Relevance of Enantiomeric Separations in Food and Beverage Analyses

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A series of new derivatized cyclodextrins have been developed for use as chiral stationary phases in capillary gas chromatography. As a result, a large number of the enantiomeric components in food and beverage products can be resolved relatively quickly and easily. The study focuses on compounds other than amino acids (e.g., malic acid, lactic acid, tartaric acid, esters, alcohols, lactone flavors or fragrances, and so on). The ability to separate and quantitate enantiomers at low levels should be useful for detecting adulterated products, for evaluating fermentation processes, and for the accurate characterization of enantiomeric flavor components, growth regulators, pesticides, and herbicides as well as their chiral environmental degradation products and metabolites.

A large number of the organic components of foods and beverages are chiral molecules. In addition, a significant number of additives, flavors, fragrances, preservatives, growth regulators, fumigants, pesticides, herbicides, and so on used in the industry also are chiral molecules. Chiral or enantiomeric molecules are known to rotate plane polarized light, and they cannot be superimposed on their mirror image isomer. It is well-known that enantiomers have exactly the same physical and chemical properties in an isotropic environment. This can make it very difficult to separate them from one another or even to discern that there is a mixture of the two. An equimolar mixture of two enantiomers is referred to as a racemic mixture or racemate. Most synthetically produced chiral compounds are racemates. Spectroscopically (polarimetry, circular dichroism, or optical rotatory dispersion), racemates appear to be achiral.

When the various organic components of food and beverage products are analyzed, it is rare to consider the enantiomeric makeup of the chiral components. However, Sandra et al. (1984) and Kuneman et al. (1988) pointed out the value of separating L- and D-amino acids because the presence of the synthetically produced D enantiomer could be used to detect adulterated fruit juices. Indeed, the identification of adulterated consumer products is one of a number of important areas where enantiomeric separations can make a significant contribution. Table I lists a few of the areas in which enantiomeric separations may be relevant to food science. For example, amino acids are not the only components of foods and beverages that can be analyzed to identify adulterated products. There are a variety of chiral organic acids, alcohols, diols, esters, lactones, aldehydes, and ketones that not only are useful but also may be more specific markers than some amino acids. Consequently, we will focus on compounds other than amino acids, such as malic acid, lactate esters, tartaric acid, and butylene glycol.

Fermentation processes sometimes can alter the enantiomeric excess of certain solutes in addition to producing other chiral molecules. Specific examples are lactic acid and acetoin (3-hydroxy-2-butanone) in milk and dairy products (Alm, 1982). It is well-known that L-amino acids racemize slowly with time (Smith et al., 1978). Depending on the pH, temperature, state (i.e., liquid or solid), and other factors, many chiral molecules can racemize at different rates. Thus, it may be possible to use enantiomeric purity to evaluate the age, past treatment, or storage effects of a variety of alcoholic and nonalcoholic beverages (and other food products). A variety of natural and synthetic chiral flavor and fragrance components can be found in most foodstuffs. There are a variety of receptors on the human tongue and nasal membrane that are stereoselective for certain compounds. One well-known example is carvone; the *R* enantiomer smells like mint, and the *S* enantiomer smells like caraway. Enantiomeric analyses allow a more accurate evaluation of flavors and fragrances.

Table I. Ways in Which Enantioselective Separations Can Be Used in Food and Beverage Studies

identifying adulterated foods and beverages
more exact control and monitoring of fermentation processes and products
evaluation and identification of age, past treatment, and storage effects
more exact evaluation of some flavor and fragrance components
fingerprinting complex mixtures
analysis of chiral metabolites of many chiral and prochiral constituents of foods and beverages
50% less material (e.g., flavors, fragrances, preservatives, additives, regulators, biocides, etc.) can be used in some cases
decrease environmental persistence of some compounds

Many complex raw materials, such as extracts and concentrates, have characteristic chromatographic profiles or "fingerprints". Often, these fingerprints can be used to identify different sources for the same raw material or to determine if one material has been treated differently from another. This technique, coupled with pattern recognition, has been used in the past to identify crude oil sources, air pollution sources, and so on (McClenny et al., 1989). This method can be used to monitor raw material used in the food and beverage industry as well (e.g., grape juice, pulp concentrates, extracts, bulk wines). An enantioselective fingerprint would be more definitive and much more difficult to duplicate in an adulterated product.

