RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR THE ESTIMATIONOF OSILODROSTAT IN API FORM.

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ABSTRACT

For the management of Cushing Syndrome, osilodrostat is used. For the estimation of Osilodrostat, a straightforward, accurate, quick, and stability indicating RP-HPLC method was devised. C18 (250 4.6 mm, 5 mm) column and Phos. Buffer pH 3.2- MeOH (40:60) as the mobile phase were used to separate the samples at a flow rate of 1.0 ml/min. The detection process took place at 240 wavelengths. Osilodrostat was discovered to have a retention time of 5.160 minutes. For linearity, accuracy, precision, robustness, LOD, and LOQ, the technique has been validated. Osilodrostat has been found to be linear. Linearity observed for Osilodrostat 10-30 µg/ml. The LOD and LOQ were found 0.0015 µg/ml and 0.048 µg/ml respectively. The estimation of Osilodrostat in API form was successfully implemented using this technique.The RP-HPLC technique was discovered to be straightforward, precise, reliable, and repeatable.

Keywords:*Osilodrostat (OSI), RP-HPLC, Method development, Stability Study, Analytical Method Validation, ICHQ2 (R1) guideline.*

INTRODUCTION [1-4]

Cushing syndrome, a condition brought on by excessive adrenal cortical activity. Cushing syndrome is the term used when the pituitary gland tumor is the cause. Likewise known as hypercortisolism. Cushing syndrome is the name given to this endocrine condition. Later on, it was discovered that many individuals with comparable symptoms and manifestations did not actually have a pituitary tumor.

Because of this, the word "Cushing syndrome" has been changed to refer to all patients who exhibit the condition's classic symptoms and signs, regardless of the underlying cause, You acquire Cushing's syndrome when your body consistently produces too much cortisol. Some of the hallmarks of Cushing syndrome,
-Fatty hump between the shoulders,
-A round face,
-Pink or purple stretch marks on the skin,
In rare cases, Cushing syndrome can also lead to 1- type 2 diabetes, 2- high blood pressure, 3- bone loss.

Pathophysiology Of Cushing Syndrome:

The adrenal gland produces cortisol and other steroid chemicals in response to ACTH stimulation. Corticotropin-releasing hormone (CRH) from the brain stimulates the pituitary gland, causing it to release ACTH into the petrosal venous sinuses. (Figure 1).
In women- 1- Periods that are irregular
2- nonexistent Hirsutism
In men – 1-Sexual dysfunction 2- a decreasedecreased fertility found.

Experimental work:

PRELIMINARYANALYSIS OF OSI

1- **Description -**The colour and texture of the Osilodrostat sample were scrutinised.
2- **Identification Test-**

Step-1 Using a hydraulic pellet press and 1 mg of OSI, a potassium bromide IR disc was created.

Step-2 This disc was FTIR scanned in the 4000-400cm-1 range, and the IR spectra that was produced was compared to the OSI reference spectrum.

3- **Solubility-** The OSI sample was placed in test tubes, and its solubility in several solvents including water, methanol, 0.1 N HCl, and 0.1 N NaOH was evaluated.

Apparatus and Instruments:(Table 1)

Procurement of API(Table 3)

Identification of Drug

Determination of Solubility (Table 4)
Identification by IR:(Figure 2)
Interpretation of sample IR of OSI (Table 5)

Development and validation of an analytical technique for estimating OSI using RP-HPLC.[5]

Preparation of standard solutions:

(A) **OSI standard stock solution: (200µg/mL) -**A 100 mL volumetric flask was filled to the appropriate level with methanol after 20 mg of osilodrostat were weighed and transferred.

(B) **Preparation of working solution OSI (20µg/mL)-** Transfer 1 mL of the OSI stock solution into a volumetric flask with a capacity of 10 mL, and then add the appropriate amount of the mobile phase, which was used in the relevant trials.

(C) Phosphate Buffer preparation- 6.8 gm of Phosphate buffer was transferred to 1000 ml beaker, into this 200 ml of water added and shaken for few minutes than volume was made up with water, pH was adjusted by adding 1% o-Phosphoric acid or 0.1M KOH.

(D) **Diluent:** Mobile phase

Selection of wavelength: The HPLC method's susceptibility to UV detection depends on how accurately the detection wavelength is chosen.

-The wavelength that responds well to the medications being identified is the optimum wavelength.

-Between 200 and 400 nm, a standard OSI solution (20 g/mL) was examined using a UV-visible spectrophotometer.

