A NOVEL, RAPID RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DOLUTEGRAVIR BY QbD APPROACH

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ABSRACT

Background: Dolutegravir is a HIV integrase inhibitor, and multidrug and toxin extrusion transporter 1 inhibitor, and organic cation transporter 2 inhibitor.

Method:

A simple, rapid, accurate, robust and reproducible RP-HPLC method was developed and validated for the estimation of Dolutegravir. The quantitative analysis was carried out by using SpursilC18 (250*4.6mm, 5 µm) particle size with mobile phase composing of Phosphate buffer: Acetonitrile (70:30) ratio at a flow rate of 1.0ml/min detection wave length was carried out at 260 nm using PDA detector with injection volume 10µl, and the retention time was found to be 2.352 mins. This method was performed as per ICH guidelines and it produce linear response in the concentration ranges 20-100 ppm (R^2=0.9996). The recovery studies was carried out and found to be within limits. The %RSD was found to be <2. The proposed method was statistically evaluated and can be applied for the routine analysis of Dolutegravir.

Results: The Dolutegravir obeyed Beer Lambert's law in the concentration range of 20-100ppm with regression 0.9996 at 260 nm. The overall % recovery was found to be 99.69% for Dolutegravir which reflects that the method was free from the interference of the impurities and other excipients, used in the bulk and marketed dosage form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be 0.8 for Dolutegravir respectively which is <2% hence proved that method is precise.

Conclusion: The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Dolutegravir in bulk and tablet dosage form. The proposed method was found to be rapid, accurate, precise, specific, robust, rugged and economical.

Keywords: Dolutegravir, RP-HPLC, Accuracy, Precision.

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INTRODUCTION

Dolutegravir [1] is an HIV-1antiviral agent. It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of retroviral DNA integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in the inhibition of viral activity.

The field of pharmaceutical research continually seeks innovative methods to improve drug development, formulation, and analysis. Dolutegravir, a novel and potent antiretroviral agent, has shown great promise in the treatment of HIV/AIDS. Accurate and reliable estimation of this drug's concentration is crucial to ensure its efficacy and safety. High-Performance Liquid Chromatography (HPLC) is a widely employed analytical technique for drug quantification due to its high sensitivity, specificity, and reproducibility.

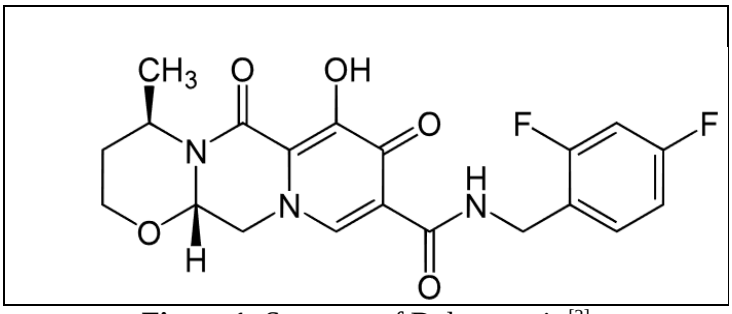

Figure 1: Structure of Dolutegravir [2]

The significance of this research lies in its potential to provide a more sensitive and efficient HPLC method for the estimation of Dolutegravir, thus contributing to the quality control of this important antiretroviral drug. Additionally, the utilization of Design of Experiment principles ensures a systematic and thorough exploration of the experimental space, leading to robust and transferable analytical methods.

The remainder of this paper will develop into the methodology, results, and discussion of the experimental findings. Furthermore, the validation and application of the optimized RP-HPLC method for the estimation of Dolutegravir in real pharmaceutical samples will be presented, along with a comparison to existing methods. Ultimately, this research aims to offer a valuable contribution to the field of analytical chemistry and pharmaceutical analysis.

Experimental:

Chemicals and reagents:

Dolutegravir, Water HPLC Grade, Acetonitrile, Sodium hydroxide, Phosphate buffer.

