Original Article

Stability Indicating RP-UPLC Method Development & Validation for Quantitation of Doravirine in Tablet Formulation

Tandrima Majumder[1], Shiva Kumar Gubbiyappa[2]

[1,2]Department of Pharmacy, Gitam Deemed to be University, Patancheru, Telangana, India

Abstract

The goal of this study is to provide a simple, accurate, and precise stability indicating technique for quantifying doravirine (DRVN) in tablet dosage form using UPLC. Chromatography was executed on an HSSC18 (100 mm x 2.1 mm, 1.8mm) reverse phase column with an isocratic elution of buffer acetonitrile, methanol and 0.01 N Potassium dihyrogen orthophosphate (pH-4.5) in the composition of 40:25:35% V/V at a flowing rate of 0.30ml/min. The column oven temperature was kept at 30.0°C, as well as the wavelength detection was processed at 238 nm. Retention time of DRVN was found to be 0.91min. Repeatability of the method was determined in the form of %RSD and the value was 0.33. The percentage mean recovery of the method was found to be 99.57%. LOD, LOQ values obtained from regression equation of DRVN were 0.71 and 2.31 mg/ml respectively. Regression equation and correlation coefficient values of DRVN were y = 17003x + 7203.1 and 0.9997. Drug was subjected for acid, peroxide, photolytic, alkali, neutral and thermal degradation study and the results shown that the percentage of degradation was found between 6.12% and 8.96%. Retention time and total run time of the drug was decreased and the developed method was simple and economical. So, the developed method can be adopted in industries as a regular quality control test for the quantification of DRVN.

Keywords: Doravirine, UPLC, Accuracy, Linearity, Validation, Stability study.

Introduction

DRVN is a non-nucleoside reverse transcriptase inhibitor (NNRTI) for HIV-1 that is used in conjunction with other antiretroviral drugs. DRVN is available as a single drug or as a combination of DRVN (100 mg), lamivudine (300 mg), and tenofovir disoproxil fumarate (tenofovir disoproxil fumarate) (300 mg). DRVN is now fully approved for the treatment of HIV-1 infection in adult patients who have never received antiretroviral therapy before, broadening the range of therapeutic options for HIV-1 infection control [1-3].

In patients who are virologically suppressed (HIV-1 RNA fewer than 50 copies per mL) on a stable antiretroviral regimen with no history of treatment failure and no known substitutions linked with DRVN resistance, it is also suggested to change the present antiretroviral regimen. HIV manufactures complementary DNA (cDNA) to its RNA genome via reverse transcriptase, and this cDNA is subsequently incorporated into the host cell genome, where it may be transcribed into viral RNA for reproduction [4,5].

Address for correspondence: Tandrima Majumder
Department of Pharmacy, Gitam Deemed to be University, Patancheru, Telangana, India
Email: tandrima.agt@gmail.com

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.04.243

How to cite this article: Tandrima M, Shiva Kumar G, Stability Indicating RP-UPLC Method Development & Validation for Quantitation of Doravirine in Tablet Formulation, J PHARM NEGATIVE RESULTS 2022;13(4): 1776-1782.

DRVN suppresses HIV-1 replication by inhibiting HIV-1 reverse transcriptase in a non-competitive manner. 5 DRVN, on the other hand, has no effect on the human cellular DNA polymerases, ß, or mitochondrial DNA polymerase.

DRVN chemically designated as 3-Chloro-5-({1-[(4-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]-2-oxo-4-(trifluoromethyl)-1,2-dihydro-3-pyridinyl}oxy)benzonitrile with molecular weight and formula of 425.75 g/ mole and C17H11ClF3N5O3 respectively (Fig. 1) [1-4]. The literature of DRVN unveils that, few analytical methods were reported for simultaneous evaluation of DRVN with other antiviral drugs [6-9]. Based on the literature, there is a need to develop a stability indicating RP-UPLC method for the quantification of DRVN in bulk and dosage forms.

