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Review Article

Overview of Forced Degradation Analysis for FDA Approved Antiretroviral agents: A Review

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ABSTRACT

Forced degradation and its analysis play a crucial role in the development and production of pharmaceutical products at various stages. Such studies include the assessment of appropriate drug candidates, product development, as well as comparability studies, clarification of probable intermediates and identification of by-products, and the development of stability-indicating technologies. The stability of drugs can be analyzed with Forced degradation study, which gives perspective knowledge of the molecule's stability as well as the degradant that is produced during the drug's shelf life. In this review on stability-indicating methods for FDA approved antiretroviral drugs from (1987-2021) such as Zidovudine, Lamivudine, Nevirapine, Ritonavir, Abacavir, Efavirenz, Tenofovir, Atazanavir, Emtricitabine, Enfuvirtide, Fosamprenavir, Tipranavir, Darunavir, Maraviroc, Raltegravir Etravirine, Nevirapine, Rilpivirine, Dolutegravir, Cobistat, Doravirine, Fostemsavir, and Cabotegravir is mentioned and analytical tools like HPLC, LC-MS/MS, UPLC their different parameters for the chromatographic condition are mentioned. No such guideline is available that mentions the pH for hydrolysis, the temperature at which thermal deterioration occurs, and the concentration of oxidation agent for oxidative degradation. The regulatory necessities for the shortcomings of the above-mentioned methods were highlighted. As a result, this study addresses recent approaches for forced degradation by presenting techniques for accessing studies on degradation mechanisms, this review helps researchers to get information on stability-indicating methods for FDA Approved antiretroviral agents for their development and validation of stability indicating methods and the characterization of degradation products produced during degradation studies.

Keywords: Stability indicating methods, Anti-retroviral, Chromatographic technique, HPLC, LC-MS/MS, UPLC.

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INTRODUCTION

The chemical stability of active pharmaceutical ingredients plays an important and diverse role from the process of selection of raw materials, excipients and formulation till the shelf life of drug or formulated products. FDA and ICH standards demand stability testing record to establish how the purity of raw materials and formulated drug product alteration throughout time as a result of a variety of environmental factors.[1-4]

The stability of molecules provides assistance in the choice of proper manufacturing and packaging conditions, including the provision of suitable warehousing conditions till expiration date. Which are all required at the period of governing documentation in various stages of IND and NDA submission. Forced debasement is a process wherein drug formulation and raw materials are degraded further under stressful condition than accelerated conditions, yielding degradants that may be assessed to determine the molecule's stability. According to the ICH recommendation, stress testing is used to identify potential degradation products, which aids in determining the molecule's inherent stability and developing degradation routes, in addition to validate the stability-indicating methodologies used.[1] HIV Virus is a member of the retrovirus family which belongs to the subgroup of lentivirus, HIV affects and destroys the immune system of the body leads to Acquired Immune Deficiency Syndrome (AIDS). To be diagnosed with AIDS CD4 Cells counts less than 200 cells/mm3. The total count of People existing with HIV worldwide in 2020 is 37.7 million, where 6,80,000 People died of HIV in 2020.[2] As one of the most essential enzymes for converting single-stranded RNAs into double-stranded DNAs, HIV-1 transcriptase (RT) is critical for HIV replication control and a prime target for antiviral research.[3] The fundamental goal of the Stability indicating method is to note the outcome throughout the period of degradation study of drugs, in order to secure the drug's quality, safety, and efficacy.[4] A stress testing study is known as forced degradation study. It is the behavior of introducing the drug into accelerated chemical and environmental conditions, for the detection of product breakdown level and its preliminary degradation kinetics, which can be identified and characterized using various analytical techniques.[5] Stability testing of API and drug products are discussed by ICH Q1A(R2) Guidelines 2003 for the determination of the storage conditions, maximum expiring dating period, and appropriate packaging conditions needed for the protection of product during its transportation and storage. The recommended conditions according to ICH Q1A(R2) needed for forced degradation study are discussed in Table 1. And different stress conditions applied in different types of formulations and drug substances are illustrated in Figure 1.[6]

