Food & Flavor Analysis: Sample Extraction and Introduction

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Food & Flavor Analysis: Sample Extraction and Introduction

I. Extraction Techniques
   A. Solvent Extraction
   B. Solid Phase Extraction (SPE)
   C. SPME
   D. SBSE
II. Headspace Sampling
   A. Static Headspace
   B. Dynamic Headspace
   C. Thermal Desorption

Sampling Challenges

- Very Low Concentrations
  - ppm to ppt
- Matrix Issues
  - Volatiles can be intracellular
  - Disruption might be necessary
- Very Complex Volatile Profiles
  - e.g. coffee has ~800 components
- Wide Range of Volatilities
- Air + Heat Instability

Solvent Extraction Techniques

- Solid-Liquid
  - Soxhlet extraction
- Liquid-Liquid
  - Separatory funnel
  - Liquid-liquid extractors
- Solvents
  - Methylene chloride – good all-purpose
  - Diethyl ether, pentane, hydrocarbons, Freons
- Concentration Techniques
  - Dry over sodium or magnesium sulfate
  - Concentrate on steam bath

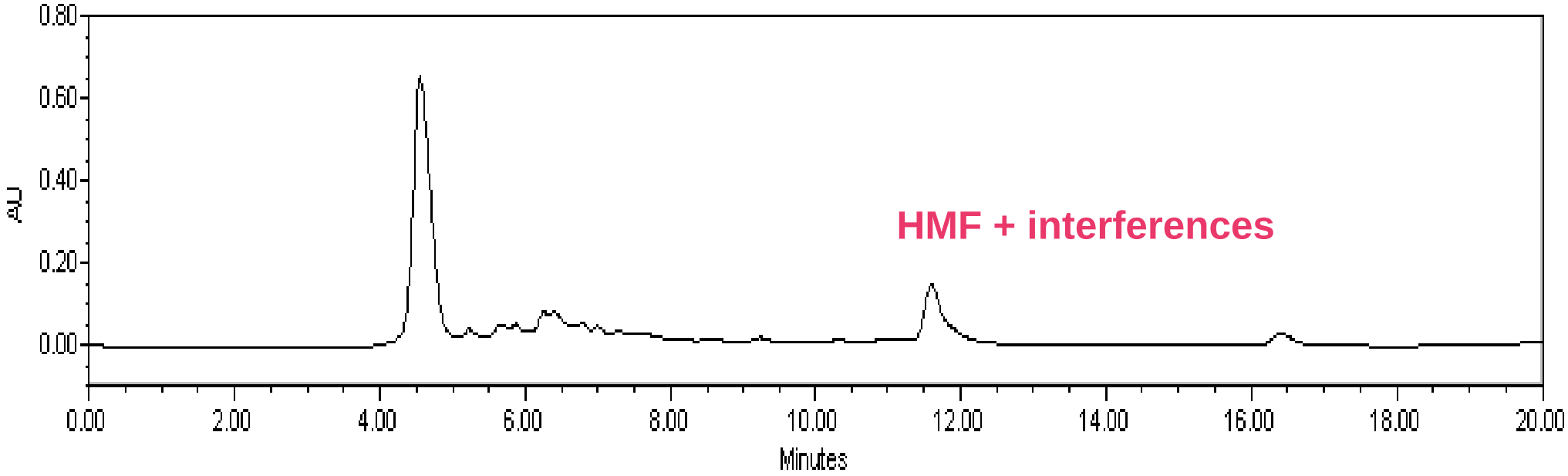
Distillation Techniques

- Prior to liquid-liquid extraction
  - Distillation can remove nonvolatile compounds
  - Can be done under vacuum – less heat required
- *Supercritical CO_2 extractions are gaining in popularity – "cleaner" final sample*

Solid Phase Extraction (SPE)

- "Trap" specific compounds
  - Compound of interest
  - Interferences
- Wide range of materials available
  - Non-polar – C18
  - Polar – carbon materials, fluorisil
- Standardized procedures
  - i.e. EPA test methods
- Validation is very important
  - Recoveries, breakthrough levels

