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Analytical method development and Validation for the Estimation of Avacopan in bulk and capsuledosage form by RP-HPLC

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Abstract
In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity. A new method was established for estimation of Avacopan RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Avacopan. The maximum absorbance was found to be at 257 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm 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: Water (70:30% 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 2.21 min and Retention Time found to be 2.286, % RSD was reported as 0.663419. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.05 & 0.15 µg/ml respectively.. Avacopan was degraded in acidic, basic & 3 % hydrogen peroxide & stable at thermal & light stress conditions.The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. The suggested RP-HPLC method can be used for routine analysis of Avacopan in API and Pharmaceutical dosage form
Keywords: Acetonitrile, Water, Avacopan, LOD, LOQ, API, RP-HPLC

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1. Introduction

Avacopan is an orally bioavailable complement 5a receptor (C5aR) antagonist for the treatment of severe anti-neutrophil cytoplasmic (auto) antibody (ANCA)-associated vacuities.

IUPAC Name: (2R, 3S)-2-[4-(cyclopentylamino) phenyl]-1-(2-fluoro-6-methyl benzoyl)-N-[4-methyl-3-(trifluoromethyl) phenyl] piperidine-3-carboxamide

Molecular formula: $C_{33}H_{35}F_4N_3O_2$
Molecular weight: 581.656
Phase: solid (STP).
Appearance: white.
Storage temperature: 2-8º.
Solubility in water: Soluble in water.

Molecular Structure:

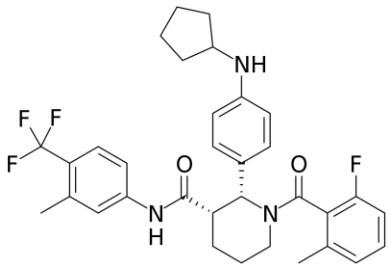

Fig.1Avacopan

IUPAC Name:(2R, 3S)-2-[4-(cyclopentylamino) phenyl]-1-(2-fluoro-6-methyl benzoyl)-N-[4-methyl-3-(trifluoromethyl) phenyl] piperidine-3-carboxamide
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2. Methodology

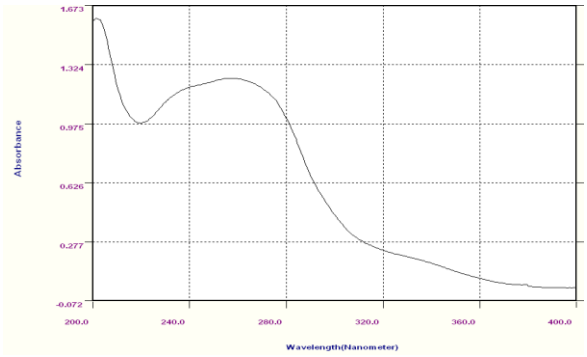

Fig.2. UV Spectrum of Avacopan

Standard, sample preparation for UV-spectrophotometer analysis:
25mg of Avacopan standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.
The standard & sample stock solutions:
The Samples are prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Avacopan, so that the same wave number can be utilized in HPLC UV detector for estimating the Avacopan. While scanning the Avacopan solution we observed the maxima at 257 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.
Mobile Phase Preparation
The mobile phase used in this analysis consists of a mixture of Buffer (0.05 M potassium dihydrogen phosphate & pH adjusted to 3.4 with orthophosphoric acid) and Acetonitrile in a ratio of 60:40.600 ml of this buffer solution was added and properly mixed with 400 ml of acetonitrile and a homogenous solution is achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment.
Sample & Standard Preparation for the Analysis
10 mg of Avacopan standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.The sample was analyzed by HPLC by using the above method and a very nicely resolved peak has been obtained at a Retention Time of about 2.26min. The respective chromatogram is attached in the following page.
Summary of Optimized Conditions
The Optimum conditions obtained from experiments can be summarized as below:

Table.1

Mobile phase	phosphate buffer(pH 3.4): acetonitrile (60:40)
Wavelength	257 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm

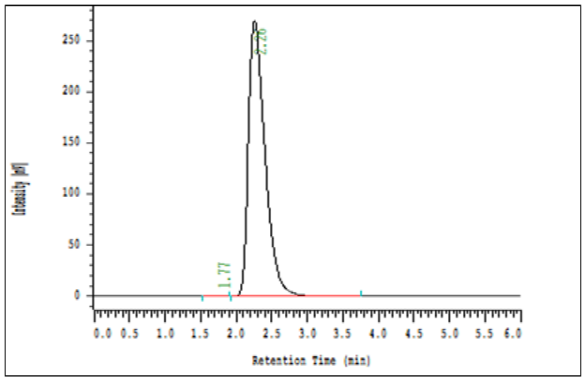

Fig.3. HPLC spectrum of Avacopan (50 ppm) in optimized conditions (RT 2.26 min.)

