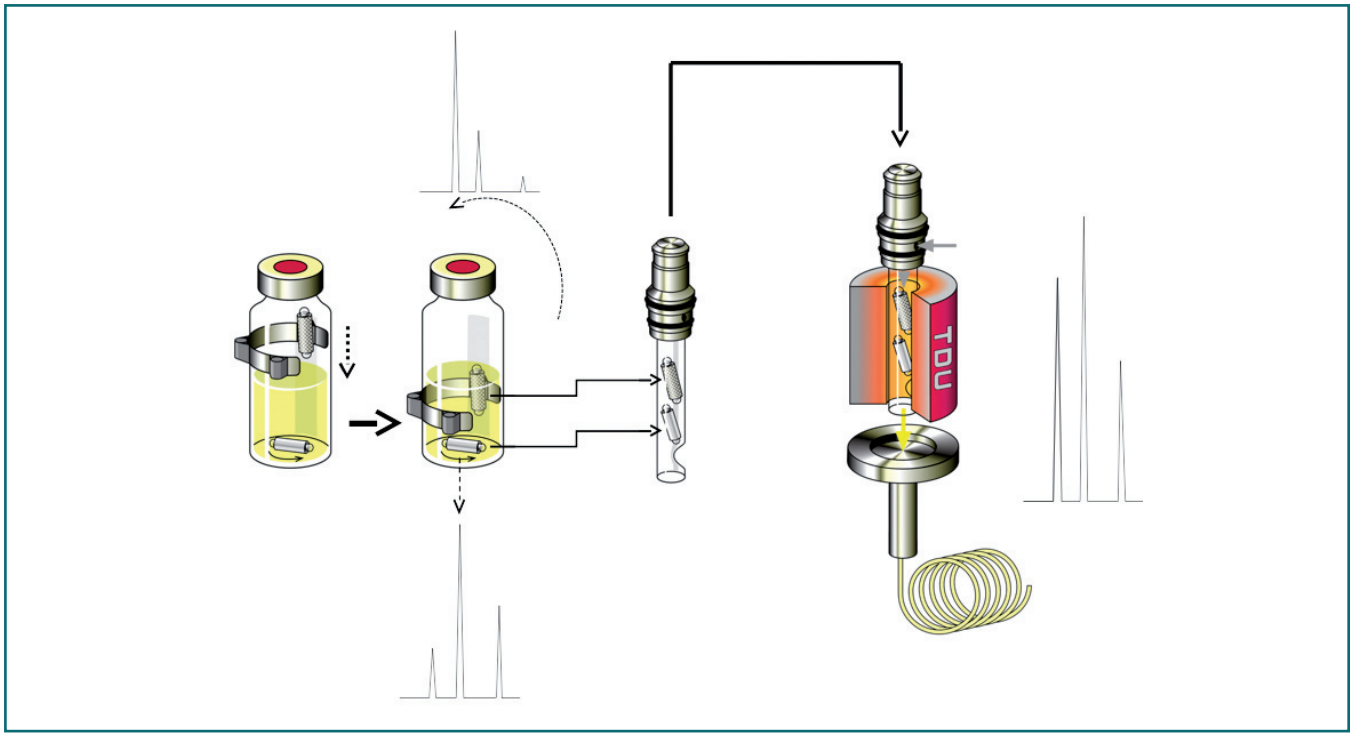

Figure 1: Multi-SBSE (^{m}SBSE) procedure.

