Application Note 02219

Evaluation of a New EPA Drinking Water LC/MS/MS Method for the Determination of Non-Volatile Compounds on the Varian 325-MS Triple Quadrupole Mass Spectrometer

Emma E. Rennie and Ed George
Varian, Inc.

Introduction

The U.S. EPA National Exposure Research Laboratory (NERL) in Cincinnati, OH is responsible for the development of methods for drinking water contaminants in response to requirements under the Safe Drinking Water Act. A regulation within this Act known as the Unregulated Contaminant Monitoring Rule (UCMR)[1] is meant to gather occurrence data from public water supplies on select contaminants of concern. Based on the data collected, the Agency can make decisions about whether or not a certain contaminant will be regulated and promulgate a permanent requirement for monitoring,

In recent years, the EPA has been utilizing LC/MS/MS for the analysis of semi-volatile and non-volatile pollutants. Example methods developed for the UCMR that use LC/MS/MS are EPA Methods 535 and 536. Both of these methods were aimed at the detection of pesticide residue degradation products that are typically more polar than the parent compound. These polar degradates are less likely to be absorbed onto particles and can make their way into ground water sources and run-off into surface waters.

A new method for the analysis of semi-volatile and non-volatile contaminants by LC/MS/MS has been recently developed at NERL.[2] As part of the method validation process, the EPA has established a Secondary Laboratory Validation Study, which gives participating laboratories the opportunity to set up the new method and evaluate it. This provides important feedback to the EPA, giving them an opportunity to correct or streamline the new method before it is finalized and published in the Code of the Federal Register. In this application note, the Varian 325-MS LC/MS/MS was used to evaluate the new method. The advanced vortex ESI (vESI™) source, together with the ability to program the temperature and the flow rate of the atmospheric pressure interface (API) gases, provides excellent results in terms of sensitivity, precision and accuracy.

Instrumentation

- Varian 325-MS triple quadrupole mass spectrometer with vortex ESI (vESI™) source
- Varian 212-LC binary solvent delivery system
- CTC Analytics HTS PAL™ autosampler with cooling option

Materials and Reagents

- The EPA kindly provided the analytes and internal standards (NERL in Cincinnati, OH).
- HPLC grade methanol was purchased from Fisher Scientific (Pittsburgh, PA).
- 99.99+% purity ammonium acetate and 2-mercaptopyridine-1-oxide sodium salt solution (40% in H_2O) were purchased from Sigma-Aldrich (St. Louis, MO).

Sample Preparation

This LC/MS method is a direct aqueous injection method. Samples are prepared by adding the recommended method preservatives prior to analysis (ammonium acetate for removing free chlorine, and 2-mercaptopyridine-1-oxide to inhibit microbial decomposition of the target compounds). Samples prepared in this manner are stable for up to 14 days at 4 °C.

Standard Stock Solutions

Stock solutions of 2-mercaptopyridine-1-oxide (32 g/L) and ammonium acetate (2 M) were prepared using HPLC grade water. The aqueous analyte primary dilution standard (water PDS) solution (0.125-10.7 ng/µL) was prepared in HPLC grade water containing 10% HPLC grade methanol from the CH_3OH PDS provided by the EPA. Seven aqueous calibration standards were prepared spanning concentrations between 0.050-5 ppb for seven of the analytes of interest. Due to low response factors, the remaining four analytes (quinoline, aldicarb, aldicarb sulfoxide and thiofanox) were present in higher concentration levels in the CH_3OH PDS, producing final calibration (CAL) levels between 0.1-214 ppb. All calibration standards were spiked with internal standards at 4 ppb (except for quinoline-D7 which had a concentration of 125 ppb), and contained 20 mM ammonium acetate and 64 mg/L 2-mercaptopyridine-1-oxide (chemical preservation reagent).