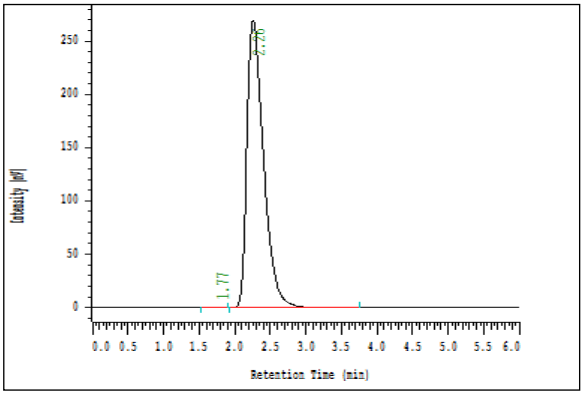

Fig.4. HPLC spectrum of Avacopan (50 ppm) in optimized conditions (RT 2.26 min.)

3. Results and Discussion

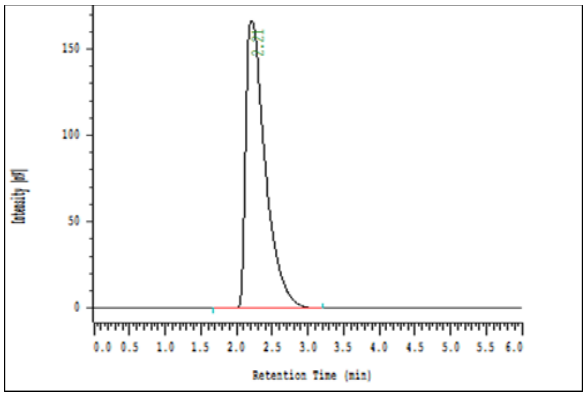

Fig.5. Accuracy S1 Solution -40 PPM

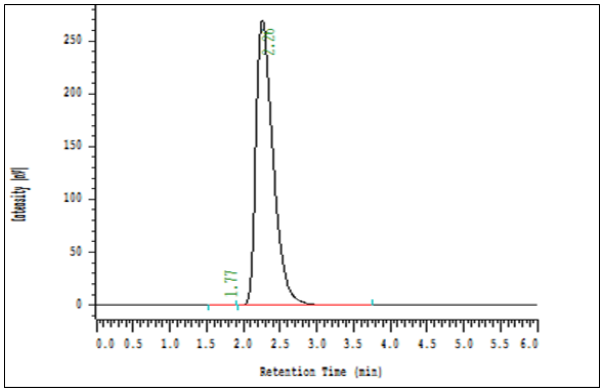

Fig.6. Accuracy S2 Solution- 50PPM

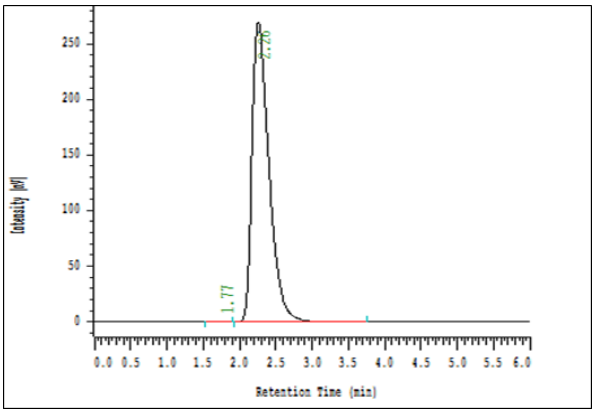

Fig.7 Precision

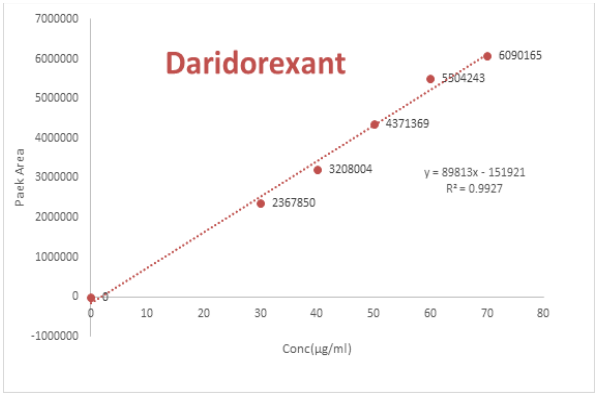

Fig.8. Calibration curve of .Avacopan (API).

