DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR EPERISONE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

Aneesha. K*, Prof. Raju .R

Department of Chemistry, National college of Pharmacy, Calicut.

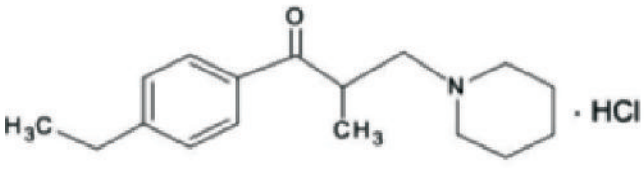
ABSTRACT:A simple, fast, accurate and precise method has beendeveloped for the determination of Eperisone Hydrochloride from pharmaceutical formulation by Reverse-phase High Performance Liquid Chromatography. The separation was carried out on C_{18} column using mobile phase consisting of methanol and phosphate buffer P^H 6 (90:10 V/V) at a flow rate of 1ml/min. The UV detection was carried out at wave length of 256nm. The retention time was found to be 3.46min. The method was found to be linear (r^2>0.999) in the range of 5-25µg/ml. The proposed method has validated according to ICH guideline with respect to linearity, precision, accuracy, limit of detection, limit of quantification and robustness. The proposed method can be successfully applicable to pharmaceutical preparation.

KEYWORDS:- **Eperisone, Validation**

I. INTRODUCTION

EPE is chemically (2RS)-1-(4Ethyl phenyl)-2-methyl-3(piperidine-1-yl) propan –one[1]. EPEexhibits both skeletal muscle relaxant and vasodilator properties because of its action with in the central nervous system and on vascular smooth muscle and demonstrates a variety of pharmacological effect such as cervical spondylosis, head ache and low back pain[2]. EPE is official in Japanese Pharmacopoeia and described potentiometric method for its estimation[3]. Literature survey reveals ESI-MS method for estimation of EPE in human plasma[4], HPLC/MS, GC-MS, NMR, UV and IR analytical technique to identify a degradation product for EPE in the tablet dosage form are available[5].

II. MATERIAL AND METHODS:



Eperisone Hydrochloride

Material-

Pure drug of Eperisone Hydrochloride was kindly gifted by Macleods Pharmaceuticals Pvt.Ltd , Mumbai. The tablet formulation containing Eperisonehydrochloride (Myosone 50mg tablet, marketed by Macleodspharma), was purchased from local market. All chemicals used are of HPLC/AR grade and the reagent solutions were prepared using double distilled water.

Method-

INSTRUMENTATION

HPLC system (waters HPLC) consisting of waters 515 HPLC pump, UV detector(waters PDA-2998) with a Spherisorb C18, 5µ silica , 4 × 250mm column was employed for analysis. Chromatographic data was acquired using empower 2 software.

CHROMATOGRAPHIC CONDITIONS

Methanol : Phosphate buffer (PH 6) in the ratio 90:10 v/v was used as mobile phase and was filtered before use through 0.45µ membrane filter. A constant flow of 1.0ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 256nm

PREPARATION OF SOLUTIONS

STANDARD STOCK SOLUTION

Weigh accurately 10mg of Eperisone Hydrochloride in to 10ml standard flask, dissolve and made up the volume with mobile phase (methanol: phosphate buffer 90:10v/v). The solution has a concentration of 1mg/ml. further dilution was made to get the final concentration of 100µg/ml.

From the above stock solution0.5ml, 1ml, 1.5ml, 2ml, 2.5ml were pipette out into each of 10ml standard flask, made up the volume with mobile phase. To obtain final concentrations of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml & 25µg/ml respectively.

SAMPLE SOLUTION

Twenty tablets (MYOSONE) each containing 50mg of Eperisone Hydrochloride were weighed. Their average weight was determined and finely powdered using glass mortar andpestle. The tablet powder equivalent to 10mg of Eperisone Hydrochloride was transferred to 10ml volumetric flask and dissolvein approximate 8ml of mobile phase, sonicated for 15min. The flask was allowed to cool and filter the solution using whatmann No.1 filter paper to 10 ml standard flask with the washings make up the solution. The stock solution was further diluted with mobile phase to obtain working sample solution of 15µg/ml

II. METHOD VALIDATION

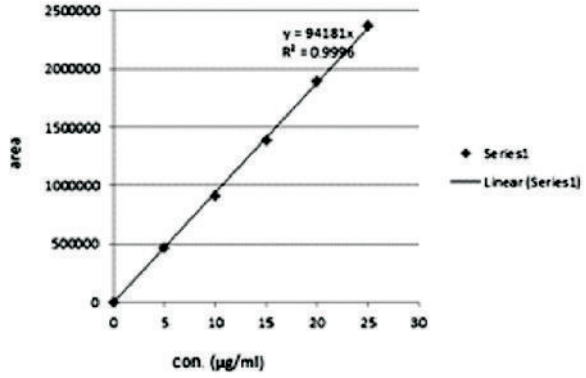
Validation of proposed method was as per ICH guidelines by means of the following parameters.

LINEARITY

Linearity was observed in the concentration range of 5-25µg/ml. calibration curve was plotted over the concentration range.

PRECISION

Precision of the instrument was checked by



repeated scanning and measurement of absorbance of solution of Eperisone and without changing the parameters of the proposed method. The Percent standard deviation (%RSD) was found to within the limit (Not more than 2%).