HPLC Conditions

Column:	Pursuit™ XRs C18, 5 µm, 150 x 2.0 mm (Varian Part Number A6001150X020)
Guard Column:	Pursuit XRs C18, 3 µm, MetaGuard™ 2.0 (Varian Part Number A3001-MG2)
Solvent A:	5 mM ammonium formate and 0.2% formic acid in water
Solvent B:	90% methanol: 10% solvent A
Column Heater:	MetaTherm™ HPLC column heater (Varian Part Number A9540)

HPLC Conditions (continued)

LC Program:

Time (min:sec)	%A	%B	Flow (µL/min)
0:00	90	10	200
3:00	90	10	200
5:00	70	30	200
8:00	70	30	200
20:00	30	70	200
25:00	10	90	200
30:00	90	10	200
33:00	90	10	200

Injection Volume: 50 µL

MS Parameters

Ionization Mode: vESI (positive)
API Nebulizing Gas: 70 psi
Detector: Extended Dynamic Range (EDR)
Needle: +2000 V
Shield: +650 V
CID Gas Pressure: 2.0 mTorr

	Segment 1	Segment 2	Segment 3	Segment 4
Dyring Gas Pressure:	35 psi	60 psi	35 psi	60 psi
Dyring Gas Temp:	300 °C	150 °C	150 °C	300 °C
Vortex Gas Pressure:	25 psi	60 psi	25 psi	60 psi
Vortex Gas Temp:	250 °C	100 °C	100 °C	250 °C

Table 1. Compounds in new EPA method with 325-MS. MS/MS segment parameters.

Peak Number	Compound	Segment	MRM	Capillary Voltage (V)	Collision Energy (V)
3	Quinoline	1	130 → 77	100	29
3	*Quinoline-D7	1	137 → 81	100	29
1	Methamidophos/monitor	1	142 → 94	55	12.5
1	*Methamidophos/ monitor-D6	1	148 → 97	55	12.5
2	Acephate	1	184 → 143	30	6.5
2	*Acephate-D6	1	190 → 149	30	6.5
4	Aldicarb sulfoxide	1	207 → 105 207 → 132	30	10.0 5.0
6	Dicrotophos	1	238 → 112	68	10.5
5	Oxydemeton-methyl	1	247 → 169	38	11.5
5	*Oxydemeton-methyl-D6	1	253 → 175	38	11.5
8	DIMP**	3	181 → 97	20	11
8	*DIMP-D14	3	195 → 99	20	11
7	Aldicarb	3	208 → 89 208 → 116	15	14.5 6.0
11	Thiofanox	3	219 → 57	20	6.5
9	Fenamiphos sulfoxide	3	320 → 233 320 → 292	100	21.5 13.0
10	Fenamiphos sulfone	3	336 → 266 336 → 308	85	17.0 12.5

*Denotes an internal standard
**Diisopropyl methylphosphonate (DIMP)
See Figure 2 for labeled chromatogram relating to the peak number.

Results and Discussion

The EPA Validation Study requires participating labs to perform the following tasks:

a. Optimize the LC/MS/MS conditions and establish initial calibration in a range of 0.05 to 5 ppb

b. Analyze four to seven laboratory fortified blank (LFB) replicates in reagent water at a low level (0.05 ppb) and near mid-calibration level (1-3 ppb)

c. Analyze four to seven laboratory fortified matrix (LFM) spikes at mid-level using a local water source

d. Establish a single laboratory Lowest Concentration Minimum Reporting Level (LCMRL) along with a detection limit (DL) determination in reagent water

All of these tasks are to be analyzed with appropriate quality control samples, method preservatives added and within the bounds of permitted changes that analysts can make to method conditions. Typically, analysts are allowed to change mobile phase and HPLC columns as long as the method quality control criteria are met.

