



Stability Indicating Method Development and Validation of Cenobamate in Bulk and Dosage form by Liquid Chromatography

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A simple, Precise, Accurate method was developed for the estimation of Cenobamate by RP-HPLC technique. Chromatographic conditions used are stationary phase symmetry C18 (150 mm* 4.6 mm 5 µm), mobile phase Acetonitrile: 0.01NKH₂PO₄ in the ratio of 55:45 and flow rate was maintained at 1.0ml/min, detection wave length was 272.0 nm; column temperature was set to 30°C. Retention time was found to be 2.908 min. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150 % levels, R² value was found to be as 0.999. Precision was found to be 0.5 for repeatability and 0.8 for intermediate precision. LOD and LOQ are 0.01µg/ml and 0.03µg/ml respectively. By using above method assay of marketed formulation was carried out 100.32% was present. Degradation studies of Cenobamate were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

Keywords: HPLC; cenobamate; method development; ICH guidelines.

ABBREVIATIONS

API	: Active Pharmaceutical Ingredient
ICH	: International Conference on Harmonization
LOD	: Limit of Detection
LOQ	: Limit of Quantitation
RP-HPLC	: Reverse Phase High Performance Liquid Chromatography
RSD	: Relative Standard Deviation
SD	: Standard Deviation
USP	: United State Pharmacopoeia

1. INTRODUCTION

The antiepileptic medication cenobamate, also known as YKP-3089, is used to treat partial onset seizures. On November 21, 2019, the FDA approved cenobamate. Cenobamate has an unclear mode of action; however it regulates GABAA and inhibits voltage-gated sodium channels. Because Cenobamate is only taken once a day, its effect lasts a long time. The therapeutic window is broad, as 750mg dosages are well tolerated. The danger of DRESS syndrome, QT interval shortening, suicidal behavior, and neurological side effects should all be discussed with patients [1,2]. Structure and drug profile of Cenobamate are shown in Fig. 1 and Table 1.

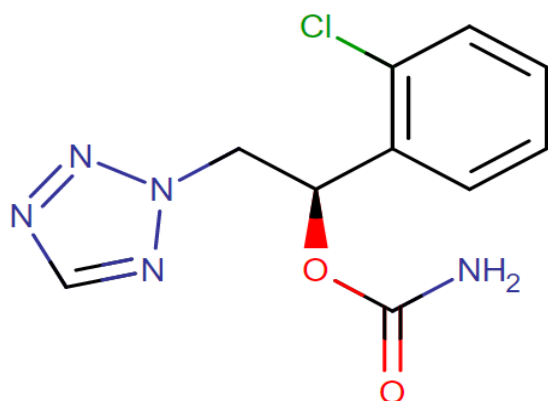


Fig. 1. Structure of Cenobamate

According to the findings of the literature review, a few analytical techniques for estimating Cenobamate have been established. The goal of this study is to design a novel, precise, accurate, and easy technique, validate it as per the ICH guideline Q2 (R1) and conduct a degradation study to determine the drug's stability under diverse circumstances [3,4,5].

2. METHODOLOGY

2.1 The used Chemicals

Cenobamate pure drugs (API), Combination Cenobamate tablets (Xcopri), Distilled water, Acetonitrile, Phosphate buffer, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

2.2 The Used Instruments

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz was be used for measuring absorbance for Cenobamate solution, Ultrasonic sonicator, pH meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump.

2.3 Method Development

2.3.1 Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and buffer taken in the ratio of 50:50

2.3.2 Preparation of Standard stock solutions

Accurately weighed 25mg of Cenobamate transferred 50ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (500µg/ml of Cenobamate).

2.3.3 Preparation of Standard working solutions (100% solution)

1ml of Cenobamate from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50µg/ml of Cenobamate).

2.3.4 Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (500 µg/ml of Cenobamate).

Table 1. Drug profile of Cenobamate

CAS Number	913088-80-9
Molecular Weight	267.67g/mol
Monoisotopic	267.0523023
Chemical formula	C ₁₀ H ₁₀ ClN ₅ O ₂
Powder	White
Physical State	Solid
Solubility	Water - 0.936 mg/mL
Melting Point	96.8 to 98.3°C
pKa Values	14.28 (strongest acidic), -1.7 (Strongest basic)
IUPAC Name	(1R)-1-(2-chlorophenyl)-2-(2H-1,2,3,4-tetrazol-2-yl)ethyl carbamate

2.3.5 Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (50 µg/ml of Cenobamate).

2.3.6 Buffer: 0.01N Potassium dihydrogen ortho phosphate

Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Ortho phosphoric acid solution.

