Solid Phase Microextraction for Food Analysis

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SUPELCO™ Analytical

SPME SUPELCO

SIGMA-ALDRICH®

Example Applications SPME

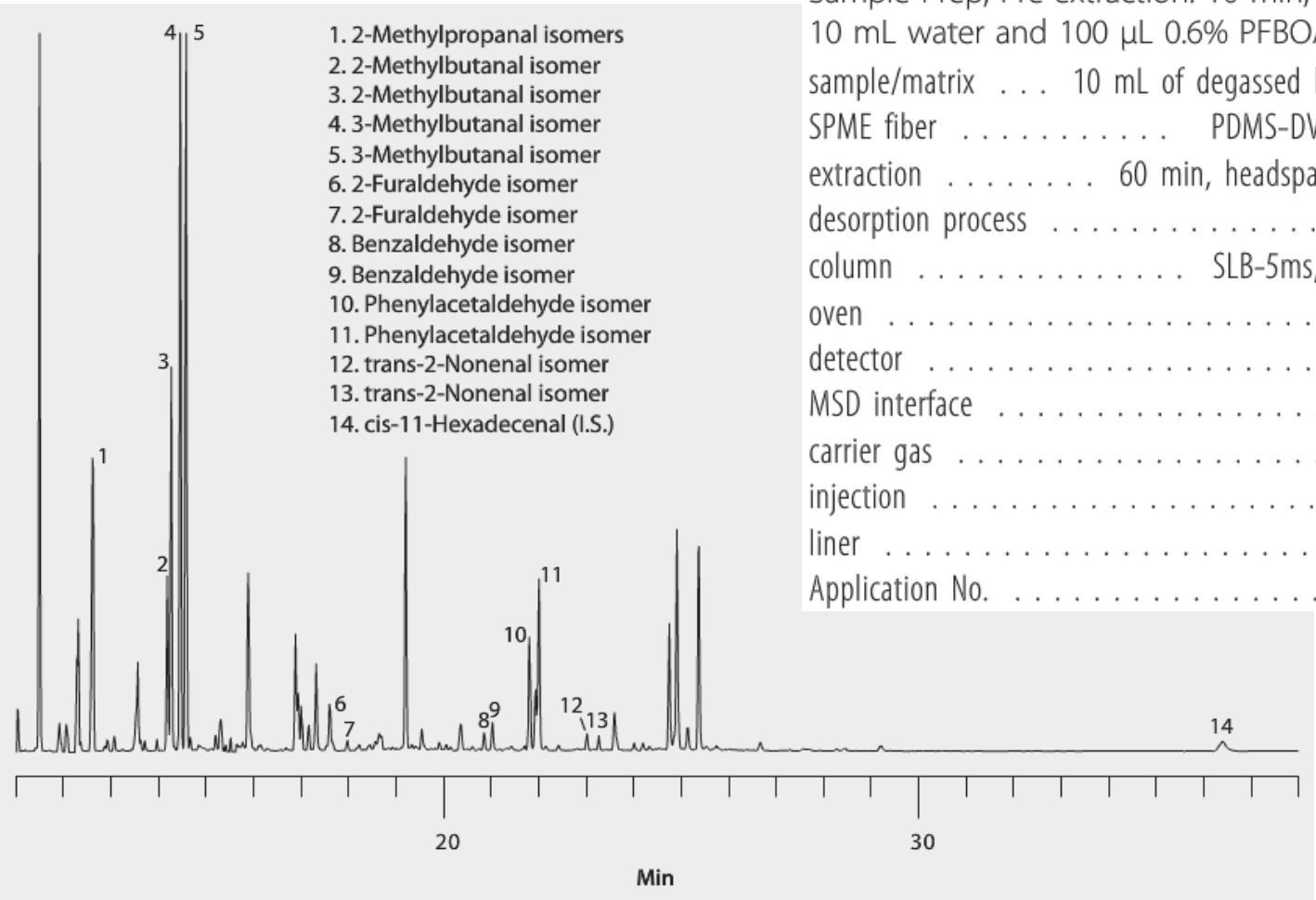
- Aldehydes in Beer
- Analysis of Volatile Acids in Parmesan Cheese
- Limoncello
- Determination of Phthalate Esters in Ramen Noodle Flavour Packets by SPME and GC/MS
- Milk off-flavors
- Resveratrol in red wine
- TCA & precursors in red wine (cork taint)
- Agricultural pesticides in wine
- Peanut Butter Flavors by SPME
- Regular Coffee Grounds by SPME
- Peppermint oil in chocolate bar

Aldehydes in Beer
with On Fiber Derivatisation

GC Analysis of Aldehydes (PFBOA Derivatives) in Beer on SLB®-5ms after SPME using 65 µm PDMS/DVB Fiber

The formation of aldehydes is a major contributor to the deterioration in flavor or "staling" of beer upon storage. Research indicates that the source of these aldehydes is the type of malt used and quality of the wort produced during the brewing process. This application illustrates the use of SPME to extract aldehydes from beer, followed by on-fiber derivatization with 0-(2,3,4,5,6- pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA). The aldehydes are then analyzed by GC/MS-SIM as their corresponding PFBOA derivatives. The aldehydes exist as geometric isomers, resulting in two derivatives for each. In the case of several of the analytes, these two derivatives were resolved chromatographically.

Aldehydes in Beer
with On Fiber Derivatisation



Sample Prep, Pre-extraction: 10 min, headspace, 20 mL vial containing 10 mL water and 100 µL 0.6% PFBOA in water

sample/matrix	10 mL of degassed beer, 3.5 gm sodium chloride in 20 mL vial
SPME fiber	PDMS-DVB, 23 guage, 65 µm, Auto, Pk/3 (57345-U)
extraction	60 min, headspace, 50 °C, agitation (5 min on, 30 min off)
desorption process	0.10 min, 250 °C
column	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm (28473-U)
oven	40 °C, 7 °C/min to 250 °C (14 min)
detector	MSD, SIM
MSD interface	275 °C
carrier gas	helium, 1.1 mL/min
injection	splitless
liner	0.75 mm I.D. SPME liner
Application No.	G005807

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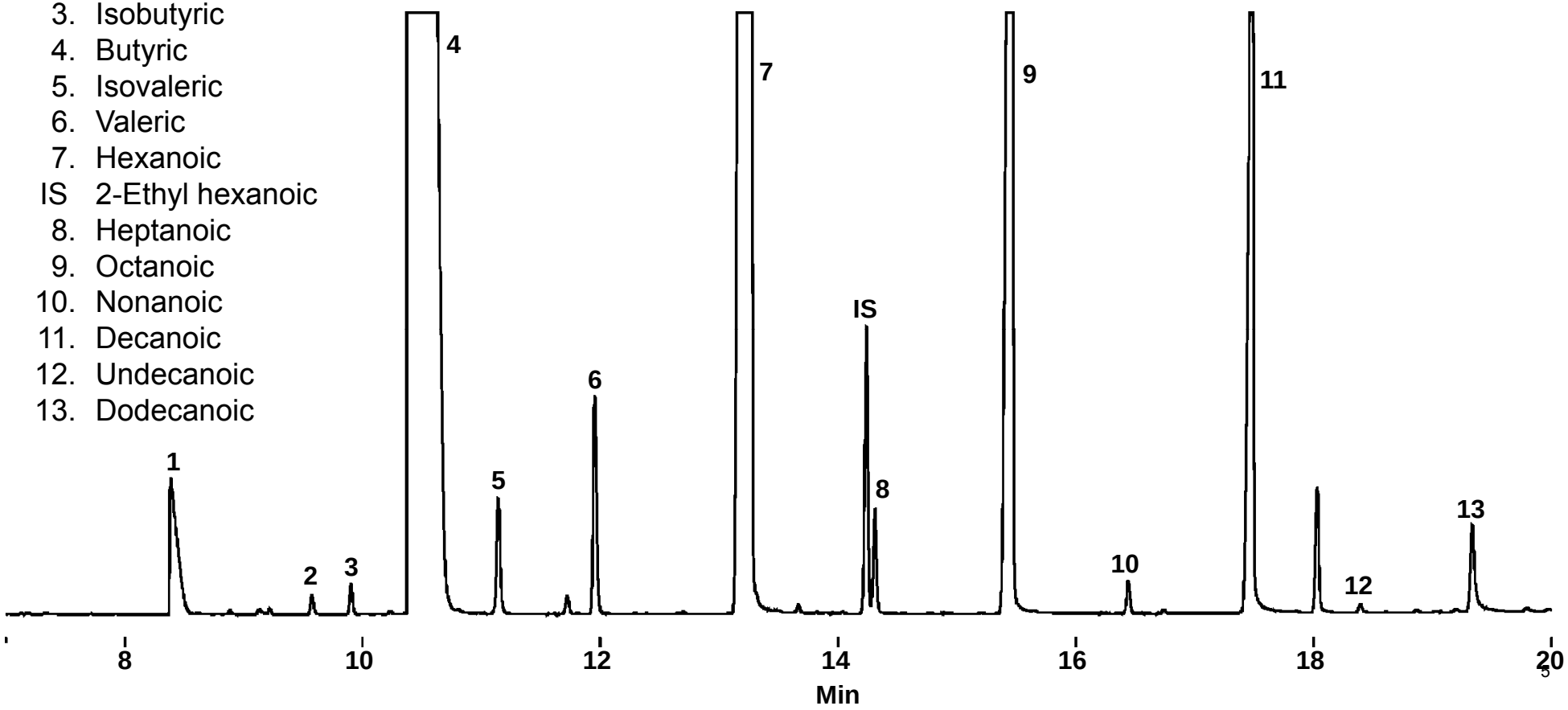
Analysis of Volatile Acids in Parmesan Cheese (Headspace 15 min at 65 °C)

