Flavors & Fragrances by Gas and Liquid Chromatography

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Flavors & Fragrances by Gas and Liquid Chromatography

I. Flavor Compounds by HPLC

II. Flavor Compounds by GC

III. Flavor Profiling Techniques

IV. Fragrance Analysis

Analysis of Flavors by HPLC

- Suitable for less volatile, thermally unstable compounds
- Flavor Compounds by HPLC
  - Vanillin & ethyl vanillin
  - Capsaicins (heat)
  - Spice compounds
  - Browning reaction products

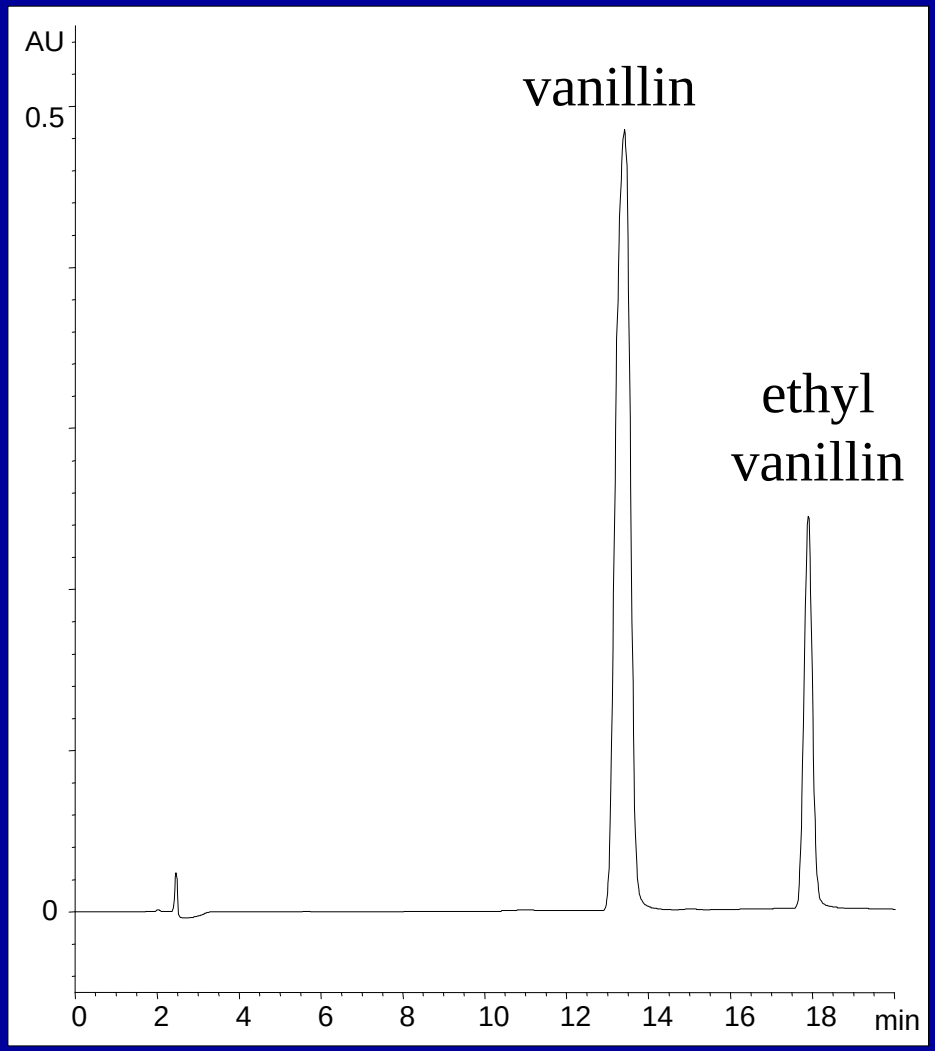
Flavor Compounds by HPLC

Vanillin & Ethyl Vanillin

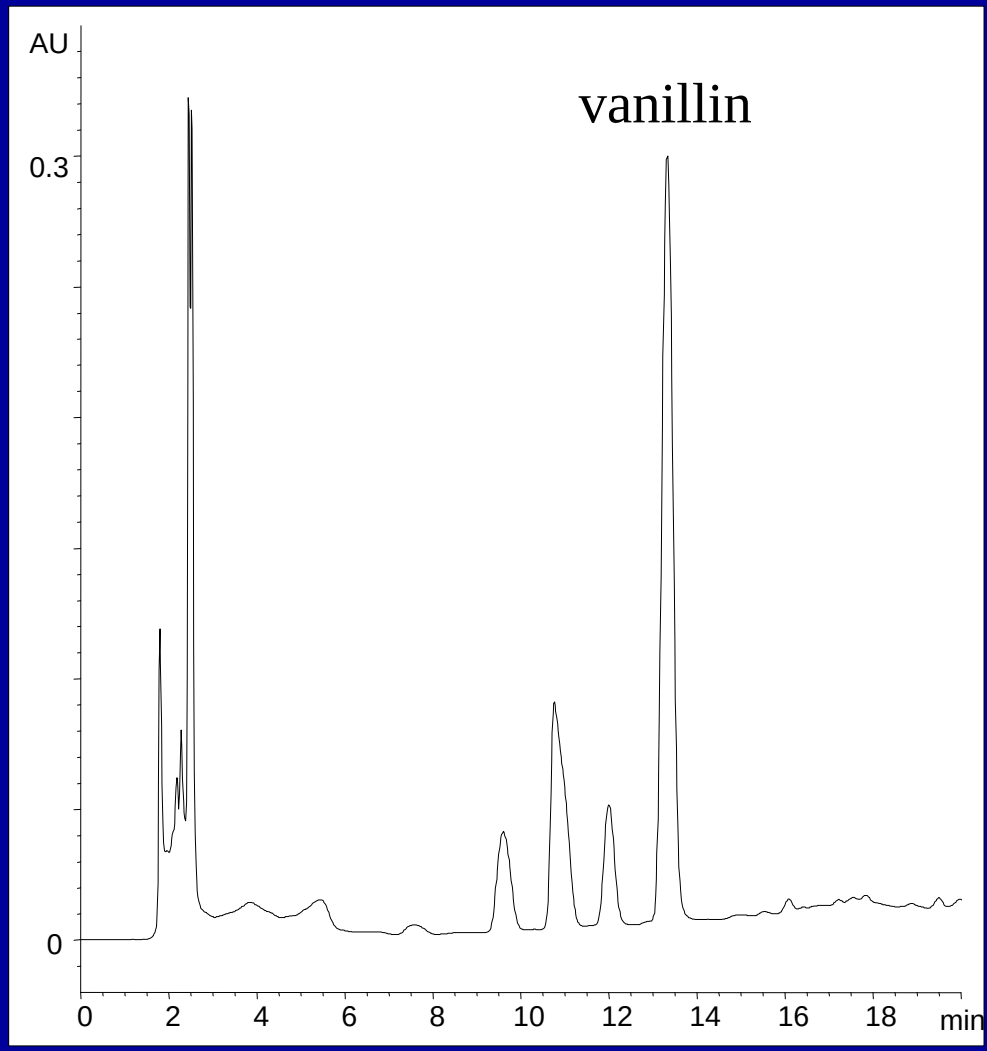
- Real Vanilla Extract
  - Vanillin, + many other flavor compounds
  - >35% alcohol by volume
- Vanilla Flavorings
  - Vanillin & ethyl vanillin
- HPLC Analysis
  - AOAC method 990.25
  - Reversed phase with UV detection

Flavor Compounds by HPLC

Vanillin & Ethyl Vanillin



Vanillin / Ethyl Vanillin Standard



Vanilla Extract

Flavor Compounds by HPLC

Vanillin & Ethyl Vanillin by HPLC

Separation Conditions:

Column: Ultra C8, 150 x 4.6 mm, 5µm

A: 1.2% acetic acid B: methanol

Time	%B
0	20
5	20
15	40
20	40
25	20

Flow Rate: 1mL/min UV@ 254 nm

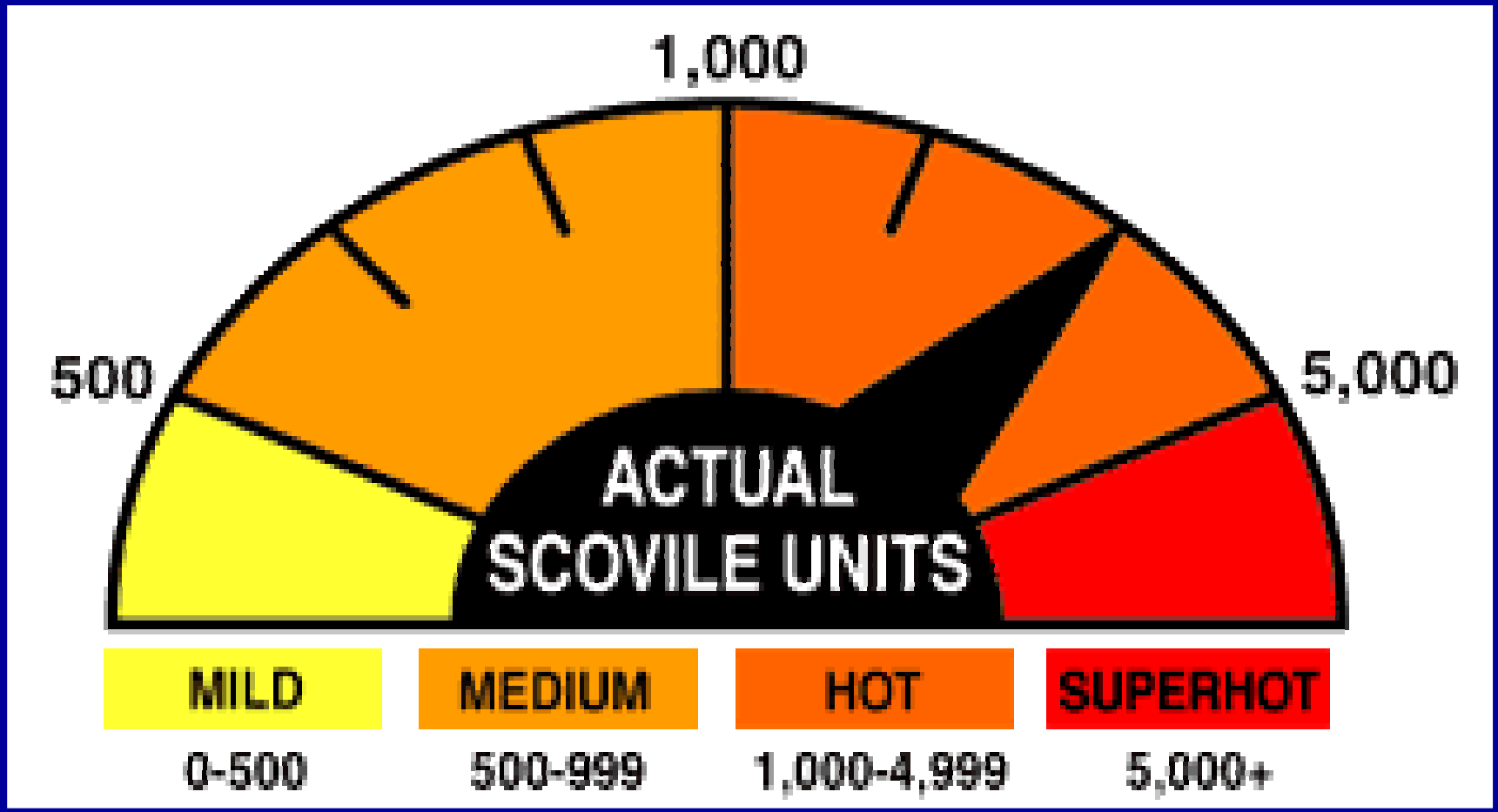
Flavor Compounds by HPLC

Heat Content in Foods

- Heat Content
  - Sensory – Scoville Heat Units
- Traditionally Measured by Trained Panelists
  - Can be subjective
  - Involved procedure
- HPLC Procedures
  - American Spice Trade Association (ASTA)
  - AOAC International
  - Calculate Scoville Heat Units - relate to sensory

