APPNOTE

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# A Comparison between SPME, SPME Arrow, SBSE and SA-SBSE for the Extraction of Polar Aroma Compounds from Aqueous Samples

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## Keywords
SPME, SPME Arrow, SBSE, Solvent-assisted SBSE (SA-SBSE), Polar aroma compounds, Aqueous samples, tea.

## Abstract
In this application note four PDMS based sorptive extraction methods, including SPME, SPME Arrow, SBSE and SA-SBSE are compared for the extraction of model aroma compounds from water and for aroma profiling of roasted green tea. The comparison was made using the same samples and keeping the analytical conditions constant for all techniques.

The comparison clearly demonstrates that the highest recoveries are obtained using SA-SBSE with a PDMS Twister®, swollen in dichloromethane/diisopropylether, especially for the most polar, hydrophilic compounds with octanol-water partitioning coefficients below 2. SA-SBSE also results in the most extended coverage of aroma compounds in real matrices and in more accurate identification.

## Introduction
Stir bar sorptive extraction (SBSE), based on the same fundamental principles as solid phase microextraction (SPME), was developed as an extraction and enrichment sample preparation technique for organic compounds in various liquid (and semi-solid) samples. SBSE is performed using dedicated "GERSTEL Twister®" devices consisting of magnetic rods imbedded in glass and coated with a 0.5 to 1 mm layer of polydimethylsiloxane (PDMS). As sorptive extraction technique, SBSE has many advantages such as (1) miniaturization of sample size, (2) simple operation, (3) combining extraction and concentration in a single step, (4) reduction or elimination of solvent use, and (5) very high sensitivity since the entire extracted amount can be introduced into GC-MS by thermal desorption.

Sorptive extraction of organic compounds from an aqueous sample using PDMS as extracting phase can fundamentally be considered as a liquid-liquid partitioning process that is controlled by two parameters: the distribution coefficient of the compound between the PDMS extraction phase and the sample ($K_{PDMS/sample}$) and the phase ratio (ß = $Volume_{PDMS}/Volume_{sample}$) [1]. In general, $K_{PDMS/sample}$ approaches the well-known octanol-water partitioning coefficient $K_{ow}$ for most organic compounds. The higher $K_{ow}$ and the lower the phase ratio (ß), the higher the extraction recovery of a given compound from a sample (theoretical recovery (%) = $[(K_{ow}/ß)/(1 + K_{ow}/ß)] \times 100$).

Since the volume of the PDMS phase of a GERSTEL Twister® is about 50 to 250 times larger than that of a classical PDMS coated SPME fiber (e.g. with 100 µm film thickness), the recovery of hydrophobic solutes is significantly improved due to the much lower phase ratio.

Recently SPME Arrow devices have been introduced to increase solute recovery based on an increased amount of PDMS extraction phase. However, the moderately larger extraction phase volume (~20x) is still below the PDMS volume available on GERSTEL Twister®s. Moreover, implementing SPME Arrow devices also requires a significant modification of standard GC

inlets due to the larger outer diameter of the arrow-shaped rod of the SPME Arrow device.

It can be calculated that for all PDMS based sorptive extraction methods, the relative recovery of organic compounds with a log $K_{ow}$ > 2 is lowest for classical SPME fibers, higher for SPME Arrow and highest for SBSE. For relatively polar solutes with an octanol-water partition coefficient $K_{ow}$ < 2, however, recovery remains low for all methods [1].

To improve the limited recovery of hydrophilic/polar solutes, associated with conventional SPME and SBSE using PDMS, solvent-assisted SBSE (SA-SBSE) was developed in 2016 [2]. This technique uses a solvent swollen PDMS extraction phase. By swelling PDMS with an organic solvent such as dichloromethane (DCM), or ether, the volume and polarity of the extracting phase are increased and significantly improve the recovery of hydrophilic/polar solutes while maintaining the original high affinity for hydrophobic solutes [3-5].

Direct comparisons of SPME, SPME Arrow, SBSE and SA-SBSE for the extraction of polar compounds from aqueous samples are not well documented in the literature. In this AppNote, we present the results of a comparative study using (1) a mixture of polar aroma model compounds in water and (2) roasted green tea. The sorption-based extraction techniques, performed in immersion mode) were evaluated in terms of recovery and extraction power.

## Experimental

### *Instrumentation*

To allow a fair comparison between the four sorptive extraction techniques, instrumentation and analytical parameters were kept the same for all methods. The thermal desorption (TD)-GC-MS analyses were performed with a thermal desorption unit (TDU 2) in combination with an MPS Robotic pro auto-sampler and a Peltier cooled CIS 4 programmed temperature vaporization (PTV) inlet installed on an Agilent 7890A gas chromatograph with a 5977 single quadrupole MS (QMS). For SPME and SPME Arrow, Hot Injection and Trapping (HIT) mode [6] using TDU 2/CIS 4 was used to ensure that the TD and injection conditions were equivalent to those of SBSE and SA-SBSE. It can be noted that the HIT mode using a TDU-GC-MS configuration is a very efficient and flexible approach to perform SPME Arrow analyses without the need for additional inlet modification. This configuration also enables optimized flow and thermal conditions for SPME and SPME Arrow.

### *Samples*

A spiked ultrapure water and a ready-to-drink roasted green tea were used for the analyses. For a spiked water sample, an aliquot of a standard solution containing sixteen aroma/flavor compounds (Table 1) was spiked at 10 ng/mL per solute. Five milliliter samples were placed in a 10 mL HS vial, salted (30 % NaCl), and extracted.

### *SA-SBSE and SBSE*

A FLEX Twister with 63 µL PDMS (1 cm length × 1.0 mm thickness) (Part No.: 021075-010-00) was used for both SBSE and SA-SBSE.

