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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC AND UV METHOD FOR PROTEASE INHIBITOR: A REVIEW

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Abstract:
A technique for the analysis of various duds using RP-HPLC and UV is an essential tool in chromatography. The HPLC method is able to separate, detect and selectively quantify the various drugs. After the method development using HPLC validation is done for the performance and reproducibility and repeatability and accuracy. Also, it will help to find out the limitations of the method and influence of factors to quantify the drug. Further, according to ICH guidelines, the established method processed with stability indicating evaluation to find out the robustness of the method. It includes forced degradation with acidic, basic, neutral condition, oxidation, photolytic degradation also done.In this review, we presented a protease inhibitor class drug and its quantification using HPLC and UV.

Keyword: *RP-HPLC, UV-spectrophotometry, Validation, Stability indicating.*

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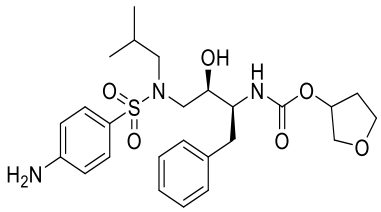
INTRODUCTION:

Acquired immunodeficiency syndrome (AIDS) is a disease caused by human immunodeficiency virus (HIV), represent the fifth most of the common cause of death in adult between the range of age is 25 and 44 years. The condition was first reported in the year 1981 by Centre for Disease Control and Prevention (CDCP), where 5 homosexual men presented with Pneumocystis cariniipneumonia. The condition was originally considered to be controlled to male homosexuals. Subsequently, it was also detected in male and female heterosexuals and bisexuals who participated in unprotected sexual behavior or who abused injected drugs. Currently, sexual activity and drug abuse remain the primary way of transmission(Chatterjee et al., 2018).

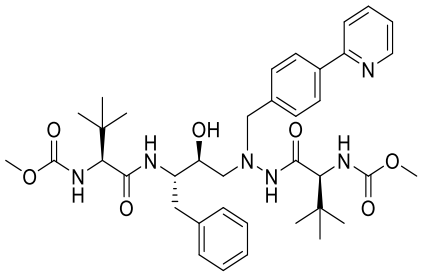
HIV most frequently enters the body via mucosal surfaces and is elated by dendritic cells to lymphoid organs, where it is then delivered to activated CD4+ T cells. Productive infection of CD4+T cells leads to viremia and dissemination of the virus to other sites in the body. The usually allied with high plasma viral loads and progressive decline in CD4+ T cell in untreated HIV infection(Parboosing et al., 2012).

Atazanavir (ATV) is a new azapeptide protease inhibitor of the HIV-1 infection. ATV possesses exclusive HIV resistance profile and good pharmacokinetic properties allowing one-day dosing(Cateau et al., 2005). The Reyataz is a brand name of atazanavir and is administered by oral antiretroviral protease inhibitors used in the healing of HIV/AIDS. ATV is categorized in Biopharmaceutical classification system (BCS) class-II with high permeability low solubility. Further, it is classified into protease inhibitors class(Konidala and Sujana, 2012)and general side effects reported with atazanavir include infection, diarrhea, abdominal pain, headache, peripheral neuropathy, nausea, vomiting, and rash(A.J. et al., 2004). Its chemical name is (3S, 8S, 9S, 12S)-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl] methyl]-2,5,6,10,13-pentaazate-tradecanedioic acid dimethyl ester, sulfate (1/1), with a molecular formula of C38H52N6O7H2SO4 and a molecular mass of 802.9. ATV is a white to pale yellow crystalline powder relatively insoluble in water. The pH of a saturated solution in water is about 1.9 at 24 ± 3°C(Bentué-Ferrer et al., 2009).

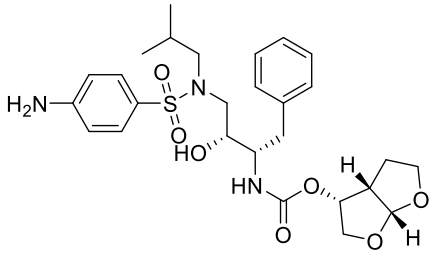
1.1 Chemistry:



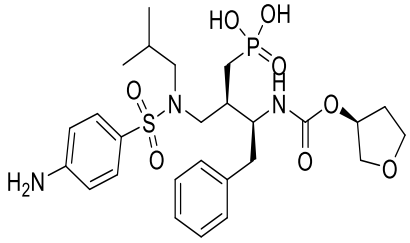
Amprenavir



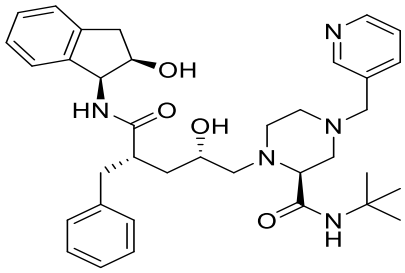
Atazanavir



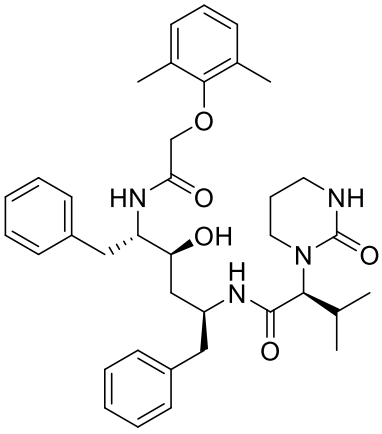
Darunavir



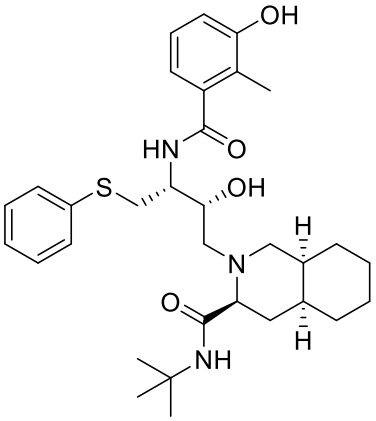
Fosamprenavir



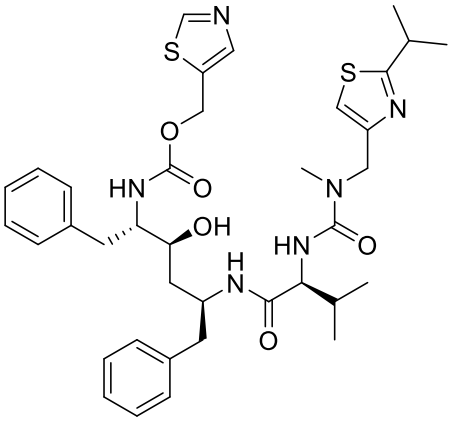
Indinavir



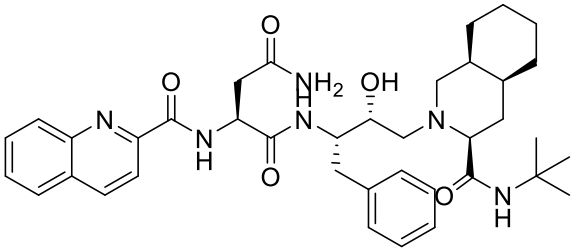
Lopinavir



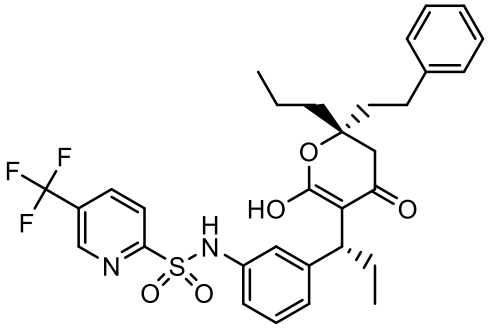
Nelfinavir



Ritonavir



Saquinavir



Tipranavir

Dose: 300 mg OD with ritonavir 100 mg taken at mealtime. ATAZOR 100, 150, 200, 300 mg caps.

