

Demo Report for UPLC-MSMS Tender Evaluation (NCFS/Outram)

Customer: Singapore Food Agency

Samples: Unknown A1, A2, A3, Blank A
Unknown B1, B2, B3, Blank B
7 Calibrants, 1 Calibrant Blank
Standards A-H, Internal Standards A-G

Instruments: ACQUITY UPLC I-Class FTN
Xevo TQ-XS Triple Quadrupole Mass Spectrometer

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1 Executive Summary

1. In this demo, sample analysis using the ACQUITY UPLC I-Class FTN system with CORTECS T3 UPLC column demonstrated excellent peak separation with consistent retention time (RSD < 0.5%).
2. The optimized MRM transitions delivered linear quantitation across the entire calibration range, with $R^2 > 0.998$ for all eight standards.
3. The ACQUITY UPLC I-Class coupled to Xevo TQ-XS Triple Quadrupole MS system provided reproducible quantitation with RSD < 10% across ten injections of Cal 1 = 1 ppt for all standards, except for Standard G, which achieved excellent repeatability for Cal 2 = 5 ppt.
4. Analytes that were identified and quantitated from the samples also demonstrated target ion ratios that are within $\pm 15\%$ range of the predicted target ion ratio values.
5. Analytes were also identified from blanks A and B, with their values provided in the requested summary report.

2 Summary Report

Mobile phase A used: Water with 0.1% Formic Acid

Mobile phase B used: Acetonitrile with 0.1% Formic Acid

Column used: CORTECS UPLC T3 Column, 120Å, 1.6 µm, 2.1 mm X 100 mm

Sample Vial	Analyte(s) identified	#Concentration of Analyte(s) in ppt
Blank A	A, B, C, D, E, F, G	A: 0.7 B: 0.2 C: 0.1 D: 0.3 E: 0.4 F: 0.2 G: 1.4
Unknown A-1	A, B, C, D, E, F, G, H	A: 5.0 B: 4.9 C: 5.0 D: 5.2 E: 3.7 F: 6.5 G: 6.0 H: 4.6
Unknown A-2	A, B, C, D, E, F, G, H	A: 9.8 B: 9.7 C: 9.9 D: 10.2 E: 9.2 F: 11.0 G: 14.0 H: 9.4
Unknown A-3	A, B, C, D, E, F, G	A: 3.1 B: 1.7 C: 4.0 D: 4.7 E: 0.1 F: 10.1 G: 2.8
Blank B	A, B, C, D, E, F, G, H	A: 293.0 B: 14.8

		C: 308.4 D: 14.3 E: 20.4 F: 5.1 G: 268.8 H: 1.2
Unknown B-1	A, B, C, D, E, F, G, H	A: 276.3 B: 15.7 C: 253.0 D: 16.5 E: 21.6 F: 5.6 G: 350.5 H: 4.4
Unknown B-2	A, B, C, D, E, F, G, H	A: 255.1 B: 21.3 C: 240.3 D: 21.5 E: 25.9 F: 13.3 G: 296.7 H: 13.0
Unknown B-3	A, B, C, D, E, F, G, H	A: 188.4 B: 8.9 C: 192.2 D: 18.5 E: 16.1 F: 4.2 G: 168.4 H: 1.1

Concentration of each analyte was determined from triplicate analyses.

All quantified analytes exhibited target ion ratio within $\pm 15\%$ range of the predicted ion ratio values (Table X – Table X), except for quantitation of G in samples A.

3 Instrument Methods

3.1 UPLC Method

The following UPLC method was developed for separation and quantitation of the standards in the provided calibrants and samples. The UPLC system coupled to the MS system was ACQUITY UPLC I-Class System (SM-FTN).

Binary Solvent Manager

- a. Column: CORTECS UPLC T3 Column, 120Å, 1.6 µm, 2.1 mm X 100 mm
- b. Injection volume: 40 µL
- c. Flow rate: 0.4 mL/min
- d. Mobile phase: A – Water with 0.1% Formic Acid
B – Acetonitrile with 0.1% Formic Acid
- e. Mobile phase gradient used:

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.4	88.0	12.0	Initial
2.3	0.5	85.0	15.0	6.0
4.2	0.5	0.0	100.0	4.0
4.3	0.5	88.0	12.0	6.0
6.3	0.5	88.0	12.0	6.0

Sample Manager FTN

- a. Wash solvent: 10/90 Water/Acetonitrile
- b. Purge solvent: 90/10 Water/Acetonitrile
- c. Sample temperature: 10 °C
- d. Column temperature: 45 °C

2.2 MS Method

MS Source parameters

- a. Capillary Voltage: 2.0 kV
- b. Source Temperature: 150 °C
- c. Desolvation Temperature: 450 °C

- d. Desolvation Gas Flow: 900 L/Hr
- e. Cone Gas Flow: 150 L/Hr
- f. Nebulizer Pressure: 7.0 Bar

3.3 MRM Development

To develop optimized conditions and MRM transitions for accurate and reproducible quantitation of each analyte, the formula of each compound was predicted based on their corresponding exact mass values. Using this information, literature search was performed to identify putative compounds with matched molecular formulas. This information was utilized to postulate an initial LC method with appropriate column chemistry for resolving the analytes.

An LCMS analysis via full scan mode was subsequently performed to identify the retention time of each analyte, followed by a daughter ion scan to select for suitable daughter ions. Cone voltage and collision energy (CE) were optimized for each MRM transition to obtain enhanced sensitivity for each transition.

Table 1: Optimized source conditions for each MRM transition. Retention time and target ion ratio of each MRM transition is also listed accordingly. Quantifier daughter ion is depicted in red font.

Formula	Compound	Parent ion (m/z)	Daughter ion (m/z)	Cone (V)	CE (eV)	Retention time (min)	Target Ion Ratio	Type of adduct
C ₁₀ H ₁₅ N	Standard A	150.0	90.9 118.9	16 16	15 10	1.7	2.65	[M+H] ⁺
C ₁₁ H ₁₅ NO ₂	Standard B	193.9	163.0 105.0	24 16	23 13	1.8	2.14	[M+H] ⁺
C ₁₈ H ₂₁ NO ₃	Standard C	300.0	215.2 165.2	16 20	43 25	1.2	1.11	[M+H] ⁺
C ₁₃ H ₁₆ ClNO	Standard D	238.1	125.1 207.1	6 28	25 13	2.5	3.35	[M+H] ⁺
C ₉ H ₁₃ N	Standard E	136.0	90.9 118.9	16 20	13 10	1.4	2.64	[M+H] ⁺
C ₁₀ H ₁₃ NO ₂	Standard F	180.0	163.1 105.0	20 20	20 10	1.5	2.54	[M+H] ⁺
C ₂₃ H ₂₁ FN ₂ O	Standard G	361.1	213.0	23	20	3.9	1.32	[M+H] ⁺

			233.0	23	15			
C19H26FN3O3	Standard H	364.1	318.2 145.0	3 7	43 13	3.8	1.20	[M+H] ⁺
C10D8H7N	Internal standard A	158.0	93.0 124.0	16 16	15 10	1.7	1.54	[M+H] ⁺
C11D5H10NO2	Internal standard B	199.0	165.0 107.0	24 16	23 13	1.7	2.39	[M+H] ⁺
C18D3H18NO3	Internal standard C	303.0	215.2 165.0	16 20	43 25	1.2	1.20	[M+H] ⁺
C13H12D4ClNO	Internal standard D	242.0	129.0 211.0	6 28	25 13	2.5	3.59	[M+H] ⁺
C9H8D5N	Internal standard E	141.0	92.8 124.0	16 20	13 10	1.4	1.11	[M+H] ⁺
C10H8D5NO2	Internal standard F	185.1	168.0 110.0	20 20	20 10	1.5	2.52	[M+H] ⁺
C24H17D5FNO	Internal standard G	382.0	155.0 127.0	20 20	20 20	3.8	42.29	[M+NH ₄] ⁺

Chromatograms of individual MRM transition

Images of individual MRM chromatograms were obtained after processing the raw data using TargetLynx XS, which is a software that automates data processing and reporting for quantitation. Figures of all MRM chromatograms are shown below.

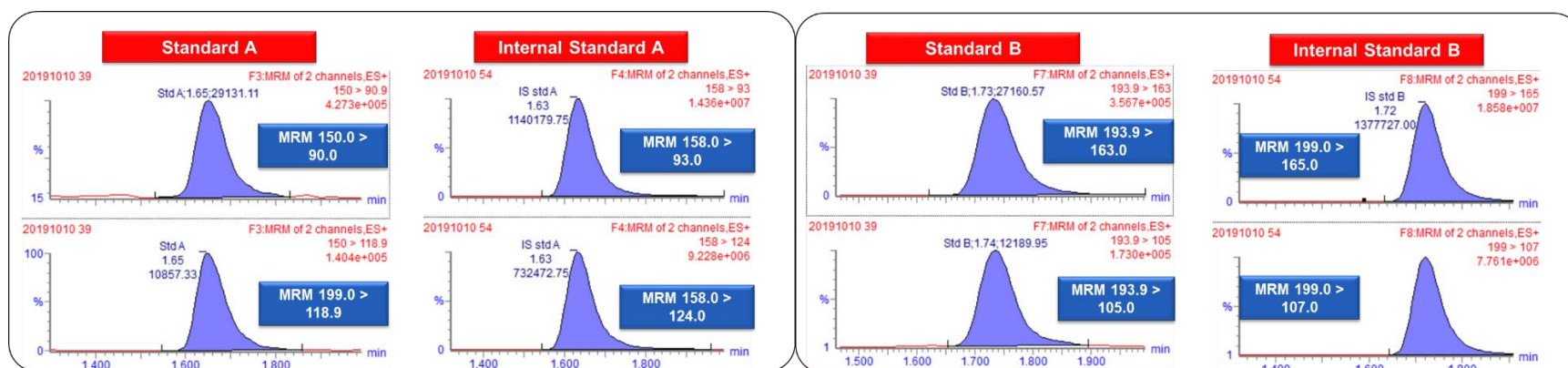


Figure 1: MRM chromatograms of standards (A and B) and internal standards (A and B). Chromatograms were obtained from LC-MSMS analysis of standard solution using optimized LC and MS parameters.

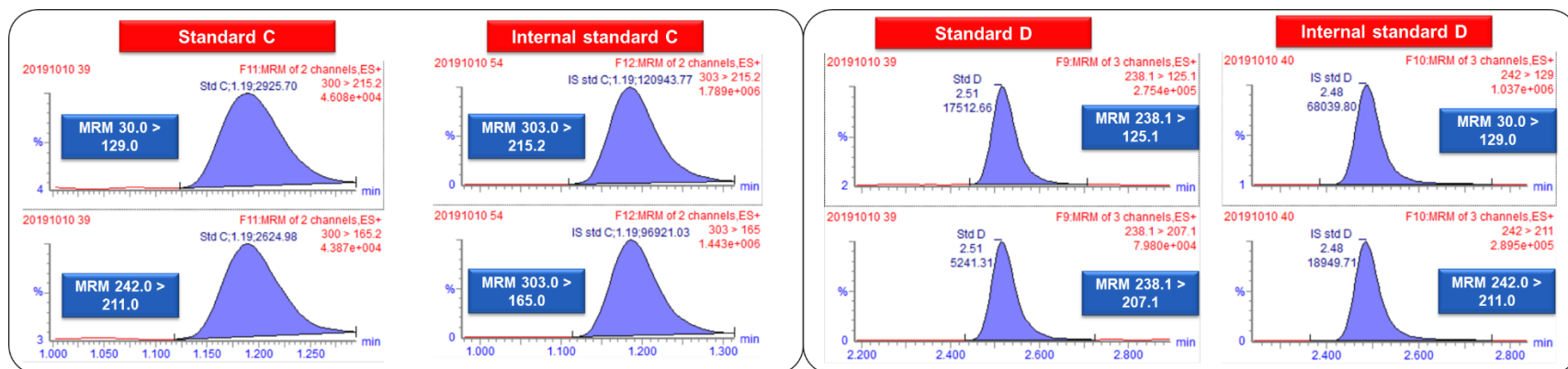


Figure 2: MRM chromatograms of standards (C and D) and internal standards (C and D). Chromatograms were obtained from LC-MSMS analysis of standard solution using optimized LC and MS parameters.

