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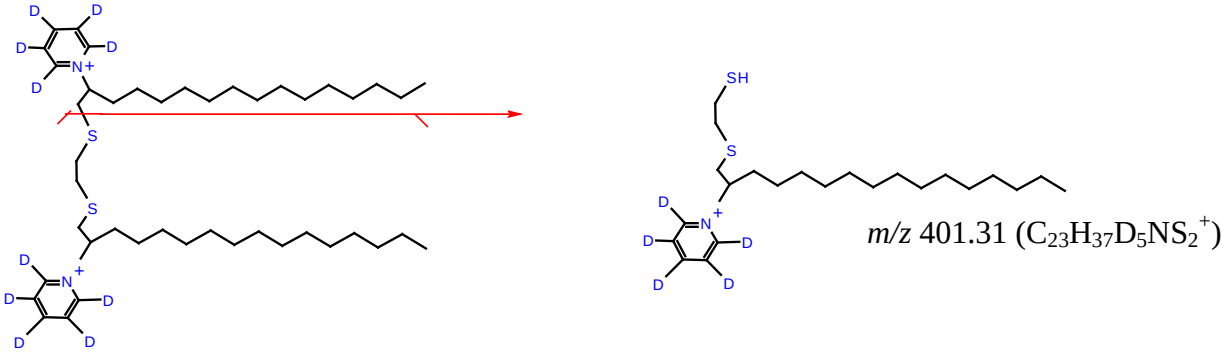
SUPPLEMENTARY INFORMATION

16(Py)-S-2-S-(Py)16-***d*₁₀**

[M]$^{2+}$: *m/z* 354.31 ($C_{44}H_{68}\mathbf{D_{10}}N_2S_2^{2+}$)