1.2 Mode of Action

The mode of action of protease inhibitor that inhibits the HIV type 1 (HIV-1) protease enzyme, which is required for the processing of viral *gag* and *gag-pol* precursor polyproteins. The interruption of this latter step in the HIV life cycle prevents the efficient processing of viral structural proteins, thus preventing the formation of an infectious and mature viral particle(A.J. et al., 2004).

Their mechanism of action is inhibition of the human immunodeficiency virus protease, an enzyme necessary for viral maturation and replication. In the presence of protease inhibitors, cells produce viral particles that are immature and non-infectious. Most HIV protease inhibitors display poor bioavailability. They are extensively metabolized by microsomal cytochrome P450 enzymes, mainly CYP3A4. Their effectiveness is influenced by the administration of other agents that induce or inhibit these metabolizing enzymes(Pereira et al., 2002).

1.3 Pharmacokinetics

Each drug belongs to protease inhibitor has distinct pharmacokinetic and metabolic properties. They differ with regard to dosing regimens and the potential for interactions with food and other drugs. Most of the protease inhibitors have poor oral bioavailability and low solubility because of the first-pass metabolism. Food also decreases their bioavailability. These drugs are metabolized primarily in the liver and by intestinal epithelial cells. They undergo oxidative metabolism by CYP3A4 and other cytochrome P450 enzymes. The active metabolite of a protease inhibitor is generated by CYP2C19. The protease inhibitors are also substrates for P-glycoprotein, the multidrug efflux pump encoded by MDR1. The protease inhibitors bind widely to plasma proteins, in particular, alpha1-. although the percentage binding varies with then individual agent, very high concentrations of these glycoproteins may substantially decrease the activity of some protease inhibitors.

1.3.1 Absorption

Drugs from Protease inhibitor class has an oral bioavailability of approximately 60–68% and must be taken with food. Bioavailability of this drug is highly dependent on pH. Administration with food increases protease inhibitor bioavailability, for example, Amprenavir, Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Ritonavir, Nelfinavirand reduces interpatient pharmacokinetic variability(A.J. et al., 2004). All protease inhibitor drugs are biotransformed by the liver via CYP3A and also inhibits both CYP3A and the UGT1A1. CYP3A is similarly affected by and an important enzyme in the metabolism of many commonly prescribed drugs. ATV with other protease inhibitor is rapidly absorbed with a median T_{max} of 2 (range: 0.5 – 6) h (400 mg un-boosted) to 3 h (300 mg boosted).

ATV demonstrated the nonlinear pharmacokinetics with bigger than dose- proportional increase in AUC and C_{max} values over the dosage range of 200- 800 mg once daily. Steady-state is achieved between day 4 and 8. Administration of ATV with food enhances bioavailability and reduces pharmacokinetic variability.

Administration of a single 400 mg dose with a light snack resulted in a 70% increase in AUC and a 57% increase in C_{max} relative to the fasting state. Administration of a single 400 mg dose with a high-fat snack resulted in a 35% increase in AUC with no change in C_{max} relative to the fasting state; these results allow recommending taking ATV with food(Bentué-Ferrer et al., 2009).

1.3.2 Distribution

Protease inhibitor is highly protein bound at 86%to both α-1-acid glycoprotein and to albumin (86%). This binding is concentration independent(Bentué-Ferrer et al., 2009).

1.3.3 Metabolism

Protease inhibitor class of drugs is extensively metabolized in the human body. Atazanavir, Tipranavir, Saquinavir Mesylate competitively inhibits the cytochrome P450 (CYP) 3A4 isoenzyme, CYP1A2, and CYP2C9 isoenzymes, uridine di phosphate -glucuronosyltransferase (UGT) 1A1 enzyme, and may be a substrate of pgp (A.J. et al., 2004).Biotransformation pathways for protease inhibitors or its metabolites consist of glucuronidation, N-dealkylation, hydrolysis, and oxygenation with dehydrogenation. *In vitro* studies using human liver, microsomes suggested that ATV, RTV, TPV, NFV, is metabolized by CYP3A.ATV is a weak CYP3A inhibitor. As predictable, ATV has not been shown to induce its own metabolism or to increase the biotransformation of other drugs metabolized by CYP3A. It is also a direct inhibitor for UDP-glycosyltransferase 1A1 (UGT1A1). Finally, in HIV-1 infected patients with end-stage liver disease, un-boosted protease inhibitors showed a pharmacokinetic profile which was parallel to that of patients with boosted ATV and was able to maintain efficacy(Bentué-Ferrer et al., 2009).

1.3.4 Elimination

The major route of elimination of protease inhibitor class of anti-retroviral drugs is biliary, as 79% of a labeled dose was recovered in the feces. An increase of 42% in the AUC was noted in patients with hepatic impairment compared with the AUC of healthy subjects (A.J. et al., 2004).The unaffected drug accounted for ~ 20 and 7% of the administered dose in the feces and urine, respectively. The mean elimination half-life of ATV in HIV-1 infected patients was ~8 h when boosted with RTV and 4 h without RTV.

2 Instrumentation

2.1 High-Performance Liquid Chromatography (HPLC)

The HPLC is a superior separation system of the liquid chromatography and is used for differentiate or separating out a complex mixture of analyte come into the contact chemical and biological systems, in order to recognize increase the role of individual molecules. It was in the year 1980, The HPLC method as detected visible for 1st time for assay of bulk drug materials. This has the most important process in USP and to a lesser amount but one of the most widely useful techniques.

During the review of the journals it was observed that HPLC chromatographic techniques have been most widely used in the estimation of bulk drug, drug product and combination of drug, drug in plasma.

The choice of detection approach is critical to assure that all the components are detected. UV detector is generally used in HPLC system which is able to monitor numerous wavelength in tandem; this is possible by only applying for several wavelength scanning programs. All UV- absorbing compounds are identified in the UV detector, are present in enough amount. A photodiode array (PDA) is a distinct of line array photodiode on an integrated circuit (IC) chip for spectroscopy. The image is situated at the plane of a spectrophotometer to allow the collection of wavelengths to be sensed parallel while an unpredictable wavelength detector (VWD) is used as a sample should be injected various times, with shifting wavelength, to every peak are detected. In the case of PDA, when all the compound that absorbs within this range can be recognized in a single analysis and it is used a wavelength range can be programmed. HPLC offers a chief service in answering many difficulties posed by the pharmaceutical industry.

However, the restrictions of HPLC include the cost of columns, purity of solvents and a need for long time reproducibility due to the proprietary environment of column packing. Liquid Chromatography is combined with Mass Spectroscopy (LC-MS) is considered as one of the most essential techniques of the previous 20th century. It happens to the method of selection for analytical support carry in many levels of quality control and assurance inside the pharmaceutical industry.

2.2 Spectrophotometry

One more vital group of methods which finds an important place in the pharmacopoeia are spectrophotometric techniques is based on natural UV absorption and chemical reactions. The function of the wavelength of spectrophotometry is a quantitative method is depending on the basis of reflection or transmission properties of a material.

The precision method of the Spectroscopy is very helpfully and outstanding.

Thes UV- VIS Spectroscopy is used as particularly applied in the analysis of pharmaceutical dosage form has increased rapidly. The main advantages of these methods are low time, low cost and labor consumption.

