METHOD DEVELOPMENT AND VALIDATION OF DARUNAVIR AND RITONAVIR DRUGS BY THE APPLICATION OF GREENER CHROMATOGRAPHIC TECHNIQUE

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Introduction

Traditional methods used for the analysis of pharmaceutically active compoun- ds require large volumes of organic solvents and generate large amounts of waste. The main aim of green analytical chemistry is to find new analytical methodology or to modify an old method to incorporate procedures that use less hazardous chemicals. There are several methods is available to achieve this goal such as using environmentally benign solvents and reagents, reducing the chromatographic separation times and miniaturization of analytical devices. Green chemistry which is mainly based on the principle where the usage or generation of hazardous chemicals or substances is minimized. It's also called as programmed chemistry in chemistry and related areas. In modern analytical chemistry, the development of greener methods necessitates considering the green aspects at the stage of method development

The main aim of the Green chromatography is to reducing the amount of the toxic chemicals consumed and wastages generated during the method development. Green solvents or the bio solvents which are more environmental friendly and cost effective compared to the petrochemicals is recommended the solvents like ethyl acetate and ethanol [1]

Liquid chromatography is generally considered less green than gas chromatography, as it requires solvents for the separation. The interest in green analytical chemistry is growing. Liquid chromatography is generally considered less green than gas chromatography, as it requires solvents for the separation. On the flip side, this offers more possibilities for "greening". The next strategy for greening liquid chromatography is the search for "green" components of the mobile phase.[2]

Typical mobile phases used in RPLC include acetonitrile/water and methanol/water mixtures. Although both acetonitrile and methanol are toxic, the latter has lower disposal costs; therefore it should be selected over acetonitrile whenever possible. According to the guide on "green" mobile phases, water, acetone, methanol, and ethanol can be treated as environmentally friendly LC phase. Ethanol is a particularly desirable solvent for green liquid chromatography. Chromatographic techniques have the potential to be greener at all steps of the analysis, from sample collection through its preparation to separation and final determination. An ideal chromatographic method would yield analytical results without using any consumables; it would be performed in *in-line* mode, without sample preparation. Unfortunately, sample preparation prior to chromatographic analysis is required in most cases. Consequently, solvent less, miniaturized sample preparation techniques should be used whenever possible to minimize solvent consumption. As most chromatographic determinations performed are routine ones, it is extremely important to perform them with methodologies that have small environmental impact. [3,4]

The retention mechanism of the drugs in MLC depends on several experimental factors, such as the column type, concentration of SDS, kind and proportion of organic solvent, and pH. Therefore, it is desirable to investigate their effects on the main chromatographic parameters of the resulting peaks, such as retention time, efficiency and asymmetry. Indeed, they must be set during the development of the method to adequately resolve the analytes. The stability and reproducibility of the retention mechanism in MLC under an isocratic mode have allowed the prediction of these parameters from the composition of the mobile phase by means of a mathematical model, based on the three-phase theory. This can expedite the resolution of complex mixtures.

The remarkable properties of SDS-micellar solutions are considerably useful for the sample pretreatment of biological fluids .Hydrophobic and non-water soluble endogenous compounds can be dissolved and do not need to be removed before the injection Micelles tend to bind proteins and other macromolecules competitively, provoking their denaturation, solubilization and the release of linked drugs. When these compounds are injected, they are free to interact with the stationary phase and can be determined, whereas micelle-bound proteins are harmless eluted near the dead time, rather than precipitating into the column. After a simple dilution in a pure micellar medium and filtration, these biological fluids can be directly injected, without the aid of extraction or cleanup steps. Thus, the sample is quantitatively introduced in the chromatographic system [8].

Antiviral drugs indicate that those are the drugs that combat viral infections are called antiviral drugs. Antiviral drugs work by interfering with viral replication.[10] Because viruses are tiny and replicate inside cells using the cells' metabolic pathways, there are only a limited number of metabolic functions that antiviral drugs can target. Because viruses hijack many of the metabolic processes of the host cell itself, it is difficult to find drugs that are selective for the pathogen. However, some enzymes are virus-specific, and these have proved to be useful drug targets.[11]

