Stir bar sorptive extraction (SBSE) for Trace Analysis of Aroma Compounds in Beverages

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Outline

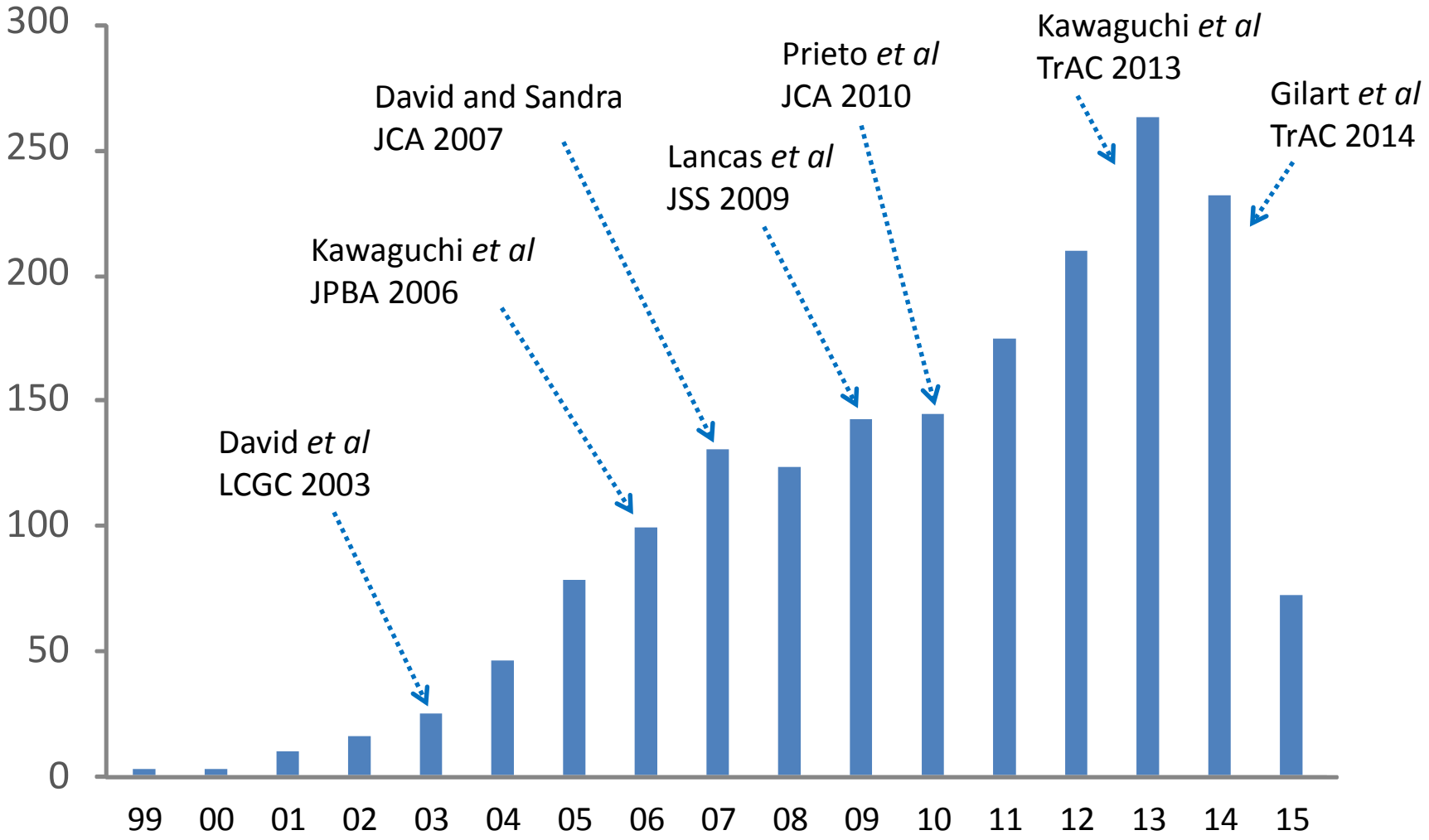
Introduction

Multi-stir bar sorptive extraction (^{m}SBSE) for non-targeted analysis of aroma compounds

SBSE with *in-situ* derivatization (*der*-SBSE) for targeted analysis of off-flavors and key aroma compounds

Conclusion

SBSE research papers and review papers



www.sciencedirect.com
Keyword: SBSE

SBSE for Aroma analysis

For aroma analysis,

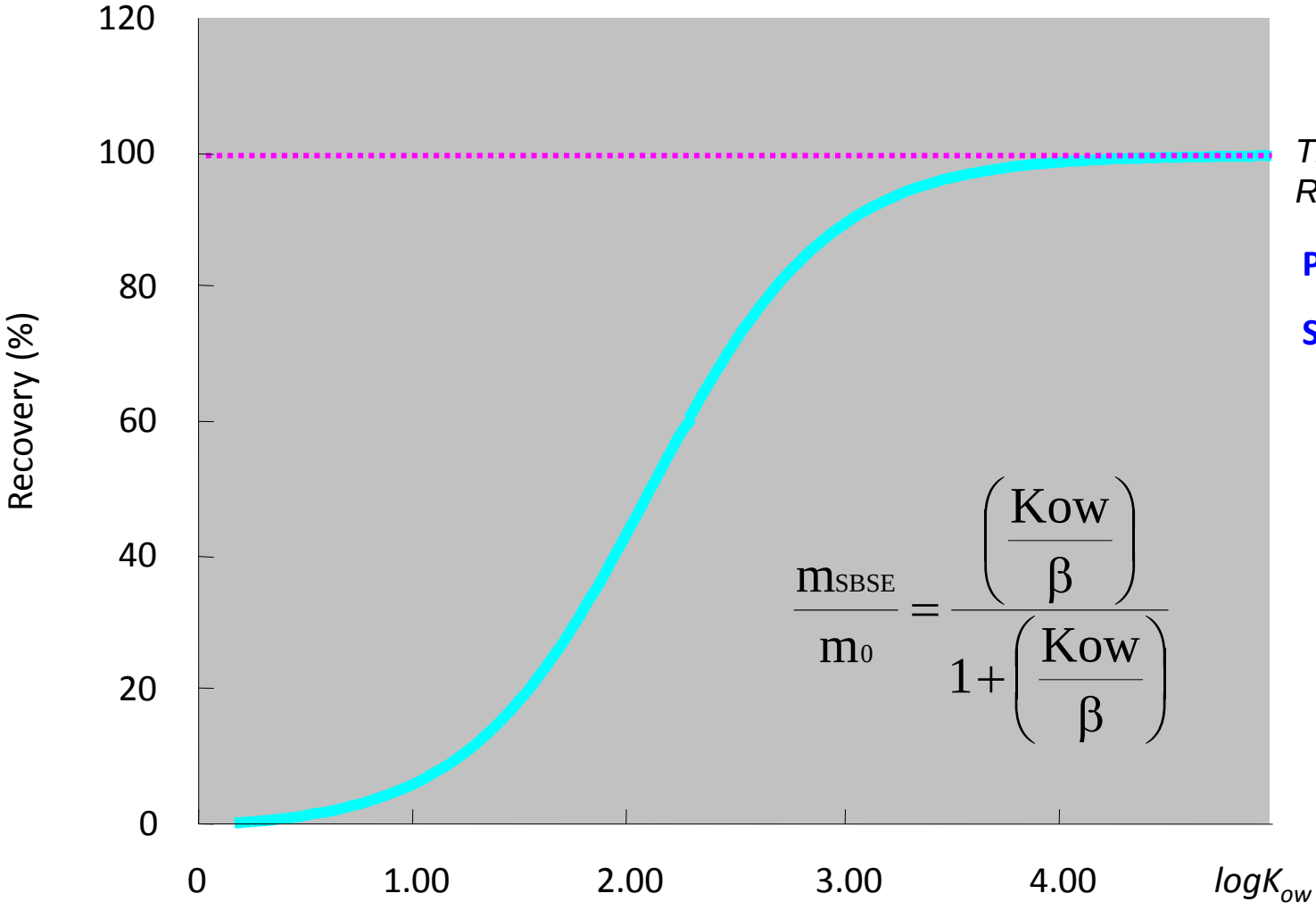
Beverages, Vinegar,
Fruits, Vegetables,
Herbs, Plant material,
Essential oils



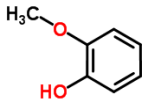
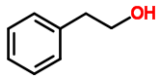
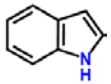
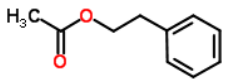
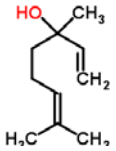
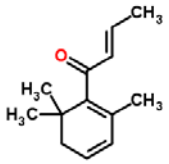

PDMS stir bar (Twister)

David and Sandra, JCA 2007
Lancas *et al,* JSS 2009
Prieto *et al,* JCA 2010
Kawaguchi *et al,* TrAC 2013
Gilart *et al,* TrAC 2014

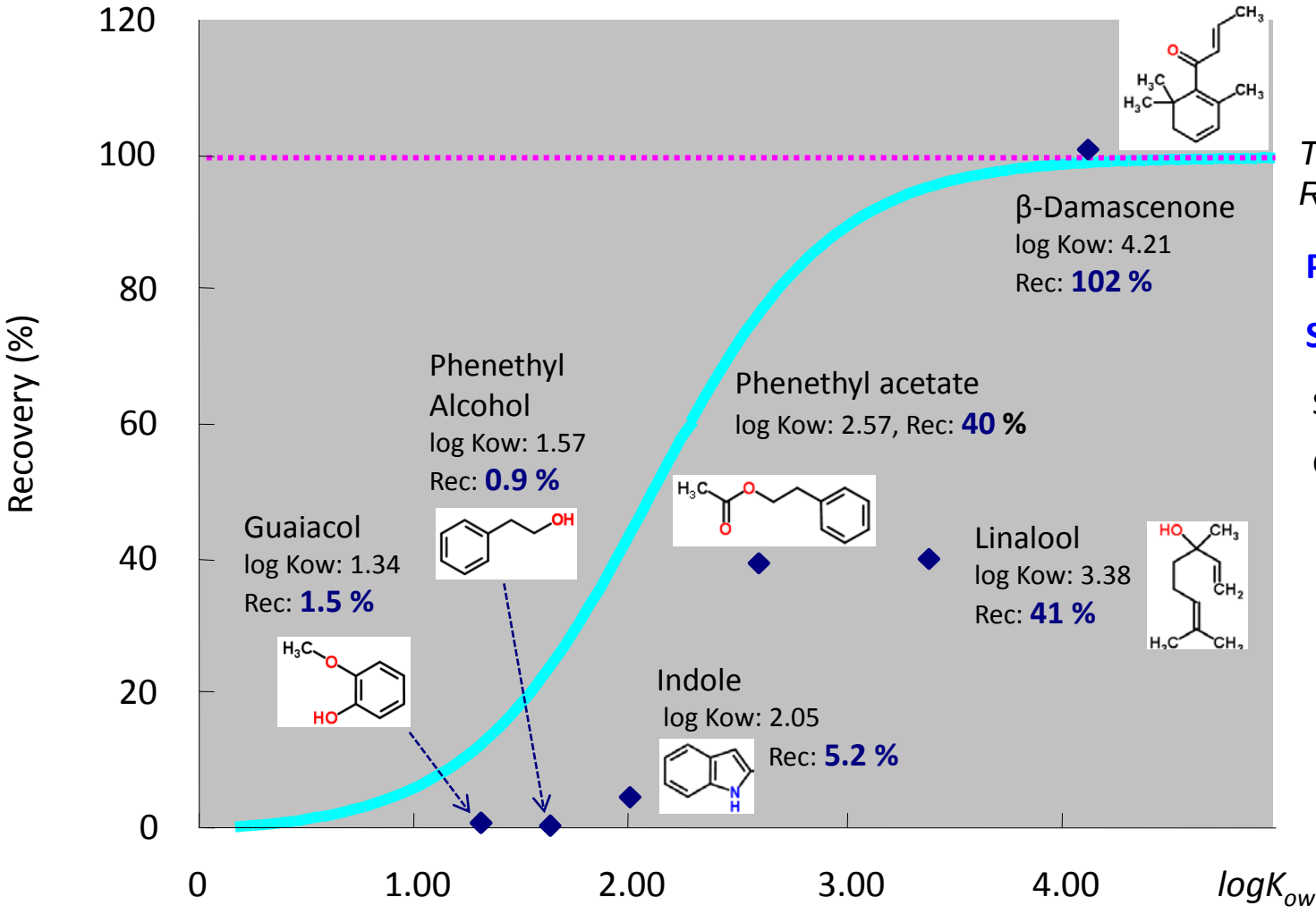
Theoretical recovery as a function of log K_{ow}



