Versatile New SBSE Techniques for Flavor, Fragrance, and Odor Analysis

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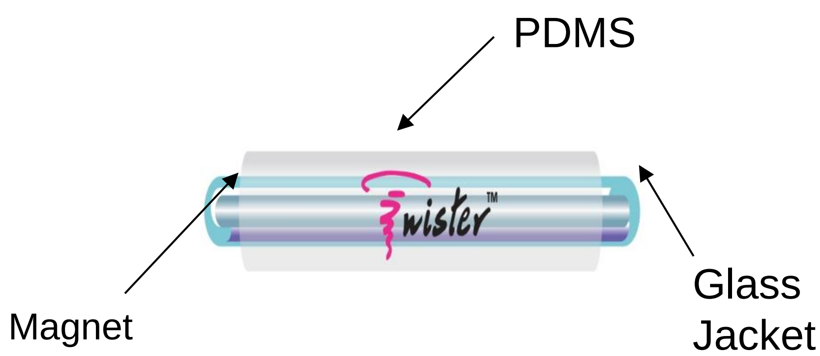
Objectives and Goals

- To demonstrate versatility of SBSE and HSSE for flavor, fragrance and odor analysis.
- To show application potential of EG-Silicone Twister for unusual sample types and analytes.
- To explain advantages over other analytical sample preparation techniques prior to GC-MS analysis.

Stir Bar Sorptive Extraction: SBSE



SBSE is by nature an equilibrium technique.
The extraction mechanism is similar to that of SPME, but the Enrichment Factor is much higher [1)] because of larger amount of sorptive phase on the Twister.

After sampling, absorbed compounds are thermally desorbed.

EG-Silicone Twister

1) E. Baltussen *et al*, *J. Microcol. Sep.*, 11 (1999) 737

The GERSTEL Twister

Automated Extraction from Liquid Samples

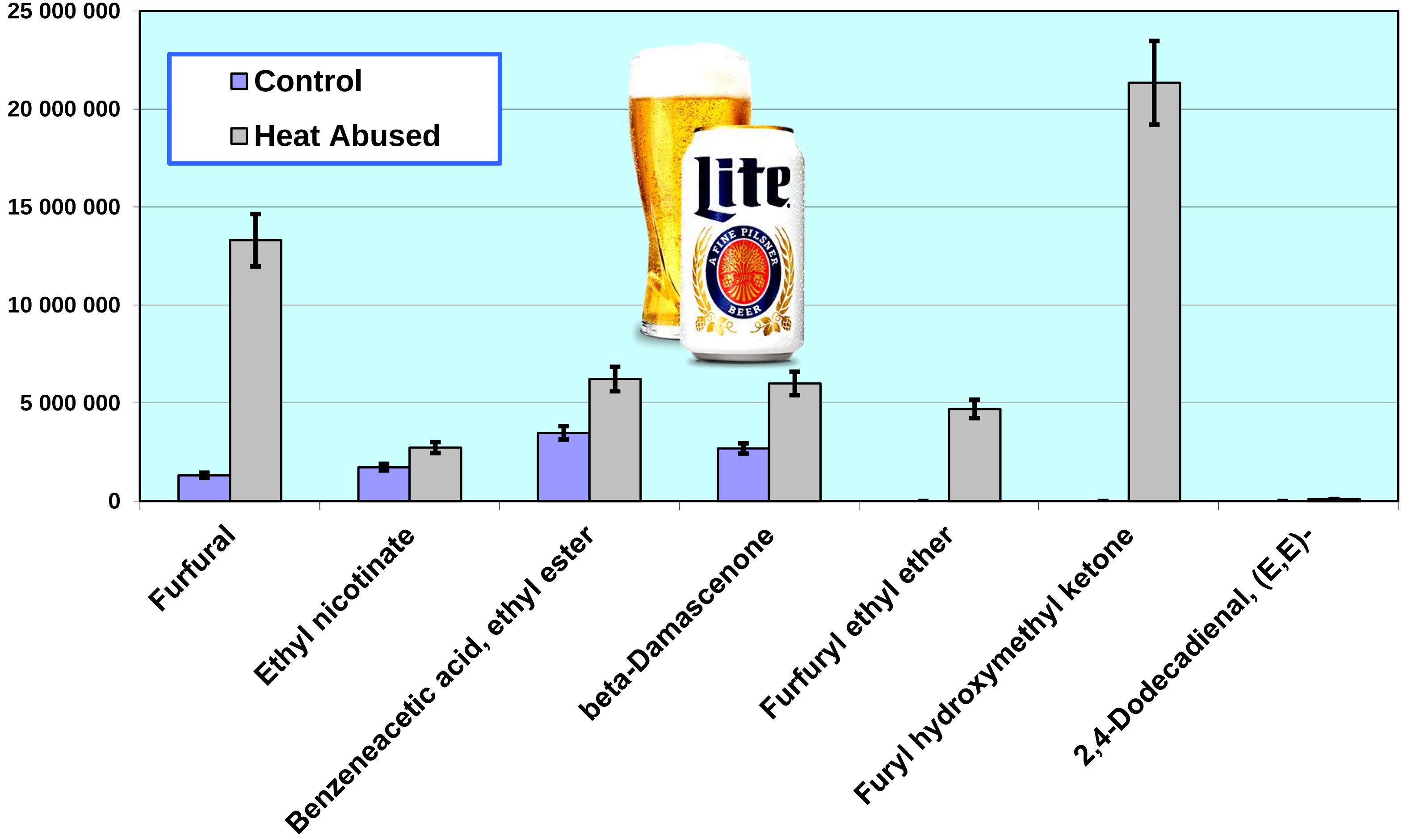
Magnetic core, sealed in glass and coated with PDMS

l = 10 mm, df = 0.5 mm → 24 µl
df = 1.0 mm → 63 µl
l = 20 mm, df = 0.5 mm → 47 µl
df = 1.0 mm → 126 µl

New Polar Phase: EG-Silicone 32 µL 10 mm
SPME : max. 0.5 µL

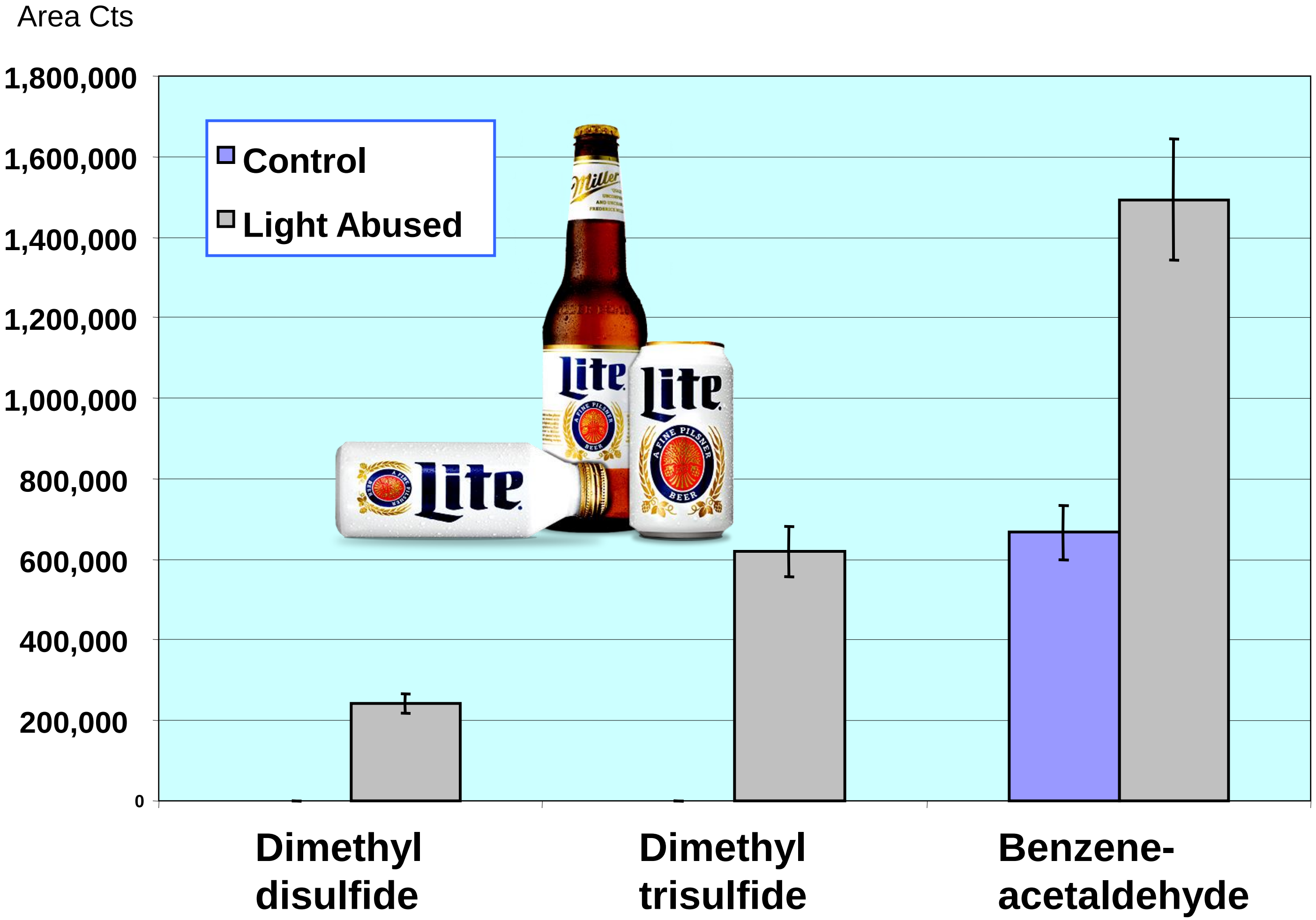
GERSTEL

Increases in Off-Flavor Chemicals in Heat-Abused Vs. Control Beer by PDMS SBSE/GC-TOFMS (Miller Lite Heat Abused: 30°C for 12 wks, 10 mL Extracted 1 hr)



