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REVIEW ARTICLE

Ritonavir and Lopinavir: A Review on Novel Analytical Techniques

Huma Sulthana [1*], Chetan MB [2], Prakash Kumar B [3]
[1,3*]Department of Pharmaceutical Analysis, Sri Adichunchanagiri College of pharmacy, B.G Nagara, Karnataka-571448, India.
[2]Department of Pharmaceutical Chemistry, Sharada Villas College of Pharmacy, Mysore, Karnataka-570004, India.
Email: huma776040@gmail.com

ABSTRACT
Antiretroviral drugs like ritonavir and lopinavir are used to treat HIV/AIDS.RTV and LPV are frequently used in combination with a variety of antiretroviral medications. As of now, several investigations on the analysis of LPV &RTV in bulk, pharmaceutical formulations, and biological fluids have been reported on analytical methodologies. The current review provides in-depth information on the types of various analytical methods, including chromatography and spectrophotometry, which are both investigated for the quantification and detection of metabolites as well as for stability research on RTV and LPV.This study provides brief and comprehensive information on the analytical validation parameters for the analysis of RTV, LPV alone or in combination with other medications, such as Limit of detection (LOD), Limit of Quantification (LOQ), Standard Curve, Accuracy & Precision. This review facilitates conducting more analytical research on the drugs indicated.
Keywords: Antiretroviral, Ritonavir, Lopinavir, Spectroscopic methods, Chromatographic methods, HIV, AIDS.

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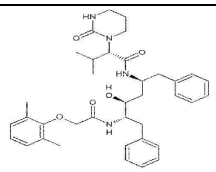
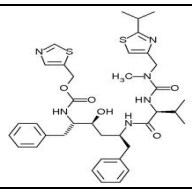
INTRODUCTION

In general, viruses are far smaller than bacteria and are microscopic parasites. They require a host body to survive and reproduce. Medical Microbiology states that the main function of the virus or virion is to "transport its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell [1]. Drugs that treat viral infections are known as antiviral drugs. Drugs used as antivirals prevent the reproduction of viruses [2]. Antiviral medications can only target a certain number of metabolic processes since viruses are small and multiply inside cells via the cells by metabolic pathways. It is challenging to identify medications that are selective for the virus since viruses' control many of the metabolic processes of the host cell. However, several enzymes have been shown to be valuable as therapeutic targets because they are virus-specific [3-5].

History and development of LPV&RTV:

The US Food and Medication Administration (USFDA) authorized LPV/RTV (Kaletra®) as the sixth HIV-1 protease inhibitor (PI) drugby USFDA, was the first and only PI to be co-formulated, and it was approved in September 2000 in the US (April 2001 in Europe) for the treatment of HIV infection in adults and children older than 6 months.The FDA granted its approval for the present LPV/RTV tablet formulation in October 2005 [6].In 1997, during the 4th Conference on Retroviruses and Opportunistic Infections, Abbott Laboratories revealed ABT-378, a novel protease inhibitor that would ultimately be renamed lopinavir. Low dosages of RTV, a strong inhibitor of cytochrome P450 3A4, significantly raise the blood levels of lopinavir. The LPV/RTV (Kaletra®) was first produced as a soft-gel capsule with 133.3 mg of LPV and 33.3 mg of RTV [7]

Table number-01: Drug profile [8-11].

