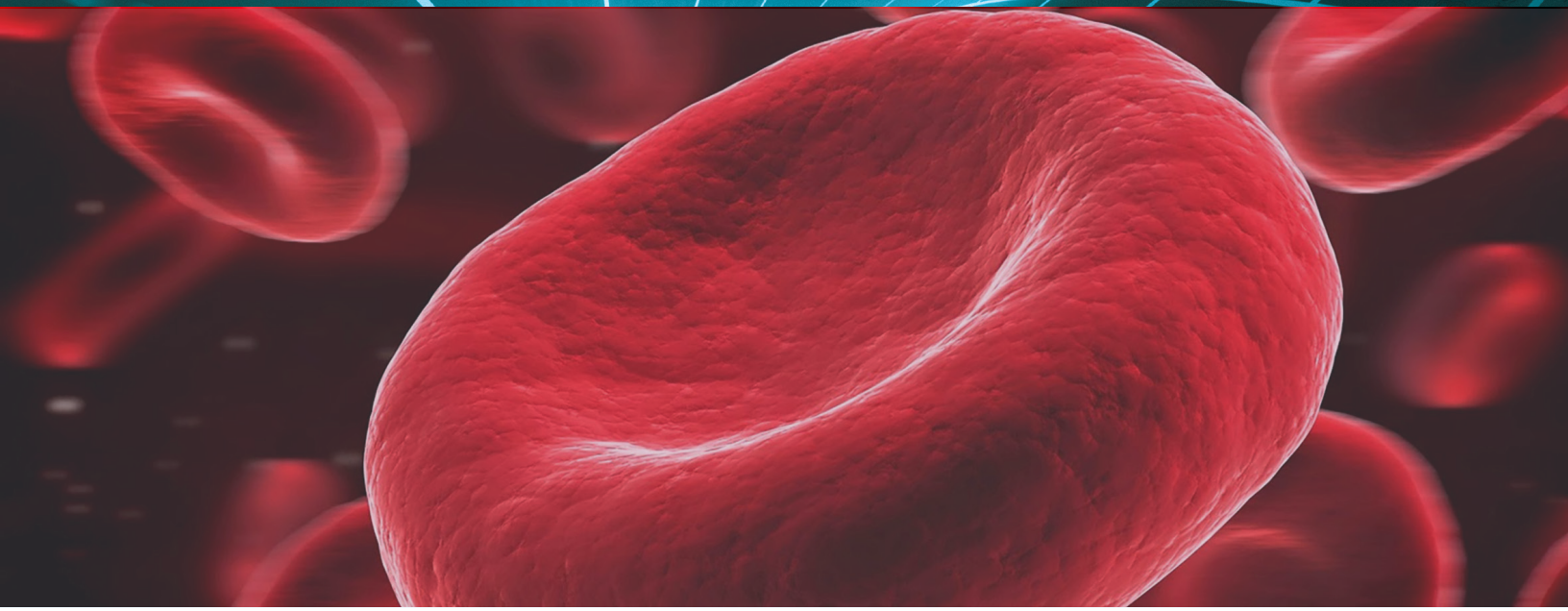

Authors: C. Derrick Quarles, Jr., Nathan Saetveit, Nick Bohlim, and Daniel R. Wiederin

Fully Automated Inline Sample Introduction System for Total Metals and Elemental Speciation in Clinical Samples

Introduction

The elemental analysis of biological samples (e.g. blood, serum, or urine) has many current applications, including biomonitoring studies, controlling industrial exposure, therapeutic monitoring, and medical diagnosis. Laboratories performing these clinical analyses must achieve excellent accuracy while maintaining high throughput. Although laboratories with a high number of samples may have the resources to analyze each sample type on a dedicated ICPMS, many labs must analyze all sample types with a single ICPMS. In addition to the downtime required to manually switch the ICPMS between different matrices, manual sample dilution is often the bottleneck that limits laboratory productivity.

This work demonstrates the use of the prep*FAST* IC as the front-end sample introduction system for analyzing undiluted blood, serum, plasma, and urine. The undiluted biological samples are loaded by vacuum or syringe, depending on the matrix and the amount of sample available. For total metals analysis, each sample type is automatically diluted inline by an appropriate dilution factor. Urine may also be automatically analyzed in speciation mode to determine the arsenic species present in the sample. The automated features of the system allow for multiple methods to be queued and run unattended, resulting in more efficient use of the ICPMS. Table 1 displays the sample loading strategies for each biological matrix.

Table 1. Sample loading strategies for urine, blood, serum, plasma, nails, and hair matrices.

	Sample Loading	Sample Uptake Volume*	Sample Loop**	Dilution Loop***	Sample-Inline Dilution
Urine	Vacuum or Syringe	300 µL	200 µL	1 mL	10X
Urine As Speciation	Vacuum or Syringe	300 µL	200 µL	1 mL	30X
Blood	Syringe	60 µL	50 µL	0.5 mL	50X
Serum, Plasma	Syringe	60 µL	50 µL	0.5 mL	25X
Hair, Nails	Syringe	1.2 mL	1 mL	1 mL	5X

* Sample uptake volume can be adjusted depending on the amount of sample available.
** Sample loop volume can vary depending on the amount of sample available (e.g. 100 µL sample loop).
*** Dilution loop size is dictated by the measurement time of the ICPMS method.

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Instrumentation

A NexION 2000 ICPMS in combination with the prep*FAST* IC as part of an integrated analytical station (Diagram 1) was used to analyze blood, serum, plasma, urine, hair, and nails for the elements listed in Table 2. For each sample type, calibrations were prepared inline by autodiluting a single stock standard and automatically matrix-matching with ESI's Clinical Matrix (p/n CLIN-0500, Elemental Scientific). NYDOH proficiency testing (PT) samples from 2018 were used as reference samples to demonstrate accuracy. Undiluted samples were automatically stirred for 5 seconds using an adjustable probe-stir function in the *FAST* method, followed by sample loading (vacuum or syringe) and subsequent dilution steps. The direct mode diagram shows the sample loading steps for total metals mode, which bypasses the chromatographic column, while the speciation mode diagram shows the sample being sent through the chromatographic column for speciation analysis. In total metals mode, the carrier and diluent were chosen to be compatible with each matrix, and the diluent and eluent in speciation mode were DI water and ammonium carbonate, respectively.

