SPME SUPELCO

Solid Phase Micro Extraction Quantification and Troubleshooting

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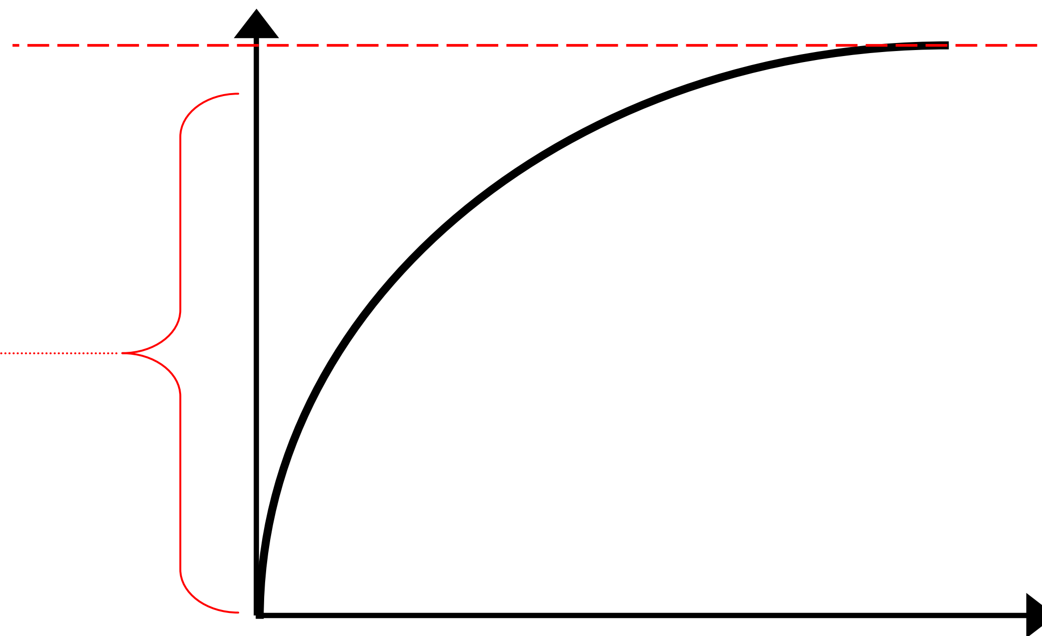
First:

SPME is an Equilibrium Technique!

- Influence on Equilibrium

- Influence on Kinetic
  - Stiring
  - (Temperature)



Reproducibility / Quantification

- Sample-Parameter
  - Temperature
  - Time
  - Volumes
- Equilibrium
  - Stirring
- Matrix
  - Salt
  - Organic content
  - pH value
  - .....
- Linearity
  - Dynamic Range
- Calibration
  - Internal
  - Standard addition
- Background
  - Cross contamination
  - Lab Air
- Loss of analyte
  - Transport

Handling

- Fiber Breakage
  - Septa
  - Stableflex, Metal
- Fiber Durability
  - Headspace
  - Conditioning
- Septum pieces
  - Septum free injection
- Peak shape
  - Liner
  - Column
  - Temp. Program
- Accessories
  - Autosampler
  - Stirbar / flea
  - Vials/Septa

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Quantification

SPME is a <u>quantitative</u> Technique.

However

" SPME does not solve the calibration problem!"

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Quantification / Calibration in SPME

- External Calibration
  - For Samples with simple matrix e.g. drinking water
- Standard addition
  - Calibration into the sample
- Internal Standard (always recommended)
  - Use of compound with similar distribution constant
  - Compound should not be present in the sample
  - Deuterated Standards (MSD required, GC/MS)

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Important aspects of a calibration for proper quantification:

- Calibration must cover all steps
- Work with idealy diluted aqueous solutions.
  - to prevent inter analyte interaction
- Work with lager Volumes (minimize wall interactions and prevent exhaustive extraction)
- Optimal simulation of the sample (pH, T, V, Salt content, etc.) by adjusting the calibration standards properly

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Extraction parameter must be constant

- Stir velocity, fiber position in the sample
- Temperature
- Sample
  - Matrix (Salt?)
  - Volume (small samples!)
  - Vial size
- Extraction time

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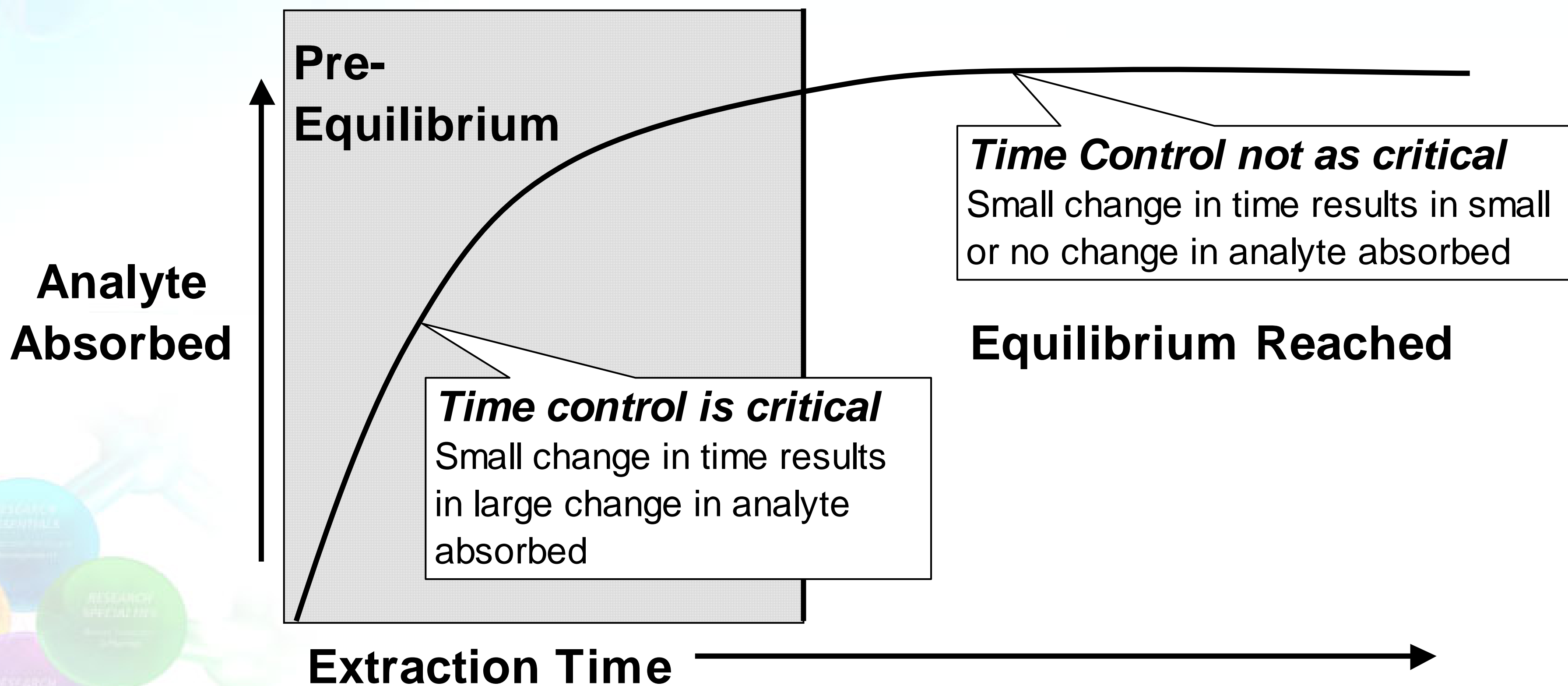
SPME - Extraction Time



