Assessment of ZenoTOF 7600 system robustness for quantitative proteomics workflows

ZenoTOF 7600 system evaluation using Zeno SWATH data-independent acquisition (DIA)

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This technical note demonstrates the performance and robustness of the ZenoTOF 7600 system for extended microflow proteomics LC-MS analysis. The system was run consecutively for 28 weeks without instrument cleaning or maintenance apart from routine tuning and calibration. Robustness was evaluated using quality control (QC) tests performed at regular intervals over a continuous 21-week period, in which replicate injections of 5 ng and 50 ng loadings of a human K562 tryptic digest standard were analyzed with Zeno SWATH DIA methods to assess quantitative proteomics performance. The evaluation period included an accelerated robustness test performed from weeks 24–28 consisting of >1,000 injections (on-column loadings between 0.5-1 ng per injection) and >1,020 µg of K562 digest injected into the system to simulate a high-throughput proteomics study. More than 1,200 µg of protein digest was analyzed over 28 weeks. The QC results show that the system performance remained stable over the 21-week testing period and the numbers of identified and quantified protein groups and precursors were consistent with coefficients of variation (CVs) <20%. These data indicate that the ZenoTOF 7600 system is well-suited for high-throughput studies and the analysis of large sample cohorts that are important for systems biology and biomarker discovery work.

Key features of the robustness assessment of the ZenoTOF 7600 system

- The ZenoTOF 7600 system ran continuously for 28 weeks, with >1,200 µg total protein digest injected into the system
- The numbers of protein groups and precursors identified and quantified were reproducible (CVs <20%) across >1,000 QC injections over 21 consecutive weeks
- Peptides monitored during the QC tests showed consistent peak areas over time and across a wide range of intensities, with peptide peak area CVs <20%

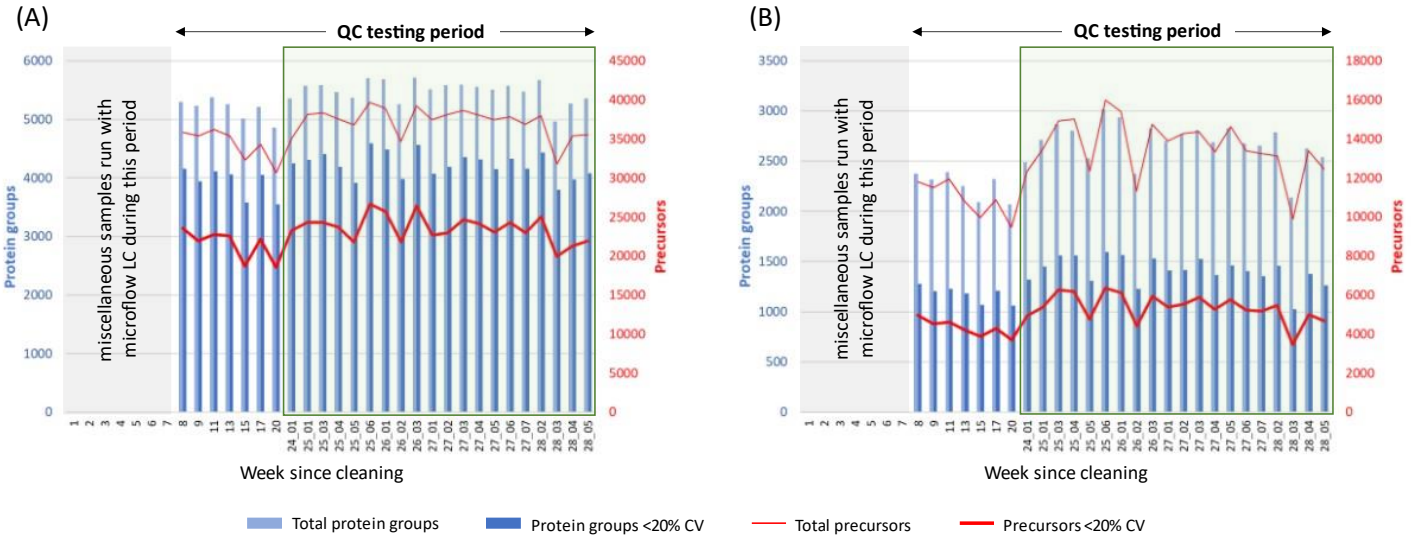

Figure 1. Numbers of protein groups and precursors identified and quantified (with CVs <20%) from triplicate injections of (A) 50 ng and (B) 5 ng of K562 digest standard over a 21-week instrument testing period (28 total weeks of continuous use). From weeks 24 to 28 (green shaded box), >1,020 µg of total K562 digest was injected in a simulated high-throughput experiment. The numbers of protein groups identified and quantified are shown by the light- and dark-shaded blue bars, respectively. The light and heavy red lines show the number of precursors identified and quantified, respectively. During weeks 24 to 25 (inclusive), K562 digest QC standards were run at intervals of every 36 µg of total protein injected. For weeks 26 to 28 (inclusive), K562 digest QC standards were run at intervals of every 72 µg of total protein injected. The CV values for protein groups and precursors identified and quantified over the 21-week testing period were below 20% (summarized in Table 1).

