

A Combination of Standard (SBSE) and Solvent-Assisted (SA-SBSE) Stir Bar Sorptive Extraction for Comprehensive Analysis of Flavor Compounds in Beverages

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KEYWORDS

Standard and Solvent-Assisted Stir Bar Sorptive Extraction, polar aroma compounds, smoothie beverage, Aroma Office ²D.

ABSTRACT

Standard (SBSE) and Solvent-Assisted (SA-SBSE) Stir Bar Sorptive Extraction were both applied for profiling of flavor compounds in a matrix-heavy beverage sample. Unlike standard SBSE, SA-SBSE uses a solvent swollen polydimethylsiloxane (PDMS) stir bar for extraction and enhanced recovery of polar compounds. After extraction compounds were recovered from both stir bars by liquid desorption (LD) – large volume injection (LVI) and transferred to the analytical system for GC-MS analysis, with each sample run consecutively to give duplicate data files. The extra solvent dimension in SA-SBSE allows detection of more polar compounds, but the increased response of these compounds can sometimes obscure compounds which are more readily detected by standard SBSE. This leads to a result where a common set of compounds could be detected by both SBSE and SA-SBSE, but also each separate mode giving a unique set of compounds. This additional interpretation complexity can be simplified by employing both mass spectrometric (MS) and retention index (RI) information for compound detection. Aroma Office ²D applies automatic searching of a total ion

chromatogram (TIC) and has a built-in requirement for positive detection only with agreement of both MS spectral information and corresponding RI values for compounds.

INTRODUCTION

Stir Bar Sorptive Extraction was first introduced by Baltussen et al. in 1999 [1,2] and has since gained wide acceptance as a highly efficient sample preparation technique for enrichment of solutes from aqueous samples. SBSE-TD-GC-MS has been applied for such diverse applications as pesticides and flavor compounds in wine [3,4], organic solutes from biological fluids [5] and fast screening of pesticides in aqueous solutions [6]. SBSE using polydimethylsiloxane (PDMS) as an extraction phase allows high recoveries and extremely low limits of detection (LOD) down to sub-ng/L level for the extraction and enrichment of relatively apolar solutes ($\log K_{ow} > 3.0$) from aqueous samples. One of the advantages of SBSE using PDMS (Twister) is the extraction of relatively GC amenable solutes from aqueous food matrices without enrichment of non-volatile solutes such as amino acids, sugars, glycosides, polyphenols, etc. In fact after extraction from such complex matrices stir bars can be rinsed briefly in ultrapure water to remove any adhering materials and then dried with a lint-free tissue.

In 2016, a new SBSE method using a solvent-swollen

PDMS stir bar, namely “Solvent-Assisted SBSE (SA-SBSE) was introduced [7]. In SA-SBSE, the solvent absorbed in the swollen PDMS phase acts not only as a modifier of the PDMS phase (increasing diffusion), but also as an additional extraction medium, resulting in enhanced recovery of solutes from the aqueous phase. Recoveries are significantly improved, especially for relatively polar solvents with $\log K_{ow}$ ranging from 1.0 to 2.0. SA-SBSE allows both thermal desorption and liquid desorption and offers high robustness comparable to conventional SBSE using a standard PDMS stir bar. In this paper a matrix-heavy beverage -“smoothie”- is extracted by both SBSE and SA-SBSE and the corresponding GC-MS data files then processed by Aroma Office 2D [8]. A smoothie is a thick beverage made from blended raw fruit or vegetables with other ingredients such as water, ice or sweeteners. Examination of the corresponding data files reveal subtle complexities and differences where each mode separately can identify certain groups of compounds. Aroma Office processing, which matches MS and RI data for positive identification, significantly speeds up and improves the data interpretation.

EXPERIMENTAL

Reagents and materials. Acetone, dichloromethane, and diisopropyl ether, were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Instrumentation. The thermal desorption (TD)-GC-MS analysis was performed with a thermal desorption unit (TDU) equipped with an MPS autosampler and a Peltier cooled CIS 4 programmed temperature vaporization (PTV) inlet installed on an Agilent 7890A gas chromatograph with a 5975C single quadrupole mass selective detector (MSD).

Sample preparation. The smoothie sample was a mixture of apple, grape, lemon, strawberry, raspberry, carrot and beet and was centrifuged for 5 min at 3000 rpm. A five milliliter aliquot of the supernatant was transferred to a 10 ml headspace vial and 30% NaCl was added before SA-SBSE and SBSE.