Before SA-SBSE, solvent swelling of the FLEX-Twister was done in a 2 mL-vial. First, 105 µL of 1/1 dichloromethane (DCM)/di-isopropyl ether (DIPE) mixed solvent is added into the 2 mL-vial containing the FLEX-Twister. The sealed vial is laid down and left for minimal 30 min. The solvent swollen FLEX-Twister can be stored in the 2 mL vial at room temperature (typically for a week).

Both SBSE and SA-SBSE extractions were performed at room temperature (25 °C) during 60 min while stirring at 750 rpm. After extraction, the stir bars were removed with a magnetic rod (Twister taking tool, Part No.: 013820-000-00) and forceps, rinsed 10 seconds in ultrapure water, and dried with a lint-free tissue.

### *SPME and SPME Arrow*

A SPME fiber with 0.5 µL PDMS (1 cm length × 0.1 mm thickness) (Part No.: 093639-001-00) and a SPME Arrow needle with 10 µL PDMS (2 cm length × 0.25 mm thickness) (Part No.: 100100-235-00) were used for SPME and SPME Arrow, respectively.

SPME and SPME Arrow extractions were performed in immersion mode at room temperature (25 °C) during 60 min while stirring at 750 rpm (5s on and 2s off) using a dedicated agitator for SPME Arrow. The temperature of the agitator was not controlled and was set in "OFF" mode.

After extraction, the SPME fiber and SPME Arrow needle were rinsed 10 seconds in ultrapure water before TD using the HIT mode. The rinsing was performed automatically by immersion in ultrapure water using the "derivatization mode (post-extraction)" feature of the MAESTRO software.

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*Analysis Conditions*

| | |
|---|---|
| TDU 2 | splitless<br>240 °C (3 min) (HIT-SPME/HIT-SPME Arrow)<br><br>30 °C (0.5 min); 720 °C/min;<br>280 °C (3 min) (SBSE/SA-SBSE) |
| CIS 4 | Tenax TA packed liner<br>low split (3 mL)<br>20 °C (1.5 min); 12 °C/sec; 240 °C |

*Analysis Conditions Agilent 7890A GC*

| | |
|---|---|
| Column | 20 m DB-WAX UI (Agilent)<br>$d_i$ = 0.18 mm, $d_f$ = 0.30 µm |
| Oven | 40 °C (3 min); 5 °C/min; 240 °C (7 min)<br>Backflush @240 °C (10 min) |

*Analysis Conditions 5977*

Scan 28.7 – 300 m/z

*Data Analysis*

MassHunter Quantitative Analysis Ver. B.10.0 (Agilent), MassHunter Unknowns Analysis Version B.10.0 (Agilent), Chem Station F.01.03 (Agilent), and Aroma Office database version 7.00.01 (Gerstel KK) were used for data analysis.

## Results and Discussion

*Comparison of Recovery of Aroma Compounds in Water*

To investigate actual recoveries in water samples, 16 model aroma compounds were spiked in ultrapure water at 10 ng/mL each. For the extraction of aqueous samples using PDMS, the partition coefficient between water and octanol ($K_{ow}$) is often used to estimate the theoretical maximum recovery at equilibrium [1]. The theoretical recovery (in %), defined as the ratio between the extracted solute amount $m_{PDMS}$ versus the total solute amount originally present in the water sample $m_0$, can be calculated as:

$$\text{Recovery (\%)} = m_{PDMS}/m_0 \times 100 = [(Kow/ß)/(1 + Kow/ß)] \times 100.$$

For a given extraction method (with a given phase ratio ß), the theoretical recovery can be plotted in function of (log) $K_{ow}$, as shown in Figure 1. These curves show that theoretical recoveries are lowest for classical SPME, higher for SPME Arrow, higher for SBSE and the highest for SA-SBSE, due to the respectively higher amounts of extracting phase: 0.5 µL PDMS for SPME fiber, 10 µL PDMS for SPME Arrow needle, 63 µL PDMS for a 1 cm x 1 mm Twister® in SBSE, and 168 µL for SA-SBSE (with 63 µL PDMS + 105 µL DCM/DIPE). The sample volume was kept constant at 5 mL for each method.

The experimental results obtained in the comparative study are shown by the datapoints in Figure 1 and are also listed in Table 1. Keeping in mind that the calculation of the theoretical recoveries is only an estimation since other parameters might play a role (stirring conditions, extraction time, pH, salt concentration,…), the experimental recoveries match relatively well with the theoretical values. For each solute the order in recovery is clearly respected: SPME < SPME Arrow < SBSE < SA-SBSE. Especially for the solutes with log $K_{ow}$ up to 2, the gain in recovery corresponds very well to the difference in phase ratio (for instance a factor 20 for SPME Arrow versus classical SPME, and a factor 127 for SBSE versus classical SPME).

For phenolic type compounds such as guaiacol (5), phenol (7), phenylethylalcohol (8), 4-methyl guaiacol (10), p.cresol (11), 4-ethyl guaiacol (13) and 4-ethylphenol (14), all important aroma compounds in beer, wine and alcoholic beverages, the experimental recoveries obtained by SPME, SPME Arrow and SBSE are typically lower than the theoretically predicted. This could be explained by the fact that recovery of these solutes depends on pH and a slight pH shift can result in a significant change in extraction efficiency. Interestingly, the measured recoveries for these compounds are higher and match better with theoretical predictions if SA-SBSE is applied.