Beverage Analysis

The Twister Sisters pick up the Flavors

By utilizing Multi-SBSE and SBSE with in-situ derivatization, you can determine non-target analytes across a wide polarity range as well as target off-flavors and key flavor compounds. If a high-end GC/MS system is used, the amount of information gained is significantly increased

By Nobuo Ochiai, GERSTEL K.K., Tokyo, Japan

Stir bar sorptive extraction (SBSE) has been successfully applied to food analysis including aroma analysis. For aroma analysis, SBSE has been applied to various sample matrices, such as water, beverages, fruits, herbs, plant material, essential oils, and vinegar [1–3]. These applications have been mostly performed using polydimethylsiloxane (PDMS) coated stir bars, because this was the only available phase for commercial stir bars (Twister®) before 2011. SBSE recovery can be estimated if the octanol–water distribution coefficient (K_{ow}) of the analyte is known. Hydrophobic solutes with a high K_{ow} can be extracted with high recovery, while hydrophilic solutes with a low K_{ow} show lower recovery [1]. Therefore, SBSE using PDMS phase is generally more selective for hydrophobic solutes, often resulting in a partial chromatogram biased towards less hydrophilic solutes. In 2011, a new Twister phase coated with polyethyleneglycol-modified silicone (EG Silicone) on a metal mesh support was introduced and applied to various sample types including whisky, wine, essential oils, and brewed coffee [4, 5]. This new polar coating showed good performance for the extraction of polar/hydrophilic solutes. A novel SBSE procedure termed multi-SBSE (^{m}SBSE) was developed in 2013 [6]. ^{m}SBSE consists of the PDMS Twister stirring at the bottom of the vial and the EG Silicone Twister attached to the inner side wall of the vial (a magnetic clip is used for the set-up). Compared to conventional SBSE, ^{m}SBSE provides more uniform enrichment of a wide range of aroma compounds in aqueous medium since the two Twister phases complement each other.

For the extraction (and analysis) of specific hydrophilic/polar solutes, SBSE in combination with *in-situ* derivatization (e.g. acylation, esterification, and oximation) can also be used (derivat-SBSE). For polar/hydrophilic solutes with low K_{ow} values, the corresponding derivatives generally have higher K_{ow} values, resulting in higher recovery and

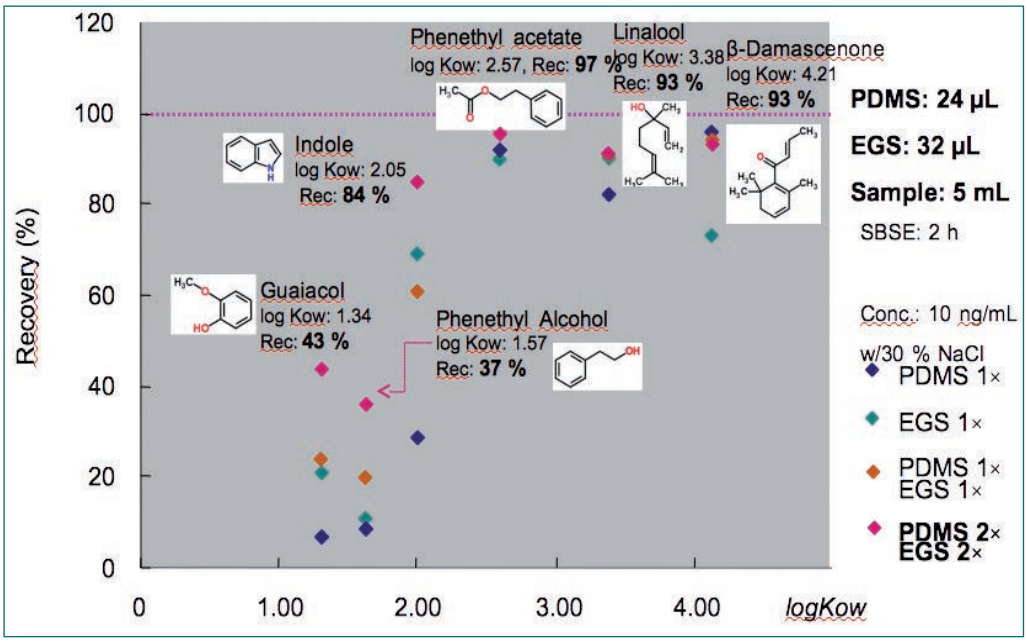

Figure 2: Comparison of recovery for test aroma compounds between single SBSE and ^{m}SBSE.

sensitivity [1]. Also, the higher molecular weights of derivatives provides higher selectivity in GC-MS analysis.