16-3-16-***d*₆₆**

[M]$^{2+}$: *m/z* 323.51 ($C_{39}H_{18}\mathbf{D_{66}}N_2^{2+}$)
m/z 388.61 ($C_{23}H_{18}\mathbf{D_{33}}N_2^{+}$)

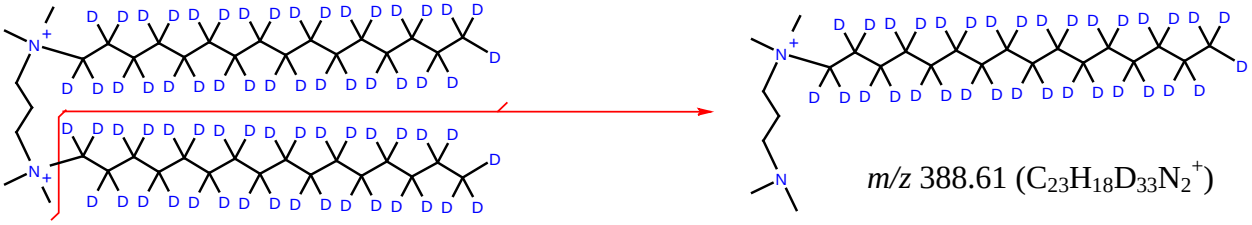
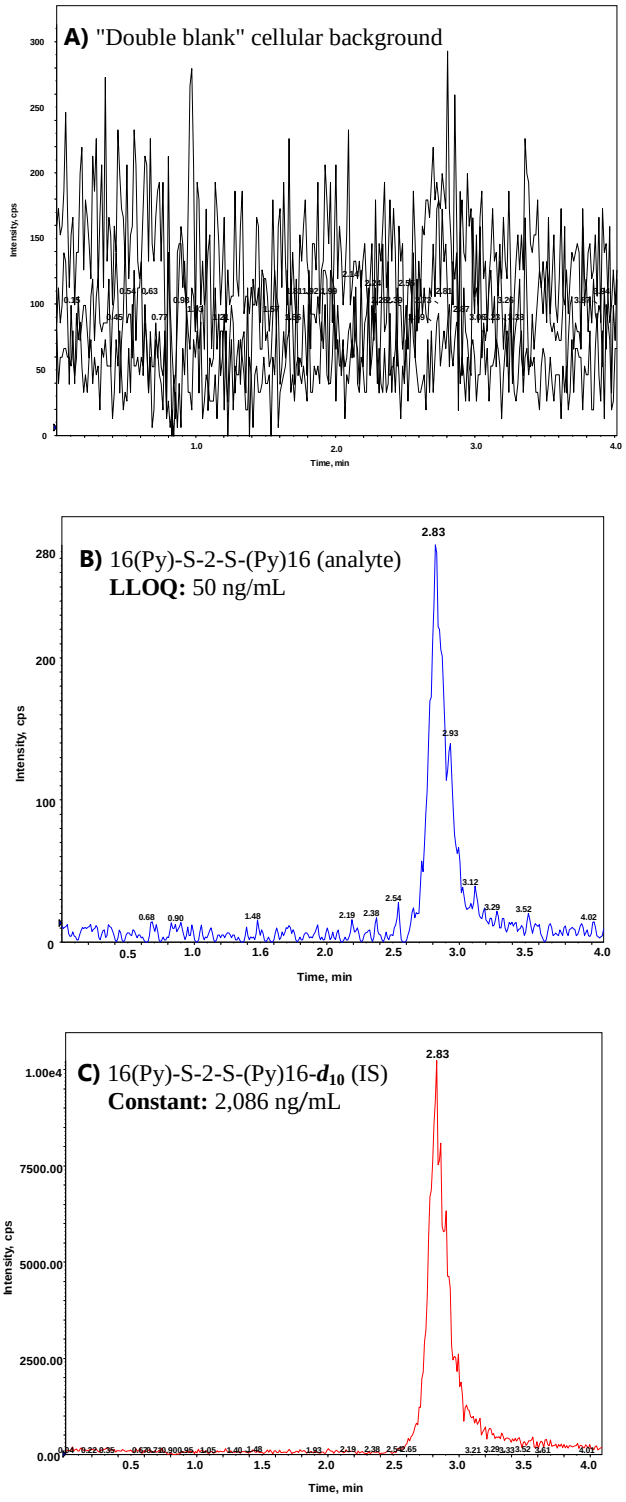


