Enantiomer separation of non-steroidal anti-inflammatory drugs

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

Application Note

Pharmaceuticals

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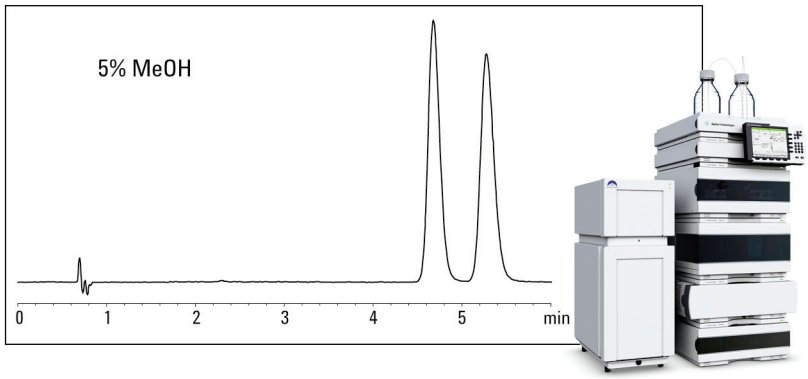

Abstract

The enantiomer separation by supercritical fluid chromatography (SFC) of six profen drugs was investigated on the new generation of chiral columns from Daicel Corporation, that are packed with the immobilized polysaccharide-derived chiral stationary phases CHIRALPAK IA, CHIRALPAK IB, CHIRALPAK IC, and CHIRALPAK ID. Methanol, ethanol, 2-propanol and acetonitrile were used in combination with trifluoroacetic acid as the mobile phase modifier in the supercritical CO_2. Using the Agilent 1260 Infinity Analytical SFC System, systematic studies were carried out in terms of the mobile phase additive, the modifier nature and its percentage to achieve complete resolution of these profen drugs on the given column set.

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Introduction

Profens (2-arylmethylpropionic acids), such as Flurbiprofen, Ibuprofen, and Ketoprofen, have been widely used as non-steroidal anti-inflammatory drugs (NSAIDs). The enantiomers of these chiral drugs are reported to have different pharmacological and pharmacokinetic effects and undergo dissimilar metabolic processes[1-2]. As a consequence, the enantiomer resolution of profens and method optimization for diverse purposes have been the subject of intensive investigations by chromatography[3].

The columns packed with polysaccharide-based chiral stationary phases (CSPs) have proved suitable for enantiomer resolution of a number of profen drugs[4-8]. The advanced immobilized version of the polysaccharide-based CSPs offers the possibility of enhancing the application scope and enantioselective performance. Specifically, these new generation columns have the advantages of robustness, universal solvent compatibility and creation of new selectivity profiles[9]. They have now been successfully integrated into the tool box for chiral separation by LC and SFC.

The objective of the current study is to investigate the analytical separation feasibility of the typical profen enantiomers using a set of columns packed with the immobilized polysaccharide-derived CSPs in combination with advanced technology of the Agilent 1260 Infinity Analytical SFC System. The structures of the profen molecules under investigation are shown in Figure 1.

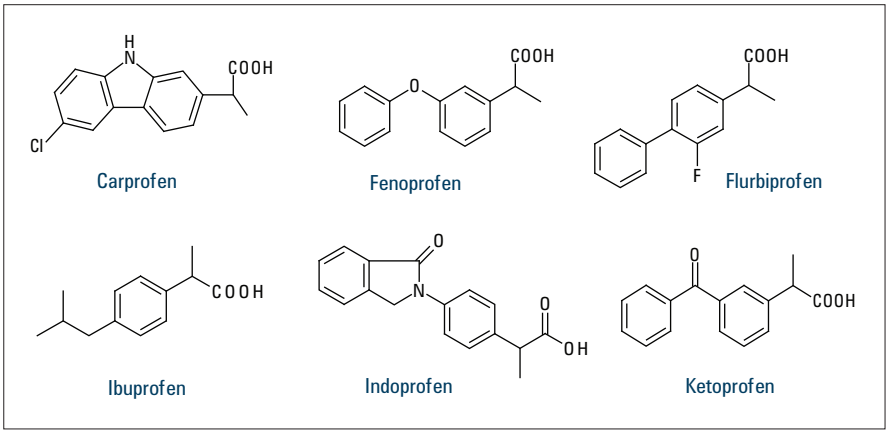

Figure 1
Structures of the profen molecules under investigation.

