APPNOTE

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Double Consecutive Stir Bar Sorptive Extraction (D-SBSE) Analysis for Total Extraction Calculation

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Keywords

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Abstract

Accurate quantification of VOCs/SVOCs in complex matrices is critical, but traditional extraction techniques face limitations like inconsistent compound extraction efficiencies and strong matrix effects. This study introduces a double consecutive stir bar sorptive extraction (D-SBSE) method for exhaustive extraction of odor-active compounds in aqueous samples, evaluated against four consecutive SBSE extractions. The method was found to enhance efficiency via theoretical total peak area calculation by extrapolation, using double consecutive extraction data integration. Experimental optimization of extraction time (2 h for NaCl solutions, 4 h for aqueous solutions) and salt addition (NaCl) enhanced partition coefficients via salting-out effects. Comparative analysis showed that D-SBSE achieved relative deviations comparable to total extraction (0.02–21.53% in NaCl solutions, 0.19–29.79% in aqueous solutions). The method overcame matrix dependency by integrating dual extraction data-enabling robust quantification with simplified workflow and improved throughput.

Introduction

Accurate determination of volatile organic compounds (VOCs) and semi-volatile organic compounds (semi-VOCs) in various sample matrices using appropriate extraction techniques coupled with gas chromatography–mass spectrometry (GC-MS) is critical for quality control, regulatory compliance, and product development [1]. Traditional extraction approaches, however, face several limitations, including excessive solvent consumption, matrix complexity, and insufficient overall method sensitivity [2]. To address these challenges, various solvent-free or solvent-minimized techniques based on the equilibrium partitioning of analytes between the sample and a polymer-coated sorptive phase have been developed. These include solid-phase microextraction (SPME), SPME Arrow, thin-film SPME (TF-SPME), stir bar sorptive extraction (SBSE, GERSTEL Twister®), and dynamic headspace (DHS) [3]. These methods are widely applied for the extraction and pre-concentration of target analytes from complex environmental [4], food, and biological matrices prior to chromatographic analysis.

Sorptive extraction techniques are inherently based on equilibrium processes. Therefore, quantitative analysis requires accurate recovery assessment [5]. Several calibration strategies have been employed to address matrix effects and ensure reliable quantitation, such as the use of internal standards, matrix-matched calibration, stable isotope dilution analysis (SIDA) [6], and full evaporation dynamic headspace (FEDHS) [7]. Multiple headspace solid-phase microextraction (MHS-SPME) [8], a variant of SPME, enables quantitative estimation of total analyte content by performing successive extractions and modeling the resulting peak area decay using an exponential correlation. This is an extension of the Multiple Headspace Extraction (MHE) technique, used especially in the polymer industry to demonstrate equilibrium with, and calculate total analyte amounts in, complex samples [8].

Stir bar sorptive extraction (SBSE) is a prominent equilibrium-based sample preparation technique that relies on the partitioning of analytes between an aqueous sample and a polydimethylsiloxane (PDMS)-coated stir bar. Both SPME and SBSE can be operated in immersion or headspace modes. Compared to SPME, SBSE employs a significantly larger sorptive phase volume - typically

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50 to 250 times greater - resulting in superior enrichment factors and enhanced analyte recovery, even from substantially larger samples. This higher capacity makes SBSE particularly suitable for trace- and ultra-trace level analysis and has led to its widespread application across diverse fields.

In this study, we investigate the enrichment of a broad spectrum of odor-active compounds in aqueous samples using a double consecutive stir bar sorptive extraction approach (D-SBSE). The proposed method is evaluated through a comparative study with four consecutive extractions using SBSE. The applicability and advantages of D-SBSE are demonstrated by its ability to achieve exhaustive extraction, offering an efficient, robust, solvent-minimized alternative to traditional techniques for quantitative analysis of aroma compounds.

