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Short communication

A validated chiral CE method for Frovatriptan, using cyclodextrin as chiral selector

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Abstract

A cyclodextrin modified capillary zone electrophoretic method has been developed for the evaluation of chiral purity of Frovatriptan using sulfobutyl ether beta cyclodextrin (SB-beta-CD) as the chiral selector. The method is highly specific, accurate and reproducible. The method was optimized with a systematic method development approach by optimizing the pH of electrolyte, attempting the separation in different classes of chiral selectors and modifying parameters such as cyclodextrin concentration and the organic modifier type and concentration. The optimized method was validated for specificity, precision, linearity, accuracy and stability in solution using Imidazole as the internal standard. The limit of detection (LOD) and limit of quantification (LOQ) were 1.0 μg/mL and 5.0 μg/mL respectively for each isomer. The method was applied for estimating the chiral purity of various batches of Frovatriptan.

Keywords: Frovatriptan; Chiral separation; Capillary electrophoresis; Cyclodextrin; Validation and internal standard

1. Introduction

The stereochemical resolution of optically active molecules is an important and essential step in the development of biologically active potential drugs because many biological interactions and reactions are subject to varying degrees of stereoselectivity. Stereoselectivity in nature is evident in a number of biological processes, e.g., absorption, protein binding, selective tissue uptake of renal or biliary excretion in addition to metabolism [1]. The application of CE to the separation of enantiomers offers speed, simplicity, high resolution, low cost and small sample volume requirements. With the use of chiral selectors, resolution can be achieved even when there is a typical difference of only 0.03 kJ/mol of free energy of interaction between the enantiomers and the chiral selector [2].

Frovatriptan, administered as a single enantiomer (*R*)-(+)-3-(methylamino)-1,2,3,4-tetrahydro-9H-carbazole-6-carboxamide is a potent 5-hydroxytryptamine receptor agonist, one with a long duration of action and good tolerability. Frovatriptan reverses cerebral vasodilation by activating 5-HT_{1B}, and it prevents neurogenic inflammation by activating 5-HT_{1D} [3]. Frovatriptan is not only more potent but also unlike sumatriptan, zolmitriptan and naratriptan which fall into the category of triptamine derivatives, does not appear to constrict human coronary and peripheral arteries [3].

In synthetic process the yield of (*R*)-Frovatriptan is dependant on the configuration of pyroglutamic acid, which is a commercially available reagent. It can be seen from the synthetic scheme (Fig. 1) that (*R*)-Frovatriptan is predominantly the major product in the presence of L-pyroglutamic acid. Our method is not only capable of determining the chiral purity but also quantifying the unwanted ((*S*)-Frovatriptan) isomer during the synthesis of (*R*)-Frovatriptan succinate and in pharmaceutical formulations. So far to our current knowledge no chiral CE methods using cyclodextrin have been reported in the literature for the