-Both solutions were scanned between 200 and 400 nanometers.

The overlay spectra of the answers were used to select a wavelength

Chromatographic separation-

Step-1 With a 20µl micro- syringe, a standard OSI solution containing 20 µg/ml l was introduced into the column.

Step-2 The chromatogram was performed with mobile phase Buffer, pH 3.2: MeOH for the necessary number of minutes. (40:60). The measurement was done at 243 nm wavelength.

Step-3 After complete separation was accomplished, the chromatogram was terminated. Software was used to capture information about the peak, such as its area, height, retention time, and resolution.

Validation of RP-HPLC method:

Linearity- By analysing a standard solution of OSI in the range of 10-330 g/ml, the linearity for Osilodrostat was evaluated.

Step 1- Pipette out 0.5, 0.75, 1, 1.25, and 1.5 ml solutions from the OSI stock solution (200 g/ml)

Step 2 - transfer them to a 10 ml volumetric flask, and then fill them up with mobile phase to get 10, 15, 20, 25, and 30 mg/ml of OSI. The graph of peak area obtained verses respective concentration was drawn in terms of slope, intercept, and correlation co-efficient value.

Precision- Results should be expressed using relative standard deviation (RSD) or the measure of variance.

Repeatability- OSI (20 g/ml) was injected six times into a standard solution. The areas of the peaks were measured, and the percent RSD was computed.

(A) Intraday Precision- Three injections of a standard solution containing OSI (10, 20, and 30 g/ml) were made on the same day. The areas of the peaks were then measured, and the percent RSD was computed.

(B) Interday Precision- OSI standard solutions (10, 20, and 30 g/ml) were injected three times on various days, and the areas of the peaks were measured and the percent RSD was computed.

Accuracy-

Step-1 Three distinct flasks labelled A, B, and C each contained a 10 g/ml drug solution.

Step-2 It was spiked with 80%, 100%, and 120% of the reference solution and

diluted to a maximum of 20ml. At 240nm, the size of each solution peak was measured. At each level, the amount of OSI was determined, and the percentage recoveries were calculated**.**

LOD and LOQ- The LOD was calculated using the same collection of three calibration curves that were used to assess linearity.

The LOD may be calculated as**,LOD = 3.3 × (SD/Slope)**

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves, Slope = Mean slope of the 3 calibration curves.

The set of three calibration curves that were used to determine linearity were used to estimate the LOD. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves

Robustness

1 Flow rate of mobile phase : (± 0.2 ml/min)- 0.8 ml/min and 1.2 ml/min.

2 Ratio of Mobile phase was (±2) Buffer - 3.2: MeOH (42:58) and Buffer 3.2: MeOH(38:62)

3 pH of Mobile phase : (±0.2) - 3.0 and 3.4.

Result and Discussion

RP-HPLC Method Development

Selection of wavelength

- OSI (20 ppm) medication solutions were made in methanol for the current study. -
- After that, this drug solution was scanned in the UV area between 190 and 400 nm, and the maximum absorbance was noted.(Figure 3)
- Solution was scanned between 190 - 400 nm.
- Wavelength What Gives Maximum Absorbance was selected from the above Spectra.

Selection of Mobile Phase

Selection of Mobile phase for OSI

- The trial includes mobile phases that are thought of as Methanol and Water in varying ratios, volumes, and flow rates.
- Based on numerous tests, it was determined that the Phos. Buffer pH 3.2- MeOH (40:60) mixture at 1 mL/min flow rate was superior to other mixtures in terms of peak shape, theoretical plate, and asymmetry.Trials are summarizing in following table 6.
    - System suitability parameter are shown in table 7

RP-HPLC optimized chromatographic conditions - table 8

Final chromatogram - figure 4.

Method Validation:

Linearity- By analysing a standard solution with a concentration between 10 and 30 g/ml and adding mobile phase to a 100 ml volumetric flask, the linearity for OSI was evaluated.

Correlation co-efficient for calibration curve OSI was found to be 0.9908.

For OSI: $y = 435.5x – 2721.4$, $R^2 = 0.9819$.(Figure 5)

Linearity data for OSI (Table 9)

Overlay Chromatogram of Osilodrostat(Figure 6)

Accuracy:

A known standard concentration was added to the pre-analyzed sample before it was submitted to the proposed HPLC analysis. The recovery study findings are displayed in the table below. There were three concentration levels used in the study.(table 10)

LOD and LOQ: The standard deviation of the intercepts was calculated after five repetitions of the calibration curve. Following that, LOD and LOQ were determined as follows.