SA-SBSE. The most widely used Gerstel Twister with 24 μL PDMS (1 cm length \times 0.5 mm thickness) gives poor results in SA-SBSE because



only a small volume of solvent is taken up when swelling the PDMS. Therefore, a dedicated Twister with 63 μL PDMS (1 cm length \times 1.0 mm thickness), namely FLEX-Twister (Part No.: 021075-010-00), is used for better sensitivity and reproducibility for extraction of polar solutes. FLEX-Twister has a narrower tolerance width of PDMS volume for more uniform solvent volume in the swollen PDMS.

Before SA-SBSE, solvent swelling of the FLEX-Twister was done in a 2 mL-vial. First, using a syringe, a known amount of solvent (typically 100-150 μL) is added into the 2 mL-vial containing the FLEX-Twister. The sealed vial is laid down and left for more than 30 min. The solvent swollen FLEX-Twister can be stored in the 2 mL vial at room temperature (typically for a week).



Figure 1. FLEX-Twister and Solvent swelling procedure.

1. Using a syringe, a known amount of solvent (typically 100-150 μL) is added into the 2 mL-vial containing the FLEX-Twister.
2. The sealed vial is laid down and left for > 30 min.
3. The solvent swollen FLEX-Twister can be left at room temperature (typically for a week).

Both individual SA-SBSE and SBSE extractions were performed at room temperature (25°C) for 60 min while stirring at 800 rpm. After extraction, both stir bars were removed with a magnetic rod (Twister taking tool, Part No.: 013820-000-00) and forceps, rinsed for 10 seconds in ultrapure water, and dried with a lint-free tissue.

For liquid desorption (solvent back extraction), each stir bar was placed in the sealed 10 mL HS vial containing 0.5 mL of acetone. The stir bars were stirred at room temperature (25°C) for 30 min at 800 rpm. After solvent extraction, also called Twister Back Extraction (TBE), the acetone extract was transferred to a 2 mL vial. The sealed 2 mL vial was placed in the MPS tray.

*Analysis conditions.***SA-SBSE**

FLEX-Twister 10 mm x 1 mm (63 μ L PDMS)
 Solvent DCM/DIPE (1/1), 105 μ L
 Extraction 60 min @ 800 rpm

Twister back extraction

Solvent Acetone, 500 μ L
 Back extraction 30 min @ 800 rpm

Large volume Injection MPS/TDU-ATEX/CIS

Injection 200 μ L, 0.85 μ L/s
 TDU temperature 30°C (0.5 min), at 140°C/min to 80°C (7 min)

Desorption 100 mL/min @25 kPa (splitless)
 CIS 4 Liner Type Tenax TA
 Pneumatic Mode low split 1:3
 Temperature 20°C (0.5 min), at 12°C/sec to 240°C (hold)

GC

Column Agilent 7890A
 DB-Wax Ultra Inert (Agilent),
 20 m x 0.18 mm x 0.30 μ m
 Temperature 40°C (3 min), at 5°C/min to 240°C (7 min)
 backflush @ 240°C (10 min)

MS

Mode Agilent 5975C
 SIM/Scan (m/z 28.7 - 300)

Data Analysis. MSD ChemStation version E.02.02.1431 (Agilent), and Aroma Office ²D database version 5.01.00 (Gerstel KK, Tokyo, Japan) were used for data analysis. Aroma Office ²D contains the most comprehensive database of aroma compounds available (>101,000 entries). This software is a searchable database which contains linear retention indices (LRI) information for a wide range of aroma compounds from many literature references [8]. The log K_{ow} values were calculated with a EPI SUITE version 4.11 software.

RESULTS AND DISCUSSION

Fig 2 shows the TIC comparison between SA-SBSE (a) and conventional SBSE (b) for the smoothie sample.

