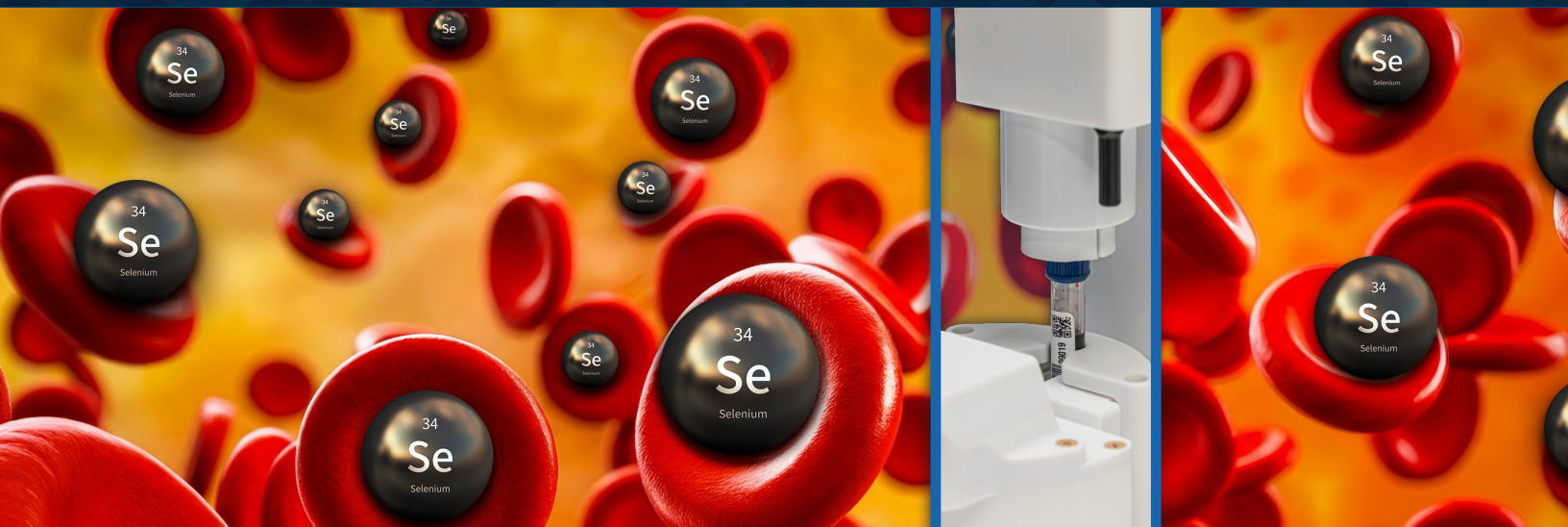




Authors: Patrick Messina, Owen Madigan, Libbie Smith

TRAXStation Clinical – Fully Automated Preparation of Whole Blood Samples for the Analysis of Selenium

Introduction

Trace element analysis plays a critical role in modern clinical diagnostics, providing essential information for evaluating nutritional status, toxic exposures, and metabolic disorders. Traditionally, the workflow for preparing and analyzing clinical samples by inductively coupled plasma mass spectrometry (ICPMS) has relied heavily on manual liquid handling steps. These steps – such as uncapping, rocking, pipetting, and dilution are time-consuming, prone to operator variability, and present ongoing risks of contamination, especially at trace and ultra-trace concentrations. As clinical demand grows, laboratories processing hundreds to thousands of patient samples per day face the mounting pressure to increase throughput while maintaining uncompromising analytical quality.

The **TRAXStation** Automated Multifunctional Liquid Handling System directly addresses these challenges by automating routine sample preparation tasks within a controlled, contamination free environment. Constructed from chemically inert materials, the platform is engineered to reliably process complex clinical matrices while minimizing external contamination and manual handling variability. Advanced capabilities including automated vial uncapping, liquid-level

detection, barcode tracking, sample mixing, and precise dilution routines ensure consistent and reproducible preparation across large sample sets.

In addition, the integrated SampleConfirm™ functionality verifies each aspiration event in real time, detecting incomplete or failed liquid handling steps and preventing compromised samples from advancing to analysis. For high-volume laboratories, this combination of automation and verification improves reproducibility, reduces operator workload, and provides a reliable, traceable pathway from raw sample to analysis-ready specimen.



Figure 1. TRAXStation Clinical 422.

Introduction (Continued)



Figure 2. TRAXStation Clinical takes up a whole blood aliquot for automated preparation in a 96-well microplate.

TRAXStation Clinical automates the full preparation workflow for whole blood samples collected in metal free collection tubes. The compact platform integrates all key steps into a single system, improving laboratory efficiency, traceability, and operator safety while ensuring

high-quality, reproducible results. By replacing labor-intensive manual steps, TRAXStation Clinical reduces errors, shortens turnaround times, and increases throughput while maintaining strict regulatory compliance and sample integrity.

TRAXStation Clinical Workflow

Barcode Reading & Sample Tracking

Integrated barcode scanning provides complete traceability and seamless connection to instruments and LIMS.

Sample Tube Rocker

Gentle rocking of tubes prior to processing guarantees homogeneous samples for accurate downstream analysis.

Automated Uncapping

Hands-free uncapping reduces manual handling, contamination risk, and biohazard exposure.

Sample Level Detecting

Sound waves are used to detect sample liquid levels. The probe will aspirate from just below the liquid surface.

SampleConfirm™

Sample integrity and verification function designed to confirm sample presence and total sample volume.

Precision Sampling & Dilution

High-accuracy pipetting enables aliquoting into tubes or 96-well plates with programmable dilutions for flexible assay workflows.

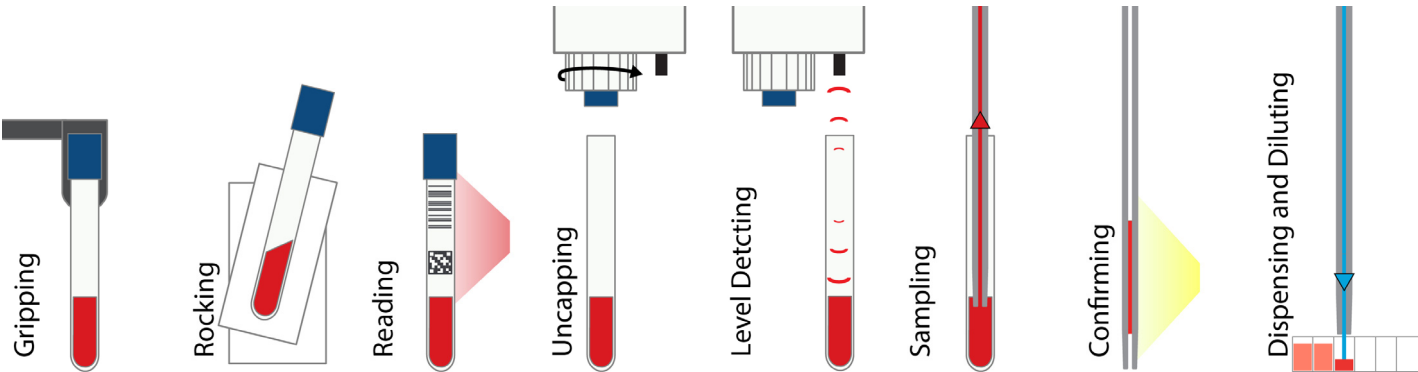


Figure 3. TRAXStation Clinical automated workflow for whole blood sample preparation.