2.4 Method Validation

2.4.1 System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Cenobamate (50 ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

2.4.2 Specificity

Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

2.4.3 Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are

made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.27 ml/min), Flow plus (0.33 ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

2.4.4 LOD sample Preparation

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Cenobamate, were transferred to 10ml volumetric flasks and made up with the same diluents

2.4.5 LOQ sample Preparation

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml Cenobamate, were transferred to 10ml volumetric flasks and made up with the same diluents

2.4.6 Assay Methodology

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system.

2.5 Degradation Procedure

2.5.1 Oxidative Degradation studies

To 1 ml of stock solution of Cenobamate 1 ml of 20% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 min at

60⁰c. For HPLC study, the resultant solution was diluted to obtain (50 ppm) solution and 10 μ l were injected into the system and the chromatograms were recorded to assess the stability of sample.

2.5.2 Acidic Degradation Studies

To 1 ml of stock solution Cenobamate 1ml of 2N Hydrochloric acid was added and refluxed for 30mins. The resultant solution was diluted to obtain (50 ppm) solution and 10 μ l solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

2.5.3 Alkaline Degradation Studies

To 1 ml of stock solution Cenobamate 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60⁰c. The resultant solution was diluted to obtain (50 ppm) solution and 10 μ l were injected into the system and the chromatograms were recorded to assess the stability of sample.

2.5.4 Thermal Degradation Studies

The standard drug solution was placed in oven at 105⁰C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (50 ppm) solution and 10 μ l were injected into the system and the chromatograms were recorded to assess the stability of the sample.

2.5.5 Photo Stability Degradation studies

The photochemical stability of the drug was also studied by exposing the (500 ppm) solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m² in photo stability chamber. For HPLC study, the resultant solution

was diluted to obtain (50 ppm) solutions and 10 μ l were injected into the system and the chromatograms were recorded to assess the stability of sample.

2.5.6 Hydrolytic Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60⁰c. For HPLC study, the resultant solution was diluted to (50 ppm) solution and 10 μ l were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3. Results and discussion

3.1 Optimized Chromatographic Condition

Based on drug solubility and pKa Value following conditions has been used to develop the method estimation of Cenobamate. Chromatographic conditions used are stationary phase symmetry C18 (150 mm*4.6 mm 5 μ m), Mobile phase Acetonitrile:0.01N kh2po4 in the ratio of 55:45 and flow rate was maintained at 1.0ml/min, detection wave length was 272.0 nm, column temperature was set to 30⁰C. The retention time was found to 2.908 min. Optimized chromatogram is shown in Fig. 2.

3.2 System Suitability Parameters

The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in Table 2.

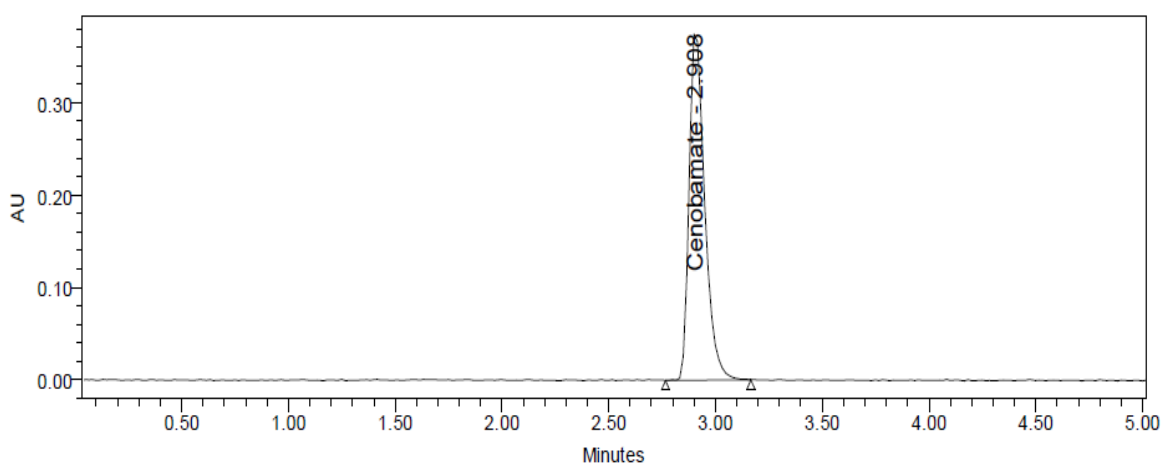


Fig. 2. Optimized chromatogram of cenobamate

Table 2. system suitability parameter data of Cenobamate

Parameters	Values	Accepted limit
USP plate count	7688	>2000
Tailing factor	1.36	<2
Rt (min)	2.908min	≥2