METHOD DEVELOPMENT BY RP- HPLC [3-5]:

HPLC system (Shimadzu) with PDA detector was used. The software LC-Solution can be used and a Rheodyne injection with a 10µL loop was used for injection of the sample. Spursil C18 (250*4.6mm, 5 µm) was used. The mobile phase was composed of phosphate buffer and acetonitrile in the various ratios with flow rate of 1 ml/min. HPLC system was operated at ambient temperature.

Preparation of the Dolutegravir Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 25 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 25 mg of Dolutegravir sample is taken into a 25ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Design of Experiment[6-8]: The standard drug sample of Dolutegravir was subjected to the design of experiment process. 2 Level Factorial design was employed to identify the underlying facts of effects of factors and their interaction effects on selected method responses. A total of 8 runs were conducted.

Statistical analysis:

- By using ANOVA, the statistical calculations were processed for variables screening and optimization of the method

- The statistical tools provide the numerical verification of variables and its effect on responses.

Method operable design region:
The different amalgamation and reciprocity of input factors produces the space referred as Design space. The establishment of design space was made by utilizing the contour graphs of Sigma tech software.

Method Verification[7,8]:
The optimized method conditions were proposed by the software in order to reach the desired method goals. The method was verified to check the predictability of the proposed model.

Table 1: 2 Level Factorial Design

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	4.60	4.95	-0.3500	0.125	-0.277	-0.258	0.011	-0.097	5
2	3.40	4.95	-1.55	0.125	-1.226	-1.281	0.215	-0.484	6
3	4.90	4.95	-0.0500	0.125	-0.040	-0.037	0.000	-0.014	4
4	6.40	4.95	1.45	0.125	1.147	1.179	0.188	0.446	8
5	7.50	4.95	2.55	0.125	2.018	2.887	0.581(1)	1.091(1)	7
6	3.90	4.95	-1.05	0.125	-0.831	-0.810	0.099	-0.306	2
7	4.30	4.95	-0.6500	0.125	-0.514	-0.485	0.038	-0.183	1
8	4.60	4.95	-0.3500	0.125	-0.277	-0.258	0.011	-0.097	3

(1) Exceeds limits.

METHOD VALIDATION [9-11]

1. Linearity:
Accurately weigh and transfer 25 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of stock solution:
Accurately weigh and transfer 25 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
From the above standard stock solution pipetted out 0.2, 0.4, 0.6, 0.8 and 1ml into a five 10ml volumetric flask and made up to the volume 10ml to get 20, 40, 60, 80 and 100 ppm concentrated solutions of Dolutegravir was filtered and injected. The calibration curve was plotted between concentration and peak area. Correlation coefficient was determined by regression analysis.

2. Precision:
From the standard stock solution an aliquot of 0.6ml was added into a six 10ml volumetric flasks, made up to 10ml with diluent. Later it was filtered and six replicates were injected into HPLC.

3. Accuracy:
Preparation of Standard stock solution:
Accurately weigh and transfer 25 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation Sample solutions:
Accuracy solutions at 50% solution (With respect to target Assay concentration):
Accurately weigh and transfer 12.5 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Accuracy solutions at 100% solution (With respect to target Assay concentration):
Accurately weigh and transfer 25 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Accuracy solutions at 150% solution (With respect to target Assay concentration):
Accurately weigh and transfer 37.5 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

4. Limit of Detection
Preparation of 0.36 µg/ml solution:
Accurately weigh and transfer 25 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 0.45ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

5. Limit of Quantification:
Preparation of 1.20 µg/ml solution:
Accurately weigh and transfer 25 mg of Dolutegravir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)
Further pipette 0.6 ml of Dolutegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

6. Robustness:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1 ml/min.
Standard solution 60 ppm of Dolutegravir prepared and analysed using the varied flow rates along with method flow rate.