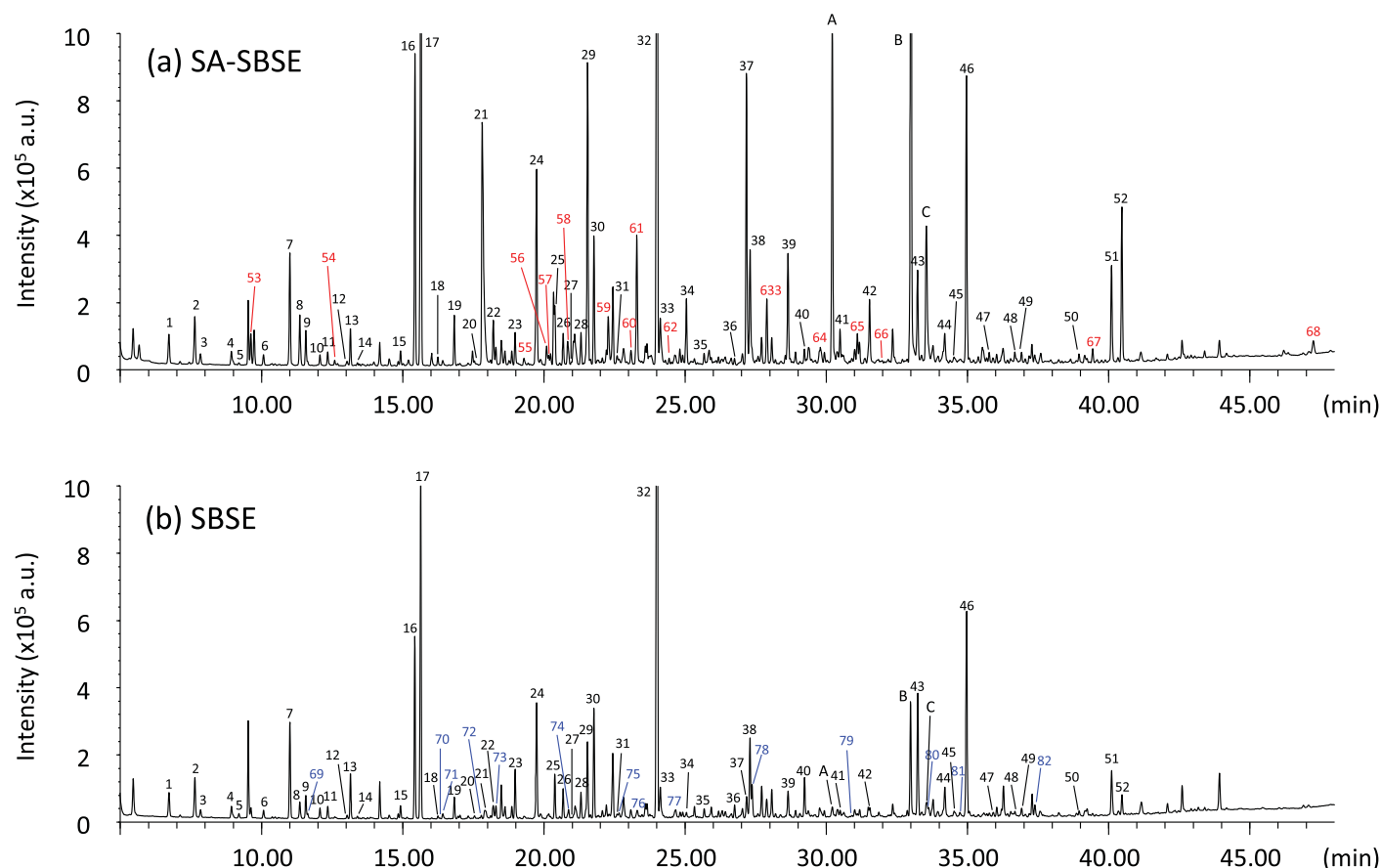


Figure 2. Comparison of TICs from SA-SBSE (a) and conventional SBSE (b) of smoothie .

The compounds numbered from 1 to 52 in black color were detected with both methods. Compounds from 53 to 68 in red color were only detected with SA-SBSE. Compounds from 69 to 82 in blue color were only detected with conventional SBSE. Compound names are listed in Table 1-3.

Table 1 shows Aroma Search results for compounds detected in both modes. This search is an automated identification requiring MS spectra and RI agreement for positive detection. Allowable criteria for identification are RI deviation of ± 15 units and PBM score of > 80 . Log K_{ow} values were obtained from EPI SUITE version 4.11 software. In this case 52 compounds were identified with both methods.

Table 1. Aroma Search results obtained from both SA-SBSE and conventional SBSE .

