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## Stability Indicating HPTLC Method for Determination of Eletriptan Hydrobromide

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### ABSTRACT

Eletriptan hydrobromide is used for the acute treatment of attacks of migraine with or without aura in adults. It is also used in Vascular Headaches. To develop stability indicating method, the technique of forced degradation studies was adopted. Eletriptan hydrobromide solution was spotted on plates precoated silica gel 60F<sub>254</sub>. The mobile phase used was Methanol: Toluene (9.5: 0.5 v/v) and quantification was carried out at wavelength 225 nm. The system showed a peak for Eletriptan hydrobromide at R<sub>f</sub> value of 0.23 ± 0.03. The Drug was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. Stress testing of Eletriptan hydrobromide was carried out according to the International Conference of Harmonization (ICH) guideline Q1A (R2). The method was successfully validated according to ICH guidelines Q2 (R1). The data of linear regression analysis indicated a good linear relationship over the range of 500–2500 ng/band concentrations with correlation coefficient value of 0.9979. The accuracy of the method was established based on the recovery studies. The LOD and LOQ of Eletriptan hydrobromide was found to be 31.03 ng/band and 94.06 ng/band respectively. Among various stress conditions, Eletriptan hydrobromide showed considerable degradation under alkali and acid catalyzed hydrolysis, oxidative and photolytic condition.

**Keywords:** Eletriptan Hydrobromide, High Performance Thin Layer Chromatography, Stability indicating method, Forced Degradation.

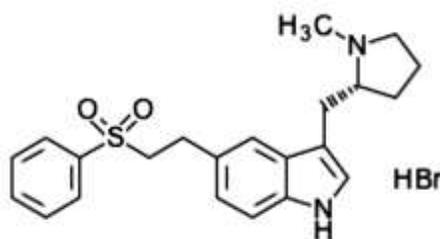
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## INTRODUCTION

Eletriptan hydrobromide is an antimigraine agent; Selective Serotonin agonist. <sup>1</sup>It is used for the acute treatment of migraine with or without aura in adults. Plasma protein binding for Eletriptan is moderate, approximately 85 %. <sup>2</sup>Eletriptan Hydrobromide reduces swelling of the blood vessels surrounding the brain, which is associated with the severe pain of a migraine attack. The mechanism of action involves blocking of substance release from nerve endings that cause more pain and other symptoms like nausea, and sensitivity to light and sound. <sup>3</sup> It is a selective 5-hydroxytryptamine 1B/1D (5-HT<sub>1B/1D</sub>) receptor agonist. Eletriptan is chemically designated as (R)-3-[(1-Methyl-2-pyrrolidinyl)methyl]-5-[2-(phenylsulfonyl)ethyl]1H-indole Monohydrobromide, and it has the following chemical structure:



**Figure 1: Structure of Eletriptan hydrobromide<sup>(4)</sup>**

The empirical formula is C<sub>22</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>S.HBr, representing a molecular weight of 463.40 gm. Eletriptan hydrobromide is a white to light pale coloured powder that is readily soluble in water. <sup>5</sup>Literature survey for Eletriptan hydrobromide reveals, UV<sup>6,7</sup>, HPLC<sup>3-8</sup>, SIM RP-HPLC<sup>9,10</sup>, UHPLC<sup>11</sup>, UPLC<sup>12</sup>, SIM LC and LC-MS<sup>13</sup> methods reported for estimation of Eletriptan hydrobromide, but there is no stability indicating HPTLC method was reported. Hence, Densitometric HPTLC method has been developed in the present work for quantitation of Eletriptan hydrobromide.

## MATERIALS AND METHOD

### Chemicals and Reagents

Working Standard Eletriptan hydrobromide was obtained as gift sample from USV Pharmaceuticals, Pvt. Ltd. Mumbai. The Eletriptan hydrobromide was used as received without purification. TLC plates precoated with silica gel (60F<sub>254</sub>, 250 μm) were purchased from E-Merck. Methanol (AR grade), Toluene (AR grade), Hydrochloric acid (HCl), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30% v/v), NaOH Pellets, were purchased from S. D. fine chemical Laboratories, Mumbai.

### Chromatographic conditions and instrumentation

Samples were applied on the plate as a band with 6mm width using 100 µl sample syringe (Hamilton, Switzerland) with Linomat 5 applicator (Camag, Switzerland). Methanol: Toluene in the ratio 9.5: 0.5 v/v was the optimized mobile phase. CAMAG twin trough glass chamber (10 cm × 10 cm) was used for linear ascending development saturation time was 15 minutes. Densitometric scanning was performed using Camag TLC scanner 3, operated by win CATS software (Version 1.4.3, Camag).

