Development and Validation of a RP-HPLC Method for the Analysis of Darolutamide in Bulk and Pharmaceutical Dosage form

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Abstract

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Darolutamide in bulk form and marketed pharmaceutical dosage forms. Separation of Darolutamide was successfully achieved on a Symmetry ODS C_{18} (4.6 x 250mm, 5µm) column in an isocratic mode of separation utilizing Acetonitrile: Methanol in the ratio of 80: 20% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 272nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 10-50mcg/mL for Darolutamide. The correlation coefficient was found to be 0.999 for Darolutamide. The LOD and LOQ for Darolutamide were found to be 1.1µg/mL and 3.2µg/mL respectively. The proposed method was found to be good percentage recovery for Darolutamide, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: Darolutamide, RP-HPLC, Accuracy, Precision, Robustness, Ich Guidelines

Introduction

Darolutamide is a third generation, oral nonsteroidal antiandrogen used to treat nonmetastatic castration-resistant prostate cancer. Darolutamide is associated with a low rate of serum enzyme elevation during therapy, but has not been linked to cases of clinically apparent liver injury with jaundice [1]. Darolutamide is indicated for the treatment of adults with non-metastatic castration-resistant prostate cancer (nmCRPC) and metastatic hormone-sensitive prostate cancer (mHSPC) in combination with docetaxel. Darolutamide, through its downstream effects on cancer cell growth, treats castrate-resistant prostate cancer. It inhibits cancer cell growth and markedly lowers prostate specific antigen (PSA) levels through potent androgen receptor antagonism [2]. Darolutamide is used to treat patients with non-metastatic castration-resistant prostate cancer (prostate cancer that is resistant to medical or surgical treatments that lower testosterone and has not yet spread to other parts of the body) [3]. The IUPAC name of Darolutamide is N-[(2S)-1-[3-(3-chloro-4-cyano phenyl) pyrazol-1-yl] propan-2-yl]-5-(1-hydroxy ethyl)-1H-pyrazole-3-carboxamide. The Chemical Structure of Darolutamide is shown in following figure-1.

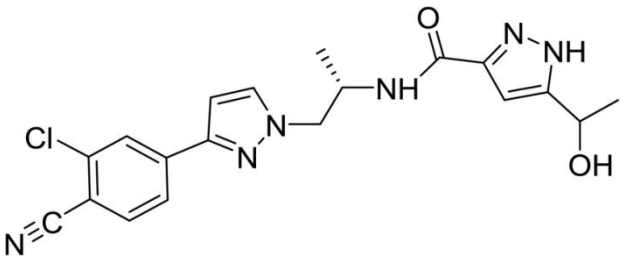

Fig 1: Chemical Structure of Darolutamide

Experimental Methods

Instruments Used

Table 1: Instruments Used

S. No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Ultra Sonicator	Labman

Chemicals Used

Table 2: Chemicals Used

S. No.	Chemical	Brand Names
1	Darolutamide (Pure)	Synpharma Research Lab, Hyderabad
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC Method Development

- **Preparation of Standard Solution:** Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol [4].
  Further pipette 0.3ml of the above Darolutamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
- **Preparation of Sample Solution:** Take average weight of the Powder and weight 10 mg equivalent weight of Darolutamide sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
  Further pipette 0.3ml of the above Darolutamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
- **Procedure:** Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [32-33].
- **Mobile Phase Optimization**: Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Methanol 80: 20% v/v) respectively.
- **Optimization of Column:** The method was performed with various C18 columns like Symmetry, Zodiac and Xterra. Symmetry ODS C18 (4.6 x 250mm, 5µm) Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.
- **Preparation of Mobile Phase:** Accurately measured 800 ml (80%) of HPLC Acetonitrile and 200 ml of Methanol (20%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
- **Diluent Preparation:** The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent [5]. (Stock solution)

Further pipette 0.3ml of the above Darolutamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Specificity Study of Drug:

- **Preparation of Standard Solution:** Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
  Further pipette 0.3ml of the above Darolutamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
- **Preparation of Sample Solution:** Take average weight of the Powder and weight 10 mg equivalent weight of Darolutamide sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
  Further pipette 0.3ml of the above Darolutamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
- **Procedure:** Inject the five replicate injections of standard and inject the three replicate injections sample solutions and calculate the assay by using formula [6-8]:

$$\%ASSAY = \frac{\text{Sample area} \times \text{Weight of standard} \times \text{Dilution of sample} \times \text{Purity} \times \text{Weight of tablet}}{\text{Standard area} \times \text{Dilution of standard} \times \text{Weight of sample} \times 100 \times \text{Label claim}} \times 100$$

- **Preparation of Drug Solutions for Linearity:** Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
- **Preparation of Level – I (10ppm of Darolutamide):** Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
- **Preparation of Level – II (20ppm of Darolutamide):** Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
- **Preparation of Level – III (30ppm of Darolutamide):** Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
- **Preparation of Level – IV (40ppm of Darolutamide):** Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent [9].
- **Preparation of Level – V (50ppm of Darolutamide):** Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
- **Procedure:** Inject each level into the chromatographic system and measure the peak area.
  Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [10-11].

