APPLICATION NOTE

HPLC-ICP-MS

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Quantification of Low Levels of Hexavalent Chromium in Water Using a NexSAR Inert HPLC-ICP-MS

Introduction

Due to rising concerns over the carcinogenic properties of hexavalent chromium (Cr^{6+}) in drinking water, many national and regional water standards are looking to lower the maximum allowable levels of total chromium and hexavalent chromium in potable waters. The California Office of Environmental Health Hazard Assessment (OEHHA), for example, established a Public Health Goal (PHG) for Cr^{6+} of 0.02 ppb to represent the level of hexavalent chromium which, based upon animal studies, has been found to not pose as significant a risk to health.[1] Hexavalent chromium is also regulated in China and Japan. The World Health Organization (WHO/SDE/WSH/03.04/4) and the European Union-Drinking Water Directive (98/83/EC) established a 50 ppb drinking water standard for total chromium in water from European countries. However, given that many natural surface waters have a slightly basic pH and are well-oxygenated, hexavalent Cr is often the prevalent form of Cr in such systems, and as such, this limit is expected to be re-evaluated to include the characterization of Cr^{6+} in the near future.[2]

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In order to assess trace concentrations of Cr^{6+} in drinking water, it is necessary to have an instrument capable of measuring ppt concentrations of Cr and possessing a wide linear dynamic range. For this reason, HPLC-ICP-MS is often the instrumentation of choice in such applications. Since most drinking water sources have low chromium concentrations, it is essential that the chromatographic baselines are also low in order to be able to accurately characterize and quantify the Cr species. Consequently, it is important to consider the fluid path of the HPLC being used. Despite the typical passivation processes employed in metal-based systems, over time many of these systems will experience corrosion.[3] This corrosion cannot only block and permanently damage expensive columns but can also raise the chromatographic baseline for Cr and adversely impact the detection limits. To mitigate this effect, it is imperative to use an HPLC with a completely metal-free fluid path, thereby dramatically lowering detection limits for a number of analytes, such as Cr, which are typically found in high abundance in metal-based LC systems.

A further challenge often encountered in the HPLC-ICP-MS analysis of Cr is that all mobile phases have some level of carbon, where $^{40}Ar^{12}C$, in addition to $^{35}Cl^{16}OH^{+}$, poses as a potential interferent on ^{52}C, significantly increasing the chromatographic baseline. There is a great need to reduce the baseline without sacrificing on sensitivity, hence, using a reaction cell which is able to actively control the reaction and prevent side-reactions from taking place is highly beneficial. Moreover, a cell which is able to chemically target interferences through the use of pure gases, such as ammonia, is far more efficient at removing interferences than passive cells, such as hexapoles or octopoles. It is through this effective removal of the interfering ions that ultra-low detection limits for Cr can be achieved.

In this work, we report a method for the measurement of hexavalent Cr in drinking water. Different types of drinking water, namely twelve drinking water samples from spring, tap and groundwater sources, were analyzed to demonstrate the wide applicability of the method. Trivalent Cr was not analyzed in the samples since it is considered to be an essential micronutrient and not of toxicological relevance, however it was added to the calibration standards to ensure that peak separation had been achieved. The analysis was performed using a PerkinElmer NexSAR™ HPLC-ICP-MS solution, which is comprised of an inert NexSAR Speciation Analysis Ready HPLC system coupled to a PerkinElmer NexION® ICP-MS. Due to the narrow temperature tolerance of the column and the need for reproducible retention times, elution was thermostatically controlled using a NexSAR Column Oven.

Experimental

Sample Preparation

Twelve drinking water samples comprised of commercial spring water, tap water and groundwater (well water) collected from various sources were filtered through 0.45 µm PTFE filters (hydrophilic, Millex, Sigma Aldrich™, St. Louis, Missouri, USA) to remove unwanted particulate matter. This variety of water samples was used to demonstrate the wide applicability and suitability of the proposed analytical method for the characterization of hexavalent Cr. After filtration, samples were diluted two-fold (1:1) in mobile phase (Table 1) and allowed to complex for three hours at room temperature prior to analysis. This mobile phase was prepared to pH 7 because a neutral pH has been shown to promote the stability of chromium species when complexed with ethylenediaminetetraacetic acid (EDTA).[3]

Calibration standards with concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 and 10 ppb were prepared in the mobile phase (Table 1) to reflect the required range between the PHG and the maximum contaminant level (MCL) for Cr VI as set by the State of California in 2014. Calibration standards were prepared from the following reagents: Cr (III) from 1000 ± 4 mg/L trivalent chromium (Inorganic Ventures, Christiansburg, Virginia, USA) and Cr (VI) from 996 ± 3 mg/L hexavalent chromium (Inorganic Ventures).