The motive of the stability-indicating method to acquire complete knowledge about the behaviour of drug products or drug substances by the impact of various environmental and chemical factors so that it can remain the same until its shelf-life. Many Regulatory agencies and authorities like FDA (Food and Drug Administration), International Council on Harmonization recommended the relevance of impurities that is present beyond or acceptable limits. Contaminants present in excess of the acceptable limits should be reported, according to the International Conference on Harmonization (ICH) and the Food and

Table 1: Conditions applied for purposing forced degradation study. Degradation type

	Investigational Environments	circumstances of storage	Sampling frequency
Hydrolysis	Control API (acid or base)	40°C,60°C	1,3,5
	HCL 0.1M,	40°C,60°C	1,3,5
	NaOH 0.1M,	40°C,60°C	1,3,5
	(No-API) Acid Control,	40°C,60°C	1,3,5
	(No-API) Base Control,	40°C,60°C	1,3,5
	Ph:2,4,6,8	40°C,60°C	1,3,5
Oxidation	3% H_2O_2 Peroxide Control	25°C,60°C	1,3,5
	Azobisisobutyronitrile (AIBN)	25°C,60°C	1,3,5
		40°C,60°C	1,3,5
	AIBN Control	40°C,60°C	1,3,5
Photolysis	Light1 × ICH	NA	1,3,5
	Light3 × ICH	NA	1,3,5
	Light-Control	NA	1,3,5
Thermal	Heat-Chamber,	60°C	1,3,5
	Heat-Chamber,	60°C/75%R.H	1,3,5
	Heat-Chamber,	80°C	1,3,5
	Heat-Chamber,	80°C/75%R.H	1,3,5
	Heat-Control,	Room temperature	1,3,5

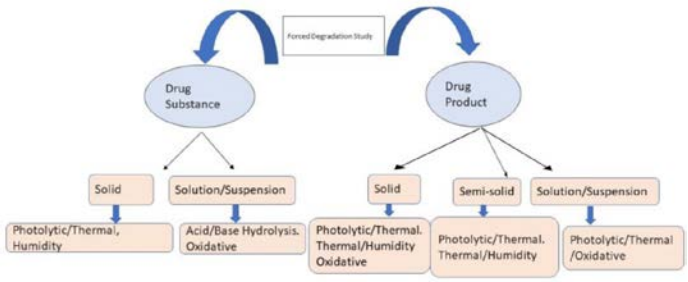

Figure 1: various ways of performing forced degradation study on drug products and drug substance.

Drug Administration (FDA). The British Pharmacopeia (BP) and the United States Pharmacopeia (USP) both have impurity limitations for API and formulation (USP).[7] It will provide knowledge about the development and validation of stability-indicating methods. Structural elucidation of degraded products. Determination of the drug product's and drug substance's degradation route. The inherent stability of the drug formulation and the drug material can be determined.[8] Biosimilar molecules like Adalimumab, having a complex physiochemical character with various functional groups are suspectable to instability through various degradation Pathways.[9]

FORCED DEGRADATION STUDY

Forced degradation or stress testing can be used to establish specificity when developing stability-indicating techniques, especially if less amount of information about possible degradant products is accessible. These works also disclose the processes of degradation as well as the degradation products that may develop during storage. Forced degradation study supports pharmaceutical formulations in areas including dosage forms development, production, and packaging, in which chemical behavior can be leveraged to enhance a therapeutic product. The stability test, also known as forced degradation, is used to demonstrate the significance of a stability-indicating analytical method developed with high-performance liquid chromatography (HPLC), that is, a single analytic method capable of distinguishing degradant peaks from drug substance/drug product peaks. Three types of stability studies must be undertaken for the purpose to assess the storage time of a formulation which are accelerated stability, intermediate stability, and regulated room temperature stability. There are many alternatives available, including a 6-month expedited study, another 12- to 24-month intermediate stability study, as well as a 12- to 24-month controlled room temperature stability study. The active pharmaceutical ingredient or drug formulation will degrade and develop new compounds, known as impurities, throughout the stability study. This is used to determine the inherent stability of a molecule.[10-11] Several types of stress are applied to deteriorate the drug component and yield contaminants, that should be separated out from the primary component and one another during forced degradation tests. For a reason, forced degradation studies are conducted to estimate the impurities that would arise during stability testing to estimate the shelf life of API or formulations. To estimate all of the degradant substances that are likely to be predicted during forced degradation investigation, numerous analytical methods can be used. HPLC with UV spectrophotometer (High-performance liquid chromatography) and HPLC with photodiode array detector (HPLC-PDA) are well-known procedures employed in the pharmaceutical industry during forced degradation studies as part of the project and validation of stability-indicating methods. Approaches to achieving the highest standards of results several analytical methods are coupled such as High-performance liquid chromatography with mass spectrometry (LC-MS), gas-chromatography employing hyphenated techniques as mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR).[12]