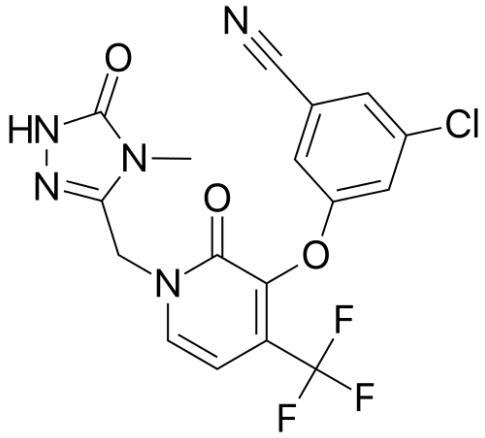

Figure 1: Structure of doravirine.

MATERIALS AND METHODS

Reagent and Chemicals

API of DRVN was obtained from spectrum Pharma Research Solutions, Hyderabad. HPLC-grade methanol and acetonitrile were procured from Rankem, avantor performance material india limited. Potassium dihydrogen orthophosphate and HPLC-grade water were bought from Merck chemical division, Mumbai, India. Pifeltro 100 mg tablets were obtained from local pharmacy.

Chromatography instrument and conditions

Chromatography was executed on an HSSC18 (100 mm x 2.1 mm, 1.8µm) reverse phase column with an isocratic elution of buffer acetonitrile, methanol and 0.01 N Potassium dihyrogen orthophosphate (pH-4.5) in the composition of 40:25:35% V/V at a flowing rate of 0.30ml/min. The column oven temperature was kept at 30.0°C, as well as the wavelength detection was processed at 238 nm. Integration of output signals were monitored and processed by waters Empower software-2.0.

Diluting solvent

The diluent was chosen based on the solubility of the medicines. Initially dissolved in acetonitrile and diluted with methanol and water (60:40).

Processing of standard stock solution

Accurately Weighed and transferred 20mg of DRVN working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. (400µg/ml of DRVN)

1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (40µg/ml of DRVN)

Processing of sample stock solution

5 tablets were weighed and the average weight of each tablet was calculated. The weight equivalent to 40 mg was transferred into a 100 ml volumetric flask and 25 ml of diluent was added and sonicated for 25 min. Further the volume was made up with diluent and filtered through 0.45 µ filter (400 µg/ml DRVN). 1 ml of the resultant solution was poured in to a 10 ml volumetric flask and made up with diluent (40 µg/ml DRVN).

Method validation

The developed method for DRVN was subjected for validation for the parameters like system suitability, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy as per the guidelines of ICH [10-14].

RESULTS AND DISCUSSION

Method development and optimization

We tried with different mobile phase combinations with methanol, water, acetonitrile and buffer. At all the combinations the resulting chromatograms got poor resolution, theoretical plates and peak shape [15]. Finally, excellent chromatographic efficiency parameters were obtained with the mobile phase composition of buffer acetonitrile, methanol and 0.01 N Potassium dihyrogen orthophosphate (pH-4.5) in the composition of 40:25:35% V/V at a flowing rate of 0.30ml/min thru an HSSC18 (100 mm x 2.1 mm,1.8 µm) reverse phase column. The column oven temperature was kept at 30.0°C, as well as the wavelength detection was processed at 238 nm. Based on the solubility, all the dilutions were made with initially dissolved in acetonitrile and diluted with methanol and water (60:40). Retention time of DRVN was found to be 0.91. An injection volume of 10 µL was infused through an UPLC system to get the better performance.

Method validation

System suitability

The system suitability variables were estimated by preparing standard solution of DRVN and the same were injected 6 times in to the chromatographic system. The variables like peak tailing, and USP plate count were estimated [16]. The results were shown in Fig. 2 and Table 1.

