

Separation of enantiomers and conformers of Tofisopamon

Using Daicel immobilized polysaccharide-derived chiral columns using the Agilent 1260 Infinity Analytical SFC System

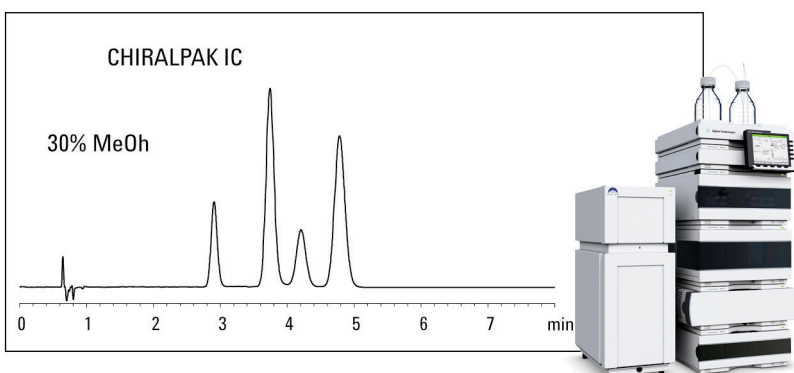
Application Note

Pharmaceuticals

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Abstract

Due to different physiological activities, the chromatographic separation of enantiomers is an important task in drug discovery and development. The determination of the enantiomeric excess (ee) is often done with supercritical fluid chromatography (SFC) in combination with chiral stationary phases. Since finding the right stationary phase that provides separation of the enantiomers is difficult to predict, automated screening of different stationary and mobile phases is advantageous. In this Application Note, the screening of different mobile and stationary phases for the separation of the enantiomers and conformers of Tofisopam is shown using the Agilent 1260 Infinity Analytical SFC and Daicel immobilized polysaccharide-derived chiral columns.



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Introduction

Tofisopam [1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine] is a member of the 2,3-benzodiazepine compound family. It has been applied as a pharmaceutical in the treatment of anxiety and alcohol withdrawal^{1,2}.

Due to its stereogenic center at C(5)-atom, Tofisopam exists as two enantiomers (R(+)) and S(-)). Upon dissolution, its diazepine ring system will exist in two boat conformations, leading to two conformers for each enantiomer (Figure 1). The driving force for conformer transition is attributed to the steric repulsion effect between C(4)-methyl and C(5)-ethyl groups.

As a result of the pharmacological interest in Tofisopam³, it is essential to have an easy and robust method to separate the four isomers in order to individually evaluate their different biological activities, to understand the kinetics and thermodynamics, and to control the quality of the drug.

In this Application Note, we have evaluated the separation of all enantiomers and conformers of Tofisopam with a single, robust, and fast chromatographic method using columns packed with the immobilized polysaccharide-derived chiral stationary phases (CSPs) in combination with the advanced SFC technology of the Agilent 1260 Infinity Analytical SFC System.

Experimental

Chemicals

The chiral columns CHIRALPAK IA, CHIRALPAK IB, CHIRALPAK IC, and CHIRALPAK ID used in this study are manufactured by Daicel Chemical Industries, Ltd. They are sized 4.6 × 150 mm id and packed with 5- μ m particles of immobilized amylose–or cellulose-derived CSPs.

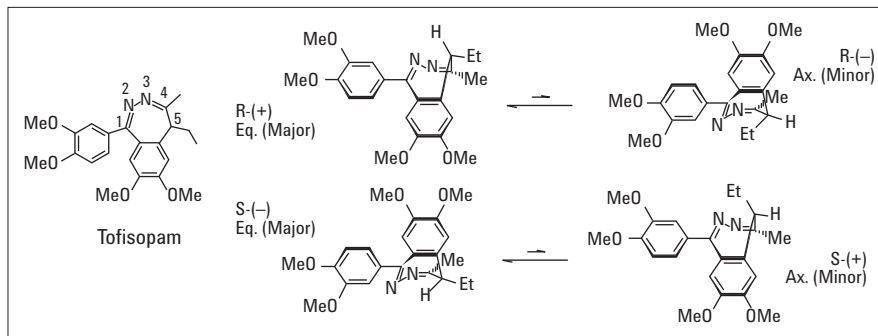


Figure 1
Conformational equilibria of Tofisopam enantiomers.