1. Acetic
2. Propionic
3. Isobutyric
4. Butyric
5. Isovaleric
6. Valeric
7. Hexanoic

IS 2-Ethyl hexanoic

8. Heptanoic
9. Octanoic
10. Nonanoic
11. Decanoic
12. Undecanoic
13. Dodecanoic

SPME Fiber: 65µm CW-DVB



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Conditions for Analysis of Volatile Acids in Parmesan Cheese

Sample:	100mg cheese in 40mL vial
SPME Fiber:	65µm CW-DVB
Extraction:	headspace, 15 min, 65° C
Desorption:	1 min, 250° C
Column:	Nukol™, 15m x 0.25mm, 0.25µm film
Oven:	50° C (2 min) to 220° C at 10° C/min
Carrier:	helium, 30cm/sec
Inj.:	splitless/split (closed 1 min), 250° C
Det.:	FID, 260° C

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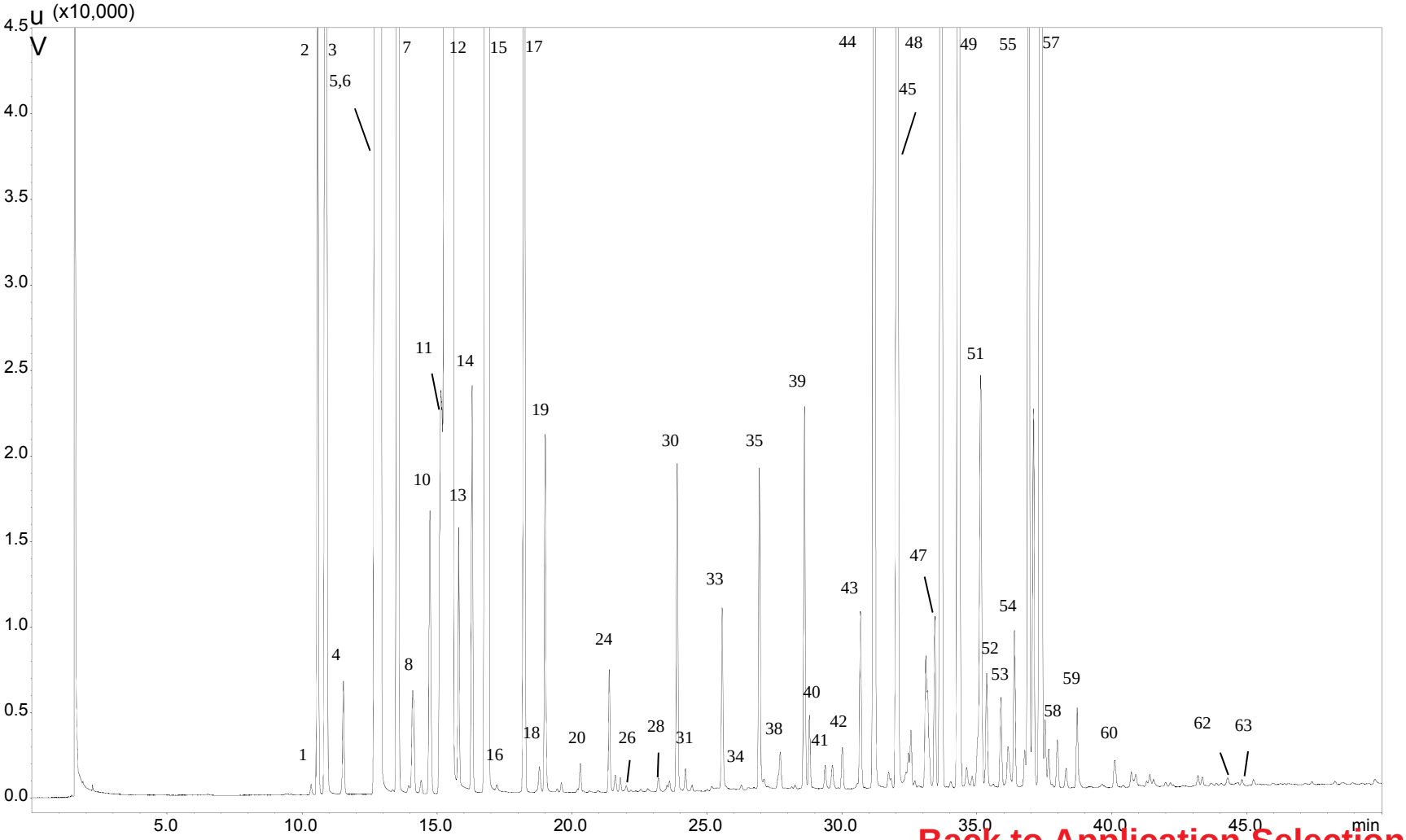
Limoncello

- Field: Flavors & Fragrances
- Sample Prep and Analysis Tools: Headspace SPME, GC
- Abstract: ~70 volatile compounds from limoncello captured by headspace SPME, desorbed and analyzed on SLB-5ms column.
- References:

Limoncello

- Headspace SPME
- Analysis on SLB-5ms



Chromatogram courtesy of Prof. Luigi Mondello (Univ. of Messina, Italy)

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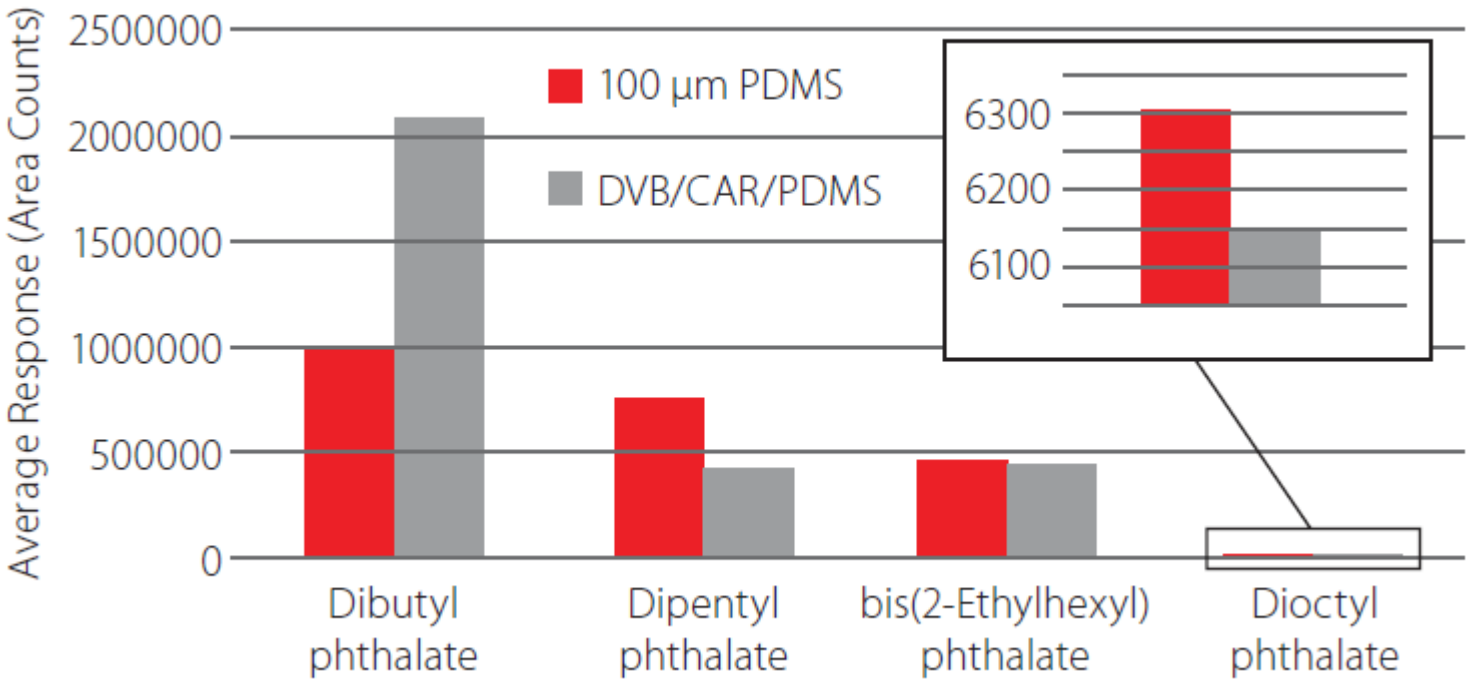
Determination of Phthalate Esters in Ramen Noodle Flavour Packets by SPME and GC/MS

- June 2011: Food Safety Authorities in Hong Kong found phthalate ester contamination in a variety of imported food and drink products including several brands of ramen noodle kits
- Concern with presence of bis(2-ethylhexyl) phthalate (DEHP) in the oil-based flavour packets in these kits
- Current method in China: gel permeation chromatography (GPC) as a cleanup step prior to GC-MS analysis [1].
  - Extremely effective
  - Time-consuming technique

[1] Determination of phthalate esters in foods. Chinese Official Method GB21911

Determination of Phthalate Esters in Ramen Noodle Flavour Packets by SPME and GC/MS

