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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF AVACOPAN BY RP-HPLC IN ITS BULK AND CAPSULE DOSAGE FORM

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ABSTRACT

In the research analysis a rapid, accurate and reliable High Performance Liquid Chromatography (HPLC) method was developed and validated by selecting chromatographic parameters for the estimation of Avacopan in bulk and capsule dosage form.The HPLC method was developed using reverse phase Agilent (4.6×150mm) 5µ column with mobile phases containing Methanol:water:Acetonitrile (55:40:5). The flow rate was 0.6 ml / min with PDA detection at λ max 220 nm with 10min run time. This method has been validated by the use of different validation parameters such as accuracy, precision, linearity and robustness. Such findings showed that the system could find practical use in its bulk and capsule dosage forms as a quality assurance tool for evaluating the drug in pharmaceutical industries.

INTRODUCTION

Avacopan, sold under the brand name Tavneos, is a medication used to treat anti-neutrophil cytoplasmic autoantibody-associated vasculitis. The most common side effects include nausea, headache, decrease in white blood cell count, upper respiratory tract infection, diarrhea, vomiting and nasopharyngitis.

METHOD DEVELOPMENT

Solution preparation

Twenty tablets were triturated in a glass mortar. The powder equivalent to 10 mg of active ingredient in 20 tablets (1534..5mg) was transferred into a 10 ml clean, dry volumetric flask, 7 ml of diluent was added, and the flask was shaken by mechanical stirrer and sonicated for about 30 minutes at five-minute intervals. It was diluted up to the mark with diluent to give a concentration of 1000 g/ml and allowed to stand until the residue (stock solution) was removed. 0.8 ml of the upper clear solution was put into a 10 ml volumetric flask and diluted to make standard concentrations.

LINEARITY

10 mg of Avacopan was carefully weighed and deposited into a 10-ml clean dry volumetric flask, 7 ml of diluent was added, sonicated to dissolve it completely, and the volume was made up to the mark using the same solvent to give a 1000 g/ml concentration.

Level I (60ppm AVACOPAN) 0.6 ml stock solution in 10 ml

Level II (70 ppm AVACOPAN): 0.7 ml in 10 ml.

Level III (80 ppm AVACOPAN): 0.8 ml in 10 ml.

Level IV (90 ppm AVACOPAN): 0.9 ml in 10 ml.
Level V (100 ppm AVACOPAN): 10 ml stock solution.

PRECISION

10 mg of Avacopan was carefully weighed and deposited into a 10-ml clean dry volumetric flask, 7 ml of diluent was added, sonicated to dissolve it completely, and the volume was made up to the mark using the same solvent to give a 1000 g/ml concentration. (Off-the-shelf)
Using the aforementioned stock solution, transfer 0.8 ml to a 10-ml volumetric flask and dilute to 80 g/ml. HPLC was used to measure the area of six injections of reference solution.

Intermediate Precision/Ruggedness:

10 mg of Avacopan was carefully weighed and deposited into a 10-ml clean dry volumetric flask, 7 ml of diluent was added, sonicated to dissolve it completely, and the volume was made up to the mark using the same solvent to give a 1000 g/ml concentration. (Off-the-shelf)
Using the aforementioned stock solution, transfer 0.8 ml to a 10-ml volumetric flask and dilute to 80 g/ml. HPLC was used to measure the area of six injections of reference solution.

ACCURACY

5 mg of Avacopan was accurately weighed and loaded into a 10-ml clean dry volumetric flask. About 7 ml of diluent was poured, sonicated to dissolve it completely, and the volume was made up to the mark using the same solvent to achieve a 1000 g/ml concentration. (Off-the-shelf) Using the aforementioned stock solution, pour 0.8 l to a measuring cylinder flask and dilute to 40 g/ml.
10 mg of Avacopan was carefully weighed and deposited into a 10-ml clean dry volumetric flask, 7 ml of diluent was added, sonicated to dissolve it completely, and the volume was made up to the mark using the same solvent to give a 1000 g/ml concentration. (Off-the-shelf)
Using the aforementioned stock solution, transfer 0.8 ml to a 10-ml volumetric flask and dilute to 80 g/ml.
15 mg of Avacopan was accurately weighed and deposited into a 10-ml clean dry volumetric flask. About 7 ml of diluent was added, sonicated to dissolve it completely, and the volume was made up to the mark using the same solvent to achieve a 1000 g/ml concentration. (Off-the-shelf)
Using the aforementioned stock solution, transfer 0.8 ml to a 10-ml volumetric flask and dilute to 120 g/ml.