OBJECTIVES OF FORCED DEGRADATION STUDY

1. Stability-indicating methodology development and validation
2. Determination of drug substance and drug product breakdown processes.
3. Distinguishing breakdown of products linked to pharmacological compounds and those related to non-drug chemicals in formulations (e.g., excipients).
4. Helps in the elucidation of degraded products.
5. The intrinsic stability of a pharmaceutical composition is determined.[13]

THE CURRENT STATE OF REGULATORY REQUIREMENTS FOR FORCED DEGRADATION STUDIES AROUND THE GLOBE

The International Council on Harmonization (ICH), the Food and Drug Administration (FDA), the European Medicines Agency (EMA), the United States Pharmacopeia (USP), the Japanese Pharmacopeia (JP), and the Agencia Nacional de Vigilancia Sanitaria (ANVS) are just a handful of good national and international regulatory bodies that provide information on forced degradation (ANVISA).[5,11,14]

VARIOUS STABILITY INDICATING METHODS

Due to the emergence of novel viruses this year, the development and treatment of antivirals agents has gained equal importance. Antiviral medications should go through a validation process before being used to ensure that the formulations are safe and effective. Antiviral medicines have been quantified using a variety of methods, including UV, capillary electrophoresis, and chromatographic methods such as GC and HPLC, LC-MS, and GC-MS. The authors of this review focus on stability, recommending HPLC/RP-HPLC technologies for the precise

and effective development and validation of antiviral medicines such as. Many formulations, as well as fixed dose combination formulations using various APIs, are storming the marketplace. Every of these might contain various sorts of contaminants and will be subjected to various mechanisms of degradation, resulting in the development of degradation products. All of these will be present in the form of contaminants. Though the impurities will be under regulatory limits, in the event of diseases like Acquired Immune Deficiency Syndrome or long-term therapy drugs, the patient will be on medicine for the rest of his/her life, and thus will be bound to get exposed with these degradants in excess.[34] Due to the emergence of novel viruses this year, the development and treatment of antivirals agents has gained equal importance. Antiviral medications should go through a validation process before being used to ensure that the formulations are safe and effective. Antiviral medicines have been quantified using a variety of methods, including UV, capillary electrophoresis, and chromatographic methods such as GC and HPLC, LC-MS, and GC-MS. The authors of this review focus on stability, recommending HPLC/RP-HPLC technologies for the precise and effective development and validation of antiviral medicines such as Zidovudine,[15-20] Lamivudine,[20-23] Nevirapine,[24-27] Ritonavir,[28-31] Abacavir,[32-34] Efavirenz,[35-38] Tenofovir,[39-41] Fosamprenavir,[42-46] Atazanavir,[47-48] Emtricitabine,[35,35,39,49] Fosamprenavir,[42-46] Tipranavir,[50-51] Darunavir,[52-55] Raltegravir,[56-58] Maraviroc,[59-60] Etravirine,[61-63] Rilpivirine,[64-66] Dolutegravir,[67-70] Cobistat,[71-72] Doravirine,[20,73-75] Fostemsavir,[75-76] and Cabotegravir.[77-78] Profile for the above-mentioned drug is represented in Table 2 and Stability indicating methods for FDA approved antiretroviral agents (1987-2021) is available in Table 2. For the analysis of degradant materials, hyphenated analytical tools such as (LC-MS, GC-MS, CE-MS, LC-MS/MS, LC-NMR) are available. For the determination, quantification, and characterization of stress study degradation products, further analytical techniques such as HPLC, GC, EC, NMR, and FT-IR are used.

