Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information for

Improved ELISA for linoleate-derived diols in human plasma utilizing a polyHRP-based secondary tracer

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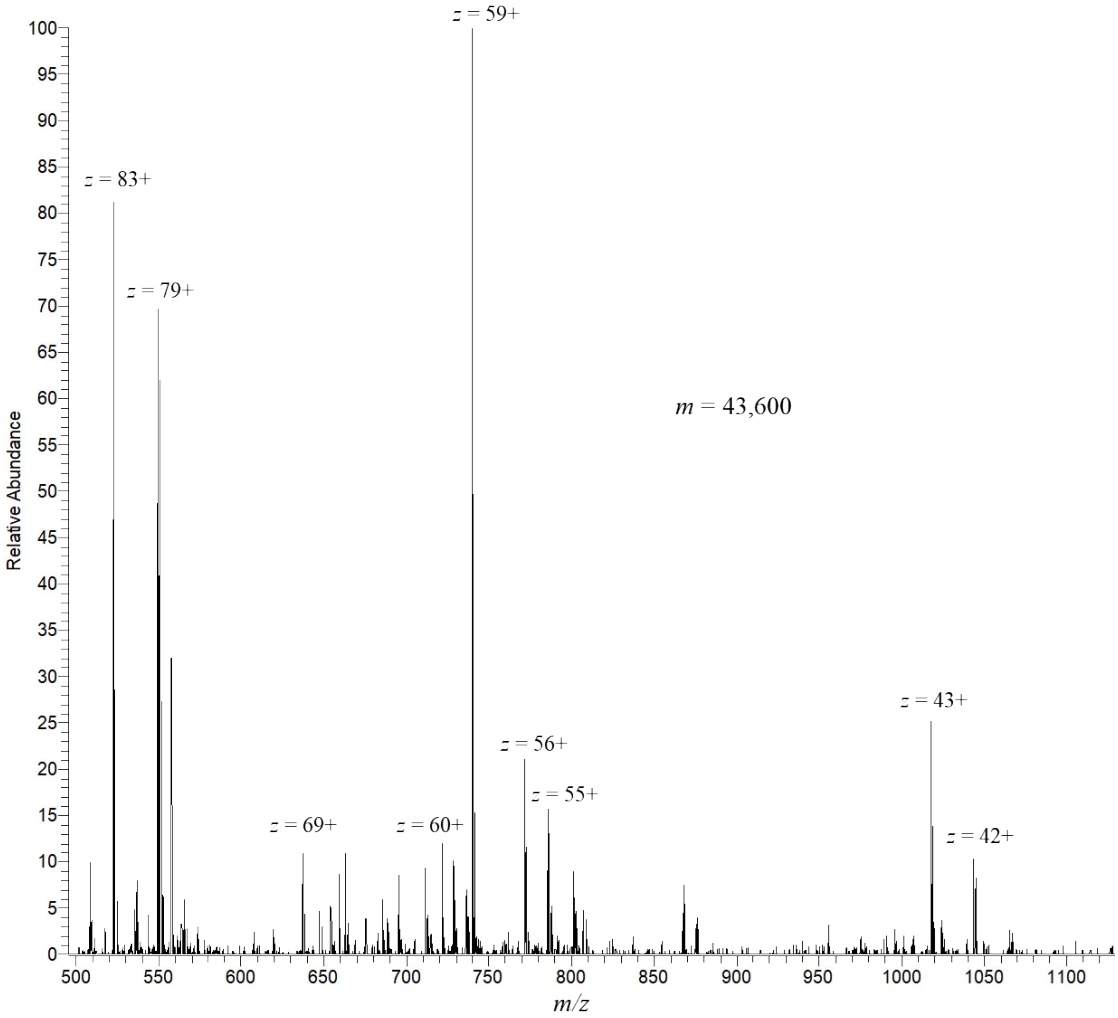
Dihydroxyoctadecenoic acids (regioisomer mixture). ^{1}H NMR (400 MHz, CDCl3) δ 6.25-5.96 (2H, broad singlet, O**H**), 5.61-5.54 (1H, multiplet (m), **vinylic**), 5.47-5.40 (1H, m, **vinylic**), 3.52-3.46 (2H, m), 2.38-2.27 (3H, m), 2.11-2.02 (3H, m), 1.69-1.59 (2H, m), 1.53-1.23 (16H, m), 0.90 (3H, triplet, J = 6.8 Hz, **Me**); HRESIMS observed *m/z* 313.2388 $[M - H]^-$ (calculated *m/z* for $C_{18}H_{33}O_4^-$, 313.2384).