Precision

Repeatability

Preparation of Darolutamide Product Solution for Precision: Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits [12].

Intermediate Precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

- **Analyst 1:** The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.
- **Analyst 2:** The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

- **For Preparation of 50% Standard Stock Solution:** Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
  Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
- **For Preparation of 100% Standard Stock Solution:** Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
  Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
- **For Preparation of 150% Standard Stock Solution:** Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
  Take 0.45ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent [13].
- **Procedure:** Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Darolutamide and calculate the individual recovery and mean recovery values.
- **Robustness:** The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.
- **For Preparation of Standard Solution:** Accurately weigh and transfer 10 mg of Darolutamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
  Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
- **Effect of Variation of Flow Conditions:** The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.
- **Effect of Variation of Mobile Phase Organic Composition:** The sample was analyzed by variation of mobile phase i.e. ACN: Methanol was taken in the ratio and 75: 25, 85: 15 instead of 80: 20, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded [14].

Results and Discussion

Development of a New Analytical Method

Optimized Chromatographic Conditions

Column: Symmetry ODS C18 (4.6 x 250mm, 5µm)
Column temperature: Ambient
Wavelength: 272 nm
Mobile phase ratio: ACN: Methanol (80: 20% v/v)
Flow rate: 1.0mL/min
Injection volume : 20 µl
Run time: 8 minutes

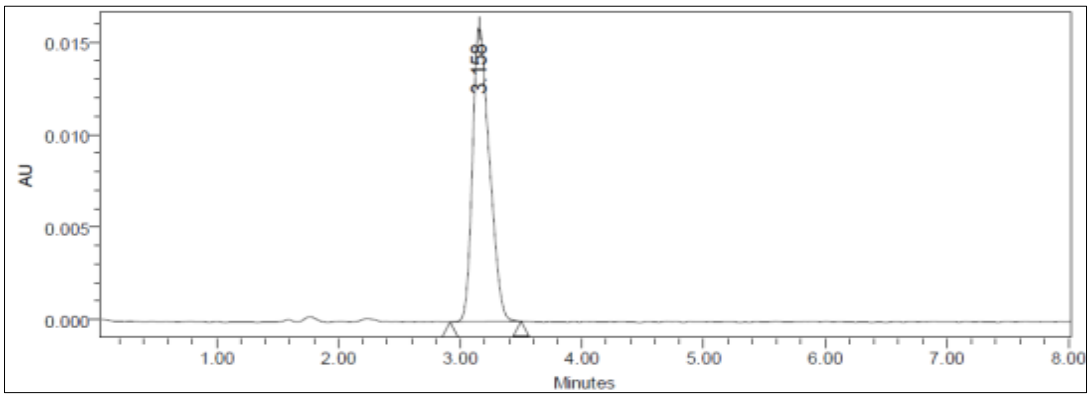

Fig 2: Optimized Chromatographic Condition

Validation of Analytical Method

The developed chromatographic method was validated for Specificity, Linearity, Precision, Accuracy, Sensitivity, Robustness and System suitability [14-17].

System Suitability

Table 3: Results of System Suitability for Darolutamide

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Darolutamide	3.192	225645	20584	6286	1.38
2	Darolutamide	3.146	225847	20965	6358	1.39
3	Darolutamide	3.123	228656	20758	6285	1.41
4	Darolutamide	3.167	228547	20859	6278	1.40
5	Darolutamide	3.158	229658	20968	6395	1.42
Mean			227670.6			
Std. Dev.			1810.899			
% RSD			0.795403			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Darolutamide in drug product [18-19].

$$\%ASSAY = \frac{\text{Sample area} \times \text{Weight of standard} \times \text{Dilution of sample} \times \text{Purity} \times \text{Weight of tablet}}{\text{Standard area} \times \text{Dilution of standard} \times \text{Weight of sample} \times 100 \times \text{Label claim}} \times 100$$

= 99.24%

The% purity of Darolutamide in pharmaceutical dosage form was found to be 99.24%.

Linearity

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [19-21]. The results shown in Table 4.