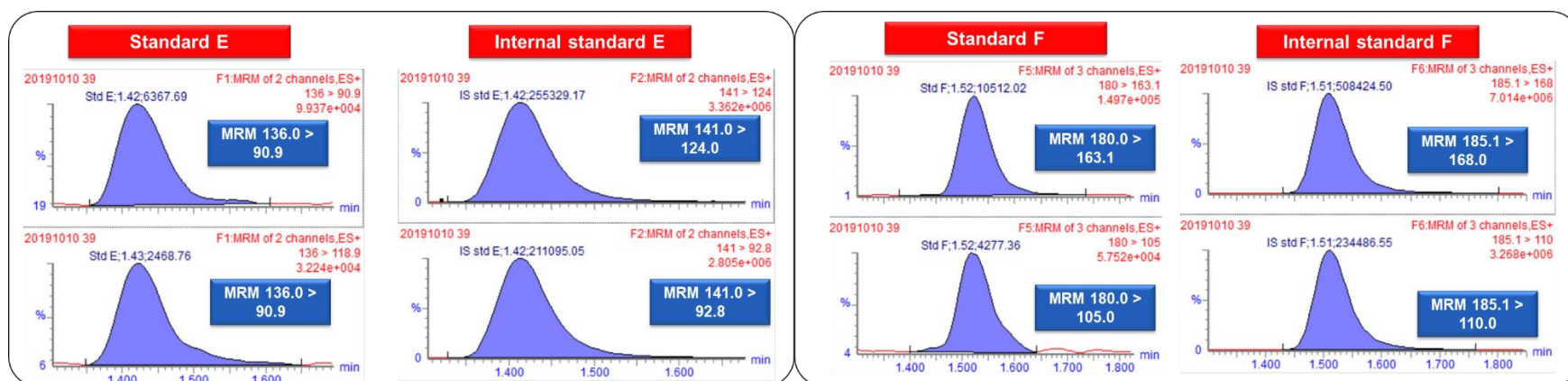


Figure 3: MRM chromatograms of standards (E and F) and internal standards (E and F). Chromatograms were obtained from LC-MSMS analysis of standard solution using optimized LC and MS parameters.

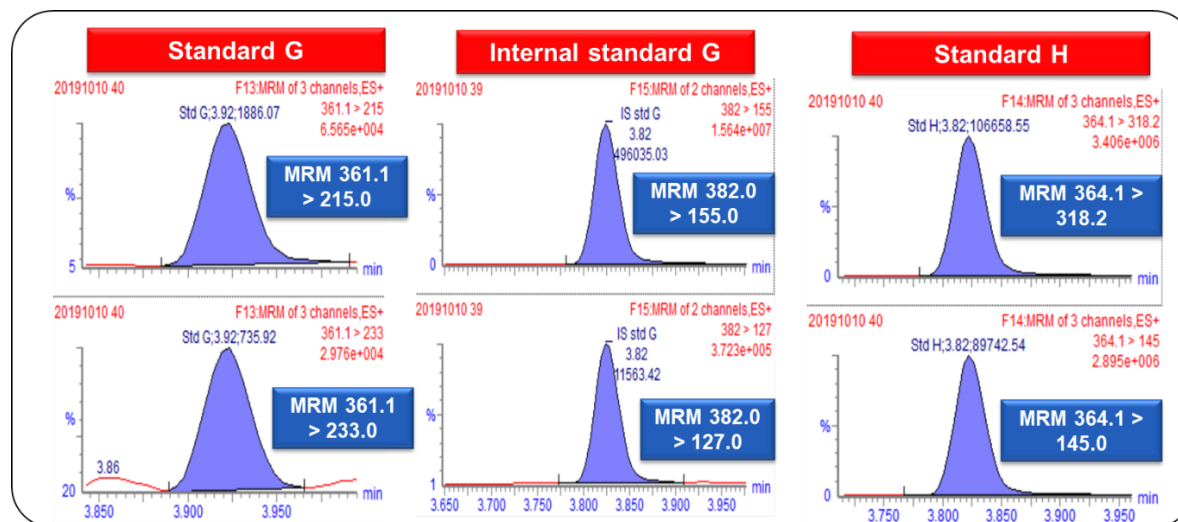


Figure 4: MRM chromatograms of standards (G and H) and internal standard G. Chromatograms were obtained from LC-MSMS analysis of standard solution using optimized LC and MS parameters.

4 Results and Discussion

4.1 Calibration of individual analyte using provided calibrants

Calibration of all analytes was performed using the seven calibrants, along with the provided calibrant blank. Observation of MRM peaks with target ion ratios within $\pm 15\%$ of the predicted target ion ratios suggests the presence of analytes in the calibrant blank. Hence, calibration curves were generated without intercepting the origin. Resultant calibration curves of all analytes exhibit excellent linearity (**Table 2**) for the provided calibration range (0 – 250 ppt).

Table 2: Summary of linearity values obtained for all standards.

Standard	Linearity (R^2)
A	0.999
B	0.999
C	0.998
D	0.999
E	0.999
F	0.999
G	0.998
H	0.998

Compound name: Std A
Correlation coefficient: $r = 0.999720$, $r^2 = 0.999441$
Calibration curve: $0.00254184 * x + 0.00238908$
Response type: Internal Std (Ref 9), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

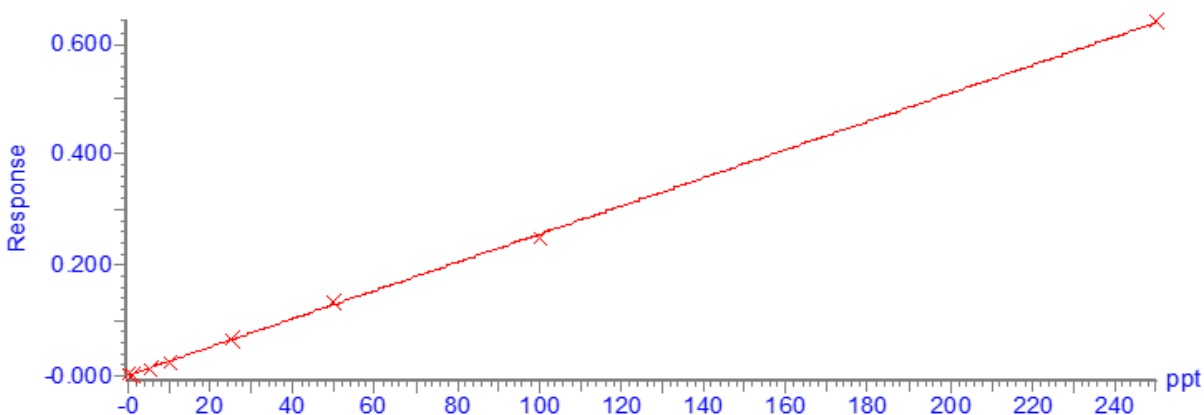


Figure 5: Calibration curve of Standard A.

Compound name: Std B
Correlation coefficient: $r = 0.999855$, $r^2 = 0.999710$
Calibration curve: $0.00197424 * x + 0.000822174$
Response type: Internal Std (Ref 10), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

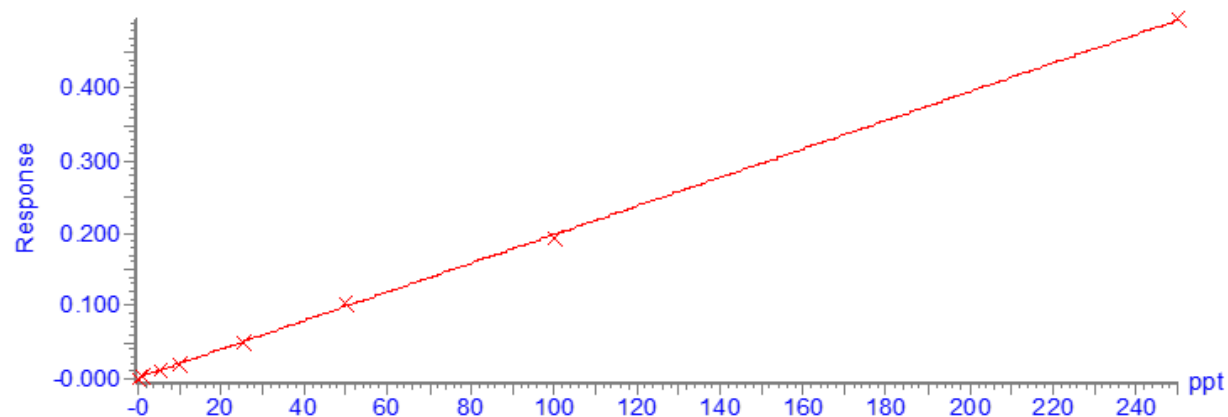


Figure 6: Calibration curve of Standard B.

Compound name: Std C
Correlation coefficient: $r = 0.999279$, $r^2 = 0.998559$
Calibration curve: $0.00255785 * x + 0.00228368$
Response type: Internal Std (Ref 11), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

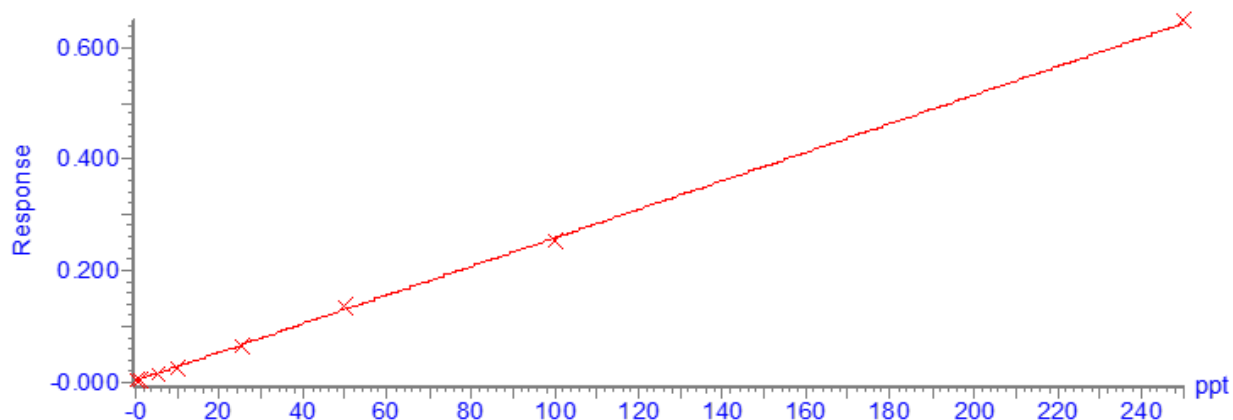


Figure 7: Calibration curve of Standard C.

Compound name: Std D
Correlation coefficient: $r = 0.999571$, $r^2 = 0.999143$
Calibration curve: $0.0231443 * x + 0.0097276$
Response type: Internal Std (Ref 12), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

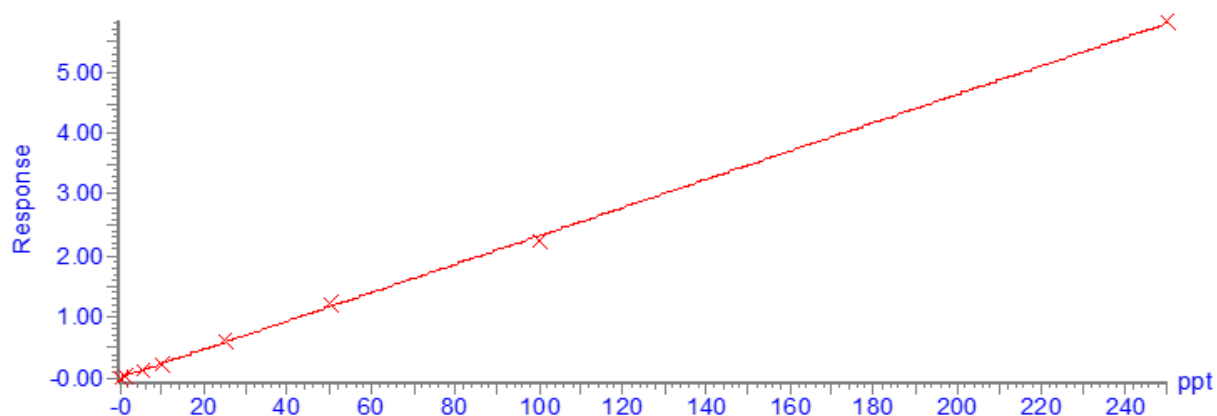


Figure 8: Calibration curve of Standard D.