Flavor Compounds by HPLC

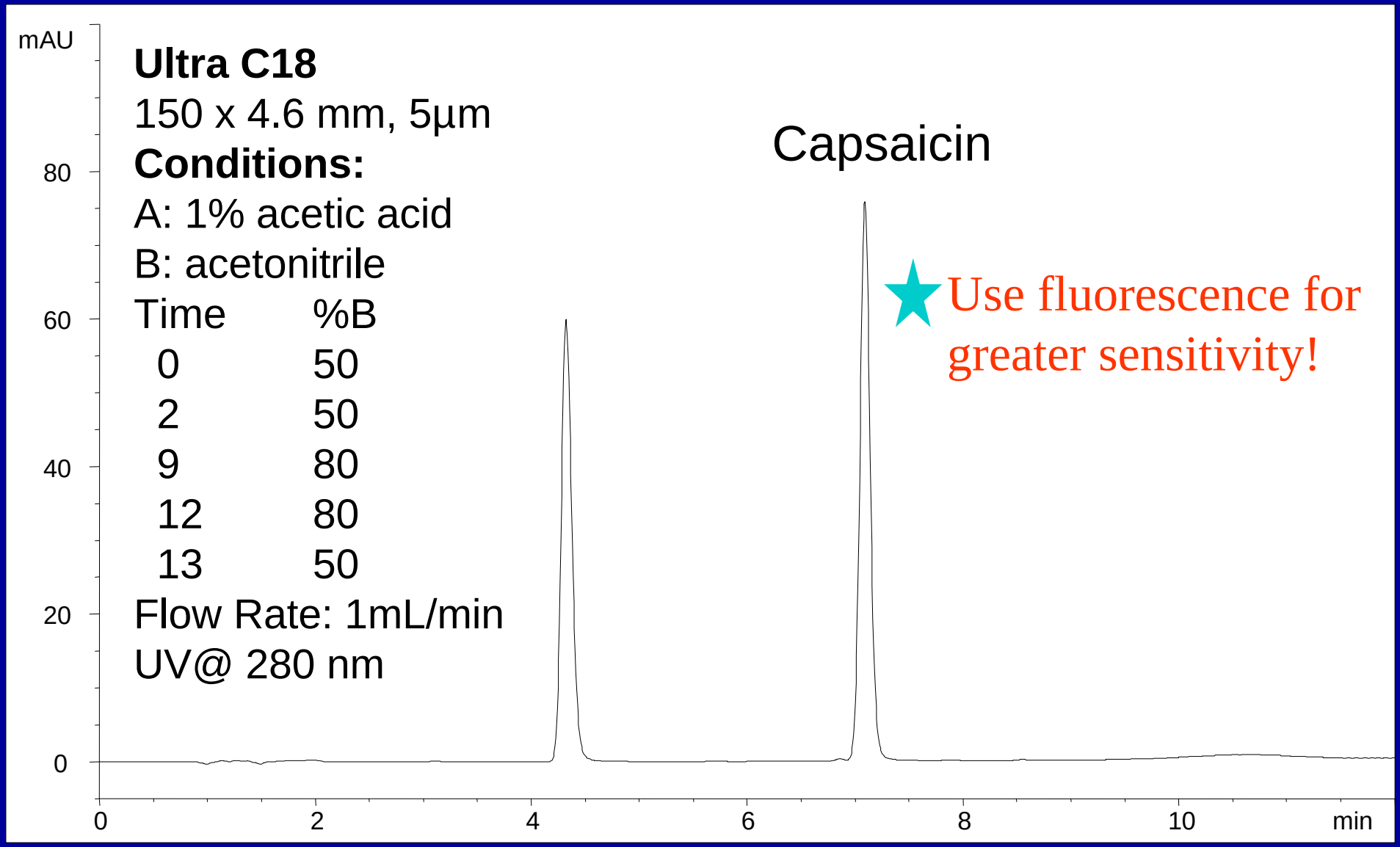
Heat Content in Foods



Source: http://www.mohotta.com

Flavor Compounds by HPLC

Heat Content in Foods



Ultra C18
150 x 4.6 mm, 5µm
Conditions:
A: 1% acetic acid
B: acetonitrile

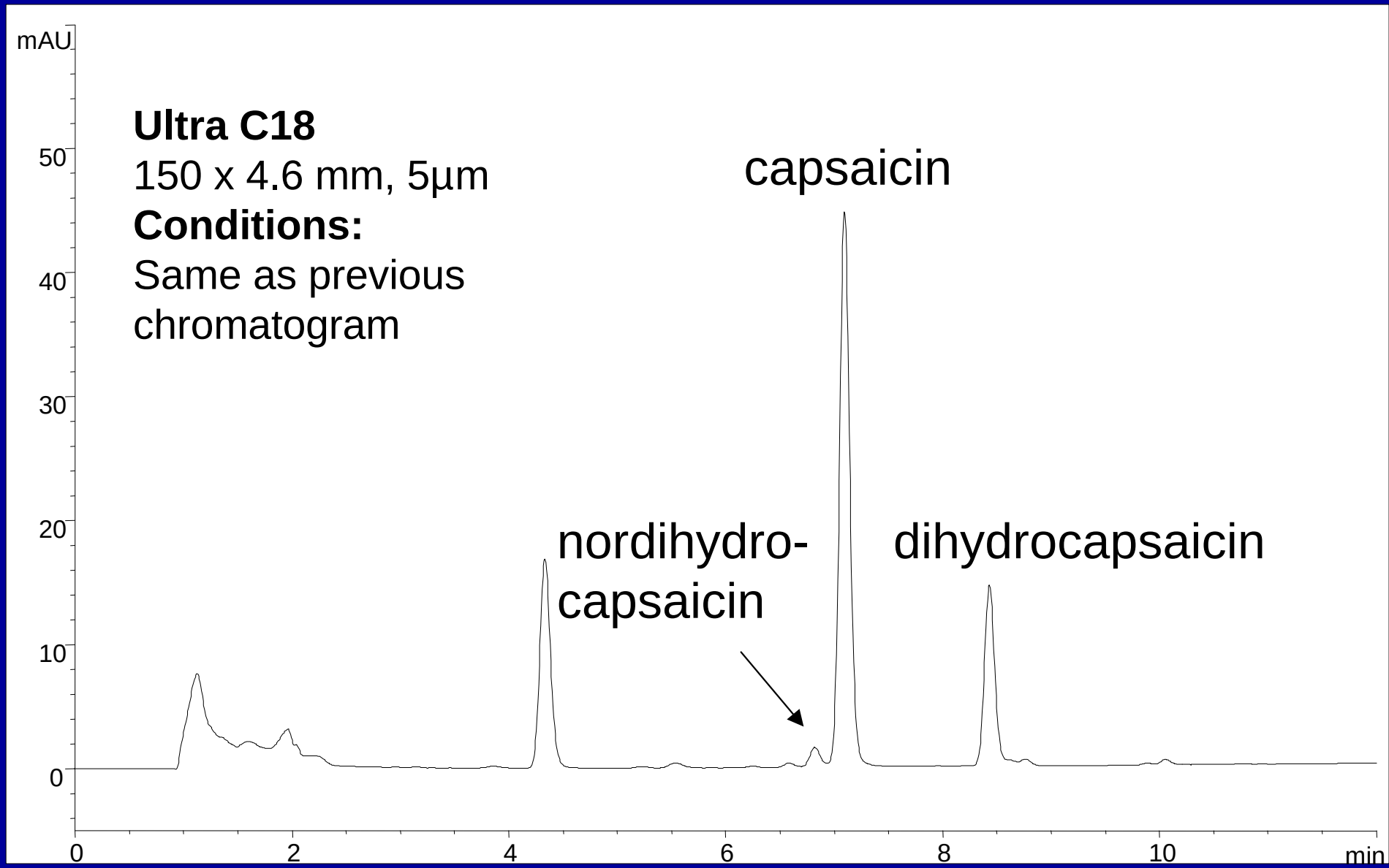
Time	%B
0	50
2	50
9	80
12	80
13	50

Flow Rate: 1mL/min
UV@ 280 nm

★ Use fluorescence for greater sensitivity!

Flavor Compounds by HPLC

Heat Content of Habenero Nuggets



Flavor Compounds by HPLC

Analyzing Spice Components

Monitoring the Chemical Compounds Responsible for the Distinctive Flavor/Aroma

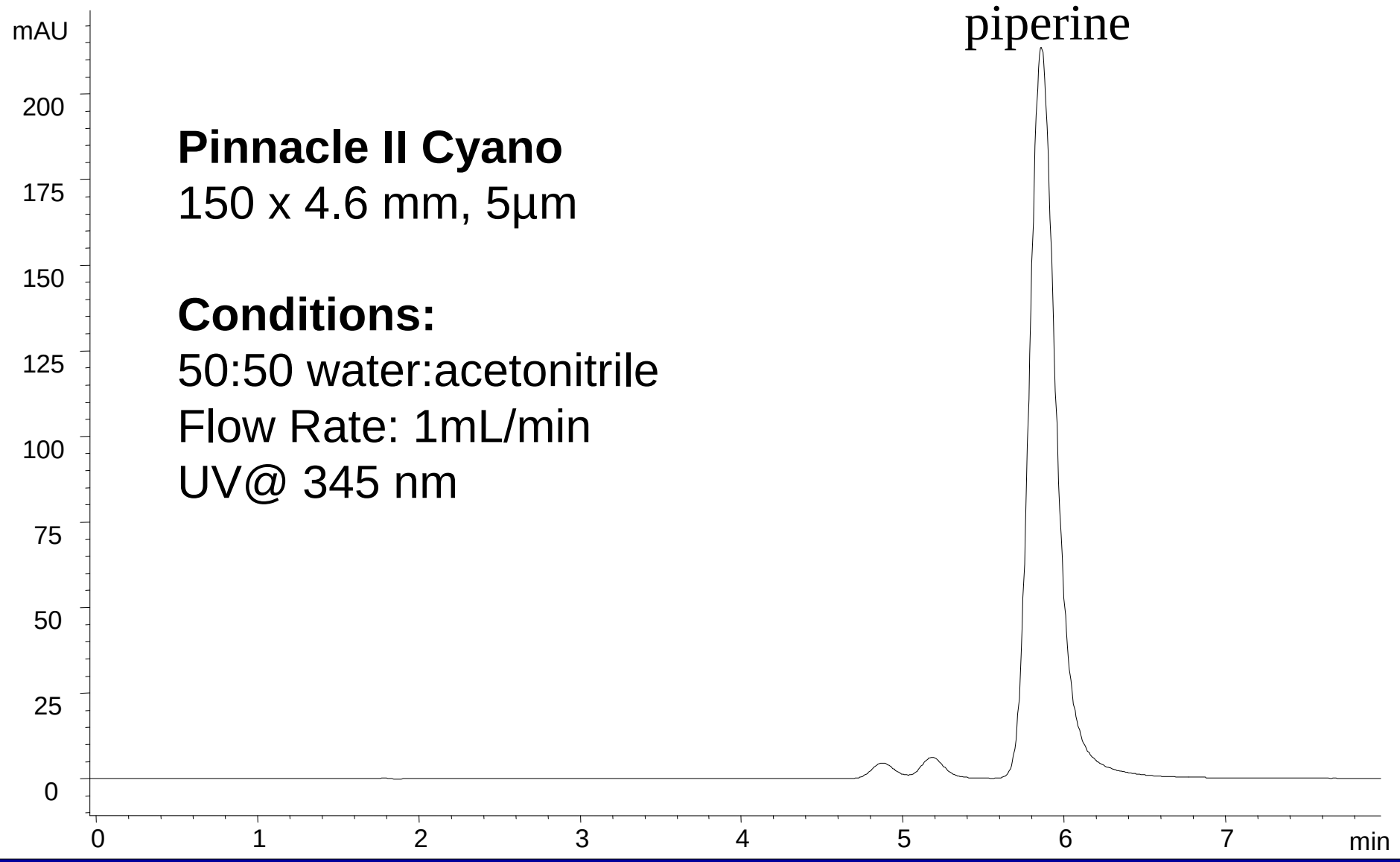
Spice	Compound(s)
Anise	(*E*)-Anethole, methyl chavicol
Caraway	*d*-Carvone, carvone derivatives
Cinnamon	Cinnamaldehyde, eugenol
Fennel	(*E*)-Anethole, fenchone
Nutmeg	Sabinine, α-pinene, myristicin
Saffron	Safranal
Turmeric	Turmerone, zingeriberene, 1,8-cineole

