Chiral Separations of Pesticide Enantiomers by High-Performance Liquid Chromatography Using Cellulose Triphenylcarbamate Chiral Stationary Phase

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Abstract

The direct chiral separations of pesticide enantiomers by high-performance liquid chromatography by applying self-prepared cellulose triphenylcarbamate chiral stationary phase are performed. The mobile phase is *n*-hexane modified by isopropanol as a polar modifier. Nine chiral pesticides (benalaxyl, vinclozolin, diclofop-methyl, tebuconazole, quizalofop-ethyl, hexaconazole, lactofen, isocarbophos, and paclobutrazol) show enantioselectivity on the chiral stationary phase. An online circular dichrorism detector is used for identifying the pesticide enantiomers. The influences of the volume content of isopropanol and column temperature on the separations are investigated. The thermodynamic parameters related to the chiral distinguish mechanisms are also calculated.

Introduction

The scientific and economic relevance of chiral substances has favored the outstanding development in separation techniques in the last two decades (1). A large number of chromatographic methods for the chiral separations have been developed, in which high-performance liquid chromatography (HPLC) has become increasingly important. Direct separation of enantiomers by HPLC based on chiral stationary phases has become one of the most useful methods in many fields dealing with drugs, natural products, agrochemicals, etc. This method is preferred because it is rapid and suitable to separate racemates both on analytical and preparative scales. Among a wide variety of natural and synthetic chiral stationary phases (CSPs) designed for chiral separations, the phenylcarbamates and esters of polysaccharides, such as cellulose and amylose, exhibit the most universal chiral recognition ability in HPLC (2–6).

Pesticides play an important role in agriculture for protecting crops, and approximately 25% of the often used pesticides are chiral (7). Enantioselective biological recognition or biodiscrimination of chiral pesticide enantiomers is often observed in biological systems. The R-(+) enantiomers of acephate and methamidophos are approximately 6-fold more toxic to houseflies than the S-(–) enantiomers (8). The (+)-fenamiphos is more toxic than (–)-fenamiphos to nontarget organisms (9). Despite of the fact that the individual enantiomers of chiral pesticides may show different bioactivity, toxicity, and environmental behaviors, most of the chiral pesticides are sold and used in the form of racemates. There will be legislative requirements for the analysis of individual enantiomers during registration of techniques in the future. There is a need to develop methods for the determination of the optical purity and analysis of the enantiomers of pesticides.

This manuscript describes the HPLC separation of nine chiral pesticides enantiomers (see Figure 1) on self-prepared cellulose triphenylcarbamate CSPs. The mobile phase was n-hexane, and isopropanol was applied as the polar modifier. The effect of isopropanol concentration on resolution was investigated, and the impact of temperature was also studied as an important factor for improving the separations. An online circular dichroism (CD) detector was used to identify the eluting enantiomers. The results

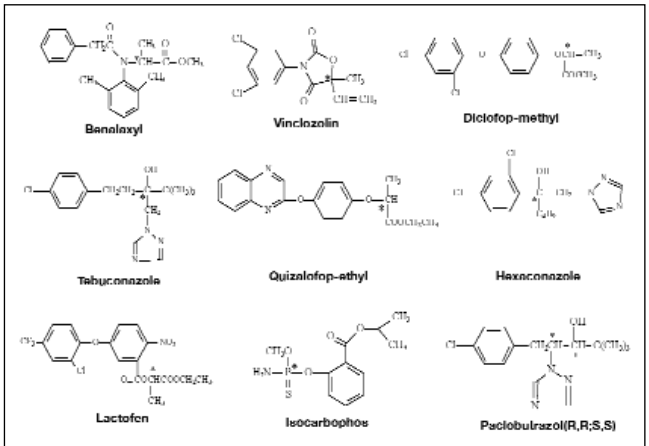

Figure 1. Chemical structures of the chiral pesticides for which resolutions were obtained. The asterisk denotes the chiral center.

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in the manuscript were not reported before, according to the previous references. Several samples in the manuscript were separated by other methods. Hexaconazole and tebuconazole were resolved by capillary electrophoresis (10) and HPLC with cellulose tri(3,5-dimethylphenylcarbamate) (OD) CSPs (11), and diclofop-methyl was resolved on cellulose tris-(4-methylbenzoate) CSP (12).

