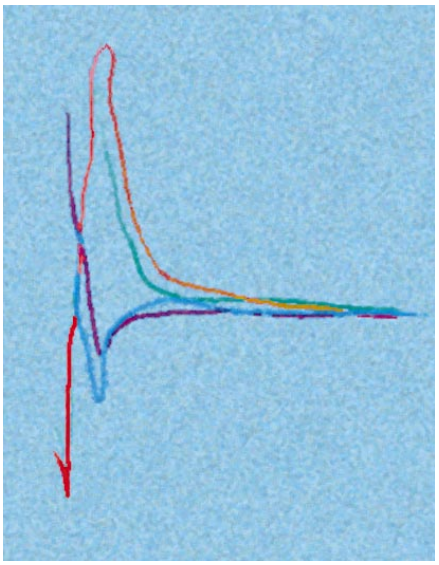

HPLC Detection and Evaluation of Chiral Compounds with a Laser-Based Chiroptical Detector

The sensitivity and ruggedness of chiroptical detectors have improved considerably during the past decade. In this article, the authors examine more than 230 chiral compounds using the latest laser-based polarimetry detector for high performance liquid chromatography (HPLC). They also examine the relationship between optical rotation at the detector wavelength of 675 nm and the sodium D line. In addition, the authors consider the sensitivity, linear dynamic range, and effect of solvent composition on rotation and its general use as an HPLC detector for chiral compounds.

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The high performance liquid chromatography (HPLC) separation of enantiomers has developed into a mature field used in many branches of science and technology (1–3). Its great success has spurred development in a variety of other areas, including in detectors for optically active or chiral compounds. Chiroptical detectors are based on optical rotation (polarimetry) or circular dichroism. Since 1980 several research groups have adopted various forms of micropolarimetry (4–14) and circular dichroism (15–24) into HPLC detector formats. Researchers have published a few reviews on chiroptical detection and the general analytical use of polarimetry and circular dichroism (25–28).

Over the past 10 years, a few commercial versions of these detectors have appeared. During the course of our research we had the opportunity to use or evaluate many of these detectors. Using chiroptical detectors provides both advantages and disadvantages. Some of the beneficial aspects are listed in the accompanying sidebar, "Uses and Beneficial Aspects of Chiroptic Detection." Of these, the most important benefits seem to be the validation of enantiomeric separations and quality control applications. Also, because only chiral compounds are detected, a chromatogram can be simplified greatly. The elimination of interfering peaks from nonchiral compounds allows analysts to focus on the chiral analytes of interest.

Far and away, the major disadvantage of all chiroptical detectors has been their poor sensitivity. Baseline drift and occasional artifact peaks often were related problems. The earliest chiroptical detectors also had difficulties with the instability of seals and other instrumental parts in the presence of certain solvents. Indeed, we considered the earliest chiroptical detectors to be two to three orders of magnitude less sensitive than necessary for most routine analytical separations. This sensitivity left a lot of room for improvement. Most of the advances in commercial chiroptical detectors during the past decade focused on increasing their sensitivity and ruggedness. These improvements have come from using better light sources that provide higher power and greater stability, better optics, and electronic systems optimized to reduce noise.

In this article we examine one of the latest chiroptical detectors. In addition to the usually reported parameters considered in studies involving these detectors, we evaluated its use and sensitivity for a very wide variety of chiral compounds; the relationship, if any, between optical rotation at 675 nm and that of the sodium D line at 589.3 nm; the linear dynamic range of this chiroptical detector; and the effect of different solvents on the relative response of this detector.

EXPERIMENTAL

Materials: We obtained all HPLC columns from Advanced Separation Technologies, Inc. (Whippany, New Jersey). The liquid chromatography (LC) columns we used included 25 cm × 4.6 mm native β-cyclodextrin Cyclobond I 2000, 25 cm × 4.6 mm 2-hydroxypropyl-β-cyclodextrin Cyclobond I 2000 RSP, and 5 cm × 4.6 mm, 5-μm d_p C18 columns. We purchased methanol, acetonitrile, glacial acetic

USES AND BENEFICIAL ASPECTS OF CHIROPTIC DETECTION

- Validation of enantioseparations
- Quality control applications
- Reasonable sensitivity for chiral compounds that lack a UV chromophore; for example, carbohydrates and certain amino acids
- Determining enantiomeric excess without separation, at moderate levels only
- Monitoring conformation of chiral polymers
- Selectivity for chiral analytes simplifies chromatograms by not detecting nonchiral compounds
- Follow or assess racemization
- Demonstrating that an asymmetric synthetic transformation has occurred

TABLE I: Alphabetical Listing of Chiral Compounds Detected After Reversed-Phase HPLC and Their Measured Relative Response Optical Rotations, Specific Rotations at 675 nm*, and Specific Rotations at the Sodium D Line†

