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Parallel SFC: A Faster Approach to Chiral Column Screening

Introduction

Supercritical fluid chromatography (SFC) is increasingly utilized for the separation and purification of optical isomers (chiral compounds) due to its advantages over high-performance liquid chromatography (HPLC), including faster analysis times, reduced consumption of organic solvents, and simplified post-processing. SFC systems enable rapid identification of optimal separation conditions through automated variation of column chemistries (column screening) and modifier solvent compositions, facilitating efficient chiral separations at the analytical scale. Once suitable conditions are established, methods can be readily scaled up for preparative purification. Since SFC is faster and more environmentally friendly compared to HPLC, it has been widely adopted in pharmaceutical drug discovery, particularly in response to increasingly stringent regulations governing the use and disposal of organic solvents.

There are two primary approaches used for column screening in SFC. In the first approach, the desired column is selected by switching valves positioned before and after the column. In the second approach, a parallel screening method is employed in which the flow path is divided at a manifold, allowing the mobile phase and the sample to be delivered simultaneously to multiple columns. This parallel configuration enables concurrent analyses, significantly reducing overall screening time. In this application note, we demonstrate the separation of flavanone, a chiral compounds, using the parallel SFC system.

Keywords

SFC, supercritical fluid chromatography, Chiral compounds, Column screening, CHIRALPAK, UV detector

Experimental

Instruments

CO_2 pump:	PU–4386
Modifier pump:	PU–4086*
Heater:	HE–02
Heater controller:	HC–4068-01
Autosampler:	AS–4350
Column oven:	CO–4060*
UV detector:	UV–4075* x 4
BP regulator:	BP–4340

* with option units

SFC Conditions

Column:	CHIRALPAK IA, IB, IC, ID* (4.6 mm I.D. x 150 mm L, 5 µm)
Eluent A:	Carbon dioxide
Eluent B:	Acetonitrile, methanol
Flow rate:	Eluent A; 8.0 mL/min
	Eluent B; 3.0 mL/min
Column temperature:	40 °C
Wavelength:	250 nm
Back pressure:	15 MPa
Injection volume:	10 µL
Sample:	1 mg/mL flavanone in MeOH

* CHIRALPAK is a trademark or registered mark of Daicel Corporation.

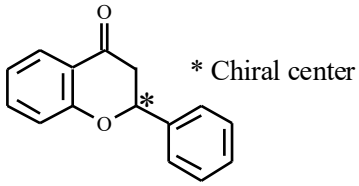
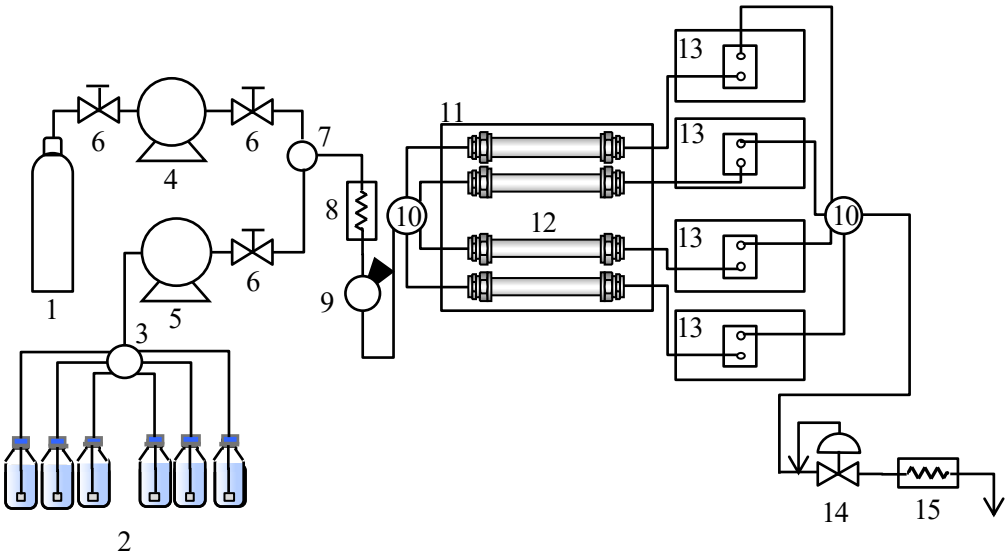


