

	Sample Name	Sample Type	Analyte Peak Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (pmol/l)	Analyte Retention Time (min)	Analyte Signal To Noise	Area Ratio	IS Peak Name	IS Peak Area (counts)	IS Peak Height (cps)	IS Retention Time (min)	Calculated Concentration (pmol/l)
1	St 0 pM	Standard	E1 145.0	8.24e+002	4.78e+002	0.00	7.62	N/A	0.00097	E1-13C3 148.0	8.42e+005	2.95e+005	7.63	0.0000000000

Sample Name: St 0 pM File: 200420\_14500.msdc Run Date: 10/26/2020 Run Time: 10:00:00  
 Peak Name: E1 145.0 Masses: 246, 100.040, 686.000 Da  
 Comment: " Annotation:  
 Sample Type: Standard  
 Concentration: 0.00 pmol/l  
 Calculated Conc: 0.000000000000 pmol/l  
 Acq. Date: 4/27/2020  
 Acq. Time: 12:29:37 AM

Modified: Yes  
 Param. Algorithm: Split Parameters - HQ III  
 Minus Percentages: 50  
 Base Corr. Window: 1.00 min  
 Peak-Split. Factors: 8  
 Report Target Peaks: 8  
 Min. Peak Width: 0.00 cps  
 Min. Peak Width: 0.00 sec  
 Sample Type: Standard  
 PE Window: 30.0 sec points  
 Reported RT: 7.63 min  
 Use Relative RT: No

Int. Type: Manual  
 Retention Time: 7.62 min  
 Height: 4.78e+002 cps  
 Raw Time: 0.00 min  
 End Time: 7.64 min

Sample Name: St 0 pM File: 200420\_14500.msdc Run Date: 10/26/2020 Run Time: 10:00:00  
 Peak Name: E1-13C3 148.0 Masses: 272, 100.040, 686.000 Da  
 Comment: " Annotation:  
 Sample Type: Standard  
 Concentration: 1.00 pmol/l  
 Calculated Conc: 0.00 pmol/l  
 Acq. Date: 4/27/2020  
 Acq. Time: 12:29:37 AM

Modified: Yes  
 Param. Algorithm: Split Parameters - HQ III  
 Minus Percentages: 50  
 Base Corr. Window: 1.00 min  
 Peak-Split. Factors: 8  
 Report Target Peaks: 8  
 Min. Peak Width: 0.00 cps  
 Min. Peak Width: 0.00 sec  
 Sample Type: Standard  
 PE Window: 30.0 sec points  
 Reported RT: 7.63 min

7.62

7.63

7.62

7.63

	Sample Name	Sample Type	Analyte Peak Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (pmol/l)	Analyte Retention Time (min)	Analyte Signal To Noise	Area Ratio	IS Peak Name	IS Peak Area (counts)	IS Peak Height (cps)	IS Retention Time (min)	Calculated Concentration (pmol/l)
1	St 0 pM	Standard	E1 145.0	8.24e+002	4.78e+002	0.00	7.62	N/A	0.00097	E1-13C3 148.0	8.42e+005	2.95e+005	7.63	0.0000000000

Sample Name: St 0 pM 13C3 148.0  
Peak Name: "E1 145.0"  
Concentration: 0.00 pmol/l  
Acq. Date: 4/27/2025  
Acq. Time: 12:29:37 AM

File: 200426\_13c3\_std\_mscw.mzml

Method: Acquisition

Sample Index: 21

Base: Sub. Window: 1.00 min

Report Largest Peaks: No

Min. Peak Width: 0.00 cps

Max. Peak Width: 0.00 sec

RT Window: 30.0 sec

Expected RT: 7.63 min

Size Relative RT: No

Int. Type: Manual

Detection Time: 7.62 min

Area: 8.24e+002 counts

Height: 4.78e+002 cps

Start Time: 7.62 min

End Time: 7.64 min

Sample Name: St 0 pM 13C3 148.0  
Peak Name: "E1-13C3 148.0"  
Concentration: 1.00 pmol/l  
Acq. Date: 4/27/2025  
Acq. Time: 12:29:37 AM

File: 200426\_13c3\_std\_mscw.mzml

Method: Acquisition

Sample Index: 21

Base: Sub. Window: 1.00 min

Report Largest Peaks: No

Min. Peak Width: 0.00 cps

Max. Peak Width: 0.00 sec

RT Window: 30.0 sec

Expected RT: 7.63 min

Size Relative RT: No

Int. Type: Manual

Detection Time: 7.63 min

Area: 8.42e+005 counts

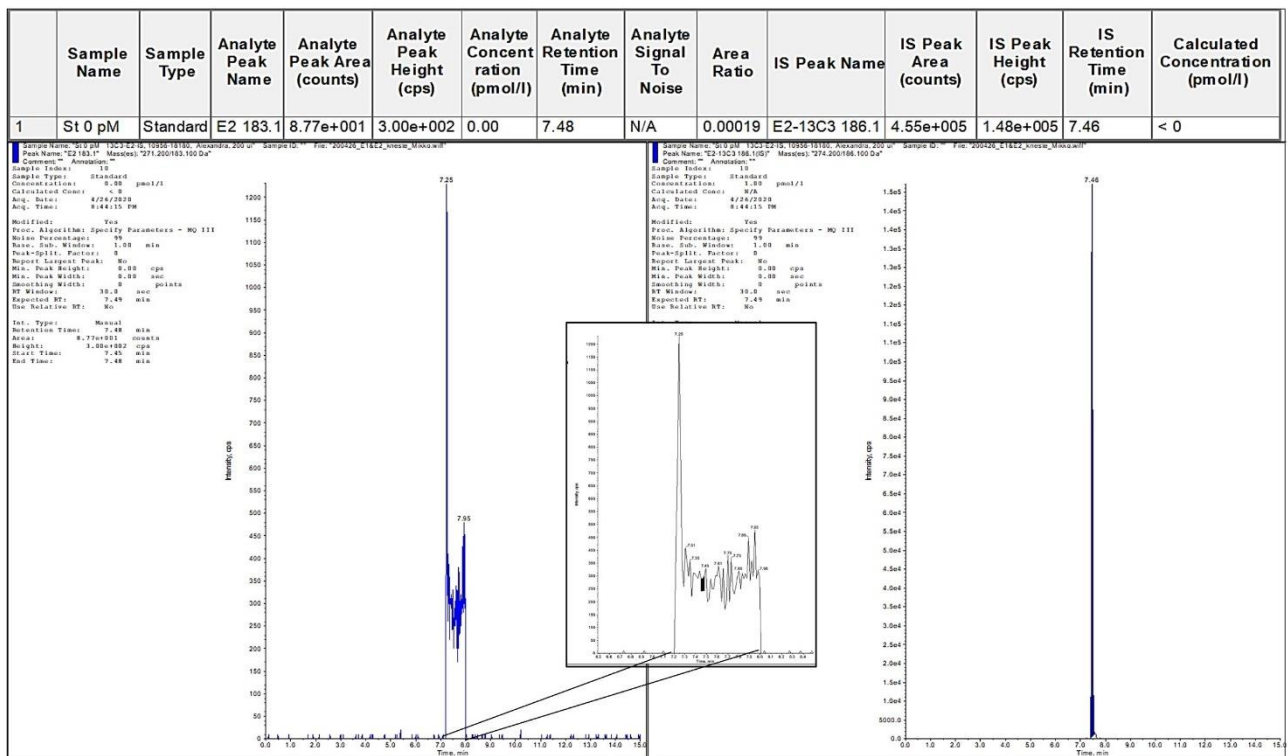
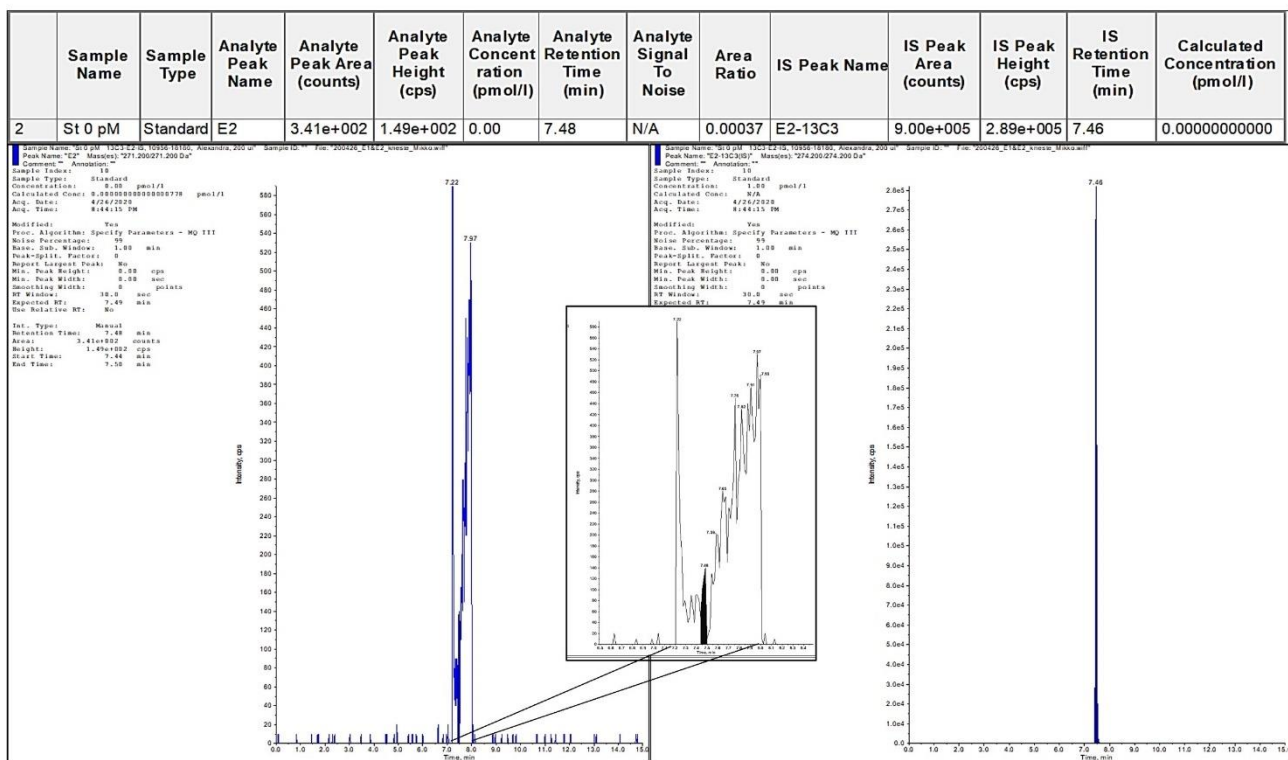
Height: 2.95e+005 cps

