majority of efforts have been directed toward identifying medicines capable of reversing the most serious COVID-19 fatal effects, especially hypercoagulation and hypercytokinemia [22]. In March 2020, Micallef et al. discouraged the administration of non-steroidal anti-inflammatory drugs (NSAIDs) and ibuprofen as supposed worsening COVID-19 [23].These cautions were based on multiple studies reviewed [24] that showed that the use of NSAIDs for fever and pain treatments, increases undesirable bacterial complications risk, especially on lungs tissues.

Paracetamol (PAR) has been identified as a secure recommended option for local therapy of pain and fever in COVID-19 patients. It is the only medicine that has been used continually to control COVID-19 in a timely way and without any safety assessment [25].

With the combination of a microcomputer and a spectrophotometer, it is now usually acceptable to employ mathematical methods to obtain derived spectra fast, simply, as well as reproducibility. This directly enlarged the scope of spectrophotometry technology use. The UV spectrophotometric technique had the opportunity to gain a significant position in the pharmacopoeia due to its advantages over HPLC in terms of cost and time savings, and the excellent results obtained for both precision and accuracy led to its widespread use in the assessment of dosage forms for drugs, which has risen rapidly in the past few years.

Multi-component formulations are also a challenge, especially in critical ratios where one or more of the components exist in an extremely low ratio. This makes it difficult to perform UV spectroscopy analysis using the same dosage form extract when the minor components in the extract are outside of the linear range and deviate from Beer's law while all the other components are within the quantitative limits. The key components of the combination are at excessively high concentrations, therefore using the concentrated extract to identify the minor components in the drugs mentioned previously could result in an error. Additionally, variations in absorption occur owing to the close proximity of the solution molecules as the solution concentration rises. At higher concentrations, the refractive index will consequently alter. Due to the increased interest in UV spectroscopy of formulations with critical ratio issues in recent years, several methods have been developed for enhancing minor components in synthesized extracts in a proportion that is

hard to achieve their quantitative limit while preserving the concentration of other components which exist in the major proportions [26]. Therefore, advancements have been made in both of the sample enrichment approaches; one relies on additional spectral manipulation of a specific concentration of the drug pure form by using spectrophotometer software in Silico, whereas the second relies on laboratory spiking of the minor component at a specific concentration for its pure form [27].

Medhat et al. [28] conducted a clinical investigation (July 2020–October 2021) that demonstrated a beneficial benefit of sofosbuvir/ledipasvir combination therapy compared to standard care treatment (including paracetamol administration) in the treatment of COVID-19. By the end of the research, about 85.7% of patients tested negative (day 14) [28]. This enabled the use of the aforementioned medications as part of a COVID-19 therapy protocol. Unfortunately, the three drugs SOF, LDV, and PAR are not available in the same dose form, yet they are administered together. Based on the pharmacological impact of commonly studied dosed tablets of the drugs mentioned in COVID-19 management, this work has adopted the simultaneous identification of SOF, LDV, and PAR present in critical ratios (4.4:1.0:5.5) (where LDV is considered a minor component) as prescribed for COVID-19 patients.

In order to identify and quantify LDV as a minor component, as well as to simultaneously determine SOF and PAR, the current work intends to achieve sensitive, clear and accurate as well as valid spectrophotometric technique.

Methods

Apparatus and software

UV–Vis spectrophotometer Shimadzu Double beam (UV-1800, Japan) and double beam (Libra, biochrome, UK) were utilized with matching 1 cm quartz cells. Shimadzu UV-probe 2.43 software was employed for obtaining the spectra. The Sonicator (Branson Model 3510 Ultrasonic Cleaner, UK) as well as Analytical Balance (Sartorius CPA225D, Italy) were both used.

Samples and solvents

SOF and LDV Pure samples were provided by AL-Rowad Industrial Pharmaceutical Company (RPIC) in Cairo, Egypt. According to the company certifications, the purities were 99.76 ± 1.44 and 99.07 ± 1.33, respectively. Al-Amriya Pharmaceutical Industries in Alexandria, generously provided a pure sample of PAR. According to the described procedure, its purity was confirmed to be 99.07 ± 1.33[29]. Mpiviropack plus® tablets (batch number 1830150) and Panadol® tablets (batch number 1830150) were purchased from the market from Marcyrl

Company and GSK, Cairo, Egypt, respectively. Each Mpiviropack plus® tablet has 400 mg SOF and 90 mg LDV, whereas each Panadol® tablet provides 500 mg of PAR. Sigma-Aldrich in Germany provided spectroscopic analytical grade methanol.