Compound name: Std E
Correlation coefficient: $r = 0.999569$, $r^2 = 0.999137$
Calibration curve: $0.00306889 * x + 0.00211535$
Response type: Internal Std (Ref 13), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

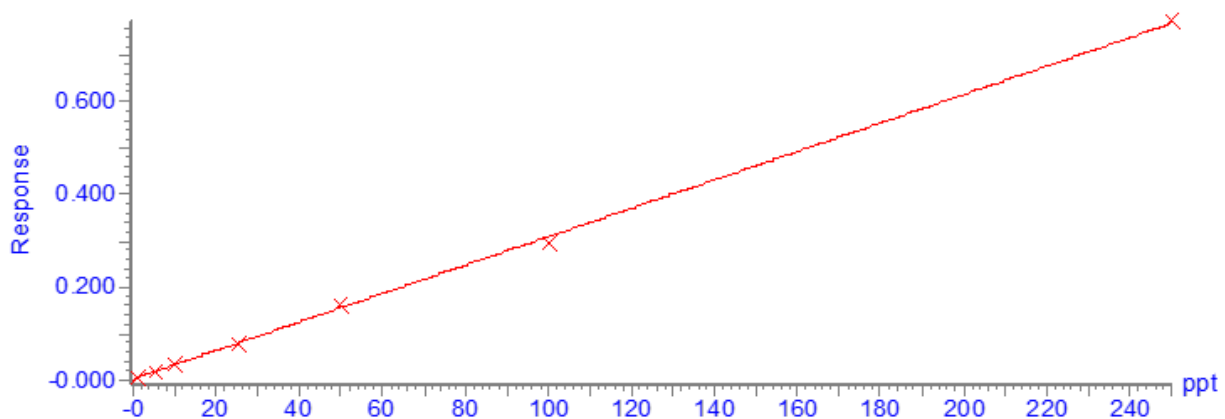


Figure 9: Calibration curve of Standard E.

Compound name: Std F
Correlation coefficient: $r = 0.999634$, $r^2 = 0.999267$
Calibration curve: $0.00174579 * x + 0.01424$
Response type: Internal Std (Ref 14), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

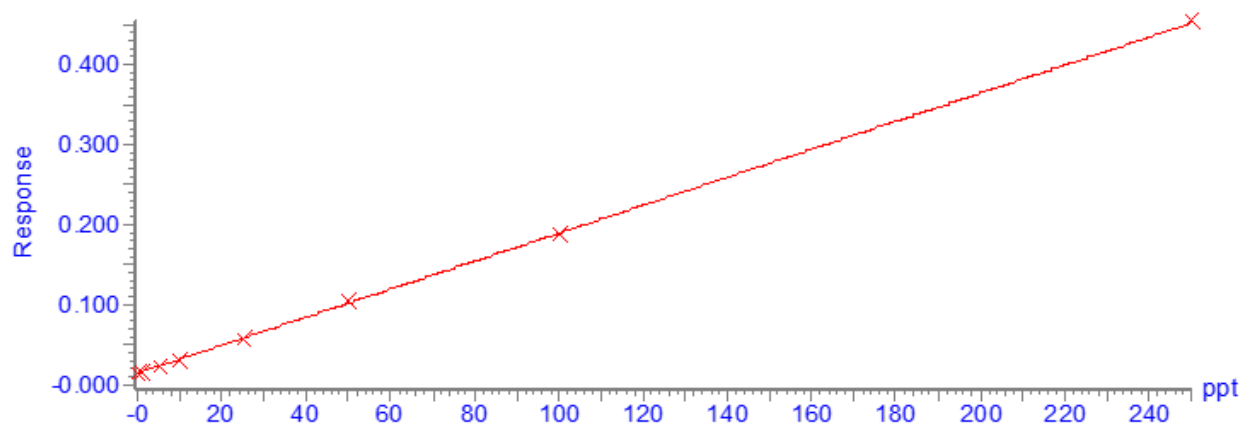


Figure 10: Calibration curve of Standard F.

Compound name: Std G
Correlation coefficient: $r = 0.999216$, $r^2 = 0.998433$
Calibration curve: $2.77154e-005 * x + 1.96302e-005$
Response type: Internal Std (Ref 15), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

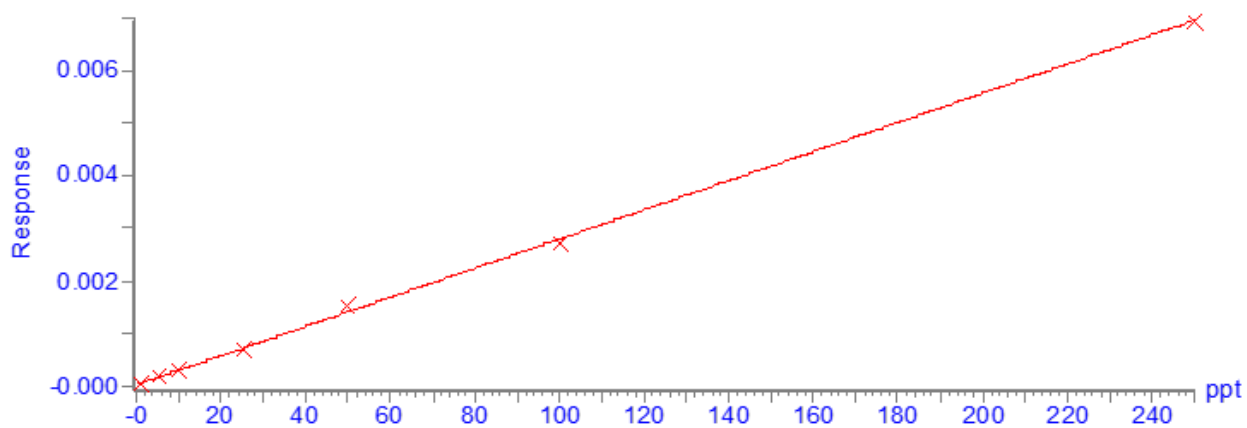


Figure 11: Calibration curve of Standard G.

Compound name: Std H
 Correlation coefficient: $r = 0.999078$, $r^2 = 0.998157$
 Calibration curve: $0.00321527 * x + 0.0047291$
 Response type: Internal Std (Ref 15), Area * (IS Conc. / IS Area)
 Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

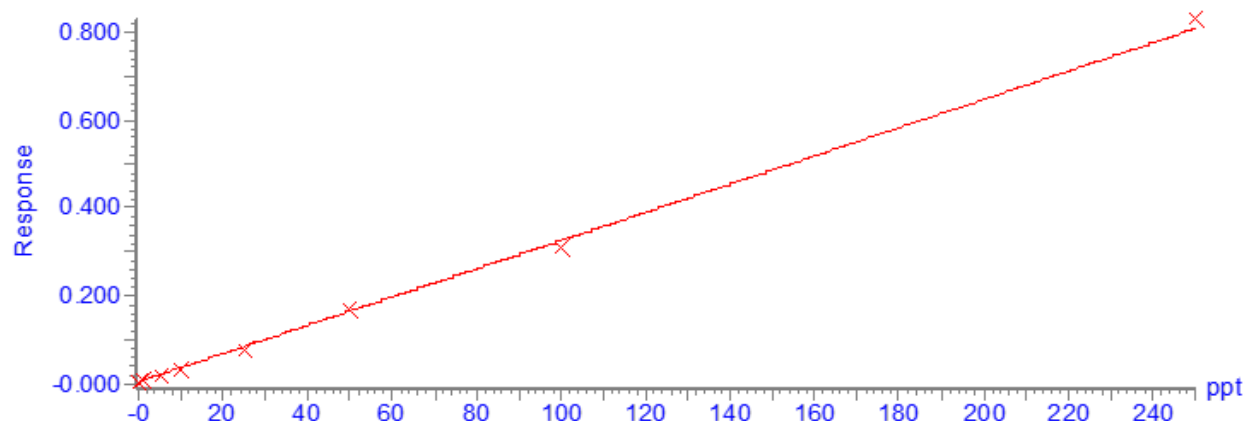


Figure 12: Calibration curve of Standard H.

4.2 Repeatability test of all standards

Repeatability test of all standards was determined by referencing the generated responses of the individual standards across ten injections. All standards exhibited excellent reproducibility at 1 ppt (Cal 1), except for standard G, which provided reproducible quantitation at 5 ppt (Cal 2). %RSD for each standard was calculated automatically using Trendplot, a software for statistical analysis of sample analysis results. Repeatability plots generated by Trendplot are provided below.

Table 3: Summary table of repeatability test for all standards. % RSD for each standard was calculated based on response values obtained from ten injections of calibrant 1 or 2.

Standard	Calibrant used	% RSD
A	Cal 1	5.7
B	Cal 1	3.9
C	Cal 1	6.0
D	Cal 1	2.2
E	Cal 1	9.0
F	Cal 1	4.4
G	Cal 2	9.3
H	Cal 1	7.0

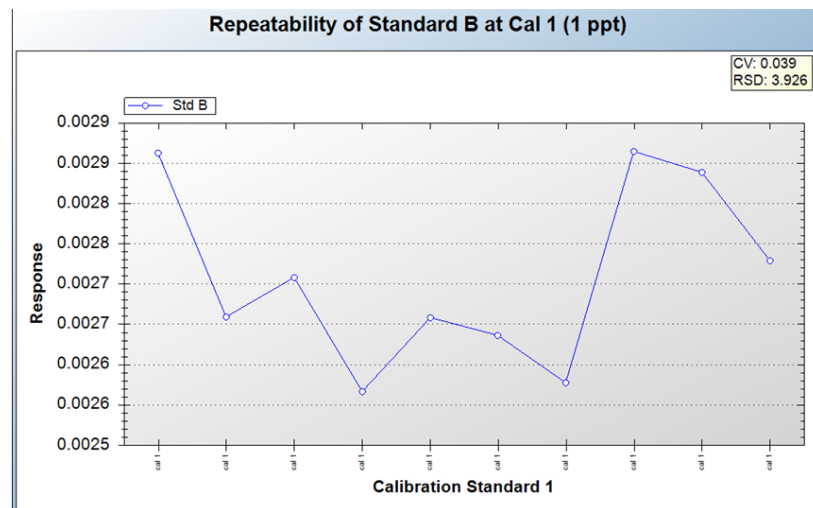
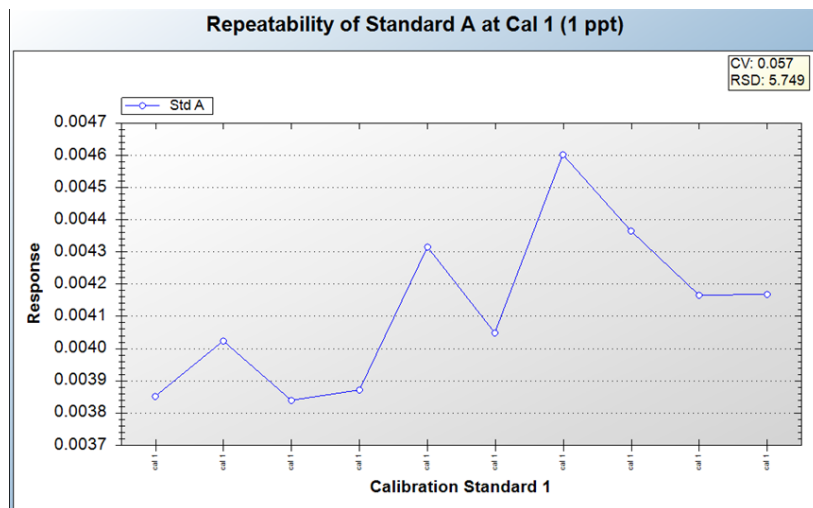


Figure 13: Trendplot of standard A and B across ten injections of Calibrant 1.