Method Optimization and Initial Calibration

Each analyte was infused using a post-column "T" so that the compounds could be infused at the analytical flow rate (200 µL/min) at the approximate mobile phase elution composition. Optimized capillary voltages and collision energies are shown in Table 1. The Total Ion Chromatogram (TIC) in Figure 1 shows excellent separation and peak shape with the Pursuit™ XRs 5 µm C18 column. The mobile phase listed above provided the best overall sensitivity and peak shape when compared to the method recommended conditions.

It was quickly discovered that the temperature of the drying and vortex gasses have a huge impact on sensitivity, as shown in Figure 1. The earlier eluting compounds clearly show preference to the higher temperatures (peaks in red trace). The large peak in the green trace near 18 minutes is aldicarb, which shows a dramatic improvement in sensitivity at the lower gas temperatures. The last compound eluting is thiofanox, and it also shows significant improvement at the lower temperatures.

Fortunately, the Varian 325-LC/MS/MS can take advantage of both gas temperature and flow rate programming in the API source. These are patented features known as SelecFlow™ and SelecTemp™. Figure 2 represents the end result of gas temperature and pressure programming in both the vortex gas and counter-current drying gas. This provides maximum sensitivity for all of the compounds in a single run.

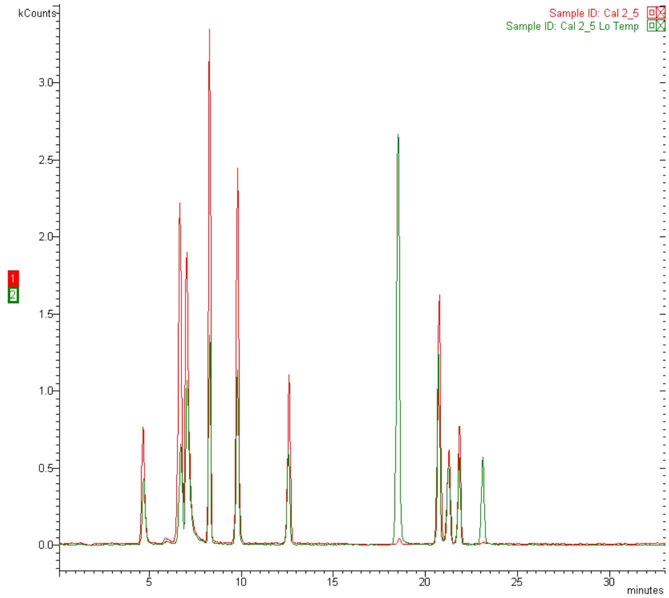


Figure 1. Overlaid TIC at a concentration of 2.5 ppb (CAL 6).
Red: drying gas 35 psi at 300 °C, vortex gas 25 psi at 250 °C.
Green: drying gas 35 psi at 150 °C, vortex gas 25 psi at 100 °C.

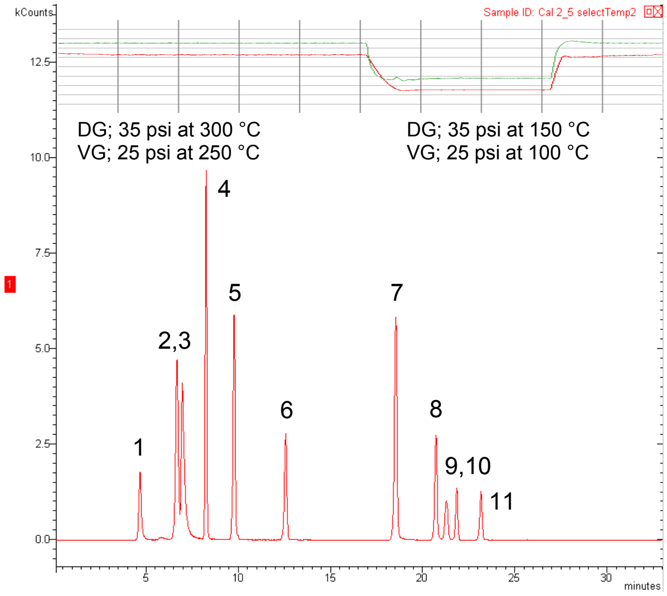


Figure 2. TIC at concentration of 2.5 ppb (CAL 6) using the SelecTemp™ and SelecFlow™ gas program as detailed in the MS parameter section. The gas temperature traces (on a scale of 0-400 °C at top of plot) have been superimposed at the top of the graph. This sample did not contain internal standards.