Background

Selenium measurement in whole blood is important because it provides a more complete and biologically relevant assessment of selenium status vs serum alone. The majority of circulating selenium is located within red blood cells, where it is incorporated into functional selenoproteins such as glutathione peroxidase that reflects long-term nutritional and antioxidant status. Because red blood cells have a lifespan of approximately 120 days, whole blood selenium integrates dietary intake and physiological status over weeks to months, making it less susceptible to short-term fluctuations caused by recent meals, supplementation, or acute illness. This longer integration period results in lower biological variability and improved comparability for long term patient monitoring and population surveillance.

For deficiency detection, whole blood selenium is especially valuable because it reflects depleted intracellular selenium reserves rather than short term changes in circulating selenium. Early or chronic selenium deficiency may not be apparent in serum due to short-term dietary intake or acute phase responses, whereas whole blood measurements better correlate with reduced selenoprotein activity and long-term insufficiency. This improves sensitivity for

identifying individuals or populations at risk of deficiency and supports more accurate public health surveillance.

In the context of toxicity assessment, whole blood selenium can help identify sustained excessive exposure by reflecting cumulative selenium burden rather than recent intake alone. Chronic high selenium intake leads to elevated selenium incorporation into red blood cells, making whole blood analysis useful for distinguishing ongoing overexposure from short term dietary supplementation increases seen in serum. This is particularly relevant in environmental, occupational, or supplementation related investigations.

From a clinical and public health perspective, whole blood selenium more closely reflects intracellular selenium reserves and functional sufficiency, which are critical for assessing chronic deficiency or marginal status. Serum selenium concentrations can change rapidly in response to supplementation or inflammatory states and may not accurately represent tissue selenium availability. As a result, whole blood selenium is frequently preferred for nutritional assessment, epidemiologic studies, and public health programs where stable, reproducible measurements are required.

Table 1. Selenium in whole blood reference ranges. The reference range was established based on the mean values reported by multiple major U.S. clinical laboratories.

Selenium Status	Concentration	Interpretation
Normal Range	125-250 µg/L	Laboratory and methodology dependent. The normal concentration range represents an average range from multiple U.S. laboratories.
Deficiency	<125 µg/L	Deficiency may impair glutathione peroxidase activity. Symptoms include fatigue, immune issues, hair/nail changes, and cardiomyopathy.
Toxicity (Selenosis)	>400 µg/L	Toxicity typically comes from chronic exposure. Symptoms include garlic odor breath, GI symptoms, hair loss, neurological signs, and skin lesions.

Experimental – TRAXStation Automated Sample Prep

Evaluation that the combination of **TRAXStation** Clinical preparation & ICPMS measurement produces acceptable precision (repeatability and reproducibility) and accuracy (bias vs assigned concentrations) for selenium across the clinical range using three pooled sample levels: Low (below normal levels), Normal (clinically relevant mid-range), and High (above normal range that may represent toxicity).

Table 2. Materials and manual preparations.

Item	Description
Matrix	Bovine Whole Blood EDTA (pooled)
Pool Levels	Low, Normal, and High (based on reference range)
Target Conc.	Low ≈ 120 µg/L Normal ≈ 200 µg/L High ≈ 400 µg/L
Aliquoting	Aliquot each pool into 25 identical tubes
Calibrators	Aqueous – 6 Levels from 25 µg/L to 1000 µg/L
Diluent	1% Nitric Acid (v/v) 2% Methanol (v/v) 10 ppb Tellurium (Internal Standard)
Additional Materials	UPW, Calibration Standards, Blanks, and Negative

Table 3. Run structure and experimental design.

Item	Description
Samples per Run	83 (25 each level + Calibration)
Total Runs	25 (1875 data points) Multiple runs per day
Run Format	Blank, Calibrators, Negative, 25 Low, 25 Mid, 25 High
Data Collection	Accuracy and Precision monitored within and across runs
Data Analysis	Inter and Intra Assay Performance presented as a percent CV. (See Data Analysis Criteria below)

Table 4. TRAXStation Clinical sample automation.

Item	Description
Rinse 1	0.4% TMAH, 1% EtOH, 0.05% TritonX
Rinse 2	DI Water
Diluent	(See Table 2 for prep) Connect and prime system
“Source” Samples	Load all calibrators and whole blood samples to “source” racks
Destination Racks	Load all destination racks with 12 mL plastic conical tubes
Dilution Factor	100x
Aspiration Volume	25 µL sample
Diluent Volume	2475 µL
Final Volume	2500 µL (allows for reanalysis)

Data Analysis Criteria

For clinical whole blood selenium testing, good precision and accuracy are demonstrated through rigorous statistical validation consistent with CLIA, CAP, and CLSI guidelines. Precision is evaluated by repeated analysis of whole blood pools at low, normal, and high concentrations relative to reference ranges, with 25 replicates per level over 25 runs/ batch and expressed as coefficient of variation (%CV). For trace metals such as selenium, intra-assay and inter-assay %CVs should generally be ≤5% at normal and high levels and ≤10% at low levels. Accuracy is verified by comparison with mean recovery required to fall within ±10% of target values. According to CLIA proficiency

testing criteria, there is no established allowable total error (TEa) for selenium in whole blood; however, we will use 15% as was established for both copper, zinc, and selenium in serum evaluations. Both short-term and long-term reproducibility must be assessed across runs, days, and operators to ensure robustness. Adherence to Good Laboratory Practice (GLP) principles and use of trace-element-free collection and processing materials are essential to minimize contamination. Together, these statistical and procedural controls ensure that whole blood selenium values are analytically valid, reproducible, and compliant with regulatory standards for clinical testing.

Instrument Prep and Parameters

All samples were prepared using **TRAXStation** Clinical, and analyzed using 4DXCi SampleSense *FAST* UHT-C in combination with a single quad ICPMS. Evaluation done

Table 5. Instrument information.

Parameter	Value
Sample Introduction System	ESI 4DCXi Autosampler SampleSense <i>FAST</i> UHT-C TRAXStation Clinical
Instrument	Single Quad ICPMS
Valve	Magnetically-Coupled Inert 6-Port Valve
Probe	Carbon Fiber 0.8 mm ID Probe SC-5037-3755-100
Nebulizer	High Solids PFA Microflow Nebulizer ES-2030-79
Rinse 1	1% Nitric Acid
Rinse 2	1% Nitric Acid
Carrier	1% Nitric Acid (v/v) 2% MEOH (v/v)
Sample-to-sample Time	20-21 sec/sample analysis

using both mass m/z 82 in Standard Mode and mass m/z 78 in KED Mode.

Table 6. Instrument parameters.

Parameter	Value
Analytes (Standard Mode)	Se (m/z 82) or
Analytes (KED)	Se (m/z 78)
ISTD	Te (m/z 82)
Sweeps per Replicate	20
Replicates per Sample	3
Integration Time	100 ms per analyte
KED Gas	He (3 mL/min)
Total Acquisition Time	3 sec/sample

Figure 4. The AutoBench4 platform provides an integrated and organized workspace for ICPMS analysis, combining the autosampler, rinse stations, and fluid management components into a single, controlled sampling environment. This design simplifies system setup, improves laboratory ergonomics, and helps maintain consistent sample handling during extended analytical runs. By centralizing key sampling components, the AutoBench platform supports stable operation, efficient workflow, and reliable performance in high-volume clinical and public health laboratories.



Selenium Calibration – Analytical Measurement Range (AMR) 25-1000 µg/L

Selenium was analyzed at both m/z 82 and m/z 78 to evaluate potential spectral interferences and to confirm the accuracy of the analytical method across different isotopes and acquisition modes. Calibration data for both masses are presented on this page of the application note, allowing direct comparison of calibration performance and response behavior. Agreement between calibration curves at m/z 82 and m/z 78 demonstrates effective interference control and supports the suitability of both masses for reliable selenium quantification in whole blood.

Table 7. Representative calibration raw data.