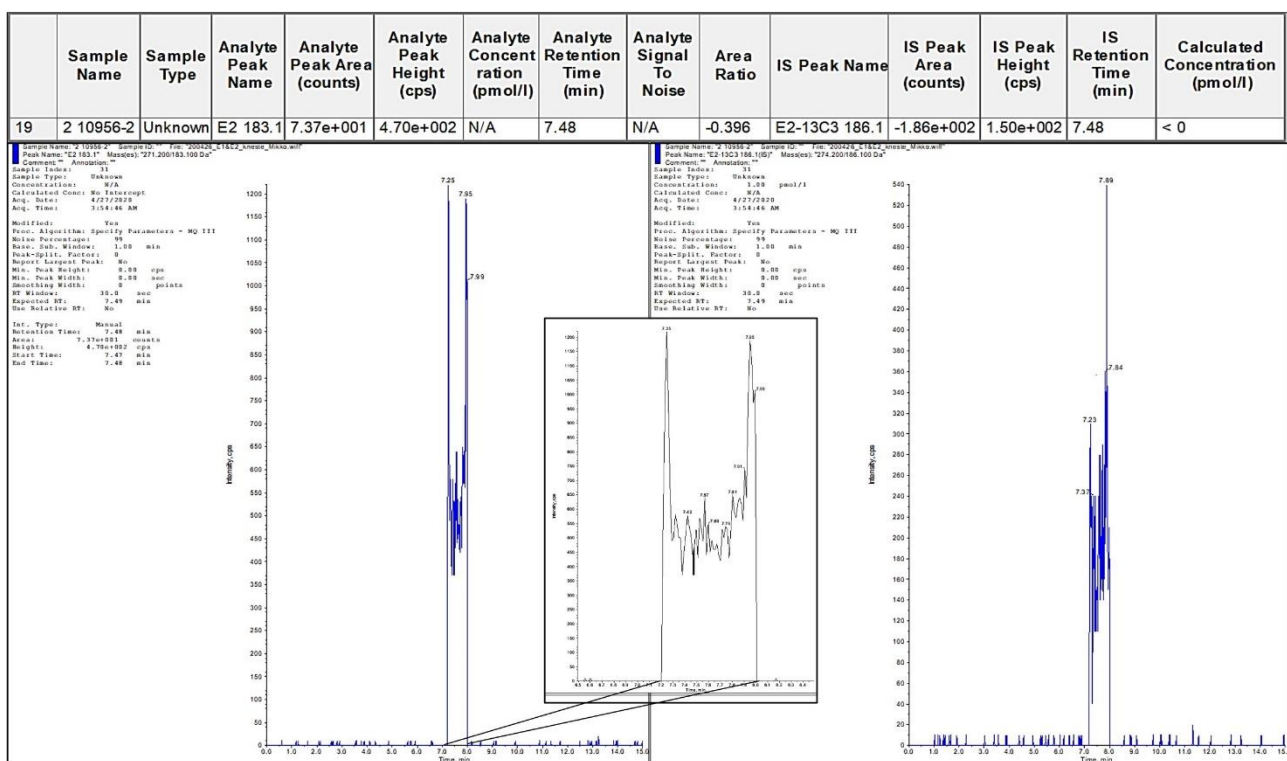
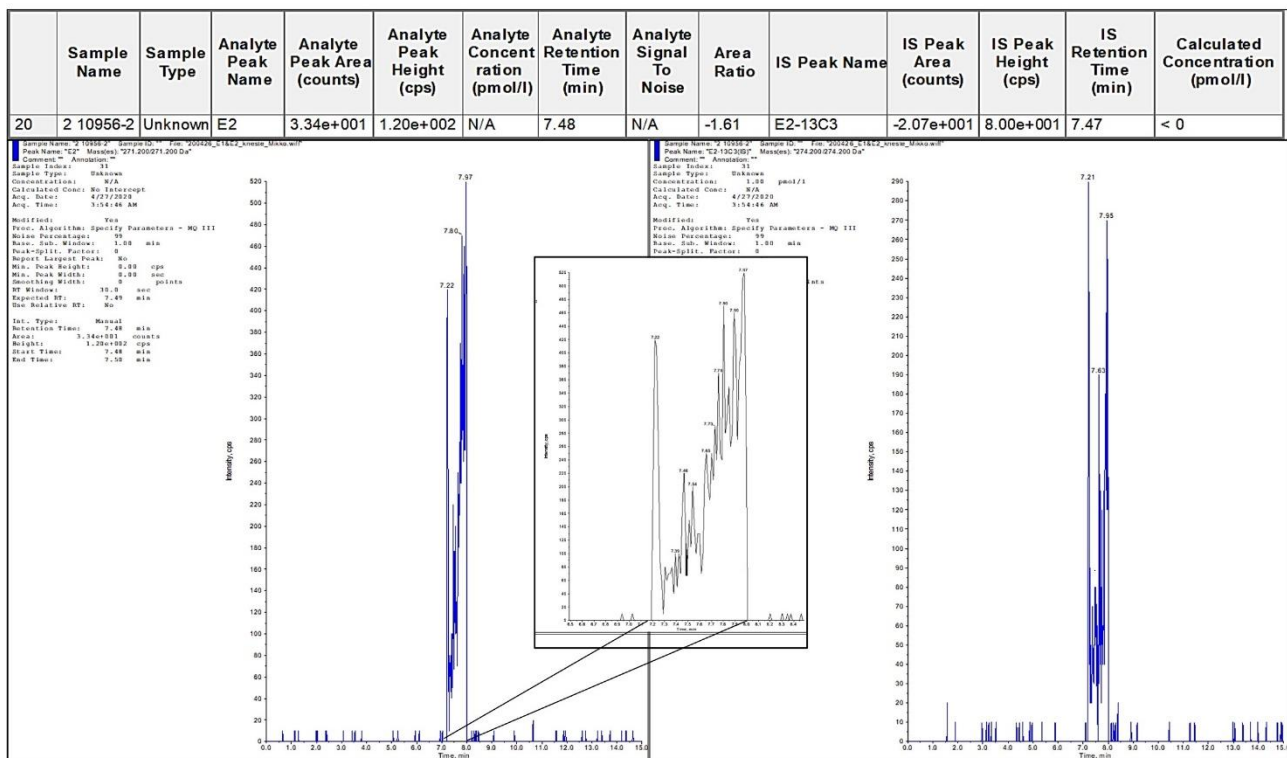
Start Time: 7.63 min

End Time: 7.64 min

The figure displays two chromatograms side-by-side, comparing a standard sample (left) with a sample containing E1-13C3 (right). Both plots show intensity in counts per second (cps) on the y-axis against time in minutes on the x-axis. The left plot has a y-axis range from 0 to 3400 and an x-axis from 0.0 to 15.0. It features a prominent peak at 7.62 minutes with an area of 8.24e+002 counts and a height of 4.78e+002 cps. The right plot has a y-axis range from 0.0 to 2.9e5 and an x-axis from 0.0 to 15.0. It shows a corresponding peak at 7.63 minutes with an area of 8.42e+005 counts and a height of 2.95e+005 cps. A central inset provides a magnified view of the baseline and the two peaks, highlighting their retention times and relative intensities.

**Supplementary figure S1B: E2 chromatograms in blank samples.** Parent and transition ions E2 [M-H]<sup>-</sup> m/z 271.2 to m/z 271.2 and m/z 183.1 in 0 pmol/L standard sample and water blank sample. No internal standard was added to the water blank.





**Supplementary figure S1C: P4 chromatograms in blank samples.** Parent and transition ions P4 [M+H]<sup>+</sup> m/z 315.1 to m/z 109.1 and m/z 96.9 in 0 pmol/L standard sample and water blank sample. No internal standard was added to the water blank.



**Sample Name:** "S:\S-08.Pxy"    **Sample ID. #**: "000004\_PROG\_knox\_bkx.wit"    **Peak Name:** "PROG-12C3 112.1037"    **Mass(es)**: "315.100/100.100 Da"

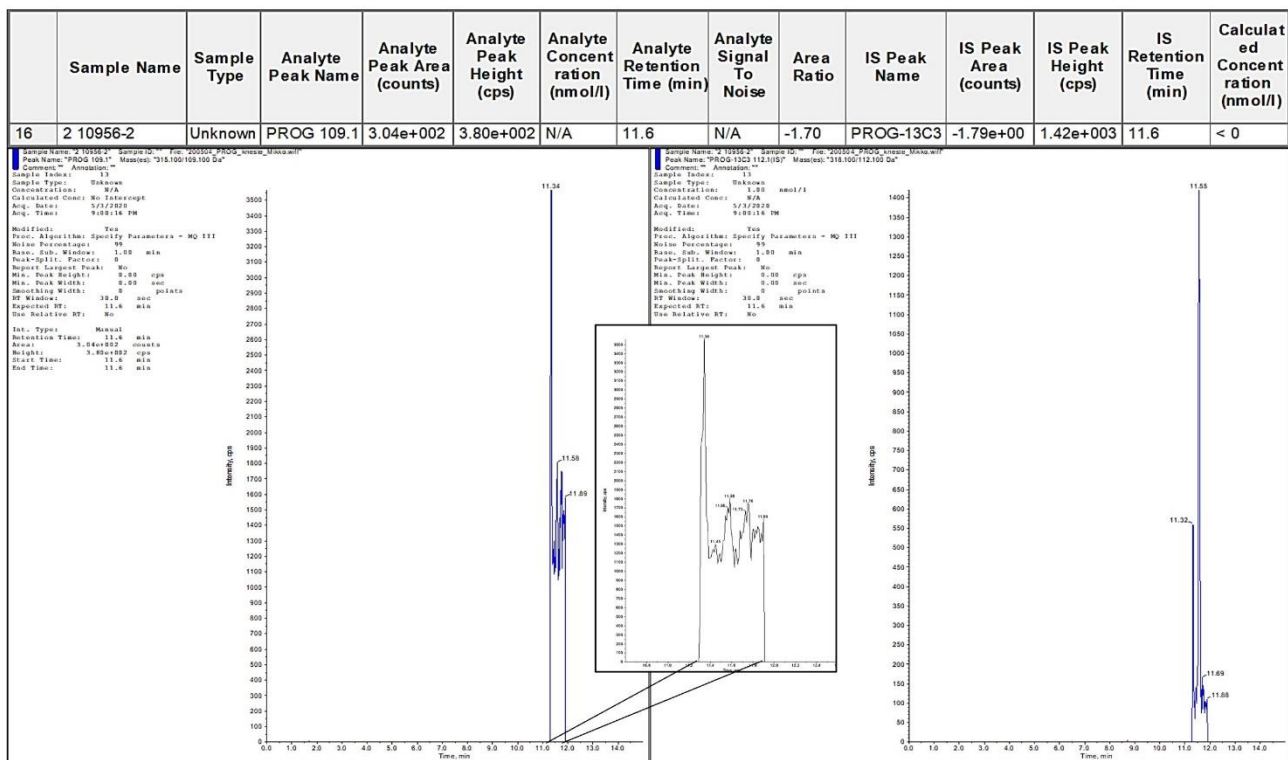
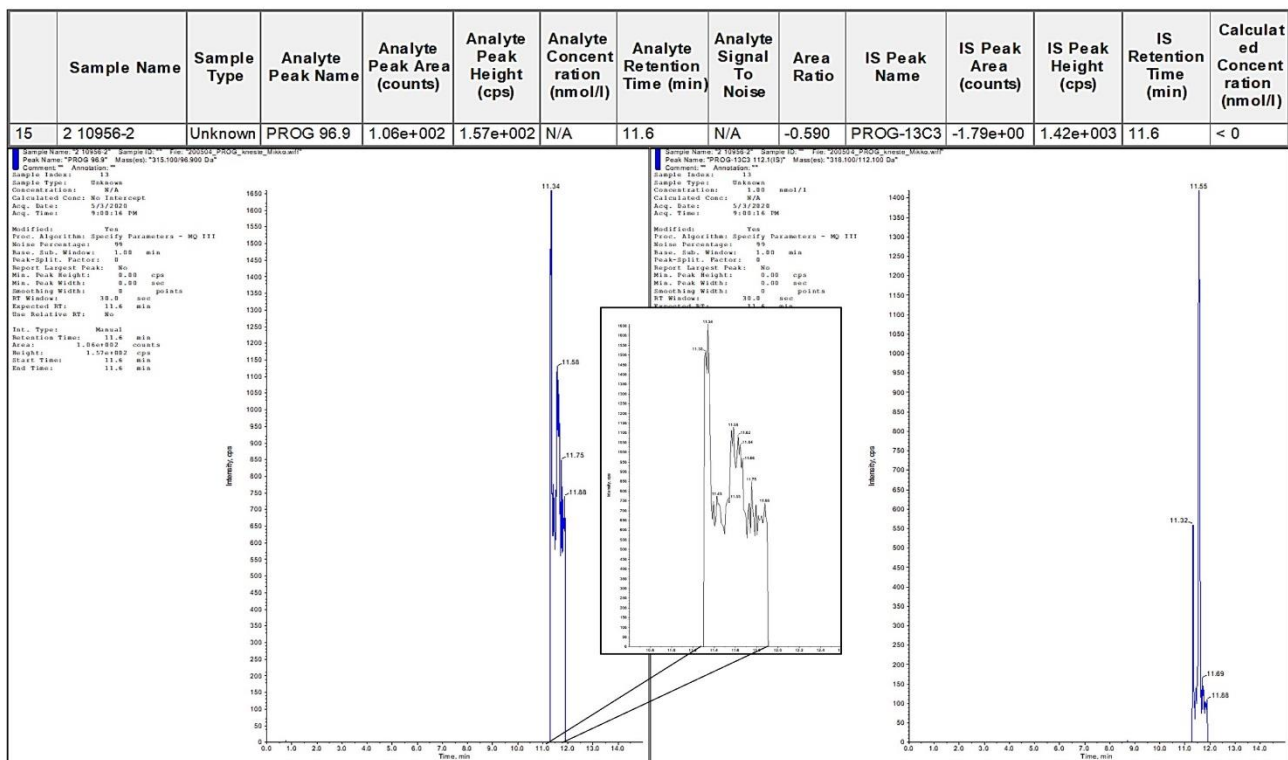
**Comments:** "Annotation."

Parameter	Value
Sample Index:	4
Sample Type:	Standard
Calculated Conc:	8.80000000000000 nmcl/l
Avg. Ret:	5/35/2028
Acq. Time:	5/35:42 PM
Modified:	Yes
Param. Algorithms Specify Parameters - HQ III	
Noise Percentage:	95
Base. Chn. Window:	1.00 min
PeakSplit. Factor:	0
Report Target Peak:	No
Min. Peak Height:	0.00 cps
Min. Peak Width:	0.00 sec
Smoothing Width:	0 points
HF Window:	30.0 sec
Repetited HF:	11.6 min
Use Relative HF:	No
Int. Type:	Manual
Integration Time:	11.6 min
Area:	7.31e+002 counts
Height:	2.34e+002 cps
Start Time:	11.6 min
End Time:	11.6 min

