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"ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES OF ANTI-CONVULSANT DRUG BY RP-HPLC METHOD"

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Abstract:

The present work is to develop and validate a simple, cost effective, sensitive and fast RP-HPLC method for the analysis of Perampanel Oral Suspension. The proposed RP-HPLC method utilizes Hypersil ODS, 250mm × 4.6 mm, 5µ, isocratic run (using Buffer: Acetonitrile in ratio of 500: 500 v/v as mobile phase), effluent flow rate (1.0 ml/min), and UV detection at 230 nm for analysis of drug. The proposed method is fully validated and found to be linear over a workable drug concentration, accurate, precise and robust. This fast and inexpensive method and also this method is HPLC Equivalent to UPLC. It is suitable for research laboratories as well as for quality control analysis in pharmaceutical industries.

Key Words: Perampanel, RP-HPLC, Waters system, validation etc.

I. INTRODUCTION

CHROMATOGRAPHY

The term Chromatography (Greek: Khromatos – color and Graphos – written) means, "color writing". The beginning of Chromatography started with the work of botanist Michael Tswett in the year 1896. The term chromatography and its principles were first discovered in 1903 by Michael Tswett.

Chromatography is the most powerful and versatile analytical technique available to the modern chemist, its power arises from its capacity to determine quantitatively many individual components present in mixture in one, single analytical procedure. Its versatility comes from its capacity to handle a very wide variety of samples that may be gaseous, liquid or solid in nature. The sample can range in complexity from a single substance to multi component mixture containing widely differing chemical species. Another aspect of the versatility of the techniques is that the analysis can be carried out, at one extreme, on a very costly and complex instrument, and at the other, on simple, inexpensive thin layer plate.[1]

Classification of Chromatography:

A. Based on principle of separation[2]

1. Adsorption chromatography
2. Ion exchange chromatography
3. Ion pair chromatography
4. Size exclusion or Gel permeation chromatography
5. Affinity chromatography
6. Chiral phase chromatography.

B. Based on elution technique

1. Isocratic separation:

In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

2. Gradient separation:

In this technique, a mobile phase combination of lower polarity or strength is used followed by gradually increasing the polarity or elution strength.

C. Based on the scale of operation

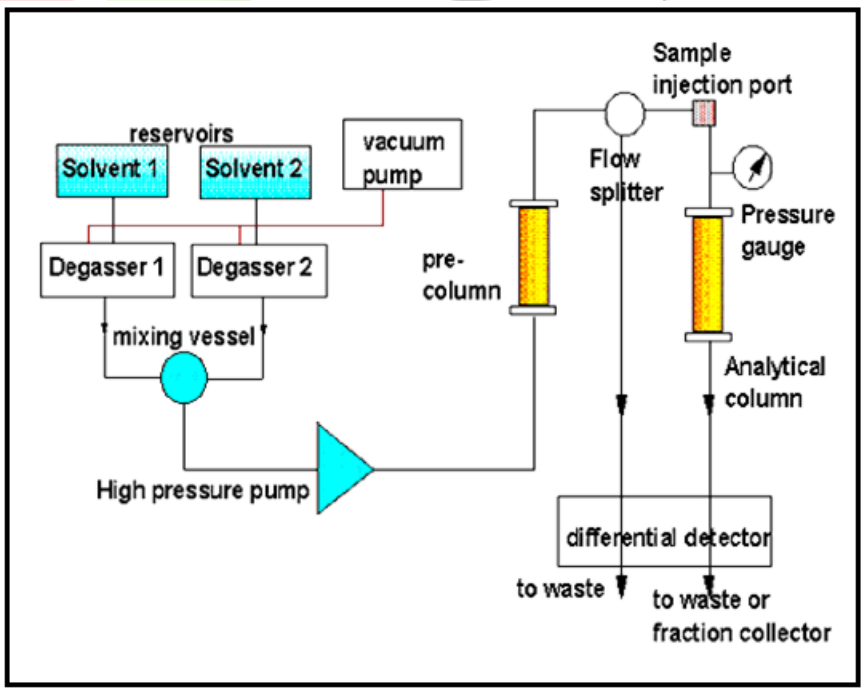
1. Analytical HPLC:

Where only analysis of the samples are done. Recovery of the samples for reusing is normally not done, since the sample used is very low.

2. Preparative HPLC:

Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused.[3]

Fig 1: Instrumentation of binary gradient HPLC system



INSTRUMENTATION:

1) HPLC Gradient mixers:

HPLC gradient mixers must provide a very precise control of solvent composition to maintain a reproducible gradient profile. This can be complicated in HPLC due to the small elution volumes required by many systems. It is much more difficult to produce a constant gradient when mixing small volumes than mixing large volumes. For low pressure systems this requires great precision in the operation of the miniature mixing valves used and low dispersion flows throughout the mixer. For multi-pump high pressure systems it requires a very precise control of the flow rate while making very small changes of the flow rate.

2) HPLC pumps:

Because of the small particles used in modern HPLC column packing, modern LC pumps need to operate reliably and precisely at pressures of 10,000 p.s.i. or at least 6,000 p.s.i. To operate at these pressures and remain sensibly inert to the wide variety of solvents used, HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats. For analytical proposes, HPLC pumps should have flow rates that range from 0 to 10 ml/min., but for preparative HPLC, flow rates in excess of 100 ml/min may be required. It is extremely difficult to provide a very constant flow rate at very low flow rate.

Pump pressure: - Pumps vary in pressure capacity, but their performance is measured on their ability to yield a consistent and reproducible flow rate. Pressure may reach as high as 6000 lbf/in² (~40 MPa, or about 400 atmospheres). Modern HPLC systems have been improved to work at much higher pressures, and therefore be able to use much smaller particle sizes in the columns (< 2 micrometers). These “Ultra High Performance Liquid Chromatography” systems or UHPLCs can work at up to 15,000 lbf/in² (~100 Mpa or about 1000 atmospheres). (Note that the term “UPLC”, sometimes found instead is a trademark of Waters Corporation and not the name for the technique in general).