### **Preparation of standard solutions**

Stock solution was prepared in methanol which was diluted appropriately to get 100 µg/ml of concentration of Eletriptan hydrobromide UV spectrum of the working standard solution was obtained as shown in Figure 2. From the spectrum of Eletriptan hydrobromide, 225 nm was selected as absorbance maxima. Standard Densitogramat  $\lambda_{\max}$  225nm is as shown Figure 3.

### **STRESS DEGRADATION STUDY OF ELETRIPTAN HYDROBROMIDE<sup>14-16</sup>**

Various stress conditions used were hydrolysis under different pH, oxidation, photolysis and thermal stress. Q1A (R2) guidelines were referred. Forced degradation conditions were optimized to attain about 10-30% degradation.

#### **Alkaline hydrolysis**

To 1 ml of 1000 µg/mL drug solution, 1 N NaOH was added for alkaline stress.

#### **Acidic hydrolysis**

To 1 ml of 1000 µg/ml solution of Eletriptan Hydrobromide, 1ml of 0.1N HCl was added. The volume was made up to 10 ml with methanol, spotted after half an hour.

#### **Neutral Hydrolysis**

1 ml standard solution of Eletriptan Hydrobromide (1000 µg/ml) was mixed with 1 ml distilled water and volume was made up to 10 ml with methanol. The solution was kept for 2 days

#### **Oxidation**

1 ml standard solution of Eletriptan Hydrobromide (1000 µg/ml) was mixed with 1 ml of 3% v/v H<sub>2</sub>O<sub>2</sub> solution. The volume was made up to 10 ml with methanol. Solution was kept for 30 min. at room temperature in dark place.

#### **Degradation under dry heat**

Dry heat studies were performed by keeping drug sample in oven (80<sup>0</sup> C) for 6 hrs.

#### **Photo-degradation studies**

As per ICH guidelines, bulk drug was spread out as thin layer and then exposed to UV light and cool white fluorescent light upto prescribed exposure limit.

## METHOD VALIDATION

For the developed method, validation parameters checked were specificity, Linearity, Accuracy, Precision, Sensitivity i.e., LOD and LOQ, Robustness as per ICH Q2A (R1) guideline.

### Specificity

The win CATS software has a provision to compare peak UV spectra as peak start, middle and end position. The matching of these spectra indicates the homogeneity of peak. Thus purity value more than 0.995 reflects noninterference by any other degradant, impurity or excipient. This proves specificity of the method.

### Linearity

The Linearity studies were performed in the concentration range of 500-2500 ng/band an accurate correlation was found between peak area and amount spotted. Result are tabulated in Table 2

### Precision

#### Interday precision

Precision of the system was evaluated by analyzing three independent standard preparations on three different days and % RSD value obtained was calculated to determine system precision. Result are tabulated in Table 3

#### Intraday precision

Precision of the system was evaluated by analyzing six independent standard preparations in a day and % RSD value obtained was calculated to determine system precision. Result are tabulated in Table 4

### Assay

Assay was performed by dissolving amount of crushed tablet powder equivalent to 100 mg Eletriptan hydrobromide in 100 ml 0.1 N HCl, sonicated the solution for half an hour then filtered through whatmann filter paper. The peak area was extrapolated in linearity equation.

Serially Diluted 1 ml solution to 10 ml to get 100 µg/ml, spotting was done of 10 µl. The assay was found to be 98.62% in Table 5

### Accuracy

Accuracy studies were performed by adding 80%, 100%, and 120% with respect to target assay concentration (1000ng/band).The amount of Eletriptan hydrobromide was calculated and % recovery is tabulated in Table 6

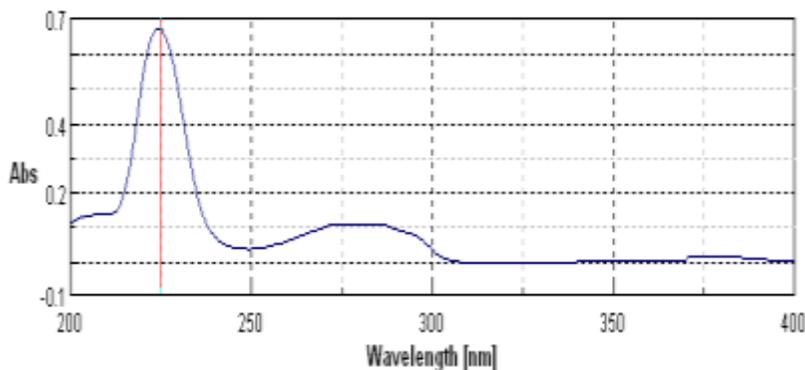
### Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of Eletriptan hydrobromide was found to be 31.03 ng/band and 94.06 ng/band respectively.

