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Research Article

METHOD DEVELOPMENT AND STABILITY STUDY BY CHROMATOGRAPHIC METHOD FOR PERAMPANEL IN API AND TABLET DOSAGE FORM

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Abstract

To develop precise , accurate and reproducible stability assay method by RP-HPLC for estimation of Perampanel in API and Tablet dosage form.The adequate separation was carried using whatman ODS partisil column (250mm*4.6mm* 5µm) using ACN:Buffer (0.5 ml Triethylamine in 500 ml water adjust pH 2.5 with OPA) (60:40v/v).Detecting wavelength is 227 nm and Rt- 2.960 for API and 3.007 min for Tablet respectively.Linearity for Perampanel was found in the range of 50 -150 µg/ml (R^2 = 0.99) respectively. The Accuracy of the present method was evaluated at 50 %,100% and 150%. The % recoveries of Perampanel API and Tablet were found to be in the range of 95.57 – 98.51 % and 94.57 – 97.57 % respectively. Precision studies were carried out and the RSD values were less than two. The method was found to be robust.The proposed method was found to be specific, accurate, precise and robust can be used for estimation of Perampanel in API and Tablet dosage form.

Keywords: HPLC, Perampanel, API, Tablet dosage form, OPA, TEA.

Introduction[1][2][3],[4],[5][6],[7],[16]

Introduction

Perampanel is chemically a 2- {6′-oxo-1′-Phenyl-1′,6′-dihydro-[2,3′-bipyridine]-5′-yl}benzonitrile with molecular weight of 349.38 g /mol.Perampanel is antiepileptic drug. The first and only non-competitive AMPA receptor antagonist that targets glutamate activity at postsynaptic neuronsGlutamate is the primary excitatory neurotransmitter in the central nervous system-specifically engineered to block glutamate activity at postsynaptic AMPA receptors. Various analytical method has been reported for Perampanel alone. They include Spectrophotometric method, HPLC .

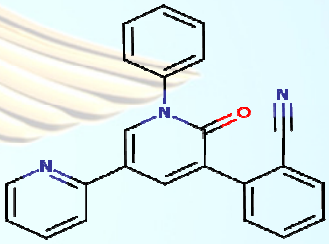

Figure 1.Structure of Perampanel

However an extensive literature search didn't reveal any estimation method for Perampanel in API and Tablet dosage form.Therefore an attempt has been made to develop and validate simple ,precise, accurate HPLC method for estimation of Perampanel in API and Tablet dosage form.

Materials and Method

Drugs, chemicals and solvents: Perampanel in API was kindly given by Advanced Analytical Research and Training Institute, Gujarat. All the chemicals and solvents used were of analytical grade.

Instruments

The analysis was performed on Agilent HPLC ,fitted with a gradient pump PDA detector and whatman ODS partisil column (250mm*4.6mm* 5µm) using ACN:Buffer (0.5 ml Triethylamine in 500 ml water adjust pH 2.5 with OPA) (60:40v/v) with flow rate of 1.5 ml/min. The injection volume

was 20µl. The chromatographic run time was adjusted to 7 min. The wavelength of the detector was set at 227 nm for analysis of the drug.

Preparation of buffer
Add 0.5 ml Triethylamine in 500 ml of HPLC water adjust pH 2.5 with OPA. and degas before use.

Preparation of mobile phase
Prepare a mixture of Buffer (0.5 ml Triethylamine in 500 ml of HPLC water adjust pH 2.5 with OPA) and Acetonitrile in the ratio of 40:60. This solution was sonicated for 5 min for degassing and filtered through 0.45 µ Millipore filter.

Diluent
The drug was dissolved in acetonitrile:water (50:50)

Preparation of Standard stock solution(API)
Accurately weighed 10 mg of Perampanel was taken in a 10 ml standard volumetric flask and dissolved in few ml of Diluent. Then the volume was made up to the mark with Diluent. From the above solution ,1 ml was diluted to 10 ml with to get a concentration of 100 µg/ml of Perampanel.

Preparation of Standard stock solution(Tablet)
The average of 10 Tablet was determined and grounded in mortar.An accurately weigh amount of powder equivalent to 10 mg of Perampanel was taken. It was transferred to 10 ml of volumetric flask .Add 5 ml of diluents and sonicated for 5 minutes to ensure complete solubilization of drug after sonication ,volume was made up to the mark with diluent(1000 µg/ml of Perampanel stock solution).Pipette out 1 ml from above solution and dilute up to 10 ml with diluent(100 µg/ml of Perampanel).

Calibration Curve
Aliquots of different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations. **Figure 20**

Forced Degradation Study
Forced Degradation Study of Perampanel in API and Tablet dosage form was carried out under different stress conditions as mentioned in ICH guideline Q1A (R2).The standard solution containing 100 µg/ml of Perampanel API and 100 µg/ml of Perampanel tablet were subjected to acid, alkali hydrolysis, peroxide ,thermal and photolytic degradation .

