APPLICATION NOTE

HPLC-ICP-MS


**AUTHOR**

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# Determination of Selenium Species in Drinking Water by HPLC-ICP-MS

## Introduction

Selenium (Se) is an essential element at trace levels, toxic at higher levels, and exists in several oxidation states in a variety of inorganic and organic compounds. Consequently, the chemistry of selenium is complex in both the environment and biota.

For the determination of selenium species in water, the most practical approach is the use of a separation technique combined with a specific and sensitive detection system. Ion exchange (cation and anion) chromatography is often used for analyte separation with a highly sensitive detector, such as inductively coupled plasma mass spectrometry (ICP-MS), and has proven to be an effective hyphenated system in recent years[1].

The formation of polyatomic ions in the argon plasma causes some interferences with many selenium isotopes. One such major interference is the formation of the dimer $^{40}Ar^{40}Ar^{+}$ which overlaps with the most abundant selenium isotope, $^{80}Se$ (49.8% abundance). An effective way to eliminate polyatomic interferences in ICP-MS is by means of collision/reaction cell technology. Though helium is often used as an inert collision gas for many interferences, it is not ideally suited to polyatomic interferences larger than four orders of magnitude. $CH_4$, in contrast, can be used as an effective reaction gas to remove the $^{40}Ar^{40}Ar^{+}$ interference, which can achieve much lower detection limits compared to that when using helium as a collision gas. When using reaction gases in the cell, it is highly beneficial to use a cell design which allows the control of reactions inside the cell, for reliable and reproducible reaction chemistry.

In this study, five selenium species in drinking water were characterized in under 13 minutes using a simple isocratic anion-exchange method on PerkinElmer's NexSAR™ HPLC-ICP-MS speciation solution.

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## Experimental

### Standard and Sample Preparation

Calibration standards with concentrations of 0.2, 1, 2.5, 5, 10, 25 and 50 µg/L were prepared from the following reagents: Se (VI) from selenate (41.5 ± 1.3 µg/g), Se (IV) from selenite (42.9 ± 0.9 µg/g), seleno-cystine (44.2 ± 1.0 µg/g), (methyl) seleno-cysteine (34.8 ± 1.0 µg/g) and selenomethionine (39.4 ± 1.0 µg/g) all from the National Institute of Metrology, China (NIM, Beijing, China). These species were selected for evaluation due to the requirements of the Chinese standard examination methods for drinking water. Concentrations of the calibration standards were chosen according to the recommended concentrations per the regulation GB/T 5750.6[2]. Each species was identified according to its elution time by analyzing each selenium species separately.

Drinking water samples were collected in Shanghai, China and used directly. In the absence of a certified reference material, a spiked sample (spiked with 10 µg/L mixed selenium species) was prepared to verify the analytical accuracy of the method.

The mobile phase was prepared from citric acid (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) and pH adjusted to 4.7 by using $NH_3H_2O$ (≥ 25%, Sigma Aldrich, Burlington, Massachusetts, USA). During this work, the calibration standards and blanks were decanted into metal-free polypropylene HPLC vials and analyzed without dilution.

### Instrumentation

All analyses were performed using a NexSAR speciation analysis ready HPLC system (PerkinElmer, Shelton, Connecticut, USA) comprised of the NexSAR 200 Inert HPLC Pump, Cooled-Inert Autosampler and Solvent Tray with Degasser. The system was coupled to a NexION ICP-MS (PerkinElmer Inc.), a robust, high-performance system able to control the reaction inside the cell, a feature unique to quadrupole-based cells. Details pertaining to the HPLC and ICP-MS conditions are shown in Tables 1 and 2, respectively. Samples and standards were run in Reaction mode with $CH_4$ as a reaction gas to remove the $^{40}Ar^{40}Ar^+$ dimer interference. All analyses and the collection of data were performed using Clarity™ chromatography software for speciation analysis.

