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Combining SPME and Derivatization for Analysis of THC and Metabolite in Surface Waters

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NEMC, 2015

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Agenda

1. What is Solid Phase Microextraction?
2. On-fiber derivatization
3. Quantitative analysis of THC and metabolite in surface waters.

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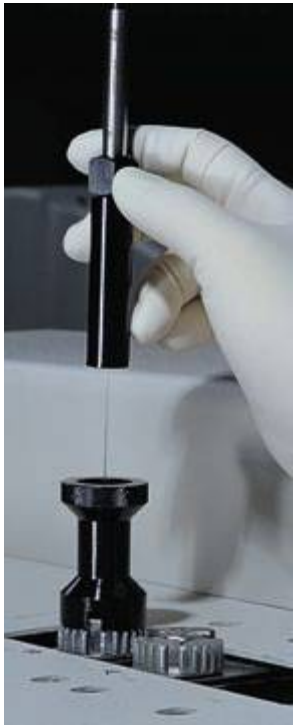
SPME Overview

- **Solvent-free extraction technique for nearly any sample or matrix**
- **Alternative to head-space GC and solid phase extraction (SPE) techniques**
- **Directly interfaced with GC analysis**
- **Non-destructive to sample**
- **Reusable (100+ times)**
- **Inexpensive**
- **Fast**

Assembled SPME fiber and holder with fiber immersed in a liquid sample.



Manual SPME holder and inlet guide.

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The SPME Concept

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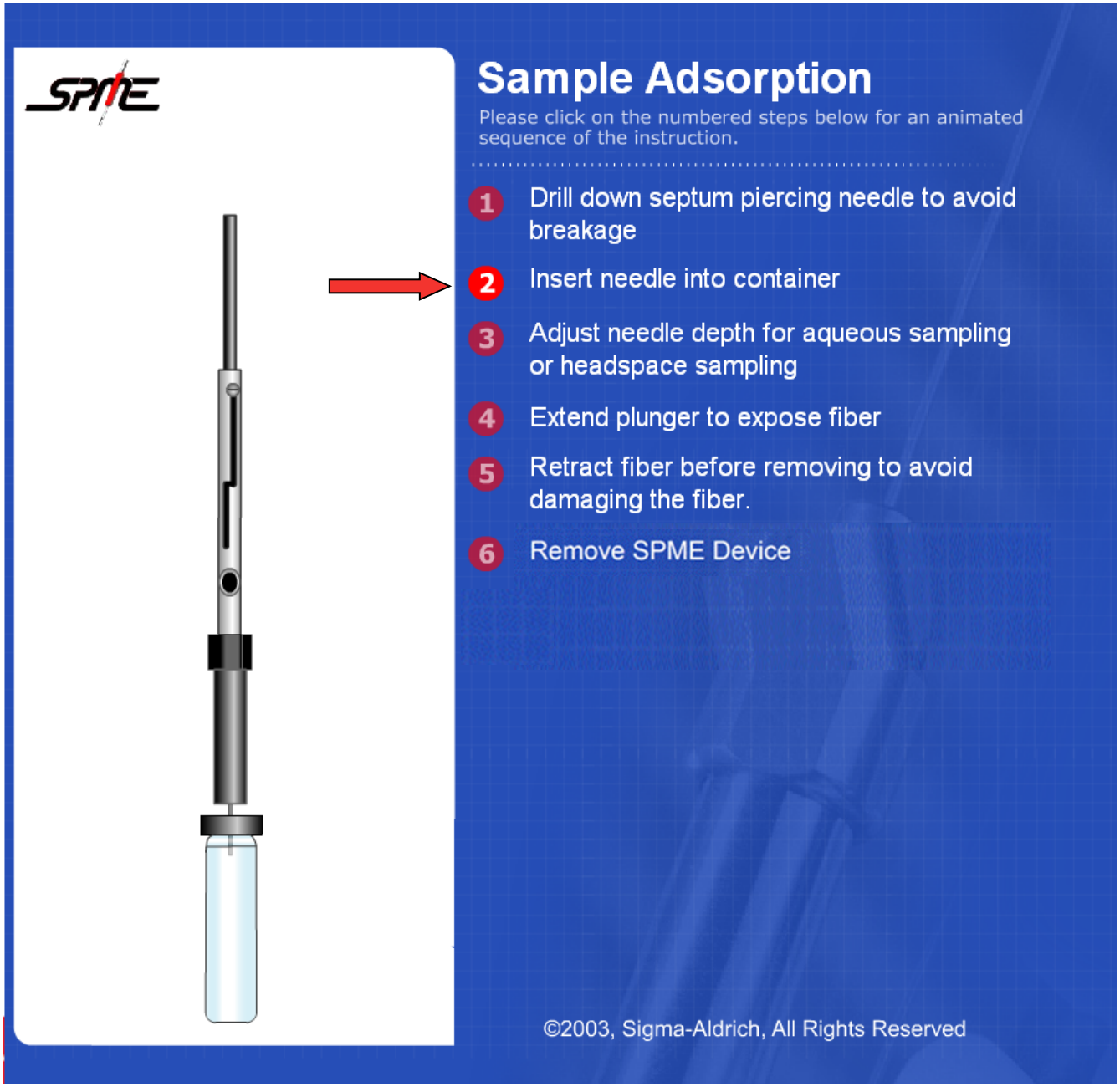
The SPME Concept



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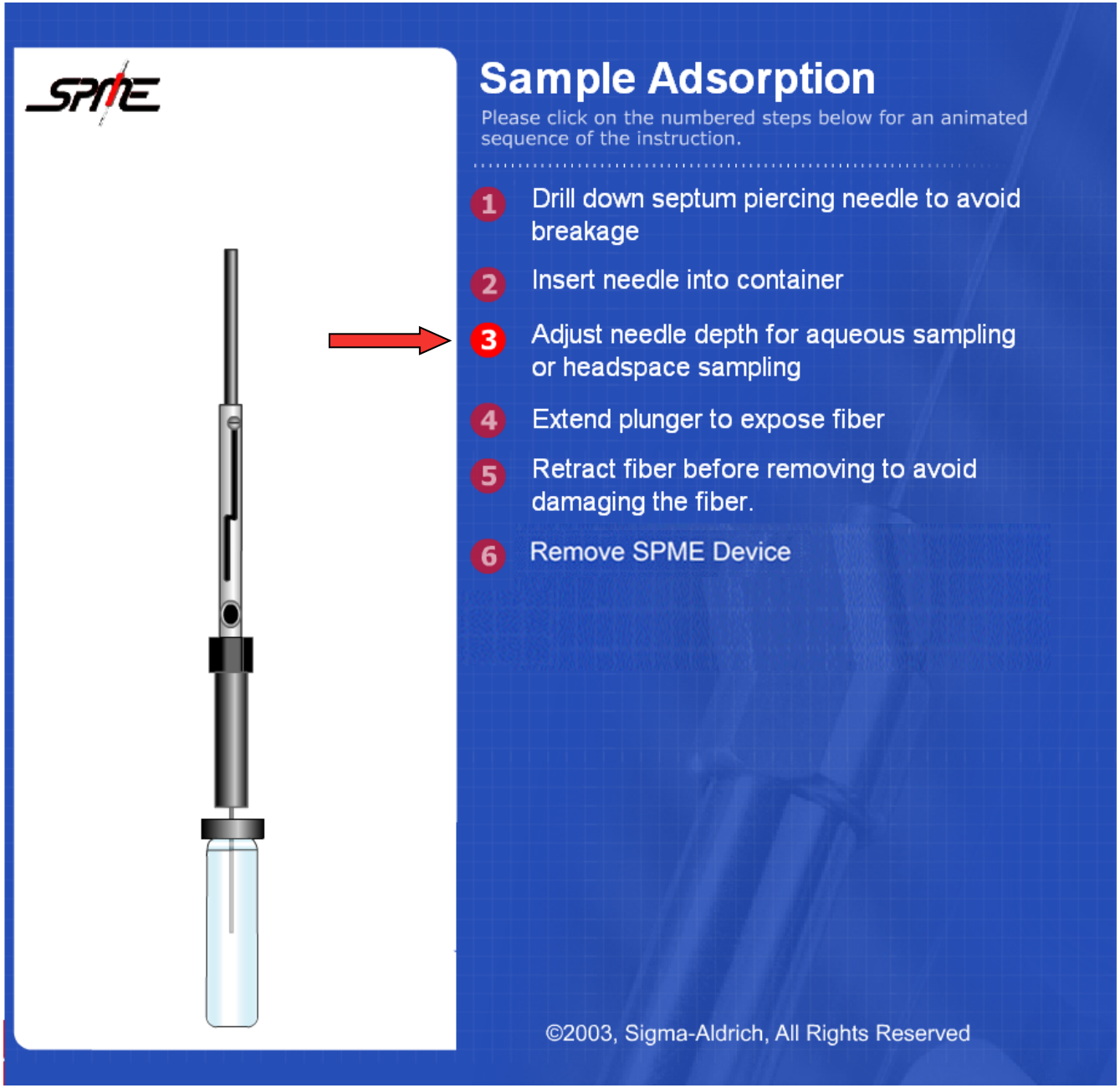
The SPME Concept