	Standard Mode (m/z 82)		KED Mode (m/z 78)	
	CPS	Conc. (µg/L)	CPS	Conc. (µg/L)
Blank	291	0.0	501	0.0
Cal 1	1,991	23.5	1,103	28.4
Cal 2	4,029	51.4	1,459	46.4
Cal 3	7,516	99.2	2,457	97.3
Cal 4	18,783	255.2	5,440	256.8
Cal 5	35,946	494.2	10,313	500.6
Cal 6	73,817	1001.7	19,551	998.4

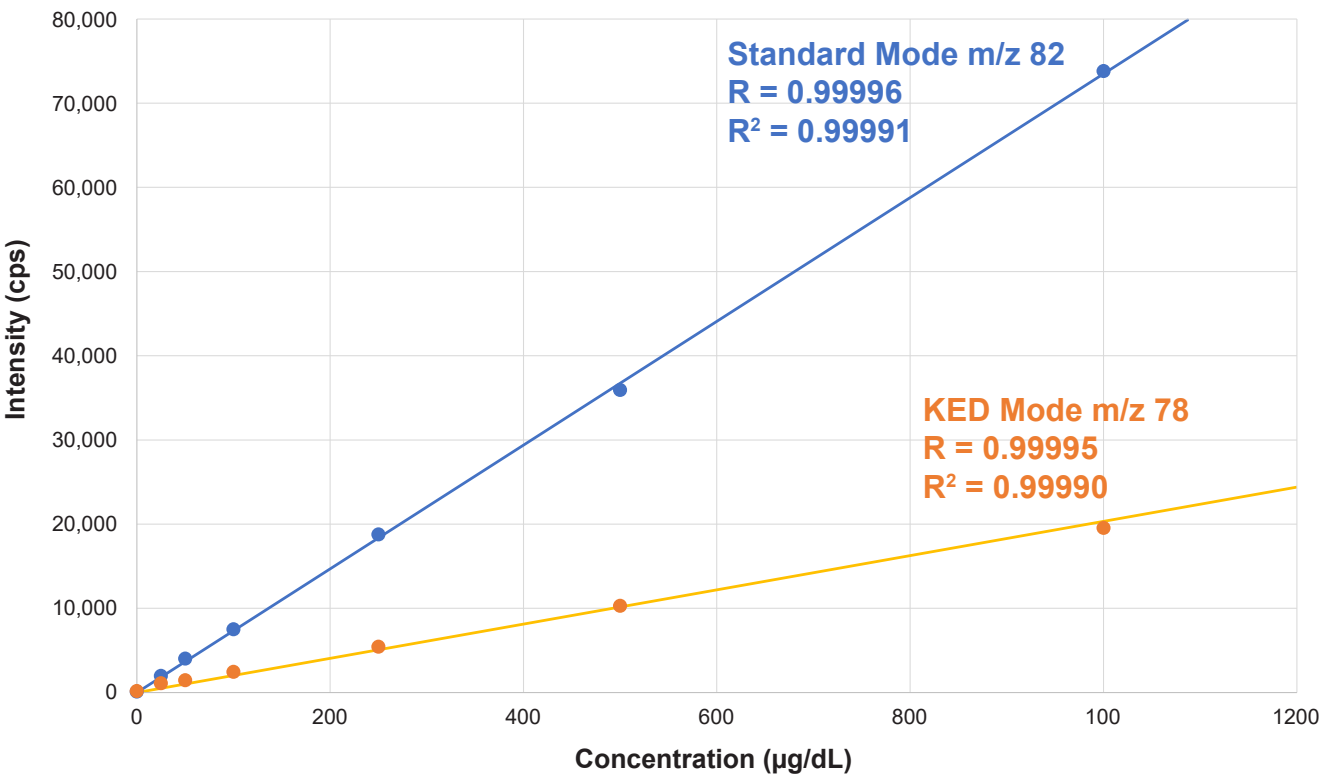


Figure 5. Calibration results demonstrated excellent precision and accuracy for selenium when aqueous calibrators were pipetted and prepared using the TRAXStation Clinical automated platform. Measured concentrations closely matched target values, and all correlation coefficients were 0.999 or better, indicating minimal bias and strong agreement with expected results. Together, these findings validate that TRAXStation delivers consistent, contamination-free preparation, ensuring reliable quantitation of selenium in clinical testing workflows.

Results Summary of m/z 82 Data

Selenium at m/z 82 in whole blood was quantified using a validated ICPMS analytical methodology following automated sample preparation on the TRAXStation Clinical platform. The automated workflow ensured consistent dilution, minimized manual handling, and reduced the potential for contamination across all samples. Analytical results demonstrated exceptional method performance, with selenium concentrations closely matching target values and exhibiting excellent precision and accuracy across the full reportable range. Performance was maintained throughout extended analytical runs, indicating robust method stability and reproducibility suitable for routine clinical and public health selenium testing in complex whole blood matrices. These results highlight the TRAXStation Clinical platform’s strong capability to deliver highly consistent, reproducible, and contamination-controlled sample preparation that directly supports high-quality ICPMS selenium analysis.

Table 8. Selenium in whole blood results, showing mass m/z 82 analyzed in Standard Mode.

	Low Concentration	Normal Concentration	High Concentration
Average (µg/L)	122.5	205.6	392.3
Standard Deviation (µg/L)	4.04	7.06	14.08
Intra-Assay Precision	3.16%	2.68%	2.52%
Inter-Assay Precision	3.30%	3.43%	3.59%

The TRAXStation Clinical 221 platform is also available. This compact version delivers the full power of automated sample preparation in a small footprint – ideal for laboratories with limited bench space. It automates uncapping, mixing, pipetting, and dilution steps to eliminate manual variability, improve reproducibility, and protect sample integrity through contamination-free, metal-inert processing. With intuitive LabSymphony software, barcode tracking, and flexible rack configurations, the TRAXStation Clinical 221 integrates seamlessly into existing ICPMS workflows while reducing labor, consumable costs, and operator exposure. Despite its small size, it provides high-throughput performance, superior consistency, and enhanced laboratory safety – all in a compact, efficient design.



Figure 6. TRAXStation Clinical 221.

See raw data on the following pages.

Selenium m/z 82 Inter- and Intra-Assay Data – Low Concentration (625 data points)



Selenium m/z 82 Inter- and Intra-Assay Data – Low Concentration (Continued)

