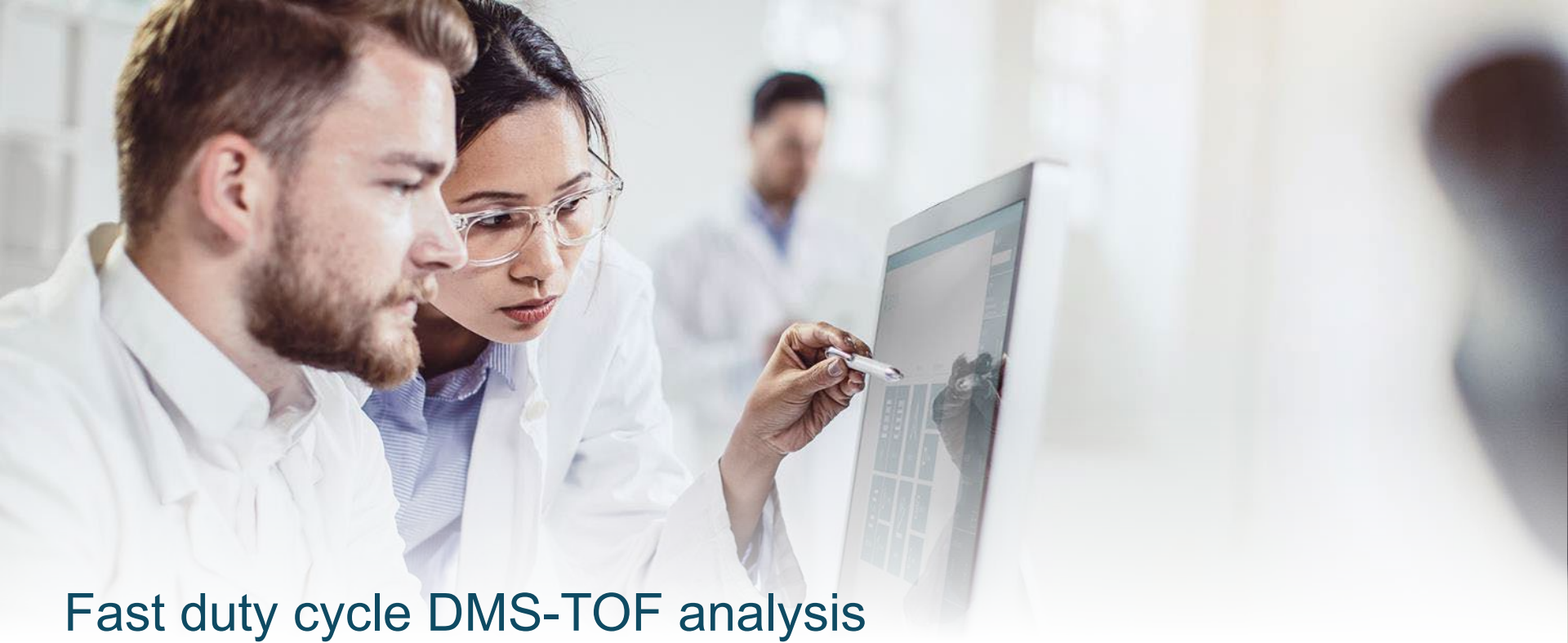


Fast duty cycle DMS-TOF analysis to increase selectivity during accurate mass LC analysis

J.C. Yves Le Blanc, Igor Chernushevich and Nic Bloomfield, SCIEX Canada

70th ASMS Conference, Minneapolis, 2022

SCIEX
The Power of Precision

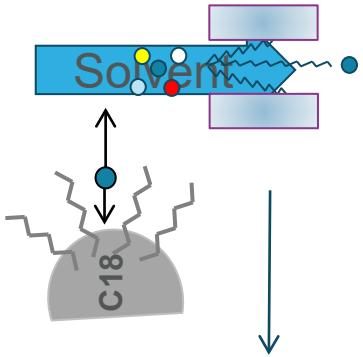
Differential Mobility Separation (DMS) as the 4th Dimension

