Applications note

Food & Flavor

Grape Flavor Analysis Using an Rt-γDEXsa™ GC Column

The enantiomeric ratios of certain chiral compounds found in flavorings can sometimes reveal adulteration. Two chiral indicators in grape flavor are methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate. Methyl-3-hydroxybutyrate is racemic and (R)-ethyl-3-hydroxybutyrate is at least 77% predominant in natural grape flavor.[1] Extreme alterations of these ratios can indicate that the flavor is not completely authentic.

Although the exact mechanisms of compound-cyclodextrin interaction are not known, it is evident that the size of the cyclodextrin cavity is important. Perhaps some compounds may be too large to effectively interact with the cavity size of beta-cyclodextrin, which is composed of seven glucopyranose units. Gamma-cyclodextrins are composed of eight glucopyranose units and so possess a larger cavity, which may be more interactive with larger chiral molecules (see **Figure 1**).

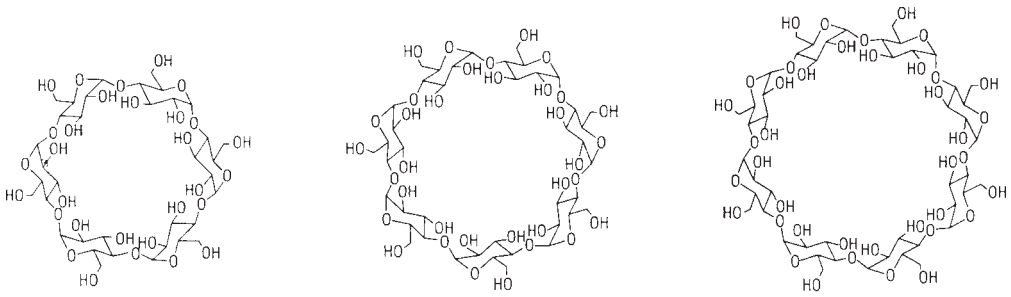
Beta-cyclodextrin stationary phases provide enantioselectivity for a variety of chiral compounds, but not for methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate. **Figure 2** (on next page) illustrates the analysis of these compounds on three beta-cyclodextrin stationary phases: the Rt-βDEXm™ column (2,3,6-tri-O-methyl-β-cyclodextrin), the Rt-βDEXsm™ column (2,3-di-O-methyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin), and the Rt-βDEXsa™ column (2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin). The peaks tail and exhibit no chiral separation on the Rt-βDEXm™ column. Different derivatives on the cyclodextrin molecule can help increase selectivity of enantiomers. Although the Rt-βDEXsm™ and Rt-βDEXsa™ columns show improved enantioselectivity for methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate, the peaks still tail and separation of the optical isomers is not baseline-resolved.

Analysis of methyl-3-hydroxybutyrate and ethyl-3-hydroxybutyrate on the new Rt-γDEXsa™ column reveals excellent chiral selectivity for both, as shown in two types of grape juice in **Figure 3** (on next page). The Rt-γDEXsa™ contains 2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-γ-cyclodextrins that are dissolved into cyanopropyl phenyl stationary phase (Rtx®-1701). This composition promotes thermal stability to a maximum temperature of 230°C and longevity that is comparable to other capillary columns. This column is available in 0.32mm ID and 0.25mm ID for direct interfacing into a mass spectrometer.

Previous articles have demonstrated the utility of beta-cyclodextrin columns for flavor analysis using gas chromatography (GC), but sometimes the larger gamma-cyclodextrin cavities provide better enantioselectivity for chiral indicating compounds. Restek's Rt-γDEXsa™ column provides better separation of specific chiral compounds in grape flavor than most beta-cyclodextrin phases.