Experimental

Apparatus and reagents

The chromatography was performed on two HPLC systems. The first was a JASCO 2000 HPLC (JASCO Corporation, Tokyo Japan), equipped with PU-2089 plus pump and CD-2095 plus CD detector, with a 20-µL sample loop and Chrompass workstation (Jasco). The second was an Agilent 1100 Series HPLC (Agilent, Palo Alto, CA) equipped with a G1311A pump and G1322A degasser, with 10-µL sample loop and G1316A COLCOM and a 20-µL sample loop and G1315B DAD. The signal was acquired and processed by an HP1100 workstation (Agilent).

Phenylisocyanate was purchased from Merk (Darmstadt, Germany) and 3-aminopropyltriethoxysilane was from ACROS ORGANICS (Geel, Belgium). Macro spherical silica was made in house with the following properties: particle size, 5 µm; average pore diameter, 6.7 nm; and specific surface area, 90 m^2/g. The chiral pesticide samples were provided by Institute for the control of Agrichemicals, Ministry of Agriculture, and Lab of Pesticide Analysis and Environmental Toxicology of China Agricultural University (Beijing, China). All eluents were of analytical grade (Beijing Yili Fine Chemicals, Beijing, China) and were distilled before use.

Chromatographic conditions

The mobile phase consisted of n-hexane and isopropanol. The injection volume was 20 µL. Wavelengths are listed in Table I. The influence of column temperature was studied at a range of 0–40°C. Capacity factor (k_1'), separation factor (α) and resolution factor (Rs) were calculated.

For the capacity factor (k_1) of the first eluted enantiomer:

$$(t_1 - t_0)/t_0 \qquad \text{Eq. 1}$$

For the capacity factor (k_2) of the second eluted enantiomer:

$$(t_2 - t_0)/t_0 \qquad \text{Eq. 2}$$

For the separation factor (α):

$$k_2/k_1 \qquad \text{Eq. 3}$$

For the resolution factor (Rs):

$$2(t_2 - t_1)/(w_1 + w_2) \qquad \text{Eq. 4}$$

Table I. Elution Orders of the Chiral Pesticides Enantiomers*

Sample	1	2	3	4	5	6	7	8	9
Wave length (nm)	230	210	230	230	230	230	254	230	230
Pk1	+	+	+	–	+	+	+	+	+
Pk2	–	–	–	+	–	–	–	–	–

* *n*-hexane–isopropanol (85:15); flow rate, 1.0 mL/min; Pk1, first eluting enantiomer; Pk2, second eluting enantiomer.

Preparation of chiral column

The CSP was synthesized according to the procedure described in the literature (13,14). Macrocrystalline cellulose was refluxed in pyridine for 12 h and reacted with phenylisocyanate for another 24 h to synthesize cellulose-trisphenylcarbamate. Aminopropylsilica (APS) was synthesized by treating spherical silica with 3-aminopropyltriethoxysilane in toluene at 110°C for 24 h. Cellulose-trisphenylcarbamate (0.55 g) was dissolved in 30 mL tetrahydrofuran, and then APS (2.45 g) was added. The mixture was stirred for 15 min and dried at 60°C for 8 h a under vacuum after evaporating the solvent. The slurry of the CSP in n-hexane–isopropanol (90:10, v/v) solution was packed into a stainless steel column (250 × 4.6-mm i.d.) under 4.0×10^7 Pa.

Results and Discussion

Enantiomer identification by CD detector

All the chiral pesticides in the manuscript consist of a pair of enantiomers, and the chemical structures are listed in the Figure 1, in which the chirality of isocarbophos was because of the chiral phosphorus atom and that of others because of the asymmetric carbon. Although paclobutrazol contains two chiral centers, the technical product is a mixture of (R,R)- and (S,S)-enantiomers. The elution orders of the chiral pesticides on the CSP was determined by a CD detector using the JASCO HPLC system and applying the mobile phase of n-hexane–isopropanol (85:15) at a flow rate of 0.8 mL/min. Table I shows the elution orders of the eluting enantiomers of the nine samples on the cellulose triphenylcarbamate chiral stationary phase. The (–)-enantiomer of tebuconazole eluted first, but the (+)-enantiomer eluted first for the other chiral pesticides.