Time separation

Chemical interactions

1 4

LCDMS

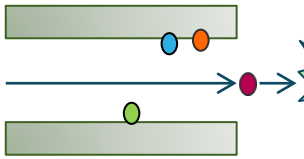


Mass separation

Chemical formula

2

MS



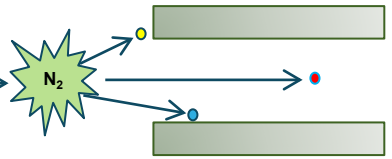
$C_xH_yO_mN_n$

Fragment separation

Chemical structure

3

MS



$C_{x-2}H_{y-3}O_{m-1}N_n$

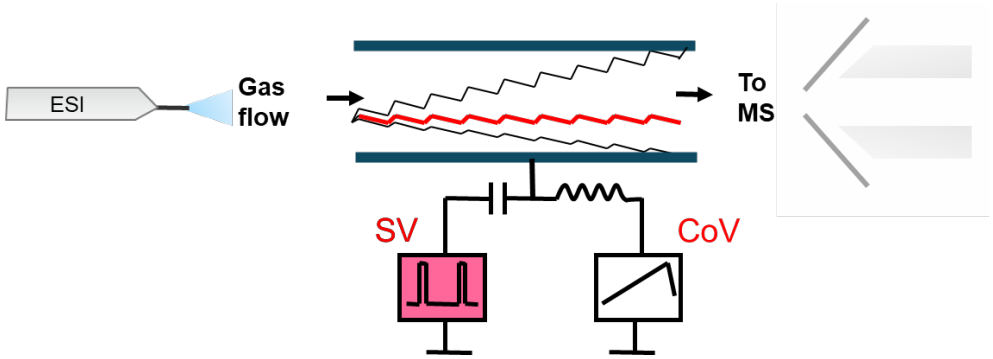
Differential mobility separation

Based on chemical interactions

Liquid/Gas phase interface

Gas phase / vacuum

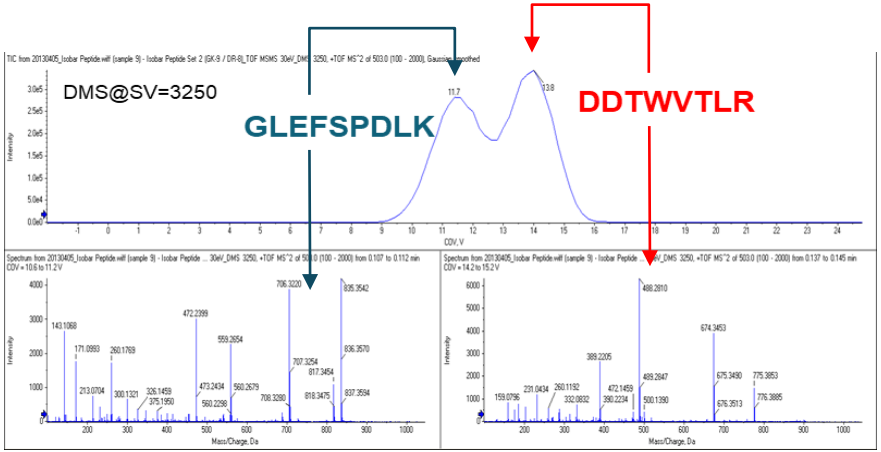
DMS Coupling to Mass Spectrometer

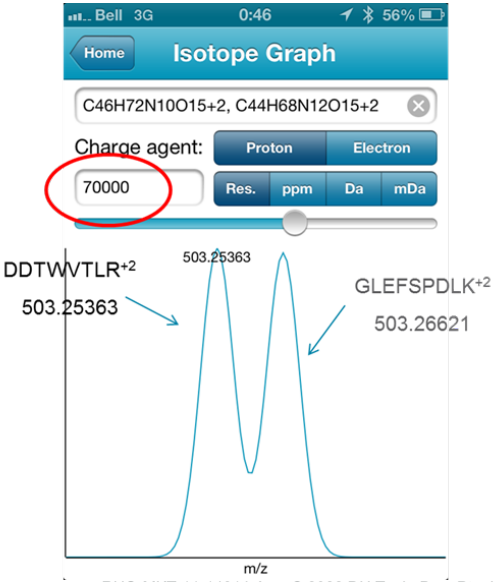


Separation due to difference between high and low field mobility

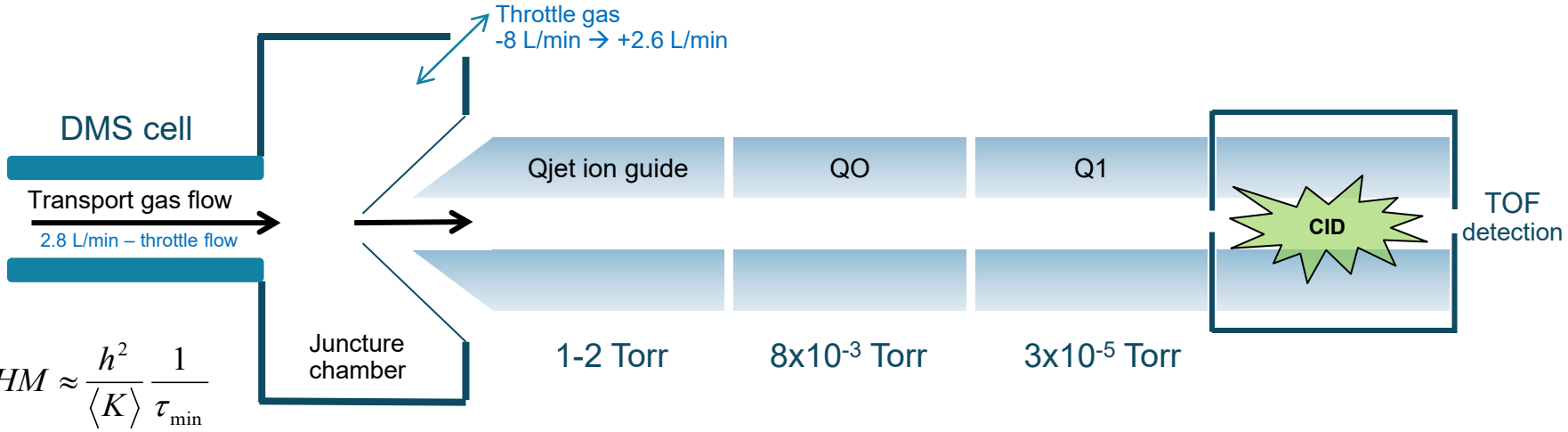
$$\alpha\left(\frac{E}{N}\right) = \frac{K\left(\frac{E}{N}\right) - K(0)}{K(0)}$$

SCIEX TripleTOF 6600 system, equipped with the SelexION™ technology





DMS coupling to a mass spectrometer with adjustable residence time



$$FWHM \approx \frac{h^2}{\langle K \rangle} \frac{1}{\tau_{\min}}$$

DMS residence time varies with throttle gas

Setting (psi)	Res.Time (ms)
Off (0)	7
Low (5)	15
Med (10)	20
High (25)	30

With an MS system, these values can increase, particularly in the high-pressure region ahead of Q1, and length of the region can further increase transit time to the detector region

This induces a further increase in pause/settling time
→ Increases cycle time
→ Leads to compromised data collection

Reserpine response versus Settling Time

Toggle between 2 SV voltage

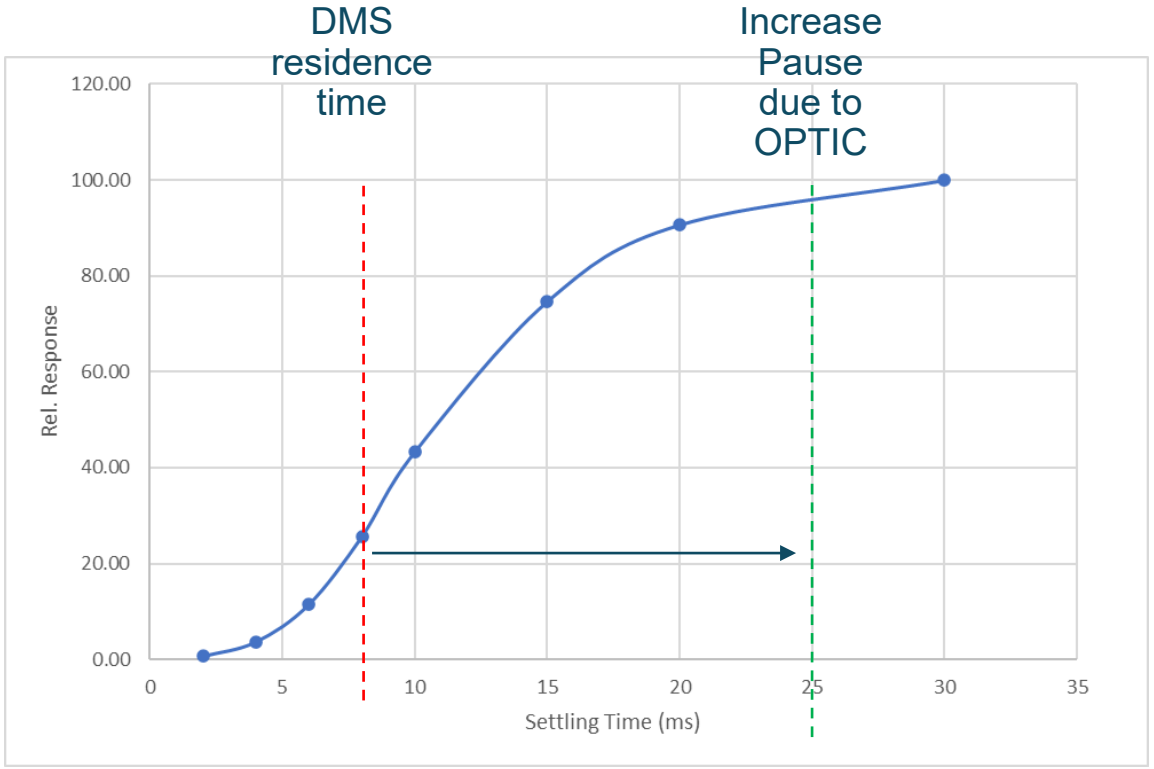
No resolving gas used

At expected transit time of DMS, ~25% of signal is observed.

Additional settling time is required to get maximum signal due to an additional delay in transmission under “depleted” ion beam conditions (DMS filtered conditions)