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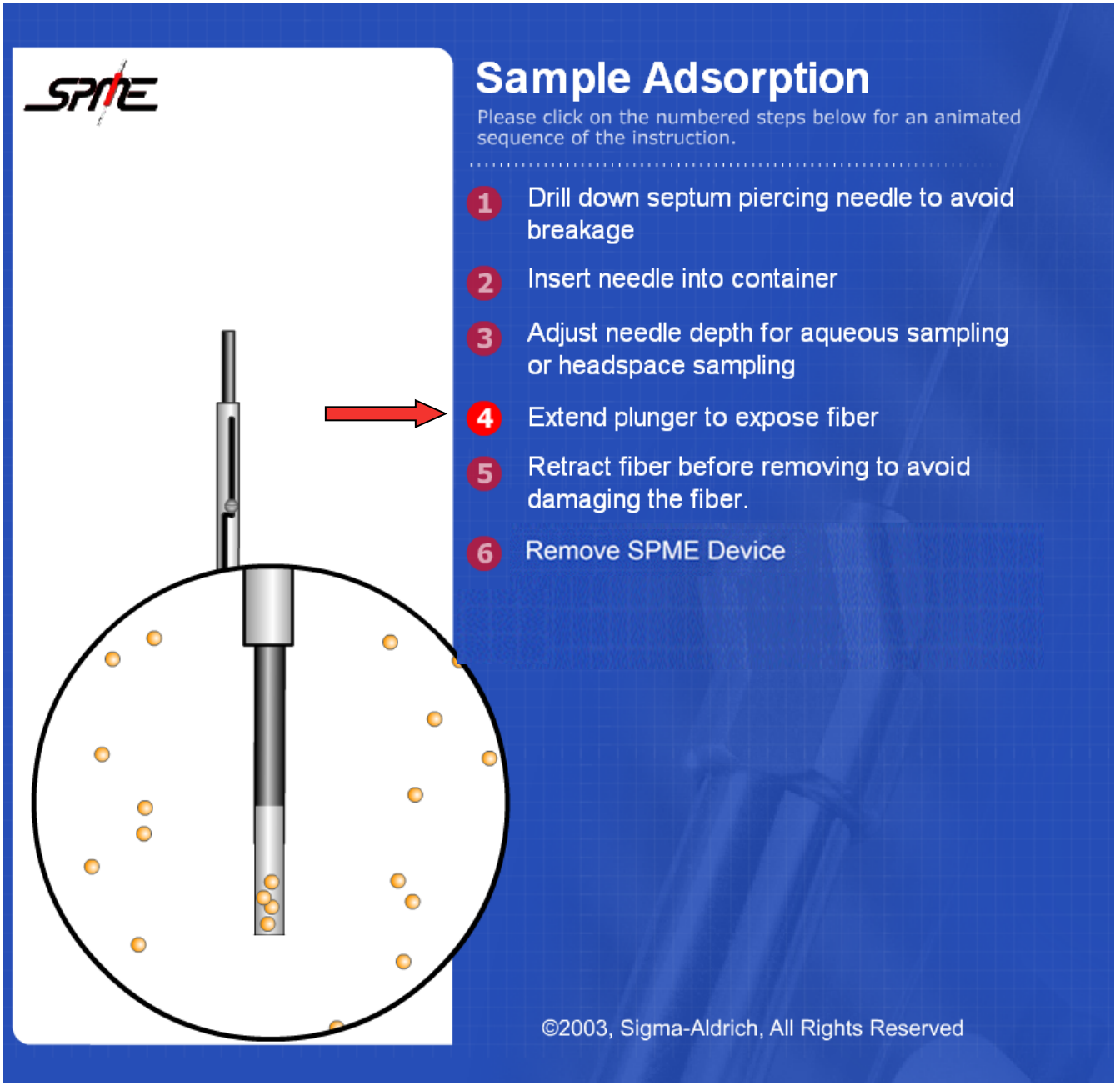
The SPME Concept



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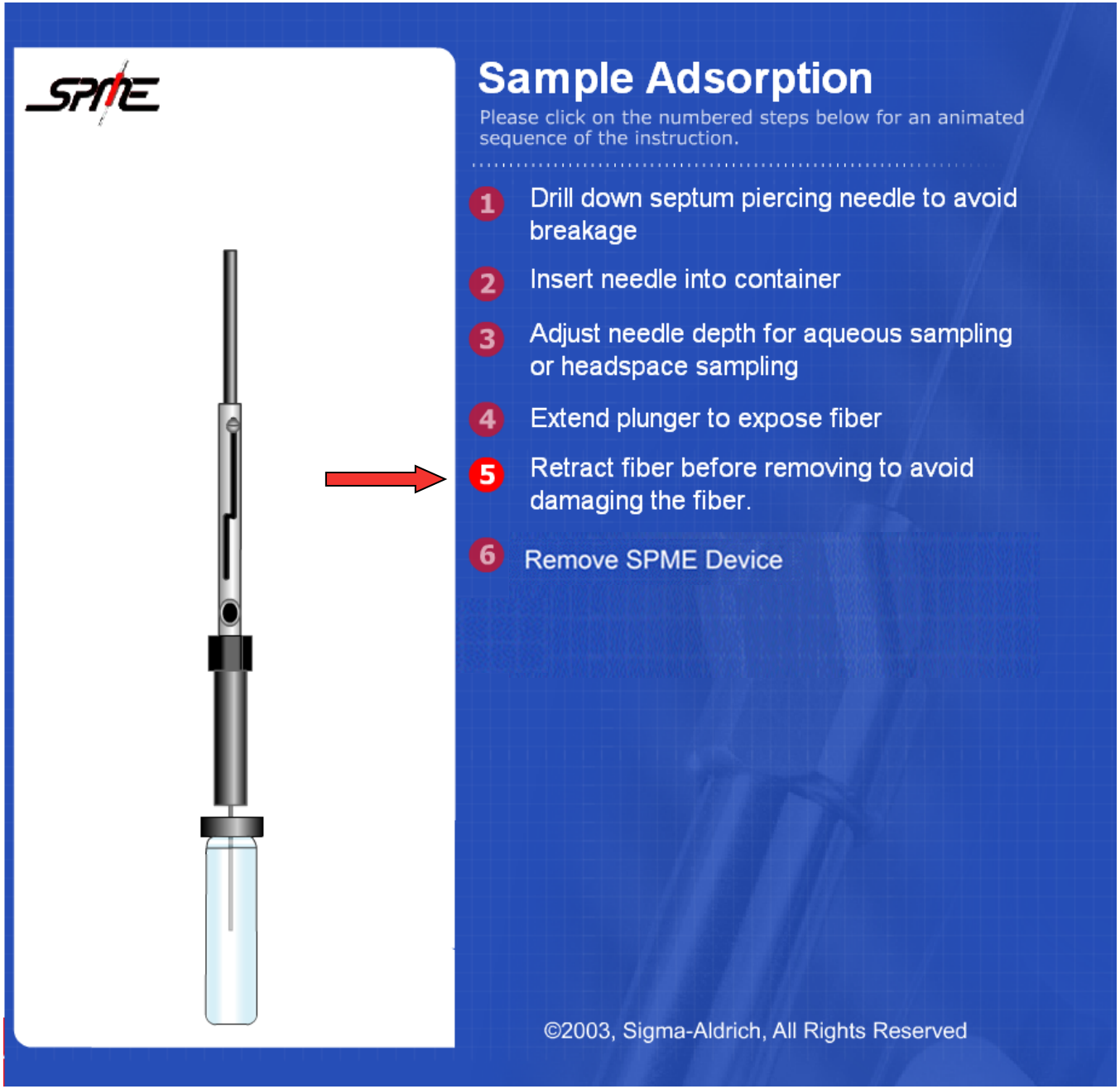
The SPME Concept



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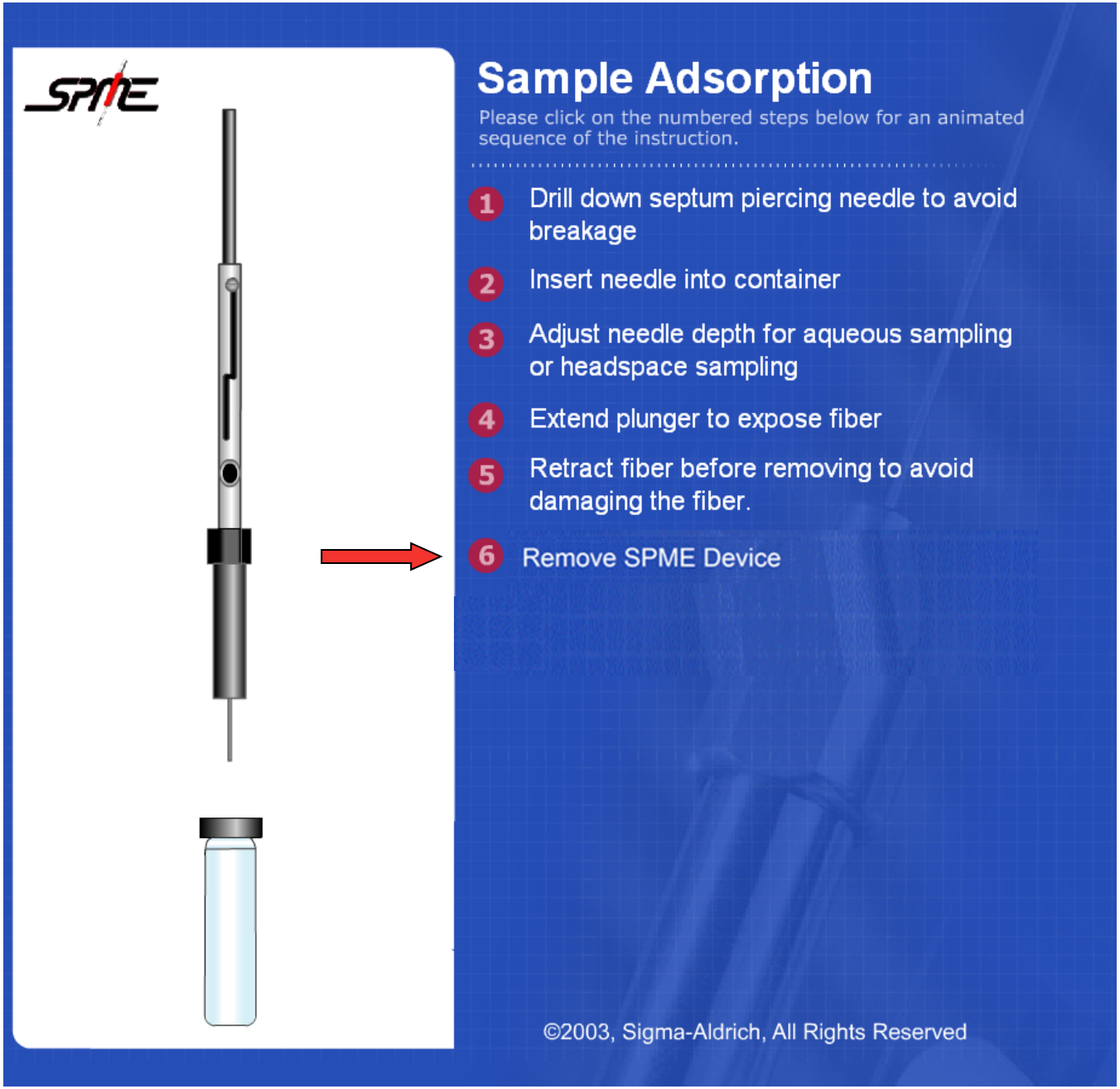
The SPME Concept



