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Supplementary Information

Development of the detection threshold concept from a close look at sorption occurrence inside a glass vial based on the in-vial vaporization of semi-volatile fatty acids

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2. Material and methods

In our experiments conducted in two different stages, recoveries of target compounds were assessed in relation to liquid (or gas) standards through direct injection of extracted samples into sorbent tubes. All analyses were done by thermal desorption (TD) – gas chromatography (GC) – mass spectrometry (MS). As the basic analytical procedures of the two experiment types described above were identical, the results can be compared on a parallel basis without considering major source(s) of experimental biases, especially matrix effect. Based on these comparative experiments, we were able to describe the effect of material types on the sorptive loss when analyzing HS samples of VFA (or VOCs) generated from vial samplers.

2.1. Preparation of liquid- and gas-phase standards

In this study, recovery of seven VFAs in liquid standards injected and vaporized in a 25 mL septum sealed glass vial (screw top, clear glass, product number: 27173, Supelco, USA) was determined (Table S1). In some experiments, previously used vials were used; the vials were cleaned with deionized water with a soft brush and dried. There was no impact on the results whether the vials were used as received or reused. The vial septum cap seal was coated with polytetrafluoroethylene (PTFE). Isobutyl alcohol was added as a reference compound (Table S1).

In order to assess the recovery in VL analysis (Exp 1), seven VFAs were selected as target analytes with i-BuAl as the reference compound (Table S1): (1) propionic acid (PPA), (2) i-butyric acid (IBA), (3)

n-butyric acid (BTA), (4) i-valeric acid (IVA), (5) n-valeric acid (VLA), (6) Hexanoic acid (HXA), (7) Heptanoic acid (HPA), and (8) isobutyl alcohol (i-BuAl). The L-WS containing seven VFA and i-BuAl was prepared by the dilution of each reagent grade chemical (RGC) with methanol (≥99.8%, Burdick & Jackson, USA). To quantify recovery of VFA (or VOC) after VL, their concentrations in the L-WS of the final calibration point were controlled to maintain similar mass range for GC-MS detection between DL and VL analyses (Table S2).

The RGCs were purchased with purities ≥99.0% from Sigma-Aldrich (USA). Initially, for the preparation of the primary standard (PS), 270 µL aliquots of each RGC (10 µL in the case of i-BuAl) were pooled together to make a final volume of 1.9 mL in a 2 mL vial to yield a mean concentration (± SD) of (1) 133 ± 3.76 µg μL^{-1} (7 VFA) and (2) 4.17 µg μL^{-1} (i-BuAl). The first L-WS was then prepared by mixing the 4 µL of the PS and the 0.6 µL of i-BuAl (RGC) with methanol to make a final volume of 4 mL (mean concentration: (1) 7 VFA = 133 ± 3.76 ng μL^{-1} and (2) i-BuAl = 123 ng μL^{-1}).

The concentrations of the final L-WS for the calibration by DL and VL were prepared in two distinct concentration ranges to allow for differences in the actual VFA detection range by the two approaches. In case of the VL analysis, the 1st L-WS was diluted with methanol for the seven-point calibration in a 2 mL vial (concentration range of the final L-WS for VL analysis: (1) 7 VFA = 666 ± 18.8 ng μL^{-1} to 9,987 ± 282 ng μL^{-1} and (2) i-BuAl = 20.9 ng μL^{-1} to 313 ng μL^{-1}). In case of DL analysis, the 1st L-WS was diluted with methanol for the five-point calibration in a 2 mL vial (concentration range of final L-WS for DL analysis: (1) 7 VFA = 1.66 ± 0.05 ng μL^{-1} to 66.6 ± 1.88 ng μL^{-1} and (2) i-BuAl = 1.54 ng μL^{-1} to 61.6

ng μL^{-1}). In contrast, the primary standard of four gaseous VOCs (MEK, MIBK, BuAc, and i-BuAl) in Exp 2 was purchased in a cylinder (Rigas, Korea).

2.2. Instrumental system

In this research, all target analytes for all different types of experiments ((1) Exp 1-DL, (2) Exp 1-VL, (3) Exp 2-DG, (4) Exp 2-VG, and (5) Exp 2-SG) were loaded on to sorbent tube for thermal desorption. Hence, all these sorbent tube samples were analyzed identically by the same GC (Shimadzu GC-2010, Japan) - MS (Shimadzu GCMS-QP2010 Ultra, Japan) with TD (Unity II, Markes International, Ltd, UK) system and operational settings. The TD focusing trap (ID (2 mm) and total sorbent bed length (50 mm)) was packed with an equi-volume ratio of Carbopack C and B (Markes International, Ltd, UK) (Table S3).

Ten sorbent tubes were prepared identically by packing 70 mg each of Carbopack C, B, and X (Supelco, USA) in empty quartz holders and conditioned before use at 350 °C for 2 hours in a flow (100 mL min^{-1}) of 99.999% N_2 gas. The sampling method of VFAs using this 3-bed sorbent tube had already been reported in our previous study.[12] The analytes loaded on the sorbent tube were thermally desorbed, transferred to GC, and separated on a CP-wax column (diameter: 0.25 mm, length: 60 m, and thickness: 0.25 µm) for MS detection. These analytes were initially examined in total ion chromatographic (TIC) mode over a mass range of 35 to 150 m/z. Extracted ion chromatographic (EIC) mode was also applied subsequently to eliminate the influence of the potential interferences using the information of identified ions based on the mass spectral data of each VOC (Table 1). Representative extracted ion chromatograms

are shown in Fig. S1.