No.	Compound	log k_{ow}	RI	Ave RI	PBM	Character
1	Ethyl butyrate	1.85	1039	1036	97	acid fruit
2	Butyl acetate	1.85	1076	1073	86	apple
3	Hexanal	1.8	1084	1082	93	apple
4	2-Methylbutyl acetate	2.26	1125	1118	87	banana
5	4-Methyl-3-penten-2-one	1.37	1135	1127	81	almond-like
6	Ethyl (E)-2-butenolate	1.63	1167	1155	81	cashew
7	Limonene	4.83	1200	1199	99	lemon
8	2-Methylbutanol	1.26	1213	1208	91	fruity
9	E-2-Hexenal	1.58	1221	1215	97	almond
10	Ethyl hexanoate	2.83	1239	1237	98	acid fruit
11	γ -Terpinene	4.75	1249	1245	96	camphor-like
12	p-Cymene	4	1273	1267	94	carrot top
13	Hexyl acetate	2.83	1277	1273	90	apple
14	Terpinolene	4.88	1286	1280	98	citrus
15	Methylheptenone	2.06	1342	1339	96	lemongrass
16	Hexanol	1.82	1361	1357	83	alcoholic
17	4-Hydroxy-4-methyl-2-pentanone	-0.34	1369	1363	83	-
18	Z-3-Hexenol	1.61	1392	1386	97	alcohol
19	E-2-Hexenol	1.61	1414	1407	91	green
20	p-Cymenene	3.99	1441	1437	90	camphor-like
21	Acetic acid	0.09	1452	1450	91	acetic
22	Furfural	0.83	1467	1462	92	alcoholic
23	2-Ethylhexanol	2.73	1497	1492	90	comparatively mild
24	Benzaldehyde	1.71	1528	1522	95	almond
25	Linalool	3.38	1554	1550	96	althea
26	Octanol	2.81	1566	1560	90	aldehydic
27	5-Methylfurfural	1.38	1578	1575	87	almond-like
28	Fenchol	2.85	1592	1588	96	earthy
29	Mesifuran	0.62	1602	1596	91	maple
30	4-Terpineol	3.33	1611	1605	97	apple
31	Phenyletanal	1.54	1646	1644	94	apple
32	α -Terpineol	3.33	1706	1700	91	anise
33	Borneol	2.85	1711	1703	86	camphor
34	E,E-Farnesene	7.1	1753	1748	96	green grass
35	4-Methylacetophenone	2.22	1781	1781	91	hay
36	E- β -damascenone	4.21	1831	1820	97	baked apple-like

RI deviation $< \pm 15$, PBM > 80

Table 1 (cont.). Aroma Search results obtained from both SA-SBSE and conventional SBSE.

No.	Compound	log k_{ow}	RI	Ave RI	PBM	Character
37	Hexanoic acid	2.05	1851	1850	90	acidic
38	p-Cymenol	2.49	1857	1849	91	cucumber
39	Benzeneethanol	1.57	1922	1917	97	floral
40	β -Ionone	4.42	1949	1947	96	balsamic
41	Phenol	1.51	2011	2003	94	acid
42	Octanoic acid	3.03	2065	2064	98	acid
43	4-Decanolide	2.57	2154	2149	90	candy
44	5-Decanolide	2.57	2205	2201	95	burnt
45	Methyl palmitate	7.25	2223	2220	90	green
46	Methyl anthranilate	2.26	2248	2253	97	aromatic
47	Cinnamic alcohol	1.84	2294	2296	96	floral
48	Chavicol	2.91	2345	2344	96	anise-like
49	Dihydroactinidiolide	2.3	2359	2354	96	sweet
50	4-Methoxyphenylpropanol	2.14	2474	2487	97	sweet
51	3-Hydroxy- β -damascone	-	2543	2539	93	-
52	Vanillin	1.05	2560	2559	97	fruity

RI deviation < ± 15 , PBM > 80

Figure 3 shows relative responses for a range of selected compounds detected by both methods from Aroma Search results.

