Preservatives

Preservatives

Preservation techniques are used in foods and beverages to maintain the quality of the product. Food preservation can be done by both physical and chemical means. Physical techniques might involve drying, heating, freezing, pasteurization, or irradiation; chemical techniques include adding sugar, salt, or preservatives. Several common chemicals, such as acetic acid and citric acid, can be used to prevent the growth of food-spoiling microorganisms. Calcium propionate can be used to prevent mold growth. In addition, benzoate and sorbate salts can be used as mold inhibitors in a range of food and beverage products.

Benzoate and sorbate salts can be analyzed in their protonated form (i.e., as benzoic acid and sorbic acid) by reversed phase HPLC using an **Ultra Phenyl** column and acidified water:methanol (80:20, v/v) as the mobile phase. By monitoring the UV absorbance at 245nm, sensitive detection of benzoic and sorbic acids can be achieved. For optimum sensitivity, monitor benzoic acid at 230nm and sorbic acid at 254nm.

Analyze phenolic antioxidants by reversed phase HPLC using a **Pinnacle II™ C18** column and an acidic mobile phase.

for **more** info

Request Flyer **High Performance Silica Products** (cat.# 59901).

Sorbic Acid and Benzoic Acid
Ultra Phenyl

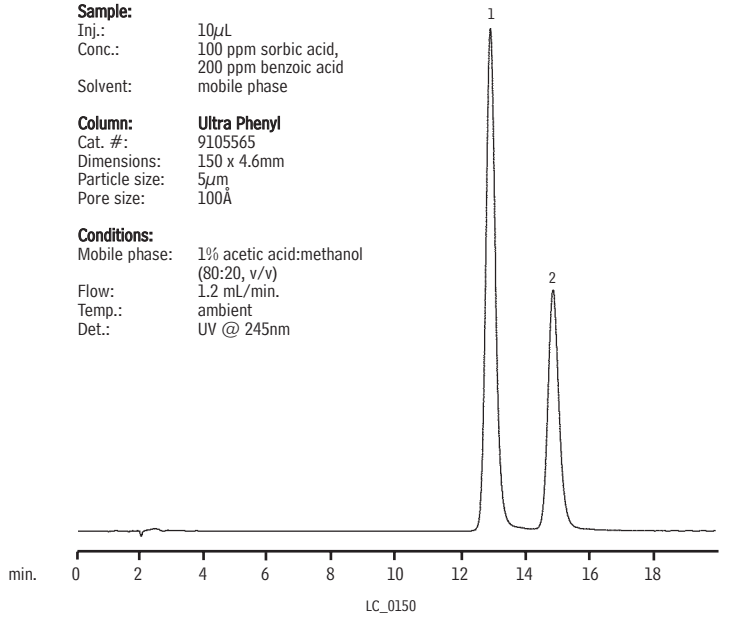
HPLC

Peak List:
1. sorbic acid
2. benzoic acid

Sample:
Inj.: 10µL
Conc.: 100 ppm sorbic acid, 200 ppm benzoic acid
Solvent: mobile phase

Column: **Ultra Phenyl**
Cat. #: 9105565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 1% acetic acid:methanol (80:20, v/v)
Flow: 1.2 mL/min.
Temp.: ambient
Det.: UV @ 245nm



LC_0150

Phenolic Antioxidants
Pinnacle II™ C18

HPLC

Peak List:	**conc.: (ppm)**
1. propyl gallate	168
2. TBHQ	182
3. 2-BHA + 3-BHA	197
4. BHT	193

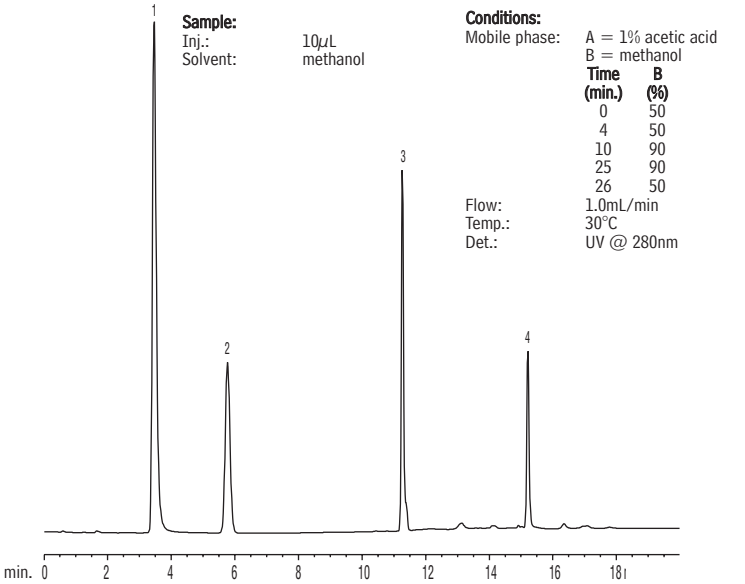
Sample:
Inj.: 10µL
Solvent: methanol

Column: **Pinnacle II™ C18**
Cat. #: 9214565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: A = 1% acetic acid
B = methanol

Time (min.)	**B (%)**
0	50
4	50
10	90
25	90
26	50

Flow: 1.0mL/min
Temp.: 30°C
Det.: UV @ 280nm



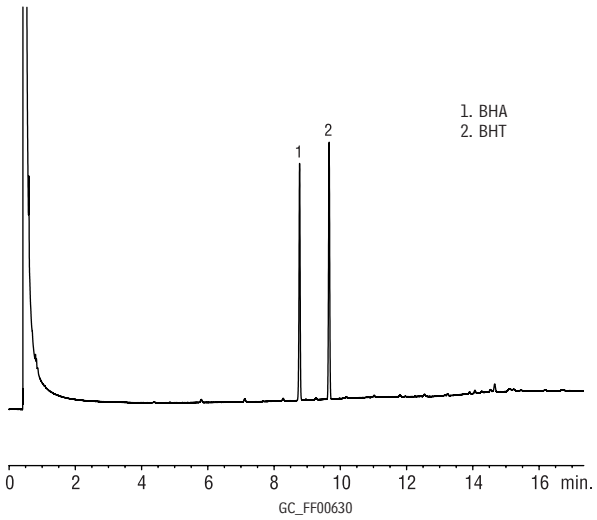
LC_0198

Foods containing fats and oils are prone to lipid oxidation, which can promote off-flavors and limit shelf-life. To inhibit lipid oxidation, antioxidants can be added to the product. Phenolic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ), are used in a variety of products. Phenolic antioxidants are regulated by the FDA and can be added to the product at levels up to 200ppm based on the fat content. Another approach is to use "natural" antioxidants, such as tocopherols and tocotrienols. These compounds inhibit lipid oxidation and promote general health in the consumer.

Phenolic antioxidants can be analyzed by GC using intermediate polarity **Rtx®-50** or **Rtx®-20** capillary columns. Coelutions that can occur with less polar columns can be avoided. Using direct injection and a flame ionization detector, BHA and BHT can be separated in less than 10 minutes. Using an **Rtx®-20** column, tocopherols from the unsaponified fraction of animal and vegetable fats and oils can be analyzed in their free form without derivatization. Baseline resolution is possible, with analyses times of less than 10 minutes.

BHA and BHT
Rtx®-50

GC



Rtx®-50, 30m, 0.53mm ID, 0.50μm (cat.# 10540)

Sample:	50ppm each in methanol
Inj.:	1.0μL direct injection, gooseneck splitless inlet liner, 4mm (cat.# 20798)
Inj. temp.:	280°C
Carrier gas:	helium, constant pressure
Linear velocity:	60cm/sec. @ 50°C
Oven temp.:	50°C to 240°C @15°C/min. (hold 3 min.)
Det.:	FID @280°C

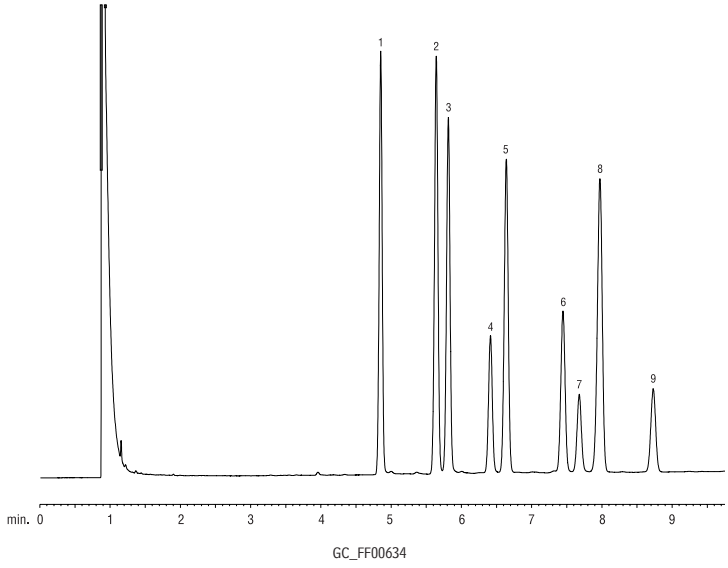
Tocopherols and Tocotrienols
Rtx®-20

GC

1. δ-tocopherol
2. β-tocopherol
3. γ-tocopherol
4. dl-δ-tocotrienol
5. α-tocopherol
6. dl-β-tocotrienol
7. dl-γ-tocotrienol
8. hexadecyl hexadecanoate
9. dl-α-tocotrienol

Rtx®-20, 30m, 0.53mm ID, 0.5μm (cat.# 10340)

Sample:	1mg/mL each component in isooctane
Inj.:	1.0μL split (split ratio 20:1), 4mm inlet liner (cat.# 20814)
Inj. temp.:	320°C
Carrier gas:	hydrogen, constant flow
Flow rate:	5.2mL/min.
Oven temp.:	270°C to 290°C @ 2°C/min. 290°C to 320°C @ 10°C/min. (1 min. hold)
Det.:	FID @ 320°C



for **more** info

Request Application Note **HPLC Analysis of Preservatives Using Ultra Aqueous and Pinnacle II™ Columns** (cat. # 59398).