Table no.1 HPLC methods of protease inhibitor

Sr no	Drug/ Formulation	Column.	Mobile Phase	Detector	Lamda max(λ)	Linearity/ Retation Time	Flow Rate	Reference
1	ATV	Ultrasphereoctyl, 4µm beads, 4.6mmx 15 cm	water, methanol, & ACN.(45:20:35)	UV Detector	260 nm	20-10,000 ng/mL 15 min.	1 mL/min.	(Cattaneo et al., 2008)
2	ATV	KromasilC18 150mmx3 mm, 5µm	acetonitrile & water (38:62v/v)	Waters 2487 UV detector	210 nm.	0.156-10µg/mL 18.5 min.	1 mL/min.	(Cateau et al., 2005)

3	ATV	Kromasil column C18 250 · 4.6 mmID	Ammonium acetate buffer & acetonitrile (45:55)	UV Detector	280 nm	250-750 µg mL 0.44 and 0.61 min	1.0 mL/ min	(Seshachalam et al., 2008)
4	ATV	Nucleosil 100-5 µm C18 AB		UV-diode array detection.	201 nm.	0.25-10.0µg/mL	1 mL/min	(Colombo et al., 2006)
5	ATV Tablet	C 18 column Phenomenix 250mm x 4.6 mmID 5µm	methanol & water (90:10)	SPD-20A Prominence UV–visible detector	249nm	10 - 90µg/mL 13 min	0.5mL/min.	(Dey et al., 2013)
6	TABLET CONTATV & COBICISTAT	Durashell C 18 column 250 X 4.6 mm	water, methanol and acetonitrile (30:20:50)	photodiode array detector.	272 nm.	19.07 to 29.30 mcg/mL 2.3 & 7.7 minutes	1 mL/min	(Devi and Kannappan, 2018)
7	ATV Capsule	X-terra RP- C8 (150 mm x 4.6 mm I.D; particle size 5 µm.	Sodium dihydrogen phosphate buffer (pH-4.5) & ACN (700:300)	Waters 2996 photodiode array	230 nm	2.0-10.0 µg/mL.	1.0 mL/min	(Chakraborty et al., 2010)K.
8	ATV &(INV, APV, SQV, NFV, RTV, and LPV, NVPN and EFV)	Zorbax C-18 150µ 4.6 mm, 3.5 mm	methanol: water.(50:50)	VWD-UV-detector	210 nm.	25 - 10,000 ng/mL	0.9mL/min	(Rezk et al., 2006)
9	ATV	Ascentis Express C8, 150mmx4.6mm, 2.7µm	water/ammonium acetate (0.025M) and CAN	photodiode array (PDA) detector	250nm	0.020µg/mL to 3.00µg/mL	1.0 mL/min.	(Chitturi et al., 2011)
10	ATV	C18 RP Column 250 mm x 4.6mm x 5 µm	Methanol and 5mM Tetra Butyl Ammonium Hydrogen Sulphate(50:50)	UV-Visible Detector	299nm	0.5-100 µg/mL 1.8 to 1.9 min	1.0 mL	
11	ATV	C18 RP Column, 250 mm x 4.6 mm	Buffer (triethylamine & pH adjusted to 3.0 with glacial acetic acid) and Acetronitrile in a ratio of 80 : 20(v/v)	UV detector	212 nm.	10-100 µg/mL 3.1min.	1.0 mL min-1.	(Nazia Hasan, Shyamala, JVC Sharma, 2015)
12	ATV bulk dosage form	C18 250 x 4.6 mm, 5 µ column	water: ACN(20:80 v/v)	DAD Detector	255 nm	10-80 µg/mL. 3.7 min	1mL/min	(Konidala et al., 2012)
13	ATV	Uptisphere HDO C , 18 3334.6 mm, 3	methanol 2.1 and CAN	MS-MS	250nm	1.00 to 1000 ng/mL	0.8 mL/min	(Schuster et al., 2003)

		mm						
14	ATV	XTerra RP18 150mm×4.6 mm, 3.5 µm column,	phosphate buffer (pH 6, 52.5 mM) & ACN (43:57v/v).	UV Detector	203 nm.	0.04 to 10 µg/mL 30 min	1.5 mL/min	(Loregian et al., 2006)
16	ATV &tipranavir	Inertsil ODS3 column 50mm x2.0mm i.d., particle size 5µm	Methanol-10mM ammonium acetate buffer pH 5.0 (35:65, v/v)	UV Detector	260 nm	0.05-10µg/mL 5.5 min	0.5 mL/min.	
17	ATV Bulk Dosage Form	Agilent TC C18 (2) 250 µ 4.6 mm, 5 m column	0.02 M ammonium dihydrogen phosphate buffer:acetonitrile: methanol (30:25:45 v/v)	UV Detector	288 nm.	550 mg/mL.	1mL/min	(Nanda et al., 2011)
18	ATV	Hypersil BDS C18 column 150mm x 4.6 mm, 5µ	buffer and acetonitrile in the ratio of 55:45% v/v	UV Detector	248 nm.	25-150 µg/mL. 2.11 min	1 mL/min	(Chandra et al., 2017).
19	ATV with amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, efavirenz, and nevirapine.	X-TERRA RP18 150 3 4.6 mm, particle size 5 µm	58% of water and 42% acetonitrile. KOH used to adjust pH to 11.7.	ultraviolet microarray spectrophotometer detector UV6000LP	240 nm	15.43 nm	1 mL/min.	(O. et al., 2005)
20	ATV	Phenomenex Gemini C18 4.6×150mm, 5µ.	Methanol: Water (90:10% v/v)	PDA Detector 996	249nm	150-450µg/mL and 37.5-112.5 µg/mL 2.11 min	1.0 mL/min	(Chandra et al., 2017)
21	TPV	Luna 5 µ C18 column 150mm×4.6mm i.d., Phenomenex,	buffer (pH 3.2) and CAN	UV detector	260 nm	(0.703–180 µg/mL) 14 min		(D'Avolio et al., 2007)
	TPV	C8 plus Satisfaction column 250mm×3 mm, 5 µm	sodium acetate buffer (pH 5):methanol:acetonitrile (35:30:35, v/v/v)	UV detector	260 nm.	2–80 mg/l.	0.5 mL/min.	(Giraud et al., 2006)
	TPV, APV, RTV, IDV, SQV, NFV, LPV, ATV, NVP, EFV	Nucleosil C18 AB column		UV– DAD.	201 nm	1.875–75µg/m,	1 mL/min	(Colombo et al., 2006)
22	APV	Aquasil C5 mm, 15032.1 mm	acetonitrile–water (55:45, v/v)	UV detector		1.6	0.3 mL/min.	(Pereira et al., 2002)

