DIRECT ANALYSIS OF ACYLCARNITINES AND AMINO ACIDS IN DRIED BLOOD SOTS WITHOUT PUNCHING OR DERIVATIZATION USING AN AUTOSAMPLER

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PAL DBS Autosampler coupled with Waters Xevo TQD

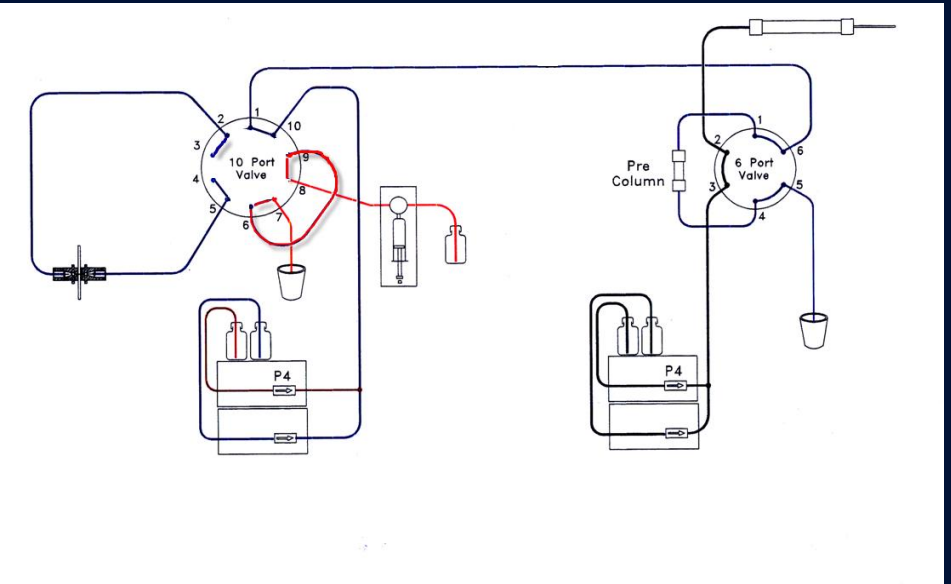
- Autosampler manufactured in Switzerland by CTC Analytics; distributed in US by Leap technologies
- Designed to hold up to 160 DMPK cards (FTE, GE); each has 4 circles (10 mm diam)
- Controlled by mass spectrometer inlet system software
- Inserts card into a clamp; pumps solvent plus internal standards directly through a 3mm diam area of the DBS and into the MS/MS system
- Can accommodate SPE/HPLC columns

Operation of flow-through HTS PAL Autosampler

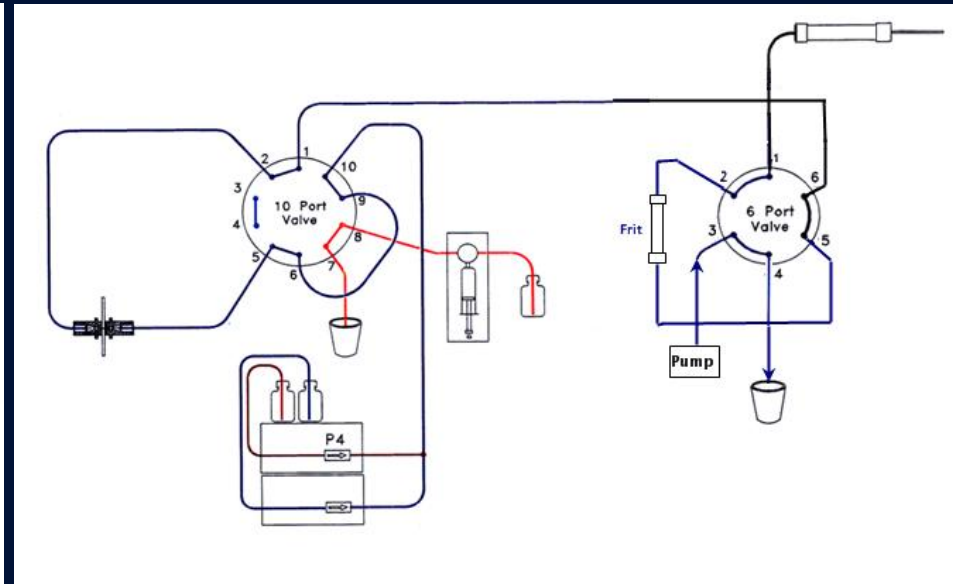
Variables

- Clamp Pressure (May depend on DBS Card)
- Clamp diameter (3mm and up)
- Extraction solvent; flow rate & time
- Internal standard – on card or in loop

Pre-load IS solution in 20 µL loop:



Solvent flow carries IS through DBS:



PHASE I: WILL IT WORK FOR ACYLCARNITINES?

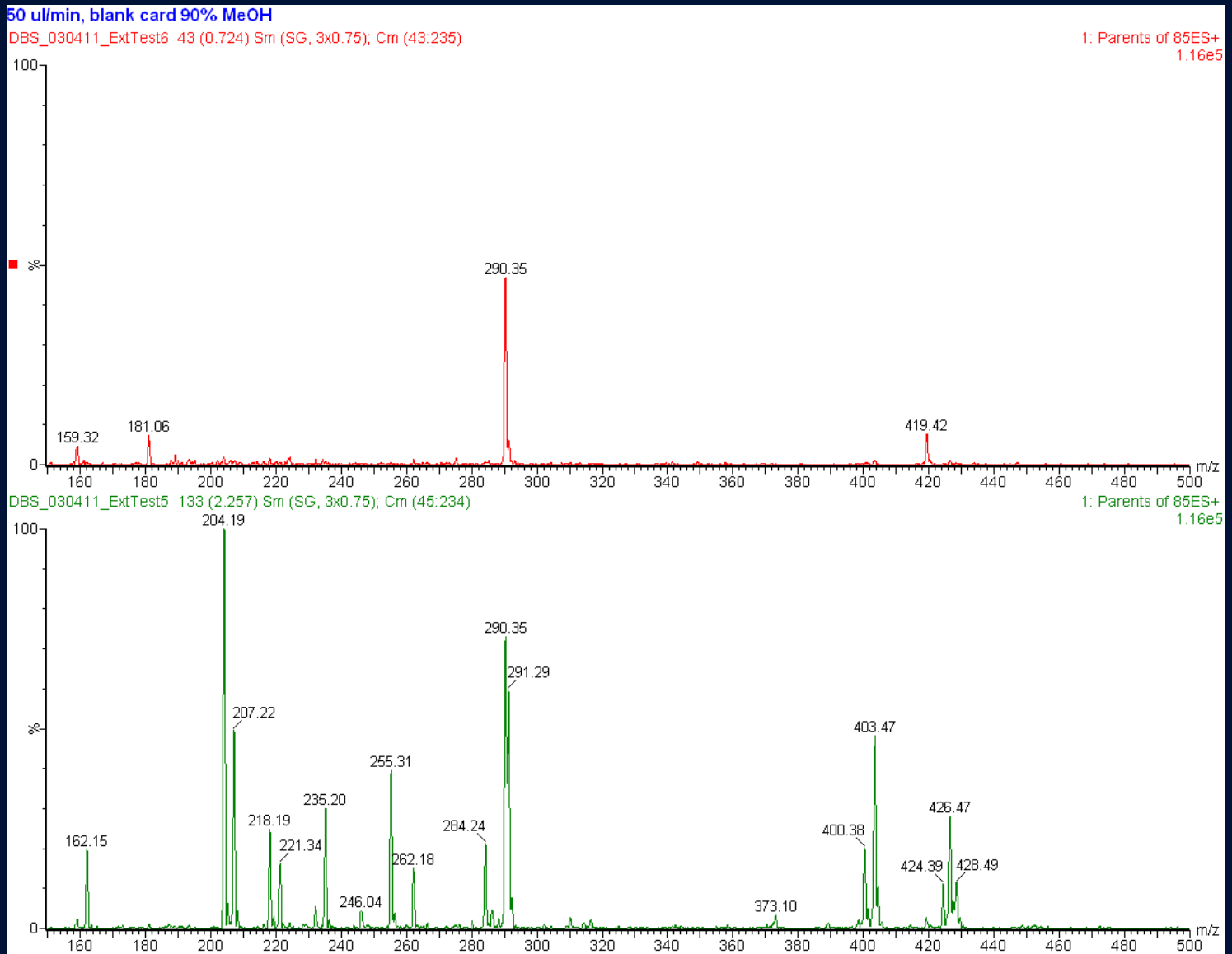
Initial Research Plan:

- 200 µL aliquots of whole blood (EDTA)
- Spiked with C2, C8 & C16 acylcarnitines (3 levels) in quadruplicate
- 35 µL aliquots spotted onto Whatman FTA® DMPK cards, allowed to dry
- Eluant - 90:9.9:0.1 v/v/v methanol:water: formic acid at a flow rate 50 µL/min
- Internal standards in 20 µL loop
- Direct coupling to Waters Xevo TQD
- Cycle time ~ 2.5 min

Standard Procedure for Analysis of Acylcarnitines in DBS

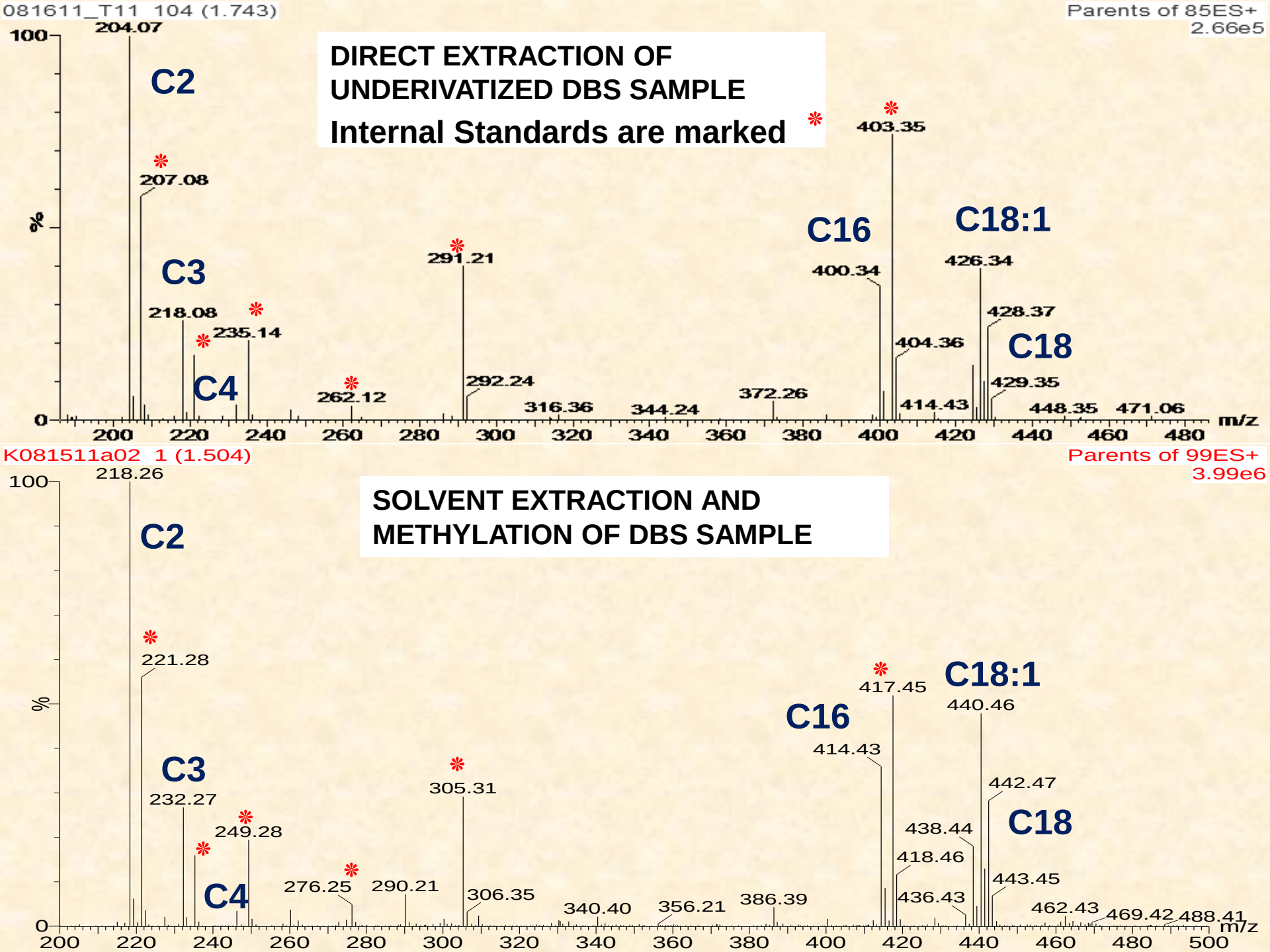
- Punch 6 mm diam DBS sample into well of 96-well microplate
- Extract with MeOH plus internal stds for 30 min
- Evaporate to dryness
- Derivatize (incubation with MeOH/HCl or BuOH/HCl for 30 min @ 55-60 °C)
- Cool; evaporate to dryness
- Add final matrix
- Place 96-well plate in autosampler for analysis by flow-injection MS/MS

Summed Spectra (precursor ion scans of m/z 85 from 150-500): 90% MeOH at 50 µL/min



BLANK (NO SAMPLE ; NO INT STDS)