J. Am. Soc. Brew. Chem. 65(3):129-137, 2007

Increases in Off-Flavor Chemicals in Light-Abused Vs. Control Beer by PDMS SBSE/GC-TOFMS (Miller Lite Light Abused: 8 hr Sunlight, 10 mL Extracted)



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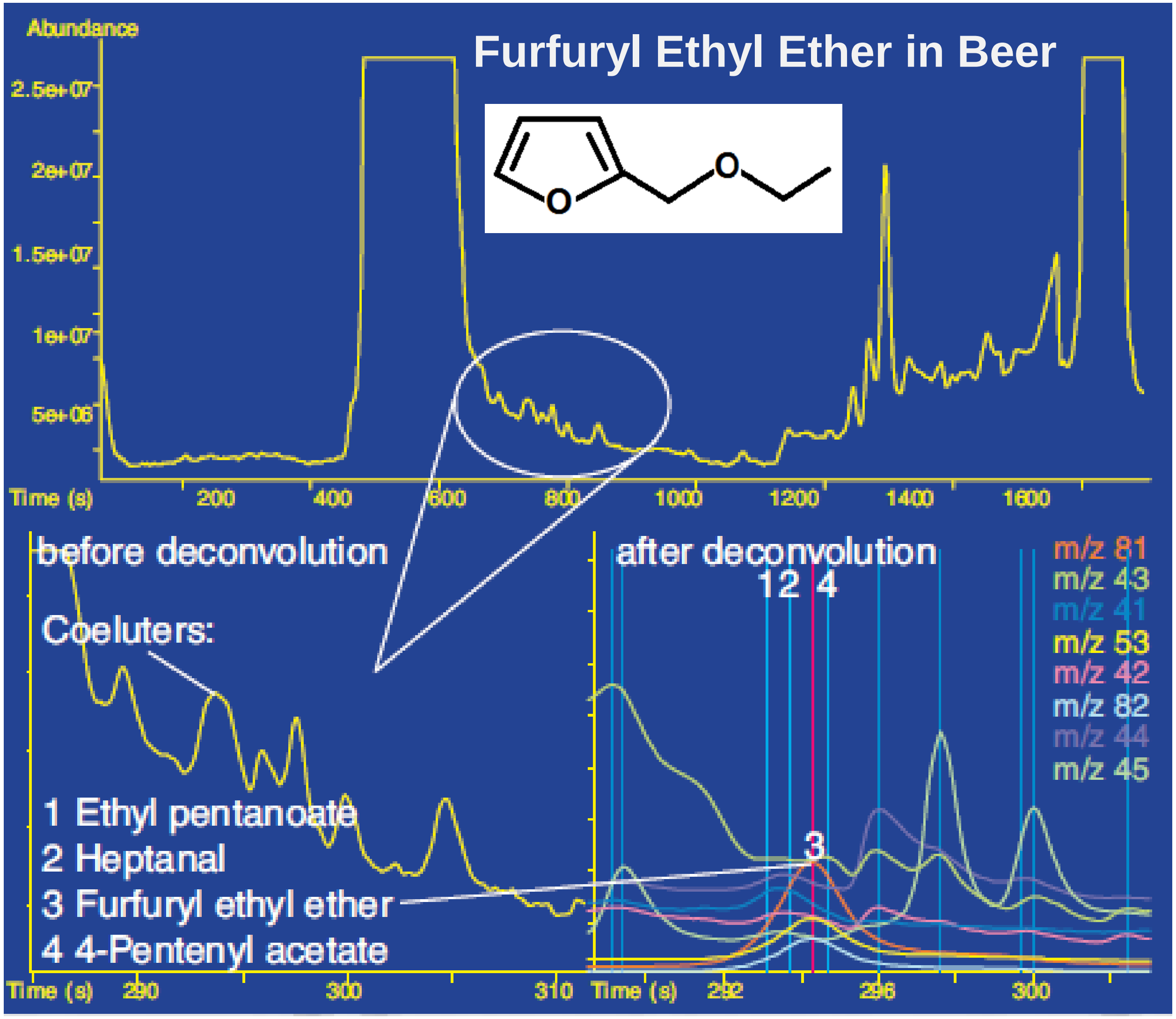


10 mL beer sample by PDMS Twister (1 cm x 0.5 mm) showing coelution of furfuryl ethyl ether and how peak deconvolution with Leco Pegasus HT GC-TOFMS can detect furfuryl ethyl ether coeluting with three other chemicals.

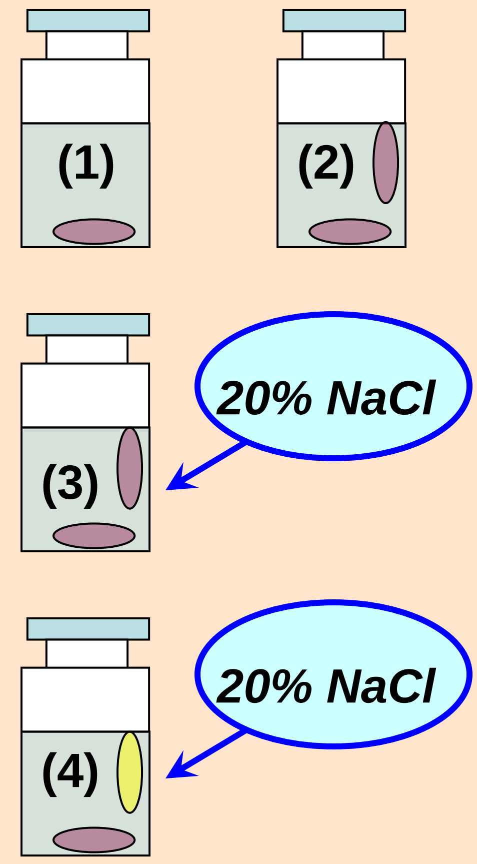
Analysis of Beer by Four SBSE Techniques

1) SBSE with one PDMS Twister 2 hr

2) SBSE with two PDMS Twisters 2 hr

3) Sequential SBSE with two PDMS Twisters
   - One stirring, one suspended in beer 1 hr
   - Stir an addition hr after adding 2 gm NaCl

4) Sequential SBSE with one PDMS Twister and one EG-Sil Twister
   - Stir one hr with PDMS Twister and EG-Sil Twister suspended in beer
   - Stir an additional hr after added 2 gm NaCl

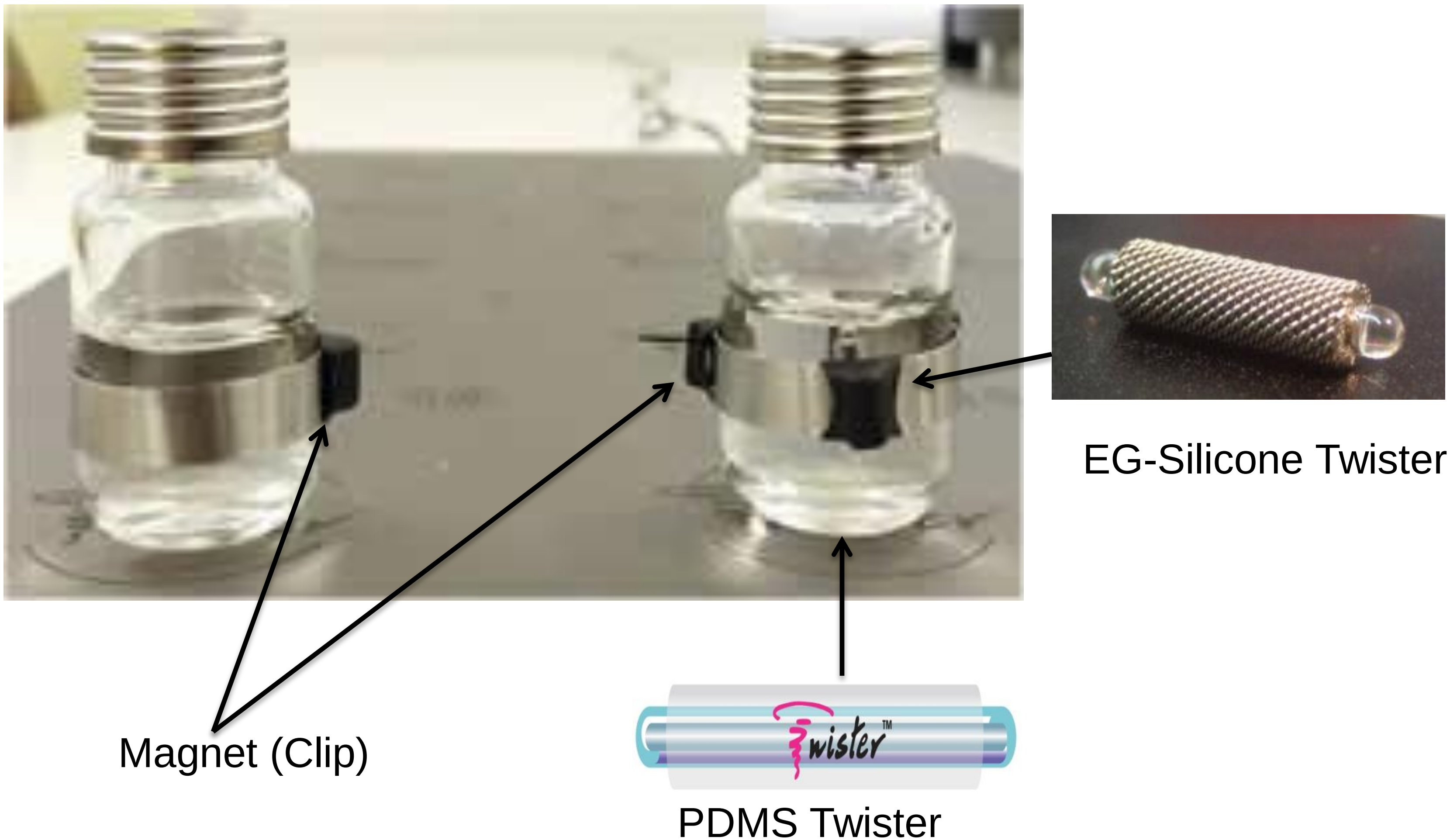


Note: 10 mL beer and stirring at 1000 rpm.

