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HPLC Method for the Enantiomeric Purity of Eletriptan Hydrobromide

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An isocratic chiral stationary phase high-performance liquid chromatographic (CSP-HPLC) method has been developed and validated for the quantitation of (S)-isomer in eletriptan hydrobromide. Separation was achieved with a Chiralpak AD column. The ratio of *n*-hexane, ethanol, diethyl amine and trifluoroacetic acid in the mobile phase were optimized to obtain the best separation. UV detection was performed at 223 nm. The described method is linear over a range of limit of quantification - 1.5 µg/mL of (S)-isomer. The mean recovery of (S)-isomer was found to be in the range of 101-102 %. The method is simple, rapid, accurate, selective and precise, useful in the quality control of bulk manufacturing.

Key Words: Column liquid chromatography, Elitriptan, (S)-isomer, Enantiomeric purity, Validation.

INTRODUCTION

Eletriptan (also known as eletriptan hydrobromide), a single enantiomer (R)-5-[2-(phenylsulfonyl)ethyl]-3-[(1-methyl-2-pyrrolidinyl)methyl]-1*H*-indole, is a second generation triptan drug marketed and manufactured by Pfizer Inc. for the treatment of migraine headaches. It is sold in the US under the brand name RELPAX®. Eletriptan was approved by the U.S. Food and Drug Administration (FDA) on December 26, 2002 for the acute treatment of migraine with or without aura in adults.

Separation of enantiomers has become very important in analytical chemistry, especially in the pharmaceutical and biological fields, because some stereoisomers of racemic drugs have quite different pharmacokinetic properties and different pharmacological or toxicological effects[1,2]. This is one of the most vital reasons why the regulatory authorities insist more on stringent investigation for evaluating the safety and the effectiveness of drugs containing chiral centers. Most of the pharmaceutical industries are now concentrating towards the study of the therapeutic effect of pure enantiomers of the existing drug molecules. A control and accurate quantification of undesired enantiomers in active pharmaceutical ingredient is essential[3], in this connection HPLC is generally opted for this purpose.

Till now, a few procedures based on liquid chromatography have been reported for the quantitative determination of eletriptan. Biljana *et al.*[4] have published a study of forced degradation behaviour of eletriptan hydrobromide by LC and LC-MS. Cooper *et al.*[5] have suggested a method, for the determination of eletriptan in plasma and saliva using automated sequential trace enrichment of dialysate and high-performance liquid chromatography. Sagirli *et al.*[6] have reported a method for liquid chromatography assay of eletriptan in tablets and *in vitro* dissolution studies. To the best of our knowledge no chiral HPLC method is reported in the literature for the enantiomeric separation of eletriptan isomers. Therefore, the aim of this study is to develop a chiral HPLC method for the determination of enantiomeric purity by accurate quantification of un-required (S)-isomer of eletriptan and validate as per the ICH guideline[7].

EXPERIMENTAL

Samples of eletriptan hydrobromide and eletriptan (S)-isomer (Fig. 1) were synthesized at SMS Pharma Research Centre (Hyderabad, India). HPLC grade *n*-hexane is obtained from Merck (India). HPLC grade ethanol is obtained from Brampton, Ontario L6T 3Y4 (Canada). Analytical grade diethyl amine (DEA) and trifluroacetic acid (TFA) are purchased from Merck (India).

The HPLC system consisted of quaternary gradient pump, auto sampler, column oven and a variable wavelength detector. The output signal was monitored and integrated using EZ-Chrom Elite Chromatography Data Software (1200 series HPLC, Agilent, USA).

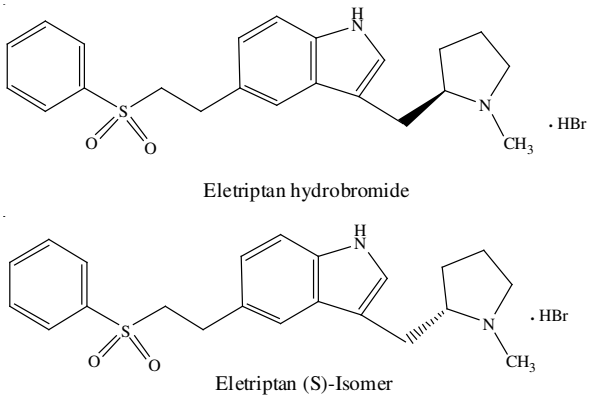

Fig. 1. Structural formula for eletriptan hydrobromide and (S)-isomer

Preparation of standard solutions: Dissolved an accurately weighed quantity of (S)-isomer working standard in minimum quantity of ethanol and made up with mobile phase to obtain a solution having known concentration of about 0.001 mg/mL and injected in to the system. Dissolved an accurately weighed quantity of eletriptan hydrobromide sample in minimum quantity of ethanol and made up with mobile phase to obtain a solution having known concentration of *ca.* 0.2 mg/mL and injected in to the system.