LOD of Osilodrostat,

LOD = 3.3 × SD / Slope
= 3.3 × 0.209 / 435.5
= 0.0015 µg/ml

LOQ of Osilodrostat,

LOQ = 10 × SD / Slope
= 10 × 0.209 / 435.5
= 0.0048 µg/ml

Precision:

1 Repeatability- The information for Osilodrostat's repeatability of peak area measurement is based on six measurements of the same Osilodrostat solution and is shown in the table below. The procedure should be carried out quickly within a laboratory by the same analyst using the same tools, and it was expressed as %RSD. %RSD of OSI was discovered to be 0.210.
Repeatability data for OSI (Table 11)

2 Intra-day Precision- OSI standard solution (10, 20, 30 g/ml) was examined three times on the same day, and %RSD was computed. Table 12 displays the date for Osilodrostat's intraday precision. Osilodrostat standard solution (10, 20, 30 g/ml) was examined three times on the same day, and %RSD was computed. Table 12 displays the date for Osilodrostat's intraday precision.

3 Inter-day Precision- Three separate analyses of a standard solution containing 5, 10, and 15 g/ml of osilodrostat were performed, and %RSD was determined. Table 13 displays the date for Osilodrostat's intraday precision.

Robustness:- The mobile phase's pH value, flow rate, and composition were used to assess the robustness. Retention duration, area, and peak symmetry did not alter significantly during the course of the robustness studies. The results of the adjustments were found to meet the standards for acceptance, which are listed in the table below. Less than 2% should be the %RSD. Table 14 displays OSI robustness data.

Assay: By analysing the standard solution and sample solution, the assay for osilodrostat was conducted, and the findings were compared to determine the SD and %RSD. Osilodrostat's%RSD was discovered to be 0.01373%.
Figures 7,8,9, and 10 illustrate the chromatograms for the samples 1, 2, and 3 of the Osilodrostat Standard, respectively.

% RSD is displayed in table no.15

Conclusion: To estimate Osilodrostat in its API form, a reverse phase high performance liquid chromatographic method was created. The.C18 (250nm 4.6 mm 5 m) column and buffer (Phosphate buffer 3.2 pH): MeOH (40:60), at a flow rate of 1 ml/min and detection wavelength at 240 nm with retention time (RT) 5.160, were used to get the response. The approach has been approved for 1-precision, 2- robustness, 3- linearity, 4- accuracy,5- robustness. Osilodrostat 10–30 g/ml showed linearity. The developed method for estimating osilodrostat in its API version was determined to be Accurate, Precise, Quick, and simple.

