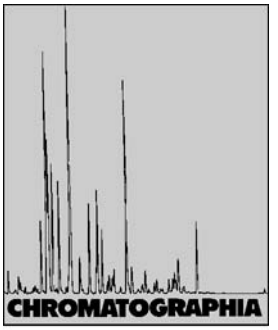
Optimization of the Reversed-Phase High-Performance Liquid Chromatographic Separation of the Enantiomers of a Cationic Chiral Drug (Tolperisone) on a Heptakis(6-Azido-6-deoxy) Perphenylcarbamated β-Cyclodextrin Column



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Key Words

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Summary

Heptakis(6-azido-6-deoxy) perphenylcarbamated β-cyclodextrin has been synthesized and chemically immobilized on silica gel for use as a chiral stationary phase (PC-CSP) for analytical separation of the enantiomers of chiral drugs. Separation of the enantiomers of tolperisone was studied by high-performance liquid chromatography under reversed-phase conditions. The chromatographic conditions were optimized by varying mobile phase pH, composition, ionic strength, and velocity; 40:60 methanol-1% triethylammonium acetate (TEAA) buffer, pH 5.5, was found to be the most suitable for this separation.

Introduction

In recent years the direct separation of enantiomers by chiral chromatography has been the target of intense research. The suitability and availability of cyclodextrins, and good economic reasons, have played a decisive role in the growing interest in these compounds chiral stationary phases in the pharmaceutical industry, and the use of cyclodextrins has resulted in the separation of a wide variety of structural, positional, and optical isomers. In this work derivatized cyclodextrins have been prepared and used effectively as chiral stationary phases for the separation of enantiomers, because inclusion-complex-formation both in solution and/or with the solid phase improves the aqueous solubility and physicochemical stability of many drug molecules.

Tolperisone (2,4-dimethyl-3-piperidinopropiophenone monohydrochloride; Figure 1) is a renowned chiral drug used as a centrally acting muscle relaxant for symptomatic treatment of spasticity and muscle spasm. The enantiomers of tolperisone have different biological effects. (+)-Tolperisone is a stronger muscle relaxant than the (–) isomer whereas the latter has greater broncho and peripheral vasodilatory activity than the dextrorotatory enantiomer.

Several chromatographic methods have been published for separation of the enantiomers of tolperisone on different chiral columns. The separation has been achieved on protein columns [1-6] and on a naphthylethylcarbamate-β-cyclodextrin column [7], both by reversed-phase HPLC.

In our research a novel chiral stationary phase, PC-CSP, has been developed by chemical immobilization of heptakis(6-azido-6-deoxy) perphenylcarbamated β-cyclodextrin on amino-functionalized silica gel [8]. It has been found to be effective for both normal and reversed-phase separation of enantiomers. In addition, because PC-CSP is covalently bonded to the silica gel, it is compatible with all commonly used mobile phases and can be used to prepare durable columns with excellent preparative capacity. Reversed-phase separation conditions have been especially useful for the separation of pharmaceutical compounds [9, 10]. There are several advantages to the use of reversed-phase conditions, for example, good solubility of polar compounds, easier sample preparation, and use of less costly solvents. Reversed-phase mobile phases are also extremely useful for preparative-scale separations, because of the high solubility of polar analytes in these mobile phases

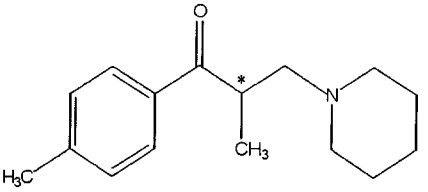

Figure 1. The chemical structure of tolperisone.

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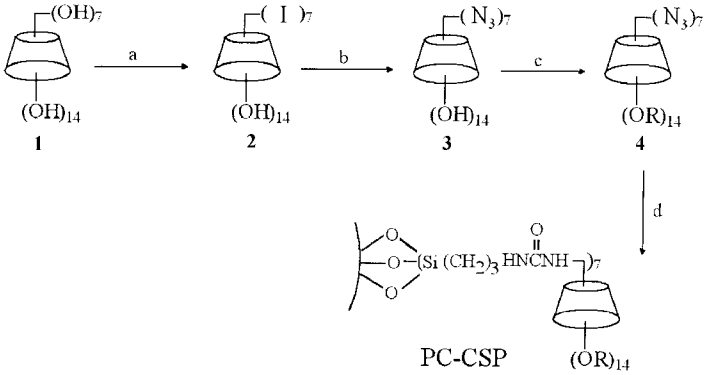