B. The Organic composition in the Mobile phase was varied from ±10%.
Standard solution 60 ppm of Dolutegravir was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

RETENTION TIME:

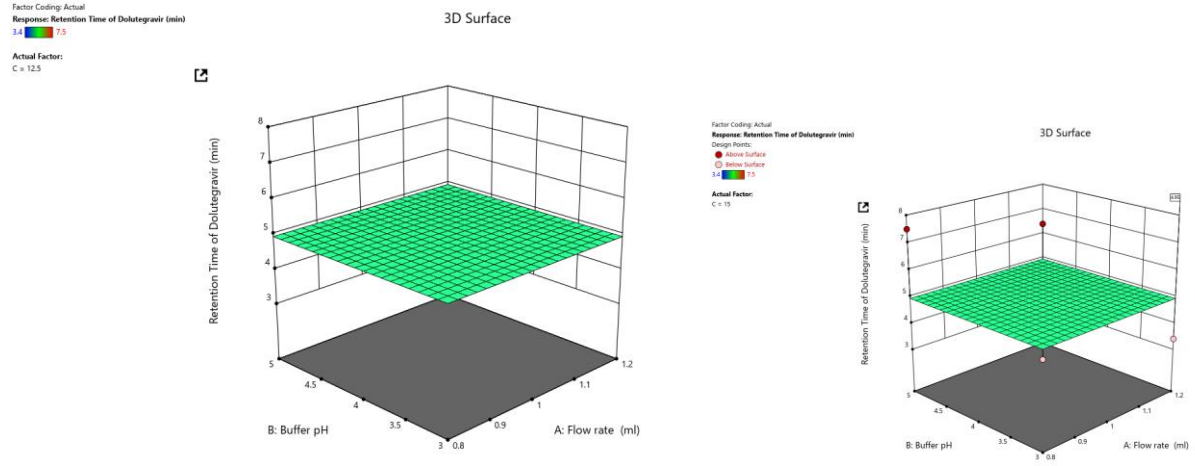

Figure 2: 3D RSM plots for retention time

Table 2: Factors

Factor	Name	Units	Type	SubType	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Flow rate	ml	Numeric	Continuous	0.8000	1.20	-1 ↔ 0.80	+1 ↔ 1.20	1.0000	0.2138
B	Buffer pH		Numeric	Continuous	3.00	5.00	-1 ↔ 3.00	+1 ↔ 5.00	4.00	1.07
C	Column Length	cm	Numeric	Continuous	10.00	15.00	-1 ↔ 10.00	+1 ↔ 15.00	12.50	2.67

Table 3: Responses

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	Retention Time of Dolutegravir	min	8.00	3.4	7.5	4.95	1.35	2.21

Optimization and development of RP-HPLC-PDA method using 2 Level Factorial Design

- In the proposed investigation, 8 experimental runs were performed and analyzed for obtained results of retention time in accord with the 2 Level Factorial Design
- Further investigation was performed using response surface methodology (RSM) to evaluate the relationship between the dependent responses and independent variables (Factors) using obtained data was reported in Table 2 & 3.
- The model was also validated by analysis of variance (ANOVA) using design expert software, and the results are as presented in Table 4. Based on press value, a quadratic model was selected for responses such as retention time.
- The significant effects showed p value less than 0.05, while the low standard deviation (% C.V) and a high adjusted R-square value indicated a good relationship between the experimental data and those of the fitted model.
- The predicated R-square value was in acceptance concordance with the adjusted R-square value for all responses.
- The final equation in terms of actual components and factors which can be used to make predictions about the response for given levels of each factor,

Table 4 : ANOVA Table.

S.NO	Response	±S. D	Mean	%CV	R^2	Adjusted R^2	Predicated R^2	Adequate precision	P value
1	Retention time	0.4313	2.51	17.16	0.4771	0.3564	0.0179	6.5602	0.0332

Coded Equations:

Retention time:

+2.20-0.3030A-0.4034B-0.1066C

Positive values represent an effect that favours optimization while a negative value indicates an inverse relationship between the factors and responses. 3-D response surface plots revealed that effect of factors such as Phosphate buffer and Acetonitrile (70:30) composition in mobile phase, pH of aqueous phase and flow rate of HPLC system on the responses Retention time.

