
Chiral separation using Supercritical Fluid Chromatography



K. Miyazawa, T. Ishiguro, H. Oda*

FPC production, Production DEPT., CPI Company, Daicel Chemical Industries, LTD.,
1-1 Shinko-cho, Myoko, Niigata 944-8550, Japan

Internet: <http://www.daicelchiral.com/>

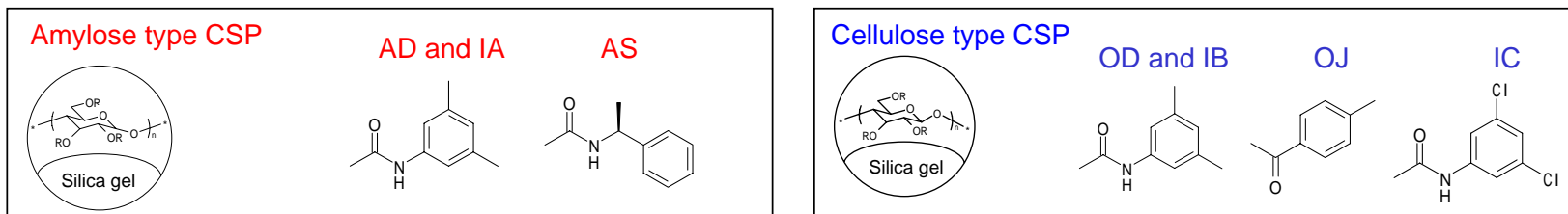
Since 1980's, chiral chromatography has been recognized as the fastest method for separation of chiral compounds. High-Performance Liquid Chromatography (HPLC) with a chiral column has become a global standard for analysis of chiral compounds. It is also widely used for preparative purposes as a simple and reliable technique compared to the conventional ones such as asymmetric synthesis and diastereometric crystallization. Nowadays, Simulated Moving Bed method (SMB) has been also regarded as a suitable tool for industrial-scale separation of chiral compounds.

In recent years, Supercritical Fluid Chromatography (SFC) has attracted much attention from many researchers. Supercritical fluid (in some cases, including Subcritical fluid) has some unique and favorable properties for chromatography. For example, diffusivity of supercritical fluid is higher than that of conventional organic solvents used for HPLC by several to tens of times while its viscosity is one third or less. These properties lead to higher column efficiency (i.e. larger number of theoretical plates), which enables us higher throughput separation with higher flow rate.

We have studied SFC preparative separation of some pharmaceutical compounds using CHIRALPAK® and CHIRALCEL® chiral stationary phases (CSPs) of Daicel, including CHIRALPAK® IA, IB and IC immobilized polysaccharide type CSPs. As a result, we found that many pharmaceutical compounds could be sufficiently separated in a very short period of time. In some cases, extremely efficient separation was achieved compared to the conventional HPLC method.

Materials and Methods

■ Chiral stationary phase used CHIRALPAK® AD, AD-H, AD-3, AS-H, IA, IB, IC and CHIRALCEL® OD, OD-H, OJ-H.



■ 3 type SFC system were used for Analysis and Preparative separation.



← Analytical SFC (JASCO)

CO₂ supply : 7kg gas cylinders
Flow rate : max 10mL/min.
Back Pressure max 30MPa
Temperature : 4-80degC (ambient)
detection : UV-VIS (multiwavelength)
Column : 0.46cm I.D. x 25cmL



→ Semi-Prep SFC (JASCO)

CO₂ supply : 7kg gas cylinders
Flow rate : max 60mL/min.
Back Pressure : 10MPa - 30MPa
Temperature : 4 - 80degC (ambient)
Detection : UV-VIS
Column: Up to 2cm I.D. x 25cmL
Fraction collector x 6



↗ Bench-scale Prep SFC (in-house designed)

CO₂ supply : 7kg & 30kg gas cylinders
Flow rate : max 230mL/min.
Back Pressure : max 20MPa
Temperature : 25degC (ambient)
Detection : UV-VIS
Column: 2-5cm I.D. x 25cmL
CO₂ recycle system (recycle rate :60-80%)
Fraction collector x 5

Case study of SFC analytical separation

■ SFC separation using coated-type CSP and alcohols

1)alpha-propionic acid derivatives (acidic compound)

Compound	Column	Mobile Phase	Flow rate	k'1	Alpha	Rs
Flurbiprofen	AD-H	CO ₂ /Methanol=90/10	2.5ml/min	2.00	1.90	8.17
Naproxen	AD-H	CO ₂ /Methanol=80/20	2.5ml/min	1.54	1.24	2.32
Suprofen	AD-H	CO ₂ /Methanol=80/20	2.5ml/min	2.42	1.47	4.38
Alminoprofen	AD-H	CO ₂ /Methanol=90/10	2.5ml/min	3.96	1.36	4.69