Finally, for the most polar solutes diethylmalate (1), mesifurane (2), 2-acetylthiazole (3) and diethylsuccinate (4), the experimental recoveries are much higher than theoretically predicted. The results obtained for the phenolic compounds and the most polar solutes clearly demonstrate that extraction in SA-SBSE mode is not only controlled by increasing the volume of the extracting phase (lower ß), but also by modification of the nature of the extraction phase (increased polarity = higher affinity for more polar solutes). In fact, for none of the 16 model compounds the experimental recovery was found to be lower than the theoretical predicted value.

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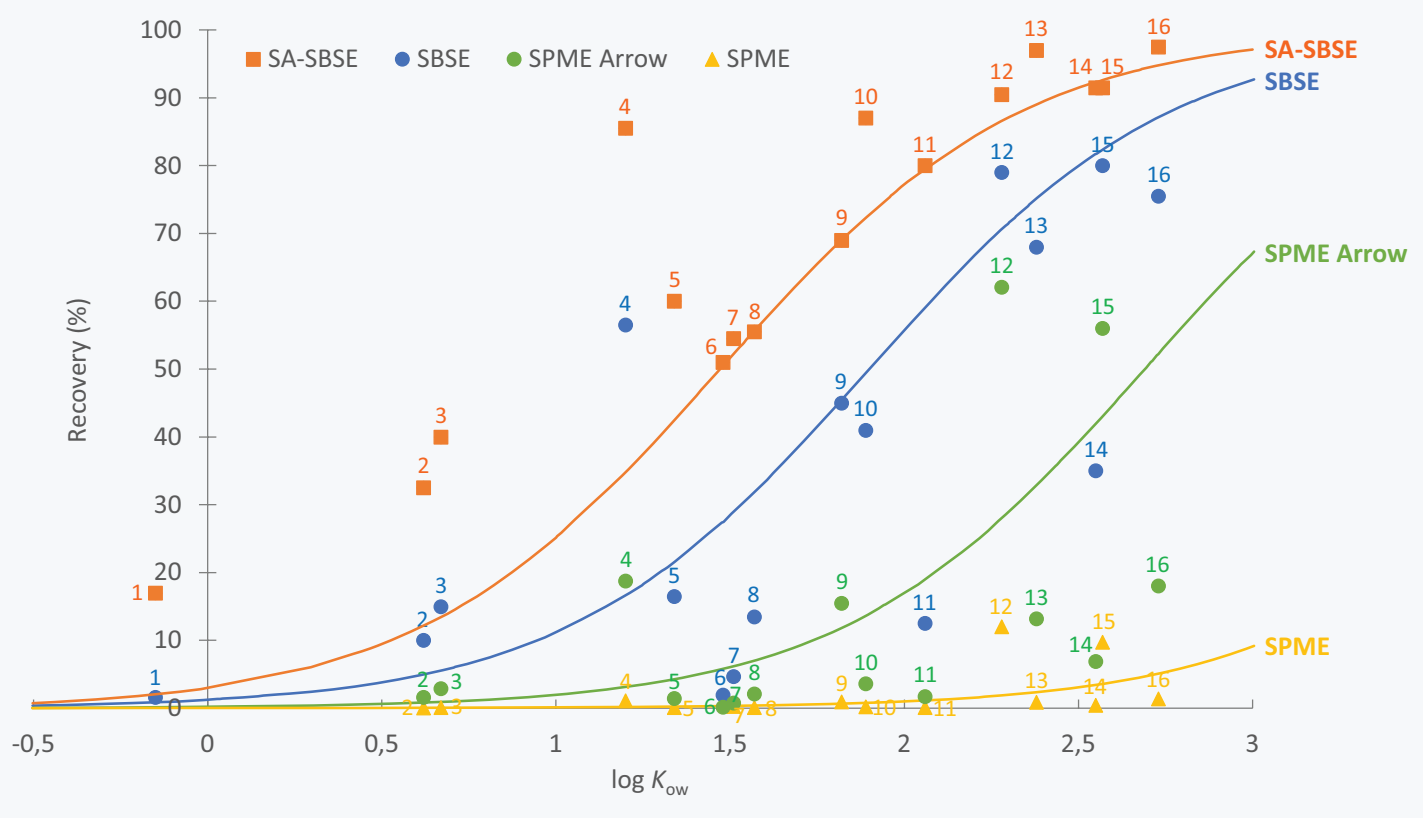


**Figure 1:** Theoretical recovery curves as a function of log $K_{ow}$ and actual recoveries of model aroma compounds for each extraction method.

**Table 1:** Actual recoveries of model aroma compounds for each extraction method.

| No. | Compound | log $K_{ow}$ | Recovery (%) | | | |
|---|---|---|---|---|---|---|
| | | | SA-SBSE | SBSE | SPME Arrow | SPME |
| 1 | Diethyl malate | -0.15 | 17 | 1.6 | 0.0 | 0.0 |
| 2 | Mesifurane | 0.62 | 33 | 10 | 1.6 | 0.08 |
| 3 | 2-Acetylthiazole | 0.67 | 40 | 15 | 2.9 | 0.14 |
| 4 | Diethyl succinate | 1.20 | 86 | 57 | 19 | 1.1 |
| 5 | Guaiacol | 1.34 | 60 | 17 | 1.5 | 0.09 |
| 6 | Raspberry ketone | 1.48 | 51 | 2.0 | 0.18 | 0.0 |
| 7 | Phenol | 1.51 | 55 | 4.7 | 0.80 | 0.26 |
| 8 | Phenethyl alcohol | 1.57 | 56 | 14 | 2.1 | 0.10 |
| 9 | 1-Hexanol | 1.82 | 69 | 45 | 15 | 0.94 |
| 10 | 4-Methyl guaiacol | 1.89 | 87 | 41 | 3.6 | 0.23 |
| 11 | p-Cresol | 2.06 | 80 | 13 | 1.8 | 0.13 |
| 12 | Ethyl phenylacetate | 2.28 | 91 | 79 | 62 | 12 |
| 13 | 4-Ethyl guaiacol | 2.38 | 97 | 68 | 13 | 0.89 |
| 14 | 4-Ethyl phenol | 2.55 | 92 | 35 | 6.9 | 0.45 |
| 15 | Phenethyl acetate | 2.57 | 92 | 80 | 56 | 9.7 |
| 16 | Eugenol | 2.73 | 98 | 76 | 18 | 1.4 |