To test for contamination, samples were divided among different polypropylene HPLC vials for analysis. Due to the absence of a certified reference material, validation of the method was achieved through the implementation of sample spikes (0.05 ppb and 1 ppb Cr VI) to cover the range of Cr VI typically found in potable waters.

Instrumentation

All analyses were performed using a NexSAR Speciation Analysis Ready HPLC system (PerkinElmer, Inc., Shelton, Connecticut, USA) comprised of the NexSAR 200 Inert HPLC Pump, Cooled Inert Autosampler, Solvent Tray and Degasser, Column Oven and Switching Valve. The NexSAR Autosampler hardware consisted of a 200 µL PEEK sample loop, 500 µL syringe with 1000 µL buffer tubing and 15 µL inert injection needle, all of which ensure a metal-free fluid path which will not leach metals into the mobile phase that could impact the chromatographic baseline and consequently the limit of detection (LOD). The system was coupled to a NexION ICP-MS (PerkinElmer, Inc.). Details pertaining to the HPLC and ICP-MS conditions and method of analysis are shown in Tables 1 and 2, respectively, and were based upon previous work.[4] All analyses and the collection of data were performed using Clarity™ software v 8.2 (DataApex, Prague, The Czech Republic).

Table 1. NexSAR Inert HPLC System Conditions

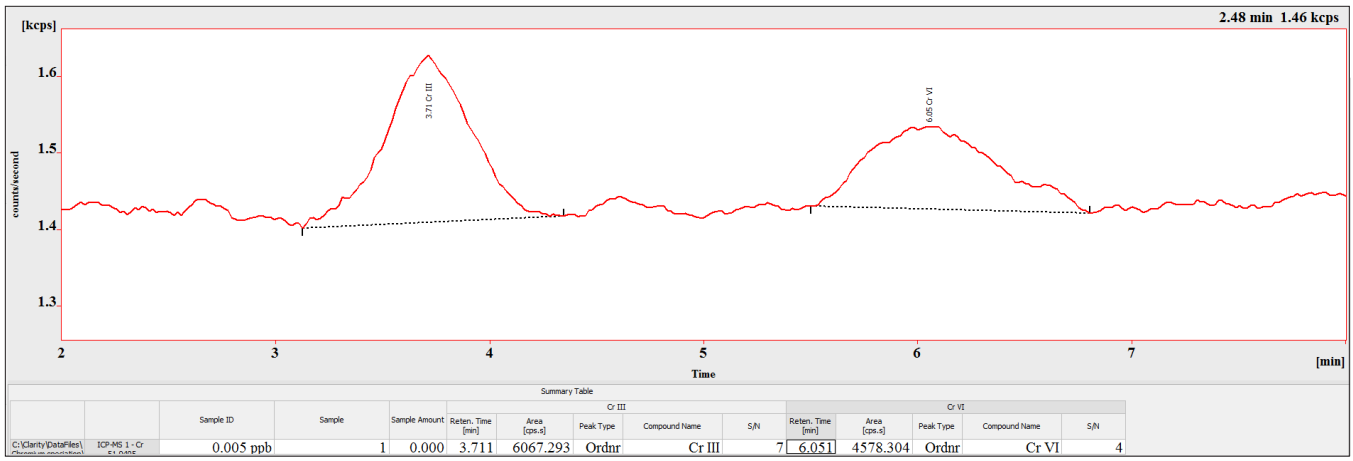
Parameter	Value
Chromatography	Ion exchange chromatography
Mobile Phase	Ion-exchange reagent
Flow Rate	1.5 mL/min
Separation Scheme	Isocratic
Injection Volume	200 µL, full loop mode
Column Temperature	30 °C
LC Vials	HPLC tested PP vials, 1.5 mL

Table 2. NexION ICP-MS Instrument Conditions.