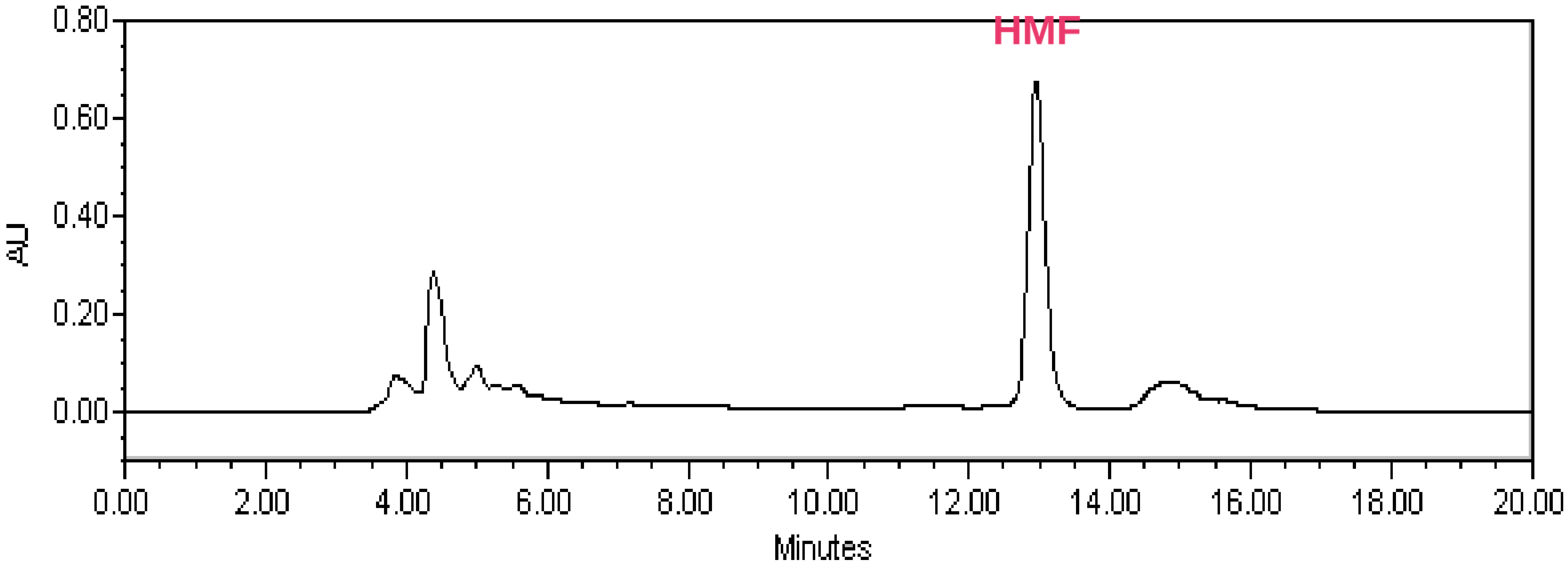
HMF in Grape Juice: No SPE Clean-up



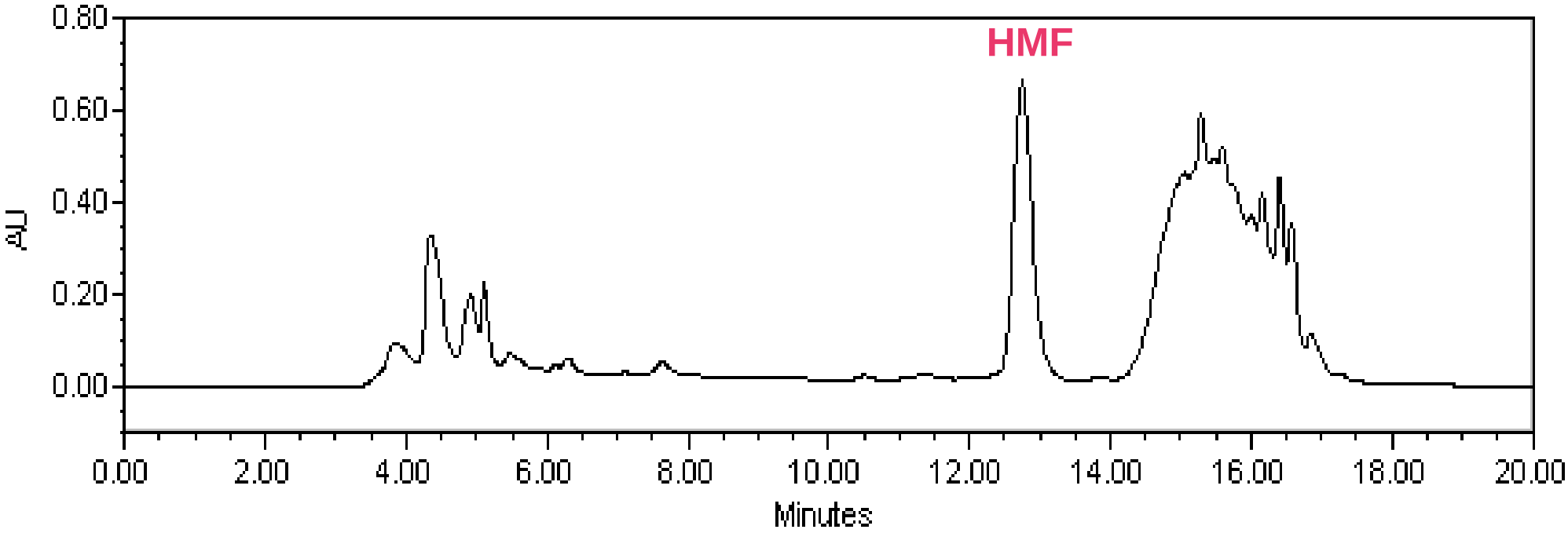
SPE Clean-up Procedure

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 in Grape Juice
C18 SPE, HMF Eluted with 20% Methanol



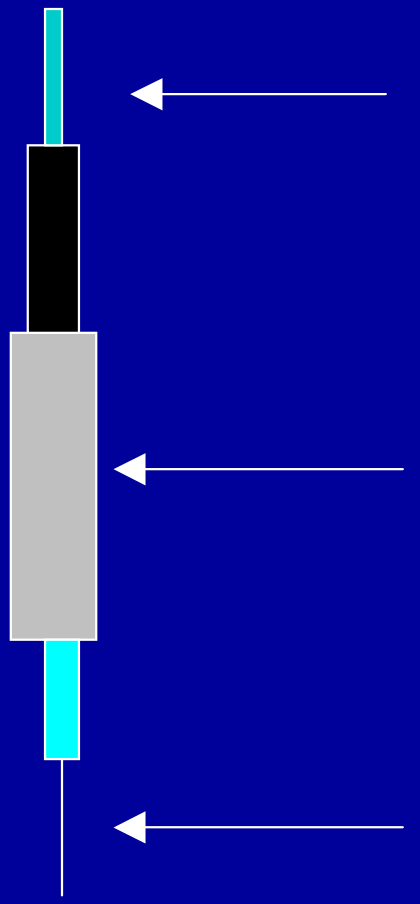
HMF in Grape Juice
C18 SPE, HMF Eluted with 50% Methanol



Solid Phase Microextraction (SPME)

- Solvent-less Extraction Technique
  - Partitioning of organic components between an aqueous or a vapor phase and a thin polymeric film
  - Independent of the matrix
  - Applicable to liquids, solids, and gases
- Factors Affecting the Extraction
  - Organic/water partition coefficient
  - Polarity & volatility
  - Volume of sample/headspace, pH, temperature, etc.

Principle of SPME



Plunger

SPME holder

Fused-silica fiber

Fiber types:

PDMS
Carboxen
Carbowax
Divinylbenzene
Combinations

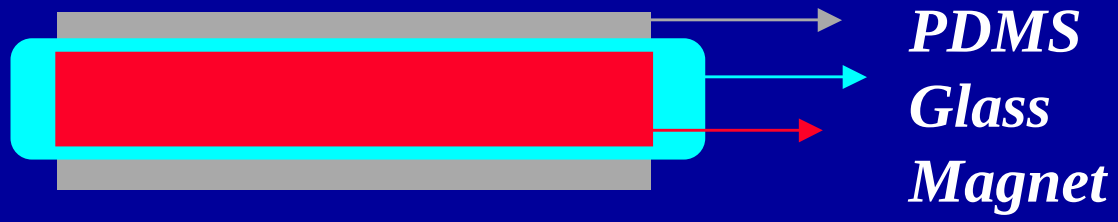
-expose fiber to liquid, headspace
-thermally desorb or solvent extract

SBSE: The Gerstel Twister™

- 1.5cm long magnetic stir bar sealed in glass
- High capacity PDMS phase on glass
- Extremely rugged
- Preconditioned for low background
- Stirs and extracts in one step
- Splitless desorption of stir bar gives low detection limits

Slide courtesy of Gerstel Inc.

Stir Bar Sorptive Extraction (SBSE)

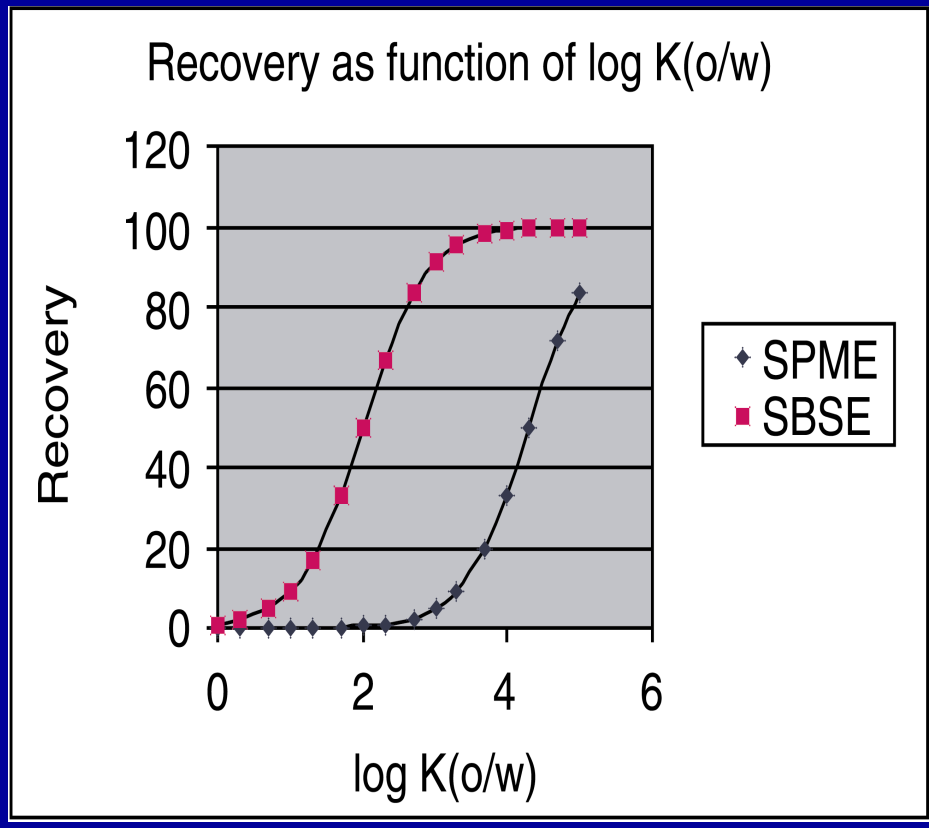


- Construction:
  - Magnetic core material, sealed in glass (1.5 cm)
  - Glass deactivated and persilylated
  - PDMS phase (0.5 mm thick)
  - Solvent washed and thermally conditioned
- Amount of PDMS on stir bar:
  - Minimum, 1 cm bar: 24 µl
  - Maximum, 2 cm bar: 126 µl

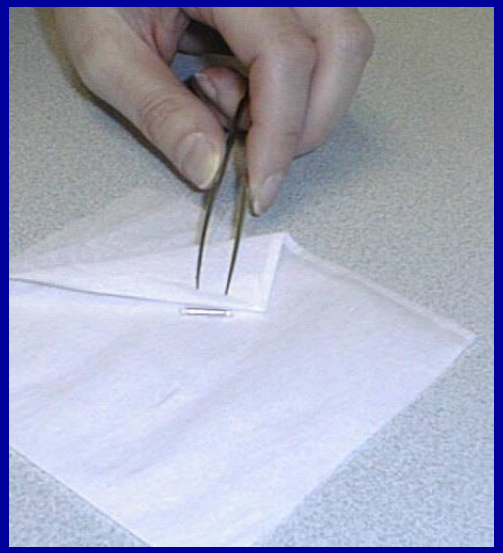
SBSE:

