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Development of the Detection Threshold Concept from a Close Look at Sorption Occurrence Inside a Glass Vial Based on the In-Vial Vaporization of Semivolatile Fatty Acids

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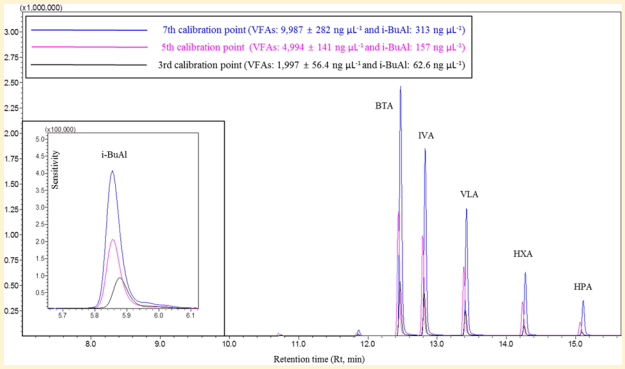
Supporting Information

ABSTRACT: Headspace (HS) analysis has been recommended as one of the most optimal methods for extracting and analyzing volatile organic compounds from samples in diverse media such as soil and water. Short-chain volatile fatty acids (VFA, C_3–C_7) with strong adsorptivity were selected as the target compounds to assess the basic characteristics of the HS analysis through simulation of HS conditions by in-vial vaporization of liquid-phase standards (VL) in 25 mL glass vials. The reliability of the VL approach was assessed by apportioning the in-vial VFA mass into three classes: (1) vaporized fraction, (2) dynamic adsorption on the vial walls (intermediate stage between vaporization and irreversible absorption), and (3) irreversible absorptive loss (on the vial wall). The dynamic adsorption partitioning inside the vial increased with n-VFA carbon number, e.g., 43% (C_2: acetic acid, extrapolated value), 65% (C_3: propanoic acid), and 98% (C_7: heptanoic acid). The maximum irreversible losses for the studied n-VFAs exhibited a quadratic relationship with carbon number. If the detection threshold limit (DTL: the onset of mass detection after attaining the maximum irreversible loss) is estimated, the DTL values for target VFAs were in the range of 101 ng for i-valeric acid to 616 ng for propionic acid, which are larger than the method detection limit by about 3 orders of magnitude. Consequently, quantitation of VFAs using the VL approach should be critically assessed by simultaneously considering the DTL criterion and the initial VFA masses loaded into the vial.



1. INTRODUCTION

Static headspace (HS) analysis is one of the most common methods for extracting and analyzing volatile organic compounds (VOC) from samples of diverse environmental media such as soil and water.[1−4] Once the environmental samples are placed in a container (e.g., a vial), the volatile compounds are volatilized from the sample into the HS. This volatilization process can occur under static (no air flow) or dynamic (with steady purging air flow) conditions through the container. If the concentration of VOCs in HS samples is not restricted by instrumental detectability, the HS samples can be analyzed directly by the detection system.[5] However, the analysis of HS samples is more commonly preceded using a number of preconcentration options such as solid phase microextraction (SPME),[6] purge and trap (PT) method,[7] and close loop stripping analysis (CLSA).[8,9]

The HS analysis is generally susceptible to experimental bias because of the many complexities involved in the extraction and analysis procedure. According to an extensive literature survey, there is a paucity of data on the absolute or relative loss of target analytes to glass surfaces in HS analysis. In some situations, silanization of glass surfaces is used to reduce sample losses by converting reactive surface−OH groups into silyl derivatives.[10] Hence, information concerning the absolute loss of analytes during HS extraction can be used as a valuable gauge to understand the fundamental nature of HS analysis.

If HS analysis is made for highly sorptive components like volatile fatty acids (VFAs), the occurrence of large ad-/absorptive loss is expected. VFAs are one of the most representative malodorous and semivolatile compounds, as some of them have significantly low odor thresholds in the parts per billion level.[11] To detect ambient concentration levels of VFAs in sub-ppb or sup-ppt range, the reliability of the sampling and analytical approach needs to be validated to overcome limitations in their collection (e.g., high sorptivity). The use of a sorbent tube made of quartz was recommended as an optimal sampling method to quantify ambient VFAs, as it was proven to effectively reduce their sorptive loss at the

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sampling stage.[12] Nonetheless, the HS-SPME method is recommended and employed as the official method for VFA analysis, e.g., the experimental protocol recommended in the malodor prevention law enforced by the Korean Ministry of Environment (KMOE). The principle of the KMOE method is that a gas sample is brought into contact with an alkaline solution to initially induce the absorption of gaseous VFAs. Then, the HS extraction of absorbed VFAs is done by SPME for subsequent analysis through acidification (pH 1) and heating (90 °C). For the accurate quantitation of VFAs based on such an approach, it is desirable to assess the absolute recovery of VFAs in the sample to ensure the objectivity of analysis. However, due to technical difficulties, the absolute recovery or reliability of the HS analysis (e.g., HS-SPME, HS-absorption, and HS-direct analysis) has not been properly evaluated according to our knowledge.

