

A Guide to the Analysis of Chiral Compounds by GC.....



Inside:

Definitions of Chirality and Chiral Chromatography
.....

Chiral Columns Offer Unique Selectivity
.....

Optimization of Chiral Separations
.....

Chiral Specific Applications of Essential Oils, Flavors, and Pharmaceuticals
.....

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A team of researchers at the University of Neuchâtel developed β -cyclodextrins with superb enantiomeric selectivity. They joined forces with Restek, a manufacturer of top quality columns, to provide a unique line of commercially available β -cyclodextrin stationary phases with enhanced capabilities for chiral capillary gas chromatography.

The Rt- β DEXsm and Rt- β DEXse chiral capillary columns offer extensive enantiomeric separation of monoterpenes, monoterpene alcohols, and monoterpene ketones that cannot be matched by permethylated β -cyclodextrin columns. Rt- β DEXsp and Rt- β DEXsa are secondary columns that best resolve specific flavor and fragrance chiral components. The Rt- β DEXcst provides excellent resolution of some complex flavor compounds and has demonstrated great potential with pharmaceutical substances as well.

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Dr. Raphael Tabacchi
 Born in Ticino, Switzerland, Dr. Tabacchi has been a professor for Analytical and Organic Structure at the University of Neuchâtel, Switzerland, since 1978. His research interests focused upon natural product chemistry, and development of HPLC and GC stationary phases. He has developed β -cyclodextrins with unique substitutions to create novel chiral phases for capillary GC.



Dr. Georges Claude Saturnin
 Born in St. Joseph, Martinique, Dr. Saturnin became a Senior Assistant and Assistant Professor in 1990 at the University of Neuchâtel. He is involved in the development of HPLC phases. His focus is the synthesis of these new cyclodextrin materials that characterize the new chiral columns.



Claire-Lise Porret
 Born in Neuchâtel, Switzerland, Ms. Porret was previously a technician at Nestlé and joined Dr. Tabacchi's team in 1991. She is also involved in the synthesis of organic compounds and with the development of GC and HPLC stationary phases.



Maurus Biedermann
 Maurus has been with Dr. Konrad Grob's GC/LCGC group at the Kantonales Laboratorium Zurich (Official Food Control Authority in Switzerland) since 1990 and has participated in the development of several LCGC methods for food analysis. During his sabbatical at Restek, he demonstrated the ability of these new and unique chiral phases for many applications such as the authenticity of essential oils, "natural" flavor extracts, and the analysis of drugs for enantiomeric composition.



Sherry Sponsler
 Sherry is an Applications Chemist and has been with Restek since 1990. She conducts method and product development for analysis of foods, flavors and fragrances, as well as some pharmaceutical samples. Frequent communication with customers has helped Sherry to identify many important chiral applications in these industries. She has demonstrated many of these key separations, especially for fragrances and amphetamines, using the new cyclodextrin capillary columns.



Lori Bitzer
 After completing her Chemistry degree at West Virginia University in 1995, Lori joined Restek as a Fused Silica Manufacturing Chemist. She is involved with the design and production of new products including capillary chiral columns. Lori ensures product quality and consistency that are characteristic of all Restek products.

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WHAT ARE CHIRAL COMPOUNDS?

Any carbon atom that is bonded to four different functional groups is termed a chiral or an asymmetric carbon. Molecules containing one or more of these carbon centers are considered chiral molecules. Chiral centers can exist in two forms called enantiomers. These two forms are non-superimposable mirror images of each other, but both have similar properties. For example, both enantiomers will have the same boiling point, densities, and reaction rates as achiral molecules. They do, however, generally possess different aroma and flavor characteristics; more impor-

tantly, they possess differences in toxicity and biological activity.

Enantiomers are also known as optical isomers because they rotate plane polarized light in different directions. Optical isomers that rotate plane polarized light to the right, or clockwise, are termed dextrorotary (denoted as (d) or (+)), Optical isomers that rotate in the left direction are termed levorotary (denoted (l) or (-)).

Enantiomers can be denoted by the specific configuration around the chiral center. Groups on the carbon center are assigned a "priority" based on atomic number of the first bonded atom (Cahn-Ingold-Prelog rules). The group with the highest atomic num-

ber is rated first. If priority cannot be established with the first atom, work outward until priority differences can be determined. Once priorities have been established for all four groups, specific configuration can be determined. An R configuration is designated when the priority around the asymmetric carbon is in a clockwise direction, whereas a counterclockwise direction is denoted as S. (Figure 1A)¹

A chiral compound can possess multiple chiral centers and many combinations of configurations. Linalool oxides possess two chiral centers, resulting in four enantiomers. (Figure 1B) Note that configuration (R or S) is independent from optical activity (+ or -) or interaction with plane-polarized light.

Figure 1A

Linalool is a chiral compound because it contains an asymmetric carbon center. The mirror images are not superimposable and so they are enantiomers.



Enantiomers can be distinguished by configuration. Following groups from high to low priority in the clockwise direction is denoted R, and S for the counterclockwise direction.

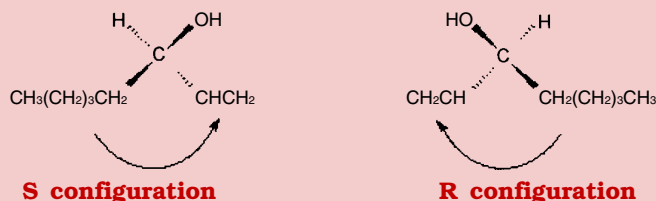
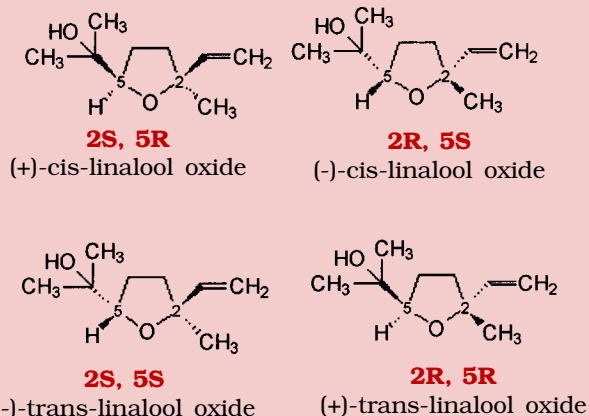


Figure 1B

Linalool oxides have two chiral centers at carbon numbers 2 and 5 and exist as four enantiomers.



WHAT ARE CHIRAL COMPOUNDS?

Any carbon atom that is bonded to four different groups is termed a chiral or an asymmetric carbon. Molecules containing one or more of these carbon centers are considered chiral molecules. Chiral centers can exist in two forms called enantiomers. These two forms are non-superimposable mirror images of each other, but both have similar properties.

WHAT IS CHIRAL CHROMATOGRAPHY?

Chiral chromatography is the separation of enantiomeric compounds. Common liquid stationary phases used in gas chromatography resolve components from one another, but they do not possess adequate selectivity for enantiomeric separation. Addition of derivatized cyclodextrin macromolecules to common stationary phases creates capillary columns with the ability to separate enantiomers as well.

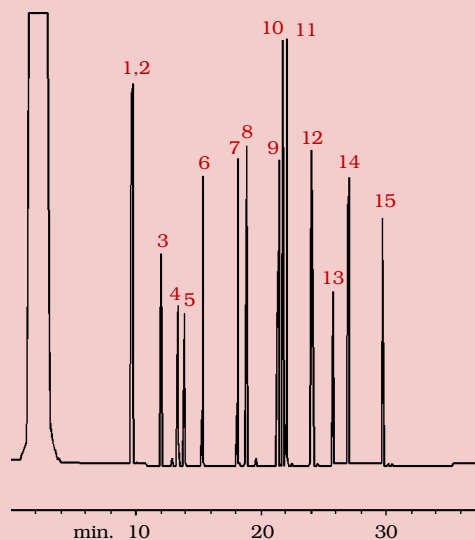
The permethylated derivative of beta-cyclodextrin in cyanopropyl-dimethylpolysiloxane liquid stationary phase is commonly used for such stereochemical separations, but it exhibits limited applications. Beta-cyclodextrins derivatized with alkyl substituents can enhance the enantiomeric resolution of various compound classes. Restek's five capillary columns incorporate various combinations of alkylated beta-cyclodextrins into a cyanopropyl-dimethyl polysiloxane liquid stationary phase to achieve significant separation.

These columns also exhibit stability and extended lifetime. From the first injection to the 250th injection on a chiral column, enantiomeric separation is maintained with almost no loss in resolution (**Figures 2A and B**).

Chiral chromatography is the separation of enantiomeric compounds. Common liquid stationary phases do not possess adequate selectivity for enantiomeric separation. Addition of derivatized cyclodextrin macromolecules to common stationary phases creates capillary columns with the ability to separate many enantiomers.

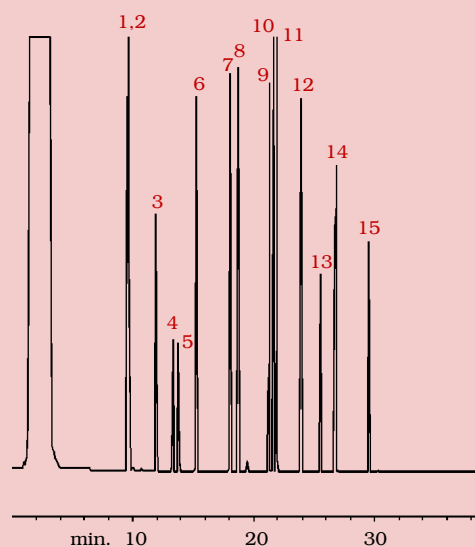
Figure 2
Restek's chiral columns demonstrate exceptional lifetime and stability for more than 250 injections without loss of resolution.

A: 1st injection



- | | | |
|-------------------------|-----------------------|------------------------|
| 1. (-) α -pinene | 6. undecane | 11. (-) phenylethanol |
| 2. (+) α -pinene | 7. nonanal | 12. methyl decanoate |
| 3. decane | 8. 1-octanol | 13. dicyclohexylamine |
| 4. (-) 2,3-butanediol | 9. 2,6-dimethylphenol | 14. methyl undecanoate |
| 5. (+) 2,3-butanediol | 10. (+) phenylethanol | 15. methyl dodecanoate |

B: 250th injection



30m, 0.32mm ID, 0.25 μ m Rt- β DEXsa (cat.# 13108)
Oven temp.: 40°C (hold 1 min.) to 230°C @ 2°C/min.;
Carrier gas: hydrogen; 80cm/sec. set @ 40°C; Detector: FID set @ 220°C

RESTEK'S CHIRAL COLUMNS OFFER UNIQUE SELECTIVITY

Figure 3

The Rt- β DEXsm column provides the best chiral separation of isoborneol and α -terpineol.

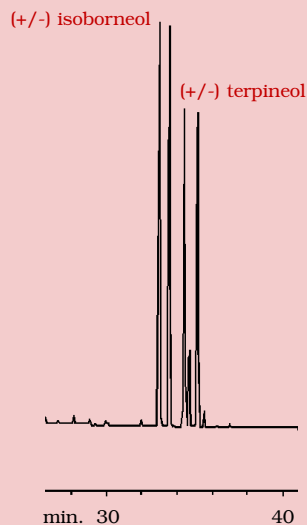
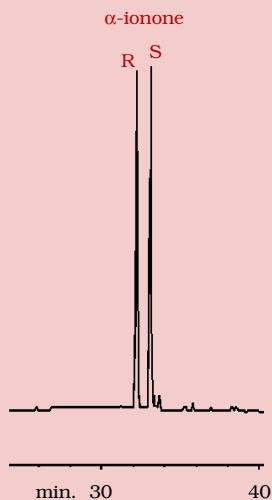


Figure 4

The Rt- β DEXsm column offers complete resolution of α -ionone.