						min		
23	APV	Symmetry C column 10034.6 mm	ACN–sodium phosphate buffer (pH 6.8) (40:60, v/v)	Fluorescence Detector	270 nm		1.5 mL/min.	(Sparidans et al., 2000)
24	IDV	Zorbax SB-CN column 5 mm, 8034 mm	ACN in 10 m*M*orthophosphoric acid,	UV detector	210 nm	2 to 2000 ng/mL	1.2 mL/min.	(Wang et al., 2013)
25	IDV	Inertsil ODS-2 C column 5 mm particle size; 15034.6 mm	ACN–water (40:60, v/v) PBS pH 6	UV Detector	210 nm	6.7 and 8.0 min,	1 mL /min	(Burger et al., 1997)
26	IDV	Delta-pak C4 chromatography –mass spectroscopy (LC–MS). reversed-phase column, 150 mm33.9 mm (I.D.) 5 mm	35:65 (v/v) ACN and buffer	PDA detector	210 nm	2 to 2000 ng/mL	1 mL/min	(Jayewardene et al., 1998)
27	IDV			UV detector		9.5 min	1 mL/min	(Prasanna et al., 2009)
28	IDV, RTV, and LPV		ACN–10 m*M*potassium phosphate buffer (50:50, v/v)	UV detector	205 nm	50 to1000 mg/ l	1.0 mL/min.	(Ray et al., 2002)
29	IDV, NFV, RTV, and SQV	Keystone BetaBasic C4 column (250 3 3 mm i.d.)	ACN-50 mmol/L ammonium formate buffer, pH 4.1 (52:48)	UV detector	218 nm	4 to 2000 ng/mL	0.5mL/min.	(Remmel et al., 2000)
30	NFV mesylate	Kromasil-CN column 250mm×4.6 mm, 5µm	ACN and 25mM monobasic ammonium phosphate. (40:60, v/v)	UV detector	210 nm.	5–150µg/mL,	1.0 mL/min	(Jing et al., 2006)
31	IDV, NFV, SQV and RTV	ChrompackInertsil ODS-2 C18 column 5 mm particle size; 15034.6 mm	ACN–50 m*M*phosphate buffer, pH 5.63 (40:60, v/v).	UV Detector	215 nm	45–30 ng/mL	1 mL/min	(Hugen et al., 1999)
32	NFV	Waters Symmetry C *i*18 analytical column 5 mm, 25034.6 mm I.D	monobasic sodium phosphate buffer-acetonitrile (58:42, v/v)	UV Detector	220 nm	0.0300 mg/mL to 10 mg/ mL 8.2 and 9.9 min	1.0 mL/min	(Wu et al., 1997)
34	NFV Mesylate	C18 Hypersil BDS C18, 250 mm µ 4.6 mm, 5 mm	Sodium phosphate buffer, acetonitrile, and methanol in the ratio of 30:50:20.	UV detection	220 nm.	50 to1000 mg/ l	1.0 mL/min	(Seshachalam et al., 2007)
35	NFV Mesylate tablet	Inertsil ODS C18 250X4.6 mm, 5µ	acetonitrile and phosphate buffer pH 6	UV Detector	230 nm.	1-20 mcg/mL.	1.2 mL/min	

			(90:10 v/v)			6.68 min.		
36	SQV	VydacC18 monomeric column (250mm×4.6mm i.d.x5µm particle size	acetonitrile and potassium dihydrogen phosphate buffer (45:55, v/v).	Fluorescence detection	237 nm	0.005 to 1.000 µgmL−1	1 mL/min	(Pathak et al., 2007)
37	LPV and RTV Tablet	Agilent TC C18 (2) 250 µ 4.6 mm, 5 m	acetonitrile: 0.05 M phosphoric acid (55: 45, v/v)	DAD UV detector	240 nm.	848 mg/mL for lopinavir and 212 mg/mL for ritonavir. 4.35 min & 6.68 min.	1.2 mL/ min.	(Hiremath and Bhirud, 2015)
38	LPV	phenomenex-Luna C18column 250×4.6mm i.d, 5u	Acetonitrile and Triethylamine (0.5%) (67:33 % v/v)	Prominence Diode array detector	240 nm		1.2 mL/ min	(Ponnilavarasan et al., 2010)I.
39	RTV Capsule	Phenomenex C18 reversed phase column 5µm, 15 cm×4.6 mm	acetonitrile:water: methanol (53:40:7, v/v/v).	UV detector	210 nm.	4.0–124.0 µg/mL	1.5 mL/min	(Rossi et al., 2007)
41	RTV	Symmetry C184.6 x 100mm, 3.5 µm	Buffer: Acetonitrile (50:50)	UV detector	239 nm.	50-150µg/mL. 5.1 min.	1.0 mL/min.	
42	DRV& NVP, IDV, APV, SQV, M-8, NFV, ATV	Atlantis dC-18 3µ column (150mmx 2.1mm i.d.)	Buffer A (HPLC grade water + 0.05% formic acid) and Buffer B (HPLC grade acetonitrile + 0.05% formic acid).	UV detector	250nm		1 mL/min	(D'Avolio et al., 2007)
43	Fosamprenavir Calcium	ODS AQ column, 150µ 4.6 mm, 3.0 mm	0.05 M Potassium dihydrogen orthophosphate monohydrate (pH 6.8) buffer and Acetonitrile in the ratio 60:40 (v/v)	UV detector.	265 nm	80 to 120%	0.8 mL min	(Chilukuri et al., 2014)
44	APV, IDV, NFV, RTV and SQV	ZorbaxSB-C column (7534.6 mm I.D. / particle size 3.5mm;		diode array UV detector	210 nm	25 ng/mL to 25 mg/mL	1.5 mL/min.	(Van Heeswijk et al., 1998)
45	IDV, RTV and LPV	Phenomenex Luna phenyl hexyl column	acetonitrile–10m*M*potassium phosphate buffer	diode array UV detector.	205 nm	50 to1000 mg/ l and 100 to	1.0 mL/min.	(Ray et al., 2002)

		(25034.6 mm I.D., particle size 5 mm;	(50:50, v/v).			15000 mg/ l.		

Dario Cattaneoet al., reported a validated HPLC method of atazanavir in Human Blood Plasma. The chromatographic separation was achieved as Ultrasphereoctyl, 4µm beads, 4.6mm× 15 cm column using mobile phase composition of water, methanol, & CAN (45:20:35). The flow rate was fixed at 1 mL/min at ambient temperature. Quantification was obtained by UV detector at 260 nm. The run time was obtained at15 min. The linearity was found in the absorption range of 20-10,000 ng/mL. This process has been validated to be linear, accuracy rugged, specific, sensitive, precise, robust and fast.

Estelle Cateauandet al. reported as simple and easyHPLC method by UV detection asa monitor at 210 nm was developed and validated for the analysis ofatazanavir in human blood plasma. The analysis was achieved on a Kromasil C18 (150mmx3 mm, 5µm) column with water and ACN (62:38 v/v) as mobile phase is delivered to the isocratic system. The flow rate 1 mL/min as maintained and retention time of ATZ and IS were 18.5 and 22.0 min. The assay was linear over the concentration range of 0.156 to 10 µg/mL and the limit of quantification was 0.156µg/mL. The method was also validated with respect to recovery, precision, accuracy and specificity. This method is suitable for the therapeutic drug monitoring of atazanavir and can be easily reproduced with standard equipment of HPLC.

U. Seshachalam et al., was reported a validated HPLC method for stability-indicating assay for the atazanavir in the presence of its degradation products. By using Kromasil column C18 4.6 mm ID with mobile phase containing ammonium acetate buffer & acetonitrile (45:55). Flow rate was maintained as 1.0 mL/ min with UV detection at 280 nm, 0.44 and 0.61 min retention time was obtained and the method was found to be linear at range 250–750 µg mL, The drug substances was found almost stable in peroxide degradation leaving two minute impurities in the levels of 0.1% at relative RT of 0.44 and 0.61, when treated with 30% peroxide solution.

S. Colombo et al., was reported the simultaneous assay was described for APV, RTV, IDV, SQV, NFV and EFV is proposed here for the simultaneous analysis in human plasma, by HPLC using Nucleosil 100–5 µm C18 column with 201 nm at UV-diode array detection. The methanol and phosphate buffer as mobile phase constituted of using a gradient elution program with retention times for clozapine and ATV are 8.9 and 24.4 min and RT was obtained for the simultaneous assay of APV, RTV,IDV, SQV, NFV and EFV in blood plasma, gives a radiant separation for ATV and is eluted in between SQV and RTV (retention time: 22.3 and 27.3 min, respectively) And flow rate maintained up to 1 mL/min. The calibration curve was linear up to 10 µg/mL, with a lower limit of quantification of 0.2 µg/mL. The Bio-analytical method has been successfully validated and is currently applied to the monitoring of ATV in HIV patients.