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Figures and Tabels

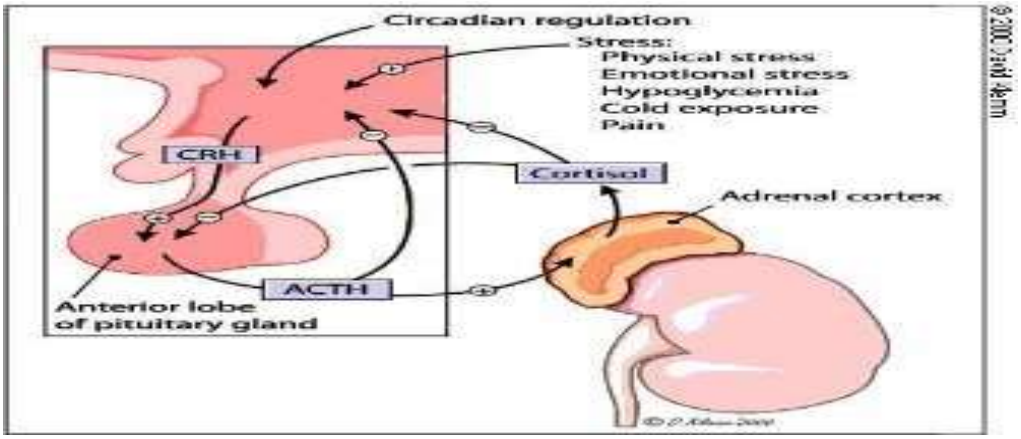

Figure 1- Pathophysiology Of Cushing Syndrome

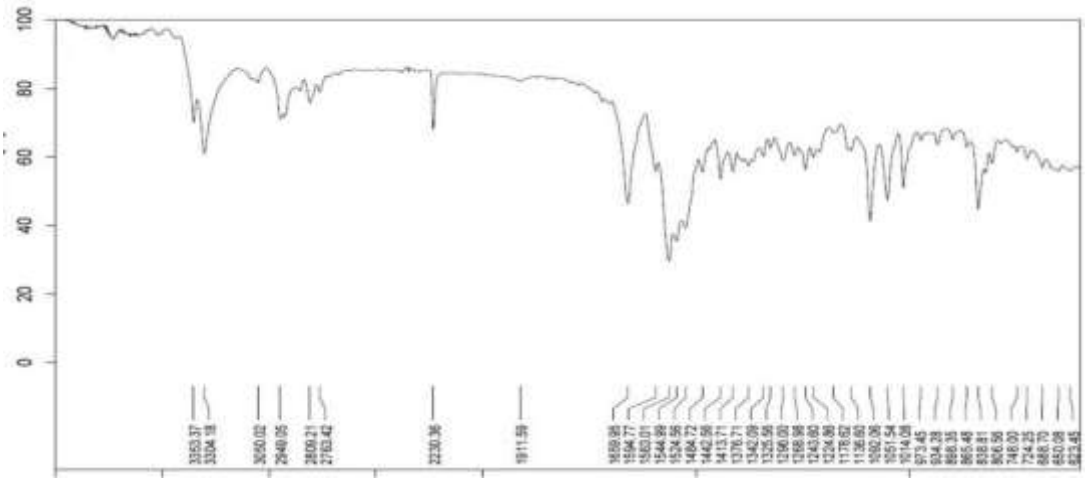

Figure 2: IR spectra of sample

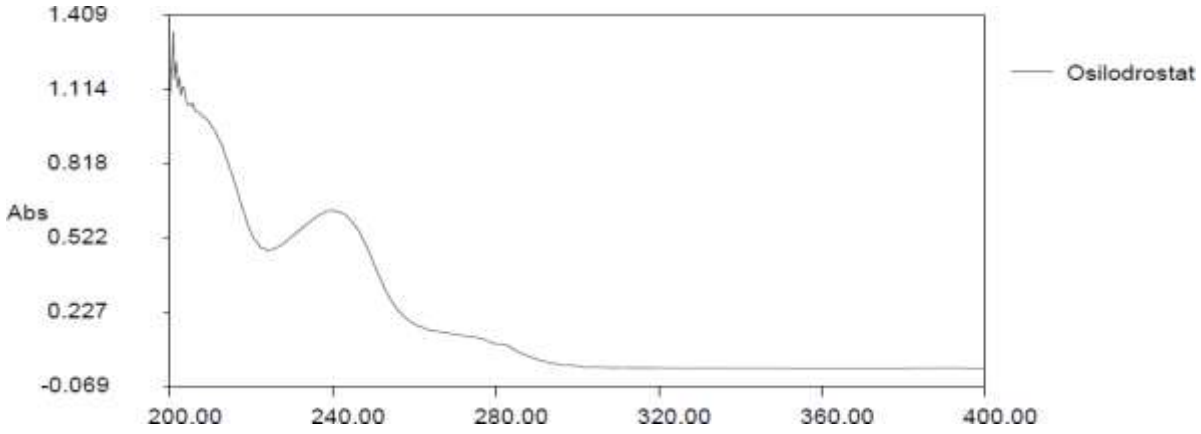

Figure 3: UV Spectra of Osilodrostat (20 ppm) (Maximum Absorbance 240 nm)