Fig. 1 *Structure of Flavanone*



1. CO_2 cylinder	6. Stop valves	11. Column oven
2. Modifier solvents	7. Mixing unit	12. Columns
3. Solvent switching valve	8. Heater	13. UV detector
4. CO_2 pump	9. Autosampler	14. Back pressure regulator
5. Modifier pump	10. Manifolds	15. Heater

Fig. 2 *Schematic diagram of SFC system*

Results

Figure 3 shows chromatograms of flavanone obtained using the parallel SFC system. Acetonitrile and methanol were evaluated as modifier solvents, and both achieved baseline separation within 4 minutes. The results indicate that, with both solvents, racemic flavanone can be successfully resolved on CHIRALPAK IA and CHIRALPAK ID columns.

On the CHIRALPAK IA column, shorter retention times were observed with acetonitrile compared to methanol, whereas the opposite trend was observed on the CHIRALPAK ID column. The reproducibility of retention times and peak areas for both columns and modifier solvents is summarized in Tables 1 to 4.

For comparison, performing the same analyses using the column-switching method required approximately 46 minutes to obtain four chromatograms, including column equilibration time. In contrast, the parallel method reduced total analysis time by more than an order of magnitude. Furthermore, although only two modifier solvents were evaluated in this study, expanding the number of solvents (up to ten) would further enhance the time-saving advantages of the parallel screening approach over conventional column switching.