Experimental

Instrumentation

PDMS Twister® stir bars (10 mm length, 1 mm phase thickness) were used to extract aroma compounds from aqueous samples. Inert glass thermal desorption tubes fitted with transport adapters were used to contain the loaded Twisters with the extracted analytes in a sealed position in the autosampler, as well as during transport to, and thermal desorption in, the Thermal Desorption Unit (TDU 2) all from GERSTEL. Analytes were refocused in a GERSTEL Cooled Injection System (CIS 4), PTV-type inlet, at low temperatures before being transferred to the GC column. An 8890A/5977B GC/MS system from Agilent Technologies was used for separation and detection of the analytes of interest. Thermal desorption tubes containing the samples were delivered to the TDU automatically using a GERSTEL MultiPurpose Sampler (MPS robotic)

Standard/Sample Preparation

A standard containing 17 compounds (Hexanal, Heptanal, 2-Pentylfuran, Sulcatone, Nonanal, 2-Octenal, Decanal, 2-Nonenal, Benzeneacetaldehyde, 2,4-Nonadienal, Ethyl phenylacetate, 2,4-Decadienal, Geranylacetone, γ-Octalactone, γ-Nonalactone, γ-Decalactone, δ-Decalactone) was purchased from Alta-Scientific (Tianjin, China). A working solution containing all compounds at a concentration of 10 µg/mL in methanol was prepared.

Samples were prepared using pure water or saturated sodium chloride solution by adding the working solution containing all target compounds in methanol at 20 ng/mL.

10 mL of sample and a GERSTEL Twister with 24 µL PDMS (10 mm length, 1 mm thickness) were transfer to a 20 mL headspace vial and closed with a seal (aluminum seals with PTFE septa). The SBSE extractions were performed at room temperature (25 °C) for 0.5-4 h stirring at 1000 rpm. Twisters were then removed with a magnetic rod, rinsed briefly in ultrapure water, dried with a clean, lint-free cloth, and then transferred to an inert glass thermal desorption tube for subsequent TD-GC-MS analysis.

Analysis Conditions

TDU	splitless mode 30 °C (1.0 min); 100 °C/min; 280 °C (8 min)
CIS	splitless mode; Tenax liner splitless; 50 mL/min, 3 min -30 °C (0.5 min), 12 °C/sec; 260 °C (3 min)

Analysis Conditions Agilent GC 7890A

Column	30 m HP-Innowax (Agilent) d_i = 0.25 mm, d_f = 0.25 µm
Pneumatics	He (> 99.999%), constant flow 1.8 mL/min
Oven	40 °C (3 min); 5 °C/min; 250 °C (20 min)
MSD	scan (33-450)

(note: MSD/ODP split ratio is with 1:1, Gerstel ODP splitter used)

Analysis Conditions Agilent 5975C MSD

EI	70 eV
Interface	250 °C
Ion source	230 °C
Quadrupole	150 °C
Full scan	33-450 amu

Optimization Procedure

Extraction time (0.5 h, 1 h, 2 h, 4 h) in aqueous solution and saturated sodium chloride solution were evaluated to achieve the equilibrium extraction.

Consecutive Stir Bar Sorptive Extractions: After completion of sampling, three consecutive extractions were performed on the sample under identical conditions as described above. All analyses were performed in duplicate (n=2).

Quantification of Volatile Compounds using D-SBSE

Sorptive extraction is inherently an equilibrium technique. For aqueous solution samples, the extraction of solutes from the aqueous phase into the extraction medium is governed by the partition coefficient of solutes between the polydimethylsiloxane (PDMS) phase and the aqueous phase. Absorption equilibrium also depends on the phase ratio, i.e., the amount of PDMS applied [9], as described by equation (1):

$$\frac{K_{PDMS}}{C_m} = \frac{m_{PDMS}}{m_w} = \frac{V_w}{V_{PDMS}} = \frac{m_{PDMS}}{m_w}\beta \quad (1)$$

$K_{PDMS/w}$: The distribution coefficient between polydimethylsiloxane and water