CONCLUSION

Force degradation assays of drug substances provide insight into the molecule's intrinsic stability as well as potential degradants that occur over the medicine's shelf life, assisting in the creation of a stable formulation. The present regulatory standards for the practical performance of forced deterioration and its use in the development of stability-indicating methods are summarised in this review. For the quantitative evaluation of antiviral medicines, a variety of methodologies have been used. This study will provide a comprehensive review of the literature on stability-indicating framework for the development and validation of FDA-approved antiretroviral agents (1987-2021), as well as provide a foundation for researchers working in the areas of new product development and quality control testing. Impurities or Degradents products should be within their threshold limit as per regulatory body and the Stability indicating method must be performed as recommended by ICH Guidelines. It Summarizes the various stability indicating analytical techniques such as HPLC, LC-MS, LC-MS/MS, UHPLC and NMR were apply for seperation of degradation product, dosage form, and raw material.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ARV: Anti-retroviral; **BCS:** Biopharmaceutics classification system; **BP:** British Pharmacopoeia; **CD4:** Cluster of Differentiation 4; **ESI:** Electron spray ionization; **FDA:** Food and Drug Administration; **FTIR:** Fourier transmission infrared; **HPLC:** High performance liquid chromatography; **ICH:** International Council on Harmonization; **INSTI:** Integrase strand transfer inhibitor; **LC-MS/MS:** Liquid Chromatography-Mass Spectroscopy; **NA:** Not Available; **NNRTI:** Non-nucleoside Reverse transcriptase inhibitor; **NRTI:** Nucleoside reverse transcriptase inhibitor; **UPLC:** Ultra performance liquid chromatography; **USP:** United States Pharmacopoeia.

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Table 2: Drug profile of FDA approved (1987-2021) antiretroviral drugs.

ARV Drug with FDA Approved Year	Matrix and Method	Stationary phase	Mobile phase (V/V) Flow rate (ml min^{-1})	Detection (nm)	Linearity (µg/ml)	Retention time(min)	Reference
Zidovudine (1987)	HPLC (API)	C_{18}(250*4.6mm* 5µm)	Buffer pH 4.5: Methanol (77:33) V/V 1 (ml min^{-1})	265	25-500	11.502	[15]
	LCMS/MS (API)	Waters Xbridge C_{18}(150*4.6mm, 3.5 µm)	10mM Ammonium Acetate: ACN(V/V) Gradient elution mode 0.8(ml min^{-1})	285	10-100	11.872	[16]
	HPLC LCMS/MS (API)	XRsLC C_{18} Column (5µ,250*4.6mm)	Methanol and 10mM ammonium formate(pH 3.5) 1.0(ml min^{-1})	254	NA	24	[17]
	RP-HPLC (Tablet)	RP-18 Terra Column (250*4.6mm* 5µm)	Water: Methanol (80:20) V/V 1.0(ml min^{-1})	266	40-220	9.102	[18]
	UPLC (API/Tablet)	Intersil ODS- 3V (250*4.6MM,5.0 µm) Column	Ammonium dihydrogen phosphate and Diammonium hydrogen phosphate buffer(pH3.9): methanol 1.0(ml min^{-1})	270	NA	45.464	[19]
Lamivudine	RP-HPLC-DAD (API/Tablet)	C_{18}(150*4.6mm*5 µm)	0.1% ortho phosphoric acid: ACN (70:30) 1.0(ml min^{-1})	260	NA	2.9	[20]
	LC-MS, (API)	C_{18}((250*4.6mm *5 µm)	Methanol: buffer (pH3.8) (5:95) 1.0(ml min^{-1})	277	50-500	20	[21]
	RP-HPLC (API)	C_{18}(150*4.6mm*3 µm)	ACN: Hexane-1-sulfonic acid (pH2.5) (50:50) V/V 0.8(ml min^{-1})	243	5-100	2.42	[22]
	UPLC (API/Tablet)	Intertsil ODS- 3V (250*4.6MM,5.0 µm)	Ammonium dihydrogen phosphate and Diammonium hydrogen phosphate buffer(pH3.9):(methanol)V/V 1.0(ml min^{-1})	270	NA	19	[23]
Ritonavir (1996)	RP-HPLC (Tablet)	Agilent C_{18}(250*4.6mm *5 µm)	ACN: Phosphoric acid(55:45)V/V 1.2(ml min^{-1})	240	2-12	4.35	[28]
	RP-UPLC LCMS/MS (Tablet)	Waters Acquity BEH RP18(100*2.1mm,1.7 µm)	0.1N KH_2PO_4 Buffer pH (3.5)and ACN(80:20)as A Water:ACN(20:80) V/V As B. 0.5(ml min^{-1})	240	NA	10.323	[29]
	LCMS/MS	Waters Xterra C_{18} column (250mm*4.6mm i.d,5 µm)	Water:Methanol:ACN (40:20:40)V/V/V 1.0(ml min^{-1})	210	10-200	9.0	[30]
	LC Capsule	Lichrospher 100RP-18 (250mm*4.6mm i.d,5 µm Merck)	ACN:Water:Methanol (53:37:10) V/V/V 1.0(ml min^{-1})	210	40-360	8.8	[31]
	RP-UPLC (Tablet)	Acquity BEH C_{18}(100*2.1mm*1.7 µm)	0.01M monobasic potassium hydrogen phosphate(pH03.6): ACN V/V 0.4(ml min^{-1})	240	NA	11.970	[32]

