Challenges in quantifying 8-OHdG and 8-Isoprostane in exhaled breath condensate

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Table S1. Description of the standard preparation with the corresponding concentrations

Label	volume of working solution at 500 pg/ml [µL]	Volume of H$_2$O [µl]	Volume of working solution of IS 2,5ng/ml [µL]	Volume of BHT [µL]	Total volume [µl]	Final concentration Std [pg/ml]	Final concentration IS [ng/ml]	BHT (mg/ml)
Std 0	0	800	200	10	1010	0	0,495	0,099
Std 15	30	770	200	10	1010	15	0,495	0,099
Std 50	100	700	200	10	1010	50	0,495	0,099
Std 100	200	600	200	10	1010	99	0,495	0,099
Std 300	600	200	200	10	1010	297	0.495	0.099
Before concentration								
H20 0	0	985	15	10	1010	0	0,037	0,099
H20 5	10	975	15	10	1010	5	0,037	0,099
H20 15	30	955	15	10	1010	15	0,037	0,099
H20 25	50	935	15	10	1010	25	0,037	0,099
H20 50	100	885	15	10	1010	50	0,037	0,099
						After concentration and reconstitution in 75µl		
						0	0,495	1,320
						67	0,495	1,320
						200	0,495	1,320
						333	0,495	1,320
						666	0.495	1.320

Std = not evaporated standards; H20 = standards that will be evaporated.
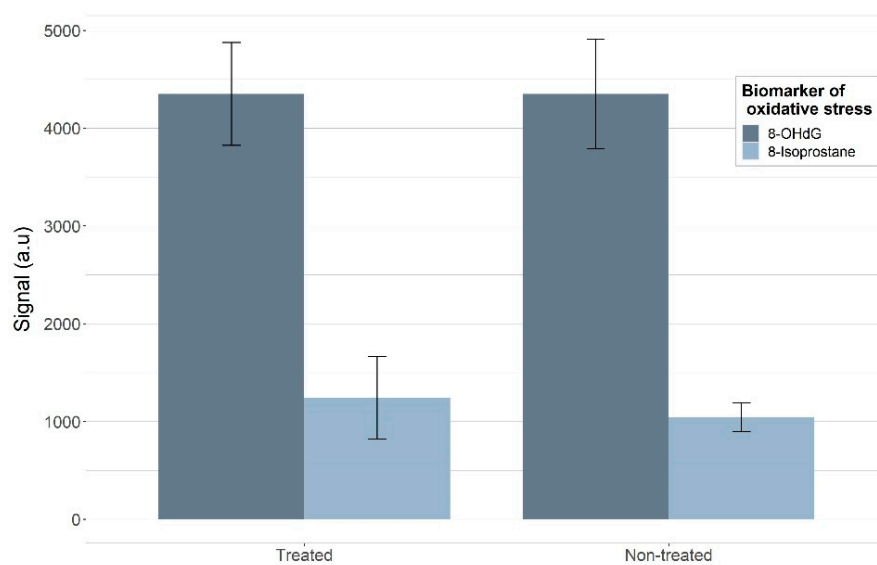
Figure S1. Effect of Tween-20 treatment on material surface on the signal (not corrected with IS) of 8-OHdg and 8- isoprostane. Error bars correspond to a minimum of nine independent repetitions.