Flavors & Fragrances

Flavor consists of the taste, the aroma, and the trigeminal response to a compound. The aroma of a compound can be exceedingly complex, with several hundred volatiles playing a role. Because the nose can be extremely sensitive to some odorants, trace-level analyses may be necessary. Off-flavors can result from chemical changes in foods, microbial growth, or contamination. Chemical changes include lipid oxidation, nonenzymatic browning, and enzymatic action in the food.

Vanilla Extracts and Flavorings

One example of flavor analysis is the determination of the compounds present in vanilla extracts and flavorings. Vanilla extracts and flavorings are used in a wide range of food products, including dairy products, beverages, baked goods, and confections. In AOAC Method 990.25, flavor compounds in vanilla extract and artificial vanilla flavor are analyzed using HPLC. The analytes are separated on a C8 column and quantified by comparing their UV absorbance at 254nm to an external standard. An efficient separation can be performed using an **Ultra C8** reversed phase HPLC column and a gradient elution program, with acidified water:methanol as the mobile phase. By using a gradient program and flow rate of 1mL/min., the analysis time can be reduced to 25 minutes.

for **more** info

Request Application Note **Analysis of Vanillin and Ethyl Vanillin in Vanilla Flavors Using Ultra C8 Column** (cat. # 59186).

Vanillin and Ethyl Vanillin
Ultra C8

HPLC

Sample:
Inj.: 10mL
Solvent: 40% ethanol

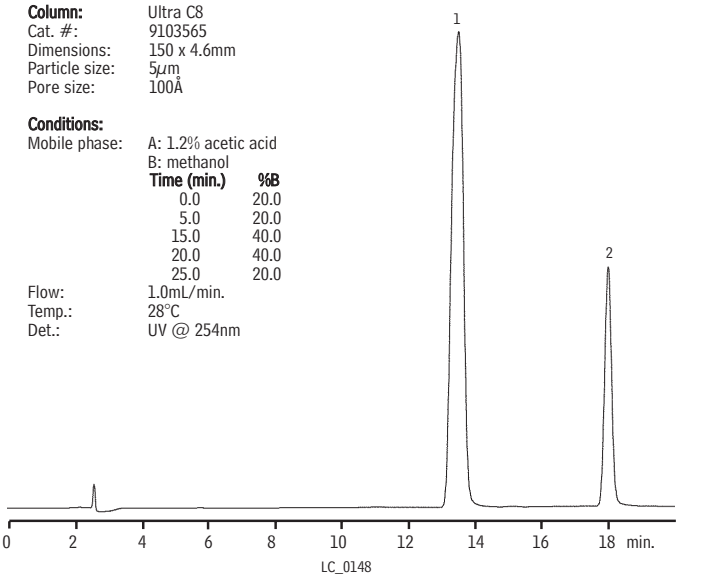
Column: Ultra C8
Cat. #: 9103565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: 1.2% acetic acid
B: methanol

Time (min.)	%B
0.0	20.0
5.0	20.0
15.0	40.0
20.0	40.0
25.0	20.0

Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm

Peak List: **Conc. (mg/mL)**
1. vanillin 0.12
2. ethyl vanillin 0.04



Vanilla Extract
Ultra C8

HPLC

Peak List:
1. vanillin

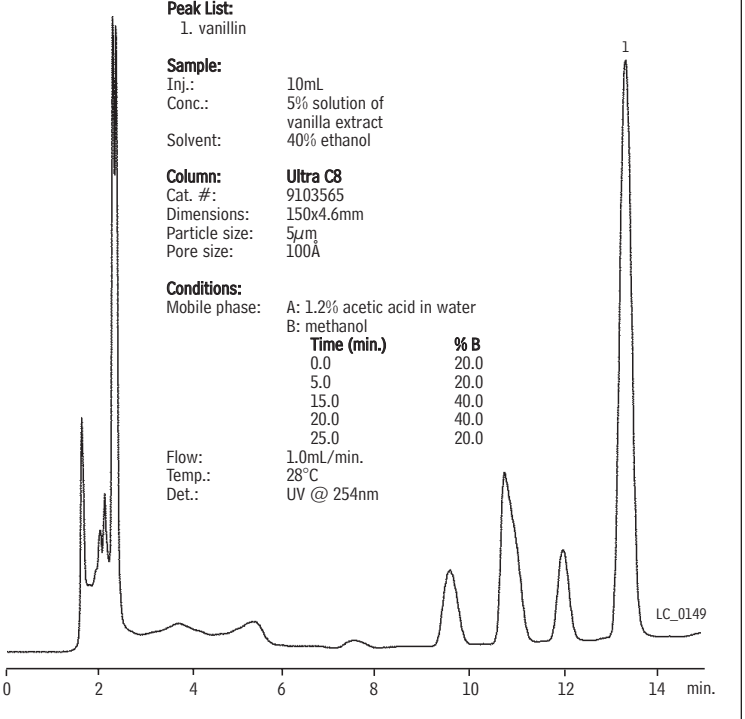
Sample:
Inj.: 10mL
Conc.: 5% solution of vanilla extract
Solvent: 40% ethanol

Column: **Ultra C8**
Cat. #: 9103565
Dimensions: 150x4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: 1.2% acetic acid in water
B: methanol

Time (min.)	% B
0.0	20.0
5.0	20.0
15.0	40.0
20.0	40.0
25.0	20.0

Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm



Capsaicinoids: Heat Level Assay Ultra C18

HPLC

Column: Ultra C18
Cat. #: 9174565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

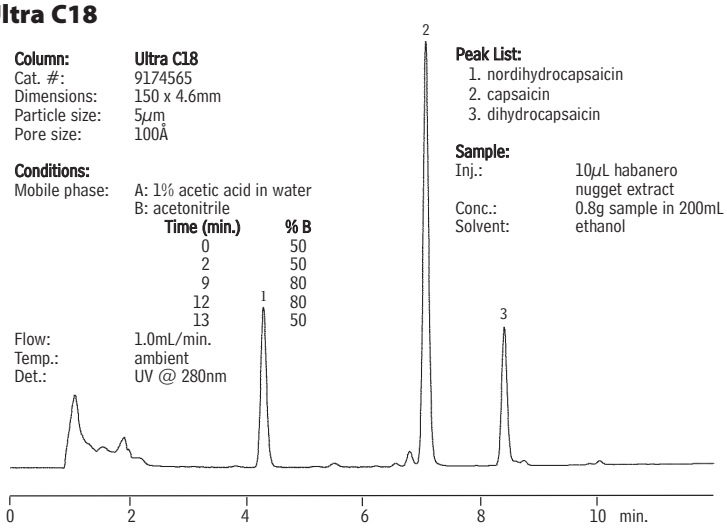
Conditions:
Mobile phase: A: 1% acetic acid in water
B: acetonitrile

Time (min.)	% B
0	50
2	50
9	80
12	80
13	50

Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 280nm

Peak List:
1. nordihydrocapsaicin
2. capsaicin
3. dihydrocapsaicin

Sample:
Inj.: 10µL habanero nugget extract
Conc.: 0.8g sample in 200mL
Solvent: ethanol

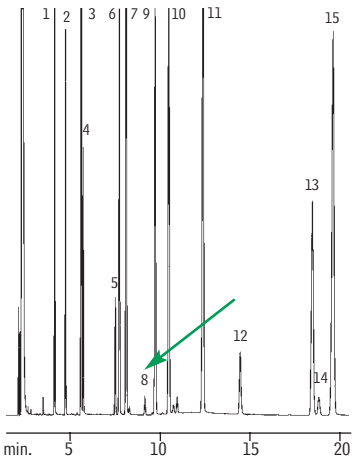


LC_0156

Flavor & Fragrance Compounds Rt-CW20M™ F&F

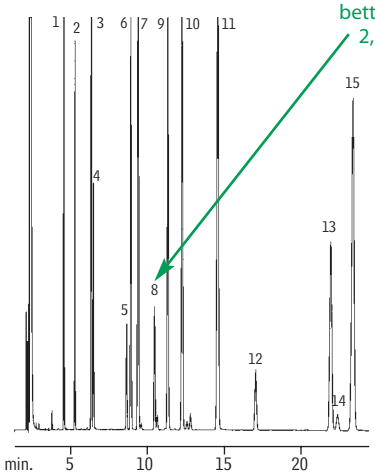
GC

Traditional Carbowax® column



1. methyl heptanoate
2. hexanol
3. methyl octanoate
4. nonanal
5. menthone
6. citronellal
7. methyl nonanoate
8. 2,3-butanediol
9. linalool
10. linalyl acetate
11. methyl decanoate
12. menthol
13. α-terpineol
14. γ-terpineol
15. methyl undecanoate

Rt-CW20M™ F&F column



50m, 0.32mm ID, 0.33µm, Carbowax® 20M or Rt-CW20M™ F&F (cat.# 12539)
On-column injection of 5ng to 150ng each compound in methylene chloride, split 10:1

Carrier gas: hydrogen, 40cm/sec.
Inj. & det. temp.: 220°C
Oven temp.: 110°C

GC_FF00433

Heat Levels of Spicy Foods

The hotness of chili peppers, salsas, and other spicy foods can be monitored using HPLC. By measuring the levels of three different capsaicinoid species in the sample, the heat level in Scoville Heat Units (SHUs) can be calculated. AOAC Method 995.03 is a reversed phase HPLC method that calls for a C18 column and acidified water:acetonitrile as the mobile phase to separate nordihydrocapsaicin, capsaicin, and dihydrocapsaicin. This separation can be performed using an **Ultra C18** column. Using a gradient elution program, an efficient separation can be performed in less than 10 minutes. The high percentage of organic in the mobile phase at the end of the analysis helps elute any strongly retained species.