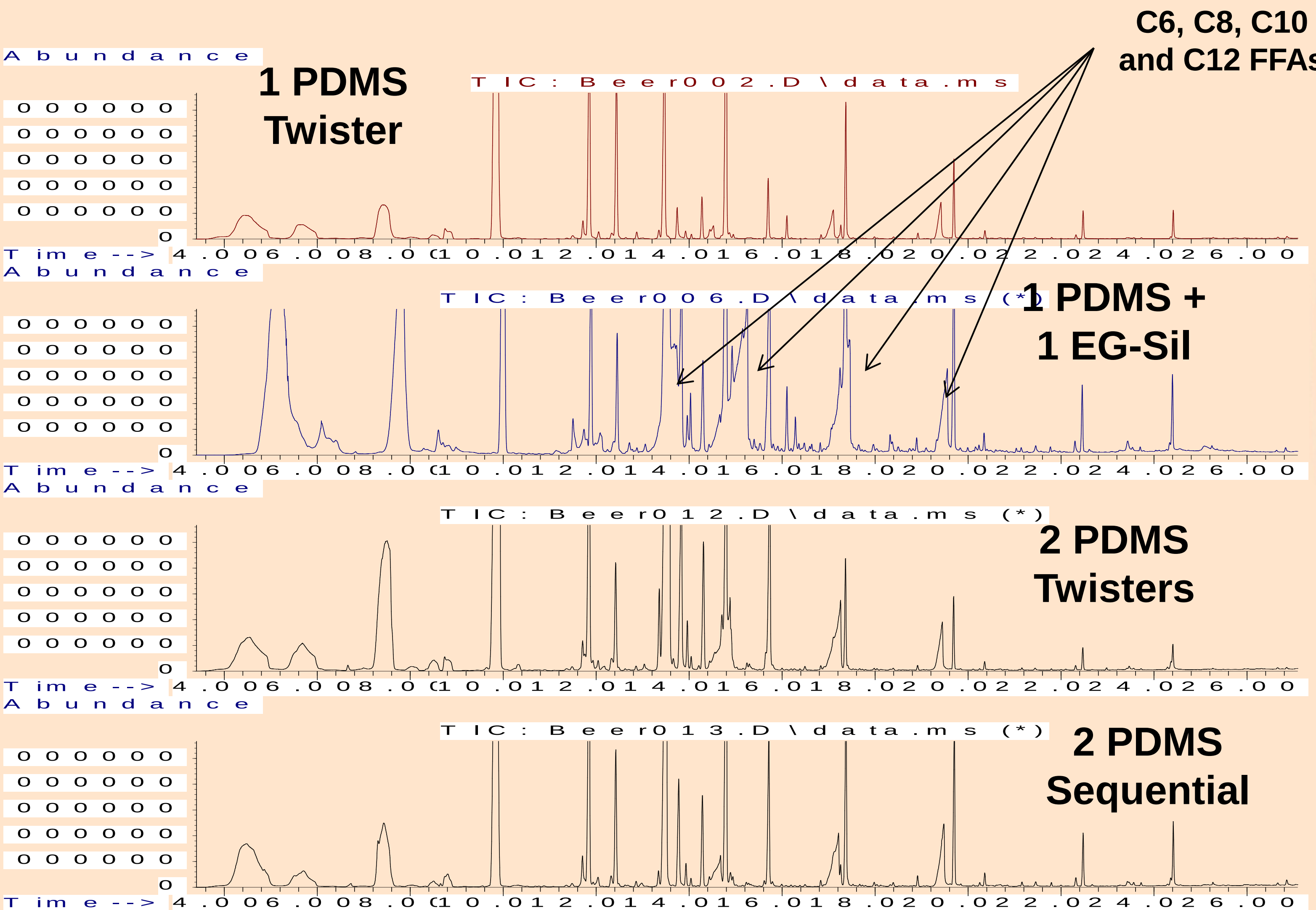
EG-Sil

PDMS 1cm x 0.5 mm

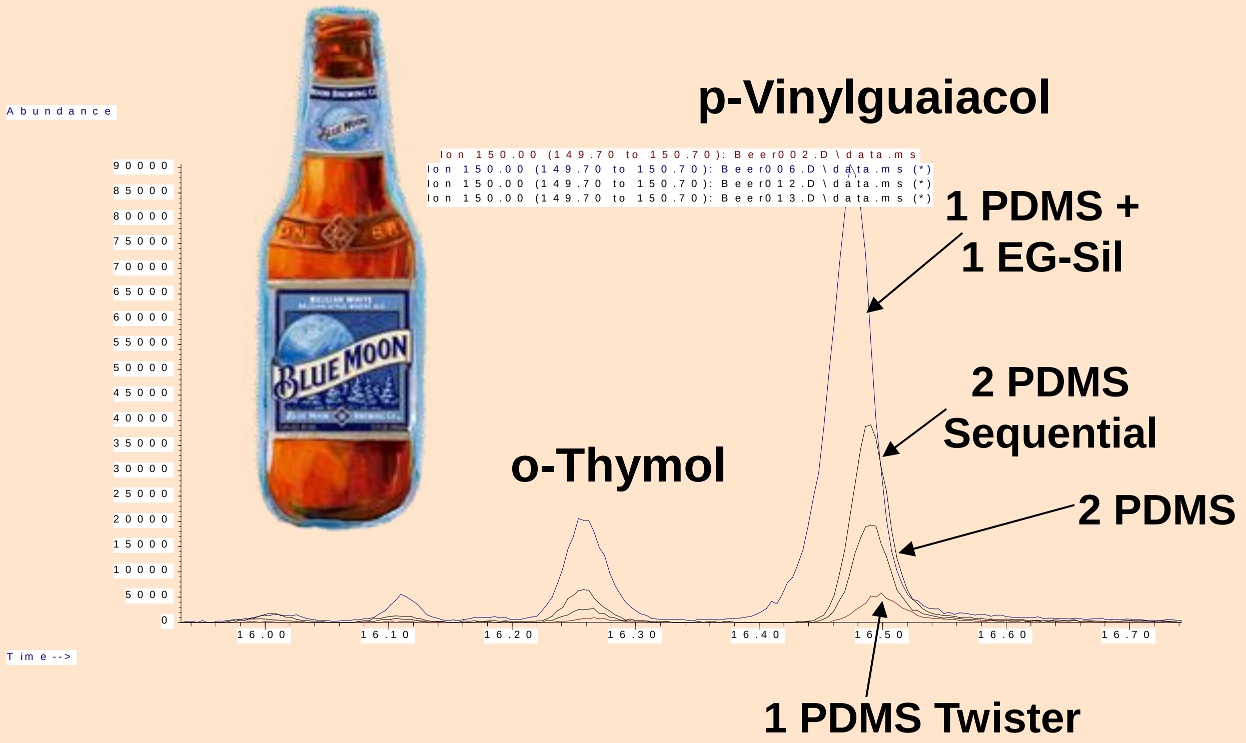
Technique for Extracting a Liquid Sample with Two Twisters Simultaneously (Twicester®)

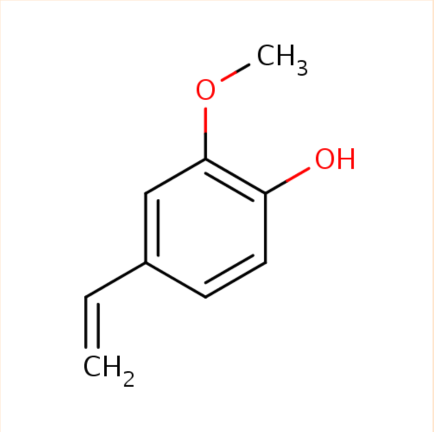


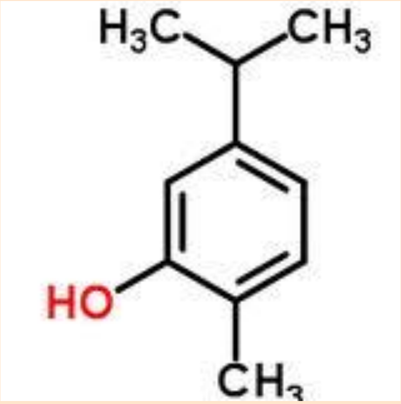
Comparison of Flavor Chemicals in Beer by Four SBSE Techniques



