National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 877

Chiral Selectivity Test Mixtures for Liquid Chromatography[1]

SRM 877 consists of five solutions of chiral compounds in ethanol designed primarily to indicate enantioselectivity of chiral stationary phases for liquid chromatography and supercritical fluid chromatography. SRM 877 is also suitable for (1) use as a control material for monitoring column performance, (2) comparisons of columns having similar chiral selectors, and (3) use in quality control for column manufacturing. A unit of SRM 877 consists of 5 ampules, each containing 1.1 mL of a solution of one racemic or enantiomerically enriched (nonracemic) compound. The five compounds are ketoprofen, indapamide, N-carbobenzyloxy-phenylalanine (N-CBZ-phenylalanine), propranolol hydrochloride, and warfarin (see Figure 1 for structures).

Expiration of Certification: SRM 870 is valid for its intended purpose until **30 September 2010**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see Instructions for Use). The certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

NOTICE AND WARNING TO USER

Toxicity: These test mixtures contain small amounts of organic compounds known to be toxic. Care should be exercised during handling and use (see Instructions for Use). Use proper methods for disposal of waste.

Preparation and analytical determinations were carried out by K.W. Phinney of the NIST Analytical Chemistry Division. The coordination of the technical measurements leading to certification was performed under the direction of L.C. Sander and S.A. Wise of the NIST Analytical Chemistry Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald.

Willie E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD
Certificate Issue Date: 30 October 2000

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[1]Certain commercial equipment, instruments, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. Tabulations of commercial LC columns are not intended to be all inclusive.

INSTRUCTIONS FOR USE

Storage: Sealed ampules, as received, should be stored in the dark at temperatures between 10 °C to 30 °C.

Choice of Appropriate Test Mixture: At least one of the five test compounds should be enantioresolved on most chiral stationary phases. The results in Figures 2 through 11 can be used as a guide for compound selection. In rare instances, none of the five compounds will be resolved on a particular chiral stationary phase. Under such circumstances, SRM 877 can provide evidence of retention behavior but should not be used to evaluate chiral selectivity, and an alternative method may be desired for monitoring column performance.

Chromatographic Conditions: Consistency of chromatographic parameters is essential for effective column evaluations with the test mixtures. Suggested mobile phases are listed in Appendix A for liquid chromatography and Appendix B for supercritical fluid chromatography. These mobile phase compositions are provided as a guide. Other mobile phase compositions might also yield successful enantioseparation of the test compounds. For comparison to results in this Certificate of Analysis, liquid chromatographic separations should be performed with a column temperature of 25 °C ± 2 °C**,** a flow rate of 1.0 mL/min, and an injection volume of 20 μL. A solvent exchange to dissolve the test compound in mobile phase may be necessary to avoid peak distortion with certain mobile phases. Supercritical fluid chromatography should be performed with a column temperature of 30 °C ± 2 °C, a flow rate of 2.0 mL/min, a pressure of 15 MPa, and an injection volume of 5 μL. Detection by measurement of UV absorbance at 254 nm is recommended for both liquid and supercritical fluid chromatography.

INTERPRETATION OF RESULTS

Separations of each of the five test mixtures are illustrated in Figures 2 through 6 for liquid chromatography, and in Figures 7 through 11 for supercritical fluid chromatography. Eight commercial columns were selected as representative of different types of chiral stationary phases. The same columns were used for liquid and supercritical fluid chromatography. These chromatograms are representative of the variations in behavior that can be anticipated for each of the test mixtures on different chiral stationary phases.

Chiral resolution of the test compounds can be verified by repeating the analysis under the same chromatographic conditions and monitoring another wavelength. The relative sizes of the two peaks should remain constant if enantiomeric separation has been achieved. Other useful wavelengths for detection include 220 nm, 240 nm, and 280 nm.

The solutions of ketoprofen, N-CBZ-phenylalanine, and propranolol hydrochloride were enriched in one of the enantiomers to facilitate confirmation of enantiomeric separation and to allow determination of enantiomer elution order. The enantiomeric ratio of each of the test solutions and the identity of the major enantiomer are listed in Table 1.

DISCUSSION

Chromatographic separations of enantiomers depend upon the formation of transient diastereomeric complexes between the enantiomers and a chiral ligand incorporated into the column packing material. Separation of the enantiomers occurs when one enantiomer forms a more stable complex and is retained longer than the other enantiomer [1]. The specific interactions responsible for chiral recognition vary with both the nature of the chiral selector and the structural features of the analyte.

Separation of each of the five test compounds was attempted on chiral stationary phases having a variety of different chiral selectors (see Appendix C). The results are tabulated in Table 2 for liquid chromatography and Table 3 for supercritical fluid chromatography. In some instances, poor peak shape precluded reliable determination of resolution (R_s). Not all chiral stationary phases developed for liquid chromatography are suitable for use in supercritical fluid chromatography.

The test mixtures are not intended to demonstrate the superiority of one stationary phase over another. The choice of the best chiral stationary phase for a particular application must be based upon a number of criteria, including the structural features of the analyte as well as the analyte solubility. The desired elution order of the enantiomers may also be an important factor. The test mixtures can be used to assess changes in chiral stationary phase performance over time and to evaluate lot-to-lot variability in column manufacturing. In addition, the test mixtures can be used for column comparisons as new chiral stationary phases are developed and commercialized.

Performance of chiral stationary phases can be affected by a number of variables, including the type of chiral selector, mobile phase composition, temperature, flow rate, and the amount of sample injected. In addition, gradual leaching of the chiral selector under certain mobile phase conditions and adsorption of sample components can adversely impact column performance.

Chiral Selector: The nature of the chiral selector has the most dramatic effect on enantioselectivity. This impact is exemplified by the range of selectivities observed for the test compounds on various chiral stationary phases (Tables 2 and 3). The chiral selector is either covalently bonded to the silica substrate or adsorbed on silica. The immobilization process can affect stereoselectivity and introduce nonstereospecific interactions. Hence, columns having the same chiral selector may behave differently because of disparities in immobilization procedures.

Mobile Phase Composition: Selection of appropriate mobile phase parameters is particularly important for enantiomeric separations. Successful enantiomeric resolutions are often obtained within a narrow range of mobile phase compositions. Inappropriate mobile phase selection may dramatically reduce or eliminate chiral selectivity. The mobile phases used to obtain the separations in Figures 2 through 6 were based on product literature from the column vendors. Replacement of traditional liquid eluents with carbon dioxide-based eluents in supercritical fluid chromatography can also produce changes in enantioselectivity for a given chiral stationary phase [2].

Temperature: Temperature plays an important role in chiral selectivity, and selectivity often decreases as temperature increases. Table 4 illustrates temperature-related effects on the separation of propranolol enantiomers. Both retention (k') and resolution (R_s) also decreased as the temperature increased. The magnitude of temperature-induced changes in stereoselectivity will not be equivalent for all chiral stationary phases.

Flow Rate: Typical flow rates for enantiomeric separations vary from 0.2 mL/min to 2.0 mL/min. Satisfactory selectivity can be obtained on most chiral stationary phases at a flow rate of 1.0 mL/min, and flow rate generally has a greater impact on resolution than on enantioselectivity.

Sample Loading: The sample capacity of chiral stationary phases depends upon the bonding density of the chiral selector. Overloading the column tends to degrade peak shapes and reduce enantioselectivity. Injection of sample volumes greater than 20 µL can also cause peak distortion in supercritical fluid chromatography.

