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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR DAROLUTAMIDE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise, accurate, high-performance thin-layer chromatography (HPTLC) method for determination of Darolutamide tablet dosage form has been developed and validated. Carbamazepine was used as Internal Standard (IS) in order to make the method more accurate and precise. Chromatographic separation of Darolutamide in tablet dosage fromulation and Carbamazepine was achieved on Silica gel 60 F_{254} HPTLC plates with mobile phase contain Tolune: Methanol: Ethyl Acetate: Acetic Acid in the proportion of (6:2:2:0.1 *V/V/V/V*). Darolutamide quantification was performed at 285 nm. Well-resolved bands were obtained with RF value 0.48. The method was validated for precision, accuracy, and specificity according to ICH guidelines. The calibration curve was found to be linear in the concentration range of 100-350 ng/band respectively. Correlation co-efficient by area was 0.9992. The method is selective and specific, with potential application in pharmaceutical analysis of these drugs in tablet dosage form.

KEYWORDS: Darolutamide, Carbamazepine, High Performance Thin Layer Chromatography (HPTLC), Internal Standard (IS), Method development and Method Validation.

INTRODUCTION

Darolutamide is chemically N-[(2S)-1-[3-(3-chloro-4-cyanophenyl)pyrazol-1-yl]propan-2-yl]-5-(1-hydroxyethyl)-1H-pyrazole-3-carboxamide. May be a formulation containing associate degree androgenic hormone receptor (AR) antagonist with potential antineoplastic activity. Darolutamide binds to ARs in target tissues; later, inhibiting androgen-induced receptor activation and facilitating the formation of inactive complexes that can't translocate to the nucleus. This prevents binding to and transcription of AR-responsive genes that regulate glandular cancer cell proliferation. This ultimately results in associate degree inhibition of growth in AR-expressing glandular cancer cells.[1]

Darolutamide may be a nonsteroidal androgenic hormone receptor antagonist for the treatment of castrate-resistant, non-metastatic glandular cancer (nmCRPC). This condition happens within the majority of patients with advanced glandular cancer WHO are treated with androgenic hormone receptor antagonists. although previous treatment for glandular cancer has been no-hit for these patients, the cancer eventually progresses to become immune to existing therapies.[1]

Darolutamide, sold under the brand name Nubeqa, is an antiandrogen medication which is used in the treatment of non-metastatic castration-resistant prostate cancer in men.[2-3] It is specifically approved to treat non-metastatic castration-resistant prostate cancer (nmCRPC) in conjunction with surgical or medical castration.[2] The medication is taken by mouth twice per day with food.[2] Side effects of darolutamide added to castration may include fatigue, asthenia, pain in the arms and legs, and rash. Darolutamide is a nonsteroidal antiandrogen (NSAA), and acts as a selective antagonist of the androgen receptor (AR).[4-5] It has been referred to as a second- or third-generation NSAA.[7-8]

A literature survey reveals that there is, High-Performance Liquid chromatography [HPLC], method for the estimation of Darolutamide form pharmaceutical formulation has been developed. However, there is no HPTLC method has been reported so far pharmaceutical dosage form. Hence trails were made to develop reliable and accurate HPTLC method for the Darolutamide in tablet dosage form.[9]

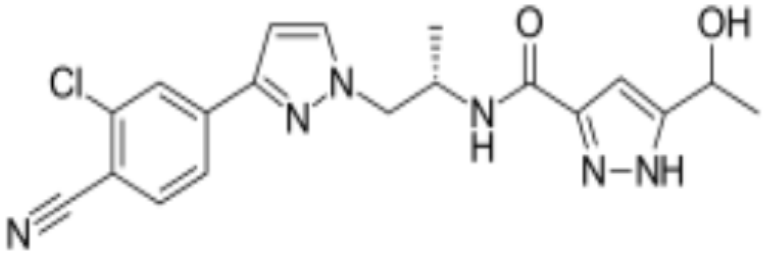

Fig 1: Chemical Structure of Darolutamide.