Chromatographic conditions: A Chiralpak AD chiral stationary phase analytical column (250 mm × 4.6 mm, 10 µm packing) (Diacel) was used. A mixture of *n*-hexane, ethanol, diethylamine and trifluoroacetic acid in the ratio of 80:20:0.1:0.1 (v/v/v/v) was used as mobile phase. It was filtered through a 0.45 µm-nylon membrane using a Millipore vacuum filtration system. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 20 µL. The detector was set to a wavelength of 223 nm.

RESULTS AND DISCUSSION

The aim of this work is to separate the enantiomers of eletriptan and accurate quantification of unrequired (S)-isomer. The racemic mixture, prepared by dissolving an accurately weighed quantities of eletriptan hydrobromide and (S)-isomer in minimum quantity of ethanol and made up with mobile phase to obtain a solution having known concentration of 0.2 and 0.05 mg/mL, respectively, was used during the method development. To develop a rugged and suitable LC method for the enantiomeric separation of eletriptan, different mobile phases and stationary phases were employed. Three different chiral stationary phase columns were employed during method development namely chiralcel OJ, chiralcel OD and chiralpak AD of Daicel. All the columns chosen were of 250 mm length, 4.6 mm internal diameter and 10 µm particle size. The chiral stationary phase in chiralcel OJ, chiralcel OD and chiralpak AD are cellulose *tris* (4-methyl benzoate), cellulose *tris*(3,5-dimethylphenyl carbamate) and amylose *tris*(3,5-dimethylphenyl carbamate), respectively, coated on silica gel. The mechanism of separation in direct chiral separation method is the interaction of chiral stationary phase (CSP) with analyte enantiomers to form a short lived, transient diastreomeric complexes[7]. Various experiments were conducted, to select the best combination of stationary and mobile phase that would give optimum resolution and selectivity for the enantiomers. Baseline chromatographic separation was not achieved on a chiralcel OD column using the mobile phase *n*-hexane:ethanol: diethyl amine:trifluoroacetic acid (80:20:0.1:0.1, v/v/v/v) and (S)-isomer eluted after eletriptan. Very good separation was achieved on chiralcel OJ with a resolution greater than 2.5 using the same mobile phase, but the peaks are not symmetrical. When the same mobile phase was employed on chiralpak AD, symmetrical peaks are found with a resolution greater than 3.5 and interestingly, (S)-isomer was eluted prior to eletrptan. Since both chiralcel OJ and chiralcel OD columns have same cellulose-based stationary phase, it showed same chiral recognition abilities for the enantiomers of eletriptan. The reversal of elution order of eletriptan enantiomers on chiralpak AD could be due to amylose-based stationary phase. Having obtained better chromatographic results on the chiralpak AD column, the method validation was carried out on the same. The enantiomeric separation of eletriptan hydrobromide on chiralcel OJ, chiralcel OD and chiralpak AD columns is shown in Fig. 2.