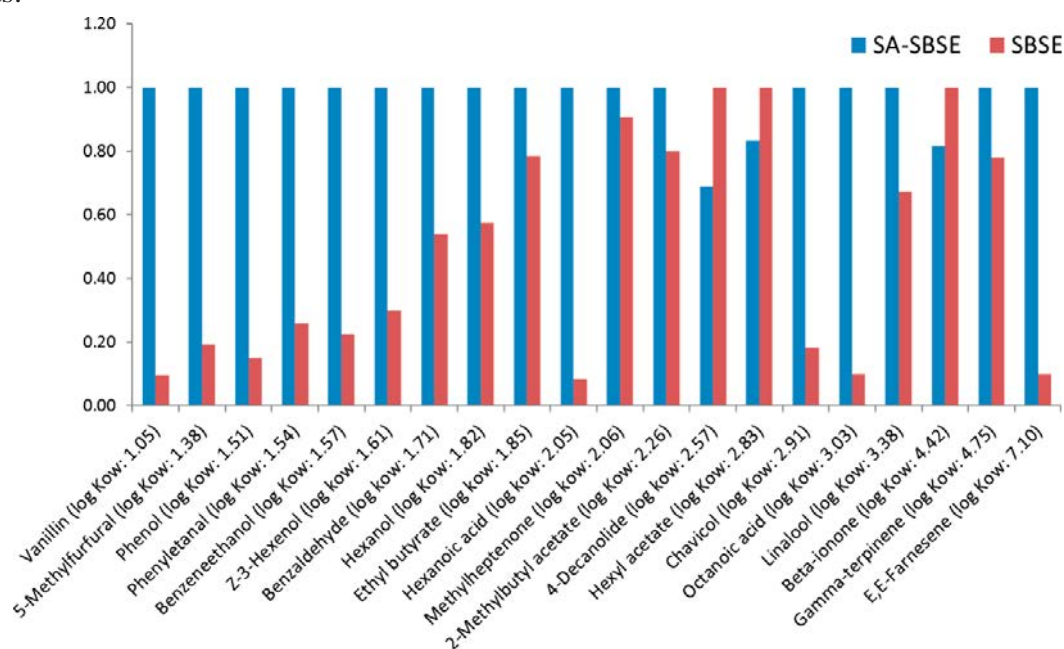


Figure 3. Relative responses of the selected aroma compounds from the Aroma Search results.

A wide range of aroma compounds (e.g. from vanillin with log K_{ow} of 1.05 to E,E-farnesene with log K_{ow} of 7.10) were identified with both methods. However, the relative responses are quite different especially for the compounds with low log K_{ow} , i.e. fatty acids, and phenolic compound such as chavicol. Several apolar aroma compounds such as 4-decanolide, hexyl acetate, and β -ionone shows higher relative responses with conventional SBSE while SA-SBSE shows more than 10 times higher relative response for E,E-farnesene with the highest log K_{ow} of 7.10. Although salt addition in conventional SBSE decreases the extraction efficiencies of more hydrophobic (apolar) solutes [9], SA-SBSE conditions compensate for the negative effect of salt addition.

Table 2 shows Aroma Search results obtained uniquely from SA-SBSE.

Sixteen (polar) aroma compounds with $\log K_{ow}$ in the range of -0.36 to 2.06 were identified with only SA-SBSE. Several important polar aroma compounds such as short chain fatty acids (C3-C5), 2,3-butanediol (diol), methionol (sulfur), p-cresol (phenol), and furaneol (hetero-cyclic/multifunctional) are seen in the list.

Table 2. Aroma Search results obtained from only SA-SBSE.

No.	Compound	$\log K_{ow}$	RI	Ave RI	PBM	Character
53	Butanol	0.84	1150	1144	87	alcoholic
54	Pentanol	1.33	1257	1254	80	acid
55	Acetylfuran	0.8	1510	1502	87	balsamic
56	Propionic acid	0.58	1542	1534	91	acidic
57	2,3-Butanediol	-0.36	1548	1541	83	butter
58	Isobutyric acid	1.0	1572	1569	80	acid
59	Butyric acid	1.07	1632	1629	91	aged cheese
60	Furanmethanol	0.45	1667	1662	96	burned
61	2-Methylbutyric acid	1.49	1675	1668	90	acidic
62	Methionol	0.44	1725	1721	97	baked cabbage
63	Benzyl alcohol	1.08	1886	1877	97	aromatic
64	5-Octanolide	1.59	1976	1973	97	burnt sugar
65	Furaneol	0.82	2043	2042	95	caramel
66	p-Cresol	2.06	2089	2085	89	animal
67	5-Hydroxymethyl-2-furfural	-0.09	2502	2496	93	cardboard
68	4-Hydroxybenzaldehyde	1.23	2945	2931	93	almonds

RI deviation < ± 15 , PBM > 80

Table 3 shows “Aroma Search” results obtained uniquely from conventional SBSE. Fourteen aroma compounds were identified with conventional SBSE. Although 10 compounds have apolar characteristics with $\log K_{ow}$ values in the range of 2.57 to 4.29, 4 compounds show low $\log K_{ow}$ values in the range of 1.44 to 2.08. This is due to the more complex chromatogram (thereby more co-elution with polar compounds) obtained using SA-SBSE.