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*Comparison of Extraction Methods for the Analysis of Aroma Compounds in Roasted Green Tea*

Fig. 2 illustrates a comparison of the total ion chromatograms (TIC) of roasted green tea obtained by each extraction method. For SA-SBSE, the peak intensities over the entire TIC are much higher than those of other three methods, and the profile is more detailed, both for the early eluting solutes (retention time < 20 min) and for late eluting compounds (retention time > 30 min). To compare the identification potential of aroma compounds in these TICs, we performed an automated data analysis workflow that combines Mass Hunter Unknown Analysis (Agilent) and AromaSearch (Aroma Office Ver. 7, GERSTEL K.K.). The automated workflow for aroma compound identification combines the deconvoluted mass spectral library search in Unknown Analysis and with retention index (RI) matching with the RI database by AromaSearch. The following three criteria were used for "Positive Identification": (1) mass spectral library search match score > 70, (2) RI deviation within ±10 units from the average RI in the Aroma Office database, and (3) area of the base peak obtained by the mass spectral deconvolution is >10000. The results are summarized in Table 2. For more details on the combination of Unknowns Analysis and AromaSearch, please refer to the GERSTEL Application Notes 227 and 235 [7, 8].

In the profile obtained by SA-SBSE 116 compounds were positively identified based on the above criteria. SBSE resulted in 92 identified compounds, and SPME Arrow in 64 compounds. Classical SPME only resulted in 29 identified compounds. Of the 24 compounds that were positively identified by SA-SBSE only, 15 have log $K_{ow}$ < 2, indicating enhanced extraction power of hydrophilic/polar aroma compounds. Five hydrophobic/apolar compounds with log $K_{ow}$ >4 were also positively identified with SA-SBSE only. Interestingly two components, dimethyl disulfide and 4,5-dimethyloxazole, which were positively identified in SPME Arrow and SBSE analyses, were not positively identified in the SA-SBSE analysis. This does not mean that these compounds were not extracted, but is related to the higher complexity of the profiles. Compared to SPME Arrow and SBSE, SA-SBSE has higher recovery for hydrophilic/polar solutes while maintaining high recovery for apolar solutes, so more compounds can be detected in the TIC. This increases the number of co-elution peaks at the same time, so even with deconvolution, interference with the mass spectrum for those compounds often occur, making identification more difficult [9]. For dimethyl disulfide and 4,5-dimethyloxazole, severe interference due to co-eluting compounds was observed and pure mass spectra were not obtained in SA-SBSE for these solutes. The use of other GC conditions and/or another type of mass spectrometer could provide a solution here.