*Table 1: NexSAR Inert HPLC Conditions*

| Component / Parameter | Type / Value |
|---|---|
| Chromatography | Anion exchange chromatography |
| Mobile phase | 5 mM citric acid |
| pH | 4.7 |
| Flow | 1.5 mL/min |
| Separation scheme | Isocratic |
| Run time | 15 min |
| Injection volume | 100 µL |
| Column temperature | 30 °C |
| Injection type | Full loop |
| LC vials | HPLC tested PP vials, 1.5 mL |

*Table 2: NexION ICP-MS Instrument Parameters*

| Component / Parameter | Type / Value |
|---|---|
| Nebulizer | MEINHARD® Plus Glass Type C |
| Spray chamber | Glass Cyclonic |
| RF power | 1600 W |
| Injector | 2.0 mm I.D. quartz |
| Dwell time | 999 ms |
| Reaction gas | $CH_4$ |
| Flow rate (mL/min) | 0.7 |
| RPq | 0.4 |

## Results and Discussion

The correlation coefficients for the standards (0.2-50 µg/L, n=7) of seleno-cystine, (methyl) seleno-cysteine, selenite, selenomethionine and selenate were 0.999939, 0.999933, 0.999978, 0.999906 and 0.999988 respectively (Figure 1a-e), demonstrating excellent linearity across the expected concentration range for Se in drinking water. The overlay of the calibration standards from 0.2-50 µg/L (Figure 2) demonstrates the consistent, reliable and reproducible flows which can be delivered by the NexSAR 200 Inert HPLC Pump, thereby ensuring that peaks are correctly identified and are consistently sharp, allowing good accuracy and S/N ratios to be achieved.