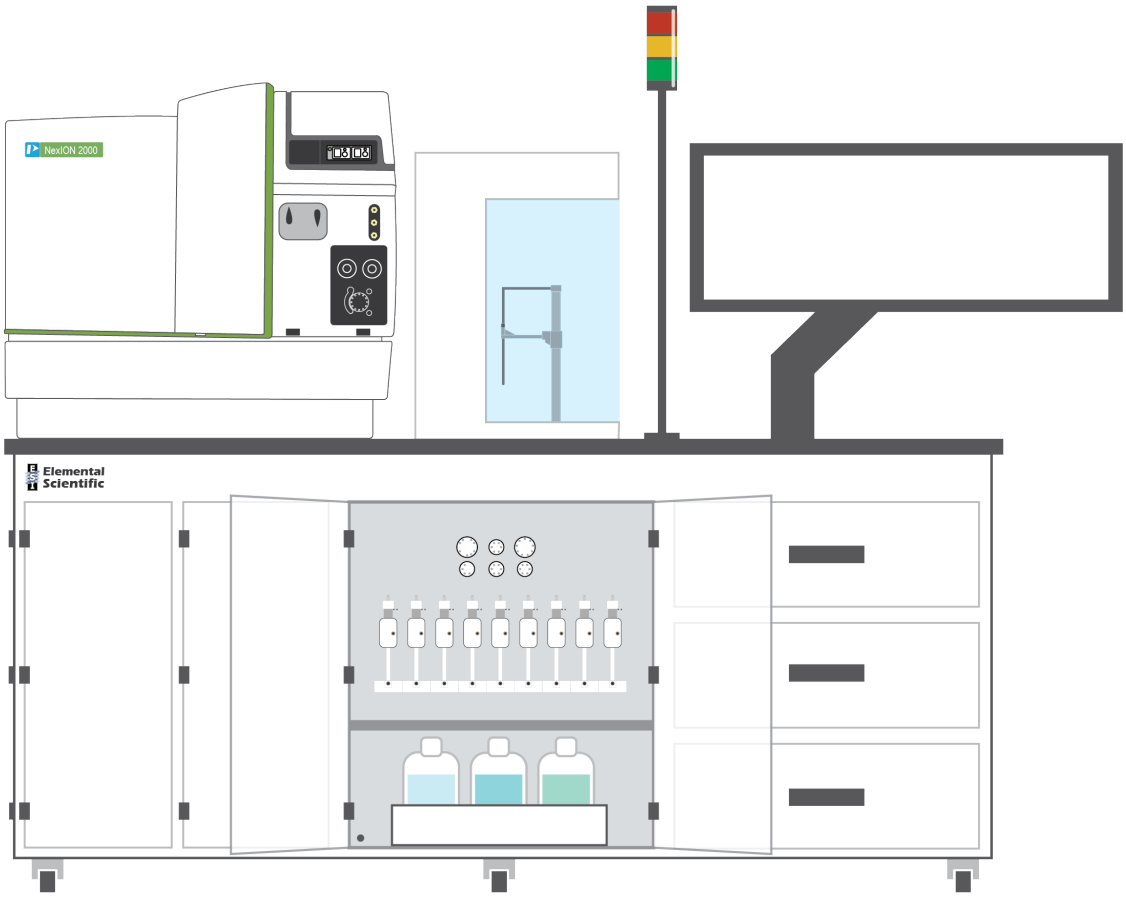
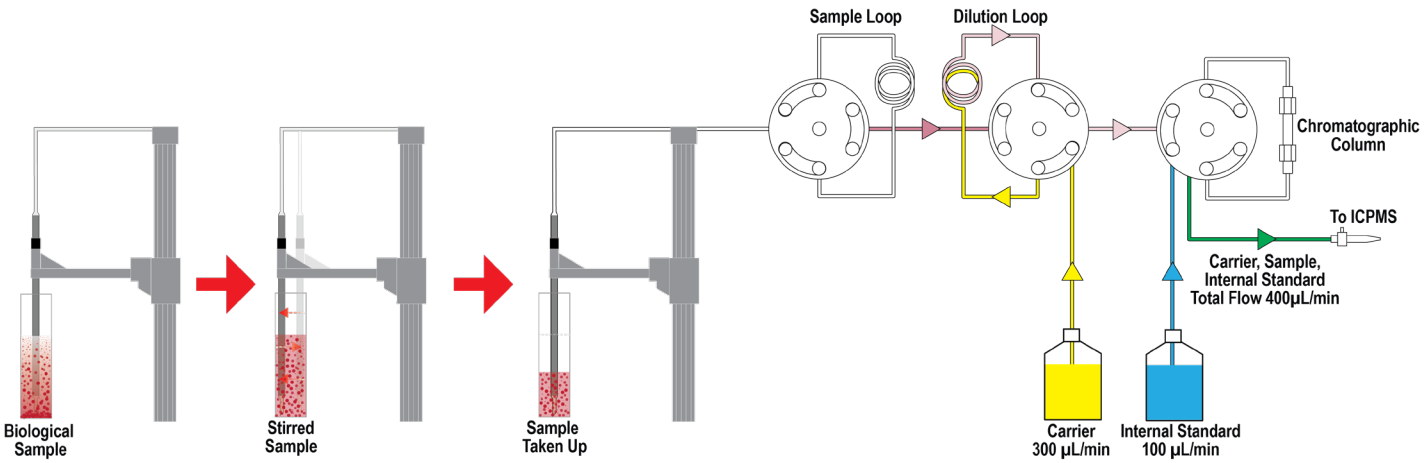