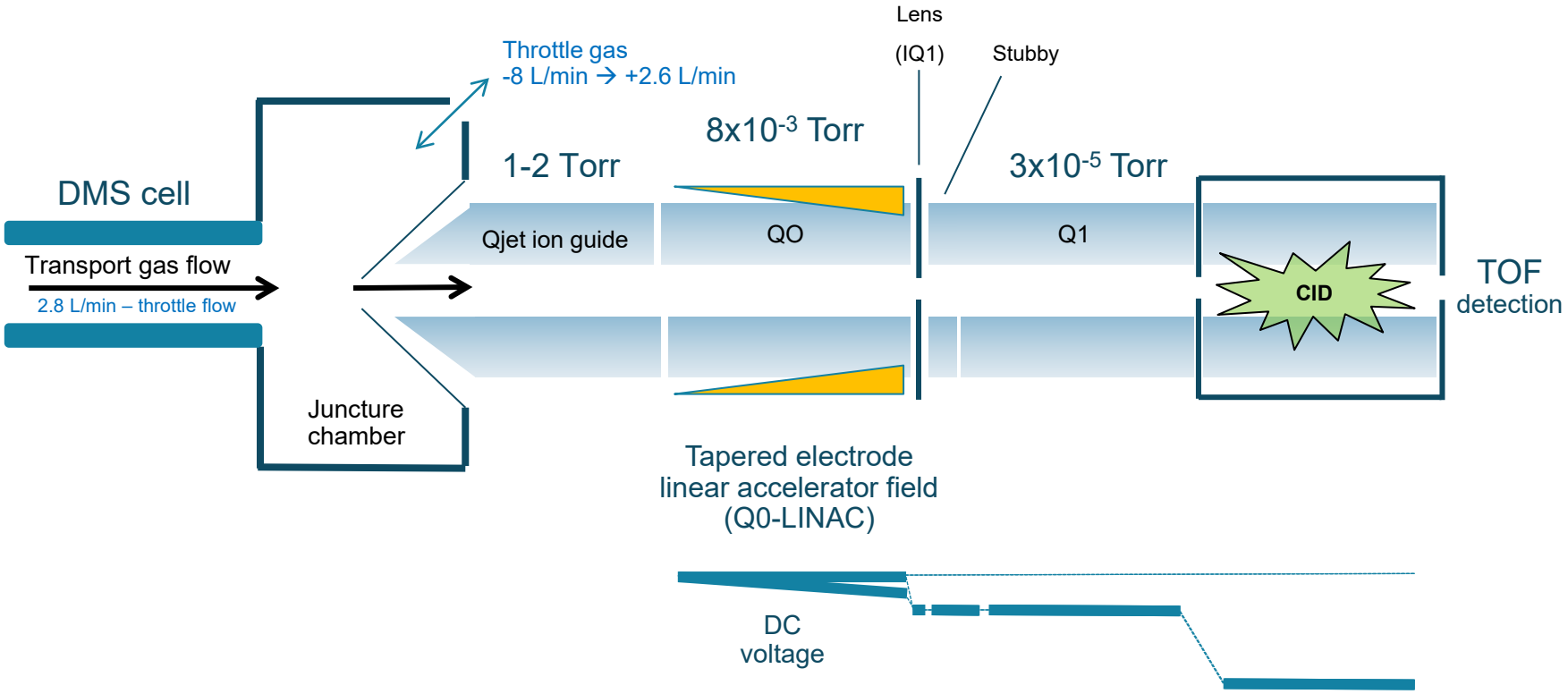
→ As a result, additional pause time is required