Chromatographic Data for Linearity Study

Table 4: Data for Linearity

Concentration µg/ml	Average Peak Area
10	78683
20	146545
30	213584
40	279895
50	346568

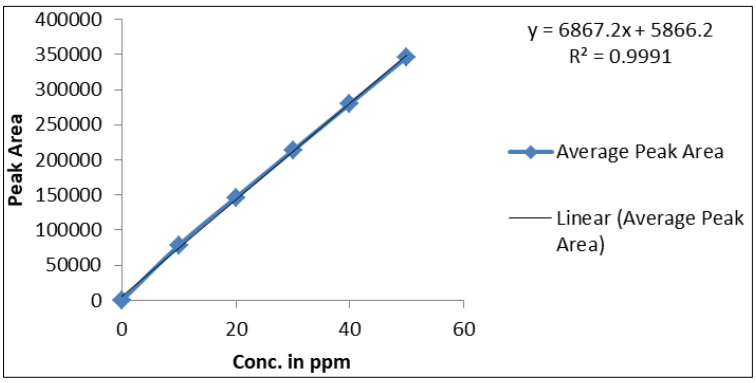

Fig 3: Calibration Curve of Darolutamide

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Darolutamide is a straight line [22].

$$Y = mx + c$$

Slope (m) =6867
Intercept (c) = 5866
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 5866. These values meet the validation criteria [23].

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [24].

Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated% RSD.

Table 5: Results of Method Precision for Darolutamide:

S. No.	Peak name	Retention time	Area (µV*sec)	Height(µV)	USP Plate Count	USP Tailing
1	Darolutamide	3.165	225645	20562	6125	1.36
2	Darolutamide	3.163	225847	20645	6129	1.36
3	Darolutamide	3.158	226542	20534	6135	1.35
4	Darolutamide	3.167	226598	20564	6189	1.36
5	Darolutamide	3.171	226584	20549	6138	1.35
6	Darolutamide	3.181	226859	20685	6179	1.37
Mean			226345.8			
Std. Dev			482.1068			
%RSD			0.212996			

Intermediate Precision [25]

Analyst 1

Table 6: Results of Ruggedness for Darolutamide

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Darolutamide	3.165	226534	20653	6235	1.35
2	Darolutamide	3.163	226542	20598	6198	1.36
3	Darolutamide	30158	225989	20653	6254	1.36
4	Darolutamide	3.167	226512	20548	6281	1.35
5	Darolutamide	3.171	226531	20653	6199	1.36
6	Darolutamide	3.171	225898	20658	6253	1.35
Mean			226334.3			
Std. Dev.			304.2622			
% RSD			0.13443			

Analyst 2

Table 7: Results of Intermediate Precision Analyst 2 for Darolutamide

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Darolutamide	3.173	225487	20542	6253	1.35
2	Darolutamide	3.134	225484	20532	6098	1.36
3	Darolutamide	3.161	225364	20541	6254	1.35
4	Darolutamide	3.174	226513	20534	6235	1.36
5	Darolutamide	3.199	225487	20549	6199	1.36
6	Darolutamide	3.199	226532	20451	6235	1.35
Mean			225811.2			
Std. Dev.			553.0524			
% RSD			0.244918			

Accuracy

The recovery studies were carried out at 50, 100 and 150% of the test concentration as per ICH guidelines [26, 32-33]. The results of the recovery studies and its statistical validation data are given in Table 8.

Table 8: The Accuracy Results for Darolutamide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109283.3	15	15.060	100.40%	100.42%
100%	212732	30	30.124	100.413%	
150%	316263.3	45	45.201	100.446%	

Limit of Detection for Darolutamide

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 [27].

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response
S = Slope of the calibration curve

Results

= 0.597µg/ml

Limit of Quantitation for Darolutamide

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined [28].

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response
S = Slope of the calibration curve

Results

= 1.811µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Darolutamide [29-31]. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Darolutamide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 9: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	225645	3.155	6125	1.36
Less Flow rate of 0.9 mL/min	236586	3.488	6452	1.38
More Flow rate of 1.1 mL/min	219865	2.877	6098	1.42
Less organic phase	235848	4.705	6126	1.43
More organic phase	241245	2.090	6324	1.39

Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 272nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry ODS C18 (4.6 x 250mm, 5µm) because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetonitrile: Methanol (80: 20% v/v) was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Run time was selected to be 8.0min because analyze gave peak around 3.158min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range of 10-50µg/ml of the Darolutamide target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