Acid degradation
Procedure for API
10 mg perampanel was taken and transferred to 10 ml volumetric flask separately and volume was made up with diluent (1000 µg/ml Perampanel). from the above solution 1 ml was taken into 10 ml volumetric flask and volume was made up by diluent (Stock solution-100µg/ml perampanel). Pipette out 1 ml from from the Stock solution and add 1 ml of 0.1N HCL and kept at 60º C in water bath for 2 hours. After that it was neutralized by adding 1 ml of 0.1N NaOH and make up with 10 ml of diluent. Filter the final solution with 0.45 µ PVDF Filter Similarly blank was prepared without adding sample. Blank and sample solution were injected in HPLC. Chromatograms are shown in figure: 3

Procedure for tablet
The Average Weight of 10 Tablet was determined and was ground in mortar. An Acurately Weighed amount of powder equivalent to 10mg of Perampanel. It was transferred 10 ml volumetric flask. Add 5 ml of diluent and sonicated for 5 minute to ensure complete solubilisation of drug After sonication volume was made up the mark with diluent (1000 µg/ml of Perampanel). Pipette out 1.0 ml from the above solution and dilute up to 10 ml of diluent. ((Stock solution 100 µg/ml Perampanel). Pipette out 1 ml from from stock solution and add 1 ml of 0.1N HCL and kept at 60º C in water bath for 2 hours. After that it was neutralized by adding 1 ml of 0.1N NaOH and make up with 10 ml of diluent. Filter the final solution with 0.45 µ PVDF Filter Similarly blank was prepared without adding sample. Blank and sample solutions were injected in HPLC. Chromatograms are shown in figure: 11

Base degradation

Procedure for API

10 mg perampanel was taken and transferred to 10 ml volumetric flask separately and volume was made up with diluent (1000 µg/ml Perampanel). from the above solution 1 ml was taken into 10 ml volumetric flask and volume was made up by diluent (Stock solution 100µg/ml perampanel). Pipette out 1 ml from from stock solution and add 1 ml of 0.1N NaOH and kept at 60º C in water bath for 2 hours. After that it was neutralized by adding 1 ml of 0.1N HCL and make up with 10 ml of diluent. Filter the final solution with 0.45 µ PVDF Filter Similarly blank was prepared without adding sample. Blank and sample solution were injected in HPLC. Chromatograms are shown in figure: 5

Procedure for tablet

The Average Weight of 10 Tablet was determined and was ground in mortar. An Acurately weighd amount of powder equivalent to 10mg of Perampanel. It was transferred 10 ml volumetric flask. Add 5 ml of diluent and sonicated for 5 minute to ensure complete solubilisation of drug After sonication volume was made up the mark with diluent (1000 µg/ml of Perampanel). Pipette out 1.0 ml from the above solution and dilute up to 10 ml of diluent. (100 µg/ml Perampanel). Pipette out 1 ml from from stock solution and add 1 ml of 0.1N NaOH and kept at 60º C in water bath for 2 hours. After that it was neutralized by adding 1 ml of 0.1N HCL and make up with 10 ml of diluent. Filter the final solution with 0.45 µ PVDF Filter Similarly blank was prepared without adding sample. Blank and sample solution were injected in HPLC. Chromatograms are shown in figure: 13

Peroxide Degradation

Procedure for API

10 mg perampanel was taken and transferred to 10 ml volumetric flask separately and volume was made up with diluent (1000 µg/ml Perampanel). from the above solution 1 ml was taken into 10 ml volumetric flask and volume was made up by diluent (Stock solution-100µg/ml perampanel). Pipette out 1 ml from from stock solution and add 1 ml of 3% H_2O_2 and kept at 60ºC in water bath for 2 hours. After that, make up with 10 ml of diluent. Filter the final solution with 0.45 µ PVDF Filter Similarly blank was prepared without adding sample. Blank and sample solutions were injected in HPLC. Chromatograms are shown in Figure: 7

Procedure for Tablet

The average of 10 Tablet was determined and grounded in mortar.An accurately weigh amount of powder equivalent to 10 mg of Perampanel was taken. It was transferred to 10 ml of volumetric flask. Add 5 ml of diluents and sonicated for 5 minutes to ensure complete solubilization of drug. After sonication ,volume was made up to the mark with diluent(1000 µg/ml of Perampanel stock solution).Pipette out 1 ml from above solution and dilute up to 10 ml with diluent(100 µg/ml of Perampanel).Pipette out 1 ml from the sample solution and add 1 ml of 3 % H_2O_2 , keep the volumetric flask in waterbath at 60ºC for 2 hours . After time period cool the contents to ambient temperature. Dilute the volume with diluent. After inject the peroxide degradation sample into HPLC, peak area and peak shape were observed. Figure No. 16

Thermal Degradation

Procedure for API

10 mg perampanel was taken and transferred to 10 ml volumetric flask separately and volume was made up with diluent (1000 µg/ml Perampanel). from the above solution 1 ml was taken into 10 ml volumetric flask and volume was made up by diluent (Stock solution-100µg/ml perampanel). Pipette out 1 ml from from stock solution and add 1 ml of diluent and kept at 60ºC in water bath for 2 hours. After that make up with 10 ml of diluent. Filter the final solution with 0.45 µ PVDF Filter Similarly blank was prepared without adding sample. Blank and sample solution were injected in HPLC. Chromatograms are shown in Figure: 8

Procedure for Tablet

The average of 10 Tablet was determined and grounded in mortar.An accurately weigh amount of powder equivalent to 10 mg of Perampanle was taken. It was transferred to 10 ml of volumetric flask. Add 5 ml of diluents and sonicated for 5 minutes to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluent(1000 µg/ml of Perampanel stock solution).Pipette out 1 ml from above solution and dilute up to 10 ml with diluent(100 µg/ml of Perampanel).Pipette out 1 ml of sample solution to 10 ml

of volumetric flask. Keep the volumetric flask in waterbath at 60 ºC for 2 hours. After time period cool the contents to ambient temperature.Make up the volume with diluent. After inject the thermal degradation sample into HPLC ,peak area and peak shape were observed.Figure No.17