If the sorptive reactivities of multiple target VFAs are different from each other, their recovery patterns should be largely distinguishable. Kim and Kim[13] investigated the recovery of gaseous VOCs using the bag sampling method by transferring the analytes from one bag to another (bag-to-bag transfer test). Ahn et al.[14] evaluated the recovery of four VFAs (propionic acid, n-butyric acid, i-valeric acid, and n-valeric acid; concentration range = 20 to 100 ppb) by the bag-to-bag transfer method and found that their recoveries generally fell below 60% across supposedly inert bag materials (i.e., 1 L polyvinyl fluoride Tedlar bags).

In this research, we developed an experimental approach based on in-vial vaporization of liquid phase VOC standards (VL) to directly measure the recovery of the vaporized VFAs from glass vials. Note that this VL method has been applied successfully to determine the Henry's law constants of several VOCs by means of the Equilibrium Partitioning in Closed Systems (EPICS) approach.[15] The objectives of this research were thus expanded to assess the recoveries of diverse target compounds from vials using both liquid- (VFA) and gas-phase (VOC) standards. Two experiments were conducted in this research. In experiment 1, liquid VFA standards were injected into glass vials, and recoveries were evaluated. In experiment 2, gaseous standards of VOC were placed into glass vials, and recoveries were evaluated similarly. The extent of analyte loss on the inner wall material of the glass syringe was also tested in a similar manner. Based on this study, we attempted to establish an accurate experimental method to assess the recovery and extractable proportion of semivolatile organics in the HS fraction. As such, we aim to provide both a theoretical and practical basis for the quantitative analysis of VFA in various environmental matrices using glass vials, purge and trap, and any other preconcentration/extraction methods.

2. MATERIAL AND METHODS

For the purpose of our study, we designed and carried out two types of experiments (expts 1 and 2), as the basic schemes of each experiment are presented in Table 1 and Figure 1. We have appended a two-letter code to the different types of experiments conducted in expt 1 and expt 2 to distinguish (1) the sample containers (first letter), D (direct injection into sorbent tube), V (vial), or S (syringe), and (2) working standard phases (second letter), L (liquid−vaporized into sorbent tube or vial) or G (gas). To eliminate the matrix effect in the sampling procedures, the sorbent tube with the same sorbents was used to collect the VOC samples.[11] In addition, the main acronyms used herein are also summarized in Table 2.

Table 1. Basic Experimental Scheme to Evaluate the Recovery of VFAs between Direct Injection (D) and Vapor Analysis (Expts 1 and 2)

A. Information of Expt Code and the Associated Sample Loading Approach	
expt code	sample loading approach (directly to or prior to sorbent tube loading)
1. expt 1-DL	1 μL of liquid standard was injected into sorbent tube
2. expt 1-VL	1 μL of liquid standard was injected into 25 mL vial
	2 mL of vapor in 25 mL vial was sampled using 10 mL gastight syringe and then injected into sorbent tube
3. expt 2-DG	1 mL of gaseous standard injected into sorbent tube
4. expt 2-VG	1 mL of gaseous standard injected into 25 mL vial
	2 mL of sample in 25 mL vial was sampled using 10 mL gastight syringe and then injected into sorbent tube
5. expt 2-SG	1 mL of gaseous standard was injected into 10 mL gastight syringe filled with nitrogen (>99.999%)
	2 mL of sample in 10 mL gastight syringe was sampled using gastight syringe and then injected into sorbent tube

B. Operation Conditions for Both DL and VL Analysis	
1. sampling tube:	Carbopack C, B, and X (70 mg each)
2. sample injection approach:	sample is injected onto sorbent tube with the constant supply of purge gas
3. purge gas:	nitrogen (>99.999%)
4. purge flow rate:	100 mL min^{-1}
5. purge time:	5 min

C. Expt 1: Calibration Conditions of VFA between Two Experimental Approaches		
sample code	Expt 1-DL	Expt 1-VL
analyte phase	liquid (sorbent tube)	gas (vial)
1. target compounds:	7 VFAs and one reference compound (i-BuAl)	
2. standard phase:	liquid	
3. calibration range (7 VFAs):	1.66 ± 0.05 ng to 66.6 ± 1.88 ng (five-point calibration)	53.3 ± 1.50 ng to 799 ± 22.6 ng (seven-point calibration)
4. analytical volume[a]:	1 μL	2 mL

D. Expt 2: Information of Gaseous VOC Standard Used in Ancillary Experiments			
sample code	Expt 2-DG	Expt 2-VG and -SG	
analyte phase	gas	gas (vial)	gas (syringe)
1. target compounds:	4 VOCs (MEK, MIBK, BuAc, and i-BuAl)		
2. standard phase:	gas		
3. concentration:	10.1 ± 0.05 ppm		
4. balance gas:	nitrogen (>99.999%)		
5. vessel:	G-WS loaded directly on sorbent tube	25 mL vial	10 mL gastight syringe
6. analytical volume[a]:	1 mL	2 mL	2 mL

[a]The amount of sample loaded on sorbent tube for the TD analysis.

Detailed descriptions of our experimental scheme including standard preparation, instrumental system, and experimental procedure are provided in the Supporting Information (SI) along with Tables S1−S5 and Figures S1−S5.

