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A validated chiral HPLC method for the determination of frovatriptan and its enantiomer in drug substance

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ABSTRACT

A simple direct Chiral HPLC method was developed for the separation of the enantiomers of Frovatriptan Succinate[R-(+)-6-Carboxamido-3-N-methylamino-1,2,3,4-tetrahydrocarbazole hemisuccinate], an antimigraine drug. The enantioseparation was achieved on a Protein based Cellobiohydrolase column (Chiral-CBH) using an isocratic mobile phase consisting of 10mM dihydrogen orthophosphate buffer and methanol in the ratio of 92:8. Flow rate was kept at 0.6 ml/min. The resolution between the enantiomers was 4.4. The developed method was capable of detecting the S-enantiomer up to 6 ng/ml. The method was validated in terms of Selectivity, Linearity, Precision, Accuracy, Robustness, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

KEYWORDS

Frovatriptan;
Enantiomer separation;
Cellobiohydrolase;
Chiral HPLC.

INTRODUCTION

Frovatriptan Succinate, a single enantiomer drug [(R)-(+)-6-Carboxamido-3-N-methylamino-1,2,3,4-tetrahydrocarbazole hemisuccinate] is used for the treatment of severe migraine. Similar to other members of triptan family, Frovatriptan is a serotonin (5-HT) receptor agonist that binds with high affinity to the 5-HT1B and 5-HT1D receptors. Frovatriptan causes vasoconstriction of meningeal, dural and cerebral vessels after binding to the 5-HT1B receptor. Unlike other triptans, Frovatriptan is a partial agonist to the 5-HT1B receptor and it is the most potent triptan in producing contraction of human basilar arteries[1]. Frovatriptan can be taken without regard for food and there is no need for dosage adjustment in the elderly or in women taking a combined oral contraceptive.

The determination of enatiomeric purity of Frovatriptan is very important as the drug is marketed as single enatiomer drug. A CE method for the estimation of chiral purity of Frovatriptan was reported[2]. As per our knowledge there is no validated chiral HPLC method reported in the literature

In the present study separation of Frovatriptan enantiomers was attempted by using protein based columns. The main reason for selecting protein based column was due to the broad applicability of these columns. Chiral AGP and CBH are the two widely used protein based chiral HPLC columns. As these columns can be used in reverse phase mode the method development is much easier by controlling the enantioselectivity by change of pH of the mobile phase and the concentration of the organic modifier in the mobile phase.

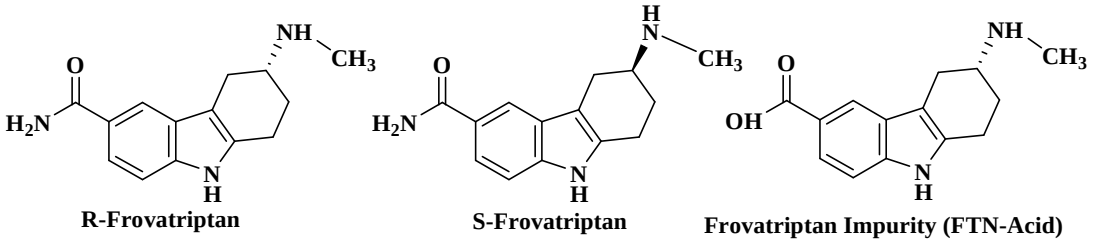

Figure 1: Chemical structure of (R)-frovatriptan, (S)-frovatriptan and frovatriptan impurity (FTN-Acid)

EXPERIMENTAL

Chemicals

Samples of Frovatriptan, (R) and (S) enantiomers of Frovatriptan and Frovatriptan impurity (FTN-Acid) were provided by Chemical Research and Development of Orchid Chemicals Ltd, R&D Center, Chennai.

Equipment

A HPLC (Waters Model Alliance 2695 separation module) equipped with an autosampler, column heater and photodiode array UV detector (Waters 996) was used for the development and validation of the method. The analysis was carried out on a Chiral-CBH column, 100×4.0mm, 5µm particle size (Chrom Tech make). Waters Millennium^{32} (ver. 4) software was used for recording and processing the data.