Test aroma compounds

	Compound	log K_{ow}	Polar function	Aroma
	Guaiacol	1.34	-OH, CH_3O-	Smoky, medicinal
	Phenethyl alcohol	1.57	-OH	Floral, sweet
	Indole	2.05	C_4H_5N (pyrrole)	Floral (high dilution)
	Phenethyl acetate	2.57	$CH_3(C=O)O$-	Fruity, sweet
	Linalool	3.38	-OH	Floral
	β-Damascenone	4.21	-C=O	Honey, Sweet

Recovery of aroma compounds in water



Theoretical Recovery (%)

PDMS: 24 µL

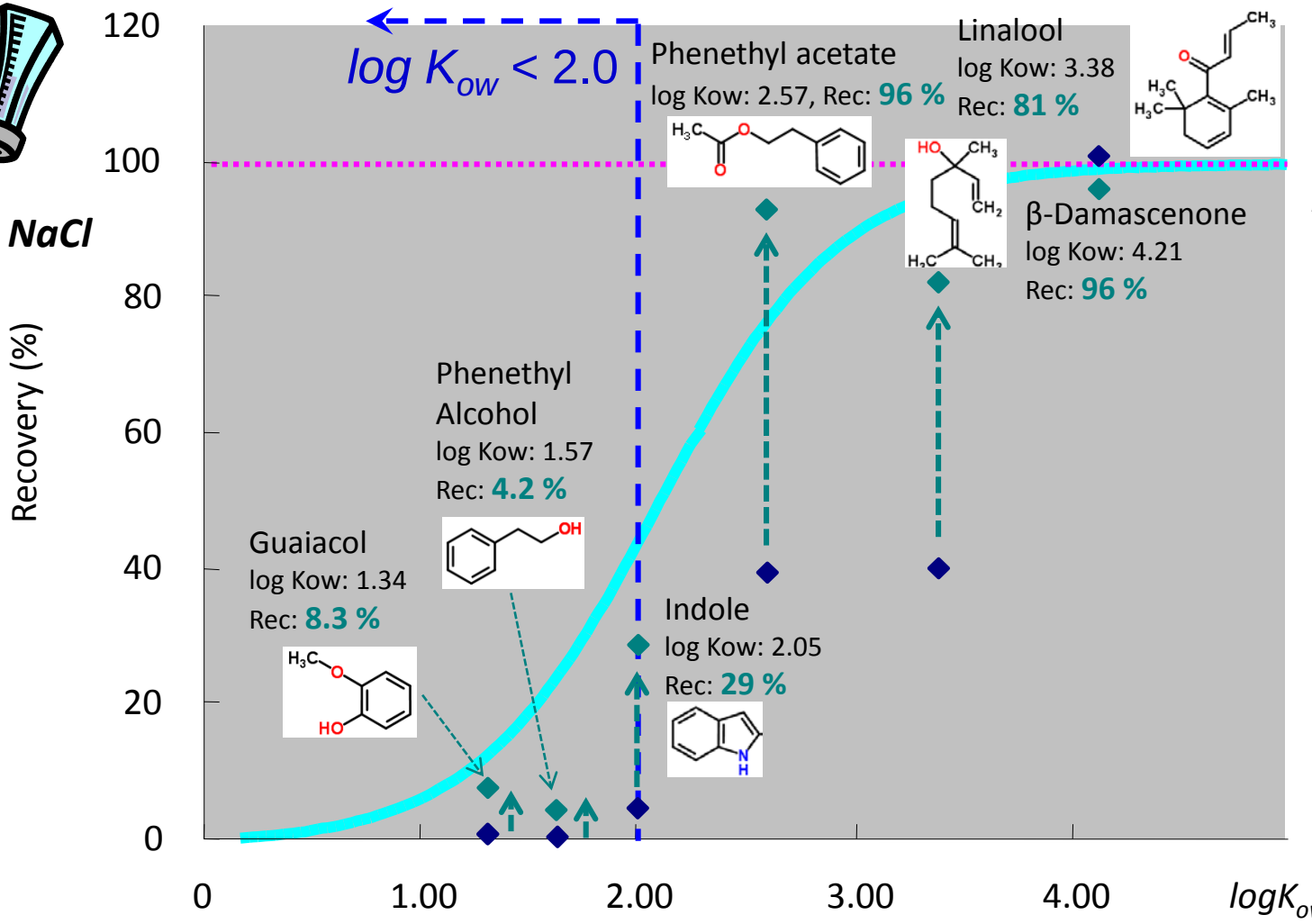
Sample: 5 mL

SBSE: 2 h (800 rpm)

Conc.: 10 ng/mL

Comparison of recovery with or without salt addition

30% NaCl



Theoretical Recovery (%)

PDMS: 24 µL

Sample: 5 mL

SBSE: 2 h (800 rpm)

Conc.: 10 ng/mL

◆ without salt

◆ **with 30% NaCl**

However, the recoveries for guaiacol and phenethyl alcohol, which have low log K_{ow} of less than 2 are still quite low. Therefore, a new SBSE coating for these polar/hydrophilic compounds would be required.

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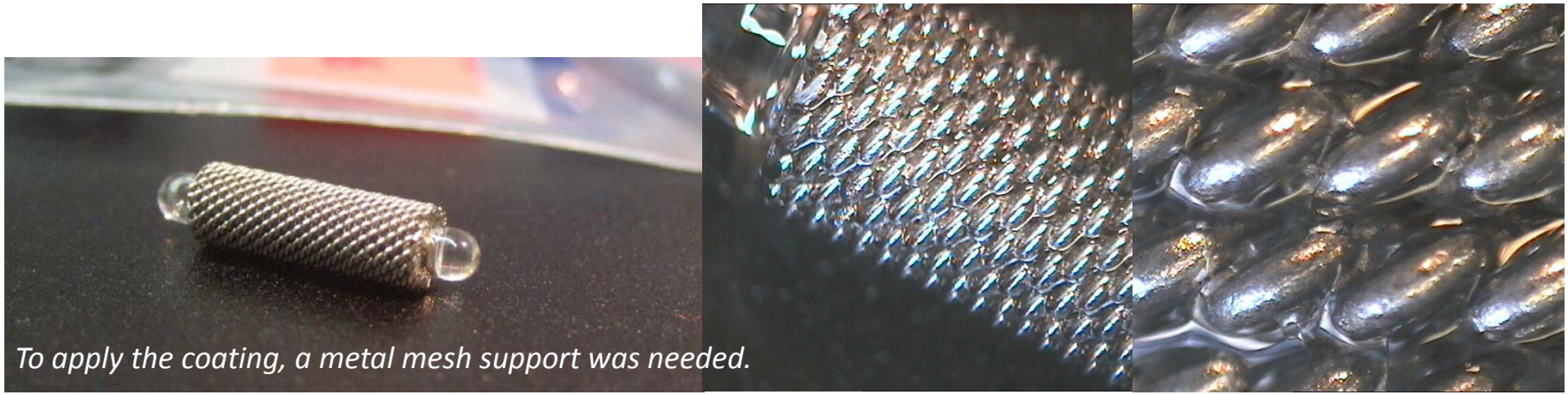
Search for new coatings for SBSE

Criteria

- Should be used with thermal desorption (TD).
- Should give a significant improvement versus PDMS.
- The production of the coating should be possible in a very reasonable way (as is the case with PDMS stir bar).