In expt 1-DL, the calibration results of VFAs were obtained through direct injection of 1 μL liquid VFA standards onto the sorbent tube using a 5 μL liquid syringe to obtain the calibration results of VFA by a TD-GC-MS system.[16] In case of expt 1-VL, 1 μL of a liquid standard was injected and vaporized into a glass vial (25 mL). Then, 2 mL of the vaporized sample

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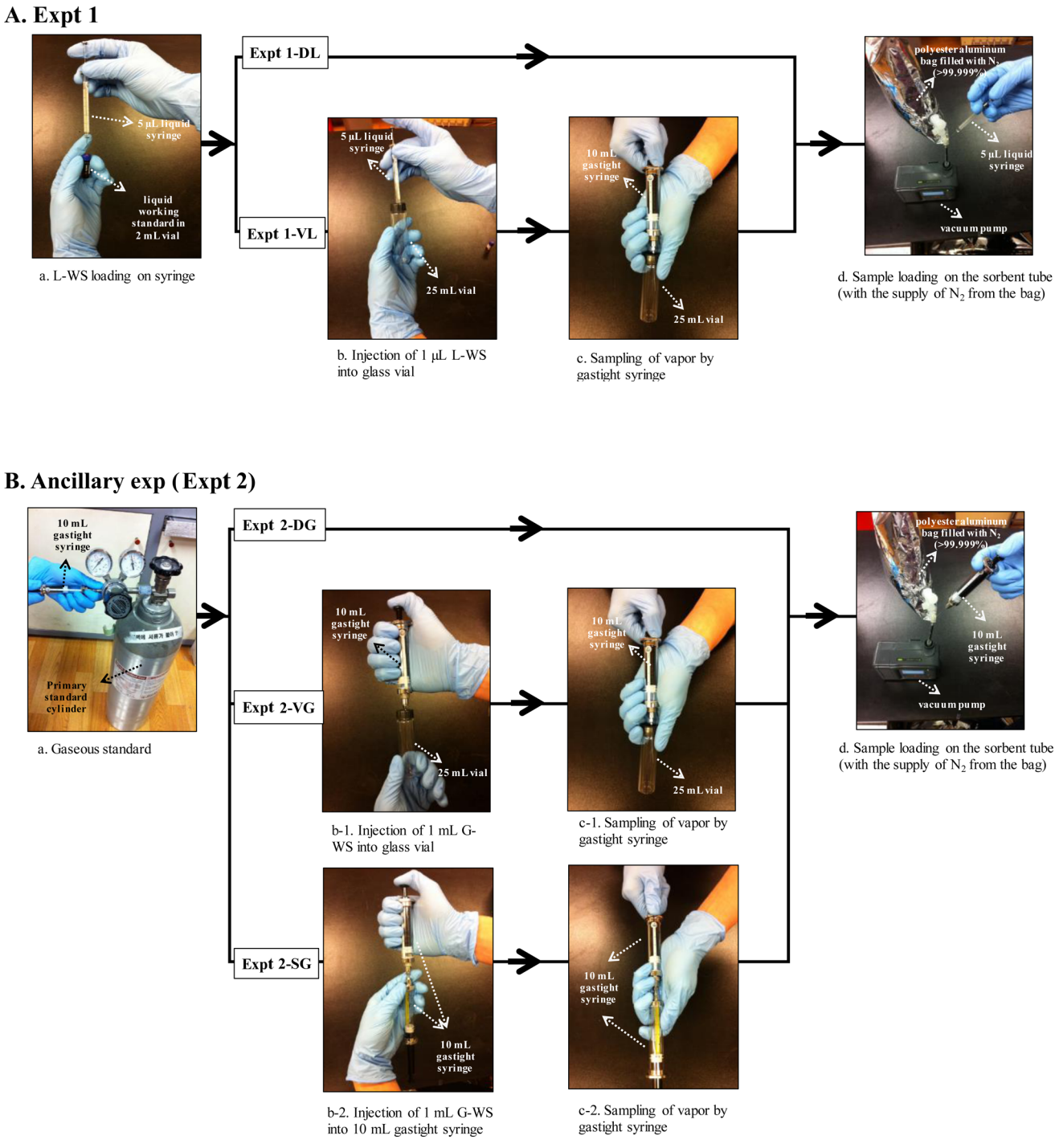

Figure 1. Flowchart showing experimental procedures to assess relative recovery of VFAs between direct liquid injection (D) and vapor approaches (expts 1 and 2).

was withdrawn from the vial and injected on the sorbent tube (using a gastight syringe). Finally, the VFA loaded on the sorbent tube (either via DL or VL) was analyzed in the same manner by TD-GC-MS.

To facilitate complete vaporization of the liquid standard in the vial, the injection volume of liquid standard was minimalized (1 μL) to ensure that the vapor partial pressure of all compounds was below the compound's saturated vapor pressure, and the vial was stirred at 2000 rpm for 1 min using a vortex mixer (Digital Vortex-Genie 2, Scientific Industries, Inc., USA). Sample perturbation was minimal as only 2 mL of HS was withdrawn by gas-tight syringe. The VL calibration data were then obtained by multiple-point analysis by the fixed standard volume approach.[17]

To thoroughly examine the sample loss patterns of VFA during VL analysis, the second stage experiments (expt 2) for relative recovery test were conducted by simulating VL analysis by gaseous phase standard of VOC (Table 1). The results of expt 2 were used to determine some experimental factors contributing to the efficiency of VL generation by treating the gas standard similarly to the VL samples derived from the liquid working standard (L-WS). To this end, gaseous standards

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Table 2. List of Symbols, Abbreviations or Parameters (Nomenclature), and Equations Using Those Parameters