Figure S1. Product ions monitored for the internal standards 16(Py)-S-2-S-(Py)16-***d*₁₀** and 16-3-16-***d*₆₆** during HILIC-LC-MS/MS analysis.

Figure S2:



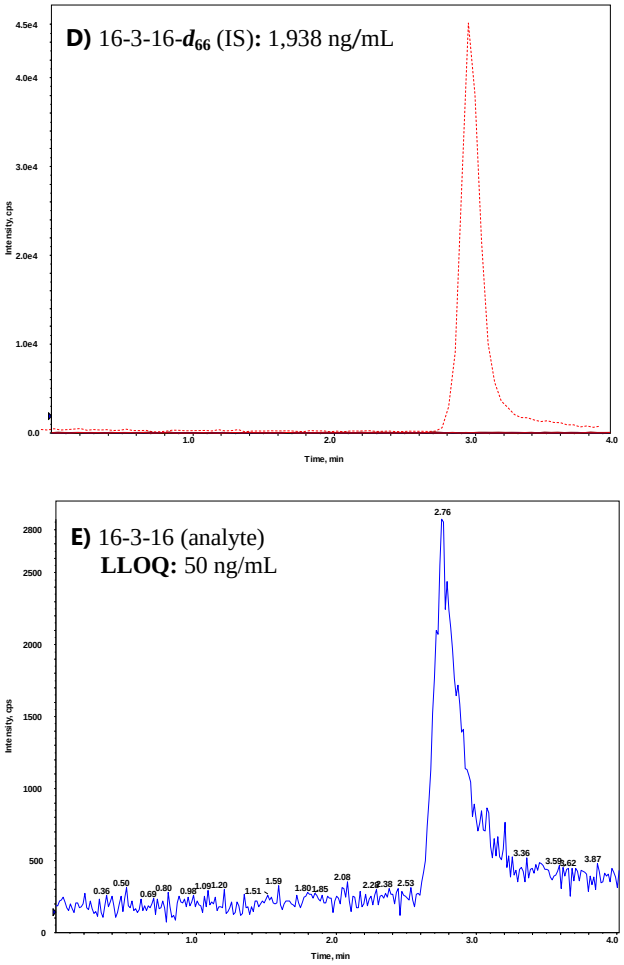


Figure S2. Chromatograms of the gemini surfactants, 16(Py)-S-2-S-(Py)16, 16-3-16, 16(Py)-S-2-S-(Py)16-d_{10}, 16-3-16-d_{66}. [Panel **A** indicates the absence of any interference against the selective determination of all four compounds by showing the detection of no signals around the established HILIC-LC-MS/MS elution times of these compounds within "double blank" cell samples. **B**) chromatogram from an injection of only 16(Py)-S-2-S-(Py)16-d_{10} and **C**) Chromatogram from an injection of only 16(Py)-S-2-S-(Py)16 at lower limit of quantitation, LLOQ. No cross-interference occurred between 16(Py)-S-2-S-(Py)16 and 16(Py)-S-2-S-(Py)16-d_{10}]. **D**) Chromatogram from an injection of only 16-3-16; and, **E**) Chromatogram from an injection of only 16-3-16-d_{66}. No cross-interference occurred between 16-3-16 and 16-3-16-d_{66} since the injections produced signal for only the respective but not both compounds. In general, the chromatograms showed symmetry and smoothness for quantitative analysis as shown.

Figure S3:

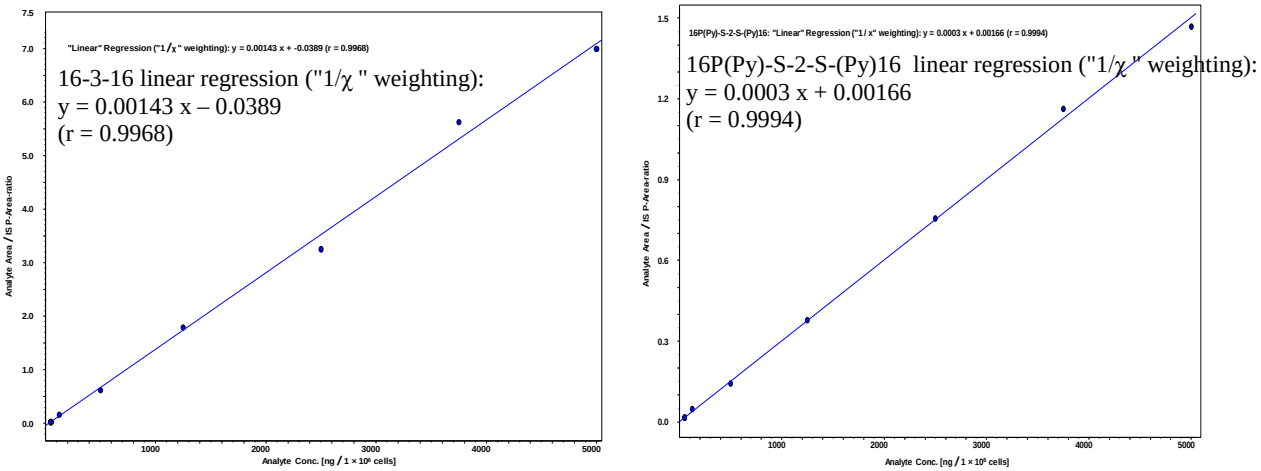


Figure S3

Calibration curves for the 16-3-16 and 16(Py)-S-2-S-(Py)16 gemini surfactants. In both cases, the linear range was 50 – 5000 ng/[1 × 10^6 cells], $r^2 \geq 0.99$.

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Figure S4:

