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Estimation of Eletriptan Hydrobromide in Oral Film Dosage Form by RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate RP-HPLC method was developed for the percentage drug release estimation of Eletriptan Hydrobromide in oral film dosage form. Eletriptan Hydrobromide is used to treat severe migraine headaches and it's associated to nausea and sensitivity to light. Eletriptan Hydrobromide oral films were prepared by solvent casting method. It is available in market as conventional tablets (Relpax). An Xterra RP18 (150 × 4.6 with 5 microns particle size) and the mobile phase, consisting of KH_2PO_4 and triethylamine in water adjusting the pH-7.0 with o-phosphoric acid: acetonitrile in ratio of 98:2 v/v & acetonitrile: methanol HPLC Grade (80:20 v/v) was used as mobile phase in isocratic mode. The flow rate was 1.0 mL/min and the effluents were monitored at 225 nm. The retention time was 7.0 minutes and the detector response was linear in the concentration of 6.60-99.02µg/mL for Eletriptan Hydrobromide. The respective linear regression equation being Y = 11447 X + 5508 for Eletriptan Hydrobromide. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were not applicable for dissolution parameter. The method was validated by determining its accuracy, precision, linearity and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, linear and accurate, which is useful for the routine estimation of percentage drug release of Eletriptan Hydrobromide in oral film dosage form.

Keywords: Eletriptan Hydrobromide, Oral Films, HPLC, Dissolution.

INTRODUCTION

Eletriptan Hydrobromide is a second generation Triptan drug intended for treatment of migraine headaches. [1-5] Its mode of action is believed to reduce swelling of the blood vessels surrounding the brain. This swelling is associated with the head pain of a migraine attack. Eletriptan Hydrobromide is classified as a selective 5-hydroxytryptamine 1B/1D (5-HT1B/1D) receptor agonist. It is used for the treatment of acute headache phase of migraine attacks with or without aura. 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. Migraines are likely due to local cranial vasodilatation and/or to the release of sensory neuropeptides (vasoactive intestinal peptide, substance P and calcitonin gene-related peptide) through nerve endings in the trigeminal system. The therapeutic activity of Eletriptan for the treatment of migraine headache is thought to be due to the agonist effects at the 5-HT1B/1D receptors

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on intracranial blood vessels (including the arterio-venous anastomoses) and sensory nerves of the trigeminal system which result in cranial vessel constriction and inhibition of pro-inflammatory neuropeptide release. Eletriptan Hydrobromide, chemically is (R)-3-[(1-Methyl-2-pyrrolidinyl) methyl]-5-[2-(phenylsulfonyl) ethyl]-1H-indole monohydro bromide (Figure 1). The Empirical Formula is $C_{22}H_{26}N_2O_2S$. HBr and the Molecular Weight is 462.43 g/mol. [6]

Literature survey reveals few chromatographic methods to determine the Eletriptan Hydrobromide [4-5, 7] in tablet dosage form and also in biological fluids. So far, no dissolution methods by liquid chromatography were reported for the percentage drug release estimation of Eletriptan Hydrobromide in oral film dosage form at the time of commencement of these investigations. Eletriptan Hydrobromide fast dissolving oral films was prepared by solvent casting method. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of percentage drug release of Eletriptan Hydrobromide in oral film dosage form. [5, 7] A detailed account of all analytical methods existing for the drug is made to avoid duplication of the method developed. The authors had made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developing analytical methods, by using HPLC System. [4-9] The results of this labor of love are set forth by developing a simple, precise and accurate reverse-phase HPLC method [10-11] for the estimation of percentage drug release Eletriptan Hydrobromide in pharmaceutical oral film dosage forms.

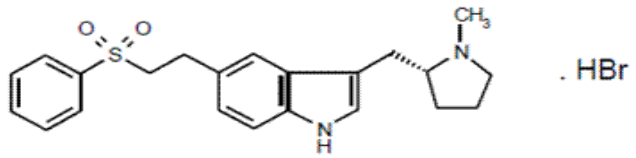


Fig. 1: Eletriptan Hydrobromide

MATERIALS AND METHODS

Materials

Eletriptan Hydrobromide was obtained as a gift sample from MSN labs Pvt Ltd, Hyderabad. Acetonitrile, Methanol and water used were of HPLC grade (Qualigens). Potassium dihydrogen phosphate, Triethylamine (TEA), Sodium hydroxide and orthophosphoric acid (88%) were obtained from SDFCL, Mumbai.

Methods

Preparation of pH 6.8 Phosphate buffer

Weigh and transfer 68.05 g of Potassium dihydrogen phosphate orthophosphate in 10 liters of water. Add 8.9 g of sodium hydroxide and mix. Adjust the pH to 6.8 ± 0.05 with 0.2 M sodium hydroxide.

