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A NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR SENSITIVE AND RAPID ESTIMATION OF OSILODROSTAT IN ISTURISA

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ABSTRACT

Osilodrostat is a steroidogenesis inhibitor widely used in the management of Cushing's syndrome, necessitating the development of reliable analytical methods for routine quality control and stability assessment. The present study focused on the development and validation of a simple, rapid, accurate, and stability-indicating RP-HPLC method for the quantitative estimation of Osilodrostat in bulk drug and pharmaceutical dosage form following ICH Q2(R2) guidelines. Chromatographic separation was achieved using a YMC Accura Triart C18 column (250 × 4.6 mm, 5 µm) with a mobile phase comprising 10 mM ammonium bicarbonate in water and acetonitrile (85:15 v/v) at a flow rate of 0.7 mL/min. Detection was performed at 245 nm with a runtime of 10 minutes. Under optimized conditions, Osilodrostat exhibited a sharp and symmetrical peak at a retention time of 3.936 minutes. System suitability studies showed an average peak area of 1268445, theoretical plate count of 5041, and tailing factor of 1.345 with %RSD for peak area of 0.058%, confirming excellent precision and chromatographic reproducibility. The method demonstrated linearity over the concentration range of 1.44–5.4 µg/mL with proportional detector response. Sensitivity studies produced signal-to-noise ratios of 5.65 for LOD and 11.30 for LOQ. Accuracy studies at 50%, 100%, and 150% levels showed mean recoveries of 100.83%, 100.46%, and 101.16%, respectively. Robustness studies under varied flow rates and temperatures showed minimal chromatographic variation. Forced degradation studies revealed stability under acidic, alkaline, and thermal conditions, while oxidative stress caused noticeable degradation. The assay of Isturisa tablets showed 101.08% drug content. Overall,

the developed RP-HPLC method was found to be precise, robust, sensitive, and suitable for routine analysis and stability studies of Osilodrostat.

KEYWORDS: Osilodrostat, YMC Accura Triart C18, ICH Q2(R2), Cushing's syndrome, steroidogenesis inhibitor.

INTRODUCTION

Osilodrostat is a synthetic steroidogenesis inhibitor belonging to the class of antihypercortisolemic agents, primarily used in the treatment of Cushing's disease. Chemically, it is identified as 3-fluoro-4-[(5R)-5H,6H,7H-pyrrolo[1,2-c]imidazol-5-yl]benzonitrile with a molecular formula of $C_{13}H_{10}FN_3$ and a molecular weight of 227.24 g/mol. The drug exhibits moderate lipophilicity (log P 2.18), a pKa of 7.15, and low aqueous solubility (0.356 mg/mL), with plasma protein binding less than 40%, making it suitable for oral therapeutic applications. It was approved by the US FDA in March 2020 under the brand name Isturisa for the management of endogenous Cushing's syndrome.The pharmacological activity of Osilodrostat is attributed to its selective inhibition of 11β-hydroxylase (CYP11B1), a key mitochondrial enzyme responsible for the final step in cortisol biosynthesis. By blocking the conversion of 11-deoxycortisol to cortisol in the adrenal cortex, the drug effectively reduces circulating cortisol levels[1-3], thereby alleviating symptoms associated with hypercortisolemia such as hypertension, hyperglycemia, obesity, and immunosuppression[4-9]. Additionally, Osilodrostat exhibits minor inhibitory effects on aldosterone synthase (CYP11B2), which may influence mineralocorticoid pathways [10-17]. The suppression of cortisol synthesis results in feedback elevation of adrenocorticotropic hormone (ACTH) and accumulation of steroid precursors, contributing to its therapeutic efficacy in long-term disease management[18-28].Analytical method development for Osilodrostat is crucial for ensuring its quality, safety, and efficacy in pharmaceutical formulations. High-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis due to its high sensitivity, precision, and capability to separate complex mixtures based on differential interactions between analytes and stationary phases . Several analytical methods have been reported for the estimation of Osilodrostat using advanced techniques[29-39]. LC-MS/MS methods have been extensively developed for quantification in biological matrices such as human plasma[40-49] and equine plasma[50-54], demonstrating high sensitivity, selectivity, and wide linear ranges. For instance, Balakirouchenane et al[55]. reported an LC-MS/MS method with excellent precision (<7.2%)

and accuracy (95.9–114.9%), while Ishii et al.[56] investigated metabolic profiling using LC/ESI–HRMS. Similarly, Fleseriu et al.[57] developed a highly sensitive LC-MS/MS method with a linear range of 0.5–500 ng/mL and precision below 8.5% RSD. In contrast, only a limited number of RP-HPLC methods have been reported for Osilodrostat in bulk form, such as the method described by Patel et al.,[58-62]which exhibited a retention time of 5.160 minutes and linearity in the range of 10–30 µg/mL .

Despite these advancements, most reported methods are focused on bio analytical applications or involve complex instrumentation such as mass spectrometry, which may not be feasible for routine quality control laboratories. Furthermore, there is a limited availability of simple, rapid, and stability-indicating RP-HPLC methods specifically designed for the estimation of Osilodrostat in its marketed dosage form (Isturisa). The need for a method with reduced run time, improved sensitivity, and compliance with regulatory validation requirements remains significant.Therefore, the rationale of the present study is to develop a simple, rapid, cost-effective, and stability-indicating RP-HPLC method for the quantitative estimation of Osilodrostat in pharmaceutical dosage form. The developed method aims to overcome the limitations of existing techniques by providing efficient chromatographic separation with shorter retention time and reliable analytical performance.The primary aim of this study is to develop and validate a novel RP-HPLC method for the estimation of Osilodrostat in both pure drug and marketed formulation (Isturisa) using PDA detection, in accordance with ICH Q2(R2) guidelines. The plan of work involved optimization of method by performing trails later performing a validation.The Validated method s applied for the assessment of presence of Osilodrostat in Isturisa thereby progressing for forced degradation studies.

MATERIALS AND METHODS

Chemicals and Reagents

Osilodrostat pure active pharmaceutical ingredient (API) was obtained as a gift sample from YMC India Pvt. Ltd., while the marketed formulation Isturisa was procured from a local pharmacy. HPLC-grade acetonitrile and methanol were purchased from Rankem. Trifluoroacetic acid (TFA) was obtained from SRL, and ammonium bicarbonate was also procured from Rankem. All chemicals and reagents used in the study were of analytical or HPLC grade to ensure accuracy and reproducibility of the results .

Instrumentation

Chromatographic analysis was performed using a Shimadzu HPLC system (P-series). Sample preparation was supported by an ultrasonic cleaner (LAB MAN Scientific, LMUC-12) to ensure proper dissolution and extraction. Weighing was carried out using a Sartorius analytical balance (SECURA225D-10IN). Purified water was obtained from a LAB JAL Millipore system (LAB NE15UV). pH adjustments were monitored using an EUTECH 700 pH meter, and thermal studies were conducted using a hot air oven (Sri Sai Scientific, SSHAO/021/2022-25).

Preparation of Diluent

A mixture of acetonitrile and water in the ratio of 1:1 (v/v) was used as the diluent throughout the study. The prepared diluent was filtered and degassed prior to use. All standard and sample solutions were prepared using this diluent, ensuring consistent solubility and stability of Osilodrostat across all analytical procedures.

Preparation of Mobile Phase

The mobile phase was prepared by dissolving approximately 514 mg of ammonium bicarbonate in about 500 mL of HPLC-grade water and making up the volume to 650 mL to obtain a 10 mM solution. To this aqueous phase, 350 mL of acetonitrile was added to achieve a final composition of water:acetonitrile (65:35 v/v). The prepared mobile phase was thoroughly mixed, filtered through a 0.45 μm membrane filter, and sonicated for 10–15 minutes to remove dissolved gases before use.

Chromatographic Conditions

Chromatographic separation was carried out using a YMC Accura Triart C18 column (250 × 4.6 mm, 5.0 μm). The mobile phase consisted of 10 mM ammonium bicarbonate in water and acetonitrile in the ratio of 85:15 (v/v). The flow rate was maintained at 0.7 mL/min, and the column temperature was set at 30°C. The injection volume was 10 μL, and the total run time was 10 minutes. Detection was performed using a PDA detector.