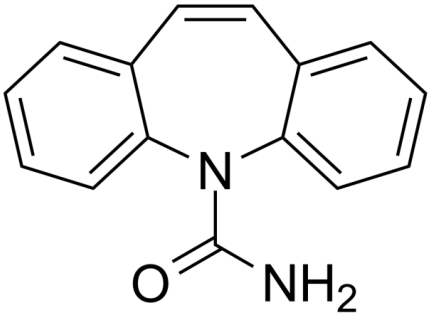

Fig. 2: Chemical Structure of Carbamazepine (Internal Standard).

EXPERIMENTAL

Instrumentation

The HPTLC system comprising of CAMAG Linomat 5 sample applicator, (CAMAG, Switzerland), coupled with Camag Hamilton Bonaduz schwetz syringe (100µl), UV chamber with dual wavelength UV lamps and CAMAG TLC scanner 4 controlled by vision CATS software (CAMAG) was used for the application and detection of spots respectively. The chromatographic separation of drugs were performed using pre-coated HPTLC plates silica gel 60 F_{254}, HPTLC plates from 250 µm thickness; (sigma- Aldrich) and a CAMAG twin-trough developing chamber was used for chromatographic method development.

Chemicals and reagents

Reference standards of Darolutamide and formulation (brand name: Nubeqa) and IS Carbamazepine was obtained from CDTL (Mumbai, India) where as Analytical grade toluene, methanol, and ammonia are from Final Chemicals (Mumbai, India) and silica gel 60 F_{254} plates from sigma- aldrich, germany.

Chromatographic conditions

Spotting was done using Camag Linomat 5 sample applicator (CAMAG, Switzerland) and Camag Hamilton Bonaduz microlitre syringe (100 µl) on HPTLC aluminium plates precoated with silica gel 60 F_{254} (20 cm × 10 cm with 250 µm thickness; Sigma-Aldrich). The plates were prewashed with methanol for 30 min in a CAMAG twin trough glass chamber closed with lid. The plates were activated at 110^{O}C for 10 min. The samples were spotted in the form of narrow bands having length of 8 mm. The application position X and Y were kept at 8 mm and 20 mm, respectively, to avoid edge effect. The distance between the two bands was 20 mm. Bands were applied at a constant rate of 15 nL/s using a nitrogen aspirator. Linear ascending development of chromatogram was carried out in a CAMAG twin trough glass chamber saturated with the mobile phase for 30 min and chromatogram run was kept up to 80 mm. Following the development, the HPTLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. Spectro densitometric analysis of the separated components was carried out using Camag TLC Scanner 4 in the reflectance–absorbance mode at 285 nm using a deuterium lamp. The slit dimension used was 6.0 mm × 0.3 mm and sensitivity was kept at auto mode. Scanning speed was 100 nm/s. Evaluation was achieved by linear regression of the peak area response against amount of drug by using vision CATS (CAMAG) software for peak area measurement and data processing.

PREPARATION OF SOLUTIONS

Standard Preparation

Ten milligrams of Darolutamide reference standards and 10 mg of Carbamazipine (Internal Standard) were accurately weighed and transferred into a 100-mL volumetric flask, each and the volume was made up to with Methanol. Further dilutions were made from the above solutions to get a final concentration of 200 µg/mL in methanol of each drug.

Analysis of marketed formulation (Sample Preparation)

Nubeqa (Darolutamide 300mg) 20 tablets were weighed, their mean weight was calculated and finely powdered. The weight of the fine powder equivalent to 100 mg of darolutamide was transferred into a 100 mL volumetric flask containing 50 mL of methanol dissoloved. The prepared solution was sonicated for 20 min, cooled and the volume was completed to 100 mL. The analysis was repeated six times and the possibility of excipients interference with the analysis was examined.