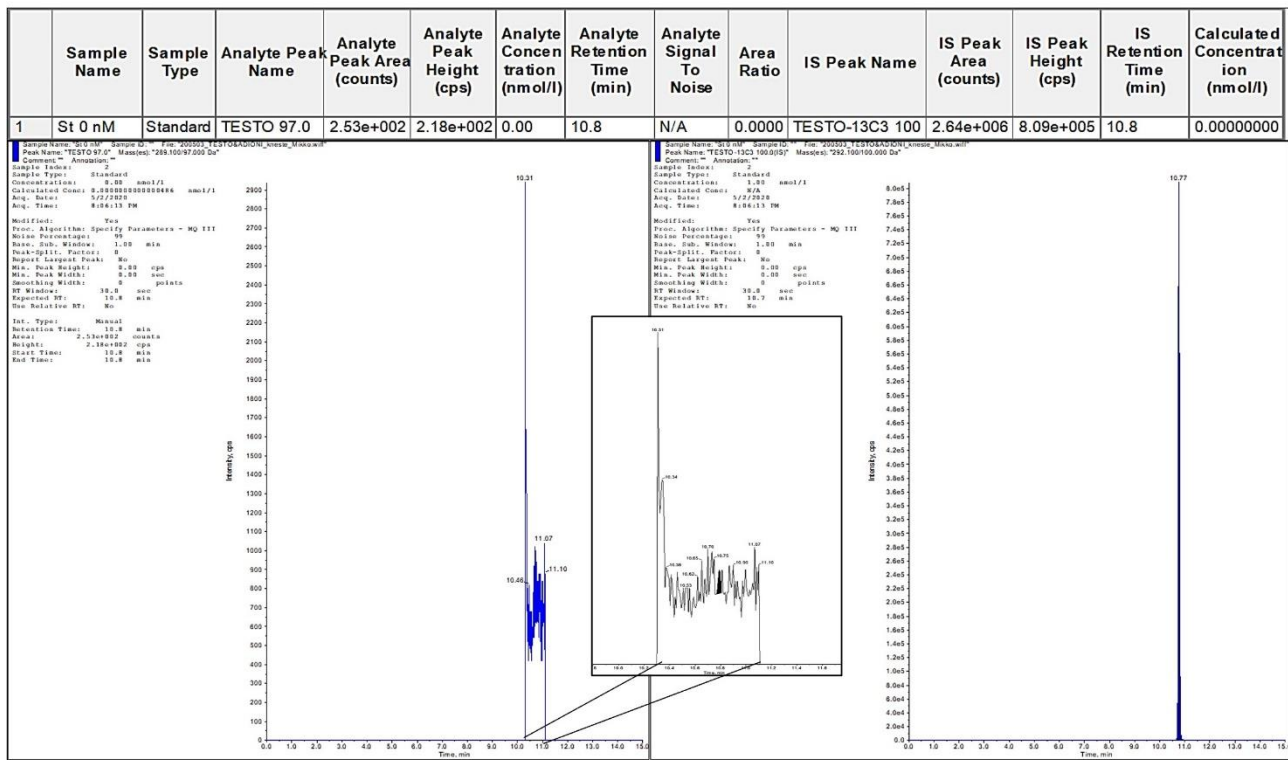
**Sample Name:** "S:\S-08.Pxy"    **Sample ID. #**: "000004\_PROG\_knox\_bkx.wit"    **Peak Name:** "PROG-12C3 112.1037"    **Mass(es)**: "315.100/100.100 Da"

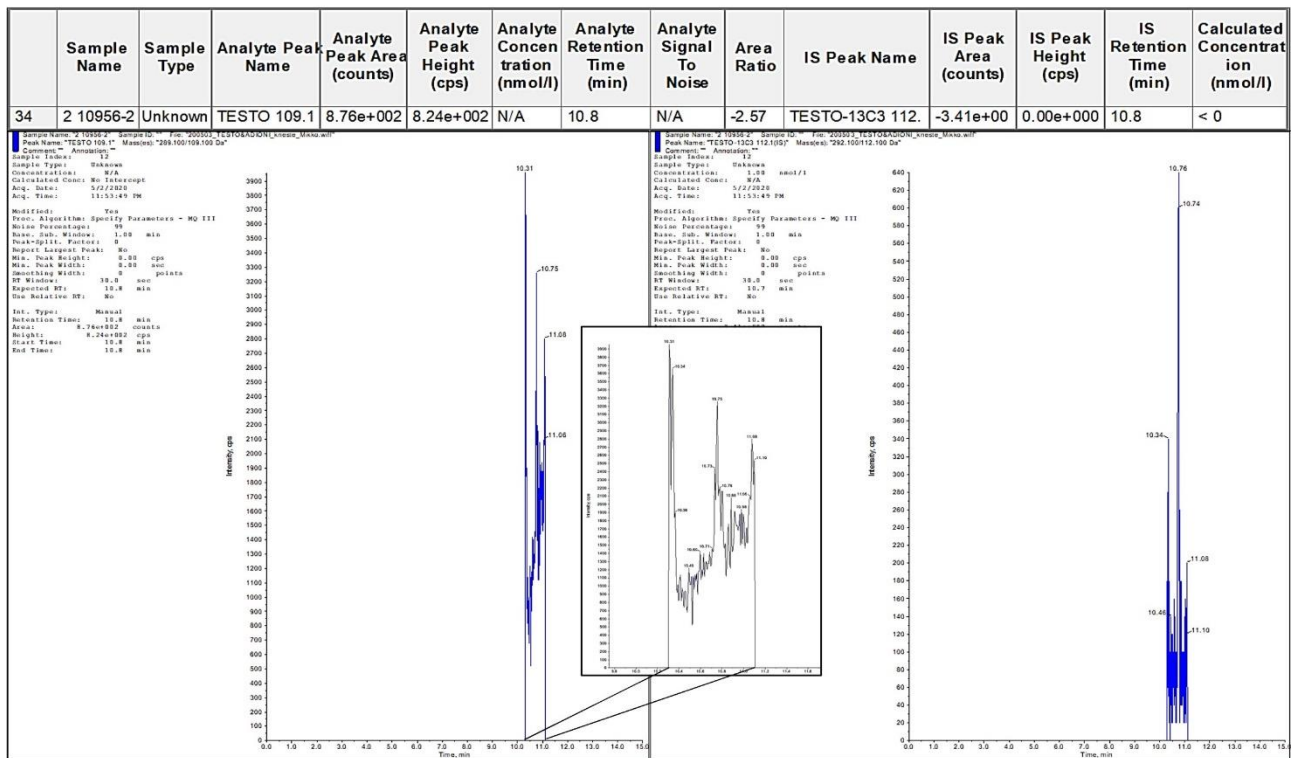
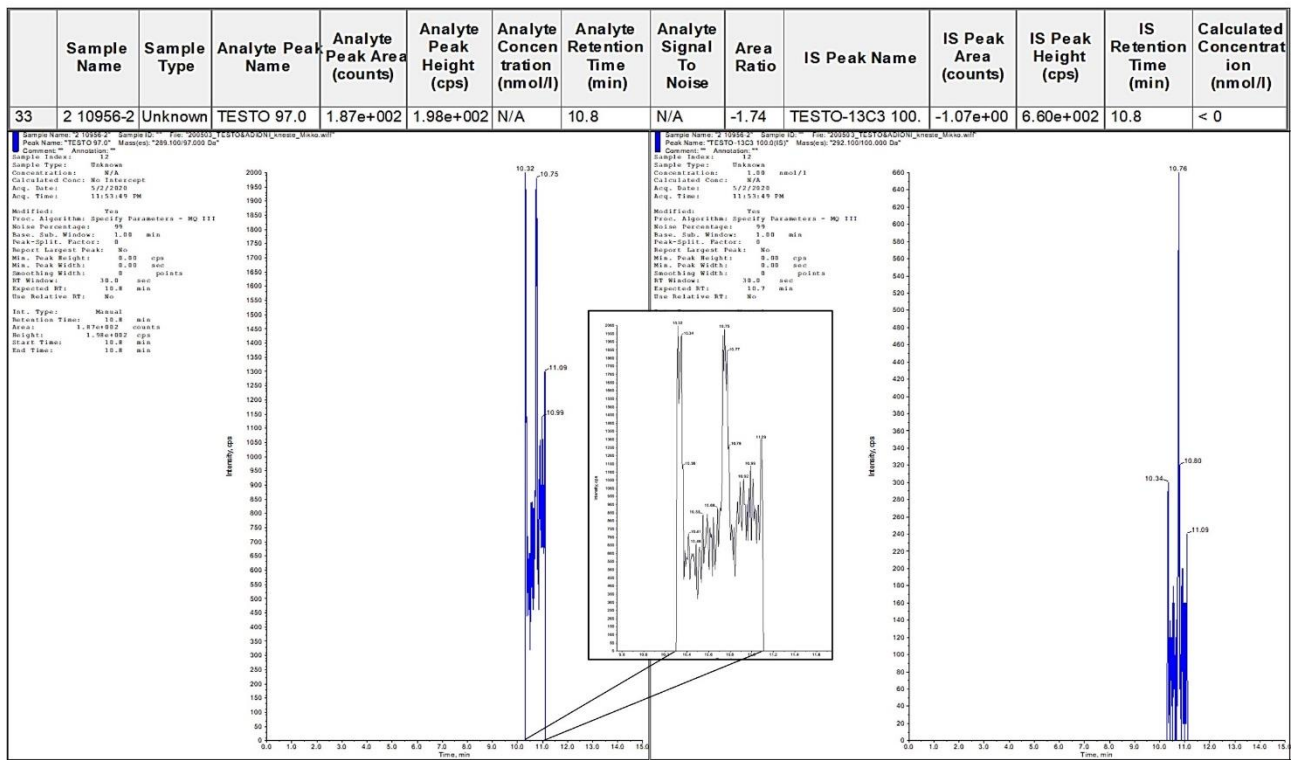
**Comments:** "Annotation."

Parameter	Value
Sample Index:	4
Sample Type:	Standard
Calculated Conc:	8.80000000000000 nmcl/l
Avg. Ret:	5/35/2028
Acq. Time:	5/35:42 PM
Modified:	Yes
Param. Algorithms Specify Parameters - HQ III	
Noise Percentage:	95
Base. Chn. Window:	1.00 min
PeakSplit. Factor:	0
Report Target Peak:	No
Min. Peak Height:	0.00 cps
Min. Peak Width:	0.00 sec
Smoothing Width:	0 points



**Supplementary figure S1D: T4 chromatograms in blank samples.** Parent and transition ions T4 [M+H]<sup>+</sup> m/z 289.1 to m/z 109.1 and m/z 97.0 in 0 pmol/L standard sample and water blank sample. No internal standard was added to the water blank.





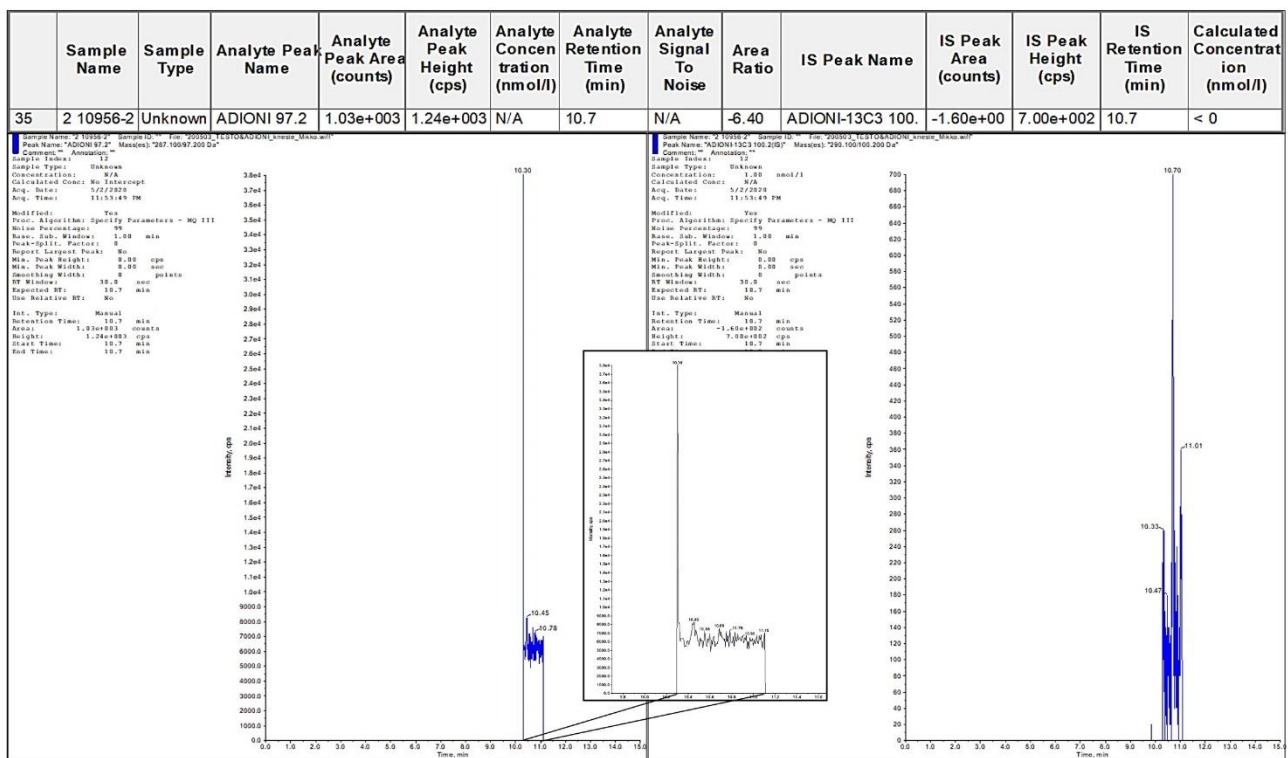
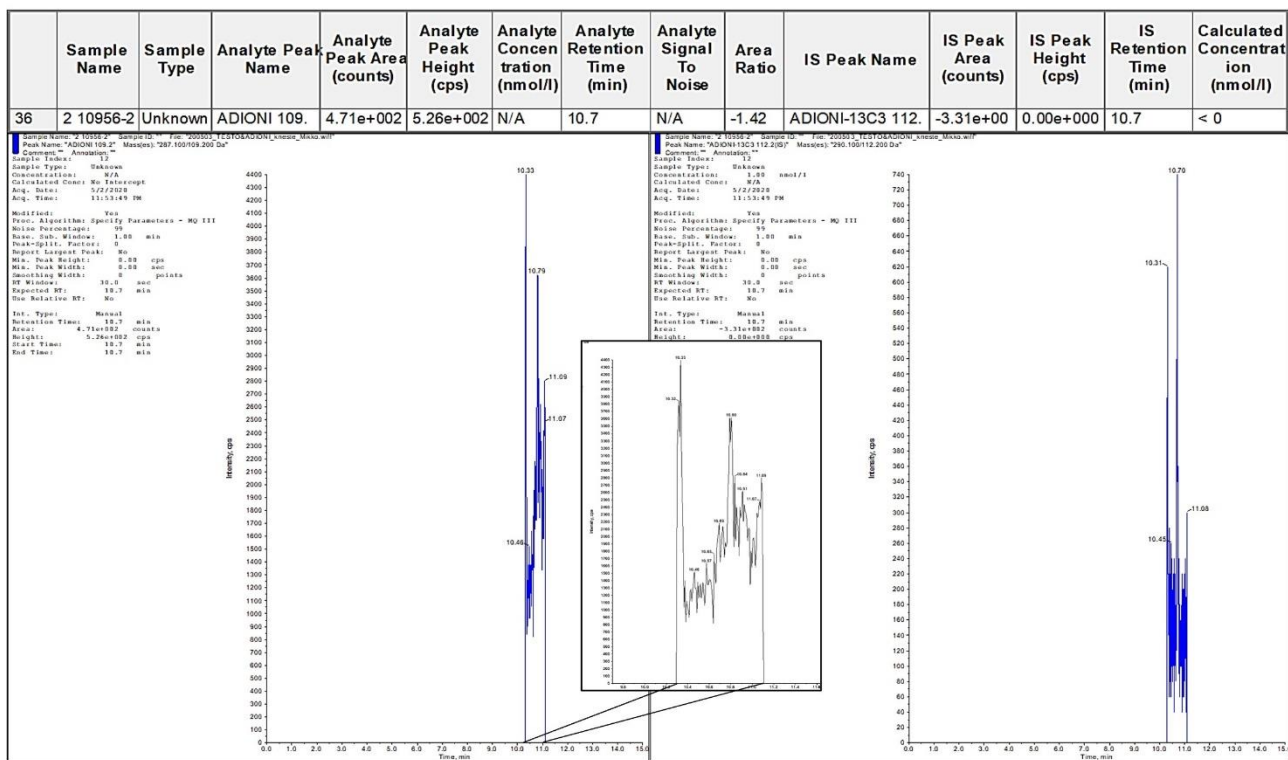
**Supplementary figure S1E: A4 chromatograms in blank samples.** Parent and transition ions A4 [M+H]<sup>+</sup> m/z 287.1 to m/z 109.2 and m/z 97.2 in 0 pmol/L standard sample and water blank sample. No internal standard was added to the water blank.



