ORGANIC LETTERS

2001
Vol. 3, No. 1
95−98

Improved Method for Rapid Evaluation of Chiral Stationary Phase Libraries†

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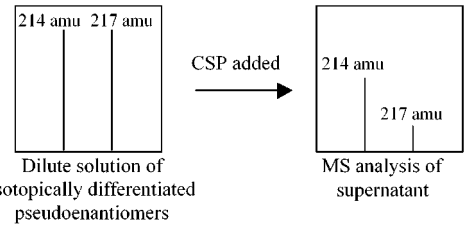
Received November 1, 2000

ABSTRACT



An improved method for rapid LC/MS screening of chiral stationary phases based on the use of isotopically labeled enantiomers is reported.

Chromatography using chiral stationary phases (CSPs) is increasingly used for the large scale separation of enantiomers.[1] While often expensive relative to other approaches, chromatographic enantioseparation can be viable, especially when the enantioselectivity and capacity of the CSP are large. While rational design has long played an important role in CSP development,[2] the use of combinatorial exploration strategies has recently become widespread in this field.[3] Many of the initial efforts at high throughput screening of CSP libraries employed a tethered version of the analyte for evaluation of libraries of candidate selectors. Such indirect approaches, while sometimes effective, are oftentimes problematic owing to the existence of "tether effects". In contrast, methods that directly probe the CSP−analyte interaction give information that can unambiguously be extrapolated to chromatographic performance. We recently described a direct method of CSP library evaluation that has the benefit of simplicity and ease but the drawback of not being generally useful for most analyte molecules.[3d] We now report an improved method based on the use of isotopically labeled enantiomers that is more rapid, more sensitive, and potentially applicable to a wider variety of analyte molecules.

In this approach, a small amount of CSP (typically 50 mg) is placed into a vial, and a dilute racemic solution of the analyte is then added (typically 500 μL of 10^{-5} to 10^{-6} M solution). The concentration of the analyte solution must be low enough to avoid saturation of the available sites on the CSP. Upon equilibration, selective adsorption of one enantiomer by the CSP leaves an excess of the opposite enantiomer in the supernatant solution. Thus, analysis of enantiomeric excess in the supernatant solution provides a rapid gauge of the enantioselectivity of the CSP in each tube.

The requirement of using a very low concentration of the racemate in the screening process makes measurement of enantioenrichment in the supernatant solution somewhat

† Dedicated to Professor Koji Nakanishi on the occasion of his 70th birthday.

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10.1021/ol0003329 CCC: $20.00
Published on Web 12/14/2000

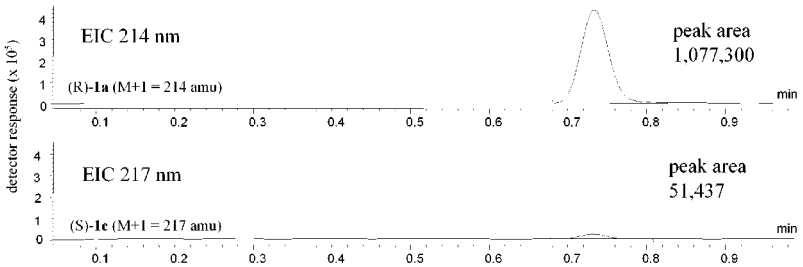

Figure 1. Measuring enantioenrichment of a 95:5 mixture of isotopically differentiated enantiomers. The peak area ratios of the extracted ion chromatograms for the two different M + 1 ions indicate an enantiomeric excess of 91% ee. Conditions: Extend C18 (4.6 × 50 mm); 3.5 μ; 1:1 acetonitrile/water (2 mM in ammonium formate and formic acid, pH 3.5); 1.5 mL/min; 30 °C; positive ion mode; Frag = 40 V; Vcap = 2000; selected ion monitoring at 164, 165, 167, 214, 215, and 217 amu.

challenging. Very few compounds have enough "signal" to allow direct measurement of enantioenrichment using polarimetry, CD, or related chiroptical spectroscopy techniques, although this method has been successful in some instances.[4] Chiral HPLC with UV detection is suitable for many compounds with good chromophores, and some compounds with poor chromophores can be analyzed following chemical derivatization or concentration. We found that the use of chiral HPLC/MS greatly broadens the range of compounds that can be evaluated at micromolar concentration, although the requirement of developing a rapid chiral separation assay using an eluent that promotes MS ionization places some additional constraints on the generality of the approach.