Photolytic Degradation

Procedure for API

10 mg perampanel was taken and transferred to 10 ml volumetric flask separately and volume was made up with diluent (1000 µg/ml Perampanel). from the above solution 1 ml was taken into 10 ml volumetric flask and volume was made up by diluent (Stock solution-100µg/ml perampanel). Pipette out 1 ml from from stock solution into 10 ml volumetric flask kept under sunlight for 2 hours. After that make up with 10 ml of diluent. Filter the final solution with 0.45 µ PVDF Filter Similarly blank was prepared without adding sample. Blank and sample solution were injected in HPLC. Chromatograms are shown in Figure: 9

Procedure for Tablet

The average of 10 Tablet was determined and grounded in mortar.An accurately weigh amount of powder equivalent to 10 mg of Perampanel was taken. It was transferred to 10 ml of volumetric flask. Add 5 ml of diluents and sonicated for 5 minutes to ensure complete solubilization of drug. After sonication ,volume was made up to the mark with diluent(1000 µg/ml of Perampanel stock solution).Pipette out 1 ml from above solution and dilute up to 10 ml with diluent(100 µg/ml of Perampanel).Pipette out 1 ml of sample solution to 10 ml of volumetric flask .It was exposed to direct sunlight for 1 hour,make up the volume with diluent.After inject the photolytic degradation sample into HPLC, peak area and peak shape were observed . Figure No.

Method validation

System suitability was carried out by injecting standard solutions of API and Tablet 5 times into the chromatographic system. The system suitability parameters were then evaluated for tailing factor, retention time and theoretical plates of standard chromatograms.

Accuracy

The accuracy of the test method was demonstrated by % recovery across its range by making three different concentrations at 50%,100% and 150 % level using standard addition method .

Intraday precision(repeatability)

Intraday precision was performed by injecting standard preparations three times on the day by maintaining the optimized chromatographic conditions and calculate % RSD of retention time and peak areas for Perampanel.

Inter-day precision

Inter-day precision was performed by injecting standard preparations three times into chromatographic system on 2 different days by maintaining the optimized chromatographic conditions and calculate % RSD of retention time and peak areas for Perampanel.

Linearity

Transfer an accurately weighed quantity about 100 mg of Perampanel in 100 ml volumetric flask ,dissolve and dilute the volume with diluents. Prepare different linearity concentration solutions in the range of 50 – 150 µg/ml .

Robustness

The robustness was studied by analyzing the sample of Perampanel by deliberate variation in method parameters. The change in response of Perampanel was noted . Robustness of the method was studied by changing flow rate ± 0.2 ml, mobile phase composition and column temperature. The change in the response of Perampanel was noted and compare with the original one.

Limit of Detection and Limit of Quantification

LOD and LOQ were determined by using the formula based on the standard deviation of the response and the slope . LOD and LOQ were calculated by using equations:

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where , σ = Standard deviation of response

S = Slope of calibration curve

Result and Discussion

The detection wavelength was carried out in the UV range of 227 nm. Chromatographic separation was carried out using mobile phase composed

ACN: Buffer (0.5 ml Triethylamine in 500 ml water adjust pH 2.5 with OPA) (60:40v/v) on whatman ODS partisil column (250mm*4.6mm* 5µm)at a flow rate of 1.5 ml/min using PDA detector .