C_{PDMS}: The concentration of a solute in the polydimethylsiloxane phase

C_w: The concentration in the aqueous solution

m_{PDMS}: The mass of the solute in the polydimethylsiloxane phase

m_w: The mass of the solute in the aqueous phase

V_{PDMS}: The volume of the polydimethylsiloxane phase

V_w: The volume of the aqueous phase

ß: The phase ratio of the aqueous phase and the polydimethylsiloxane phase

The recovery, defined as the ratio of the extracted solute amount (m_{PDMS}) to the original solute amount in water ($m_0 = m_{PDMS} + m_w$), is determined by the distribution coefficient $K_{PDMS/w}$ and phase ratio ß, as shown in equation (2):

$$R = \frac{m_{PDMS}}{m_0} = \frac{K_{PDMS/w}/\beta}{1+K_{PDMS/w}/\beta} \quad (2)$$

In consecutive stir bar sorptive equilibrium extractions, if a solute has the same partition coefficient and phase ratio in both the first and second extractions, its recovery (R) remains identical, as described in equation (3):

$$R_{1st} = R_{2nd} \quad (3)$$

Based on the concentration calculated from the first and second extractions in equation (3), the theoretical total extraction peak area is derived by combining the peak areas of dual consecutive extractions (D-SBSE), as described in equation (4).

$$\frac{C_1}{C_0} = \frac{C_2}{C_0 - C_1}$$
$$C_0 = \frac{C_1 \times C_1}{C_1 - C_2}$$
$$\frac{A_0}{m} = \frac{A_1 \times A_1}{A_1 - A_2}/m$$
$$A_0 = \frac{A_1 \times A_1}{A_1 - A_2} \quad (4)$$

The calculation equation for the recovery of the first SBSE (Stir Bar Sorptive Extraction) can be derived as equation (5).

$$R_1 = \frac{A_1}{A_0}$$
$$R_1 = 1 - \frac{A_2}{A_1} \quad (5)$$

Results and Discussion

Optimization of SBSE Condition

D-SBSE parameters influence the extraction process. Equilibrium extraction is critical for consistent recovery in successive extractions. We tested extraction at 0.5 h, 1 h, 2 h, and 4 h, with and without NaCl. NaCl addition enhanced the abundance of volatile compounds (Hexanal, Heptanal, Sulcatone, 2-Octenal, Ethyl phenylacetate, γ-Octalactone, γ-Nonalactone, γ-Decalactone, δ-Decalactone). Salt promotes "salting out" - increasing the partition coefficient of volatiles between the PDMS and solution phases, enhancing sorption into the Twister PDMS phase. However, this effect varies across compound types . Extraction time positively affected total volatiles abundance. Considering equilibrium rates, we used 2 h extraction for NaCl solutions and 4 h for aqueous solution.

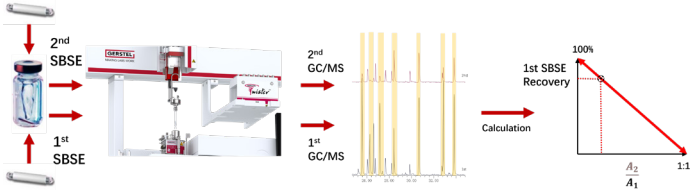

Figure 1: D-SBSE procedure.

Method Performance

The saturated sodium chloride solution and the aqueous solution, both spiked with 20 µg/L of 17 target compounds were analyzed using the SBSE method based on extraction times of 2 h and 4 h, respectively. Typical chromatograms of the two consecutive extractions of saturated sodium chloride and aqueous solutions are presented in Fig. 1. The method allowed us to successfully quantify 17 aroma compounds. Comparing the results obtained for two consecutive extractions of the saturated sodium chloride and aqueous solutions, respectively, a significant decrease in peak areas was observed for the second extraction. The greater the reduction, the higher the inferred recovery rate of the first extraction.