Table 2. Calibration (CAL) mean percent recoveries for samples including method preservation agents.

	Mean % Recovery for CAL Levels								
Compound	CAL 1 (0.05 ppb)	CAL 2 (0.12 ppb)	CAL 3 (0.25 ppb)	CAL 4 (0.50 ppb)	CAL 5 (0.99 ppb)	CAL 6 (2.50 ppb)	CAL 7 (5.00 ppb)	Acceptance Level (%)	Pass/Fail
Acephate	101.0	102.2	97.3	103.1	98.2	106.5	94.5	70-130	PASS
Aldicarb	96.3	104.5	101.4	111.8	97.4	96.8	91.1	70-130	PASS
Aldicarb sulfoxide	97.0	104.2	104.4	105.6	100.2	101.4	87.0	70-130	PASS
DIMP	98.5	102.8	96.7	101.8	99.7	101.7	96.5	70-130	PASS
Dicrotophos	94.5	103.9	97.7	109.3	93.5	103.5	90.8	70-130	PASS
Fenamiphos sulfone	96.5	107.5	103.7	101.0	96.0	104.5	91.6	70-130	PASS
Fenamiphos sulfoxide	95.5	105.0	104.0	106.6	93.9	102.8	90.8	70-130	PASS
Methamidophos/monitor	94.5	111.4	102.4	101.5	100.3	101.3	93.5	70-130	PASS
Oxydemeton-methyl	100.0	104.2	93.6	101.5	97.8	107.4	96.4	70-130	PASS
Quinoline	97.8	103.0	94.5	105.5	99.6	103.4	94.4	70-130	PASS
Thiofanox	99.3	98.8	102.9	107.6	99.6	98.8	93.1	70-130	PASS

Under these optimized conditions, calibrations were performed in both pure reagent water and reagent water containing the method specified preservatives (Table 2). The calibration is evaluated by calculating each calibration point against the curve and checking the percent recovery. The lowest calibration point must have ±50% recovery, and all other levels ±30% recovery. Even though some ion suppression occurs with the addition of the preservatives, calibration QC criteria are easily met with the 325-MS. Figure 3 shows example calibration curves for target compounds in reagent water containing the method preservation agents.

Precision and Accuracy

Laboratory Fortified Blanks (LFBs) in reagent water with method preservatives were prepared and analyzed at CAL 1 and 5. The results are listed in Tables 3 and 4. Note that the concentration of aldicarb, aldicarb sulfoxide, quinoline, and thiofanox are higher than 0.05 ppb. The EPA provided the standard at these concentrations to compensate for the differing responses observed in their LC/MS system used for method development. Mean recovery easily passes the ±30% criteria with %RSDs less than 20%.

