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Resolution of Enantiomeric Hydrocarbon Biomarkers of Geochemical Importance

Daniel W. Armstrong,* Yubing Tang, and Janusz Zukowski

Department of Chemistry, University of Missouri—Rolla, Rolla, Missouri 65401

Chiral isoprenoid and hydroaromatic compounds occur in all crude oils, coals, shales, and most sediments. Many of these compounds are referred to as biological markers since they are thought to be derived from biological sources and their presence, relative concentrations, or stereochemistry can provide information as to a geological deposit's age, maturity, diagenetic history, and so forth. Because of the previous lack of effective and efficient analytical methodologies for resolving hydrocarbon enantiomers, the stereochemical information encoded in these molecules is largely untouched. A series of derivatized α-, β-, and γ-cyclodextrin chiral stationary phases (CSPs) were used for the gas chromatographic resolution of several racemic tetralins, indans, and octahydrophenanthrenes as well as cyclic and acyclic isoprenoids. The importance of these separations to the geochemical sciences is considered.

INTRODUCTION

A variety of hydrocarbons exist in living organisms and particularly in geological deposits of biological origin. Organic extracts of geological deposits (e.g., oil, coal, and sediments) are complex mixtures containing hundreds of hydrocarbons. Some of these compounds have structures that can be related to functionalized precursors that occur in the source material (e.g., plants, fungi, bacteria, and algae). As the organic material was buried, biological, chemical, and physical processes occurred over time (diagenesis) which led to the removal of functional groups and the formation of hydrocarbons, known as biological markers or biomarkers. Biological markers in sediments, petroleum oils, coals, etc., have been the subject of considerable study since the term was first proposed by Eglinton and Calvin in 1967 (*1*). The detection of these components and their relationship to various biological precursors may provide information on paleoenvironmental conditions as well as the source, maturity, and migration of hydrocarbon resources such as oil (*2, 3*). Some of the early work by Ackman, Maxwell, and their co-workers, using diastereomeric ester separations, was instrumental in establishing biomarker stereochemistry as a means to investigate geological samples (*4–6*). Isoprenoids, tricyclic terpenes, pentacyclic terpenes, and hydroaromatics are among the most significant groups of biomarkers. Many of them can exist in two or more structural isomeric forms, and moreover, they can form pairs of enantiomeric compounds. It is well-known that enantiomers have the same physicochemical properties in an isotropic environment and cannot be separated by ordinary (achiral) means. Because of their lack of functionality, hydrocarbon enantiomers may be among the most difficult isomers to resolve by any means. The separation and identification of hydrocarbons in plant extracts and other natural and synthetic sources relies heavily on gas chromatography (GC). Even where combined gas chromatography/mass spectrometry (GC/MS) is used for analysis, isomeric assignments often cannot be made on the basis of mass spectrometric data (*7*). Many isomeric hydrocarbons, inclusive of enantiomers, have essentially identical mass spectra (*8*). Currently, a structure or stereochemically sensitive separation technique is required. In the case of volatile enantiomers, the use of a GC chiral stationary phase (CSP) may be the method of choice for the identification of these mixtures.

Several chiral stationary phases have been reported to separate enantiomers, but only CSPs derived from cyclodextrins (CDs) currently are capable of resolving chiral compounds possessing no functional groups, which is often the case with hydrocarbons (*9–11*). There have been several attempts to use cyclodextrins in gas chromatography. Smolkova-Keulemansova et al. used native α- and β-CDs in packed-column GC (*12, 13*). Schurig et al. used methylated CDs dissolved in typical achiral coatings for GC (*10, 11*). Koenig et al. found that pentylated CDs were liquid at room temperature and could be coated on capillary columns (*14, 15*). Armstrong and co-workers found that CD derivatives were "liquified" if they were mixtures of homologues and isomers, contained substituents of moderate steric bulk, and had most polar functional groups "capped" with nonpolar moieties (*16*). Also, it was shown that inclusion complexation was not always necessary for GC enantiomeric separations (*17*). A number of derivatized-CD stationary phases have now been developed for capillary GC (*14–19*). Well over three hundred racemic compounds were resolved with these CSPs in capillary GC. Another useful feature of the derivatized cyclodextrin GC stationary phases is that reversal of enantiomeric elution order has been observed for different derivatives of the same cyclodextrin as well as for the same derivative of different size cyclodextrins (*19*). This phenomenon is particularly useful when enantiomeric purities for samples that contain large excesses of one isomer are determined, as well as for mechanistic studies.