2.3. Experimental approaches (Direct injection (D) and vapor analyses)

2.3.1. Direct injection (D) analysis in both Exp 1 and 2

The inlet and outlet of the sorbent tube were connected to a 10 L polyester aluminum (PEA) bag filled with back-up gas (ultra-pure nitrogen > 99.999%) and the vacuum pump interfaced with mass flow controller (MFC) (Shibata ΣMP-30, Japan), respectively. The gaseous and liquid standards were injected onto the sorbent tube via a temporary injection port pierced in the Teflon tube that connected the inlet of the sorbent tube and the PEA bag,[16] while the back-up gas was introduced from the PEA bag to the sorbent tube (flow rate of 100 mL min^{-1} for 5 min). The injection volume of standards was fixed at 1 μL for Exp 1-DL and 1 mL for Exp 2-DG, respectively.

2.3.2. Vapor analysis: Exp 1-VL & Exp 2-VG and -SG

In Exp 1-VL and Exp 2-VG, a 25 mL glass vial was used as sample container. For calibration, the liquid (Exp 1-VL) and gaseous standards (Exp 2-VG) were injected into the vial at fixed volumes of 1 μL and 1 mL, respectively. In case of Exp 2-SG, the 10 mL borosilicate glass gas-tight syringe (SGE analytical Science, USA) was also used as the sample container (like a vial). For Exp 2-SG, 1 mL of gaseous VOC standard was initially injected into the 10 mL gas-tight syringe, and transfer of this standard was made by another syringe for further verification of sample loss.

All sample containers, whether vial or gas-tight syringe, were shaken at 2,000 rpm for 1 min using a vortex mixer (Digital Vortex-Genie 2, Scientific Industries, Inc., USA), once loaded by either liquid or gaseous standards. Finally, 2 mL gaseous samples were withdrawn from containers (vial or gas-tight syringe) and injected on the sorbent tube using a 10 mL gas-tight syringe. The sorbent tube loaded with analytes was then analyzed by TD-GC-MS. All experimental procedures are depicted in Fig. 1.

2.3.3. DL and VL data analysis: Exp 1-DL and Exp 1-VL

The calibration data shown in Table S4 and Fig. S2 were analyzed using the scheme defined below (refer to Table 2 in main manuscript) to determine the (1) vaporized fraction, (2) dynamic adsorption (intermediate stage between vaporization and irreversible absorption), and (3) absorptive loss (irreversible absorption on the wall). In addition, the partitioning co-efficient (p) for the dynamic VFA adsorption on vial walls was also determined. In our experimental scheme (in the Table 2 of main manuscript), the detected threshold limit (DTL) is equivalent to m_{iw} (maximum mass lost irreversibly on vial walls). For any mass loaded, (m_l) < m_{wv} is totally lost irreversibly. Therefore, to have gaseous analyte present in the vial, m_l has to be > m_{wv} (Eqn-A1 and A2). The results of our analysis are presented in Table 3.

Table S1. Basic information regarding (seven) target VFAs and (four) reference compounds (VOC) investigated in this study

Order	Group	Compounds	Short name	MW ($g\ mol^{-1}$)	Density ($g\ cm^{-3}$)	Boiling point (℃)	Formula	CAS number	Mass spectra[a] (m/z)
1		Propionic acid	PPA	74.08	0.99	141	$C_3H_6O_2$	79-09-4	74
2	Volatile	i-Butyric acid	IBA	88.11	0.9697	155	$C_4H_8O_2$	79-31-2	41, 42, 43
3	fatty	n-Butyric acid	BTA	88.11	0.9595	163.5	$C_4H_8O_2$	107-92-6	60
4	acid	i-Valeric acid	IVA	102.13	0.925	175-177	$C_5H_{10}O_2$	503-74-2	60
5	(VFA)	n-Valeric acid	VLA	102.13	0.930	186-187	$C_5H_{10}O_2$	109-52-4	60
6		Hexanoic acid	HXA	116.16	0.929	205.8	$C_6H_{12}O_2$	142-62-1	60
7		Heptanoic acid	HPA	130.18	0.9181	223	$C_7H_{14}O_2$	111-14-8	60
8	Alcohol	Isobutyl alcohol	i-BuAl	74.12	0.801	108	$C_4H_{10}O$	78-83-1	41, 42, 43
9	Ketone	Methyl ethyl ketone	MEK	72.11	0.8050	79.64	C_4H_8O	78-93-3	41, 42, 43
10		Methyl isobutyl ketone	MIBK	100.2	0.802	117-118	$C_6H_{12}O$	108-10-1	41, 42, 43
11	Ester	n-Butyl acetate	BuAc	116.2	0.881	126	$C_6H_{12}O_2$	123-86-4	41, 42, 43

[a]Mass spectra selected for the EIC-base analysis

Table S2. Preparation of liquid phase working standards (L-WS) of VFA and i-BuAl for the analysis by DL-TD-GC-MS or VL -TD-GC-MS system (Exp 1)

