

Supplemental File:

High-throughput and sensitive analysis of free and total 8-isoprostane in urine with isotope-dilution liquid chromatography-tandem mass spectrometry

Cory Holder^{1*}, Aaron Adams^{1,2}, Ernest McGahee¹, Baoyun Xia¹, Benjamin C. Blount¹, and Lanqing Wang^{1*}

¹Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States

²Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee 37831, USA

*Corresponding Authors:

Cory Holder - *National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States*; <http://orcid.org/0000-0002-1992-5244>

Email: mvo5@cdc.gov

Lanqing Wang - *National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States*;

Email: lfw3@cdc.gov

Contents of Supplemental File

Table S1. Matrix effects	page S2
Table S2. Enzyme Hydrolysis Optimization	page S2
Table S3. Free to Total 8-Isoprostane ratio for smokers and non-smokers	page S2
Figure S1. Representative Smoker's Urine Free 8-Isoprostane Chromatograms	page S3
Figure S2. Representative Smoker's Urine Chromatograms for Free, Total, and Standards	page S3
Figure S3. Free/Total 8-Isoprostane Geometric means adjusted for ng/g Creatinine	page S4
Figure S4. Free/Total 8-Isoprostane Geometric means adjusted for nmol/g Creatinine	page S4

Table S1. Matrix effect on calibration curves prepared in urine, water, or synthetic urine (1/x weighting)

	Urine	Water	Syn. Urine
slope	0.00282	0.00297	0.00295
y-intercept	0.00516	4.13E-09	3.61E-09
R²	0.9971	0.9999	0.998
Difference of Slope (%)	5%	ref	<1%

Table S2 Hydrolysis optimization by amount of enzyme with individual smoker urine.

Sample ID	Enzyme units/ 0.4 mL urine	Analyte Conc. (pg/mL)
Sample 1	667 units	1040
Sample 1	1000 units	1190
Sample 1	1334 units	1010
<u>Sample 1</u>	<u>2000 units</u>	<u>1000</u>
Sample 2	667 units	1310
Sample 2	1000 units	1430
Sample 2	1334 units	1490
<u>Sample 2</u>	<u>2000 units</u>	<u>1400</u>
Sample 3	667 units	151
Sample 3	1000 units	137
Sample 3	1334 units	143
<u>Sample 3</u>	<u>2000 units</u>	<u>146</u>
Sample 4	667 units	473
Sample 4	1000 units	507
Sample 4	1334 units	517
<u>Sample 4</u>	<u>2000 units</u>	<u>528</u>
Sample 5	667 units	446
Sample 5	1000 units	438
Sample 5	1334 units	451
<u>Sample 5</u>	<u>2000 units</u>	<u>383</u>
Sample 6	667 units	303
Sample 6	1000 units	309
Sample 6	1334 units	266
<u>Sample 6</u>	<u>2000 units</u>	<u>279</u>

Table S3 the ratio of Free/Total 8-Isoprostane for smokers and non-users of tobacco products. The ratios were the same before and after creatinine adjustment.