Table 01: Drug profile
DARUNAVIR

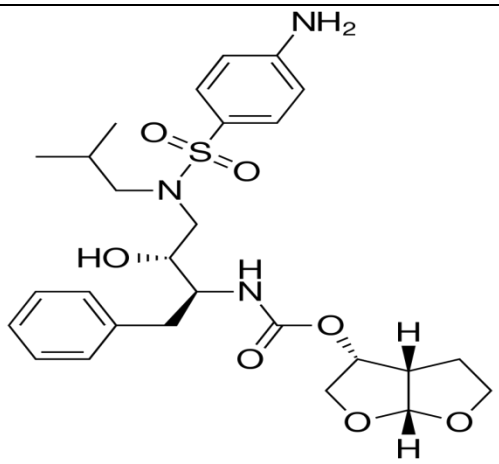
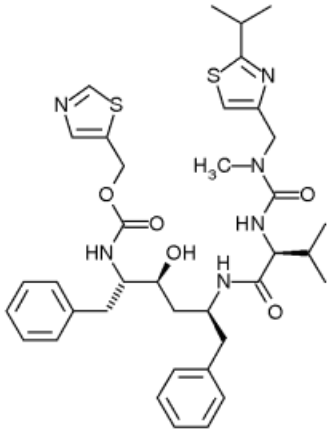
Name of the drug	DRV
Structure **(Fig. 3)**	
Brand name	DANAVIR-R(100mg of DRV and 800mg of RTV)
Chemical formulation	$C_{27}H_{37}N_3O_7S$
Molecular weight (g/mol)	547.668
IUPAC	(3R,3aS,6aR)-hexahydrofuro [2,3-b] furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)4-aminobenzenesulfonamido]-1-phenylbutan-2-yl]carbamate; ethanol
Category	Antiviral drugs, Antiretroviral, Protease inhibitors
Indication	Darunavir, co-administered with ritonavir, and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV) in children age 3 or above and adults with HIV-1 infection.

Table 02: RITONAVIR

Name of the drug	RTV
Structure	
Brand name	DANAVIR-R(100mg of DRV and 800mg of RTV)
Chemical formulation	$C_{37}H_{48}N_6O_5S_2$
Molecular weight (g/mol)	720.9
IUPAC	1,3-thiazol-5-ylmethylN-[(2S,3S,5S)-3-hydroxy-5-[[(2S)-3-methyl-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]butanoyl]amino]-1,6-diphenylhexan-2-yl]carbamate
Category	Antiviral drugs,Antiretroviral,Protease inhibitors
Indication	RTV is an HIV protease inhibitor that interferes with the reproductive cycle of HIV. Although it was initially developed as an independent antiviral agent, Indicated in combination with other antiretroviral for the treatment of HIV-1 infection in adults and pediatric patients ≥14 days old.

Methodology: -

Chemical, Reagents and Solvents

The chemicals, reagents and solvents used in the Analytical method development, validation were listed in **Table3.**

Table 3: List of Chemicals, Reagents and Solvents

Sl. No	Name of chemicals / reagents	Manufacturer
1.	Ethanol (AR grade)	S D Fine chem Pvt. Ltd., Mumbai
2.	Methanol (HPLC grade)	S D Fine chem Pvt. Ltd., Mumbai.
3.	Potassium di-hydrogen Phosphate (AR grade)	S D Fine chem Pvt. Ltd., Mumbai
4.	Orthophosphoric acid (AR)	S D Fine chem Pvt. Ltd., Mumbai
5.	NaOH (AR grade)	T F scientific Pvt. Ltd., India.
6.	Millipore water (HPLC grade)	In house, B G Nagara.

4.1 Pure Drugs

The list of the pure drugs listed in Table 4.

Table 4: List of Pure Drugs

Sl. No	Drugs	Manufacturer
1.	Ritinovir pure drug (99% pure)	Yarrow chemicals, Mumbai.
2.	Darunavir pure drug (99% pure)	Yarrow chemicals, Mumbai.

Instruments and Equipments

The instruments used in the Analytical method development, validation were listed in **Table 5.**

Table 5: List of Instruments and Equipments

Sl.No	Instruments/Equipments	Mode/Maker
1.	UFLC,LC-20AD, L20104611641 AE, UV Detector	Shimadzu, Japan
2.	Electronic Analytical Balance	Shimadzu, Japan
3.	C18 Column	Eclipse Plus
4.	Membrane Filter	160047/ AxivaSichem Biotech, Delhi.
5.	Micropipettes	Phenomenex
6.	Digital PH Meter	LI120/ Elico, India
7.	Sonicater	Labmatrix, Mumbai.

Selection of mobile phase:

The selection of ideal mobile phase is done by altering the ratio of the buffers, composition of the mobile phase solvents in the different ratio and also by trial and error principle.

Preparation of mobile phase is based on the following physicochemical properties.

- Solubility.
- Stability.
- PKa value.
- Literature survey.

Preparation of Mobile Phase: Methanol and 0.1M potassium dihydrogen phosphate are mixed as 60:40 (*v/v*) ratio. This is also employed for standard solutions preparation

Selection of analytical columns:

It is one of the most important phase in the method development. C-18 column is used for the method development because it gives the optimum resolution for the separation of the drugs and the literature surveys also suggest that C-18 column is ideal for the separation of the drugs.

Preparations of Standard Solutions:

Stock solution of darunavir and ritonavir is prepared in a 100 ml dry volumetric flask with mobile phase at a concentration of 4000 µg/ml (darunavir) and 500 µg/ml (ritonavir). For this, 400 mg of darunavir and 50 mg of ritonavir is dissolved in mobile phase of volume 100 ml.