30m, 0.32mm ID, 0.25 μ m Rt- β DEXsm (cat.# 13104)

Oven temp.: 40°C (hold 1 min.) to 230°C @ 2°C/min. (hold 3 min.);

Carrier gas: hydrogen; 80cm/sec. set @ 40°C; Detector: FID set @ 220°C

Each of the five chiral columns possesses a specific combination of alkyl substituents on the derivatized β -cyclodextrins. These unique combinations provide a wide range of utilization for each type of chiral column. Table I, on page 8, indicates that certain columns provide better resolution of specific compounds.

Rt- β DEXsm

Of the chiral columns evaluated, only the Rt- β DEXsm separates all of the 25 tested compounds, with 19 being baseline resolved. This column provides the best enantiomeric separation of α -pinene, isoborneol, α -ionone, linalool oxides, hexobarbital, and mephobarbital (**Figures 3 and 4**).

Rt- β DEXse

The Rt- β DEXse is similar in performance to the Rt- β DEXsm, but it provides better resolution for limonene, linalool, linalyl acetate, ethyl-2-methylbutyrate, 2,3-butane-diol, and styrene oxides. Sometimes extensive separation results in overlap of enantiomeric pairs, as shown in **Figures 5 and 6**.

Figure 5

The Rt- β DEXse column resolves optical isomers of ethyl-2-methylbutyrate, styrene oxide, and camphor with some overlap.

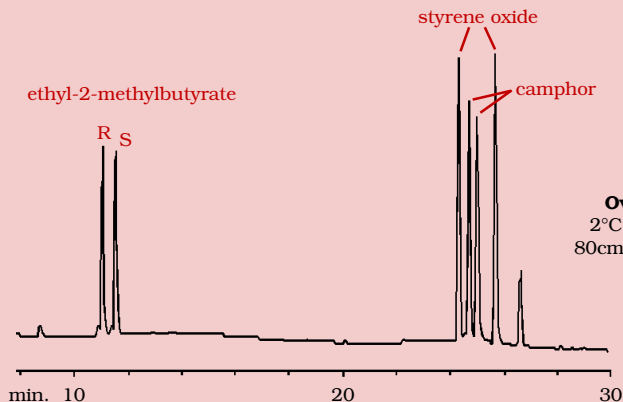
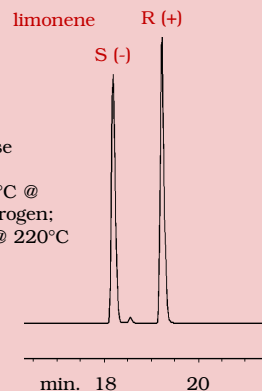


Figure 6

The Rt- β DEXse column resolves limonene enantiomers.



30m, 0.32mm ID, 0.25 μ m Rt- β DEXse (cat.# 13106)

Oven temp.: 40°C (hold 1 min.) to 230°C @ 2°C/min. (hold 3 min.); Carrier gas: hydrogen; 80cm/sec. set @ 40°C; Detector: FID set @ 220°C

Rt- β DEXsm and Rt- β DEXse

Cis and trans linalool oxides, linalool, and linalyl acetate are commonly found together in lavender oils, and resolution of all enantiomers is desirable. The Rt- β DEXsm separates the linalool oxides, but it does not resolve linalyl acetate. Conversely, Rt- β DEXse separates linalool and linalyl acetate, but does not resolve all of the linalool oxides. Combining both together in a dual column system will provide resolution for all of these enantiomers (Figure 7).

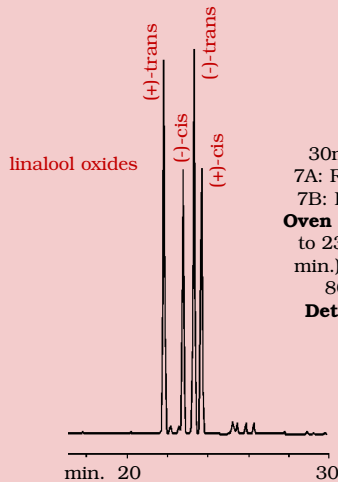
Rt- β DEXsp

Rt- β DEXsp is a specialized column that best resolves menthol. It would be useful in addition to the Rt- β DEXsm or Rt- β DEXse column for analyzing complete profiles of mint oils (Figure 8).

Rt- β DEXsa

The Rt- β DEXsa has a significantly different selectivity than the other chiral columns. It provides the best separation of 1-octen-3-ol, carvone, camphor, 1-phenylethanol, β -citronellol, and rose oxides (Figure 9).

Figure 7A
Cis and trans linalool oxide enantiomers separated on an Rt- β DEXsm column.



30m, 0.32mm ID, 0.25 μ m
7A: Rt- β DEXsm (cat.# 13104)
7B: Rt- β DEXse (cat.# 13106)
Oven temp.: 40°C (hold 1 min.)
to 230°C @ 2°C/min. (hold 3
min.); **Carrier gas:** hydrogen;
80cm/sec. set @ 40°C;
Detector: FID set @ 220°C

Figure 7B
Linalool and linalyl acetate enantiomers are resolved on the Rt- β DEXse column.

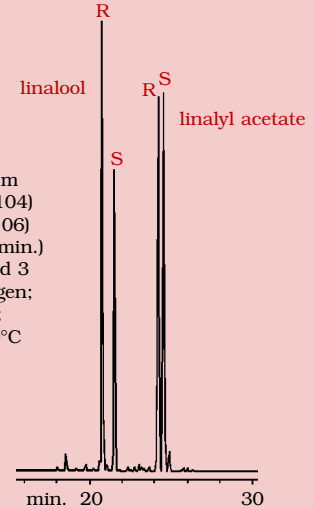


Figure 8
Menthol enantiomers are best resolved on the Rt- β DEXsp column.

30m, 0.32mm ID, 0.25 μ m Rt- β DEXsp (cat.# 13110)
Oven temp.: 60°C (hold 1 min.) to 200°C @
2°C/min.; **Carrier gas:** hydrogen; 80cm/sec. set @
40°C; **Detector:** FID set @ 220°C

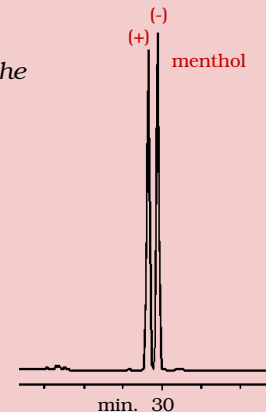


Figure 9A
1-octen-3-ol and carvone enantiomers are best resolved on an Rt- β DEXsa column.

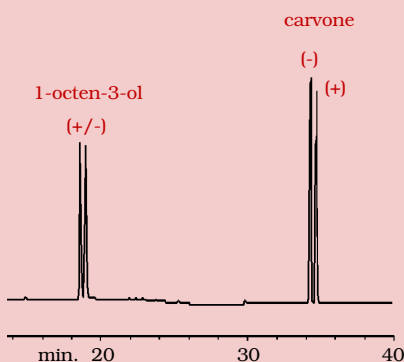
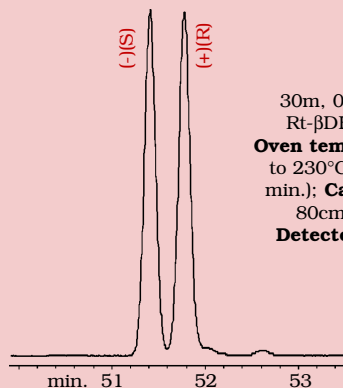


Figure 9B
An Rt- β DEXsa column provides baseline chiral resolution for β -citronellol.



30m, 0.32mm ID, 0.25 μ m
Rt- β DEXsa (cat.# 13108)
Oven temp.: 40°C (hold 1 min.)
to 230°C @ 2°C/min. (hold 3
min.); **Carrier gas:** hydrogen;
80cm/sec. set @ 40°C;
Detector: FID set @ 220°C

Figure 9C
An Rt- β DEXsa column separates racemic cis and trans rose oxides.

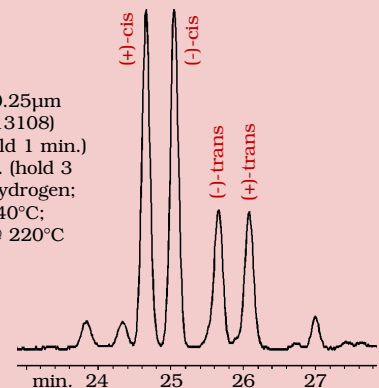


Figure 10

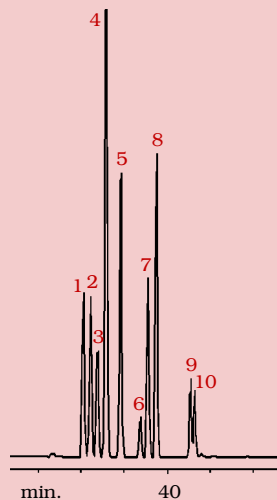
All of the irone isomers are resolved without overlapping of the enantiomeric pairs on the Rt- β DEXcst column.

1. (-)-(2R,6R)-trans- α -irone
2. (+)-(2S,6S)-trans- α -irone
3. (+)-(2R,6R)-trans- γ -irone
4. (-)-(2S,6S)-trans- γ -irone
5. (+)-(2R,6S)-cis- α -irone
6. (+)-(2R,6S)-cis- γ -irone
7. (-)-(2S,6R)-cis- γ -irone
8. (-)-(2S,6R)-cis- α -irone
9. (+)-(2R)- β -irone
10. (-)-(2R)- β -irone

30m, 0.32mm ID, 0.25 μ m
Rt- β DEXcst (cat.# 13102)

Oven temp.: 40°C (hold 1 min.) to
230°C @ 2°C/min. (hold 3 min.);

Carrier gas: hydrogen; 80cm/sec. set @ 40°C;
Detector: FID set @ 220°C



Rt- β DEXcst

This column is optimum for semi-volatile chiral compounds because lower boiling components show peak broadening (discussed in the "Optimization of Chiral Separations" section). All of the irone isomers (found in iris flowers) are resolved without overlapping of the enantiomeric pairs (**Figure 10**). This is also the best column for resolution of the γ - and δ -lactones (**Figures 11A and B**).

The Rt- β DEXcst column is good for separating the enantiomers of some barbiturates and TFA-derivatives of amphetamines as well. This is discussed in further detail on pages 22 and 23.

Figure 11

The Rt- β DEXcst column provides maximum resolution of the γ -lactones and δ -lactones.

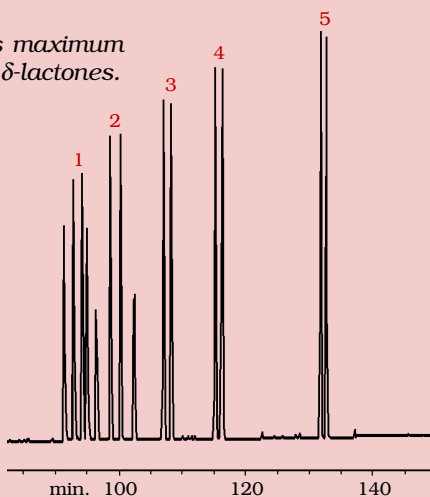
A. γ -lactones on the Rt- β DEXcst column

1. (+/-)- γ -heptalactones
2. (+/-)- γ -octalactones
3. (+/-)- γ -nonalactones
4. (+/-)- γ -decalactones
5. (+/-)- γ -dodecalactones

30m, 0.32mm ID, 0.25 μ m
Rt- β DEXcst (cat.# 13102)

Oven temp.: 60°C (hold 1 min.) to 200°C
@ 1°C/min.; Carrier gas: hydrogen;
40cm/sec. set @ 60°C;

Detector: FID set @ 220°C



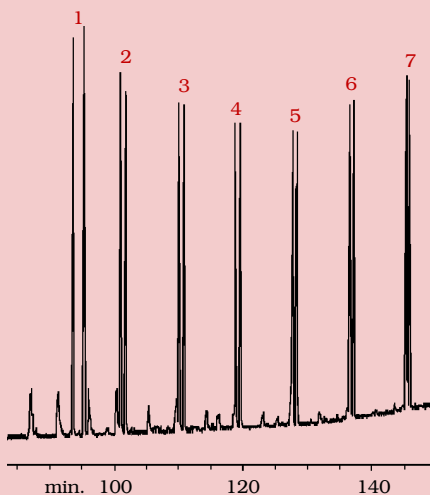
B. δ -lactones on the Rt- β DEXcst column

1. (+/-)- δ -pentalactones
2. (+/-)- δ -hexalactones
3. (+/-)- δ -heptalactones
4. (+/-)- δ -octalactones
5. (+/-)- δ -nonalactones
6. (+/-)- δ -decalactones
7. (+/-)- δ -dodecalactones

30m, 0.32mm ID, 0.25 μ m
Rt- β DEXcst (cat.# 13102)

Oven temp.: 60°C (hold 1 min.) to 200°C
@ 1°C/min.; Carrier gas: hydrogen;
40cm/sec. set @ 60°C;

Detector: FID set @ 220°C



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assistance.

