Supplementary Information for Development of Microflow Ultra High Performance Liquid Chromatography -Mass Spectrometry Metabolomic Assays for Analysis of Mammalian Biofluids

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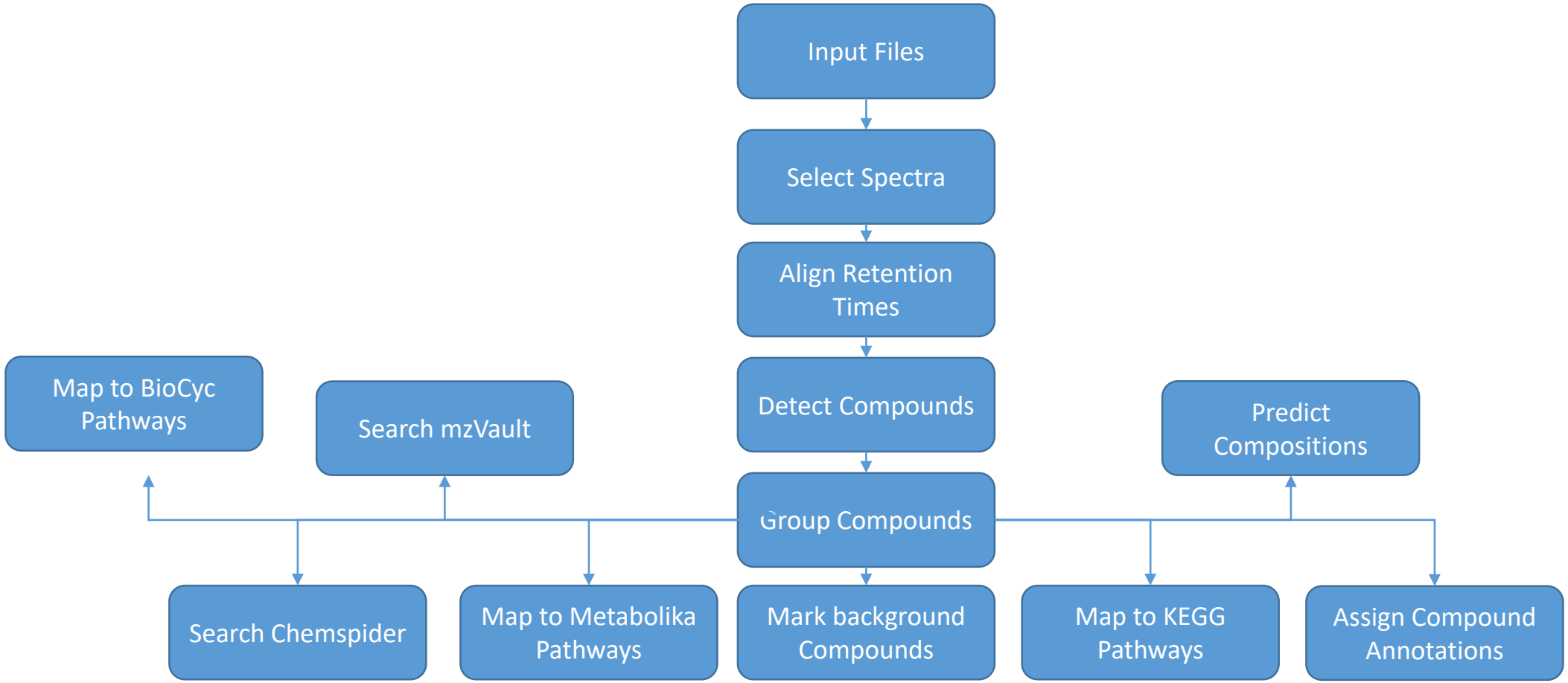
S1- serial dilution calculation table with the volumes of sample and solvent required for the serial dilution created as part of the sample preparation for all analytical methods.

Serial Dilution table			
Dilution	Sample (µL)	Solvent (µL)	Total volume (µL)
1/4	196.80	590.60	787.40
1/8	387.50	387.50	775.00
1/16	375.00	375.00	750.00
1/32	350.00	350.00	700.00
1/64	300.00	300.00	600.00
1/128	200.00	200.00	400.00

S2-A: Compound 3.0 processing workflow



S2-B: Compound 3.0 processing workflow - Select Spectra node parameters

Parameters of 'Select Spectra'

Show Advanced Parameters

1. General Settings	
Precursor Selection	Use MS(n - 1) Precursor
Provide Profile Spectra	Automatic
2. Spectrum Properties Filter	
Lower RT Limit	0
Upper RT Limit	0
First Scan	0
Last Scan	0
Ignore Specified Scans	
Lowest Charge State	0
Highest Charge State	0
Min. Precursor Mass	100 Da
Max. Precursor Mass	5000 Da
Total Intensity Threshold	0
Minimum Peak Count	1
3. Scan Event Filters	
Mass Analyzer	(Not specified)
MS Order	Any
Activation Type	(Not specified)
Min. Collision Energy	0
Max. Collision Energy	1000
Scan Type	Any
Polarity Mode	(Not specified)
4. Peak Filters	
S/N Threshold (FT-only)	1.5
5. Replacements for Unrecognized Properties	
Unrecognized Charge Replacements	1
Unrecognized Mass Analyzer Replacements	ITMS
Unrecognized MS Order Replacements	MS2
Unrecognized Activation Type Replacements	CID
Unrecognized Polarity Replacements	+
Unrecognized MS Resolution@200 Replacemer	60000
Unrecognized MSn Resolution@200 Replacem	30000

S2-C: Compound 3.0 processing workflow - Align Retention Times node parameters

Parameters of 'Align Retention Times'

Show Advanced Parameters

1. General Settings	
Alignment Model	Adaptive curve
Maximum Shift [min]	0.1
Mass Tolerance	5 ppm

S2-D: Compound 3.0 processing workflow - Detect Compounds node parameters

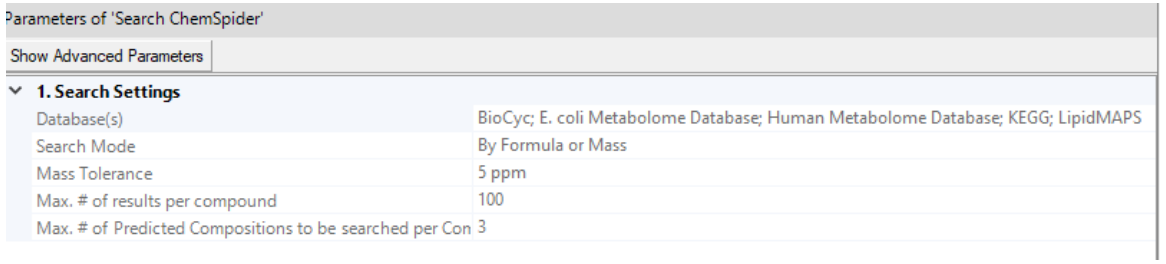
Parameters of 'Detect Compounds'

Show Advanced Parameters

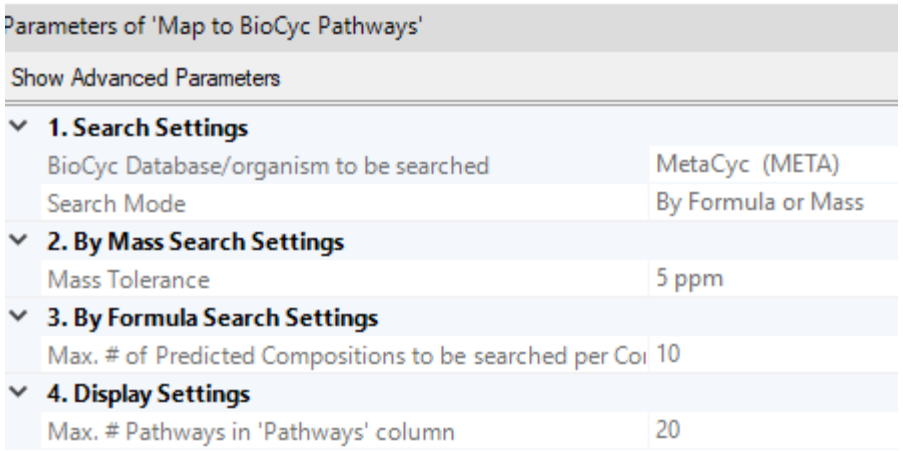
1. General Settings	
Mass Tolerance [ppm]	5 ppm
Intensity Tolerance [%]	20
S/N Threshold	3
Min. Peak Intensity	500000
Ions	[2M+ACN+H]+1; [2M+ACN+Na]+1; [2M+FA-H]-1; [2M+H]+
Min. Element Counts	C H
Max. Element Counts	C200 H600 Br3 Cl4 K2 N10 Na2 O15 P3 S5

S2-E: Compound 3.0 processing workflow Group Compounds node Parameters

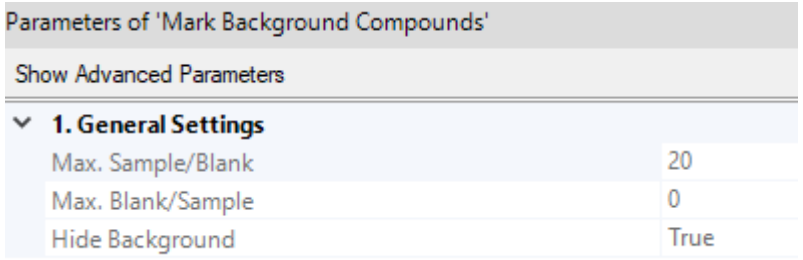
S2-G: Compound 3.0 processing workflow Search ChemSpider node Parameters



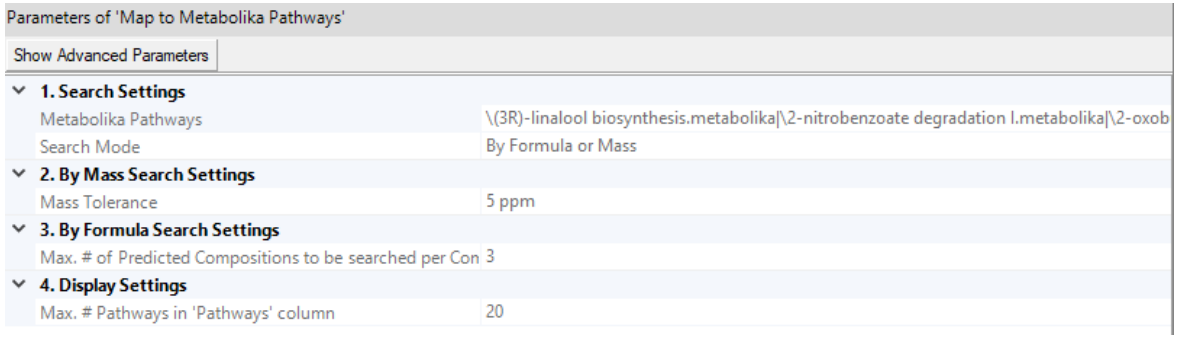
S2-F: Compound 3.0 processing workflow Map to BioCyc Pathways node Parameters



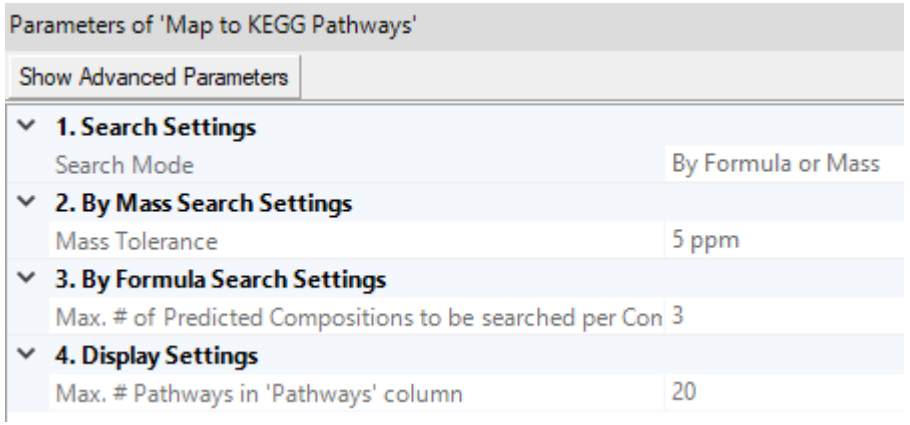
S2-H: Compound 3.0 processing workflow -Mark Background Compounds node Parameters



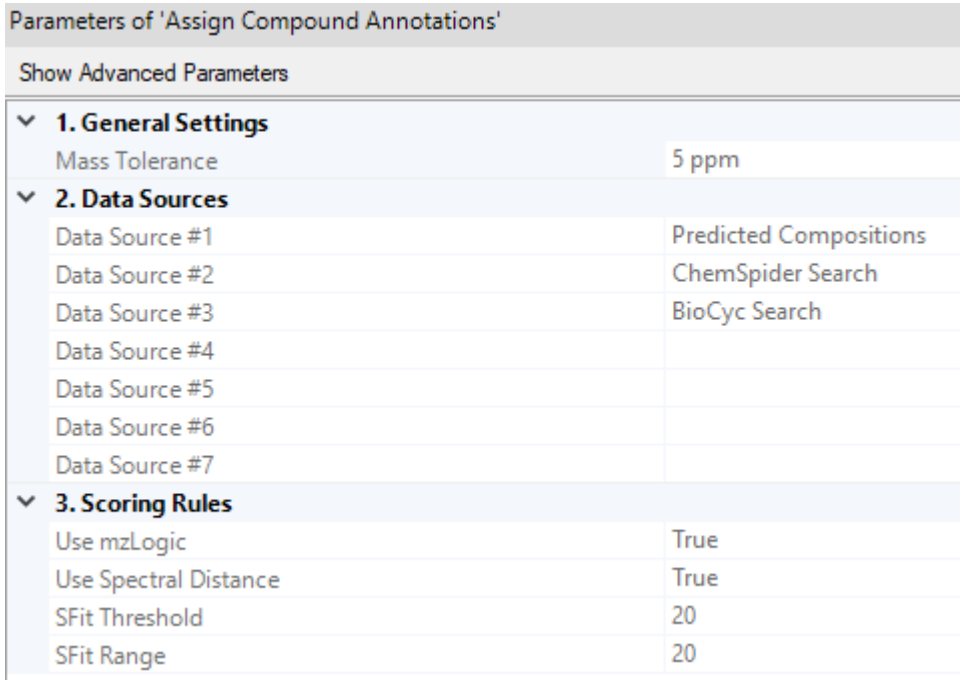
S2-I: Compound 3.0 processing workflow- Map to Metabolika Pathways node Parameters



S2-J: Compound 3.0 processing workflow – Map to KEGG pathways node Parameters



S2-K: Compound 3.0 processing workflow – Assign Compound Annotations node Paramters



S2-L: Compound 3.0 processing workflow – Predict Compositions node Parameters

Parameters of 'Predict Compositions'

Show Advanced Parameters

1. Prediction Settings	
Mass Tolerance	5 ppm
Min. Element Counts	C H
Max. Element Counts	C200 H600 Br3 Cl4 N10 O18 P3 S5
Min. RDBE	0
Max. RDBE	40
Min. H/C	0.1
Max. H/C	4
Max. # Candidates	10
2. Pattern Matching	
Intensity Tolerance [%]	20
Intensity Threshold [%]	0.1
S/N Threshold	3
Use Dynamic Recalibration	True
3. Fragments Matching	
Use Fragments Matching	True
Mass Tolerance	5 ppm
S/N Threshold	3

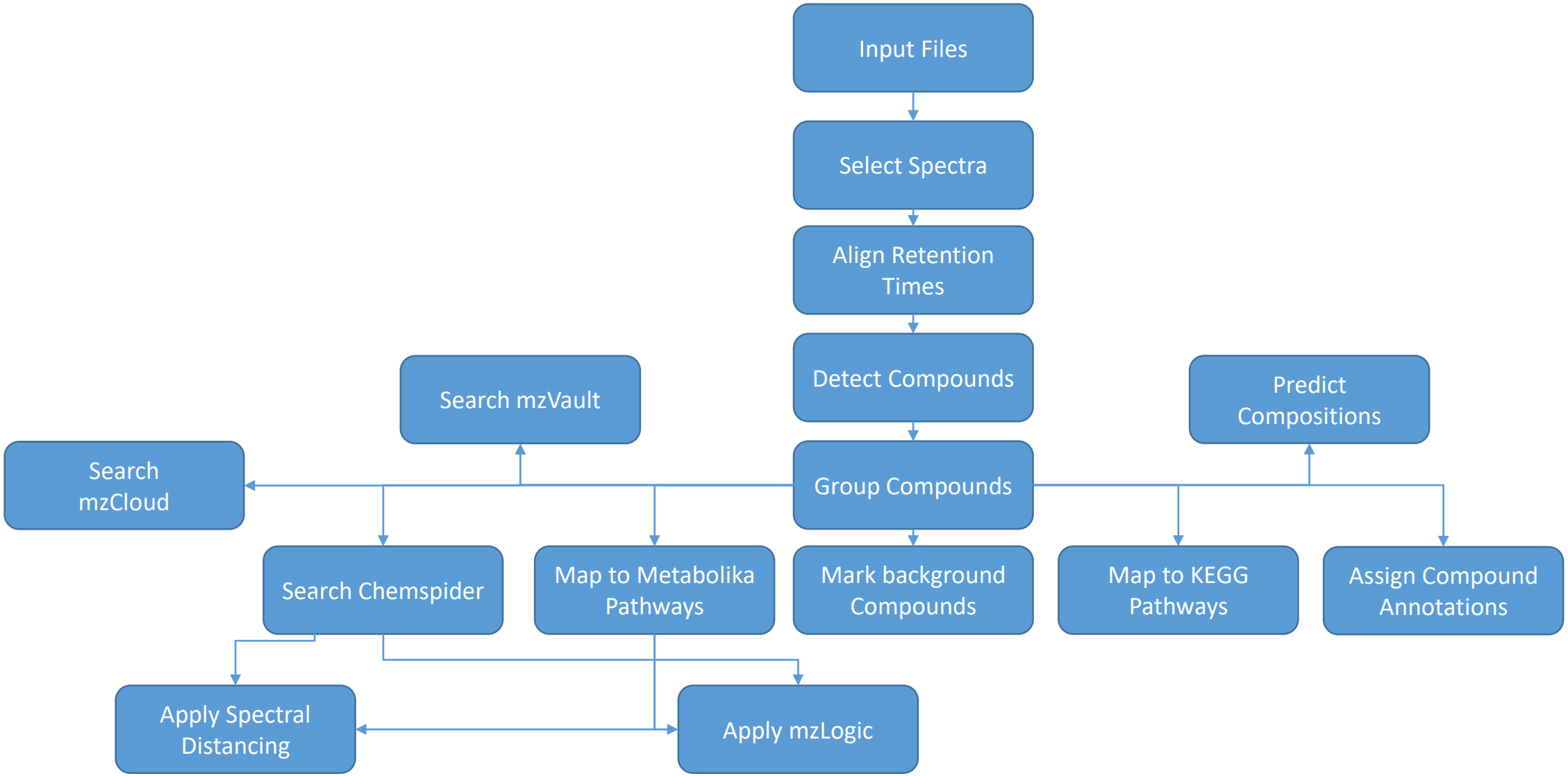
S2-M: Compound 3.0 processing workflow – Search mzVault node Parameters

Parameters of 'Search mzVault'

Show Advanced Parameters

1. Search Settings	
mzVault Library	\20190513_MetaSci_POS_Will.db
Compound Classes	All
Match Ion Activation Type	True
Match Ion Activation Energy	Match with Tolerance
Ion Activation Energy Tolerance	20
Match Ionization Method	True
Apply Intensity Threshold	True
Precursor Mass Tolerance	10 ppm
Match Analyzer Type	True
Search Algorithm	HighChem HighRes
Match Factor Threshold	50
RT Tolerance [min]	2
Use Retention Time	False

S3-A: Compound 3.1 processing workflow



S3-B: Compound 3.1 processing workflow - Select Spectra node parameters

Parameters of 'Select Spectra'

Show Advanced Parameters

1. General Settings	
Precursor Selection	Use MS(n - 1) Precursor
Provide Profile Spectra	Automatic
2. Spectrum Properties Filter	
Lower RT Limit	0
Upper RT Limit	0
First Scan	0
Last Scan	0
Ignore Specified Scans	
Lowest Charge State	0
Highest Charge State	0
Min. Precursor Mass	0 Da
Max. Precursor Mass	5000 Da
Total Intensity Threshold	0
Minimum Peak Count	1
3. Scan Event Filters	
Mass Analyzer	(Not specified)
MS Order	Any
Activation Type	(Not specified)
Min. Collision Energy	0
Max. Collision Energy	1000
Scan Type	Any
Polarity Mode	(Not specified)
4. Peak Filters	
S/N Threshold (FT-only)	1.5
5. Replacements for Unrecognized Properties	
Unrecognized Charge Replacements	1
Unrecognized Mass Analyzer Replacements	ITMS
Unrecognized MS Order Replacements	MS2
Unrecognized Activation Type Replacements	CID
Unrecognized Polarity Replacements	+
Unrecognized MS Resolution@200 Replacements	60000
Unrecognized MSn Resolution@200 Replacements	30000

S3-C: Compound 3.1 processing workflow- Align Retention Times node parameters

Parameters of 'Align Retention Times'

Show Advanced Parameters

1. General Settings	
Alignment Model	Adaptive curve
Maximum Shift [mir	0.2
Mass Tolerance	5 ppm

S3-D: Compound 3.1 processing workflow - Detect Compounds node Parameters

Parameters of 'Detect Compounds'

Show Advanced Parameters

1. General Settings	
Mass Tolerance [ppm]	5 ppm
Intensity Tolerance [%]	30
S/N Threshold	3
Min. Peak Intensity	5000
Ions	[2M+ACN+H]+1; [2M+ACN+Na]+1; [2M+FA-H]-1; [2M+H]+1; [2M+K]+1;
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5

S3-E: Compound 3.1 processing workflow - Group Compounds node Parameters

Parameters of 'Group Compounds'

Show Advanced Parameters

1. Compound Consolidation	
Mass Tolerance	5 ppm
RT Tolerance [min]	0.2
2. Fragment Data Selection	
Preferred Ions	[M+H]+1; [M-H]-1

S3-F: Compound 3.1 processing workflow- Search ChemSpider node Parameters

Parameters of 'Search ChemSpider'

Show Advanced Parameters

1. Search Settings	
Database(s)	Human Metabolome Database; KEGG; LipidMAPS; MassBank
Search Mode	By Formula or Mass
Mass Tolerance	5 ppm
Max. # of results per compound	100
Max. # of Predicted Compositions to be searched per Compound	3

S3-G: Compound 3.1 processing workflow- Mark Background Compounds node Parameters

Parameters of 'Mark Background Compounds'

Show Advanced Parameters

1. General Settings	
Max. Sample/Blank	20
Max. Blank/Sample	0
Hide Background	True

S3-H: Compound 3.1 processing workflow- Map to Metabolika Pathways Parameters

Parameters of 'Map to Metabolika Pathways'

Show Advanced Parameters

1. Search Settings	
Metabolika Pathways	\(3R)-linalool biosynthesis.metabolika|\2-nitrobenzoate degradation I.metabolika|\2-oxobutanoa
Search Mode	By Formula or Mass
2. By Mass Search Settings	
Mass Tolerance	5 ppm
3. By Formula Search Settings	
Max. # of Predicted Compositions t	3
4. Display Settings	
Max. # Pathways in 'Pathways' colu	20

S3-I: Compound 3.1 processing workflow- Map to KEGG pathways node Parameters

Parameters of 'Map to KEGG Pathways'

Show Advanced Parameters

1. Search Settings	
Search Mode	By Formula and Mass
2. By Mass Search Settings	
Mass Tolerance	5 ppm
3. By Formula Search Settings	
Max. # of Predicted Compositions to be searched per Compound	3
4. Display Settings	
Max. # Pathways in 'Pathways' column	20

S3-J: Compound 3.1 processing workflow- Assign Compound Annotations node Parameters

Parameters of 'Assign Compound Annotations'

Show Advanced Parameters

1. General Settings	
Mass Tolerance	5 ppm
2. Data Sources	
Data Source #1	mzCloud Search
Data Source #2	Predicted Compositions
Data Source #3	MassList Search
Data Source #4	ChemSpider Search
Data Source #5	
Data Source #6	
Data Source #7	
3. Scoring Rules	
Use mzLogic	True
Use Spectral Distance	True
SFit Threshold	20
SFit Range	20

S3-K: Compound 3.1 processing workflow- Apply mzLogic node Parameters

Parameters of 'Apply mzLogic'

Show Advanced Parameters

1. Search Settings	
Max. # Compounds	0
Max. # mzCloud Similarity Results to consider per Compound	10
Match Factor Threshold	30