A. Preparation of liquid phase standard

	Compound[s]	Methanol	PPA	IBA	BTA	IVA	VLA	HXA	HPA	i-BuAl	Mean (7 VFAs)	SD (7 VFAs)
a. RGC[a]	Concentration (%)		99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0		
	Density (g mL^{-1})		0.99	0.9697	0.958	0.925	0.938	0.927	0.9181	0.801		
b. PS[b]	Volume (μL)		270	270	270	270	270	270	270	10		
	Concentration (ng μL^{-1})		139,277	136,421	134,775	130,133	131,962	130,414	129,162	4,174	133,164	3,759
c. 1st L-WS[c]	Volume (μL)	3,995.4				4	(of PS)					
	Concentration (ng μL^{-1})		139	136	135	130	132	130	129	123	133	3.76

[a]RGC: Reagent grade chemical

[b]PS (primary standard): Dilution of pure chemical (RGC) to make 1,900 μL solution

[c]1st L-WS (1st liquid working standard): Dilution of PS (each 4 μL) and RGC (0.6 μL of i-BuAl) to make 4 mL solution

B. Preparation of final liquid-WS (Final L-WS) for direct injection (DL) calibration (DL-TD-GC-MS)

Order	Mixing volume (μL)		Concentration (ng μL^{-1})									
	1st L-WS	Methanol	PPA	IBA	BTA	IVA	VLA	HXA	HPA	i-BuAl	Mean (7 VFAs)	SD (7 VFAs)
1	25	1,975	1.74	1.71	1.68	1.63	1.65	1.63	1.61	1.54	1.66	0.05
2	100	1,900	6.96	6.82	6.74	6.51	6.60	6.52	6.46	6.16	6.66	0.19
3	200	1,800	13.9	13.6	13.5	13.0	13.2	13.0	12.9	12.3	13.3	0.38
4	500	1,500	34.8	34.1	33.7	32.5	33.0	32.6	32.3	30.8	33.3	0.94
5	1000	1,000	69.6	68.2	67.4	65.1	66.0	65.2	64.6	61.6	66.6	1.88

C. Preparation of final liquid-WS (Final L-WS) for vial vaporization (VL) calibration (VL-TD-GC-MS)

Order	Mixing volume (μL)		Concentration (ng μL^{-1})									
	PS	Methanol	PPA	IBA	BTA	IVA	VLA	HXA	HPA	i-BuAl	Mean (7 VFAs)	SD (7 VFAs)
1	10	1,990	696	682	674	651	660	652	646	20.9	666	18.8
2	20	1,980	1,393	1,364	1,348	1,301	1,320	1,304	1,292	41.7	1,332	37.6
3	30	1,970	2,089	2,046	2,022	1,952	1,979	1,956	1,937	62.6	1,997	56.4
4	50	1,950	3,482	3,411	3,369	3,253	3,299	3,260	3,229	104	3,329	94.0
5	75	1,925	5,223	5,116	5,054	4,880	4,949	4,891	4,844	157	4,994	141
6	100	1,900	6,964	6,821	6,739	6,507	6,598	6,521	6,458	209	6,658	188
7	150	1,850	10,446	10,232	10,108	9,760	9,897	9,781	9,687	313	9,987	282

Table S3. Operational settings of the TD-GC-MS system for the analysis of VFAs in this study

A. GC (Shimadzu GC-2010, JAPAN) and Q MS (Shimadzu GCMS-QP2010, JAPAN)			
Column: CP Wax (diameter: 0.25 mm, length: 60 m, and film thickness: 0.25 μm)			
Oven setting		Detector setting	
Oven temp:	80 ℃ (5 min)	Ionization mode:	EI (70 eV)
Oven rate:	20 ℃ min^{-1}	Ion source temp.:	230 ℃
Max oven temp:	220 ℃ (4 min)	Interface temp.:	230 ℃
Total time:	16 min	TIC scan range:	m/z 35~150
Carrier gas:	He (99.999%)		
Carrier gas flow:	1 mL min^{-1}		

B. Thermal desorber (Unity, Markes, International, Ltd, UK)			
Cold trap sorbent:	Carbopack C + Carbopack B (volume ratio=1:1)		
Split ratio:	1:5	Adsorption temp.:	0 ℃
Split flow:	5 mL min^{-1}	Desorption temp.:	330 ℃
Trap hold time:	10 min	Flow path temp:	180 ℃

C. Sorbent (Sampling) Tube			
Sorbent material:	Carbopack C + Carbopack B + Carbopack X (each 70 mg)		
Desorption flow:	100 mL min^{-1}		
Desorption time:	5 min	Desorption temp.:	320 ℃

Table S4. Comparison of calibration results obtained by VFA standard between direct injection (DL) and vial vaporization (VL) approaches (Exp 1)