Optimization of the HPTLC method

The HPTLC procedure was optimized with a view to develop a assay method, respectively. Considering the chemical nature and polarity of the molecules to be separated, Silica gel F_{254} TLC plates were used. Structurally related drug carbamazepine selected as Internal Standard to make accurate method. After few trails, symmetric peak shape with acceptable SST parameters found with the Mobile Phase comprising of good resolution as well as sharp and symmetrical peak with improved R_F values were obtained from the mobile phase containing Toluene- Ethyl Acetate-Methanol-Acetic Acid in the ratio of (6:2:2:0.1 *V/V/V/V*).

Selection of wavelength of detection

UV spectra was taken by scanning the solutions of Darolutamide between 200 to 400 nm and 285 nm was selected as the wavelength for the determination of Darolutamide because at this wavelength, the drug showed maximum absorbance. UV spectra of Darolutamide is shown in the **Fig. 7.**

METHOD VALIDATION

To fortify the suitability of the method for its intended purpose, it was validated according to the ICH guidelines Q2 (R1)[10] and validation of the optimized HPTLC method was carried out with respect to the following parameters.

Specificity

The specificity of the method was ascertained by analyzing standard drug and test samples. The band for Darolutamide in the sample was confirmed by comparing the R_F value and spectrum of the spot with that of a standard. The peak purity of Darolutamide was determined by comparing the spectrum at three different regions of the spot, i.e. peak start (S), peak apex (M) and peak end (E). *r* (S, M) 0.9998, *r* (M, E) 0.9968. **Fig 6.**

Linearity

Linearity of the method was studied by six concentrations of the drug. Calibration curves were plotted over a concentration range of 100–350 ng/band. The standard working solution of Darolutamide were applied to the plate (100–350 ng/band). The calibration curves were developed by plotting peak area versus concentrations (n = 6) with the help of the visionCATS software. **Fig 8, Table 1.**

Precision

Precision of the method was demonstrated by interday, intraday and repeatability precision studies. Repeatability (% RSD) was determined by analysis of Darolutamide at the concentration of 200 ng/band, respectively. Intra-day precision (% RSD) was determined by the analysis standard solution of darolutamide at three levels of low, medium and higher concentrations of 150, 200 and 250 ng/band for the drugs three times on the same day. Inter-day precision (% RSD) was determined by the analysis of same solution on three completely different days over a period of 1 week. **Tables 2 and 3.**

Accuracy

The accuracy of the method was determined by the analysis of standard addition at three different levels. Recovery experiments were performed by adding standard drug were spiked with 110%, 120%, and 130% of the standard drug and the mixture was re-analysed by the proposed method. This was done to verify the recovery of the drug at different levels in the formulation. **Table 4.**

Sensitivity

The sensitivity of the developed method is expressed as limit of detection (LOD), the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value under the experimental conditions, as well as limit of quantification (LOQ), which is the lowest amount of analyte that can be detected and quantified with suitable precision, accuracy, and reproducibility. The LOD and LOQ are calculated based on the standard deviation of the regression lines and slope of the calibration curves using the below equations.

$LOD = 3.3 \times \sigma/S$ *(0.90)*

$LOQ = 10 \times \sigma/S$ *(2.73)*

Where σ is the standard deviation of the regression line and S is the slope of the calibration curve.

Robustness

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as changing the mobile phase ratio, chamber saturation period and distance travelled, slight change in the solvent phase distance, the effects on the results. The amount of mobile phase was varied over the range of ±5 ml, the chamber

saturation period was varied over the range of ±5 min and the distance travelled was varied over the range of ±5 mm. **Table 6.**[11]

RESULTS AND DISCUSSION

Results of the validation studies of the method developed for Darolutamide in the current study involving Toluene-Methanol-Ethyl Acetate- Acetic Acid (6:2:2:0.1 *V/V/V*) as mobile phase using HPTLC plates (Silica Gel_{254}).