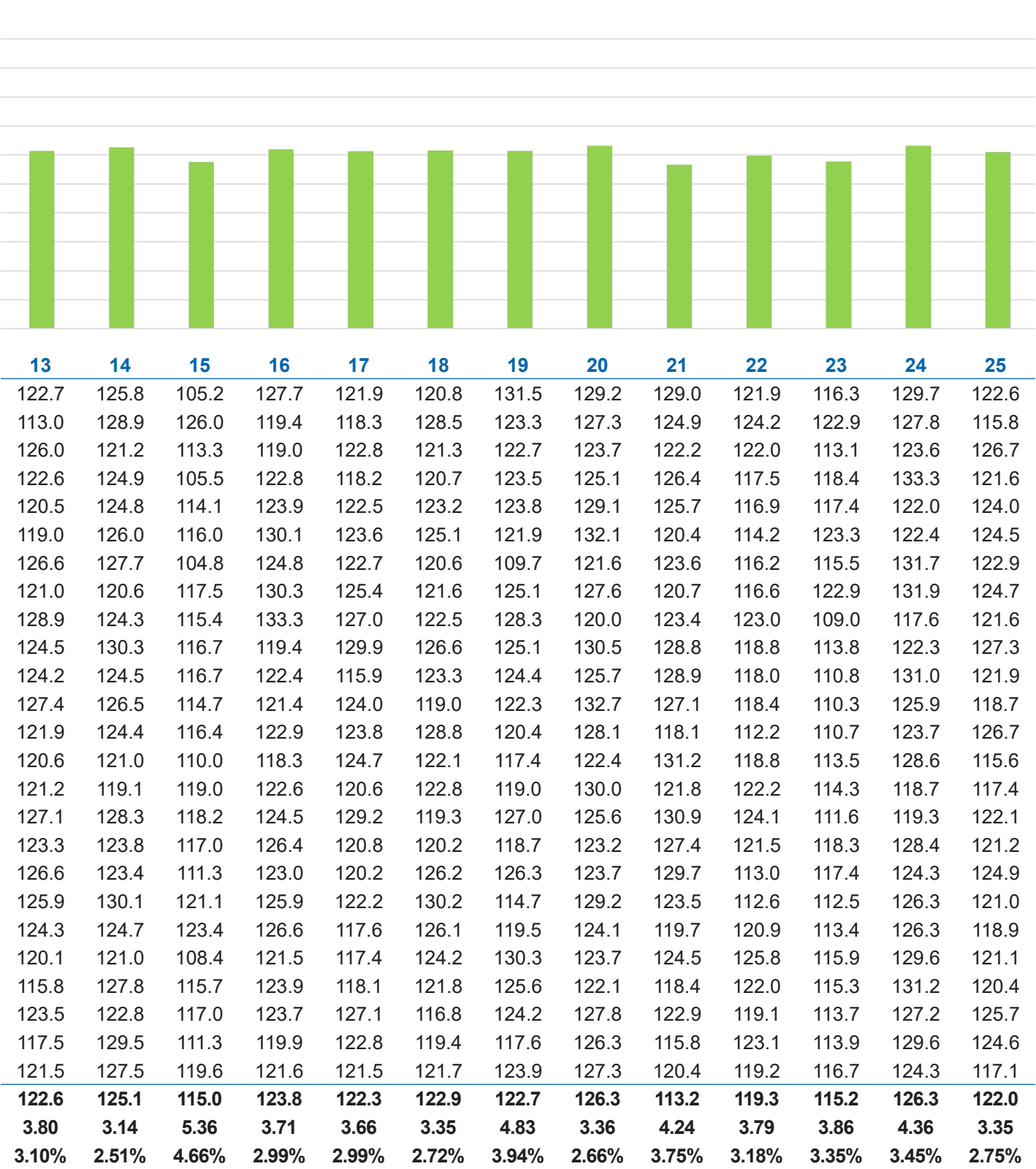
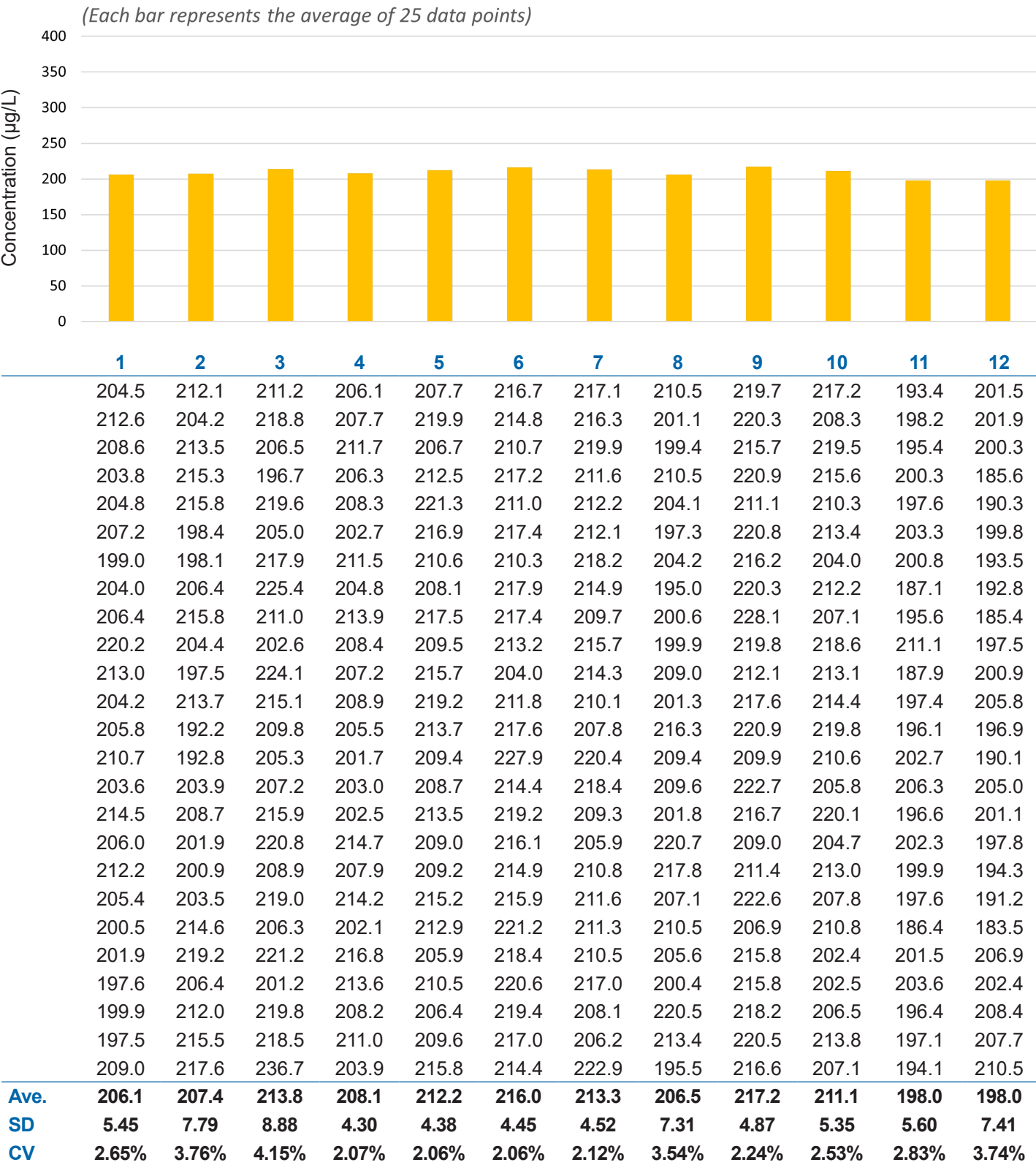


Figure 7. Low concentration for selenium measured by ICPMS (m/z 82). Each number 1-25 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Selenium m/z 82 Inter- and Intra-Assay Data – Normal Concentration (625 data points)



Selenium m/z 82 Inter- and Intra-Assay Data – Normal Concentration (Continued)