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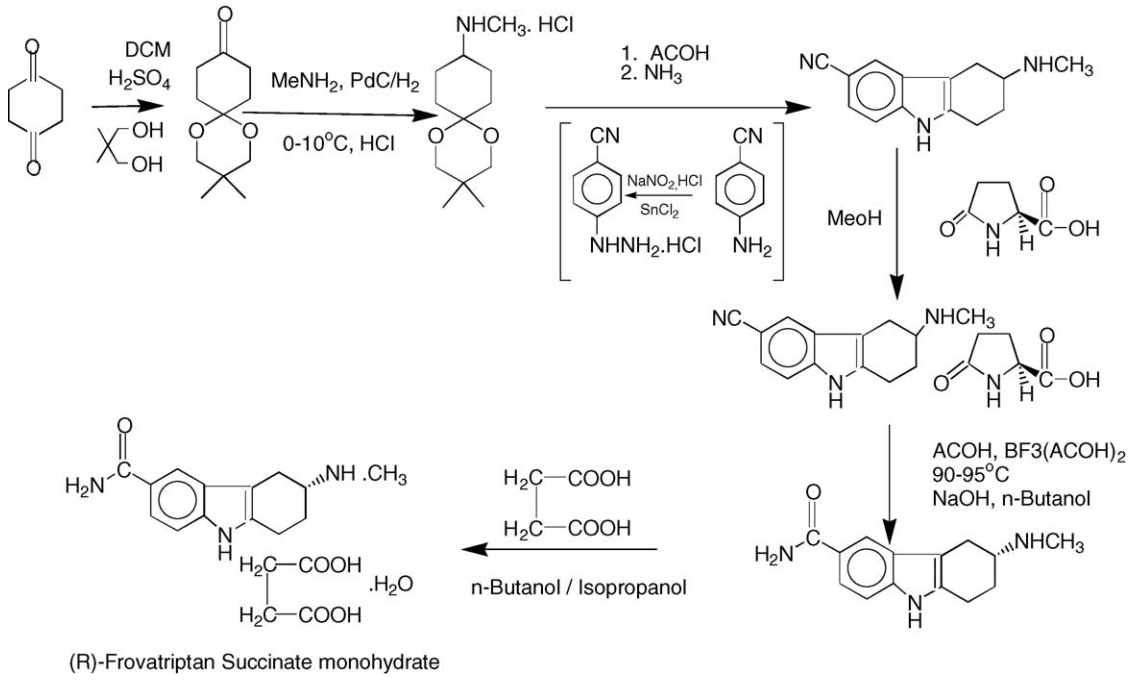

Fig. 1. Synthetic scheme of (*R*)-Frovatriptan succinate monohydrate.

enantiomeric separation of Frovatriptan in bulk drugs and pharmaceutical formulations.

2. Experimental

2.1. Instrumentation

The separation studies were performed on Agilent Technologies ^{3D}Capillary Electrophoresis system with a built-in diode-array detector. The HP ^{3D}CE ChemStation was used for system control, data acquisition and postrun processing. A 72 cm (length to detector), 50-μm i.d., bare fused silica capillary with extended light path having a bubble factor of 3 was utilized (Agilent technologies, Waldbronn, Germany).

2.2. Chemicals

Samples of Frovatriptan were kindly supplied by Bulk-Actives-III of Dr. Reddy's Laboratories Ltd. (Hyderabad, India). Imidazole (AR grade) and *iso*-propyl alcohol (AR grade) were obtained from Merck (India). SB-beta-CD was procured from Advasep® 4 (Cydex Inc., USA). Beta-cyclodextrin (Beta-CD), Heptakis (2,6-di-*O*-methyl)-beta-cyclodextrin (DM-beta-CD), Heptakis (2,3,6-tri-*O*-methyl)-beta-cyclodextrin (TM-beta-CD), (2-hydroxypropyl)-beta-cyclodextrin (HP-beta-CD), 1.0 and 0.1N sodium hydroxide (HPCE grade) were procured from Agilent technologies (Waldbronn, Germany). Disodium hydrogen orthophosphate and Sodium dihydrogen orthophosphate (AR grade) were procured from S.D. Fine chemicals (India) and Qualigens (India) respectively. Acetonitrile, ethanol and methanol (HPLC grade) were procured from Rankem (India). Water was filtered and deionized with a Milli-Q, Millipore system (Milford, MA, USA).

2.3. Preparation of buffer solution

Disodium hydrogen orthophosphate (50 mM) and sodium dihydrogen orthophosphate (50 mM) buffer solutions were prepared by dissolving appropriate amount of each buffer in Milli-Q water in separate volumetric flasks. The pH of 50 mM disodium hydrogen orthophosphate solution was titrated to 7.0 with 50 mM sodium dihydrogen orthophosphate using DMS 716 titrino autotitrator.

2.4. Preparation of background electrolyte (BGE)

The background electrolyte was prepared by dissolving 400 mg of SB-beta-CD in 10 mL of 50 mM phosphate buffer (pH 7.0) and then adding 1.0 mL of acetonitrile to 9.0 mL of this buffer. The BGE was filtered through 0.2 μm syringe filters.

2.5. Capillary preconditioning

New bare fused silica capillaries were flushed with 1.0N NaOH for 20 min, followed by CE grade water for 10 min. Prior to every use the capillary was conditioned by flushing for 5 min with water, 2 min with 0.1N sodium hydroxide, 2 min with water and then by BGE for 15 min. Between runs, the capillary was flushed with the BGE for 2 min.

