IJPSR (2022), Volume 13, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 February 2022; received in revised form, 09 April 2022; accepted, 27 April 2022; published 01 September 2022

AN ECO-FRIENDLY HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ABACAVIR, DOLUTEGRAVIR AND LAMIVUDINE IN ACTIVE PHARMACEUTICAL INGREDIENTS AND MARKETED TABLET FORMULATION

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Keywords:

Abacavir, Dolutegravir and Lamivudine, Bulk drug, Formulation, Eco-friendly, HPLC

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ABSTRACT: Selective and novel method has been optimized to evaluate Abacavir, Dolutegravir and Lamivudinein bulk and formulation by HPLC. The principle analytes were eluted with the conditions of the mobile phase having the Ethanol: Ethyl acetate (80:20, % v/v) using the Lichrosphere RP C8 column (Phenomenex, USA (250 x 4.6 mm, 5µ) analytical column with the 1.0 ml/min flow rate and 10µl sample volume at 260 nm in UV detector. The retention times of Abacavir, Dolutegravir and Lamivudine were 2.31 min, 3.120 min and 4.59min with a total run time of 6 min. The curve indicates correlation coefficient (r^2) was superior by having a value nearer to 1.000 with a linear range of 40 µg/ml-130.0 µg/ml for Abacavir, Dolutegravir and Lamivudine. The correlation coefficient (r^2) 0.9971for Abacavir, 0.9979 for Dolutegravir and 0.9947 for Lamivudine were found. The LOD and LOQ for the Abacavir, Dolutegravir and Lamivudine were found at 1.40 µg/ml, 3.01µg/ml, 5.84µg/ml, and 4.25 µg/ml, 9.12 µg/ml and 17.71 µg/ml. The developed method was applied for the bulk and formulation.

INTRODUCTION: Abacavir, which is chemically called 1S,cis-4-6- cyclopropyl-2-amino-9H-purin-9 -yl-2-cyclopentene-1-methanol sulphate (abacavir), is a C7- cyclic purine that is transformed to 9-dihydro-2 residues sulfurate and then to 9-amino-7-(dichloro)adenosine. The nano-particle transforms to the activated neurotransmitter carbovir triphosphate if the liver absorbs it. Carbovir triphosphate is an analogue that represents deoxyguanosine-5`-triphosphate (dGTP). Carbovir triphosphate is an antiviral medication used to block HIV-1 reverse transcriptase (RT).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(9).3683-89

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(9).3683-89

The drug is also a powerful inhibitor of RT and, therefore a macromolecule that gets integrated into the viral DNA. The chemical compound Dolutegravir (DTG) 1 which is chemically (4R, 12aS) - 9 - {[(2, 4difluorophenyl) methyl] carbamoyl}-4 - methyl-6, 8-dioxo-3, 4, 6, 8, 12, 12a-hexahydro-2 Hpyrido[1',2':4, 5] pyrazino [2,1-b] [1,3] oxazin-7-olate, might disrupt the retroviral DNA integration steps. This is seen as a benefit because within this chemical compound; there is a mechanism that can block retroviral DNA integration, which is essential to HIV infection.

Lamivudine (3TC) 1 is a compound that is chemically [2R, cis]4-amino-1-(2hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (*i.e.* a synthetic nucleoside analogue). Like several proteins, the HIV-1 RT is phosphorylated to the active 5′-triphosphate metabolite, lamivudine triphosphate (L-TP).

The key mode of action of L-TP is the rapid inhibition of HIV-1 RT and the subsequent termination of viral DNA chains [1-4]. Based on the literature survey, there was no Eco-friendly analytical method for this formulation, *i.e.*, Abacavir, Dolutegravir and Lamivudine. Several methods were developed for Abacavir, Dolutegravir and Lamivudine with combinations [5-25]. For the Abacavir, Dolutegravir and Lamivudine combination, there was a lack of an eco-friendly analytical method for identifying and quantifying bulk and formulation. And there was no sensitive Eco-friendly analytical method having the 40 µg/ml detectability to quantify the product traces of the manufacturing area when the product change is over.