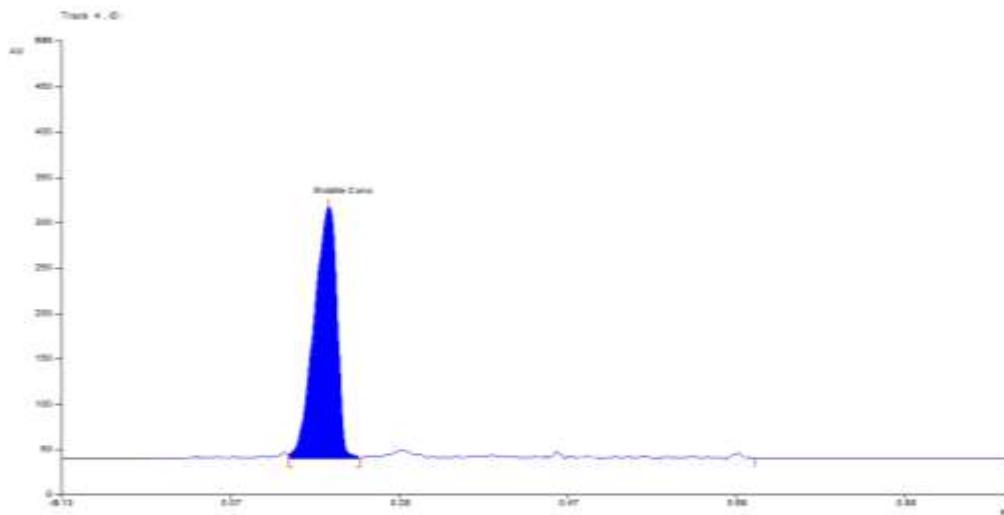
### Robustness

Robustness was determined by carrying out the analysis under conditions during which mobile phase ratio, time from application to development and time from development to scanning, chamber saturation time were altered. Impact on peak area was assessed and it was found that method is robust. Result are tabulated in Table 7

Under optimized chromatographic conditions retention factor of Eletriptan hydrobromidewas found to be  $0.23 \pm 0.03$ . Degradation was observed for Eletriptan hydrobromideduring stress conditions like Acid hydrolysis, Base hydrolysis, Neutral Hydrolysis, oxidation, dry heat and photolysis. No peak of degradation product of Eletriptan hydrobromide was observed. Peak purity results greater than 0.999 indicate that Eletriptan hydrobromide peaks are homogeneous in all stress conditions tested indicating noninterference of product of degradation. The unaffected assay of Eletriptan hy 225 nm ide confirms the stability indicating power of the method.



**Figure 2: UV Spectrum of Eletriptan hydrobromide**



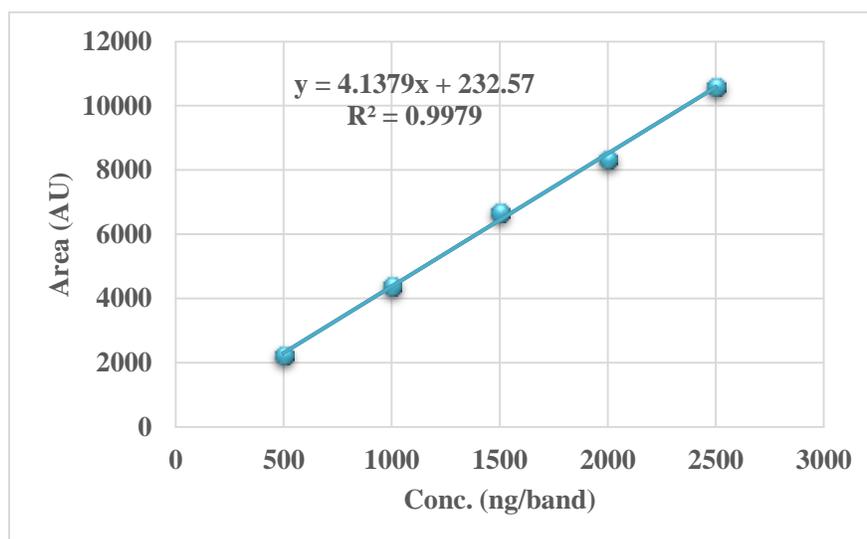
**Figure 3: Densitogram of Eletriptan hydrobromide**

**Table 1: Summary of Forced degradation of Eletriptan hydrobromide**

Forced degradation condition	Percent recovered (%)	Percent degraded (%)	Peak purity values	
			r (s,m)	r (m,e)
Initial Std. Drug	100	0	0.9999	0.9992
Acid (0.1 N HCl, 30 min.)	86.79	13.21	0.9972	0.9952
Alkali/Base (1 N NaOH)	72	28	0.9973	0.9972
Oxidation Degradation (3 % H <sub>2</sub> O <sub>2</sub> v/v 30 min)	79.39	20.61	0.9992	0.9986
Neutral/Water (2 days)	98.37	1.63	0.9997	0.9993
Dry Heat (80 <sup>0</sup> C, 6 hrs.)	83.43	16.57	0.9976	0.9971
Photo stability:	94.28	5.72	0.9994	0.9998
▪ UV, 200 watt hrs./square meter	87.74	12.26	0.9997	0.9998
▪ Florescence, 1.2 million Lux. Hrs.				