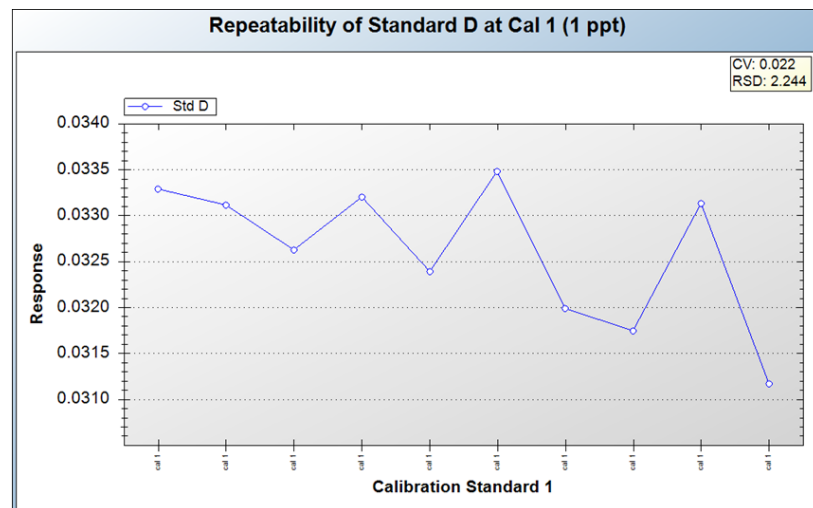
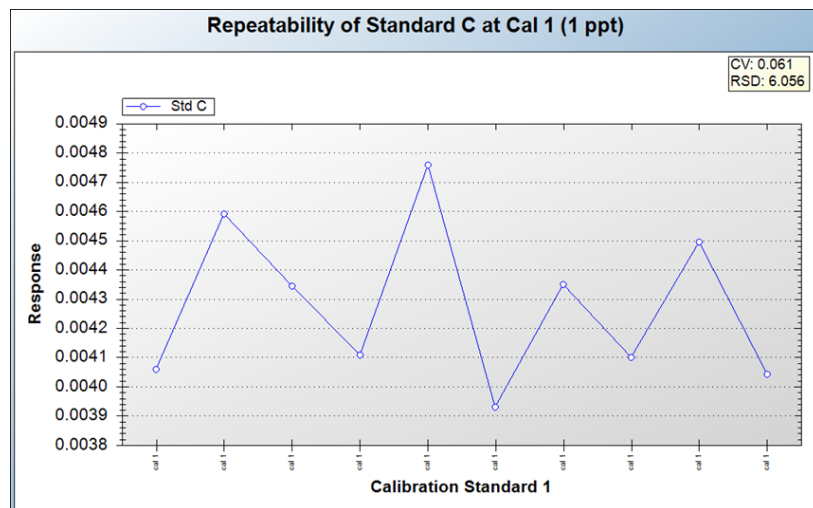


Figure 14: Trendplot of standard C and D across ten injections of Calibrant 1.

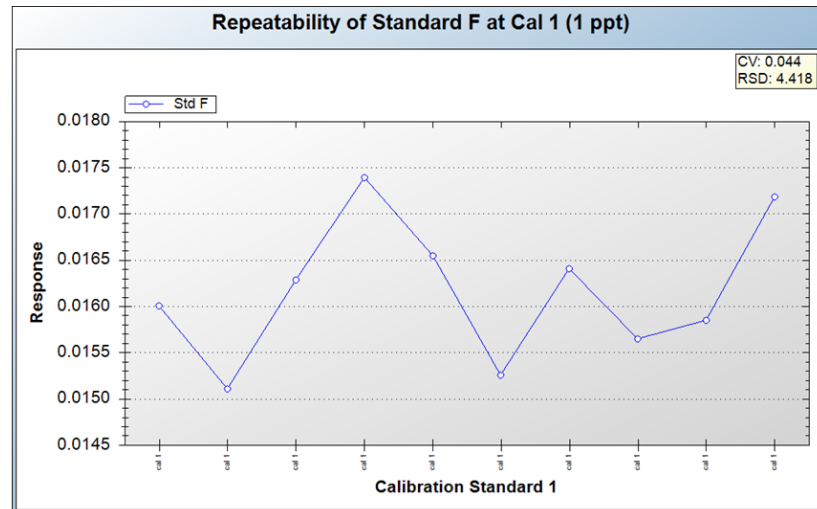
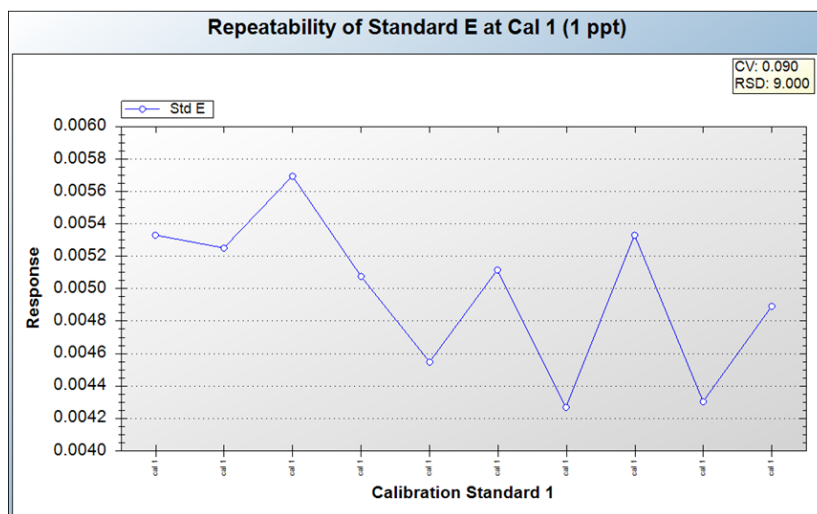


Figure 15: Trendplot of standard E and F across ten injections of Calibrant 1.

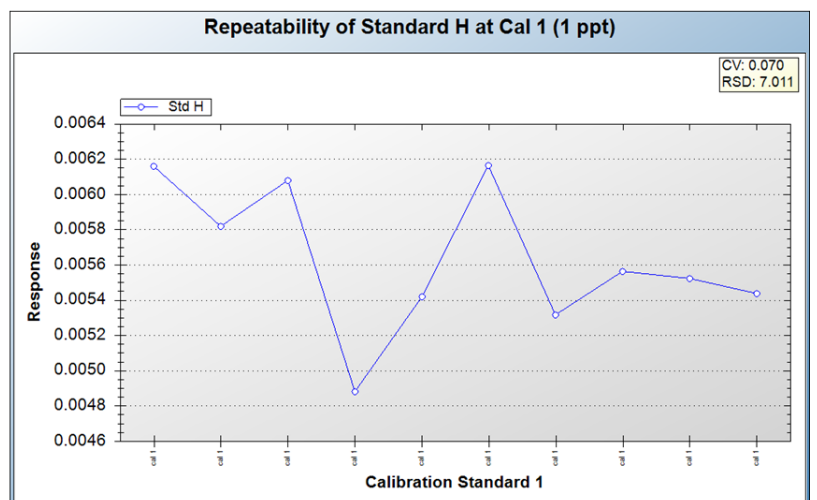
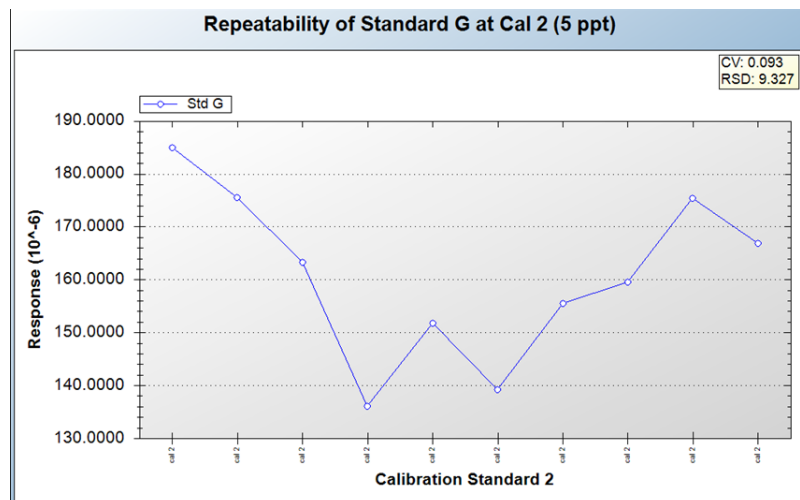


Figure 16: Trendplot of standard G and H across ten injections of Calibrant 2 and Calibrant 1, respectively.

4.3 Quantitation of analytes in provided samples

Each sample was analyzed and the quantity of individual analytes present was determined based on the generated calibration curve for each analyte. Triplicate measurements were taken for each sample and their results are shown below.

In general, reproducible measurements were obtained for all analytes in the eight samples, except for analyte G, which exhibited poor reproducibility for sample set A. Elevated baseline noise, coupled with low sensitivity of the MRM transitions for analyte G, impeded accurate and reproducible quantitation of the compound at low concentrations in the provided sample matrix. Calculated target ion ratios of the peaks also exceeded $> \pm 20\%$ range of the predicted target ion ratio.

Table 4: Summary table of analyte A quantitation in all samples.

Analyte A												
#	ID	Type	RT (min)	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	1.67	4411.7	1086248	0.004	0.7	2.59	2.65	NO	0.7	0.0
2	Blank A	Analyte	1.67	4372.6	1053646	0.004	0.7	2.92	2.65	NO		
3	Blank A	Analyte	1.67	4452.1	1058995	0.004	0.7	2.54	2.65	NO		
4	A1	Analyte	1.67	16469.6	1113941	0.015	4.9	2.85	2.65	NO	5.0	0.0
5	A1	Analyte	1.67	17373.0	1111943	0.016	5.2	2.75	2.65	NO		
6	A1	Analyte	1.67	16375.7	1123903	0.015	4.8	2.71	2.65	NO		
7	A2	Analyte	1.67	30959.0	1118848	0.028	9.9	2.60	2.65	NO	9.8	3.1
8	A2	Analyte	1.67	29624.3	1115936	0.027	9.5	2.58	2.65	NO		
9	A2	Analyte	1.67	30918.2	1104104	0.028	10.1	2.78	2.65	NO		
10	A3	Analyte	1.67	11022.8	1083068	0.010	3.1	2.48	2.65	NO	3.1	1.8
11	A3	Analyte	1.66	11318.6	1089652	0.010	3.1	2.70	2.65	NO		
12	A3	Analyte	1.66	11170.2	1058162	0.011	3.2	2.87	2.65	NO		
13	Blank B	Analyte	1.67	744936.0	1002670	0.743	291.3	2.60	2.65	NO	293.0	0.6
14	Blank B	Analyte	1.67	750934.9	999585	0.751	294.6	2.64	2.65	NO		
15	Blank B	Analyte	1.67	744970.7	997054	0.747	293.0	2.60	2.65	NO		
16	B1	Analyte	1.67	738670.7	1051743	0.702	275.4	2.63	2.65	NO	276.3	0.3
17	B1	Analyte	1.67	737915.2	1045969	0.705	276.6	2.64	2.65	NO		
18	B1	Analyte	1.67	741549.8	1050378	0.706	276.8	2.64	2.65	NO		
19	B2	Analyte	1.67	705362.4	1070162	0.659	258.4	2.59	2.65	NO	255.1	1.1
20	B2	Analyte	1.67	707475.2	1093278	0.647	253.6	2.61	2.65	NO		
21	B2	Analyte	1.67	702648.8	1087718	0.646	253.2	2.63	2.65	NO		
22	B3	Analyte	1.66	519600.7	1075874	0.483	189.1	2.63	2.65	NO	188.4	0.7
23	B3	Analyte	1.66	510081.7	1055404	0.483	189.2	2.62	2.65	NO		
24	B3	Analyte	1.66	513276.4	1074288	0.478	187.0	2.59	2.65	NO		

Table 5: : Summary table of analyte B quantitation in all samples.

