

Supplementary Information

An LC-MS/MS workflow to characterize 16 regio- and stereoisomeric trihydroxyoctadecenoic acids (TriHOMEs)

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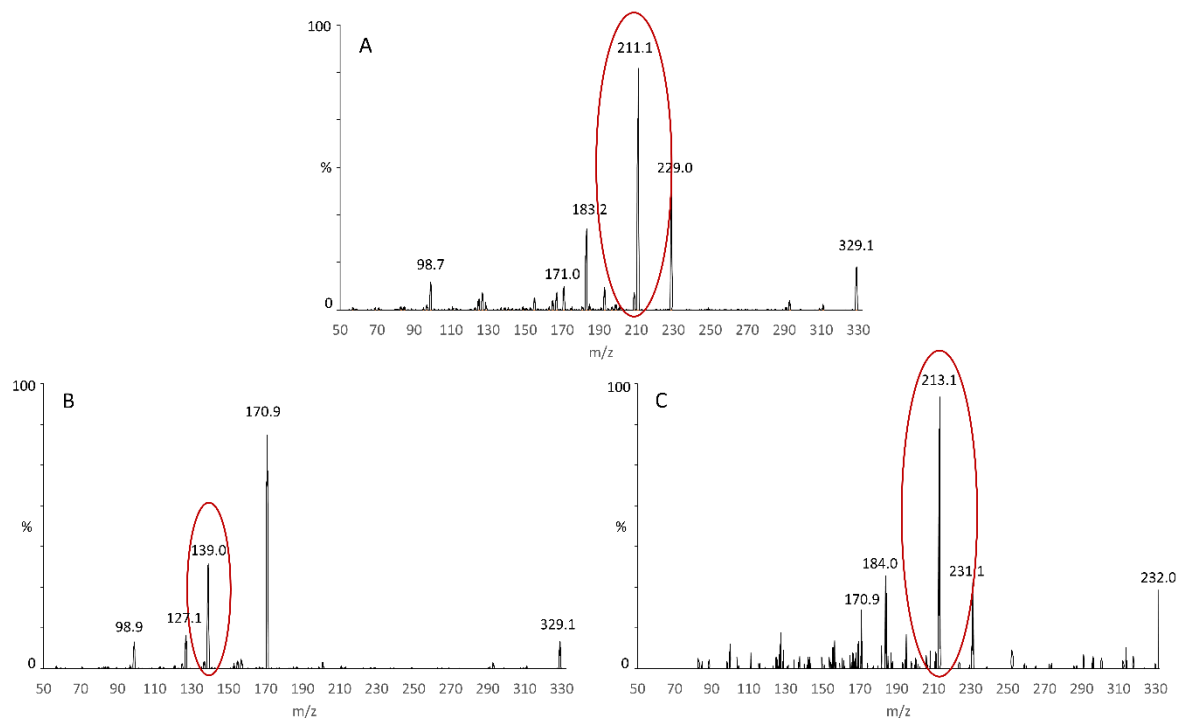
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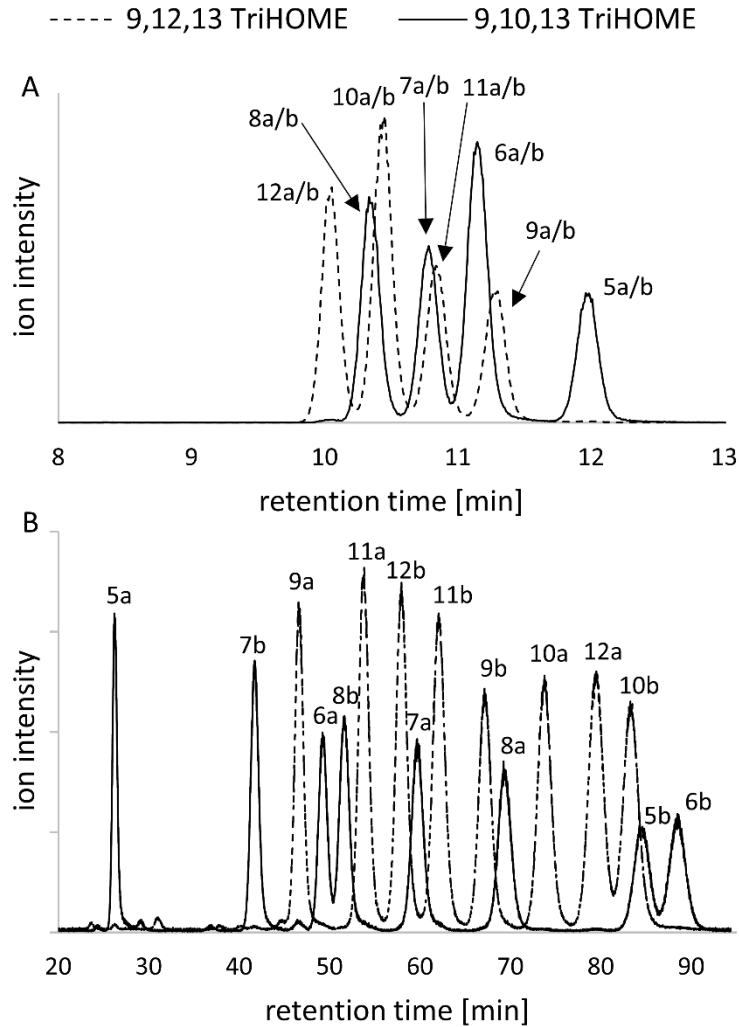
Supplemental Table S1: Median concentrations of TriHOME isomers in female healthy never smokers, healthy smokers, smoking COPD patients and COPD patients that are ex-smokers