A. Nomenclature

order	parameter	description
1	m_l	mass loaded and vaporized in 25 mL vial (initial mass in liquid standard: ng)
2	m_{vg}	net mass present in gas phase in vial (ng)
3	m_{iw}	maximum mass irreversibly lost to vial walls (ng)
4	m_{dw}	mass dynamically adsorbed on vial walls (ng)
5	p	wall/gas-phase partition coefficient (eq 5A)
6	RF_{dl}[a]	RF from direct injection of L-WS into sorbent tube (ng^{-1})
7	RF_{VL}	RF from in-vial vaporization (ng^{-1})
8	PA_{VL}	in-vial vaporization peak area of analyte
9	I_{VL}	in-vial vaporization calibration y-axis intercept

B. Equations

order	equation code	equations
1	eq A1	if $m_l > m_{iw}$, $m_{vg} = (m_l - m_{iw})/(1 + p)$ (from mass balance)
2	eq A2	if $m_l < m_{iw}$, $m_{vg} = 0$ (all analyte is lost to walls)
3	eq A3	$PA_{VL} = RF_{dl} \times (m_l - m_{iw})/(1 + p)$
4	eq A4	$RF_{VL} = RF_{dl}/(1 + p)$
5	eq A5	$m_{iw} = I_{VL} \times (1 + p)/RF_{dl})$
6	eq A6	$p = m_{dw}/m_{vg} = RF_{dl}/RF_{VL} - 1$

[a]The RF_{dl} was obtained by linear regression analysis with a forced-zero intercept

containing four VOCs, i-butyl alcohol (i-BuAl), methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK), and butyl acetate (BuAc)) were prepared from a primary standard gas cylinder (Rigas, Korea). Note that i-BuAl was also used as the reference compound in VFA liquid standards. At first, the gaseous VOC standards were injected into the sorbent tube and analyzed by TD-GC-MS (expt 2-DL). The loss of gaseous working standard (G-WS) due to the contact with vial surfaces was evaluated by taking 2 mL of the vial gaseous contents 0.5 min after injecting 1 mL of gaseous VOC standard into a sealed blank vial. The retrieved vial gaseous sample was then loaded onto the sorbent tube for subsequent analysis (expt 2-VG).

In addition, to assess the sample loss induced by the contact with any surface material (not only on inner wall of vial but also by any other surface), we examined the possible loss to the needle and surfaces of a gastight syringe. To test this possibility, two identical gastight syringes (10 mL capacity) were used to transfer samples between syringes and to check the loss after each transfer between syringes. Hence, the quantity of VOCs in gas standard was measured after transfer from one gastight syringe (10 mL size) to another (expt 2-SG). To this end, 1 mL of gaseous VOC standard was initially loaded into a 10 mL gastight syringe (A) initially filled with 10 mL of nitrogen (>99.999%) by fixing the position of plunger to simulate the condition of the sealed vial (with a 10 mL capacity). Then, the 2 mL of gaseous VOCs in syringe A was subsequently withdrawn and injected on the sorbent tube using another 10 mL gastight syringe (B). The sorbent tube loaded with these VOC samples was then analyzed by TD-GC-MS.

3. RESULTS AND DISCUSSION

3.1. Calibration Results of VFA by DL and VL Analyses Using L-WS (Expt 1). As a simple means to assess the reliability of VL analysis, the calibration results obtained by both DL and VL were compared using a number of metrics. The calibration results of VFAs in expt 1 derived by both DL and VL analyses are summarized in Table S4 in terms of (1) slope or response factor (RF), (2) coefficient of determination (R^2), and (3) relative standard error (RSE, %). The results of linear regression analysis for the two methods were first compared using intercept settings ((1) forced-zero and (2) fully floating; Figure S2). In case of DL analysis, all slopes of VFAs were comparable with or without a forced-zero intercept (mean slope values: (1) forced-zero = 54 271 ± 27 308 and (2) non-forced-zero = 54 191 ± 27 327). The percent difference (PD, %: eq 1) in slope values for each target compound (n = 8), when computed with or without forced-zero intercept, yielded very low mean values of 0.21 ± 0.25%.

$$\text{Percent difference (PD: \%)} = 100 \times \text{ABS}\{\text{slope (forced zero)} - \text{slope (non-forced zero)}\}/\text{slope (forced zero)} \quad (1)$$

For the calibration curves of DL analysis made without the forced-zero intercept, their intercept values were significantly small compared to the slope values (mean intercept (aiu) = 3878 ± 4324 (7.16% of the slope value)). The slope values from two types of calibration curves (derived with either forced-zero or nonforced zero intercept) had good linearity of above 0.999, regardless of y-axis intercept selection. In contrast, the calibration slope values of VL analysis, determined with either forced- or non-forced-zero intercept, were significantly different depending on the target compound. The PD value of slopes between two intercept types was fairly low for i-BuAl (0.84%), while PPA increased to 10.1% (mean PD value (n = 8) = 4.34 ± 3.03%).