3) HPLC columns:

Column is often referred to as the heart of the HPLC separation process. HPLC columns are packed with very fine particles (usually a few microns in diameter) to attain the low dispersion that give the high plate counts expected of modern HPLC. LC columns, in general, achieve their separation by exploiting the different intermolecular forces between the solute and the stationary phase and those between the solute and the mobile phase. The column will retain those substances that interact more strongly with the stationary phase than those that interact more strongly with the mobile phase.

C18 and C8 HPLC Columns

1. Classic reversed-phases for all general-purpose applications.
2. Excellent peak shape and efficiency compared to competitive columns.
3. Classic reversed-phase retention and selectivity.
4. C18 is generally more retentive than the C8.

4) HPLC detectors:

The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. HPLC detectors use the same detection principles with extra care being given to the small solute elution volumes that result from the combination of high column efficiencies with small volumes. In order to give an accurate chromatographic profile the detector sampling (cell) volume must be a small fraction of the solute elution volume. If the detector volume were larger than the elution volume then peaks that appeared are with flat tops as the whole peak would be resident in the detector at the same time. This means that as column volumes decrease and system efficiencies increase the volume of the detector cell must also decrease. This is of course at odds for the requirement of detector to maintain high sensitivity, as this is usually dependent on having a larger cell volume. Again, this requires careful designing of modern detectors.

1.2 ANALYTICAL METHOD VALIDATION

Validation is one element of 'quality assurance' associated with a particular process that differs so widely, there is no universal approach to validation and regulatory bodies such as FDA and EC have developed general non-mandatory guidelines. The word validation simply means "Assessment of validity" or "Action of proving" the effectiveness[19].

According to USFDA:

"Establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes.20"

According to WHO:

Defines the validation in the same way but elaborates considerably on the concept "Validation studies are essential part of good manufacturing practice and should be conducted in according with predefined protocols. A Written report summarizing results and conclusions should be recorded, prepared and stored. Process and procedures should be established based upon the validation study and undergo periodic revalidation to ensure that they remain capable of achieve the intended results.

Types of validation:

- Prospective validation
- Retrospective validation
- Concurrent validation
- Process validation

ANALYTICAL METHOD VALIDATION:

Analytical method development and validation play important roles in the discovery, development, and manufacture pharmaceuticals. The official test methods that result from these processes are used by Quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

The objective of an analytical method validation is to demonstrate for it is suitable for its intended purpose.[21]

Purpose of validation:

Setting the standards of evaluation procedures for checking compliance and taking remedial action.

As quality of the product cannot always be assured by routine quality control because of testing of statistically insignificant number of samples.

Retrospective validation is useful for trend comparison of results compliance to CGMP/CGLP.

International Pharmacopoeial harmonization particularly in respect of impurities determination and their limits.

Depending on the use of the assay, different parameters will have to be measured during the assay validation. ICH and several regulatory bodies and Pharmacopoeia have published information on the validation of analytical procedures.

Validation of analytical method is the process that establishes, by laboratory studies, that the performance characteristics of the method meet the requirements for the indented analytical applications. Typical analytical performance characteristics that should be considered in the validation of analytical methods are listed below:

a. Linearity
b. Range
c. Accuracy
d. Precision
i) Repeatability
ii) Intermediate precision
iii) Reproducibility
e. Specificity
f. Robustness
g. Detection Limit
h. Quantitation Limit

Table 1: ICH Guidelines for analytical method validation.

Type of analytical procedure	**Identification**	**Impurity testing**		**Assay**
		Quantitative	**Limit test**	
Accuracy	No	Yes	No	Yes
Precision	Yes	Yes	Yes	Yes
Repeatability	No	Yes	No	Yes
Interm. Prec.	No	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes
LOD	No	No	Yes	No
LOQ	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

1) Specificity

Specificity is the ability to assess unequivocally the analyst in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, matrix etc.

2) Accuracy

The accuracy of an analytical procedure expresses the closeness of an agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed as trueness.

3) Precision

The precision of analytical procedure expresses closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. It may be considered at three levels: It is expressed as standard deviation or coefficient of variation.

- **Repeatability**

  Repeatability expresses the precision under the same operating conditions over a small interval of time. Repeatability is also termed intra-assay precision.

- **Intermediate precision**

  Intermediate precision expresses within-laboratories variations: different days, different analyst, different equipment etc.

- **Reproducibility**

  Reproducibility expresses the precision between laboratories.

4) Detection of limit

The detection limit of an individual analytical procedure is the lowest amount of an analyte in a sample, which can be detected but not necessarily quantities as an exact value.

- **Based on visual evaluation**

  The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

- **Based on signal to noise**

  A signal to noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

- Based on standard deviation of the response and slope

  The detection limit (DL) may be expressed as

  $DL = 3.3\ \sigma/S$

  Where;

  σ = the standard deviation of the response.

  S = slope of calibration curve of analyte.

5) Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Some approaches listed below may be acceptable.

• Based on visual evaluation

The quantitation limit is determined by the analysis of samples with known concentrations of analyte.

• Based on signal to noise ratio

Signal to noise ratio between 10:1 is generally considered.

• Based on standard deviation of the response and slope

The quantitation limit (QL) may be expressed as

$QL = 10\ \sigma/S$

Where;

σ = the standard deviation of the response.

S = slope of calibration curve of analyte.

6) Linearity

The linearity of an analytical procedure is the ability to obtain test results, which are directly proportional to the concentration of an analyte in the sample.

7) Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure is of precision, accuracy and linearity.

8) Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

9) Forced degradation:

Forced degradation studies provide the approach to analyse the stability of drug samples in pharmaceutical industries. Drug product safety and efficacy is affected by the chemical stability of the molecule. Stability of molecule information provides the data for selecting proper formulation, package, proper storage conditions and shelf life. These data also play a significant role which is required in the regulatory documentation. Before filling registration dossier it is obligatory to execute stability studies of new drug molecules. International Conference on Harmonisation (ICH) guidelines, make it essential to organize the forced degradation studies and it is evidently mandated to perform forced degradation of new drug products.