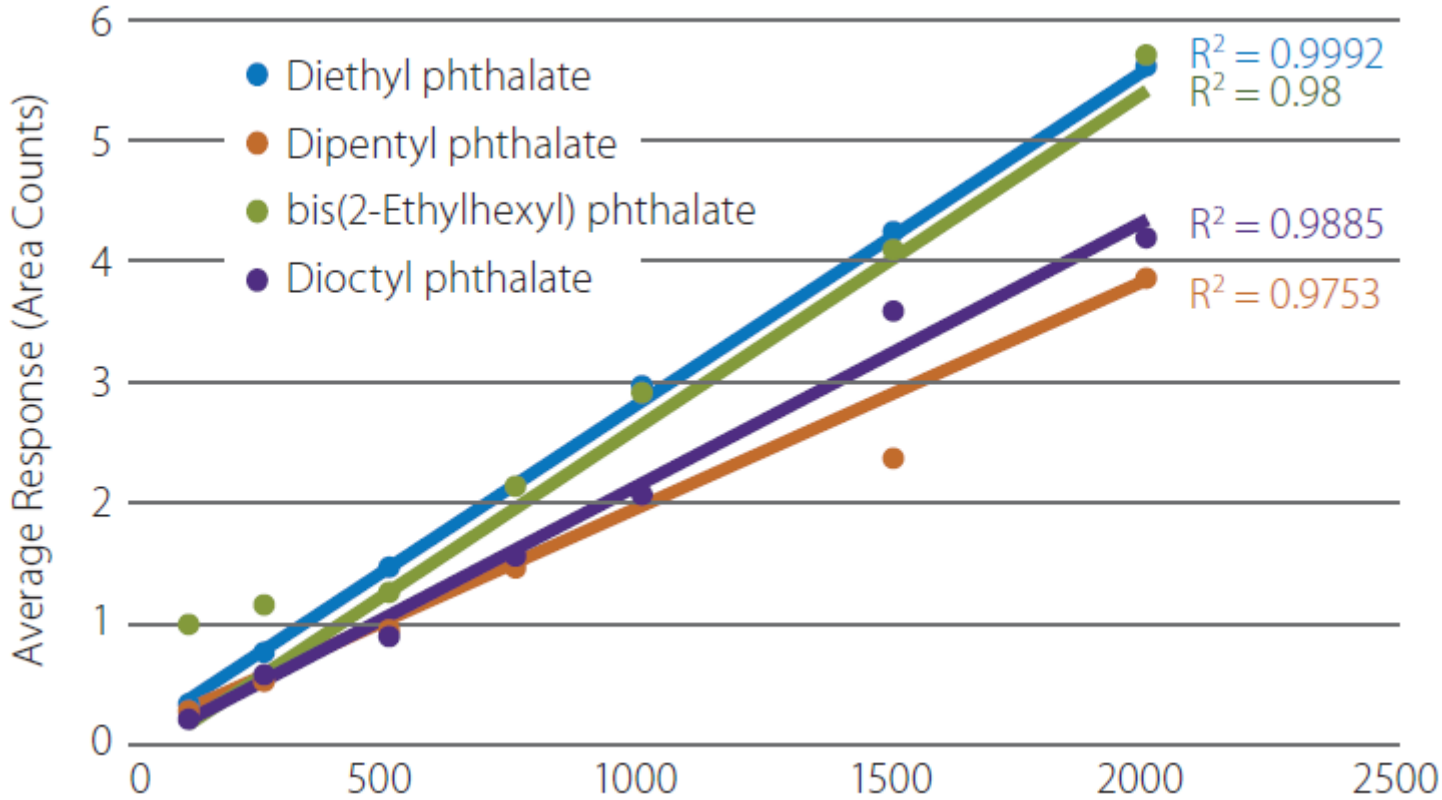
SPME Fiber Selection Study (based on ref. [2,3])



[2] Rios, J.J.; Morales, A.; Márques-Ruiz, G., *Talanta* 2010, 80, 2076–2082.
[3] Holadová, K.; Prokůpková, G.; Hajšlová, J.; Poustka,. *Analytica Chimica Acta* 2007, 24–33.

Determination of Phthalate Esters in Ramen Noodle Flavour Packets by SPME and GC/MS

Calibration Linearity



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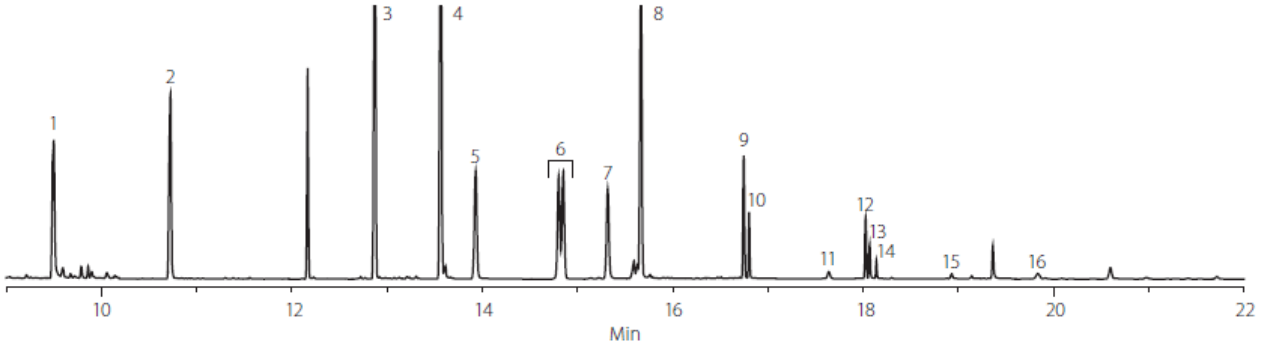
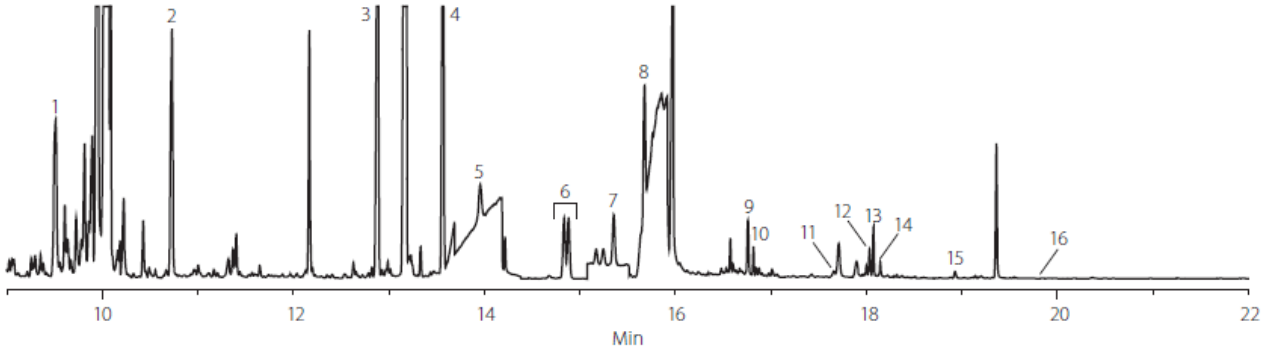
Determination of Phthalate Esters in Ramen Noodle Flavour Packets by SPME and GC/MS

Phthalate Standard, each Analyte at 500 µg/kg



Spiked Chicken-Flavoured Oil



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Determination of Phthalate Esters in Ramen Noodle Flavour Packets by SPME and GC/MS

- sample/matrix: Analytes and internal standards added to a 15 mL vial, mixed, hexane evaporated, reconstituted with 1 g corn oil, heated until headspace at 90 ° C
- SPME fibre: 100 µm PDMS fibre (57300-U)
- extraction: headspace, 90 ° C (measured in the headspace) for 30 min.
- desorption process: 260 ° C for 4 min.
- column: SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
- oven: 60 ° C (1 min.), 10 ° C/min. to 330 ° C (10 min.)
- MSD interface: 330 ° C
- scan range: SIM
- carrier gas: helium, 0.6 mL/min. constant
- liner: 0.75 mm I.D., SPME type, straight design (2637501)

- 1. Dimethyl phthalate (DMP)
- 2. Diethyl phthalate (DEP)
- 3. Diisobutyl phthalate (DIBP)
- 4. Dibutyl phthalate (DBP)
- 5. bis(2-Methoxyethyl) phthalate (DMEP)
- 6. bis(4-Methyl-2-pentyl) phthalate (BMPP)
- 7. bis(2-Ethoxyethyl) phthalate (DEEP)
- 8. Dipentyl phthalate (DPP)
- 9. Dihexyl phthalate (DHP)
- 10. Butylbenzyl phthalate (BBP)
- 11. bis(2-n-Butoxyethyl) phthalate (DBEP)
- 12. Dicyclohexyl phthalate (DCHP)
- 13. bis(2-Ethylhexyl) phthalate (DEHP)
- 14. Diphenyl phthalate
- 15. Dioctyl phthalate (DNOP)
- 16. Dinonyl phthalate (DNP)

Determination of Phthalate Esters in Ramen Noodle Flavour Packets by SPME and GC/MS

- **Official Method [1]**
  - 1. Weigh 0.5 g of sample into a 15 mL vial
  - 2. Dilute to 10 mL with ethyl acetate:hexane (1:1)
  - 3. Vortex, then filter and collect in a second container
  - 4. Perform GPC cleanup and collect in a third container
  - 5. Concentrate to 2.0 mL
  - 6. Transfer to a fourth container (autosampler vial) and
  - add internal standard
  - 7. Proceed to GC-MS analysis

150 minutes per sample
105 minutes hands-on work

- **SPME Method**
  - 1. Add internal standards into a 15 mL vial
  - 2. Evaporate hexane
  - 3. Add 1 g sample then vortex
  - 4. Heated headspace extraction (headspace at 90 ° C)
  - 5. Proceed to GC-MS analysis