Flavor & Fragrance Volatiles

Flavor and fragrance analysts have compiled retention index libraries for thousands of compounds. Unfortunately, even slight changes in column selectivity can result in misidentification of compounds. With this in mind, Restek developed the **Rtx®-1 F&F** column. This polymer matches the selectivity required by the industry, while offering higher thermal stability. Additionally, **Rt-CW20M™ F&F** columns feature a non-bonded phase designed specifically for flavor and fragrance compounds. **Rt-CW20M™ F&F** columns exhibit better inertness than other non-bonded Carbowax® columns.

for **more** info

Request Application Note **Analyzing the Heat Level of Spicy Foods Using an Ultra C18 HPLC column** (cat. # 59199).

Flavors & Fragrances

Alcoholic Beverages

The chromatographic profile of alcoholic beverages consists of a wide range of compounds, including acids, alcohols, and aldehydes. GC can be used to analyze these compounds without the need for preliminary extractions.

An **Rtx®-1301** or **MXT®-1301** capillary column provides efficient separation of the volatile organic compounds in alcoholic beverages. Packed columns, such as **CarboBlack™ B** with a **5% Carbowax® 20M** phase, are an excellent alternative for these compounds. **CarboBlack™** columns are made using SilcoSmooth™ stainless steel tubing with a deactivated silica inner layer. This improves inertness, durability, and flexibility over traditional glass packed columns.

for **more** info

Request Technical Guide **Analyzing Alcoholic Beverages by Gas Chromatography** (cat.# 59462).

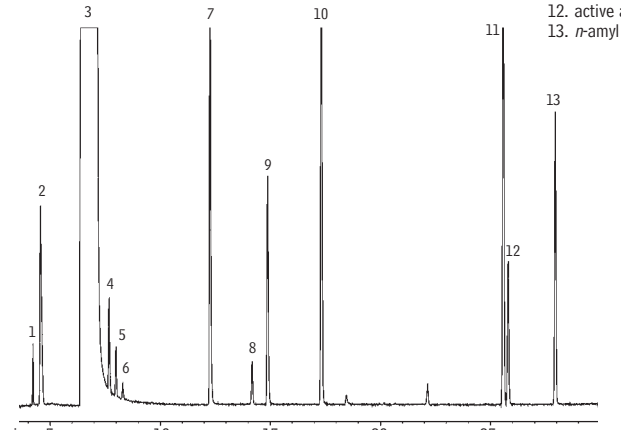
Rum
Rtx®-1301

GC

60m, 0.25mm ID, 1.4µm Rtx®-1301 (cat.# 16016)
1.0µL split injection using a Cyclosplitter® liner (cat.# 20706).
Conc.: neat

Oven temp.: 35°C (hold 5 min.) to 100°C @ 1°C/min.
Inj./det. temp.: 150°C / 200°C
Carrier gas: hydrogen @ 40cm/sec.
Split ratio: 100:1

1. acetaldehyde
2. methanol
3. ethanol
4. acetone
5. ethyl formate
6. isopropanol
7. *n*-propanol
8. ethyl acetate
9. *sec*-butanol
10. isobutanol
11. isoamyl alcohol
12. active amyl alcohol
13. *n*-amyl alcohol



GC_FF00110

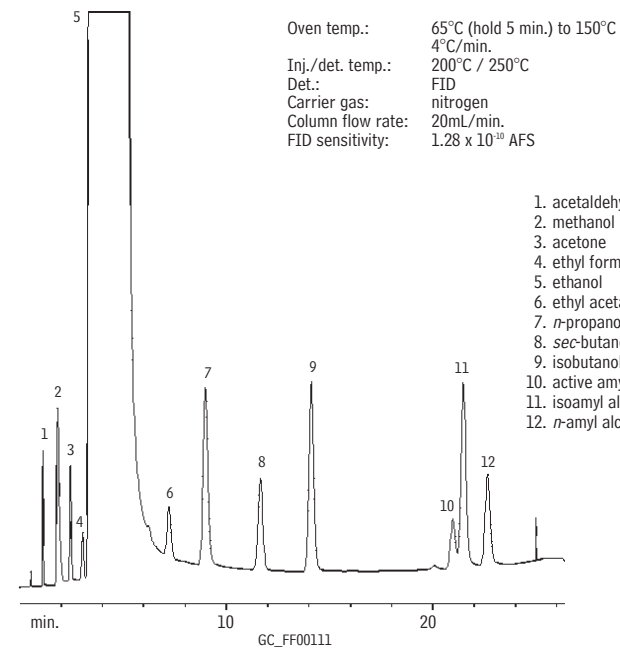
Rum
CarboBlack™ B

PACKED GC

5% Carbowax® 20M 80/120 CarboBlack™ B (cat.# 80105)
2m, 1/8" OD x 2mm ID SilcoSmooth™ tubing
0.5µL on-column injection
Conc.: neat

Oven temp.: 65°C (hold 5 min.) to 150°C @ 4°C/min.
Inj./det. temp.: 200°C / 250°C
Det.: FID
Carrier gas: nitrogen
Column flow rate: 20mL/min.
FID sensitivity: 1.28 x 10^{-10} AFS

1. acetaldehyde
2. methanol
3. acetone
4. ethyl formate
5. ethanol
6. ethyl acetate
7. *n*-propanol
8. *sec*-butanol
9. isobutanol
10. active amyl alcohol
11. isoamyl alcohol
12. *n*-amyl alcohol

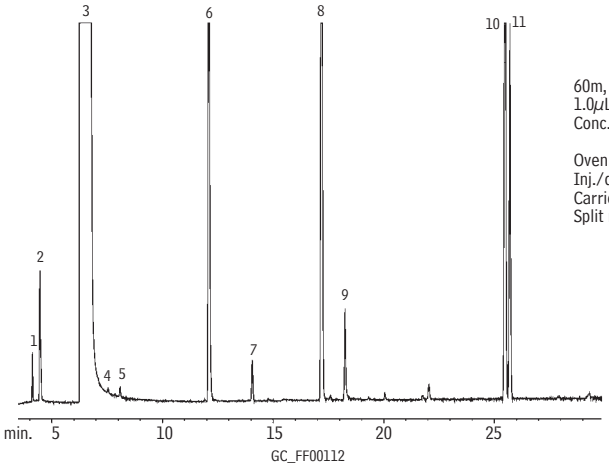


GC_FF00111

Scotch Rtx®-1301

GC

1. acetaldehyde
2. methanol
3. ethanol
4. acetone
5. isopropanol
6. *n*-propanol
7. ethyl acetate
8. isobutanol
9. acetic acid
10. isoamyl alcohol
11. active amyl alcohol



GC_FF00112

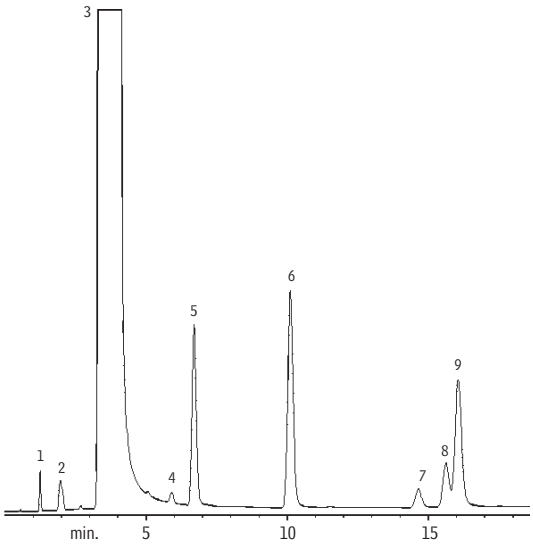
60m, 0.25mm ID, 1.4µm Rtx®-1301 (cat.# 16016)
1.0µL split injection using a Cyclosplitter® inlet liner (cat.# 20706).
Conc.: neat

Oven temp.: 35°C (hold 5 min.) to 100°C @ 1°C/min.
Inj./det. temp.: 150°C / 200°C
Carrier gas: hydrogen @ 40cm/sec.
Split ratio: 100:1

Scotch CarboBlack™ B

PACKED GC

1. acetaldehyde
2. methanol
3. ethanol
4. ethyl acetate
5. *n*-propanol
6. isobutanol
7. acetic acid
8. active amyl alcohol
9. isoamyl alcohol



GC_FF00113

5% Carbowax® 20M 80/120 CarboBlack™ B (cat.# 80105)
2m, 1/8" OD x 2mm ID Silcosmooth™ tubing
0.5µL on-column injection
Conc.: neat

Oven temp.: 70°C to 150°C @ 4°C/min.
Inj./det. temp.: 200°C / 250°C
Det.: FID
Carrier gas: nitrogen
Column flow rate: 20mL/min.
FID sensitivity: 1.28 x 10^{-10} AFS

for **more** info

Request Technical Guide **Analyzing Alcoholic Beverages by Gas Chromatography** (cat.# 59462).