Ritonavir (RTV):-The stock solution was prepared by dissolving 0.05 g of pure drug RTV in 100ml of Mobile (500µg/ml) and solutions were stored cold conditions. A series of standard solutions were prepared by the appropriate dilution of the stock standard solution of RTV with mobile to prepare the working standard solution (5µg/ml) to a concentration of 50µg/ml. (5µg/ml to 50µg/ml)

Darunavir (DRV):-The stock solution was prepared by dissolving 0.4 g of pure drug DRV in 100ml of mobile (4000µg/ml). A series of standard

solutions were prepared by the appropriate dilution of the stock standard solution of **DRV** with MP to prepare the working standard solution (25µg/ml) to a concentration of 600µg/ml.

System suitability testing:

System suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility (%RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method.

Table 6: System suitability results for Darunavir, ritonavir

Sample No. Darunavir(400µg/ml)	**RET**	**PAR**	**TP**	**PA**	**RES**
1	3.712	3434442	9186	1.57	-
2	3.711	3396372	9619	1.55	-
3	3.71	3418479	9168	1.57	-
4	3.71	3438207	9131	1.56	-
5	3.71	3424197	9097	1.58	-
Mean,	**3.711,**	**3422339,**	**9240,**	**1.566,**	-
RSD (%)	**0.024**	**0.483**	**0.322**	**0.728**	
Ritonavir (50 µg/ml)					
1	4.757	2777053	6919	1.62	5.2
2	4.755	2738973	7266	1.6	5.33
3	4.756	2770852	6885	1.62	5.21
4	4.755	2777883	6894	1.6	5.2
5	4.755	2771190	6888	1.61	5.18
Mean,	**4.756,**	**2767190,**	**6970,**	**1.610,**	**5.224,**
RSD (%)	**0.019**	**0.582**	**0.378**	**0.621**	**1.153**
Recommended limit	**RSD ≤ 2**	**RSD ≤ 2**	**> 2000**	**≤ 2**	**> 1.5**

RET-retention time; TP-theoretical plates; PAR-peak area response; PA-peak asymmetry; RES

Results And Discussion

5.1: Reverse Phase Ultra-Fast Liquid Chromatography:

In RP-UFLC method, UFLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to elute title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or factor), run time, resolution.

Table 7.0: Values obtained during different trails

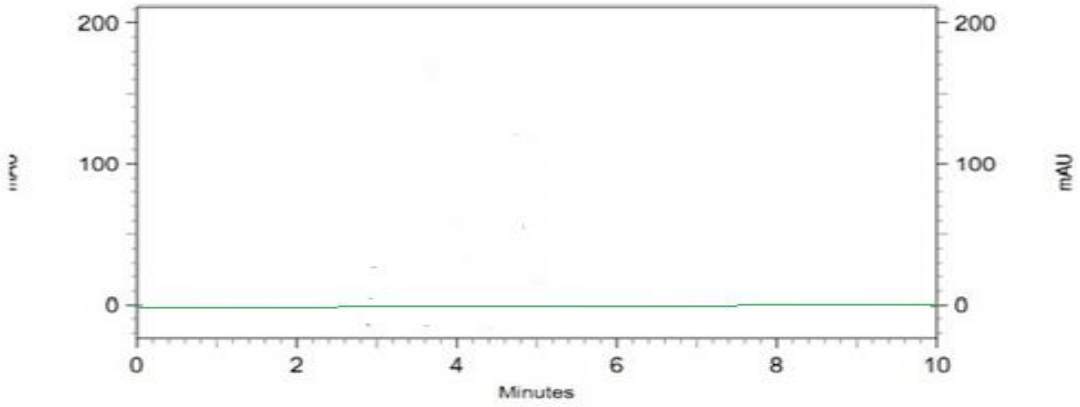
Trail	**Drug**	**RT**	**Area**	**Resolution**	**PT**	**PC**
1	DAR	3.674	1975064	-	1.97	1320
	RIT	5.325	1521996	2.94	2.27	1131
2	DAR	4.310	1909029	-	0.70	6158
	RIT	4.982	1609951	2.34	0.85	3798
3	DAR	4.335	3550084	-	1.36	3787
	RIT	4.793	996211	1.31	2.08	2244
4	DAR	4.241	3178770	-	1.77	2539
	RIT	4.960	2605361	1.80	1.86	2135
5	DAR	3.711	3506792	-	1.56	9509
	RIT	4.752	2860531	5.26	1.62	7106

DAR – darunavir; RIT – ritonavir; RT – retention time; PT – peak tailing; PC – plate count

Table.6.1: Optimized chromatographic conditions.

