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Analysis of Flavors and Off-Flavors in Foods and Beverages Using SPME

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Introduction

SPME is a convenient, solventless extraction technique that can be used to extract analytes from both liquid and solid matrices. The use of SPME for the analysis of flavors and off-flavors in food and beverages is important.

In this presentation, sample types such as non-alcoholic and alcoholic beverages, candy, and fruits are analyzed for flavor composition. The detection of off-flavors from rancid oils and fats and methods for quantifying pyrazines in peanut butter, and caffeine in coffee are presented. The ability to detect trace (low ppt) levels of odors in water is also shown. Background information concerning the fiber types typically used for these analyses is given along with guidelines on how to select the appropriate fiber for a wide variety of applications.

Fig. 1 - Extraction Procedure for SPME

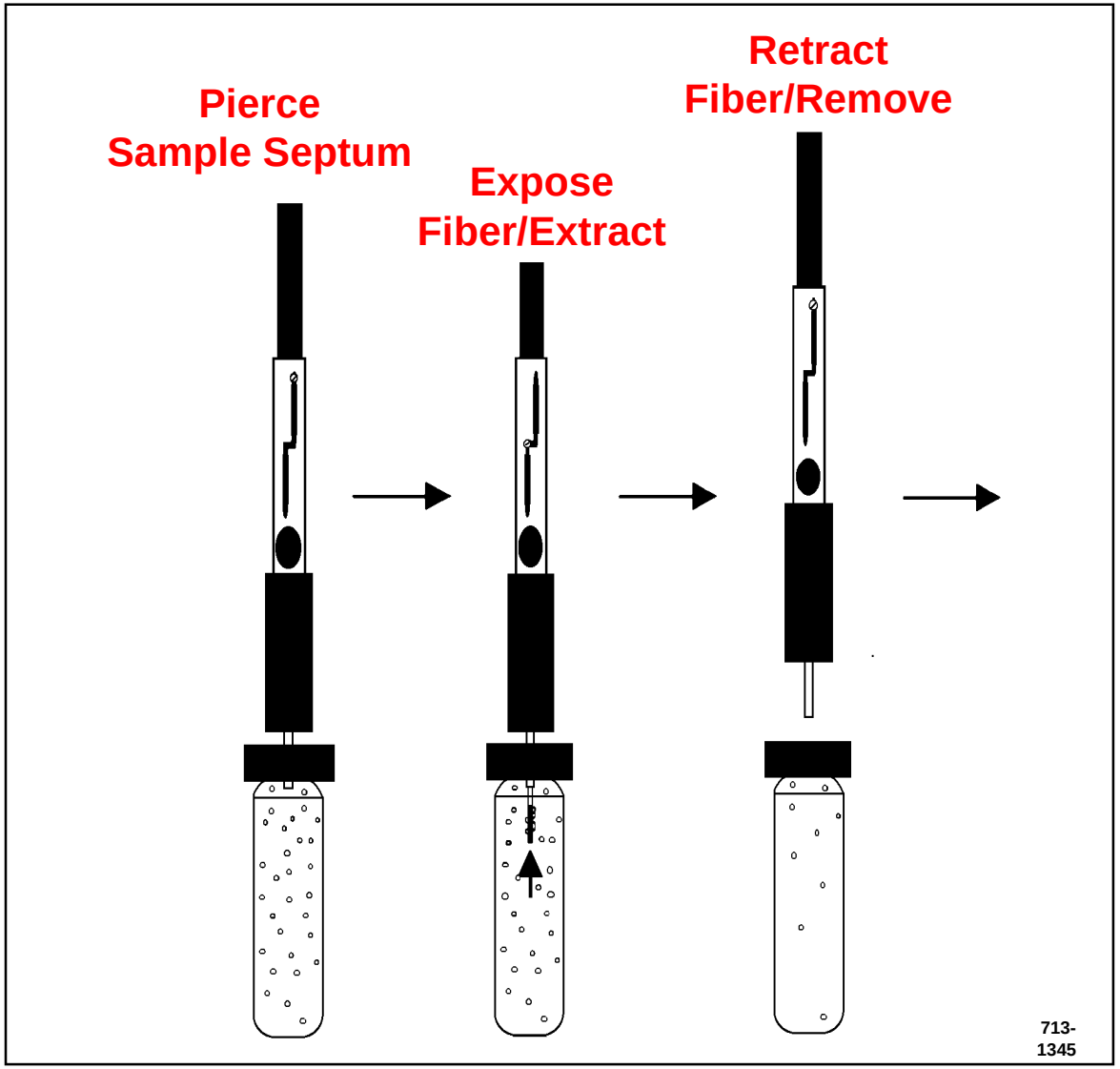
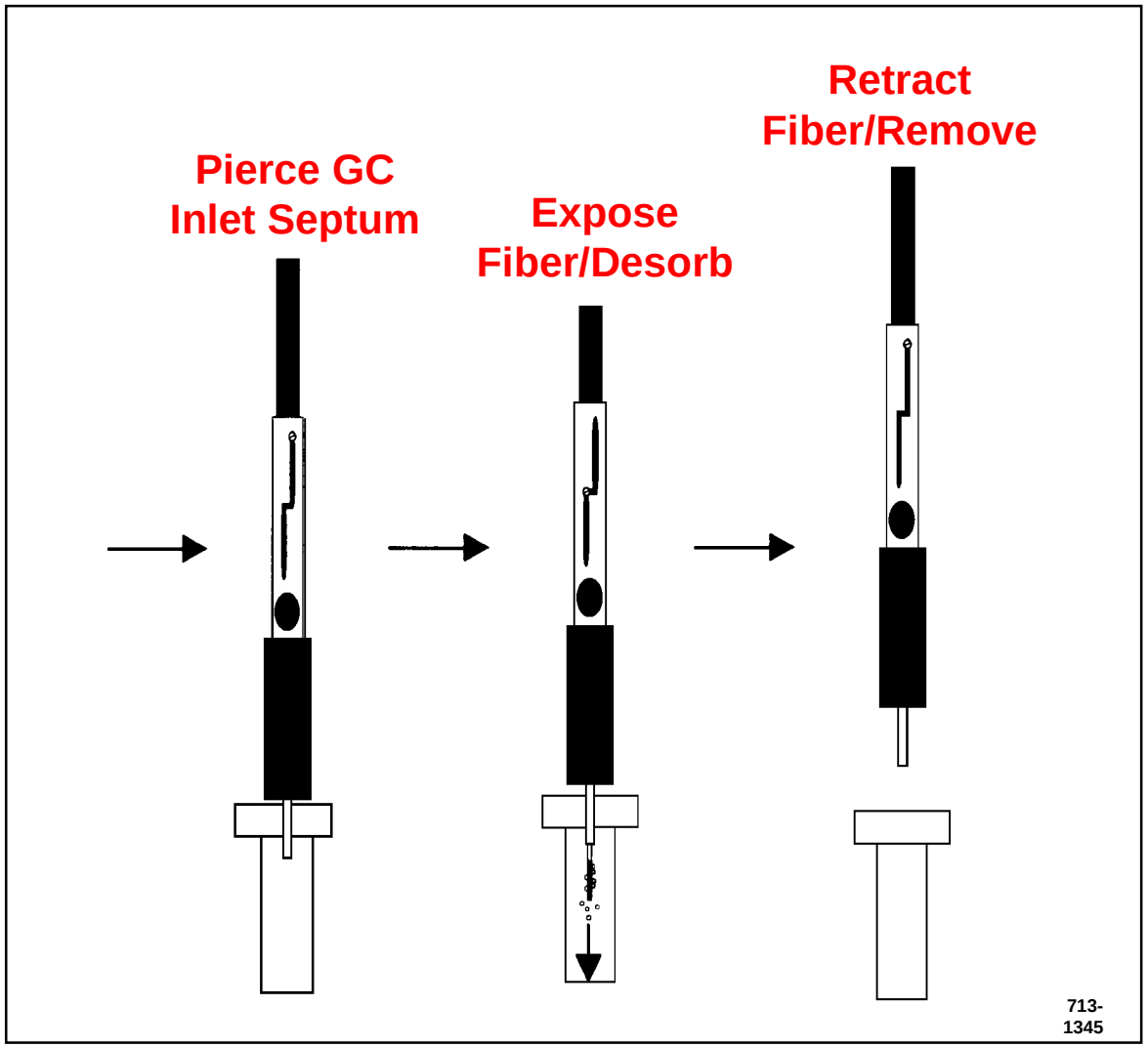


Fig. 2 - Desorption Procedure for SPME



Available SPME Fiber Coatings

Non-Polar Fibers

Polydimethylsiloxane (PDMS): 100µm, 30µm, 7µm

Polar Fibers

85µm Polyacrylate
65µm Carbowax®-divinylbenzene (CW-DVB)
50µm CW-Templated resin (CW-TPR) – HPLC only