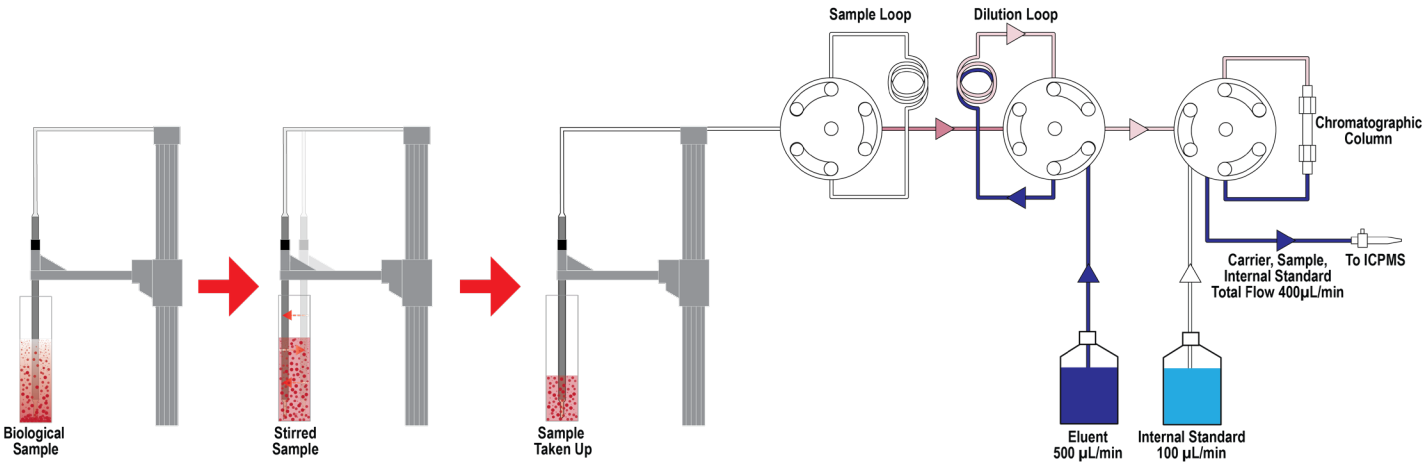
Diagram 1. prep*FAST* IC analytical station with NexION 2000.

Table 2. Elements included in each clinical method.

	ESI Part Number	Elements Included
Urine	FI-CLIN-Urine	Be, Al, Cr, Mn, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Sn, Sb, Cs, Ba, Gd, W, Pt, Tl, Hg, Pb, Bi, and U
Blood	FI-CLIN-Blood	Cr, Mn, Co, As, Se, Mo, Cd, Sb, Tl, Hg, and Pb
Serum, Plasma	FI-CLIN-Serum	Se, Al, Cr, Mn, Co, Fe, Cu, Zn, Pt, Hg, and Mg
Hair, Nails	FI-CLIN-Hair	As, Cd, Cu, Hg, Pb, Se, Zn, Ni, and Mn



Direct Mode. The probe stirs the sample followed by sample loading and sample dilution. After the sample has been diluted, it is introduced to the ICPMS, bypassing the chromatographic column.



Speciation Mode. The probe stirs the sample followed by sample loading and sample dilution. After the sample has been diluted, it is introduced to the chromatographic column. In speciation mode the carrier solution is automatically switched to the eluent for the desired elemental speciation method.

Results

Urine Multi-Element

The urine multi-element method measures 24 elements in standard and KED modes. To understand the day-to-day precision, pooled urine was spiked at two levels to create an in-house urine QC, which was measured at the beginning and end of each analysis. Figure 1 displays the day-to-day QC results for Be, As, and Pb. Over a 1-month time period, the precision for the low and high QC ranged from 3.6 – 4.6 % RSD. Figure 2 displays typical calibration curves for a few elements (Cr, As, and U) in the urine panel. These calibration curves were matrix-matched with ESI's Clinical Matrix, which simulates a urine matrix (also suitable as a clinical calibration matrix for blood, serum, plasma, nail, and hair) but has been purified for the elements of interest for these clinical methods. To validate this urine method, 2018 NYDOH PT samples were analyzed as reference materials (Table 3). The PT samples were analyzed over 3 different analytical runs with very good accuracy relative to the reference values reported by the NYDOH.

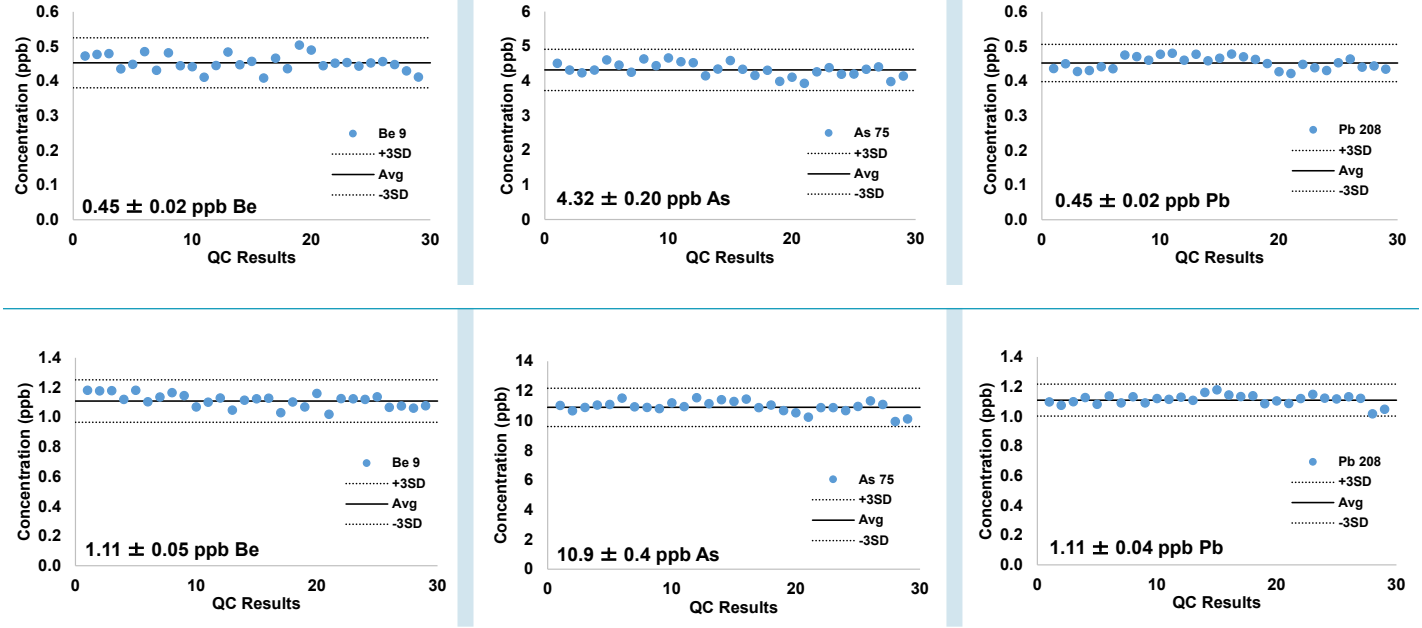


Figure 1. Precision for in-house quality control urine samples analyzed during each batch of samples. Results span over ~1 month of day-to-day analyses. Low (top) and high (bottom) QC results are displayed above for Be, As, and Pb.