Sl. no	Standard Concentration	Darunavir and Ritonavir
1.	Mobile phase	Methanol and 0.1M potassium dihydrogen phosphate are mixed as 40:60 (*v/v*) ratio
2.	Diluent	MP
3.	Pump mode	Isocratic
4.	Flow rate	1ml/ min
5.	Retention Time	5.55 min, 6.86 min
6.	Run Time	10 min
7.	Wavelength	254 nm
8.	Detector	UV
9.	Column Temperature	25^0 C
10.	Injection volume	20µl

Chromatogram of Blank:-



Chromatogram obtained during different trails:-

The chromatograms obtained during different trails are shown in the following figures.

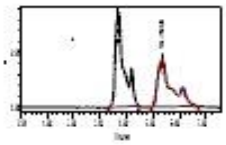


Figure 3: Chromatogram with trial 1 conditions

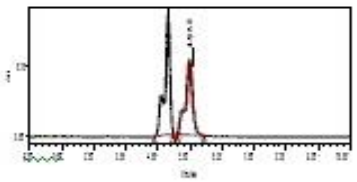


Figure 4: Chromatogram with trial 2 conditions

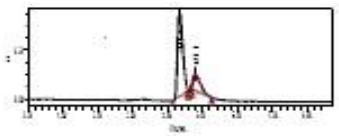


Figure 5: Chromatogram with trial 3

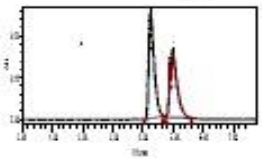


Figure 6: Chromatogram with trial 4 conditions

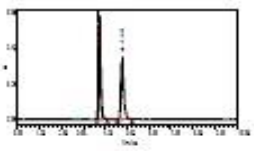


Figure 7: Chromatogram with trial 5 conditions

Figure.8.0: Standard Chromatogram

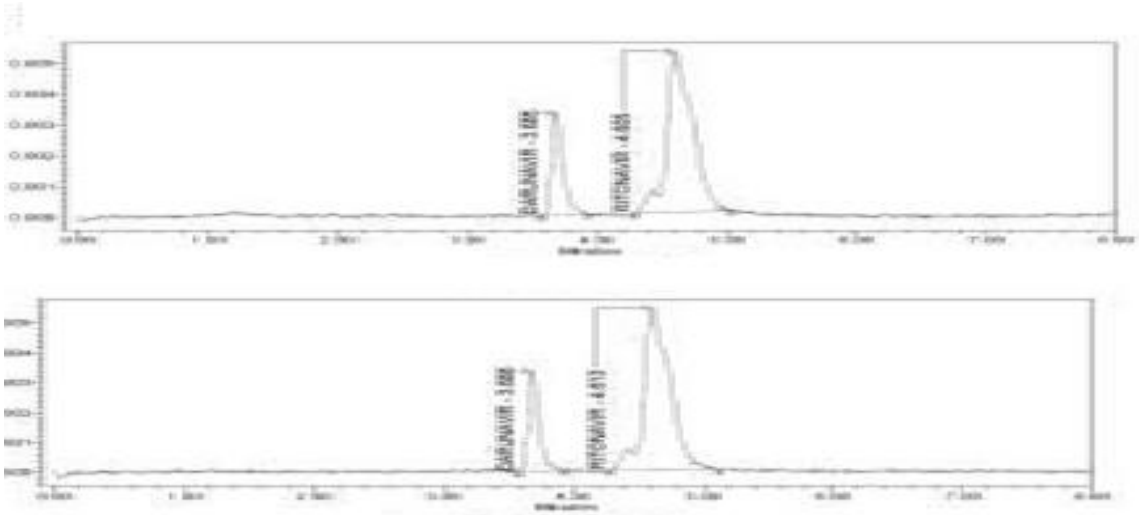


Figure.9.0:-Optimization of chromatogram in mobile phase:-

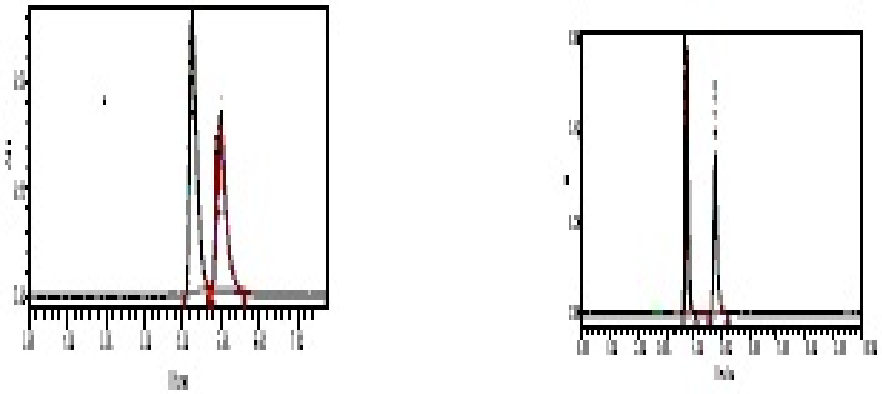


Figure.10.-chromatogram of Blank:-