Linear relationships were observed by plotting drug concentrations against peak areas for each compound. Darolutamide showed linear response in the concentration range of 100-350 ng/band. The corresponding linear regression equation was y = 0.0002x + 0.0016 with correlation coefficient of the calibration plot was 09992, respectively as shown in **Fig 8.**

Results of the repeatability and intermediate precision experiments are shown in the **Tables 2 and 3**. The developed method was found to be precise as the %RSD values for repeatability and intermediate precision studies were 1.01%, respectively as recommended by ICH guidelines.

Using the trend line equations derived from the experiments, the sensitivity of the method in terms of LOD and LOQ was calculated based on the standard deviation of the regression lines and slope of calibration curves. The LOD and LOQ were found to be 0.90, 2.73. indicating the sensitivity of the proposed method **Table 5.**

When used to evaluate the recovery after spiking with three concentrations of standard, 110%, 120%, and 130%. The proposed method showed good percentage recovery rates between 98-100%. The results of the recovery studies and its statistical validation are given in **Tables 4.**

The chromatogram of the pharmaceutical formulation obtained using the developed method showed peaks at R_F of for Darolutamide, respectively, and was found to be at the same R_F for the standard drug. The peak purity of Darolutamide was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the band. The results shown in **Table 5** demonstrate that the purity exceeded 0.999 for all peaks, indicating the specificity of the method in the presence of various excipients. Overlain peak purity spectra of Darolutamide are shown in the **Fig. 6**.

The R_F and standard deviation of peak areas were calculated for each parameter and the %RSD was found to be <2%. The low values of the %RSD and no significant changes in the R_F, as shown in the **Table 6**, indicate the robustness of the method.

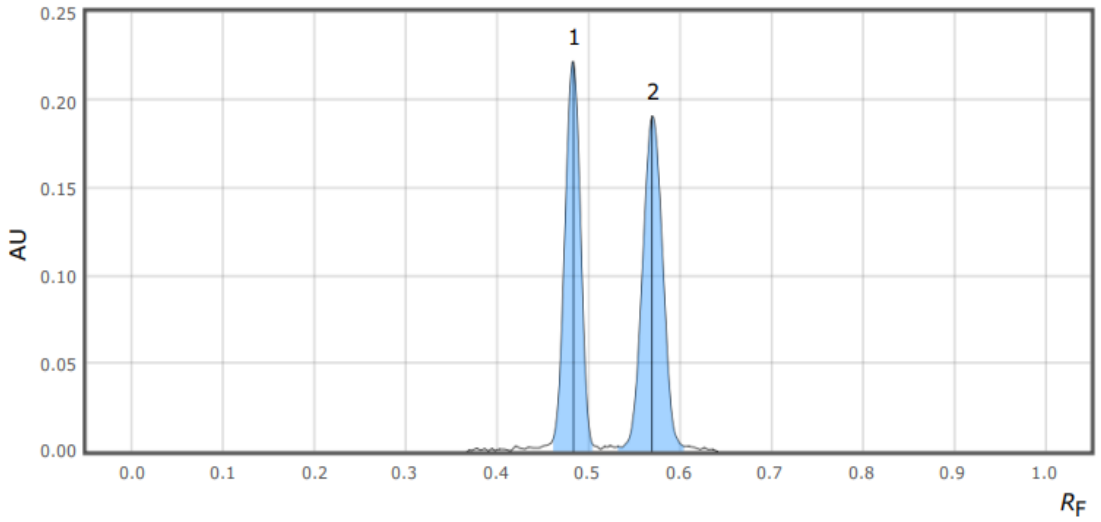

Fig. 3: HPTLC densitogram under optimized conditions showing R_F values of 0.48 for Darolutamide (200 ng/band).