Table 3. Aroma Search results obtained using only conventional SBSE.

No.	Compound	$\log K_{ow}$	RI	Ave RI	PBM	Character
69	Butyl butyrate	2.83	1223	1218	90	fruity
70	2-Nonanone	2.71	1394	1389	87	baked
71	Nonanal	3.27	1398	1392	80	aldehyde
72	Linalool oxide I	1.99	1451	1452	86	elder flower
73	2-Methyl-2-hepten-6-ol	2.57	1471	1464	74	oily-green
74	Ethyl 3-methylthiopropionate	1.44	1574	1567	95	clean
75	Menthol	3.38	1649	1641	87	fresh
76	Isoanethole	3.47	1676	1674	92	anise
77	Benzyl acetate	2.08	1735	1736	93	floral herbal
78	α -Ionone	4.29	1861	1857	99	floral
79	p-Anisaldehyde	1.79	2032	2018	92	aniseed-like
80	Eugenol	2.73	2174	2172	98	clove
81	Elemicin	2.9	2235	2226	99	spicy
82	4-Dodecanolide	3.55	2385	2377	86	cheesy

RI deviation < ± 15 , PBM > 80

The dominant peaks 2H-Pyran-2,6(3H)-dione (A) and 1,3-Butanediol (B) (Table 4) from Fig 2 (a) were tentatively identified using only manual PBM searching because they were not present in the RI database. Nonanoic acid (C) was also identified with a manual PBM search but with an automatic RI search. In this case co-eluting peaks meant a clean mass spectrum could not be obtained and the requirements of Aroma Search were not satisfied.

Table 4. Manual search results obtained from both SA-SBSE and SBSE.

No.	Compound	log K_{ow}	RI	Ave RI	PBM	Character
A	2H-Pyran-2,6(3H)-dione	1.09	1996	-	87	-
B	1,3-Octanediol	1.67	2142	-	90	-
C	Nonanoic acid	3.52	2171	2169	95	fat

The preceding Figures and Tables show that by analyzing a sample with both solvent assisted and conventional SBSE a wider range of compounds can be detected compared to either mode separately. Essentially the trade-off is that SA-SBSE allows detection of many more important polar species, but obscures or does not detect some compounds which are detected in SBSE mode only. This is due to co-elution or interference from these polar compounds detected in SA-SBSE mode. Figures 4 and 5 show examples of this situation.

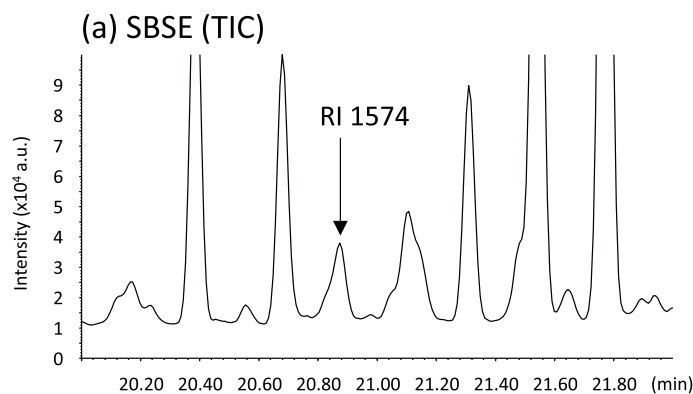


Figure 4 (a) shows the peak with RI of 1574 in the TIC obtained from conventional SBSE, which is identified as ethyl-3-methyl thiopropanoate by Aroma Search (using both RI and MS spectra match).