As the main mobile phase component, supercritical CO₂ (industrial quality 4.8) was used in this study. Different mobile phase modifiers such as methanol (MeOH), 2-propanol (2-PrOH), and acetonitrile (ACN) were screened in different concentrations. All solvents used were HPLC quality. Due to the basic nature of the Tofisopam molecule, diethylamine (DEA) was added to all mobile phases (0.1% v/v).

Instruments

All SFC experiments were carried out on an Agilent 1260 Infinity Analytical SFC System. The system contains the A5 fusion module for CO₂ pre- and post-conditioning and a modified Agilent 1260 Infinity Binary LC System for accurate and constant metering of the mobile phase. The system (G4309A) consisted of the following modules:

- SFC Fusion A5 module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity Standard Degasser
- Agilent 1260 Infinity Standard Autosampler
- Agilent 1260 Infinity Diode Array Detector with high pressure SFC flow cell

In addition, an Agilent SFC Method Development kit was used consisting of:

- Two Agilent 1260 Infinity Thermostatted Columns Compartments with built-in valve drives
- Agilent ZORBAX Method Development Valve Kit, 600 bar

Chromatographic conditions

Throughout the experimental work, the flow rate was set at 3.0 mL/min, the temperature of the column compartments at 35 °C and the back pressure of CO₂ supercritical fluid at 150 bar.

Results and Discussion

Due to their remarkable enantioselectivity and versatility, CSPs based on polysaccharide derivatives have been widely used for separation of enantiomers or stereoisomers by LC and SFC. Since 2004, the advanced immobilized version of the polysaccharide-based CSPs has been successfully integrated into the tool box for chiral separation. These new columns have the advantages of universal solvent compatibility, creation of new selectivity profiles, and material robustness⁴⁻⁶. In addition, SFC is considered as an advantageous technique over LC due to its improved diffusion properties, more favorable mass transfer characteristics, and low mobile phase viscosity which result in faster separation. The simultaneous separation of the enantiomers and conformers of Tofisopam has been evaluated in our laboratories with the combination of SFC and the four immobilized chiral columns: CHIRALPAK IA, CHIRALPAK IB, CHIRALPAK IC and CHIRALPAK ID. Various mobile phase modifiers were examined in a systematic way, including MeOH, 2-PrOH, and ACN.

As shown in Figure 1, R(+)- and S(-)-Tofisopam are the dominating conformers where the ethyl group attached to the C(5)-atom has a quasi-equatorial orientation (Eq.). Depending on the solvent in which the product is dissolved, the conformational equilibria will move in favor of the formation of R(-)- or S(+)- conformers where the ethyl group attached to the C(5)-atom will change to the axial orientation (Ax.). Our study on conformer transition of Tofisopam in various sample media (MeOH, EtOH, 2-PrOH, Methyl tert-butyl ether/MeOH 90/10 and ACN) indicate that MeOH induces the fastest formation in the highest proportion (up to 26%) of the R(-)- or S(+)- conformers, while ACN leads to the slowest

kinetics and in the lowest proportion (up to 16.5% only) of the same molecules⁷. In no case, does the presence of the R(-)- and S(+)- conformers overtake that of the R(+)- and S(-)- enantiomers in the sample mixture. As a consequence, the R(+)- and S(-)- enantiomers (Eq.) are always chromatographically eluted as major peaks and the R(-)- or S(+)- conformers (Ax.) are always eluted as minor peaks. The relative elution order between the R(+)- and S(-)- and between the R(-)- or S(+)- isomers were not determined in our study due to the lack of the reference standards. To facilitate the discussion, we denote here the minor peaks as $P_{(Ax.)}$ and the major peaks as $P_{(Eq.)}$ without assigning the absolute configuration of the molecules in each pair of enantiomer-conformers.

