Supplementary Information

Trace Analysis of Dimethoxytrityl Alcohol (DMT-OH) in Oligonucleotide Matrices Using Liquid Chromatography Coupled with Tandem Mass Spectrometry

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S1.   Analysis of DMT-OH by LC-UV

Table S1. Raw data used to evaluate LC-UV (230 nm) LOQ

DMT-OH Concentration (ng/mL)	UV230 nm peak area after blank subtraction
32	1.20
64	2.74
128	6.98
256	15.99
512	34.91

Standard deviation (σ) was calculated to be 0.365 via MS-Excel LINEST function.
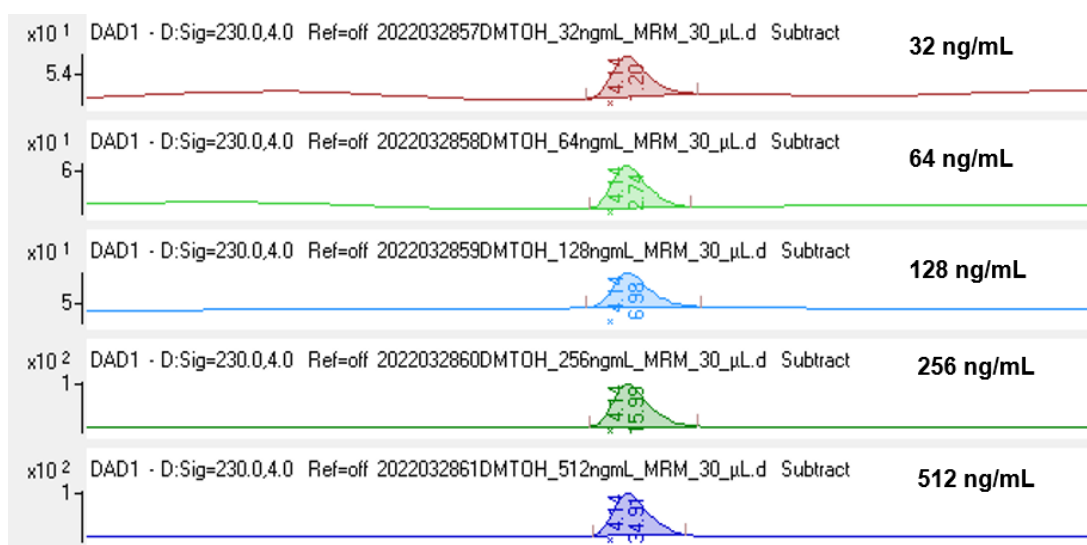LOQ = 10 * σ/slope = 10 * 0.365/0.071 = 51 ng/m

Figure S1. Absorption of UV 230 nm at various DMT-OH concentrations. Blank subtraction was performed via Agilent MassHunter Qualitative Analysis Software

S2. Analysis of DMT-OH by LC-MS (SIM)

Table S2. Raw data used to evaluate LC-MS (SIM) LOQ

DMT-OH Concentration (ng/mL)	(-) SIM 319 peak area without blank subtraction
50	167
100	404
200	846
300	1586
400	2299

Standard deviation (σ) was calculated to be 96.0 via MS-Excel LINEST function.
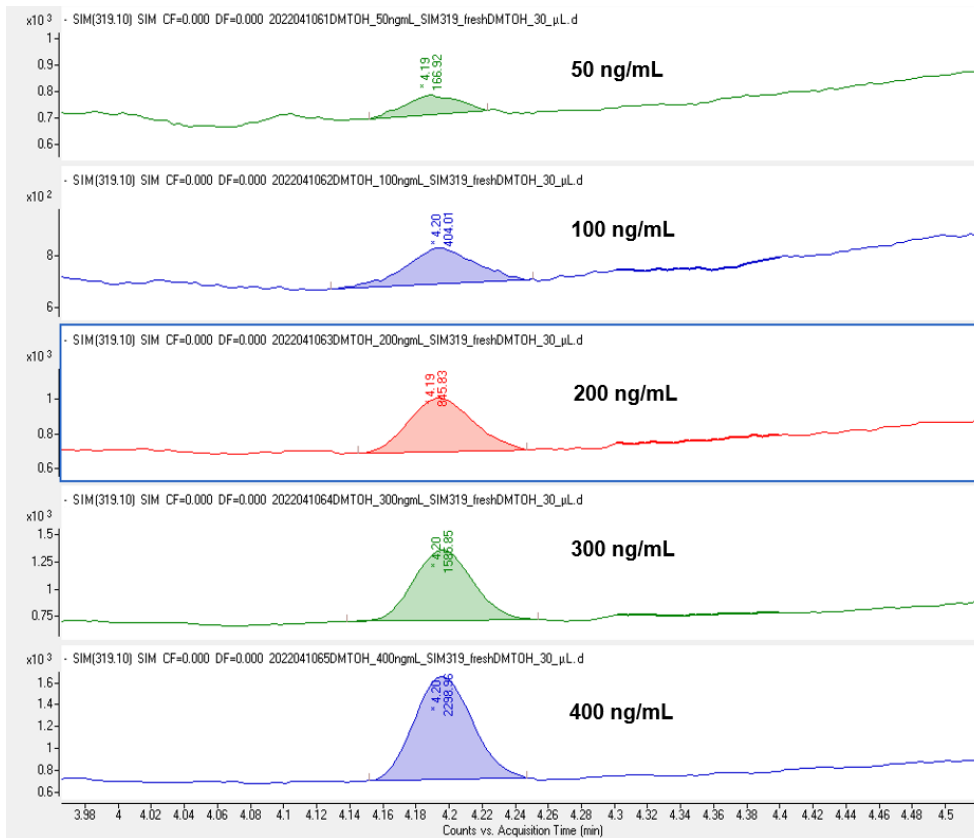LOQ = 10 * σ/slope = 10 * 96.0/6.1 = 157 ng/mL