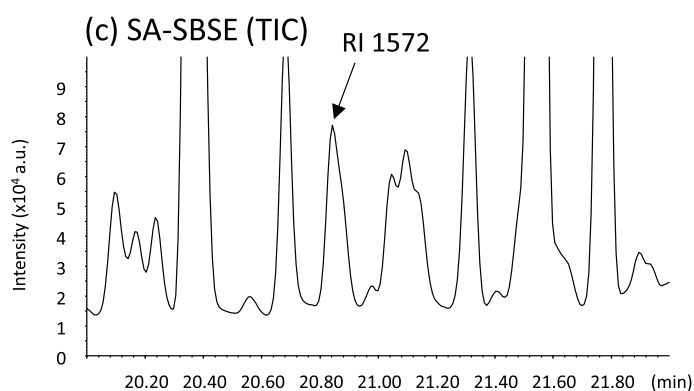


Figure 4 (c) shows the peak with RI of 1572 in the TIC obtained from SA-SBSE, corresponding to the peak with RI of 1574 in the TIC (a).

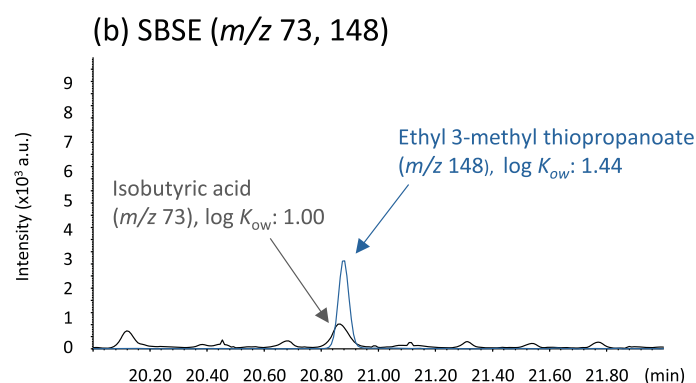


Figure 4 (b) shows the co-eluted peaks of isobutyric acid (m/z 73) and ethyl-3-methyl thiopropanoate (m/z 148) with RI around 1574 in the mass chromatograms (m/z 73 and 148) obtained from conventional SBSE.

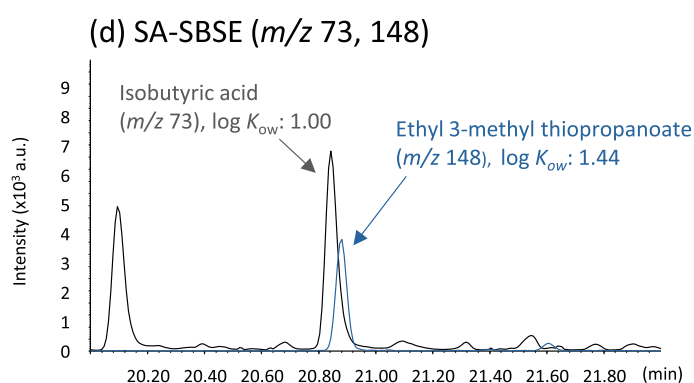


Figure 4 (d) shows the co-eluted peaks of isobutyric acid (m/z 73) and ethyl-3-methyl thiopropanoate (m/z 148) with RI around 1572 in the mass chromatograms (m/z 73 and 148) obtained from SA-SBSE.

In Aroma Search of the TIC (c) obtained from SA-SBSE, the larger peak of isobutyric acid interfered with the mass spectrum of ethyl-3-methyl thiopropanoate even with the slightly larger response of the latter compared to that of conventional SBSE.

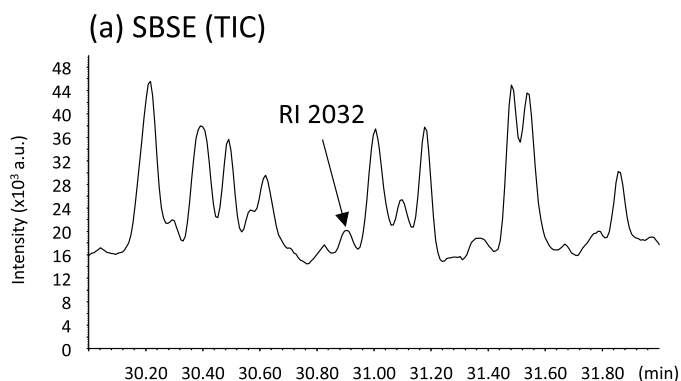


Figure 5 (a) shows the peak with RI of 2032 in the TIC obtained from conventional SBSE, which is identified as *p*-anisaldehyde by Aroma Search (using both RI and MS spectra match).