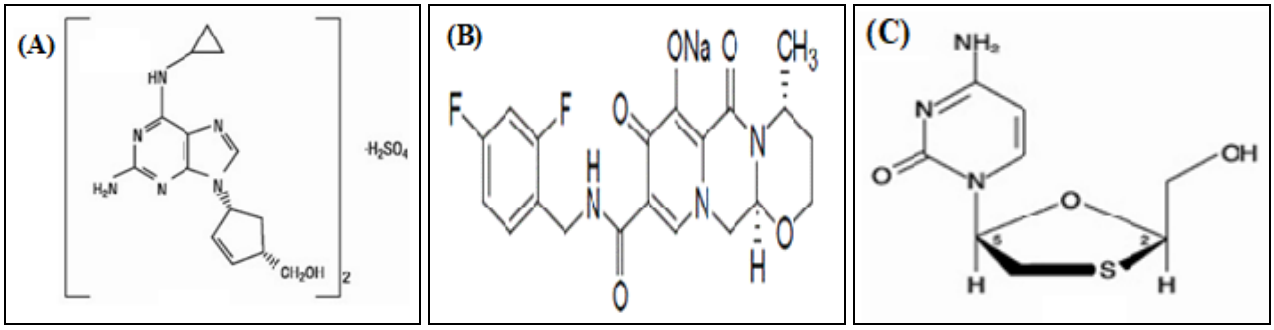

FIG. 1: CHEMICAL STRUCTURES OF ABACAVIR (A), DOLUTEGRAVIR (B), LAMIVUDINE (C)

MATERIALS AND METHODS: Abacavir, Dolutegravir and Lamivudine **Fig. 1**, high purity Ethyl acetate (J. T. Baker, Phillipsburg, NJ, USA), Ethanol (HPLC grade, Sigma Aldrich).

Preparation of Standard Solution: Abacavir, Dolutegravir and Lamivudine standards stock solution prepared by taking10 mg in 10 ml volumetric flask then adding 10 ml ethanol and sonicated for 3 min. Then makeup to 10ml with the ethanol.

Preparation of Mobile Phase: Added 500 ml of ethanol to the 500 ml of Ethyl acetate, degassed to prepare 1000 ml of the mobile phase.

Optimization of Chromatographic Conditions: After series of trials, the chromatographic conditions were accomplished with the Ethanol: Ethyl acetate (80:20, % v/v) by utilizing the stationary phase Lichrosphere RP C8 column (Phenomenex, USA (250 x 4.6 mm, 5µ) Spherisorb C_{18}, 5 µ.m, 4.6 mm x150 mm to obtain the best peak shape. The Abacavir, Dolutegravir and Lamivudine separation was good at 260nm with a column temperature 25°C and sample compartment temperature 10° C with the flow 1.0 ml/min with the sample volume 10µl.

Assay Sample Preparation: One tablet has Abacavir 60 mg, Dolutegravir 30 mg, and Lamivudine 60 mg into 1000 ml volumetric flask and dissolved in the diluent and make up to the 100 ml. This preparation is considered a stock solution. From the stock solution, take 1ml to 10 ml in a volumetric flask and make up to the mark with the diluents and filter.

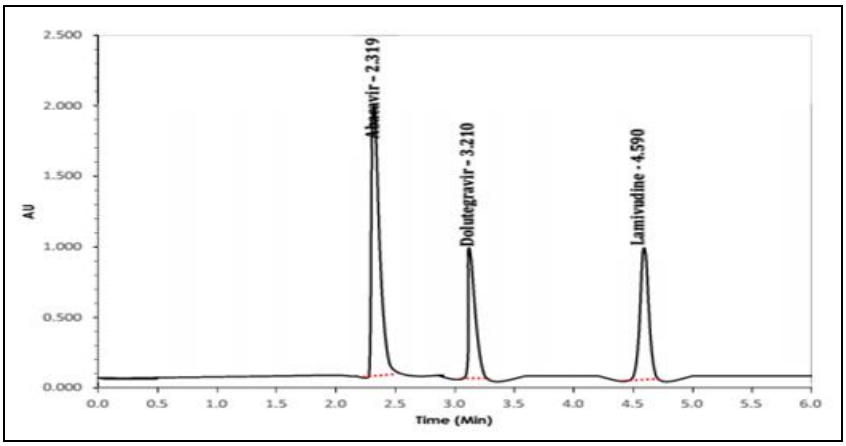

FIG. 2: TEST SAMPLE CHROMATOGRAM

Validation of Analytical Methods: Validation was performed for the developed method with the stringent limit to prove the efficiency of this method [26].