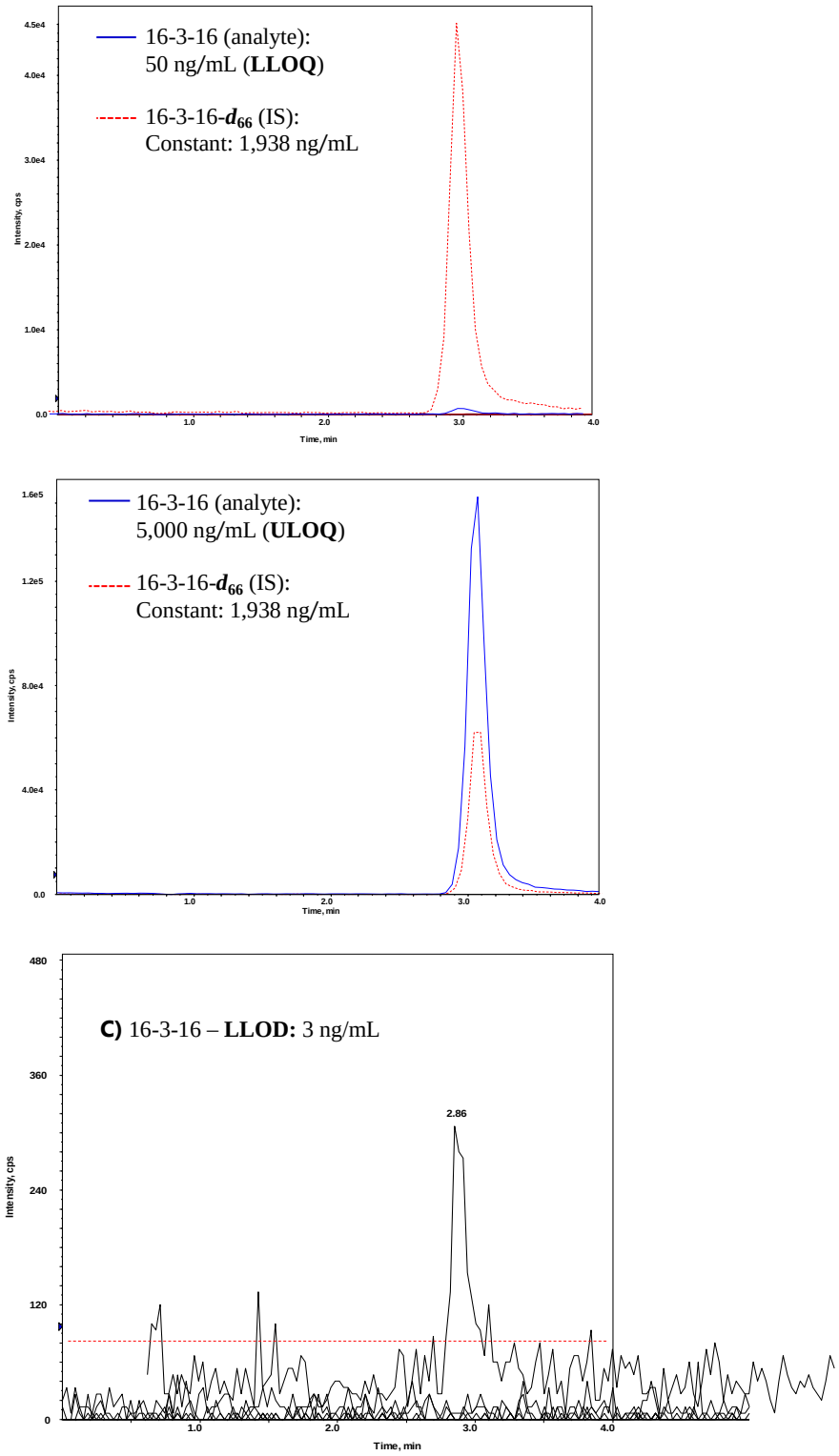
16-3-16 (analyte):
50 ng/mL (LLOQ)
16-3-16-d66 (IS):
Constant: 1,938 ng/mL
Intensity, cps
Time, min
16-3-16 (analyte):
5,000 ng/mL (ULOQ)
16-3-16-d66 (IS):
Constant: 1,938 ng/mL
C) 16-3-16 – LLOD: 3 ng/mL
2.86

Figure S4. Chromatograms of the 16-3-16 gemini surfactant at LLOQ, ULOQ and LLOD. The relative response signal is shown for the analyte at: **A**) LLOQ and **B**) ULOQ in relation to the internal standard, 16-3-16-d_{66}, which was present at a constant concentration. **C**) Extracted chromatogram for 16-3-16 at LLOD.

