Analysis of 2,4-D, 2,4,5-T, Bromoxynil, and Dinoseb Herbicides in Drinking Water Using the Agilent 6495 Triple Quadrupole LC/MS

Application Note

Environmental

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Abstract

Analysis by direct injection of four commonly monitored herbicides in water using the Agilent 6495 Triple Quadrupole LC/MS provided detection levels of 1–2 ppt in negative ion mode. Reproducibility was excellent, and matrix effects in either laboratory water or drinking water were minimal.

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Introduction

The levels of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), bromoxynil, and dinoseb herbicides in drinking water are often monitored in environmental laboratories. Of these, 2,4-D is one of the most widely used herbicides in the world, often on rangeland and pasture, with as much as 50 million pounds used each year in the US alone [1]. Bromoxynil is widely used in the Great Plains region of Canada, and it decomposes readily in soil, leading to lower levels in drinking water than other pesticides. Both 2,4,5-T and dinoseb, a dinitrophenol, have not been used in the US, Canada, or the European Union for many years, but they may still persist in the environment at low levels.

Adverse health effects have been associated with exposure to all of these herbicides, at various levels. Maximum allowable levels in drinking water have been established in several countries for these herbicides, including the US and Canada, and monitoring of these compounds is essential.

Liquid chromatography/mass spectrometry (LC/MS) methods are most often used for detection and quantification of these herbicides, with MS detection frequently done in negative ionization mode. This application note demonstrates the exceptional sensitivity and reproducibility of quantification of the Agilent 6495 Triple Quadrupole LC/MS for detection of these four herbicides at concentrations as low as 1–2 parts per trillion (ppt), in negative ionization mode, using direct injection.

Experimental

Reagents and standards

Herbicide analytical standards were obtained from Sigma-Aldrich, Oakville, Ontario. LC solvents were LC grade from Caledon Laboratories, Georgetown, Ontario.

Instruments

The system was set up using the Agilent G4226A Infinity Autosampler and an Agilent 1290 Infinity Series LC system, coupled to an Agilent 6495A Triple Quadrupole LC/MS. The LC/MS run conditions are shown in Table 1.

Table 1. HPLC and MS Conditions

HPLC	
Analytical column	Agilent ZORBAX Eclipse Plus C18, HD 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Column temperature	40 °C
Injection volume	20 µL
Mobile phase	A) 0.10 % acetic acid in water B) Acetonitrile
Flow rate	0.3 mL/min
Gradient	Time (min) %B 0 5 0.5 5 6.0 100 8.0 100
Stop time	10 minutes
Post time	2 minutes
Run time	12 minutes, injection to injection
MS	
Acquisition parameters	ESI mode, negative ionization, MRM
Sheath gas temperature	400 °C
Sheath gas flow rate	12 L/min
Drying gas temperature	200 °C
Drying gas flow rate	12 L/min
Nebulizer pressure	40 psig
Nozzle voltage	2,000 V
Vcap	2,000 V negative

Sample preparation

Samples were filtered directly into autosampler vials through 0.22-µm PTFE syringe filters.

Analysis parameters

The multiple reaction monitoring (MRM) transitions used for these herbicides and their internal standards are shown in Table 2.

Table 2. MRM Analysis Parameters

Compound	Precursor ion	Product ion	Collision energy (V)	Collision cell accelerator voltage (V)	Polarity
2,4,5-T†	254.9	196.9	10	2	Negative
2,4,5-T	252.9	194.9	10	2	Negative
2,4-D†	221	162.9	8	2	Negative
2,4-D	219	160.9	8	2	Negative
Dinoseb	239.1	192.9	25	2	Negative
		134.0	50	2	Negative
Bromoxynil†	275.8	80.9	36	2	Negative
Bromoxynil	273.8	78.9	36	2	Negative

† ^{37}Cl or ^{81}Br isotope-labeled internal standard

Results and Discussion

Sensitivity

The sensitivity of this method is such that extra care must be taken with blanks. For example, a sample of Milli-Q water showed background levels of 2,4-D and dinoseb (Figure 1). A drinking water sample actually contained 24 ppt of 2,4-D, which was easily detectable using this method (Figure 2). The background levels for the other three compounds in drinking water were comparable to those found in Milli-Q water. With the exception of 2,4-D, the herbicides were detectable at 2 ppt in drinking water (Figures 1 and 2).