Figure 2. Reaction scheme and conditions for synthesis of the chiral stationary phase. Reaction conditions: (**a**) iodine, triphenylphosphine, dry dimethylformamide, 85 °C, 12 h; (**b**) sodium azide, dimethylformamide, 95 °C, 12 h; (**c**) R = phenylisocyanate, dry pyridine, 90 °C, 12 h; (**d**) amino-functionalized silica gel, carbon dioxide, triphenylphosphine, 20 h.

and the relative simplicity of their evaporation [4].

This paper reports the direct separation of the enantiomers of tolperisone under optimized reversed-phase conditions by use of a phenylcarbamate-derivatized β-cyclodextrin (PC-CSP) column. The effect of mobile phase pH, ionic strength, composition, and flow rate on retention time, enantioselectivity, and resolution were investigated. Room temperature (22 °C) was maintained throughout the experiments. Eventually the derived optimum conditions shall be used in a simulated moving bed (SMB) for preparative-scale operation.

Experimental

Chemicals, Reagents, and Equipment

Tolperisone was purchased from Sigma (Singapore). Methanol of HPLC grade was obtained from Fisher Chemical (Singapore). Purified water was obtained from a Milli-Q system. Chemicals such as triethylamine (TEA) and glacial acetic acid were of analytical-reagent grade and were also procured from Sigma (Singapore). A Hanna Instruments H1 9021 microprocessor pH meter was used for pH readings.

Preparation of Chiral Stationary Phase

Native β-cyclodextrin (Figure 2, **1**), used as the starting material for the synthesis, was converted to heptakis(6-iodo-6-deoxy)-β-cyclodextrin (**2**) by treatment with iodine and triphenylphosphine in DMF. The heptakis(6-iodo-6-deoxy)-β-cyclodextrin was then reacted with sodium azide in DMF (95 °C, overnight) to give heptakis(6-azido-6-deoxy)-β-cyclodextrin (**3**) which was further perphenylcarbamated by treatment with phenyl isocyanate in pyridine (90 °C, overnight) to afford heptakis(6-azido-6-deoxy) perphenylcarbamated β-cyclodextrin (**4**). This product was purified by column chromatography on silica gel with hexane-chloroform, 1:4, as mobile phase.

Chemical immobilization of heptakis (6-azido-6-deoxy) perphenylcarbamated β-cyclodextrin (**4**) on amino-functionalized porous spherical 5-µm silica gel (Hypersil 300) was achieved by reaction in the presence of CO_2 and triphenylphosphine for 20 h. The chiral stationary phase obtained, PC-CSP, was packed into an analytical column (25 cm × 4.6 cm), at 7500 psi, by means of a packing machine (Alltech, US). This column was conditioned with the mobile phase used for the enantiomer separations.

Chromatography

HPLC was performed with a Perkin-Elmer Series 200 autosampler equipped with 20-µL injection loop, series 200 LC pump, 785A UV-visible detector, and Supelco mobile-phase-degassing system. The detector output was monitored and recorded by means of a Hewlett-Packard PC.

Separation of the enantiomers of tolperisone was performed at room temperature (22 °C). The UV detector was operated at 220 nm. Drug sample concentrations were in the range 0.02–0.03 mg mL^{-1}. The void volume (V_0) was determined by injecting mobile phase of different composition. The column was preconditioned by flushing overnight with 60% methanol at a flow rate of 0.1 mL min^{-1}. The volume of sample solution injected was typically 20 µL. Each run was repeated at least three times to confirm the experimental results obtained.

Preparation of Mobile Phases

A mixture of methanol and triethylammonium acetate (TEAA) buffer solution was used as mobile phase for enantiomer separations. The 1% triethylamine solution was prepared by dissolving 55.10 mg TEA in 4000 mL deionized water. The range of buffer pH used for the analysis was varied between 4 and 6.5. Buffer pH was adjusted by adding glacial acetic acid to the 1% TEA solution.