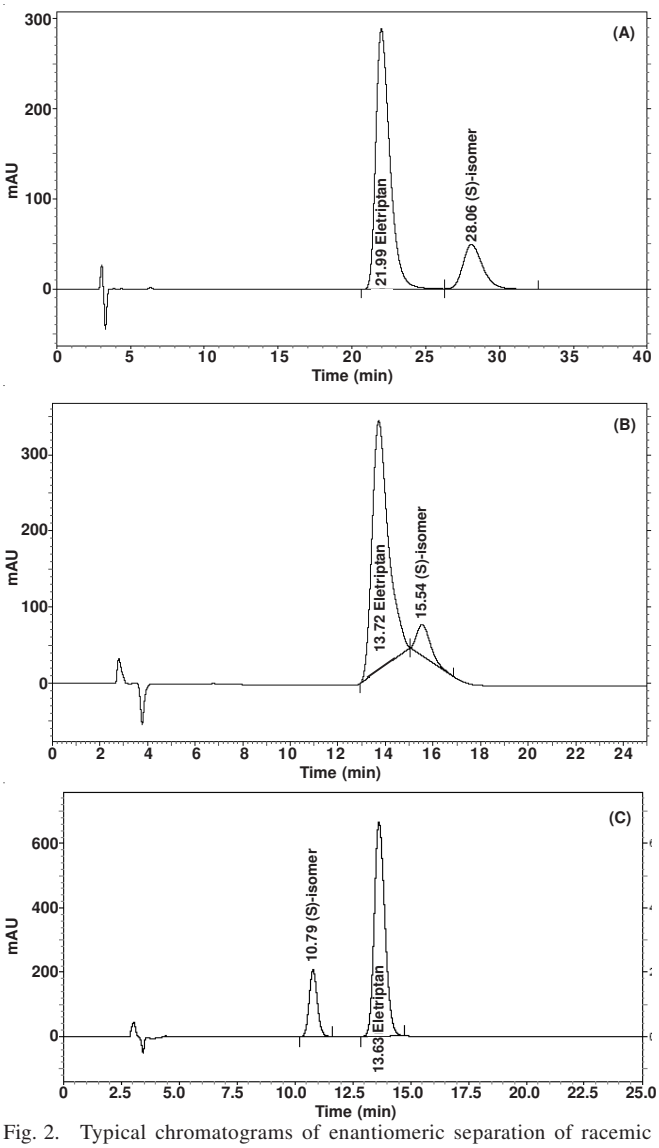

Fig. 2. Typical chromatograms of enantiomeric separation of racemic eletriptan on (A) chiralcel OJ, (B) chiralcel OD and (C) chiralpak AD columns; mobile phase composed of *n*-hexane:ethanol: diethylamine:trifluoroacetic acid (80:20:0.1:0.1, v/v/v/v); flow rate 1.0 mL/min; UV-223 nm

Quantification of (S)-isomer: Known concentration of standard solution (0.001mg/mL) was used for the quantification of (S)-isomer in eletriptan hydrobromide sample (0.2 mg/mL). Not more than 0.50 %m/m of (S)-isomer is found in eletriptan hydrobromide.

Method validation

Specificity: Eletriptan hydrobromide and (S)-isomer were injected separately to confirm the retention times. System suitability solution was then injected. (S)-isomer and eletriptan peaks are eluted at 10.57 minutes and 13.41 min, respectively (relative retention 0.8). The resolution between the peaks was found to be 3.8. The asymmetry for (S)-isomer and eletriptan peaks are 1.1 and 1.2, respectively.

Linearity: Standard solutions of 10 different concentration levels ranging from 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.75, 1.00, 1.25 and 1.50 µg/mL were prepared (0.025-0.75 % of analyte concentration of 0.2 mg/mL). Each sample solution was injected in triplicate. The mean responses recorded were plotted against concentration. The correlation coefficient for (S)-isomer was found to be 0.99996, which indicated good linearity. The calibration equation for (S)-isomer was found to be y = 106814x - 686.7.

Accuracy: Eletriptan hydrobromide sample was spiked with (S)-isomer at 0.25, 0.50 and 0.75 % of analyte concentration of 0.2 mg/mL. Each spiked solution was prepared in triplicate and injected. The mean recoveries, recovery percentage and % RSD were calculated. The mean recoveries of (S)-isomer at each spike solution with 95 % confidence interval are found to be 102 ± 0.5, 101 ± 0.5 and 101±0.4 %, respectively. Accuracy results are shown in Table-1. The acceptance criteria for recovery at each level are between 80 and 120 % as per in-house validation protocol.

TABLE-1
ACCURACY RESULTS FOR (S)-ISOMER

(S)-isomer spike level (%, m/m)	Added (µg) (n = 3)	Recovery (µg)	Recovery (%)	Mean recovery	% RSD	95 % Confidence interval
0.25	25.03	25.76	102.9	102	0.40	102 ± 0.5
		25.55	102.1			
		25.62	102.4			
0.50	50.06	50.24	100.4	101	0.44	101 ± 0.5
		50.22	100.3			
		50.62	101.1			
0.75	75.09	75.57	100.6	101	0.35	101 ± 0.4
		76.09	101.3			
		75.89	101.1			

Precision: Repeatability was demonstrated by injecting six individual spiked test preparations of eletriptan hydrobromide (0.2 mg/mL). Intermediate precision was demonstrated by analyzing same preparations of eletriptan hydrobromide by two different analysts on two different days. Intra-day variations of (S)-isomer content in eletriptan hydrobromide are expressed in terms of % RSD values. The values calculated were found to be 1.3 for repeatability, 0.9 and 0.4 % for intermediate precision. Repeatability and intermediate precision results are shown in Table-2.