2.6. Preparation of sample solution

Sample solutions were prepared by spiking appropriate amounts of racemic Frovatriptan to 30 mg of standard drug ((*R*)-Frovatriptan) in the absence of pure (*S*)-Frovatriptan, for the purpose of method validation. To this 1 mL of acetonitrile was added and sonicated. A 1.0 mL of the internal standard (Imi-

dazole) having a concentration of 5 mg/mL prepared in diluent (water:acetonitrile, 90:10 v/v) was added and finally made up to 10 mL with diluent. The sample solution was filtered through 0.2 μm filters.

2.7. Electrophoretic separation conditions

The voltage applied was 30 kV with polarity set to positive. The internal standard peak and the drug peaks were monitored with timed wavelength program using a diode array UV detector. Imidazole was monitored at 205 nm and the enantiomers of Frovatriptan were monitored at 245 nm. Samples were injected hydrodynamically by pressure at 50 mbar for 3 s followed by injection of a water plug by pressure at 50 mbar for 2 s.

3. Results and discussion

3.1. Method development and optimization

3.1.1. Effect of pH on separation

The pH of the background electrolyte directly controls the electro osmotic flow and the ionizing ability of the analyte thereby affecting the migration time and also the peak symmetry. Schmitt and Engelhardt [4] have shown that the migration order of racemic ephedrine was dependant on the pH of buffer. The effect of pH on peak symmetry and enantioresolution was investigated using various buffers at constant molar strength (50 mM) keeping the other separation parameters constant. Frovatriptan was expected to be considerably ionized at pH 9.3 and fully ionized under all other experimental conditions owing to a high pK_a value of 10.42. Symmetric peaks were obtained at pH 2.5 (phosphate buffer), pH 4.6 (citrate buffer) and pH 7.0 (phosphate buffer), however the peaks showed signs of tailing at a higher pH of 9.3 (borate buffer) which can be attributed to partial ionization. Resolutions of 1.99 and 2.9 were obtained at pH 2.5 and 4.6 respectively in negative polarity. At pH 9.3 the resolution was about 2.65 but in all the cases the peaks eluted with high migration times of 18 minutes or more. A high resolution (greater than 3.0) and short migration times could be achieved, only with the use of phosphate buffer at pH 7.0 which was finally optimized.

3.1.2. Optimization of the chiral selector type and concentration

The chiral discrimination of the enantiomers was attempted using different cyclodextrins as chiral selectors. No chiral recognition was observed with native beta-CD, DM-beta-CD, TM-beta-CD and HP-beta-CD. Total resolution of the enantiomers could be achieved only with SB-beta-CD.

The effect of SB-beta-CD concentration on separation was explored by varying the concentration from 5 to 25 mM. These experiments were performed using 10% acetonitrile as the organic modifier in the background electrolyte. The peaks for rac-Frovatriptan were found to be splitting with 5 mM SB-beta-CD. Both resolution and migration time increased with increase in the concentration of cyclodextrin. The concentration of cyclodextrin was optimized to 20 mM as higher concentrations resulted in generation of undesirable higher current without a significant increase in resolution. The impact of SB-beta-CD concentration on migration time and resolution is shown in Fig. 2a and b.