→ Mainly due to Q0 region



MSMS with 10ms Acc.Time

Reducing residence time in Q0 region

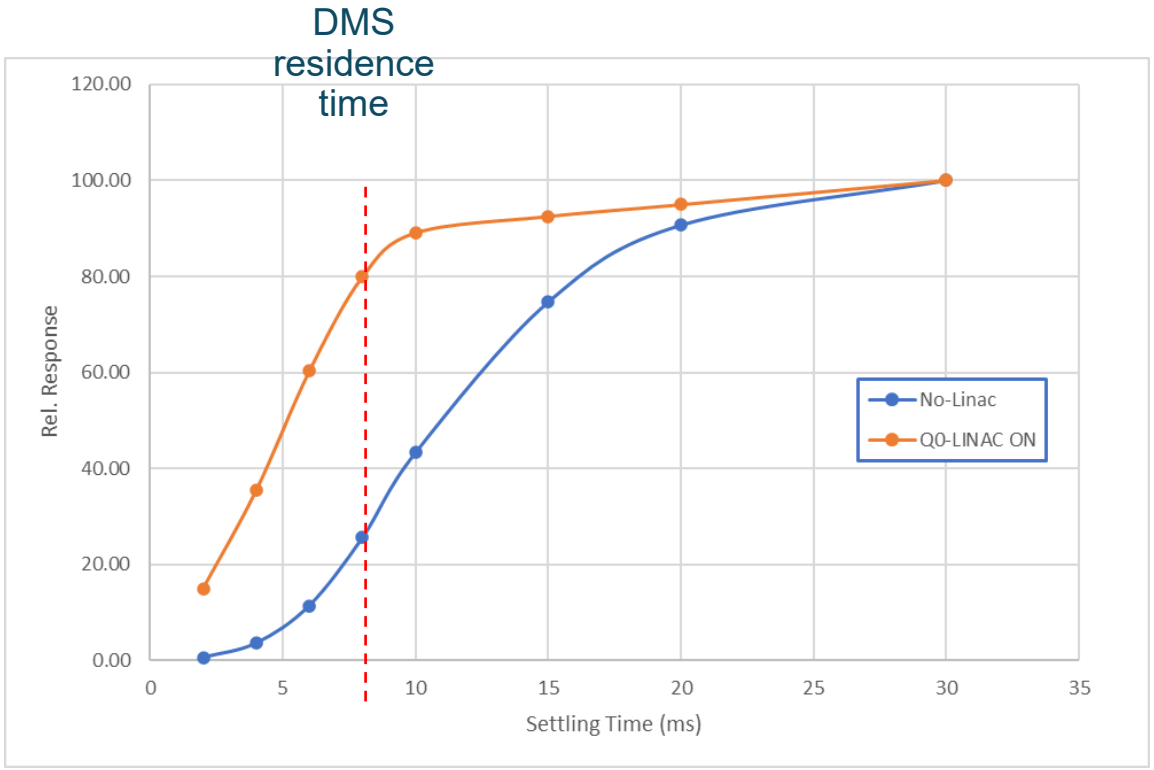


Reserpine response versus settling time

Toggle between 2 SV voltage

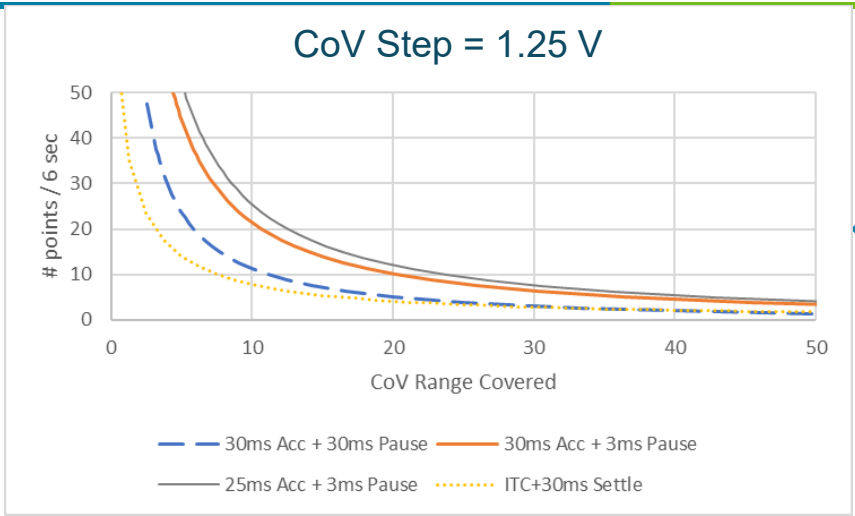
No resolving gas

At expected transit time of DMS, 80% of signal is observed



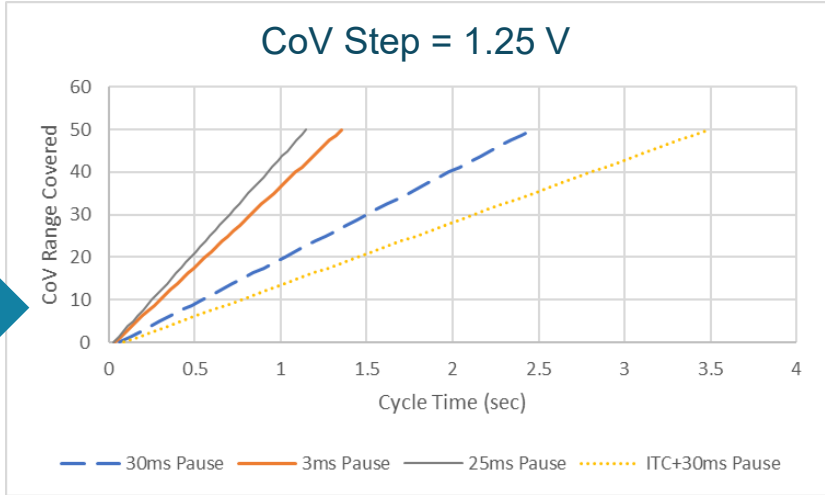
MSMS with 10ms accumulation time

Using Q0-LINAC improves cycle time

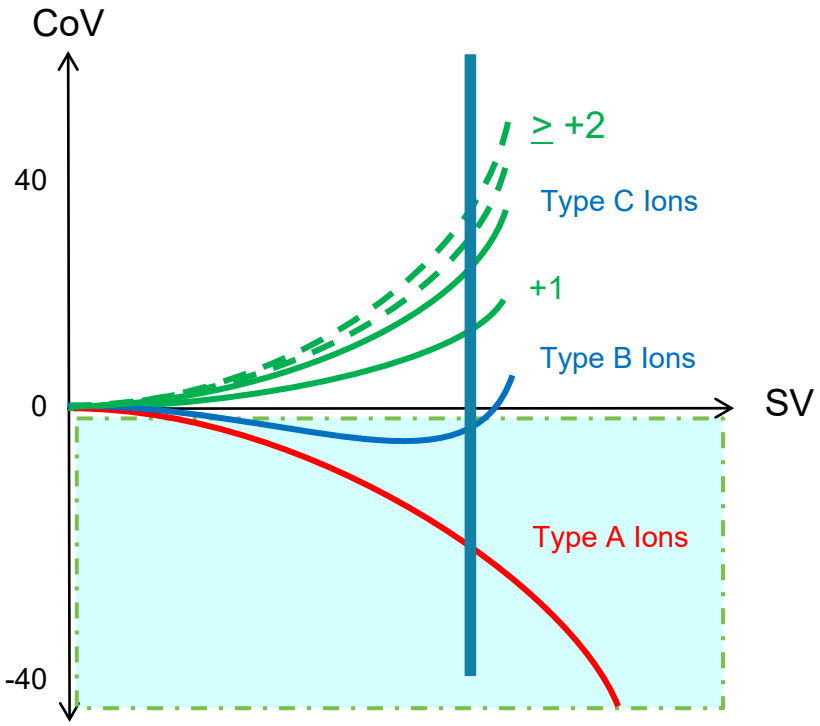


Increases the number data points for LC

Increases the number of **experiments**/unit time



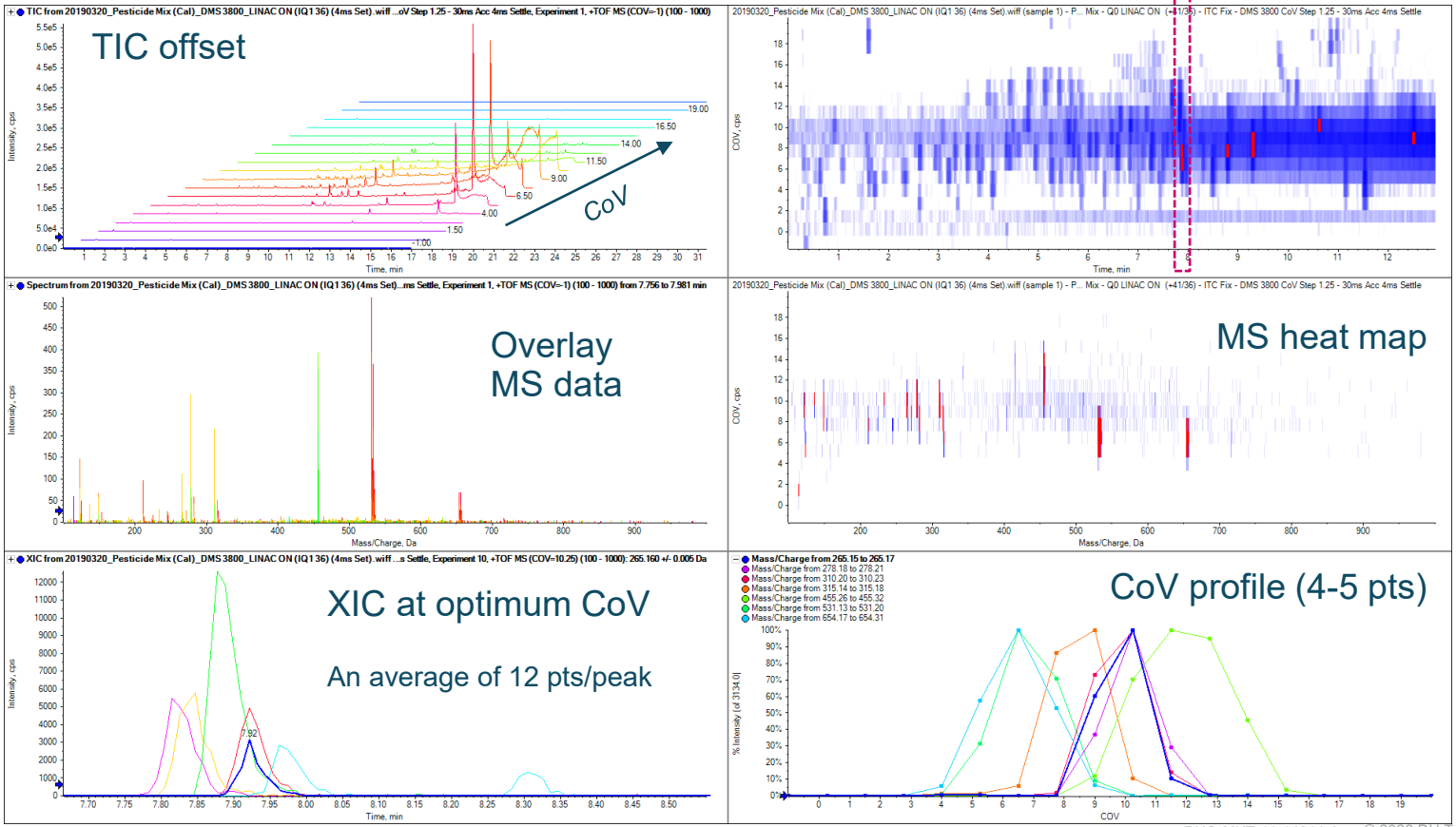
DMS operation range



Minimizing cycle time is critical because:

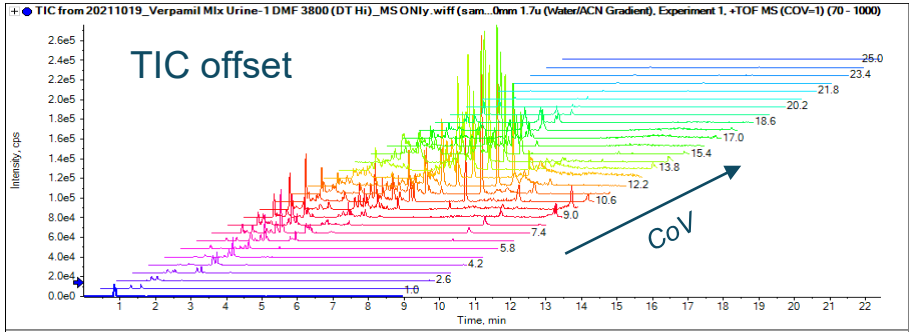
- The range of compensation voltage (CoV) to cover can be quite large for an “untargeted application”
- Want to maximize the number of CoV data points collected
- Need to balance the number of data points with the transient nature of signal with chromatographic separation (LC peak)

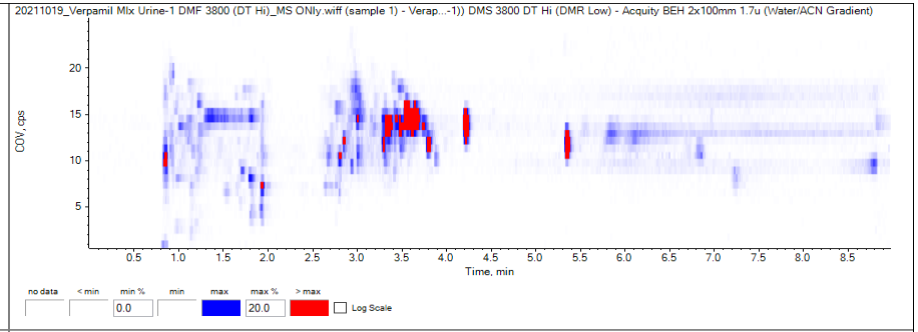
DMS @ SV 3800 (N_2) with CoV ranging from -1 to +20 V