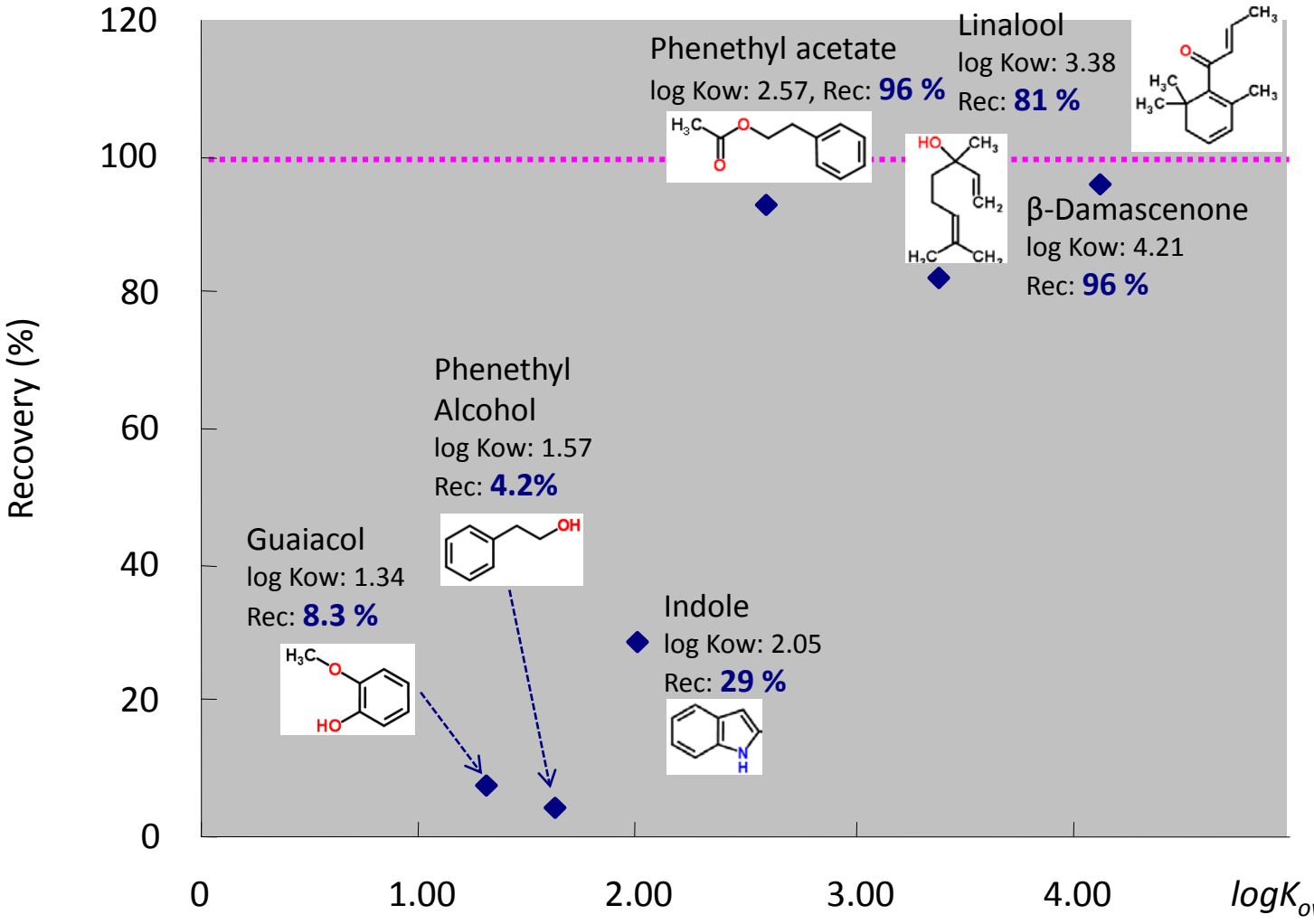
Polyethyleneglycole-modified Silicone (EG-Silicone)



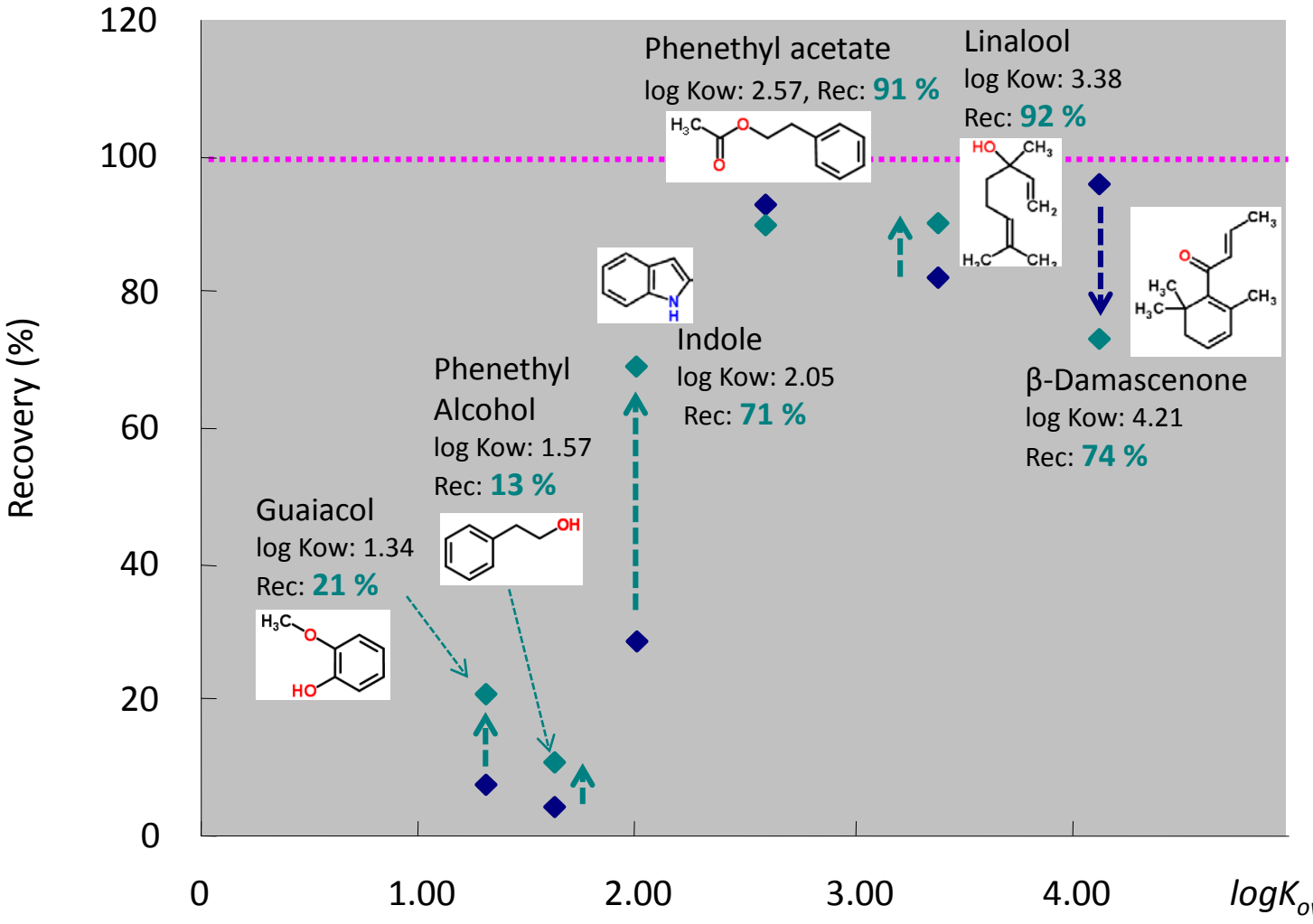
To apply the coating, a metal mesh support was needed.

Y. Nie, E. Kleine-Benne, GERSTEL AppNote 3/2011, AppNote 2/2012.
N. Ochiai: "Flavor, Fragrance, and Odor Analysis, Second edition", edited by R. Marsili, p.12 (2011), CRC press.
B. Sgorbini *et al*, J. Chromatogr. A, 1265 (2012) 39.
J. J. Cacho et al, J.Pham. Biomed. Anal. 78-79 (2013) 255.
N. Gilart et al, Anal. Chim. Acta, 774 (2013) 51.

Comparison of recovery between PDMS and EGS stir bar



Comparison of recovery between PDMS and EGS Twister



PDMS: 24 µL

EGS: 32 µL

Sample: 5 mL

SBSE: 2 h (800 rpm)

Conc.: 10 ng/mL

w/30 % NaCl

◆ **PDMS 1×**

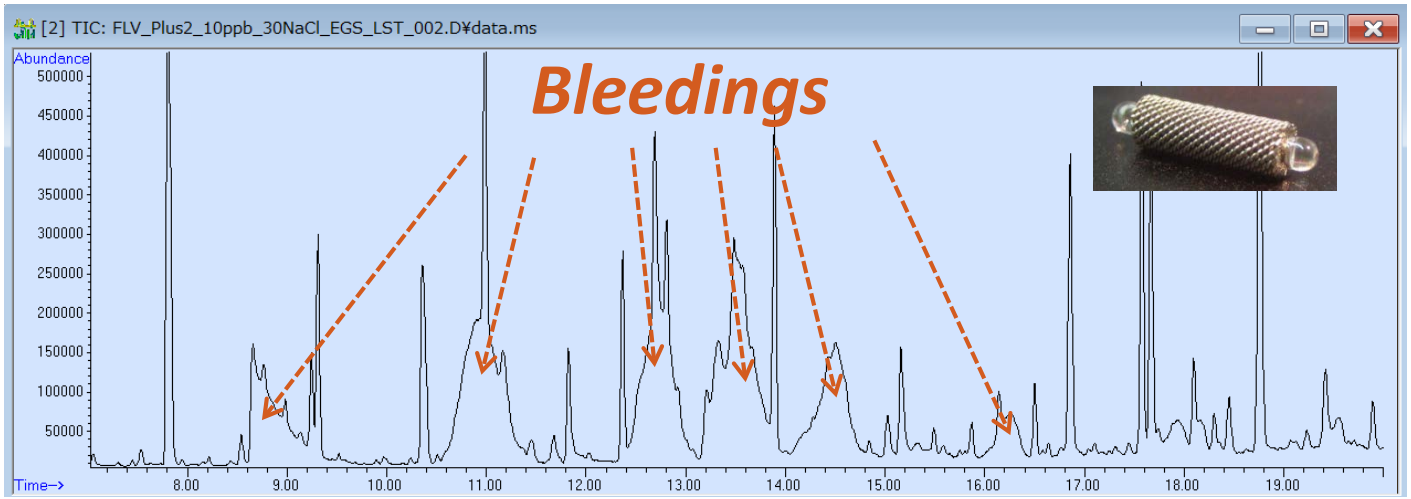
◆ **EGS 1×**

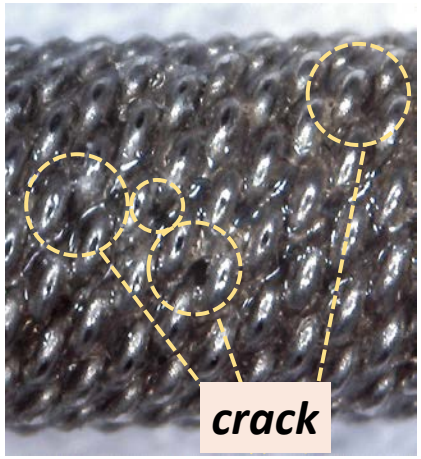
The EG Silicone stir bar provides higher recovery for polar (hydrophilic) solutes, specifically the solutes which can form hydrogen bonding.

Bleeding from the decomposed phase

Robustness:

Although the EG-Silicone stir bar showed good performance in SBSE, when re-used several times, increased bleeding from the decomposed phase and decreased repeatability were observed. For those EG-Silicone stir bars, ***numerous scratches were observed on the surface of the grid by using microscopy and tiny exposed metal surfaces appeared.***





This is mainly due to the much softer nature of EG-Silicone phase compared to the PDMS phase.

