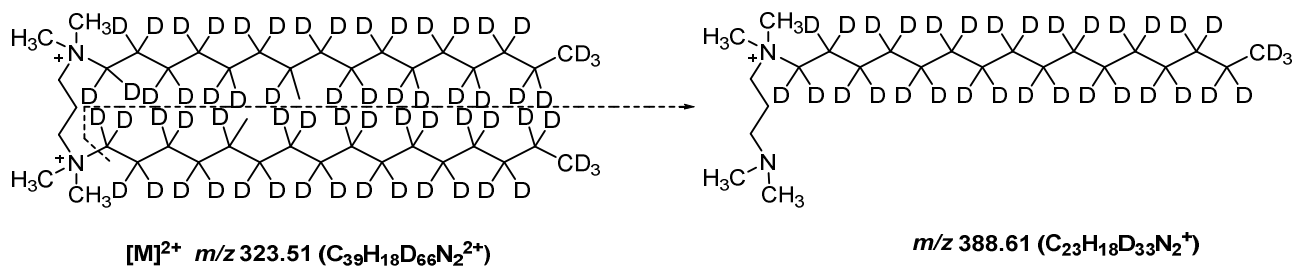
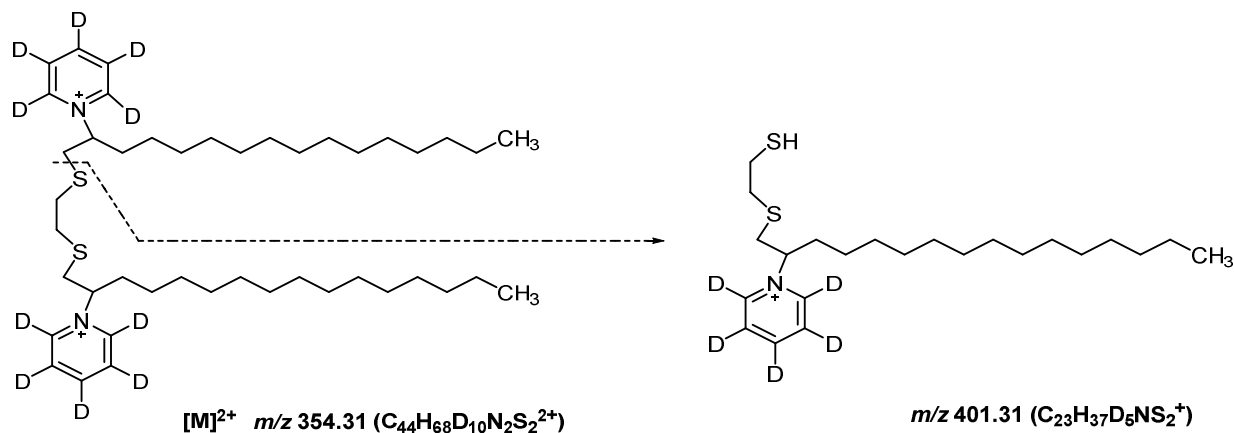