To demonstrate the abilities of the five different types of chiral columns, we analyzed twenty-five chiral compounds commonly found in flavors, fragrances, and pharmaceutical analyses. The extent to which two enantiomers are resolved (or any two peaks) can be determined by the resolution equation and are known as resolution factors, sometimes de-

noted R. An R value of 1.5 indicates baseline resolution. Resolution factors for all chiral compounds on all the β -cyclodextrin columns were compared to those obtained on the existing Rt- β DEXm (permethylated cyclodextrin) column. **Table I** shows the degree of enantiomeric separation by resolution factor for all twenty-five components on each column. The col-

umn that has the largest resolution factor provides the best separation of a particular compound. These values can easily be compared to help determine which column is optimum for specific chiral components. **Charts 1–6** illustrate the degree of enantiomeric separation of each compound on each chiral column.

Table I
Resolution of common chiral compounds on Restek's cyclodextrin columns.

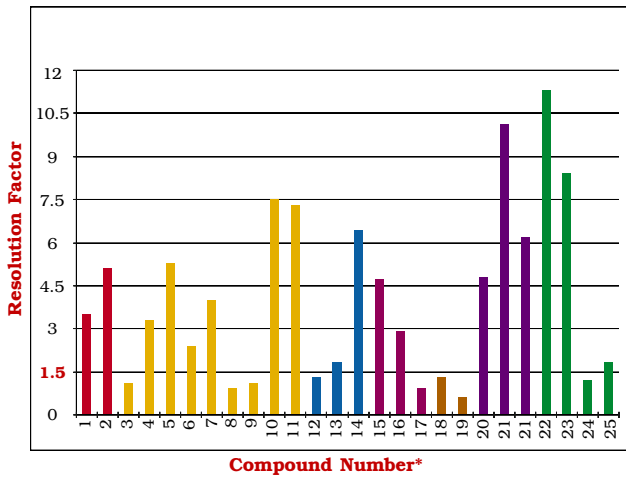
| Compounds | Formula | m.w. | Column Resolution Factors | | | | | | |
|-----------------|-----------------------------|---|---------------------------|-------------------|-------------------|-------------------|--------------------|------------------|-------|
| | | | Rt- β DEXsm | Rt- β DEXse | Rt- β DEXsp | Rt- β DEXsa | Rt- β DEXcst | Rt- β DEXm | |
| Terpenes | 1. α -pinene | C ₁₀ H ₁₆ | 136 | 3.50 | 0.80 | ns | ns | 0.90b | 3.30 |
| | 2. limonene | C ₁₀ H ₁₆ | 136 | 5.10 | 7.30 | 3.20 | ns | 2.70b | 1.40 |
| Alcohols | 3. 1-octen-3-ol | C ₈ H ₁₆ O | 128 | 1.10 | ns | 0.50 | 2.30 | 0.60b | 0.50t |
| | 4. linalool | C ₁₀ H ₁₈ O | 154 | 3.30 | 6.00 | 4.30b | 3.70 | 2.60t | 1.00 |
| | 5. α -terpineol | C ₁₀ H ₁₈ O | 154 | 5.30 | 5.50 | 2.20 | 4.00 | 4.30t | 1.70 |
| | 6. terpinen-4-ol | C ₁₀ H ₁₈ O | 154 | 2.40 | 2.20 | ns | ns | 1.20t | 1.80t |
| | 7. isoborneol | C ₁₀ H ₁₈ O | 154 | 4.00 | 3.30t | ns | ns | 1.90t | 2.00 |
| | 8. β -citronellol | C ₁₀ H ₂₀ O | 156 | 0.90 | 0.80 | ns | 1.00t | ns | ns |
| | 9. menthol | C ₁₀ H ₂₀ O | 156 | 1.10 | 1.10 | 2.20 | ns | 1.10 | 1.60t |
| | 10. 2,3-butanediol | C ₄ H ₁₀ O ₂ | 90 | 7.50 | 8.10 | 2.20 | 4.60 | 4.00 | 2.60 |
| | 11. 1-phenylethanol | C ₈ H ₁₀ O | 122 | 7.30 | 6.40 | 1.10 | 7.80 | 6.30 | 6.60 |
| Ketones | 12. carvone | C ₁₀ H ₁₄ O | 150 | 1.30 | ns | ns | 2.50 | 1.10 | ns |
| | 13. camphor | C ₁₀ H ₁₆ O | 152 | 1.80 | 2.10t | 1.30b | 4.30 | 2.50t | ns |
| | 14. α -ionone | C ₁₃ H ₂₀ O | 192 | 6.40 | 3.20 | 1.30 | 4.40 | ns | 3.20 |
| Lactones | 15. γ -nonalactone | C ₉ H ₁₆ O ₂ | 156 | 4.70 | 5.00 | 3.40 | 4.40 | 5.90 | 1.00 |
| | 16. γ -undecalactone | C ₁₁ H ₂₀ O ₂ | 184 | 2.90 | 2.90 | 1.70 | 3.70 | 4.50 | ns |
| | 17. δ -decalactone | C ₁₀ H ₁₈ O ₂ | 170 | 0.90 | ns | ns | 2.10 | 2.70 | ns |
| Esters | 18. ethyl-2-methylbutyrate | C ₇ H ₁₄ O ₂ | 130 | 1.30 | 3.50 | 1.10b | ns | ns | ns |
| | 19. linalylacetate | C ₁₂ H ₂₀ O ₂ | 196 | 0.60 | 2.20 | 1.20 | 0.10 | ns | ns |
| Epoxides | 20. styreneoxide | C ₈ H ₈ O | 120 | 4.80 | 10.20 | 6.50 | 1.00 | 1.40t | 2.50t |
| | 21. trans-linalooloxides | C ₁₀ H ₁₈ O ₂ | 170 | 10.10 | 2.40 | ns | 1.50 | 4.30b | 6.80* |
| | 21. cis-linalooloxides | | | 6.20 | 3.60 | ns | 1.00 | 3.50b | 4.20* |
| Drugs | 22. hexobarbital | C ₁₂ H ₁₆ O ₃ N ₂ | 236 | 11.30 | 4.70 | 0.90 | ns | 8.90 | 6.30 |
| | 23. mephobarbital | C ₁₃ H ₁₄ O ₃ N ₂ | 246 | 8.40 | 3.80 | 0.60 | ns | 6.20 | 6.10 |
| | 24. fenfluramine | C ₁₂ H ₁₆ F ₃ N | 231 | 1.80 | 2.90t | ad | ad | ns | ad |
| | 25. fenfluramine TFAA der. | C ₁₄ H ₁₅ F ₆ NO | 327 | 1.20 | ns | ns | 2.40 | 3.40 | ns |

ns = no separation of enantiomers
t = peak tailing
b = peak broadening

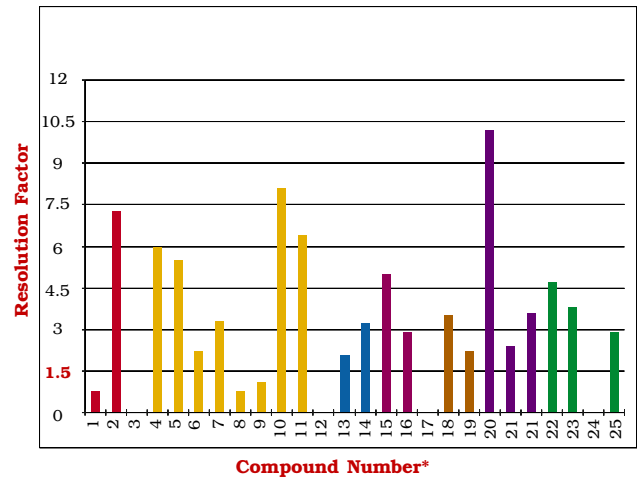
ad = adsorption of nonderivatized drug compound
* = cis and trans linalool oxides analyzed @ 9.0 psi head pressure

Charts 1-6 illustrate the unique separation capabilities of each cyclodextrin column.

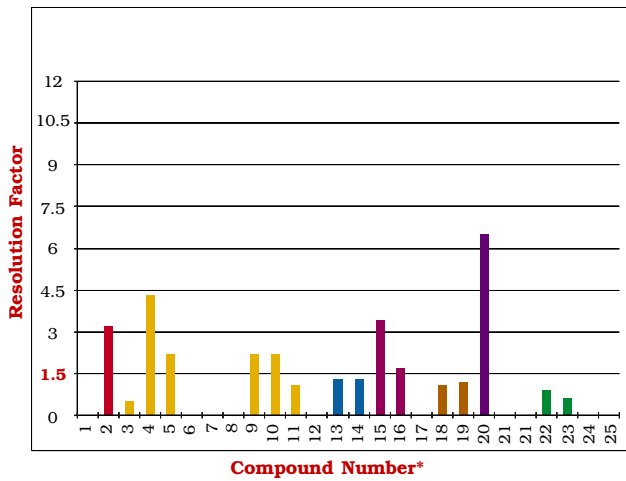
Rt-βDEXsm



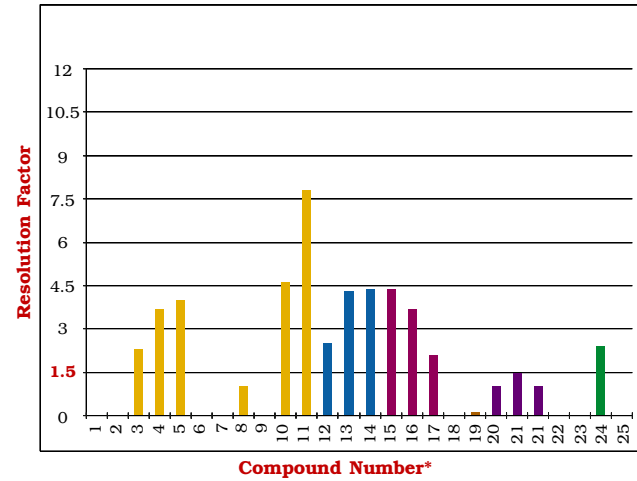
Rt-βDEXse



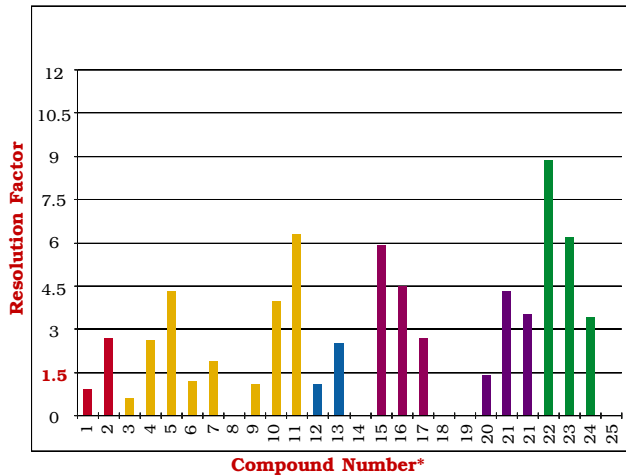
Rt-βDEXsp



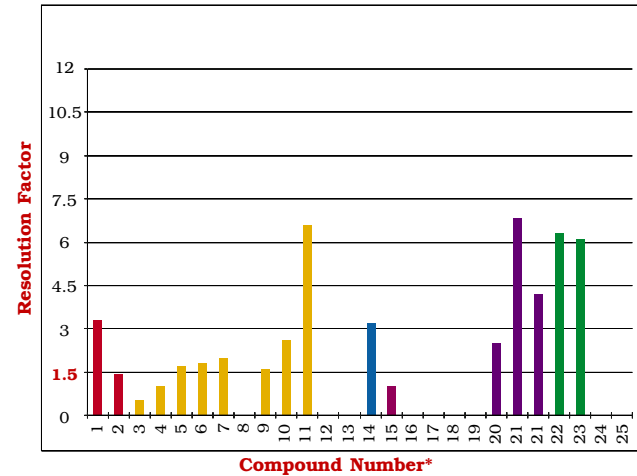
Rt-βDEXsa



Rt-βDEXcst



Rt-βDEXm



*Refer to Table I for compound identification.