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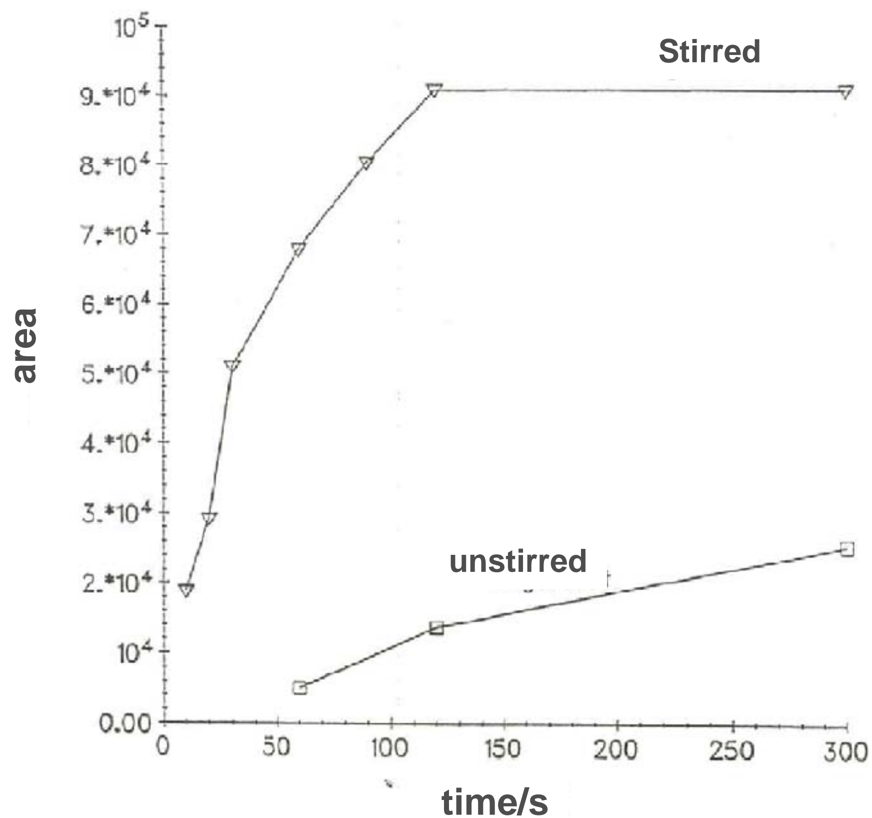
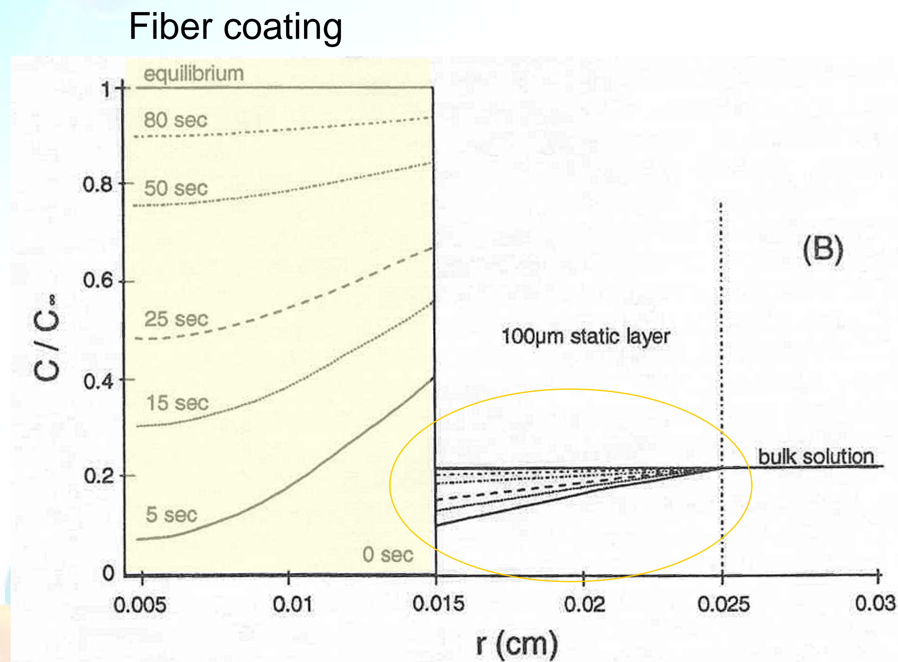
Stirring in SPME