Parameter	Value
Nebulizer	MEINHARD® plus Glass Type C
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Injector	2.0 mm I.D. quartz
Nebulizer Gas Flow Rate	Optimized for <2% oxides
Mode	Reaction mode, NH_3, 0.5 mL/min
RPq	0.8
Dwell Time	999 ms

Results and Discussion

Due to the inert fluid path of the NexSAR HPLC system, there was no undue elevation of the chromatographic baseline which is commonly seen in metal-based systems. This allowed for the quantification and accurate characterization of ultra-trace levels of Cr VI. This can be seen in Figure 1, where 0.005 ppb (5 ppt) of Cr VI was easily quantified using the specified system with a signal-to-noise of four for Cr VI. Cr III is also shown for reference. Since the LOD in chromatography is determined by S/N ratios of three, the results provide a theoretical detection limit of 3.8 ppt for Cr VI using the defined method, ensuring a limit of quantification (LOQ) of 12.5 ppt and demonstrating that concentrations around the California PHG can be quantified with confidence.



Summary Table

		Sample ID	Sample	Sample Amount	Cr III Reten. Time [min]	Cr III Area [cps.s]	Cr III Peak Type	Cr III Compound Name	Cr III S/N	Cr VI Reten. Time [min]	Cr VI Area [cps.s]	Cr VI Peak Type	Cr VI Compound Name	Cr VI S/N
C:\Clarity\DataFiles\	ICP-MS 1 - Cr	0.005 ppb	1	0.000	3.711	6067.293	Ordnr	Cr III	7	6.051	4578.304	Ordnr	Cr VI	4

Figure 1. Chromatogram (moving average) of 0.005 ppb (5 ppt) standard of Cr III and Cr VI in mobile phase without blank subtraction.

The correlation coefficient for the standards (0.005 – 10 ppb, n=8) of Cr VI was 0.99999 (Figure 2), showing good linearity across the range for the PHG and MCLs, as defined by California water standards. The overlay of the calibration standards from 0.005-10 ppb (Figure 3) demonstrates the consistency of the pump flow rate as well as the indispensability of the column oven, where these components work together to ensure that retention times are consistent and that peaks are not incorrectly identified. An added benefit of the NexSAR Column Oven is the line pre-heater, which ensures that later-eluting peaks are sharper, improving detection limits and making sure that there is little dispersion due to fluctuations in the column temperature over the column length.

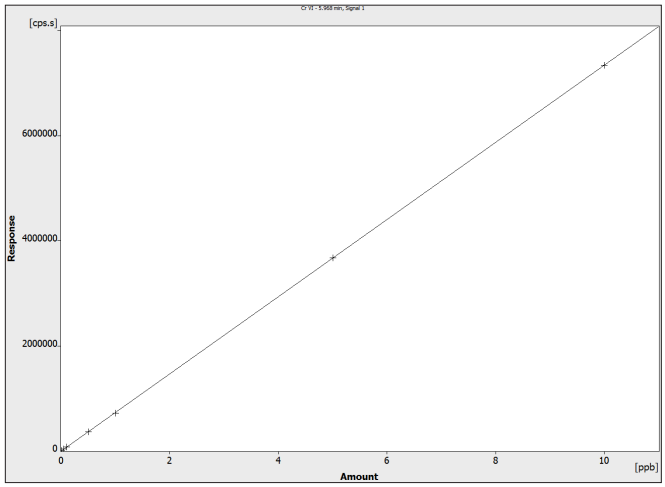


Figure 2. Linear regression of calibration standards (blank subtracted) for Cr VI ranging in concentration from 0.005-10 ppb (n=8).