Figure S2. Chromatograms of SIM (m/z: -319.0) at various DMT-OH concentrations

S3.   Analysis of DMT-OH by LC-MS/MS (MRM)

Table S3. Raw date used for LC-MS/MS (MRM) linearity

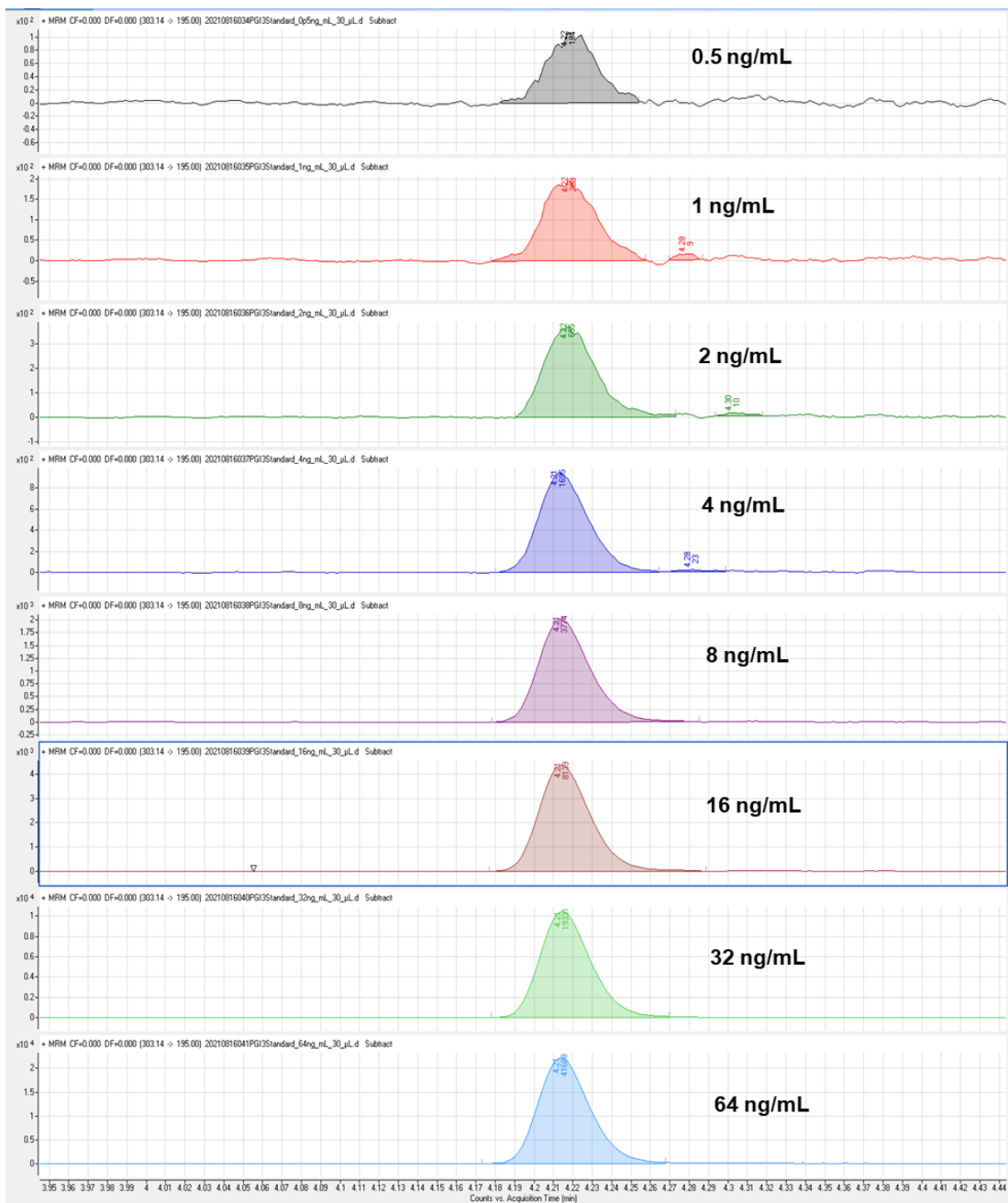| DMT-OH standard conc. (ng/mL)	DMTOH MRM195 Results after Blank Subtraction	
	RT (min)	MRM Peak Area
0.5	4.2160	194
1	4.2142	388
2	4.2160	695
4	4.2142	1695
8	4.2142	3774
16	4.2142	8133
32	4.2142	19331
64	4.2142	41688

Figure S3. MRM (m/z: 303 --> 195) at various DMT-OH concentrations. Blank subtraction was performed via Agilent MassHunter Qualitative Analysis Software

LOQ was evaluated based on five concentrations (0.5 to 8 ng/mL). The standard deviation (σ) was calculated to be 77.5 via MS-Excel LINEST function.

LOQ = 10 * σ/slope = 10 * 77.5/482.1 = 1.6 ng/mL