INTRODUCTION OF EPILEPSY

Epilepsy is a neurological disorder characterized by recurrent, unprovoked seizures. A seizure is a sudden, uncontrolled electrical disturbance in the brain that can cause changes in behavior, movements, feelings, or levels of consciousness. Epilepsy can affect people of all ages, and it can have various causes and manifestations.

Here are key points about epilepsy:

Seizures:

Seizures are the hallmark of epilepsy. They can vary widely in their presentation, ranging from brief lapses of attention or muscle jerks to severe and prolonged convulsions. The type and intensity of seizures can differ from person to person.

Causes:

Epilepsy can have various causes, including genetic factors, brain injuries, infections, developmental disorders, or structural abnormalities in the brain. In many cases, the cause may not be identified.

Diagnosis:

Diagnosis is typically based on a detailed medical history, including a description of the seizures and their frequency, along with neurological exams and various diagnostic tests. Electroencephalogram (EEG) is commonly used to record the electrical activity of the brain during seizures.

Treatment:

Treatment of epilepsy often involves antiepileptic medications to control or reduce the frequency and severity of seizures. The choice of medication depends on factors such as the type of seizures, the individual's age, overall health, and potential side effects.

Surgery:

In some cases, surgery may be considered, especially if seizures are not controlled with medications. Surgical options may include removing or disconnecting the part of the brain responsible for the seizures.

Lifestyle Management:

Lifestyle modifications can also play a role in managing epilepsy. This may include getting enough sleep, avoiding triggers, and adhering to the prescribed medication regimen.

Prognosis:

Many people with epilepsy can effectively manage their condition with appropriate treatment. Some individuals may outgrow epilepsy, while others may require lifelong management.

II. RESEARCH METHODOLOGY

Material and equipment

A. Chemicals

Table 2. List of chemicals used in the study

Sr .no	List of chemicals	Make	Grade
1	Perampanel Standard	R&D Lab	NA
2	Potassium dihydrogen phosphate	Merck	Emparta
3	Ortho-phosphoric acid	Merck	Emparta
4	Acetonitrile	Honey well	HPLC
5	Methanol	J. T. Baker	HPLC
6	Sodium hydroxide	Merck	AR
7	Tetrahydrofuran	Merck	HPLC
8	Water	Milli Q	HPLC

B. Instruments

Table3. list of the Instruments utilized in the research

Sr. No	**Equipment's**	**Make**	**Model**
1	HPLC	Waters	Alliance 2489
2	Analytical Balance	Mettler Toledo	XS 64
4	All glassware's	Borosil glass works	NA
5	Ultrasonicator	Labman Scientific	LMUC3
6	PH Meter	Mettler Toledo	FP20

Table 4. Chromatographic Conditions

HPLC Column	Hypersil ODS, 250mm × 4.6 mm, 5µ
Flow rate	1.0 ml/min
Column oven temperature	45°C
Sample Compartment temperature	5°C
Injection volume	10µL
Wavelength	230 nm
Run time	15 min
Retention Time	About 9.0 min (For information only)

A) METHOD DEVELOPMENT:

Optimization of chromatographic parameters:

The process of identifying a set of conditions that adequately analyse the quantity of the analyte with appropriate accuracy, precision, sensitivity, specificity, cost, ease, and speed of analysis is known as optimization in HPLC.

Selection of Detection Wavelength:

In case of degradation study, wavelength detection by UV method must be accurately chosen because in presence of active drug and its impurity, the absorption spectra shifted to shorter wavelength compare to parent compound.

In present study solution of Perampanel was prepared in diluent at concentration 10µg/ml and UV visible spectra were acquired. The optimal wavelength selected for detection was 230 nm. The first UV absorption maxima of Perampanel, was at approximately 230 nm, so detection at 230nm was selected for HPLC method-development on basis of appropriate intensity of Perampanel.

Preparation of diluted ortho-phosphoric solution:

Transferred 10 ml of ortho phosphoric acid in 100ml of volumetric flask, added 70ml of water , mixed well and make volume with water.

Preparation of sodium hydroxide solution:

Weighed and transferred about 2.0 gm of sodium hydroxide pellets into 20ml of wide mouth volumetric flask, added 15ml of water, sonicated to dissolve, mixed well and made up the volume with water.

Mobile Phase:

Preparation of Phosphate Buffer (pH=4.5):

Dissolved 2.72 g of monobasic potassium phosphate in 1000 ml of water and mixed well. Adjusted to pH of 4.50±0.05 with diluted sodium hydroxide solution or diluted Ortho-phosphoric acid solution and mixed well. And Filter the buffer through 0.45µ nylon membrane filter.

Preparation of Mobile phase:

Prepared a mixture of Buffer: Acetonitrile in ratio of 500: 500 v/v, sonicated for 10 minutes to degas.

Preparation of Diluent:

Prepared a mixture of Water: Methanol in ratio of 300:700 v/v, sonicated for 10 minutes to degas. Allow it cool at room temperature before use.

Preparation of Standard solution:

a. Standard Stock Solution-I (250mcg/mL):

Weighed and transfer about 50mg of Perampanel working standard/reference standard to 200mL dry volumetric flask, added 160mL of diluent and sonicated till dissolve with intermittent shaking, cool to room temperature and diluted up to mark with diluent and Mixed well.

b. Standard Solution (50mcg/mL)

Further pipette out and transferred 10ml above standard stock solution to 50ml of volumetric flask and diluted up to the mark with diluent and Mixed well.

Note: Standard solution is found stable for 36 hours at 5°C.