There was a large difference in absolute slope (RF) values (for target analytes) between DL and VL analysis (e.g., depending on the analytical approaches; Figure S1). The RF values of DL were also generally 3 to 50 times higher than those of VL analysis (mean slope values: ng^{-1}): (1) DL = 54 271 ± 27 308 (zero offset) and 54 191 ± 27 327 (nonzero offset) vs (2) VL = 12 055 ± 17 808 (zero offset) and 12 339 ± 17 931 (nonzero offset). In VL analysis, the negative y-axis intercept values tend to occur consistently in the calibration curves of seven VFAs. The absolute magnitude of those negative intercept values was 100 times higher than their slope values. It is thus likely that the VFA losses in VL analysis should be reflected most noticeably by the presence of large negative y-axis intercept values in the linear regression analysis. Despite such differences in slope values, the reproducibility of both analytical approaches (DL and VL analysis) was fairly robust with their RSE values < 5% (mean RSE values (%): (1) DL = 0.39 ± 0.33 and (2) VL = 2.30 ± 1.53). In the case of VL analysis, some VFAs with relatively high molecular weights (HXA and HPA) had RSE values slightly larger than others (3.49% and 4.94%).

3.2. Assessment of the VL-Based Recovery Using L-WS (Expt 1). As defined by Konieczka and Namieśnik,[18] the recovery value of an analytical procedure (such as the recovery of an analyte from a sample and the recovery dependence on the accuracy of final measurements) is defined as the key elements of uncertainty in chromatographic analysis. To learn more about the trueness of the data obtained by our approach, the calibration results of the VL approach (with noticeable reduction in sensitivity or sample loss) are evaluated in this

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section in reference to those derived from DL approach. As the DL-based analysis was carried out without any extra treatment on L-WS (relative to VL sample), it is reasonable to assume that the DL results have the least negative bias due to minimal sample losses.[16] The amount of VFA (or VOC) in the VL samples was initially estimated by using the RF values (forced-zero intercept) of the DL analysis as the first criterion. According to this estimation, the mass of VFA in VL samples was significantly low with the VL recovery range of 1.69 (HPA) to 25.2% (PPA), if the loaded mass (m_l) was much larger than m_{iw} (m_{iw} is the maximum VFA mass irreversibly lost to surfaces). The variation of m_{iw} vs carbon number of VFAs is shown as a quadratic regression line (Figure S3A). The i-VFAs are shown as separate points. The m_{iw} values for formic (1740 ng) and acetic acids (900 ng) could in principle be estimated by extrapolation (see Table 3). Comparison between the detected (ng) and loaded mass (ng) of L-WS in a 25 mL vial showed significant correlations ($R^2 > 0.99$; Figure S4). To estimate the absolute loss in the collection of the VL sample, the offset value of the regression curve was used further as the second criterion for correction (Table S5).

As shown in Figure S4, the reduction in detected mass (compared to loaded mass) can be explained as the combined effects of both the negative offset and the reduced slope values in the regression equation of VL analysis. Hence, these two terms are arbitrarily named in this study "Loss-O" and "Loss-S," respectively. (Here, O and S for loss terms denote offset and slope, respectively.) As shown in Figure S4, all regression curves consistently yielded negative offset values (Loss-O).

$$\text{Loss-O (ng)} = \text{negative of } y\text{-intercept value in linear plot of detected mass in vial HS vs loaded mass in vial} \quad (2a)$$

$$\text{Loss-O (ng)} = -I_{VL}/\text{RF}_{dl} \quad (2b)$$

The Loss-O term of VFAs by the VL approach varies systematically with VFA molecular weight. For instance, PPA had the highest Loss-O of 216 ng, while HPA recorded the lowest Loss-1 of 8.91 ng (mean Loss-O (ng) of seven VFAs = 50.7 ± 74.9 ng). In contrast, Loss-O of i-BuAl was very low at 0.84 ng, which is almost negligible compared to the others. If the Loss-S term is computed by relative differences in the slope values, the percentage of loss due to sensitivity reduction (Loss-S; %) can also be estimated in relation to that of Loss-O.

$$\text{Loss-S (\%)} = (1 - \text{slope of linear plot of mass detected in vial vs. mass loaded } (m_l) \text{ in vial}) \times 100 \quad (3a)$$

From the eq A2 in Table 2, Loss-S can be written in terms of the dynamic loss partition coefficient (p) as

$$\text{Loss-S (\%)} = 100/(1 + p) \quad (3b)$$

The percentage (%) of Loss-S increased gradually with increasing VFA molecular weight (the range of Loss-S (%) in seven VFAs = 64.9% (PPA) to 97.9% (HPA)). The Loss-S term for i-BuAl (49.8%) was, however, moderately lower than those of VFAs. A plot of ln(p) vs carbon number for n-VFAs yielded an excellent linearity (R^2 = 0.9938; Figure S3B). The extrapolated p values for formic and acetic acids were relatively low at 0.32 and 0.74, respectively, if compared to the studied VFAs. In theory, HS quantification of aqueous formic and

Table 3. Comparison of Partition Coefficient (p), Maximum Mass Lost Irreversibly to Walls (m_{iw}), and Analyte Surface Density for Studied Compounds and Extrapolated Data for Formic and Acetic Acids[a]