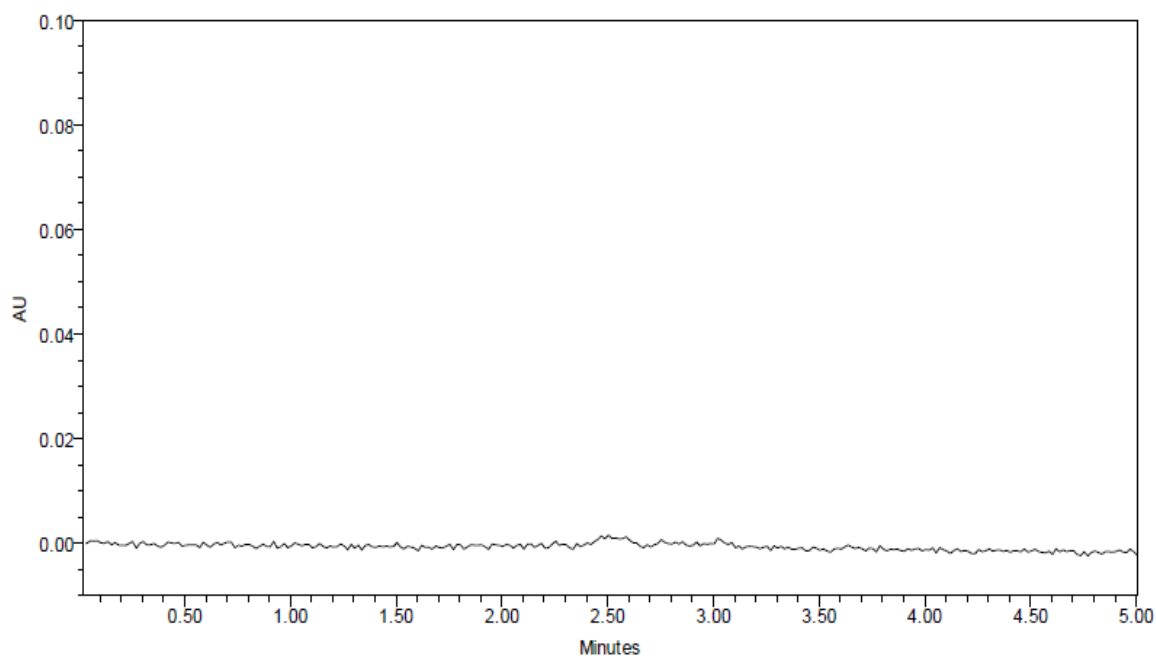


Fig. 3. Chromatogram of blank

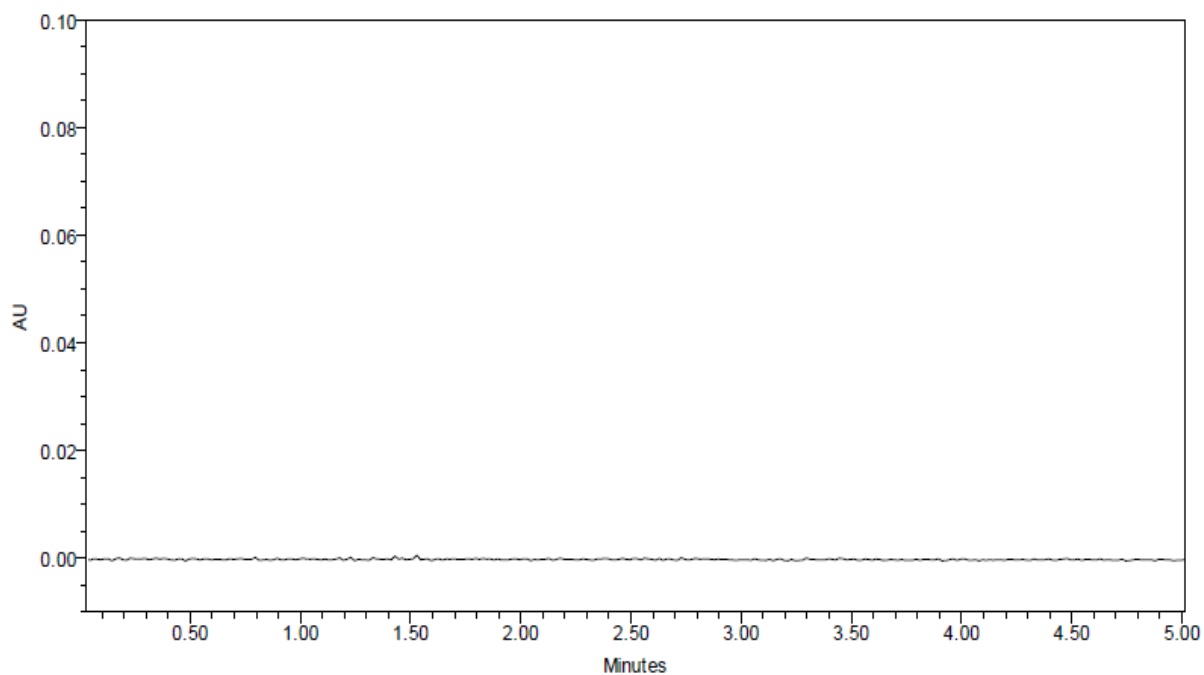


Fig. 4. Chromatogram of placebo

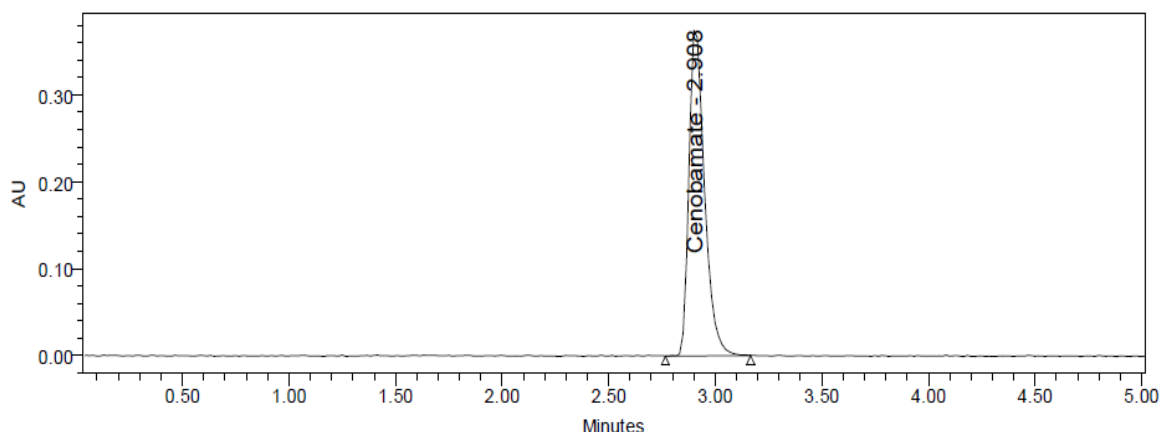


Fig. 5. Chromatogram of Cenobamate

3.3 Specificity

The chromatogram of Blank, placebo and drugs are shown in Fig. 3, 4, 5.

3.4 Precision

3.4.1 Repeatability

Six working sample solutions of 60ppm are injected and the % Amount found was calculated and %RSD was found to be 0.5 and data is shown in Table 3.

Intermediate precision

Six working sample solutions of 60 ppm are injected on the next day of the preparation of samples and the percentage amount found was calculated and %RSD was found to be 0.8 and Table 4 shows the data of intermediate precision

Linearity

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 12.5 ppm to 75 ppm of Cenobamate. Plot

a graph to concentration versus peak area. Slope obtained was $y = 45610x + 24737$ and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Fig. 6 and data is shown in Table 5.

Accuracy

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and % Recovery was calculated as 99.38. Accuracy data are depicted in Table 6.

LOD: Detection limit of the Cenobamate in this method was found to be 0.89 µg/ml.

LOQ: Quantification limit of the Cenobamate in this method was found to be 2.68 µg/ml.

Robustness

Small Deliberate change in the method was made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated and expressed in Table 7.

Table 3. Repeatability data of Cenobamate

S.No	Peak Area
1	1731413
2	1739179
3	1728377
4	1712527
5	1733942
6	1726766
AVG	1728701
STDEV	9056.0
%RSD	0.5

Table 4. Intermediate precision data of Cenobamate

S.No	Peak Area
1	1629615
2	1622241
3	1615225
4	1627602
5	1653768
6	1626628
AVG	1629180
STDEV	13089.3
%RSD	0.8

Table 5. linearity data of cenobamate

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	12.5	577527
50	25	1175193
75	37.5	1760731
100	50	2348317
125	62.5	2906421
150	75	3377476