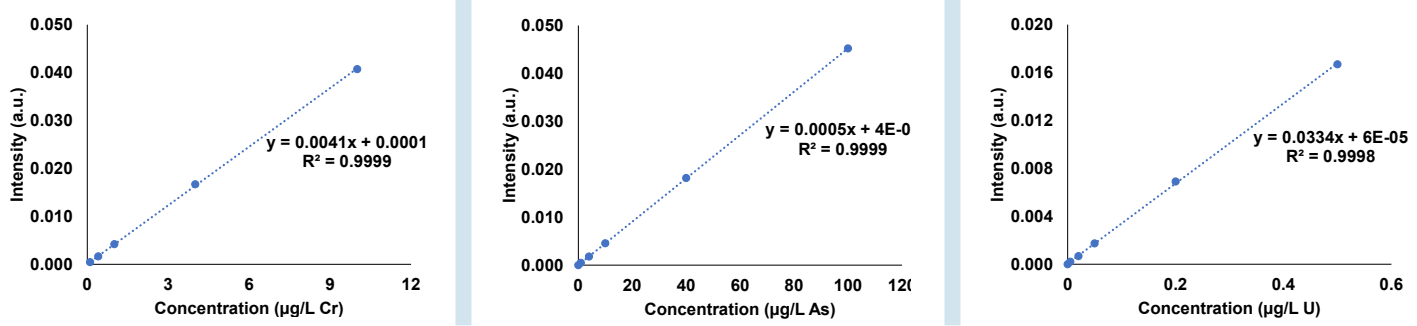


Figure 2. Example matrix-matched calibration curves (Cr, As, U) for the urine analysis panel using the synthetic calibration matrix.

Table 3. prep*FAST* IC results from 2018 NYDOH urine proficiency testing samples.

		As	Ba	Be	Cd	Co
UE 18-08	Reference Value (µg/L)	175	4.2	5.1	0.45	3.2
	Reference Range (µg/L)	140 - 210	3.2 - 5.2	4.1 - 6.1	0 - 1.45	1.7 - 4.7
	prep*FAST* IC - Measured Value (µg/L)	179	4.0	5.0	0.46	3.3
		Cr	**Hg**	**Mn**	**Pb (µg/dL)**	**U**
	Reference Value (µg/L)	1.8	7.9	7.5	2.2	0.138
	Reference Range (µg/L)	0 - 4.8	4.9 - 10.9	5.6 - 9.4	1.2 - 3.2	0.108 - 0.168
	prep*FAST* IC - Measured Value (µg/L)	2.6	6.3	7.7	2.3	0.140
		As	**Ba**	**Be**	**Cd**	**Co**
UE 18-10	Reference Value (µg/L)	81.7	0.9	0.76	3.63	0.73
	Reference Range (µg/L)	65.4 - 98.0	0 - 1.9	0 - 1.76	2.63 - 4.63	0 - 2.23
	prep*FAST* IC - Measured Value (µg/L)	85.4	0.9	0.73	3.76	0.75
		Cr	**Hg**	**Mn**	**Pb (µg/dL)**	**U**
	Reference Value (µg/L)	6.6	3.5	0.8	13.5	0.042
	Reference Range (µg/L)	3.6 - 9.6	0.5 - 6.5	0.25 - 1.35	10.8 - 16.2	0.012 - 0.072
	prep*FAST* IC - Measured Value (µg/L)	7.86	3.5	0.8	14.0	0.043
		As	**Ba**	**Be**	**Cd**	**Co**
UE 18-15	Reference Value (µg/L)	7.57	1.19	1.63	4.42	0.6
	Reference Range (µg/L)	1.57 - 13.57	0.19 - 2.19	0.63 - 2.63	3.42 - 5.42	0 - 2.10
	prep*FAST* IC - Measured Value (µg/L)	8.24	1.16	1.69	4.61	0.6
		Cr	**Hg**	**Mn**	**Pb (µg/dL)**	**U**
	Reference Value (µg/L)	3.43	4.8	3.24	3.2	0.0300
	Reference Range (µg/L)	0.43 - 6.43	1.8 - 7.8	2.43 - 4.05	2.20 - 4.20	0.0007- 0.0607
	prep*FAST* IC - Measured Value (µg/L)	4.31	3.8	3.26	3.3	0.0340

prep*FAST* IC - Measured values represent the average of 3 results (n = 3).
NYDOH PT urine samples were measured in 3 separate analyses.

Blood Multi-Element

The blood multi-element method measures 11 elements in standard and KED modes (optional use of DRC with O_2 for Mn). Figure 3 displays some example matrix-matched calibration curves for Mn, Pb, and Se using the Clinical Matrix. Validation of the blood method was done using UTAK reference materials and 2018 NYDOH PT samples. The results for the 2018 NYDOH PT samples are shown in Table 4. There was very good agreement between the measured values and the reference values except for the Mn results, which were all on the high end of the reference range. Typically, KED mode would be used to determine Mn; however, the combination of high Fe concentration and decreased abundance sensitivity (more tailing) of $^{56}Fe^{+}$ in KED mode causes a large interference at m/z 55 with a concentration equivalent approximately 10x the typical Mn concentration in whole blood. Therefore, standard mode was selected for the analysis. In standard mode there was a high bias when using Rh as an internal standard in the first set of experiments, but switching to Ga in a second set of experiments led to more accurate results. To confirm the absence of other significant interferences in standard mode, DRC mode using oxygen gas was also investigated and resulted in similar accurate recoveries for Mn when using Ga as an internal standard. These results can be found in Table 5.