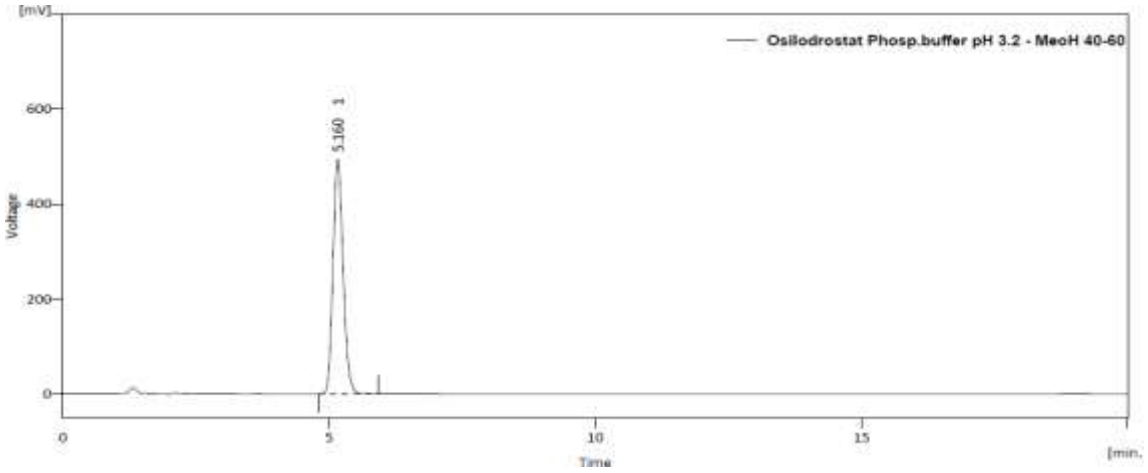

Figure 4: Final HPLC Chromatogram of Osilodrostat 20 ppm in Phos. Buffer pH 3.2:MeOH (40:60)

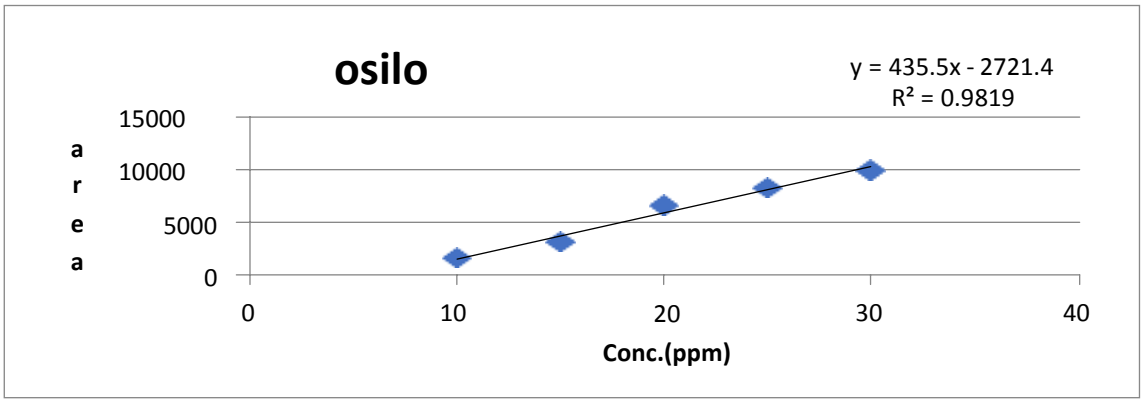

Fig 5: Calibration curve of Osilodrostat (10-20 µg/ml)