References

1. Drug Bank. Darolutamide [Internet]. [cited 2025 Jul 29]. Available from: https://go.drugbank.com/drugs/DB12941
2. PubChem. Darolutamide [Internet]. [cited 2025 Jul 29]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Darolutamide
3. Wikipedia. Darolutamide [Internet]. [cited 2025 Jul 29]. Available from: https://en.wikipedia.org/wiki/Darolutamide
4. Sharma BK. Instrumental methods of chemical analysis: Introduction to analytical chemistry. 23rd ed. Meerut: Goel Publishing House; 2004. p. 12-23.
5. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed. New Delhi: CBS Publishers and Distributors; 1986. p. 518-21, 580-610.
6. Adamovies J. Chromatographic analysis of pharmaceuticals. 2nd ed. New York: Marcel Dekker Inc.; 1997. p. 74, 5-15.
7. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. 5th ed. New Delhi: Himalaya Publishing House; 2002. p. 1.1-1.8, 2.566-2.570.
8. Skoog DA, Holler FJ, Nieman TA. Principles of instrumental analysis. 5th ed. Saunders College Publishing; 1998. p. 778-87.
9. Skoog DA, Holler FJ, Nieman TA. Principles of instrumental analysis. 5th ed. Harcourt Publisher's International Company; 2001. p. 543-54.
10. Kemp W. Organic spectroscopy. New York: Palgrave; 2005. p. 7-10, 328-30.
11. Sethi PD. HPLC: Quantitative analysis pharmaceutical formulations. New Delhi: CBS Publishers and Distributors; 2001. p. 3-137.
12. Schartz ME, Krull IS. Analytical method development and validation. 2004. p. 25-46.
13. Snyder R, Kirkland J, Glajch L. Practical HPLC method development. 2nd ed. New York: Wiley International Publication; 1997. p. 235, 266-8, 351-3, 653-600, 686-95.
14. Basic education in analytical chemistry. Anal Sci. 2001;17(1).
15. International Conference on Harmonization. Validation of analytical methods - definition and terminology. Q2A. Geneva: ICH; 1996.
16. Berry RI, Nash AR. Pharmaceutical process validation,

analytical method validation. In: Swarbrick J, Boylan JC, editors. Encyclopedia of pharmaceutical technology. New York: Marcel Dekker Inc.; 1993. p. 411-28.
17. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons. London: Pharmaceutical Press; 2004. p. 1109-10, 1601-2.
18. Florey K. Analysis profile of drugs substances. New York: Academic Press; 2005. p. 406-35.
19. Arora PN, Malhan PK. Biostatistics. India: Himalaya Publishers House; 2006. p. 113, 139-40, 154.
20. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. New York: John Wiley & Sons; 2009.
21. Dong MW. Modern HPLC for practicing scientists. Wiley; 2006.
22. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. New York: John Wiley & Sons; 1997.
23. Ahuja S, Rasmussen HT, editors. HPLC method development for pharmaceuticals. Academic Press; 2007.
24. Ahuja S, Dong MW, editors. Handbook of pharmaceutical analysis by HPLC. Elsevier/Academic Press; 2005.
25. Kazakevich YV, LoBrutto R, editors. HPLC for pharmaceutical scientists. Wiley; 2007.
26. Neue UD. HPLC columns: Theory, technology, and practice. New York: Wiley-VCH; 1997.
27. McMaster MC. HPLC, a practical user's guide. Wiley; 2007.
28. Doserge. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry. 8th ed. Lippincott Company; 1982. p. 183-97.
29. Snyder LR, Dolan JW. High-performance gradient elution: The practical application of the linear-solvent-strength model. Wiley Interscience; 2006.
30. Giddings JC. Dynamics of chromatography, Part I. Principles and theory. New York: Marcel Dekker, Inc.; 1965. p. 281.
31. Robards K, Haddad PR, Jackson PE. Principles and practice of modern chromatographic methods. Amsterdam: Elsevier/Academic Press; 1994.
32. ICH Guidance. Validation of analytical methods - definition and terminology. Q2A. Geneva: International Conference on Harmonization; 2005.
33. ICH Guidance. Validation of analytical procedures - methodology. Q2B. Geneva: International Conference on Harmonization; 2005.
34. Nawle Y, Bandre A, Kumar MV, Singh RM, Daroi P, Bhaskar V. Development and validation of darolutamide in pharmaceutical dosage form by RP-HPLC. Int J Pharm Sci Rev Res. 2023;80(1):60-5.
35. Kamani VG, Sujatha M, Daddala GB. Development and validation of a novel stability indicating HPLC method for the separation and determination of darolutamide and its impurities in pharmaceutical formulations. Res Gate. 2021;104(4):57-68. doi:10.31489/2021Ch4/57-68.
36. Venkatesh P, Kulandaivelu U, Rao GSK, Chakravarthi G, Alavala RR, Rajesh B. Development and validation of a stability indicating UPLC method for determination of darolutamide in its tablet formulation. Res J Pharm Technol. 2022;15(1):165-70. doi:10.52711/0974-360X.2022.00027.
37. Harmani NJ, Patel OB, Patel NS, Patel HU. Stability indicating analytical method development and validation for the estimation of darolutamide in pharmaceutical dosage form. World J Pharm Pharm Sci. 2020;9(6):1133-46.
38. Nawle Y, Daroi P, Sonawane B, Bandre A, Munipalli VK, Warde SU, Singh RM. Development and validation of HPTLC method for darolutamide in tablet dosage form. World J Pharm Res. 2022;11(6):868-81.