Chromatographic conditions

The Chromatographic conditions were optimized using a Chiral-CBH (100 × 4.0 mm, 5µ) connected through a guard column of 10 × 4 mm length. The mobile phase was 10 mM potassium dihydrogen orthophosphate buffer and methanol (92:8 v/v). The flow rate was 0.6 ml/min. The detection was carried out at a wavelength of 245 nm. The injection volume was 5µl.

3. RESULTS AND DISCUSSION

3.1 Method development

Separation of the enantiomers of Frovatriptan by Chiral HPLC column was the objective of the present study. Racemic Frovatriptan was used for the initial separation study. The sample was prepared in mobile phase with an analyte concentration of 0.1 mg/ml. Initially separation attempts were made using carbohydrate based chiral columns such as CHIRALCEL OD-H and CHIRALPAK AD-H[2-3] in normal phase mode, using various combinations of n-hexane and 2-propanol. As these columns were not showing any chiral selectivity, separation was then tried with protein based Chiral-AGP[4] column in reverse phase mode with a combination of buffer, methanol and acetonitrile. The enantiomeric separation could not be achieved in this case also. Trials were then made with Chiral-CBH (Cellobio hydrolase) column[5-9]. Chiral-CBH was found to be showing selectivity between the two enantiomers. A mobile phase with a mixture of 10 mM potassium dihydrogen orthophosphate and methanol was used in various ratios to optimize the resolution of the enantiomers. It was found that content of methanol in the mobile phase was playing a major role in the resolution of enantiomers. An increase of methanol content in the mobile phase increased the resolution. As the content of methanol in the mobile phase is restricted to a maximum of 10% by the column manufacturer, a composition of 92:8(v/v) of buffer and methanol was fixed. The flow rate was fixed as 0.6 ml/min. The elution was monitored with UV detector at a wavelength of 245nm.

In the optimized method the resolution between the enantiomers was found to be about 4.4 with a USP tailing factor of about 1.1 for both the enantiomers. Typical retention times of (S) and (R) enantiomers were found to be 6.5 and 10.5 minutes, respectively. A potential impurity of Frovatriptan (FTN-Acid) was injected in this optimized condition and found to be separating well from the analyte peaks. The structure of R and S enatiomers and Frovatriptan impurity (FTN-Acid) are displayed in figure 1. The chromatograms of mixture of R and S enatiomers, R enatiomer and R enantiomer spiked with impurity are displayed in figures 2-4 respectively. As per ICH guidelines[10] the method was validated in terms of the following parameters.

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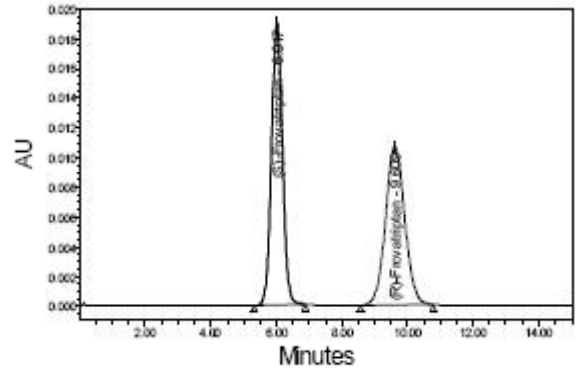