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journal homepage: www.elsevier.com/locate/chroma

Multi-stir bar sorptive extraction for analysis of odor compounds in aqueous samples

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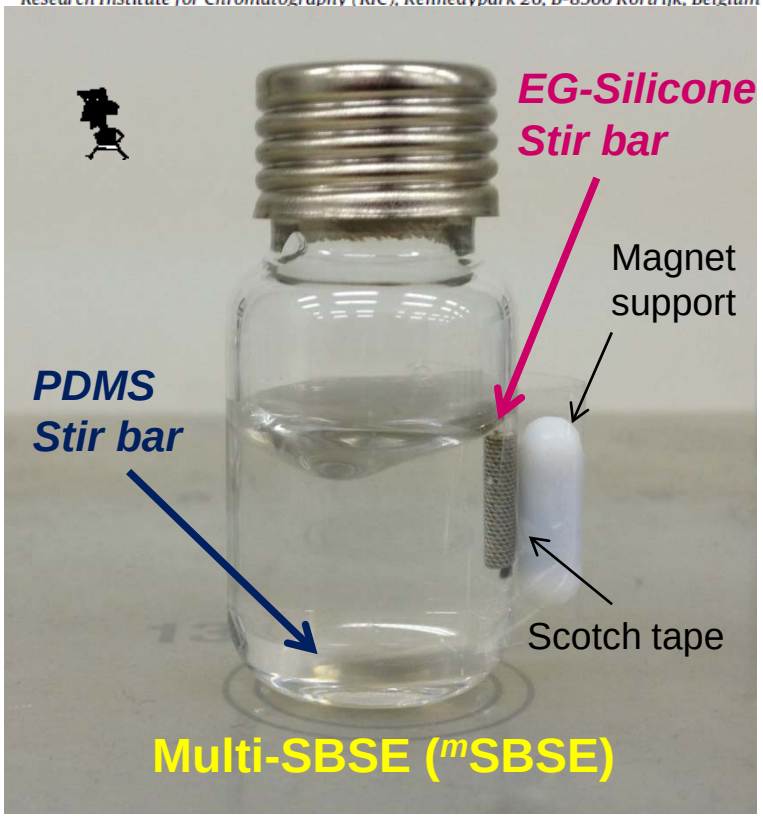
[b] Research Institute for Chromatography (RIC), Kennedypark 26, B-8500 Kortrijk, Belgium



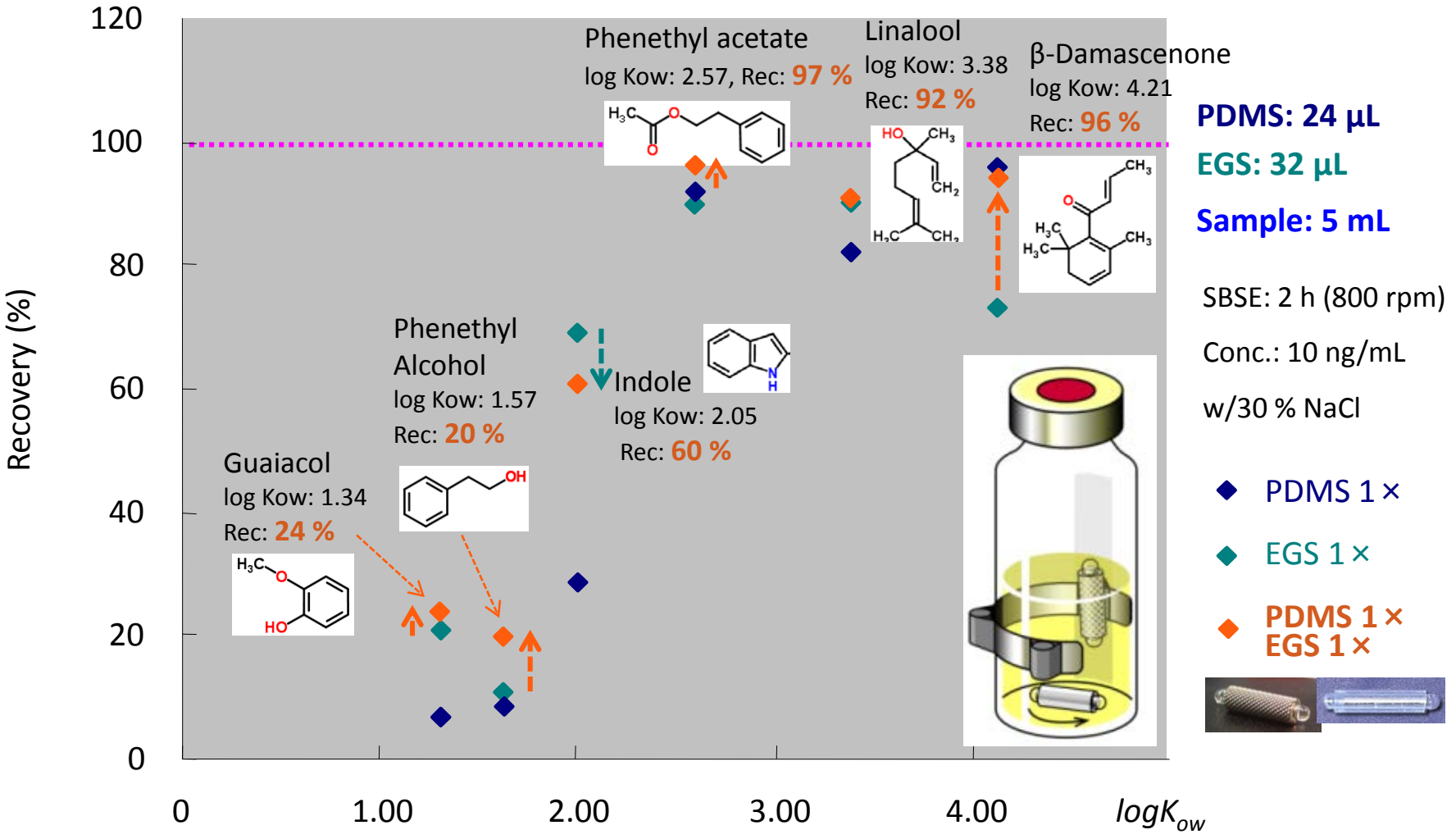
The EG-Silicone stir bar is attached to the inner side wall of the vial.

A robust PDMS stir bar is stirring at the bottom of the vial for agitation as well as extraction of the solutes.

Both stir bars can complement each other for more uniform enrichment of a wide range of solutes without any damage of EG Silicone phase.

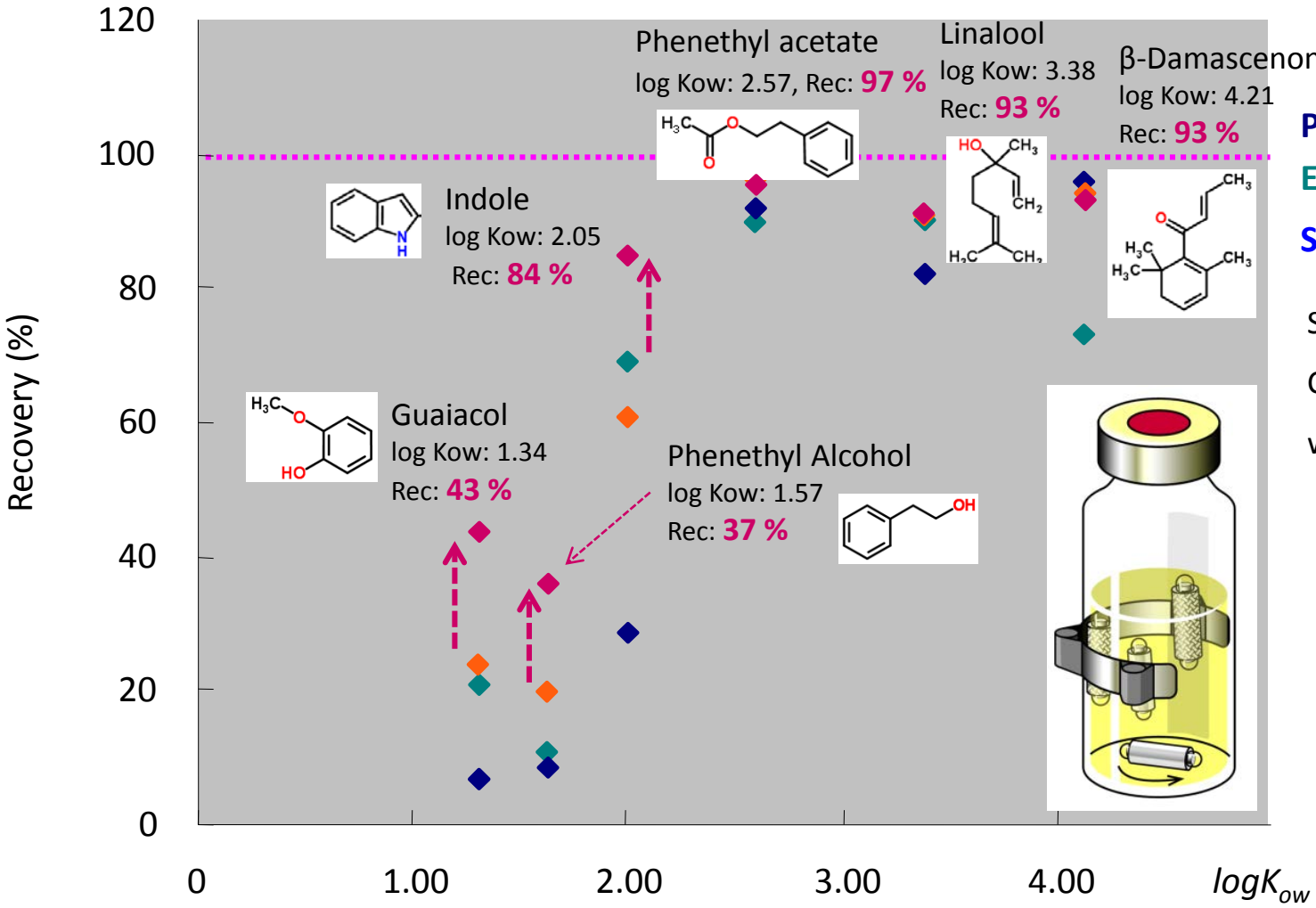
After extraction, the two stir bars are placed in a single TD liner and are simultaneously thermally desorbed.

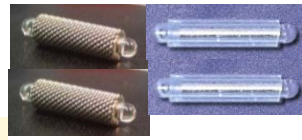
Comparison of recovery between single SBSE and ^{m}SBSE



Multi-SBSE compensated the recovery of β-damascenone, while improving the recovery of phenethyl alcohol. However, the recovery for indole was decreased from 71% to 60%.

Comparison of recovery between single SBSE and ^{m}SBSE





The ^{m}SBSE approach not only combines the extraction power of the PDMS stir bar with the EG-Silicone stir bar, but also results in higher recovery due to increased phase volume (and hence the smaller phase ratio).

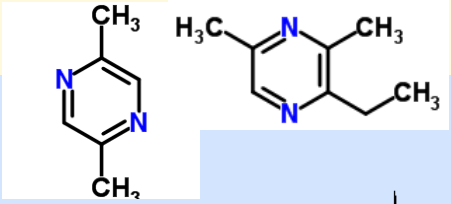
Aroma compounds in roasted green tea (Houji-cha)

The Maillard reaction roasting process of Houji-cha replaces the fine green and vegetative tones of standard green tea with more complex aroma (e.g. toasty, nutty, and caramel-like), but those additional aroma compounds including numerous polar/hydrophilic compounds are still only present at trace level.