Bi-Polar Fibers

65µm PDMS-DVB
65µm PDMS-DVB StableFlex™
75µm Carboxen™-PDMS
50/30µm DVB-Carboxen-PDMS StableFlex

Fibers for the Analysis of Flavors and Fragrance

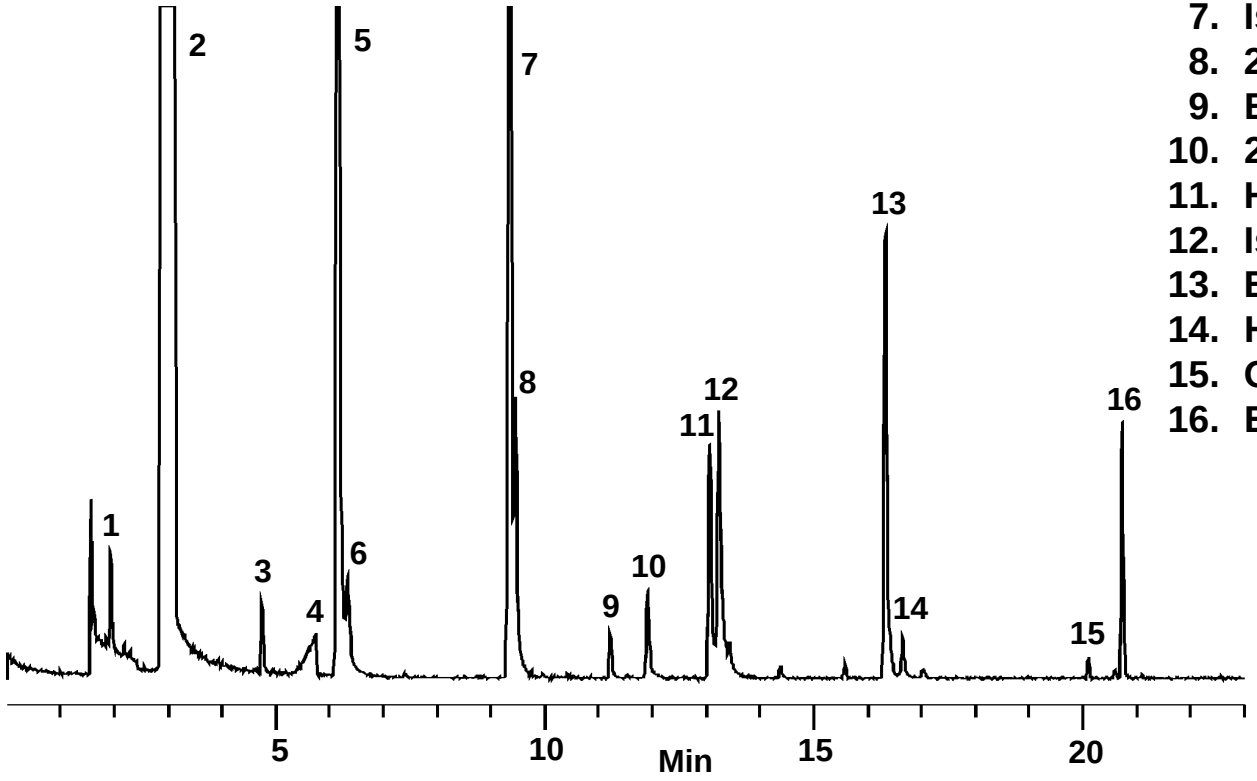
Fiber Types	Types of Analytes	Concentration Range
75µm Carboxen-PDMS	most gases, volatiles	low ppt to high ppb
50/30µm DVB-Carboxen-PDMS	some gases, volatiles and semivolatiles	low ppt to high ppb
65µm PDMS-DVB	volatiles and semivolatiles	high ppt to low ppm
100µm PDMS	volatiles and semivolatiles	low ppb to high ppm
65µm Carbowax-DVB	volatile free acids, polar oxygenates	mid ppt to mid ppm

Comparison of SPME to other Extraction Techniques

Extraction Technique	Types of Analytes and Matrix	Conc.	Range	Ease of use
Static headspace analyzer	gases, volatiles & some semivolatiles, liquids & solids	wide	high	medium
Dynamic headspace P&T	some gases, volatiles liquids only	narrow	high	easy
SPE	semivolatiles liquids only	wide	low	hard
SPME	some gases, volatiles &semivolatiles liquids and solids	wide	low	easy

Fig. 3 - Volatiles in White Wine by GC/MS Using SPME

Sample: white wine + 25% NaCl
SPME Fiber: Carboxen™/PDMS
Extraction: 10 min headspace, 40°C
Desorption: 3 min at 290°C
Column: VOCOL™, 30m x 0.25mm ID, 1.5µm
Detector: GC/MS, Quadrapole, m/z = 31-240



1. Sulfur dioxide
2. Ethanol
3. Methyl formate
4. Acetic acid
5. Ethyl acetate
6. Isobutanol
7. Isopentanol
8. 2-Methyl-1-butanol
9. Ethyl butyrate
10. 2,3-Butanediol
11. Hexanol
12. Isoamyl acetate
13. Ethyl hexanoate
14. Hexyl acetate
15. Octanoic acid
16. Ethyl octanoate

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Fig. 4A - Artificial Cherry Flavored Candy by SPME