Force degradation in API

Acid Degradation

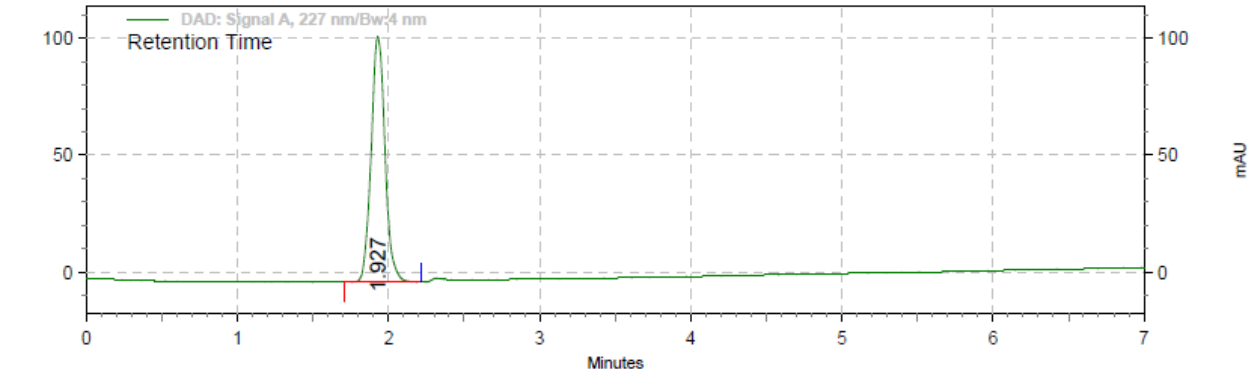

Figure 2 Chromatogram of Blank solution for Acid Degradation

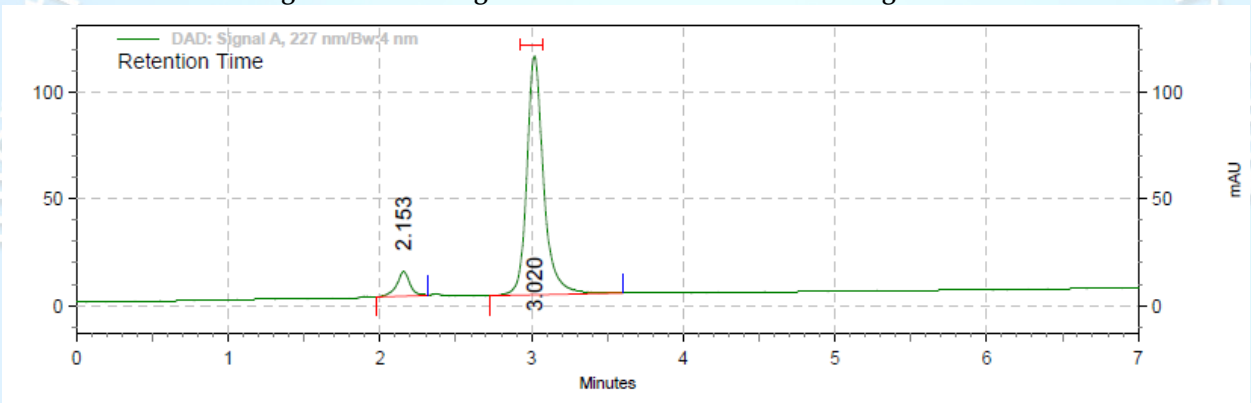

Figure 3 Chromatogram of Perampanel (100 µg/ml) for Acid Degradation

Base Degradation

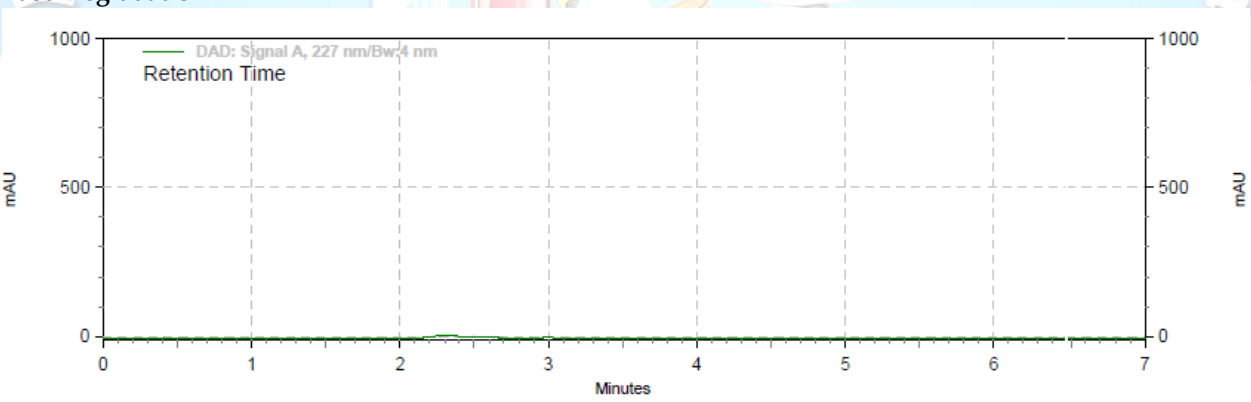

Figure:4 Chromatogram of Blank solution for Base Degradation

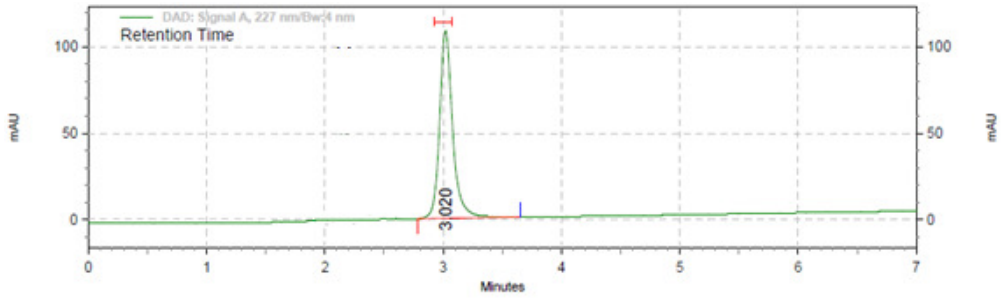

Figure:5 Chromatogram of Perampanel (100 µg/ml) for Base Degradation

Peroxide Degradation

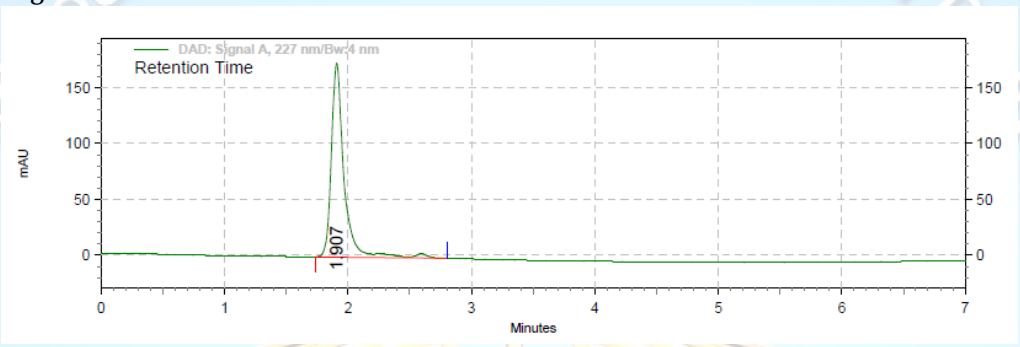

Figure: 6 Chromatogram of Blank solution for Peroxide Degradation

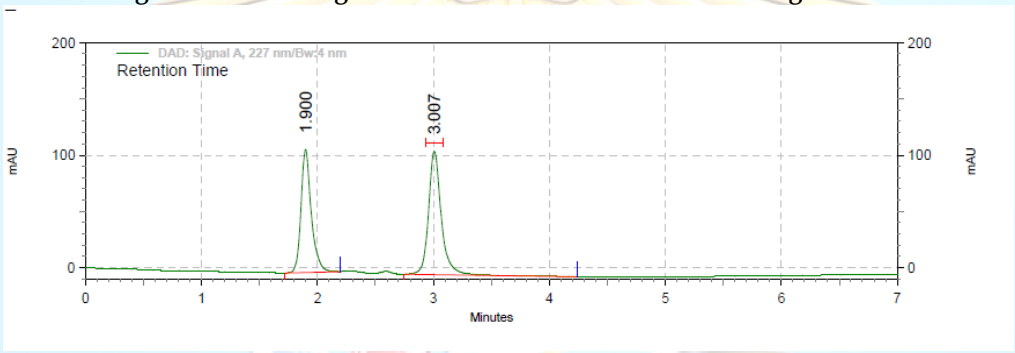

Figure: 7 Chromatogram of Perampanel (100 µg/ml) for Peroxide Degradation