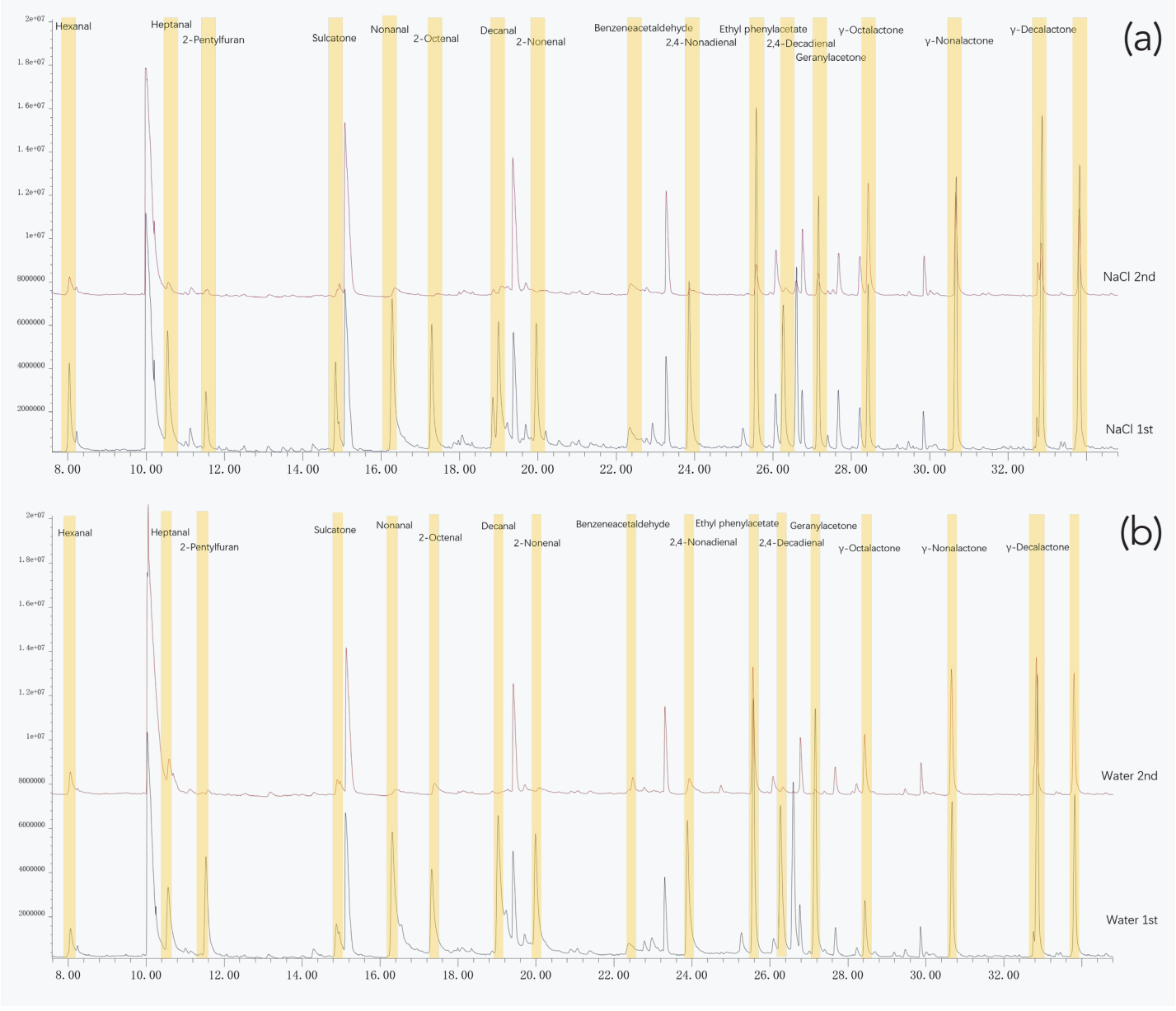

Figure 2: Chromatograms of two consecutive extractions of saturated sodium chloride solution (a) and aqueous solution (b).