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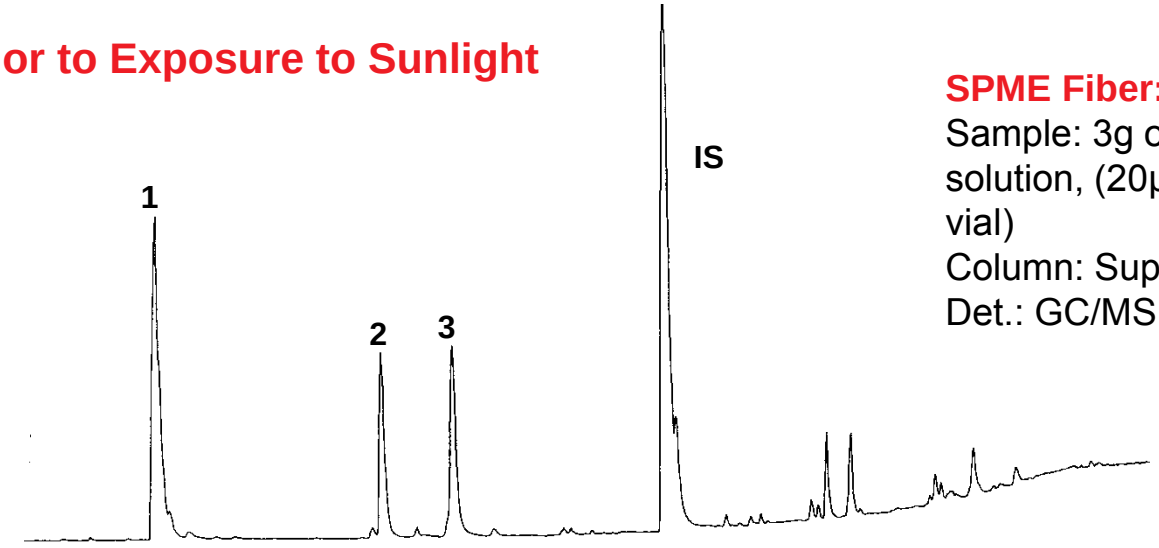
42 minutes per sample
12 minutes hands-on work

Milk off-flavors

- Field: Flavors & Fragrances
- Sample Prep and Analysis Tools: SPME, GC-MS
- Abstract: SPME was used to detect the formation of aldehydes that are formed when unsaturated fatty acids are exposed to UV light. SPME was a useful tool for monitoring shelf life of milk.
- References: SPME Applications CD

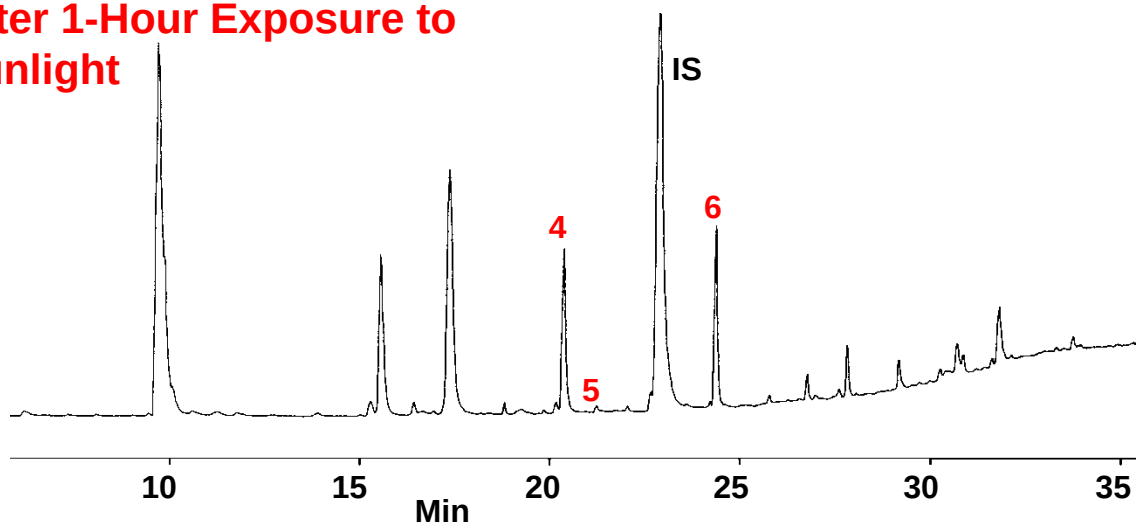
Milk Sample Off-Flavors by SPME-GC/MS

Prior to Exposure to Sunlight



SPME Fiber: 75 µm PDMS/Carboxen
Sample: 3g of 2% milk + 10µL internal standard solution, (20µg/mL 4-methyl-2-pentanone) (9mL GC vial)
Column: Supel-Q™ PLOT, 30m x 0.32mm ID
Det.: GC/MS ion trap, m/z = 33-300

After 1-Hour Exposure to Sunlight



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

IS. 4-Methyl-2-pentanone

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

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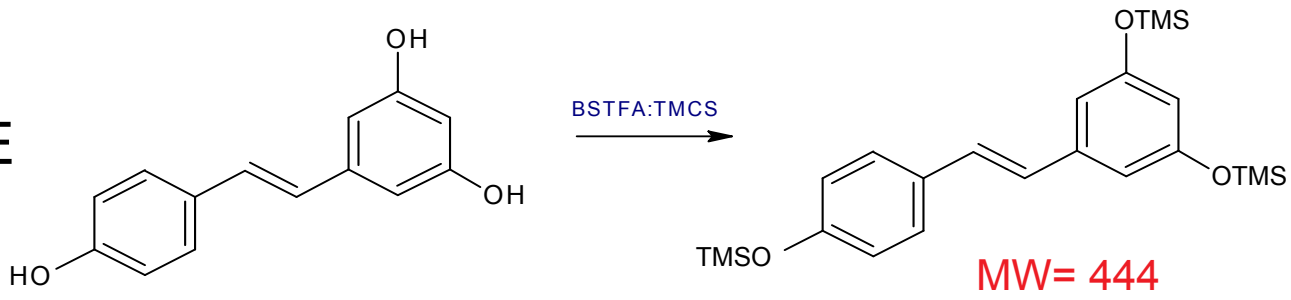
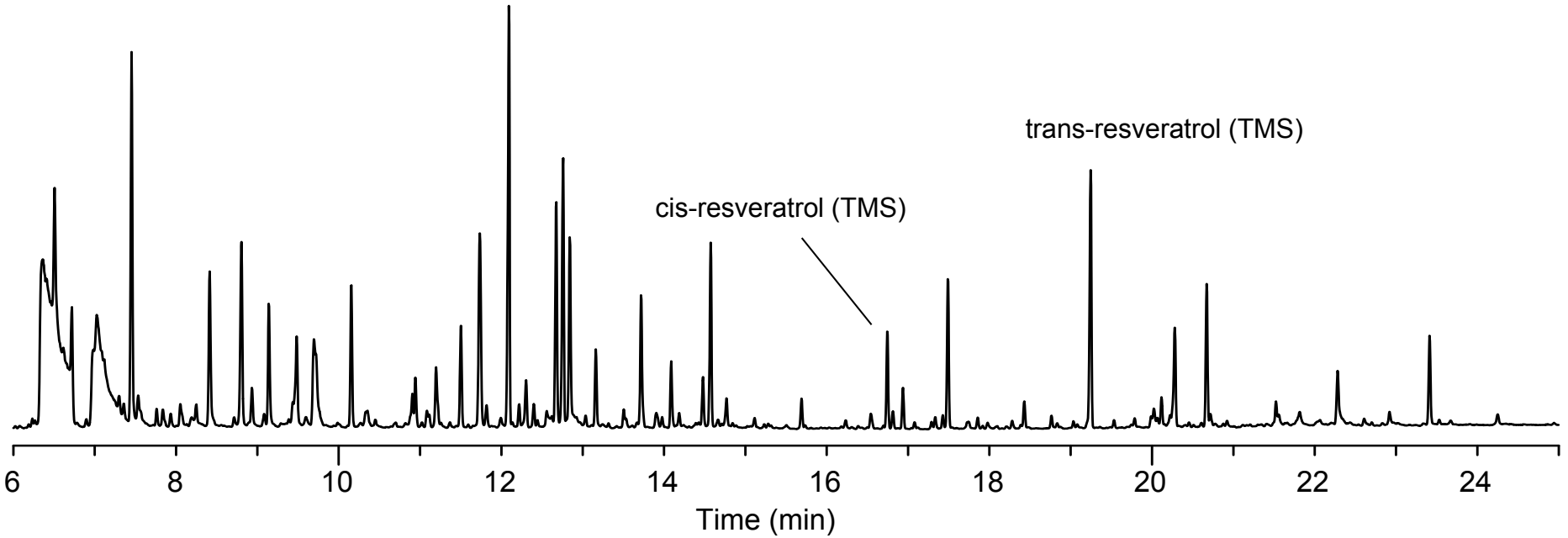
Resveratrol in red wine

- Field: Food & Beverage
- Sample Prep and Analysis Tools: SPME, GC (SLB-5ms), on-fiber derivatization
- Abstract: SPME when used in combination with on-fiber derivatization was found to be applicable to the extraction of resveratrol from red wine. The technique was found to be highly sensitive, simple, and quantitative. The polyacrylate fiber was also found to withstand exposure to the vapors of the silylating reagent without damage resulting from swelling.
- References: Supelco Reporter, vol. 27.4, pg. 18