In this paper two SBSE approaches, ^{m}SBSE and derivat-SBSE will be described for aroma/off-flavor analysis of beverages. ^{m}SBSE shows more uniform enrichment of aroma compounds covering a wide polarity range in roasted green tea. Also, derivat-SBSE will be used to demonstrate trace analysis of key aroma compounds and off-flavors in beer.

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

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

Fig. 1 describes the multi-SBSE (^{m}SBSE) procedure. The extraction was performed by using a 24 µL PDMS Twister and a 32 µL EG Silicone Twister on a 5 mL sample after addition of 30 % NaCl. After extraction, the Twisters were thermally desorbed in split mode with a split ratio of 1:1 using the low split option controlled by the pneumatic box of the TDU system, and analyzed on a 30 m length × 0.25 mm i.d. × 0.25 µm df DB-Wax column using MS detection in scan mode.

Recoveries obtained by ^{m}SBSE for test aroma compounds in water, including various types of chemical classes (e.g. alcohol, ester, hetero-cyclic, ketone, and phenol), were compared with those obtained by conventional single SBSE. The log K_{ow} values of the test compounds were in the range of 1.34 (guaiacol) to 4.21 (β-damascenone). The concentration of the test compounds was 10 ng/mL each. Fig. 2 demonstrates a recovery comparison between 4 different SBSE conditions; (a) single SBSE using the PDMS Twister (1×), (b) single SBSE using the EG Silicone Twister (1×), (c) ^{m}SBSE using the PDMS Twister (1×) and the EG Silicone Twister (1×), (d) ^{m}SBSE using two PDMS Twisters (2×) and two EG Silicone Twisters (2×) (one PDMS Twister is stirring, while another PDMS Twister and two EG Silicone Twisters are attached on the inner side wall of the vial). For the condition (a) single SBSE using the PDMS Twister (1×), the solutes with log K_{ow} > 2.5 showed more than 80 % recoveries, while the solutes with log K_{ow} < 2.5 showed low recoveries, especially for guaiacol (log K_{ow}: 1.34, recovery: 8.3 %) and phenethyl alcohol (log K_{ow}: 1.61, recovery: 10 %). For the condition (b) single SBSE using the EG Silicone Twister (×1), the solutes with log K_{ow} > 2 showed higher than 70 % recovery, while the solutes with log K_{ow} < 2 also showed low recovery. However, compared to the condition (a), the recoveries for guaiacol (log K_{ow} 1.34) and indole (log K_{ow} 2.05) increased from 8.3 % to 21 %, and 29 % to 71 %, respectively, while the recovery for β-damascenone (log K_{ow} 4.21) decreased from 96 % to 74 %. Meanwhile, the condition (c) ^{m}SBSE using the PDMS Twister (1×) and the EG Silicone Twister (1×) showed higher recoveries for all test solutes except for indole (log K_{ow} 2.05, recovery 60 %) compared to single SBSE approaches. Moreover, the condition (d) ^{m}SBSE using two PDMS Twisters (2×) and two EG Silicone Twisters (2×), which has the highest phase ratio, showed the highest recoveries for the solutes with log K_{ow} of less than 2.5. Consequently, the ^{m}SBSE approach not only combines the extraction power of the PDMS Twister with the EG Silicone Twister, but also results in higher recovery due to increased phase volume (smaller phase ratio).