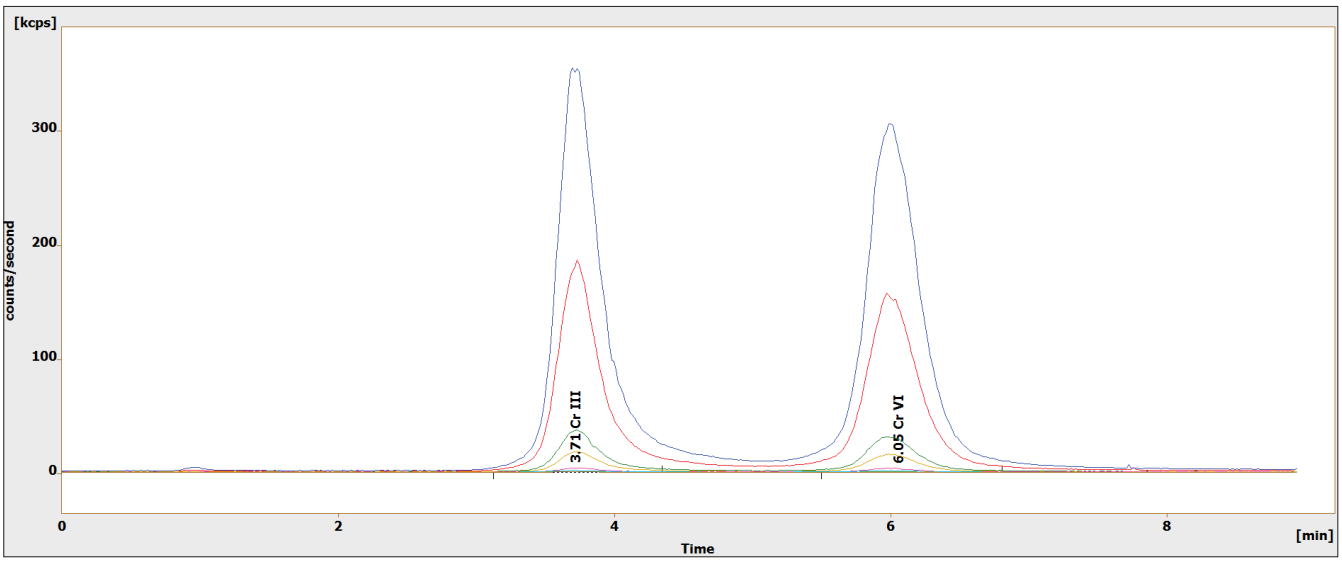


Figure 3. Chromatogram showing overlay of calibration standards (0.005 – 10 ppb, blank subtracted) in the mobile phase at pH 7.0.

In order to assess the impact of the matrix upon analytical accuracy, three groundwater, spring water and tap water samples were diluted (1:1) with the mobile phase, and a low-end (0.05 ppb) and high-end (1 ppb) spike of Cr VI was added to two of each sample matrix. The undiluted and spiked samples were thereafter quantified. As shown in Figure 4, the spike recoveries for Cr VI for the different sample matrices were excellent, ranging between 96% and 99% for the groundwater sample, 92% and 106% for the tap water sample, and 98% and 97% for the spring water sample for the low and high concentration spikes, respectively. These results prove the accuracy of the method across a larger linear dynamic range, encompassing the PHG and MCL, and its applicability in a wide range of potable drinking waters for the quantitation of Cr VI. Since Cr VI is the species of analytical interest, it is possible to use the NexSAR Switching Valve to divert the sample to waste for the first part of the analysis where salts and matrix ion elute and return in time for the acquisition of the analyte of interest, Cr VI, all of which can be easily automated through Clarity™ chromatography software.

As can be seen in Figure 5, all samples evaluated contained less than 0.12 ppb (120 ppt) of Cr VI, and can be outlined as follows:

- Highest concentrations were found in three of the four spring water samples which were evaluated;
- The groundwater samples were all below the detection limits of the method (BD);
- Tap water samples had the second highest concentrations of Cr VI, where the filtered tap water sample had lower concentrations of Cr VI than the other tap water samples.

These results, coupled with excellent spike recoveries and calibration correlation coefficients, demonstrate the applicability of the proposed method in the evaluation of hexavalent Cr in a variety of different potable waters and shows that the NexSAR Inert HPLC, coupled to the NexION ICP-MS, is able to quantify low concentrations of Cr VI with confidence. Based upon the PHG of 0.02 ppb (20 ppt) Cr VI as set by the State of California, all samples except for the groundwater samples exceeded this concentration, but were all below the current MCL of 10 ppb for Cr VI.