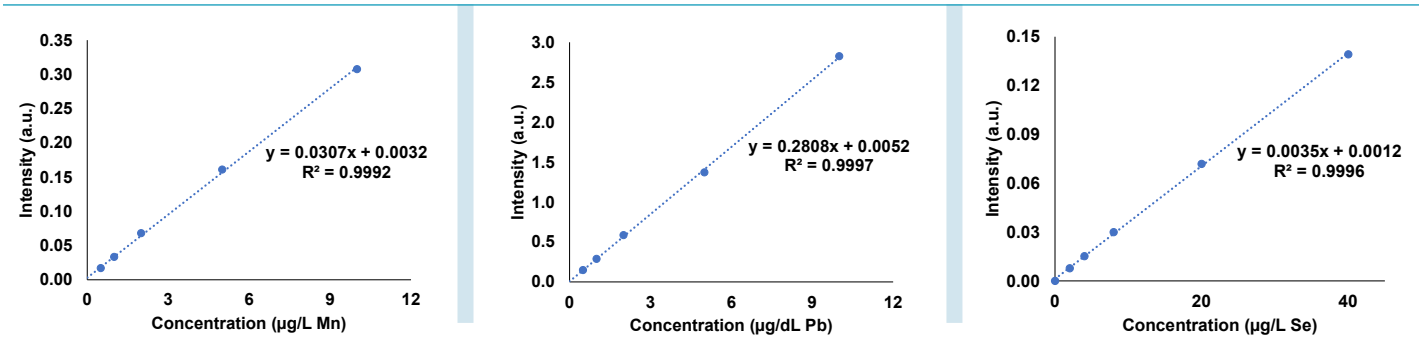


Figure 3. Example matrix-matched calibration curves (Mn, Pb, Se) for the blood analysis panel using the synthetic calibration matrix.

Table 4. prep*FAST* IC results from 2018 NYDOH blood proficiency testing samples.

		As	Cd	Co	Cr	Hg	Mn	Pb (µg/dL)
BE 18-04	Reference Value (µg/L)	40.3	3.4	13.6	10.1	18.5	12.4	42.7
	Reference Range (µg/L)	32.2 - 48.4	2.4 - 4.4	10.9 - 16.3	8.1 - 12.1	13.0 - 24.1	9.4 - 15.4	38.4 - 47.0
	prep*FAST* IC - Measured Value (µg/L)	39.5	3.0	14.3	11.6	21.4	15.0	40.5
BE 18-11	Reference Value (µg/L)	50.8	8.0	2.79	15.7	28.5	34.8	0.8
	Reference Range (µg/L)	40.6 - 61.0	6.83 - 9.23	1.29 - 4.29	12.6 - 18.8	20.0 - 37.1	28.9 - 40.7	0 - 2.832
	prep*FAST* IC - Measured Value (µg/L)	57.4	7.7	2.77	18.0	32.4	40.2	0.7
BE 18-15	Reference Value (µg/L)	22.9	11.2	12.2	4.6	22.1	28.5	3.55
	Reference Range (µg/L)	16.9 - 28.9	9.5 - 12.9	9.8 - 14.6	2.6-6.6	15.5 - 28.7	23.7 - 33.3	1.55 - 5.55
	prep*FAST* IC - Measured Value (µg/L)	25.8	10.7	12.4	4.4	25.0	33.0	3.42

prep*FAST* IC - Measured values represent the average of 3 results (n = 3).
NYDOH PT blood samples were measured in 3 separate analyses.

Table 5. Comparison of detection modes for Mn measurements in the 2018 NYDOH blood proficiency testing samples.

		Mn (Rh, Std Mode)	Mn (Ga, Std Mode)	Mn (Ga, DRC Mode)
BE 18-11	Reference Value (µg/L)	34.8	34.8	34.8
	Reference Range (µg/L)	28.9 - 40.7	28.9 - 40.7	28.9 - 40.7
	prep*FAST* IC - Measured Value (µg/L)	40.2	33.0	34.6
BE 18-15	Reference Value (µg/L)	28.5	28.5	28.5
	Reference Range (µg/L)	23.7 - 33.3	23.7 - 33.3	23.7 - 33.3
	prep*FAST* IC - Measured Value (µg/L)	33.0	27.2	27.7

DRC Mode = O_2 gas
Ga and Rh were compared as internal standards for Mn in Std mode.

Serum/Plasma Multi-Element

The serum/plasma multi-element method measures 11 elements in standard and KED modes. Figure 4 displays some typical matrix-matched calibration curves for Co, Cu, and Zn using the Clinical Matrix. Validation for the serum/plasma method was undertaken by measuring UTAK serum and NYDOH PT serum samples. The results for the 2018 NYDOH PT serum samples can be found in Table 6. The accuracy for all of the elements was very good; however, the Co results came back ~ 1 ppb lower than the target value but well within the reference range reported.

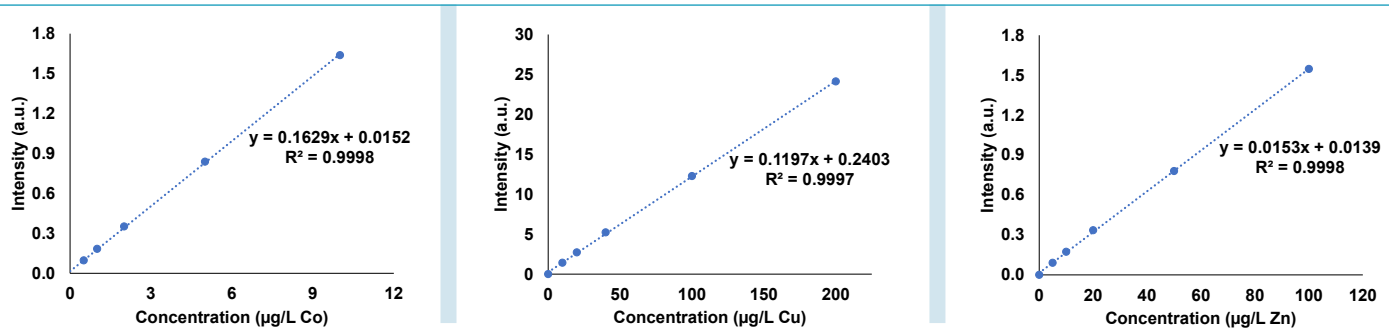


Figure 4. Example matrix-matched calibration curves (Co, Cu, Zn) for the serum/plasma analysis panel using the synthetic calibration matrix.

Table 6. prep*FAST* IC results from 2018 NYDOH serum proficiency testing samples.

		Co	Cr	Se	Zn	Cu
SE 18-02	Reference Value (µg/L)	1.2	9.2	310	1691	2011
	Reference Range (µg/L)	0.0 - 2.7	7.2 - 11.2	248 - 372	143 - 1945	1709 - 2313
	prep*FAST* IC - Measured Value (µg/L)	0.2	9.8	319	1663	2152
SE 18-12	Reference Value (µg/L)	6.7	3.0	116	2890	1990
	Reference Range (µg/L)	5.2 - 8.2	1.0 - 5.0	93 - 139	2460 - 3320	1690 - 2290
	prep*FAST* IC - Measured Value (µg/L)	5.8	3.3	117	2932	2079

prep*FAST* IC - Measured values represent the average of 3 results (n = 3).
NYDOH PT urine samples were measured in 3 separate analyses.

