High Sensitivity HPLC Analysis of Perchlorate in Tap Water Using an Agilent 6460 Triple Quadrupole LC/MS System

Application Note

Environmental

Authors

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Abstract

A modification of EPA method 331.0 for analysis of perchlorate in tap water with a method detection limit of 0.009 µg/L has been developed using an Agilent 6460 Triple Quadrupole LC/MS system. It provides very similar overall performance to EPA 331.0, including method detection limit, recovery and repeatability.

Agilent Technologies

Introduction

Perchlorate is used as an oxidizer in flares, pyrotechnics, and explosives; and is often associated with release from defense and military operations. Natural sources include certain fertilizers and potash ores. Perchlorate has been detected in drinking and surface waters in the United States and Canada, as well as in soil, vegetation, and groundwater. In addition to its ubiquity, perchlorate is also very persistent in the environment.

Although perchlorate has been detected in groundwater since the 1950s, its toxicological properties have only recently been studied in detail. At high concentrations, perchlorate can affect the thyroid gland by inhibiting the uptake of iodine, and it has the potential to cause hypothyroidism. Perchlorate contamination is, therefore, of increasing concern, due to its widespread presence in drinking water aquifers.

While no national drinking water regulation for perchlorate has been set in the United States, the EPA has adopted a guideline of 15 µg/L [1], based on a report by the National Research Council Committee to Assess the Health Implications of Perchlorate Ingestion. Also, the State of California has set a maximum allowed level of 6 µg/L, and Massachusetts has set the level at 2 µg/L.

The EPA developed method 331.0 [2] for the detection of perchlorate using liquid chromatography/electrospray ionization mass spectrometry (LC/MS). This application note describes a modification of method 331.0 using an Agilent 1290 Infinity LC System coupled to a 6460 Triple Quadrupole LC/MS. Without cleanup or concentration, this approach provides a method detection limit (MDL) of 0.009 µg/L, which is well below the guideline of 15 µg/L, or the maximum state-allowed doses of 2 and 6 µg/L. The overall method performance is very similar to that of EPA method 331.0, while using only 20-µL injections instead of 100 µL as specified in the EPA method.

Experimental

Reagents and Standards

Reagent/Standard/Consumable	Source
$NaClO_4$ (99+ %)	Acros Organics
$Cl^{18}O_4^-$ internal standard	Provided by K'Prime Technologies
Reagent water (LC grade)	Caledon Labs
KP-RPPX, 4.6 × 250 mm, 5-µm column	K'Prime Technologies (p/n KP-RPPX250)
0.2-µm PTFE Filter (13 mm)	Canadian Life Sciences (SF6500-06)

The calibration standard solution was prepared by dissolving $NaClO_4$ in LC grade water to a final concentration of 1 mg/L. Calibrators were prepared through 1:5 serial dilutions of the 1-mg/L solution down to 0.06 µg/L in LC grade water. Fortification of samples for method validation was accomplished using 1 mg/L and 1.6 µg/L intermediate solutions, yielding final fortification levels of 40, 0.32, and 0.032 µg/L. All calibrators and fortified samples contained the $Cl^{18}O_4^-$ internal standard and were passed through a 13 mm, 0.2 µm PTFE filter prior to analysis.

Instruments

This method was developed on an Agilent 1290 Infinity LC System coupled to an Agilent 6460 Triple Quadrupole LC/MS system. The instrument conditions are listed in Table 1.

Table 1. HPLC and MS Instrument Conditions

HPLC conditions	
Analytical column	KP-RPPX, 4.6 × 250 mm, 5 µm (K'Prime Technologies p/n KP-RPPX250)
Column temperature	30 °C
Injection volume	20 µL
Mobile phase	A = 0.1% (v/v) acetic acid B = Acetonitrile, 0.1% (v/v) acetic acid
Run time	16.0 minutes
Flow rate	0.8 mL/min
Method type	Isocratic (35% B)
MS conditions	
Acquisition parameters	ESI mode, negative ionization; MRM
Sheath gas temperature	375 °C
Sheath gas flow rate	12 L/min
Drying gas temperature	325 °C
Drying gas flow rate	5 L/min
Nebulizer pressure	45 psig
Nozzle voltage	0 V
Vcap	2,400 V
Δ EMV	400 V

Table 2. Multiple Reaction Monitoring (MRM) Analysis Parameters

Compound	Precursor (*m/z*)	Product ion (*m/z*)	Dwell (msec)	Fragmentor (V)	Collision energy (V)	Cell accelerator voltage (V)
ClO_4^-	98.9	83.0*	250	79	30	7
		67.0	250	79	46	7
		51.0	250	79	50	7
$Cl^{18}O_4^-$	107.0	89.0*	250	120	26	7

*Quantifier ion

Sample Collection and Preparation

Tap water samples were collected from the city of Calgary in 40-mL EPA vials with PTFE lined septa. No preservative was added at the time of collection. Internal standard was added to all samples, after which they were filtered using a 13-mm, 0.2-µm PTFE filter, then injected directly into the HPLC system.