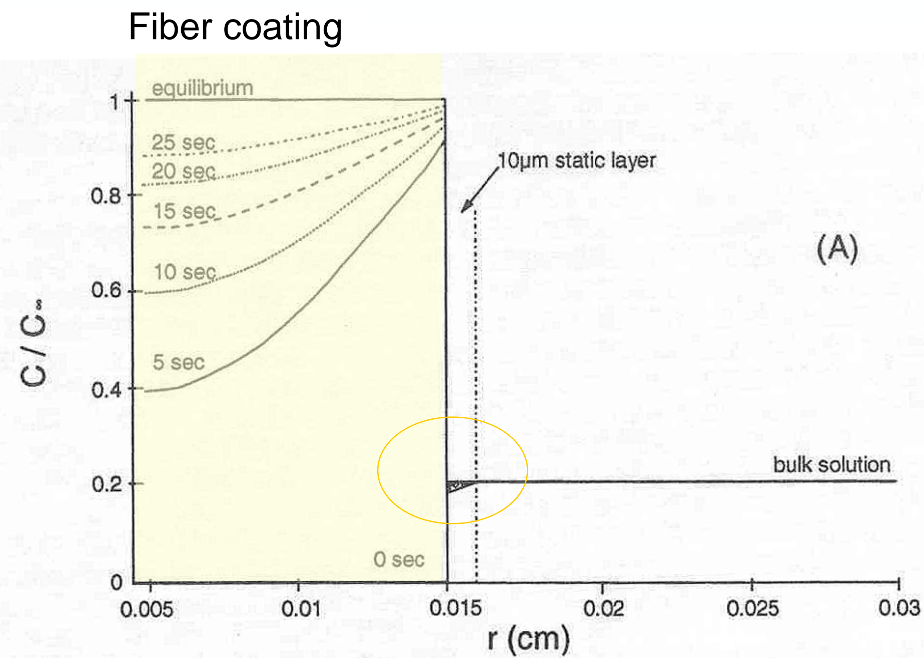
Stir effects – Static Layer

Fiber coating



Without Stirring

Fiber coating



With Stirring

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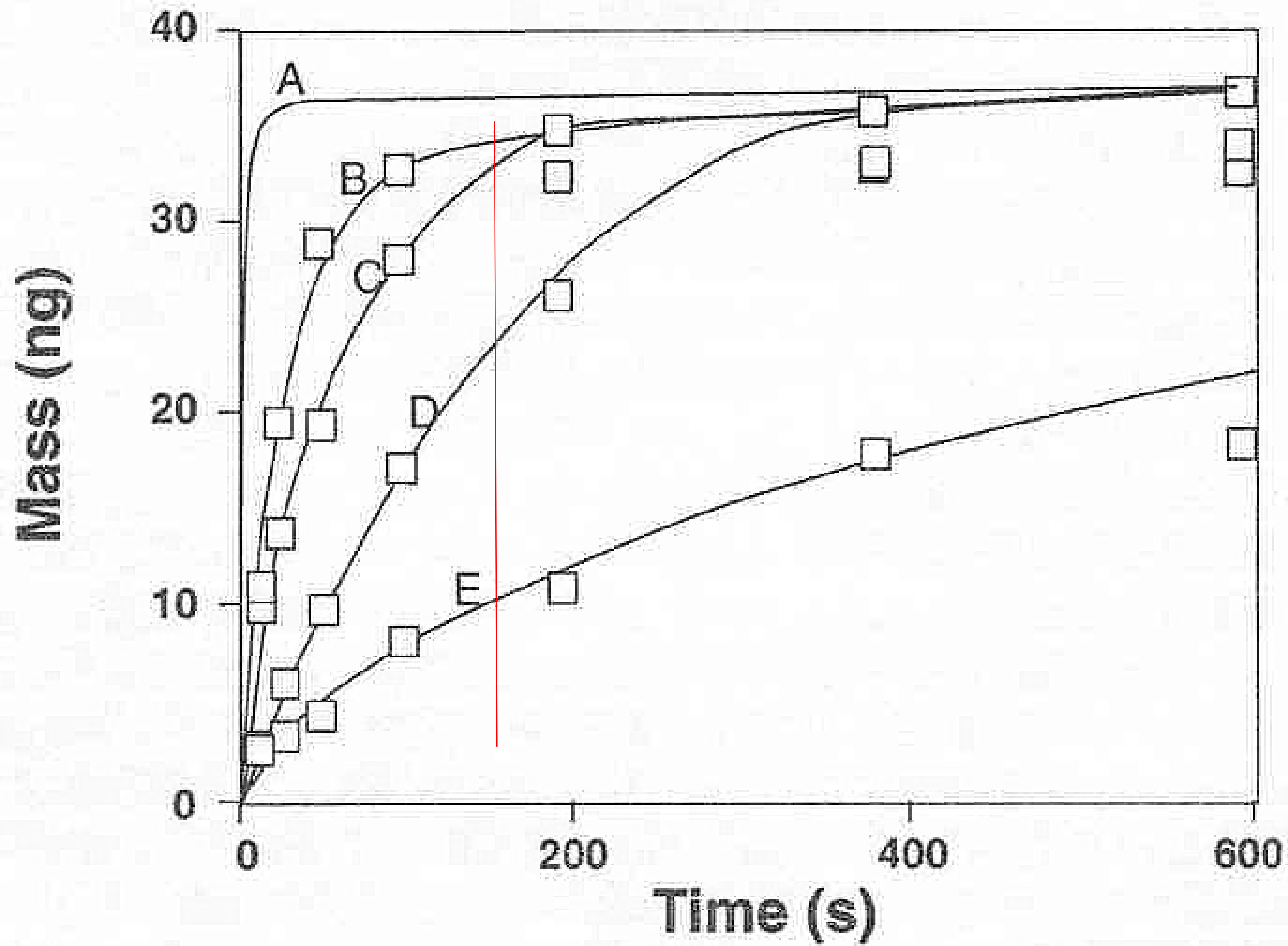
Stirring influences the extraction efficiency

- Stirring greatly decreases Equilibrium Time
- Stirring reduces Variability between Extractions
- Inconsistent Stirring may cause worse results than no Stirring
- Ultra sonication might be suitable, but it heats sample (const. T?)

Stirring in manual SPME
Fiber position?