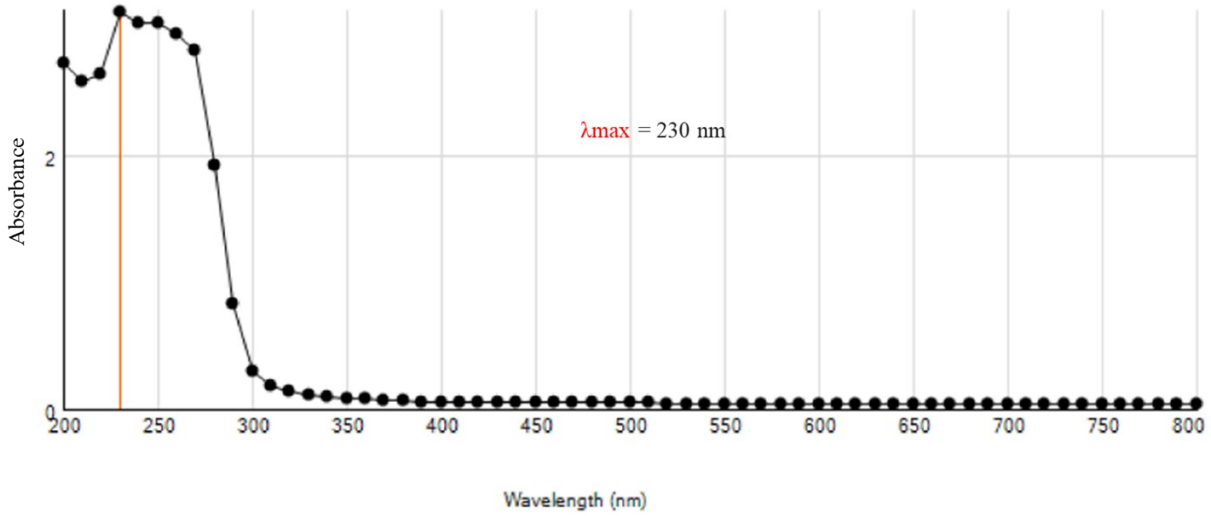


Fig. S1 MS and UV-vis characterization of OLE-OVA (coating antigen)

Table S1 HPLC gradient for elution of DiHOMEs

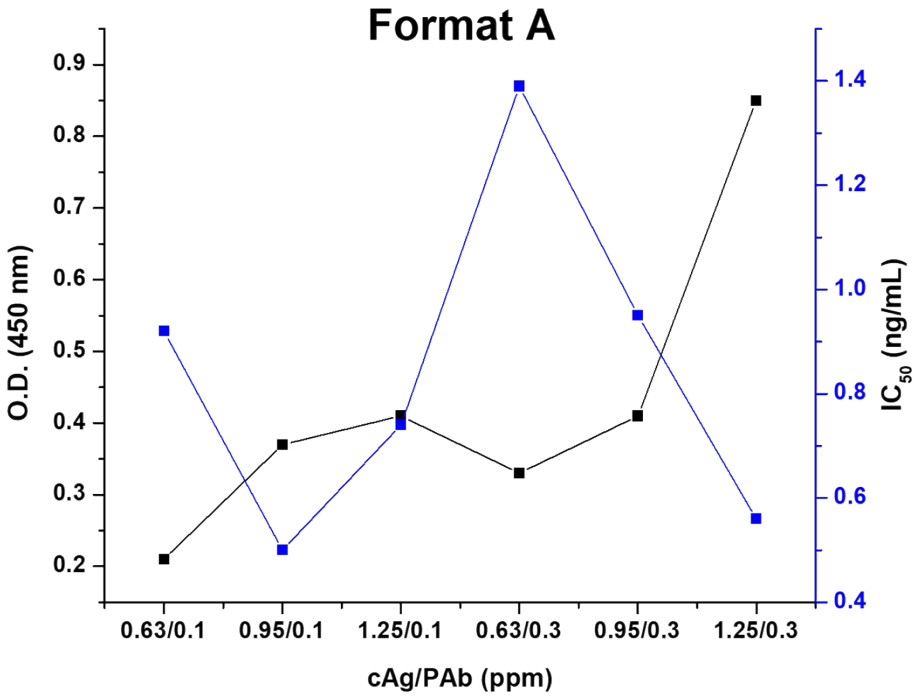
Time (min)	B (%)
0	20
1	70
5	95
6	95
7	20