order	VFA/VOC	RF_{dl} (DL) (ng^{-1})	RF_{VL} (VL) (ng^{-1})	intercept I_{VL} (VL)	peak area (PA_{VL}) (VL)	partition coefficient (wall/gas)	least mass loaded (m_l) (VL) (ng)	mass (m_{vg}) remaining in gas phase (ng)	mass (m_{dw}) dynamically adsorbed on walls (ng)	maximum mass lost irreversibly to wall (m_{iw}) (ng)	carbon number	VFA wall density for m_{iw} ng lost on wall (molec·cm^{-2})	area per molecule for m_{iw} ng lost on wall (Å^2)	ln(p)
1	*formic*	nd	nd	nd	nd	0.32	nd	nd	nd	1740	1	4.14×10^{14}	24.2	*−1.14*
2	*acetic*	nd	nd	nd	nd	0.74	nd	nd	nd	900	2	1.64×10^{14}	60.9	*−0.30*
3	PPA	14 337	5039	−3 102 148	477 038	1.85	696	28.4	52.4	616	3	9.10×10^{13}	110	0.61
4	IBA	72 715	18 377	−3 402 915	7 616 138	2.96	682	126	371	185	4	2.30×10^{13}	435	1.08
5	BTA	42 574	7748	−1 957 217	2 716 071	4.49	674	76.7	345	253	4	3.14×10^{13}	319	1.50
6	IVA	47 952	5795	−587 327	2 519 807	7.27	651	66.4	483	101	5	1.09×10^{13}	920	1.98
7	VLA	49 528	3903	−633 035	1 719 978	11.7	660	39.2	458	162	5	1.74×10^{13}	575	2.46
8	HXA	43 880	2013	−520 029	660 950	20.8	652	18.1	376	258	6	2.44×10^{13}	411	3.03
9	HPA	54 335	1141	−484 081	388 750	46.6	646	4.66	217	424	7	3.57×10^{13}	280	3.84
10	i-BuAl	108 844	54 691	−91 674	1 143 453	0.99	20.9	9.64	9.55	1.68	4	2.48×10^{11}	40 383	−0.01

[a]nd: no data, not studied in present work. Italic data entries: extrapolated data based on present work data.

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Table 4. Method Detection Limit (MDL) and Detection Threshold Limit (DTL) of VFA Computed for Both DL and VL Approaches[a]

compounds	PPA	IBA	BTA	IVA	VLA	HXA	HPA	*i*-BuAl
A. Expt 1-DL								
mass of analyte[b] (ng)	0.87	0.85	0.84	0.81	0.82	0.82	0.81	0.77
peak area 1	11 135	50 156	37 565	41 212	42 546	33 265	41 544	90 546
peak area 2	13 564	48 956	35 214	40 215	45 585	34 658	41 668	95 654
peak area 3	11 235	49 563	34 554	44 365	41 215	35 445	43 558	92 654
peak area 4	9986	53 258	35 858	45 554	40 545	33 235	47 565	91 012
peak area 5	12 115	52 354	38 120	42 558	42 655	31 254	40 986	88 656
peak area 6	14 465	50 215	35 212	41 112	42 002	33 265	42 457	93 654
peak area 7	13 545	52 569	34 889	41 585	41 254	30 998	43 448	89 656
mean	12 292	51 010	35 916	42 372	42 257	33 160	43 032	91 690
SD	1618	1681	1383	1929	1654	1623	2221	2436
MDL (peak area)	5082	5280	4343	6059	5192	5095	6973	7650
MDL[c] (ng)	0.36	0.07	0.10	0.13	0.10	0.12	0.13	0.07
B. Expt 1-VL								
mass of analyte[b] (ng)	696	682	674	651	660	652	646	20.9
peak area 1	8135	153 256	61 565	50 212	32 546	13 265	13 544	20 546
peak area 2	13 564	142 454	50 546	60 454	35 585	14 658	8668	25 654
peak area 3	11 235	142 565	58 554	51 284	31 215	25 445	13 558	22 654
peak area 4	29 986	133 565	55 858	55 468	40 545	13 235	9565	21 012
peak area 5	22 115	153 254	48 120	48 456	42 655	21 254	15 986	25 656
peak area 6	29 154	157 101	48 558	53 114	36 447	17 500	12 255	25 100
peak area 7	12 545	142 154	48 212	60 555	44 098	25 976	16 935	20 678
mean	18 105	146 336	53 059	54 220	37 584	18 762	12 930	23 043
SD	8920	8382	5550	4825	4969	5509	3058	2380
MDL (peak area)	28 010	26 318	17 428	15 150	15 603	17 299	9601	7473
MDL[c] (ng)	5.56	1.43	2.25	2.61	4.00	8.59	8.41	0.14
DTL[d] (ng)	616	185	253	101	162	258	424	1.68

[a]Sample loading approach of Expt1-DL and-VL: Refer to Table 1A. [b]Mass of analyte loaded per 1 μL injection of liquid standard. [c]The product of the standard deviation of seven replicates multiplied by the Student's t-value at the 99.9% confidence level (six degrees of freedom, $t = 3.14$). [d]Actual detection limit of VL approach was predicted by the combination of "Loss-O" and "Loss-S" as follows (refer to Table S5 and Figure S4): Detection threshold limit (DTL: ng) = Loss-O (ng)/(100 − Loss-S(%)) × 100.

acetic acids should have much reduced bias or low sorptive losses (relative to the heavier VFAs).

The basic aspects of VFA sorptive loss had been critically assessed in our recent study of relative performance between different sorbent tube materials.[12] We analyzed the sorptive loss of five VFAs (acetic acid (ACA), PPA, BTA, IVA, and VLA) on the inner wall surface of a tube sampler by passing their vapor-phase standards through empty tubes (quartz vs stainless steel). The sorptive losses (%) of VFAs on the stainless steel tube were fairly high in the range of 33.6% (ACA) to 97.5% (VLA). Although quartz tubes showed the least losses, losses for certain VFAs (IVA and VLA (with relatively high molecular weight)) exceeded 30%. In light of those results, the significant loss of VFAs in this study is suspected to reflect sorptive reaction on the inner wall material of a vial (or gastight syringe) during VL analysis. However, we also have to consider the possible role of other factors to explain the full extent of sample loss in VL analysis such as diffusion loss in the VL approach in the container during extraction of VL samples by syringe, the efficiency of VL generation by L-WS, the reactivity of raw materials used for the container, etc.