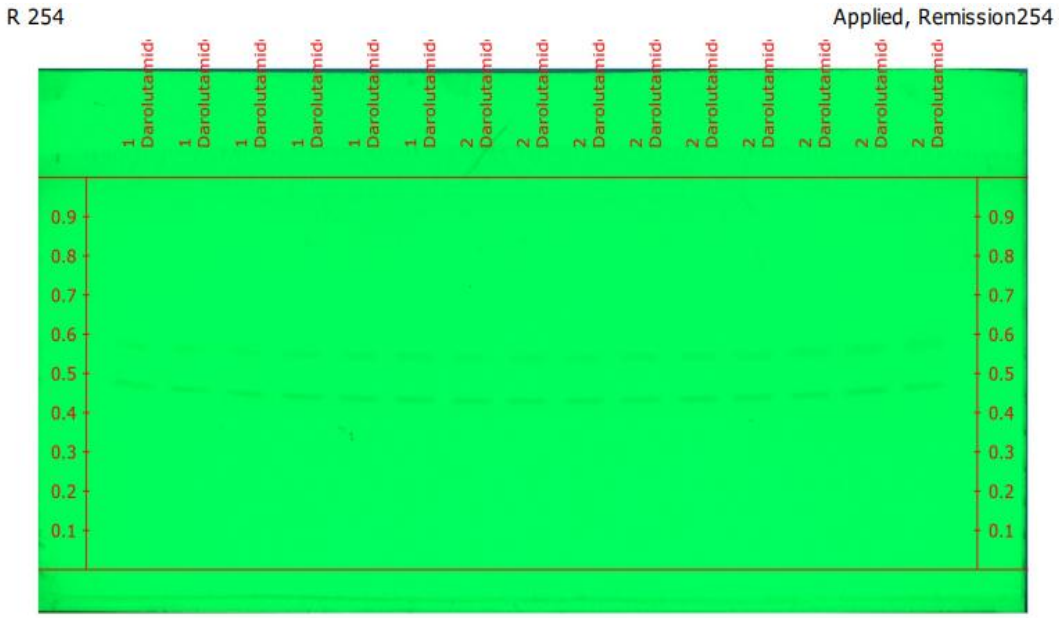

Fig 4: Images of the HPTLC plates taken at 254 nm.

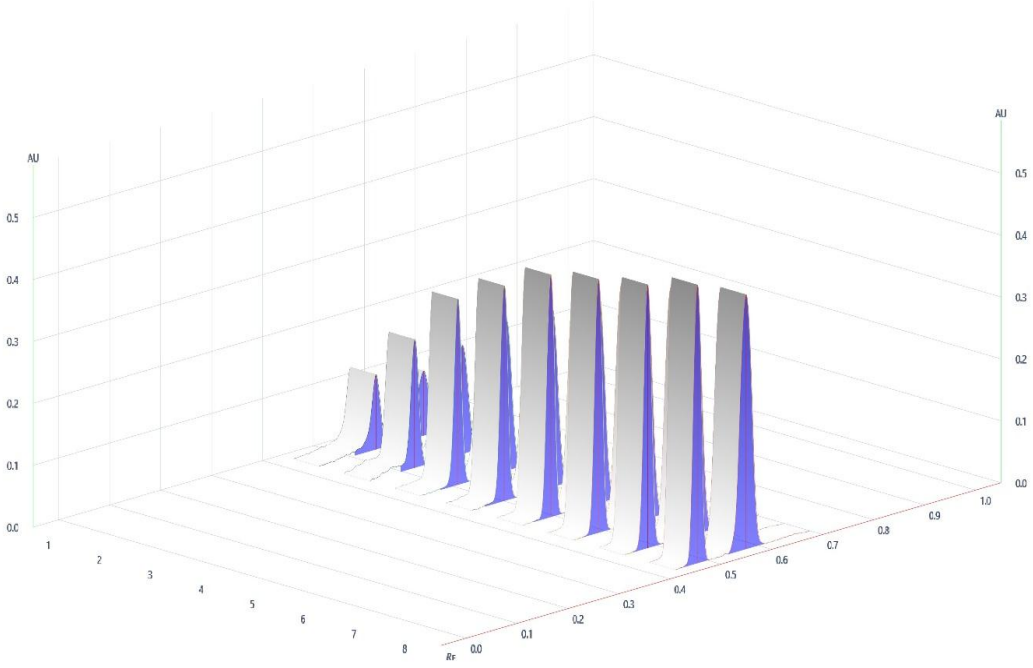

Fig 5: Three-dimensional densitogram for the linearity of Darolutamide at 285 nm.

