

Supporting Information: Mitigating Matrix Effects in LC-ESI-MS/MS Analysis of a Urinary Biomarker of Xylenes Exposure

Brett A. Bowman, Elizabeth Ejzak, Christopher M. Reese, Benjamin C. Blount, and Deepak Bhandari*

*Tobacco and Volatiles Branch
Division of Laboratory Sciences
National Center for Environmental Health
Centers for Disease Control and Prevention
Atlanta, GA 30341*

***Corresponding Author:**

Deepak Bhandari, PhD
Centers for Disease Control and Prevention
Division of Laboratory Sciences, Tobacco and Volatiles Branch
Email: dbhandari@cdc.gov
Phone: 770-488-0939
Fax: 770-488-0181

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Supplementary Information

Supplementary Table I. The individual data points for the analytical method comparison. Data points with asterisks () were excluded because results were <LOD or >ULOQ.*

Urine Sample	Analytical Result			
	2MHA-[² H ₇] (ng/mL)	2MHA-[¹³ C ₆] (ng/mL)	4MHA-[² H ₇] (ng/mL)	4MHA-[¹³ C ₂ - ¹⁵ N] (ng/mL)
Smoker-1	21.3	41.3	225	226
Smoker-2	114	198	1110	1070
Smoker-3	24.7	32.7	185	181
Smoker-4	841	1990	8140*	9430*
Smoker-5	113	257	1300	1380
Smoker-6	72.5	110	618	617
Smoker-7	69.2	161	873	896
Smoker-8	618	1190	5680*	6230*
Smoker-9	52.6	83.6	423	430
Smoker-10	67.0	148	1040	1010
Smoker-11	7.31	16.0	153	157
Smoker-12	195	405	1290	1360
Smoker-13	17.4	20.4	182	178
Smoker-14	50.5	79.5	410	402
Smoker-15	28.2	52.2	323	326
Smoker-16	171	389	2350	2390
Smoker-17	82.7	163	938	981
Smoker-18	73.6	133	756	763
Smoker-19	107	159	637	583
Smoker-20	3.63*	5.61*	36.1	33.6
Smoker-21	34.6	65.5	368	362
Smoker-22	117	143	742	735
Smoker-23	76.0	177	965	964
Smoker-24	34.7	57.0	342	325
Smoker-25	83.3	243	653	662
Non-smoker-1	17.3	31.9	76.1	72.0
Non-smoker-2	7.46	9.70	4.92*	3.14*
Non-smoker-3	13.2	28.2	95.1	90.9
Non-smoker-4	4.60*	7.95*	34.0	32.9
Non-smoker-5	54.0	100	205	216
Non-smoker-6	<0*	0.343*	3.11*	1.35*
Non-smoker-7	39.3	69.8	3.70*	1.86*
Non-smoker-8	<0*	<0*	<0*	<0*
Non-smoker-9	70.6	163	90.3	87.1
Non-smoker-10	27.2	62.3	174	186
Non-smoker-11	11.3	18.5	<0*	<0*
Non-smoker-12	7.43	12.4	47.8	45.4

Supporting Information: Mitigating Matrix Effects in LC-MS/MS – Xylenes Metabolites

Supplementary Table II. The individual spike accuracy data for 2MHA internal standard comparison collected for 3 spike levels across 14 unknowns.

Sample ID	Internal Standard	Spike 1			Spike 2			Spike 3		
		Spiked Concentration (ng/ml)	Resulting Concentration (ng/mL)	Spike Error (%)	Spiked Concentration (ng/ml)	Resulting Concentration (ng/mL)	Spike Error (%)	Spiked Concentration (ng/ml)	Resulting Concentration (ng/mL)	Spike Error (%)
Non-smoker 1	2MHA- ² H ₇	47.0	26.1	-44.5	312	182	-41.7	2740	1680	-38.7
	2MHA- ¹³ C ₆		43.8	-6.8		290	-7.1		2680	-2.2
Non-smoker 2	2MHA- ² H ₇	47.0	41.1	-12.6	312	250	-19.9	2740	2230	-18.6
	2MHA- ¹³ C ₆		47.5	1.1		297	-4.8		2670	-2.6
Non-smoker 3	2MHA- ² H ₇	47.0	23	-51.1	312	128	-59.0	2740	1280	-53.3
	2MHA- ¹³ C ₆		49.7	5.7		279	-10.6		2720	-0.7
Non-smoker 4	2MHA- ² H ₇	47.0	30.6	-34.9	312	220	-29.5	2740	2050	-25.2
	2MHA- ¹³ C ₆		43.5	-7.4		296	-5.1		2760	0.7
Non-smoker 5	2MHA- ² H ₇	47.0	24.9	-47.0	312	161	-48.4	2740	1540	-43.8
	2MHA- ¹³ C ₆		46	-2.1		290	-7.1		2600	-5.1
Non-smoker 6	2MHA- ² H ₇	47.0	29.8	-36.6	312	176	-43.6	2740	1620	-40.9
	2MHA- ¹³ C ₆		52.6	11.9		284	-9.0		2650	-3.3
Non-smoker 7	2MHA- ² H ₇	47.0	33.0	-29.8	312	211	-32.4	2740	1930	-29.6
	2MHA- ¹³ C ₆		44.1	-6.2		286	-8.3		2760	0.7
Non-smoker 8	2MHA- ² H ₇	47.0	28.5	-39.4	312	183	-41.3	2740	1710	-37.6
	2MHA- ¹³ C ₆		42.1	-10.4		288	-7.7		2700	-1.5
Smoker 1	2MHA- ² H ₇	47.0	26.6	-43.4	312	172	-44.9	2740	1520	-44.5
	2MHA- ¹³ C ₆		47.1	0.2		291	-6.7		2670	-2.6
Smoker 2	2MHA- ² H ₇	47.0	39.3	-16.4	312	247	-20.8	2740	2320	-15.3
	2MHA- ¹³ C ₆		45.5	-3.2		288	-7.7		2780	1.5
Smoker 3	2MHA- ² H ₇	47.0	30.9	-34.3	312	182	-41.7	2740	1810	-33.9
	2MHA- ¹³ C ₆		45.2	-3.8		286	-8.3		2780	1.5
Smoker 4	2MHA- ² H ₇	47.0	27.5	-41.5	312	154	-50.6	2740	1540	-43.8
	2MHA- ¹³ C ₆		48.7	3.6		279	-10.6		2670	-2.6
Smoker 5	2MHA- ² H ₇	47.0	25.3	-46.2	312	156	-50.0	2740	1460	-46.7
	2MHA- ¹³ C ₆		43.1	-8.3		285	-8.7		2720	-0.7
Smoker 6	2MHA- ² H ₇	47.0	24.9	-47.0	312	156	-50.0	2740	1530	-44.2
	2MHA- ¹³ C ₆		50.7	7.9		288	-7.7		2640	-3.6