Four consecutive extractions were performed for each analysis, and the total peak area was calculated by summing the results of all four extractions - provided that the peak area of the fourth extraction was less than 7% of that of the first. When the fourth extraction peak area exceeded 7% of the first extraction peak area, the total peak area was calculated [10] using equation (6):

$$LnA_i = k \times i + b$$

$$A_0 = \frac{A_1}{1-e^k} \qquad (6)$$

A_1: The first area peak of the first extraction

i: The number of extraction time

A_i: The peak area obtained in the ith extraction

k: The slope of linear regression analysis

b: The intercept of linear regression analysis

A_0: The total peak area

Table 3: Peak areas of aroma compounds from four consecutive extractions and the proportion of the fourth to the first extraction peak area.

Compounds	NaCl solutions					Aqueous solution				
	1st	2nd	3rd	4th	4th/1st	1st	2nd	3rd	4th	4th/1st
Hexanal	3943050	1396923	510760	169141	4.3%	1941420	1403484	1162544	782897	40.3%*
Heptanal	6058215	843626	270774	110483	1.8%	4836600	1893586	657381	312681	6.5%
2-Pentylfuran	5455087	414064	323591	151483	2.8%	9642392	258804	386190	156964	1.6%
Sulcatone	2795469	308055	60457	23671	0.8%	1749283	870899	412069	186784	10.7%*
Nonanal	5685040	793516	555203	350667	6.2%	6606259	679148	623129	367477	5.6%
2-Octenal	3655721	169114	13316	8749	0.2%	3889029	708132	128029	20478	0.5%
Decanal	2720046	435340	261843	178118	6.5%	3785381	244377	169287	160642	4.2%
2-Nonenal	2326381	130732	13947	7770	0.3%	3128970	169914	52799	4057	0.1%
Benzeneacetaldehyde	5229985	1862214	901706	6995	0.1%	4916478	1640396	1362348	1232044	25.1%*
2,4-Nonadienal	14761541	606917	51565	12317	0.1%	15453018	2129681	164792	19098	0.1%
Ethyl phenylacetate	12743242	1177778	107034	10279	0.1%	9756449	4208881	1309340	432008	4.4%
2,4-Decadienal	12605247	819983	217837	76755	0.6%	15092358	695197	124536	29059	0.2%
Geranylacetone	3327432	356566	87285	25492	0.8%	4036036	54730	15554	10792	0.3%
γ-Octalactone	17003809	12531738	8215503	5020088	29.5%*	7707905	6788938	6018342	6059733	78.6%*
γ-Nonalactone	26629267	10144535	2801228	615844	2.3%	16659330	12183992	7613587	5142936	30.9%*
γ-Decalactone	29804489	4499660	517544	104912	0.4%	24883358	11071640	3247584	922207	3.7%
δ-Decalactone	17056188	4944313	1130151	285762	1.7%	10037040	6976496	3936939	2578331	25.7%*

*The peak area of the fourth extraction exceeded 7% of that of the first extraction

Table 2: The comparation peak area according to total and D-SBSE method.