Comparison of SBSE Techniques in Beer: O-Thymol and p-Vinylguaiacol





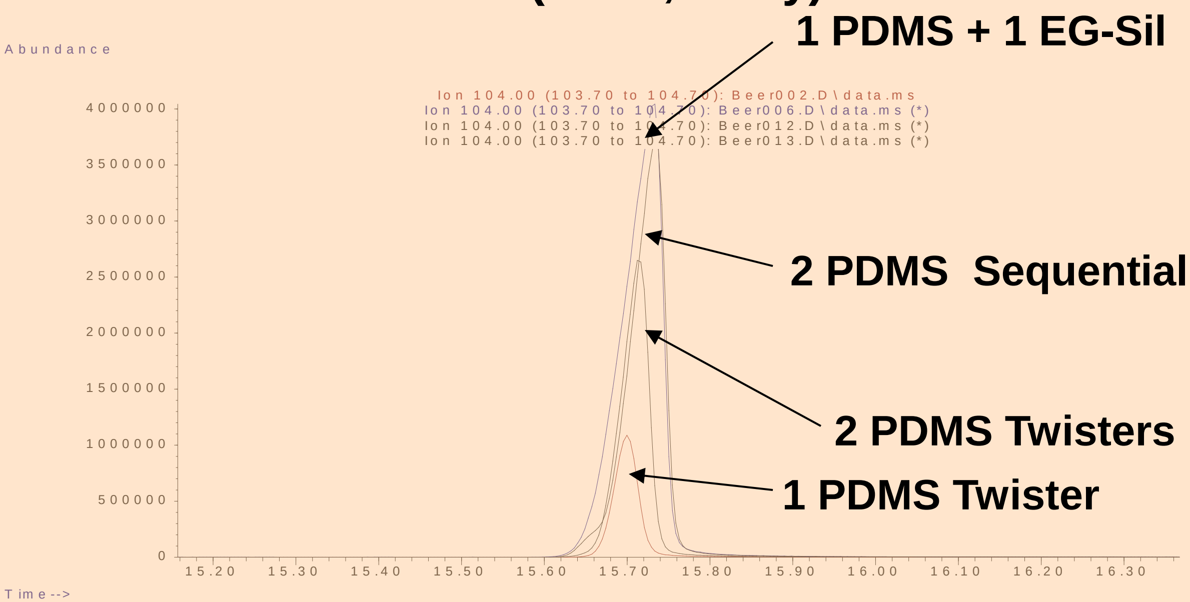


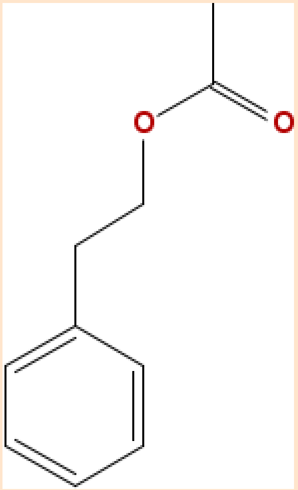
p-Vinylguaiacol (clove note) is a key flavor impact character in German-style wheat beers. With other beers, it is an off-flavor from wild yeasts.

3D Substance Structure

Comparison of SBSE Techniques in Beer: 2-Phenylethyl acetate

2-Phenylethyl acetate (floral, fruity)

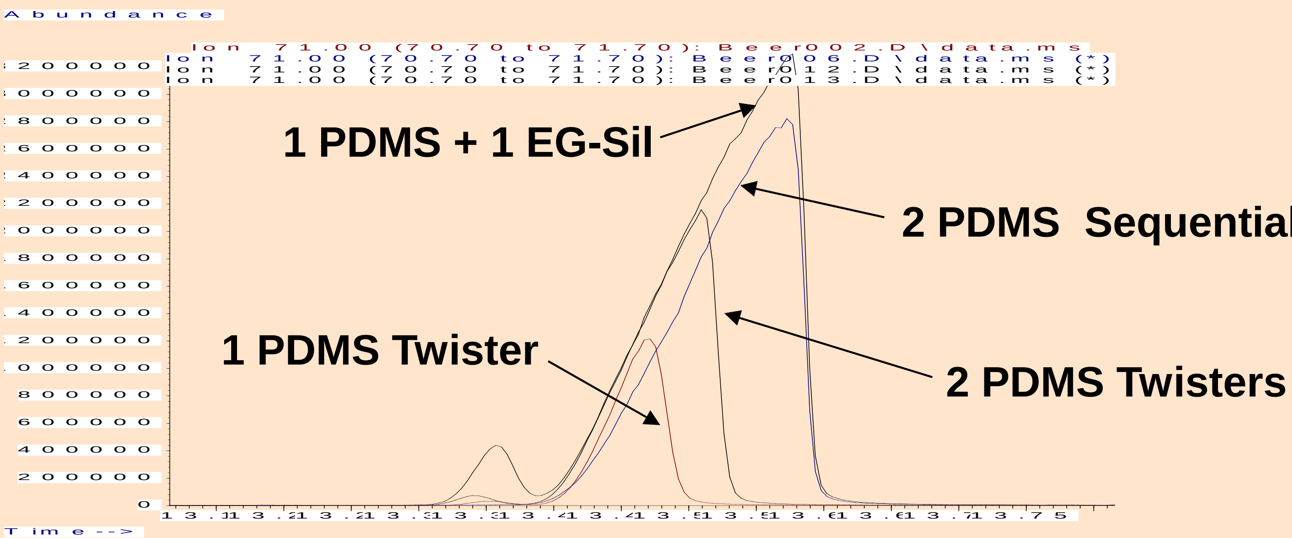


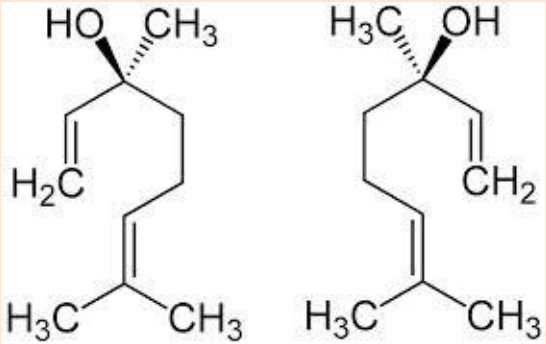


Other 2-phenylethyl esters detected include 2-phenylethyl octanoate and 2-phenylethyl decanoate.

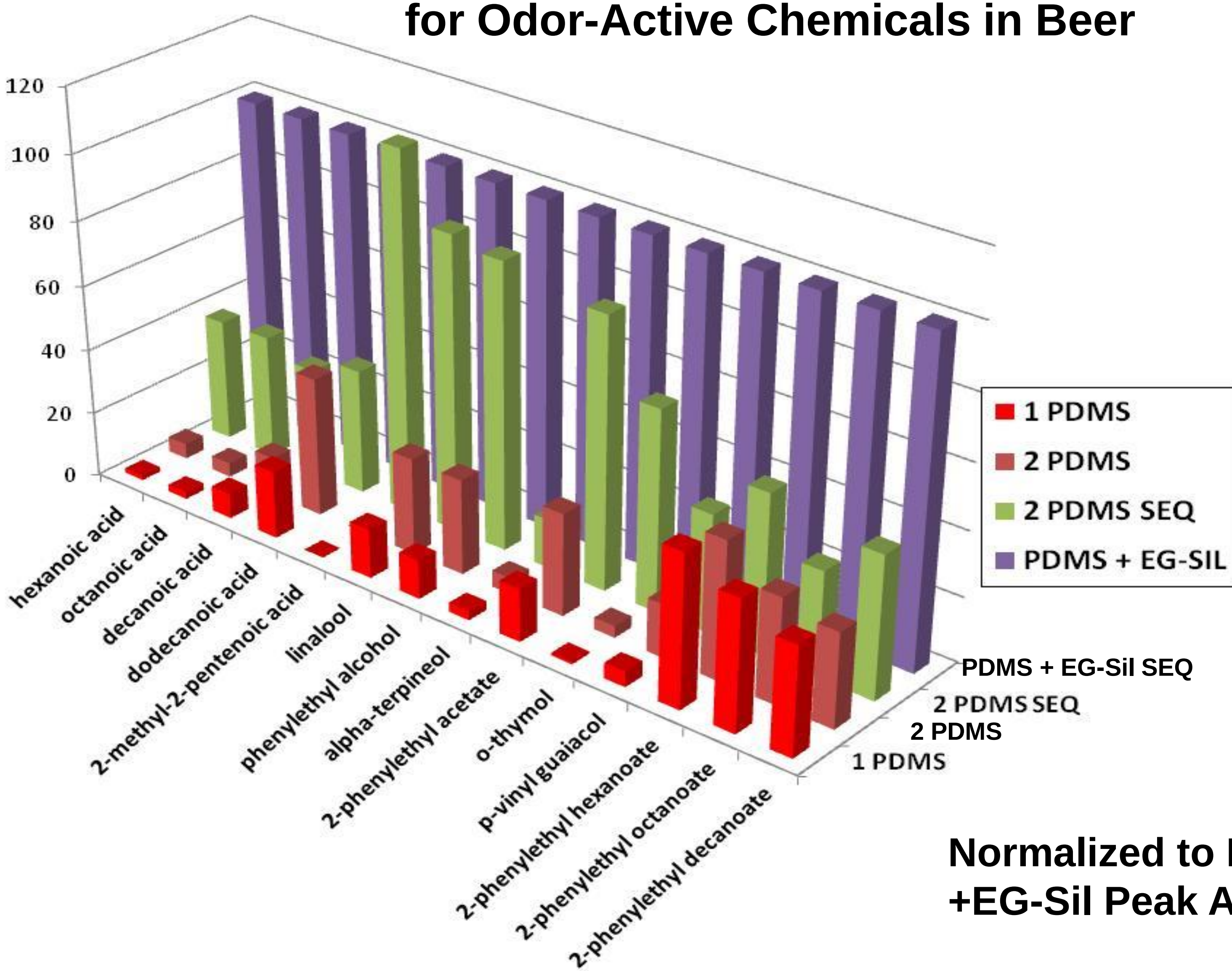
Comparison of SBSE Techniques in Beer: Linalool

Linalool (floral with touch of spiciness)





Comparison of Four SBSE Extraction Techniques for Odor-Active Chemicals in Beer



Normalized to PDMS +EG-Sil Peak Areas

Analysis of Axillary Odorants from Soiled Shirts

Odorless underarm secretions from eccrine, apocrine and sebaceous glands

Bacterial biotransformation (Staphylococcus, Micrococcus, Propionibacterium, and especially Corynebacterium)