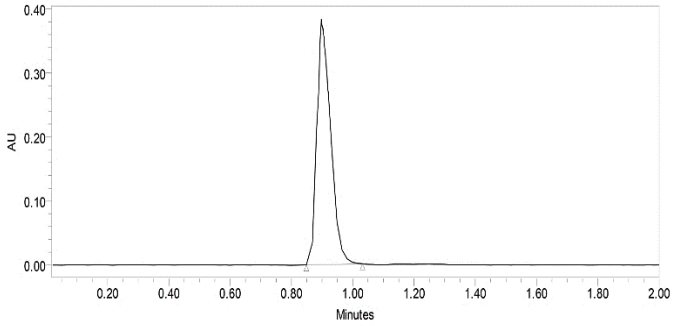

Figure 2: System suitability chromatogram of DRVN

Table 1: System suitability parameters for DRVN

SI. No	DRVN		
	RT(min)	Plate Count as per USP	Tailing
1	0.911	3701	1.17
2	0.913	3587	1.16
3	0.912	3658	1.18
4	0.912	3808	1.28
5	0.911	3659	1.27
6	0.911	3842	1.15

Specificity

Method specificity was determined by infusing the blank, placebo, standard and sample solutions in to a chromatographic system and the resulting chromatograms were evaluated for interference with the excipients, degradants and other components may expected to be present. Blank, standard, formulation and placebo chromatograms were represented in Fig. 3.

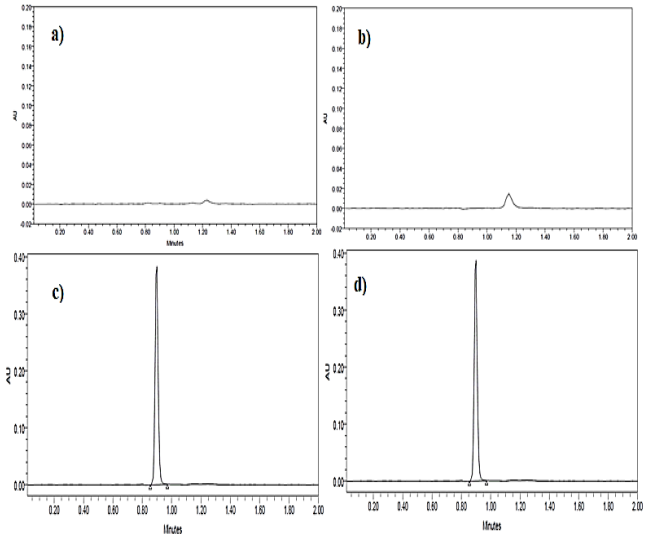

Figure 3: Chromatograms of a) blank, b) placebo, c) standard and d) sample

Precision

Precision of the method was evaluated in terms of method precision and intermediate precision. The method precision (repeatability) was estimated by infusing 6 standard solutions and 6 sample solutions. Intermediate precision was evaluated by infusing 6 standard solutions and 6 sample solutions on different days by different employees on different chromatographic systems [16, 17]. The peak responses of all the chromatograms were taken and standard deviation, % RSD (relative standard deviation) and percentage assay of sample solutions were calculated. The findings were represented in Table 2.

Table 2: Repeatability and intermediate precision results of DRVN

SI. No	Area of DRVN	
	Day one	Day two
1.	615464	623135
2.	610345	625323
3.	613197	622733
4.	616754	625083
5.	611930	622016
6.	612846	625527
Mean	613423	623970
SD	2338.7	1519.2
%RSD	0.41	0.33

SD: standard deviation; RSD: relative standard deviation

Accuracy

Method accuracy was estimated at three variable concentrations of 50%, 100%, and 150% level by spiking the known amount of the analyte [18]. The % recovery at each level was calculated and the findings were represented in Table 3.