System Suitability: To verify the system produces the consistent results with the optimized method injected the standard six times with the criteria of % RSD for retention time and area NMT 2.0%, theoretical plates NLT 3000 plates, tailing factor NMT 1.5 and resolution NLT 4.

TABLE 1: SYSTEM SUITABILITY PARAMETERS

Parameter	Compound	Result
Retention Time	Abacavir	2.31 min
	Dolutegravir	3.21 min
	Lamivudine	4.59 min
Peak Area	Abacavir	294621
	Dolutegravir	867991
	Lamivudine	512422
Theoretical plates	Abacavir	5120
	Dolutegravir	4200
	Lamivudine	12013
Tailing factor	Abacavir	0.72
	Dolutegravir	0.64
	Lamivudine	0.32
Resolution	Abacavir	-
	Dolutegravir	5.61
	Lamivudine	6.53
% Rsd	Abacavir	0.53
	Dolutegravir	0.17
	Lamivudine	0.23

Selectivity: To verify the method validation in terms of the selectivity and exactness, injected triplicate preparations of 100 % concentration, *i.e.*, 100 µg/ml of Abacavir, Dolutegravir and Lamivudine.

Then injected one blank to prove the method did not have the carryover issue. The specificity's limit is that it should pass the system suitability criteria, and there should not be an RT shift for all three preparations.

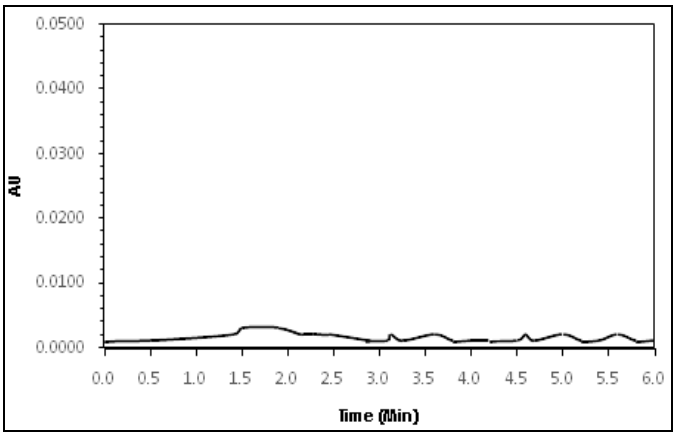

FIG. 3: BLANK CHROMATOGRAM

Precision: After passing the specificity and system suitability criteria, the method was verified for the system precision and method precision with the limit of % RSD for the retention time and area NMT 2%.

The intermediate precision was verified the next day with another column by following the limit as % RSD for the retention time and the area should be NMT 2%.

TABLE 2: PRECISION AND ACCURACY OF DATA

Intraday precision						
	Abacavir		Dolutegravir		Lamivudine	
Mean	293930	97.48	861297	97.40	502985	99.80
SD	1222.49	0.74	8167.69	1.20	8934.62	1.80
% RSD	0.42	0.76	0.95	1.23	1.78	1.80
Intermediate precision						
	Abacavir		Dolutegravir		Lamivudine	
Mean	294904	96.61	865243	96.20	507801.17	98
SD	2154.36	0.74	2056.04	1.22	5110.41	1.02
% RSD	0.73	0.76	0.24	1.27	1.01	1.04

Accuracy and Recovery: To verify the method's accuracy, triplicate preparations were prepared at 80% and 100%, and 120% levels of 100 % concentration by spiking the standard into the diluent. Calculated the recovery with the acceptance criteria of 95-105%.

