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REVIEW ARTICLE **OPEN ACCESS**

A Review: Analytical Development and Validation of Lopinavir in Bulk and Pharmaceutical Dosage Form

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ABSTRACT
Lopinavir is approved in 2000 by USFDA. Highly active antiretroviral therapy (HAART) is recognized as the most effective treatment method for AIDS, and protease inhibitors play a very important role in HAART. This paper could be a review of analytical HPLC techniques that are widely used in determining common provision issues. These reviews involve information about HPLC method development like mobile phase, mobile phase ratio, column, retention time, flow rate and wavelength of UV detector, run time, and Validation parameters is Linearity, percentage recovery, the limit of detection, and limit of quantification. Pharmaceutical analysis plays an important role in quality assurance as an internal control of pure and pharmaceutical dosage forms. Analytical method development has become an important activity of study.
Keywords: *Lopinavir, HAART, HPLC, UV detector*

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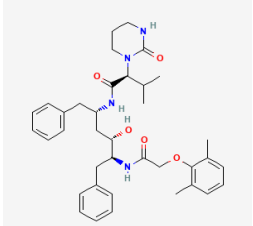
INTRODUCTION

Abbott Laboratories' lopinavir (brand name: Kaletra), it combines lopinavir and ritonavir, was approved by the FDA in 2000 as a ritonavir-based drug. Lopinavir is an antiretroviral protease inhibitor which is used in the prevention and treatment of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) (AIDS). Lopinavir can cause transient, generally asymptomatic increases in blood aminotransferase levels, as well as clinically obvious acute liver damage in rare cases. Highly active antiretroviral treatment with lopinavir in HBV or HCV coinfected individuals may worsen the underlying chronic hepatitis B or C [1-3].

Table1. Drug Profile

Drug	Lopinavir
Structure	
IUPAC Name	(2*S*)-*N*-[(2*S*,4*S*,5*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
Molecular Formula	$C_{37}H_{48}N_4O_5$
Molecular Mass	628.8
Melting Point	124-127 °C
Physical State	Solid
Solubility	Freely soluble in Methanol and Ethanol; soluble in Isopropanol. Practically insoluble in water.
pKa	Strongest Acidic, 3.7
Half-Life	The elimination half-life of lopinavir is 6.9 ± 2.2 hours.
Use	Lopinavir is an antiretroviral protease inhibitor used in combination with retinovir in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS).

MECHANISM OF ACTION

The HIV lifecycle is divided into three stages: assembly, which involves the production and packaging of essential viral components; budding, which occurs when the viral particle crosses the plasma membrane of the host cell and forms a lipid envelope; and maturation, which takes place when the viral particle constantly changes and becomes infectious. The Gag polyprotein, along with the products of its proteolysis, is at the heart of its lifecycle, coordinating various phases and serving as the virus's key structural proteins. The HIV-1 protease enzyme, a dimeric aspartic protease, is responsible for cleaving the Gag polyprotein and consequently is involved in many phases of the HIV viral lifecycle. The HIV-1 protease enzyme is inhibited by lopinavir. Its structure is based on the "peptidomimetic" theory, in which a hydroxyl ethylene scaffold mimics the typical peptide linkage (cleaved by HIV protease) but cannot be cleaved. Lopinavir causes the generation of immature, non-infectious viral particles by inhibiting HIV-1 protease activity and thus the proteolysis of the Gag polyprotein[4,5].