OPTIMIZATION OF CHIRAL SEPARATIONS

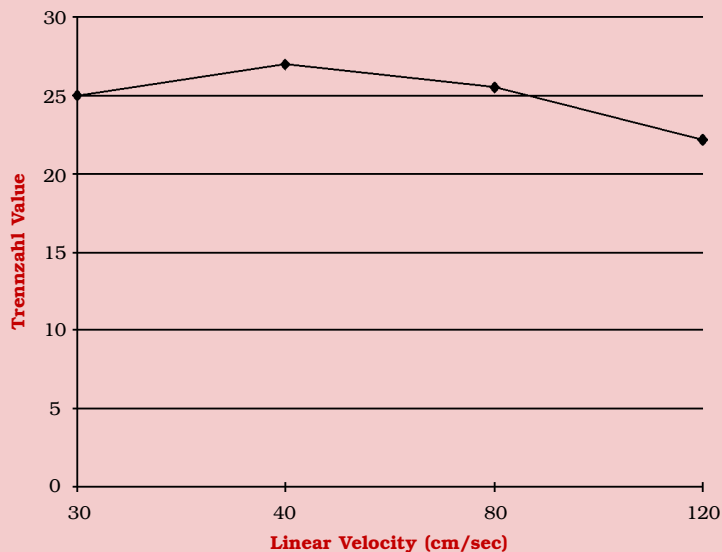
Although the new β -cyclodextrin columns can resolve a variety of chiral compounds, certain parameters must be optimized to obtain maximum separation and column performance. Variation in linear velocity and temperature ramp rate can greatly affect the resolution of enantiomers. Depending on the type of chiral column, initial GC oven temperature can affect peak width. Column sample capacity varies with different compounds, and overloading results in broad tailing peaks and reduced enantiomeric separation.

Linear Velocity (Column Flow)

The resolution between the enantiomeric pairs can be improved by increasing the linear velocity. This is especially important if the resolution factor is below two for optical isomers (see Table I). Trennzahl values are measurements of column separation efficiency, which are often optimum at a linear velocity of 40 cm/sec with hydrogen carrier gas. This is illustrated in **Figure 12A**. Although optimal linear velocity can be different for each chiral compound and column, the typical optimum linear velocity for maximum enantiomeric separation is around 80 cm/sec with hydrogen carrier gas, as illustrated with six chiral compounds on the Rt- β DEXsa column in **Figure 12B**. This is twice the linear velocity required to achieve maximum efficiency as indicated by the Trennzahl values of 1-octen-3-ol enantiomers in **Figure 12A**.

Figure 12A

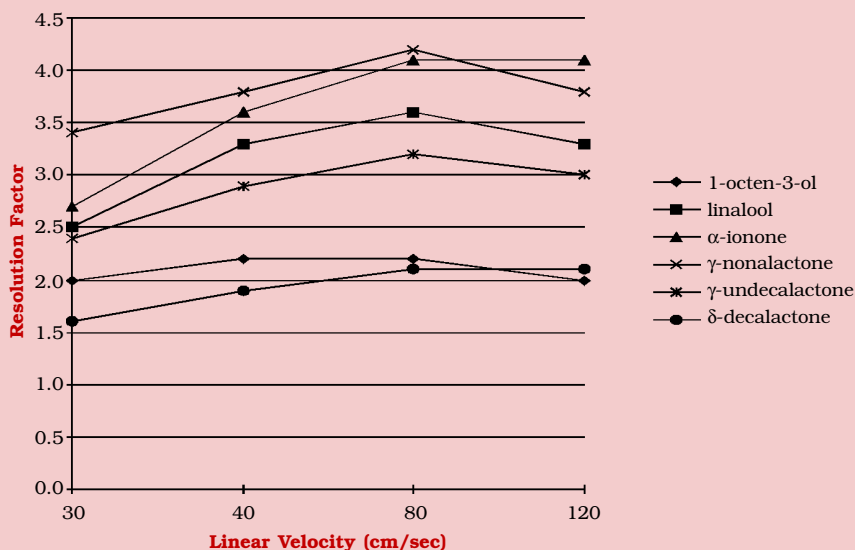
Higher Trennzahl values are obtained at 40 cm/sec with hydrogen carrier gas.



Rt- β DEXsa: 40°C to 200°C @ 2°C/min. (hold 1 min.).
Hydrogen carrier gas.

Figure 12B

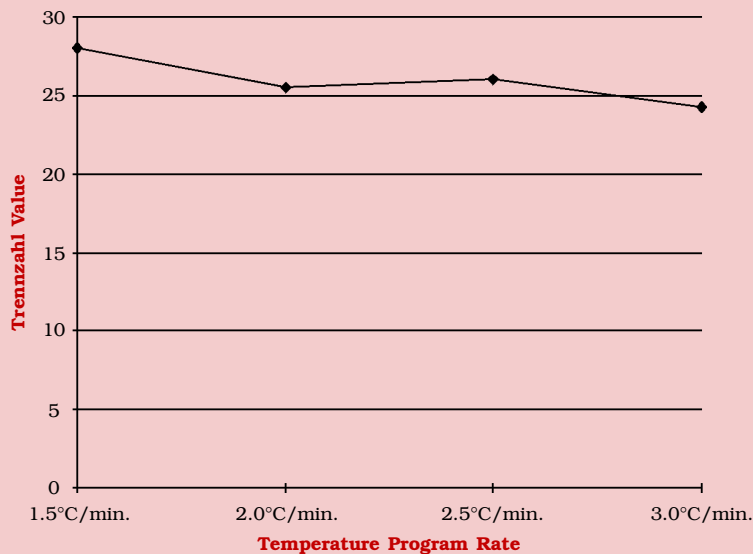
Faster linear velocities provide maximum resolution of chiral pairs.



Rt- β DEXsa: 40°C (80°C for lactones) to 200°C @ 2°C/min. (hold 1 min.). Hydrogen carrier gas.

Figure 13A

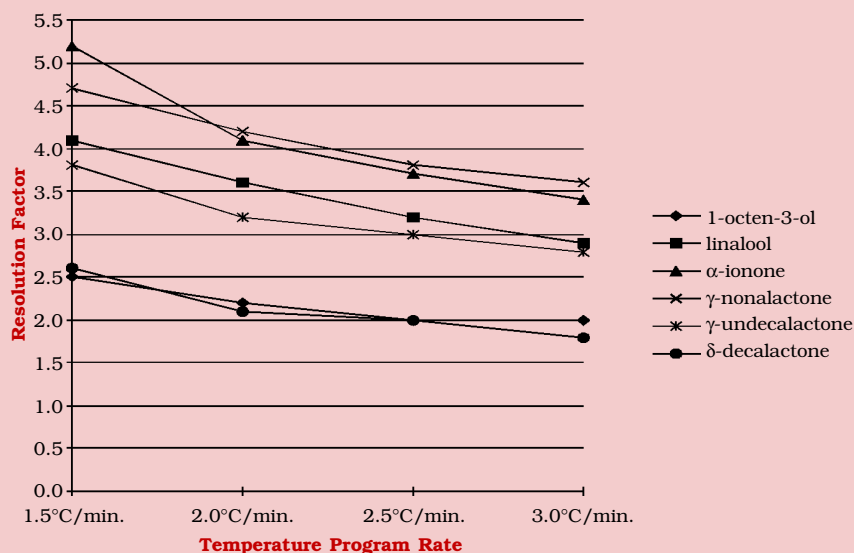
Trennzahl values increase with enantiomeric resolution factors as temperature ramp rates decrease.



Rt- β DEXsa: 40°C to 200°C @ 2°C/min. (hold 1 min.).
Hydrogen carrier gas.

Figure 13B

Lower temperature ramp rates provide maximum resolution of chiral pairs.



Rt- β DEXsa: 40°C (80°C for lactones) to 200°C @
2°C/min. (hold 1 min.). Hydrogen carrier gas.

Temperature Program

The resolution between the enantiomeric pairs can be improved by using slow temperature ramp rates. The best temperature ramp rates are 1-2°C/min. Trennzahl values improve along with enantiomeric resolution as the temperature ramp rate is decreased (**Figures 13A and B**).

Remember, to optimize chiral separation use:

- 1) Faster linear velocities (80 cm/sec.) with hydrogen carrier gas.
- 2) Slower temperature ramp rates (1-2°C/min.).
- 3) Appropriate minimum operating temperature (40 or 60°C).
- 4) On-column concentrations of 50ng or less.

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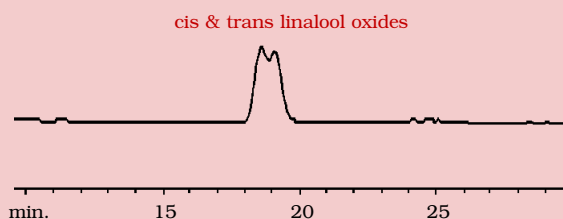
Minimum Temperature

For maximum resolution of chiral compounds with low boiling points (below 100°C), initial temperatures of 35-40°C are recommended for the Rt-βDEXsm, Rt-βDEXse, and Rt-βDEXsa columns. In contrast, the same volatile compounds exhibit a very broad peak shape on the Rt-βDEXsp and Rt-βDEXcst columns at these initial oven temperatures. Linalool oxides are volatile compounds that exhibit peak broadening and almost no resolution on the Rt-βDEXcst column with an initial oven temperature of 40°C (**Figure 14A**). The peak shapes and overall resolution of the linalool oxides improve when initial temperature is increased to 70°C, even though the individual enantiomers of both the cis and trans isomers are not separated (**Figure 14B**). Higher initial temperatures do not always completely eliminate peak broadening for some components. However, the improvement in solvent peak shape indicates a physical transition of the cyclodextrin macromolecules from a crystalline structure to a liquid at this higher temperature. Thus the recommended minimum operating temperature of Rt-βDEXsp and Rt-βDEXcst columns is 60°C.

Higher initial temperatures do not always completely eliminate peak broadening for some components. However, the improvement in solvent peak shape indicates a physical transition of the cyclodextrin macromolecules from a crystalline structure to a liquid at this higher temperature.

Figure 14A

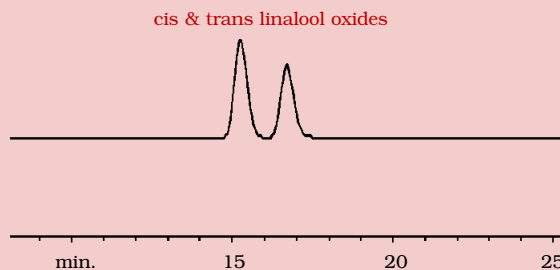
Linalool oxides exhibit extreme peak broadening and poor resolution on the Rt-βDEXcst column with an initial oven temperature of 40°C.