Comparison of TICs of the roasted green tea by single SBSE and ^{m}SBSE-TD-GC-QMS

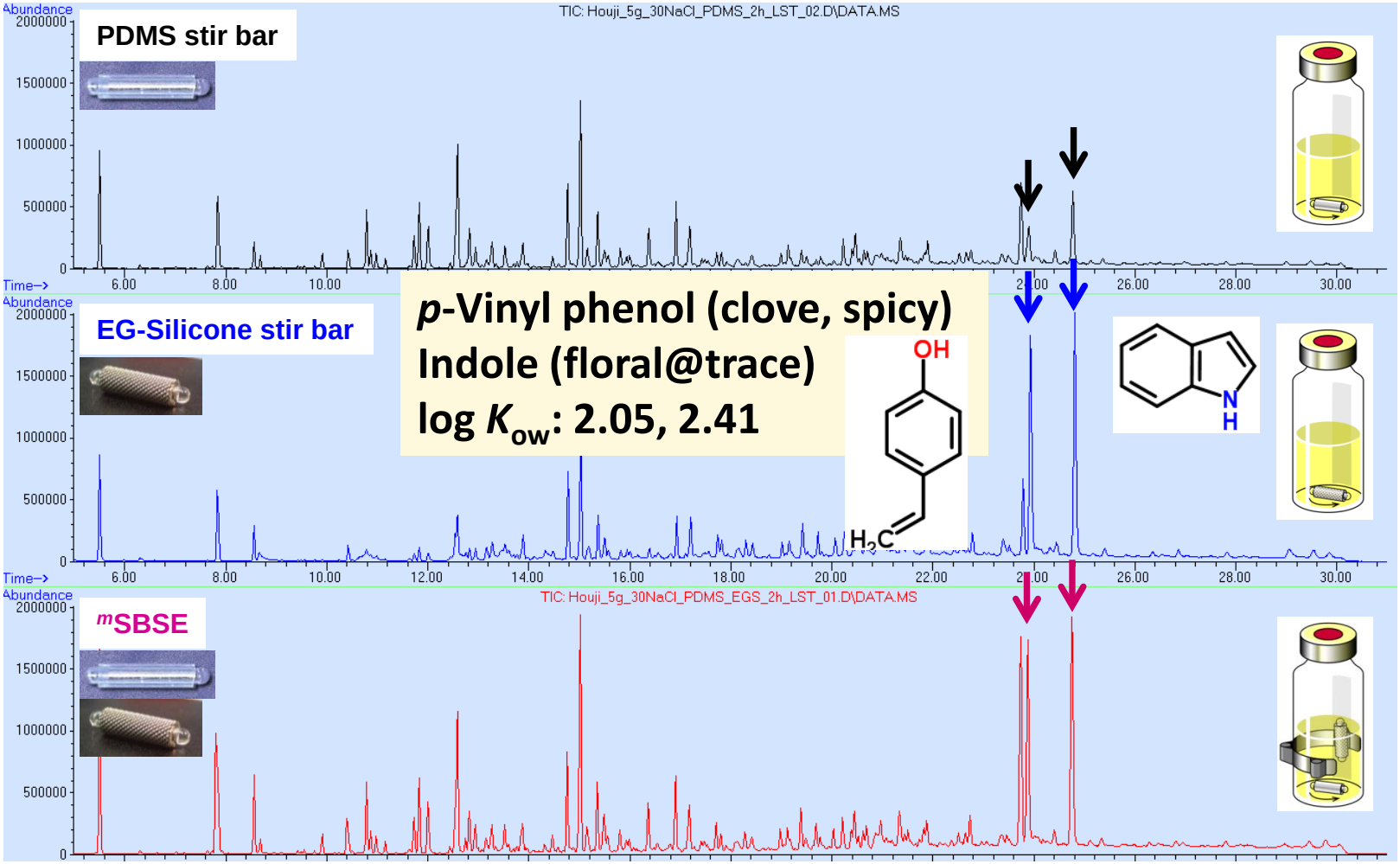
Alkyl pyrazine congeners (Nutty, roasty)

log K_{ow}: 1.03-2.07



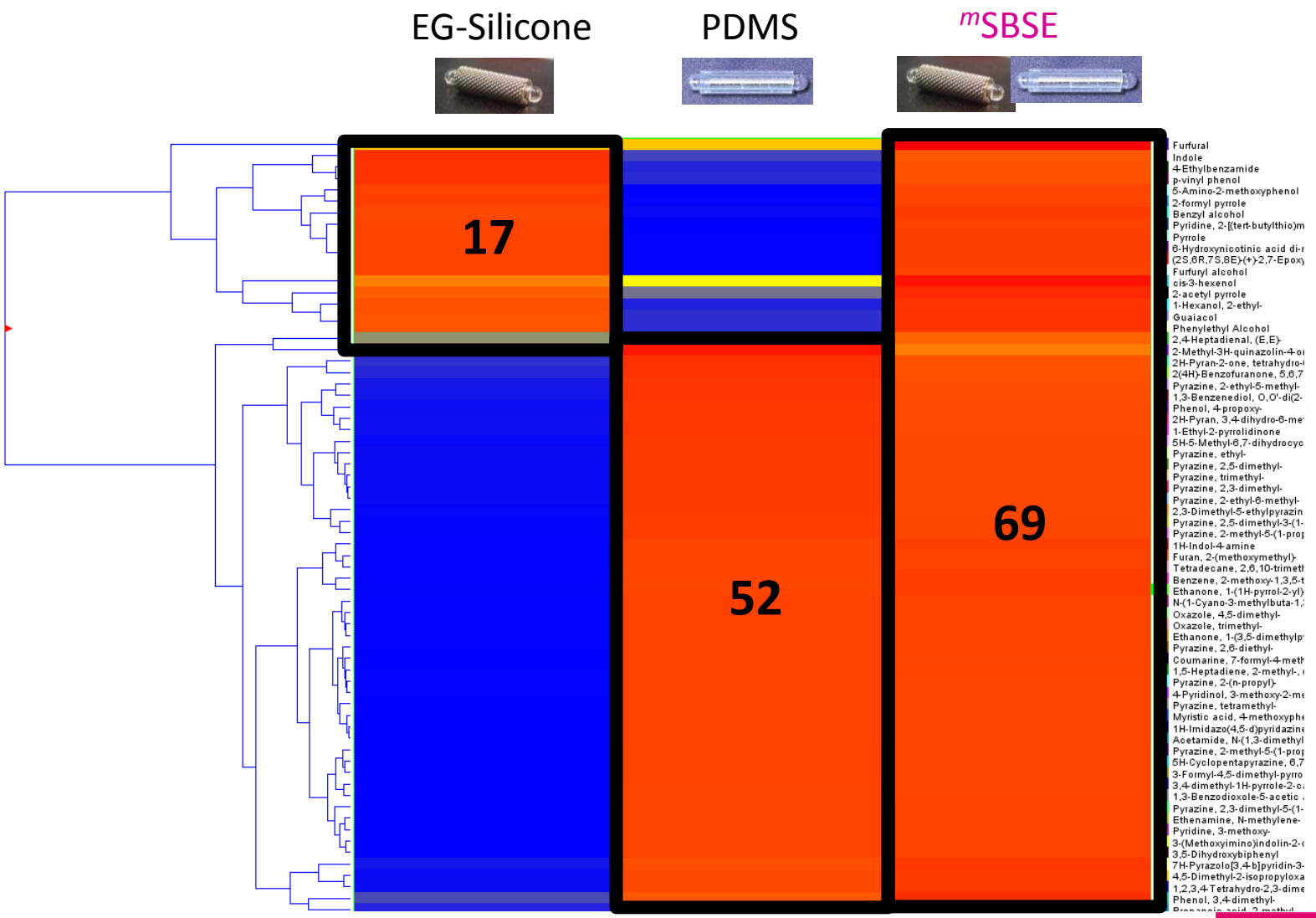
It is interesting to observe that several alkyl pyrazine congeners detected with the single SBSE using the PDMS stir bar, are much smaller peaks with the single SBSE with the EG-Silicone stir bar. But they are clearly detected again with multi-SBSE.

Comparison of TICs of the roasted green tea by single SBSE and ^{m}SBSE-TD-GC-QMS



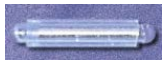
It is also clear that dominant late eluting peaks such as p-vinyl phenol and indole which can form hydrogen bonding are more intense with the single SBSE using the EG-Silicone stir bar than with the single SBSE using the PDMS stir bar. And they are also clearly present with multi-SBSE.

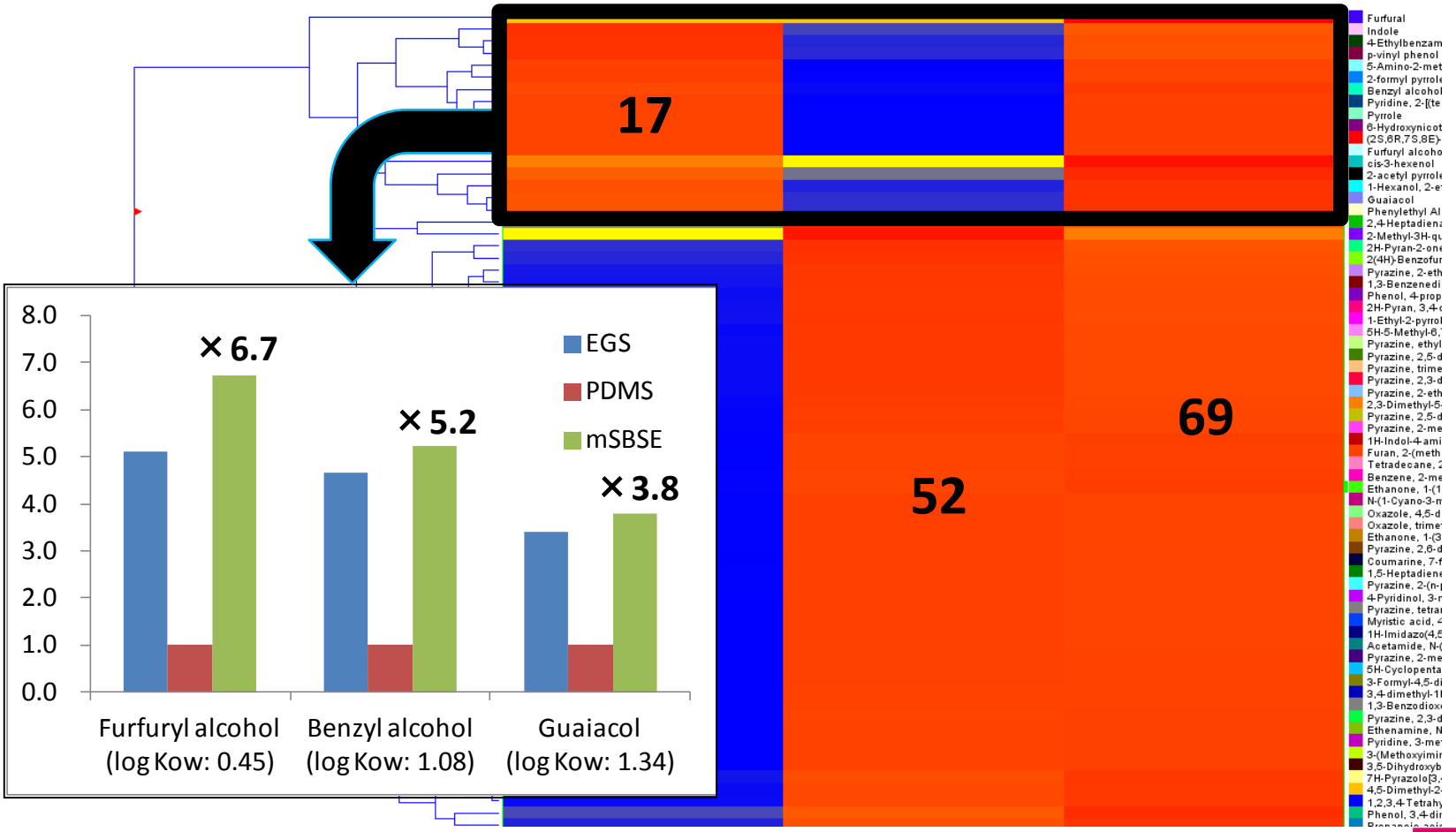
Hierarchical cluster analysis of aroma compounds in roasted green tea by single SBSE and *m*SBSE