Stir Velocity



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Headspace vs. Direct Immersion

- Immersion:
  - 2 Phases / 1 Equilibrium

- Headspace:
  - 3 Phases / 2 Equilibriums

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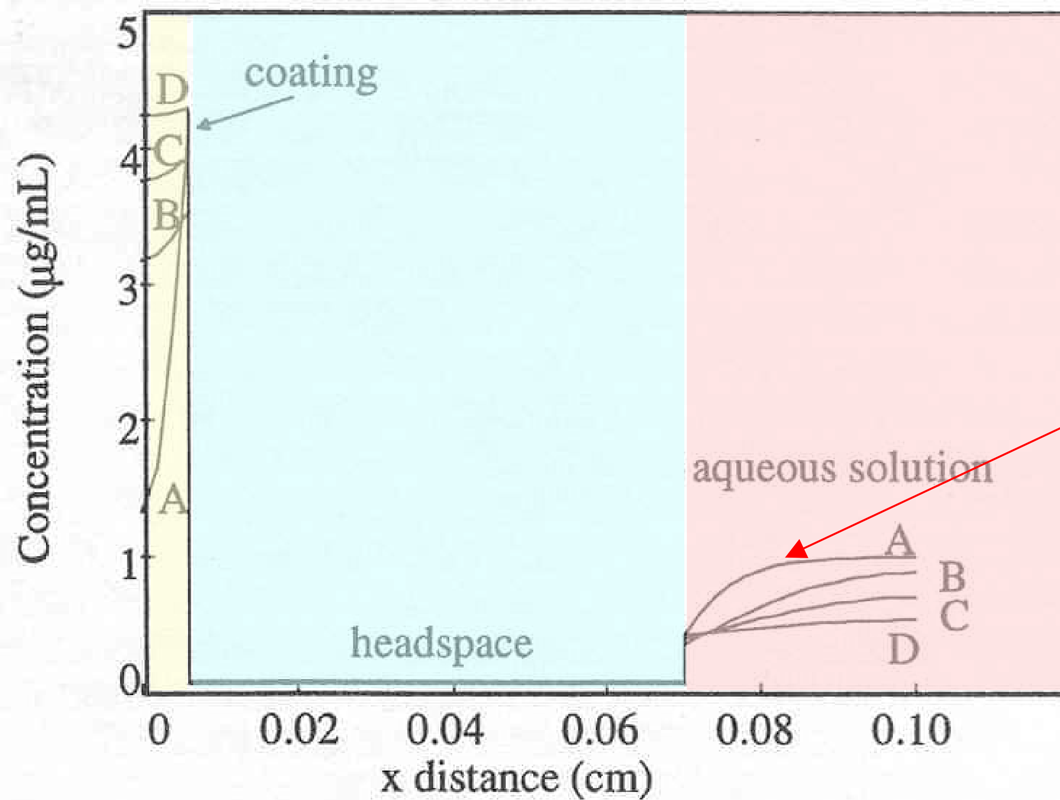
Headspace Sampling



Formation of a static layer

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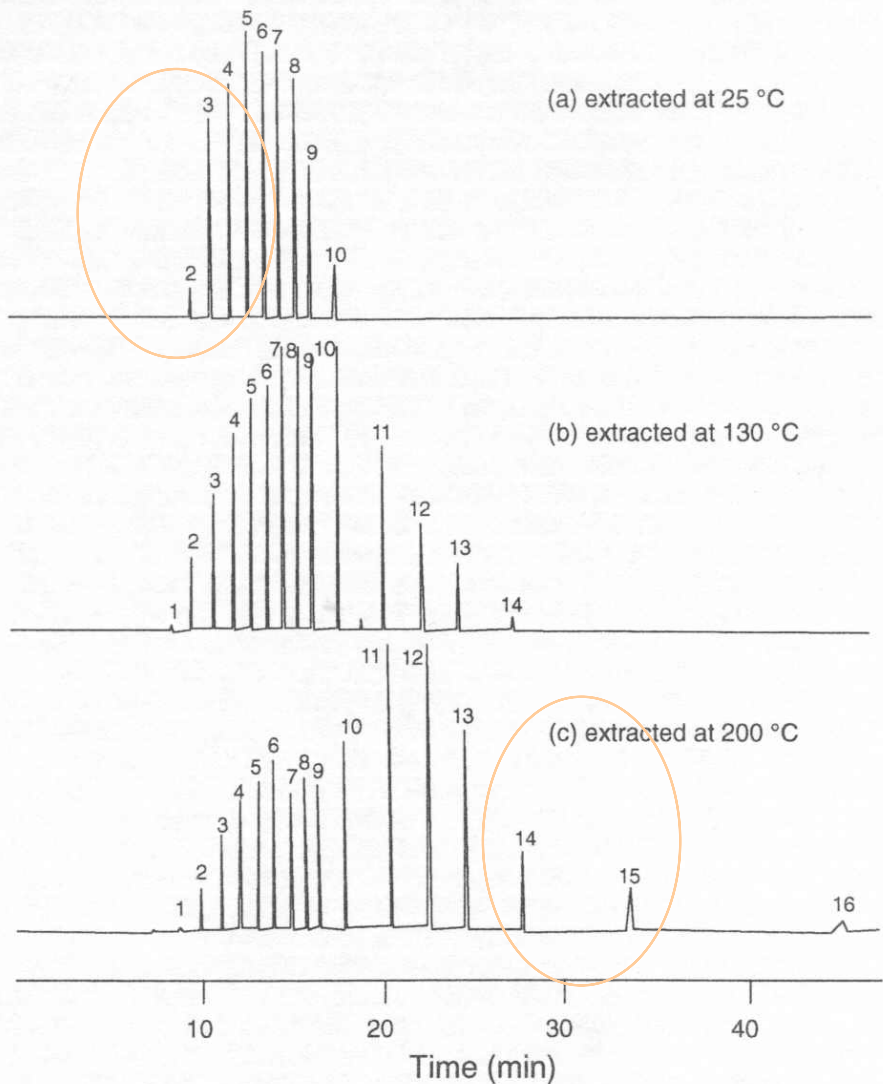

Figure 3.33 Total ion current chromatogram of 16 straight chain hydrocarbons sampled by Headspace SPME from spiked sand at 25°C (a), 130°C (b) and 200°C for 60 minutes: 1, C10; 2, C11; 3, C12; 4, C13; 5, C14; 6, C15; 7, C16, 8, C17; 9, C18; 10, C20; 11, C24; 12, C28; 13, C32; 14, C36; 15, C40.

