Supporting Information

Application of screening experimental designs to assess chromatographic isotope effect upon isotope-coded derivatization for quantitative liquid chromatography–mass spectrometry

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Table S-1. Factors and levels chosen for the asymmetrical and Plackett-Burman experimental designs

no.	factor	Levels			
		1	2	3	4
1	stable isotope labeling[a]	d_3	$^{15}N_2$	$^{15}N_4$	$^{13}C_6$
2	column temperature (°C)	25	50	40	
3	gradient time (min)	2	8	4	
4	stationary phase chemistry	PhenHex[b]	C18[c]		
5	aqueous mobile phase pH	3.3	8.2		
6	organic solvent	MeOH	ACN		
7	analyte concentration (μg/mL)[d]	0.1	1.0		

[a]Factor examined only in the asymmetrical design. [b]Phenyl-hexylsilica. [c]Octadecylsilica. [d]Factor examined only in the Plackett-Burman design.

Table S-2. Asymmetrical experimental design constructed from Addelman's basic plan[25]

experiment	isotope labeling	column temperature (°C)	gradient time (min)	stationary phase[a]	pH	organic solvent	dummy 1	dummy 2	dummy 3
1	d_3	25	8	PhenHex	3.3	MeOH	–1	–1	–1
2	$^{15}N_4$	50	4	PhenHex	3.3	MeOH	–1	1	1
3	$^{15}N_2$	40	4	PhenHex	3.3	MeOH	1	–1	1
4	$^{13}C_6$	40	2	PhenHex	3.3	MeOH	1	1	–1
5	$^{15}N_4$	40	4	PhenHex	8.2	ACN	–1	–1	–1
6	d_3	40	2	PhenHex	8.2	ACN	–1	1	1
7	$^{13}C_6$	50	8	PhenHex	8.2	ACN	1	–1	1
8	$^{15}N_2$	25	4	PhenHex	8.2	ACN	1	1	–1
9	$^{15}N_2$	50	2	C18	3.3	ACN	–1	–1	–1
10	$^{13}C_6$	25	4	C18	3.3	ACN	–1	1	1
11	d_3	40	4	C18	3.3	ACN	1	–1	1
12	$^{15}N_4$	40	8	C18	3.3	ACN	1	1	–1
13	$^{13}C_6$	40	4	C18	8.2	MeOH	–1	–1	–1
14	$^{15}N_2$	40	8	C18	8.2	MeOH	–1	1	1
15	$^{15}N_4$	25	2	C18	8.2	MeOH	1	–1	1
16	d_3	50	4	C18	8.2	MeOH	1	1	–1

[a]PhenHex denotes phenylhexylsilica, C18 denotes octadecylsilica

Table S-3. Plackett-Burman experimental design constructed according to Vander Heyden et al.[26]

experiment	analyte conc. (μg/mL)	dummy 1	organic solvent	temperature (°C)	dummy 2	dummy 3	dummy 4	pH	stationary phase[a]	gradient time (min)	dummy 5
1	0.1	1	MeOH	50	1	1	-1	3.3	PhenHex	2	–1
2	1	1	ACN	25	1	1	1	3.3	PhenHex	8	1
3	0.1	–1	ACN	50	–1	1	1	8.2	PhenHex	8	–1
4	1	1	MeOH	50	1	–1	1	8.2	C18	8	–1
5	1	–1	ACN	25	1	1	–1	8.2	C18	2	–1
6	1	–1	MeOH	50	–1	1	1	3.3	C18	2	1
7	0.1	–1	MeOH	25	1	–1	1	8.2	PhenHex	2	1
8	0.1	1	MeOH	25	–1	1	–1	8.2	C18	8	1
9	0.1	1	ACN	25	–1	–1	1	3.3	C18	2	–1
10	1	1	ACN	50	–1	–1	–1	8.2	PhenHex	2	1
11	0.1	–1	ACN	50	1	–1	–1	3.3	C18	8	1
12	1	–1	MeOH	25	–1	–1	–1	3.3	PhenHex	8	–1

[a]PhenHex denotes phenylhexylsilica, C18 denotes octadecylsilica column.