		median concentration (interquartile range) [ng/mL]			
		n=20	n=19	n=11	n=6
Compound*		healthy never smokers	healthy smokers	COPD smokers	COPD ex-smokers
9,10,13-TriHOMEs	5 a/b	0.009 (0.012 - 0.008)	0.008 (0.011 - 0.006)	0.015 (0.018 - 0.012)	0.010 (0.012 - 0.007)
	6 a/b	0.011 (0.013 - 0.009)	0.009 (0.011 - 0.007)	0.018 (0.020 - 0.014)	0.011 (0.013 - 0.009)
	7 a/b	0.021 (0.026 - 0.018)	0.017 (0.023 - 0.014)	0.028 (0.035 - 0.014)	0.021 (0.026 - 0.019)
	8 a/b	0.018 (0.023 - 0.013)	0.016 (0.025 - 0.013)	0.026 (0.042 - 0.21)	0.018 (0.028 - 0.017)
	sum	0.059 (0.067 - 0.051)	0.049 (0.070 - 0.041)	0.088 (0.111 - 0.071)	0.060 (0.079 - 0.054)
9,12,13-TriHOMEs	9 a/b	0.007 (0.009 - 0.006)	0.006 (0.008 - 0.004)	0.011 (0.013 - 0.010)	0.007 (0.009 - 0.005)
	10 a/b	0.011 (0.014 - 0.006)	0.011 (0.013 - 0.010)	0.020 (0.021 - 0.017)	0.013 (0.014 - 0.012)
	11 a/b	0.013 (0.017 - 0.012)	0.015 (0.013 - 0.011)	0.019 (0.027 - 0.016)	0.013 (0.017 - 0.012)
	12 a/b	0.016 (0.020 - 0.012)	0.017 (0.022 - 0.013)	0.024 (0.035 - 0.020)	0.015 (0.022 - 0.015)
	sum	0.047 (0.059 - 0.042)	0.049 (0.061 - 0.039)	0.074 (0.093 - 0.063)	0.049 (0.062 - 0.042)