Name of the drug	LPV	RTV
Structure		
Brand name	Kaletra(100mg of LPV and 25mg of RTV)	
Chemical formulation	$C_{37}H_{48}N_4O_5$	$C_{37}H_{48}N_6O_5S_2$
Molecular weight (g/mol)	628.8	720.9
Melting point range(°C)	124-127°C	126-132°C
Boiling point (±65.0°C at 760 mmHg)	924.2°C	947.0°C
IUPAC	(2S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide	1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[[(2S)-3-methyl-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]butanoyl]amino]-1,6-diphenylhexan-2-yl]carbamate
pKa(Strongest Acidic)	13.39	13.68
Category	Antiviral drugs Antiretroviral Protease inhibitors	Antiviral drugs Antiretroviral Protease inhibitors
Indication	Advised for use in combination with other antiretroviral medications to treat HIV-1 infection.	The HIV reproductive cycle is disrupted by the HIV protease inhibitor drug RTV. It is advised in combination with another antiretroviral for the treatment of HIV-1 infection in adults and children who are at least 14 days old, despite the fact that it was first designed as an independent antiviral drug.
Side effects	Diarrhea, Headache, Weakness, Nausea, Vomiting, Stomach Upset, Drowsiness, and Dizziness.	
Contraindication	Diabetes is the high number of triglycerides in the blood High cholesterol and high triglycerides Low amount of magnesium in the blood Low amount of potassium in the blood Hemophilia.	
Mechanism of action	HIV-1 protease is an aspartic protease that catalyzes the breakdown structural and functional proteins from precursor viral polypeptide strands, playing a critical role	

	in the viral life cycle. Immature, non-infectious virion is produced when the protease is inhibited. The HIV-1 protease is effectively inhibited by LPV. The LPV/RTV co-formulation inhibits the generation of infectious virions, preventing successive rounds of cellular infection, and as a result, has an antiviral impact.

Compilations of quantification methods for Ritonavir and Lopinavir by UV-Spectrophotometry:

Due to its simplicity and efficiency in drug analysis, UV spectroscopy is a popular method for analysing drug compounds. The identification and quantification of the drug ingredient are aided by the use of spectroscopy, which provides short information on the solubility, max of the entity, and UV absorbance pattern[12-13].

Table number-02: Quantification methods for Ritonavir and Lopinavir by UV-Spectrophotometer

Reference	[12]				[13]		[14]
Formulation type, Brand name	Tablet (lopiumune RTV-50mg LPV-200mg). Bulk.		Tablet (Lopiumune RTV-50mg LPV-200mg)		Syrup 80mg/ml LPV 20mg /ml RTV		Tablet (lopimunefrom cipla ltd, LPV-200mg RTV-50mg)
Parameters	Accuracy Linearity Precision		Accuracy Linearity Precision		Accuracy		Linearity Precision Accuracy Ruggedness
Beer' s law limit /concentration range µg/ml	10-35µg/ml	100-500µg/ml	10-35µg/ml	100-500µg/ml	4-24 µg/ml	1-6 µg/ml	80-160 µg/ml
λ max in nm	238nm	260 nm	228-248 nm	250-270 nm	259 nm	239 nm	257.5 nm
Sample solvent/ diluents	Distilled water		Distilled water		50% Methanol		Acetonitrile: distilled
Name of the instrument	A Jasco double beam UV visible spectrophotometer, model:V-630,		Jasco double beam UV visible spectrophotometer, model: V-630,		Shimadzu UV-160A		UV visible double beam
Guideline followed	ICH Q2 R1		ICH Q2 R1		ICH		ICH
Method type (Single /simultaneous drug analysis)	Simultaneous Absorption maxima method		The area under the curve method		LPV & RTV in syrup		Simultaneous in bulk and tablet
Name of the sample	RTV &LPV				LPV & RTV		LPV & RTV

[15]	Tablet (RITOVIR and EMPETUS 100mg of RTV)	Sensitivity Precision Accuracy	10-50 µg/ml	10-50 µg/ml	239 nm	Methanol	Shimadzu UV-1800 UV visible	Absorption maxima method	RTV
			10-50 µg/ml	10-50 µg/ml	232 nm	Methanol	Shimadzu UV-1800 UV visible spectrophotometer	First-order derivative spectroscopic method	
[16]	Tablet (RTV-50mg LPV-20mg)	Accuracy Precision Specificity Ruggedness	5-40 µg/ml	10-50 µg/ml	240 nm	Methanol	Shimadzu UV-1800 UV visible	Absorption maxima method	
			20-120 µg/ml		246 nm	Acetonitrile	Shimadzu 1601UV-visible spectrophotometer with r of 10mm quartz cell	Simultaneous. First-order derivative spectroscopic method	RTV& LPV
[17]	Tablet (Norvir 100mg)	Precision Accuracy Linearity	10-50 µg/ml		278 nm				
				203		Methanol	UV spectrophotometer –UV	Lopinavir bulk and tablet dosage form. Absorption maxima method	LPV