Figure 1

*Structures of beta- and gamma-2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-cyclodextrins.*



α-Cyclodextrin *β-Cyclodextrin* *γ-Cyclodextrin*

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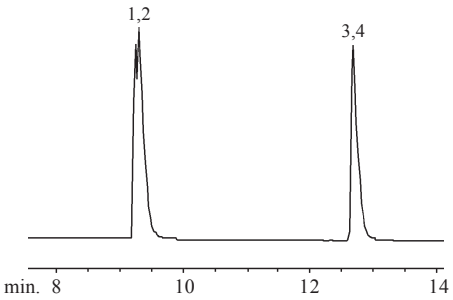
Figure 2

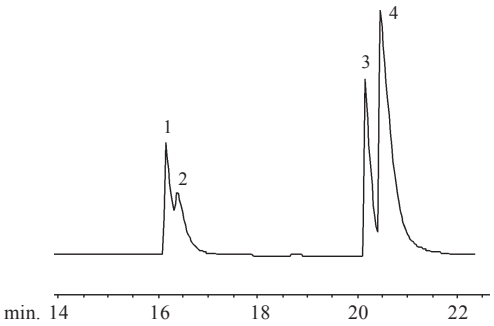
Beta-cyclodextrin columns do not provide optimum separation of methyl- and ethyl-3-hydroxy butyrate.

A) The Rt-βDEXm™ column exhibits no enantiomeric selectivity.

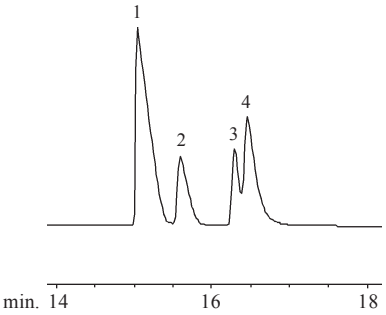
1. (S)-(+)-methyl-3-hydroxybutyrate
2. (R)-(–)-methyl-3-hydroxybutyrate
3. (S)-(+)-ethyl-3-hydroxybutyrate
4. (R)-(–)-ethyl-3-hydroxybutyrate



B) The Rt-βDEXsm™ column exhibits poor enantiomeric separation.



C) The Rt-βDEXsa™ column exhibits improved enantiomeric separation.

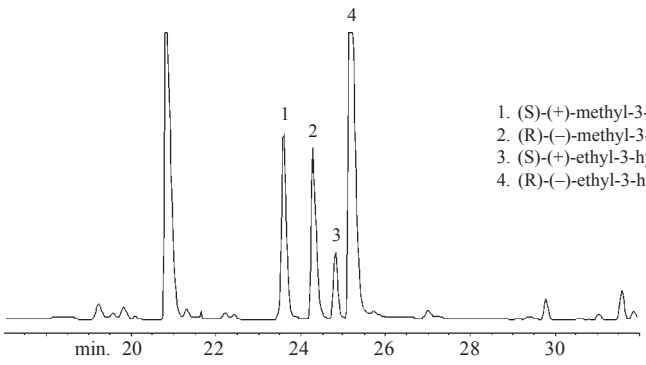


30m, 0.32mm ID, 0.25µm a) Rt-βDEXm™; b) Rt-βDEXsm™; and c) Rt-βDEXsa™ columns (cat.#s 13101, 13104, and 13108 respectively). 1.0µL split injection of methyl- and ethyl-3-hydroxybutyrate; **On-column concentration:** ~150ng/enantiomer; **Oven temp.:** 40°C (hold 1 min.) to 200°C @ 2°C/min.; **Inj. / det. temp.:** 200°C/230°C; **Carrier gas:** H_2; **Linear velocity:** 80cm/sec. set @ 40°C; **Split ratio:** 25:1.