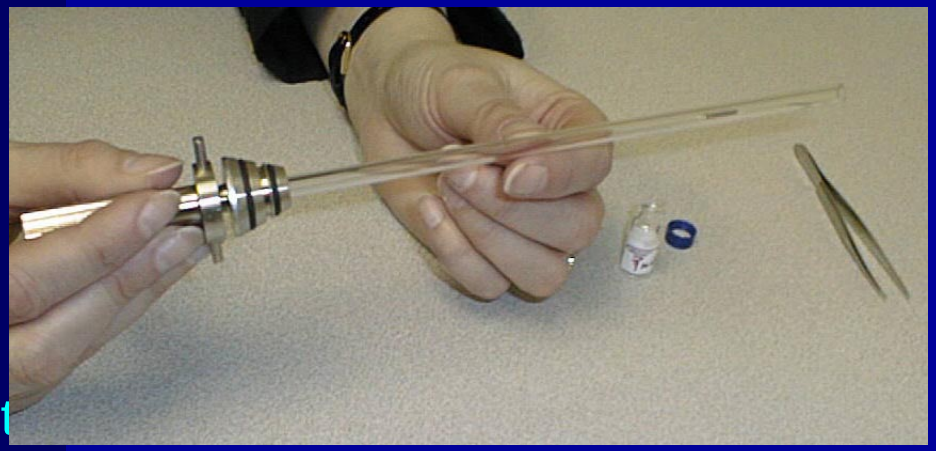
Lower Detection Limits due to Higher Analyte Recovery

- Typical SPME phase: <0.5µl
- Typical Twister phase:
  24-126µl
- Greater SBSE capacity gives higher recovery of analytes relative to SPME as polarity increases



SBSE: How to use





- Add stir bar to vial
- Stir 1hr-overnight
- Remove stir bar with forceps
- Rinse briefly in distilled water
- Dry with lint-free tissue
- Transfer bar into thermal desorption tube
- Thermally extract
- Refocus analytes in cold inlet

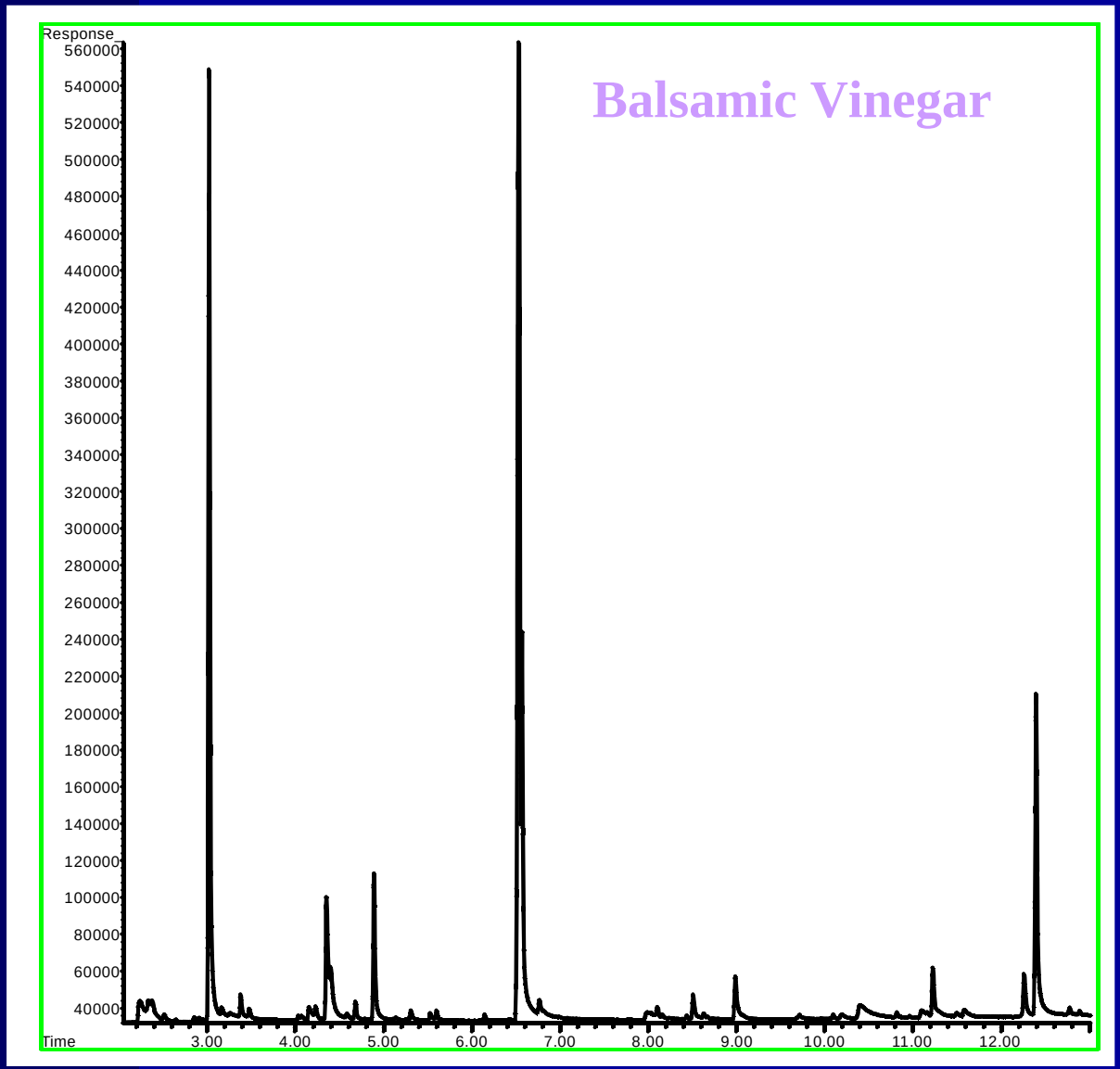
Slide courtesy of Gerstel Inc.

Stir Bar Sorptive Extraction
Features/Benefits

- Immobilized liquid PDMS phase retains negligible water
- Linear sorption isotherms with minimal displacement effects
- PDMS phase selectivity enhances extraction of nonpolar analytes
- Selectivity eliminates polar matrix interference
- Extractions from lipophilic matrices (e.g. dairy, soaps) possible
- High recovery and splitless sample introduction provide extremely low detection limits
- Predictable analyte recovery
- Sample extraction done in parallel with minimal labor