30m, 0.32mm ID, 0.25µm Rt-βDEXcst (cat.# 13102)
Oven temp.: 40°C (hold 1 min.) to 200°C @
 2°C/min.; **Carrier gas:** hydrogen; 80cm/sec. set @ 40°C;
Detector: FID set @ 220°C

Figure 14B

Linalool oxides exhibit improved peak shape and resolution on the Rt-βDEXcst column when initial oven temperature is increased to 70°C.

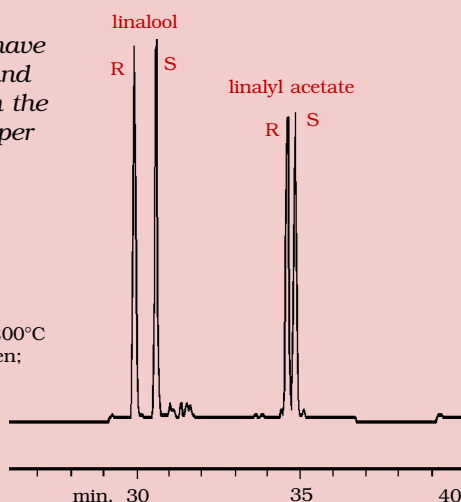


30m, 0.32mm ID, 0.25µm Rt-βDEXcst (cat.# 13102)
Oven temp.: 70°C (hold 1 min.) to 200°C @
 2°C/min.; **Carrier gas:** hydrogen; 80cm/sec. set @ 40°C;
Detector: FID set @ 220°C

Figure 15A

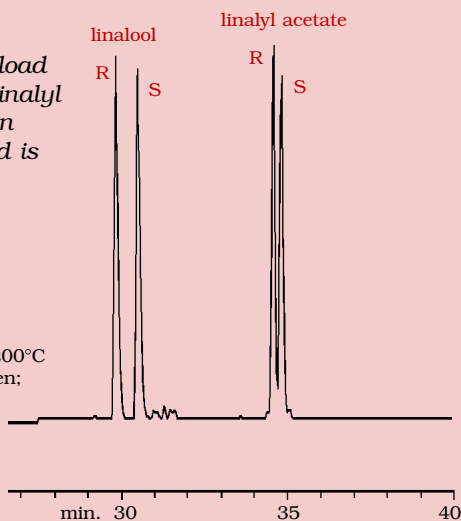
Linalool and linalyl acetate have symmetrical peak shapes and excellent chiral separation on the Rt- β DEXse column at 25ng per component on-column.

30m, 0.32mm ID, 0.25 μ m
Rt- β DEXse (cat.# 13106)
Oven temp.: 40°C (hold 1 min.) to 200°C
@ 2°C/min.; **Carrier gas:** hydrogen;
80cm/sec. set @ 40°C;
Detector: FID set @ 220°C

**Figure 15B**

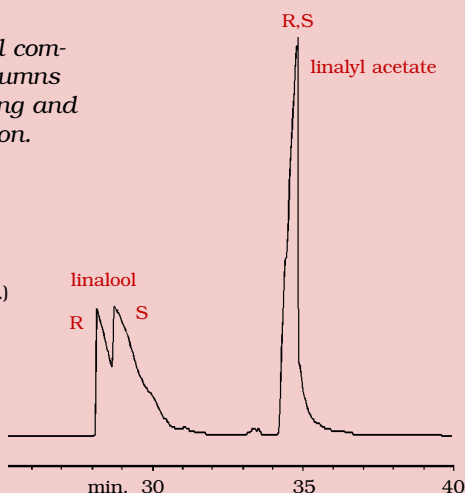
Linalool shows signs of overload with slight peak tailing, and linalyl acetate has a small loss in resolution when sample load is increased to 160ng.

30m, 0.32mm ID, 0.25 μ m
Rt- β DEXse (cat.# 13106)
Oven temp.: 40°C (hold 1 min.) to 200°C
@ 2°C/min.; **Carrier gas:** hydrogen;
80cm/sec. set @ 40°C;
Detector: FID set @ 220°C

**Figure 15C**

Excessive overload of chiral compounds on cyclodextrin columns results in extreme peak tailing and complete loss in resolution.

30m, 0.32mm ID, 0.25 μ m
Rt- β DEXse (cat.# 13106)
Oven temp.: 40°C (hold 1 min.)
to 200°C @ 2°C/min.;
Carrier gas: hydrogen;
80cm/sec. set @ 40°C;
Detector: FID set @ 220°C



Overloading and Tailing

Some chiral compounds show overloading at lower concentrations than achiral compounds. One reason is the different amounts of cyclodextrin (5-50%) dissolved in the stationary phase. Unlike the classical fronting peaks of normal stationary phases, the characteristic of an overloaded peak on cyclodextrin stationary phases is indicated by a tailing peak. Overloading chiral compounds results in loss of resolution, even when column capacity has not been exceeded. **Figure 15A** shows the enantiomeric separations of linalool and linalyl acetate on the Rt- β DEXse. The amount for each component in the column is about 25 ng. **Figure 15B** shows the same components at a higher concentration of 160ng on-column. Note that the linalool enantiomers are beginning to tail, and there is a small loss in chiral resolution for linalyl acetate. Even though the maximum sample capacity for 0.32mm ID capillary columns is normally 400-500ng per component, the peak shapes of chiral compounds indicate overload at one-third of the sample amount. Again, there is much less cyclodextrin for which a chiral compound can interact. **Figure 15C** shows pronounced overloading of these compounds at 5 μ g on-column. Extreme tailing and complete loss in resolution are the result.

CHIRAL SPECIFIC APPLICATIONS OF ESSENTIAL OILS, FLAVORS, AND PHARMACEUTICALS

ESSENTIAL OILS

Chiral capillary GC has proven to be a convenient method for characterizing essential oils and differentiating natural flavors from those of synthetic origin. Chiral compounds from natural origins usually exist as one predominant optical isomer. Also, the inspection of enantiomeric ratios can characterize regional differences between oils. Although sometimes a result of processing, the presence of racemic pairs (one-to-one ratios of each enantiomer) most often indicates adulteration or unnatural origin.

Since most chiral compounds naturally exist as one predominant isomer, resolution is more challenging, especially for components in higher concentrations. For primary constituents in essential oils, select a chiral column that provides a resolution factor value greater than two to overcome possible loss of resolution.

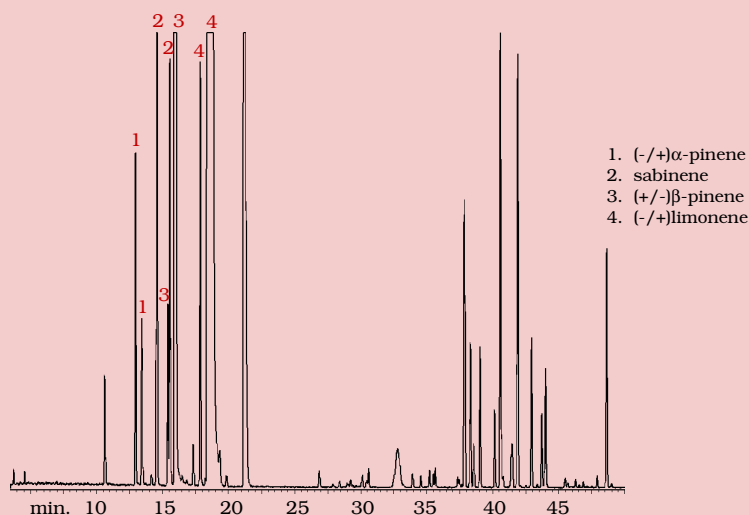
Since essential oils are mixtures of many compounds, coelution of peaks and overlapping of certain optical pairs are sometimes hard to avoid. Not all of the chiral compounds found in an essential oil or flavor extract may separate on the same column. Connecting two different columns together is possible, but the elution order of some enantiomers may reverse with this combination, resulting in loss of separation. Dual column analysis is a logical alternative to obtain a more complete enantiomeric profile and to provide confirmational identification of individual constituents. To reduce analysis time, both columns can be installed into the same injection port for simultaneous confirmation. (Consult Restek's Chromatography Products Guide for more information about dual column analysis.)



For primary constituents in essential oils, select a chiral column that provides a resolution factor value greater than two to overcome possible loss of resolution.

Figure 16

The Rt- β DEXsm column provides chiral resolution of the primary terpenes in Artificial Lemon oil.

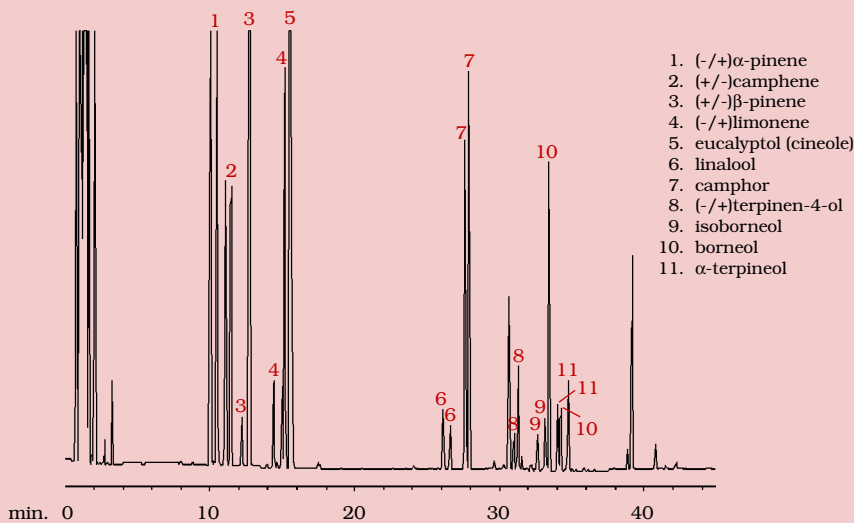


1. (-/+) α -pinene
2. sabinene
3. (+/-) β -pinene
4. (-/+limonene

30m, 0.32mm ID, 0.25 μ m Rt- β DEXsm (cat.# 13104);
Oven temp.: 40°C (hold 1 min.) to 200°C @ 2°C/min. (hold 3 min.);
Carrier gas: hydrogen; 80cm/sec.; **Detector:** FID set @ 220°C

Figure 17

The Rt- β DEXsm, the most versatile β -cyclodextrin column for essential oil analysis, resolves enantiomers of α -pinene, β -pinene, camphene, limonene, linalool, camphor, terpinen-4-ol, α -terpineol, borneol, and isoborneol in rosemary oil.



1. (-/+) α -pinene
2. (+/-)camphene
3. (+/-) β -pinene
4. (-/+limonene
5. eucalyptol (cineole)
6. linalool
7. camphor
8. (-/+terpinen-4-ol
9. isoborneol
10. borneol
11. α -terpineol

30m, 0.32mm ID, 0.25 μ m Rt- β DEXsm (cat.# 13104);
Oven temp.: 40°C (hold 1 min.) to 200°C @ 2°C/min. (hold 3 min.);
Carrier gas: hydrogen; 80cm/sec.; **Detector:** FID set @ 220°C

Lemon Oil

The Rt- β DEXsm is the optimum column for obtaining chiral profiles of Lemon and other citrus oils since it provides enantiomeric separation for the main terpene constituents like α - and β -pinenes, sabinene (these enantiomers overlap with those of β -pinene) and limonene (**Figure 16**).

The Rt- β DEXsm is the optimum column for obtaining chiral profiles of Lemon and other citrus oils.