REFERENCES

[1] Armstrong, D.W., "Optical Isomer Separation by Liquid Chromatography," Anal. Chem., **59**, pp. 84A-91A (1987).

[2] Williams, K.L., Sander, L.C. and Wise, S.A., "Comparison of Liquid and Supercritical Fluid Chromatography for the Separation of Enantiomers on Chiral Stationary Phases," J. Pharm. Biomed. Anal., **15**, pp. 1789-1799, (1997).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.

Table 1. Components and Compositions of SRM 877 Solutions

Compound	CAS Number[a]	Mass Fraction mg/g[b]	Enantiomeric Ratio[b,c]	Major Enantiomer
Indapamide	26807-65-8	1.28	1.0	N/A[d]
Ketoprofen	22071-15-4	1.28	2.3	R
N-CBZ-phenylalanine	2448-45-5 (D) 1161-13-3 (L)	1.28	4.0	L
Propranolol hydrochloride	13071-11-9 (R) 4199-10-4 (S)	1.28	2.3	S
Warfarin	81-81-2	1.27	1.0	N/A[d]

[a] Chemical Abstract Registry Number
[b] Data are provided for information purposes only and are not to be used for quantitation
[c] Enantiomeric ratio (er) = mass E_1/mass E_2, where E_1 is the major enantiomer
[d] Not applicable

Table 2. Liquid Chromatographic Data for Selected Commercial Chiral Stationary Phases

Column	Chiralcel	Chiralpak	Chirex	Chirex	Chirobiotic	Chirobiotic	Cyclobond I	Cyclobond I 2000
Compound								
Indapamide								
Mobile	A	A	G	G	K	M	H	H
k′(1)[a]	1.15	0.64	8.66	9.17	3.38	2.58	0.76	5.43
α	1.88	1.15	1.00	1.10	1.00	1.05	1.00	1.00
R_s	3.66	0.44	0.00	1.15	0.00	0.21	0.00	0.00
Ketoprofen								
Mobile	D	E	P	F	K	N	H	J
k′(1)	3.23	1.22	3.39	1.67	3.73	0.94	3.12	4.97
α	1.00	1.20	1.10	1.00	1.07	1.11	1.00	1.00
R_s	0.00	1.56	0.99	0.00	0.41	0.33	0.00	0.00
Phenylalani								
Mobile	E	E	P	F	K	H	S	J
k′(1)	1.39	1.78	3.15	2.08	3.11	2.97	2.31	2.65
α	1.16	1.13	1.00	1.00	1.09	1.08	1.00	1.00
R_s	1.26	1.41	0.00	0.00	N.D.[b]	N.D.[b]	0.00	0.00
Propranolol								
Mobile	C	B	G	G	T	R	S	S
k′(1)	1.84	2.01	3.77	7.00	3.20	8.99	4.72	4.19
α	1.88	1.00	0.00	1.09	1.13	1.11	1.00	1.00
R_s	5.16	0.00	0.00	0.85	2.53	1.77	0.00	0.00
Warfarin								
Mobile	A	A	F	F	K	H	Q	L
k′(1)	0.38	0.52	6.59	5.71	5.94	4.46	0.40	4.87
α	2.47	1.46	1.00	1.09	1.10	1.48	1.14	1.00
R_s	3.63	2.11	0.00	0.78	0.68	3.24	0.59	0.00

[a] Capacity factor of first eluting enantiomer
[b] Not determined

Table 3. Supercritical Fluid Chromatographic Data for Selected Commercial Chiral Stationary Phases

Column	Chiralcel OD	Chiralpak AD	Chirex 3005	Chirex 3022	Chirobiotic T	Chirobiotic V	Cyclobond I	Cyclobond I
Compound								
Indapamide								
mobile phase	EE	CC	CC	DD	CC	CC	CC	CC
k′(1)[a]	3.16	12.14	14.15	11.56	19.16	22.59	9.31	18.08
α	1.35	1.00	1.00	1.05	1.03	1.04	1.00	1.00
R_s	5.55	0.00	0.00	1.40	1.08	1.12	0.00	0.00
Ketoprofen								
mobile phase	BB	AA	CC	CC	CC	CC	CC	CC
k′(1)	4.28	11.83	9.89	7.67	8.50	10.95	7.73	6.10
α	1.00	1.11	1.08	1.00	1.00	1.00	1.00	1.00
R_s	0.00	3.17	1.78	0.00	0.00	0.00	0.00	0.00
Phenylalanine								
mobile phase	BB	BB	DD	DD	CC	CC	CC	CC
k′(1)	8.38	5.50	15.91	12.62	18.69	19.20	19.00	16.26
α	1.10	1.11	1.00	1.00	1.04	1.00	1.00	1.00
R_s	2.18	2.66	0.00	0.00	1.33	0.00	0.00	0.00
Propranolol								
mobile phase	DD	BB	CC	CC	CC	CC	CC	CC
k′(1)	4.36	4.07	11.94	8.87	25.50	23.80	7.66	9.45
α	1.70	1.30	1.00	1.00	1.09	1.07	1.00	1.00
R_s	12.41	7.19	0.00	0.00	3.51	2.45	0.00	0.00
Warfarin								
mobile phase	DD	DD	CC	CC	CC	CC	CC	CC
k′(1)	2.80	2.12	9.95	12.14	9.93	10.64	7.54	8.31
α	1.72	1.94	1.07	1.00	1.00	1.02	1.00	1.00
R_s	5.64	14.72	1.33	0.00	0.00	0.73	0.00	0.00

[a] Capacity factor of the first eluting enantiomer

Table 4. Effect of Temperature on the Separation of Propranolol Enantiomers

Temperature (°C)	K′(1)	k′(2)	a	R_s
15	3.60	4.18	1.16	3.00
20	3.57	4.11	1.15	2.83
25	3.45	3.94	1.14	2.73
30	3.40	3.85	1.13	2.59
35	3.36	3.77	1.12	2.48

APPENDIX A. Suggested Mobile Phase Compositions for Liquid Chromatographic Separations of SRM 877

Mobile Phase	Composition
A	Hexane:ethanol (50:50)
B	hexane:2-propanol (95:5) with 0.1 % diethylamine
C	hexane:2-propanol (80:20) with 0.1 % diethylamine
D	hexane:2-propanol (95:5) with 1 % trifluoroacetic acid
E	hexane:2-propanol (80:20) with 1 % trifluoroacetic acid
F	hexane:1,2-dichloroethane:ethanol-trifluoroacetic acid (85:10:5) [a]
G	hexane:1,2-dichloroethane:ethanol-trifluoroacetic acid (60:35:5) [a]
H	1 % triethylammonium acetate, pH 4.1: acetonitrile (90:10)[b]
J	1 % triethylammonium acetate, pH 4.1: acetonitrile (80:20)
K	1 % triethylammonium acetate, pH 4.1: methanol (80:20)
L	1 % triethylammonium acetate, pH 6.9: acetonitrile (90:10)
M	1 % triethylammonium acetate, pH 7.0: acetonitrile (90:10)
N	20 mmol/L sodium citrate, pH 6.3: tetrahydrofuran (90:10)
P	20 mmol/L ammonium acetate in methanol
Q	Acetonitrile with 0.3 % acetic acid and 0.2 % triethylamine
R	Methanol with 0.01 % trifluoroacetic acid and 0.01 % ammonium hydroxide
S	Acetonitrile:methanol (95:5) with 0.3 % acetic acid and 0.2 % triethylamine
T	Acetonitrile:methanol (55:45) with 0.3 % acetic acid and 0.2 % triethylamine

[a] Ethanol and trifluoroacetic acid were premixed 20:1 (v/v)
[b] 1 % triethylammonium acetate was prepared by adding 1 % (v/v) triethylamine to water and adjusting pH to the desired value with glacial acetic acid.