SPECIFICITY

A) AVACOPAN identification: Standard and sample solutions were loaded into HPLC.
criterion d'acceptance
Standard and sample chromatograms should have similar retention times.
B) Placebo interference was studied. The test protocol called for injecting placebo into HPLC.
The placebo Chromatogram should not peak at the analyte peak retention time. No placebo interference with analyte retention time. Method is specific.
Blank interference was studied. As per method, diluent was introduced into HPLC.

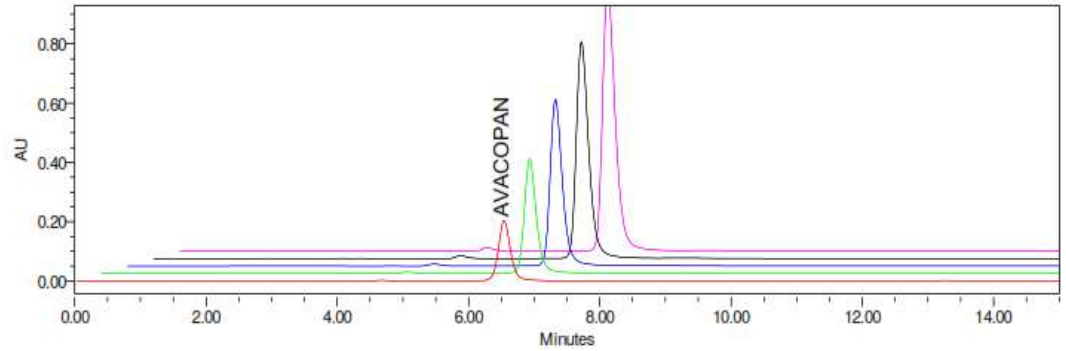
ROBUSTNESS

The robustness of the suggested approach was evaluated by analysing aliquots from homogenous batches with varying physical parameters such flow rate, mobile phase composition, and temperature. Responses were still within the assay's limits.
Variation in flow rate was studied. Flow rate was 0.5-0.7 ml/min. 80 ppm Avacopan solution
Based on the results, flow rate change affected the approach greatly. The approach is resilient even with 10% flow rate fluctuation.
Only under certain flow circumstances is the approach robust.
b) Effect of modification in mobile phase composition: Changing the mobile phase ratio was studied. 55 to 65% organic component in mobile phase

LOD& LOQ

Based on blank signal-to-noise ratio, 0.2 microgram per millilitre solution was created.
Based on blank signal-to-noise ratio, 0.76 g/mL LOD solution was created.

LINEARITY:

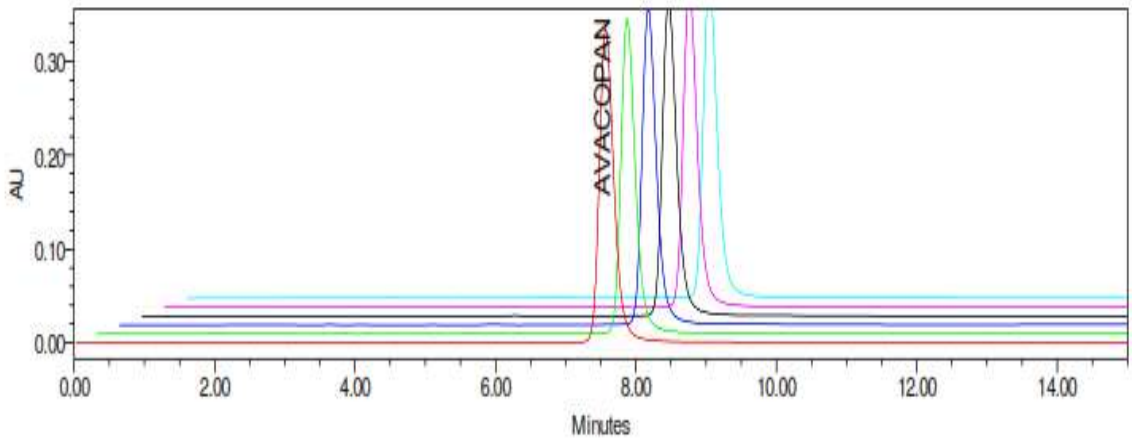


Linearity results:

S.No	Linearity Level	Concentration	Area
1	I	60 ppm	593412
2	II	70 ppm	834616
3	III	80 ppm	1101599
4	IV	90 ppm	1348563
5	V	100 ppm	1583765