Standard solutions
Stock standard solutions comprising 1000.0 μg/mL of SOF, LDV and PAR were prepared separately in 100 mL volumetric flasks by dissolving 0.1 gm of each standard powder in methanol and then completed to the volume by the same solvent.

Working standard solutions (100.0 μg/mL) were prepared by transferring 10 ml from each of SOF, LDV and PAR standard stock solutions (1000.0 μg/mL) to 100 ml volumetric flask and completing the volume with methanol.

Calibration graph construction and resolving spectra
Different aliquots were prepared in three independent series of 10 mL volumetric flasks by appropriately diluting to each working standard solutions (100 μg/mL of each drug) using methanol. These aliquots were equivalent to 8.0–60 μg/mL for SOF, 4–22 μg/mL for LDV, and 2–14 μg/mL for PAR.

The results of prepared solutions were saved in the computer after being scanned in the 200–400 nm range against methanol as a blank. Three trials were averaged to produce the calibration graph.

To assess their effect on the shape and resolution of the produced spectra, it was essential to study some of the instrumental parameters, which are scaling factor and the wavelength increment ($\Delta\lambda$). Additionally, various scale factors (10 and 100), $\Delta\lambda$ (4, 8, and 16), and solvents including deionized water, DMSO, and ethanol were explored as solvents for all drugs. All of the solvents previously listed displayed a high amount of noise and poor resolution. When methanol was used as the solvent, the best spectra for every drug were obtained using a scaling factor of 10 and $\Delta\lambda = 4$.

Zero order spectrophotometric method:
A calibration graph was developed that correlated the absorbance of the zero order spectra at the maximum wavelength of 333 nm for LDV with the corresponding concentrations, and a regression equation was computed.

First derivative spectrophotometric method
Calibration graphs' regression equations have been calculated to relate the first derivative spectra's peak amplitudes of PAR and SOF at $P_{260.8}$ and $P_{247.2}$ versus their concentrations, respectively, by using $\Delta\lambda$ equal 4 and scaling factor equal 10.

The resolving spectra preparation
Zero order spectrum for LDV factorized
It was calculated by dividing the (D^0) of any concentration of pure LDV within its linearity range by the numerical value of absorbance maxima at 333.0 nm using spectrophotometer software.

First derivative spectrum for PAR factorized
It was obtained by dividing the derivative spectrum of pure PAR at any concentration within the linearity range by the peak amplitude at 260.8 nm using the spectrophotometer software with scaling factors equal 10.0 and $\Delta\lambda$ equal 4.

Laboratory prepared mixtures analysis
The specificity of suggested spectrophotometric approaches has been investigated through the preparation of solutions with various ratios of SOF, LDV, and PAR. The formulated mixtures' spectra were recorded and saved in the computer at (200.0–400.0 nm). The following data processing is then applied.

Zero order spectrophotometric method (D^0)
Sample enrichment in silico: Prior to undergoing the related processing ways, LDV concentration in the zero order of combinations containing low LDV concentrations was enhanced by adding the stored spectrum of pure standard LDV (10 μg/mL) through spectrophotometer software. The absorbance maximum for LDV was determined at 333.0 nm using the stored zero order spectra of each lab-labeled combination. To produce the D^0 spectrum of LDV in the combination, the absorbance value was multiplied by the prepared factorized D^0 spectrum of LDV. The enhanced concentration of LDV (claimed and added) was estimated at 333.0 nm by utilizing the relevant regression equation. Using the same testing and manipulation techniques, the added concentration of pure LDV (10μg/mL) was also measured. The mentioned LDV concentration in each combination was then estimated by calculating difference between the enhanced and added LDV. Spectrum subtraction of the obtained D^0 spectrum of LDV was applied to obtain a binary mixture's D^0 of SOF and PAR.

First derivative spectrophotometric method (D^1)
For each resolved D^0 binary mixture, derivative manipulation has been applied using $\Delta\lambda$ equal 4 nm and scaling factor equal 10 where, the obtained amplitude at 260.8 nm was recorded and multiplied by the prepared factorized D^1 spectrum of PAR to obtain D^1 spectrum of PAR in the mixture with P_{max} at 260.8 nm. The concentration of PAR was calculated separately from the related regression equation. Spectrum subtraction of the obtained D^1

spectrum of PAR was used to get D^1 of SOF, and its concentration was calculated using the related computed regression equation at $P_{\max}$ at 247.2 nm.