Resveratrol in Merlot wine

- Spiked wine sample
- Extracted with SPME
- Analyzed by GC-MS

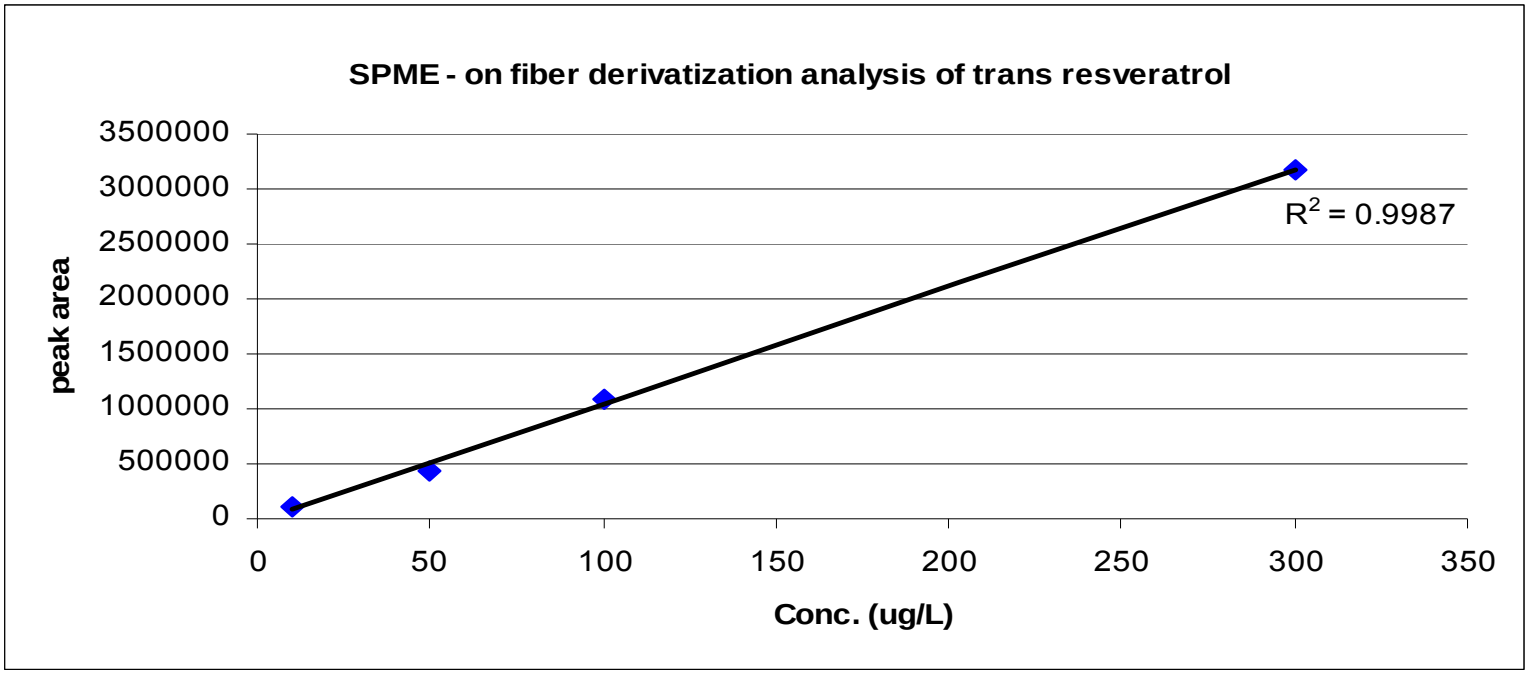




SPME fiber: 85 µm polyacrylate
Column: SLB-5ms; 30 m x 0.25 mm I.D., 0.25 µm

Linearity of Method

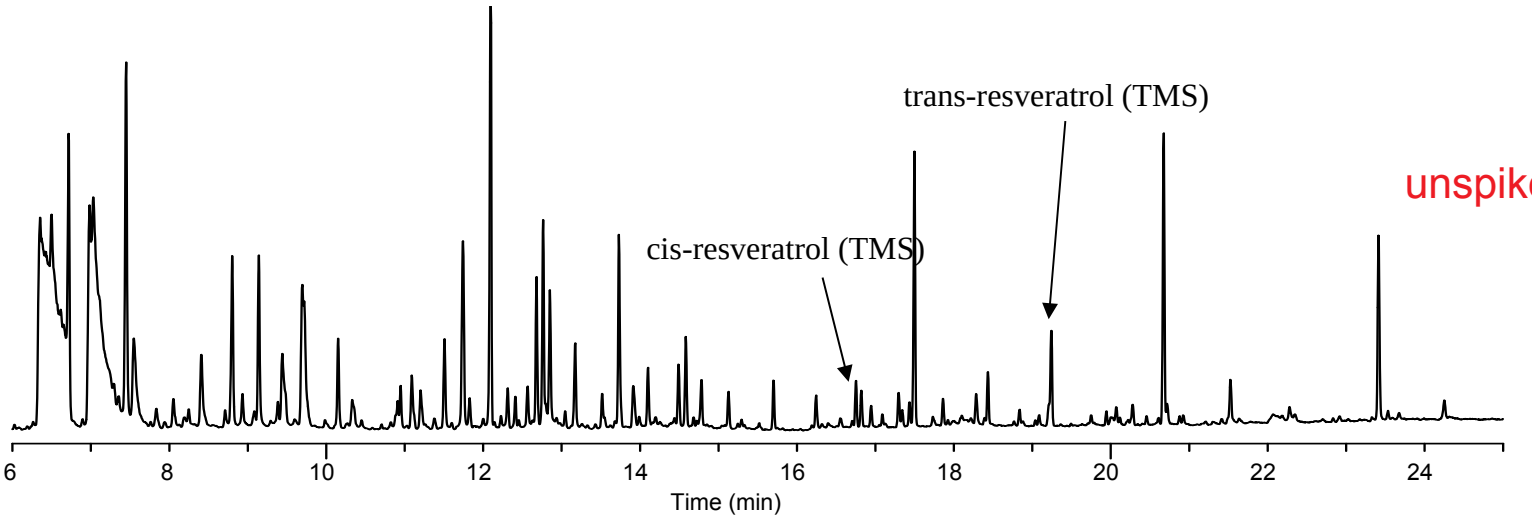
Trans-resveratrol standards prepared in 12% ethanol in water



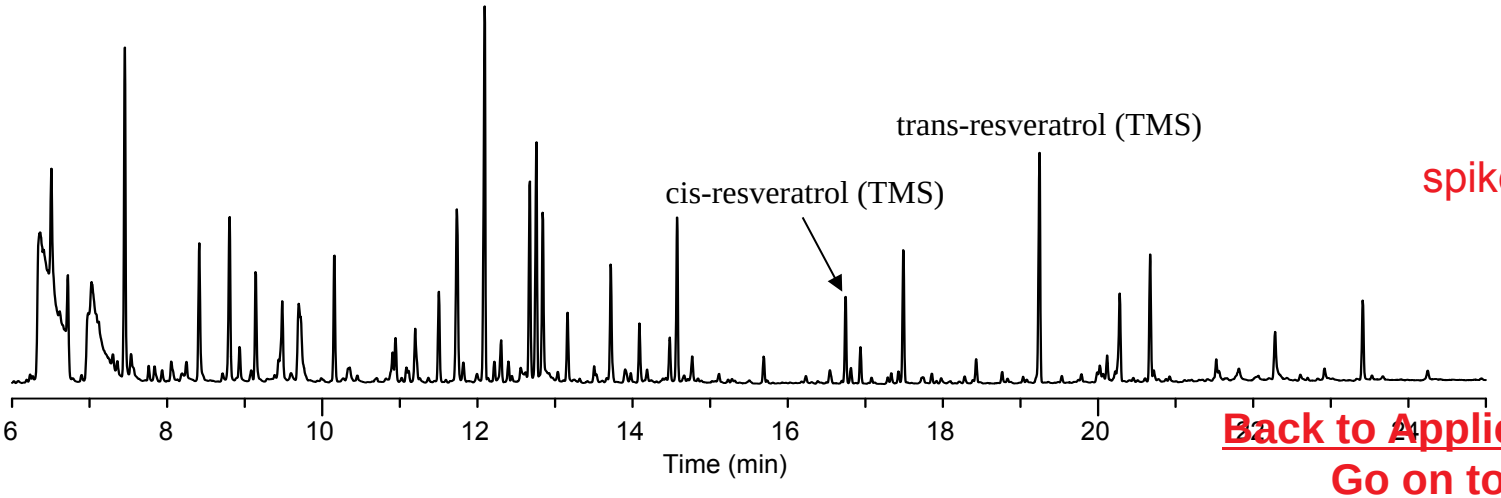
Quantification of spiked wine sample

	Unspiked red wine	Spiked red wine (100 ug/L)
Conc. of trans-resveratrol (ug/L)	22.6	134.7
% recovery	---	110%

SPME-GC/MS Analysis of red wine samples



unspiked wine



spiked wine

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TCA & precursors in red wine (cork taint)

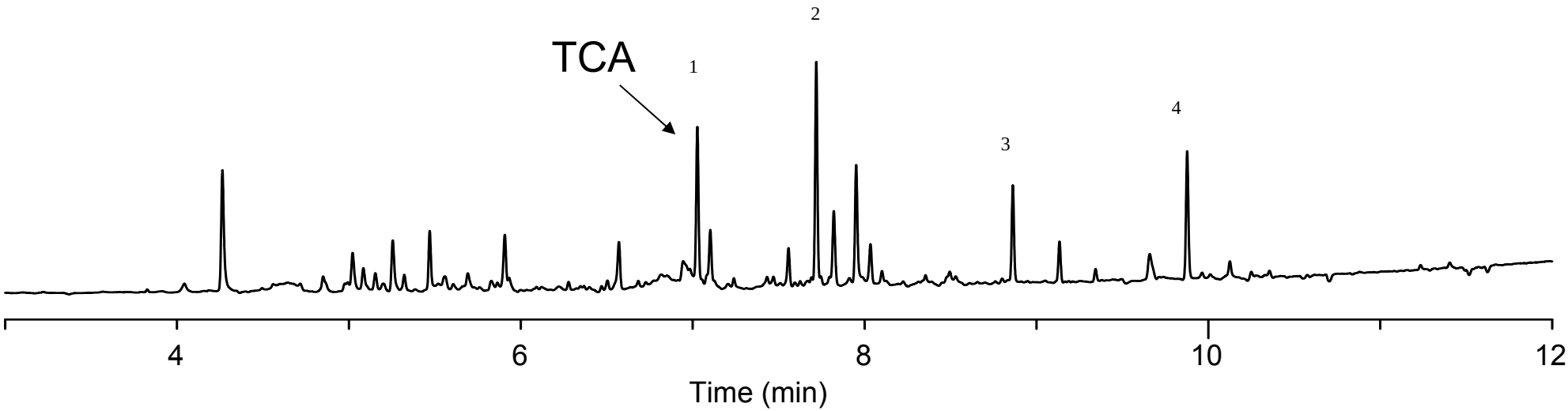
- Field: Food & Beverage

- Sample Prep and Analysis Tools: Headspace SPME, GC (SLB-5ms)

- Abstract: The ability of headspace SPME, in combination with analysis on the SLB-5ms, to detect a low level of 2,4,6- trichloroanisole (TCA) in wine is shown here. TCA is often the source of the musty smell in wine resulting from tainted corks. This is another example of how the SLB-5ms column provides low bleed and inertness to meet the demands of today's sensitive GC-MS and GC applications.