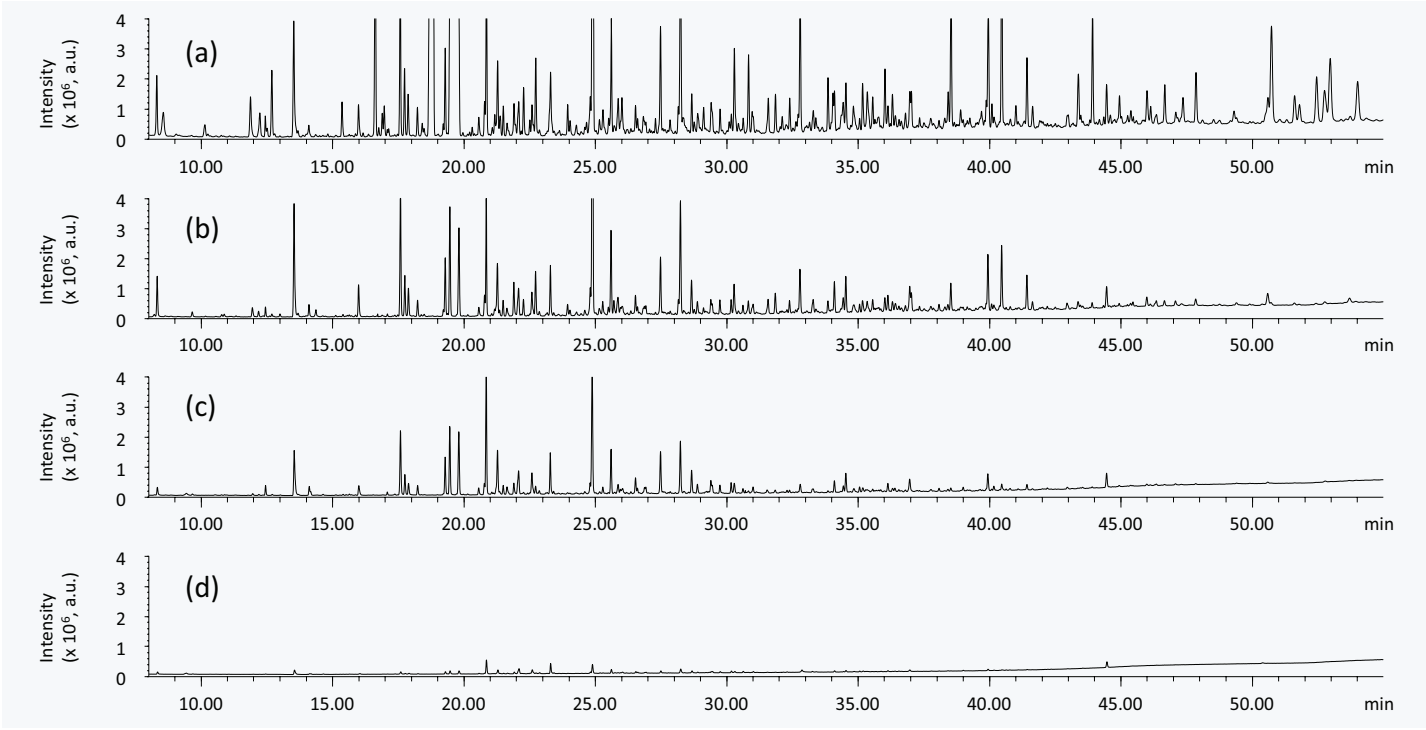


**Figure 2:** Comparison of TIC of roasted green tea obtained by four sorption-based methods. (a) SA-SBSE, (b) SBSE, (c) SPME Arrow, (d) SPME.

**Table 2:** Comparison of positively identified aroma compounds in roasted green tea by four extraction methods.

| Compound Name | RI | log $K_{ow}$ | SPME | SPME Arrow | SBSE | SA-SBSE |
|---|---|---|---|---|---|---|
| 2-Methylbutanal | 914 | 1.23 | ✓ | ✓ | ✓ | ✓ |
| 2-Butanone, 3-methyl | 930 | 0.67 | - | ✓ | ✓ | ✓ |
| Dimethyl disulfide | 1075 | 1.87 | - | ✓ | ✓ | - |
| Hexanal | 1084 | 1.80 | - | ✓ | ✓ | ✓ |
| 4-Methyl-3-penten-2-one | 1136 | 1.37 | - | ✓ | ✓ | ✓ |
| 4,5-Dimethyloxazole | 1155 | 1.31 | - | ✓ | ✓ | - |
| 1-Ethylpyrrole | 1185 | 1.92 | ✓ | ✓ | ✓ | ✓ |
| 2-Heptanone | 1187 | 1.73 | ✓ | ✓ | ✓ | ✓ |
| Pyridine | 1191 | 0.80 | - | ✓ | ✓ | ✓ |
| Trimethyloxazole | 1206 | 1.86 | ✓ | ✓ | ✓ | ✓ |
| Isopentanol | 1215 | 1.26 | - | - | - | ✓ |
| Furfuryl methyl ether | 1242 | 1.14 | - | ✓ | ✓ | ✓ |
| 2-Methylthiazole | 1247 | 1.54 | - | - | ✓ | ✓ |
| 3-Methylbutanol | 1258 | 1.26 | - | - | ✓ | ✓ |
| 2-Methyltetrahydrofuran-3-one | 1270 | -0.20 | - | ✓ | ✓ | ✓ |
| methylpyrazine | 1274 | 0.48 | ✓ | ✓ | ✓ | ✓ |
| Acetol | 1306 | -0.78 | ✓ | ✓ | ✓ | ✓ |
| Z-2-Pentenol | 1329 | 1.12 | - | - | ✓ | ✓ |
| 2,5-Dimethylpyrazine | 1333 | 1.03 | ✓ | ✓ | ✓ | ✓ |
| 2,6-Dimethylpyrazine | 1339 | 1.03 | ✓ | ✓ | ✓ | ✓ |
| Ethylpyrazine | 1344 | 0.98 | ✓ | ✓ | ✓ | ✓ |
| 2,3-Dimethylpyrazine | 1357 | 1.03 | ✓ | ✓ | ✓ | ✓ |
| Isobutyric anhydride | 1366 | 1.24 | - | - | ✓ | ✓ |
| Z-3-Hexenol | 1393 | 1.61 | - | - | ✓ | ✓ |
| 2-Ethyl-3-methylpyrazine | 1403 | 1.53 | ✓ | ✓ | ✓ | ✓ |
| Trimethylpyrazine | 1416 | 1.58 | ✓ | ✓ | ✓ | ✓ |
| Propylpyrazine | 1429 | 1.47 | - | ✓ | ✓ | ✓ |
| 2-Cyclohexene-1-one | 1447 | 1.20 | - | - | - | ✓ |
| Linalool oxide I | 1454 | 1.99 | - | - | - | ✓ |
| 3-Ethyl-2,6-dimethylpyrazine | 1457 | 2.07 | ✓ | ✓ | ✓ | ✓ |
| Methional | 1462 | 0.41 | - | - | - | ✓ |
| 2,3-Diethylpyrazine | 1466 | 2.02 | - | ✓ | ✓ | ✓ |
| Furfural | 1470 | 0.83 | - | ✓ | ✓ | ✓ |
| 2-Ethyl-5,6-dimethylpyrazine | 1474 | 2.07 | ✓ | ✓ | ✓ | ✓ |
| Linalool oxide II | 1482 | 1.99 | - | ✓ | ✓ | ✓ |
| Tetramethylpyrazine | 1488 | 2.13 | ✓ | ✓ | ✓ | ✓ |
| 2-Ethylhexanol | 1498 | 2.73 | ✓ | ✓ | ✓ | ✓ |
| 2-Methyl-5-vinylpyrazine | 1499 | 1.39 | - | - | ✓ | ✓ |
| Acetylfuran | 1513 | 0.80 | - | ✓ | ✓ | ✓ |
| Pyrrole | 1522 | 0.88 | - | - | ✓ | ✓ |
| Benzaldehyde | 1532 | 1.71 | ✓ | ✓ | ✓ | ✓ |
| 3,5-Dimethyl-2-isobutylpyrazine | 1538 | 2.51 | ✓ | ✓ | ✓ | ✓ |