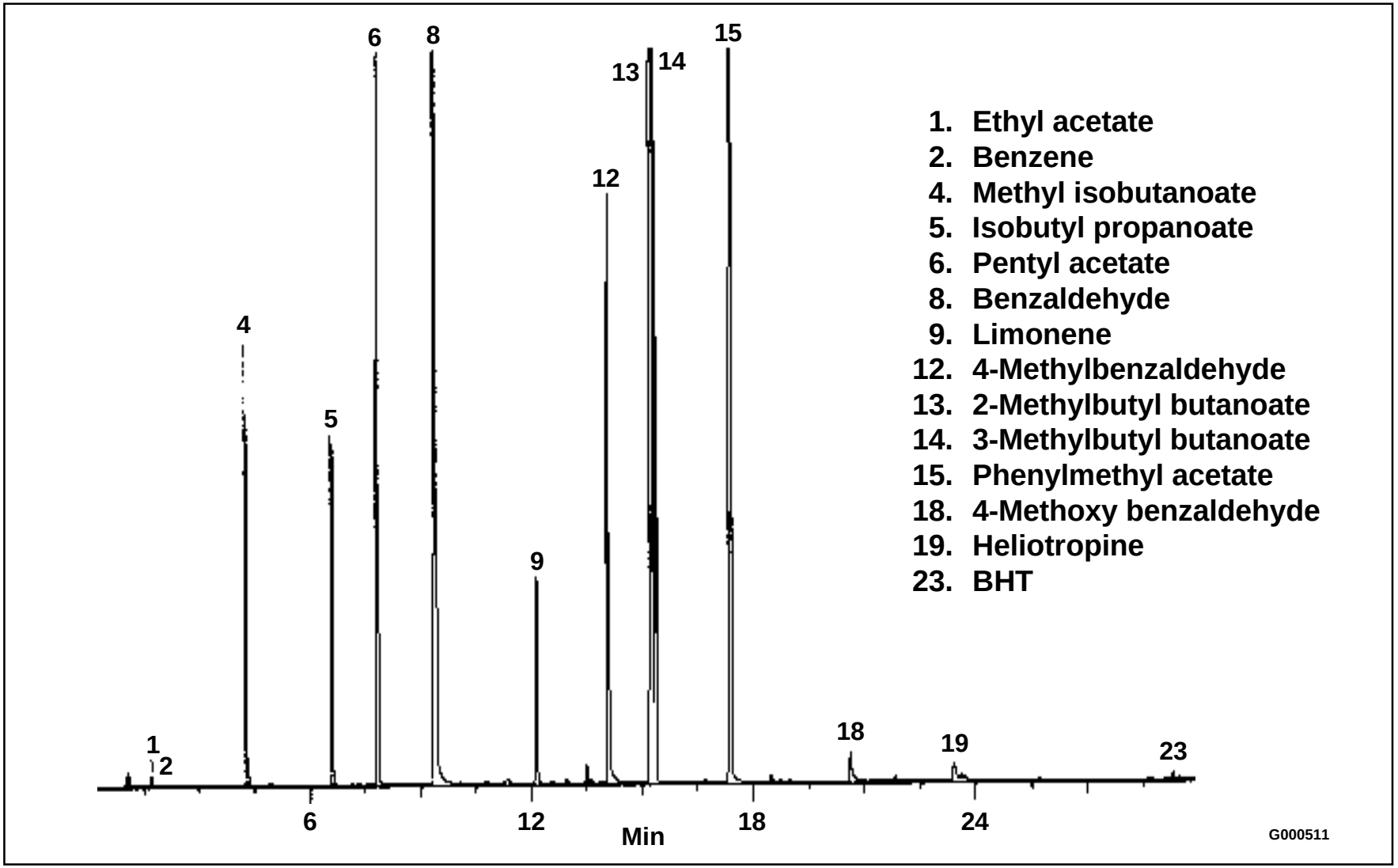
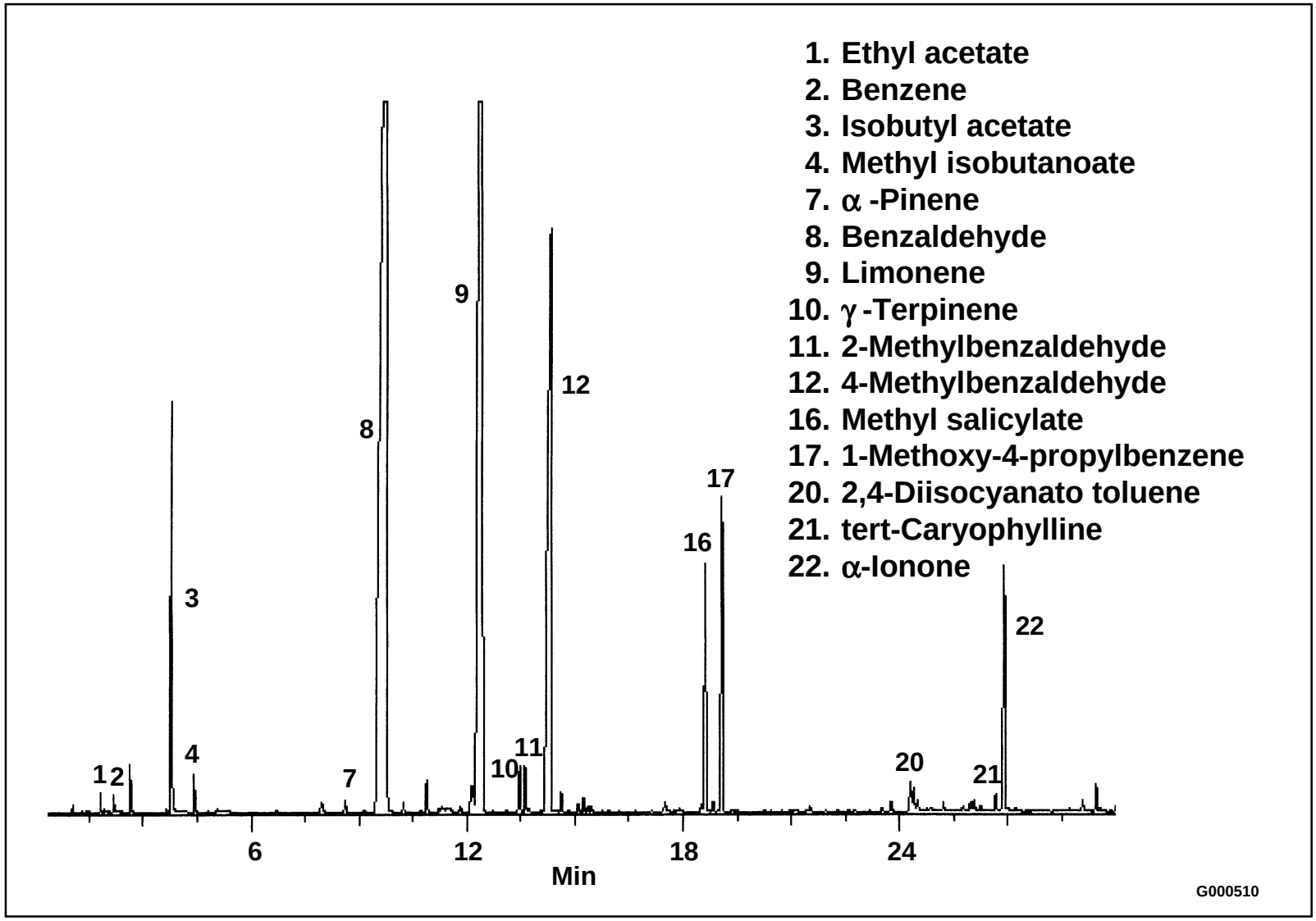


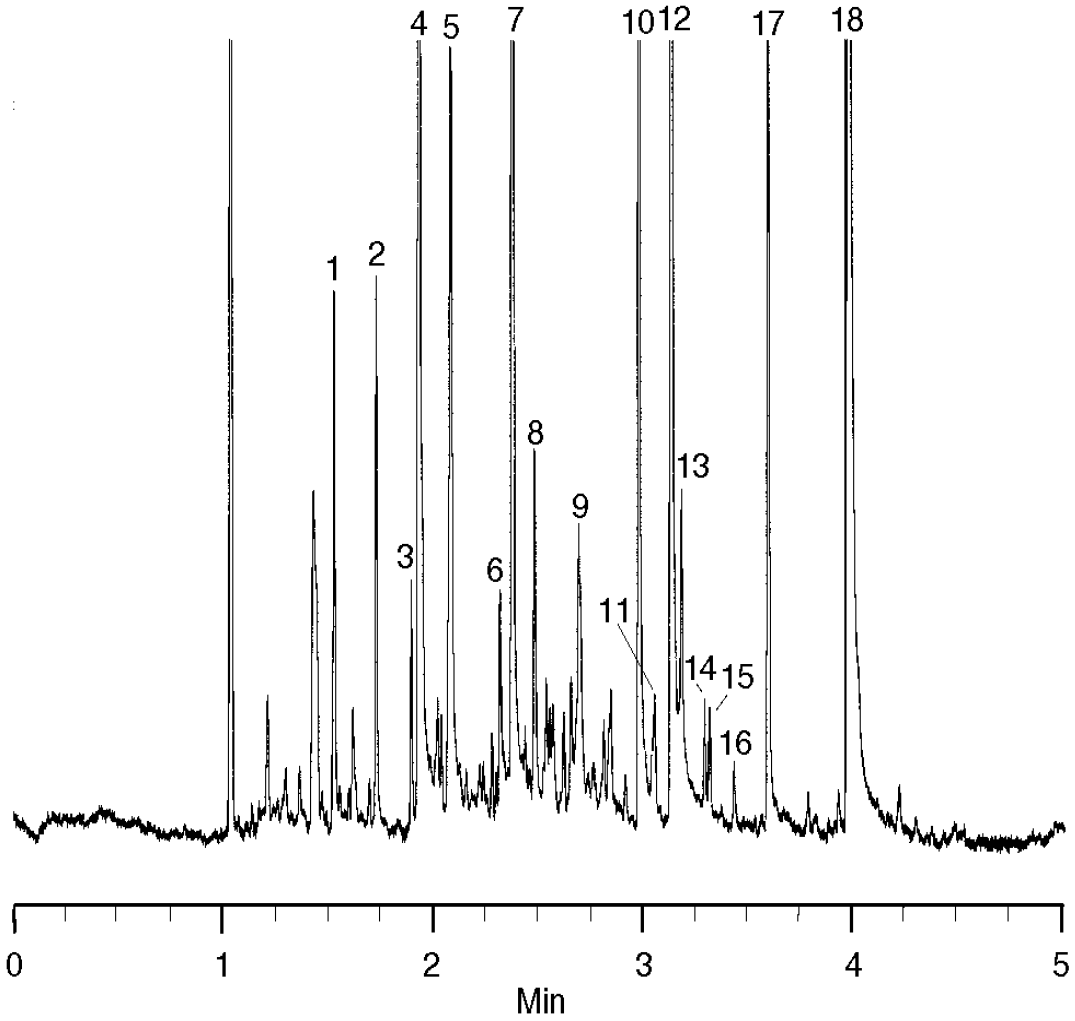
Fig. 4B - Artificial and Natural Cherry Flavored Candy



Conditions for Analysis of Hard Candy by SPME

Sample:	**0.5g candy in 5mL water in a 15mL vial**
SPME Fiber:	**DVB-Carboxen™-PDMS StableFlex™**
Extraction:	**headspace, 30 min at 40°C**
Desorption:	**270°C for 5 min**
Column:	**Meridian MDN-5, 30m x 0.25mm x 0.25µm**
Oven:	**45°C (1.5 min) to 260°C at 4°C/min**
Inj.:	**split or splitless with 0.75mm liner, 270°C**
Det.:	**ion trap mass spectrometer, m/z = 33-400 at 0.6 sec/scan**
	Selected ions used for quantitation.