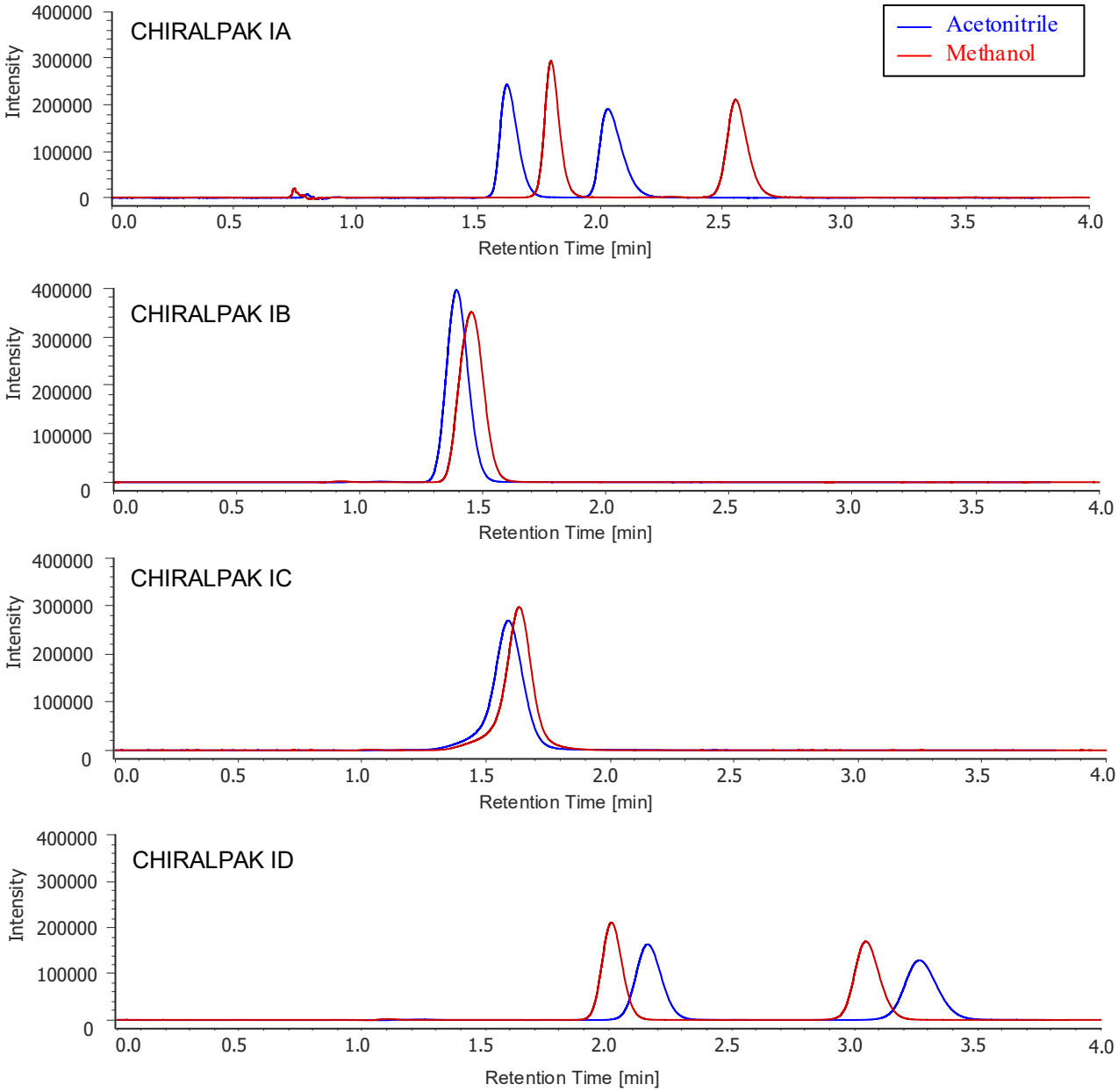


Fig. 3 *Chromatograms of flavanone obtained using the parallel SFC system (at UV 250 nm)*

Table 1. *Reproducibility of measurement results obtained using parallel SFC system (modifier solvent: acetonitrile, column: CHIRALPAK IA)*

Injection Number	Flavanone 1		Flavanone 2	
	tR [min]	Peak Area	tR [min]	Peak Area
1	1.627	1,232,807	2.046	1,232,651
2	1.633	1,258,190	2.045	1,248,113
3	1.617	1,241,883	2.029	1,240,881
4	1.624	1,241,476	2.039	1,230,797
5	1.627	1,258,181	2.040	1,246,964
Average	1.626	1,246,507	2.040	1,239,881
SD	0.005	10,071	0.006	7,124
RSD [%]	0.320	0.808	0.296	0.575

Table 2. *Reproducibility of measurement results obtained using parallel SFC system (modifier solvent: acetonitrile, column: CHIRALPAK ID)*

Injection Number	Flavanone 1		Flavanone 2	
	tR [min]	Peak Area	tR [min]	Peak Area
1	2.177	1,231,384	3.309	1,231,448
2	2.176	1,255,521	3.288	1,253,962
3	2.157	1,238,788	3.261	1,241,872
4	2.165	1,241,112	3.288	1,245,737
5	2.163	1,247,816	3.275	1,254,756
Average	2.168	1,242,924	3.284	1,245,555
SD	0.008	8,200	0.016	8,578
RSD [%]	0.357	0.660	0.485	0.689

Table 3. *Reproducibility of measurement results obtained using parallel SFC system (modifier solvent: methanol, column: CHIRALPAK IA)*

Injection Number	Flavanone 1		Flavanone 2	
	tR [min]	Peak Area	tR [min]	Peak Area
1	1.782	1,272,380	2.531	1,270,850
2	1.797	1,266,372	2.553	1,272,041
3	1.787	1,262,603	2.538	1,264,940
4	1.788	1,249,751	2.545	1,253,775
5	1.797	1,256,198	2.554	1,263,684
Average	1.790	1,261,461	2.544	1,265,058
SD	0.006	7,867	0.009	6,504
RSD [%]	0.330	0.624	0.346	0.514

Table 4. *Reproducibility of measurement results obtained using parallel SFC system (modifier solvent: methanol, column: CHIRALPAK ID)*

Injection Number	Flavanone 1		Flavanone 2	
	tR [min]	Peak Area	tR [min]	Peak Area
1	1.995	1,240,478	3.026	1,244,109
2	2.010	1,251,413	3.045	1,253,958
3	2.000	1,238,216	3.028	1,241,664
4	2.000	1,218,774	3.035	1,234,367
5	2.010	1,236,875	3.042	1,241,633
Average	2.003	1,237,151	3.035	1,243,146
SD	0.006	10,520	0.007	6,315
RSD [%]	0.300	0.850	0.246	0.508

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