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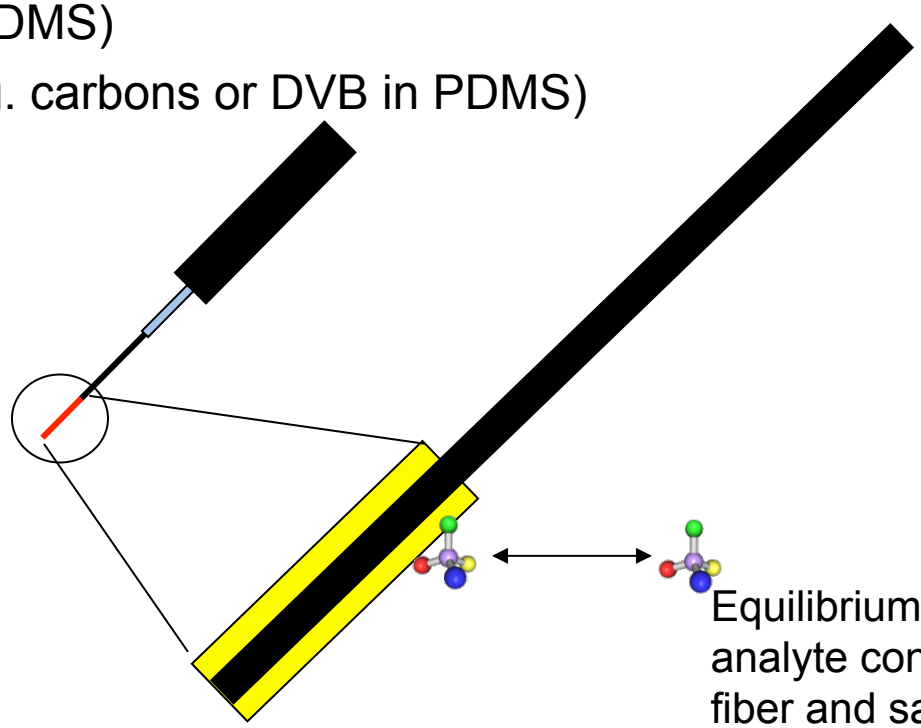
SPME Fiber Coating: The Business End

- Not an exhaustive extraction technique
- An equilibrium is set up between analytes dissolved in the sample (solution or gas phase) and in the liquid coating on the fiber.
- The fiber coatings consist of:
  - Polymer films (e.g. PDMS)
  - Particles + binder (e.g. carbons or DVB in PDMS)



Enlargement of the SPME fiber coating

Equilibrium of analyte conc. in fiber and sample

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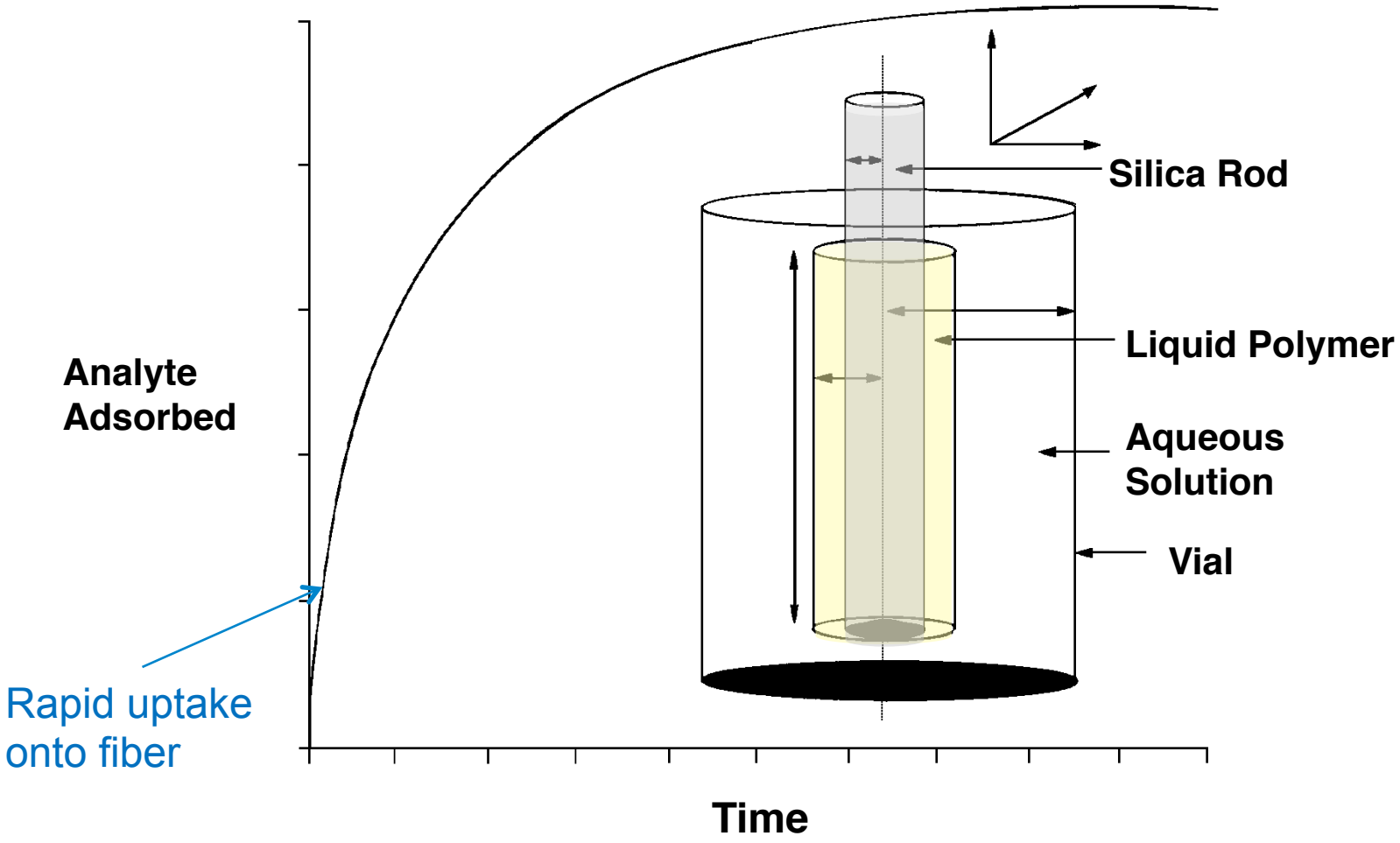
Adsorption Mechanism for SPME



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Number of Moles of Analyte Extracted by Fiber (n)

$$n = (K_{fs} V_f V_s C_0) / (K_{fs} V_f + V_s)$$

K_{fs} = Distribution constant between fiber and sample

C^{∞}_f = Equilibrium concentration on fiber

V_f = Volume of fiber coating

C_0 = Initial concentration of sample

V_s = Sample volume

$$K_{fs} = C^{\infty}_f V_f / C^{\infty}_s V_s$$

K ⟶ affinity of analyte for stationary phase on fiber

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Absorbent vs. Adsorbent Fibers

Absorbent-type fibers

(Film-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

Adsorbent-type fibers

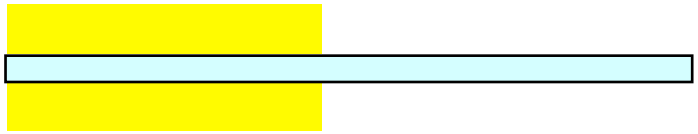
(Particle-type fibers)

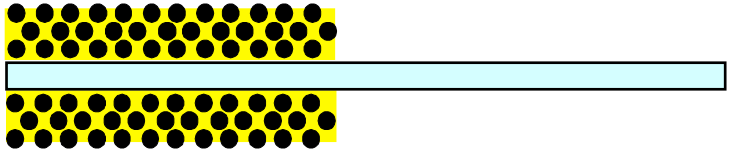
Physically traps or interacts with analytes