Compounds	NaCl solutions			Aqueous solutions		
	Total Peak area	D-SBSE	RD	Total Peak area	D-SBSE	RD
Hexanal	6019874	6106390	1.42%	8079192*2	7006620	13.28%
Heptanal	7283097	7038324	3.48%	7700247	7948551	3.22%
2-Pentylfuran	6344226	5903162	7.47%	10444350	9908334	5.13%
Sulcatone	3187652	3141676	1.46%	3227522*3	3483660	7.94%
Nonanal	7384426	6607282	11.76%	8276013	7363226	11.03%
2-Octenal	3846900	3833037	0.36%	4745669	4754806	0.19%
Decanal	3595347	3238338	11.02%	4359687	4046623	7.18%
2-Nonenal	2478830	2464898	0.57%	3355740	3308640	1.40%
Benzeneacetaldehyde	8000901	8121914	1.49%	9151267	7378252	10.73%
2,4-Nonadienal	15432340	15394481	0.25%	17766589	17923120	0.88%
Ethyl phenylacetate	14038332	14040959	0.02%	15706677	17158564	9.24%
2,4-Decadienal	13719822	13482282	1.76%	15941150	15821124	0.75%
Geranylacetone	3796775	3726794	1.88%	4117113	4091519	0.62%
γ-Octalactone	50732172*1	64652270	21.53%	92077788*4	64650634	29.79%
γ-Nonalactone	40190875	43016646	6.57%	48233447*5	62013919	28.57%
γ-Decalactone	34926605	35104271	0.51%	40124788	44830158	11.73%
δ-Decalactone	23416413	24018869	2.51%	26122430*6	32916424	26.01%

*The fourth extraction peak area is more than 7% of first extraction peak area, the total peak area was calculated by the equation (5,6), the D-SBSE total peak area was caculated by the equation (4). The coefficient of determination (R^2) *1: 0.994, *2: 0.995, *3: 0.999, *4: 0.899, *5: 0.997, *6:0.996

RD: Relative deviation between total peak area and D-SBSE peak area.

The performance of the D-SBSE method was evaluated. Peak areas from four consecutive extractions of aroma compounds and the proportion of the fourth to first extraction peak area (Table 1) were determined. Total peak areas (exhaustive extraction) were calculated by summing the four extractions or using equation (5), depending on whether the fourth extraction peak area exceeded 7% of the first. When extraction was not exhaustive, total peak area was mathematically derived using the first extraction peak area and a "k" constant - calculated from repeated four-extraction analyses of the sample. D-SBSE total peak areas were computed using Equation (4). Relative deviations in NaCl and aqueous solutions are presented in Table 2. NaCl solutions showed better relative deviations (0.02–21.53%), whereas aqueous solutions exhibited deviations ranging from 0.19 to 29.79%. Table 3 lists the 1st SBSE extraction recoveries for both matrices. The experimental values in aqueous solution were close to theoretical predictions.

Table 3: SBSE extraction recovery and theoretical extraction recovery.

Compounds	NaCl solutions	Aqueous solutions	Log $K_{o/w}$	Theoretical Extraction
Hexanal	64.6%	27.7%	1.78	27.5%
Heptanal	86.1%	60.8%	2.29	55.1%
2-Pentylfuran	92.4%	97.3%	3.87	97.9%
Sulcatone	89.0%	50.2%	2.06	42.0%
Nonanal	86.0%	89.7%	3.27	92.1%
2-Octenal	95.4%	81.8%	2.57	70.1%
Decanal	84.0%	93.5%	3.76	97.3%
2-Nonenal	94.4%	94.6%	3.06	87.9%
Benzeneacetaldehyde	64.4%	66.6%	1.78	27.5%
2,4-Nonadienal	95.9%	86.2%	2.84	81.3%
Ethyl phenylacetate	90.8%	56.9%	2.28	54.6%
2,4-Decadienal	93.5%	95.4%	3.33	93.1%
Geranylacetone	89.3%	98.6%	4.04	98.6%
γ-Octalactone	26.3%	11.9%	1.59	19.7%
γ-Nonalactone	61.9%	26.9%	1.59	19.7%
γ-Decalactone	84.9%	55.5%	1.59	19.7%
δ-Decalactone	71.0%	30.5%	1.59	19.7%

Log $K_{o/w}$: Calculate using the SRC-KOWWIN software
Theoretical extraction: calculate using the Gerstel TwisterCalc software

Conclusions

A double consecutive stir bar sorptive extraction (D-SBSE) method was developed and validated for the efficient exhaustive extraction of aroma compounds. The optimization of extraction time is critical for achieving stable extraction efficiencies across different matrices. Results from this study demonstrate that D-SBSE enables precise and accurate quantification of aroma compositions in NaCl solutions and aqueous matrices.