Preparation of Test solution (50mcg/mL)

Taken perampanel oral suspension bottle and shake well for about 20 seconds and then immediately transferred 70 ml of suspension in a 100 ml of clean and dry beaker. Used a separate 50-mL syringe for sample and withdraw more than 50.0mL of suspension. Removed air bubbles from the syringe. Adjusted the volume to 50.0mL. Weigh the syringe (T1) in mg and transferred 50.0mL of the sample (equivalent to about 25mg of Perampanel) to a 500-mL volumetric flask. Added 250 mL of methanol sonicated for 15 min with intermittent shaking, put suitable size of magnetic needle into the flask and stir at about 700rpm for 30min. on magnetic stirrer, then rinsed the magnetic needle with 20ml of water while removing from the flask and again added 80ml of water, sonicate for 30min with intermittent shaking. Then added 50ml methanol and allow the solution to attain room temperature and dilute up to the mark with methanol, Mix well. Centrifuge this solution 4000rpm for 5 min. Then filtered solution slowly dropwise through 0.45µm PVDF filter (Millipore millex HV- Hydrophilic) discarding first 1mL of filtrate. Use the filtrate.

Weigh the empty syringe (T2) in mg after transferring the sample and determine the weight of the sample (WT) in mg.

Note: Sample solution is found stable for 15 hours at 5°C.

Calculations:

$$\%\ \text{W/W of assay of Perampanel} = \frac{AT}{AS} \times \frac{WS}{200} \times \frac{10}{50} \times \frac{500}{WT} \times \frac{V}{L} \times \frac{P}{100} \times \frac{D}{1} \times 100$$

Weight of sample taken for test preparation in mg (WT)

Weight of syringe +sample = T1

Weight of empty syringe after transferring sample + T2

Weight of sample (WT) = (T1-T2)

Where,

AT = Average area count of Perampanel in the chromatogram of the Test solution.

AS = Average area count of Perampanel in the chromatogram of the Standard solution.

WS = Weight of perampanel working standard taken in mg.

WT = Weight of Oral suspension taken for test preparation in mg.

V = 1 ml (Volume of Suspension claimed in ml).

L = Labelled amount 0.5 mg of Perampanel in 1 ml of oral suspension.

P = % Potency of Perampanel WS on as is basis.

D = Density of Suspension i.e. Wt./ml in mg/ml.

Table No. 5 Acceptance criteria for evaluation of experimental result

Sr. No.	Parameter	Acceptance limit
1	Number of theoretical plates or Efficiency (N)	NLT 2000
2	Tailing factor or Asymmetry(T)	NMT 2.0
3	Relative standard deviation (RSD)	NMT2.0

B) METHOD VALIDATION:

1) Precision:

i) System precision:

Prepared standard solution as per test method and injected for 6 times into HPLC system. The mean, SD and %RSD for peak areas of Perampanel were calculated. **Note: Weight of standard is used 50.01 mg.**

ii) Method Precision:

Prepared six sample solutions of 0.5mg/ml strength and analyzed as per the test method. Calculated the %RSD for assay of six preparations. The %assay for Perampanel in six samples was calculated. **Note: Weight per ml of sample is 1065.34**

iii) Intermediate Precision (Ruggedness):

Ruggedness of the method was verified by analysing the six samples of 0.5mg/ml strength of same batch which was used for method precision as per test method by using different column, different system and on different day. The % assay of Perampanel was determined. Calculated %RSD for % assay of Perampanel six samples and overall %RSD for ruggedness results with the method precision results.

2) Specificity:

Blank (diluent), placebo (triplicate preparation), standard, individual all specified identified products, Perampanel standard were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of Perampanel peak. Peak purity data reveals that Perampanel peak was homogeneous and there were no co-eluting peaks at the retention time of Perampanel peak.

Calculated the % assay difference between the mean of method precision sample of method precision results.

3) Linearity:

The linearity of Perampanel was performed using Perampanel standard solution the range of 20% to 200% of finished product test concentration of 0.5 mg/ml strength (finished product test concentration is 50 mcg/mL for Perampanel) i.e. 9.92 mcg/mL to 99.22 mcg/mL for perampanel. A graph was plotted with concentration (in mcg/mL) on x-axis and peak areas of perampanel on y-axis. Slope, y-intercept, correlation coefficient (R-values) and residual sum of squares (RSS) were determined.

Table no.6 Linearity study procedure

Sr. No.	Spike level in %	Concentration of Perampanel in mcg/ml	Vol. added from Linearity std. stock (ml)	Diluted to (ml)
1	20	9.92	1	25
2	50	24.80	2.5	25
3	80	39.69	4	25
4	100	49.61	5	25
5	150	74.41	7.5	25
6	200	99.22	10	25

4) Accuracy:

Known amount of Perampanel standard was spiked with placebo in triplicate at 50%, 100% and 200% for finished product test concentration of 0.5 mg/ml strength (finished product test concentration is 50mcg/ml for Perampanel). The amount of Perampanel was quantified as per the test method. The % recovery was calculated from the amount found and actual amount added.

- **Preparation of standard stock solution for accuracy:**

Weighed 312.65 mg of perampanel API in 100 ml volumetric flask dissolved with diluent and made up the volume with diluent.

Table no.7 Accuracy study procedure

Sr. No.	Level no/Spike level in %	Volume taken of standard stock solution in ml	Diluted to volume	Conc. (µg/ml)
1	Level-1 (50%)	4	500	25.012
2	Level-2 (100%)	8		50.024
3	Level-3 (200%)	16		100.048

5) Range:

Range inferred from the data of linearity, accuracy and precision experiments.

6) Robustness:

Robustness of the method was verified by deliberately varying the following instrumental conditions.

a. By changing the mobile phase flow rate by ± 10%.

b. By changing the column oven temperature by +5°C.

c. By changing the mobile phase buffer pH by ± 0.2 units.

d. Changing the organic content in mobile phase by ± 2%.

System suitability was evaluated in each condition and sample was analyzed in triplicate. The results were compared with the method precision data.

7) Forced degradation study by HPLC method.