- **Porous particles**
- **High surface area**

Analytes may compete for sites

Fibers have limited capacity





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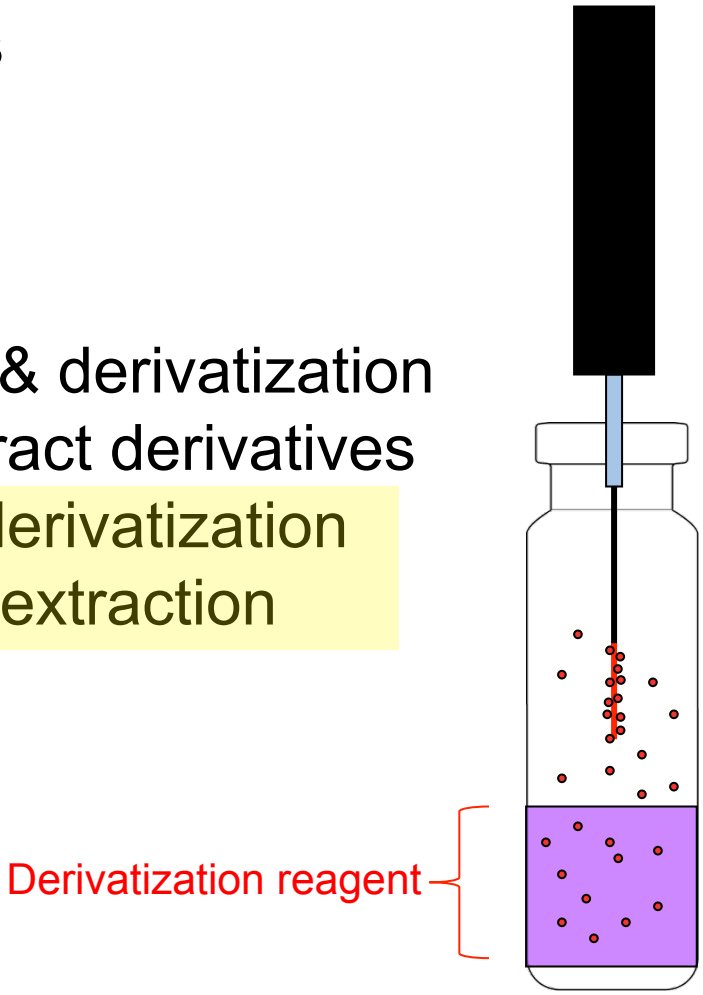
Derivatization & SPME

- Necessary for some compounds
  - Enhance thermal stability
  - Enhance detection

- Three approaches using SPME & derivatization
  1. Derivatize in-matrix and extract derivatives
  2. Simultaneous extraction & derivatization
  3. On-fiber derivatization after extraction

On-fiber derivatization techniques



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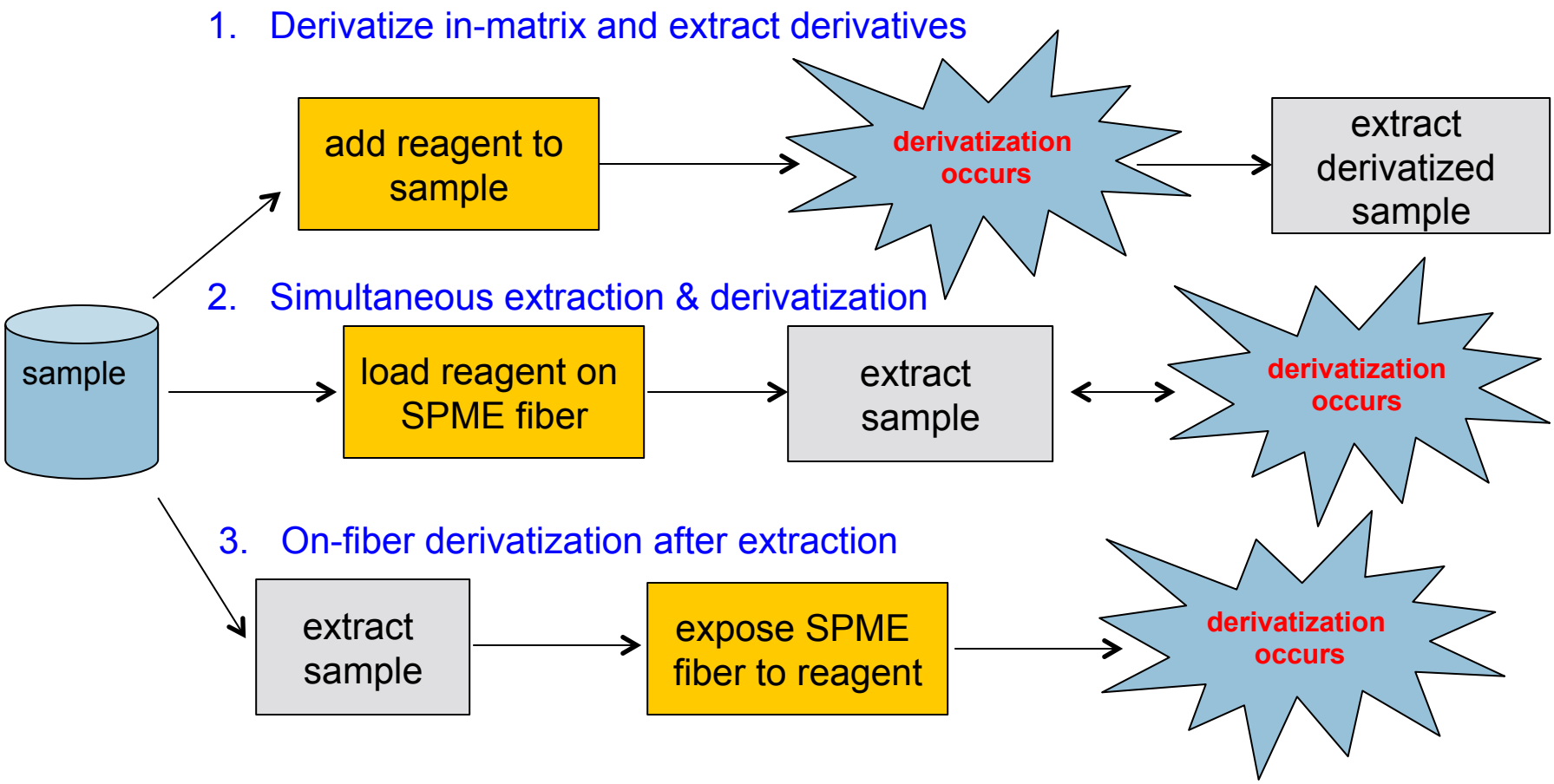
Or, put another way…..
approaches to derivatization with SPME:



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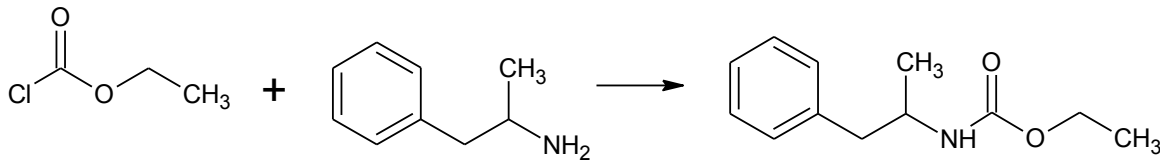
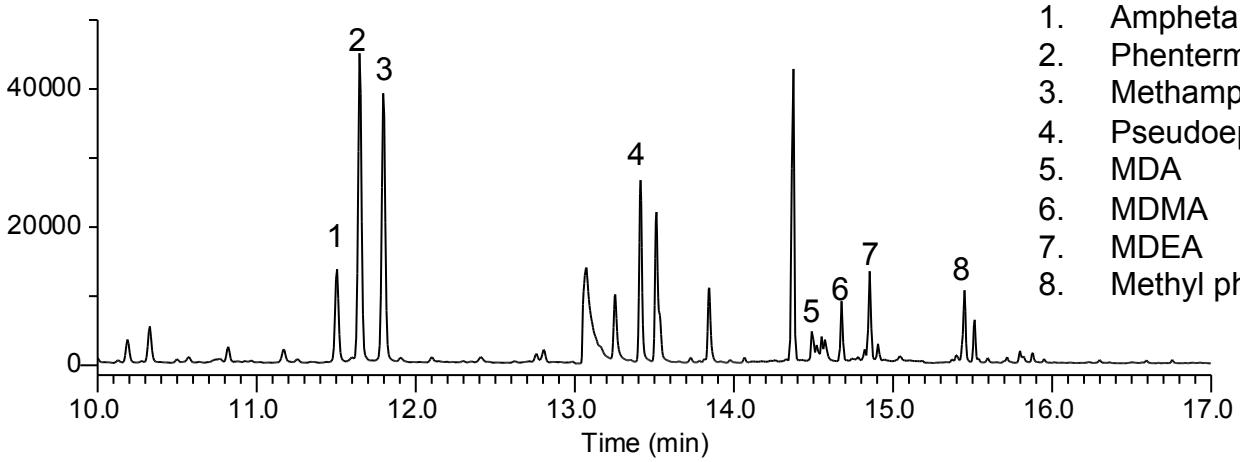
SPME & Derivatization;

Example 1: in-matrix derivatization followed by SPME

- Amphetamines in plasma, 50 ng/L
- Pre-extraction derivatization with ethyl chloroformate
- SPME headspace extraction & GC-MS analysis





1. Amphetamine
2. Phentermine
3. Methamphetamine
4. Pseudoephedrine, Ephedrine
5. MDA
6. MDMA
7. MDEA
8. Methyl phenidate