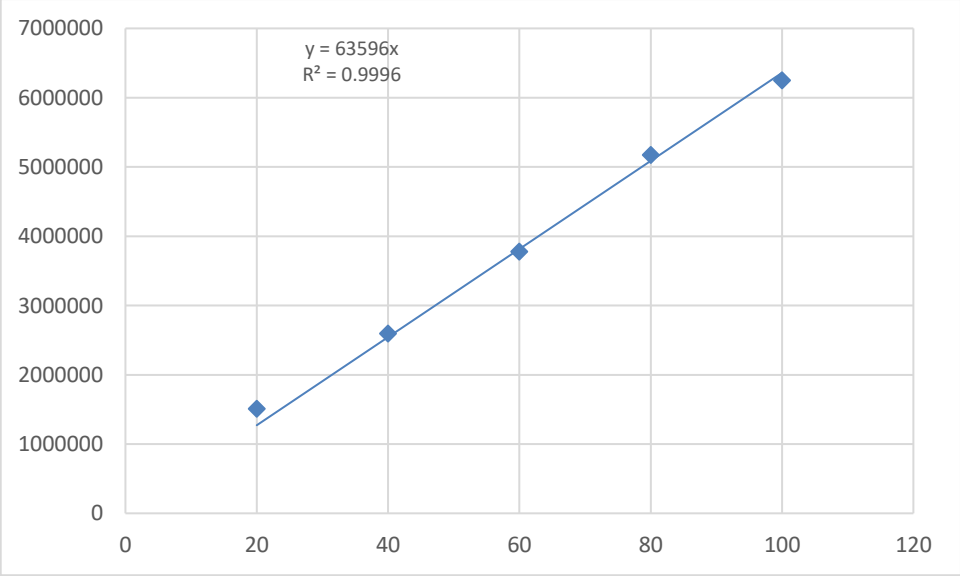

Figure 3: Linearity Curve of Dolutegravir

Name	Retention time	Peak area	Theoretical plates	Tailing Factor
Dolutegravir	2.335	7896091	2107.7	1.66

Observation:
The separation was good; peak shape was good, the retention of peak was good, resolution was good, tailing factor was less than 2, theoretical plates were more than 2000, and this trial was taken as optimized method.

METHOD VALIDATION:

Table 5 : Linearity studies of Dolutegravir

S. No	Linearity Level	Concentration	Area
1	I	20	3741269
2	II	40	7464246
3	III	60	10756450
4	IV	80	14611120
5	V	100	18059489
Correlation Coefficient			0.9996

Acceptance criteria: Correlation coefficient should be not less than 0.9996.

Accuracy:
The accuracy is the method of closeness of the measured value to true value for the sample. Accuracy is usually determined by recovery studies.

Table 6: Accuracy results of Dolutegravir

Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3985452	12.5	12.49	99.53	99.69
100%	7992625	25	25.11	99.80	
150%	11981438	37.5	37.80	99.74	

Acceptance criteria: The % recovery for each level should be between 98.0 to 102.0%.

Precision:
The precision studies were carried out by 6 replicate injections of Dolutegravir.

Table 7: Precision (Repeatability) results of Dolutegravir

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Dolutegravir	2.378	7777473	877732	2078.98	1.66
2	Dolutegravir	2.306	7855543	885773	2156.18	1.69
3	Dolutegravir	2.306	7803038	885055	2162.29	1.67
4	Dolutegravir	2.335	7958430	870854	2049.47	1.68
5	Dolutegravir	2.335	7896091	870081	2054.40	1.65
6	Dolutegravir	2.348	7881223	867202	2070.58	1.66
Mean		2.30.0	7861966.4			
Std. Dev.		1.0	65564.8			
% RSD			0.8			

Acceptance criteria: The % RSD for the area of six standard injections results should not be more than 2.

Robustness
As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

Table 8: Robustness results for Dolutegravir (Change in Flow rate)

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3106.22	1.44
2	**1.0**	**3477.23**	**1.65**
3	1.2	2795.68	1.38

Table 9: Robustness results for Dolutegravir (Change in Organic Composition in the Mobile Phase)

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3256.8	1.24
2	*Actual	**3477.23**	**1.65**
3	10% more	2895.68	1.20

Assay:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

$$\frac{7892987}{79926255} * \frac{25}{25} * \frac{0.6}{10} * \frac{25}{198} * \frac{10}{0.6} * \frac{198}{25} * \frac{99.8}{100} * 100 = 98.56\%$$

Discussion: The percentage assay of Dolutegravir was found to be 98.56%.