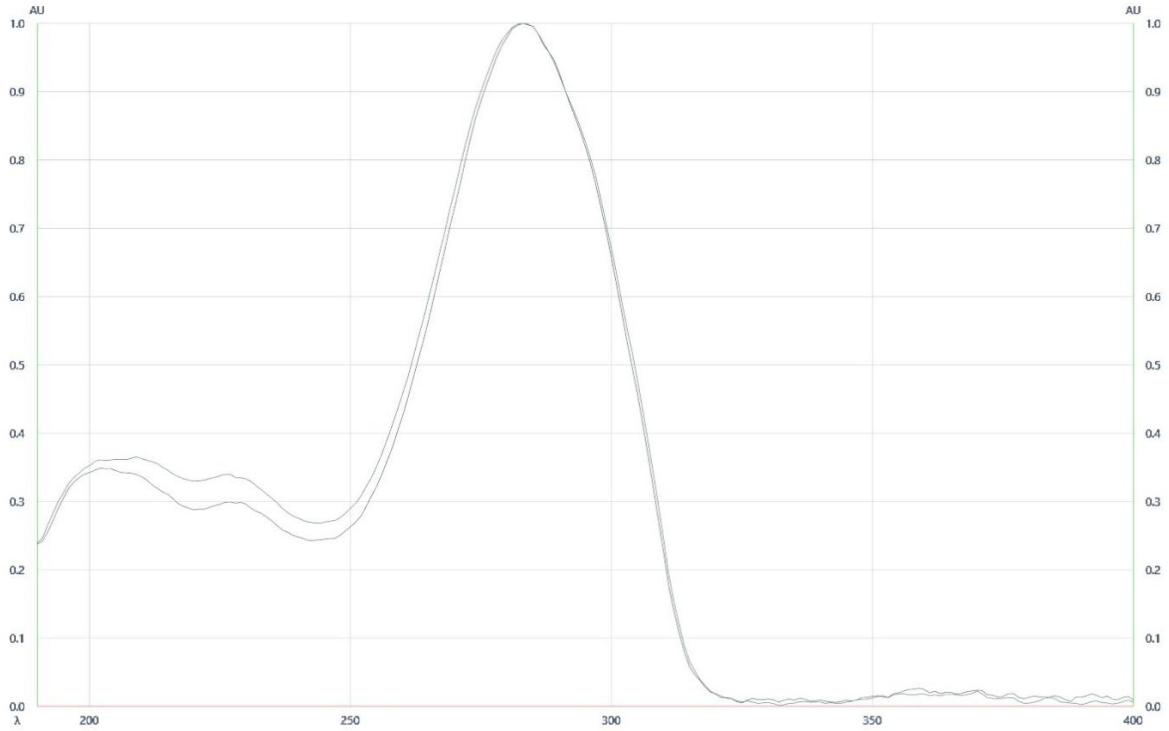

Fig 6: Overlain peak purity spectra of Darolutamide.