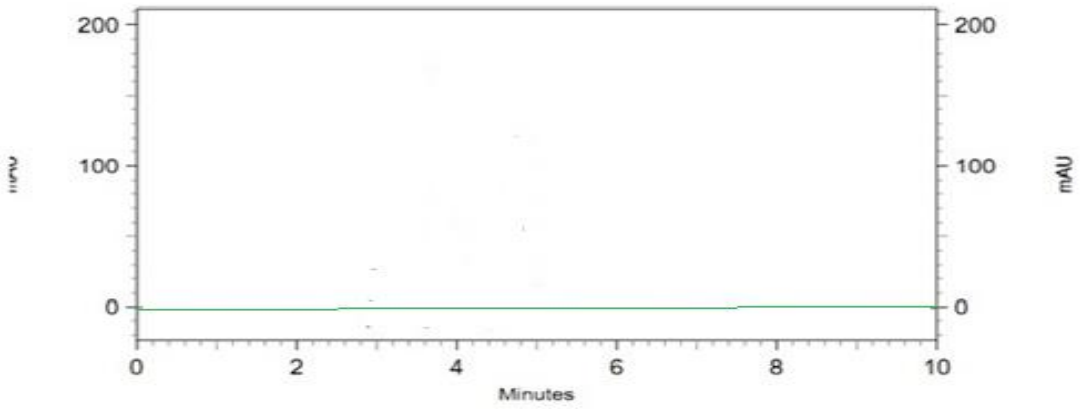
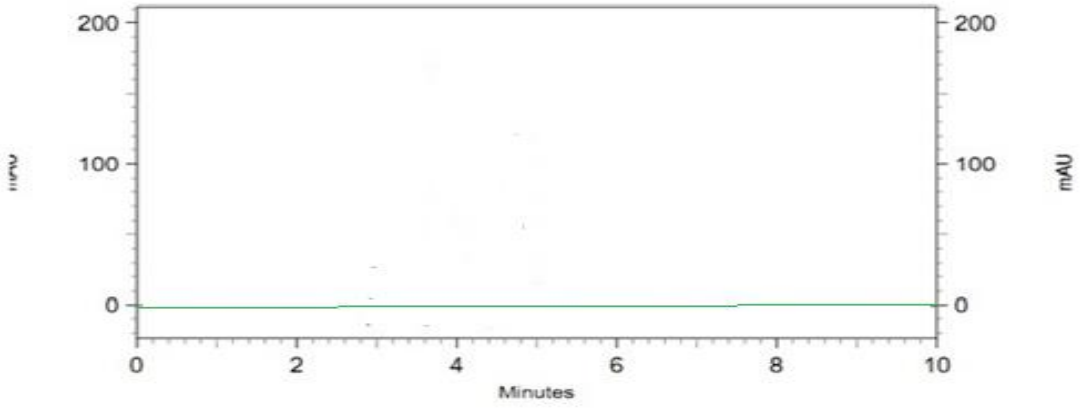


Fig.4: chromatogram of Blank

Validation of the Developed Method as per ICH Guidelines

6.1: Specificity

The Specificity of the method showed good separation of the standard Ritonavir and Darunavir. It is show that it is not affected by the other exipients and additives. This indicates that the analytical method is specific to drug .The investigation of RTV &DRV 25µg/ml & 200 µg/ml easily detected from matrices (n=6) at 10 min run time. All the chromatograms were analyzed and found to be free from interference with RTV & DRV.



Linearity and Calibration Curve of Ritonavir

The linearity was tested in the concentration range of 5-50 µg/ml and the calibration curve was constructed and evaluated by its correlation coefficient. The correlation coefficient (r^2) for all the calibration curves was consistently greater than 0.997 represented in **Figure11.**

Table 7: Standard curve data for Ritonavir

Sl. No	Concentration(µg/ml)	Peak Area	Correlation coefficient (r^2)
1.	25	1381833	1.0
2.	35	2070296	
3.	45	2767626	
4.	55	3455671	
5.	65	4158907	
6.	75	2070296	

