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IMPURITY PROFILING AND STABILITY-INDICATING UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF AVACOPAN

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Abstract:

A simple, rapid, precise, sensitive and reproducible ultra performance liquid chromatography (UPLC) method has been developed for the quantitative analysis of Avacopan and its impurities. Chromatographic separation of Avacopan and its impurities was achieved on Waters Acquity-e2695, by using Waters acquity UPLC BEH Shield RP-18 100mmx2.1, 1.7µm column and the mobile phase containing Ammonium formate pH-3.0/OPA & Acetonitrile in the ratio of 30:70% v/v. The flow rate was 0.2 ml/min; detection was carried out by absorption at 241nm using a photodiode array detector at ambienttemperature. The number of theoretical plates and tailing factor for Avacopan and its impurities were NLT 2000 and shouldnot more than 2 respectively. %Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Avacopan and its impurities and study of its stability.

Keywords: UPLCAvacopan,Impurity-A,Impurity-B

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INTRODUCTION:

Ultra-High Performance Liquid Chromatography (UHPLC), an advancement of HPLC, offers faster, more efficient, and high-resolution separations. Introduced by Waters in 2004 as UPLC®, this technology allows the use of columns with sub-2 µm particle sizes and operates under pressures exceeding 1000 bars, reducing analysis time while enhancing sensitivity and efficiency. The technique benefits from innovations in particle chemistry, system hardware, and detector design, making it both cost-effective and environmentally friendly due to lower solvent use.

The separation mechanism in UHPLC, like HPLC, is based on the interaction between the stationary and mobile phases. The Van Deemter equation underpins the technique's principle, showing how smaller particles enhance efficiency and resolution. The core components of UHPLC instrumentation include sample injection systems capable of handling extreme pressures, specialized columns, and various detectors such as PDA (Photodiode Array), TUV (Tunable UV), FLR (Fluorescence), and ELS (Evaporative Light Scattering). In this work, the PDA detector is emphasized for its high sensitivity and 2D/3D spectral analysis capability.

Columns used in UHPLC are typically BEH-type (Bridged Ethylene Hybrid) and come in various bonded phases such as C18, C8, Shield R18, Phenyl, and Amide, offering broad pH stability and selectivity. These columns, due to their small particle size and high chemical stability, are central to the improved resolution in UHPLC.

Advantages of UHPLC : include increased selectivity, sensitivity, faster analysis, lower solvent usage, reduced run times, and high throughput, making it ideal for real-time monitoring and quality assurance. However, it also presents **disadvantages**, primarily high back pressure which reduces column life, and the non-regenerability of sub-2 µm particles.

Applications of UHPLC span a wide range of fields:

- Pharmaceutical analysis (e.g., plasma drug analysis, statins in wastewater)
- Herbal and natural product research
- Food safety (e.g., analysis of vitamins, coumarin, antibiotics in eggs)
- Environmental monitoring (e.g., pesticide residues in groundwater)
- Stability studies and kinetic analysis of energetic materials

Analytical Method Validation: is essential for generating reliable, reproducible data and involves parameters like specificity, accuracy, precision, LOD, LOQ, linearity, range, and robustness. These validations align with ICH guidelines and are required for identification tests, impurity analysis, and API quantification.

Stability studies, especially forced degradation tests, help evaluate drug stability under stress conditions (light, heat, oxidation, hydrolysis) and support method development and regulatory compliance. These studies are vital for understanding degradation pathways and ensuring product efficacy and safety.

Impurity profiling is critical in ensuring drug quality and involves identifying organic, inorganic, and residual solvent impurities. Impurities may arise from manufacturing processes, degradation, or environmental exposure (light, temperature, humidity). Proper impurity control ensures product safety, efficacy, and regulatory acceptance. Organic impurities often stem from synthesis, degradation, or excipient interactions, while inorganic ones may come from reagents or equipment. Residual solvents are categorized based on toxicity and must comply with ICH limits.