Analysis Parameters

Table 2 shows the 6460 Triple Quadrupole LC/MS multiple reaction monitoring (MRM) analysis parameters.

Results and Discussion

Method Performance

The method provides highly accurate calibration curves over a concentration range of 0.06 to 200 µg/L, with R^2 values > 0.999 for quadratic fit and a relative standard deviation (RSD) of 0.01% for the R^2 values over three days (Figure 1).

The method was tested on six tap water samples on each of three days, for a total of 18 replicates. Table 3 shows that recoveries were excellent, ranging from 99.5% to 103% for fortification levels of 0.32 and 40.0 µg/L, respectively. Same–day RSDs, referred to here as repeatability, were less than 3.5% for the two fortification levels, and RSDs were less than 5% across multiple days, referred to here as reproducibility. The method detection limit (MDL) was 0.009 µg/L, which is well below the EPA guideline of 15 µg/L, as well as the 2 µg/L maximum limit required in the state of Massachusetts. Table 3 shows that the performance of this method is very similar to that of Method 331.0 [2].

Table 3. Method Performance

Parameter	**Current method**		**EPA 331.0**	
Matrix	**Tap water**		**Ground water**	
Replicates	**18 (6 reps × 3 days)**		**7**	
Fortification level (µg/L)	0.32	40.0	0.5	5.0
Repeatability (%)	3.5	1.8	3.6	1.3
Reproducibility (%)	5.0	3.1	-	-
Recovery (%)	99.5	103.0	100	103
MDL (µg/L)	0.009[a]		0.005[b]	

[a] Fortification level = 0.032 µg/L

[b] Fortification level = 0.075 µg/L

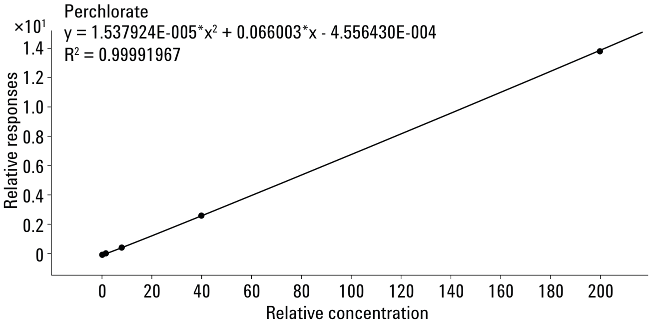

Figure 1. Calibration curve for perchlorate from 0.06 to 200 µg/L with an R^2 value of 0.9999. The % RSD for the R^2 value over three days was 0.01%.

Method Considerations

The method exhibits some matrix effects in reagent versus tap water (Figure 2). Shifts in retention time (RT) and changes in absolute response were observed. Given the lack of sample cleanup, these differences likely result from chemical composition differences between the two matrices. Using an internal standard compensates for these effects.

