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Combining subsidiary and synchronous approaches for concurrent spectrofluorimetric assurance of lopinavir and ritonavir in tablets utilized in convention for treatment of coronavirus infection (COVID-19) and biological fluids

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HIGHLIGHTS

- Determination of lopinavir and ritonavir down to biological concentration level has been carried out. The determination is based on increasing the selectivity of the spectrofluorimetric technique by combining both derivative and synchronous spectrofluorimetric approaches.
- Application of of the method was successfully carried out in the commercial tablets and spiked plasma with good agreement with the comparison methods.

ARTICLE INFO

Article history:
Received 18 March 2021
Received in revised form 4 June 2021
Accepted 7 June 2021
Available online 10 June 2021

Keywords:
Lopinavir
Ritonavir
COVID-19
Synonymous
Fluorescence spectroscopy
Spiked human plasma

ABSTRACT

In this think about, assurance of lopinavir and ritonavir down to organic concentration level has been carried out. The assurance is based on expanding the selectivity of the spectrofluorimetric procedure by combining both subordinate and synchronous spectrofluorimetric approaches, which allow effective estimation of lopinavir at 248.8 nm and ritonavir at 300.1 nm within the nearness of each other at Δλ of 60 nm. Worldwide Conference on Harmonization approval rules were taken after to completely approve the strategy, and linearity was gotten for the two drugs over the extend of 0.4–2.4 μg mL⁻¹ for Lopinavir and 0.1–0.6 μg mL⁻¹ for ritonavir. Application of of the strategy was successfully carried out within the commercial tablets with great understanding with the comparison strategies. As the detection limits were down to 0.133 and 0.022 μg mL⁻¹ and quantitation limits were 0.395 and 0.068 μg mL⁻¹ for lopinavir and ritonavir, individually; the in vivo assurance of lopinavir and ritonavir in spiked plasma tests was pertinent. The rate recuperations in natural tests were 99.10 ± 0.77 and 99.54 ± 0.60 for lopinavir and ritonavir, individually. Water was utilized as the ideal weakening dissolvable within the proposed strategy which includes an eco-friendly justify.

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1. Introduction

Lopinavir (LPV) is an HIV (human immunodeficiency infection) protease inhibitor (PI) co-administered with a low dose measurements of ritonavir (RTV) in healthy volunteers expanded the range beneath the lopinavir plasma concentration-time bend >100 crease; beneath the brand title Kaletra (LPV/r) as portion of antiretroviral treatment (Craftsmanship) in individuals influenced by HIV. The combination was affirmed by the U.S. Nourishment and Medicate Organization (FDA) two decades prior (Oldfield & Plosker, 2006). Since 2006, the World Wellbeing Organization (WHO) rules have reliably prescribed LPV/r as one of PIs in second-line regimens (World Wellbeing Organization, 2006). Within the most recent WHO rules (2019), LPV/r is still prescribed as the favored PI treatment for second-line Craftsmanship regimen, elective first-line Craftsmanship regimen in children and in uncommon circumstances in neonates (World Wellbeing Organization, 2019). Most as of late, due to the worldwide flare-up of the serious intense respiratory disorder coronavirus 2 (SARS-CoV-2) contamination driving to COVID-19 infection, LPV/r is being considered as one of the potential candidates, with numerous clinical trials started to test its viability [1–3]. Lopinavir is as of now beneath examination in combination with ritonavir for the treatment of COVID-19 caused by SARS-CoV-2 [4]. Lopinavir chemical name is (2S)-N-[[25,43,55]-S-5-[[2-(2,6-dimethylphenoxy)acetyl]amino]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[(2-oxo-1,3-diazinan-1-yl)butanamide (Fig. 1, a). A survey of the writing appeared that strategies detailed for the assurance of Lopinavir alone or in combinations were HPLC [1,5–7] and LC-MS [8–10]. Ritonavir (RTV) could be a peptidomimetic HIV protease inhibitor outlined to complement the C2 hub of symmetry of the protein dynamic location. Ritonavir is dynamic against both HIV-1 and HIV-2, in spite of the fact that it may be somewhat less dynamic against the last mentioned. Ritonavir is for the most part utilized as a pharmacokinetic enhancer (CYP 3A4 inhibitor) [3]. Ritonavir chemical name is 1,3-thiazol-5-ylmethyl N-[[25,43,55]-S-3-hydroxy-5-[[25]-3-methyl-2-[[methyl-2-(propan-2-yl)-1,3-thiazol-4-yl]methyl]carbamoyl]amino] butanoyl[amino]-1,6-diphenylhexan-2-yl] carbamate (Fig. 1, b). Ritonavir was decided alone or in combinations by assortment of strategies as electrochemical strategies [11], spectrophotometry [12–14], HPLC [15–17], TLC-densitometry [18–20] and LC-MS [21–24]. There are detailed strategies for the assurance of the two drugs together either in their twofold blend or within the nearness of other antiviral drugs as spectrophotometry [25–27], HPLC [28–31], TLC-densitometry [32–34] and LC-MS [35–38]. The contrasted with each other working arrangements for the assurance of the two drugs together either in their twofold blend or within the nearness of their debasement items by synchronous spectrofluorimetry. Our point was to create a modern delicate and keen technique for their concurrent quantitation. The display think about is proposing a unused spectrofluorimetric strategy for LPV and RTV assurance for the primary time utilizing first derivative SFS. It may be a fruitful way to quantitate them with fulfilled precision and exactness in tablets and biological fluids. Moreover, the strategy is simple and cost-effective because it employs a procedure accessible in most research facilities.