Flavors & Fragrances

Essential Oils

Essential oil samples are very complex; hundreds of components can be present and some are present at ppm levels. **Rtx®-1, Rtx®-5** and **Stabilwax®** capillary GC columns are very effective for these analyses. A comprehensive list of retention times for flavor & fragrance compounds on **Rtx®-1** and **Stabilwax®** columns is on pages 28–29.

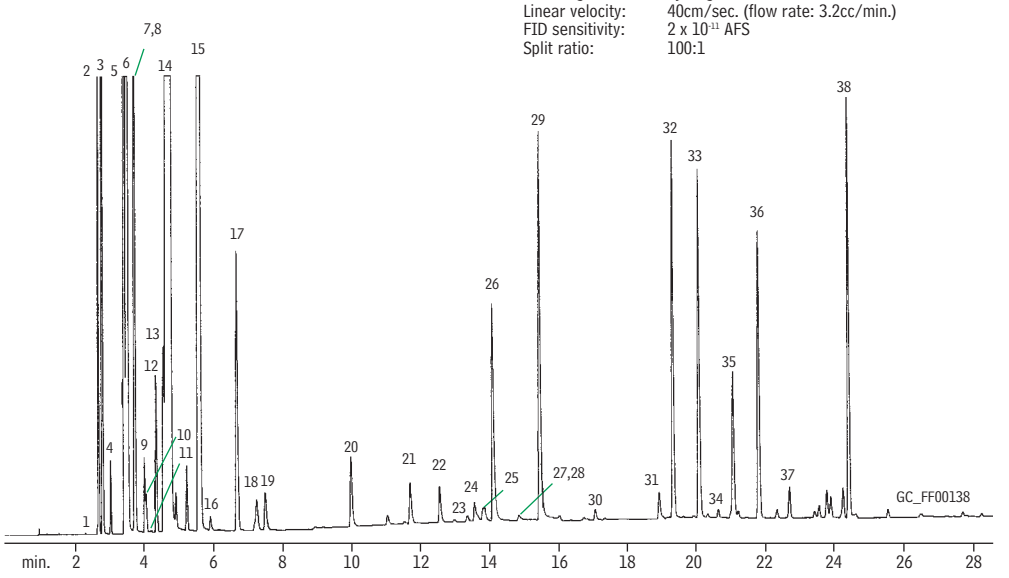
To determine the enantiomeric ratios of volatile components in essential oils, see Chiral Separations (pages 23–26).

Lemon Oil
Rtx®-5

GC

30m, 0.32mm ID, 0.25µm Rtx®-5 (cat.# 10224)
Wet needle split injection of a neat lemon oil

Oven temp.:	75°C (hold 8 min.) to 250°C @ 4°C/min.
Inj. & det. temp.:	250°C
Carrier gas:	hydrogen
Linear velocity:	40cm/sec. (flow rate: 3.2cc/min.)
FID sensitivity:	2 x 10^{-11} AFS
Split ratio:	100:1



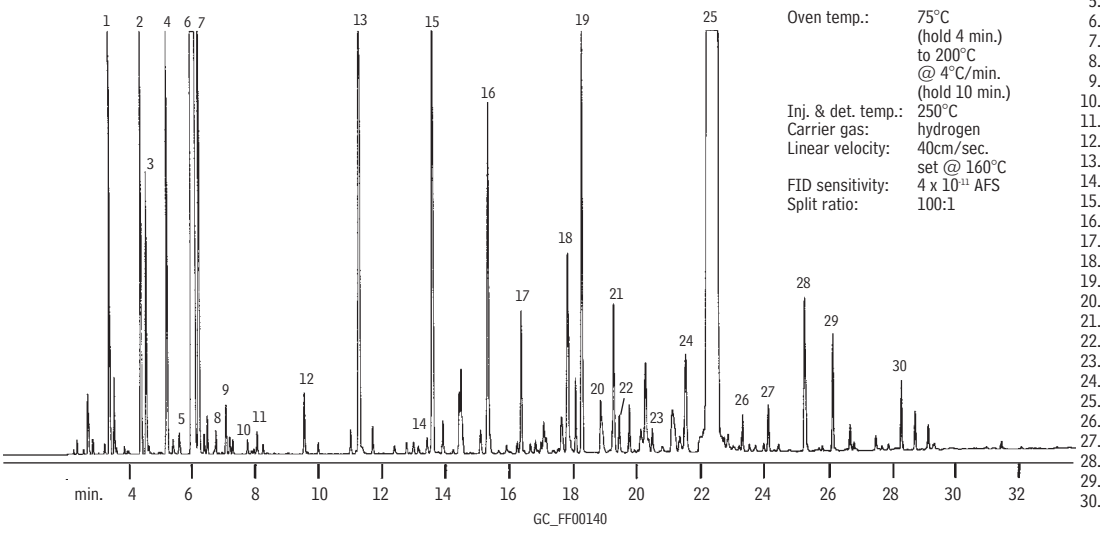
1. heptanol
2. α-thujene
3. α-pinene
4. camphene
5. sabinene
6. β-pinene
7. 6-methyl-5-hepten-2-one
8. myrcene
9. octanal
10. α-phellandrene
11. 3-carene
12. α-terpinene
13. *p*-cymene
14. limonene
15. γ-terpinene
16. octanol
17. terpinolene
18. linalool
19. nonanal
20. citronellal
21. terpinene-4-ol
22. α-terpineol
23. decanol
24. octyl acetate
25. nerol
26. neral
27. carvone
28. geraniol
29. geranial
30. nonyl acetate
31. citronellyl acetate
32. neryl acetate
33. geranyl acetate
34. dodecanal
35. β-caryophyllene
36. *trans*-α-bergamotene
37. α-humulene
38. β-bisabolene

Spearmint Oil (Native)
Stabilwax®

GC

60m, 0.25mm ID, 0.25µm
Stabilwax® (cat.# 10626)
0.2µL split injection of a neat spearmint oil

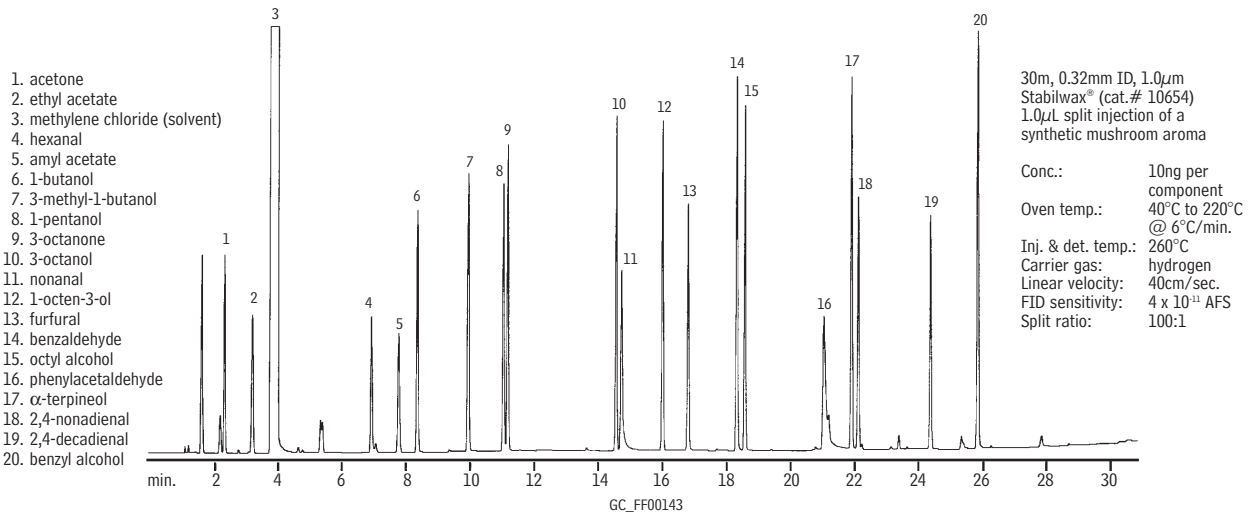
Oven temp.:	75°C (hold 4 min.) to 200°C @ 4°C/min. (hold 10 min.)
Inj. & det. temp.:	250°C
Carrier gas:	hydrogen
Linear velocity:	40cm/sec. set @ 160°C
FID sensitivity:	4 x 10^{-11} AFS
Split ratio:	100:1