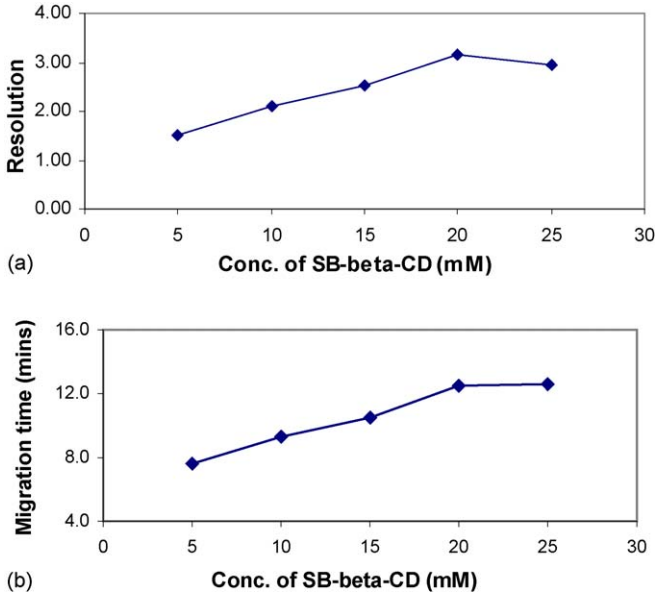

Fig. 2. (a) Effect of SB-beta-CD concentration on resolution. (b) Effect of SB-beta-CD concentration on migration time.

3.1.3. Effect of organic modifiers and their concentration on separation

Different organic modifiers acetonitrile, methanol, ethanol and isopropyl alcohol were added to the BGE at 10% (v/v) concentration (Fig. 3a and b) and it was found that acetonitrile produced the highest resolution within shortest run times. The addition of organic solvent to the buffer affects several variables including viscosity, dielectric constant and zeta potential. Tsuda et al. [5] have reported that electroosmotic velocities obtained with acetonitrile, water and methanol were proportional to the ratios of their dielectric constants to their viscosities. Wren and Rowe [6] have proposed a model to explain the affect of addition of organic solvent on separation. The analyte is assumed to form a complex with cyclodextrin with the hydrophobic part of it sitting inside the hydrophobic part of the cyclodextrin. The addition of organic solvents reduces the affinity of the analyte for the cyclodextrin cavity, due to the decreased polarity of the solvent i.e. the formation of an inclusion complex will be less favored and there will be an increased affinity of the analyte for the buffer. This change in analyte cyclodextrin binding ability will depend on the polarity of the organic solvent and also on its ratio in the buffer. Solvent, which is comparatively non-polar, will be expected to have a more pronounced affect in decreasing the affinity of analyte for the cyclodextrin.

The effect of acetonitrile concentration on separation was studied by changing its concentration from 5 to 20%. Both resolution and migration times decreased with increase in acetonitrile concentration. The decrease in resolution can be explained by the fact that the concentration of cyclodextrin was at the optimum value for an organic free buffer. Acetonitrile at 10% (v/v) concentration was chosen as the optimum concentration as it

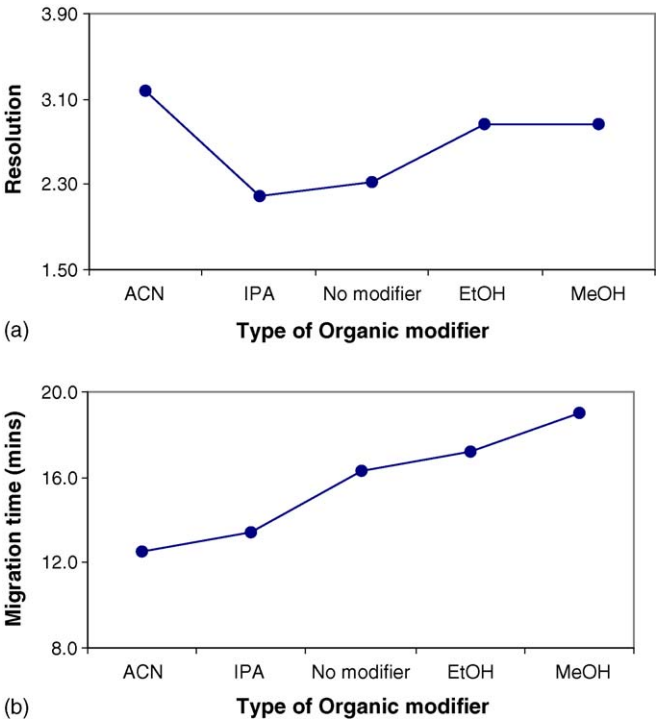

Fig. 3. (a) Effect of organic modifier type on resolution. (b) Effect of organic modifier type on migration time.

provided a fair compromise between resolution and run time (Fig. 4a and b).

