Development of a method for separation of the four stereoisomers of troglitazone using capillary electrophoresis

Application Note

Pharmaceutical

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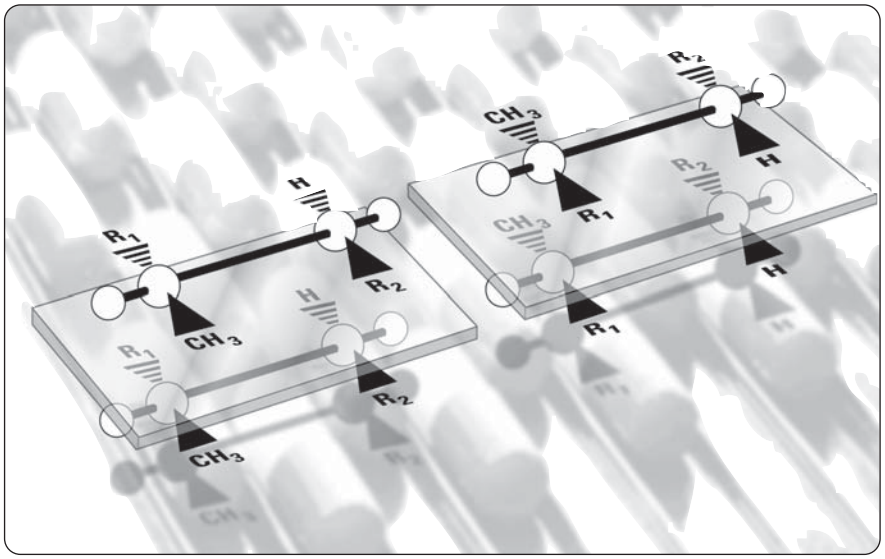

Abstract

This Application Note describes the development of a method using capillary electrophoresis (CE) to separate the four stereoisomers of troglitazone. Excellent resolution between the stereoisomers was achieved by using a borate buffer with SDS (sodium dodecyl sulfate) and Heptakis (2,3,6-tri-Omethyl)-β-cyclodextrin and by optimizing the capillary temperature. Other factors such as the reproducibility of migration times and peak areas were also investigated.

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Introduction

Troglitazone, an enhancer of the action of insulin and inhibitor of hepatic gluconeogenesis, was discovered in the research laboratories of Sankyo Co. Ltd., Tokyo, Japan,[1,2] and has been shown to be orally effective in both non-insulin dependent and insulin-dependent diabetes mellitus.[3-6] Troglitazone is an equal mixture of four stereoisomers involving two asymmetric centers at the 2 position of the chroman ring and the 5 position of the thiazolidine ring (figure 1).

In one of the racemates the two enantiomers have the R,R and S,S configuration, respectively, and the enantiomers of the other racemate are the R,S and S,R configuration, respectively. Two of the four stereoisomers that are not mirror images of each other (i.e. S,S and S,R) are diastereoisomers. The chemical name for troglitazone is (±)-5-[4-(6-hydroxy-2,5,7,8- tetramethylchroman-2- ylmethoxy)benzyl]-2,4-thiazolidinedione.

As the pharmacodynamic activities of enantiomers of a drug may differ drastically there is a need to study the pharmacological and toxicological properties of optically active compounds. A reliable and accurate assay is therefore necessary to isolate the stereoisomer of interest.

Chromatography is well suited for the stereospecific analysis of free drugs and drugs in biological fluids and is widely used for this purpose. Chromatographic techniques including thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and supercritical fluid chromatography (SFC) have all been employed for chiral recognition. In recent years, capillary electrophoresis has become a powerful technique for the separation of a variety of complex mixtures. The main advantages of CE are its high separation efficiency and low consumption of sample and solvents. Therefore the technique is a very attractive separation tool.

Micellar electrokinetic chromatography (MEKC), an important mode of CE is widely used for the separation of non-polar molecules. This technique uses buffers containing micelles which form a non-polar phase into which molecules can partition. This type of partitioning is equivalent to that in reversed-phase HPLC.