Table 3: Accuracy results of DRVN

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	20	19.90	99.48	99.57%
	20	19.82	99.10	
	20	19.94	99.71	
100%	40	39.86	99.59	
	40	39.97	99.95	
	40	39.92	99.79	
150%	60	59.61	99.30	
	60	59.55	99.25	
	60	59.47	99.13	

Linearity

Linearity of the developed method was evaluated by processing 6 different concentration levels of DRVN over the concentration of 10.0 to 60.0 µg/ml. Each concentration level was processed in triplicates [19, 20]. The linearity plot was acquired by plotting peak response (on X-axis) versus concentration (on Y-axis). The results of the linearity were represented in Fig. 4 and Table 4.

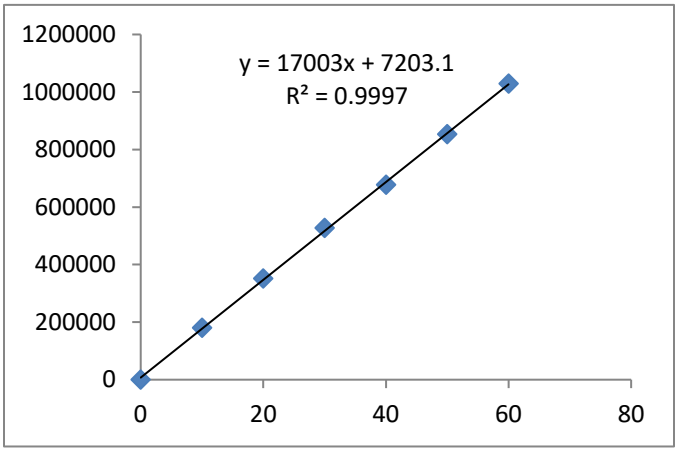

Figure 4: Calibration curve of DRVN

Table 4: Linearity results of DRVN

S. No	Concentration (µg/ml)	Peak area
1	0	0
2	10	180652
3	20	351425
4	30	526977
5	40	677785
6	50	853069
7	60	1029005

LOD and LOQ

LOD is lowest quantity of drug in a sample that can be identified but cannot be quantify exactly. LOQ is the lowest quantity of a drug in an analyte which can be quantitatively estimated with a suitable accuracy and precision [21]. The LOD and LOQ values were calculated from the linearity data by utilizing standard deviation and slope of the curve and the values were 0.71 and 2.31 mg/ml respectively.

Robustness

The method robustness was processed by introducing small variation in the optimized LC conditions such as organic phase in mobile phase (±5%), flow rate (-0.1 and +0.1 ml/ min) and column temperature (±5°C). The findings were shown in the Table 5.

Table 5: Robustness data for DRVN

S. No.	Variation in LC conditions	DRVN % RSD
1	Organic phase -5%	0.91
2	Organic phase + 5%	1.11
3	Temperature at 25°C	0.61
4	Temperature at 35°C	0.89
5	Flow rate -0.1ml/min	0.92
6	Flow rate +0.1ml/min	0.76

Degradation study

Study of alkali degradation

To 1 ml of stock solution of DRVN, 1 ml of 2N NaOH was added in to a 10 ml volumetric flask and kept at 60°C for 30 min. Further, the resulting solution was made up to the mark to get 40 µg/ml DRVN. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte [22, 23]. The findings were represented in Table 6 and Fig. 5.

Table 6: Degradation data of DRVN

Type of degradation	DRVN		
	Area	%Recovered	% Degraded
Neutral	577263	92.08	7.92
UV light	589577	93.84	6..16
Thermal	565739	92.24	7.76
Acid	573001	91.40	8.60
Peroxide	587048	93.84	6.16
Alkali	567048	91.04	8.96