Preparation of Standard Stock Solution

The standard stock solution was prepared by accurately weighing 1.8 mg of Osilodrostat API and transferring it into a 25 mL volumetric flask. The drug was dissolved in a small quantity of diluent (acetonitrile:water, 1:1 v/v), and the volume was made up to the mark with the same diluent to obtain a final concentration of 72 μg/mL .

Preparation of Secondary Stock Solution

From the prepared standard stock solution, 1 mL was transferred into a 10 mL volumetric flask and diluted up to the mark with the diluent to obtain a secondary stock solution of concentration 7.2 µg/mL. This solution was used for further dilution and calibration studies .

Preparation of Sample Solution (Isturisa)

The sample solution was prepared by transferring an amount equivalent to approximately 10 mg of Osilodrostat from the marketed formulation into a 100 mL volumetric flask. About 70 mL of diluent was added, and the mixture was sonicated for 15 minutes with intermittent shaking to ensure complete extraction of the drug from the formulation matrix. After cooling to room temperature, the volume was made up to 100 mL with the diluent to obtain a stock solution of approximately 100 µg/mL. Further, 5 mL of this stock solution was diluted to 10 mL with diluent to obtain a working concentration of 50 µg/mL. The solution was filtered through a 0.45 µm membrane filter, discarding the initial filtrate, and the final filtrate was used for HPLC analysis.

Preparation of Linearity Solutions

Linearity solutions were prepared from the secondary stock solution by transferring aliquots of 0.20 mL, 0.30 mL, 0.40 mL, 0.50 mL, 0.60 mL, and 0.75 mL into individual volumetric containers and diluting to volume with diluent. This resulted in final concentrations of 1.44, 2.16, 2.88, 3.6, 4.32, and 5.4 µg/mL, respectively, which were used for constructing the calibration curve.

Preparation of Stability-Indicating (Forced Degradation) Solutions

Forced degradation studies were carried out under various stress conditions including acidic, basic, oxidative, and thermal environments to evaluate the stability-indicating capability of the developed RP-HPLC method.

For acidic degradation, 5 mL of Osilodrostat standard solution (72 µg/mL) was transferred into 50 mL of 0.1 N hydrochloric acid and incubated at 60°C for 2 hours to induce degradation. After completion of the degradation period, the solution was cooled to room temperature and neutralized using 0.1 N sodium hydroxide. The resulting solution was then diluted to the required concentration with diluent, filtered through a 0.22 µm membrane filter, and subjected to chromatographic analysis.

For basic degradation, 5 mL of Osilodrostat standard solution (72 µg/mL) was mixed with 50 mL of 0.1 N sodium hydroxide and incubated at 60°C for 2 hours. After the degradation process, the solution was cooled and neutralized with 0.1 N hydrochloric acid. The final

solution was diluted with diluent to the required concentration, filtered through a 0.22 µm membrane filter, and analyzed using the developed HPLC method.

For oxidative degradation, 5 mL of Osilodrostat stock solution was treated with 50 mL of 3% hydrogen peroxide (H_2O_2) and allowed to stand at room temperature for 2 hours. After completion of the reaction, the solution was diluted appropriately with diluent to achieve the desired concentration and filtered through a 0.22 µm membrane filter prior to chromatographic analysis.

For thermal degradation, approximately 1 mg of Osilodrostat API was exposed to dry heat at 105°C in a hot air oven for 24 hours. After the exposure period, 0.5 mg of the degraded sample was accurately weighed and dissolved in 10 mL of diluent to obtain a stock solution with a concentration of 72 µg/mL. The solution was filtered through a 0.22 µm membrane filter and analyzed using the developed RP-HPLC method.Each of the degraded samples was evaluated for peak purity, resolution, and formation of degradation products to confirm the stability-indicating nature of the developed analytical method.

RESULTS AND DISCUSSION

The development of the RP-HPLC method for Osilodrostat was initiated through systematic optimization trials to achieve a sharp, symmetrical, and well-resolved peak. In the first assessment, performed using a YMC Triart Bio C4 column with a mobile phase of ammonium acetate and acetonitrile (20:80 v/v), the chromatographic peak was found to be blunt and lacked symmetry, indicating inadequate separation efficiency (Figure 1). In the second assessment, the column was changed to YMC Accura Triart C18 (150 mm), and the mobile phase composition was modified to ammonium bicarbonate buffer (70:30 v/v); although peak formation was observed, baseline disturbances and noise were evident (Figure 2). Further optimization in the third trial using a YMC Accura Triart C18 column (250 mm) with a mobile phase composition of ammonium bicarbonate and acetonitrile (85:15 v/v), at a flow rate of 0.7 mL/min and column temperature of 30°C, resulted in a sharp, symmetrical peak with stable baseline and optimal retention time. These optimized chromatographic conditions were finalized for further validation studies (Figure 3) .

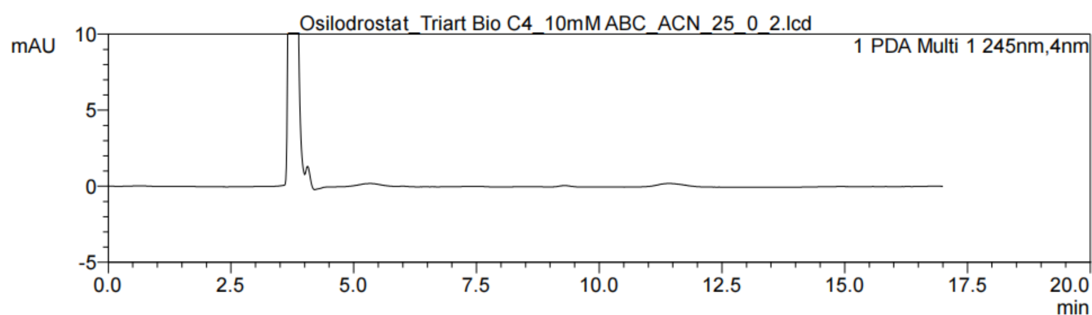


Figure 1: Tracking of Osilodrostat's first assessment.

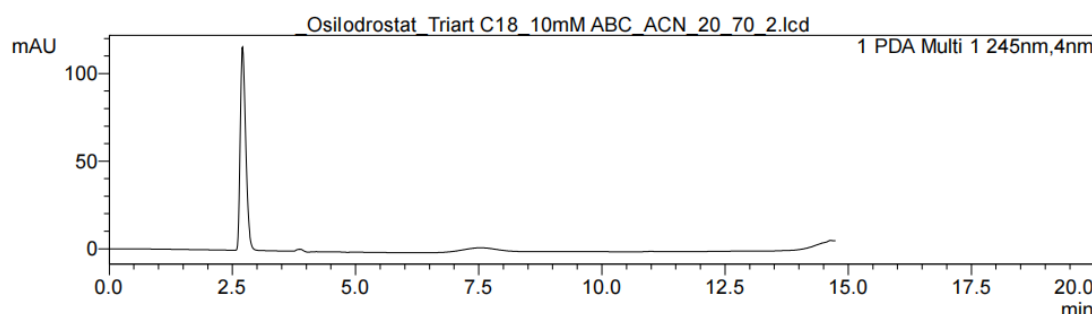


Figure 2: Tracking of Osilodrostat's second assessment.