Results and Discussion

Systemic analysis of tolperisone on the heptakis(6-azido-6-deoxy) perphenylcarbamated β-cyclodextrin (PC-CSP) column with aqueous buffer solutions as mobile phase was performed to investigate the effect of mobile phase conditions such as pH, composition, ionic strength, and flow rate on chromatographic properties such as retention time, resolution, and selectivity.

The time between sample injection and the sample peak reaching the detector at the end of the column is termed the retention time (t_R) and the time taken for the mobile phase to pass through the column is called t_M. A term known as the retention factor (also called as capacity factor), k', is used to describe the rate of migration of the sample through the column. The retention factor for a compound A is defined as:

$$k'_A = (t_R - t_M)/t_M$$

t_R and t_M are easily obtained from the chromatogram. A quantity called the selectivity factor, a, which describes the separation of two species, A and B, on the column is defined as:

$$\alpha = k'_B/k'_A$$

The retention times of the enantiomers depend on their retention behavior on the column. The selectivity expresses the separating power of the adsorbent for the enantiomers but although it describes the

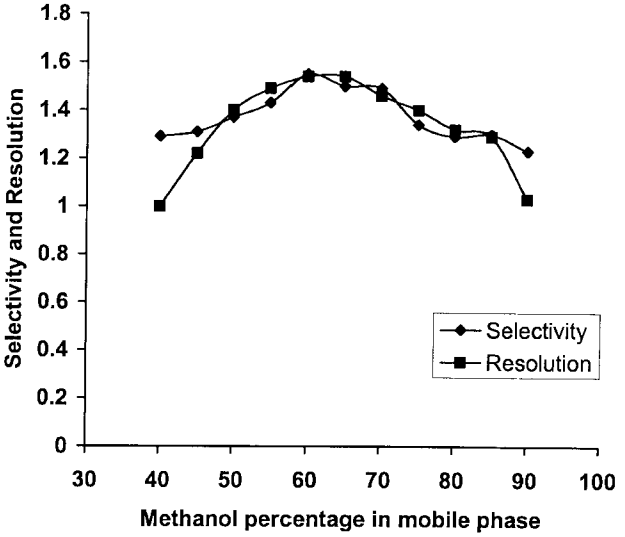

Figure 3. Effect of mobile-phase methanol content on resolution and selectivity.

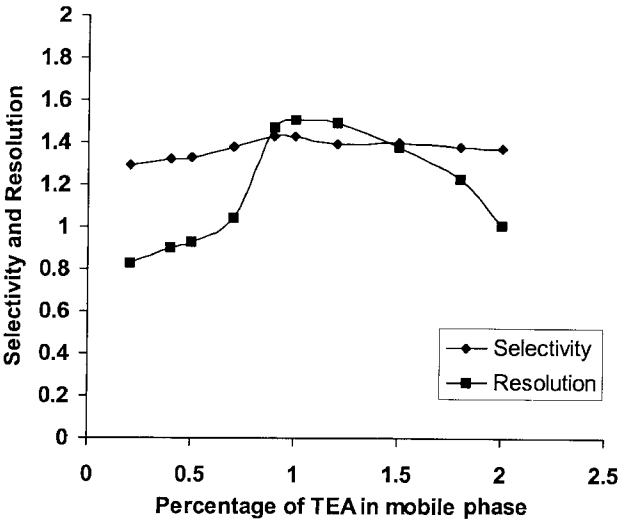

Figure 4. Effect of mobile-phase TEA content on resolution and selectivity.

separation of the band centers, it does not take peak widths into account. Another measure of how well species have been separated is provided by measurement of the resolution, which depends on both retention times and peak broadening.. The resolution of two species, A and B, is defined as:

$$R_S = 2[(t_R)_B - (t_R)_A]/(W_A + W_B)$$

where $(t_R)_B - (t_R)_A$ is the difference between the retention times of the two peaks and W_A and W_B are the widths of the peaks.

From this study the optimum conditions were derived for the best enantiomeric separation.

Effect of Mobile-Phase Composition

The methanol-to-buffer ratio was varied from 40:60 to 90:10 to study the effect of mobile-phase composition on retention time, resolution, and selectivity. The retention time increased as the amount of methanol was increased. As the amount of methanol was increased the resolution improved until the composition was 60:40. Selectivity and resolution then decreased as the mobile-phase methanol content was increased further (Figure 3). Methanol-buffer in the ratio 60:40, i. e. that at which selectivity and resolution were highest, was therefore used throughout the experiments.