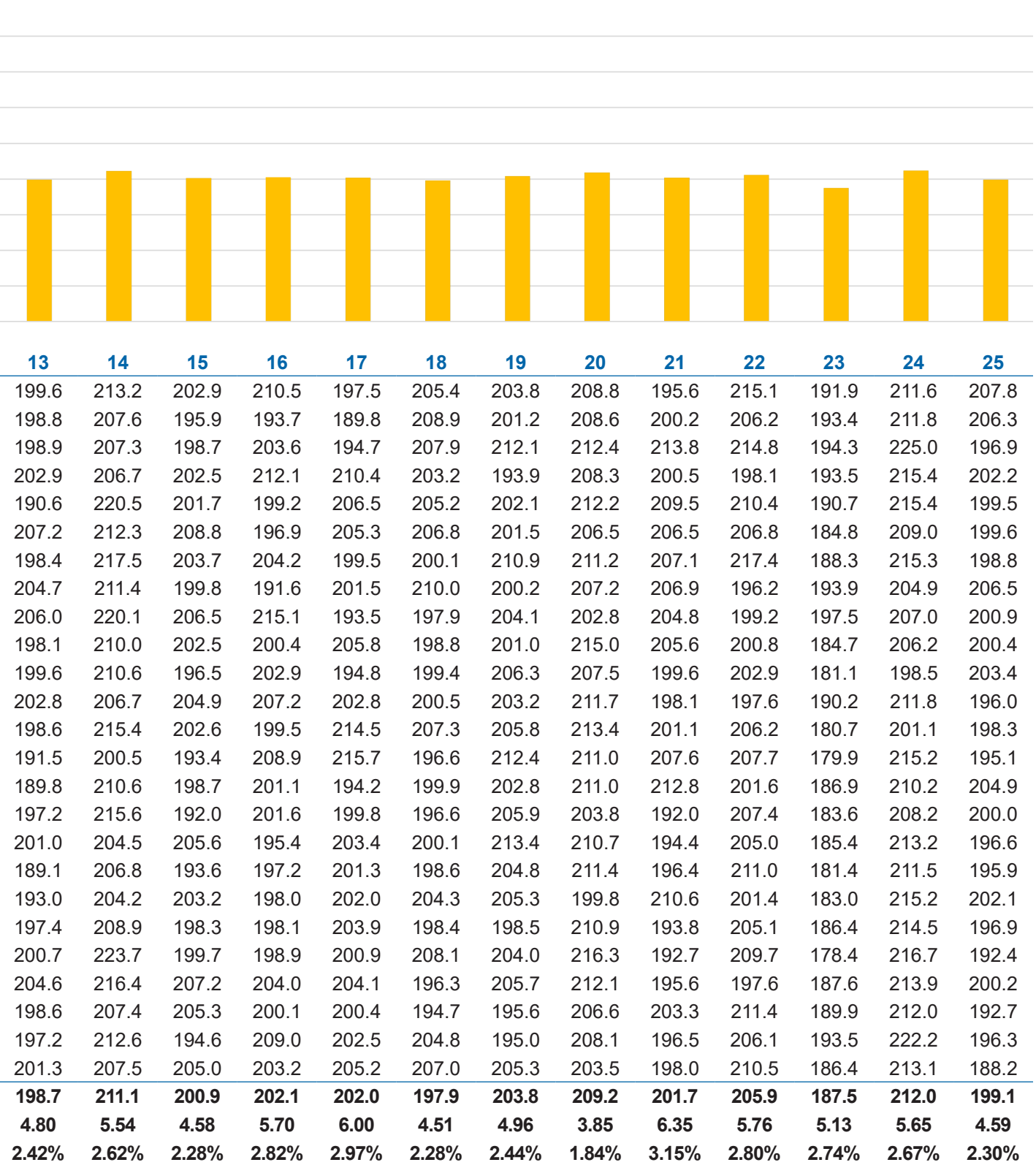
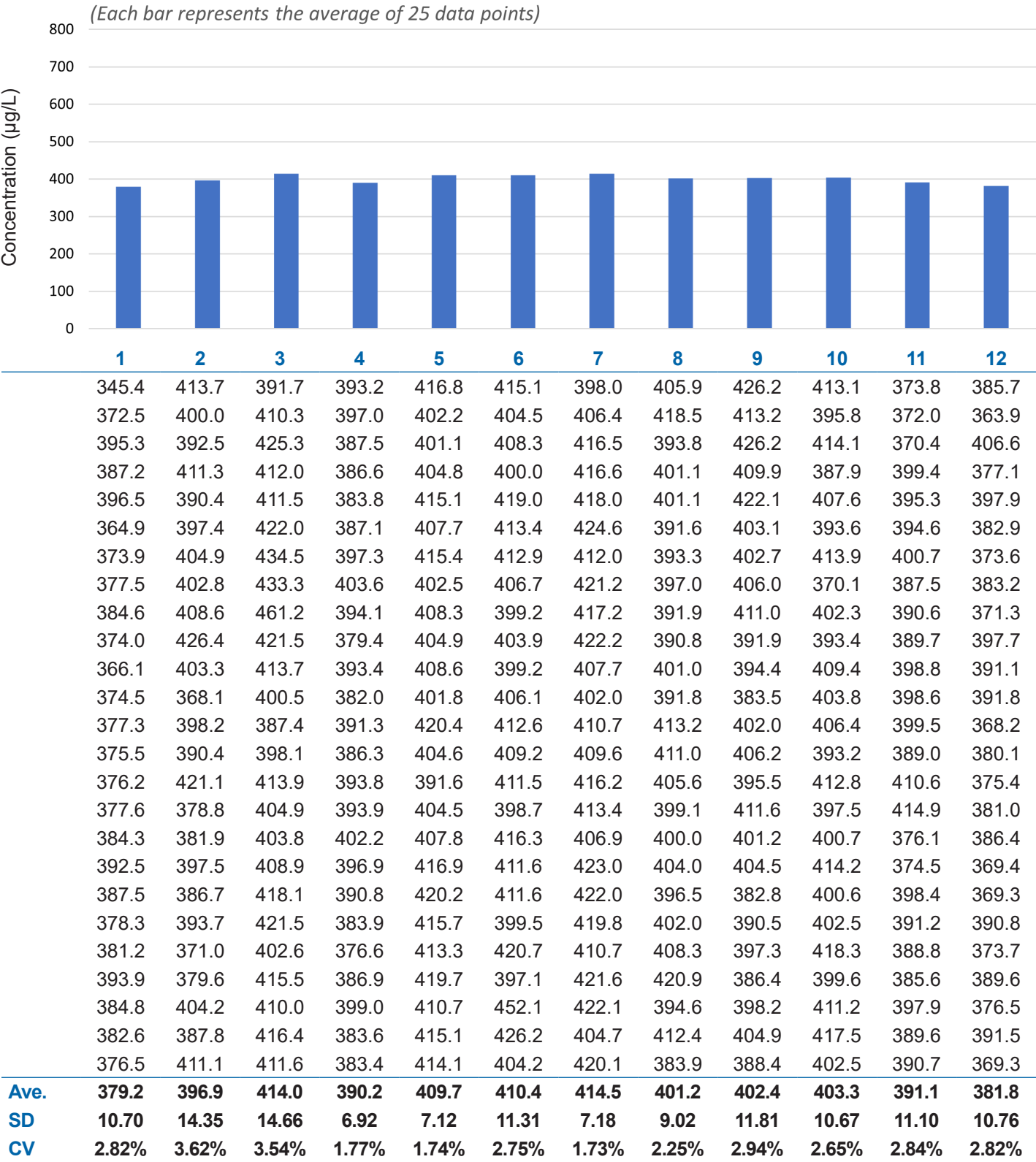


Figure 8. Normal concentration for selenium measured by ICPMS (m/z 82). Each number 1-25 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Selenium m/z 82 Inter- and Intra-Assay Data – High Concentration (625 data points)



Selenium m/z 82 Inter- and Intra-Assay Data – High Concentration (Continued)