Compound	Relative Response‡	$[\alpha]^{22}_{675}$ (degrees)	Published $[\alpha]^{20}_{D}$ Value (degrees)	Solvent Used to Obtain Published Value
(1*R*,4*S*)-*cis*-4-Acetoxy-2-cyclopenten-1-ol	−12.36	−59.4	−67	Chloroform
(1*S*,4*R*)-*cis*-4-Acetoxy-2-cyclopenten-1-ol	12.59	63.5	68	Chloroform
(*R*)-(−)-*O*-Acetylmandelic acid	−22.14	−115.6	−147.5	Acetone
(*S*)-(+)-*O*-Acetylmandelic acid	21.80	105.3	147.5[19]	Acetone
N-(4-Aminobenzoyl)-L-glutamic acid diethyl ester	5.45	28.5	17.9[21]	Chloroform
(*S*)-(+)-2-Amino-1-butanol	2.09	8.9	10	Neat
L−α-Amino-*n*-butyric acid	1.83§	9.76§	20.4	5 N Hydrochloric acid
D−α-Amino-*n*-butyric acid	−1.94§	−10.57§	−7.94	Water
(*S*)-2-Amino-4-butyrolactone hydrobromide	−10.34	−50.9	−21	Water
(1*S*,2*R*)-(+)-2-Amino-1,2-diphenylethanol	2.85	16.4	7	Ethanol
(1*R*,2*S*)-(−)-2-Amino-1,2-diphenylethanol	−2.89	−14.5	−7	Ethanol
(1*R*,2*S*)-(+)-2-Amino-1-phenyl-1,3-propanediol	4.09	24.4	28	Methanol
(*R*)-(+)-2-Amino-3-phenyl-1-propanol	4.30	15.1	23	1 N Hydrochloric acid
(*S*)-(−)-2-Amino-3-phenyl-1-propanol	−4.64	−16.6	−22.8[22]	1 N Hydrochloric acid
(*R*)-(−)-1-Amino-2-propanol	−8.63	−38.4	−23.5	Methanol
(*S*)-(+)-1-Amino-2-propanol	8.78	39.5	23.5	Methanol
(*R*)-(+)-3-Aminoquinuclidine dihydrochloride	9.28	43.1	24	Water
(*S*)-(−)-3-Aminoquinuclidine dihydrochloride	−9.31	−42.5	−24	Water
1,6-Anhydro-3,4-*O*-isopropylindene-2-tosyl-D-galactose	−14.05	−64.4	−60[23]	Chloroform
(*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol	−7.95	−39.2	−25.5	Chloroform
(*S*)-(+)-1-(9-Anthryl)-2,2,2-trifluoroethanol	7.77	42.5	25.5	Chloroform
L-Arginine	3.30b	16.7	26.1	6 N Hydrochloric acid
D-Asparagine monohydrate	−2.15§	−10.12§	−27	1 N Hydrochloric acid
(1*R*)-(−)-2-Azabicyclo[2.2.1]hept-5-en-3-one	−125.74	−629.7	−565	Chloroform
(1*S*)-(+)-2-Azabicyclo[2.2.1]hept-5-en-3-one	126.44	634.8	565	Chloroform
(*S*)-(−)-2-Azetidine carboxylic acid	−31.14§	−152.9§	−120	Water
(*S*)-(+)-Benzoin	41.96	235.6	115[19]	Acetone
(*S*)-(+)-Benzoyl-2-*tert*-butyl-3-methyl-4-imidazolidinone	27.17	133.1	125[26]	Methylene chloride
(*R*)-(+)-4-Benzyl-5,5-dimethyl-2-oxazolidinone	31.94	128.1	96	Chloroform
(*S*)-(−)-4-Benzyl-5,5-dimethyl-2-oxazolidinone	−33.03	−135.8	−98[22]	Chloroform
Benzyl (*R*)-(−)-glycidyl ether	−3.10	−15.2	−5.4	Toluene
(+)-2,3-*o*-Benzylidene-D-threitol	3.32	15.2	11[18]	Methanol
(−)-2,3-*o*-Benzylidene-L-threitol	−3.41	−14.6	−10.5	Methanol
N-Benzyl-α-methylbenzylamine	−6.74	−32.4	−40[19]	Neat
(*S*)-(+)-*N*-Benzyl-1-(1-naphthyl)-ethylamine hydrochloride	8.17	59.1	61	Methanol
(*R*)-(−)-*N*-Benzyl-1-(1-naphthyl)-ethylamine hydrochloride	−8.77	−56.8	−61	Methanol
(*R*)-(+)-4-Benzyl-2-oxazolidinone	4.02	24.5	64[18]	Chloroform
(*S*)-(−)-4-Benzyl-2-oxazolidinone	−3.91	−23.4	−63	Chloroform
(*R*)-(+)-3-(Benzyloxycarbonyl)-4-oxazolidine carboxylic acid	20.30	101.5	92	Chloroform
(*R*)-(−)-Benzyloxy-3-(*p*-tosyloxy)-2-propanol	−2.88	−14.5	−7	Toluene
(*S*)-(+)-Benzyloxy-3-(*p*-tosyloxy)-2-propanol	2.87	11.6	7	Toluene
(*R*)-(−)-4-Benzyl-3-propionyl-2-oxazolidinone	−34.50	−179.0	−102	Ethanol
(*S*)-(+)-4-Benzyl-3-propionyl-2-oxazolidinone	33.54	177.6	97	Ethanol
(*R*)-(+)-1,1′-Bi-2-naphthol	5.03	30.2	34[21]	Tetrahydrofuran
(*S*)-(−)-1,1′-Bi-2-naphthol	−4.93	−30.5	−34[22]	Tetrahydrofuran
(*R*)-(−)-1,1′-Bi-2-naphthol bis(trifluoromethanesulfonate)	−34.87	−176.5	−146[23]	Chloroform
(*S*)-(+)-1,1′-Bi-2-naphthol bis(trifluoromethanesulfonate)	34.53	174.5	148[21]	Chloroform
(*R*)-(−)-Binaphthyl-2,2′-diyl hydrogen phosphate	−138.25	−622.1	−607.1[19]	Methanol
(*S*)-(+)-Binaphthyl-2,2′-diyl hydrogen phosphate	139.22	626.5	595[22]	Methanol
2,6-Bis[(4*R*)-(+)-isopropyl-2-oxazolin-2-yl]pyridine	31.68	163.4	118	Methylene chloride
2,6-Bis[(4*S*)-(−)-isopropyl-2-oxazolin-2-yl]pyridine	−31.89	−156.1	−118[25]	Methylene chloride
{Bis-[(2*S*,3*aR*,4*S*,7*aR*)-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl]-ether}	−43.49	−229.6	−202	Tetrahydrofuran
[(1*S*)-*endo*]-(−)-Borneol	−9.05	−45.0	−35.3	Ethanol
(+)-Borneol	9.75	48.9	36	Ethanol
(2*R*,3*R*)-3-(4-Bromophenyl)-glycidol	11.09	51.1	33	Chloroform
(2*S*,3*S*)-(+)-2,3-Butanediol	2.14	10.2	13	Neat
(2*R*,3*R*)-(−)-2,3-Butanediol	−2.07	−9.2	−13.2[23]	Neat
(*R*)-(+)-1,2,4-Butanetriol	8.78	42.5	26	Methanol
(*S*)-(−)-1,2,4-Butanetriol	−9.70	−47.6	−27[19]	Methanol
(*R*)-(−)-2-Butanol	−11.28	−58.7	−12.6[22]	Neat
tert-Butyl-(*R*)-(+)-lactate	2.82	13.1	7.3	Methylene chloride
(+)-Camphor	10.29	53.9	44.1[25]	Ethanol
(−)-Camphor	−10.32	−52.1	−30.7	Methanol
(1*S*,3*R*)-(−)-Camphoric acid	−13.18	−67.2	−48	Ethanol
(1*R*)-(−)-Camphorquinone	−20.49	−94.4	−101	Toluene
(1*R*)-(+)-2,10-Camphorsultam	7.73	54.3	32	Chloroform
(1*S*)-(−)-2,10-Camphorsultam	−8.08	−49.0	−32[19]	Chloroform
(1*S*)-(+)-3-Carene	6.05	29.5	17	Neat
D-Carnitine	4.24	22.8	30.9	Water
(*R*)-(−)-Carvone	−16.95	−80.4	−61	Neat
(*S*)-(+)-Carvone	16.66	80.6	61	Neat
(+)-β-Cedrene	4.28	21.6	13	Neat
(*R*)-(+)-4-Chloro-3-hydroxybutyronitrile	5.52	31.2	11[25]	Neat
(*S*)-(−)-4-Chloro-3-hydroxybutyronitrile	−5.37	−25.2	−8[25]	Neat
Cholic acid	10.15	50.2	36[23]	95% Ethanol
(+)-β-Citronellene	11.16	54.0	9	Neat
(*S*)-(−)-β-Citronellol	−1.26	−6.3	−5.3	Neat
(1*R*,2*R*)-*trans*-1,2-Cyclopentanediol	−8.43	−41.7	−21	Chloroform
(1*S*,2*S*)-*trans*-1,2-Cyclopentanediol	8.22	45.8	19	Chloroform
(1*R*,3*S*)-(+)-4-Cyclopentane-1,3-diol-1-acetate	13.03	67.3	68	Chloroform
3,4-Dehydro-2-proline	−79.31	−375.1	−385	Water
(*R*)-(+)-1,2-Diaminopropane dihydrochloride	8.42	46.2	4	Water
(*S*)-(−)-1,2-Diaminopropane dihydrochloride	−8.51	−42.4	−4	Water
(−)-2,3-Dibenzoyl-L-tartaric acid	−24.32	−109.5	−116	Ethanol
(+)-2,3-Dibenzoyl-D-tartaric acid	24.31	109.4	116[28]	Ethanol

* All samples were measured in a methanol mobile phase (unless mentioned otherwise) at a concentration of 3 mg/mL, and with 1 μL of solution injected onto the reversed-phase HPLC column.
† All values of $[\alpha]^{20}_{D}$ were obtained from references 30 and 31.
‡ Relative responses of peak area of the chiral detector at 675 nm compared with L-valine.
§ Responses measured in water.