Forced degradation study was carried out on Perampanel oral suspension 0.5mg/mL strength, by treating the blank, placebo and sample under following conditions. Assay forced degradation study were performed along with related substances forced degradation study. Assay samples (Untreated and treated) were prepared by diluting organic impurities sample stock solution in assay diluent. Solid state sample was used for UV-Visible light, Thermal and Humidity stress conditions. Acid, Base, Peroxide and Hydrolysis treatment was given after extraction of sample.

a) Degradation by Hydrochloric acid (Acid treated sample):

Sample, blank and placebo were treated with 2mL of IN Hydrochloric acid and kept on bench top for about 88 hours and neutralized with 2mL of IN Sodium hydroxide solution. Treated Sample, blank and placebo solutions were analyzed as per the test method.

b) Degradation by Sodium hydroxide: (Alkali treated sample):

Sample, blank and placebo were treated with 2mL of IN Sodium hydroxide and kept on bench top for about 88 hours and neutralized with 2mL of IN Hydrochloric acid solution. Treated Sample, blank and placebo solutions were analyzed as per the test method.

c) Degradation by Hydrogen peroxide: (Peroxide treated sample):

Blank, Sample and Placebo treated with 2mL of 30% solution of Hydrogen peroxide and kept on bench top for about 88 hours. Treated blank, sample and placebo solutions were analyzed as per the test method.

d) Degradation by UV-Visible light: (UV-Visible light treated sample):

Sample and placebo were exposed to UV light of about 200 watt hours/square meter and to visible light for about 1.2 million lux hours in photo stability chamber. Treated Sample and placebo solutions were analyzed as per the test method.

e) Degradation by Humidity:

Sample and Placebo were exposed with 25°C temperature and 90% relative humidity for about 96 hours. Treated Sample and placebo solution were analysed as per the test method.

f) Degradation by Heat: (Thermal treated sample):

Sample and placebo were kept in oven at 80°C for about 96 hours. Treated Sample and placebo solutions were analyzed as per the test method.

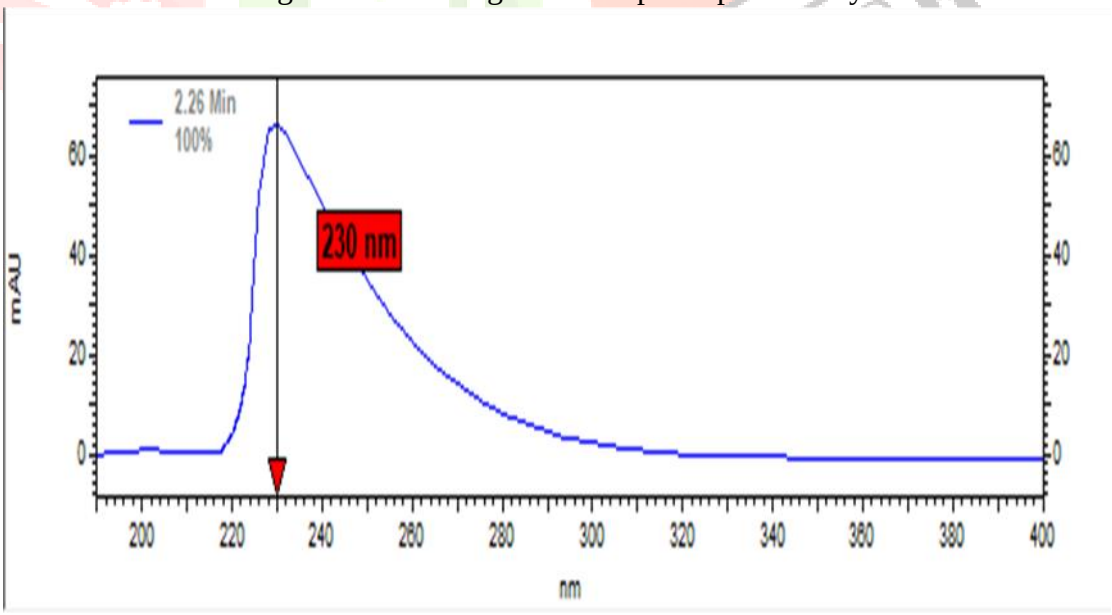
g) Degradation by Hydrolysis:

Blank, Sample and Placebo were treated with 2mL of water and kept on bench top for about 88 hours. Treated Sample, blank and placebo solution were analyzed as per the test method.

III. RESULT AND DISSCUSION

Selection of wavelength by UV spectrophotometry:

Fig.No 2. wavelength of UV spectrophotometry



Mobile phase Buffer: ACN (50:50) was found to be satisfactory retention time and symmetry factor for further resolution from degradation products.

