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RP-HPLC Method for the Estimation of Eletriptan in Pharmaceutical Dosage Forms

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ABSTRACT

A reverse phase high performance liquid chromatographic method has been described for the estimation of eletriptan in its pharmaceutical formulations using an inertsil ODS C-18, 5 µm column having 250 mm × 4.6 mm I.D., in isocratic mode using acetonitrile:methanol:0.01M phosphate buffer in the ratio of 40:40:20 v/v/v. The detection was carried out using UV detector at 251 nm. Linearity of eletriptan was found to be in the concentration range of 200 to 1000 µg/ mL. The flow rate was 1.0 mL/min and the run time was 10 min. The developed method was validated with respect to linearity, precision, accuracy and specificity as per the International Conference on Harmonisation (ICH) guidelines. The mean recoveries were found to be with in the limits. The developed method was simple, fast, accurate and precise and has been successfully applied for the analysis of eletriptan in bulk sample and in pharmaceutical dosage forms.

Keywords: Eletriptan, HPLC, Estimation, Linearity.

INTRODUCTION

Eletriptan hydrobromide is a novel, orally active, selective serotonin 5-$HT_{1B/1D}$ receptor agonist and is second generation anti-migraine drug [1]. Eletriptan hydrobromide is chemically designated as (R)-3-[(1-methyl-2-pyrrolidinyl)methyl]-5-[2-(phenylsulfonyl)ethyl]-1*H*-indole monohydrobromide (Fig. 1). Eletriptan hydrobromide used for the treatment of acute migraine headaches. Its pharmacological effects include the constriction of cerebral blood vessels and neuropeptides secretion blockade which eventually relieves the pain [2]. The pharmacokinetics and metabolism of eletriptan have been investigated in the rat, dog and human. In all three species, eletriptan was rapidly absorbed and extensively cleared by metabolism. The pathways of eletriptan metabolism are similar in the rat, dog and human and principal routes include pyrrolidine *N*-demethylation to *N*-desmethyl eletriptan, together with *N*-oxidation, oxidation of the pyrrolidine ring and formation of tetracyclic quaternary ammonium metabolites [3].

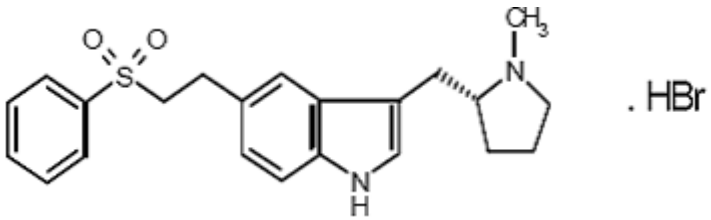

Fig.-1: Structure of Eletriptan hydrobromide

Literature survey revealed that very few analytical methods have been reported for the determination of eletriptan in pure drug, pharmaceutical dosage forms and in biological samples using HPLC [4,5] and LC-MS [6] techniques. The aim of the present work is to develop and validate a simple, fast, reliable and appropriate chromatographic method with UV detection for the determination of eletriptan in bulk drug and in pharmaceutical formulations. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) guidelines [7] for the determination of eletriptan in bulk sample and in tablet dosage forms.

MATERIALS AND METHODS

Drugs and Chemicals

Acetonitrile and methanol (HPLC grade) were purchased from Merck Specialities Pvt. Ltd, Mumbai, India. Water (HPLC grade) was purchased from Loba Chemie, Mumbai, India. All other reagents used in the study were of AR grade. Eletriptan hydrobromide was kindly supplied by R.V. Labs, Guntur, India.

Instruments

A high performance liquid chromatograph (Shimadzu HPLC class VP series) with binary LC-20 AT VP pumps, variable wave length detector SPD-20 A VP, SCL-20 A VP system controller (Shimadzu) and a reverse phase inertsil ODS C-18 column (250 mm × 4.6 mm I.D., 5 µm particle size) was used for the estimation. The HPLC system was equipped with the software Class VP series version 5.03 (Shimadzu). All weighings were done on electronic balance (Shimadzu AY-120).

Chromatographic conditions

The mobile phase consisting of acetonitrile, methanol and 0.01M phosphate buffer (KH_2PO_4, pH adjusted to 4.4 with orthophosphoric acid) were filtered through 0.45 µ membrane filter, degassed and were pumped from the solvent reservoir in the ratio of 40:40:20 % v/v/v and was pumped into the column. The flow rate of mobile phase was maintained at 1.0 mL/min and detection was done by using UV detector at 251 nm with a run time of 10 min. The volume of injection loop was 20 µL prior to injection of the drug solution the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Procedure

About 1 gm of eletriptan hydrobromide was accurately weighed and dissolved in methanol and finally makes up the volume up to 100 mL in volumetric flask with methanol so as to give 10 mg/mL solution. Subsequent dilution of this solution was made to obtain 100 µg/ mL. Linearity solutions containing 200, 400, 600, 800 and 1000 µg/mL were prepared from the above stock solution. Initially the mobile phase was pumped for 30 min to saturate the column there by to get the baseline corrected. Then solutions prepared as above filtered through 0.45 µ membrane filter and then 20 µL of the filtrate was injected each time in to the column at a flow rate of 1.0 mL/min. Evaluation of the drug was performed with UV detector at 251 nm. The peak area for each of the drug concentrations was calculated. The plot of peak area vs the respective drug concentration gives the calibration curve. The recovery studies were carried out by adding a known amount of eletriptan hydrobromide to the pre analyzed samples and subjecting them to proposed HPLC method.