Analyte B												
#	ID	Type	RT (min)	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	1.75	1292.7	1096982	0.001	0.2	2.21	2.14	NO	0.2	0.0
2	Blank A	Analyte	1.75	1256.6	1077631	0.001	0.2	2.13	2.14	NO		
3	Blank A	Analyte	1.75	1320.1	1071014	0.001	0.2	2.16	2.14	NO		
4	A1	Analyte	1.75	11849.2	1120051	0.011	4.9	2.37	2.14	NO	4.9	3.1
5	A1	Analyte	1.75	11577.8	1145817	0.010	4.7	2.06	2.14	NO		
6	A1	Analyte	1.75	12057.8	1121642	0.011	5.0	2.11	2.14	NO		
7	A2	Analyte	1.75	22930.5	1123455	0.020	9.9	2.35	2.14	NO	9.7	2.2
8	A2	Analyte	1.75	22657.2	1147279	0.020	9.6	1.98	2.14	NO		
9	A2	Analyte	1.75	22152.3	1135368	0.020	9.5	2.12	2.14	NO		
10	A3	Analyte	1.75	4539.0	1124464	0.004	1.6	2.15	2.14	NO	1.7	5.9
11	A3	Analyte	1.74	4942.9	1153014	0.004	1.8	2.22	2.14	NO		
12	A3	Analyte	1.74	4789.2	1157222	0.004	1.7	2.23	2.14	NO		
13	Blank B	Analyte	1.75	30059.6	991405	0.030	14.9	1.94	2.14	NO	14.8	1.6
14	Blank B	Analyte	1.75	29602.6	977225	0.030	14.9	2.10	2.14	NO		
15	Blank B	Analyte	1.75	28805.5	981087	0.029	14.5	2.04	2.14	NO		
16	B1	Analyte	1.75	34139.9	1088929	0.031	15.5	2.02	2.14	NO	15.7	1.7
17	B1	Analyte	1.75	34699.6	1096580	0.032	15.6	2.30	2.14	NO		
18	B1	Analyte	1.75	35502.8	1092661	0.032	16.0	2.19	2.14	NO		
19	B2	Analyte	1.75	47893.5	1095200	0.044	21.7	2.29	2.14	NO	21.3	1.8
20	B2	Analyte	1.75	46612.8	1100419	0.042	21.0	2.14	2.14	NO		
21	B2	Analyte	1.75	46963.5	1103758	0.043	21.1	2.14	2.14	NO		
22	B3	Analyte	1.75	21670.9	1151525	0.019	9.1	2.44	2.14	NO	8.9	1.7
23	B3	Analyte	1.75	20313.6	1113562	0.018	8.8	2.37	2.14	NO		
24	B3	Analyte	1.75	20242.5	1097137	0.018	8.9	1.97	2.14	NO		

Table 6: Summary table of analyte C quantitation in all samples.

Analyte C												
#	ID	Type	RT	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	1.22	81.1	35485.8	0.002	0.0	1.04	1.11	NO	0.1	173.2
2	Blank A	Analyte	1.22	68.1	34466.5	0.002	0.0	1.11	1.11	NO		
3	Blank A	Analyte	1.22	94.7	34984.4	0.003	0.2	1.04	1.11	NO		
4	A1	Analyte	1.22	552.6	36367.7	0.015	5.0	1.17	1.11	NO	5.0	1.2
5	A1	Analyte	1.22	519.5	34704.4	0.015	5.0	1.12	1.11	NO		
6	A1	Analyte	1.22	523.9	35333.7	0.015	4.9	0.94	1.11	NO		
7	A2	Analyte	1.22	987.3	36039.9	0.027	9.8	1.23	1.11	NO	9.9	0.6
8	A2	Analyte	1.22	1028.2	37174.2	0.028	9.9	1.18	1.11	NO		
9	A2	Analyte	1.22	1004.8	36560.4	0.027	9.9	1.19	1.11	NO		
10	A3	Analyte	1.22	451.9	36159.6	0.012	4.0	1.28	1.11	NO	4.0	1.4
11	A3	Analyte	1.22	468.7	36440.1	0.013	4.1	1.08	1.11	NO		
12	A3	Analyte	1.22	479.0	38445.6	0.012	4.0	1.23	1.11	NO		
13	Blank B	Analyte	1.23	27370.6	34820.7	0.786	306.4	1.16	1.11	NO	308.4	0.7
14	Blank B	Analyte	1.23	26892.6	34028.2	0.790	308.1	1.17	1.11	NO		
15	Blank B	Analyte	1.23	26833.2	33681.9	0.797	310.6	1.16	1.11	NO		
16	B1	Analyte	1.23	24654.5	38016.5	0.649	252.6	1.13	1.11	NO	253.0	0.6
17	B1	Analyte	1.23	24933.7	38559.3	0.647	251.9	1.14	1.11	NO		
18	B1	Analyte	1.23	25198.8	38556.3	0.654	254.6	1.16	1.11	NO		
19	B2	Analyte	1.22	24031.3	39470.8	0.609	237.1	1.14	1.11	NO	240.3	1.1
20	B2	Analyte	1.23	24594.0	39638.3	0.620	241.7	1.17	1.11	NO		
21	B2	Analyte	1.23	24255.4	39040.4	0.621	242.0	1.15	1.11	NO		
22	B3	Analyte	1.22	19289.2	38939	0.495	192.8	1.16	1.11	NO	192.2	0.9
23	B3	Analyte	1.22	19343.7	38883.8	0.497	193.6	1.15	1.11	NO		
24	B3	Analyte	1.22	19355.9	39572.9	0.489	190.3	1.16	1.11	NO		

Table 7: Summary table of analyte D quantitation in all samples.

Analyte D												
#	ID	Type	RT	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	2.52	1196.8	71218.5	0.017	0.3	3.01	3.35	NO	0.3	0.0
2	Blank A	Analyte	2.52	1148.3	71903.6	0.016	0.3	3.05	3.35	NO		
3	Blank A	Analyte	2.52	1277.4	70974.9	0.018	0.3	3.70	3.35	NO		
4	A1	Analyte	2.52	9380.8	71258.5	0.132	5.3	3.41	3.35	NO	5.2	2.2
5	A1	Analyte	2.52	9364.8	72952.1	0.128	5.1	3.35	3.35	NO		
6	A1	Analyte	2.52	9443.5	71451.2	0.132	5.3	3.39	3.35	NO		
7	A2	Analyte	2.52	17603.3	72019.1	0.244	10.1	3.40	3.35	NO	10.2	1.5
8	A2	Analyte	2.52	17866.7	71556.2	0.250	10.4	3.49	3.35	NO		
9	A2	Analyte	2.52	17784.2	72538.8	0.245	10.2	3.46	3.35	NO		
10	A3	Analyte	2.52	7803.6	66100.4	0.118	4.7	3.34	3.35	NO	4.7	1.2
11	A3	Analyte	2.52	7960.4	68227.3	0.117	4.6	3.21	3.35	NO		
12	A3	Analyte	2.51	8089.9	68061.4	0.119	4.7	3.37	3.35	NO		
13	Blank B	Analyte	2.52	24426.5	72234.8	0.338	14.2	3.33	3.35	NO	14.3	2.1
14	Blank B	Analyte	2.52	23715.6	71180.9	0.333	14.0	3.26	3.35	NO		
15	Blank B	Analyte	2.52	24344.2	69879.8	0.348	14.6	3.34	3.35	NO		
16	B1	Analyte	2.52	28298.5	73567.9	0.385	16.2	3.22	3.35	NO	16.5	1.6
17	B1	Analyte	2.52	28856.8	73390.3	0.393	16.6	3.29	3.35	NO		
18	B1	Analyte	2.52	29413.0	74416	0.395	16.7	3.30	3.35	NO		
19	B2	Analyte	2.52	38405.0	74901.8	0.513	21.7	3.33	3.35	NO	21.5	0.9
20	B2	Analyte	2.52	38450.6	75914.5	0.506	21.5	3.29	3.35	NO		
21	B2	Analyte	2.52	38237.0	75915	0.504	21.3	3.32	3.35	NO		
22	B3	Analyte	2.52	20088.0	73732.5	0.272	11.4	3.27	3.35	NO	11.5	1.0
23	B3	Analyte	2.52	20225.2	72567.9	0.279	11.6	3.31	3.35	NO		
24	B3	Analyte	2.52	20489.6	73369.1	0.279	11.6	3.36	3.35	NO		

Table 8: Summary table of analyte E quantitation in all samples.

Analyte E												
#	ID	Type	RT	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	1.45	485.8	148437	0.003	0.4	2.36	2.64	NO	0.4	15.7
2	Blank A	Analyte	1.44	438.4	141602	0.003	0.3	3.01	2.64	NO		
3	Blank A	Analyte	1.44	468.6	139211	0.003	0.4	2.91	2.64	NO		
4	A1	Analyte	1.45	1906.7	143459	0.013	3.6	2.91	2.64	NO	3.7	12.4
5	A1	Analyte	1.45	1800.0	146855	0.012	3.3	2.76	2.64	NO		
6	A1	Analyte	1.44	2163.5	144222	0.015	4.2	2.68	2.64	NO		
7	A2	Analyte	1.44	4226.4	147077	0.029	8.7	2.69	2.64	NO	9.2	4.4
8	A2	Analyte	1.44	4592.9	147659	0.031	9.4	2.74	2.64	NO		
9	A2	Analyte	1.44	4514.4	145948	0.031	9.4	2.84	2.64	NO		
10	A3	Analyte	1.44	399.4	175991	0.002	0.1	2.46	2.64	NO	0.1	0.0
11	A3	Analyte	1.45	439.5	173547	0.003	0.1	2.99	2.64	NO		
12	A3	Analyte	1.44	405.3	170990	0.002	0.1	2.71	2.64	NO		
13	Blank B	Analyte	1.45	9271.4	146797	0.063	19.9	2.93	2.64	NO	20.4	2.5
14	Blank B	Analyte	1.45	9777.4	147268	0.066	20.9	3.02	2.64	NO		
15	Blank B	Analyte	1.45	9464.3	145936	0.065	20.4	2.94	2.64	NO		
16	B1	Analyte	1.45	9644.1	141564	0.068	21.5	2.86	2.64	NO	21.6	2.6
17	B1	Analyte	1.45	10095.1	143494	0.070	22.2	2.89	2.64	NO		
18	B1	Analyte	1.45	9648.2	144594	0.067	21.1	2.98	2.64	NO		
19	B2	Analyte	1.45	11933.1	149128	0.080	25.4	3.01	2.64	NO	25.9	6.4
20	B2	Analyte	1.45	11807.6	152303	0.078	24.6	2.63	2.64	NO		
21	B2	Analyte	1.45	13184.6	150746	0.087	27.8	2.91	2.64	NO		
22	B3	Analyte	1.44	7832.1	153504	0.051	15.9	2.85	2.64	NO	16.1	2.0
23	B3	Analyte	1.44	8033.6	152252	0.053	16.5	2.45	2.64	NO		
24	B3	Analyte	1.44	7882.0	153713	0.051	16.0	2.80	2.64	NO		

Table 9: Summary table of analyte F quantitation in all samples.