1. α-pinene
2. β-pinene
3. sabinene
4. myrcene
5. α-terpinene
6. l-limonene
7. 1,8-cineole
8. *cis*-ocimene
9. γ-terpinene
10. *p*-cymene
11. terpinolene
12. 3-octyl acetate
13. 3-octanol
14. l-menthone
15. *trans*-sabinenehydrate
16. β-bourbonene
17. linalool
18. terpinene-4-ol
19. β-caryophyllene
20. dihydrocarvone
21. *trans*-dihydrocarvyl acetate
22. *trans*-β-farnesene
23. α-terpineol
24. germacrene-Δ
25. carvone
26. *cis*-carvyl acetate
27. *trans*-carveol
28. *cis*-carveol
29. *cis*-jasmone
30. viridiflorol

Synthetic Mushroom Aroma
Stabilwax®

GC



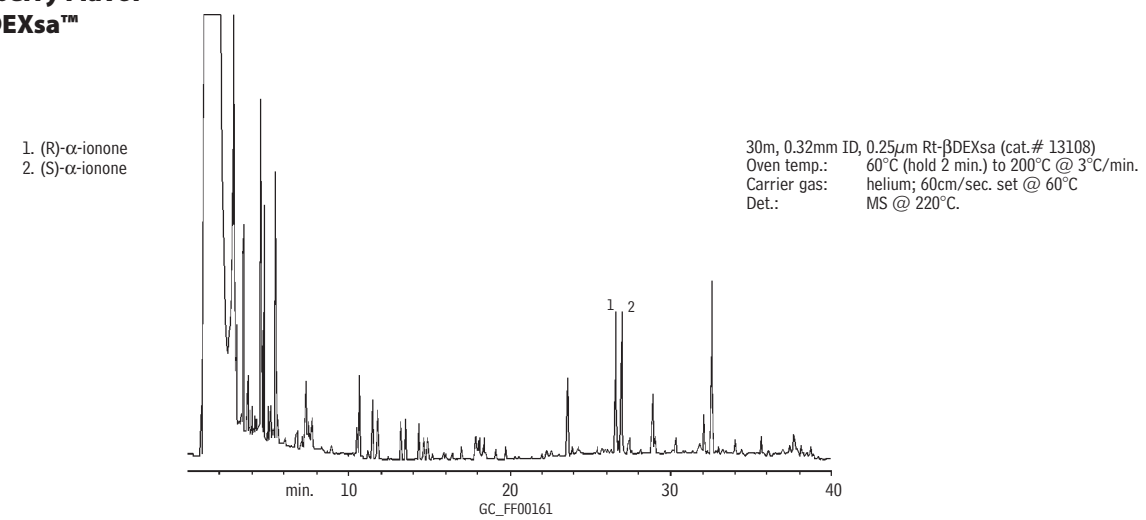
Chiral Separations

Chiral chromatography is the separation of enantiomeric compounds, which are mirror images of each other that are not superimposable. Common liquid stationary phases used in GC typically are not able to resolve enantiomeric compounds. However, the addition of derivatized cyclodextrin molecules to common stationary phases makes such separations possible. Restek's five chiral capillary columns incorporate various combinations of alkylated β-cyclodextrins into a cyanopropyl-dimethyl siloxane liquid stationary phase. The unique combinations of cyclodextrins allow analysis of a wide range of enantiomeric compounds.

Chiral capillary chromatography is a relatively new technique for determining the enantiomeric ratios of volatile components in essential oils. Enantiomeric ratios can be used for determining the authenticity of an essential oil or for characterizing regional differences among oils. The separation of enantiomeric compounds in flavor and fragrance samples can be optimized through column selection. Each of the five Restek chiral columns offers a different selectivity. The **Rt-βDEXsp™** column is optimized for menthol analysis, while the **Rt-βDEXsa™** column provides the best separation for 1-octen-3-ol, carvone, camphor, 1-phenylethanol, β-citronellol, and rose oxides. **Rt-βDEXsm™** and **Rt-βDEXse™** columns, used in combination, provide the best resolution for *cis*- and *trans*-linalool oxides, linalool, and linalyl acetate. The **Rt-βDEXcst™** column is ideal for semivolatile chiral compounds, including the irone isomers and γ- and δ-lactones.

Raspberry Flavor
Rt-βDEXsa™

GC



Chiral Separations

Flavor chemists can use chiral chromatography to monitor the ratios of various enantiomeric compounds. γ-lactones, for example, can be monitored to determine if a peach flavor has been adulterated. Ethyl-2-methylbutyrate and 2-methylbutyrate are important contributors to apple flavor, and both are naturally present in predominantly the (S) form in apple juices. The enantiomers of these two compounds can be resolved on an **Rt-βDEXsm™** column.

for **more** info

Request **Chiral Column Technical Guide** (cat.# 59889).

tech tip

To optimize chiral separations, use:

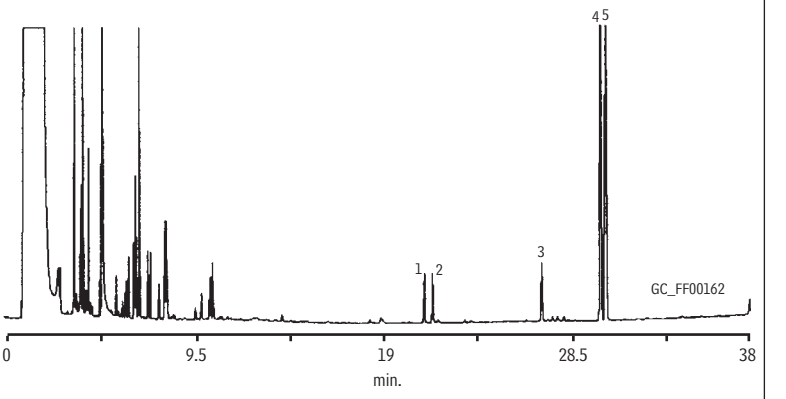
1) Faster linear velocities (80cm/sec.) with hydrogen carrier gas.
2) Slower temperature ramp rates (1–2°C/min.).
3) Appropriate minimum operating temperature (40 or 60°C).
4) On-column concentrations of 50ng or less.

Peach/Vanilla Flavor
Rt-βDEXsa™

GC

1. (R)-γ-octalactone
2. (S)-γ-octalactone
3. (R)-γ-decalactone
4. (R)-γ-undecalactone
5. (S)-γ-undecalactone

30m, 0.32mm ID, 0.25μm Rt-βDEXsa™ (cat.# 13108)
Oven temp.: 60°C (hold 2 min.) to 100°C @ 15°C/min. to 220°C @ 3°C/min.
Carrier gas: helium, 60cm/sec. set @ 60°C
Det.: MS @ 220°C

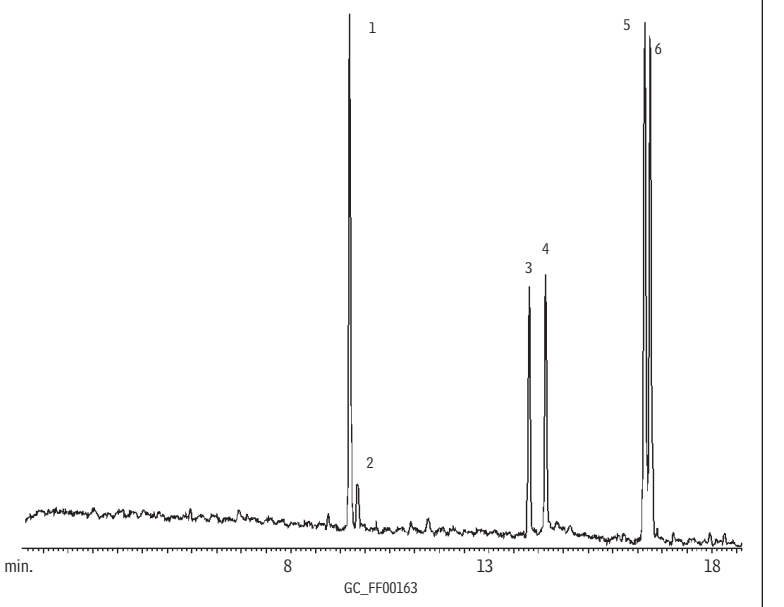


Bergamot Flavor
Rt-βDEXse™

GC

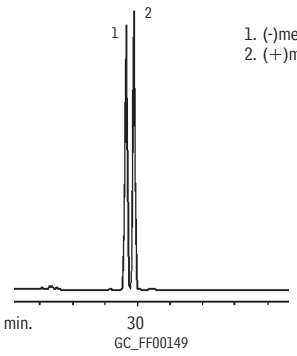
1. (S)-limonene
2. (R)-limonene
3. (R)-linalool
4. (S)-linalool
5. (R)-linalyl acetate
6. (S)-linalyl acetate

30m, 0.32mm ID, 0.25μm Rt-βDEXse™ (cat.# 13106)
Oven temp.: 40°C (hold 1 min.) to 200°C @ 4°C/min.
Carrier gas: helium, 60cm/sec. set @ 40°C
Det.: MS @ 220°C



GC

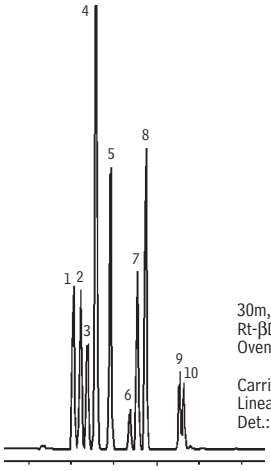
Menthol - Rt-βDEXsp™



1. (-)menthol
2. (+)menthol

30m, 0.32mm ID, 0.25μm
Rt-βDEXsp™ (cat.# 13110)
Oven temp.: 60°C (hold 1 min.) to 200°C @ 2°C/min.
Carrier gas: hydrogen
Linear velocity: 80cm/sec. set @ 40°C
Det.: FID @ 220°C