Flavor Compounds by HPLC

Piperine

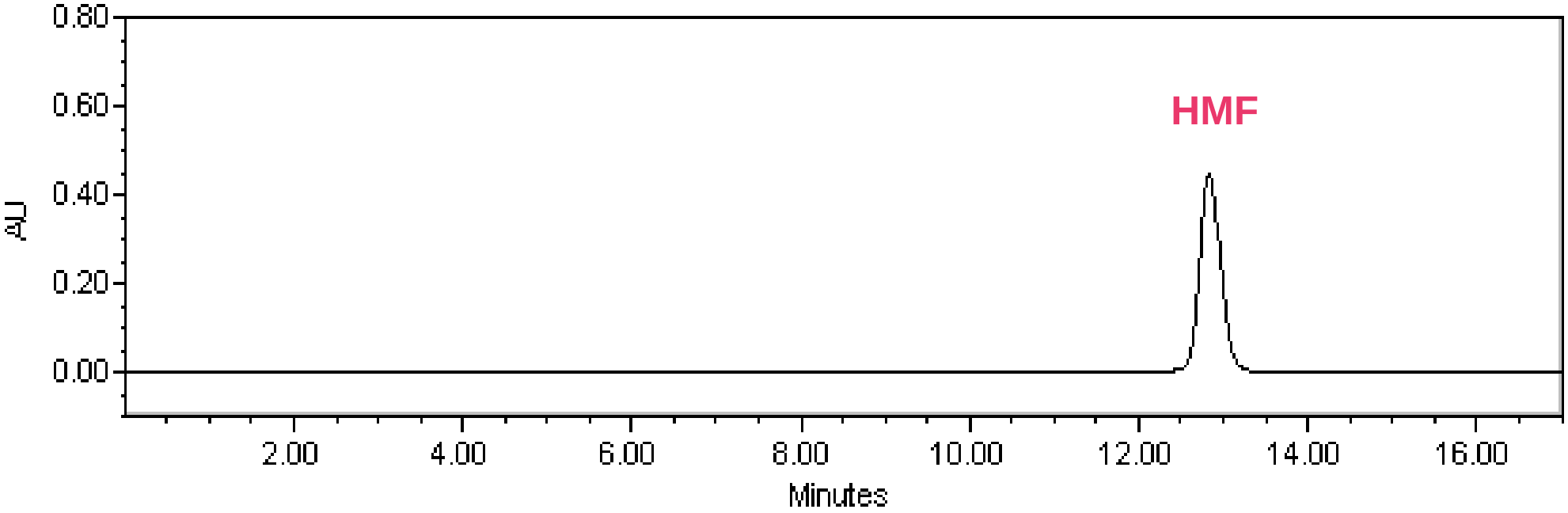
Pinnacle II Cyano
150 x 4.6 mm, 5µm

Conditions:
50:50 water:acetonitrile
Flow Rate: 1mL/min
UV@ 345 nm



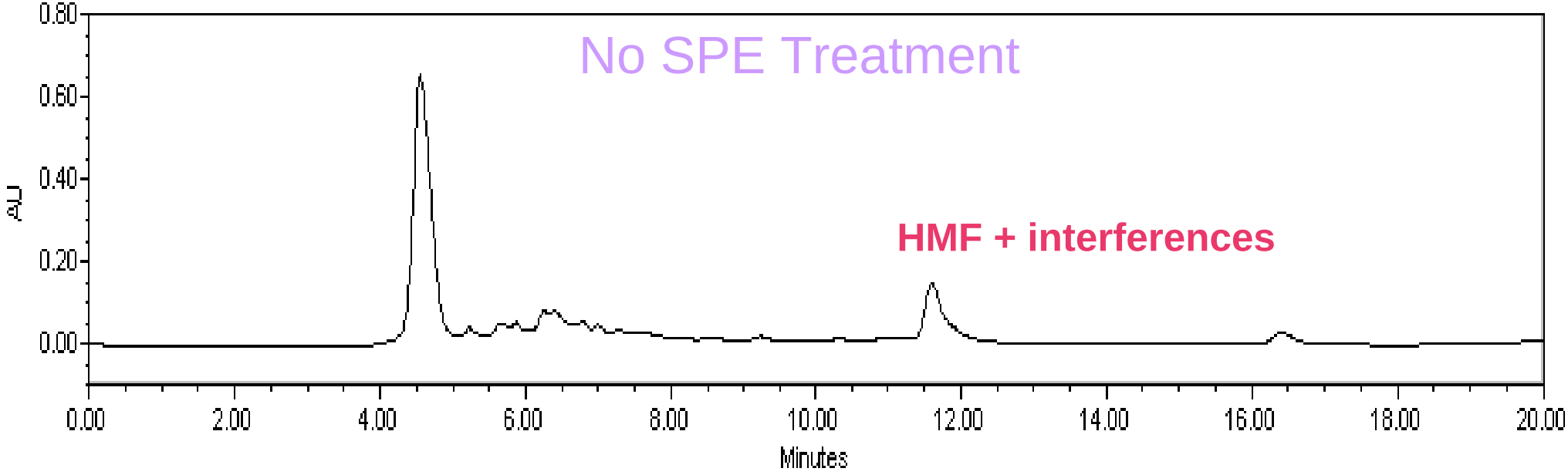
Browning Reaction Products

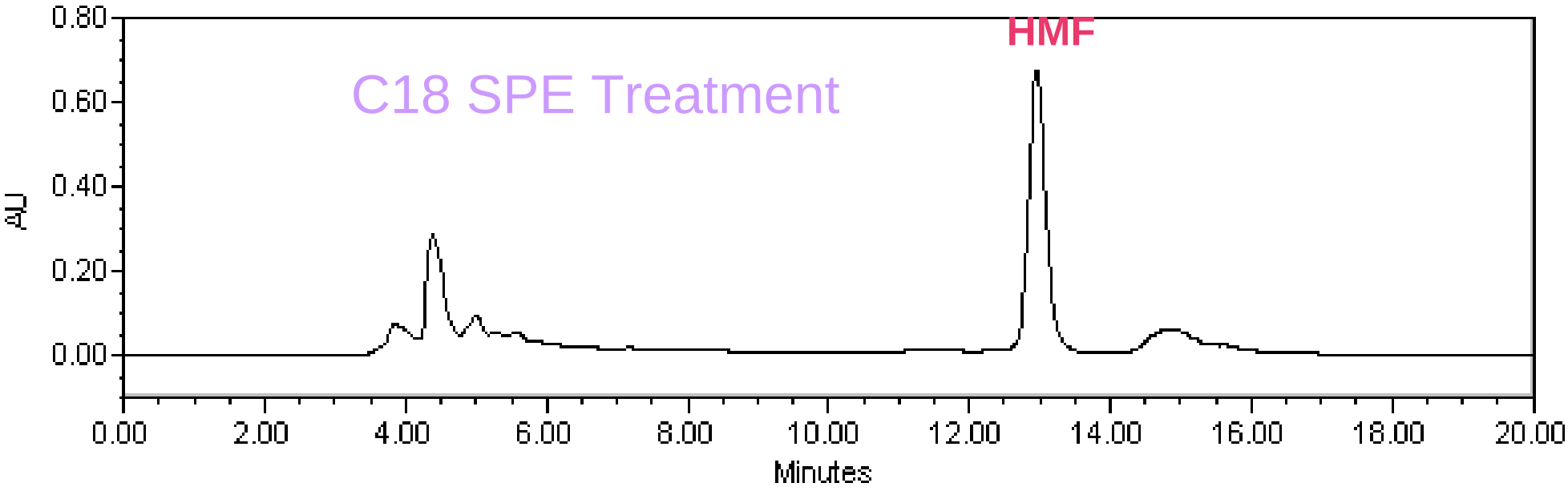
HMF Standard by HPLC



Column: Ultra C18 (Restek Corp.), 250 mm x 4.6 mm, 5 µm
Mobile Phase A: 90:10 water:methanol, 10 mM ammonium formate
Mobile Phase B: 10:90 water:methanol, 10 mM ammonium formate
Gradient: 0-5 min at 100% A, to 100% B at 10 min, 10 min. hold
Flow: 0.5 mL/min.
Temperature: ambient
Detector: UV @ 280 nm
Injection Volume: 10 µL

HMF in Grape Juice by HPLC



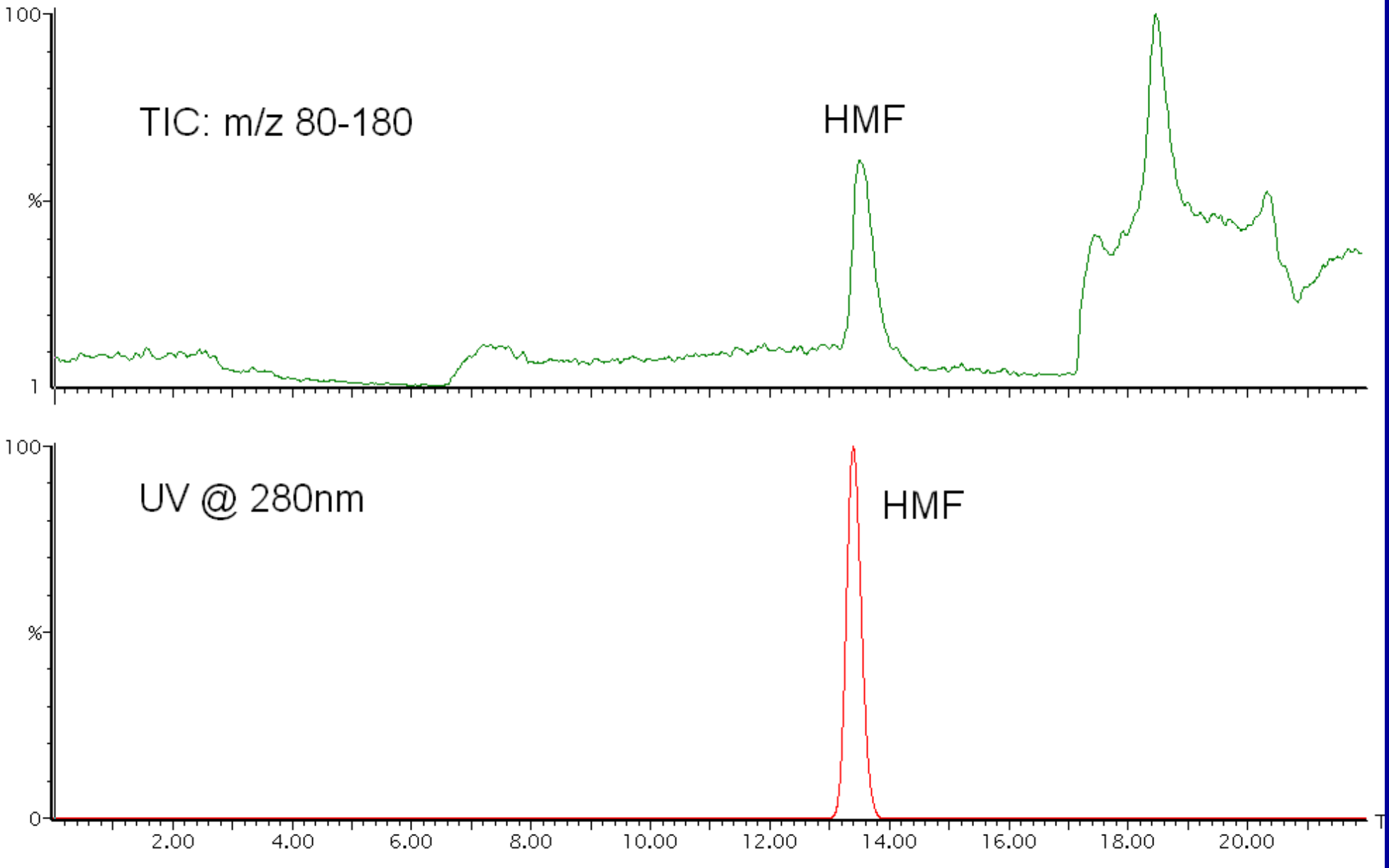


Sample Prep: HMF in Grape Juice

Extraction Procedure

1. Conditioning
   a. Apply 3 mL methanol
   b. Apply 3 mL deionized water
2. Sample Application
   a. Apply 4 mL sample to moist SPE tube, gravity feed
3. Wash
   a. Pull remaining sample through tube, using vacuum
   b. Apply 3 mL water
   c. Remove excess water from bed under vacuum
4. Elution
   a. Apply 2 mL elution solvent, gravity feed, dilute to volume

HMF by LC/MS


100
%
1
TIC: m/z 80-180
HMF
100
%
0
UV @ 280nm
HMF
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
T

Analysis of Flavors by GC

- GC is suitable for volatile, thermally stable compounds
- Higher resolution separations generally possible
- Flavor Compounds by GC
  - Alcoholic beverages
  - Volatiles

Flavor Compounds by GC

- Determine the Goals of the Analysis
  - QC / purity determinations
  - Comparison of flavors
  - Reverse engineering
- Detection
  - FID & MSD most common
  - Specialty detectors (GCO, AED)

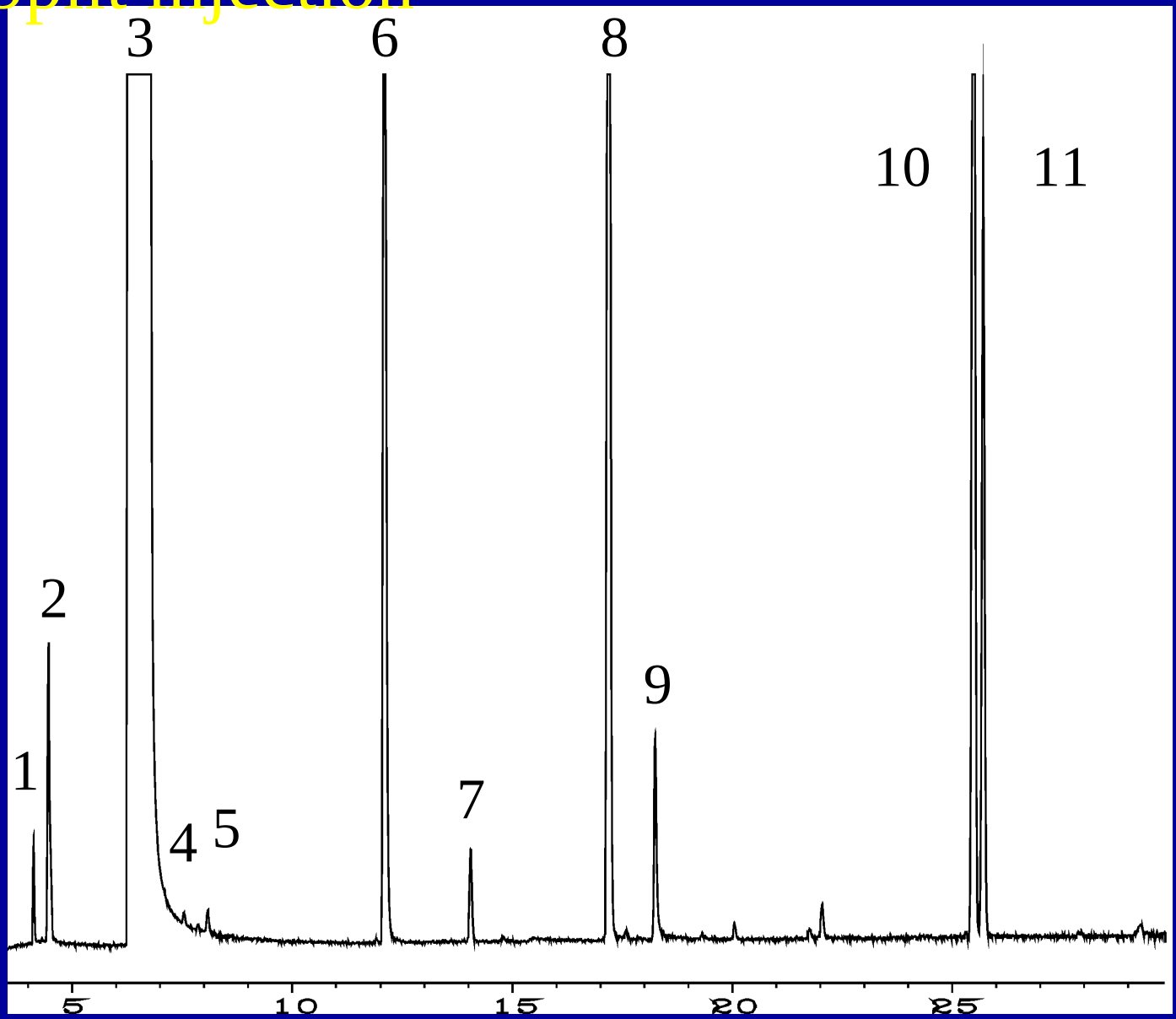
Flavor Compounds by GC

Sampling Techniques

- Liquid Sampling
  - Split/splitless injection
  - Direct or on-column injection
  - Large volume injection
- Headspace Sampling
  - Static
  - Dynamic (purge & trap)
  - Thermal desorption
- Extraction Techniques