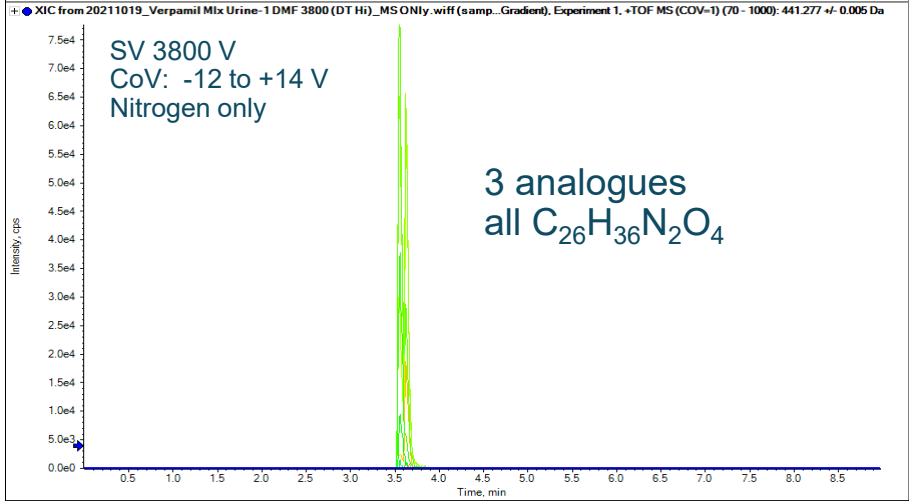


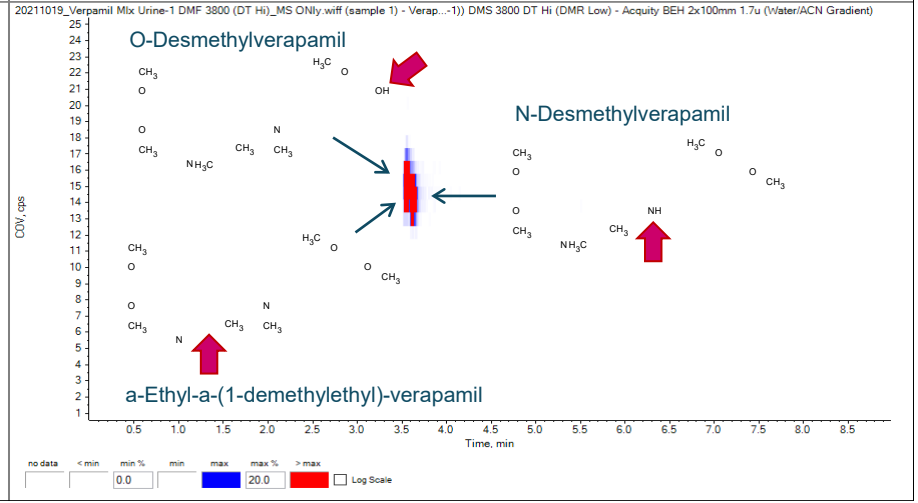
30ms accumulation time

Separating verapamil analogues

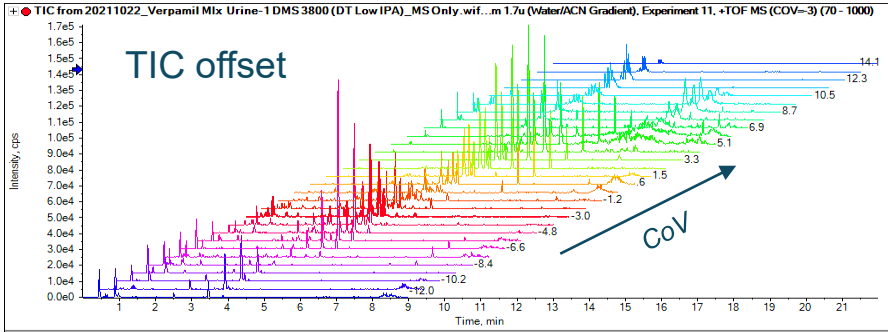


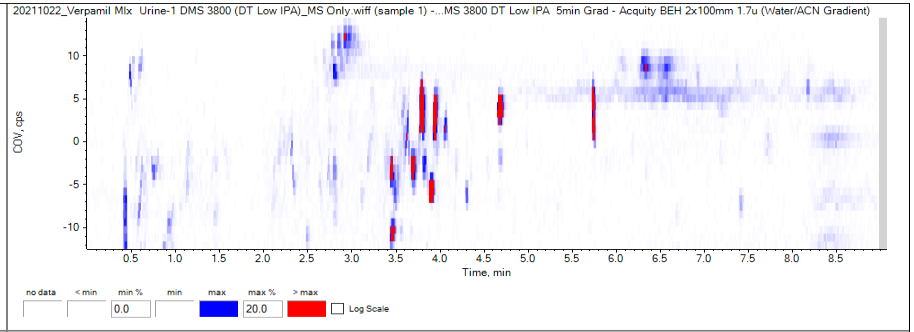


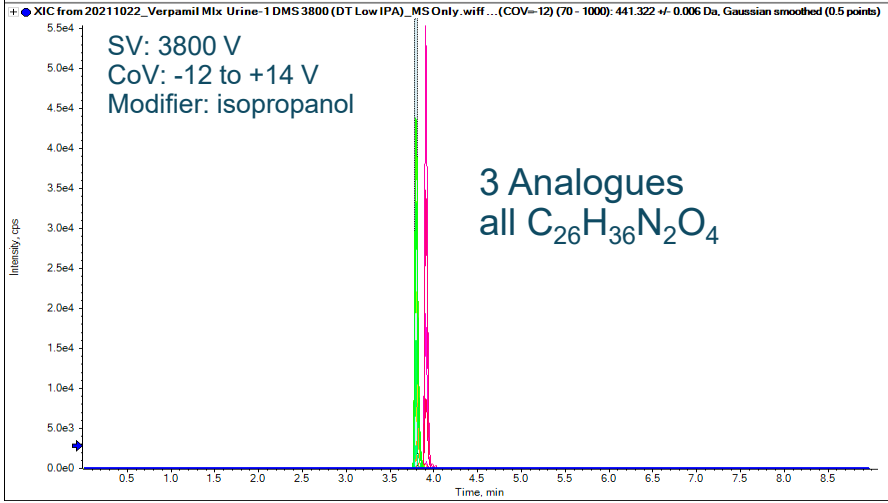


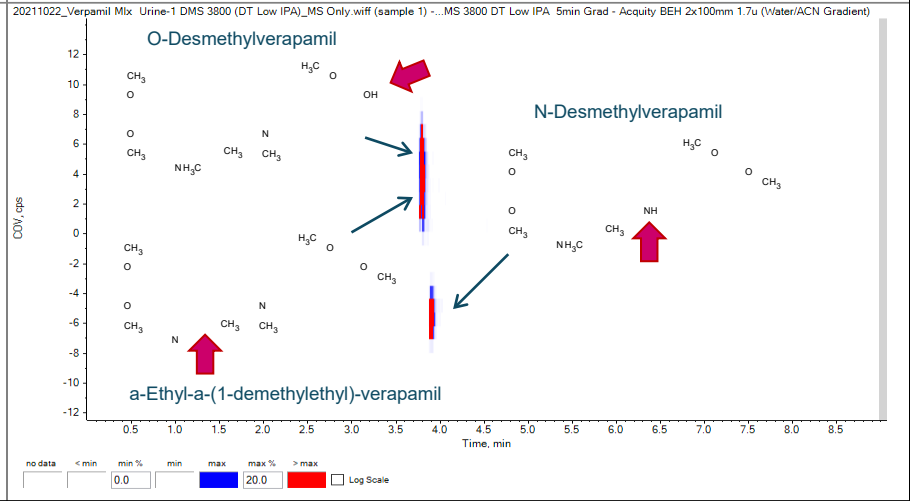


Separating analogues with gas phase modifier





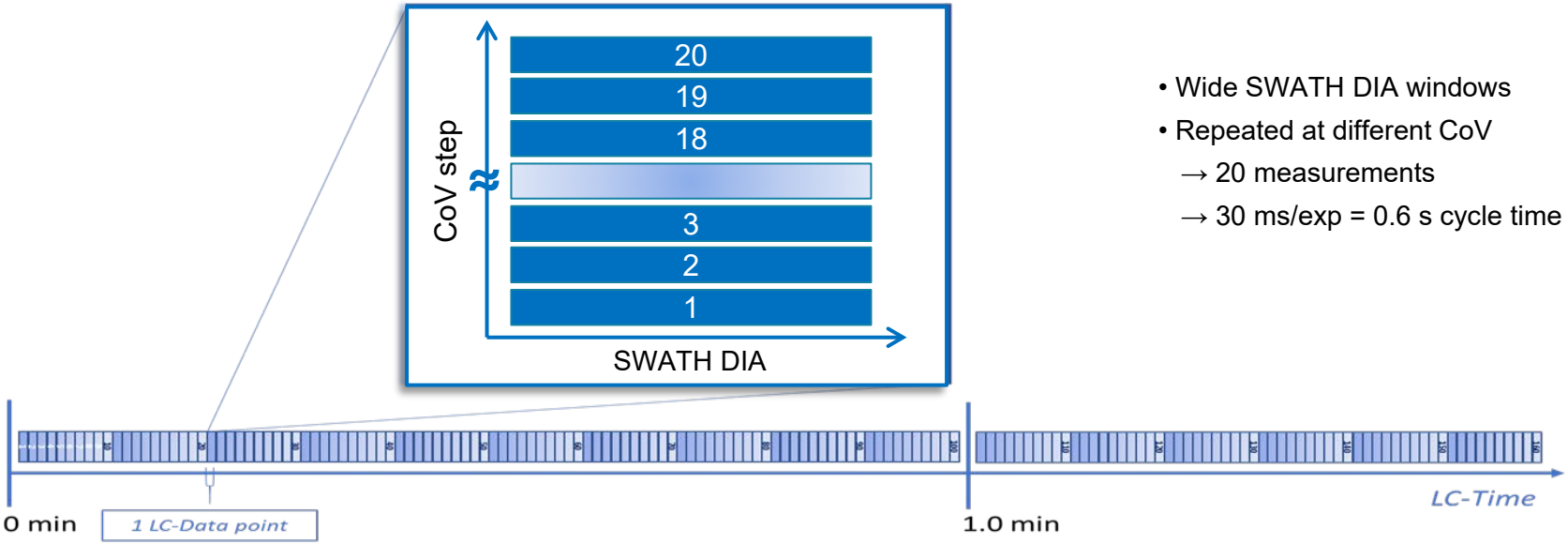




RUO-MKT-11-14814-A

Or use with DMS SWATH DIA

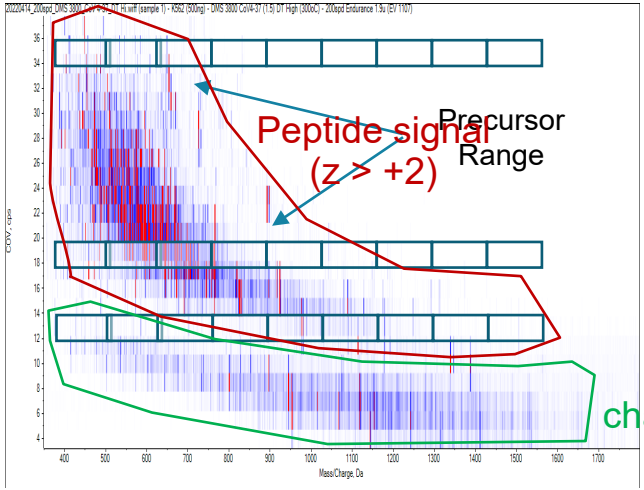
Relies on larger SWATH DIA precursor ion windows, operated at different SV-CoV combination

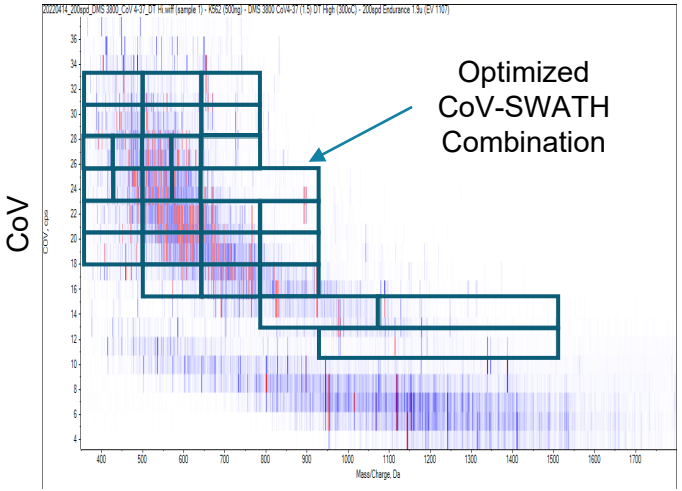


- Wide SWATH DIA windows
- Repeated at different CoV
  → 20 measurements
  → 30 ms/exp = 0.6 s cycle time

Presented ASMS 2018, San Diego; B.Seale et al, "Characterization of Biologic Compounds with Differential Mobility and SWATH" WP-454

For peptide analysis from K562 cell digest





Run as a multi-experiment to map the SV-CoV space with the goal to perform in single experiment (or a limited number of experiments)

K562 Digest with the Evosep 100 spd (11.5 min gradient)

1.5 V step

Peptide Level		# of Common IDs versus DMS conditions															
Cond.	Total # Pept.	DMS OFF	CoV 12	CoV 13.5	CoV 15	CoV 16.5	CoV 18	CoV 19.5	CoV 21	CoV 22.5	CoV 24	CoV 25.5	CoV 27	CoV 28.5	CoV 30	CoV 31.5	CoV 33