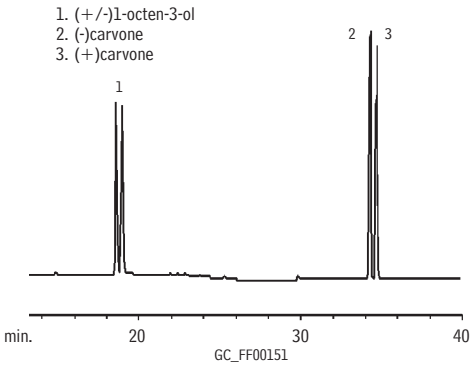
Irone Isomers - Rt-βDEXcst™



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, 0.25μm
Rt-βDEXcst™ (cat.# 13108)
Oven temp.: 40°C (hold 1 min.) to 230°C @ 2°C/min. (hold 3 min.)
Carrier gas: hydrogen
Linear velocity: 80cm/sec. set @ 40°C
Det.: FID @ 220°C

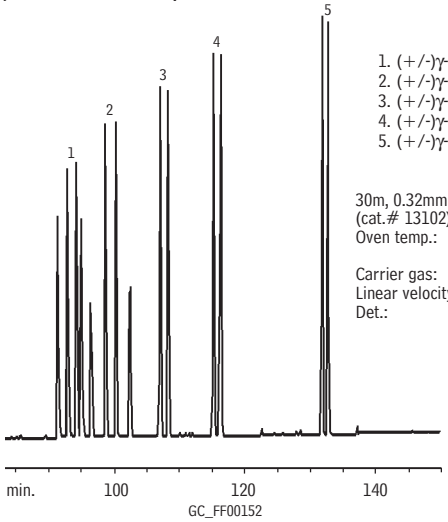
1-octen-3-ol and carvone - Rt-βDEXsa™



1. (+/-)1-octen-3-ol
2. (-)carvone
3. (+)carvone

30m, 0.32mm ID, 0.25μm
Rt-βDEXsa™ (cat.# 13108)
Oven temp.: 40°C (hold 1 min.) to 230°C @ 2°C/min. (hold 3 min.)
Carrier gas: hydrogen
Linear velocity: 80cm/sec. set @ 40°C
Det.: FID @ 220°C

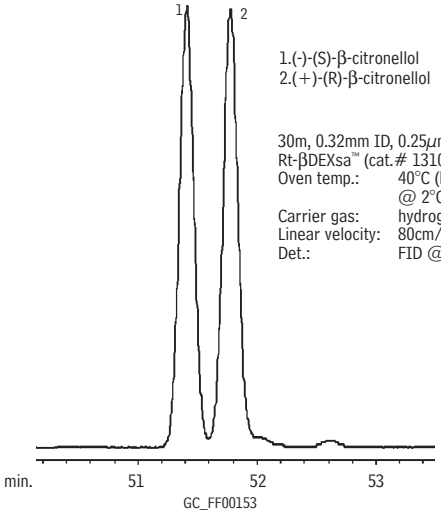
γ-lactones - Rt-βDEXcst™



1. (+/-)γ-heptalactone
2. (+/-)γ-octalactone
3. (+/-)γ-nonalactone
4. (+/-)γ-decalactone
5. (+/-)γ-dodecalactone

30m, 0.32mm ID, 0.25μm Rt-βDEXcst™ (cat.# 13102)
Oven temp.: 60°C (hold 1 min.) to 200°C @ 1°C/min.
Carrier gas: hydrogen
Linear velocity: 40cm/sec. set @ 60°C
Det.: FID @ 220°C

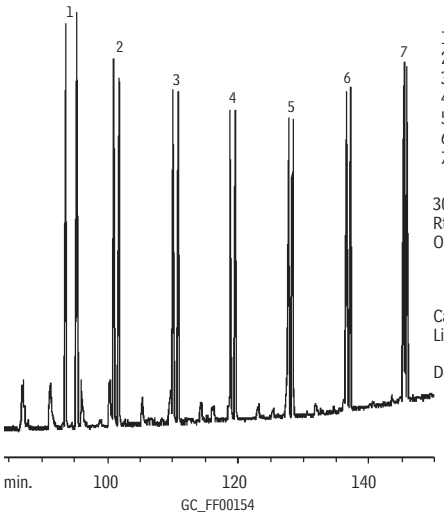
β-citronellol - Rt-βDEXsa™



1.(-)-(S)-β-citronellol
2.(+)-(R)-β-citronellol

30m, 0.32mm ID, 0.25μm
Rt-βDEXsa™ (cat.# 13108)
Oven temp.: 40°C (hold 1 min.) to 230°C @ 2°C/min. (hold 3 min.)
Carrier gas: hydrogen
Linear velocity: 80cm/sec. set @ 40°C
Det.: FID @ 220°C

δ-lactones - Rt-δDEXcst™



1. (+/-)δ-pentalactone
2. (+/-)δ-hexalactone
3. (+/-)δ-heptalactone
4. (+/-)δ-octalactone
5. (+/-)δ-nonalactone
6. (+/-)δ-decalactone
7. (+/-)δ-dodecalactone

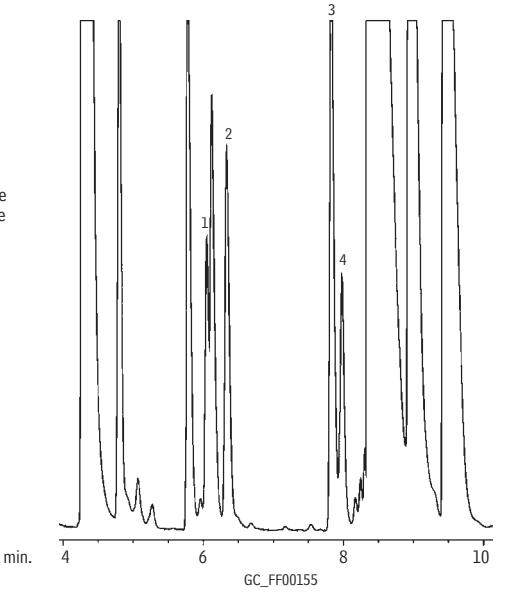
30m, 0.32mm ID, 0.25μm
Rt-βDEXcst™ (cat.# 13102)
Oven temp.: 60°C (hold 1 min.) to 200°C @ 1°C/min.
Carrier gas: hydrogen
Linear velocity: 40cm/sec. set @ 60°C
Det.: FID @ 220°C

Chiral Separations

GC

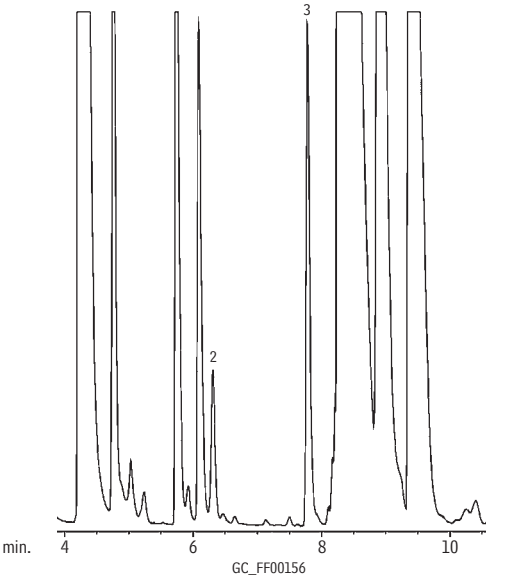
1. (R)-ethyl 2-methylbutyrate
2. (S)-ethyl 2-methylbutyrate
3. (R)-2-methylbutyrate
4. (S)-2-methylbutyrate

Apple Juice with Added Standards
Rt-βDEXsm™



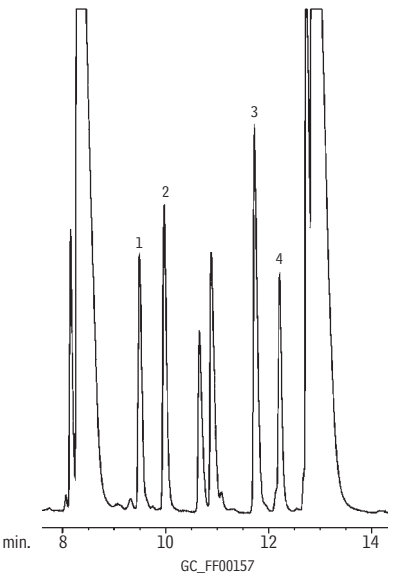
30m, 0.32mm ID, 0.25μm Rt-βDEXsm™ (cat.# 13104)
On-column conc. (standards): ~50ng

Apple Juice
Rt-βDEXsm™



30m, 0.32mm ID, 0.25μm Rt-βDEXsm™ (cat.# 13104)

Apple Juice with Added Standards
Rt-βDEXse™



30m, 0.32mm ID, 0.25μm Rt-βDEXse™ (cat.# 13106)
On-column conc. (standards): ~50ng

Apple Juice
Rt-βDEXse™

30m, 0.32mm ID, 0.25μm Rt-βDEXse™ (cat.# 13106)

1.0μL split injection.