Rosemary Oil

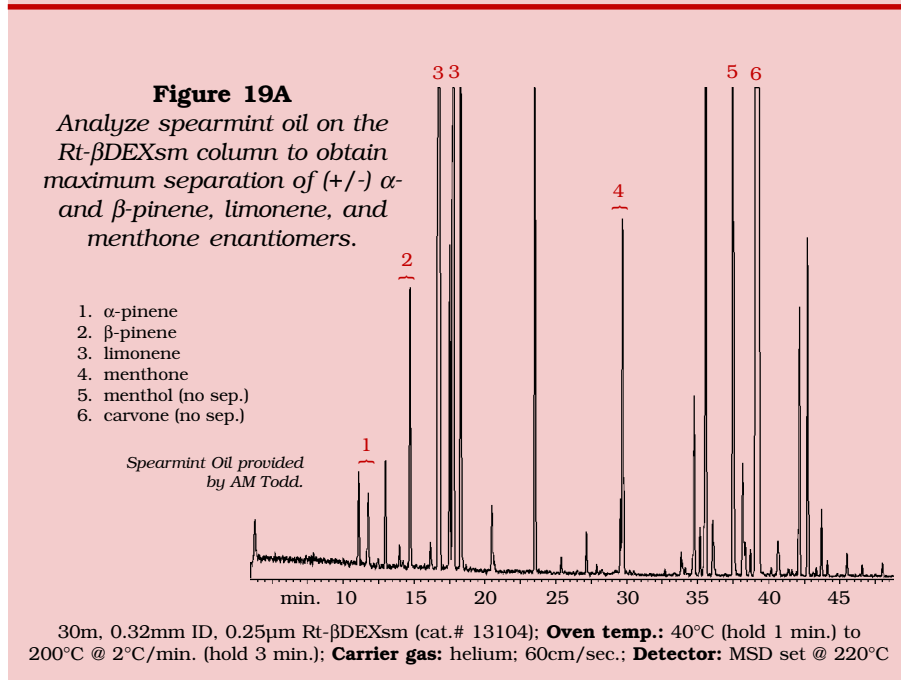
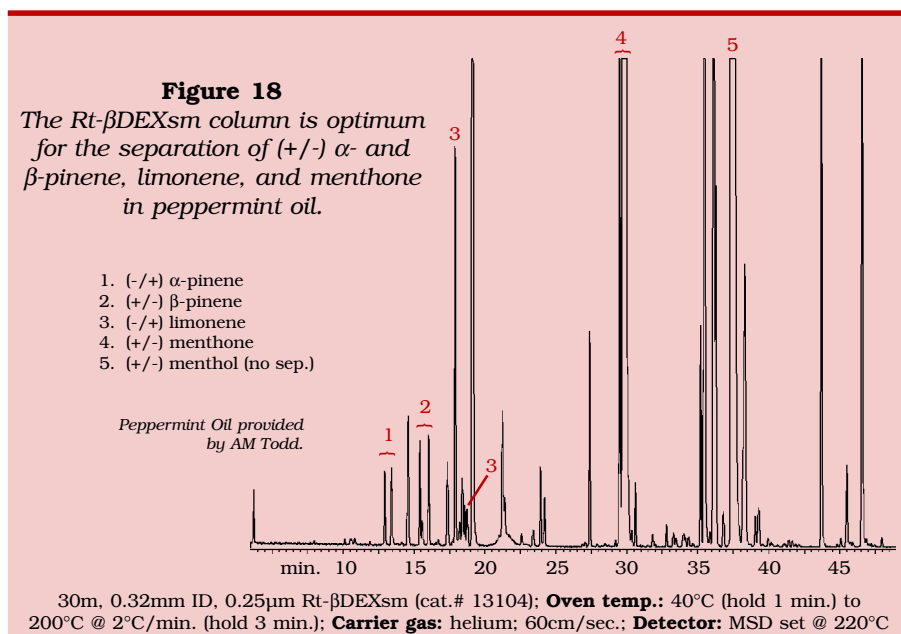
Chiral constituents in this oil include α -pinene, β -pinene, camphene, limonene, linalool, camphor, terpinen-4-ol, α -terpineol, borneol, and isoborneol. Baseline enantiomeric separation is easily achieved for all of these compounds on the new Rt- β DEXsm column. The common permethylated β -cyclodextrin column cannot completely resolve the optical isomers of limonene, linalool, and camphor (**Figure 17**).

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Peppermint Oil

The Rt- β DEXsm column is optimum for the separation of (+/-) α - and β -pinene, limonene and menthone. Since menthone and menthol enantiomers are major constituents of peppermint oil, reducing the sample size to prevent overloading of these components and provide better enantiomeric resolution may be necessary.

An alternative solution is to use an Rt- β DEXsp as a secondary column since it provides better resolution of menthol. However, do not use this column in a dual column system with an Rt- β DEXsm. The minimum temperatures of these phases differ by 20°C, which would minimize volatile terpene separation on the Rt- β DEXsm (**Figure 18**).



Spearmint Oil

The Rt- β DEXsm column yields maximum separation of (+/-) α - and β -pinene, limonene and menthone enantiomers in spearmint oil. The enantiomeric ratios of the primary chiral constituents in an artificial spearmint oil can be seen in the chromatogram shown in **Figure 19A**.

Although the optical isomers of carvone best separate on the Rt- β DEXsa column, α -pinene and limonene do not. For the separation of carvone, use a dual column system comprised of 30-meter Rt- β DEXsm and Rt- β DEXsa columns, since both have a minimum operating temperature of 40°C. **Figure 19B** compares carvone in natural sources of spearmint oil to a racemic standard on the Rt- β DEXsa column.

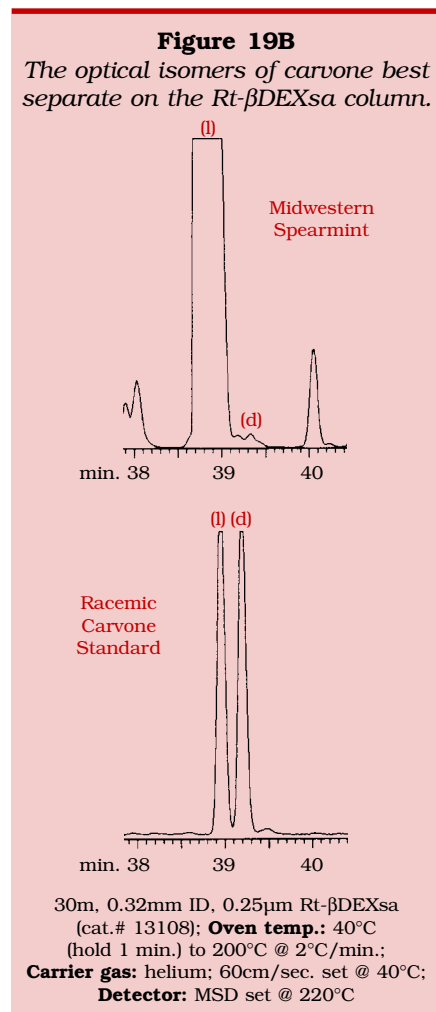
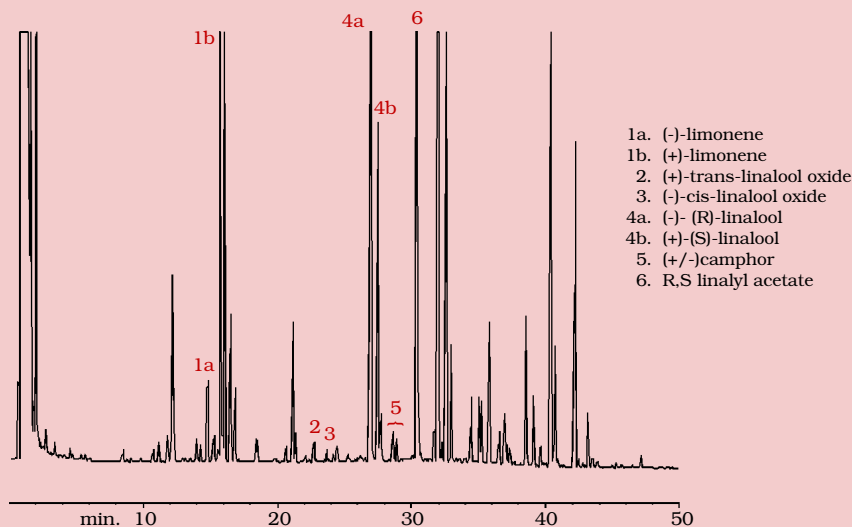


Figure 20A

Linalool and linalool oxides in lavender oils are stereochemically separated on the Rt-βDEXsm column.

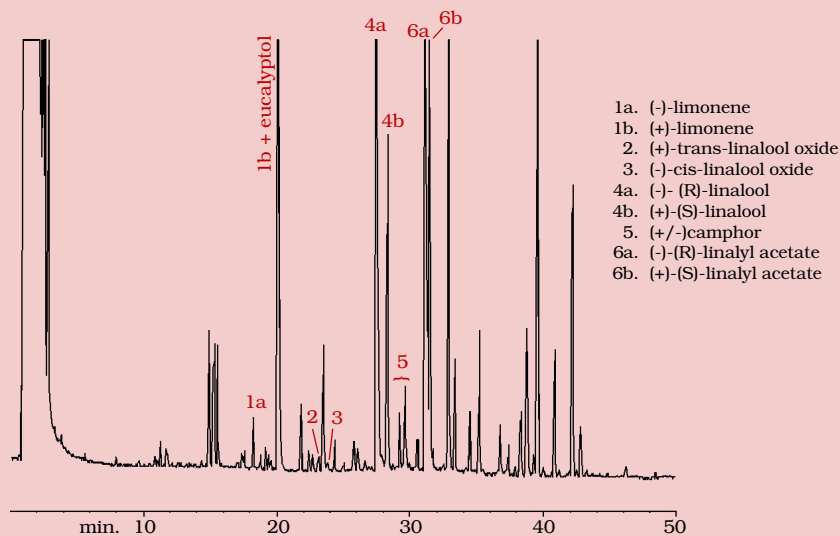


- 1a. (-)-limonene
- 1b. (+)-limonene
2. (+)-trans-linalool oxide
3. (-)-cis-linalool oxide
- 4a. (-)- (R)-linalool
- 4b. (+)- (S)-linalool
5. (+/-)-camphor
6. R,S linalyl acetate

30m, 0.32mm ID, 0.25μm Rt-βDEXsm (cat.# 13104);
Oven temp.: 40°C (hold 1 min.) to 200°C @ 2°C/min. (hold 3 min.);
Carrier gas: hydrogen; 80cm/sec.; **Detector:** FID set @ 220°C
Lavender oils provided by Belmay.

Figure 20B

The Rt-βDEXse column resolves enantiomers of linalyl acetate in lavender oils.



- 1a. (-)-limonene
- 1b. (+)-limonene
2. (+)-trans-linalool oxide
3. (-)-cis-linalool oxide
- 4a. (-)- (R)-linalool
- 4b. (+)- (S)-linalool
5. (+/-)-camphor
- 6a. (-)- (R)-linalyl acetate
- 6b. (+)- (S)-linalyl acetate

30m, 0.32mm ID, 0.25μm Rt-βDEXse (cat.# 13106);
Oven temp.: 40°C (hold 1 min.) to 200°C @ 2°C/min. (hold 3 min.);
Carrier gas: hydrogen; 80cm/sec.; **Detector:** FID set @ 220°C
Lavender oils provided by Belmay.

Lavender Oil

The Rt-βDEXsm column separates the enantiomers of the primary chiral compounds found in lavender oil, including linalool. Both the cis and trans enantiomeric pairs of the furanoid linalool oxides, which contribute characteristic odors to lavender oils and Clary sage oil, are separated on this column. (R)-Linalool is present in at least 85% enantiomeric excess. (2R)-Configured linalool oxides are present in about 77% enantiomeric excess in authentic Lavender oils.² Both the cis and trans (R)-linalool oxides are essentially enantiomerically pure in this oil, as shown in **Figure 20A** (peaks 2 and 3).