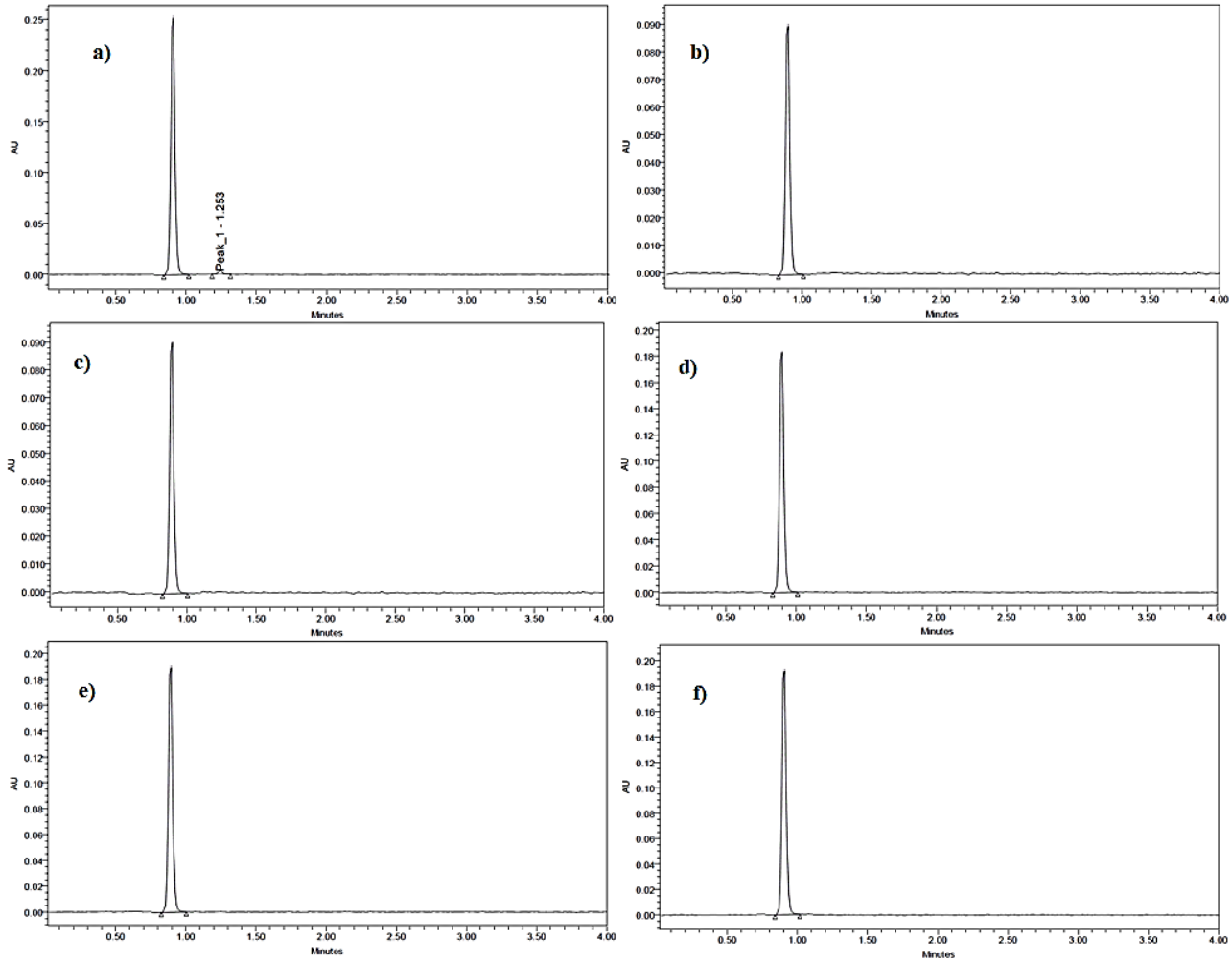

Figure 5: Representative chromatograms of a) acid degradation, b) alkali degradation, c) peroxide degradation, d) thermal degradation, e) UV-degradation and f) neutral degradation.