Methods

Sample preparation: Human K562 tryptic digest was purchased from Promega and diluted to a stock concentration of 500 ng/µL in water with 5% acetonitrile and 0.1% formic acid. This stock solution was further diluted to prepare 2 QC samples, consisting of (a) 50 ng/µL digest in water with 0.1% formic acid and (b) 5 ng/µL digest in water and 0.1% formic acid. The 5 ng/µL sample also contained 5 fmol/µL bovine serum albumin digest (Thermo Fisher Scientific) to minimize non-specific binding losses.

Chromatography and MS source conditions: Separations were performed using a Waters M-Class LC system with a Phenomenex Kinetex XB-C18 (0.3 mm x 150 mm, 2.6 µm) analytical column, using a flow rate of 5 µL/min in direct-inject mode. The ZenoTOF 7600 system used an OptiFlow Turbo V ion source fitted with a 1-10 µL/min microflow probe. The source parameters for all methods used an ionspray voltage of 5,000 V, source temperature of 200º C, curtain gas of 35 psi, gas 1 setting of 20 psi and gas 2 setting of 60 psi.

Zeno SWATH DIA methods: QC injections of 5 ng and 50 ng of K562 digest were done using a 20-minute LC gradient (30-minute total run time). The Zeno SWATH DIA method consisted of 65 variable windows (Q1 mass range 400-750 m/z) with a TOF MS accumulation time of 50 msec and TOF MS/MS accumulation time of 13 msec per SWATH window. For the accelerated robustness test injections (0.5 µg and 1 µg K562 digest loads per run), methods were run with 5-minute gradients (12-minute total runtime) and 10-minute gradients (20-minute total runtime). These shorter gradient methods used a similar 65 variable window SWATH method but did not employ the Zeno trap for MS/MS acquisition. The TOF MS/MS accumulation times were shortened here to 9 msec and 11 msec for the 5-minute and 10-minute gradient methods, respectively.

Experimental timeline: A cleaned ZenoTOF 7600 system was used for 28 consecutive weeks without any subsequent cleaning/maintenance. Beginning week 8, periodic QC injections were performed to evaluate LC-MS system stability. The QC injections consisted of 5 ng and 50 ng loadings of K562 digest analyzed by Zeno SWATH DIA using the 20-minute gradient. During weeks 24-28, an accelerated robustness test was conducted by injecting 1,020 µg of K562 digest over the 5-week period. Repeated injections of 0.5 µg or 1 µg of K562 digest were run with 5-minute and 10-minute gradients using SWATH DIA methods and without Zeno trap activation for MS/MS acquisition. During this time, replicate QC injections of 5 ng and 50 ng K562 digest loads were performed, either every 36 µg (weeks 24–25, inclusive) or 72 µg (weeks 26–28, inclusive) of total K562 digest sample loaded onto the system. In addition, single injections of 50 ng of K562 digest were performed every 36 µg of protein injected. These data were processed separately. In the 28-week period experiment, the instrument was vented 3 times, including 2 instances in which the instrument was moved to a new location (weeks 9, 26_02, and 28_03 in Figures 1, 2, and 4).

Data processing: Zeno SWATH DIA files corresponding to the triplicate QC injections of 5 ng and 50 ng of K562 digest were processed with DIA-NN software, version 1.8.1. Searches were performed against a combined spectral library previously generated from human K562 and HeLa digests with high-pH fractionation and data-dependent acquisition[1]. Default settings were used, match between runs (MBR) was turned on and cross-run normalization was set to RT-dependent. The numbers of protein groups and precursors identified at a 1% false discovery rate (FDR) and quantified with CVs <20% were reported using the resulting DIA-NN software output files (pg_matrix.tsv and pr_matrix.tsv, respectively).

The separate analysis of 38 selected injections of 50 ng of K562 digest over the 21 weeks was also performed with DIA-NN software against the same combined K562/HeLa spectral library, with MBR and cross-run normalization turned off. Protein group and precursor intensities were plotted using the output pg_matrix.tsv and pr_matrix.tsv files, respectively. Select peptides from the K562 lysate proteins were also chosen and peak areas from the resulting DIA-NN software output files were plotted over the 28-week period.

