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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP- HPLC METHOD FOR QUANTIFICATION OF ERENUMAB IN ITS FORMULATIONS

***Parbati Kirtania, Yasmeen Baig and Dr. Anupama Konneru**

Sultan-Ul-Uloom College of Pharmacy, Mount Pleasant, Road No.3, Banjara Hills, Hyderabad- 500034.

***Corresponding Author: Parbati Kirtania**

Sultan-Ul-Uloom College of Pharmacy, Mount Pleasant, Road No.3, Banjara Hills, Hyderabad- 500034.

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ABSTRACT

A simple, Reversed Phase high-performance liquid chromatographic (RP-HPLC) technique has been developed and validated for the estimation of Erenumab. Chromatographic conditions used are stationary phase Kromasil C18 (250mm*4.6mm5m), Mobile phase Water: Methanol in the ratio of 40:60 and flow rate was maintained at 1.0ml/min, detection wavelength was 235nm, column temperature was set to 30^{D} C and diluents was mobile phase Conditions were finalized as optimized method. 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, R 2 value was found to be as 0.999. Precision was found to be 0.8 for repeatability and 0.5 for intermediate precision. LOD and LOQ are 0.01µg/ml and 0.04µg/ml respectively. By using above method assay of pharmaceutical dosage form was carried out 100.36% was present. Degradation studies of Erenumab were done, in all condition's the results obtained where within the acceptable range. this method can be used for routine analysis of Erenumab.

KEYWORDS: RP- HPLC Erenumab, ICH Guidelines, validation.

INTRODUCTION

Approval is a procedure of building up reported proof, which gives a high level of affirmation that a particular movement will reliably create a coveted outcome or item meeting its foreordained determinations and quality attributes. Strategy approval is the way toward showing that diagnostic techniques are appropriate for their proposed utilize and that they bolster the character, quality, virtue, and intensity of the medication substances and medication items. The genuine objective of approval process is to challenge the strategy and decide cutoff points of permitted.

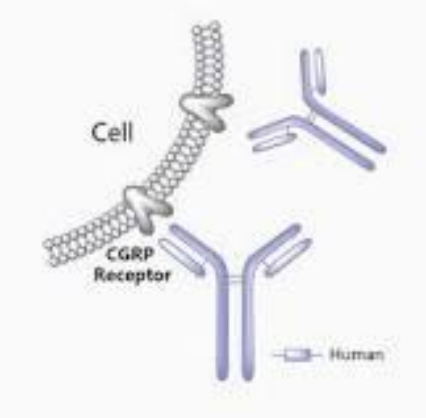
Type of analytical procedures to be validated: Validation of analytical procedures is directed to the four most common types of analytical procedures.

- Identification test.
- Quantitative test for impurities content.
- Limit test for the control of impurities.
- Quantitative test of the active moiety in samples of drug substance on drug product on other selected components in the drug product.

1) First determine the classification of the method.
2) The second step is to consider the characteristics of the analytical method.

For analytical method validation of pharmaceuticals, guidelines from the International Conference on Harmonization (ICH), United States Food and Drug Administration (USFDA), American Association of Official Analytical Chemists (AOAC), United States Pharmacopoeia (USP) and International Union of Pure and Applied Chemists (IUPAC) provide a frame work for performing such validations in efficient and productive manner.

Erenumab



It is a human monoclonal antibody intended specifically to bind and antagonize the gene-related peptide receptor of calcitonin as a means of preventing migraines(Anti-Migraine Agent). Brand name Aimovig, published and sold by Novartis and Amgen.

Table: Drug profile.

Drug	**Erenumab**
Drug category	Antimigraine agent
Molecular formula	$C_{6472}H_{9964}N_{1728}O_{2018}S_{50}$
Molecular weight	488.01 g/mol g·mol^{-1}
Brand name	Aimovig
Manufacturer	Amgen Novartis
Half life	28 days
Description (Physical state)	Liquid
Dosage form	Subcutaneous injection
Dose	70 mg
Storage	$2^{D}C$-$8^{D}C$

MATERIALS AND METHOD

MATERIALS

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

Equipment and Apparatus used

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2, Sonicator (Ultrasonic sonicator), P H meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump.

Reagents used

Methanol HPLC Grade, Acetonitrile HPLC Grade, Potassium dihydrogen ortho phosphate HPLC Grade, HPLC grade Water.

METHODS

Preparation of Standard stock solutions: Accurately weighed 17.5mg of Erenumab transferred 25ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution (700µg/ml of Erenumab).

***Preparation of Standard working solutions* (100% solution):** 1ml of Erenumab from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with diluent. (70µg/ml) of Erenumab).

***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 10 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 (700 µg/ml of Erenumab).

***Preparation of Sample working solutions* (100% solution)**

5ml of filtered sample stock solutionwas transferred to 10ml volumetric flask and made up with diluent. (70µg/ml of Erenumab).