PRECISION

A. Repeatability:



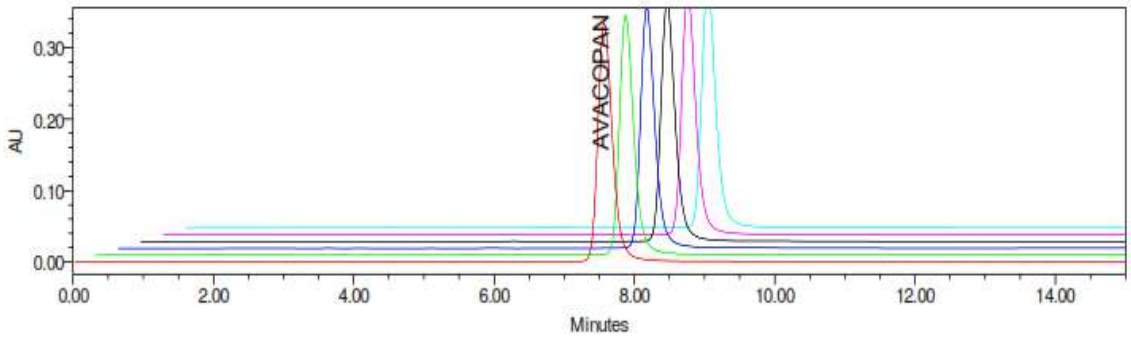
Sample Chromatograms for Repeatability

Chromatogram values for Repeatability

a) AVACOPAN

Injection No	Peak Area	R_t
1	1870855	7.781
2	1851015	7.804
3	1817754	7.799
4	1823745	7.799
5	1865619	7.799
Avg	1845798	7.802
SD	24087.1	0.0044
% RSD	1.30	0.141

B) Intermediate precision (Analyst to Analyst variability): Analyst 2
Chromatograms for Intermediate precision



Chromatogram values for intermediate Precision
a)AVACOPAN

Injection No	Peak Area	R_t
1	1121577	7.785
2	1129193	7.782
3	1134378	7.804
4	1132712	7.807
5	1125645	7.804
Mean	1128701	7.785
SD	5211.9	0.0076
% RSD	0.46	0.24

AVACOPAN Chromatogram Accuracy

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Each level's % Recovery must be between 98.0% and 102.0

ROBUSTNESS

Robustness results for Avacopan (flow rate):

S.No	Drug	Flow Rate ml/min		
		0.5 ml/min R_t	0.6 ml/min	0.7 m l/min
1	**Avacopan**	3.915	3.109	2.519
T Plate Count of method		2196.8	2037.2	2014.1
Tailing		1.7	1.7	1.7

Acceptance Criteria: • Each level's % Recovery should be 98.0 to 102.0.

REFERENCES:

1. Ahmed, A; Oswald, R(2010)." Methylcobalamine Defines a New Binding Site for Allosteric Modulators of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors". ***JMed.Chem***.**53** (5):2197-2203.
2. KG. Baheti* and VR. Galande, Validated Simultaneous Estimation of Gabapentin in the Presence of Methylcobalamin in Tablet by HPTLC Method. International Journal of Research in Pharmaceutical and Biomedical Sciences, Vol. 2 (3) Jul – Sep 2011 ISSN: 2229-3701.
3. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. Vol. II. New Delhi: ***CBS Publisher and Distributors***; 1986. p. 13-17.
4. Chhaya K. Chauhan, Development And Validation Of Analytical Method For Simultaneous Estimation Of Gabapentin And Methylcobalamin In Pharmaceutical Dosage Form. An International Journal of Advances in Pharmaceutical Sciences, Volume 6|Issue 2|March-April 2015|Pages 2815-2817.
5. Binder, S; Doddabela, "The efficacy of Methylcobalamine on the mental functional capacity of chronic alcoholics (author's transl)".
6. Saravanan. J*, 2 Shajan A, 1 Joshi NH, 3Varatharajan R and 3Valliappan K, A Simple and validated RP-HPLC method for the estimation of methylcobalamin in bulk and capsule dosage form. International Journal of Chemical and Pharmaceutical Sciences 2010, Dec., Vol.1 (2).
7. Brown, Phyllis R, *Journal of advances in chromatography*, volume 35, Page No:498-501.nautical Chemists, 12th edition, Page No: 489.
8. Chatten LG. Pharmaceutical Chemistry. Vol. II. New York: *Marcel Dekker Inc;* 1996. p. 23-25.
9. Conant, R; Schauss, AG (2004 Mar). "Therapeutic applications of citicoline for stroke and cognitive dysfunction in the elderly: a review of the literature.". *Alternative medicine review: a journal of clinical therapeutic* 9 (1): 17-31.
10. David CL, Michael Webb. Pharmaceutical Analysis. London: *Black good publishing;* 1994. p. 2-4.
11. Day RA, Underwood AL. *Quantitative Analysis*. 4th ed. New Delhi: Prentice Hall; 1986. p. 14-19.