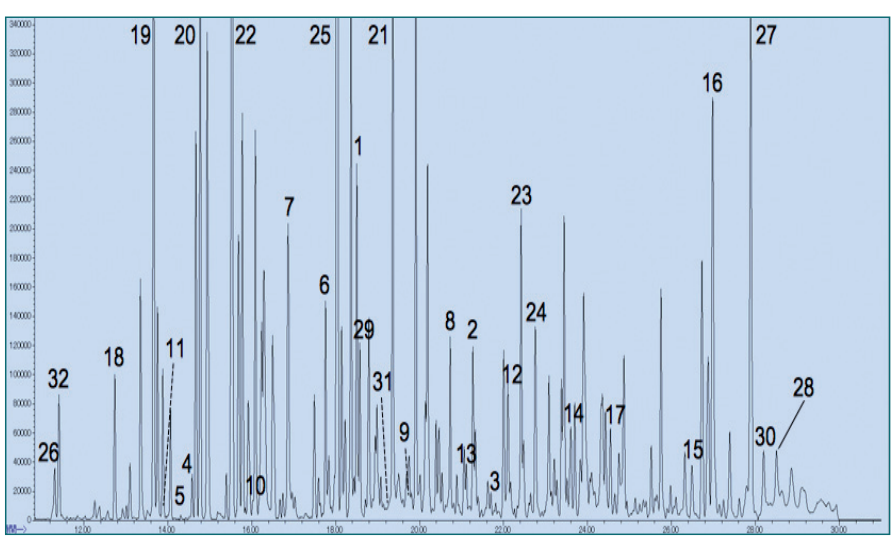

Figure 3: Total ion chromatogram of roasted green tea obtained from ^{m}SBSE-TD-GC-MS.

1. Furfuryl alcohol (log K_{ow} 0.45), 2. Benzyl alcohol (log K_{ow} 1.08), 3. Phenethyl alcohol (log K_{ow} 1.57), 4. *cis*-3-Hexenol (log K_{ow} 1.61), 5. 1-Hexanol (log K_{ow} 1.82), 6. 2,6-Dimethyl-1,3,7-octatrien-6-ol (log K_{ow} 3.24), 7. Linalool (log K_{ow} 3.38), 8. Geraniol (log K_{ow} 3.47), 9. Citronellol (log K_{ow} 3.56), 10. Furfural (log K_{ow} 0.83), 11. 6-Methyl-5-hepten-2-one (log K_{ow} 2.06), 12. *cis*-Jasmone (log K_{ow} 3.55), 13. Guaiacol (log K_{ow} 1.34), 14. *p*-Cresol (log K_{ow} 2.06), 15. Vinyl Guaiacol (log K_{ow} 2.24), 16. *p*-Vinyl phenol (log K_{ow} 2.41), 17. *p*-Ethyl phenol (log K_{ow} 2.55), 18. 2-Methyl pyrazine (log K_{ow} 0.49), 19. 2,5-Dimethyl pyrazine (log K_{ow} 1.03), 20. 2-Ethyl-5-methyl pyrazine (log K_{ow} 1.53), 21. 5,6,7,8-Tetrahydroquinoxaline (log K_{ow} 1.90), 22. 2-Ethyl-3,5-dimethyl pyrazine (log K_{ow} 2.07), 23. 2-Acetyl pyrrole (log K_{ow} 0.56), 24. 2-Formyl pyrrole (log K_{ow} 0.60), 25. 1-Ethyl-2-formyl pyrrole (log K_{ow} 1.14), 26. 1-Ethyl pyrrole (log K_{ow} 1.92), 27. Indole (log K_{ow} 2.05), 28. 2-Methyl indole (log K_{ow} 2.60), 29. 2-Acetyl thiazole (log K_{ow} 0.67), 30. Coumarin (log K_{ow} 1.51), 31. 2-Formyl thiophene (log K_{ow} 1.53), 32. 2,4,5-Trimethyl oxazole (log K_{ow} 1.86).