Preparation of 0.2 M sodium Hydroxide

Weigh and dissolve 0.8 g of sodium hydroxide in 100 mL of water.

Chromatography Instrument

Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 10µL. An Xterra RP18 (150 × 4.6 mm i.d; particle size 5µ) was used. The HPLC system was equipped with Empower 2 Software. The column was maintained at 25°C and eluted under isocratic conditions over 12.0 min at a flow rate of 1.0 mL/min.

HPLC Conditions

For the preparation of the mobile phase; weigh accurately and transfer 2.72 g of potassium dihydrogen phosphate and add 2 mL of triethylamine in 1000 mL of milli–Q water and adjust pH 7.0 ± 0.05 with orthophosphoric acid (88%). Filter this solution through 0.45µ filter.

Mobile Phase- A: Buffer pH 7.0: acetonitrile (98:2 v/v)

Mobile Phase-B: Acetonitrile: methanol (80:20 v/v)

Preparation of the Primary Standard/Stock Drug Solution

Weigh and transfer Eletriptan Hydrobromide standard 50 mg into 25 mL volumetric flask, add 20 mL of diluent sonicate to dissolve and dilute to volume with diluent. Further dilute 4 mL of above solution to 100 mL with dissolution medium.

Dissolution parameters

Dissolution medium: pH 6.8 Phosphate buffer 0.05M

Volume: 300 mL

Apparatus: Basket

Speed: 50 RPM

Temperature: 37°C ± 0.5°C

Time points: 1, 2, 3, 5, 7 and 10 mins

Preparation of Sample solution

Arrange the dissolution apparatus as per above dissolution conditions, add one film to each of the six vessels and run the system for 10 minutes. Withdraw 10 mL of sample at the end of specific time interval and discard first 5 mL of the filtrate through 0.45µ Nylon filter, and collect sample solutions directly into vials from each of the bowl.

Linearity

Aliquots of standard Eletriptan Hydrobromide stock solution was taken in 25 mL volumetric flasks and diluted up to the mark with the dissolution medium. From the above standard stock solutions diluted to different concentrations such that the final concentrations of Eletriptan Hydrobromide was in the range of 6.60 -99.02µg/mL. Each of these drug solutions (10µL) was injected, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 225 nm and the calibration graph was obtained by plotting peak area versus concentration in µg/mL of Eletriptan Hydrobromide (Figure 2). The plot of peak areas of each sample against respective concentration of Eletriptan Hydrobromide was found to be linear in the range of 6.60-99.02µg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table 1. The regression characteristics, such as

slope, intercept & % RSD were calculated for this method and given in Table 1.

Accuracy

Accuracy was evaluated in triplicate by addition of three different amounts of Eletriptan Hydrobromide, to a previously analyzed sample and comparing the amounts of analytes recovered with the amounts added. The amounts added were equivalent to 50, 100, and 150% of the amount originally present. % Recovery and RSD (%) were calculated for amount added. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in Table 2.

Precision

The precision of the method was ascertained, separately from the peak area obtained by actual determination of six replicas of a fixed amount of the drug and formulation.

The HPLC system was set up, describing chromatographic conditions, mentioned as above and following the system equilibration of the working standard solution containing 66µg/mL of Eletriptan, by injecting six times and recording the response peak areas. The precision was repeated with the formulated sample for the same concentrations by injecting the working sample solutions containing 66.6µg/mL. The test sample was processed six times for the response of peak area. The % Relative Standard Deviation (RSD) were calculated and presented in Tables 3 & 4 respectively.

Method Applicability

The present developed method was evaluated by applying to oral film dosage forms for the estimation of Eletriptan Hydrobromide by our research group.

Dissolution

10µl of sample solution (Eletriptan oral film-20 mg) was injected into the injector of liquid chromatograph. The retention time was found to be 7.0 min for Eletriptan Hydrobromide. The amount of drug present per oral film was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

HPLC Method Development and Optimization [11]

In response to lack of simple, reliable and easy-to-use method for the determination of Eletriptan Hydrobromide concentrations in Oral films, a gradient RP-HPLC method was developed for quantification of Eletriptan Hydrobromide. We examined several HPLC method variables with respect to their corresponding effects on the result of analysis. To optimize the chromatographic conditions, different combinations of phosphate buffer with different pH 4.0, pH 7.0 with acetonitrile and methanol were used. From above Buffer peak shape was not satisfactory so triethylamine have been used. Based on several trials phosphate buffer with triethylamine pH 7.0 : acetonitile, acetonitrile: methanol were preferred for better peak shape and early retention time of Eletriptan Hydrobromide after several preliminary investigatory runs, compared with other mobile phases. The other parameters in this factorial design were different column, temperature, variation in flow rate, detection wavelength, buffer pH variation in mobile phase and injection volume. At 225 nm, λ max was observed and there are no interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram (Standard & Working Sample) of Eletriptan Hydrobromide has been shown in Figure 3 & 4. Parameters that were studied to evaluate system suitability were discussed and presented in Table 5.