MeOH appears to be the most versatile co-solvent in resolution of Tofisopam isomers. As indicated in Table 1, the best resolution of the four peaks was achieved with CHIRALPAK IA and CHIRALPAK ID. Analysis times can be as short as 3 minutes (Figure 2 (a)) or 5 minutes (Figure 2 (b)), respectively. Interestingly, the apparent elution order is the same on CHIRALPAK IA, CHIRALPAK IC, and CHIRALPAK ID, that is, $P_{(Ax.)}$ - $P_{(Eq.)}$ - $P_{(Ax.)}$ - $P_{(Eq.)}$. Co-elution of two $P_{(Ax.)}$ and one $P_{(Eq.)}$ is observed on CHIRALPAK IB.

	CHIRALPAK IA	CHIRALPAK IB	CHIRALPAK IC	CHIRALPAK ID
tr_1 (min)	1.26	2.55	2.90	1.56
tr_2 (min)	1.48	2.55	3.74	1.90
tr_3 (min)	2.03	2.55	4.20	2.50
tr_4 (min)	2.45	2.72	4.77	3.91
$RS_{(12)}$	2.72		4.21	3.33
$RS_{(23)}$	4.44		1.90	4.19
$RS_{(34)}$	2.57	1.25	2.04	5.90

Table 1

Resolution of Tofisopam enantiomers and conformers with MeOH/CO₂. Co-solvent: 20% (MeOH/DEA 100/0.5 v/v) for CHIRALPAK IA and IC; 10% (MeOH/DEA 100/1.0 v/v) for CHIRALPAK IB; 40% (MeOH/DEA 100/0.25 v/v) for CHIRALPAK ID