Table S-4. SRM table

Analyte	SRM transition	CE (V)
ACR-DNPH	235 → 158	16
ACR-$^{13}C_6$-DNPH	241 → 164	16
ACR-d_3-DNPH	238 → 161	16
ACR-$^{15}N_2$-DNPH	237 → 158	16
ACR-$^{15}N_4$-DNPH	239 → 160	16
HNE-DNPH	335 → 182	23
HNE-$^{13}C_6$-DNPH	341 → 188	23
HNE-d_3-DNPH	338 → 185	23
HNE-$^{15}N_2$-DNPH	337 → 184	23
HNE-$^{15}N_4$-DNPH	339 → 185	23
MDA-DNPH	235 → 189	18
MDA-$^{13}C_6$-DNPH	241 → 195	18
MDA-d_3-DNPH	238 → 192	18
MDA-$^{15}N_2$-DNPH	237 → 190	18
MDA-$^{15}N_4$-DNPH	239 → 192	18
ONE-DNPH	333 → 182	23
ONE-$^{13}C_6$-DNPH	339 → 188	23
ONE-d_3-DNPH	336 → 185	23
ONE-$^{15}N_2$-DNPH	335 → 184	23
ONE-$^{15}N_4$-DNPH	337 → 185	23

Table S-5. Absolut effects of various factors on the retention time difference between light and $^{15}N_4$-labeled heavy pair of DNPH-derivatives obtained from the Plackett-Burman design. Critical effects were estimated according to Vander Heyden et al.[26]

factor	MDA	ACR	HNE	ONE
temperature	0.023	0.101	0.024	0.000
gradient time	0.006	0.035	0.027	0.065[a]
stationary phase	0.096	0.034	0.024	0.065[a]
pH	0.062	0.034	0.016	0.000
organic solvent	0.345	0.034	0.044	0.000
analyte concentration	0.272	0.036	0.065	0.000
dummies				
dummy1	0.162	0.030	0.000	0.065
dummy2	0.231	0.096	0.000	0.065
dummy3	0.006	0.100	0.066	0.000
dummy4	0.027	0.030	0.070	0.000
dummy5	0.137	0.034	0.069	0.065
critical effects				
$E_{critical}$	0.362	0.171	0.136	0.129
ME	0.367	0.130	0.099	0.001
SME	0.590	0.209	0.159	0.001

[a]Effect is not considered significant because dummy factors (dummy1, 2, and 5) demonstrated the same effect value.