A 16-3-16-D₆₆



B 16(Py)-S-2-S-(Py)16-D₁₀



C 16-7N(GK)-16-D₄

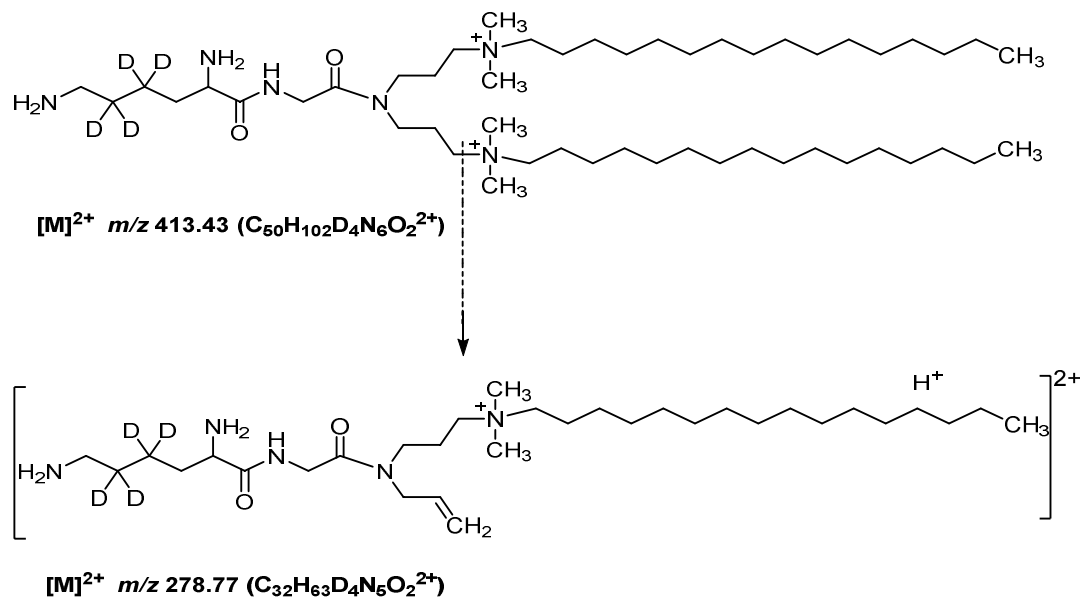


Figure S1. (A) Structure of 16-3-16-D₆₆ and the monitored product ion, (B) structure of 16(Py)-S-2-S-(Py)16-D₁₀ and the monitored product ion, and (C) structure of 16-7N(GK)-16-D₄ and the monitored product ion.

