An Agilent Guide to SPME Analysis

Jessica Westland
Sample Preparation Application Scientist
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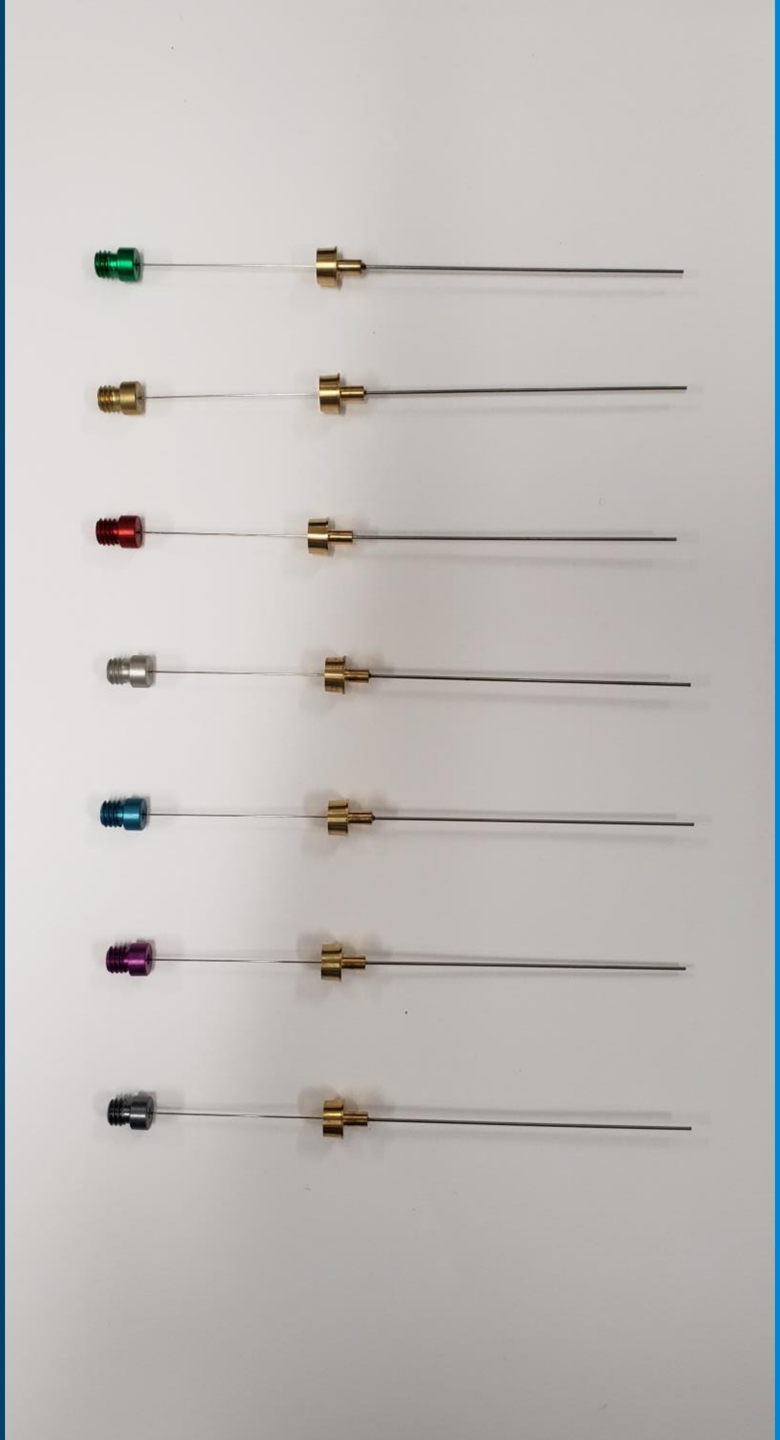
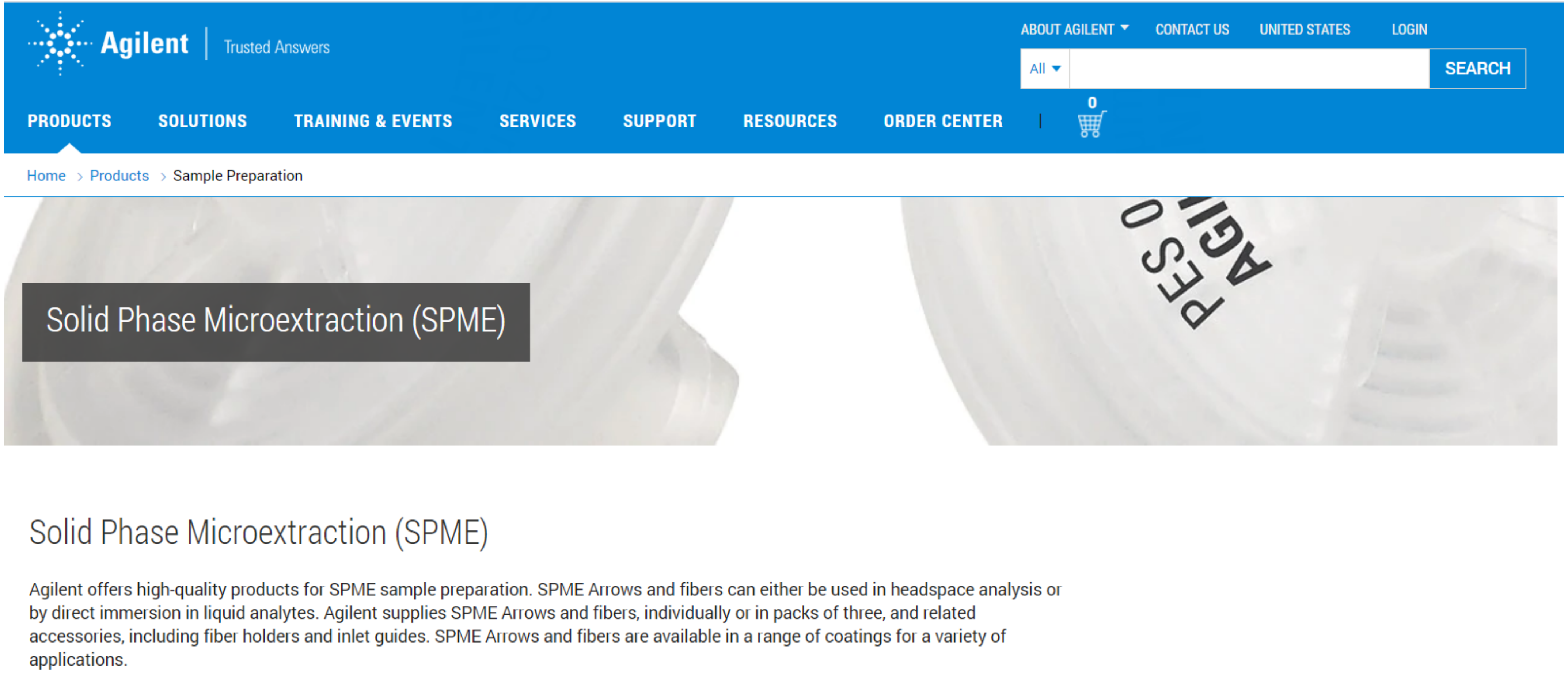


Agilent offers high-quality products for SPME sample preparation



What is Solid Phase Microextraction (SPME)

Sample preparation technique

Solid Phase Microextraction (SPME) is a fast, solvent-less sample extraction technique.

It is used for extracting organics from a matrix (solid, liquid, or gaseous) into or onto a stationary phase immobilized on a fiber.

In SPME, analytes establish equilibria among the sample matrix, the headspace above the sample, and a polymer-coated fused fiber.

Two types of extraction:

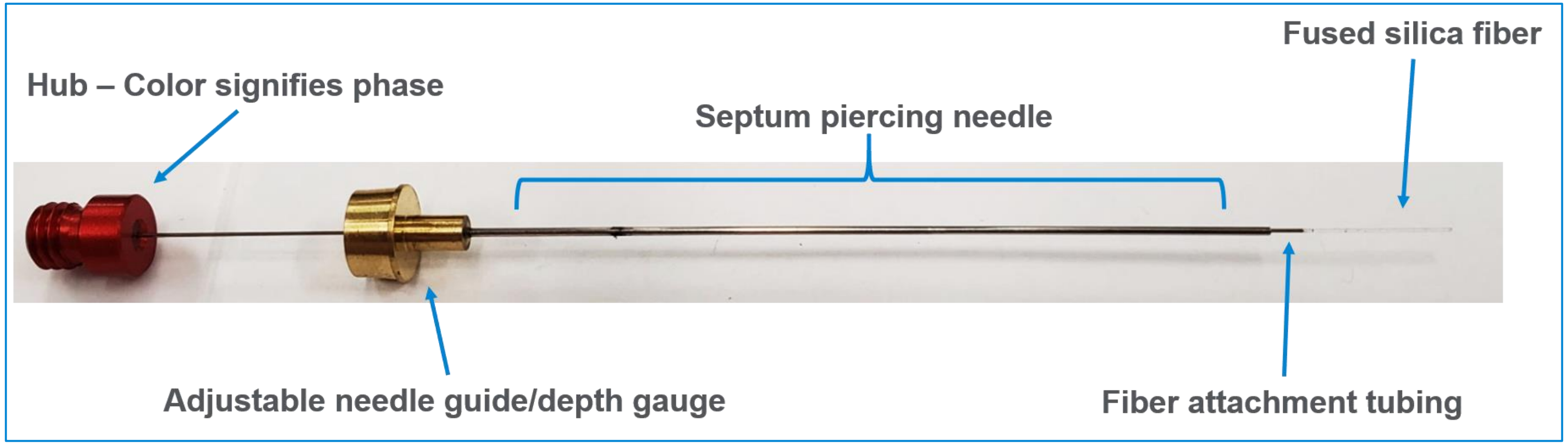
- Headspace (most common)
- Direct immersion (application specific) – SPME fiber is immersed into the aqueous sample