METHOD AND METHODOLOGY:

a) Equipment Used:

S. No	Name	Model	Manufacturer
1	UPLC	ACQUITY	Waters (Empower software v2.0)
2	pH Meter	-	Eutech
3	Weighing Balance	-	Sartorius
4	UV/VIS Spectrophotometer	UV-1700	-
5	Pipettes, Beakers and Burettes	-	Borosil
6	Ultrasonicator	UCA701	Unichrome
7	Pump	Isocratic Model	-

b) Reagents & Chemicals

List of Chemicals Used in UPLC Method

S. No	Name	Grade	Manufacturer
1	Acetonitrile	HPLC	Rankem
2	Water (Milli-Q)	HPLC	In-house production
3	Ortho Phosphoric Acid	HPLC	Analytical Reagent
4	Ammonium Formate	HPLC	Rankem

Determination of Working Wavelength (λmax):

The wavelength of maximum absorption of Avacopan in a mixture of Acetonitrile and Ammonium Formate buffer pH 3.0 (70:30 v/v) was determined using a PDA detector over the range of 200–400 nm. The maximum absorbance was observed at 241 nm, which was selected as the detection wavelength for the UPLC analysis.

Chromatographic Conditions

Parameter	Condition
Column	Waters Acquity UPLC BEH Shield RP-18 (100 mm × 2.1 mm, 1.7 µm)
Mobile Phase Ratio	Acetonitrile : Ammonium Formate buffer (pH 3.0/OPA) = 70:30
Detection Wavelength	241 nm
Flow Rate	0.2 mL/min
Injection Volume	5 µL
Runtime	4 minutes

Solution Preparations

1. Standard Solution:

10 mg of Avacopan working standard was accurately weighed and transferred into a 10 mL volumetric flask. It was dissolved using diluent (mobile phase), sonicated, and made up to volume. From this, 1 mL was further diluted to 10 mL to obtain a 100 ppm solution.

2. Sample Solution:

334 mg of Avacopan sample was weighed and transferred into a 10 mL volumetric flask. It was dissolved using diluent, sonicated for 30 minutes, centrifuged, and volume was made up. The solution was filtered through a 0.45 µm filter.

3. Impurity Stock Solution:

5 mg each of Impurity-A and Impurity-B were transferred into a 10 mL volumetric flask, dissolved with 7 mL diluent, sonicated for 30 minutes, and made up to volume. Then, 2 mL of this solution was further diluted to 10 mL.

4. Spiked Sample Solution:

1 mL each of sample solution and impurity stock solution were mixed in a 10 mL volumetric flask and diluted to volume with diluent. Filter through 0.45 µm filter.

5. Ammonium Formate Buffer Solution:

6.3 g of Ammonium Formate was dissolved in 1 L of water. pH was adjusted to 3.0 with OPA and filtered through 0.45 µm nylon membrane.

6. Mobile Phase:

Ammonium Formate buffer pH 3.0 and Acetonitrile were mixed in 30:70 ratio and filtered through 0.45 µm membrane.

7. Diluent:

Mobile phase.

METHOD VALIDATION

1. System Suitability

- The tailing factor for Avacopan and its impurities in the standard solution should not exceed 2.0.
- The number of theoretical plates for both Avacopan and its impurities in the

standard solution should not be less than 2000.

2. Specificity

Specificity refers to the ability of an analytical method to measure the analyte of interest without interference from other components such as the blank or known impurities. Blank, standard, and sample chromatograms were recorded. The blank chromatogram exhibited no response at the retention times of the analyte, confirming the specificity of the method.

3. Linearity

Preparation of Avacopan Stock Solution

- Weigh accurately 10 mg of Avacopan working standard into a 10 mL volumetric flask. Add diluent, sonicate to dissolve, and make up the volume with the same solvent.

Preparation of Impurity Stock Solution

- Weigh 5 mg each of impurity-A and impurity-B into a 10 mL volumetric flask. Add diluent, sonicate, and make up to volume. Pipette 2 mL of this solution into a 10 mL volumetric flask and make up to volume with diluent.