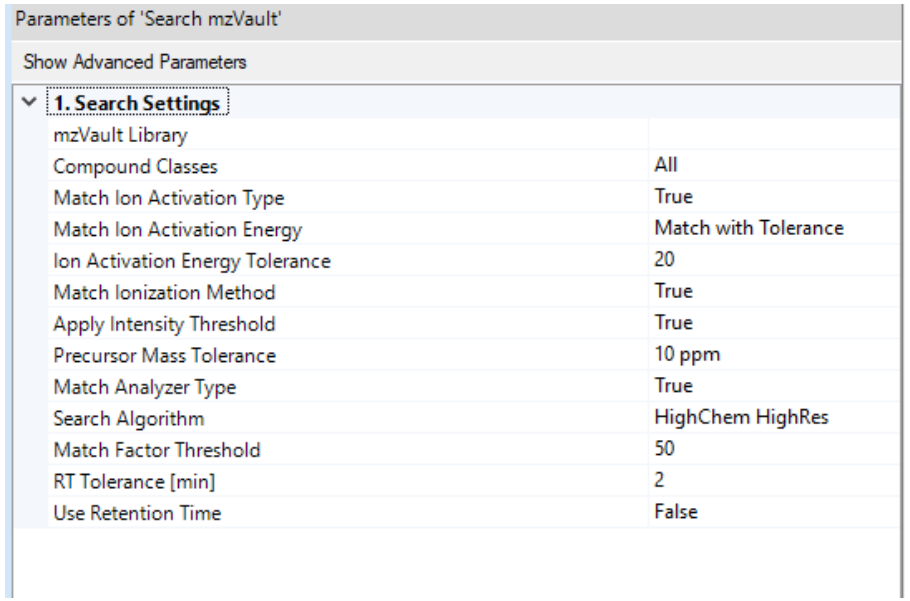
S3-L: Compound 3.1 processing workflow – Predict Compositions node Parameters

Parameters of 'Predict Compositions'

Show Advanced Parameters

1. Prediction Settings	
Mass Tolerance	5 ppm
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl4 N10 O18 P
Min. RDBE	0
Max. RDBE	40
Min. H/C	0.1
Max. H/C	3.5
Max. # Candidates	10
2. Pattern Matching	
Intensity Tolerance [%]	30
Intensity Threshold [%]	0.1
S/N Threshold	3
Use Dynamic Recalibration	True
3. Fragments Matching	
Use Fragments Matching	True
Mass Tolerance	5 ppm
S/N Threshold	3

S3-M: Compound 3.1 processing workflow- Search mzVault node Parameters

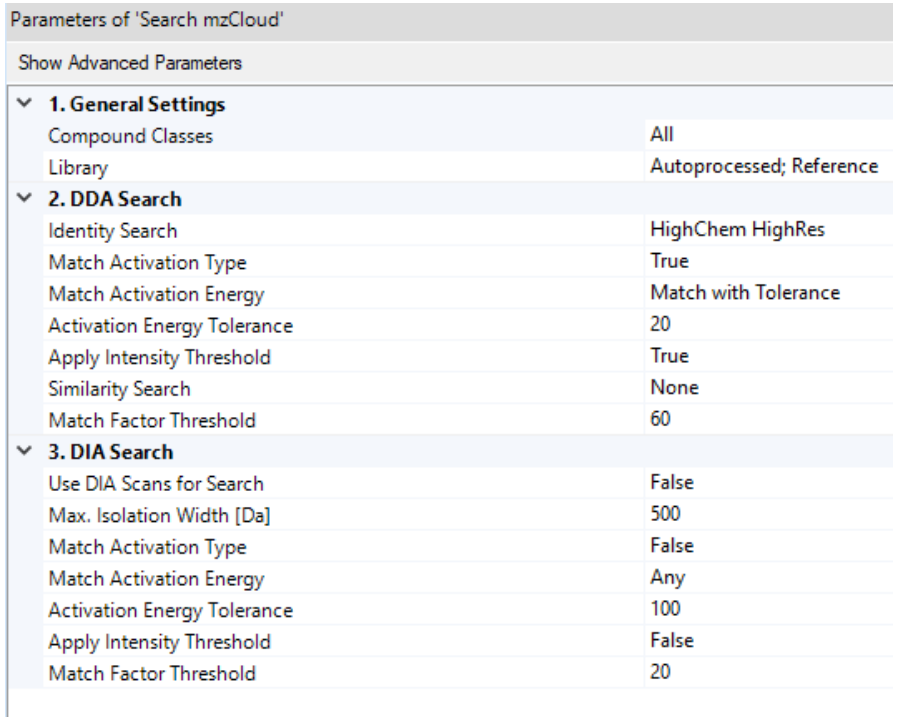


Parameters of 'Search mzVault'

Show Advanced Parameters

1. Search Settings	
mzVault Library	
Compound Classes	All
Match Ion Activation Type	True
Match Ion Activation Energy	Match with Tolerance
Ion Activation Energy Tolerance	20
Match Ionization Method	True
Apply Intensity Threshold	True
Precursor Mass Tolerance	10 ppm
Match Analyzer Type	True
Search Algorithm	HighChem HighRes
Match Factor Threshold	50
RT Tolerance [min]	2
Use Retention Time	False

S3-N:Compound 3.1 processing workflow- Search mzCloud node Parameters



Parameters of 'Search mzCloud'

Show Advanced Parameters

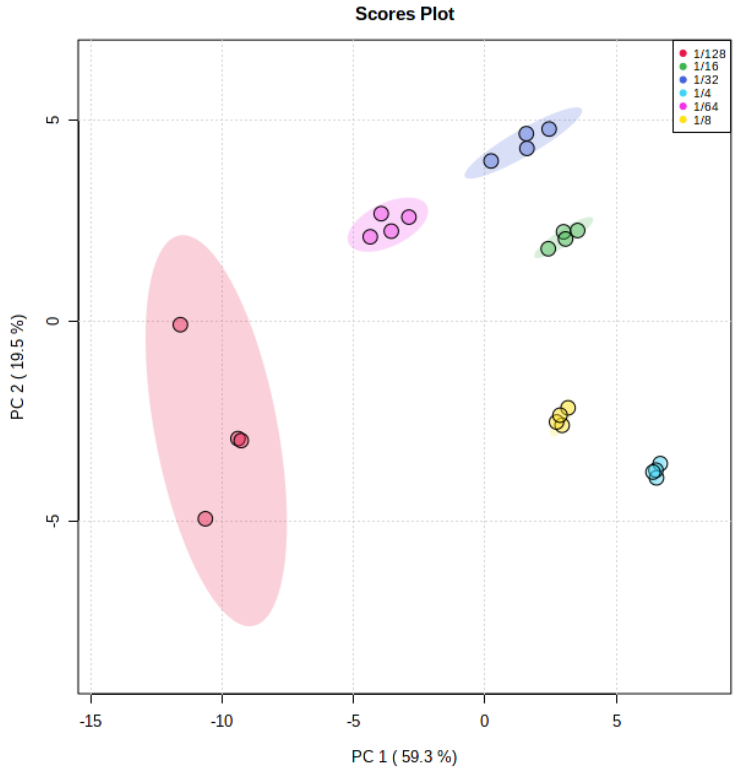
1. General Settings	
Compound Classes	All
Library	Autoprocessed; Reference
2. DDA Search	
Identity Search	HighChem HighRes
Match Activation Type	True
Match Activation Energy	Match with Tolerance
Activation Energy Tolerance	20
Apply Intensity Threshold	True
Similarity Search	None
Match Factor Threshold	60
3. DIA Search	
Use DIA Scans for Search	False
Max. Isolation Width [Da]	500
Match Activation Type	False
Match Activation Energy	Any
Activation Energy Tolerance	100
Apply Intensity Threshold	False
Match Factor Threshold	20

S3-O: Compound 3.1 processing workflow- Apply Spectral Distance node Parameters

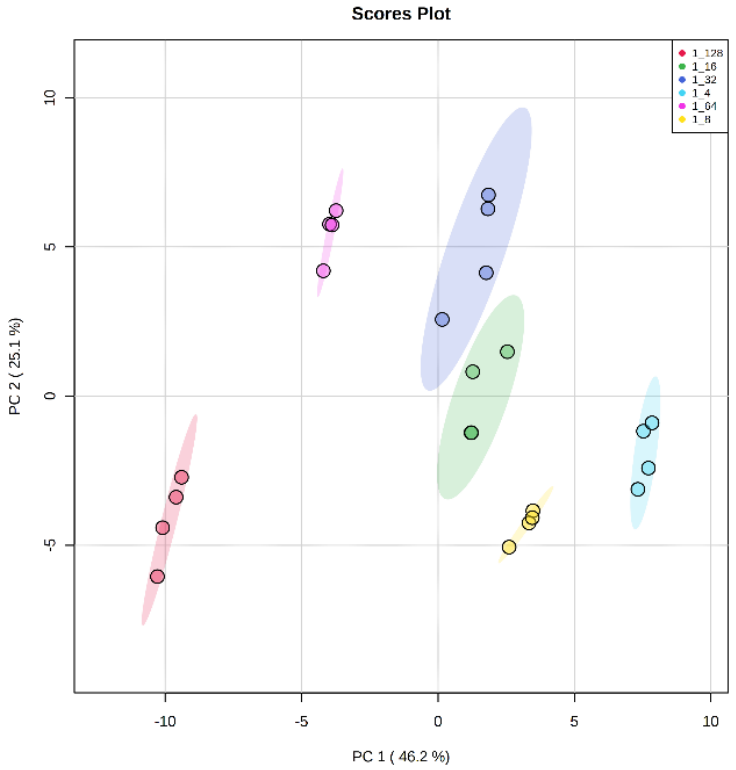
Show Advanced Parameters

1. General Settings	
Compound Classes	All
Library	Autoprocessed; Reference
2. DDA Search	
Identity Search	HighChem HighRes
Match Activation Type	True
Match Activation Energy	Match with Tolerance
Activation Energy Tolerance	20
Apply Intensity Threshold	True
Similarity Search	None
Match Factor Threshold	60
3. DIA Search	
Use DIA Scans for Search	False
Max. Isolation Width [Da]	500
Match Activation Type	False
Match Activation Energy	Any
Activation Energy Tolerance	100
Apply Intensity Threshold	False
Match Factor Threshold	20

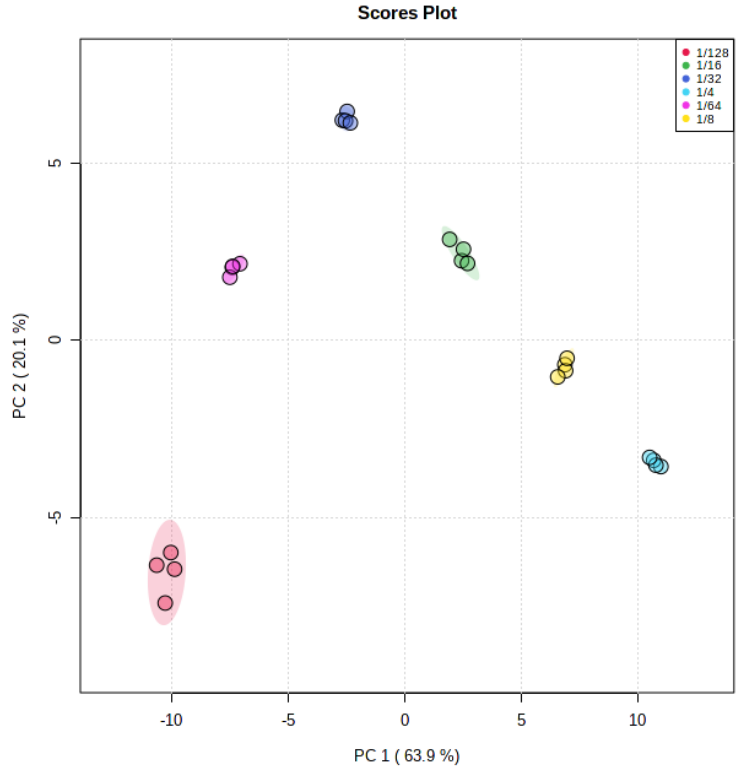
S4-A: Variation between different dilutions when applying the 2.1mm ID column to HILIC assays in negative ion mode using Plasma samples



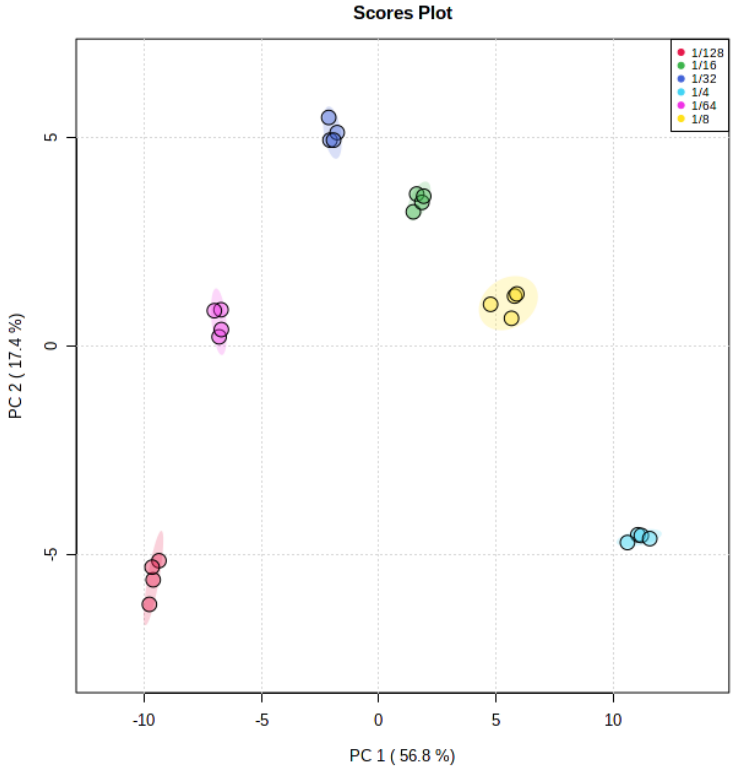
S4-B: Variation between different dilutions when applying the ID column to HILIC assays in negative ion mode using Plasma samples



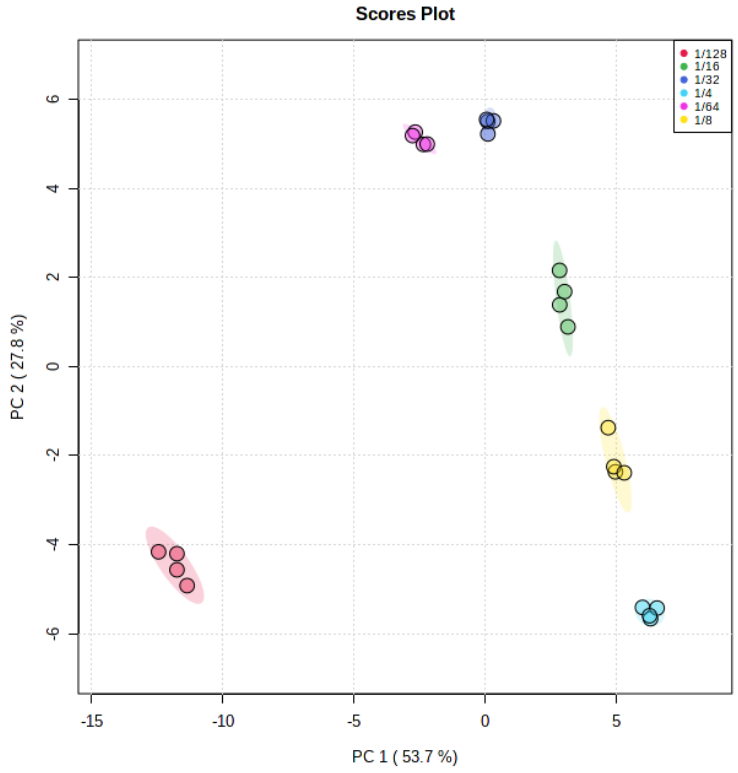
S5-A: Variation between different dilutions when applying the 2.1mm ID column to HILIC assays in positive ion mode using Plasma samples



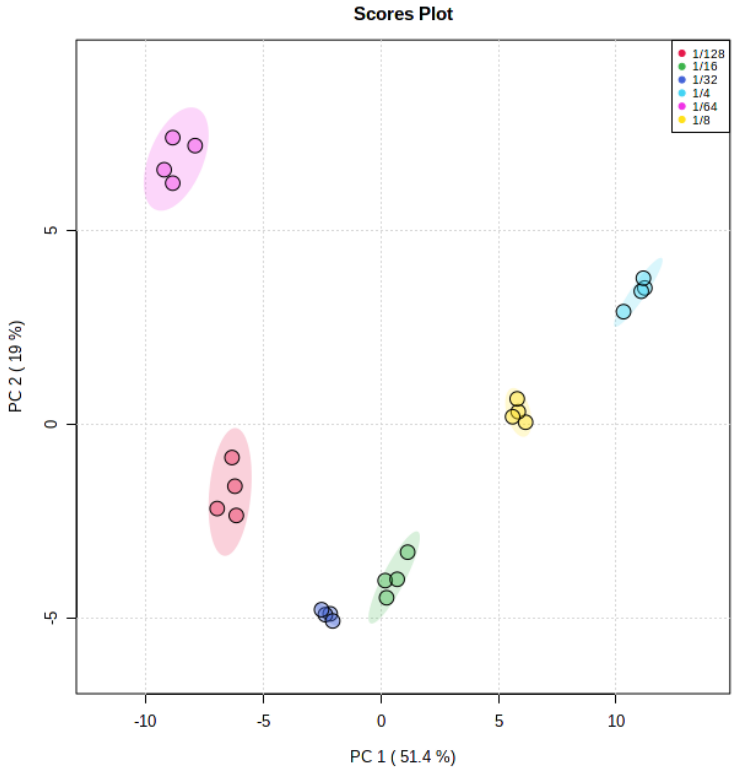
S5-B:Variation between different dilutions when applying the 1mm ID column to HILIC assays in positive ion mode using Plasma samples



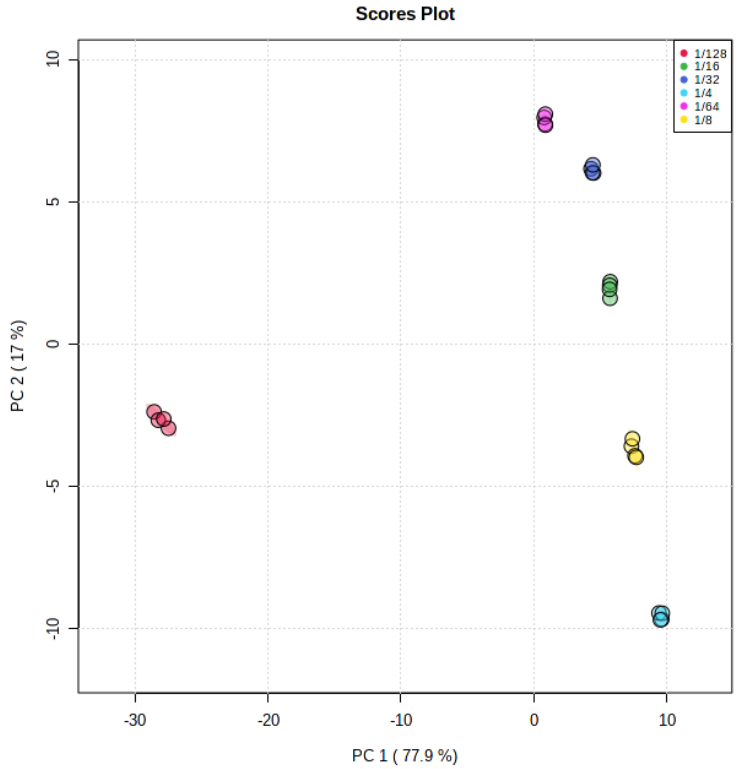
S6-A: Variation between different dilutions when applying the 2.1mm ID column to HILIC assays in negative ion mode using urine samples



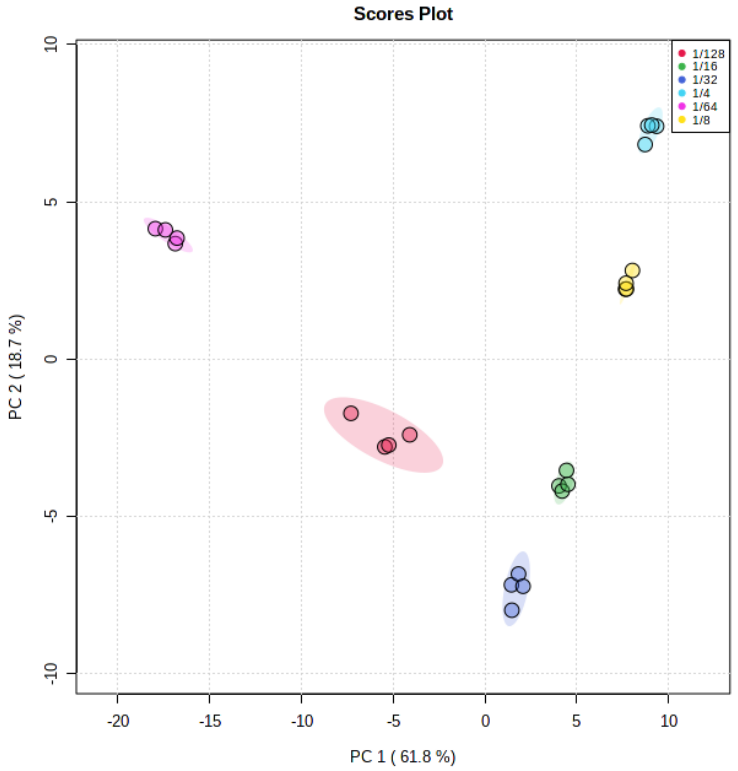
S6-B: Variation between different dilutions when applying the 1mm ID column to HILIC assays in negative ion mode using urine samples



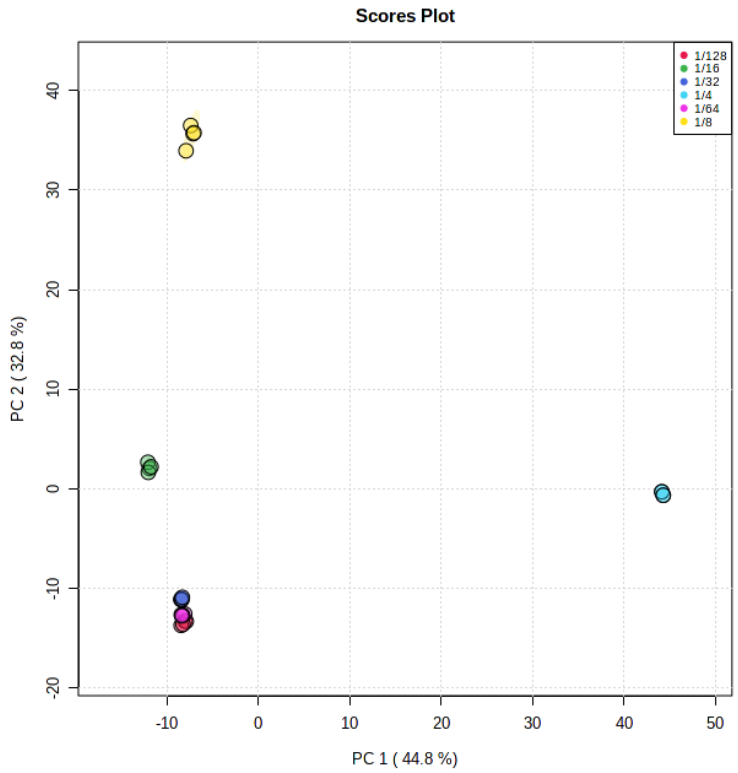
S7-A: Variation between different dilutions when applying the 2.1mm ID column to HILIC assays in positive ion mode using urine samples



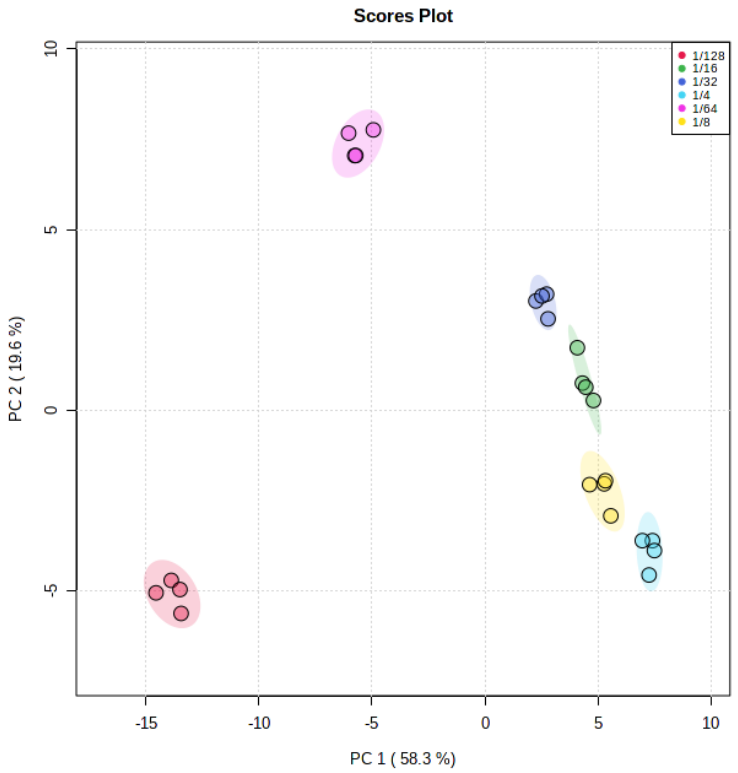
S7-B: Variation between different dilutions when applying the 1mm ID column to HILIC assays in positive ion mode using urine samples