Experimental

Chemicals

The chiral columns CHIRALPAK IA, CHIRALPAK IB, CHIRALPAK IC, and CHIRALPAK ID used in this study were manufactured by Daicel Corporation, Tokyo, Japan. Columns with dimensions of 4.6 × 150 mm and packed with 5-µm particles of immobilized amylose- or cellulose-derived CSPs were used.

The liquid carbon dioxide (industrial quality 4.8) in a cylinder of B50 was used for CO_2 supply. Different mobile phase modifiers such as methanol (MeOH), ethanol (EtOH), 2-propanol (2-PrOH), and acetonitrile (ACN) were employed in different proportions. All solvents used were HPLC quality. Trifluoroacetic acid (TFA) was used as the acidic additive and added to the modifier solvents equivalent to 0.06% by volume in the mobile phase.

Instrumentation

All SFC experiments were carried out on an Agilent 1260 Infinity Analytical SFC System. The system (G4309A) consists of the following modules:

- Aurora SFC Fusion A5 module for CO_2 pre- and post-conditioning
- Agilent 1260 Infinity SFC Binary Pump for accurate and constant metering of the mobile phase
- Agilent 1260 Infinity Degasser
- Agilent 1260 Infinity Autosampler
- Agilent 1260 Infinity Diode Array Detector with high pressure SFC flow cell

In addition, the Agilent SFC Method Development Kit was integrated into the system consisting of two Agilent 1260 Infinity Thermostatted Column Compartments with built-in valve drive and the 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 supercritical fluid carbon dioxide ($SF\text{-}CO_2$) at 150 bar.

Results and Discussion

The first screening of the profen molecules was undertaken by running a gradient (Figure 2). This allowed the determination of the modifier percentage to be used in isocratic mode so that the compounds were eluted in a reasonable time window (for example, 2 to 10 minutes). With this approach, isocratic runs delivering no enantiomer separation were avoided. The guideline for method transfer from gradient to isocratic is given in Table 1. In certain cases, fine-tuning regarding the modifier concentration may be necessary to optimize the separation.

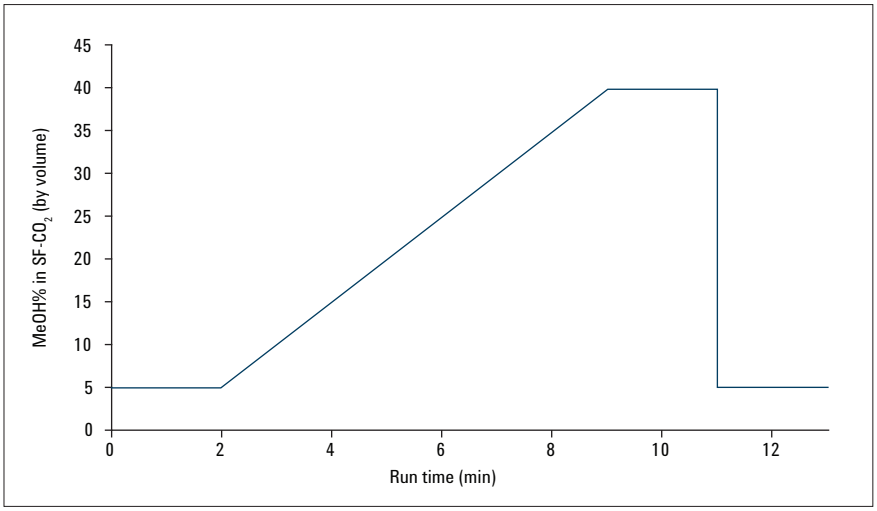

Figure 2
MeOH gradient program.