Flavor Compounds in Scotch

Split injection



Peak List

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

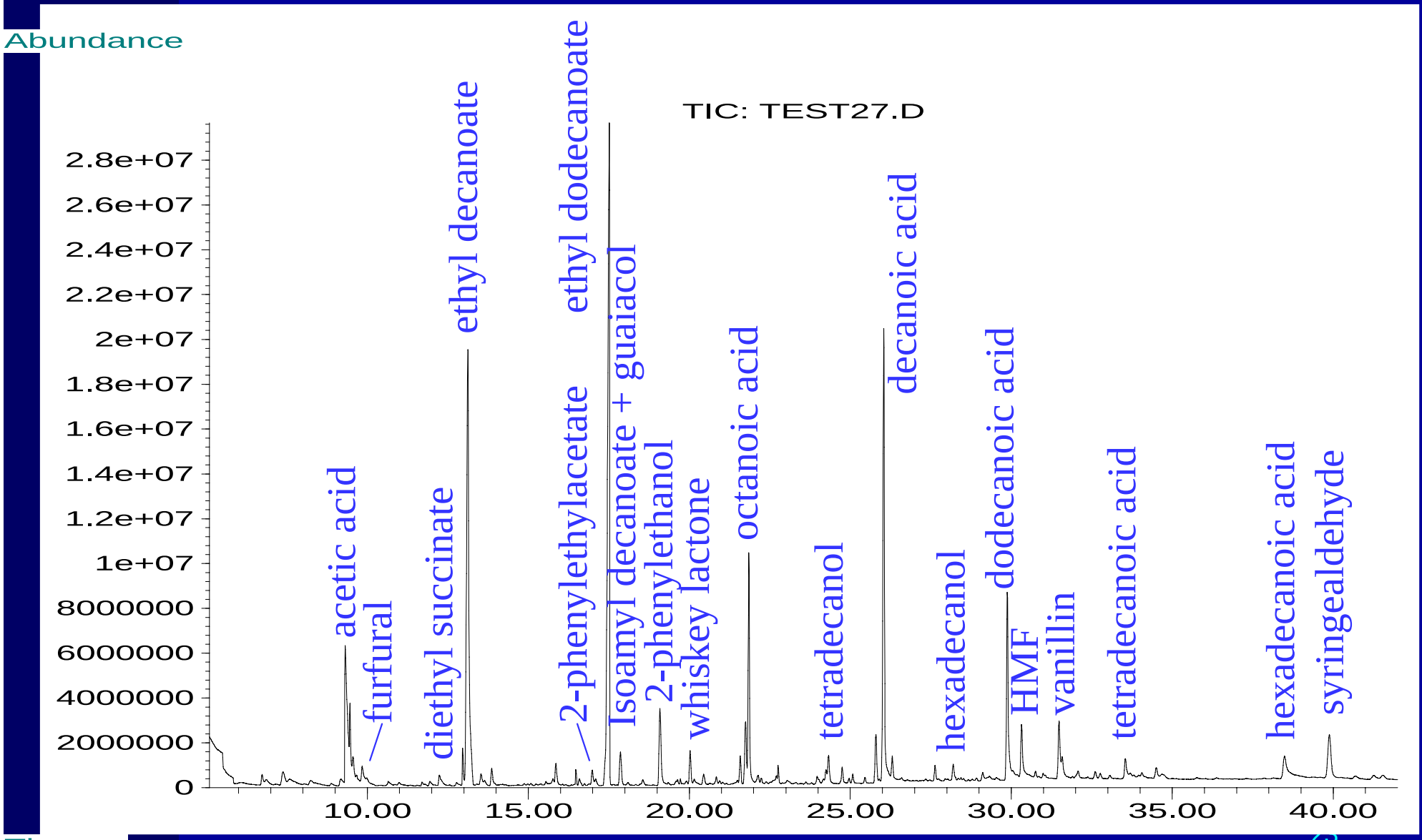
Flavor Compounds in Scotch by GC

- Analytical Column
  - Rtx®-1301, 60m x 0.25mm x 1.4µm
  - Linear velocity: H_2 @ 40 cm/sec.
- Temperature Program
  - 35°C (5 min. hold) to 100°C @ 1°C/min.
- Injector
  - 100:1 split
  - 1.0 µL neat injection using a Cyclosplitter® sleeve
- Detector
  - FID @ 200°C

Large Volume Injectors (LVI)

- Large volume injected at low temperature
  - Must have early solvent vent
  - Injector rapidly heated after venting
- Injection must not over-fill the pre-column
  - Injection rate must match the evaporation rate
- Used in the analysis of trace level components

Large Volume Injectors (LVI)
Malt Whiskey



Chromatogram courtesy of Kevin MacNamara, Irish Distilleries

Chromatogram Conditions

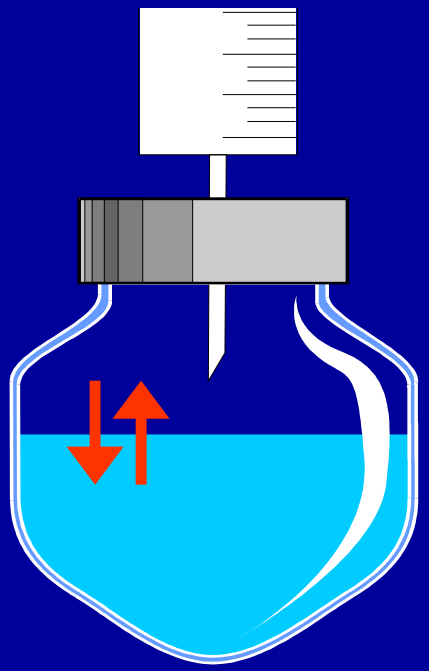
Large Volume Injectors – Malt Whiskey

Column:	Stabilwax®-DA, 30m x 0.18mm, 0.18µm
Oven temp.:	60°C(2 min. hold) to 100°C at 20°C/min, to 240°C at 5°C/min, 10 min. hold
Carrier gas:	Helium @ 45 cm/sec
Quad Temp:	150°C
Source Temp:	230°C
Scan range:	30-400AMU
Ionization:	70eV EI
Inj Volume:	10µL LVI (splitless) @ 10 µL/min
Injector:	Gerstel CIS Injector
	35°C for 2 min, then 10°C/sec to 300°C, hold 5 min.
He vent flow:	600 mL/min with 1.8 min. vent end time

Flavor Profiling
Headspace Sampling Techniques

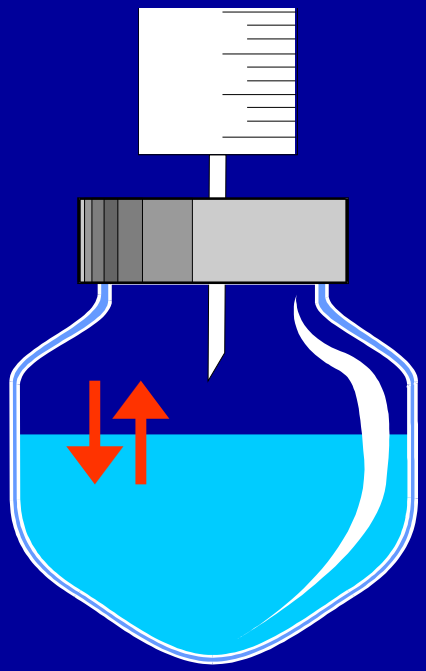
- Advantages
  - “Cleaner” samples
  - Minimal sample preparation
- Types
  - Static headspace
  - Dynamic headspace
  - Thermal desorption

Flavor Profiling
Static Gas Extraction



- Advantages
  - Excellent screening tool
  - Inexpensive
  - Minimal sample carryover
  - Easy to perform
- Disadvantages
  - Less sensitive
  - Involves preparing calibration in sample matrix

Flavor Profiling: Static Gas Extraction



- Constant ratio between liquid & gas phases at equilibrium

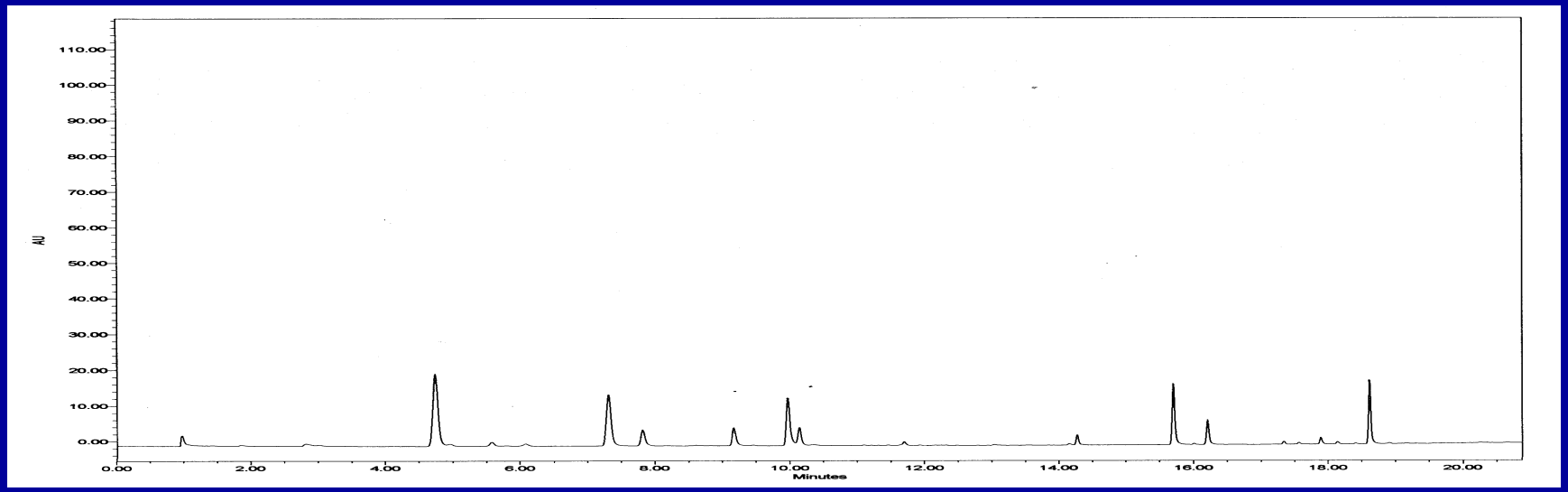
Where $K = (C_{gas} / C_{liquid})$

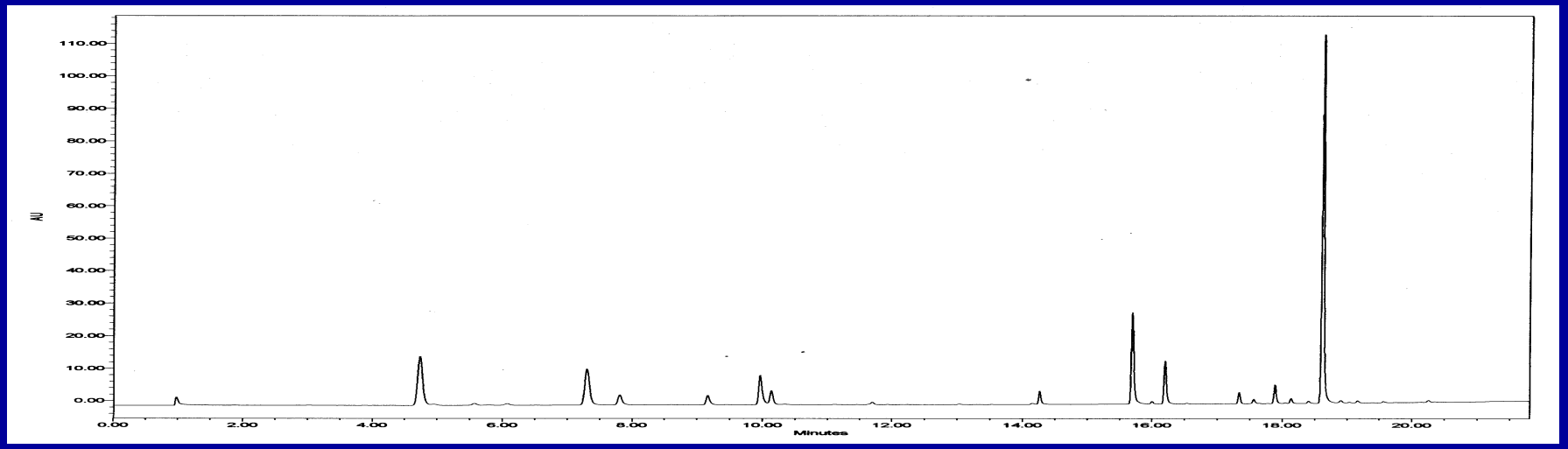
K = distribution coefficient

- Large K values favor the gas state
- C_{gas} is directly proportional to peak area