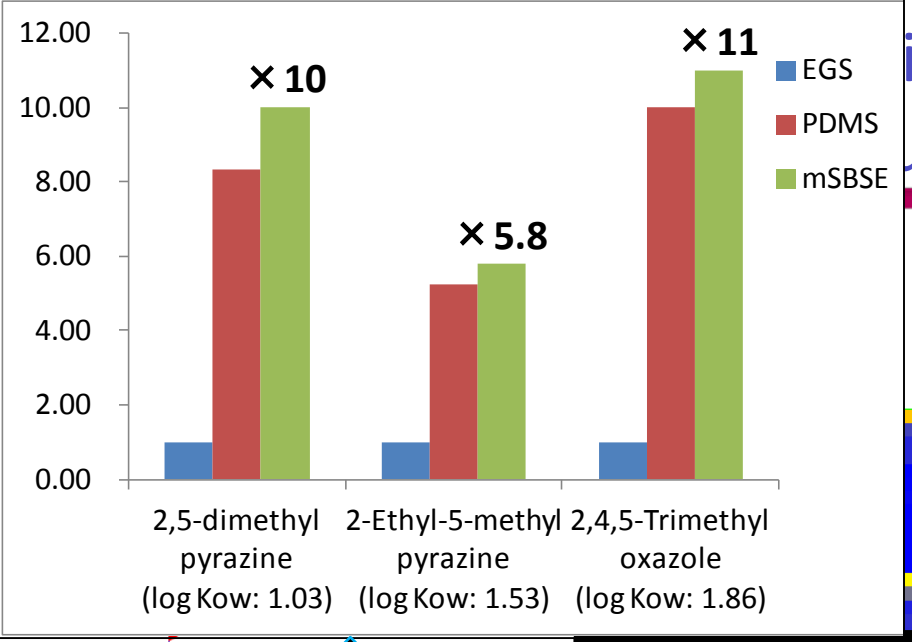
Comparison of normalized peak area of the representative compounds

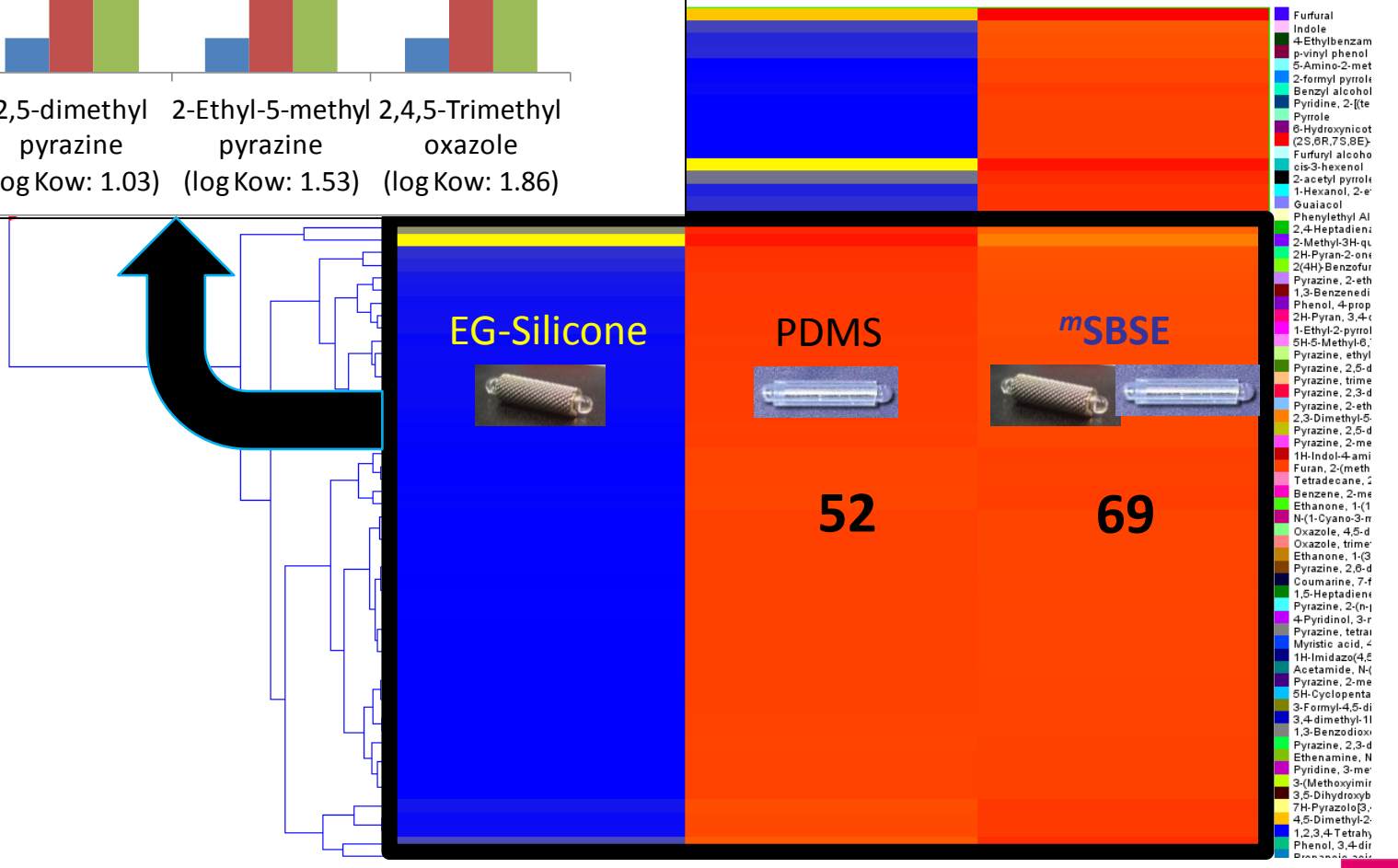
EG-Silicone PDMS ^{m}SBSE

ized peak area of the unds

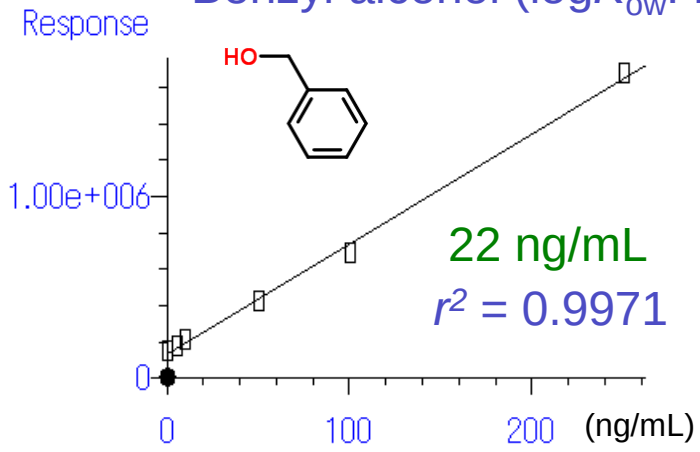




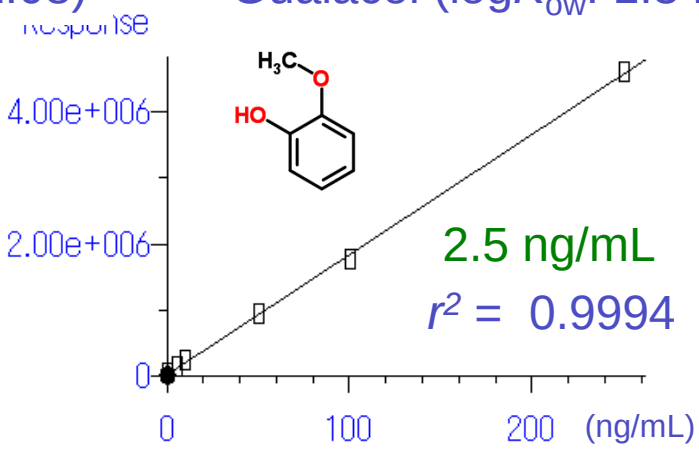
GERSTEL

Determined values obtained by ^{m}SBSE using standard addition calibration (5-250 ng/mL)

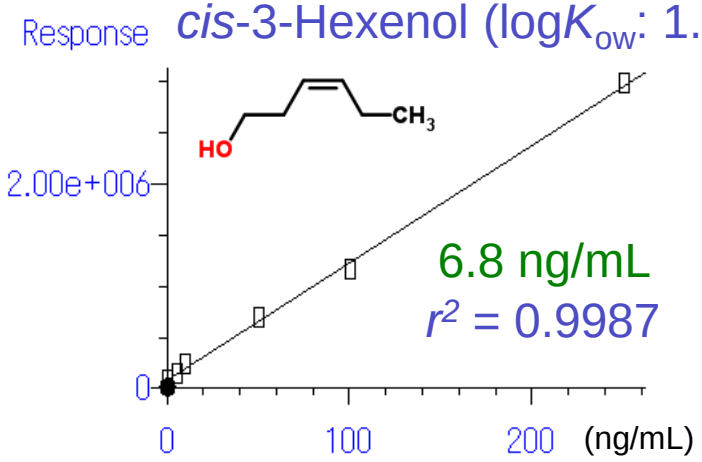
Benzyl alcohol (logK_{ow}: 1.08)



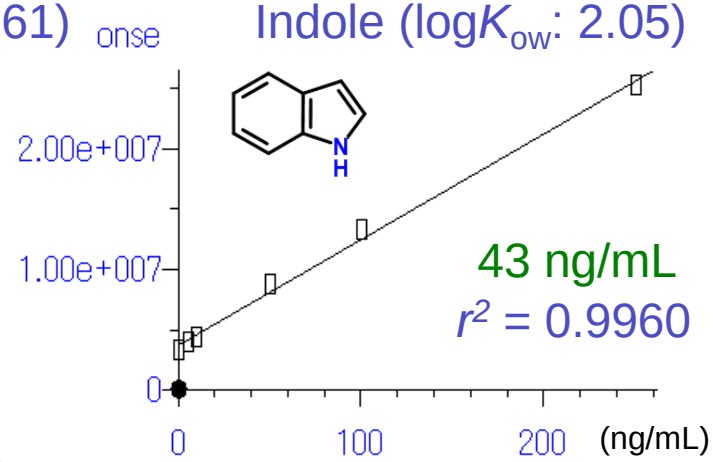
Guaiacol (logK_{ow}: 1.34)

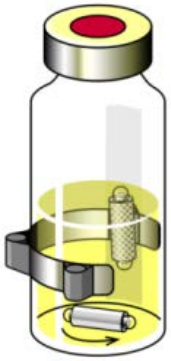


cis-3-Hexenol (logK_{ow}: 1.61)



Indole (logK_{ow}: 2.05)





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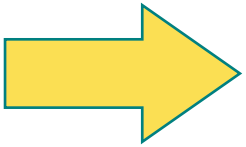
SBSE with in-situ derivatization (*der*-SBSE)

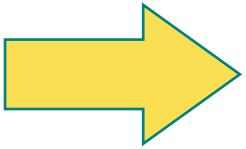
For specific hydrophilic/polar solutes,

SBSE with *in-situ* derivatization (e.g. acylation, esterification, and oximation) can be also used.