Malodorants

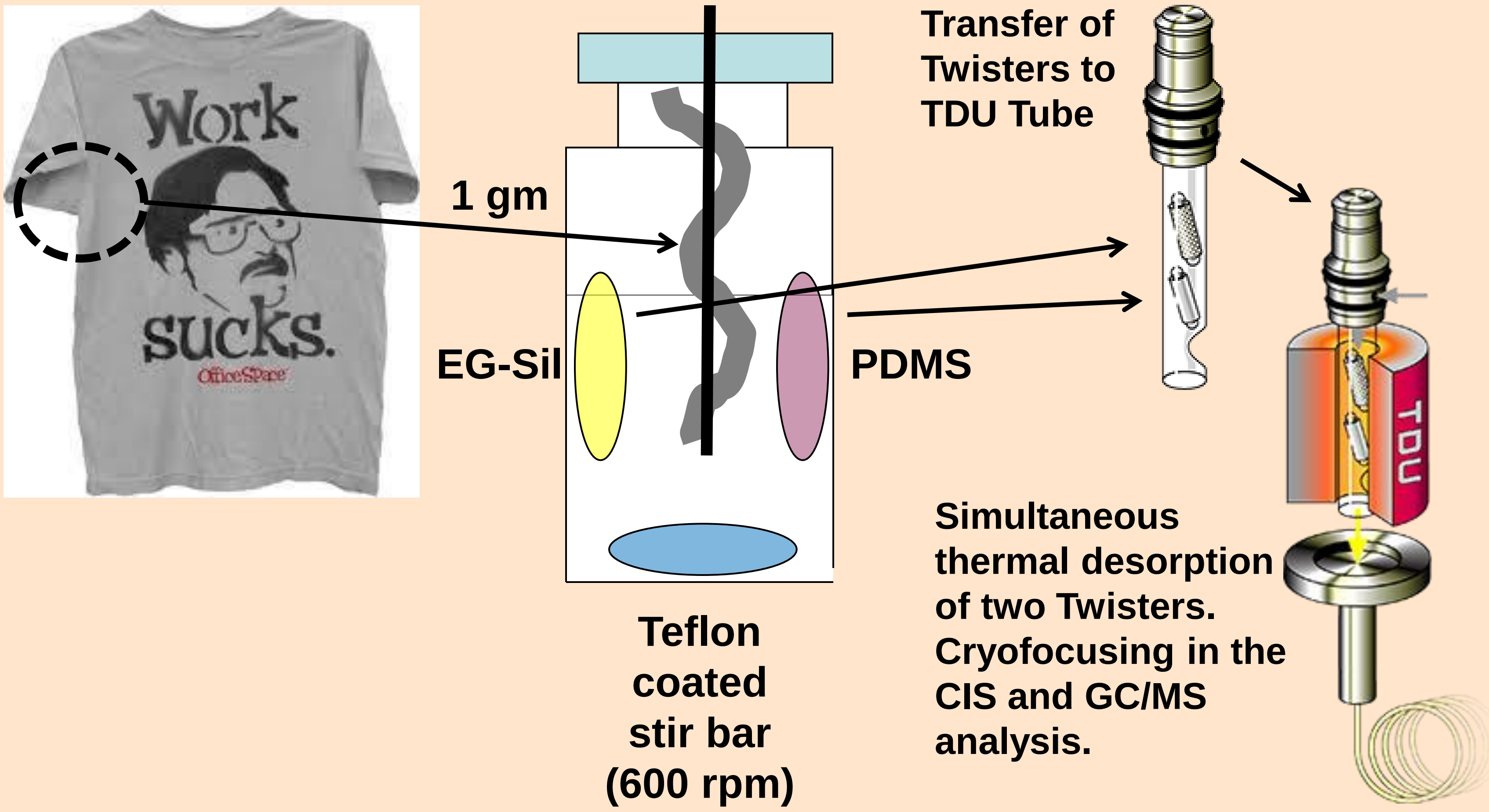
- Propanoic acid
- 2-Methyl propanoic acid
- Butanoic acid
- Pentanoic acid
- 2-Methyl butanoic acid
- 3-Methyl butanoic acid
- Hexanoic acid
- Octanoic acid
- (E)-3-methyl-2-hexenoic acid
- 3-Hydroxy-3-methyl hexanoic acid
- Other acids

Also interested in 2-nonenal and 5α-androst-16-en-3-one (androstenone) .

Analysis of Axillary Odorants from Soiled Shirts

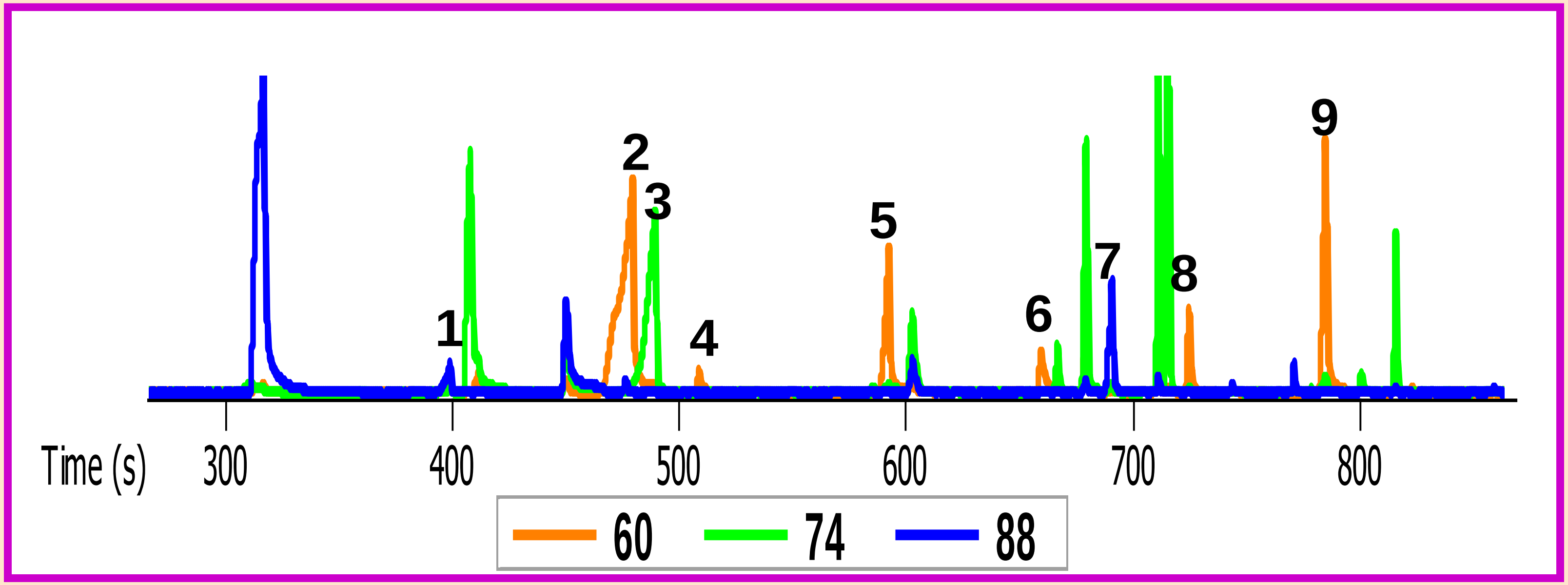
HSSE Using Combination of PDMS and EG-Sil Twisters (50°C 2 hrs)

If EG-Sil worked so well for acids in beer, why not try it for axillary malodors?



Analysis of Axillary Odorants from Soiled Shirts

Chromatogram of cotton shirt (1 gm) after exercising. Analyzed by HSSE dual Twister method (1 PDMS + 1 EG-Sil), 50°C 2 hrs



Peak identities:(1) isobutanoic acid; (2) 3-methyl butanoic acid; (3) 2-methyl butanoic acid; (4) pentanoic acid; (5) hexanoic acid; (6) heptanoic acid; (7) 2-ethyl hexanoic acid; (8) octanoic acid; (9) nonanoic acid. None of these acids were present in Control shirt.

Analysis of Axillary Odorants from Soiled Shirts

Calibration curve results for five levels of standards in the 10-500 μg/L range anlyzed by HSSE using one PDMS and one EG-Sil.

Standards	Mass Ion	Detect Limit (ppb)	R^2	Analytical Method
butyric acid	60 (XIC)	5	0.9921	HSSE
isovaleric acid	60 (XIC)	5	0.9731	HSSE
pentanoic acid	60 (XIC)	5	0.9737	HSSE
hexanoic acid	60 (XIC)	5	0.9972	HSSE
octanoic acid	60 (XIC)	10	0.9911	HSSE
2-methybutanoic acid	74 (XIC)	5	0.9655	HSSE
3-methyl-2-hexenoic acid	113 (XIC)	10	0.9901	HSSE
2-nonenal	70 (XIC)	5	0.9900	HSSE
androstenone	272 (SIM)	0.4	0.9914	SBSE

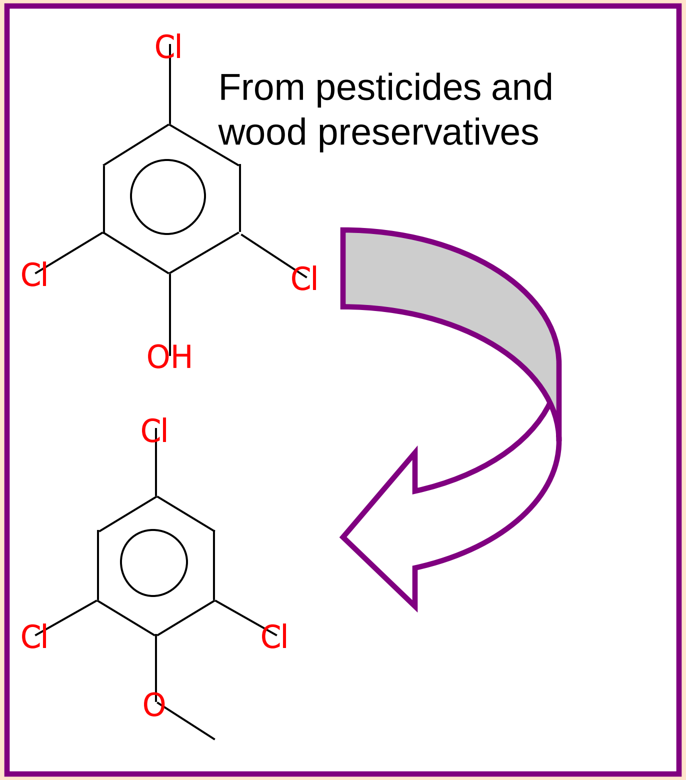
XIC = extracted ion mode; SIM = selected ion mode

Androstenone (log Ko/w =3.69), isopropanol/water extract of shirt, PDMS SBSE.