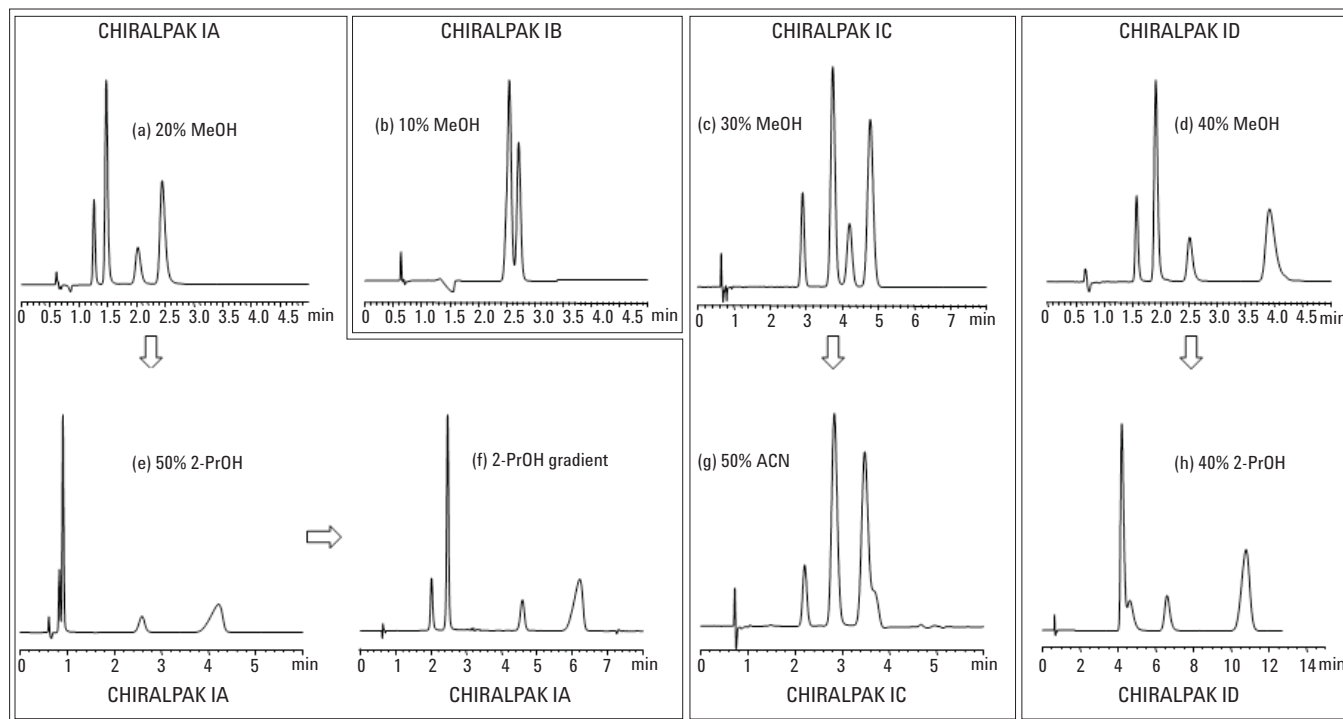


Figure 2

Separation examples. Modifier (solvent channel B, by volume):

20% (MeOH/DEA 100/0.5 v/v) for (a); 10% (MeOH/DEA 100/1.0 v/v) for (b); 30% (MeOH/DEA 100/0.33 v/v) for (c); 40% (MeOH/DEA 100/0.25 v/v) for (d);

50% (2-PrOH/DEA 100/0.2 v/v) for (e); Gradient* with (2-PrOH/DEA 100/0.2 v/v) for (f); 50% (ACN/DEA 100/0.2 v/v) for (g); 40% (2-PrOH/DEA 100/0.25 v/v) for (h)

*Gradient: 0-1.5 min 20% B; 1.5-2.5 min 20%-50% B; 2.5-6.5 min 50% B; 6.5-8.5 min 20% B

The nature of the modifier in SFC can greatly impact the resolution of the four peaks. For instance, the complete resolution on CHIRALPAK IA between the two first eluting peaks is compromised when changing from MeOH (Figure 2 (a)) to 2-PrOH (Figure 2 (e)). In order to achieve full resolution of the first two eluting peaks with this modifier, a gradient run proved to be a good approach (Figure 2, $Rs_{(12)} = 1.27$ in (e); $Rs_{(12)} = 4.18$ in (f)).

Modifiers also have an effect on elution profile of Tofisopam peaks. This is demonstrated by the separation examples on CHIRALPAK IC and CHIRALPAK ID. On CHIRALPAK IC, the apparent elution order is $P_{(Ax.)}^- - P_{(Eq.)}^- - P_{(Ax.)}^- - P_{(Eq.)}^-$ with 20% MeOH (Figure 2 (c)), but becomes $P_{(Ax.)}^- - P_{(Eq.)}^- - P_{(Eq.)}^- - P_{(Ax.)}^-$ with 50% ACN (Figure 2 (g)). On CHIRALPAK ID, the four species are eluted in the order of $P_{(Ax.)}^- - P_{(Eq.)}^- - P_{(Ax.)}^- - P_{(Eq.)}^-$ with 40% MeOH (Figure 2 (d)), but changes to $P_{(Eq.)}^- - P_{(Ax.)}^- - P_{(Ax.)}^- - P_{(Eq.)}^-$ while switching to 40% 2-PrOH (Figure 2 (h)).

The separation of Tofisopam isomers by SFC is perfectly reproducible, as demonstrated by 120 injections of Tofisopam over 20 hours on CHIRALPAK IC under the given chromatographic conditions (Figure 3). This can be attributed to the high performance of the Agilent 1260 Infinity SFC system providing superior CO₂ pre- and post-conditioning in combination with precise mobile phase metering. Such a system performance is depicted in Figure 4 for the CO₂ back (or outlet) pressure (a) and the pressure of the Agilent 1260 Infinity SFC Binary Pump (b).

Conclusion

The Daicel columns packed with immobilized polysaccharide-derived chiral stationary phases are highly robust for multiple separation solutions for enantiomers and conformers of Tofisopam.

The complete resolution of Tofisopam isomers in a single chromatographic run can be achieved in SFC and HPLC on the same set of chiral columns⁷. SFC is the preferred choice for faster separation of Tofisopam. The high instrument performance of the Agilent 1260 Infinity Analytical SFC System renders the analytical separation of Tofisopam robust and reproducible.