Higher K_{ow} values

Higher Recovery & Sensitivity



Higher molecular weight

Higher selectivity in GC-MS analysis

Analysis of stale flavor aldehydes in beer

Oxidatively produced unsaturated aldehyde such as E-2-nonenal plays a major role in the development of stale-flavor in beer.

_E_-2-nonenal (logK_{ow}: 3.06)

Odor threshold: 0.1 ng/mL

Analysis of E-2-nonenal and similar congeners in beer is generally rather challenging taking into account the relatively high levels of matrices (e.g. fusel alcohols, fatty acids, and esters).

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Determination of stale-flavor carbonyl compounds in beer by stir bar sorptive extraction with in-situ derivatization and thermal desorption–gas chromatography–mass spectrometry

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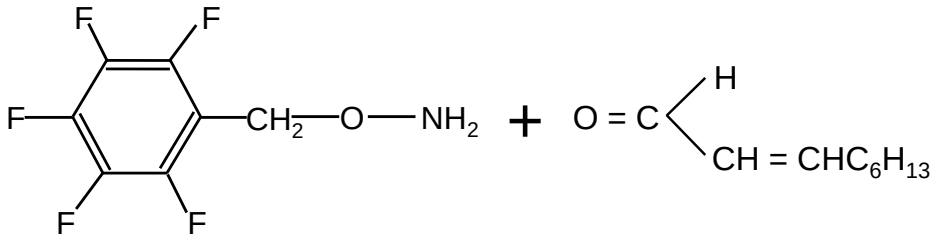
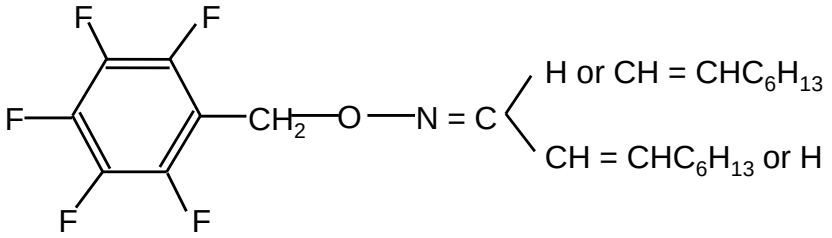
[b]*GERSTEL GmbH and Co. KG, Aktienstrasse 232-234, D-45473 Mülheim an der Ruhr, Germany*



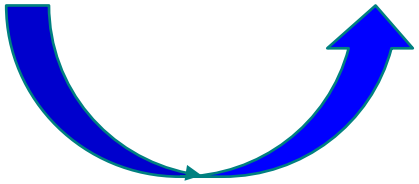
O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine

E-2-nonenal



E-2-nonenal PFBHA derivatives

PFBHA



der-SBSE with PFBHA of *E*-2-nonenal

30 mL beer (10-fold dilution)

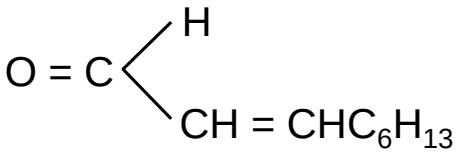
0.45 mL PFBHA (10 mg/mL)

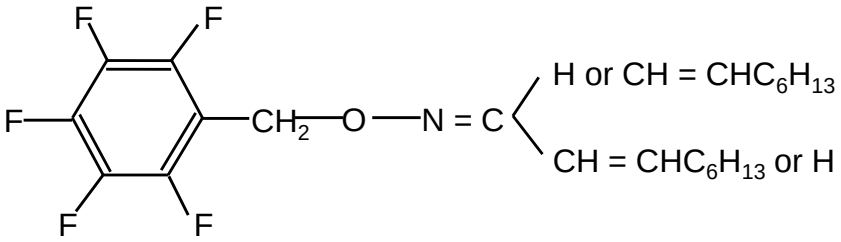
47 µL PDMS stir bar

der-SBSE@1000 rpm (1 h)

TD-GC-QMS (SIM)

Comparison between *E*-2-nonenal and its oxime



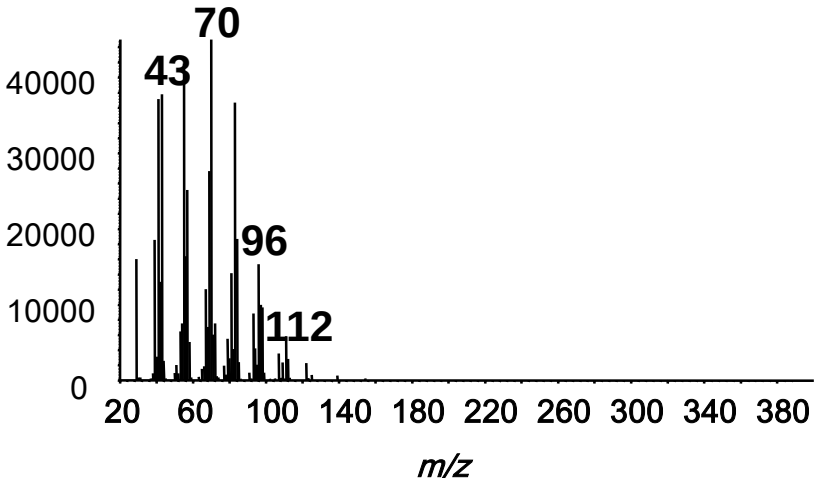


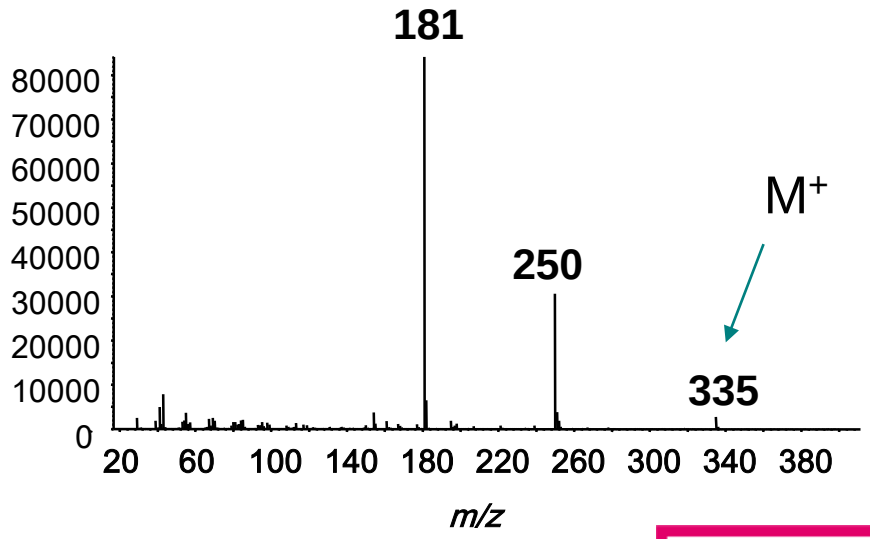
E-2-nonenal

MW: 140, logK_{ow}: 3.06

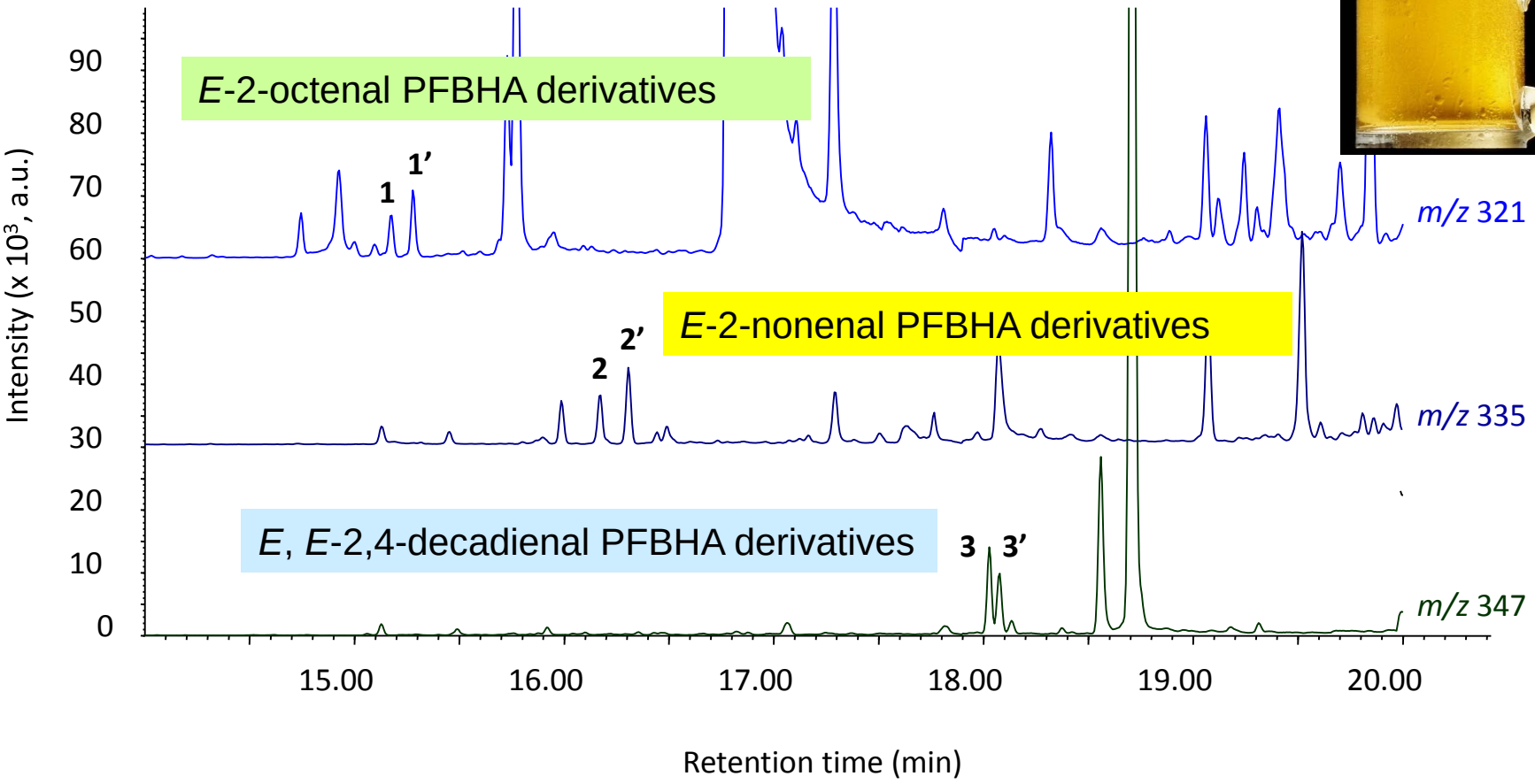
E-2-nonenal PFBHA derivatives

MW: **335**, logK_{ow}: **5.85**





SIM chromatograms of spiked beer at 0.5 ng/mL



Method performance

***E*-2-nonenal**

Linearity: r^2 = 0.9993 (0.1-10 ng/mL)