Thermal Degradation

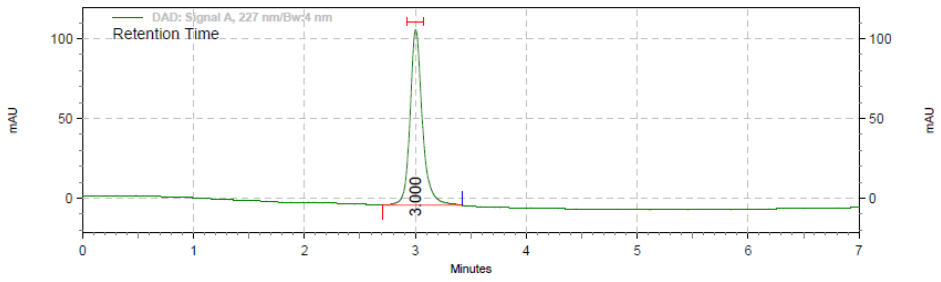

Figure: 8 Chromatogram of Perampanel (100 µg/ml) for Thermal Degradation

Photolytic Degradation

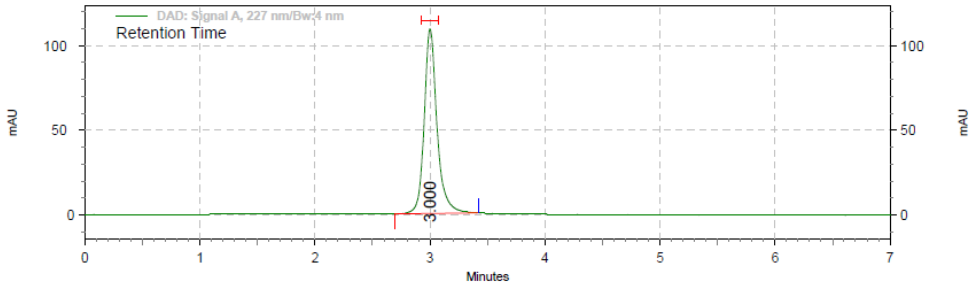

Figure: 9 Chromatogram of Perampanel (100 µg/ml) for photolytic Degradation

Force degradation (for Tablet)
Acid Degradation

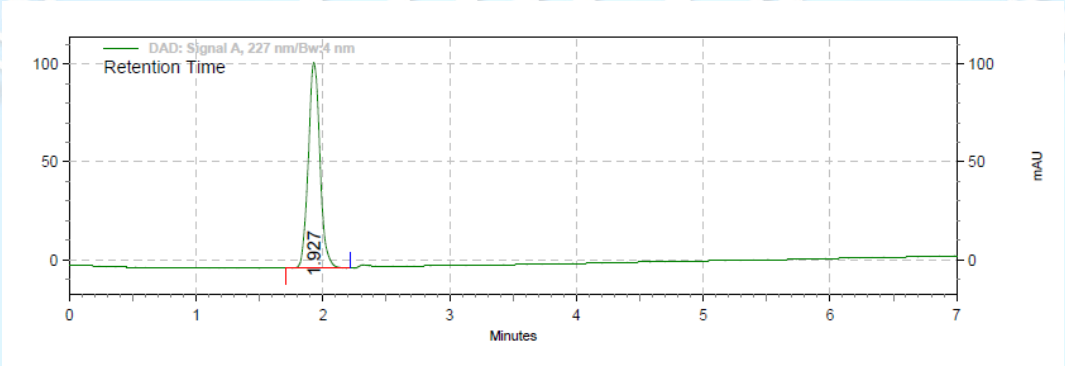

Figure 10 Chromatogram of Blank solution for Acid Degradation

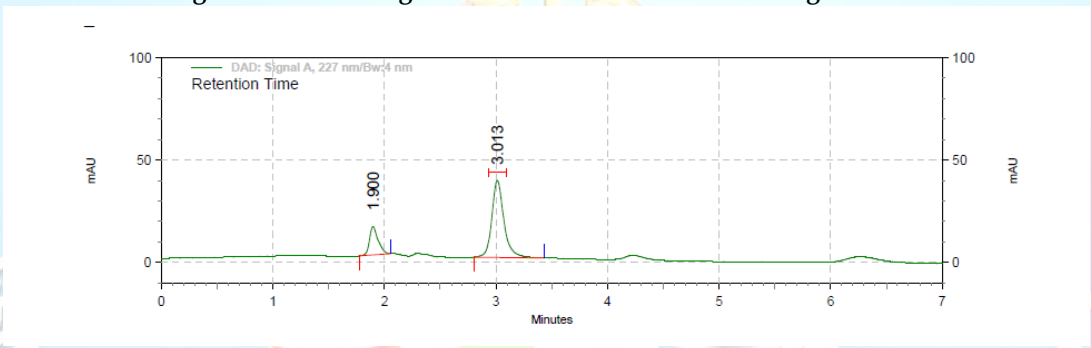

Figure 11 Chromatogram of Perampanel (100 µg/ml) for Acid Degradation

Base Degradation

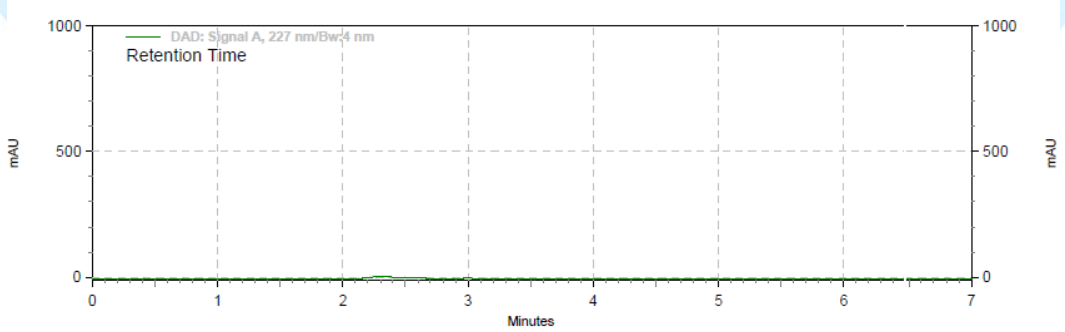

Figure 12 Chromatogram of Blank solution for Base Degradation

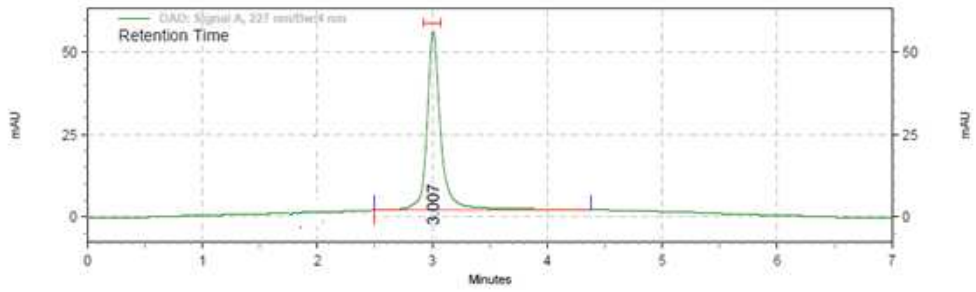