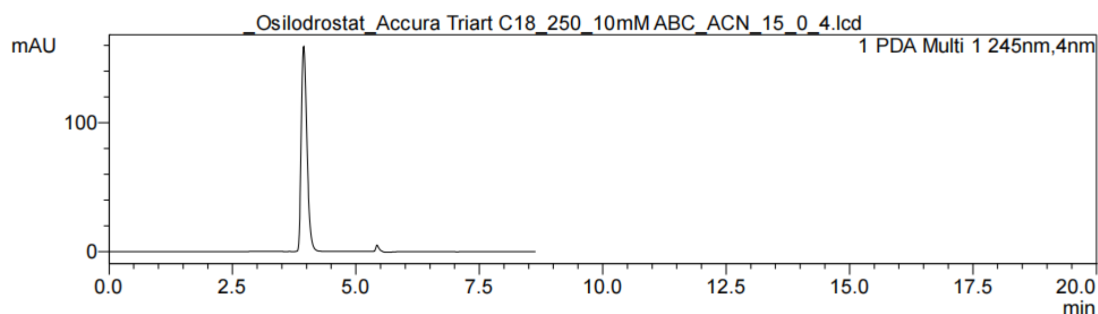


Figure 3: Tracking of third assessment

Specificity studies confirmed the absence of interference from diluent or blank, indicating that the method is selective for Osilodrostat Figure 4.System suitability results demonstrated excellent performance of the developed method. The retention time was consistently observed at 3.936 minutes with a %RSD of 0.000%, while the average peak area was 1268445 with a %RSD of 0.058%. Theoretical plate count averaged 5041 and tailing factor was 1.345, confirming good column efficiency and peak symmetry, as summarized in Table 1 and chromatograms in Figures 5. Precision studies revealed excellent repeatability and intermediate precision. In intra-day precision, %RSD for peak area was 0.058%, while in inter-day precision, %RSD was found to be 0.018%, demonstrating high reproducibility of the method. The retention times ranged between 3.893 and 3.904 minutes with consistent

peak area response, as presented in Table 2. Linearity was established over a concentration range of 1.44–5.4 µg/mL, with peak areas increasing proportionally from 24457 to 96545. The calibration curve exhibited excellent linear correlation, confirming direct proportionality between concentration and detector response (Table 3; Figure 6).

Table 1:System Success Statistics accompanied. ***(n=6)***

Data File Name	Sample Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_002.lcd	Blank	Blank	0.000	0	--	--
13042026_003.lcd	System Precision	Inj_01	3.936	1267445	5114	1.383
13042026_004.lcd	System Precision	Inj_02	3.936	1269173	5100	1.374
13042026_005.lcd	System Precision	Inj_03	3.936	1268113	5023	1.343
13042026_006.lcd	System Precision	Inj_04	3.936	1268570	5008	1.347
13042026_007.lcd	System Precision	Inj_05	3.936	1269368	4979	1.332
13042026_008.lcd	System Precision	Inj_06	3.936	1268001	5025	1.293
Average			3.936	1268445	5041	1.345
%RSD			0.000	0.058	1.064	2.377
Standard Deviation			0.000	735	54	0.032

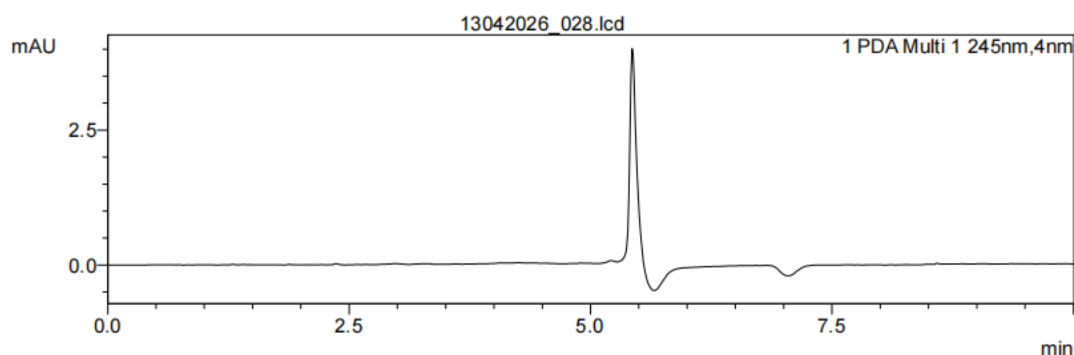


Figure 4: Accomplishment of (ACN:Water)

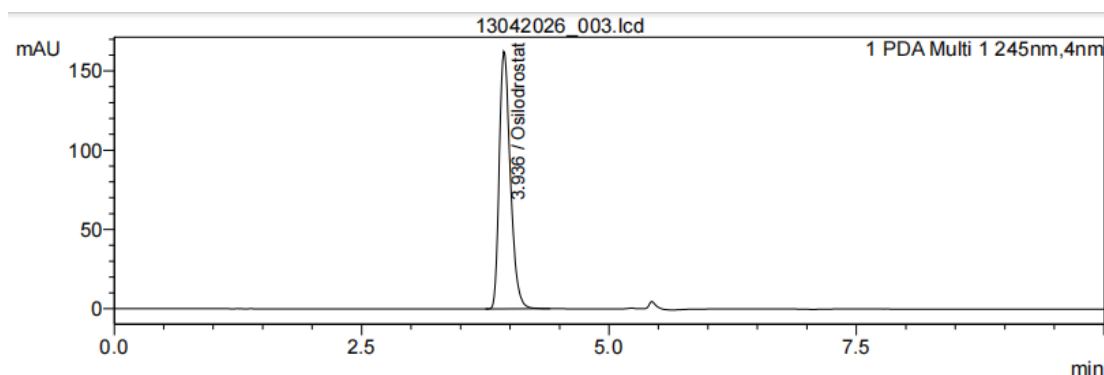


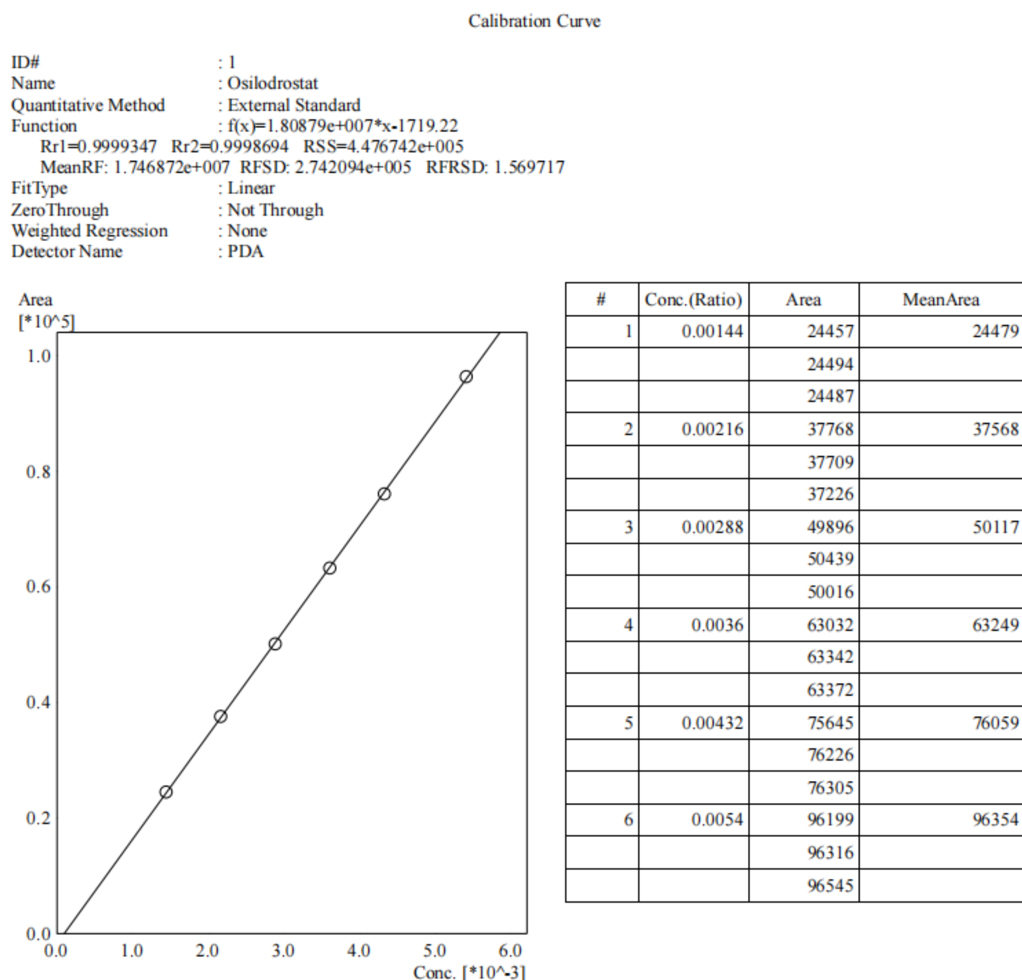
Figure 5: Accomplishment for Osilodrostat.