Sodium dodecyl sulfate (SDS), the most widely used anionic surfactant, is an attractive choice because it gives excellent selectivity for a wide range of compounds. It is available in high purity, and is inexpensive.

The separation of diastereoisomers can be successfully achieved by MEKC with achiral micellar solution alone. However, for the separation of enantiomers by MEKC with achiral micelles, a chiral selector is added to the separation buffer. The chiral selectors most used in this method are cyclodextrins (CD). This technique has been called “cyclodextrin modified

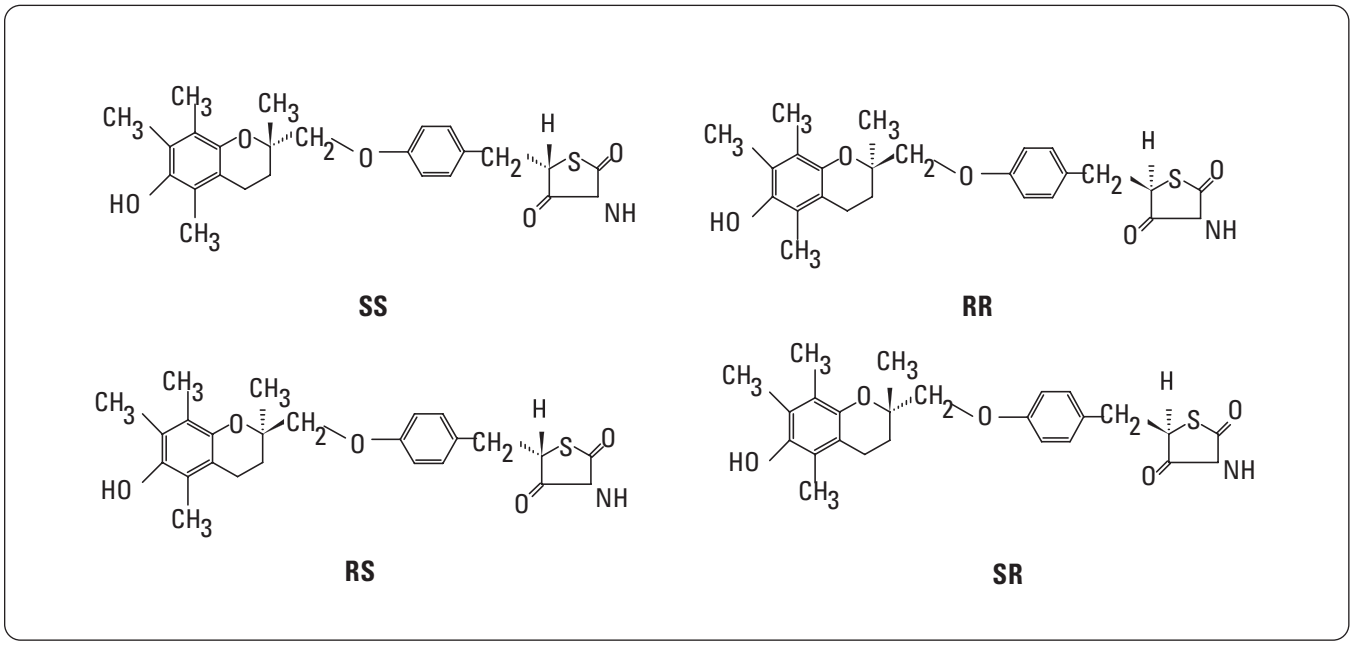

Figure 1
Structures of the four stereoisomers of troglitazone.

micellar electrokinetic chromatography" (CDMEKC). Chiral separation occurs due to the formation of transient non-covalent structures of CD and the diastereoisomers which differ in their energy of formation. In CDMEKC, the solutes are distributed among three phases: an aqueous phase, the micelle and the CD.

Numerous reports on chiral resolution by CE use one of the cyclodextrins as the optically active reagent. CDs are relatively inexpensive, easy to use, and readily available. CE with its two major modes, capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography is gaining acceptance in the pharmaceutical field for the separation of drugs.