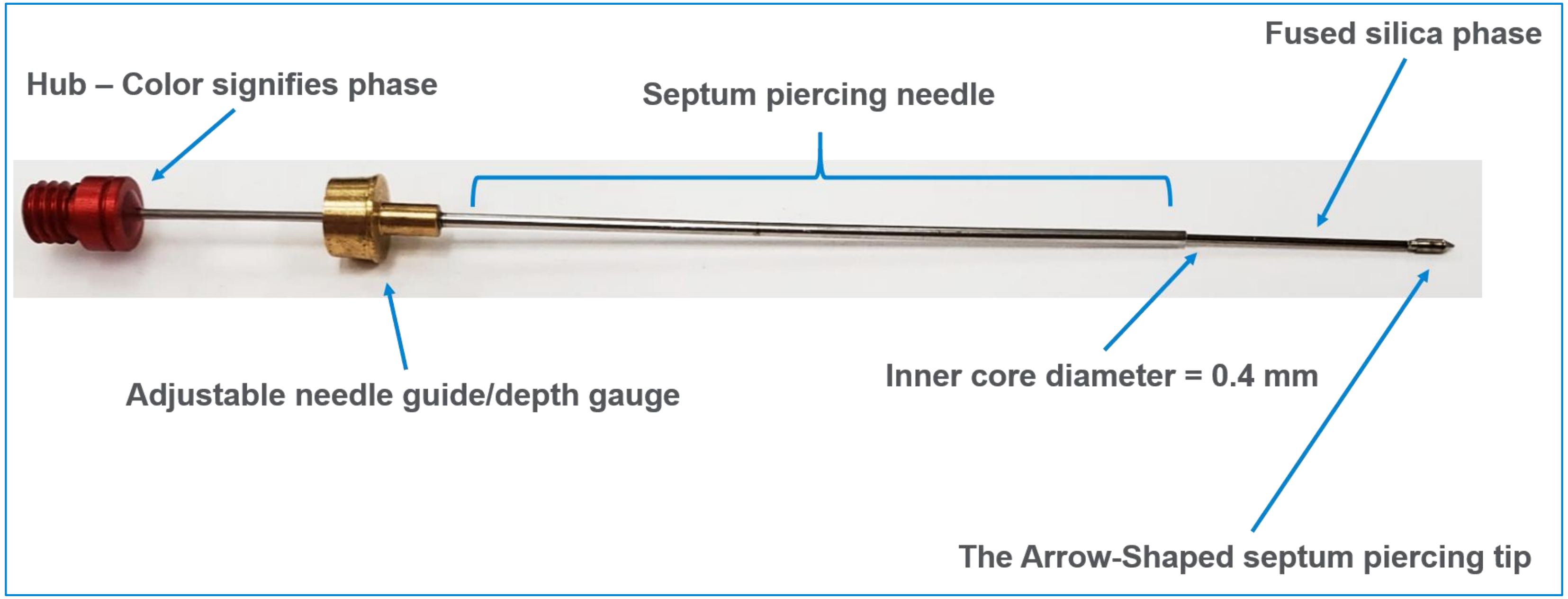
Headspace

Immersion

Parts of the SPME Fiber/Arrow

SPME characteristics





Key Component – The Phase

SPME fiber hub colors

7 µm PDMS	Green
30 µm PDMS	Gold
100 µm PDMS	Red
85 µm Polyacrylate	Grey
95/10 µm CAR-WR/PDMS	Dark Blue
65 µm DVB/PDMS	Violet
80/10 µm DVB/CAR-WR/PDMS	Dark Grey

Acronym	Full Name
PDMS	Polydimethylsiloxane
PA	Polyacrylate (aka acrylate)
DVB	Divinylbenzene
C-WR	Carbon Wide Range

Key Component – The Phase

SPME Arrow hub colors

Acronym	Full Name
PDMS	Polydimethylsiloxane
PA	Polyacrylate (aka acrylate)
DVB	Divinylbenzene
C-WR	Carbon Wide Range

100 µm PDMS	Red
100 µm Polyacrylate	Grey
120 µm CAR WR/PDMS	Light Blue
120 µm DVB/PDMS	Violet
120 µm DVB/CAR WR/PDMS	Dark Grey
250 µm PDMS	Black

SPME Functionality

Fiber/Arrow Conditioning

SPME functionality

Fibers/Arrows **MUST** be preconditioned prior to first use (or after sitting)

- Done at a specified temperature in an inert gas phase environment (He or N2)

Fibers/Arrows **MUST** be conditioned prior to and/or after desorption of sample – cleans the fiber

- Done at a hotter temperature than the injection/inlet temperature

<table>
<tr><th>Stationary Phase Fiber Type</th><th>Maximum Temperature (°C)</th><th>Recommended Operating Temperature (°C)</th><th>Preconditioning Temperature (°C) Min. | Max</th><th>Preconditioning Time (min.) Min. | Max | Recom.</th><th>Conditioning Temperature (°C) Min. | Max</th><th>Conditioning Time (min.) Min. | Max | Recom.</th><th>Fiber Rinsing Solvent</th><th>Fiber Rinsing Time (min.) Min. | Max | Recom.</th></tr>
<tr><td colspan="9">PDMS Fiber (Polydimethylsiloxane)</td></tr>
<tr><td>7 µm</td><td>340</td><td>200-340</td><td>200 | 340</td><td>15 | 120 | 30</td><td>200 | 340</td><td>1 | 60 |5</td><td>MeOH | EtOH iProp</td><td>0.5 | 10 | 2</td></tr>
<tr><td>30 µm</td><td>280</td><td>200-280</td><td>180 | 280</td><td>15 |120 | 30</td><td>180 | 280</td><td>1 | 60 | 5</td><td>MeOH | EtOH iProp</td><td>0.5 | 10 | 2</td></tr>
<tr><td>100µm</td><td>280</td><td>200-280</td><td>180 | 280</td><td>15 |120 | 30</td><td>180 | 280</td><td>1 | 60 | 5</td><td>MeOH | EtOH iProp</td><td>0.5 | 10 | 2</td></tr>
<tr><td colspan="9">Acrylate Fiber (Polyacrylate) 300</td></tr>
<tr><td>85 µm</td><td>300</td><td>200-280</td><td>180 | 280</td><td>15 |120 | 30</td><td>180 | 280</td><td>1 | 60 | 5</td><td>MeOH aliphatic HC</td><td>0.5 | 10 | 2</td></tr>
<tr><td colspan="9">Carbon WR Fiber / PDMS (Carbon Wide Range / PDMS)</td></tr>
<tr><td>95 µm</td><td>300</td><td>220-300</td><td>200 | 300</td><td>15 | 120 | 60</td><td>200 | 300</td><td>1 | 60 | 10</td><td>MeOH | EtOH iProp</td><td>0.5 | 10 | 2</td></tr>
</table>

Table 2. Operational Parameters for PAL SPME Fibers.

MeOH = Methanol
iProp = Iso-Propanol (2-Propanol)
EtOH = Ethanol
aliphatic HC = aliphatic hydrocarbons (example n-Hexane)

<table>
<tr><th>Stationary Phase Arrow Type</th><th>Maximum Temperature (°C)</th><th>Recommended Operating Temperature (°C)</th><th>Preconditioning Temperature (°C) Min. | Max</th><th>Preconditioning Time (min.) Min. | Max | Recom.</th><th>Conditioning Temperature (°C) Min. | Max</th><th>Conditioning Time (min.) Min. | Max | Recom.</th><th>Arrow Rinsing Solvent</th><th>Arrow Rinsing Time (min.) Min. | Max | Recom.</th></tr>
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<tr><td colspan="9">DVB SPME Arrow / PDMS (Divinylbenzene / PDMS)</td></tr>
<tr><td>120 µm</td><td>300</td><td>220-300</td><td>200 | 300</td><td>15 | 120 | 60</td><td>200 | 300</td><td>1 | 60 | 10</td><td>MeOH | EtOH iProp</td><td>0.5 | 10 | 2</td></tr>
<tr><td colspan="9">PDMS SPME Arrow (Polydimethylsiloxane)</td></tr>
<tr><td>250 µm</td><td>300</td><td>220-300</td><td>200 | 300</td><td>15 | 120 | 60</td><td>200 | 300</td><td>1 | 60 | 10</td><td>MeOH | EtOH iProp</td><td>0.5 | 10 | 2</td></tr>
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Table 2. Operational Parameters for PAL SPME Arrows.

MeOH = Methanol
iProp = Iso-Propanol (2-Propanol)
EtOH = Ethanol
aliphatic HC = aliphatic hydrocarbons (example n-Hexane)

SPME Manual Sampling

SPME functionality

The Agilent manual injection kit (p/n 5191-5877) will allow the end user to extract samples using SPME fibers or arrows and then inject the samples into a GC inlet.

Part	Component
1	Extraction guide
2	Injection guide
3	Arrow/fiber syringe
4	Large inner diameter (id) locking screw
5	Small inner diameter (id) locking screw

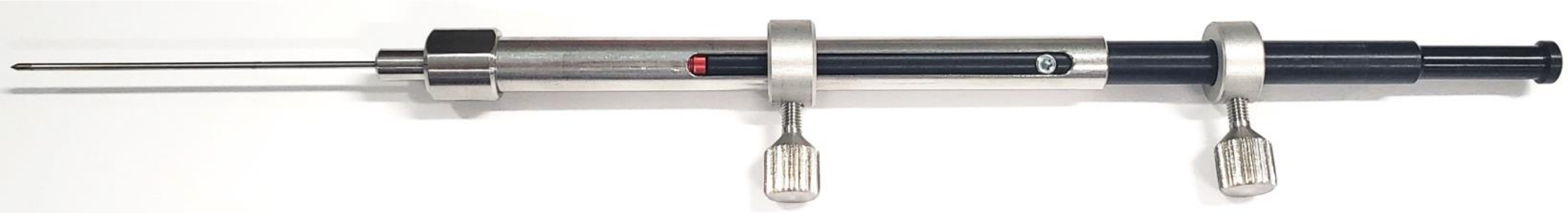
Alignment ring (p/n G7371-67001)
**Not included in kit*

SPME Manual Sampling

Agilent demonstration guide: 5994-1732EN

Manual SPME sampling: SPME fibers and Arrows

A detailed step-by-step guide (with images) to assist with the installation of a SPME fiber or Arrow into the manual sampling holder for either headspace or immersion sampling and adjustments for the injector penetration depth.