Table 2: Accomplishment in arbitrary injections.

Data File Name	Sample Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_056.lcd	Blank	Blank	0.000	0	--	--
13042026_057.lcd	Interday Precision	Inj_01	3.904	1270576	4686	1.293
13042026_058.lcd	Interday Precision	Inj_02	3.904	1270581	4698	1.286
13042026_059.lcd	Interday Precision	Inj_03	3.904	1270515	4692	1.289
13042026_060.lcd	Interday Precision	Inj_04	3.904	1271016	4691	1.284
13042026_061.lcd	Interday Precision	Inj_05	3.893	1270987	4657	1.401
13042026_062.lcd	Interday Precision	Inj_06	3.893	1270856	4649	1.368
Average			3.900	1270755	4679	1.320
%RSD			0.141	0.018	0.439	3.870
Standard Deviation			0.006	225	21	0.051

Table 3: Extensive availability Vs Concentration.

Data File Name	Sample Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_009.lcd	Blank	Blank	0.000	0	--	--
13042026_010.lcd	Linearity_01	Inj_01	3.936	24457	5029	1.263
13042026_011.lcd	Linearity_01	Inj_02	3.925	24494	4950	1.395
13042026_012.lcd	Linearity_01	Inj_03	3.925	24487	4938	1.366
13042026_013.lcd	Linearity_02	Inj_01	3.936	37768	4926	1.319
13042026_014.lcd	Linearity_02	Inj_02	3.925	37709	4930	1.391
13042026_015.lcd	Linearity_02	Inj_03	3.925	37226	4900	1.343
13042026_016.lcd	Linearity_03	Inj_01	3.925	49896	4935	1.370
13042026_017.lcd	Linearity_03	Inj_02	3.925	50439	4830	1.334
13042026_018.lcd	Linearity_03	Inj_03	3.925	50016	4835	1.310
13042026_019.lcd	Linearity_04	Inj_01	3.925	63032	4869	1.346
13042026_020.lcd	Linearity_04	Inj_02	3.925	63342	4852	1.356
13042026_021.lcd	Linearity_04	Inj_03	3.925	63372	4855	1.284
13042026_022.lcd	Linearity_05	Inj_01	3.925	75645	4886	1.276
13042026_023.lcd	Linearity_05	Inj_02	3.915	76226	4822	1.414
13042026_024.lcd	Linearity_05	Inj_03	3.915	76305	4817	1.412
13042026_025.lcd	Linearity_06	Inj_01	3.915	96199	4833	1.399
13042026_026.lcd	Linearity_06	Inj_02	3.915	96316	4819	1.398
13042026_027.lcd	Linearity_06	Inj_03	3.915	96545	4797	1.398
Average			3.924	57971	4879	1.354
%RSD			0.168	42.428	1.265	3.551
Standard Deviation			0.007	24596	62	0.048



#	Conc.(Ratio)	Area	MeanArea
1	0.00144	24457	24479
		24494	
		24487	
2	0.00216	37768	37568
		37709	
		37226	
3	0.00288	49896	50117
		50439	
		50016	
4	0.0036	63032	63249
		63342	
		63372	
5	0.00432	75645	76059
		76226	
		76305	
6	0.0054	96199	96354
		96316	
		96545	

Figure 6: An Ordinal contour across 1.44-5.4 µg/mL.

Sensitivity of the method was confirmed through LOD and LOQ studies. At the LOQ level, the average peak area was 25013 with %RSD of 0.485% and signal-to-noise ratio of 11.30, while at the LOD level, the average peak area was 12339 with %RSD of 0.237% and signal-to-noise ratio of 5.65. These results confirm that the method is sufficiently sensitive for quantification and detection of Osilodrostat at low concentrations (Table 4 and 5).

Table 4: A Measure of Method Sensitivity: Defining the Thresholds for Osilodrostat Quantification.

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor	S/N
13042026_028.lcd	Blank	0.000	0	--	--	0.00
13042026_029.lcd	Inj_01	3.915	24942	4801	1.352	11.23
13042026_030.lcd	Inj_02	3.915	25153	4772	1.349	11.44
13042026_031.lcd	Inj_03	3.915	24944	4806	1.357	11.24
Average		3.915	25013	4793	1.352	11.30
%RSD		0.000	0.485	0.390	0.313	1.038
Standard Deviation		0.000	121	19	0.004	0.12

Table 5: A Measure of Method Sensitivity: Defining the Thresholds for Osilodrostat Detection.

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor	S/N
13042026_032.lcd	Blank	0.000	0	--	--	0.00
13042026_033.lcd	Inj_01	3.915	12368	4765	1.325	5.67
13042026_034.lcd	Inj_02	3.915	12309	4837	1.304	5.59
13042026_035.lcd	Inj_03	3.915	12341	4798	1.306	5.71
Average		3.915	12339	4800	1.312	5.65
%RSD		0.000	0.237	0.746	0.880	1.088
Standard Deviation		0.000	29	36	0.012	0.06

Accuracy studies showed mean recovery values of 100.83%, 100.46%, and 101.16% at 50%, 100%, and 150% levels, respectively, indicating excellent accuracy and absence of matrix interference. The individual chromatograms corresponding to each level further confirmed consistent peak response and reproducibility (Table 6).

Table 6: Readings for recovered Osilodrostat.

	PS ^{x}	Isturisa ^{y}	RA^{y1}	
50%	1.44	2.88	100.5	100.83^{z}
			100.6	0.7023^{z1}
			101.0	0.696^{z2}
100%	2.88	2.88	100.1	100.46^{z}
			101.6	1.159^{z1}
			100.2	1.153^{z2}
150%	4.32	2.88	101.2	101.16^{z}
			100.5	0.3785^{z1}
			101.2	0.374^{z2}

Robustness studies demonstrated that minor variations in chromatographic conditions did not significantly affect the performance of the method. Variation in flow rate (0.6 and 0.8 mL/min) resulted in retention times of 4.533 and 3.435 minutes, respectively, while maintaining acceptable %RSD values and peak symmetry (Table 7 and Table 8). Similarly, variation in column temperature (25°C and 35°C) showed consistent chromatographic performance with minimal deviation in retention time and peak area (Table 9 and Table 10). Ruggedness studies further confirmed reproducibility under different analysts and instruments, with %RSD values well within acceptable limits (Table 11 and Table 12).

Table 7: Condensed pace of flow is gauged by the equipment's execution (0.6 mL/min)

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_040.lcd	Blank	0.000	0	--	--
13042026_041.lcd	Inj_01	4.533	1455397	5148	1.293
13042026_042.lcd	Inj_02	4.533	1452028	5166	1.336
13042026_043.lcd	Inj_03	4.533	1452074	5134	1.278
Average		4.533	1453166	5149	1.302
%RSD		0.000	0.133	0.304	2.340
Standard Deviation		0.000	1932	16	0.030

Table 8: Strengthened flow is gauged by the equipment's execution (0.8 mL/min).