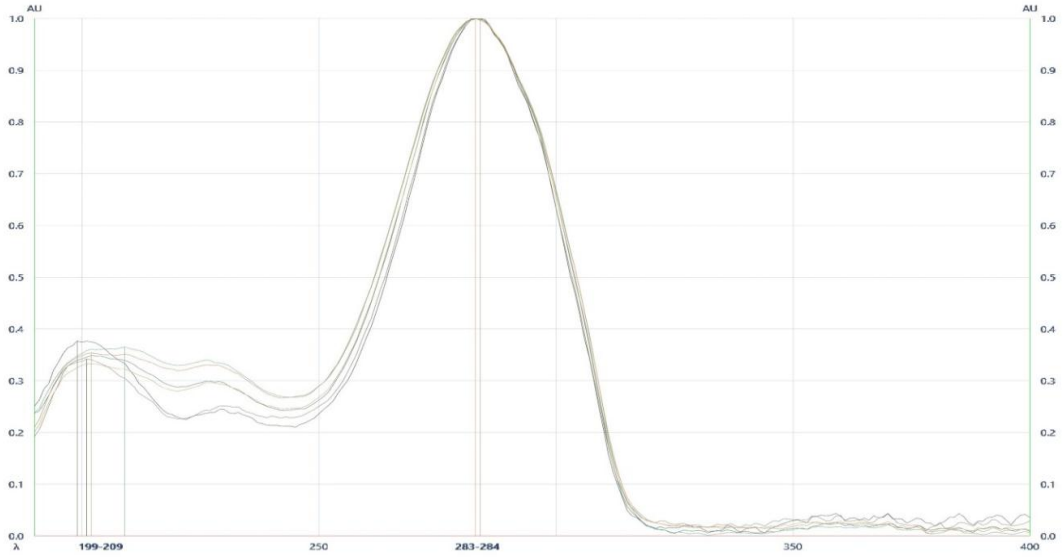

Fig 7: UV Spectra of Darolutamide.

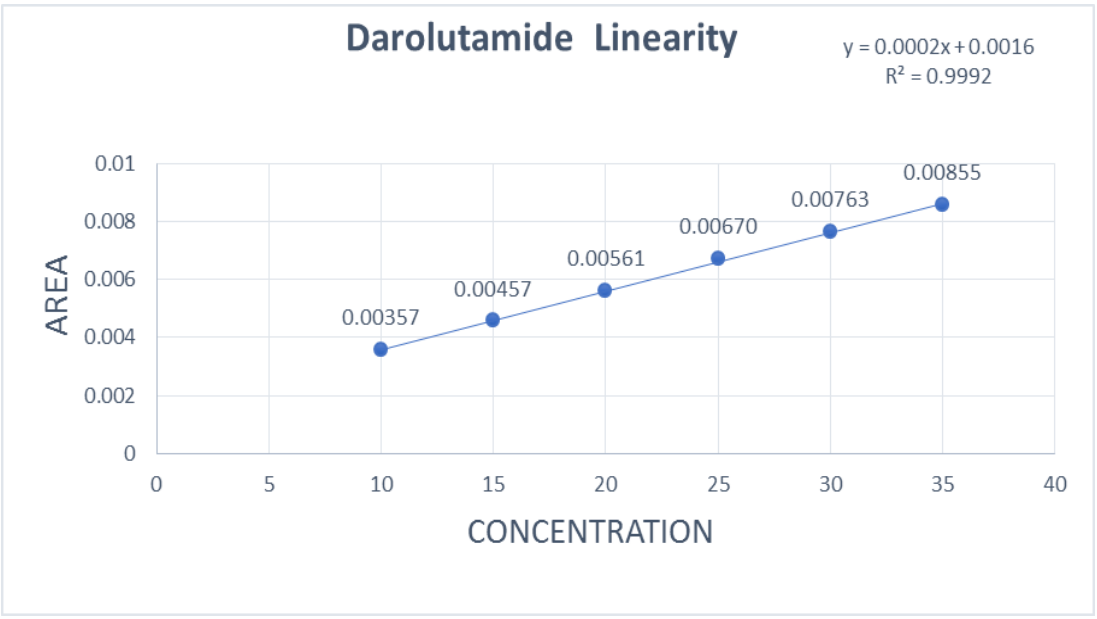

Fig 8: Calibration plot for Darolutamide.

