



# SFC Separation of Tofisopam Isomers

Tong ZHANG<sup>1</sup>, Dung NGUYEN<sup>1</sup>, Pilar FRANCO<sup>1</sup>, Martin VOLLMER<sup>2</sup>

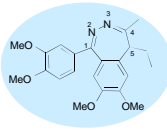
<sup>1</sup>CHIRAL TECHNOLOGIES EUROPE  
 Parc d'Innovation, Bd. Gonthier d'Andernach, B.P. 80140, 67404 Illkirch Cedex, FRANCE  
<sup>2</sup>AGILENT TECHNOLOGIES GmbH  
 Hewlett-Packard Str. 8, 76337 Waldbronn, Germany

tzhang@chiral.fr

## INTRODUCTION

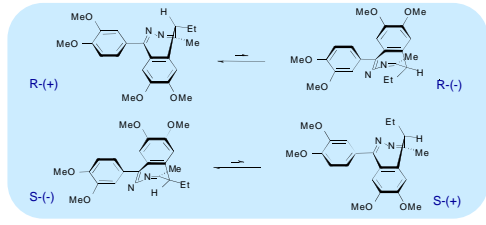
### Tofisopam

[1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-methyl-5H-2,3-benzodiazepine]



Tofisopam is a member of the 2,3-benzodiazepine compound family and has been used as a drug in the treatment of anxiety and alcohol withdrawal [1,2].

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



On count of the pharmacological interests of Tofisopam [3], it is essential to develop an efficient method for separating the four isomers in view of the individual study of their different biological activities, for understanding the kinetic and thermodynamic aspects of Tofisopam and for the quality control of the drug as well.

The current study focuses on efficient separation of the enantiomers and the conformers of Tofisopam in a single chromatographic run. Both HPLC and SFC techniques are explored for the purpose by using a set of columns packed with immobilised polysaccharide-derived chiral stationary phases.

## EXPERIMENTAL

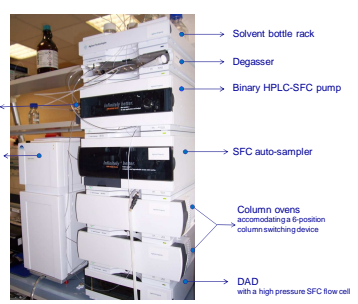
### Analytical chiral columns

Amylose phenylcarbamates	Cellulose phenylcarbamates
<b>CHIRALPAK IA</b> R:	<b>CHIRALPAK IB</b> R:
<b>CHIRALPAK ID</b> R:	<b>CHIRALPAK IC</b> R:

The above amylose and cellulose derivatives are immobilised on spherical 5µm/3µm silica matrix by Daicel Chemical Industries, Ltd. using proprietary technologies.

### Instruments

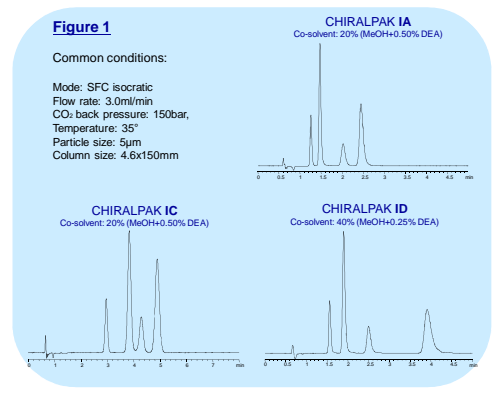
HPLC: Agilent 1100 equipped with a column switch device  
 SFC: Agilent 1260 Infinity Analytical SFC system



## SFC SEPARATIONS

The simultaneous separation of the enantiomers and conformers of Tofisopam was attempted by SFC on the four immobilised chiral columns using various co-solvents such as MeOH, EtOH, 2-PrOH, ACN and THF. Among them, MeOH proved to be the most helpful for the purpose in terms of selectivity, resolution degree and eluting strength.

As shown in Figure 1, three of the four columns in test could afford complete resolution of the four isomers within a very short time (5-6 minutes) using 20-40% MeOH containing diethylamine (DEA) additive. Unfortunately, the elution order in these separations was not determined due to the lack of the reference standards and the current challenge in detector hyphenation in the SFC system for DAD-CD or DAD-Polarimeter.



## HPLC SEPARATIONS

Owing to the immobilised nature, CHIRALPAK IA, IB, IC and ID columns could be extensively tested with a whole series of organic mobile phase systems (including the non-standard solvents such as THF, MtBE and DCM) with no risk of compromising the column stability and the method reproducibility [4]. The mobile phase systems investigated for separating Tofisopam isomers include:

- Hexane/EtOH and Hexane/2-PrOH
  - EtOH, MeOH and ACN
  - THF-containing
  - MtBE-containing
  - DCM-containing
- "Non-standard" mobile phases

As indicated by the data in Table 1 and Table 2, the separation degree and the elution order (determined by the optical rotation via a polarimeter) of the four Tofisopam isomers depend on the column as well as the mobile phase in use.