A much improved approach to the problem of rapidly evaluating the enantioenrichment of dilute solutions is to use in the screening process a "pseudoracemate" made up of a pair of isotopically differentiated pseudoenantiomers. The enantioenrichment of each of the resulting solutions can then be rapidly estimated by comparing the MS abundance of the ions corresponding to the two enantiomers. The use of isotopically labeled enantiomers for the study enantioselective processes has a long history.[5] Reetz and co-workers have recently used this approach for the high throughput screening of kinetic resolution catalysts,[6] and Sawada and co-workers have reported the use of isotopically labeled enantiomers to evaluate enantioselective crown ether hosts by direct FAB-MS evaluation of the diastereomeric complexes.[7] We have found that the use of isotopically labeled enantiomers is well suited for the improved rapid microscale screening of CSP libraries, although it does, of course, require the preparation of the labeled enantiomers. Isotopic substitution is perhaps the least obtrusive method of differentially labeling two enantiomers. The presence of a label on only one of the enantiomers means that some difference in enantioselective adsorption owing to the presence of the label is theoretically possible. In practice, the chromatographic separation of enantiomers that are chiral by virtue of isotopic substitution is a supremely challenging feat,[8] suggesting that the presence of an isotopic label will have little influence on the approximate estimation of enantioenrichment in this application.

Acetamide **1a** was prepared by acylation of the commercially available (*R*)-1-(1-naphthyl)ethylamine with acetic

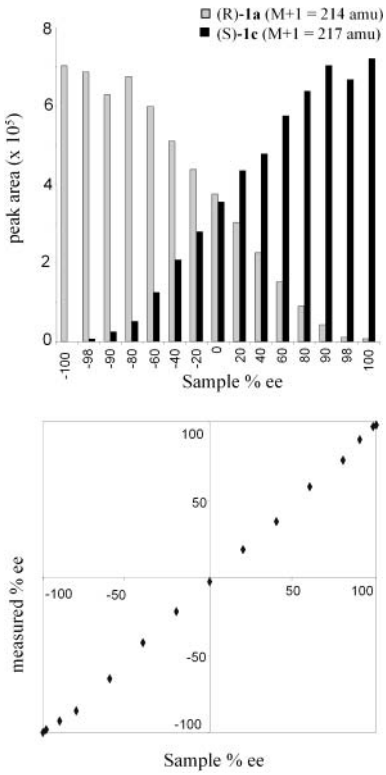

Figure 2. LC/MS determinations of enantiomeric enrichment of mixtures of **1a** and **1c** using ratios of peak areas in the extracted ion chromatograms at 214 and 217 amu.

anhydride. The isotopically labeled compounds **1b** and **1c** were prepared by acylation of (*S*)-1-(1-naphthyl)ethylamine with acetic-2-^{13}C anhydride or hexadeuterioacetic anhydride, respectively. The phenyl ethylacetamides **2a**−**c** were prepared in an analogous fashion.

Mixing stock solutions of the isotopically labeled enantiomers affords samples of known concentration and enantioenrichment. Analysis by LC/MS with selected ion monitoring for the component pseudoenantiomers affords a measure of enantioenrichment that agrees well with expected values (Figures 1 and 2).

The situation is somewhat complicated when the two pseudoenantiomers differ by only a single mass unit. In the

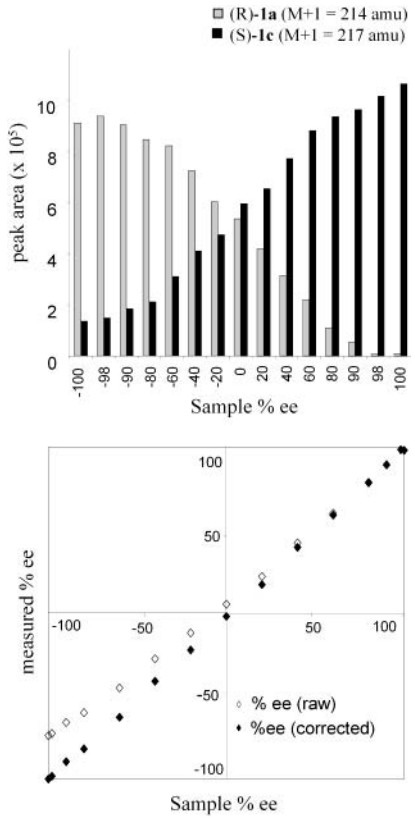

Figure 3. LC/MS determinations of enantiomeric enrichment of mixtures of **1a** and **1b** using ratios of peak areas in the extracted ion chromatograms at 214 and 215 amu. The analysis is complicated by the presence of natural abundance 215 amu signal in **1a** (about 15%).