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Figure S5:

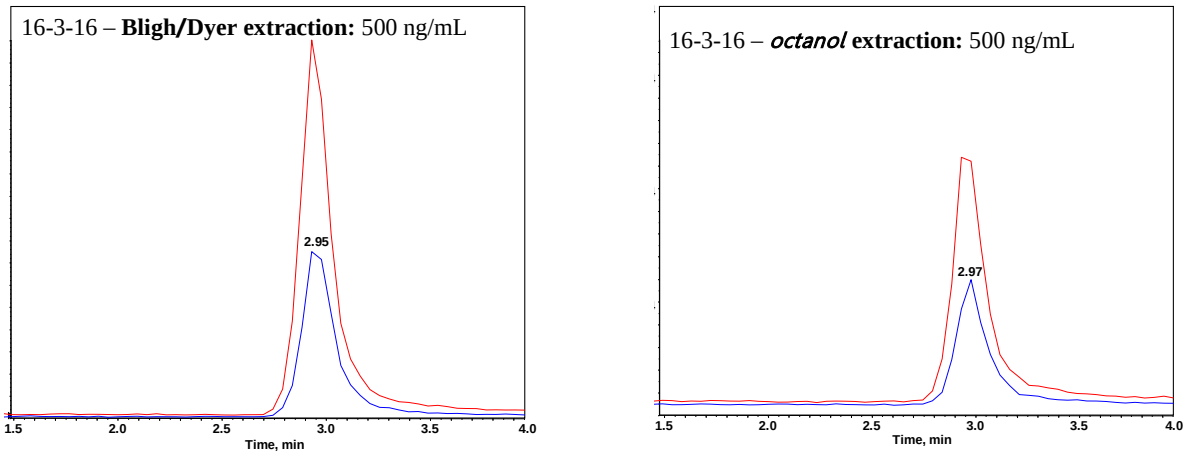

Figure S5. Contrasting analyte recovery efficiencies for Bligh/Dyer lipid extraction vs *octanol* extraction. Bligh/Dyer lipid extraction gave a better recovery (typically 98%) of the analyte and was the chosen liquid-liquid extraction method, departing from a recent report in which octanol extraction (70% efficiency) was used.

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TABLES & TABLE CAPTIONS:

Table S1. Gemini surfactant mass concentrations equivalent to 3 mM molarity

Gemini surfactant	Mass concentration (≈ 3 mM)
12(Py)-S-2-S-(Py)12	2.24 mg/mL
14(Py)-S-2-S-(Py)14	2.40 mg/mL
16(Py)-S-2-S-(Py)16	2.57 mg/mL
18(Py)-S-2-S-(Py)18	2.74 mg/mL
16-3-16	2.22 mg/mL
16-7-16	2.39 mg/mL
12-3-12	1.88 mg/ml
12-4-12	1.92 mg/mL
12-8-12	2.09 mg/mL
12-10-12	2.18 mg/mL
12-12-12	2.26 mg/mL
12-16-12	2.43 mg/mL
18-3-18	2.39 mg/ml
18-7-18	2.55 mg/mL
18:1-3-18:1	2.37 mg/mL
18:1-6-18:1	2.50 mg/mL
12-7NH-12	2.05 mg/mL
Py-3-12	2.24 mg/mL

Table S2. Overview of the HILIC-LC-MS/MS method validation parameters

Analyte	16(Py)-S-2-S-(Py)16	16-3-16
Internal standard (IS)	16(Py)-S-2-S-(Py)16-d_{10}	16-3-16-d_{66}
Method description	LLE, HILIC-LC-MS/MS	LLE, HILIC-LC-MS/MS
QC range	150 – 4375 ng/mL	150 – 4375 ng/mL
Calibration curve range	50 – 5000 ng/mL	50 – 5000 ng/mL
Lower limit of detection (LLOD)	6 ng/mL	3 ng/mL
Average analyte recovery	97.7% (Bligh/Dyer method)	98.2% (Bligh/Dyer method)
Intra-day accuracy range	92 – 110%	92 – 110%
Intra-day precision range	3 – 11%	3 – 11%
Inter-day accuracy range	92 – 110%	92 – 110%
Inter-day precision range	3 – 11%	3 – 11%
Bench-top stability (hrs)	24	24
Autosampler stability (hrs)	48	48
Freeze-thaw stability (cycles)	3	3
Long-term stability (days)	105	105
Specificity (i.e., matrix effects)	No net matrix effects	No net matrix effects