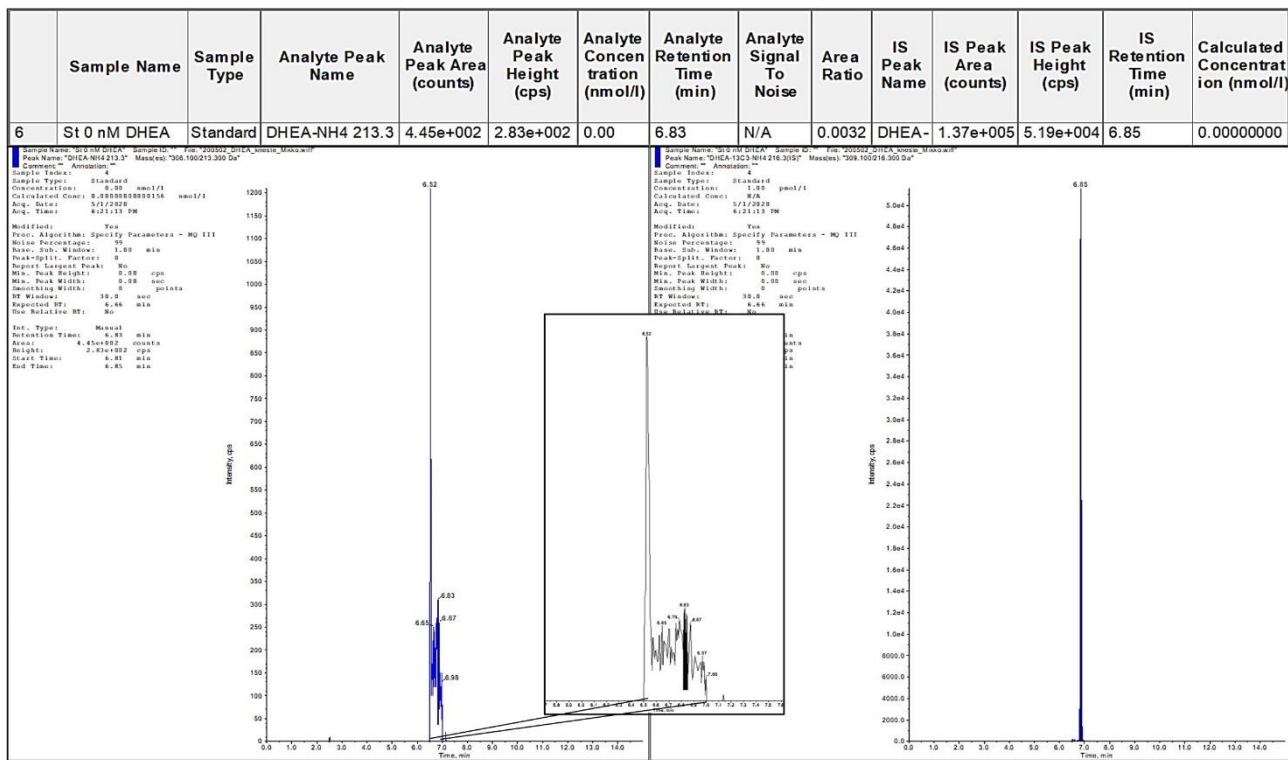
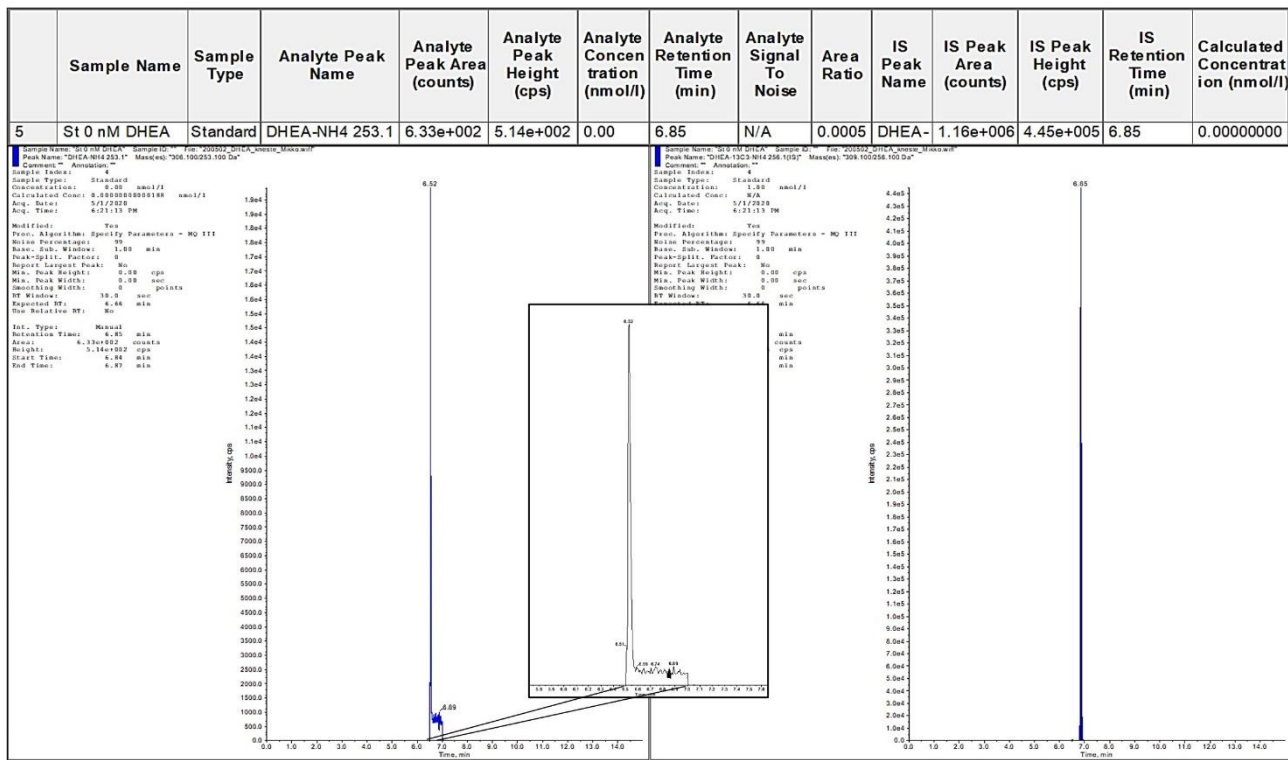
	Sample Name	Sample Type	Analyte Peak Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (nmol/l)	Analyte Retention Time (min)	Analyte Signal To Noise	Area Ratio	IS Peak Name	IS Peak Area (counts)	IS Peak Height (cps)	IS Retention Time (min)	Calculated Concentration (nmol/l)
3	St 0 nM	Standard	ADIONI-97.2	3.15e+003	1.90e+003	0.00	10.7	N/A	0.0012	ADIONI-13C3 10	2.52e+006	7.81e+005	10.7	0.00000000

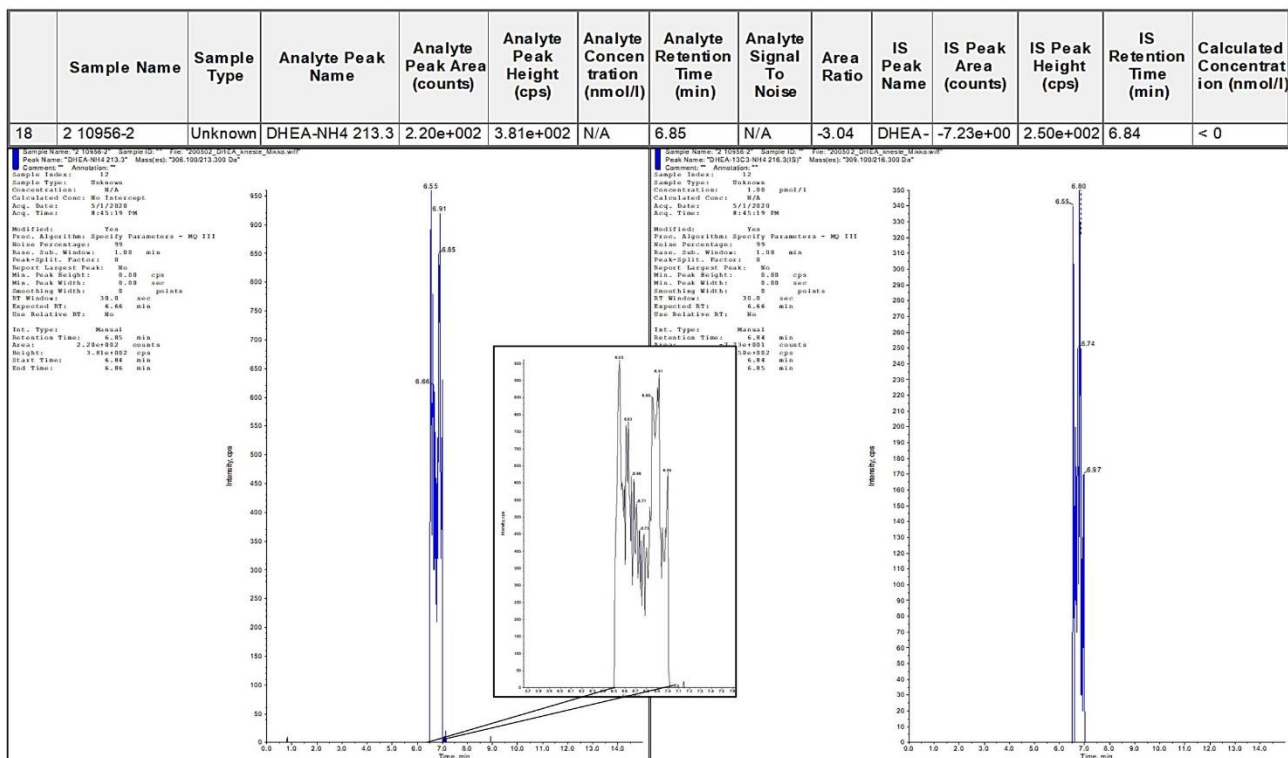
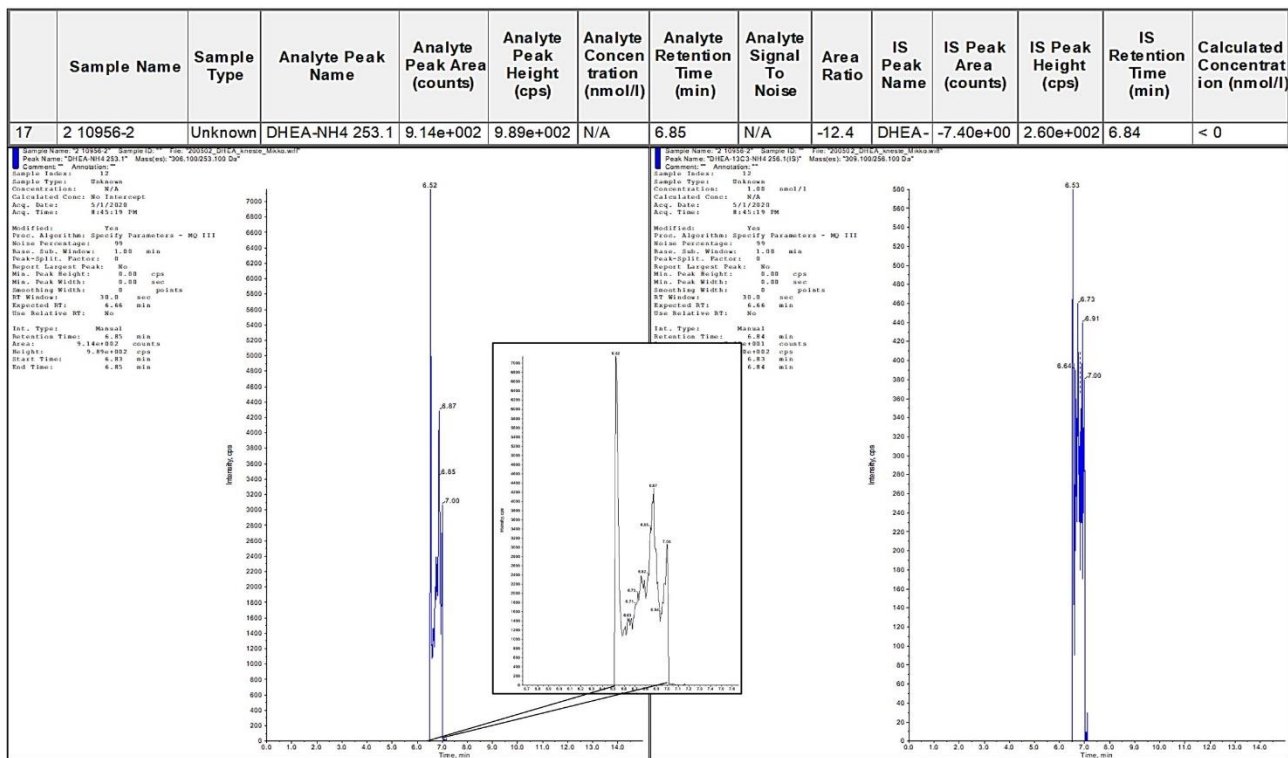
Sample Name: St 0 nM Sample ID: File: 000003\_123.D\\DATA\\ADIONI\_97.2\_Massvol  
Peak Name: "ADIONI-97.2" Masses: "287.100/67.200/284"  
Concave: "Amidation,"  
Sample Index: 2  
Sample Type: Standard  
Concentration: 0.00 nmol/l  
Calculated Conc: 0.0000000000000000 nmol/l  
Acq. Date: 5/2/2028  
Acq. Time: 8:08:13 PM  
  