3.3. Comparison between Detection Limit and Detection Threshold Limit (Expt 1). In this study, the quality assurance (QA) of VFA analysis was initially assessed for both DL and VL approaches in terms of method detection limits (MDL; Table 4). In the DL approach, the MDL values were determined by heptaplicate analyses of a diluted L-WS (0.82 ± 0.03 ng μL^{-1}) which was made by 2-fold dilution of the first-point calibration standard for the DL approach (Tables S2 and 4). It is relatively simple to calculate the detection limit of the DL analysis in terms of MDL. However, in the case of VL analysis, the original concept of MDL was no longer valid, as the detection of VFA from VL samples did not occur until m_l exceeded m_{iw} (the maximum irreversible adsorption loss on the vial wall). In fact, the extent of sample loss expressed as two loss terms of Loss-O and -S (as mentioned in section 3.2) in VL analysis was significantly larger than the MDL values (determined as lower than 10 ng). Thus, to describe this situation, we employed a new concept, namely a detection threshold limit (DTL, ng) which can be estimated from the calibration results of expts 1-DL and -VL. More specifically, the DTL values were predicted using the aforementioned Loss-O and Loss-S in VL analysis as follows (refer to Figure S4):

$$\text{detection threshold limit (DTL: ng)} = \text{Loss-O (ng)}/(100 - \text{Loss-S (\%)}) \times 100 \quad (4)$$

The DTL can be expressed in terms of y-axis intercept value (I_{VL}) from a linear regression plot of the VL peak area (PA_{VL}) vs m_l and direct injection RF value (RF_{dl}); $m_{iw} = I_{VL} \times (1 + p)/RF_{dl}$ (eq A5 in Table 2). In the case of VL analysis, due to the notable sample losses occurring in VL analysis, the first-point calibration standard of the VL approach with relatively high concentration (mean 585 ± 229 ng μL^{-1}) was used for MDL

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determination. The MDL values of the VL approach were also determined by heptaplicate analyses of these first-point VL calibration standards. Note that the major fraction of VFA standard added into a vial was initially removed due to the irreversible adsorptive losses (see Table 3) defined as DTL; the actual amounts of VFA used for the MDL test which overcome such adsorptive losses can thus be estimated to range from 6.46 (PPA) to 43.9 ng (IVA) (computed as (initially injected mass (m_l) − DTL)/12.5) per 2 mL vial sample.

Both MDL values for DL and VL approaches were calculated according to U.S. EPA guidelines as the product of the standard deviation of seven replicates and the Student's t-value at the 99% confidence level (6 df, t = 3.14).[19] The MDL values for seven VFAs used in DL analysis were found from 0.07 ng (IBA) to 0.36 ng (PPA; mean = 0.14 ± 0.10 ng). In contrast, those of VL analysis were 1.43 ng (IBA) to 8.59 ng (HXA) with a mean of 4.69 ± 2.92 ng, which were about 30 times higher than those of the DL analysis. It should however be noted that the difference in MDL values between DL and VL approaches for i-BuAl was relatively smaller (MDL values of i-BuAl: (1) DL = 0.07 ng and (2) VL = 0.14 ng).

In the case of our VL analysis, the DTL values for seven VFAs were about 60 times higher than their MDL values: (1) mean DTL value = 286 ± 178 ng and (2) mean MDL value = 4.69 ± 2.92 ng. Among seven VFAs, PPA had the highest DTL (or m_{iw}) value with 616 ng, while IVA recorded the lowest DTL value of 101 ng. In the case of i-BuAl, the difference between the DTL (1.68 ng) and MDL value (0.14 ng) was about an order of magnitude, which was far smaller than those of VFAs. It is well-known that silica and bulk glass surfaces contain silanol sites.[20,21] These silanol sites will react irreversibly with VFAs (e.g., formic and acetic acids) to give surface silyl esters. D'Souza et al.[22] determined silanol concentrations on amorphous silica surfaces to be in the range 1.3−4.1 OH groups per 100 Å^2. On the basis of the present VL experiments to determine m_{iw}, we estimated that ∼1 PPA molecule is irreversibly lost per 100 Å^2 (Table 3) which is consistent with the D'Souza et al.[22] data.