Application to pharmaceutical formulation

Mpiviropackplus® and Panadol®, each ten tablets, were crushed separately. Accurate amount of powder equivalent to 44.44 mg SOF, 10 mg LDV, and 55.55 mg PAR was transferred to a 100 mL volumetric flask, the volume was completed with methanol then sonicated for 10 min. A filter paper was used to filter the solution (11 cm, grade 102) the obtained stock solution concentration were equal to 444.4 µg/mL SOF, 100 µg/mL LDV, and 555.5 µg/mL PAR. Accurate volume (0.96 ml) were transferred from the stock solution to 10 ml volumetric flask then volume completed with methanol to obtain The working standard solution concentration 9.6 µg/mL SOF, 2.16 µg/mL LDV, and 12 µg/mL PAR. The corresponding regression equations were used to compute the drug concentrations in the pharmaceutical dosage form.

Results

Spectrophotometric measurement

SOF, LDV, and PAR in methanol have been examined independently and overlaid in the dose form ratio. Figure 2 depicts the LDV being extended over the remaining components in the UV region. Meanwhile, LDV revealed an absorbance measurement of 333.0 nm vs zero for the other components. Moreover, both SOF and PAR had severely overlapped spectra, which hampered their identification in zero order spectra. An essential approach in spectrophotometric study of complex mixtures is the coupling of resolution technique followed by data processing of the resolved less complicated spectra. As a result, the first derivative spectra of these compounds modified and displayed, demonstrating that PAR has a peak amplitude opposing a zero-crossing with SOF at 260.8 nm; Fig. 3. This allows for the simultaneous determination of the drugs demonstrated.

For LDV

Regarding the study of the compound's spectral characteristics, it was found by analyzing various spectra concentrations of the suggested drugs that LDV does not fall within its linearity range and will deviate from Beer's law once it is present in the ratio of the studied components, preventing its accurate determination. As a result, LDV was calculated using sample enrichment technique; by using the In Silico technology, which involves scanning the spectrum of 10µg/mL of pure standard LDV added using spectrophotometer software to each recorded spectrum before applying the corresponding processing
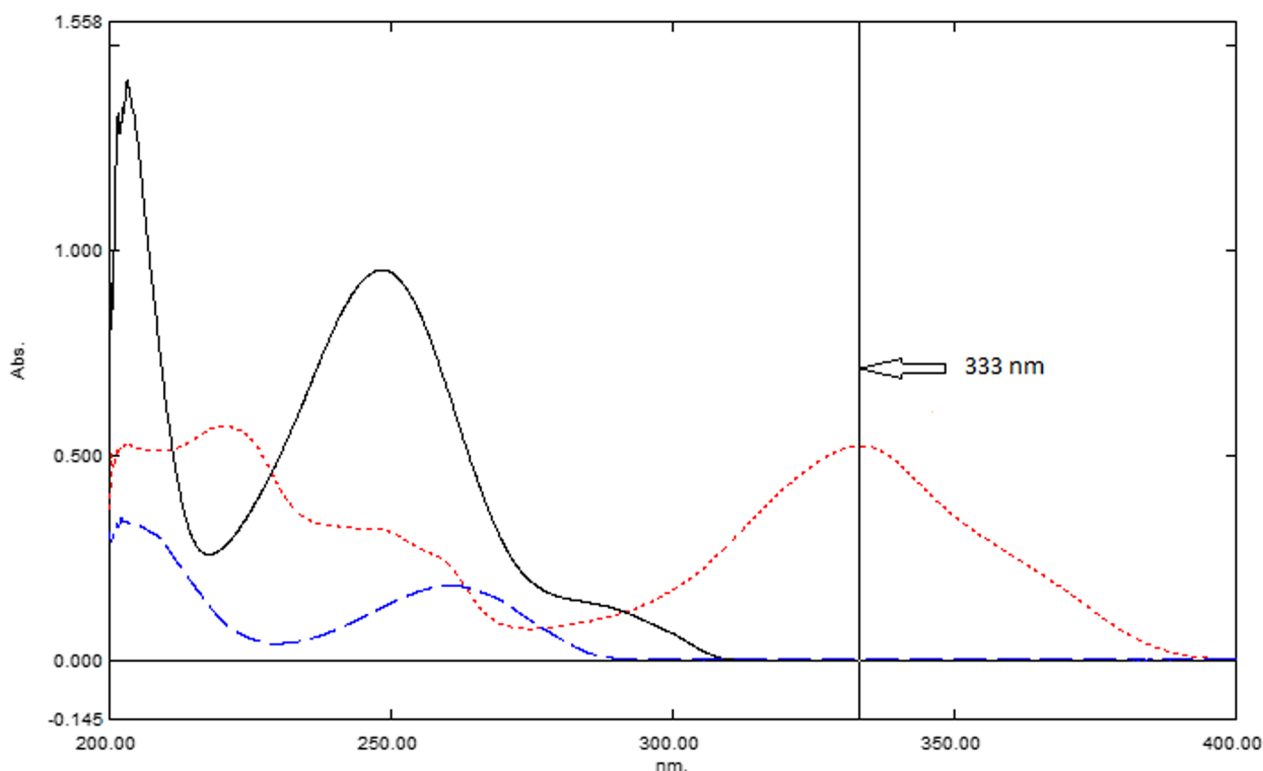