Fig. No. 4 Chromatogram of perampanel WS

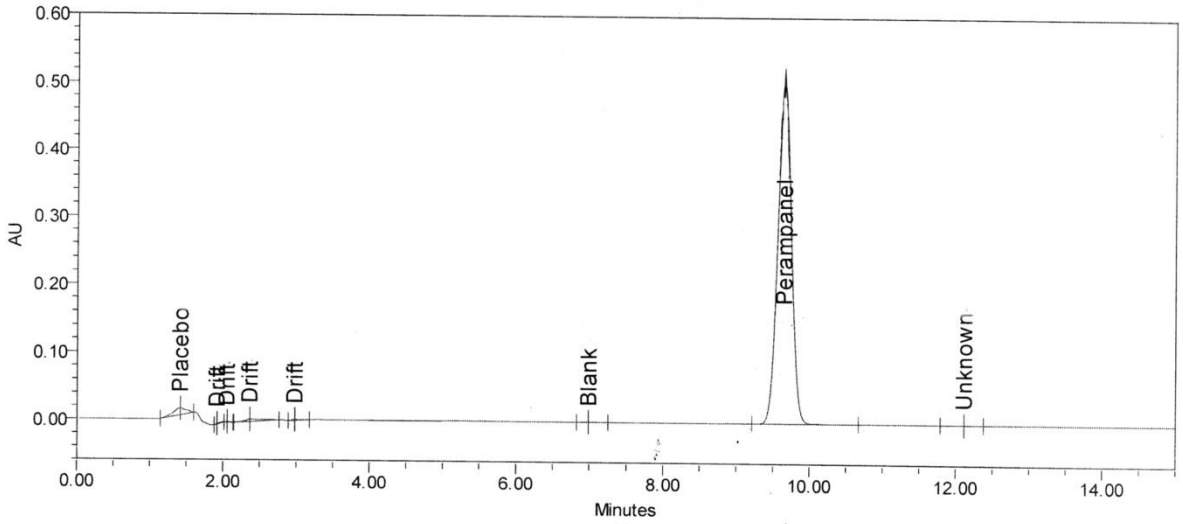


Fig. No. 5 Chromatogram of perampanel Oral Suspension sample

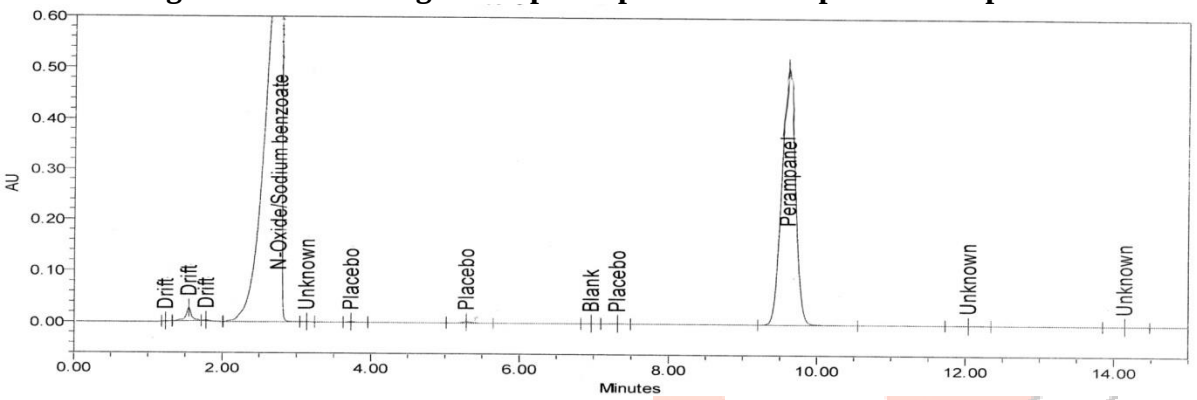


Fig. No. 6 Chromatogram of blank.