Musty Taint in Casein Powder

MUSTY COMPOUNDS	Odor threshold	Odor type	Molecular mass
2-MIB (2-methyl isoborneol)	5-10 ng/l	earthy	152
Geosmin	1 - 10 ng/l	musty	182
2,4,6-TCA (2,4,6-trichloroanisole)	0,1 - 2 ng/l	musty	212
2,3,6-TCA (2,3,6-trichloroanisole)	0,1 - 2 ng/l	musty	212
2,3,4-TCA (2,3,4-trichloroanisole)	0,2 - 2 ng/l	musty	212
2,4,6-TBA (2,4,6-tribromoanisole)	0,15 - 2 ng/l	musty	346

Musty Taint in Casein Powder



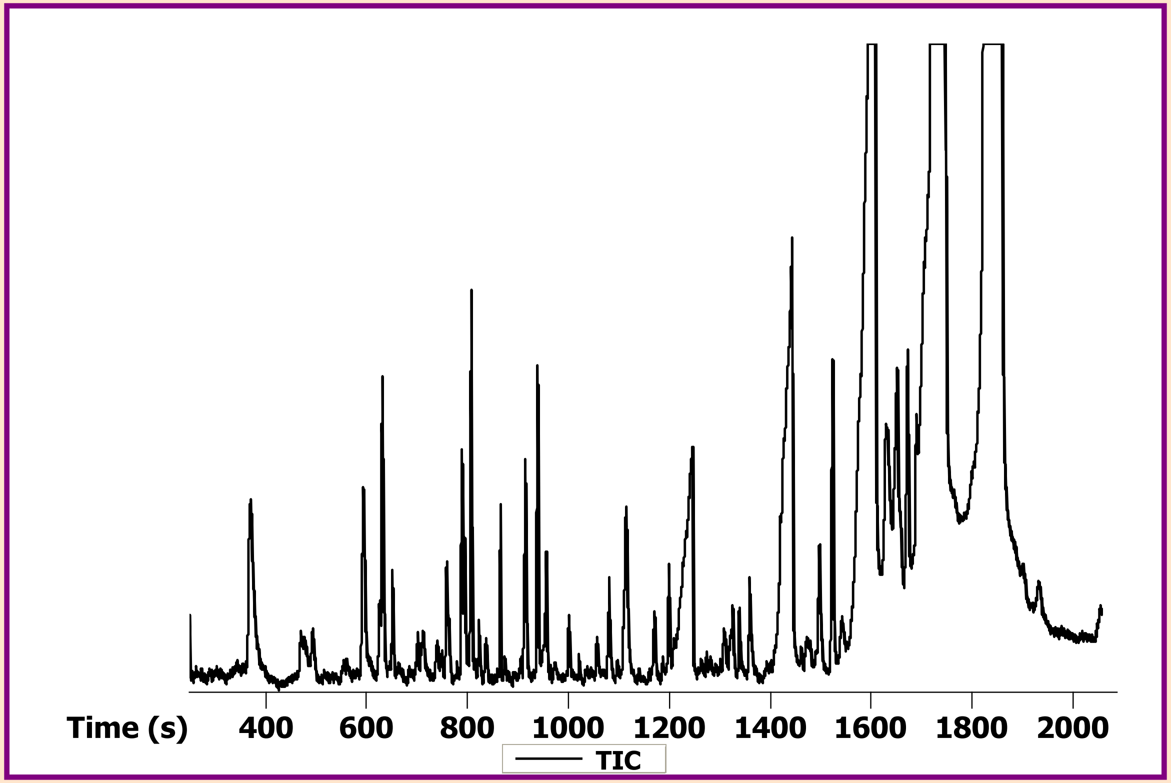
Microbial (i.e., fungal enzymatic) o-methylation to form the strong musty trichloroanisole from the trichlorophenol.

<u>Problem:</u> Two instances of TCA contamination of casein powder and one instance of TBA contamination of casein powder occurred in summer of 2007. Tainted (musty) caseins were used to make nutritional beverages. Several law suits resulted.

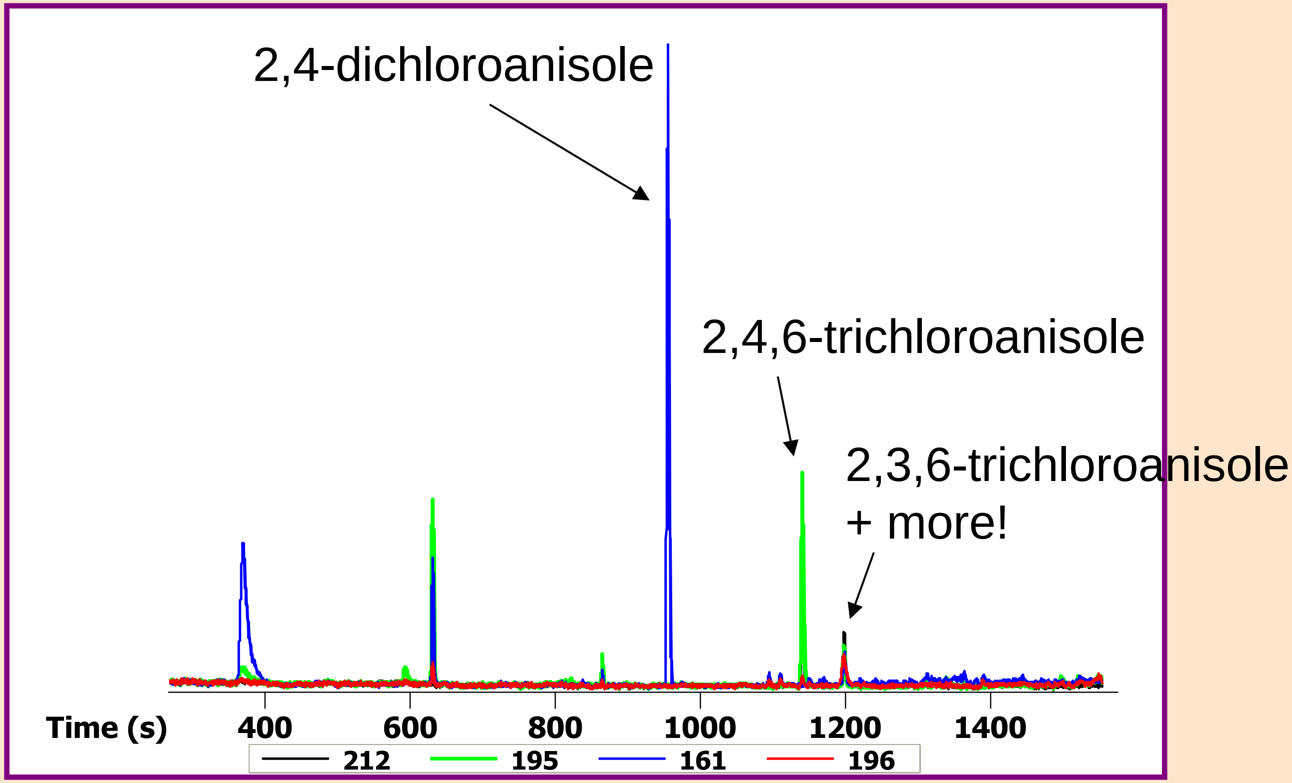
Musty Taint in Casein Powder

1gm musty casein + 25 mL water, stir 30 min @ RT; SBSE for 3 hr with 2 cm x 0.5 mm Twister®

Note for 2,4,6-TCA: log $K_{o/w}$ = 4.11.