Preparation of buffer

0.01N Potassium dihydrogen ortho phosphate Buffer: Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000mlof 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. Orthophosphoric solution.

0.1%OPA Buffer

1ml of Perchloric was diluted to 1000ml with HPLC grade water.

Experimental conditions

Chromatographic separation achieved using an analytical Kromasil C18 250mm x 4.6 mm, Mobile phase consisted of Methanol: Water (60:40% v/v). The elution was achieved at a flow rate of 1.0ml/min with injection volume of 10µl. the column temperature was set at ambient temperature and chromatograph was recorded at wavelength 235nm.

ANALYTCAL METHOD VALIDATION

HPLC method was validated according to the International Conference on Harmonization (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

Linearity: Inject each level (10, 20,30,40,50 and 60µg/ml) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area. in the concentration range of 10 – 60µg/ml. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

Precision

Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Intermediate precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different analysts by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy
Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found, and Amount added for Erenumab and calculate the individual recovery and mean recovery values.

Robustness
Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for injections peak area values of each change in condition.

System suitability: This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of erenumab and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity
Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

Limit of detection
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
LOD= 3.3 × σ / s

Limit of Quantitation
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
LOQ=10×σ/S

Assay Methodology
Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae.

Calculate the percentage purity of Erenumab present in tablet.
Calculation:

$$\text{Assay} = \frac{\text{Spl area}}{\text{Std area}} \times \frac{\text{Std. Dil. Fac}}{\text{Spl. Dil.}} \times \frac{\text{Avg. Wt of Tab}}{\text{Fac L.C}} \times \text{Potency of Std}$$

STABILITY INDICATING STUDIES
A Stability-indicating assay method can be defined as "Validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug products are specific so that the content of active ingredients and degradation products can be accurately measured without interference".

Generally forced degradation/stress testing is used to generate the samples for stability-indicating assay methods. Forced degradation/stress testing is defined as "the stability testing of drug substance and drug product under conditions exceeding those used for accelerated stability testing". Degradation can be achieved by exposing the drug, for extended period of time, to extremes of, Oxidation, Acid Degradation, Alkali Degradation, Dry Heat Degradation, Photo Stability, Neutral Degradation to achieve degradation to an extent of 5–20%. Generally, trial and error experimentation are used during these experiments.

Degradation procedure
Oxidation
To 1 ml of stock solution of Erenumob 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60 0 c. For HPLC study, the resultant solution was diluted to obtain (70ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies
To 1 ml of stocks solution Erenumob 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 1c. The resultant solution was diluted to obtain (70ppm) solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies
To 1 ml of stock solution Erenumob 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60 0 c. The resultant solution was diluted to obtain (70ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105 0 c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (70ppm) solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

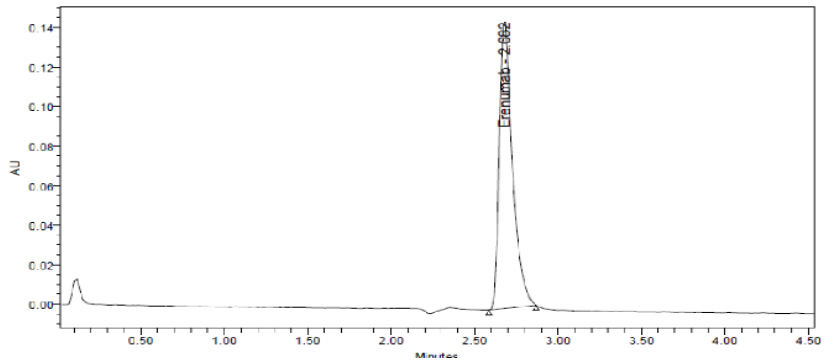
The photochemical stability of the drug was also studied by exposing the (700ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200-Watt hours/m 2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (70ppm) solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to (70ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Optimized Chromatographic Conditions
Column : Kromasil C18 250mm x 4.6 mm, 5 (.
Mobile phase: Water: Methanol (40:60)
Flow rate : 1.0 ml/min
Detector: PDA 235nm
Temperature : 30^0C
Injection Volume: 10µl



Optimized chromatogram

Observation: All the system suitability specifications are in range and satisfactory as per ICH guidelines.

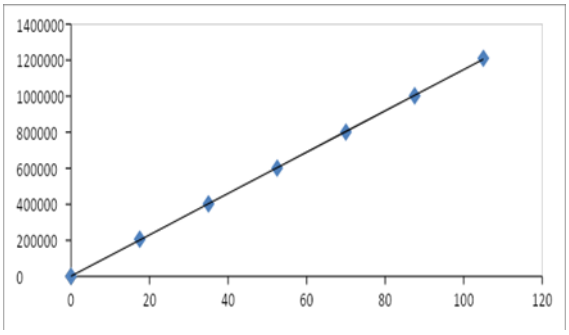
SYSTEM SUITABILITY PARAMETERS

S no.	Peak Name	Rt	Area	USP Rate count	USP Tailing
1	Erenumab	2.673	806863	6081	1.67
2	Erenumab	2.674	791976	5735	1.54
3	Erenumab	2.676	799725	5875	1.57
4	Erenumab	2.679	790958	5829	1.57
5	Erenumab	2.679	810419	5677	1.57
6	Erenumab	2.680	800813	5578	1.53
Mean	Erenumab		801126		
Std Dev	Erenumab		6669.8		
% RSD	Erenumab		0.8		

Discussion: As per ICH rules plate count should be > 2000, tailing factor should be < 2 and resolution must be > 2.