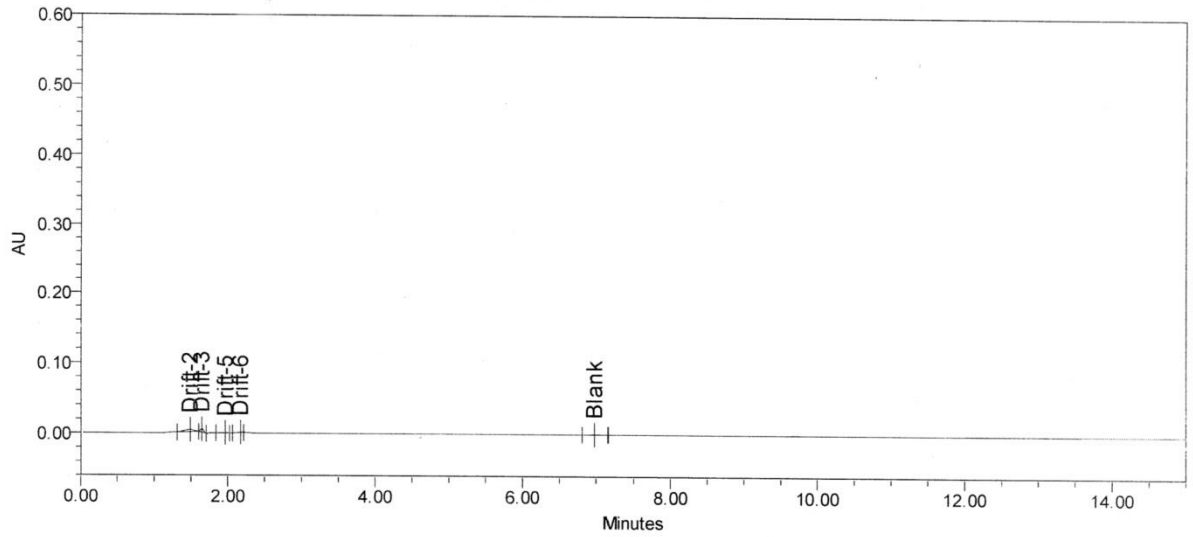


Fig. No. 7 Chromatogram of placebo

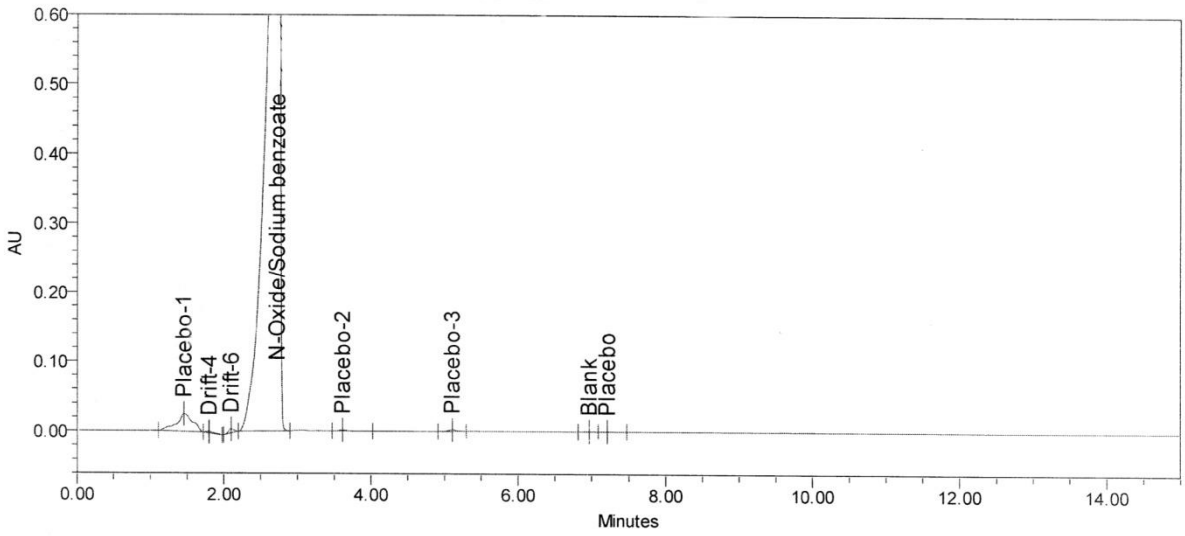


Table no. 8 Results of system precision

Sr.No.	Peak area of Perampanel Standard	Retention Time (min)
1	6480646	9.298
2	6477355	9.301
3	6482695	9.301
4	6471920	9.295
5	6464001	9.280
6	6468661	9.318
Mean	6474213	
SD	7260.4	
% RSD	0.11	

Table no. 9 Results of Method precision

Sample No.	Area and RT (Min)					% Assay
	Injection-1	RT(Min)	Injection-2	RT(Min)	Average Area	
1	6613444	9.300	6619307	9.301	6616376	102.84
2	6714127	9.298	6724774	9.300	6719451	104.38
3	6658345	9.299	6668541	9.299	6663443	103.75
4	6634775	9.301	6639802	9.310	6637289	102.76
5	6665384	9.310	6655968	9.310	6660676	103.64
6	6622833	9.290	6630515	9.299	6626674	103.29
					Average	103.44
					SD	0.611
					%RSD	0.59

Table no. 10 Results of intermediate precision:

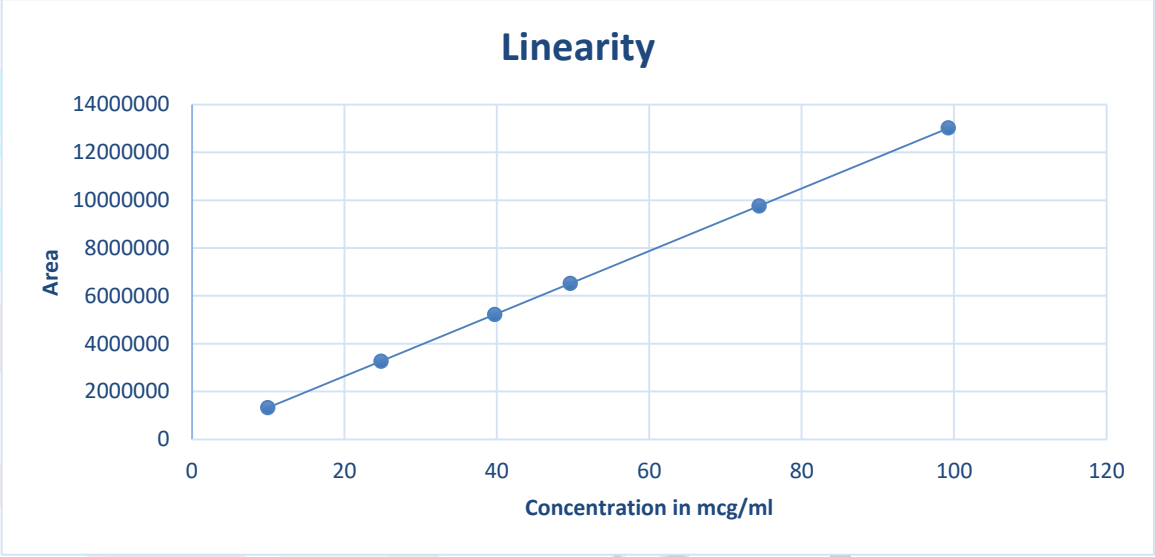
Sample No.	Area and RT (Min)					% Assay
	Injection-1	RT(Min)	Injection-2	RT(Min)	Average Area	
1	6711303	9.301	6713067	9.300	6712185	102.77
2	6754761	9.300	6755410	9.298	6755086	102.40
3	6819330	9.299	6815732	9.299	6817531	103.56
4	6794611	9.310	6797059	9.301	6795835	102.97
5	6730583	9.310	6726998	9.310	6728791	102.94
6	6669258	9.299	6684020	9.290	6676639	101.99
					Average	102.77
					SD	0.536
					%RSD	0.52

The developed method was found to be precise as the RSD value for method precision and intermediate precision studies were <2.0%, respectively as recommended by ICH guideline. Separation of the drug and different degradation products in stressed sample was found to be similar when analysis was performed on different chromatographic system on different days.

Table no. 11 : Data of linearity

Level	Spike level in %	Area Inj-1	Area Inj-2	Average area
1	20	1324999	1324164	1324582
2	50	3258738	3263657	3261198
3	80	5208311	5216899	5212605
4	100	6506727	6514364	6510546
5	150	9750716	9761189	9755953
6	200	13016021	13028900	13022461
		Slope		130999
		Intercept		19925
		Correlation coefficient (cc)		1.00000

Fig. No. 8 Linearity plot for perampanel



The detector response of perampanel is directly proportional to test concentration ranging from 20% to 200%.