Fig. 5 - Volatile Aroma Compounds in Apple Fruit



1. 2-Methyl butanol
2. Butyl acetate
3. Hexanol
4. 2-Methyl butyl acetate
5. Butyrolactone
6. Methylpropylbutanoate
7. Hexyl acetate
8. Butyl-2-methylbutanoate
9. Pentyl butanoate
10. Butyl hexanoate/Hexyl butanoate
11. p-Methyoxyallylbenzene
12. Hexyl-2-methylbutanoate
13. 3-Methylbutylhexanoate
14. Pentyl hexanoate
15. 2-Pentenylhexanoate
16. 2-Methylpropyl-2-methylbutenoate
17. Hexyl hexanoate
18. α-Farnesene

Figure provided by Jun Song, Department of Horticulture, Michigan State University, East Lansing, MI, USA.

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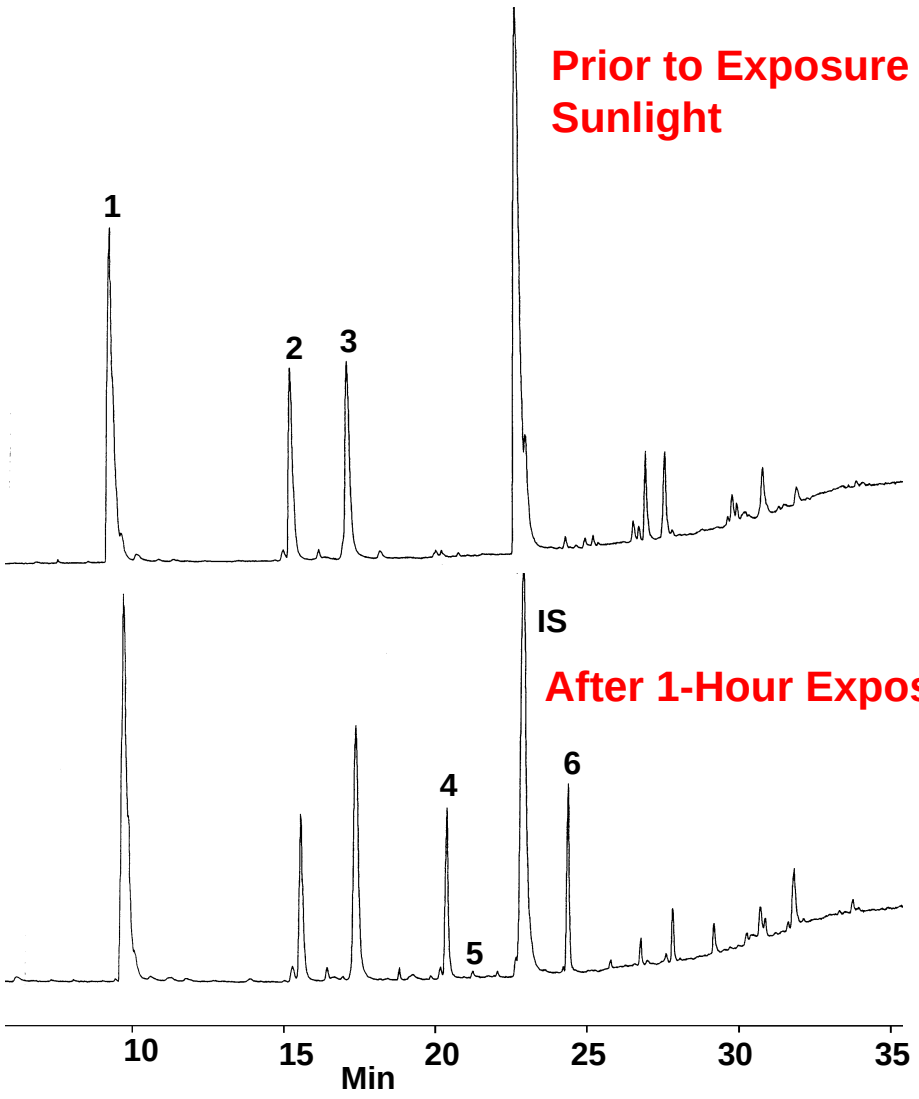
Conditions for Analysis of Apple Aromas

Sample:	**“Mutsu” apple fruit, 300-400g in a 3 liter flask**
SPME Fiber:	**65µm PDMS/DVB**
Sampling:	**headspace, 4 min, under a stream of N_2**
Desorption:	**250°C for 90 sec, then cryofocused at -100°C**
GC Column:	**(5% phenyl) polydimethylsiloxane, 25m x 0.1mm ID, 0.34µm film**
Oven:	**40°C (1.5 min) to 250°C at 50°C/min**
Carrier:	**helium, 1.5mL/min**
Det.:	**mass spectrometer, m/z = 40-300**

Fig. 6 - Milk Sample Off-Flavors by SPME-GC/MS



1. Acetone
2. 2-Butanone
3. 3-Methylpentane
4. Pentanal
5. Dimethyldisulfide
6. Hexanal

IS 4-Methyl-2-pentanone (int. std.)

Chromatogram provided by Ray Marsili, Dean Foods Technical Center, Rockford, IL, USA.

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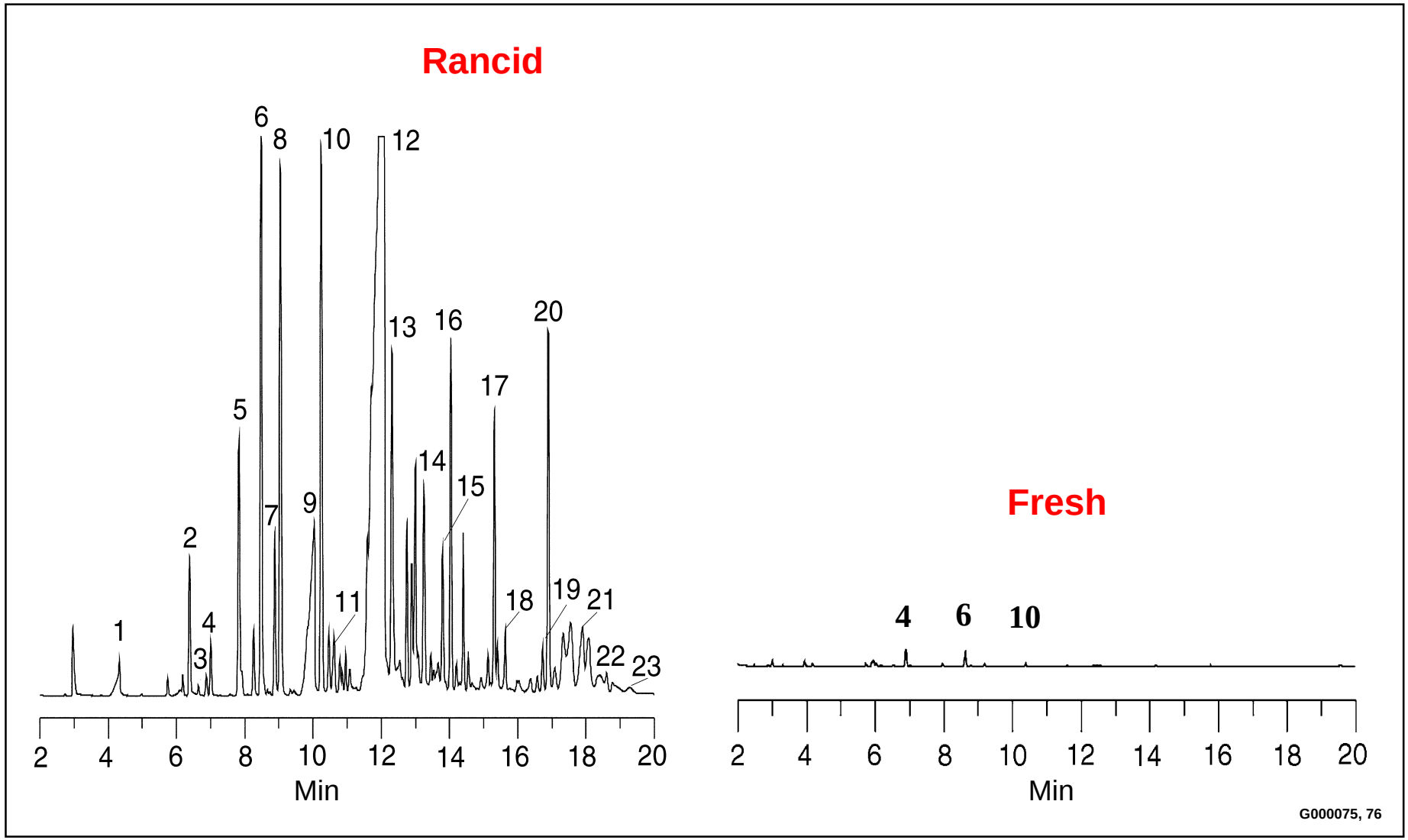
Conditions for Analysis of Milk Off-Flavors

