Extending Linear Dynamic Range on a TripleTOF ® 6600

Feng Zhong[1]; Doug Simmons[1]; J. C. Yves Leblanc[1]; Nic Bloomfield[1]
[1]AB SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada

ABSTRACT

On the current TripleTOF® 5600 platform, a combination of a TDC acquisition and signal processing are employed to provide 3 to 4 order intra-scan linear dynamic range. The signal processing considers the number of TOF pulses for which any given ion is not present and applies a Poisson statistical correction for each channel and transmission window. Using this method, in combination with 4 parallel channels, the system maintains linearity to an average of 4 ions per TOF pulse per channel which is comparable to a 1 channel 8 bit ADC detection system. Furthermore, this statistical technique assumes that the ion flux is reasonably constant during the scan. However, expanding inter-scan LDR beyond this value is difficult and requires a new detection technology.

INTRODUCTION

To increase the LDR, a 10 bit 5 Giga-sample ADC with an on board TDC was interfaced in the TripleTOF® 6600. The four channel configuration was maintained by splitting the 5 G/S into 4 X 1.25 G/S channels. In order to accommodate the much higher ion currents which could be recorded, the detector was modified to include a first stage of amplification and conversion to differential signaling. Outside of vacuum further amplification was introduced before processing with an ADC. In addition, dynamic attenuation of the ion beam, which considers both total ion current on the detector and individual ion signals, was adapted with higher limits (~7x) to match the capabilities of the new digitizer.

MATERIALS AND METHODS

Sample Preparation:
100 µg/mL stock solutions of alprazolam and atorvastatin were prepared in methanol individually. Alprazolm_D5 and atrovastatin_D5 were spiked in the diluent, 50/50 water/acetonitrile, at 10 ng/mL, respectively. 17 calibration standards were prepared by making 3-fold serial dilutions of the stock solutions for both alprazolam and atorvastatin.

LC Conditions:
A Shimadzu Prominence LC system (one system controller CBM-20A, two Isocratic pumps LC-20ADXR, one autosampler SIL-20ACXR and a column oven CTO-20AC) combined with a Phenomentex Kinetex, C18 2.6 µm 100A (50 x 2 mm) (PN 00B-4462-AN) was used to achieve 5-6 second LC peak using a blast gradient profile.

Mobile Phase A : 98/2 H20/Acetonitrile + 0.1% formic acid + 2mM ammonium acetate
Mobile Phase B : 2/98 H20/Acetonitrile + 0.1% formic acid + 2mM ammonium acetate

Gradient Profile:

Total Time(min)	Flow Rate (ul/min)	A (%)	B (%)
0.1	500	90	10
2	500	2	98
2.5	500	2	98
2.55	500	90	10
3.55	500		stop

MS and MS/MS Conditions:
Three different instruments with Standard interface DuoSpray™ Ion Source CDS (Calibrant Delivery System, APCI positive Calibrant and APCI negative Calibrant) with Analyst® TF 1.7 were used to run TOF MS and looped MS/MS scans with the following conditions to study alprazolam linearity:

MS Experiment	Start Mass	Stop Mass	Accumulation Time (ms)	CE positive	DP positive
TOF MS	150	650	200	10	80

MS Experiment	Start Mass	Stop Mass	Accumulation Time (ms)	CE positive	Product of	CES	DP positive
TOF MS2	100	650	100	45	309.1	0	80
TOF MS2	100	650	100	45	314.1	0	80

RESULTS

In the MRM[HR] looped full scan MS/MS are acquired across the LC peak at high resolution. Single or multiple high resolution XIC's are generated post acquisition yielding data similar to MRM's.