Hair/Nails Multi-Element

The hair/nails multi-element method measures 9 elements in standard and KED modes. Figure 5 displays typical matrix-matched calibration curves for Ni, Hg, and Cd using the Clinical Matrix. Validation for the hair/nails method was performed using in-house spike recovery studies. The results suggest that the method is both accurate and precise for the 9 elements of interest.

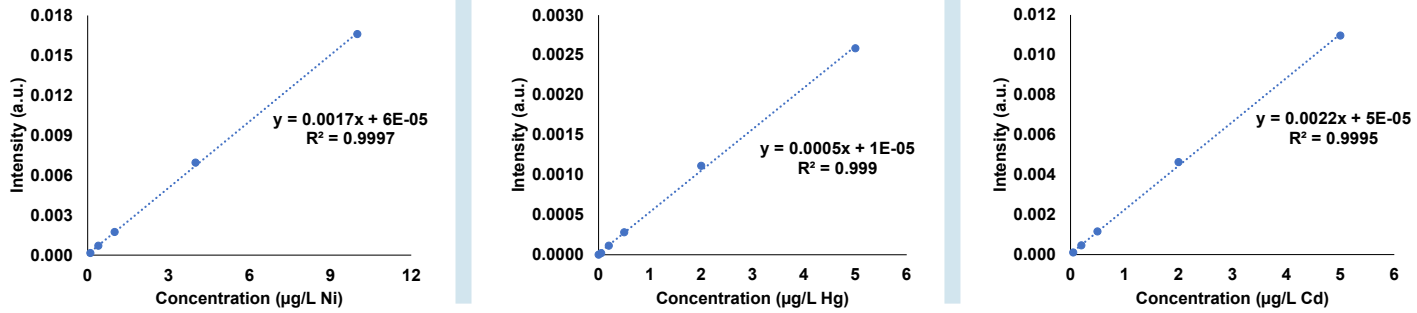


Figure 5. Example matrix-matched calibration curves (Ni, Hg, Cd) for the hair/nail analysis panel using the synthetic calibration matrix.

Urine Arsenic Speciation

All of the multi-element methods mentioned previously were operated with the prep*FAST* IC in "total metals" mode. For the urine arsenic speciation method, the prep*FAST* IC is operated in "speciation" mode; thus, the carrier is switched over to ammonium carbonate (eluent 1) and the diluent to DI water. This switching of modes is automated as part of the sample sequence in the ICPMS method. Figure 6 shows typical chromatograms for arsenic species calibration standards. The optimized separations and timings are displayed for the prep*FAST* IC. The prep*FAST* IC is capable of separating 6 arsenic species in under 2 minutes. The accuracy of this method has been previously demonstrated in JAAS (Quarles, et. Al., J. Anal. At. Spectrom., 2018, 33, 745-751). Table 7 displays some example patient results for arsenic species in urine. This particular laboratory only measures AsB, As(III), DMA, MMA, and As(V); therefore, there are no AsC results listed.