Demonstration Guide

Agilent
Trusted Answers

Manual SPME Sampling

SPME fibers and Arrows

SPME Fiber or Arrow Manual Injection Kit

SPME manual sampling

The Agilent manual injection kit will allow the end user to extract samples using SPME fibers or Arrows. They can then inject the samples into a GC inlet.

p/n 5191-5877

Part	Component
1	Extraction guide
2	Injection guide
3	Arrow/fiber syringe
4	Large inner diameter (id) locking screw
5	Small inner diameter (id) locking screw

SPME Automated Sampling

SPME functionality

Agilent's SPME fibers and Arrows work directly with your current rail system.

PAL-xt Holder
For PAL and PAL-xt systems with CombiPAL and PAL Combi-xt - Fibers Only

PAL SPME tool
For older PAL system Models (or corresponding OEM rails). Fibers only.

PAL SPME fiber tool
PAL3 Series RSI and PAL RTC

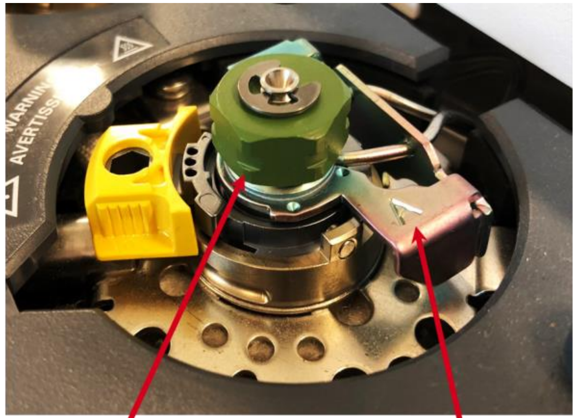
PAL SPME Arrow tool
PAL3 Series RSI and PAL RTC

SPME Automated Sampling

Changes for SPME Arrows

Due to their larger diameter, SPME Arrows require instrumental changes in addition to obtaining the SPME Arrow option on the PAL3 rail system.

You need an enlarged GC turn top assembly



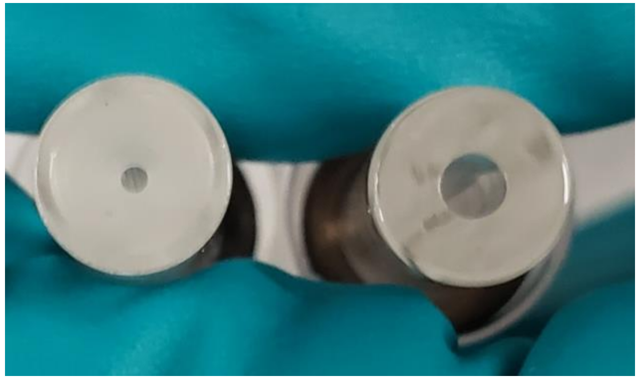
SPME Arrow modified Septum Nut is notched

The letter "A" stamped on metal bracket indicates Arrow modified inlet.

GC Model	Agilent Part No.
7890B GC	G3452-60930
8890/8860 GC	G3450-60638
Intuvo 9000	G4585-60633

You must use an inlet liner with a larger diameter

0.75 mm id 2 mm id



Inner Diameter	Requirement	Agilent Part No.
0.75 mm	SPME fibers **only**	5190-4048
2 mm	Both SPME fibers and Arrows	5190-6168