2)beta-blocker (basic compound)

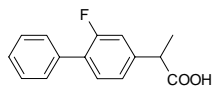
Compound	Column	Mobile Phase	Flow rate	k'1	Alpha	Rs
Atenolol	OD-H	CO ₂ /Methanol/DEA=80/20/0.1	2.5ml/min	1.69	2.84	7.73
Metoprolol	OD-H	CO ₂ /Methanol/DEA=80/20/0.1	2.5ml/min	0.50	4.29	7.85
Oxprenolol	OD-H	CO ₂ /IPA/DEA=80/20/0.1	2.5ml/min	1.23	5.03	8.76
Pindolol	OD-H	CO ₂ /Methanol/DEA=70/30/0.1	3.0ml/min	1.04	5.93	10.23

3)others

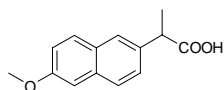
Compound	Column	Mobile Phase	Flow rate	k'1	Alpha	Rs
Warfarin	OD-H	CO ₂ /Ethanol=80/20	2.5ml/min	1.98	2.51	7.47
Guaifenesin	OD-H	CO ₂ /Ethanol=85/15	2.0ml/min	0.87	1.90	5.94
Naringenin	AD-H	CO ₂ /Methanol=60/40	2.5ml/min	2.99	1.62	4.32

■ SFC separation using immobilised-type CSP

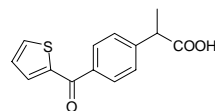
Compound	Column	Mobile Phase	Flow rate	k'	Alpha	R_s
Benzoin	IA	CO ₂ /THF=80/20	2.5ml/min	4.25	1.21	3.67
BINOL	IA	CO ₂ /THF=80/20	2.5ml/min	5.59	1.30	3.28
Flavanone	IA	CO ₂ /MTBE=70/30	3.0ml/min	4.03	1.76	3.17
Tröger's base	IB	CO ₂ /DMF=90/10	2.5ml/min	1.28	1.75	6.93
trans-Stilbene Oxide	IA	CO ₂ /Dioxane=80/20	2.5ml/min	0.65	2.36	4.93
TFAE	IB	CO ₂ /Dioxane=80/20	2.5ml/min	1.90	3.36	4.40
Wieland-Miescher ketone	IA	CO ₂ /Dioxane=80/20	2.5ml/min	1.70	1.21	1.20
Wieland-Miescher ketone	IC	CO ₂ /Acetonitrile=80/20	2.5ml/min	6.01	1.49	7.26
Co(acac) ₃	IC	CO ₂ /IPA=80/20	2.5ml/min	1.50	1.53	3.77