Modified: Yes  
Proc. Algorithm: Specify Parameters - HQ ITI  
Ratio Percentages: 95  
Base, Sub. Window: 1.00 min  
Peak-Split, Points: 0  
Report Largest Peak: No  
Min., Peak Height: 0.00 cps  
Max., Peak Width: 0.00 sec  
Smoothing Width: 0 points  
ST Window: 10.0 sec  
Expected RT: 10.7 min  
Use Relative RT: No  
  
Int. Type: Manual  
Retention Time: 10.7 min  
Area: 3.15e+003 counts  
Height: 1.90e+003 cps  
Start Time: 10.7 min  
End Time: 10.7 min

Sample Name: St 0 nM Sample ID: File: 000003\_123.D\\DATA\\ADIONI\_97.2\_Massvol  
Peak Name: "ADIONI-13C3 100.2087" Masses: "290.160/100.200/284"  
Concave: "Amidation,"  
Sample Index: 2  
Sample Type: Standard  
Concentration: 1.00 nmol/l  
Calculated Conc: N/A  
Acq. Date: 5/2/2028  
Acq. Time: 8:08:13 PM  
  
Modified: Yes  
Proc. Algorithm: Specify Parameters - HQ ITI  
Ratio Percentages: 95  
Base, Sub. Window: 1.00 min  
Peak-Split, Points: 0  
Report Largest Peak: No  
Min., Peak Height: 0.00 cps  
Max., Peak Width: 0.00 sec  
Smoothing Width: 0 points  
ST Window: 10.0 sec  
Expected RT: 10.7 min  
Use Relative RT: No  
  
Int. Type: Manual  
Retention Time: 10.7 min  
Area: 2.52e+006 counts  
Height: 7.81e+005 cps  
Start Time: 10.7 min  
End Time: 10.7 min



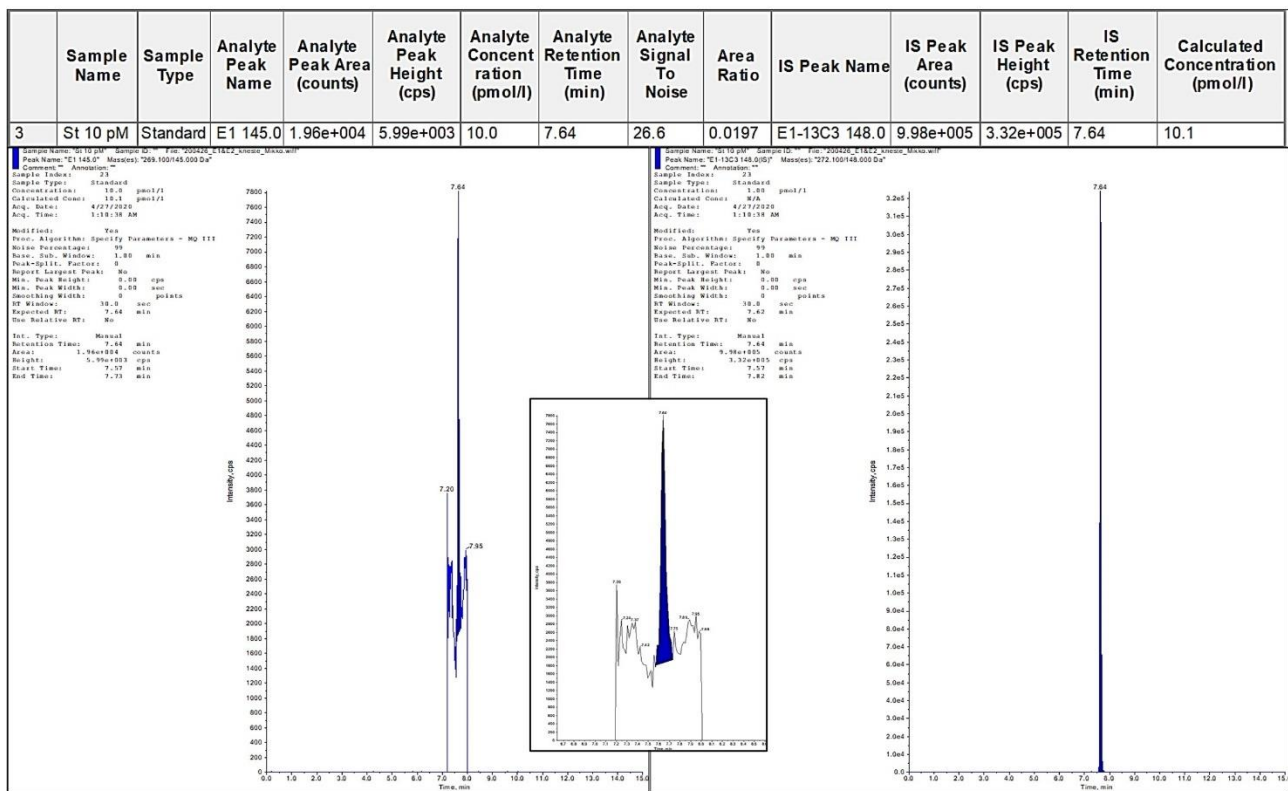
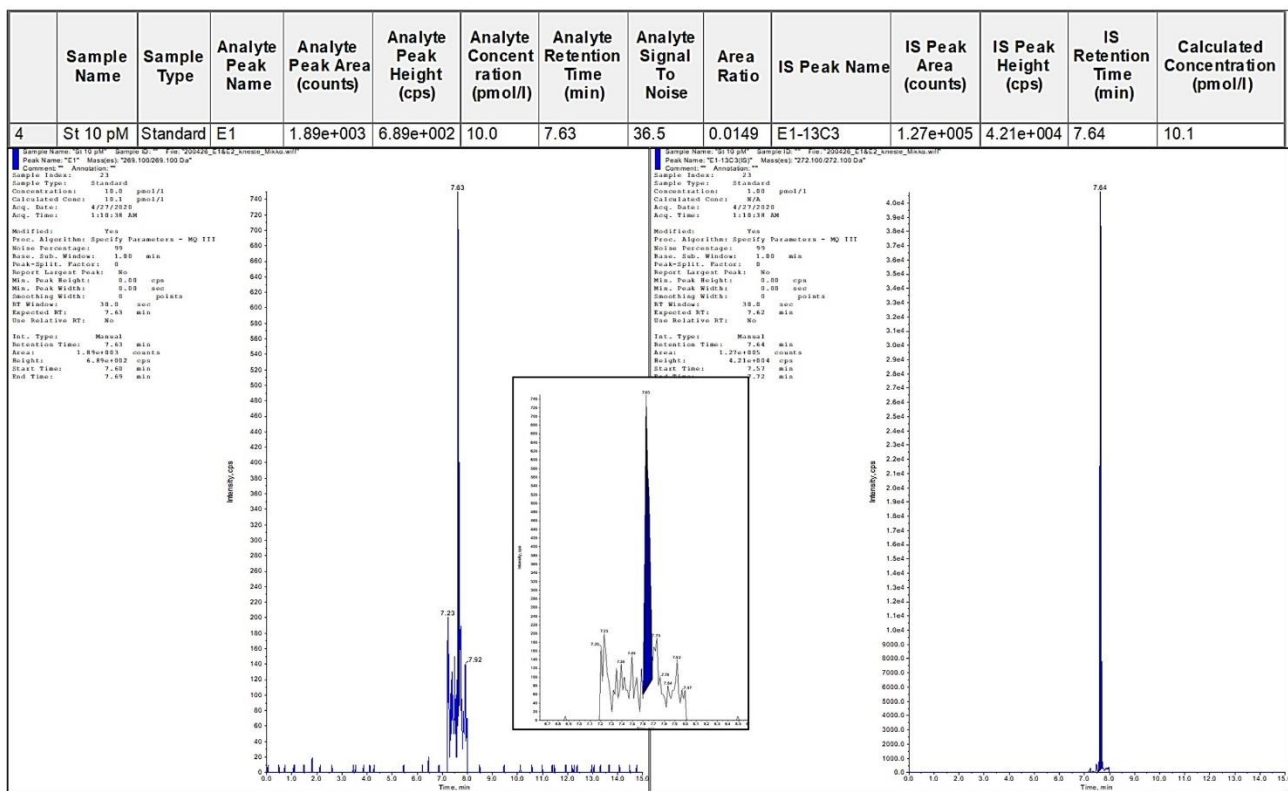
**Supplementary figure S1F: DHEA chromatograms in blank samples.** Parent and transition ions [M+NH<sub>4</sub>]<sup>+</sup> m/z 306.1 to m/z 253.1 and m/z 213.3 in 0 pmol/L standard sample and water blank sample. No internal standard was added to the water blank.