Table 1. Separation of Tofisopam isomers by MeOH/DEA 100/0.1

Column	Elution order*	Separation	RS(min)	RS(max)	Run time
CHIRALPAK IA	(-)-(+)-(+)-(-)	Partial-Full	1.38	3.23	8 minutes
CHIRALPAK IB	(+)-(+)-(+)-(-)	Null-Partial	0.00	1.73	6 minutes
CHIRALPAK IC	(+)-(+)-(+)-(-)	Full	2.46	3.87	12 minutes
CHIRALPAK ID	(-)-(+)-(+)-(-)	Partial-Full	1.72	10.44	14 minutes

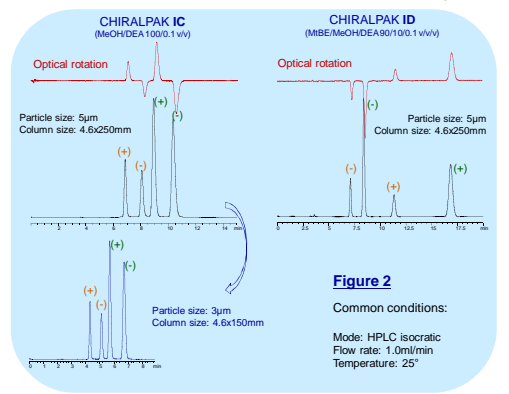
Table 2. Separation of Tofisopam isomers on CHIRALPAK IC

Bulk mobile phase	Elution order*	Separation	RS(min)	RS(max)	Run time
EtOH**	(+)-(+)-(+)-(-)	Partial-Full	1.68	4.01	20 minutes
MeOH	(+)-(+)-(+)-(-)	Full	2.46	3.87	12 minutes
ACN	(+)-(+)-(+)-(-)	Partial-Full	1.28	2.45	8 minutes
MtBE/MeOH 90/10	(+)-(+)-(+)-(-)	Null-Full	0.00	3.51	10 minutes

**Common conditions:**  
 Columns 4.6x250mm packed with 5µm particles;  
 Flow rate: 1.0ml/min (\*0.5ml/min); T=25°C

\* Peak separation and elution order.  
 \* or \*\*: fused or non-fused peaks  
 (+): Minor peak-positive optical rotation signal (C(5)-ethyl group at quasi-axial position)  
 (-): Minor peak-negative optical rotation signal (C(5)-ethyl group at quasi-axial position)  
 (+): Major peak-positive optical rotation signal (C(5)-ethyl group at quasi-equatorial position)  
 (-): Major peak-negative optical rotation signal (C(5)-ethyl group at quasi-equatorial position)

Two of the best HPLC separations obtained on CHIRALPAK IC and ID columns are presented in Figure 2.

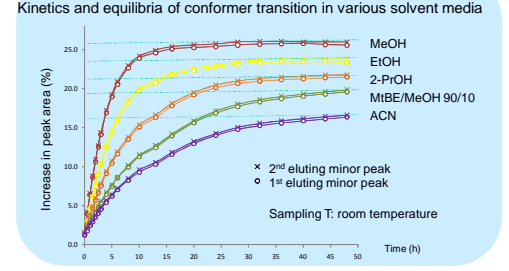


As demonstrated in the same figure, the method transfer from the 5µm-packed the same figure, the method transfer from the 5µm-packed 4.6x250mm-sized CHIRALPAK IC column to the 3µm-packed 4.6x150mm-sized one was straightforward. It led to the shortened analysis time (~40%) with no loss in resolution degree of the isomers. Taking the advantages of the short analysis and potentially high accuracy for quantitative analysis, this later method was used to investigate the conformer transition kinetics.

## CONFORMER TRANSITION

In solid state, R-(+)- and S(-)-Tofisopam are the dominating conformers in which the ethyl group attached to C(5)-atom has quasi-equatorial orientation. They correspond to the two last eluted major peaks on CHIRALPAK IC (Figure 2). Upon dissolution of solid Tofisopam in a solvent, the inter-conversion of the conformers occurs, leading to the two first eluted minor peaks in the same chromatogram. Depending on the solvent in use for dissolution of Tofisopam, the conformer transition shows different kinetics and equilibria (Figure 3). Curiously, the transition of one pair conformers is always at a slightly higher level than the other pair regardless of the sample dissolution medium.

Figure 3. Kinetics and equilibria of conformer transition in various solvent media



## CONCLUSION

The columns packed with immobilised polysaccharide-derived chiral stationary phases afford multiple separation solutions for complete resolution of Tofisopam isomers in a single chromatographic run. The SFC methods offer faster analyses than the HPLC ones.

The choice of the separation method can be guided by the chromatographic technique (SFC or HPLC), the elution profile and the sample dissolution-injection media. The column set in use constitute a useful tool for studying kinetics and thermodynamics in certain chiral/chemical processes.

## REFERENCES

- [1] T. Seppala, E. Palva, M.J. Mattila, R.C. Shrotriya, *Psychopharmacology* 69 (1980) 209-218.
- [2] A. Bond, M. Lader, *European Journal of Clinical Pharmacology*, 22 (1982) 137-142.
- [3] M. Hu, P. He, Y. Chen, G. Carr, J. Guo, N. Ye, *Journal of Chromatography A*, 1129 (2006) 47-53.
- [4] T. Zhang, D. Nguyen, P. Franco, *Journal of Chromatography A*, 1191 (2008) 214-222.