Preparation of Calibration Levels

- Level I (25 ppm Avacopan, 2.5 ppm Imp-A & Imp-B): 0.25 mL of each stock solution in 10 mL flask
- Level II (50 ppm Avacopan, 5 ppm Imp-A & Imp-B): 0.5 mL of each stock solution
- Level III (75 ppm Avacopan, 7.5 ppm Imp-A & Imp-B): 0.75 mL of each stock solution
- Level IV (100 ppm Avacopan, 10 ppm Imp-A & Imp-B): 1 mL of each stock solution
- Level V (125 ppm Avacopan, 12.5 ppm Imp-A & Imp-B): 1.25 mL of each stock solution
- Level VI (150 ppm Avacopan, 15 ppm Imp-A & Imp-B): 1.5 mL of each stock solution

Procedure Inject each level into the UPLC system, record the peak area, and plot a calibration graph (concentration vs. peak area). Calculate the correlation coefficient.

4. Range

The method's range is determined by its ability to provide accurate, precise, and linear responses over the specified interval.

- Acceptance Criterion: Correlation coefficient should be ≥ 0.999

5. Accuracy

Preparation of Accuracy Sample Solutions

- 50% Level:
  - Weigh 334 mg of Avacopan sample and 5 mg each of Imp-A & Imp-B into separate 10 mL flasks.
  - Dissolve and make up with diluent. From impurity solution, pipette 2 mL into a 10 mL flask, make up with diluent.
  - Pipette 0.5 mL each from Avacopan and impurity stock solutions into a 10 mL flask and make up (50 ppm Avacopan, 5 ppm each impurity).
- 100% Level: Same as above, but use 1 mL each stock solution (100 ppm Avacopan, 10 ppm each impurity).
- 150% Level: Use 1.5 mL each stock solution (150 ppm Avacopan, 15 ppm each impurity).

Procedure Inject spiked sample solutions (50%, 100%, 150%) into the system.

- Acceptance Criteria: % Recovery should be within 98.0–102.0%

6. Precision

Precision evaluates repeatability under normal conditions and includes:

1. System Precision
2. Method Precision
3. Intermediate Precision (Intraday and Interday)

- Inject six replicates of a solution containing 100 ppm Avacopan and 10 ppm each of Imp-A and Imp-B.
- Acceptance Criteria: % RSD of peak areas should be $\leq 2\%$

7. Robustness

To evaluate robustness, deliberate changes in analytical conditions were studied.

A. Flow Rate Variation

- Evaluated at 0.18 mL/min and 0.22 mL/min.
- No significant impact on results observed.

Method is robust to ±10% flow rate variation.

B. Mobile Phase Composition Variation

- Analyzed the impact of changes in organic phase composition.
- No significant change was observed.

8. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Calculated as per ICH guidelines using the formula:

- LOD = 3.3 × σ/S
- LOQ = 10 × σ/S
- LOD: Avacopan – 0.3 µg/mL, Imp-A & Imp-B – 0.03 µg/mL
- LOQ: Avacopan

Degradation Studies

Stock Solution:

10 mg of Avacopan in 10 mL of diluent, sonicated and diluted.

Stress Condition	Procedure
Acid Degradation	Add 1 mL of 1N HCl, heat at 60°C for 1 hour, neutralize with 1N NaOH, dilute to 10 mL, filter.
Alkali Degradation	Add 1 mL of 1N NaOH, heat at 60°C for 1 hour, neutralize with 1N HCl, dilute to 10 mL, filter.
Peroxide Degradation	Add 1 mL of 3% H2O2, heat at 60°C for 1 hour, stand 15 min at room temp, filter.
Reduction	Add 1 mL of 10% Sodium Bisulphite, heat at 60°C for 1 hour, stand 15 min at room temp, filter.
Hydrolysis	Add 1 mL HPLC water, heat at 60°C for 1 hour, stand 15 min at room temp, filter.
Photolytic	UV exposure for 12 hours, dilute with diluent, inject and analyze.
Thermal	Expose sample at 105°C for 24 hours in hot air oven, dilute and analyze.

