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

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ABSTRACT: A simple, fast, accurate and precise method has been developed for the determination of Eperisone Hydrochloride from pharmaceutical formulation by Reverse-phase High Performance Liquid Chromatography. The separation was carried out on C18 column using mobile phase consisting of methanol and phosphate buffer pH 6 (90:10 V/V) at a flow rate of 1ml/min. The UV detection was carried out at wavelength 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]. EPE exhibits both skeletal muscle relaxant and vasodilator properties because of its action within 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:

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 0.45μm 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 solution 0.5ml, 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 and pestle. The tablet powder equivalent to 10mg of Eperisone Hydrochloride was transferred to 10ml volumetric flask and dissolve in 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.

\[
LOD = 3.3 \times \frac{\sigma}{S} \\
LOQ = 10 \times \frac{\sigma}{S}
\]

Where \( \sigma \) = 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±0.1 ml/min. The mobile phase ratio was changed to ±2%. Wavelength of detection was changed to 256±5 nm.

**RESULTS AND DISCUSSION**

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

The linearity was observed in the concentration range of 5-25 µg/ml for Eperisone hydrochloride (fig no-1). Commercial formulation containing Eperisone hydrochloride was analysed 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 3.46 min at a flow rate of 1 ml/min (fig no-2). The chromatogram was recorded at 256 nm.

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.

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

The proposed method of analysis was found to be precise, accurate, rapid and reproducible. The developed method provides simple mobile phase composition, flow rate (1.0 ml/min) and short retention time i.e 3.4 min. 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, rapid and quality control tool for analysis of drug, in both research and industrial quality control laboratories.

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REFERENCE