Figure 3

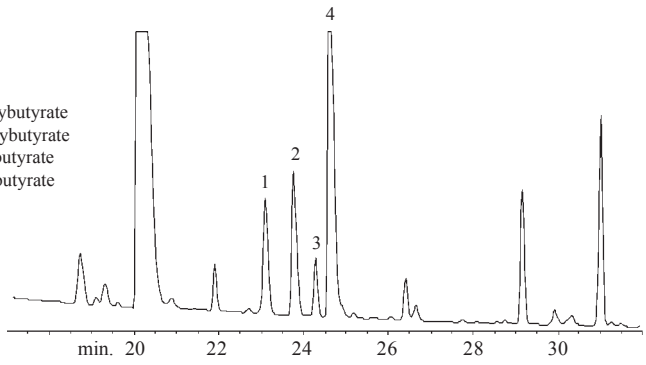
The Rt-γDEXsa™ column provides excellent chiral selectivity for both methyl- and ethyl-3-hydroxybutyrate in the flavor extracts of grape juice and white grape juice.

Fig. 3a - grape juice



1. (S)-(+)-methyl-3-hydroxybutyrate
2. (R)-(–)-methyl-3-hydroxybutyrate
3. (S)-(+)-ethyl-3-hydroxybutyrate
4. (R)-(–)-ethyl-3-hydroxybutyrate

Fig. 3b - white grape juice



30m, 0.32mm ID, 0.25µm Rt-γDEXsa™ (cat.# 13112) 10µL direct injection of grape juice (Fig. 3a) and white grape juice (Fig. 3b) using a 4mm open-top Uniliner® sleeve (cat.# 20843). **Oven temp.:** 40°C (hold 1 min.) to 230°C @ 2°C/min.; **Inj. / det. temp.:** 200°C/230°C; **Carrier gas:** H_2; **Linear velocity:** 80cm/sec. set @ 40°C.

Comparing Grape Juices and Grape-Flavored Beverages

Extraction Procedure

Grape juice, white grape juice, grape drink, grape-flavored soda, and a grape-flavored sport drink were evaluated. Each 16–20oz. beverage was added to a 500mL separatory funnel. Thirty milliliters of methylene chloride were added to the sample in the separatory funnel, which was shaken for 3–5 minutes. The extract was then collected into a beaker. This procedure was repeated three times. The organic extract was then funneled through anhydrous sodium sulfate to eliminate water and transferred to a Kuderna-Danish collector with a Snyder column. This was immersed into a hot water bath of 65°C until the extract was concentrated to 4mL.

Analysis

Ten microliters of sample was introduced via direct injection. A 1.5m x 0.53mm guard column was connected to the 4mm open-top Uniliner® sleeve and to the 30m, 0.32mm ID, 0.25µm Rt-γDEXsa™ column, to accommodate the large volume injection and to protect the analytical column. Some spectral confirmation was conducted by GC/MS on a 30m, 0.25mm ID, 0.25µm Rt-γDEXsa™ column, using splitless analysis.

Results

The methyl-3-hydroxybutyrate was essentially racemic in both the grape and white grape juices, as shown in **Figure 3a** and **3b**. It was also racemic in one grape-flavored drink, but at much lower concentrations. Analysis on a 0.25mm ID Rt-γDEXsa™ column with a slower linear velocity provides resolution of benzaldehyde and (R)-methyl-3-hydroxybutyrate (**Figure 4**). The (R)-ethyl 3-hydroxybutyrate was predominant in both juices and grape-flavored drink. Neither chiral compound was detected in the grape-flavored soda or sport drink.