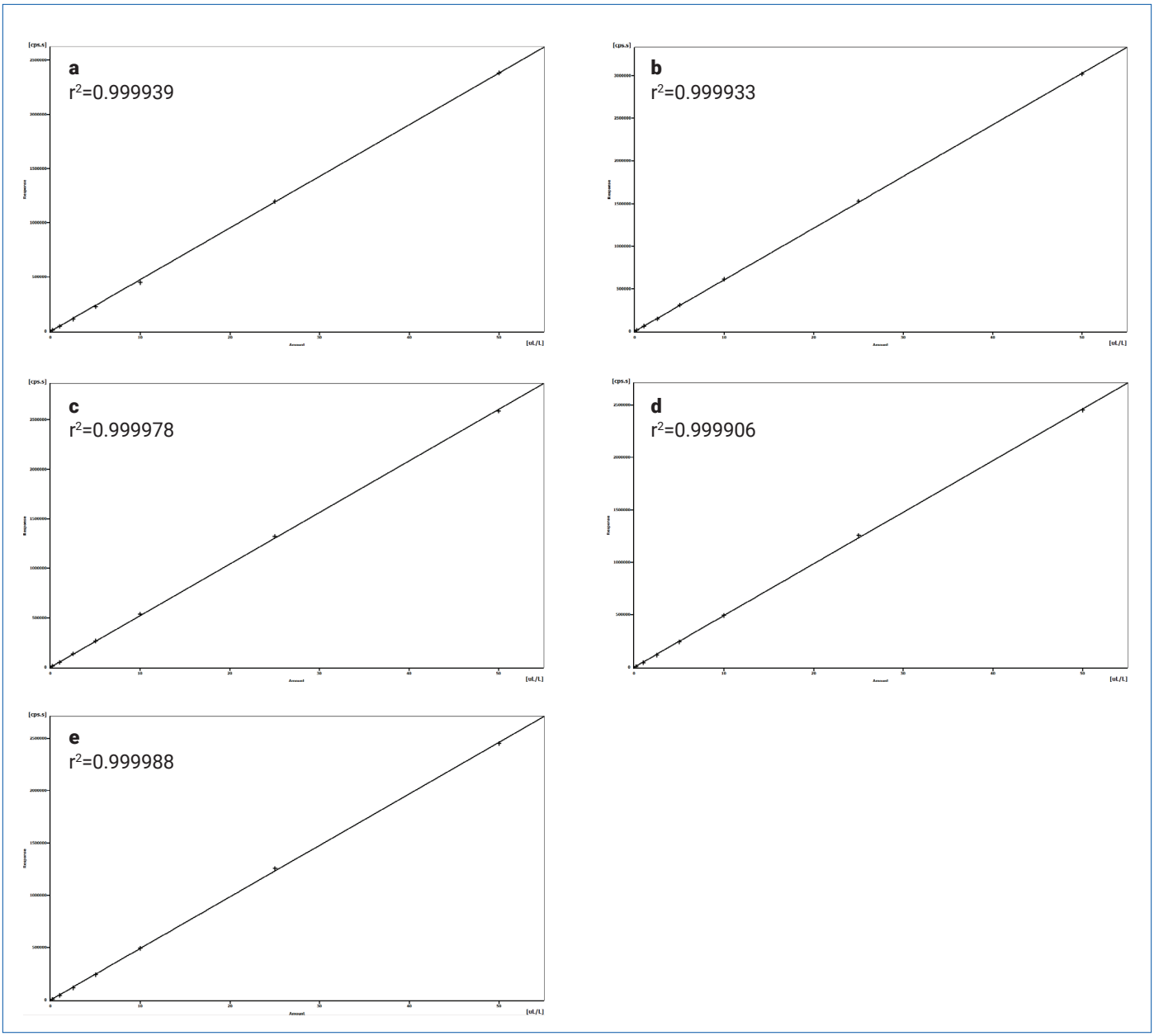


*Figure 1. Linear regression of calibration standards ranging in concentration from 0.2-50 µg/L for (a) seleno-cystine, (b) (methyl) seleno-cysteine, (c) selenite, (d) selenomethionine, (e) selenate in ultra-pure water, and the respective correlation coefficients.*

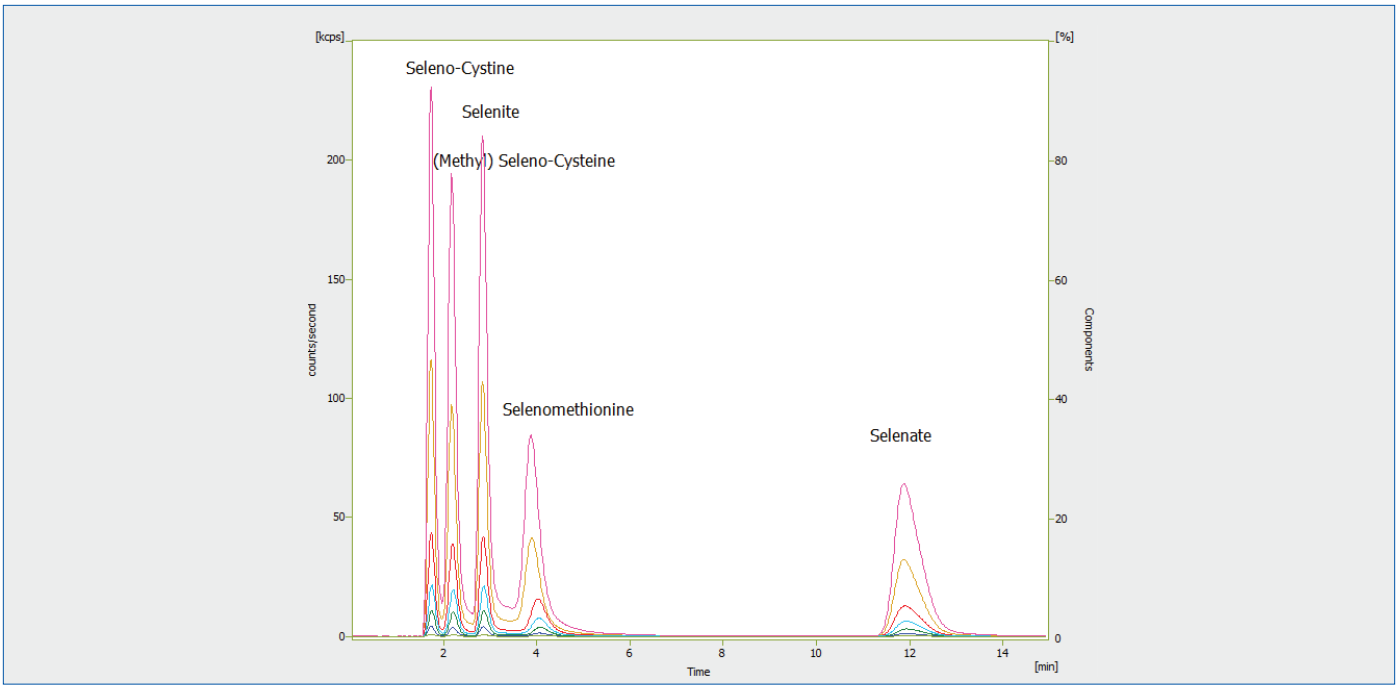


*Figure 2. Overlay of calibration standards (0.2-50 µg/L, n=7) in the mobile phase at pH 4.7.*