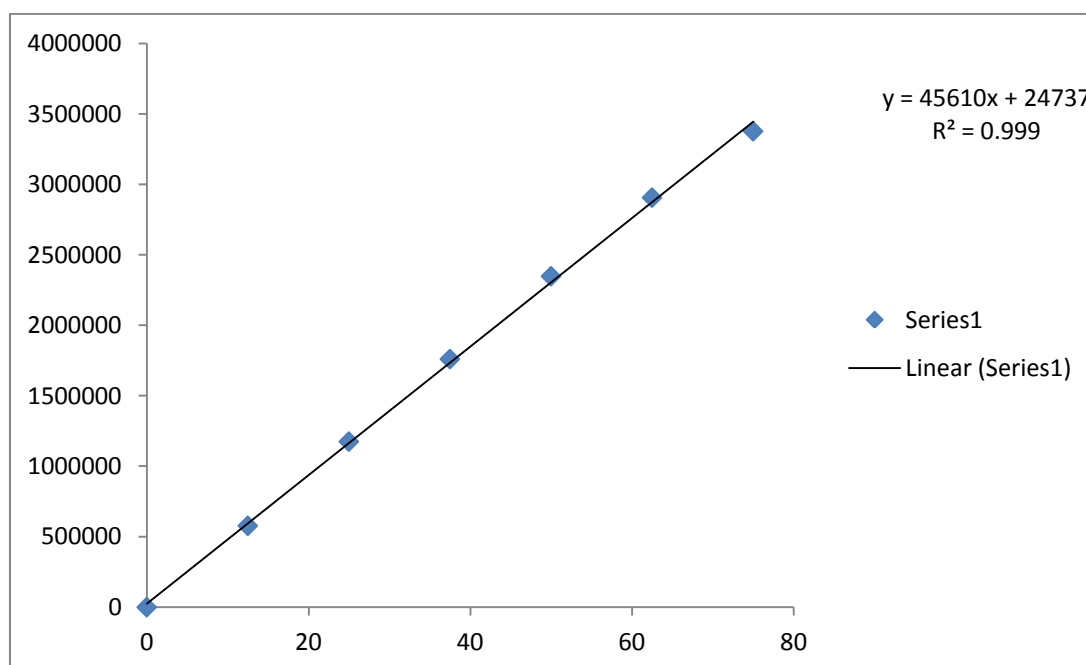


Fig. 6. Linearity plot of cenobamate

ASSAY OF MARKETED FORMULATION

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula. Table 8 shows the Assay data of Formulation.

Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation and data shown in Table 9 and chromatograms are shown in Fig. 7-12.

Table 6. Accuracy data of Cenobamate

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	24.73703	98.95	99.88%
	25	24.80035	99.20	
	25	24.70941	98.84	
100%	50	49.49597	98.99	
	50	49.3456	98.69	
	50	49.43241	98.86	
150%	75	75.1184	100.16	
	75	75.34304	100.46	
	75	75.19798	100.26	

Table 7. Robustness data of cenobamate

Parameter	%RSD
Flow Minus(0.9ml/min)	0.5
Flow Plus(1.1ml/min)	0.7
Mobile phase Minus(50B:50A)	0.4
Mobile phase Plus(40B:60A)	0.3
Temperature minus (25 ^o C)	0.5
Temperature plus (35 ^o C)	0.5

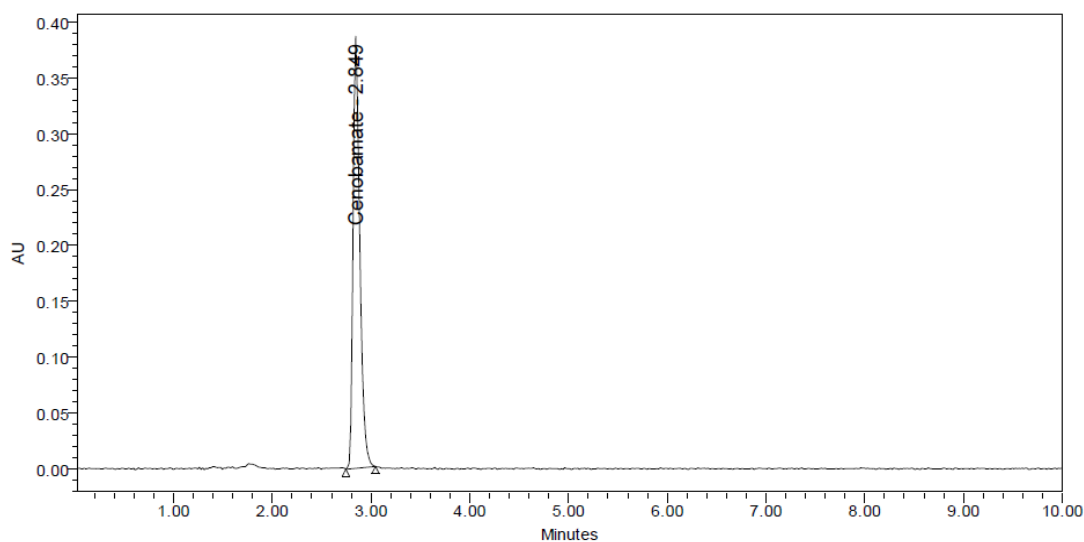


Fig. 7. Acidic Degradation Chromatogram of Cenobamate

Table 8. assay data of Cenobamate

Sample	Standard	Sample	%Assay
1	1721225	1731413	100.48
2	1728281	1739179	100.93
3.	1700836	1728377	100.30
4.	1727168	1712527	99.38
5.	1716436	1733942	100.63
6.	1734483	1726766	100.21
AVG	1721405	1728701	100.32
STDEV	11826.5	9056.0	0.526
%RSD	0.7	0.5	0.52

