Development and validation of RP-HPLC method for the estimation of Eletriptan hydrobromide in bulk and tablet dosage form

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ABSTRACT

A simple, precise, rapid, and reproducible RP-HPLC method was developed and validated for the determination of Eletriptan Hydrobromide in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a PhenomenaxLunaC18 (ODS) column (250 X 4.6 mm i.d., particle size 5µ). The mobile phase consisted of 60:40 v/v mixture of phosphate buffer pH 6.0 and Acetonitrile v/v. An isotropic elution at a flow rate of 1 ml/ min at ambient temperature. The detection was carried out at 221nm using Waters HPLC with UV-Visible detector. The retention time of Eletriptan is found to be 4.1 min and the calibration curve was linear in the concentration range of 5–30 µg/ml (r2= 0.9995). The limit of detection and the limit of quantification were found to be 0.6327 µg/ml and 1.963 µg/ml respectively. The amount of Eletriptan present in the formulation RELPAX Tablets containing 20mg of Eletriptan manufactured by Pfizer was found to be 99.55. The method was validated statistically using the SD, %RSD and SE and the values are found to be within the limits and the recovery studies were performed and the percentage recoveries was found to be 99.53± 0.6327 %. So, the proposed method was found to be simple, specific, linear, and rugged. Hence it can be applied for routine analysis of Eletriptan in the Pharmaceutical formulations.

Key words: Eletriptan, UV detection, RP-HPLC method; Tablet dosage forms.

INTRODUCTION:

Eletriptan is a new drug and it is used as is chemically 3-methoxy-8-{[4-methoxy-3,5-dimethyl-pyridin-2-yl]methyl sulfinyl} 2,7,9-triazabicyclo[4.3.0] nona-2,4,8,10-tetraene. It is newer drug used in the treatment of Anti-ulcer agent. The review of literature revealed that no methods were reported for the estimation of in bulk and tablet formulations. But there is no method was reported for the estimation of Eletriptan in bulk drug and in formulation.

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of Eletriptan in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 5 min) Eletriptan good precision (R.S.D.< 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH) for the determination of Eletriptan in bulk and in tablet dosage form.

Fig.1. Chemical structure of Eletriptan

Experimental:

Chemicals and reagents

HPLC grade Acetonitrile and water was purchased from Loba fine Chemicals (Mumbai, India). Eletriptan standard sample was provided by Dr. Reddy’s Laboratories (Hyderabad, India). RELPAX Tablets containing 20mg of Eletriptan manufactured by Pfizer Ireland pharmaceuticals® commercial formulation was procured from local market. The tablet dosage forms containing obtained was 20 mg of Eletriptan for oral administration. The molecular weight is 463.43 for Eletriptan.

Instrumentation and analytical conditions:

The validated method utilized a Waters HPLC system and UV-Visible detector with an isocratic elution technique at a flow rate of 1 ml / min on a Phenomenax Luna C18 column (150 X 4.6 mm i.d., 5µ) at ambient temperature. The mobile phase consisted of phase consisted of 60:40 v/v mixture of phosphate buffer pH 6.0 and Acetonitrile. The UV detection wavelength was at 221 nm. The retention time for Eletriptanwas found to be 3.4 minutes.

Stock and working standard solutions:

Stock standard solution of 1000µg/ml of Eletriptan was prepared freshly by accurately weighing 25mg of Eletriptan into 25ml volumetric flask. Dissolved and made up to the volume with Phosphate buffer (pH 6.0). Further diluted by pipetting 1ml into 25ml volumetric flask to obtain 40 µg/ ml solution. The solution was further diluted with mobile phase in 10ml volumetric flask to obtain six working standards in the concentration range 5-30 µg/ml of Eletriptan. Chromatogram was recorded thrice for each dilution. All the solutions were prepared in triplicates.

Assay of sample preparation:

Twenty commercial (RELPAX) Tablets containing 20mg of Eletriptan manufactured by Pfizer Ireland pharmaceuticals®. Were weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 25 mg of Eletriptan was quantitatively transfer into a 25 ml volumetric flask with about 20 ml of Ammonium acetate buffer at pH 6.0. The solution was
sonicated for 10 min, brought to the volume with phosphate buffer, mixed well and 1 ml of filtered test solution was transferred into 25 ml volumetric flask and made up to the volume with mobile phase 40 µg/ml 1.5 ml aliquot solution was transferred into a 10 ml volumetric flask. The theoretical Eletriptan concentration after dilution was 6 µg/ml 100% of Eletriptan. An aliquot of this solution was filtered through a 13mm membrane syringe filter (Pore size 0.2 µm) prior to the injection into the HPLC system. Peak area of Eletriptan was measured for the determinations.

Finally the method was validated as per ICH guide lines for precision, accuracy, specificity, linearity, reproducibility, LOD and LOQ. Sample solution short term stability was tested at ambient temperature (20 ± 1°C) for 3 days.

**Validation procedure:**
The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, short term stability and system suitability.

Standard plots were constructed with six concentrations in the range of 5-30 µg/ml prepared in triplicates to test linearity. The peak area of Eletriptan was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method.

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Eletriptan test solution in the same equipment at a concentration of 100% (12 µg/ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of Eletriptan was determined and precision was reported as % R.S.D.

Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analyzing samples of Eletriptan at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Eletriptan recovered in the samples.

