IJBPAS, November, 2023, 12(11): 5062-5071

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A NOVEL ENVIRONMENT-FRIENDLY HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ANTIVIRAL DRUGS IN BULK AND PHRMACEUTICAL DOSAGE FORM

GOLLAPALLI NAGARAJU[1*], RITESH AGRAWAL[2] AND RAMA RAO NADENDLA[3]

1: Research Scholar, Department of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Pacific Hills, Pratap Nagar Extension, Debari, Udaipur-313024, Rajasthan

2: Faculty of Pharmacy, Pacific Academy of Higher Education and Research, Pacific Hills, Airport Road, Pratap Nagar Extension, Debari, Udaipur- 313024, Rajasthan

3: Principal, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh-522 034

[*]Corresponding Author: Mr. Gollapalli Nagaraju: E Mail: rajaneeraja@gmail.com

Received 19th Sept. 2022; Revised 16th Oct. 2022; Accepted 27th Feb. 2023; Available online 1st Nov. 2023

https://doi.org/10.31032/IJBPAS/2023/12.11.7545

ABSTRACT

A novel, simple method was developed to evaluate Abacavir, Dolutegravir, and Lamivudinein bulk and formulation by RP-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 injection volume at 260 nm in UV detector. The retention times of Abacavir, Dolutegravir, and Lamivudine were 2.31min, 3.120 min, and 4.59min with a total run time of 6 min. The curve indicates correlation coefficient (*r2*) 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 (*r2*) 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 in routine analysis.

Keywords: Abacavir, Dolutegravir, Lamivudine, HPLC

INTRODUCTION

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 **[5-21]**. 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.

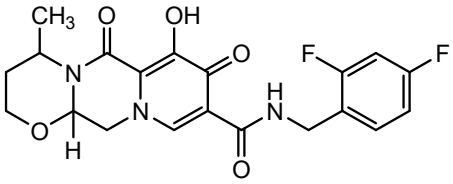


Figure 1: Dolutegravir

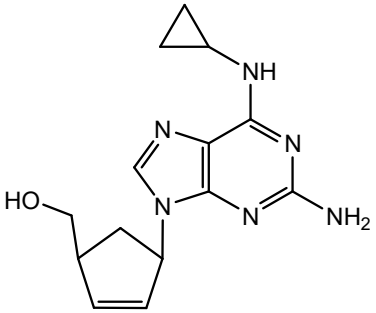


Figure 2:Abacavir

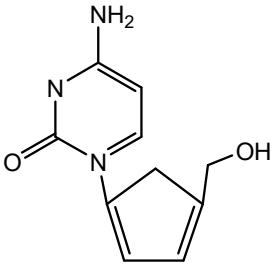


Figure 3:Lamivudine

MATERIALS AND METHODS:

Dolutegravir, Abacavir and Lamivudine **Figure 1, 2, 3**, 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 C18, 5 µm, 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.