Chiral separations and the effect of IPA content

The chiral separations and the determination of the effect of isopropanol content were performed using the Agilent HPLC system. The void time (t_0) of the column was 2.20 min, determined by 1,3,5-tri-t-butylbenzene. Table II shows the results and the effect of isopropanol content at 20°C. Benalaxyl achieved the best separation (Rs = 2.68, 5%) among the samples. Hexaconazole and paclobutrazol could also obtain complete resolutions with Rs values of 1.57 and 1.87, respectively, using 5% isopropanol. Figure 2 show the chromatograms for the resolutions of the benalaxyl, hexaconazole, and paclobutrazol. Vinclozolin, tebuconazole, lactofen, isocarbophos, diclofop-methyl, and quizalofop-ethyl obtained partial separations. Capacity factors (k')

and resolution factors (Rs) increased with the decreasing content of isopropanol. Separation factors (α) were also increased when the content of isopropanol decreased, except benalaxyl and diclofop-methyl, which obtained a higher α value at 10% isopropanol. The separation factor usually did not change much; however, capacity factor and resolution factor did change. The CSP showed strong retentions towards most samples. The fact that the separation factor did not change much with the isopropanol content may be because of the strong retention of the CSP. When the content of isopropanol was too low, the enantiomers would not elute from the column.

Table II. Effect of IPA Content on the Separations*

Samples	Wavelength	IPA concentration %	k_1	k_2	α	Rs
Benalaxyl	230 nm	15	3.53	4.86	1.38	1.82
		10	5.31	7.49	1.41	2.21
		5	9.24	12.86	1.39	2.68
Vinclozolin	210 nm	15	2.07	2.39	1.16	1.04
		10	2.74	3.21	1.17	1.12
		5	3.35	3.92	1.17	1.26
Diclofop-methyl	230 nm	15	2.07	2.19	1.06	0.41
		10	2.79	3.01	1.08	0.58
		5	3.49	3.74	1.07	0.60
Tebuconazole	230 nm	15	4.04	4.52	1.12	0.79
		10	9.29	10.49	1.13	0.95
		5	22.94	26.20	1.14	1.27
Quizalofop-ethyl	230 nm	15	3.22	3.59	1.11	0.74
		10	4.88	5.47	1.12	0.85
		5	7.49	8.50	1.14	1.00
Hexaconazole	230 nm	15	2.06	2.48	1.20	0.89
		10	4.21	5.16	1.22	1.25
		5	9.22	11.48	1.24	1.57
Lactofen	254 nm	15	3.75	4.05	1.08	0.49
		10	6.23	6.96	1.12	0.87
		5	11.16	12.67	1.14	0.95
Isocarbophos	230 nm	15	4.22	4.22	1.00	0
		10	7.65	8.01	1.05	0.53
		5	14.83	15.72	1.06	0.59
Paclobutrazol	230 nm	15	1.70	2.17	1.28	1.15
		10	3.84	5.01	1.30	1.45
		5	9.42	12.67	1.35	1.87

* Flow rate, 1.0 mL/min; temperature, 20°C.

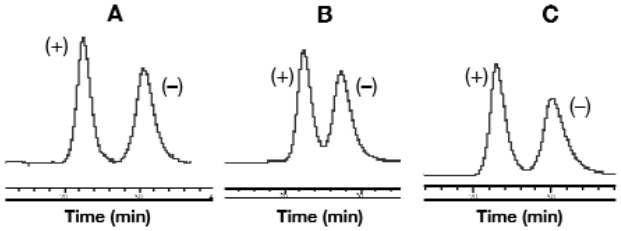

Figure 2. Chromatograms for the chiral resolutions of benalaxl (A), hexaconazole (B), and paclobutrazol (C), *n*-hexane–IPA (95:5), 1.0 mL/min, 20°C, 23 nm.

It is commonly considered that the enantiomeric discrimination on phenylcarbamates of polysaccharide CSPs is mainly because of the difference of a combination of attractive forces, such as hydrogen bonding, dipole–dipole interactions, and π–π interactions, between the enantiomers and polar carbamate groups of the CSP. Although the chiral resolution mechanism was extensively investigated, the exact mechanism was not clearly elucidated at the molecular level.