2. Experimental

2.1. Apparatus

Agilent Cary Overshadow Fluorescence Spectrofluorimeter (USA); prepared using 150 W xenon streak light and 1 cm quartz cell. The opening width of excitation and emanation was 10 nm, worked with version 1.2 of the Cary overshadow search application software. The HANNA pH 211 Chip pH Meter with double intersection glass anode was used to make pH estimates. Syringe filters (Minisart RC25)-0.45 μm pore size were purchased from Sartorius-Stedim (Göttingen, Germany).

2.2. Materials, solvents and reagents

Lopinavir and ritonavir were given by Abbott Co., USA. kaletra® tablets (Abbott Co., USA) containing 200 mg of LPV and 50 mg RTV as named [25]. Human plasma tests were compassionate given by Minia College Healing centers (Minia, Egypt) and kept solidified. The tests were subjected to delicate defrosting some time recently utilize. Methanol, acetonitrile and n-propanol were obtained from Sigma-Aldrich (Germany). Surfactants as sodium dodecyl sulfate (SDS), cetrimide and Tween 80 and chemicals utilized for buffer arrangements were bought from El Nasr Co., Egypt. Acetic acid derivation and borate buffers were arranged at concentration of 0.2 M for each.

2.3. Standard solutions

In order to prepare stock solutions with concentration of 100.0 μg mL⁻¹, 10.0 mg of each LPV and RTV were dissolved separately in 100 mL methanol. Subsequently dilution with methanol was carried out to get the working solutions.

In arrange to plan stock arrangements with concentration of 100.0 μg mL⁻¹ for each LPV and RTV were broken down independently in 100 mL methanol in volumetric flasks. In this way dilution with methanol was carried out to induce the working arrangements.

2.4. Procedures

2.4.1. Construction of calibration graphs

Aliquots from the working arrangements of LPV and RTV were exchanged into a arrangement of 10 mL volumetric jars. The arrangements were at that point completed with refined water to.
volume to reach ranges of 0.4–2.4 \( \mu \text{g mL}^{-1} \) and 0.1–0.6 \( \mu \text{g mL}^{-1} \) for LPV and RTV, separately. Synchronous estimations were carried out at \( \Delta \lambda = 60 \) with filtering run 200–500 nm. The primary – arrange subsidiary spectra (1D) were worked utilizing channel estimate 20.0 and 1.0 nm interval. The plentifulness were evaluated at 248.8 and 300.1 nm for LPV and RTV, separately, considering the clear perusing. In arrange to differentiate the calibration charts; (1D) amplitudes were plotted against the ultimate medicate concentration in \( \mu \text{g mL}^{-1} \) and relapse examination was carried out.

