Simple and Economical UV Spectrophotometric Area under Curve Method for Estimation of Eletriptan Hydrobromide

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ABSTRACT
Objective: Simple and economical area under curve method was developed and validated for the estimation of Eletriptan Hydrobromide. Method: The solvent implemented for estimation of Eletriptan Hydrobromide was water. The λ max was found to be 221 nm. The area under curve of UV spectrum between 216 to 226 nm. The method is validated according to ICH guideline Q2(R1). Results: The linearity range of Eletriptan HBr was found to be 5-25 µg/ml. The correlation coefficient was found to be 0.999. The values of %RSD for inter and intra-day precisions were within the acceptable limit. LOD and LOQ were found to be 5.77 µg/ml and 1.90 µg/ml respectively. % Recovery of Eletriptan HBr was 99.66 to 99.95. The values of ruggedness shows the method is rugged. Conclusion: The developed method can be implemented for routine analysis of Eletriptan HBr in bulk as well as formulation as per ICH Q2 (R1) guidelines.

Key words: Eletriptan HBr, Water, Area under Curve, UV-Spectrophotometer, ICH Q2(R1).

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INTRODUCTION
Eletriptanhydrobromide is a 5- Hydroxytryptamine1B/1D receptor agonist. Eletriptan binds with high affinity to 5-HT1B, 5-HT1D and 5-HT1F receptors, has modest affinity for 5-HT1A, 5-HT1E, 5-HT2B and 5-HT7 receptors. Eletriptan is chemically a designated as (R)-3-[(1-Methyl-2-pyrrolidinyl) methyl]-5-[(2-(Phenyl sulfonyl) ethyl]-1H indol mono hydrobromide.

Its pharmacological effects include the constriction of cerebral blood vessels and neuropeptides secretion blockade which eventually relieves the pain. Eletriptanhydrobromide was rapidly absorbed and extensively cleared by metabolism. Literature survey shows that there are HPLC, UV methods available for estimation of Eletriptan HBr in bulk as well as formulation as per ICH Q2 (R1) guidelines. The developed method can be implemented for routine analysis of Eletriptan HBr in bulk as well as formulation as per ICH Q2 (R1) guidelines.

VALIDATION OF THE METHOD
The method was validated in terms of linearity, precision, Sensitivity, repeatability and ruggedness, accuracy.

Linearity
The linearity was determined by using working standard solutions between 5-25 µg/ml. The spectrums of these solutions were recorded and area under curve was calculated in wavelength range 216-226 nm. Calibration curve of Area under curve vs. Corresponding concentration was plotted over which area should be calculated.

Selection of wavelength range:
The standard solution of 10µg/ml was scanned between 400 nm to 200 nm in UV spectrophotometer against water as blank after baseline correction. Wavelength range was selected around wavelength maxima (221 nm). Various working standards were prepared between 5-25 µg/ml. Various wavelength range were tried and final range between 216-226 nm was selected based on linear relationship between area and corresponding concentration.

Method: Area Under Curve
The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area should be calculated.

MATERIALS AND METHODS
Active pharmaceutical ingredient (API) of Eletriptan Hydrobromide was supplied as a gift sample from Enaltec Lab, Abernathy, (Mumbai, India). Commercially available tablets (Elipran containing 20 mg of Eletriptan Hydrobromide) were obtained from local pharmacy. Shimadzu UV 1800 (Japan) with matched quartz cells, connected to computer loaded with UV Probe Software, Single pan electronic balance, Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath.

Preparation of standard stock and working standard solution:
The standard stock solution of Eletriptan Hydrobromide was prepared by dissolving accurately weighed 10mg of the drug in water and diluted to 100 ml with the same solvent to obtain a final concentration of 100µg/ml.
range of solution has been decided according to statistical parameters of generated equation.

**Precision**

Precision of the method was studied as intraday and inter-day variations. Intra-day precision was determined by analyzing the 5, 10, 15, 20, and 25 μg/ml of Eletriptan Hydrobromide solutions for three times in the same day. Inter-day precision was determined by analyzing the 5, 10, 15, 20, and 25 μg/ml of Eletriptan Hydrobromide solutions daily for three days over the period of week.

**LOD and LOQ**

The sensitivity of measurements of Eletriptan Hydrobromide by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x S.D. /m and LOQ = 10 x S.D. /m, where, ‘m’ is the slope of the corresponding calibration curve.

**Repeatability**

Repeatability was determined by analyzing 15 μg/ml concentration of Eletriptan Hydrobromide solution for six times.

**Ruggedness**

Ruggedness of the proposed method is determined for 15 μg/ml concentration of Eletriptan Hydrobromide by analysis of dilution from homogeneous slot by two analysts using same operational and environmental conditions.

**Accuracy**

The accuracy for the analytical procedure was determined at 80 %, 100 % and 120 % levels of standard solution. Area under curve was measured in the range of 216-226 nm and results were expressed in terms of % recoveries. Three determinations at each level were performed and % RSD was calculated.

**Determination of Eletriptan Hydrobromide In Bulk**

Accurately weighed 10 mg of Eletriptan Hydro bromide was transferred to a 100-ml volumetric flask and 50 ml water was added. After shaking for 2min, the mixture was diluted up to mark with water. From stock solution, correct dilution was taken in such a way that the final concentration is 100μg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 221 nm.

**Application of proposed method for Pharmaceutical formulation**

For analysis of commercial formulation two tablet of 20 mg Elitriptan hydro bromide was transferred to 100 ml volumetric flask 50 ml water was added. After ultrasonic vibration for 15 min, the mixture was diluted up to mark with water. The whole solution filtered using whatman filter paper no. 42. From filtrate, correct dilution was taken in such a way that the final concentration is 100 μ/ml. The concentration of the drug was calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 221 nm.