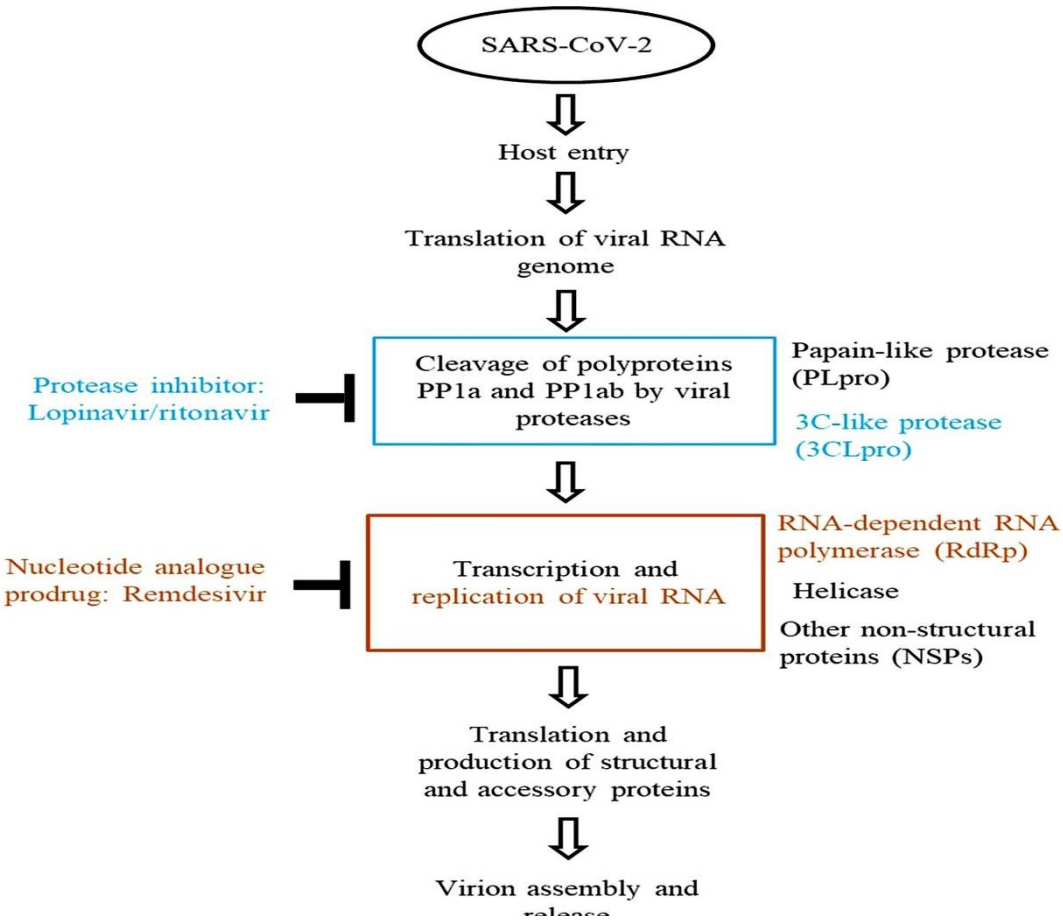


Figure 1. Inhibition of viral infection by protease inhibitor [6].

The HIV protease inhibitor lopinavir has previously been shown to have selective toxicity against HPV-positive cervical cancer cells via an unknown mechanism. To establish that lopinavir suppresses the proteasome in these cells, SiHa cervical cancer cells were transfected with the proteasome sensor vector pZsProSensor-1. After that, the Panorama Xpress profiler 725 antibody array was used to look for specific changes in protein expression in lopinavir-treated vs control untreated SiHa cells, which was followed by PCR and western blotting. Lopinavir-treated E6/E7 immortalised human keratinocytes were compared to control keratinocytes in colorimetric growth tests.The researchers also utilised targeted small interfering RNA gene silencing followed by a growth assay comparison of lopinavir-treated or untreated SiHa cells. The fluorescence of pZsProSensor-1 transfected SiHa cells increased after treatment with lopinavir, indicating proteasomal suppression. The protein ribonuclease L (RNASEL) was shown to be up-regulated in lopinavir-treated SiHa cells, as evidenced by PCR and western blot. SiHa cells were less sensitive to lopinavir when RNASEL was silenced. Lopinavir caused selective toxicity in E6/E7 immortalised keratinocytes compared to control cells, which was linked to increased RNASEL expression.These findings support the theory that lopinavir's toxicity against HPV-positive cervical cancer cells is linked to its capacity to prevent viral proteasome activity and generate an increase in the antiviral protein RNASEL.