Compilations of quantification methods for RTV and LPV by RP-HPLC Technique:

Chromatography is an essential biophysical technique for separating, identifying, and purifying mixture components for qualitative and quantitative research. There are several chromatographic techniques, such as affinity chromatography, thin-layer chromatography (TLC), column chromatography, paper chromatography, gas chromatography, ion-exchange chromatography, gel permeation chromatography, and high-pressure liquid chromatography. Chemical compound separation frequently made use of HPLC. New methods greatly outperformed older ones in terms of separation, identification, purification, and quantification. In chromatography, the stationary phase is either a solid phase or a liquid phase deposited on top of a solid phase.The stationary phase is covered by a liquid or gaseous mobile phase. When the mobile phase is liquid, the method is known as liquid chromatography (LC) Analytes are separated using this method, known as normal phase HPLC (NP-HPLC), based on polarity. In NP-HPLC, both the polar stationary phase and non-polar mobile phase are used. The polar stationary phase reacted with the polar analyte and absorbed it.A non-polar stationary phase and an aqueous, moderately polar mobile phase are the components of reversed-phase HPLC (RP-HPLC or RPC). As a result of repulsive forces between a polar eluent, the comparatively non-polar analyte, and the non-polar stationary phase, RPC works on the theory of hydrophobic interactions [18-19]. The most used liquid chromatography (LC) method for drug analysis is HPLC, which has the advantage of a high separation capacity for frequent examination. The most sophisticated type of LC, known as HPLC, is used to separate certain compounds from complicated mixtures like biological fluids. The majority of methods are used in pharmaceutical drug analysis and method development using reverse phase HPLC (RP-HPLC)[20-24].

Table number-03: Quantification methods for RTV and LPV by RP-HPLC Technique

Reference	[20]	[21]	[22]	[23]
Regression coefficient	R^2 = 0.9999 R^2 = 0.9999	R^2 = 1.0000	R^2 = 0.99982	R^2 = 0.9 999
LOQ	12.7 and 50 µg LPV and RTV	99 µg LPV and 24 µg RTV	0.521407 µg/ml	36.121 µg/ml
LOD	12.7 and 50µg LPV and RTV	99 µg LPV and 24 µg RTV	0.172064 µg/ml	11.92 µg/ml
Linearity range (µg/mL) and Accuracy	50-300 Lopinavir and 12-76 Ritonavir & 98.6% - 101.00%	200 Lopinavir& 25 Ritonavir: 99.6% & 100.3%	8.0 & 240 : 98.08 & 01.9%	150-350 : 99.85%
Isosbestic point (λ Max nm)	210	210	239	215
Mobile phase	A mixture of Ammonium acetate buffer and acetonitrile (55:45%v/v)	A mixture of water and Acetonitrile pH 7.9 with sodium dihydrogen dihydrate	ACN: OPA in the ratio 55:45	Acetonitrile and phosphate buffer pH 7.8 (85:15v/v)
Sample solvent/ diluents	Acetonitrile	Acetonitrile	Methanol: Water (70:30)	Methanol
Name of the instrument & column specification	LC -10AT VP series model chromatograph Zorbax C18 column (150 x 4.6mm, 5 µm)	Water e2695 alliance HPLC&agilent1100 and1200 system connected with PDA detector G1315B Chemostation ver.3.02,EZchrom software	RP HPLC ,Zodiac C18, 150mm x 4.6mm, 5µm	RP-HPLC-SHIMADZU LC 20 AD C18 column 250×4.6mm, 5µ particle size, Injector-Rheodyne, UV-Visible Spectrophotometer- Perkin Elmer
Method type (isocratic/ gradient analysis)	Isocratic	Isocratic	Isocratic	Isocratic
Name of the sample &RT (min)	LPV & RTV : 4.323 & 5.656	Lopinavir& Ritonavir: 8.452 & 10.169	Ritonavir 13	Lopinavir 4.4