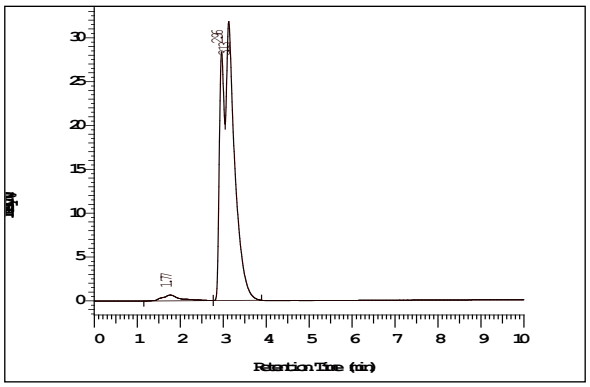

Fig.9. Chromatogram showing degradation for Avacopan in 0.1N HCl

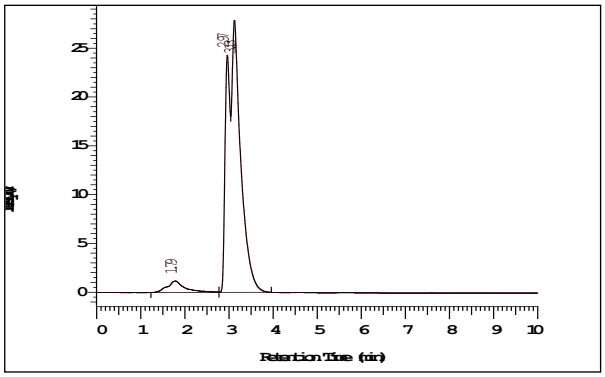

Fig.10. Chromatogram showing degradation related impurity in 0.1 N NaOH

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of AVACOPAN were taken and added to the pre-analyzed formulation of concentration 10□g/ml. From that percentage recovery values were calculated

Precision:

Repeatability:

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug.

Intra-assay & inter-assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Avacopan revealed that the proposed method is precise

Linearity & Range: The calibration curve showed good linearity in the range of 00 – 70 µg/ml, for Avacopan (API) with correlation coefficient (r2) of 0.992. A typical calibration curve has the regression equation of y = 89813x + 15192 for Avacopan.

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LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.05 & 0.15 µg/ml respectively.

Stability related impurity studies:

Following protocol was strictly adhered to for forced degradation of Avacopan Active Pharmaceutical Ingredient (API).The API (Avacopan) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing.The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

Table 2: Accuracy Readings

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	40	50	99.18	Mean= 98.97667% S.D. = 0.200083 % R.S.D.= 0.202152
S_2 : 80 %	40	50	98.78	
S_3 : 80 %	40	50	98.97	
S_4 : 100 %	50	50	99.87	Mean= 99.54% S.D. = 0.33 % R.S.D.= 0.331525
S_5 : 100 %	50	50	99.54	
S_6 : 100 %	50	50	99.21	
S_7 : 120 %	60	50	99.32	Mean= 99.567% S.D. = 0.33 % R.S.D. = 0.331159
S_8 : 120 %	60	50	99.65	
S_9 : 120 %	60	50	99.98	

Table 3: Precision

HPLC Injection Replicates of Avacopan	Retention Time	Area
Replicate – 1	2.26	4371369
Replicate – 2	2.29	4327048
Replicate – 3	2.29	4372696
Replicate – 4	2.29	4283857
Replicate – 5	2.3	4340455
Average	0.015166	36636.23285
Standard Deviation	2.286	4339085
% RSD	0.663419	0.844330841

Table.4 Results of intra-assay & inter-assay

Conc. of Avacopan (API) (µg/ml)	Observed Conc. of Avacopan (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.08	0.96	10.03	0.97
20	20.04	0.40	30.03	0.42
40	39.97	0.33	39.95	0.14

Table 5: Calibration results of .Avacopan (API)

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
30	2367850
40	3208004
50	4371369
60	5504243
70	6090165

Table. 6: Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.07
Flow (0.9 ml/min)	0.02
Temperature (27^0C)	0.09
Temperature (23^0C)	0.13

Wavelength of Detection (270 nm)	0.04
Wavelength of detection (266 nm)	0.01

Table 7: Assay of Avacopan tablets

Brand name of tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Avacopan	0.5	0.49 (±0.06)	98.00 (±0.48)

Table 8: Chromatogram showing degradation for Avacopan in 0.1 N HCl

Sl. No	Rt	Peak Area	Peak Concerntartion
1	1.77	135348	1.46
2	2.96	2034249	48.73
3	3.13	2203146	49.79

Table 9: Chromatogram showing degradation related impurity in 0.1 N NaOH

Sl. No	Rt	Peak Area	Peak Concerntartion
1	1.79	87453	1.76
2	2.96	1934167	47.91
3	3.13	2308574	49.51

Table 10: Results of force degradation studies of Avacopan API

Stress condition	**Time**	**Assay of active substance**	**Assay of degraded products**	**Mass Balance (%)**
Acid Hydrolysis (0.1 M HCl)	24Hrs.	43.75	54.61	98.36
Basic Hydrolysis (0.IM NaOH)	24Hrs.	43.32	55.02	98.32
Thermal Degradation (50 ^{0}C)	24Hrs.	97.39	----------	97.39
UV (254nm)	24Hrs.	95.19	04.34	99.53
3 % Hydrogen peroxide	24Hrs.	95.75	04.28	100.03

4. Conclusion

A sensitive and selective RP-HPLC method has been developed & validated for the analysis of Avacopan API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Avacopan in different formulations. Hence the suggested RP-HPLC method can be used for routine analysis of Avacopan in API and Pharmaceutical dosage form.

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