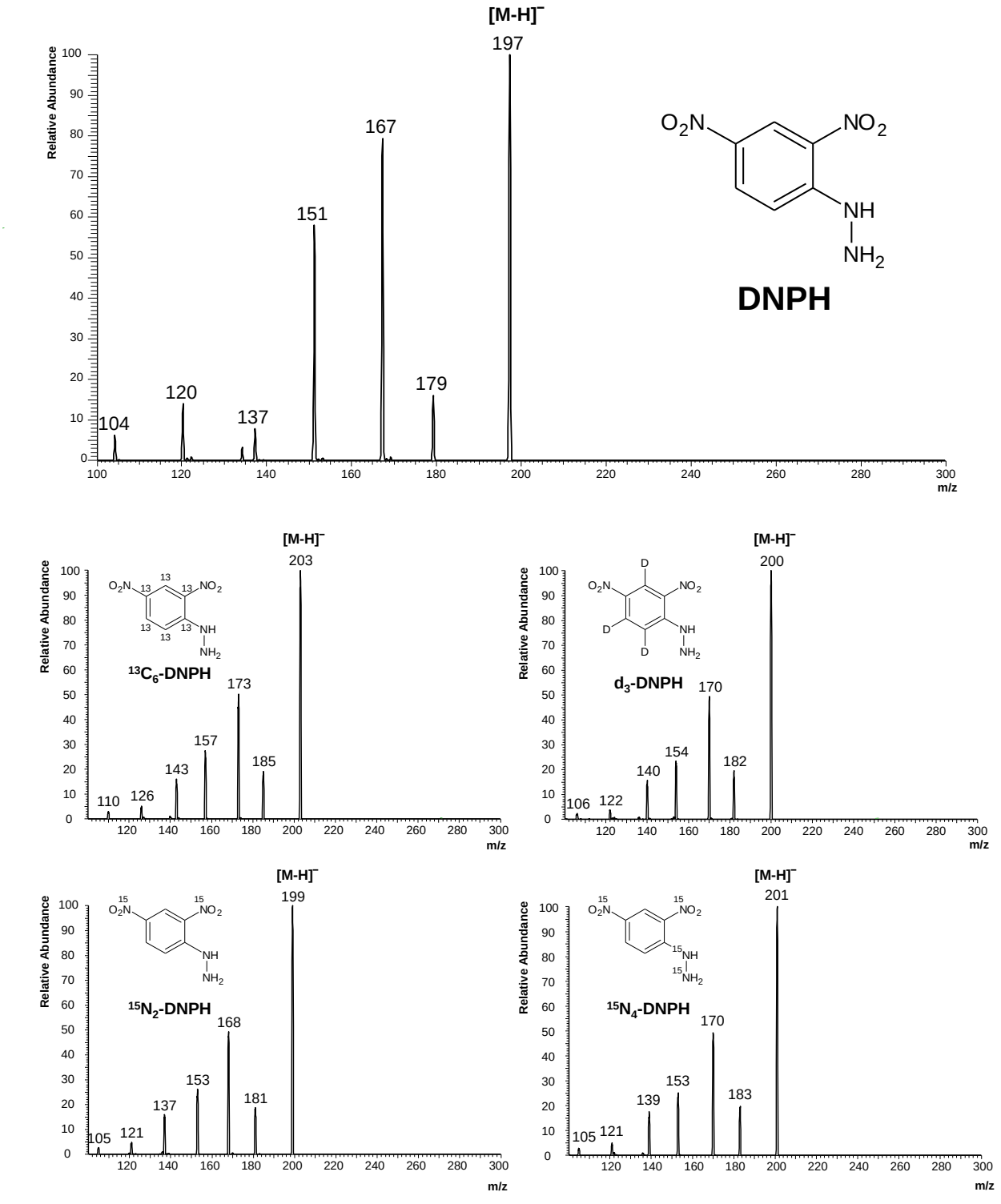

Figure S-1. Negative ion mode MS^2 product ion scan spectra and chemical structures of the DNPHs labeled with various stable isotopes on different atoms.

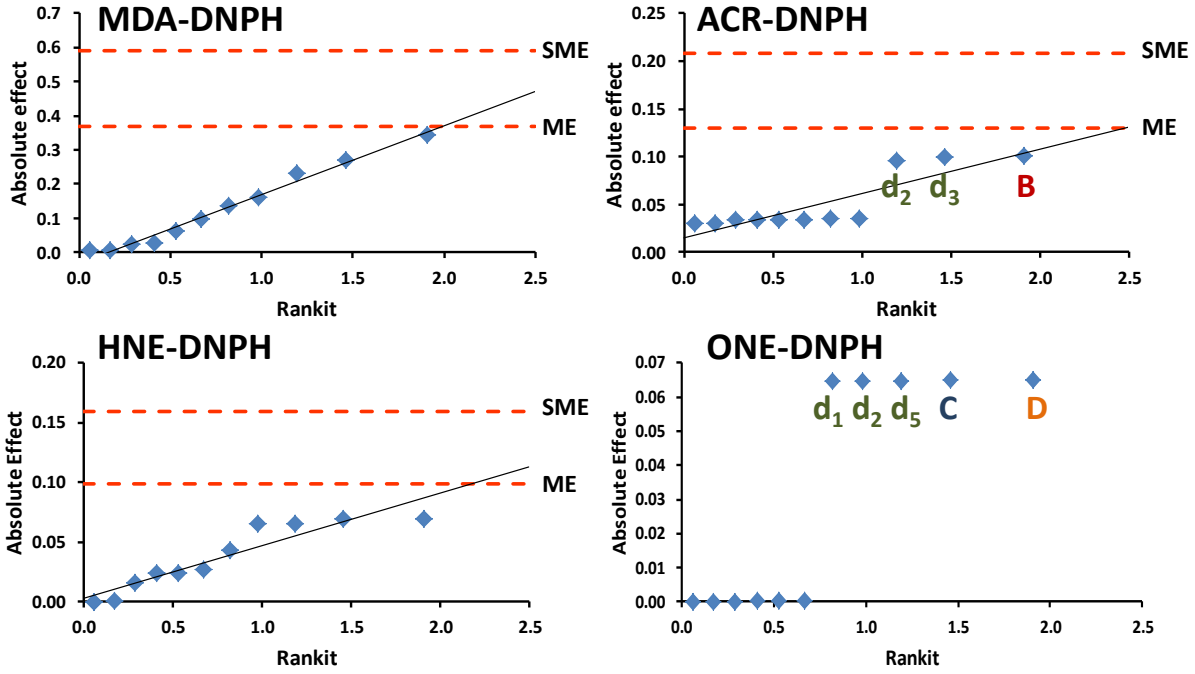

Figure S-2. Half-normal probability plots of the absolute effects on the retention time difference measured between light and heavy $^{15}N_4$-labeled aldehyde hydrazones in the Plackett-Burman design with identification of margin of error (ME) and simultaneous margin of error (SME) as critical effects. B, C, D, and d_x denote column temperature, gradient time, stationary phase chemistry, and dummy factors, respectively.