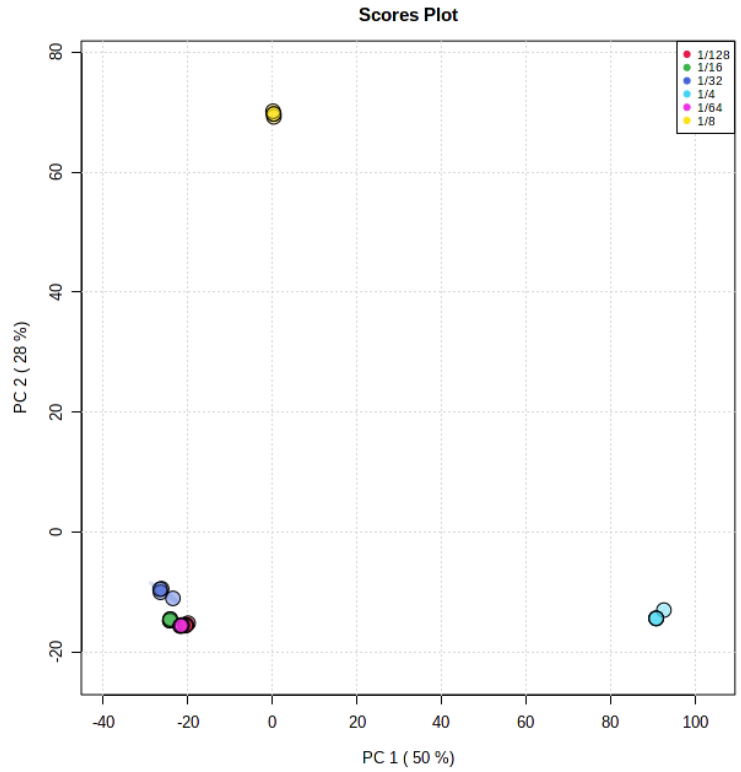
S8-A: Variation between different dilutions when applying the 2.1mm ID column to Lipidomic assays in negative ion mode using Plasma samples



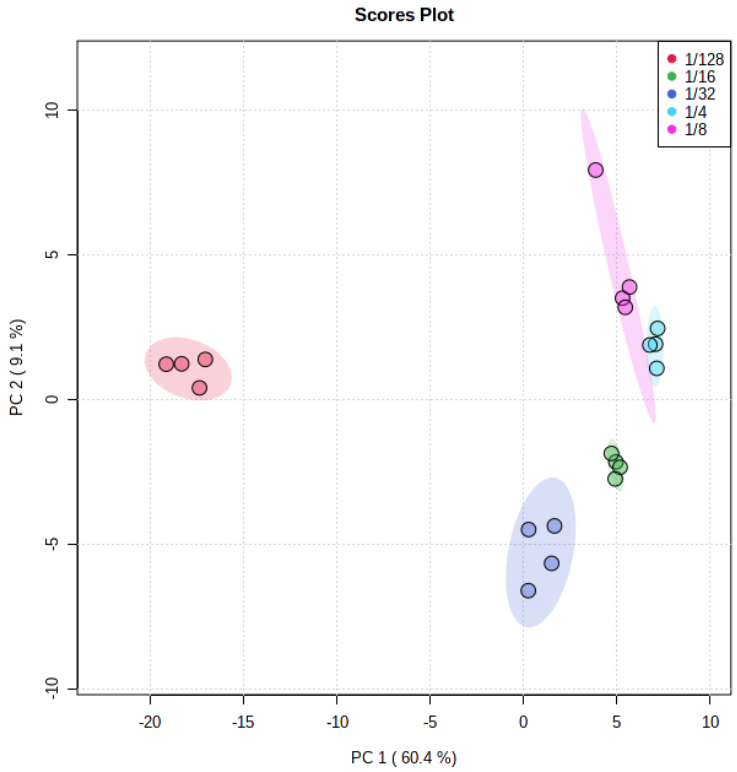
S8-B: Variation between different dilutions when applying the 1mm ID column to Lipidomic assays in negative ion mode using Plasma samples



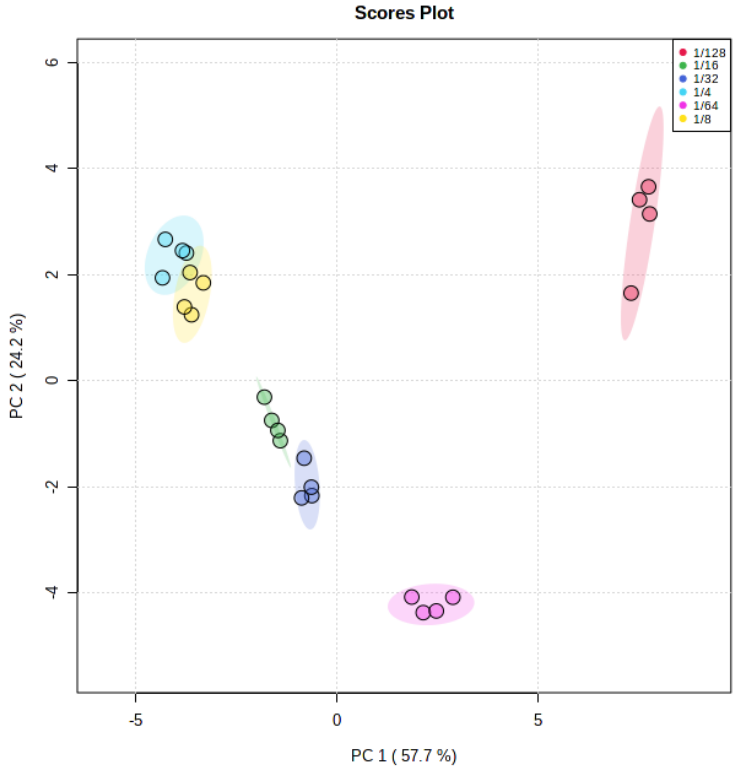
S9-A: Variation between different dilutions when applying the 2.1mm ID column to Lipidomic assays in positive ion mode using Plasma samples



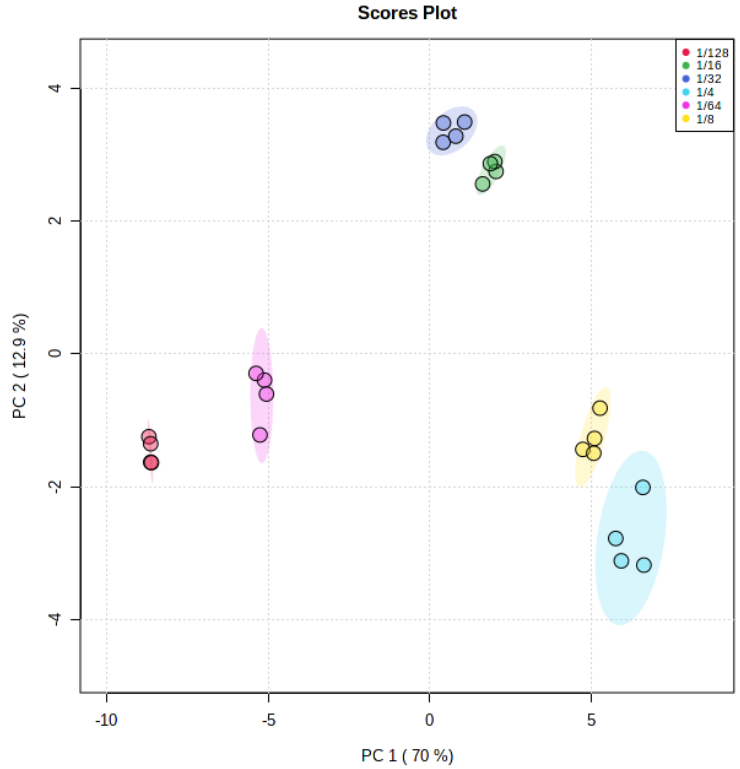
S9-B: Variation between different dilutions when applying the 1mm ID column to Lipidomic assays in positive ion mode using Plasma samples



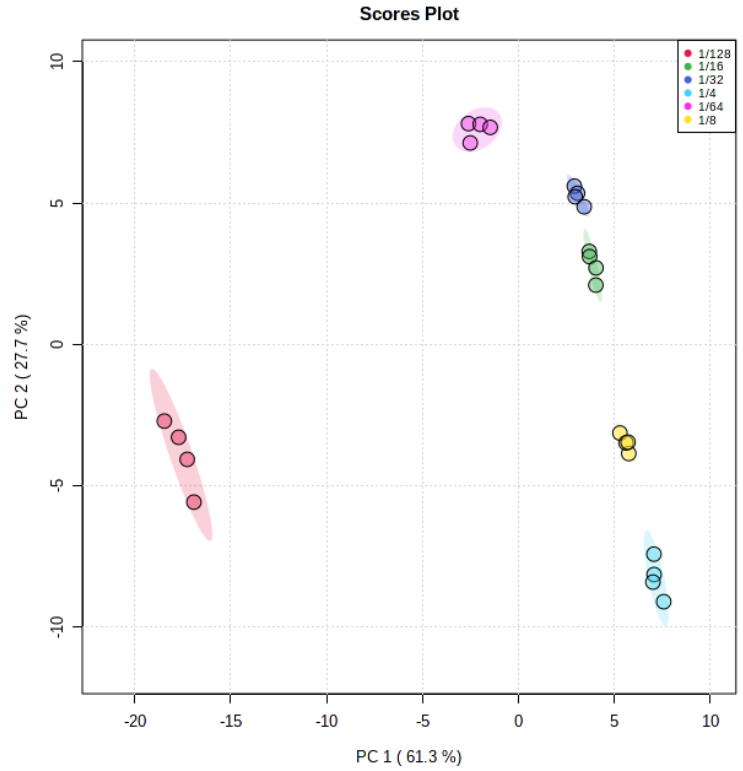
S10-A: Variation between different dilutions when applying the 2.1mm ID column to Reversed Phase aqueous assays in negative ion mode using urine samples



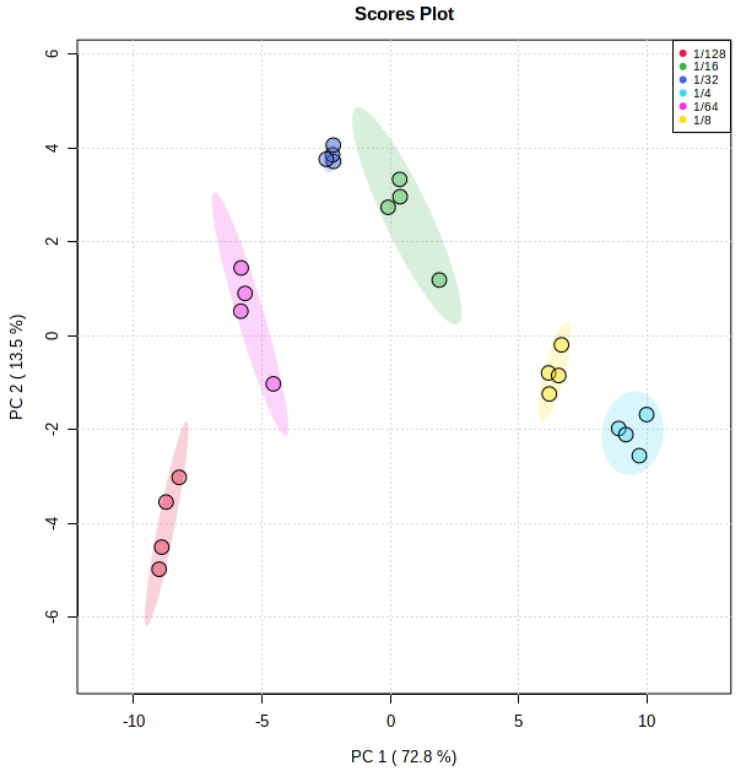
S10-B: Variation between different dilutions when applying the 1mm ID column to Reversed Phase aqueous assays in negative ion mode using urine samples



S11-A: Variation between different dilutions when applying the 2.1mm ID column to Reversed Phase aqueous assays in positive ion mode using urine samples



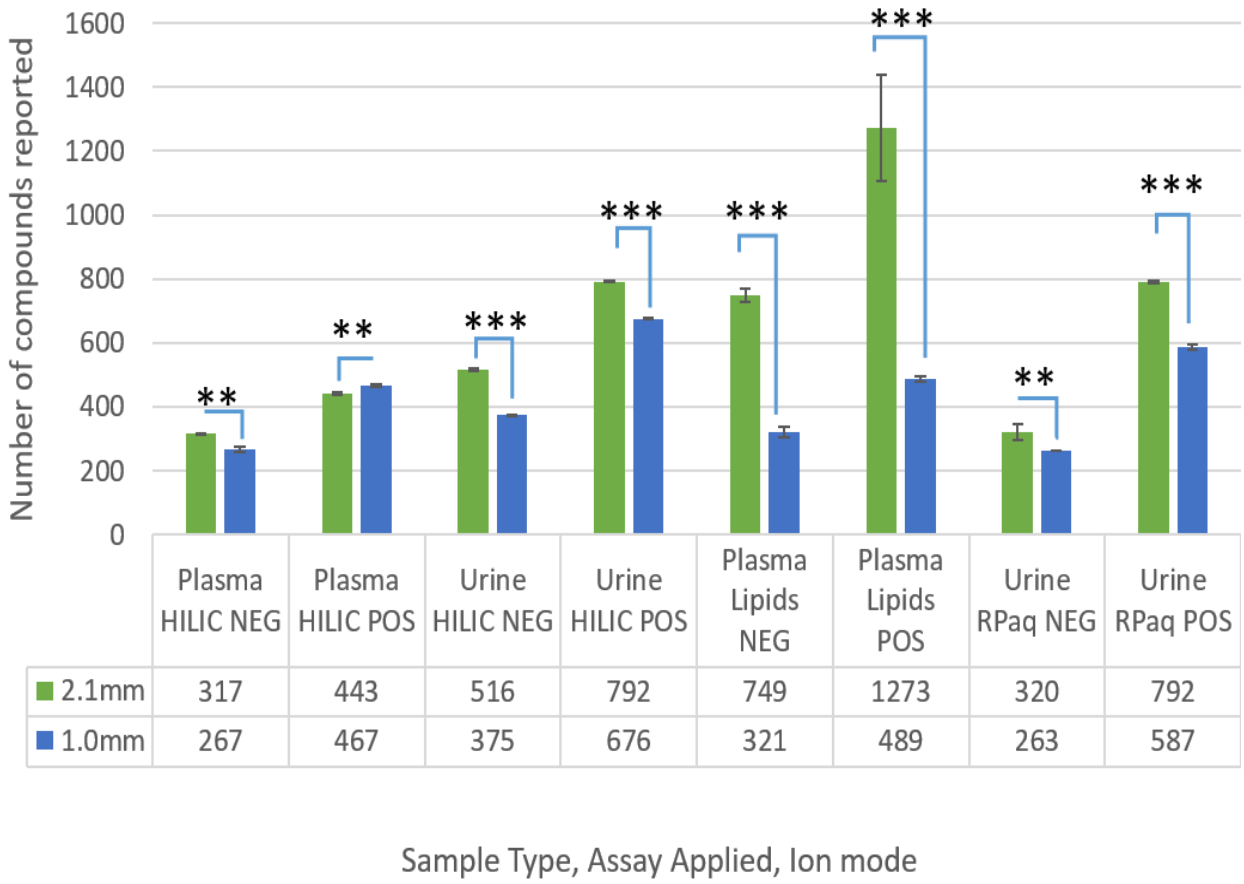
S11-B: Variation between different dilutions when applying the 1mm ID column to Reversed Phase aqueous assays in positive ion mode using urine samples



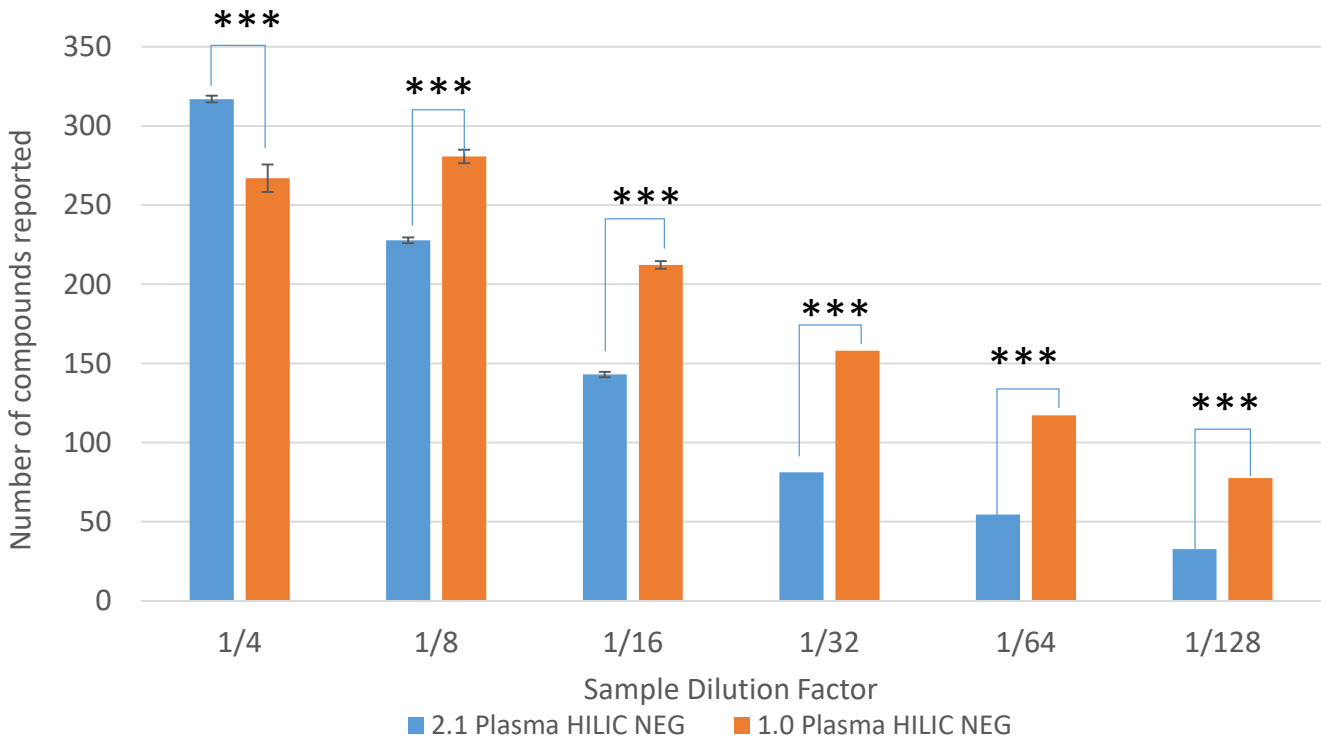
S12: RSD range in each Dilution for each assay before filtering

	1/4	1/8	1/16	1/32	1/64	1/128
2.1 Plasma HILIC NEG	0.27-83.4	0.20-98.2	0.14-111	0.52-84.5	1.0-79	1.93-55
1.0 Plasma HILIC NEG	0.23-136	0.28-138	0.19-123	0.006-126	0.65-68	0.075-136
2.1 Plasma HILIC POS	0.02-104	0.21-128	0.15-119	0.11-110	0.18-115	0.36-127
1.0 Plasma HILIC POS	0.08-107	0.003-122	0.13-119	0.24-139	0.31-87.9	0.31-109
2.1 Urine HILIC NEG	0.19-87.8	0.12-97.9	0.30-126	0.40-99.8	0.47-42.1	0.44-48.5
1.0 Urine HILIC NEG	0.1-122	0.02-143	0.09-104	0.1-99	0.55-105	0.7-112
2.1 Urine HILIC POS	0.21-140	0.17-137	0.23-133	0.21-169	0.07-99.8	0.05-115
1.0 Urine HILIC POS	0.003-131	0.19-114	0.23-124	0.21-144	0.2-134	0.2-132
2.1 Plasma LIPID NEG	0.21-140	0.17-137	0.23-133	0.21-170	0.07-99.8	0.05-115
1.0 Plasma LIPID NEG	0.02-192	0.12-194	0.31-193	0.07-168	0.09-191	0.04-166
2.1 Plasma LIPID POS	0.1-141	0.16-166	0.02-160	0.02-159	0.02-172	0.02-188
1.0 Plasma LIPID POS	0.13-185	0.08-196	0.18-154	0.003-158	0.52-150	0.43-190
2.1 Urine RPaq NEG	0.06-73.2	0.03-77.4	0.61-68	0.57-116	0.54-30.7	0.08-64.1
1.0 Urine RPaq NEG	0.45-86.5	0.85-84.0	0.91-68.9	0.46-49.3	1.34-85.1	0.44-37.3
2.1 Urine RPaq POS	0.08-120	0.02-95.4	0.39-136	0.29-149	0.07-117	1.00-127
1.0 Urine RPaq POS	0.11-139	0.20-120	0.07-112	0.39-114	0.04-129	0.65-129

S13: - The average number of reported compounds in human plasma and urine samples following a 1 in 4 dilution for each of three UHPLC-MS assays.
Compounds which were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. 2.1mm ID column (green) and 1.0mm ID column (Blue) *** has a significance of <0.01, ** has a significance of <0.05.



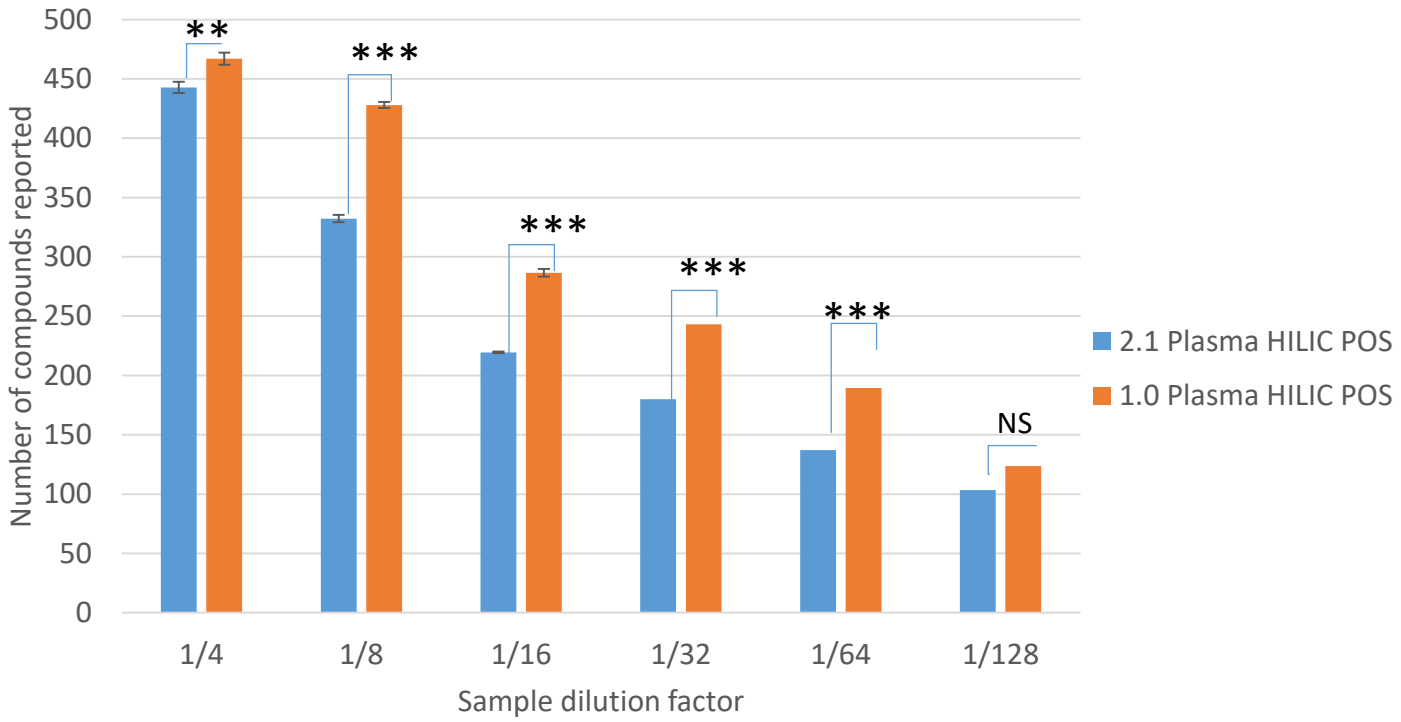
S14-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in negative ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds at each dilution was of a very significant (P<0.001)



S14-B: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in negative ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Plasma HILIC NEG	1.0 Plasma HILIC NEG
1/4	317	267
1/4 SD	2.12	8.79
1/8	228	281
1/8 SD	2.49	1.92
1/16	143	212
1/16 SD	1.87	4.21
1/32	81	158
1/32 SD	2.17	3.81
1/64	55	117
1/64 SD	1.66	2.38
1/128	33	78
1/128 SD	1.64	1.66