In this work we evaluate the ability of derivatized-cyclodextrin GC chiral stationary phases to directly resolve hydrocarbon enantiomers which are biological markers. To our knowledge these are the first successful direct separations of any of the enantiomeric hydrocarbon biomarkers. Most of the past studies involving the stereochemistry of biomarkers involved diastereomeric separations (*2–6*).

EXPERIMENTAL SECTION

Materials. 2,6-Di-*O*-pentyl-3-*O*-(trifluoroacetyl)-γ-cyclodextrin (G-TA) was made as described previously (*19, 20*). Permethyl derivatives of *O*-((*S*)-(2-hydroxypropyl))cyclodextrin (A-PH and B-PH) were made in two steps as outlined in previous papers (*16, 19, 20*). First, the desired cyclodextrin was functionalized with an average of seven hydroxypropyl groups (*16*). The second step involved permethylation with methyl iodide. The capillary coating procedure was reported previously as well (*16, 19*). All-fused-silica capillary tubing (0.25-mm i.d.) was obtained from Supelco Co. (Bellefonte, PA). All of the derivatized-cyclodextrin fused-silica GC capillary columns can now be obtained from Advanced Separation Technologies, Inc. (Whippany, NJ).

All biomarker hydrocarbons except substituted indans were purchased from Chiron, Inc. (Chiron Laboratories, A.S. Jerlevelen 4, 7041 Trondheim, Norway). The concentrations of these samples were 1 mg/mL in isooctane and they were injected without further treatment. Substituted indans were the generous gift of Daniel

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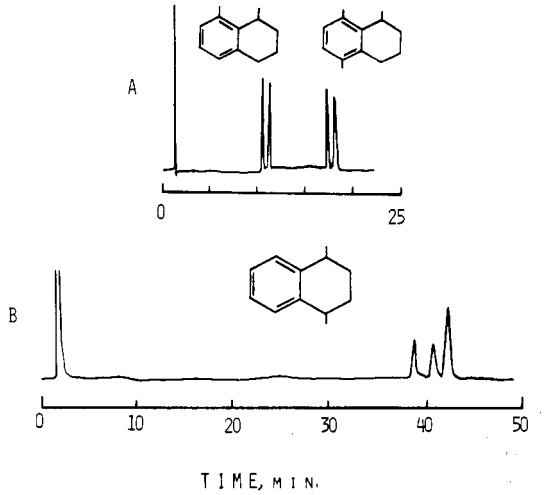


Figure 1. (A) Resolution of racemic 1,8-dimethyltetralin and 1,5,8-trimethyltetralin. Chromatogram B shows the separation of the three stereoisomers (one pair of enantiomers) of 1,4-dimethyltetralin. Both separations were done on a 10-m B-PH capillary GC column at 110 °C for (A) and 80 °C for (B).

A. Netgel of the Wyoming Research Institute, University Station, Laramie, WY (21). About 1 mg/mL solutions of the derivatized indans were made by dissolving samples in isooctane. All native cyclodextrins were obtained from Advanced Separation Technologies.

Methods. A Varian Model 3700 gas chromatograph was used for all separations. Flame ionization detector was utilized. Split injections of 0.1–0.2 μL of samples were done with a split ratio 1/100. The injection port and detector temperature was set at 250 °C. Nitrogen was used as the carrier gas with a linear velocity of ~10 cm/s for the 20-m columns (gas pressure inlet of 3.5 psi) and 9 cm/s for 10-m columns (gas pressure inlet of 2.5 psi).

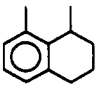
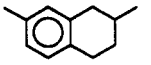
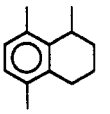
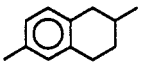
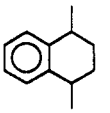
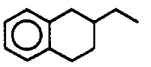
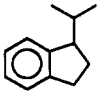
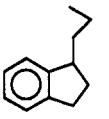
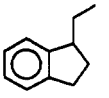
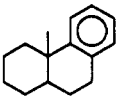
A Hewlett-Packard Model 5890, series II GC connected to a 5970 series mass selective detector was used to provide supplemental evidence for enantiomeric separation and to eliminate the possibility of identifying a nonisomeric impurity as an enantiomer. Enantiomers had identical MS parent ion peaks and fragmentation patterns. Diastereomers often had identical parent ion peaks, but slightly different fragmentation patterns. Nonisomeric contaminant peaks were found in some standards.