**Table 2 (cont.):** Comparison of positively identified aroma compounds in roasted green tea by four extraction methods.

| Compound Name | RI | log $K_{ow}$ | SPME | SPME Arrow | SBSE | SA-SBSE |
|---|---|---|---|---|---|---|
| Propanoic acid | 1545 | 0.58 | - | - | - | ✓ |
| Linalool | 1555 | 3.38 | ✓ | ✓ | ✓ | ✓ |
| 2-Propionylfuran | 1581 | 1.29 | - | - | ✓ | ✓ |
| 5-Methylfurfural | 1582 | 1.38 | - | ✓ | ✓ | ✓ |
| Methyl furoate | 1585 | 0.95 | - | ✓ | ✓ | ✓ |
| 3-Methoxypyridine | 1596 | 0.89 | - | ✓ | ✓ | ✓ |
| Dimethyl succinate | 1602 | 0.40 | - | - | ✓ | ✓ |
| Isophorone | 1609 | 2.62 | - | ✓ | ✓ | ✓ |
| 2-Acetylpyridine | 1614 | 0.49 | - | - | ✓ | ✓ |
| Hotrienol | 1618 | 3.24 | ✓ | ✓ | ✓ | ✓ |
| 2-Formyl-1-methylpyrrole | 1632 | 1.14 | - | - | ✓ | ✓ |
| Methyl benzoate | 1632 | 1.83 | - | - | ✓ | ✓ |
| Butyrolactone | 1641 | -0.31 | - | ✓ | ✓ | ✓ |
| 4-Pyridinyl acetate | 1647 | 0.40 | - | - | ✓ | ✓ |
| 2-Acetylthiazole | 1658 | 0.67 | - | - | - | ✓ |
| Acetophenone | 1662 | 1.67 | ✓ | ✓ | ✓ | ✓ |
| Furanmethanol | 1669 | 0.45 | - | - | ✓ | ✓ |
| 2,3-Dimethyl-5-isopentylpyrazine | 1670 | 3.04 | ✓ | ✓ | ✓ | ✓ |
| α-Terpineol | 1709 | 3.33 | - | ✓ | ✓ | ✓ |
| Dimethyl glutarate | 1710 | 0.90 | - | - | - | ✓ |
| r-Hexalactone | 1716 | 0.60 | - | - | ✓ | ✓ |
| Methyl phenylacetate | 1771 | 2.08 | - | ✓ | ✓ | ✓ |
| 1-(3-Methylphenyl)ethanone | 1787 | 2.22 | - | ✓ | ✓ | ✓ |
| Methyl salicylate | 1788 | 2.60 | ✓ | ✓ | ✓ | ✓ |
| 2,2,6-Trimethyl-1,4-cyclohexanedione | 1791 | 0.36 | - | - | - | ✓ |
| 2,5-Dimethoxytoluene | 1798 | 2.70 | - | - | - | ✓ |
| 2-Tridecanone | 1807 | 4.68 | - | - | - | ✓ |
| N-Furfurylpyrrole | 1838 | 2.50 | ✓ | ✓ | ✓ | ✓ |
| Cyclotene | 1842 | 1.29 | - | - | - | ✓ |
| Calamenene | 1845 | 6.25 | - | - | - | ✓ |
| Hexanoic acid | 1854 | 2.05 | - | - | - | ✓ |
| Geraniol | 1858 | 3.47 | ✓ | ✓ | ✓ | ✓ |
| Guaiacol | 1871 | 1.34 | - | - | ✓ | ✓ |
| Bnzyl alcohol | 1889 | 1.08 | - | ✓ | ✓ | ✓ |
| Benzeneethanol | 1926 | 1.57 | - | ✓ | ✓ | ✓ |
| Benzyl cyanide | 1939 | 1.56 | - | ✓ | ✓ | ✓ |
| Isoquinoline | 1957 | 2.14 | - | ✓ | ✓ | ✓ |
| 2-Ethylhexanoic acid | 1959 | 2.96 | - | - | - | ✓ |
| Z-Jasmone | 1961 | 3.55 | - | ✓ | ✓ | ✓ |
| Maltol | 1981 | -0.19 | - | - | ✓ | ✓ |
| 2-Acetylpyrrole | 1985 | 0.56 | - | ✓ | ✓ | ✓ |
| beta-Ionone-5,6-epoxide | 2009 | 2.93 | ✓ | ✓ | ✓ | ✓ |

**Table 2 (cont.):** Comparison of positively identified aroma compounds in roasted green tea by four extraction methods.