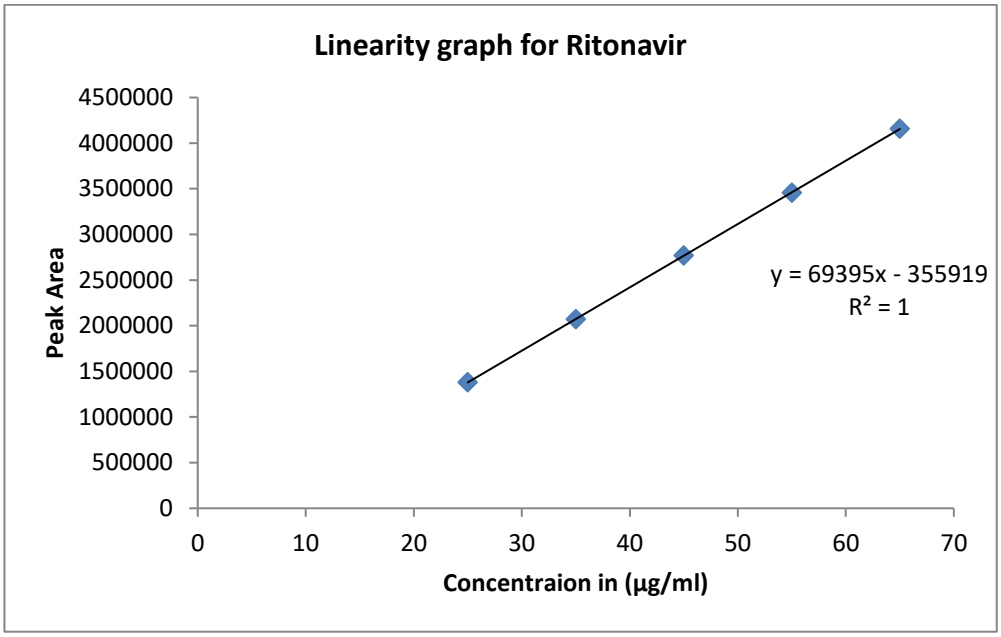


Fig.11: Linearity graph for Ritonavir

Observation:

The standard linearity for Ritonavir was generated from 25µg/ml to 75 µg/ml. R^2 was found to be 1.0 with y = 69395.x - 35591..

Linearity and Calibration Curve of Darunavir

The linearity was tested in the concentration range of 200-600 µg/ml and the calibration curve was constructed and evaluated by its correlation coefficient. The correlation coefficient (r^2) for all the calibration curves was consistently greater than 0.997 represented in **Figure12.**

Table 8: Standard curve data for Darunavir

Sl. no	Concentration(µg/ml)	Area	Correlation coefficient (r^2)
1.	**200**	**1713323**	1.0
2.	**300**	**2566960**	
3.	**400**	**3429686**	
4.	**500**	**4277480**	
5.	**600**	**5135870**	
6.	**200**	**2566960**	

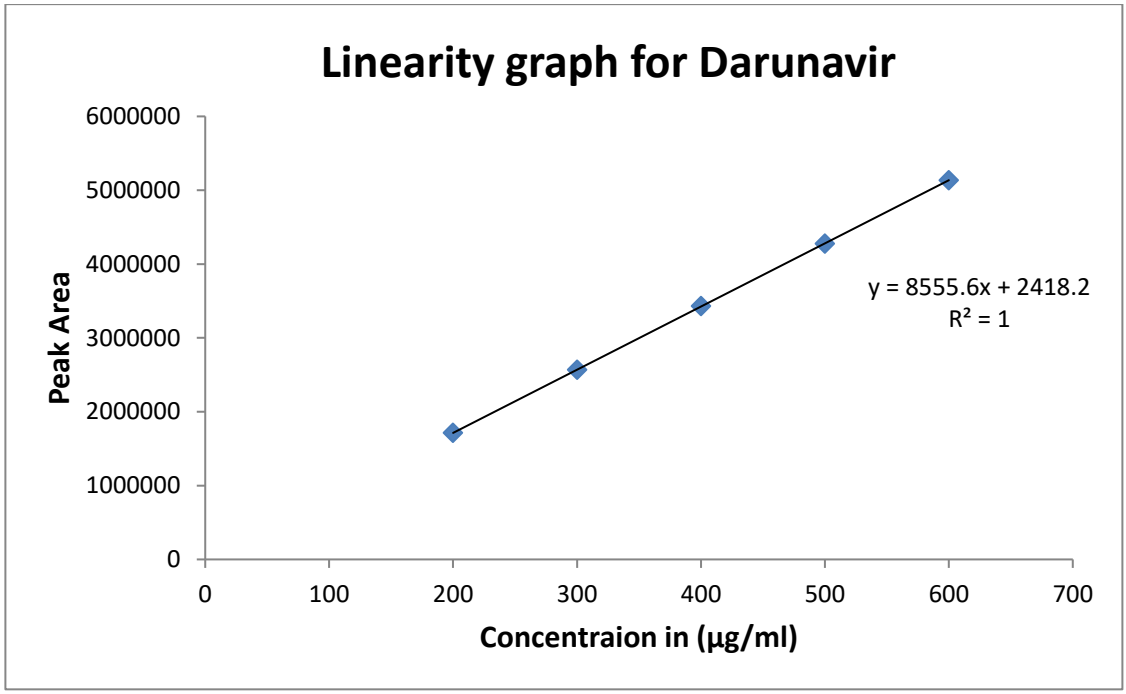


Fig.12: Linearity graph for Darunavir

Observation:
The standard linearity for Darunavir was generated from 25µg/ml to 200 µg/ml. R^2 was found to be 1.0 with y = 8555x + 2418.