Temperature effects in HS-SPME

Higher Pick Up
for less volatiles.

Desorption of Volatiles
during Extraction?

Tips for Headspace SPME

- Reduce the Headspace volume
- Minimize temperature for heated samples
  - in general 40°C to 60°C are sufficient
- Salt addition commonly increases sensitivity
- Stirring of aqueous samples

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Dynamic Range
Recommendations for Using Particle Fibers

- Particle fibers for trace level analyses (ppt and ppb range)
  - Dynamic range 10^2-10^3
- Use Headspace if possible
- Clean fiber prior to use
  - Blanks
  - Check for carry over

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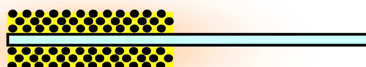
Adsorbent vs. Absorbent Fibers

Adsorbent type fibers

- Physically traps or chemically reacts bonds with analytes
  - -porous material
  - -high surface area
- Analytes may compete for sites
- Fibers have limited capacity

Absorbent type fibers

- Analytes are extracted by partitioning
  - -liquid phase
  - -retains by thickness of coating
- Analytes do not compete for sites
- Fibers can have high capacity



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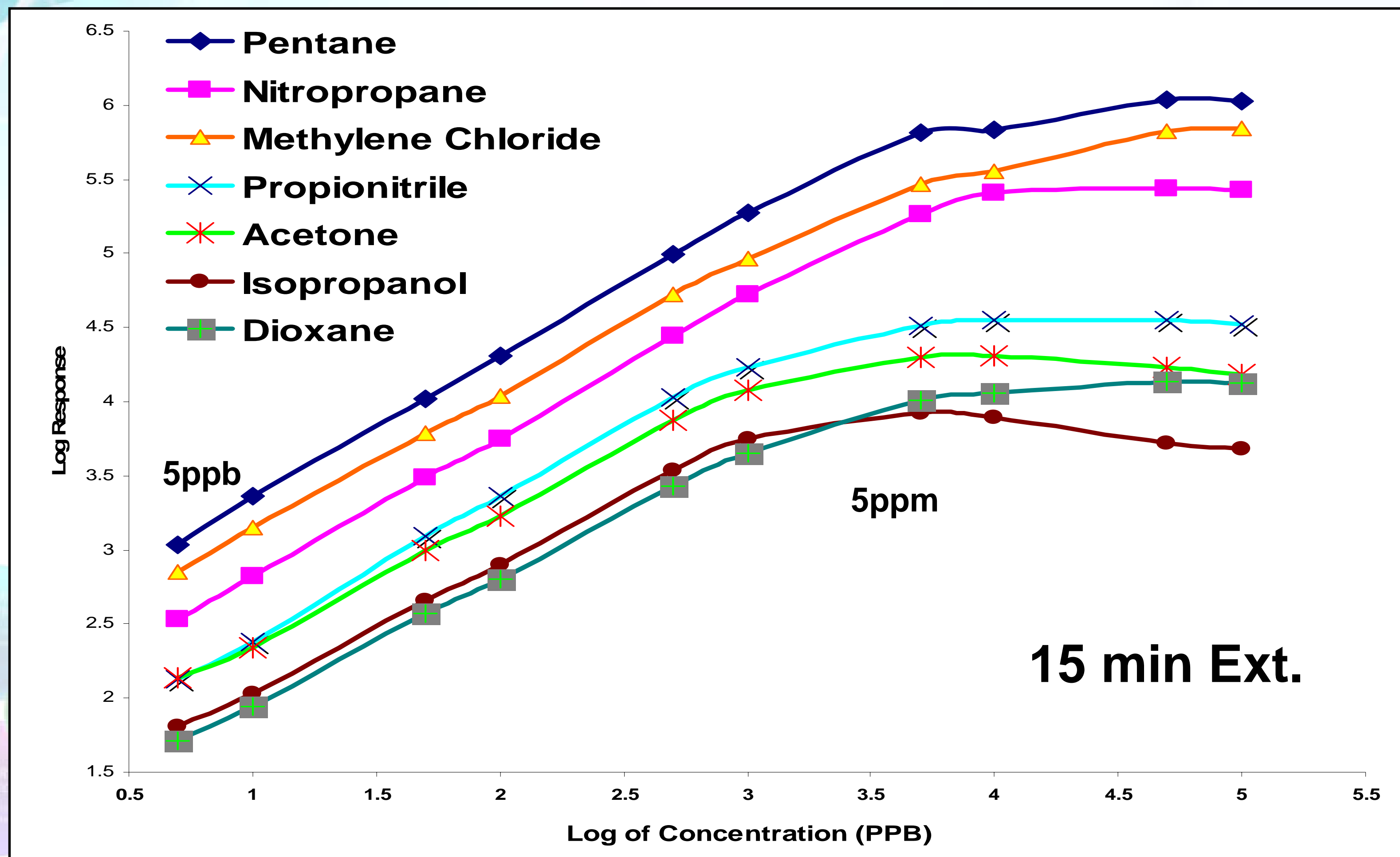
Analyte recovery vs. conc. (Carboxen-PDMS)