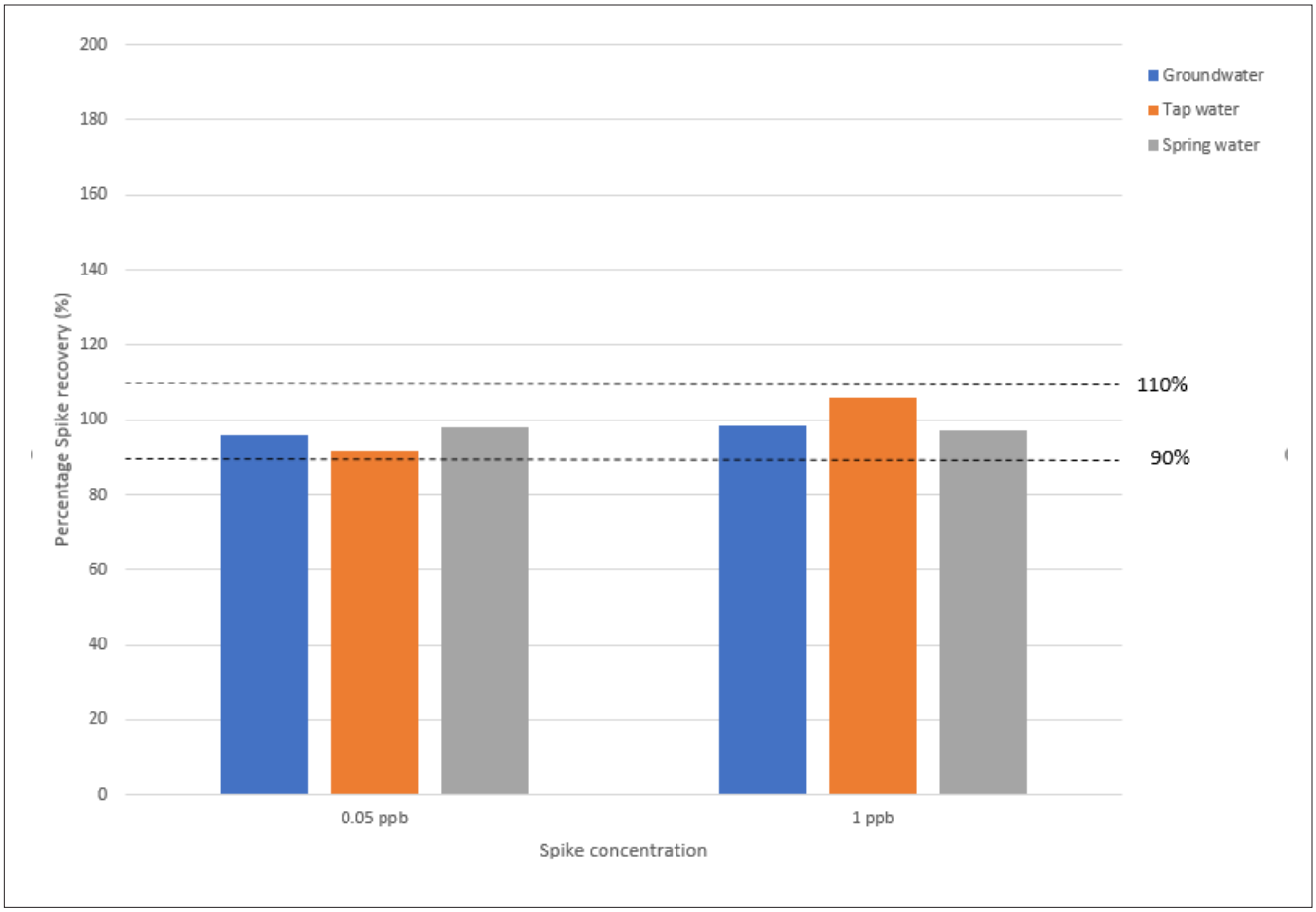


Figure 4. Spike recoveries for low concentration (0.05 ppb) and high concentration (1 ppb) Cr VI in groundwater, tap water and spring water samples.