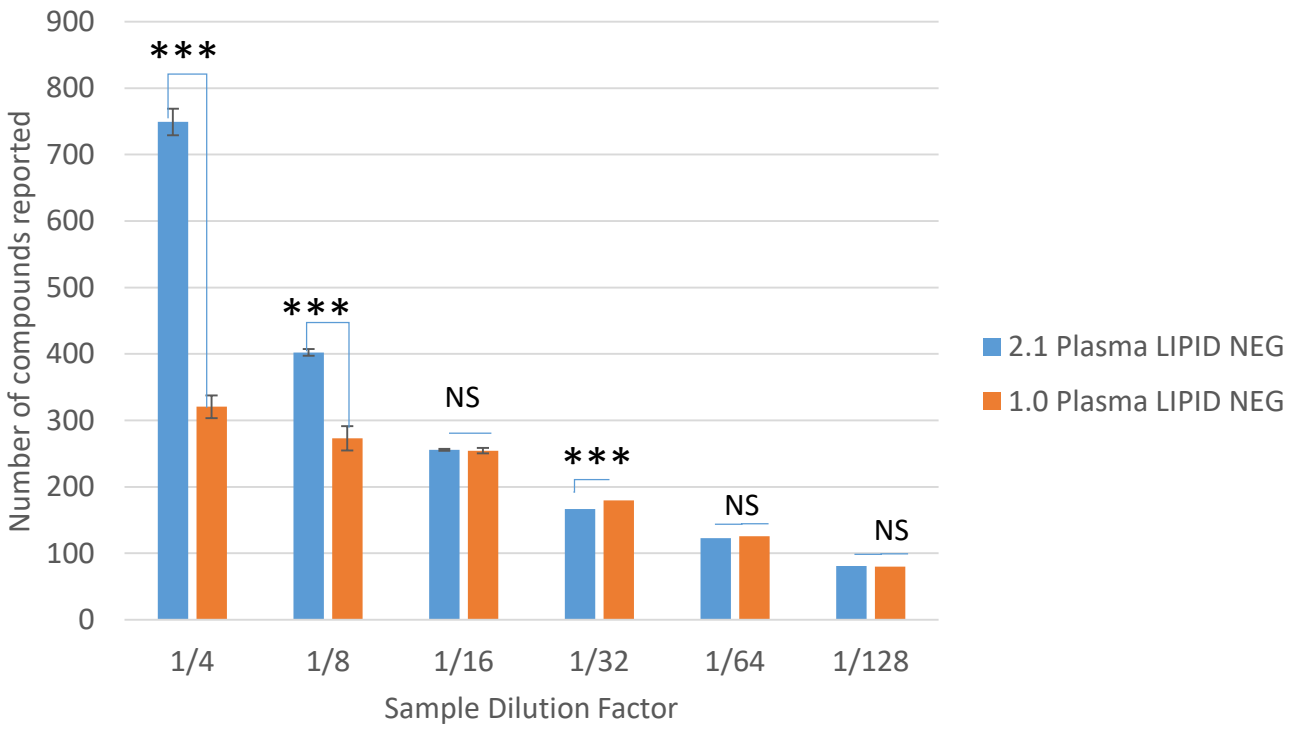
S15-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in positive ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds detected by each column ID was moderately significant (P<0.01), and then each dilution was of a very significant (P<0.001), until the final one which was not significant (P>0.05)



S15-B: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in positive ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Plasma HILIC POS	1.0 Plasma HILIC POS
1/4	443	467
1/4 SD	4.71	5.10
1/8	332	428
1/8 SD	2.77	2.12
1/16	220	287
1/16 SD	3.20	2.50
1/32	180	243
1/32 SD	3.00	1.41
1/64	137	190
1/64 SD	0.71	3.20
1/128	104	124
1/128 SD	3.50	0.87

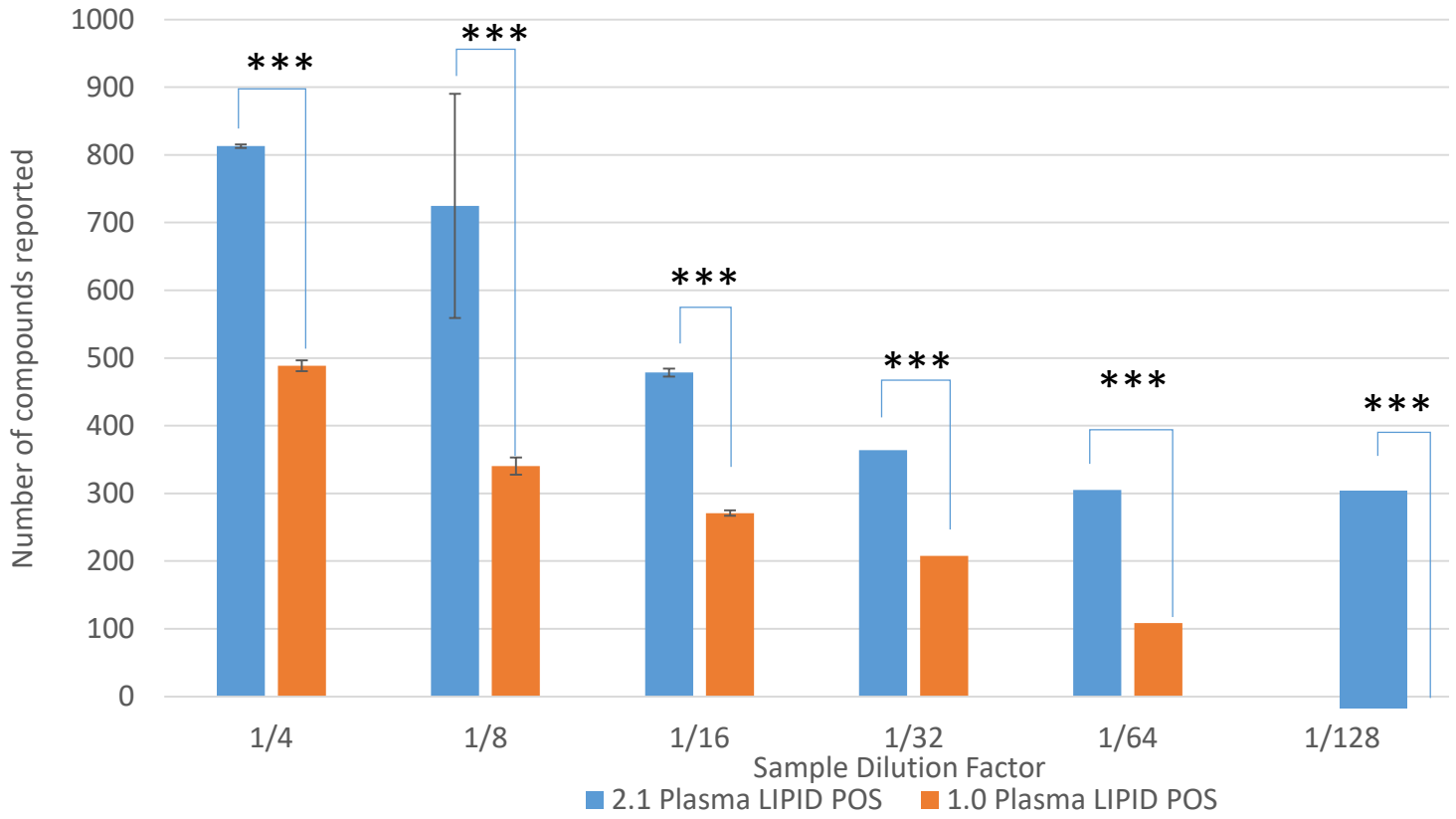
S16-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Lipidomic assays in negative ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds detected from each column ID was very significant (P<0.001), or not significant (P>0.05).



S15-6: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Lipidomic assays in negative ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Plasma LIPID NEG	1.0 Plasma LIPID NEG
1/4	749	321
1/4 SD	20.00	17.17
1/8	402	273
1/8 SD	8.70	5.79
1/16	256	254
1/16 SD	5.12	18.23
1/32	167	180
1/32 SD	3.11	1.66
1/64	123	126
1/64 SD	1.50	4.03
1/128	81	80
1/128 SD	0.71	2.74

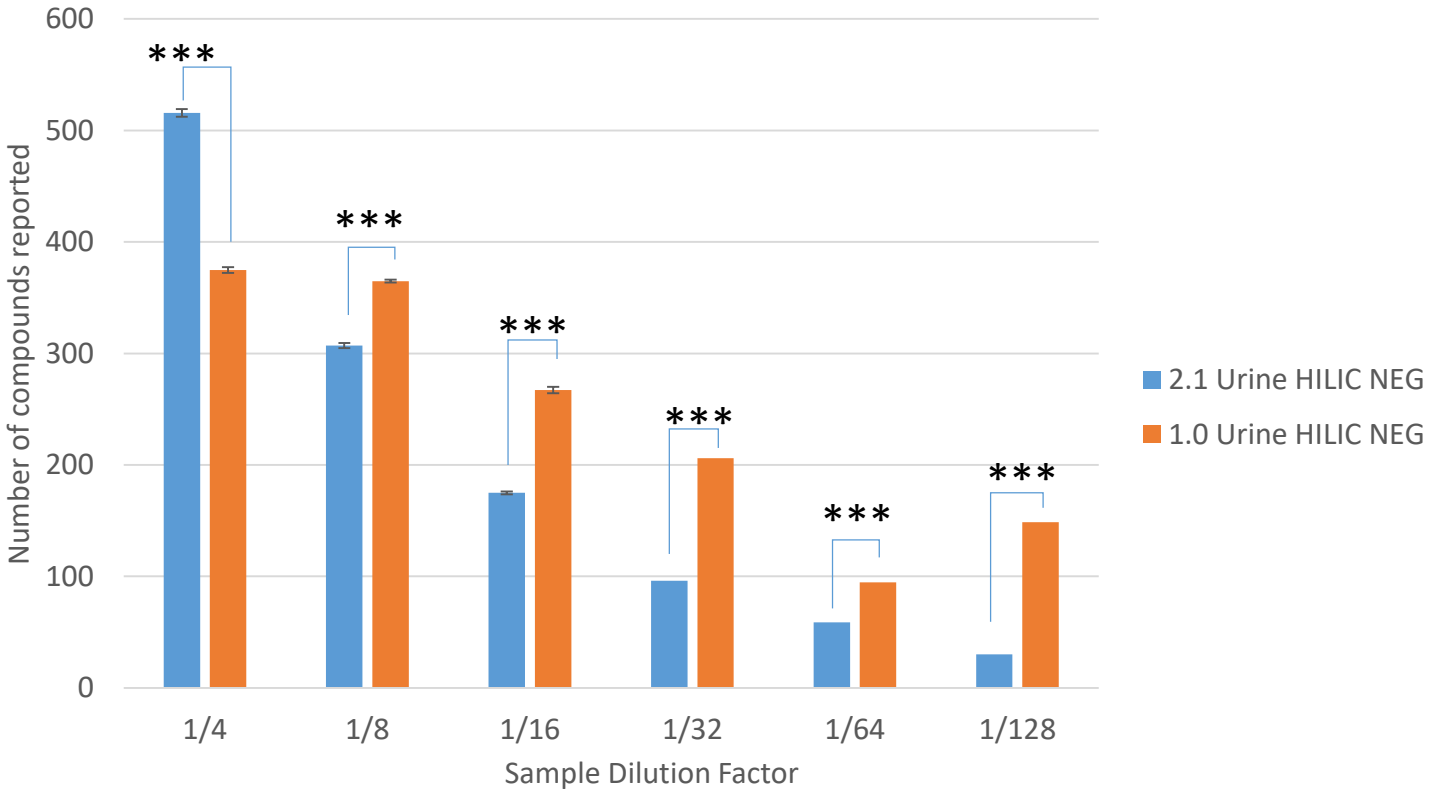
S17-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Lipidomic assays in positive ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds detected from each column ID was very significant (P<0.001).



S17-B: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Lipidomic assays in positive ion mode using Plasma samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Plasma LIPID POS	1.0 Plasma LIPID POS
1/4	1273	271
1/4 SD	2.05	7.89
1/8	813	340
1/8 SD	7.12	21.98
1/16	725	489
1/16 SD	165.55	12.75
1/32	479	208
1/32 SD	31.58	2.87
1/64	364	108
1/64 SD	6.04	4.15
1/128	305	NA
1/128 SD	2.92	NA

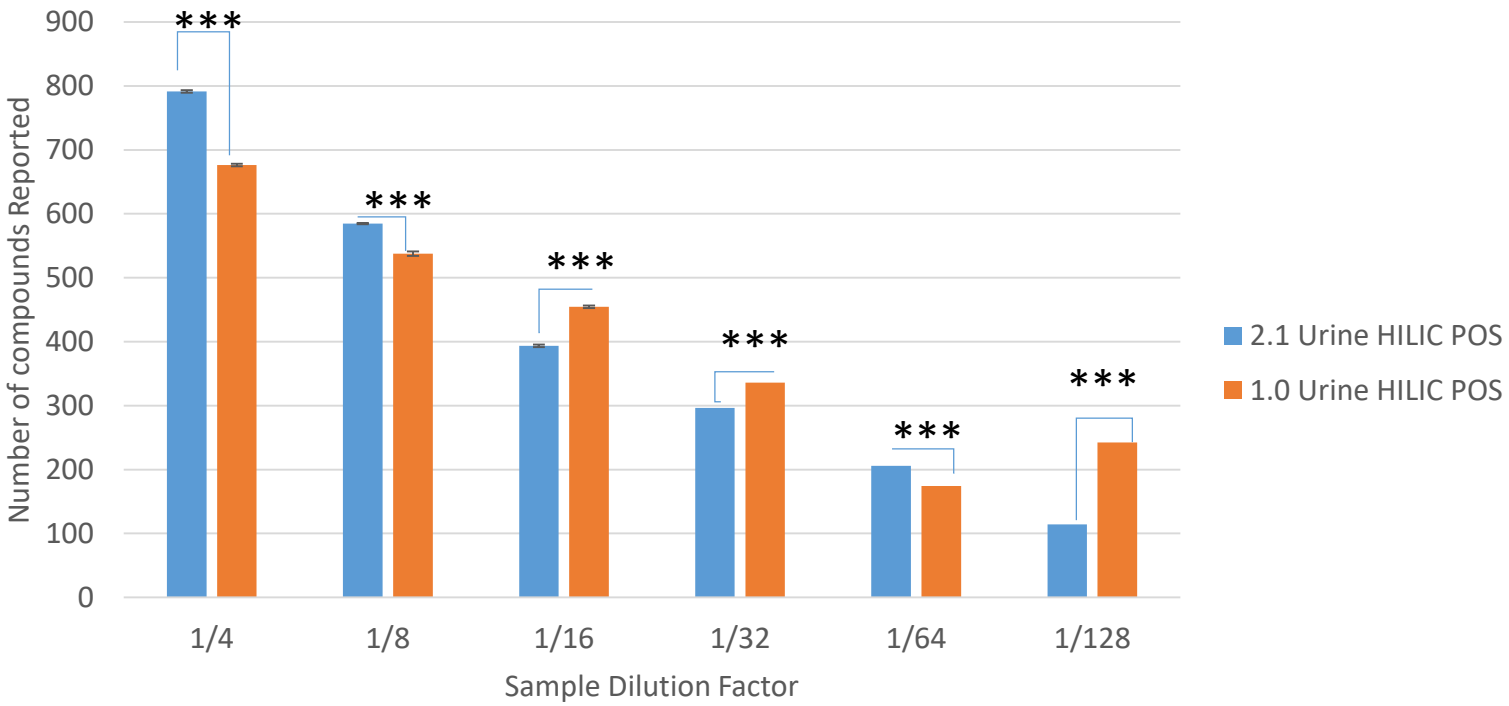
S18-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in negative ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds at each dilution was of a very significant (P<0.001)



S18-B: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in negative ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Urine HILIC NEG	1.0 Urine HILIC NEG
1/4	516	375
1/4 SD	3.49	2.49
1/8	307	365
1/8 SD	1.87	3.03
1/16	175	267
1/16 SD	2.24	1.30
1/32	96	206
1/32 SD	0.83	1.58
1/64	59	95
1/64 SD	1.30	2.86
1/128	30	149
1/128 SD	0.43	2.96

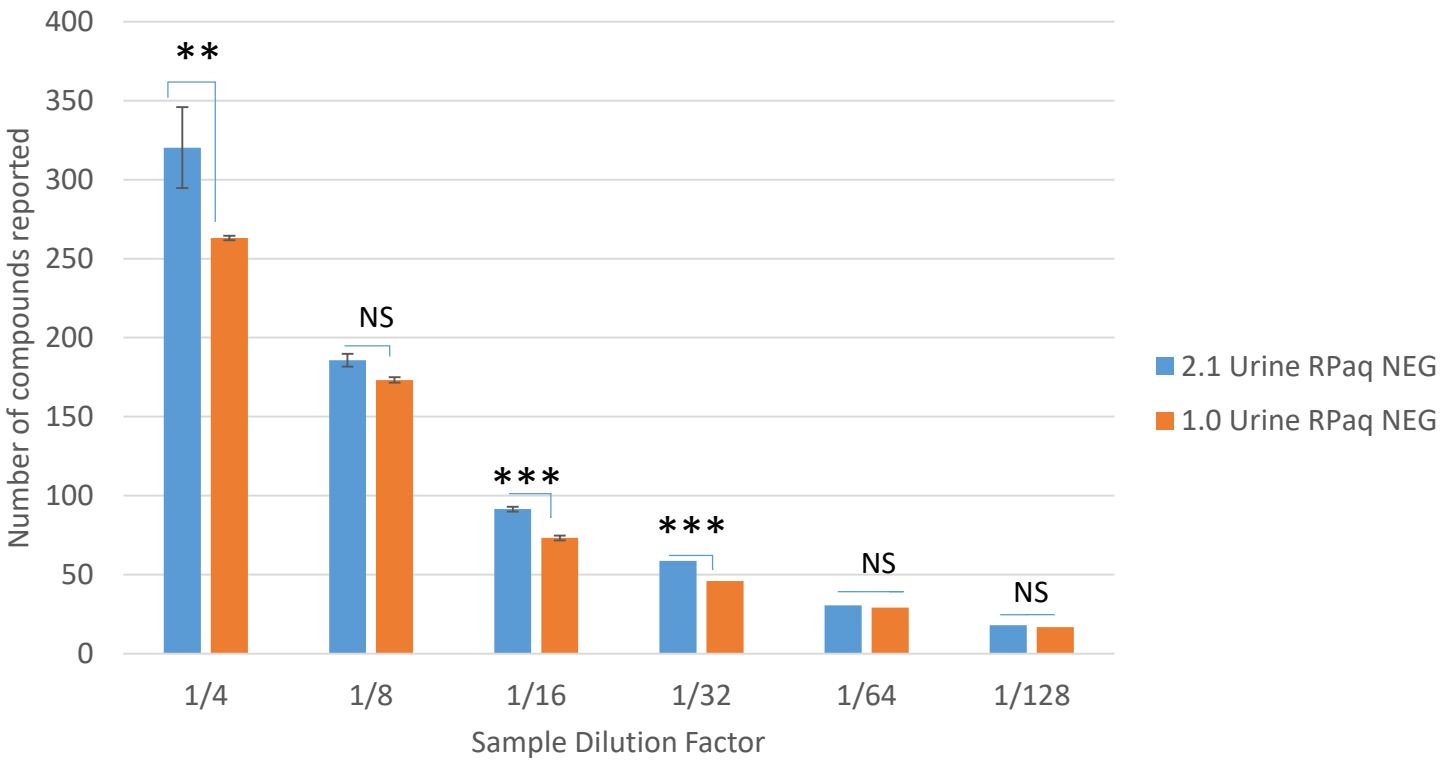
S19-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in positive ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds at each dilution was of a very significant (P<0.001)



S19-B: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to HILIC assays in positive ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Urine HILIC POS	1.0 Urine HILIC POS
1/4	792	676
1/4 SD	2.06	1.92
1/8	585	538
1/8 SD	1.48	4.49
1/16	394	455
1/16 SD	1.12	3.49
1/32	296	336
1/32 SD	2.17	2.45
1/64	206	175
1/64 SD	1.92	2.06
1/128	114	243
1/128 SD	1.30	2.96

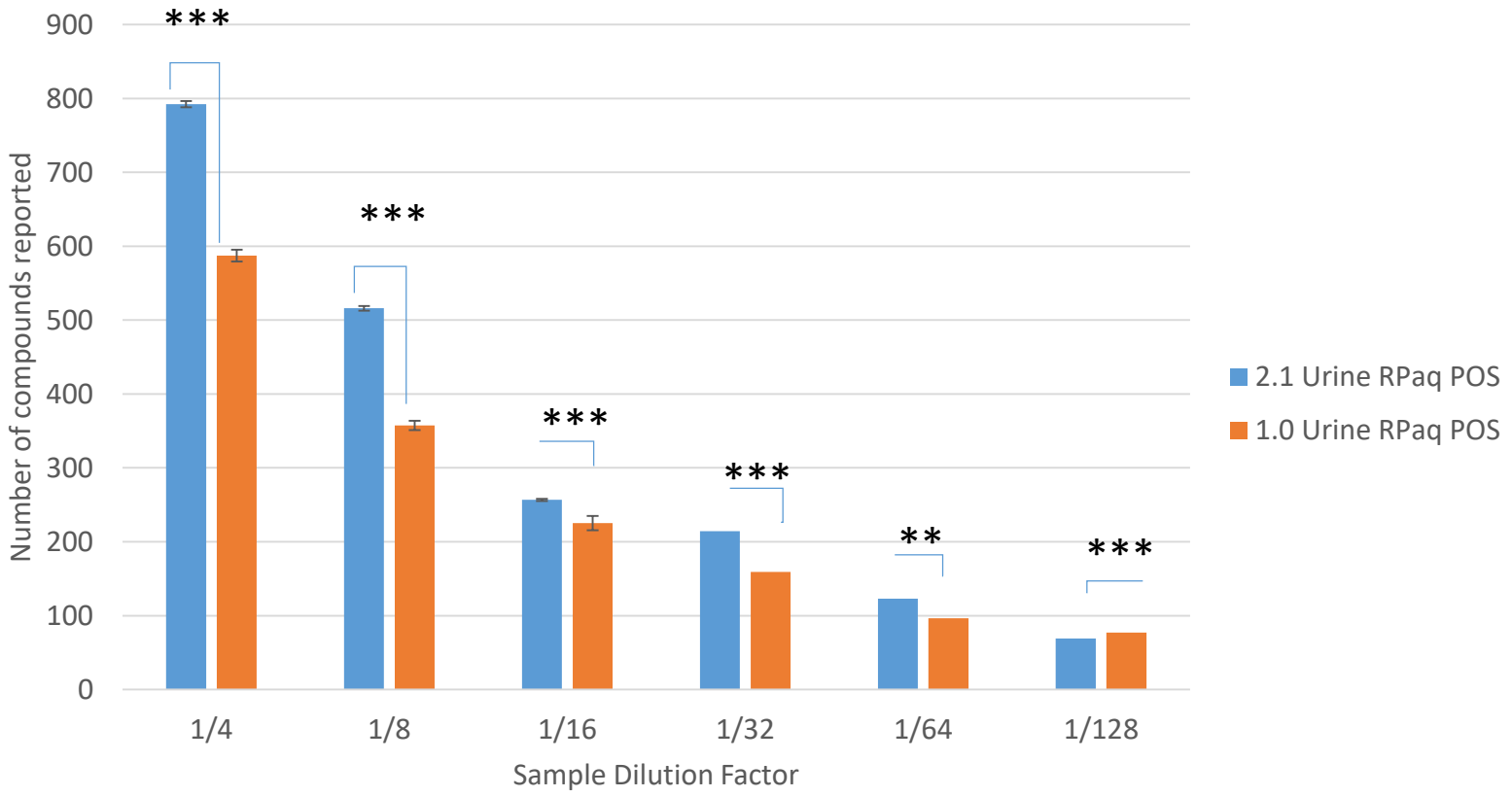
S20-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Reversed Phase aqueous assays in negative ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds at each dilution was of a very significant (P<0.001)



S20-B: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Reversed Phase aqueous assays in negative ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Urine RPaq NEG	1.0 Urine RPaq NEG
1/4	320	263
1/4 SD	25.62	1.41
1/8	186	173
1/8 SD	13.75	2.05
1/16	92	73
1/16 SD	4.09	1.79
1/32	59	46
1/32 SD	2.77	1.22
1/64	31	29
1/64 SD	1.50	1.48
1/128	18	17
1/128 SD	0.71	0.43

S21-A: Graph of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Reversed Phase aqueous assays in positive ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained. The difference in the number of compounds at each dilution was of a very significant (P<0.001)



S21-B: Table of number of compounds reported for both 2.1mm and 1mm ID columns when applied to Reversed Phase aqueous assays in positive ion mode using Urine samples. Compounds were filtered so that only peaks with FWHM <0.2 minutes, were present in ¾ injections in each dilution and had an RSD of <30% remained.

	2.1 Urine RPaq POS	1.0 Urine RPaq POS
1/4	792	587
1/4 SD	4.15	7.89
1/8	516	358
1/8 SD	3.08	2.06
1/16	257	225
1/16 SD	3.11	6.22
1/32	215	159
1/32 SD	4.72	1.22
1/64	123	96
1/64 SD	1.41	9.65
1/128	69	77
1/128 SD	3.40	3

S22: Summery table for the number of reported compounds for each dilution in each assay. All compounds reported have a FWHM <0.2 minutes, were present in ¾ of injections and RSD <30% in each separate injection.