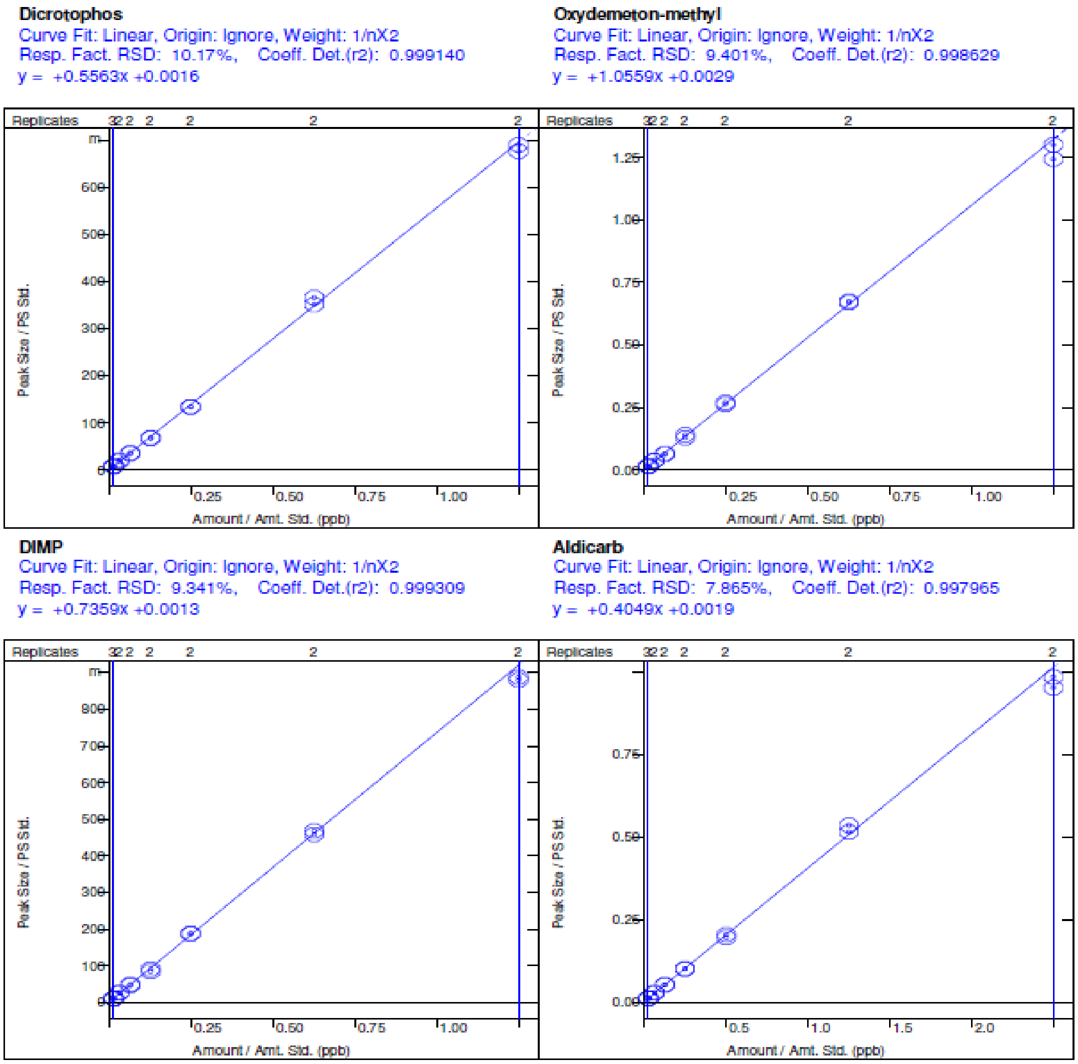


Figure 3. Calibration curves for dicrotophos (top left), oxydemeton-methyl (top right), DIMP (bottom left) and aldicarb (bottom right) in reagent water including method preservative agents.

Table 3. Precision and accuracy in reagent water including preservative agents fortified at 0.05 ppb[a] (n=8) for CAL 1.

Compound	Fortified Concentration (ppb)	Mean % Recovery	%RSD
Acephate	0.05	101	6.1
Aldicarb	0.10	92	9.1
Aldicarb sulfoxide	0.10	100	4.9
DIMP	0.05	95	10.0
Dicrotophos	0.05	79	11.2
Fenamiphos sulfone	0.05	96	7.6
Fenamiphos sulfoxide	0.05	90	17.5
Methamidophos/monitor	0.05	109	10.2
Oxydemeton-methyl	0.05	98	12.8
Quinoline	2.10	106	9.5
Thiofanox	0.20	111	13.5

[a] Except where otherwise noted

Table 4. Precision and accuracy in reagent water including preservative agents fortified at 0.99 ppb[a] (n=8) for CAL 5.