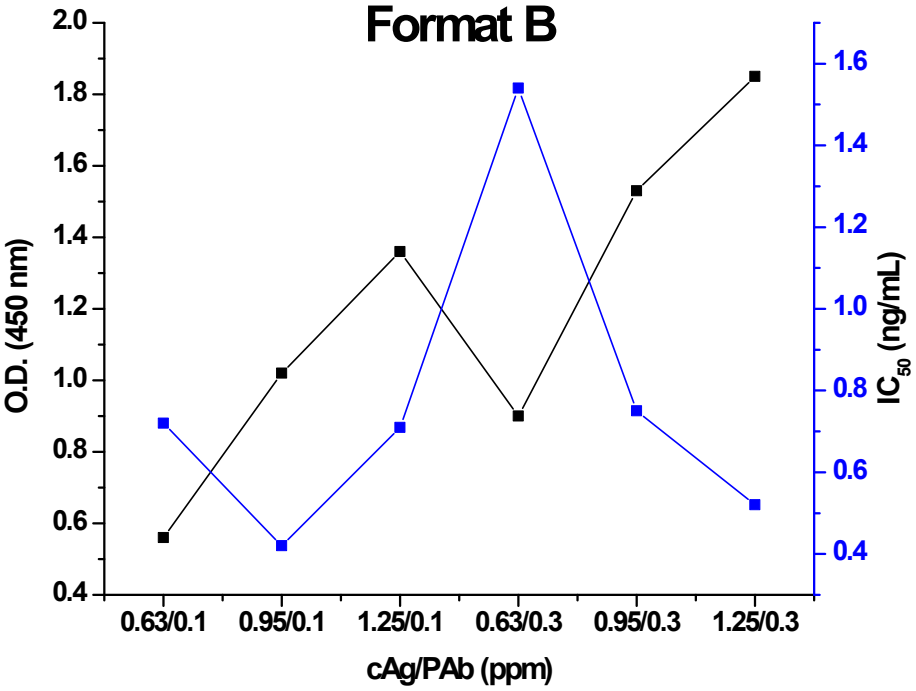
Table S2 Q-TRAP mass spectrometer conditions

Parameter	Value
Curtain Gas (psi)	30
Collision Gas	Medium
IonSpray Voltage (V)	-4500
Temperature (°C)	450
Ion Source Gas 1 (psi)	50
Ion Source Gas 2 (psi)	50
Interface Heater (ihe)	ON

Table S3 Tandem MS parameters for DiHOMEs

Analyte	Declustering potential (V)	Entrance potential (V)	Collision energy (V)	Collision cell exit potential (V)
9,10-DiHOME	-50	-10	-30	-5
12,13-DiHOME	-50	-10	-30	-5


Format A
O.D. (450 nm)
IC50 (ng/mL)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
1.4
1.2
1.0
0.8
0.6
0.4
0.63/0.1
0.95/0.1
1.25/0.1
0.63/0.3
0.95/0.3
1.25/0.3
cAg/PAb (ppm)


Format B
O.D. (450 nm)
IC50 (ng/mL)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.63/0.1
0.95/0.1
1.25/0.1
0.63/0.3
0.95/0.3
1.25/0.3
cAg/PAb (ppm)

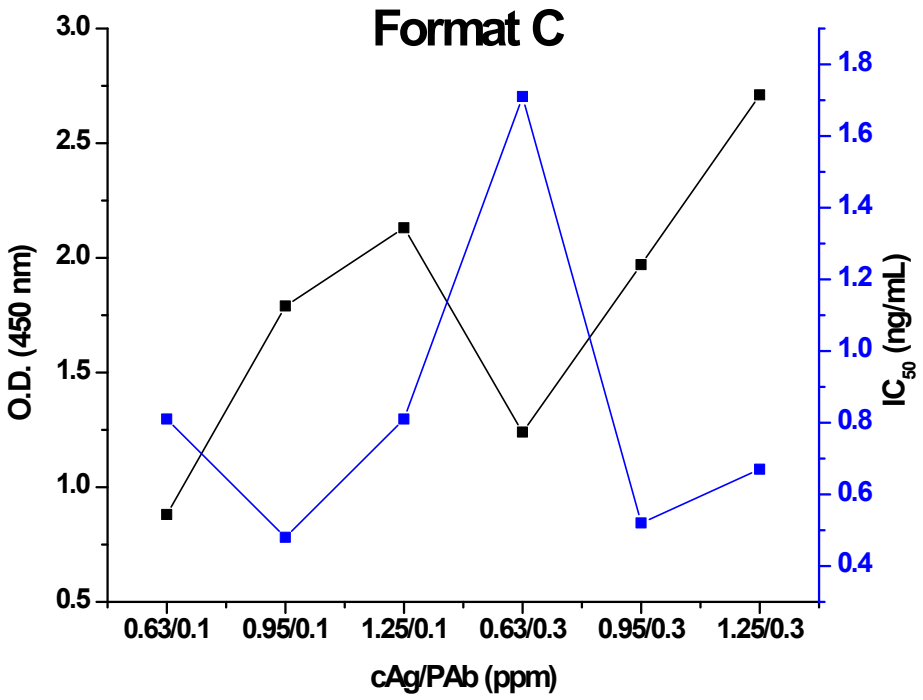


Fig. S2 Checkerboard titration-based optimization of coating antigen (cAg) and primary antibody (PAb) concentrations (ppm) for DiHOME ELISA formats A-C