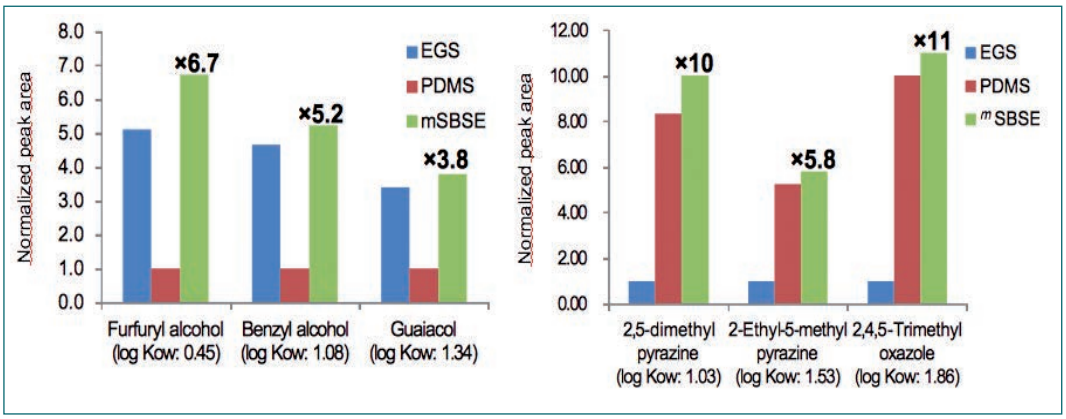

Figure 4: Comparison of the normalized areas of some aroma compounds between single SBSE and ^{m}SBSE.

Analysis of roasted green tea

Aroma compounds in green tea are present at trace level (from pg/mL to ng/mL), and therefore analytical methods should include powerful extraction and enrichment steps before GC analysis. Several sample preparation techniques, e.g. liquid phase extraction, gas phase extraction/distillation, and solid phase extraction, have been proposed for isolation and extraction of aroma compounds in green tea. The major drawbacks are, however, large sample volumes, e.g. 3-30 L [7], and the fact that the enrichment factor (original sample amount versus final extract volume) obtained with these techniques are rather limited and require additional evaporative concentration to a very small volume (<1 mL).

Roasted green tea (Houji-cha) was analyzed as an example of trace analysis of a wide variety of aroma compounds. 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) [8], but those additional aroma compounds are still only present at trace level. Fig. 3 demonstrated a total ion chromatogram (TIC) of roasted green tea obtained from ^{m}SBSE using the PDMS Twister (1×) and the EG Silicone Twister (1×). A variety of solutes which contribute to the aroma of roasted green tea were detected in the chromatogram from only 5 mL of sample, including coumarin (log K_{ow} 1.05), guaiacol (log Kow 1.34), *p*-cresol (log K_{ow} 2.06), indole (log K_{ow} 2.05), 2-ethyl-3,5-dimethyl pyrazine (log K_{ow} 2.07), linalool (log K_{ow} 3.38), geraniol (log K_{ow} 3.47), and *cis*-jasmone (log K_{ow} 3.55). Most of these solutes were determined in the range of 7.0 to 43 ng/mL with the standard addition calibration method [6].

Fig. 4 shows a comparison of the normalized areas of some aroma compounds between single SBSE using the PDMS Twister, single SBSE using the EG Silicone Twister, and ^{m}SBSE using both the PDMS Twister and the EG Silicone Twister. These data suggest that solutes which form a hydrogen bond were mainly recovered by EG Silicone Twister, while some heterocyclic solutes (2,5-dimethyl pyrazine, 2-ethyl-5-methyl pyrazine, and 2,4,5-trimethyl oxazole) were mainly recovered by PDMS Twister.