- References: Supelco Reporter, vol. 24.4, pg. 14

TCA & precursors in red wine (cork taint)



SPME of “Cork Taint” and its precursors

- “Cork taint” or a musty odor sometimes detected in wine, is the result of 2,4,6-trichloroanisole (TCA). The source of TCA is thought to be the fungal methylation of chorophenols present in the wine, with these compounds emanating from the cork or other sources such as biocides, fungicides, and exposure of processing equipment to antiseptic cleaning products containing halophenols (1).

- This application demonstrates the use of solid phase microextraction (SPME) for the analysis of TCA and several chlorophenolic precursors from wine. The chlorophenols were derivatized in matrix using acetic anhydride, and the acylated derivatives extracted from the headspace. The TCA, which is not derivatized, was simultaneously extracted with the halophenols. Final analysis was performed by GC-ECD on the SLB-5ms capillary column.
- Target analytes were:

  2,4,6-Trichloroanisole

  2,4,6-Trichlorophenol

  2,3,4,6-Tetrachlorophenol

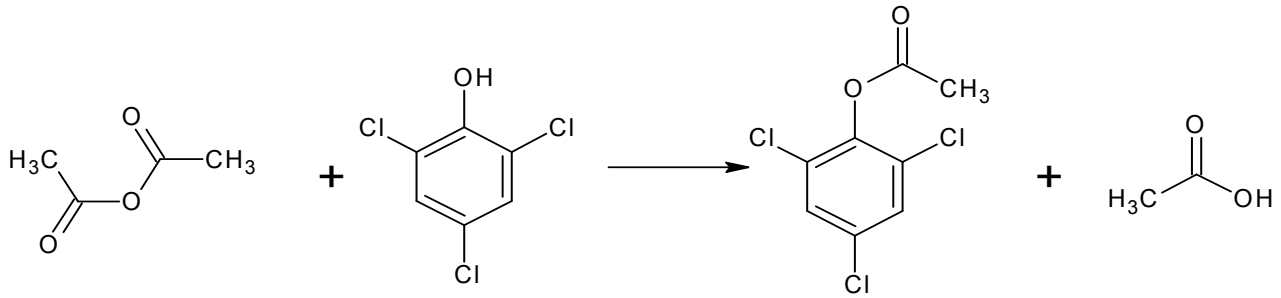
  Pentachlorophenol

Extraction and analysis conditions

- sample: 1.5 mL sample + 600 µL 5% K2CO3 + 240 mg NaCl + 60 µL acetic anhydride
- SPME fiber: metal fiber coated with 100 µm PDMS (57928-U)
- extraction: headspace, 50 ° C, 30 min., with stirring
- desorption temp.: 250 ° C, 3 min.
- column: SLB-5ms; 30 m x 0.25 mm I.D. x 0.25 µm (28471-U)
- oven: 50 ° C (1 min.), 25 ° C/min. to 280 ° C
- detector: ECD, 290 ° C
- carrier gas: helium, 1.5 mL/min constant flow
- liner: 0.75 mm I.D. SPME

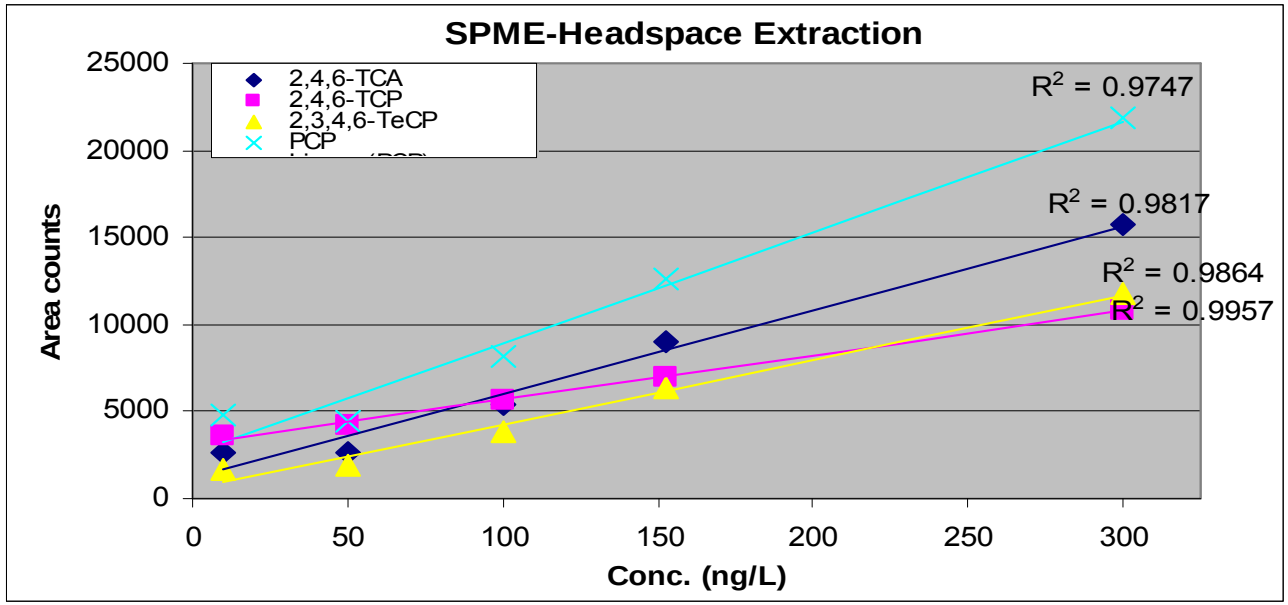
Derivatization reaction

- The halophenols were acylated with acetic anhydride prior to extraction. Acetic anhydride will hydrolyze in the presence of water, however the phenolic groups present on the analytes are more reactive, making it possible to conduct derivatization in an aqueous matrix (2). The addition of K_2CO_3 drives the reaction by removing the acetic acid that is formed:



Linearity

- Standards from 10-300 ng/L prepared in 12% ethanol in water were derivatized, extracted, and the response vs. concentration was plotted:



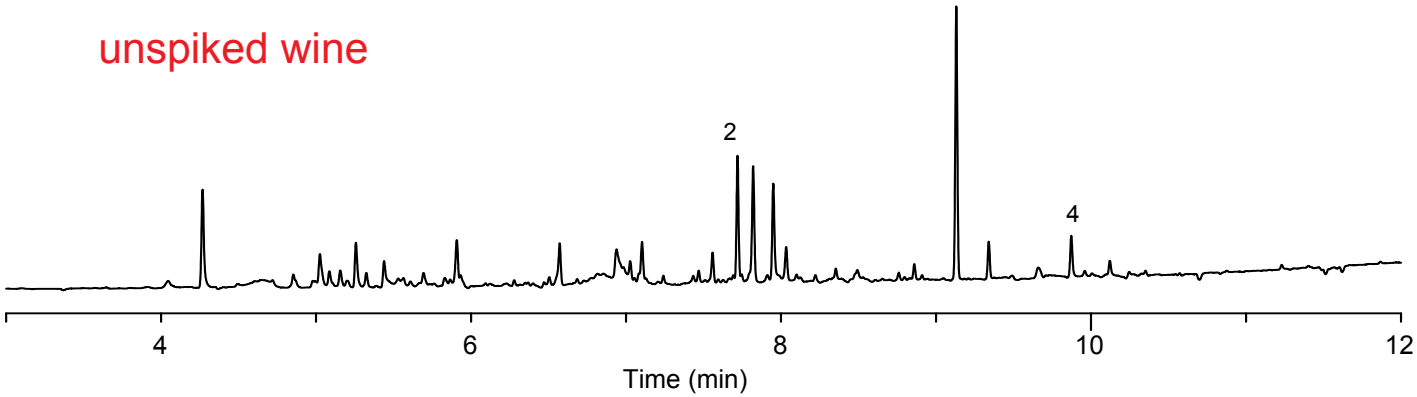
Standards in 12% ethanol in water

- Good linearity was obtained, indicating the method to be quantitative

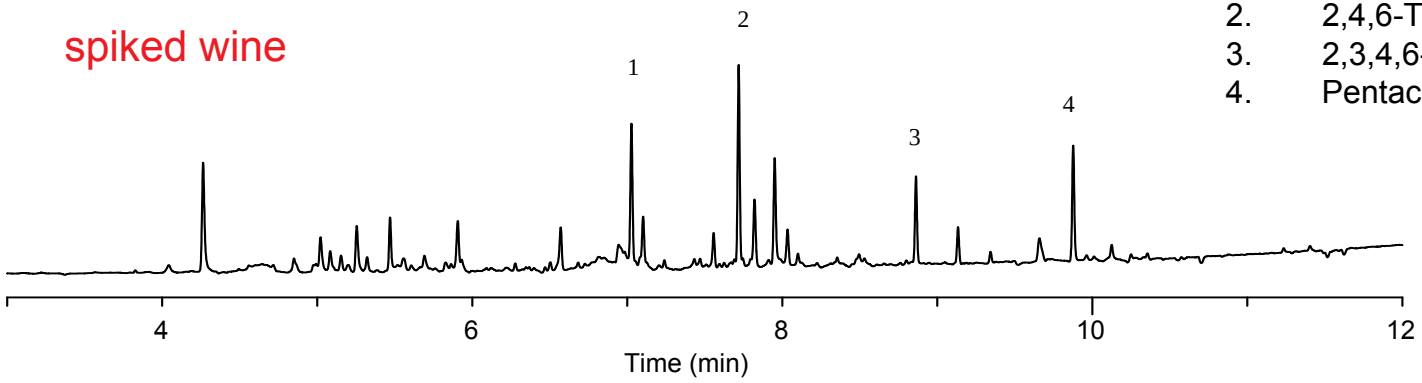
SPME-GC/ECD Analysis of wine sample

- The wine sample used for extraction was a California shiraz in a wax-lined carton-type container with a plastic closure. This type of packaging was chosen to minimize the presence of “cork taint” compounds.