Suddhasattya Dey et al., reported RP- HPLC method for stability-indicating method, validated and developed for the determination of ATV in the form of tabletbyusing C 18 column Phenomenix (250mm × 4.6 mm, 5µm particle size) with a mixture of methanol and water as mobile phase pH was adjusted to 3.55 with Acetic acid to get the proportion of 90:10 v/v. the flow rate was set as0.5mL/min and analyte monitored at 249 nm using UV–visible detector, the RT of atazanavir sulfate was achieved to be 8.323. The Linearity was observed in the range of10- 90µg/mL (Coefficient of determination R^2= 0.999). Atazanavir sulfate was subjected to stress conditions of acidic, alkaline, oxidation, photolysis and thermal degradation. This method has been validated and shown to be specific, sensitive, precise, linear, accurate, rugged, robust and The method was validated as per ICH guidelines.

K. Devi et al., was developed and validated RP-HPLC method for simultaneous study of ATV and Cobicistat (COB) in a tablet dosage form. This method was optimized with Durashell C18 column (250 X 4.6 mm, particle size- 5µm) with 20:30:50 of methanol, water, and acetonitrile respectively as mobile phase and pH adjusted to 5.5 with triethylamine. The flow rate was set at 1 mL/min and then UV detector was used for chromatographic detection at 272 nm, the ATV and COB were eluted with retention times of 2.3 and 7.7 minutes respectively. The concentration was found in the range of 38 to 58 mcg/mL for ATV and COB for 19 to 29 mcg/mL. The LOQ was found 10.0 and 4.28 mcg/mL for COB and ATV respectively. LOD was found in the range between 3.3 and 1.41 mcg/mL. The forced degradation study was performed by using acid, base, oxidation with hydrogen peroxide, increase temperature and photolytic environment

exposed the stability of solution for the first and third day. This method was validated as per the ICH norms. This method has been simple, rapid. specific, accurate, precise, dependable and reproducible.

K. Parameswara Rao et al., was reported a validated and developed RP-HPLC method for the determination of ATV in API (active pharmaceutical ingredient) and formulations. The mobile phase composition of buffer (pH-4.5) and acetonitrile in the ratio of 75:25 v/v with X-terra C_8 column (150 mm x 4.6 mm I.D; PS- 5 µm). at 1.0 mL/min as a flow rate. The effluents were monitored at 230 nm on the PDA detector. The retention time was 5min, the linearity was obtained 2.0-10.0 µg/mL. The method has been developed thought to be easy, accurate and selective and is helpful for the assay of Atazanavir in dosage forms and can be newly employed in the quality control analysis of bulk manufacturing and formulations units.

Naser L. Rezket al., reported a complete validation and development of an analytical method to facilitate that combines ATV with 6 HIV-protease inhibitors (IDV, APV, SQV, NFV, RTV, and LPV) and 2 non-nucleoside reverse transcriptase inhibitors (NVP and EFV). Using 200 mL of plasma and a simple liquid–liquid extraction method. The column was used as Zorbax C-18 (150µ4.6 mm, 3.5 mm),the analyte was monitored at 210 nm on UV detector. This method linearity range was found to be25 to 10,000 ng/mL and is accuracy was found 90.4% to 110.5% and precision within a day and between days ranged from 2.3% to 8.3% and flow rate was maintained at 0.9 mL/min.

Sreenivasa Rao Chitturi et al., reported a new and simple reverse-phase- high-performance liquid chromatography method for find theimpurities and degradation products have been developed and validate of atazanavir sulfate (ATV) drug material. Chromatographic partition was performed in the stationary phase of Ascentis Express C8 (150mm×4.6mm, 2.7µm) column under the thermostatic at 30ºC, with mobile phase containing PDP(potassium dihydrogen phosphate pH 3.5, 0.02M) and ACN, at a flow rate was maintained at 1.0 mL/min. Quantification at 250nm on photodiode array (PDA) detector was used for forced degradation, The linearity of detection response to different concentrations was evaluated for ATV and all the unidentified components in ranging from 0.020µg/mL to 3.00µg/mL is equivalent to 0.002 (% w/w) to 0.300 (% w/w) with respect to sample absorption. The reported method has been developed and validated with deference to LOD and LOQ, accuracy, linearity, precision and robustness and can be implemented for habitual quality control investigation and stability test of ATV.

Upender Rao Eslawathet al., was reported a method of development for an active constituent of ATV and its dosage forms. ATV was subjected to a range of force degradation conditions using acid, alkali, oxidative and photolytic degradation. From the forced degradation studies, it was found that Atazanavir was stable during acid degradation when compared to alkali, hydrogen peroxide and through sunlight. The retention time of Atazanavir was altered from 1.8 to 1.9 minutes. The C18 RP Column (250 mm x 4.6mm x 5 µm) with mobile phase containing Methanol and 5mM Tetra Butyl Ammonium Hydrogen Sulphate 50:50 v/v and then UV detector was used at 299nm and the linearity was found within 0.5-100 µg/mL for Atazanavir. From the results exposed, it was found that % RSD is less than 2%; which indicates that the planned method has superior reproducibility.

Nazia Hasan et al., was reported a method for the resolve of Atazanavir in the solid dosage form. The evaluation was carried out on a Waters ODS (C18) RP Column, 250 mm x 4.6 mm column with mobile phase consisting of a mixture of Buffer and ACN in a ratio of 80: 20 v/v, with pH adjusted to 3.0 by glacial acetic acid. UV detector is used at 212 nm. The method has been validated and developed for precision, specificity, linearity, accuracy, LOD and LOQ and robustness as per ICH norms. The retention time was achieved at 3.1min and the flow rate was set 1.0 mLmin-1. The linearity in the range of 10-100 µg/mL, for Atazanavir (API) with correlation coefficient value(R^2) of 0.998. The LOD and LOQ values were established to be 0.05 g/mL and 0.15 g/mL. The developed and validated method has been successfully used for representative the constancy of the drug and quantitative analysis of commercially accessible dosage form.

Sathish Kumar Konidala et al., reported as the developed and validated RP-HPLC method for the evaluation ATV in capsule on stationary phase as Agilent C18 (250 x 4.6 mm, 5 µ) column is used and then mobile phase it contained water and ACN (20:80 v/v) pH adjusted to 3. The Flow rate was set at 1 mL/min at ambient temperature. Quantification was found at 255 nm by UV detection. The 3.7 min retention time was found. The detector response was linear in the concentration range of 10 – 80 µg/mL. This method was validated and developed to be specific, sensitive, precise, linear, specific, sensitive, accurate, rugged, strong and rapid.

A. Schuster et al., was developed the precise, selective LC-MS assay for the determination of the ATV in blood plasma samples. The column used here a Uptisphere HDOC, 4.6 mm, 3 mm column with methanol and ACN as a mobile phase. The linearity range from 1.00 to 1000 ng/mL with a LOQ of 1.00 ng/ mL. The inter- and intra-day coefficients of deviation for the evaluate were 4% and 99–102% accuracy as found. Quantification was established with MS-MS absorption at 250 nm.

Arianna Loregianet al., was developed and validated a reported RP-HPLC method for the analysis of ATV in human blood plasma samples. The isocratic stationary phase on an XTerra RP18 (150mm×4.6 mm, 3.5 µm) column is used with 203 nm of UV detection. The mobile phase contained phosphate buffer (pH 6) and ACN (43:57, v/v). the retention time was found to be 30min and the flow rate was set at1.5 mL/min. The linearity ranges from 0.04 to 10 µg/mL with a LOQ of0.04 µg/mL. The method was precise, with both intra-day and inter-day coefficients of difference 3.0%, and correct This method has been providing a simple, responsive, precise and repeatability assay for the therapeutic drug monitoring of atazanavir in clinical everyday of laboratories with standard equipment.