LINEARITY

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 17.5 ppm to 105 ppm of Erenumab. Plot a graph to concentration versus peak area. Slope obtained was 11471Y-Intercept was 1017 and Correlation Co-efficient was found to 0.999 and linearity plot was shown.



Linearity Graph

PRECESION: *Repeatability:* six working sample solutions of 70ppm are injected and the % amount found was calculated and %RSD was found to be 0.5.

S. No	Peak Area
1	809808
2	801855
3	809667
4	809568
5	803070
6	809285
AVG	807209
STDEV	3700.5
%RSD	0.5

***Intermediate precision*:** Five working sample solutions of 70ppm are injected on next day of the preparation of samples and the % amount found was calculated and %RSD was found to be 0.9.

Table Intermediate precision data.

S. No	Peak Area
1	760370
2	778377
3	760697
4	770445
5	773532
6	768623
AVG	801125
Std DEV	7120.1
%RSD	0.9

ACCURACY: Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 99.77.

Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	35	34.66	99.01	99.77%
	35	34.66	99.02	
	35	34.82	99.49	
100%	70	69.86	99.80	
	70	70.43	100.61	
	70	70.41	100.58	
150%	105	105.12	100.11	
	105	104.34	99.37	
	105	104.95	99.96	

LOD: Detection limit of the Erenumab was found to be 0.01µg/ml.

Parameter	Obtained value	Limit
LOD	0.01µg/ml.	NMT3

LOQ: Quantification limit of the Erenumab in this method was found to be 0.04µg/ml.

Parameter	Obtained value	Limit
LOQ	0.04µg/ml.	NMT10

ROBUSTNESS

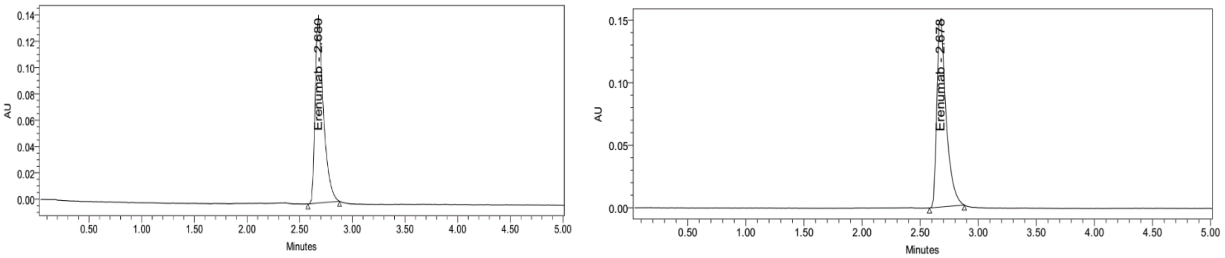
Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated.

Table Robustness Data.

Parameter	% RSD
Flow (2ml\min)	0.4
Flow (1ml\min)	0.4
Mobile phase (40:60)	0.2
Mobile phase (60:40)	0.4
Temperature ($30+5^{D}$)	0.7
Temperature ($30-5^{D}$)	0.5

ASSAY METHODOLOGY

S No.	Assay%
1.	99.69
2.	100.66
3.	100.65
4.	99.84
5.	100.61
6.	100.36
7.	0.46
8.	0.5



Standard chromatogram Sample chromatogram

STABILITY INDICATING STUDIES

Stability 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.

Table Degradation Data of Erenumab.

S no.	Degradation Conditions	% drug degraded	Purity Angle	Purity Threshold
1	Acid	6.17	0.397	0.399
2	Alkali	4.88	0.247	0.335
3	Oxidation	4.93	0.246	0.322
4	Thermal	1.50	0.313	0.326
5	UV	1.05	0.282	0.326
6	Water	1.05	0.247	0.321

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 neutralization of Acid sample with 2N basesolution and neutralization of Base sample with 2N acid solution there will be no change in retention time.

ACKNOWLEDGMENT

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CONCLUSION

In the present investigation a simple, sensitive, precise and accurate RP-HPLC method was developed for quantitative estimation of erenumab in pharmaceutical dosage form. The results expressed in tables for RP-HPLC method were promising. This method can be used for the routine quantitative determination of erenumab. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. erenumab was freely soluble in methanol, water and sparingly soluble in ethanol. Methanol: Water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. This method can be used for the routine determination of erenumab in Pharmaceutical dosage forms.

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