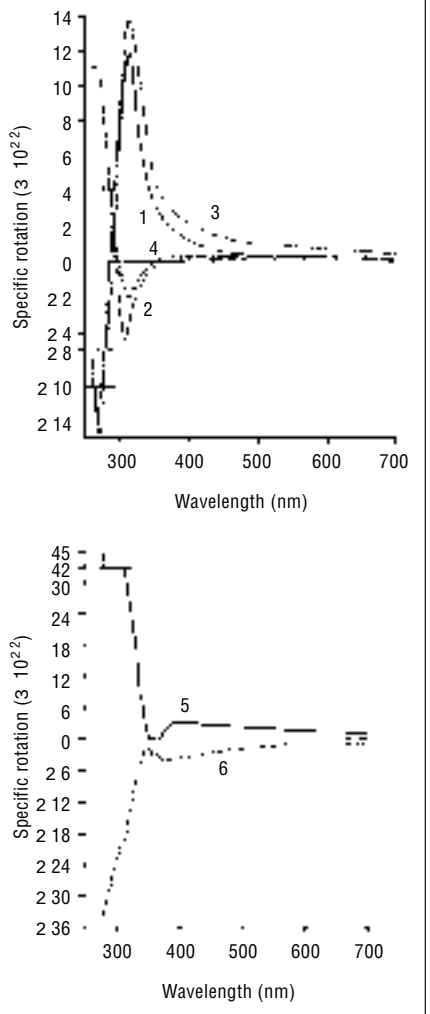

FIGURE 1: Optical rotatory dispersion curves. 1 = B-norcholestan-3-one in methanol, 2 = B-norcoprostan-3-one in methanol, 3 = *trans*-8-methylhydrindan-5-one in methanol, 4 = *cis*-8-methylhydrindan-5-one in dioxane, 5 = (5*S*,10*S*)-5-hydroxy-10-methyl-$\Delta^{1(9)}$-2-octalone in dioxane, 6 = (5*S*,10*R*)-5-hydroxy-10-methyl-$\Delta^{1(9)}$-2-octalone in dioxane. (Reproduced with permission from reference 33.)

acid, and triethylamine from Fisher Scientific (St. Louis, Missouri). All chiral compounds were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin); Sigma Chemical Co. (St. Louis, Missouri); and Fluka Chemical Corp. (Ronkonkoma, New York).

Apparatus: We performed LC separations using a LC-6A pump, a CR601 Chromatopac integrator, a SPD-2AM spectrophotometric detector, and a RID-10A refractive index detector (all from Shimadzu Scientific Instruments, Columbia, Maryland) and an Advanced laser polarimeter (PDR-Chiral, Inc., Palm Beach Gardens, Florida). The specifications for this laser-based polarimeter–chiral detector indicate that it routinely provides sensitivity levels of less than 25×10^{-6} ° using a 675-nm diode laser, a new modulation scheme, and window materials that enable performance independent

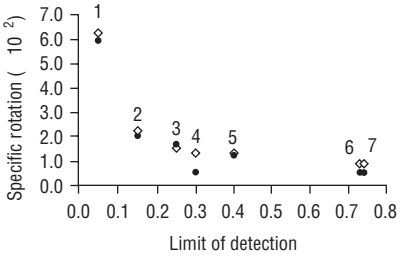

FIGURE 2: Plots of specific rotation versus limit of detection obtained using LC with the chiral detector. The limit of detection is considered to be the amount of compound in micrograms needed to produce a signal-to-noise ratio of 3. 1 = (*S*)-(−)-binaphthyl-2-2′-diyl hydrogen phosphate (0.05 µg, $[\alpha]_D^{22}$ = 595°, $[\alpha]_{675}^{22}$ = 626.5°); 2 = bis-[(2*S*,3a*R*,4*S*,7a*R*)-octahydro-7,8,8-trimethyl-4-7-methanobenzofuran-2-yl]-ether (0.15 µg, $[\alpha]_D^{20}$ = 202°, $[\alpha]_{675}^{22}$ = 229°); 3 = (4*R*,5*S*)-(+)-methyl-5-phenyl-2-oxazolidinone (0.25 µg, $[\alpha]_D^{18}$ = 168°, $[\alpha]_{675}^{22}$ = 155°); 4 = (*S*)-(+)-4-isobutyl-α-methylphenylactic acid (0.30 µg, $[\alpha]_D^{20}$ = 59°, $[\alpha]_{675}^{22}$ = 132.4°); 5 = (*S*)-(+)-benzoyl-2-*tert*-butyl-3-methyl-4-imidazolidinone (0.40 µg, $[\alpha]_D^{26}$ = 125°, $[\alpha]_{675}^{22}$ = 133°); 6 = (*S*)-(+)-4-phenyl-2-oxazolidinone (0.73 µg, $[\alpha]_D^{20}$ = 48°, $[\alpha]_{675}^{22}$ = 79.7°); 7 = (*R*)-(+)-*sec*-phenethyl alcohol (0.74 µg, $[\alpha]_D^{20}$ = 42°, $[\alpha]_{675}^{22}$ = 63.4°). ● = $[\alpha]_D^{20}$; ◇ = $[\alpha]_{675}^{22}$.

of operating pressure, solvent composition, or flow rate (29).

The UV detection wavelength was set at 190 nm for cyclodextrins and sugars or 254 nm for the enantiomeric separations of aromatic compounds. The LC chromatograms for all chiral compounds were obtained by using two LC-10AT pumps, a SIL-10A autoinjector, a SLC-10A system controller, and a CR501 Chromatopac integrator (all from Shimadzu) and the chiral detector (PDR-Chiral). All chromatograms were obtained at ambient temperature (22 °C). Table I lists the experimental conditions for more than 200 commercially available chiral compounds (30,31).

Specific rotation measurements: We calculated each compound's specific rotation at 675 nm using chromatographic peaks generated with the chiral detector and equation 1, outlined by Bobbitt and co-workers (32) as

$$[a] = \frac{v\,a}{l m F_G} \qquad [1]$$

where [α] is the specific rotation in degrees, *v* is the detector flow cell volume in milliliters, α is the observed rotation in degrees, *l* is the detector path length in decimeters, *m* is the injected mass in grams, and F_G is the Gaussian fraction (that is, the fraction of injected mass present in the flow cell [32]).

The chiral detector's flow cell volume and path length are 56 µL and 5.17 cm, respectively.