The drug's selective toxicity and up-regulation of RNASEL in E6/E7 immortalised keratinocytes, as well as improved resistance to lopinavir in SiHa cells after suppression of RNASEL gene expression, corroborate this theory [7].

Lopinavir reduces HIV type 1 (HIV-1) replication by interfering with HIV protease. HIV protease cleaves viral polypeptide products of the gag and gag-pol genes during HIV replication to produce structural proteins for the virion core and important viral enzymes. Lopivir limits the maturation of the virus by interfering with the synthesis of these critical proteins and enzymes, resulting in nonfunctional, immature, and non-infectious virions. In vitro, lopinavir has modest effect against HIV type 2. (HIV-2) [8].

PHARMACOKINETIC

Absorption - Lopinavir has a limited oral bioavailability (25%) when taken alone; as a result, it is only given in combination with ritonavir, which greatly improves bioavailability, inhibits drug metabolism, and enables for therapeutic lopinavir concentrations to be achieved. Maximum plasma concentrations of lopinavir/ritonavir are reached at about 4.4 hours (Tmax), with Cmax and AUCtau of 9.8 3.7 - 11.8 3.7 g/mL and 92.6 36.7 - 154.1 61.4 g•h/L, respectively. When compared to fasted administration, meal administration modestly increases the AUC of the tablet formulation (19%) but considerably increases the AUC of the oral solution formulation (130%).

Volume of Distribution - The volume of distribution of lopinavir following oral administration is approximately 16.9 L.

Metabolism - Lopinavir is metabolised extensively in the liver, almost entirely by CYP3A isozymes. Co-administration with ritonavir, a severe inhibitor of CYP3A enzymes, prevents lopinavir from being biotransformed and increases plasma levels of active antiviral medicines. In vitro, twelve metabolites were discovered, with the C-4 oxidation products M1, M3, and M4 being the most prevalent in plasma. The structures of these primary metabolites have been determined, but the structures of the remaining minor metabolites have yet to be determined.

Lopinavir was metabolised largely by hepatic CYP3A4 isoenzymes in rats, dogs, and humans. After oral delivery, the radioactivity in rat and dog faeces mostly comprised of unmodified parent chemicals. Although the metabolite patterns of rats, dogs, and humans were comparable, there were qualitative and quantitative variances. The rat's metabolism of lopinavir was sensitive to the inhibition.

In vitro studies with human hepatic microsomes show that lopinavir is largely metabolised via oxidation. The hepatic cytochrome P450 system metabolises lopinavir extensively, nearly entirely through the CYP3A isozyme. Ritonavir is a powerful CYP3A inhibitor that prevents lopinavir from being metabolised, resulting in higher plasma levels of the drug.In a human (14)C-lopinavir research, the parent drug was responsible for 89 percent of the plasma radioactivity following a single 400/100 mg Kaletra dose. In humans, at least 13 lopinavir oxidative metabolites have been discovered. Ritonavir has been demonstrated to activate metabolic enzymes, causing its metabolism to be induced. During multiple dosage, pre-dose lopinavir concentrations decrease over time, stabilising after 10 to 16 days.

Route of Elimination - The majority of lopinavir is excreted in the faeces. Approximately 10.4 2.3 percent of the administered dose is eliminated in the urine, and 82.6 2.5 percent is excreted in the faeces after oral administration. In urine and faeces, unchanged parent medicines accounted for 2.2 percent and 19.8 percent of the administered dose, respectively.

Biological half-life The elimination half-life of lopinavir is 6.9 ± 2.2 hours.

The average elimination half-life after a single dose was 2 to 3 hours, and it appeared to be longer when multiple doses were administered (about 4-6 hr) [9].

Therapeutic use

Lopinavir is an antiretroviral protease inhibitor used in combination with retinovir in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS) [10].