| Compound Name | RI | log $K_{ow}$ | SPME | SPME Arrow | SBSE | SA-SBSE |
|---|---|---|---|---|---|---|
| Phenol | 2015 | 1.51 | ✓ | ✓ | ✓ | ✓ |
| 2-Pyrrolecarbaldehyde | 2039 | 0.60 | - | ✓ | ✓ | ✓ |
| Isopropyl myristate | 2043 | 7.17 | - | - | ✓ | ✓ |
| Furaneol | 2049 | 0.82 | - | - | ✓ | ✓ |
| Methyl pyrrole-2-carboxylate | 2065 | 0.71 | - | ✓ | ✓ | ✓ |
| 2-Methyl-4-quinazolinone | 2070 | 1.73 | - | ✓ | ✓ | ✓ |
| Methyl cinnamate | 2091 | 2.36 | - | - | - | ✓ |
| 4-Ethylphenol | 2107 | 2.55 | - | ✓ | ✓ | ✓ |
| Parabanic acid | 2133 | -0.54 | - | - | - | ✓ |
| Nonanoic acid | 2175 | 3.52 | - | - | ✓ | ✓ |
| 4-Vinylguaiacol | 2207 | 2.24 | - | - | ✓ | ✓ |
| Methyl palmitate | 2225 | 7.25 | - | - | ✓ | ✓ |
| Methyl anthranilate | 2255 | 2.26 | - | ✓ | ✓ | ✓ |
| 2,6-Dimethoxyphenol | 2278 | 1.16 | - | - | - | ✓ |
| Decanoic acid | 2282 | 4.02 | - | - | - | ✓ |
| DDMP * | 2288 | 0.03 | - | - | - | ✓ |
| 4-Methyl-5-thiazoleethanol | 2329 | 1.11 | - | - | - | ✓ |
| Methyl jasmonate | 2354 | 2.76 | - | - | ✓ | ✓ |
| Isoeugenol | 2362 | 2.65 | - | ✓ | ✓ | ✓ |
| Dihydroactinidiolide | 2374 | 2.30 | ✓ | ✓ | ✓ | ✓ |
| 3-Ethyl-4-methyl-2,5-pyrrolidinedione | 2382 | 0.48 | - | - | - | ✓ |
| Methyl stearate | 2434 | 8.23 | - | - | - | ✓ |
| 3-Hydroxypyridine | 2437 | 0.32 | - | - | ✓ | ✓ |
| Indole | 2462 | 2.05 | ✓ | ✓ | ✓ | ✓ |
| Coumarin | 2475 | 1.51 | - | ✓ | ✓ | ✓ |
| Skatole | 2509 | 2.60 | - | ✓ | ✓ | ✓ |
| 5-Hydroxymethylfurfural | 2517 | -0.09 | - | ✓ | ✓ | ✓ |
| 3-Hydroxy-beta-damascone | 2559 | 2.89 | - | - | - | ✓ |
| Vanillin | 2583 | 1.05 | - | - | ✓ | ✓ |
| Methyl vanillate | 2625 | 1.82 | - | - | - | ✓ |
| Acetovanillone | 2661 | 1.02 | - | - | ✓ | ✓ |
| Myristic acid | 2707 | 5.98 | - | - | - | ✓ |
| Palmitic acid | 2919 | 6.96 | - | - | ✓ | ✓ |
| Raspberry ketone | 3013 | 1.48 | - | - | - | ✓ |

* DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

A ✓ mark indicates a positive identification.

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To further illustrate the extraction power of the four extraction methods, the sensitivities (signal-to-noise ratios, S/N) and MS library search match scores for eight important aroma compounds, present at low ppb (ng/mL) level in roasted green tea are compared in Table 3. To this, quantitative analysis of the selected aroma compounds with log $K_{ow}$ 0.48 to 3.38 was performed using SA-SBSE standard addition method. Concentrations between 1.6 to 15 ng/mL were measured. The deconvoluted mass spectra of these eight compounds for each extraction method were then compared to those in the library (using MassHunter Unknowns Analysis) and to estimate the sensitivities obtained by each extraction method, the signal-to-noise (S/N) ratio was calculated from the extracted ion chromatograms (using the target ion for quantification). Table 3 shows the log $K_{ow}$, target ions for quantification, measured concentration, S/N ratio for each extraction method, and MS library match score for the target compounds.

**Table 3:** Log $K_{ow}$ values, target ions, concentrations, S/N ratio, and MS library search match score for the selected aroma compounds in each extraction method.

| Compound | log $K_{ow}$ | m/z | Conc. | S/N Ratio | | | | MS library search match score | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | [ng/mL] | SA-SBSE | SBSE | SPME Arrow | SPME | SA-SBSE | SBSE | SPME Arrow | SPME |
| Methional | 0.48 | 104 | 1.6 | 260 | 34 | - | - | 87 | - | - | - |
| r-Hexalactone | 0.60 | 85 | 2.7 | 810 | 200 | 10 | - | 96 | 78 | - | - |
| Vanillin | 1.05 | 152 | 15 | 2100 | 390 | 64 | - | 91 | 87 | 68 | - |
| Cyclotene | 1.29 | 112 | 13 | 470 | 62 | 18 | - | 77 | - | - | - |
| Raspberry ketone | 1.48 | 164 | 6.8 | 550 | 24 | 4.3 | - | 88 | - | - | - |
| Indole | 2.05 | 117 | 6.5 | 5700 | 2600 | 640 | 72 | 98 | 98 | 96 | 87 |
| Methyl salicylate | 2.60 | 152 | 4.7 | 2300 | 2400 | 1800 | 400 | 97 | 98 | 98 | 96 |
| Linalool | 3.38 | 93 | 5.1 | 3800 | 2600 | 2200 | 610 | 95 | 96 | 97 | 94 |

For indole (log $K_{ow}$ 2.05), methyl salicylate (log $K_{ow}$ 2.60), and linalool (log $K_{ow}$ 3.38), which have relatively high affinity to PDMS, the S/N ratios of the ions for quantification in all methods were very high (hundreds to thousands) even at the low ng/mL level, and good library matches (score 87-98) were obtained. However, for hydrophilic and polar aroma compounds with log $K_{ow}$ <1.5, the S/N ratios differed significantly among the extraction methods, with all five compounds being not-detected in SPME. For SPME Arrow, only vanillin (15 ng/mL), with a S/N ratio of 64, showed a library match score of 68, but the other four compounds with S/N ratios of 18 or less did not yield candidate compounds/library match score in the search results. For SBSE, only γ-hexalactone (2.7 ng/mL) with a S/N ratio of 200, in addition to vanillin with a S/N ratio of 390, showed a library match score above 70. SA-SBSE, on the other hand, showed S/N ratios above 260 for all five hydrophilic and polar compounds, with library match scores ranging clearly above 70. Fig. 3 illustrates the extracted ion chromatogram and deconvolution mass spectrum of cyclotene (log $K_{ow}$ 1.29, 5.1 ng/mL) for each extraction method.