DMS OFF	5537	5537	164	370	890	1642	1800	1738	1585	1289	949	712	521	361	205	88	44
12	352		352	291	151	148	6	0	1	0	0	0	0	0	0	0	1
13.5	895			895	545	551	31	4	4	2	1	1	1	0	0	0	1
15	1880				1880	1857	359	27	11	8	3	2	5	1	1	1	1
16.5	3622					3622	1514	380	40	16	5	4	7	3	2	1	1
18	3245						3245	1477	350	48	15	11	6	7	2	1	2
19.5	3313							3313	1417	304	38	22	13	12	4	0	1
21	3147								3147	1199	234	42	21	19	8	3	2
22.5	2669									2669	956	202	41	24	7	3	1
24	2015										2015	771	171	37	5	4	1
25.5	1744											1744	699	153	21	7	2
27	1687												1687	582	98	8	4
28.5	1383													1383	458	59	4
30	918														918	222	31
31.5	407															407	99
33	237																237
Unique vs DMS OFF		na	188	525	990	1980	1445	1575	1562	1380	1066	1032	1166	1022	713	319	193
Unive as % of DMS OFF			3.40	9.48	17.88	35.76	26.10	28.45	28.21	24.92	19.25	18.64	21.06	18.46	12.88	5.76	3.49
Common as % of DMS OFF			2.96	6.68	16.07	29.66	32.51	31.39	28.63	23.28	17.14	12.86	9.41	6.52	3.70	1.59	0.79

- 18 separate experiments, including one with DMS off in MS only, performed with triplicate injection
- Processed using DIA-NN
- Pair-wise comparison shows a narrow range of CoV where similar peptides are found
- Wide separation at peptide level
- Minimal overlap between adjacent CoV steps

K562 Digest with the Evosep 100 spd (11.5 min gradient)