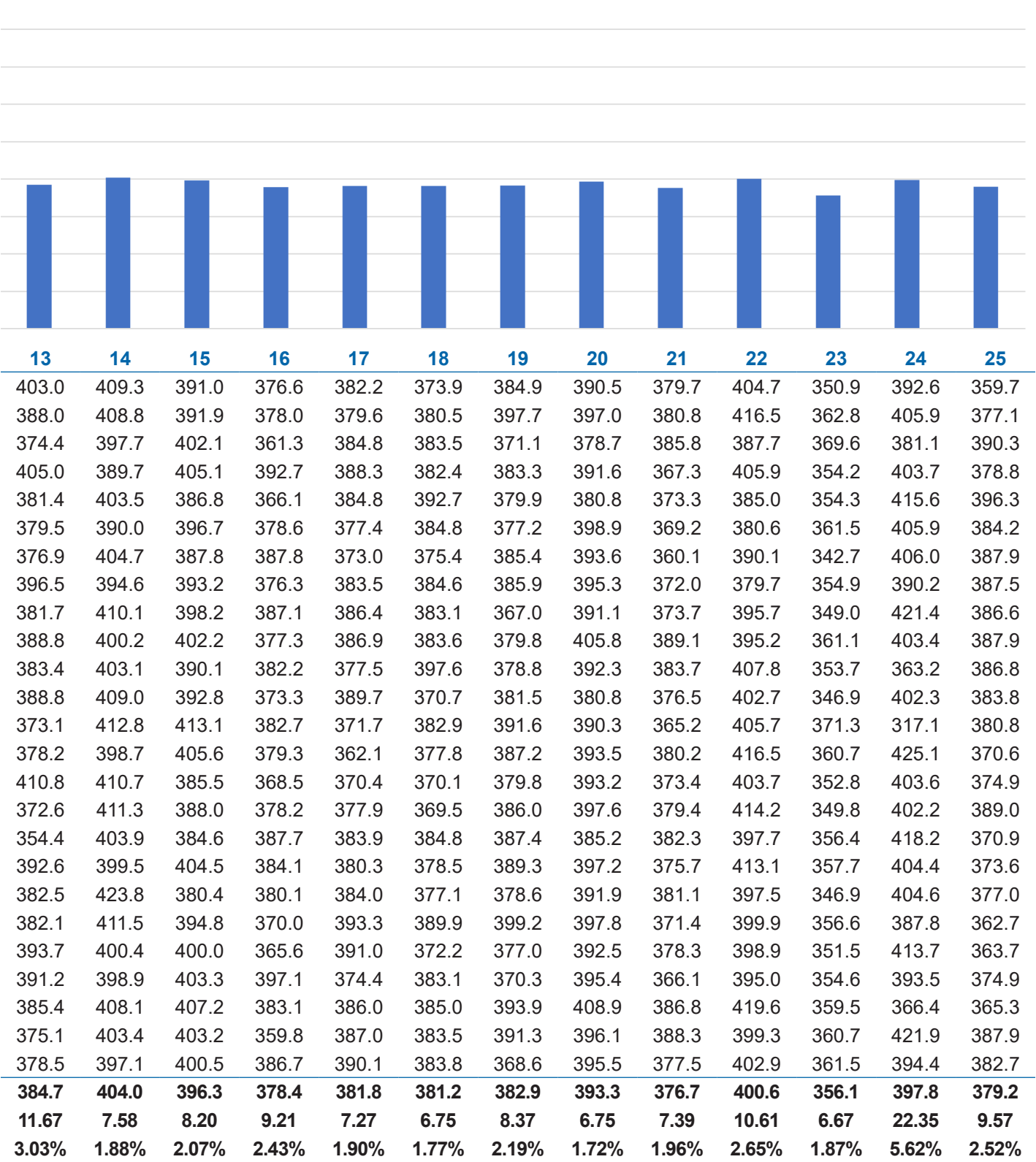


Figure 9. High concentration for selenium measured by ICPMS (m/z 82). Each number 1-25 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Results Summary of m/z 78 Data

Analysis of selenium at m/z 78 was performed to address known spectral interferences that can affect selenium determination and to establish an interference controlled reference for method evaluation. In standard mode, m/z 78 is susceptible to argon-based polyatomic interferences; therefore, acquisition in KED mode applies collisional energy discrimination to reduce these matrix and plasma-derived species. By generating selenium data at m/z 78 under interference minimized conditions, a reliable comparison dataset was established against which results from m/z 82

in standard mode could be evaluated. Agreement between concentrations measured at m/z 78 (KED) and m/z 82 (standard) provides strong evidence that interferences are effectively controlled and that the primary analytical mass is producing accurate results. Running m/z 78 in KED mode provides a more meaningful comparison to m/z 82 in standard mode than simply comparing m/z 82 in standard vs. KED mode, because it isolates interference control from isotope-specific behavior and avoids masking real analytical differences.

Table 9. Selenium in whole blood results, showing mass m/z 78 analyzed in KED Mode.

	Low Concentration	Normal Concentration	High Concentration
Average (µg/L)	121.1	205.6	392.3
Standard Deviation (µg/L)	4.72	7.88	10.88
Intra-Assay Precision	3.21%	2.72%	2.53%
Inter-Assay Precision	3.90%	3.80%	2.72%

	1	2	3	4	5	6	7	8	9	10	11	12
Ave.	118.0	120.9	125.3	122.6	127.9	111.7	116.5	111.2	119.5	124.8	108.1	118.0
SD	128.3	119.6	127.1	124.8	120.0	120.9	122.0	112.4	115.2	124.7	104.6	118.7
CV	124.7	121.1	119.8	120.2	122.4	117.6	110.9	116.9	122.8	115.1	113.2	123.3
	122.4	123.6	124.7	116.2	125.8	119.0	116.8	118.6	118.1	112.3	108.9	121.5
	126.7	124.2	133.6	124.7	125.7	119.3	117.2	113.2	117.2	117.0	111.5	117.3
	136.9	127.5	121.2	124.7	124.3	124.7	115.8	112.7	126.6	119.7	115.7	113.3
	122.6	120.9	127.8	126.3	129.7	119.3	114.8	107.5	118.7	121.1	109.4	119.3
	127.1	120.0	126.2	130.7	122.1	128.7	122.3	114.6	116.0	113.5	109.1	117.0
	122.0	123.5	128.5	117.3	121.3	121.4	117.9	115.8	115.3	114.3	112.1	112.8
	126.7	115.1	124.9	125.7	124.1	119.9	116.3	114.2	125.3	120.1	114.8	116.6
	124.2	130.2	134.6	126.2	128.7	119.2	119.5	116.8	124.2	115.6	115.0	117.6
	122.3	122.5	127.7	124.4	134.9	123.5	120.4	115.4	113.6	120.9	111.9	114.8
	124.8	124.9	130.7	121.5	128.8	124.8	124.0	115.5	118.1	119.8	111.5	118.4
	124.1	131.7	126.8	125.5	127.4	128.0	112.9	117.1	119.7	122.4	114.8	116.9
	122.9	123.3	134.4	125.3	130.2	119.5	123.4	112.4	119.7	123.1	114.6	118.3
	117.2	125.6	128.2	131.1	126.0	122.4	117.9	112.7	123.1	124.8	110.2	116.5
	125.4	124.1	124.5	120.5	132.0	127.9	115.0	115.1	124.7	103.5	117.0	121.1
	121.0	125.7	129.6	126.2	128.3	117.7	125.8	121.5	122.0	111.6	118.7	121.2
	128.8	121.5	138.0	122.1	123.8	123.6	119.5	118.4	125.8	120.1	116.0	119.9
	120.8	131.8	123.8	123.4	130.5	121.8	125.7	115.3	118.5	119.8	111.9	118.0
	121.8	136.2	132.5	125.4	129.5	120.9	121.9	122.2	122.9	117.5	114.9	119.0
	115.9	118.4	123.4	129.5	125.6	116.7	121.1	118.0	119.8	118.3	113.1	113.6
	123.5	132.6	136.7	125.5	129.2	118.6	117.7	114.3	118.2	124.7	113.5	118.3
	121.7	125.2	128.7	124.4	132.5	126.2	125.2	114.9	121.8	122.3	116.3	122.9
	127.5	124.8	128.7	120.0	132.3	117.2	119.9	116.1	117.3	121.9	107.5	119.1
Ave.	123.9	124.6	128.3	124.2	127.3	121.2	119.2	115.3	120.2	118.8	112.6	118.1
SD	4.19	4.80	4.55	3.54	3.76	3.95	3.99	3.15	3.59	5.06	3.37	2.72
CV	3.38%	3.86%	3.55%	2.85%	2.95%	3.26%	3.34%	2.73%	2.99%	4.26%	2.99%	2.30%

See raw data on the following pages.