RESULTS AND DISCUSSION

Three different cyclodextrin CSPs were used to resolve hydrocarbon enantiomers. They were the permethyl-*O*-(2-hydroxypropyl)-α- and β-cyclodextrins (A-PH and B-PH) as well as trifluoroacetylated heptakis(2,6-*O*-dipentyl)-γ-CD (G-TA). One useful aspect of these somewhat more polar cyclodextrin derivatives is that they can be directly coated on undeactivated fused-silica capillaries.

Four different types of compounds were examined: (1) substituted tetralins, (2) substituted indans, (3) partially hydrogenated 4a-methylphenanthrenes, and (4) isoprenoids. The first three types of molecules are all hydroaromatic compounds. Their structures are given in Table I. They have been found in petroleum, coal, shale, and sedimentary organic matter (*21–24*). The distribution patterns of the di-and tricyclic compounds has been used to obtain information about the thermal maturity of sedimentary organic matter (*23*). It is possible that a sensitive stereochemical analysis of these enantiomeric biomarkers would provide additional useful information. In addition, hydroaromatics can constitute up to 80% of some synthetic fuels (*24*). There is concern about the mutagenic effects of these and related compounds. As in other biologically active chiral compounds, it is likely that hydroaromatic enantiomers have different physiological effects (*24, 25*). Hence a true understanding of the physiological properties of these compounds will require enantiomeric separation and testing.

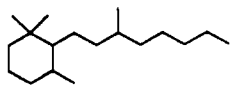
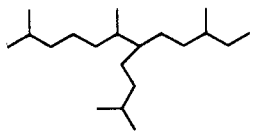
Table I. Relevant Structure Information and Separation Data for the Gas Chromatographic Separation of Enantiomeric Hydroaromatic Biomarkers

compound					
name	structure	k'^a	α^b	temp, °C	column[c]
tetralin					
1,8-dimethyl-		6.9	1.03	110	10 m, B-PH
2,7-dimethyl-		38.4	1.02	70	10 m, B-PH
		21.1	1.05	90	10 m, A-PH
1,5,8-trimethyl-		10.3	1.05	120	10 m, B-PH
2,6-dimethyl-		35.2	1.03	70	10 m, B-PH
		12.42	1.04	100	10 m, A-PH
1,4-dimethyl-		21.5	1.06[d]	80	10 m, B-PH
		58.4	1.01[d]	70	10 m, G-TA
2-ethyl-		40.5	1.01	70	10 m, B-PH
		12.9	1.02	100	10 m, A-PH
indan					
1-isopropyl-		19.4	1.02	90	10 m, A-PH
1-propyl-		19.4	1.02	90	10 m, A-PH
1-ethyl-		15.0	1.05	90	10 m, A-PH
4a-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene		34.3[e]	1.01[e]	105	10 m, B-PH
		48.9	1.03		
		20.12[e]	1.01[e]	120	10 m, A-PH
		29.22	1.03		

[a] The k' value is for the first eluted enantiomer. [b] The separation factor (α) is the ratio of the capacity factor of the second peak (k'_2) divided by the capacity factor of the first peak (k'_1). [c] Column abbrevations refer to the type of functionalized cyclodextrin stationary phase and are explained in the Experimental Section. [d] This compound exists as a pair of enantiomers and a meso compound. This α value is for the enantiomeric pair only. The complete separation is shown in Figure 1. [e] This compound exists as two pair of enantiomers, hence two values are given (see Figure 3).