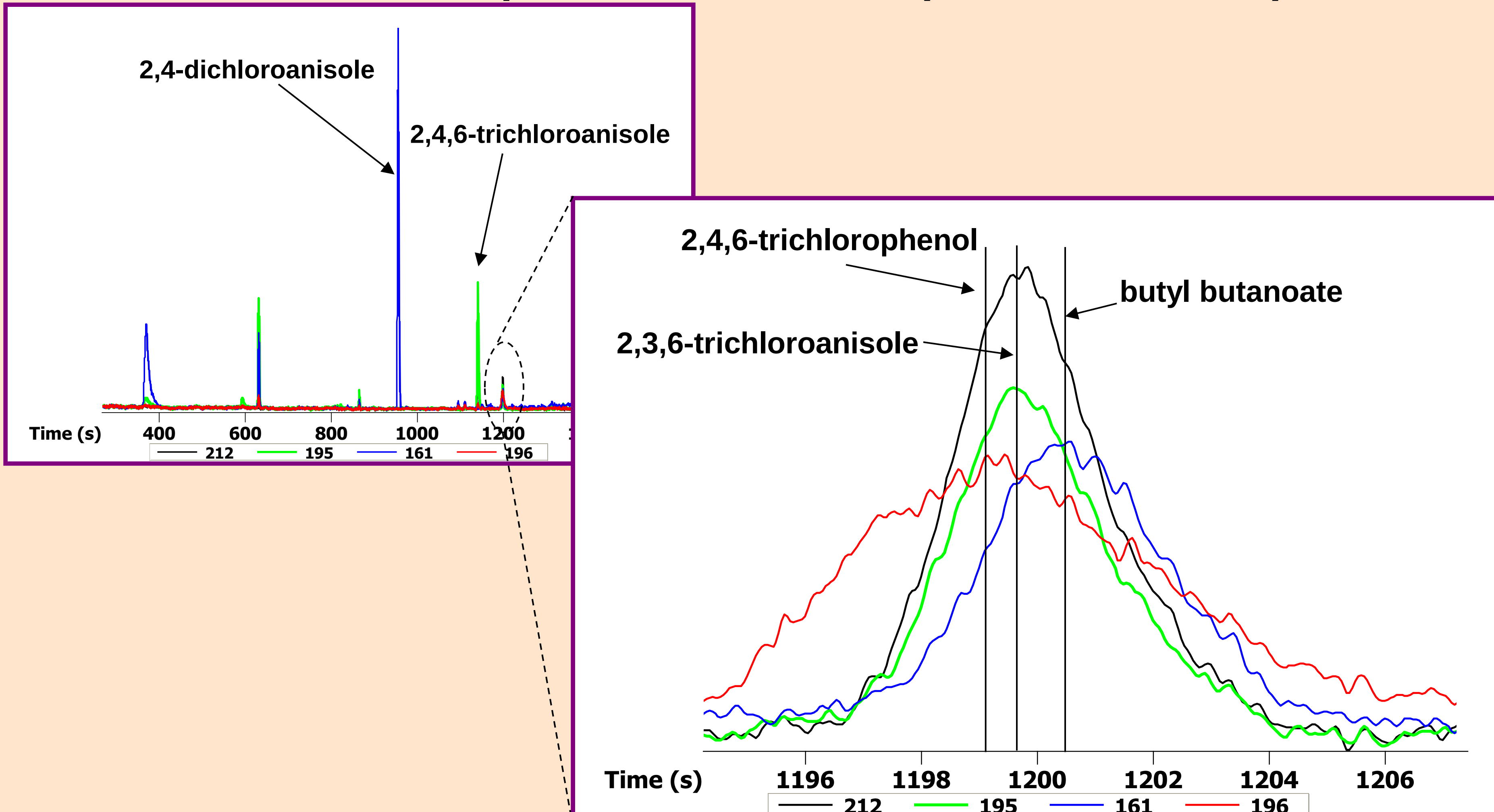


Finding chloroanisoles in the chromatogram…

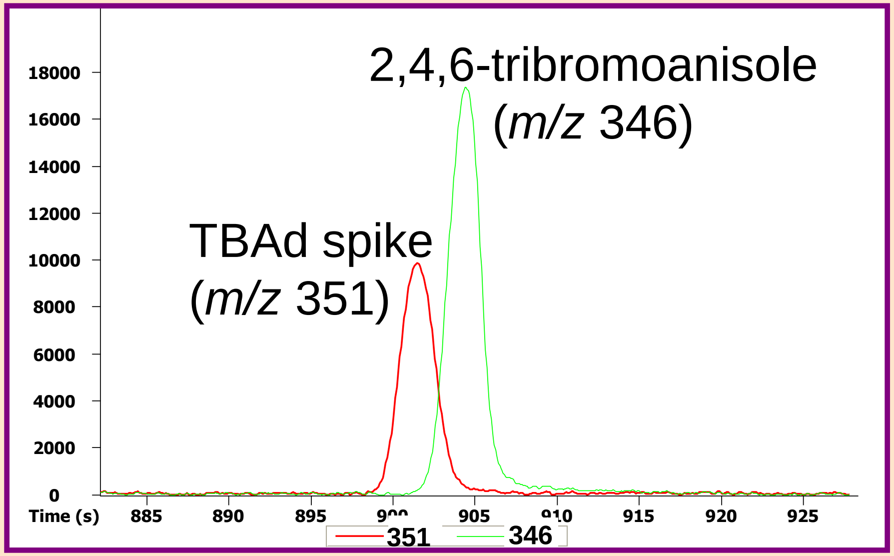


Musty Taint in Casein Powder

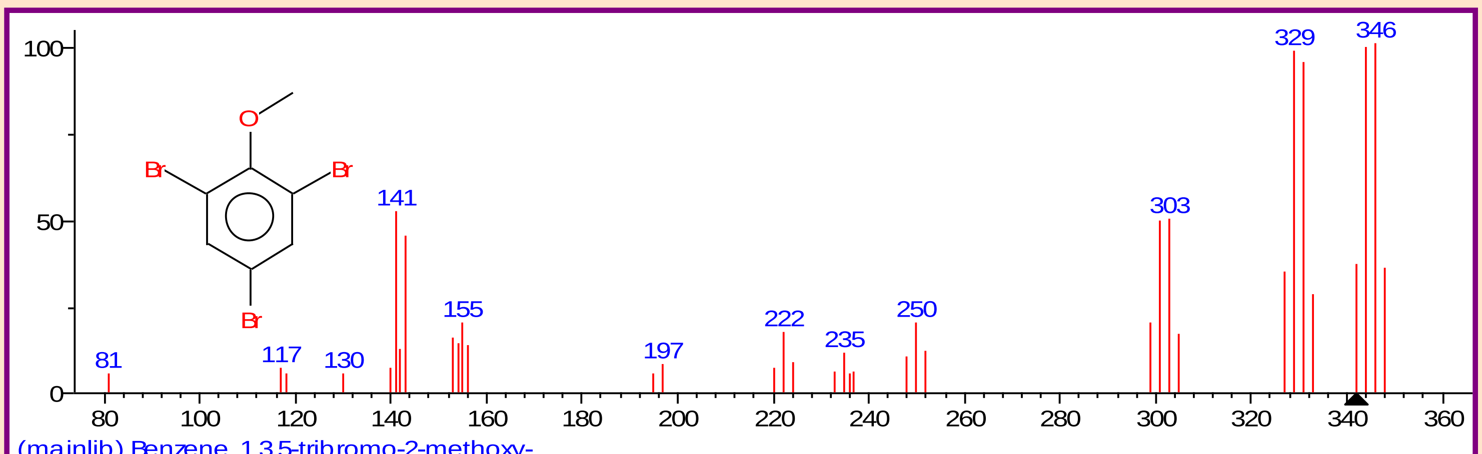
The value of peak deconvolution (Leco GC-TOFMS)



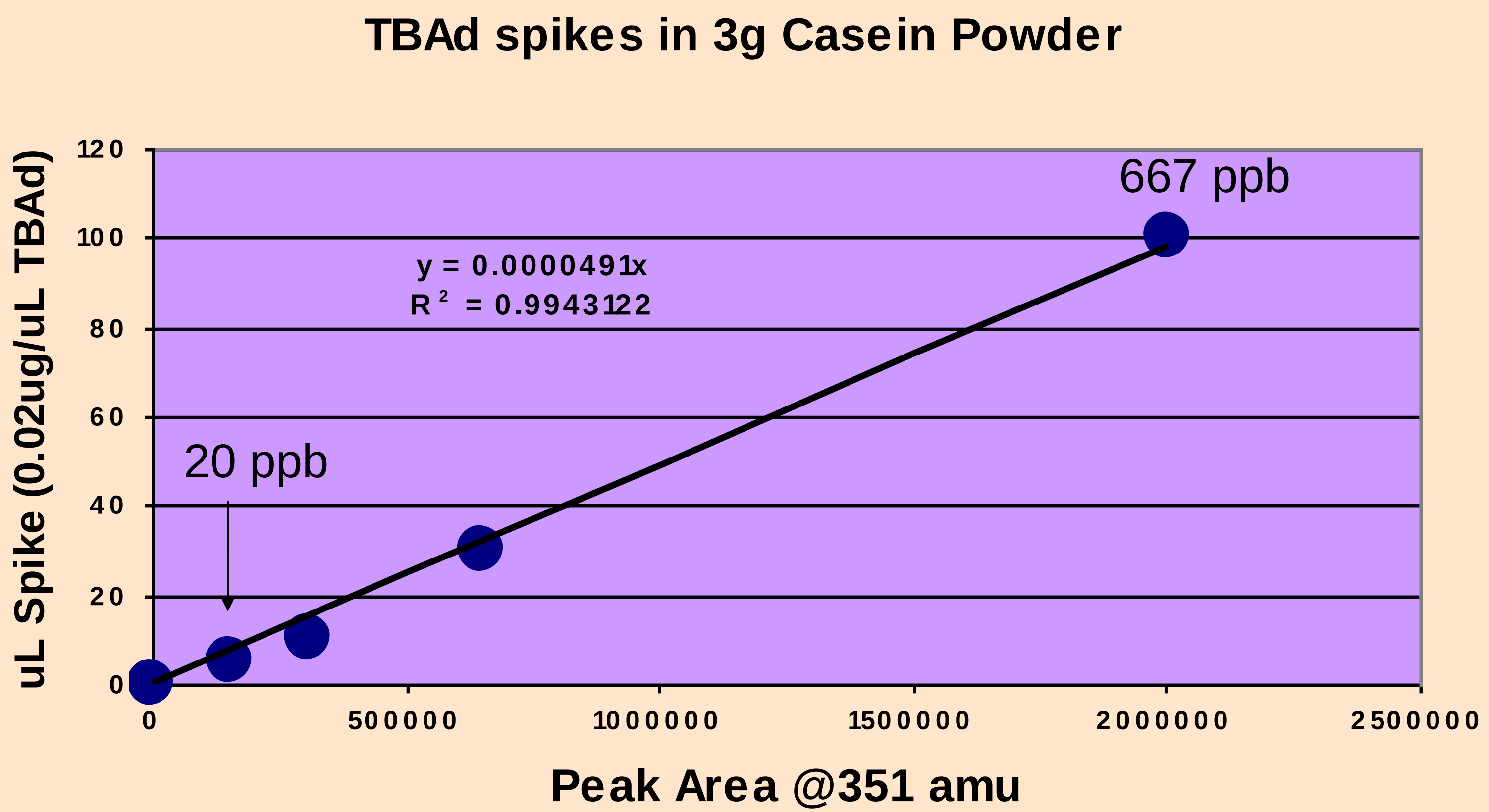
TBA and TBAd Spike (133 ppb) in Musty Casein



Technique is called SIDA (Stable Isotope Dilution Analysis). "SIDA is arguably the most accurate technique for quantitative determination of organic compounds." (Alan D. Harmon in *Flavor, Fragrance and Odor Analysis*.)