RESULTS AND DISCUSSION:

Development of UPLC Method for Simultaneous Estimation of Avacopan and Its Impurities

A robust and precise UPLC method was successfully developed for the simultaneous estimation of Avacopan and its related impurities using a Waters Acquity UPLC BEH Shield RP-18 column (100 mm × 2.1 mm, 1.7 µm particle size). The optimized mobile phase consisted of ammonium formate buffer (pH 3.0, adjusted with orthophosphoric acid) and acetonitrile in the ratio of 30:70 v/v. The flow rate was maintained at 0.2 mL/min, and the detection wavelength was set at 241 nm using a PDA detector. Column temperature was kept at ambient conditions throughout the analysis.

Method Development

Selection of Wavelength for Simultaneous Estimation

To determine the most suitable detection wavelength, the UV absorption spectra of Avacopan and its impurities were recorded using a photodiode array (PDA) detector. The optimal wavelength selected was 241 nm, as it provided maximum absorbance and optimal response for both the drug and its impurities.

Preparation of Standard Solutions for Wavelength Determination

- Avacopan Standard:
  - Accurately weighed 10 mg of Avacopan was transferred into a 10 mL volumetric flask.
  - Dissolved in buffer and diluted to volume with solvent to obtain a 1000 µg/mL stock solution.
  - From this stock, 1 mL was pipetted into another 10 mL volumetric flask and diluted to volume with solvent (final concentration: 100 µg/mL).
- Impurity-A and Impurity-B Standards:
  - Accurately weighed 5 mg each of Impurity-A and Impurity-B were transferred to a 10 mL volumetric flask.

- Dissolved in 7 mL of diluent (mobile phase) and volume made up to 10 mL.
- From this solution, 2 mL was pipetted into a 10 mL volumetric flask and diluted to volume.
- Further, 1 mL of this diluted solution was transferred to another 10 mL volumetric flask and diluted to volume with diluent.

Chromatographic Analysis

The prepared solutions were loaded into the auto sampler, and auto-injection was performed using the UPLC system with PDA detection. The spectra confirmed adequate absorbance at 241 nm, with no significant interference from the blank or diluent. The retention times of Avacopan and its impurities were well-resolved under the optimized chromatographic conditions.

DISCUSSION:

The developed method exhibited high resolution between Avacopan and its known impurities, demonstrating excellent specificity. Sharp, symmetrical peaks were observed for all analytes with acceptable tailing factors (<2.0) and theoretical plate counts (>2000), indicating column efficiency. The method was found to be suitable for routine analysis of Avacopan in pharmaceutical formulations and for stability-indicating studies. The chosen wavelength (241 nm) offered strong sensitivity and consistent response for all analytes, supporting the method's robustness and reliability.

Further validation parameters such as linearity, accuracy, precision, robustness, LOD, and LOQ were assessed and met ICH Q2(R1) acceptance criteria, confirming the method's suitability for impurity profiling and quality control of Avacopan. SS