Relevant chromatographic data for the resolution of chiral, substituted tetralins, indans, and octahydrophenanthrenes is shown in Table I. Separation factors ranged from 1.01 to 1.1. Figures 1–3 show enantiomeric separations for each of the three classes of hydroaromatic compounds used in this study. In all cases a relatively short 10-m "scout" column was used to generate these separations. Note in Figure 1 the base-line separation of the cis meso compound and both enantiomers of *trans*-1,4-dimethyltetralin. In some cases (i.e., 1,4-dimethyltetralin and the methyl-substituted octahydrophenanthrene, Table I) the permethyl-*O*-(hydroxypropyl)-β-CD (B-PH) column showed the greatest enantioselectivity. For the remaining substituted racemic tetralins, the B-PH and

Table II. Relevant Structural Information and Separation Data for the Gas Chromatographic Separation of Isoprenoid Biomarkers

compound					
name	structure	k'^a	α	temp, °C	column[b]
1,1,3-trimethyl-2-(3-methyloctyl)cyclohexane		80.2[c]	*d*	70	20 m, B-PH
2,6,10-trimethyl-7-(3-methylbutyl)dodecane		70.4	1.01[e] 1.01	100	10 m, A-PH

[a] The k' value is for the first eluted enantiomer. [b] Column abbreviations refer to the type of functionalized cyclodextrin stationary phase and are explained in the Experimental Section. [c] There are three stereogenic centers (four pairs of enantiomers) for this sample. The k' value is for the first eluted enantiomer of the first pair. Additional data can be derived from Figure 4. [d] See Figure 4 for details. [e] This compound also contains three stereogenic centers. However, all eight stereoisomers could not be resolved. The two α values are for the eluted peaks.

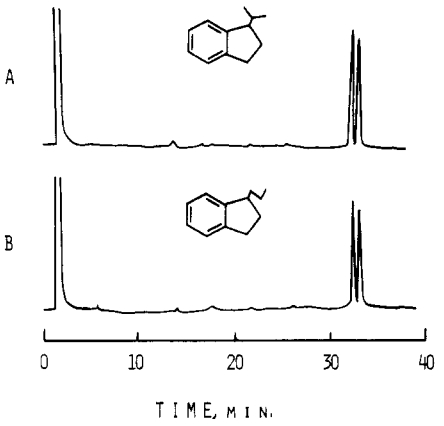


Figure 2. Enantiomeric separation of (A) 1-isopropylindan and (B) 1-*n*-propylindan. Both gas chromatographic separations were done on a 10-m A-PH capillary column at 90 °C.

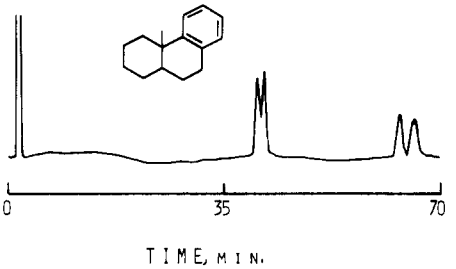


Figure 3. Gas chromatographic separation of both pairs of enantiomers of 4a-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene on a 10-m B-PH column at 105 °C.

the G-TA columns gave similar enantioselectivities. However, retention times were consistently longer for all of these compounds on the G-TA column. Figure 2 shows the resolution of racemic 1-*n*-propylindan and 1-isopropylindan. Unlike the substituted tetralins, the substituted indans were best resolved on an α-cyclodextrin stationary phase (i.e., permethyl-*O*-(hydroxypropyl)-α-CD).

Perhaps most interesting are the stereoselective separations of the isoprenoid compounds (Table II and Figure 4). These are saturated hydrocarbons with none of the functional groups that typically enhance chiral recognition. The branched C20 isoprenoid (2,6,10-trimethyl-7-(3-methylbutyl)dodecane) has been found in several recent sediments as well as in Rozel Point crude oil. Its structure has been confirmed by synthesis. The presence of this hydrocarbon in algae suggests a possible source for this compound in sediments and other geological samples (*23*).

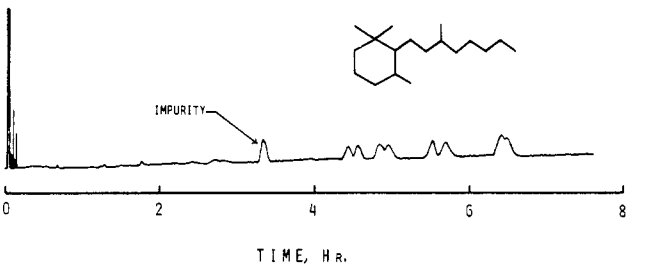


Figure 4. Gas chromatographic separation of the stereoisomers of 1,1,3-trimethyl-2-(3-methyloctyl)cyclohexane on a 20-m B-PH column: temperature 70 °C; split ratio 100/1.