In this work, the ASTM noise evaluation function in Clarity™ software was used to determine quantitation limits for each species, according to Figure 3, using the criteria that the quantitation limit is the concentration which produces a peak which is 10 times the amplitude of the baseline noise i.e., 10 S/N, the same as specified by GB/T 5750.6. The quantitation limits for seleno-cystine, (methyl) seleno-cysteine, selenite, selenomethionine and selenate were 17, 10, 17, 76 and 60 ng/L respectively by using the defined mobile phase. As shown in Figure 4, the quantitation limits are more than an order of magnitude lower than specified in GB/T 5750.6 for all species. If lower quantitation limits are required, there are multiple ways they can be achieved, such as increasing the dwell times which can improve the signal-to-noise ratios, higher column temperatures (if allowed by the column specifications), different column (i.e., narrow bore), mobile phase, etc.

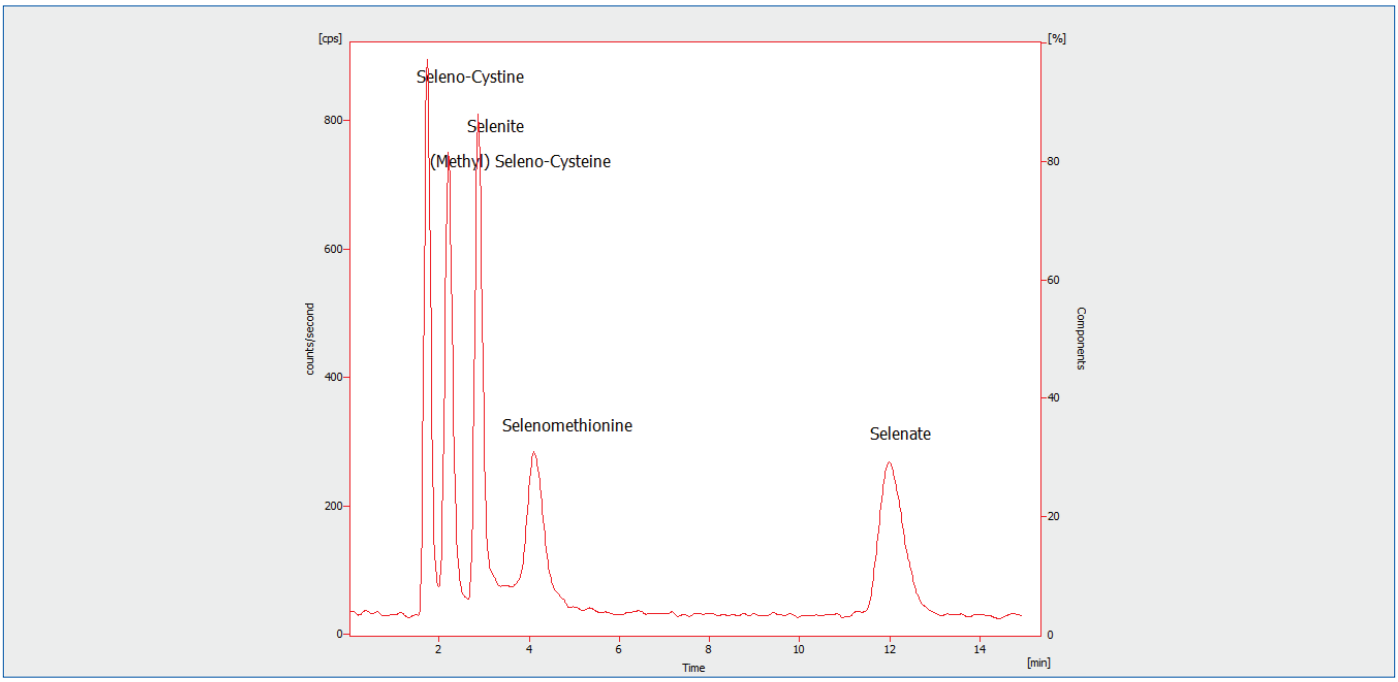


*Figure 3. 200 ng/L standard of five species of selenium to determine the theoretical detection limits.*

