SPME-GC: Not Just for Volatiles Anymore

Nicholas H. Snow, Department of Chemistry and Biochemistry, Center for Academic Industry Partnership, Seton Hall University, South Orange, NJ 07079, nicholas.snow@shu.edu
SPME in 2010

• 20 years of SPME
  – Some “inside” stories
  – Fundamentals

• SPME works for semi- and non-volatiles
  – Drugs and preservatives: SPME-IMS
  – Headspace?? GCxGC-ToFMS??
  – Post-extraction Derivatization
    • Drugs: steroids, estrogens, challenges

• Discussion and ideas
Solid phase micro extraction

- SPME device consists of a silica fiber coated with phase material. The fiber is mounted into a microsyringe, which protects the fiber.

- Coating on the fiber is non-volatile polymeric phase, e.g. PDMS, PA.

- An equilibrium technique - analyte is distributed between the fiber coating and sample.

- After the extraction concentrated analytes are desorbed into the analytical instrument for separation and quantitation.
Solid phase microextraction (SPME)

- Fiber holder for automated sampling/HPLC
- Fiber holder for manual sampling
- Portable field sampler
- Plunger
- Barrel
- Z-slot
- Adjustable Needle
- Sealing Septum
- Septum Piercing Needle
- Coated Fused Silica Fiber
Solid phase micro extraction

SPME procedures for:
A. direct ext.
B. headspace ext.

The distribution coefficient determines the amount of analyte that is extracted.

\[ n = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + V_s} \quad K_{fs} V_f \ll V \quad n = K_{fs} V_f C_0 \]
DIRECT ABSORPTION
“LIQUID-LIQUID” EXTRACTION

- Thermodynamics, $K$
- Kinetics, $D$
- pH
- Temperature
- Fiber, Sample and Headspace Volume
- Agitation
HEADSPACE ABSORPTION

- Thermodynamics, $K$
- Kinetics, $D$
- pH
- Temperature
- Fiber, Sample and Headspace Volume
- Agitation
Effect of Incubation Temperature
Effect of Incubation Temperature

Incubation Temperature Effect

peak height

toluene  | chlorobenzene  | ethylbenzene  | m-xylene  | o-xylene

60°C  | 70°C  | 80°C

0  | 1000  | 2000  | 3000  | 4000  | 5000  | 6000  | 7000  | 8000

compound
DESORPTION
GC INJECTION

• Expose Fiber
• Hot Injector
• Fiber --->
  Mobile Phase
  GC Column
• Splitless, Direct or
  On-column
Fiber Position

- Maximum liquid velocity
- Protects against water in the needle
- Small headspace maintained in vial

SPME Trace Analysis

400 part per trillion
toluene, ethylbenzene, o-xylene, p-xylene

extraction: 3 mL, 30 min, direct
desorption: 220°C, 5 min

Column: 30m x 0.32mm x 1 mm SPB-1, 40°C (5min), 10°C/min.
Detector: FID, 250°C.
Conditions: 400 ppb each; Liner: 4mm; Fiber: 100mm PDMS; Tinj: 220°C; TP: 0°C/5min; 10°C/min; Desorb: 5 min.
Column: 15m by 0.25 mm x 0.25µm DB-5.

SPME-IMS Injection/Desorption Procedure

The direct coupling of SPME to IMS has not been optimized in the past to quantitate multiple analytes in complex matrices.

- The exposed fiber is placed on the desorption tray in the center of the sampling region.
- The desorber rises, sealing the SPME fiber against the heated IMS inlet.
- Air is drawn through the sampling region to transfer the sample into the IMS detector.

Extraction profile of ephedrine in water and urine, sampled directly by five SPME fibers.
Extraction time profile ephedrine by SPME

Ephedrine response as a function of sample temperature
Ephedrine response as a function of sample pH
A 2-D Plasmagram of ephedrine spiked in water
A 2-D plasmagram of ephedrine in urine sample

Mode: Positive; Desorber temperature: 260°C; Inlet Temperature: 260°C; Drift Tube Temperature: 230°C; Flow Rate: 300 cc/min
Desorption time: 30 s; Spectra collection delay: 1ms; Shutter grid width of 0.2 ms
Parabens

- A paraben mix - four different paraben esters: methyl-, ethyl-, propyl-, butyl-parahydroxybenzoic acids.
- Exhibits antimicrobial characteristics
- Parabens are the most commonly used preservatives in topical pharmaceutical preparations.