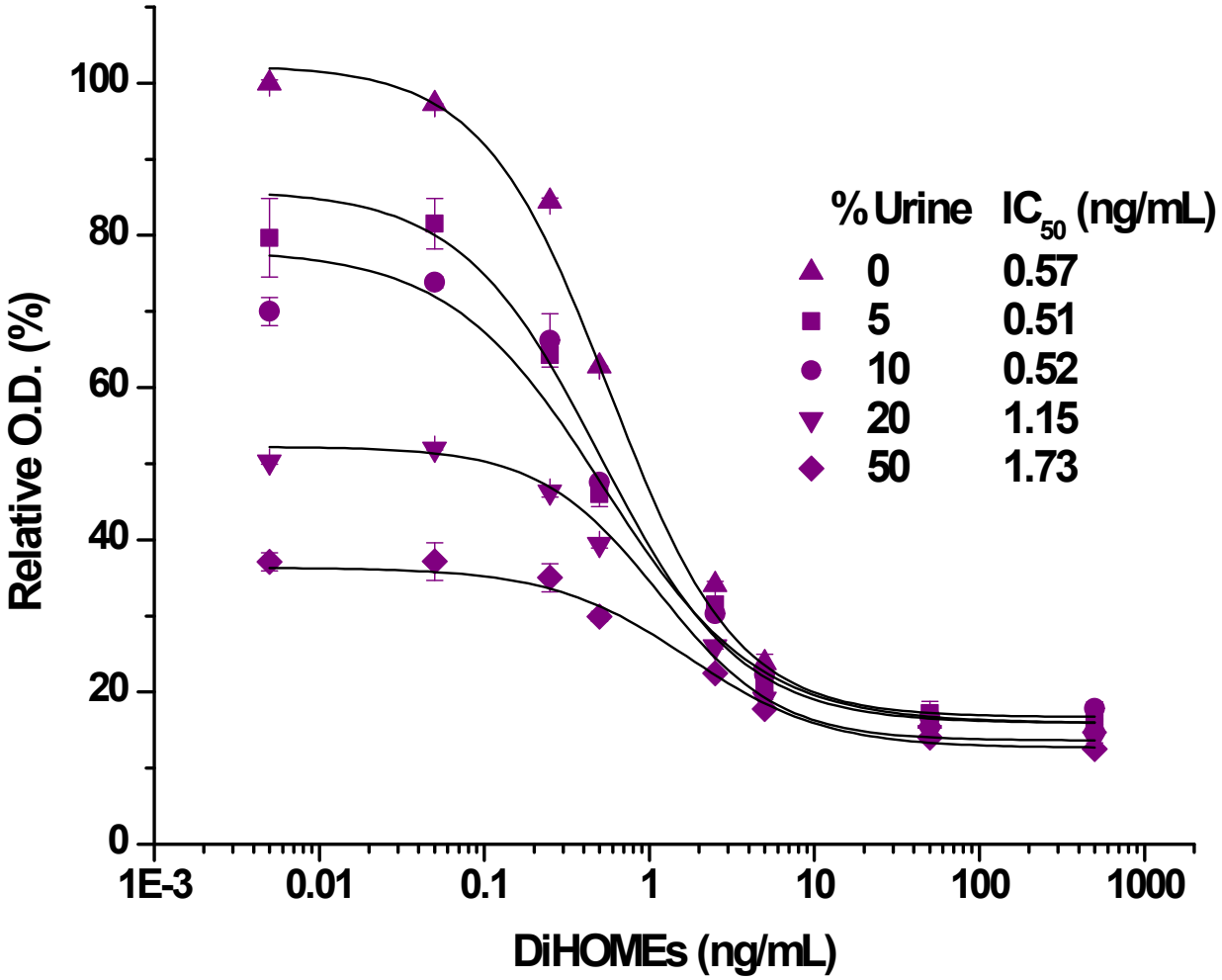


Fig. S3 Calibration curves (and IC_{50} values) for DiHOMEs in varying concentrations (0-50% in neat assay buffer) of human urine