Many of the flavors, fragrances, preservatives, growth regulators, additives, biocides, etc. used in the food industry are racemic mixtures in which only one enantiomer

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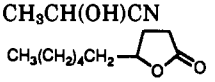
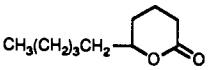
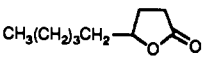
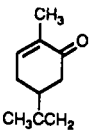
Table II. Structural and Chromatographic Data for Enantiomeric Solutes

enantiomeric compounds	structure	R	column[a]	temp, °C	k' [b]	α [c]
malic acid	$ROC(O)CH_2CH(OR)C(O)OR$	TMS[d]	PMHP-β-CD	95	28.0	1.10
lactic acid	$CH_3CH(OH)C(O)OR$	CH_3	PMHP-β-CD	55	13.0	1.05
		CH_2CH_3	PMHP-β-CD	50	12.9	1.03
		$(CH_2)_3CH_3$	PMHP-β-CD	60	15.0	1.02
		TMS[d]	PMHP-β-CD	90	21.3	1.25
acetoin	$CH_3C(O)CH(OH)CH_3$		PMHP-β-CD	45	5.7	1.03
tartaric acid dimethyl ester	$CH_3OC(O)CH(OR)CH(OR)C(O)OCH_3$	$C(O)CF_3$	PMHP-β-CD	90	2.8	1.04
tartaric acid diisopropyl ester	$CH(CH_3)_2OC(O)CH(OR)CH(OR)C(O)OCH(CH_3)_2$	$C(O)CF_3$	PMHP-β-CD	90	8.6	1.07
2,3-butylene glycol	$CH_3CH(OR)CH(OR)CH_3$	$C(O)CF_3$	DPTFA-γ-CD	70	1.4	1.58
2-hydroxybutyric acid	$CH_3CH_2CH(OH)C(O)OR$	CH_3	PMHP-β-CD	60	16.3	1.10
2-hydroxyvaleric acid	$CH_3CH(OH)CH_2CHC(O)OR$	CH_3	PMHP-β-CD	60	8.7	1.25
2-hydroxycaproic acid	$CH_3(CH_2)_3CH(OH)C(O)OR$	CH_3	PMHP-β-CD	75	28.0	1.02
mandelic acid	$C_6H_5CH(OH)C(O)OR$	CH_3	PMHP-β-CD	120	4.0	1.04
		CH_2CH_3	PMHP-β-CD	120	3.6	1.03
lactonitrile	$CH_3CH(OH)CN$		DPTFA-β-CD	100	5.0	1.30
γ-decalactone			DPTFA-γ-CD	140	7.1	1.06
δ-decalactone			DPTFA-γ-CD	140	8.5	1.04
γ-nonalactone			DPTFA-γ-CD	160	4.07	1.06
carvone			DPTFA-β-CD	110	7.1	1.09
	Halogenated Carboxylic Acids					
chlorosuccinic acid	$ROC(O)CH_2CH(Cl)C(O)OR$	CH_3	PMHP-β-CD	70	15.7	1.15
bromosuccinic acid	$ROC(O)CH_2CH(Br)C(O)OR$	CH_3	PMHP-β-CD	80	5.0	1.25
2-bromobutyric acid	$CH_3CH_2CH(BrC(O)OR$	CH_3	PMHP-β-CD	75	7.3	1.10
2-bromo-3-methylbutyric acid	$(CH_3)_2CHCH(Br)C(O)OR$	CH_3	PMHP-β-CD	70	11.5	1.20
2-chloropropionic acid	$CH_3CH(Cl)C(O)OR$	CH_3	PMHP-β-CD	42	6.0	1.12

[a] All capillary columns were 9 m long. The abbreviation PMHP-β-CD stands for permethyl-(*S*)-hydroxypropyl derivatized β-cyclodextrin. The abbreviation DPTFA-γ-CD stands for octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin. [b] This is the capacity factor (k') of the first eluted enantiomer. It is calculated from $k' = (t_1 - t_0)/t_0$, where t_1 is the retention time of the first eluted enantiomer and t_0 is the retention time of an unretained solute. [c] The separation factor (α) is equal to the ratio of the two enantiomeric k' values. [d] Trimethylsilyl group.

produces the desired effect. If it is known which optical antipode is active and the pure enantiomer is used, 50% less material can be applied. Furthermore, the environmental persistence of the unneeded enantiomers can be relatively longer than that of the useful antipode. This can cause problems with unwanted residues in various food products.