Uses:
- Cosmetics
- Skin care products – OTC and Rx
- Foods
- Industrially in oils, fats, shoe polishes, textiles
- Oral dosage pharmaceuticals
A plasmagram of a standard solution spiked with MP, EP, PP, BP

Plasmagram shows sharp Gaussian-shaped peaks. Separation for the five parabens achieved in less than 16 ms.

A plot of reduced mobilities vs. molecular weight for the MP, EP, PP and BP ions

The mass-mobility correlation exhibits an $r^2$ of 0.996 for the parabens.
Application of SPME/IMS Method to Real Samples

Products containing various combinations of parabens were tested by the SPME/IMS using external standards.

- Cream A
- Cream B
- Cream C
- Solution A
- Lotion A
- Ointment A

Duplicate samples were tested and the mean results calculated in mg/g.

Samples tested by an HPLC method for comparison
- injection volume 25 μL
- mobile phase – 50% acetonitrile; Flow rate – 1.5 mL/min
- column – C$_{18}$ 5μm 4.6 x 250mm
- detection - UV 254 nm
A plasmagram of a standard solution spiked with MP, EP, PP, BP and benzyl paraben as an internal standard.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Drift time (ms)</th>
<th>Reduced Mobility cm²V⁻¹s⁻¹</th>
<th>Resolution</th>
<th>Theoretical Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl paraben</td>
<td>12.746</td>
<td>1.4412</td>
<td>N/A</td>
<td>11163</td>
</tr>
<tr>
<td>Ethyl paraben</td>
<td>13.538</td>
<td>1.3569</td>
<td>1.09</td>
<td>11778</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>14.295</td>
<td>1.285</td>
<td>1.04</td>
<td>13023</td>
</tr>
<tr>
<td>Butyl paraben</td>
<td>15.053</td>
<td>1.2204</td>
<td>1.00</td>
<td>11342</td>
</tr>
<tr>
<td>Benzyl paraben</td>
<td>16.140</td>
<td>1.1382</td>
<td>1.31</td>
<td>12035</td>
</tr>
</tbody>
</table>

Contents of MP, EP, PP and BP determined in commercial topical formulations determined by SPME-IMS quantitated using an internal standard. Results are compared with to HPLC data.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Preservatives</th>
<th>Quantitation by HPLC (mg/g)</th>
<th>Quantitation by SPME/IMS (mg/g)</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream A</td>
<td>Methyl paraben</td>
<td>0.98</td>
<td>1.00</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Propyl paraben</td>
<td>0.27</td>
<td>0.24</td>
<td>11.1</td>
</tr>
<tr>
<td>Cream B</td>
<td>Methyl paraben</td>
<td>1.98</td>
<td>1.90</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Propyl paraben</td>
<td>0.21</td>
<td>0.19</td>
<td>9.5</td>
</tr>
<tr>
<td>Cream C</td>
<td>Methyl paraben</td>
<td>1.92</td>
<td>2.17</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>Ethyl paraben</td>
<td>0.41</td>
<td>0.37</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>Propyl paraben</td>
<td>0.23</td>
<td>0.20</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>Butyl paraben</td>
<td>0.45</td>
<td>0.50</td>
<td>11.1</td>
</tr>
<tr>
<td>Lotion A</td>
<td>Methyl paraben</td>
<td>1.42</td>
<td>1.44</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Propyl paraben</td>
<td>0.21</td>
<td>0.18</td>
<td>14.3</td>
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<tr>
<td>Solution A</td>
<td>Methyl paraben</td>
<td>1.65</td>
<td>1.68</td>
<td>1.8</td>
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<tr>
<td>Ointment A</td>
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</table>
SPME-GCxGC-TOFMS

Fiber: 100µm PDMS, Direct Immersion, 30 min.
Desorb: 250°C, 2 min, 2mm liner

TP(1): 35°C, hold 1 min,
3°C/min to 65°C then 10°C/min

TP(2): 40°C, hold 1 min,
3°C/min to 70°C then 10°C/min

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The “Difficult Six”