Fig. 2 Zero order interference of LDV 10 µg/mL (red curve) at 333nm with SOF10 µg/mL (blue curve) and PAR 10 µg/mL (black curve)
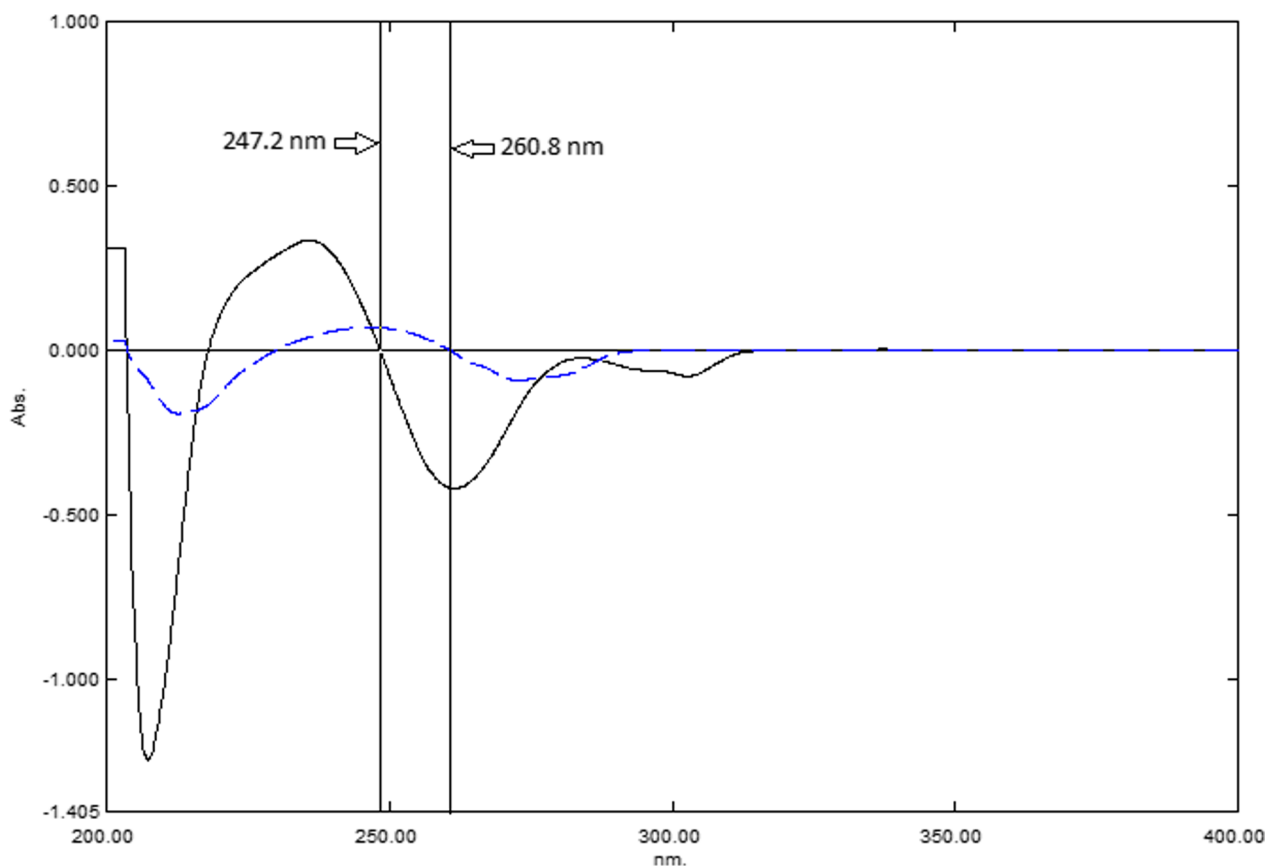
Fig. 3 First derivative spectra showing zero-crossing of SOF 10 μg/mL (black curve) at 247.2 nm and PAR 10 μg/mL (blue curve) at 260.8 nm