example illustrated in Figure 3, the natural abundance ^{13}C isotopic content of (*R*)-**1a** gives rise to significant signal at 215 amu (about 15% of the signal at 214 amu). Correction for this contribution allows the actual enantiomeric excess to be determined surprisingly well, certainly well enough to perform preliminary screenings of CSP libraries. The ability to use pseudoenantiomers that differ by only a single mass unit makes this technique much more general, since synthesis of the requisite isotopically differentiated enantiomers can be as simple as incorporation of a single deuterium or ^{13}C.

An interesting and potentially useful aspect of this approach is the fact that several pseudoracemates can be evaluated simultaneously. This approach, related to Kagan's method for simultaneously screening several different substrates with a single asymmetric catalyst,[9] has been shown to work with chiral HPLC analysis of CSP libraries by Roussel and co-workers.[10] A drawback of the chiral HPLC approach is that it requires a chromatographic method where there is no peak overlap. In contrast, the simultaneous evaluation of several different isotopically labeled pseudoenantiomer mixtures by LC/MS seems particularly well

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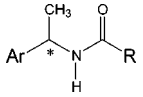
Table 1. Isotopically Labeled Enantiomers Used in the Study



compd	Ar	*abs config	R	M + H (amu)
1a	1-naphthyl	*R*	CH_3	214
1b	1-naphthyl	*S*	$^{13}CH_3$	215
1c	1-naphthyl	*S*	CD_3	217
2a	phenyl	*R*	CH_3	164
2b	phenyl	*S*	$^{13}CH_3$	165
2c	phenyl	*S*	CD_3	167

Table 2. HPLC Separation of (*R*)-**1a** and (*S*)-**1c** Using Columns Containing CSPs Used in the Screening Study[a]

CSP	*k*1	*k*2	α	retained[b]
Chiralpak AD	0.9	0.9	1.0	
Chiralcel OD	1.7	9.4	5.6	*S*
Chiralpak AS	2.8	3.2	1.2	*R*
Chiralcel OJ	1.5	1.5	1.0	
(*S*,*S*) Whelko	9.1	105.8	11.7	*S*
TBB	0.9	0.9	1.0	
Chiris AD1	13.8	22.3	1.6	*S*
Chirobiotic V	14.7	17.4	1.2	*S*
Chirobiotic T	7.5	7.8	1.0	
Cyclobond I	4.9	4.9	1.0	

[a] Conditions: 10% IPA/hexane, 1.5 mL/min; DAD UV at 210 and 254 nm. [b] Indicates more retained enantiomer.

suited for this type of study, since one must only avoid analytes with overlapping masses that are not chromatographically resolved.

Armed with the ability to measure enantioenrichment in dilute solutions by LC/MS, we turned our attention to using this method for evaluating CSP performance. In a preliminary experiment, 50 mg of (*S*,*S*) Whelko CSP was placed into autosampler vials and a 10^{-6} M pseudoracemate solution (**1a** + **1c** mixture) in 10% IPA/hexane was added to each vial. A timecourse evaluation showed that the system reaches equilibrium after only a few minutes of shaking, with an enantiomeric excess of about 70% being observed in the supernatant solution.

We next turned our attention to the screening of libraries of commercially available preparative CSPs. Library screening was carried out as described above using a 10^{-6} M solution of the **1a** + **1c** pseudoracemate in 10% IPA/hexane and allowing 30 min of shaking before measuring the enantioenrichment in the supernatant solution. Several CSPs in the study showed so little adsorption of the pseudoracemate that estimation of the apparent enantioselectivity of the CSP was difficult. Therefore, a second screening experiment was performed with a pseudoracemate solution in 2% IPA/hexane. Increased adsorption was observed in all cases, and significant apparent enantioselectivity was observed with the Whelko, Chiralcel OD, and Chiris-AD1 CSPs (Figure 4).