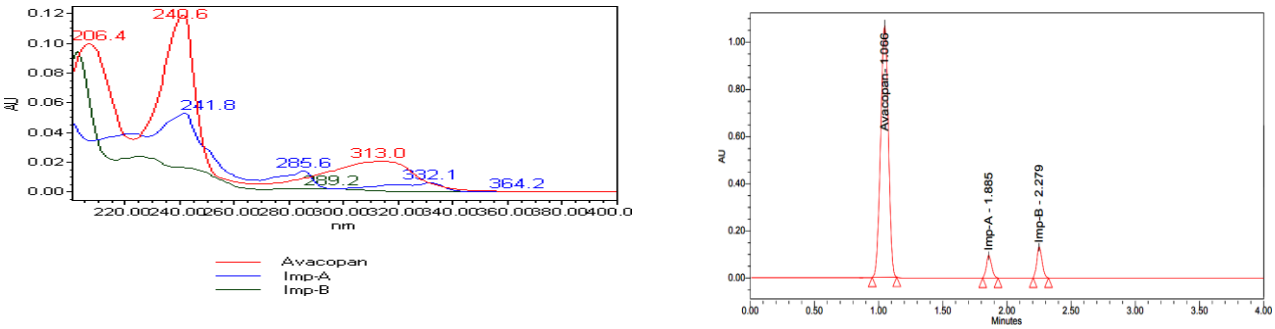


Fig-PDASpectrum

From the above spectra of Avacopan and its impurities, a wavelength was selected at which the drugs showed maximum absorbance. The wavelength selected was 241 nm.

Table: Summary of UPLC Method Development Trials for Avacopan

Trial No	Column Used	InjVolume (µL)	Flow Rate (mL/min)	Wavelength (nm)	Observation
1	C18 (100 × 2.1 mm, 1.7 µm)	2	0.3	241	Broad peaks, poor resolution
2	C18 (100 × 2.1 mm, 1.7 µm)	2	0.3	241	Tailing observed, low sensitivity
3	BEH Shield RP-18 (100 × 2.1 mm, 1.7 µm)	2	0.2	241	Sharp peaks, good resolution
4	BEH Shield RP-18 (100 × 2.1 mm, 1.7 µm)	2	0.2	241	Slight peak overlap
5	BEH Shield RP-18 (100 × 2.1 mm, 1.7 µm)	2	0.2	241	Best separation, sharp peaks, reproducible

	Name	Retention Time	Area	USP Resolution	USP Tailing	USPPlate Count
1	Avacopan	1.066	8972494		0.97	5369
2	Imp-A	1.885	614536	8.13	1.02	7890
3	Imp-B	2.279	801298	4.06	1.00	11374

Method Validation: The validation of the UPLC method for the determination of Avacopan, Impurity-A (Imp-A), and Impurity-B (Imp-B) was conducted as per ICH guidelines to demonstrate its suitability for the intended use. The method was evaluated across various validation parameters:

1. System Suitability

System suitability tests were performed to confirm the analytical system's capability to produce accurate and precise results. A standard solution of Avacopan and its impurities was prepared according to the test procedure and injected six times into the UPLC system.

System suitability parameters such as retention time, tailing factor, and theoretical plates were calculated from the standard chromatograms. The results are summarized below:

Acceptance Criteria:

- The tailing factor (T) should not be more than 2.0.
- The number of theoretical plates (N) should not be less than 2000.

The system suitability was verified using a mixture containing 100 µg/mL Avacopan, 10 µg/mL Imp-A, and 10 µg/mL Imp-B. The method met the required acceptance criteria.

Table : Results of System Suitability

Parameter	Avacopan	Imp-A	Imp-B
Retention Time (min)	1.066	1.885	2.279
Theoretical Plates	5369	7890	11374
Tailing Factor	0.97	1.02	1.00
USP Resolution	--	8.13	4.06

Observation: All parameters were found to be within acceptable limits, confirming the suitability of the instrument, reagents, and column for further validation.

2. Linearity and Range

Linearity was established across the following concentration ranges:

- Avacopan: 25–150 µg/mL
- Imp-A: 2.5–15 µg/mL
- Imp-B: 2.5–15 µg/mL

Aliquots of standard stock solutions were prepared and labeled as Solution 1 through Solution 6. These were injected into the UPLC system as per the test procedure.