Sample:	**3g of 2% milk + 10µL IS (20µg/mL 4-methyl-2-pentanone) (9mL GC vial)**
SPME Fiber:	**PDMS/Carboxen™, 75µm film**
Extraction:	**headspace, 15 min with constant stirring at 45°C**
Desorption:	**5 min, 250°C**
Column:	**Supel-Q™ PLOT, 30m x 0.32mm ID**
Oven:	**70°C (2 min) to 140°C at 6°C/min (2 min hold) then to 220°C at 6°C/min (5 min hold)**
Carrier:	**helium, 35cm/sec**
Inj.:	**splitless (closed 2 min)**
Det.:	**GC/MS ion trap, m/z = 33-300**

The exposure of unsaturated fatty acids to UV light can result in cleavage at the double bond. The resulting products are easily oxidized to form aldehydes such as hexanal and heptanal as shown in **Figure 6**. These components produce an off-flavor that is undesirable. The analysis of these by-products was traditionally done by purge and trap. Marsili of Dean Foods noted that SPME with the Carboxen-PDMS was not only as sensitive as purge and trap, but SPME provided a wider linear range compared to purge and trap. Also, SPME was suitable for detecting dimethylsulfide another off-flavor from oxidation of fats.

Fig. 7 - Analysis of Potato Chips



Identified Components in Rancid and New Potato Chips

1. Acetic acid
2. Pentanal
3. Butanoic acid
4. Propyl acetate
5. Methyl butyrate
6. Hexanal
7. Octane
8. Methyl hexanal
9. Hexanoic acid
10. Heptanone
11. Heptanal
12. Heptanoic acid
13. Octanal
14. Octanoic acid
15. Nonanone
16. Nonanal
17. Butyl hexanoate
18. Decanal
19. Undecanone
20. Pentyl hexanoate
21. Dodecanone
22. Methyl heptanol
23. Dodecanal

Conditions for Analysis of Chips

Extraction Conditions:

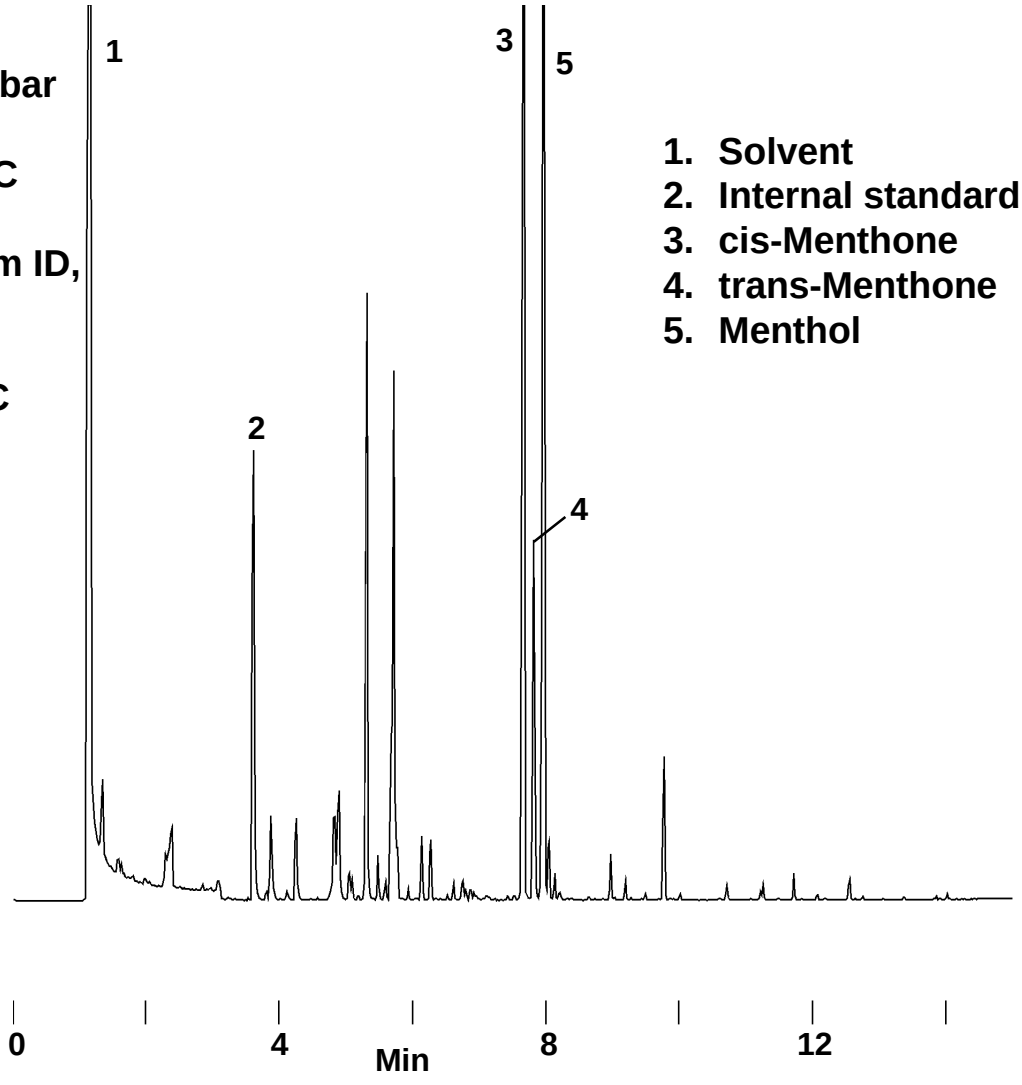
Fiber:	**DVB-Carboxen-PDMS StableFlex or 100µm PDMS**
Sample:	**3 grams of crushed potato chips in 15mL vial**
Extraction:	**heated headspace, 65°C for 20 min**
Desorption:	**3 min at 250°C**

GC/MS Conditions:

Column:	**SPB™-1 SULFUR, 30m x 0.32mm ID, 4.0µm film**
Oven:	**45°C (hold 1.5 min) to 250°C at 12°C/min (hold 10 min)**
Carrier Gas:	**helium, 40cm/sec**
Injection Port:	**splitless/split, closed for 2 min at 250°C**
Detector:	**quadrupole mass spectrometer, m/z = 35-290 @ 0.6 sec/scan**

Fig. 8 - Peppermint Oil in Chocolate Cookie Bar

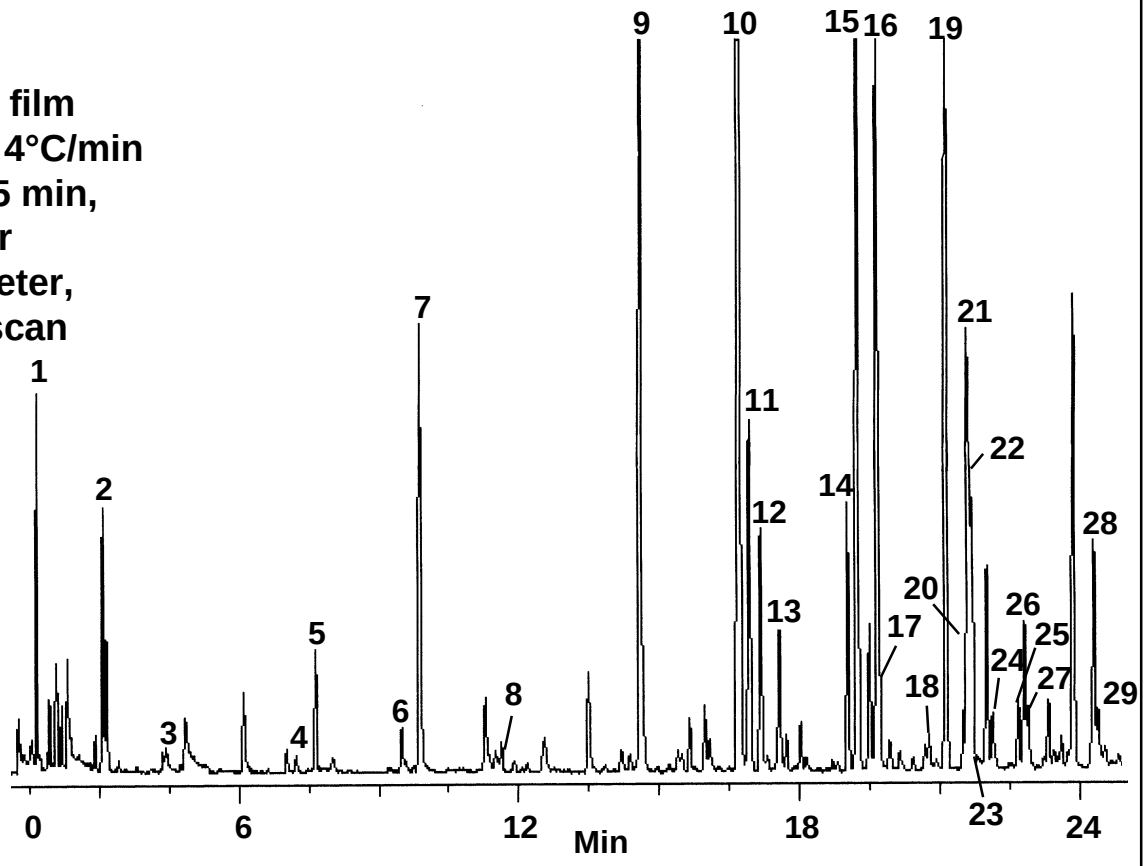
Sample:	**4g peppermint cookie bar**
SPME Fiber:	**100µm PDMS**
Extraction:	**headspace, 1 min, 45°C**
Desorption:	**5 min at 250°C**
Column:	**PTE™-5, 30m x 0.25mm ID, 0.25µm film**
Detector:	**FID, 250°C**
Injector:	**splitless (3 min), 250°C**