	Creatinine Adjusted	Unadjusted
smoker	0.653	0.653
non-user	0.683	0.683

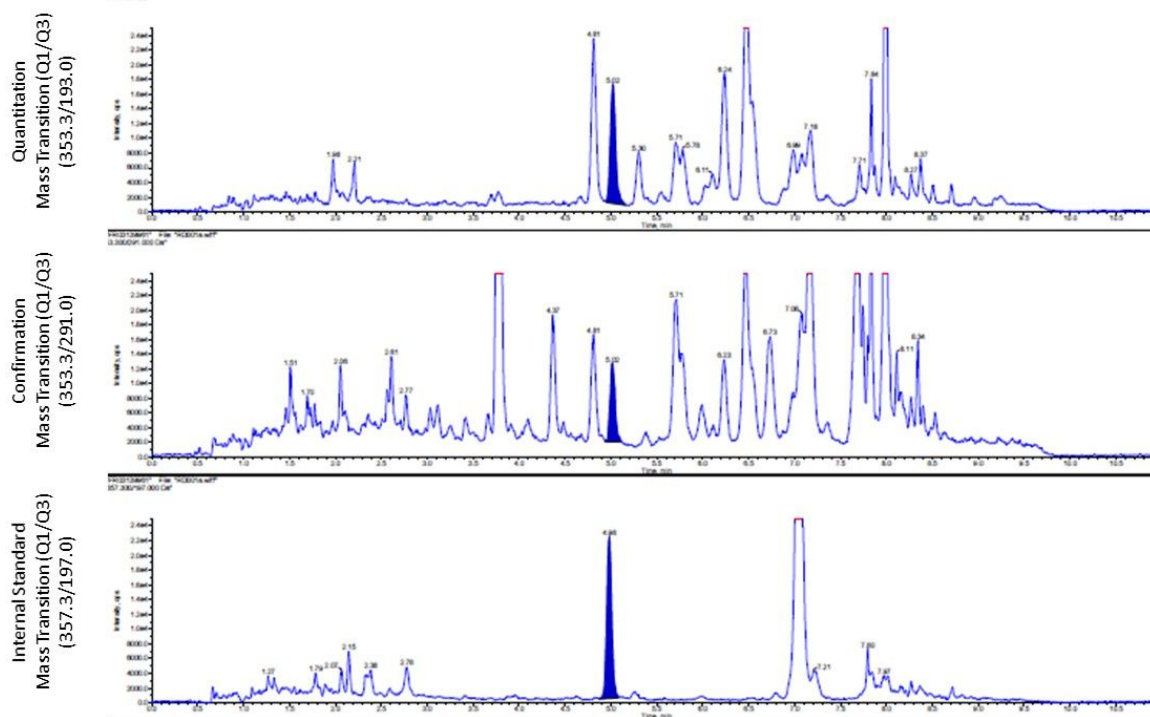


Figure S1 Total 8-Isoprostane integrated quantitation (mass transition: 353.3/193.0 Da), confirmation (mass transition: 353.3/291.0 Da), and internal standard (mass transition: 357.3/197.0 Da) peaks for a smoker’s urine sample (622 pg/ml). Chromatogram shows resolution of peaks over the 11-minute run time.