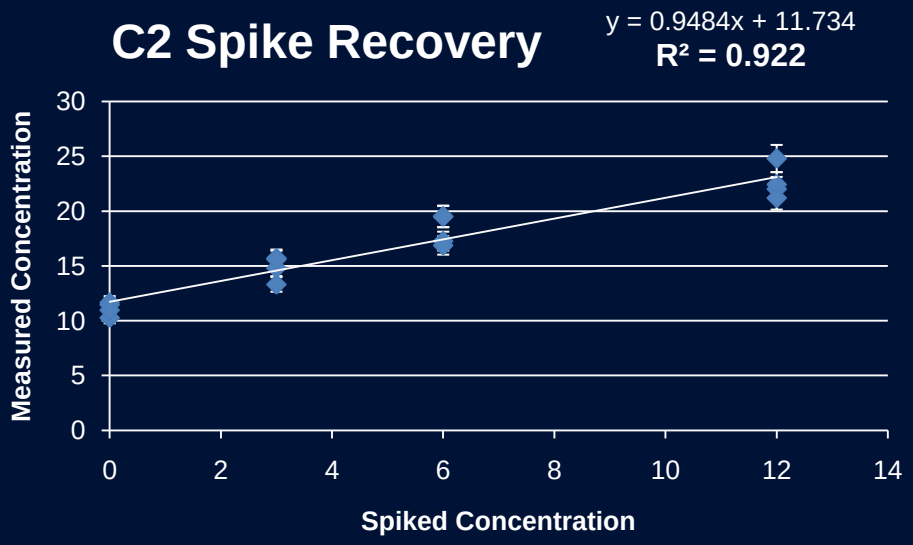
DBS (SAMPLE A)

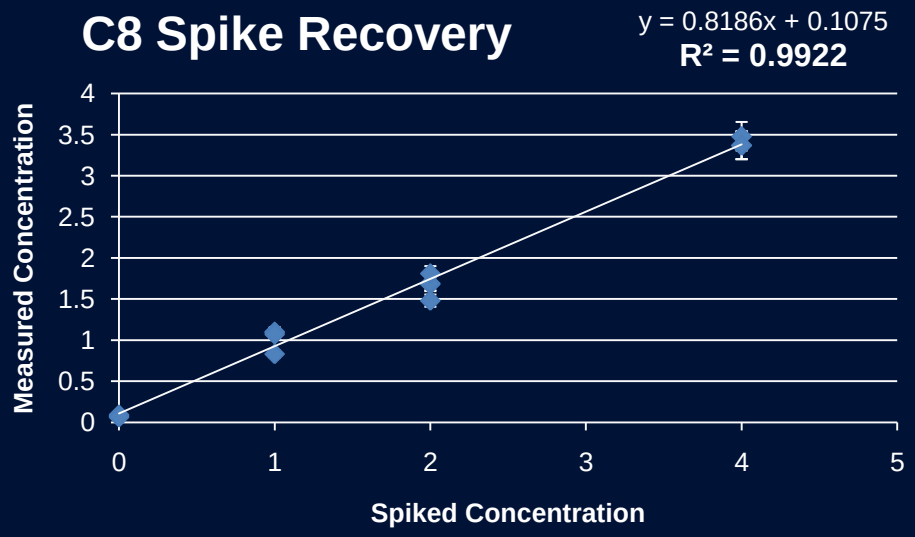

081611_T11 104 (1.743)
Parents of 85ES+
2.66e5
DIRECT EXTRACTION OF UNDERIVATIZED DBS SAMPLE
Internal Standards are marked
C2
C3
C4
C16
C18:1
C18
204.07
207.08
218.08
235.14
262.12
291.21
292.24
316.36
344.24
372.26
400.34
403.35
404.36
414.43
426.34
428.37
429.35
448.35
471.06
K081511a02 1 (1.504)
Parents of 99ES+
3.99e6
SOLVENT EXTRACTION AND METHYLATION OF DBS SAMPLE
C2
C3
C4
C16
C18:1
C18
218.26
221.28
232.27
249.28
276.25
290.21
305.31
306.35
340.40
356.21
386.39
414.43
417.45
418.46
436.43
438.44
440.46
442.47
443.45
462.43
469.42
488.41
%
m/z

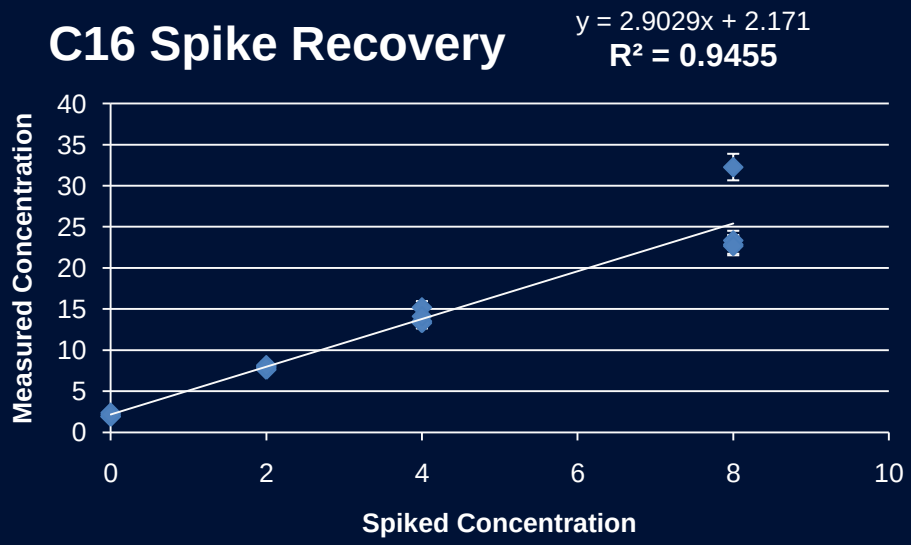
Replicate Analyses

	C2	C3	C4	C16	C18:1	C18
DBS_RepTest_A1	6.17	1.18	0.21	0.64	0.82	0.31
DBS_RepTest_A2	5.86	1.29	0.18	0.60	0.69	0.42
DBS_RepTest_A3	5.73	1.09	0.16	0.74	0.85	0.43
DBS_RepTest_A4	6.43	0.93	0.20	0.79	0.97	0.37
MEAN	6.05	1.12	0.19	0.69	0.83	0.38
STDDEV	0.31	0.15	0.02	0.09	0.11	0.06
CV (%)	5	14	12	13	14	14