3.2. Method validation

The optimized method was validated for quantification of *S*-Frovatriptan, using imidazole as the internal standard, with the assessment of specificity, precision in migration time and peak area, linearity, accuracy and stability of drug in solution. The validation was performed keeping in mind the ICH guidelines for analytical method validation [7].

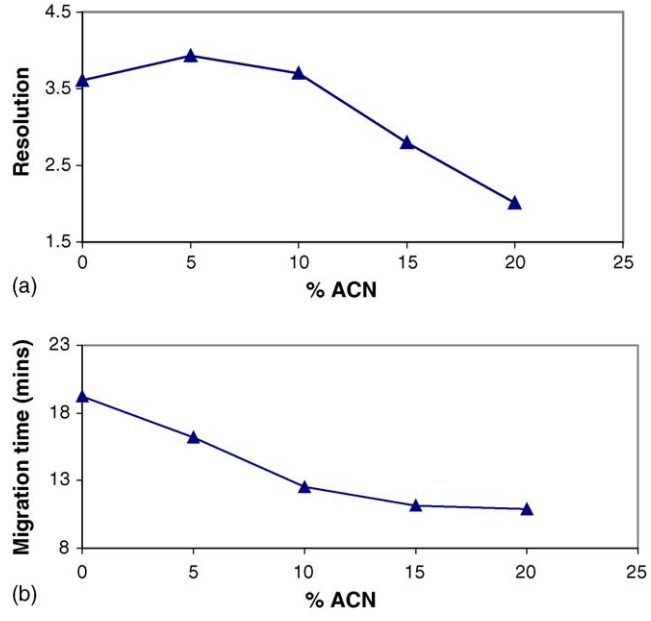

Fig. 4. (a) Effect of acetonitrile concentration on resolution. (b) Effect of acetonitrile concentration on migration time.

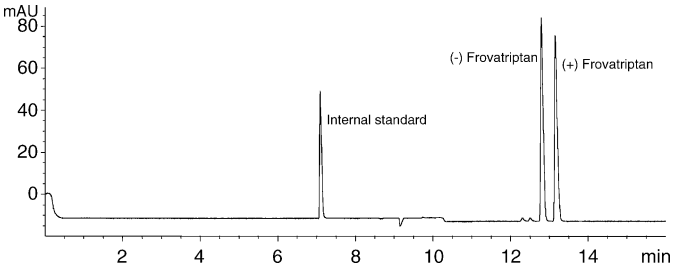

Fig. 5. A representative electropherogram of Frovatriptan enantiomers with the internal standard.

3.2.1. Specificity

Specificity of a method is its ability to detect and separate impurities present in the drug. Specificity can be demonstrated in the form of spectral and peak purity data of the drug and its related impurities. In the present method complete resolution of enantiomers of Frovatriptan and its related substances has been achieved. Moreover no other component of significant response co eluted establishing the specificity of the method. Photodiode array detection method was applied to prove the specificity of the method and to evaluate homogeneity of the peaks. A typical electropherogram of racemic Frovatriptan in the presence of internal standard is shown in Fig. 5.

3.2.2. Precision

The precision of the method was determined as repeatability, intraday and inter-day precision (intermediate precision) of migration times and corrected peak area ratios. Since the area of a peak is dependant on its electrophoretic mobility in capillary electrophoresis the corrected areas i.e. the ratio of peak area and migration time which remains constant is considered. Corrected peak area ratio is the ratio of the corrected peak area of analyte peak and internal standard.

Repeatability was determined by making six replicate injections of a standard solution spiked with the unwanted isomer at Limit of Quantitation (LOQ) level and at 1% level. The relative standard deviation (R.S.D.) values for migration time, correct peak area and corrected peak area ratios are given in Table 1. The use of internal standard is necessary in order to compensate the poor precision observed with the hydrodynamic injection, and hence to achieve good method precision [8].

The intermediate precision was evaluated over 3 days by performing six successive injections on each day. The results of intermediate precision in Table 1 suggest that precision in corrected peak area ratio was as good as repeatability and intraday precision. In all the cases the R.S.D. was less than 1.61% in migration time and 3.53% in corrected peak area ratios.