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_044.lcd	Blank	0.000	0	--	--
13042026_045.lcd	Inj_01	3.435	1093652	4387	1.308
13042026_046.lcd	Inj_02	3.435	1094652	4354	1.310
13042026_047.lcd	Inj_03	3.435	1093921	4354	1.327
Average		3.435	1094075	4365	1.315
%RSD		0.000	0.047	0.436	0.815
Standard Deviation		0.000	518	19	0.011

Table 9: The equipment's efficacy checks for hike in column oven temperature.(+5°C)

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_052.lcd	Blank	0.000	0	--	--
13042026_053.lcd	Inj_01	3.861	1246267	4734	1.418
13042026_054.lcd	Inj_02	3.872	1248269	4687	1.351
13042026_055.lcd	Inj_03	3.861	1247439	4716	1.415
Average		3.865	1247325	4712	1.395
%RSD		0.159	0.081	0.505	2.688
Standard Deviation		0.006	1006	24	0.037

Table 10: The equipment's efficacy checks for depreciation in column oven temperature.(-5°C)

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_048.lcd	Blank	0.000	0	--	--
13042026_049.lcd	Inj_01	3.947	1250601	4691	1.335
13042026_050.lcd	Inj_02	3.947	1251185	4664	1.304
13042026_051.lcd	Inj_03	3.947	1249843	4667	1.389
Average		3.947	1250543	4674	1.343
%RSD		0.000	0.054	0.323	3.189
Standard Deviation		0.000	673	15	0.043

Table 11: The equipment's efficacy checks for hike in column oven Temperature.(+5°C)

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_052.lcd	Blank	0.000	0	--	--
13042026_053.lcd	Inj_01	3.861	1246267	4734	1.418
13042026_054.lcd	Inj_02	3.872	1248269	4687	1.351

13042026_055.lcd	Inj_03	3.861	1247439	4716	1.415
Average		3.865	1247325	4712	1.395
%RSD		0.159	0.081	0.505	2.688
Standard Deviation		0.006	1006	24	0.037

Table 12: The equipment's efficacy checks for depreciation in column oven temperature.(-5°C)

Data File Name	Sample ID	Ret. Time	Area	NTP	Tailing Factor
13042026_048.lcd	Blank	0.000	0	--	--
13042026_049.lcd	Inj_01	3.947	1250601	4691	1.335
13042026_050.lcd	Inj_02	3.947	1251185	4664	1.304
13042026_051.lcd	Inj_03	3.947	1249843	4667	1.389
Average		3.947	1250543	4674	1.343
%RSD		0.000	0.054	0.323	3.189
Standard Deviation		0.000	673	15	0.043

Assessing Osilodrostat in Isturisa

To make an estimate, a sample was injected into an HPLC at a concentration of 72 µg/ml and the following calculations were applied.By the obtained peak areas as shown in figure 7a & 7b yielding assay value of 101.08%.

Sample area	Weight of standard	Dilution of sample	Purity of API	Weight of tablet	*100
Standard area	Dilution of standard	Weight of sample	100	label claim	

1268413	1.8	1	100	1	99.9	10.049
1268570	25	1	7.15	1	100	10

0.999876239*0.072*1*13.98601399*1*0.999*1.0049

=1.010790233 *100

=101.08 %

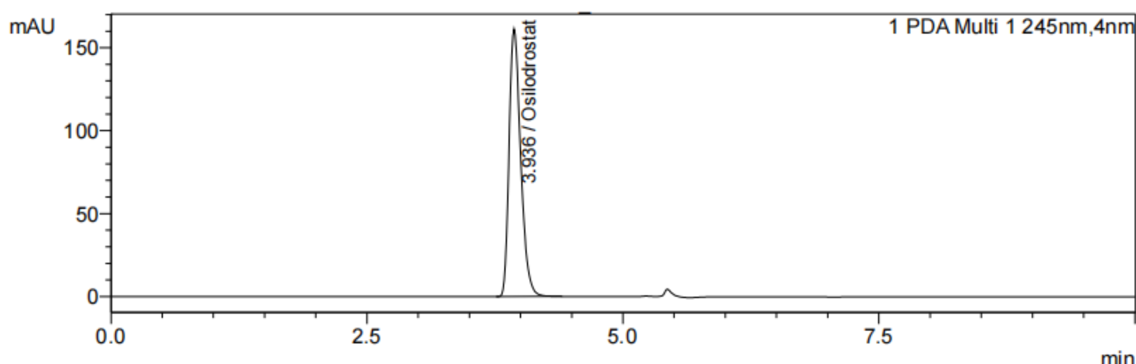


Figure 7a: Showing the total amount of Osilodrostat benchmark input and Total Accomplishment.

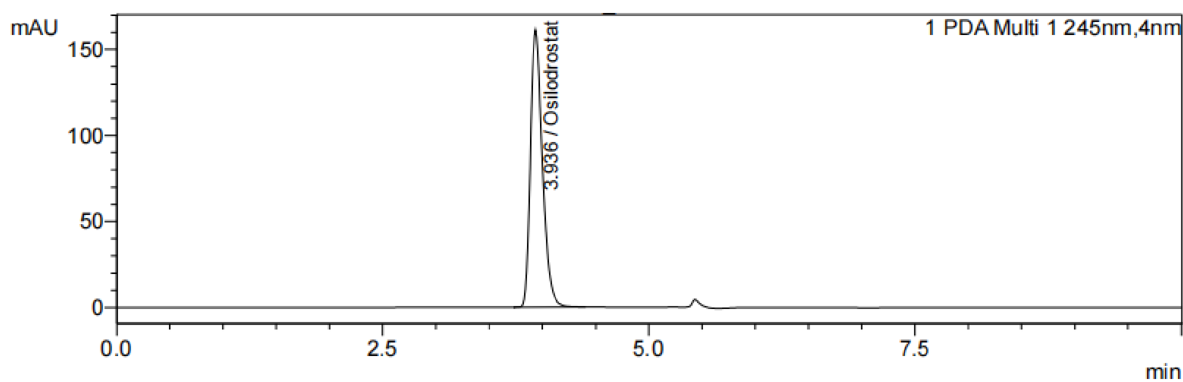
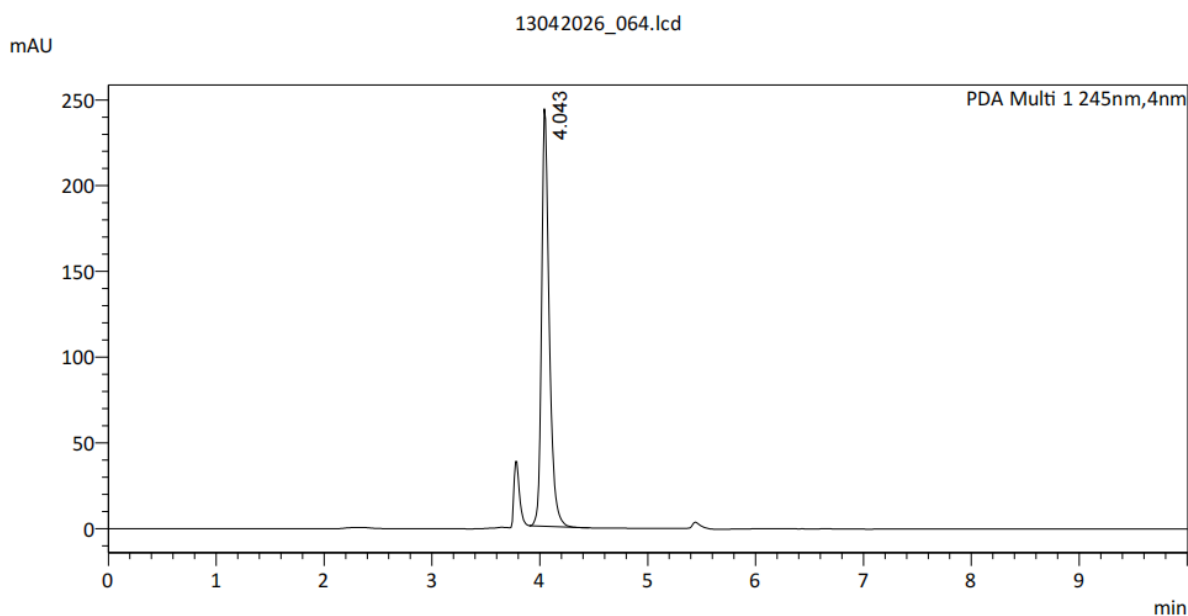


Figure 7b: Showing the total amount of Isturisa 10 mg tablets input and Total Accomplishment.

The stability-indicating capability of the method was confirmed through forced degradation studies under acidic, basic, oxidative, and thermal conditions. The chromatograms showed distinct separation of degradation products from the main analyte peak, confirming the specificity of the method. No interference at the retention time of Osilodrostat was observed, and peak purity analysis indicated that the method is capable of accurately quantifying the drug in the presence of degradation products. These results demonstrate that the developed method is stability-indicating and suitable for stability studies.