K.M.L. Crommentuyn et al reported as developed and validated the method by using liquid chromatography attached with electrospray tandem mass spectrometry (LC-MS/MS), for the quantification of the new protease inhibitors ATV and TPV. Chromatographic separation was performed in an Inertsil ODS3 column (50mm × 2.0mm i.d., particle size 5µm), with methanol and ammonium acetate buffer pH adjusted to 5.0 (35:65, v/v) mobile phase. Flow rate as maintained at 1mL/min and analytical run time was 5.5 min. The assay was linearity rangeabout a 0.05-10µg/mL for ATV and 0.1-75µg/mL for TPV. Saquinavir was used as an internal standard. Accuracyinside±7.3 and ±7.2% for ATV and TPV, The developed technique can easily be mutual with a before developed LC-MS/MS assay for the quantification of protease inhibitors.

Charushila H. Bhirudet al., as reported easy, precise and validated the stability indicating RP-HPLC technique for the estimation of ATV in a capsule.The chromatographic separation was achieved in Agilent TC C18 250µ 4.6 mm, column using mobile phase it contained 0.02M PBS: acetonitrile: methanol (30:25:45 v/v) and pH attuned at 2.5 among ortho-phosphoric acid. The flow rate was setat 1mL/min at ambient temperature. Quantification was obtained at 288 nm on ultraviolet detection. The retention time achieved for Atazanavir sulfate at 3.0 min. the result wasfound to be linearity in the range of 50 mg/mL. This method was concluded the validated and revealed to be definite, sensitive, precise, linear, accurate, rugged, fast and robust.

K. Poorna Chandra Rao et al., reported as developed for the estimation of atazanavir sulfate in bulk and pharmaceutical dosage form. A reverse was used as Hypersil BDS C18 column (150mm X 4.6mmm 5µ) with mobile phase containing buffer and ACN in the ratio of 55:45% V/V was used, the flow rate was maintained 1mL/min and the UV detector was monitored at 248 nm. The retention time was obtained 2.11min, the linearity was found to be in the range of 25-150 µg/mL.

Antonio D'Avolioet al., as reported developed and validated. Quinoxaline,as IS, as added to 50 µl of blood plasma before a liquid-liquid extraction by 600 µl of protein precipitate solution. Chromatographic severance was achieved on Luna 5 µ C18 column (150mm×4.6mm i.d., Phenomenex) with gradient mobile phase it contained potassium phosphate buffer (pH 3.2) and acetonitrile and Detection was performed by a UV detector at 260 nm. LOD and LOQ were 0.035 µg/ml and 0.090 µg/mL respectively. Mean recovery was 87.1%±2.4%. thelinearity range up to 180µg/mL. Concentration range when optimized (0.703–180 µg/mL).

Arlene S. Pereira et al., as reported an HPLC-MS-MS method to the determination of APV in human seminal plasma has been developed and validated. The chromatographic separation was Aquasil (C 5 mm, 15032.1 mm) column used with a mobile phase consisting of acetonitrile-water (55:45, v/v). the MS detection was used and the flow rate is 0.3 mL/min. This method is accurate less than 7.2% and precise less than4.2% over the linearity range of 30-4000 ng/ mL. and retention time was 1.6min.

Ling Zhong et al.,as reported a fast, susceptible and robust method for the quantitative determination of IDV in human cerebrospinal fluid (CSF) and blood plasma is determined. while the chromatographic separation was Zorbax SB-CN column (5 mm, 8034 mm) with ACN in 10 mM ortho-phosphoric acid as mobile phase.The UV detection was set at 210 nm. The standard curve was linearity over the concentration range of 2 to 2000 ng/mL in CSF and 5 to 2000 ng/mL in plasma, and the flow rate was maintained 1.2 mL/min. The method was utilized to support clinical pharmacokinetic studies.

D.M. Burger et al., A sensitive HPLC method has been developed to determine the concentration of the IDV in human blood plasma. Chromatographic separation was passed out on anInertsil ODS-2 C column (5 mm particle size; 15034.6 mm). with mobile phase is consisted acetonitrile-water (40:60, v/v). Ultraviolet detection at 210 nm was used. The flow rate was maintained at1mL/min and retention time was 6.7 and 8.0 min, the detection limit was 12.5 ng/ mL, This method it was validated with observe to specificity, detection limit, lower and upper LOQ, recovery, accuracy, and intra- and inter-day assay precision. Stability tests under various conditions were performed.

Qiufang Jing et al. reported as isocratic, precise and stability representative HPLC method of determination of NFV both as an API and in formulations, it has been developed and validated. Kromasil-CN column (250mm×4.6 mm,5µm) with mobile phase consisted acetonitrile and 25mM monobasic ammonium phosphate. (40:60, v/v) was used for chromatographic separation, a flow rate delivered at 1.0 mL/min with UV detection at 210 nm. The linear concentration was found in the range of 5–150µg/mL. The developed method was validated in conditions of selectivity, linearity, the limit of quantification, precision, accuracy and solution stability.

S. Colombo et al., as a report for the resolve of the narrative non-peptidic HIV protease inhibitor tipranavir (TPV) in human plasma, by off-line solid-phase extraction (SPE), follow via HPLC. Nucleosil 100-5µm C18 AB column by means of gradient elution program constitute methanol and phosphate buffer solution adjusted to pH 5. at UV/DAD detection at 201 nm. The flow rate was maintained at 1mL/min, clozapine and TPV retention times of 8.3 and 32.2 min were obtained and Over the concentration range from 1.875 to 75µg/mL for TPV by way of a LOQ of 0.125 µg/mL. The signify absolute recovery of TPV is 77.1±4.0%. The methods have been validated and area t present useful to the monitor of TPV plasma levels in HIV patients.

Shriram M. Pathak et al., as reported the simple development and validation for the study of SQV pharmacokinetics in rat plasma. For the chromatographic analysis was performed in Vydac C18 monomeric (250mm×4.6mm i.d.×5µm particle size) column with using a mobile phase it contained acetonitrile and potassium dihydrogen phosphate buffer (45:55v/v). The Fluorescence detection was performed at 237 nm (excitation) and 380 nm (emission). The validity of the method was studied and the method was found to be precise and accurate with a linearity range from 0.005 to 1.000 µgmL^{-1} (r > 0.9980). The limit of detection (LOD) was found to be 0.001µgmL^{-1}. The developed method was applied successfully to monitor the pharmacokinetic profile following oral administration of SQV to rats.

Shivan and N. Hire et al. reported as A simple, accurate and fast constancy indicating High-performance liquid chromatography method for instantaneous determination of LPV and RTV in combined dosage forms. The chromatographic process has been developed for the quantitative determination of two antiviral drugs. LPV and RTV on Agilent C18 (2) 250µ 4.6 mm, 5 m column with using mobile phase consisted of 0.05 phosphoric acid: acetonitrile (45: 55,v/v) a flow rate was set as1.2 mL/min. absorption was achieved with UV detection at 240 nm and retention time was found for LPV at 6.68 min and for RTV at 4.35min. the method to be linearity in the concentration range of848 µg/mL for LPV and 212 µg/mL for RTV. This method was validated and evaluate to be there specific, approachable, precise, linear, truthful, rugged, robust and fast it concludes.