Sample code	Compounds									
	PPA	IBA	BTA	IVA	VLA	HXA	HPA	i-BuAl	Mean	SD
A. DL approach										
a. Slope value (ng^{-1})										
Zero offset (RF)	14,337	72,715	42,574	47,952	49,528	43,880	54,335	108,844	54,271	27,308
Non-zero offset	14,254	72,708	42,414	47,679	49,524	43,834	54,336	108,776	54,191	27,327
PD value[a]	0.58	0.01	0.37	0.57	0.01	0.10	0.001	0.06	0.21	0.25
b. Coefficient of determination (R^2)										
Zero offset	0.99982	0.99966	0.99984	0.99983	0.99979	0.99988	0.99975	0.99947	0.9998	0.0001
Non-zero offset	0.99989	0.99983	0.99994	0.99995	0.99989	0.99994	0.99988	0.99974	0.9999	0.0001
PD value[a]	0.007	0.017	0.010	0.012	0.010	0.006	0.013	0.027	0.013	0.007
c. Intercept (unitless)										
Non-zero offset	4,156	321	7,663	12,681	186	2,132	900	2,982	3,878	4,324
d. Relative standard error[b] (RSE, %)										
	0.23	0.42	0.14	0.37	0.09	0.12	0.96	0.83	0.39	0.33
B. VL approach										
a. Slope value (ng^{-1})										
Zero offset (RF)	4,578	17,681	7,448	5,702	3,803	1,930	1,064	54,237	12,055	17,808
Non-zero offset	5,039	18,377	7,748	5,795	3,903	2,013	1,141	54,691	12,339	17,931
PD value[a]	10.1	3.94	4.04	1.64	2.61	4.28	7.29	0.84	4.34	3.03
b. Coefficient of determination (R^2)										
Zero offset	0.9828	0.9963	0.9961	0.9964	0.9983	0.9929	0.9855	0.9987	0.9934	0.0060
Non-zero offset	0.9957	0.9975	0.9985	0.9968	0.9993	0.9955	0.9927	0.9988	0.9968	0.0022
PD value[a]	1.302	0.123	0.234	0.040	0.101	0.261	0.718	0.010	0.349	0.445
c. Intercept (unitless)										
Non-zero offset	-3,102,148	-3,402,915	-1,957,217	-587,327	-633,035	-520,029	-484,081	-91,674	-1,347,303	1,296,749
d. Relative standard error[b] (RSE, %)										
	0.80	2.50	3.09	1.41	1.89	3.49	4.94	0.28	2.30	1.53

[a] Percent difference (PD: %) = ABS{slope (forced-zero) — slope (non forced-zero)} / slope (forced-zero) x 100

[b] Triplicate analyses of the 3rd (VL) or the 4th (DL) calibration point

Table S5. Evaluation of the VFA losses in VL sample relative to DL-based calibration (Exp 1)

Calibration point	Compounds							
	PPA	IBA	BTA	IVA	VLA	HXA	HPA	i-BuAl
A. Mass (ng) of VFA injected into 25 mL vial for VL calibration analysis (Exp-1-VL)								
1st	696	682	674	651	660	652	646	20.9
2nd	1,393	1,364	1,348	1,301	1,320	1,304	1,292	41.7
3rd	2,089	2,046	2,022	1,952	1,979	1,956	1,937	62.6
4th	3,482	3,411	3,369	3,253	3,299	3,260	3,229	104
5th	5,223	5,116	5,054	4,880	4,949	4,891	4,844	157
6th	6,964	6,821	6,739	6,507	6,598	6,521	6,458	209
7th	10,446	10,232	10,108	9,760	9,897	9,781	9,687	313
B. Mass (ng) of VFA detected from VL sample (quantified against the calibration results[a] of DL method)								
1st	33.3	105	63.8	52.5	34.7	15.1	7.15	10.5
2nd	306	274	185	127	87.1	51.2	20.4	21.1
3rd	576	501	331	214	142	71.8	27.8	30.6
4th	931	818	586	409	258	128	65.3	47.9
5th	1,489	1,219	855	607	375	228	81.7	77.9
6th	2,338	1,758	1,222	784	516	298	128	106
7th	3,458	2,496	1,770	1,142	760	426	198	156
C. Computation of 'Loss-O' by the negative offset value[b] (between injected and detected mass)								
mass (ng)	216	46.8	46.0	12.2	12.8	11.9	8.91	0.84
D. Computation of 'Loss-S' by the relative difference in slope values[c] (between VL and DL approaches)								
a. percent (%)	64.9	74.7	81.8	87.9	92.1	95.4	97.9	49.8
b. mass (ng)								
1st	452	510	551	572	608	622	632	10.4
2nd	903	1,019	1,102	1,144	1,216	1,244	1,264	20.8
3rd	1,355	1,529	1,654	1,716	1,823	1,866	1,897	31.1
4th	2,258	2,549	2,756	2,860	3,039	3,111	3,161	51.9
5th	3,387	3,823	4,134	4,290	4,559	4,666	4,742	77.9
6th	4,516	5,097	5,512	5,720	6,078	6,222	6,322	104
7th	6,774	7,646	8,268	8,580	9,117	9,332	9,484	156

Table S5. (Continued)

E. Total loss of VFA calculated by the correlation (linear) equation [d] (between injected and detected mass)

1st	668	557	597	584	621	634	641	11.2
2nd	1,120	1,066	1,148	1,156	1,228	1,256	1,273	21.6
3rd	1,571	1,576	1,700	1,728	1,836	1,878	1,906	32.0
4th	2,475	2,595	2,802	2,872	3,052	3,123	3,170	52.8
5th	3,604	3,870	4,180	4,302	4,571	4,678	4,751	78.7
6th	4,733	5,144	5,558	5,733	6,091	6,233	6,331	105
7th	6,991	7,693	8,314	8,593	9,130	9,344	9,493	157