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Table S3. Recovery of 16-3-16 (analyte) from the aqueous cellular matrix

	Bligh/Dyer extraction			***Octanol*** extraction		
Theoretical concentration	**Extracted cells** (mean ± SD, ng/mL)	**Spiked extracted cells** (mean ± SD, ng/mL)	Recovery (%)	**Extracted cells** (mean ± SD, ng/mL)	**Spiked extracted cells** (mean ± SD, ng/mL)	Recovery (%)
16-3-16						
50 ng/mL (LLOQ)	48.24 ± 5.73	49.38 ± 3.74	97.7	36.38 ± 5.02	52.21 ± 4.74	69.7
150 ng/mL (LQC)	147.19 ± 13.61	150.53 ± 15.31	97.8	114.51 ± 8.72	159.26 ± 11.38	71.9
375 ng/mL (MQC)	371.57 ± 51.33	383.46 ± 47.32	96.9	277.87 ± 33.16	385.06 ± 37.92	72.2
4375 ng/mL (HQC)	4359.45 ± 359.41	4368.17 ± 212.57	100.2	3210.64 ± 179.11	4528.22 ± 182.33	70.9

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Table S4. Intra- and inter-day accuracy and precision in the analysis of 16-3-16

Quality Control		Observed concentration (mean ± SD, ng/mL)	Accuracy (%)	Precision (%RSD)
INTRA-DAY:				
	Analysis Day (#)			
LLOQ: 50 ng/mL	Day 1	47.45 ± 4.22	94.9	8.9
	Day 2	49.61 ± 3.77	99.2	7.6
	Day 3	50.19 ± 3.31	100.4	6.6
LQC: 150 ng/mL	Day 1	160.95 ± 14.65	107.3	9.1
	Day 2	159.45 ± 13.39	106. 3	8.4
	Day 3	163.78 ± 14.58	109.2	8.9
MQC: 375 ng/mL	Day 1	343.88 ± 34.73	91.7	10.1
	Day 2	392.62 ± 33.37	104.7	8.5
	Day 3	336.75 ± 32.33	89.8	9.6
HQC: 4375 ng/mL	Day 1	4480.01 ± 456.96	102. 4	10.2
	Day 2	4536.88 ± 426.47	103.7	9.4
	Day 3	4738.13 ± 355.36	108.3	7.5
INTER-DAY:				
	Concentration			
LLQC	50 ng/mL	55.05 ± 5.84	110.1	10.6
LQC	150 ng/mL	145.955 ± 11.09	97.3	7.6
MQC	375 ng/mL	393.03 ± 34.19	104.8	8.7
HQC	4375 ng/mL	4475.63 ± 434.14	102.3	9.7

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Table S5. Stability of the 16-3-16 gemini surfactant within the sample matrix

Quality Control	Storage condition/ period	Observed concentrations (mean ± SD, ng/mL)	Accuracy (%)	Precision (%RSD)
LQC: 150 ng/mL	0 h	150.31 ± 11.57	100.2	7.7
	24 h on bench top	164.42 ± 10.69	109.6	6.5
	Three freeze/thaw cycles	135.45 ± 14.22	90.3	10.5
	–20 ˚C for 105 days (LT)	134.85 ± 8.23	89.9	6.1
	48 h extract in autosampler	144.15 ± 13.84	96.1	9.6
MQC: 375 ng/mL	0 h	362.63 ± 34.45	96.7	9.5
	24 h on bench top	353.62 ± 34.31	94.3	9.7
	Three freeze/thaw cycles	338.63 ± 32.17	90.3	9.5
	–20 ˚C for 105 days (LT)	345.38 ± 36.26	92.1	10.5
	48 h extract in autosampler	399.75 ± 37.58	106.6	9.4
HQC: 4375 ng/mL	0 h	4147.51 ± 443.78	94.8	10.7
	24 h on bench top	4379.38 ± 381.01	100.1	8.7
	Three freeze/thaw cycles	4038.13 ± 415.93	92.3	10.3
	–20 ˚C for 105 days (LT)	4239.38 ± 390.02	96.9	9.2
	48 h extract in autosampler	4392.51 ± 347.01	100.4	7.9

LT (long term): –20 ˚C for 105 days

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