*t grad. (min)	Modifier%
>11	40 - 50
8 - 11	30
5.5 - 8	20
4 - 5.5	10
< 4	2 - 5

Table 1
Guideline from gradient to isocratic.
(* t grad. = (tr1+tr2)/2)

Effect of acidic additive

Due to its acidic character, SF-CO_2 has been used as the major mobile phase component for enantiomer resolution of acidic compounds with no additional acidic additives[10].

Using this approach, the racemic profens were first injected on the columns using MeOH as modifier without any additives. However, some comparative trials in the presence of TFA at trace level (0.06%) led to improved separation of the enantiomers. As indicated by the data in Table 2 and Table 3, the addition of 0.06% TFA in the mobile phase systematically resulted in:

- Shorter retention times (tr)
- Slightly higher enantioselectivity (α)
- Increased plate counts (N)
- Better peak symmetry (S)

These four benefits led to increased resolution (Rs) of the enantiomers.

	MeOH%	TFA %	tr_1	tr_2	α	Rs
Carprofen	40	-	1.72	1.93	1.19	1.86
	40	**0.06**	**1.67**	**1.88**	**1.20**	**2.21**
Flurbiprofen	10	-	2.05	2.34	1.20	2.55
	10	**0.06**	**1.84**	**2.14**	**1.25**	**3.40**
Ibuprofen	5	-	5.62	5.90	1.08	0.85
	5	**0.06**	**5.22**	**5.51**	**1.09**	**0.96**
Indoprofen	30	-	3.10	3.23	1.05	0.64
	30	**0.06**	**2.94**	**3.08**	**1.06**	**0.84**

Table 2
Chromatographic results with and without TFA in the mobile phases.
Column: CHIRALPAK IA

	MeOH%	TFA %	N_1	N_2	S_1	S_2
Carprofen	40	-	4478	4418	0.75	0.77
	40	**0.06**	**5643**	**5511**	**0.89**	**0.92**
Flurbiprofen	10	-	6250	6643	0.67	0.73
	10	**0.06**	**7797**	**8199**	**0.95**	**0.97**

Table 3
Effect of TFA on plate counts (N) and peak symmetry factors (S).
Column: CHIRALPAK IA

The effect of TFA on enantiomer separation of Carprofen and Flurbiprofen is demonstrated in Figure 3. The presence of TFA in the mobile phase, even at very low concentration, can more efficiently suppress the undesirable achiral interaction between the acidic molecules and the silica matrix therefore enhancing the enantiomeric resolution of acidic compounds. As a consequence, 0.06% TFA was added to the mobile phases throughout the following experiments.

