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Analysis of Hydroxyzine by SFC-PDA-CD Method Scouting System – 10 columns x 3 solvents for rapid screening

Introduction

Supercritical fluid chromatography (SFC) employs carbon dioxide as the primary component of the mobile phase and is characterized by its ability to maintain high separation efficiency even at high flow rates (linear velocities). This capability makes SFC particularly well suited for the rapid separation and fractionation of chiral compounds. To determine optimal separation conditions, method scouting approaches are commonly used, in which analytes are systematically screened across a range of modifier solvents and chiral stationary phases. In recent years, chiral columns packed with 3 µm particles have been developed, offering improved efficiency and significantly faster separations compared to conventional 5 µm particle size columns traditionally used in SFC.

The circular dichroism (CD) detector used in this study provides simultaneous acquisition of UV chromatograms, CD chromatograms, and the g-factor (CD/UV ratio) in real time. In CD chromatograms, optical isomers produce peaks with opposite signs, enabling unambiguous identification of enantiomers even when the elution order is reversed during method screening.

In this application note, rapid method scouting for the chiral separation of hydroxyzine was performed using a JASCO SFC system equipped with the Method Scouting Assistant Program, an add-in software package for ChromNAV Ver. 2. Hydroxyzine, an anti-allergic and sedative agent, was evaluated using three modifier solvents and ten chiral columns with a 3 µm particles, enabling high-speed and high-efficiency screening. Diethylamine was added to the modifier to improve peak shape and enhance separation performance since hydroxyzine is a basic compound.

Keywords

Hydroxyzine, supercritical fluid chromatography, SFC, method screening, method scouting, chiral separation, circular dichroism detector, CD detector, CHIRALPAK, 3 µm

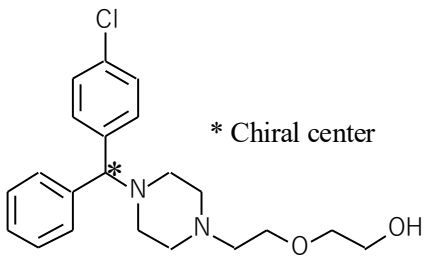


Fig.1 *Structure of Hydroxyzine*

Experimental

Instruments

CO_2 pump:	PU–4380
Modifier pump:	PU–4185*
Autosampler:	AS–4350
Column oven:	CO–4065*
PDA detector:	MD–4010*
CD detector:	CD–4095*
BP regulator:	BP–4340

* with option units

SFC Conditions

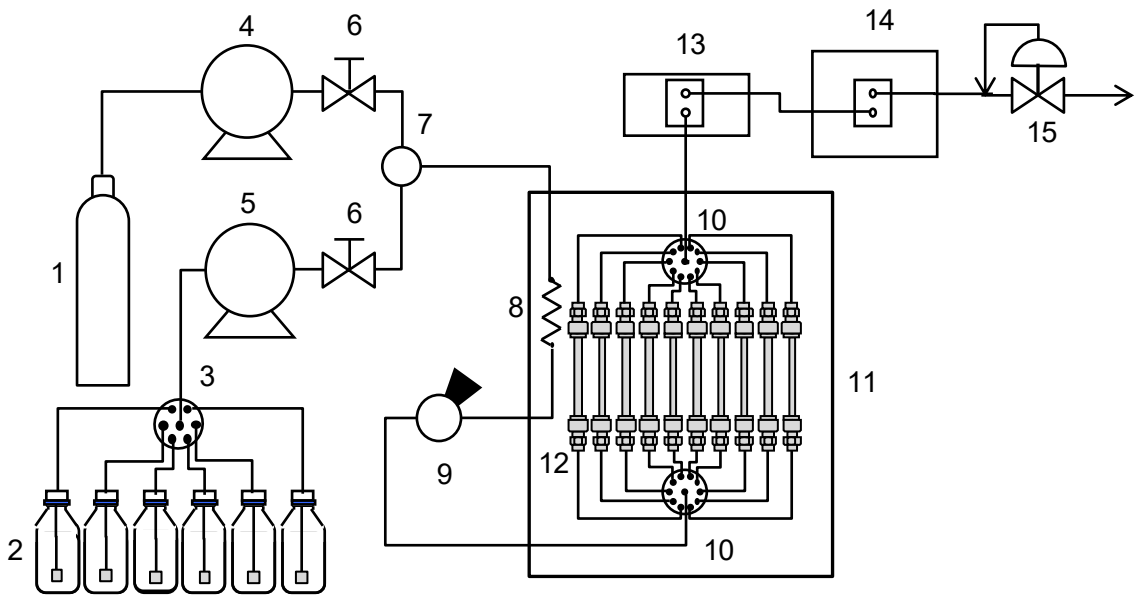
Column: CHIRALPAK IA/SFC, IB-N/SFC,
IC/SFC, ID/SFC, IE/SFC, IF/SFC,
IG/SFC, IH/SFC, IJ/SFC, IK/SFC
(3.0 mm I.D. x 50 mm L, 3 µm)*