INTERMEDIATE PRECISION-

The intraday and interday precision of the proposed method was determined by analysing the corresponding responses times on the same day and on three different days over a period of one week. The percent standard deviation (%RSD) was found and was within limit.

ACCURACY

The accuracy of the method was determined by calculating the recovery of Eperisone Hydrochloride by the standard addition method. Recovery studies were carried out by

the addition of standard drug solution to sample at three different (80 %, 100 % , 120%) concentration level.

The recovery study was conducted by adding known amount of pure drug in to the sample solution of fixed concentration and then analysed.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of detection (LOD) and limit of quantification (LOQ) of the drug was derived using the following equation as per ICH guidelines.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ = Standard deviation of the response and S= slope of the calibration curve.

ROBUSTNESS

Robustness of the method was determined by small change in flow rate, mobile phase ratio, and wavelength detection. Flow rate was changed to 1± 0.1ml/min. The mobile phase ratio was changed to ±2%. Wavelength of detection was changed to 256±5nm.

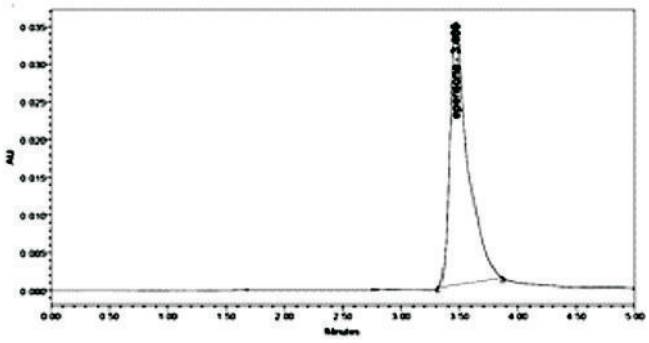
RESULTS AND DISCUSSION

The method was found to be simple, accurate and rapid for routine estimation of Epeisone hydrochloride, in tablet dosage form.

The linearity was observed in the concentration range of 5-25µg/ml for Eperisone hydrochloride (fig no-1). Commercial formulation containing Eperisone hydro chloridewasanalysed by the proposed method. Five replicate analysis of formulation were carried out and mean assay value was found close to 100%. The retention time of Eperisone hydrochloride was 3.46min at a flow rate of 1ml/min (fig no-2). The chromatogram was recorded at 256nm.

The accuracy of the proposed method was determined by recovery studies. Precision was determined as repeatability and intermediate precision, the results shows that it was within the acceptable limit.

The analysis of the marketed sample (myosone) of Eperisone hydrochloride was carried out the % amount found is 98.76.



Parameters	Eperisone Hydrochloride
Analytical wavelength	256nm
Linearity range (µg/ml)	5-25
Regression equation	Y =94181x
Correlation co-efficient (R2)	0.999
LOD (µg/ml)	0.635
LOQ (µg/ml)	1.924
Precision Repeatability (%RSD) Intermediate (%RSD)	 0.204 0.139
Accuracy (mean % recovery)	98.96-100.38
Robustness	Robust

CONCLUSION

The proposed method of analysis was found to be precise, accurate, rapid and reproducible. The developed method provide simple mobile phase composition, flow rate (1.0ml/min) and short retention time i.e 3.4min. The method was validated as per ICH guidelines.

The resulted method should not interfere with excipients in the tablet dosage form. Therefore the reported method could find practical application as an economical, cost effective and rapid quality control tool for analysis of drug, in both research and industrial quality control laboratories.

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REFERENCE

1 Maryadele JN. The Merck Index: An encyclopedia of chemicals, drugs and biological. 14th ed. New Jersey: Merck INC., Whitehouse Station; 2006.

2. Cabitza P, Randelli P. Efficacy and safety of eperisone in patients with low back pain: A double blind randomized study. Eur Rev Med PharmacolSci2008;12:229-35.

3. Society of Japanese Pharmacopoeia. The Japanese Pharmacopoeia. 15th ed. Shibuya Tokyo Japan: The stationery office; 2006.

4. Ding L, Wei X, Zhang S, Sheng J, Zhang Y. Rapid and sensitive liquid chromatography –electrospray ionization-mass spectr-ometry method for the determ-ination of eperisone in human plasma: method and clinical applications. J Chromatogr Sci2004;42:254-8.

5. Ding L, Wang X, Yang Z, Chen Y. The use of HPLC/MS, GC/MS, NMR, UV and IR to identify a degradation product of eperisone hydrochloride in the tablets.J Pharm Biomed Anal 2008;46(2):282-7.

6. ShantharamGajananKhanage, Popat BabanMohite, SandeepJadhav, develop-ment and validation of UV-Visible spectro-photometric method for simultaneous determination of Eperisone and Paracetamol in solid dosage form. Advanced pharmaceutical bulletin 2013;3 (2): 447-451.

7. Bhati Sanjay singh, PadaliyaHardik, Mujapara Ashwin, S SPancholi, developed and validate first order derivative spectrophotometric method for simultaneous estimation of EPE and Diclofenac sodium in pharmaceutical dosage form. International journal of universal pharmacy and bio-sciences 2013; 267-276.

8. Bhatt DivyangJ,Akthar Jawed, Banghel Madhuri, development and validation of spectrophotometric area under curve method for simultaneous estimation ofEperisone Hydrochloride and Diclofenac sodium in combined capsule dosage form: international research journal of pharmacy 2013;4(2): 71-75.