The CE approaches mentioned, are relatively simple when compared with HPLC in which expensive chiral stationary phases are frequently used.

Experimental

All experiments were performed using an Agilent Capillary Electrophoresis system equipped with a diode array detector. An Agilent ChemStation was used for system control, data acquisition and data analysis. Buffer components were of the highest available purity. The following chemicals must be ordered from 3rd party vendors. For ordering please refer to http://www.sigald.sial.com/fluka for Sigma and to www.cyclolab.hu for Cyclolab.

Component	Vendor	Order Number
(±)-Epinephrine Free Base	Sigma	E1635
β-cyclodextrin	Cyclolab	CY-2001
(2-hydrooxyl)propylate-β-cyclodextrin	Cyclolab	CY-2005.2
Heptakis(2,6-di-0-methyl-β-cyclodextrin	Cyclolab	CY-2004.0
Heptakis(2,3,6-tri-0-methyl)-β-cyclodextrin	Cyclolab	CY-2001
Gamma-cyclodextrin	Cyclolab	CY-3001

The fused-silica capillaries, 50 µm id, with effective lengths of either 56 or 72 cm, as noted, were from Agilent Technologies. The capillary was cooled with the Peltier cooling unit built into the CE system. The applied voltage in all cases was 30 kV. Separation conditions for the final method were as follows:

- Inlet buffer = 2.5 mM sodium borate pH 9.4, 20 mM SDS, 56 mM Heptakis (2,3,6-tri-Omethyl)-cyclodextrin;
- Outlet buffer = 2.5 mM sodium borate pH 9.4, 20 mM SDS
- Injection by pressure = 50 mbar for 3 seconds
- Capillary length = 72 cm effective, 50 µm id
- Operating temperature = 10 °C
- Detection by UV at 200 nm

New capillaries were conditioned by rinsing with 1 N sodium hydroxide, 0.1 N sodium hydroxide, and inlet buffer. The CE instrument was programmed to rinse with inlet buffer for 5 minutes prior to each injection. In order to maintain satisfactory reproducibilities of peak response and migration time, new inlet and outlet vials were used after every five injections.

All buffer solutions and samples were filtered through 0.2 µm filters before use.

The samples used were prepared by dissolution in ethanol, and either injected directly or further diluted with water.

Experimental conditions are given in the individual figure captions.

Results and discussion

Initially an HPLC method was used which gave a resolution of about 1 between the first two eluting stereoisomers. It also had a fairly long run time (35 minutes). Therefore, it was decided to try to develop a method using cyclodextrin- modified MEKC in order to improve the resolution and shorten the run time.

In MEKC the minimum surfactant concentration is defined by the critical micelle concentration (CMC) which is 8 mM for SDS. The optimum concentration is best determined experimentally because the actual CMC is affected by pH and ionic strength of the buffer.

One of the first steps in method development for chiral drugs with cyclodextrins is the selection of the appropriate cyclodextrin. The size of the CD cavity in conjunction with its chemical modifications determines the degree of solute interaction. Chiral recognition is dependent on both the size of the cavity and the lipophility of the rim substituents on the CD.

Using γ-cyclodextrin and Heptakis (2,6-di-O-methyl)-β-cyclodextrin no resolution was obtained. Use of Heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin showed clear advantages and a partial resolution of the diastereisomers was achieved. Figure 2 shows the impact of cyclodextrin concentration on the resolution of troglitazone stereoisomers.

The higher cyclodextrin concentration naturally favors complex formation so the drug spends more time in complexed form. The charge to mass ratio is reduced and likewise its mobility. If the cyclodextrin concentration is too high, valuable separation time may be wasted without meaningful gain in resolution. In some cases there can be an optimum cyclodextrin concentration, below and above which resolution decreases.

The influence of the capillary length is shown by comparing the rightmost panel in figure 2 and figure 3. The longer the solutes remain in the capillary, the better the differentiation based on the difference of the mobility of the enantiomers.

By lowering the temperature to 10 °C, but using the same concentrations of sodium borate buffer, SDS and cyclodextrin, the separation of all four stereoisomers was achieved with baseline resolution of all peaks (figure 4).