Method Validation Tests

Recommended method validation characteristics including Method precision (RSD, %), Method accuracy (Recovery % and RSD, %) and Linear range (Correlation Coefficient were investigated systematically.

Table 1: Linear regression data of calibration curves

Parameter	Eletriptan Hydrobromide
Concentration range (µg/mL)	6.60 -99.02
Slope (m)	11447
Intercept (Y)	-4875
Standard error of estimate (c)	5508
Correlation coefficient (r)	0.9999
Linear regression (r^2)	0.9998

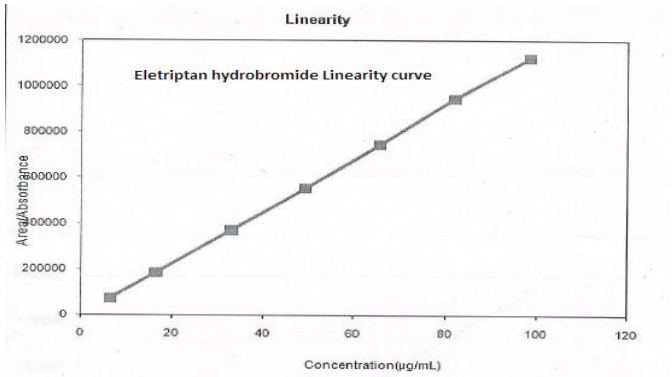
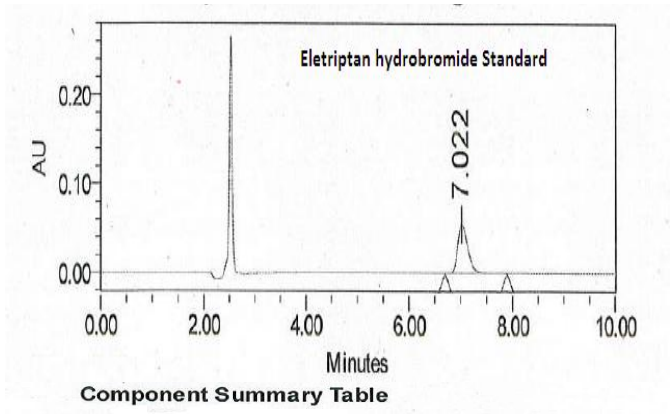


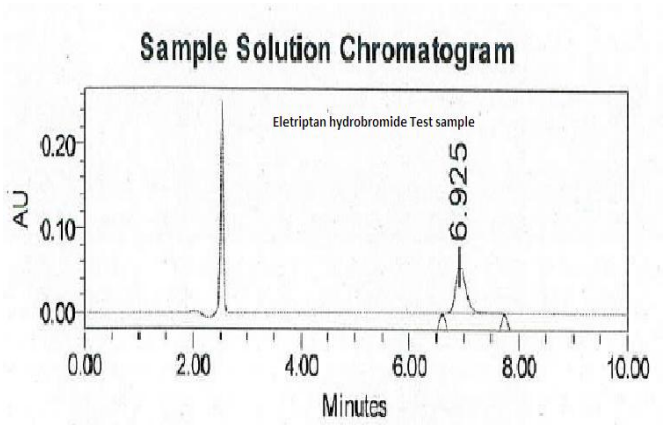
Fig. 2: Calibration curve of the Eletriptan Hydrobromide by RP-HPLC



Component Summary Table

Vial	Injection	Name	RT	Area	EP Plate Count	Symmetry Factor
1:2	2	Eletriptan	7.02	747431	6521	1.34

Fig. 3: Typical Chromatogram of Eletriptan Hydrobromide (Standard) by RP-HPLC



Component Summary Table

	Vial	Injection	RT	Area	EP Plate Count	Symmetry Factor
1	1:2	1	6.93	745218	6360	1.33

Fig. 4: Typical Chromatogram of Eletriptan Hydrobromide (Test Sample)

Linearity

The plot of peak areas of each sample against respective concentrations were found to be linear, in the range of 6.60-99.02µg/ml for Eletriptan Hydrobromide with correlation coefficient of 0.9999 (Table 1). Linear regression least square fit data obtained from the measurements are given in Table 1. The regression characteristics, such as slope and intercept were calculated for this method and given in Table 1. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1. These results show that there was an excellent correlation between peak areas and analyte concentration.