TABLE-2
PRECISION RESULTS FOR (S)-ISOMER

Repeatability	
Mean of (S)-isomer content (%, m/m) (n = 6)	0.237
Standard deviation (SD)	0.003
%RSD	1.3
Intermediate Precision	
Analyst-1/Day-1	
Mean of (S)-isomer content (%, m/m) (n = 6)	0.237
Standard deviation (SD)	0.003
%RSD	1.3
Analyst-2	
Mean of (S)-isomer content (%, m/m) (n = 6)	0.234
Standard deviation (SD)	0.002
%RSD	0.9
Overall %RSD (n = 12)	1.3
Day-2	
Mean of (S)-isomer content (%, m/m) (n = 6)	0.232
Standard deviation (SD)	0.001
%RSD	0.4
Overall %RSD (n = 12)	1.7

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection and limit of quantification for (S)-isomer was calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve. A typical S/N ratio of 2-3 and 9-10 are generally considered to be acceptable for LOD and LOQ respectively. LOD and LOQ values are found to be 0.0147 and 0.0489 µg/mL, respectively.

Robustness: In order to demonstrate the robustness of the method, chromatographic conditions were deliberately altered and the resolution was checked between (S)-isomer and eletriptan peaks. To study the effect of variation of flow rate on the resolution, 0.1 units of flow were changed from 1.0 mL min^{-1} (*i.e.* 0.9 and 1.1 mL min^{-1}). The effect of column temperature on resolution was studied at 22 and 32 ºC instead of 27 ºC. In all the above varied conditions, the composition of the mobile phase was held constant as those of the initial condition. To study the robustness in terms of variation in the mobile phase composition, the ethanol composition was varied (± 2 %) keeping the chiral mobile phase additive solution composition as such.

In all the deliberate varied chromatographic conditions carried out (flow rate, temperature and mobile phase composition) the resolution between (S)-isomer and eletriptan was greater than 3.5, illustrating the good robustness of the method.

Batch analysis: The (S)-isomer content in three different batch samples of eletriptan hydrobromide was determined and found to be less than 0.5 % m/m. Other related substances were evaluated by a reverse phase HPLC method, known and unknown impurities (any other impurity) are less than 0.10 % and total impurities are less than 1.0 % (excluding (S)-isomer content by chiral stationary phase HPLC). The chromatograms showing real sample and real sample spiked with (S)-isomer (0.5 %, m/m) are shown in Fig. 3.

Conclusion

The present paper describes the development of a new HPLC method for the quantitation of (S)-isomer in eletriptan hydrobromide and its validation. The method was found to be

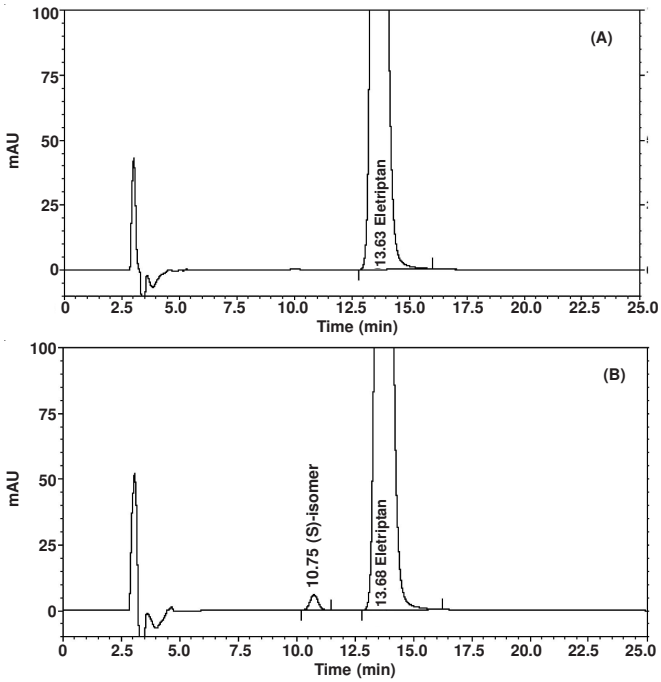

Fig. 3. Typical chromatograms for eletriptan hydrobromide (A) real sample and (B) real sample spiked with (S)-isomer (0.5 %, m/m)

selective, sensitive, precise and accurate for the quantitation of (S)-isomer. This method can be used for the routine analysis as well as for stability studies to evaluate S→R, R→S isomerization in pharmaceutical quality control.

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