Oven temp.:	40°C (hold 1 min.) to 220°C @ 2°C/min.
Inj. & det. temp.:	220°C
Carrier gas:	hydrogen
Linear velocity:	80cm/sec.

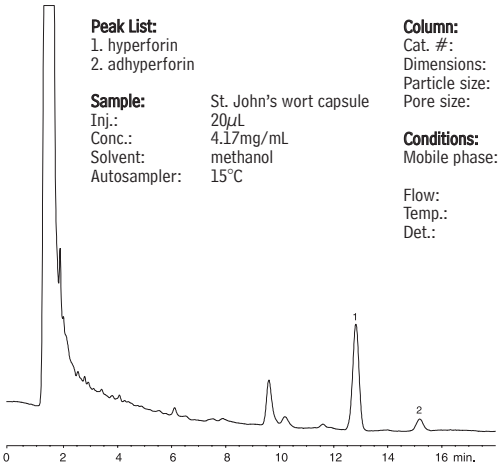
Hyperforin in St. John's Wort
Pinnacle II™ C18

HPLC

Peak List:
1. hyperforin
2. adhyperforin

Sample:	St. John's wort capsule
Inj.:	20µL
Conc.:	4.17mg/mL
Solvent:	methanol
Autosampler:	15°C

Column:	**Pinnacle II™ C18**
Cat. #:	9214565
Dimensions:	150 x 4.6mm
Particle size:	5µm
Pore size:	110Å
Conditions:	
Mobile phase:	0.01N phosphate buffer, pH 2.5: acetonitrile (15:85, v/v)
Flow:	1.2mL/min.
Temp.:	30°C
Det.:	UV @ 270nm



Phenolics in *Echinacea*
Pinnacle II™ C18

HPLC

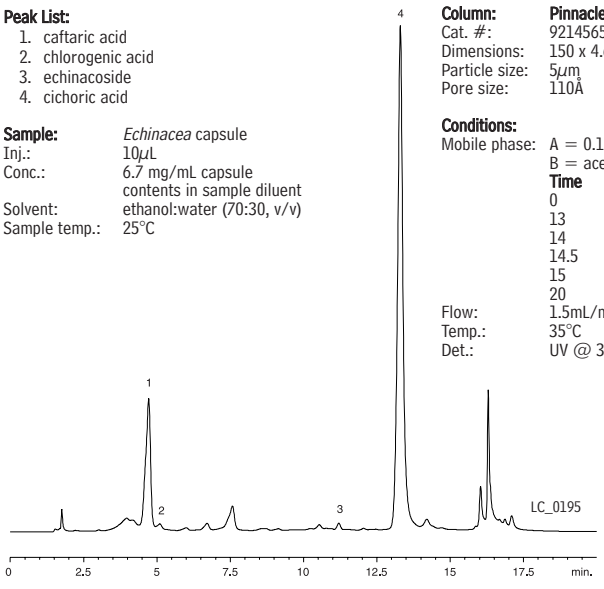
Peak List:
1. caftaric acid
2. chlorogenic acid
3. echinacoside
4. cichoric acid

Sample:	*Echinacea* capsule
Inj.:	10µL
Conc.:	6.7 mg/mL capsule contents in sample diluent
Solvent:	ethanol:water (70:30, v/v)
Sample temp.:	25°C

Column:	**Pinnacle II™ C18**
Cat. #:	9214565
Dimensions:	150 x 4.6mm
Particle size:	5µm
Pore size:	110Å
Conditions:	
Mobile phase:	A = 0.1% phosphoric acid B = acetonitrile

Time	%B
0	10
13	22
14	40
14.5	40
15	10
20	10

Flow:	1.5mL/min.
Temp.:	35°C
Det.:	UV @ 330nm



for **more** info

Request Applications Note **Analyzing Nutraceutical Products by Liquid and Gas Chromatography** (cat.# 59364).

Dietary Supplements

Over the past decade, the nutraceutical industry has seen rapid growth as more people add flowers, leaves, roots, and fruits of botanicals to their diets in hope of gaining health benefits.

Herbal products are very complex, often containing hundreds of compounds, and it is not always clear which compounds are responsible for the beneficial properties. Marker compounds—phytochemicals that have been identified and are known to have some relationship to the reported health benefit—can be evaluated qualitatively to identify a raw material or to verify purity. To determine the concentration or strength of a material, quantitative analysis is necessary.

Hyperforin in St. John's Wort

According to the Institute for Nutraceutical Advancement (INA), INA Method 112.001—the HPLC analysis of hyperforin and adhyperforin in St. John's wort—the samples are extracted with methanol in an ultrasonic bath. Chromatographic separation is performed on a C18 reversed phase column with acetonitrile and phosphate buffer as the mobile phase. Analysis of encapsulated St. John's wort using a **Pinnacle II™ C18** column shows excellent peak shapes for the active ingredients in this herbal product.

Phenolics in *Echinacea*

The active compounds in *echinacea* are thought to be caffeic acid derivatives such as caftaric acid, cichoric acid, chlorogenic acid, and echinacoside.

Conclusion

Gas and liquid chromatography are powerful tools for the analysis of foods, flavors, and fragrances. This technical guide describes columns and analytical conditions that will help you achieve more accurate identification and quantitation of target analytes. However, if you have questions, please don't hesitate to contact Restek's technical service by e-mail (support@restekcorp.com) or by phone (extension 4), or contact your Restek representative. We will do everything we can to help you find a solution.

References
1. Fennema, O. R. Food Chemistry (1996), 3rd edition.
2. Bensinger, M. "How Hot is that 'Devil' Sauce?" in Fiery Foods Magazine (1997), Sept/Oct.
3. Brandt, Laura. "The Creation and Use of Vanilla", Food Product Design (1996), editorial archives.
4. AOAC Official Methods of Analysis (2000), 17th edition, AOAC International.
5. Official Methods and Recommended Practices (1998), 5th edition, American Oil Chemists' Society.
6. AACC Approved Methods (2000), 10th edition, American Association of Cereal Chemists.

References are not available from Restek.

Flavor and Fragrance Compounds Retention Time Index

Retention time data collected using 60m, 0.25mm ID, 0.25µm Rtx®-1 and Stabilwax® columns.
Oven temp.: 100°C to 260°C @ 4°C/min.; **Carrier gas:** helium; **Linear velocity:** 27.2cm/sec. @ 100°C; **Dead time:** 3.68 min. @ 100°C.

Component	Retention Time (min.) Rtx®-1	Stabilwax®
isopropyl alcohol	3.66	3.93
allyl alcohol	3.67	4.05
tert-butyl alcohol	3.68	3.89
1-propanol	3.70	4.13
3-buten-2-ol	3.71	4.12
ethyl formate	3.71	3.84
acetone	3.72	3.86
methyl acetate	3.75	3.86
2-butanol	3.76	4.03
propyn-1-ol	3.77	6.26
tert-amyl alcohol	3.79	4.14
isobutyraldehyde	3.79	3.84
2-methyl-3-buten-2-ol	3.82	4.12
methyl ethyl ketone	3.84	3.83
cis-2-buten-1-ol	3.84	5.01
ethyl acetate	3.86	3.90
propyl formate	3.87	3.83
2-methylfuran	3.88	3.87
isobutyl alcohol	3.89	4.72
methyl propanoate	3.89	4.03
3-buten-1-ol	3.91	4.76
3-methyl-2-butanol	3.92	4.25
2-butenal	3.94	4.26
2-pentanol	3.95	4.42
isopropyl acetate	3.96	4.03
1-butanol	3.96	4.57
neopentanol	3.99	4.60
methyl isobutyrate	4.03	4.08
2-pentanone	4.03	3.95
isoamyl alcohol	4.04	4.96
allyl acetate	4.05	4.14
ethyl acrylate	4.06	4.08
3-pentanone	4.07	4.08
pentanal	4.07	4.08
tert-butyl acetate	4.09	3.93
pinacolone	4.09	4.00
propyl acetate	4.10	4.04
2-ethylfuran	4.10	3.99
2,5-dimethylfuran	4.12	3.98
3-methyl-1-butanol	4.18	4.88
3-penten-2-one	4.18	4.45
2-methyl-1-butanol	4.20	4.88
pinacol alcohol	4.20	4.38
thiophene	4.21	4.16
methylisobutylketone (MIBK)	4.22	4.15
methyl butanoate	4.22	4.09
2-methyl-3-pentanone	4.27	4.15
ethyl isobutyrate	4.28	4.03
cis-2-penten-1-ol	4.29	5.85
3-methyl-3-pentanol	4.29	4.37
1-pentanol	4.30	5.24
3-hexanone	4.30	4.28
3-methyl-2-buten-1-ol	4.32	5.81
isobutyl acetate	4.34	4.13
butryolactone	4.34	7.07
2-methyl-3-pentanol	4.36	4.59

