EPA Method 509.1 Using an Agilent 1290 Infinity HPLC and an Agilent 6460 Triple Quadrupole LC/MS: Initial Demonstration of Capability

Application Note

Environmental

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Abstract

Performing the EPA draft Method EPA 509.1 using the Agilent 6460 Triple Quadrupole LC/MS resulted in performance that exceeded the method requirements. The calculated LCMRL was 5.2 ng/L with 5 % precision at a spiking level of 4 ng/L, and accuracy ranged from 97 to 105 % over a calibration range of 4 to 2,000 ng/L.

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Introduction

Environmental contamination by ethylene thiourea (ETU) is due primarily to the use of several fungicides categorized as ethylene bisdithiocarbamates (EBDCs), which can be applied to ornamental plants, vegetables, fruits, and field crops. ETU is an environmental degradation product, metabolite, and synthesis contaminant of EBDCs [1]. ETU is also used as an accelerator for vulcanizing neoprene and polyacrylate rubbers as well as in electroplating baths, synthetic resins, pharmaceuticals, dyes, and as a scavenger in waste water treatment.

ETU has the potential for contaminating drinking water from both surface water and groundwater sources, due to its high solubility in water, and its mobility in the environment. According to US Environmental Protection Agency (EPA) reports, ETU has been measured in one public drinking-water well at 0.21 parts per billion (ppb), but it was not detected in any of 84 finished drinking water sources that were sampled. In addition, a targeted study did not find ETU in surface water at a detection limit of 0.1 ppb [2,3,4]. Following testing of US groundwater wells, the EPA has estimated that 0.1 % of rural wells are contaminated with ETU.

There is adequate evidence of carcinogenicity from experimental animal studies for ETU to be reasonably anticipated to be a human carcinogen. Epidemiological study data in humans are not adequate to evaluate the relationship between exposure specific to ETU and human cancer [5]. However, the US EPA has established a Drinking Water Equivalent Level of 0.7 ppb in drinking water. In addition, EPA Method 509.1 has recently been released as a draft for the direct detection of ETU in water using liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS). The method allows flexibility in LC columns, LC conditions, and MS conditions, as long as method performance is not affected.

This application note describes the use of the Agilent 1290 Infinity Series LC and the Agilent 6460A Triple Quadrupole LC/MS with Agilent Jet Stream technology to meet the stringent quality control requirements of EPA draft Method 509.1, using direct injection of the sample. This analysis platform provided Lowest Concentration Minimum Reporting Levels (LCMRLs) and Detection Limits (DLs) that exceeded EPA requirements. Accuracy and precision were also well within the requirements of the draft EPA method.

Experimental

Reagents and materials

Ethylene thiourea, glycine hydrochloride, cysteine hydrochloride, and ammonium fluoride were obtained from Sigma-Aldrich, Oakville, Ontario. ETU-d4 was obtained from CDN Isotopes in Pointe-Claire, Quebec. Solvents were LC grade, obtained from Caledon Laboratories, Georgetown, Ontario.

Instruments

The draft EPA Method 509.1 was run using an Agilent 1260 Infinity High Performance Autosampler and an Agilent 1290 Infinity LC system, which was coupled to an Agilent 6460A Triple Quadrupole LC/MS with Agilent Jet Stream technology. The instrument conditions are shown in Table 1.

Table 1.   HPLC and MS Conditions

HPLC

Analytical column	Agilent ZORBAX SB-Aq, 3.0 × 150 mm, 3.5 µm (p/n 863954-314)
Column temperature	40 °C
Injection volume	60 µL
Mobile phase	A) 1 mM Ammonium fluoride B) MeOH
Flow rate	0.5 mL/min
Elution	0 %B isocratic
Column flush	100 %B, for 20 minutes following each batch of samples
Run time	4 minutes, injection to injection

MS

Acquisition parameters	ESI mode, positive ionization, MRM
Sheath gas temperature	380 °C
Sheath gas flow rate	12 L/min
Drying gas temperature	200 °C
Drying gas flow rate	4 L/min
Nebulizer pressure	40 psig
Nozzle voltage	0 V
Vcap	2,000 V positive

Sample preparation

Preservatives (0.5 mM glycine hydrochloride and 20 mM L-cysteine hydrochloride) were added to 10 mL water samples per the draft EPA Method 509.1. The samples were then spiked with internal standard at 0.27 ng/mL (and target analyte as appropriate), and passed through a PVDF filter directly into autosampler vials.

Analysis parameters

The multiple reaction monitoring (MRM) transitions used for ethylene thiourea and the deuterated ethylene thiourea internal standard are shown in Table 2.

Results and Discussion

Draft Method 509.1 requirements

Draft EPA Method 509.1 calls for at least five calibration standards, with the lowest concentration being at or below the minimum reporting limit (MRL). The precision of all measurements expressed as percent relative standard deviations (RSDs) must be $\leq 20\,\%$ and accuracy expressed as recovery must be between 80 and 120 %. The goal of this implementation of Method 509.1 is to generate LCMRLs and DLs equal to or lower than those reported in the draft EPA method.