Linalyl acetate is another primary constituent in lavender oils. The (R)-(-) enantiomer is predominant in authentic lavender oils.³ A dual column system comprised of both the Rt-βDEXsm and Rt-βDEXse columns can be used to resolve the enantiomers of linalyl acetate as well (peak 6 in **Figure 20B**). Note that the (-)-(R)-enantiomer constitutes >92% of linalyl acetate in this lavender oil.

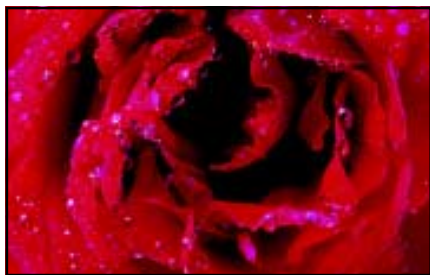
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 or call 800-356-1688,
 ext. 4, for technical
 assistance.**

Geranium Oil

Chiral constituents in geranium oils include cis and trans rose oxides, linalool, and β -citronellol. The Rt- β DEXsa column provides chiral resolution for all of these compounds. In authentic samples of geranium oil, (-)-(4R)-configured diastereomers of cis- and trans-rose oxides predominate over their (+)-enantiomers.³ The (-)-(S) form of β -citronellol is 74-80% of the enantiomeric ratio.⁴ Note that cis- and trans-rose oxides and β -citronellol are racemic in this particular commercial geranium oil, as shown in **Figure 21A**. These racemic compounds indicate that this oil is not authentic.

Rose Oil

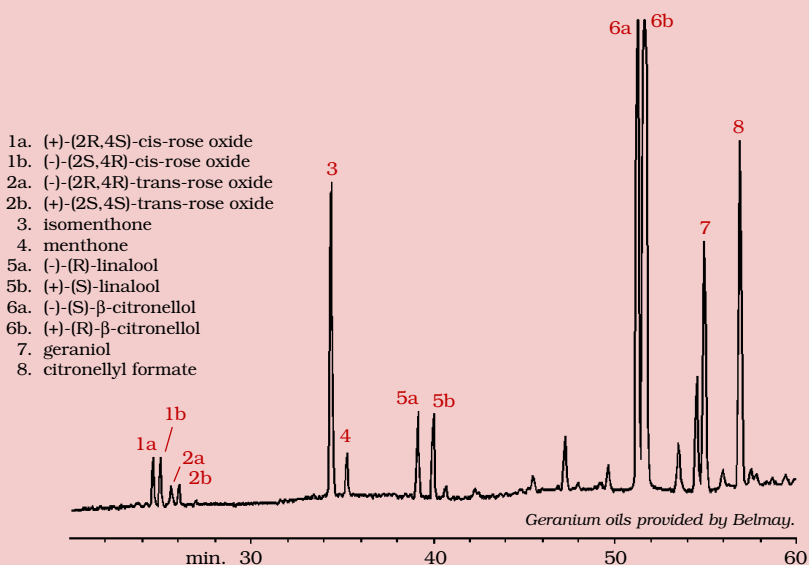
As with geranium oils, (-)-(2S,4R)-cis and (-)-(2R,4R)-trans rose oxides and (-)-(S)- β -citronellol are specific indicators of genuine rose oils.⁵ Note the enantiomeric purity of these compounds in Rose Oil Maroc, as shown in **Figure 21B**.



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 ext. 4, for technical
 assistance.

Figure 21A

The Rt- β DEXsa column provides chiral resolution for cis and trans rose oxides, linalool, and β -citronellol in Chinese Geranium oil.

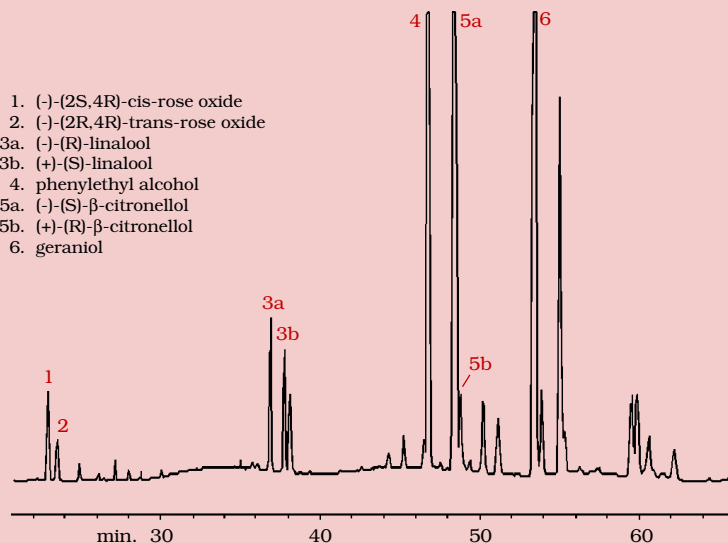


- 1a. (+)-(2R,4S)-cis-rose oxide
- 1b. (-)-(2S,4R)-cis-rose oxide
- 2a. (-)-(2R,4R)-trans-rose oxide
- 2b. (+)-(2S,4S)-trans-rose oxide
3. isomenthone
4. menthone
- 5a. (-)-(R)-linalool
- 5b. (+)-(S)-linalool
- 6a. (-)-(S)- β -citronellol
- 6b. (+)-(R)- β -citronellol
7. geraniol
8. citronellyl formate

30m, 0.25mm ID, 0.25 μ m Rt- β DEXsa (cat.# 13109);
Oven temp.: 60°C to 110°C @ 1°C/min. (hold 30 min.);
Carrier gas: hydrogen; 40cm/sec. set @ 60°C; **Detector:** FID set @ 220°C

Figure 21B

The Rt- β DEXsa column reveals authenticity of Rose oil Maroc.

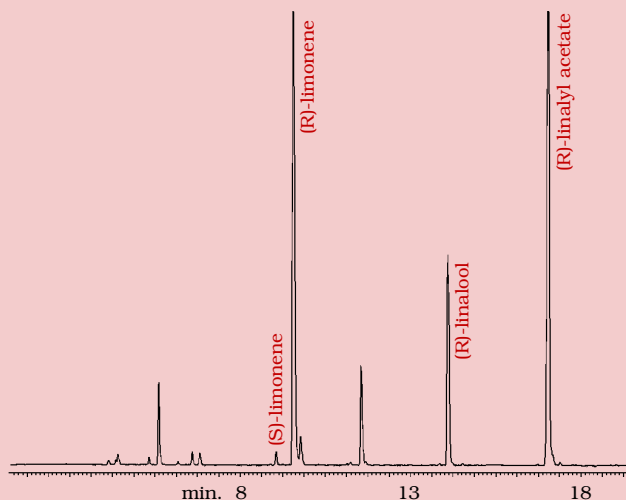
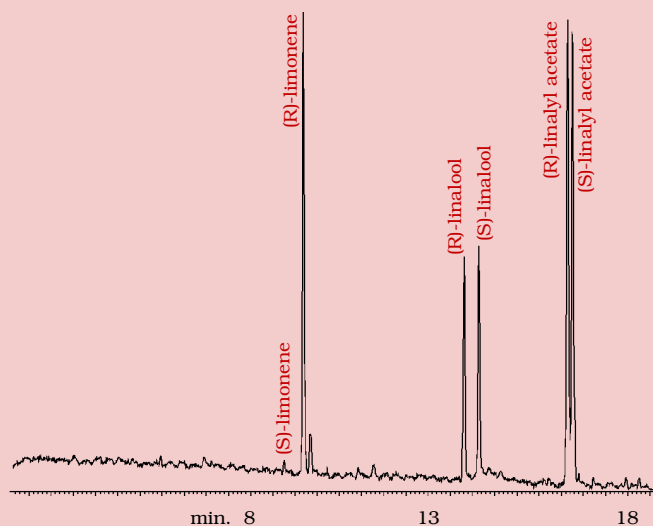


1. (-)-(2S,4R)-cis-rose oxide
2. (-)-(2R,4R)-trans-rose oxide
- 3a. (-)-(R)-linalool
- 3b. (+)-(S)-linalool
4. phenylethyl alcohol
- 5a. (-)-(S)- β -citronellol
- 5b. (+)-(R)- β -citronellol
6. geraniol

30m, 0.25mm ID, 0.25 μ m Rt- β DEXsa (cat.# 13109);
Oven temp.: 60°C to 110°C @ 1°C/min. (hold 30 min.);
Carrier gas: hydrogen; 40cm/sec. set @ 60°C; **Detector:** FID set @ 220°C

Figure 22

The Rt- β DEXse column can differentiate Bergamot extract from Bergamot flavor.

A: Bergamot Extract**B: Bergamot Flavor**

30m, 0.32mm ID, 0.25 μ m Rt- β DEXse (cat.# 13106);

Oven temp.: 40°C (hold 1 min.) to 200°C @ 4°C/min.;

Carrier gas: helium; 60cm/sec. set @ 40°C; Detector: MSD set @ 220°C

FLAVORS

Bergamot oil and a few of the popular fruit flavorings such as raspberry, strawberry, and peach were examined. The composition of extracts from natural sources were compared to those from commercially available flavored teas and drinks. Some target chiral compounds examined were linalool and linalyl acetate in bergamot oil, α -ionone and δ -decalactone in raspberry, and γ -lactones in peach extracts.

Bergamot Flavor

A genuine cold-pressed bergamot oil should contain only the (R)-isomers of linalool and linalyl acetate.⁶ The enantiomeric purity of (R) limonene should also be considered.⁷ Chromatogram A in **Figure 22** is a natural source of bergamot oil. Only the (R)-enantiomers of limonene, linalool and linalyl acetate were present. Chromatogram B illustrates an extract from an artificially flavored tea. Both samples were analyzed on an Rt- β DEXse column. The presence of racemic linalool and linalyl acetate indicates bergamot flavor of unnatural origin.

Peach Flavor

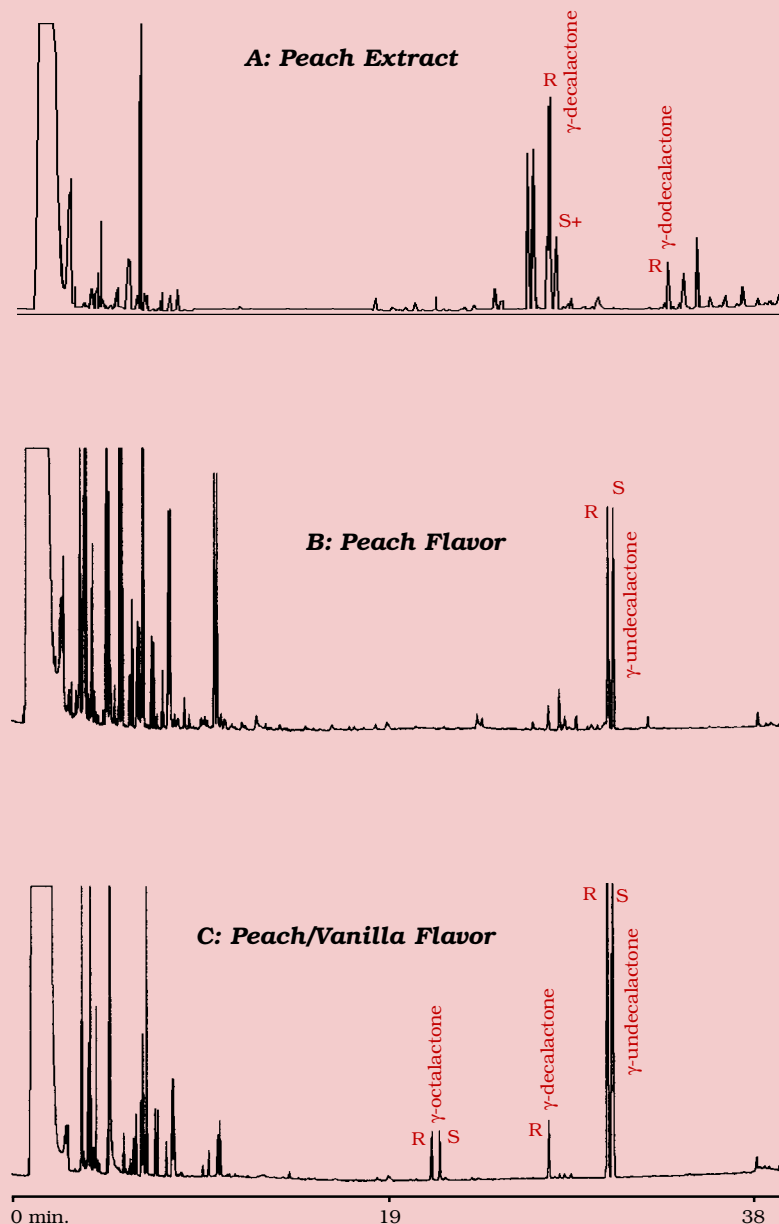
Both δ - and γ -lactones are present in peaches, but only the γ -lactones are analyzed for the adulteration of peach flavor. Gamma-decalactone occurs in the 89% (R) : 11%(S)- enantiomers in natural Peach flavor.⁸ In **Figure 23**, Chromatogram A is a peach extract. A significant amount of the (R)- γ -decalactone was present, along with the (S)-enantiomer, which coeluted with an unknown. A small amount of (R)- γ -dodecalactone was also detected. Chromatogram B is an extract from a beverage with “all natural” peach flavor. Gamma-decalactone was not present, but racemic γ -undecalactone was found in a 1:1 ratio. This was the same result with another peach-flavored beverage. Chromatogram C is from an “all natural-flavored beverage,” with peach and vanilla flavors. Although only the (R)-enantiomer of γ -decalactone was present, the amount is very small. Both γ -octalactone and γ -undecalactone were found to be racemic, indicating adulteration.