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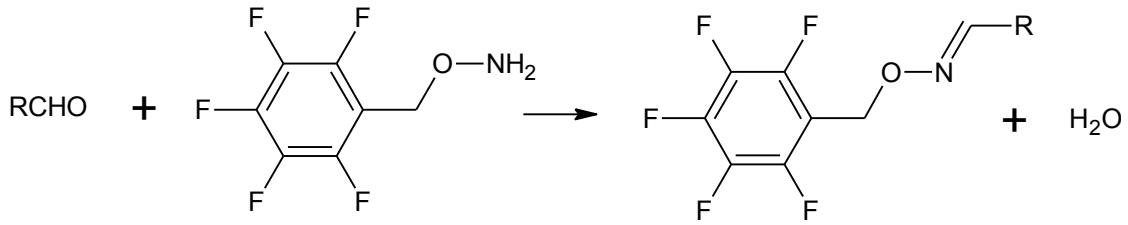
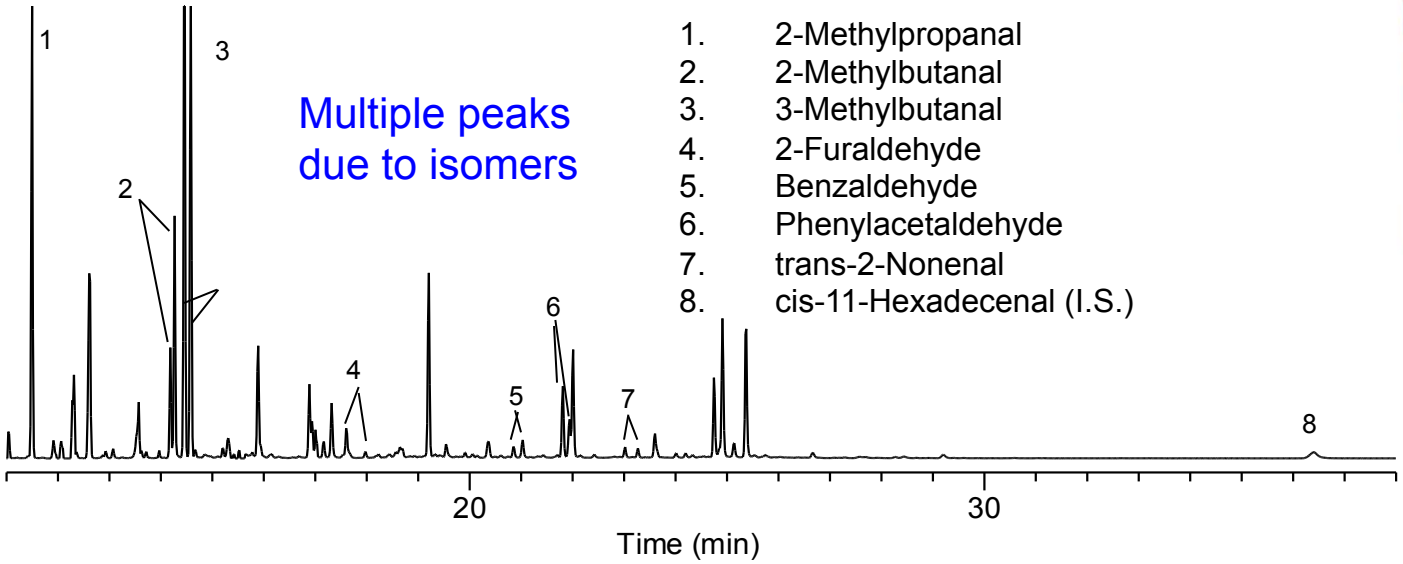
SPME & Derivatization;

Example 2: on-fiber derivatization, reagent loaded pre-extraction

- Analysis of aldehydes in beer
- Load derivatization reagent (PFBOA*) on fiber prior to extraction
- Headspace extraction of aldehdyes and derivatization on PDMS-DVB fiber

*pentafluorobenzyl)hydroxylamine hydrochloride





Multiple peaks due to isomers

1. 2-Methylpropanal
2. 2-Methylbutanal
3. 3-Methylbutanal
4. 2-Furaldehyde
5. Benzaldehyde
6. Phenylacetaldehyde
7. trans-2-Nonenal
8. cis-11-Hexadecenal (I.S.)

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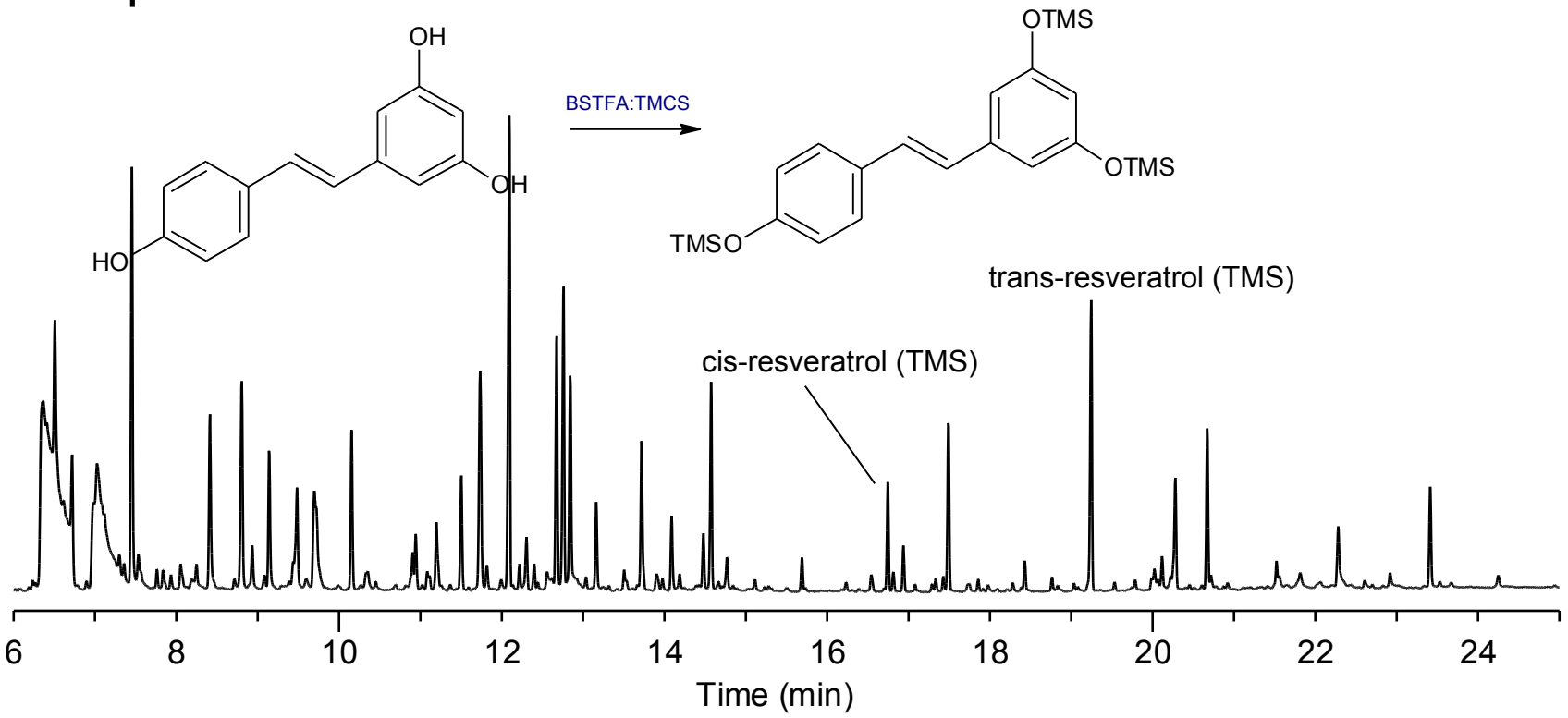
SPME & Derivatization;

Example 3: on-fiber derivatization, reagent loaded post-extraction

- Analysis of resveratrol in red wine
- Extracted resveratrol by immersion, 85 um polyacrylate SPME fiber
- Expose fiber to BSTFA:TMCS after extraction



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Fiber Selection & On-Fiber Derivatization

- Some fiber coatings may swell when exposed to certain derivatization reagents
  - Example: PDMS can swell when exposed to silylating reagents
- PEG fiber could be damaged by derivatization reagents that react with hydroxyl groups
- Best coating choices for on-fiber derivatization:
  - Polyacrylate : resists swelling
  - Adsorptive coatings (DVB, Carboxen)

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Cannabis

- Schedule 1 substance under federal law
  - High potential for abuse
  - No currently accepted medical treatment in the US
- Legal for recreational and medical use in CO, WA, AK, OR.
- Of interest in effluent as part of sewage epidemiology to study drug use.
- Principal psychoactive constituent is **tetrahydrocannabinol (THC).**
  - Identified by chemist Raphael Mechoulam in 1963
- Major metabolite is **11-nor-9-Carboxy-THC (THCCOOH)**

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Why SPME instead of SPE?

- No organic solvents required
- More easily automated
- Less “hands-on” sample preparation time
- Highly sensitive
- Compatible with existing equipment

(i.e. GC/MS)