Table 2.   MRM ESI Analysis Parameters

Compound	Retention time	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (V)	Dwell (msec)	Polarity	Type
Ethylene thiourea	2.95	103.0	86.1	89	20	150	Positive	Target
			44.2*	89	20	150	Positive	Target
Ethylene thiourea-d4[†]	2.92	107.1	48.2	92	20	150	Positive	ISTD

[†] Internal standard
* Transition used for quantitation

Method performance

Calibration curves were constructed by spiking Laboratory Fortified Blanks (LFBs) with ETU standard and run from 0.004 to 2.000 ng/mL (4 to 2,000 ng/L) using the following seven concentrations: 0.004, 0.008, 0.020, 0.067, 0.200, 0.667, and 2.000 ng/mL. Excellent linearity was obtained, as shown in Figures 1 and 2, with a correlation coefficient of 1.000 with linear fit, ignoring the origin, and no weighting. Precision and accuracy were also excellent for four to seven replicates of

each of the calibration curve concentrations, as shown in Table 3. The accuracy ranged from 97 to 105 %, well within the ± 20 % specified by Method 509.1. Precision ranged from RSDs of 0.3 for the highest concentration, to only 5 % for the lowest concentration, 0.004 ng/mL. Three of the calibrator concentrations were analyzed as seven replicates, to insure proper determination of a MRL, as described in Method 509.1.
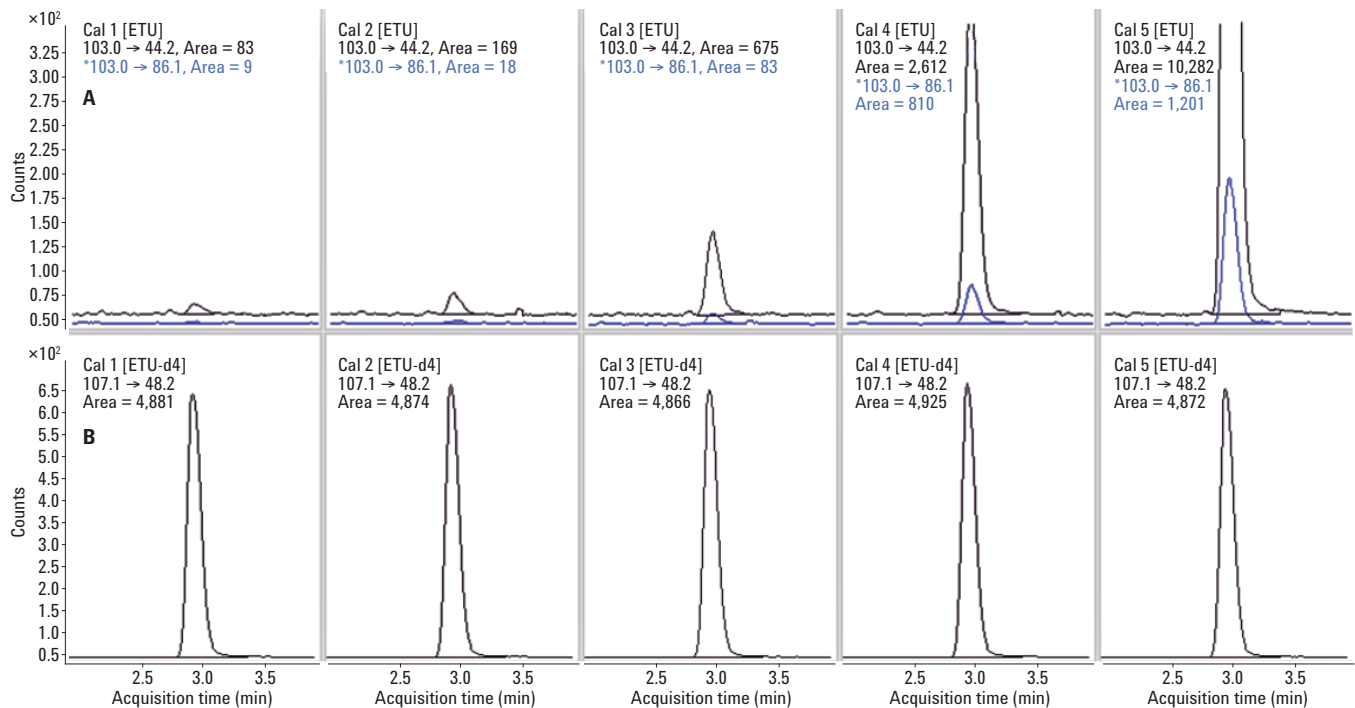