Table 1: Linearity data of Darolutamide.

Concentration (ng/band)	Peak Area
100	0.00357
150	0.00457
200	0.00561
250	0.00670
300	0.00763
350	0.00855

Table 2: Result from determination of precision Darolutamide as repeatability.

Concentration (ng/band)	Peak Area
200	0.00551
200	0.00539
200	0.00536
200	0.00537
200	0.00540
200	0.00538
Average (n=6)	0.00540
SD	5.49241902
RSD (%)	1.01680081

**Average Mean of Six determination, SD = Standard Deviation, % RSD = Percentage relative standard deviation, NMT = Not more than, NLT = Not less than.*

Table 3: Result from determination of precision Darolutamide.

Concentration (ng/band)	Intra-day precision		Inter-day precision	
	Peak area SD (n=3)	%RSD	Peak area SD (n=3)	%RSD
150	0.00510 ±0.0000208	0.40	0.00512 ±0.0000288	0.62
200	0.00598 ±0.000416	0.69	0.00602 ±0.0000300	0.49
250	0.00732 ±0.0000137	1.87	0.00738 ±0.0000126	1.71

Table 4: Result from recovery study of Darolutamide.

% LEVEL	Amount Spiked (ng/band)	Amount Recovered (ng/band)	%Recovery	%Mean Recovery	% RSD
110	220	216	98.20	98.28	0.2172
110	220	216	98.52		
110	220	215	98.12		
120	240	235	98.23	99.09	0.8981
120	240	240	100.01		
120	240	237	99.04		
130	260	264	101.88	100.19	1.6424
130	260	260	100.09		
130	260	256	98.59		

Table 5: Analysis of marketed formulation.

Label claim (mg/tablet)	Amount found (mg)	Label claim estimated (%)	(%) RSD
300	296.31	98.77	0.46

Table 6: Robustness results of the proposed HPTLC method Change in the mobile phase ratio (6:2:2:0.1% ± 0.2 in toluene content)

Ratio	R_F	Area ± SD (ng/band)	%RSD
5.8:2:2:0.1%	0.47±0.02	0.00585±0.00001.52	0.26
6.2:2:2:0.1%	0.44±0.02	0.00587±0.00002.64	0.45

Change in chamber saturation time (40 min ± 5)

Saturation time (min)	R_F	Area ± SD (ng/band)	%RSD
35	0.47±0.02	0.00581±0.00001.52	0.26
45	0.44±0.02	0.00581±0.00002.00	0.34

Change in distance travelled (80mm ± 5)

Distance travelled (mm)	R_F	Area ± SD (ng/band)	%RSD
75	0.46±0.02	0.00588±0.00002.64	0.44
85	0.47±0.02	0.00591±0.00001.00	0.16

Table 7: Analytical validation parameters for Darolutamide using the HPTLC method.

Parameters	Darolutamide
Linearity	
Linearity range (ng/band)	100-350
Correlation coefficient (r^2)	0.9992
Precision (%RSD)	
Repeatability	1.01
Intra-day precision	0.40-1.87
Inter-day precision	0.49-1.71
Sensitivity	
LOD (ng/band)	0.90
LOQ (ng/band)	2.73
Specificity	
r (S, M)	0.9998
r (M, E)	0.9968
Accuracy	
110	98.28±0.21
120	99.09±0.89
130	100.19±1.64

CONCLUSIONS

This study reports a simple, fully validated HPTLC protocol for the quantification Darolutamide in pharmaceutical formulation. It demonstrates that the method can accurately quantify the drug content of the tablet formulation without excipient interference or the necessity of a drug extraction step prior to analysis. The data obtained from various parameter indicates the accuracy of the method, hence the method can be applied for the routine in quality control of Darolutamide in tablet dosage form. Given the ease of sample preparation, HPTLC's high capacity (with up to 15 samples per plate) and the flexibility of running qualitative and quantitative assays simultaneously, the approach should be considered.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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