continued...

Table 2: Cont'd.

ARV Drug with FDA Approved Year	Matrix and Method	Stationary phase	Mobile phase (V/V) Flow rate (ml min^{-1})	Detection (nm)	Linearity (µg/ml)	Retention time(min)	Reference
Nevirapine (1996)	UHPLC (API)	Poroshell 120 C_{18}(100*2.1mm *2.7 µm)	ACN: WaterV/V 0.2(ml min^{-1})	254	4-48	9.706	[24]
	RP-HPLC (Tablet)	C_{18} Phenomenex (250*4.6mm*5 µm)	Methanol:1%tetrah ydofuran: ACN (50:30:20)v/v/v) 1.0(ml min^{-1})	286	10-60	4.80	[25]
	LCMS/MS (API)	Zorbax(5µm*4.6mm* 150mm)	0.1% formic acid:ACN with 0.1% formic acid V/V 1.0(ml min^{-1})	NA	NA	NA	[26]
	RPLC-UV (API)	Luna C_{18}(150mm*4.6mm) Column	50mM Ammonium acetate Buffer (pH 6.8):methanolV/V NA	265	10-80	15.7	[27]
Abacavir-1998	(UHPLC) (API)	Waters C_8(50mm*2.1*1.7 µm)	A (0.1% V/V Ortho phosphoric acid in water) B(0.1% Ortho phosphoric acid in methanol) 0.40(ml min^{-1})	255	50-150	NA	[32]
	HPLC LC-MS(API)	Zorbax C_{18}(250*4.6mm*5 µm)	Buffer:methanol:ACN (75:10:15)V/V/V 1.2(ml min^{-1})	214	NA	5.0	[33]
	LCMS/MS (API)	Waters Xterra C_{18}(250mm*4.6*5 µm)	20mM Ammonium acetate:ACN 1.0(ml min^{-1})	220	0.5-10.0	18.0	[34]
Efavirenz-1998	RP-HPLC (Tablet)	C_{18} column	(Phosphate buffer pH 3.5: ACN) 1.5(ml min^{-1})	256	60-900	8.2	[35]
	RP-HPLC (API /Tablet)	C_{18}(4.6mm*250*5µm)	Methanol:10mM Ammonium-Acetate buffer(pH3.1) (70:30) 1.0(ml min^{-1})	247	5-25	7.73	[36]
	HPLC (API)	Novapak phenyl column (150*3.9mm*4 µm)	Buffer pH6.0:ACN (55:45)V/V 1.0(ml min^{-1})	247	NA	6.59	[37]
	RP-HPLC (API/Capsule)	Zorbac SB-CN,15cm*4.6mm i.d	(A)90% water,with 0.05% trifluoroacetic acid:10%methanol(B)90% methanol:10%water,with 0.05% trifluoroacetic acidV/V 1.5 (ml min^{-1})	250	120-360	NA	[38]
	RP-HPLC-DAD	C_{18}(150*4.6mm*5 µm)	0.1% ortho phosphoric acid:ACN(70:30) 1.0(ml min^{-1})	260	NA	3.6	[39]
	RP-HPLC (API&FDC)	ODS Column(250*4.6mm,*5 µm)	A(potassium dihydrogen orthophosphate pH2.5) B(ACN)55:45 1.0(ml min^{-1})	250	3-8	4.17	[40]
Tenofovir Disoproxil fumarate 2001	RP-HPLC (API)	ODS Column (250*4.6mm,*5 µm)	0.2% triethylamine buffer:methanol(40:60)V/V 1.2(ml min^{-1})	260	12.5-62.5	4.53	[41]