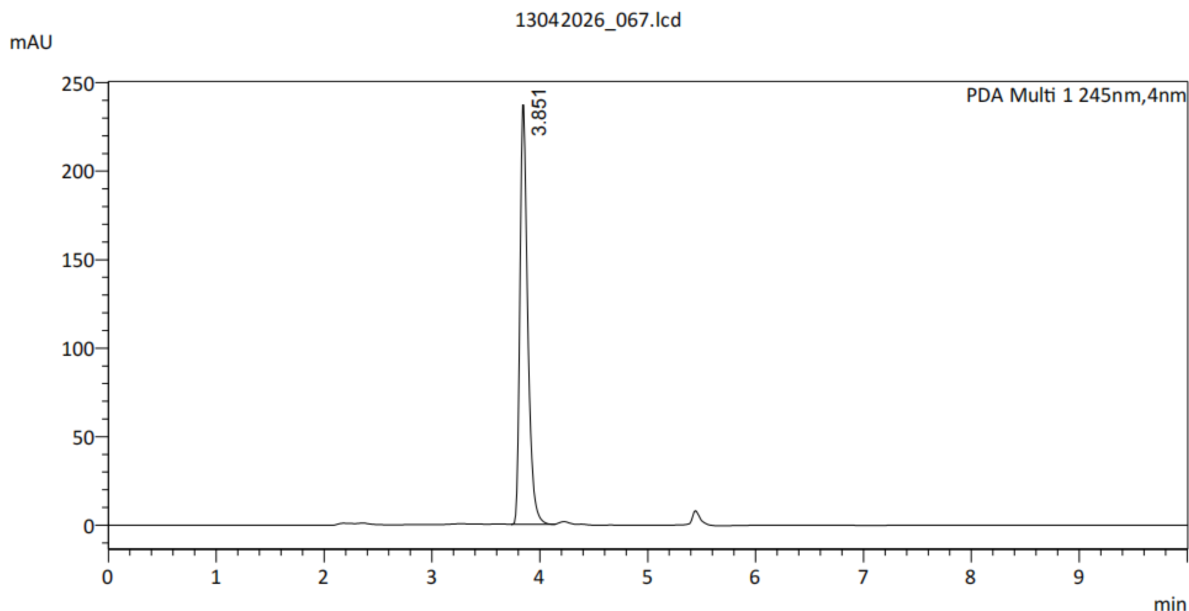
<Peak Table>

13042026_064.lcd

PDA Ch1 245nm

Peak#	Ret. Time	Area	Height	Area%	Tailing Factor	NTP
1	4.043	1206093	243570	100.000	1.434	12414
Total		1206093	243570	100.000		

Figure 8: Depiction of Osilodrostat by acid depletion.



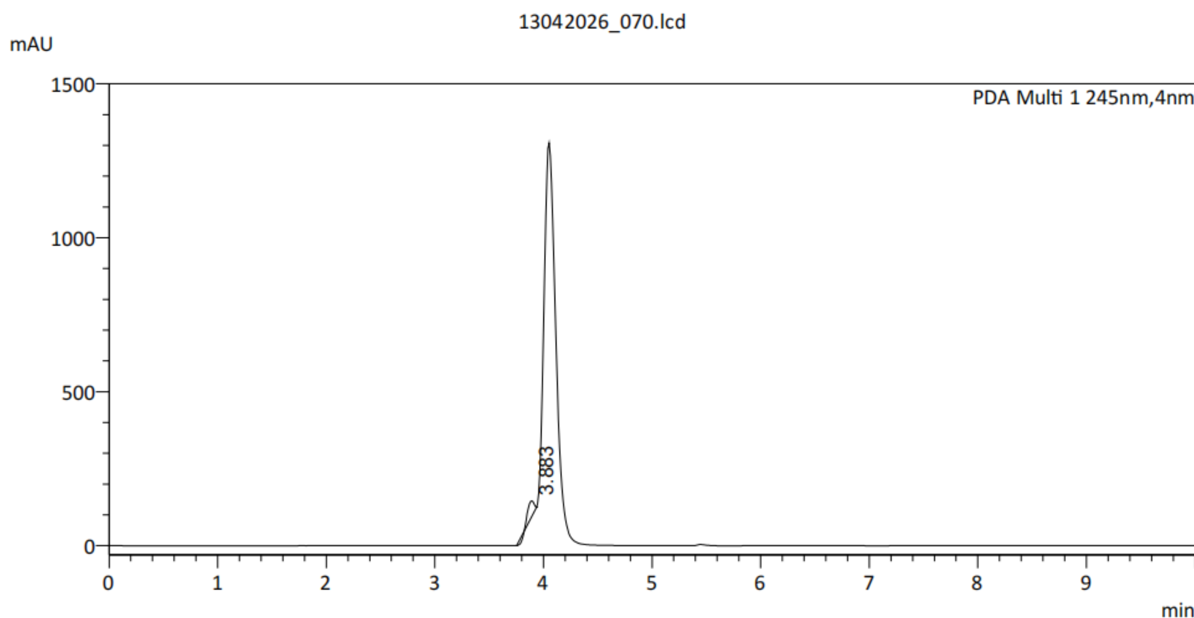
<Peak Table>

13042026_067.lcd

PDA Ch1 245nm

Peak#	Ret. Time	Area	Height	Area%	Tailing Factor	NTP
1	3.851	1200279	236904	100.000	1.310	10924
Total		1200279	236904	100.000		

Figure 9: Depiction of Osilodrostat by base depletion.



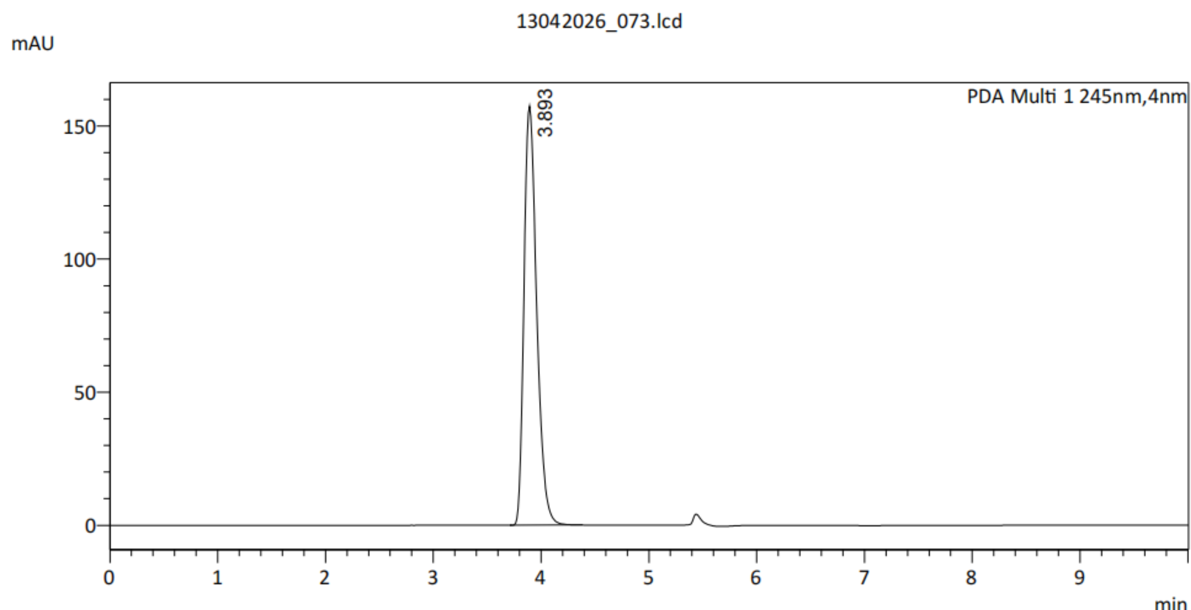
<Peak Table>

13042026_070.lcd

PDA Ch1 245nm

Peak#	Ret. Time	Area	Height	Area%	Tailing Factor	NTP
1	3.883	184428	55384	100.000	0.704	13742
Total		184428	55384	100.000		

Figure 10: Depiction of Osilodrostat by peroxide depletion.