Effect of Ionic Strength

The solvent strength of the organic modifier will influence the solvophobic effect. It is common to add a buffer to the mobile phase to ensure that the solutes are uncharged during the separation, i. e. to avoid possible ionic interactions that might affect the separation. Because it is important to control the ionic behavior of solute, the effect of ionic strength on the separation of the enantiomers of tolperisone was studied by varying the ionic strength from 0.2% to 2%. Selectivity was not substantially affected but resolution increased as the ionic strength was increased to 1.4% (Figure 4) then decreased, possibly because of suppression of direct interaction between the CSP and the solute at higher ionic strengths. To obtain better chromatographic separation 1% TEAA buffer was used in subsequent experiments.

Effect of Buffer pH

pH is of fundamental importance in the optimization of separations on chiral β-cyclodextrin stationary phases. In this work glacial acetic acid was used to adjust the pH to suppress ionization of the silanol groups on the silica-based support. The effect of pH on the enantiomeric separation was investigated by varying the pH of the aqueous component of the mobile phase from 4 to 6.8. Increasing the pH above 5.5 increased the retention of the enantiomers and resulted in peak broadening. Resolution and selectivity were maximum at pH 5.5, and then decreased. Reducing the pH resulted in retention as a result of weak ionic binding. It is apparent from Figure 5 that pH 5 was the optimum for this separation.

Effect of Mobile-Phase Flow Rate

The effect of mobile phase flow rate on the enantiomeric separation was also examined. When the flow rate was varied from 0.1 to 1 mL min^{-1} resolution and enantioselectivity were found to be maximum at a flow rate of 0.5 mL min^{-1} and this was selected as the optimum for separation of the enantiomers.

Optimization of chromatographic processes includes optimization of running conditions to yield the best economically feasible separation. After examining the liquid-chromatographic separation and retention behavior of the enantiomers of tolperisone on a column containing heptakis (6-azido-6-deoxy) perphenylcarbamated β-cyclodextrin immobilized on silica gel, and its dependence on mobile phase composition, ionic strength, pH, and flow rate, the optimized isocratic conditions for the efficient, economical, and time-saving separation were: 60:40 (%, *v/v*) methanol-buffer, with the pH 5 buffer containing 1% TEAA, at a flow rate of 0.5 mL min^{-1}. A typical separation obtained under these conditions, with UV detection at 220 nm, is illustrated in Figure 6.

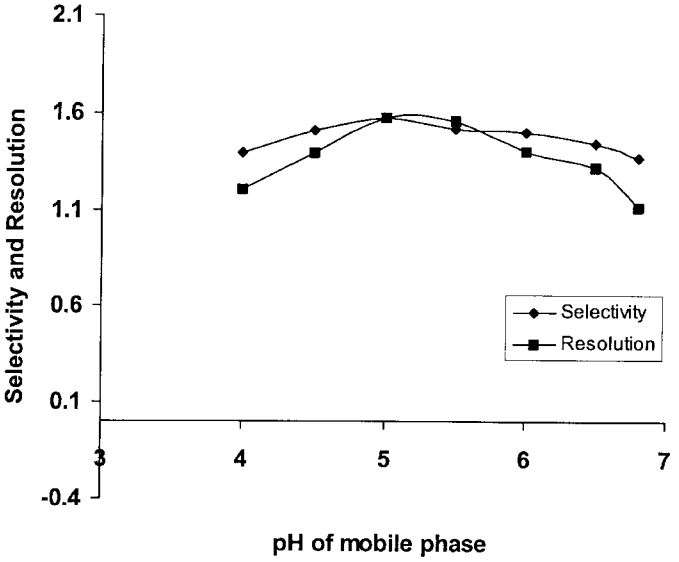

Figure 5. Effect of mobile-phase pH on resolution and selectivity.