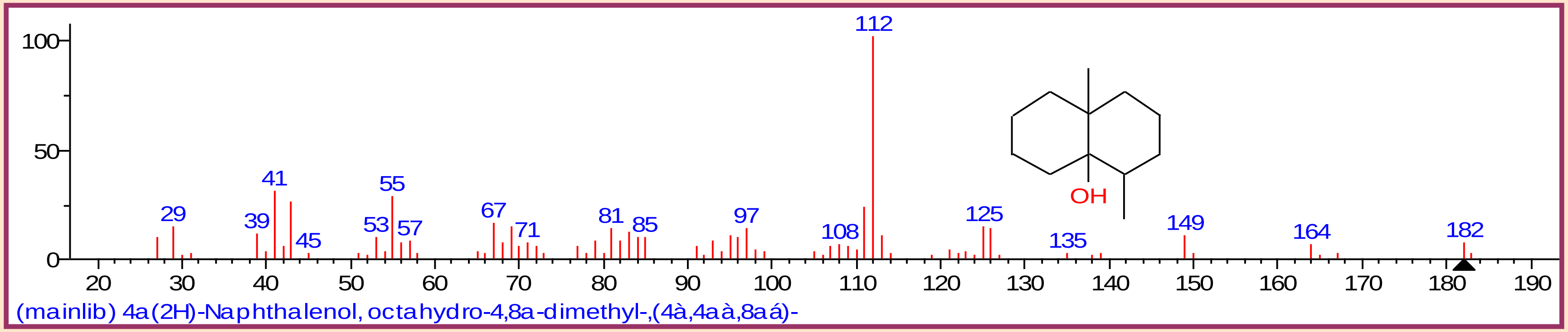


TBAd Standard Curve in Control Casein



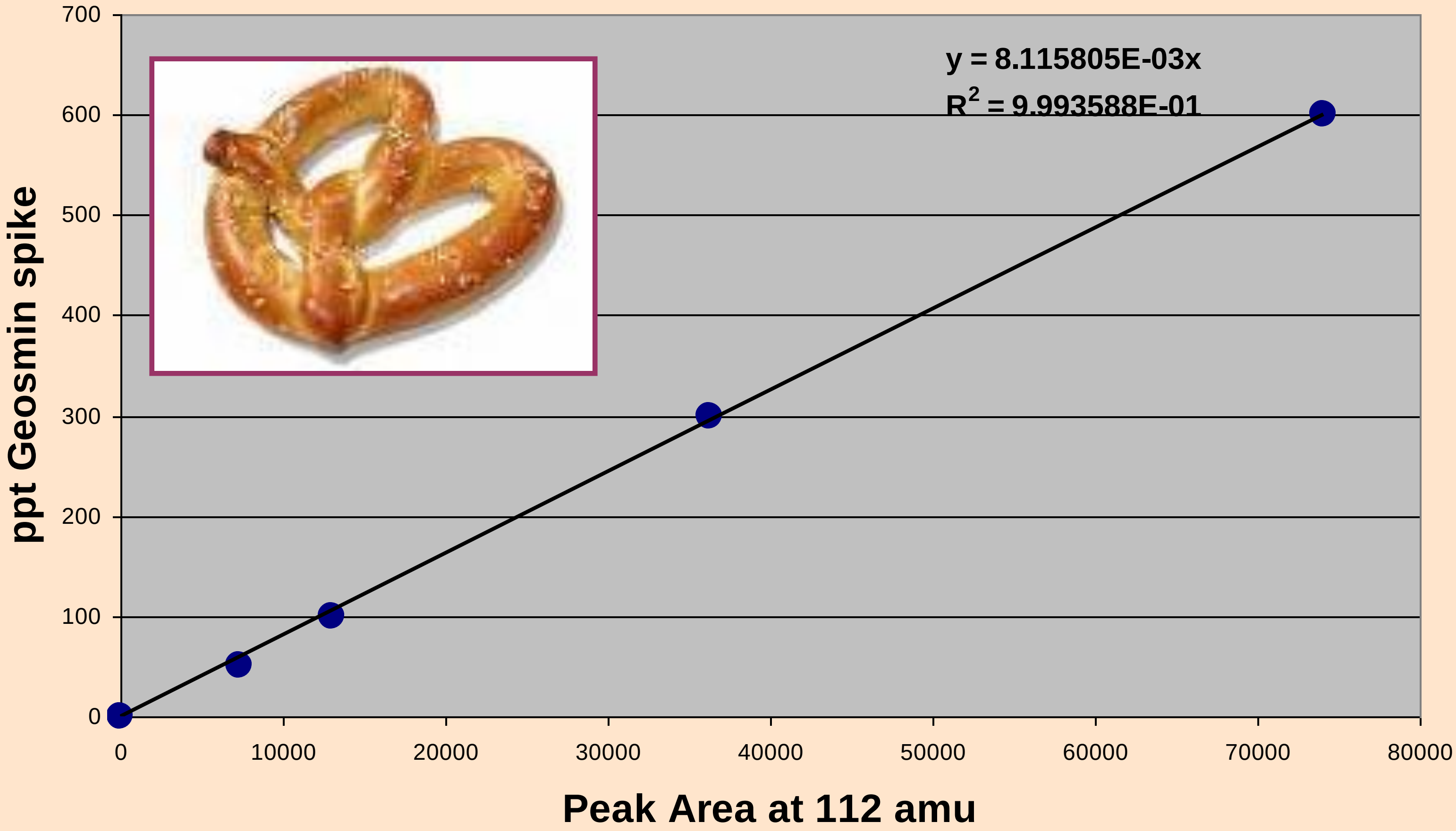
Geosmin in Pretzels

<u>Method:</u> 5gm pretzels + 100mL 15% EtOH and mix with a handheld blender for 3 min. Allow solids to settle and extract 25mLs with 2cm x 0.5mm Twister for 2hr. Analyze with thermal desorption GC-MS in SIM (112 and 182 amu). Note for Geosmin: log $K_{o/w}$ = 3.57.



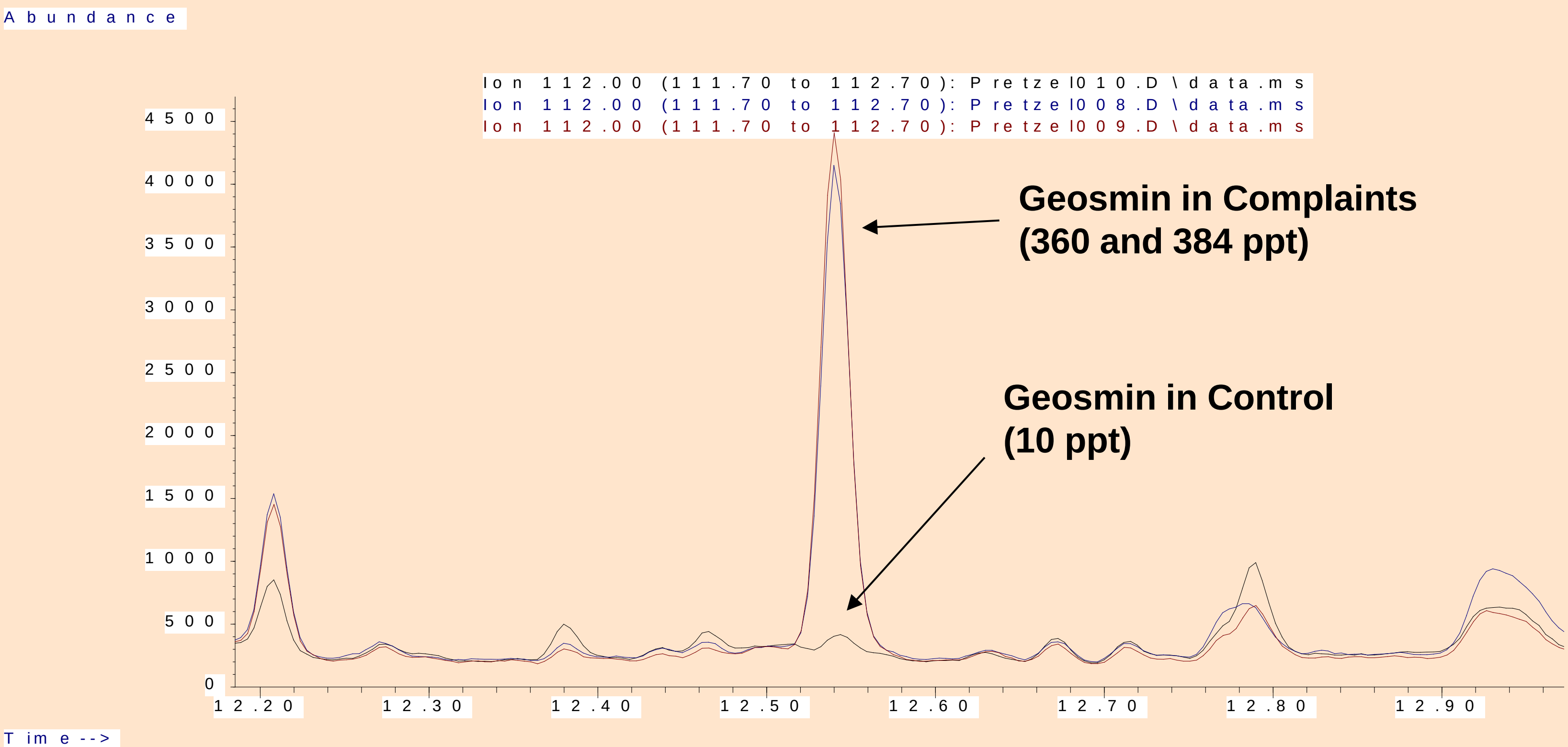
Geosmin in Pretzels

Geosmin Standard Calibration Curve



Geosmin in Pretzels

Source of geosmin in the pretzels is the water that was used to manufacture the pretzels. Water treatment was necessary to eliminate the problem.



Note: Ratio of peak areas at *m/z* 112/182 is approximately 21 in standards and sample.

Acknowledgements

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Intelligent Automation for GC/MS and LC/MS

Ed Pfannkoch, Baltimore, MD USA
Nobuo Ochiai, GERSTEL K.K., Japan

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