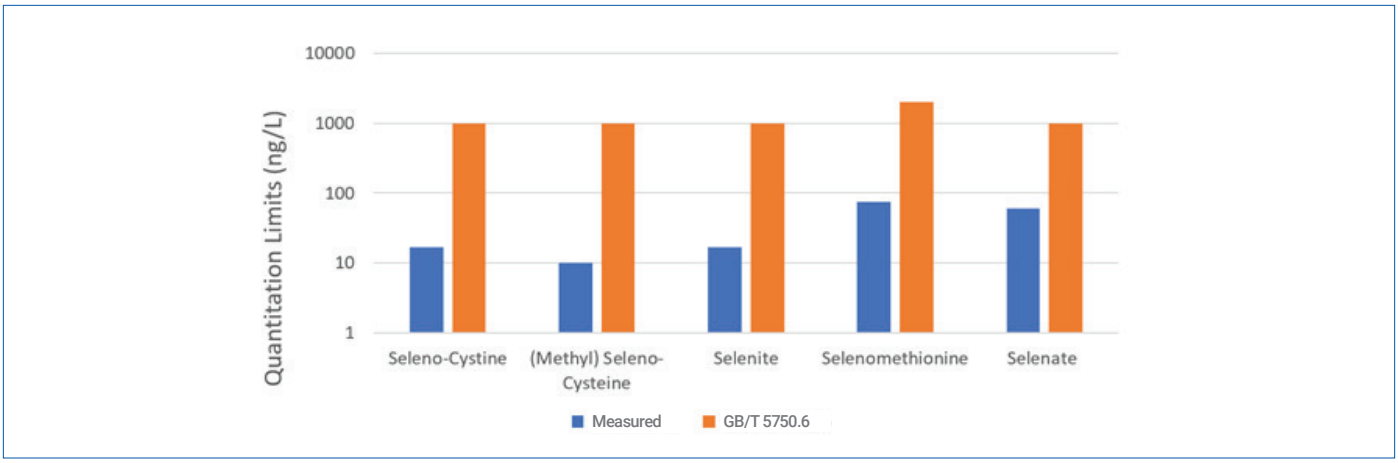


*Figure 4. Quantitation limits specified in GB/T 5750.6 and measured.*

The overlay of the unspiked drinking water, the spiked (2.5 µg/L) drinking water sample and the 5 µg/L standard can be seen in Figure 5. Seleno-cystine, (methyl) seleno-cysteine, selenite and selenomethionine were below the quantitation limits in the drinking water samples, and selenate was measured to be 0.263 µg/L in the drinking water sample. This overlay demonstrates that the sample matrix does not have an effect on the retention time of different Se species, proving the robustness of the method which is enhanced through the accuracy of the pump flows. Excellent spike recoveries of 109, 100, 108, 91 and 103% were achieved for seleno-cystine, (methyl) seleno-cysteine, selenite, selenomethionine and selenate, further validating the method and proving its accuracy for each individual Se species in the sample matrix.