HPLC separation of a mixture of **1a** and **1c** using columns corresponding to the CSPs used in the study afforded the results presented in Table 2. These results agree well with

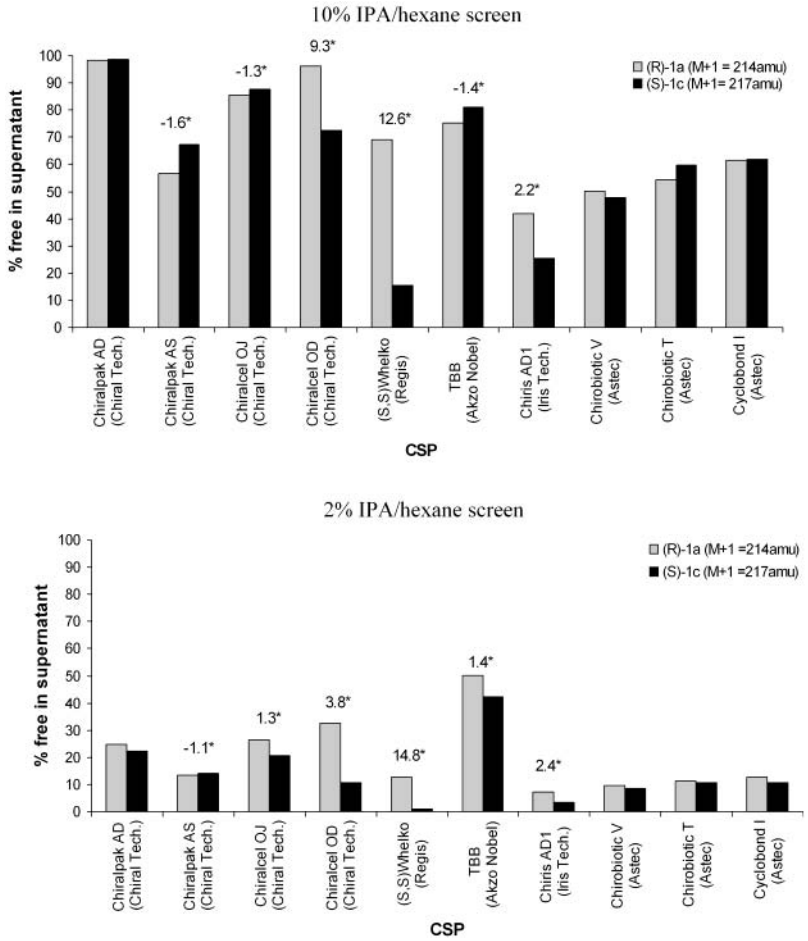


Figure 4. Results of screening a library of commercially available preparative CSPs. A 50 mg portion of each CSP is placed in a 2 mL vial, 1 mL of a 10^{-6} M solution of **1a** + **1c** is added, and the mixture is shaken for 30 min. After settling, a 100 μL aliquot of the supernatant solution is withdrawn, diluted with 200 uL of acetonitrile, and analyzed by LC/MS using selected ion monitoring at 214 and 217 amu. *α_{app} = the apparent enantioselectivity; if X_R and X_S represent the percentage of the (*R*) and (*S*) enantiomers free in the supernatant solution, the apparent enantioselectivity of the CSP is given by the expression

$$\alpha_{app} = \frac{\dfrac{1 - X_S}{X_S}}{\dfrac{1 - X_R}{X_R}}$$

Care should be exercised in using the apparent enantioselectivity values in cases where the enantiomers are either very strongly or very weakly adsorbed by the CSP.

the predicted enantioselctivity for the CSPs, with the Whelko being best, followed by the Chiralcel-OD and then the Chiris-AD1.

These data clearly show the utility of isotopically labeled pseudoenantiomers for the evaluation of microscale CSP libraries. The potential for ultrafast screening using an approach of this general type is apparent. While the library used in the current demonstration is of limited size, the technique will clearly be useful for rapid analysis of larger CSP libraries.

Acknowledgment. We would like to thank Koji Nakanishi, Yasuhiro Itagaki, Nina Berova, David Weingarten, Ernest Eliel, and Ed Roberts for valuable discussions.

Supporting Information Available: Figure showing simultaneous determination of enrichment of two differentiated pseudoenantiomers. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0003329