Finally, enantioselective separations will be essential in scientific studies involving metabolites of chiral and prochiral food and beverage food components. These studies are analogous to those currently being required by the FDA for chiral and prochiral pharmaceutical products (DeCamp, 1989).

Thus far, relatively few of the aforementioned studies (Table I) have been done. One reason for this was the lack of facile, efficient methods of enantiomeric analysis. Over the past few years the situation has changed dramatically. Over 40 different liquid chromatographic columns containing highly selective chiral stationary phases have been introduced (Armstrong, 1987; Armstrong and Han, 1988). Most recently, König et al. (1988) and Armstrong et al. (1990) introduced derivatized cyclodextrin stationary phases for the capillary gas chromatographic separation of enantiomers. In this study, we show that cyclodextrin-based capillary gas chromatography is particularly effective in resolving enantiomeric molecules found in a variety of foods and beverages. Many of these solutes are difficult to resolve by other means.

MATERIALS AND METHODS

Cyclodextrin Derivative GC Phases. The nonpolar chiral stationary phases were made with hexakis-, heptakis-, and octakis(2,6-di-*O*-pentyl)-α-, β-, and γ-cyclodextrins, respectively. All of these were synthesized by using a Williamson reaction. A solution of the appropriate cyclodextrin hydrate (1.0 g) in dimethyl sulfoxide (20 mL) was treated with finely ground NaOH (2.0 g, 50.0 mmol) and 1-bromopentane (5.97 g, 39.5 mmol). The reaction was cooled slightly, stirred for 48 h, and then quenched by the addition of chloroform (60 mL) and water (60 mL). The organic phase was separated and treated with $MgSO_4$ and filter cel, stirred for 20 h, and filtered. The pale yellow clear solution was concentrated under vacuum. In this reaction, the 2- and 6-hydroxy groups of each individual glucose unit of the cyclodextrin can react to produce a viscous liquid which is a mixture of dipentylcyclodextrin isomers and homologues (Armstrong et al., 1990). The 2,6-di-*O*-pentyl-3-*O*-trifluoroacetylcyclodextrins were made by trifluoroacetylation of the 3-hydroxy group of each glucose unit of the dipentylcyclodextrins, with trifluoroacetic anhydride in tetrahydrofuran for 2 h at room temperature. The final product was extracted with chloroform which was subsequently evaporated.

The hydrophilic chiral stationary phases were made with *O*-permethyl-(*S*)-hydroxypropyl derivatized cyclodextrins. These were made in two steps. First, the desired cyclodextrin was dissolved in aqueous sodium hydroxide (5% w/w), and the solution was cooled in an ice bath; then (*S*)-propylene oxide was slowly added while stirring. After about 6 h in the ice bath, the reaction was left to proceed for a day at room temperature, neutralized, and dialyzed briefly to remove the contaminating salts (Armstrong et al., 1990). The retained solution was filtered and the product obtained by freeze-drying. The second step was performed in an ice bath at 0 °C. An excess of methyl iodide was added dropwise to a solution of cyclodextrin derivative and sodium hydride (50% suspension in paraffin oil) in dimethyl sulfoxide. The mixture was allowed to react under gentle stir-