<Peak Table>

13042026_073.lcd

PDA Ch1 245nm

Peak#	Ret. Time	Area	Height	Area%	Tailing Factor	NTP
1	3.893	1289751	157419	100.000	1.327	4583
Total		1289751	157419	100.000		

Figure 11: Depiction of Osilodrostat by thermal depletion

CONCLUSON

The present study successfully developed and validated a simple, rapid, precise, accurate, robust, and stability-indicating RP-HPLC method for the quantitative estimation of Osilodrostat in bulk drug and Isturisa tablet formulation in accordance with ICH Q2(R2) guidelines. The optimized chromatographic conditions employing a YMC Accura Triart C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of 10 mM ammonium bicarbonate in water and acetonitrile (85:15 v/v) at a flow rate of 0.7 mL/min, detection at 245 nm, and column temperature of 30°C resulted in a sharp, symmetrical peak with a retention time of approximately 3.936 minutes. System suitability parameters demonstrated excellent chromatographic performance with an average peak area of 1268445, theoretical plate count of 5041, tailing factor of 1.345, and %RSD of 0.058%, confirming high precision and system reliability. The method exhibited excellent specificity with no interference from blank or excipients, while precision studies showed %RSD values of 0.058% (repeatability) and 0.018% (intermediate precision), indicating outstanding reproducibility. Linearity was established over the concentration range of 1.44–5.4 µg/mL with proportional detector response, and sensitivity studies confirmed reliable detection and quantification with signal-

to-noise ratios of 5.65 (LOD) and 11.30 (LOQ). Accuracy studies demonstrated mean recoveries of 100.83%, 100.46%, and 101.16% at 50%, 100%, and 150% levels, respectively, confirming the reliability of the method. Robustness studies under varied flow rates (0.6 and 0.8 mL/min) and temperatures (25°C and 35°C) showed minimal variation in chromatographic parameters, while ruggedness studies confirmed reproducibility across different analysts and instruments. The assay of Isturisa tablets showed a drug content of 101.08%, validating the applicability of the method for routine pharmaceutical analysis. Forced degradation studies revealed that Osilodrostat remained stable under acidic, alkaline, and thermal stress conditions, whereas significant degradation was observed under oxidative conditions, confirming the stability-indicating nature of the method. Overall, the developed RP-HPLC method proved to be reliable, sensitive, reproducible, and suitable for routine quality control as well as stability studies of Osilodrostat in its pharmaceutical formulations.

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REFERENCES

1. Skoog, Douglas A.; West, Donald M.; Holler, F. James; Crouch, Stanley R. (2014). Fundamentals of Analytical Chemistry. Belmont: Brooks/Cole, Cengage Learning.1. ISBN978-0-495-55832-3.
2. Atole, d.M. And RAJPUT, H.H., 2018. Ultraviolet Spectrophotometry and its pharmaceutical applications-a brief review. Asian J pharm Clin res, 11(2), 59-66.
3. Tissue, B.M., 2002. Ultraviolet and visible absorption Spectrophotometry. Characterization of materials, 1-13.
4. Shah, R.S., Shah, R.R., Pawar, R.B. And gayakar, P.P., 2015. Uv-visible Spectrophotometry-a review. International journal of institutional pharmacy and life sciences, 5(5), 490-505.
5. Choudhary, A, 2017. The principle of ultraviolet (UV) spectrophotometer.
6. Weckhuysen, B.M., 2004. Ultraviolet-visible Spectrophotometry.
7. Howell, J.A. And Sutton, R.E., 1998. Ultraviolet and absorption light spectrometry. Analytical chemistry, 70(12), pp.107-118.
8. J.B. Calvert (2002-01-16). "Electronics 30 - Phototubes". University of Denver.

9. Susha, C. and Afsaneh, K., 2018. High Performance Liquid Chromatography (HPLC). J.New Medicals Life science.
10. Vidushi, Y. and Meenakshi, B., 2017. A review on HPLC method development and validation. Res J Life Sci, Bioinform, Pharm Chem Sci, 2(6), p.178.
11. Snyder, Lloyd R.; Dolan, John W. (2006). High-Performance Gradient Elution: The PracticalApplication of the Linear-Solvent-Strength Model. Wiley Interscience. ISBN 978-0470055519.
12. Hostettmann K, Marston A, Hostettmann M (1998). Preparative Chromatography Techniques Applications in Natural Product Isolation (Second ed.). Berlin, Heidelberg: SpringerBerlin Heidelberg. p. 50. ISBN 9783662036310.
13. Henry, Richard A. (1 February 2009) "The Early Days of HPLC at Dupont". ChromatographyOnline. Avanstar Communications Inc.
14. Engelhardt, H., 2012. High performance liquid chromatography. Springer Science & Business Media.
15. Shinde, S., 2016. SA nirmal. A review on force degradation study for pharmaceutical product development. International journal of pharmaceutical and chemical sciences, 5(2).
16. Blessy, M.R.; Patel, R.D.;Prajapati, P.N. and Agrawal,Y.K. (2014).Development of forced degradation and stability indicating studies of drugs-A Review. J. Pharm. Anal.4(3):159-65.
17. Forsyth, R.J. and Haynes, D.C. (1998). Cleaning validation in Pharmaceutical research facility. Pharmaceutical Technology. 22(9): 104-112.
18. Ramalingam P, Bhadraya K, Reddy YP. Analytical Quality by Design: A Tool for Regulatory Flexibility and Robust Analytics. Int J Anal Chem. 2015;2015:868727.
19. D. Parmar, K. Kansagra, J. Patel, S. Joshi, N. Sharma, A. Shelat, N. Patel, V. Nakrani, F. Shaikh and H. Patel (2019). Trial investigators. outcomes of desidustat treatment in people with anemia and chronic kidney disease: a phase 2 study, Am. J. Nephrol. 49, 470-478.
20. Q. Zheng, H. Yang, L. Sun, R. Wei, X. Fu, Y. Wang, Y. Huang, N. Liu and W. Liu (2020). Efficacy and safety of HIF prolyl-hydroxylase inhibitor vs epoetin and darbepoetin for anemia in chronic kidney disease patients not undergoing dialysis: A network meta-analysis, Pharmacol. Res. 159. doi: 10.1016/j.phrs.2020.105020
21. S. Hasegawa, T. Tanaka and M. Nangaku (2018). Hypoxia-inducible factor stabilizers for the anemia of chronic kidney disease, Curr. Opin. Nephrol. Hypertens. 27, 331-338.

22. M. Sugahara, T. Tanaka and M. Nangaku (2022). Future perspectives of anemia management in chronic kidney disease using hypoxia-inducible factor-prolyl hydroxylase inhibitors, Pharmacol. Ther. 239,doi: 10.1016/j.pharmthera.2022.108272
23. R. Pandya, H. Kachhiya, D. Solanki, J. Tandel, U. Chhalotiya and D. Shah (2023). Sensitive, precise, accurate, and economic planar chromatographic method developed and validated for quantification of Desidustat as per guideline, Sep Sci plus. 6. doi:10.1002/sscp.202300069
24. J. Prajapati (2023). Stability Indicating RP-HPLC method development and validation for the analysis of desidustat in tablet dosage form, IJNRD 8, 55-73.
25. R. Patel and P. Yadav (2023). Stability indicating RP-HPLC method development and validation for the estimation of desidustat in tablet dosage form, World J. Pharm. Pharmaceut. Sci. 4(8), 1145-1152.
26. M. Mazzarino, I. Perretti, C. Stacchini, F. Comunita, X. Torre and F. Botre (2021). UPLC–MS-based procedures to detect prolyl-hydroxylase inhibitors of HIF in urine, J. Anal. Toxicol. 45, 184–194.
27. International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use ICH harmonized tripartite guideline validation of analytical procedures: text and methodology Q2(R1) Validation.
28. M. Topal (2020). Secondary metabolites of ethanol extracts of pinus sylvestris cones from eastern Anatolia and their antioxidant, cholinesterase and alpha-glucosidase activities, Rec. Nat. Prod. 14, 129-138.
29. Z. Ozer, S. Carikci, H. yilmaz, T. Kilic, T. Dirmenci and A. Goren (2020). Determination of secondary metabolites of Origanum vulgare subsp. hirtum and O. vulgare subsp. vulgare by LC-MS/MS, J. Chem. Metrol. 14, 35-34.
30. A. Chokshi, R. Prajapati, P. Desai and N. Vyas (2022). HPTLC- Densitometric method for assay of chlorthalidone, metoprolol succinate and telmisartan in combined pharmaceutical formulation, J. Chem. Metrol. 14, 35-34.
31. A. Kul (2022). Simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol by HPLC-PDA in pharmaceutical dosage forms, J. Chem. Metrol. 16, 102-110.
32. H. Kiziltas, A.C. Goren, Z. Bingöl, S.H. Alwasel and I.Gulcin (2021). Anticholinergic, antidiabetic and antioxidant activities of Ferula orientalis L. determination of its polyphenol contents by LC-HRMS, Rec. Nat. Prod. 15, 513-528.