### 2.4.2. Analysis of LPV and RTV synthetic mixtures and pharmaceutical preparations

Aliquots with changed proportions of LPV and RTV were analyzed as portrayed beneath (development of calibration charts). At that point, the rate recuperations were calculated by alluding to the to the calibration bends or the relapse conditions. For tablet test, 10 kaletra\(^{9}\) tablets were subjected to weighing, blending and crushing. Ten milligrams were exchanged into a 100 mL volumetric flask, 70 mL methanol was included and sonicated for 30 min. The volumetric flasks were completed to volume and sifted to be measured at outlined in (development of calibration charts). (1D) were calculated and the substance of tablets was computed by the relapse conditions.

### 2.4.3. Analysis of LPV and RTV in spiked human plasma

Concurrent assurance of LVP and RTV in spiked human plasma was carried out alluding to their restorative levels [39]. One milliliter plasma was exchanged independently into a set of centrifugation tubes. Aliquots from LPV and RTV stock arrangements were included to reach last concentrations 0.4–2.4 and 0.1–0.6 \( \mu \text{g mL}^{-1} \) for both drugs, individually. Four milliliters methanol were included and after that the tubes were subjected to vortex blending for 5 min and centrifuged for another 30 min at 4000 r.p.m. to permit total partition of the drugs from plasma substance. The upper clear layer was sifted through 0.45 \( \mu \text{m} \) syringe channels. One milliliter aliquots from the filtrate were quantitatively moved into a set of 10 mL volumetric jars and weakened with water to the volume. Estimation of the sedate concentration was carried out as the method characterized beneath (development of calibration charts) with clear test in parallel. The amplitudes were plotted versus the concentration of each medicate in \( \mu \text{g mL}^{-1} \).

### 3. Results and discussion

Based on the significance of synchronous fluorimetry in selectivity and determination improvement, we pointed to utilize this approach to measure the commonly co-administered drugs LVP and RTV at the same time in their distinctive networks. Way better execution was watched when combining both synchronous fluorimetry and subsidiary approaches. Fig. 2 outlines the covering spectra of LVP and RTV, where LVP excitation and outflow wavelengths are 250.1 and 450.3 nm, individually, and RTV has an outflow band at 400.2 nm when energized at 300.2 nm. The pH was investigated utilizing 0.2 M acetic acid derivation buffer (pH 3.6–5.6) and 0.2 M borate buffer (pH 7.0–10.0). Diverse pH values did not improve the synchronous fluorescence concen-
3.2. Validation of the method

To consider the proposed strategy a approved strategy, the rules of Universal Conference on Harmonization (ICH) Q2 (R1) were taken after [41]. The calculated information are summarized in Table 1.

It was found that straight relationship between crest amplitudes and concentration was given over the run 0.4–2.4 µg mL\(^{-1}\) for LVP and 0.1–0.6 µg mL\(^{-1}\) for RTV at 248.8 and 300.1 nm, separately. Limits of quantitation and limits of location are calculated and condensed in Table 1. Conditions that speak to straight relapse examination are:

\[ 1D = 79.63C - 3.02 \quad (r = 0.9999) \quad \text{for LVP at 248.8 nm} \]

\[ 1D = 15.01C - 4.11 \quad (r = 0.9998) \quad \text{for RTV at 300.1 nm} \]

Where \(1D\) is the peak amplitude, \(C\) is the concentration of the drug in µg mL\(^{-1}\) and \(r\) is the correlation coefficient.

To test the accuracy of the proposed synchronous spectrophotometric method, the obtained results were compared with reported method [25]. Additionally, statistical of data [42] indicated no significant difference between the methods as shown in Table 2.

The proposed strategy was too tried with respect to intra-day and inter-day exactness (Table 3). Three concentrations inside the calibration bend for each sedate were inspected and the relative standard deviations were found to be little demonstrating the repeatability and middle of the road exactness of the proposed strategy.