The cyclic isoprenoid 1,1,3-trimethyl-2-(3-methyloctyl)-cyclohexane is believed to be derived from carotenoids and has been found in many fossil materials (*24*). Stereochemical data may clearly indicate where stereogenic centers have been retained unchanged and where new ones have been created and others epimerized. Thus enantiomeric compositions of the isoprenoids carry "original" information about maturation processes and the origin of fossils, oils, and sediments. The cyclic isoprenoid analyzed in this study (Table II and Figure 4) contains three stereogenic centers and therefore can exist in eight stereoisomeric forms (four pairs of enantiomers). The acyclic compound also contains three stereogenic centers.

The separation of the cyclic isoprenoid was particularly interesting in that very different stereoselective separations were obtained with different cyclodextrin CSPs. The best separation was obtained with the B-PH column. Eleven peaks were obtained. Of these, three peaks were shown to be impurities by GC/MS (see Experimental Section). The eight peaks representing the stereoisomers of the cyclic isoprenoid (Figure 4) eluted between a less retained impurity and two higher retention impurities (not shown in Figure 4). All stereoisomers of 1,1,3-trimethyl-2-(3-methyloctyl)cyclohexane gave a distinct m/z parent ion peak of 252 while the impurities did not. This separation is particularly impressive in view of the number of stereoisomers present and the compound's lack of functionality (Figure 4). The branched isoprenoid showed only a partial separation under analogous separation conditions (Table II).

Capillary GC is particularly useful for the analysis of hydrocarbon enantiomers because it permits the complete resolution of peaks with relatively low separation factors. The use of helium rather than nitrogen carrier gas and the use of longer capillaries can tremendously enhance the resolving power of this method if need be. This may be necessary when complex samples of crude oil and coal or shale extracts are analyzed. The enantioselective separation of biomarkers is

a new and potentially useful approach in the areas of geochemistry and paleoarcheology. Gas chromatographic separations on functionalized cyclodextrin CSPs constitute the most viable approach for these studies at this time. Currently, studies are underway to evaluate the enantioselective separation mechanism for chiral hydrocarbons. In addition, multidimension techniques are being developed for the efficient direct analysis of chiral biomarkers contained in geological samples.

LITERATURE CITED

(1) Eglinton, G.; Calvin, M. *Sci. Am.* **1967**, *216*, 32-43.
(2) Didyk, B. M.; Simoneit, B. R. T.; Brassell, S. C.; Eglinton, G. *Nature* **1978**, *272*, 216-222.
(3) Philp, R. P. *Chem. Eng. News* **1986**, *64* (6), 28-43.
(4) Ackman, R. G.; Cox, R. E.; Eglinton, G.; Hooper, S. N.; Maxwell, J. R. *J. Chromatogr. Sci.* **1972**, *10*, 392-401.
(5) Cox, R. E.; Maxwell, J. R.; Eglinton, G.; Pillinger, C. T.; Ackman, R. G.; Hooper, S. N. *J. Chem. Soc.* **1970**, *23*, 1639-1641.
(6) Maxwell, J. R.; Cox, R. E.; Eglinton, G.; Pillinger, C. T.; Ackman, R. G.; Hooper, S. N. *Geochim. Cosmochim Acta* **1973**, *37*, 297-313.
(7) Yon, D. A.; Maxwell, J. R.; Rybak, G. *Tetrahedron Lett.* **1982**, *23*, 2146.
(8) Miller, D. J.; Hawthorne, S. B. *ASTM Spec. Tech. Publ.* **1989**, *No. 1019*, 38-46.
(9) Sybilska, D.; Jurczak, J. *Carbohydr. Res.* **1989**, *192*, 243-250.
(10) Schurig, V.; Novotny, H. P.; Schmalzing, D. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 736-737.
(11) Schurig, V.; Novotny, H. P. *J. Chromatogr.* **1988**, *441*, 155-163.
(12) Smolkova-Keulemansova, E.; Feltl, L.; Krysl, S. *J. Inclusion Phenom.* **1985**, *3*, 183-196.
(13) Smolkova-Keulemansova, E.; Kralova, H.; Kripl, S.; Feltl, L. *J. Chromatogr.* **1982**, *241*, 3-8.
(14) König, W. A.; Lutz, S.; Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 979-980.
(15) König, W. A.; Krebber, R.; Wenz, G. *J. High Resolut. Chromatogr.* **1989**, *12*, 641-644.
(16) Armstrong, D. W.; Li, W.; Chang, C.-D.; Pitha, J. *Anal. Chem.* **1990**, *62*, 914-923.
(17) Li, W. Y.; Jin, H. L.; Armstrong, D. W. *J. Chromatogr.* **1990**, *509*, 303-324.
(18) Armstrong, D. W.; Li, W.; Stalcup, A. M.; Secor, H. V.; Izac, R. R.; Seeman, J. I. *Anal. Chem. Acta* **1990**, *234*, 365-380.
(19) Armstrong, D. W.; Li, W.; Pitha, J. *Anal. Chem.* **1990**, *62*, 214-217.
(20) Pitha, J.; Pitha, J. *Pharm. Sci.* **1985**, *74*, 987-990.
(21) Adamczyk, M.; Watt, D. S. and Netzel, D. A. *J. Org. Chem.* **1984**, *49*, 4226-4237.
(22) Radke, M.; Welte, D. H.; Willsch, H. *Geochim. Cosmochim. Acta* **1982**, *46*, 1-10.
(23) Radke, M.; Willsch, H.; Welte, D. H. *Anal. Chem.* **1984**, *56*, 2538-2546.
(24) Wozniak, T. J.; Hites, R. A. *Anal. Chem.* **1985**, *57*, 1320-1327.
(25) Wozniak, T. J.; Hites, R. A. In *Advanced Technologies in Synthetic Fuel Analysis*; Wright, C., Weimer, W. C., Felix, W. D., Eds.; Technology Information Center: Oak Ridge, TN, 1983.
(26) Rowland, S. J.; Yon, D. A.; Lewis, C. A.; Maxwell, J. R. *Org. Geochim.* **1985**, *8*, 207-213.
(27) Hall, P. B.; Douglas, A. G. *Adv. Org. Geochem.* **1983**, 576-587.