APPENDIX B. Suggested Mobile Phase Compositions for Supercritical Fluid Chromatographic Separations of SRM 877

Mobile Phase	Composition
AA	carbon dioxide:methanol (95:5) with 0.5 % isopropylamine
BB	carbon dioxide:methanol (90:10) with 0.5 % isopropylamine
CC	carbon dioxide:methanol (85:15) with 0.5 % isopropylamine
DD	carbon dioxide:methanol (80:20) with 0.5 % isopropylamine
EE	carbon dioxide:methanol (70:30) with 0.5 % isopropylamine

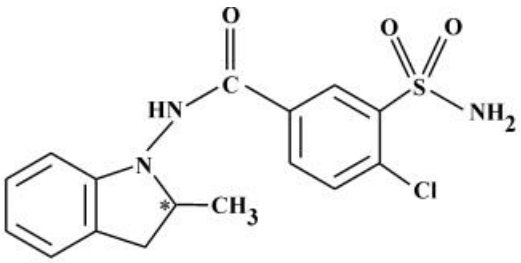
APPENDIX C. Column Identification

Column	Configuration (i.d. x length, mm)	Particle size (μm)	Chiral selector	Serial or lot number	Manufacturer
Chiralcel OD	4.6 x 250	10	cellulose tris (3,5-dimethylphenylcarbamate)	555-026-50303	Chiral Technologies, Inc., Exton, PA
Chiralpak AD	4.6 x 250	10	amylose tris (3,5-dimethylphenylcarbamate)	AD00CE-G1020	Chiral Technologies, Inc., Exton, PA
Chirex 3005	4.6 x 250	5	(R)-naphthylglycine and N-3,5-dinitrobenzoic acid	341223	Phenomenex, Torrance, CA
Chirex 3022	4.6 x 250	5	(S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl)-ethylamine	341224	Phenomenex, Torrance, CA
Chirobiotic T	4.6 x 250	5	Teicoplanin	A145-3	Advanced Separation Technologies Inc. (Astec), Whippany, NJ
Chirobiotic V	4.6 x 250	5	Vancomycin	92-3	Advanced Separation Technologies Inc. (Astec), Whippany, NJ
Cyclobond I 2000	4.6 x 250	5	β-cyclodextrin	7726	Advanced Separation Technologies, Inc. (Astec), Whippany, NJ
Cyclobond I 2000 RSP	4.6 x 250	5	(R,S)-2-hydroxypropyl-β-cyclodextrin	A145-5C	Advanced Separation Technologies, Inc. (Astec), Whippany, NJ

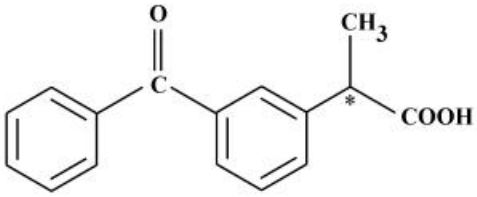
APPENDIX D. Participants

The following individuals and organizations participated in the development of SRM 877:

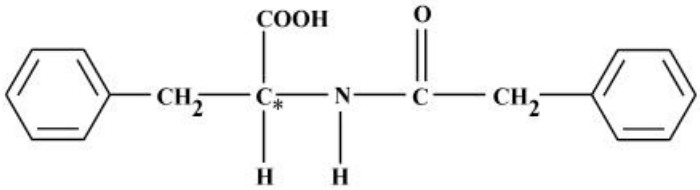
T. Beesley, Advanced Separation Technologies, Inc., Whippany, NJ
R. Bopp, Chiral Technologies Inc., Exton, PA
D. Handley, Sepracor Inc., Marlborough, MA
J.T. Lee, Advanced Separation Technologies Inc., Whippany, NJ
T. Rossi, Phenomenex, Torrance, CA
A. Stalcup, University of Cincinnati, Cincinnati, OH



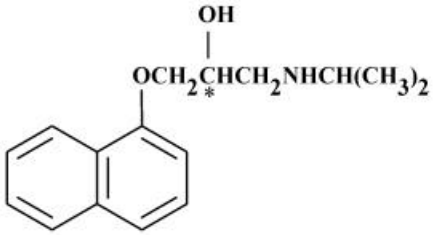
indapamide



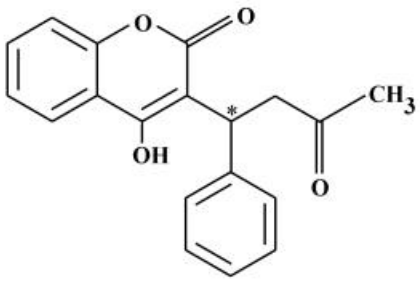
ketoprofen



N-CBZ-phenylalanine



propranolol



warfarin

Figure 1. Structures of components of SRM. The chiral center of each compound is indicated by an asterisk.