Eluent: Carbon dioxide/modifier (75/25)

Modifier: A; Methanol/diethylamine (100/0.4)
B; Acetonitrile/ethanol/diethylamine (80/20/0.4)
C; t-Butyl methyl ether/ethanol/diethylamine (80/20/0.4)

Flow rate:	1.2 mL/min
Column temperature:	40 °C
Wavelength:	230 nm (MD–4010)
	230 nm (CD–4095)
Back pressure:	15 MPa
Injection volume:	1 µL
Sample:	0.5 mg/mL hydroxyzine in MeOH

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



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. PDA detector
4. CO_2 pump	9. Autosampler	14. CD detector
5. Modifier pump	10. Column switching valve	15. Back pressure regulator

Fig. 2 *Schematic diagram of SFC system*

Results

During method scouting, the column stabilization time was set to 10 minutes for the first column following solvent changeover and 3 minutes for subsequent columns to allow sufficient equilibration. The analysis time for each run was set to 3.0 minutes, resulting in a total screening time of approximately 3.4 hours per compound.

Figure 3–1 shows the screening results obtained using a photodiode array (PDA) detector at a wavelength of 230 nm, while Figure 3–2 shows the corresponding results obtained using a circular dichroism (CD) detector at a wavelength of 230 nm. In these figures, each row represents a different modifier solvent, and each column represents a different chiral column. In the CD chromatograms, enantiomers produce signals of opposite sign, enabling clear and immediate differentiation of optical isomers.

Table 1 summarizes the resolution for hydroxyzine under each condition. Results with a resolution of 1.0 or less were classified as incomplete separation (I.S.). When methanol containing 0.4% diethylamine was used as the modifier, resolutions of 2.0 or greater were achieved with the IF and IJ columns. Among the conditions evaluated, the best overall performance in terms of resolution, analysis time, and peak shape was obtained using the CHIRALPAK IJ/SFC column with a t-butyl methyl ether/ethanol (80/20) modifier containing 0.4% diethylamine.

Complete separation was also achieved under certain conditions using the IA and IF columns, whereas incomplete separation (I.S.) was observed for several other stationary phases. For preparative or scale-up applications, careful optimization of separation conditions is required, as increased sample loading may adversely affect peak shape and resolution.

Conclusion

In this application note, the use of 3 µm particle size chiral columns in SFC enabled rapid and efficient method scouting, allowing optimal chiral separation conditions for hydroxyzine to be identified within approximately 3.4 hours. Conditions that yielded partial or near-baseline separation may be further optimized through adjustments to column dimensions, modifier composition or additive concentration to achieve baseline separation.

For the chiral separation of basic compounds by SFC, the use of basic additives, such as diethylamine, is essential to improve peak shape and enhance resolution.

Compared to conventional HPLC, SFC offers significantly faster method development as well as reduced solvent consumption. The SFC method scouting system used in this application note provides a powerful and efficient approach for the rapid screening and optimization of chiral separations, making it highly valuable for both analytical and preparative applications.