TABLE I: Continued

Compound	Relative Response‡	$[\alpha]_{675}^{22}$ (degrees)	Published $[\alpha]_D^{20}$ Value (degrees)	Solvent Used to Obtain Published Value
(−)-*N,N′*-Dibenzyl-D-tartramide	−22.73	−109.3	−83	Pyridine
(+)-*N,N′*-Dibenzyl-D-tartramide	24.27	117.9	83	Pyridine
(−)-3,9-Dibromocamphor	−20.33	−106.0	−100[19]	Chloroform
(+)-3,9-Dibromocamphor	20.56	110.2	100[19]	Chloroform
(1*R*,2*S*)-(+)-2-(Dibutylamino)-1-phenyl-1-propanol	3.01	14.7	21	Chloroform
(−)-10-Dicyclohexylsulfamoyl-D-isoborneol	−6.39	−30.1	−25	Ethanol
(+)-10-Dicyclohexylsulfamoyl-L-isoborneol	5.92	33.9	25	Ethanol
(3*R-cis*)-(−)-2,3-Dihydro-3-isopropyl-7*a*-methylpyrrolo-[2,1-β]oxazol-5(7*aH*)-one	18.59	94.9	41	Chloroform
(3*S-cis*)-(+)-2,3-Dihydro-3-isopropyl-7*a*-methylpyrrolo-[2,1-β]oxazol-5(7*aH*)-one	−18.44	−89.4	−37[22]	Chloroform
(3*S-cis*)-(+)-2,3-Dihydro-7*a*-methyl-3-phenylpyrrolo-[2,1-β]oxazol-5(7*aH*)-one	38.10	195.8	124	Chloroform
(3*R-cis*)-(−)-2,3-Dihydro-7*a*-methyl-3-phenylpyrrolo-[2,1-β]oxazol-5(7*aH*)-one	−39.67	−199.9	−124	Chloroform
Diisopropyl-D-tartrate	−5.98	−23.0	−17[23]	Neat
Diisopropyl-L-tartrate	5.81	24.1	17[24]	Neat
(1*S*)-(+)-Dimenthyl succinate	24.81	125.1	89	Chloroform
(1*R*)-(−)-Dimenthyl succinate	−24.59	−131.6	−89	Chloroform
(+)-*trans*-α,α′-(2,2-Dimethyl-1,3-dioxolane-4,5-diyl)-bis(diphenylmethanol)	17.23	90.6	67[19]	Chloroform
(−)-*trans*-α,α′-(2,2-Dimethyl-1,3-dioxolane-4,5-diyl)bis-(diphenylmethanol)	−17.37	−86.4	−62.6[19]	Chloroform
(*R*)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol	−3.75	−17.6	−13.7	Neat
(+)-Dimethyl-2,3-*o*-isopropylidene-D-tartrate	18.79	81.7	55	Neat
(−)-Dimethyl-2,3-*o*-isopropylidene-L-tartrate	−18.92	−82.5	−54	Neat
(1*R*,exo,exo)-3-[*N*-(3,5-Dimethylphenyl)-benzene-sulfonamido]-isoborneol	10.59	77.4	65	Chloroform
(4*S*,5*R*)-(+)-1,5-Dimethyl-4-phenyl-2-imidazolidinone	16.58	81.7	45	Methanol
(4*R*,5*S*)-(−)-1,5-Dimethyl-4-phenyl-2-imidazolidinone	−16.43	−74.6	−42	Methanol
(*S*)-(+)-*N,S*-Dimethyl-*S*-phenylsulfoximine	37.92	187.9	140[24]	Methanol
(*R*)-(−)-*N,S*-Dimethyl-*S*-phenylsulfoximine	−37.63	−182.3	−140[24]	Methanol
(7*S*)-(−)-10,10-Dimethyl-5-thia-4-azatricyclo[5.2.1.0]-dec-3-ene-5,5-dioxide	−9.86	−46.4	−34	Chloroform
(7*R*)-(+)-10,10-Dimethyl-5-thia-4-azatricyclo[5.2.1.0]-dec-3-ene-5,5-dioxide	9.75	52.1	34	Chloroform
N-(3,5-Dinitrobenzoyl)-L-leucine	−3.28	−17.3	−14.3	Ethanol
(*R*)-(−)-*N*-(3,5-Dinitrobenzoyl)-α-methylbenzylamine	−7.95	−37.3	−46[18]	Acetone
(*S*)-(+)-*N*-(3,5-Dinitrobenzoyl)-α-methylbenzylamine	6.27	36.2	46.2	Acetone
(*R*)-(−)-*N*-(3,5-Dinitrobenzoyl)-α-phenylglycine	−18.86	−94.3	−98.1[19]	Tetrahydrofuran
(4*R*,5*R*)-(+)-*cis*-4,5-Diphenyl-2-oxazolidinone	17.69	88.2	56[22]	Chloroform
(4*S*,5*R*)-(−)-*cis*-4,5-Diphenyl-2-oxazolidinone	−17.09	−86.4	−56[22]	Chloroform
(+)-(*S*)-1-[(*R*)-2-(Diphenylphosphino)ferrocenyl]ethyl methyl ether	65.89	335.4	337	Chloroform
(−)-(*R*)-1-[(*R*)-2-(Diphenylphosphino)ferrocenyl]ethyl methyl ether	−64.78	−331.0	−337	Chloroform
(1*S*,2*R*)-(+)-Ephedrine hydrochloride	8.95	43.9	34.3[23]	Water
(*S*)-(+)-Epichlorohydrin	9.96	46.6	34	Methanol
(*R*)-(−)-Epichlorohydrin	−9.95	−47.2	−34	Methanol
Ethyl (*S*)-(−)-2-(*tert*-butyl-dimethylsilyloxy)propionate	−8.63	−41.0	−30	Chloroform
(1*R*)-(−)-Fenchone	−18.18	−79.4	−50.5[24]	Neat
β-D-(+)-Glucose	16.77§	82.1§	18.7–~52.7	Water
(*R*)-(+)-Glycidyl triryl ether	1.46	7.8	10.5	Chloroform
(*S*)-(+)-4,4*a*,5,6,7,8-Hexahydro-4*a*-methyl-2(3*H*)-naphthalenone	39.15	200.6	211.1	Ethanol
(2*S*,5*S*)-(+)-Hexanediol	8.57	40.4	34.5	Chloroform
L-Homoserine	−2.69§	−13.3§	−8.8[26]	Water
(*R,R*)-(+)-Hydrobenzoin	25.81	132.6	93[28]	Ethanol
(*S,S*)-(−)-Hydrobenzoin	−28.23	−141.8	−94[24]	Ethanol
(*R*)-(−)-2-Hydroxy-3,3-dimethyl-γ-butyrolactone	−5.70	−30.9	−51	Water
L−α-Hydroxyisovaleric acid	1.55	5.7	18	Chloroform
(*S*)-(+)-5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexane-1-one	9.07	46.6	41[19]	Ethanol
(*S*)-(−)-5-(Hydroxymethyl)-2(5*H*)-furanone	−44.68	−226.8	−144	Water
(*R*)-(−)-5-(Hydroxymethyl)-2-pyrrolidinone	−10.79	−50.6	−31	Ethanol
(*S*)-(+)-3-Hydroxy-3-methyl-4,4,4-trichlorobutyric β-lactone	1.57	6.8	6[26]	Ethanol
(*R*)-(−)-3-Hydroxy-3-methyl-4,4,4-trichlorobutyric β-lactone	−1.64	−7.4	−6[27]	Ethanol
(*R*)-(+)-4-Hydroxy-2-pyrrolidinone	15.62	77.8	43[23]	Ethanol
(*S*)-(−)-4-Hydroxy-2-pyrrolidinone	−14.82	−75.9	−43[23]	Ethanol
(*S*)-(+)-3-Hydroxytetrahydrofuran	5.40	31.1	17.5	Methanol
(*R*)-(−)-3-Hydroxytetrahydrofuran	−5.47	−32.6	−18	Methanol
(*R*)-(−)-1-Indanol	−7.74	−24.6	−29[20]	Chloroform
(*S*)-(+)-1-Indanol	7.63	27.8	30	Chloroform
(*S*)-(+)-4-Isobutyl-α-methylphenylacetic acid	25.94	132.4	59	Ethanol
(+)-Isopinocampheol	10.82	48.6	36.2	Ethanol
(−)-Isopinocampheol	−10.53	−47.4	−36	Ethanol
(+)-2,3-*O*-Isopropylidene-L-threitol	−7.44	−32.0	3.1[42]	Ethanol
(−)-2,3-*O*-Isopropylidene-D-threitol	7.43	31.2	−2.1[26]	Chloroform
(*S*)-(−)-4-Isopropyl-2-oxazolidinethione	−3.52	−16.5	−23	Chloroform
(4*R*)-(+)-4-Isopropyl-2-oxazolidinone	7.20	36.7	17	Ethanol
(4*S*)-(−)-4-Isopropyl-2-oxazolidinone	−7.32	−35.3	−18	Ethanol
(*R*)-(−)-3-Isopropyl-2,5-piperazinedione	−10.12	−49.6	−31.5	Water
(+)-Isopulegol	4.93	24.4	22	Neat
(−)-Isopulegol	−5.06	−25.3	−22	Neat