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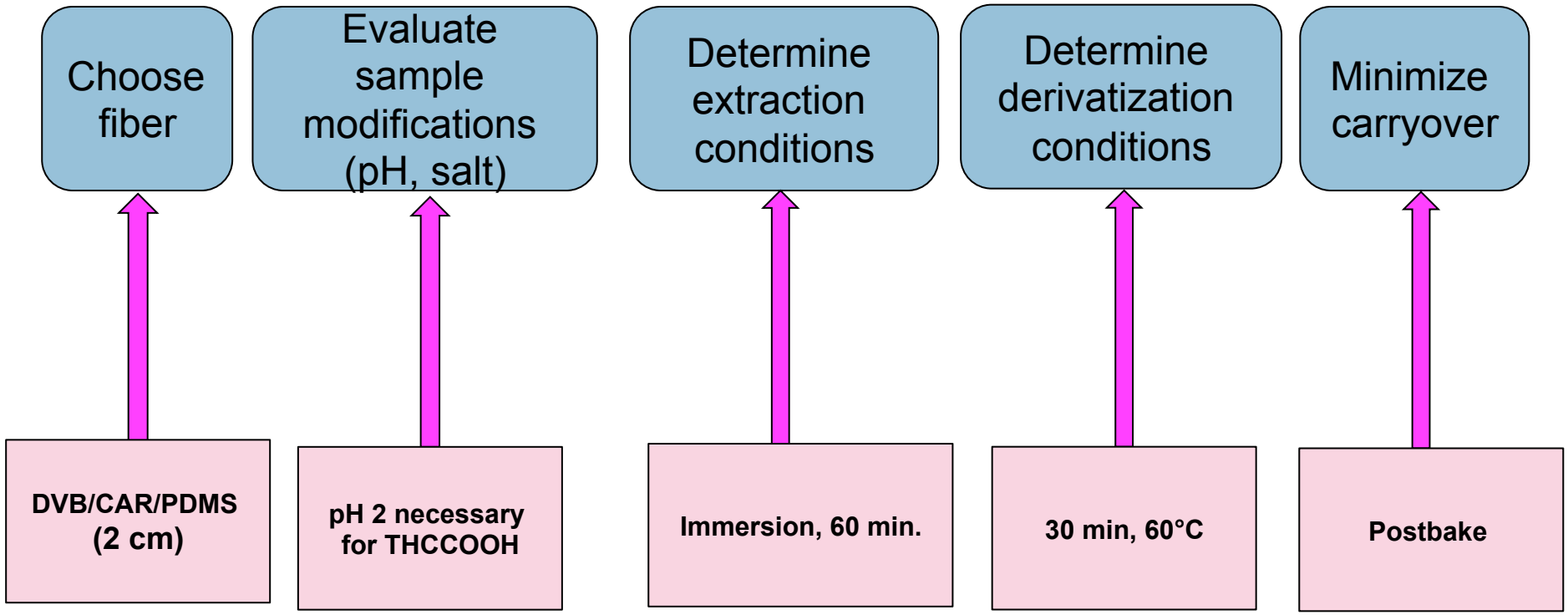
SPME Method Development for THC & THCCOOH

Approach: On-fiber derivatization after extraction



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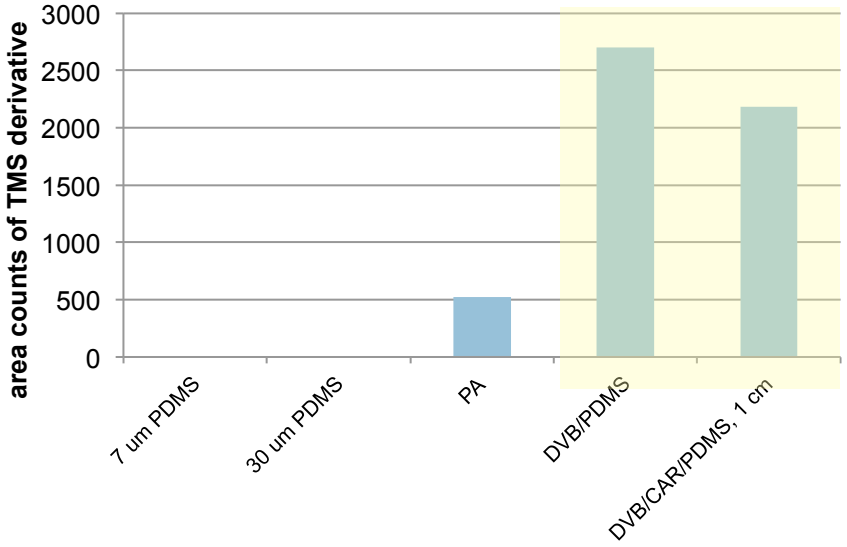
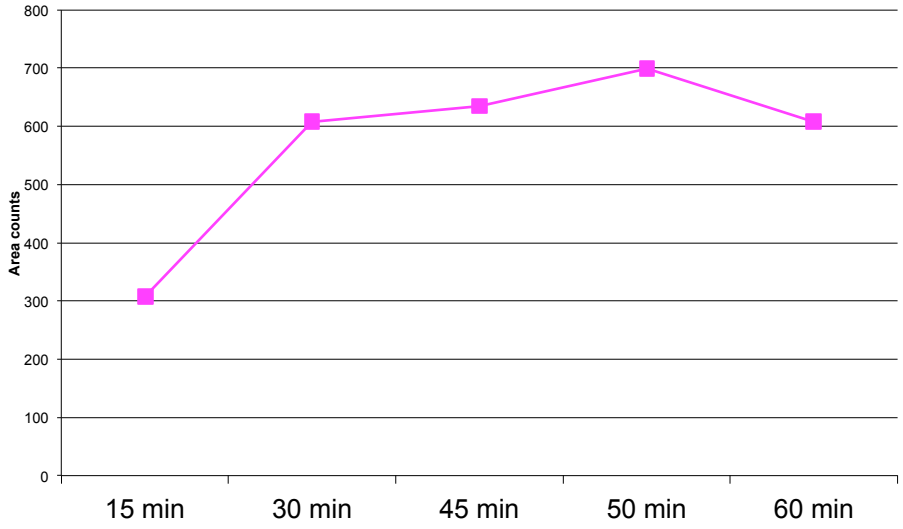
Method Optimization Studies; THCCOOH response



Fiber selection

- Best response using adsorbent fibers
- 2 cm DVB/CAR/PDMS was the final selection



Extraction time

- High K value = long extraction time
- 60 minutes chosen for method

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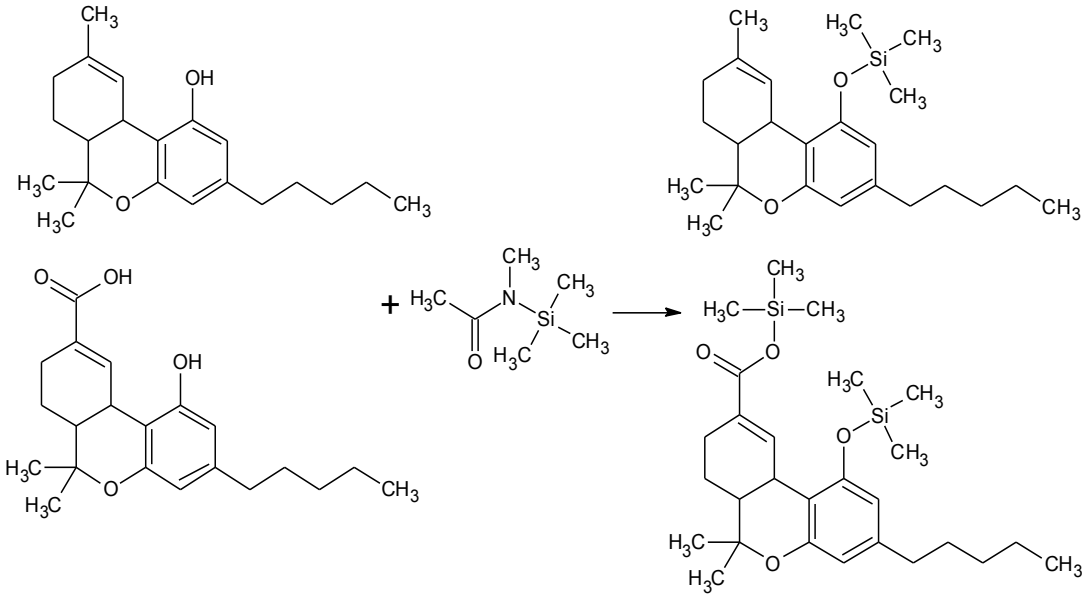
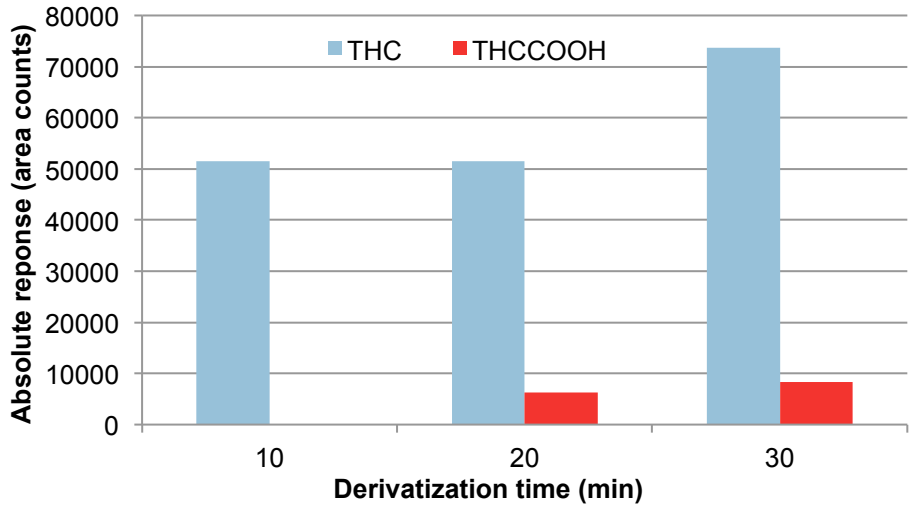
Method Optimization Studies; Derivatization



- Silylation using MSTFA; forms TMS derivatives
- Derivatives are stable
- Water can affect reaction



- Derivatization time limited to 30 min.