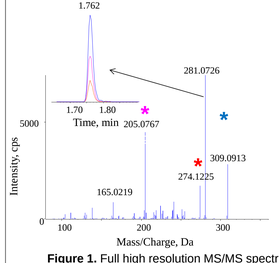

Figure 1. Full high resolution MS/MS spectrum

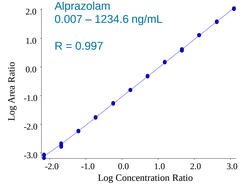

Figure 2. Alprazolam (309.09) fragment 281.07 concentration curve

Component N..	Actual Concentra..	Num. V..	Mean	Standard Devi..	Percent CV	Accuracy	Value #1	Value #2	Value #3
Alprazolam 2..	0.00697	3 of 3	7.197e-3	1.171e-3	16.27	103.26	5.847e-3	7.804e-3	7.940e-3
Alprazolam 2..	0.02091	3 of 3	1.889e-2	2.600e-3	13.77	90.33	1.854e-2	1.648e-2	2.164e-2
Alprazolam 2..	0.06272	3 of 3	6.190e-2	1.091e-3	1.76	98.70	6.269e-2	6.069e-2	6.222e-2
Alprazolam 2..	0.18817	3 of 3	1.909e-1	6.110e-3	3.20	101.48	1.839e-1	1.943e-1	1.945e-1
Alprazolam 2..	0.56450	3 of 3	5.818e-1	1.415e-2	2.43	103.07	5.981e-1	5.724e-1	5.749e-1
Alprazolam 2..	1.69351	3 of 3	1.718e0	1.140e-2	0.66	101.47	1.709e0	1.715e0	1.731e0
Alprazolam 2..	5.08053	3 of 3	5.237e0	1.687e-1	3.22	103.08	5.250e0	5.062e0	5.399e0
Alprazolam 2..	15.24158	3 of 3	1.600e1	4.637e-1	2.84	104.97	1.570e1	1.577e1	1.652e1
Alprazolam 2..	45.72474	3 of 3	4.375e1	1.913e0	4.37	95.67	4.156e1	4.513e1	4.456e1
Alprazolam 2..	137.17421	3 of 3	1.406e2	1.065e0	0.76	102.47	1.395e2	1.405e2	1.419e2
Alprazolam 2..	411.52263	3 of 3	4.125e2	4.320e0	1.05	100.24	4.156e2	4.143e2	4.076e2
Alprazolam 2..	1234.56790	3 of 3	1.176e3	1.985e1	1.69	95.26	1.173e3	1.197e3	1.158e3

Figure 3. Percent CV and Accuracies' across the dilution series.

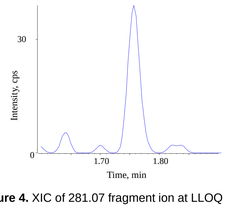

Figure 4. XIC of 281.07 fragment ion at LLOQ

Figure 5. Summary of MRM[HR] Alprazolam (309.09) quantitation statistics from 3 different instruments. Fragment 281.07 of was processed with MultiQuant™ 3.0

TOF MS2	Two MS2, 100 ms accumulation time per MS2, 200 ms cycle time							
Instrument	LLOQ (ng/mL)	Accuracy at LOQ	CV at LLOQ	Reps	ULOQ (ng/mL)	Accuracy at ULOQ	Orders LDR	R coefficient
Instrument 1								
Alprazolam 281 (with IS 286)	0.007	103.68	11.75	3	1235	98.01	5.2	0.99776
Instrument 2								
Alprazolam 281 (with IS 286)	0.007	103.26	16.27	3	1235	95.26	5.2	0.99734
Instrument 3								
Alprazolam 281 (with IS 286)	0.007	103.93	7.53	3	1235	85.13	5.2	0.99643

Figure 6. Summary of Atrovastatin (559.26) MRM[HR] quantitation statistics acquired on 3 different instruments. Fragment 440.22 was processed with MultiQuant™ 3.0

TOF MS2	Two MS2, 100 ms accumulation time per MS2, 200 ms cycle time							
Instrument	LLOQ (ng/mL)	Accuracy at LOQ	CV at LLOQ	Reps	ULOQ (ng/mL)	Accuracy at ULOQ	Orders LDR	R coefficient
Instrument 1								
Atorvastatin 440 (with IS 445)	0.002	100.66	16.45	3	1235	102.95	5.7	0.99782
Instrument 2								
Atorvastatin 440 (with IS 445)	0.007	96.26	19.57	3	3704	89.25	5.7	0.99701
Instrument 3								
Atorvastatin 440 (with IS 445)	0.021	101.67	12.1	3	3704	106.56	5.2	0.99663

As well as MRM[HR] both compounds were run in full scan high resolution mode and quantified solely on precursor intensity to evaluate the quantitative capability of the TripleTOF 6600®. The tests were also run in triplicate and again replicated on three different instruments. Except for the change to the single 100 ms TOF MS scan from looped product ion the experimental conditions were unchanged.