Analytical HPLC method development for Lopinavir

High-performance liquid chromatography is a type of chromatography used to separate, identify, and quantify active chemicals in organic chemistry and analysis. The type of column chromatography known as high performance liquid chromatography is the most widely utilised technique in the pharmaceutical sector. The sample is injected at the top of a porous material column (stationary phase) and pumped and pressurised through the chromatographic column with the mobile phase (liquid). The separation of sample is based on the differences between in the rates of migration through the column arising from different partition of the solution between the stationary phase and mobile phase and flow rate of mobile phase. High Performance Liquid Chromatography is much versatile than gas chromatography since it is not limited to unpredictable and thermally stable samples, and the choice of stationary phaseand mobile phase is wider. High performance liquid chromatography is an important analytical tool in assess of drug

product. This article reviews the use of HPLC for chromatographic analysis of antiretroviral protease inhibitor [11-12].

Method Validation

Analytical methods should be validated as per International Conference on Harmonization (ICH) guidelines. Method validation has ICH guideline's defines eight steps for validation:

1. Accuracy
2. Precision
3. Specificity
4. Linearity
5. Range
6. Detection limit
7. Quantitation limit
8. Robustness
9. Ruggedness
10. Sensitivity
11. Repeatability
12. Reproducibility

Table 2. Review of papers [13-34]

Sr. No.	Author	Mobile phase	Flow Rate	Retention Time	Detection	column	Linearity
1	Suneetha. A. *et al.*(2011)	Potassium hydroxide: Acetonitrile: Methanol (50:35:15 v/v)	1.0 ml/min	6.0 min	Phenomenex	Phenomex Gemini C_{18} Column	400-600 ug/ml
2	Shivanand N. *et al.*(2015)	Acetonitrile: 0.05 M Phosphoric acid (55:45 v/v)	1.2 ml/min	6.68 min	240 nm	Agilent TC C_{18} Column	2 - 12 ug/ml
3	C. M. Phechkrajang*et al.*(2009)	10 mM ammonium acetate, pH 7 and acetonitrile (50:50, v/v)	1.0 ml/min	14.7 min	245 nm	BDS Hypersil C18 (250 × 4.6 mm i.d., 5µm)	2-18 ug/ml
4	Deepthi D K*et al.* (2019)	Acetonitrile: Water (70: 30)	1.0 ml/min	4.6 min	198 nm	Phenomenex lunar (250x4.6mm , 5µ) column	80 – 120 ug/ml
5	Madhukar A.*et al.*(2011)	Methanol: Acetonitrile: Buffer (50:30:20)	1.0 ml/min	7.26 min	210 nm	C_{18} Column	5 – 150 ug/ml
6	P. S. Raghu *et al.*(2018)	Orthophosphoric acid buffer: Acetonitrile (44:6 v/v)	1.0 ml/min	2.214 min	310 nm	Inertsil C18 (250mm 4.6mm, 5µ)	25 – 150 ug/ml
7	J. Faux *et al.* (2001)	acetonitrile and water (41:59, v/v)	1.0 ml/min	6.8 min	210 nm	Xterra, C_8 (150×3.9 mm I.D.) column	0.187 to 10.0 µg/ml
8	Rao B Venkateswara *et al.*(2014)	Acetonitrile: Phosphate buffer (60:40v/v, pH 3)	1.5ml/ min	2.1min	220 nm	C18 column [ODS UG column. 250mm× 4.5 mm]	20-100 µg/ml
9	Shreenivasaraochitturi*et al.*(2008)	0.02 M KH_2PO_4 (pH 2.5): acetonitrile	1.0 ml/min	4.4 min	210 nm	YMC Pack ODS-AQ (250 mm × 4.6 mm, 5 µm)	0.028-0.063 µg/ml