Compound	Fortified Concentration (ppb)	Mean % Recovery	%RSD
Acephate	0.99	100.6	2.2
Aldicarb	2.00	103.6	4.1
Aldicarb sulfoxide	2.00	107.3	3.8
DIMP	0.99	102.4	2.2
Dicrotophos	0.99	97.2	4.2
Fenamiphos sulfone	0.99	91.1	4.0
Fenamiphos sulfoxide	0.99	92.6	4.6
Methamidophos/monitor	0.99	94.9	3.1
Oxydemeton-methyl	0.99	102.9	3.0
Quinoline	43.0	101.1	5.4
Thiofanox	4.00	106.7	2.9

[a] Except where otherwise noted

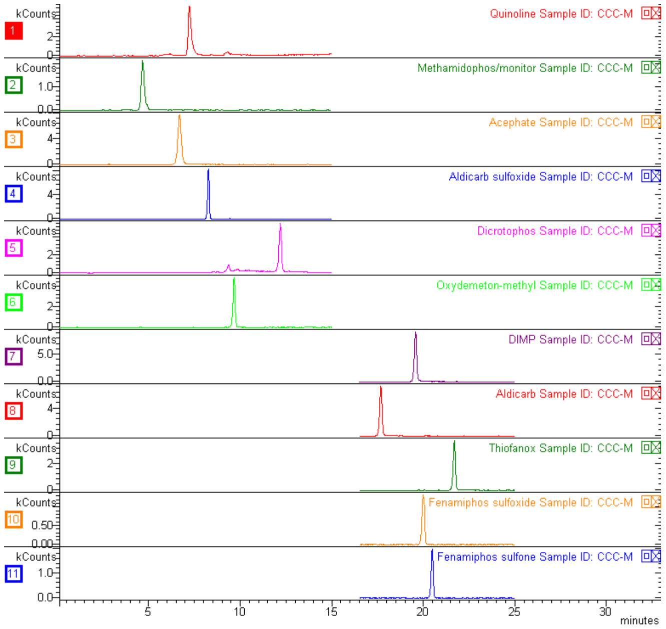


Figure 4. MRM traces for analytes with preservative agents at a concentration of 0.99 ppb[a]. [a]Except where otherwise noted in Table 4.

Figure 4 represents the Multiple Reaction Monitoring (MRM) traces for each target compound at CAL 5. For all analytes, excellent peak shape and sensitivity were observed from 0.05 ppb (50 ppt) upwards on the 325-MS. The majority of analytes were detected with good peak shape at 5 ppt in reagent water containing method preservative agents. MRM traces for acephate and DIMP are shown from 5-50 ppt in Figures 5 and 6.

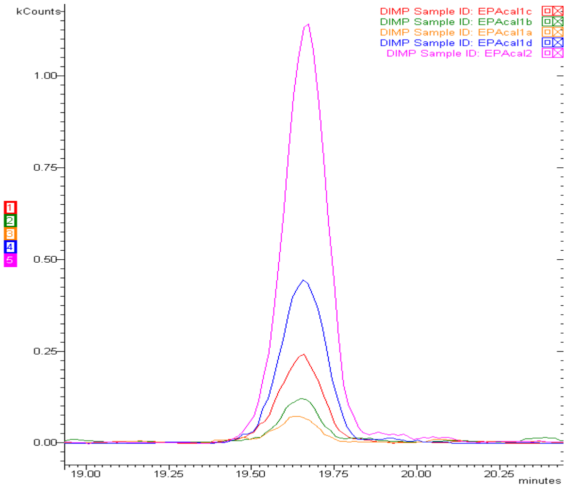


Figure 5. Overlaid MRM traces for DIMP with preservative agents at concentrations 5, 12, 25, 50 and 120 ppt.