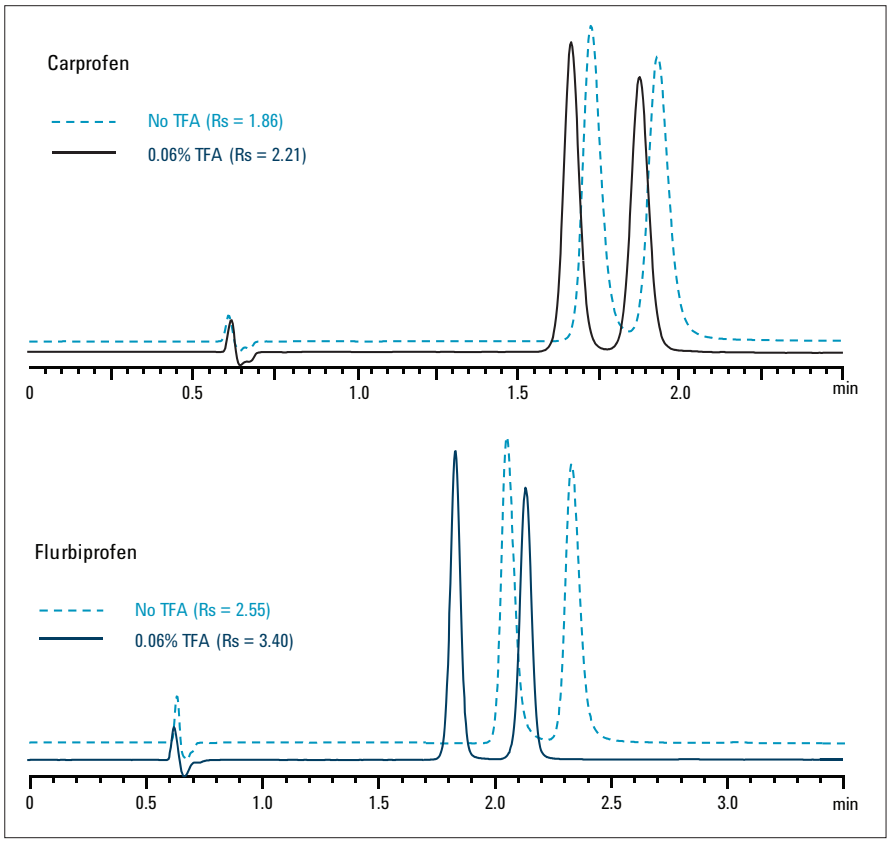

Figure 3
Improved enantiomer resolution of profens with 0.06% TFA.
Column: CHIRALPAK IA

Effect of modifier

It has been reported that the organic modifier may have an important effect in chiral SFC with packed columns of polysaccharide-derived CSPs[11].

This was also observed with the profen molecules. As summarized in Table 4, the most suitable modifier for the separation varied depending on the molecule structure. For instance, the highest resolution of Carprofen enantiomers was obtained with 2-PrOH (Figure 4a) while no separation was observed when ACN was used as modifier (Figure 4b). In the case of Flurbiprofen, MeOH resulted in the highest enantio-selectivity and the largest resolution. For the separation of Ketoprofen enantiomers, however, ACN delivered the best results. Considering these findings, the automated screening of modifiers is the most effective approach for SFC method development.

It should be noted that, in comparison with the alcoholic modifiers, ACN appeared to be a relatively poor modifier. It has considerably weaker eluting strength (needing higher percentage) and sometimes induces broader peak shape associated with compromised peak symmetry, even in the presence of TFA.

	CHIRALPAK	Modifier and its %		tr_1	tr_2	α	Rs
Carprofen	IA	MeOH	30	3.44	3.96	1.19	2.88
		EtOH	30	2.67	2.79	1.06	0.67
		2-PrOH	**30**	**3.53**	**5.23**	**1.59**	**7.38**
		ACN	50	3.16	3.16	1.00	0.00
Flurbiprofen	ID	**MeOH**	**5**	**2.06**	**2.75**	**1.50**	**4.33**
		EtOH	5	1.97	2.31	1.27	2.01
		2-PrOH	5	2.35	2.65	1.18	1.36
		ACN	20	2.21	2.88	1.45	3.66
Ketoprofen	ID	MeOH	10	2.08	2.25	1.12	1.44
		EtOH	10	2.16	2.26	1.07	0.74
		2-PrOH	10	2.98	2.98	1.00	0.00
		ACN	**30**	**2.10**	**2.68**	**1.42**	**2.73**

Table 4
Chromatographic results with various modifiers.

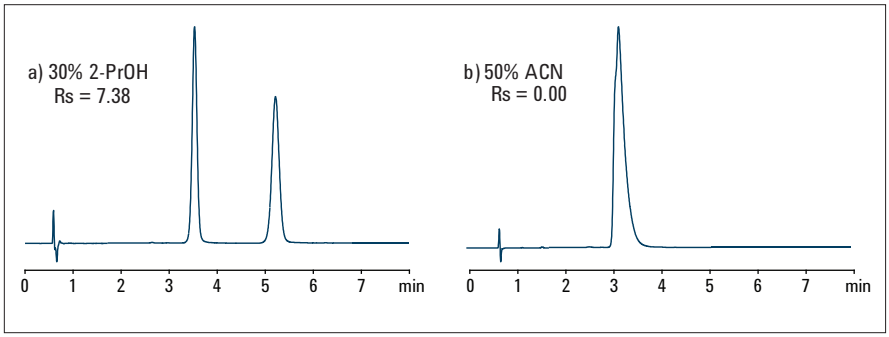

Figure 4
Separation of Carprofen enantiomers with different modifiers.
Column: CHIRALPAK IA

Effect of modifier percentage

In SFC, the eluting strength of the mobile phase increases with the concentration of the polar organic modifiers. As a general rule, the retention times decrease with the increase in percentage of a given modifier.

