Food & Flavor Applications note

GC Analysis of Chiral Flavor Compounds in Apple Juices Using the Rt-βDEXsm™ and Rt-βDEXse™ Columns

The fruit market has been estimated at over $12 billion per year in the U.S., and the incidence of adulterated/mislabeled juices is at least 10%, costing over $1 billion per year. This small percentage means that sugar, color, organic acids, minerals, and flavors are being sold either partially or wholly in place of the juice. Many natural flavoring materials are much more expensive to produce than the corresponding artificial materials and they command much higher prices.

Many flavor constituents are chiral compounds that usually exist as one predominant enantiomer in nature; whereas those from a synthetic origin often occur as a racemic mixture. Chiral gas chromatography (GC), which determines enantiomer ratio, is a good method to detect synthetic adulterants. Ethyl-2-methylbutyrate and 2-methylbutyrate are important constituents contributing to apple flavor, and both are naturally predominant as the (S) isomer in apple juices. Analysis of apple essence on modified cyclodextrin chiral columns can detect additions of synthetic ethyl-2-methylbutyrate. The Rt-βDEXsm™ column (2,3-di-O-methyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin) and the Rt-βDEXse™ column (2,3-di-O-ethyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin) can separate the enantiomers of 2-methylbutyrate and ethyl-2-methylbutyrate (Figure 1). In addition, the Rt-βDEXsm™ column also can resolve the optical isomers of 2-methylbutyric acid, a chiral acid that is used in significant quantities as a flavor substance. The enantiomers are not well resolved on the Rt-βDEXse™ column (Figure 2). Natural 2-methylbutyric acid has been investigated in many fruits such as apples, apricots, pineapples, and strawberries, and in all cases was found to be present in the almost enantiopure (S) form.

**Figure 1**

*Enantiomeric resolution of racemic ethyl-2-methylbutyrate and 2-methylbutyrate is achieved on Rt-βDEXsm™ and Rt-βDEXse™ columns.*

<table>
<thead>
<tr>
<th>Rt-βDEXsm™</th>
<th>1. (R)-EMB</th>
<th>2. (S)-EMB</th>
<th>3. (R)-MB</th>
<th>4. (S)-MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>min. 6</td>
<td>8</td>
<td>3</td>
<td>4</td>
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</table>

30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#'s 13104 and 13106). 10µL direct injection; Oven temp.: 40°C (hold 1 min.) to 220°C @ 2°C/min.; Inj. & det. temp.: 220°C; Carrier gas: hydrogen @ 80cm/sec.

**Figure 2**

*Enantiomeric resolution of racemic 2-methylbutyric acid is provided by the Rt-βDEXsm™ column, but not by the Rt-βDEXse™ column.*

<table>
<thead>
<tr>
<th>Rt-βDEXsm™</th>
<th>5. (S)-2-methylbutyric acid</th>
<th>6. (R)-2-methylbutyric acid</th>
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<tbody>
<tr>
<td>min. 18</td>
<td>24</td>
<td>26</td>
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30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#'s 13104 and 13106). 10µL direct injection; Oven temp.: 40°C (hold 1 min.) to 220°C @ 2°C/min.; Inj. & det. temp.: 220°C; Carrier gas: hydrogen @ 80cm/sec.
Analysis of Apple Juices

Sample Preparation
The flavor compounds were obtained by liquid-liquid extraction. Twenty milliliters of methylene chloride were added to 16–20 oz. of juice in a 500mL separatory funnel and were then shaken. After the organic layer (on the bottom) was removed, the previous step was repeated two more times. The final organic extract was then funneled through a bed of sodium sulfate and concentrated to 4mL in a Kuderna-Danish concentrator with a hot water bath at 70°C.

Analysis
The enantiomers of ethyl-2-methylbutyrate, 2-methylbutyrate, and 2-methyl butyric acid for several brands of apple juices were analyzed on both Rt-βDEXsm™ and Rt-βDEXse™ columns. Ten microliters of sample were introduced via direct injection. A 1.5m guard column was connected to the 4mm open-top Uniliner® sleeve and to the beta-cyclodextrin column to accommodate the large volume injection and to protect the analytical column. Some spectral confirmation was conducted by GC/mass spectrometry (MS).