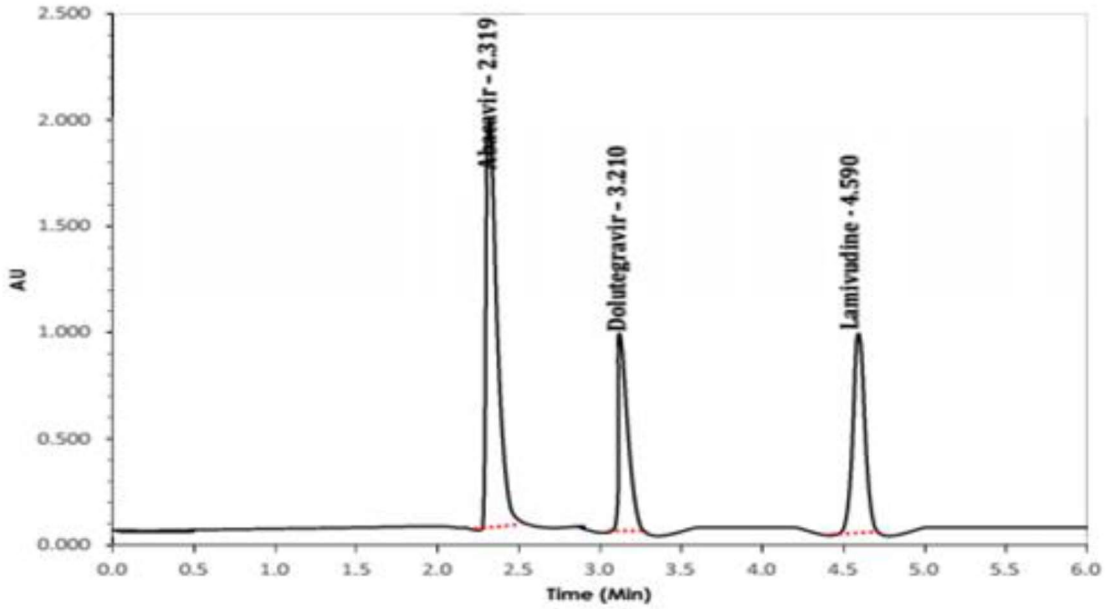


Figure 4: Test sample chromatogram

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

Compound	Retention Time	Peak Area	Theoretical plates	Tailing factor	%RSD
Abacavir	**2.31 min**	**294621**	**6524**	**1.12**	**0.54**
Dolutegravir	**3.21 min**	**867991**	**7589**	**1.04**	**0.85**
Lamivudine	**4.59 min**	**512422**	**4265**	**0.92**	**0.12**

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.

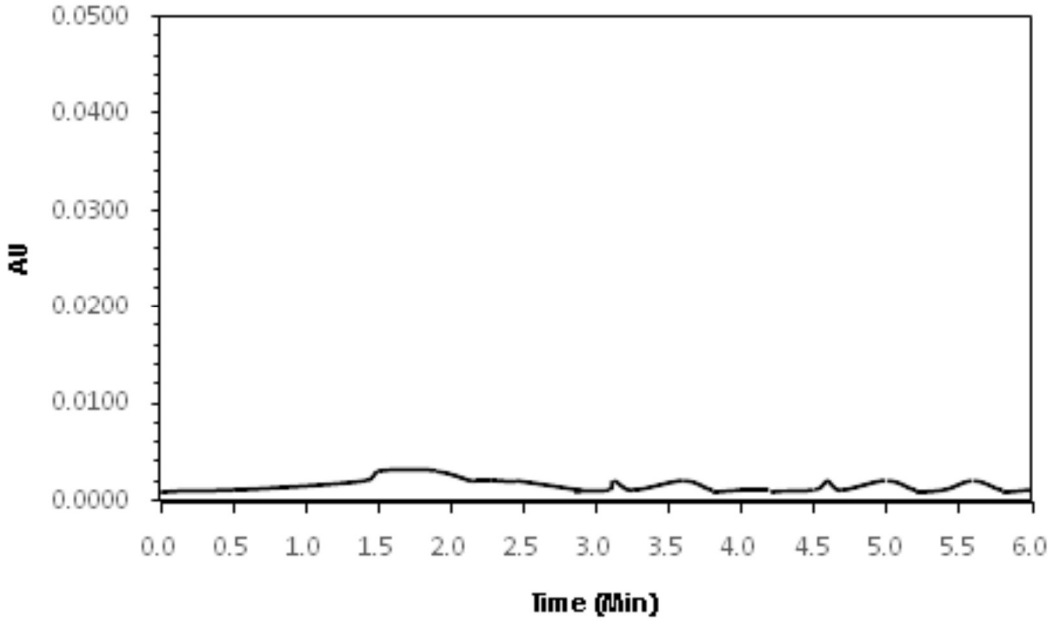


Figure 5: Blank chromatogram

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

Parameter	Abacavir	Dolutegravir	Lamivudine
Mean	293930	861297	502985
SD	1222.49	8167.69	8934.62
% RSD	0.42	0.95	1.78