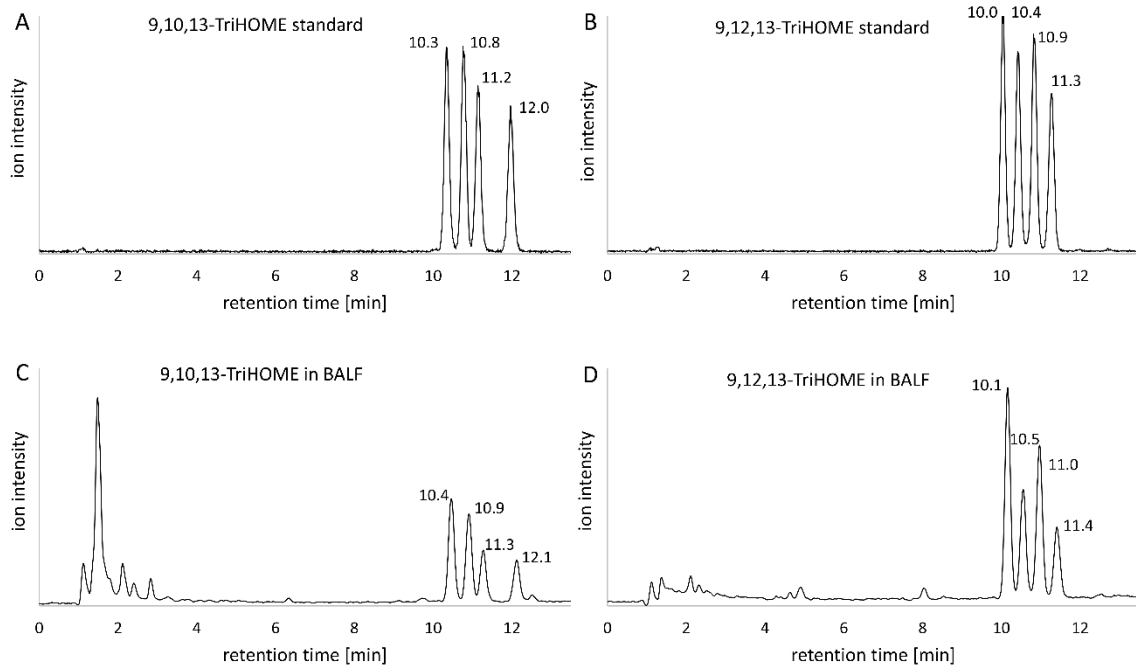
*See Figure 1 for compound structure and nomenclature



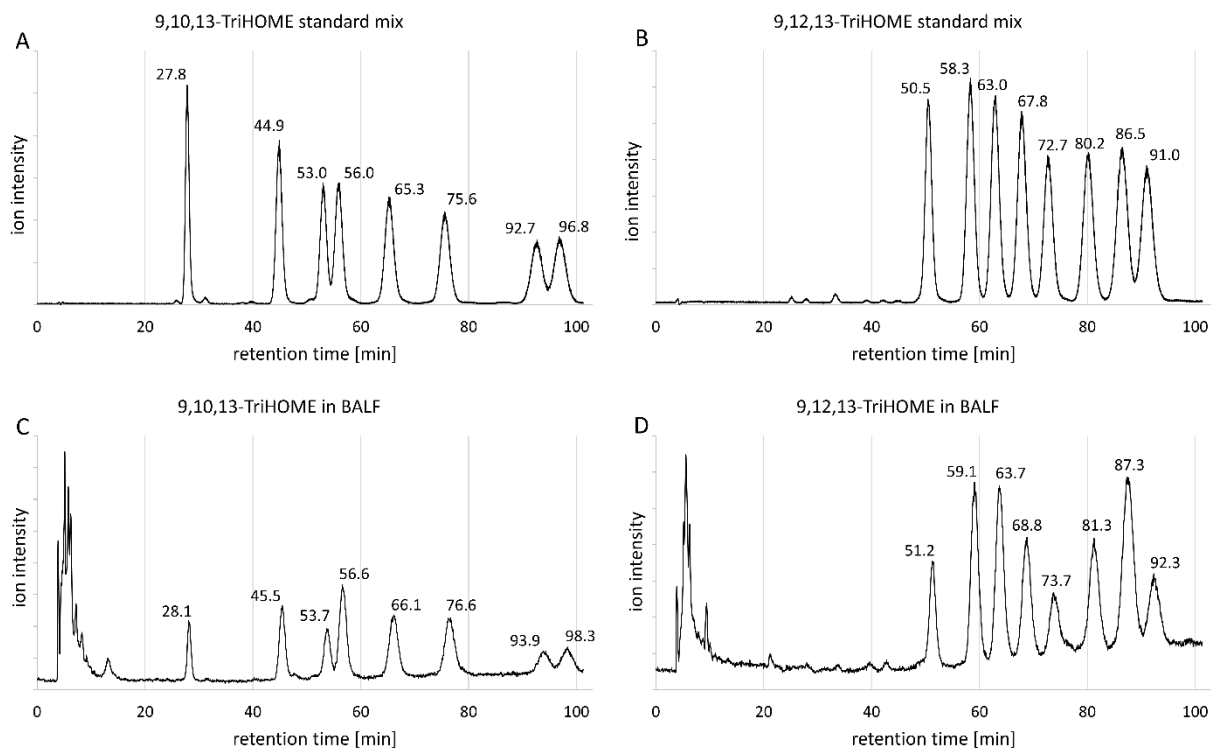
Supplemental Figure S1: Product ion scans of A: 9,12,13-TriHOME (parent mass: 329.1, CE: 25 eV), B: 9,10,13-TriHOME (parent mass: 329.1, CE: 21 eV) and C: [11,12,13-¹³C₃] 9,12,13-TriHOME internal standard (parent mass: 332.1, CE 25 eV). Red circles indicate fragmentation ions used for MRM scans.