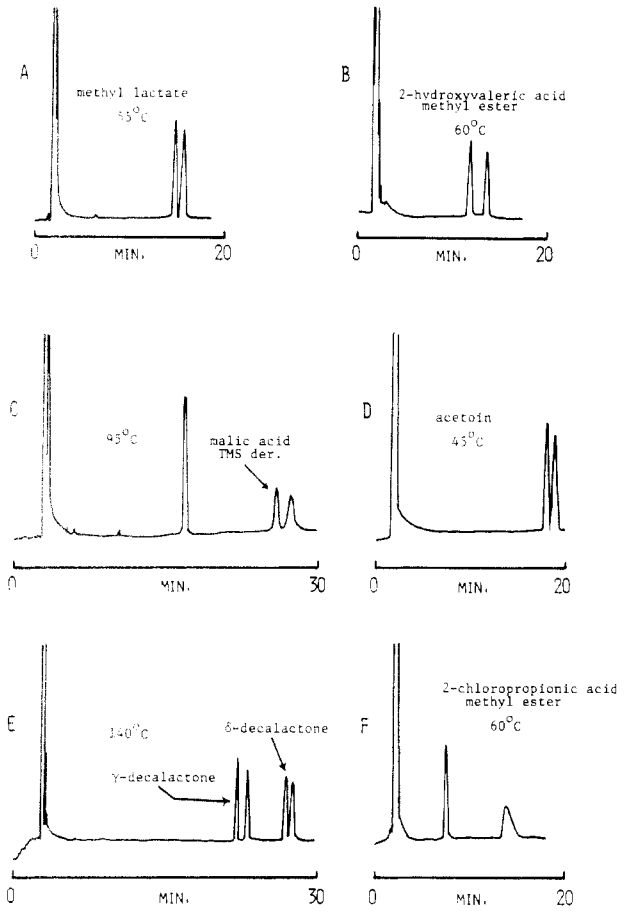


Figure 1. Six gas chromatograms showing enantiomeric separations of seven racemic mixtures of interest to those involved in food science and agrochemicals. Chromatograms A–D were generated on a 9-m permethyl-(*S*)-hydroxypropyl derivatized β-cyclodextrin coated fused silica capillary column. Chromatograms E and F were generated on the analogous 9-m 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-γ-cyclodextrin column. In chromatogram E, two different racemic lactones were injected simultaneously. For further experimental details see Materials and Methods and Table II.

ring for 1 h. After 24 h at room temperature, the product was extracted with chloroform which, upon evaporation, yielded the product.

Medium (20 m) and short (9 m) fused silica capillary (0.25 mm i.d.) columns were coated following the static method described by Bouche and Verzele (1968). A 0.2% w/v ether solution of the derivatized cyclodextrin filled the capillary. After evaporation of ether at 36 °C under reduced pressure, the coating was estimated to be about 0.5 μm thick, producing around 4000 plates per column meter (solute decane) and only about 2000 plates per column meter with the carbohydrate solutes. All of the derivatized cyclodextrin fused silica GC capillary columns can now be obtained from Advanced Separation Technologies, Inc. (Whippany, NJ).

Reagents. All enantiomeric compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO). *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide and trifluoroacetic anhydride were obtained from Aldrich as well. Methanol and sulfuric acid were obtained from Fisher (Fairlawn, NJ). Fused silica capillaries were obtained from Supelco Co. (Bellefonte, PA). All cyclodextrins were obtained from Advanced Separation Technologies.

Apparatus. A Varian Model 3700 and a Shimadzu Model GC-8A gas chromatograms were used. Flame ionization and electron capture detectors were utilized. Split injections of 0.2–0.5 μL of sample were done with a split ratio 1/100. The injection port and detector temperature was 200 °C. Nitrogen was used as the carrier gas with a linear velocity of about 10 cm/s for the 20-m columns (gas pressure inlet of 7 psi or 0.5 kg/cm^2) and 7.5 cm/s for the 9-m columns (gas pressure inlet of 3 psi or 0.2 kg/cm^2).

Procedure. To carry out the trimethylsilylation reaction, approximately 1.0 mg of the racemic analytes was dissolved in 0.3 mL of acetonitrile along with 0.1 mL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide. After 20 min of reaction, dry nitrogen gas was bubbled through the solution to remove excess reagent. Methyl esters were made by dissolving 1.0 mg of the racemic analyte in 1.0 mL of methanol with 0.05 mL of concentrated sulfuric acid. The reaction was heated at 50–60 °C for 4–6 h in a closed vial. Trifluoroacetyl esters were made by the same procedure as in trimethylsilylation except that trifluoroacetic anhydride was used as the reagent. Some compounds were derivatized only to increase their volatility. Generally, enantioselectivity is more pronounced at lower temperatures. There was no evidence of racemization for these particular compounds during any of the aforementioned procedures.

RESULTS AND DISCUSSION

Data for a variety of enantiomeric separations on the *O*-permethyl-(*S*)-hydroxypropyl derivatized cyclodextrin columns and the 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl derivatized cyclodextrin columns are presented in Table II. The first 15 solutes are common to a number of food and beverage products either as a natural constituent or as an additive. For example, malic acid is found in many fruits, vegetables and juices, e.g., apple, pear, cherry, orange, strawberry, and carrot (Junge and Spadinger, 1982). Dairy products and many others contain lactic acid, which is altered during the fermentation process. Wine contains lactate and tartrate esters as well as a variety of other chiral components such as acetoin and 2,3-butylene glycol. Several of the hydroxy acids are well-known metabolites. Many lactones and carvone occur naturally and also are used as additives to enhance fragrance and/or flavor in a variety of products. γ-Decalactone has a "fruity-peach" fragrance, δ-decalactone a coconut fragrance, and γ-nonalactone an anise or licorice fragrance. The final five compounds in Table II are halogenated carboxylic acids that are metabolic degradation products or, in some cases, synthetic precursors to a variety of chiral biocides used in the agrochemical industry. For example, 2-chloropropionic acid is used in the synthesis of a variety of herbicides. Although these compounds are usually racemic, only the D enantiomer is thought to have the desired activity.