In SFC, the decrease in modifier percentage to a reasonable extent may be favorable for the enantiomer separation. The experimental results with variation of the modifier content in SF-CO_2 are presented in Table 5. It can be observed that the lower percentage of the modifier resulted in increased enantiomer resolution,. However, such variations did not significantly impact the enantio-selectivity. The separation examples in Figure 5 show that the incomplete separation of Ketoprofen enantiomers was transformed into full separation by simply reducing MeOH concentration from 10% to 5%. However, the gain in resolution in this case was at the expense of a significantly longer analysis time.

	CHIRALPAK	Modifier and its %		tr_1	tr_2	α	Rs
Carprofen	IA	MeOH	40	1.67	1.88	1.20	2.21
			30	3.44	3.96	1.19	2.88
			20	**6.58**	**7.74**	**1.19**	**3.34**
Ibuprofen	IA	2-PrOH	5%	2.78	2.82	1.02	0.19
			2%	**9.49**	**10.15**	**1.08**	**0.80**
Indoprofen	IC	MeOH	30%	2.51	2.67	1.08	1.12
			20%	**6.22**	**6.70**	**1.09**	**1.61**
Ketoprofen	ID	MeOH	10%	2.08	2.25	1.12	1.44
			5%	**4.67**	**5.27**	**1.15**	**2.29**

Table 5
Chromatographic results of different modifier percentages.

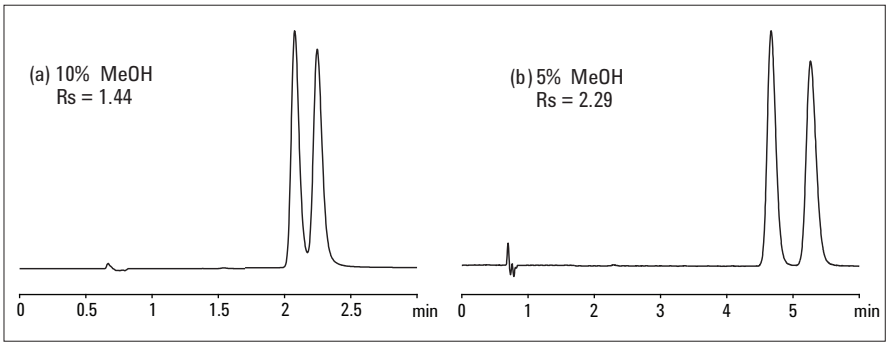

Figure 5
Separation of Ketoprofen enantiomers with different modifier percentage.
Column: CHIRALPAK ID

Conclusions

The complete enantiomer separations of the six racemic profen drugs are readily achieved by SFC using the columns packed with Daicel immobilized polysaccharide-derived CSPs. The best separations of the profen enantiomers are obtained alternately on CHIRALPAK IA, CHIRALPAK IC, and CHIRALPAK ID (Table 6) depending on the individual compound. CHIRALPAK IB certainly merits its involvement in the general chiral screening by offering some specific separations, although it does not deliver the best separation results with the current series of compounds.

Applying automated method development and solvent selection with the Agilent 1260 Infinity Analytical SFC System is fast, straightforward and highly successful for the selected profen drug examples.

	CHIRALPAK	Modifier and its %		tr_1	tr_2	α	Rs
Carprofen	IA	2-PrOH	30	3.53	5.23	1.59	**7.38**
Fenoprofen	IC	ACN	20	1.89	2.12	1.19	**2.15**
Flurbiprofen	ID	MeOH	5	2.06	2.75	1.50	**4.33**
Ibuprofen	IC	ACN	15	2.05	2.33	1.21	**2.46**
Indoprofen	ID	MeOH	30	2.66	3.19	1.27	**2.95**
Ketoprofen	ID	MeOH	5	4.67	5.27	1.15	**2.29**

Table 6
Optimized SFC separations of the profen enantiomers.

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