• Class 2 residual solvents not readily detected by the headspace injection conditions:

  – formamide: 11.3-ppm
  – 2-ethoxyethanol: 8.1-ppm
  – 2-methoxyethanol: 2.5-ppm
  – ethylene glycol: 30.9-ppm
  – N-methylpyrrolidone: 26.6-ppm
  – sulfolane: 8.2-ppm
The “Difficult Six”

1. n-methyl pyrolidone
2. 2-methoxyethanol
3. 2-ethoxyethanol
4. DMSO (diluent)
5. ethylene glycol
6. formamide
7. sulfolane

Agilent 5890-5972 GCMS
HP Innowax Column, 30m x 0.25mm x 0.25μm
Carboxen/PDMS fiber, 30 min
HS extraction at 60°C
SPME-GC of Caffeine

Starbucks

Seton Hall

Acknowledgment: Katie Andreski, SHU Class of 2010
SPME DERIVATIZATION: A SIMPLE THREE STEP PROCEDURE

Extract - Direct, 30 min

Derivatize - Headspace, 1 hr.

Inject - Splitless, 280°C
RESULTS - 17-β-ESTRADIOL
TOTAL ION CHROMATOGRAM

bistrimethylsilyl 17-β-estradiol

RESULTS - STEROID MIXTURE

Steroids- TMS Derivatives
1. estrone
2. 17-β-estradiol
3. endogenous
4. norethisterone
5. ethinyl estradiol
6. ethinyl estratriol

SPME of Estrogens

SPME of Estrogens

Analysis of a single contraceptive tablet. Peak identification: 1. syn-norgestimate-TMS. 2. anti-norgestimate-TMS.

Higher temperatures favored formation of the di-TMS product.
Derivatization Time

Derivatization Time Effect

Time (min)
Derivatization
Time/Temperature

Recovery losses noted
Chromatogram of
second blank fiber in
90°C derivatization for 1
hour

Mono and di-derivatives
present
Degradation products of diphenhydramine

SPME/GC-MS SCHEME FOR DIPHENHYDRAMINE HCl DEGRADATION STUDY

Diphenhydramine Hydrochloride (DPH)

1000 ppm in MeOH/pH 2 & pH 12 Buffer (50/50)
Reflex 1 - 5 hrs.

1/100 Dilution (10 ppm DPH)
Adjust to pH 2 and pH 7

Acidic (pH 2) and Neutral (pH 7)
Degr. Products

Direct SPME PDMS/DVB
Polyacrylate CAR/DVB

in situ GC-MS
On-Fiber BSTFA Derivatization
GC-MS

1/10 Dilution (100 ppm DPH)
Adjust to pH 13

Basic (e.g. Amines)
Degradation Products

Headspace SPME 80C 15 min PDMS/DVB

in situ GC-MS
On-Fiber BSTFA Derivatization
GC-MS

250 mg in 10 mL Sealed Vial
170C/20 min.

2 mg in 2 mL Sealed Vial

Headspace SPME 60C 15 min PDMS/DVB

in situ GC-MS
On-Fiber BSTFA Derivatization
GC-MS

1000 ppm in MeOH/pH 2 & pH 12 Buffer (50/50)
Reflex 1 - 5 hrs.

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1000 ppm in MeOH/pH 2 & pH 12 Buffer (50/50)
Reflex 1 - 5 hrs.
SPME-GC/MS of diphenhydramine and degradants
SPME-GC/MS with derivatization

(A)

(B)

Average of 5.494 to 5.592 min. 1 DPAEDS: D (−)

Chemical structure:

\[ \text{CH}_3\text{SiO-CH}_2\text{CH}_2\text{NCH}_3 \]
SPME-derivatization applications

- Estrogens and steroids
- Amphetamines
- Fatty acids
- Haloacetic acids
- Low molecular weight aldehydes and ketones
- Formaldehyde
- Bisphenol A
- Herbicides
SPME in 2010 - Conclusions

• 20 years of SPME
  – Some “inside” stories
  – Fundamentals

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