SPME Applications

SPME Sampling

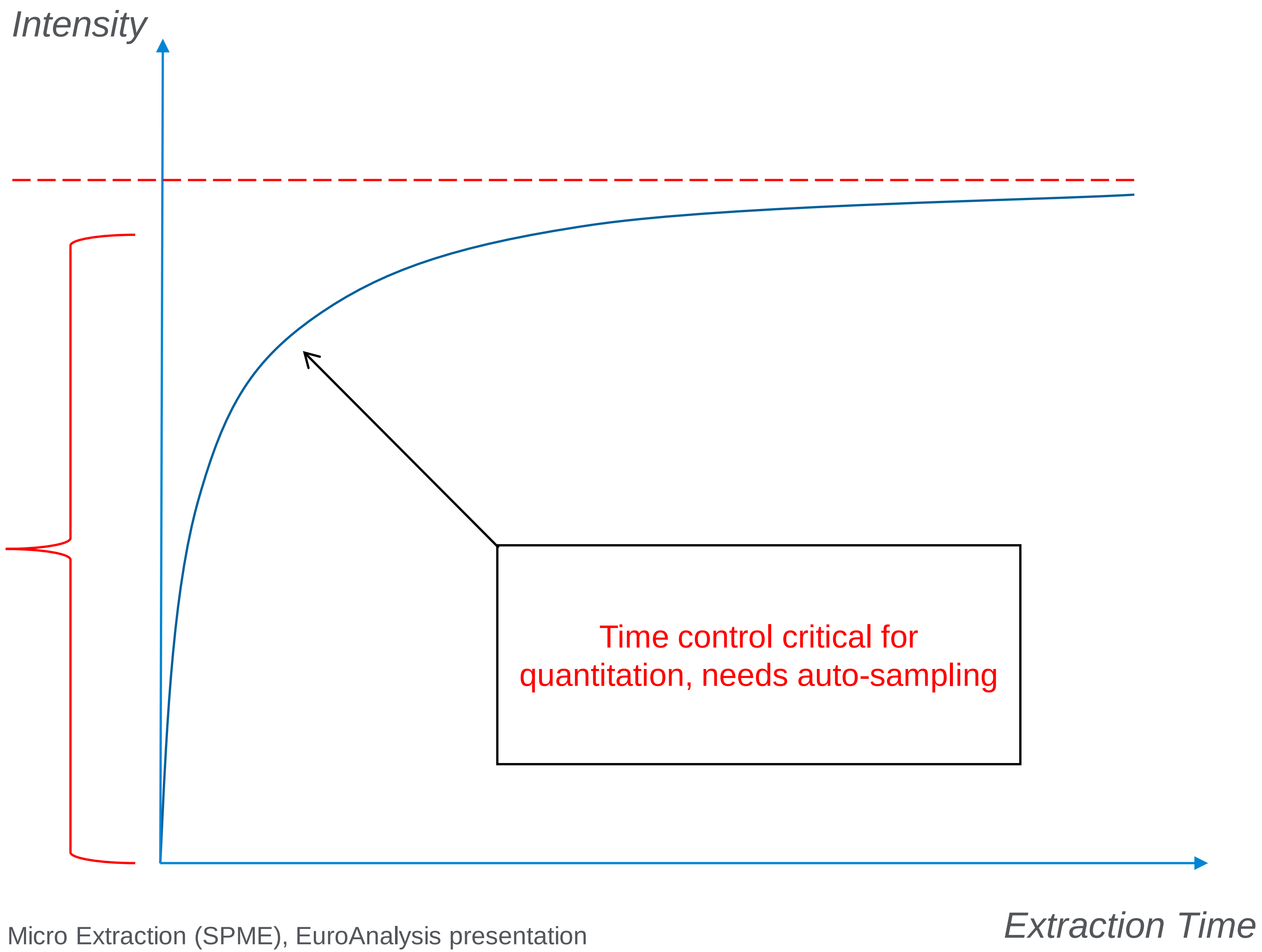
Applications

Equilibrium condition

- Sorbent material
- Temperature
- Time

Kinetics influence

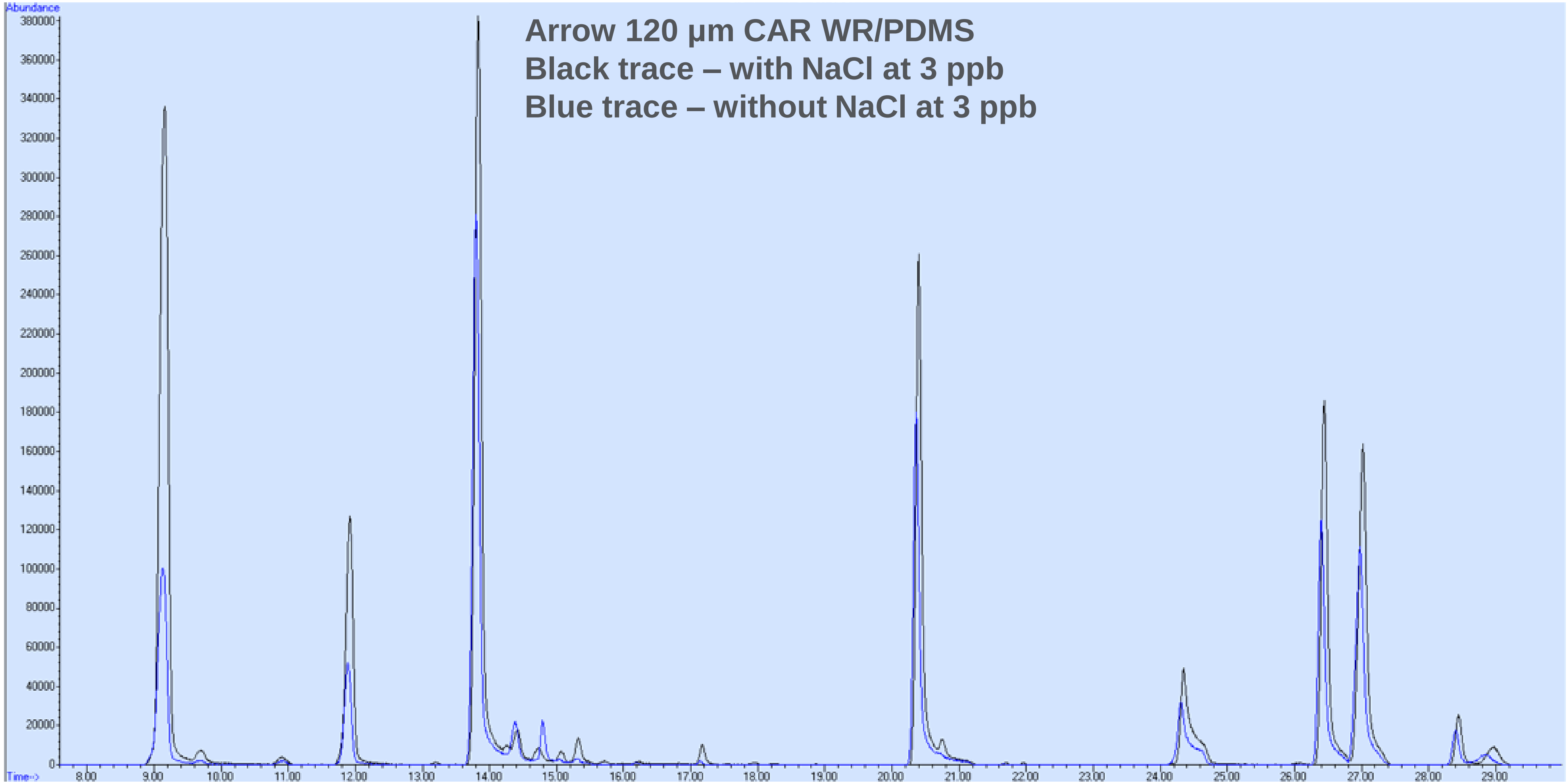
- Stirring/agitation
- Salting out
- pH Value
- Temperature



Supelco 2011 - Michel, F., Buckendahl, K., Basics in Solid Phase Micro Extraction (SPME), EuroAnalysis presentation

SPME Sampling – Salting out

Applications



SPME Fiber or Arrow

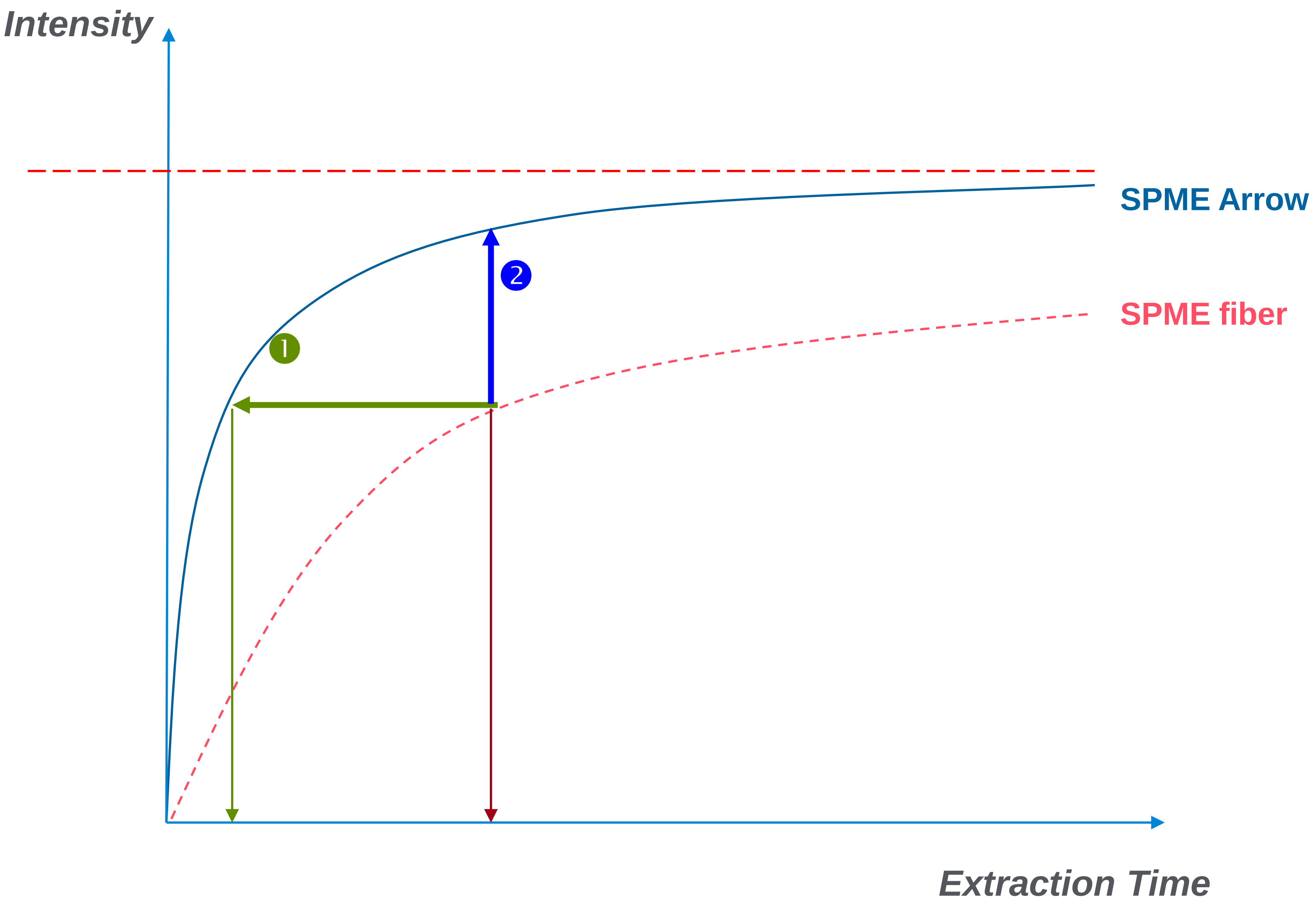
Applications

Speed

- Shorter extraction speed
- For same response as fibers
  - Improved productivity
  - Higher sample throughput

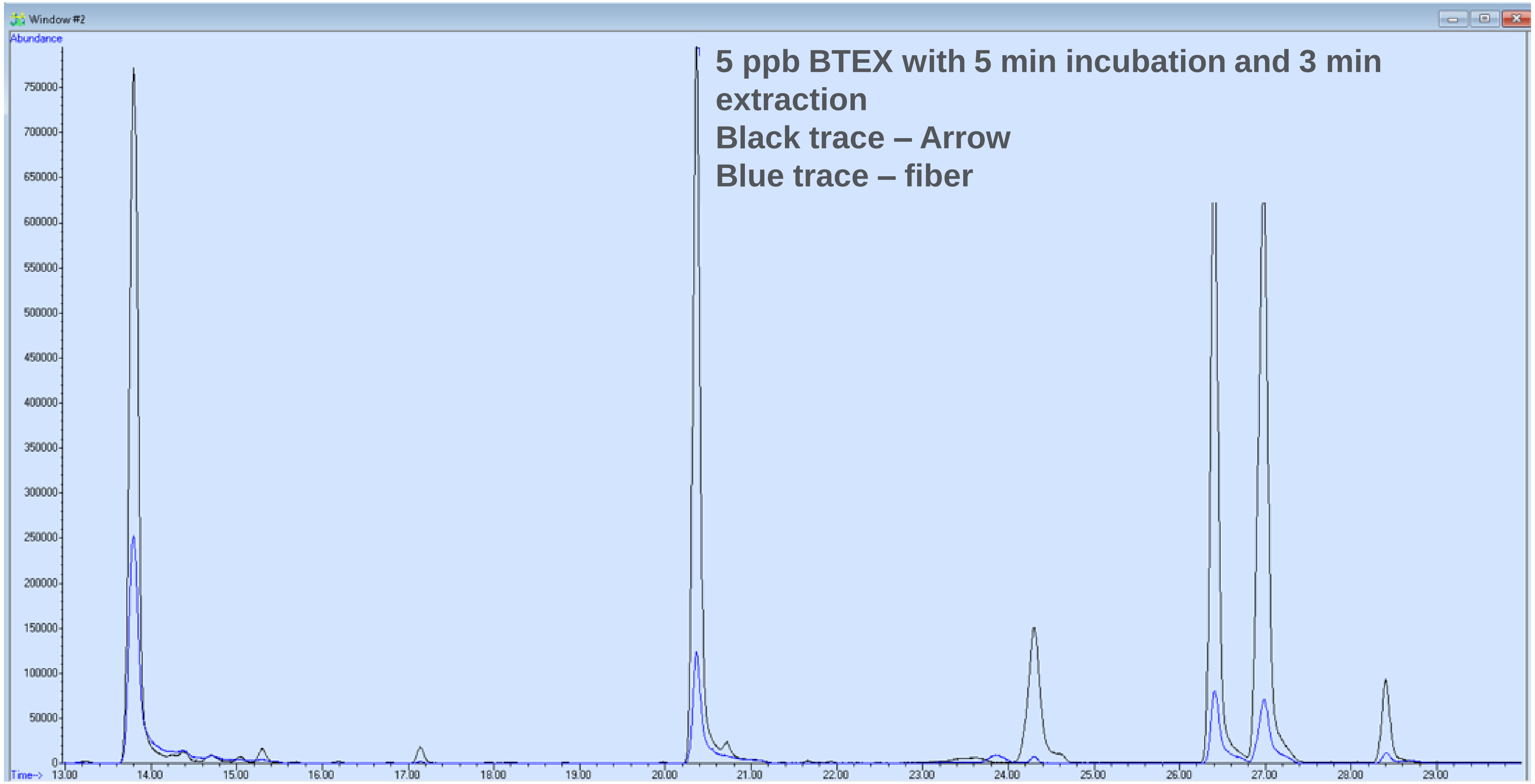
Sensitivity

- Higher response
- At same extraction time as fibers
  - Lower detection limits
  - Improved precision