Accuracy

Recovery of the individual substances at 50%, 100%, and 150% of specified concentrations were between 98.9%-100.9%, which proves the accuracy of the method. From these data, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (Table 2).

Precision

The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (Table 3 & 4).

Robustness

Robustness was studied out to evaluate the effect of small but deliberate variations in the chromatographic conditions at three different levels, i.e. –2, 0, +2. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the columns, mobile phase flow rate by 10% of actual flow, mobile phase pH by ± 0.5 units (pH 6.5 and pH 7.5) had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. The results are shown in Table 6.

Specificity

No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical formulations were tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms.

Table 2: Assay & recovery accuracy studies of Eletriptan Hydrobromide in oral film dosage forms

Film formulation	Amount claim (mg/film) Eletriptan Hydrobromide	Amount Obtained (mg)* by proposed method Eletriptan Hydrobromide	** % Recovery by the Proposed method Eletriptan Hydrobromide
150%	30	29.8	99.33
100%	20	20.1	100.5
50%	10	10.13	101.3
Average Mean	20	20.06	100.38

*Average of three determinations

Accuracy parameter	Eletriptan hydrobromide
Assay (150%)	149.00 %
Assay (100%)	100.50%
Assay (50%)	50.65%

Table 3: Precision of Recommended Procedure Using API- { Eletriptan Hydrobromide } & its Oral Film

Sr. No	Inj. No	Name of the Standard Drug & Conc. (20.5 µg/mL)	Retention time in minutes	Peak Area	Name of the Sample Drug & Conc. (20.5 µg/mL)	Retention time in minutes	Peak Area
		API (Eletriptan Hydrobromide)			Formulation (Oral Film)		
1	1	Eletriptan Hydrobromide	7.02	747431	Eletriptan Hydrobromide oral film	6.93	745218
2	2	Eletriptan Hydrobromide	7.01	749824	Eletriptan Hydrobromide oral film	6.90	759830
3	3	Eletriptan Hydrobromide	7.02	739578	Eletriptan Hydrobromide oral film	6.89	750180
4	4	Eletriptan Hydrobromide	7.00	742650	Eletriptan Hydrobromide oral film	6.81	739028
5	5	Eletriptan Hydrobromide	7.01	741891	Eletriptan Hydrobromide oral film	6.94	739182
6	6	Eletriptan Hydrobromide	7.02	738690	Eletriptan Hydrobromide oral film	6.85	746102
7		Mean	7.01	743344	Mean	6.88	746590
8		Standard Deviation	0.008	4407.47	Standard Deviation	0.04	7776.48
9		% RSD	0.11	0.59	% RSD	0.71	1.04

RESULTS AND DISCUSSION

In the present investigation the estimation of percentage drug release in Eletriptan Hydrobromide oral films was successfully developed by using RP-HPLC. The proposed method was found to be simple, fast, robust, more precise and accurate under given experimental conditions. Therefore the developed method can be used for routine analysis of estimation of percentage drug release Eletriptan Hydrobromide in oral film dosage forms.

Table 4: Validation Summary / System Suitability

Parameter	Eletriptan hydrobromide (Standard API Drug)	Formulation (oral film)
Theoretical Plates(N)	6521	6360
Tailing factor	1.34	1.33
Retention time(min)	7.02	6.93
Area	747431	745218

Table 5: Results from testing of the Robustness of the method

Condition Studied in Robustness	Modification In analysis	Parameter Fixation	Mean Peak Area ± S.D	% RSD (Peak Area)	Mean Retention Time (in min) ± S.D	% RSD (Retention time)
			Eletriptan Hydrobromide	Eletriptan Hydrobromide	Eletriptan Hydrobromide	Eletriptan Hydrobromide
Column(s)	Intertsil C8 &	Standard	704321	0.5	7.31 ± 0.051	0.82
Xterra RP18	Hypersil BDS	Sample	694128	0.8	7.38 ± 0.068	0.90
		Standard-decrease	750213	0.4	7.12 ± 0.045	0.52
Flow rate	0.90 ml/min &	Standard-increase	731428	0.2	6.89 ± 0.056	0.42
(1.0 ml/min)	1.10 ml/min	Sample-decrease	749702	0.5	7.09 ± 0.031	0.49
		Sample-increase	729841	0.4	6.91 ± 0.019	0.68
		Standard-decrease	739015	0.8	7.12 ± 0.009	0.64
pH	6.8 & 7.2	Standard-increase	748952	0.8	6.98 ± 0.013	0.78
(7.0)		Sample-decrease	730019	0.6	7.09 ± 0.011	0.82
		Sample-increase	740268	0.5	7.01 ± 0.037	0.85

The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the oral films. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of Eletriptan Hydrobromide and can be used for routine analysis in pharmaceutical quality control within a short time.

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