Protein Group	# of Common IDs versus DMS conditions															
Cond.	Total # Prot.	DMS OFF	CoV 12	CoV 13.5	CoV 15	CoV 16.5	CoV 18	CoV 19.5	CoV 21	CoV 22.5	CoV 24	CoV 25.5	CoV 27	CoV 28.5	CoV 30	CoV 31.5	CoV 33
DMS OFF	2626	2626	244	492	933	1405	1430	1435	1371	1193	995	892	797	685	482	246	139
12	321		321	274	214	231	177	190	190	174	157	142	141	135	93	61	39
13.5	754			754	547	580	371	375	371	353	310	284	274	280	194	106	60
15	1425				1425	1410	713	634	617	566	484	456	446	428	310	167	89
16.5	2385					2385	1365	1002	882	798	676	632	619	578	409	218	117
18	2137						2137	1327	905	758	633	598	569	540	364	187	105
19.5	2181							2181	1282	806	637	598	585	523	373	188	112
21	2068								2068	1123	691	582	547	490	350	186	108
22.5	1787									1787	897	577	502	450	300	172	100
24	1429										1429	766	503	414	277	149	80
25.5	1281											1281	708	423	258	142	75
27	1260												1260	605	288	141	81
28.5	1094													1094	472	152	80
30	769														769	235	81
31.5	371															371	112
33	222																222
Unique vs DMS OFF		na	77	262	492	980	707	746	697	594	434	389	463	409	287	125	83
Unive as % of DMS OFF			2.93	9.98	18.74	37.32	26.92	28.41	26.54	22.62	16.53	14.81	17.63	15.58	10.93	4.76	3.16
Common as % of DMS OFF			9.29	18.74	35.53	53.50	54.46	54.65	52.21	45.43	37.89	33.97	30.35	26.09	18.35	9.37	5.29

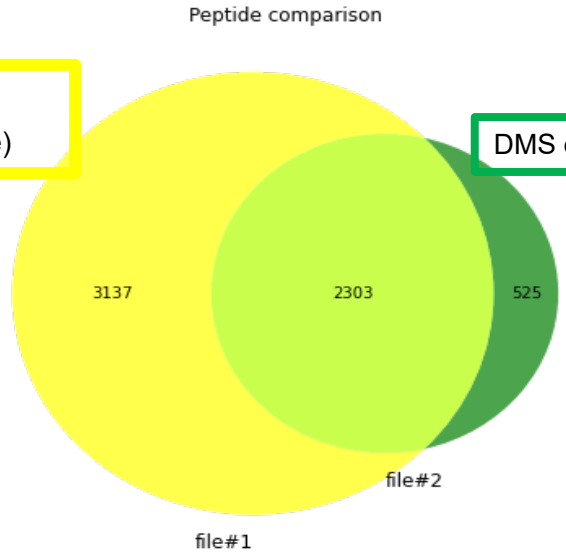
- 18 separate experiments, including one with DMS off in MS only, performed with triplicate injection
- Processed using DIA-NN
- At the protein level, each CoV contribute a significant number of unique proteins, so a wide CoV range is required
- But if we look at the precursor distribution ….

Significant increase in unique peptides/proteins identified with DMS

100 samples per day (spd) method

DMS 3800 – Cov from 12-34.5 V

→ Extra 3,137 unique peptide (>2x increase)



Version: 1.0.0

Venn Diagram Generator

Please Note this is used and your own risk

This works on the full result from DIANN

Please select the first full *.tsv result -- file #1

then select the comparitors file -- file#2

There is no update / progress of loading and for very large this may take some time.

Compounds File#1:

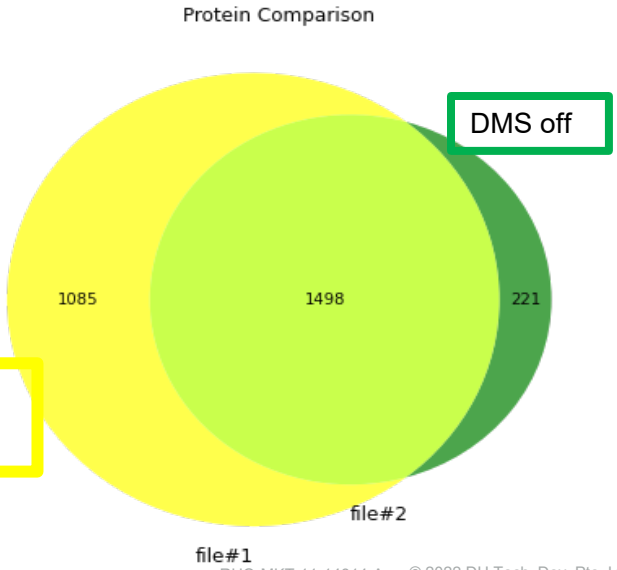
20220407_100spd_DMS OFF_01-03.tsv

Compounds File#2:

20220407_100spd_DMS 3800_A-03-05-07-09-11

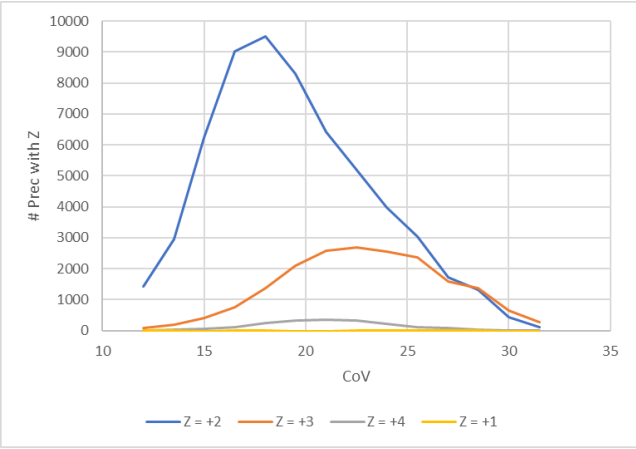
DMS 3800 – Cov from 12-34.5 V

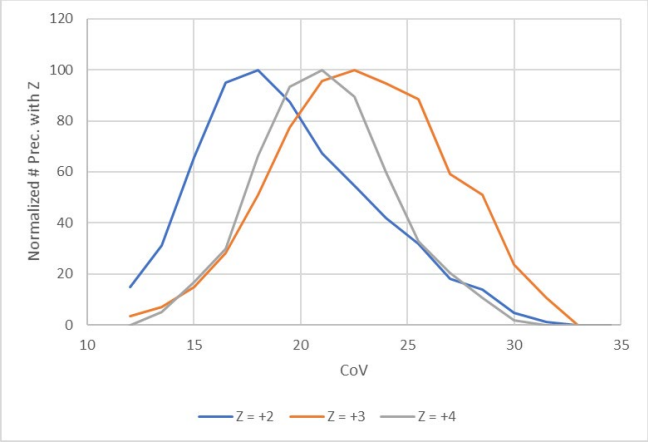
→ Extra 1,085 unique protein (>2x increase)



K562 Digest with the Evosep 100 spd (11.5 min gradient)

CoV	12	13.5	15	16.5	18	19.5	21	22.5	24	25.5	27	28.5	30	31.5
m/z bin	CoV 12	CoV 13.5	CoV 15	CoV 16.5	CoV 18	CoV 19.5	CoV 21	CoV 22.5	CoV 24	CoV 25.5	CoV 27	CoV 28.5	CoV 30	CoV 31.5
<400	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400-450	0.0	0.0	2.2	14.1	32.6	46.7	37.0	53.3	97.8	100.0	71.9	51.1	7.4	0.0
450-500	0.0	0.0	2.1	6.2	12.7	30.4	58.6	91.7	100.0	92.5	58.1	54.9	21.4	5.3
500-550	0.0	0.2	1.9	8.4	23.0	52.0	75.5	98.7	100.0	88.4	54.8	42.4	14.4	4.2
550-600	0.7	1.8	7.5	19.3	46.5	77.5	91.6	100.0	81.0	68.2	40.6	30.5	11.2	3.0
600-650	1.3	3.8	15.3	38.1	67.1	91.8	100.0	74.0	52.7	38.7	22.0	16.3	6.6	2.8
650-700	2.1	7.0	25.7	57.7	92.9	100.0	73.6	43.9	27.7	18.3	11.9	10.4	4.2	2.3
700-750	6.8	14.8	46.1	81.4	100.0	87.4	45.6	26.6	16.2	13.5	9.8	9.3	5.3	2.9
750-800	12.3	24.6	66.0	100.0	90.7	48.0	23.2	13.2	9.1	8.1	7.1	8.1	4.7	1.9
800-850	23.7	46.5	87.1	100.0	70.5	27.7	14.4	13.8	13.1	10.8	4.7	1.5	0.6	0.3
850-900	38.9	77.5	100.0	86.9	36.6	15.7	12.4	12.7	10.3	5.7	1.0	0.3	0.0	0.0





- Eliminating regions with less than 20% of precursor are detected – yields 70 windows
- With 20 ms of accumulation time, a total cycle time of 1.47 s
- With 3 groups of 23 CoV SWATH DIA, the cycle time is 0.56 s
- Or use Variable SWATH windows in conjunction with DMS to further reduce the number of groups

K562 Cell Digest with Evosep 200spd (5 min. gradient)

Venn Diagram Generator

Please Note this is used and your own risk

This works on the full result from DIANN

Please select the first full *.tsv result -- file #1

then select the comparitors file -- file#2

There is no update / progress of loading and for very large this may take some time.