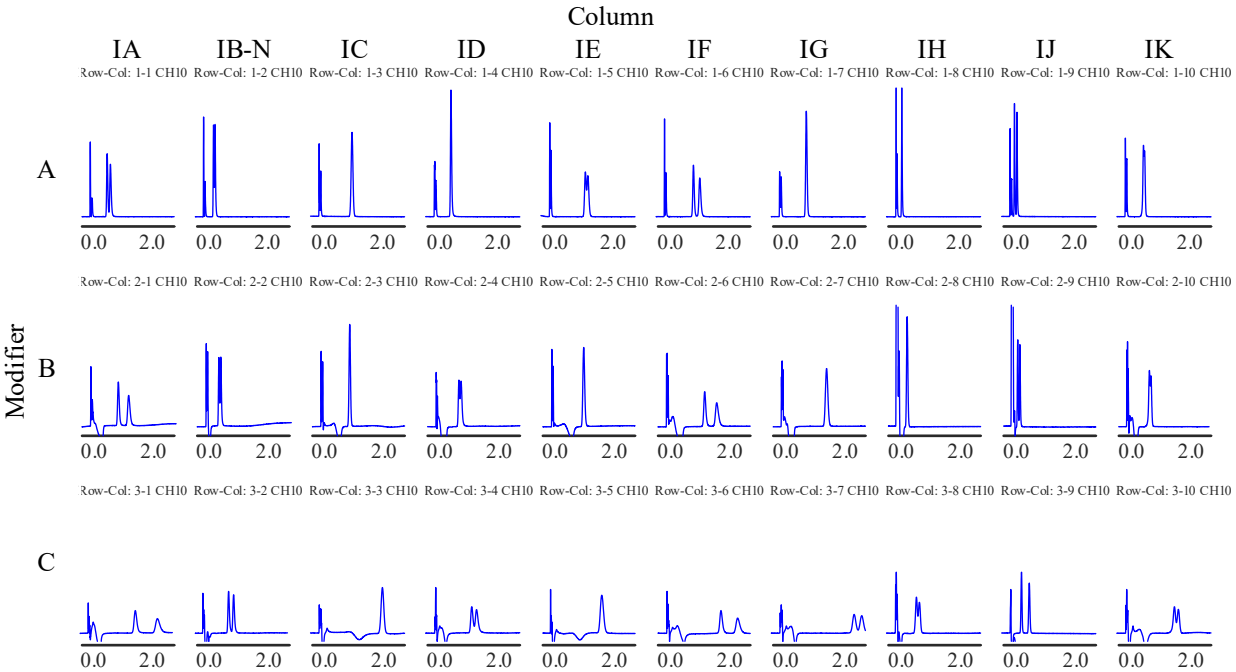


Fig. 3-1 *Chromatograms of hydroxyzine obtained using method scouting system (PDA detection wavelength: 230 nm)*

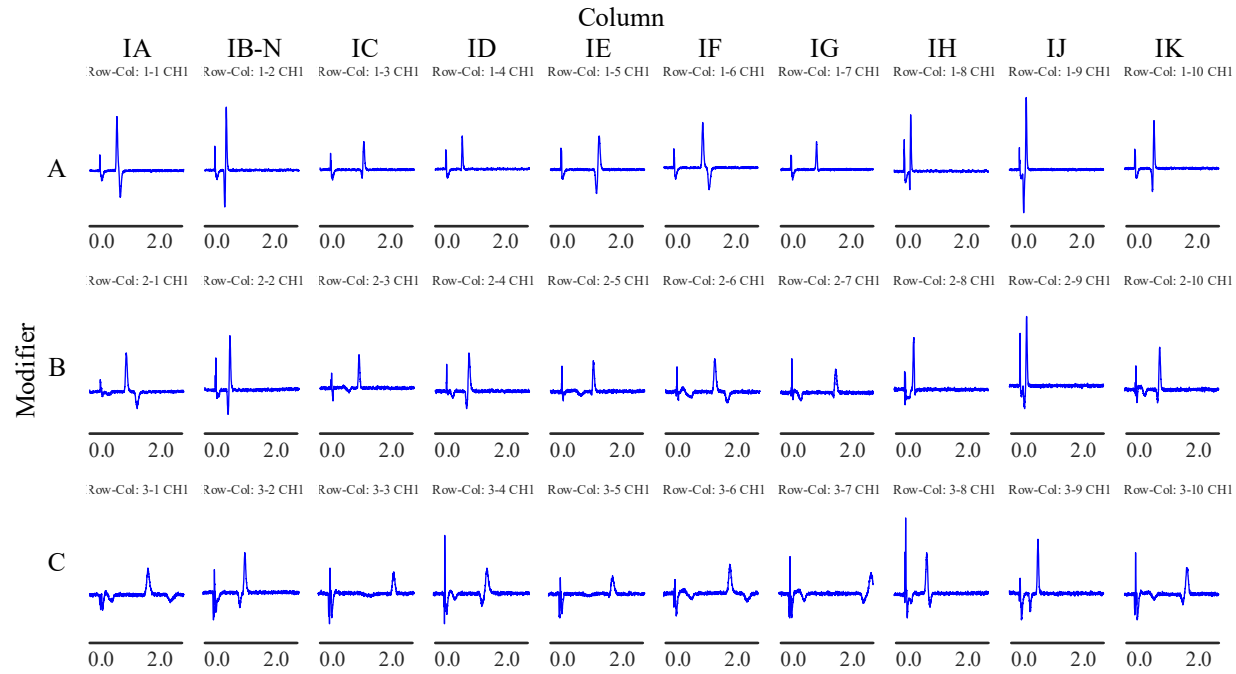


Fig. 3-2 *Chromatograms of hydroxyzine obtained using method scouting system (CD detection wavelength: 230 nm)*

Table 1. *Comparison of resolution based on method scouting conditions*

Column / Modifier	IA	IB-N	IC	ID	IE	IF	IG	IH	IJ	IK
Methanol / diethylamine (100/0.4)	1.38	0.96	N.S.	N.S.	I.S.	2.02	N.S.	N.S.	2.05	I.S.
Acetonitrile / ethanol / diethylamine (80/20/0.4)	2.74	0.93	N.S.	I.S.	N.S.	2.56	N.S.	N.S.	1.44	I.S.
t-Butyl methyl ether / ethanol / diethylamine (80/20/0.4)	3.34	1.78	N.S.	1.00	N.S.	2.77	1.27	I.S.	3.57	0.84

I.S.: Incomplete Separation, N.S.: Not Separated

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