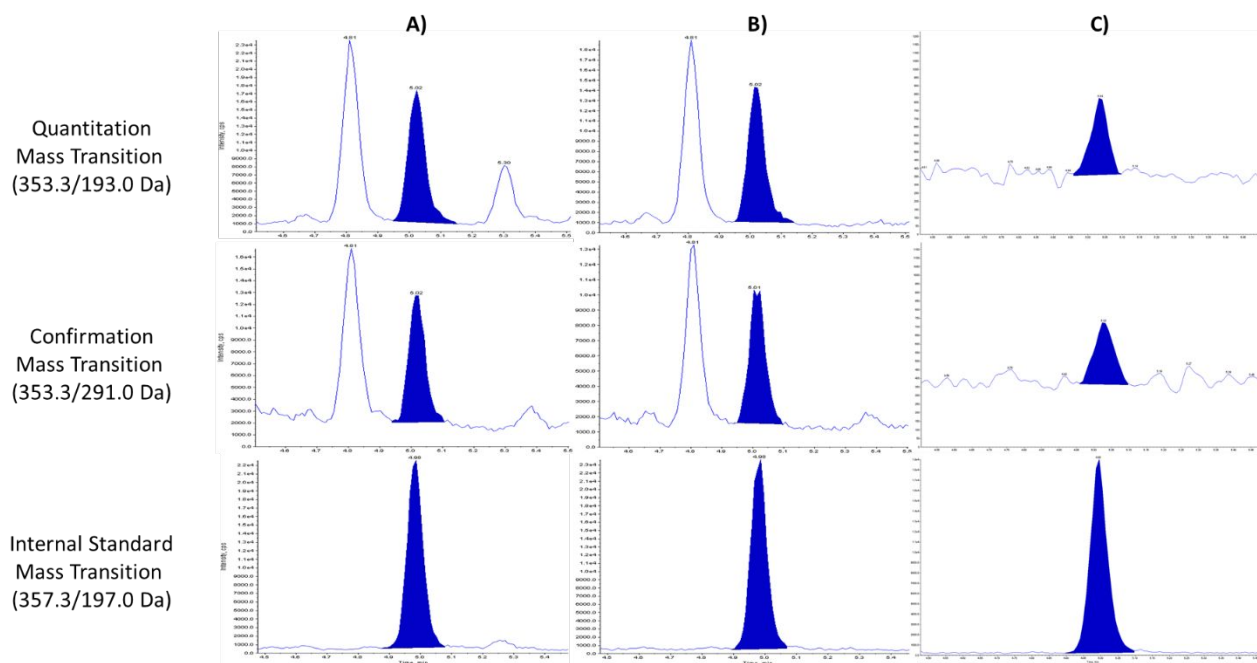


Figure S2 Representative chromatograms for **A)** total 8-isoprostane (left) (622 pg/mL) and **B)** free 8-isoprostane (middle) (491 pg/mL) of a cigarette smoker, and **C)** a standard injection (50% methanol and water solution) (right) (8.8 pg/mL). The mass transitions for each channel are as follows: quantitation (353.3/193.0 Da), confirmation (353.3/291.0 Da), and internal standard (357.3/197.0 Da). **A)** and **B)** are chromatograms obtained from the same donor.

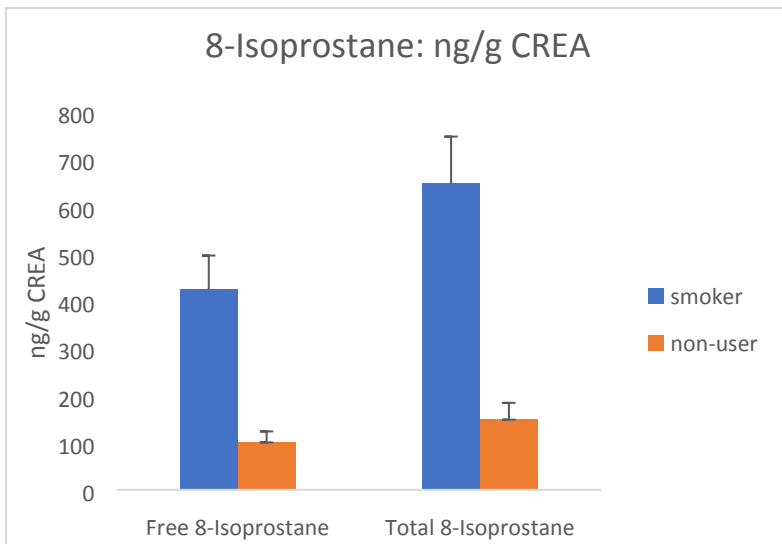


Figure S3 Comparison of geometric means \pm standard errors of free and total urinary 8-isoprostane concentrations adjusted for creatinine in smokers (free: 423 \pm 72.5 ng/g CREA; total: 648 \pm 99.1 ng/g CREA) and non-users (free: 101 \pm 22.3 ng/g CREA; total: 148 \pm 35.6 ng/g CREA).

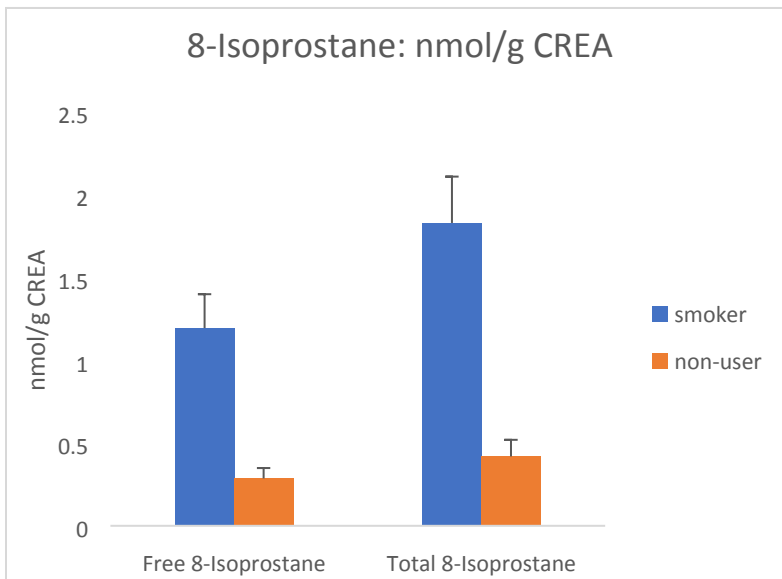


Figure S4 Comparison of geometric means \pm standard errors of free and total urinary 8-isoprostane molar concentrations adjusted for creatinine in smokers (free: 1.19 \pm 0.204 nmol/g CREA; total: 1.83 \pm 0.280 nmol/g CREA) and non-users (free: 0.286 \pm 0.0628 nmol/g CREA; total: 0.418 \pm 0.100 nmol/g CREA).