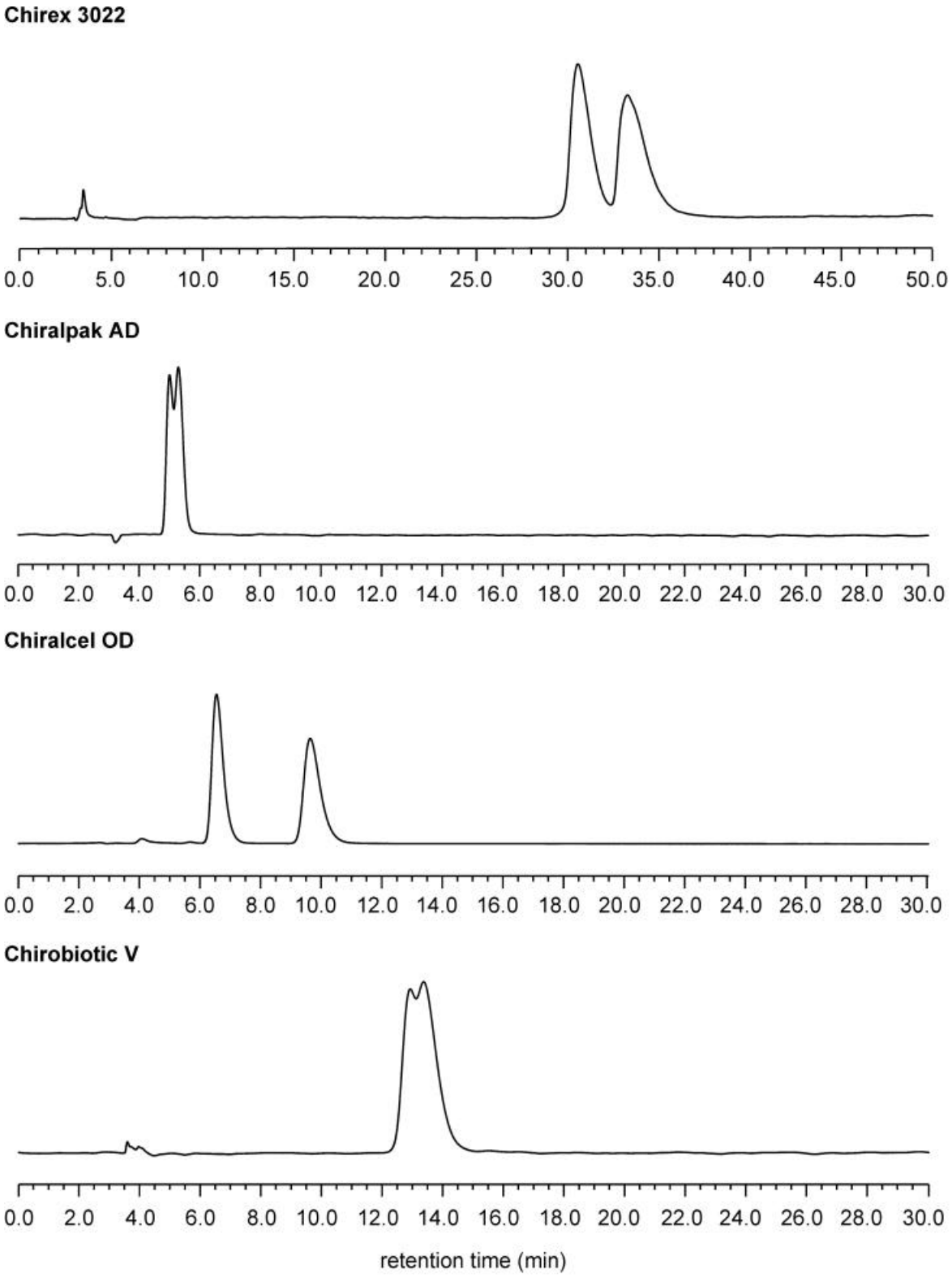


Figure 2. Separations of indapamide enantiomers on commercial chiral stationary phases by liquid chromatography.

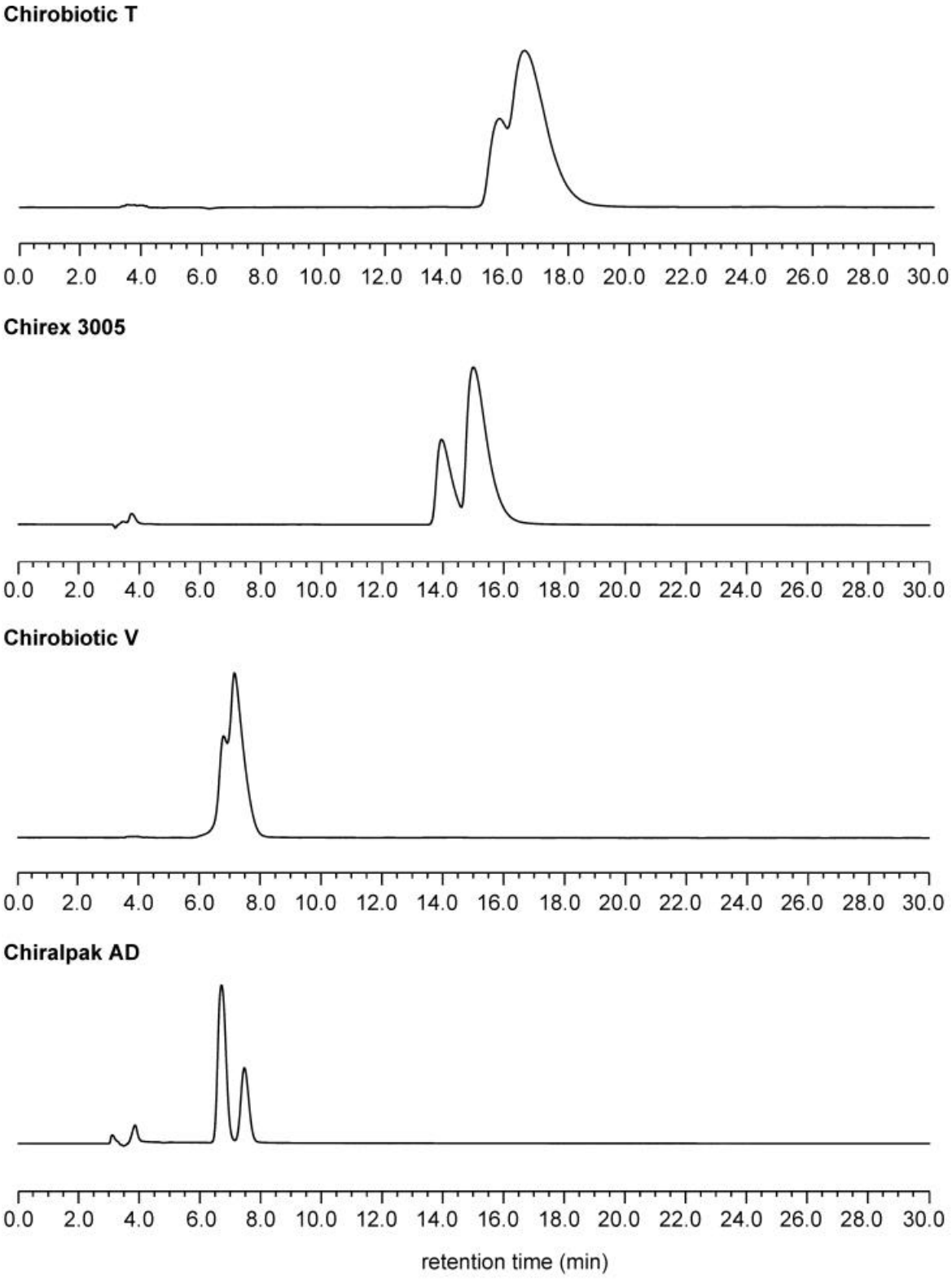


Figure 3. Separations of ketoprofen enantiomers on commercial chiral stationary phases by liquid chromatography.