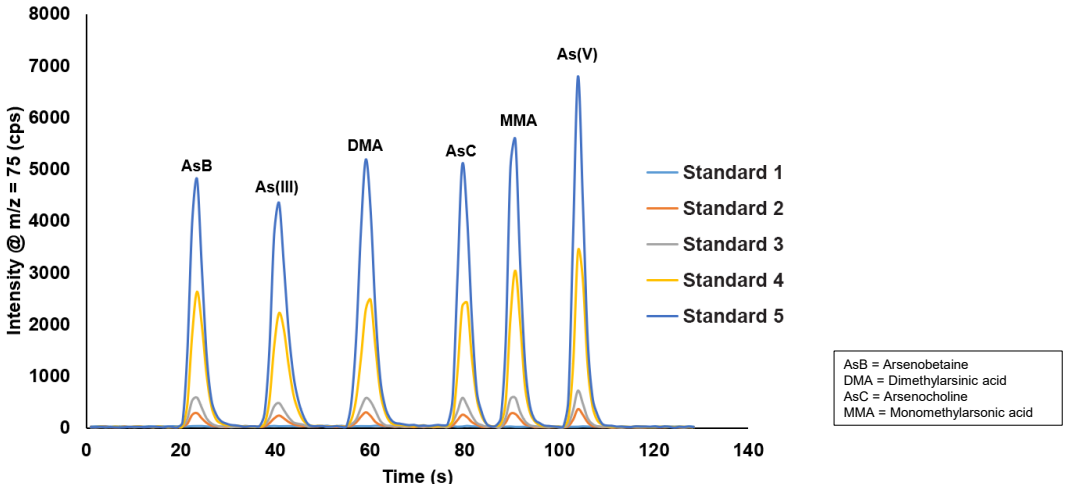


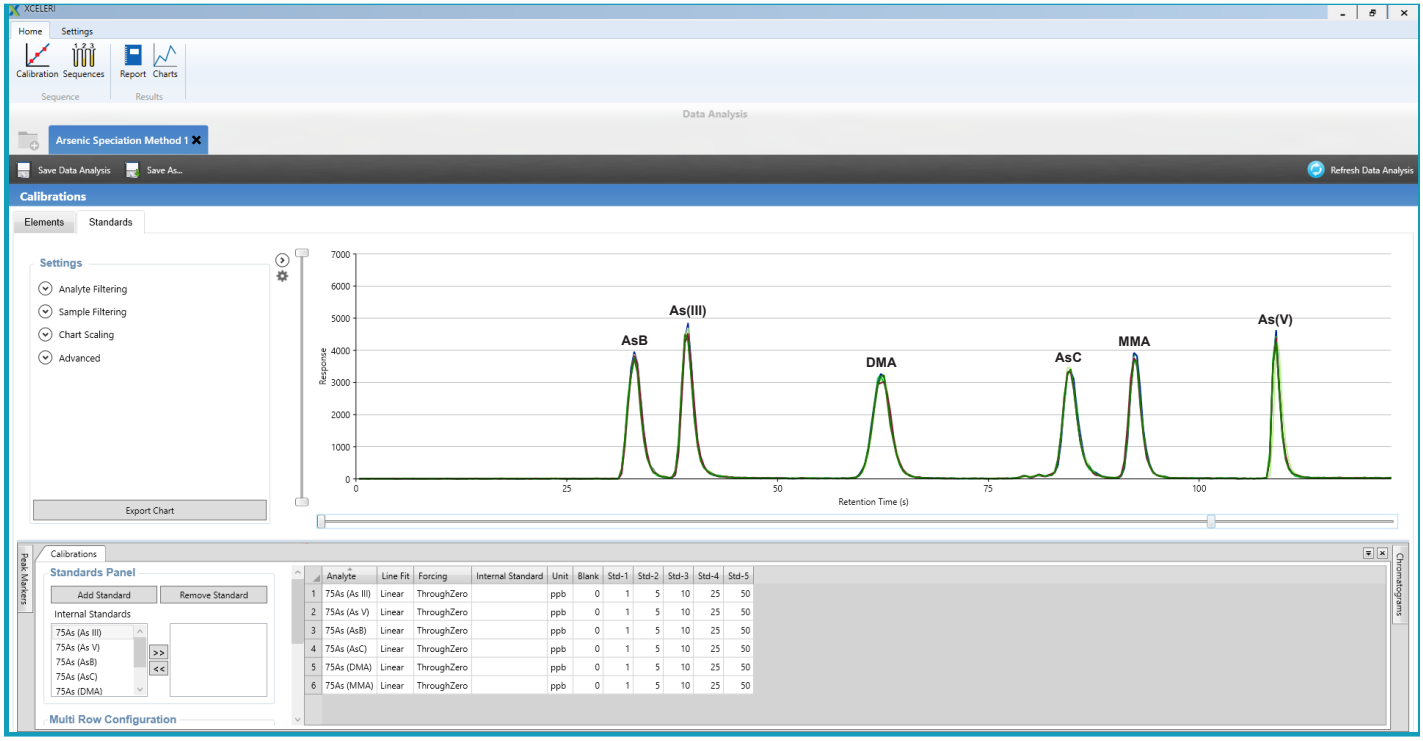
Figure 6. Example chromatograms for urine arsenic speciation obtained using the prep*FAST* IC.

Table 7. Example patient results obtained in a clinical laboratory for arsenic species using the Xceleri software package.

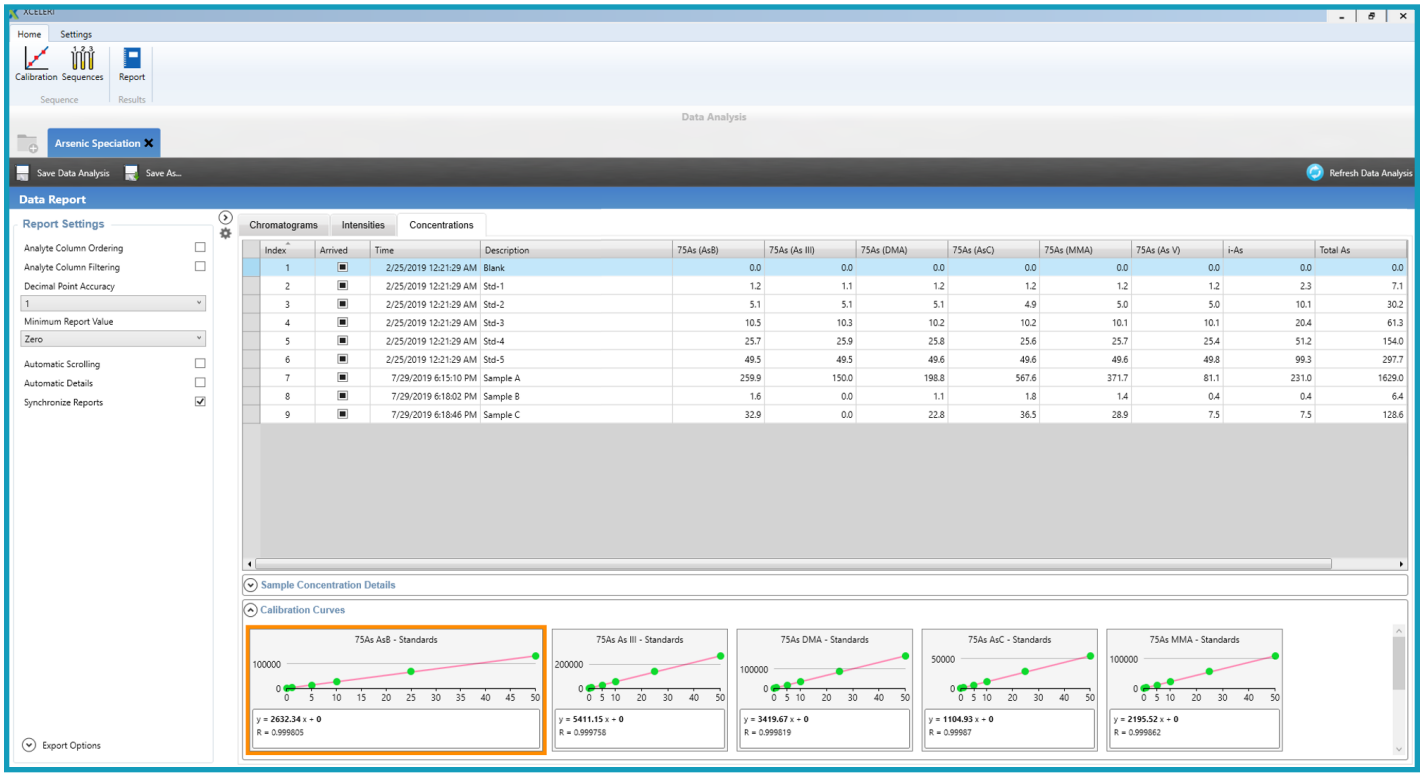
	ppb						
	AsB	As III	DMA	MMA	As V	Total As	i-As
Urine QC	**19.0**	**0.7**	**9.6**	**0.9**	**2.2**	**32.4**	**2.9**
Sample A	10.8	2.4	28.0	2.5	5.5	49.2	7.9
Sample B	14.2	0.9	23.0	1.1	2.1	41.3	3.0
Sample C	5.9	0.0	5.6	0.0	4.9	16.4	4.9
Sample D	0.3	0.4	3.2	0.0	3.6	7.5	4.0
Sample E	470	13.0	34.1	0.0	1.5	519	14.5
Sample F	0.4	16.2	65.7	23.8	6.0	112	22.2
Sample G	3.3	0.0	23.3	0.7	1.7	29.0	1.7
Sample H	9.3	1.1	9.3	2.3	2.4	24.4	3.5
Sample I	0.0	0.4	5.9	0.0	5.0	11.3	5.4
Sample J	5.0	0.2	4.4	0.0	2.1	11.7	2.3
Urine QC	**19.2**	**0.6**	**9.4**	**0.9**	**2.1**	**32.2**	**2.7**