steps to each method. LDV used an absorbance reading at its maximum; 333.0 nm, for each stored D^0 of each enriched ternary mixture, as opposed to a zero-absorbance value for both SOF and PAR. The augmented D^0 of LDV was calculated via measuring the absorbance at 333 nm and multiplying it by the prepared D^0 factorized LDV spectrum. The absorbance values of LDV at 333 nm and its associated concentrations in the range (4–22 μg/mL) were found to be linearly correlated. The concentration of LDV present in the mixture could then be calculated by subtracting the LDV concentration added from the total concentration obtained by substituting LDV with the relevant regression equation. By subtracting the obtained D^0 of LDV from the ternary mixture, a binary mixture comprising SOF and PAR will be obtained.

For PAR and SOF

For each resolved D^0 of binary mixture comprising SOF and PAR, a severe overlapping between the studied drugs was detected hindering their ease determination. Thus, derivative spectrophotometry can be used for resolving this problem satisfactorily. Figure 3 shows the first derivative spectra (D^1), which permits PAR's determination at 260.8 nm (zero-crossing of SOF).

The peak amplitude reading at 260.8 nm and the produced D^1 factorized PAR's spectrum was multiplied to obtain the first derivative PAR spectrum for every mixture. The peak amplitude values of PAR at 260 nm and

Table 1 Validation parameters of the spectrophotometric determination of SOF, LDV, and PAR

Parameter	SOF(first der. 247.2 nm)	LDV(zero order 333 nm)	PAR(first der. 260.8 nm)
Range(μg/mL)	8–60	4–22	2–14
Slope	0.007	0.055	0.041
Intercept	−0.007	-0.024	0.008
Correlation coefficient (*r*)	0.9997	0.9998	0.9998
Precision (RSD %)			
Intraday*	0.715	1.268	1.759
Interday*	0.597	1.1504	1.306

* Relative standard deviation (*n* = 3), average of three different concentrations of SOF, LDV, and PAR

Table 2 Accuracy results for spectrophotometric determination of SOF, LDV, and PAR in bulk powder

SOF			LDV(zero order 333 nm)			PAR		
Taken (µg/mL)	Found (µg/mL)	Recovery (%)[a]	Taken (µg/mL)	Found (µg/mL)	Recovery (%)[a]	Taken (µg/mL)	Found (µg/mL)	Recovery (%)[a]
15	14.95	99.72	8	8.1	101.35	3	2.96	98.79
30	29.55	98.51	12	11.8	98.40	5	4.92	98.40
45	45.36	100.81	16	15.99	99.97	12	12.11	100.98
Mean ± SD	99.68 ± 1.149		Mean ± SD	99.91 ± 1.47		Mean ± SD	99.39 ± 1.391	

[a] Average of three determinations

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Table 3 Determination of the studied drugs in laboratory prepared mixtures by the proposed methods

Binary mixture SOF: LDV: PAR ratios	%Recovery[a]		
	SOF(first derivative) (%)	LDV(zero order) (%)	AR(first derivative) (%
4: 0.9(+ spiking 10ug/mL):5	100.92	101.86	101.18
2: 1:1	100.47	100.21	98.23
1: 2:1	99.59	100.74	98.71
Mean ± SD	100.33 ± 0.67	100.93 ± 0.84	99.38 ± 1.58

[a] Average of three determinations

Table 4 Analysis of pharmaceutical dosage form (Mpiviropack plus®) and application of standard addition technique by the proposed spectrophotometric methods for determination of SOF and LDV

SOF(first derivative)				LDV(zero order)			
Mpiviropack plus® %recovery* ± SD	Standard addition			Mpiviropack plus® %recovery* ± SD	Standard addition		
	Pure taken (µg/mL)	Pure found (µg/mL)	Recovery (%)*		Pure taken (µg/mL)	Pure found (µg/mL)	Recovery (%)*
99.05 ± 0.81	1.92	1.90	98.95	101.26 ± 0.148	0.432	0.422	97.68
	3.84	3.81	99.21		0.864	0.872	100.92
	7.68	7.56	98.43		1.728	1.69	97.80
	Mean ± SD		98.87 ± 0.397		Mean ± SD		98.80 ± 1.83