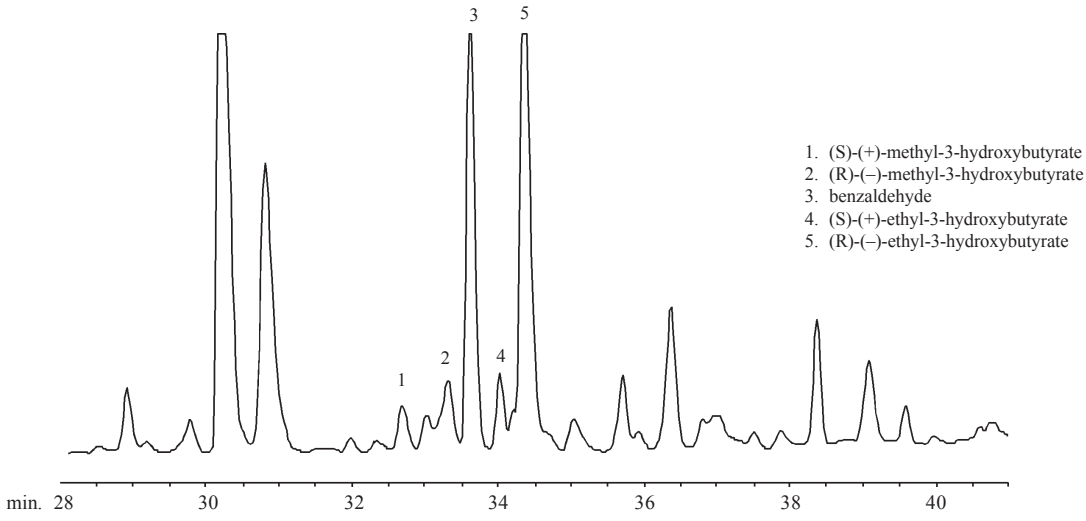
Beta-cyclodextrin phases can separate a variety of chiral indicating compounds in flavors, but are not effective in all applications. Using different cyclodextrin derivatives can help chiral selectivity, but going to a larger cyclodextrin sometimes is necessary. Switching from a 2,3-di-O-methyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin to a 2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-β-cylcodextrin column partially improved enantiomer separation for chiral indicating compounds in grape flavor. However, the 2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl-γ-cyclodextrin column provided the best enantiomeric profile of these compounds. The Rt-γDEXsa™ column allows detection of racemic methyl-3-hydroxybutyrate and (R)-ethyl-3-hydroxybutyrate in juices and drinks that contain authentic grape flavor. This column can offer certain separations that cannot be achieved by beta-cyclodextrin columns and may be a more suitable alternative for your chiral analysis.

References

1. Dr. Joulain, Robertet Corp., “private communication.”

Figure 4

The 0.25mm ID Rt-γDEXsa™ column provides detection of benzaldehyde, and methyl-and ethyl-3-hydroxybutyrate.



30m, 0.25mm ID, 0.25µm Rt-γDEXsa™ (cat.# 13113) 10µL splitless injection of a grape-flavored drink. **Oven temp.:** 40°C (hold 1 min.) to 200°C @ 2°C/min.; **Inj. / det. temp.:** 200°C/230°C; **Carrier gas:** He; **Linear velocity:** 35cm/sec. set @ 40°C; **Splitless hold time:** 1 min.

Product Listing

Rt-γDEXsa™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13113
0.32mm	0.25	40 to 230°C	13112

Rt-βDEXsa™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13109
0.32mm	0.25	40 to 230°C	13108

Rt-βDEXm™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13100
0.32mm	0.25	40 to 230°C	13101

Rt-βDEXsm™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13105
0.32mm	0.25	40 to 230°C	13104

To Optimize Chiral Separations Use:

1) Faster linear velocities (80cm/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.

Direct Injection Sleeves for HP/Finnigan GCs

Description	Each	5-pk.
4mm ID Uniliner®	20335	20336
4mm ID Cyclo-Uniliner®	20337	20338
4mm ID Open-top Uniliner® w/wool	20843	20844

Restek offers a wide range of cyclodextrin columns for the analysis of many chiral compounds.

Rt-βDEXse™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13107
0.32mm	0.25	40 to 230°C	13106

Rt-βDEXsp™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13111
0.32mm	0.25	40 to 230°C	13110

Rt-βDEXcst™

ID	df (µm)	Temp. Limits	30-Meter
0.25mm	0.25	40 to 230°C	13103
0.32mm	0.25	40 to 230°C	13102

RESTEK
Freude an Chromatografie
Restek GmbH
Schaberweg 23 * 61348 Bad Homburg
Tel: 06172 / 27 97 0 * Fax: 06172 / 27 97 77
info@restekgmbh.de * www.restekgmbh.de