Stir Bar Sorptive Extraction

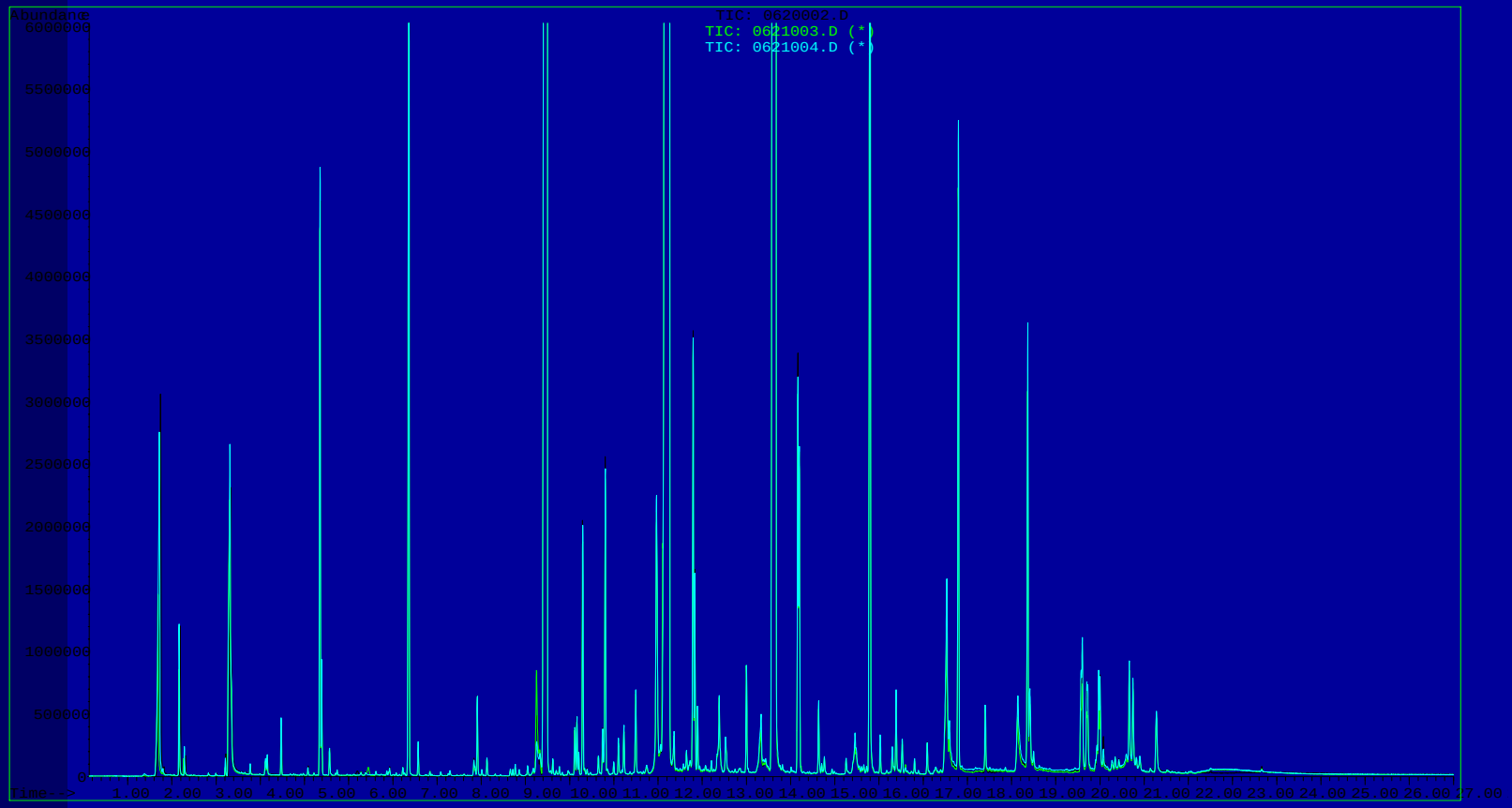
Eliminates Matrix Interferences



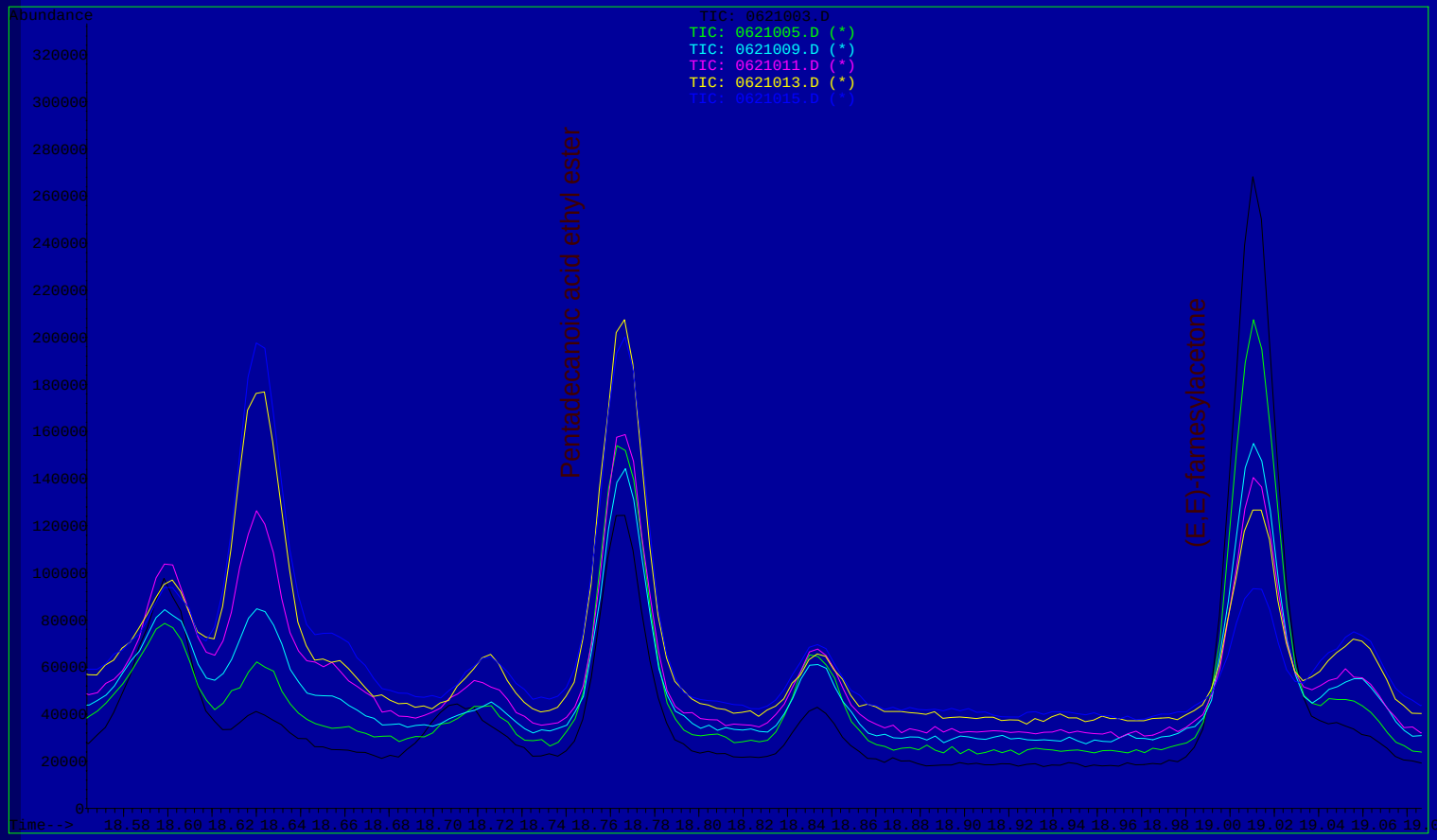
- Polar matrix components (water, acetic acid, ethanol, PG, glycerol) do not partition into PDMS.

- Volatile components (ethanol, methanol, acetone, etc.) can be further reduced by allowing brief evaporation time.

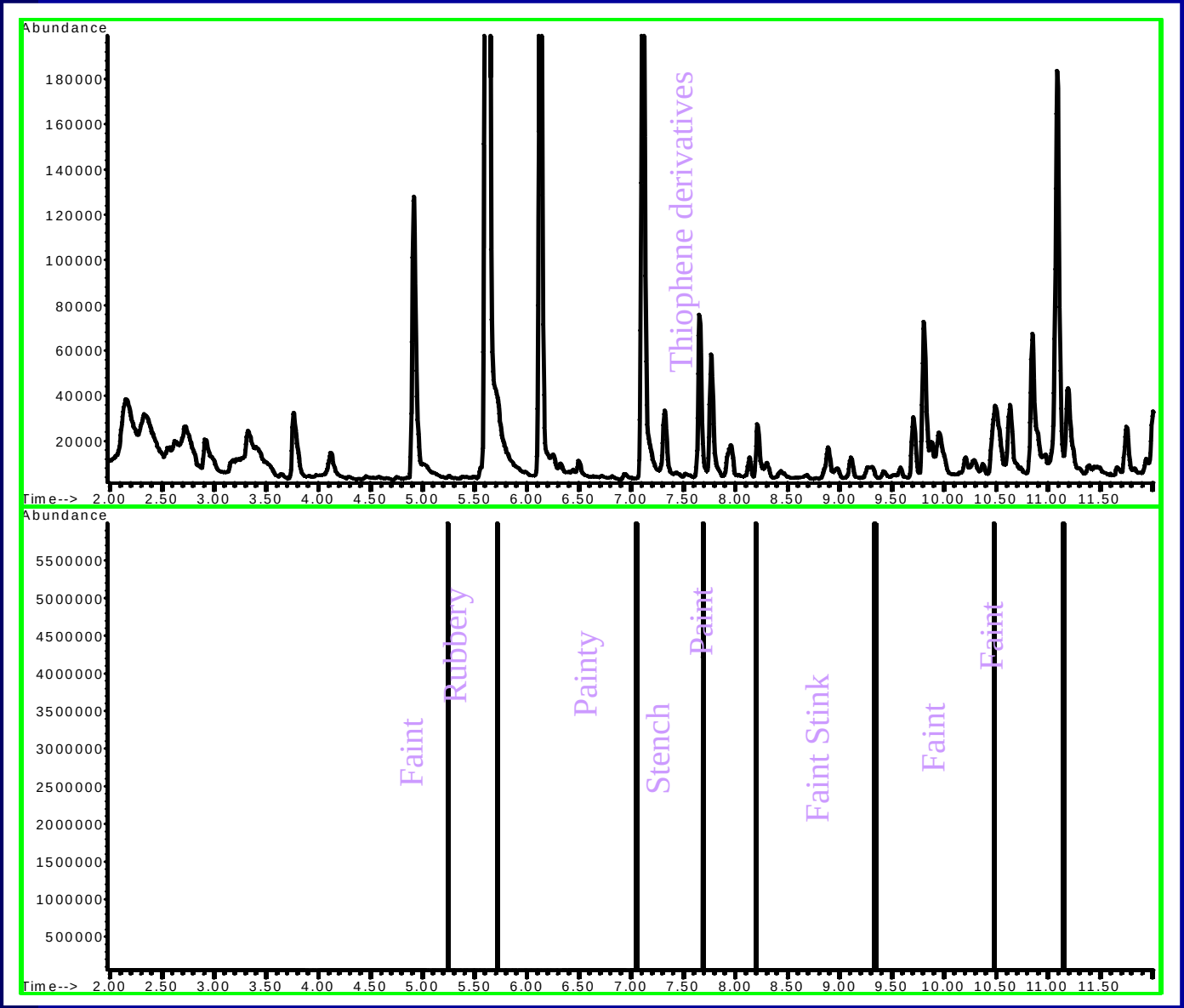
Overlays of 1 yr old Bourbon, Triplicate SBSE