	1/4	1/4 SD	1/8	1/8 SD	1/16	1/16 SD	1/32	1/32 SD	1/64	1/64 SD	1/128	1/128 SD
2.1 Plasma HILIC NEG	317	2.12	228	2.49	143	1.87	81	2.17	55	1.66	33	1.64
1.0 Plasma HILIC NEG	267	8.79	281	1.92	212	4.21	158	3.81	117	2.38	78	1.66
2.1 Plasma HILIC POS	443	4.71	332	2.77	220	3.20	180	3.00	137	0.71	104	3.50
1.0 Plasma HILIC POS	467	5.10	428	2.12	287	2.50	243	1.41	190	3.20	124	0.87
2.1 Urine HILIC NEG	516	3.49	307	1.87	175	2.24	96	0.83	59	1.30	30	0.43
1.0 Urine HILIC NEG	375	2.49	365	3.03	267	1.30	206	1.58	95	2.86	149	2.96
2.1 Urine HILIC POS	792	2.06	585	1.48	394	1.12	296	2.17	206	1.92	114	1.30
1.0 Urine HILIC POS	676	1.92	538	4.49	455	3.49	336	2.45	175	2.06	243	2.96
2.1 Plasma LIPID NEG	749	20.00	402	8.70	256	5.12	167	3.11	123	1.50	81	0.71
1.0 Plasma LIPID NEG	321	17.17	273	5.79	254	18.23	180	1.66	126	4.03	80	2.74
2.1 Plasma LIPID POS	725	2.05	813	7.12	1273	165.55	479	31.58	364	6.04	305	2.92
1.0 Plasma LIPID POS	489	7.89	340	21.98	271	12.75	208	2.87	108	4.15	NA	NA
2.1 Urine RPaq NEG	320	25.62	186	13.75	92	4.09	59	2.77	31	1.50	18	0.71
1.0 Urine RPaq NEG	263	1.41	173	2.05	73	1.79	46	1.22	29	1.48	17	0.43
2.1 Urine RPaq POS	792	4.15	516	3.08	257	3.11	215	4.72	123	1.41	69	3.40
1.0 Urine RPaq POS	587	7.89	358	2.06	225	6.22	159	1.22	96	9.65	77	3

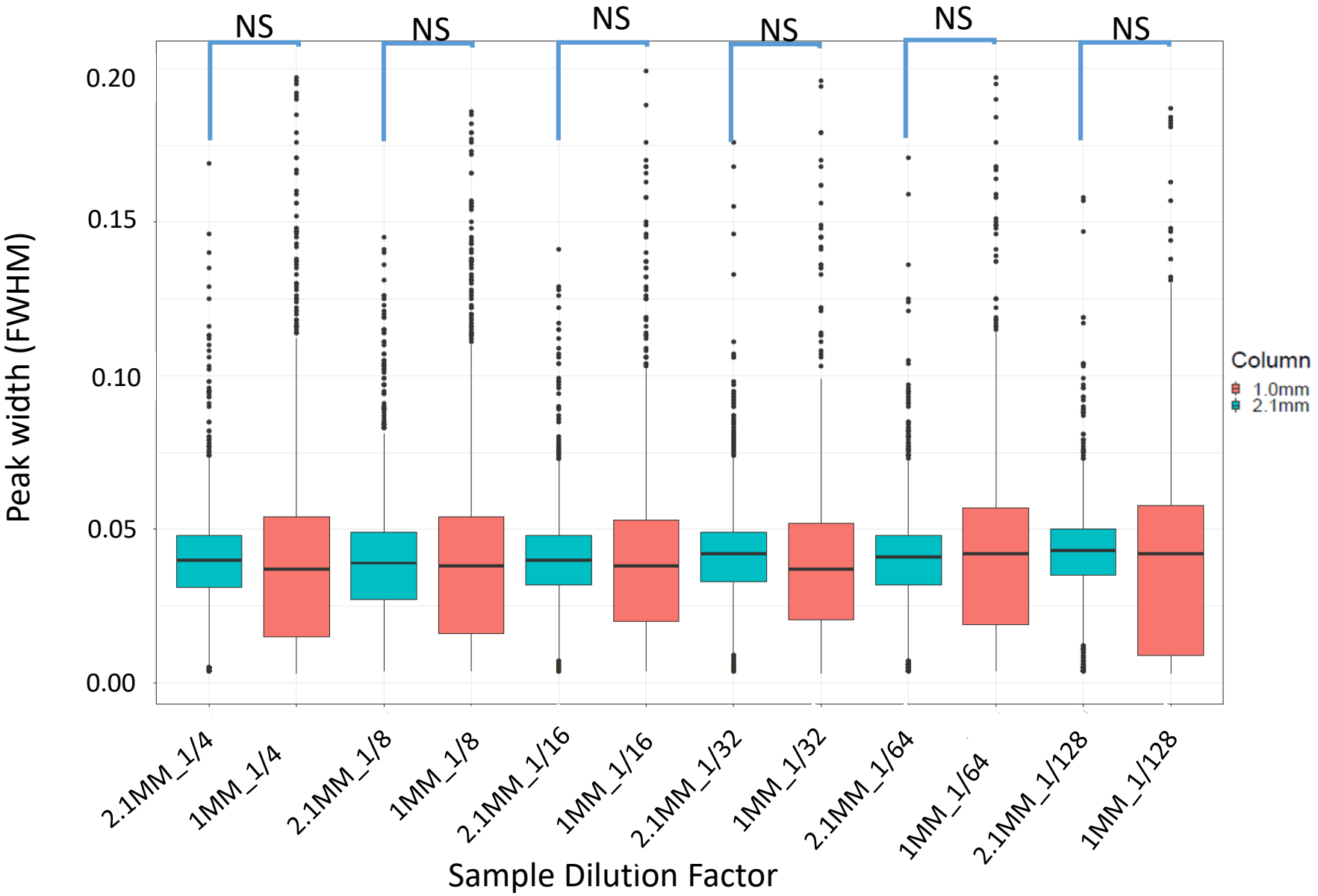
S23-B: A summary of the full width half max for each dilution applying 1mm ID column to a Lipidomics assay in positive ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	1189	0.0406	0.0184	0.0310	0.0400	0.0480	1.03E+08	5.53E+08
1/8	2192	0.0394	0.0199	0.0270	0.0390	0.0490	1.48E+08	9.67E+08
1/16	1312	0.0397	0.0182	0.0318	0.0400	0.0480	1.02E+08	5.11E+08
1/32	1231	0.0418	0.0189	0.0330	0.0420	0.0490	1.22E+08	5.67E+08
1/64	1410	0.0401	0.0180	0.0320	0.0410	0.0480	1.31E+08	5.58E+08
1/128	937	0.0422	0.0185	0.0350	0.0430	0.0500	9.91E+07	3.31E+08

S23-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a Lipidomics assay in positive ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	2181	0.0397	0.0296	0.0150	0.0370	0.0540	1.53E+08	9.23E+08
1/8	1521	0.0401	0.0306	0.0160	0.0380	0.0540	1.05E+08	6.28E+08
1/16	1154	0.0402	0.0293	0.0200	0.0380	0.0530	6.28E+08	5.39E+08
1/32	815	0.0394	0.0299	0.0205	0.0370	0.0520	6.94E+07	3.44E+08
1/64	734	0.0445	0.0331	0.0190	0.0420	0.0570	6.19E+07	2.22E+08
1/128	754	0.0417	0.0322	0.0090	0.0420	0.0578	6.51E+07	2.60E+08

S23-C: Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to Lipidomics assay in positive ion mode on plasma samples. Comparisons between columns were not statistically significant (P>0.05) at any dilution.



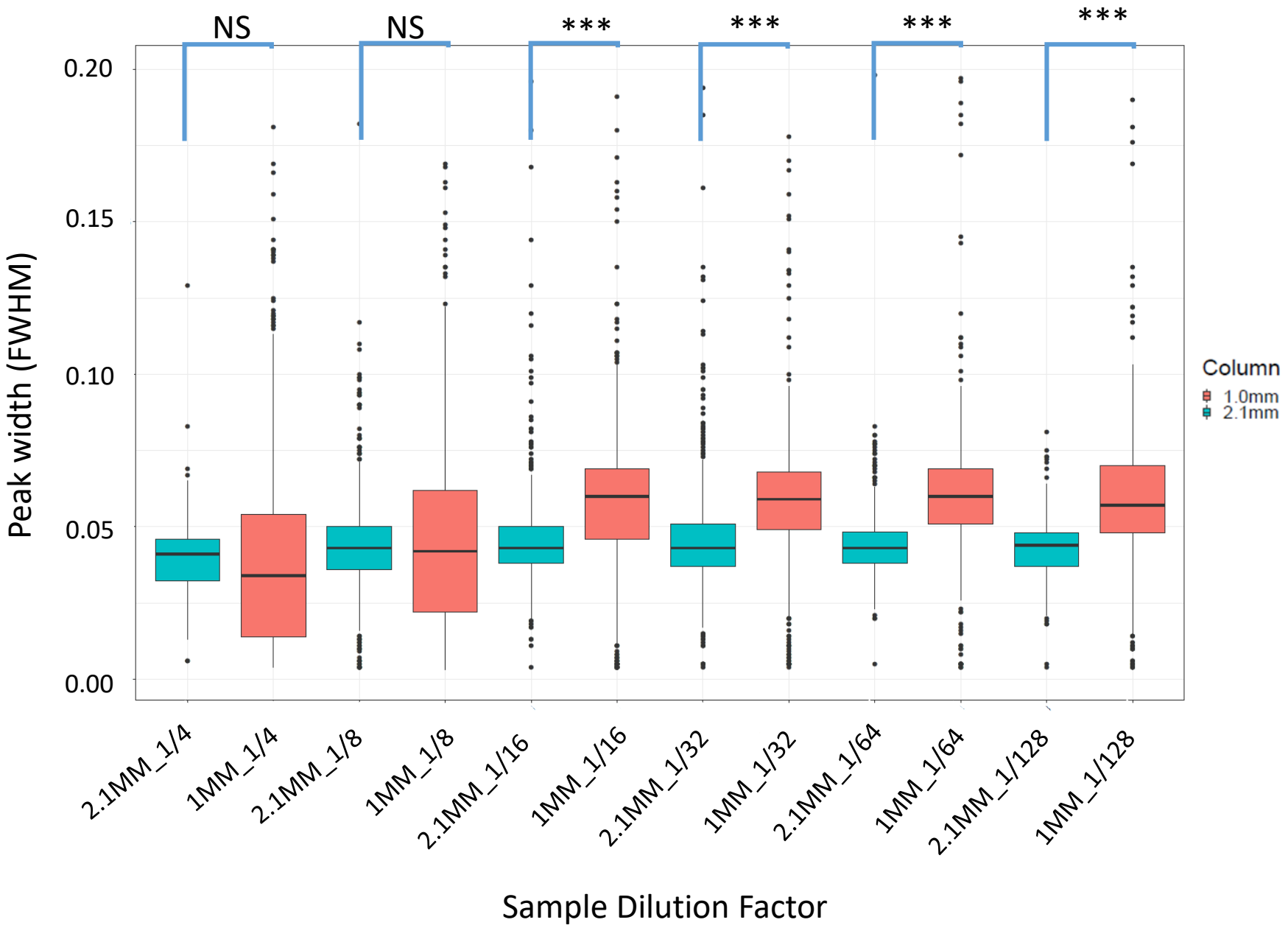
S24-B: A summary of the full width half max for each dilution applying 1mm ID column to a Lipidomics assay in negative ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	1251	0.0374	0.0277	0.014	0.034	0.054	4.59E+07	2.01E+08
1/8	960	0.0451	0.0298	0.022	0.042	0.062	3.92E+07	1.42E+08
1/16	535	0.0569	0.0279	0.046	0.06	0.069	5.46E+07	1.68E+08
1/32	463	0.0586	0.0242	0.049	0.059	0.068	4.91E+07	1.41E+08
1/64	364	0.0608	0.0261	0.051	0.06	0.069	4.01E+07	9.86E+07
1/128	342	0.0586	0.0258	0.048	0.057	0.07	4.49E+07	1.14E+08

S24-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a Lipidomics assay in negative ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	319	0.0403	0.0120	0.03225	0.041	0.046	2.14E+07	6.58E+07
1/8	675	0.0443	0.0161	0.036	0.043	0.05	2.70E+07	1.11E+08
1/16	529	0.0464	0.0179	0.038	0.043	0.05	2.32E+07	6.98E+07
1/32	526	0.0464	0.0201	0.037	0.043	0.051	2.26E+07	4.78E+07
1/64	284	0.0450	0.0145	0.038	0.043	0.04825	1.69E+07	3.60E+07
1/128	129	0.0439	0.0130	0.037	0.044	0.048	1.43E+07	2.49E+07

S24-C:Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to Lipidomics assay in negative ion mode on plasma samples. Comparisons between columns were very significant (P≤0.001) from 1 in 16 onwards. The two most concentrated dilutions were not statistically significant (P>0.05)



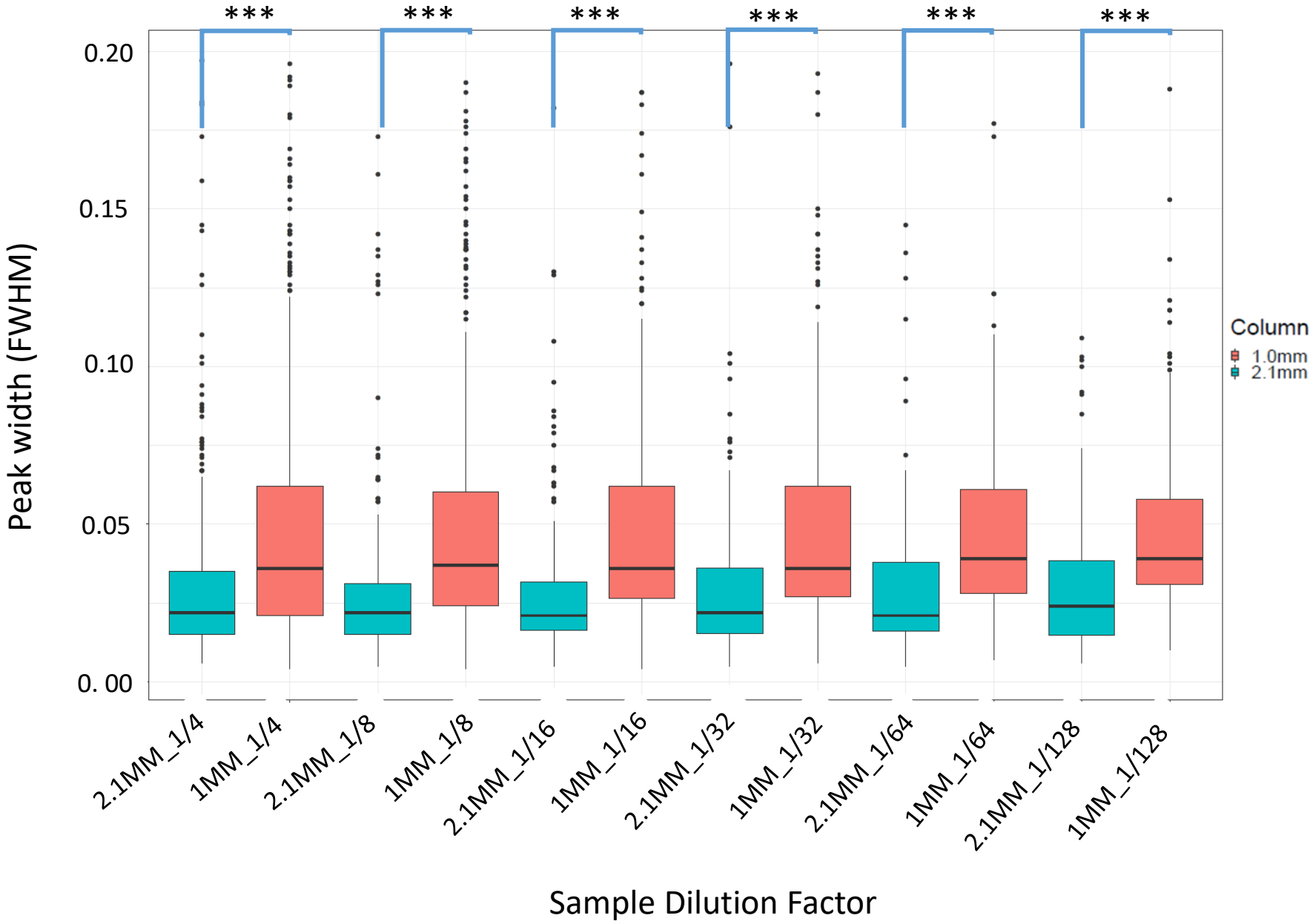
S25-B: A summary of the full width half max for each dilution applying 1mm ID column to a HILIC assay in negative ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	484	0.0482	0.0383	0.021	0.036	0.062	4.21E+07	1.87E+08
1/8	460	0.0471	0.0369	0.024	0.037	0.06025	7.55E+07	4.32E+08
1/16	331	0.0472	0.0335	0.0265	0.036	0.062	6.99E+07	3.48E+08
1/32	255	0.0493	0.0351	0.027	0.036	0.062	7.92E+07	3.95E+08
1/64	175	0.0474	0.0285	0.028	0.039	0.061	6.65E+07	2.84E+08
1/128	122	0.0492	0.0300	0.031	0.039	0.058	5.70E+07	3.01E+08

S25-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a HILIC assay in negative ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	398	0.0299	0.0269	0.015	0.022	0.035	2.07E+07	1.29E+08
1/8	296	0.0279	0.0243	0.015	0.022	0.03125	1.50E+07	8.04E+07
1/16	199	0.0279	0.0231	0.01625	0.021	0.03175	1.24E+07	6.45E+07
1/32	142	0.0302	0.0276	0.01525	0.022	0.036	8.98E+06	3.79E+07
1/64	99	0.0312	0.0271	0.016	0.021	0.038	1.06E+07	3.34E+07
1/128	84	0.0317	0.0246	0.01475	0.024	0.0385	7.26E+06	2.16E+07

S25-C: Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to HILIC assay in negative ion mode on plasma samples. Comparisons between columns were very significant (P≤0.001) at all dilutions.



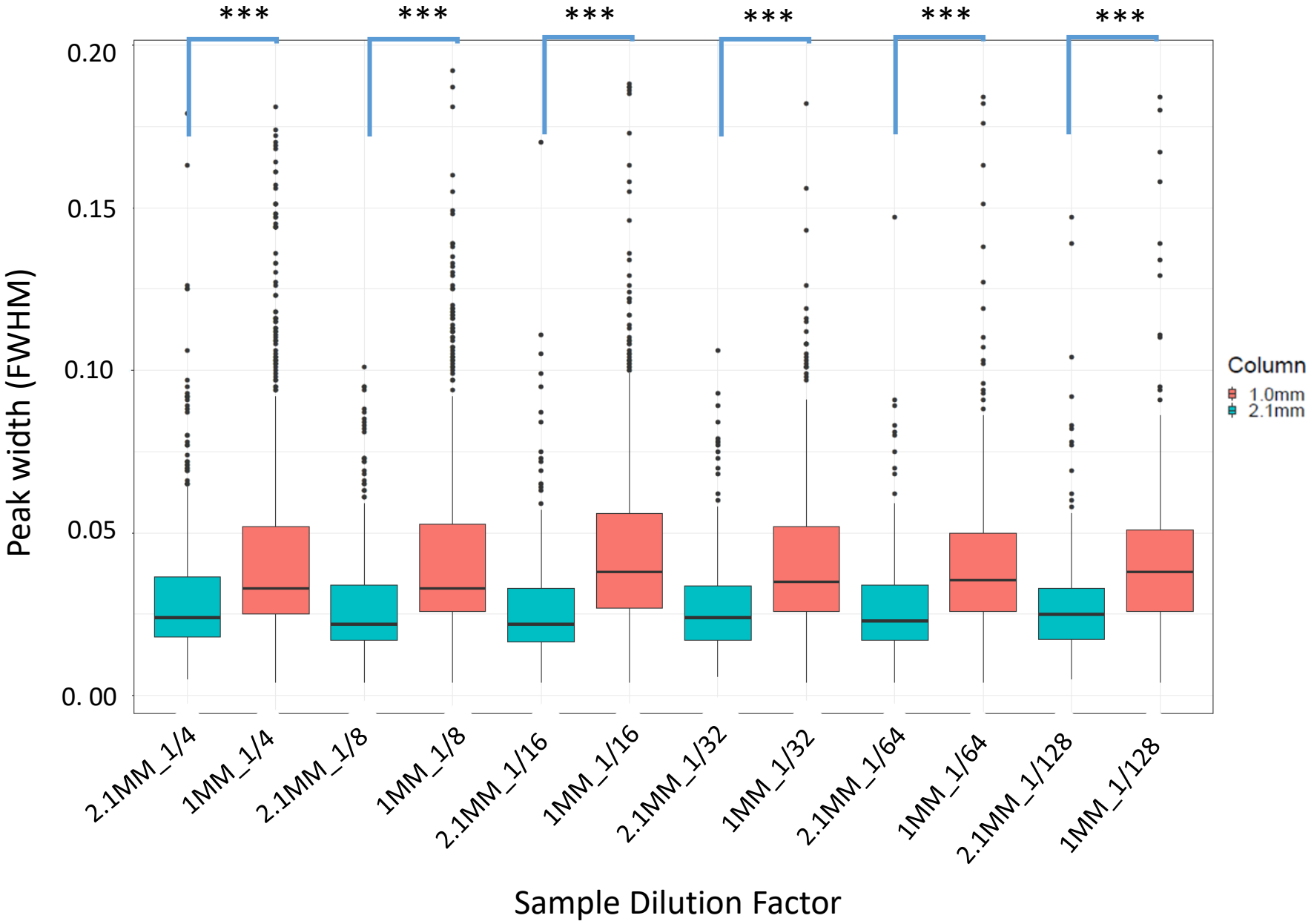
S26-B: A summary of the full width half max for each dilution applying 1mm ID column to a HILIC assay in positive ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	741	0.0437	0.0321	0.025	0.033	0.052	8.53E+07	4.76E+08
1/8	606	0.0444	0.0299	0.026	0.033	0.05275	6.93E+07	3.76E+08
1/16	567	0.0460	0.0302	0.027	0.038	0.056	5.41E+07	2.53E+08
1/32	374	0.0428	0.0253	0.026	0.035	0.052	4.53E+07	1.92E+08
1/64	322	0.0420	0.0264	0.026	0.0355	0.05	3.72E+07	1.59E+08
1/128	229	0.0435	0.0273	0.026	0.038	0.051	2.99E+07	1.01E+08

S26-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a HILIC assay in positive ion mode using Plasma samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	579	0.0301	0.0205	0.018	0.024	0.0365	4.79E+07	2.88E+08
1/8	495	0.0270	0.0162	0.017	0.022	0.034	3.58E+07	2.07E+08
1/16	371	0.0268	0.0171	0.0165	0.022	0.033	2.97E+07	1.58E+08
1/32	350	0.0271	0.0157	0.017	0.024	0.03375	2.27E+07	1.14E+08
1/64	243	0.0277	0.0171	0.017	0.023	0.034	1.87E+07	8.47E+07
1/128	246	0.0282	0.0183	0.01725	0.025	0.033	1.54E+07	6.57E+07

S26-C: Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to HILIC assay in positive ion mode on plasma samples. Comparisons between columns were very significant (P≤0.001) at all dilutions.