Lit. #59895 — Headspace Guide

Flavor Profiling: Static Gas Extraction

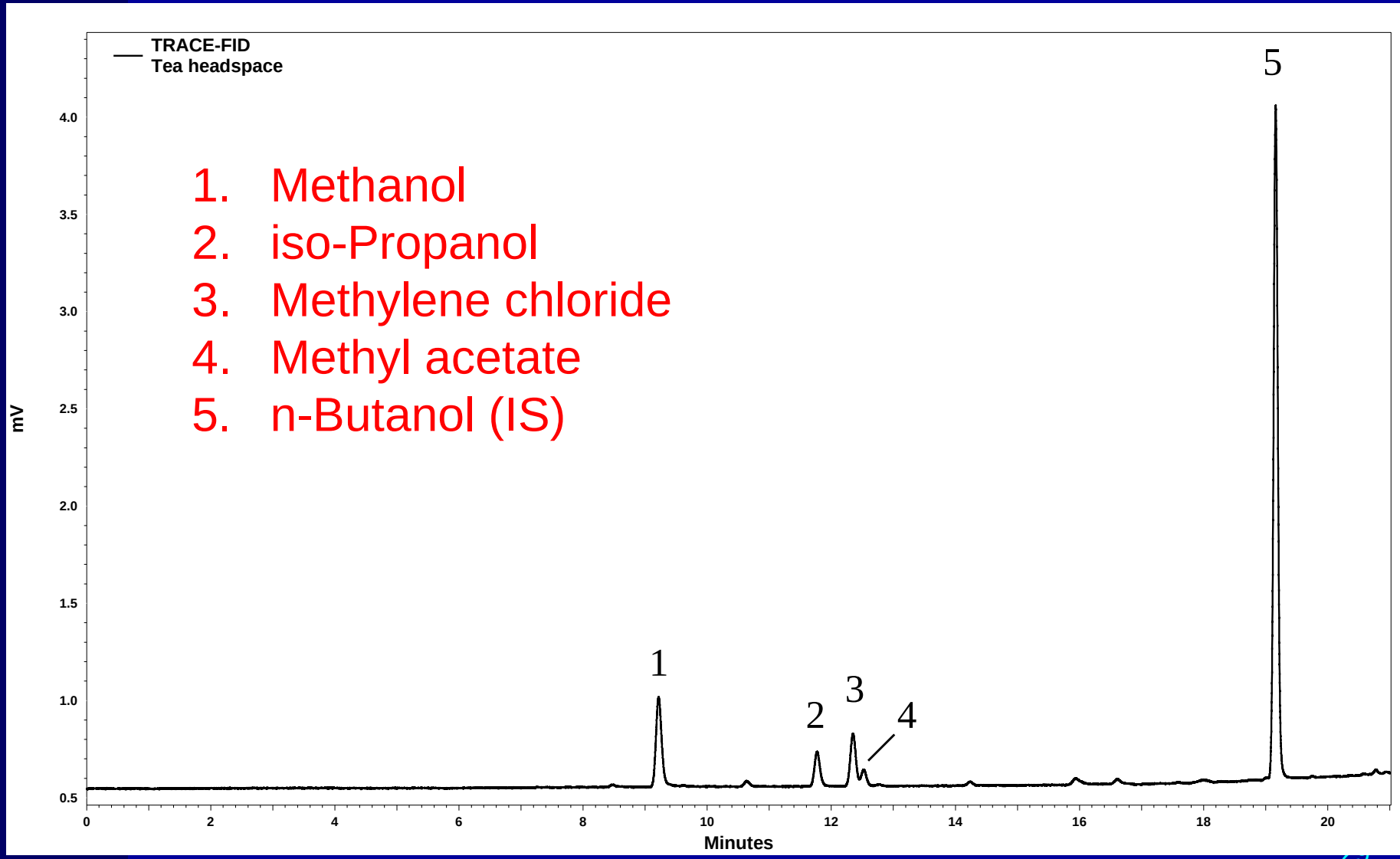




Volatiles from 2 different batches of chewing gum. The headspace was sampled after heating to 60°C.

Flavor Profiling: Static Gas Extraction

Residual solvents in decaffeinated lemon tea



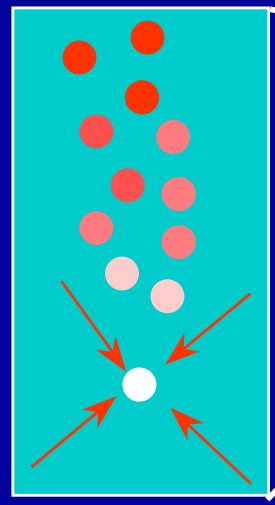
1. Methanol
2. iso-Propanol
3. Methylene chloride
4. Methyl acetate
5. n-Butanol (IS)

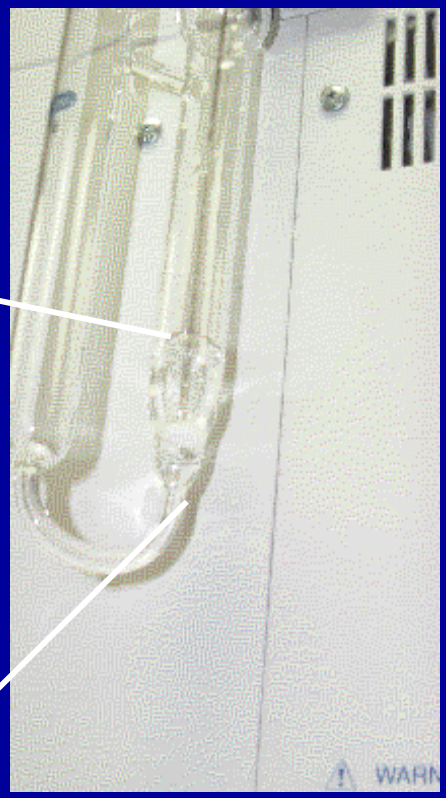
Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Inlet System

- Concentrates volatiles
- Dynamic extraction of solids and liquids
- Adsorbent trap
- Desorb (10-80 mL/min)
- Narrow bore column (split flow)
- Part of GC system

Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sampling

1. Wet Purge

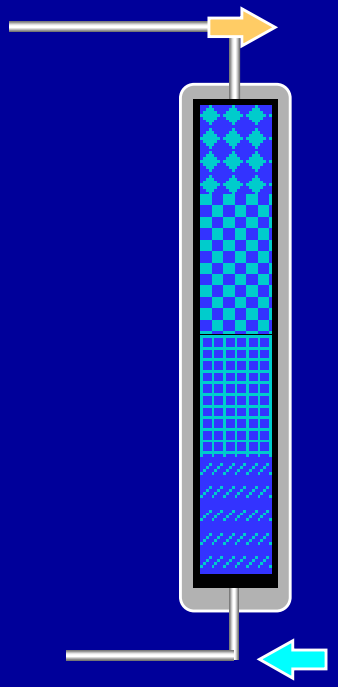




- Carrier gas bubbled through the matrix
- Volatiles in matrix diffuse into carrier gas & are carried away
- Typical flows: 40-50 mL/min. (can heat)
- Typical purge time: 10-12 min.

Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

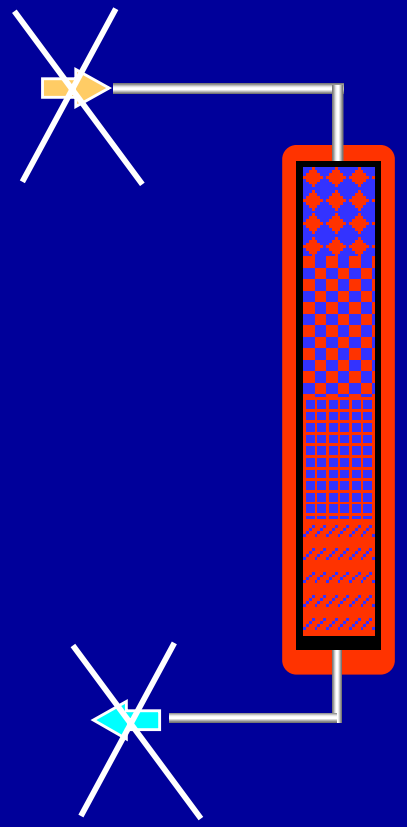
2. Dry Purge



- Trap is dried by purging with gas only
- Typical time: 1-4 min.

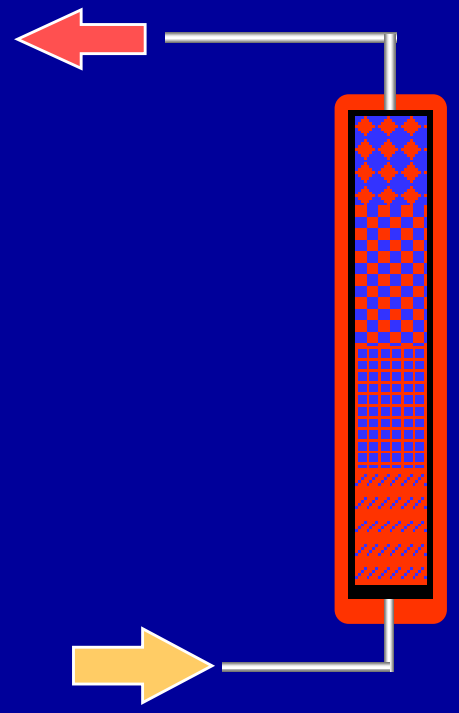
Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

3. Desorb Preheat



- Trap is heated without flow
- Typical temp: 5^{o} below desorb temperature
- Minimizes retention on the trap

Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

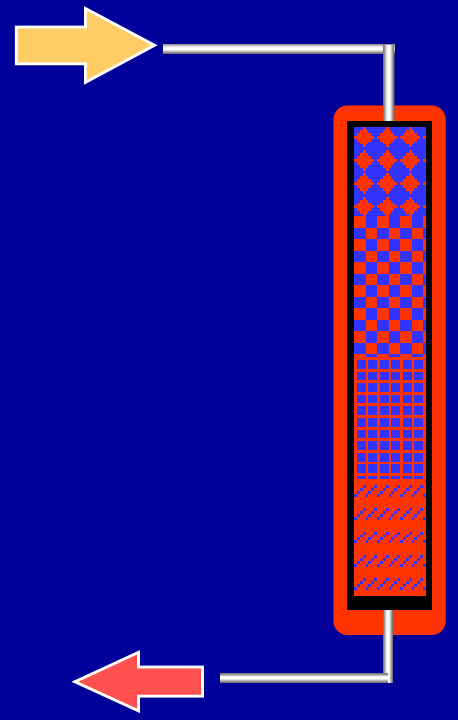


4. Desorb

- Trap is backflushed into column
- Typical time: 2 - 4 minutes
- Typical flow: 10-80 mL/min.
- Typical temp: 180° - 250°C

Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

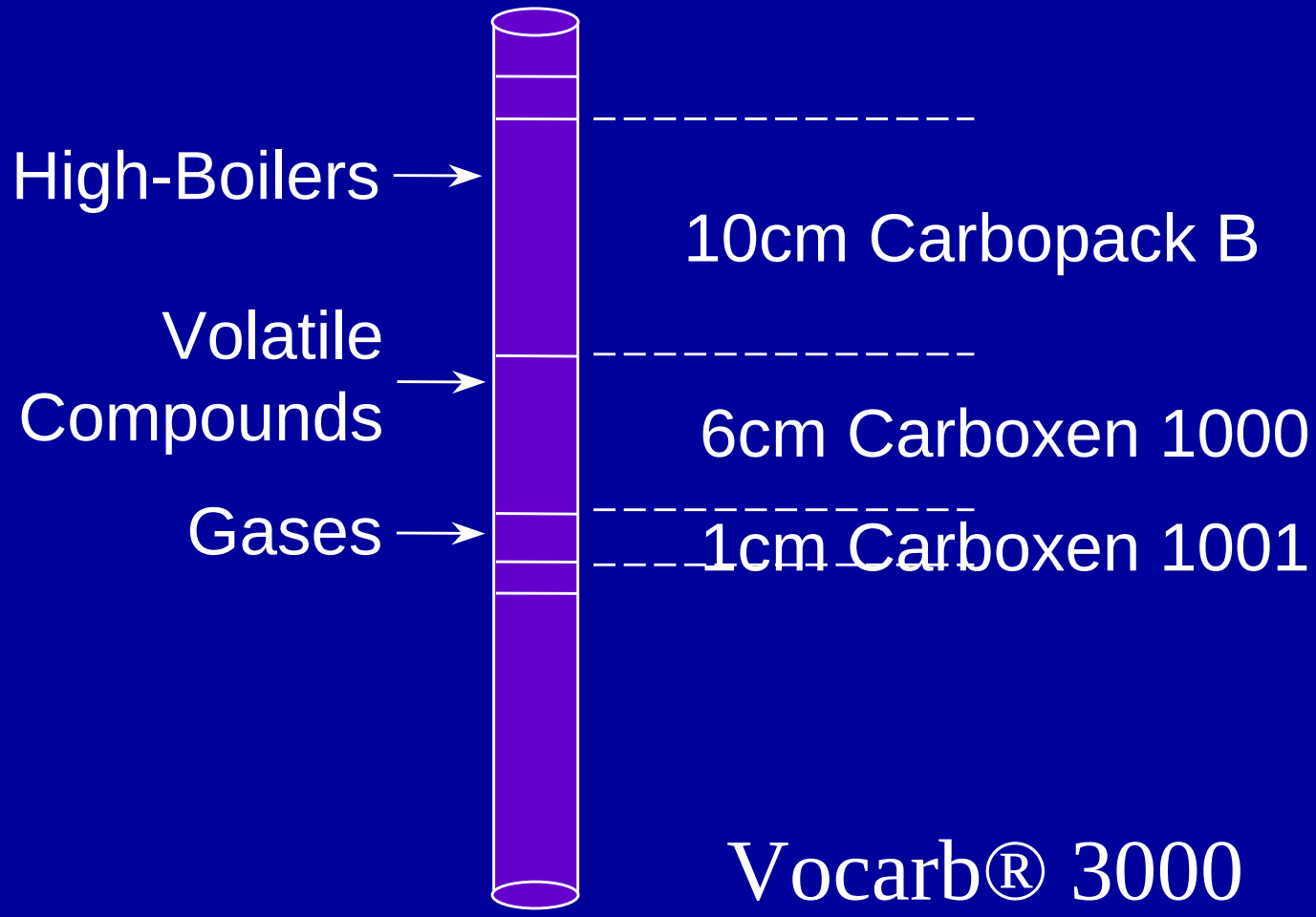
5. Trap Bake



- Trap is baked clean with flow
- Typical time: 8^{+} minutes
- Typical temp: higher than desorb temperature
- Avoid overheating adsorbents

Flavor Profiling: Dynamic Gas Extraction

Typical Adsorbents for Purge & Trap



Vocarb® 3000

Flavor Profiling: Dynamic Gas Extraction

Requirements of a Trap

- Retention of polar & non-polar compounds
- Hydrophobic characteristics
- Reproducible desorption
  - Increasing levels of adsorbency
- Able to withstand a broad temperature range

Flavor Profiling: Dynamic Gas Extraction

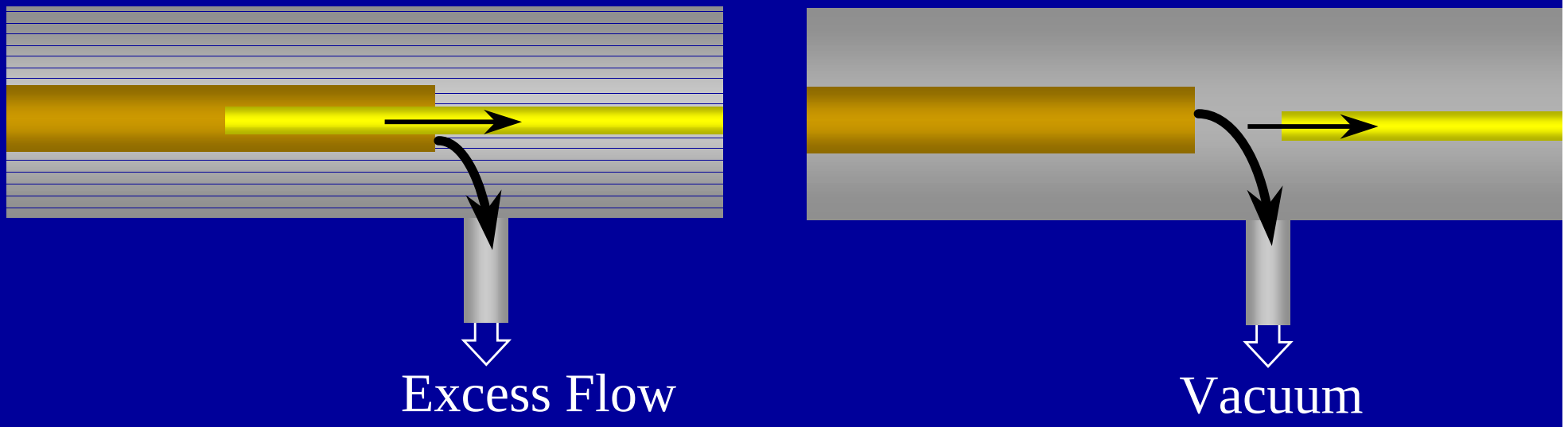
Connecting a Purge & Trap to the GC Column

- Via the injection port
- Directly to the column
  - 1/16" union
  - Silica transfer line from 6 port valve directly to the column
- Low volume injector

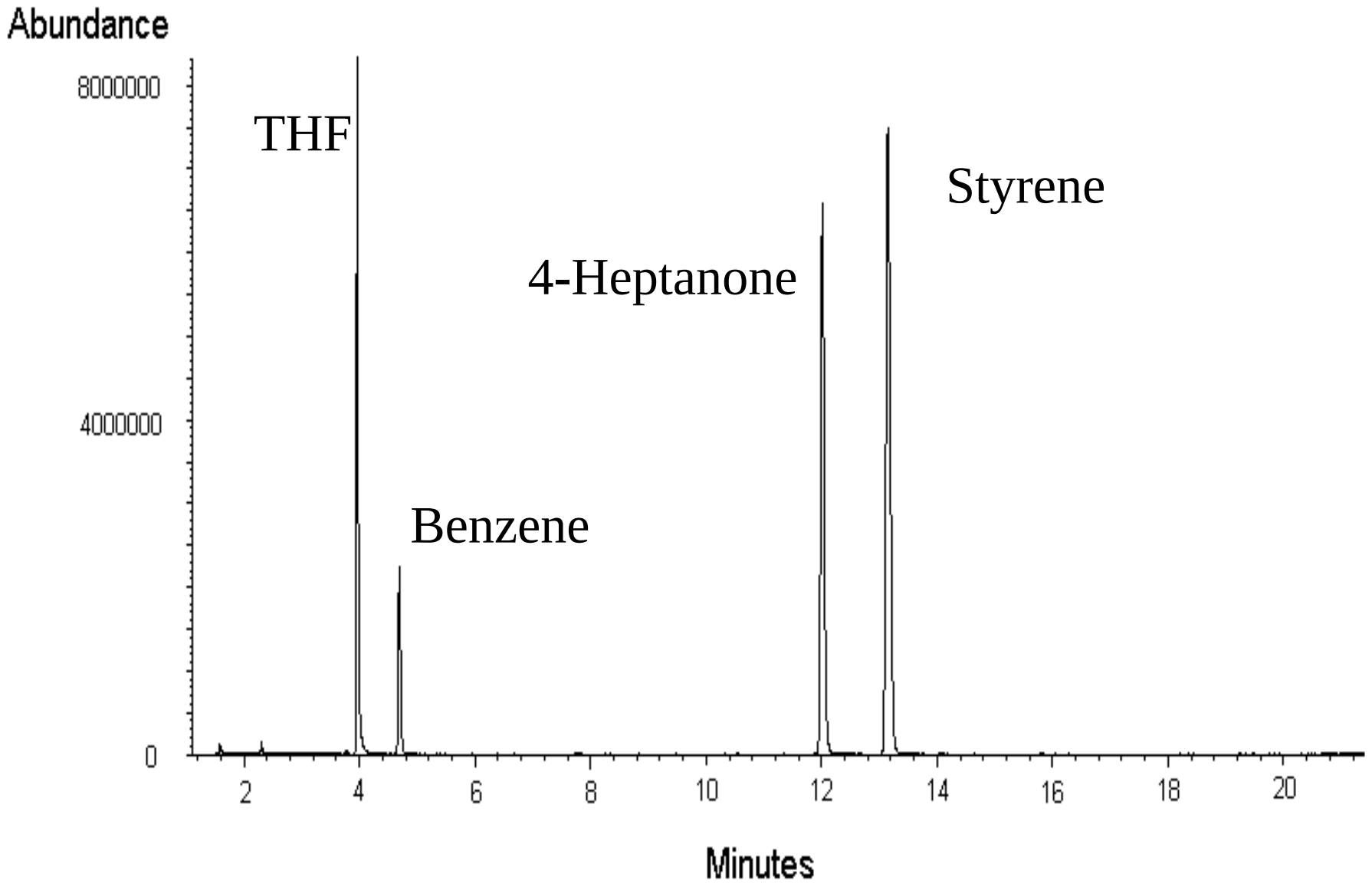
Interfacing to a GC/MS System

- Narrow bore column flows (0.5-1.3 mL/min.) permit a direct interface
- 0.53mm ID columns require:

Open Split Interface or Jet Separator



Purge & Trap GC/MS of Volatiles



Purge & Trap GC/MS of Volatiles

GC Parameters

- Column: Rtx-5MS, 30m x 0.25mm x 1.0um
- Injector: 250°C, 20:1 split
- Carrier gas: Helium at 1 mL/min, constant flow
- Oven: 50 °C to 92 °C at 3 °C/min, to 220°C/min. at 20°C/min. (1 min. hold)

MSD Parameters

- Temperature: 280°C
- Scan Range: 35-260, 1 min. solvent delay
- Ionization: EI @ 70eV

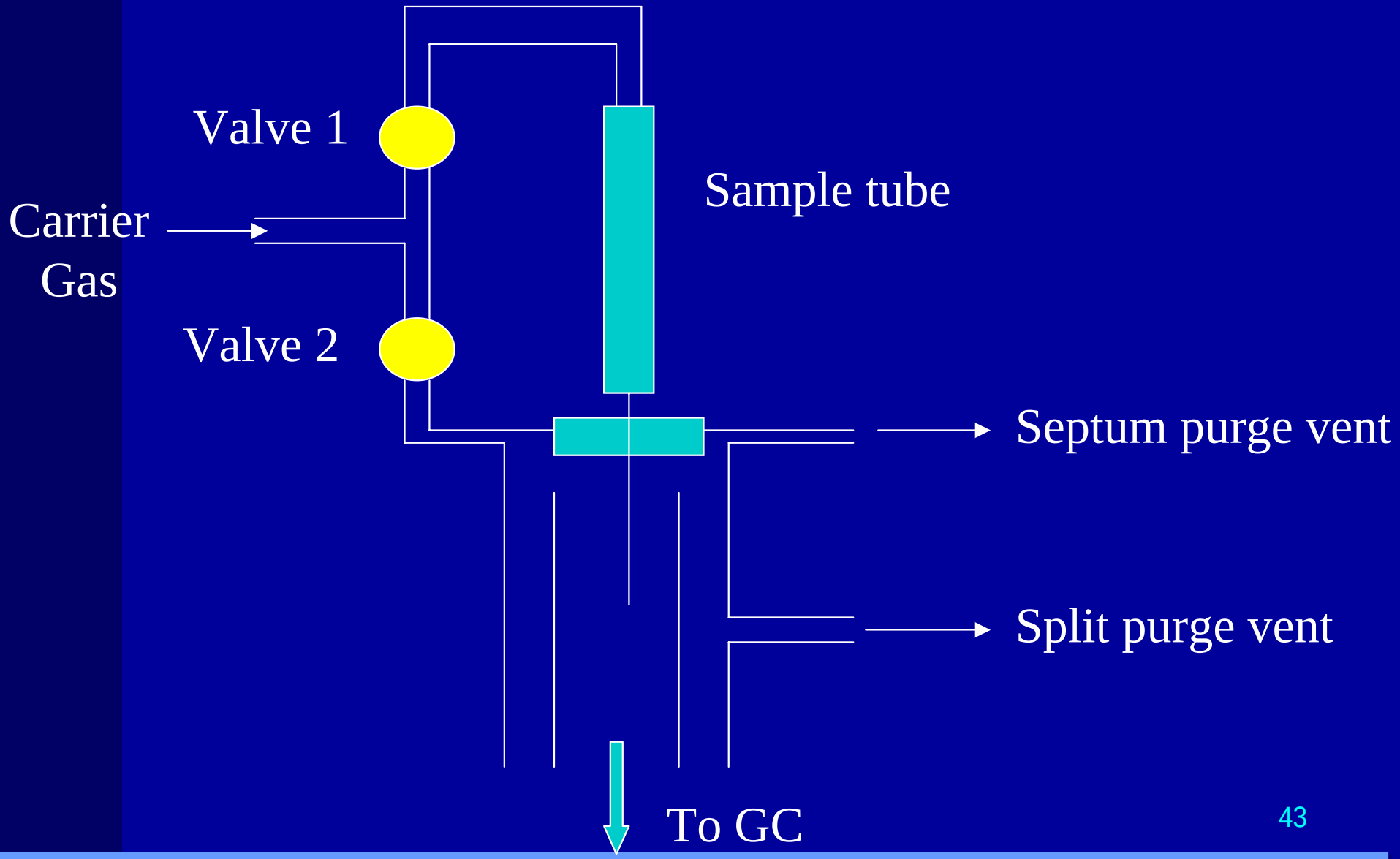
Purge & Trap Parameters

- Concentrator: Tekmar LSC-3100 with Vocarb 3000 (type K) trap
- Purge: 10 min. at 40 mL/min, 60°C
- Dry purge: 3 min. at 40 mL/min.
- Desorb: 2 min. at 40 mL/min, 245°C