Figure13 Chromatogra of Perampanel(100 µg/ml) for Base Degradation

Peroxide degradation

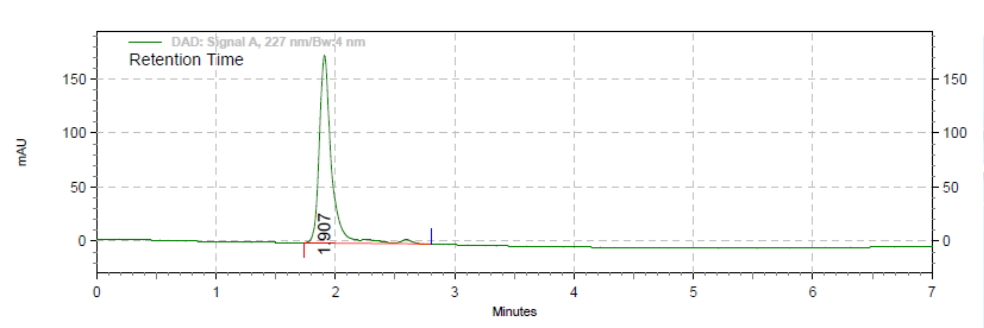

Figure 14 Chromatogram of Blank solution for peroxide Degradation

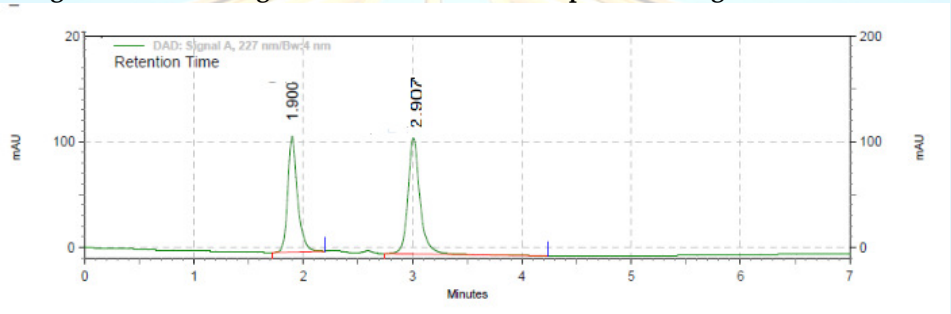

Figure15 Chromatogram of Perampanel (100 µg/ml) for Peroxide Degradation

Thermal degradation

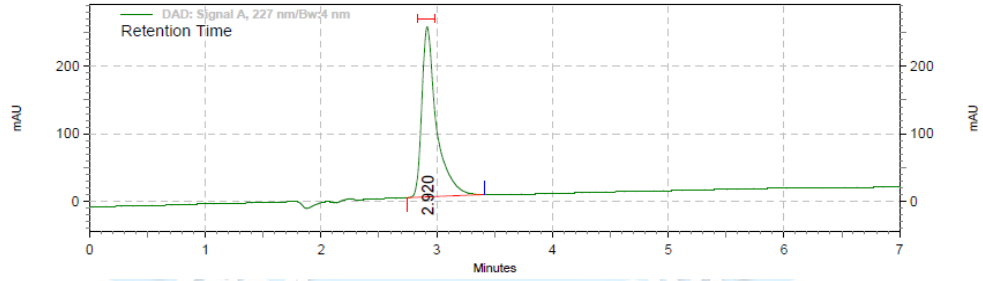

Figure 16 Chromatogram of Perampanel (100 µg/ml) for Thermal Degradation

Photolytic degradation

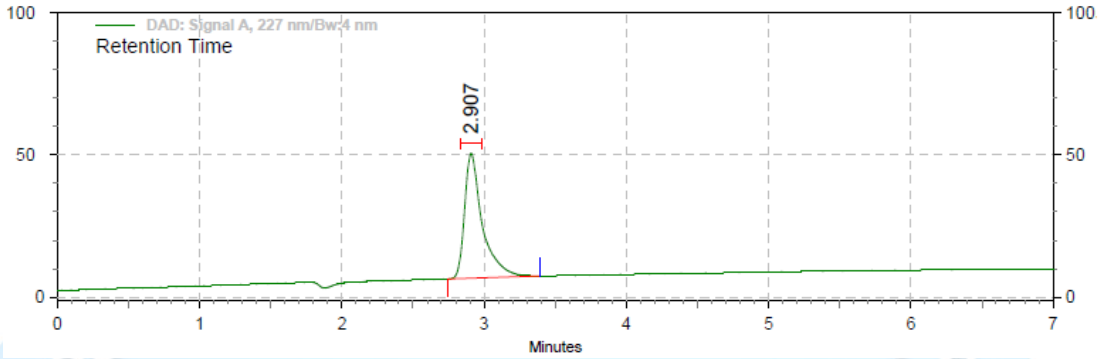

Figure 17 Chromatogram of Perampanel (100 µg/ml) for Photolytic Degradation

Table 6.5 Degradation Summary for API

Type	Solutions	Area	% Degradation
As Such	Perampanel	17701755	-
Acid 0.1 N HCL at 60ºc for 2 hrs	Perampanel	1811629	10.23%