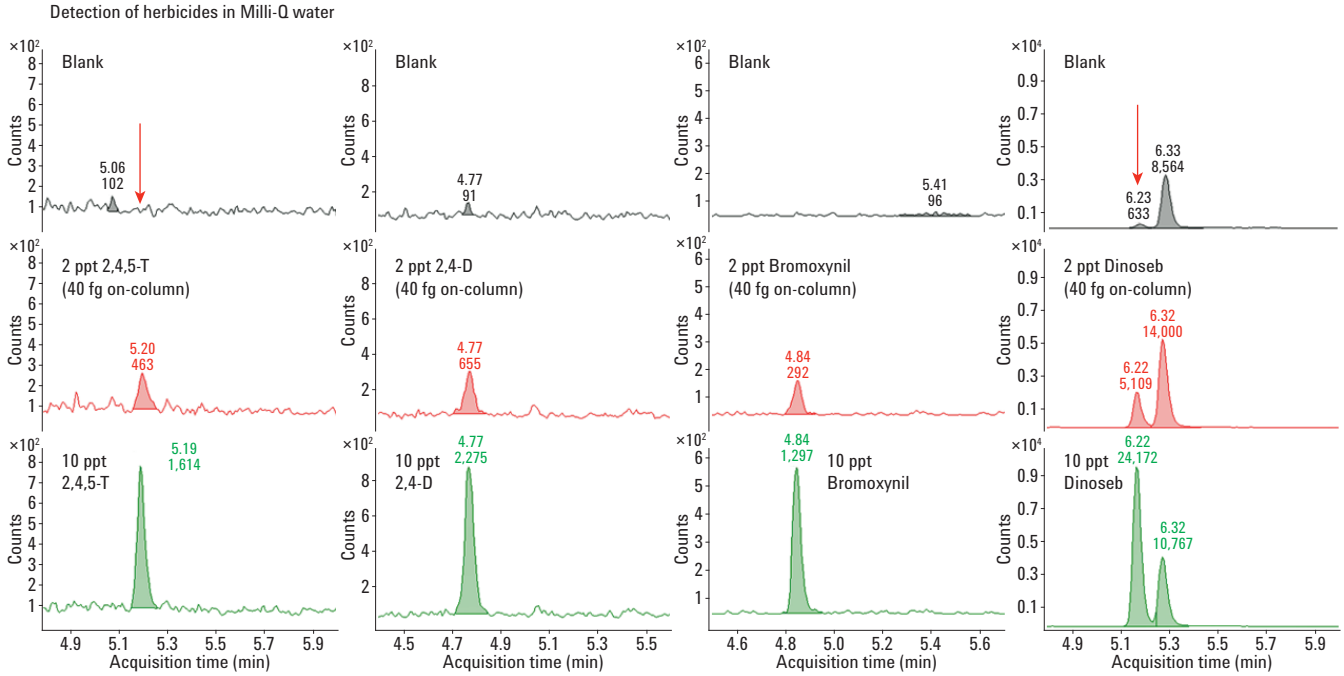

Figure 1. Extra care must be taken with blanks due to the high sensitivity of the method. Background levels can be seen in Milli-Q water for 2,4-D and dinoseb. However, all four herbicides can be detected at 2 ppt.