1. **Solvent**
2. **Internal standard**
3. **cis-Menthone**
4. **trans-Menthone**
5. **Menthol**

Fig. 9 - Analysis of Peanut Butter Flavors by SPME

Sample:	**5g peanut butter in 40mL vial**
SPME Fiber:	**DVB-Carboxen™-PDMS StableFlex™**
Extraction:	**headspace, 30 min at 65°C**
Desorption:	**5 min, 270°C**
Column:	**SUPELCOWAX™ 10, 30m x 0.25mm x 0.25µm film**
Oven:	**40°C (5 min) to 230°C at 4°C/min**
Inj.:	**splitless/split, closed 0.5 min, 270°C, with 0.75mm liner**
Det.:	**ion trap mass spectrometer, m/z = 30-350 at 0.6 sec/scan Selected ions used for quantitation.**



Flavor Components in Peanut Butter

Some Volatile Components in Peanut Butter

1. Carbon disulfide
2. 3-Methylbutanal
3. Pentanal
4. Dimethyl disulfide
5. Hexanal
6. 4-Methyl-pentene-2-one
7. 1-Methyl pyrrole
8. Heptanal

Pyrazines in Peanut Butter

9. 2-Methyl pyrazine
10. 2,5-Dimethyl pyrazine
11. 2,3-Dimethyl pyrazine
12. 2-Ethyl pyrazine
13. 2,6-Dimethyl pyrazine
14. 2-Ethyl-6-methyl pyrazine

Pyrazines in Peanut Butter (contd.)

15. 2-Ethyl-5-methyl pyrazine
16. Trimethyl pyrazine
17. 2-Ethyl-3-methyl pyrazine
18. 2,6-Diethyl pyrazine
19. 2-Ethyl-3,5-dimethyl pyrazine
20. 2,3-Diethyl pyrazine
21. 2-Methyl-5-isopropyl pyrazine
22. 3-Ethyl-2,5-dimethyl pyrazine
23. 5-Methyl-2-propyl pyrazine
24. 2-Methyl-5-propyl pyrazine
25. 2-Ethenyl-6-methyl pyrazine
26. 3,5-Diethyl-2-methyl pyrazine
27. 2-Ethenyl-5-methyl pyrazine
28. 2-Methyl-6-cis propenyl pyrazine
29. 2-Allyl-5-methyl pyrazine

Quantitation of Pyrazines in Peanut Butter

$$\left(\begin{matrix}\text{Area counts}\\ \text{spiked pb}\end{matrix}\right) - \left(\begin{matrix}\text{Area counts}\\ \text{unspiked pb}\end{matrix}\right) \times \left(\frac{\text{g spiked pb}}{\text{g unspiked pb}}\right) = \left(\begin{matrix}\text{Area counts}\\ \text{pyrazine spike}\end{matrix}\right)$$

Area counts (spiked pyrazine) = ng/g for each pyrazine

$$\text{Pryazines in ppb} = \frac{\text{ng/g x area counts (unspiked p.b.)}}{\text{area counts (spiked pyrazine)}}$$

Analytes	ppb
2-Methyl pyrazine	158
2,5-Dimethyl pyrazine	526
2,3-Dimethyl pyrazine	47
2,6-Dimethyl pyrazine	16

The roasting of peanut butter (PB) produces the formation of pyrazines from the Maillard reaction. The nutty flavor and aroma in PB are the result of the pyrazines. By heating the peanut butter to 65°C, the pyrazines are released from the fat and transferred into the headspace. The DVB-Carboxen-PDMS fiber was ideal for extracting the pyrazines along with some of the smaller flavor components as shown in **Figure 9**.

Quantitation of peanut butter can be accomplished by spike addition. An equal weight of a peanut butter sample was place into 2 vials. One vial was spiked with a known weight of pyrazines. Both the unspiked and spiked vials of PB were extracted with the same fiber using identical conditions. The difference in area counts between the two samples provided the area counts of the spiked pyrazines. By determining the amount of the spiked pyrazines per gram of PB, the amount of each pyrazine could be determined in the unspiked PB. The results obtained are within published results for pryazines in PB.

Fig. 10A - Analysis of Regular Coffee Grounds by SPME

Sample: 5g coffee grounds in 40mL vial
SPME Fiber: DVB/Carboxen™/PDMS StableFlex™
Extraction: headspace, 30 min at 65°C
Desorption: 270°C for 5 min

Column: SUPELCOWAX™ 10, 30m x 0.25mm x 0.25µm film
Oven: 40°C (5 min) to 230°C at 4°C/min
Inj.: splitless/split, closed 0.5 min, 270°C, with 0.75mm liner
Det.: ion trap mass spectrometer, m/z = 30-350 at 0.6 sec/scan
Selected ions used for quantitation.