TABLE 3: RECOVERY DATA

Abacavir			Dolutegravir			Lamivudine		
Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
97.71	1.11	1.14	97.87	1.42	1.45	98.36	1.10	1.12
98.59	0.96	0.97	96.59	1.30	1.35	97.24	1.06	1.09
97.27	1.78	1.83	96.60	1.42	1.47	96.12	1.60	1.66

Linearity: The method linearity was verified with the six concentrations of 100 % concentration as 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml, 120 µg/ml, and 130 µg/ml for the Abacavir, Dolutegravir and Lamivudine with the acceptance criteria of the regression coefficient (R^2) NLT 0.99.

To verify the method efficiency when the minor changes happened in the optimized method parameters like mobile phase composition, flow rate and wavelength parameters were performed with the criteria, it should pass the system suitability criteria.

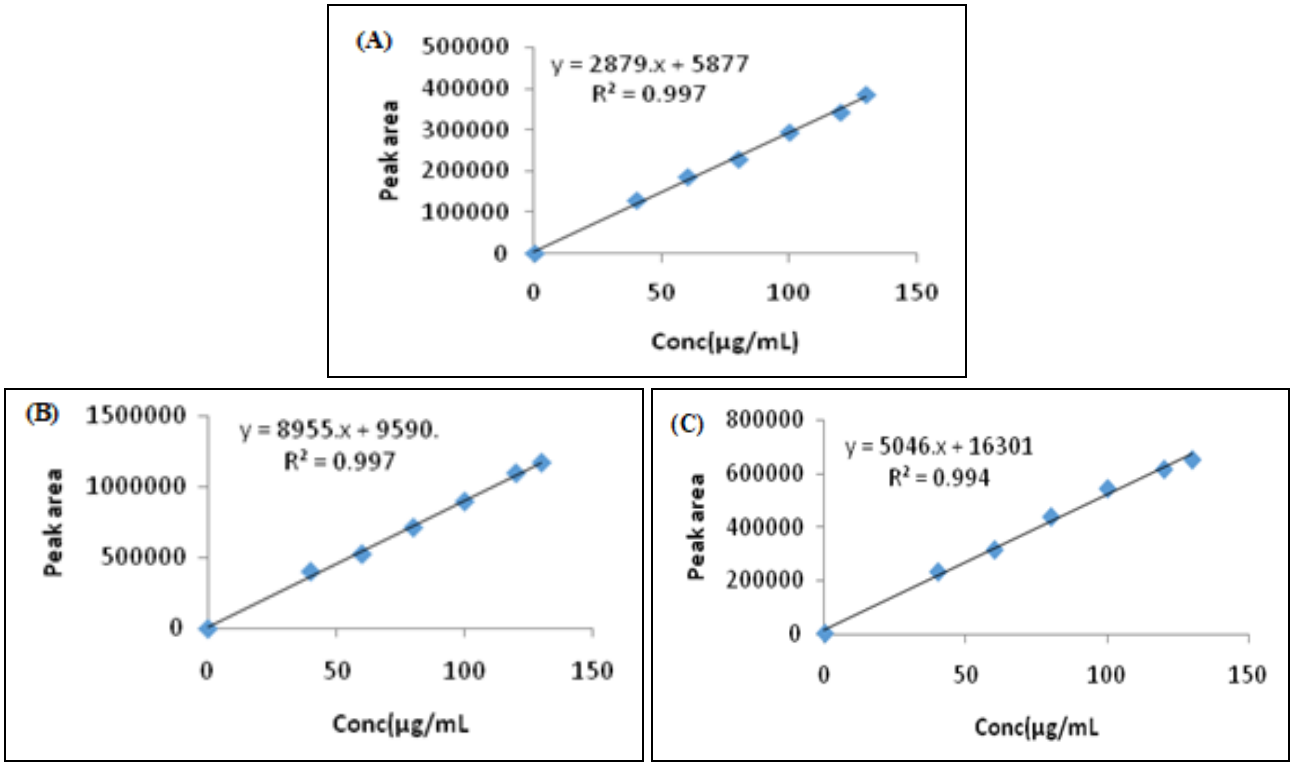

FIG. 4: LINEARITY DATA OF ABACAVIR (A), DOLUTEGRAVIR (B), LAMIVUDINE (C) ROBUSTNESS

TABLE 4: ROBUSTNESS EVALUATION OF CHROMATOGRAPHIC METHOD

Compound	Altered conditions	Condition	System suitability parameters							
			Area	% Change	RT	% Change	N	% Change	Tailing factor	% Change
Abacavir	Mobile Phase composition (±2%)	A = 80%	294621	0.38	2.310	-0.43	5120	-0.04	0.710	-1.41
		a = 70%	293511		2.320		5122		0.720	
	Wavelength(±2nm)	A = 260 nm	289512	-1.76	2.310	0.87	5110	-0.20	0.730	1.37
		a = 262 nm	294611		2.290		5120		0.720	
	Flow rate ±0.2mL	A = 1.0 mL/min	294621	0.34	2.300	-0.87	5210	-0.38	0.710	-1.41
		a = 1.2 mL/min	293620		2.320		5230		0.720	
Dolutegravir	Mobile Phase composition (±2%)	A = 80%	856814	0.11	2.300	-0.87	5140	-0.21	0.640	1.56
		a = 70%	855914		2.320		5151		0.630	
	Wavelength (±2nm)	A = 260 nm	854814	-0.23	2.300	-0.87	5260	-0.19	0.610	-1.64
		a = 262 nm	856814		2.320		5270	-0.19	0.620	
	Flow rate ±0.2mL	A = 1.0 mL/min	853814	-0.35	2.330	0.43	5180		0.642	-0.16
		a = 1.2 mL/min	856814		2.320		5190		0.643	
Lamivudine	Mobile Phase composition (±2%)	A = 80%	512422	0.19	2.300	-0.43	5300	-0.19	0.320	-0.31
		a = 70%	511432		2.310		5310		0.321	
	Wavelength (±2nm)	A = 260 nm	520442	0.57	2.330	0.43	5220	-0.19	0.323	0.93
		a = 262 nm	517472		2.320		5230		0.320	
	Flow rate ±0.2mL	A = 1.0 mL/min	515481	-0.19	2.300	-0.43	5240	-0.19	0.311	-1.29