Compounds File#1:

20220503_K562 200spd_DMS OFF (400-900 only

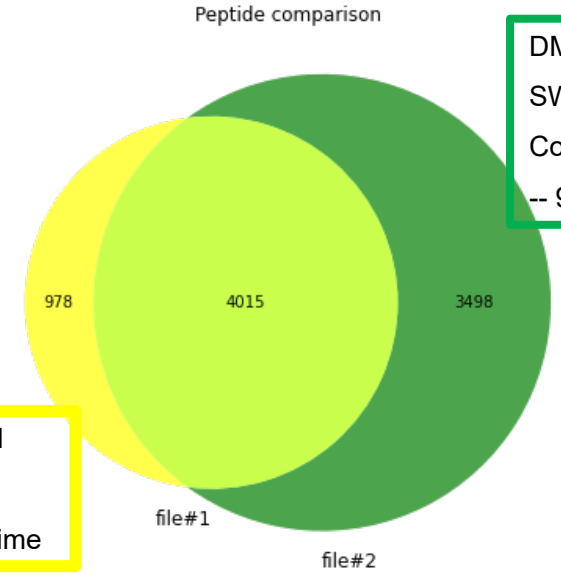
Compounds File#2:

20220503_K562 200spd All CoV Split (TB4)_01-03

DMS OFF – 3 replicated

5 min Gradient

< 20 min total analysis time

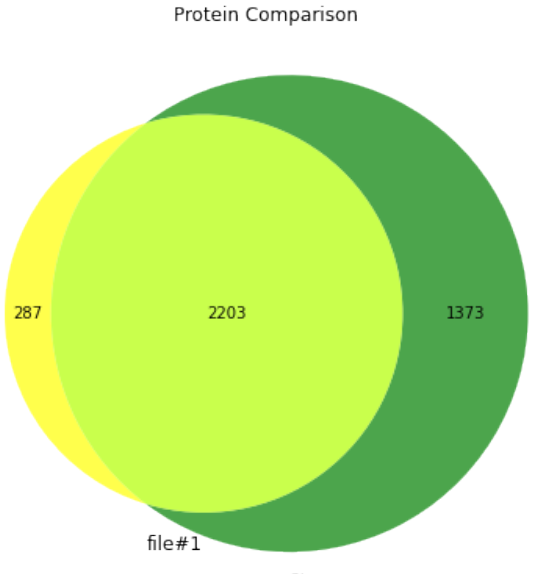


DMS @ SV 3800 – CoV range from 12-30

SWATH fixed to 50amu

CoV range divided in 3 groups – each analyzed in triplicate

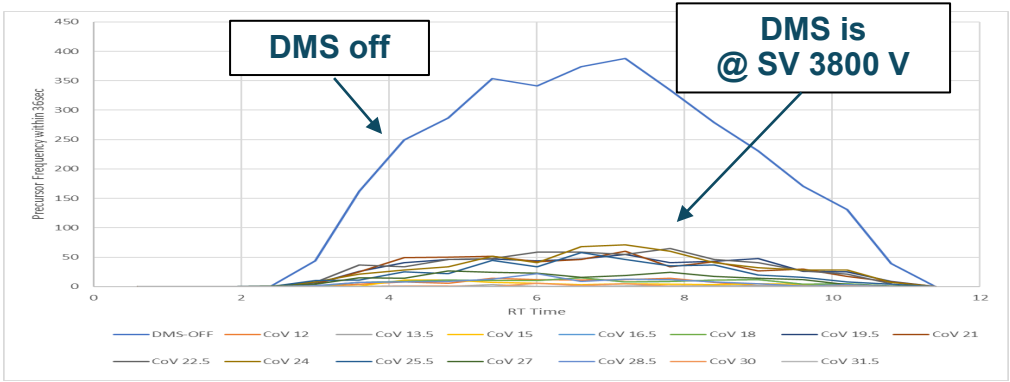
-- 9 injections x 5min < 50 min total analysis time



Using DMS allowed to extract from the data
>70% more unique peptide
>50%more unique proteins

Supplementing LC Peak Capacity with DMS

RT-Bin	DMS OFF	CoV 12	CoV 13.5	CoV 15	CoV 16.5	CoV 18	CoV 19.5	CoV 21	CoV 22.5	CoV 24	CoV 25.5	CoV 27	CoV 28.5	CoV 30	CoV 31.5
Bin	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency	Frequency
0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.4	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
3	44	0	0	0	1	3	5	4	7	8	10	4	2	0	0
3.6	162	4	0	1	7	8	26	25	37	21	11	15	7	0	0
4.2	249	8	1	11	9	19	41	49	34	28	25	14	8	1	0
4.8	287	6	0	11	11	24	46	50	46	34	22	27	9	1	0
5.4	354	14	3	7	10	39	47	52	48	52	45	24	13	0	0
6	341	12	0	6	10	42	44	42	59	41	34	23	22	6	0
6.6	374	11	0	3	15	40	47	46	59	68	58	16	9	2	0
7.2	388	12	1	5	8	38	55	60	55	71	46	19	13	5	0
7.8	334	14	1	4	9	34	41	34	65	60	35	24	12	1	0
8.4	279	7	1	3	11	23	43	42	46	41	37	17	8	0	0
9	231	2	2	5	12	23	48	27	41	32	20	14	5	0	0
9.6	171	2	0	4	4	21	26	30	27	28	16	12	1	0	0
10.2	131	1	0	1	5	18	25	17	21	28	8	3	3	1	0
10.8	39	0	0	1	1	3	9	8	5	7	4	1	0	0	0
11.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



RT-Min	AVG	Min	Max	Avg Cap.Incr.	Min.Cap.Incr.
3	4.89	1	10	9.00	4.40
3.6	13.50	1	37	12.00	4.38
4.2	20.58	1	49	12.10	5.08
4.8	23.83	1	50	12.04	5.74
5.4	29.50	3	52	12.00	6.81
6	27.92	6	59	12.21	5.78
6.6	31.00	2	68	12.06	5.50
7.2	31.92	1	71	12.16	5.46
7.8	27.75	1	65	12.04	5.14
8.4	23.25	1	46	12.00	6.07
9	19.25	2	48	12.00	4.81
9.6	14.25	1	30	12.00	5.70
10.2	10.83	1	28	12.09	4.68
10.8	3.25	1	9	12.00	4.33

- Distribution of precursor co-elution within 36 sec window for the SWATH window of 550-600
- When compared to DMS in transmission mode, the number of co-eluting precursors is reduced by a factor of 12 over the entire CoV range, and by a factor of 5 where maximum signal is detected.

Faster Duty Cycle with Q0-LINAC

- Using Q0-LINAC enables significant reduction in settling time when using DMS on QTOF configuration
- This enables a significantly wider range of compensation voltage (CoV) on an LC-time scale
- Opens the potential for additional orthogonality with the use of modifier on transport gas
- DMS could potentially allow the use of wider SWATH without sacrificing selectivity under various LC conditions

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