Lowering the capillary temperature increases the migration times since

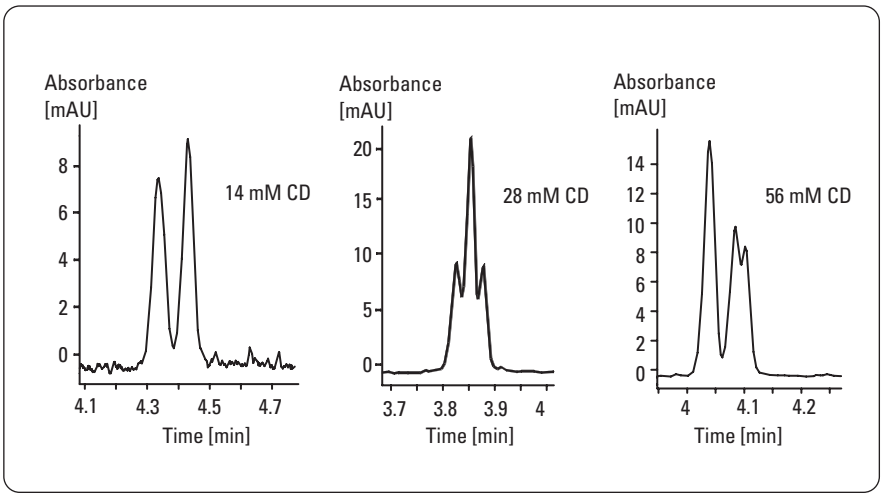

Figure 2
The impact of cyclodextrin concentration on the resolution of Troglitazone stereoisomers.

Chromatographic conditions

Buffer:	2.5 mM borate pH 9.4, 20 mM, SDS, Heptakis (2,3,6-tri-Omethyl)-β-cyclodextrin
Voltage:	30 kV
Capillary:	l = 56 cm, 50 µm id (Agilent part number G1600-62211)
Temperature:	25 °C
Detection:	200 nm, 10 nm Bw

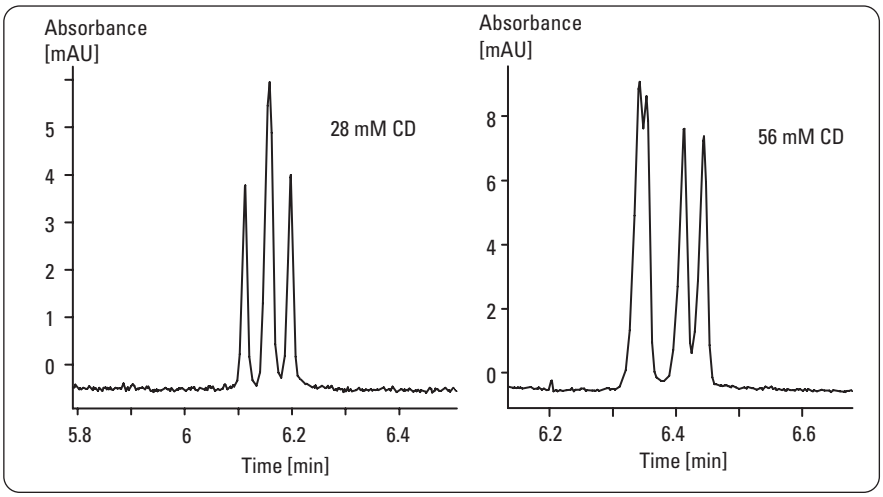

Figure 3
Effects of increasing capillary effective length (compare with figure 2).

Chromatographic conditions

Buffer:	2.5 mM borate pH 9.4, 20 mM, SDS, Heptakis (2,3,6-tri-Omethyl)-β-cyclodextrin
Voltage:	30 kV
Capillary:	l = 72 cm, 50 µm id (Agilent part number G1600-62211)
Temperature:	25 °C
Detection:	200 nm, 10 nm Bw

mobility always decreases as viscosity increases. A secondary effect might be an increase in the equilibrium constant since complex stability may be enhanced at low temperatures.