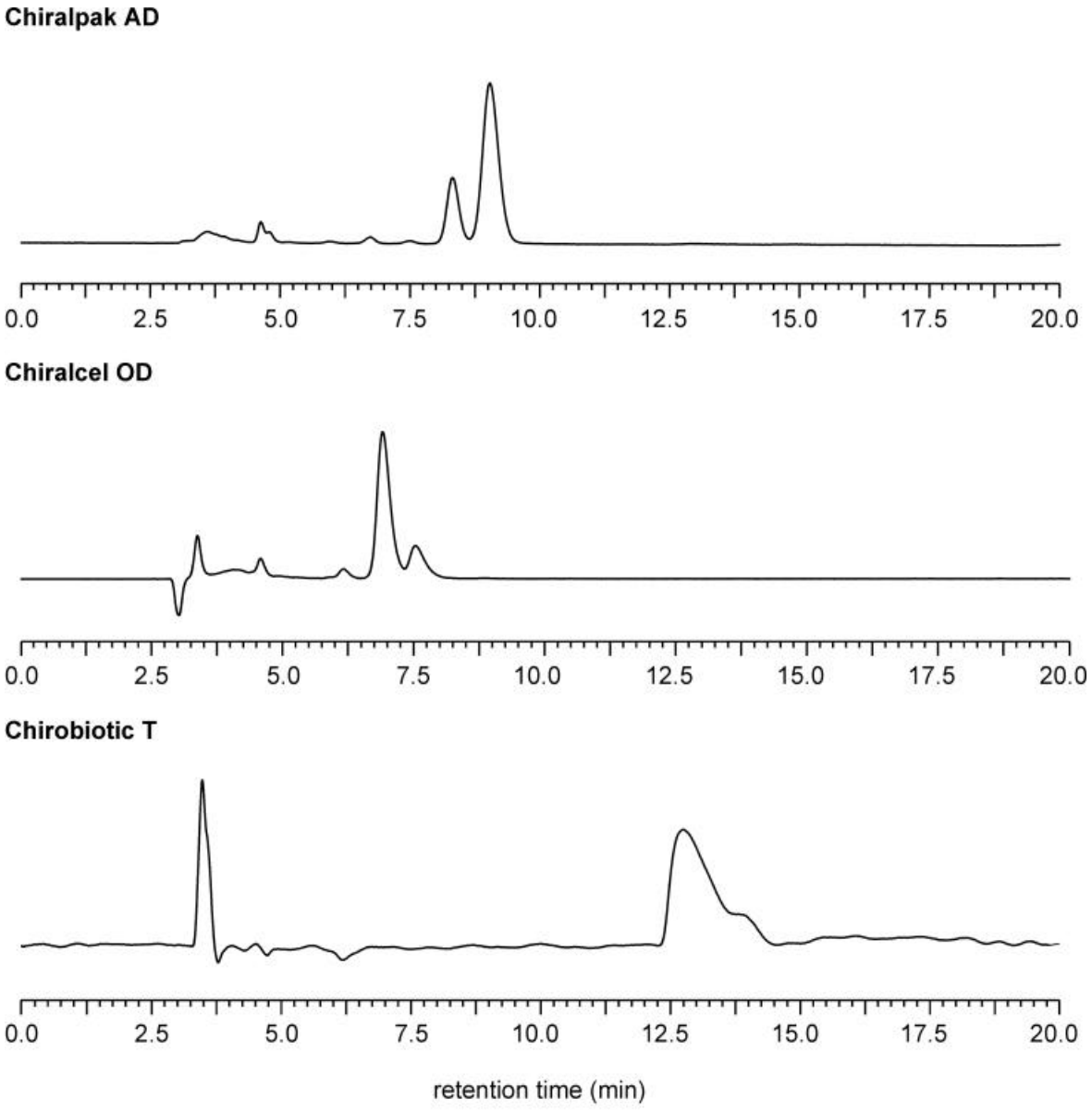


Figure 4. Separations N-CBZ-phenylalanine enantiomers on commercial chiral stationary phases by liquid chromatography.

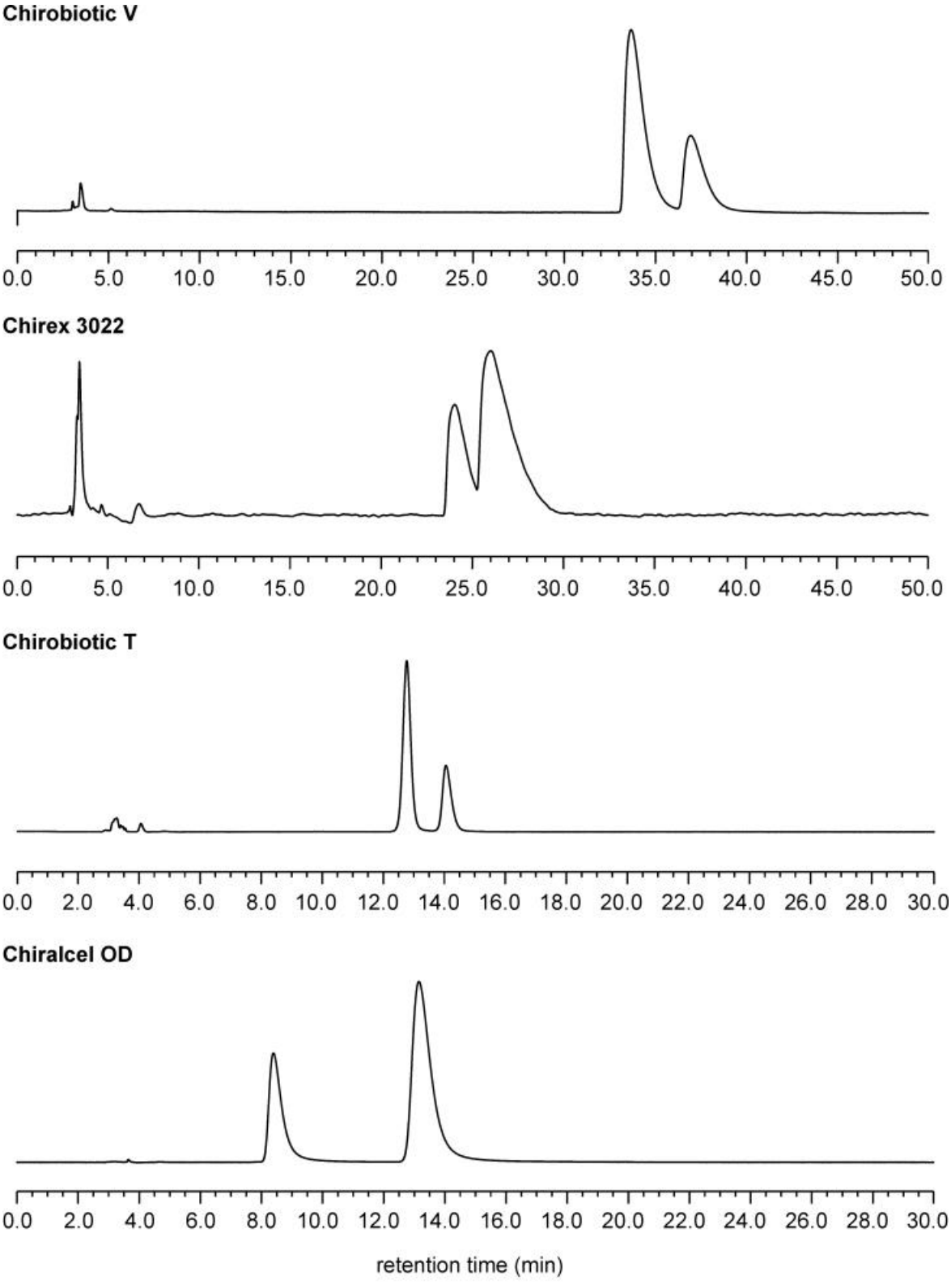


Figure 5. Separations of propranolol enantiomers on commercial chiral stationary phases by liquid chromatography.