Effect of temperature on the separations

Temperature was an important factor affecting the chromatographic separations, especially chiral separation. Many previous works have been done to investigate the effect of temperature as a variable in improving chiral resolution. The effect of temperature on the separations of the chiral pesticides was performed at a range of 0–40°C in this manuscript. The chromatographic conditions and the results are listed in Table II.

The capacity factors (k') for all the samples decreased as the temperature increased, and the separation factors and resolution factors of benalaxyl, tebuconazole, and isocarbophos also decreased with continuously increasing temperature. Temperature seems to have no obvious impact on the α and Rs values for diclofop-methyl, lactofen, hexaconazole and quizalofop-ethyl. The separation factor of vinclozolin increased at low temperature, and the resolution factor decreased because of peak broadening, and a better separation was obtained at a higher temperature. The enantiomeric separations of benalaxyl, tebuconazole, and isocarbophos were affected by temperature significantly. The Rs values for benalaxyl were 2.27 at 0°C and 1.59 at 40°C, and the values for tebuconazole were 1.06 at 0°C and decreased to 0.64 at 40°C. The two enantiomers of isocarbophos obtained no separation at 40°C.

It is usually considered that temperature affects the chiral separation mainly in kinetic and thermodynamic ways (15). The kinetic effect was the influence on the viscosity and on the diffusion coefficient of the solute. An increase of temperature reduced the viscosity of the mobile phase and increased the diffusion coefficient of the solute in both the mobile and stationary phase. The thermodynamic effect was the influence on the separation factor (α), which usually decreases as temperature increases. The reason was that the partition coefficients and the Gibbs free energy change (ΔG) of transfer of the analyte between the stationary phase and the mobile phase vary with temperature.

The thermodynamic parameters including ΔH, ΔS*, ΔΔH, and ΔΔS of the chiral resolutions were calculated according to the van't Hoff equation(15–17):

$$\ln k = \frac{-\Delta H}{RT} + \frac{-\Delta S}{R} + \ln\phi$$

or

$$\ln \alpha = -\frac{\Delta_{R,S}\Delta H}{RT} + \frac{-\Delta_{R,S}\Delta S}{R}$$

Where the ΔH and ΔS are the standard enthalpy and entropy of transfer of the solute from the mobile phase to the stationary phase. If plots of lnk versus 1/T (van't Hoff plots) are linear, the slope and intercept are $-\Delta H/R$ and $\Delta S/R + \ln(\Delta S^*)$, respectively. $\Delta_{R,S}\Delta H$ and $\Delta_{R,S}\Delta S$ are the differences $\Delta H_2 - \Delta H_1$ and $\Delta S_2 - \Delta S_1$, respectively. For a linear plot of ln Δ versus 1/T, the slope and

Table III. The Impact of Temperature on the Resolutions of Chiral Pesticides by Cellulose Triphenylcarbamate CSP, van't Hoff Equations and the Thermodynamic Parameters