Base 0.1 N NaOH at 60ºc for 2 hrs	Perampanel	1751791	9.89%
Peroxide 3% H_2O_2 at 60ºc for 2 hrs	Perampanel	1780296	10.05%
Thermal At 60°C for 2hrs	Perampanel	1795105	10.14%
Photolytic In sun light for 2 hr	Perampanel	2283080	12.89%

Table 6.6 Degradation Summary for Tablet

Type	Solutions	Area	% Degradation
As Such	Perampanel	8261178	-
Acid 0.1 N HCL at 60°C for 2 hrs	Perampanel	752540	9.10%
Base 0.1 N NaOH at 60°C for 2 hrs	Perampanel	961297	11.63%
Peroxide 3% H_2O_2 at 60ºc for 3 hrs	Perampanel	821987	9.95%
Thermal At 60°C for 2hrs	Perampanel	909555	11.01%
Photolytic In sun light for 2 hr	Perampanel	829495	10.29%

Validation Data

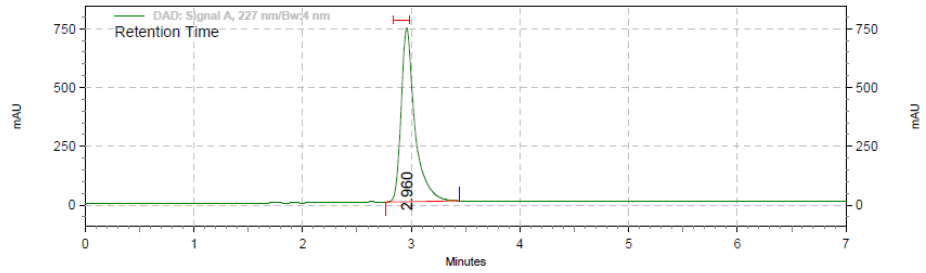

Figure 18: Chromatogram of API

Table 3 : System Suitability results (API)