Calibration curves for Avacopan, Imp-A, and Imp-B were plotted using concentration versus peak area. The

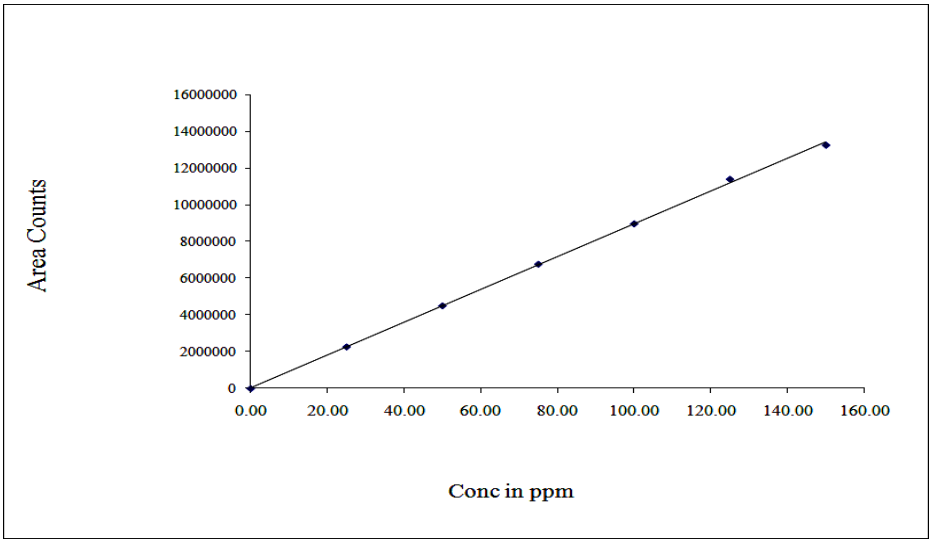
method exhibited good linearity within the specified ranges.

Acceptance Criteria:

- Correlation Coefficient (R^2) should not be less than 0.999.

Table-LinearityresultsofAvacopan

S.No.	Conc(µg/ml)	PeakArea
1	25.00	2261741
2	50.00	4502133
3	75.00	6760898
4	100.00	8965641
5	125.00	11387446
6	150.00	13239647
Regression equation	y=89191.23x +41730.25	
Slope	89191.23	
Intercept	41730.25	
R^2	0.99973	



- Figure-Calibration curveofAvacopan

Table: Linearity Parameters for Avacopan, Imp-A and Imp-B

Parameters	Avacopan	Imp-A	Imp-B
Linearity Range	25–150 µg/mL	2.5–15 µg/mL	2.5–15 µg/mL
Correlation Coefficient	0.99973	0.99983	0.99977

Observation: The correlation coefficient values for all three compounds were within the acceptable limits,

confirming good linearity.

3. Precision

Precision of the analytical method was evaluated by analyzing multiple samples from a homogeneous mixture. It was expressed as standard deviation (SD) or relative standard deviation (%RSD). According to ICH guidelines, precision must be assessed at three levels:

- System Precision (Repeatability)
- Method Precision (Reproducibility)

(a) System Precision (Repeatability)

System precision was evaluated by injecting six replicates of the same standard preparation. %RSD of peak areas was calculated for Avacopan, Imp-A, and Imp-B.

Acceptance Criteria:

%RSD for peak areas of Avacopan, Imp-A, and Imp-B from six replicate injections should be ≤ 2.0%.

Table: System Precision Values

S. No.	Avacopan	Imp-A	Imp-B
1	8963261	614127	801695
2	8861689	613805	803077
3	8858213	615208	800731
4	8867057	612933	804632
5	8870622	613451	802563
6	8862914	614699	801024
Mean	8880626	614037	802287
SD	40712.18	828.69	1453.69
%RSD	0.46	0.13	0.18

Observation:

%RSD values for peak areas were well within acceptable limits, confirming the repeatability of the method.

(b) Method Precision (Intraday Precision)

Method precision was evaluated by analyzing six independent sample preparations from a homogeneous blend of the same sample. %RSD of peak areas was calculated.

Acceptance Criteria:

%RSD for peak areas of Avacopan, Imp-A, and Imp-B should be ≤ 2.0%.