Overlays of 1yr, 2yr, 4yr, 6yr, 8yr and 10yr Bourbon



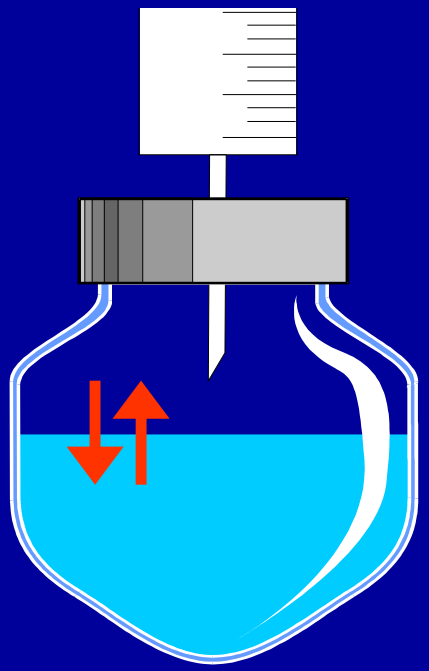
SBSE of Baking Soda Impurities



Flavor Profiling
Headspace Sampling Techniques

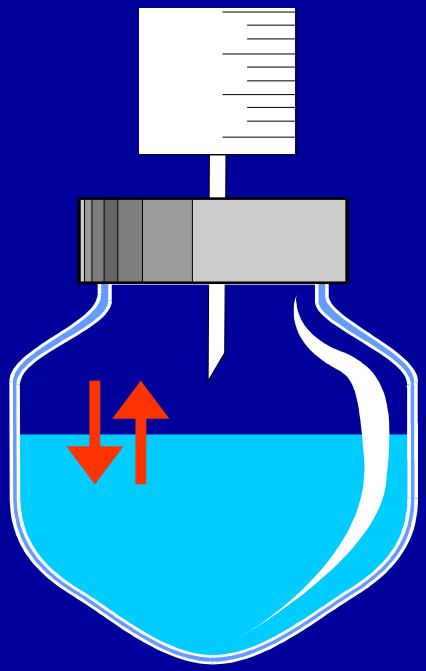
- Advantages
  - “Cleaner” samples
  - Minimal sample preparation
- Types
  - Static headspace
  - Dynamic headspace
  - Thermal desorption

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



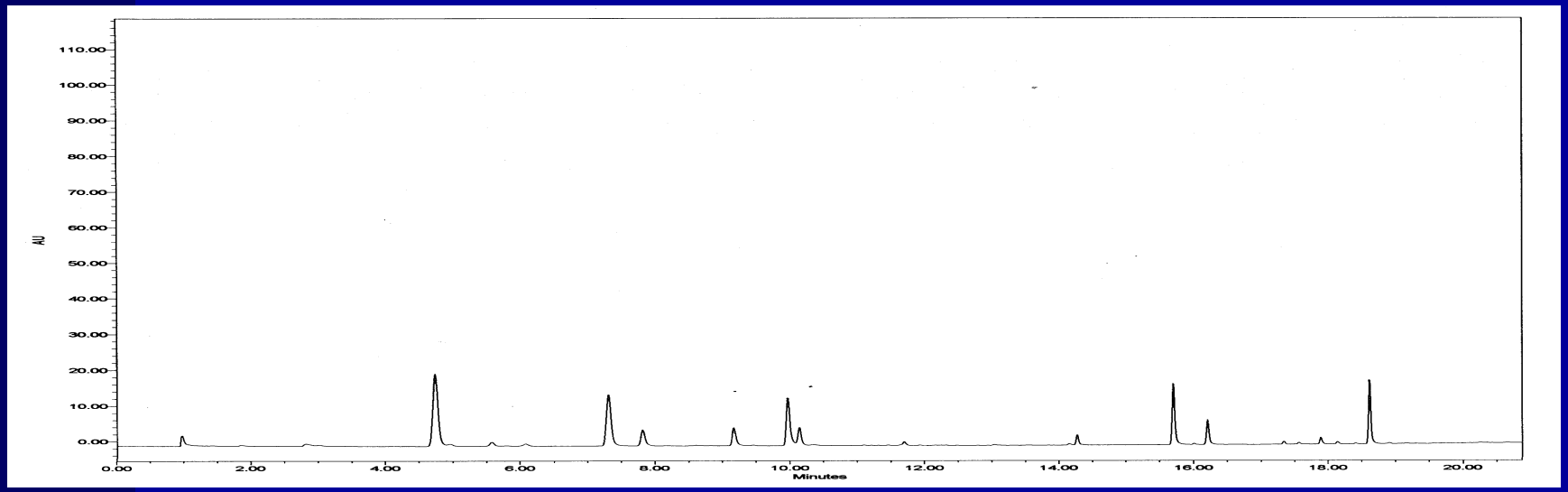
- Constant ratio between liquid & gas phases at equilibrium