Estimation of eletriptan hydrobromide in pharmaceutical dosage forms

Commercial formulations of eletriptan hydrobromide were not available in local market. Two formulations of eletriptan hydrobromide were prepared in-house. Twenty tablets each containing 40 mg of eletriptan hydrobromide were accurately weighed and powdered. From this powder mixture, an amount of the tablet powder equivalent to 40 mg of eletriptan hydrobromide was transferred to a 100 mL standard volumetric flask. A small amount of methanol was added and sonicated to dissolve. The volume was made up with methanol, filtered with a 0.45 µ membrane filter and the above filtrate solution was diluted to 100 mL with methanol and 20 µL of tablet sample solution was injected each time into the HPLC system and a chromatogram was obtained at a flow rate of 1.0 mL/min. The injections were repeated six times and the peak areas were recorded. The mean value of the peak area was calculated and the drug content in each tablet was quantified using the regression equation. The same procedure was followed for the estimation of eletriptan hydrobromide for both in-house formulations of tablet dosage forms.

RESULTS AND DISCUSSION

The typical chromatogram for the proposed method is shown in Fig. 2.

Linearity

A good linear relationship (r=0.9998) was observed with a concentration range of 200-1000 µg/mL. The regression equation was constructed by linear regression fitting and its mathematical expression was y=1219.9+199.19x, where y is the peak area and x is the concentration of eletriptan (µg/mL). It was found that correlation coefficient and

regression analysis are within the limits. The peak areas of different concentrations are shown in Table 1. The peak areas of eletriptan hydrobromide were reproducible, as indicated by a low coefficient of variation.

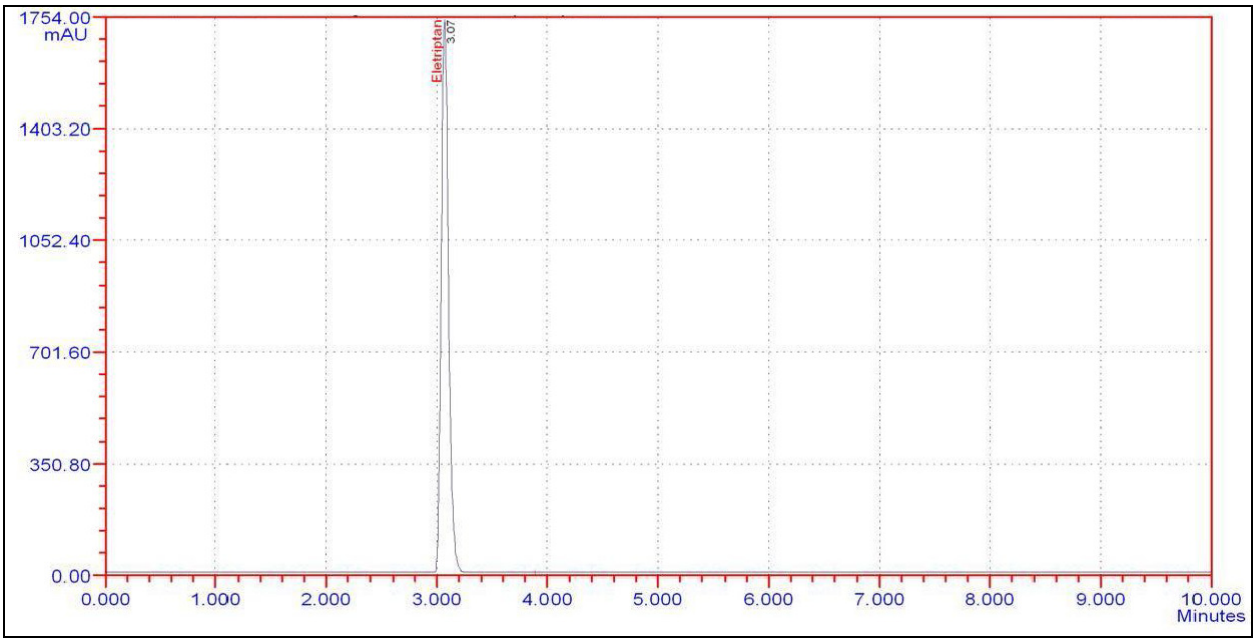

Fig.-2: RP-HPLC chromatogram for eletriptan hydrobromide

Table-1: Standard graph for the estimation of eletriptan hydrobromide

Concentration of eletriptan hydrobromide (µg/mL)	Peak area
200	41003
400	81120
600	119227
800	163151
1000	199182

Specificity

Placebo, blank and sample run were carried out to determine the specificity of the chromatographic method developed for eletriptan. The chromatograms indicate that the placebo (which had the excipients of the tablet formulation but not the drug) did not show any peak, indicating that there was no interference with or suppression of the peak at the retention time of eletriptan due to the commonly used tablet excipients.