Rochelle C. Rossi et al., reported as to validated and developed a dissolution method for RTV flexible gelatin capsules (Norvir) based on in vivo records. The dissolution test was validated by using an HPLC. For this formulation, the best dissolution environment was maintained by using the paddle, 900 mL of the medium it contained water with 0.7% (w/v) of SLS at a 25 rpm. The chromatographic separation was obtained in Phenomenex C18 RP-column (5µm, 15 cm×4.6 mm) with mobile phase containing acetonitrile: water: methanol (53:40:7, v/v/v). The UV detector was used as 210 nm and the flow rate was set at 1.5 mL/min, the linear concentrations were found to be in the range of 4.0-124.0 µg/mL, In the HPLC method a relative RSD for inter-day precise was 1.4% and for intra-day precise was 1.6%. Accurate has been from 98.5% -101.6% above the concentration range mandatory for the dissolution analysis (4.0–124.0 µg/mL).

K. Chiranjeevi et al., The reported method as easy, precise, explicit and accurate RP-HPLC technique has been developed for the determination of RTV in bulk and pharmaceutical dosage forms. The chromatographic separation was performed on Symmetric C18, 100mm X 4.6, 3.5µ column is used with mobile phase composition of Buffer: Acetonitrile (50:50) as the mobile phase at a flow rate was set1.0 mL/min. The analysis was monitored by

using a UV detector at 239 nm. at a flow rate was set 1.0 mL/min. Linearity was observed in the concentration range of 50-150µg/mL. The retention time found for RTV at 5.1 min. The method has validated as per ICH norm with reverence to specificity, linearity, accuracy, precision, and robustness.

Rolf W. Sparidanset al., A responsive bio-analytical method of determination for amprenavir, based on RP-HPLC and fluorescence detection is reported. The chromatographic analysis was achieved as Symmetry C column (10034.6 mm) with mobile phase it contained ACN and sodium phosphate buffer (pH 6.8) (40:60, v/v) The sample is detected by fluorescence detector at 270 and 340 nm for excitation and release, The method was validated in linearity range of 1-1000 ng/mL for a 50-mL of blood plasma and in the 0.5-50 ng/Ml linear for a 100-mLamount of CSF and semen. The LOQ was 0.5 ng/mL in CSF (cerebra-spinal fluid) and 1 ng/mL in equally plasma and semen. Precision and accuracy equally assemble the current requirements for a bio-analytical method and are,15% in the validated ranges. The method was effectively used to obtain a concentration-time curve of APV in plasma.

Ellen Y. Wu et al., The announced analytical technique was developed to determine the pharmacokinetic parameters of the free base, The investigation was by means of UV detection at 220 nm with Waters Symmetry C I 18 analytical column (5 mm, 25034.6 mm I.D.). The mobile phase comprising monobasic sodium phosphate cushion acetonitrile (58:42, v/v), The connoted mean from 92.4 to 83.0% for NFV and was 95.7% for the IS. The technique was linearity over absorption of 0.0300 mg/mL to 10 mg/mL, with a base test dimension of 0.0500 mg/mL for NFV. The technique was approved under Good Laboratory Practice (GLP) conditions for specificity, inter and intra-assay accuracy and precision, total recovery and stability.

Antonio D'Avolioet al., reported as (HPLC– MS) was developed and validate, for the determination of plasma deliberation of the novel protease inhibitors DRV and other 11 anti-HIV agents (RTV, APV, ATV, LPV, SQV, IDV, NFV and its metabolite M-8, NFV, EFV and TPV). chromatographic partition of drug and Internal Standard was used as quinoxaline was accomplished with a gradient mobile phase it consisted ACN and water with formic acid 0.05% on an Atlantis DC-18 3µ column (150mm×2.1mm i.d.) and run time at 25 min. The calibration curve was optimized according to the range of drug concentrations in volunteer, and linearity (correlation coefficient *r*2) was superior to 0.998 for all compounds. Represent intra- and inter-day precision for all mix compounds were 8.4 and 8.3%, respectively, and the mean accuracy was 3.9%. Extracted improvement ranged within 93 and 105% for all drugs evaluations. This new HPLC-MS method allows a precise, responsive and reliable resolve of DRV and 11 other antiretrovirals.

U. Seshachalam et al., as reported trouble-free, isocratic RP-HPLC method was developed to find the impurities from its NFV. This method chromatographic separation was achieved byC18 (Hypersil BDS C18, 250 mm µ4.6 mm, 5 mm) column with The mobile phase it contained a mixture of 50mM of sodium phosphate buffer, methanol and acetonitrile in the ratio of 30:20:50. The method was validated above the range of LOQ to 120% of impurity requirement limit and LOQ to 150% of compound concentration for assay. The flow rate set at 1.0 mL/min with ultraviolet detector was used for absorption monitored at 220 nm. The has been method and validated for linearity, range, precision, accuracy, and specificity.

Emmanuelle Giraud et al., reported an as an assay method for the determination of TPV, An IS for-prazepam was added. The chromatographic separation was performed by C8 plus column (250mmx3 mm, 5µm) with The flow set at of 0.5 mL/min with mobile phase it contained acetate buffer with pH 5 :methanol:acetonitrile (35:30:35, v/v/v). theUVdetection was used at 260 nm wavelength. The method was linearity was validated above a linearity range of 2-80 mg/l. The indicate precise and accuracy of the methodin that order, 10.5 and 9.1%. The mean recovery was 70.8%.

Ponnilavarasan, A. Rajasekaran, et al., reportedas an uncomplicated RP-HPLC process was developed and validated for the determination of anti-HIV drugs Lopinavir and Ritonavir. Chromatography was carried out by dual gradient system phenomenex-Luna C18 column(250×4.6mm i.d, 5u) using Ambroxol as the internal standard. With mobile phase it contained Acetonitrile and Triethylamine (0.5%) (67:33 % v/v).the photoDiode array detector was used as 240nm with flow rate at 1.2mL/min. The calibration curve for each compound in the preferred concentration range (r2 > 0.999) was established to be linear. The improvement values were obtained to be 99.9 and 100.24% and RSD was greater than 2% for Lopinavir and Ritonavir correspondingly. The predictable method is greatly responsive, specific and correct, which was obvious from the LOD value of 30 ηg/mL for LPV and 25 ηg/mL for RTV, therefore,

the nearby method practically for the determination of active pharmaceutical component content in the mutual formulations of LPV and RTV.

K. Vanitha Prakash et al., reported as an RP-HPLC method is established for the resolve of NFV in tablet formulation. Chromatography was carried on Inertsil ODS C18 (250X4.6 mm, 5µ) using a combination of acetonitrile and phosphate buffer pH 6 (90:10 v/v) at a flow rate maintained of 1.2 mL/min with UV detection at 230 nm. The run time was found 6.68 min. The detector response was linearity in the range of 1-20 mcg/mL. The LOD and LOQ were 1.0 and 10.0 mcg/ mL correspondingly. The percentage examine of NFV was 99.77 %. The method was validated by Examine its sensitivity, accuracy, and precision. The anticipated method is simple, rapid, accurate and precise and therefore can be useful for everyday quality control of Nelfinavir Mesylate in mass and tablet dosage form.

Anura L. Jayewardene et al., reported as HPLC assay was urbanized and validated for the estimation of indinavir in human plasma. IDV and the internal standard were inaccessible from the plasma by ether withdrawal. Chromatography was used Delta-pak C4chromatography-mass spectroscopy (LC-MS). reversed-phase column, 150 mm33.9 mm (I.D.) 5 mm with eluted isocratically with a mobile phase consisting of 35:65 (v/v) of acetonitrile and buffer. A wavelength of 210 nm was found to be PDA detection. The calibration range of this assay was from 10 to 5000 ng/mL. This HPLC method was established to be a simple and reproducible technique for monitoring IDV levels in human plasma obtained through clinical trials of the drug.