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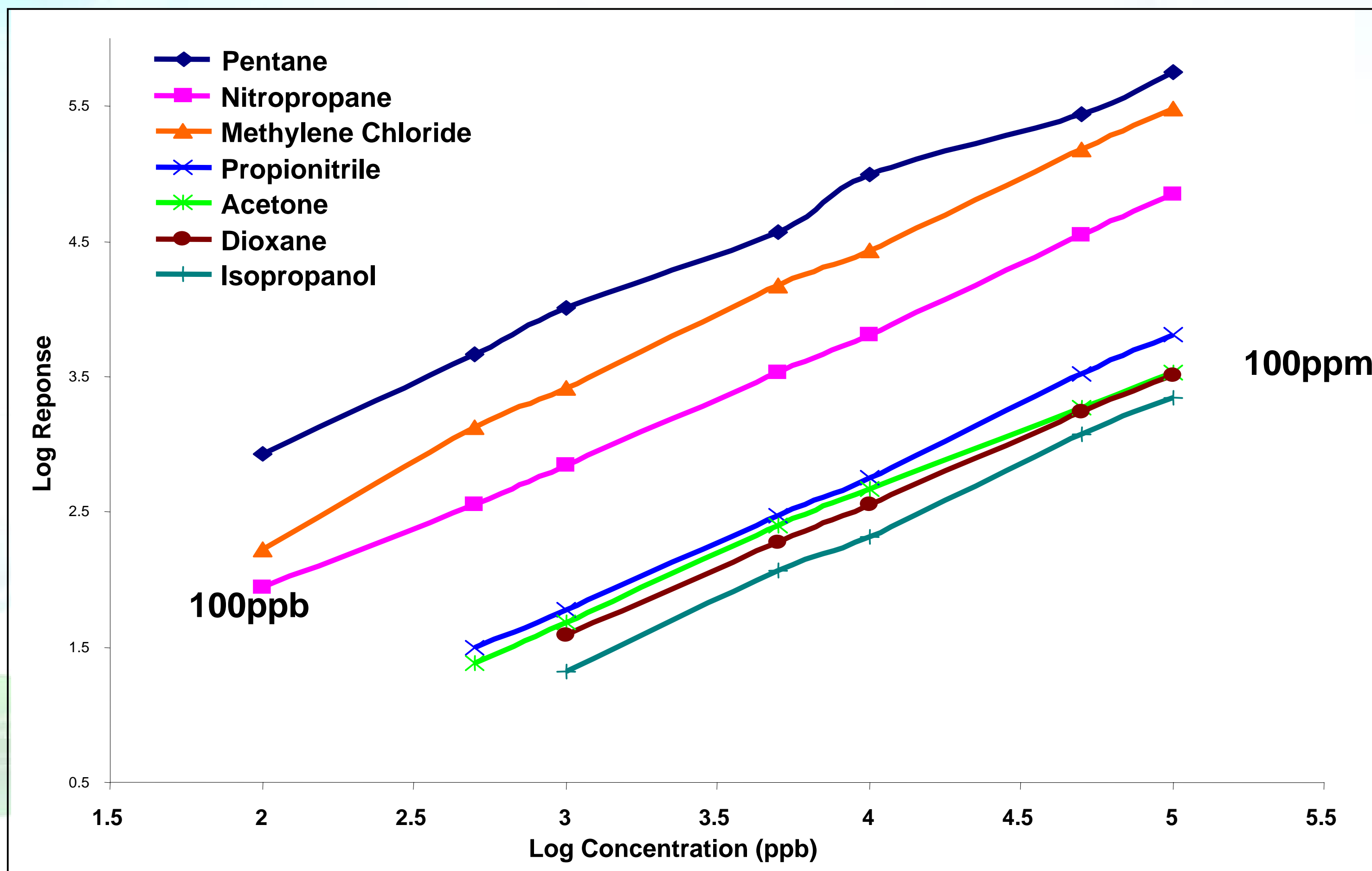
Analyte recovery vs. conc. (100µm PDMS)



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Recommendations for use of Carboxen™/PDMS Fibers

- For trace analysis (ppt and ppb range)
- If possible use headspace sampling
- For analytes with boiling points < 220°C
- If possible extraction times below 30min
- Keep injector hot (above 280°C)
- Cleaning of Fiber prior use
  - by blank desorption if fiber is not used for more than 1h