Ruggedness

Ruggedness was established by determining eletriptan in the tablet formulation using two different chromatographic systems (Shimadzu, HPLC binary LC-20 AT VP pumps with SPD-20 A VP UV detector) and two different analysts. The RSD for analyst and inter-system variations were 0.63-1.22 % (limit < 2.0 %) and 0.84-1.38 % (limit < 2.0 %), respectively. This indicates that the method was rugged.

Robustness

Robustness of a method is its ability to remain unaffected by small deliberate variations in the method parameters. The following changes in the optimum parameter values were examined, the flow rate of the mobile phase (adjusted by ± 0.02 mL/min) and the detection wave length (adjusted by ± 1 nm).

System suitability

For the determination of eletriptan, different compositions of mobile phases were employed. Finally the ratio was fixed to acetonitrile, methanol and 0.01M phosphate buffer (KH_2PO_4, pH adjusted to 4.4 with orthophosphoric acid) in the ratio of 40:40:20 % v/v/v, where eletriptan was eluted at 3.07 minutes with symmetric peak shape and shorter retention time. The system suitability parameters were given in Table 2.

Table-2: Summary of validation parameters

System suitability	Results
Theoretical plates (N)	8284
Linearity range (µg/mL)	200-1000
Retention time (min)	3.07
Tailing factor	1.68
Correlation coefficient	0.9998
LOD (µg/mL)	0.080
LOQ (µg/mL)	0.120

Intra-day and inter-day precision

The precision was determined in terms of intra-day and inter-day precision. For intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and RSD was 1.14 % (limit RSD < 2.0 %). In addition, the day-to-day (inter-day) precision was studied by injecting the same concentration of standard solution on consecutive days and the RSD was 1.14 % (limit RSD < 2.0 %). The results are provided in Table 3.

Table-3: Intra- and inter-day precision

Concentration of eletriptan (1000 µg/mL)	Peak area	
	Intra-day	Inter-day
Injection 1	183705	183891
Injection 2	184352	184635
Injection 3	186732	186947
Injection 4	184869	184963
Injection 5	184640	184831
Average	184859	185053
Standard Deviation	1134.1	1136.8
% RSD	1.14	1.14

**RSD= relative standard deviation*

Accuracy

The accuracy of the method was assessed by recovery of eletriptan in the dosage formulation at three different concentration levels (50, 100 and 150 %) with reference to label claim of tablet. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the percentage recovery. Recoveries ranged from 98.36 to 99.55 %. The results are furnished in Table 4.

Table-4: Accuracy data

Concentration (Spike level)	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50 %	300	305	98.36	99.29 %
100 %	600	602	99.66	
150 %	900	904	99.55	

Limit of detection (LOD) and limit of quantification (LOQ)
The limit of detection (LOD) for eletriptan was 0.080 µg/mL while the limit of quantification (LOQ) for eletriptan was 0.120 µg/mL.

Analysis of in-house formulations
The amount of the drug present in the tablet dosage forms were calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 5.

Table-5: Assay of tablet formulations

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Formulation-1	40	40.12	99.70
Formulation-2	40	39.86	100.35

In the proposed method, the retention time for eletriptan hydrobromide was found to be 3.07 minutes. Quantification was linear in the concentration range of 200-1000 µg/mL. The regression equation of the linearity plot of concentration of eletriptan over its peak area was found to be y=1219.9+199.19x, where y is the peak area and x is the concentration of eletriptan (µg/mL). The number of theoretical plates calculated was 8284, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.080 µg/mL and 0.120 µg/mL respectively, which indicate the sensitivity of the method. The use of acetonitrile, methanol and 0.01M phosphate buffer pH 4.4 in the ratio of 40:40:20 % v/v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. The absence of additional peaks indicates no interference of the excipients used in the tablets.

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and sensitive. This developed method can be used for routine analysis of drug in bulk sample as well as in tablet dosage forms.

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REFERENCES

1. Milton K.A., Scott N.R., Allen M.J., Abel S., Jenkins V.C., James G.C., Rance D.J., Eve M.D., J Clin Pharmacol, **42**(2002)528.
2. Evans D.C., O'Connor D., Lake B.G., Evers R., Allen C., Hargreaves R., Drug Metab Dispos., **31**(2003)861
3. Morgan P., Rance D., James C.G., Milton K.A. ,Headache, **37**(1997)324
4. Zecevic M., Jocic B., Agatovonic-Kustrin S., Zivanovic L., J. Serb. Chem. Soc.,**71**(2006) 1195.
5. Cooper J.D.H., Muirhead D.C., Taylor J.E., J. Pharm. Biomed. Anal.,**21**(1999) 787.
6. Jocic B., Zecevic M., Zivanovic L., Protic A., Jadranin M., Vajs V., J. Pharm. Biomed. Anal.,**50**(2009) 622
7. ICH topic Q2 (R1), Validation of analytical procedures: text and methodology ,**62**(1997)27463.

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