* All samples were measured in a methanol mobile phase (unless mentioned otherwise) at a concentration of 3 mg/mL, and with 1 µL of solution injected onto the reversed-phase HPLC column.
† All values of $[\alpha]_D^{20}$ were obtained from references 30 and 31.
‡ Relative responses of peak area of the chiral detector at 675 nm compared with L-valine.
§ Responses measured in water.

TABLE I: Continued

Compound	Relative Response‡	$[\alpha]^{22}_{675}$ (degrees)	Published $[\alpha]^{20}_{D}$ Value (degrees)	Solvent Used to Obtain Published Value
(+)-Limonene oxide	17.47	90.9	69^{22}	Neat
(*S*)-(+)-Mandelic acid	27.94	156.0	154	Water
(*R*)-(+)-Mandelonitrile	6.52	31.7	42	Chloroform
(+)-Menthol	13.76	68.8	49	Ethanol
(*R*)-(+)-4-(Methoxymethyl)-1,3-dioxolan-2-one	10.48	55.2	44	Neat
(*S*)-(−)-4-(Methoxymethyl)-1,3-dioxolan-2-one	−10.53	−51.2	−44	Neat
(4*S*,5*S*)-Methoxymethyl-2-methyl-5-phenyl-2-oxazoline	−35.17	−175.6	-113.2^{22}	Chloroform
(+)-6-Methoxy-α-methyl-2-naphthaleneacetic acid	14.39	71.9	66^{25}	Chloroform
(*S*)-(+)-α-Methoxyphenyl acetic acid	23.81	125.7	150^{17}	Ethanol
(*S*)-(+)-2-Methoxy-2-phenylethanol	35.03	178.0	133^{19}	Acetone
(*S*)-(−)-*N*-(α-Methylbenzyl)phthalic acid monoamide	−11.27	−59.5	-56^{20}_{546}	Ethanol
(*R*)-(+)-*N*-(α-Methylbenzyl)phthalic acid monoamide	11.47	57.0	56^{20}_{546}	Ethanol
Methyl (*R*)-(+)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidinecarboxylate	18.37	93.9	54	Chloroform
Methyl (*S*)-(−)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxazolidinecarboxylate	−18.52	−90.3	−55	Chloroform
[3a*R*-[2(3′a*R*,8′a*S*),3′a*b*,8′a*b*)]-(+)-2,2′-Methylenebis-[3a,8a-dihydro-8*H*-indenol[1,2-*d*]-oxazole]	60.49	295.6	353^{22}	Chloroform
[3a*S*-[2(3′a*R*,8′a*S*),3′aα,8′aα)]-(−)-2,2′-Methylenebis-[3a,8a-dihydro-8*H*-indenol[1,2-*d*]-oxazole]	−59.20	−264.1	-355^{22}	Chloroform
(*R*)-(+)-Methyl lactate	3.64	18.2	8.4^{21}	Neat
(*R*)-(−)-Methyl mandelate	−43.72	−200.2	−144	Methanol
(*S*)-(+)-Methyl mandelate	41.74	180.9	141.4	Methanol
(1*S*,2*R*)-(+)-*trans*-2-(1-Methyl-1-phenylethyl)cyclohexaneol	9.20	36.3	29^{24}	Methanol
(1*R*,2*S*)-(−)-*trans*-2-(1-Methyl-1-phenylethyl)cyclohexaneol	−8.28	−33.6	-29^{24}	Methanol
(4*R*,5*S*)-(+)-4-Methyl-5-phenyl-2-oxazolidinone	30.98	155.0	168^{18}	Chloroform
(*S*S,5*R*)-(−)-4-Methyl-5-phenyl-2-oxazolidinone	−31.30	−150.4	-168^{25}	Chloroform
(*R*)-(+)-Methyl-*p*-tolylsulfoxide	40.10	184.1	145	Acetone
(*S*)-(−)-Methyl-*p*-tolylsulfoxide	−40.60	−188.5	−145	Acetone
Mono-(1*S*)-(+)-menthyl phthalate	21.64	111.2	93	Chloroform
(−)-Noe-lactol dimer	−49.22	−266.5	−200	Tetrahydrofuran
(1*R*,5*SS*)-(+)-2-Oxabicyclo[3.3.0]oct-6-en-3-one	29.99	148.6	102.5	Methanol
(2*S*,4*S*)-(+)-Pentanediol	18.64	83.9	39.8	Chloroform
(*R*)-(+)-Perillyl alcohol	15.14	75.7	109	Neat
(*S*)-(−)-Perillyl alcohol	−15.57	−77.8	-88^{22}	Methanol
(*S*)-(−)-*sec*-Phenethyl alcohol	−12.67	−63.4	-41.3^{23}	Neat
L-Phenylalanine	−5.08	−27.9	−33.7	Water
(*R*)-(+)-1-Phenyl-1-butanol	8.19	54.4	55	Chloroform
(*S*)-(−)-1-Phenyl-1-butanol	−8.15	−59.3	-48.6^{21}	Chloroform
(*R*)-(−)-2-Phenylbutyric acid	−23.73	−120.5	−93	Toluene
(*S*)-(+)-2-Phenylbutyric acid	23.43	125.1	92^{19}	Toluene
(1*S*,2*R*)-(+)-*trans*-2-Phenyl-1-cyclohexanol	14.33	71.6	58	Methanol
(*R*)-(+)-4-Phenyl-1,3-dioxane	9.51	49.0	54.5	Neat
(*S*)-(−)-4-Phenyl-1,3-dioxane	−10.69	−53.1	−54.5	Neat
(*R*)-(−)-1-Phenyl-1,2-ethanediol	−15.01	−61.9	−69	Chloroform
(*S*)-(+)-1-Phenyl-1,2-ethanediol	14.70	67.7	69	Chloroform
(*S*)-(−)-*N*-(1-Phenylethyl)maleimide	−21.02	−79.3	-61^{24}	Ethanol
(2*R*,3*R*)-(+)-3-Phenylglycidol	16.95	79.2	49	Chloroform
(2*S*,3*S*)-(−)-3-Phenylglycidol	−16.55	−73.9	−49	Chloroform
D-3-Phenyllactic acid	6.65	31.6	19	Ethanol
L-3-Phenyllactic acid	−5.87	−28.1	-20.8^{24}	Water
(*R*)-(−)-4-Phenyl-2-oxazolidinone	−16.43	−80.6	-48^{25}	Chloroform
(*S*)-(+)-4-Phenyl-2-oxazolidinone	16.56	79.7	48	Chloroform
(1*R*)-(+)-*cis*-Pinane	5.06	25.2	24	Neat
(1*S*)-(−)-*cis*-Pinane	−5.13	−24.1	−24	Neat
(1*R*)-(+)-*trans*-Pinane	2.74	14.4	17	Neat
(1*S*)-(−)-*trans*-Pinane	−2.75	−12.3	−17	Neat
(1*R*,2*R*,3*S*,5*R*)-(−)-Pinanediol	25.54	120.3	-8.6^{21}	Toluene
(1*S*,2*S*,3*R*,5*S*)-(+)-Pinanediol	−25.26	−123.6	8.5	Toluene
(1*S*)-(−)-β-Pinene	−3.29	−16.5	−21	Neat
D-Pipecolic acid	8.27§	38§	27^{25}	Water
L-Pipecolic acid	−7.26§	−35.6§	-26.4^{25}	Water
L-Proline	−22.33	−106.8	−84	Water
(*R*)-(+)-Propylene carbonate	15.42	74.5	2	Neat
(*S*)-(−)-Propylene carbonate	−15.15	−71.8	−2	Neat
(*S*)-(−)-Pulegone	−4.80	−23.7	−22	Neat
L-Pyroglutamic acid	1.59	8.1	−10	Water
(+)-Sabinene	26.34	131.3	107	Neat
D-Serine	2.98	12.9	6.83	Water
(*R*)-(+)-Styrene oxide	1.84	9.0	33^{18}	Neat
[(−)-2-(2,4,5,7-Tetranitro-9-fluorenylideneaminooxy)-propionic acid]	−12.67	−63.4	−91	Dioxane
(*S*)-(+)-Tetrahydrofurfurylamine	2.04	10.1	12	Chloroform
(*R*)-(−)-1,2,3,4-Tetrahydro-1-naphthol	−10.64	−44.8	-32^{17}	Chloroform
Tetrakis[1-[(4-*tert*-butylphenyl)-sulfonyl]-(2*S*)-pyrrolidinecarboxylate]dirhodium(II)	−42.44	−221.7	−187	Chloroform
D-Threitol	−3.48	−19.9	−14	Ethanol
L-Threonine	−8.51§	−38.3§	−27.4	Water
D-Threonine	8.58§	40.6§	27	Water
(−)-3-(Trifluoroacetyl)-camphor	−12.33	−94.7	-148^{19}	Methylene chloride
(*S*)-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol	8.84	40.3	29^{25}	Chloroform
(*R*)-(−)-2,2,2-Trifluoro-1-(9-anthryl)ethanol	−9.28	−44.9	-30^{25}	Chloroform
(*R*)-(−)-γ-Trityloxymethyl-γ-butyrolactone	−9.47	−40.1	−26	Chloroform
L-Tryptophan	9.12§	45.2§	2.4	0.5 N Hydrochloric acid
D-(+)-Turanose	21.87§	109.6§	75	Water
L-Valine	1.00§	4.9§	27.5	6 N Hydrochloric acid