6.3: Accuracy:
Accuracy of Ritonavir:
A known quantity of standard solution has been added to the sample solutions previously analyzed at three different levels (50%, 100% and 150%). The amount recovered for RTV and LPV has been calculated for three concentrations. 20 µL of each working standard solution ranging from 25µg/ml, 40µg/ml & 60µg/ml of Ritonavir were injected into UFLC. Retention time and peak area were obtained were recorded and Direct recovery study was calculated:

Table 9: Accuracy data of Ritonavir.

Accuracy data of Ritonavir										
Sl.No	**Level of % recovery**	**Amount of drug taken (µg/ml) (STD)**	**Amount of drug added (µg/ml) (sample)**	**Total amount of drug (n=3)**	**Peak area**	**Conc. found**	**SD**	**Mean conc.**	**% Assay**	**% RSD**
1.	50	25	12.5	37.5	1381833	37.14	0.14	37.14	99.04	0.37
					1381733	36.88				
					1382933	37.1				
2.	100	25	25	50	1381833	49.5	0.56	50.11	100.22	1.11
					1381833	50.25				
					1381833	50.60				
3.	150	25	36.5	61.5	1381833	61.5	0.56	61.52	100.03	0.91
					1381833	60.98				
					1381833	62.1				

STD- Standard, SD- Standard Deviation, RSD- Relative Standard Deviation

Accuracy of Darunavir:
A known quantity of standard solution has been added to the sample solutions previously analyzed at three different levels (50%, 100% and 150%). The amount recovered for DRV has been calculated for three concentrations. 20 µL of each working standard solution ranging from 200µg/ml, 400µg/ml & 500µg/ml of DRV were injected into UFLC. Retention time and peak area were obtained were recorded and direct recovery study was calculated.

Table 10: Accuracy data of Darunavir.

Accuracy data of Darunavir										
Sl.no	**Level of % recovery**	**Amount of drug taken (µg/ml) (STD)**	**Amount of drug added (µg/ml) (sample)**	**Total amount of drug(n=3)**	**Peak area**	**Conc. found**	**SD**	**Mean conc.**	**% Assay**	**% RSD**
1.	50	200	100	300	**1713323**	300.15	0.62	299.72	99.9	0.20
					1713323	300.02				
					1713323	299.01				
2.	100	200	200	400	3418686	400.2	0.47	400.33	100.0	0.11
					3329686	400.5				
					3459686	400.30				
3.	150	200	300	500	4287480	500.19	0.52	500.76	100.1	0.103
					4197480	500.91				
					4259480	501.2				

STD- Standard, SD- Standard Deviation, RSD- Relative Standard Deviation

Precision:
Successive six injections of 20 µL of working standard solution of RTV and DRV (25µg/mL and 200µg/mL) were injected simultaneously at different intervals in to a UFLC.

The chromatograms obtained recorded and peak area was noted. From the peak area and concentration of RTV & DRV was determined and % RSD also calculated. The result table is show in **Table 11 & Table 12**

Table 11: Repeatability Data Ritonavir

Repeatability	**Conc. of Ritonavir = 25µg/mL**	
Injection No	**Retention Time**	**Peak Area**
Replicate 1	4.697	1381833
Replicate 2	4.785	1371833
Replicate 3	4.776	1331833
Replicate 4	4.765	1328833
Replicate 5	4.755	1391833
Replicate 6	4.657	1381733
Mean	4.73	1364650
SD	0.050	27340.11
%RSD	1.057	2.003

Observation:
The % RSD for the repeatability study was found to be 1.057 for Retention Time and 2.003 for Peak Area of Ritonavir respectively. Therefore, the precision of the analytical method was found to be within the acceptance limits. **Acceptance Criteria:** The %RSD for repeatability not more than 2.0%

Table 12: Repeatability Data Darunavir

Repeatability	**Conc. of Darunavir = 200µg/mL**	
Injection No	**Retention Time**	**Peak Area**
Replicate 1	3.83	**1713323**
Replicate 2	3.86	**1715623**
Replicate 3	3.81	**1713823**
Replicate 4	3.81	**1713923**
Replicate 5	3.81	**1714323**
Replicate 6	3.81	**1716323**
Mean	3.82	21949.33
SD	0.022	1163.901
%RSD	0.575	0.8962

SD- Standard Deviation, RSD- Relative Standard Deviation

Observation:
The % RSD for the repeatability study was found to be 0.575 for Retention Time and 0.8962 for Peak Area of Darunavir respectively. Therefore, the precision of the analytical method was found to be within the acceptance limits.