F. Ancillary exp (Exp 2): relative loss (RL, %) of VOC: simulation of VL by gaseous working standard of four reference compounds[e]

Compounds	MEK	MIBK	BuAc	i-BuAl
Exp 2-VG	36.6	50.3	34.1	48.9
Exp 2-SG	0.14	0.13	5.26	4.30

[a] Detected mass (ng) = peak area (VL, PA_{VL}) / response factor (DL, RF_{dl}: ng^{-1})

[b] Loss-O (ng) = negative of y-intercept value in linear plot of detected mass in vial HS vs. loaded mass in vial

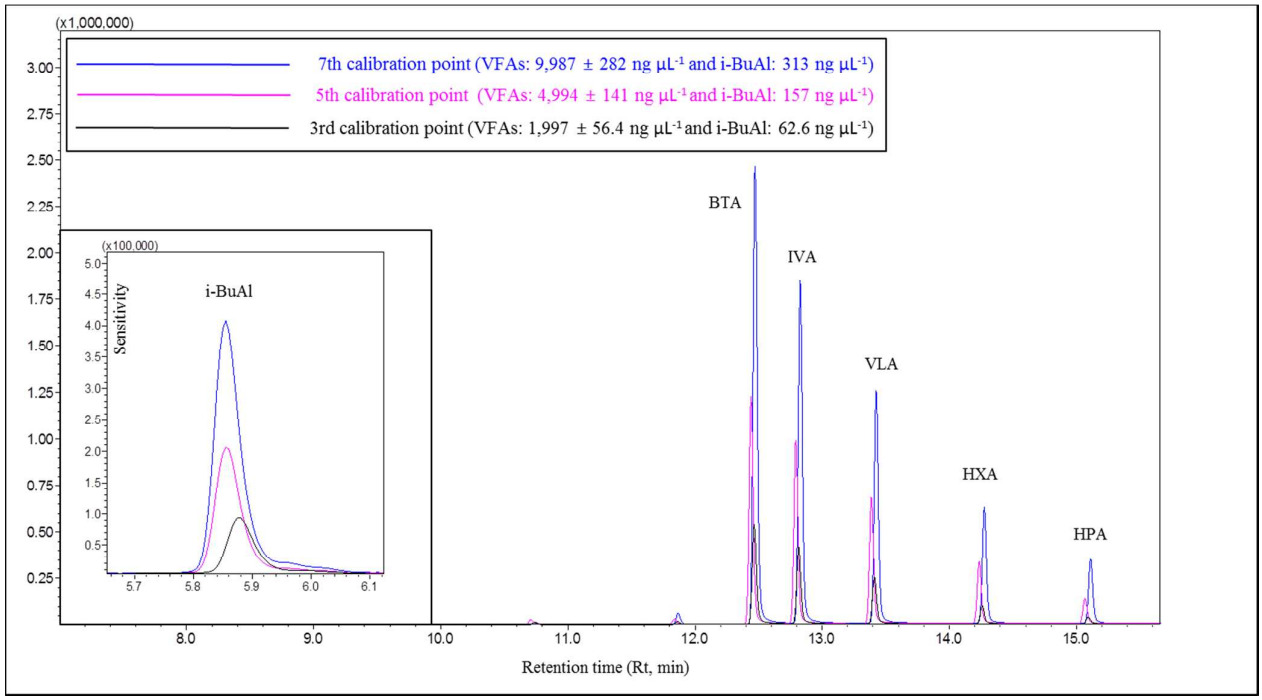
Loss-O (ng) = — I_{VL}/RF_{dl} (Refer to Table 2 in main manuscript)

[c] Loss-S (%) = (1 – slope of linear plot of mass detected in vial vs. mass loaded (m_l) in vial) ×100

Loss-S (%) = 1/(1 + p) (Refer to Table 2 in main manuscript)

[d] Total loss (ng) = (Total loaded mass (ng) into 25 mL vial x Loss-S (%)) / 100 + Loss-O (ng)

[e] Relative loss (RL: %) = (peak are (DG) — peak area (VG or SG)) / peak area (DG) x 100