Figure 2: Chromatogram of (R) and (S) frovatriptan

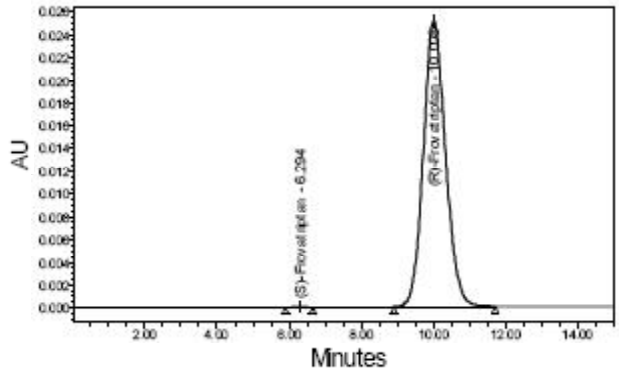

Figure 3: Chromatogram of (R)-frovatriptan

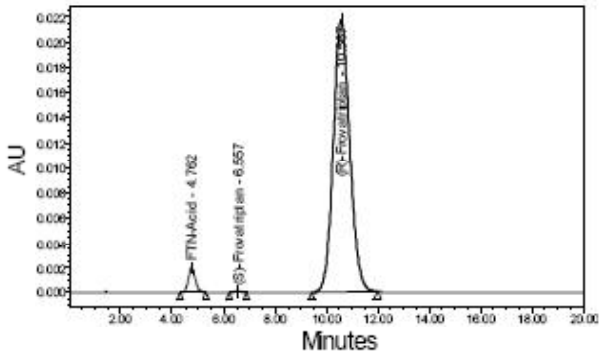

Figure 4: Chromatogram of (R)-frovatriptan spiked with FTN-acid

3.2 Validation

1. System suitability

The resolution between (S) and (R) enantiomers has been fixed as the criteria for evaluating the suitability of the test method. The resolution was found to be 4.4 between the two enantiomers. Other parametes like USP tailing and USP tangent were also evaluated during the course of study. A value of 1.11 and 1.17 was obtained as tailing factor for (S) and (R) enantiomers, respectively. The tangents were found to be about 2000 for (S)-enantiomer and 1700 for (R)-enantiomer.

Selectivity

The ability of the method to measure the analyte response in the presence of its potential impurities was evaluated in this study. The selectivity of the method in presence of one of the major impurity (FTN-Acid) was evaluated. Forced degradation studies were conducted with Frovatriptan (solid sample) sample under UV light at 254 nm and heat (105 deg C) for 10 days. Content of (S)- enantiomer was checked in Frovatriptan sample exposed under UV light and heat at the end of the study period. The peak purity of the degraded samples was determined using a photodiode array detector. The peaks were found to be pure. Further the stressed samples were evaluated under varied chromatographic conditions by changing the methanol composition in the mobile phase. No degradation impurities were found merging with the analyte peaks.

Also the stability of the sample solution was checked for a period of 12 hrs at room temperature and injecting the solution at various time intervals. A cumulative percentage RSD value of 2.24% for (R)-enantiomer and 1.98% for (S)-enantiomer peak areas after 12 hrs showed that analyte did not degrade in solution.

Precision

The precision of the method is the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample.

The precision of the method was checked by analyzing six replicate samples of Frovatriptan (at the analytes concentration of 0.1 mg/ml). The RSD of (S)-enantiomer was found to be 0.42%. The allowed limit of (S)-enantiomer in Frovatriptan was fixed as 0.50% based on the profile of few batches of Frovatriptan synthesized in the laboratory. However the sample used for validation was found to contain 0.86% of (S)-enatiomer.

Linearity

The linearity of the method is a measure of how well a calibration plot of response versus concentration approximates a straight line.

The linearity of the (S)-enantiomer was evaluated with nine working solutions of (S)-enantiomer ranging

TABLE 1: Precision of (S)-enantiomer at LOQ level

Preparation	Area
1	896
2	895
3	841
4	937
5	898
6	903

%RSD = 3.46

TABLE 2: Precision of (S)-enantiomer at LOD level

Preparation	Area
1	581
2	674
3	509
4	486
5	484
6	518

%RSD = 13.65

TABLE 3: Recovery of (S)-enantiomer

Level	Added(ng)	Recovered (ng)	%Recovery	%RSD*
50%	252	253	100.4	1.6
100%	505	495	98.1	1.5
150%	757	795	105.1	1.8