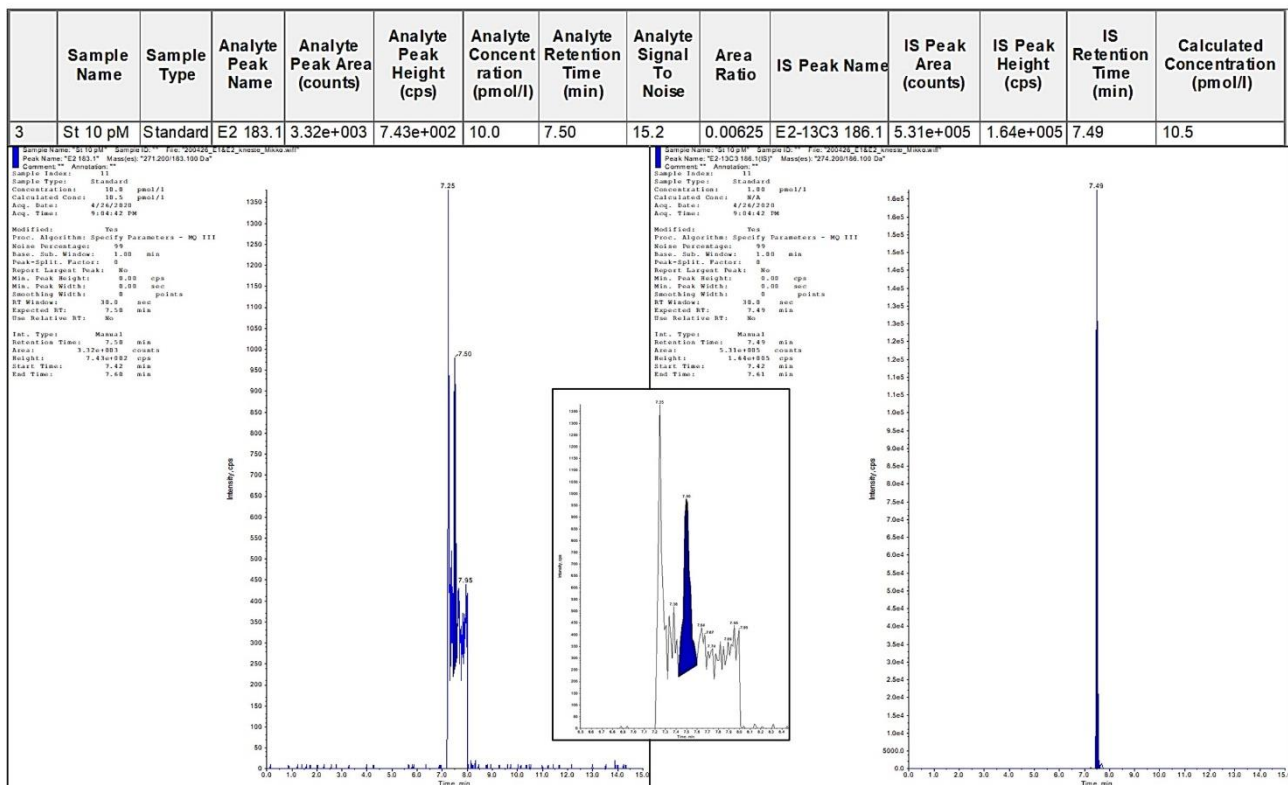
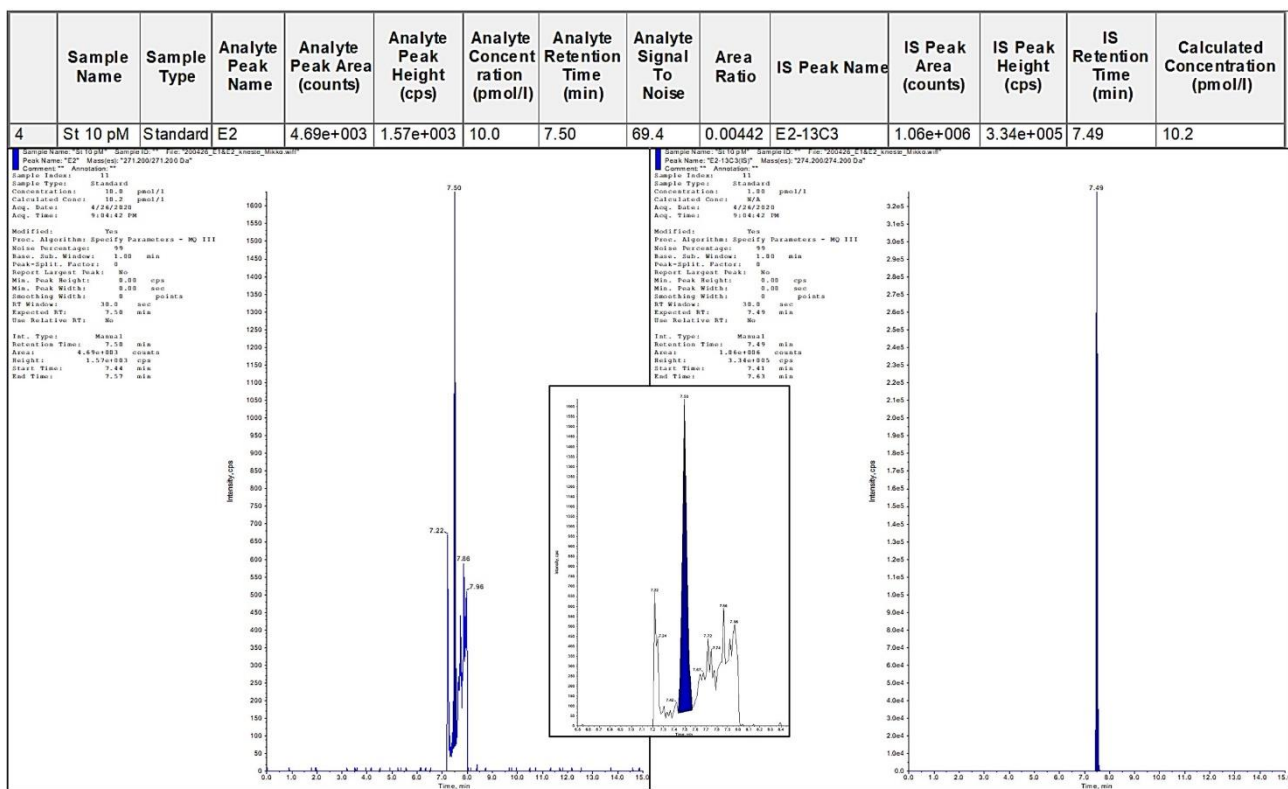


**Supplementary figure S2A: E1 LOQ chromatogram and S/N ratios.** Parent and transition ions E<sub>1</sub> [M-H]<sup>-</sup> m/z 269.1 to m/z 269.1 and m/z 145.0 in 10 pmol/L standard sample. Left panel shows calibrator chromatogram, while on the right is presented <sup>13</sup>C-estrone IS. The high S/N ratio (36.5 and 26.6, respectively) permitted extrapolation of the LOQ to 5 pmol/L.

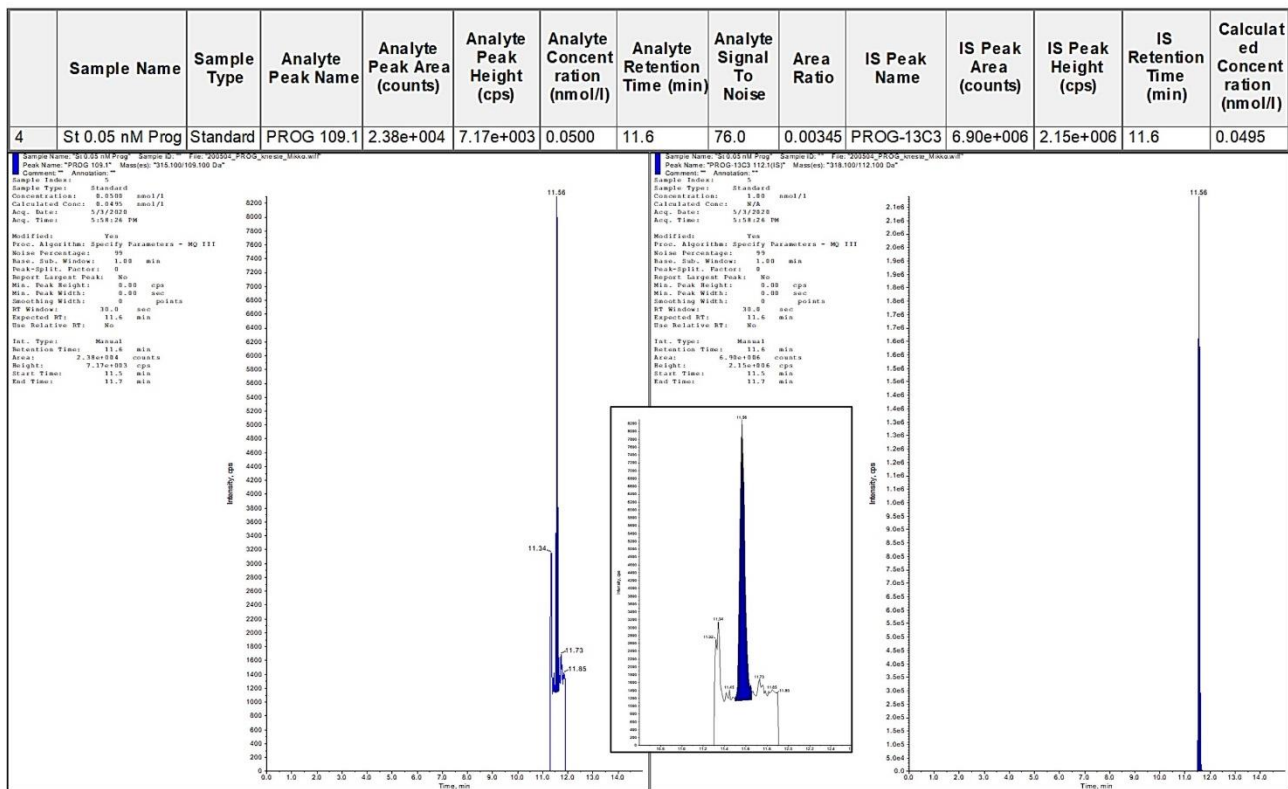
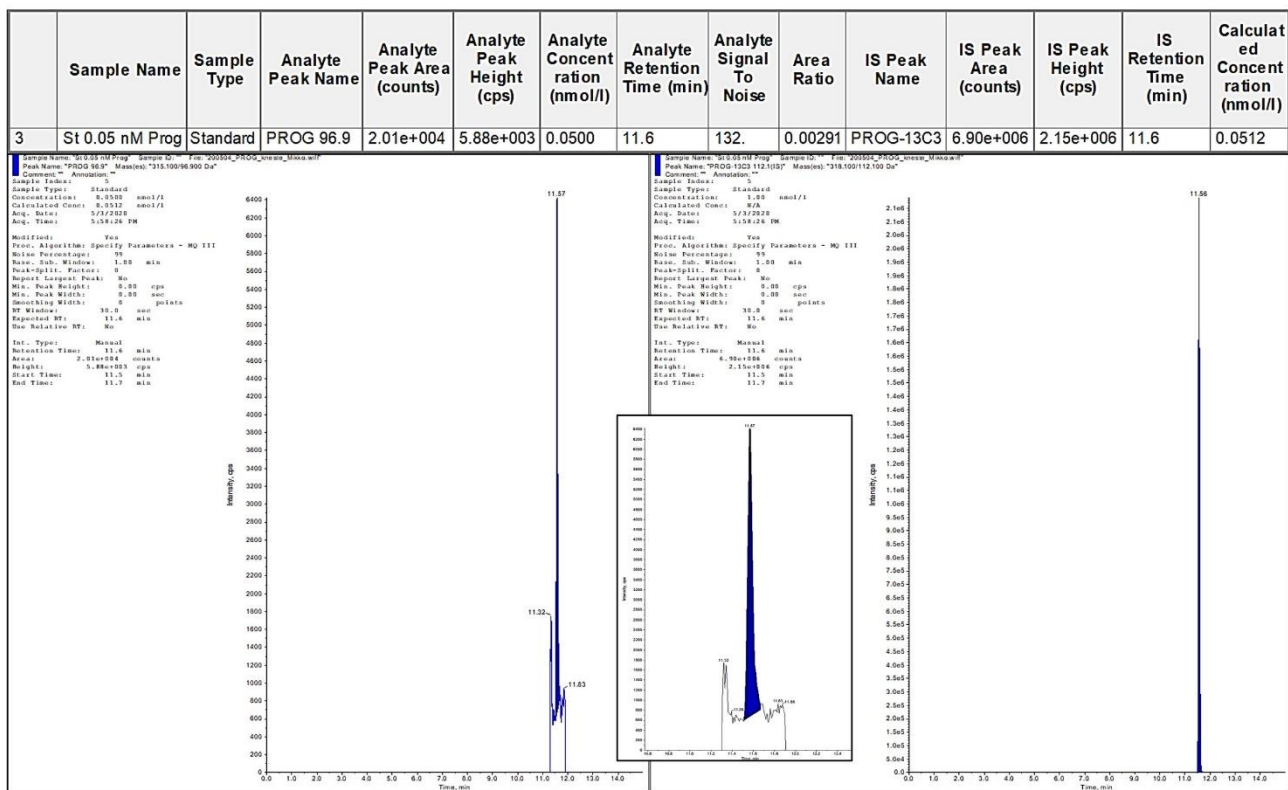