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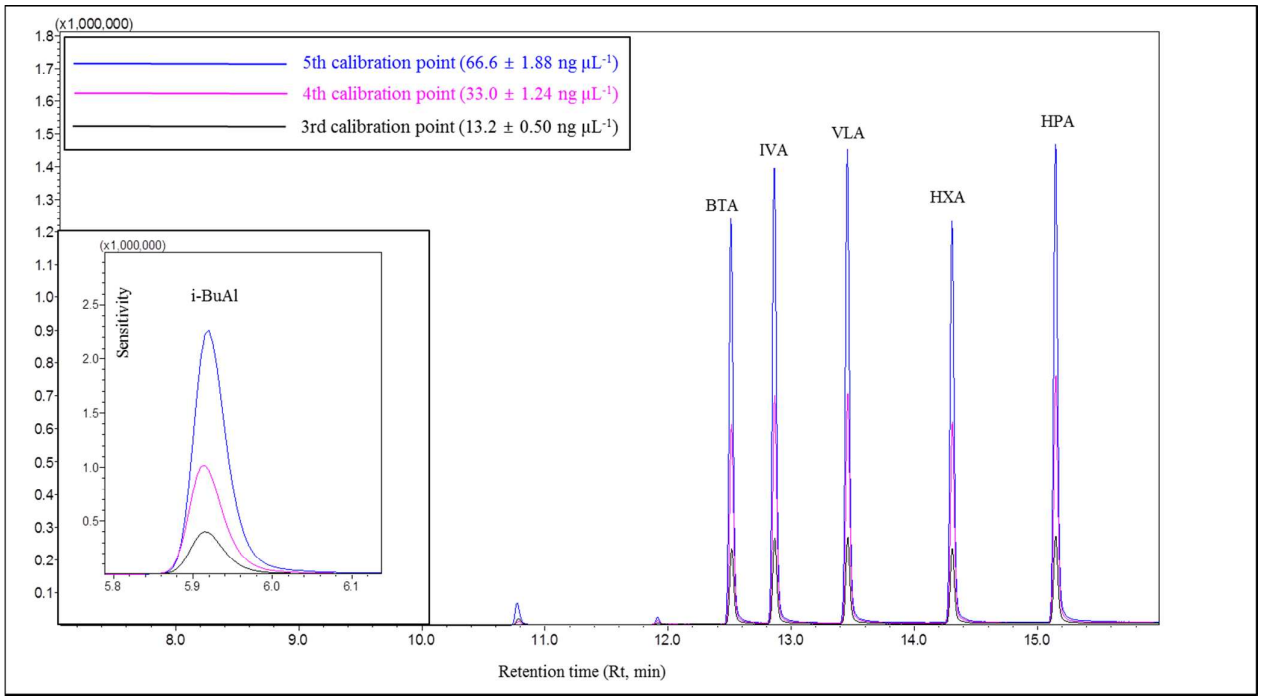
127 A. VL approach