Intermediate precision

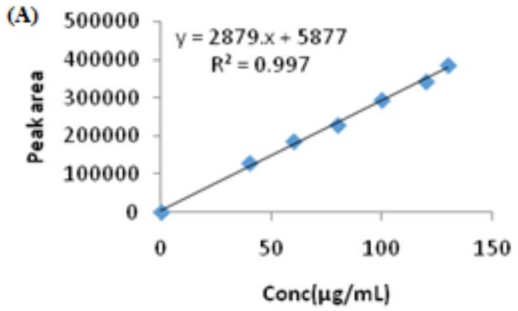
Parameter	Abacavir	Dolutegravir	Lamivudine
Mean	294904	865243	507801.17
SD	2154.36	2056.04	5110.41
% RSD	0.73	0.24	1.01

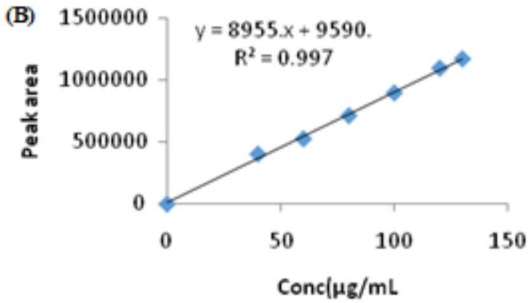
Accuracy and Recovery: To verify the method's accuracy, triplicate preparations were prepared at 80% and 100%, and 120% levels of 100 %concentration by spiking the standard into the diluent. Calculated the recovery with the acceptance criteria of 98-102%.

Table 3: Recovery data

%RECOVERY	Abacavir			Dolutegravir			Lamivudine		
	Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
80% Level	98.71	1.11	1.14	99.87	1.42	1.45	98.36	1.10	1.12
100% Level	99.59	0.96	0.97	99.59	1.30	1.35	99.24	1.06	1.09
120% Level	99.27	1.78	1.83	99.60	1.42	1.47	99.12	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 (R2) NLT 0.99.





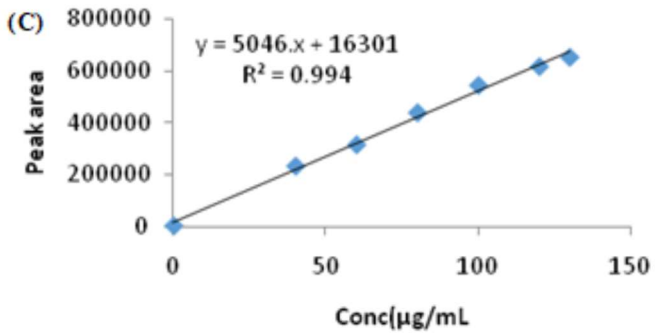


Figure 6: Linearity data of Abacavir (A), Dolutegravir (B), Lamivudine (C)

ROBUSTNESS

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.

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

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.

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 usedin 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 Pacific Academy of Higher Education and Research University, Pacific Hills, Pratap Nagar Extension, Debari, Udaipur- 313024, Rajasthan and Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, A.P, India, for providing technical assistance and necessary facilities.

REFERENCES:

[1] http://www.drugbank.ca/drugs/DB00709.

[2] http://www.drugbank.ca/drugs/DB08930.

[3] https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Dovato/pdf.

[4] Vii V Healthcare. D-ovato (dolutegravir and lamivudine). https: //www.centerwatch.com. Accessed 7 Aug 2019.

[5] Ashok G and Mondal DS: Development and validation of stability indicating method for the simultaneous estimation of batcaver sulphate, lamivudine and dolutegravir sodium in pharmaceutical dosage forms by RP-HPLC; Saudi. J Med Pharm Sci 2018; 4: 289–296.

[6] Khaleel N and Sharmila Nurbhasha: A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form; Future Journal Pharmaceutical Sciences 2020; 6(1): 1-10.

[7] Tata Prasanna Kumari, Venkateswara Rao, B, Sri Lakshmi N, Rajeswari P and Tharunkumar B: A Novel Stability Indicating RP-HPLC Method Development and Validation for The Determination of Dolutegravir, Lamivudine AndabacavirIn Bulk And Pharmaceutical Formulations; High Technology Letters 2021; 27(1): 478-505.