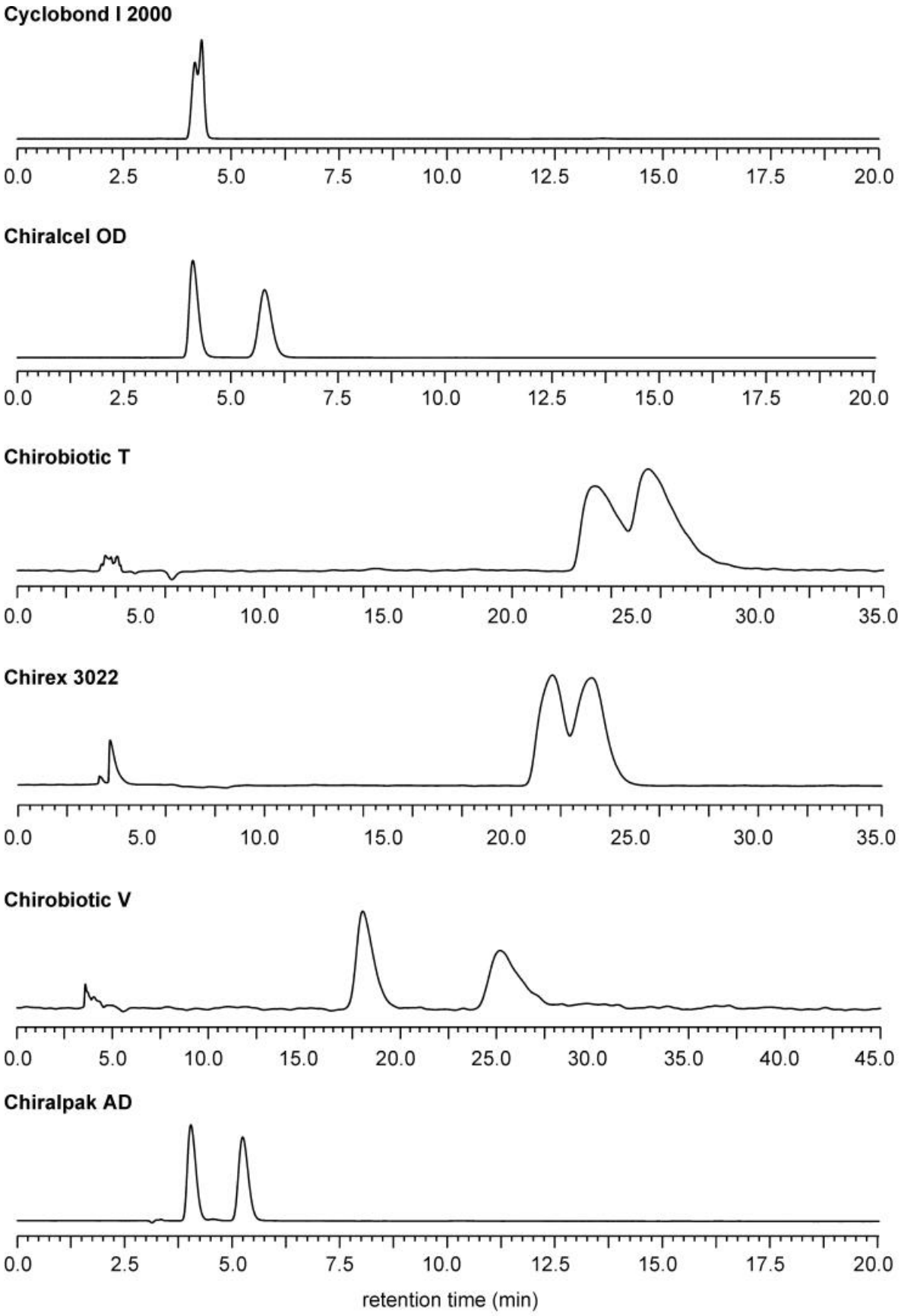


Figure 6. Separations of warfarin enantiomers on commercial chiral stationary phases by liquid chromatography.

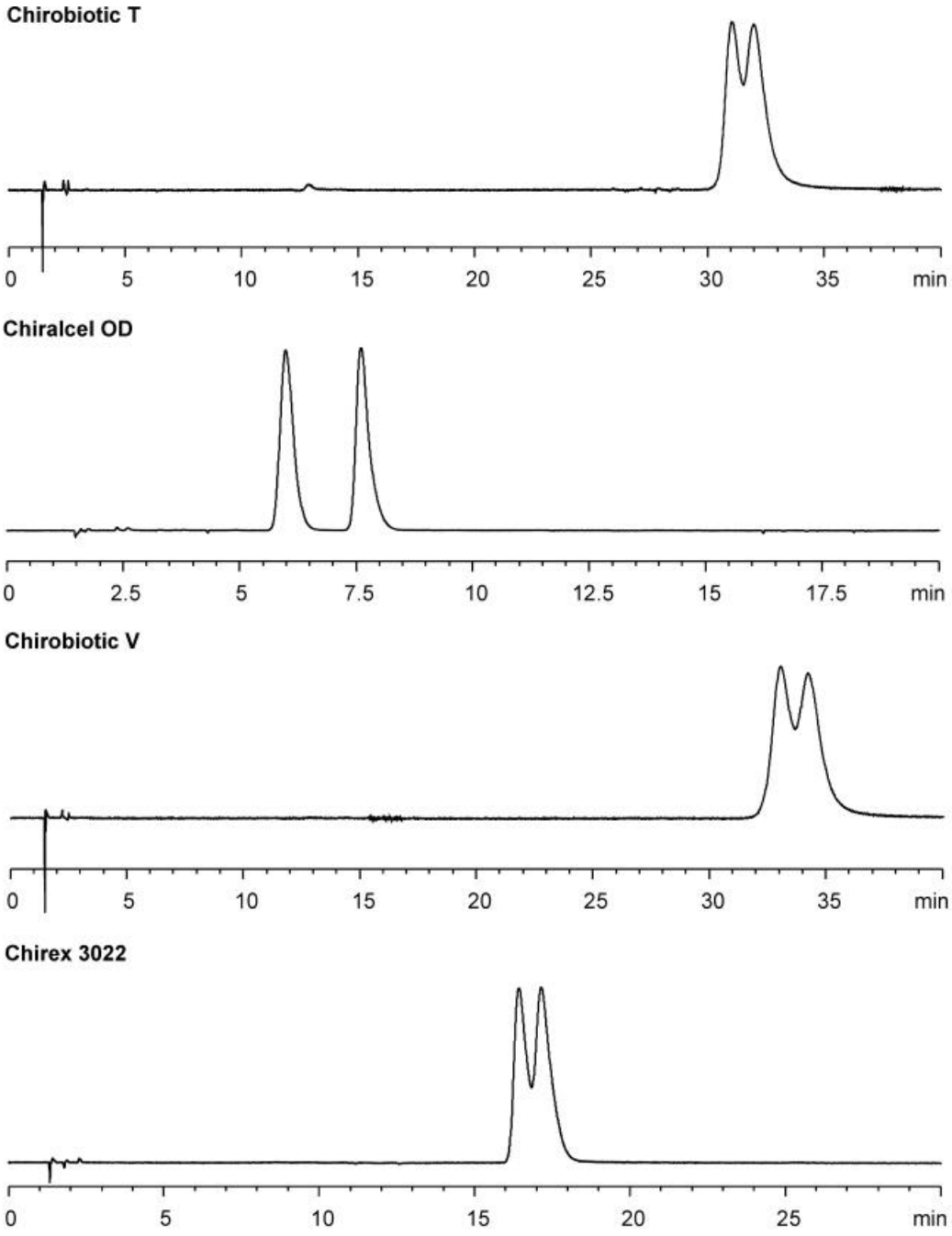


Figure 7. Separations of indapamide enantiomers on commercial chiral stationarey phases by supercritical fluid chromatography.