Component	Retention Time (min.) Rtx®-1	Stabilwax®
2-hexanone	4.41	4.11
ethyl butanoate	4.41	4.18
cyclopentanol	4.42	5.74
cyclopentanone	4.46	4.96
2,4-dimethyl-2-pentanol	4.47	4.45
3-hexanol	4.47	4.84
2-hexanol	4.48	5.01
4-methyl-3-penten-2-one	4.48	4.61
hexanal	4.48	4.41
2,4-dimethyl-3-pentanone	4.49	3.95
pyridine	4.50	4.91
propyl propanoate	4.51	4.22
a-angelicalactone	4.51	7.26
butyl acetate	4.54	4.29
methyl pentanoate	4.61	4.36
furfural	4.64	7.65
2,2-dimethyl-3-pentanol	4.65	4.63
2-methyl-1-pentanol	4.65	5.63
4-hexen-3-one	4.67	4.88
isopropyl butyrate	4.72	4.19
furfuryl alcohol	4.74	11.07
2,4-dimethyl-3-pentanol	4.75	4.76
trans-2-hexenal	4.78	5.08
pinacol	4.79	7.91
ethyl-2-methyl butanoate	4.80	4.26
2-ethyl-1-butanol	4.80	5.72
trans-2-hexenol	4.82	6.56
5-methyl-2-hexanone	4.84	4.54
1-hexanol	4.92	6.16
3-ethyl-3-pentanol	4.94	4.94
isoamyl acetate	4.98	4.49
cis-3-hexen-1-ol	4.98	6.58
4-heptanone	4.99	4.45
trans-2-hexen-1-ol	5.01	6.81
anisole	5.06	6.13
3-heptanone	5.08	4.62
1,3-xylene	5.09	4.67
1,4-xylene	5.09	4.64
2-heptanone	5.10	4.85
4-heptanol	5.14	5.45
propyl butyrate	5.16	4.49
ethyl pentanoate	5.17	4.54
cyclohexanone	5.19	5.85
2-heptanol	5.20	5.63
heptanal	5.21	4.35
butyl propionate	5.24	4.56
amyl acetate	5.29	4.73
1,2-xylene	5.30	4.93
nonane	5.32	3.80
isobutyl isobutyrate	5.34	4.39
methyl hexanoate	5.41	3.81
tetrahydro-2-fufanmethanol	5.50	8.53
d-valerolactone	5.51	9.57
cumene	5.62	4.84
5-methyl-3-heptanone	5.65	4.94
ethyl amyl ketone	5.65	4.94

Component	Retention Time (min.) Rtx®-1	Stabilwax®
5-methylfurfural	5.73	9.64
a-pinene	5.81	4.84
benzaldehyde	5.90	8.76
tricyclene	5.91	4.16
1-heptanol	5.95	7.54
camphene	6.02	4.24
2,6-dimethyl-4-heptanone	6.03	4.81
1-octen-3-ol	6.09	7.33
furfuryl acetate	6.09	8.77
3-octanone	6.16	5.35
2-octanone	6.20	6.35
sec-butylbenzene	6.28	5.37
tert-butylbenzene	6.32	5.15
myrcene	6.32	4.53
butyl butyrate	6.34	5.02
b-pinene	6.38	4.40
octanal	6.39	5.73
2-octanol	6.39	7.00
hexyl acetate	6.49	5.41
decane	6.58	4.08
2-methylanisole	6.60	6.97
a-phellandrene	6.69	6.13
3-methylanisole	6.74	7.39
4-methylanisole	6.78	7.42
2-ethyl-1-hexanol	6.85	8.10
benzyl alcohol	6.86	16.48
3-carene	6.89	4.67
p-cymene	6.91	5.38
a-terpinene	6.93	12.38
limonene	7.09	4.84
salicaldehyde	7.09	11.93
camphor	7.11	8.77
trans-ocimene	7.13	4.72
1,8-cineole	7.16	5.06
eucalyptol	7.16	5.06
cis-ocimene	7.21	5.06
a-methylbenzylalcohol	7.34	18.34
p-cresol	7.45	21.40
g-terpinene	7.54	5.26
1-octanol	7.55	9.37
2,6-dimethylanisole	7.55	7.16
5-nonanone	7.57	6.39
tetrahydrofurfuryl acetate	7.60	9.84
fenchone	7.68	7.08
linalool oxide	7.81	7.56
3-nonanone	7.82	6.38
2-nonanone	7.89	7.17
methyl benzoate	8.03	10.72
linalool	8.13	9.02
2-nonanol	8.15	8.65
nonanal	8.16	6.86
terpinyl acetate	8.18	12.51
maltol	8.23	18.48

Flavor and Fragrance Compounds Retention Time Index

Retention time data collected using 60m, 0.25mm ID, 0.25µm Rtx®-1 and Stabilwax® columns.
Oven temp.: 100°C to 260°C @ 4°C/min.; **Carrier gas:** helium; **Linear velocity:** 27.2cm/sec. @ 100°C; **Dead time:** 3.68 min. @ 100°C.

Component	Retention Time (min.) Rtx®-1	Stabilwax®
trans-sabinene hydrate	8.37	9.06
2,4-dimethylanisole	8.39	8.45
2,5-dimethylanisole	8.39	8.42
undecane	8.41	4.38
a-thujone	8.46	15.01
methyl octanoate	8.57	6.69
b-thujone	8.68	7.63
2,3-dimethylanisole	8.87	9.30
citronellal	9.19	7.99
benzyl acetate	9.31	13.02
menthone	9.42	7.97
borneol	9.53	12.52
ethyl benzoate	9.62	11.74
1-nonanol	9.70	11.56
isoborneol	9.72	11.69
menthofuran	9.73	8.14
isomenthone	9.75	8.57
neomenthol	9.85	10.22
a-terpineol	9.91	5.58
menthol	9.95	11.16
dihydrocarveol	10.09	13.64
terpinen-4-ol	10.11	10.31
2-decanone	10.14	8.41
a-terpinolene	10.32	7.36
4-allylanisole	10.35	11.75
estragole	10.35	11.75
decanal	10.48	8.51
trans-dihydrocarvone	10.59	23.12
verbenone	10.67	12.88
dodecane	10.75	4.90
cis-dihydrocarvone	10.80	10.80
linalyl acetate	10.93	9.36
b-citronellol	11.06	13.93
cis-nerol	11.14	13.82
carveol	11.24	16.29
benzyl acetone	11.27	16.25
citral a	11.39	12.13
geranial	11.39	12.13
cuminaldehyde	11.43	14.40
pulegone	11.43	11.46
p-anisaldehyde	11.47	20.15

Component	Retention Time (min.) Rtx®-1	Stabilwax®
r-carvone	11.48	13.28
s-carvone	11.51	13.36
geraniol	11.74	15.84
trans-cinnamaldehyde	11.97	20.50
citral b	12.12	13.25
neral	12.12	13.25
propyl benzoate	12.16	13.82
1-decanol	12.36	13.99
perillaldehyde	12.37	14.52
citronellyl formate	12.38	10.69
trans-menthyl acetate	12.56	9.01
indole	12.57	29.33
trans-anethole	12.67	15.41
cumin alcohol	12.68	21.88
thymol	12.71	23.82
2-undecanone	12.81	10.45
carvacrol	12.87	24.56
bornyl acetate	12.88	10.09
trans-cinnamyl alcohol	12.94	26.11
cis-menthyl acetate	13.02	9.62
perillyl alcohol	13.02	19.36
tridecane	13.47	5.72
2-methylcinnamaldehyde	13.59	19.52
triacetin	13.67	21.33
methyl decanoate	13.69	10.26
cis-carvyl acetate	14.09	13.23
cumic acid	14.40	34.95
g-valerolactone	14.48	10.55
citronellyl acetate	14.49	11.81
eugenol	14.55	23.53
thiazole	14.67	5.23
neryl acetate	14.76	13.08
trans-carvyl acetate	14.88	14.05
dihydrocoumarin	14.91	26.02
geranyl acetate	15.26	13.08
dihydrojasmone	15.34	16.24
vanillin	15.34	32.21
ethyl decanoate	15.69	11.22
2-dodecanone	15.72	12.83
cis-jasmone	15.75	18.34
trans-cinnamyl acid	16.07	37.16

Component	Retention Time (min.) Rtx®-1	Stabilwax®
carvone hydrate	16.25	27.44
tetradecane	16.38	6.95
coumarin	16.54	29.90
cis-carvyl propionate	16.63	14.68
a-ionone	16.88	16.23
trans-cinnamyl acetate	16.88	23.12
ethyl vanillin	17.08	31.44
isoeugenol	17.09	27.57
3-methyl-*p*-anisaldehyde	17.23	20.08
b-caryophyllene	17.32	10.56
trans-carvyl propionate	17.65	15.76
a-methylcinnamic acid	18.15	4.64
a-humulene	18.29	12.05
2,3-dimethylanisaldehyde	18.46	25.74
b-ionone	18.57	18.37
vanillin acetate	18.93	30.85
pentadecane	19.34	8.60
valencene	19.40	13.15
2,5-dimethylanisaldehyde	19.50	21.72
6-methylcoumarin	20.22	32.66
carvone acetate	20.50	26.09
7-methylcoumarin	20.65	32.52
ethyl laurate	21.69	16.17
caryophyllene oxide	21.88	19.47
hexadecane	22.27	10.64
cinnamide	22.36	45.53
amyl cinnamaldehyde	23.08	25.95
cis-trans-farnesol	24.61	27.48
heptadecane	25.10	12.94
trans-trans-farnesol	25.28	28.09
guaiazulene	27.04	28.98
nootketone	27.69	31.89
octadecane	27.83	15.40
nonadecane	30.44	17.92
eicosane	32.94	20.44
heneicosane	35.32	22.91
docosane	37.62	25.35
tricosane	39.79	27.68
tetracosane	42.02	29.95
hexacosane	47.40	34.26

See **page 30** for product listing.