Analyte F												
#	ID	Type	RT	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	1.54	3736.3	256383	0.015	0.2	2.46	2.54	NO	0.2	34.6
2	Blank A	Analyte	1.54	3647.3	244639	0.015	0.4	2.71	2.54	NO		
3	Blank A	Analyte	1.54	3519.6	243468	0.014	0.1	2.19	2.54	NO		
4	A1	Analyte	1.54	6362.8	250370	0.025	6.4	2.74	2.54	NO	6.5	3.2
5	A1	Analyte	1.54	6342.1	250688	0.025	6.3	2.69	2.54	NO		
6	A1	Analyte	1.54	6454.6	248485	0.026	6.7	2.57	2.54	NO		
7	A2	Analyte	1.54	8806.2	251350	0.035	11.9	2.81	2.54	NO	11.0	7.2
8	A2	Analyte	1.54	8364.4	258825	0.032	10.4	2.35	2.54	NO		
9	A2	Analyte	1.54	8196.2	248610	0.033	10.7	2.44	2.54	NO		
10	A3	Analyte	1.54	9038.7	284046	0.032	10.1	2.50	2.54	NO	10.1	2.5
11	A3	Analyte	1.54	10514.8	334439	0.031	9.9	2.74	2.54	NO		
12	A3	Analyte	1.54	10233.9	315499	0.032	10.4	2.54	2.54	NO		
13	Blank B	Analyte	1.54	7379.4	326515	0.023	4.8	2.87	2.54	NO	5.1	6.0
14	Blank B	Analyte	1.54	7588.5	314844	0.024	5.6	2.77	2.54	NO		
15	Blank B	Analyte	1.54	7282.2	317725	0.023	5.0	2.52	2.54	NO		
16	B1	Analyte	1.54	7690.8	319375	0.024	5.6	2.52	2.54	NO	5.6	5.4
17	B1	Analyte	1.54	7645.6	325938	0.023	5.3	2.34	2.54	NO		
18	B1	Analyte	1.54	8002.2	325582	0.025	5.9	2.54	2.54	NO		
19	B2	Analyte	1.54	12340.0	327787	0.038	13.4	2.60	2.54	NO	13.3	7.5
20	B2	Analyte	1.54	11927.7	333671	0.036	12.3	2.49	2.54	NO		
21	B2	Analyte	1.54	12895.3	329527	0.039	14.3	2.66	2.54	NO		
22	B3	Analyte	1.54	6907.6	319226	0.022	4.2	2.89	2.54	NO	4.2	1.4
23	B3	Analyte	1.54	6941.5	320795	0.022	4.2	2.79	2.54	NO		
24	B3	Analyte	1.54	6946.5	324808	0.021	4.1	2.89	2.54	NO		

Table 10: Summary table of analyte G quantitation in all samples.

Analyte G												
#	ID	Type	RT	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	3.91	8.3	550236	0.000	< 0	0.11	1.32	YES		
2	Blank A	Analyte	3.9	34.0	555519	0.000	1.5	1.39	1.32	NO	1.4	10.1
3	Blank A	Analyte	3.94	31.4	553365	0.000	1.3	0.45	1.32	YES		
4	A1	Analyte	3.93	178.4	547509	0.000	11.0	1.61	1.32	YES		
5	A1	Analyte	3.91	38.8	560360	0.000	1.8	0.19	1.32	YES	6.0	78.1
6	A1	Analyte	3.91	88.7	551631	0.000	5.1	0.67	1.32	YES		
7	A2	Analyte	3.91	324.9	561771	0.001	20.2	1.33	1.32	NO		
8	A2	Analyte	3.91	75.9	547476	0.000	4.3	0.52	1.32	YES	14.0	60.8
9	A2	Analyte	3.91	282.4	558139	0.001	17.6	2.86	1.32	YES		
10	A3	Analyte	3.92	68.2	541648	0.000	3.8	0.70	1.32	YES		
11	A3	Analyte	3.91	48.9	523739	0.000	2.7	0.20	1.32	YES	2.8	36.2
12	A3	Analyte	3.89	35.3	513882	0.000	1.8	0.22	1.32	YES		
13	Blank B	Analyte	3.9	2460.9	311806	0.008	284.1	1.37	1.32	NO		
14	Blank B	Analyte	3.9	2311.4	322865	0.007	257.6	1.49	1.32	NO	268.8	5.1
15	Blank B	Analyte	3.9	2312.5	314486	0.007	264.6	1.46	1.32	NO		
16	B1	Analyte	3.9	3202.6	331871	0.010	347.5	1.47	1.32	NO		
17	B1	Analyte	3.9	3223.4	332433	0.010	349.2	1.40	1.32	NO	350.5	1.1
18	B1	Analyte	3.9	3246.1	329369	0.010	354.9	1.54	1.32	NO		
19	B2	Analyte	3.9	2967.4	358713	0.008	297.8	1.36	1.32	NO		
20	B2	Analyte	3.9	3036.4	364629	0.008	299.8	1.53	1.32	NO	296.7	1.3
21	B2	Analyte	3.9	2957.8	363805	0.008	292.6	1.46	1.32	NO		
22	B3	Analyte	3.9	1965.7	395127	0.005	178.8	1.49	1.32	NO		
23	B3	Analyte	3.9	1789.3	400462	0.004	160.5	1.25	1.32	NO	168.4	5.6
24	B3	Analyte	3.9	1870.4	404810	0.005	166.0	1.53	1.32	NO		

Table 11: Summary table of analyte H quantitation in all samples.

Analyte H												
#	ID	Type	RT	Area	IS Area	Response	Concentration (ppt)	1° Ratio (Actual)	1° Ratio (Pred)	1° Ratio Flag	Average Concentration (ppt)	%RSD
1	Blank A	Analyte	3.82	1290.4	550236	0.002	< 0	1.02	1.2	YES		
2	Blank A	Analyte	3.81	1515.1	555519	0.003	< 0	1.49	1.2	YES	< 0	
3	Blank A	Analyte	3.81	1589.5	553365	0.003	< 0	1.35	1.2	NO		
4	A1	Analyte	3.82	11395.8	547509	0.021	5.0	1.32	1.2	NO		
5	A1	Analyte	3.81	11163.5	560360	0.020	4.7	1.32	1.2	NO	4.6	11.2
6	A1	Analyte	3.81	9683.1	551631	0.018	4.0	1.20	1.2	NO		
7	A2	Analyte	3.81	19136.4	561771	0.034	9.1	1.18	1.2	NO		
8	A2	Analyte	3.81	19411.3	547476	0.035	9.6	1.21	1.2	NO	9.4	2.7
9	A2	Analyte	3.81	19442.4	558139	0.035	9.4	1.22	1.2	NO		
10	A3	Analyte	3.81	2099.5	523739	0.004	< 0	1.27	1.2	NO		
11	A3	Analyte	3.81	2331.8	513882	0.005	< 0	1.14	1.2	NO	< 0	
12	A3	Analyte	3.81	2228.6	515901	0.004	< 0	1.13	1.2	NO		
13	Blank B	Analyte	3.92	2463.4	311806	0.008	1.0	1.06	1.2	NO		
14	Blank B	Analyte	3.83	3042.4	322865	0.009	1.5	1.08	1.2	NO	1.2	22.0
15	Blank B	Analyte	3.92	2599.9	314486	0.008	1.1	1.20	1.2	NO		
16	B1	Analyte	3.82	5847.0	331871	0.018	4.0	1.25	1.2	NO		
17	B1	Analyte	3.81	6422.4	332433	0.019	4.5	1.21	1.2	NO	4.4	8.2
18	B1	Analyte	3.81	6521.1	329369	0.020	4.7	1.31	1.2	NO		
19	B2	Analyte	3.81	17846.5	358713	0.050	14.0	1.26	1.2	NO		
20	B2	Analyte	3.81	16431.3	364629	0.045	12.5	1.16	1.2	NO	13.0	6.4
21	B2	Analyte	3.81	16426.6	363805	0.045	12.6	1.23	1.2	NO		
22	B3	Analyte	3.81	3159.6	396702	0.008	1.0	1.18	1.2	NO		
23	B3	Analyte	3.81	3355.8	395603	0.008	1.2	1.33	1.2	NO	1.1	10.2
24	B3	Analyte	3.81	3492.9	400462	0.009	1.2	1.35	1.2	NO		

MRM chromatograms of samples

Blank A

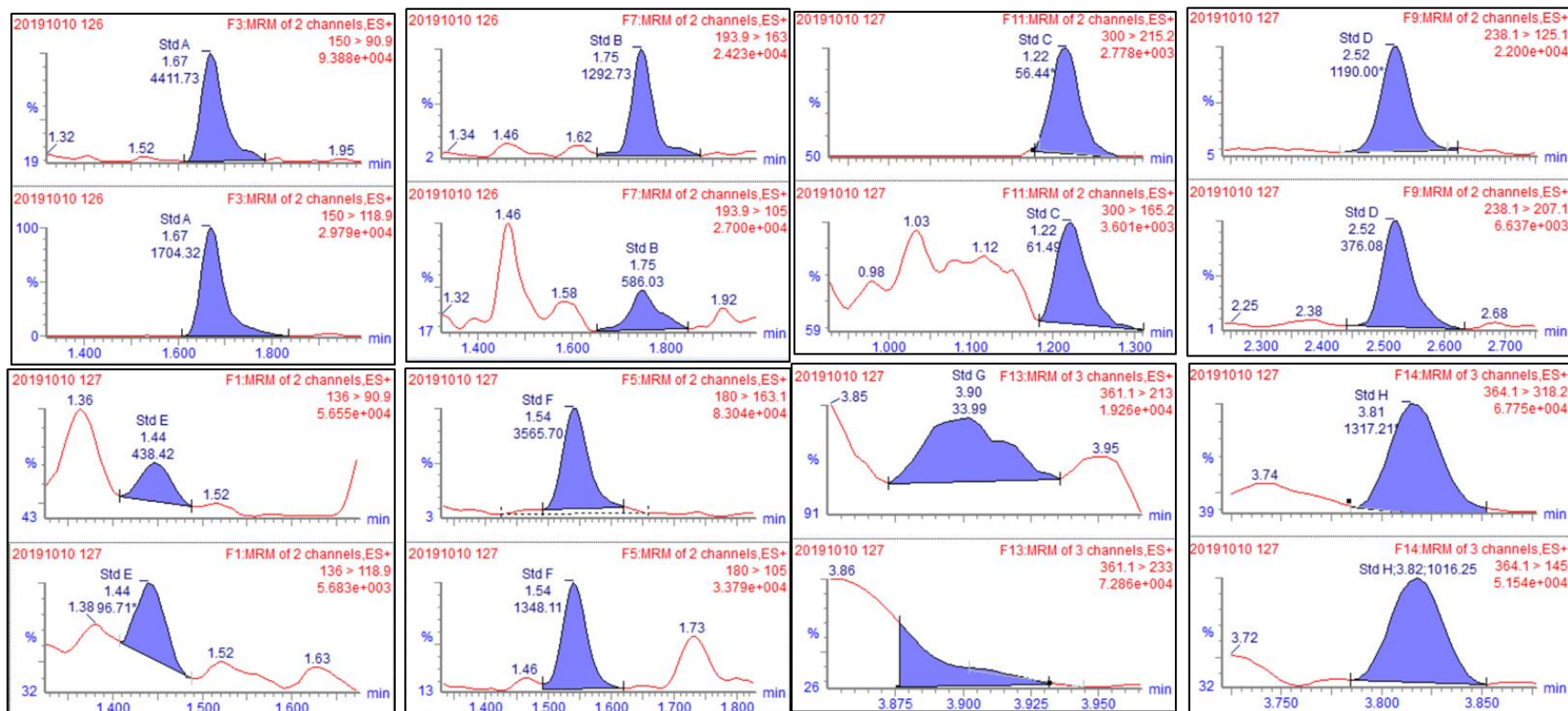


Figure 17: Compilation of MRM chromatograms from Blank A. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