Sample solution short term stability was tested at ambient temperature (20 ± 1°C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re injected after 24 and 48 hrs at ambient temperature and compared with freshly prepared solutions.

**RESULTS AND DISCUSSION:**

**Screening and optimization**

**Selection of the detection wavelength**
The UV spectra of Eletriptan in phase consisted of 60:40 v/v mixture of phosphate buffer pH 6.0 and Acetonitrile in the region between 200 and 400 nm are shown in Fig 2. It shows that at 221 nm, Eletriptan have maximum absorbance. Hence λ max of Eletriptan in mobile phase was selected as an optimum detection wavelength for the quantification of Eletriptan.

**Optimization of the chromatographic conditions:**
Proper selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. The drug Eletriptan is non polar. Non polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase.

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Validation of methods:

Linearity
Six point’s calibration graphs were constructed covering a concentration range 5-30 µg/ml (Three independent determinations were performed at each concentration. Linear relationships between the of peak area signal of Eletriptan the corresponding drug concentration was observed as shown in Fig 4. The standard deviations of the slope and intercept were low. The determination coefficient (r²) exceeded 0.9995. The statistical analysis of calibration is shown in table 1.

Table 2: Repeatability of Eletriptan

<table>
<thead>
<tr>
<th>S.No</th>
<th>Labelled amount (µg/tab)</th>
<th>Amount found (µg/ tab)</th>
<th>Percentage obtained (%)</th>
<th>Average</th>
<th>S.D.</th>
<th>% RSD</th>
<th>S.E</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>19.97</td>
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<td></td>
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<tr>
<td>2</td>
<td>20</td>
<td>19.95</td>
<td>98.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>19.95</td>
<td>98.33</td>
<td>99.60</td>
<td>1.538</td>
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<td>5</td>
<td>20</td>
<td>19.97</td>
<td>99.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>20.08</td>
<td>102.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean of six observations

Table 3: Intraday and interday precision of the method

<table>
<thead>
<tr>
<th>Amount Found (Percentage Obtained)</th>
<th>% RSD</th>
<th>Intraday*</th>
<th>Interday*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

99.23 100.28 0.0325 0.01935

* Mean ± SD of three observations

Precision:
The validated method was applied for the assay of commercial tablets containing 20 mg of Eletriptan: Relpax. Sample was analyzed in for six times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, was found to be 99.60 ± 1.538 for Relpax showing that the content of Eletriptan in tablet formulations confirmed to the content requirements (95 - 105 %) of the label claim. Low values of standard deviation denoted very good repeatability of the measurement.

Thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day and on three consecutive days (n=3) indicated a R.S.D. of 0.0355 and 0.01955% respectively. Both values were far below to 2%, the limit percentage indicated a good method precision. The results of analysis are shown in table 2 and table 3.

Table 4: Accuracy study for Eletriptan (n =9)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Amount Present (µg/ ml)</th>
<th>Amount Added (µg/ ml)</th>
<th>Amount Found* (µg/ ml)</th>
<th>Amount Recovered (µg/ ml)</th>
<th>Percentage Recovery*</th>
<th>S.D</th>
<th>% RSD</th>
<th>S.E</th>
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<tr>
<td>1</td>
<td>1.491</td>
<td>3.6</td>
<td>18.59</td>
<td>3.59</td>
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</tr>
<tr>
<td>2</td>
<td>1.491</td>
<td>7.2</td>
<td>22.11</td>
<td>7.19</td>
<td>99.86 0.3195 0.3207 0.1304</td>
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<td></td>
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</tr>
<tr>
<td>3</td>
<td>1.491</td>
<td>10.8</td>
<td>25.65</td>
<td>10.72</td>
<td>99.25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Accuracy:
The data for accuracy were expressed in terms of percentage recoveries of Eletriptan in the real samples. These results are summarized in table 4. The mean recovery data of Eletriptan in real sample were within the range of 98.72 and 99.82 %. Mean % R.S.D. was 1.348 %, satisfying the acceptance criteria for the study. It proved that there is no interference due to excipients used in tablet formulation. Hence the accuracy of the method was confirmed.

Stability:
The stability of Eletriptan in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 1°C). The solutions were checked in triplicate after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that Eletriptan is stable in standard and sample solutions for at least 2 days at ambient temperature.

Table 5: System suitability study of Eletriptan

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Eletriptan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tailing factor</td>
<td>1.11</td>
</tr>
<tr>
<td>2</td>
<td>Asymmetrical factor</td>
<td>1.20</td>
</tr>
<tr>
<td>3</td>
<td>Theoretical plates</td>
<td>5622</td>
</tr>
<tr>
<td>4</td>
<td>Capacity factor</td>
<td>1.50</td>
</tr>
<tr>
<td>5</td>
<td>HETP</td>
<td>0.0266</td>
</tr>
</tbody>
</table>

System suitability:
The system suitability parameter like capacity factor, asymmetric factor, tailing factor, HETP and No. of theoretical plates also calculated. It was observed that all the values are within the limits (table 5).

The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Eletriptan in tablet formulation.

CONCLUSION:
A validated isocratic HPLC - UV method has been developed for the determination of Eletriptan in tablet dosage form. The proposed method is simple, rapid, accurate, precise, and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of Eletriptan in pharmaceutical dosage form. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as GC-MS that is complicated, costly and time consuming rather than a simple HPLC-UV method. Hence the proposed method could be useful for the national quality control laboratories in developing countries.

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