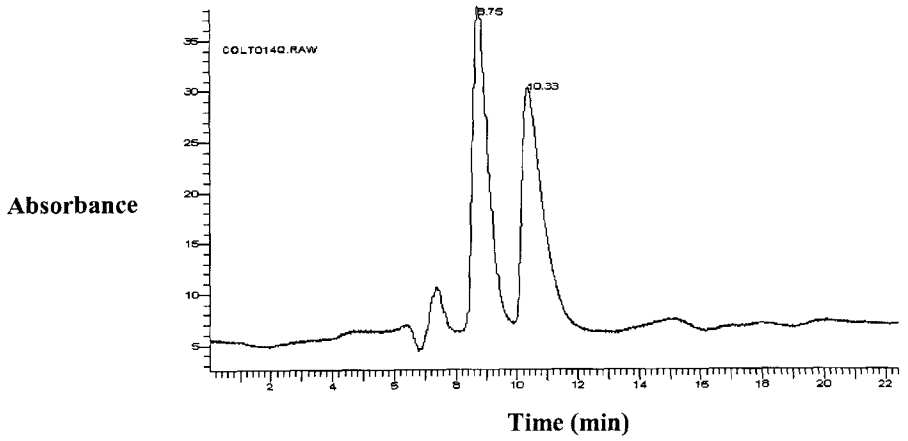

Figure 6. Chromatographic separation of the enantiomers of tolperisone.

Conclusions

It has been shown that heptakis(6-azido-6-deoxy) perphenylcarbamated β-cyclodextrin stationary phase (PC-CSP) chemically bonded via urea linkages can have significant advantages in chiral HPLC separations. The key advantages of the PC-CSP can be summarized as:

– first, high selectivity ($a > 1.55$) under reversed-phase conditions, partly attributable to the partitioning and binding of many hydrophobic, amphiphilic, and hydrophobic organic molecules in the β-cyclodextrin cavity;
– second, the use of this novel phenylcarbamated CSP eliminates most of the solubility problems typically associated with the use of organic solvents under reversed-phase conditions. Problems normally arise when solutes are added to control or vary experimental conditions such as pH, ionic strength, and buffer capacity. Because the chiral selector of this CSP is covalently bonded to silica gel, it can be used with a variety of mobile phases without significant deterioration of its chiral recognition properties.

The enantiomer separation can be affected by changing the pH, the concentration of ions in the mobile phase, and the flow rate. Reducing the pH of the mobile phase reduces the influence of ionic bonding. The chromatographic behaviour of the basic drug tolperisone was highly dependent on pH and the best separation was obtained by use of a pH between 4 and 5.5.

Low retention means it is not necessary to add modifier to the mobile phase to elute the enantiomers within a reasonable time. If the concentration of modifier in the mobile phase is low, higher enantioselectivity is obtained and this results in high resolution. Under the optimized conditions very good separation of the tolperisone enantiomers was obtained on PC-CSP, with the advantages of low cost and simple and rapid analysis. This (PC-CSP) column will be useful for application of continuous chromatography, which requires CSP materials of high efficiency and reproducibility.

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References

[1] Haginaka, J.; Kagawa, C.; Matsunaga, H. *J. Chromatogr. A* **1999**, *858*, 155–165.
[2] Rudaz, S.; Veuthey, J.L. *Chirality* **1999**, *11*, 319–325.
[3] Zhang, L.F.; Chen, L.; Lee, T.C.; Ng, S.C. *Tetrahedron: Asymmetry* **1999**, *10*, 4107–4113.
[4] Schulte, M.; Ditz, R.; Devant, R.M.; Kinkel, J.N.; Chaton, F. *J. Chromatogr. A* **1997**, *769*, 93–100.
[5] Felix, G.; Cachau, C.; Thienpont, A.; Soulard, M.H. *Chromatographia* **1996**, *42*, 583–590.
[6] Hennansson, J.; Grahn, A. *J. Chromatogr. A* **1995**, *694*, 57–69.
[7] Armstrong, D.W.; Chang, C.D.; Li, S.H. *J. Chromatogr.* **1991**, *539*, 83–90.
[8] Chen, L.; Zhang, L.F.; Ching, C.B.; Ng, S.C. *J Chromatogr. A* **2002**, *950*, 65–74.
[9] Zhang, L.F.; Chen, L.; Lee, T.C.; Ng, S.C. *Tetrahedron: Asymmetry* **1999**, *10*, 4107.
[10] Mosheni, R.M.; Hurtubise, R.J. *J Chromatogr. A* **1990**, *499*, 395–410.

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