The data in Table II indicate that the permethyl-(*S*)-hydroxypropyl derivatized β-cyclodextrin column efficiently resolves a number of mono- and dicarboxylic acid enantiomers. The dipentyltrifluoroacetyl derivatized γ-cyclodextrin stationary phase is more effective in separating lactones, nitriles, and alcohols. Both stationary phases were able to resolve the halogenated carboxylic acids, although data are reported only for the permethylhydroxypropyl β-cyclodextrin column (Table II).

Figure 1 is a compilation of several chromatograms that illustrate the enantiomeric selectivity of the derivatized cyclodextrin chiral stationary phases. From a comparison of gas chromatography (GC) to liquid chromatography (LC) approaches to the separation of enantiomers, it appears the GC may be the preferred analytical technique for the types of compounds evaluated in this study. Most liquid chromatographic methods work best for enantiomers containing one or more aromatic rings as well as hydrogen-bonding groups (Armstrong and Han, 1988). In addition, these functional groups should be located α or β to the chiral center for optimal results. Interestingly, cyclodextrin-based GC can be used to resolve enantiomers that contain none of the requisite groups that are important for LC. Indeed, with these chiral GC stationary phases, enanti-

omers containing no aromatic rings or hydrogen-bonding groups have been resolved. Detection and sensitivity also can be a problem in LC if the analyte does not contain a good chromophore or fluorophore. This was the case for most of the solutes in this study. Also, the high efficiency, peak capacity, and sensitivity of capillary GC would be particularly useful when low levels of chiral substrates in the complex matrices that constitute most foodstuffs and beverages are analyzed.

Currently there is some question as to the role of inclusion complexation between the derivatized cyclodextrin and the chiral analyte in gas–liquid systems (König et al., 1988); Armstrong et al., 1990). We are evaluating mechanisms of enantioselective retention and chiral recognition by measuring the ΔH, ΔS, and ΔG values of transfer of specific solutes between gas and liquid phases, as well as analyzing size selectivity trends and column overloading behavior. These studies should help our basic understanding of chiral recognition in derivatized cyclodextrin mediated GC.

In conclusion, it appears that the GC separation of enantiomers can be extended to a greater variety of compounds contained in many foods and beverages. This will allow the detection of a greater number of adulterants, thereby greatly expanding the scope of these evaluations. Also, stereoselective GC may give rise to more elegant and accurate ways to evaluate fermentation, aging and storage effects, flavors and fragrances, fingerprinting extracts, and complex mixtures and other related scientific studies. Since the Food and Drug Administration has become aware of the new enantioselective chromatographic procedures and has begun to require greater accountability of pharmaceutical firms in the area of chiral and racemic products (DeCamp, 1989), it is likely that the food and agrochemical industries will be expected to follow suite in due time.

ACKNOWLEDGMENT

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Registry No. Octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin, 128165-34-4; β-cyclodextrin, 7585-39-9; γ-cyclodextrin, 17465-86-0; 2,6-di-*O*-pentyl-γ-cyclodextrin, 125474-95-5; 1-bromopentane, 110-53-2; malic acid, 617-48-1; lactic acid, 598-82-3; acetoin, 52217-02-4; dimethyl tartrate, 608-69-5; diisopropyl tartrate, 58167-01-4; 2,3-butylene glycol, 107-88-0; 2-hydroxybutyric acid, 600-15-7; 2-hydroxyvaleric acid, 6450-97-1; 2-hydroxycaproic acid, 6064-63-7; mandelic acid, 611-72-3; lactonitrile, 42492-95-5; γ-decalactone, 2825-92-5; δ-decalactone, 705-86-2; γ-nonalactone, 57084-16-9; carvone, 22327-39-5; chlorosuccinic acid, 16045-92-4; bromosuccinic acid, 923-06-8; 2-bromobutyric acid, 2385-70-8; 2-bromo-3-methylbutyric acid, 565-74-2; 2-chloropropionic acid, 62138-52-7.