Acceptance Criteria:
The %RSD for repeatability not more than 2.0%

Detection Limit (Lod):
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD and LOQ of Darunavir, Ritonavir, are performed and calculated by below formula.
The detection limit (DL) may be expressed as:
$DL = 3.3\ \sigma/S$
Where σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte. The results are show in **Tab 13 & 14**

Quantitation Limit (Loq):
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The quantitation limit (QL) may be expressed as:
$QL = 10\ \sigma/S$
Where σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte. The results are show in **Table 13 & 14.**

Table no 13: Ritonavir

Sl. No		
1	Standard deviation	27340.11
2	Slope	69395.23
3	LOD	1.30µg/ml
4	LOQ	3.94µg/ml

Table no 14: Darunavir

Sl. No		
1	Standard deviation	1163.901
2	Slope	8555.64
3	LOD	0.44µg/ml
4	LOQ	1.36 µg/ml

Range:
Observation:
RTV and DRV 25-60 µg/ml and 200-500 µg/ml range corresponding to 50% to 150% was used for Accuracy and Precision study and %RSD was found to be less than 2.

Acceptance criteria:
The acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained as per the above criteria, in addition to that yields of precision is ≤ 2% RSD

Summary:
The developed method was validated as according to the ICH guidelines for the quantification of **Ritonavir**, and **Darunavir**, in pharmaceutical substance.
In this RP-HPLC greener method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with Methanol and 0.1M potassium dihydrogen phosphate (60:40, v/v) at flow rate of 1.0 mL/min was found to be robust method.
A suitability test was performed to various system suitability parameters and the results found within acceptable limits of tailing factor ≤ 2.0 and theoretical plates >2000.
The calibration curve was constructed with series of concentration for the ritonavir and Darunavir in the range from 25-75 µg/mL ,and 200-600 µg/mL This calibration data made the conclusion that the method was linear throughout the range selected.
Specificity was studied for the quantification of interference substances /excipients in diluent. From the results it was indicated that none of interference substances /excipients were interfere at analytes retention time. Hence the developed method was specific.
The precision of the method was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases % RSD was calculated and results were the acceptable limits. The low values of RSD indicate that the method was precise.
A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be within acceptance limits.

The results of total hazard values (tHV) and total analytical hazard values (taHV) calculation

	Compound name	CAS number	tHV	TaHV
Hydrocarbons	Pentane	109-66-0	23.4	36.4
	Hexane	110-54-3	53.8	81.4
	Cyclohexane	110-82-7	57.9	79.8
	Heptane	142-82-5	15.2	20.9
	Isooctane	540-84-1	57.1	79.2
	Benzene	71-43-2	84	122
	Toluene	108-88-3	43.9	60.5
	Xylenes	1330-20-7	51.5	68.2
Alcohols	Methanol	67-56-1	8.8	15.7
	Ethanol	64-17-5	4.1	7.2
	Isopropanol	67-63-0	3.9	6.8
	Heptanol	111-70-6	22.9	28.6
	Octanol	111-87-5	38.3	45.7
	Nonanol	143-08-8	39.8	46.7
	Benzyl alcohol	100-51-6	45.2	55.1
Ethers	Diethyl ether	60-29-7	8.7	16
	Methyl tert-butyl ether	1634-04-4	2.7	3.8
	Tetrahydrofuran	109-99-9	9.1	14.9
Aldehydes	Furfural	98-08-1	47.7	69.2
	Benzaldehyde	100-52-7	56.9	77.5
Ketones	Acetone	67-64-1	1.5	2.6
Organic acids	Formic acid	64-18-6	25.8	43
	Acetic acid	64-19-7	1.3	2.1
	Propionic acid	79-19-4	27	41.2
Esters	Ethyl acetate	141-78-6	5	7.3
Chlorinated hydrocarbons	Dichloromethane	75-09-2	39.3	59.8
	Chloroform	67-66-3	70.7	103.8
	Carbon tetrachloride	56-23-5	80	109.7
	Trichloroethylene	79-01-6	90.9	125.1
	Tetrachloroethylene	127-18-4	82.7	107.7
	1.1.1-Trichloroethane	71-55-6	34.7	49
	1.1.2.2-Tetrachloroethane	79-34-5	76.9	97.3

By the above table we can make concluded that the use of methanol as mobile phase in the determination of ritonavir and Darunavir is less toxic to environment and analyst as it has the tHV of 8.8 and TaHV of 15.7, which fulfills the principle of the Green chromatography. By reducing the amount of the toxic chemicals consumed and wastages generated during the method development find more environmental friendly and cost effective compared to the other toxic.

Thus, we recommended the solvents like, ethyl acetate, ethanol, and methanol as safer solvent in term of safety in total analytical hazard.

Conclusion:

The method developed in present investigation is novel UFLC method for Ritonavir and Darunavir, using eco friendly solvent by Greener chromatography. This method is simple, precise and accurate and safer to analyst, environment which is adopdetd for the determination of Ritonavir and Darunavir with the absence of additional peaks in the chromatogram indicating that there is no interference of the common impurities in the manufacturing of drugs. This method is useful in quantification of Ritonavir and Darunavir in marketed formulation. This methodology can be applied to the quantification of drugs in other physiological matrices, such as plasma and urine. This method can reduce the environmental impact from other mobile phases like acetonitrile and methanol.

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