continued...

Table 2: Cont'd.

ARV Drug with FDA Approved Year	Matrix and Method	Stationary phase	Mobile phase (V/V) Flow rate (ml min^{-1})	Detection (nm)	Linearity (µg/ml)	Retention time(min)	Reference
Fosamprenavir 2003	RP-HPLC (API)	YMC Pack ODS AQ (150*4.6*3.0 µm) 40°C	0.05M potassium dihydrogen ortho phosphate monohydrate (pH6.8):ACN(60:40)V/V 0.8(ml min^{-1})	265	80-120	NA	[42]
	HPLC (Tab)	RP18 Column	Sodium acetate buffer:ACN V/V 1.2(ml min^{-1})	264	NA	NA	[43]
	HPLC LC-MS LC-NMR(API)	Interstil phenyl column (250*4.6mm*5 µm)	Methanol:10mM ammonium formate buffer pH4.0 V/V 1.0(ml min^{-1})	265	NA	23	[44]
	RP-HPLC (API&Tab)	Interstil C8-3(250*4.6mm*5 µm)	A(Ammonium formate and triethyalamine pH 3.2) B(Methanol and ACN 60:40) 60:40V/V 1.0(ml min^{-1})	266	22.4-134.4	4.1	[45]
	HPLC (API)	Xbridge C_{18} (250*4.6mm*5 µm)	Phosphate buffer Ph6.8:ACN(70:30)V/V 1.0(ml min^{-1})	267	50-150	7.97	[46]
Atazanavir 2003	(RP-HPLC) (API)	C_{18} Phenomenex (250mm*4.6 mm*5µm)	Methanol:Water(90:10)V/V 0.5(ml min^{-1})	245	10-90	8.323	[47]
	(RP-HPLC) MS-MS H-NMR (API)	Express C8 (150mm* 4.6mm*2.7µm)	Ammonium acetate(pH3.5 0.02mM)with ACN V/V 1.0(ml min^{-1})	250	0.020-3.005	19	[48]
Emtricitabine 2003	HPLC (API)	Interstil ODS 3V-Column(150*4.6*5 µm)	10mM sod.phosphate Buffer:Methanol(85:15) V/V 1.0(ml min^{-1})	280	0.05-0.15	12.695	[35]
	RP-HPLC (API&FDC)	ODS Column(250*4.6mm,*5 µm)	A(potassium dihydrogen orthophosphate pH2.5) B(ACN)55:45V/V 1.0(ml min^{-1})	250	2-12	2.287	[39]
	RP-HPLC (API)	ODS Column(250*4.6mm,*5 µm)	0.2% triethylamine buffer:methanol(40:60)V/V 1.2(ml min^{-1})	260	100-500	2.805	[40]
	RP-HPLC (API)	Interstil ODS(150* 4.6mm,5 µm)	Phosphate buffer pH3.5: ACN V/V 1.5(ml min^{-1})	256	20-300	2.0	[49]

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Table 2: Cont'd.