Numerous parameters such as the viscosity of the buffer solution, the pk_a of the solute and the complexation constant with cyclodextrin are directly affected by variations in the temperature. Therefore, it is not surprising that the temperature exerts a great influence on the separation. In some cases, separation can only be obtained at sub-ambient temperatures.

The resolution between the enantiomers was 1.3 for peaks 1 and 2 and 2.5 for peaks 3 and 4. The corrected peak area reproducibilities for eight injections for each peak were < 2 % RSD, and the migration times < 0.5 % RSD. The percentage of each isomer was calculated using corrected peak areas and were comparable to the label claim (table 1).

Conclusions

Troglitazone was best resolved into its four stereoisomers by using CD-MEKC with SDS and Heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin dissolved in borate buffer pH 9.4. The advantages of this method over HPLC are that the analysis is less expensive, there is less organic waste, improved resolution and a shorter run time.

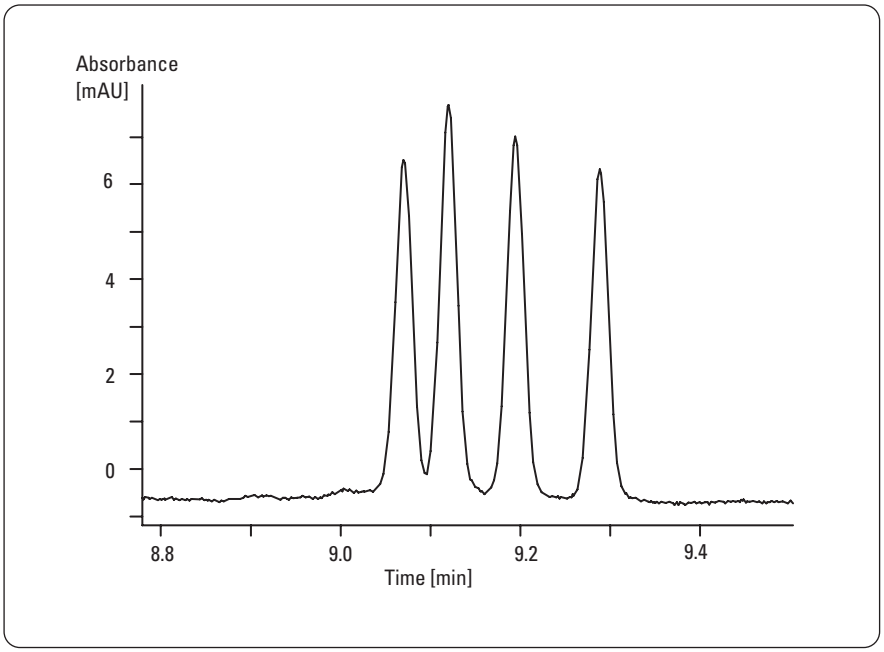

Figure 4
Optimized separation of the four steroisomers of troglitazone.

Chromatographic conditions

Buffer:	2.5 mM borate pH 9.4, 20 mM, SDS, 56 mM Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin
Voltage:	30 kV
Capillary:	l = 72 cm, 50 µm id (Agilent part number G1600-62211)
Temperature:	10 °C
Detection:	200 nm, 10 nm Bw

	% isomer corrected peak area	label claim
Peak 1	23.8	21.9
Peak 2	27.2	28.3
Peak 3	25.7	26.3
Peak 4	23.4	23.6

Table 1
The percentage isomer using corrected peak area compared to label claim.

The best conditions for good separation as well as ruggedness and reproducibility of the method were found to be 2.5 mM sodium borate buffer, 20 mM SDS, and 56 mM Heptakis (2,3,6-tri-Omethyl)-β-cyclodextrin at a capillary temperature of 10 °C. Resolution of the four stereoisomers was dependent on the type of CD used, the CD concentration and the capillary temperature. The precision and day-to-day reproducibility of the method was good. This method can be used to determine the relative amounts of the four stereoisomers in troglitazone tablets.

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Published November 1, 2008
Publication Number 5989-9809EN

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