Standard Addition Curves

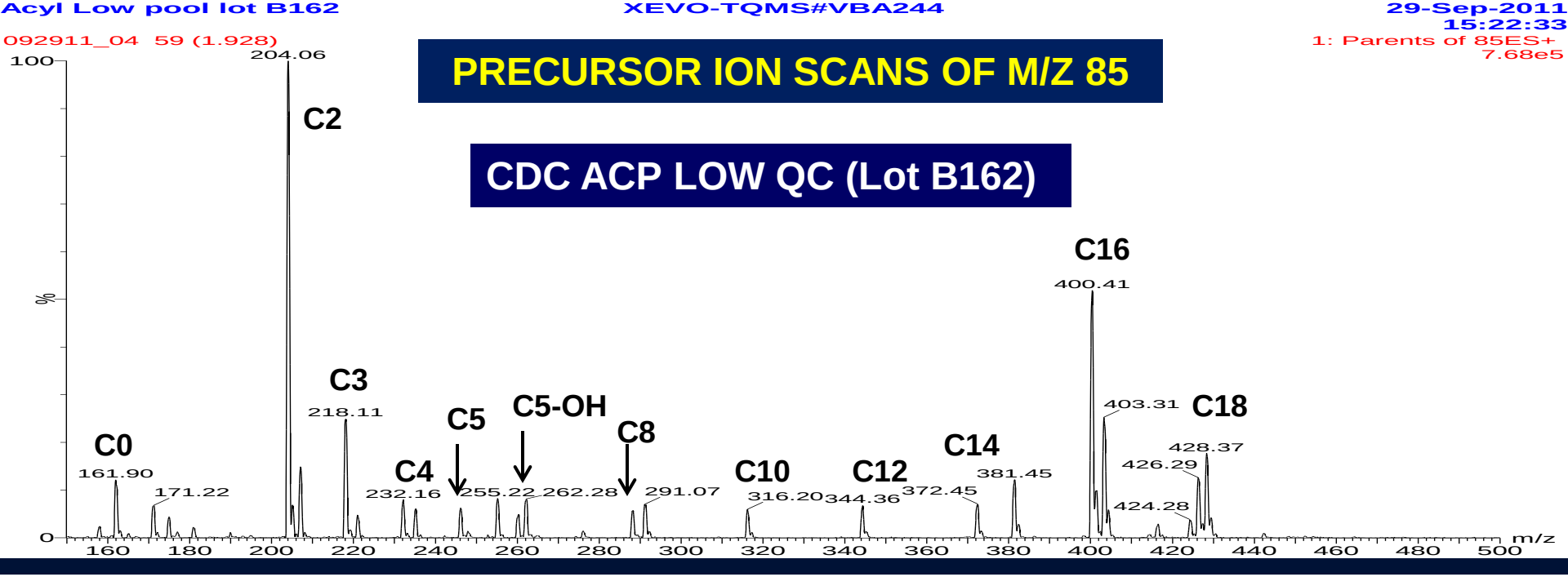


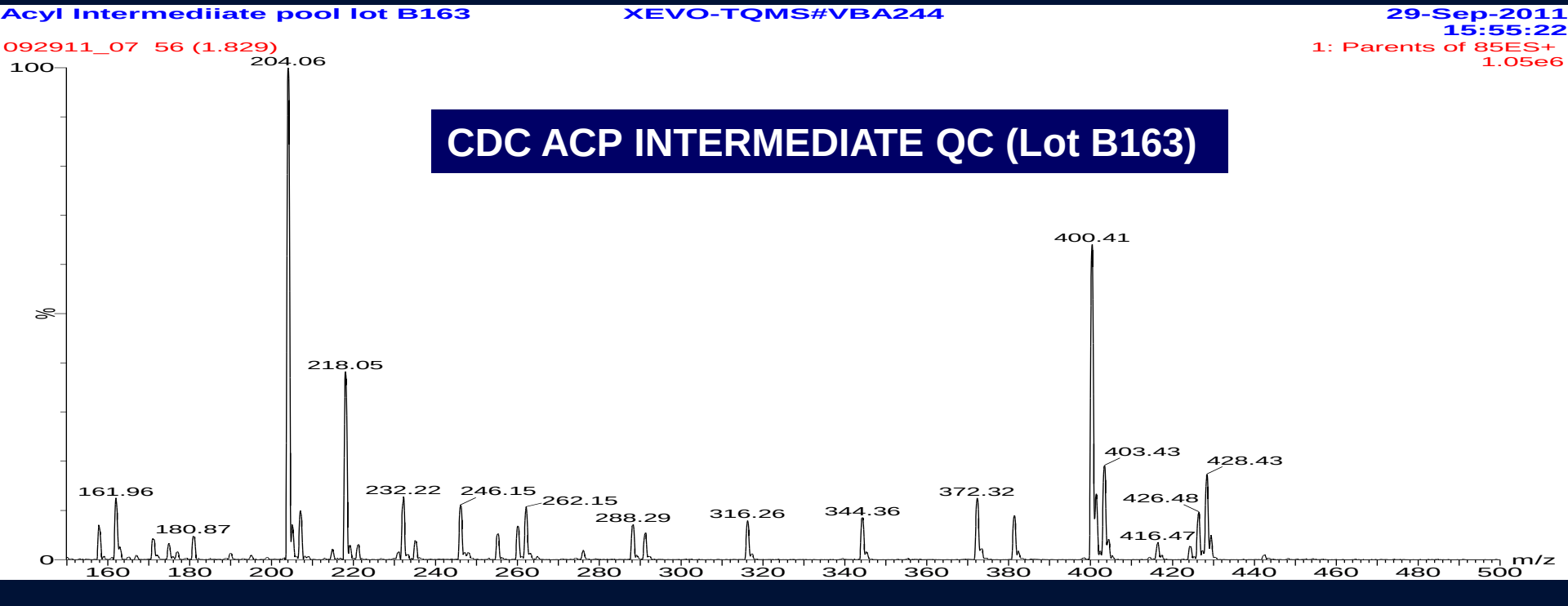


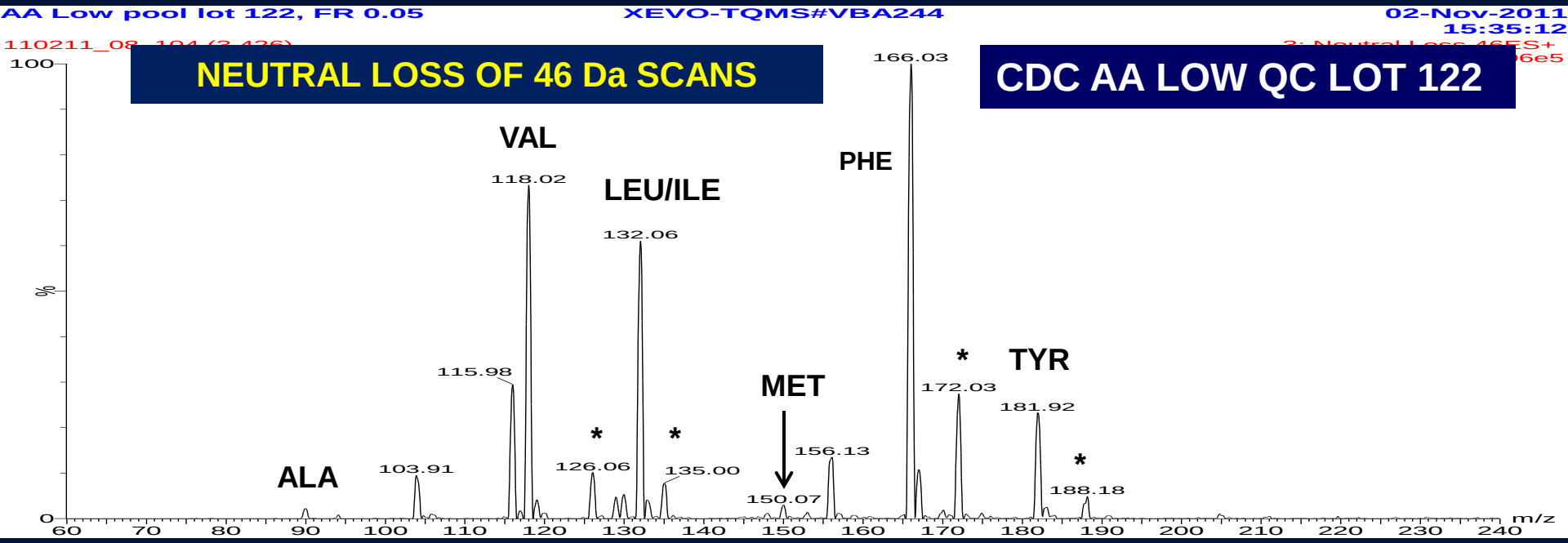


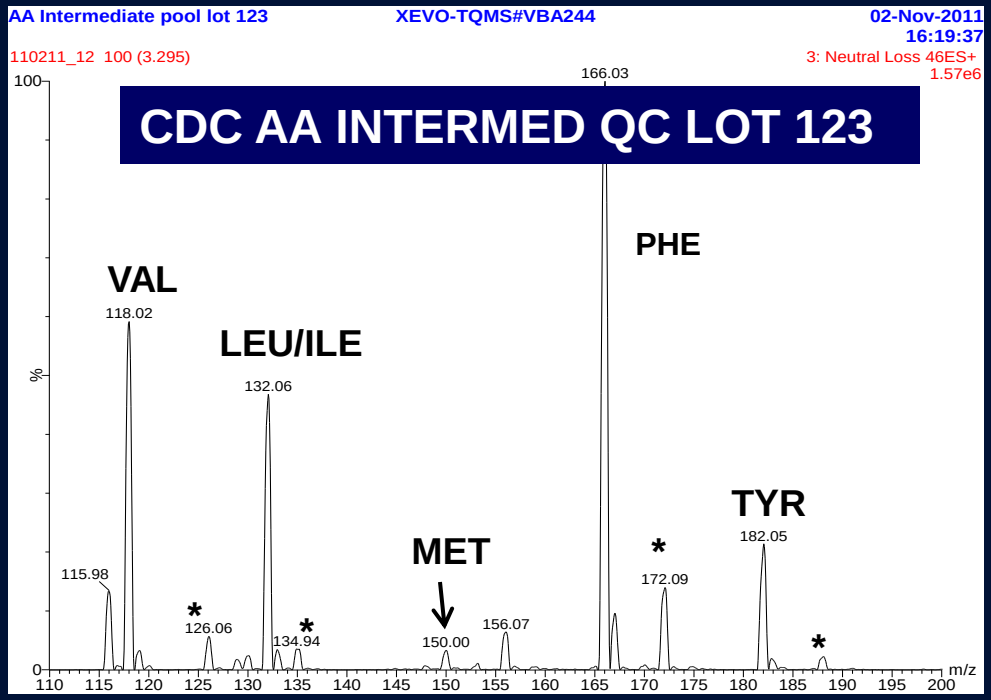
Phase II: CDC QC Controls

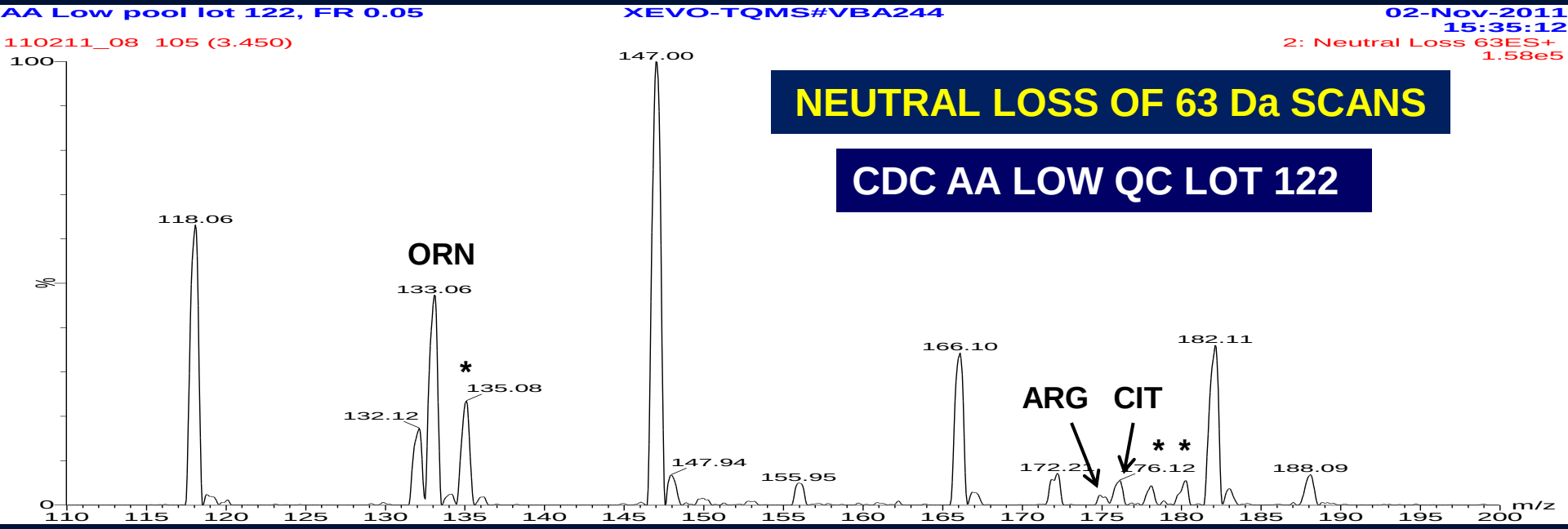
- Direct analysis of CDC control DBS (blinded) for acylcarnitines and amino acids in a single, batched analysis
- Use of Cambridge Isotopes Internal Standard Kit (kindly supplied by Victor DeJesus, CDC)
- Include wash cycle to minimize carry-over
- Comparison of data with CDC reports