33. B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics, (2nd ed. 2014). ISBN 978-91-87461-59-0
34. Kansagra KA, Parmar D, Jani RH, Srinivas NR, Lickliter J, Patel HV, et al. (January 2018). "Phase I Clinical Study of ZYAN1, A Novel Prolyl-Hydroxylase (PHD) Inhibitor to Evaluate the Safety, Tolerability, and Pharmacokinetics Following Oral Administration in Healthy Volunteers". Clinical Pharmacokinetics. 57 (1): 87–102. doi:10.1007 /s40262-017-0551-3. PMC 5766731. PMID 28508936.
35. Mazzarino M, Perretti I, Stacchini C, Comunità F, de la Torre X, Botrè F. UPLC-MS-Based Procedures to Detect Prolyl-Hydroxylase Inhibitors of HIF in Urine. J Anal Toxicol. 2021;45(2):184-194. doi:10.1093/jat/bkaa055
36. Mr. Jaivik Prajapati Mansukhbhaia Stability Indicating RP-HPLC Method Development and Validation for the Analysis of Desidustat in Tablet Dosage Form. IJNRD.ORG.2023;8(6)
37. Sharma S, Jain S. A Novel Spectrophotometric Method Development for Quantification of Desidustat in Bulk and Pharmaceutical Dosage Form. Drug Metab Bioanal Lett. 2023;16(2):133-139. doi: 10.2174/2949681016666230828150206. PMID: 37642003.
38. Rutvik Pandya , Heta Kachhiya, Krunal Solanki, Jinal Tandel, Usmangani Chhalotiya and Dimal Shah. Reversed phase-HPLC-PDA method for quantification of Desidustat. Chem. Metrol.2023; 17:2 215-224. DOI:http://doi.org/10.25135/jcm.95.2307.2839
39. Riya Patel and Priyanka Yadav Stability indicating RP-HPLC Method Development and Validation for Estimation of Desidustat in Tablet Dosage Form. World Journal of Pharmacy and Pharmaceutical Sciences. 2015;12(7):1290-1304
40. Dr. S. Naazneen. A Validated Rp-HPLC Method for the Determination of Desidustat in Pharmaceutical Dosage Forms. International Journal Of Innovative Research In Technology.2023; 10(5): 142-147.
41. Rupali Mahajan, Gananadhamu Samanthula, Saurabh Srivastava, Amit Asthana. A critical review of Roxadustat formulations, solid state studies, and analytical methodology. Heliyon. 2023 (2023) e16595 https://doi.org/10.1016/j.heliyon
42. M. V. Basaveswara Rao, V. Prasanthi, G. Venkata Rao, and B. V. Raman Development and validation of new RP-HPLC method for the determination of dexrazoxane. Indian J Pharm Sci,2012, 74 (6) : 588-591

43. Mr.Jaivik Prajapati Mansukhbhai , Ms.Seju Patel , Ms.Vanita Marvaniya , Dr. Chaitanya Bhatt.Stability Indicating RP-HPLC Method Development and Validation for the Analysis of Desidustat in Tablet Dosage Form.2023;8(6): b55-b73
44. Zothanpuii, Rajesh. R , K. Selvakumar. Stability Indicating RP-HPLC Method Development and Validation for the Analysis of Tizanidine in Bulk and Pharmaceutical Formulation, Journal of Pharmaceutical Sciences and Research. 2020;12(9):1162-1169.
45. Feng, Xiangling & Cao, Yan & Ding, Yufeng & Zheng, Heng. Development and validation for the quantitative determination of xanthine oxidoreductase inhibitor topiroxostat by LC-MS/MS and its clinico-pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis. 189,2020.
46. Iram, Farah & Iram, Huma & Iqbal, & Husain, Asif. (2016). Forced Degradation Studies. J. Analytical Pharm. Res. 3. 1-5.
47. Varade SA, Kawade JV, Deshmukh YR. Development and validation of an analytical method for estimation of Finerenone in bulk and pharmaceutical dosage form by RP-HPLC. GSC Biol Pharm Sci. 2024;06(02):14–21.
48. Murugesan M, Mathrusri AN. Stability indicating RP-HPLC method for the estimation of Finerenone in pharmaceutical dosage forms. Acta Sci Pharm Sci. 2021;5(7):61–67.
49. Illendula R, Sanapalli V, Narala S, Pothuganti B, Jasti B. Development and validation of RP-HPLC method for the estimation of Finerenone in bulk and tablet dosage form. World J Pharm Life Sci. 2023;9(3):123–128.
50. Jilla SR, Aleti AV, Erusalagandi S. Method development and validation of a rapid RP-HPLC method for estimation of Finerenone in pharmaceutical dosage form. Int J Adv Res Eng Sci Manag. 2022;4(6):13–19.
51. Imran M, Shakeel S, Hussain A, Usman M, Jameel N, Ibrahim M, et al. A validated stability-indicating RP-HPLC method for quantitative estimation of Finerenone in pharmaceutical tablets. Res Sq . 2023.https://doi.org/10.21203/rs.3.rs-3015659/v1
52. Rohde G, Mahler HC, Carlier T, Kleinjung F, Fischer V. Validation and application of a sensitive HPLC–MS/MS method for the quantification of Finerenone in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2021;1175:122760. doi:10.1016/j.jchromb.2021.122760
53. Santhosh P, Kiran Kumar K, Ramesh T. RP-HPLC method development and validation for the estimation of Finerenone in bulk and pharmaceutical dosage form.
54. Somase K, Rishipathak D. Finerenone API Estimation Using RP-HPLC Technique and LC-MS Characterization of its Degradation Products. Orient J Chem 2025;41(2).

55. Jyothi Mirjapuram, Gade Sammaiah. Development and validation of a new analytical RP-HPLC method for the quantitative estimation of Finerenone in API form and marketed pharmaceutical dosage form. Int. J. of Allied Med. Sci. and Clin. Res. 2023 Jun. 27 ;11(2):210-8. https://ijamscr.com/ijamscr/article/view/1331
56. Balakirouchenane D, Castinetti F, Dufour H, Brue T, Guillet B, Boudou-Rouquette P. LC-MS/MS method for simultaneous quantification of Osilodrostat and metyrapone in human plasma from patients treated for Cushing's syndrome. J Pharm Biomed Anal. 2023;226:115238.
57. Patel J, Patel R, Raval U, Patel A, Patel Y, Patel D. RP-HPLC method development and validation for the estimation of Osilodrostat in API form. Eur Chem Bull. 2023;12(Special Issue 4):728-743
58. Panchal CV, Patil SS, Ghadge SS, Kumbhar SP, Usnale VD. Method development and validation of RP-HPLC Osilodrostat in bulk and dosage form.Int J Res Anal Rev. 2024;11(3):562-576.
59. Reddy P, Kumar V, Sharma S. A comprehensive review on analytical methods used for analysis of steroidogenesis inhibitors. Int J Pharm Res Appl. 2023;8(5):39-47.
60. Ishii H, Ishikawa Y, Mizobe F, Leung GNW. Pharmacokinetic study of osilodrostat and identification of mono-hydroxylated metabolite in equine plasma for the purpose of doping control. Rapid Commun Mass Spectrom. 2024;38(2):e9695.
61. Li W, Luo S, Rebello S, Tse FLS. A semi-automated LC–MS/MS method for the determination of LCI699, a steroid 11β-hydroxylase inhibitor, in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2014;958:119-124.
62. Fleseriu M, Auchus R, Bancos I, Ben-Shlomo A, Bertherat J, Biermasz NR, et al. Analytical and clinical applications of LC–MS/MS methods for steroidogenesis inhibitors used in Cushing's syndrome therapy. Clin Chim Acta. 2022;533:115-123.