Table : Method Precision Values

S. No.	Avacopan	Imp-A	Imp-B
1	8871618	615264	802854
2	8848720	613396	801736
3	8863554	614025	804025
4	8884212	610627	806848
5	8859433	612810	803230
6	8826789	611600	805511
Mean	8859054	612954	804034
SD	19793.91	1670.18	1866.54

S. No.	Avacopan	Imp-A	Imp-B
%RSD	0.22	0.27	0.23

Observation:
All %RSD values were within acceptable limits, confirming the reproducibility of the method.

4. Accuracy

The accuracy of the UPLC method was evaluated by recovery studies at three concentration levels: 50%, 100%, and 150% for Avacopan, Impurity-A (Imp-A), and Impurity-B (Imp-B). The study was performed in triplicate at each level by spiking known quantities of the analytes into the matrix. The percentage recovery and %RSD were calculated to determine the closeness of the measured value to the true value.

Acceptance Criteria:
The mean percentage recovery of Avacopan, Imp-A, and Imp-B at each level should be between 98.0% and 102.0%.

Table: Accuracy Results for Avacopan

Recovery Level	Amount Taken (mg)	Area	Average Area	% Recovery	%RSD
50%	50	4472989			
	50	4455905	4447073	100.1	0.70
	50	4412324			
100%	100	8962357			
	100	8969024	8962123	100.9	0.08
	100	8954987			
150%	150	13194553			
	150	13264125	13307601	99.8	1.05
	150	13464125			

Table : Accuracy Results for Imp-A

Recovery Level	Amount Taken (µg/mL)	Area	Average Area	% Recovery	%RSD
50%	5	307754			
	5	305557	305981	99.7	0.52
	5	304632			
100%	10	614959			
	10	614135	614937	100.2	0.13
	10	615718			
150%	15	915640			
	15	913132	915301	99.4	0.22
	15	917132			

Table: Accuracy Results for Imp-B

Recovery Level	Amount Taken (µg/mL)	Area	Average Area	% Recovery	%RSD
50%	5	404786			
	5	402786	402978	100.4	0.43
	5	401361			

Recovery Level	Amount Taken (µg/mL)	Area	Average Area	% Recovery	%RSD
100%	10	802354			
	10	801395	801797	99.9	0.06
	10	801641			
150%	15	1213287			
	15	1203035	1213119	100.8	0.82
	15	1223035			

Observation:
The accuracy results for Avacopan, Imp-A, and Imp-B were found to be within the acceptable range of 98–102% recovery. The %RSD values at all three levels were within 2%, indicating that the method is accurate and reliable for quantifying the analytes

Table: Robustness, Ruggedness, LOD & LOQ Summary

Parameter	Condition	Analyte	Peak Areas (n=3)	%RSD	Observation
Robustness (Flow Rate)	0.18 mL/min	Avacopan	9126347, 9133688, 9119152	0.08	Within limits
		Imp-A	635284, 634468, 634951	0.06	Within limits
		Imp-B	818456, 817234, 818802	0.10	Within limits
	0.22 mL/min	Avacopan	8726185, 8735994, 8748301	0.13	Within limits
		Imp-A	608347, 607621, 609452	0.15	Within limits
		Imp-B	788152, 787044, 785723	0.15	Within limits
Robustness (Mobile Phase)	Organic Minus (30:70→23:77)	Avacopan	9323961, 9339455, 9310248	0.16	Within limits
		Imp-A	643348, 641820, 642437	0.12	Within limits
		Imp-B	840891, 842274, 841465	0.08	Within limits
	Organic Plus (30:70→37:63)	Avacopan	8616423, 8630995, 8609207	0.13	Within limits
		Imp-A	585410, 583936, 584278	0.13	Within limits
		Imp-B	771289, 772547, 773024	0.12	Within limits
Ruggedness	Analyst 1 & 2 Comparison	Avacopan	8981523 (A1), 8962401 (A2)	0.15	Within limits
		Imp-A	613865 (A1), 614273 (A2)	0.05	Within limits
		Imp-B	800961 (A1), 802547 (A2)	0.14	Within limits