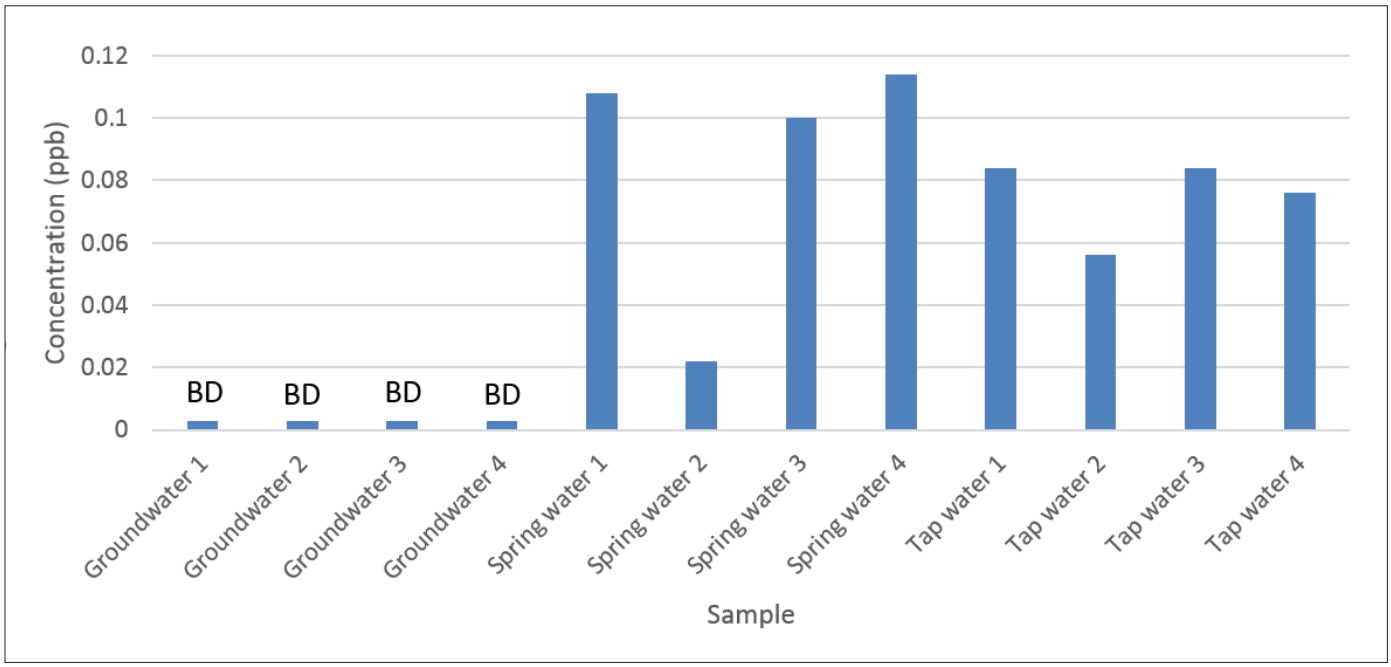


Figure 5. Hexavalent chromium concentrations in 16 samples of groundwater, commercial spring water and tap water origins, where concentrations below the detection limits are shown by 'BD'.

Conclusion

In this study we presented a method for the ultra-low detection and characterization of hexavalent chromium in potable waters in less than eight minutes with excellent accuracy. The inert and metal-free fluid path of the NexSAR Inert HPLC, coupled with the NexION ICP-MS' Universal Cell Technology™, which controls reactions and can rapidly eject interfering ions and reaction byproducts from the cell, were able to ensure that a calculated LOD of 3.8 ppt could be attained. This allowed a limit of quantification as low as 12.5 ppt and provided confidence that concentrations around the PHG of California could be easily assessed. As demonstrated, hexavalent chromium could be observed in all samples except for groundwater, which had come from a pristine location, and the Cr VI in these samples was below the method detection limit.

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
200 µL Loop	200 µL PEEK sample loop	N8152913
PEEK Guard Column	Anion exchange guard column	N8122254

References

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