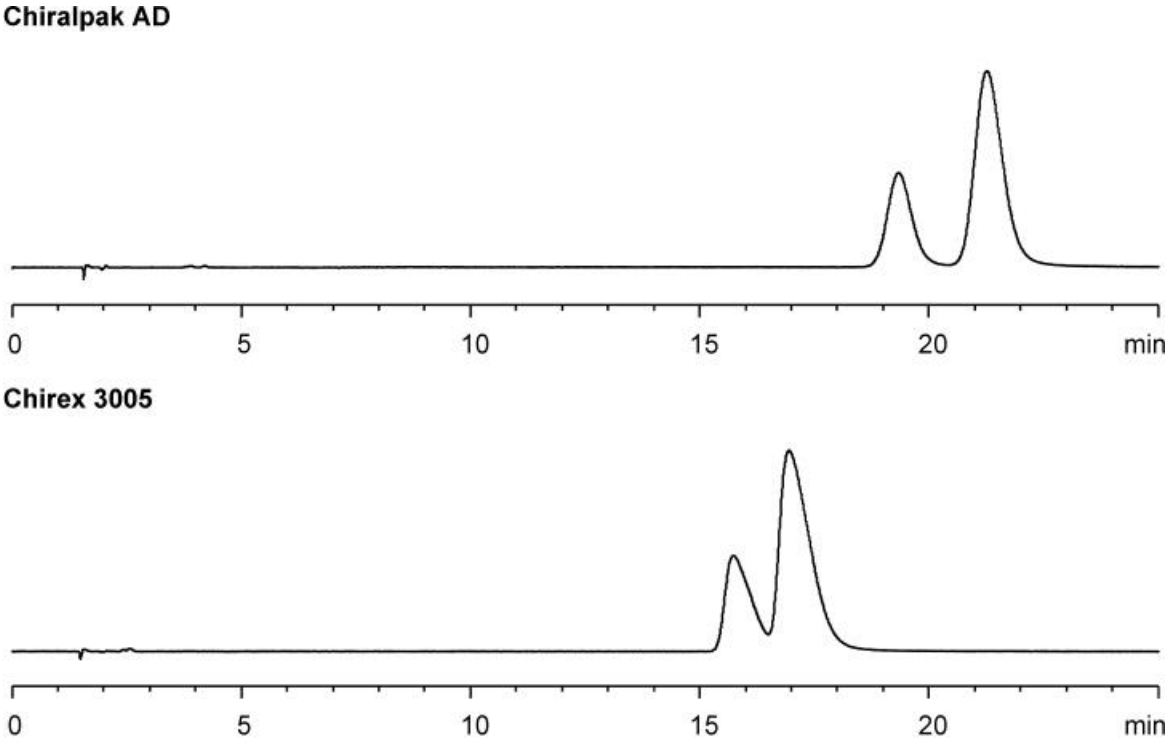


Figure 8. Separations of ketoprofen enantiomers on commercial chiral stationary phases by supercritical fluid chromatography.

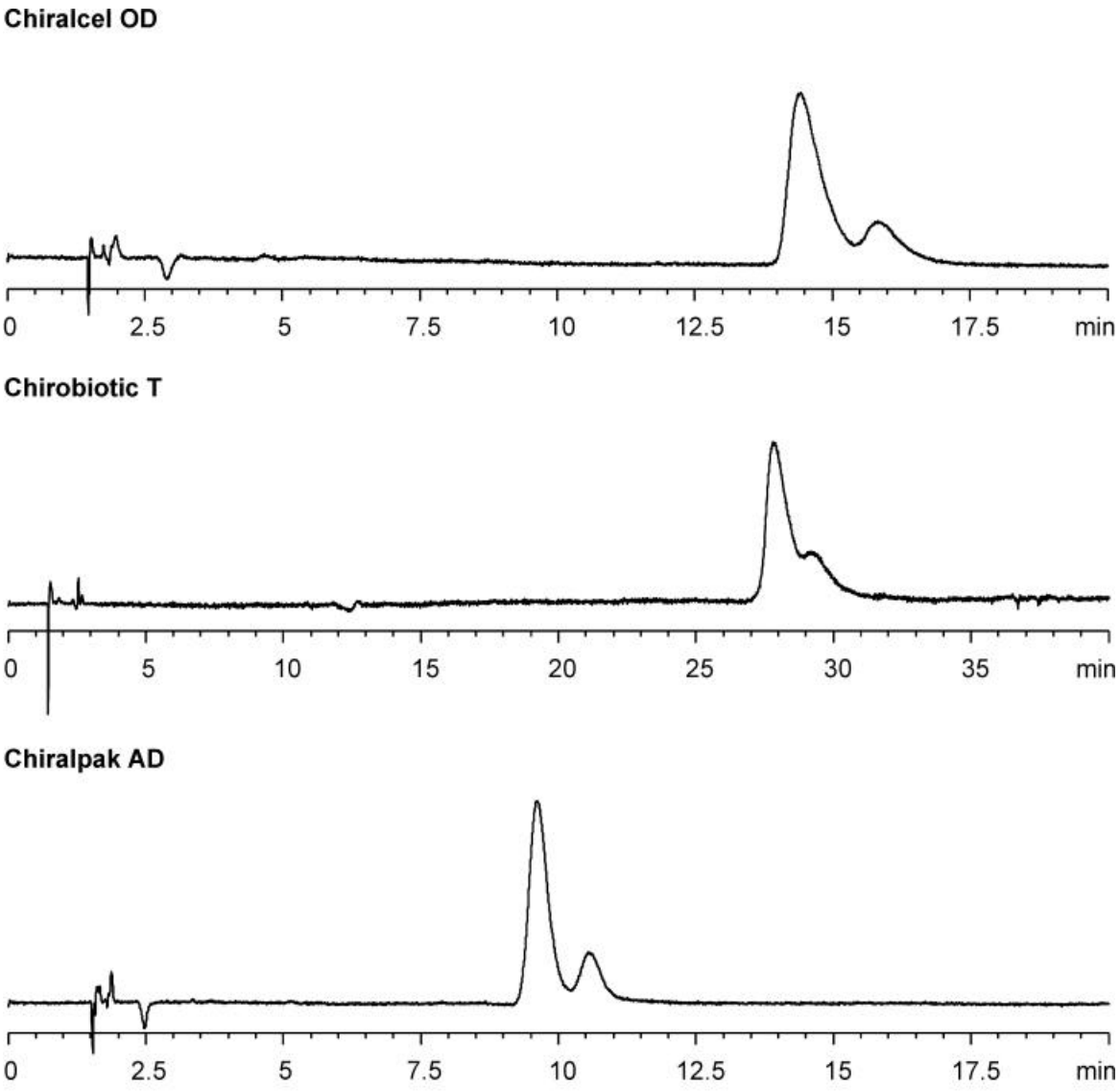


Figure 9. Separations of N-CBZ-phenylalanine enantiomers on commercial chiral stationary phases by supercritical fluid chromatography.