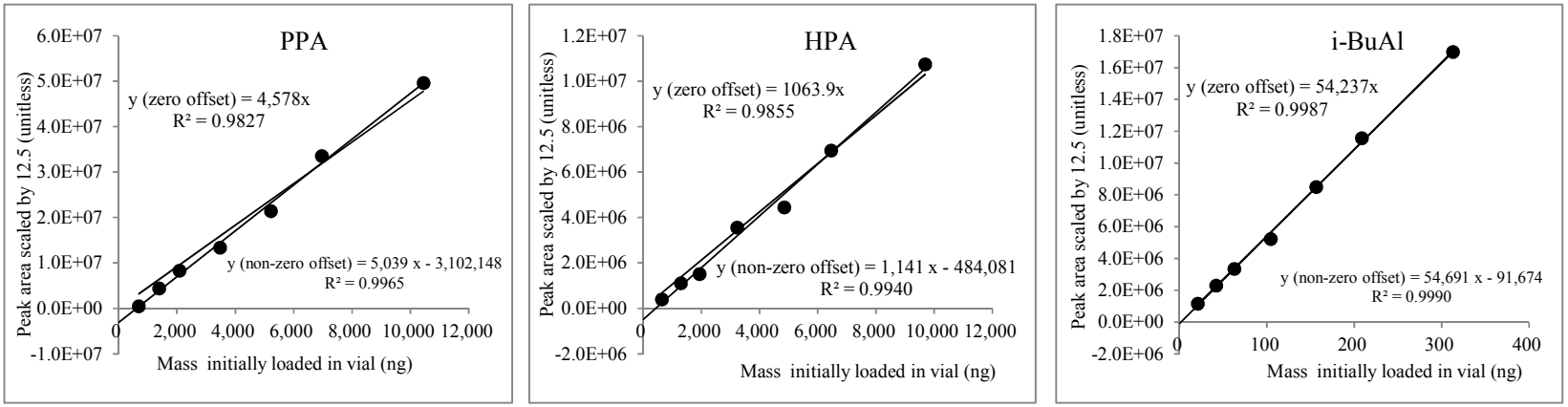
128



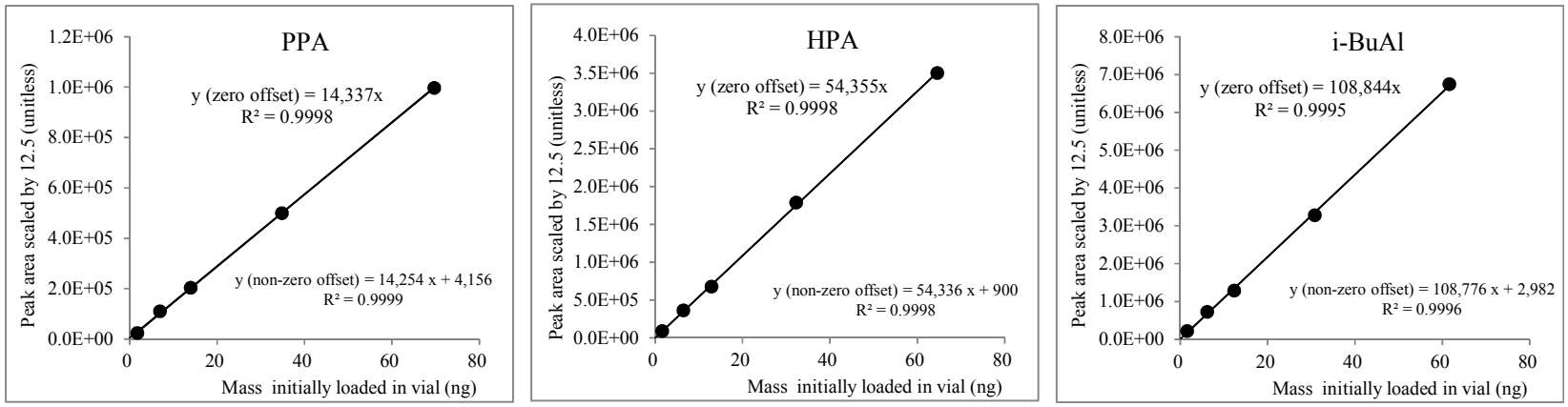
129 B. DL approach

130

131 **Fig. S1. Comparison of chromatograms of VFA standards between VL and DL approaches (Exp 1)**

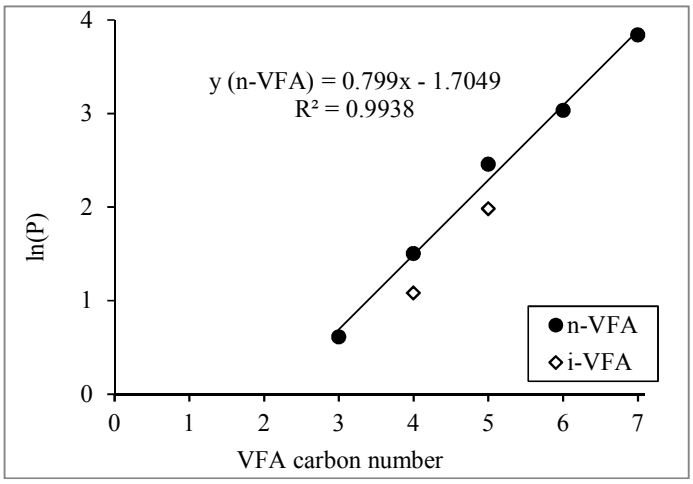


A. VL analysis

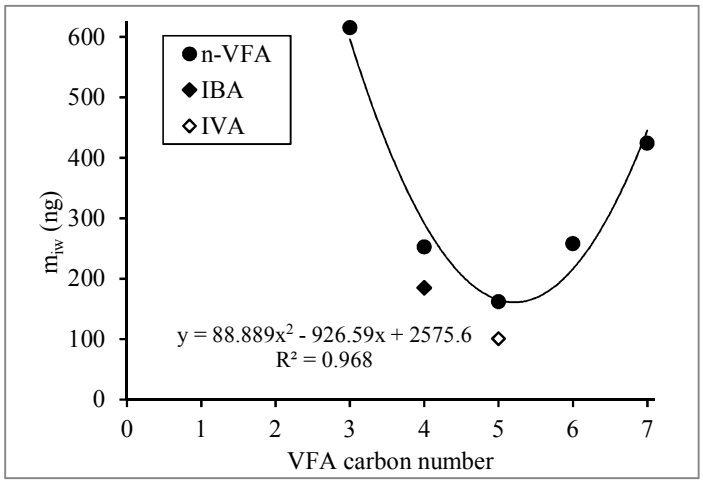


B. DL approach

Fig. S2. Comparison of VFA calibration curves with and without forced zero offset (Exp 1: DL vs VL approaches)



A. Plot of ln(p) vs. volatile fatty acid carbon number



B. Plot of volatile fatty acid mass (m_{iw}) irreversibly lost to walls to vial walls vs. volatile fatty acid carbon number: DL and VL approached (Exp 1)

Fig. S3. Plots of: A) ln(p) as a function of volatile fatty acid carbon number and B) volatile fatty acid mass (m_{iw}) irreversibly lost to vial walls