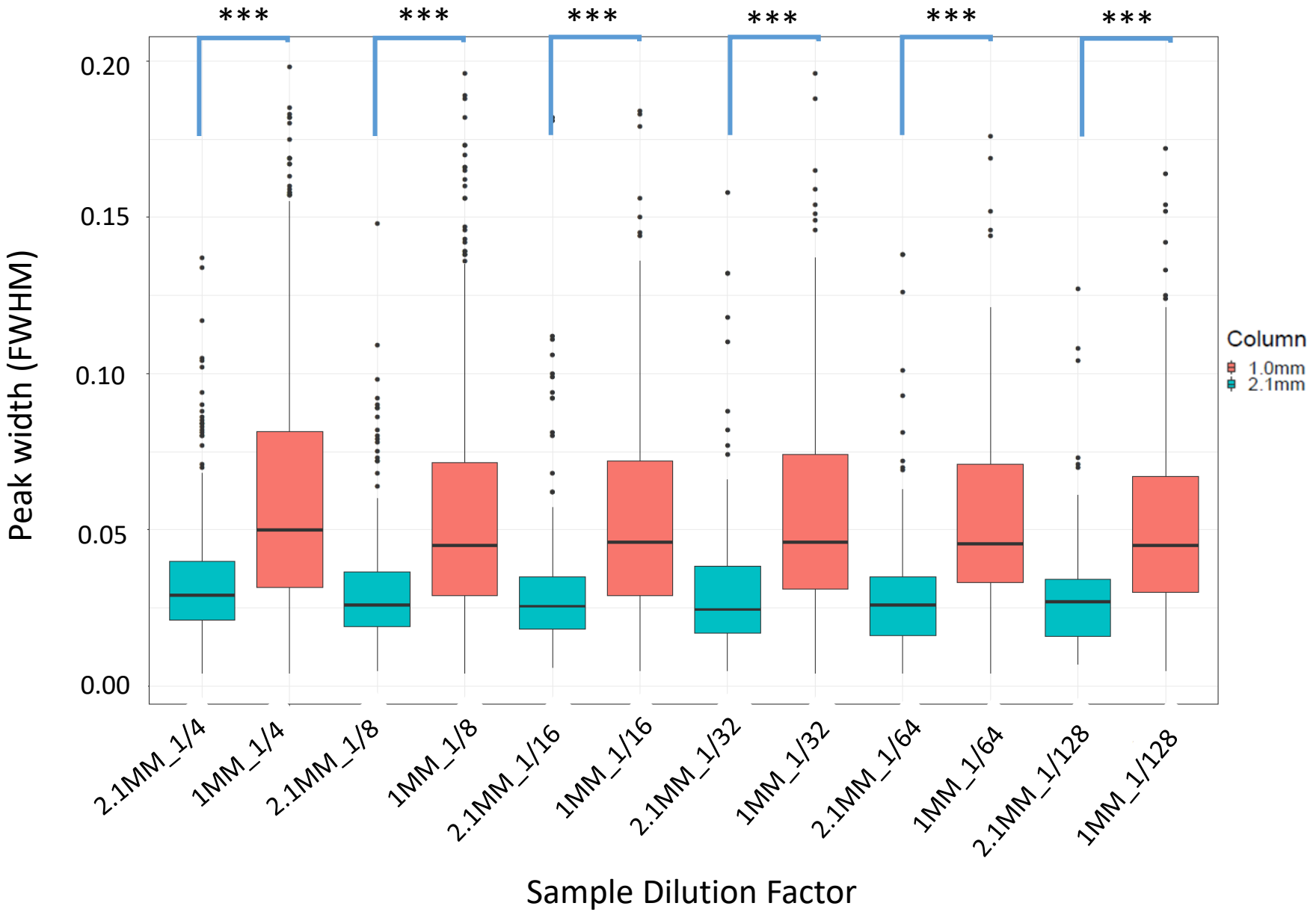
S27-B: A summary of the full width half max for each dilution applying 1mm ID column to a HILIC assay in negative ion mode using urine samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	585	0.0664	0.0872	0.031	0.05	0.08	1.51E+08	7.03E+08
1/8	534	0.0645	0.0977	0.03	0.047	0.077	9.89E+07	4.86E+08
1/16	388	0.0667	0.1524	0.03	0.048	0.075	7.44E+07	2.94E+08
1/32	304	0.0675	0.1080	0.031	0.049	0.078	6.25E+07	2.32E+08
1/64	219.75	0.0642	0.1284	0.034	0.049	0.0715	4.03E+07	1.31E+08
1/128	222	0.0716	0.1724	0.033	0.05	0.073	5.16E+07	1.81E+08

S27-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a HILIC assay in negative ion mode using urine samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	616	0.0322	0.0181	0.021	0.029	0.04	1.79E+07	7.86E+07
1/8	423	0.0295	0.0172	0.019	0.026	0.0365	1.19E+07	4.32E+07
1/16	261	0.0306	0.0220	0.01825	0.0255	0.035	9.09E+06	2.70E+07
1/32	172	0.0312	0.0232	0.017	0.0245	0.03825	7.77E+06	2.02E+07
1/64	119	0.0314	0.0244	0.016	0.026	0.035	6.99E+06	1.69E+07
1/128	84	0.0295	0.0218	0.01575	0.027	0.03425	5.75E+06	1.19E+07

S27-C: Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to HILIC assay in negative ion mode on urine samples. Comparisons between columns were very significant (P≤0.001) at all dilutions.



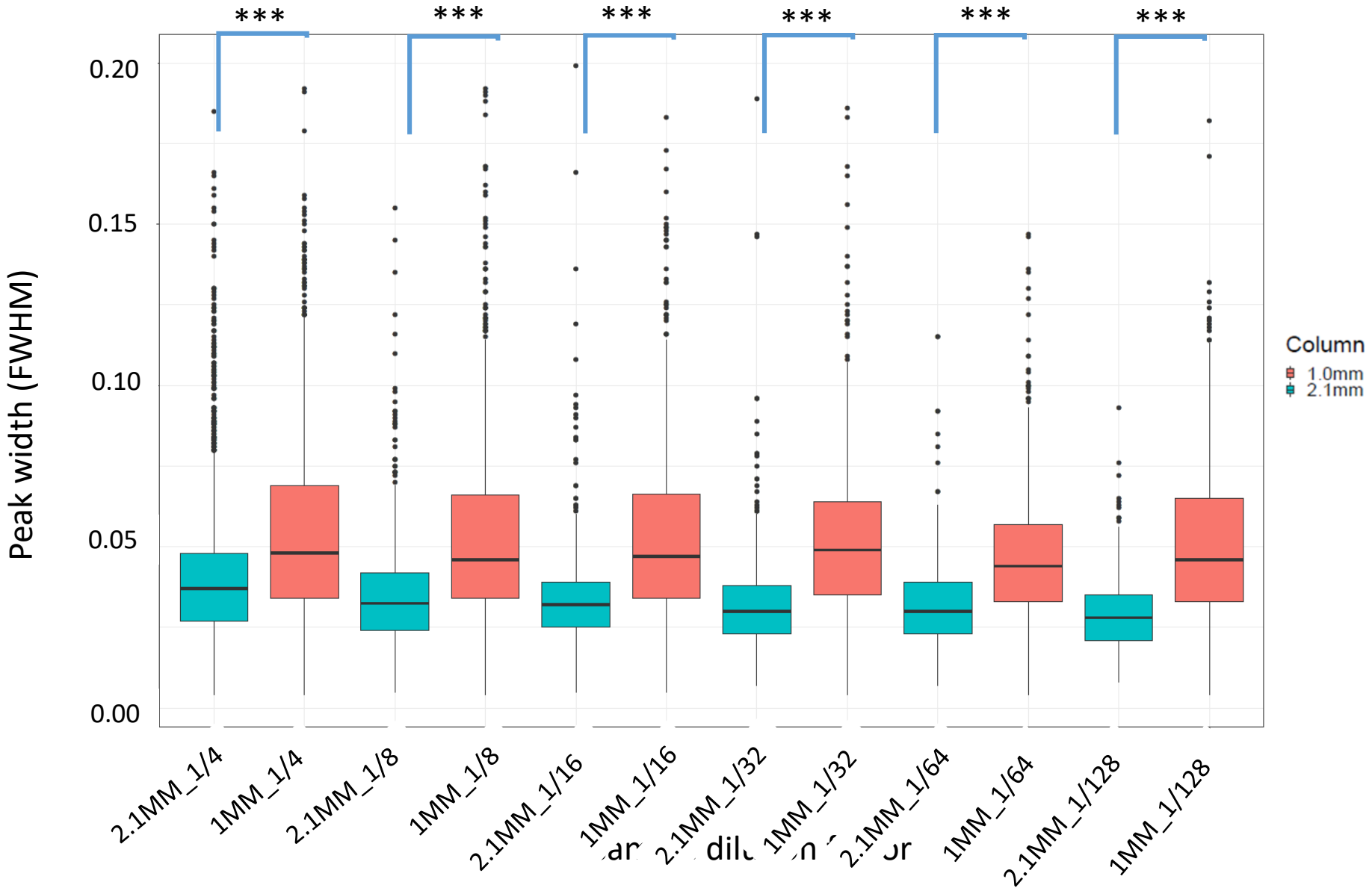
S28-B: A summary of the full width half max for each dilution applying 1mm ID column to a HILIC assay in positive ion mode using urine samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	966	0.0540	0.0306	0.034	0.048	0.069	1.20E+08	7.45E+08
1/8	824	0.0536	0.0308	0.034	0.046	0.066	1.41E+08	1.72E+09
1/16	636	0.0536	0.0293	0.034	0.047	0.06625	1.10E+08	1.34E+09
1/32	525	0.0525	0.0274	0.035	0.049	0.064	8.77E+07	9.67E+08
1/64	336	0.0476	0.0245	0.033	0.044	0.057	4.91E+07	5.11E+08
1/128	379	0.0512	0.0277	0.033	0.046	0.065	7.26E+07	7.84E+08

S28-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a HILIC assay in positive ion mode using urine samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	967	0.0393	0.0208	0.027	0.036	0.0475	7.53E+07	6.76E+08
1/8	772	0.0346	0.0178	0.024	0.0325	0.042	5.67E+07	5.01E+08
1/16	528	0.0341	0.0182	0.025	0.032	0.039	5.12E+07	4.34E+08
1/32	410	0.0330	0.0184	0.023	0.03	0.038	4.03E+07	3.27E+08
1/64	284	0.0322	0.0159	0.023	0.03	0.039	3.64E+07	2.85E+08
1/128	280	0.0289	0.0131	0.021	0.028	0.035	2.66E+07	1.95E+08

S28-C: Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to HILIC assay in positive ion mode on urine samples. Comparisons between columns were very significant (P≤0.001) at all dilutions.



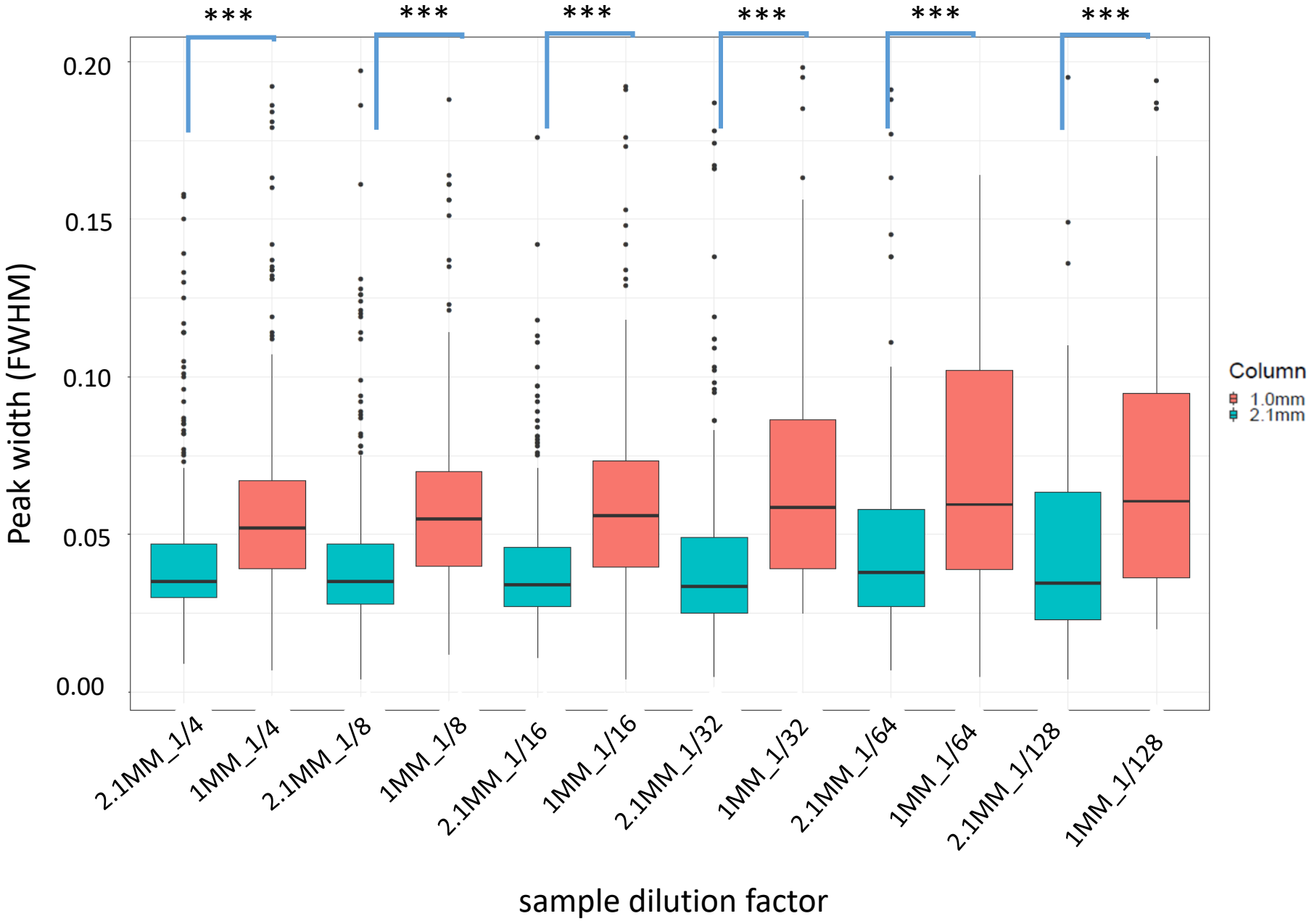
S29-B: A summary of the full width half max for each dilution applying 1mm ID column to a Reversed Phase aqueous assay in negative ion mode using urine samples.

`	No. metabolites	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	370	0.0572	0.0284	0.039	0.052	0.067	1.56E+07	3.20E+07
1/8	245	0.0592	0.0275	0.04	0.055	0.07	1.44E+07	2.89E+07
1/16	136	0.0633	0.0353	0.03975	0.056	0.07325	1.76E+07	3.79E+07
1/32	100	0.0693	0.0385	0.039	0.0585	0.08625	1.98E+07	3.58E+07
1/64	84	0.0694	0.0371	0.03875	0.0595	0.102	2.02E+07	4.03E+07
1/128	74	0.0722	0.0439	0.03625	0.0605	0.09475	2.19E+07	4.25E+07

S29-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a Reversed Phase aqueous assay in negative ion mode using urine samples.

`	No. metabolites	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	471	0.0414	0.0207	0.03	0.035	0.047	1.02E+07	2.54E+07
1/8	329	0.0410	0.0256	0.028	0.035	0.047	1.08E+07	2.48E+07
1/16	195	0.0402	0.0240	0.027	0.034	0.046	1.18E+07	2.65E+07
1/32	178	0.0445	0.0338	0.025	0.0335	0.049	1.23E+07	2.92E+07
1/64	141	0.0481	0.0348	0.027	0.038	0.058	1.53E+07	3.31E+07
1/128	124	0.0448	0.0315	0.023	0.0345	0.0635	1.27E+07	2.63E+07

S29-C: Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to Reversed Phase aqueous assay in negative ion mode on urine samples. Comparisons between columns were very significant ($P \leq 0.001$) at all dilutions.



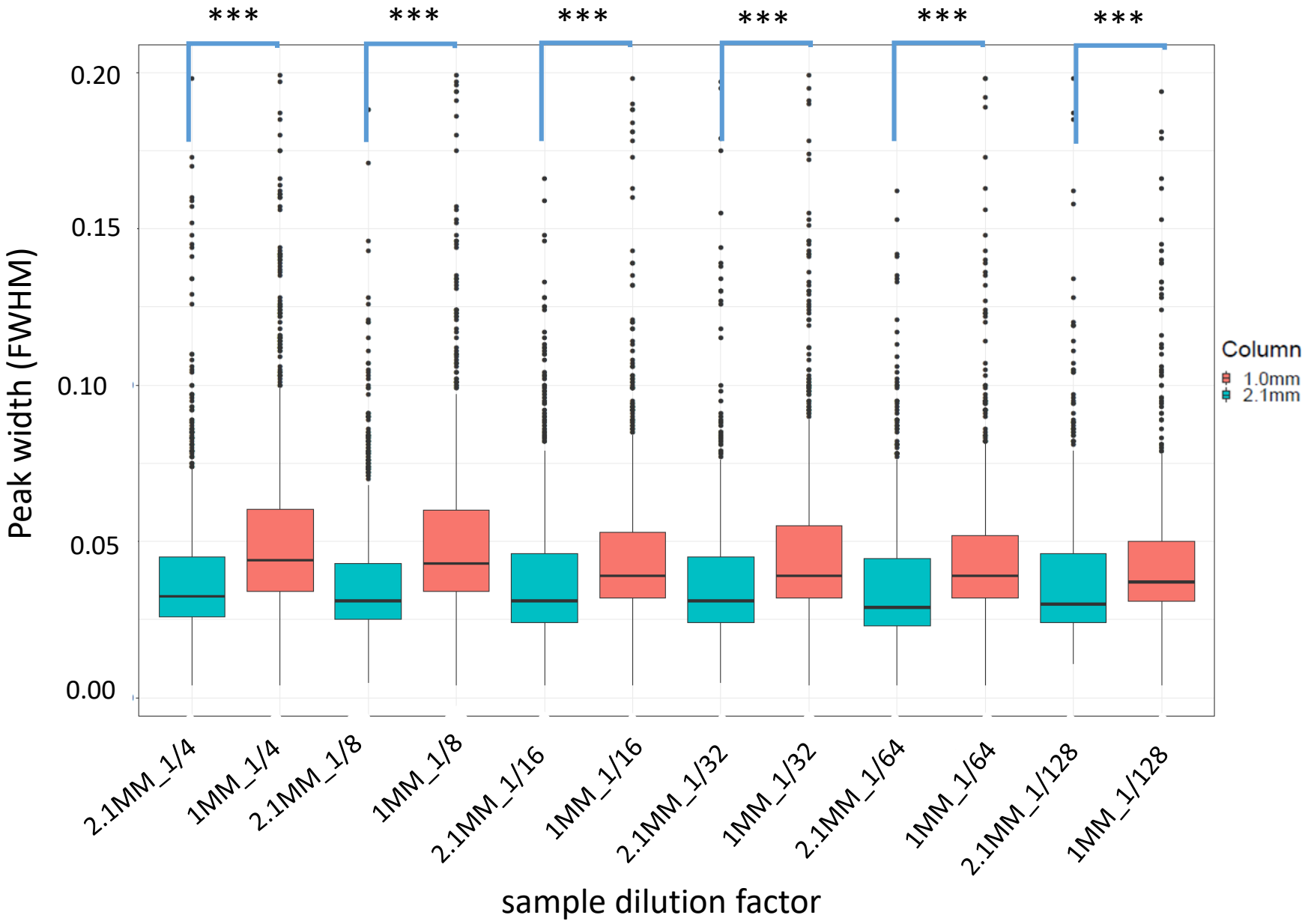
S30-B: A summary of the full width half max for each dilution applying 1mm ID column to a Reversed Phase aqueous assay in positive ion mode using urine samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	1344	0.0502	0.0276	0.034	0.044	0.06025	2.71E+07	2.58E+08
1/8	1039	0.0499	0.0272	0.034	0.043	0.06	2.58E+07	1.82E+08
1/16	910	0.0461	0.0255	0.032	0.039	0.053	2.26E+07	1.03E+08
1/32	813	0.0461	0.0255	0.032	0.039	0.053	2.26E+07	1.03E+08
1/64	761	0.0451	0.0251	0.032	0.039	0.052	2.24E+07	6.09E+07
1/128	703	0.0441	0.0246	0.031	0.037	0.05	2.23E+07	5.45E+07

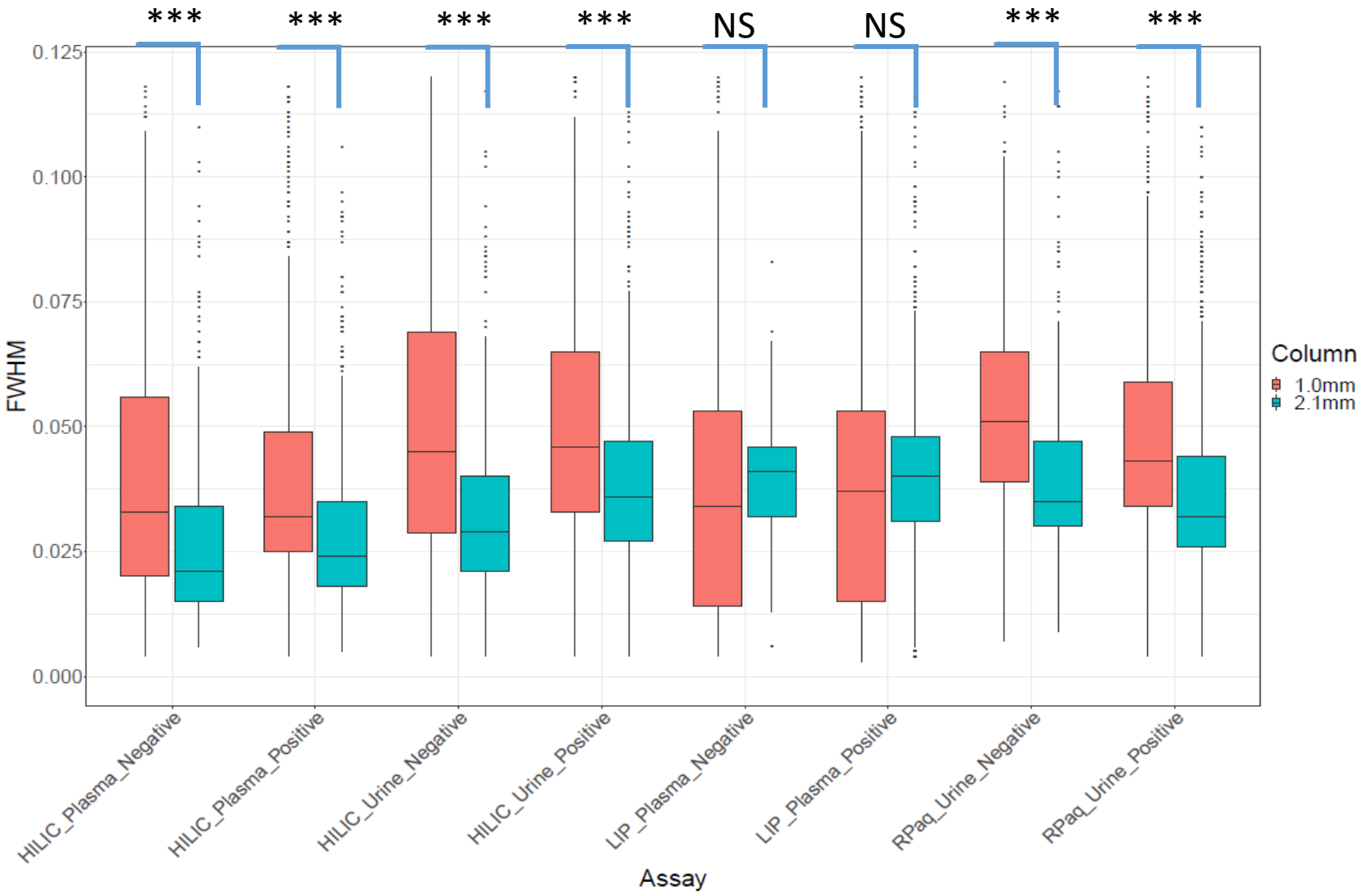
S30-A: A summary of the full width half max for each dilution applying 2.1mm ID column to a Reversed Phase aqueous assay in positive ion mode using urine samples.

	No. compounds	FWHM (mean)	FWHM (SD)	FWHM (QUART1)	FWHM (QUART/ median)	FWHM (QUART3)	Total peak area (mean)	Total peak area (SD)
1/4	1448	0.0381	0.0200	0.026	0.0325	0.045	1.71E+07	8.62E+07
1/8	1164	0.0372	0.0202	0.025	0.031	0.043	1.60E+07	6.52E+07
1/16	829	0.0387	0.0227	0.024	0.031	0.046	1.71E+07	5.25E+07
1/32	798	0.0381	0.0234	0.024	0.031	0.045	1.64E+07	4.69E+07
1/64	765	0.0372	0.0219	0.023	0.029	0.0445	1.69E+07	4.58E+07
1/128	654	0.0387	0.0241	0.024	0.03	0.046	1.71E+07	4.43E+07

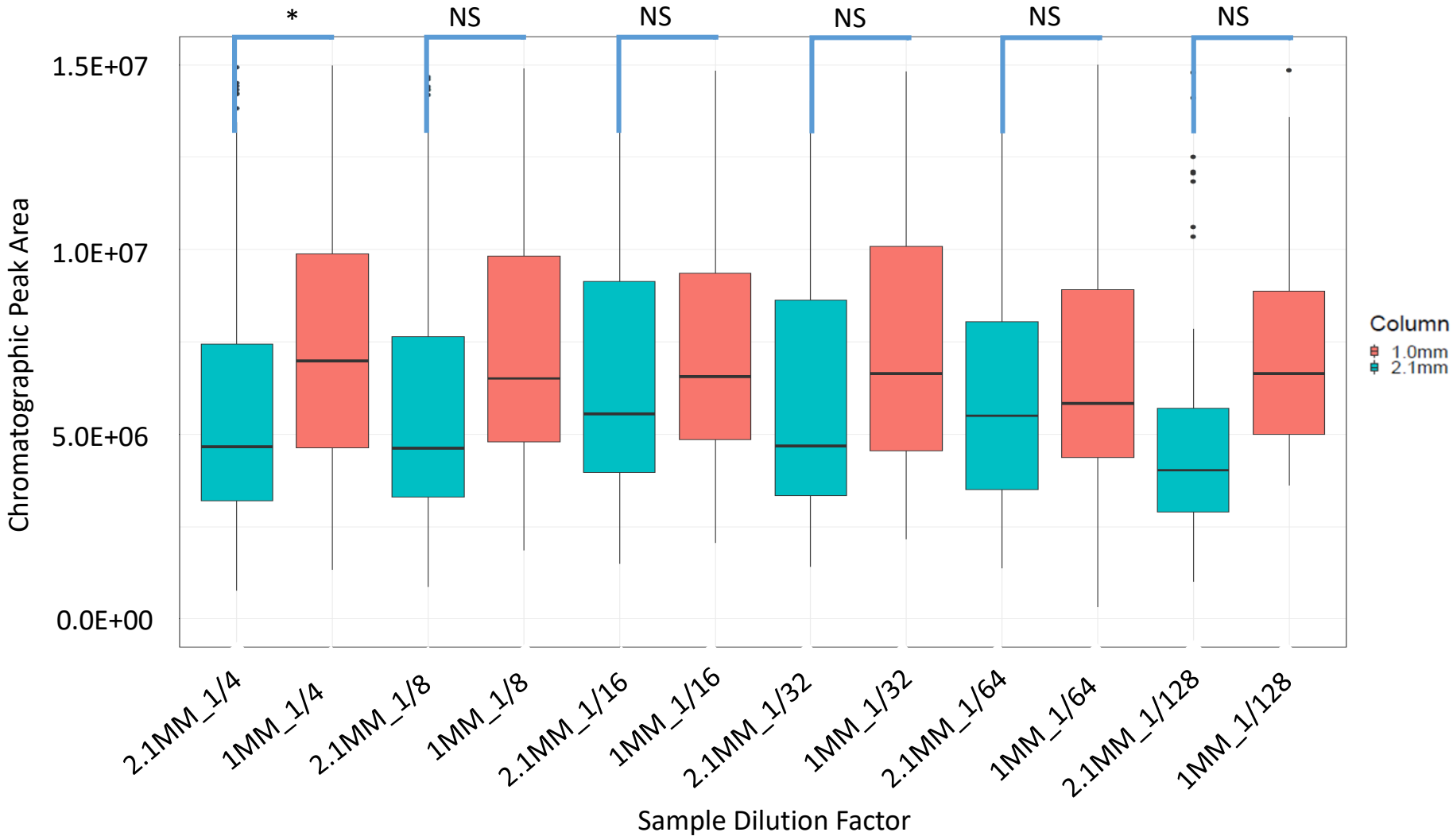
S30-C: Distribution of Full Width Half Max between all dilutions applying both the 2.1mm ID and 1mm ID columns to Reversed Phase aqueous assay in positive ion mode on urine samples. Comparisons between columns were very significant (P ≤ 0.001) at all dilutions.