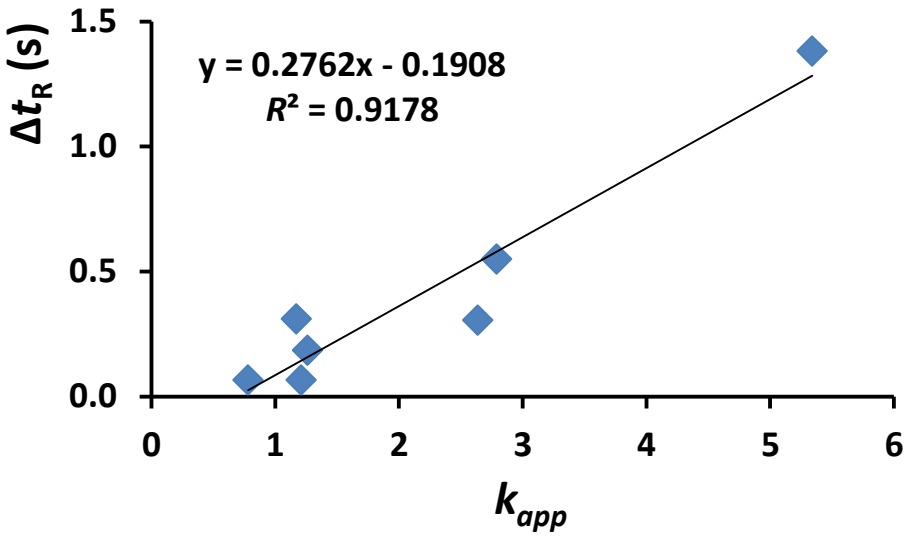

Figure S-3. Retention time differences for MDA-DNPH unlabeled and d_3-labeled isotopologue pair plotted against apparent retention factor (k_{app}) obtained by Plackett-Burman design experiments. The legend shows the linear regression equation and correlation coefficient (R^2). Note that MDA-DNPH was not detected in five out of 12 experiments, therefore seven data points are shown.

			Exp.	$Rs_{critical}$	Analysis time (min)	Normalized signal response			
						MDA-DNPH	ACR-DNPH	HNE-DNPH	ONE-DNPH
Phenyl-hexyl column	MeOH	pH 3.3	1	1.7	6.2	66	100	100	100
			2	1.1	3.8	77	91	88	86
			3	1.1	4.1	96	96	94	97
			4	0.8	3.0	100	91	81	82
	ACN	pH 8.2	5	4.2	2.1	0	41	30	38
			6	3.5	2.0	0	46	35	38
			7	4.4	2.2	0	36	30	39
			8	4.6	2.4	0	42	32	43
C18 column	ACN	pH 3.3	9	3.2	1.9	5	70	51	66
			10	4.4	2.5	6	73	59	73
			11	3.9	2.4	8	74	50	74
			12	4.6	2.7	8	72	56	75
	MeOH	pH 8.2	13	0.3	3.6	1	53	33	48
			14	0.3	5.0	4	50	35	57
			15	0.2	2.9	2	58	30	41
			16	0.3	3.4	2	54	32	48

Figure S-4. Heat map created from the dataset obtained by the asymmetric experimental design showing important parameters of the LC–MS/MS assay. $Rs_{critical}$ denotes the resolution between the worst-resolved pair of peaks, HNE-DNPH and ONE-DNPH in the present assay; analysis time denotes the retention time of the last-eluting analyte of interest, ONE-DNPH in the present assay; gradient color scale ranging from red to green is assigned to unacceptable/suboptimal and acceptable/optimal values, respectively.