Supporting Information: Mitigating Matrix Effects in LC-MS/MS – Xylenes Metabolites

Supplementary Table III. The individual spike accuracy data for 4MHA internal standard comparison collected for 3 spike levels across 14 unknowns.

Sample ID	Internal Standard	Spike 1			Spike 2			Spike 3		
		Spiked Concentration (ng/ml)	Resulting Concentration (ng/mL)	Spike Error (%)	Spiked Concentration (ng/ml)	Resulting Concentration (ng/mL)	Spike Error (%)	Spiked Concentration (ng/ml)	Resulting Concentration (ng/mL)	Spike Error (%)
Non-smoker 1	4MHA- ² H ₇	45.0	43.6	-3.1	295	296	0.3	2760	2690	-2.5
	4MHA-[¹³ C ₂ - ¹⁵ N]		35.6	-20.9		276	-6.4		2680	-2.9
Non-smoker 2	4MHA- ² H ₇	45.0	52.3	16.2	295	297	0.7	2760	2660	-3.6
	4MHA-[¹³ C ₂ - ¹⁵ N]		49.8	10.7		287	-2.7		2660	-3.6
Non-smoker 3	4MHA- ² H ₇	45.0	55.8	24.0	295	290	-1.7	2760	2770	0.4
	4MHA-[¹³ C ₂ - ¹⁵ N]		51.2	13.8		275	-6.8		2730	-1.1
Non-smoker 4	4MHA- ² H ₇	45.0	47.4	5.3	295	296	0.3	2760	2720	-1.4
	4MHA-[¹³ C ₂ - ¹⁵ N]		50.7	12.7		285	-3.4		2750	-0.4
Non-smoker 5	4MHA- ² H ₇	45.0	38.2	-15.1	295	276	-6.4	2760	2630	-4.7
	4MHA-[¹³ C ₂ - ¹⁵ N]		39.4	-12.4		273	-7.5		2730	-1.1
Non-smoker 6	4MHA- ² H ₇	45.0	43.0	-4.4	295	280	-5.1	2760	2690	-2.5
	4MHA-[¹³ C ₂ - ¹⁵ N]		47.0	4.4		278	-5.8		2720	-1.4
Non-smoker 7	4MHA- ² H ₇	45.0	45.0	0	295	279	-5.4	2760	2660	-3.6
	4MHA-[¹³ C ₂ - ¹⁵ N]		45.4	0.9		278	-5.8		2750	-0.4
Non-smoker 8	4MHA- ² H ₇	45.0	46.6	3.6	295	292	-1.0	2760	2730	-1.1
	4MHA-[¹³ C ₂ - ¹⁵ N]		44.3	-1.6		282	-4.4		2700	-2.2
Smoker 1	4MHA- ² H ₇	45.0	46.4	3.1	295	262	-11.2	2760	2740	-0.7
	4MHA-[¹³ C ₂ - ¹⁵ N]		43.9	-2.4		252	-14.6		2760	0.0
Smoker 2	4MHA- ² H ₇	45.0	43.3	-3.8	295	274	-7.1	2760	2730	-1.1
	4MHA-[¹³ C ₂ - ¹⁵ N]		42.5	-5.6		266	-9.8		2760	0.0
Smoker 3	4MHA- ² H ₇	45.0	49.8	10.7	295	284	-3.7	2760	2770	0.4
	4MHA-[¹³ C ₂ - ¹⁵ N]		44.0	-2.2		271	-8.1		2760	0.0
Smoker 4	4MHA- ² H ₇	45.0	57.3	27.3	295	274	-7.1	2760	2610	-5.4
	4MHA-[¹³ C ₂ - ¹⁵ N]		58.7	30.4		267	-9.5		2640	-4.3
Smoker 5	4MHA- ² H ₇	45.0	42.3	-6.0	295	265	-10.2	2760	2730	-1.1
	4MHA-[¹³ C ₂ - ¹⁵ N]		31.0	-31.1		266	-9.8		2800	1.4
Smoker 6	4MHA- ² H ₇	45.0	40.7	-9.6	295	297	0.7	2760	2630	-4.7
	4MHA-[¹³ C ₂ - ¹⁵ N]		54.7	21.6		294	-0.3		2670	-3.3