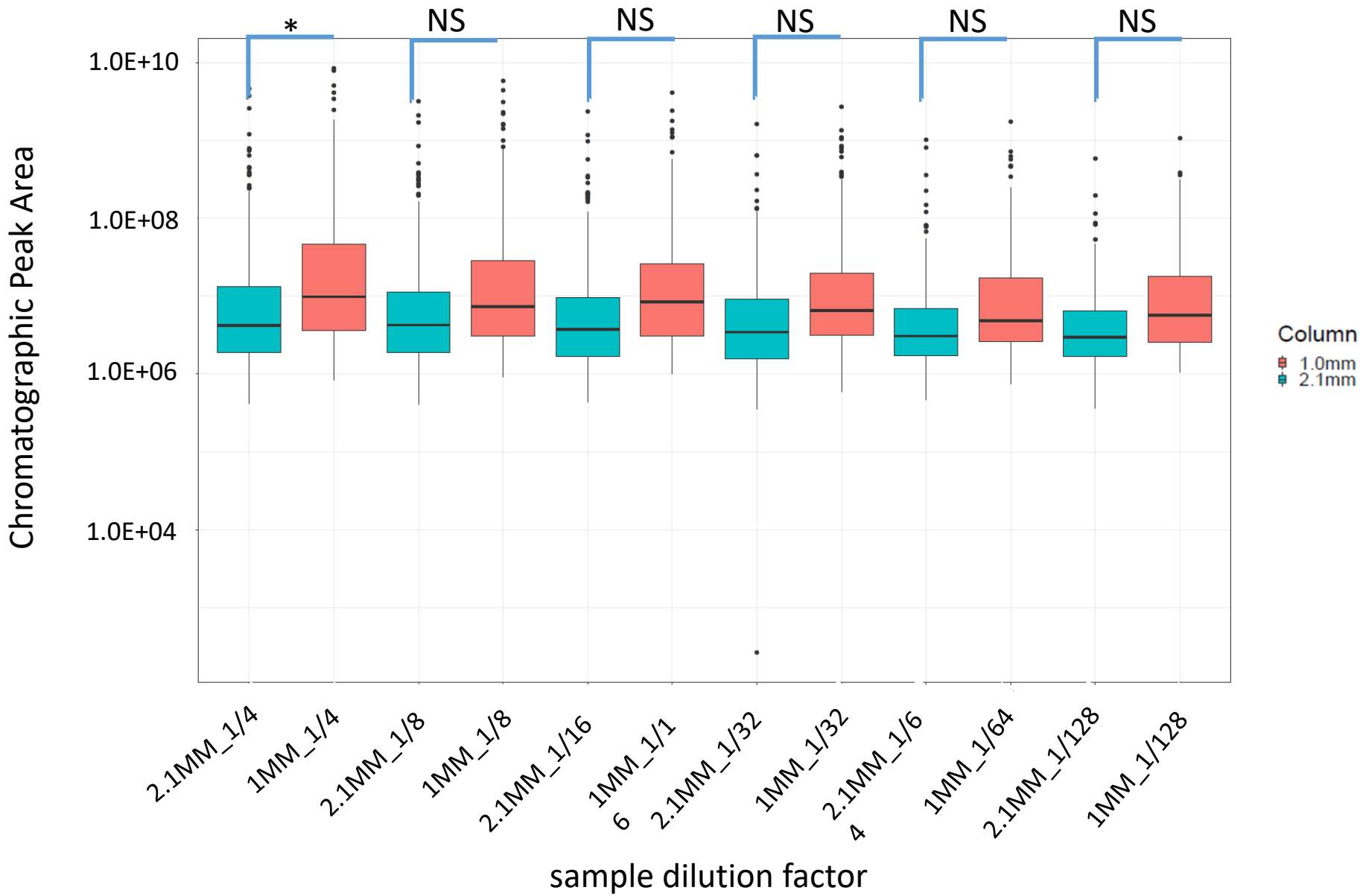
S31: Distribution of Full Width Half Max between the for 1 in 4 dilution across all assays, sample types and ion modes applying both2.1mm (blue) and 1mm ID (red). Most comparisons between columns were very significant (P ≤ 0.001) whilst two were not significant at all(P > 0.05).



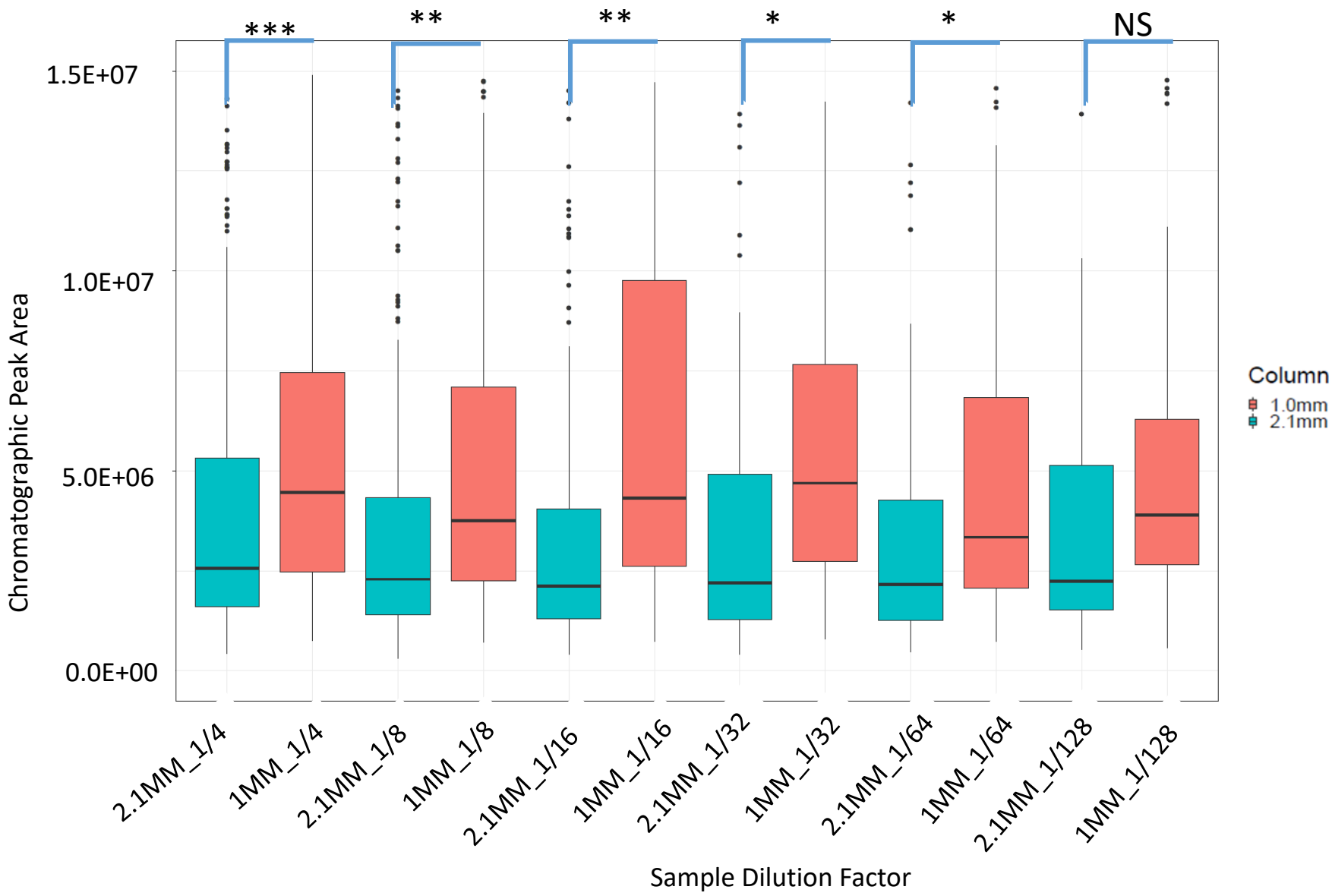
S32: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) applying the HILIC assay in negative ion mode on Plasma samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. All but one comparison between columns was not significant ($P > 0.05$) instead it was statistically significant ($P \leq 0.05$).



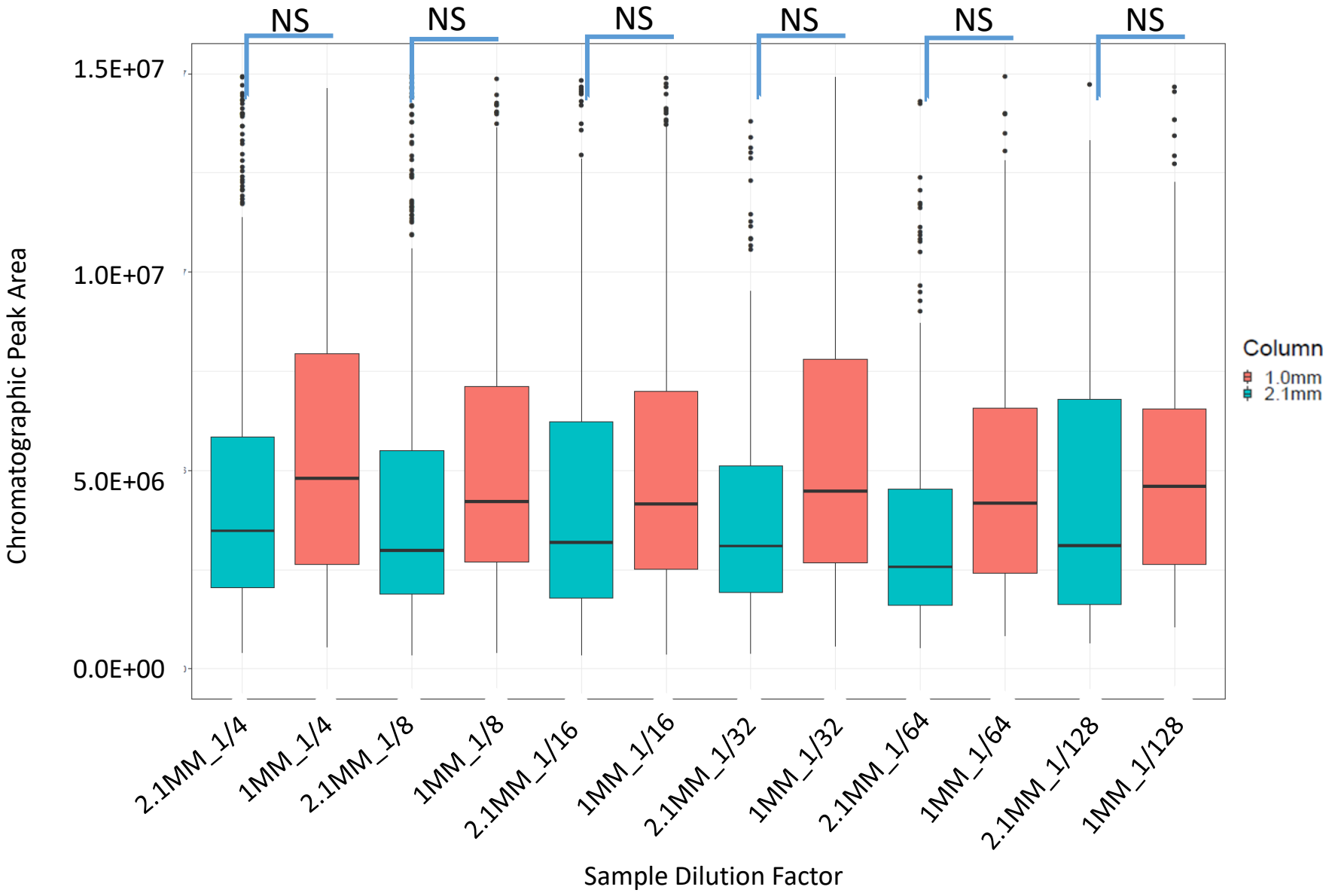
S33: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) applying the HILIC assay in positive ion mode on Plasma samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. All but one comparison were not significant (P > 0.05), whilst one was statistically significant (P ≤ 0.05).



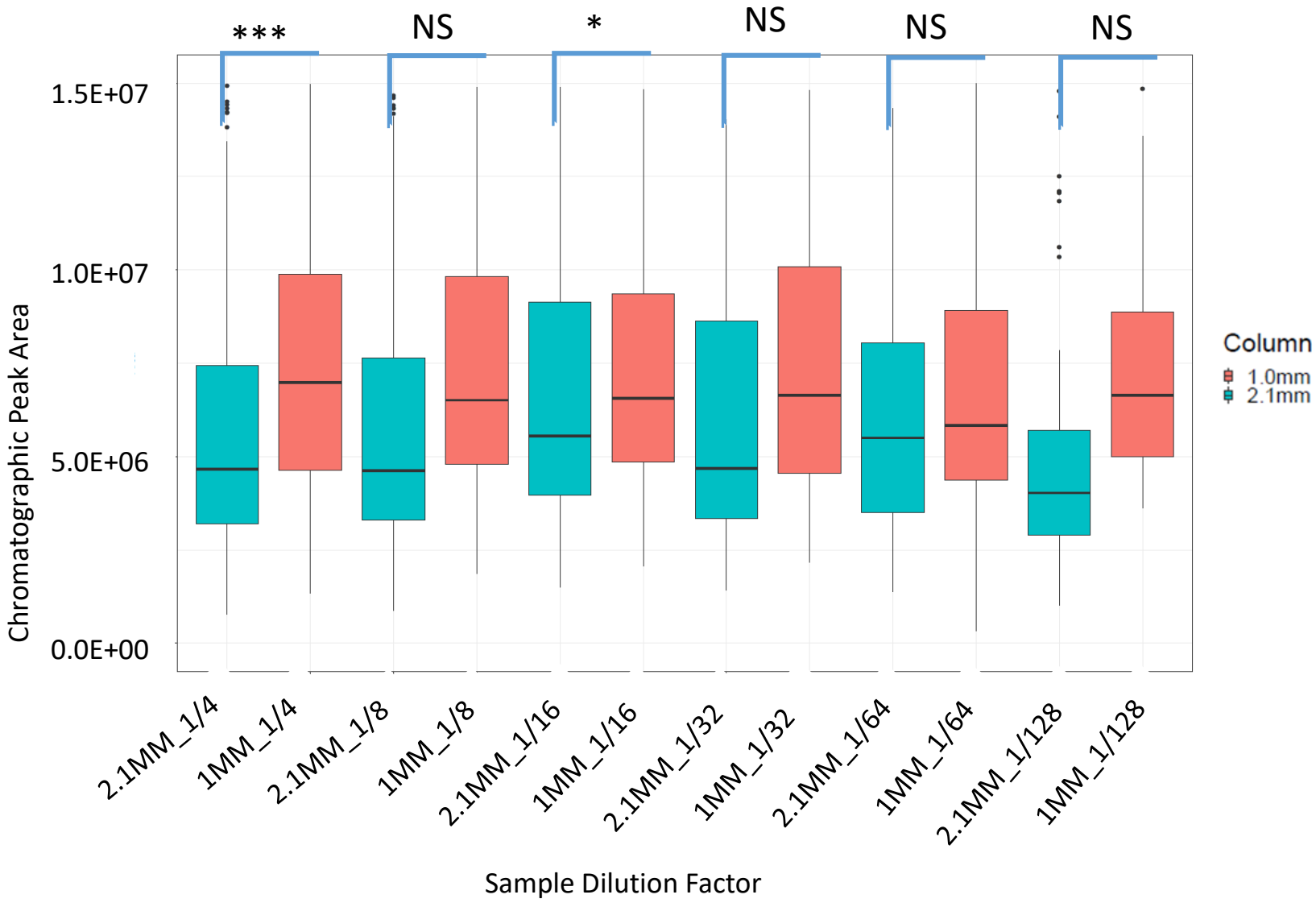
S34: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) applying the HILIC assay in negative ion mode on Urine samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. One comparison between columns was not significant ($P > 0.05$). Two comparisons were statistically significant ($P \leq 0.05$), two were moderately significant (($P \leq 0.01$) and one was very statistically significant ($P \leq 0.001$).



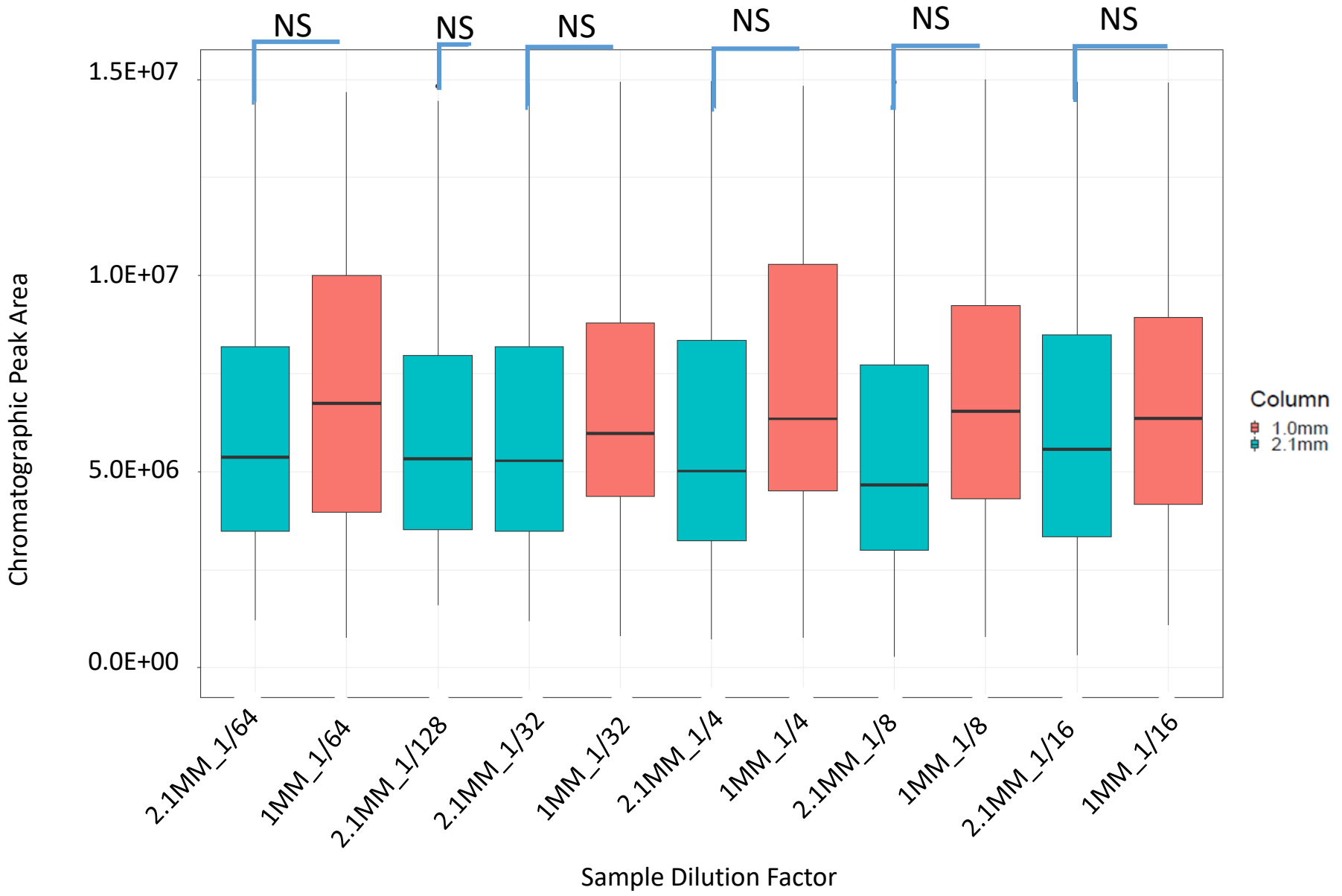
S35: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) applying the HILIC assay in positive ion mode on Urine samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. All comparisons were not significant (P > 0.05).



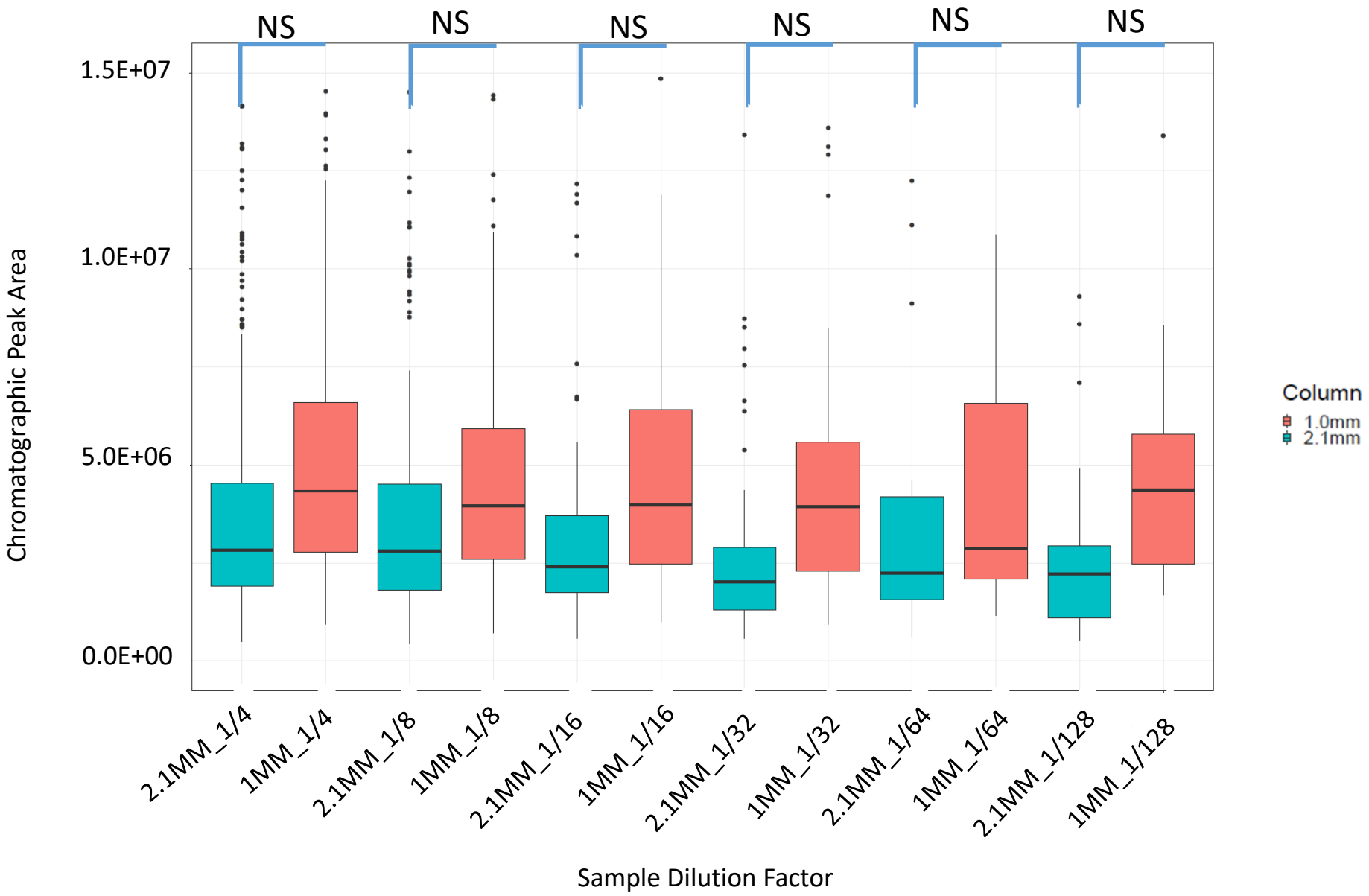
S36: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) applying the lipidomics assay in negative ion mode on plasma samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. All comparisons between columns were not significant. Most comparisons were not significant ($P > 0.05$), one comparison was statistically significant ($P \leq 0.05$), one was very statistically significant ($P \leq 0.001$).