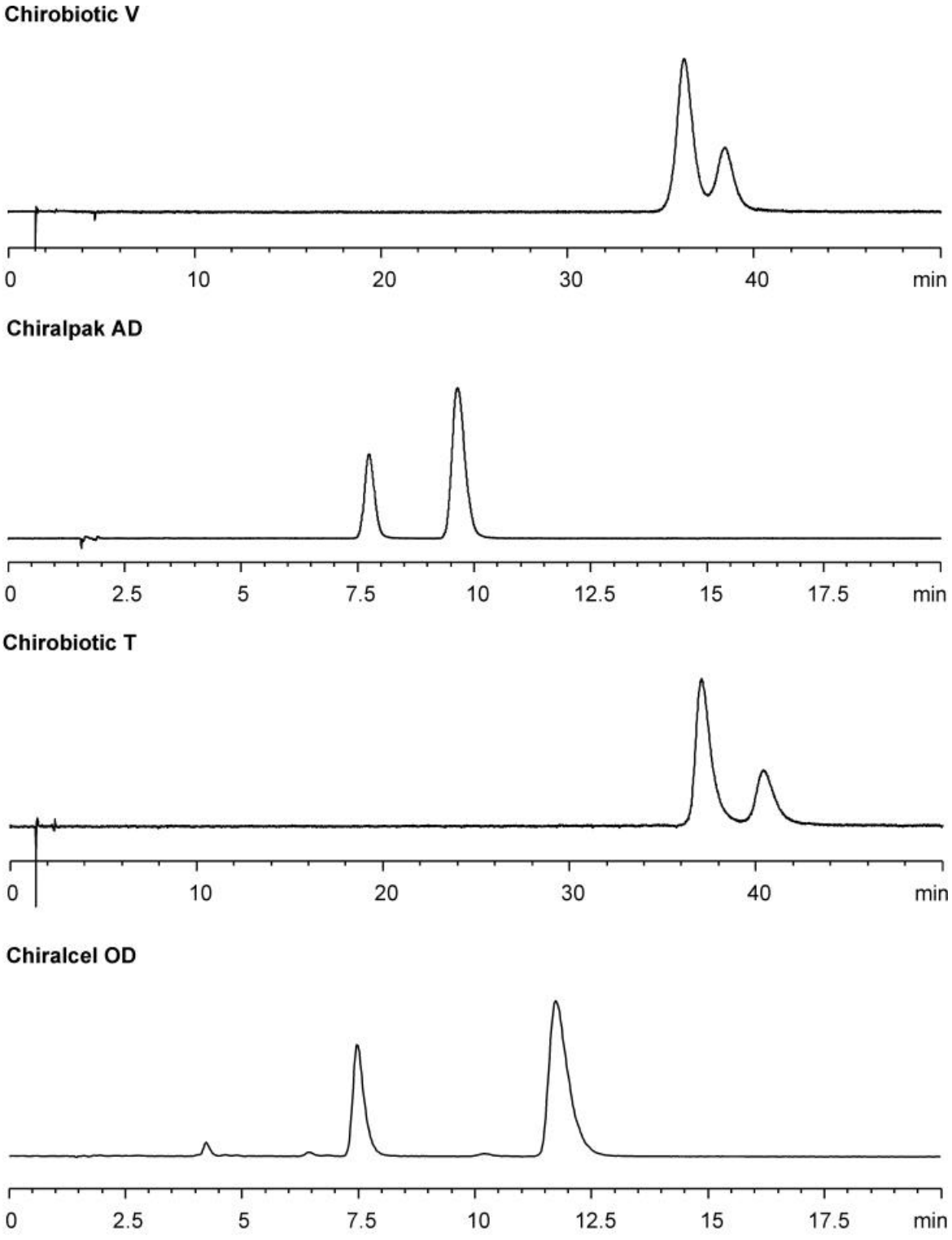


Figure 10. Separations of propranolol enantiomers on commercial chiral stationary phases by supercritical fluid chromatography.

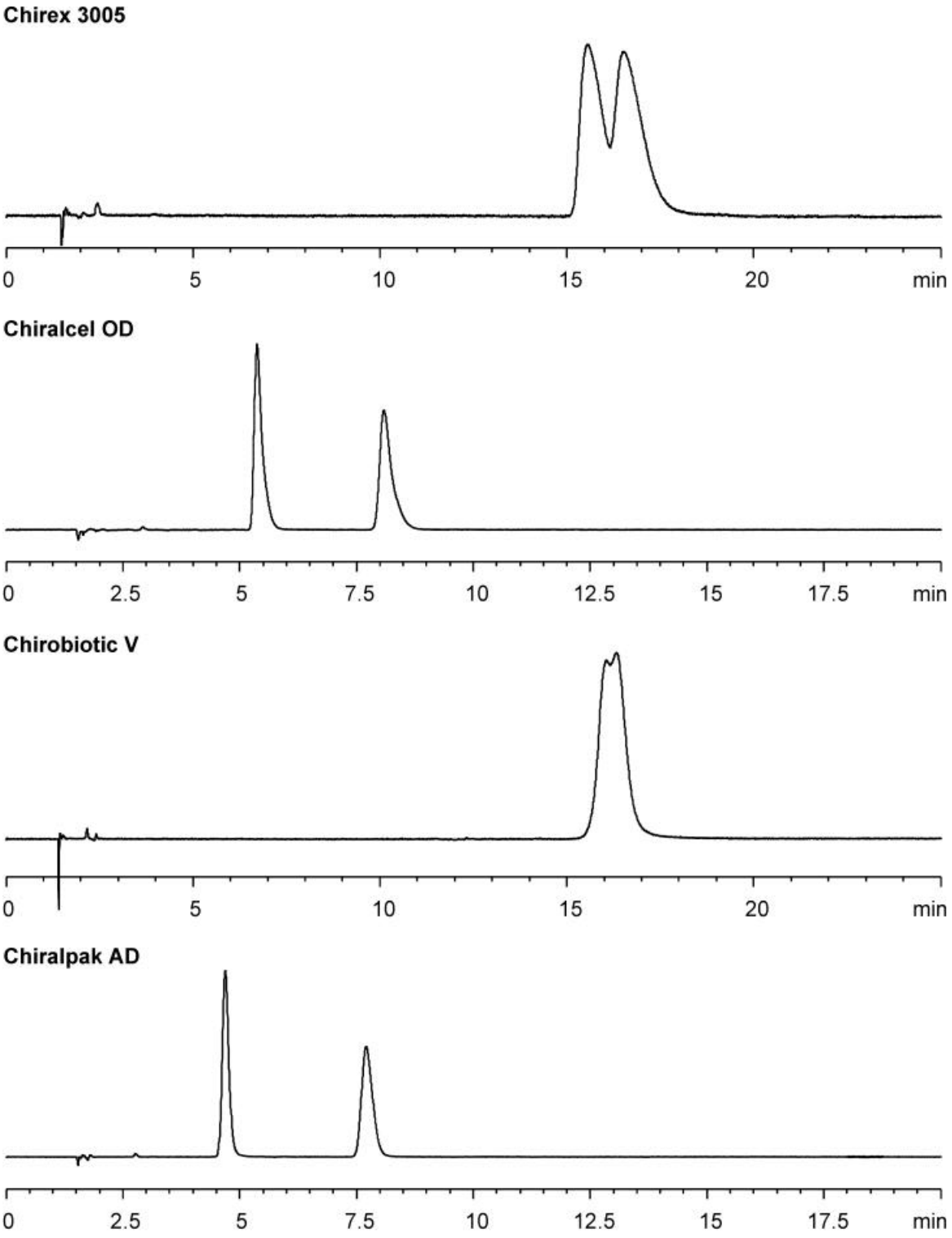


Figure 11. Separations of warfarin enantiomers on commercial chiral stationary phases by supercritical fluid chromatography.