Thermal Desorption Techniques

- Heat sample to drive volatiles into headspace
- Can trap and concentrate volatiles
  - Cryofocusing step
- Minimal sample preparation
- Commercially available units

Short-Path Thermal Desorption System



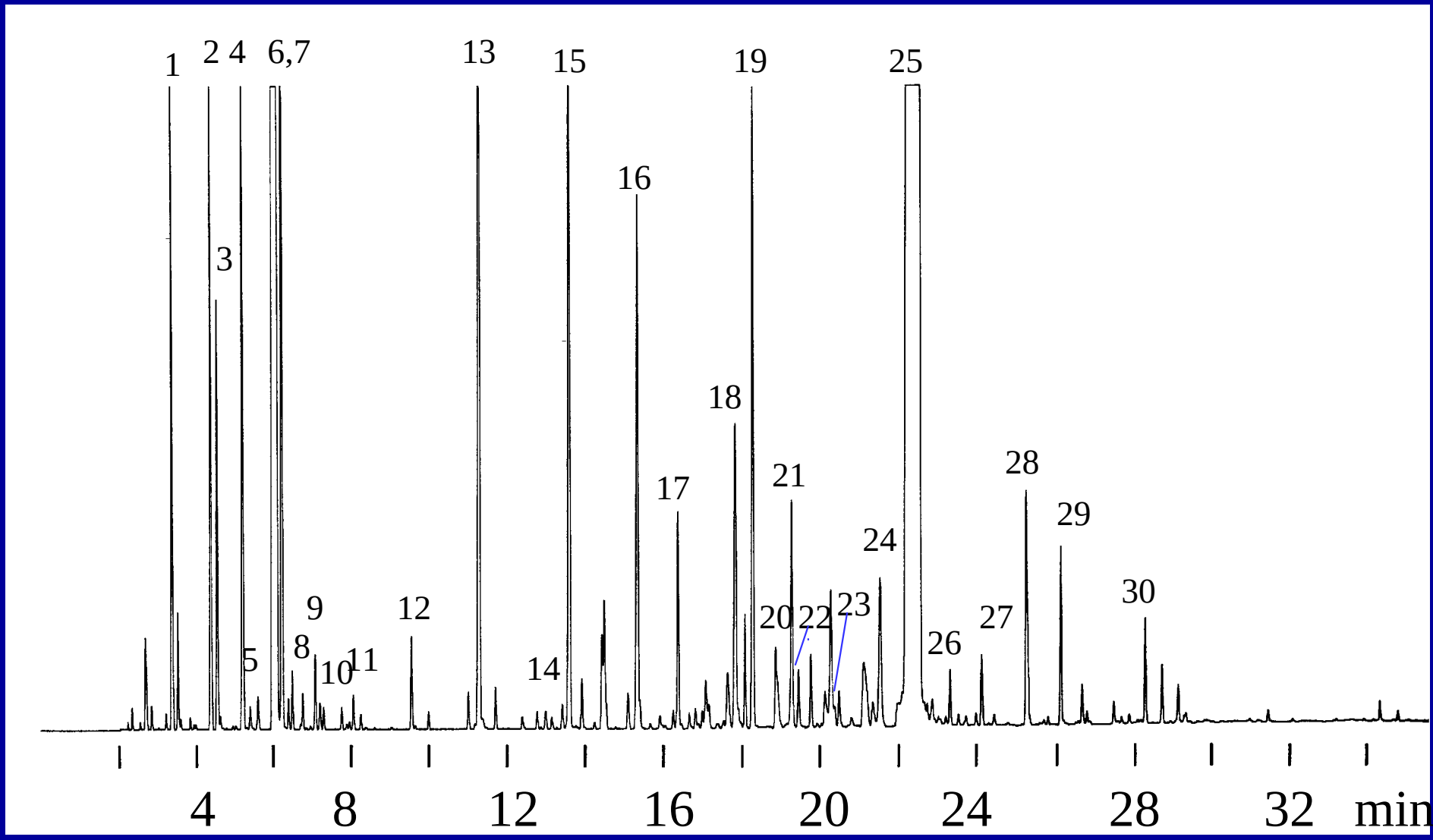
Analysis of Fragrances by GC

- Typically Very Complex
  - 100+ Components
  - Range of compound types, volatilities
- Fragrance Compounds by GC
  - Essential Oils
  - Chiral Compounds

Essential Oil Analysis by GC

- Complex Samples
  - Hundreds of volatiles
  - Many at trace levels
- Multiple Uses / Products
  - Spices, perfumes, fragrances, medicinals
- Multiple Phases Can be Used
  - Low polarity (OV1)
  - High polarity (Waxes, trifluoropropyl, cyano phases)

Spearmint Oil Analysis by GC



60m x 0.25mm x 0.25um Stabilwax®, 0.2uL neat oil, 100:1 split.
75°C (4 min hold) to 200°C @4°C/min (10 min. hold), H2@40cm/s.

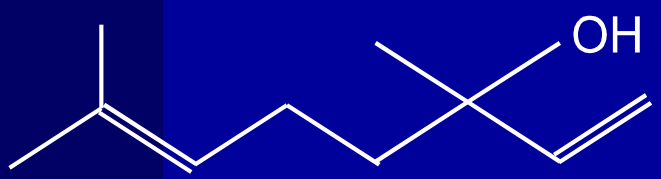
Peak List for Spearmint Oil Analysis

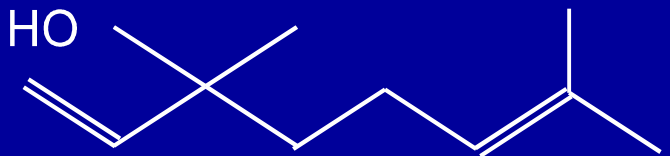
1. α-pinene
2. β-pinene
3. sabinene
4. myrcene
5. α-terpinene
6. l-limonene
7. 1,8-cineole
8. cis-ocimene
9. γ-terpinene
10. p-cymene
11. terpinolene
12. 3-octyl acetate
13. 3-octanol
14. l-menthone
15. trans-sabinene hydrate
16. β-bourbonene
17. linalool
18. terpinene-4-ol
19. β-caryophyllene
20. dihydrocarvone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

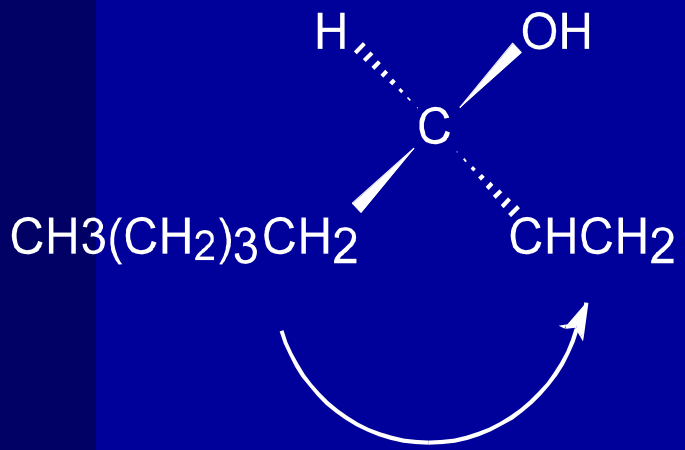
Chiral GC: Definition of Chirality

- Any compound that does not contain a plane of symmetry
- Compound can exist as enantiomers
  - Non-superimposable mirror images
- Typically a carbon center with 4 different substituents