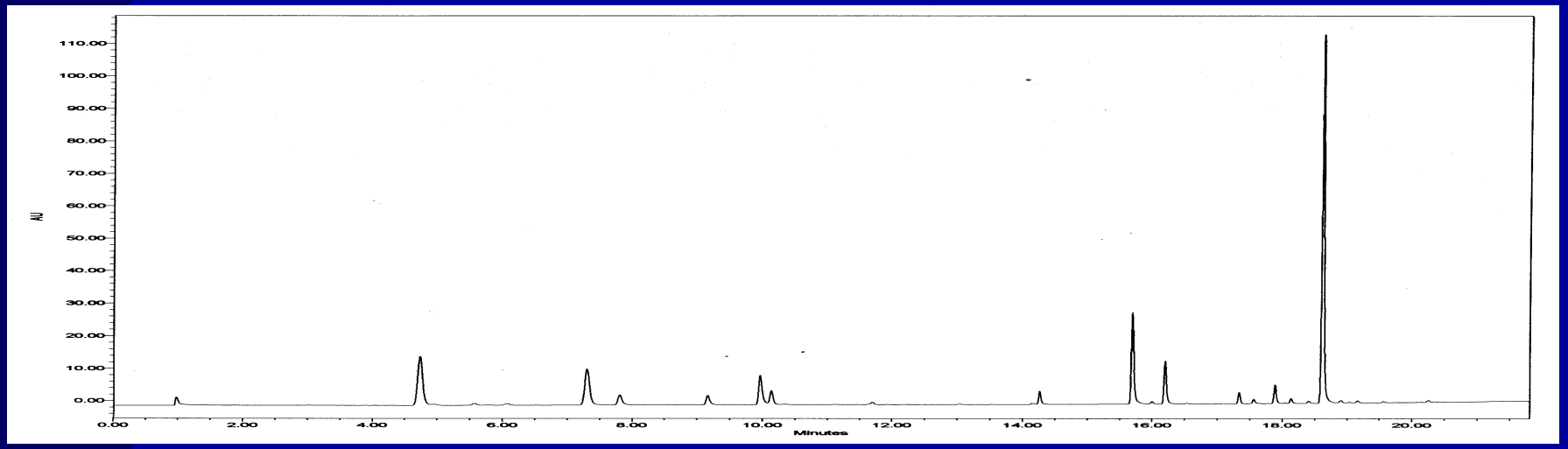
Where $K = (C_{gas} / C_{liquid})$

K = distribution coefficient

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

Flavor Profiling: Static Gas Extraction

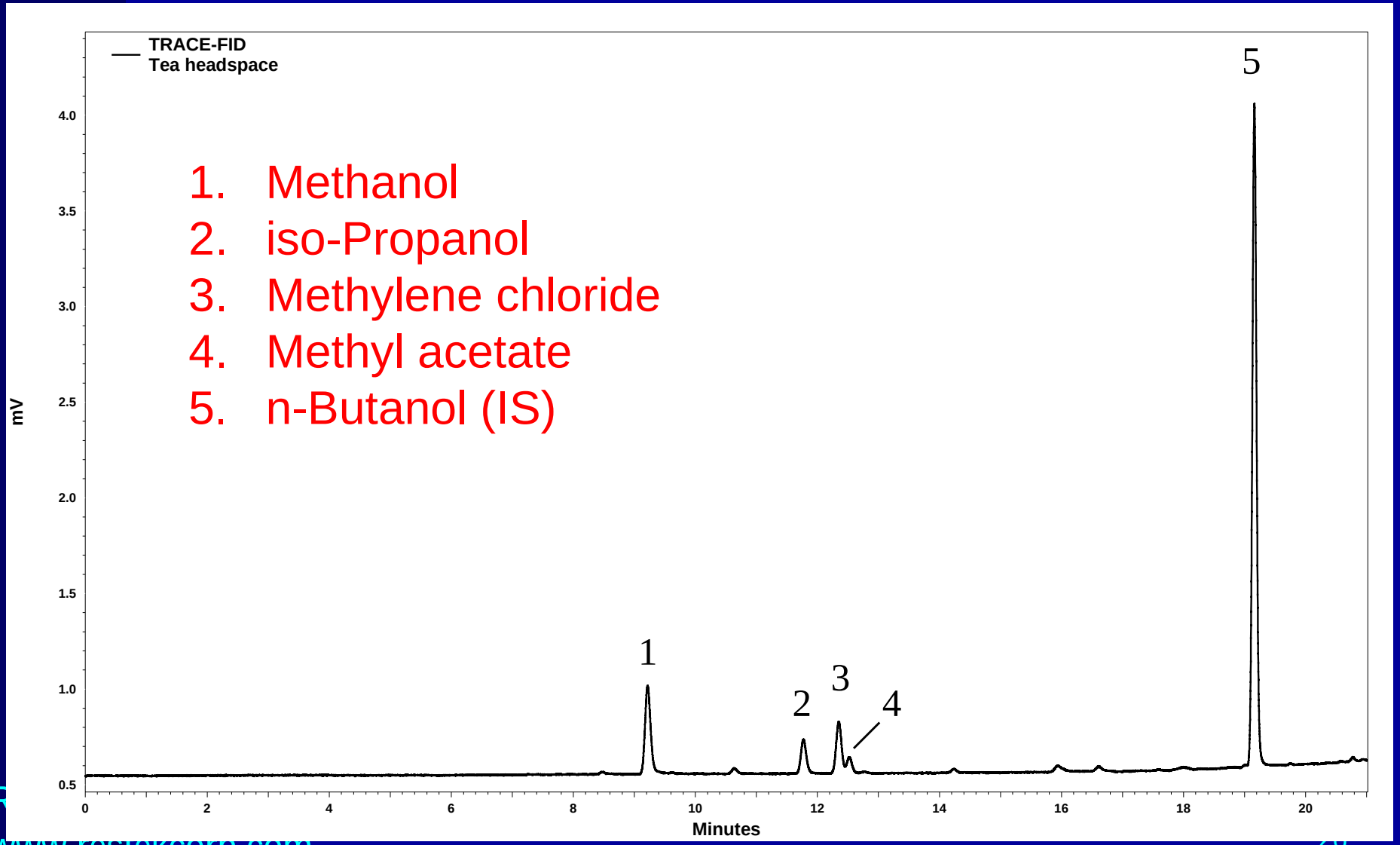




Volatiles from 2 different batches of chewing gum. The headspace was sampled after heating to 60°C.

Flavor Profiling: Static Gas Extraction

Residual solvents in decaffeinated lemon tea



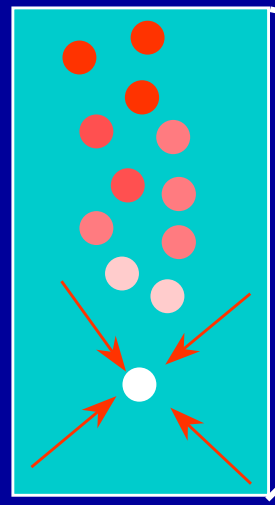
1. Methanol
2. iso-Propanol
3. Methylene chloride
4. Methyl acetate
5. n-Butanol (IS)

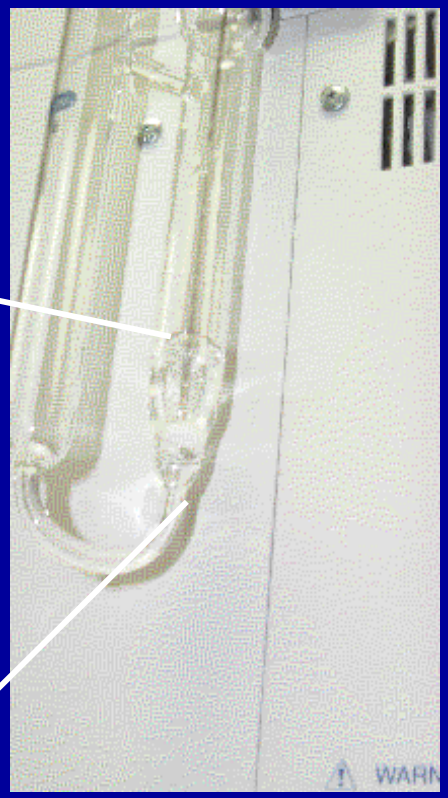
Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Inlet System

- 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

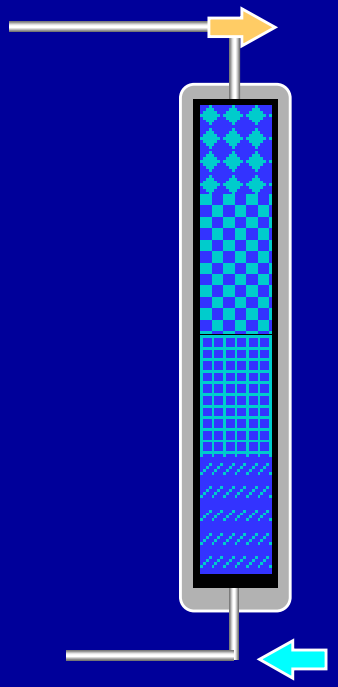




- Carrier gas bubbled through the matrix
- Volatiles in matrix diffuse into carrier gas & are carried away
- Typical flows: 40-50 mL/min. (can heat)
- Typical purge time: 10-12 min.

Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

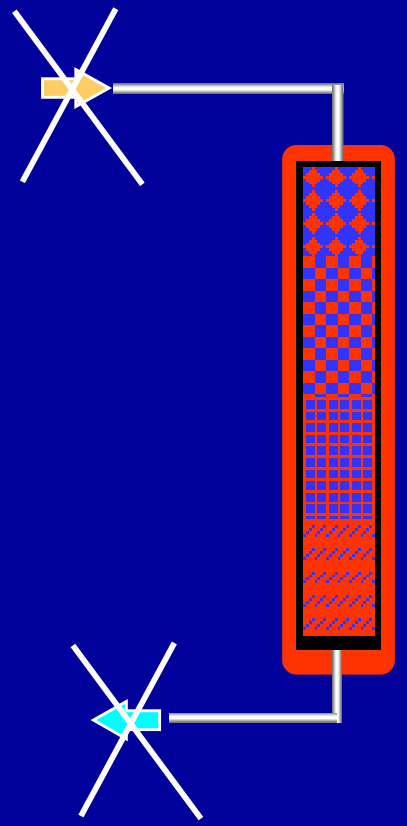
2. Dry Purge



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

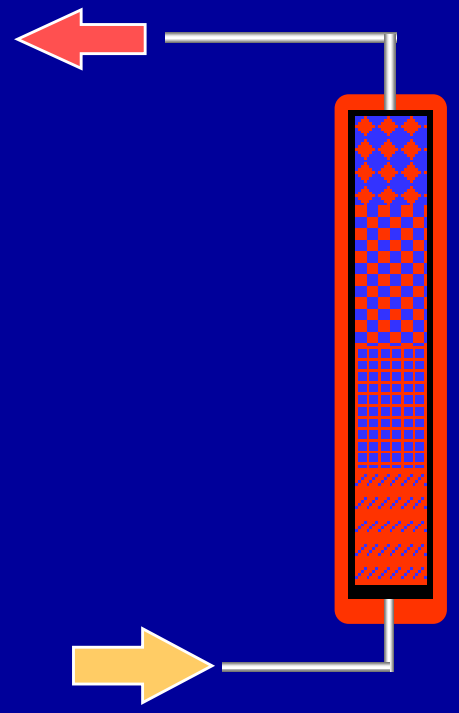
Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

3. Desorb Preheat



- Trap is heated without flow
- Typical temp: 5° below desorb temperature
- Minimizes retention on the trap

Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

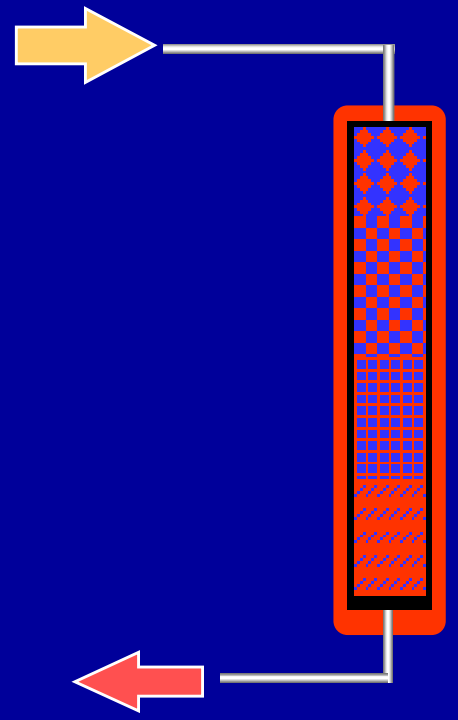


4. Desorb

- 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

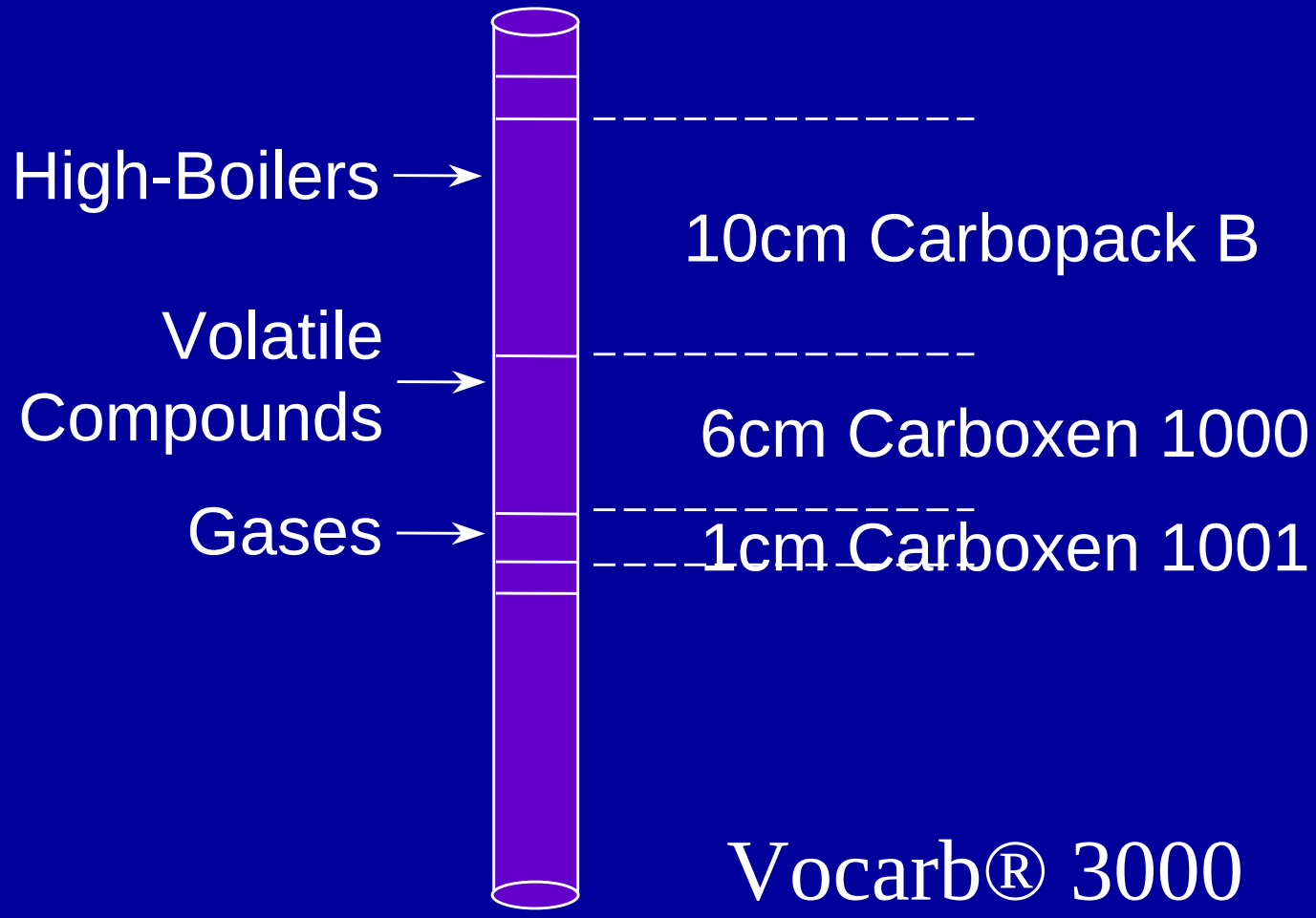
5. Trap Bake



- 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

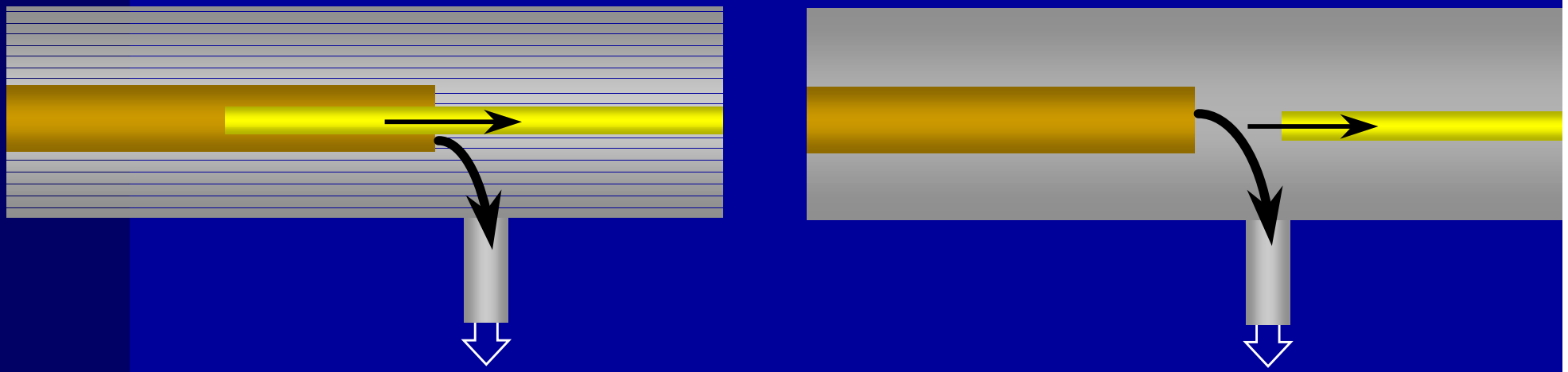
Connecting a Purge & Trap to the GC Column