The γ -lactones are inspected for the adulteration of peach flavor.

Figure 23

The γ -lactones are analyzed for the adulteration of peach flavor on the Rt- β DEXsa column.

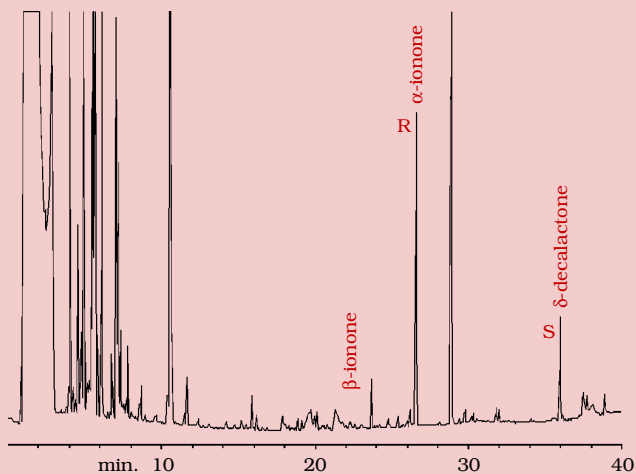


30m, 0.32mm ID, 0.25 μ m Rt- β DEXsa (cat.# 13108); **Oven temp.:** 60°C (hold 2 min.) to 100°C @ 15°C/min., then to 220°C @ 3°C/min.;

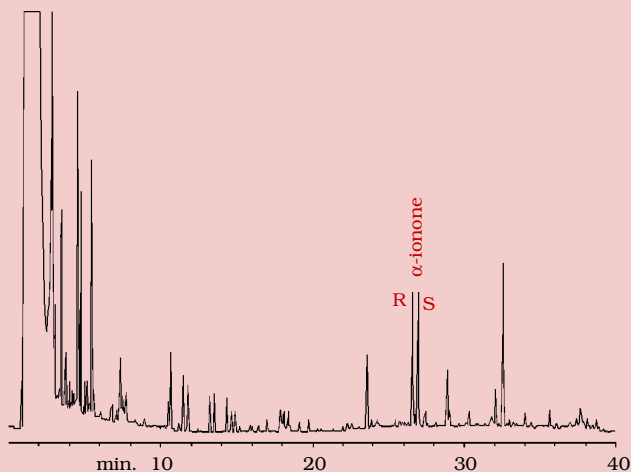
Carrier gas: helium; 60cm/sec. set @ 60°C; **Detector:** MSD set @ 220°C.

Figure 24

The Rt- β DEXsa column resolves isomers of α -ionone to determine raspberry authenticity.

A: Raspberry Extract

30m, 0.32mm ID, 0.25 μ m Rt- β DEXsa (cat.# 13108);
Oven temp.: 60°C (hold 2 min.) to 200°C @ 3°C/min.;
Carrier gas: helium; 60cm/sec. set @ 60°C; **Detector:** MSD set @ 220°C.

B: Raspberry Flavor

30m, 0.32mm ID, 0.25 μ m Rt- β DEXsa (cat.# 13108);
Oven temp.: 60°C (hold 2 min.) to 200°C @ 3°C/min.;
Carrier gas: helium; 60cm/sec. set @ 60°C; **Detector:** MSD set @ 220°C.

Raspberry Flavor

Alpha-ionone from raspberries occurs as an enantiopure (R)(+)-enantiomer, illustrated on an Rt- β DEXsa column in **Figure 24A**. Chromatogram B represents a “naturally flavored” raspberry iced tea. A racemic mixture of α -ionone was present, indicating that it is not a completely natural raspberry flavor. Thus, α -ionone serves as a good marker compound for determining raspberry authenticity.



Alpha-ionone serves as a good marker compound for determining raspberry authenticity.

DRUGS

Stereochemical properties of chiral drugs have been found in many instances to be the controlling factor concerning activity. One enantiomer may provide a biological function. The other may be inactive or exhibit another functionality, which could result in side effects. In some cases, one optical isomer may be harmful. The FDA requires drug manufacturers to test the individual enantiomers of new drugs for toxicity.

Fenfluramine

Fenfluramine is an appetite suppressant to promote weight loss with obese patients.⁹ Although it is structurally similar to amphetamines, it differs somewhat pharmacologically. Norfenfluramine is a metabolite that is found in urine and serum of patients. The purpose of the chiral isolations, like many analyses in the pharmaceutical industry, is of proprietary nature. The TFA derivatives of both fenfluramine and norfenfluramine are separated into their enantiomers on the Rt- β DEXcst column (Figure 25).

Barbiturates

Mephobarbital and Hexobarbital are barbiturates with sedative, hypnotic and anticonvulsant properties.¹⁰ Because psychological and physical dependence may occur with continuing use, they are controlled substances in the U.S. Code of Federal Regulations. The optical isomers of these barbiturates can be simultaneously resolved on an Rt- β DEXcst column (Figure 26).

Amphetamines

Dextroamphetamine (*d*-amphetamine), *d,l*-amphetamine, and *d*-methamphetamine are sympathomimetic amines with central nervous system stimulant activity.¹¹ They are significant drugs of abuse in the United States and are included among the drugs to be tested under

Figure 25

The optical isomers of TFA-fenfluramine and its metabolite are well resolved on the Rt- β DEXcst column.

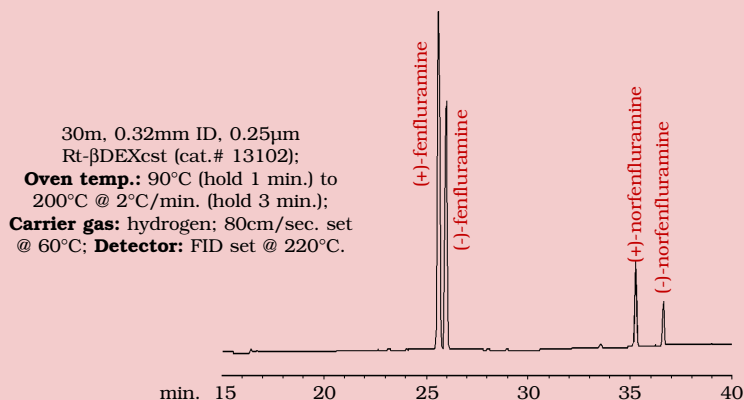


Figure 26

The Rt- β DEXcst column can resolve the enantiomers of common barbiturates.

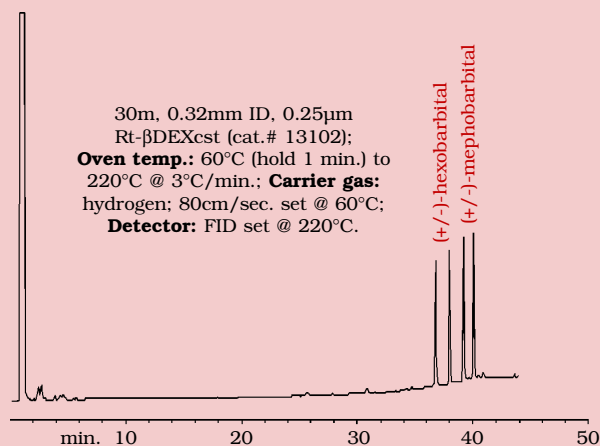
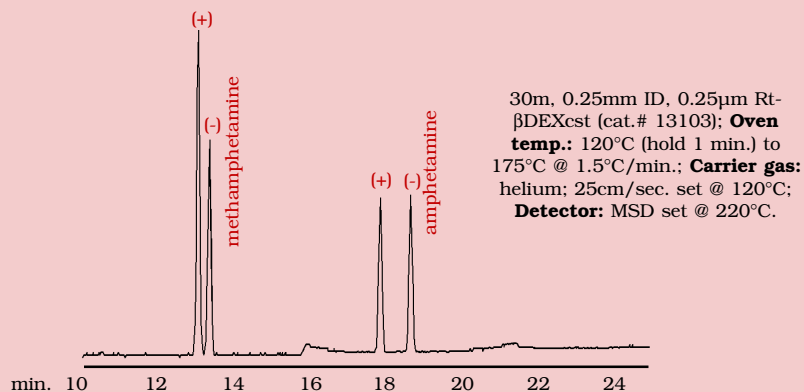


Figure 27

The Rt- β DEXcst column simultaneously resolves the enantiomers of TFA-amphetamine and TFA-methamphetamine.



the federal guidelines for workplace drug testing.¹² However, *l*-methamphetamine (deoxyephedrine) is found in over-the-counter decongestants and is not a controlled substance.¹³ Enantiomeric separation of these

compounds, which is necessary for accurate interpretation of drug tests, is easily achieved on the Rt- β DEXcst chiral capillary GC column (**Figure 27**).



Stereochemical properties of chiral drugs have been found in many instances to be the controlling factor concerning activity. One enantiomer may provide a biological function and the other may be inactive or may exhibit another functionality, which could result in side effects.

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PRODUCT LIST

| Rt- β DEXsm (30m, 0.25 μ m) | | | | Rt- β DEXse (30m, 0.25 μ m) | | | |
|--|-------|------------|------------|---------------------------------------|-------|------------|------------|
| mm ID | cat.# | min. temp. | max. temp. | mm ID | cat.# | min. temp. | max. temp. |
| 0.25 | 13105 | 40°C | 230°C | 0.25 | 13107 | 40°C | 230°C |
| 0.32 | 13104 | 40°C | 230°C | 0.32 | 13106 | 40°C | 230°C |
| Rt- β DEXsa (30m, 0.25 μ m) | | | | Rt- β DEXsp (30m, 0.25 μ m) | | | |
| 0.25 | 13109 | 40°C | 230°C | 0.25 | 13111 | 60°C | 230°C |
| 0.32 | 13108 | 40°C | 230°C | 0.32 | 13110 | 60°C | 230°C |
| Rt- β DEXcst (30m, 0.25 μ m) | | | | Rt- β DEXm (30m, 0.25 μ m) | | | |
| 0.25 | 13103 | 60°C | 230°C | 0.25 | 13100 | 40°C | 230°C |
| 0.32 | 13102 | 60°C | 230°C | 0.32 | 13101 | 40°C | 230°C |

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