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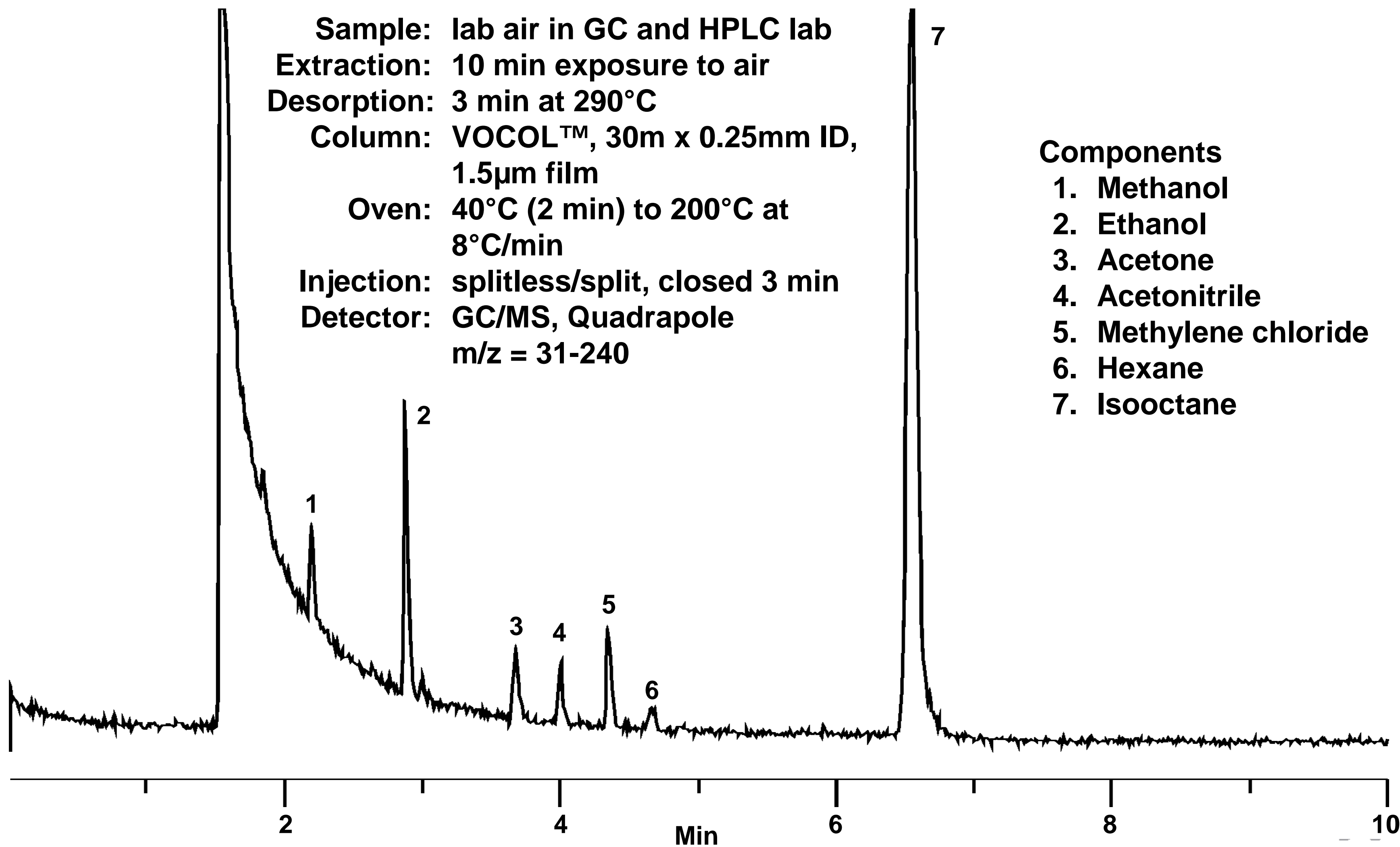
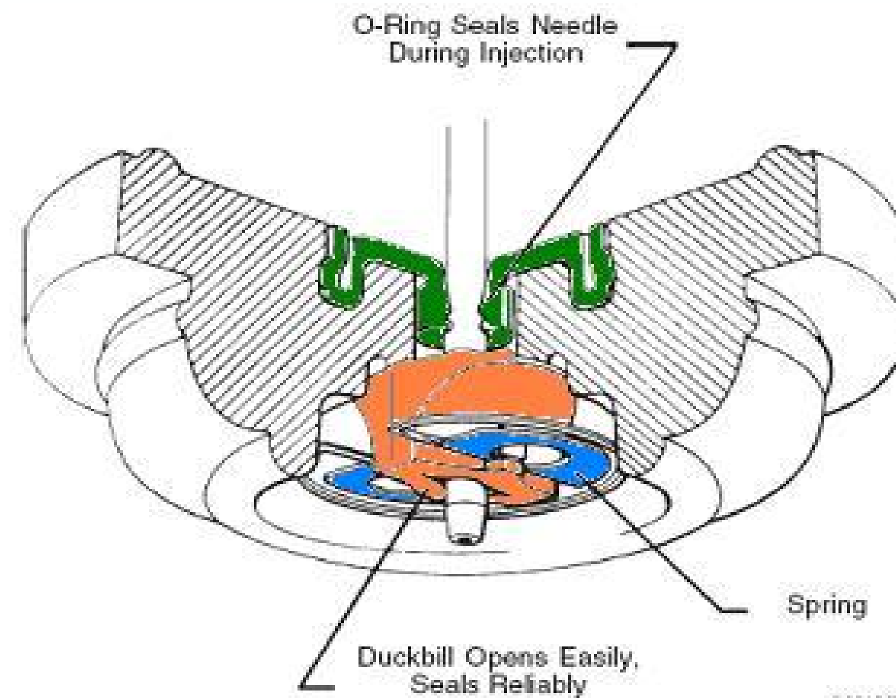
Exposure of SPME Fiber in a Research Lab



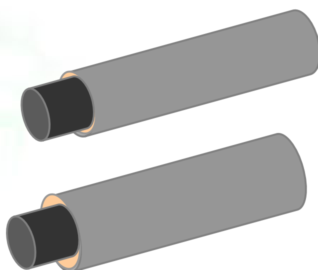
Septum free Injections systems - Merlin Microseal



Requires 23 gauge needles for tight seal!

24 Gauge

23 Gauge



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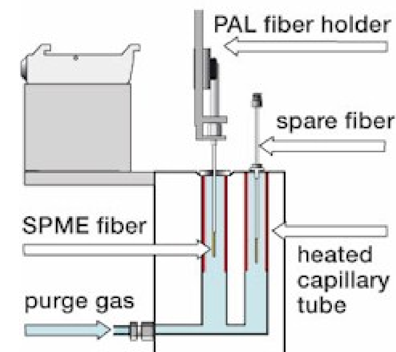
Autosampler - CTC Analytics Combi PAL

Fiber Conditioning Station



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Summary - Desorption Temperature, Time, and Injection Depth (These variables are interrelated)

- Select minimum temperature necessary to produce sharp peaks and minimize carryover:
  - To reduce septum & fiber bleed
  - To increase life of fiber
- Temperature guidelines:

PDMS fibers:	200-260°C,	7µm PDMS up to 320°C
Polyacrylate fibers:	250-300°C	
Divinylbenzene fibers:	220-265°C	
Carboxen™ fibers:	240-310°C	

- Desorption time depends on sample and matrix. Longer desorption times:
  - Remove contaminants (2-10 min)
  - Minimize carryover
  - Keep fiber clean for next extraction
- Depth of fiber should be in the hottest zone in the injection port.
  - For most instruments, expose 5-6cm of the fiber or set the top of the black depth gauge between the 2.5 and 3.5 markings. Depth of the fiber into injection port must be consistent. Set o-ring or use numbered scale on fiber holder to ensure consistency.