- 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

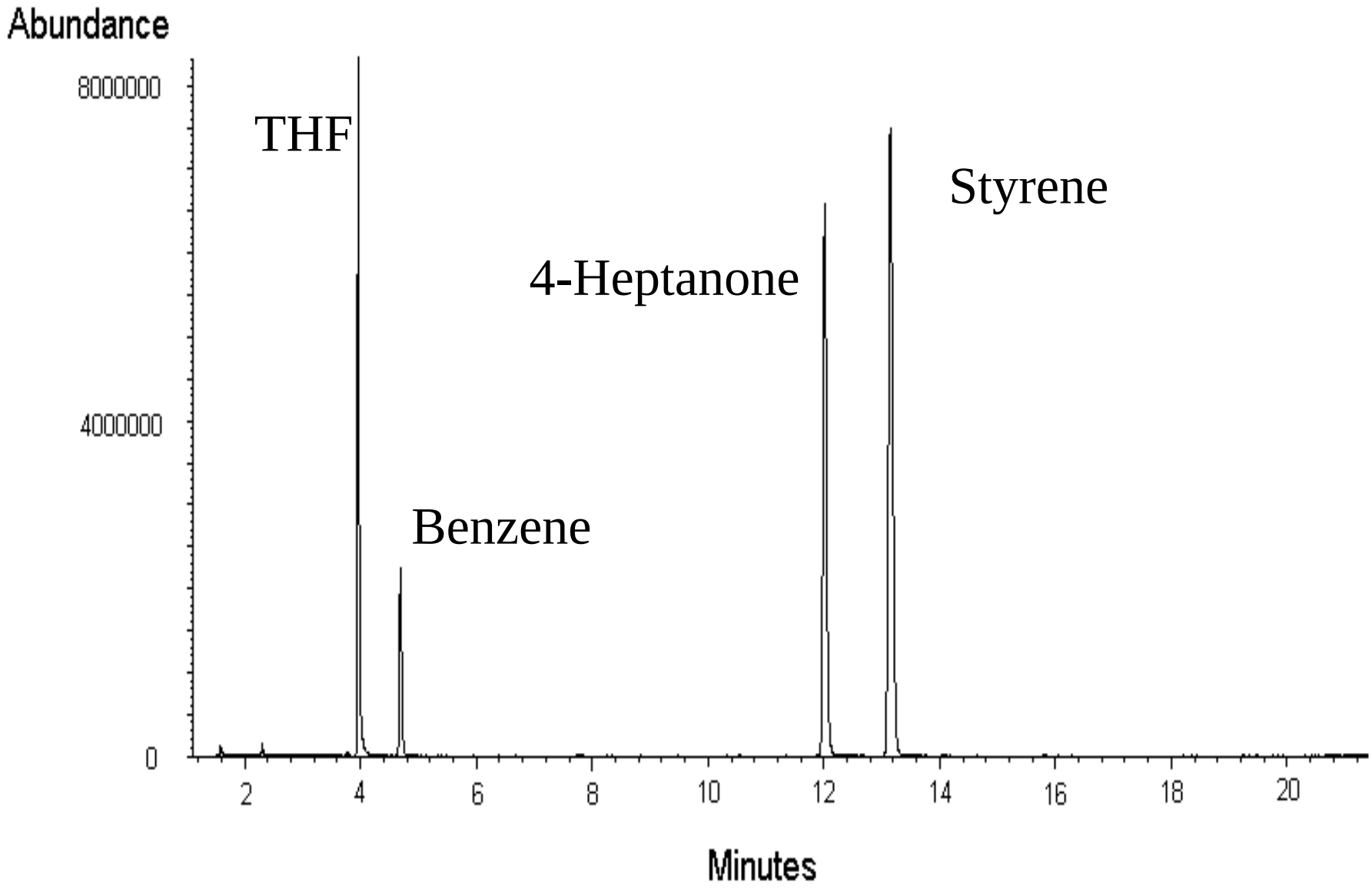
Interfacing to a GC/MS System

- 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

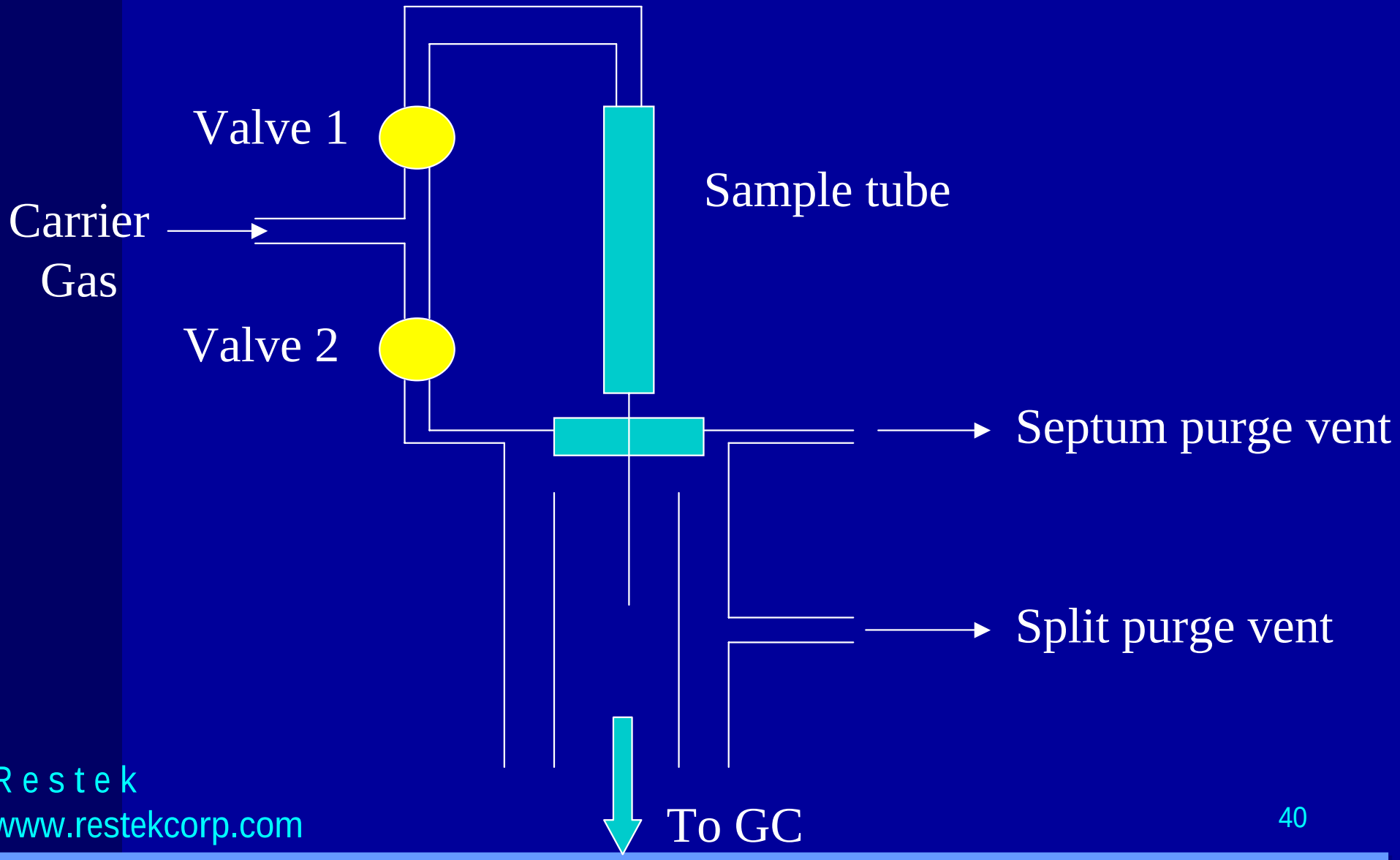
Purge & Trap Parameters

- 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



Summary of Extraction & Introduction Techniques

- Sampling techniques for solid and liquid samples
- Food & flavor samples often have extremely challenging matrices
  - Need to sample trace level components
  - Matrix can vary significantly from sample to sample
- Newer technologies focus on extracting flavor compounds from sample matrix without significant dilution