Results
The data revealed that most of the tested samples of apple juice contained almost enantiomerically pure (S)-ethyl-2-methylbutyrate and (S)-2-methylbutyrate, as shown in Figure 3, concluding that there was no flavor adulteration of the juice. Figure 4 shows the same brand spiked with racemic ethyl-2-methylbutyrate and 2-methylbutyrate.

Figure 5B illustrates the example of matrix interference in some juices. The 2-methylbutyrate may at first appear to be racemic, because in this case (R)-2-methylbutyrate coelutes with 3-methylbutyrate on the Rt-βDEXse™ column. In Figure 4, the 3-methylbutyrate is not present in Brand “X” apple juice, but is in Brand “Y”.

Figure 3

Detection of (S)-ethyl-2-methylbutyrate and (S)-2-methylbutyrate in Brand “X” apple juice, on the Rt-βDEXsm™ and Rt-βDEXse™ columns respectively, indicates enantiomerically pure flavor.

A) Rt-βDEXsm™

B) Rt-βDEXse™

Figure 4

The analysis of Brand “X” apple juice spiked with both racemates of ethyl-2-methylbutyrate and 2-methylbutyrate shows how the presence of racemic mixtures would look.

A) Rt-βDEXsm™

B) Rt-βDEXse™

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A second column, the Rt-βDEXsm™ column, confirms the presence of 3-methylbutyrate and not racemic 2-methylbutyrate. Figure 4A shows what the presence of both racemates would look like from Brand “X” apple juice on the Rt-βDEXsm™ column. Figure 5A reveals the enantiomerically pure (S)-2-methylbutyrate on the Rt-βDEXsm™ column. This second column does not have the interference of 3-methylbutyrate and, therefore, is a good confirmational column, especially when identification confirmation by GC/MS is not available.

Inspection of 2-methylbutyric acid in all samples of juice revealed almost complete enantiomeric purity of (S)-2-methylbutyric acid. Figure 6 illustrates 2-methylbutyric acid in Brand “Z” apple juice and in cranberry/apple juice.

The Rt-βDEXsm™ and Rt-βDEXse™ columns reveal the enantiomeric ratio of key chiral flavor components in apple juice, especially in a dual-column system. Inspection of ethyl-2-methylbutyrate, 2-methylbutyrate, and 2-methylbutyric acid do not reveal any flavor adulteration of the five brands of juices analyzed, but the ability to do so has been demonstrated with chiral capillary GC.

**References**

2. ibid.

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**Figure 5**

_Dual-column analysis confirms the enantiomeric purity of (S)-2-methylbutyrate._

**A) Rt-βDEXsm™**

![Graph A](image1)

**B) Rt-βDEXse™**

![Graph B](image2)

30m, 0.32mm ID, 0.25µm a) Rt-βDEXsm™ and b) Rt-βDEXse™ columns (cat.#’s 13104 and 13106). 10µL direct injection; _Oven temp._: 40°C (hold 1 min.) to 220°C @ 2°C/min.; _Inj. & det. temp._: 220°C; _Carrier gas_: hydrogen @ 80cm/sec.

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**Figure 6**

_The Rt-βDEXsm™ column confirms enantiomeric purity of (S)-2-methylbutyric acid in apple juice and cranberry/apple juice._

**A) Brand “Z” Apple Juice**

![Graph A](image3)

**B) Cranberry/Apple Juice**

![Graph B](image4)

30m, 0.32mm ID, 0.25µm Rt-βDEXsm™ column (cat.# 13104). 10µL direct injection; _Oven temp._: 40°C (hold 1 min.) to 220°C @ 2°C/min.; _Inj. & det. temp._: 220°C; _Carrier gas_: hydrogen @ 80cm/sec.
### 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.

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

#### Rt-βDEXsm™

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#### Rt-γDEXsa™

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### Direct Injection Sleeves for HP/Finnigan GCs

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For more information on chiral analysis, visit our web site at [www.restekcorp.com](http://www.restekcorp.com) to view *A Guide to the Analysis of Chiral Compounds by GC.*

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