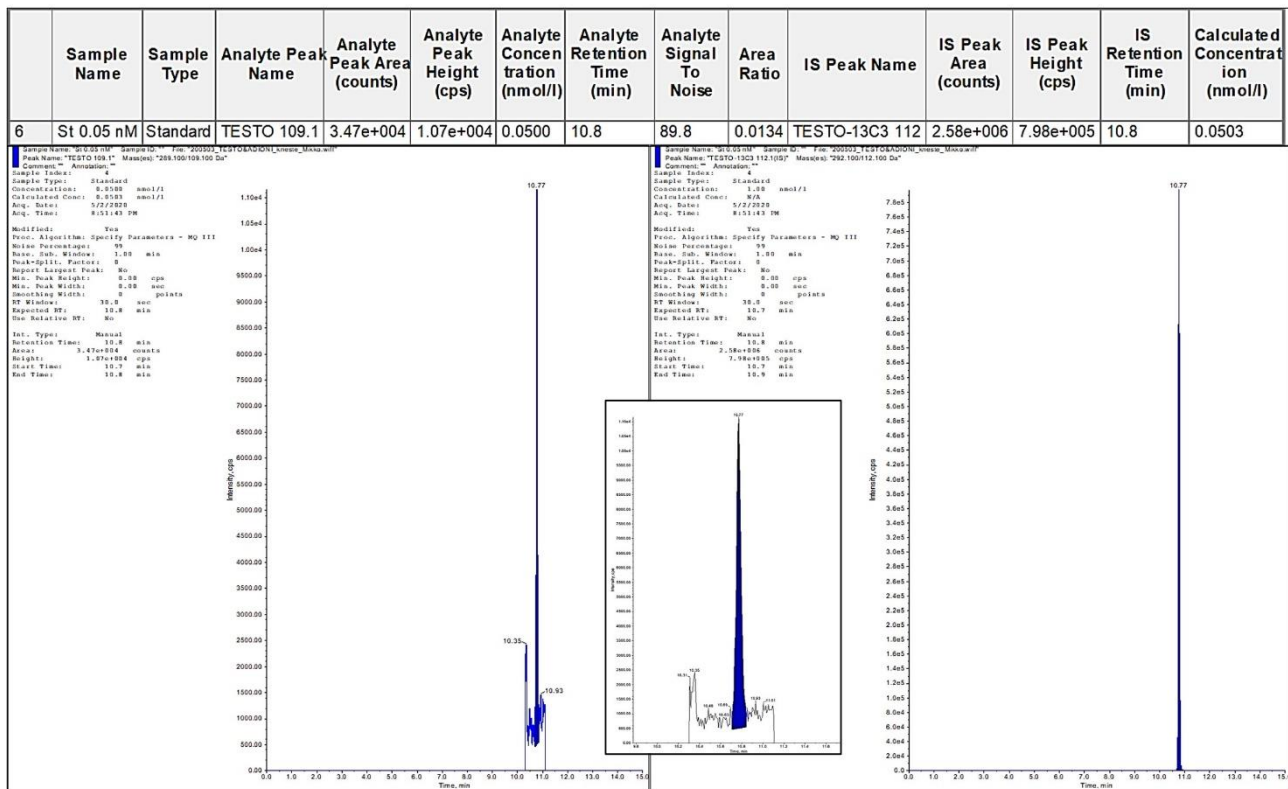
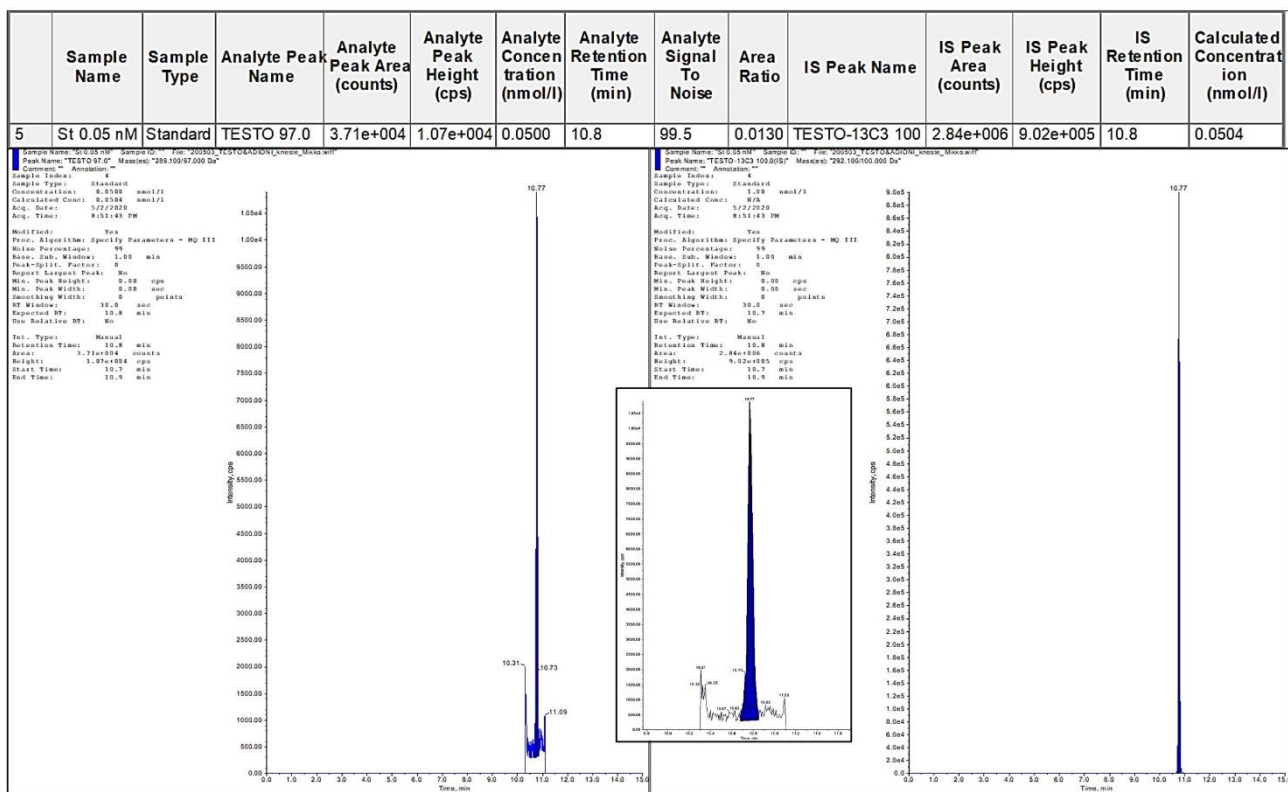
**Supplementary figure S2B: E2 LOQ chromatogram and S/N ratios.** Parent and transition ions E<sub>2</sub> [M-H]<sup>-</sup> m/z 271.2 to m/z 271.2 and m/z 183.1 in 10 pmol/L standard sample. Left panel shows calibrator chromatogram, while on the right is presented <sup>13</sup>C-estradiol IS. The high S/N ratio permitted extrapolation of the LOQ to 5 pmol/L.



**Supplementary figure S2C: P4 LOQ chromatogram and S/N ratios.** Parent and transition ions P4 [M+H]<sup>+</sup> m/z 315.1 to m/z 109.1 and m/z 96.9 in 50 pmol/L standard sample (S/N=132). Left panel shows calibrator chromatogram, while on the right is presented <sup>13</sup>C-progesterone IS. The high S/N ratio permitted extrapolation of the LOQ to 10 pmol/L.

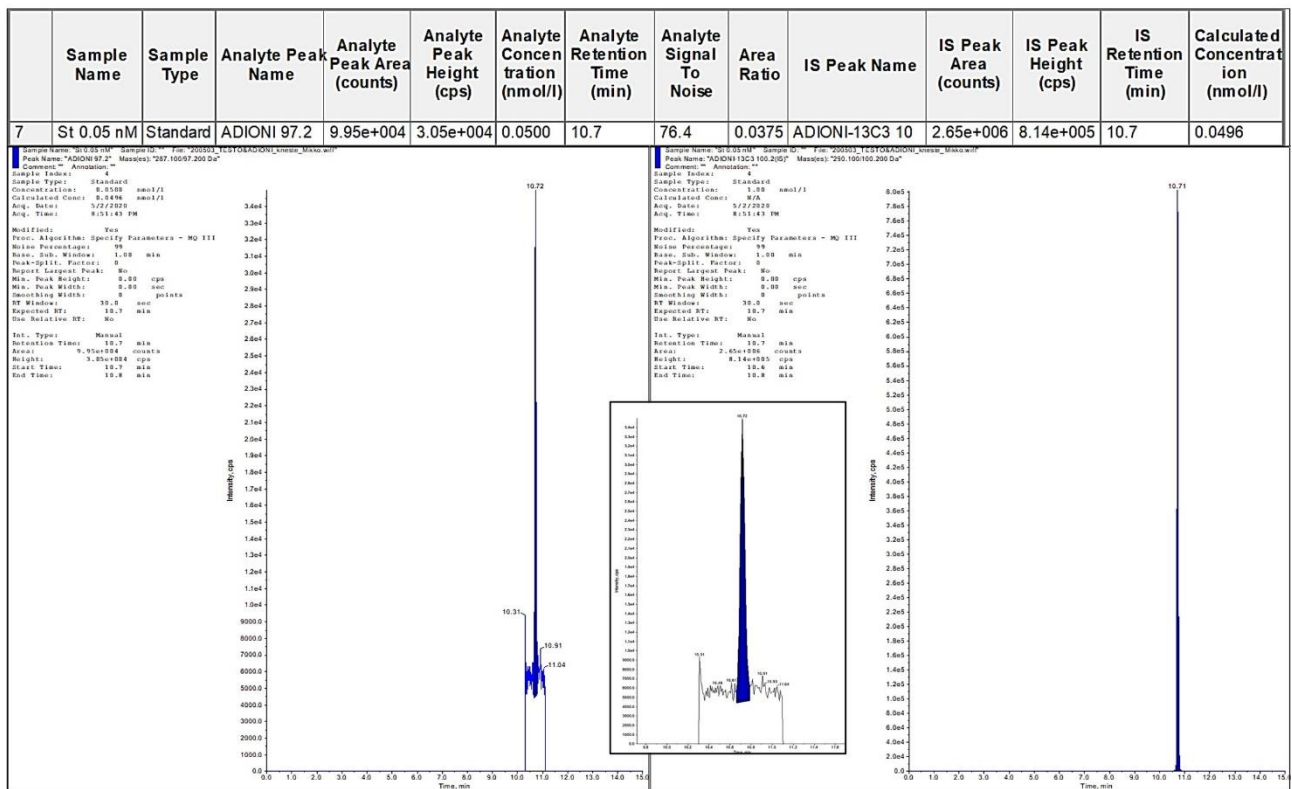
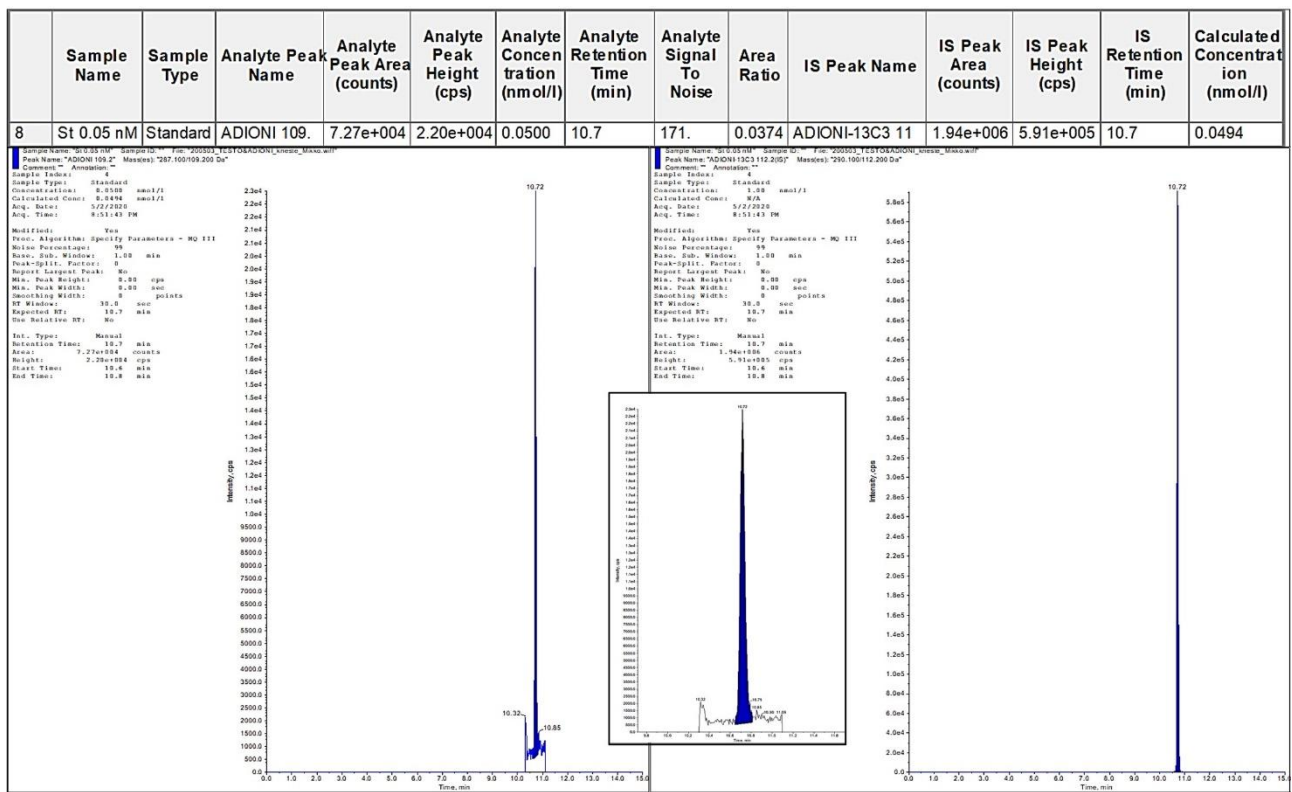


**Supplementary figure S2D: T4 LOQ chromatogram and S/N ratios.** Parent and transition ions T4 [M+H]<sup>+</sup> m/z 289.1 to m/z 109.1 and m/z 97.0 in 50 pmol/L standard sample (S/N=99.5). Left panel shows calibrator chromatogram, while on the right is presented <sup>13</sup>C-testosterone IS. The high S/N ratio permitted extrapolation of the LOQ to 10 pmol/L.