		a = 1.2 mL/min	516482		2.310		5250		0.315	

A= Average values obtained at nominal concentration; a = Average values obtained at altered conditions.

Lower Level of Quantification: By considering the 10% concentration of the target concentration, injected the sample into the system with the acceptance criteria S/N ration NLT 10. The LOQ concentration was injected with the different concentration preparation to identify the detectability with the acceptance criteria 3:1 and minimum detectability five times out of six injections from the same concentration.

Lower Level of Quantification Precision: LOQ precision verified with the limit NMT 2.0% for the RT and area.

Assessment of Stability of Standard: The prepared standards were verified up to 72 h for stability at room temperature and refrigerated conditions.

Filter Compatibility: To evaluate the impact of PVDF and Nylon filters on the assay results, the samples were analyzed after passing through the filters.

TABLE 5: FILTER COMPATIBILITY AND ASSAY OF TEST FORMULATION

Filter type	Label claimed (mg/Tab)		
PVDF	**Abacavir**	**Dolutegravir**	**Lamivudine**
	600	50	300
	Conc. found (mg/Tab)		
	594.26	49.32	296.18
	% Assay		
	99.04	98.64	98.73
Filter type	**Label claimed (mg/Tab)**		
Nylon	**Abacavir**	**Dolutegravir**	**Lamivudine**
	600	50	300
	Conc. found (mg/Tab)		
	593.94	48.75	298.82
	% Assay		
	98.99	97.50	99.61

RESULTS: There was a clear separation and good resolution, and without any carryover was achieved with this method, as shown in **Fig. 1** and **2**. The system suitability acceptance criteria were also satisfactory, as shown in **Table 1**. For the system precision parameter, the %RSD of RT and area for the Abacavir, Dolutegravir and Lamivudine achieved 0.53%, 0.17, and 0.23% as shown in **Table 3** against the limit NMT 2.0%. The linearity parameter was quantified by peak area *vs.* concentration methodology. Different concentrations from 40µg/ml to 130 µg/ml standard solutions for Abacavir, Dolutegravir and Lamivudine were prepared and injected into the system. The calculated regression coefficient for Abacavir, Dolutegravir and Lamivudine is nearer to 1.000 as shown in **Fig. 4.**