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Direct Interfacing of High-Speed Countercurrent Chromatography to Frit Electron Ionization, Chemical Ionization, and Fast Atom Bombardment Mass Spectrometry

Hisao Oka,* Yoshitomo Ikai, Norihisa Kawamura, and Junko Hayakawa

Aichi Prefectural Institute of Public Health, Tsuji-machi, Kita-ku, Nagoya 462, Japan

Ken-ichi Harada, Hideaki Murata, and Makoto Suzuki

Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan

Yoichiro Ito

Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20892

Direct interfacing of analytical high-speed countercurrent chromatography (HSCCC) to mass spectrometry (MS) was demonstrated for the first time, and its performance was evaluated in terms of chromatography and mass spectrometry. HSCCC/MS interface was based upon Frit electron ionization (EI), chemical ionization (CI), and fast atom bombardment (FAB). Separations were conducted by newly developed HSCCC-4000 with a 2.5-cm revolutional radius and 0.3 mm or 0.55 mm i.d. multilayer coiled column which is capable of operating at a maximum speed of 4000 rpm. To demonstrate the potential capability of HSCCC/frit MS, three indole auxin mixtures, two mycinamicin (macrolide antibiotics) mixtures, and a colistin complex (peptide antibiotics) were analyzed under HSCCC/frit EI, CI, and FABMS conditions, respectively. The data obtained indicated that interfacing to frit/MS does not adversely affect the chromatographic resolution and mass spectra provide structural information. The HSCCC system interfaced with a frit-equipped mass spectrometer will offer a new dimension in the separation of biologically important substances.

INTRODUCTION

Because analysis methods combining chromatography with mass spectrometry (MS) can identify and determine analytes in a complex sample matrix without an isolation process, various methods coupled with MS such as thin-layer chromatography/MS, high-performance liquid chromatography/MS, gas chromatography/MS, supercritical fluid chromatography/MS, and capillary electrophoresis/MS have been developed and they are useful tools in the fields of natural products and analytical chemistry. However, all of them use solid supports as stationary phases except capillary electrophoresis/MS, so they do not eliminate various complications arising from the use of solid supports.

High-speed countercurrent chromatography (HSCCC) using a multilayer coiled column is a unique liquid–liquid partition technique that does not require the use of a solid support (*1*). The use of two immiscible solvent phases in an open column free of solid support matrix can eliminate various complications associated with conventional liquid chromatography such as tailing of solute peaks, adsorptive sample loss and deac-

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