Sr.no	System suitability parameters	Results
1	USP Tailing	1.6
2	Rt minutes	2.960
3	USP Plate count	3108
4	Area	13561098
5	Correlation coefficient	0.99
6	LOD	0.32 µg/ml
7	LOQ	0.98 µg/ml

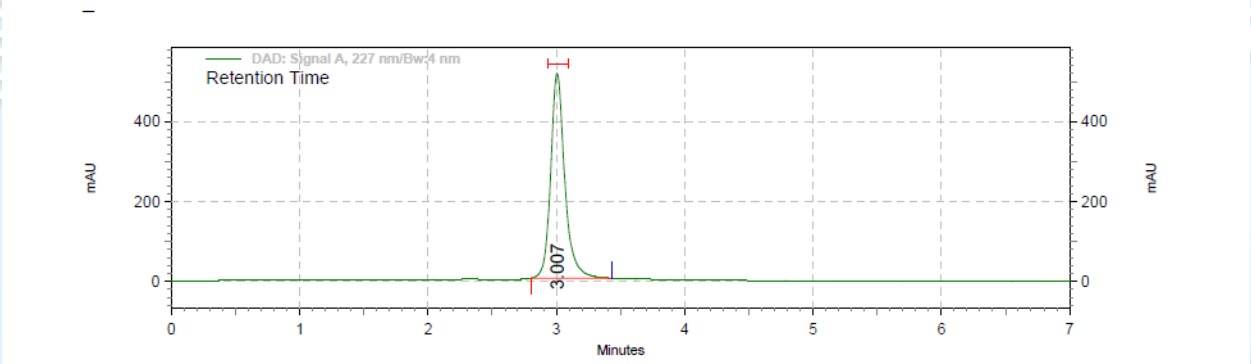

Figure 19:Chromatogram of Tablet

Table 4: System Suitability results (Tablet)

Sr.no	System suitability parameters	Results
1	USP Tailing	1.36
2	Rt minutes	3.007
3	USP Plate count	3938
4	Area	8261178

Table 5: Linearity data for Perampanel

Sr. no	Linearity level	Area
1	50	5045770
2	80	8045766
3	100	10619980
4	120	12881430
5	150	15344875

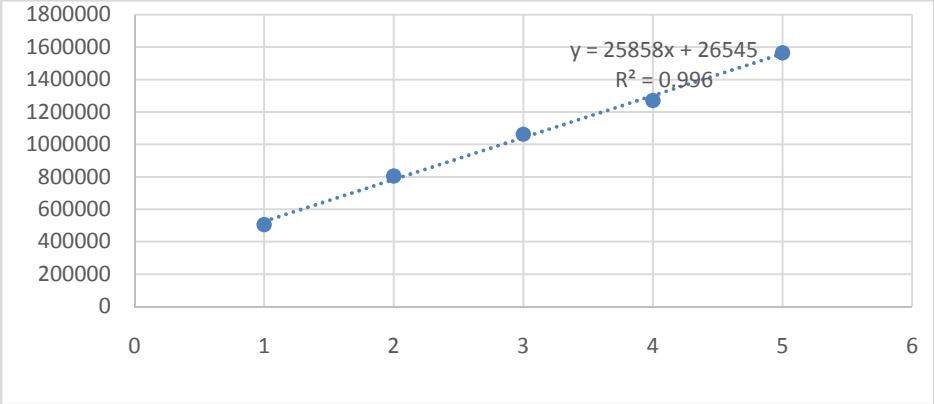

Figure 20 : Calibration plot of Perampanel

Table 6 :Accuracy for API and Tablet

Sample	Level (%)	Amount Recovered(µg/ml)	Mean % recovery ± SD
Perampanel	50%	97.57 ± 0.1	451.38 ± 0.14
API	100%	95.48 ± 0.1	599.74 ± 0.15
	150%	94.57 ± 0.22	149.39 ± 0.21
Perampanel	50 %	398.65±0.55	98.67±0.13
Tablet	100 %	501.98±0.871	99.40±0.17
	150 %	605.89±0.98	99.98±0.16

Table 7 : Precision Study Results

Concentration (500 µg/ml)	Repeatability	Intra-day study Day 1	Inter-day study Day 2	
Avg. Area	10578754	10667615	10736376	10958519
SD	25378.8696	115536.9	80158.9	87698.47
% RSD	0.24	1.08	0.74	0.80

Table 8: Robustness Study

Concentration (500 µg/ml)	Flow rate 1.3ml	1.7 ml	Temperature (ºC) 22º	32º	Mobile Phase +5 ml	-5 ml
Avg. Area	41002067	61245678	49802480	41690278	18539569	12201653
SD	98378.66	103609.9	74560.48	186902.5	1368000	124058.7
% RSD	0.20	0.10	0.10	0.40 0.70	1.01	

Conclusion:

In the present study we have developed a new , rapid RP-HPLC method and Validated for different parameters (System suitability, linearity, accuracy, precision, LOD,LOQ Robustness). By studying all these we have concluded that the method was linear, accurate, precise, robust and rapid for determination of Perampanel in API and Tablet dosage form.hence the method was successfully applied for the estimation of Perampanel in API and Tablet dosage form.

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