Samples *n*-hexane–isopropanol	T/°C	k_1	k_2	α	Rs	van't Hoff equation (R^2)	$\Delta H/\Delta_{R,S}\Delta H$ kJ mol^{-1}	$\Delta S^*/\Delta_{R,S}\Delta S$ J mol^{-1} K^{-1}
Benalaxyl (90:10)	0	8.74	13.65	1.56	2.27	$\ln k_1 = 1520.3/T - 3.49(0.94)$	–12.64	–3.49
	10	5.91	8.42	1.43	1.87	$\ln k_2 = 1880.3/T - 4.38(0.94)$	–15.63	–4.38
	20	5.31	7.49	1.41	2.21	$\ln\alpha = 359.95/T - 0.89(0.94)$	–2.99	–7.40
	30	4.69	6.35	1.35	1.87			
	40	4.06	5.28	1.30	1.59			
Vinclozolin (95:5)	0	4.24	5.02	1.18	1.16	$\ln k_1 = 925.05/T - 1.93(0.99)$	–7.69	–1.93
	10	3.89	4.60	1.18	1.23	$\ln k_2 = 962.6/T - 1.90(0.99)$	–8.00	–1.90
	20	3.35	3.92	1.17	1.26	$\ln\alpha = 37.5/T + 0.03(0.92)$	–0.31	0.25
	30	3.10	3.63	1.17	1.24			
	40	2.76	3.21	1.16	1.25			
Diclofop–methyl (95:5)	0	4.52	4.85	1.07	0.58	$\ln k_1 = 972.71/T - 2.05(0.99)$	–8.09	–2.05
	10	4.13	4.41	1.07	0.58	$\ln k_2 = 992.08/T - 2.05(0.99)$	–8.25	–2.05
	20	3.49	3.74	1.07	0.60	nonlinear plot of lnα to 1/T		
	30	3.23	3.44	1.07	0.58			
	40	2.89	3.08	1.06	0.58			
Tebuconazole (90:10)	0	10.90	12.81	1.18	1.02	$\ln k_1 = 633.2/T + 0.08(0.99)$	–5.26	0.08
	10	10.30	11.95	1.16	1.02	$\ln k_2 = 781.7/T - 0.30(0.98)$	–6.50	–0.30
	20	9.29	10.49	1.13	0.95	$\ln\alpha = 148.5/T - 0.38(0.91)$	–1.24	–3.16
	30	8.86	10.03	1.13	0.72			
	40	8.11	8.85	1.09	0.62			
Quizalofop–ethyl (95:5)	0	9.48	10.86	1.15	0.96	$\ln k_1 = 890.5/T - 1.00(0.97)$	–7.40	–1.00
	10	8.95	10.23	1.14	0.97	$\ln k_2 = 934.0/T - 1.02(0.98)$	–7.77	–1.02
	20	7.49	8.50	1.14	1.00	$\ln\alpha = 43.47/T - 0.02(0.94)$	–0.36	–0.17
	30	6.92	7.84	1.13	0.96			
	40	6.40	7.18	1.12	0.94			
Hexaconazole (95:5)	0	10.67	13.55	1.27	1.47	$\ln k_1 = 592.2/T + 0.22(0.94)$	–4.92	0.22
	10	10.58	13.32	1.26	1.51	$\ln k_2 = 653.5/T + 0.24(0.95)$	–5.43	0.24
	20	9.22	11.48	1.24	1.57	$\ln\alpha = 61.3/T + 0.01(0.94)$	–0.51	0.08
	30	8.85	11.02	1.25	1.36			
	40	8.24	10.15	1.23	1.44			
Lactofen (95:5)	0	15.37	17.39	1.13	0.98	$\ln k_1 = 1293.7/T - 1.98(0.97)$	–10.76	–1.98
	10	14.07	15.95	1.13	1.02	$\ln k_2 = 1288.8/T - 1.83(0.97)$	–10.72	–1.83
	20	11.16	12.67	1.14	0.95	nonlinear plot of lnα to 1/T		
	30	10.34	11.72	1.13	0.88			
	40	8.39	9.52	1.14	0.98			
Isocarbophos (90:10)	0	10.39	11.09	1.07	0.57	$\ln k_1 = 1020/T - 1.40(0.96)$	–8.48	–1.40
	10	9.30	9.83	1.06	0.55	$\ln k_2 = 1155.7/T - 1.82(0.97)$	–9.61	–1.82
	20	7.65	8.01	1.05	0.53	$\ln\alpha = 135.64/T - 0.43(0.93)$	–1.13	–3.57
	30	7.51	7.70	1.03	0.30			
	40	6.37	6.37	1.00	0			
Paclobutrazol (90:10)	0	4.56	6.11	1.34	1.41	$\ln k_1 = 780.1/T - 1.32(0.97)$	–6.48	–1.32
	10	4.31	5.75	1.33	1.39	$\ln k_2 = 935.2/T - 1.59(0.97)$	–7.78	–1.59
	20	3.84	5.01	1.30	1.45	$\ln\alpha = 155.1/T - 0.27(0.94)$	–1.29	–2.25
	30	3.61	4.63	1.28	1.43			
	40	3.15	3.92	1.25	1.34			

* Flow rate was 1.0 mL/min; wavelengths were the same as in Table I.

intercept are $-\Delta_{R,S}\Delta H/R$ and $\Delta_{R,S}\Delta S/R$, respectively.

Linearities [linear correlation coefficient $(R)^2 > 0.94$] of lnk versus 1/T were established for all the samples. The plots of ln α versus 1/T for most samples (except for samples 3 and 7) were approximately linear with R^2 value higher than 0.91; however, the linearity for lactofen and diclofop-methyl were not found. The thermodynamic parameters calculated based on the linear van't Hoff equation are listed in Table III.