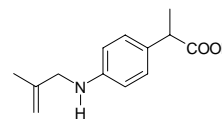
Flurbiprofen



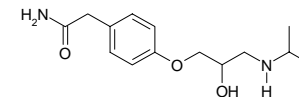
Naproxen



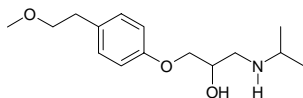
Suprofen



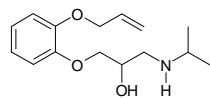
Alminoprofen



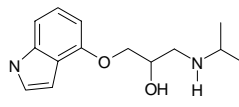
Atenolol



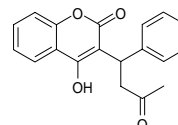
Metoprolol



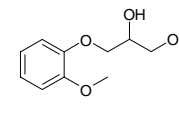
Oxprenolol



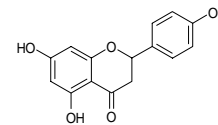
Pindolol



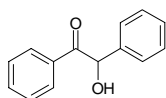
Warfarin



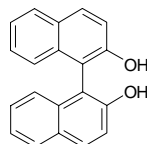
Guaifenesin



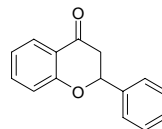
Naringenin



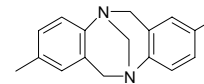
Benzoin



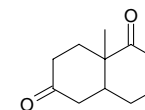
BINOL



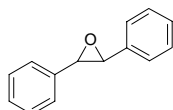
Flavanone



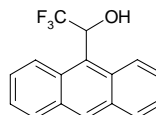
Tröger's base



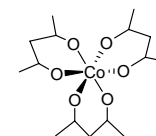
Wieland-Miescher ketone



trans-Stilbene Oxide



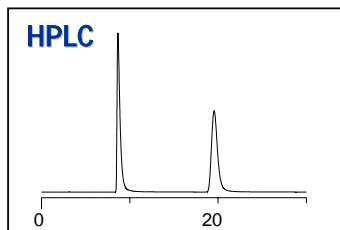
TFAE



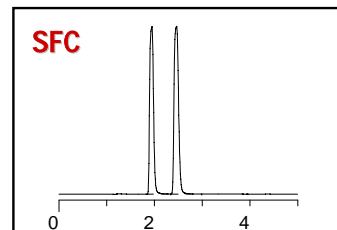
Co(acac)₃

SFC performance relative to HPLC

■ Guaifenesin



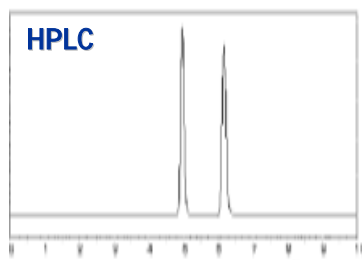
OD-H 0.46cmID x25cmL
Hex/Ethanol=80/20
Flow rate: 1.0mL/min
Temperature: 25degC



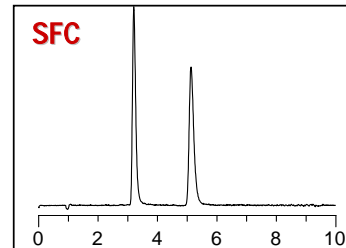
OD-H 0.46cmID x25cmL
CO₂/Ethanol = 70/30
Flow rate: 3.0mL/min
Temperature: 25degC
Back Pressure.: 15MPa

7.5-fold reduction in analysis time

■ Flurbiprofen



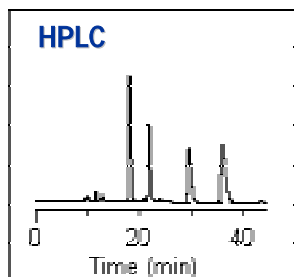
AD-H 0.46cmID x25cmL
Hex/IPA/TFA=80/20/0.1
Flow rate: 1.0mL/min
Temperature: 25degC



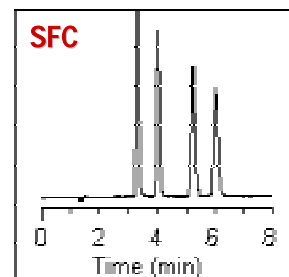
AD-H 0.46cmID x15cmL
CO₂ /Methanol=90/10
Flow rate: 2.5mL/min
Temperature: 25degC
Back Pressure.: 15MPa

**Higher resolution
Could separate without
TFA additives**

■ Diastereomer compound X (Acidic compound)



AD-H 0.46cmID x25cmL
Hex/Ethanol/AcOH=90/10/0.1

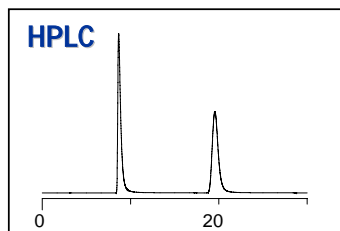


AD-H 0.46cmID x25cmL
CO₂/Ethanol=75/25

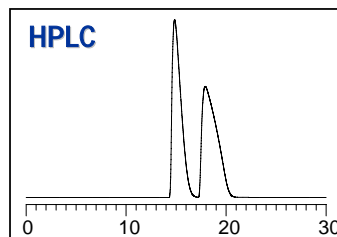
**5.6-fold reduction in analysis time
Could separate without
AcOH additives**

Preparation of Guaifenesin

■ Loading study

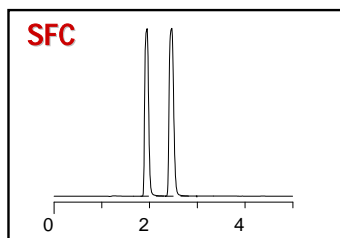


Adjusted for Preparative Condition

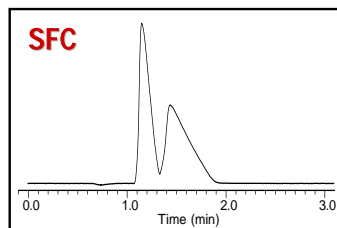


Mobile Phase: Hex/Ethanol = 50/50 (v/v)
 Flow rate: 0.3mL/min
 Temperature: 25degC

Solubility : 30g/L in mobile phase
 Injection : 7.5mg
 Interval : 8 min.



Adjusted for Preparative Condition



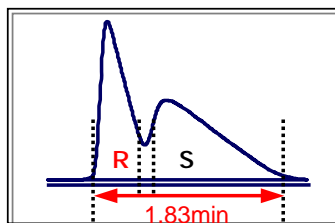
Mobile Phase: CO₂/Ethanol = 70/30 (v/v)
 Flow rate: 5.6mL/min
 Temperature: 25degC
 Back Pressure.: 15MPa
 Solubility : 200g/L in Ethanol
 Injection : 19.4 mg
 Interval : 1.4 min.