**Table 2: Linearity of Eletriptan hydrobromide**

Replicate	Concentrations of Eletriptan Hydrobromide (ng/band)				
	500	1000	1500	2000	2500
	Peak Area				
1.	2275.4	4344.1	6733.8	8263	10461.5
2.	2192.9	4312.8	6693.8	8320.6	10595.6
3.	2234.1	4404.6	6684.3	8354	10745.5
4.	2212.7	4396.7	6530.8	8291.6	10508.3
5.	2175	4486.1	6702.8	8471	10615.3
Mean	2218.02	4384.86	6669.1	8340.04	10585.24
STDEV	38.93	66.26	79.51	80.63	109.46
%RSD	1.75	1.51	1.19	0.96	1.03

**Figure 4: Calibration curve of Eletriptan hydrobromide**

**Table 3: Interday precision of Eletriptan hydrobromide**

Sr. no.	Concentration (ng/band)	Inter-day area	Inter-day mean area*	% recovery	SD	% RSD
1.	1000	4353.6 4446.8 4363.7	4388.7	100.53	50.67	1.15
2.	1500	6325.9 6410.5 6436.3	6390.9	99.07	57.75	0.90
3.	2000	8431.5 8340.4 8243.1	8338.3	98.11	94.21	1.12

**Table 4: Intraday precision of Eletriptan hydrobromide**

Replicate	Intraday Precision
1	4343.6
2	4446.8
3	4345.7
4	4358.4
5	4438.2
6	4326.3
Mean Area	<b>4376.5</b>
Standard Deviation	<b>52.20666624</b>
% RSD	<b>1.19</b>

**Table 5: Assay of Eletriptan hydrobromide**

Sr. no.	Peak area of Eletriptan Hydrobromide for 1000 ng/band	Amount Recovered (ng/band)	% Recovery
1.	4286.884	979.8	97.98
2.	4343.987	993.6	99.36
3.	4449.918	1019.2	101.92
4.	4293.919	981.5	98.15
5.	4233.919	967	96.7
6.	4272.402	976.3	97.63
<b>Mean</b>	<b>4313.505</b>	<b>986.233</b>	<b>98.62</b>
<b>SD</b>	<b>75.701</b>	<b>18.294</b>	<b>1.829</b>
<b>% RSD</b>	<b>1.755</b>	<b>1.854</b>	<b>1.854</b>

**Table 6: %Recovery Eletriptan hydrobromide**

Level	Theoretical Conc. (ng/band)	Area	Recovered Conc. (ng/band)	% Recovery (%)
80%	1800	8034.173	1785.18	99.18
100%	2000	8858.373	1973.8	98.69
120%	2200	9839.853	2175.507	99.92

**Table 7:Results of Robustness**

Sr. no.	Parameters	Robust condition	% RSD
1.	Chamber saturation time(15min) $\pm$ 2 min.	13 min 17 min	1.27 1.07
2.	Mobile phase composition Methanol : Toluene (9.5: 0.5 v/v) $\pm$ 0.2 Methanol	Methanol : Toluene (9.3: 0.7 v/v) $\pm$ 0.2 Methanol Methanol : Toluene (9.7: 0.3 v/v) $\pm$ 0.2 Methanol	1.11 1.56
3.	Time from Application to development (immediate)	After 30 Min. After 2 hrs.	1.61 1.81
4.	Time from Development to Scanning(immediate)	After 30 Min. After 2 hrs.	1.48 1.67

## DISCUSSION

This developed method is stability indicating. HPTLC method with 'high through put' advantage. There is no any SIM HPTLC method for Eletriptan Hydrobromide. Some stability indicating methods are reported on this drug by RP-HPLC but the percentage of degradation is not clearly mentioned. Considerable degradation for oxidative stressed condition was observed in one paper which was similar to that of our result. No degradation for acid stressed condition was observed in other paper where as we found the significant degradation. Current study confirms that Eletriptan hydrobromide does not degrade at mild conditions and degrades with 0.1 N HCl and H<sub>2</sub>O<sub>2</sub>(3%) conditions. Whereas it is thermo labile and degrades at higher temperature. Eletriptan hydrobromide is sensitive to UV radiation.

## CONCLUSION

The developed method was found to be simple, time saving, economic. This method can be used for stability study of Eletriptan hydrobromide.

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