Laboratory Fortified Matrix Replicates

Laboratory tap water was used for the analysis. The sample was properly preserved to ensure that no residual chlorine was present. Table 5 lists the results obtained for the study.

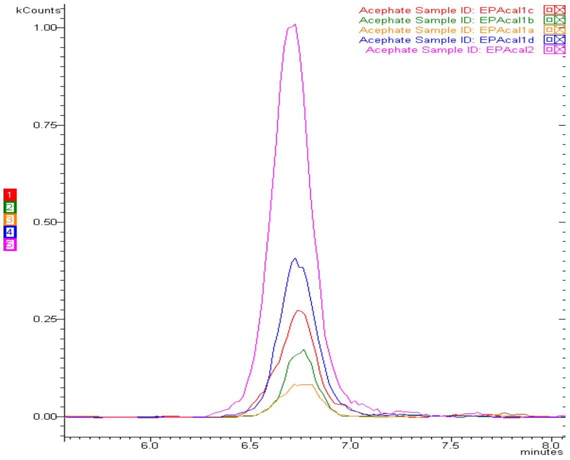


Figure 6. Overlaid MRM traces for acephate with preservative agents at concentrations 5, 12, 25, 50 and 120 ppt.

Table 5. Precision and accuracy in tap water including preservative agents fortified at 2.5 ppb[a] (n=8).

Compound	Fortified Concentration (ppb)	Mean % Recovery	%RSD
Acephate	2.50	102	2.8
Aldicarb	5.00	95	3.4
Aldicarb sulfoxide	5.00	91	5.7
DIMP	2.50	101	2.4
Dicrotophos	2.50	95	3.5
Fenamiphos sulfone	2.50	98	3.1
Fenamiphos sulfoxide	2.50	95	2.6
Methamidophos/monitor	2.50	96	2.9
Oxydemeton-methyl	2.50	93	2.3
Quinoline	107.00	104	3.5
Thiofanox	9.90	99	3.8

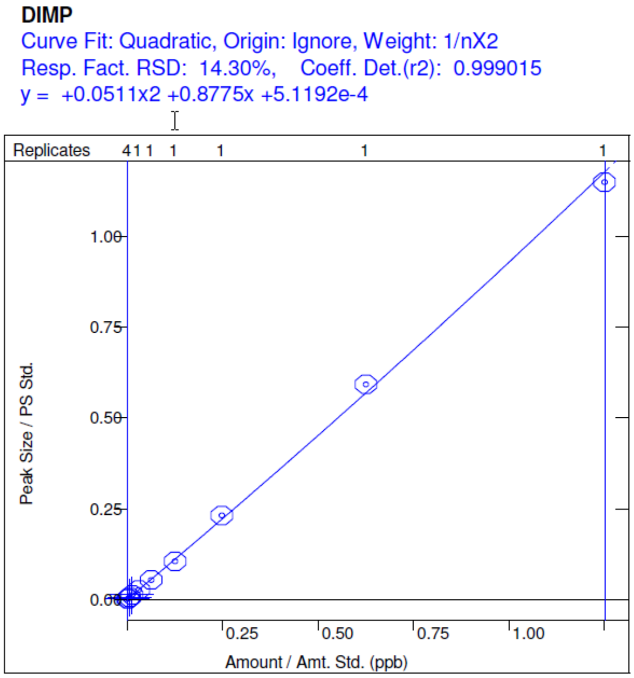
[a]Except where otherwise noted

LCMRL and Detection Limit (DL) Determination

For the Secondary Laboratory Demonstration, the Agency has asked participating labs to perform a Lowest Concentration Minimum Reporting Level (LCMRL) experiment. The LCMRL is described as the true concentration for which future recovery is predicted to fall with high confidence (99%) between 50 and 150% recovery.[3] This gives the user more accurate information on the reporting limit and true detection limit for analytes in the method. The procedure involves taking multiple concentration replicate data and processing it through a downloadable LCMRL calculator as measured concentrations versus the true concentrations.[4] The LCMRL calculator was developed through a collaboration of Shaw and EPA contract statisticians. Table 6 and Figure 8 are results obtained from the LCMRL calculator. In order to obtain valid calculations, it was necessary to prepare calibration levels below 0.05 ppb (CAL 1) for most analytes. Figure 7 displays a typical curve used for this process, showing excellent linearity down to 0.005 ppb (5 ppt).