ARV Drug with FDA Approved Year	Matrix and Method	Stationary phase	Mobile phase (V/V) Flow rate (ml min^{-1})	Detection (nm)	Linearity (µg/ml)	Retention time(min)	Reference
Tipranavir 2005	LC-UV Capsule	Lunar(150*4.6mm*5 µm)	Methanol:ACN: Acidified water pH3.5(40:31:29)V/V/V 1.0(ml min^{-1})	254	10-100	9.5	[50]
	HR-MS	C_{18} column (250 x 10 mm i.d., particle size 10 µm)	Methanol, acetonitrile and acidified water, pH 3.5 (45:40:15 v/v/v) 2.0(ml min^{-1})	254	20-200	2.8	[51]
Darunavir-2006	API +Tab	X-bridge C_{18} (150*4.6*mm3.5µm)	0.01M ammonium formate buffer pH(3.0):ACN(55:45)V/V 1.0(ml min^{-1})	265	0.6-0.24	NA	[52]
	HPLC (Tab)	RP C_{18}(symmetry 5µm,mm, Waters)	ACN : Water (50/50)V/V 1.0(ml min^{-1})	267	6.0-21.0	NA	[53]
	LC-MS/MS HRMS(API)	C8(250*4.6mm.,5 µm)	10mM ammonium acetate :ACN(52:48)V/V 1.0(ml min^{-1})	210	50-250	8.99	[54]
	UPLC MS-MS (API)	C_{18}(50*2.1mm*1.7 µm)	A(ACN:Methanol,80:20) B(5.0mM ammonium acetate with 0.01% formic acid)V/V 0.4(ml min^{-1})	NA	NA	6.0	[55]
Raltegravir 2007	RPHPLC Tablet	Zorbax SB phenyl (150*4.6mm*3.5 µm)	(0.1% v/v Phosphoric acid in water): ACN (40:60)V/V 1.0(ml min^{-1})	260	60-140 µg/ml	5.4	[56]
	UPLC (API)	BEH Sheild RP18 (2.1mm*100mm*1.7 µm)	Potassium dihydrogen phosphate buffer pH3.0: Methanol (30:70)V/V 0.23(ml min^{-1})	254	15-150	1.04	[57]
	RP-HPLC LCMS/MS	C_{18}(100*4.6*5 µm)	Buffer:ACN(60:40)V/V	213	25-150	NA	[58]
Maraviroc 2007	HPLC (API)	Xbridge C_{18}(250*4.6mm*5 µm)	Potassium phosphate buffer:Methanol (70:30)V/V 0.8(ml min^{-1})	NA	NA	37.7	[59]
	UPLC LC-MS (API)	RP-18 column (100*2.1mm*1.7 µm)	0.01mM ammonium acetate in water pH6.5: ACN (63:37)V/V 0.4(ml min^{-1})	210	50-200	3.194	[60]

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Table 2: Cont'd.