Table 1
Precision data

	LOQ level (% R.S.D.)	1% level (% R.S.D.)
Repeatability[a]		
Migration time	0.68	0.25
Corrected peak area	8.33	1.74
Corrected peak area ratio	3.53	1.50
Intra-day precision[a]		
Migration time	0.83	0.88
Corrected peak area	5.86	5.21
Corrected peak area ratio	3.42	2.13
Inter-day precision		
Migration time	1.35	1.61
Corrected peak area	4.70	3.33
Corrected peak area ratio	1.86	1.22

[a] $n = 6$ determinations.

3.2.3. Linearity

Linearity of the detector response to sample concentration was assessed by spiking (*S*)-Frovatriptan to the standard drug at five calibration points ranging from LOQ to 60 μg/mL (2% of the nominal analyte concentration). Each sample was injected in triplicate with the internal standard. Regression coefficients were obtained by plotting the corrected peak area ratios versus concentration, using the least squares method. The straight-line equation for (*S*)-Frovatriptan was $y = 0.0326x + 0.081$ with a coefficient of regression (R^2) of 0.9986.

3.2.4. Limits of detection (LOD) and quantification (LOQ)

Limit of detection of a compound is defined as the lowest concentration that can be detected. It demonstrates the detection sensitivity of the compound. A signal-to-noise ratio of approximately 2–3 is considered to be acceptable for estimation of detectable limit. The LOD of each of the enantiomers was found to be 1.0 μg/mL. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. A concentration with a typical signal-to-noise ratio of 9–12 is regarded as the LOQ level. The LOQ level for each of the enantiomers was found to be 5.0 μg/mL.

3.2.5. Accuracy

Accuracy of the method was established by performing recovery experiments. Samples were independently prepared in triplicate by spiking (*S*)-Frovatriptan at LOQ, 0.8%, 1.0% and 1.2% levels to the standard drug. The percentage recoveries were calculated from the slope and intercept obtained from the standard curve. The percentage recovery ranged from 98.75 to 105.38% as shown in Table 2.

3.2.6. Stability

Stability of the sample in solution was evaluated by injecting the sample immediately after preparation and then reanalyzed after storing at bench-top for 24 h. There was no significant change either in the peak area ratios or in enantiomeric composition confirming that no degradation or inter conversion of the enantiomers took place for at least 24 h.

Table 2
Accuracy data

Amount spiked (μg/mL)	Amount recovered[a] (μg/mL)	Percentage recovery
5.02	5.29 ± 0.10	105.38
24.03	24.32 ± 1.46	101.21
30.04	30.84 ± 3.03	102.66
36.05	35.60 ± 0.73	98.75

[a] $n = 3$ determinations.

Table 3
Results for quantification of Frovatriptan (*S*)-isomer in bulk samples

Batch no.	Percentage of (*S*)-Frovatriptan[a]
Batch-A	0.51 ± 0.0003
Batch-B	0.07 ± 0.0010
Batch-C	0.10 ± 0.0058
Batch-D	32.11 ± 0.0058
Batch-E	0.09 ± 0.0002
Batch-D	0.07 ± 0.0056

[a] $n = 3$ determinations

3.2.7. Quantification of Frovatriptan (S)-isomer in bulk samples

Bulk samples of Frovatriptan were analyzed for the content of (*S*)-isomer. About 30 mg of each test sample was transferred into a separate 10 mL volumetric flask. The sample was dissolved in 1.0 mL of acetonitrile by sonication. The volume was finally made up to 10 mL with Milli-Q water. Samples solutions were filtered through 0.2 μm syringe filters before injection. The results of the analysis of various batch samples of Frovatriptan are shown in Table 3.

4. Conclusion

The developed CZE method was found to be simple and efficient for estimating the unwanted enantiomer. The impact of various parameters like pH of BGE, the cyclodextrin concentration, type and concentration of organic modifier and temperature were studied during method optimization process and the best separation conditions were chosen. The optimized method was validated and was found to be sensitive, precise and reproducible. The method was applied to the bulk samples of Frovatriptan for estimating the content of (*S*)-isomer.

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