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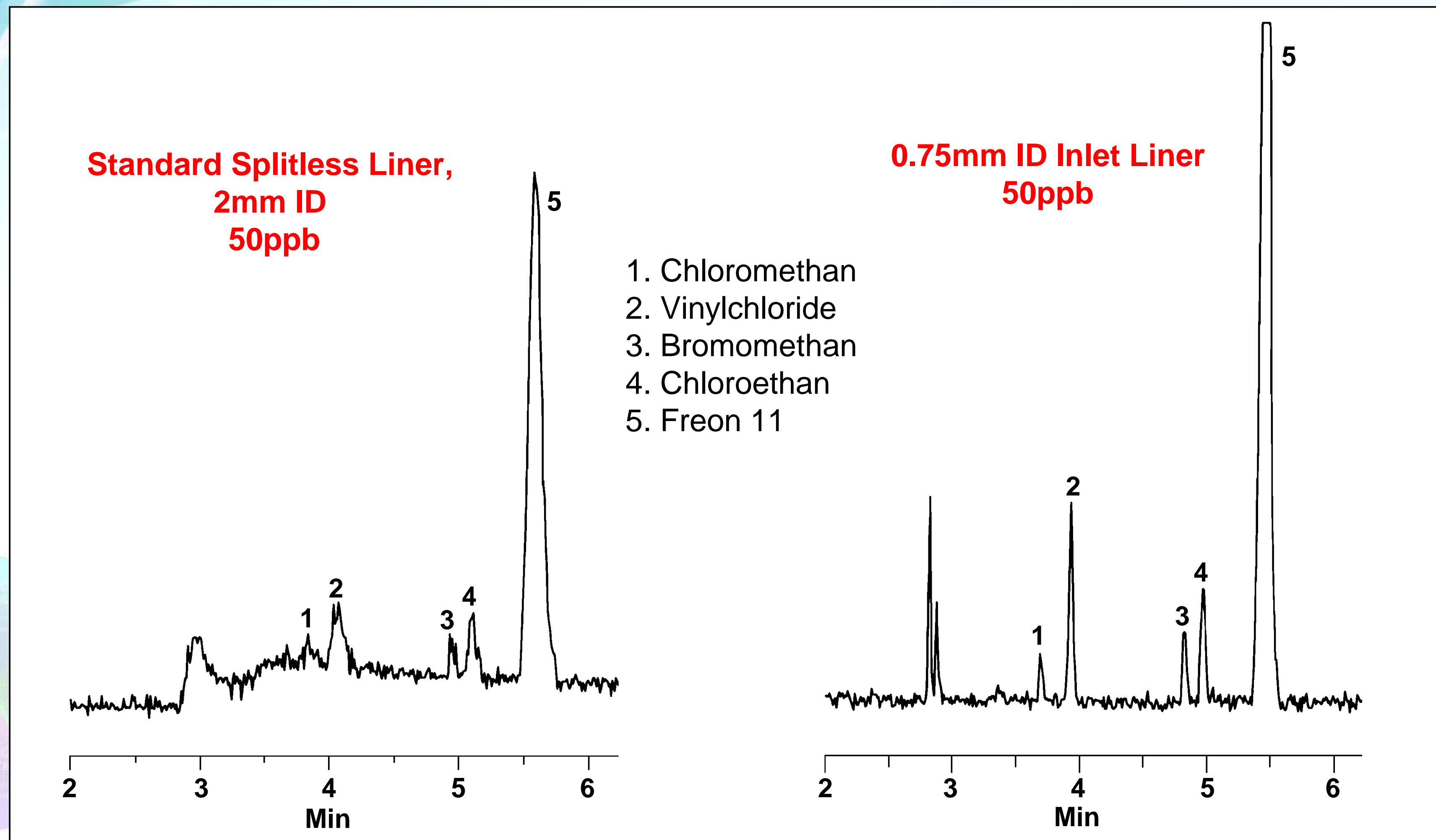
Injector and Liner

- SPME is generally compatible with all heated injector ports
- Temperature programmable injectors should be kept hot
- Keep the fiber in the hottest part of the injector block
- Straight unpacked liner (split/splitless o. direct)
  - 0.78mm ID is the optimum
- Reducing the inlet volume leads to sharper peaks and reduces the need for cryogenic

Inlet-Liner comparison for Analysis of VOCs by SPME



Troubleshooting Suggestions

- Observe and Record Conditions and Changes
  - Fiber,Sampling, Desorption, Inlet, Column, Detector Response, etc.
- Spare Parts at Hand
  - Backup Fibers & Pre-tested Fiber w/ known Performance
  - Backup Column & Pre-tested Column w/ known Performance
  - Spare Injection Port Septa and Liner
  - Spare Sampling Vials and Septa
- Instruction Sheets and Manuals
  - „If every Trail fails, read the Manual“

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Troubleshooting Steps

- Step 1 - Inject Standard directly
- Step 2 - Sample a clean Matrix
- Step 3 - Analyze Fiber under previous used Conditions
  - No problem - Investigate the Sample Matrix
  - Still problem → Step 4
- Step 4 - Check
  - Sampling vial, Fiber, Fiber Position

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SPME Troubleshooting Guide
Bulletin 928

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Bulletin 928

Fiber holder for automated sampling/HPLC

Fiber holder for manual sampling

Portable field sampler

Solid Phase Microextraction
Troubleshooting Guide

How to Locate & Solve Problems

Solid phase microextraction (SPME) is an innovative, solvent free technology that is fast, economical, and versatile. SPME has gained wide spread acceptance as the technique of preference for many applications. As with any analytical process however, problems occur on occasion. The most important step in correcting a problem when it occurs is identifying the root cause of the problem without wasting time. The systematic approach to troubleshooting described in this guide will allow you a quick solution to many problems. The guide contains helpful tips to prevent problems before they occur, as well as, a troubleshooting table listing the symptoms of the common problems, the possible causes, and suggested remedies. By following these recommendations, you can save valuable time and money.*

Troubleshooting Suggestions 2
Isolating the Problem Source 2
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- Troubleshooting Suggestions
- Isolating the Problem
- Tips for Problem Prevention
- Trouble Shooting Table
- Helpful Products

or

- Call our Technical Service for advice

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Practical Guide to Quantification SPME Bulletin 929

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Bulletin 929

A Practical Guide
to Quantitation with
Solid Phase Microextraction

Solid Phase Microextraction (SPME) is an innovative, solvent free technology that is fast, economical, and versatile. SPME has gained wide spread acceptance as the technique of preference for many applications. This guide presents a practical introduction to quantitation using the technique based on your type of sample. We present the factors that will influence your accuracy and precision and the different quantitation approaches that you can use. To help you further, we provide specific examples for each of the different approaches discussed and suggested references for additional reading.*

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Quantitation Guide Table 2
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We are committed to the success of our Customers, Employees and Shareholders through leadership in Life Science, High Technology and Service.

- Quantification Guide Table
- Approaches to Quantification
- Tips for Improving Quantification

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Summary

- Keep Parameters constant
  - Equilibrium / Kinetics
  - “Watch your T’s!” – Time, Technique, Temperature
- Adjust System for SPME
  - Liner, Column, Temp. program, Sample (ionic strength), Handling of fiber
- Calibration
  - Check matrix influence (external calibration or standard addition)
  - Calibration over whole sample system
  - Use internal Standards
- Autosampler Use

→ Get support from Supelco

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Thank you!

Sample
Prep
Made
Easy