Elemental Scientific has developed a data analysis package, Xceleri, which is capable of automating the peak integration process for elemental speciation (Display 1), overlaying chromatograms for comparison (Display 1), building calibration curves (Display 2), and custom report building (Display 2). Using the Xceleri software, the user can obtain real-time chromatograms while the sample is being collected within Syngistix using normal operating procedures. Once the sample or sample set is completed, it is easily loaded into Xceleri for data processing. The software is designed with ease of use in mind but is powerful enough to ensure accurate and reliable data. Using the custom report building function, the end user can add calculated columns, such as inorganic As (i-As = As(III) + As(V)) or total As, which is the sum of all the species detected. This allows the user to see at a quick glance if the inorganic As is the major constituent in the results.

Display 1. Screenshot from ESI's Xceleri software displaying overlaid chromatograms for a typical arsenic speciation analysis.



Display 2. Screenshot from ESI's Xceleri software displaying sample results and calibration curves for a typical arsenic speciation analysis.



Limits of Detection for Clinical Methods

The limits of detection (LOD) using the prep*FAST* IC in combination with a NexION 2000 ICPMS for each of the aforementioned multi-element methods are listed in Table 8. Except for Se (1.3 – 2.6 µg/L), all of the determined elements have detection limits in the ng/L range. The LODs for the six arsenic species in urine range from 0.3 - 1.7 ng/L As (Table 9).

Table 8. Estimated limits of detection in urine, blood, serum, plasma, hair, and nails with the prep*FAST* IC + NexION 2000 ICPMS.

Urine				Blood		Serum/Plasma		Hair/Nail	
Element	LOD (µg/L)	Element	LOD (µg/L)	Element	LOD (µg/L)	Element	LOD (µg/L)	Element	LOD (µg/L)
Be	0.003	Sn	0.01	Cr	0.6	Se	1.3	Mn	0.05
Al	0.5	Sb	0.02	Mn	0.2	Al	0.4	Ni	0.03
Cr	0.04	Cs	0.003	Co	0.04	Cr	0.3	Cu	0.04
Mn	0.07	Ba	0.1	As	0.09	Mn	0.08	Zn	0.2
Co	0.006	Gd	0.001	Se	2.6	Co	0.02	As	0.04
Ni	0.06	W	0.005	Mo	0.1	Fe	0.2	Se	1.5
Cu	1.1	Pt	0.01	Cd	0.05	Cu	0.1	Cd	0.02
Zn	0.1	Tl	0.009	Sb	0.02	Zn	0.1	Hg	0.1
As	0.3	Hg	0.05	Tl	0.01	Pt	0.007	Pb	0.03
Sr	0.7	Pb	0.02	Hg	0.1	Hg	0.07		
Mo	0.03	Bi	0.009	Pb	0.005 µg/dL	Mg	0.2		
Cd	0.02	U	0.001						

Table 9. Estimated limits of detection for arsenic species in urine with the prep*FAST* IC + NexION 2000 ICPMS.

	AsB	As III	DMA	AsC	MMA	As V
LOD (ppt)	1.7	0.6	0.7	0.8	1.0	0.3

Summary

- The prep*FAST* IC is capable of inline sample preparation for undiluted biological matrices (urine, blood, serum, plasma) and digested hair/nails.
- Biological samples can be automatically stirred on the autosampler deck and then loaded by syringe or vacuum, depending on the sample volume limitations.
- Validation of each method was successfully demonstrated using NYDOH PT samples.
- The prep*FAST* IC automatically switches between total metals and speciation, allowing for urine total and urine arsenic speciation to be combined into a single sample sequence.
- The prep*FAST* IC can be setup to automatically switch between two total metals methods, for example blood method (basic matrix) and urine method (acidic matrix).
- Elemental Scientific's Xceleri software package simplifies the data analysis process for speciation.
    - Easily overlay and compare standards/samples
    - Automated integration of peaks
    - Build calibration curves
    - Custom report building
    - Easily export chromatograms, raw data, concentrations, and calibration curves to Excel

prep*FAST* IC Publications

1. Quarles, Jr., C.D., Sullivan, P., Field, M.P., Smith, S., Wiederin, D.R., Use of an inline dilution method to eliminate species interconversion for LC-ICP-MS based applications: focus on arsenic in urine, J. Anal. At. Spectrom., 2018, 33, 745-751.
2. Quarles, Jr., C.D., Szoltysik, M., Sullivan, P., Reijnen, M., A fully automated total metals and chromium speciation single platform introduction system for ICP-MS, J. Anal. At. Spectrom., 2019, 34, 284-291.
3. Quarles, Jr. C.D., Manard, B.T., Wylie, E.M., Xu, N., Trace elemental analysis of bulk uranium materials using an inline automated sample preparation technique for ICP-OES, Talanta, 2018, 190, 460-465.
4. Manard, B.T., Metzger, S.C., Quarles, Jr., C.D., Rogers, K.T., Ticknor, B.W., Bostick, D.A., McBay, E.H., Hexel, C.R., Evaluation and specifications for in-line uranium separations using inductively coupled plasma optical emission spectroscopy (ICP-OES) detection for trace elemental analysis, Appl. Spectr., 2019, 73, 927-935.
5. Quarles, Jr., C.D., Toms, A.T., Smith, Jr., R.S., Sullivan, P., Bass, D., Leone, J., Automated ICP-MS method to measure bromine, chlorine, and iodine species in total metal content in drinking water, Talanta Open, 2020, 1, 100002.
6. Quarles, Jr., C.D., Macke, M., Michalke, B., Zischka, H., Karst, U., Sullivan, P., Field, M.P., LC-ICP-MS method for the determination of "extractable copper" in serum, Metallomics, 2020, 12, 1348-1355.

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