ARV Drug with FDA Approved Year	Matrix and Method	Stationary phase	Mobile phase (V/V) Flow rate (ml min^{-1})	Detection (nm)	Linearity (µg/ml)	Retention time(min)	Reference
Etravirine 2008	RP-UPLC API	Shimpack ODS-II (100*2.1mm*1.7 µm)	A(3.08 gm ammonium acetate in 1000 ml of water pH6.0) B(Methanol,ACN:1:1) 50:50.V/V 0.6 ml/min	303	50-150	5.80	[61]
	RP-HPLC (Tab)	Zorbax C_{18}(250*4.6mm*5 µm)	ACN:10mM ammonium acetate buffer pH4.5(90:10)V/V 1.0(ml min^{-1})	271	15-45	4.74	[62]
	RP-HPLC (Tab)	Inertsil ODS 3V C_{18} (250*4.6mm*5 µm)	0.03M potassium dihydrogen orthophosphate as buffer pH3.2:ACN (30:70)V/V 1.0(ml min^{-1})	309	80-240	9.11	[33]
Rilpivirine 2011	RP-UPLC (API&Tab)	Thermosil octa decyl (4.6*50mm*1.7 µm) 0.3	0.1M triethyl amine buffer :ACN(35:65)V/V	260	12.5-62.25	2.18	[64]
	RP-HPLC (API&Tab)	ODS RP C_{18} (15cm*4.6mm*5 µm) 1.0	ACN:Potassium dihydrogen phosphate buffer pH2.2(40:60)V/V	282	NA	4.50	[65]
	RP-HPLC (API&Tab)	Thermosil C_{18}(4.6*150mm*5 µm) 0.8	ACN:Phosphate buffer pH3.5(45:55)V/V	260	30-70	2.42	[66]
Dolutegravir 2013	RPHPLC API/Tablet	Kinetex C_{18}(250*4.6mm*5 µm) 1.0	Methanol with orthophosphoric acid(50:50)v/v	260	1.5-210	7.70	[67]
	RP-HPLC (API&Tab)	Thermosil C_{18}(4.6*150mm*5 µm) 0.8	ACN:Phosphate buffer pH3.5(45:55)V/V	260	80-120	2.24	[68]
	UPLC/MSMS (API)	Phenyl hexyl column (100*2.1mm*1.7 µm) 0.3	10mM acetate buffer pH 4.0:ACN in gradient mode V/V	257	NA	8.62	[69]
	RP-HPLC (Tab)	C_{18} phenyl column (250*4.6mm*5 µm) 1.0	NA	260	4.5-28	11.4	[70]
Cobistat 2013	RP-HPLC (API/Tab)	C_{18}(150*4.6mm*5 µm)	Water:ACN(90:10)V/V 1.0(ml min^{-1})	240	7.5-45	4.09	[71]
	UPLC API/Tab)	C8(50*2.1mm*1.8 µm)	0.01N Potassium dihydrogen orthophosphate pH4.8	NA	18.75-112.5	1.34	[72]

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Table 2: Cont'd.

ARV Drug with FDA Approved Year	Matrix and Method	Stationary phase	Mobile phase (V/V) Flow rate (ml min^{-1})	Detection (nm)	Linearity (µg/ml)	Retention time(min)	Reference
Doravirine 2018	RP-HPLC (API/Tab)	C_{18} (150*4.6mm*5 µm)	0.1%orthophosphoric acid: ACN (70:30) V/V 1.0(ml min^{-1})	260	12.5-75	2.4	[73]
	RP-HPLC (API)	C_{18} (150*4.6mm*3 µm)	ACN: Hexane-1-sulfonic acid(pH2.5) (50:50) V/V 0.8(ml min^{-1})	243	1.75-35	8.6	[74]
	UPLC (API/Tab)	C_{18} ODS (2.1*50mm*1.7 µm)	ACN: Triethylamine buffer pH3(60:40) V/V 1.0(ml min^{-1})	260	20-100	0.80	[75]
	RP-HPLC (Tab)	C_{18} column (150 × 4.6 mm, 2.7 µm)	Phosphate buffer : ACN (50:50) V/V 1.0(ml min^{-1})	230	NA	2.22	[76]
Fostamsavir 2020	RP-HPLC(API)	X-Bridge phenyl column (150*4.6mm*3.5 µm)	Buffer 0.1% ortho phosphoric acid: ACN (50:50) V/V 1(ml min^{-1})	NA	2.5-37.5	NA	[76]
	RP-HPLC (API)	C_{18} (150*4.6*5 µm)	0.01N disodium ortho phosphate:ACN(55:45)V/V 1(ml min^{-1})	278	15-90	2.47	[77]
Cabotegravir 2021	UPLC (API)	Phenyl column	10mM ammonium formate buffer:ACN V/V	258	0.25-1.0	5.38	[78]
	UPLC (API)	C_{18} (150*4.6mm*5 µm)	0.1% Orthophosphoric acid: ACN (60:40) V/V	245	25-150	1.66	[79]

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