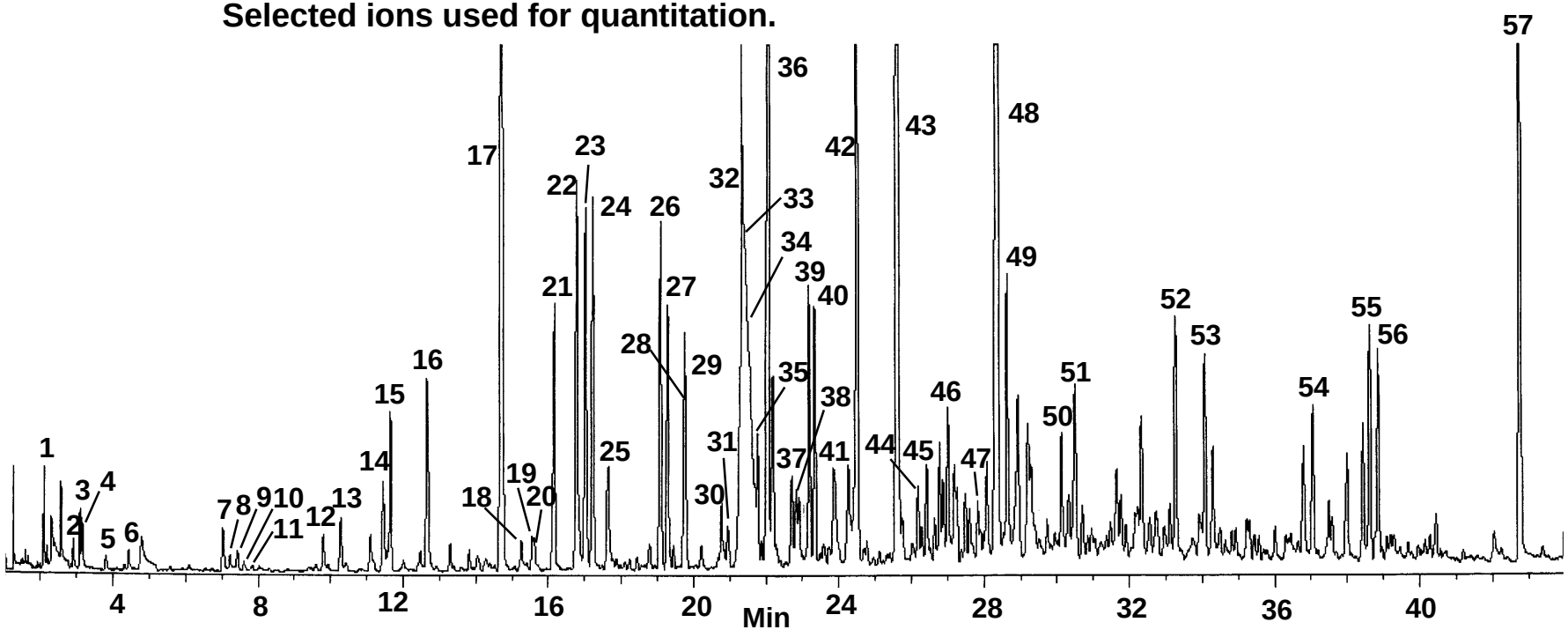
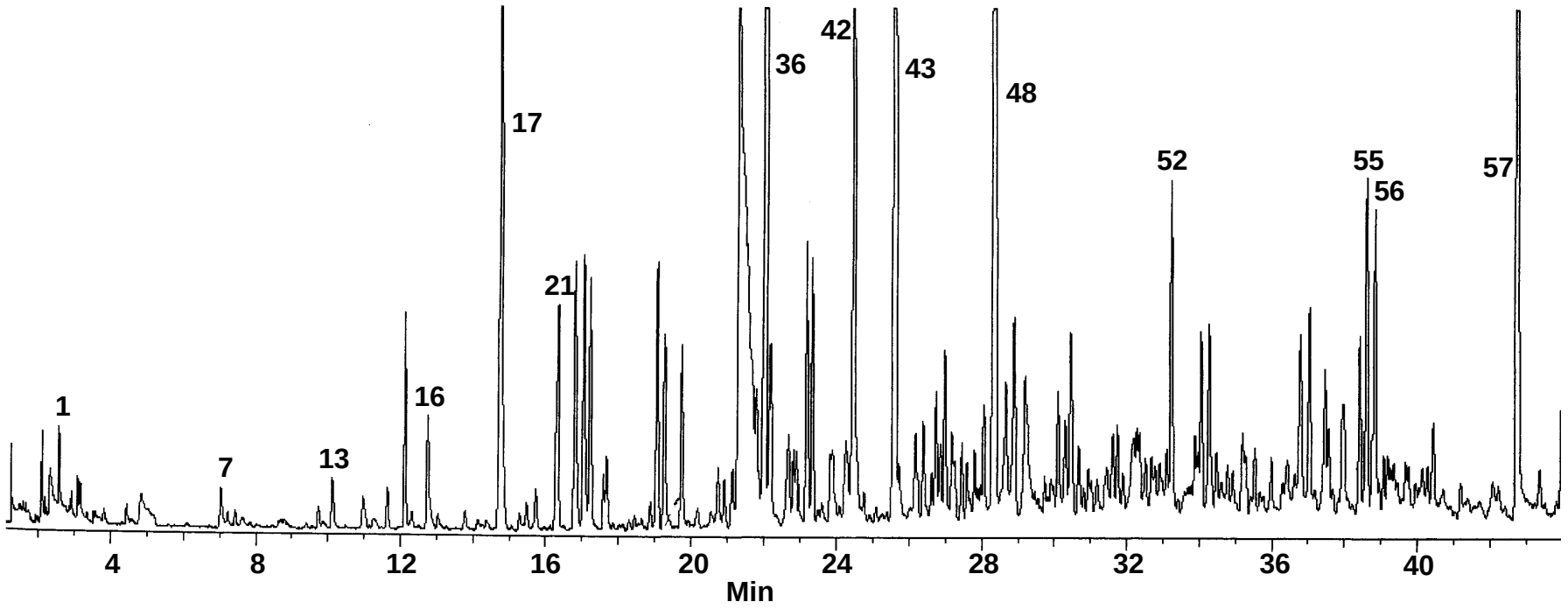


Fig 10B - Analysis of Decaffeinated Coffee Grounds by SPME

Sample:	**5g coffee grounds in 40mL vial**
SPME Fiber:	**DVB/Carboxen™/PDMS StableFlex™**
Extraction:	**headspace, 65°C, 30 min**
Desorption:	**5 min, 270°C**
Column:	**SUPELCOWAX™ 10, 30m x 0.25mm x 0.25µm film**
Oven:	**40°C (5 min) to 230°C at 4°C/min**
Inj.:	**splitless/split, closed 0.5 min, 270°C, with 0.75mm liner**
Det.:	**ion trap mass spectrometer, m/z = 30-350 at 0.6 sec/scan**
	Selected ions used for quantitation.



Components in Coffee

1. 2-Methyl furan
2. 2-Butanone
3. 2-Pentanone
4. 3-Methyl butanal
5. 2,5-Dimethylfuran
6. 2-Acetyloxy-2-propanone
7. 2-Ethyl hexanol
8. Dimethyldisulfide
9. Phenol
10. Hexanal
11. 2-Methyl thiophene
12. n-Methyl pyrrole
13. 4-Methylphenol
14. 2-Ethyl pyrrole
15. Pyridine
16. Pyrazine
17. Methyl pyrazine
18. 4-Methyl thiazole
19. 3-Hydroxy butanone
20. Dimethyl phenol (isomer)
21. 1,2-Ethanediol, monoacetate
22. 2,5-Dimethylpyrazine
23. 2,3-Dimethylpyrazine
24. 2-Ethylpyrazine
25. 2,6-Dimethylpyrazine
26. 2-Ethyl-6-methylpyrazine
27. 2-Ethyl-5-methylpyrazine
28. Trimethylpyrazine
29. 2-Ethyl-3-methylpyrazine
30. 2,6-Diethylpyrazine
31. 2-Ethenylpyrazine
32. 2-Ethyl-3,5-dimethylpyrazine
33. Glycerol
34. 2,3-Diethylpyrazine
35. 2-Ethyl-3,6-dimethylpyrazine
36. 2-Furancarboxaldehyde
37. 2-Isopropenylpyrazine
38. 3,5-Diethyl-2-methylpyrazine
39. Furfural formate
40. 2-Furonyl ethanone
41. Methyl benzoylformate
42. Furanmethanol acetate
43. 5-Methyl-2-furancarboxaldehyde
44. Furanmethanol proprionate
45. Furfanyl furan
46. Pyridine methanol
47. 2-Methyl-5-propenylpyrazine
48. Furanmethanol
49. 3-Ethyl-4-methyl-2,5-furandione
50. Pyrazinecarboxamide
51. 2-Ethyl-3-hydroxy-4H pyran-4-one
52. 1-(2-Furanylmethyl)-pyrrole
53. 2-Methoxyphenol
54. 1-(1H-pyrrole-2-yl)-ethanone
55. 4-Ethyl-2-methoxy phenol
56. 3-Phenylpropenal or 2-Methylbenzofuran
57. 3,5-Dimethylbenzoic acid

Comparison of Caffeine Levels in Coffee and Extraction Type

Coffee and Extraction	Regular	Decaffeinated	%Decaffeinated
Grounds HS	1202079	207422	83%
Brewed Immersed	13623252	1567167	88%
Brewed HS	77431	8347	89%

1 hour extraction time with DVB-Carboxen-PDMS StableFlex Fiber
HS = headspace at 65°C

Fig. 11A - Odor Agents at 1ppt in Water by SPME-GC/MS

Sample: 30mL water containing MIB and geosmin at 1ppt and 25% NaCl in a 40mL vial, at 65°C
SPME Fiber: 2cm DVB/Carboxen™/PDMS
Extraction: heated headspace, 30 min, 65°C, with rapid stirring
Desorption: 3 min, 250°C, splitter closed

Column: Meridian MDN-5, 30m x 0.25mm x 0.25µm film
Oven: 60°C (1 min) to 250°C at 15°C/min
Det.: mass spectrometer, m/z = 75-180 at 0.6 sec/scan (quantitation ions 95 and 112)