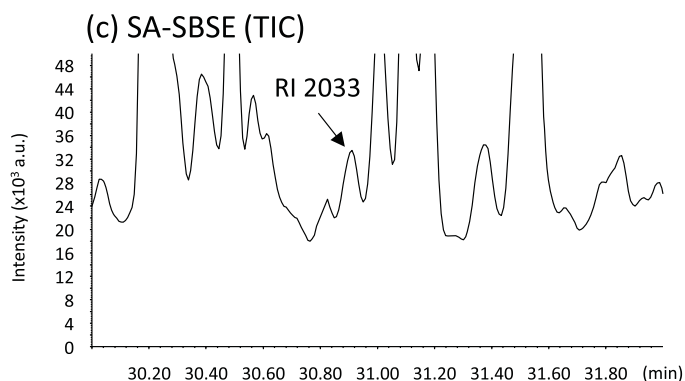


Figure 5 (c) shows the peak with RI of 2033 in the TIC obtained from SA-SBSE, corresponding to the peak with RI of 2032 in the TIC (a).

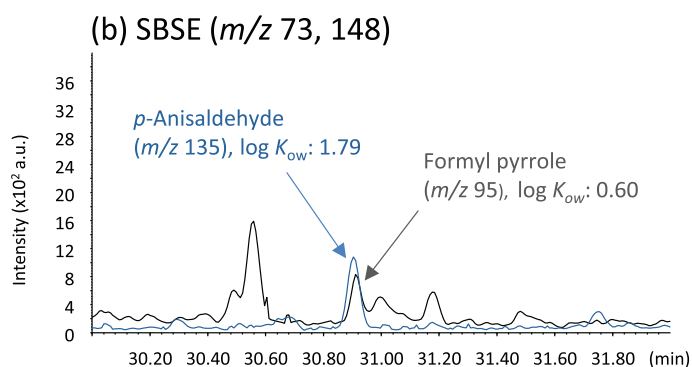


Figure 5 (b) shows the co-eluted peaks of formyl pyrrole (*m/z* 95) and *p*-anisaldehyde (*m/z* 135) with RI around 2032 in the mass chromatograms (*m/z* 95 and 135) obtained from conventional SBSE.

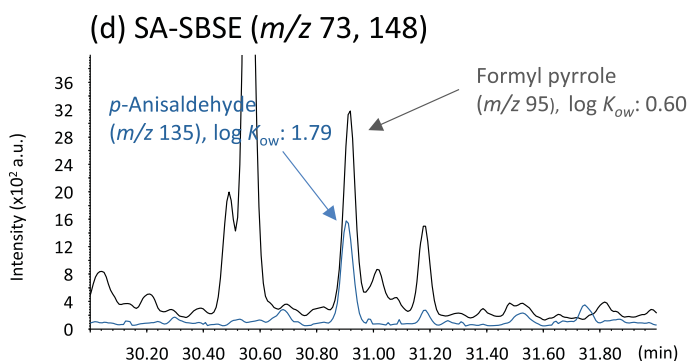


Figure 5 (d) shows the co-eluted peaks of formyl pyrrole (*m/z* 95) and *p*-anisaldehyde (*m/z* 135) with RI around 2033 in the mass chromatograms (*m/z* 95 and 135) obtained from SA-SBSE.

In Aroma Search of the TIC (c) obtained using SA-SBSE, the larger peak of formyl pyrrole interfered with the mass spectrum of *p*-anisaldehyde even with the slightly larger response of the latter compared to that of conventional SBSE.

CONCLUSIONS

It is clear that by using the complimentary contributions of both standard and solvent-assisted SBSE, a wider range of compounds can be detected than when either approach used individually. SA-SBSE allows detection of many important polar compounds which are not compatible with the apolar nature of a standard stir bar, but these same compounds can interfere by co-elution with compounds detected with the standard stir bar. Both modes of operation use the same injection and analytical conditions and so automated sequence running is simply a matter of sample duplication.

Finally, the additional interpretational efforts required for confident compound detection in this dual-mode operation are significantly helped by use of Aroma Office ²D. A problem with many important flavor compounds is their mass spectral similarity and this often leads to errors in PBM ranking results. Aroma Office also gives an RI value and since the software is integrated into Chemstation software, both the CAS No. of any hit and the RI value can be sent to the Aroma Office database for screening. In this way suspect PBM results can be effectively disregarded with important savings in data processing time.

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