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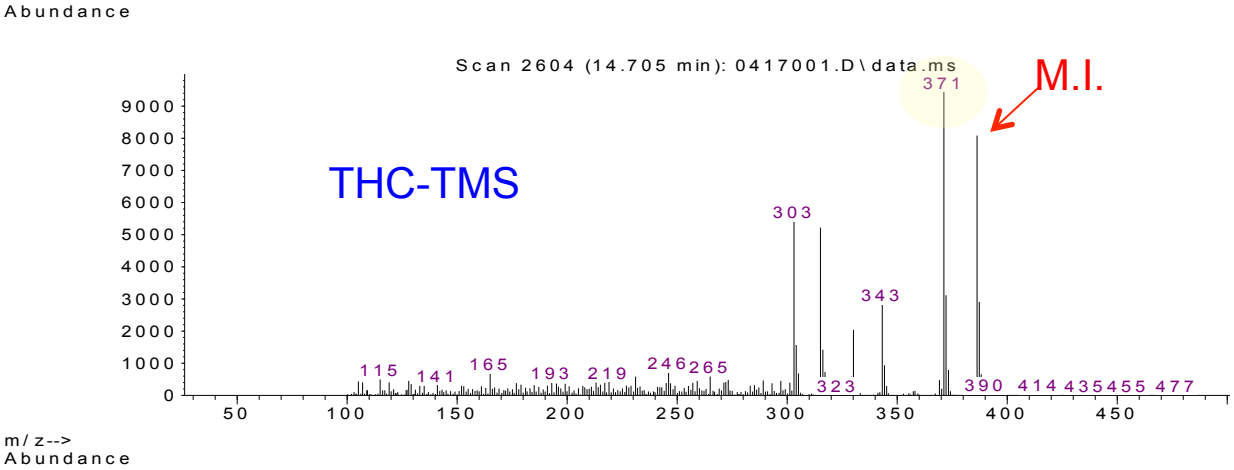
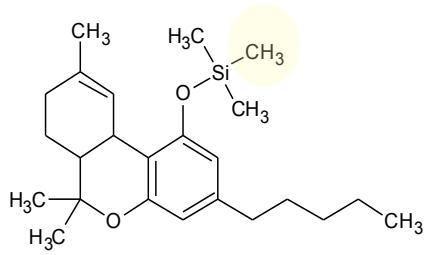
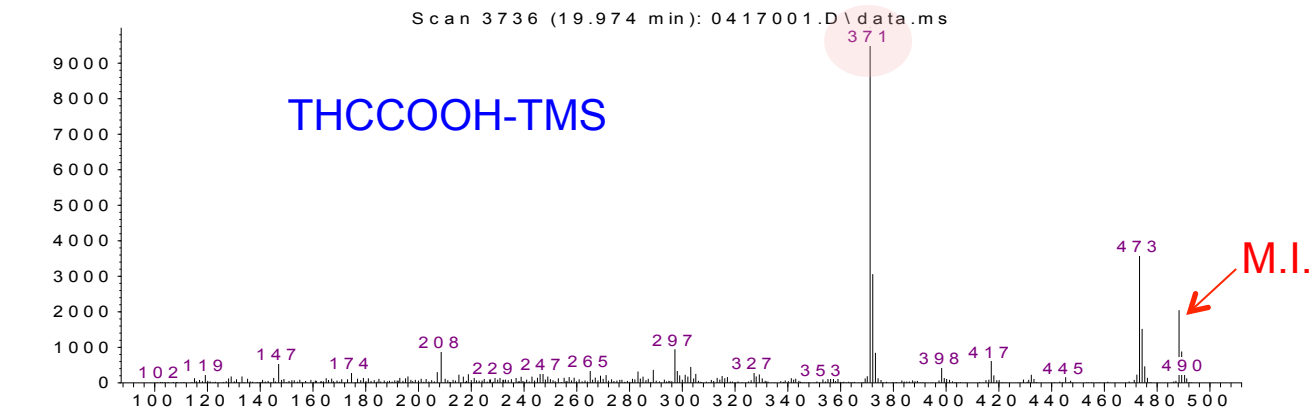
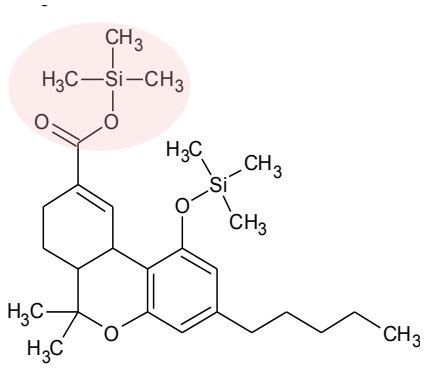
EI Spectra of TMS Derivatives





MW 386





MW 488

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Final SPME method

Fiber: DVB/CAR/PDMS, 2 cm
Sample: 8 mL, pH=2 in 10 mL vial
Extraction: immersion, 60 min w/agitation
Derivatization: 30 min at 60°C, MSTFA (500 µL in 10 mL vial)
Desorption: 260 °C, 3 min
Fiber post-bake: 260°C, 10 min
Analysis: GC-MS/SIM on SLB-5ms (20 x 0.18mm x 0.18µm)

Excess reagent to compensate for water

To reduced carryover:
THC < 0.1%
THCCOOH < 0.01% (usually not detected)

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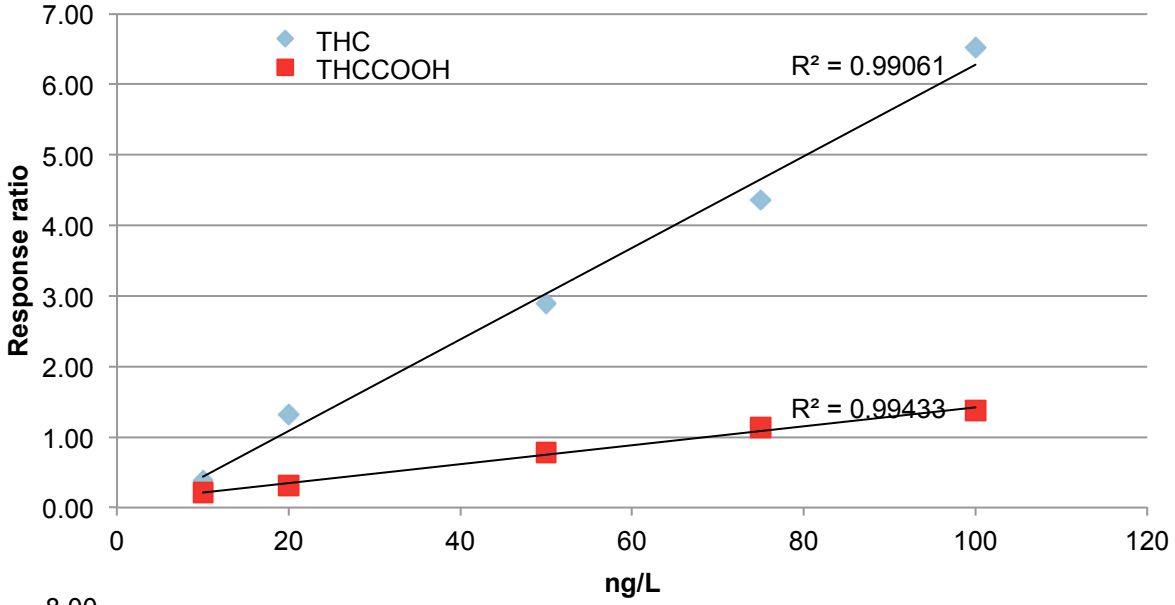
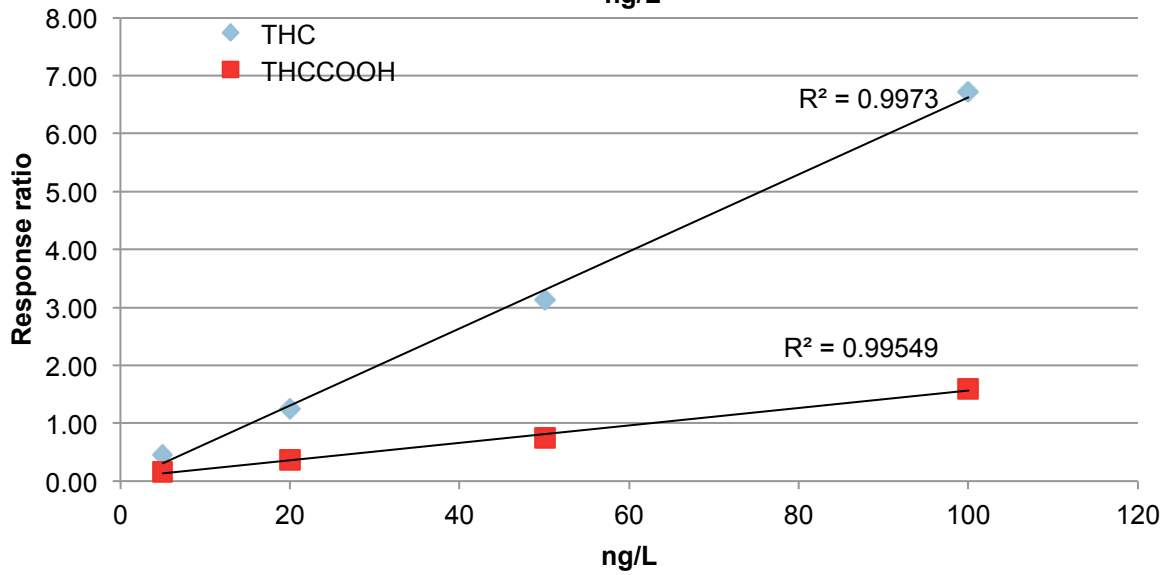
Method Linearity



10-100 ng/L (Feb. 2015)



5-100 ng/L (April 2015)

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Method Detection Level Study

- Deionized water spiked at 10 ng/L
- *n*=8
- Calculated LOD using students t value (99% confidence level) x std. dev. Calculated LOQ using 10 x std. dev.