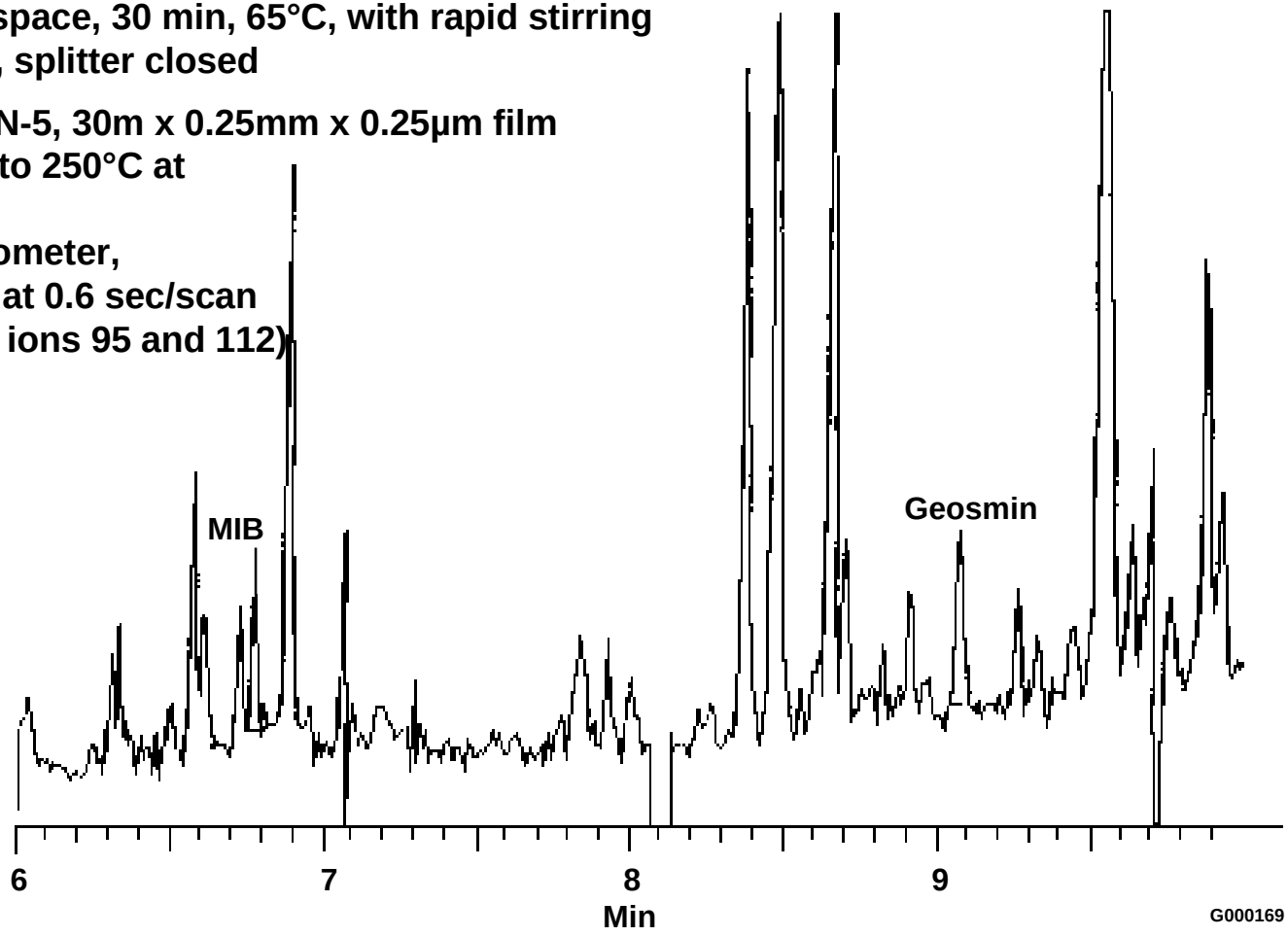
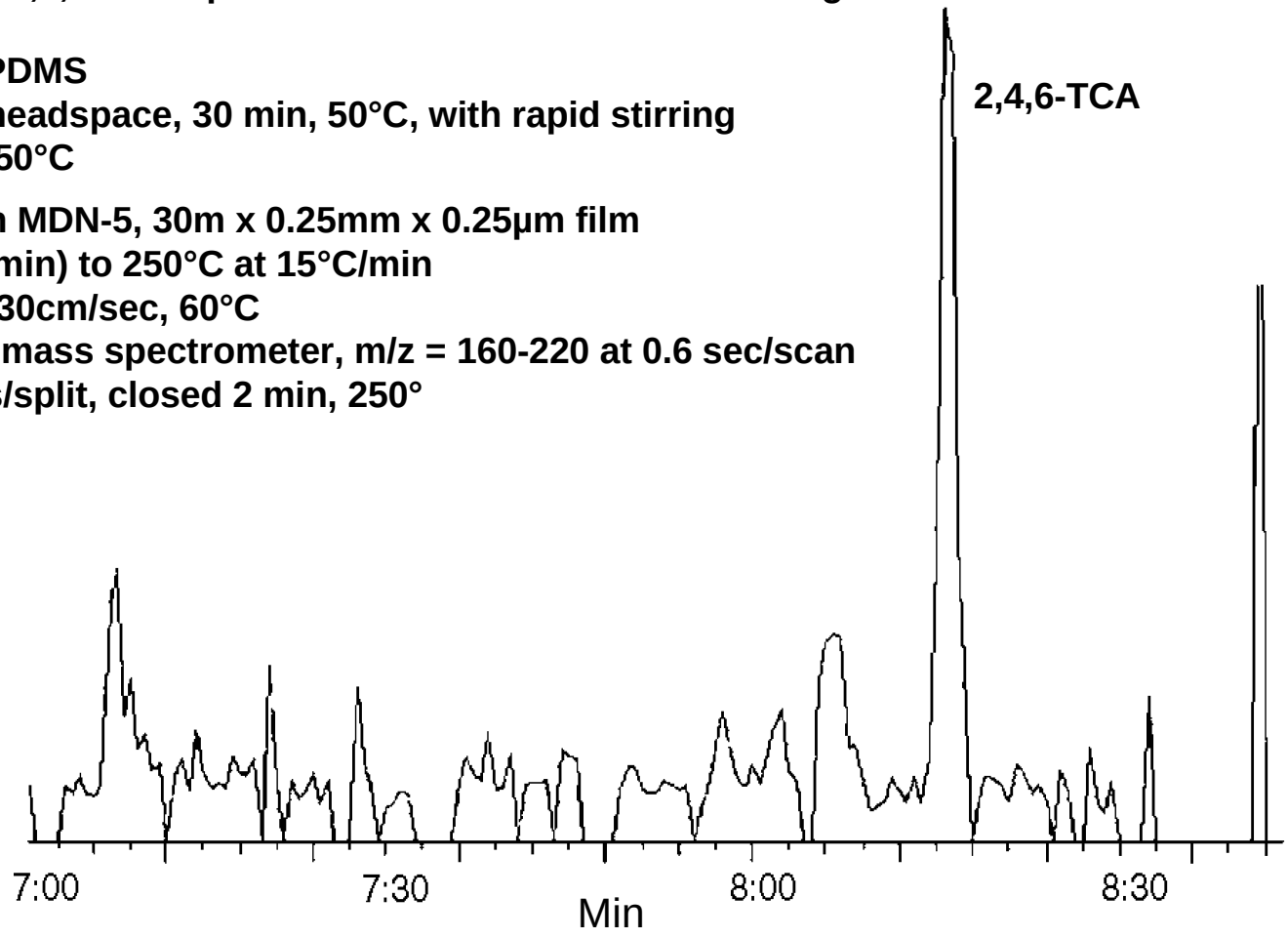


Fig. 11B - 2,4,6-Trichloroanisole in White Wine by SPME

Sample:	**10ppt of 2,4,6-TCA spiked in 12mL of white wine and 2.5g of NaCl**
SPME Fiber:	**100µm PDMS**
Extraction:	**heated headspace, 30 min, 50°C, with rapid stirring**
Desorption:	**5 min, 250°C**
Column:	**Meridian MDN-5, 30m x 0.25mm x 0.25µm film**
Oven:	**60°C (1 min) to 250°C at 15°C/min**
Carrier.:	**helium, 30cm/sec, 60°C**
Det.:	**Ion trap mass spectrometer, m/z = 160-220 at 0.6 sec/scan**
Inj.:	**splitless/split, closed 2 min, 250°**



Drinking water that comes from reservoirs may contain blue-green algae. This algae produces by-products that have a highly undesirable odors.These by-products, geosmin and methylisoborneol (MIB), produce a musky odor that is easily detected at 10 ppt by the human nose. In some cases, the threshold is less than 5 ppt. Even though these odors are not harmful, they can produce many customer complaints when detected. As a result, the water utilities monitor for MIB and geosmin at levels less than 5 ppt. **Figure 11A** shows the capability of heated headspace SPME and selected ion MS to detect these odor components at 1 ppt. A special 2 cm-SPME fiber is used to enhance sensitivity.

In wine, the bleaching of cork can cause the formation of 2,4,6-trichloroanisole. Like geosmin and MIB this by-product also has a low odor threshold around 10-20 ppt. Using headspace SPME this odor can be detected at 10 ppt in from wine as shown in **Figure 11B**.

CONCLUSIONS

- **SPME can be used to detect flavors in both solid and liquid foods.**
- **Both volatile and semivolatile compounds can be analyzed .**
- **SPME can easily detect a wide range of analytes with one fiber.**
- **Specificity can be obtained with different types of fibers.**
- **Quantification is possible with analyte addition.**
- **SPME can detect analytes at trace and high concentration levels in one sample.**