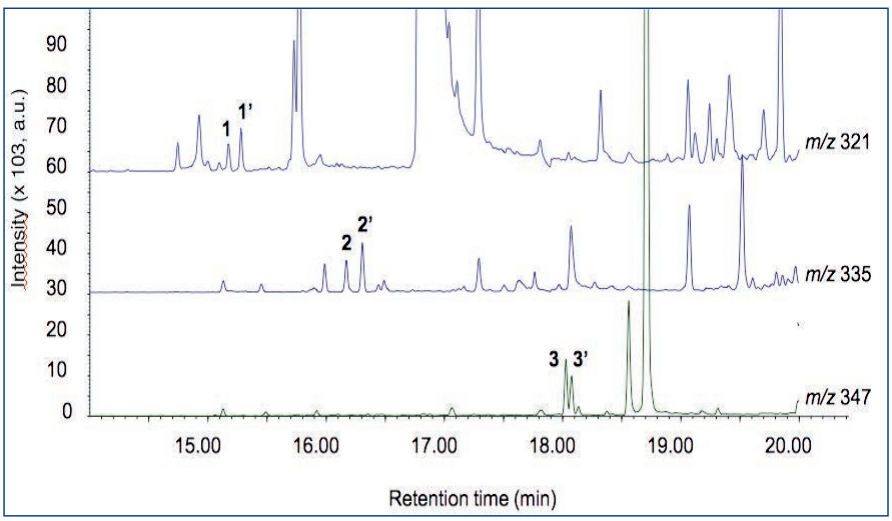

Figure 5: Selected ion monitoring (SIM) chromatograms of spiked beer at 500 pg/mL obtained from derivat-SBSE-TD-GC-MS. 1,1': *E*-2-Octenal, 2,2': *E*-2-Nonenal, 3,3': *E,E*-2,4-Decadienal

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

Analysis of stale flavor aldehydes in beer

Oxidatively produced unsaturated aldehydes play a major role in the development of stale-flavor in beer. *E*-2-Nonenal has been considered as the major source of the papery/cardboard stale-flavor in beer because of its very low odor threshold level at 0.1 ng/mL [9]. 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). A simple and effective method to decrease the interference caused by beer matrices during both sample preparation and GC analysis is to use derivatization. *E*-2-nonenal and similar congeners can be enriched and selectively detected by GC-MS using SBSE with in-situ derivatisation (derivat-SBSE). For *in-situ* derivatization, pentafluorobenzylhydroxylamine (PFBHA) was used to derivatize the targeted aldehydes (log K_{ow} 2.57-3.33) into the corresponding oximes (log K_{ow} 5.36-6.13), resulting in a highly selective and sensitive method (LOD 21-32 pg/mL) [10]. This is illustrated in Fig. 5 showing the analysis of a beer sample spiked at 500 pg/mL with a mixture of 3 aldehydes (*E*-2-octenal, E-2-nonenal, and *E*, *E*-2,4-decadienal). The extraction was performed by derivat-SBSE using a 47 µL PDMS Twister on a 30 mL sample (10-fold diluted with water) after addition of 0.45 mL of PFBHA solution (10 mg/mL). After extraction, the oximes were thermally desorbed in splitless mode and analyzed on a 30 m length × 0.25 mm i.d. × 0.25 µm df HP-5MS column using MS detection in selected ion monitoring (SIM) mode. The excellent sensitivity is clearly illustrated.

Analysis of tropical aroma thiols in beer

Polyfunctional thiols in food and beverages have received special attention due to their extremely low odor threshold levels and high sensory impact. Several thiols, e.g. 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), are well known for their contributions to the

fruity/citrus/tropical aroma. For strongly hopped beer, the odor threshold levels are in the low ng/L range [11], and consequently very sensitive and selective methods are needed. A derivat-SBSE method was developed for these thiols by Ochiai et al. [12] using a 24 µL PDMS Twister and the simple ester of propiolic acid as derivatization reagent. After extraction, thermal desorption (TD)-GC-QQQ-MS in selected reaction monitoring mode (SRM) was performed. In Fig. 6, SRM chromatograms of a beer sample spiked in the range from 1-10 ng/L are superimposed on the SRM chromatogram obtained for the non-spiked beer sample. At 17.18 min, thioacrylates of 3MHA (*cis*-derivative) appears in the SRM chromatogram of the beer samples. This compound was detected below its odor threshold level of 5.0 ng/L.