* Average of three determinations

Table 5 Analysis of pharmaceutical dosage form (Panadol®) and application of standard addition technique by the proposed spectrophotometric methods for determination of PAR

PAR(first derivative)			
Panadol® %recovery* ± SD	Standard addition		
	Pure taken (µg/mL)	Pure found (µg/mL)	Recovery (%)*
103.82 ± 1.89	2.4	2.37	98.75
	4.8	4.88	101.66
	9.6	9.48	98.75
	Mean ± SD		99.72 ± 1.68

* Average of three determinations

Table 6 Robustness results of the proposed methods

Parameters	Zero order	First derivative	
	LDV	SOF	PAR
	Recovery ± RSD	Recovery ± RSD	
Different device	100.5 ± 0.321	101.3 ± 0.425	98.7 ± 0.377
Wavelength*	99.6 ± 0.445	99.91 ± 0.753	100.16 ± 0.663
Different solvent suppliers	101.2 ± 1.182	100.8 ± 0.482	101.1 ± 1.033

* peak amplitude was recorded at the selected wavelengths ± 0.2 nm for the proposed methods

the associated concentrations range from 2 to 14 µg/mL were found to be linearly related.

The binary mixture was subtracted from the obtained D^1 of PAR to obtain D^1 of SOF, whose concentration was then calculated by substituting the peak amplitude reading at 247.2 nm in the regression equation relating the peak amplitude values of SOF at 247.2 nm and the corresponding concentrations range from 8 to 60 µg/mL.

Method validation

As indicated in Tables 1 and 2, the suggested spectrophotometric procedures were validated according to the parameters of sensitivity, linearity range and accuracy as

well as precision in accordance with some of the ICH recommendations. When acceptable results were obtained over the calibration range, analytical results of laboratory prepared mixtures containing different drug ratios confirmed the specificity of the suggested procedures as reported in Table 3. The drugs contained in Mpiviropack plus® and Panadol® tablets were also determined using the suggested procedures utilizing the standard addition technique, and satisfactory results were obtained, as shown in Tables 4 and 5, respectively.

Robustness

Robustness was assessed by examining several different parameters such as device (Libra, Biochrom, England), different solvent (methanol) suppliers (Merck and Sigma), and wavelengths. The reported findings were acceptable, as shown in Table 6.

Discussion

Simplicity of use, operation, stability, sensitivity, and economy of spectrophotometry techniques are demonstrated. In developing these analytical methods, most analysts concentrate mostly on how to initiate new mathematical methodologies. The major benefits of spectrophotometric approaches are their versatility and adaptability to the circumstances of each combination, allowing analysts to use the most appropriate way of analysis and resolution.

The concentration ratio was discovered to be a critical ratio (4.4: 1: 5.5); furthermore determining the concentration of each compound simultaneously in zero order spectra was observed to be extremely difficult, because minor LDV concentrations were outside of the linearity range to achieve this ratio in its dosage form content. Beer's Law was followed since each drug's spectral characteristics and absorption limited the concentration range.

A unique potential for spectrophotometric approaches to enable accurate, precise, and easy quantitative measurement of recommended drugs has been presented by the combined study of SOF, LDV, and PAR, particularly considering that these ternary drug companies are frequently repurposed for treating the symptoms of COVID-19 patients.

Conclusion

Simultaneous analysis of LDV, SOF, and PAR as the most reused medication mixture for COVID-19 treatment using advanced simple and intelligent spectrophotometric methods. The procedures were clear, precise, and accurate, and no special hardware or software was needed. These techniques are simple and relatively easy to use in quality control laboratories and can therefore be appropriately used for continuous analysis and monitoring of the examined drugs in both pure bulk powder and dosage form in quality control laboratories lacking liquid chromatographic equipment and without preliminary separation procedures.

Abbreviations

SOF	Sofosbuvir
LDV	Ledipasvir
PAR	Paracetamol
DAAS	Directly acting antiviral agents
RdRp	RNA-dependent RNA polymerase
HCV	Hepatitis C virus
NSAIDs	Non-steroidal anti-inflammatory drugs
$\Delta\lambda$	Wavelength increment

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors declare no conflict of interest.

Competing Interests
The authors declare that they have no competing interests.

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