10	RishikesanRathnasam *et al.*(2018)	Buffer: Methanol: Acetonitrile (40:50:10)	1.1 ml/min	6.6 min	220 nm	Xbridge C18 (250 mm × 4.6 mm i.d., 3.5 µm	20-100 µg/ml
11	I. Ponnilavarasan*et al.*(2010)	Acetonitrile: Triethylamine (0.5%) (67:33 % v/v)	1.0 ml/min	9.1 min	210–250 nm	phenomenex-Luna C18 column	40-200 µg /ml
12	Varaprasad B *et al.*(2012)	Water: acetonitrile (30 :70)	2 ml/min	8.452 min	210nm	thermo hypresil BDS C18 column	-
13	S. M. Varma *et al.*(2012)	Acetonitrile: phosphate buffer (7.8) 85:15 v/v	1.0 ml/min	4.4 min	215 nm	phenomenex C18	150-350 µg/ml
14	Chaolong Qin *et al.*(2020)	Acetonitrile: *n*-hexane–ethyl acetate (70:30 *v/v*)	0.3 mL/ min	-	211 nm	Phenomenex Gemini column (C_{18}, 150 mm × 2.0 m m, 5 µm)	0.1-100 µg/m L
15	SalinthipJarusintanak orn*et al.*(2013)	Acetonitrile: 25 mM phosphate buffer pH 6 (50:50 v/v)	1.0 ml/min	-	215–249 nm	Hypersil Gold C18	3–18 µg/mL
16	A. Kiran Kumar *et al.*(2012)	Buffer: acetonitrile 55:45%v/v	1.5 mL/mi n	4.323 min	210 nm	Zorbax C18 column (150 x 4.6mm, 5 µm)	50-300µg/m L
17	A. Indira *et al.*(2022)	Phosphate buffer: acetonitrile 30:70v/v	1.0 ml/min	8.0 min	220nm	Kromasil C18 HPLC Column (250 x 4.6mm; 5µm)	50 – 250 µg/mL
18	MasanamJyothiBindu*e t al.*(2017)	Buffer: Acetonitrile (55:45 %V/V)	1.5 ml/min	20 min	255 nm	Phenomenex Luna C-18 (250*4.6 mm, 5um)	20-120µg/m L
19	Sarika R. Jadhav*et al.*(2013)	Acetonitrile: water: Methanol (55: 30: 15 v/v/v)	1 ml/ min	10.82.±1 min	260 nm	octadecyl column (C18)	50 – 250 µg/mL
20	Dasari Varun *et al.* (2012)	Acetonitrile: buffer (0.05M Potassium dihydrogen orthophosph ate)	2.0ml/ min	12.49 min	215 nm	C18 column	-
21	R.B. Mardia*et al.*(2014)	acetonitrile: 10 mM ammonium acetate buffer (pH 4.5): Methanol (40:30:30)	1 ml/ min	12.58 min	210 nm	Brownlee C18 column	5-35 µg/ml
22	Jaiprakash N. Sangshetti *et al.*(2014)	Sodium dihydrogen phosphate buffer: acetonitrile 45:55 v/v	1 ml/ min	8.90 min	262 nm	Phenomenex Gemini C18 (250 mm×4.6 mm, 5 µ)	5-30 µg/ml

CONCLUSION

Lopinavir is an HIV-1 protease inhibitor used in combination with ritonavir to treat human immunodeficiency virus (HIV) infection. The above study gives the analytical high performance liquid chromatography methods for study and analysis of lopinavir in pure form and pharmaceutical dosage form. A literature survey reveals that many methods are reported for the method development and validation parameter of various drugs. A present review illustrates various analytical approaches for the evaluation of lopinavir had performed including HPLC in pure form and pharmaceutical dosage form. These methods are reported for the method development and validation parameter of lopinavir drugs. Analysis of the drug plays an important role after formulation to identify the drug and its metabolites.

Conflict of Interest

No conflict of interest

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None

Declaration of competing interest

The authors confirm that the conclusions of this review paper are not influenced by personal or financial interests.

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