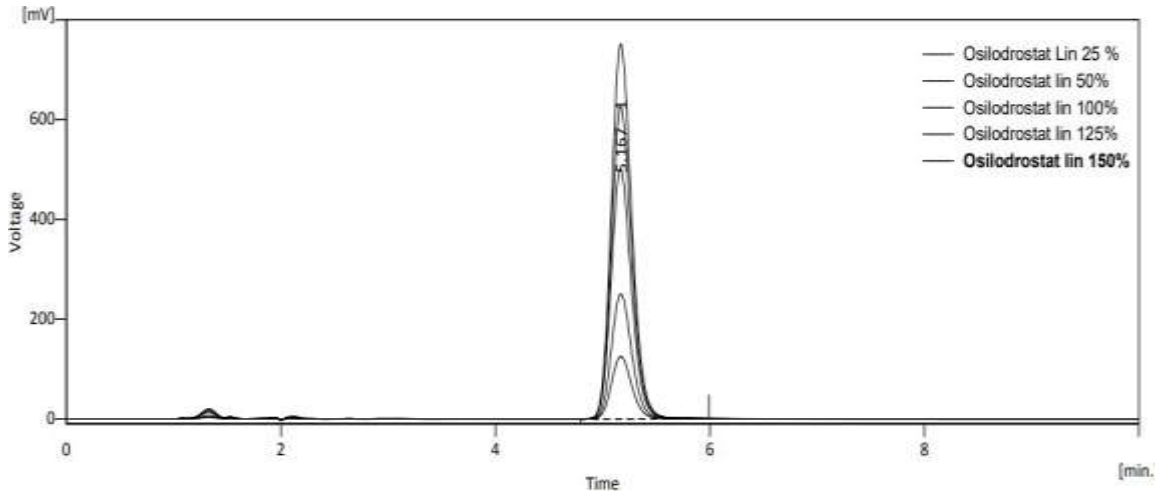

Fig. 6 Overlay Chromatogram of Osilodrostat

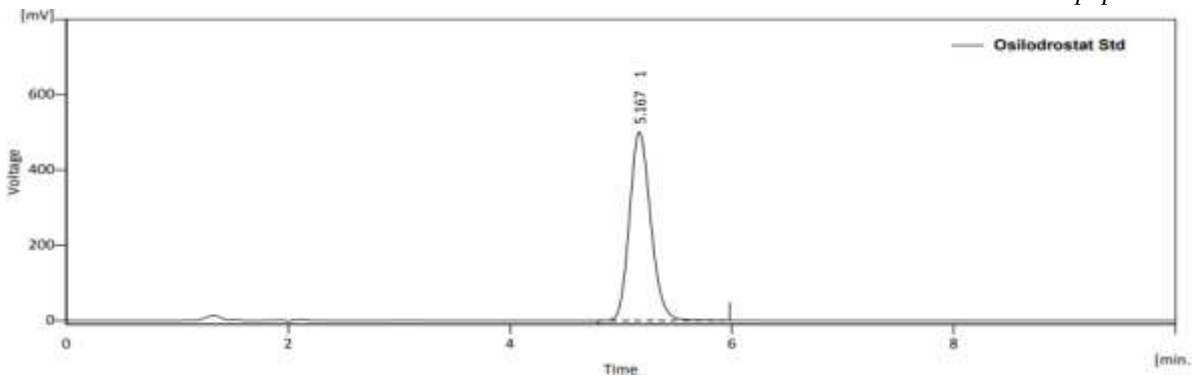

Fig 7: Chromatogram of Osilodrostat Standard

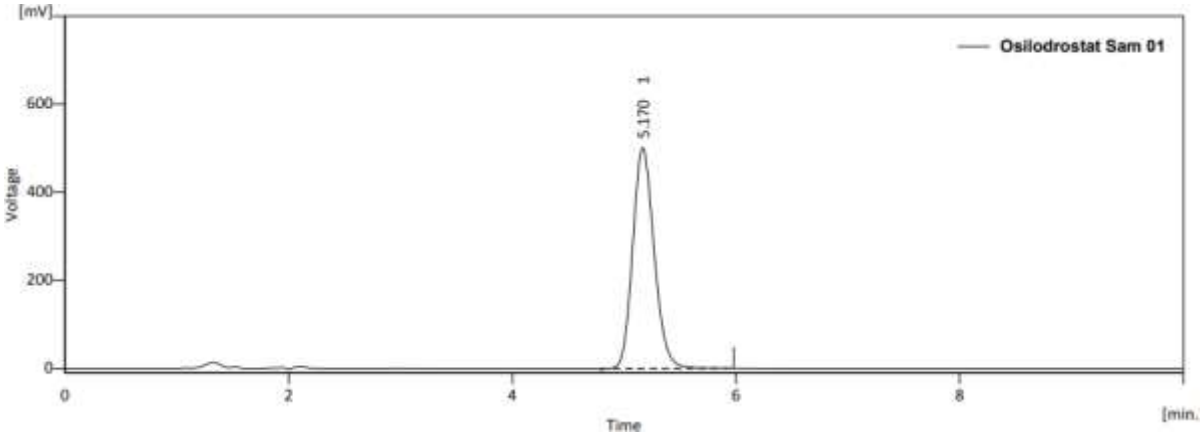

Fig 8: Chromatogram of Osilodrostat Sample 1

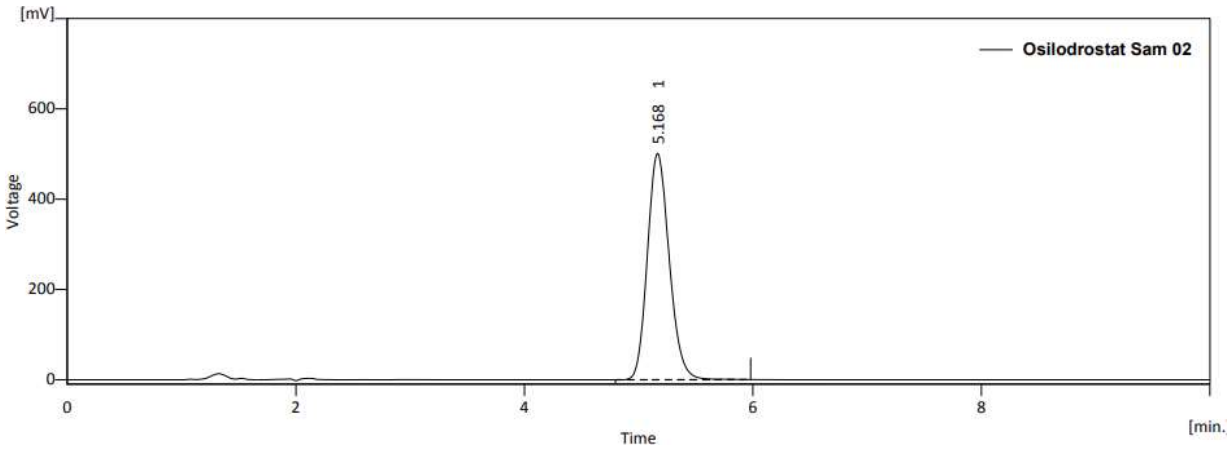

Fig 9: Chromatogram of Osilodrostat Sample 2

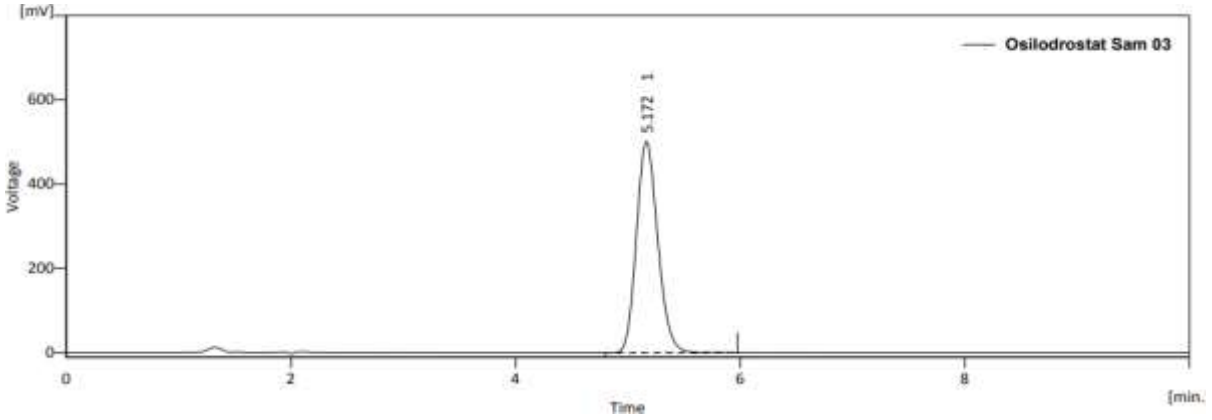

Fig 10: Chromatogram of Osilodrostat Sample

Table 1 Apparatus and Instruments

Sr. no.	Name of equipment / Instrument	Manufacturer
1	UV Spectrophotometer	Shimadzu
2	HPLC	Model: Shimadzu HPLC System Pump: -LC-20 AT Column: Cosmosil C18 (250 mm × 4.6 mm, 5 µm) columnInjector: 20µL fixed loop. Detector: UV Detector Software: Spin chrom
3	Analytical balance	Electronic Balance (Shimadzu AUX220)
4	pH Meter	LabMan
5	Melting Point Apparatus	LabMan
6	Ultrasonicator	Dolphin
7	Hot air oven, MITEC-784	Patel Scientific
8	FT-IR	Bruker, Alpha-II

Tablet 2: Reagents and Materials

Sr. no	Name of Chemicals	Grade of Chemicals	Supplier
1	Methanol	HPLC Grade	Finar Limited, Gujarat
2	Water	HPLC Grade	Finar Limited, Gujarat
3	Acetonitrile	HPLC Grade	Finar Limited, Gujarat
4	Potassium Dihydrogen Phosphate	AR Grade	Ranbaxy chemicals
5	Potassium hydroxide	AR Grade	Ranbaxy chemicals

Tablet 3: Procurement of API

Sr. no	API Name	Soure	% Purity
1	Osilodrostat	Gift sample	99.58%

Table 4: Determination of Solubility

Solvent	Osilodrostat
Distilled Water	Slightly Soluble
0.1 M HCl	Soluble
0.1 M NaOH	Soluble
Methanol	Sparingly Soluble
Acetonitrile	Sparingly Soluble