***n = 3 determinations**

TABLE 4: Robustness of the chiral HPLC method

Parameter	Changes made	USP Resolution between (R) and (S) enantiomer
Flow rate (ml/min)		
0.54	-10% Flow rate	4.3
0.6	Control	4.4
0.66	+10% Flow rate	4.6
Methanol percentage(%v/v)		
6	-2% Absolute	3.8
8	Control	4.4
10	+2% absolute	5.0
Column oven temp (Deg C)		
27	Ambient	4.4
35	+8 Deg C	4.2
pH of Mobile Phase		
4.3	-0.2 pH	4.5
4.5	Control	4.4
4.7	+0.2 pH	4.5

from 13 ng/ml (LOQ) to 829 ng/ml (13, 26, 52, 104, 207, 414, 622 and 829 ng/ml), prepared by suitably diluting from the stock solution of 0.1 mg/ml of (S)-enantiomer in mobile phase. The linear regression equation y=107x+8 was obtained with a correlation coefficient of 0.999, indicating a good correlation between the peak area and concentration of the (S)-enantiomer. Also the linearity of (S)-enatiomer was studied in the range of 0.1% to 10% of analyte concentration in presence of (R)-enantiomer. The response of (S)-enantiomer was found to be linear with a correlation coefficient of 0.999.

Limit of detection and limit of quantitation of (S)-enantiomer

The limit of detection (LOD) of a test method is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated. The limit of quantitation (LOQ) of a test method is the lowest amount of analyte in a sample, which can be reliably quantified.

LOD and LOQ for (S)-enantiomer were predicted using the slope and the residual standard deviation values of the linearity curve. A LOQ value of 51 ng/ml was predicted initially. However, a further lower concentration of 13 ng/ml was found to give a relative standard deviation (RSD) of 3.46% (TABLE 1), which is well accepted; the LOQ of (S)-enantiomer by this method was fixed as 13 ng/ml for an injection volume of 5µl. The LOD level was established by six replicate injections of 6 ng/ml solution of (S)-enantiomer with a relative standard deviation of 13.65%. (TABLE 2).

Accuracy

The accuracy of an analytical method expresses the closeness of the agreement between the acceptable value and the value found. The accuracy of the method was established by performing recovery experiments in triplicate each at 50, 100 and 150% of the specification level of (S)-enantiomer (0.5%). The recoveries at all the three levels were in the range of 96% to 106%(TABLE 3).

3.2.7 Ruggedness

The ruggedness of the method was evaluated under different lots of columns/reagents, different labs, different analysts, different instruments and different days.

The %RSD of (S)-enantiomer content calculated by using the values obtained from the above experiments, was found to be 2.17%.

3.2.8 Robustness

Robustness is the measure of the ability of the method to remain unaffected by small changes in the parameters such as flow rate, mobile phase composi-

tion, wavelength of detection and the temperature of the column oven.

A set of 9 experiments was conducted with small deliberate changes in the method to assess the robustness of the method. The first set of experiments consisted change of flow rate by +10% (0.66 ml/min) and -10% (0.54 ml/min), while keeping other parameters constant. The second set of experiments consisted of change of wavelength of detection by -5nm (240 nm) and +5 nm (250 nm). The third set of experiments was conducted by changing the methanol composition of the mobile phase by +2% absolute (90:10) and –2% absolute (94:6). The fourth set of experiments was conducted by changing the pH of the buffer by +0.2 units (4.6 pH) and -0.2 units (4.2 pH). The last experiment was conducted by setting the column oven temperature at 35^0C.

In all the experiments except for the change in methanol composition, there was no major change in the enantiomer resolution. Experiment 5 with 2% increased methanol in the mobile phase resulted in an increase of resolution to 5.0 and the experiment 6 with a decrease of methanol content by 2% resulted in a decrease of resolution to 3.8.

4. CONCLUSION

A simple chiral HPLC method was developed for the enantiomer separation of Frovatriptan. Chiral-CBH was found to be selective for the enantiomers of Frovatriptan. The method was completely validated and the results found to be well within the acceptance criteria of ICH. The developed method is stability indicating and suitable for the estimation of (S)-enantiomer in frovatriptan drug substance.

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