Table 6. DLs and LCMRLs in reagent water.

Compound	DL (ppb)	LCMRL (ppb)
Acephate	0.0038	0.019
Aldicarb	0.043	0.013
Aldicarb sulfoxide	0.0093	0.022
DIMP	0.0026	0.020
Dicrotophos	0.019	0.076
Fenamiphos sulfone	0.0071	0.015
Fenamiphos sulfoxide	0.0166	0.014
Methamidophos/monitor	0.0086	0.071
Oxydemeton-methyl	0.020	0.033
Quinoline	0.63	1.1
Thiofanox	0.024	0.076



DIMP

Curve Fit: Quadratic, Origin: Ignore, Weight: 1/nX2

Resp. Fact. RSD: 14.30%, Coeff. Det.(r2): 0.999015

y = +0.0511x2 +0.8775x +5.1192e-4

Figure 7. Calibration curve for DIMP in reagent water including method preservative agents spanning concentration levels from 5 ppt – 2.5 ppb.

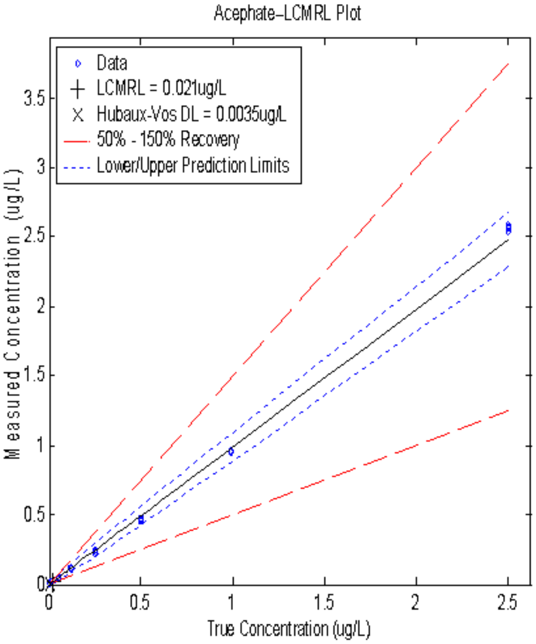


Figure 8. Acephate LCMRL regression plot.

Conclusion

The Varian 325-MS triple quadrupole mass spectrometer with vESI™ source provides excellent results for the EPA's new method Secondary Laboratory Validation Study. All quality control criteria are met. Only Varian can provide the complete solution for this method at the highest possible sensitivity:

- Vortex ESI (vESI) API for optimal desolvation and focusing of ions into the MS
- Patented features SelecFlow™ and SelecTemp™ of **both** the drying and vortex gasses for optimum sensitivity in a **single injection**
- Pursuit™ XRs C18 column for excellent peak shape

References

1. URL for EPA Unregulated Monitoring Rule Program: http://www.epa.gov/safewater/ucmr/index.html.
2. At the time of writing, the new method was in draft form and had no official assigned EPA method number.
3. Guidance Document for Determining Lowest Concentration Minimum Reporting Levels (LCMRLs), Document No. TSC-3-0344, Rev 2.0, Stephen Winslow.
4. Downloadable LCMRL calculator: http://www.cadmusweb.com/LCMRL/Beta.htm.

Varian, Inc.
www.varianinc.com
North America: 800.926.3000, 925.939.2400
Europe *The Netherlands*: 31.118.67.1000
Asia Pacific *Australia*: 613.9560.7133
Latin America *Brazil*: 55.11.3238.0400