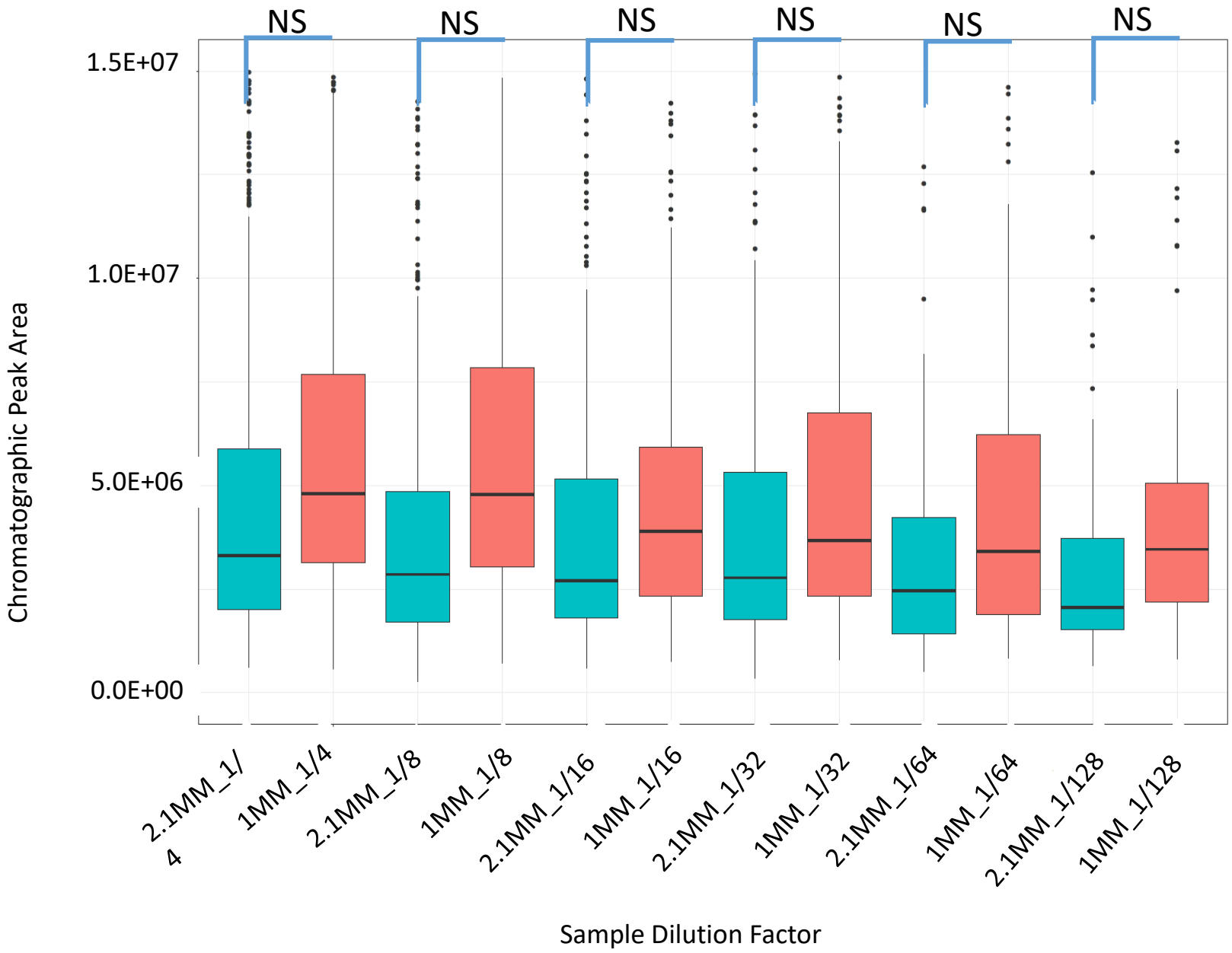
S37: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) applying the lipidomics assay in positive ion mode on plasma samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. All comparisons between columns were not significant. All comparisons were not significant ($P > 0.05$).



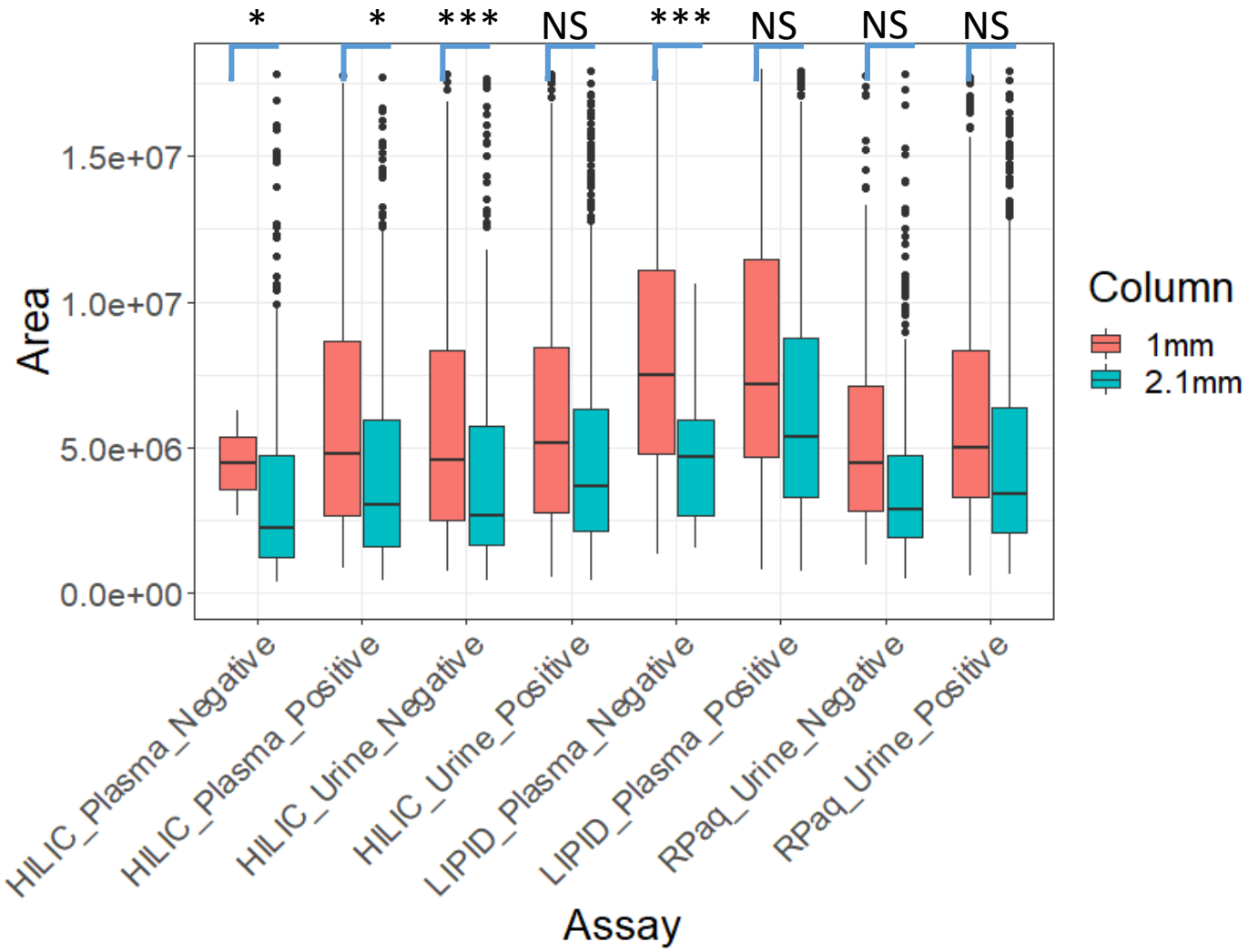
S38: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) ID applying the Reversed phase aqueous assay in negative ion mode on urine samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. All comparisons between columns were not significant. All comparisons were not significant ($P > 0.05$).



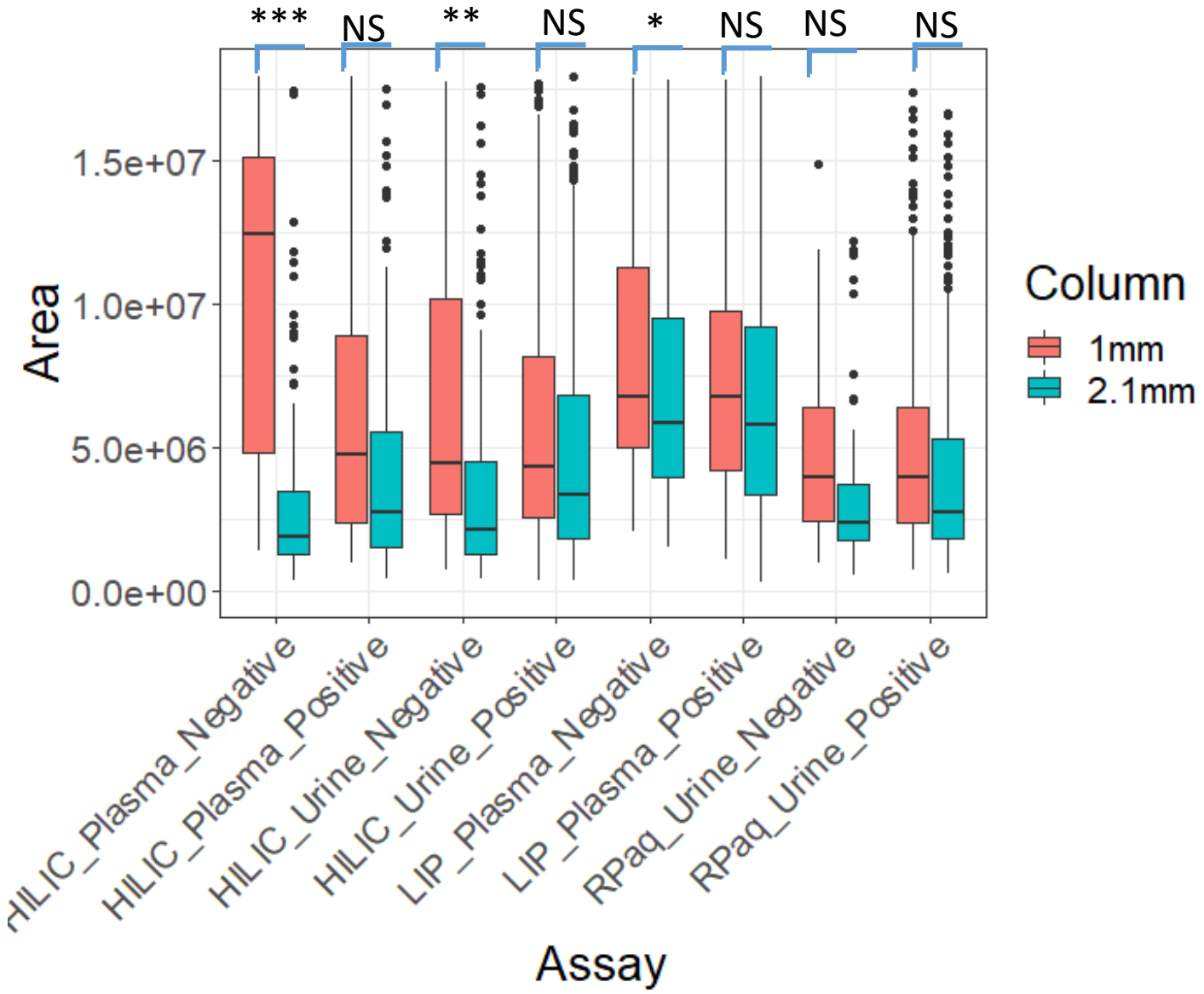
S39: Distribution of chromatographic peak areas for all dilutions of 2.1mm ID (blue) vs. 1mm ID (red) ID applying the Reversed phase aqueous assay in positive ion mode on urine samples. Compounds used were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. All comparisons between columns were not significant. All comparisons were not significant (P > 0.05).



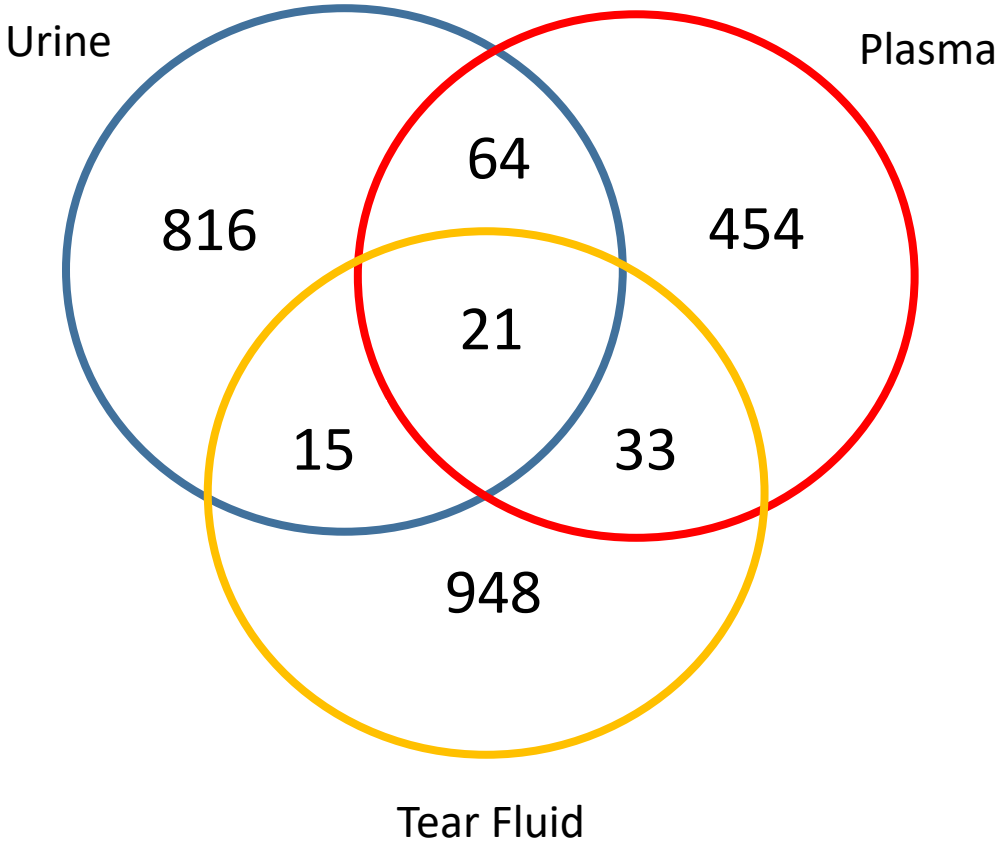
S40: Distribution of chromatographic peak areas in the 1 in 4 dilution of each assay type for both the 2.1mm ID (blue) and 1mm ID (red) columns. Compounds which were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. Most comparisons were not significant ($P > 0.05$), whilst two were significant ($P \leq 0.05$) and two were very significant. ($P \leq 0.001$)



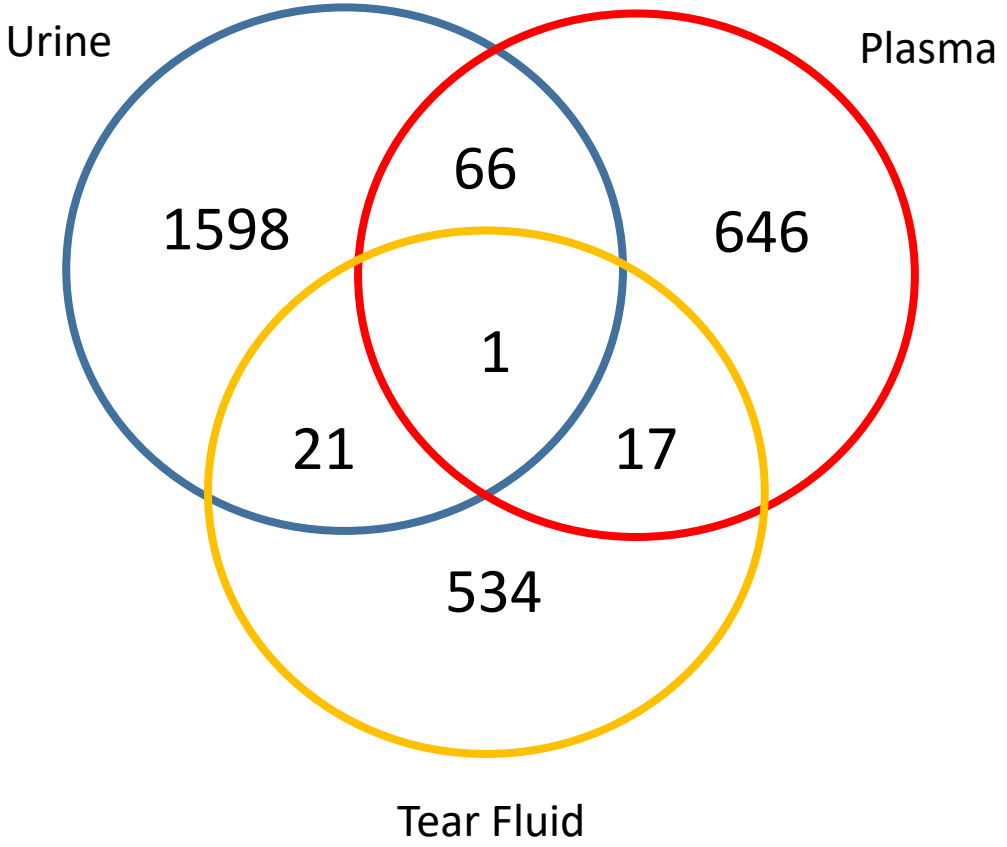
S41: The distribution of chromatographic peak areas in the 1 in 16 dilution of each assay type for both the 2.1mm ID (blue) and 1mm ID (red) columns. Compounds which were present in 75% of replicates and had a relative standard deviation (RSD) <30% are reported. Most comparisons were not significant (P > 0.05), whilst one was significant (P ≤ 0.05), one was moderately significant (P≤ 0.01) and one was very significant (P ≤ 0.001).



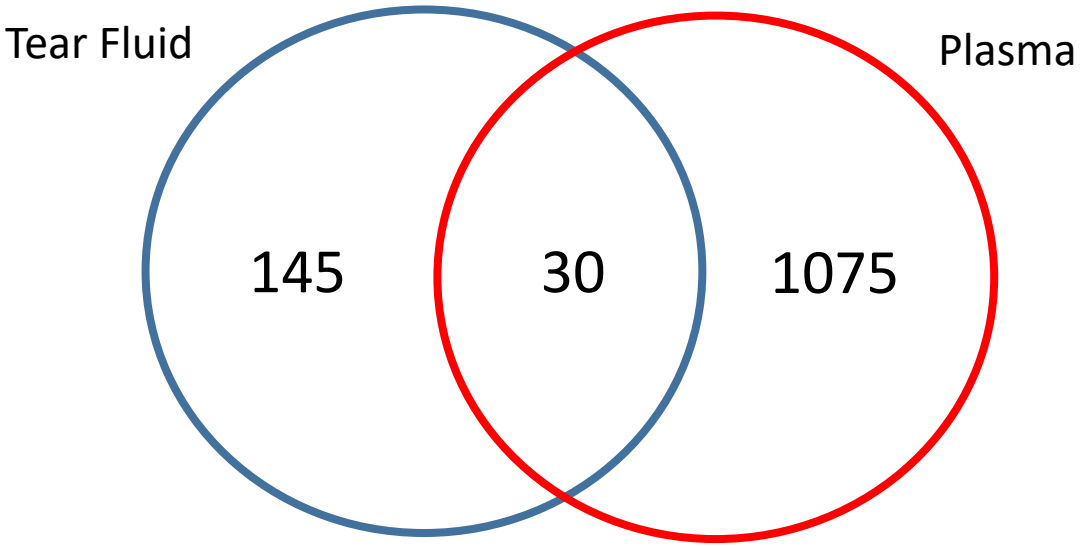
S42: Overlap of porcine plasma (red), urine (blue), and tear fluid (yellow) compounds detected during analysis using HILIC assay in negative ion mode



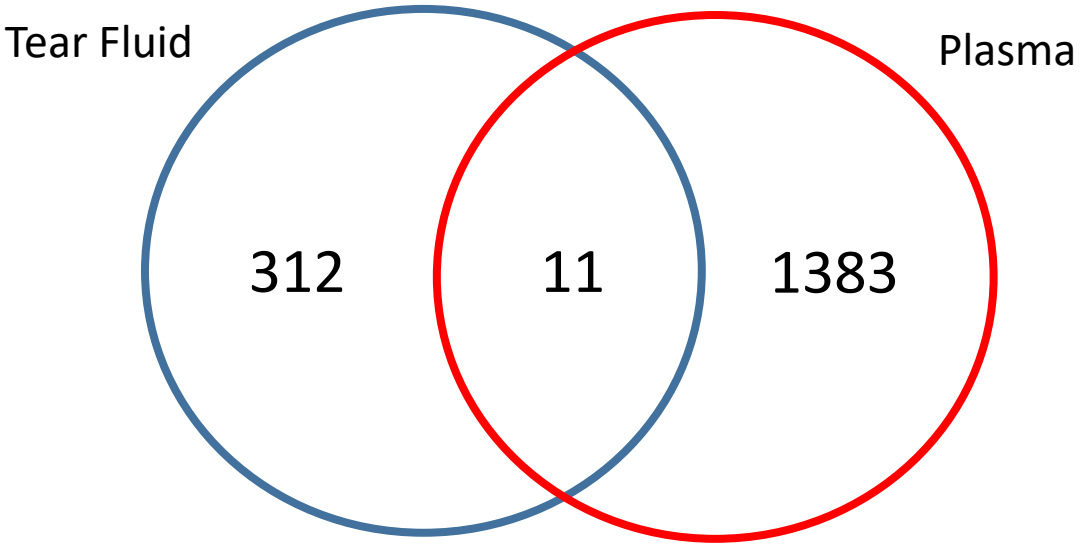
S43: Overlap of porcine plasma (red), urine (blue), and tear fluid (yellow) compounds detected during analysis using HILIC assay in positive ion mode



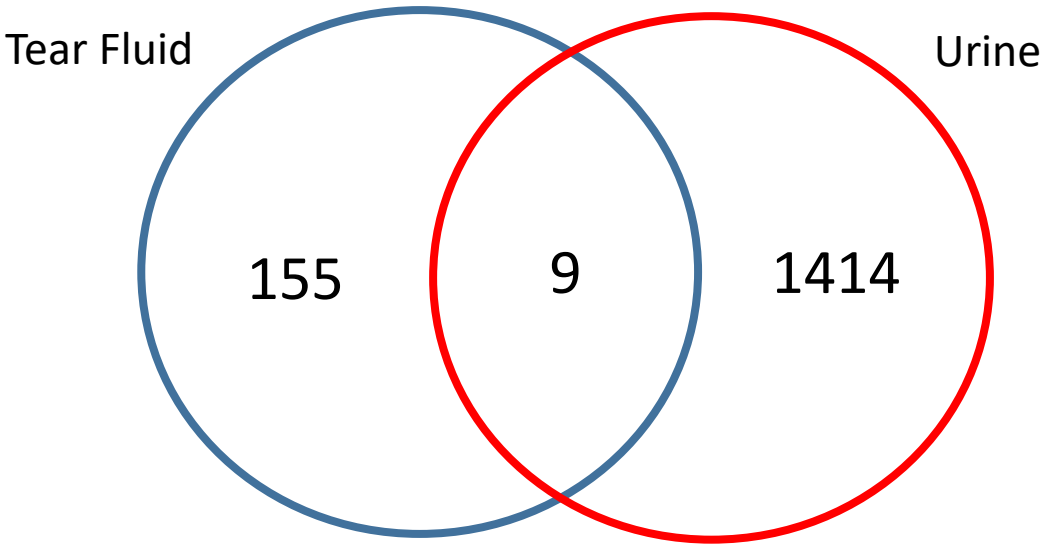
S44: Overlap of porcine plasma (red) and tear fluid (blue) compounds detected during analysis using lipidomics assay in negative ion mode



S45: Overlap of porcine plasma (red) and tear fluid (blue) compounds detected during analysis using lipidomics assay in positive ion mode



S46: Overlap of porcine urine (red) and tear fluid (blue) compounds detected during analysis using reversed phase aqueous assay in negative ion mode



S47: Overlap of porcine urine (red) and tear fluid (blue) compounds detected during analysis using reversed phase aqueous assay in positive ion mode