unspiked wine



spiked wine



1. 2,4,6-Trichloroanisole
2. 2,4,6-Trichlorophenol (acylated)
3. 2,3,4,6-Tetrachorophenol (acylated)
4. Pentachlorophenol (acylated)

Quantification of spiked wine sample

- Unspiked and spiked (100 ng/L) red wine samples were extracted and recovery was determined against the calibration curves generated using the extracted standards in 12% ethanol.

Spike level of 100 ng/L	Unspiked wine (ng/L)	Spiked wine (ng/L)	%Rec.
2,4,6-Trichloroanisole	ND	60.7	61
2,4,6-Trichlorophenol	22.7	96.3	74
2,3,4,6-Tetrachlorophenol	ND	55.7	56
Pentachlorophenol	3.3	33.5	30

- All four analytes were recovered, however it appears that the wine matrix may have interfered with accuracy to some extent, as indicated by the % recovery values.

Reproducibility

- A check of reproducibility was performed by doing extractions of sets of three spikes prepared in the 12% ethanol in water and red wine.

(area counts)	2,4,6-TCA	2,4,6-TCP	2,3,4,6-TeCP	PCP
12% EtOH 100	4272	4495	2775	5011
12% EtOH 100	4063	4374	3074	5766
12% EtOH 100	4235	4299	3168	6055
Avg; 12% EtOH	**4190**	**4389**	**3006**	**5611**
std. Dev	**112**	**99**	**205**	**539**
% RSD, 12% ETOH	**3%**	**2%**	**7%**	**10%**
wine 100	3298	4231	1977	2750
wine 100	2986	3916	1613	2335
wine 100	2727	2341	1843	2745
Avg.; wine	**3004**	**3496**	**1811**	**2610**
std. Dev	**286**	**1013**	**184**	**238**
% RSD, spiked wine	**10%**	**29%**	**10%**	**9%**

Reproducibility for the 12% ethanol in water samples was good, with %RSD values of <10%. The data indicates the effect of matrix, with average area counts lower and more variable overall for the wine samples.

Conclusions

- SPME can be used for the extraction of 2,4,6-trichloroanisole and its halophenolic precursors from wine.
- Derivatization makes the analytes easier to extract and analyze by GC.
- Headspace extraction in combination with ECD can be used to reduce background interference.
- The method appears to be quantitative, although further work would be necessary to optimize extraction efficiency from wine matrix.

References

- 1. Insa, S., Salvado, V., Antico, E., Development of solid-phase extraction and solid-phase microextraction methods for the determination of chlorophenols in cork macerate and wine samples. J. Chromatogr. A, 2004, 1047: 15-20.
- 2. K. Blau; J. Halket, Handbook of Derivatives for Chromatography, Second Edition, John Wiley & Sons, New York, 1993. pp 38.

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Agricultural pesticides in wine

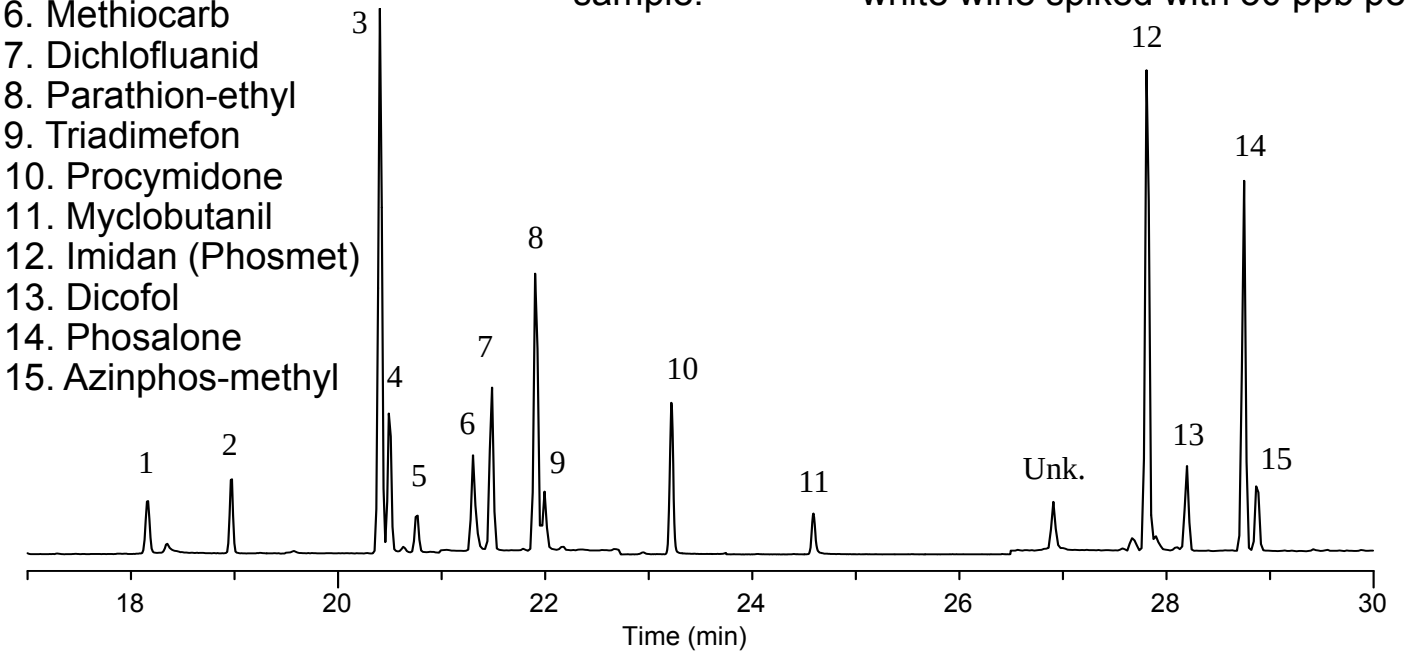
- Field: Food Safety
- Sample Prep and Analysis Tools: SPME, GC
- Abstract: This application demonstrates the usefulness of SPME in the low-level extraction of agricultural pesticides from wine, and the use of the SLB-5ms in the subsequent analysis. The pesticides chosen for the analysis represent a group of insecticides and fungicides that could be found in commercial wines. These compounds contain a variety of polar functional groups, and the polyacrylate fiber provided the selectivity necessary for extraction from a wine matrix. The inertness and low bleed of the SLB-5ms enabled subsequent low-level analysis of these compounds by GC-MS.
- References: Supelco Reporter, vol. 24.4, pg. 14

Agricultural pesticides in wine

SPME-GC/MS Analysis of spiked wine sample

column:	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
SPME fiber:	85 µm polyacrylate (57304)
extraction:	immersion, room temp. (30 min.)
desorption:	5 min. at 250 ° C
oven:	60 ° C (1 min.), 15 ° C/min to 100 ° C, 7 ° C/min. to 300 ° C (1 min.)
MSD interface:	325 ° C
scan range:	SIM
carrier gas:	helium, 0.7 mL/min., constant
liner:	0.75 mm I.D. SPME liner
sample:	white wine spiked with 50 ppb pesticides

Peak IDs:
1. Dicloran
2. Diazinon
3. Chloropyrifos-methyl
4. Vinclozolin
5. Carbaryl
6. Methiocarb
7. Dichlofluanid
8. Parathion-ethyl
9. Triadimefon
10. Procymidone
11. Myclobutanil
12. Imidan (Phosmet)
13. Dicofol
14. Phosalone
15. Azinphos-methyl