Study of photolytic stability

For the photolytic stability study, DRVN 400 µg/ml solution was exposed to UV-light by placing the solutions in UV cabinet for 7 days or 200 Watt hours/m2 in photo stability chamber. The resulting solution was transferred in to a 10 ml volumetric flask and made up to the mark with diluent to get 40 µg/ml DRVN. From that 10 µl of solution was infused in to an UPLC system and the resultant chromatograms were analyzed for the stability of analyte. The findings were represented in Table 6 and Fig. 5.

Study of acid degradation

To 1 ml of stock solution of DRVN, 1ml of 2N Hydrochloric acid was added in to a 10 ml volumetric flask and refluxed at 60°C for 30 min. Further, the resulting solution was made up to the mark to get 40 µg/ml DRVN. From that 10 µl of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table 6 and Fig. 5.

Study of neutral degradation

To 1 ml of stock solution of DRVN, 5 ml of water was added in to a 10 ml volumetric flask and kept for refluxing at 60°C for 1 h. Further, the resulting solution was made up to the mark to get 40 µg/ml DRVN. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table 6 and Fig. 5.

Study of oxidation

To 1 ml of of stock solution of DRVN, 1 ml of 20% hydrogen peroxide (H2O2) were added in to a 10 ml volumetric flask and kept at 60°C for 30 min. Further, the resulting solution was made up to the mark to get 40 µg/ml DRVN. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table 6 and Fig. 5.

Thermal degradation study

Standard stock solution of DRVN was monitored at 105°C for 6 h in a hot air oven to perform the dry heat stability study. Further, the resulting solution was subjected for dilution to get 40 µg/ml DRVN. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table 6 and Fig. 5.

CONCLUSION

A simple, accurate and precise method was developed for the estimation of DRVN in Tablet dosage form by RP-UPLC technique. Retention time of DRVN was found to be 0.91min and chromatographic elution was processed through a HSSC18 (100 x 2.1 mm, 1.8mm) reverse phase column and the mobile phase composition of acetonitrile, methanol and 0.01 N Potassium dihyrogen orthophosphate (pH-4.5) in the composition of 40:25:35% V/V at a flowing rate of 0.30ml/min. Repeatability of the method was determined in the form of %RSD and the value was 0.33. The percentage mean recovery of the method was found to be 99.57%. LOD, LOQ values obtained from regression equation of DRVN were 0.71 and 2.31 mg/ml respectively. Regression equation and correlation coefficient values of DRVN were y = 17003x + 7203.1 and 0.9997. Drug was subjected for acid, peroxide, photolytic, alkali, neutral and thermal degradation study and the results shown that the percentage of degradation was found between 6.12% and 8.96%. Retention time and total run time of the drug was decreased and the developed method was simple and economical. So, the developed method can be adopted in industries as a regular quality control test for the quantification of DRVN.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of the paper.