[24]	[25]	[26]
R^2 = 0.99995 R^2 = 0.99991	R^2 = 0.9999 R^2 = 0.9999	R^2 = 1.0000 R^2 = 0.9999
50ppm& 10ppm	0.91 µg/ml & 0.30 µg/ml	0.060 & 0.010
50ppm& 10ppm	0.30 µg/ml & 0.10 µg/ml	0.060 & 0.010
5 & 4.8-15: 98.50% & 98.70%	8-48 & 2-12 : 100.04% & 99.64%	120.3/ 20.8µg/ml &100 - 110% & 101 - 116%
220	240	205
potassium dihydrogen phosphate buffer (pH 3.5): Acetonitrile: Methanol (40:50:10)	Acetonitrile: 0.05 M phosphoric acid (55: 45, v/v)	45 : 5 : 50 (v/v/v) of acetonitrile/methanol/0.02M TMAP in 0.2% TFA
potassium dihydrogen phosphate buffer (pH 3.5): Acetonitrile: Methanol (40:50:10	methanol	water/ethanol
Waters RP-HPLC equipped with software(Empower 2,695 separation module) and a UV-Visible or DAD the detector, manual injector with 100 µl loop, and X-Bridge C18 (150 mm × 4.6 mm i.d., 5 µm particle size).	Agilent technologies 1260 LC system with gradient pump connected to DAD UV detector and Agilent TC C18 250 X 4.6 mm, 5 m column	Waters pump model 6000A, 484 tunable absorbance detector, 741 data module, and WISP 710B auto sample processor Radial-Pak Nova-Pak C18 column (4m m, 83100 mm, Waters)
Isocratic	Isocratic	Isocratic
Lopinavir& Ritonavir : 5.7 & 6.6	Lopinavir& Ritonavir: 4.35 & 6.68	Lopinavir& Ritonavir :13 & 14

Compendia of bio-analytical quantification methods for RTV and LPV by RP-HPLC Technique:

Determine the concentration of a drug or its metabolite, or both, in the biological matrix, such as plasma, serum, urine, etc., through using bio-analytical approach. Studies on human clinical pharmacology, bioavailability, and bioequivalence, as well as pharmacokinetic assessment, all make use of bioanalytical data. Additionally, non-human pharmacological and toxicological research employ the bio-analytical approach (preclinical studies). The goal of establishing a bio-analytical technique is to use available resources to create a method that is more accurate and precise for the target analyte under the specified lab circumstances. to make it possible to measure the levels of medicines and their metabolites in biological matrices. Qin c et al., developed a sensitive and cost-effective bioanalytical HPLC-UV method for estimation of LPV in rat and human plasma. In this method only 10µL of cannabidiol was used as internal