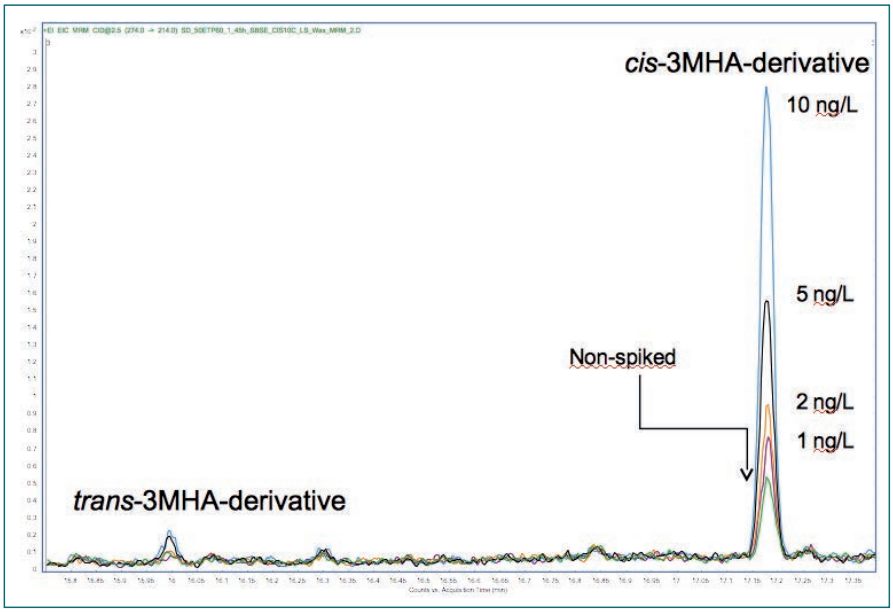

Figure 6: Selected reaction monitoring (SRM) chromatograms of spiked (1-10 ng/L) and non-spiked beer obtained from derivat-SBSE-TD-GC-MS/MS.

Conclusion

Multi-SBSE (^{m}BSE) and SBSE with *in-situ* derivatization (derivat-SBSE) can be successfully applied to the non-targeted analysis of a wide range of aroma compounds, and target 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 even more information content and/or improved sensitivity/selectivity when combined with high-end GC-MS (e.g. ^{m}SBSE with GC-high-resolution TOF-MS, and derivat-SBSE with GC-QQQ-MS).

Acknowledgements

All co-authors of the references [6, 10, 12] and Dr. Kevin MacNamara (Dublin, Ireland) are thanked for their contributions.

References

[1] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54.
[2] F.M. Lancas, M.E.C. Queiroz, P. Grossi, I.R.B. Olivares, J. Sep. Sci. 32 (2009) 813.
[3] C. Bicchi, E. Liberto, C. Cordero, B. Sgorbini, P. Rubiolo, LCGC NA (2009) 376.
[4] Y. Nie, E. Kleine-Benne, GERSTEL AppNote 3/2011, 2011.
[5] B. Sgorbini, C. Cagliero, C. Cordero, E. Liberto, P. Rubiolo, M. Rosanna, C. Bicchi, J. Chromatogr. A 1265 (2012) 39.
[6] N. Ochiai, K. Sasamoto, T. Ieda, F. David, P. Sandra, J. Chromatogr. A 1315 (2013) 70.
[7] K. Kumazawa, H. Masuda, J. Agric. Food Chem. 47 (1999) 5169.
[8] M. Kawakami, A. Kobayashi, K. Kator, J. Agric. Food Chem. 41 (1993) 633.
[9] M. Meilgaard, A. Elizondo, E. Moya, MBAA Tech. Q. 7 (1970) 143.
[10] N. Ochiai, K. Sasamoto, S. Daishima, A.C. Heiden, A. Hoffmann, J. Chromatogr. A 986 (2003) 101.
[11] T. Kishimoto, A. Wanikawa, K. Kono, K. Shibata, J. Agric. Food. Chem. 54 (2006) 8855.
[12] N. Ochiai, K. Sasamoto, T. Kishimoto, in preparation.