■ Expected productivity

	Productivity [kg-en/kgCSP/day]	Production rate using 2cmID column [g/day]	Solvent consumption rate [L/g-en]
HPLC	0.3	25	0.34
SFC	4.0	400	0.11

■ Experimental proof

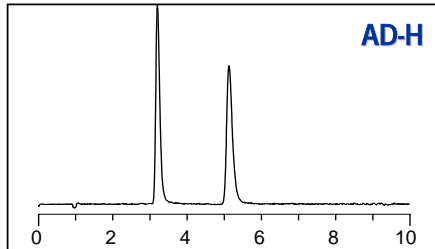
Column : OD (1cmID x 25cmL)
 Mobile phase : CO₂/Ethanol = 80/20(v/v)
 Flow rate : 60 g/min
 Back pressure : 15 MPa
 Injection : 460 ml (200 mg/ml)
 Temperature : 25 degC



	optical purity[%e.e.]	yield[%]
R-component	99.0	94.9
S-component	98.8	98.8

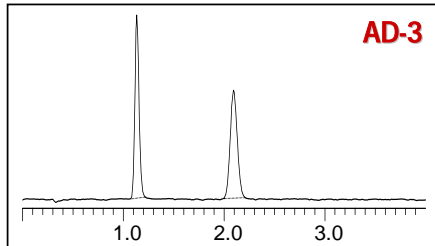
Comparison of efficiency between 3micron CSP and 5micron CSP

■ AD-3 analysis and loading study of Flurbiprofen



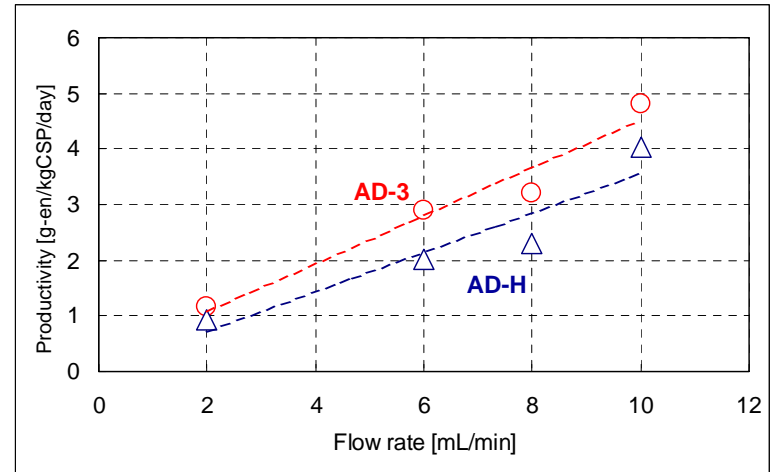
AD-H 0.46cmID x15cmL
CO₂ /Methanol=90/10
Flow rate: 2.5mL/min
Temperature: 25degC
Back Pressure.: 15MPa

Alpha = 1.90
Rs = 8.17



AD-3 0.46cmID x15cmL
CO₂ /Methanol=90/10
Flow rate: 8.0mL/min
Temperature: 25degC
Back Pressure.: 10MPa

Alpha = 2.12
Rs = 9.42



Productivity of Flurbiprofen

Column : AD-H(5micron) and AD-3(3micron)

Mobile phase : CO₂/methanol= 80/20 (AD-H), CO₂/methanol= 85/15 (AD-3)

Comparison of estimative production with 3 types AD column

	Particle size [micron]	Length [cm]	@MeOH [%]	Flow rate [mL/min]	Pressure drop [MPa]	Productivity [kg-en/kgCSP/day]
AD	20	25	20	8.0	4.0	2.21
AD-H	5	25	20	6.0	9.3	2.02
AD-3	3	15	15	6.0	8.0	2.89

	Production [g-en/day]	CO ₂ Consumption [kg-CO ₂ /g-en]	MeOH Consumption [kg-MeOH/g-en]
AD 2cmID x 25cmL	104	1.5	0.33
AD-H 2cmID x 25cmL	95	1.3	0.27
AD-3 2cmID x 15cmL	82	1.6	0.24

Conclusion

- SFC is an alternative and powerful tool for chiral separation.
- SFC have higher productivity , but less solvent consumption.
- 3micron Column is effective tool to progress the SFC high throughput analysis and prep separation.

Appendix : Past results of SFC in Daicel

Past results of productivity - SFC vs HPLC

	%
HPLC's productivity was unknown	24.1
Obtained no resolution with HPLC	34.5
Productivity : HPLC < SFC	3.4
Productivity : SFC > HPLC	
~ 2 times	3.4
~ 10 times	17.2
~ 50 times	10.3
over 50 times	6.9

*29 projects have been performed from 2003 to 2007