Conclusion

In this study, the chiral separations of nine pesticides were studied on the cellulose triphenylcarbamate CSP by HPLC. The influence of temperature was also studied, and the thermodynamic parameters were, thus, calculated according to the previously described van't Hoff equation. Linear van't Hoff plots were not established for all of the samples. The elution orders were determined by a CD detector. Cellulose triphenylcarbamate is not a very robust CSP for the chiral separations of the chiral pesticides. Three samples obtained complete resolution, and others achieved partial separations. Most enantiomers showed relatively long retentions, and some peak broadening occurred on the CSP.

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References

1. N.M. Maier, P. Franco, and W. Lindner. Separation of enantiomers: needs, challenges, perspectives. *J. Chromatogr. A* **906:** 3–33 (2001).
2. K. Tachibana and A. Ohnishi. Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases. *J. Chromatogr. A* **906:** 127–54 (2001).
3. G. Felix. Regioselectively modified polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography. *J. Chromatogr. A* **906:** 171–84 (2001).
4. E. Yashima. Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation. *J Chromatogr. A* **906:** 105–25 (2001).
5. H.Y. Aboul-Enein. High-performance liquid chromatographic enantioseparation of drugs containing multiple chiral centers on polysaccharide-type chiral stationary phases. *J. Chromatogr. A* **906:** 185–93 (2001).
6. P. Franco, A. Senso, L. Oliveros, and C. Minguillon. Covalently bonded polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography. *J. Chromatogr. A* **906:** 155–70 (2001).
7. A. Williams. Opportunities for chiral agrochemicals. *Pestic. Sci.* **46:** 3–9 (1996).
8. J.J. Ellington, J.J. Evans, and K.B. Prickett, W.L. Champion, Jr. High-performance liquid chromatographic separation of the enantiomers of organophosphorus pesticides on polysaccharide chiral stationary phases. *J Chromatogr. A* **928:** 145–54 (2001).
9. Y.S. Wang, K.T. Tai, and J.H. Yen. Separation, bioactivity, and dissipation of enantiomers of the organophosphorus insecticide fenamiphos. Ecotox. *Environ. Safe.* **57:** 346–56 (2004).
10. Y.S. Wu, H.K. Lee, and S.F.Y. Li. High performance chiral separation of fourteen triazole fungicides by sulfated β-cyclodextrin mediated capillary electrophoresis. *J. Chromatogr. A* **912:** 171–79 (2001).
11. P. Wang, S.R. Jiang, D.H. Liu, P. Wang, and Z.Q. Zhou. Direct enantiomeric resolutions of chiral triazole pesticides by high-perforrnance liquid chromatography. *J. Biochem. Bioph. Meth.* **62:** 219–30 (2005).
12. P. Chen, P.J. Na, X.Q. Han, C.P. Pan, J.G. Hou, X.Z. Du, and J.Z. Gao. Enantiomeric resolution of diclofop-methyl by HPLC with chiral stationary *Phase. J. Instrum. Anal.* **22:** 39–41 (2003).
13. Z.Q. Zhou, P. Wang, S.R. Jiang, M. Wang, and L. Yang. The Preparation of Polysaccharide-based chiral stationary phases and the direct separation of five chiral pesticides and related intermediates. *J. Liq. Chrom. Rel. Technol.* **26:** 2873–80 (2003).
14. Y. Okamoto, M. Kawashima, and K. Hatada. Chromatographic resolution. *J. Chromatogr.* **363:** 173–86 (1986).
15. A. Peter, E. Vekes, and D.W. Armstrong. Effects of temperature on retention of chiral compounds on a ristocetin A chiral stationary phase. *J Chromatogr. A* **958:** 89–107 (2002).
16. I. Slama, E. Jourdan, A. Villet, C. Grosset, A. Ravel, and E. Peyrin. Temperature and solute molecular size effects on the retention and enantioselectivity of a series of D, L dansyl amino acids on a vancomycin-based chiral stationary phase. *Chromatographia* **58:** 399–404 (2003).
17. J.A. Blackwell and R.W. Stringham. Temperature effects for chiral separations using various bulk fluids in near-critical mobile phases. *Chirality* **9:** 693–98 (1997).

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