Detection and quantitation of protein groups and precursors from triplicate QC injections of K562 digest

Periodic QC injections of K562 digest with Zeno SWATH DIA were performed on the ZenoTOF 7600 system over a 21-week timeframe beginning on week 8. Between weeks 8 and 24, a variety of different proteomics samples were analyzed in

Table 1. Reproducibility of protein groups and precursors identified and quantified (with CVs <20%) from the triplicate injections of K562 digest across the 21-week testing period.

	50 ng K562		5 ng K562	
	Average	%CV	Average	%CV
Total protein groups	5,411	4.2	2,578	10.3
Protein groups with CVs <20%	4,149	6.3	1,348	12.1
Total precursors	36,445	6.2	12,887	13.8
Precursors with CVs <20%	23,019	8.7	5,093	15.6

microflow LC-MS mode in between the QC injections. On week 24, an accelerated robustness test was initiated by performing continuous injections of 0.5 µg or 1 µg of K562 digest with SWATH DIA methods to simulate a multi-week, high-throughput LC-MS experiment. During the first 23 weeks, approximately 130 µg of protein digest was injected, whereas >1,020 µg of protein was injected during weeks 24-28 for the accelerated robustness testing. The entire set of QC injections was processed with DIA-NN software against a combined K562/HeLa spectral library (Figure 1). These data indicate consistent performance over the testing period, despite the injection of >1,200 µg of protein and no cleaning procedures. Triplicate injections of 50 ng of K562 digest QC samples (Figure 1A) were analyzed with a 20-minute gradient using a Zeno SWATH DIA method. Over 21 weeks of QC tests, between 4,859 and 5,717 (5,411 average) total protein groups were identified at 1% FDR. Of these, between 3,554 and 4,595 were quantifiable with CVs <20%. The system showed minimal variability in protein groups identified and quantified across the testing period, with CVs of 4.2% and 6.3%, respectively. Similarly, between 30,725 and 39,661 (36,445 average) precursors were identified, whereas between 18,567 and 26,673 (23,019 average) precursors were quantified with CVs <20%. Across the testing period, the variability in precursors identified and quantified was minimal, with CVs of 6.2% and 8.7%, respectively.

Similar evaluations were performed with the 5 ng loadings of K562 digest (Figure 1B). Between 2,068 and 3,014 (2,578 average) protein groups were identified during the testing period and between 1,029 and 1,592 (1,348 average) were quantified with CVs <20%. Between 9,469 and 16,000 precursors (12,887 average) were identified and between 3,477 and 6,356 (5,093 average) were quantified with CVs <20%. As expected, the data collected from the 5 ng K562 digest load (with numbers of protein groups and precursors identified having CVs of 10.3% and 13.8%, respectively) was more variable than those from the 50 ng load. Data are summarized in Table 1.

Detection and quantitation of protein groups and precursors from single QC injections of 50 ng of K562 digest

Single QC injections of 50 ng of K562 digest during weeks 8 to 23 and QC injections performed every 36 µg of K562 loaded in weeks 24 to 28 (38 total datafiles) were processed using DIA-NN software without MBR or cross-run normalization to evaluate performance using unbiased processing conditions. The processing results are shown in Figure 2. In this dataset, between 4,732 and 5,426 (5,218 average) protein groups were identified and between 27,329 and 35,224 (32,717 average) precursors were identified. The CVs for this dataset were 3.7% and 6.3% for the protein groups and precursors, respectively. The average and median CVs for the peak areas of 46,465 total precursors identified between weeks 8 and 28 were 38% and 28.7%, respectively (Figure 3).

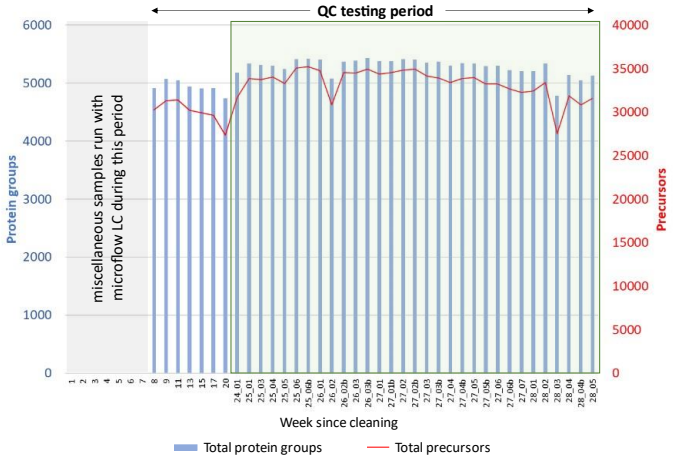

Figure 2. Total protein groups and precursors identified from the analysis of periodic single QC injections of 50 ng of K562 digest over 21 continuous weeks of testing. Blue bars and red lines represent the number of protein groups and precursors identified, respectively. Between weeks 24 and 28 (green shaded box), >1,020 µg of protein digest was injected for the accelerated robustness test.