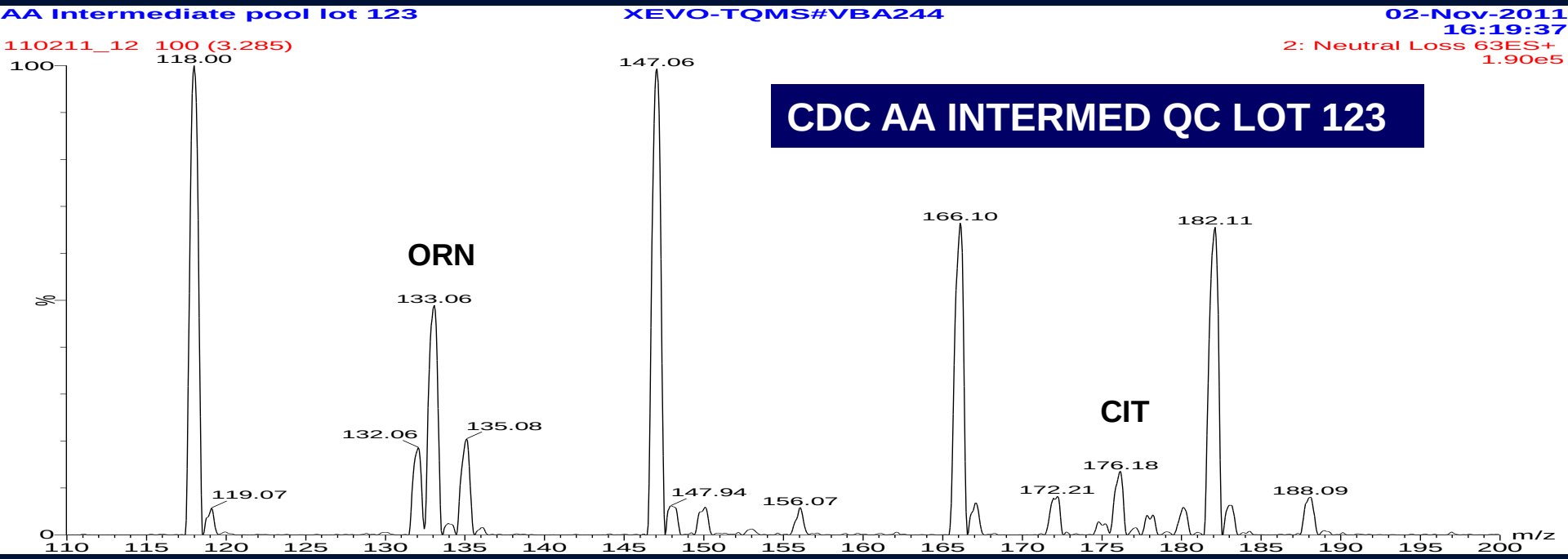

Acyl Low pool lot B162
XEVO-TQMS#VBA244
29-Sep-2011
15:22:33
092911_04 59 (1.928)
1: Parents of 85ES+
7.68e5
PRECURSOR ION SCANS OF M/Z 85
CDC ACP LOW QC (Lot B162)
C0
C2
C3
C4
C5
C5-OH
C8
C10
C12
C14
C16
C18
204.06
218.11
161.90
171.22
232.16
255.22
262.28
291.07
316.20
344.36
372.45
381.45
400.41
403.31
424.28
426.29
428.37
%
m/z


Acyl Intermediiate pool lot B163
XEVO-TQMS#VBA244
29-Sep-2011
15:55:22
092911_07 56 (1.829)
1: Parents of 85ES+
1.05e6
CDC ACP INTERMEDIATE QC (Lot B163)
204.06
218.05
161.96
180.87
232.22
246.15
262.15
288.29
316.26
344.36
372.32
400.41
403.43
416.47
426.48
428.43
%
m/z


AA Low pool lot 122, FR 0.05
XEVO-TQMS#VBA244
02-Nov-2011
15:35:12
NEUTRAL LOSS OF 46 Da SCANS
CDC AA LOW QC LOT 122
VAL
PHE
LEU/ILE
MET
TYR
ALA
166.03
118.02
132.06
115.98
172.03
181.92
156.13
103.91
126.06
135.00
150.07
188.18
%
m/z


AA Intermediate pool lot 123
XEVO-TQMS#VBA244
02-Nov-2011
16:19:37
110211_12 100 (3.295)
3: Neutral Loss 46ES+
1.57e6
CDC AA INTERMED QC LOT 123
PHE
VAL
LEU/ILE
MET
TYR
166.03
118.02
132.06
115.98
182.05
172.09
126.06
134.94
150.00
156.07
%
m/z


AA Low pool lot 122, FR 0.05
XEVO-TQMS#VBA244
02-Nov-2011
15:35:12
110211_08 105 (3.450)
2: Neutral Loss 63ES+
1.58e5
NEUTRAL LOSS OF 63 Da SCANS
CDC AA LOW QC LOT 122
ORN
ARG
CIT
118.06
133.06
132.12
135.08
147.00
147.94
155.95
166.10
172.21
176.12
182.11
188.09
%
m/z


AA Intermediate pool lot 123
XEVO-TQMS#VBA244
02-Nov-2011
16:19:37
110211_12 100 (3.285)
2: Neutral Loss 63ES+
1.90e5
CDC AA INTERMED QC LOT 123
ORN
CIT
118.00
119.07
133.06
132.06
135.08
147.06
147.94
156.07
166.10
172.21
176.18
182.11
188.09
%
m/z

COMPARISON OF DATA FROM CDC PT SAMPLES: DATA FROM LEAP DBS-A/S v CDC EXPECTED VALUES

CDC CODE	C0		C3		C4		C5:1		C5		C4-OH/ C3-DC		C6		C4-DC/ C5-OH		C5-DC	
PT 4061	8.03	5.66	0.20	0.25	0.13	0.09	0.00	0.02	0.09	0.05	0.04	0.04	0.00	0.02	0.49	0.55	0.06	0.03
PT 4062	28.03	23.11	1.27	1.55	0.15	0.12	0.00	0.02	0.11	0.08	0.04	0.05	2.68	3.02	0.45	0.46	0.04	0.02
PT 4063	29.77	34.99	1.65	2.25	0.18	0.18	1.36	1.99	1.47	2.12	0.10	0.13	0.05	0.07	0.50	0.60	0.00	0.03
PT 4064	31.45	28.40	5.73	11.10	0.18	0.21	0.00	0.02	0.15	0.12	0.28	1.57	0.03	0.05	0.42	0.55	0.47	2.10
PT 4065	11.66	11.35	0.51	0.60	0.14	0.12	0.01	0.01	0.07	0.07	0.05	0.03	0.01	0.02	0.40	0.55	0.01	0.08