SPME Fiber or Arrow

Applications



Analyte Relation to Phase Type

Applications

Thinner coatings
For higher MW compounds
Can accept higher conditioning temperature

7 µm PDMS

30 µm PDMS

100 µm PDMS

85 µm Polyacrylate

95/10 µm CAR-WR/PDMS

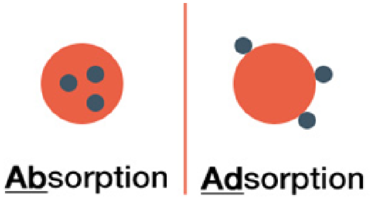
65 µm DVB/PDMS

80/10 µm DVB/CAR-WR/PDMS

Thicker coatings
Require longer extraction times
Use lower conditioning temperature

Absorbent

Adsorbent



Analyte Relation to Phase Type

Applications

Nonpolar high MW compounds → 7 µm PDMS

Nonpolar semi-volatiles → 30 µm PDMS

Volatiles → 100 µm PDMS

Polar semi-volatiles → 85 µm Polyacrylate

Gases and volatiles → 95/10 µm CAR-WR/PDMS

Polar volatiles → 65 µm DVB/PDMS

Odors and flavors → 80/10 µm DVB/CAR-WR/PDMS

Nonpolar

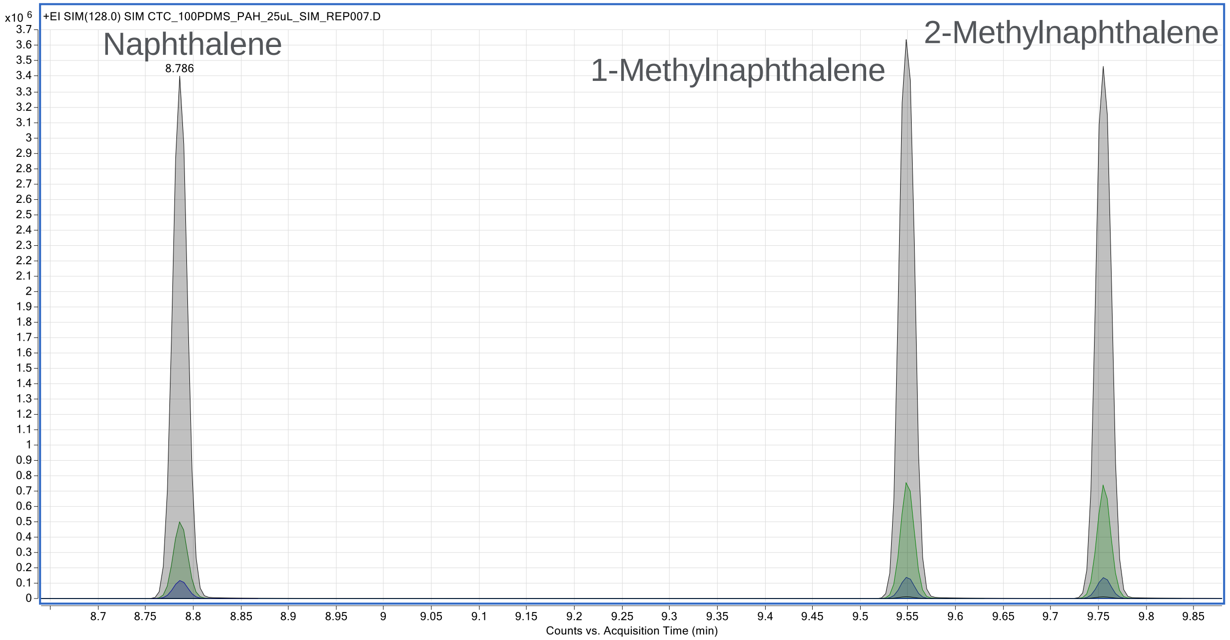
Polar

Bipolar

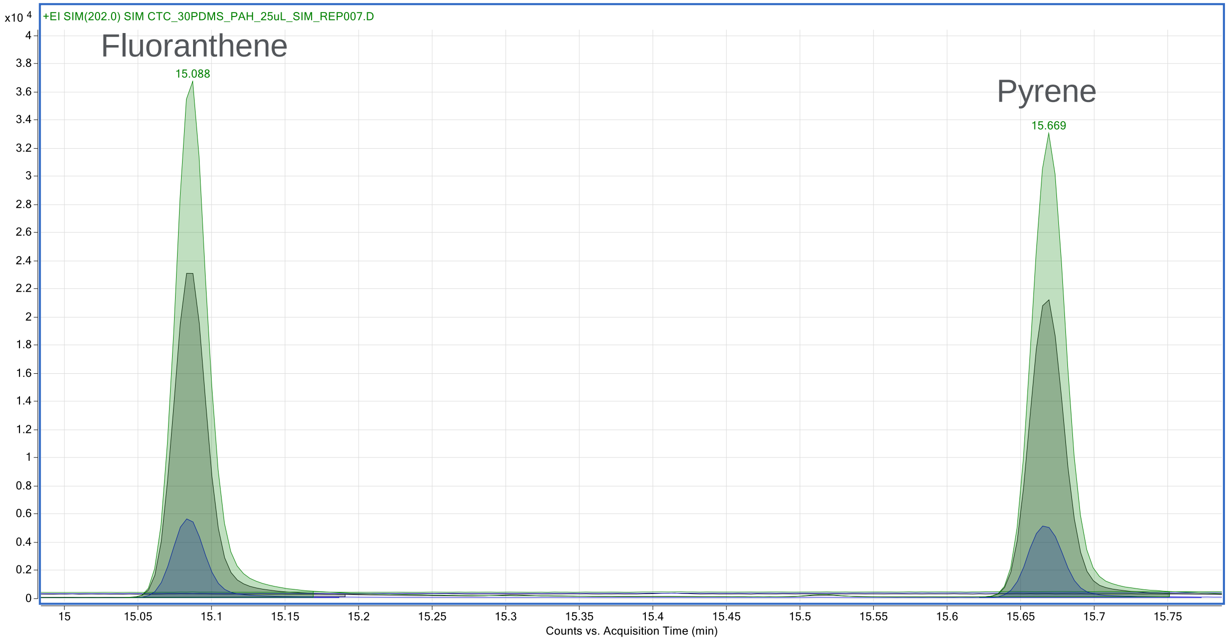
Examination of Lower Molecular Weight PAHs in Drinking Water Using Agilent PDMS SPME Fibers

Learn more, search 5994-1301EN at www.agilent.com

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic rings. PAHs are considered compounds of concern by every environmental organization; their concentration in water is strictly regulated.



SIM chromatogram of naphthalenes with PDMS fibers
(black trace = 100 µm; green trace = 30 µm; blue trace = 7 µm)

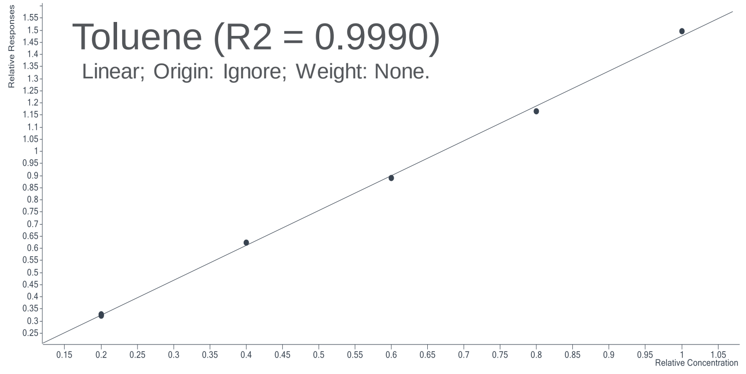


SIM chromatogram of fluoranthene and pyrene with PDMS fibers
(black trace = 100 µm; green trace = 30 µm; blue trace = 7 µm)

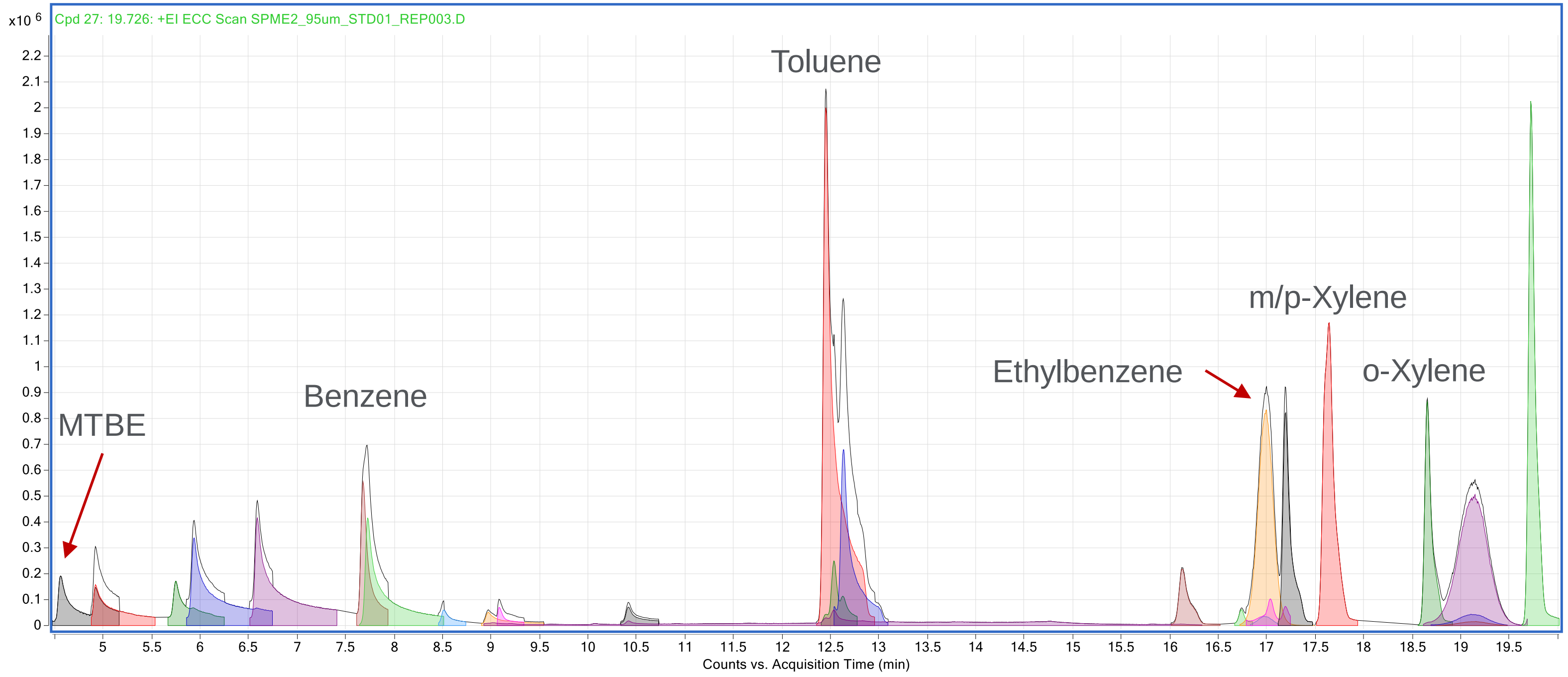
Analysis of BTEX in Water with a CAR-WR/PDMS 95 µm SPME Fiber