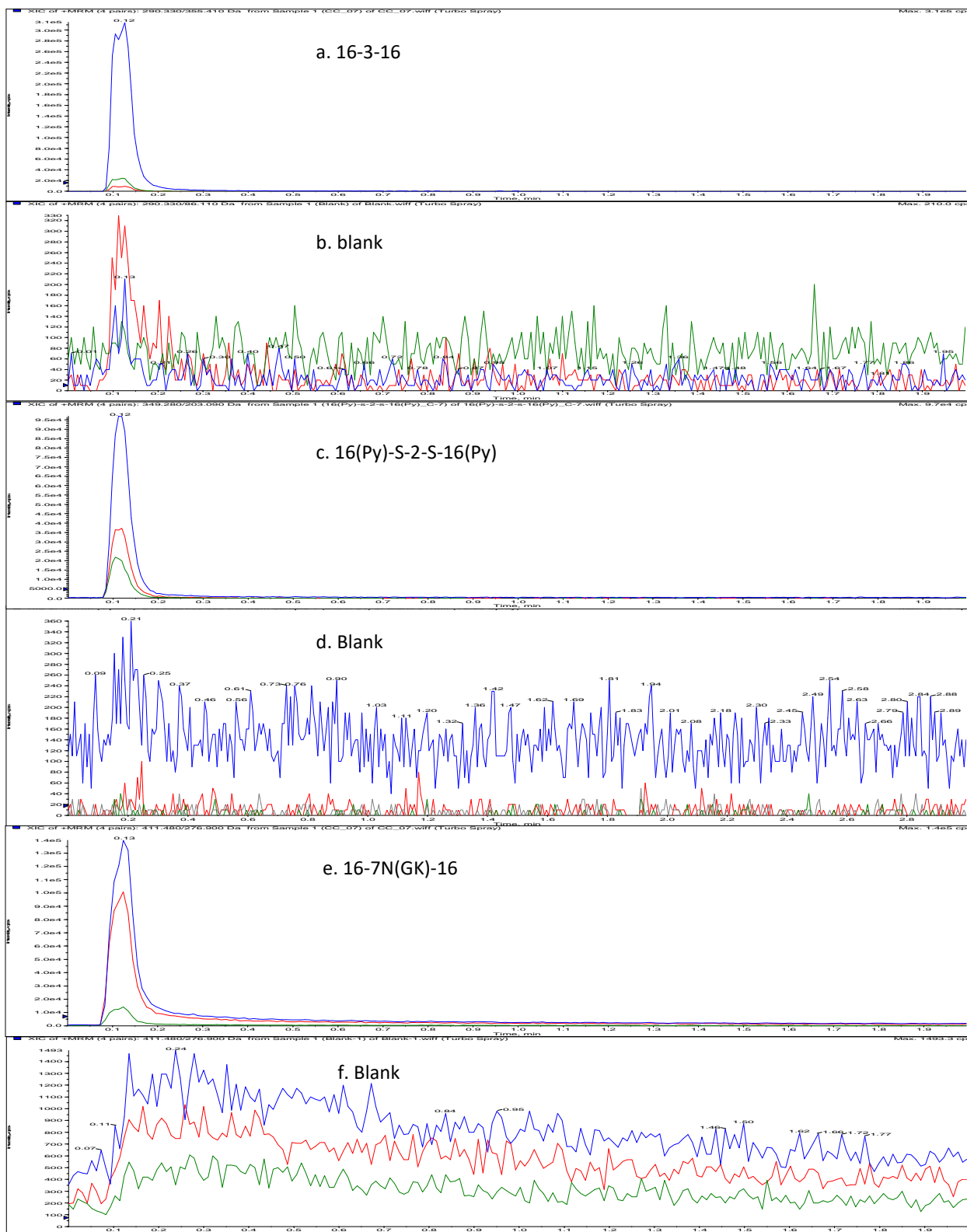


Figure S2. Illustration of no carry over. FIA-MS/MS chromatograms of the highest curve point of (a) 16-3-16, (c) 16(Py)-S-2-S-16(Py) and (e) 16-7N(GK)-16, and the following blank samples of (b) 16-3-16, (d) 16(Py)-S-2-S-16(Py) and (f) 16-7N(GK)-16.

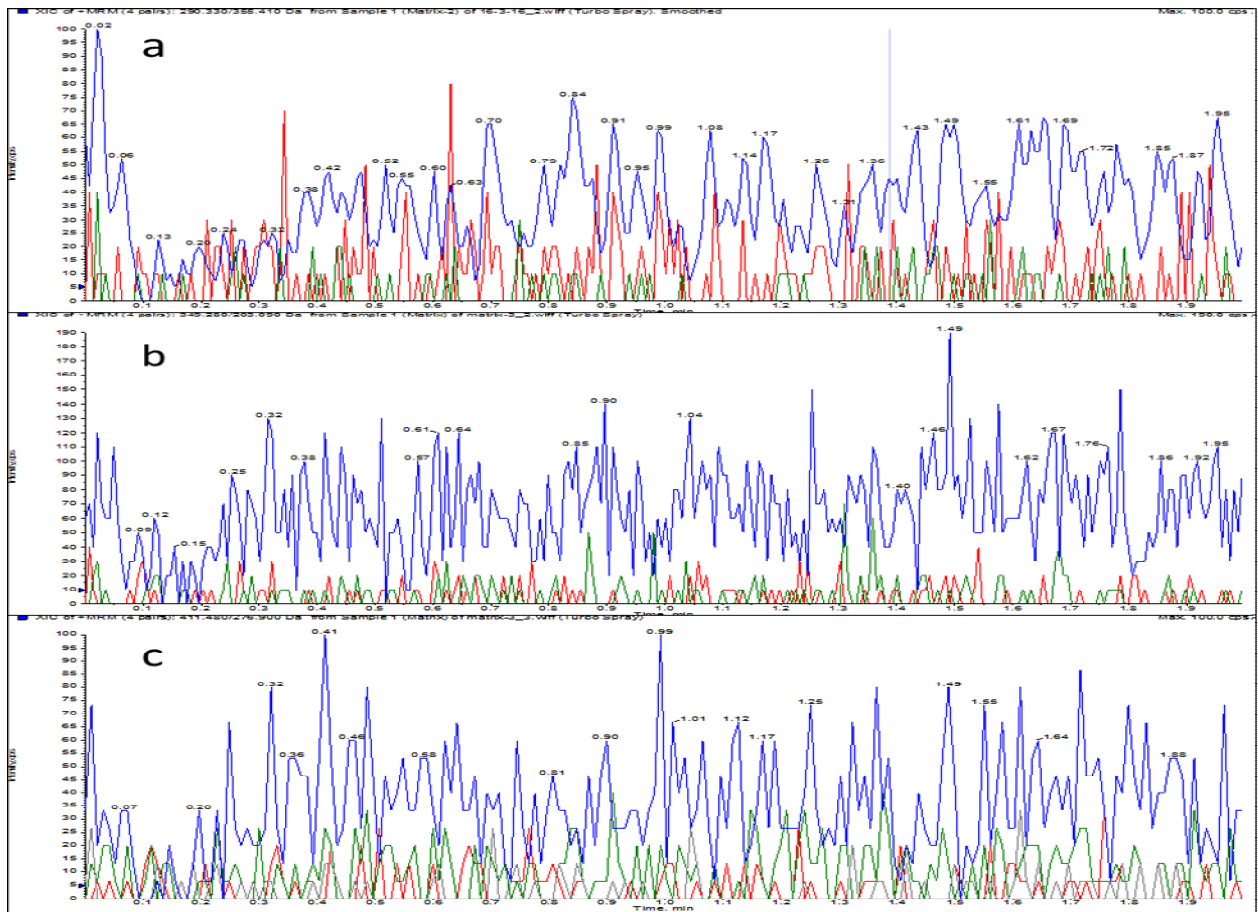


Figure S3. FIA-MS/MS chromatogram of blank cell matrix for MRM transitions: (a) 16-3-16, (b) 16(Py)-S-2-S-16(Py) and (c) 16-7N(GK)-16