[8] Sapna M Rathod and Paresh U Patel: Development and validation of RP-HPLC method for estimation of lamivudine and dolutegravir sodium in synthetic mixture; Research Journal of Pharmacy and Technology 2020; 13(6): 2864-2868.

[9] Ponnamalla Jasmine Carey, Devanaboyina Narendra and Gadi Vijaya Lakshmi: Method development and validation for simultaneous estimation of abacavir, lamivudine & dolutegravir by RP-HPLC method; World J of

Pharmaceutical and Med Research 2020; 6(7): 229-235.

[10] Tamilarasan S and Malathi S: Analytical Method Development and Validation for the Estimation of Dolutegravir and Lamivudine in Bulk Form by RP- HPLC; Journal of Pharmaceutical Sciences and Research 2021; 13(4): 227-231.

[11] Srinivas Lella, Venkateswararao P and Srinivasrao N: A stability indicating RP-HPLC methods for simultaneous estimation of the abacavir, lamivudine and dolutegravir in bulk and pharmaceutical dosage form; International Journal of Research in Pharmacy and Chemistry 2019; 9(3): 146-153.

[12] Gorja Ashok and Sumanta Mondal: Development and Validation of Stability Indicating Method for the Simultaneous Estimation of Batcaver Sulfate, Lamivudine and Dolutegravir Sodium in Pharmaceutical Dosage forms by RP-HPLC; Saudi Journal of Medical and Pharmaceutical Sciences 2018, 4(2): 289-296.

[13] Khushboo Patel, Ujashkumar Shah, Darshak Patel, Jayvadan K. Patel and Tejas B. Patel: Development and Validation of RP-HPLC Stability Indicating Method for Simultaneous Estimation of Dolutegravir and Lamivudine in Bulk and Pharmaceutical Dosage Form; Journal of Pharmaceutical Research International 2021; 33(60): 570-584.

[14] Padmavathi Sakinala, Pujitha G, Sharmila J, Sireesha M and Nikitha K: HPLC method development and validation of abakavir, lamivudine and dolutegravir in human plasma; Journal of Global Trends in Pharmaceutical Sciences 2020; 11(2): 7854 –7865.

[15] Chandramadherange and Kanchan Chauhan: Development and validation of RPHPLC method for dolutegravir in bulk and solid dosage form; Journal of Emerging Technologies and Innovative Research 2020; 7(9): 811-816.

[16] Yusuff Ismail, Vijaya Vara Prasad M, M. Shaheedha S and Mohammad Habeeb: A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and its Dosage

Forms; Iranian Journal of Pharmaceutical Sciences; 2019: 15 (4): 53-72.

[17] Shalini, PushpendraSoni and Lavakesh Kumar Omray: New Analytical Method Development and Validation for Estimation of Dolutegravir Sodium In Synthetic Mixture By Using RP-HPLC; Asian Journal of Pharmaceutical Education and Research 2018; 7(4): 48-56.

[18] Priya and Sankar: Simultaneous stability-indicating method for the determination of abacavir, dolutegravir and lamivudine by RP-HPLC; IJPSR 2016; 7(7): 2905-16.

[19] NachiketDighe, Prachi P. Kanawade and Ganesh S. Shinde: A Review on Development and Validation of RP-HPLC Method for Simultaneous Estimation of Lamivudine, Tenofovir Disproxil and Dolutegravir in Bulk and Combined Dosage; World Journal of Pharmaceutical Research 2020; 9(5): 847-854.

[20] Phoebe Esinam Goku, Emmanuel Orman, Anna NaaKwarley Quartey, Joseph Kwasi Adu,andReimmel Kwame Adosraku. A Simple RP-HPLC Method to Simultaneously Assay the Contents of Lamivudine, Tenofovir and Nevirapine in Fixed Dose Combined Oral Antiviral Medicines; Journal of Chemistry 2020; 1-9.

[21] Srivastava B, Sheeja VK and Haribabu Y: Analytical Techniques for Dolutegravir: A Review; International Journal of Pharmaceutical Sciences Review and Research 2020; 60(1): 109-114.