standard 100µL of plasma sample was used & samples were liquid-liquid extracted, the separation was achieved by using following chromatographic condition C18 reversed-phase analytical column with a mobile phase of acetonitrile-water with gradient elution. Column oven was set at 40^0C and UV detection was conducted at 211 nm, flow rate 0.3ml min^{-1}. Linearity was found in the range of 10- 10000 ng ml^{-1} of LPV and the mean extraction recoveries in accuracy were found to be 88.7-96.5%. This assay allowed for preclinical pharmacokinetic and drug administration investigations in rats in both developed and developing nations while increasing sensitivity while requiring less plasma. It can be utilised for clinical research and clinical TDM of HIV-infected and perhaps SARS-Cov-2-infected patients undergoing LPV due to its high selectivity, sensitivity, and stability [27]. Kou H J, et al, have developed a novel, specific, accurate, and reproducible HPLC-UV-VIS method for the quantitative determination of LPV & RTV in human plasma in this assay method only 200µL of plasma sample was used & samples were liquid-liquid extracted, and diazepam was used as an internal standard. The separation was achieved by using the following chromatographic conditions C18 reversed-phase analytical column with a mobile phase of acetonitrile-sodium dihydrogen phosphate buffer(10mmol L-1, pH-4.80)(60:40, V/V). column oven was set at 40^oC and UV detection was conducted at 205 nm. Linearity was found in the range of 0.5-20µg mL^{-1} and 0.05-5 µg mL^{-1} of LPV& RTV respectively and the mean extraction recoveries in accuracy were found to be 79.17%, 52.26%, and 91.35% LPV, RTV, and diazepam respectively after a successful method development and validation is done later it was applied to human plasma samples from HIV-Positive Chinese patients and because of its good features like simple, robust and inexpensiveness it could be used in pharmacokinetic studies and routine therapeutic drug monitoring of LPV & RTV[28]. R.Vats, et al, have developed a novel, rapid, sensitive, and reproducible HPLC-UV method for the determination of LPV in Wister rat plasma in this assay method only 100µL of drug spiked plasma sample was used &the sample was extracted by single-step protein precipitation. The separation was achieved by using the following chromatographic condition C18 reverse phase column with a mobile phase of acetonitrile – ammonium acetate buffer(10mmol L-1, pH- 6.5) (65:35, V/V).UV detection was conducted at 210nm.linearity was found in the range of 250- 4000 ng mL^{-1} of LPVand the mean extraction recoveries in accuracy were found to be 97.5-100.19% after a successful method development and validation is done on rat plasma and because of its good features like simple, rapid, precise and cost-effective. it was used to determine the pharmacokinetic parameters of the drug following IV bolus administration in rats with LPV[29].

Infrared spectroscopic interpretation of RTV and LPV:

Infrared Spectroscopy is the absorption of lower energy radiation that excites groups of atoms inside molecules in both a rotational and vibrational manner. It is simple to identify functional groups because of their distinctive absorptions. In pharmacological compounds, an IR spectrum—which provides precise information on the infrared absorptions found for various bound atoms and groups—is frequently displayed. Organic compound structure elucidation benefits the most from infrared interpretation [30]. RTVIR(KBr)v(cm^{-1}) values expected for various functional groups are as follows: 3550-3200 (OH-Hydroxyl group), 1595-470 (Aromatic C=C), 1700-1680cm (Carbonyl C=O), 1465-1150 (Aliphatic C-H), and 3520-3400(Amide NH) and the obtained values of RTV IR(KBr) v(cm^{-1})2964(OH-Hydroxyl group), 1645.87 (Aromatic C=C), 1723.18 cm^{-1} (Carbonyl –C=O), 1530.23 (CH_2-aliphatic), 3484.82 (Amide-NH)[30]. LPV IR(KBr)v(cm^{-1})values expected for various functional groups are as follows: 3436.22 (OH-Hydroxyl group), 1450.49(Aromatic C=C), 1653.30(Carbonyl C=O), 1085(Aliphatic C-H), and 3399(Amide NH) and the obtained values of LPV IR(KBr) v (cm^{-1}) 3436.22 (OH- Hydroxyl group), 1450.49(Aromatic C=C), 1653.30(Carbonyl C=O), 1085(Aliphatic C-H), and 3399(Amide -NH) [31].

CONCLUSION

The techniques mentioned above provide concise overall data about the analysis of the RTV and LPV when combined with various anti-viral medicines. All of the methodologies indicated have been confirmed to be in accordance with the ICH/USFDA criteria and are helpful in the examination of the medications specified. The RP-HPLC technique is the preferred method for analysing the drug. Acetonitrile with potassium dihydrogen phosphate buffer, methanol with sodium phosphate buffer, and acetonitrile with phosphoric acid are the three main solvents employed in these techniques. In UV spectroscopic methods acetonitrile, water, and methanol solvents were used, and the lambda max for RTV and LPV changed into determined to be 238 nm and 260nm.

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CONFLICT OF INTEREST

There is no Conflict of interest regarding the publication of this review paper.

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