For the method precision parameter, the % RSD of area for the Abacavir, Dolutegravir and Lamivudine achieved 0.73%, 0.24 and 1.01 % against the limit NMT 2.0%. The method was verified for the ruggedness as intraday and interday precision. For the intermediate precision parameter, the %RSD of area for the Abacavir, Dolutegravir and Lamivudine achieved in day-1 as 0.76%, 1.23%, and 1.80% on the next day (Day-2)0.76%, 1.27% and 1.04% against the limit NMT 2.0% as shown in **Table 2.**

The recovery for the 80%, 100%, and 120% was more than 95% against the acceptance criteria of 95-105% as shown in **Table 3**. To evaluate the method's capability of producing precise results with the minor variations of flow, mobile phase composition, and wavelength variations as robustness was performed. The results were shown in **Table 4**. The results proved that the method was stable to produce consistent results with the minor variation of the method parameters.

The LOQ and LOD were identified by injecting the lower concentration of 40 µg/ml with the S/N ratio criteria. The LOQ for the Abacavir, Dolutegravir and Lamivudine was 1.40 µg/ml, 3.01 µg/ml and 5.84 µg/ml. The LOD for the Abacavir, Dolutegravir and Lamivudine was 4.25 µg/ml, 9.12 µg/ml and 17.71 µg/ml. Based on the stability results, the standards were stable up to 72 h at room temperature and refrigerated conditions. The compatibility of the filters was verified with the PVDF and Nylon filters. The assay of the Abacavir, Dolutegravir and Lamivudine was more accurate (99.04% for Abacavir, 98.64% for Dolutegravir and 98.73% for Lamivudine) with the PVDF filter compared to Nylon filter (98.99% for Abacavir, 97.50% for Dolutegravir and 99.61% for Lamivudine) as shown in **Table 5.**

DISCUSSION: During method optimization, organic solvents were initially used as mobile phases in different compositions. But three compounds were not detected. Then, an organic solvent such as ethanol and ethyl acetate were used

in different ratios with the Lichrosphere RP C8 column (Phenomenex, USA (250 x 4.6 mm, 5µ). Finally, the method was found to be optimized with the conditions of mobile phase (ethanol and ethyl acetate (80:20 % v/v), wavelength 260 nm, flow rate of 1.0 ml/min, column temperature of 25°C, sample compartment temperature of 10°C, a sample volume of 10µl. With this method, both actives *i.e.* Abacavir, Dolutegravir and Lamivudine eluted at 2.31 min, 3.120 min, and 4.59 with good resolution and symmetry.

After the method optimization, the method was validated as per ICH guidelines. As per the results obtained in the method validation, there was no interference of the blank and carryover problem even at the LOQ level quantification. Both LOQ and LOD of this method were verified practically in the instrument with S/N ratio criteria. The results were found satisfactory. Based on the recovery results, it proves that the method has the capability of high extraction efficiency (NLT 90%). The method was successfully applied to the assay of dosage forms to verify filter capability. The assay results show satisfactory and free from the interference of nylon and PVDF filters.

CONCLUSION: Based on the results obtained, the developed method was very sensitive, accurate, linear, and economical. Due to the short time of the chromatographic program, more samples can be analyzed within the short period, which will be helpful in the industry at the time of multiple products manufacturing continuously. The method met all the predefined acceptance criteria. The bulk and formulation samples can be analyzed in various dosage forms containing Abacavir, Dolutegravir and Lamivudine.

ACKNOWLEDGEMENT: The authors are grateful to The authors are thankful to Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, A.P, India, for providing technical assistance and necessary facilities.

CONFLICTS OF INTEREST: Authors assuring that there are no conflicts of interest.

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How to cite this article:
Nagaraju G, Agarwal R and Nadendla RR: An eco friendly HPLC method for the simultaneous estimation of abacavir, dolutegravir and lamivudine in active pharmaceutical ingredient and marketed tablet formulation. Int J Pharm Sci & Res 2022; 13(9): 3683-89. doi: 10.13040/IJPSR.0975-8232.13(9).3683-89.