C8		C10:1		C10		C14:1		C14		C16		C16-OH		C18	
0.05	0.02	0.00	0.01	0.02	0.02	0.04	0.03	0.08	0.07	0.41	0.63	0.00	0.02	0.24	0.45
4.80	6.04	2.30	3.77	2.25	3.05	0.03	0.06	0.04	0.07	0.43	0.85	0.00	0.01	0.22	0.65
0.13	0.11	0.09	0.13	0.17	0.19	0.04	0.11	0.06	0.13	0.78	1.19	0.00	0.03	0.41	0.99
0.09	0.09	0.05	0.07	0.05	0.10	0.04	0.05	0.06	0.11	0.56	0.85	0.01	0.02	0.32	0.62
0.02	0.02	0.01	0.02	0.01	0.03	0.04	0.04	0.06	0.10	0.82	0.93	0.02	0.02	2.29	6.39

COMPARISON OF DATA FROM CDC PT SAMPLES: DATA FROM LEAP DBS-A/S v CDC PEER GROUP - CODE 60

CDC CODE	C0		C3		C4		C5:1		C5		C4-OH/C3-DC		C6		C4-DC/C5-OH		C5-DC	
PT 1161	9.96	5.84	0.25	0.21	0.19	0.10	0.01	0.01	0.14	0.06	0.05	0.05	0.03	0.03	0.90	0.49	0.03	0.05
PT 1162	43.27	33.29	1.41	1.03	3.36	2.61	0.03	0.02	2.47	2.02	0.12	0.12	0.06	0.06	0.57	0.61	0.03	0.11
PT 1163	33.52	33.59	11.80	10.16	0.21	0.19	0.02	0.02	0.13	0.12	0.25	0.48	0.04	0.07	0.68	0.61	0.47	2.26
PT 1164	28.55	17.13	1.11	0.74	0.17	0.11	2.19	1.28	0.11	0.07	0.06	0.07	0.02	0.05	0.88	0.42	0.00	0.07
PT 1165	16.57	11.05	1.43	0.93	0.16	0.11	0.01	0.02	0.10	0.07	0.08	0.07	0.03	0.04	0.46	0.31	0.00	0.05

C8		C10:1		C10		C14:1		C14		C16		C16-OH		C18:1		C18	
0.02	0.02	0.01	0.02	0.02	0.02	0.03	0.02	0.10	0.06	0.77	0.67	0.01	0.01	0.57	0.65	0.42	0.51
0.12	0.09	0.07	0.07	0.12	0.09	0.05	0.05	3.22	2.89	10.99	11.79	0.01	0.03	0.89	1.32	0.73	0.85
0.06	0.09	0.07	0.07	0.07	0.09	0.07	0.05	0.10	0.07	0.82	1.02	0.03	0.01	0.99	1.34	0.53	0.78
0.02	0.05	0.95	0.57	0.10	0.05	7.38	5.87	1.17	0.88	1.03	1.10	0.01	0.01	1.75	2.15	0.56	0.78
0.03	0.11	0.01	0.02	0.11	0.11	0.03	0.04	0.14	0.11	11.88	11.19	0.40	0.56	3.51	4.44	4.56	5.36

AMINO ACID VALUES IN CDC QC SAMPLES: COMPARISON OF LEAP DBS-A/S WITH CDC TARGETS

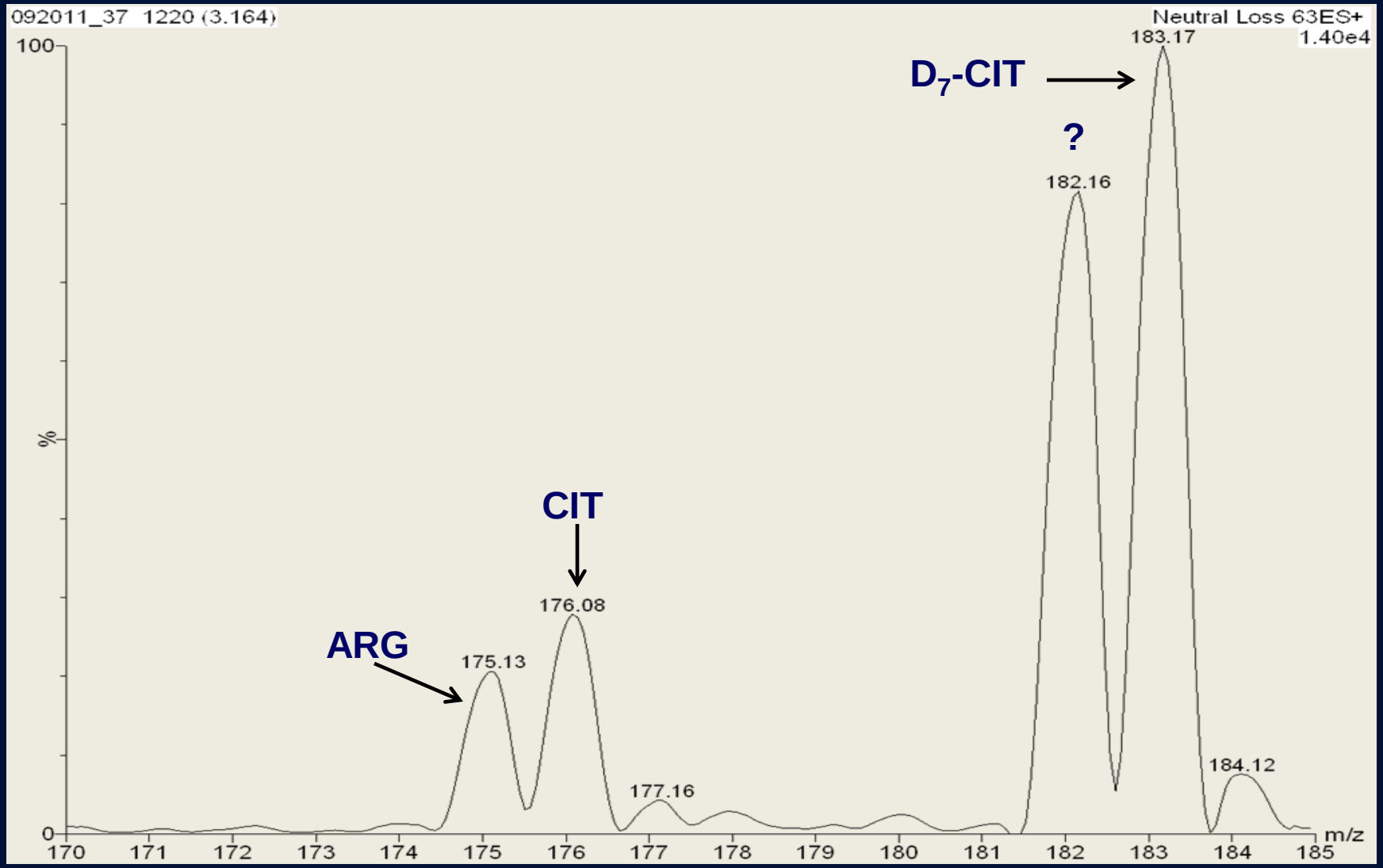
CDC LOT#	VAL			LEU/ILE			MET			PHE			TYR			CIT		
		- ENDOG			- ENDOG			- ENDOG			-ENDOG			- ENDOG			- ENDOG	
BP (#121)	191	0	0	253	0	0	24	0	0	93	0	0	75	0	0	38	0	0
BP (#121)	195	0	0	216	0	0	30	0	0	84	0	0	69	0	0	53	0	0
LP (#122)	452	259	250	490	255	200	137	110	100	229	140	150	302	230	250	84	38	50
LP (#122)	457	264	250	404	169	200	115	88	100	254	165	150	346	274	250	76	30	50
IP (#123)	674	481	600	835	600	500	191	164	250	446	357	400	607	535	500	218	172	150
IP (#123)	670	477	600	750	515	500	203	176	250	472	383	400	614	542	500	199	153	150
HP (#124)	1088	895	1000	1022	787	800	485	458	500	661	572	600	773	701	750	293	247	300
HP (#124)	1161	968	1000	1087	852	800	380	353	500	706	617	600	937	865	750	263	217	300