* All samples were measured in a methanol mobile phase (unless mentioned otherwise) at a concentration of 3 mg/mL, and with 1 µL of solution injected onto the reversed-phase HPLC column.
† All values of $[\alpha]^{20}_{D}$ were obtained from references 30 and 31.
‡ Relative responses of peak area of the chiral detector at 675 nm compared with L-valine.
§ Responses measured in water

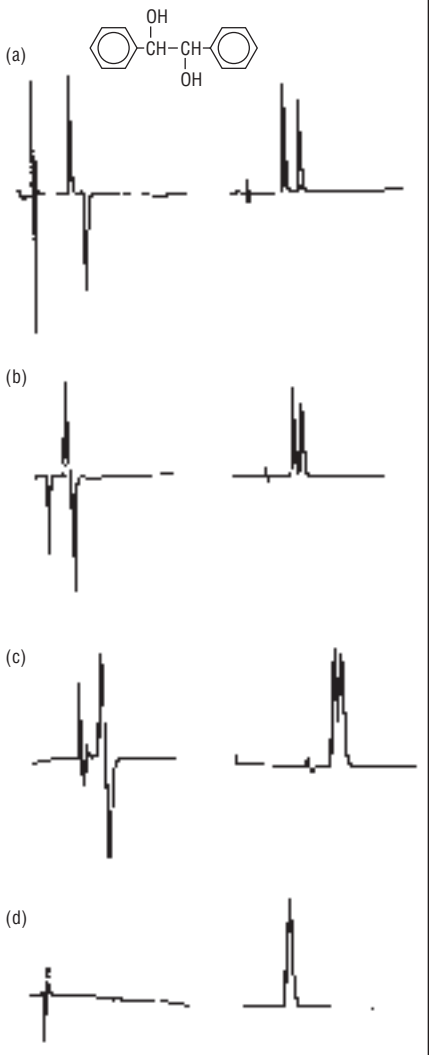

FIGURE 3: Chiral separation of hydrobenzoin enantiomers using optical rotation (left) and UV (right) detection. Column: (a–c) 25 cm × 4.6 mm Cyclobond I 2000 RSP, (d) 5 cm × 4.6 mm C18; mobile phase: (a) 10:90 (v/v) methanol–1% TEAA (pH 4.1), (b) 30:70 (v/v) methanol–1% TEAA (pH 4.1), (c) 50:50 (v/v) methanol–1% TEAA (pH 4.1), (d) methanol; flow rate: 1 mL/min; UV detection wavelength: 254 nm. Resolution: (a) 1.9, (b) 1.4, (c) 0.6, (d) no separation.

RESULTS AND DISCUSSION

As a starting point, we chromatographed more than 230 chiral compounds under identical conditions — or as close to identical as possible given each compound's solubility in the mobile phase. Instead of providing ideal separation conditions for this great variety of compounds, the purpose of chromatography in this exercise was providing fairly rapidly eluted peaks (that is, $0 < k < 1.5$) that could be compared directly with others in terms of sensitivity and could be used to calculate the specific rotation of each compound (see Experimental). Table I lists these results. The first column gives the relative response of each compound relative to