Figure 1.   Extracted ion chromatograms (EICs) for five calibration levels (0.004, 0.008, 0.020, 0.067, 0.200, ng/mL), showing both the ethylene thiourea qualifier and quantifier ions (A) and the ethylene thiourea-d4 internal standard quantifier ion (B).
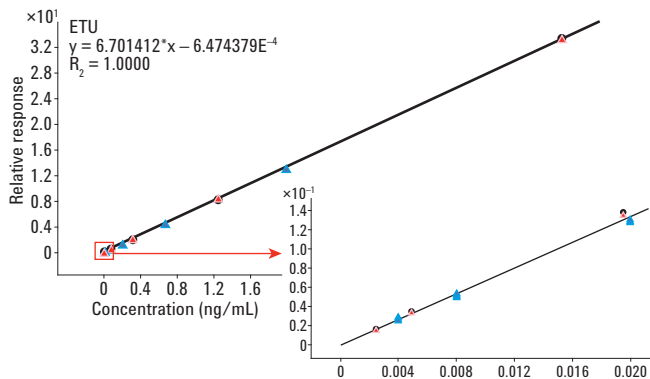

Figure 2.   Calibration curve for ethylene thiourea, using seven calibration standards from 0.004 to 2.000 ng/mL (4 to 2,000 ng/L), illustrating an R^2 value of 1.000 with no weighting and ignoring the origin. The expanded region from 0 to 0.02 ng/mL demonstrates that the excellent linearity extends to these very low concentrations.

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Table 3. *Accuracy and Precision for Ethylene Thiourea Calibration Standards*

Replicate no.	Experimental concentration (ng/L)						
	4	8	20	66.7	200	666.7	2,000
1	111.1 %	101.6 %	98.7 %	99.4 %	96.3 %	99.0 %	97.0 %
2	105.9 %	94.1%	97.2 %	97.1 %	97.3 %	98.8 %	97.6 %
3	105.5 %	102.3 %	97.9 %	96.6 %	96.7 %	101.4 %	97.0 %
4	102.6 %	100.1 %	96.2 %	99.5 %	96.4 %	99.0 %	96.9 %
5	99.6 %	100.2 %			96.1 %		
6	98.9 %	100.1 %			96.9 %		
7	112.4 %	96.4 %			96.9 %		
Accuracy	105.2 %*	99.3 %*	97.5 %*	98.1 %*	96.7 %*	99.6 %*	97.1 %*
RSD[†]	5.0 %	3.0 %	1.1 %	1.5 %	0.4 %	1.3 %	0.3 %

* Average recovery values as a percentage of the experimental concentration
[†] Precision expressed as % RSD

LCMRL and DL calculations

Method 509.1 requires the calculation of the LCMRL, which is accomplished by analyzing seven replicates spiked at the proposed MRL and then entering the values into an EPA-supplied LCMRL Calculator [6]. The LCMRL is defined by this method as the lowest spiking concentration at which recovery of between 50 and 150 % is expected 99 % of the time by a single analyst. This range is determined by first calculating the Half Range for the Prediction Interval of Results, or HR_{PIR}, using the following formula:

$$HR_{PIR} = 3.963S$$

where S is the standard deviation and 3.963 is a constant value for seven replicates.

The Upper and Lower PIR limits for recovery are then calculated using the following formulas:

$$Lower\ PIR\ limit = \frac{Mean - HR_{PIR}}{Fortified\ concentration} \times 100$$

$$Upper\ PIR\ limit = \frac{Mean + HR_{PIR}}{Fortified\ concentration} \times 100$$

The LCMRL was calculated in this study using a spiking level of 4 ng/L, rather than the 10 ng/L used by the EPA. The calculated HRPIR was 0.8 ng/L, which resulted in a Lower PIR Limit of 84.4 %, and an Upper PIR Limit of 126 %. This is a narrower range than is required by Method EPA 509.1 (50 to 150 %). The resulting LCMRL is shown in Table 4, along with the precision and accuracy obtained from the seven replicates, compared to the EPA values.

The DL, the determination of which is optional for Method 509.1, is defined as the statistically calculated minimum concentration that can be measured with 99 % confidence that the reported value is greater than zero, and the method provides a formula for it calculation. This implementation of Method 509.1 using the 6460 Triple Quadrupole LC/MS provides performance that exceeds all of the EPA values, including the DL (Table 4).

Table 4. *Comparison of Performance Values to Those in the EPA 509.1 Method*

Compound	Determined LCMRL	EPA LCMRL	Determined precision	EPA precision	Determined DL	EPA DL
ETU	5.2 ng/L	6.1 ng/L	5.0 %	6.8 %	1.2 ng/L	2.8 ng/L

Conclusions

Using an Agilent 1290 Infinity LC coupled to the Agilent 6460 Triple Quadrupole LC/MS with Agilent Jet Stream technology for the analysis of ethylene thiourea in drinking water can enable laboratories to exceed the stringent QC requirements of draft EPA Method 509.1. Accuracy ranged from 97 to 105 %, well within the ± 20 % required by the EPA. Precision ranged from 0.3 to 5 %, also well below the EPA limit of 20 %. The calculated LCMRL (5.2 ng/L) was lower than the EPA value (6.1 ng/L), and the calculated DL (1.2 ng/L) was also lower than the EPA value (2.8 ng/L).

References

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