Learn more, search 5994-1104EN at www.agilent.com

The production of safe drinking water is an important issue, and legislation has established allowed levels of chemical substances in drinking water, whether they occur naturally, as deliberate additions, or as contaminants.



Toluene linear calibration curve

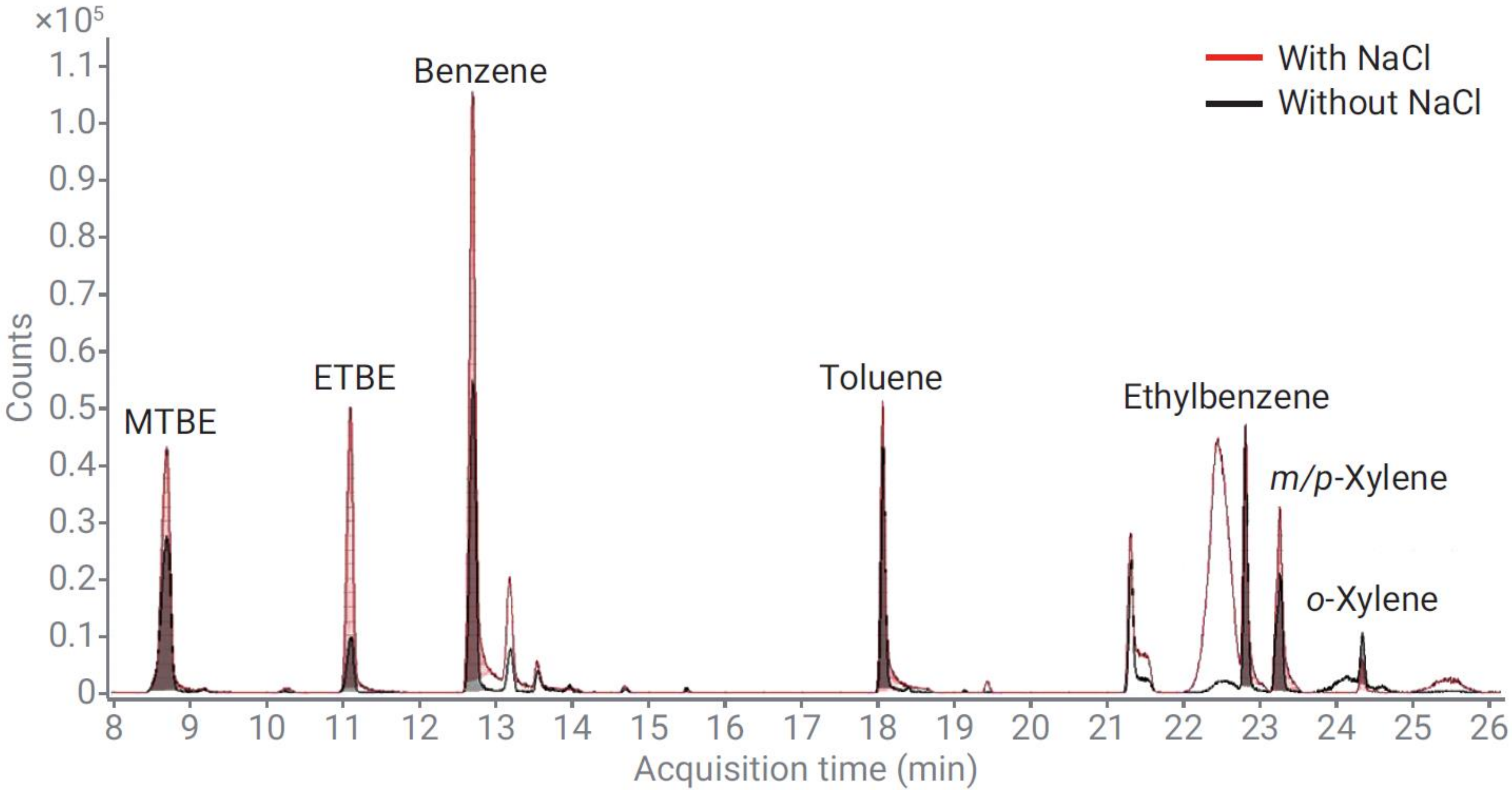


1.25 ppb on column (Replicate #3; CAR-WR/PDMS 95 µm SPME Fiber)

Determination of VOCs in Water by GC/MS after Headspace-Solid-Phase Microextraction (HS-SPME)

Learn more, search 5994-1045EN at www.agilent.com

The addition of NaCl increased the extraction efficiency for the analytes of interest.

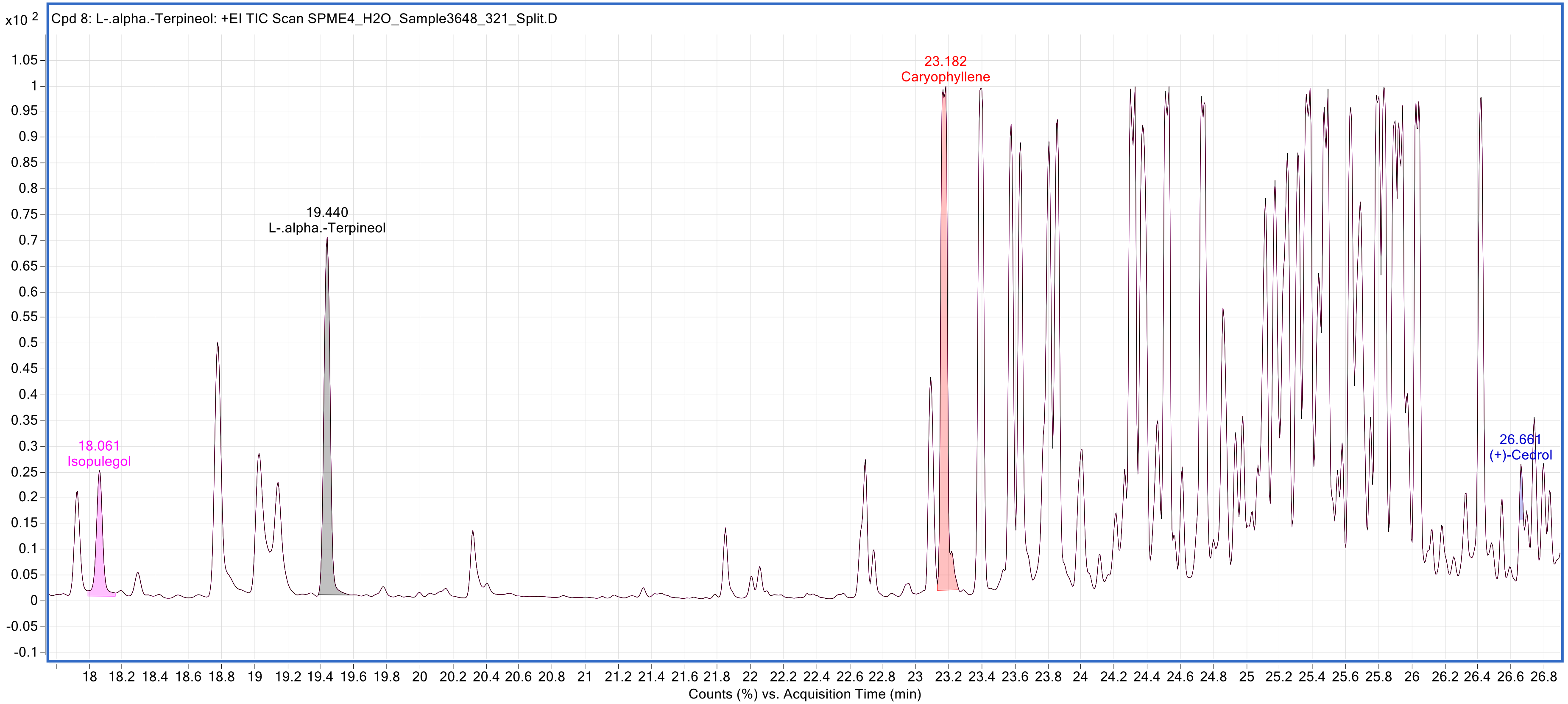


Chromatogram of 0.8 ppb BTEX standard analyzed by a 120 µm CAR WR/PDMS; SPME Arrow with NaCl (red trace) and without NaCl (black trace)

SPME-GC/MS of Selected Terpenes using Agilent DVB/CAR-WR/PDMS SPME Fiber

Learn more, search 5994-1411EN at www.agilent.com

Since terpenes have high vapor pressures, and are extremely volatile, they are excellent candidates for static headspace (HS) gas chromatography/mass spectrometry (GC/MS) analysis.