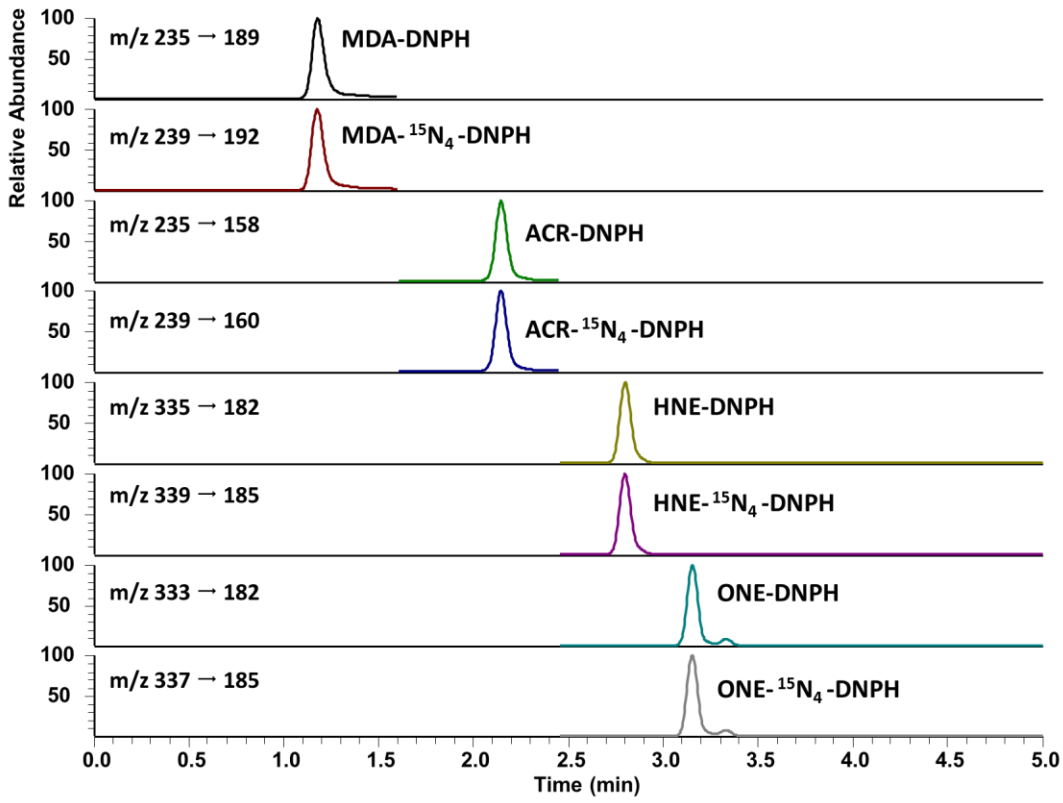

Figure S-5. Representative SRM traces of the selected lipid peroxidation-derived reactive aldehyde derivatives (MDA-DNPH, ACR-DNPH, HNE-DNPH, and ONE-DNPH, at a concentration of 1 μg/mL each) and their corresponding $^{15}N_4$-labeled isotopologues obtained by the optimized LC–MS/MS assay. Separation was achieved using a Phenomenex Kinetex phenyl-hexyl column (50 × 2.1 mm i.d., 5 μm particles) with a mobile phase of (A_1) 0.1% acetic acid in water and (B_1) acetonitrile. Gradient profile was 40% B to 100% B in 4 min with a flow rate set at 0.4 mL/min, and the column oven temperature was maintained at 30 °C.

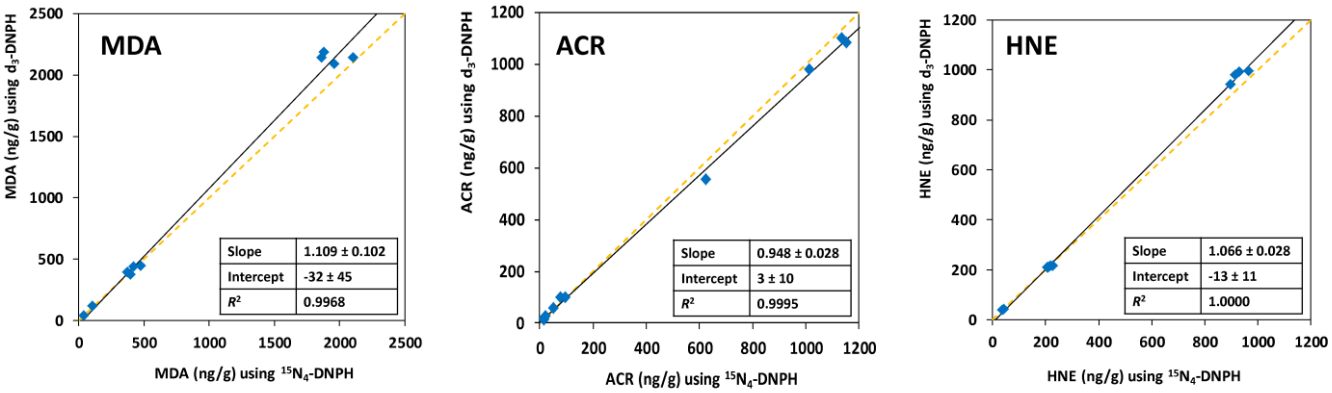

Figure S-6. Scatter plots of quantities of selected aldehydes measured in fortified mouse tissue extracts by AIDA using d_3- or $^{15}N_4$-labeling. Solid line represents the Deming regression line with regression statistics included in the inset table (slope and intercept data are expressed as mean ± 95% confidence limit); the diagonal dashed line is the line of equality indicating the perfect agreement between the methods.