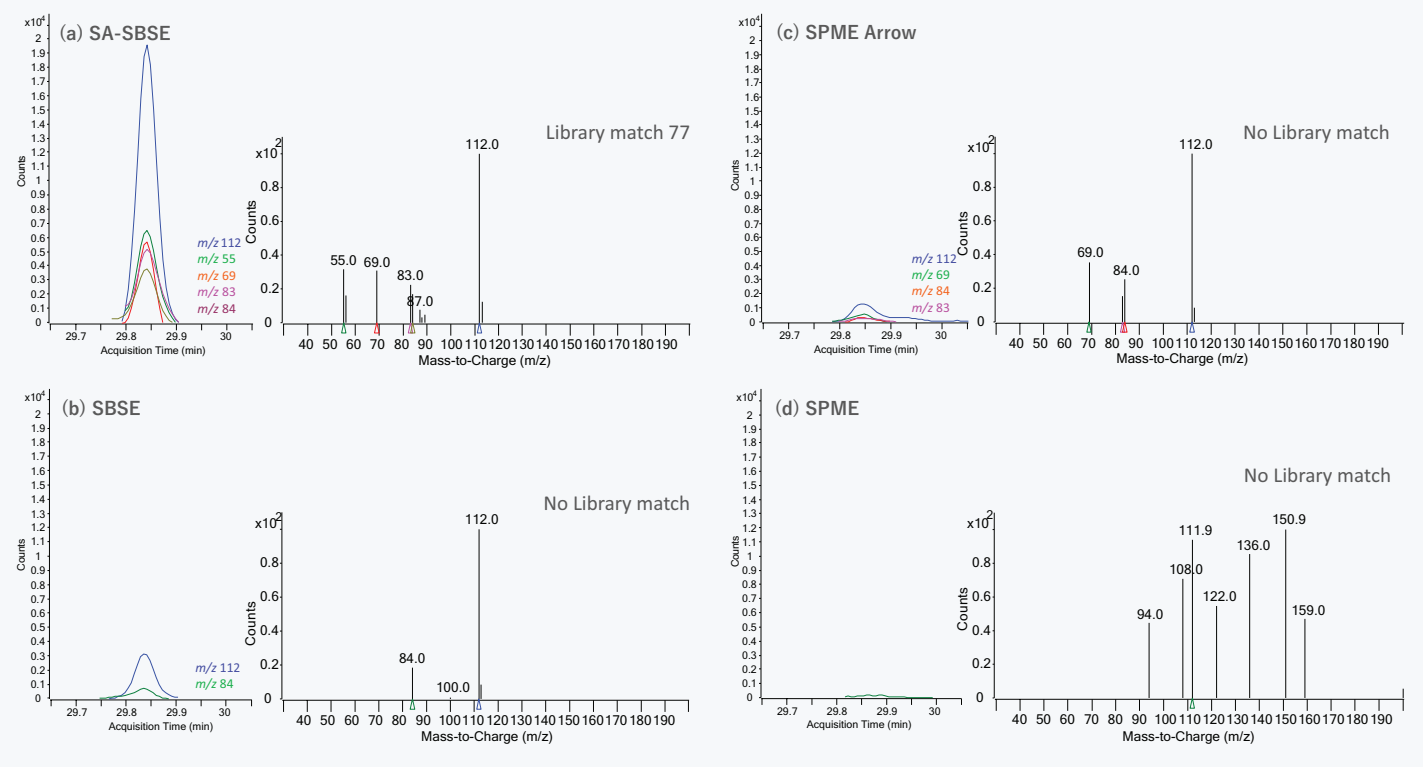


**Figure 3:** Comparison of mass chromatograms and mass spectra of cyclotene (log $K_{ow}$ 1.29, concentration 13 ng/mL) obtained by MassHunter Unknowns Analysis in each extraction method.

## Conclusion

Comparison of the recoveries of model aroma compounds from an aqueous sample using four PDMS based sorptive extraction techniques showed that the order of the recoveries was SPME < SPME Arrow < SBSE < SA-SBSE. For all solutes, the highest recovery was obtained using SA-SBSE. This is consistent with prediction based on the octanol-water partitioning coefficient of the solute and on the ratio of the PDMS volume over sample volume.

However, even in SBSE, where the PDMS volume is the largest, the recoveries tended to be much lower than the theoretical values for phenolic compounds and multifunctional hydrophilic/polar compounds with hydroxyl groups. In contrast, SA-SBSE, which uses a PDMS swollen with DCM/DIPE, showed recoveries often exceeding the predicted values. This is a clear illustration that SA-SBSE is based both on increased volume of the extraction phase and on polarity modification. The increased polarity results in a higher affinity for more polar solutes, especially notable for the hydrophilic and polar compounds.

In a comparison of typical aroma compounds in roasted green tea, SA-SBSE provided the highest extraction power, especially for compounds with log $K_{ow}$ <1.5, which was about 5 to 10 times higher than for SBSE, which showed the second highest extraction power. For hydrophilic and polar aroma compounds present at low ng/mL level, MS library search match scores for deconvoluted mass spectra obtained by SA-SBSE were the highest, while library search or detection itself were sometimes difficult with SPME, and SPME Arrow and SBSE.

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