Table 10 : Summary data of validation parameters

S.NO	VALIDATION PARAMETERS		ACCEPTANCE CRITERIA	RESULTS
1.	System suitability		%RSD for 5 replicate injections of standard solution NMT 2.0%	0.4
2.	Linearity		The correlation coefficient should be NLT 0.999	R^2=0.9996
3.	Accuracy		The %Recovery at each level should be NLT 80.0% and NMT 120% of the amount added	%Recovery – 99.69
4.	Precision		The %RSD of peaks obtained from the 6 replicate injections should be NMT 2.0%	%RSD- 0.2
5.	LOD		-	0.0021µg/ml
6.	LOQ		-	0.0065µg/ml
7.	Robustness	Variation in Flow rate(0.9ml/mn,1.1ml/min)	-	USP Tailing 0.9ml/min=1.44 1.1ml/min=1.38
		Variation in Organic Composition (10% less and 10% more)	-	USP Tailing 10% less =1.24 10% more =1.20
8.	Assay		-	98.56%

CONCLUSION

A Novel, rapid, isocratic RP-HPLC method was developed and validated for the estimation of Dolutegravir in pure and Pharmaceutical dosage form by using Phosphate buffer and acetonitrile in the ratio 0f 70:30. The proposed method was validated as per ICH guidelines and the method was found to be simple, linear, accurate, precise, robust and reproducible. So it is concluded that the developed RP-HPLC method was statistically evaluated and can be applied for the Routine analysis of Dolutegravir in pure and pharmaceutical dosage form. The use of DOE approach is a flexible strategy for reducing the no. of trial experimental runs required for a method to be created in a short period of time. The proposed method was found to be rapid, accurate, precise, specific, robust, rugged and economical.

BIBLIOGRAPHY

1. https://www.rxlist.com/consumer-tivicay-dolutegravir/drugs-condition.htm.
2. Available from https://go.drugbank.com/drugs/DB08930.

3. Taleuzzaman.M, Alis, Gilani.SJ, Imam's, Hafeez. Ultra Performance Liquid Chromatography (UPLC) - A Review. Austin J Anal pharm 2015; 2(6):1056.
4. Mahesh Mukund Deshpande, Veena Sanjay Kasture, Mahalaxmi Mohan and Macchindra J. Chavan. Bioanalytical Method Development and Validation: A Review. Recent advances in analytical chemistry. 2019.
5. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development, Wiley Inter Science; New York,1997; 2nd Ed,1-56, 234-289,685-712.
6. Available from: https://www.science/article/pii/S2405722315300360
7. Available from: http://shodhganga.inflibnet.ac.in:8080/jspui/handle/10603/286929
8. https://asq.org/quality-resources/design-of-experiments.
9. ICH Q 2 B Validation of Analytical Procedure. International Conference on Harmonization; Geneva1996; 1-8.
10. ICH Q 2 A Text on Analytical Method Validation. International conference on Harmonization; Geneva, 1991; 1-5.
11. ICH Q 8 (R2) Pharmaceutical Development. International Conference on Harmonization; Geneva, 2009.
12. Sk Mastanamma, J Asha Jyothi, P Saidulu, M Varalakshmi Pharmaceutical Methods 9(2), 49-55, 2018.
13. Narottam Pal, Avanapu Srinivasa Rao, Pigilli Ravikumar Asian journal of chemistry 28 (2), 273, 2016.
14. Khaleel Noorbasha & Sharmila Nurbhasha Future Journal of Pharmaceutical Sciences 6, Article number:39 (2020). Pg.no 1-10.
15. Ismail Yusuff, M vijaya vara Prasad, SM Shaheedha, Mohammad Habeeb Iranian journal of pharmaceutical Sciences 15 (4),53-72,2019.
16. Venkatanarayana M, Siva Jyothi N Journal of chromatography & separation techniques 11(1) ;1-7,2020