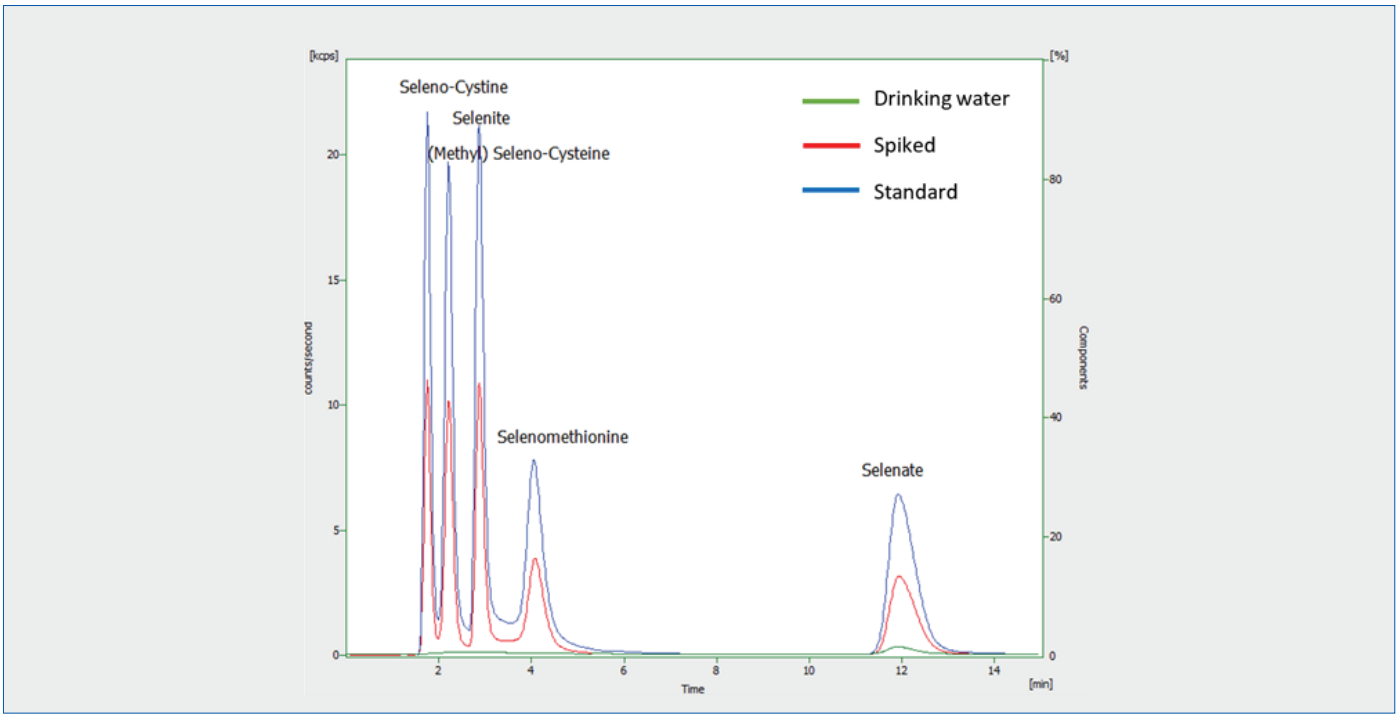


*Figure 5. Overlay of chromatograms for the drinking water sample, spiked drinking water sample and 5 µg/L standard.*

## Conclusion

This study evaluated Se species in drinking water using a NexSAR HPLC-ICP-MS speciation solution comprising of an inert NexSAR HPLC system coupled to a NexION ICP-MS. The isocratic method allowed for the complete baseline separation of all species in less than 13 minutes. The robust plasma of the NexION ICP-MS and accurate flow of the mobile phase delivered by the NexSAR HPLC pump are critical to achieving consistent, repeatable separations and analyses, as demonstrated in this work. The ability to use reaction gases in a true quadrupole reaction cell allowed to selectively control the reactions taking place in the cell and actively eject interfering ions. The measured quantitation limits far exceed those specified in GB/T 5750.6. Therefore, it can be concluded that determination of seleno-cystine, (methyl) seleno-cysteine, selenite, selenomethionine and selenate in drinking water using the NexSAR HPLC-ICP-MS speciation solution meets and exceeds the requirements of GB/T 5750.6.

## References

1. C. B'Hymer, et al, "Selenium speciation analysis using inductively coupled plasma-mass spectrometry", Journal of Chromatography A, Vol. (1114) 1-20, 2006.
2. GB/T 5750.6 Standard examination methods for drinking water— Part 6: Metal and Metalloid Indices.

## Consumables Used

| Component | Description | Part Number |
|---|---|---|
| HPLC Vials | HPLC Tested Plastic Vials, 1.5 mL PP | N9301736 |
| PEEK Tubing | Yellow, 0.007" ID, 1/16" OD (5 feet) | N9302678 |
| PEEK Fittings | Fingertight for 1/16" OD PEEK Tubing | 09920513 |
| Nebulizer Connector | Column-to-Glass Concentric Nebulizer Connector | N8152484 |

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