Development of new DBS assays (e.g. second-tier tests based on MS/MS)

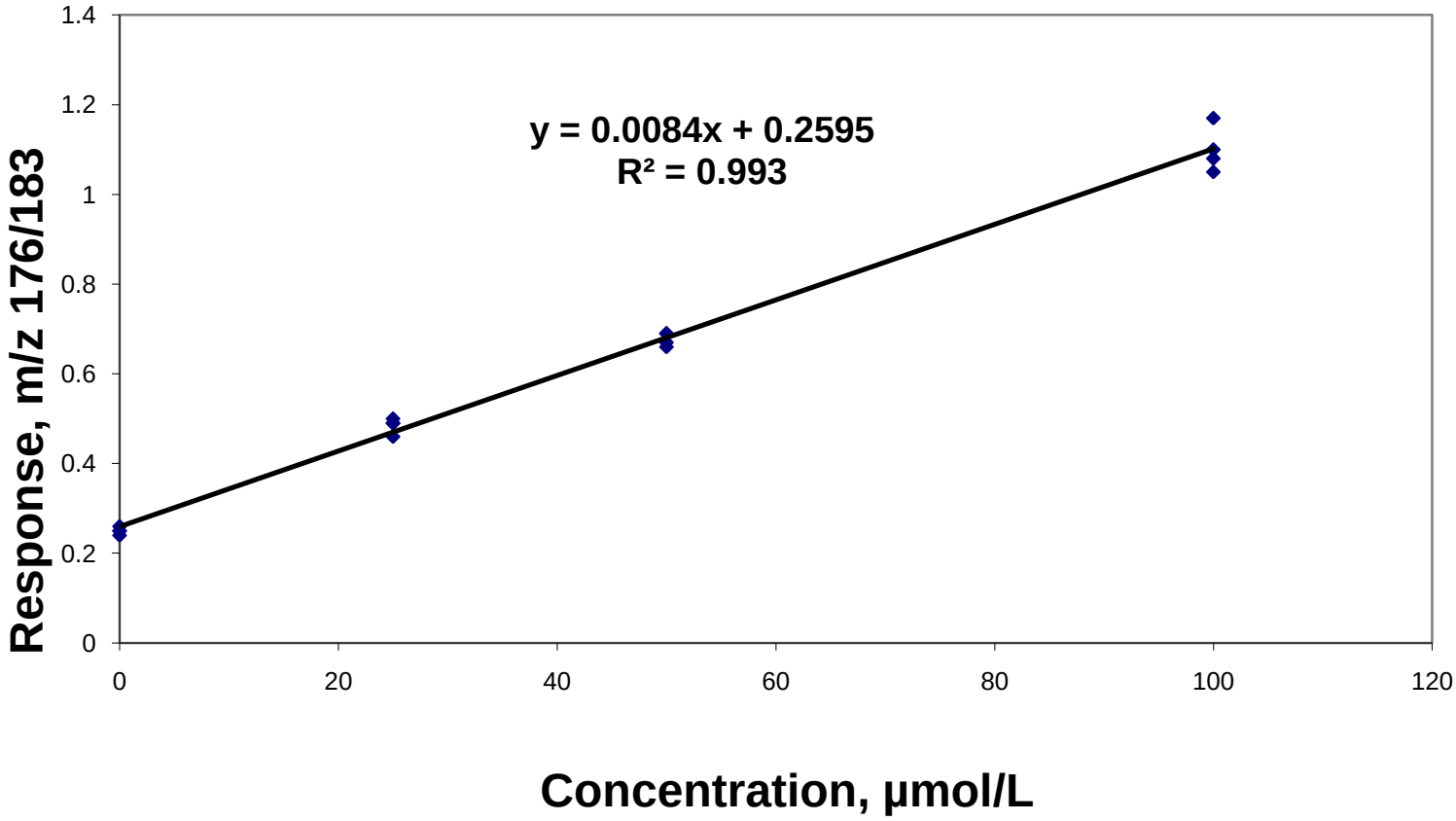
Example: Citrulline in DBS - Research Plan

- Make 4 calibrators from normal whole blood spiked with 0, 25 50 and 100 µmol/L citrulline; spot onto DMPK DBS cards & dry
- Make up internal standard solution: L-citrulline-2,3,3,4,4,5,5 d_7 (50 µmol/L) for loop injection via DBS autosampler
- Analyze calibrators in triplicate; specimens with known citrulline concentration

Neutral loss (63 Da) scan for dibasic amino acids



Citrulline Standard Curve



Citrulline Results

Sample ID	Citrulline µmol/L	Mean	Citrulline by AAA
Subject A, #1	30.7	30.1	
Subject A, #2	29.4		
Subject B1 #1	33.0	34.2	
Subject B1 #2	35.4		
Subject B2 #1	30.6	30.8	
Subject B2 #2	31.0		
Control 1 #1	32.9	30.9	30.0
Control 1 #2	28.0		
Control1 #3	31.7		
Subject C #1	18.3	18.9	16.7
Subject C #2	19.5		

Summary

- Device is robust, requires little maintenance
- Time and cost-saving features are attractive
- Contamination of MS system needs to be managed (e.g. by using flow diverter valve during system back-flush & wash cycles)
- Overall, results of QC testing are good, with exception of poor recovery of dicarboxylic ACPs – may be improved by using higher flow-rate
- Will require PKU cards designed to fit system
- May be valuable for second-tier tests that use MS/MS

Acknowledgements

- Peter Smith, Leap Technologies for loan of PAL DBS autosampler & technical support
- Jared James, Waters Inc. (technical support)
- Victor DeJesus, CDC, Atlanta (QC samples, internal standard kit)