Selenium m/z 78 Inter and Intra-Assay Data – Low Concentration (300 data points)



Figure 10. Low concentration for selenium measured by ICPMS (m/z 78). Each number 1-12 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Selenium m/z 78 Inter and Intra-Assay Data – Normal Concentration (300 data points)

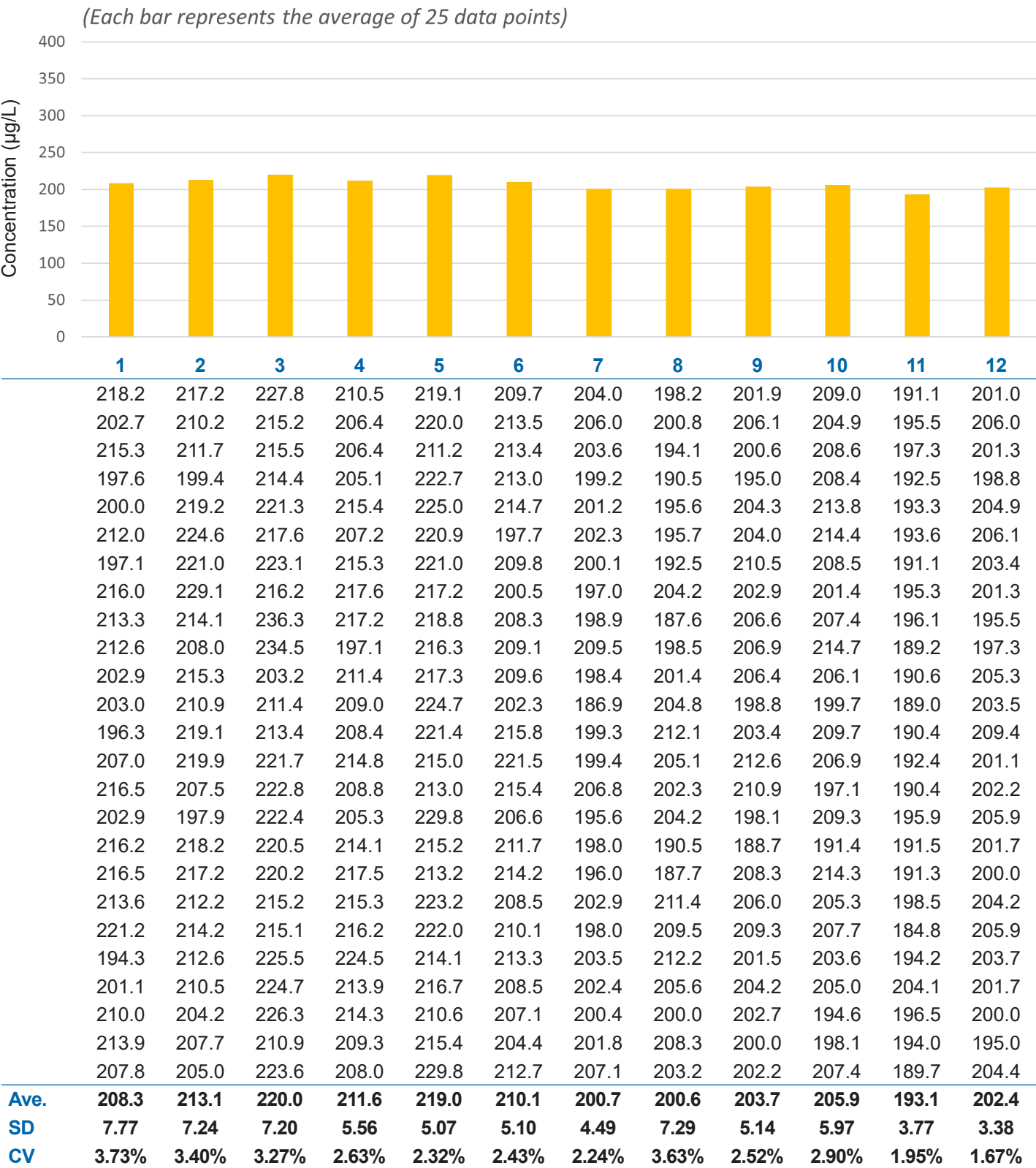


Figure 11. Normal concentration for selenium measured by ICPMS (m/z 78). Each number 1-12 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Selenium m/z 78 Inter and Intra-Assay Data – High Concentration (300 data points)

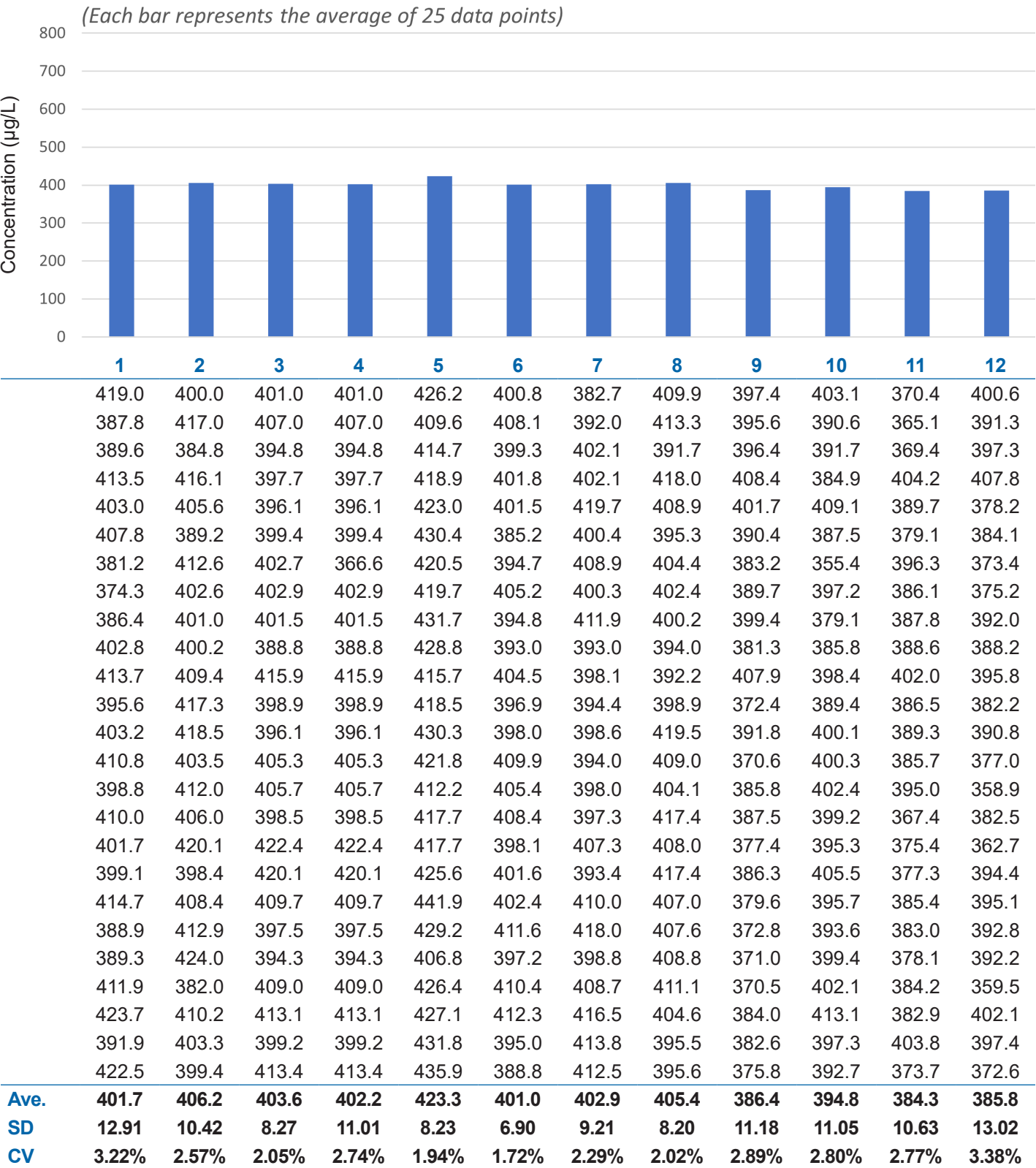


Figure 12. High concentration for selenium measured by ICPMS (m/z 78). Each number 1-12 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Comparison Results – m/z 82 in Standard Mode to m/z 78 in KED Mode