REFERENCES

1. Pifeltro- doravirine tablet, film coated. DailyMed. 10 October 2019. Retrieved 22 September 2020.
2. Collins, Simon; Horn, Tim. The Antiretroviral Pipeline (PDF). Pipeline Report. p. 10. Archived from the original (PDF) on 11 March 2016. Retrieved 6 December 2015.
3. Drug Approval Package: Pifeltro (doravirine). U.S. Food and Drug Administration (FDA). 9 October 2018. Retrieved 22 September 2020.
4. Sanchez RI, Fillgrove KL, Yee KL, Liang Y, Lu B, Tatavarti A, Liu R, Anderson MS, Behm MO, Fan L, Li Y, Butterton JR, Iwamoto M, Khalilieh SG. Characterisation of the absorption, distribution, metabolism, excretion and mass balance of doravirine, a non-nucleoside reverse transcriptase inhibitor in humans. Xenobiotica. 2018;28:1-11.
5. Wilby KJ, Eissa NA. Clinical Pharmacokinetics and Drug Interactions of Doravirine. Eur J Drug Metab Pharmacokinet. 2018; 25:497-3.
6. Hu WS, Hughes SH. HIV-1 reverse transcription. Cold Spring Harb Perspect Med. 2012;2(10):a006882.
7. Gollu G, Gummadi S (2020) Simultaneous quantifcation of lamivudine, tenofovir disoproxil fumarate and doravirine in pharmaceutical dosage form by liquid chromatography with diode array detection. Pharm Chem J 54:526–535.
8. Tiruveedhi VLNBG, Battula VR, Bonige KB (2021) RP-HPLC (stabilityindicating) based assay method for the simultaneous estimation of doravirine, tenofovir disoproxil fumarate and lamivudine. Int J Appl Pharm 13:153–159.
9. Desai R, Roadcap B, Goykhman D, Woolf E. Determination of doravirine in human plasma using liquid-liquid extraction and HPLC-MS/MS. Bioanalysis 2019;11:1495-508.
10. ICH: Q2 (R1), Validation of analytical procedures: text and methodology; 2005.
11. ICH: Q2B. Harmonized Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva; 1996.
12. Swathi P, Vidyadhara S, Sasidhar RLC, Kalyan Chakravarthi K. Method development and validation for the estimation of entecavir in bulk and pharmaceutical dosage forms by RP-HPLC. Int J Curr Pharm Res 2017;9:107-11.
13. Shweta Mishra, Patel CJ, Patel MM. Development and validation of stability indicating chromatographic method for simultaneous estimation of sacubitril and valsartan in pharmaceutical dosage form. Int J App Pharm 2017;9:1-8.
14. Prasanthi Chengalva, Latha Lavanya Peddavengari, Madhavi Kuchana. A validated analytical method for the simultaneous estimation of cytarabine and daunorubicin in bulk and infusion formulation by reverse phase high performance liquid chromatography. Asian J Pharm Clin Res 2019;12:128-31.
15. Kafiya Suroor, Kudaravalli Sreedevi. RP - HPLC method development & validation for the simultaneous estimation of encorafenib and binimetinib in API & tablet dosage form. International Journal of Science and Research 2019;8:184-190.
16. Ramesh Guguloth, Madhukar A, Kannappan N, Ravinder A. Method development and validation of new RP- HPLC method for the determination of sofosbuvir tablet. J. Pharma Re 2016;5:161-163.
17. Charde M S, Welankiwar A S, Cajole R D. Development of validated RP-HPLC method for the simultaneous estimation of atenolol and chlorthalidone in combine tablet dosage form. Int Jl of Advs in Pharmaceutics 2014;3:1-11.
18. Estella Hermoso de Mendoza A, Imbuluzqueta I. Development and validation of ultra-high performance liquid chromatography-mass spectrometry method for LBH589 in mouse plasma and tissues. J Chromatogr B: Anal Technol Biomed Life Sci 2011;79:3490–6.
19. Kishore kumar L Mule. Rapid analytical method for assay determination for prochlorperazineedisylate drug substances by Ultra performance liquid chromatography. Int J Curr Pharm Res 2017;9:118-22.
20. Baki Sharon, Meruva Sathish Kumar, Marakatham S, Kanduri

Valli Kumari. A New RP-UPLC method development and validation for the simultaneous estimation of ivacaftor and lumacaftor. J. Global Trends Pharm Sci 2018;9: 5730-7.
21. Madhavi S, Prameela Rani A. Simultaneous reverse phase ultra-performance liquid chromatography method development and validation for estimation of Grazoprevir and Elbasvir. Asian J Pharm ClinRes 2018;11:100.
22. Ngwa G. Forced degradation study as an integral part of HPLC stability indicating method development. Drug Delivery Technol 2010;10:56-9.
23. Balaswami B, Ramana PV, Rao BS, Sanjeeva P. A new simple stability indicating RP-HPLC-PDA method for simultaneous estimation of triplicate mixture of sofosbuvir, voxilaprevir and velpatasvir in tablet dosage form. Res J Pharm Technol 2018;11:4147-56.