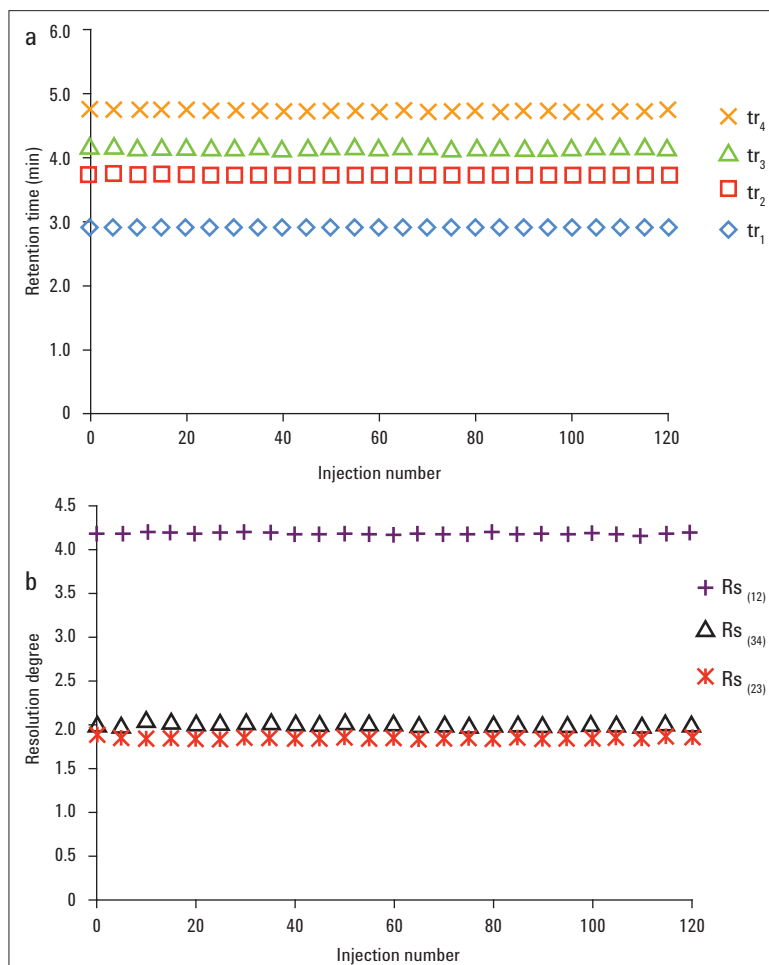


Figure 3
Reproducibility of the separation on CHIRALPAK IC
Modifier: 30% (MeOH/DEA 100/0.33 v/v), Flow rate: 3 mL/min, Temperature: 35 °C
(a) Retention time; (b) Resolution degree

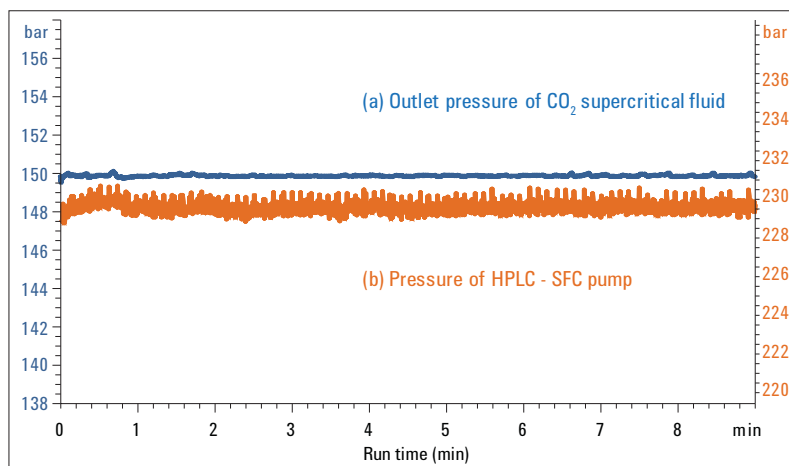


Figure 4
Pressure stability of the Agilent 1260 Infinity Analytical SFC System
Modifier: 30% (MeOH/DEA 100/0.33 v/v), Flow rate: 3 mL/min, Temperature: 35 °C

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