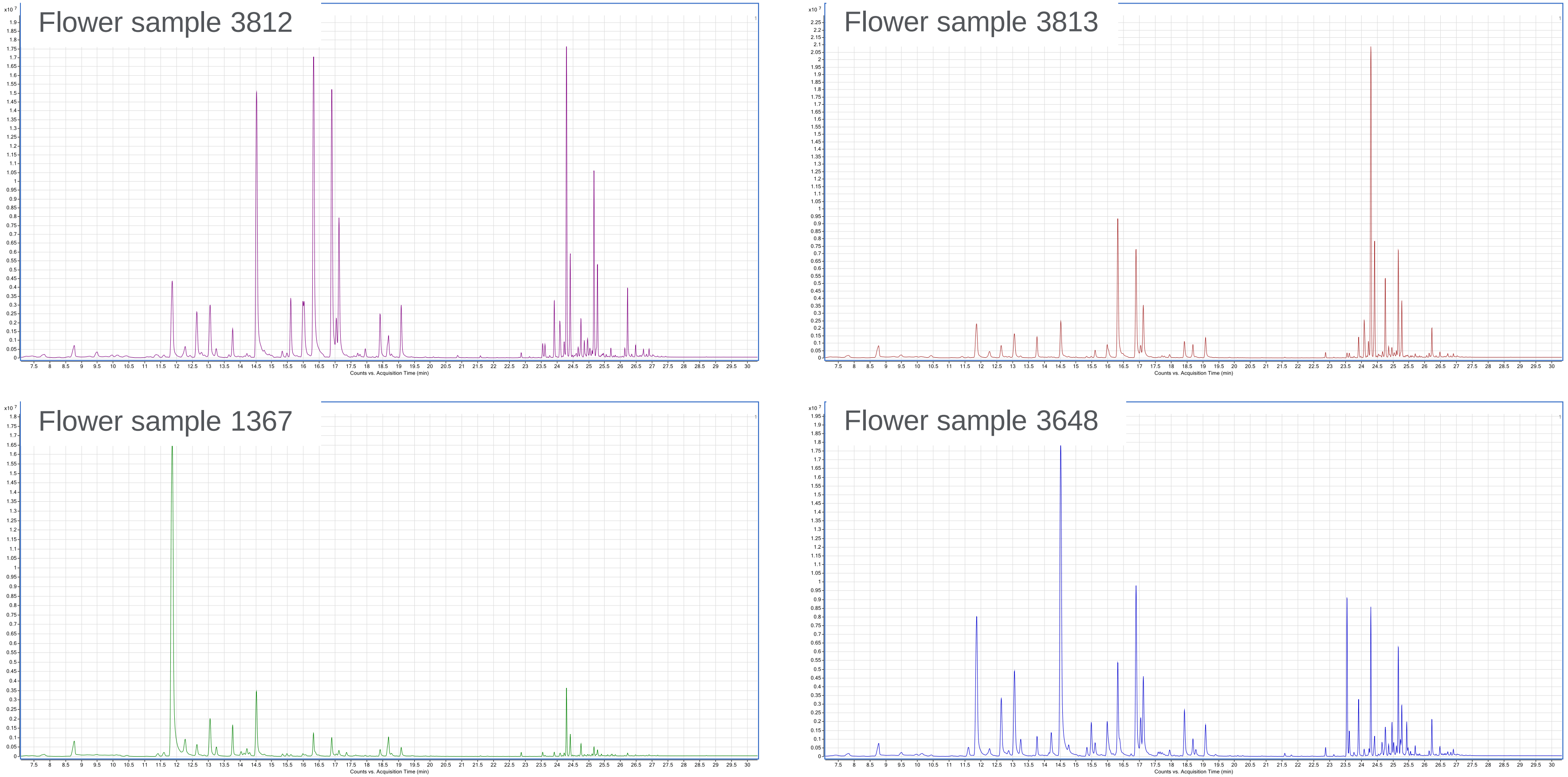
Selected terpenes in cannabis flower sample 3648: (-)-Isopulegol, Terpineol, b-Caryophyllene, and (+)-Cedrol

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

SPME Arrow Sampling of Terpenes in Cannabis Plant Material

Learn more, search 5994-1046EN at www.agilent.com

PAL SPME Arrows provided the sensitivity and robustness needed to profile the predominant terpenes in an unknown variety of cannabis plant samples



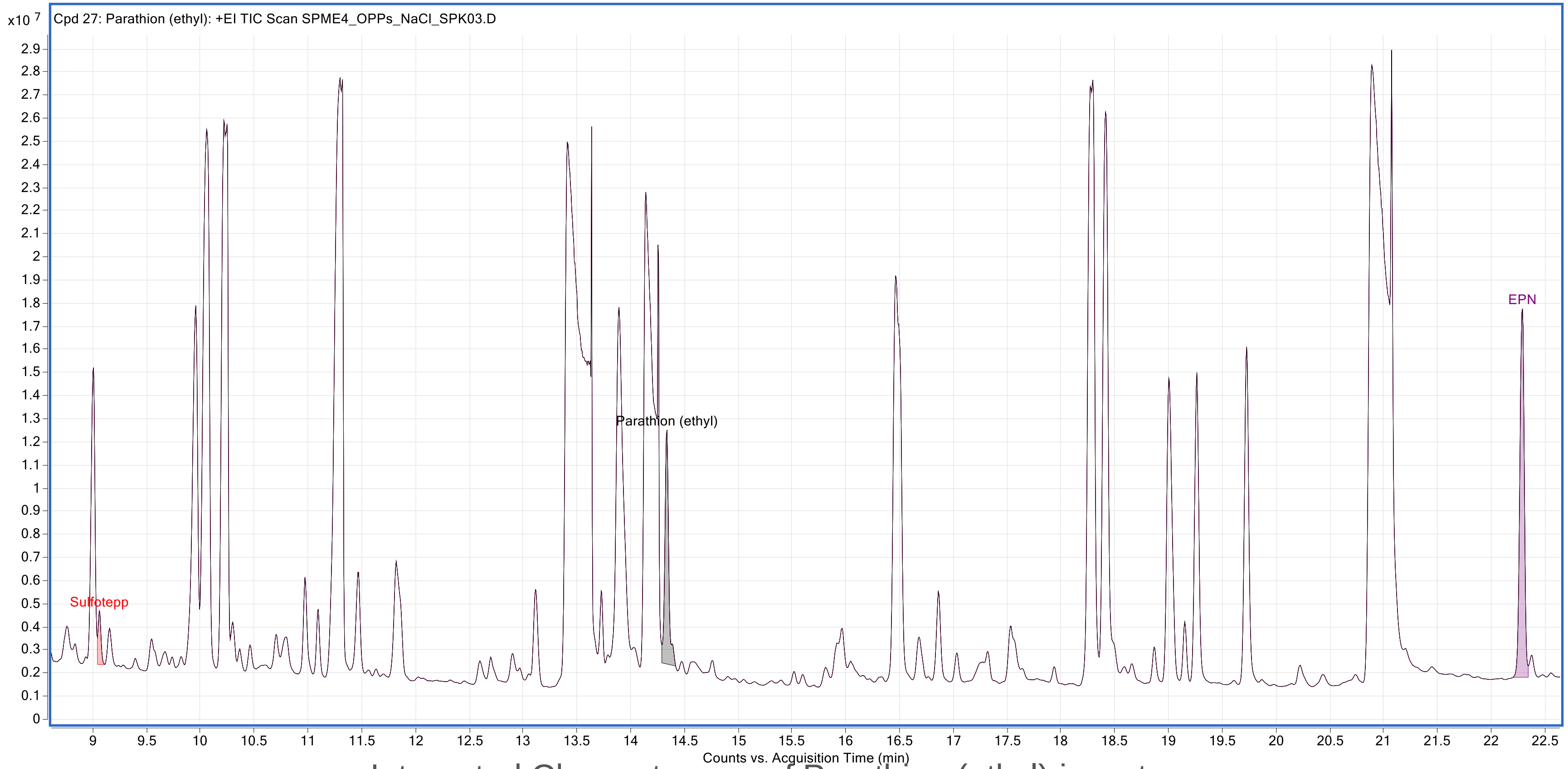
Flower samples profiled with the use of the 120 µm DVB/CAR WR/PDMS (p/n 5191-5861) Arrow

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

Analysis of Parathion-Ethyl in Water with an 85 µm Polyacrylate SPME Fiber

Learn more, search 5994-1546EN at www.agilent.com

Organophosphorus insecticides are among the most widely used pesticides throughout the world. As a result of their widespread use their residues have been detected in samples such as surface waters, soil, and agricultural products.