Unknown A1

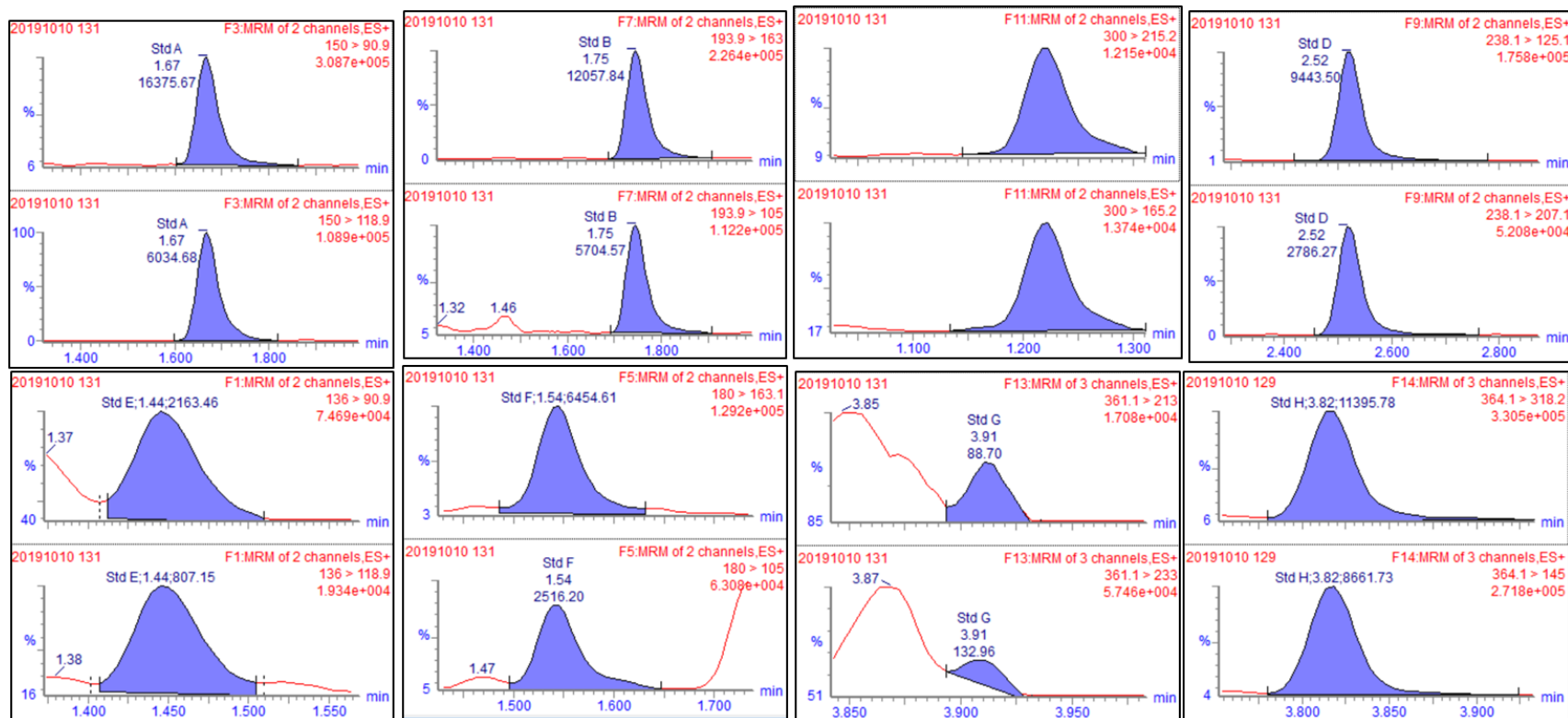


Figure 18: Compilation of MRM chromatograms from Unknown A1. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

Unknown A2

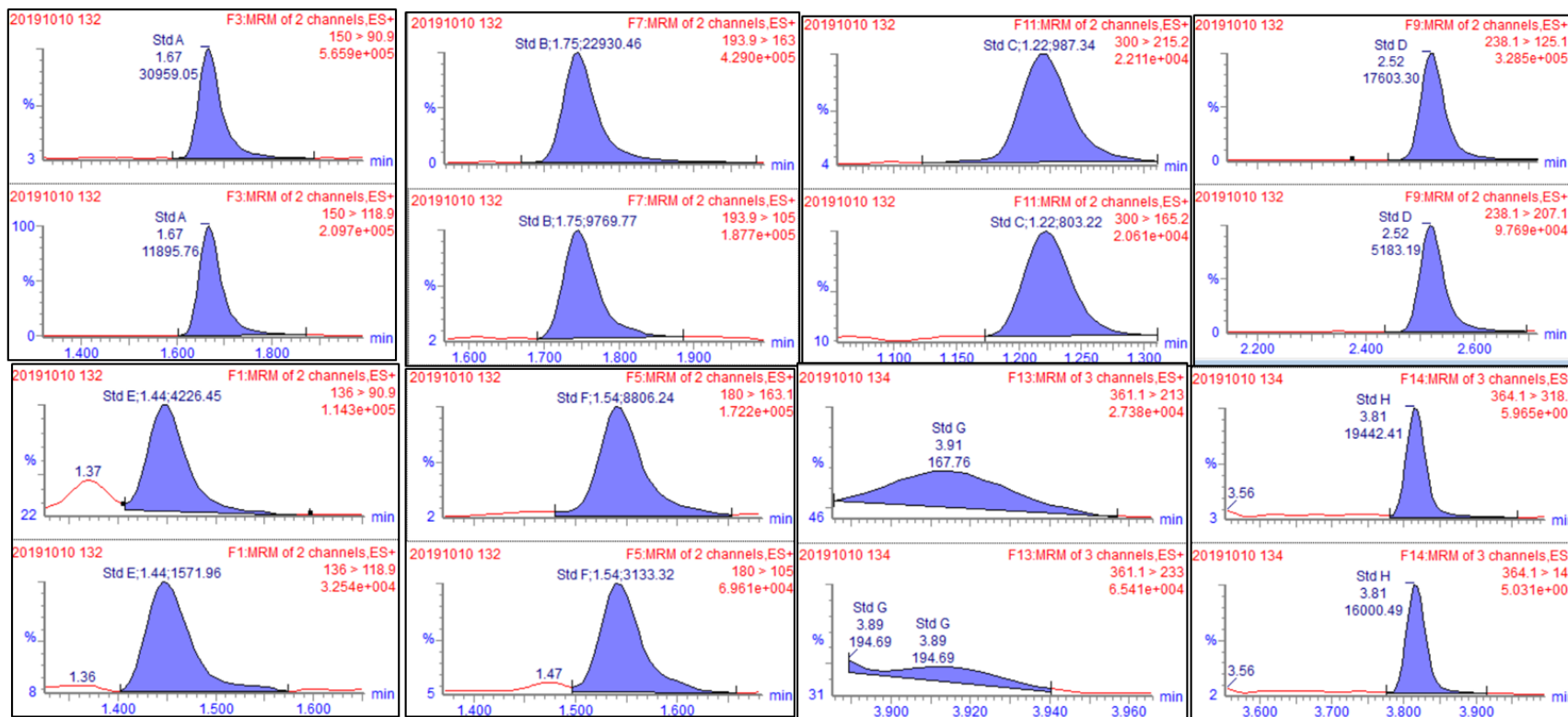


Figure 19: Compilation of MRM chromatograms from Unknown A2. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

Unknown A3

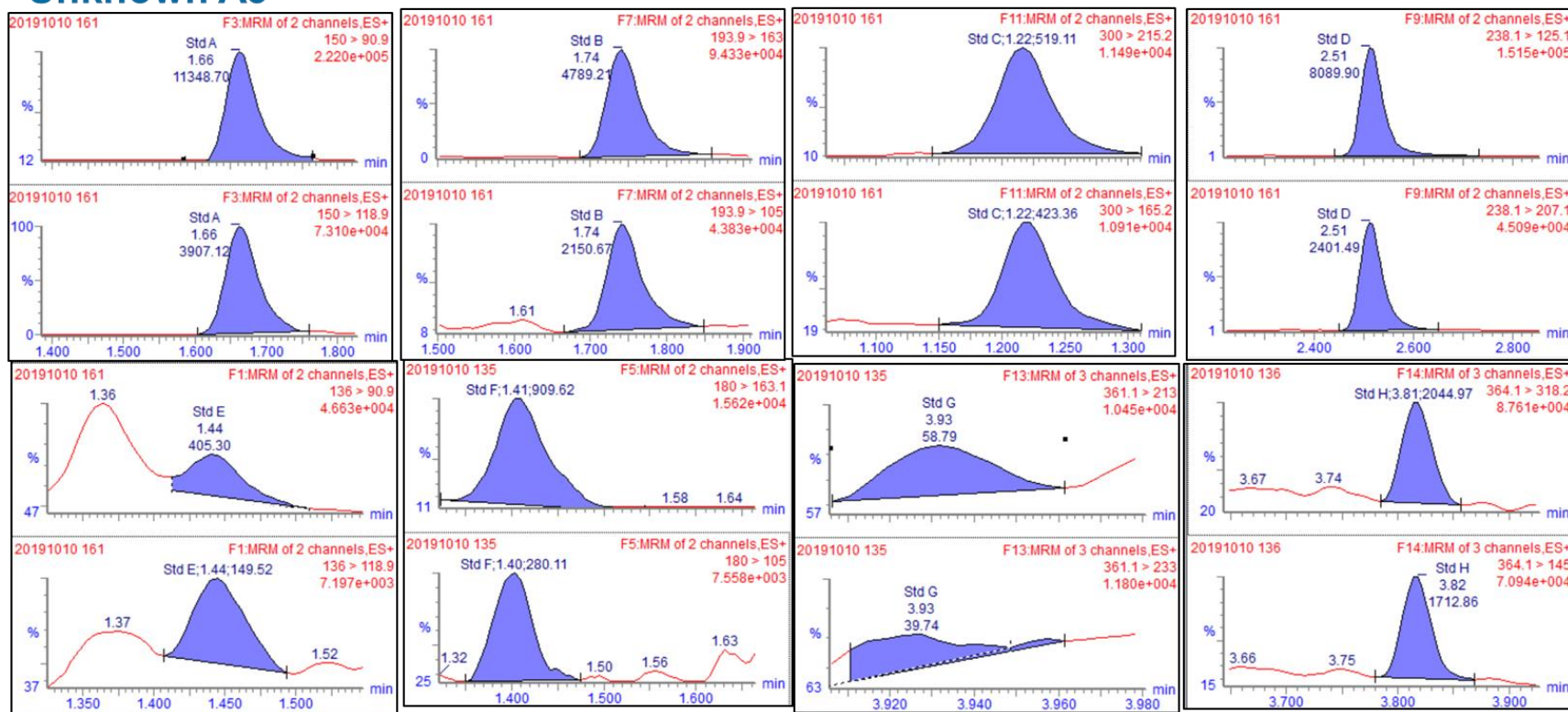


Figure 20: Compilation of MRM chromatograms from Unknown A3. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