Parameter	Condition	Analyte	Peak Areas (n=3)	%RSD	Observation
LOD/LOQ		Avacopan	LOD: 0.3 µg/mL, LOQ: 1.0 µg/mL	–	Satisfactory
		Imp-A	LOD: 0.03 µg/mL, LOQ: 0.1 µg/mL	–	Satisfactory
		Imp-B	LOD: 0.03 µg/mL, LOQ: 0.1 µg/mL	–	Satisfactory

Forced Degradation Study of **Avacopan**:

Table: Summary of Forced Degradation Studies for Avacopan

Condition	Area		% Label Claim	% Degradation	Purity Angle	Purity Threshold	Observation
Control	8,880,254	100.0	0.0	3.542	7.543	No degradation, purity angle < threshold	
Acid	7,801,463	87.8	12.2	3.527	7.518	Purity angle < threshold	
Alkali	7,909,516	89.0	11.0	3.556	7.554	Purity angle < threshold	
Peroxide	7,541,036	84.9	15.1	3.529	7.524	Purity angle < threshold	
Reduction	8,744,712	98.4	1.6	3.549	7.568	Purity angle < threshold	
Hydrolysis	8,807,589	99.1	0.9	3.563	7.544	Purity angle < threshold	
Thermal	7,949,477	89.5	10.5	3.527	7.554	Purity angle < threshold	
Photolytic	7,845,640	88.3	11.7	3.581	7.523	Purity angle < threshold	

Purity angle should be less than purity threshold. Avacopan, Imp-A and Imp- B and its degraded substances should not have any flag in purity results table.

Figure:Representative chromatogram for Control degradation degradation

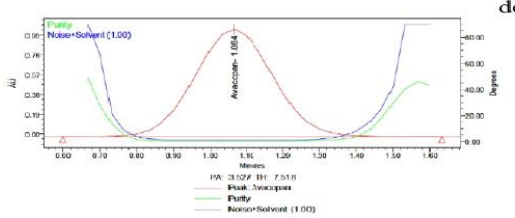
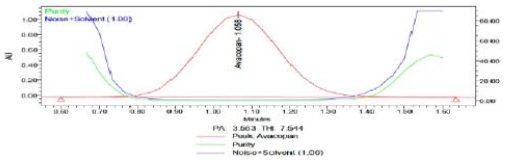


Figure-:Purity Plot of Control



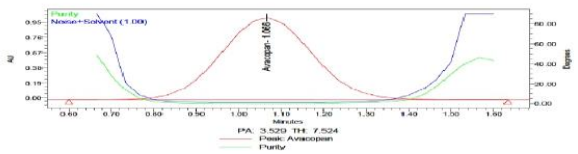


Figure:Purity Plot of Peroxide degradation
Figure-:Purity Plot of Hydrolysis degradation

CONCLUSION:

The developed UPLC method for the estimation of Avacopan and its impurities is simple, rapid, accurate, precise, robust, and cost-effective. The mobile phase and solvents used are easy to prepare, economical, and allow for a sensitive and time-efficient analysis.

The sample recovery results were consistent with their respective label claims, indicating no interference from formulation excipients. This confirms the method's suitability for routine analysis of Avacopan in pharmaceutical laboratories.

The method effectively separates Avacopan and its impurities with good resolution and a short run time of approximately 4 minutes. This makes it highly suitable for the routine testing of impurities during commercial batch manufacturing.

Validation parameters demonstrated satisfactory performance in terms of accuracy, precision, and reproducibility, both for the pure drug and in the presence of excipients. These results support the reliability and applicability of the method for analytical purposes.

In conclusion, the proposed UPLC method is a stability-indicating assay that is specific, accurate, and free from interference by placebo or degradation products. Therefore, it can be confidently applied for the routine quality control and stability testing of Avacopan and its impurities in bulk and pharmaceutical dosage forms.

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