	Avg. amount measured (ng/L)	Std. Dev. (ng/L)	RSD	Accuracy	LOD (ng/L)	LOQ (ng/L)
THC	9.84	1.12	11%	98%	3.4	11.2
THCCOOH	9.52	1.35	14%	95%	4.0	13.5

RSD = Relative standard deviation
LOD = Limit of detection
LOQ = Limit of quantitation

- Accuracy of > 95% for both compounds
- LODs < 5 ng/L, LOQs < 15 ng/L

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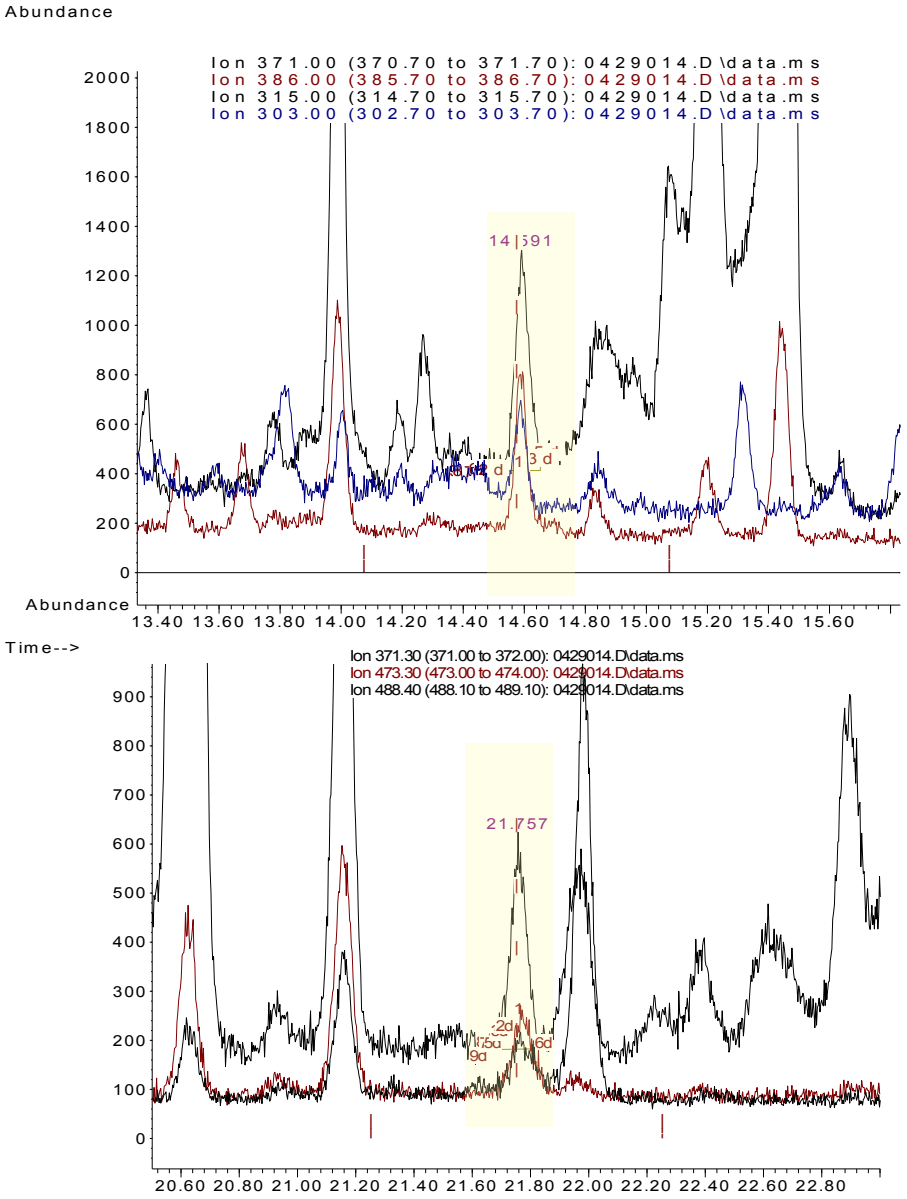
10 ppT THC and THCCOOH in deionized water



THC - TMS derivative

THCCOOH - TMS derivative

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Analysis of Surface Water Samples

- Analyzed 2 sample sets
  1. **Creek** in Colorado; adjacent to discharge point for treated effluent from wastewater treatment plant
  2. **Drainage basin** in State College/University Park, PA
- Samples collected at two sampling spots
- Analyses done in duplicate
- MS/MSD run with each set; prepared from one sample for each.
  - 50 ng/L spiking level for creek water set
  - 10 ng/L spiking level for drainage basin set

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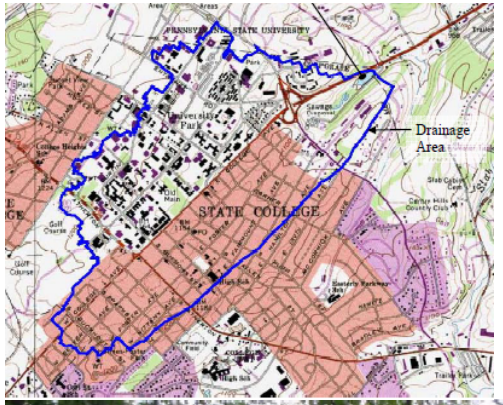
Sampling sites

Drainage area



Source: opp.psu.edu

Source: bouldercolorado.gov

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Results: Drainage Basin Samples

	Site 1 avg. n=2 (ng/L)	RPD	Site 2 avg. n=2 (ng/L)	RPD	Site 1 avg. MS/MSD (ng/L)	Accuracy	RPD
THC	4.1	85%	0	--	9.1*	91%	1%
THCCOOH	0	--	0	--	10.3*	103%	3%

RPD= reproducibility of 2 measurements
MS/MSD = matrix spike and matrix spike duplicate, spiked at 10 ng/L;
**amount reported after sample subtraction*

- THC detected at site 1; just above LOD
- THCCOOH not detected at either site
- MS/MSD good accuracy and reproducibility

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Results: Creek Samples

	Site 1 avg. n=2 (ng/L)	RPD	Site 2 avg. n=2 (ng/L)	RPD	Site 1 avg. MS/MSD (ng/L)	Accuracy	RPD
THC	8.8	2%	7.9	1%	42.0*	84%	5%
THCCOOH	4.5	50%	---		41.5*	83%	5%

**less unspiked*

- THC detected at both sites > LOD but < LOQ; good reproducibility of measurement
- THCCOOH detected at site 1
- MS/MSD good accuracy and reproducibility

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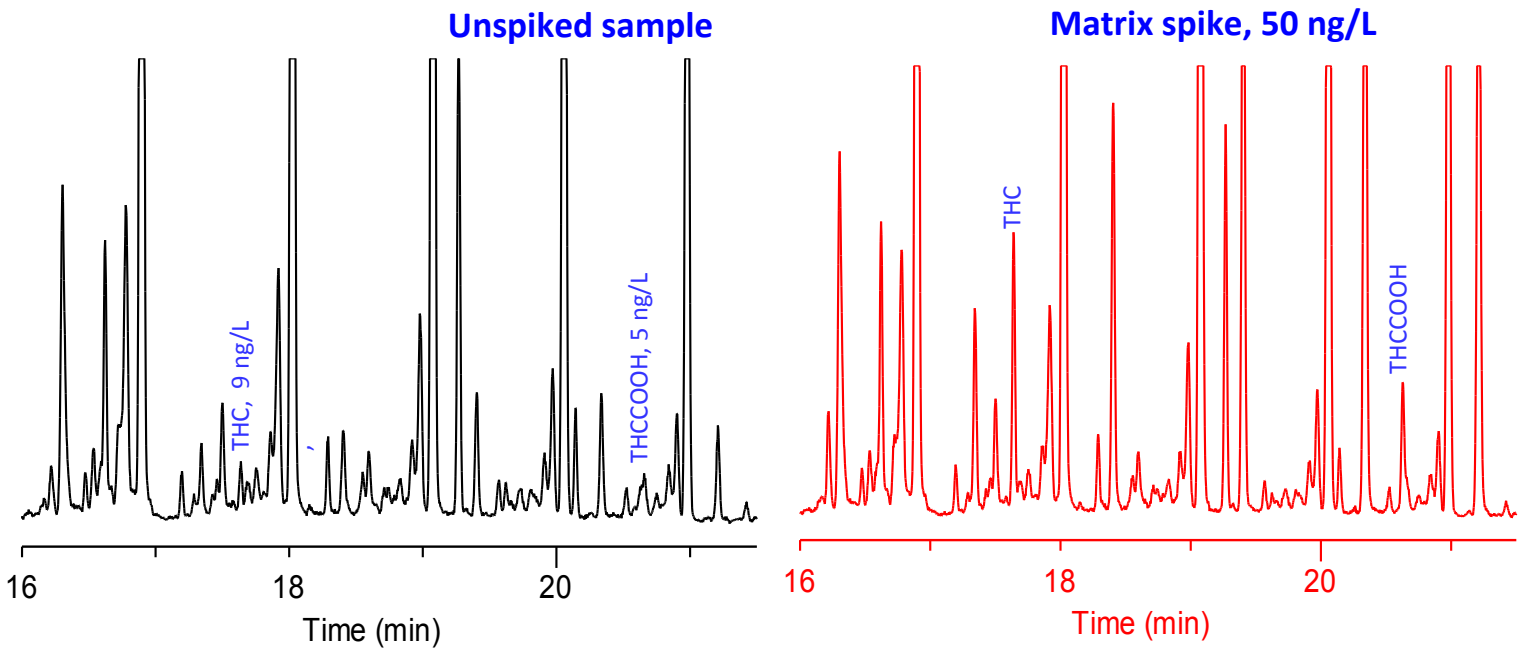
Results: Creek Samples



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Conclusions

- SPME with on-fiber derivatization can be used for simultaneous analysis of THC and the metabolite THCCOOH.
- The detection limit from water was 3-4 ng/L for both compounds with a quantitation limit of 10-15 ng/L. When applied to surface water samples, the method was able to detect both THC and THCCOOH at low ng/L levels.
- The method requires minimal hands-on time, and is cost effective with minimal waste in that it does not require the use of organic solvents or single-use extraction cartridges.
- In addition to THC and THCCOOH, the SPME method could possibly be extended to include additional cannabinoids.

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Acknowledgements

Bob Shirey
Yong Chen
Gary Oishi

Thank you!

Questions?

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