Table 9. Degradation data of Cenobamate

Degradation Condition	%Drug undegraded	%Drug Degraded
Acidic	94.17	5.83
Alkaline	97.80	2.20
Oxidative	93.74	6.26
Thermal	97.84	2.16
Photo stability	98.15	1.85
Hydrolytic	97.57	2.43

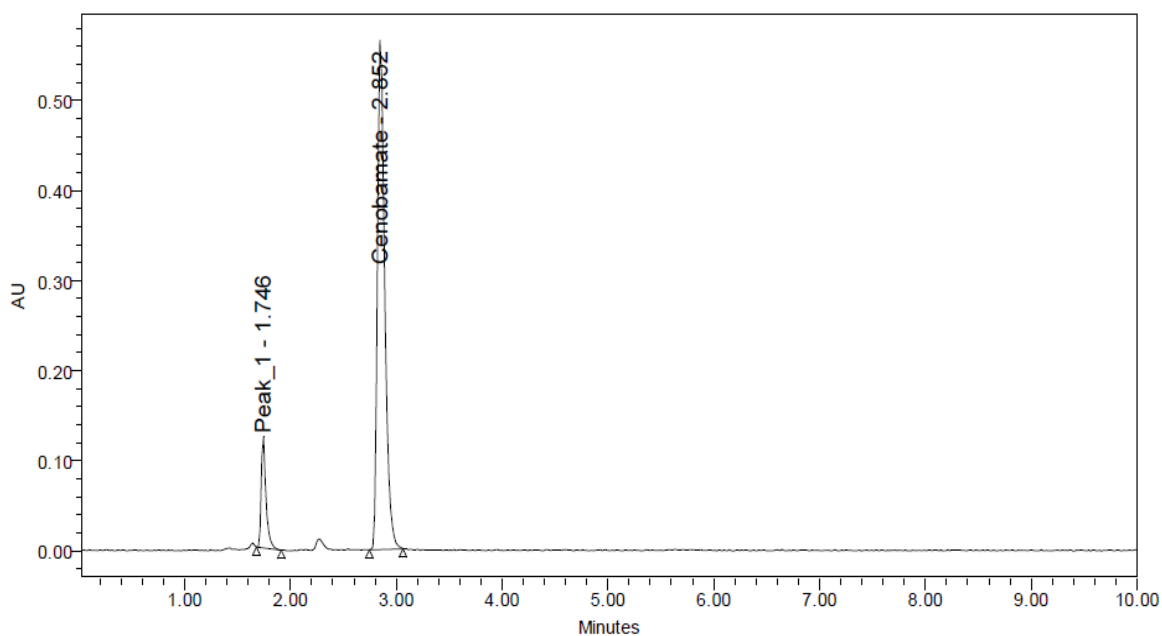


Fig. 8. Alkaline Degradation Chromatogram of Cenobamate

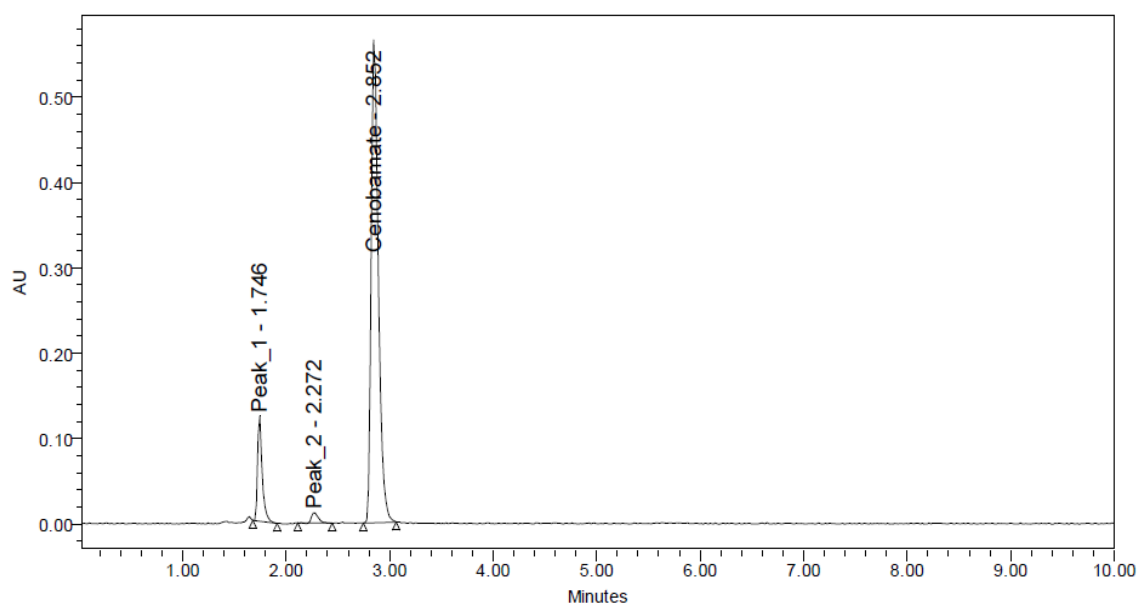


Fig. 9. Oxidative Degradation Chromatogram of Cenobamate

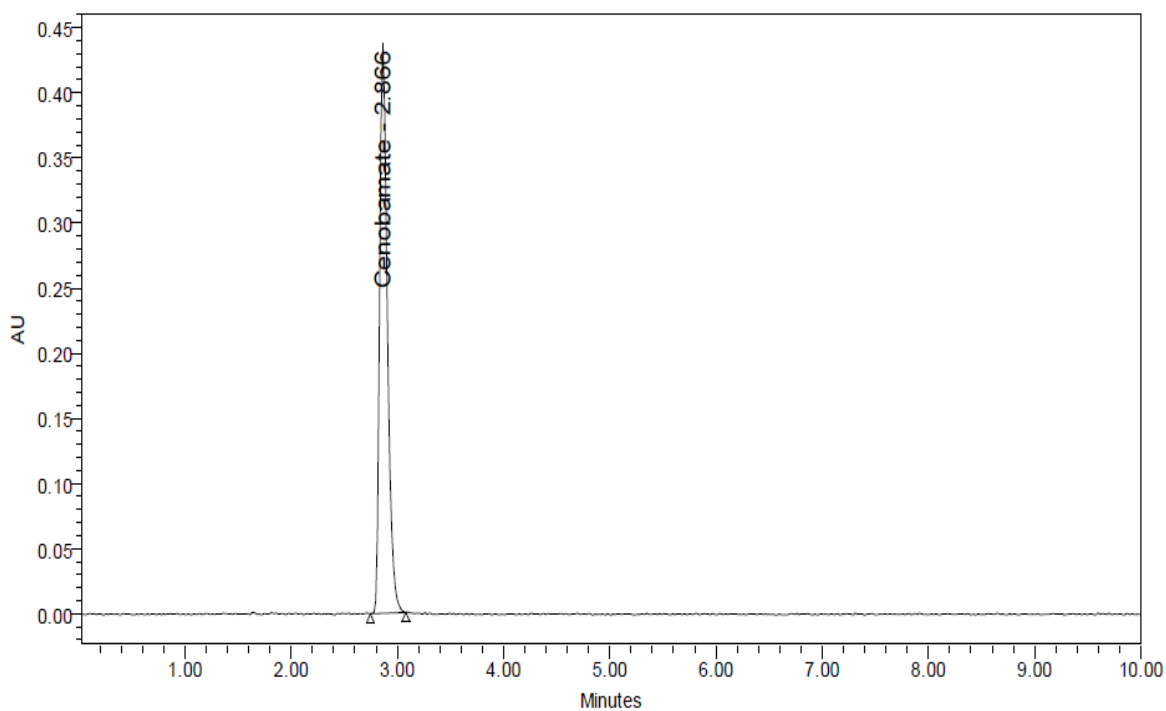


Fig. 10. Thermal Degradation Chromatogram of Cenobamate

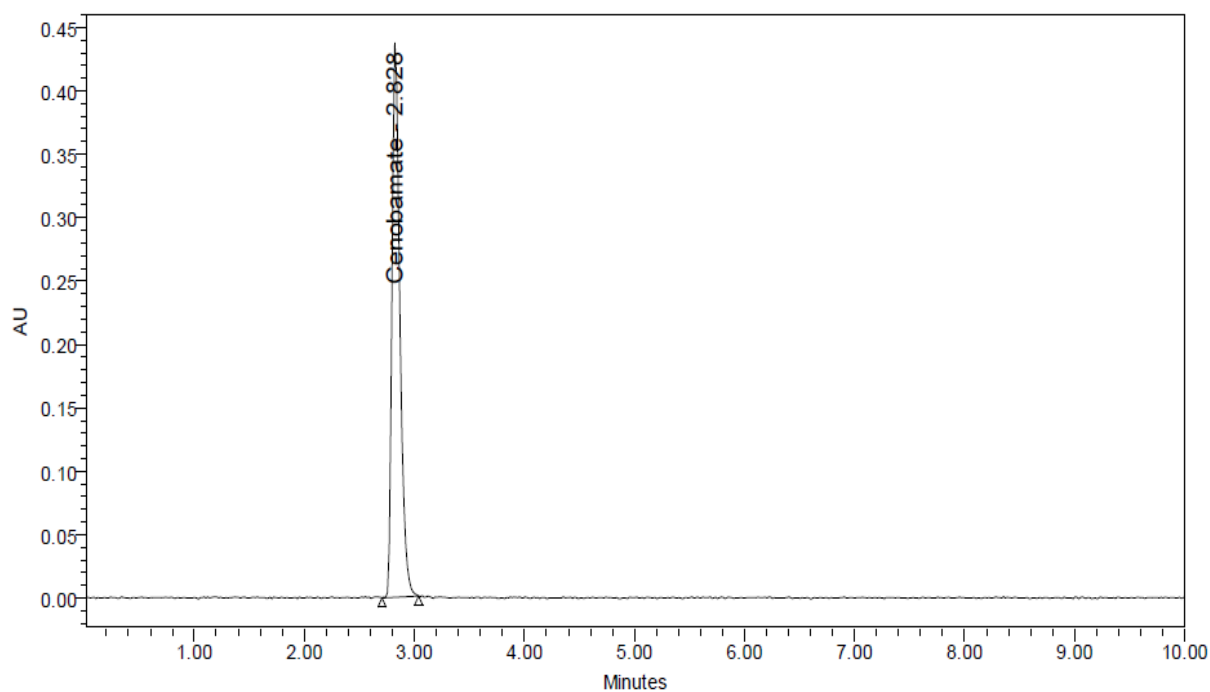


Fig. 11. Photo stability Degradation chromatogram by Cenobamate

4. DISCUSSION