Selenium results obtained at m/z 82 in standard mode and m/z 78 in KED mode were independently evaluated against their respective target concentrations and directly compared to one another across the full analytical range. Agreement to target values and close concordance between the two masses demonstrated consistent accuracy and precision for both analytical modes. This dual evaluation provides an effective and scientifically sound means of assessing method performance, as m/z 78 in KED mode serves as an interference controlled confirmation while m/z 82 in standard mode represents routine analytical conditions. Comparison of selenium results obtained from whole blood samples analyzed at m/z 82 in standard mode and from the same samples analyzed at m/z 78 in KED mode demonstrated excellent agreement across all concentration levels evaluated.

Measured selenium concentrations were statistically indistinguishable between the two approaches, with no observable bias or systematic deviation. This strong concordance indicates that potential spectral interferences commonly associated with m/z 82, including argon-based, calcium-related, and bromine-related polyatomic species, were effectively controlled under the applied analytical conditions. The absence of divergence between standard-mode and KED-mode results provides clear evidence that interferences at m/z 82 were negligible in the whole blood matrix. Based on these findings, selenium quantification at m/z 82 in standard mode is demonstrated to be accurate and acceptable for whole blood analysis, supporting its use as a reliable primary mass for routine clinical and public health selenium testing.

Table 10. Selenium in whole blood results, showing mass m/z 78 compared to mass m/z 82.

Mass Comparison	Low Concentration	Normal Concentration	High Concentration
m/z 82 vs. Target	102.08%	102.80%	98.08%
m/z 78 vs. Target	100.92%	103.70%	99.95%
m/z 82 vs. m/z 78	101.16%	99.13%	97.50%
m/z 78 vs. m/z 82	98.86%	100.88%	101.91%

Conclusion

The **TRAXStation** Clinical Automated Multifunctional Liquid Handling System demonstrated performance that surpassed expected analytical targets for both accuracy and precision. Measured values consistently aligned over extended periods of time indicating minimal systematic error and confirming high method accuracy. Replicate analyses showed very low variability, with relative standard deviations well below acceptance criteria, reflecting exceptional precision. Collectively, the data provide strong evidence of method robustness and reliability, exceeding established quality benchmarks and reinforcing confidence

in both the measurement process and resulting conclusions. Selenium results obtained at m/z 82 and m/z 78 showed no measurable differences, confirming consistent accuracy across isotopes and analytical modes and demonstrating effective control of potential spectral interferences. The **FAST 4DXCi** UHT-C autosampler supported reliable ultra-high-throughput operation, achieving 21-second sample-to-sample analysis with no observable carryover. These results confirm a robust, high-performance workflow suitable for routine whole-blood selenium analysis in high-volume clinical and public health laboratories.

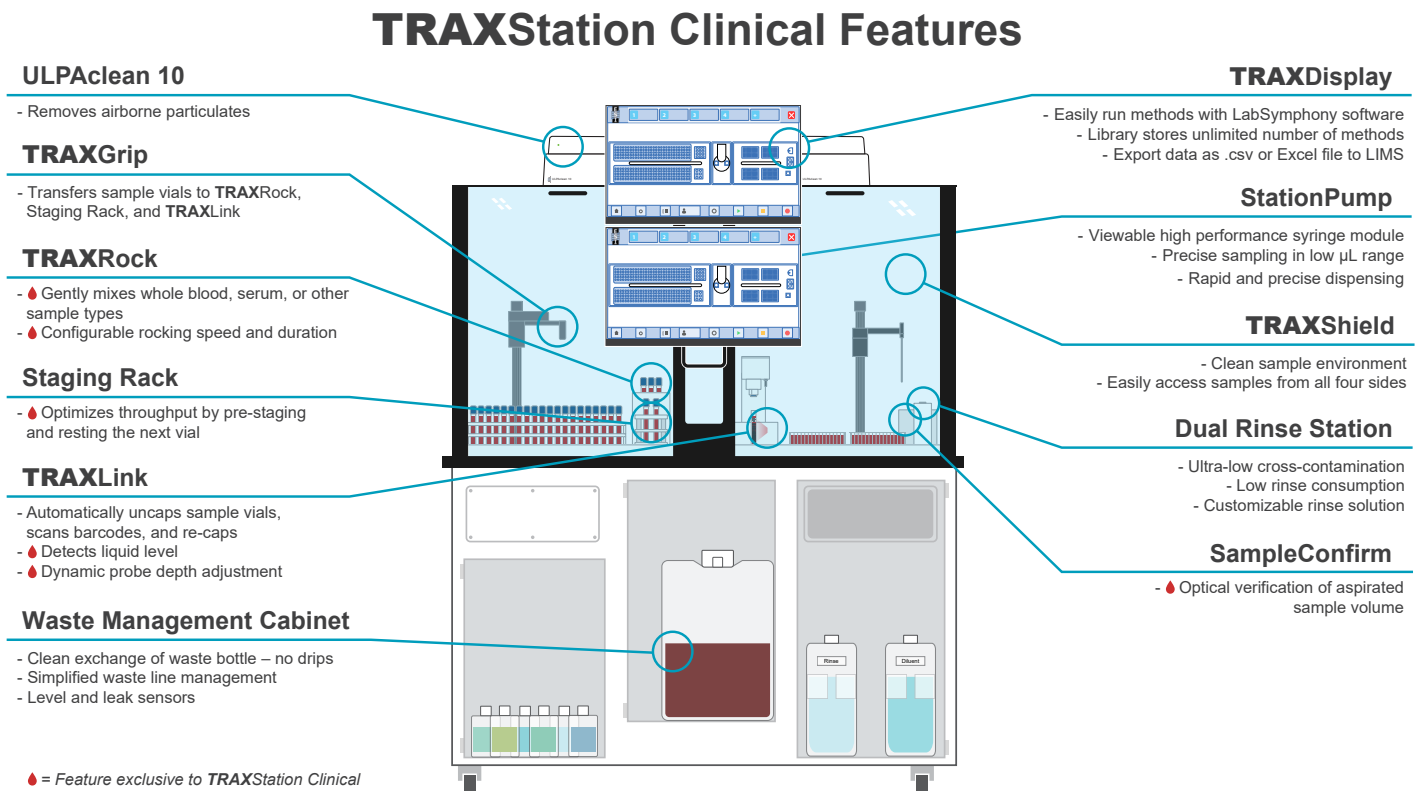


Figure 13. TRAXStation Clinical features diagram. TRAXStation Clinical is available in several models and configurations to match laboratory methods and throughput requirements. These include TRAXStation 221, 422, and 442 models.



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