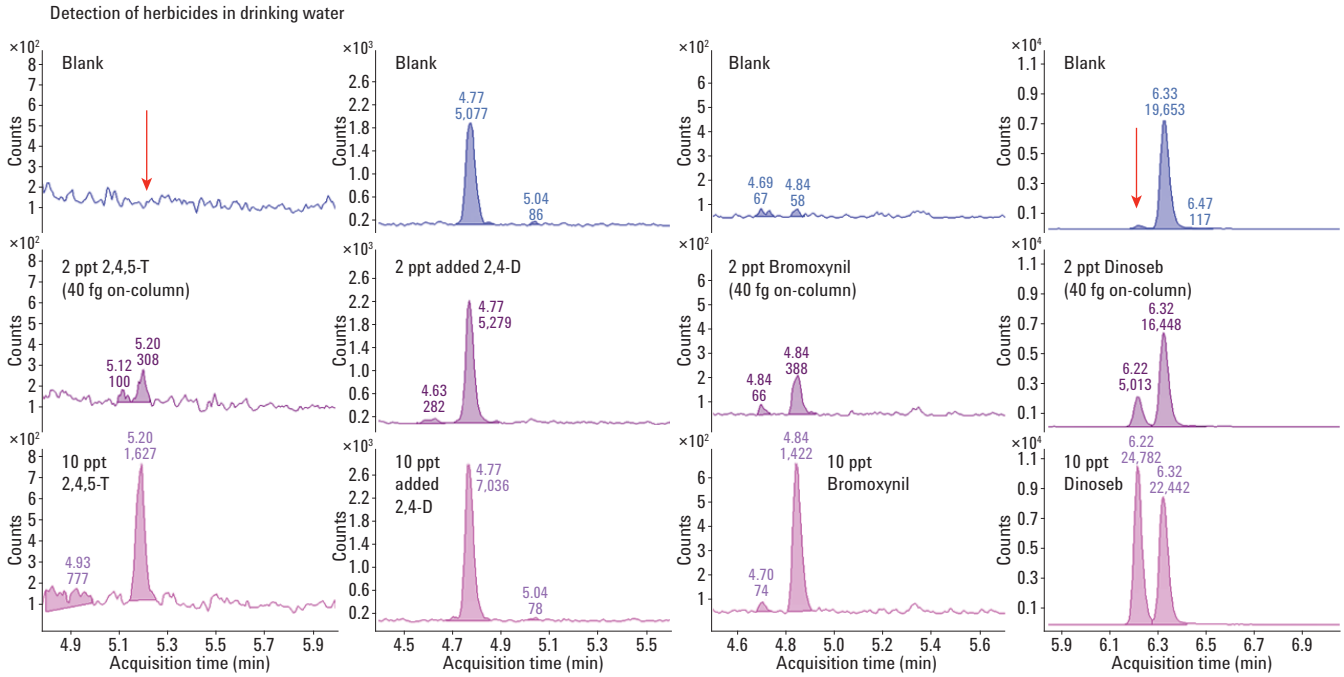

Figure 2. The drinking water sample contained easily detectable levels of 2,4-D, at approximately 24 ppt. However, all three of the other herbicides could be detected at 2 ppt when spiked into drinking water.

Minimal matrix effects

The responses for 2,4,5-T and bromoxynil in Milli-Q and drinking water were very similar, indicating that the method exhibits minimal matrix effects (Table 3, Figure 3). The peak areas, reproducibility, and limits of detection (LODs) for 2-ppt spiked samples of 2,4,5-T and bromoxynil were very similar in both matrices (Table 3).

Reproducibility and LODs

Figure 3 shows excellent reproducibility for detection of bromoxynil and 2,4,5-T in drinking water, with relative standard deviations (RSDs) of 12.9 and 21.9 %, respectively. The LODs were very low as well, at 0.8 and 1.4 ppt, respectively. These values were calculated using a t-stat value for seven replicates (3.143) and the following formula:

LOD = t-stat × % RSD × Spike Level

Table 3. Minimal Matrix Effects at 2 ppt

	2,4,5-T		Bromoxynil	
Replicate no.	**Milli-Q**	**Drinking water**	**Milli-Q**	**Drinking water**
1	463	308	292	388
2	339	384	326	349
3	258	272	351	386
4	296	328	302	431
5	256	179	314	391
6	290	261	258	333
7	476	299	272	287
Average peak area	340	290	302	366
% RSD*	27.4 %	21.9 %	10.5 %	12.9 %
LOD†	1.7	1.4	0.7	0.8

* Relative standard deviation
† Limit of detection (ppt)