Blank B

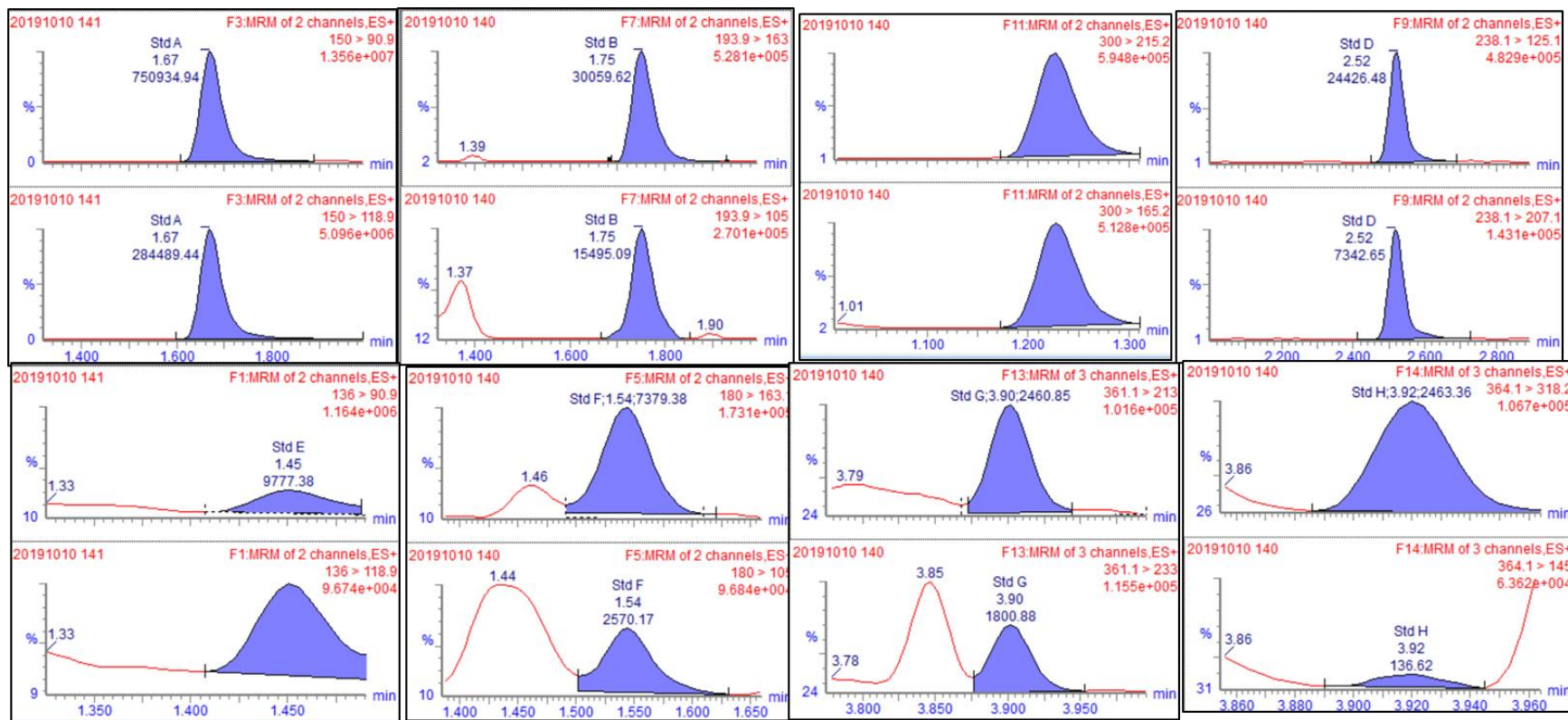


Figure 21: Compilation of MRM chromatograms from Blank B. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

Figure 1 displays 16 chromatograms arranged in a 4x4 grid, showing the separation of 14 standards (A through N) using two different HPLC methods. The top two rows show results for a 2-channel ES+ method, and the bottom two rows show results for a 3-channel ES+ method. Each plot displays the chromatogram (red line) and the fitted peak (blue area). The x-axis is time in minutes, and the y-axis is relative intensity (%). The plots are arranged in a 4x4 grid, with the first two rows showing results for the 2-channel ES+ method and the last two rows showing results for the 3-channel ES+ method. The standards are labeled A through N, with their retention times and peak areas provided for each. The 3-channel ES+ method shows improved resolution and peak fitting compared to the 2-channel method.

Standard	Method	Retention Time (min)	Peak Area
Std A	2-channel ES+	1.67	741549.75
Std B	2-channel ES+	1.75	35502.84
Std C	2-channel ES+	1.23	25198.79
Std D	2-channel ES+	2.52	29413.03
Std E	2-channel ES+	1.45	280845.78
Std F	2-channel ES+	1.75	16236.32
Std G	2-channel ES+	1.23	21729.10
Std H	2-channel ES+	2.52	8912.65
Std I	3-channel ES+	1.52	136 > 90.9
Std J	3-channel ES+	1.54	180 > 163.1
Std K	3-channel ES+	3.90	361.1 > 213
Std L	3-channel ES+	3.81	364.1 > 318.2
Std M	3-channel ES+	1.45	136 > 118.9
Std N	3-channel ES+	1.54	180 > 105
Std O	3-channel ES+	3.90	361.1 > 233
Std P	3-channel ES+	3.81	364.1 > 145

Figure 22: Compilation of MRM chromatograms from Unknown B1. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

Unknown B2

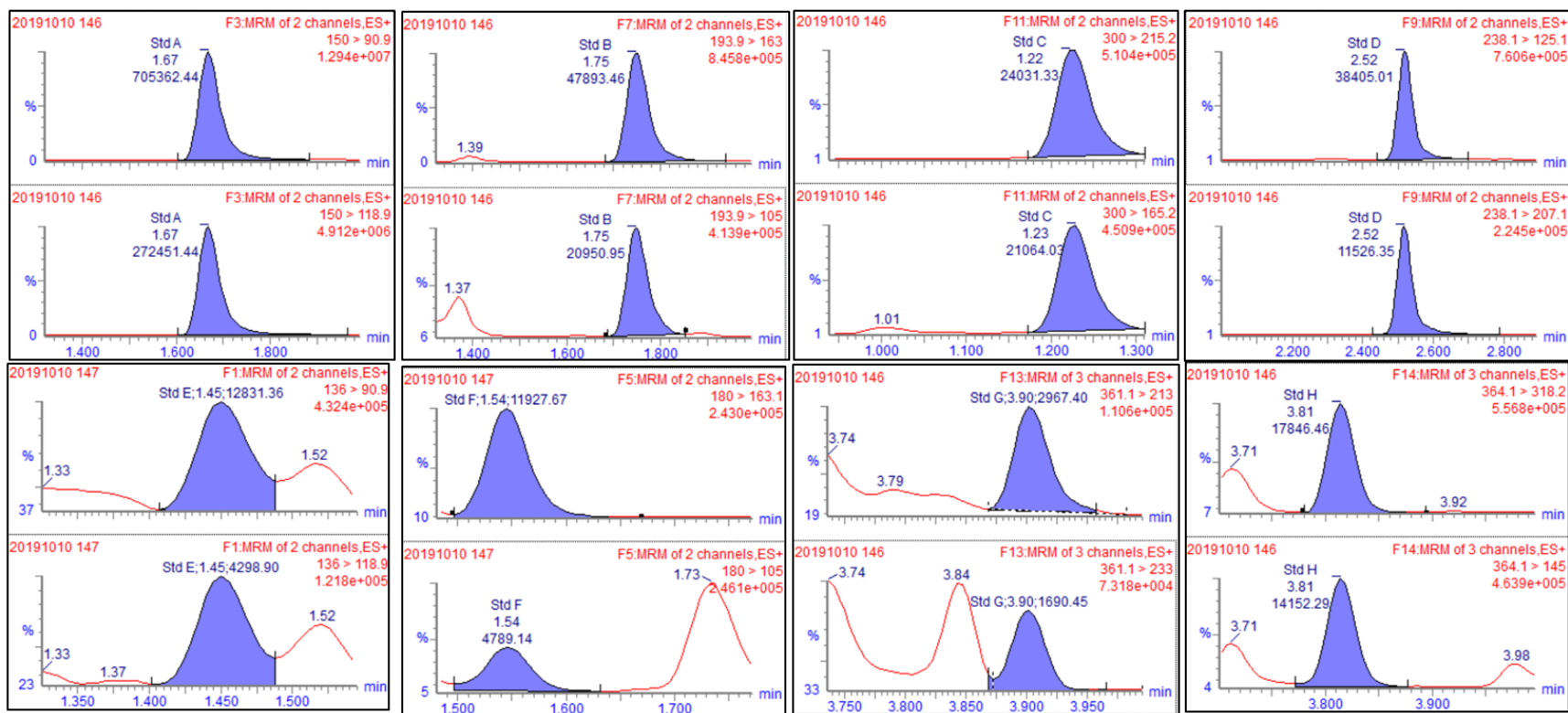


Figure 23: Compilation of MRM chromatograms from Unknown B2. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

Unknown B3

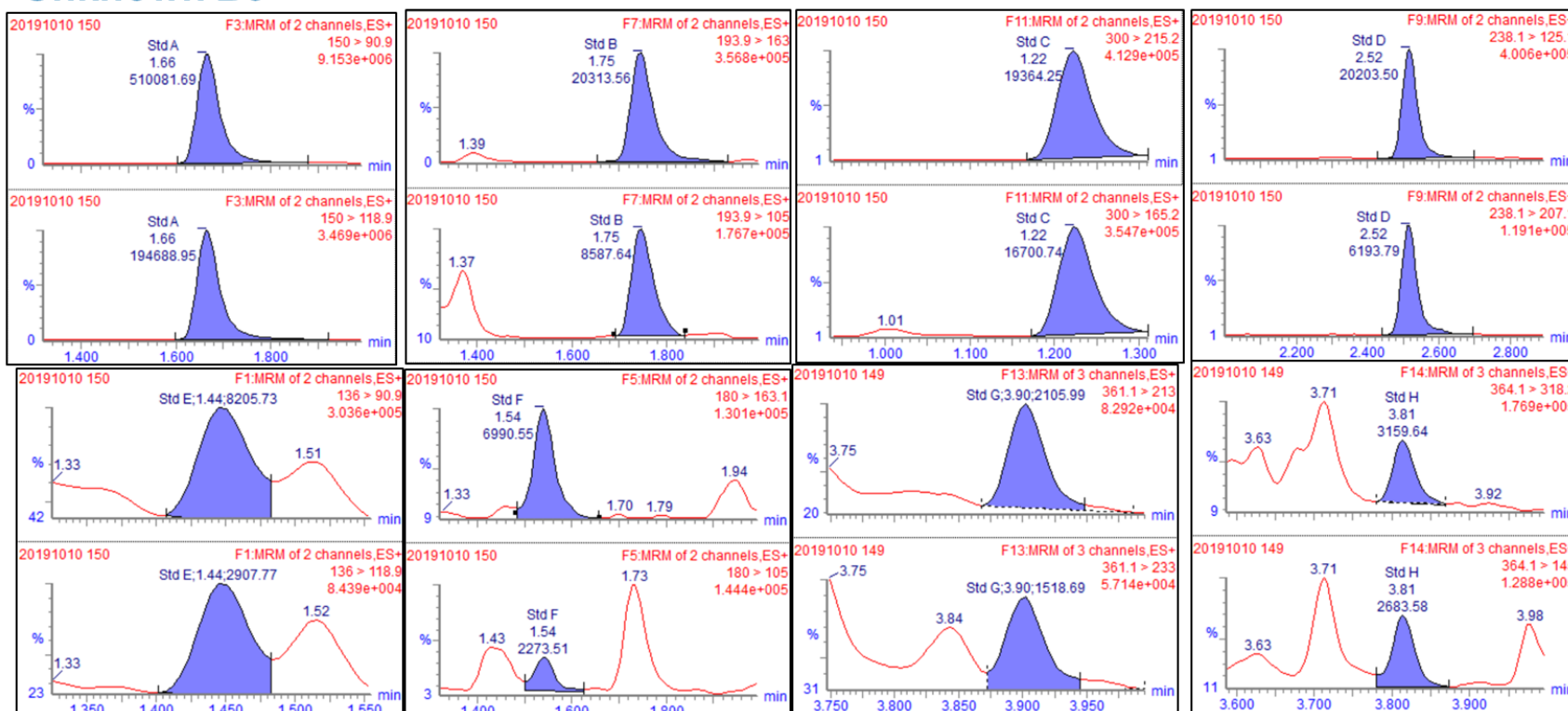


Figure 24: Compilation of MRM chromatograms from Unknown B3. Chromatograms are displayed in sequential order, with analyte A to D on the top panel (left to right) and analyte E to H on the bottom panel (left to right).

5 Conclusion

The combination of ACQUITY UPLC I-CLASS with Xevo TQ-XS offers desirable peak resolution and sensitivity for accurate and reproducible quantitation of trace analytes of interest.

MassLynx and its integrated software, Trendplot and TargetLynx facilitates data acquisition and processing for statistical analysis of generated results.

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