### Table 1

Analytical performance data of the proposed first derivative synchronous spectrophotometric method.

| Parameter                        | LPV                  | RTV                  |
|----------------------------------|----------------------|----------------------|
| Wavelength (nm)                  | 248.8                | 300.1                |
| Linearity range (µg mL\(^{-1}\)) | 0.4–2.4              | 0.1–0.6              |
| Intercept (a)                    | -3.02                | -4.11                |
| Slope (b)                        | 79.63                | 15.01                |
| Correlation coefficient (r)      | 0.9999               | 0.9998               |
| S.d. of residuals (S_r)          | 0.33                 | 0.50                 |
| S.d. of intercept (S_a)          | 0.34                 | 0.41                 |
| S.d. of slope (S_b)              | 0.84                 | 0.167                |
| Percentage relative standard deviation, %RSD | 1.38 | 1.54 |
| Percentage relative error, % error | 0.55 | 0.58 |
| Limit of detection, LOD (µg mL\(^{-1}\)) | 0.133 | 0.022 |
| Limit of quantitation, LOQ (µg mL\(^{-1}\)) | 0.395 | 0.068 |

### Table 2

Application of the proposed method to the determination of LVP and RTV in their pure form. Each result is the average of three separate determinations. The values between parentheses are the tabulated \(t\) and \(F\) values at \(p = 0.05\) [25].

| Compound | Proposed method % Found | Comparison method % Found |
|----------|-------------------------|---------------------------|
| LVP      | 99.78                   | 99.70                     |
| ± SD.    | 1.00                    | 1.12                      |
| N        | 6                       | 3                         |
| \(t\) (2.36) | 0.105                   |                           |
| F (19.3) | 1.254                   |                           |
| RTV      | 100.27                  | 99.93                     |
| ± SD.    | 1.32                    | 1.67                      |
| N        | 6                       | 3                         |
| \(t\) (2.36) | 0.308                   |                           |
| F (19.29) | 1.6                     |                           |

Each result is the average of three separate determinations.

3.3. Applications

#### 3.3.1. Analysis of LVP and RTV in their synthetic mixtures and pharmaceutical preparations

Distinctive concentrations from the two considered drugs were decided in changed proportions in manufactured blends. Investigation of these blends appears the appropriateness of the outlined strategy for their particular assurance, as spoken to in Table 4. Fig. 5 illustrates the great ghastly determination for LVP and RTV in their engineered blend. Besides, single fixing tablets for each medicate were analyzed by the proposed strategy to test the specificity and the likelihood of obstructions from the excipients, maize starch, powder and lactose monohydrate. The comes about in Table 5 uncover great rate recuperations and standard deviations. In addition, the comparison of the comes about with already distributed ones get worthy factual information with respect to Student’s \(t\)-test and change proportion \(F\)-test [42].

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Fig. 5. First derivative synchronous spectra of: (a) LPV (1.2 µg mL\(^{-1}\)) and (b) RTV (0.3, 0.4, 0.5 and 0.6 µg mL\(^{-1}\)) at 300.1 nm.

Fig. 6. First derivative synchronous fluorescence spectra of: (a) LPV (1.2 µg mL\(^{-1}\)), (2) RTV (0.5 µg mL\(^{-1}\)) and (3) Mixture of 1.2 µg mL\(^{-1}\) LPV and 0.5 µg mL\(^{-1}\) RTV.
3.3.2. Analysis of LVP and RTV in spiked human plasma
Top plasma levels of around 1.0 mg L⁻¹ of RTV happen 2 h after 250 mg single verbal measurements and the restorative concentration of LVP is detailed to be more noteworthy than 0.25 mg L⁻¹ [26]. The affectability of the proposed strategy was down to 0.412 and 0.115 for LVP and RTV, individually; hence, it was pertinent to assess the two drugs in natural level. Utilizing the proposed direction, a straight relationship was built in plasma tests spiked with LVP and RTV by plotting the amplitudes of the primary subsidiary spectra versus the medicate concentration (Table 6).