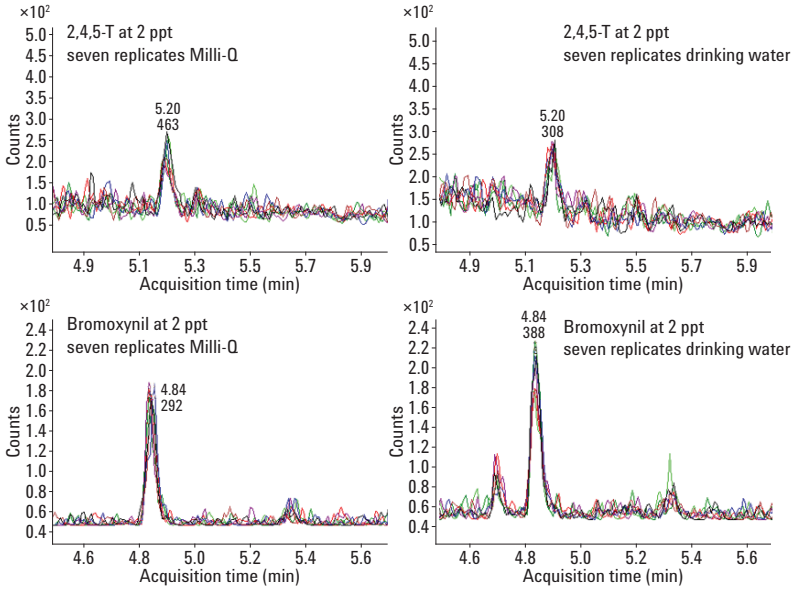

Figure 3. Overlaid traces of seven replicate analyses of 2,4,5-T and bromoxynil at 2 ppt in Milli-Q and drinking water, illustrating reproducibility and the lack of matrix effects (see Table 3).

The method was also tested with two surface water samples received from an external lab (Figure 4, Table 4). One sample was taken from a national park (Sample 1), and the other an urban source (Sample 7). The peak areas for seven replicates are shown in Table 4, along with the average areas, % RSDs, and calculated concentrations. The reference values for the concentrations obtained by the external lab with a different method are also shown for comparison. The method used by the external lab was not sensitive enough to determine concentrations below 10 ppt (Sample 1). However, the concentrations for Sample 2 were essentially identical when determined using either the method developed here, or the method used by the external lab.

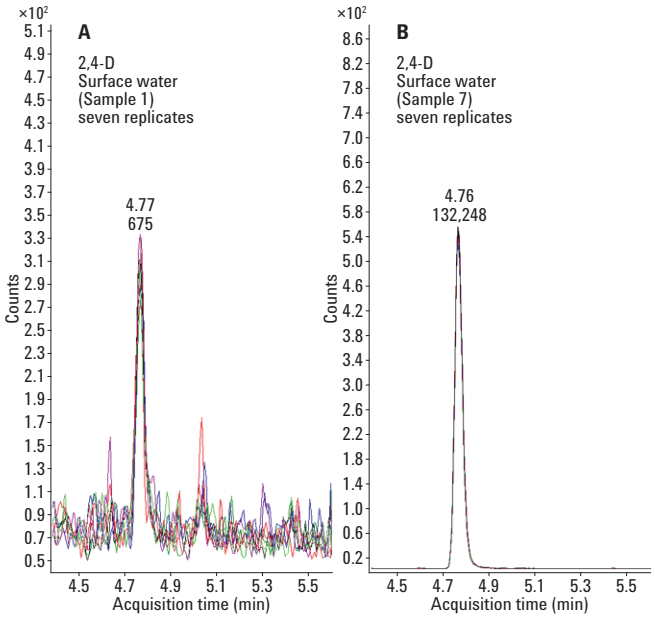

Figure 4. Analysis of 2,4-D in two surface water samples obtained from an external laboratory, showing the overlaid results of seven replicates. The sample in A (Sample 1) was from a national park, and the sample in B (Sample 7) was from an urban source.

Table 4. Determination of 2,4-D in Surface Water

	2,4-D Peak area	
Replicate	**Sample 1**	**Sample 2**
1	578	132,248
2	509	132,741
3	584	136,599
4	521	132,161
5	675	135,684
6	747	134,390
7	539	131,950
Average	593	133,682
% RSD	14.7 %	1.4 %
Concentration (ppt)	2	599
Reference concentration	< 10	592

Conclusions

The Agilent 6495 Triple Quadrupole LC/MS, when used in negative ionization mode, and in combination with the Agilent 1290 Infinity LC, is capable of detecting these four herbicides using direct injection at 1–2 ppt. Some of these compounds could be detected at even lower levels, subject to having clean blanks. Matrix effects in drinking water are minimal, when compared to Milli-Q water, and reproducibility is excellent, with % RSDs of approximately 15–20 % at 1–2 ppt.

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