TABLE II: The Change in Sign with Solvent Composition

			Signal in Different Solvents*				
Compounds	$[\alpha]_D^{20}$ (°)†	**Solvent**	**Toluene**	**Chloroform**	**Methanol**	**Acetone**	**Tetrahydrofuran**
(*R*)-(−)-Benzyloxy-3-(*p*-tosyloxy)-2-propanol	−7	Toluene	−	−	−	−	−
(*S*)-(+)-Benzyloxy-3-(*p*-tosyloxy)-2-propanol	+7	Toluene	+	+	+	+	+
(*S*)-(+)-3-Hydroxy-3-methyl-4,4,4-trichlorobutyric β-lactone	$+6.0^{26}$	Ethanol	−	+	+	+	+
(*R*)-(−)-3-Hydroxy-3-methyl-4,4,4-trichlorobutyric β-lactone	-6.0^{27}	Ethanol	+	−	−	−	−
(+)-2,3-*O*-Isopropylidene-L-threitol	$+3.1^{24}$	Ethanol	+	−	−	+	+
(−)-2,3-*O*-Isopropylidene-D-threitol	-2.1^{26}	Chloroform	−	+	+	−	−
(1*R*,2*R*,3*S*,5*R*)-(−)-Pinanediol	-8.6^{21}	Toluene	−	−	+	N/D	−
(1*S*,2*S*,3*R*,5*S*)-(+)-Pinanediol	+8.5	Toluene	+	+	−	N/D	+
(*R*)-(+)-Propylene carbonate	+2	Neat	+	−	+	N/D	+
(*S*)-(−)-Propylene carbonate	−2	Neat	−	+	−	N/D	−
(7*S*)-(−)-10,10-Dimethyl-5-thia-4-azatricyclo[5.2.1.0] dec-3-ene-5,5-dioxide	−34	Chloroform	−	−	−	−	−
(7*R*)-(+)-10,10-Dimethyl-5-thia-4-azatricyclo[5.2.1.0] dec-3-ene-5,5-dioxide	+34	Chloroform	+	+	+	+	+
(1*R*)-(−)-2-Azabicyclo[2.2.1]hept-5-en-3-one	−565	Chloroform	−	−	−	−	−
(1*S*)-(+)-2-Azabicyclo[2.2.1]hept-5-en-3-one	+565	Chloroform	+	+	+	+	+

* This notation refers to the direction of rotation of plane polarized light at 675 nm, levorotatory = (−), and dextrorotatory = (+).
† All values of $[\alpha]_D^{20}$ were obtained from references 30 and 31.

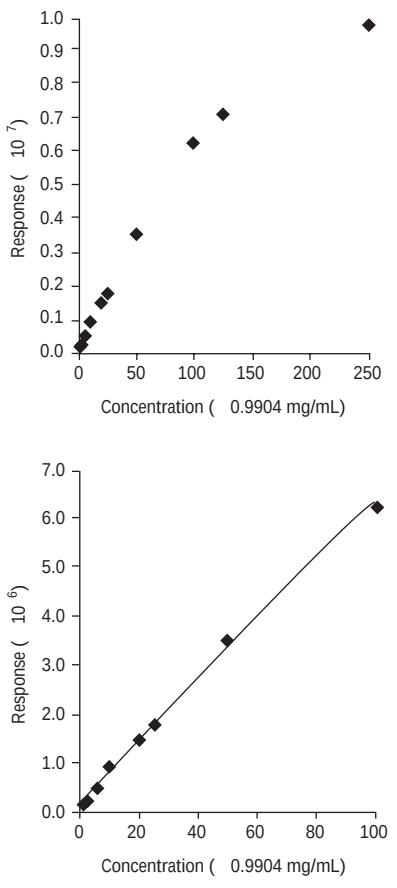

FIGURE 4: Plots of detector response versus concentration for (4*R*)-(+)-4-isopropyl-2-oxazolidinone. $[\alpha]_D = +17°$, $[\alpha]_D^{22} = +36.7°$, $R^2 = 0.997$.

L-valine, which was assigned the normalized value of +1. The second column lists the calculated specific rotations for each compound at 675 nm and 22 °C. The third and fourth columns provide published values of the specific rotation at the sodium D line and the solvent in which they were measured.

The specific rotation of a chiral compound changes with the wavelength of light used and other environmental conditions. Optical rotatory dispersion spectra essentially are plots of specific rotation versus wavelength, as shown in Figure 1 (33). However, most of the literature about chiral compounds discusses using 589.3 nm (the sodium D line) when giving specific rotations. Modern laser-based micropolarimeters use different wavelengths (λ), which are dictated by the nature of the laser light source used.

Generally, analysts would expect no relationship between the direction of rotation of plane polarized light at 589.3 and 675 nm. As Table I shows, however, more than 98% of the examined compounds had the same direction, although different magnitude, of rotation at 589.3 and 675 nm. The main reason for this correlation is that none of the compounds examined had chromophores in the visible region of the spectrum, although most had absorbance in the UV region.

As Figure 1 shows, the optical rotatory dispersion spectra of compounds far from any absorption bands (that is, the portion in the visible region) tend to be flat and featureless, nearly parallel to the line of zero specific rotation. In fact, theory indicates that rotation changes slowly with wavelength at wavelengths far from an absorption band (29). Hence if the specific rotation of a compound is measured under identical conditions at two wavelengths that are not too distant from one another but are far from an adsorption band, there is a high probability that they will have the same sign of rotation (Table I).

Two pairs of enantiomers in Table I did not rotate plane polarized light in the same direction at both 589.3 and 675 nm. They were the enantiomers of 2,3-*O*-isopropylidenethreitol and pinanediol. In both of these cases, the optical rotation at 589.3 nm was quite small. Indeed, the smaller the optical rotation at either of the wavelengths being compared, the more likely is the occurrence of a discrepancy in their direction of rotation. In addition, compounds with small optical rotations are more likely to change their sign with environmental changes such as solvent, pH, and temperature changes.

Table II shows the solvent effect on the direction of plane polarized light for several compounds. Those compounds with small rotations are most likely to show changes in sign with different solvent types. This result, of course, is eminently logical. If the absolute value of a compound's change in rotation is less than the absolute value of its specific rotation, then its sign or direction of rotation must always be the same. Conversely, if the absolute value of a compound's change in rotation is greater than the absolute value of its specific rotation, then it may or may not show the opposite sign depending on the direction of change.

Sensitivity is one of the most important factors when considering a chiroptical detector. Obviously, the magnitude of a chiral molecule's specific rotation at the wavelength of detection will affect its detectability. Figure 2 shows the limit of detection of seven compounds versus their specific rotation at 675 nm. Also included is the analogous plot that uses

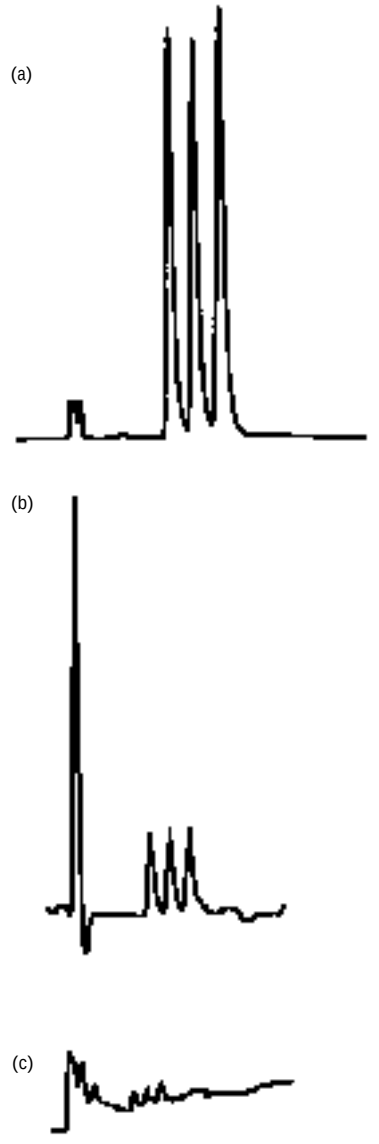

FIGURE 5: Separation and detection of α-, β-, and γ-cyclodextrins using (a) the chiral detector, (b) a refractive index detector, and (c) a UV-absorbance detector at 190 nm. Column: 25 cm × 4.6 mm Cyclobond I 2000; mobile phase: 75:25 (v/v) acetonitrile–water; flow rate: 1 mL/min.