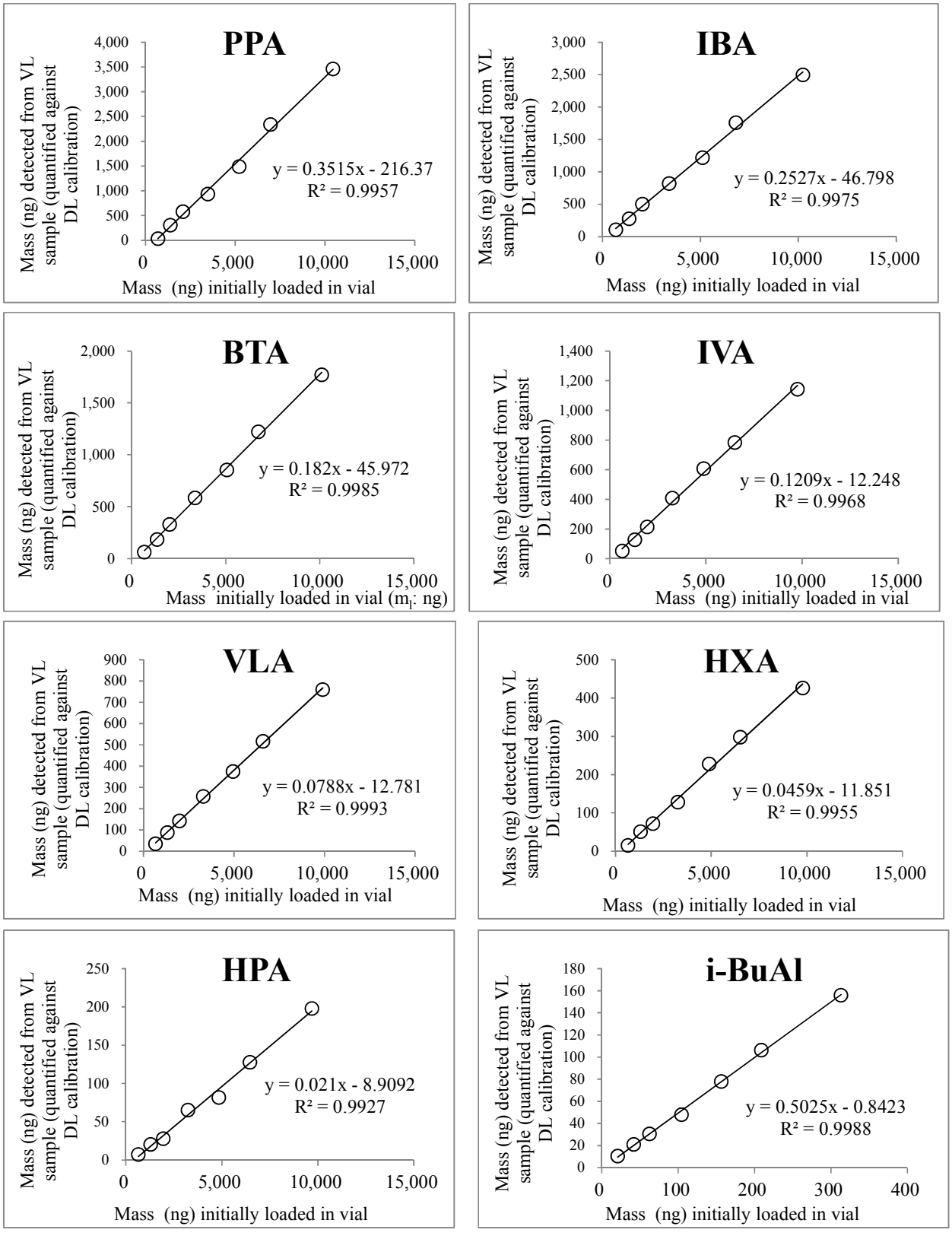


Fig. S4. Relationship between mass (ng) of VFA loaded into 25 mL vial and their mass (ng) (in 25 mL VL) computed by the RF value of DL approach (Exp 1)

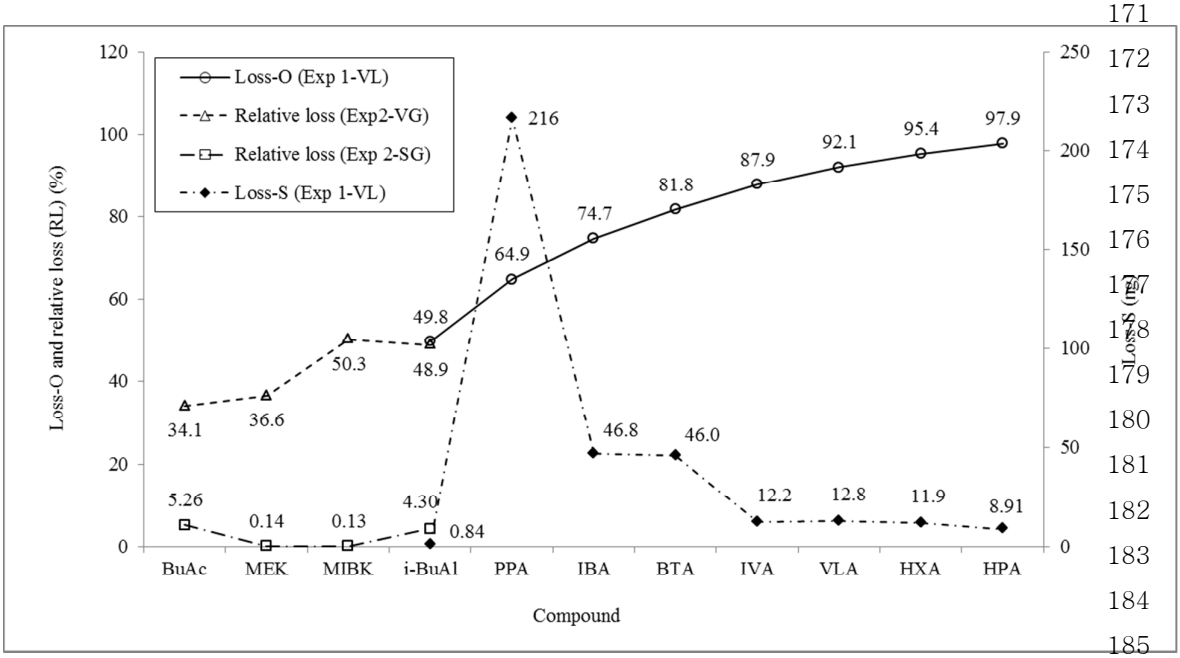


Fig. S5. Comparison of all types of (relative) loss terms of VFAs and VOCs (Exp 1 and Exp 2)