**RESULTS AND DISCUSSION**

**Method Validation**

The proposed method was validated as per ICH Guidelines. The solution of drugs was prepared as per the earlier adopted procedure given in the experiment.

**Linearity studies**

The linear regression data for the calibration curves showed good linear relationship over the concentration range 5-25 μg/ml for Eletriptan Hydrobromide (Figure 1). Linear regression equation was found to be $Y=0.7532x + 1.262$ ($r^2 = 0.999$). The result is expressed in Table 1.

**Precision**

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation. The results are given in Table 2.

**Sensitivity**

The linearity equation was found to be $Y= 0.7532x + 1.262$ ($r^2 = 0.999$). The LOQ and LOD for Eletriptan Hydrobromide were found to be 5.77 μg and 1.90 μg, respectively. The results are given in Table 3.

**Repeatability**

Repeatability was determined by analyzing 15 μg/ml concentration of Eletriptan Hydrobromide solution for six times and the % amount found was between 97% to 101% with % R.S.D. less than 2. The results are given in Table 4.

**Ruggedness**

The peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 5. The results showed that the % R.S.D. was less than 2%.

**Figure 1: Structure of Eletriptan Hydrobromide.**
Deshpande et al: UV-Spectrophotometric AUC method for estimation of Eletriptan HBr

Table 1: Linearity study of Eletriptanhydrobromide

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance Mean (n=3)</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.467</td>
<td>0.4393</td>
</tr>
<tr>
<td>10</td>
<td>0.832</td>
<td>0.4298</td>
</tr>
<tr>
<td>15</td>
<td>1.203</td>
<td>0.4358</td>
</tr>
<tr>
<td>20</td>
<td>1.548</td>
<td>0.4315</td>
</tr>
<tr>
<td>25</td>
<td>1.910</td>
<td>0.4353</td>
</tr>
</tbody>
</table>

Figure 2: Calibration Curve of EletriptanHydrobromide (5-25 µg/ml).

Table 2: Results of Precision studies

<table>
<thead>
<tr>
<th>Conc.(µg/ml)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amt.found</td>
<td>% R.S.D.</td>
</tr>
<tr>
<td>5</td>
<td>100.06</td>
<td>0.2437%</td>
</tr>
<tr>
<td>10</td>
<td>98.97</td>
<td>0.2410%</td>
</tr>
<tr>
<td>15</td>
<td>99.50</td>
<td>0.2423%</td>
</tr>
</tbody>
</table>

Average of three estimations.

Table 3: Sensitivity studies

<table>
<thead>
<tr>
<th></th>
<th>L.O.D. (µg/ml)</th>
<th>L.O.Q. (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.90</td>
<td>5.77</td>
</tr>
</tbody>
</table>

Table 4: Repeatability studies

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount taken (µg/ml) (n=6)</th>
<th>Amount found %</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eletriptanhydrobromide</td>
<td>15</td>
<td>15.12</td>
<td>0.2441%</td>
</tr>
</tbody>
</table>

Table 5: Ruggedness studies

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount taken (µg/ml) (n=3)</th>
<th>Amount found (%)</th>
<th>Analyst-I ±SD</th>
<th>Analyst-II ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eletriptanhydrobromide</td>
<td>15</td>
<td>99.421±0.2437</td>
<td>99.680±0.2430</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Accuracy studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Initial amount (µg/ml)</th>
<th>Amount added (µg/ml)</th>
<th>% Recovered</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eletriptanhydrobromide</td>
<td>10</td>
<td>8</td>
<td>98.01%</td>
<td>0.2387%</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>100.05%</td>
<td>0.2437%</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12</td>
<td>99.72%</td>
<td>0.2429%</td>
</tr>
</tbody>
</table>

Table 7: Analysis of Eletriptanhydrobromide in Formulation

<table>
<thead>
<tr>
<th>Conc.(µg/ml)</th>
<th>Amount found (%)</th>
<th>Mean Amount found (%)</th>
<th>(% R.S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>99.5</td>
<td>99.8</td>
<td>99.4</td>
</tr>
</tbody>
</table>

Figure 3: UV spectrum of EletriptanHydrobromide (10 µg/ml) in water.

Accuracy

The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2 which showed that the % amount found was between 97.00% to 101.00% with %R.S.D.>2. The results are given in Table 6.

Application of Proposed Method For Pharmaceutical Formulation

The spectrum was recorded at 221 nm. The concentrations of the drug were calculated from linear regression equation. The % amount found was between 98.00% to 102.00%. The results are given in Table 7.

CONCLUSION

This UV Spectrophotometric method is quite simple, accurate, precise, reproducible and sensitive. The UV method has been developed for quantification of Eletriptanhydrobromide in tablet formulation. The validation procedure confirms that this is an appropriate technique for their quantification in the formulation. It is also used in routine quality control of the formulation containing this entire compound.
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Conflict of Interest
The author is not having any conflict of interest associated with this work.

Abbreviation Used
HBr: Hydrobromide; ICH: International council for Harmonization; LOD: Limit of detection; LOQ: Limit of Quantitation; RSD: Relative Standard deviation.

References

Summary
The objective of study is to develop and validate simple, economical method for estimation of Eletriptan HBr. The current method is really simple, economical, accurate, and reproducible for estimation of Eletriptan HBr. This method follows ICH guidelines Q2 (R1). The analysis is in 200-400nm range and AUC was taken 216-226 nm.

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