Table S1. Matrix effect of gemini surfactants: 16-3-16, 16(Py)-S-2-S-16(Py) and 16-7N(GK)-16 at various concentrations

Sample	Replicates	Matrix effect (Mean± SD)		
		16-3-16	16(Py)-S-2-S-16(Py)	16-7N(GK)-16
LQC	3	47.1%±1.5%	42.9%±4.9%	31.9%±1.5%
MQC	3	33.4%±5.8%	35.4%±6.0%	40.5%±1.5%
HQC	3	44.0%±5.2%	36.1%±2.4%	34.6%±2.5%
Mean	3	41.5%±7.2%	40.2%±7.8%	35.7%±4.4%

Table S2. Stability of gemini surfactants 16-3-16 and 16(Py)-S-2-S-16(Py) at various conditions

Samples	Stability	Measured concentration (Mean ± SD, nM)		Precision		Accuracy	
		16-3-16	16(Py)-S-2-S-16(Py)	16-3-16	16(Py)-S-2-S-16(Py)	16-3-16	16(Py)-S-2-S-16(Py)
LQC (30nM)	0h	32.1±1.1	29.0±0.3	3.4%	1.1%	106.9%	96.8%
	6 freeze-thaw	32.7±0.9	29.5±1.5	2.8%	5.1%	109.1%	98.4%
	8h (bench top)	33.2±0.3	32.2±0.9	0.8%	2.9%	110.8%	107.4%
	20h (autosampler)	33.6±0.5	32.1±1.3	1.5%	4.1%	111.8%	107.1%
	90 days at -80°C	28.0±0.9	28.2±1.3	3.2%	4.6%	93.4%	93.9%
MQC (150nM)	0h	152.7±3.9	144.0±2.8	2.5%	2.0%	101.8%	96.0%
	6 freeze-thaw	162.7±3.8	139.2±5.5	2.3%	4.0%	108.4%	92.8%
	8h (bench top)	155.3±2.7	153.7±2.3	1.7%	1.5%	103.6%	102.4%
	20h (autosampler)	156.0±2.2	155.3±3.5	1.4%	2.3%	104.0%	103.6%
	90 days at -80°C	138.8±3.4	138.2±2.8	2.5%	2.0%	92.6%	92.1%
HQC (800nM)	0h	823.7±8.0	804.5±16.3	1.0%	2.0%	103.0%	100.6%
	6 freeze-thaw	860.5±24.3	790.3±15.4	2.8%	1.9%	107.6%	98.8%
	8h (bench top)	824.8±14.7	792.7±11.4	1.8%	1.4%	103.1%	99.1%
	20h (autosampler)	821.8±10.3	818.7±17.6	1.2%	2.2%	102.7%	102.3%
	90 days at -80°C	748.0±15.6	712.5±43.7	2.1%	6.1%	93.5%	89.1%

Table S3. Stability of gemini surfactants 16-7N(GK)-16 at various conditions

Samples	Stability	Measured concentration (Mean± SD, nM)	Precision	Accuracy
LQC (60nM)	0h	56.9±2.5	4.5%	94.9%
	6 freeze-thaw	62.7±4.2	6.7%	104.4%
	8h (bench top)	58.0±4.2	7.3%	96.7%
	20h (autosampler)	58.2±2.5	4.4%	96.9%
	90 days at -80°C	55.2±2.0	3.6%	91.9%
MQC (400nM)	0h	388.8±11.8	3.0%	97.2%
	6 freeze-thaw	394.2±23.9	6.1%	98.5%
	8h (bench top)	399.8±10.5	2.6%	100.0%
	20h (autosampler)	410.2±11.5	2.8%	102.5%
	90 days at -80°C	386.7±27.1	7.0%	96.7%
HQC (1600nM)	0h	1600.0±87.2	5.4%	100.0%
	6 freeze-thaw	1515.0±53.6	3.5%	94.7%
	8h (bench top)	1605.0±50.9	3.2%	100.3%
	20h (autosampler)	1618.3±91.7	5.7%	101.1%
	90 days at -80°C	1518.3±52.3	3.4%	94.9%