Integrated Chromatogram of Parathion (ethyl) in water

Immersion Sampling

Best practices

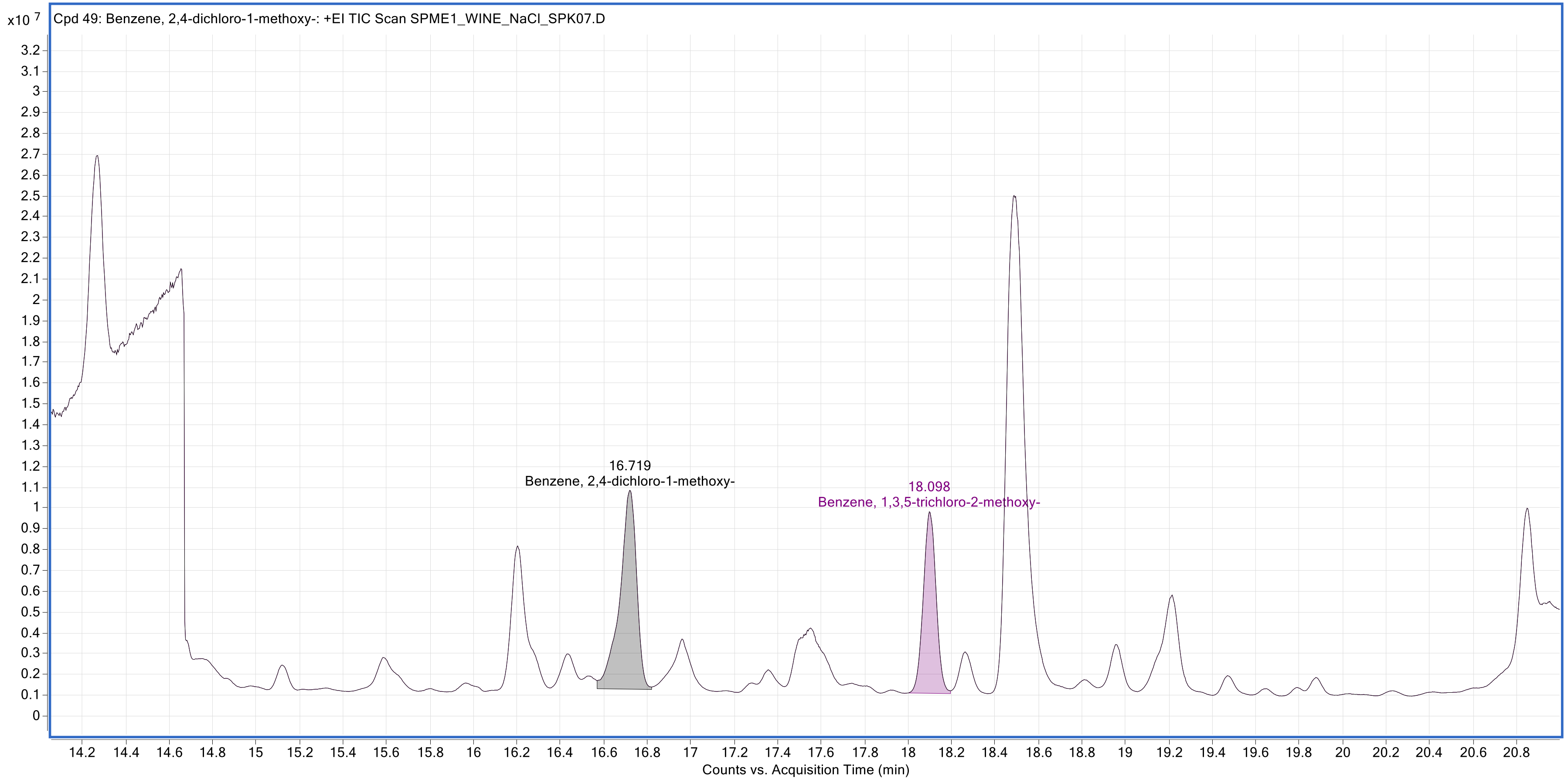
- Immersion sampling sensitivity is improved by filling the sampling vial to a minimum of 80%
- Immersion sampling works best for low concentration water-based sample matrices
- When using immersion for samples containing sugars, proteins, and particulate matter, rinse the fiber in water before desorption.
  - This will extend fiber life and reduce injection port contamination.
- Position the fiber just below the sample surface for immersion sampling and maintain this position consistently for all extractions.

Immersion

SPME-GC/MS of 2,4,6-trichloroanisole Using Agilent DVB/PDMS SPME Fiber

Learn more, search 5994-1564EN at www.agilent.com

The main compound responsible for cork taint is 2,4,6-trichloroanisole. It is one of the most odor intense compounds known and has a distinct musty, moldy aroma.



2,4-Dichloroanisole and 2,4,6-Trichloroanisole @ 25 ppb in Red Wine

SPME Troubleshooting

Make troubleshooting faster and easier by closely observing and keeping complete records of your analytical methodologies

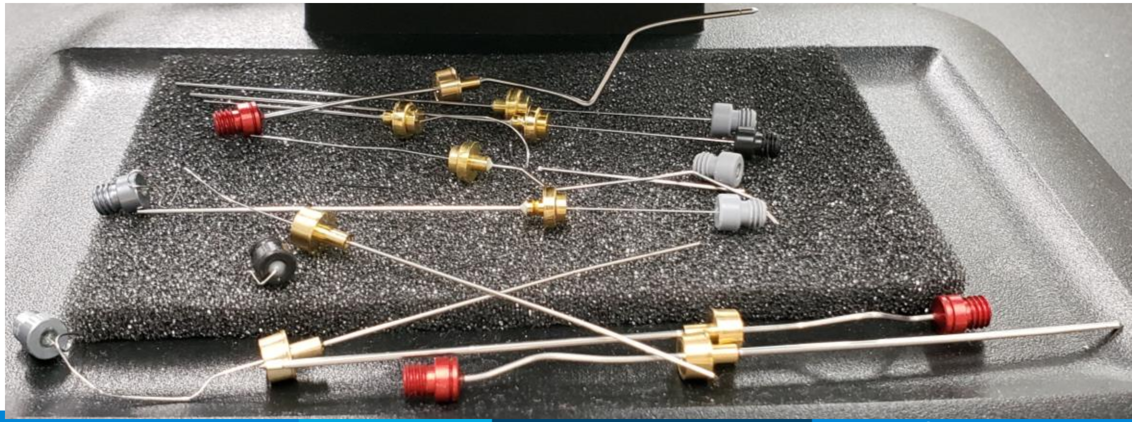
Needle Bends/Fiber Breaks

SPME troubleshooting

Improper alignment is one of the main causes of fiber/arrow damage.

- Follow Agilent's Demonstration Guide (5994-1732EN) to ensure proper alignment and set up of the manual sample holder
- Retract fiber into protective needle during insertion into vial/injection port and removal
- Check for proper alignment of the PAL3 fiber/arrow needle with the injector, vial trays, and vials
- Make sure that the insertion depths into the vials and injection port are properly set
- Use larger splitless inlet liners without glass wool or adsorbents
  - p/n 5190-4056 (5/pk): splitless, straight, 0.75 mm id
  - p/n 5190-4047 (1/pk): splitless, straight, 1 mm id
  - p/n 5190-6168 (1/pk): splitless, straight, 2 mm id (use for SPME Arrows)
- Avoid stress of the needle by keeping agitation speed low
  - Agitator module: < 600 rpm
  - Heatex Stirrer module: < 1300 rpm

Note: All Agilent SPME fiber assemblies use 23 gauge needles for increased strength and less bending



Septum Issues

SPME troubleshooting

Septum of sample vial cap is too thick

- Use on Agilent screw headspace magnetic caps (p/n 5188-2759; 18 mm) PTFE/silicone septa (top white, bottom blue) on Agilent amber screw top headspace sampling vials (p/n 5188-6537; 20 mL)

Septum in injection port is too thick or too tight

- Loosen the nut slightly to allow for improved injection

Septum coring

- Use a septum-less injector system (i.e. Merlin Microseal)
  - Fibers:
    - p/n 5182-3445: Merlin Microseal 100 psi nut
    - p/n 5182-3444: Merlin Microseal general-purpose replacement Micros

Discolored Coatings

SPME troubleshooting

Coatings will discolor when heated too high or there are leaks in the flow path

- Minimize oxygen in carrier gas, condition fibers in oxygen free gas flow
  - p/n CP17970: Gas Clean oxygen purifier
  - p/n CP17973: Gas Clean carrier gas purifier
- Reduce the injection port temperature to the recommended maximum setting
- Carbowax/DVB may slightly darken during use
  - Sensitive to temperature (< 260 ˚C is recommended)
- The polyacrylate coated fiber will discolor above 280 °C

Data Analysis

SPME troubleshooting

Endogenous peaks in chromatogram

- Precondition fiber/arrow prior to sampling
- Use low-bleed septa to minimize injection port septum bleed or a septum-less injector system (i.e. Merlin Microseal)
- Replace the inlet liner

Interfering peaks

- Change GC column or temperature program rate

Carryover from previous analysis of the fiber

- Bake out fiber/Arrow at the recommended conditions for several more minutes

Poor reproducibility

- Time of extraction and temperature are the two most critical conditions to control
- Make sure the positioning of the fiber/arrow is consistent from sample to sample
- Apply uniformly across all extractions with any pH or salt adjustments
- Use internal standards, surrogates, or the standard addition technique to compensate for variations in sample matrix
- Maintain the same headspace volume and agitation conditions across all extractions

SPME Summary

Solid phase microextraction (SPME)

Agilent offers high-quality products for SPME sample preparation

Agilent puts together the supplies for both manual and automated SPME sampling

Agilent follows the market trends to keep you up to date with application notes and technical collateral

Columns, Sample Prep and Supplies Questions

Do you have questions about the following during a potential lab shutdown?

- Column storage
- What to do with mobile phase, buffers, and other HPLC solvents
- Chemical standards storage
- What supplies to stock up on to get your instrument back up and running in a timely manner

Take advantage of Agilent's best-in-class columns, sample prep, and supplies technical support!

Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 Option 3, Option 3:

Option 1 for GC/GCMS Columns and Supplies

Option 2 for LC/LCMS Columns and Supplies

Option 3 for Sample Preparation, Filtration and QuEChERS

Option 4 for Spectroscopy Supplies

Option 5 for Chemical Standards

800 Phone lines available 8-5 in all US time zones

gc-column-support@Agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

Looking to increase your knowledge during your lab shutdown?

Check out on-demand and live webinars on GC/GCMS, LC/LCMS and Sample Prep consumables.

https://www.agilent.com/en/training-events/eseminars/gc-gc-ms-webinars

https://www.agilent.com/en/training-events/eseminars/lc-lc-ms-column-e-seminars

Thank You