John Ray et al., reported as an isocratic RP-HPLC technique UV detector is used for λmax at 205 nm was validated for the resolve of indinavir, ritonavir, and lopinavir (ABT 378) in human plasma. superior chromatographic separation was obtained by using a Phenomenex Luna phenyl-hexyl column (25034.6 mm I.D., particle size 5 mm) and a mobile phase it contained ACN -PBS (50:50, v/v). The calibration for IDV was linearity above the range of 50 to1000 mg/ l while the RTV and LPV of linearity above the range of 100 to 15000 mg/ l. The lower LOQ for IDV, RTV and LPV were 50, 100 and 100 mg/ l, in that order by using 500 mL of human blood plasma. The validation data showed that the assay is responsive, precise and reproducible for evaluation of indinavir, ritonavir (RTV) and lopinavir (LPV).

Mohanreddy Chilukuri et al., reported as A new stability-indicating RP-HPLC method was developed for quantitative estimation of Fosamprenavir Calcium, Chromatographic separation was achieved using a YMC Pack ODSAQ (150 mm 34.6 mm3 3.0 mm) HPLC column in isocratic mode employing 0.05 M Potassium dihydrogen orthophosphate monohydrate (pH 6.8) buffer and Acetonitrile in the ratio 60:40 (v/v) with a flow rate of 0.8mL/ min. UV Detector λmax was monitored at 265 nm. The HPLC method was validated as for each ICH norm with reverence to specificity, accuracy, linearity, precision, and strength. Regression study showed a correlation coefficient value greater than 0.999.

S.G. Khanageet al., Aeasy, precise and cheap spectrophotometric method was developed for the estimation of Atazanavir sulfate in bulk and formulations. With solvent using Distilled Water. The quantitative estimation of the drug was agreed on the first order derivative technique. Atazanavir sulfate shows λmax at 254.0 nm, The drugs follows Beer-Lambert's law in the linearity range of 10-50µg mL with a correlation coefficient of 0.9986. Results of the analysis were validated statistically and found to be satisfactory.

Table no.2 UV methods of Protease inhibitor

Sr	Compound	Method	Solvent	Lamda Max	Linearity/ LOD	Reference
1	ATV Bulk	Sensitive extraction spectrophotometric methods of determination	methanol	480nm	20-50µg/mL	
2	ATV and RTV in tablets	Simultaneous	Methanol	249.5 nm and 238.5 nm	10-50 µg/mL and 10-50 µg/mL	(Nanda et al., 2011)
3	ATV and RTV in tablet	simultaneous	Methanol	280.01 nm and 286.12 nm	15-75 µg/mL and 5-25 µg/mL	(Patel et al., 2010)
4	ATV	STABILITY INDICATING ASSAY	Methanol	249.6 nm	2-12 µg/mL	(B et al., 2017)

		METHOD				
5	ATV	first order derivative method	D W	254.0 nm	10-50µg mL	(Khanage et al., 2010)
6	ATV and RTV in combined tablet	simultaneous determination	*methanol*	*246.97-252.03 nm & 240.78-244.16 nm*	15-75 µg/mL and 5-25 µg/mL	
7	Lopinavir	methanol	Acetonitrile	220 nm,	5 to 35 µg/mL.	(Thakkar and Patel, 2010)
8	Ritonavir in Bulk	Development, Validation and Stability study	methanol.	239nm	4 - 20 µg/mL	(Behera et al., 2011)

K. Parameswararao et al., was developed and validated a simple and responsive pulling out spectrophotometric methods for the determination of ATV in bulk and formulations. For the analysis solvent was used as methanol and the λ max of the drug was obtained to be 480 nm. Linearity was observed in the range of 2.5 -12.5 µg/mL. The planned methods were applied effectively for the estimation of atazanavir in marketable tablets dosage forms and no important interfering was observed from the excipients usually used as pharmaceutical aids with the analytical procedure.

R. K. Nanda et al., was developed two uncomplicated, sensitive, fast spectrophotometric methods for concurrent determination of ATV and RTV in tablets with solvent using methanol. Solve the simultaneous equations on the basis of the dimension of absorbance at two wavelengths 249.5 nm and 238.5 nm λ max of ATV and RTV, correspondingly. Next method analysis on the basis of area under the curve and the absorption maxima chosen for analysis at 254-244 nm for Atazanavir Sulfate and 243-233 nm for Ritonavir. Beer's lamberts law was obeyed in the linearity concentration of 10-50 µg/mL and 10-50 µg/mL for ATV and RTV, Statistical analysis prove that the methods were accurate, and reproducible linearity range, sensitivity, precision, and simple method involve for analysis of ATV and RTV in tablets. The methods were validated as per ICH guidelines.

Disha A. Patel et al., was reported a sensitive, simple and precise method development for simultaneous estimation of ATV and RTV in the tablet dosage form. The present work was carried out on Shimadzu Ultraviolet (UV)-1700 double beam spectrophotometer with solvent using methanol. the absorption maxima for ATV and RTV were found to be 279 and 240 nm, The drugs chase Beers-Lambert's law in the linearity is 30-90 and 10-30 µg/mL for ATV and RTV, correspondingly. The percentage revival was obtained to be 100-100.33% and 100-101.5% for ATV and RTV, respectively. The process was validated for different parameters as per the ICH norms.

Shinde Yogesh Bet al. was established and developed a noncomplicated, susceptible, rugged, cheap, effectual UV-VIS method for the determination of ATV in bulk and Tablet method. The solvent for used was methanol and the λ max of the drug was found to be 249.6 nm. Linearitywas obtained in the range of 2-12 µg/mL with a regression coefficient of 0.999. The process was then validated for dissimilar as per the ICH.

HetalP. Thakkar et al., as reported the development and validation of a first-derivative ultraviolet spectrophotometric technique for the determination of LPV in formulation and API (active pharmaceutical ingredient). the first-derivative spectrum showed at 220 nm, The solvent it contained acetonitrile. The linearity was found in the range of 5 to 35 µg/mL. The method was established to enclose a high level of accuracy and precision, together with inter- and intra-day. The LOQ and LOD were obtained to be 2.558 and 0.844 µg/mL, respectively.

Anindita Behera et al.,reported as to developed and validated are responsive, fast, simple and precise methods are developed for the determination of RTV in bulk and tablet forms. Stability analysis of RTV is done in UV-Visible Spectrophotometer under diverse stress conditions suggested by ICH guidelines. The λmax is found to be 239nm in methanol as solvent. the linearity is obtained involving 4 - 20 µg/mL with a coefficient of relationship value 0.9981. the range for the area under the curve (AUC)preferred is 237-242nm. The linearity is obtained between 4 -20 µg/mL with a coefficient of correlation value 0.9992. The developed methods were validated and

optimized to be easy, rapid, accurate and cheap. The degradation study in a tablet dosage form can be used as a stability indicating method.

CONCLUSION:

The proposed review of analytical methods such as HPLC and UV Spectrometry methods and its validation. In this review chemistry of various drugs are discussed. The pharmacokinetic behavior of protease inhibitors is discussed as it relates with in the method development. The solubility in a various organic solvent is crucial in method development, pH and pKa value are also plays a role in method development which is important for the selective separation process. The optimized method is validated with validation parameters according to ICH guidelines. The stability indication evaluation is done with the acidic, basic and neutral environment. Oxidation and photolytic stability evaluation performed for the stability behavior of drugs. All the parameters were performed in accordance with ICH guidelines.

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