4. Conclusion
The commonly endorsed drugs LVP and RTV are utilized within the convention for treatment contamination leading to COVID-19 illness. To achieve the objective of their synchronous assurance, easy and quick strategy was created based on to begin with subsidiary synchronous spectrofluorometry. The strategy grants fast measuring utilizing water as an financial and secure weakening dissolvable. Investigation of engineered blends and single fixing tablets was carried out to demonstrate selectivity of the strategy.

### Table 3
Inter-day and intra-day precision of the developed method. Each result is the average of three separate determinations.

| Drug | Conc. (µg ml⁻¹) | Intra-day Mean ± SD. | % RSD | % error | Inter-day Mean ± SD. | % RSD | % error |
|------|----------------|----------------------|-------|---------|----------------------|-------|---------|
| LPV  | 0.8            | 99.15 ± 1.37         | 1.36  | 1.01    | 100.57 ± 1.46        | 1.45  | 0.97    |
|      | 1.6            | 98.96 ± 0.93         | 0.94  | 0.35    | 99.67 ± 1.28         | 1.27  | 0.76    |
|      | 2.4            | 100.87 ± 0.38        | 0.37  | 0.22    | 99.45 ± 0.88         | 0.88  | 0.45    |
| RTV  | 0.2            | 100.21 ± 1.21        | 1.21  | 0.83    | 99.48 ± 1.32         | 1.32  | 0.84    |
|      | 0.3            | 100.34 ± 1.03        | 1.02  | 0.72    | 100.35 ± 0.89        | 0.88  | 0.45    |
|      | 0.4            | 99.34 ± 0.48         | 0.48  | 0.15    | 99.43 ± 0.49         | 0.49  | 0.16    |

Each result is the average of three separate determinations.

### Table 4
Application of the proposed method to the determination of LPV and RTV in their synthetic mixtures.

| Synthetic mixture (ratio) | Amount taken (µg ml⁻¹) | LPV | RTV | % Found LPV | % Found RTV |
|---------------------------|------------------------|-----|-----|------------|------------|
|                           |                        |     |     |            |            |
| 4:1                       | 0.8                    |     |     | 99.06      | 100.12     |
| 1:1                       | 0.5                    |     |     | 100.43     | 99.23      |
| 3:1                       | 1.8                    |     |     | 100.24     | 100.12     |
| Mean ± SD.                |                        |     |     | 99.91 ± 0.57 | 99.85 ± 0.45 |

Each result is the average of three separate determinations.

### Table 5
Application of the proposed method to the determination of LPV and RTV in their pharmaceutical preparation. Values between parentheses are the tabulated t and values at p = 0.05 [25].

| Compound | Proposed method % Found | Comparison method % Found | LPV | RTV |
|----------|-------------------------|---------------------------|-----|-----|
| Kaletra * tablets | LPV | RTV | 100.03 | 100.72 |
| Mean ± SD. | 1.11 | 1.39 | 1.61 | 3 |
| N | 6 | 6 | 3 | 3 |
| t (2.77) | 0.996 | 1.046 | 99.98 | 99.96 |
| F (19.00) | 1.872 | 1.073 | 1.47 | 1.47 |

Each result is the average of three separate determinations.

### Table 6
Assay results for the determination of the studied drugs in spiked human plasma samples using the proposed method.

| Parameter | Amount taken (µg ml⁻¹) | Amount found (µg ml⁻¹) | % Found LPV | % Found RTV |
|-----------|------------------------|------------------------|-------------|-------------|
|           |                        |                        |             |             |
| 0.4       | 0.3                    | 0.396                  | 98.89       | 100.04      |
| 0.8       | 0.4                    | 0.806                  | 100.74      | 99.86       |
| 1.2       | 0.5                    | 1.17                   | 97.67       | 98.72       |

% RSD | 0.77 | 0.60 |
% error | 0.39 | 0.30 |

Each result is the average of three separate determinations.
As the two drugs are commonly endorsed together, the strategy was pertinent to their measure in spiked human plasma utilizing the optimized direction. The preferences of the proposed strategy incorporate improving selectivity, determination and m.o.i. investigation time. This makes our proposed strategy an perfect one for the investigation of both drugs.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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