Regarding the pH adjustment in mobile phase for the acid and base degradation studies have

movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time

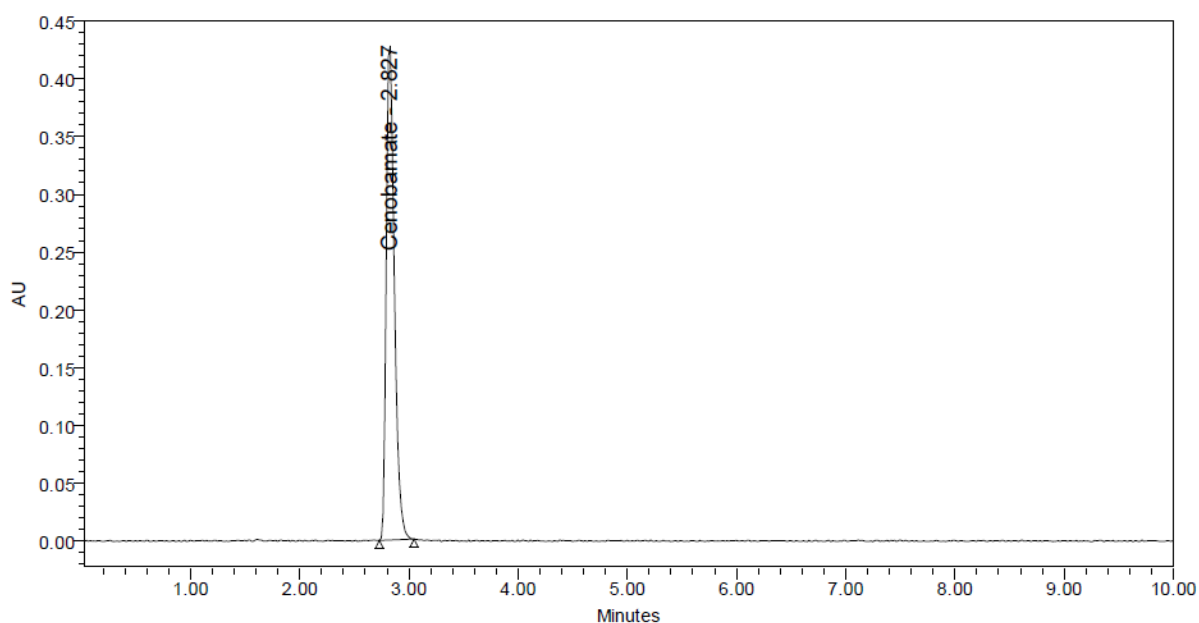


Fig. 12. Hydrolytic Degradation Chromatogram of Cenobamate

5. CONCLUSION

An antiepileptic medication Cenobamate is used to treat partial onset seizures. The separation was achieved by using stationary phase symmetry C₁₈ (150 mm*4.6 mm 5 μm), mobile phase Acetonitrile: 0.01NKH₂PO₄ in the ratio of 55:45 and flow rate was maintained at 1.0ml/min, detection wave length was 272.0 nm; column temperature was set to 30°C. Conditions were finalized as optimized method with a retention time of 2.908min. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, R² value was found to be as 0.999. Precision was found to be 0.5 for repeatability and 0.8 for intermediate precision. LOD and LOQ are 0.89μg/ml and 2.68μg/ml respectively. By using above method assay of marketed formulation was carried out 100.32% was present. Degradation studies of Cenobamate were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Method was found to be Precise, Accurate, simple and easy to perform and it follows all the ICH guideline Q2 (R1).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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