Although the log $K_{o/w}$ values of individual compounds can partially predict their extraction efficiencies in aqueous matrices, the complexity of different sample matrices presents challenges. The D-SBSE approach addresses this by using double consecutive extractions to calculate the total amount, whether extracted or not , providing an effective strategy for the quantitative analysis by SBSE-TD-GC-MS. This method overcomes matrix-dependent limitations and establishes a reliable model for the exhaustive extraction of aroma compounds in complex systems. A fully automated SBSE/Twister (GERSTEL) workflow enables seamless extraction automation, eliminating manual intervention and enhancing operational efficiency. This technology allows precise control over extraction parameters (e.g., extraction time, stirring speed) during consecutive extractions, providing a more effective automated approach for D-SBSE to achieve consistent extraction recoveries across cycles. It is anticipated that integrating this automated workflow will further optimize the application performance of D-SBSE in analytical practice. This technique allows for accurate quantitative analysis of environmental water samples and water-containing food samples such as wines, coffee, tea, and milk. Given the high sensitivity and reproducibility of SBSE, D-SBSE boasts promising application prospects.

References

[1] He M, Wang Y, Zhang Q, Zang L, Chen B, Hu B. *Stir bar sorptive extraction and its application*. J Chromatogr A. **2021** Jan;1637:461810.

[2] Ochiai N, Sasamoto K, David F, Sandra P. *Recent Developments of Stir Bar Sorptive Extraction for Food Applications: Extension to Polar Solutes*. J Agric Food Chem. **2018** Jul 18;66(28):7249–55.

[3] Ochiai N, Sasamoto K, David F, Hoffmann A. *A Comparison between SPME, SPME Arrow, SBSE and SA-SBSE for the Extraction of Polar Aroma Compounds from Aqueous Samples*. GERSTEL AppNote 286.

[4] Lerch O, Zboron J, Brady K, Aybar JL. *Highly Sensitive Determination of Contaminants in Surface Water in the Context of the EU Water Framework Directive using Stir Bar Sorptive Extraction (SBSE) and GC-MS/MS*. GERSTEL AppNote 196. **2018**;(196).

[5] Ochiai N, Sasamoto K, Ieda T, David F, Sandra P. *Multi-stir bar sorptive extraction for analysis of odor compounds in aqueous samples*. J Chromatogr A. **2013** Nov;1315:70–9.

[6] Hao W, Kingston HM 'Skip,' Dillard A, Stuff J, Pamuku M. *Quantification of persistent organic pollutants in dietary supplements using stir bar sorptive extraction coupled with GC-MS/MS and isotope dilution mass spectrometry*. Food Addit Contam Part A. **2020** Jul 2;37(7):1202–15.

[7] Ochiai N, Sasamoto K, Hoffmann A, Okanoya K. *Full evaporation dynamic headspace and gas chromatography–mass spectrometry for uniform enrichment of odor compounds in aqueous samples*. J Chromatogr A. **2012** Jun;1240:59–68.

[8] Ezquerro Ó, Ortiz G, Pons B, Tena MT. *Determination of benzene, toluene, ethylbenzene and xylenes in soils by multiple headspace solid-phase microextraction*. J Chromatogr A. **2004** Apr;1035(1):17–22.

[9] David F, Sandra P. *Stir bar sorptive extraction for trace analysis*. J Chromatogr A. **2007** Jun;1152(1–2):54–69.

[10] Xu Z, Zhou C, Shi H, Zhang H, Bi Y, Xu X. *Processing of Flavor-Enhanced Oils: Optimization and Validation of Multiple Headspace Solid-Phase Microextraction-Arrow to Quantify Pyrazines in the Oils*. Life. **2021** Apr 26;11(5):390.