In order to test the effectiveness of control techniques for odorants (such as VFAs with low odor threshold of below 1 ppb), one needs an analytical system to reliably detect VFA in the sub-ppb range. Thus, if one intends to analyze gaseous VFA in the ppb level by this type of VL approach, a significantly large volume of sample (over 100 L) should be obtained to match sorptive loss conditions so that sufficient quantities of samples are obtained to exceed the DTL value. If the mean DTL value is applied to 100 L of air sample, the concentration of VFA is computed as 0.75 ± 0.60 ppb. If one considers that the collection of odor samples should be made instantly or over a fairly short interval (e.g., less than an hour), the applicability of the VL method is by no means practical to measure VFAs due to the significantly large DTL values of the method. In our laboratory, the quantitation of volatile fatty acids in processed wastewater (sewage) samples was assessed by direct injection into sorbent tubes or dynamic headspace sampling followed by sorbent tube preconcentration and compared to the reported literature metrological paraments (e.g., MDL). In summary, our results were comparable to the literature.[23,24]

3.4. Simulation of Sorptive Loss in VL Analysis Using G-WS (Expt 2). The recovery and detection limit of VL analysis were assessed using the L-WS containing VFAs in expt 1. The results of expt 1 showed that the significant sorptive loss of VFA occurred with the generation of VL with dramatically high DTL values for seven VFAs (286 ± 178 ng). The sorptive reaction between samples and the inner wall of containers (i.e., vial, syringe, etc.) is suspected as a major source of sample losses in the VL analysis. However, one may not rule out the possible role of other factors that can lead to sample loss such as the diffusion loss in the handling of syringe and/or the efficiency of VL generation from L-WS in the VL analysis.

In order to examine the possible role of other factors contributing to the sample losses of the VL analysis, its experimental conditions were simulated using gaseous VOC standards (expt 2; Figure 1). To this end, the gaseous standards containing four VOCs (MEK, MIBK, BuAc, and i-BuAl) were injected into a sealed vial (25 mL: expt 2-VG) or gastight syringe (10 mL: expt 2-SG). The gaseous samples in either the vial (VG) or syringe (SG) were then loaded on the sorbent tube for analysis. In expt 2-VG, the recovery of four VOCs in VL samples was determined using gaseous VOC standards in relation to L-WS. In the case of expt 2-SG, the sorptive reaction of the gastight syringe, used as the transfer media of gaseous samples, was evaluated against that of a vial system used for VL analysis. In this experiment, the possibility of diffusion loss (during collection and injection of VL sample between two syringes) was also tested. The relative loss of samples was then computed by the following equation:

$$\text{Relative loss (RL: \%)} = 100 \times (\text{peak area (DG)} - \text{peak area (VG or SG)})/\text{peak area (DG)} \quad (5)$$

Interestingly, the relative loss (RL, %) of i-BuAl in expt 2-VG (48.9%) was similar to that of expt 1-VL (49.8%). In light of this comparability, we can infer that vaporization of VOCs (or VFAs) in L-WS proceeded effectively in expt 1-VL (Table S5C). In expt 2-SG, the RL values of four gaseous VOCs were fairly low, e.g., below 5% (Figure S5). Although the relatively high RL values (range: 36.6% (MEK) to 50.3% (MIBK)) were seen consistently in expt 2-VG, it was not the case for those of expt 2-SG with fairly small mean values of 2.46 ± 2.71%. This noticeable difference between the two experiments thus implies that the sample losses due to the use of a gastight syringe are almost negligible compared to the sorptive loss occurring on a vial (Table S5C). Thus, we can explain that most of the sample loss in VL analysis was caused mainly by the sorptive reaction on the inner wall material of a vial. The observed loss patterns may be accounted for by the raw material properties of containers we tested. Although the vial was made of untreated glass, the gastight syringe was made of borosilicate glass with high chemical resistance (leading to low sorptive loss compared with a vial).[25] Large sorptive losses of acetic acid to glass surfaces of IR cells have been reported.[26] The sorptive losses increased as the temperature decreased and also increased when glass beads were added to the IR cell to increase surface area. In light of the fact that most commonly produced vials are intended for one-time use, this reactivity issue (e.g., silanization of the vial surfaces) is unlikely to be considered in order to keep production costs low.

4. CONCLUSIONS

In-vial vaporization can be used as an efficient tool to analyze extracted volatile compounds from diverse media such as soil and water. However, the extent of sample loss occurring in VL analysis can vary greatly due to differences in the sorptive

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reactivity of target compounds and materials used to produce a container. If the analysis of highly reactive compounds like volatile fatty acids involves in-vial vaporization of liquid samples, the quantitation of target compounds can be biased significantly by the sample losses to surfaces. To help establish a reliable experimental approach to quantify semivolatile compounds, we designed and conducted a series of laboratory experiments to account for the sorptive loss behavior using both liquid- (VFA) and gas-phase standards (VOC) using an in-vial vaporization approach or through its simulation. On the basis of our study, we were able to account for the VFA losses by the combined effects of dynamic/equilibrium adsorption on the vial walls (intermediate stage between vaporization and irreversible absorption) and irreversible absorptive loss (on the vial wall). Because of the occurrence of excessive losses to the vial walls (ranging from 101 ng (IVA) to 616 ng (PPA)), we were able to confirm the existence of a detection threshold limit (DTL) as the onset of mass detection after attaining the maximum irreversible loss. As a result, this DTL concept can be used to estimate the minimum mass to be loaded in a vial to have any VFA (or other analyte) in the vapor phase.

As such, the quantitation of VFA in ambient samples is unlikely to be possible under the common sampling conditions of odorants. Hence, the HS analysis of VFA or VOC, if made using vial sampler, should be made cautiously by considering the extent of sample loss on the container media. In this respect, the use of the DTL concept introduced in this study can be a good barometer to initially evaluate the feasibility of HS analysis and to account for a unique loss mechanism of semivolatile compounds like VFA.

■ ASSOCIATED CONTENT

Supporting Information

Material and methods, Tables S1−S5, and Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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