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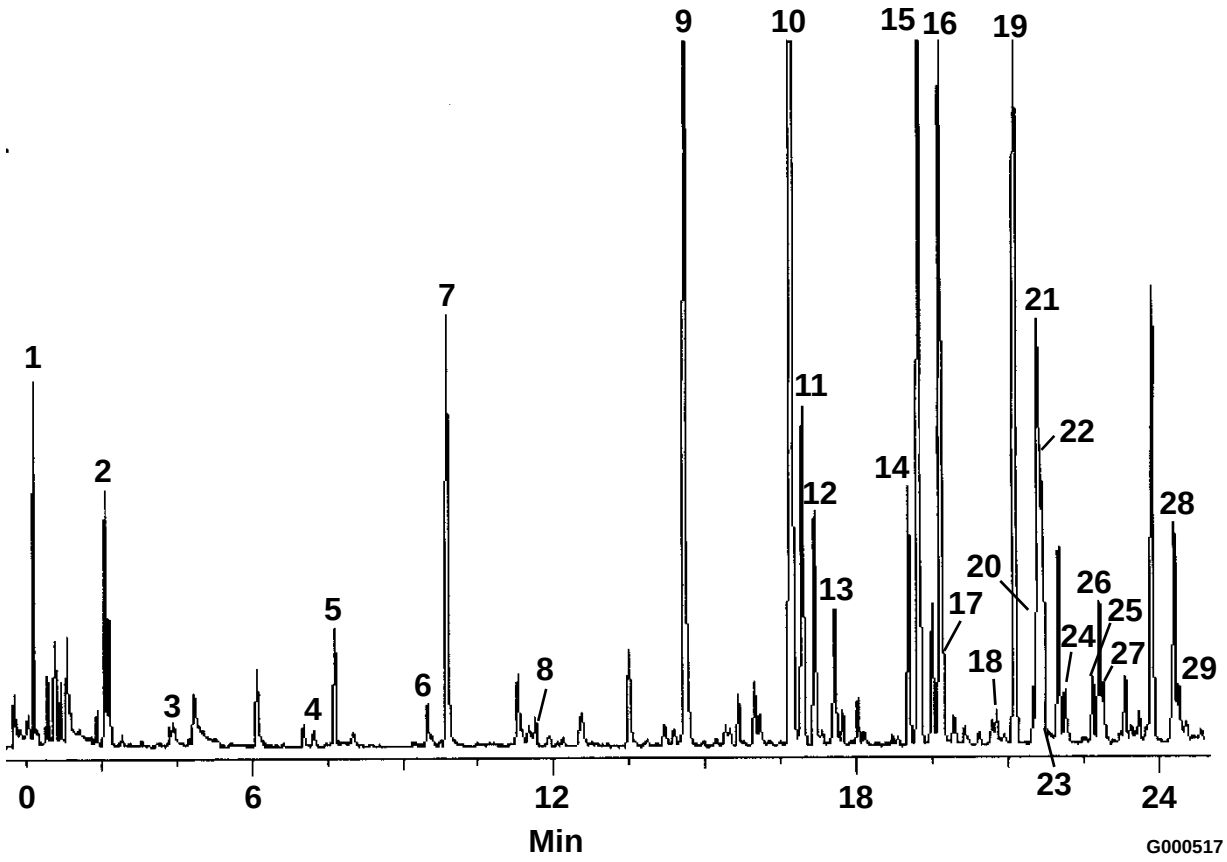
Peanut Butter Flavors by SPME

Sample:	5g peanut butter in 40mL vial
SPME Fiber:	DVB-Carboxen™-PDMS (StableFlex™ Fiber)
Extraction:	headspace, 30 min at 65° C in heating block
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.



SIGMA-ALDRICH®

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

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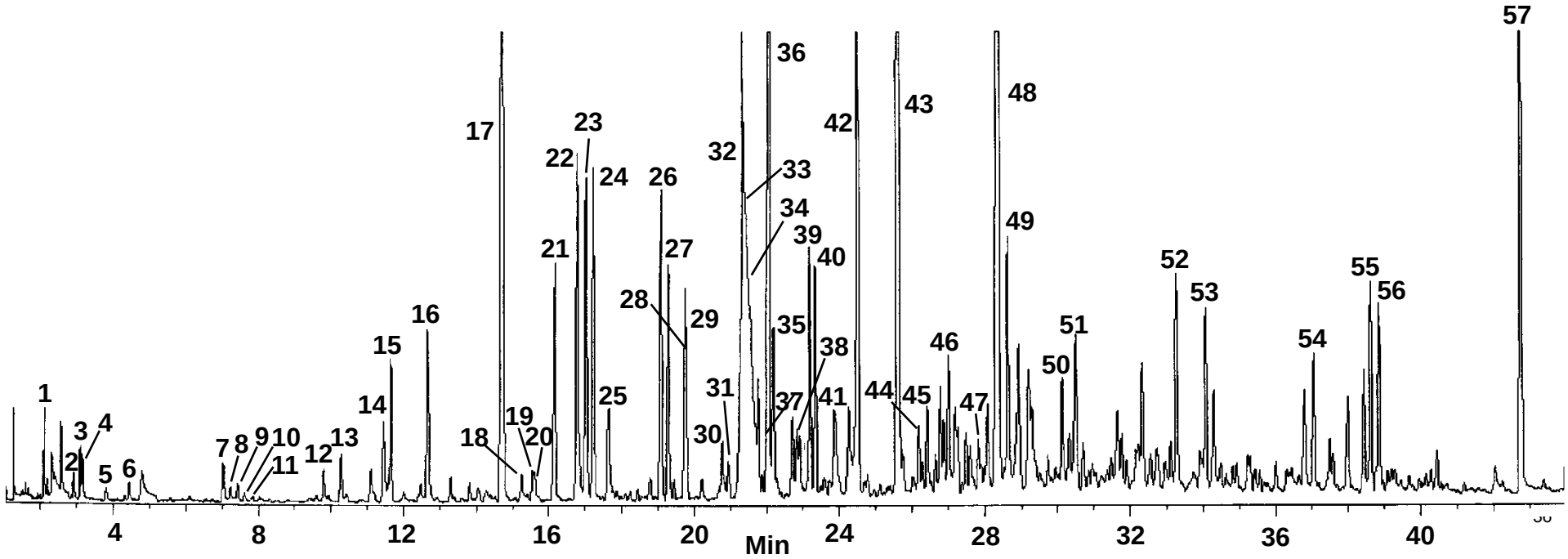
Regular Coffee Grounds by SPME

Sample: 5g coffee grounds in 40mL vial
SPME Fiber: DVB/Carboxen™/PDMS (StableFlex™ Fiber)
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.



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

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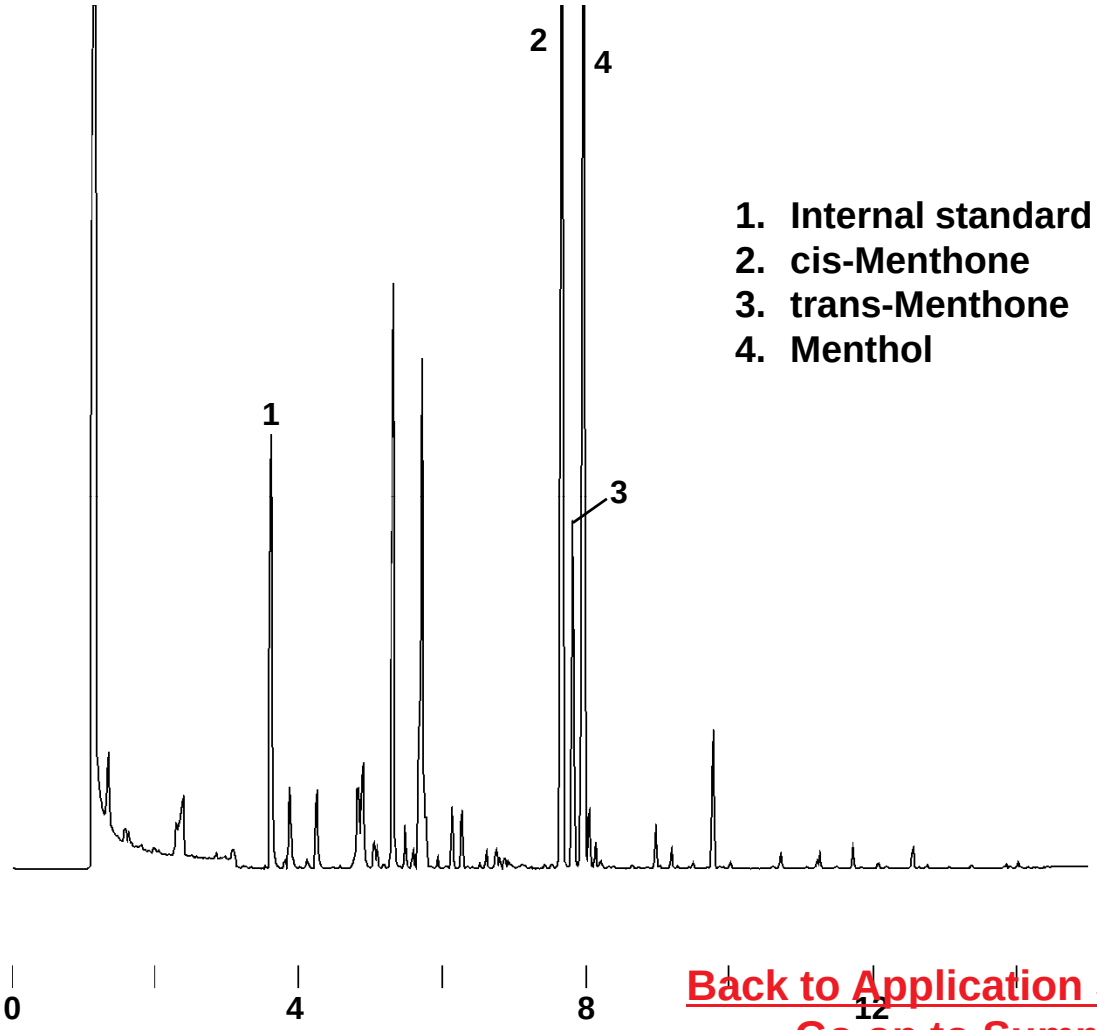
Peppermint oil in chocolate bar

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. Internal standard
2. cis-Menthone
3. trans-Menthone
4. Menthol

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SIGMA-ALDRICH®

Supelco SPME Bulletins

925 “SPME-Applications Guide” **(only on CD & web)**

923 “Theory and Optimization of Conditions”

928 “Trouble Shooting guide”

929 “Practical Guide to Quantification SPME”

901 “Drugs, Alcohol, org. Solvents in Biological Fluids”

922 “Forensic Applications: Explosives, Fire Debris, and Drugs of Abuse”

869 “Flavour and Fragances”

All on the SPME-CD plus additional applications & videos

SIGMA-ALDRICH®

Thank you!

S ample
P rep
M ade
E asy