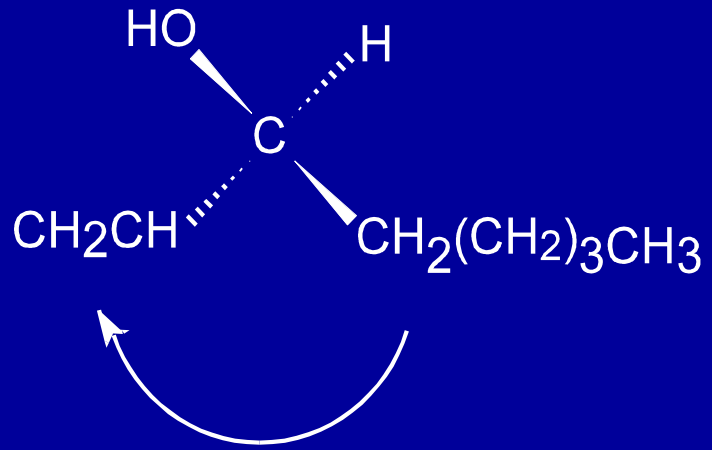
Chiral Compound: Linalool







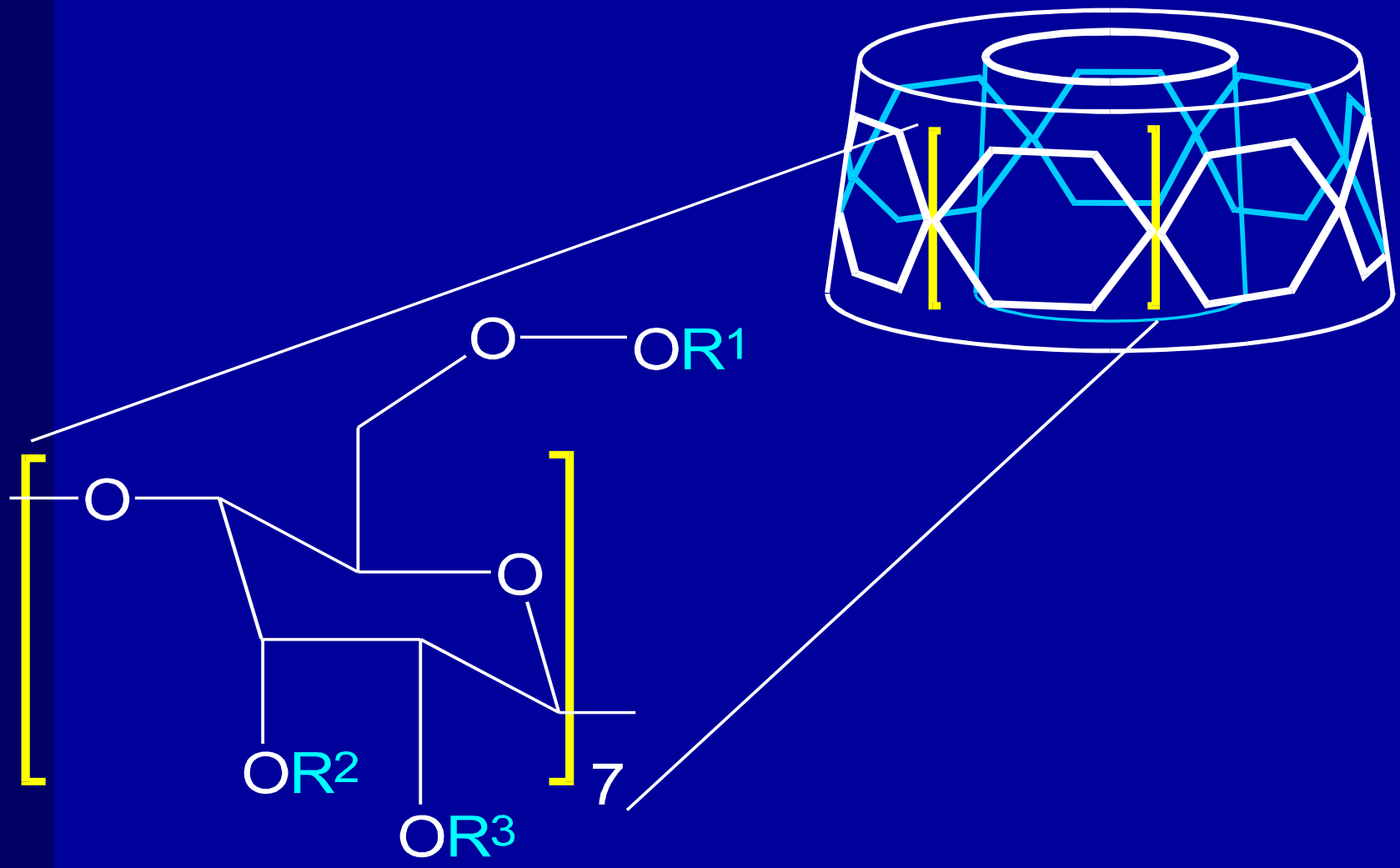
S configuration



R configuration

Beta-Cyclodextrin Phases

Derivatized with various functional groups

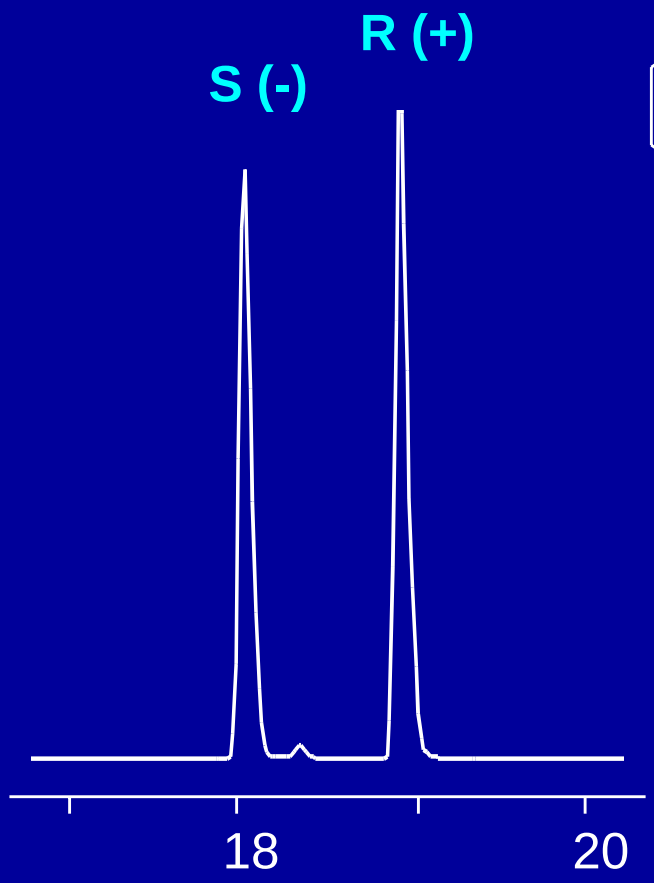


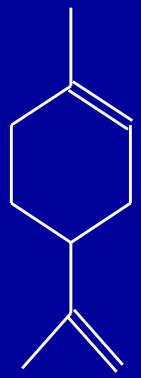
Limonene on Rt-βDEXse

Column:
30m x 0.32mm x 0.25µm
Rt-ßDEXse

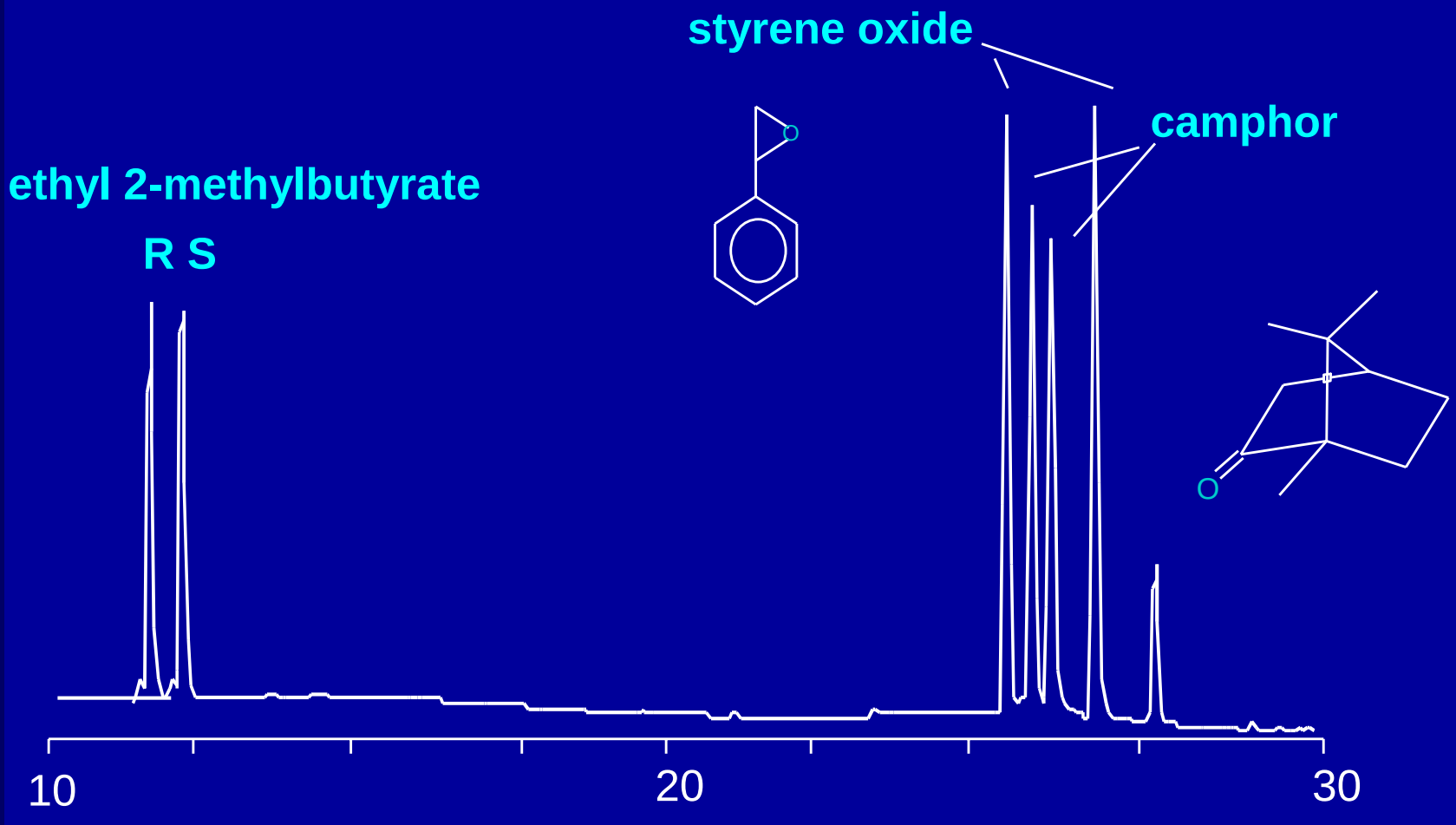
Oven temperature:
40ºC (1 min.) 2º /min to 230ºC

Linear velocity:
80 cm/sec with hydrogen carrier





Chiral compounds on Rt-βDEXse

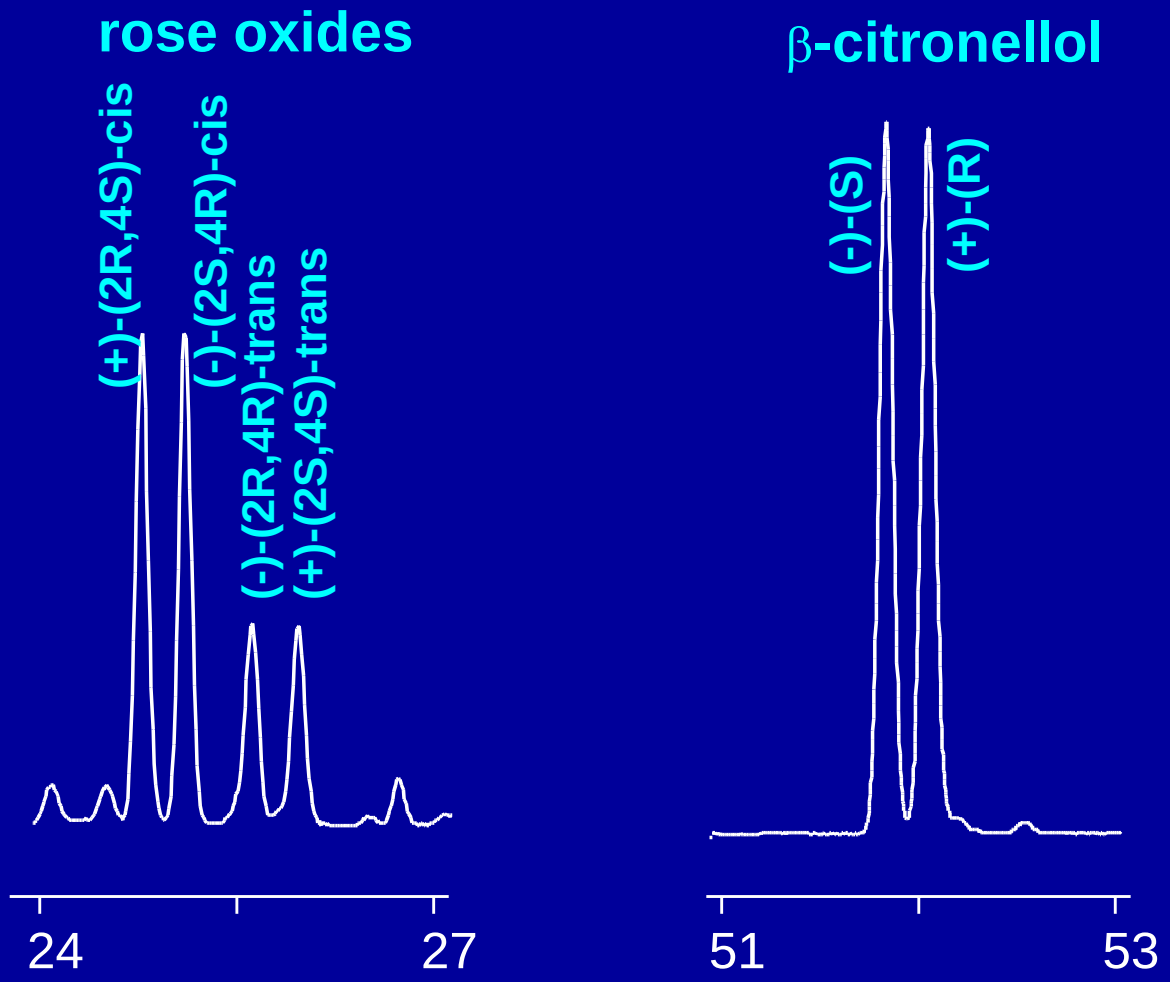


Rose oxides and β-citronellol
Commercial Geranium Oil

Column: **Rt-βDEXsa**
30m x .25mm x .25µm

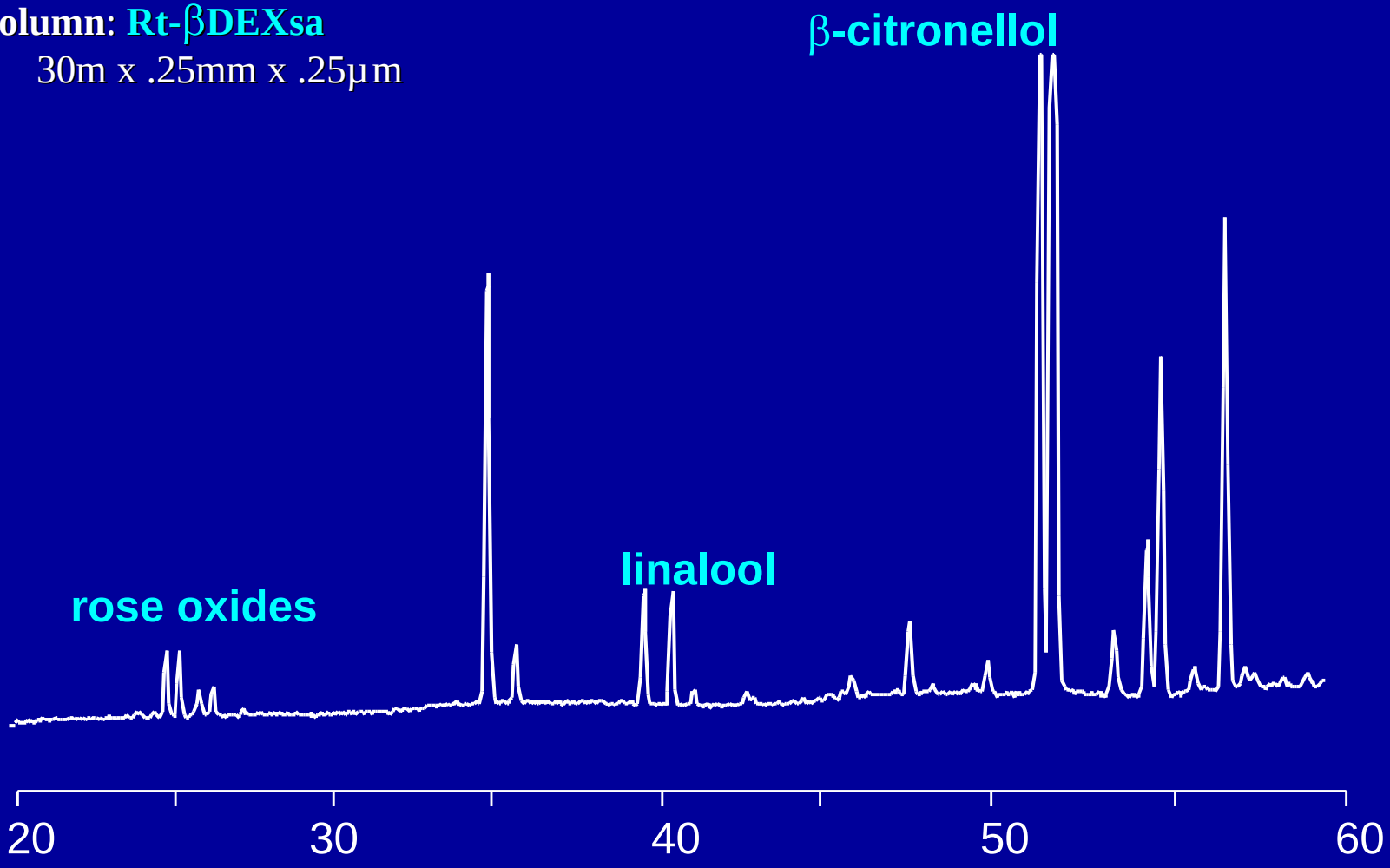
Carrier: Hydrogen @ 40cm/sec

Oven temperature program: 60°C 1°/min to 110°C; GC-FID



Commercial Geranium Oil

Column: **Rt-βDEXsa**
30m x .25mm x .25µm



Summary of Flavor & Fragrance Analysis

- Chromatography is a Powerful Tool for Flavor & Fragrance Analyses
  - Headspace GC for volatiles
  - HPLC for less thermally stable compounds
- What is the Goal of the Analysis?
  - Evaluate a flavor or fragrance material
  - Identify an off flavor