Table 5: Interpretation of sample IR of Osilodrostat

Functional Group	Wave number
Aromatic C-H Stretching	3050
Saturated CH2	2949
C≡N Stretching	2230
C=N Stretching	1659
-C-N Stretching	1325

Table 6: **List of Mobile Phase trials for Osilodrostat**

Trial	Mobile Phase	Retention time (minute)	Remark
1	Methanol	-	Peak did not Observed
2	Methanol: Water (70:30)	6.837	Peak Observed But not sharp
3	MeOH: Water (50:50)	5.103	Observed peak is not sharp
4	ACN: Water (80:20)	5.513	Resolution is not good
5	ACN- Water (70:30)	4.730	Peak shape is not good
6	Phos. Buffer pH 4.4- MeOH (80:20)	11.267	Still the peak shape is not good
7	Phos. Buffer pH 4.4- MeOH (40:60)	8.650	Retention time reduced
8	Phos. Buffer pH 3.2- MeOH (60:40)	6.273	Resolution is not good
9	Phos. Buffer pH 3.2- MeOH (40:60)	5.160	Sharp Peak of Osilodrostat Confirmed

Table 7: System suitability parameter

Parameters	Osilodrostat
Retention Time	5.160
Theoretical Plates	3454
Asymmetry	1.275

Table 8: RP-HPLC optimized chromatographic conditions

Parameters	Chromatographic Condition

Mode of elution	Isocratic
Mobile Phase	Phos. Buffer pH 3.2- MeOH (40:60)
Column	C_{18} (250× 4.6mm, 5µ)
Flow rate	1.0 ml/min
Injection volume	20 µL
Detection wavelength	240 nm

Table 9: Linearity data for Osilodrostat

Sr. No	Concentration (µg/ml)	Area
1	10	1639.831
2	15	3292.531
3	20	6670.467
4	25	8333.702
5	30	10006.827

Table 10: Accuracy data for Osilodrostat

Sr. No.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± SD
1	80%	10	8	7.98	99.82	99.87 ± 0.33
2		10	8	8.01	100.23	
3		10	8	7.96	99.56	
4	100%	10	10	9.97	99.73	99.76 ± 0.24
5		10	10	10.00	100.01	
6		10	10	9.95	99.54	

7	120%	10	12	12.00	100.00	99.98 ± 0.16
8		10	12	11.97	99.82	
9		10	12	12.01	100.14	

Table 11: Repeatability data for Osilodrostat

Sr. No	Conc.(µg/ml)	Area	Mean ± SD (n=6)	% RSD
1	20	6671.064	6654.87 ± 13.94	0.210
		6640.163		
		6656.857		
		6649.351		
		6640.804		
		6671.009		

Table 12: Intra-day Precision data for Osilodrostat

	Osilodrosta t		
Sr. No.	**Conc. (µg/ml)**	**Area Mean ± SD (n=3)**	**% RSD**
1	10	1641.325 ± 0.95	0.0579
2	20	6670.708 ± 0.89	0.0134
3	30	10005.23 ± 0.87	0.0087

Table 13: Inter-day Precision data for Osilodrostat

	Osilodrosta t

Sr. No.	Conc. (µg/ml)	Area Mean ± SD (n=3)	% RSD
1	10	1640.56 ± 0.965	0.0588
2	20	6671.752 ± 0.950	0.0142
3	30	10006.568 ± 1.273	0.0127

Table 14: Robustness data for Osilodrostat

Sr. No.	Area at Flow rate (+0.2 ml/min)	Area at Flow rate (-0.2 ml/min)	Area at pH (-0.2)	Area atpH (+0.2)	Area at Mobile Phase (-0.2)	Area at Mobile Phase (+0.2)
1	6671.021	6669.348	6669.201	6671.143	6670.517	6672.145
2	6672.146	6670.457	6670.549	6670.648	6669.014	6671.385
3	6671.986	6668.859	6668.416	6672.504	6671.867	6673.758
% RSD	0.0091	0.0122	0.0161	0.0144	0.0213	0.0181

Table 15 : Assay of Osilodrostat

Sr. No	Osilodrostat	Area	% RSD
1	Standard	6673.154	0.01373
2	Sample 01	6671.642	
	Sample 02	6670.367	
	Sample 03	6672.145	