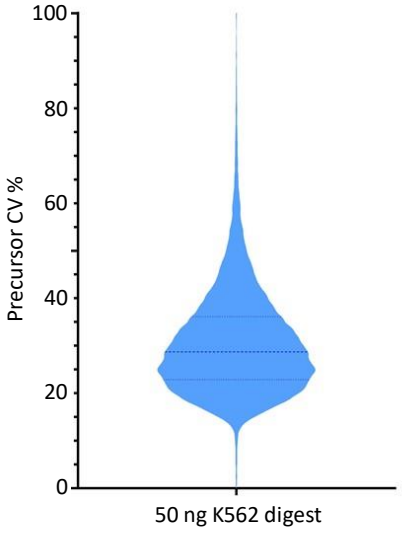

Figure 3. Violin plot showing the %CV distribution of 46,465 precursors analyzed from replicate QC injections of 50 ng of K562 digest across the 21-week testing period. Peak areas were taken from the resulting pr_matrix.tsv output files from DIA-NN software. The average and median CVs for the precursors were 38% and 28.7%, respectively.

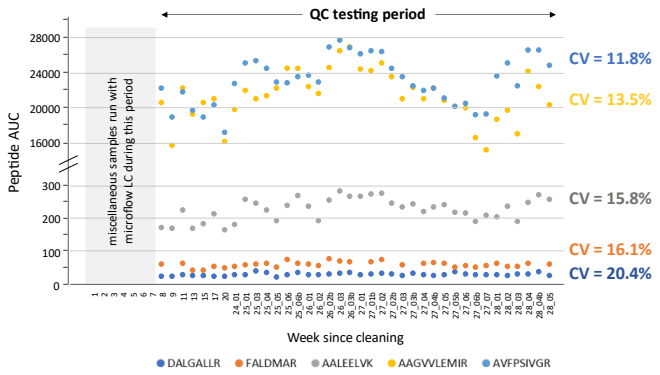

Figure 4. Non-normalized peak areas under the curve (AUC) for 5 human tryptic peptides from K562 digest over 21 weeks of system QC testing. Data were taken from the analysis of single replicates of 50 ng of K562 digest acquired periodically across the testing duration. Peak areas were taken from the resulting pr_matrix.tsv output files from DIA-NN software. The %CV values for each peptide across the testing duration are shown.

To further evaluate quantitative reproducibility over the 21-week period, the peak areas were plotted for peptides from selected proteins identified from 50 ng QC injections of K562 digest (Figure 4). Proteins with varying overall intensities were chosen for this label-free quantitation (LFQ) analysis. For example, the human protein ACTG (P63261), with 42 precursors (40 peptides) and an average LFQ value of 23,353 counts had a CV of 19.1%. Its peptide AVFPSIVGR had a CV of 11.8%. Similarly, the lower abundance protein MON1B (Q7L1V2), with 4 precursors, had an average LFQ value of 22 counts and a CV of 17.3%. The CV value for its peptide DALGALLR was 20.4%. The protein ILK (Q13418), with 8 precursors, had an LFQ intensity of 58 counts and a CV of 17.6%. Its peptide FALDMAR, which was identified in 33 of 38 runs, had a CV of 16.1%. The average and median LFQ protein intensities for the dataset from the 50 ng injection of K562 were 232.5 and 51.6 counts, respectively.

Conclusions

The data presented here demonstrate the consistent performance of the ZenoTOF 7600 system over a 21-week evaluation of microflow LC-MS quantitative proteomics (28 total weeks of continuous usage). The ZenoTOF 7600 system is ideally suited for high-throughput proteomics studies due to its robustness, rapid acquisition speed, and heightened sensitivity of MS/MS detection.

- The ZenoTOF 7600 system was operated for 28 continuous weeks without cleaning or maintenance (>1,200 µg of total protein digest injected). The system was tested for 21 consecutive weeks of microflow proteomics LC-MS QC runs (>1,000 injections) and showed stable performance over time.
- Triplicate QC injections with Zeno SWATH DIA analysis of K562 digest standards, at both 5 ng and 50 ng loadings, run at regular intervals over the 21-week performance testing period showed highly reproducible numbers of identified and quantified protein groups and precursors, all with CVs <20%.
- Analyses of 50 ng single QC injections of K562 digest across the 21-week performance testing period showed unbiased identifications of protein groups and precursors with CVs of 3.7% and 6.3%, respectively.

References

1. Large-scale protein identification using microflow chromatography on the ZenoTOF 7600 system. SCIEX technical note, RUO-MKT-02-14415-A.

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