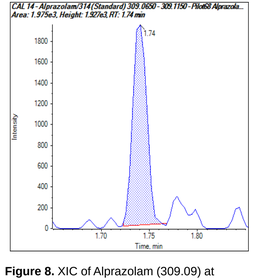

Figure 8. XIC of Alprazolam (309.09) at LLOQ

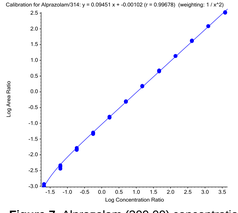

Figure 7. Alprazolam (309.09) concentration curve

Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
0.02091	3 of 3	2.20E-02	1.07E-03	4.88	105.23	2.12E-02	2.15E-02	2.32E-02
0.06272	3 of 3	5.49E-02	5.51E-03	10.03	87.54	6.05E-02	4.95E-02	5.48E-02
0.18817	3 of 3	1.74E-01	9.62E-03	5.54	92.38	1.64E-01	1.75E-01	1.83E-01
0.5645	3 of 3	5.29E-01	2.45E-02	4.64	93.66	5.01E-01	5.48E-01	5.37E-01
1.69351	3 of 3	1.67E+00	4.63E-02	2.78	98.32	1.68E+00	1.61E+00	1.70E+00
5.08053	3 of 3	5.23E+00	5.32E-02	1.02	103.03	5.29E+00	5.21E+00	5.19E+00
15.24158	3 of 3	1.62E+01	3.79E-01	2.35	106.02	1.61E+01	1.66E+01	1.58E+01
45.72474	3 of 3	4.84E+01	1.40E+00	2.89	105.79	4.85E+01	4.97E+01	4.69E+01
137.17421	3 of 3	1.45E+02	8.06E-01	0.56	105.53	1.45E+02	1.44E+02	1.46E+02
411.52263	3 of 3	4.43E+02	7.30E+00	1.65	107.64	4.48E+02	4.35E+02	4.46E+02
1234.5679	3 of 3	1.27E+03	7.90E+00	0.62	103.06	1.26E+03	1.27E+03	1.28E+03
3703.7037	3 of 3	3.40E+03	4.34E+01	1.28	91.81	3.38E+03	3.45E+03	3.37E+03

Figure 9. Percent CV and Accuracies' across the dilution series.

Figure 10. Summary of full scan Alprazolam (309.09) quantitation statistics from 3 different instruments processed with MultiQuant™ 3.0

TOFMS	TOF MS alone, 100 ms accumulation time							
Instrument	LLOQ (ng/mL)	Accuracy at LOQ	CV at LLOQ	Reps	ULOQ (ng/mL)	Accuracy at ULOQ	Orders LDR	R coefficient
Instrument 1								
Alprazolam 309/314 (with IS)	0.02091	105.84	4.23	3	3704	91.8	5.2	0.99622
Instrument 2								
Alprazolam 309/314 (with IS)	0.02091	100.05	5.7	3	3704	87.67	5.2	0.99838
Instrument 3								
Alprazolam 309/314 (with IS)	0.00697	97.44	14.73	3	3704	86.66	5.7	0.99726

Figure 11. Summary of full scan Atrovastatin (559.26) quantitation statistics from 3 different instruments processed with MultiQuant™ 3.0

TOFMS	TOF MS alone, 100 ms accumulation time							
Instrument	LLOQ (ng/mL)	Accuracy at LOQ	CV at LLOQ	Reps	ULOQ (ng/mL)	Accuracy at ULOQ	Orders LDR	R coefficient
Instrument 1								
Atorvastatin 564/559	0.00697	100.43	3.99	3	1235	90.98	5.2	0.99736
Instrument 2								
Atorvastatin 564/559	0.02091	100.45	10.19	3	3704	85.5	5.2	0.9972
Instrument 3								
Atorvastatin 564/559	0.02091	105.5	9.28	3	3704	98.68	5.2	0.99634

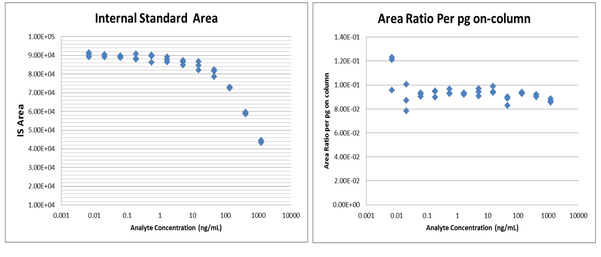

Figure 12. The role of the internal standard in compensating for source saturation in Alprazolam is clearly visible in the higher 4 concentration in the dilution series, shown in left. But Once ion source saturation is corrected by internal standard, the detector response, measured by area ratio per pg on-column, is linear within 5.2 orders of analyte concentrations, shown in right.

CONCLUSIONS

Greater than 5 orders of linear dynamic range is achievable with this system and has been demonstrated on multiple systems with multiple compounds. The combination of a 10 bit digitizer with TDC timing and 4 parallel data channels combined with careful signal handling is capable of maintaining linearity across wide quantitative range. This system configuration is highly suitable as a quantitation instrument while offering the additional benefit of high resolution and accurate mass over other traditional quantitation instruments.

TRADEMARKS/LICENSING