Table no. 12 : Area of all accuracy levels

Level No.	Area of Injection-1	RT (min)	Area of injection-2	RT (min)	Average area
50%_Set-1	3311892	9.299	3316388	9.300	3314140
50%_Set-2	3327353	9.232	3330722	9.299	3329038
50%_Set-3	3297682	9.288	3297379	9.298	3297531
100%_Set-1	6624754	9.301	6618427	9.291	6621591
100%_Set-2	6606872	9.301	6613318	9.299	6610095
100%_Set-3	6637357	9.291	6638136	9.232	6637747
200%_Set-1	12997166	9.298	13012163	9.288	13004665

200%_Set-2	13041491	9.299	13041408	9.301	13041450
200%_Set-3	13047814	9.300	13063029	9.301	13055422

Table No. 13 Results of Accuracy Study

Spike level	Reps	Amount added in mcg/ml	Amount Recovered in mcg/ml	% Recovery	Mean	SD	%RSD
50%	Set 1	25.012	25.369	101.43	101.41	0.480	0.47
	Set 2		25.483	101.88			
	Set 3		25.242	100.92			
100%	Set 1	50.24	50.687	101.33	101.35	0.211	0.21
	Set 2		50.599	101.15			
	Set 3		50.810	101.57			
200%	Set 1	100.048	99.548	99.50	99.72	0.201	0.20
	Set 2		99.829	99.78			
	Set 3		99.936	99.89			
Over all mean				100.83			
Over all SD				0.875			
Over all % RSD				0.87			

The analytical method meets the pre-established acceptance criteria for accuracy study as per protocol. Hence the method is accurate for finished product bulk homogeneity, uniformity of dosage units, dose uniformity and assay of Perampanel in Perampanel oral suspension 0.5mg/ml.

E) RANGE

Range inferred from the data of linearity, accuracy and precision experiments.

a) The method was found to be linear for Perampanel in the range of 20% to 200% of finished product test concentration i.e. 9.92 mcg/mL to 99.22 mcg/mL (finished product test concentration is 50 mcg/ml for Perampanel).

b) The method was found to be accurate in the range of 50% to 200% of finished product test concentration for 0.5mg/ml strength (finished product test concentration is 50 mcg/ml for Perampanel).

Table No. 14 The results were compared with the method precision data and robustness data.

Sr.No.	I	II	III	IV	V	VI	VII	VIII	IX
1	102.84	102.89	102.64	102.61	102.52	102.47	102.43	102.60	102.57
2	104.38	101.89	101.65	102.01	101.76	103.00	102.99	102.01	101.88
3	103.75	102.15	101.86	101.5	101.41	103.57	103.62	101.60	101.64
4	102.76	-	-	-	-	-	-	-	-
5	103.64	-	-	-	-	-	-	-	-
6	103.29	-	-	-	-	-	-		-
Overall Mean		103.07	102.98	102.98	102.93	103.30	103.30	102.99	102.97
Overall SD		0.788	0.887	0.896	0.955	0.596	0.607	0.876	0.889
Overall %RSD		0.76	0.86	0.87	0.93	0.58	0.59	0.85	0.86

The method was found robust for change in mobile phase flow rate, change in column oven temperature, change in mobile phase, buffer, pH and change in organic content.

Table No. 15 The results of forced degradation study

Sr.No.	Conditions	% w/w Assay	% Degradation w.r.t. Untreated sample	Purity angle	Purity threshold	Purity flag
1	Un-treated sample	100.83	00	0.086	0.280	No
2	Acid treated sample	99.80	1.02	0.081	0.278	No
3	Base treated sample	100.61	0.22	0.083	0.279	No
4	Peroxide treated sample	92.80	7.96	0.068	0.268	No
5	Thermal treated sample	102.45	00	0.081	0.276	No
6	UV-vi light treated sample	99.10	1.72	0.079	0.275	No
7	Hydrolysis treated sample	102.97	00	0.081	0.279	No
8	Humidity treated sample	101.90	00	0.084	0.278	No

Forced degradation study was performed using very harsh condition. For treated sample all peaks are well separated and there were no co-eluting peaks and no interference of blank and placebo at retention time of perampanel peak. Hence the method is stability indicating for assay of perampanel in perampanel oral suspension 0.5mg/ml.

Table No. 16 Summary of system suitability

Sr. No.	Name of Experiment	Tailing factor	Theoretical plate	%RSD
1	System precision	1.0	14000	0.11
2	Method precision	1.0	14070	0.59
3	Intermediate precision	1.0	14112	0.52
4	Specificity	1.0	14301	0.25
5	Linearity	1.0	14111	0.10
6	Accuracy	1.0	13413	0.02

7	Ruggedness	1.0	14690	0.05
8	Robustness plus temperature	1.0	14305	0.11
9	Robustness minus temperature	1.0	13695	0.12
10	Robustness plus pH	1.0	14073	0.09
11	Robustness minus pH	1.0	14034	0.05
12	Robustness plus flow	1.0	13930	0.04
13	Robustness minus flow	1.0	13312	0.08
14	Robustness plus organic	1.0	13928	0.06
15	Robustness minus organic	1.0	14280	0.02

IV. CONCLUSION:

The proposed RP-HPLC method utilizes Hypersil ODS, 250mm × 4.6 mm, 5µ, isocratic run (using Buffer: Acetonitrile in ratio of 500: 500 v/v as mobile phase), effluent flow rate (1.0 ml/min), and UV detection at 230 nm for analysis of drug.

The observations and result obtained for each parameter including Specificity, Linearity, Accuracy (Recovery), Method Precision (Repeatability), Intermediate precision (Ruggedness), Robustness, Solution stability and System suitability lies well within the acceptance criteria.

Specificity of the method was demonstrated by analysing Blank preparation, Placebo preparation, Standard preparation, Test preparation and Blank preparation, Placebo preparation, did not show any interference.

The data obtained from Linearity, Precision and Accuracy reveals that the method is linear, precise and accurate over the range of 70% to 130% of test concentration. Ruggedness of the method was evaluated under intermediate precision and results were found within acceptable limits.

The Standard preparation and Test preparation is found stable up to 36 Hours at (5 ± 2°C). The system suitability parameters met the acceptance criteria, which were commenced during study of each individual validation characteristics.

The proposed method can be used for routine analysis of Perampanel Oral Solution in quality control laboratories.

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