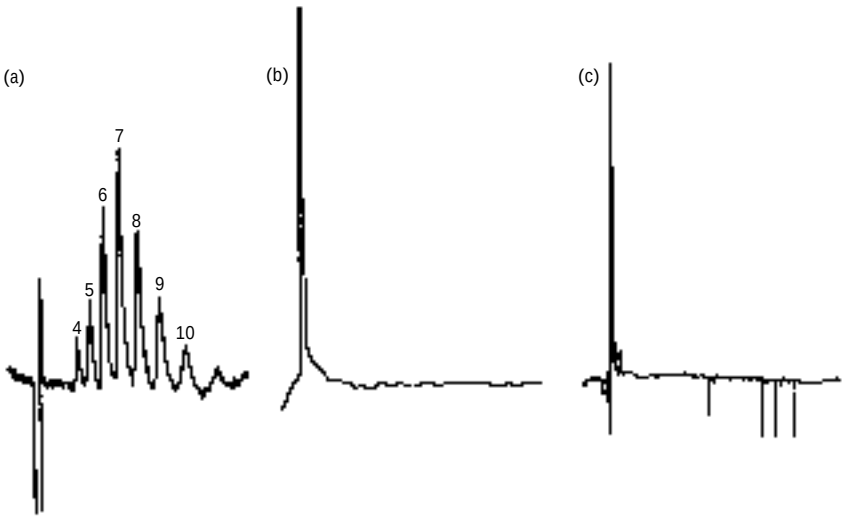

FIGURE 6: Separation and detection of a mixture of malto-oligosaccharides using (a) the chiral detector, (b) a refractive index detector, and (c) a UV-absorbance detector at 190 nm. Column: 25 cm × 4.6 mm Cyclobond I 2000; mobile phase: 70:30 (v/v) acetonitrile–water; flow rate: 1 mL/min. Peak labels correspond to degree of polymerization.

each compound's specific rotation at the sodium D line. The plot versus $[\alpha]_{675}$ shows the expected correlation of smaller limit of detection with higher specific rotation. The most sensitive determination was for (*S*)-(−)-binaphthyl-2,2′-diylhydrogen phosphate, which provided a $[\alpha]_{675}$ value of 635°. We were able to detect approximately 50 ng of this compound. Compounds 1 through 7 in Figure 2 span the range of specific rotations found for most compounds of pharmaceutical interest, as well as many chiral synthons, auxiliaries, and catalysts (34,35). The curve for $[\alpha]_D^{20}$ (Figure 2) did not correlate as well; indeed, we would not expect them to because the limits of detection were calculated using a different wavelength of light. However, we observed the same general trend. Because the specific rotation of most compounds still is reported at the sodium D line and not at other wavelengths, analysts may be able to estimate an approximate limit of detection in some cases.

Figure 3 shows several chromatograms that compare the response from the chiral detector (left) with a standard UV detector (right) for enantiomers of hydrobenzoin at various degrees of resolution. When peaks overlap, the chromatogram developed using any chiroptical detector cannot accurately calculate resolution (R_s), selectivity, or quantitate the enantiomers. As Zukowski, Tang, Berthod, and Armstrong (22) showed, analysts must use an in-line UV detector or other conventional detectors to determine these parameters correctly (22).

The linear dynamic range of the chiral detector covers approximately 2.5 orders of magnitude, although it varies with the specific rotation of each analyte. Figure 4 shows the plot for a typical case of (4*R*)-(+)-4-isopropyl-2-oxazolidinone. The detector response is no longer linear when the amount of analytes injected exceeds approximately 100 μg (Figure 4).

The chiral detector is not as sensitive for chiral aromatic molecule analysis as are typical UV HPLC detectors. However, this detector tends to be much more sensitive than UV detectors, even when used at low wavelengths, or differential refractometer detectors for various chiral nonaromatic compounds such as carbohydrates and some amino acids (6). Figures 5 and 6 clearly show these results for a chromatographic separation of different cyclodextrins and a series of linear oligosaccharides.

It has been stated in numerous publications that chiroptical detectors coupled with conventional detectors can be used to determine enantiomeric purity without having to perform a chromatographic separation of enantiomers (7,9,10,12,22,23). Although this statement may be true for mixtures of moderate enantiomeric excess, this approach should be avoided in situations involving a large excess of one antipode. Zukowski, Tang, Berthod, and Armstrong (22) demonstrated conclusively that the error for this determination is enormous for mixtures of high or low enantiomeric excess. The error in the coupled detector method is dependent on matching the sensitivity and linear range of the two detectors, as well as their precision and accuracy (22). Because the chiroptical detector always had greater limitations in these areas, it generally imposes the greatest error on this approach (22).

Using enantiomerically pure standards, analysts can construct a calibration curve of the ratio of chiroptical detector peak area to UV detector peak area versus the enantiomeric composition, as shown in Figure 7. The composition of unknown mixtures then can be determined using the standard curve. The chiral

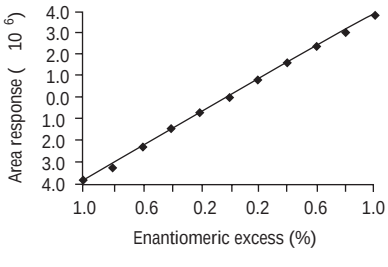

FIGURE 7: Calibration plot of peak area response versus enantiomeric excess for (*S*)-(+)-4-benzyl-3-propionyl-2-oxazolidinone (R^2 = 0.999).

detector can be used effectively in this manner if the standards are available (Figure 7). However no chiroptical detector should be used for mixtures of enantiomeric excess greater than 90% because of the escalating error for determining mixtures in that range (22). The most efficient and accurate way to determine enantiomeric excesses is by chromatography or capillary electrophoresis in some cases.

CONCLUSION

Chiroptical detectors can be exceedingly useful for the detection and analysis of various chiral molecules. They also provide a rapid, efficient way to validate enantiomeric separations. Traditionally, the shortcomings of these detectors has been their lack of sensitivity and robustness. Some early detectors also were far too large and complex to be practical HPLC detectors. The chiral detector we used in our study was small, compact, and simple to use.

We were able to couple it with most HPLC systems. Thus far, it is the most stable and sensitive of the chiroptical detectors that we have used. We could detect HPLC enantioseparations at analyte levels that do not overload most chiral stationary phases (this finding was not true of most earlier commercial detectors). However, this detector is still not as sensitive as a typical UV detector for most aromatic compounds.

One aspect of this detector that may prove useful is the unexpected high correlation between the magnitude and direction the specific rotations of molecules at 675 nm and at the sodium D line. This ability allows analysts to estimate a compound's sign of rotation and possibly detector sensitivity from literature values and vice versa. Clearly, chiroptical detector technology is rapidly approaching the point at which the detectors will be useful and perhaps routine devices in laboratories and manufacturing plants in which chiral molecules are prevalent and important compounds.

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