LOD: 0.023 ng/mL (SD × 3@0.1 ng/mL)

Recovery (%): 99 % (0.5 ng/mL)

Repeatability: RSD 2.4 (%, n=6)

Method validations for the determination of stale-flavor carbonyl compounds in beer by SBSE with in-situ derivatization and TD–GC–MS

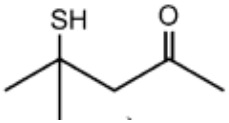
Compound	Correlation coefficient (r^2) (0.1–10 ng ml^{-1})[a]	LOD/ng ml^{-1}[b]	Recovery (%)[c]	RSD (%), $n=6$[c]
E-2-Octenal	0.9994	0.021	100	3.7
E,Z-2,6-Nonadienal	0.9995	0.032	101	7.3
E-2-Nonenal	0.9993	0.023	99	2.4
E,E-2,4-Decadienal	0.9996	0.023	98	3.4

[a] Linear range of the standard addition calibration graph.

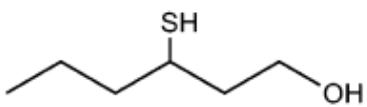
[b] The LODs were calculated as three times the standard deviation (3 s) of replicate analyses ($n=6$) of fortified samples spiked at the lowest concentrations of the calibration graph.

[c] The recoveries and precision were also examined by replicate analyses ($n=6$) of fortified samples spiked at 0.5 ng ml^{-1}. For both calibration and determination, the sum of the isomer peak area for each analyte was used.

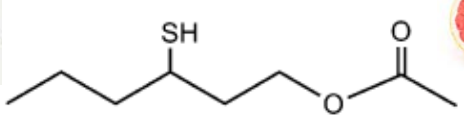
Analysis of polyfunctional thiols in beer



4MMP



3MH



3MHA

Odor threshold: **0.0015 µg/L***

Blackcurrant, Fruity, and catty.

Odor threshold: **0.055 µg/L***

Passion fruit, Grapefruit, and catty.

Odor threshold: **0.005 µg/L***

Grapefruit, Passion fruit, and sweaty.

Polyfunctional thiols have received special attentions due to their extremely low odor threshold levels and high sensory impact.

Several thiols, e.g. 4-mercapto-4-methyl pentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), are known as important aroma in some beer (e.g. strongly-hopped beer), as well as wine.

* T. Kishimoto, A. Wanikawa, K. Kono, K. Shibata,J. Agric. Food Chem. 56 (2008) 1051-1057.

Ethyl propiolate as derivatization agent

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Ethyl propiolate derivatisation for the analysis of varietal thiols in wine

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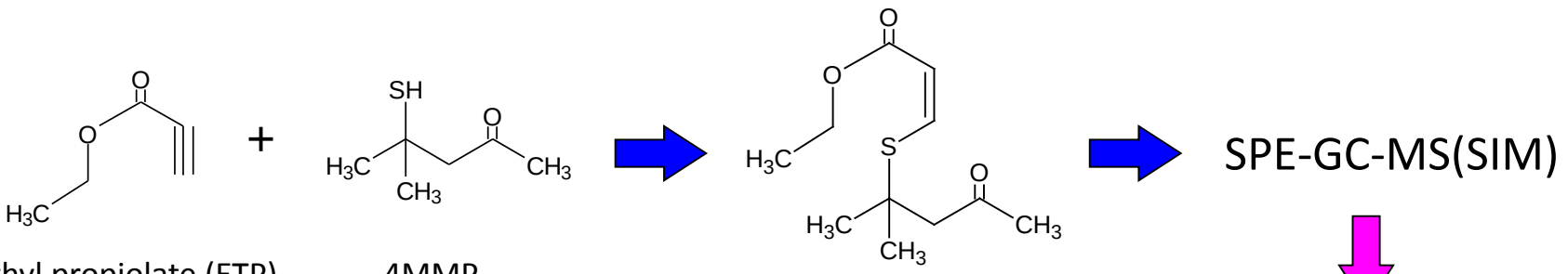


Ethyl propiolate (ETP)

4MMP
MW: 132
logK_{ow}: 1.07

cis-/*trans*-4MMP ETP derivative
MW: **230**
logK_{ow}: **1.83**

der-SBSE-GC-QQQMS(SRM)

der-SBSE-TD-GC-MS/MS*

10 mL Beer at pH 9

ETP solution

Salt addition (NaCl 30%)

24 µL PDMS stir bar

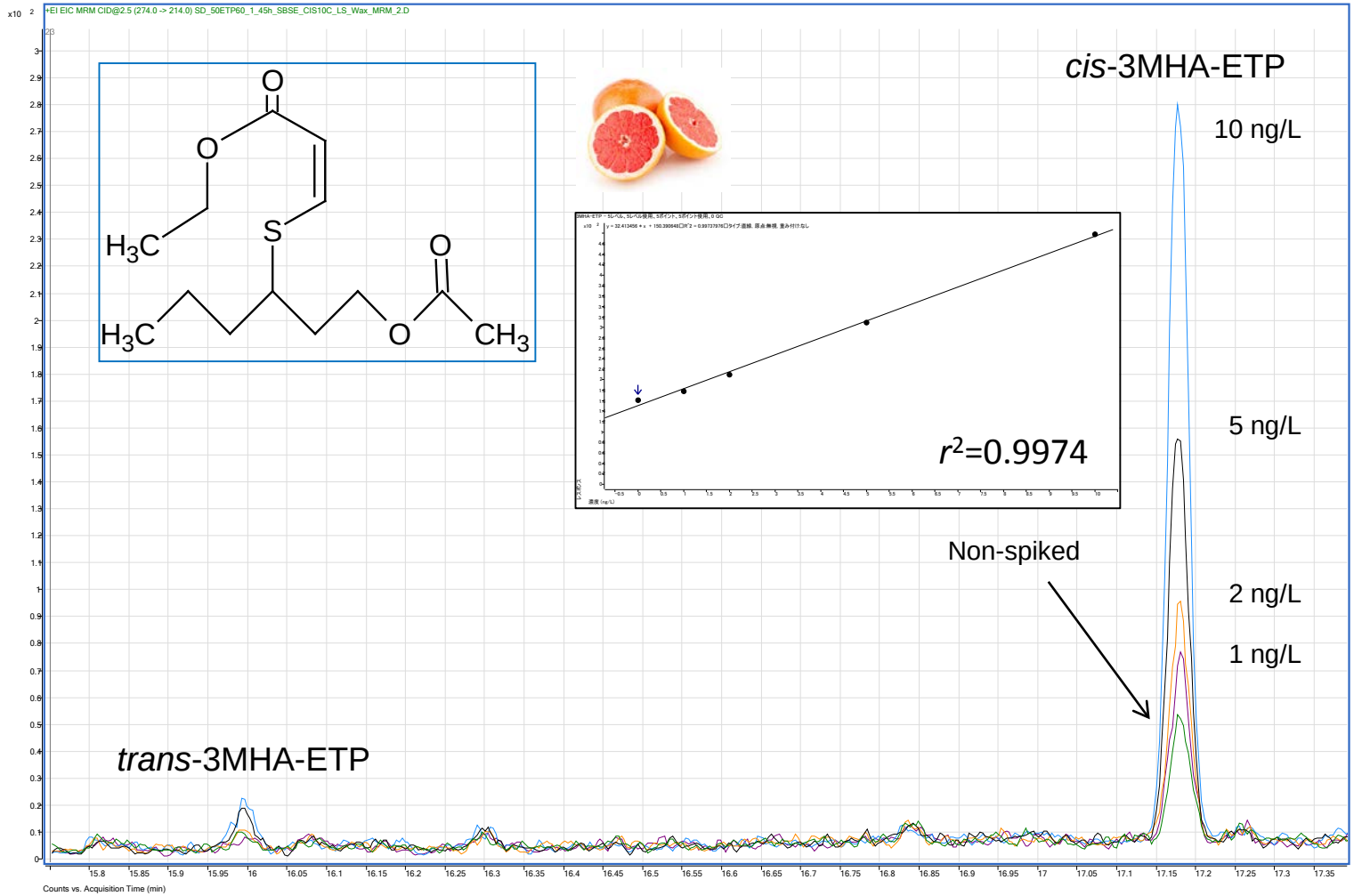
der-SBSE@1500 rpm (3 h)

TD-GC-QQQMS (SRM)

* N. Ochiai, K. Sasamoto, T. Kishimoto, in preparation (2015)

SRM chromatograms of 3MHA-ETP-derivative in beer*

(non-spiked, spiked at 1, 2, 5, 10 ng/L)



* N. Ochiai, K. Sasamoto, T. Kishimoto, in preparation (2015)

Outline

Introduction

Multi-stir bar sorptive extraction (^{m}SBSE) for non-targeted analysis of aroma compounds

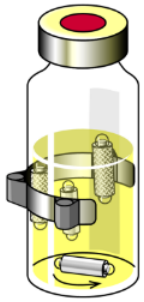
SBSE with *in-situ* derivatization (*der*-SBSE) for targeted analysis of off-flavors and key aroma compounds

Conclusion

Conclusion

Multi-SBSE (^{m}SBSE) and SBSE with *in-situ* derivatization (*der*-SBSE) can be successfully applied to the non-targeted analysis of a wide range of aroma compounds, and the targeted analysis of off-flavors and key aroma compounds, respectively.

These two SBSE modes can be considered as very complementary for aroma/off-flavor analysis of beverages, and can offer more accessible information content of the resulting data and/or improved sensitivity/selectivity when combined with TD-GC-MS(MS/MS).



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