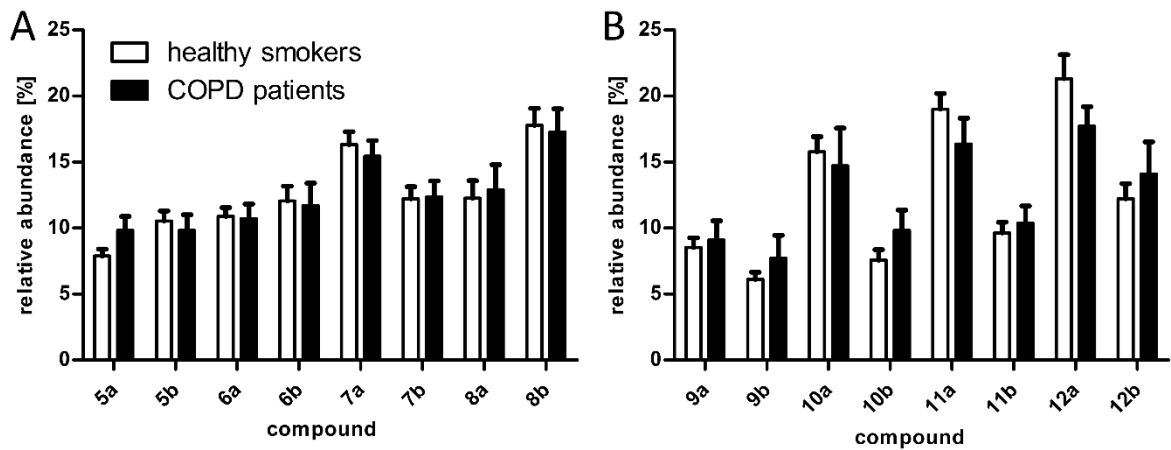
Supplemental Figure S2: Overlays of chromatograms of a solution containing TriHOME standard mix A and standard mix B. MS/MS transition for 9,10,13-TriHOME: m/z 329.1 \rightarrow 139.0; MS/MS transition for 9,12,13-TriHOME: m/z 329.1 \rightarrow 211.0. Compound nomenclature is provided in Figure 1. A: Reversed phase UHPLC-MS/MS chromatograms, B: Chiral LC-MS/MS chromatograms



Supplemental Figure S3: Reversed phase UHPLC-MS/MS chromatograms of TriHOME standards (A and B) and chromatograms of TriHOMEs in a representative human BALF sample (C and D). Exact retention times are indicated above the respective TriHOME peaks. MS/MS transition of 9,10,13-TriHOME: m/z 329.1 \rightarrow 139.0, MS/MS transition of 9,12,13-TriHOME: m/z 329.1 \rightarrow 211.0.



Supplemental Figure S4: Chiral LC-MS/MS chromatograms of TriHOME standards (A and B) and chromatograms of TriHOMEs in a representative human BALF sample (C and D). Exact retention times are indicated above the respective TriHOME peaks. MS/MS transition of 9,10,13-TriHOME: m/z 329.1 \rightarrow 139.0, MS/MS transition of 9,12,13-TriHOME: m/z 329.1 \rightarrow 211.0.



Supplemental Figure S5: Relative abundance of A: 9,10,13-TriHOME isomers (compounds 5a/b – 8a/b) and B: 9,12,13-TriHOME isomers (compound 9a/b – 12a/b) in healthy smokers and smoking COPD patients. Values are shown as the mean with 95 % confidence interval. healthy smokers: n = 19; smoking COPD patients: n = 11.