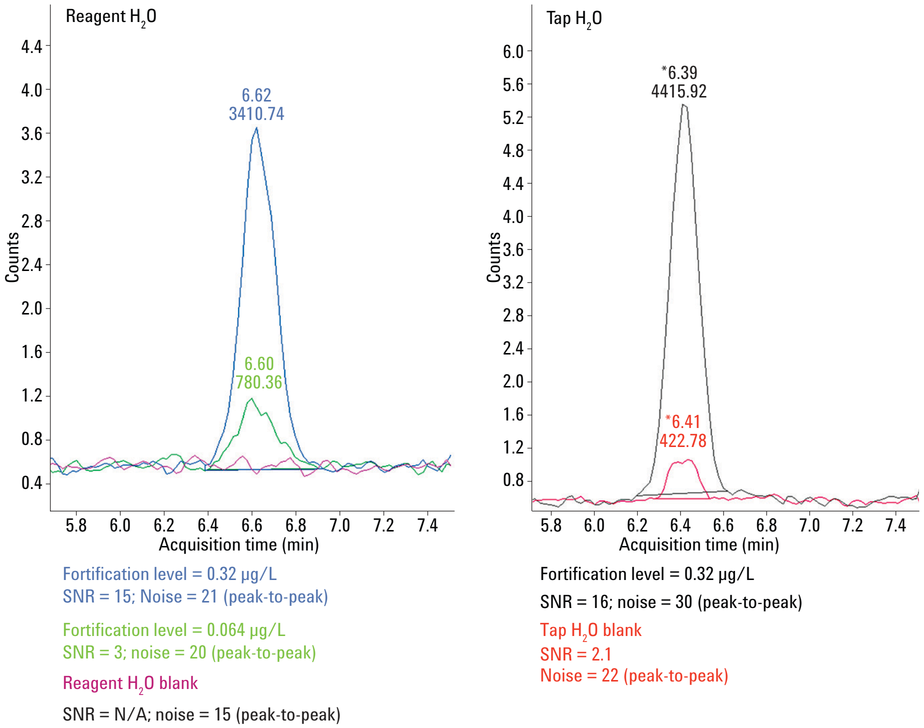

Figure 2. MRM results for reagent and tap water, illustrating the differences in RT as well absolute response due to matrix effects. These differences are corrected by use of the internal standard.

The sulfate ion is commonly found in high concentrations in ground water, and the $H^{34}SO_4^-$ isotope (nominal *m/z* = 99) is an isobaric interference in the MS analysis of ClO_4^- (nominal *m/z* = 99). A shift in RT occurs in the presence of 100 mg/L HSO_4^- , as well as a slight ion enhancement (<10%) for ClO_4^- (Figure 3). The use of MRM and chromatography mitigate the possibility of interference from the ^{34}S isotope of HSO_4^-, and the use of an internal standard corrects for both the RT and ion enhancement effects. Therefore, the presence of the HSO_4^- does not interfere with the measurement of perchlorate when using this method.

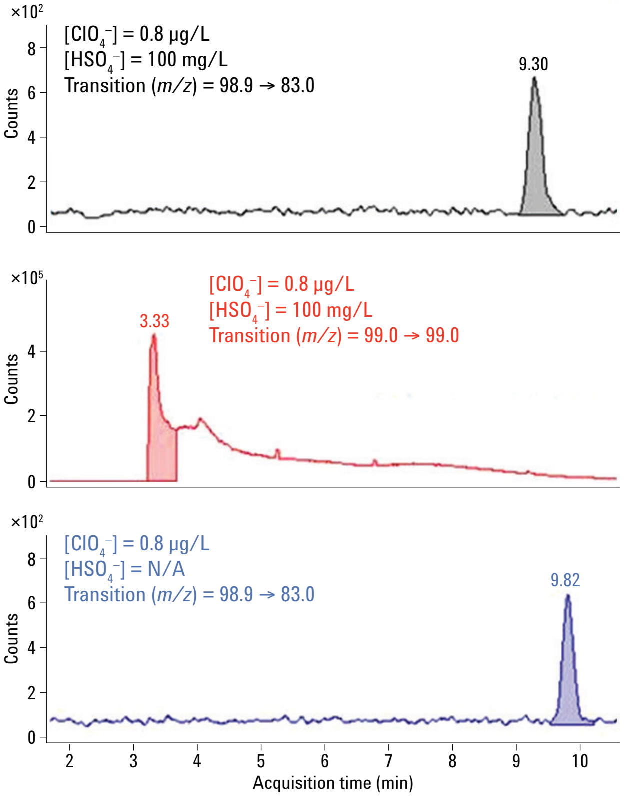

Figure 3. *Potential interference from the $H^{34}SO_4^-$ ion (m/z = 99.0), resulting in a shift in RT and slight ion enhancement. The use of MRM and an internal standard corrects for any effects from this interference. Note the different y-axis scaling in the middle chromatogram.*

Conclusions

Analyzing tap water for the presence of perchlorate using the 1290 Infinity LC System coupled to the 6460 Triple Quadrupole LC/MS system enables an MDL of 0.009 µg/L, which is well below the current EPA guideline of 15 µg/L, as well as either of the current state-regulated maximum allowable levels. This slight modification of the EPA 331.0 method provides very similar overall performance, including recovery, repeatability and MDL.

References

1. EPA Memorandum, January 8, 2009, from Susan Parker Bodine to Regional Managers.

2. METHOD 331.0 Determination Of Perchlorate In Drinking Water By Liquid Chromatography Electrospray Ionization Mass Spectrometry, Revision 1.0, January 2005, http://www.epa.gov/ogwdw/methods/pdfs/methods/met331_0.pdf

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Printed in the USA
November 8, 2012
5991-1387EN

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