Table S2. Main figure of merit of the analytical method for 8-OHdG in EBC

Method characteristics - 8-OHdG	Expected performances	Observed values	Conclusion
Calibration			
Function			linear
Concentration range [pg/ml]	0-1333		
Bias for std. 67 pg/ml	>15%	6,9%	Verified
Bias for std. 200 pg/ml	10%	4,9%	Verified
Bias for std. 333 pg/ml	10%	1,3%	Verified
Bias for std. 666 pg/ml	10%	1,0%	Verified
Bias for std. 1333 pg/ml	10%	0,4%	Verified
LOQ (a-priori estimate)	22 pg/ml (ech conc speedvac)		
Maximum accepted range	±13.2 pg/ml =60%*22 pg/ml		
Upper accepted value [pg/ml]	35,2	27,1	Verified
Lower accepted value [pg/ml]	8,8	23,5	Verified
LOD (1/3 LOQ) [pg/ml]		7,30	Verified
LOD in EBC [pg/ml]		0,5	Verified
Recovery			
Value EBC non spiked [pg/ml]	0,00		
EBC spiked 1 [pg/ml]	5,00	4,8	
EBC spiked 2 [pg/ml]	15,00	14,0	
Recovery spike 1 [%]	[90 - 110]	98±10	Verified
Recovery spike 2 [%]	[90 - 110]	89±8	Verified
Repeatability (CV)			
EBC non spiked	< LOD	n.d.	
EBC spiked 1 (5 pg/ml)	20%	19,8	Verified
EBC spiked 2 (15 pg/ml)	10%	1,0	Verified
Accuracy			
Reference value EBC spiked 1 [pg/ml]	5,00		
u_{ref}	2,0		
Maximum accepted difference	25%		
Normalized range	2	0,14	Verified
Maximum accepted range [pg/ml]	6,25	6,02	Verified
Minimum accepted range [pg/ml]	3,75	4,53	Verified
Reference value EBC spiked 2 [pg/ml]	15,00		
u_{ref}	2,0		
Maximum accepted difference	10%		
Normalized range	2	-0,52	Verified
Maximum accepted range [pg/ml]	16,50	14,82	Verified
Minimum accepted range [pg/ml]	13,50	13,10	Not verified

Table S3. Main figure of merit of the analytical method for 8-isoprostane in EBC

Method characteristics - 8-isoprostane	Expected performances	Observed values	Conclusion
Calibration			
Function			linear
Concentration range [pg/ml]	0-1333		
Bias for std. 67 pg/ml	>15%	10,0%	Verified
Bias for std. 200 pg/ml	10%	-5,9%	Verified
Bias for std. 333 pg/ml	10%	4,5%	Verified
Bias for std. 666 pg/ml	10%	-2,6%	Verified
Bias for std. 1333 pg/ml	10%	1,0%	Verified
LOQ (a-priori estimate)	40 pg/ml (ech conc speedvac)		
Maximum accepted range	±24 pg/ml =60%*40 pg/ml		
Upper accepted value [pg/ml]	63,5	27,9	Verified
Lower accepted value [pg/ml]	15,9	18,3	Verified
LOD (1/3 LOQ) [pg/ml]		13,2	Verified
LOD in EBC [pg/ml]		1,0	Verified
Recovery			
Value EBC non spiked [pg/ml]	0,00		
EBC spiked 1 [pg/ml]	5,00	5,4	
EBC spiked 2 [pg/ml]	15,00	15,1	
Recovery spike 1 [%]	[90 - 110]	104±7	Verified
Recovery spike 2 [%]	[90 - 110]	95±6	Verified
Repetability (CV)			
EBC non spiked	< LOD	n.d.	
EBC spiked 1 (5 pg/ml)	20%	11,1	Verified
EBC spiked 2 (15 pg/ml)	10%	5,9	Verified
Accuracy			
Reference value EBC spiked 1 [pg/ml]	5,00		
u_{ref}	2,0		
Maximum accepted difference	50%		
Normalized range	2	0,38	Verified
Maximum accepted range [pg/ml]	7,50	7,34	Verified
Minimum accepted range [pg/ml]	2,50	4,23	Verified
Reference value EBC spiked 2 [pg/ml]	15,00		
u_{ref}	2,0		
Maximum accepted difference	15%		
Normalized range	2	0,04	Verified
Maximum accepted range [pg/ml]	17,25	16,87	Verified
Minimum accepted range [pg/ml]	12,75	13,29	Verified

Table S4. Results obtained for the calibration, QC and EBC samples

8-OHdG	Slope	Intercept	R²	LOD (pg/ml)	Theoretical	Experimental	Average EBC Concentration
	Calibration Curve				QC 5 (pg/ml)		
Healthy (n=10)							<LOD
Asthma (n=7)	0.032	-0.0019	0.999	0.5	5	4.86 ±0.11 (n=4)	<LOD
COPD (n=9)							<LOD
8-isoprostane							
Healthy (n=10)	0.065	0.034	0.996	1.0	5	5.06±1.06 (n=4)	<LOD
Asthma (n=7)							<LOD
COPD (n=9)							<LOD

n = the number of measures.