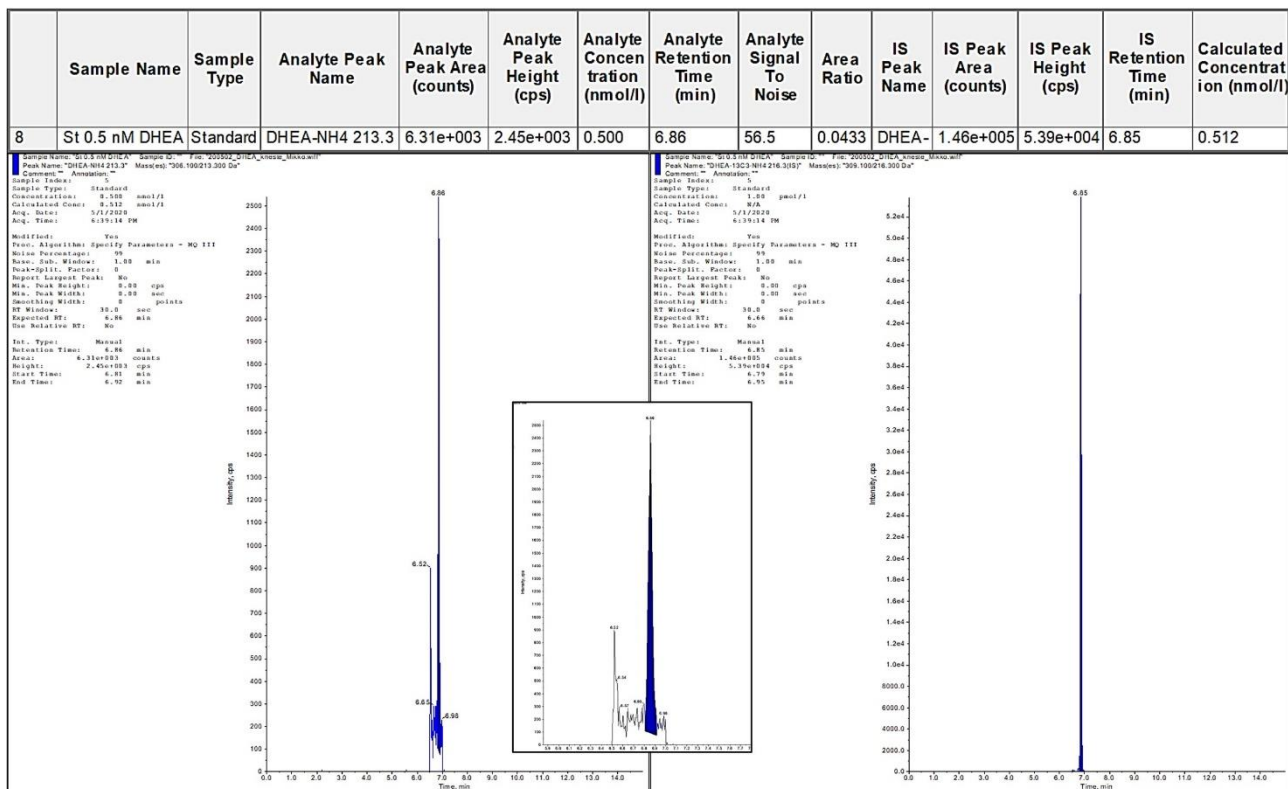
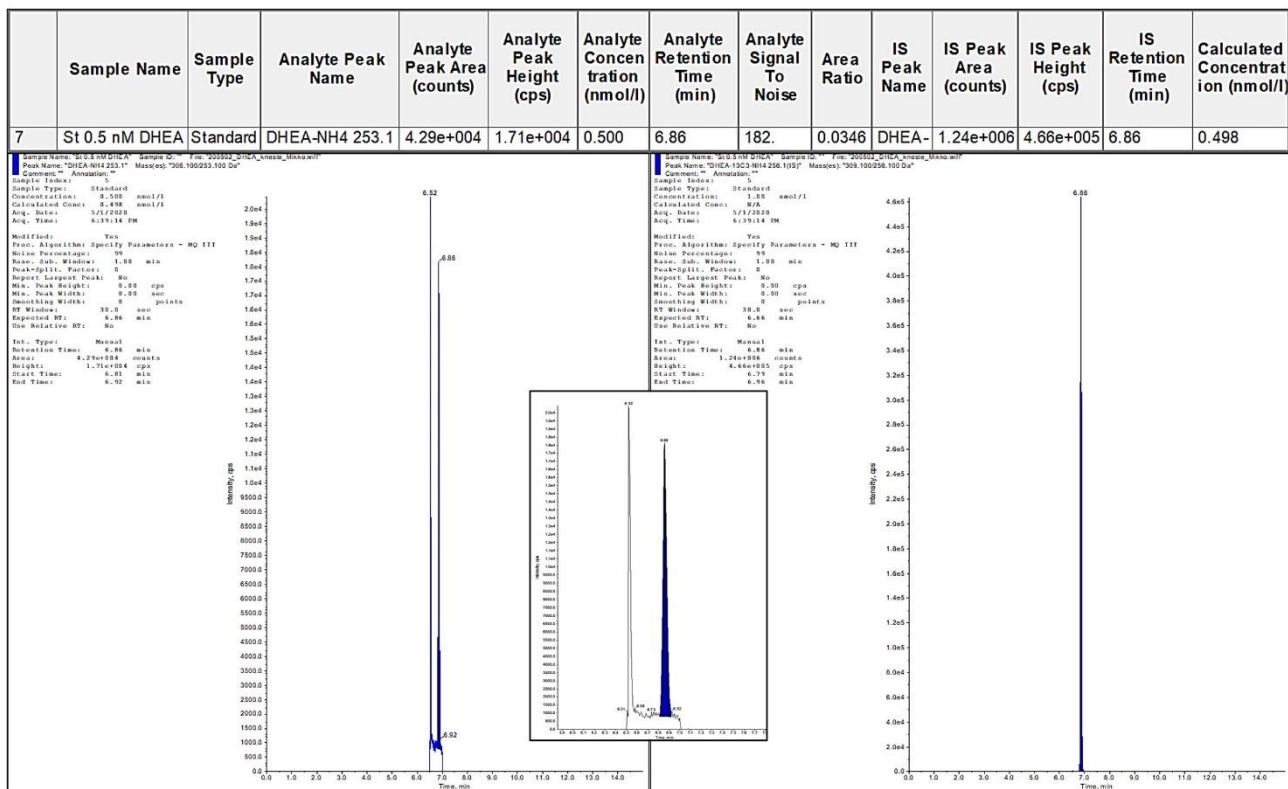


**Supplementary figure S2E: A4 LOQ chromatogram and S/N ratios.** Parent and transition ions A4 [M+H]<sup>+</sup> m/z 287.1 to m/z 109.2 and m/z 97.2 in 50 pmol/L standard sample (S/N=171.0). Left panel shows calibrator chromatogram, while on the right is presented <sup>13</sup>C-androstenedione IS. The high S/N ratio permitted extrapolation of the LOQ to 10 pmol/L.





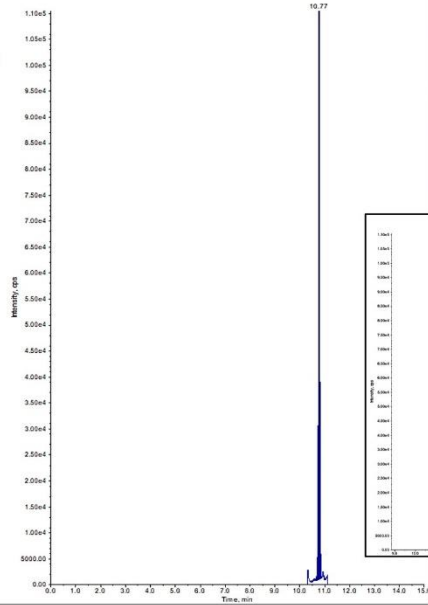
**Supplementary figure S2F: DHEA-LOQ chromatogram and S/N ratios.** Parent and transition ions – ammonium adduct [M+NH<sub>4</sub>]<sup>+</sup> m/z 306.1 to m/z 253.1 and m/z 213.3 in 0.5 nmol/L standard sample (S/N=182.0). Left panel shows calibrator chromatogram, while on the right is presented <sup>13</sup>C-DHEA IS. The high S/N ratio permitted extrapolation of the LOQ to 0.1 nmol/L.



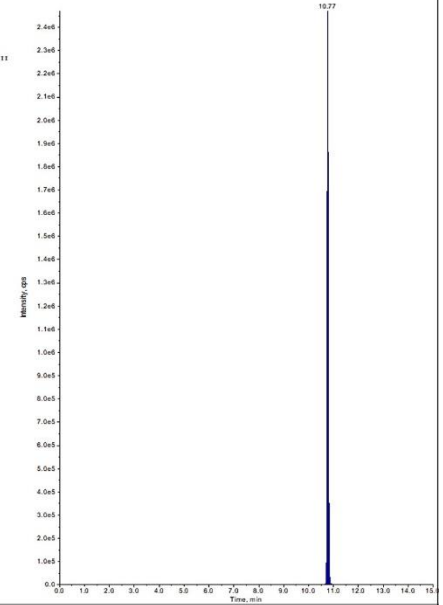
**Supplementary figure S3: T4 .** Parent and transition T4 [M+H]<sup>+</sup> m/z 289.1 to m/z 109.1 and m/z 97.0. Left panel shows analyte chromatogram, while on the right <sup>13</sup>C-testosterone IS.

a	Sample Name	Sample Type	Analyte Peak Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (nmol/l)	Analyte Retention Time (min)	Analyte Signal To Noise	Area Ratio	IS Peak Name	IS Peak Area (counts)	IS Peak Height (cps)	IS Retention Time (min)	Calculated Concentration (nmol/l)
85	15 10956-15	Unknown	TESTO 97.0	3.67e+005	1.10e+005	N/A	10.8	954.	0.0472	TESTO-13C3	7.77e+006	2.48e+006	10.8	0.801

Sample Name: 15 10956-15 Sample ID: 200593\_1510956-15\_Unknown\_Lineset\_Mass.wiff  
Peak Name: TESTO 97.0 Mass(es): 289.100/97.000 Da  
Comment: Annotation:  
Sample Index: 28  
Sample Type: Unknown  
Concentration: N/A  
Calculated Count: 8.883 nmol/l  
Acq. Date: 5/3/2023  
Acq. Time: 5:58:19 AM  
Modified: Yes  
Proc. Algorithm: Specific Parameters - HQ III  
Noise Percentage: 95  
Base. Sub. Window: 1.00 min  
Peak-Split. Factor: 0  
Report Largest Peak: No  
Min. Peak Height: 0.00 cps  
Min. Peak Width: 0.00 sec  
Smoothing Width: 0 points  
RT Window: 39.8 sec  
Expected RT: 10.8 min  
Use Relative RT: No  
Int. Type: Manual  
Retention Time: 10.8 min  
Area: 3.67e+005 counts  
Height: 1.10e+005 cps  
Start Time: 10.7 min  
End Time: 10.9 min

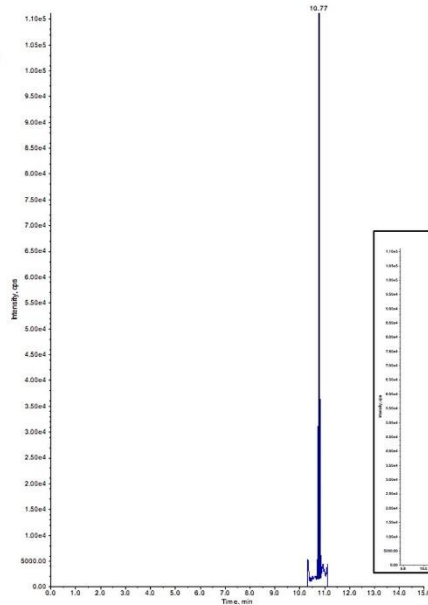


Sample Name: 15 10956-15 Sample ID: 200593\_1510956-15\_Unknown\_Lineset\_Mass.wiff  
Peak Name: TESTO-13C3 100.0157 Mass(es): 282.100/100.000 Da  
Comment: Annotation:  
Sample Index: 28  
Sample Type: Unknown  
Concentration: N/A  
Calculated Count: 9.94  
Acq. Date: 5/3/2023  
Acq. Time: 5:58:19 AM  
Modified: Yes  
Proc. Algorithm: Specific Parameters - HQ III  
Noise Percentage: 95  
Base. Sub. Window: 1.00 min  
Peak-Split. Factor: 0  
Report Largest Peak: No  
Min. Peak Height: 0.00 cps  
Min. Peak Width: 0.00 sec  
Smoothing Width: 0 points  
RT Window: 39.8 sec  
Expected RT: 10.7 min  
Use Relative RT: No  
Int. Type: Manual  
Retention Time: 10.8 min  
Area: 7.77e+006 counts  
Height: 2.48e+006 cps  
Start Time: 10.6 min  
End Time: 11.0 min



	Sample Name	Sample Type	Analyte Peak Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (nmol/l)	Analyte Retention Time (min)	Analyte Signal To Noise	Area Ratio	IS Peak Name	IS Peak Area (counts)	IS Peak Height (cps)	IS Retention Time (min)	Calculated Concentration (nmol/l)
86	15 10956-15	Unknown	TESTO 109.1	3.48e+005	1.10e+005	N/A	10.8	405.	0.0491	TESTO-13C3	7.07e+006	2.29e+006	10.8	0.833

Sample Name: 15 10956-15 Sample ID: 200593\_1510956-15\_Unknown\_Lineset\_Mass.wiff  
Peak Name: TESTO 109.1 Mass(es): 289.100/109.100 Da  
Comment: Annotation:  
Sample Index: 28  
Sample Type: Unknown  
Concentration: N/A  
Calculated Count: 8.883 nmol/l  
Acq. Date: 5/3/2023  
Acq. Time: 5:58:19 AM  
Modified: Yes  
Proc. Algorithm: Specific Parameters - HQ III  
Noise Percentage: 95  
Base. Sub. Window: 1.00 min  
Peak-Split. Factor: 0  
Report Largest Peak: No  
Min. Peak Height: 0.00 cps  
Min. Peak Width: 0.00 sec  
Smoothing Width: 0 points  
RT Window: 39.8 sec  
Expected RT: 10.8 min  
Use Relative RT: No  
Int. Type: Manual  
Retention Time: 10.8 min  
Area: 3.48e+005 counts  
Height: 1.10e+005 cps  
Start Time: 10.7 min  
End Time: 10.9 min



Sample Name: 15 10956-15 Sample ID: 200593\_1510956-15\_Unknown\_Lineset\_Mass.wiff  
Peak Name: TESTO-13C3 112.1057 Mass(es): 282.100/112.100 Da  
Comment: Annotation:  
Sample Index: 28  
Sample Type: Unknown  
Concentration: N/A  
Calculated Count: 9.94  
Acq. Date: 5/3/2023  
Acq. Time: 5:58:19 AM  
Modified: Yes  
Proc. Algorithm: Specific Parameters - HQ III  
Noise Percentage: 95  
Base. Sub. Window: 1.00 min  
Peak-Split. Factor: 0  
Report Largest Peak: No  
Min. Peak Height: 0.00 cps  
Min. Peak Width: 0.00 sec  
Smoothing Width: 0 points  
RT Window: 39.8 sec  
Expected RT: 10.7 min  
Use Relative RT: No  
Int. Type: Manual  
Retention Time: 10.8 min  
Area: 7.07e+006 counts  
Height: 2.29e+006 cps  
Start Time: 10.7 min  
End Time: 11.0 min

